MORPHOLOGY AND SYSTEMATICS OF THE ORDER PLECTIDA MALAKHOV, 1982 (NEMATODA)

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Chapter 1

General introduction

Nematodes – general remarks

Nematodes constitute one of the largest, most widely distributed and successful phyla of multicellular animals, occupying an enormous range of habitats and displaying a fascinating array of lifestyles. About 20.000 species have been described, but the expected number of nematode species is believed to be between 100.000 and several million. Dominating soil, litter and benthos, free-living nematodes play an important role in ecosystem functioning; amoung others they feed on bacteria, protozoa, algae, enchytraeids and plants. At the same time nematodes are the feeding source of other animals and fungi. Nematode parasites of animals and plants often have a strong impact on their host populations. In spite of variations in habitat, all nematodes possess a characteristic and specialised body plan, which allows the possibility of invasion of very different niches without strong morphological modification. Small size and ecological plasticity allow nematodes to occupy the whole biosphere.

Marine nematodes are found everywhere in the sediment from the littoral zone to the deep sea. Some Monhysteridae inhabit lacunary spaces of Arctic ice flows (Tchesunov & Riemann, 1995). Freshwater *Aphelenchoides* and *Monhystera* species even occur in hot springs at temperatures exceeding 50^{o}C. Others inhabit small aquatic habitats between the leaves of pitcher plants or bromeliads including those high in trees, as well as in the narrow space of detritus under the lichens on trees and rocks. Terrestrial nematodes were found on the Antarctic continent and in the alpine meadows at about 4000 m above sea level. Entomopathogens, as members of the genera *Heterorhabditis* Poinar, 1976 and *Steinernema* Travassos, 1927, infect insects with toxic bacteria that then multiply inside the host (see review in Gaugler, 2002), while marine Stilbonematidae live in obligate symbiosis with sulphur-oxidizing bacteria, which cover their cuticle (Ott et al., 1991). Worth mentioning are the parasitic nematodes of plants and animals, which inhabit almost all multicellular organisms, including other nematodes, but also are intracellular predators or parasites of Foraminifera (Tchesunov et al., 2000).

Nematode systematic – present and future

The systematics of nematodes have some peculiarities. Having rather similar body plans, nematodes differ in a large number of small characters, some of which may be expressed independently in seemingly identical but non-homologous modifications. The limitation of light microscopy, relative to the rare use of the ultrastructural studies (TEM and SEM), often prevents the researcher from correctly establishing homology, with respect to structure, of certain features. The absence of sufficient palaeontological data forces the scientist to base phylogeny and classification schemes solely from information about existing taxa. Higher taxa are sometimes grounded on the basis of few characters, resulting in many polyphyletic taxa and groups of uncertain affinity. It is still often the case that the descriptions of new taxa are based on very few distinct diagnostic characters, whereas the structural details that may provide more phylogenetically informative features are not mentioned; this oversight often necessitates restudy of the material in question.

Detailed analysis of morphology, development, genetics, biochemistry, ecology and evolution of nematodes is supported by the application of a range of new methods in zoology. Coomans (2000) pointed out that: “the main goals (of the future nematode systematics) are **1)** further characterisation of taxa for a better assessment of biodiversity and better understanding of ecological relationships; **2)** further analyses of phylogenetic relationships, and **3)** construction of a classification that reflects these relationships”.

Present state of knowledge on the biology of the order Plectida Malakhov, 1982

The order Plectida as it is currently accepted includes a “mixture of paraphyletic and/or misplaced families” (De Ley & Blaxter, 2002). Its present systematic position and ranking was accessed mostly on the base of only one family, Plectidae Örley, 1880, whose members of the genera *Plectus* Bastian, 1865 and *Tylocephalus* Crossman, 1933 were used in molecular phylogeny (Blaxter et al., 1998, 2000). The first members of the order were described by Bastian (1965), who proposed the genus *Plectus* and included nine species. Some years later Örley contributed to the classification of nematodes by erecting the families Plectidae and Leptolaimidae Örley, 1880. Morphology, distribution and taxonomy of certain genera of Plectida were studied by Allen & Noffsinger (1968), Anderson (1966), Andrássy (1985a, 1998), Coomans & Raski (1991a, 1991b), Heyns & Coomans (1980, 1983, 1990), Maggenti (1961a, 1961b), Raski & Coomans (1990, 1991), Tchesunov & Miljutina (2002), Zell (1993) and others.

In spite of the broad distribution and relatively high number of plectids in at least freshwater and terrestrial habitats, only two species were included in studies of energy budget, growth, respiration and productivity; furthermore, only ecology of species of the genus *Chronogaster* Cobb, 1913 was analysed in detail. The influence of temperature on the respiration and productivity of *Anonchus* sp. was studied by Layborn (1979). Several scientists (Klekowski et al., 1979; Schiemer et al., 1980) published experiments on respiration, growth and productivity in relation to food uptake for *Plectus palustris* de Man, 1880. Ettema et al. (2000) described the coexistence of five *Chronogaster*-species on 0.7 hectares of wetland during ten months.

Within the Plectida, embryological development studies were restricted to members of the Plectidae and only the pattern of early embryogenesis was described by Maggenti (1961a), Drozdovsky (1978) and Tahseen et al. (1992). Early cleavage and gastrulation in seven species of Plectidae were described by Lahl et al. (2003) using staining techniques and laser micromanipulation; these were compared with previously published data on embryogenesis in other nematode species. These authors found that embryos of *Plectus*-species share a number of developmental similarities with secernentean nematodes, but they also noted some differences that suggest an early phylogenetic separation of these two taxa.

The first detailed phylogenetic analysis of the superfamily Plectoidea Örley, 1880, published by Gagarin (1975), was based on morphological characters and ecological peculiarities of the taxon. He also suggested possible evolution of the stoma, valvular apparatus, renette cell, and he proposed a phylogeny of the Plectoidea and a new family, Chronogastridae Gagarin, 1975. Later classification schemes of Andrássy (1976), Lorenzen (1981), Maggenti (1981) and Inglis (1983) dealt with all free-living nematodes. Andrássy's classification was based mainly on phenetics and an intuitive evolutionary approach, while Lorenzen and Maggenti used monophyly criteria to varying extents. The most recent classification of De Ley & Blaxter (2002, 2004) used both morphology and molecular data. Unfortunately, all the above mentioned publications deal with taxa above the (sub-)family level, and therefore did not consistently consider all available information about morphological diversity. Moreover, the use of molecular data in phylogeny (Blaxter et al, 1998; 2000; De Ley, Blaxter, 2002, 2004; Litvaitis et al., 2000) is restricted to a relatively small number of sequenced species and so its robustness is strongly dependent on the set of representative species (less than 1% of the species described) and the algorithms employed for analysis.

Future prospects

Although, the number of publications devoted to the systematics of Plectida is relatively high, many aspects of their morphology, distribution, development, phylogeny and evolution remain to be solved. The specific diversity is far from completely described, morphological details are often lacking in species descriptions and the inter- and intraspecific variability has only been studied for very few genera. Furthermore, the embryonic, post-embryonic development, ecological peculiarities, growth, reproduction and resource utilisation is only known for few representatives. There is no published information about the internal ultrastructure of the digestive and reproductive system of Plectida, and only a few species and genera have been studied using scanning electron microscopy. The only available information from transmission electron microscopy is of Ceramonematidae, whose affinity to the Plectida is questioned. The currently available amount of rRNA sequences is restricted to four published and a few additional unpublished* data sets, thus making the molecular phylogeny of Plectida very provisional. All above-mentioned tasks for study await the researcher.

Objectives of the thesis

The Order Plectida is of particular interest for investigating the origin of Rhabditida. Rhabditida has several scientifically and economically important groups of nematodes including plant and animal parasites as well as the best known model species for molecular, developmental and genetic studies, *Caenorhabditis elegans* (Maupas, 1900) Dougherty, 1955. The similarities between plectids and rhabditids were noted already by Bütschli (1873) and Örley (1880); however, only Chitwood & Chitwood (1950) and Maggenti

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* 18S rDNA sequences are now publicly available for 25 different populations of the genera *Anaplectus, Chronogaster, Plectus, Tylocephalus* and *Wilsonema*; see also the National Centre for Biotechnology information web site at (www.ncbi.nlm.nih.gov).

(1961a) discussed the relationships between Plectidae and secernentean nematodes in detail. Paramonov (1964), following the opinion of other nematologists in suggesting that Plectidae are the intermediate link between the two, then recognised subclasses of nematodes: Adenophorea (=Aphasmidia) and Secernentea (=Phasmidia; now Rhabditida).

Quite recently, Fürst von Lieven (2003) found further similarities between plectids and rhabditids in the pharynx morphology and functioning, and he hypothesised that the family Plectidae and “Secernentea” evolved from a common ancestor. Embryological studies of Plectidae by Lahl et al. (2003) also showed many basic features of early development to be similar to those of “Secernentea” but not with “Adenophorea”. Finally, the molecular phylogeny of Blaxter et al. (1998), Litvaitis et al. (2000) and De Ley & Blaxter (2002) suggests a link between *Plectus* and “Secernentea”, usually considering Plectidae as the closest sister taxon of rhabditid nematodes, and assuming that Plectidae may be a surviving representative of the group from within which the secernentean radiation began.

Therefore, the objects of the present research are the freshwater and soil-inhabiting genera of the order Plectida and their closest marine relatives, thus excluding superfamilies Ceramonematoidea and Haliplectoidea from the analysis. The aim of the present study is: **1)** to describe in detail the morphology of different species using light and scanning electron microscopy; **2)** to discern possible evolutionary changes in the structure of the sensory organs, digestive, excretory and reproductive systems; **3)** to add to knowledge about the early stages of the post-embryonic development in members of the family Plectidae; **4)** to hypothesise phylogenetic relationships within Plectida (particularly of the freshwater and soil-inhabiting families) using morphological data,; **5)** to revise the taxonomy of families, on the basis of the detailed phylogeny; **6)** to elucidate the possible morphological and behavioural adaptations, or occurrence in the freshwaters and soil. The results, obtained in the present study, will be useful in the further analysis of the origin of the Rhabditida.

Outline of the thesis

These theses consist of six chapters, including general introduction and general discussion. In the first two chapters the morphology and phylogeny of the most primitive, marine and freshwater taxa are described. The subsequent two chapters deal mainly with terrestrial taxa, and their morphological features and evolutionary pathways are presented. Although, chapters 2-5 are based on published articles, herein they were updated to include the most recent data through footnotes and, where necessary, additional references.

At the first stage I revised the genus *Anonchus* Cobb, 1913 (Chapter 2). This genus is remarkable by its diverse morphology of the female reproductive organs and male copulatory apparatus, restricted distribution, and existence in marine, brackish and freshwater habitats. Furthermore, new information, obtained in the course of this study, allowed me to update and emend the taxonomy of the family Aphanolaimidae Chitwood, 1936.

On the basis of new data on morphology of six species of the superfamilies Leptolaimoidea Örley, 1880 and Camacolaimoidea Micoletzky, 1924, supplemented with the information taken from the literature for an additional eighteen genera, we proposed a phylogeny and revised classification for both superfamilies (Chapter 3) and a possible origin of Plectidae. This work was supplemented with a discussion of taxonomy of the genus *Leptolaimus* de Man, 1876. New information on males of *Odontolaimus chlorurus* de Man, 1880 and *Tobrilia imberbis* (Andrássy, 1953) Andrássy, 1967 contributed to reconsideration of their systematic position.

Chapter 4 includes descriptions of five species of the subfamily Wilsonematinae Chitwood, 1951 on the basis of studies with light and scanning electron microscopy. Special attention was given to the morphology and homology of the complex labial region within the subfamily. With consideration of newly received information and published data, the phylogenetic analysis elucidated relationships between genera of Wilsonematinae and its possible sister taxa.

The phylogenetic analysis of the superfamily Plectoidea, based on updated and emended morphological data for 35 species, is given in Chapter 5. It is followed by new data about postembryonic development in four species of the family Plectidae and a discussion of evolutionary pathways within this group. Finally, an updated classification of the superfamily Plectoidea is proposed.

Chapter 2

Morphology and systematics of the genus *Anonchus* Cobb, 1913 and reappraisal of the family Aphanolaimidae Chitwood, 1936

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Abstract

The genus *Anonchus* is revised. The genera *Assia* and *Haconnus* are considered to be synonymous with *Anonchus* on the base of the morphological analysis. Additional descriptions of six species, *viz. A. maculatus, A. mirabilis, A. millelacunatus, A. palaeotropicus, A. coomansi* and *A. pulcher* are provided on the basis of type material or recently collected populations. The lectotype of *A. monohystera* is designated and described and the synonymy of this species with *A. maculatus* is confirmed. Two new species are described. *A. winiszewskae* from Paraguay and *A. venezolanus* from Venezuela. Several aspects of the morphology are described and the variability and diagnostic significance of the main morphological characters discussed. A study of intrageneric and suprageneric taxonomy of the genus *Anonchus* is presented. The subfamily Aphanolaiminae is reinstated for the genera *Aphanolaimus, Aphanonchus* and *Paraphanolaimus* and raised to family rank. The family Aphanolaimidae includes two subfamilies: Aphanolaiminae and Anonchinae. An emended diagnosis and a revised classification of *Anonchus* are proposed.

Nomenclatorial remarks

Following nomenclatorial suggestions and changes were proposed in the original publication: **1)** New names "*Anonchus winiszewskae* Holovachov, Zullini, Loof & Bongers, 2002" and "*Anonchus venezolanus* Holovachov, Zullini, Loof & Bongers, 2002" were proposed for two new species. **2)** The genera *Assia* Gerlach, 1957 and *Haconnus* Andrássy, 1973 were considered a junior synonyms of the genus *Anonchus* Cobb, 1913, resulting in new combinations for *Assia laureata* Gerlach, 1957 (now *Anonchus laureatus* (Gerlach, 1957) Holovachov, Zullini, Loof & Bongers, 2002) and *Haconnus millelacunatus* Andrássy, 1973 (now *Anonchus millelacunatus* (Andrássy, 1973) Holovachov, Zullini, Loof & Bongers, 2002). **3)** The subfamily Aphanolaiminae Chitwood, 1936 was raised to the family rank. The date of publication of new names and new combinations, which were introduced in the original article, is 25 October 2002.

Introduction

The subfamily Anonchinae Andrássy, 1973 is a small group of marine and freshwater nematodes containing three genera, two of which *viz. Haconnus* and *Assia* are monotypic. The third genus, *Anonchus* includes six valid species of which four, including *Haconnus millelacunatus* and *Assia laureata* were only known from the original description. The remaining two: *A. mirabilis* and *A. maculatus* (with *A. monohystera* as a junior synonym) have been rarely found in freshwater habitats and have been described by several authors (Alekseev, 1990, 1992; Andrássy, 1968; Riemann, 1970; Zullini, 1982). Detailed morphological studies were performed on specimens of *A. mirabilis* by Chitwood and Chitwood (1936, 1974). Nevertheless, some aspects of morphology, variability, distribution and taxonomy of the group remained unknown.

Material and methods

The present study is based on new and type specimens of eight species of the subfamily Anonchinae: *A. maculatus* from Paraguay (courtesy Dr G. Winiszewska) and Colombia (courtesy Dr F. Riemann), type specimen of *A. monohystera* from the collection of N. A. Cobb as well as Cobb's unpublished descriptions and notes from the USDA Nematode Collection, Beltsville, USA (courtesy Dr Z. Handoo), *A. mirabilis* from Germany (courtesy Dr W. Traunspurger) and Italy, type specimens of *H. millelacunatus* from the Museum of Natural History, Budapest, Hungary (courtesy Dr I. Andrássy), type specimens of *A. palaeotropicus* from the Zoological Institute of RAS, St. Petersburg, Russia (courtesy Dr S. Ya. Tsalolikhin), *A. coomansi* from Ivory Coast (courtesy Dr M.-N. Helleouet) plus type specimens of the same species from the Laboratory of Nematology of Wageningen University, Wageningen, The Netherlands, type specimens of *A. pulcher* from INBio, Santo Domingo de Heredia, Costa Rica (courtesy Dr A. Esquivel), *A. winiszewskae* from Paraguay (courtesy Dr G. Winiszewska) and Colombia (courtesy Dr F. Riemann), *A. venezolanus* from Venezuela from the Laboratory of Nematology of Wageningen University, Wageningen, The Netherlands.

All material was available either on permanent slides in glycerine or as a suspension of nematodes in 4% formaldehyde. In the latter case specimens were processed to pure glycerine by a slow evaporation method and mounted on permanent Cobb slides in glycerine with paraffin wax as a support for the coverslip (S'Jacob & van Bezooijen, 1984). Additional data on the morphology of types of *A. maculatus* were kindly given by Dr I. Andrássy. As S. Gerlach had not made permanent slides until 1964 (F. Riemann, *in litt.*, 10.04.2001), types of *Assia laureata* and *Anonchus mangrovi* do not exist.

The total length of the stoma was measured from the anterior body end to the stoma base. The position of the amphid was measured from the anterior end to the anterior margin of the amphidial aperture. All curved structures were measured along the curved median line. The position of the supplements (distance from cloacal opening or anterior end) was measured as proposed by Zell (1993) for plectids. On the figures, black setae are located on the side of the body facing the viewer and white setae are located on the opposite side. Epidermal glands and body pores are shown on both sides on the figures of the caudal region of *A. maculatus, A. mirabilis, A. coomansi* and *A. winiszewskae*. On the other figures epidermal glands and body pores are shown on the side of the body facing the viewer. Data in the text and tables are presented as mean and range. Standard deviation was not calculated due to the small number of specimens studied.

General morphology

General aspect: Heat relaxed body almost straight in anterior half, only slightly curved ventrad in posterior part in female or strongly curved ventrad in posterior part in male. Body almost cylindrical anteriorly to vulva, tapering evenly posteriorly but narrowing more quickly on tail. Maximum body width anterior to vulva in females in monodelphic species and at vulva in didelphic, males body more cylindrical.

Cuticle: Cuticle always annulated with narrow, ridge-like lateral field. Anteriormost body annule always appearing posterior to amphid, at level with middle of stoma or posterior to stoma base. Somatic setae absent in females, present in males. Body pores present, arising from oval to round lateral epidermal gland cells.

Labial and cephalic sensilla: Labial sensilla papilliform, four cephalic sensilla setiform. *A. pulcher* is characterised by finely, but prominently, bifurcated cephalic setae. Finely bifurcated or bilobed cephalic setae were described for six species of *Aphanolaimus* de Man, 1880 (Coomans & De Waele, 1979; Andrássy, 1989; Raski & Coomans, 1990) and four species of *Paraphanolaimus* Micoletzky, 1923 (Raski & Coomans, 1991). In the latter genus this is discernible only with SEM. Nothing is known about the variability of this character or about its function. All other studied specimens of *Anonchus* have cephalic setae with finely rounded tips as seen under the light microscope. *A. venezolanus* is characterised by the presence of four additional subcephalic setae in male. This fact can not be explained easily, since the character is unusual for Leptolaimidae as a whole. Only three of the six species of the genus *Setostephanolaimus* Tchesunov, 1994 have a pair of lateral subcephalic setae in-between the circle of cephalic setae and amphid (see review in Platt, 1983). However, the genus is different in a number of morphological characters and is distantly related to *Anonchus*. It is not known whether the subcephalic setae of *A. venezolanus* are homologous to the neck setae of *A. mirabilis*, since we did not find any connection between these subcephalic setae and epidermal glands in the single available male of the former species.

Somatic sensilla: There are two pairs of neck setae in *A. mirabilis,* arising from third and fourth pairs of the body pores and connected to respective epidermal glands. The same character was observed for males of some species of *Aphanolaimus* and *Paraphanolaimus* (Eyualem, 1996; Raski & Coomans, 1990, 1991). In *Aphanolaimus yamani* Raski & Coomans, 1990*, Aphanolaimus chilensis* Raski & Coomans, 1990*, Aphanolaimus elegans* Raski & Coomans, 1990 and *Aphanolaimus fuegoensis* Raski & Coomans, 1990, a single pair of neck setae arise from the first pair of pores, in *Aphanolaimus tudoranceai* Zullini, 1988 – two pairs arise from the first and second pairs of pores, in *P. behningi* Micoletzky, 1923– two pairs arise from the first and fourth pairs of pores, in *P. latescens* Raski & Coomans, 1991 – a single pair arises from the fourth pair of pores, in *P. anisitsi* (Daday, 1905) – three pairs arise from the first, second and third or first, second and fourth pairs of pores and in *P. tahoensis* Raski & Coomans, 1991 – two or three pairs of setae arise from the first and second or first to third pairs of pores. Neck setae were found in all studied males in both populations of *A. mirabilis* but not in any other species of the genus. This could indicate the specific character of the feature.

Amphid: Amphid with rounded to oval aperture and larger subcuticular fovea. It is always located on the unstriated labial region, level with, or posterior to, the stoma. The amphid structure in species of *Anonchus* is usually described as unispiral. An SEM study by Eyualem (1996) on *A. coomansi* showed the amphidial aperture to be small and oval in shape. We found the same situation in all studied specimens. The "outer spiral" described for some species: *A. monohystera* by Cobb (1913), *A. maculatus* and *A. millelacunatus* by Andrássy (1968, 1973 respectively) – corresponds to the edge of a subcuticular amphidial fovea that is unispiral in shape and larger than the aperture.

Digestive system: Stoma heavily sclerotised, consist of two sections: the anterior (cheilostom) with strongly sclerotised lining, with six strongly sclerotised digitate projections; the posterior usually barrel-shaped, but shallower or more elongate tubular in some species. Pharynx muscular, almost cylindrical, with slight constriction at nerve ring level, lacking radial tubules, bulbs and valves. Pharyngeal glands often obscure. Dorsal gland orifice located just posterior to stoma base. Cardia glandular, free, with posterior part surrounded by intestinal tissue.

Secretory-excretory system: The ventral secretory-excretory gland cell complex was first described in detail in *Aphanonchus intermedius* Coomans & Raski, 1991 (Coomans & Raski, 1991). A comparable structure was also found in *Aphanolaimus*, *Paraphanolaimus* and *Anonchus* (Coomans & De Waele, 1979; Eyualem, 1996; Raski & Coomans, 1990, 1991; De Waele & Coomans, 1993). Although, some specific differences do exist, the general scheme of the ventral secretory-excretory gland complex is similar in all *Anonchus* species. The cell body of the ventral secretory-excretory gland is very large, *ca* two corresponding body widths long and contains granular cytoplasm. It is located midventrally opposite the cardia or somewhat posteriorly, extending posteriad into two ventrosublateral lobes that reach the tips of the anterior ovary or testis. Posterior part of cell with nucleus, anterior part with refractive vacuole which is connected with numerous filamentous structures. Posterior part of sclerotised canal making numerous coils and loops and forming a ball-like structure just anterior to vacuole. Canal extending forward along ventral side of pharynx with its anterior part opening into anterior stoma.

Female reproductive system: Female reproductive system didelphic, amphidelphic or monodelphic, prodelphic. Ovary branches reflexed antidromously. Two offset, oval, sac-like spermathecae located on each (right and left) side of each (anterior and posterior) gonoduct. The spermathecae are not visible in all specimens. Vagina always encircled by single sphincter muscle. *Pars refringens vaginae* undeveloped. Two species of *Anonchus*

(*A. maculatus* and *A. pulcher*) possess a very characteristic anterior curvature of vagina: S-shaped in the former and bent forward in the latter. An anteriorly bent vagina was described for two species of *Paraphanolaimus*: *P. behningi* Micoletzky, 1923 and *P. embryonophorus* (Alekseev & Naumova, 1977) Andrássy, 1984. Tsalolikhin (1989) questioned the vagina shape in the former species, but subsequent studies by Eyualem (1996) showed a very constant anterior vaginal bend in an Ethiopian population of this species[*]. Species of the genus *Aphanonchus* have an even more complex sigmoid vagina with strongly sclerotised *pars refringens vaginae* (see Coomans & Raski, 1991; Wu & Liang, 2000). A V-shaped vagina with the point of V directed forward was described for *Aphanolaimus aymarae* De Waele & Coomans, 1993 and *Aphanolaimus spiriferus* Cobb, 1914 (De Waele & Coomans, 1993, Raski & Coomans, 1990 respectively). Vagina structure is a constant specific character.

Male reproductive system: Male reproductive system monorchic, with outstretched, glandular anterior part and reflexed functional posterior testis lying right subventrally from the body axis in all studied males. Spicules always paired and symmetrical curved ventrad. Male accessory apparatus usually composed of two types of supplementary structures: midventral alveolar supplements anteriorly; midventral tubular supplements posteriorly. The alveolar supplements are circular invaginations arranged in a midventral row: those located in neck region are usually arranged in a zigzag manner and are closer to each other than those located more posteriorly in an almost straight line. Sublateral precloacal and caudal setae only present in males.

Caudal region: Three rectal glands (dorsal and two ventrosublateral) present in proximal rectum in females of *A. maculatus, A. mirabilis, A. coomansi, A. pulcher* and *A. winiszewskae*. They were also seen in some males of *A. mirabilis* and *A. coomansi*, but observations were obscured by the spicules and copulatory muscles. Rectal glands were not found in both sexes of *A. millelacunatus* and *A. venezolanus*. Tail almost similar in shape in both sexes (shorter and more curved ventrad in male), elongate conoid, gradually narrowing proximally; ventrally curved distally. Caudal glands present in all species but often obscure. Spinneret well developed in all species, with protruding tube.

Differential characters for the species identification are: shape of labial region; length and proportion of stoma and its sections; distance from anterior end to anteriormost body annule; position of amphid and anteriormost body pore, beginning of lateral field; number of lateral epidermal glands; number of female genital branches; development of spermathecae; shape and length of vagina; shape and length of spicules and gubernaculum; number and arrangement of male sensory structures (tubular and alveolar supplements, subcephalic, neck and precloacal setae). The variability of quantitative characters should be considered. See also Table 2.7.

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[*] See also Holovachov & Sturhan (2004a) for the discussion of vagina shape in *P. behningi.*

Species descriptions

Anonchus maculatus (Daday, 1905) T. Goodey, 1951

(Figs 2.1 & 2.2, Tables 2.1 & 2.5).

Lectotype of *Anonchus monohystera* Cobb, 1913[*]

Male: Cuticle damaged, outer cuticular layer defoliated, annules approximately 1.3 µm wide at midbody. Lateral field demarcated by two straight lines; originating on the 40th to 45th annule, at level with middle of pharynx; terminating at middle of tail. Crystalloids not seen. Labial region damaged, cephalic setae not seen. Amphid located at level with posterior stoma section. Anteriormost body pore located posteriorly to stoma base. Neck setae absent. Stoma somewhat flattened, thus stoma length/diameter ratio is lower. Anterior stoma section ca 6 µm long and 14 µm wide, posterior ca 5 µm long and 14 µm wide, barrel-shaped with posterior part enveloped by muscular pharyngeal tissue. Cardia, nerve ring, hemizonid, ventral secretory-excretory gland and gonad not seen. Spicules ventrally curved with oval manubrium and conical, gradually narrowing body. Gubernaculum rectangular, but damaged. Anteriormost alveolar supplement 28 µm from anterior end. Anteriormost tubular supplement 421 µm from anterior end and 315 µm from cloacal opening. Posteriormost tubular supplement 52 µm from cloacal opening, at level with spicule manubrium. Two (one pair) precloacal setae situated at spicule level. Only six (three pairs) ventrosublateral caudal setae discernible on tail. Distal portion of tail may bear other setae, but these are obscure. Unstriated tail tip swollen. Caudal glands not seen.

Remarks: The following taxonomically important data can be added to the lectotype description from the more complete original unpublished descriptions and drawings of N. A. Cobb (present terminology is followed).

Adult: Labial region bluntly rounded, with four cephalic setae. The stoma length/diameter ratio is nearly 1. Cardia length equal to corresponding body width.

Female: Reproductive system monodelphic, prodelphic. Vagina “leads inward and forward to the uterus a distance nearly equal to the corresponding body diameter” which may indicate an S-shaped curvature of vagina. Rectum longer than anal body width.

Male: Seventy one alveolar and 22 tubular supplements counted on drawing of the entire male.

Population from Paraguay

Adult: Annules 2 µm wide at midbody. Lateral field demarcated by two straight lines, 2 µm wide in female and 1 µm in male; originating on 40th to 47th annule, at level with posterior two-fifths of pharynx; terminating at middle of tail in female and just posterior to cloaca in male. Elongate crystalloids present in one female. Body pores 1-2 µm

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[*] The single male specimen available for study from the type collection of N.A. Cobb, is somewhat flattened with partially damaged anterior end and obscure sexual characters. It was designated as lectotype in Holovachov, Zullini, Loof & Bongers (2002) as no holotype was designated by Cobb (1913) and no other specimens from the type locality are present in his collection.

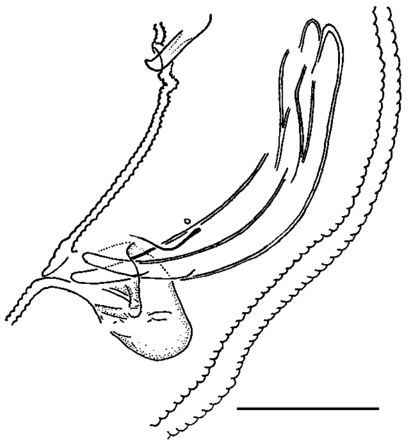

Figure 2.1. *Anonchus monohystera* Cobb, 1913. Lectotype. Male cloacal region. Scale bar 20 µm.

wide. Epidermal gland cells on average 13-15 µm long and 3-13 µm wide and filled with brown granules. Labial region bluntly rounded, continuous with body contour, with four 11-13 µm long (equal to 60-90% of labial region diam.) cephalic setae. Amphids located at level with posterior stoma section. Anteriormost body pore located posterior to stoma base on first to third annule. Anterior stoma section 7 µm long and 14 µm wide, posterior section 10-12 µm long and 11 µm wide and barrel-shaped. Posterior fourth of entire stoma length enveloped by muscular pharyngeal tissue. Cardia cylindrical, 18-23 µm long and 10.5-13 µm wide. Nerve ring surrounding pharynx at its posterior third. Hemizonid not seen. Central oval body of ventral secretory-excretory gland 65-81 µm long, situated ventrally opposite to cardia; nucleus oval, 11.5-15 µm long, nucleolus 2-4.5 µm wide. Intestinal cells filled with brown granules. Unstriated tail tip 11.5-15 µm long, swollen. Caudal glands obscured by epidermal glands.

Female: Reproductive system monodelphic, prodelphic. Ovary 163-177 µm long, located left subventrally from body axis in both females. Spermathecae 21-25.5 µm in diam., filled with round spermatozoa, 3.5 µm in diam. Vagina S-shaped: initially straight, then curved anteriad for majority of length and then curved to dorsal side to join uterus. Postvulval uterine sac present. Intra uterine egg not observed. Rectum 58-59 µm long, 2.6-2.7 times ABD long. Rectal glands present.

Male: Subcephalic and neck setae absent. Spicules 2.2 ABD long, ventrally curved with prominent rounded manubrium and conical, gradually narrowing body. Gubernaculum rectangular continuing dorsally into fine lamina. Anteriormost alveolar supplement 48 µm from anterior end. Anteriormost tubular supplement 635 µm from anterior end, 344 µm from cloacal opening. Posteriormost tubular supplement 54 µm from cloacal opening, at level with spicule manubrium. Two (one pair) precloacal setae at level with spicules. Eight (four pairs) caudal setae distributed as three ventrosublateral plus one dorsosublateral pair.

Population from Colombia: Similar to specimens from Paraguay except for shorter body, pharyngeal region, tail, narrower labial region, smaller stoma, lateral field starting more anteriorly, fewer epidermal glands, shorter vagina and shorter spicules. These differences are considered to be intraspecific variability. Anterior stoma section 4.5-7 µm long and 8-9 µm wide, posterior one 8-10.5 µm long and 7-8 µm wide. Intrauterine egg measuring 93×32 µm. Rectum 41.8 (37-46) µm long, 2.1 (1.8-2.3) times ABD long.

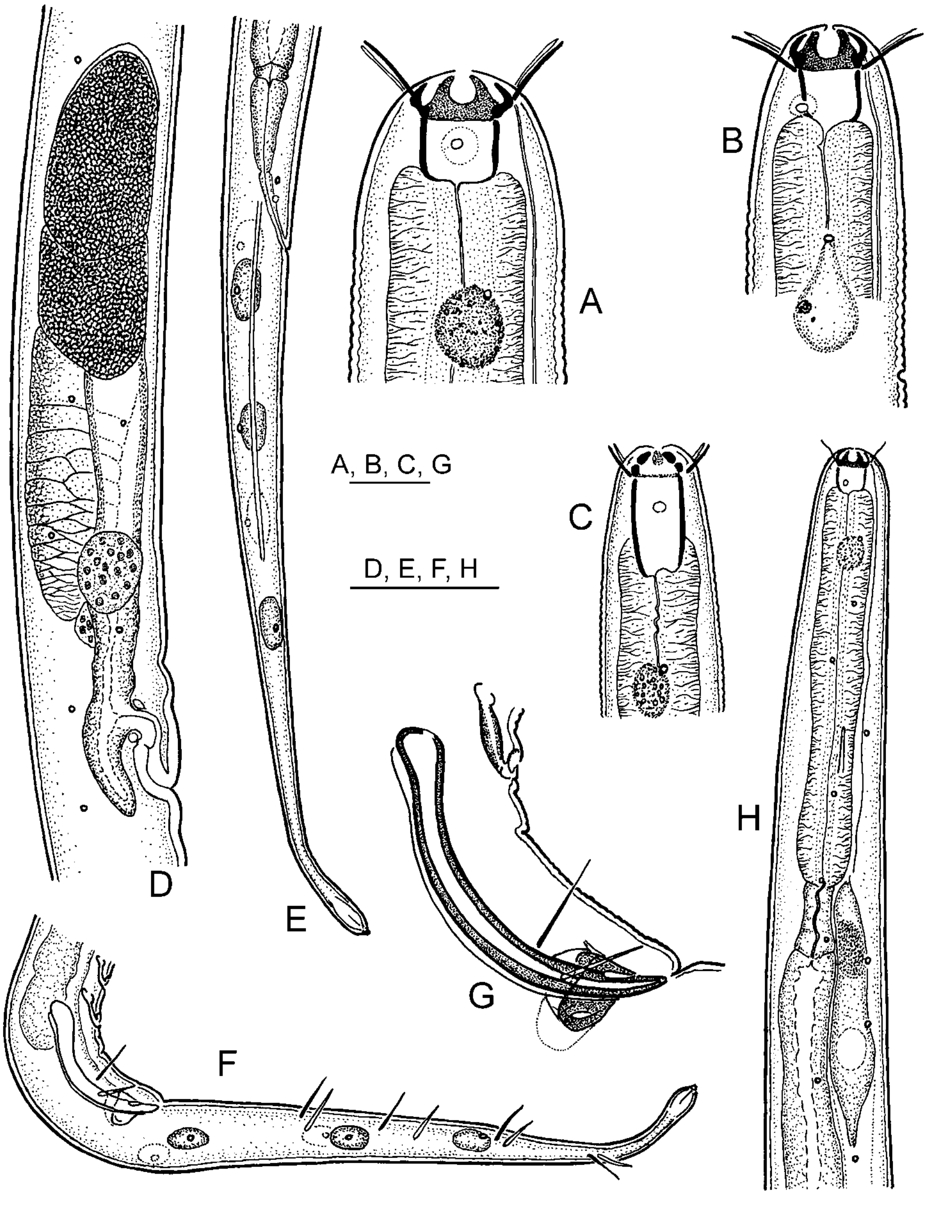

Figure 2.2. *Anonchus maculatus* (Daday, 1905) T. Goodey, 1951. A: Female anterior end; B: Male anterior end; C: Juvenile anterior end; D: Female reproductive system; E: Female tail; F: Male posterior region; G: Male cloacal region; H: Female pharyngeal region. Scale bars: A-C, G = 10 µm, D-F, H = 50 µm.

Table 2.1. Measurements of monodelphic species of the genus *Anonchus* (all measurements in µm except “Body length” in mm).

Species	*A. maculatus*					*A.* sp.
Locality	USA	Paraguay		Colombia		Colombia
	Lectotype	Female	Male	Female	Male	Male
n	1	2	1	8	6	3
Body length	0.89	1.36; 1.28	1.17	0.98 (0.85-1.11)	0.98 (0.90-1.07)	0.95-1.09
Body diam. (BD)	35	47; 46	32	37 (32-40.5)	28.8 (26.5-32)	36-39
Phar. region length	144	150; 147	147	132 (119-144)	129 (119-143)	138-153
Tail length	157	231; 233	190	165 (146-196)	152 (127-173)	156-185
Anal body diam.	32	22; 22	25	20 (17-23)	22.6 (21-25)	25-28
a	25.4	28.7; 28.1	36.5	26.7 (24.7-31.1)	34.5 (30.1-36.2)	25.7-27.9
b	6.2	7.9; 7.8	8.0	7.5 (6.3-8.4)	7.6 (7.1-8.1)	6.6-7.1
c	5.7	5.9; 5.5	6.2	6.0 (5.1-6.6)	6.5 (5.6-8.1)	5.7-6.1
c’	4.9	10.4; 10.7	7.5	8.3 (7.1-10)	6.9 (6.0-7.9)	6.2-6.6
Labial region diam.	15	18.5; 17	12.5	11.8 (10.5-14)	11 (10.5-11.5)	17-19.5
Stoma length (STL)	10.5	19; 16	12	14.9 (13-16)	14.2 (13-16)	21-25.5
Stoma diam. (STD)	14	11.5; 12	9	9 (8-9)	8.7 (8-9)	14-15
STL/STD	0.8	1.6; 1.3	1.3	1.6 (1.5-1.9)	1.7 (1.5-2)	1.4-1.8
Amphid location	13	12; 14	11.5	13.4 (13-16)	11 (10.5-13)	10.5-13
Anteriormost annule	26	26; 29	26	29 (24-37)	24.2 (22-29)	22-26.5
Anteriormost body pore	39	32; 32	28	33.6 (31-37)	30.4 (24-38)	26.5-30
Lateral field starts	69	99; 99	124	80.4 (73-87)	82.6 (78-90)	63-69
V or T	?	41.9; 41.9	58.7	45.5 (43.2-47.5)	62.8 (60.6-67.4)	61-62
G_1, %	-	17.4; 16.1	-	18.4 (15.1-20.1)	-	-
Vagina length	-	38; 41	-	27.2 (22.5-32)	-	-
Vagina/BD	-	0.8; 0.9	-	0.7 (0.6-0.8)	-	-
PUS length	-	43; 43	-	26.4 (18.5-44)	-	-
PUS/BD	-	0.9; 0.9	-	0.7 (0.5-1.2)	-	-
Spicules length	59	-	56	-	43 (40.5-45)	46-50
Gubernaculum length	8	-	9.5	-	9.7 (9-13)	9-10.5
Tubuli number	23	-	16	-	14 (13-17)*	21-23
Alveoli number	75	-	57	-	107 (94-141)*	63-100

* – n=8.

Anteriormost alveolar supplement 31.7 (25.6-40.5) µm from anterior end. Anteriormost tubular supplement 598 (522-664) µm from anterior end, 234 (219-242) µm from cloacal opening. Posteriormost tubular supplement 45.4 (40.5-49.5) µm from cloacal opening.

Diagnosis: *A. maculatus* is characterised by the 0.85-1.36 mm long body, presence of 48-73 lateral epidermal glands, bluntly rounded labial region, stoma with barrel-shaped posterior section, amphid located at level with posterior stoma section, anteriormost body pore located posteriorly to stoma base, lateral field starting at level with middle of pharynx, secretory-excretory cell body situated opposite to cardia; monoprodelphic female reproductive system, developed spermathecae, S-shaped vagina, presence of a postvulval uterine sac; male without subcephalic or neck setae and with 13-23 midventral precloacal tubular and 57-141 alveolar supplements, two precloacal and eight caudal setae, spicules 40.5-59 µm long with prominent rounded manubrium and conical, gradually narrowing, body, rectangular gubernaculum.

Justification of the identification: The following data could be obtained from the type material of *A. maculatus* (I. Andrássy, in litt., 04.12.2000): several females and one male in rather poor condition are available, in one female a postvulval uterine sac nearly half body diam. long is discernible, the rectum is anteriorly swollen (rectal glands are probably present), male with 21 tubular supplements, posteriormost lying level with anterior end of spicules, spicules equal in length, 48 µm along median curve. Recent specimens from Paraguay were collected close to one of the localities mentioned in the original description of Daday (1905), namely Asuncion. They clearly correspond with the syntype specimens in vagina structure, spicules and gubernaculum, as well as in general morphology as given in original description. Comparison of the male from Paraguay and the lectotype of *A. monohystera* shows no significant differences except for body length (1.17 *vs* 0.89 mm in the lectotype), position of anteriormost body pore (28 *vs* 39 µm respectively), starting of lateral field (124 *vs* 69 µm respectively) and number of tubular supplements (16 *vs* 23 respectively). Differences influenced by flattening were not taken into account. Riemann (1970) pointed that *A. maculatus* and *A. monohystera* differ in the position of excretory pore, which is posterior to the nerve ring in the latter. These data were taken from the Cobb formula in the original description. The excretory duct in the lectotype specimen was seen to extend along the entire pharyngeal region to stoma level. The excretory pore was not found and the excretory duct probably opens in the region of the anterior stoma. We consider our specimens from Paraguay to be identical with *A. maculatus* and conspecific with *A. monohystera*, the latter being a subjective junior synonym of the former. The number of tubular supplements strongly differs in different populations, varying from 23 in the lectotype, 22 as drawn by Cobb (unpublished), 22 given by Daday (1905), 21 in a syntype male (I. Andrássy, in litt. 04.12.2000), 13-17 in males from Colombia and 16 in a male from Paraguay. Nevertheless, the range in variation is almost continuous.

Material examined: Lectotype male, slide No G-12487 (USDA Nematode Collection) collected in USA: DC, Washington, Potomac river, in algae, May 3, 1911, *legit* N. A. Cobb. Paraguay: Encarnacion, 90 km along the road to Asuncion, in water with mud around the roots of semiaquatic plants, November 24, 1998; *legit* J. Kisielewski. Colombia: Magdalena, five kilometres along the river, 110 4.1' N / 740 50.2' W, in sand on the bank and at a depth of 20 cm in the river, August 24, 1967 (sites 28 and 29 in Riemann, 1970).

Distribution: The species was reported from Paraguay (Daday, 1908; Andrássy, 1968; present study), Argentina (Andrássy, 1968), Colombia (Riemann, 1970; present study), USA (Cobb, 1913; present study).

Anonchus mirabilis (Hofmänner in Hofmänner & Menzel, 1914) Chitwood & Chitwood, 1936

(Fig. 2.3, Tables 2.2 & 2.5).

Population from Italy

Adult: Annules 1.5-2 µm wide at midbody. Lateral field demarcated by two straight lines, 2 µm wide in female and 1-1.5 µm in male; originating on the 30th to 44th annule, at level with middle of pharynx; terminating at anterior third of tail in both sexes. Crystalloids absent. Body pores 1-2 µm wide. Epidermal gland cells on average 16-20 µm long and 8-10 µm wide and are filled with brown granules. Labial region bluntly rounded, continuous with body contour, with four 10.6 (8-12) µm long (equal to 40-70 % of labial region diam.) cephalic setae. Amphid located at level of posterior stoma section. Anteriormost body pore located posteriorly to stoma base, on second to fourth annule. Anterior stoma section 7.6 (6-8) µm long and 15.1 (11-17) µm wide, posterior section 14.4 (11-17) µm long and 11.7 (9-15) µm wide and barrel-shaped. Posterior fourth of entire stoma length enveloped by muscular pharyngeal tissue. Cardia cylindrical, 19.3 (10.5-30) µm long and 15.4 (14-17) µm wide. Nerve ring surrounding pharynx at its posterior third. Hemizonid not seen. Central oval body of ventral secretory-excretory gland 40-67 µm long, situated ventrally opposite to cardia; nucleus oval, 8-9 µm long, nucleolus 2 µm wide. Intestinal cells filled with brown granules. Unstriated tail tip 20 µm long, swollen. Caudal glands obscured by epidermal glands.

Female: Reproductive system didelphic, amphidelphic. Anterior ovary 95-118 µm long, located right subventrally from body axis in five females and left subventrally in four females. Posterior ovary 90-118 µm long, located right subventrally from body axis in four and left subventrally in five females. Both ovaries right subventrally in three, left subventrally in three, anterior right and posterior left subventrally in two, anterior left and posterior right subventrally in one female. Spermathecae 24-25 µm in diam., filled with oval spermatozoa, 3.5-4 µm in diam. Vagina straight. Intra uterine egg not observed. Rectum 43.4 (40.5-47.0) µm long, 1.7 (1.4-2.0) ABD long. Rectal glands present.

Male: Subcephalic setae absent. Two pairs of setae located in pharyngeal region: anterior pair at a distance of 106 (97-112) µm from anterior end, connected with third epidermal glands and posterior pair at a distance of 122 (116.5-133) µm from anterior end, connected with fourth epidermal glands. One male with additional third seta on right side of neck. Spicules 1.7 (1.5-1.9) ABD long, twisted along its body axis with elliptical capitular opening, almost cylindrical middle part and conical tips. Gubernaculum rectangular.

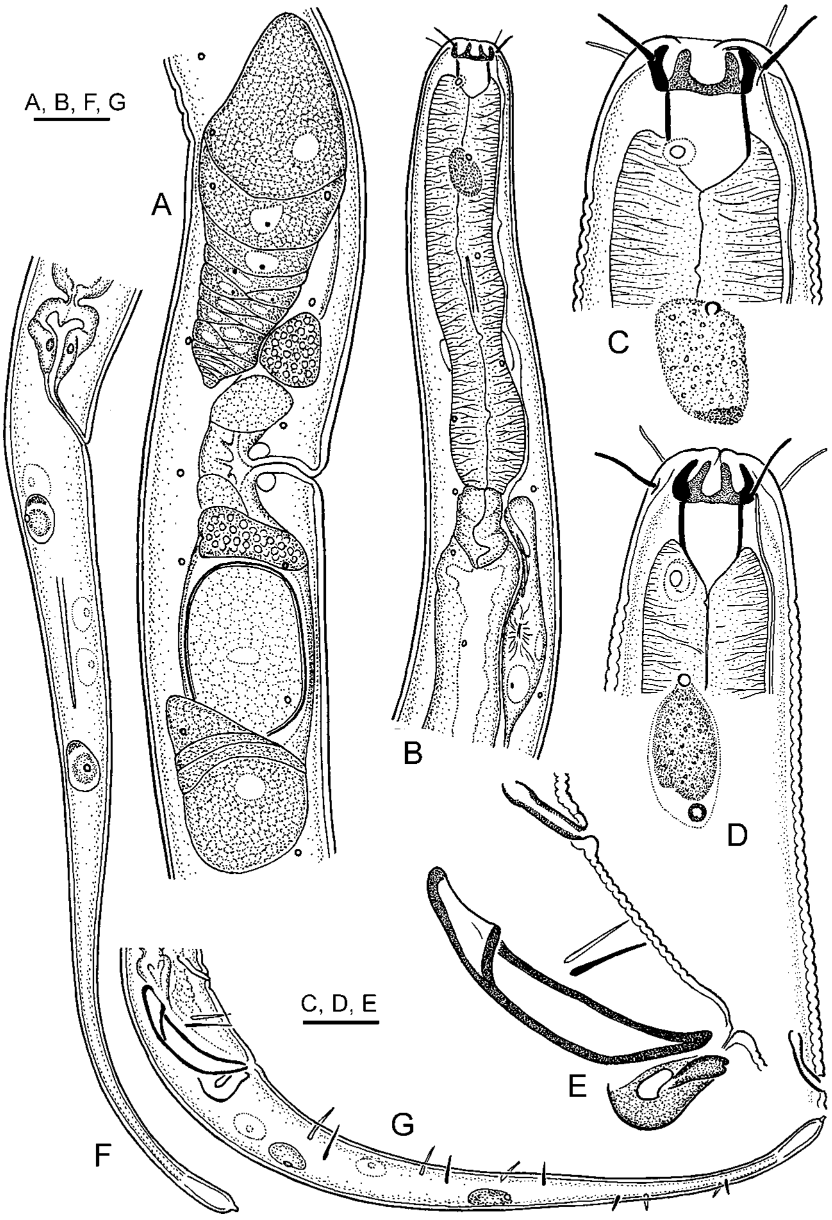

Figure 2.3. *Anonchus mirabilis* (Hofmänner in Hofmänner & Menzel, 1914) Chitwood & Chitwood, 1936. A: Female reproductive system; B: Female pharyngeal region; C: Female anterior end; D: Male anterior end; E: Male cloacal region; F: Female tail; G: Male posterior region. Scale bars: A-B, F-G = 25 µm, C-E = 10 µm.

Table 2.2. Measurements of didelphic species of the genus *Anonchus* (all measurements in µm except “Body length” in mm).

	A. mirabilis					*A. millelacunatus*	
Locality	Italy			Germany		Cuba	
	Female	Female	Male	Female	Male	Types	
						Female	Male
n	1	13	10	12	3	3	1
Body length	1.32	1.26 (1.12-1.40)	1.12 (1.10-1.17)	1.27 (1.08-1.40)	1.03-1.26	1.21-1.30	1.24
Body diam. (BD)	54	48.3 (45-53)	32.5 (28-39)	62.1 (56.5-68)	40.5-41.5	22-26.5	17
Phar. region length	186	187 (170-205)	166 (157-173)	160.6 (151-179)	137-146	194-213	161
Tail length	240	264 (242-283)	209 (185-236)	257.9 (219-278)	196-216	179-185	171
Anal body diam.	28	25.8 (23-29)	31 (27-37)	28.6 (25.5-32)	31-34.5	17-21	15
a	24.5	25.9 (24.3-27.3)	34.9 (28.5-40.6)	20.5(17.8-21.6)	24.9-30.4	49.1-55.0	73
b	7.1	6.7 (6.2-7.2)	6.8 (6.5-7.1)	7.9 (6.8-8.9)	7.4-8.8	6.0-6.2	7.8
c	5.5	4.8 (4.3-5.2)	5.4 (4.9-6.1)	4.9 (4.6-5.1)	5.0-5.7	6.5-7.0	7.2
c’	8.6	10.3 (9.8-11.5)	6.8 (5.6-7.7)	9.1 (7.5-10.4)	5.7-6.9	8.8-10.9	11.4
Labial region diam.	23	24.5 (23-25.5)	19.9 (16-22)	21.5 (19.5-24)	18.5-19.5*	15-16	13
Stoma length (STL)	25.5	23.3 (21-25.5)	19.6 (18.5-22)	21.9 (19.5-23)	21-24*	29-30	26.5
Stoma diam. (STD)	14	16.4 (14-17)	13.1 (11.5-15)	14.9 (14-16)	10.5-11.5*	10.5	9
STL/STD	1.8	1.4 (1.3-1.5)	1.5 (1.4-1.9)	1.5 (1.2-1.7)	2.0-2.1*	2.8-2.9	2.9
Amphid location	14	17.6 (15-22)	14.8 (13-17)	16.5 (11.5-19.5)	11.5-15*	7-8	6
Anteriormost annule	24	26.4 (23-30)	25.6 (23-30)	25.3 (23-31)	22-23*	8-13	8
Anteriormost body pore	37	34.5 (31-44)	31.3 (28-35)	36.3 (31-40.5)	28-33.5*	6-7	6
Lateral field start	78	75.7 (69-88)	74.2 (59-81)	74 (63-87)	59-81*	173-185	127
V or T	43.5	43.2 (40.5-45.8)	54.0 (50.5-58)	44.2 (41.0-50.0)	50.4-55.6	47.1-48.5	-
G_1, %	12.0	10.3 (8.7-11.3)	-	11.7 (8.9-13.4)	-	6.4-11.1	-
G_2, %	11.2	8.9 (7.2-10.6)	-	10.3 (8.7-11.5)	-	6.8-11.0	-
Vagina length	31	25.6 (23-31)	-	28.3 (24-33.5)	-	11.5-13	-
Vagina/BD	0.6	0.5 (0.5-0.6)	-	0.5 (0.4-0.5)	-	0.5	-
Spicules length	-	-	54.0 (48-64)	-	52-54	-	25.5
Gubernaculum length	-	-	17.5 (16-21)	-	15-19.5	-	13
Tubuli number	-	-	64 (54-72)	-	67-71*	-	1
Alveoli number	-	-	Abs	-	Abs	-	816

* – n=4.

Alveolar supplements absent. Anteriormost tubular supplement 63.5-96 µm from anterior end, 830 (792-858) µm from cloacal opening. Posteriormost tubular supplement 32-44 µm from cloacal opening, at level with spicule manubrium. Two (one pair) precloacal setae situated at spicule level. Ten-twelve (six-seven pairs) caudal setae present: three-four ventrosublateral and two dorsosublateral pairs.

Population from Germany: Similar to specimens from Italy except for shorter pharyngeal region and higher number of epidermal glands. Anterior stoma section 7.6 (7-8) µm long and 14.3 (11.5-16) µm wide, posterior 13.2 (10.5-15) µm long and 11.4 (9-13) µm wide and barrel-shaped. Intra uterine eggs (n=3) measuring 62-72×40-58 µm. Rectum 51.3 (44-57) µm long, 1.8 (1.4-2.2) times ABD long. Anteriormost tubular supplement 59-77 µm from anterior end, 770-982 µm from cloacal opening. Posteriormost tubular supplement 30-40.5 µm from cloacal opening.

Diagnosis: *A. mirabilis* is characterised by the 1.03-1.40 mm long body, presence of 60-94 lateral epidermal glands, bluntly rounded labial region, stoma with barrel-shaped posterior section, amphid located at level of posterior stoma section, anteriormost body pore located posterior to stoma base, lateral field starting level with middle of pharynx, secretory-excretory cell body situated opposite to cardia; didelphic female reproductive system, developed spermathecae, straight vagina; male without subcephalic and with four neck setae, with 54-72 midventral precloacal tubular supplements and without alveolar supplements, two precloacal and ten-twelve caudal setae, spicules 48-64 µm long, twisted along body axis with elliptical capitular opening, almost cylindrical middle part and conical tips, rectangular gubernaculum.

Justification of the identification: Since the original description of the species (Hofmänner & Menzel, 1914, 1915) lacks many important characters, identification is based mainly on the later redescription by the same author (Hofmänner, 1926). *A. mirabilis* is the only known species of *Anonchus* which possesses a row of tubular supplements extending from the cloaca anteriad towards anterior half of the neck and with alveolar supplements lacking. Recent specimens agree well with the original description in general morphology, female reproductive system, shape of stoma, spicules and gubernaculum, presence of only tubular supplements in male, but differ somewhat in some morphometric characters, such as: longer body (1.03-1.40 *vs* 0.96-1.1 mm) and b-ratio (6.2-8.9 *vs* 5.1-6.7). This is considered to be intraspecific variability.

Specimens described by Alekseev (1990, 1992) agree well with recent populations in general morphology except for shorter (42-43 along chord *vs* 48-64 µm along arc in present specimens) and differently shaped spicules, fewer tubular supplements (47-59 *vs* 54-72 respectively), shape of gubernaculum and tubular supplements, smaller stoma (13-15×7-9 *vs* 18.5-25.5×10.5-17 µm respectively), fewer lateral epidermal glands (32-54 vs 60-94 glands respectively). Taking into account the distant and discontinuous distribution we can assume that the specimens described by Alekseev (1990, 1992) may represent a separate, but closely related species (or subspecies). Since no material of the “far east” population was available for study, the question remains to be answered.

Material examined: Italy: Lecco province, Lake of Como at Colico, 199 meters above sea level (m a.s.l.), mesotrophic lake, in sediment with gravel + sand + pebbles, November 1978, *legit* A. Zullini; Varese province, Lake of Monate, mesotrophic lake, pH = 7.4, in the silt sediment, *legit* A. Zullini. Germany: Bavaria, Lake Hopfensee, in upper 2 cm of the sediment, August 1997, *legit* W. Traunspurger.

Distribution: Additional populations were found in Italy: Turin province, Lake of Viverone, 230 m a.s.l., eutrophic lake, in sediment with sand + silt + gravel, October 1979; Lecco province, Adda river near Imbersago, 300 m a.s.l., in sediment with sand + silt + gravel, December 1978 and January 1979 (all Italian specimens collected by A. Zullini); and Germany: Bavaria, Lakes Lustsee, Froschkausersee and Sulzbergersee (W. Traunspurger, in litt., 17.07.2001). The species was reported from Switzerland (Hofmänner & Menzel, 1914, 1915; Hofmänner, 1926), Italy (Zullini, 1982; present study), Germany (present study), Poland (Prejs, 1977a, 1977b, 1977c), Russia Kursk Bay (Zakhidov, 1974) and Far East (Alekseev, 1990, 1992).

Feeding habit: Diatoms, testacean amoebae and algae were observed in the intestine of several specimens of *A. mirabilis*.

Anonchus millelacunatus (Andrássy, 1973) Holovachov, Zullini, Loof & Bongers, 2002

(Figs 2.4 & 2.5, Tables 2.2 & 2.5)

Adult: Annules 1.7-1.9 µm wide at midbody. Lateral field starts as alae of unstriated cuticle; originating on the 80th annule, at level with posterior fifth of pharynx, then becoming demarcated by two straight lines, 2-3 µm wide; terminating at middle of tail in both sexes. Crystalloids absent. Body pores 1 µm wide. Epidermal gland cells on average 4.5-6 µm long and 3.5 µm wide and filled with brown granules. Labial region rounded, slightly offset from body contour, with four 11.5-13 µm long (equal to 70-90 % of the labial region diam.) cephalic setae. Amphids located at level of anterior half of posterior stoma section. Anteriormost body pore located at level of anterior stoma section, anterior to amphid, on unstriated labial region. Anterior stoma section 6-7 µm long and 9-10.5 µm wide, posterior section 21-24 µm long and 7-8 µm wide and tubular. Posterior fifth of entire stoma length enveloped by muscular pharyngeal tissue. Cardia cylindrical, 15-24 µm long and 8-13 µm wide. Nerve ring surrounding pharynx at its posterior fourth. Hemizonid not seen. Central oval body of ventral secretory-excretory gland 83 µm long, situated ventrally two corresponding body diam. posteriorly to cardia; nucleus oval, 13 µm long; nucleolus 2 µm wide. Intestinal cells filled with yellowish granules. Unstriated tail tip 13-15 µm long, almost conoid. Caudal glands obscured by epidermal glands.

Female: Reproductive system didelphic, amphidelphic. Anterior ovary 24-144 µm long, located left subventrally from body axis in two females and right subventrally in one female. Posterior ovary is 42-113 µm long, located left subventrally from body axis in two and right subventrally in one female. Spermathecae undeveloped, spermatozoa not observed. Vagina straight. Intra uterine egg not observed. Rectum 18.5-25.5 µm long, 0.9-1.5 ABD long. Rectal glands not observed.

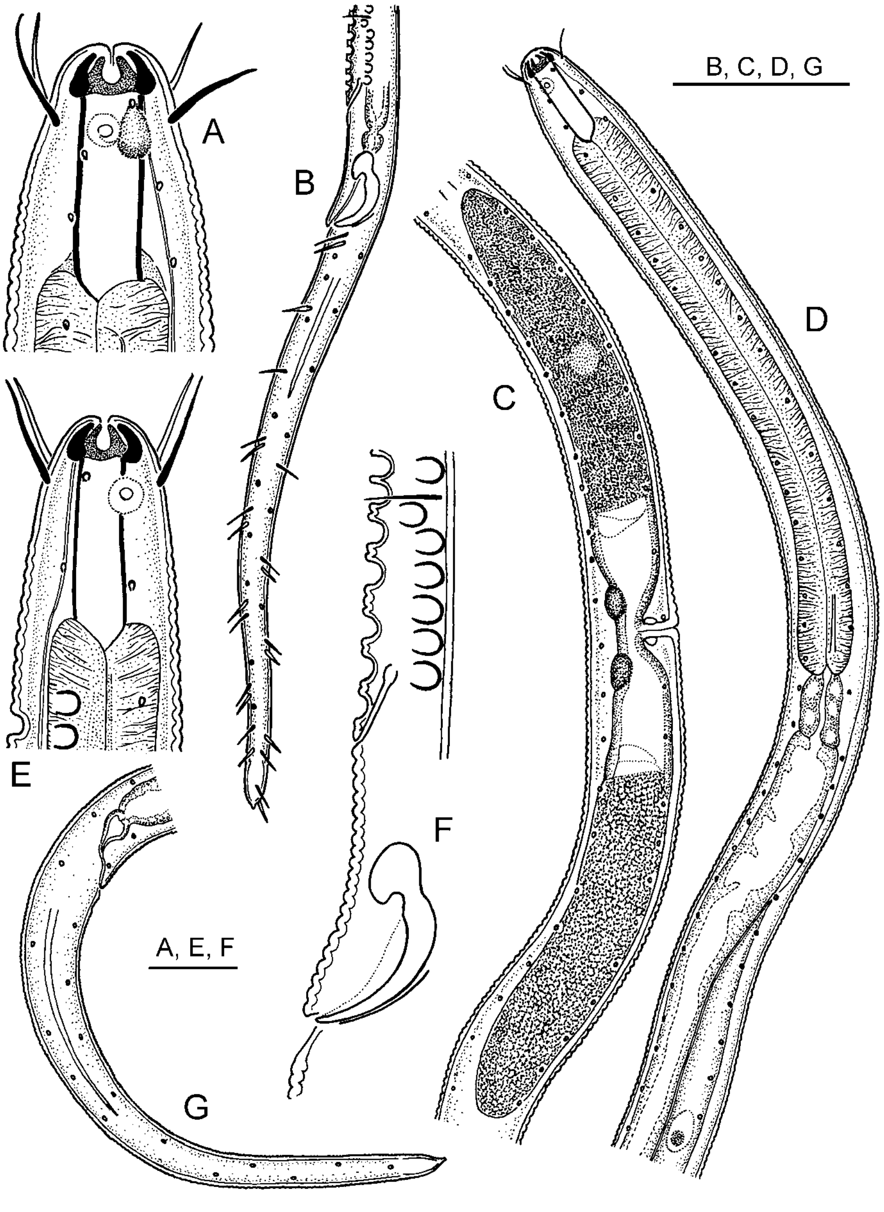

Figure 2.4. *Anonchus millelacunatus* (Andrássy, 1973) Holovachov, Zullini, Loof & Bongers, 2002. A: Female anterior end; B: Male posterior region; C: Female reproductive system; D: Female pharyngeal region; E: Male anterior end; F: Male cloacal region; G: Female tail. Scale bars: A, E, F = 10 µm, B-D, G = 50 µm.

Figure 2.5. Distribution of the alveolar supplements in male of *Anonchus millelacunatus* (Andrássy, 1973) Holovachov, Zullini, Loof & Bongers, 2002.

Male: Subcephalic and neck setae absent. Spicules 1.7 ABD long, with large oval manubrium and arcuate conoid body. Gubernaculum platelike. Alveolar supplements very conspicuous, each occupying 2-3 body annules, distributed in three longitudinal rows: midventral and two ventrosublateral (right and left). Alveoli in each row divided into two groups, anterior and posterior, separated by regularly annulated cuticle. Distribution of alveolar supplements as shown in Fig 5. Single midventral tubular supplement 10.5 µm long, located anteriorly to spicules, 32 µm from cloacal opening. Two (one pair) precloacal setae situated far above tubule, level with sixth-seventh alveoli. Twenty-three caudal setae present: seven subventral and five subdorsal on left and seven subventral and four subdorsal setae on right side of tail.

Remark: A second male on the same slide as holotype was not included in the description due to the strongly curved body not allowing accurate measurements. Small morphometrical differences between the original description and this study are due to differences in measuring and error. We could not confirm the presence of body setae posterior to stoma as depicted on Abb. 3A in Andrássy (1973). At the same level numerous filamentous particles were found, which could be misinterpreted as thin and slender body setae.

Diagnosis: *A. millelacunatus* is characterised by the 1.21-1.30 mm long body, presence of 317-387 lateral epidermal glands, rounded to slightly offset labial region, stoma with wide tubular posterior section, amphid located at level with anterior third of stoma, anteriormost body pore located anterior to amphid, lateral field starting at level with posterior part of pharynx, secretory-excretory cell body situated at or more than two corresponding body diam. posterior to cardia; didelphic female reproductive system, undeveloped spermathecae, straight vagina; male without subcephalic and neck setae, with one midventral precloacal tubular and 816 alveolar supplements, two precloacal and 23 caudal setae, spicules 25.5 µm long with large oval manubrium and arcuate conoid body, platelike gubernaculum.

Material examined: Holotype female and paratype male (latter not included) on slide Cu-8140, allotype male and two paratype females on slide Cu-8144 (Museum of Natural History, Budapest, Hungary) collected in Cuba: Matanzas province, Cárdenas, Playa Varedero, Atlantic coast, in sand with detritus, April 20, 1969, *legit* Dr. L. Botoşăneanu; Oriente province, Playa Baracoa, Atlantic coast, in sand, April 4, 1969, *legit* Dr. L. Botoşăneanu (sites 25 and 13 in Andrássy, 1973 respectively). The species is known only from the type locality.

Anonchus palaeotropicus Tsalolikhin, 1989

(Fig. 2.6, Table 2.3).

Adult: Annules 1.2-1.4 µm wide at midbody. Lateral field demarcated by two straight lines, 1.5 µm wide in both sexes; originating at level of pharynx. Crystalloids absent. Body pores 1.5 µm wide. Epidermal gland cells on average 10-15 µm long. Labial region bluntly rounded, continuous with body contour, with four cephalic setae. Amphid located level with stoma base or somewhat posteriorly. Anteriormost body pore located posteriorly to stoma base. Anterior stoma section 4-5 µm long, posterior section 15-24 µm long in female and 20 µm long in male and barrel-shaped. Posterior third of entire stoma length enveloped by muscular pharyngeal tissue. Cardia, nerve ring, hemizonid and secretory-excretory gland not seen. Unstriated tail tip 25 µm long, swollen. Caudal glands present.

Female: Reproductive system didelphic, amphidelphic. Anterior ovary 80-130 µm long. Posterior ovary 75 µm long. Spermathecae not seen. Vagina straight. Intra uterine egg 66×25 µm. Rectum and rectal glands not seen.

Male: Subcephalic and neck setae not seen. Spicules about one ABD long, arcuate, gradually narrowing. Gubernaculum platelike. Anteriormost alveolar supplement lying level with pharynx. Posteriormost tubular supplement lying at level with spicule manubrium. Precloacal and caudal setae not seen.

Diagnosis: *A. palaeotropicus* is characterised by the 0.96-1.01 mm long body, bluntly rounded labial region, stoma with barrel-shaped posterior section, amphid located at level of stoma base, anteriormost body pore located posterior to stoma base; didelphic female reproductive system, straight vagina; male with 23 midventral precloacal tubular and numerous alveolar supplements, spicules 35-40 µm long arcuate, gradually narrowing, gubernaculum platelike.

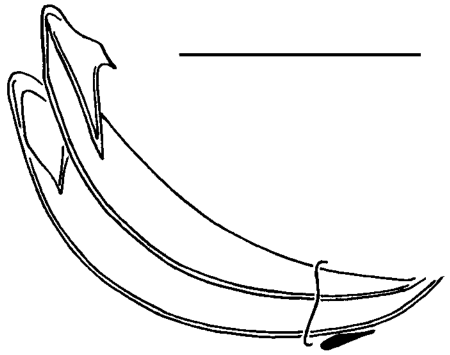

Figure 2.6. *Anonchus palaeotropicus* Tsalolikhin, 1989. Spicules and gubernaculum. Scale bar 20 µm.

Material examined: Holotype female on slide A-3956 and paratype female and male (Zoological Institute of RAS, St. Petersburg, Russia) collected Tanzania: Bujumbura town, lake Tanganyika, 1978, *legit* Dr. A. G. Perekhodtsev. The species is known only from the type locality.

Anonchus coomansi Eyualem, 1996

(Fig. 2.7, Tables 2.3 & 2.5).

Adult: Annules 1.4-1.9 µm wide at midbody. Lateral field demarcated by two straight lines, 1-2 µm wide in both sexes; originating on 28th to 36th annule, at level with middle of pharynx; terminating at middle of tail in female and anterior fifth of tail in male. Crystalloids absent. Body pores 1-2 µm wide. Epidermal gland cells on average 9-11.5 µm long and 6-7 µm wide, filled with brown granules which are more prominent in some specimens. Labial region bluntly rounded, continuous with body contour, with four 7-11.5 µm long (equal to 60-80 % of labial region diam.) cephalic setae. Amphid located level with stoma base. Anteriormost body pore located posteriorly to stoma base, on third to fourth annule. Anterior stoma section 4.8 (4.5-6) µm long and 7.9 (8-11.5) µm wide, posterior section 9.0 (7.5-10.5) µm long and 7.8 (7-9) µm wide and barrel-shaped. Posterior third of entire stoma length enveloped by muscular pharyngeal tissue. Cardia cylindrical, 15 (11.5-18.5) µm long and 11.5 (9-14) µm wide. Nerve ring surrounding pharynx at its posterior third. Hemizonid not seen. Central oval body of ventral secretory-excretory gland 41-52 µm long, situated ventrally opposite cardia; nucleus oval 10-13 µm long. Intestinal cells filled with brown or green granules. Unstriated tail tip 14 (11.5-16) µm long, swollen. Caudal glands obscured by epidermal glands.

Female: Reproductive system didelphic, amphidelphic. Anterior ovary 69-82 µm long, located right subventrally from body axis in two females. Posterior ovary 47-93 µm long, located left subventrally from body axis in two females. Spermathecae 10-19 µm in diam., filled with oval spermatozoa, 2-3.5 µm in diam. Vagina straight. Intra uterine egg not observed. Rectum 28.7 (25.5-30) µm long, 1.4 (1.3-1.7) times ABD long. Rectal glands present.

Male: Subcephalic and neck setae absent. Spicules 1.7-2.0 ABD long, twisted along its body axis with elliptical capitular opening, almost cylindrical middle part and conical tips. Gubernaculum platelike. Anteriormost alveolar supplement 18.7 (14-22) µm from anterior end. Anteriormost tubular supplement 407 (377-431) µm from anterior end, 335 (298-361) µm from cloacal opening. Posteriormost tubular supplement 40 (38-41.5) µm from cloacal opening, anterior to spicules. Two (one pair) precloacal setae situated at level with spicules. Eight-fourteen (four-seven pairs) caudal setae present: three to five ventrosublateral and one to two dorsosublateral pairs.

Diagnosis: *A. coomansi* is characterised by the 0.80-0.94 (0.54-0.83 in type population) mm long body, presence of 71-104 (45-59) lateral epidermal glands, bluntly rounded labial region, stoma with barrel-shaped posterior section, amphid located at level of stoma base, anteriormost body pore located posterior to stoma base, lateral field starting at level with middle of pharynx, secretory-excretory cell body situated opposite to cardia;

Table 2.3. Measurements of didelphic species of the genus *Anonchus* (all measurements in µm except "Body length" in mm).

	A. palaeotropicus		*A. coomansi*		*A. venezolanus*		
Locality	Tanzania		Ivory Coast		Venezuela		
	Types		Female	Male	Holotype	Paratype	
	Female	Male				Female	Male
n	2	1	9	5		1	1
Body length	0.96-1.01*	0.96*	0.87 (0.82-0.92)	0.88 (0.80-0.94)	1.05	1.04	0.97
Body diam. (BD)	39; 35	29	38.1 (33.5-44)	26.5 (23-30)	28	23	20
Phar. region length	170; 156	167	132 (123-143)	123 (119-128)	120	122	123
Tail length	178-200*	132*	147 (129-159)	139 (127-158)	142	135	146
Anal body diam.	?	?	20.1 (17-23)	20.3 (18.5-22)	20	17	24
a	25.8-31*	33*	23.2 (19.3-26.1)	33.4 (30.6-35)	37.8	45.1	49.2
b	5.9-6.5*	5.7*	6.6 (6.1-7.1)	7.2 (6.6-7.7)	8.7	8.5	7.8
c	5.4-5.5*	7.2*	6.0 (5.4-6.6)	6.4 (5.8-7)	7.4	7.7	6.6
c'	?	?	7.4 (6.6-9.1)	6.7 (6.2-7.2)	7.2	7.8	6.1
Labial region diam.	11; 10	11	14.4 (13-16)	12 (11.5-13)	21	23	21
Stoma length (STL)	21; 29	25	14.4 (14-15)	13 (12-14)	23	24	22
Stoma diam. (STD)	7; 8	9	9.6 (8-11.5)	8.3 (8-9)	15	18.5	16
STL/STD	3.1; 3.6	2.8	1.5 (1.2-1.9)	1.6 (1.4-1.7)	1.5	1.3	1.4
Amphid location	21; 35	20	14 (13-16)	12.2 (11.5-15)	9	9	9
Anteriormost annule	20; 35	20	20.9 (18.5-24)	17.8 (16-19.5)	21	23	21
Anteriormost body pore	31; 43	43	22.8 (19.5-25.5)	20.5 (18.5-25.5)	7	7	6
Lateral field start	110; 150	?	68 (63-73)	60.5 (58-63)	28	?	26
V or T	47-49*	-	46.1 (44.8-48)	54.6 (52.2-57.2)	49.4	49.9	48.9
G_1, %	13; 8	-	12.4 (9.8-14.9)	-	-	-	-
G_2, %	?; 7.4	-	12.2 (8.9-15.2)	-	-	-	-
Vagina length	13; 15	-	18.1 (15-23)	-	7	8	-
Vagina/BD	0.3; 0.4	-	0.5 (0.4-0.5)	-	0.25	0.35	-
Spicules length	-	45-48	-	36 (34.5-37)	-	-	26-28
Gubernaculum length	-	5	-	10.7 (9-11.5)	-	-	13
Tubuli number	-	23*	-	22 (21-24)	-	-	1
Alveoli number	-	?	-	88 (85-94)	-	-	Abs

* – Measurements from Tsalolikhin (1989), rest – original.

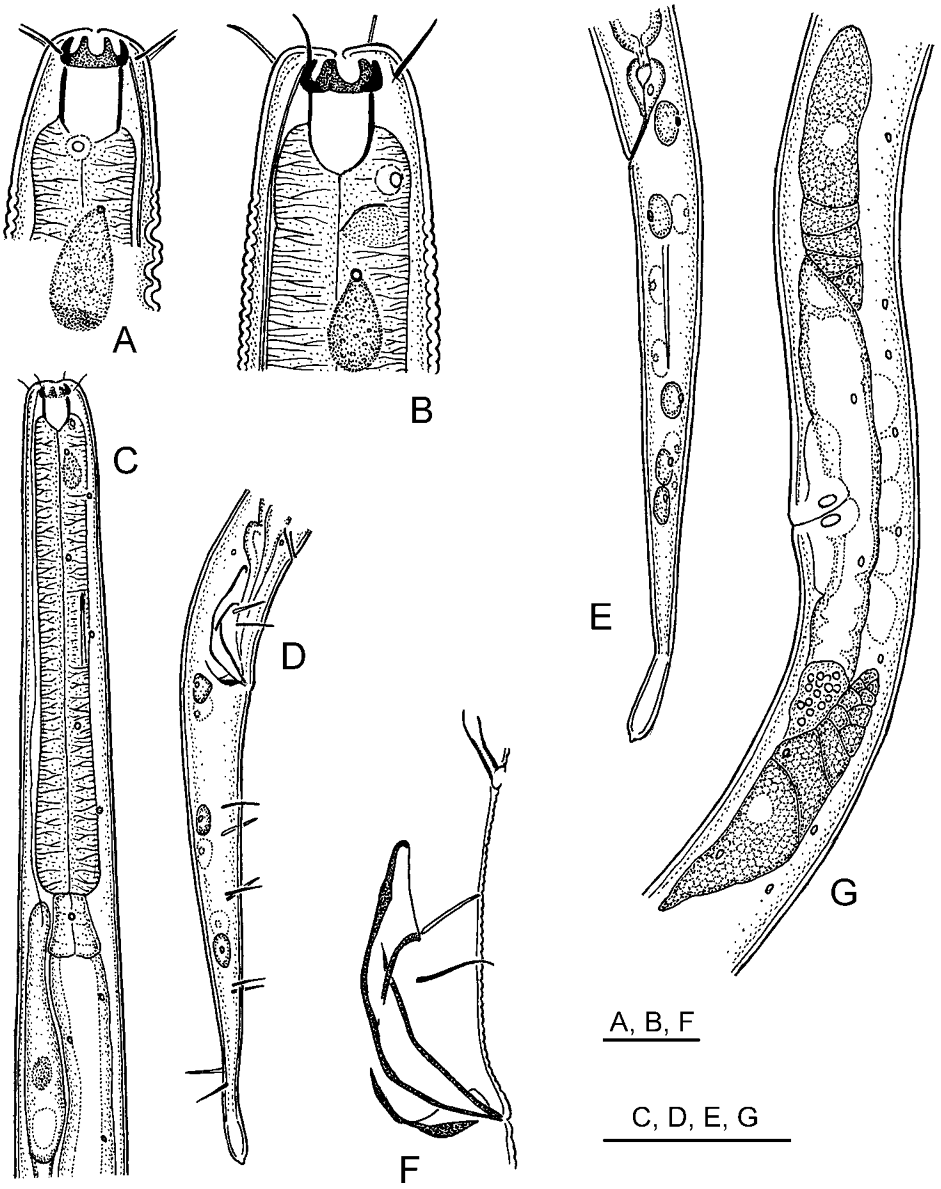

Figure 2.7. *Anonchus coomansi* Eyualem, 1996. A: Male anterior end; B: Female anterior end; C: Female pharyngeal region; D: Male posterior region; E: Female tail; F: Male cloacal region; G: Female reproductive system. Scale bars: A, B, F = 10 µm, C-E, G = 50 µm.

didelphic female reproductive system, developed spermathecae, straight vagina; male without subcephalic and neck setae, with 21-24 (20-28) midventral precloacal tubular and 85-94 (80-112) alveolar supplements, two precloacal and eight-fourteen caudal setae, spicules 34.5-37 (27-45) µm long twisted along body axis with elliptical capitular opening, almost cylindrical middle part and conical tips, gubernaculum platelike.

Justification of the identification: Present specimens agree well with the original description in general morphology, structure of stoma, vagina and male copulatory apparatus, but differ in longer body (0.80-0.94 *vs* 0.54-0.83 mm in type population), longer (12-15 *vs* 6-10 µm respectively) and wider (8-11.5 *vs* 5-7 µm respectively) stoma, higher number of epidermal glands (87-104 *vs* 45-59 – see Table 2.5), more anterior position of the posteriormost tubular supplement (38-41.5 from cloacal opening *vs* 13-17 µm). As no qualitative differences were found, we consider this to be intraspecific variability and regard our specimens as A. coomansi.

Locality and habitat: Ivory Coast: Abidjan, Ebrie lagoon, January 1992 (sites C and D in Guiral et al., 1995), Abidjan, Acadja, 300 m north by the river form the Ebrie lagoon, January 1992 (sites J and L in Guiral et al., 1995).

Distribution: The species was reported from Ethiopia (Eyualem, 1996), Ivory Coast (Guiral et al., 1995; present study).

Anonchus pulcher Zullini, Loof & Bongers, 2002

(Fig. 2.8, Tables 2.4 & 2.5).

Adult: The following data should be added to the original description. Crystalloids absent. Labial region bluntly rounded, offset by a distinct depression, with four 8-9 µm long (equal to 45% of the labial region diam.) finely bifurcated cephalic setae. Amphid located at level with posterior stoma section. Anteriormost body pore located posterior to stoma base, on third to sixth body annule. Anterior stoma section 6-8 µm long and 10.5-15 µm wide, posterior section 6-8 µm long and 9-13 µm wide and barrel-shaped. Unstriated tail tip 17 µm long, swollen. Caudal glands obscured by epidermal glands.

Female: Reproductive system monodelphic, prodelphic. Ovary 48.5-144 µm long, located right subventrally from body axis. Vagina bent anteriorly. Postvulval uterine sac present. Rectal glands present.

Male: Subcephalic and neck setae absent. Spicules 2.7-3.0 ABD long, with slightly swollen oval manubrium and nearly isodiametric tubular body. Gubernaculum a complex structure with a heavy sclerotised part 13 µm long, continuing dorsally with an even larger thin lamina. Anteriormost alveolar supplement 35-45 µm from anterior end. Anteriormost tubular supplement 420 (397-458) µm from anterior end, 488 (469-505) form cloacal opening. Posteriormost tubular supplement 54-64 µm from cloacal opening, at level of spicule manubrium. Two (one pair) precloacal setae situated at spicule level. Ten (five pairs) caudal setae present: three ventrosublateral and two dorsosublateral pairs.

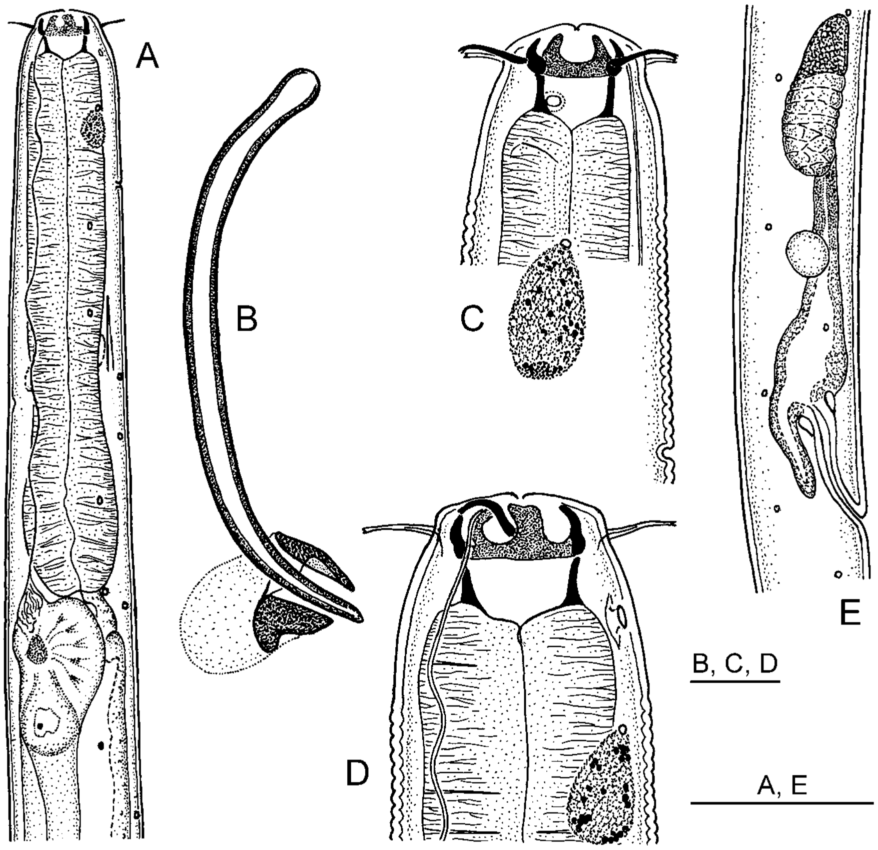

Figure 2.8. *Anonchus pulcher* Zullini, Loof & Bongers, 2002. A: Female pharyngeal region; B: Spicules and gubernaculum; C: Male anterior end; D: Female anterior end; E: Female reproductive system. Scale bars: A, E = 50 µm, B-D = 10 µm.

Diagnosis: *Anonchus pulcher* is characterised by the 1.02-1.11 mm long body, presence of 89-102 lateral epidermal glands, labial region offset by depression, stoma with barrel-shaped posterior section, amphid located at level with posterior stoma section, anteriormost body pore located posterior to stoma base, lateral field starting at level with middle of pharynx, secretory-excretory cell body situated opposite to cardia; monoprodelphic female reproductive system, developed spermathecae, anteriorly bent vagina, postvulval uterine sac present; male without subcephalic and neck setae, with 28-31 midventral precloacal tubular and 51-60 alveolar supplements, two precloacal and twelve caudal setae, spicules 72-74 µm long with slightly swollen oval manubrium and nearly isodiametric tubular body, rectangular gubernaculum.

Material examined: Complete type material from Costa Rica: Area Conservación Osa, path Samia, brook Salitre, tropical humid forest, October 1997, sand, gravel and silt sediment. For the deposition of type specimens we refer to Zullini et al. (2002). The species is known only from the type locality.

Anonchus winiszewskae Holovachov, Zullini, Loof & Bongers, 2002[*]

(Fig. 2.9, Tables 2.4 & 2.5).

Type population from Paraguay

Adult: Annules 1.5 µm wide at midbody. Lateral field demarcated by two straight lines, 1 µm wide in both sexes; originating on the 26th to 35th annule, at level with middle of pharynx; terminating at middle of tail in female and anterior fourth of tail in male. Elongate crystalloids present in all specimens. Body pores 1-2 µm wide. Epidermal gland cells on average 7-11.5 µm long and 6-8 µm wide and are filled with brown granules. Labial region bluntly rounded, continuous with body contour, with four 6 µm long (equal to 50-70 % of labial region diam.) cephalic setae. Amphid located posteriorly to stoma base. Anteriormost body pore located posteriorly to stoma base, on first to third annule. Anterior stoma section 4-4.5 µm long and 6.5-8 µm wide, posterior section 1-1.5 µm long and 6-7 µm wide and shallow. Posterior third of entire stoma length enveloped by muscular pharyngeal tissue. Cardia cylindrical, 13-16 µm long and 8-11.5 µm wide. Nerve ring surrounding pharynx at its posterior third. Hemizonid visible in one female only, 2 µm long, its anterior margin lying 71.5 µm from anterior end. Central oval body of ventral secretory-excretory gland 37-44 µm long, situated ventrally opposite to cardia; nucleus oval, 6-7 µm long; nucleolus 1 µm wide. Intestinal cells filled with brown or green granules. Unstriated tail tip 11.5-16 µm long, swollen. Caudal glands obscured by epidermal glands.

Female: Reproductive system monodelphic, prodelphic. Ovary 69-106 µm long, located right subventrally from body axis in two females and left subventrally in one female. Spermathecae 14-18.5 µm in diam., filled with oval spermatozoa, 1-2 µm in diam. Vagina straight. Postvulval uterine sac present. Intra uterine egg not observed. Rectum 22-26 µm long, 1.2-1.5 times ABD long. Rectal glands present.

Male: Subcephalic and neck setae absent. Spicules two ABD long, with prominent rounded manubrium and conical, gradually narrowing body. Gubernaculum platelike with strongly developed oval dorsal apophysis. Anteriormost alveolar supplement 30 µm from anterior end. Anteriormost tubular supplement 379 µm from anterior end, 272 µm from cloacal opening. Posteriormost tubular supplement 22 µm from cloacal opening, at level with spicule manubrium. Two (one pair) precloacal setae situated at spicule level. Ten (five pairs) caudal setae present: three ventrosublateral and two dorsosublateral pairs.

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[*] After the publication of the original article, a new population of this species came into my hands. It was collected in Costa Rica (Holovachov & Esquivel, unpublished) thus adding to the range where this species occurs.

Table 2.4. Measurements of monodelphic species of the genus *Anonchus* (all measurements in μm except "Body length" in mm).

Species	*A. pulcher*		*A. winiszewskae*			
Locality	Costa Rica		Paraguay			Colombia
	Types		Holotype	Paratypes		Female
	Female	Male		Female	Male	
n	3	5		2	1	1
Body length	1.05-1.08	1.06 (1.02-1.11)	1.01	0.82; 0.74	0.78	0.78
Body diam. (BD)	34-42	29.6 (28-31)	38	34; 31	21	30
Phar. region length	165-170*	157 (153-160)*	118	90; 76	89	89
Tail length	176-183	175 (167-181)	174	149; 92	127	143
Anal body diam.	18-20	25.8 (24-27)	18	17; 14	20	18.5
a	26-31	36 (35-37)	26.4	24.2; 23.5	37.0	25.9
b	6.1-6.3	6.4 (6.2-6.5)	7.5	7.9; 8.0	7.8	8.7
c	5.7-6.1	6.1 (5.7-6.3)	5.8	5.5; 8.0	6.1	5.4
c'	8.8-10	6.8 (6.2-7.5)	9.7	8.6; 6.5	6.0	7.7
Labial region diam.	19-20	15.3 (15.0-15.5)	12	11; 11	10.5	11.5
Stoma length (STL)	14-15*	13 (13-14)*	7	6; 6	6	7
Stoma diam. (STD)	14-15*	11.1 (10.5-11.5)*	8	8; 7	7	8
STL/STD	0.9-1.1*	1.1 (1.1-1.2)*	0.9	0.8; 0.9	0.9	0.9
Amphid location	11.5-15*	10.8 (10.5-11.5)*	10	11; 11	10	11.5
Anteriormost annule	18-27	14-18	19	15.5; 19	16.5	19.5
Anteriormost body pore	29-30*	26.3 (24-29)*	21	21; 21	19	24
Lateral field starts	77-80*	74.3 (62-80)	64	61; 40	52	52
V or T	49-50	57.0 (56.0-58.1)	42.9	44.0; 49.4	55.1	44.5
G_1, %	17-21	-	14	16; 19	-	20
Vagina length	42.5-47*	-	17	13; 12	-	14
Vagina/BD	1.0-1.3	-	0.5	0.4; 0.4	-	0.5
PUS length	21-22	-	31	20.5; 15.5	-	26.5
PUS/BD	0.6	-	0.8	0.6; 0.9	-	0.9
Spicules length	-	73.4 (72-74)	-	-	39	-
Gubernaculum length	-	9.0 (8-10.5)	-	-	15.0	-
Tubuli number	-	28-31	-	-	20	-
Alveoli number	-	51-60	-	-	53	-

* – Additional measurements of type specimens.

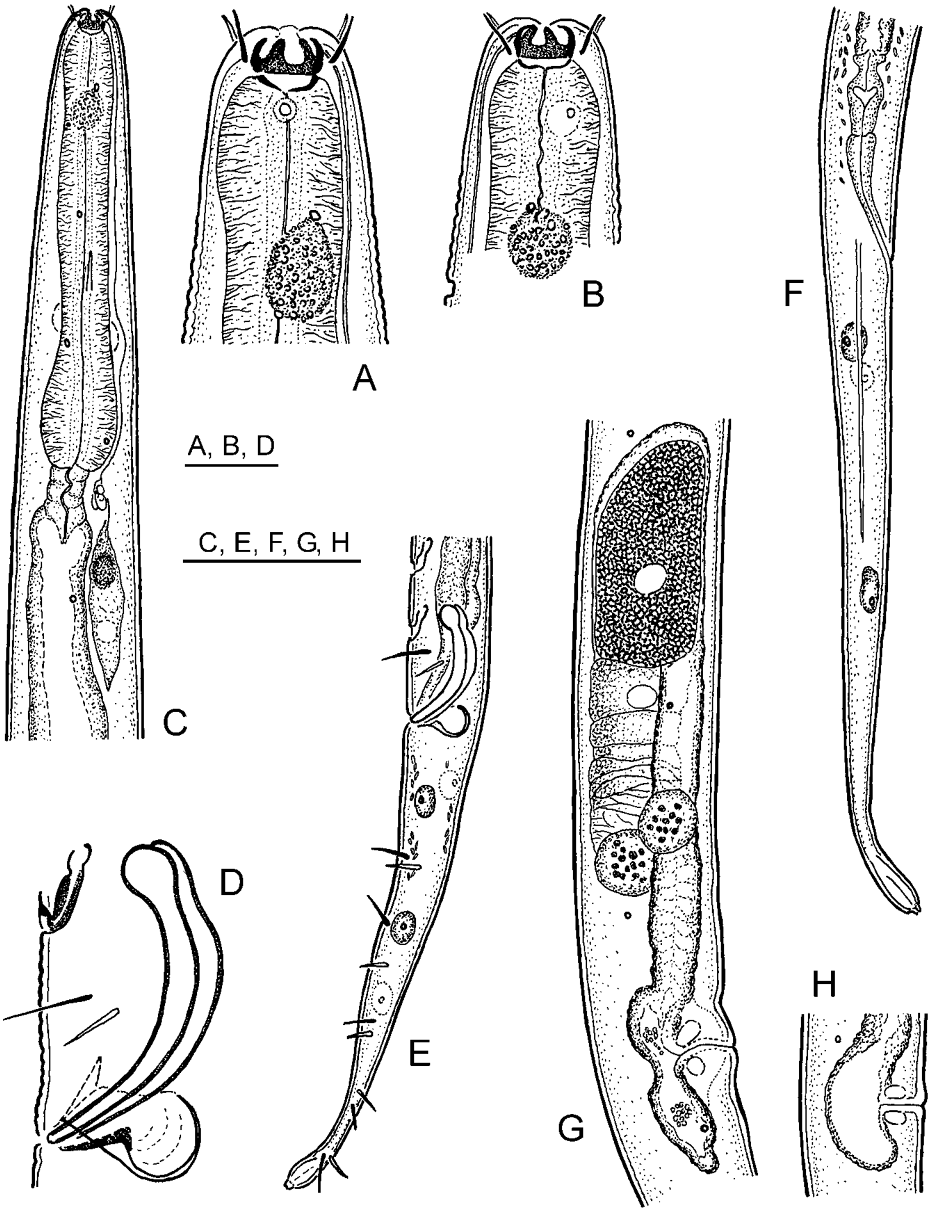

Figure 2.9. *Anonchus winiszewskae* Holovachov, Zullini, Loof & Bongers, 2002. A: Female anterior end; B: Male anterior end; C: Female pharyngeal region; D: Male cloacal region; E: Male posterior region; F: Female tail; G: Female reproductive system; H: Female vagina. Scale bars: A, B, D = 10 µm, C, E-H = 50 µm.

Population from Colombia: Single female similar to the females from type population, differing slightly in measurements. Hemizonid 2 µm long, its anterior margin lying 70 µm from anterior end. Unstriated tail tip 19.5 µm long, swollen. Rectum 31 µm long, 1.7 times ABD long.

Diagnosis: *A. winiszewskae* is characterised by the 0.74-1.01 mm long body, presence of 29-34 lateral epidermal glands, bluntly rounded labial region, stoma with shallow posterior section, amphid located posteriorly to stoma base, anteriormost body pore located posterior to stoma base, lateral field starting at level with middle of pharynx, secretory-excretory cell body situated opposite to cardia; monoprodelphic female reproductive system, developed spermathecae, straight vagina, postvulval uterine sac present; male without subcephalic and neck setae, with 20 midventral precloacal tubular and 53 alveolar supplements, two precloacal and ten caudal setae, spicules 39 µm long with prominent rounded manubrium and conical gradually narrowing body, gubernaculum platelike with dorsal apophysis.

Relationships: *A. winiszewskae* is distinguished from other *Anonchus*-species in having a short and shallow second stoma section (1-1.5 long *vs* 6-24 µm long in other species). In having only an anterior ovary *A. winiszewskae* resembles *A. maculatus* and *A. pulcher*. In addition to the characters mentioned above, the new species differs from *A. maculatus* in amphid located posterior to stoma base (*vs* level with second stoma section), fewer epidermal glands (19-24 *vs* 48-73), straight vagina (*vs* S-curved anteriad), shape of gubernaculum; from *A. pulcher* in amphid located posterior to stoma base (*vs* level with second stoma section), cephalic setae finely rounded under LM (*vs* finely bifurcated), fewer epidermal glands (19-24 *vs* 89-102), straight vagina (*vs* bent anteriad), shorter spicules (39 *vs* 72-74 µm), shape of gubernaculum.

Type locality and habitat: Paraguay: Encarnacion, 90 km along the road to Asuncion, in water with mud around the roots of semiaquatic plants, November 24, 1998; *legit* J. Kisielewski.

Other locality and habitat: Colombia: Magdalena, 5 km along the river, in sand on the bank of the river, August 24, 1967 (site 29 in Riemann, 1970).

Type specimens: Holotype female and paratype male in the nematode collection of Museum I Instytut Zoologii, PAN, Warszawa, Poland and two paratype females in the nematode collection of Laboratory of Nematology, Wageningen University, Wageningen, The Netherlands. Additional female from Colombia in the nematode collection of Institut für Meeresforschung, Bremerhaven, Germany.

Anonchus venezolanus Holovachov, Zullini, Loof & Bongers, 2002

(Fig. 2.10, Tables 2.3 & 2.5).

Adult: Annules 2.3 µm wide at midbody. Lateral field demarcated by two crenate lines, 1 µm wide in both sexes; originating on unstriated labial region, just posterior to amphid. Lateral field at level with stoma base starting as a single irregular line but

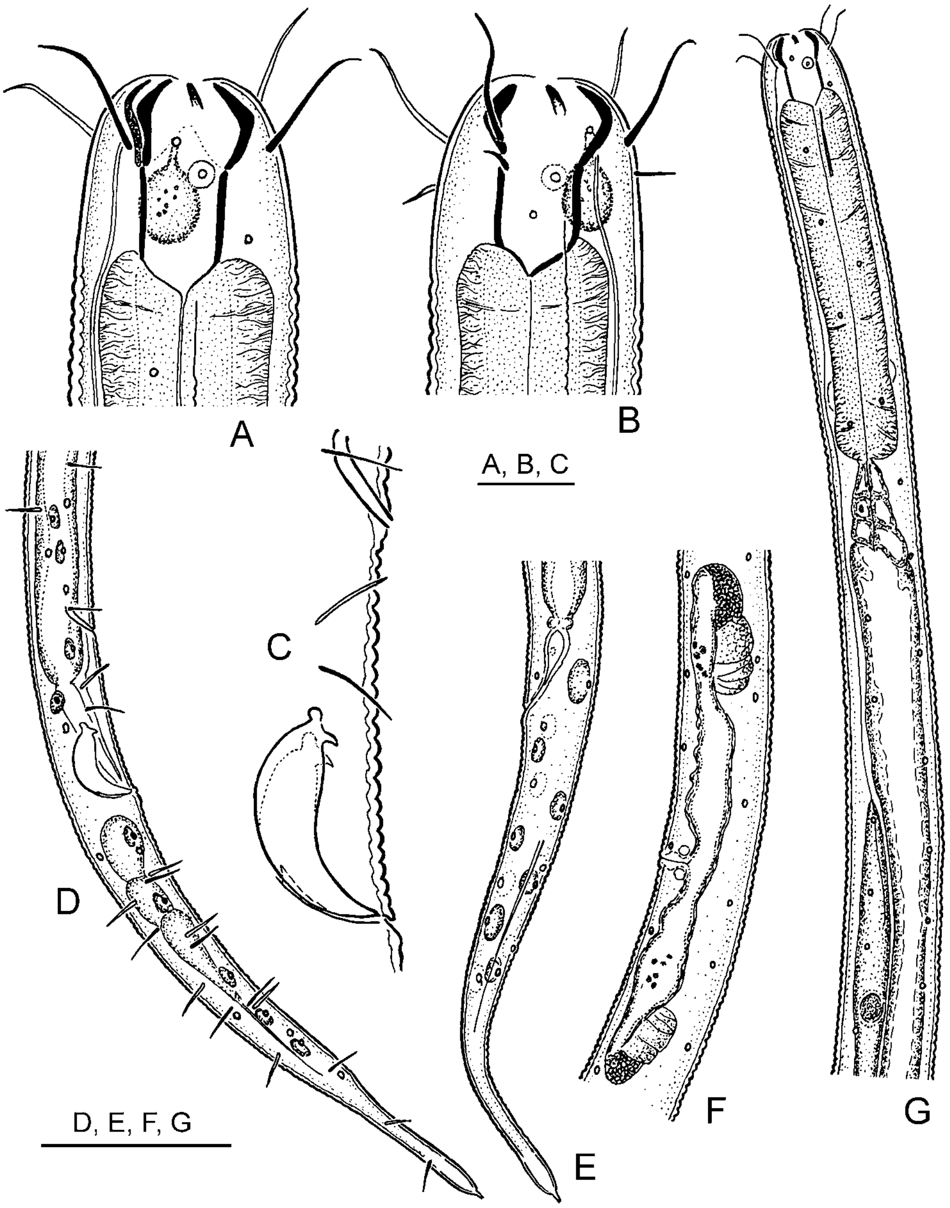

Figure 2.10. *Anonchus* venezolanus Holovachov, Zullini, Loof & Bongers, 2002. A: Female anterior end; B: Male anterior end; C: Male cloacal region; D: Male posterior region; E: Female tail; F: Female reproductive system; G: Female pharyngeal region. Scale bars: A-C = 10 µm, D-G = 50 µm.

becoming complete (with two lines) 30 µm from anterior end, at level with anterior fourth of pharynx; terminating at middle of tail in both sexes. Crystalloids absent. Body pores 1 µm wide. Epidermal gland cells on average 7-9 µm long and 4.5-6 µm wide, and filled with brown granules. Labial region bluntly rounded, continuous with body contour, with four 17 µm long (equal to 80-95 % of the labial region diam.) cephalic setae. Amphids located at level of posterior stoma section. Anteriormost body pore located at level with anterior stoma section, anterior to amphid, on unstriated labial region. Anterior stoma section 10-13 µm long and 15-18.5 µm wide, posterior section 11.5-13 µm long and 9-10 µm wide and barrel-shaped. Posterior fifth of entire stoma length enveloped by muscular pharyngeal tissue. Cardia conical, 21-27 µm long and 9-15 µm wide. Nerve ring surrounding pharynx at its posterior fourth. Hemizonid not seen. Central oval body of ventral secretory-excretory gland 85 µm long, situated ventrally three corresponding body diam. posterior to cardia; nucleus oval 9 µm long. Intestinal cells filled with brown granules. Unstriated tail tip 13-15 µm long, almost cylindrical. Caudal glands obscured by epidermal glands in female, prominent in male.

Female: Reproductive system didelphic, amphidelphic. Anterior ovary 28-37 µm long, located right subventrally in both females. Posterior ovary 27-37 µm long, located right subventrally in both females. Spermathecae absent, spermatozoa in oviduct. Vagina straight. Intra uterine egg not observed. Rectum 23-25 µm long, 1.3 times ABD long. Rectal glands not seen.

Male: Four additional 6 µm long subcephalic setae located 2 µm posterior to circle of cephalic setae. Neck setae absent. Spicules 1.1-1.2 ABD long, with small manubrium and ventral cuneus. Gubernaculum platelike. Alveolar supplements absent. Single midventral tubular supplement 14 µm long, located anterior to spicules, 45 µm from cloacal opening. Body setae 34 in number. Twenty setae located anterior to cloaca: two dorsosublateral and eight ventrosublateral on right side of body and three dorsosublateral and seven ventrosublateral on left side of body (anteriormost ones located level with secretory-excretory cell). Fourteen setae located on tail: three dorsosublateral and four ventrosublateral on right side and three dorsosublateral and four ventrosublateral on left side of tail.

Diagnosis: *A. venezolanus* is characterised by the 0.97-1.05 mm long body, presence of 114-161 lateral epidermal glands, bluntly rounded labial region, stoma with strongly developed anterior and barrel-shaped posterior section, amphid located at level with middle of stoma, anteriormost body pore located anterior to amphid, lateral field starting at level with stoma base, secretory-excretory cell body situated three body diam. posterior to cardia; didelphic female reproductive system, undeveloped spermathecae, straight vagina; male with four subcephalic and without neck setae, with one midventral precloacal tubular supplement, twenty precloacal and fourteen caudal setae, spicules 26-28 µm long arcuate with small manubrium and ventral cuneus, platelike gubernaculum.

Relationships: *A. venezolanus* is distinguished from the other species of *Anonchus* in having only one tubular supplement and no alveolar supplements, 10 pairs of precloacal setae (*vs* one pair) and four subcephalic setae in male, lateral field starting level with stoma base (*vs* at level with middle or posterior fifth of the neck). In having anteriormost body pore located anterior to amphid and single tubular supplement *A. venezolanus* comes close to *A. millelacunatus*. In addition to the characters mentioned above, the new species differs from *A. millelacunatus* in different stoma proportions (stoma length/diameter ratio 1.3-1.5 *vs* 2.8-2.9), relatively shorter male tail (c'= 6.1 *vs* c'= 11.4) and differently shaped spicules.

Type locality and habitat: Venezuela: Cata, March 1974, in algae on the rock, *legit* P. A. A. Loof.

Type specimens: Holotype female and paratype female and male in the nematode collection of the Laboratory of Nematology, Wageningen University, Wageningen, The Netherlands.

Anonchus sp.*

(Fig. 2.11, Table 2.1).

Adult: Lateral field demarcated by two straight lines. Labial region bluntly rounded, continuous with body contour, with four 11.5-14 µm long (equal to 60-70 % of labial region diam.) cephalic setae. Amphid located level with posterior stoma section. Anteriormost body pore located posteriorly to stoma base. Anterior stoma section 7-8 µm long and 14-15 µm wide, posterior section 14-18.5 µm long and 13-14 µm wide and barrel-shaped. Posterior third of entire stoma length enveloped by muscular pharyngeal tissue. Cardia cylindrical, 17-21 µm long and 10.5-11.5 µm wide. Nerve ring surrounding pharynx at its posterior third. Hemizonid not seen. Intestinal cells filled with brown granules. Unstriated tail tip 14 µm long, swollen. Caudal glands obscured by epidermal glands.

Female: Three females available, all flattened or partly damaged. Reproductive system monodelphic, prodelphic. Spermathecae present, filled with oval spermatozoa. Vagina straight. Postvulval uterine sac present. Intra uterine egg not observed. Rectal glands present.

Male: Subcephalic and neck setae absent. Spicules 1.7-1.9 ABD long, twisted along body axis, with prominent rounded manubrium and conical, gradually narrowing body. Gubernaculum rectangular. Anteriormost alveolar supplement 21-40 µm from anterior end. Anteriormost tubular supplement 475-549 µm from anterior end, 317-354 µm from cloacal opening. Posteriormost tubular supplement 44-52 µm from cloacal opening, at level with spicule manubrium. Precloacal and caudal setae present but obscure.

Relationships: In having only an anterior ovary and straight vagina *Anonchus* sp. comes close to *A. winiszewskae.* It differs from A. winiszewskae in larger stoma (21-23.5×14-15 *vs* 6-7×7-8 µm), longer (46-50 *vs* 39 µm) and differently shaped spicules, differently shaped gubernaculum. This population also resembles *A. maculatus* and

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[*] After the publication of the original article, a new population of this species came into my hands, which is therefore may be described as new to science. It was collected in Costa Rica (Holovachov & Esquivel, unpublished) thus adding to the range where this species occurs. The valid description of this species with a proposal of a name of it will be published in a separate paper.

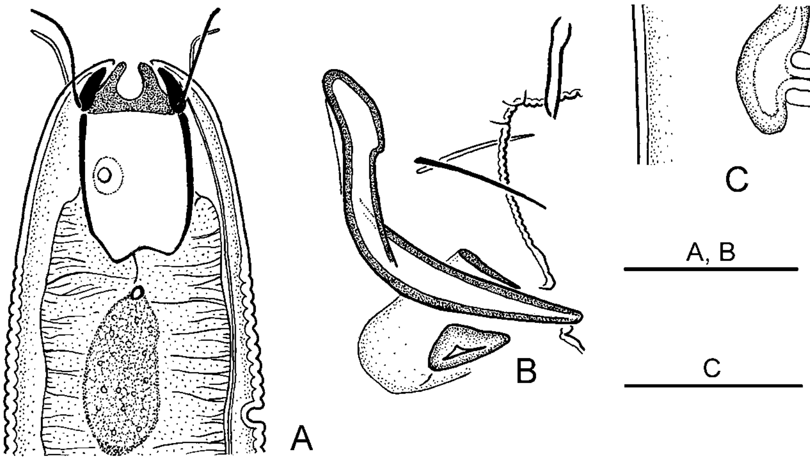

Figure 2.11. *Anonchus* sp. A: Male anterior end; B: Male cloacal region; C: Female vagina. Scale bars: A-B = 20 µm, C = 50 µm.

A. pulcher in being monodelphic, but differs from these two species in vagina structure and measurements. In view of the above mentioned differences, these specimens may represent a new species closely related to *A. winiszewskae.* Unfortunately, number and quality of available specimens do not allow us to make a complete description and comparison. For these reasons we prefer not to describe them as a new species, but to give as complete as possible description of the unnamed population. It is worth mentioning that *Anonchus* sp. was found in the same samples with *A. maculatus* and *A. winiszewskae* Differences in stoma size between the three species (21-25.5×14-15 in *Anonchus* sp., 13-19×8-12 in *A. maculatus* and 6-7×7-8 in *A. winiszewskae*) probably indicate different feeding habitats, allowing the three species to co-exist in the same locality.

Material examined: Colombia: Magdalena, five kilometres along the river, 110 4.1' N / 740 50.2' W, in sand on the bank and on the 20 cm depth in the river, August 24, 1967 (sites 28 and 29 in Riemann, 1970).

Anonchus mangrovi Gerlach, 1957

Original description based on two females and one male. Since type material was not preserved, comparison was based solely on the description. The species may be identified on the base of the following characters: female reproductive system didelphic, ovoviviparous mode of reproduction, strongly curved 30 µm long spicules with cephalated manubrium and wide velum, gubernaculum platelike, nine midventral tubular supplements and numerous alveolar supplements.

Table 2.5. Distribution of lateral epidermal glands in species of the genus *Anonchus* (left and right refers to the left and right side of the body).

			Female								
Species	Population	n	Pharyngeal region		Cardia to vulva		Vulva to anus		Tail		Total
			Left	Right	Left	Right	Left	Right	Left	Right	
A. maculatus	Paraguay	2	5-6	6-7	13	14-16	13	14-15	2	3	72-73
	Colombia	9	4-5	4-5	9-13	10-13	8-11	8-11	3	3	48-60
A. mirabilis	Italy	2	5-6	5-7	14-15	13-17	14-18	15-16	2	2	75-78
	Germany	11	4-5	4-5	13-20	15-22	14-19	15-21	2-3	2	72-94
A. millelacunatus	Cuba (type)	1	33	38	70	69	75	71	16	15	387
A. coomansi	Ivory Coast	9	5-6	6-7	15-21	15-19	16-22	16-20	3-5	3-5	87-104
	Ethiopia (type)	2	5-6	5	9-13	10-13	9-10	8-10	3	2-3	45-59
A. pulcher	Costa Rica (type)	3	8-10	8-9	19-23	18-21	14-19	15-17	3	3	89-102
A. winiszewska	Paraguay (type)	3	3-4	3-4	5-8	6-8	4-6	5	1	2	31-37
	Colombia	1	3	3	6	7	3	4	1	2	29
A. venezolanus	Venezuela (type)	2	9-10	8	28-34	28-35	30-31	28-30	6	6	146-161

			Male						
	Population	n	Pharyngeal region		Cardia to cloaca		Tail		Total
			Left	Right	Left	Right	Left	Right	
A. maculatus	USA (type)	1	7	6	27	31	2	2	65
	Paraguay	1	5	6	27	28	2	3	71
	Colombia	6	4-5	4-5	16-23	17-23	2-3	3	49-61
A. mirabilis	Italy	6	5-6	5-6	21-28	22-27	2	2	60-68
	Germany	3	4-5	5	27-30	27-31	2	2	69-74
A. millelacunatus	Cuba (type)	1	25	28	111	122	15	16	317
A. coomansi	Ivory Coast	5	6	6	27-35	26-35	3	3	71-88
	Ethiopia (type)	2	4-5	5	17-21	20-21	3	2-3	52-57
A. pulcher	Costa Rica (type)	5	7-9	6-9	34-43	34-38	3	3	89-100
A. winiszewskae	Paraguay (type)	1	3	3	13	11	2	2	34
A. venezolanus	Venezuela (type)	1	8	8	47	41	5	5	114

Anonchus laureatus (Gerlach, 1957) Holovachov, Zullini, Loof & Bongers, 2002

Original description based on two females and one male. Since type material was not preserved, comparison was based solely on the description. The species could be identified on the base of the following characters: amphid located anterior to middle of stoma, first annule just posterior to amphid or level with middle of stoma, female reproductive system didelphic, arcuate 21 µm long spicules with ventrally inclined manubrium, gubernaculum platelike, tubular supplements absent. Male was described as: "Bei dem Männchen fallen auf der Ventralseite dieser Seitenmembrane rundliche Vakuolen auf, die anscheinend in oder unter der Cuticula liegen und in ihrer Anordnung unabhängis von der Cuticularingen sind" (see Discussion).

Systematics of the genus Anonchus

The systematics of the subfamily Anonchinae was based mainly on the male sexual characters. The genus *Haconnus* was distinguished from *Anonchus* only on the basis of the male copulatory apparatus, differing from the latter in having only one midventral tubular supplement (*vs* more than nine) and eight hundred alveolar supplements arranged in three rows (*vs* up to 141 in a single row). The other character that was proposed by Andrássy (1973) was a comparatively longer and narrower stoma. Nevertheless, *A. laureatus* and *A. palaeotropicus* both have a similar stoma length/diam. ratio. The newly discovered species *A. venezolanus* has only one tubular supplement and no alveoli. Although the description of *A. laureatus* is incomplete, the male (but not female!) was described as bearing large "vacuolar" structures just ventrally to the lateral field (probably identical to ventrosublateral rows of alveoli in the male of *A. millelacunatus*) and with very similar spicule and gubernaculum morphology.

The variability of male sexual characters is high in Leptolaimidae and this organ system seems to be less useful for generic diagnosis. Alekseev & Rassadnikova (1977) proposed subdividing the genus *Leptolaimus* into four subgenera based solely on the presence-absence of tubular and/or alveolar supplements in males: subgenus *Leptolaimus s. str.* to accommodate species with males with alveolar and tubular supplements; *Dermatolaimus* Steiner, 1916 (syn. *Tubulaimus* Alekseev & Rassadnikova, 1977) – males with only tubular supplements; *Alveolaimus* Alekseev & Rassadnikova, 1977 – males with only alveolar supplements and *Boveelaimus* Alekseev & Rassadnikova, 1977 – males without both tubular and alveolar supplements. However, species of the genera *Antomicron* Cobb, 1920, *Leptolaimoides* Vitiello, 1971 and *Procamacolaimus* Gerlach, 1954 show a comparable variability in male sexual characters. Species of *Antomicron* and *Procamacolaimus* have either both tubular and alveolar supplements or only tubular supplements, while species of *Leptolaimoides* have either tubular or no supplements (Cobb, 1920; Lorenzen, 1966; Gerlach, 1953, 1954; Vitiello, 1971; Furstenberg & Vincx, 1988).

Nevertheless, among three genera closely related to *Anonchus* (i.e. *Aphanolaimus, Paraphanolaimus* and *Aphanonchus* – see below) the variability in supplements is less. Among about 30 species of *Aphanolaimus* and *Paraphanolaimus* none has alveolar supplements, while all three known species of *Aphanonchus* have a row of alveolar supplements in the male and also in the female in one species. These genera are also different in detailed structure of the stoma and secretory-excretory system.

Since the taxonomic value of the male sexual characters is difficult to access, a comparative analysis of the other morphological characters was made to find out the "clear-cut" characters, which could justify the separate generic status of *Haconnus* and clarify the position of the species from Venezuela. As a first step, the members of the subfamily Anonchinae were grouped in four species-groups solely on the male sexual characters – number and arrangement of tubular and alveolar supplements (Table 2.6). Unfortunately, three of the four groups are "monotypic." *A. laureatus* was not considered due to the lack of details in the original description, but it represents a fifth group according to the structure of the male sexual characters.

On the base of the data presented in the Table 2.6, *A. venezolanus* comes close to *A. millelacunatus* on the base of the position of the anteriormost body pore located anterior to the amphid. The females of these species have three characters in common: the more posterior location of the ventral secretory-excretory gland cell, spermathecae unobserved and rectal glands absent (*vs* ventral gland cell located almost opposite to cardia, spermathecae present and rectal glands present in the rest of the *Anonchus* species), while males of both species have only one tubular supplement. Similarities in male sexual characters are not considered. Position of the ventral secretory-excretory gland cell, as a diagnostic character, is rather variable. Spermathecae structure could be obscured in poorly preserved specimens and it can be absent in parthenogenetic forms (spermathecae were not found in presumably parthenogenetic species *Paraphanolaimus terrestris* Raski & Coomans, 1991 and *Aphanolaimus aymarae*). However, its absence in the amphimictic species is remarkable. Rectal glands were not found in several studied species of *Aphanolaimus, Aphanonchus* or *Paraphanolaimus*, but they are present in some distantly related genera of Leptolaimidae.

The position of the anteriormost body pore anterior to the amphid is unique amongst all Leptolaimidae studied in this respect. Comparison between the number of body pores (and lateral epidermal glands) in the neck region and position of the anteriormost body pore showed that the position of the latter could not be explained by the higher number of pores (and glands) in *A. venezolanus* and *A. millelacunatus*. The former species has only 8-10 pairs of pores in the neck region, as in the rest of the species (4-10 pairs), while the latter has 25-38 pairs, although the absolute and relative (b-ratio) lengths of the pharyngeal region in all species do not show essential differences and strongly overlap. These two species differs in the stoma proportions and the beginning of the lateral field, but this is considered as a specific character. The most striking difference between species is that the male of *A. millelacunatus* has one tubular supplement, three rows of very conspicuous alveolar supplements and one pair of precloacal setae, while the male of *A. venezolanus* has one tubule, no alveoli and ten pairs of precloacal setae. However, we were able to examine an as yet undescribed new species of *Aphanolaimus* from Korea that is particularly characterised by the absence of precloacal setae, while all other species of the genus have one pair of precloacal setae, indicating the possibility of exceptional patterns

Table 2.6. Morphological comparison of four species groups of the genus *Anonchus* with closely related genera.

	Anonchus s. str. (6 species)	*A. mirabilis*	*A. venezolanus*	*A. millelacunatus*	*Aphanolaimus, Paraphanolaimus*	*Aphanonchus*
Male						
Number of tubuli	9-31	47-72	1	1	1-14	10-20
Number and arrangement of alveoli	51-141, single row	absent	absent	816, three rows	absent	42-108, single row
Subcephalic setae	absent	absent	four	absent	absent	absent
Neck setae	absent	four	absent	absent	none to six	none to one**
Precloacal setae	one pair	one pair	ten pairs	one pair	none* or one pair	one pair
Female						
Spermathecae (in amphimictic species)	present	present	absent	absent	present	present
Rectal glands	present	present	absent	absent	absent	absent
General						
Anteriormost body pore	posterior to amphid	posterior to amphid	anterior to amphid	anterior to amphid	posterior to amphid	posterior to amphid
Excretory cell location	opposite to cardia	opposite to cardia	two body diam. posterior to cardia	three body diam. posterior to cardia	opposite to cardia	opposite to cardia
Lateral field start	middle of the pharyngeal region	middle of the pharyngeal region	stoma base	posterior fifth of the pharyngeal region	anterior part to middle of the pharyngeal region	middle of the pharyngeal region

* In *Aphanolaimus camerunensis* Holovachov, 2005, *A. costatus* Holovachov, 2005, and in undescribed new species fom Korea and Vietnam (Holovachov, 2005, unpublished).

** See Holovachov & Sturhan (2004) for detailes.

Table 2.7. Compendium of species of the genus *Anonchus* on the base of literature data supplemented with recent observations.

	General characters							Female characters			Male characters**		
	Body length	Stoma			Amphid location	Ant. body annule	Ant. body pore	Genital branches	Vagina structure	Sperma-thecae	Spicules length	Supplements	Setae (pairs)
		length	diam.	L/D									
A. maculatus *(A. monohystera)*	0.9-1.5	12-19	8-12	1.3-2	10-16	22-37	24-39	anterior	S-shaped	two	40.5-59	57-141 alveolar 13-23 tubular	1 precloacal 5 caudal
A. mirabilis	0.9-1.4	13-26	7-17	1.3-2.1	11.5-22	22-31	28-44	two	straight	four	42-64	0 alveolar 47-72 tubular	2 neck 1 precloacal 5-6 caudal
A. mangrovi	0.6-0.9	13	7	1.9	7*	18*	?	two	?	?	30	?(+) alveolar 9 tubular	?, present
A. laureatus	1.3-1.4	27	10*	2.7	7*	14*	?	two	?	?	21	? alveolar 0 tubular	?, present
A. millelacunatus	1.2-1.3	25-30	9-11	2.8-2.9	6-8	8-13	6-7	two	straight	absent	23-26	816 alveolar 1 tubular	1 precloacal 12 caudal
A. palaeotropicus	0.9-1.0	21-29	7-8	2.8-3.6	21-29	20-35	31-43	two	straight	?	35-40	?(+) alveolar 23 tubular	?, absent
A. coomansi	0.5-0.9	6-15	5-11.5	1.2-1.9	8-16	16-24	16-28	two	straight	four	27-45	80-112 alveolar 20-28 tubular	1 precloacal 6-8 caudal
A. pulcher	1.0-1.1	13-15	10-15	0.9-1.2	10.5-15	14-27	24-30	anterior	bent anteriorly	two	72-74	51-60 alveolar 28-31 tubular	1 precloacal 5 caudal
A. winiszewskae	0.8-1.0	6-7	7-8	0.8-0.9	10-12	15-20	19-21	anterior	straight	two	39	52 alveolar 20 tubular	1 precloacal 5 caudal
A. venezolanus	1.0-1.1	22-24	15-19	1.3-1.5	9	21-23	6-7	two	straight	absent	26-28	0 alveolar 1 tubular	4 subcephalic 10 precloacal 7 caudal

* Counted on the relevant figure.

** Shape of the spicules and gubernaculum are also of high taxonomic value, consult the drawings for comparison.

in the structure of the male copulatory apparatus in some species. The mosaic distribution of characters in-between all the species of the genus leads us to the synonymisation of the genera *Haconnus* and *Assia* with *Anonchus*.

The subfamily Anonchinae was proposed by Andrássy (1973) to accommodate two genera: *Anonchus* (type genus) and *Haconnus* (see also Andrássy, 1976). The genus *Assia* was added to the subfamily by Lorenzen (1981). Since *Haconnus* and *Assia* are considered synonymous with *Anonchus*, the subfamily Anonchinae becomes monotypic. A heavily cuticularized stoma consisting of two sections, *i. e.* anterior with six finger-like processes and posterior barrel-shaped, amphid with small oval aperture and unispiral subcuticular fovea seems to be synapomorphies of the genus *Anonchus* and subfamily Anonchinae.

Among all other genera of the family Leptolaimidae, *Anonchus* is closely related to the genera *Aphanolaimus, Paraphanolaimus* and *Aphanonchus* Coomans & Raski, 1991 in the structure of the ventral secretory-excretory gland (see Coomans & De Waele, 1979; Coomans & Raski, 1991). The character is unique for leptolaims so far described. It is considered to be synapomorphic for the taxon that includes genera *Anonchus, Aphanolaimus, Paraphanolaimus* and *Aphanonchus* and thereby establishes a monophyletic origin.

Andrássy (1976) tentatively placed these genera in the family Halaphanolaimidae De Coninck & Schuurmans Stekhoven, 1933 together with *Halaphanolaimus* Southern, 1914 (type genus) and *Stephanolaimus* Ditlevsen, 1919. The genus *Stephanolaimus* was recently revised by Tchesunov (1994) and split into two: *Stephanolaimus* s. str. and *Setostephanolaimus* Tchesunov, 1994. Both genera are characterised by the "regular" structure of the ventral secretory-excretory gland, which opens outside the body via a pore. The latter is usually located at the middle of the pharyngeal region (Tchesunov, 1994). Recent observation of *Halaphanolaimus* sp. and literature data (Timm, 1963 for *Halaphanolaimus luridus*, p. 48) shows that the structure of the ventral secretory-excretory gland in this genus is similar to the remaining genera of Leptolaimidae where such studies were carried out. The ventral secretory-excretory gland cell is small and usually obscure, located ventrally opposite the posterior end of the pharynx. The excretory duct is weakly cuticularized and is often obscure (except its distal part), opening outside via the excretory pore, which is usually located at the middle of the pharyngeal region. Therefore, the genus *Halaphanolaimus* should be considered within the family Leptolaimidae, subfamily Leptolaiminae as proposed by Lorenzen (1981).

Chitwood (1936) proposed the subfamily Aphanolaiminae Chitwood, 1936 within the family Camacolaimidae Micoletzky, 1924 to accommodate two genera: *Aphanolaimus* (type genus) and the newly described *Anguinoides* Chitwood, 1936. In later publications the subfamily Aphanolaiminae was considered synonymous with Leptolaiminae (Lorenzen, 1981) while *Anguinoides* is now placed in Camacolaiminae. We propose to raise the subfamily Aphanolaiminae to family rank to accommodate two subfamilies and four genera.

Appendix: an updated classification of the family Aphanolaimidae

Family Aphanolaimidae Chitwood, 1936

Diagnosis: Leptolaimoidea. Medium sized nematodes. Cuticle annulated with narrow ridge like lateral field. Lateral epidermal glands present, opening through prominent pores. Somatic setae present in male only. Four cephalic setiform sensilla. Amphid unispiral or derived therefrom, located on unstriated labial region. Stoma barrel-shaped or cylindrical, weakly or heavily sclerotised. Pharynx cylindrical. Cardia glandular. Secretory-excretory system well developed with large gland and cuticularized duct extending to lip region and opening into the stoma. Female reproductive system didelphic, amphidelphic or monodelphic, prodelphic. Ovary branches reflexed antidromously. Each branch with two lateral sack-like spermathecae or spermathecae undeveloped. Vagina encircled by single sphincter muscle. Male reproductive system monorchic with outstretched glandular anterior part and reflexed functional posterior testis. Spicules paired and symmetrical. Gubernaculum present. Tubular supplements present. Alveolar supplements present or absent. Tail conical. Caudal glands present, opening via a spinneret on tail tip.

Type family: Aphanolaiminae Chitwood, 1936.

Other family: Anonchinae Andrássy, 1973.

Subfamily Aphanolaiminae Chitwood, 1936

Diagnosis: Aphanolaimidae. Amphid unispiral, circular with central elevation or reniform. Stoma barrel-shaped or cylindrical, weakly or heavily sclerotised, consisting of one section. Pharynx cylindrical, weakly muscularised. Ventral secretory-excretory gland cell oval or bilobed. Female reproductive system didelphic.

Type genus: *Aphanolaimus* de Man, 1880

Other genera: *Aphanonchus* Coomans & Raski, 1990; *Paraphanolaimus* Micoletzky, 1922 (Syn.: *Bathyonchus* Kreis, 1936).

Subfamily Anonchinae Andrássy, 1973

Diagnosis: Aphanolaimidae. Amphid with rounded aperture and unispiral subcuticular fovea. Stoma heavily sclerotised, consisting of two sections: anterior with six longitudinal sclerotised ridges and posterior barrel-shaped or conical. Pharynx cylindrical, strongly muscularised. Ventral secretory-excretory gland cell bifurcated. Female reproductive system didelphic or monoprodelphic.

Type and only genus: *Anonchus* Cobb, 1913 (Syn.: *Bathylaimus* Daday, 1905 nec Cobb, 1894; *Pseudobathylaimus* Filipjev, 1918; *Dadayia* Micoletzky, 1922; *Assia* Gerlach, 1957; *Haconnus Andrássy*, 1973).

Appendix: an updated classification of the genus *Anonchus*

Genus *Anonchus* Cobb, 1913

Diagnosis: Anonchinae. Somatic (subcephalic, neck, precloacal and caudal) setae present in male only. Amphid with rounded aperture and unispiral subcuticular fovea. Stoma heavily sclerotised, consisting of two sections: anterior with six longitudinal sclerotised ridges and posterior barrel-shaped or conical, partly enveloped by pharyngeal muscular tissue in posterior part. Ventral secretory-excretory gland large with two ventrosublateral posterior appendages. Female reproductive system didelphic or monodelphic. Each branch with two lateral sack-like spermathecae or spermathecae undeveloped. Male reproductive system monorchic. Spicules paired and symmetrical. Gubernaculum present. One to 72 precloacal midventral tubular supplements. Alveolar supplements present (51-816), arranged in a single row or in three rows, or absent.

Type species:

A. maculatus (Daday, 1905) T. Goodey, 1951 (Syn.: *Bathylaimus maculatus* Daday, 1905; *Pseudobathylaimus maculatus* (Daday, 1905) Filipjev, 1918; *Dadayia maculata* (Daday, 1905) Micoletzky, 1922; *Anonchus monohystera* Cobb, 1913).

Other species:

A. mirabilis (Hofmänner in Hofmänner & Menzel, 1914) Chitwood & Chitwood, 1936 (Syn.: *Bathylaimus mirabilis* Hofmänner in Hofmänner & Menzel, 1914; *Dadayia mirabilis* (Hofmänner in Hofmänner & Menzel, 1914) Micoletzky, 1922);

A. mangrovi Gerlach, 1957;

A. laureatus (Gerlach, 1957) Holovachov, Zullini, Loof & Bongers, 2002 (Syn.: *Assia laureata* Gerlach, 1957);

A. millelacunatus (Andrássy, 1973) Holovachov, Zullini, Loof & Bongers, 2002 (Syn.: *Haconnus millelacunatus* Andrássy, 1973);

A. palaeotropicus Tsalolikhin, 1989;

A. coomansi Eyualem, 1996;

A. pulcher Zullini, Loof & Bongers, 2002;

A. winiszewskae Holovachov, Zullini, Loof & Bongers, 2002;

A. venezolanus Holovachov, Zullini, Loof & Bongers, 2002.

Chapter 3

Morphology and systematics of the superfamilies Leptolaimoidea Örley, 1880 and Camacolaimoidea Micoletzky, 1924

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Abstract

Five known and one new species of Leptolaimoidea viz. *Dagda bipapillata*, *Deontolaimus papillatus*, *Leptolaimus mixtus*, *Paraplectonema loofi*, *Rhadinema flexile* and *Stephanolaimus elegans* are described on the basis of new material from Europe and the Americas. *Dagda bipapillata* is studied by SEM for the first time. *Paraplectonema loofi* is described from Costa Rica and differs from all other species of the genus by the position of excretory pore on labial region. *Leptolaimus primitivus* is transferred to *Paraplectonema*. A revised classification of the superfamily Leptolaimoidea is proposed based on a phylogenetic analysis of a set of morphological data of most genera of the group and a reappraisal is made of the superfamily Camacolaimoidea including the families Camacolaimidae and Rhadinematidae. The taxonomy of *Leptolaimus* is discussed. First description of a male of *Odontolaimus chlorurus* is given. It is characterised by the presence of five setiform sensilla along the ventral side of the pharyngeal region and no precloacal supplementary sensilla. New information on the male of *Tobrilia imberbis* is provided. The systematic positions of the genera *Bastiania, Odontolaimus* and *Tobrilia* are reconsidered. Emended diagnoses are given for the superfamilies Leptolaimoidea and Camacolaimoidea, families Leptolaimidae, Camacolaimidae and Rhadinematidae.

Nomenclatorial remarks

Folloving nomenclatorial suggestions and changes were proposed in the original publication: **1)** New name "*Paraplectonema loofi* Holovachov & Boström, 2004" was proposed for a new species. **2)** *Leptolaimus primitivus* Gagarin, 2001 was transferred to the genus *Paraplectonema* Strand, 1934, thus resulting in a new combination *Paraplectonema primitivum* (Gagarin, 2001) Holovachov & Boström, 2004. **3)** The family Peresianidae Vitiello & De Coninck, 1938 was considered a junior synonym of the family Leptolaimidae Örley, 1880. The date of publication of new names and new combinations, which were introduced in the original article, is 29 December 2004.

Introduction

The superfamily Leptolaimoidea is a group of free-living nematodes, inhabiting marine, freshwater and terrestrial habitats, whose taxonomy has not yet been a matter of detailed morphological and phylogenetic analysis. Lorenzen (1981) considered it impossible to divide the suborder Leptolaimina Lorenzen, 1981 into monophyletic superfamilies, since no holapomorphic characters were found. In the latest phylogenetic system of the whole phylum Nematoda presented by De Ley & Blaxter (2002), the superfamily Leptolaimoidea includes nine morphologically diverse families which do not form a monophyletic taxon, viz.: Leptolaimidae, Rhadinematidae Lorenzen, 1981, Aegialoalaimidae Lorenzen, 1981, Diplopeltoididae Tchesunov, 1990, Paramicrolaimidae Lorenzen, 1981, Ohridiidae Andrássy, 1976, Bastianiidae De Coninck, 1935, Odontolaimidae Gerlach & Riemann, 1974 and Rhabdolaimidae Chitwood, 1951. Between the system of Lorenzen (1981) and that of De Ley & Blaxter (2002), the classification of some of the families listed above was reconsidered. First, the family Rhabdolaimidae (*sensu* Lorenzen, 1981) was transferred from Leptolaimina to Microlaimoidea under Chromadorina by Eyualem & Coomans (1996). Although De Ley & Blaxter (2002) did not follow this opinion, we agree with Eyualem & Coomans (1996) that Rhabdolaimidae do not belong to Leptolaimoidea. The systematic position of the genus *Tobrilia* Andrássy, 1967, which was listed in Rhabdolaimidae by Lorenzen (1981), however, will be briefly discussed in the present paper. Subsequently, Holovachov et al. (Chapter 2) on the basis of a detailed morphological analysis installed the family Aphanolaimidae Chitwood, 1936 for some genera previously included within the family Leptolaimidae. In the present paper we will reappraise the phylogeny and systematics of the superfamily Leptolaimoidea *sensu* De Ley & Blaxter (2002) based on a phylogenetic analysis of a set of morphological data of most genera of the group.

Several species are redescribed from recent material. Morphological information for other taxa was taken from the existing descriptions and verified on the additional material studied here. We will discuss most of the taxa listed within the superfamily by De Ley & Blaxter (2002), with the following exceptions: **1)** the family Paramicrolaimidae will not be treated in the analysis, since no material was available to the author; **2)** the family Rhabdolaimidae was transferred to Microlaimoidea by Eyualem & Coomans, 1996, to which we agree, but we will discuss the systematic position of the genus *Tobrilia*; and **3)** three genera currently assigned to Leptolaimidae, viz. *Cynura* Cobb, 1920 (syn. *Plectolaimus* Inglis, 1966), *Pakira* Yeates, 1967 and *Caribplectus* Andrássy, 1973, showing strong similarities to the members of the superfamily Plectoidea in the structure of the digestive tract (stoma, pharynx) and excretory system (gland, duct) will be discussed in Chapter 5.

Materials and methods

Populations of *Dagda bipapillata*, *Deontolaimus papillatus*, *Leptolaimus mixtus* (all courtesy T. Bongers), *Paraplectonema loofi* (courtesy A. Esquivel), *Rhadinema flexile* (courtesy W.D. Hope) and *Stephanolaimus elegans* (courtesy T. Bongers) were available on slides in glycerine or in vials in ethanol (only *Dagda bipapillata*). All studied specimes (also those listed below as additional material) were transferred to pure glycerine by a variety of methods (not known in many cases) and mounted on permanent slides in glycerine with paraffin wax as support for the coverslip. Five females and five males of *D. bipapillata* were prepared for SEM. They were postfixed in 1% osmium tetroxide (OsO_4) and transferred to pure acetone through an acetone/distilled water series. Specimens were critical point dried in liquid CO_2, mounted on stubs, gold-plated under vacuum to a thickness of 200 Å in a sputter, and examined in an Hitachi S-4300 SEM at an accelerating voltage of 10 kV.

Morphometric data and measurements in µm are given as mean ± standard deviation and range wherever appropriate. Coiled and curved structures were measured along the median line. Parameters (measurements) of the male copulatory apparatus were taken according to Zell (1993). Dimensions of the renette cell are given as the lengh of the oval or elongate part of the cell excluding the narrow distal part that extends anterior to the excretory ampulla. Annule width was taken according to Andrássy (1984). Definitions of stoma parts are according to De Ley et al. (1995), and terminology of nematode sensilla follows Coomans (1979).

The following terms are used in the present paper: **1)** "renette cell" is used instead of "ventral gland" or "ventral secretory-excretory gland" (Turpeenniemi & Hyvärinen, 1996); and **2)** "lateral alae" is used here for the external longitudinal bands of smooth cuticle, while "lateral chord" is used throughout the paper for the internal longitudinal bands of hypodermal cells. The term "lateral field" is used occasionally for both cases in diverse nematological literature, but is not used in the present paper.

In additions to the species described, the following specimens which belong to the superfamily Leptolaimoidea, were also studied: *Antomicron elegans* (de Man, 1922) De Coninck, 1965 (1♀, 1♂), *Manunema annulatum* (Vitiello & De Coninck, 1968) Riemann, von Thun & Lorenzen, 1971 (1♂), *Manunema proboscidis* Gerlach, 1957 (3♂♂), *Manunema* sp. (3♀♀), *Setostephanolaimus* sp. (1♀) and *Setostephanolaimus bicoronatus* Boucher & Helléouët, 1977 (1♂) from the Netherlands, Oostershelde, August 1991, *legit* R. Alkemade (courtesy Dr. T. Bongers); *Camacolaimus* sp. (1♀, 2♂♂) and *Leptolaimus* sp. 1 (1♀, 1♂) from the Netherlands (courtesy Dr. T. Bongers); *Leptolaimus* sp. 2 (1♀, 2♂♂) from Antarctica, Southern Atlantic, South Shetland Islands, Admiralty Bay (U.K.), 76 m depth, January 1991, *legit* R. Alkemade (courtesy Dr. T. Bongers); *Onchium* sp. (1♀), Curacao (courtesy Dr. T. Bongers). Type material of *Hemiplectus muscorum* Zell, 1991[*] (2♂♂ paratypes) collected in UK, Orkney Islands, Mainland, Ring of Brogar, moss, August 1986, *legit* H. Zell (courtesy Dr. P.A.A. Loof).

Material of several other species, whose systematic positions are still unsettled, are also discussed. These are: *Bastiania acarayensis* Andrássy, 1968 (2♂♂) from Mexico, Jalapa Veracruz, *legit* K. Jankiewicz (courtesy Dr. G. Winiszewska); *Dintheria tenuissima* de Man, 1921 (1♂) collected in Ukraine, Ivano-Frankivsk province, Nadvirna district,

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[*] After the acceptance of the original article for publication, new population of this species came into my hands. It was collected in Windemere, Lake District, Great Britain by Dr. B. Sohlenius (Holovachov & Boström, unpublished).

Vorokhta village, Bretskul mountain, marsh, 20.08.1993, *legit* A. Susulovsky (courtesy Dr. A. Susulovsky); *Odontolaimus chlorurus* de Man, 1880 (20♀♀, 3♂♂) from Ukraine, Lviv province, Yavoriv district, "Cholgini" ornithological reserve, moist clayish soil on the shore of the water reservoir, 10.03.2002, *legit* O. Holovachov; and *Tobrilia imberbis* (Andrássy, 1953) Andrássy, 1967 (20♀♀, 1♂), Ukraine, Lviv, "Znesinnya" park, dry meadow, grasses, soil, 24.09.2002, *legit* O. Holovachov.

For the phylogenetic analysis, "multistate" characters were recoded into "binary" using FACTOR programme of PHYLIP (Phylogeny Inference Package) Version 3.57c (Felsenstein, 1989) in the following way: **1)** multistate characters "A" and "B", that developed independently from the same primitive state "P" (A←P→B) were recoded as: "P" = 00, "A" = 01, "B" = 10; **2)** multistate characters that developed sequentially, "A" from "P" and then "B" from "A" (P→A→B) were recoded as: "P" = 00, "A" = 01, "B" = 11. Characters, that were not seen, but may be present, or unavailable from the literature were coded as "?"; not applicable data were coded as "-". A cladistic analysis of the entire dataset was performed by using the MIX programme of PHYLIP 3.57 based on WAGNER parsimony (all characters treated as "unordered"), while a strict consensus tree was obtained by using the CONSENSE programme of the same package.

We included one representative of the family Aphanolaimidae into the phylogenetic analysis, namely *Aphanonchus intermedius*, in order to settle the family position within the superfamily. For the morphological data of this species, as well as other representatives of the family we refer to Coomans & Raski (1991a), Holovachov & Sturhan (2004a, 2004b) and Chapter 2. Furthermore, we included *Plectus parietinus* Bastian, 1865 in the data matrix, trying to find the root and sister taxon for the superfamily Plectoidea using morphological data published by Maggenti (1961a) and personal unpublished observations.

Species descriptions

Dagda bipapillata Southern, 1914

(Figs 3.1-3.3, Table 3.1)

Adult: Body slender, cylindrical, gradually narrowing anteriorly in pharyngeal region and posteriorly on tail; ventrally curved upon fixation, more strongly curved ventrad in posterior part, and spirally curved in anterior part of pharyngeal region due to presence of strong muscle cells directed obliquely from ventral side posteriorly to dorsal side. Cuticle annulated; annuli 1.3-2.1 µm wide at midbody, without ornamentation. Lateral alae, hypodermal glands and body pores absent. A ventral cuticle ridge is present in the pharyngeal region as seen in the SEM (Fig. 3.1 B) and seen under LM as a thickening of the cuticle along the ventral side of the pharyngeal region. Somatic setae present, sometimes seen to be connected with gland-like cells in hypodermis, scattered sublaterally along the body. Labial region truncate, slightly separated from rest of body by slight and wide depression, lips fused. First annulus appears 5.5-8.0 µm from anterior end, posterior to cephalic setae bases and amphid, forming the base of the cephalic capsule. Inner and outer labial sensilla papilliform, located on anterior surface of lips. Cephalic sensilla setiform,

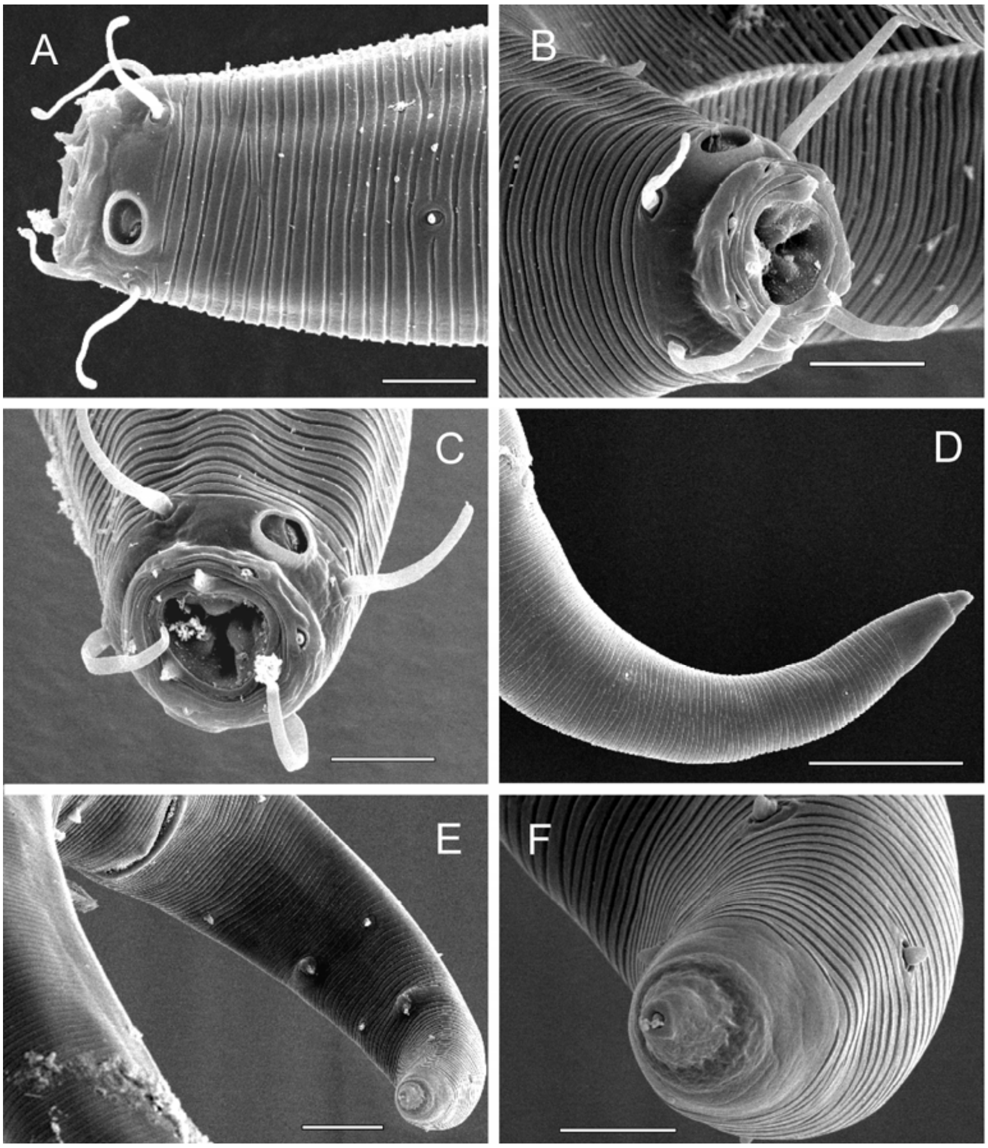

Figure 3.1. *Dagda bipapillata* Southern, 1914. A: Female anterior end, lateral view; B: Male anterior end, oblique subdorsal view; C: Female anterior end, semi-en face; D: Female tail, lateral view; E: Male tail, ventral view; F: Male tail terminus with spinneret and posterior caudal setae. Scale bars: A-C, F = 5 µm. E = 12.5 µm. D = 25 µm.

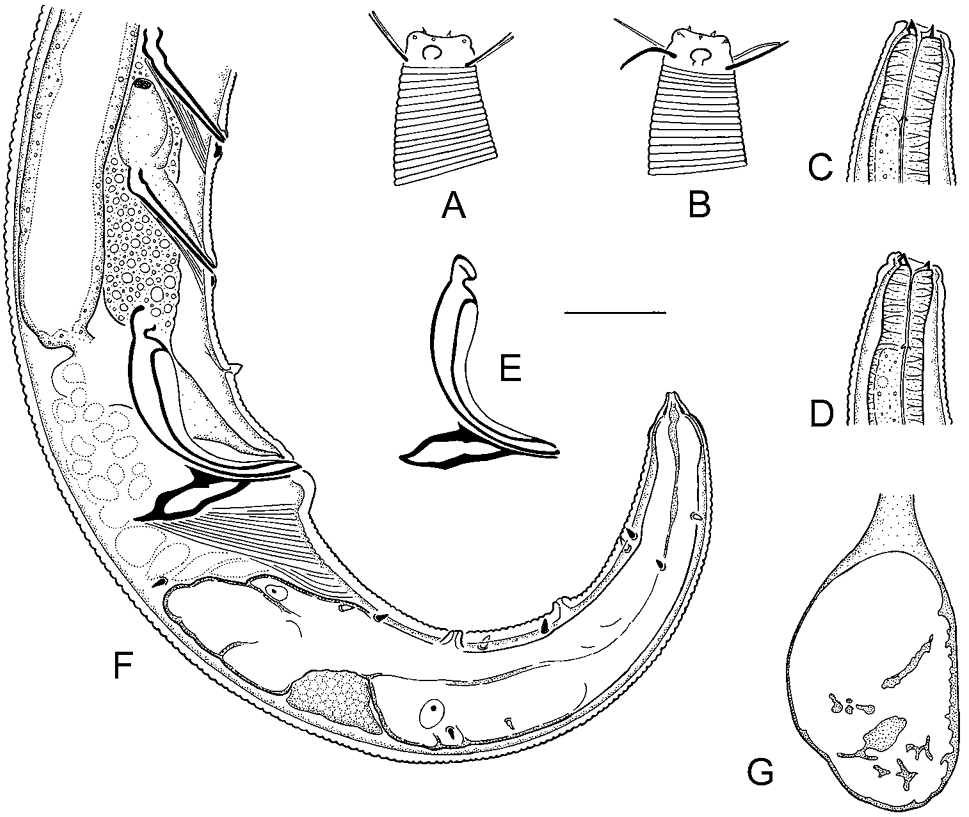

Figure 3.2. *Dagda bipapillata* Southern, 1914. A: Female anterior end, surface view; B: Male anterior end, surface view; C: Female anterior end, median section; D: Male anterior end, median section;. E: Spicule and gubernaculum; F: Male caudal region; G: Renette cell. Scale bar 20 µm.

directed outward; their bases located posterior to amphids, 4.5-8.0 µm from anterior end. Subcephalic setae absent. Amphid 3.0-4.5 µm wide, unispiral, transverse oval with a break at its posterior margin. Ocelli and deirid absent. Secretory-excretory system present; renette cell 45-89 µm long, oval, located posterior to cardia, along ventral side of anterior part of intestine; extending anteriorly along ventral side of pharynx, forming an excretory ampulla just posterior to level of nerve ring. Excretory canal weakly cuticularised, extends from excretory ampulla for a short distance anteriorly and opens to the exterior via a pore. Stoma broad, 5.5-8.0 µm wide, with funnel-shaped posterior part. Cheilostom undifferentiated. Gymnostom short, with three (one dorsal and two ventrosublateral) odontia. Stegostom funnel-shaped, undifferentiated, with weakly cuticularised walls. Pharynx cylindrical anteriorly, gradually widening posteriorly; heavily muscularized; with uniformly thickened lumen; without valves and bulbs. Dorsal and subventral (one pair) gland orifices penetrate

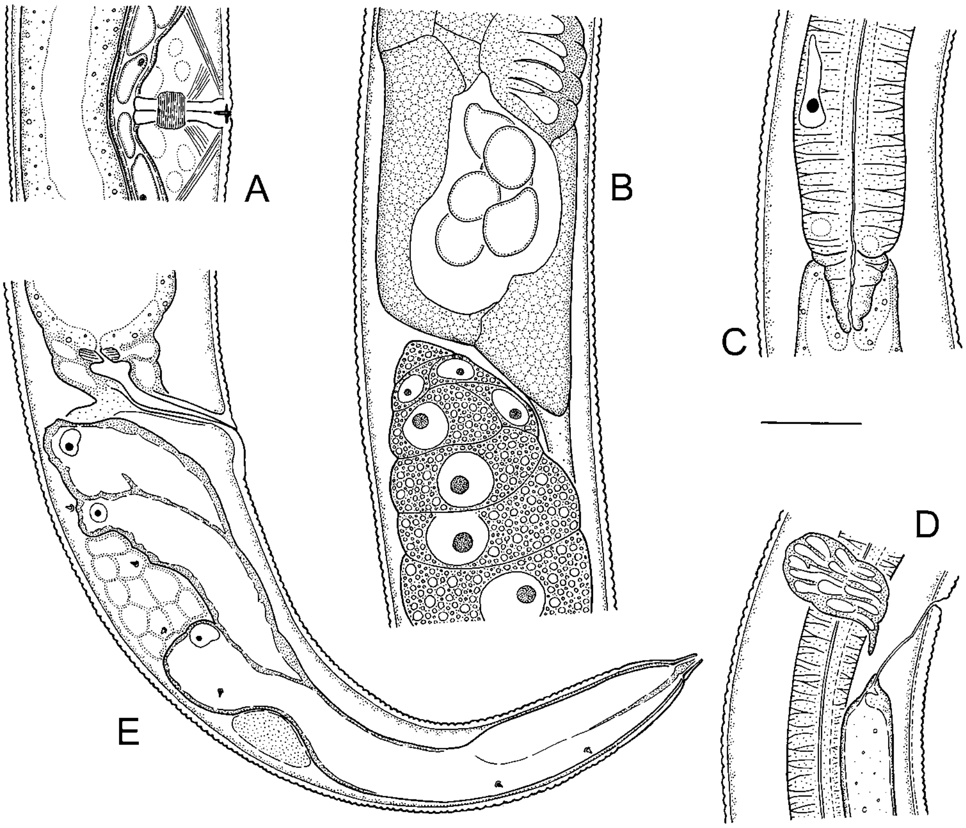

Figure 3.3. *Dagda bipapillata* Southern, 1914. A: Vulval region; B: Posterior spermatheca and ovary; C: Posterior part of pharynx; D: Nerve ring and excretory ampulla; E: Female tail. Scale bar 20 µm.

pharyngeal lumen somewhat posterior to stoma base. Nucleus of only dorsal pharyngeal gland distinct, located in posterior part of pharynx. Cardia conoid, embedded in intestine. Tail gradually narrowing, arcuate ventrad. Three caudal glands present, their nuclei are incaudal. Spinneret functional, weakly cuticularised.

Male: Reproductive system diorchic; anterior testis 429-602 µm long, outstretched; posterior testis 63-85 µm long, outstretched but with reflexed proximal part. Spicules symmetrical, with oval, ventrally inclined manubrium, arcuate conoid shaft and wide ventral velum. Gubernaculum plate-like, with rectangular dorsal apophysis. Nine to twelve equally distributed similar midventral tubular supplements, a single midventral precloacal sensillum and two subventral postcloacal sensilla. Each tubule is connected with a single gland-like cell, both lying on the right-hand side of intestine. There is a small sclerotized rod in the cuticle, caudally at distal end of each tubule. Posteriormost tubule located 40-50 µm anterior to cloacal opening. Precloacal sensillum setiform in shape, consists of a nerve

Table 3.1. Measurements (in µm) of *Dagda bipapillata* Southern, 1914, *Deontolaimus papillatus* de Man, 1880 and *Stephanolaimus elegans* Ditlevsen, 1918.

Species	*Dagda bipapillata*		*Deontolaimus papillatus*		*Stephanolaimus elegans*	
	Males	Females	Males	Females	Males	Females
n	6	6	6	6	6	5
Body length	4024±284 (3647-4365)	4103±169 (3886-4395)	698±25 (666-738)	803±165 (502-994)	2574±626 (1952-3402)	2828±356 (2250-3101)
Body diameter (BD)	39.1±2.7 (37.0-43.0)	42.2±4.1 (37.0-48.0)	14.8±1.5 (12.0-17.0)	18.0±3.5 (12.0-21.0)	43.9±9.3 (34.5-57.0)	54.7±6.5 (49.0-65.5)
Pharyngeal region length	518±23.7 (480-553)	530±41.7 (473-570)	145±6.9 (134-152)	160±22.4 (119-181)	322±49.9 (272-372)	342±39.1 (280-381)
Tail length	147±12.1 (130-166)	134±7.4 (129-148)	55.7±1.1 (54.5-57.0)	65.4±12.5 (41.0-75.5)	194±49.9 (147-252)	208±30.1 (159-227)
Anal or cloacal body diameter	40.6±2.2 (37.0-42.0)	37.0±2.4 (34.5-41.0)	12.8 (12.0-13.0)	10.9±1.9 (8.0-13.0)	42.4±7.7 (34.5-51.0)	39.3±5.4 (33.0-48.0)
a	103±8.1 (96.4-119)	97.8±7.9 (89.0-111.5)	47.6±5.8 (41.9-58.1)	44.7±3.3 (41.1-48.3)	58.5±5.2 (53.1-66.6)	52.5±10.0 (41.3-60.6)
b	7.8±0.4 (7.3-8.3)	7.8±0.6 (7.1-8.6)	4.8±0.2 (4.6-5.2)	5.0±0.6 (4.2-5.6)	7.9±0.8 (7.2-9.2)	8.3±0.2 (8.0-8.6)
c	27.5±2.8 (24.5-32.8)	30.7±2.0 (27.3-32.7)	12.5±0.5 (12.0-13.3)	12.3±0.7 (11.8-13.6)	13.3±0.6 (12.3-14.1)	13.7±0.9 (12.4-14.8)
c’	3.6±0.3 (3.1-4.1)	3.6±0.2 (3.3-3.9)	4.4±0.3 (4.1-4.6)	6.0±0.6 (5.3-6.8)	4.5±0.5 (4.0-5.3)	5.3±0.6 (4.7-6.2)
Labial region diameter	12.0 (11.0-12.0)	11.7 (11.0-12.0)	5.5	5.9±0.6 (5.5-7.0)	9.1±1.5 (8.0-11.0)	10.2±0.9 (9.0-11.0)
Cephalic setae length	11.9 (11.0-12.0)	11.3±0.8 (10.0-12.0)	1.8 (1.0-2.0)	1.4±0.5 (1.0-2.0)	23.3±1.7 (20.0-24.5)	22.7±2.7 (19.0-25.5)
Amphid location	3.5 (3.0-4.5)	3.3±0.7 (2.0-4.5)	1.0	1.3±0.5 (1.0-2.0)	6.9±1.3 (5.5-9.0)	7.3±1.3 (5.5-9.0)
1st cervical seta location	absent	absent	absent	absent	51.8±8.5 (43.0-65.5)	58.7±9.1 (47.0-67.0)
2nd cervical seta location	absent	absent	absent	absent	73.5±9.7 (63.0-84.5)	78.7±13.3 (65.5-95.5)
1st somatic seta location	23.1±3.1 (18.0-25.5)	28.3±1.4 (27.0-30.0)	33.7 (32.0-34.5)	35.6±5.5 (28.0-40.0)	?	?
1st body pore location	absent	absent	absent	absent	56.5±4.8 (49.0-63.0)	60.0±8.4 (51.0-73.0)
Stoma length	6.5±0.8 (5.5-8.0)	5.7±0.8 (4.5-7.0)	9.6 (9.0-10.0)	10.6±0.9 (9.0-11.0)	11.3±4.6 (9.0-13.0)	10.5±2.3 (7.0-12.0)
Dorsal gland orifice location	17.3 (17.0-18.0)	16.7±1.4 (15.5-19.0)	10.7 (10.0-11.0)	10.6±0.9 (9.0-11.0)	23.8±2.0 (21.0-27.0)	25.3±2.8 (22.0-29.0)
Dorsal gland nucleus location	496±14.8 (482-520)	499±41.0 (444-540)	146 (145-148)	164±12.9 (147-177)	297±45.9 (260-351)	326±41.9 (268-363)
Cardia length	16.0±3.1 (13.0-21.0)	16.5±2.4 (13.0-19.0)	5.0±0.9 (3.0-5.5)	5.9±0.6 (5.5-7.0)	11.5±1.1 (10.0-13.0)	15.6±4.5 (10.0-21.0)

Table 3.1. Continued.

Species	*Dagda bipapillata*		*Deontolaimus papillatus*		*Stephanolaimus elegans*	
	Males	Females	Males	Females	Males	Females
n	6	6	6	6	6	5
Nerve ring location	150±6.9 (139-159)	152±8.8 (139-162)	68.9±3.0 (65.5-73.0)	74.6±10.8 (58.0-85.5)	166±27.9 (133-197)	172±21.7 (139-192)
Nerve ring (% of phar. region)	28.9±1.0 (27.7-30.3)	28.7±1.9 (27.2-32.4)	48.1±2.6 (45.1-52.1)	46.8±1.8 (44.2-48.7)	51.5±1.9 (48.4-53.3)	50.1±2.0 (47.7-53.2)
Excretory pore location	144±8.7 (133-156)	149±10.2 (133-162)	67.8	80.4±12.3 (64.5-95.5)	175±25.1 (146-207)	18.4±23.1 (147-200)
Excretory pore (% of phar. region)	27.7±1.5 (26.1-29.7)	28.2±2.2 (26.6-32.4)	48.0	50.6±2.9 (47.1-54.2)	54.6±3.0 (49.5-57.6)	53.7±2.7 (51.6-58.1)
Spinneret length	6.5±0.8 (5.5-8.0)	7.0±0.6 (7.0-8.0)	2.0	2.0	29.3±5.7 (22.0-38.0)	32.7±6.7 (24.5-41.0)
Supplement number	9-12 tub	absent	22-35 alv	absent	32-43 tub	19-34 tub
V (%) or T (%)	55.7±2.4 (53.2-59.4)	54.9±1.7 (52.2-56.8)	53.4±3.3 (49.7-58.8)	50.4±2.6 (48.4-55.3)	65.2±4.4 (57.7-69.8)	51.8±1.2 (51.2-53.9)
G1 (%)	-	11.5±2.2 (7.7-13.4)	-	11.4±1.6 (9.8-13.3)	-	17.5±2.2 (14.1-19.7)
G2 (%)	-	12.4±2.3 (9.8-15.4)	-	10.3±1.2 (9.4-12.2)	-	18.9±3.0 (15.7-21.8)
Vagina or Spicule length	42.5±5.8 (37.0-52.0)	17.6±2.2 (14.5-20.0)	26.5±1.6 (24.5-29.0)	8.7±1.8 (5.5-10.0)	59.8±7.8 (52.0-70.0)	25.6±4.2 (21.0-32.0)
Rectum or gubernaculum length	26.3±2.9 (21.0-29.0)	28.9±1.9 (25.5-31.0)	7.8±1.2 (5.5-9.0)	14.1±2.1 (11.0-17.0)	19.4±3.2 (15.5-23.0)	30.2±2.9 (25.5-33.0)

ending, located 22-28 µm anterior to cloacal opening. Postcloacal sensilla stub-like, each consists of a nerve ending, located subventrally, right at 54-68 µm and left at 79-99 µm posterior to cloacal opening. Eleven to twelve caudal setae arranged in three subventral and three dorsosublateral pairs (one seta is sometimes missing).

Female: Reproductive system didelphic, amphidelphic; ovary branches reflexed antidromously. Anterior ovary 167-324 µm long, located on the right-hand side of intestine in four and on the left-hand side in two females, posterior ovary 100-283 µm long, located on the right-hand side of intestine in one and on the left-hand side in five females. Oviduct a narrow tube. Spermatheca axial, in proximal part of uterus. Vagina straight, 0.3-0.5 times vulval body diameter long; encircled by a single sphincter muscle, with a T-shaped symmetrical sclerotisation. Intrauterine egg measuring 171×42 (168-174×40-43) µm, 3.9-4.4 times its own diameter long. Rectum 0.7-0.8 times ABD long; its proximal part surrounded by three gland-like cells: one dorsal and two ventrosublateral. Four to six caudal setae arranged in two to three sublateral pairs.

Material examined: France, Ambleteuse, North Sea coast, rocky beach with sand between the rocks, 1975, *legit* T. Bongers.

Relationships: The recent population agrees fairly well with the original description in most body measurements, structure of the anterior end and male copulatory apparatus, differing only in slightly longer (3.6-4.4 mm *vs* 3.5 mm) and more slender body (a=89-119 *vs* 70).

Deontolaimus papillatus de Man, 1880

(Fig. 3.4, Table 3.1)

Adult: Body slender, cylindrical, gradually narrowing anteriorly in pharyngeal region and posteriorly on tail; ventrally curved upon fixation, more strongly curved ventrad in posterior part. Cuticle annulated; annuli 0.6-0.8 µm wide at midbody, without ornamentation. Lateral alae a narrow and smooth band of cuticle demarcated by two straight lines; originating at level of pharyngeal region; terminating posteriorly on tail. Hypodermal glands and body pores absent. Somatic setae present, scattered sublaterally along the body. Lip region continuous with body contour, rounded, lips fused. First annulus appears 2-3 µm from anterior end, posterior to cephalic setae bases and amphid, forming the base of the cephalic capsule. Inner labial sensilla indistinct. Outer labial sensilla papilliform, located on anterior surface of lips. Cephalic sensilla setiform, directed outward; their bases located posterior to amphids, 2-3 µm from anterior end. Subcephalic setae absent. Amphid 2 µm wide, unispiral, transverse oval with a break at its posterior margin. Ocelli and deirid absent. Secretory-excretory system present; renette cell 20-22 µm long, elongated, located posterior to cardia, along ventral side of anterior part of intestine; extending anteriorly along ventral side of pharynx, forming an excretory ampulla just posterior to level of nerve ring. Excretory canal weakly cuticularised, extends from excretory ampulla for a short distance anteriorly and opens to the exterior via a pore. Stoma narrow, with dorsal stomatal armament. Cheilostom undifferentiated. Gymnostom short, with a single dorsal odontium pointing forward. Stegostom funnel-shaped, with dorsal onchiostyle, enveloped by muscular pharyngeal tissue. Odontium and onchiostyle joined together. Pharynx cylindrical anteriorly, gradually widening posteriorly, heavily muscularised, with uniformly thickened lumen, without valves and bulbs, with prominent glandular dorsal sector. Dorsal gland orifice penetrates pharyngeal lumen at stoma base, subventral gland orifices not seen. Usually only nucleus of dorsal gland distinct, located at posterior part of pharynx; in one specimen nuclei of one pair of subventral pharyngeal glands were seen 18 µm anterior to dorsal gland nucleus. Cardia conoid, embedded in intestine. Tail subcylindrical, arcuate ventrad. Three caudal glands present, their nuclei are incaudal. Spinneret functional, weakly cuticularised.

Male: Reproductive system diorchic, both testes directed forward (outstretched); anterior testis 33-61 µm long; posterior testis 39-56 µm long. Spicules symmetrical, with oval ventrally inclined manubrium, arcuate conoid shaft and ventral velum along proximal part of spicula. Gubernaculum plate-like. 22 to 35 midventral alveolar supplements and two subventral postcloacal sensilla. Alveolar supplements located along ventral side of anterior part of body; anteriormost supplement located 7-10 µm from anterior end, at level of stoma; posteriormost one located 111-178 µm from anterior end, at level of posterior part of pharynx or renette cell. Postcloacal sensilla papilliform, located subventrally, at 28-30 µm

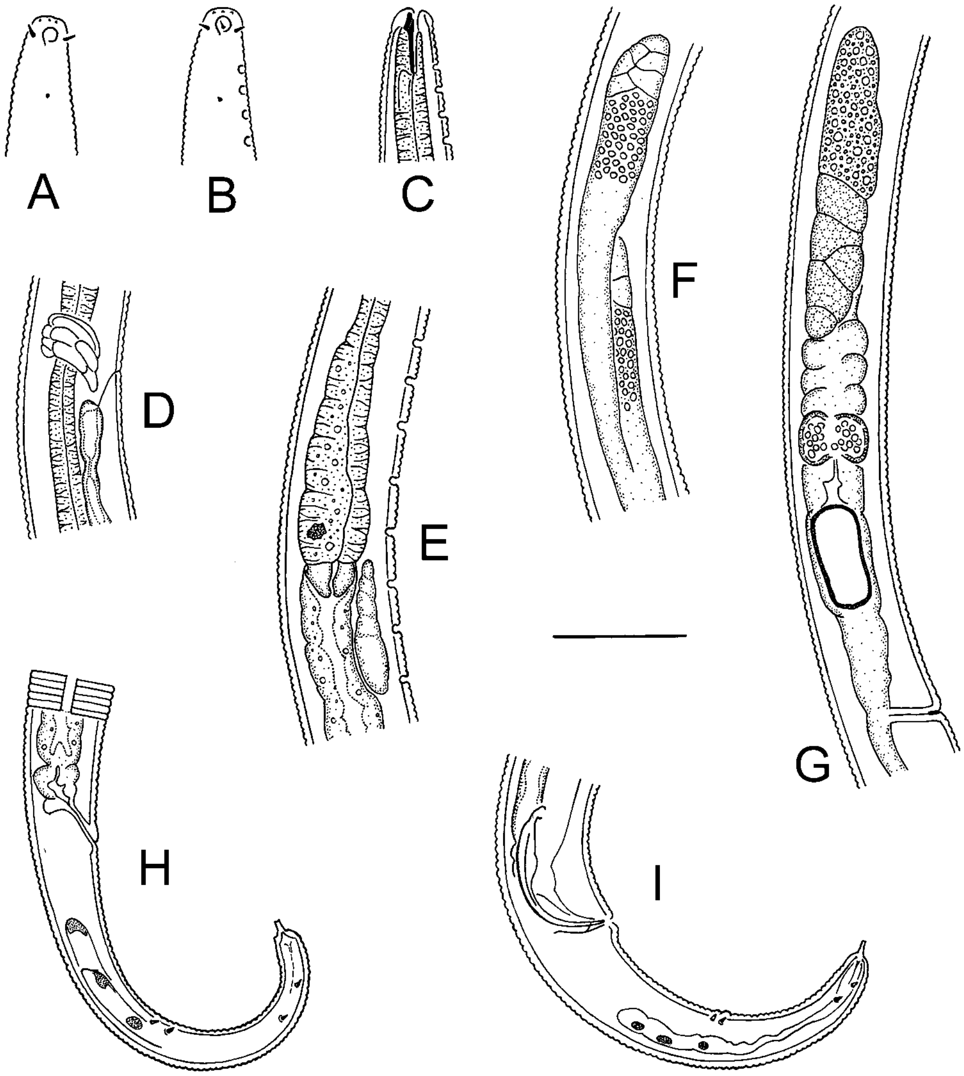

Figure 3.4. *Deontolaimus papillatus* de Man, 1880. A: Female anterior end, surface view; B: Male anterior end. surface view; C: Male anterior end, median section; D: Nerve ring and excretory ampulla; E: Posterior part of pharynx and renette cell; F: Testes; G: Anterior female gonad; H: Female tail; I: Male tail. Scale bar 20 µm.

posterior to cloacal opening. Four caudal setae arranged in two pairs: one pair subventral at mid-tail and one pair subdorsal near tail teminus.

Female: Reproductive system didelphic, amphidelphic; ovary branches reflexed antidromously. Anterior ovary 37-61 µm long, posterior ovary 38-55 µm long. Oviduct with large glandular cells. Spermatheca axial, in proximal part of uterus. Vagina straight, 0.5 times vulval body diameter long, without sclerotisations. Intrauterine egg measuring 16.5×8.0 µm, 2.1 times its own diameter. Rectum 1.2-1.4 times ABD long; its proximal part surrounded by three gland-like cells: one dorsal and two ventrosublateral. Four caudal setae arranged in two pairs: one pair subventral at mid-tail and one pair subdorsal at posterior one-fifth of tail.

Material examined: The Netherlands, salt marshes.

Relationships: A comparison between the recent specimens and the redescription of the species lectotype and para(lecto)type by Loof (1961) did not reveal any essential morphological or morphometric differences, although few morphological data were possible to observe on de Man's original material (Loof, 1961). The males described by Meyl (1954) are somewhat larger than those of the recent population (L= 814-1041 *vs* 666-738 µm) and have shorter spicules (13 *vs* 24-29 µm), for which no manubrium was drawn by the author. The specimens described by Lorenzen (1969) agree well with the recent males in morphology (particularly in stoma and spicula structure), but they are also slightly larger (L= 845-1055 *vs* 666-738 µm) and have shorter spicules (20-24 *vs* 24-29 µm). Finally, three males described by Alekseev (1981) from the Sakhalin area are longer (L= 870-1160 *vs* 666-738 µm), have shorter stoma (6 *vs* 9-10 µm), longer tails (65-76 *vs* 54.5-57 µm) and a larger number of alveolar supplements (32-38 *vs* 22-35).

Leptolaimus mixtus Lorenzen, 1972

(Fig. 3.5, Table 3.2)

Adult: Body slender, cylindrical, gradually narrowing anteriorly in pharyngeal region and posteriorly on tail; straight in the anterior half, only slightly curved ventrad in the posterior part in females and strongly curved ventrad in the posterior part in males. Cuticle annulated; annuli 2 µm wide at midbody, without ornamentation. Lateral alae a narrow and smooth band of cuticle demarcated by two straight lines, originating at level of middle of pharynx, terminating at middle of tail in females and at level of cloaca in males. Body pores present, arising from oval sublateral hypodermal gland cells, distributed as follows: 4-5 pores on each body side of pharyngeal region, 6-11 pores on each side between cardia and anus or cloaca and one pair of pores on tail. Sensilla connected with most hypodermal glands (except second and third pairs in male) and extending to the exterior through body pores. Lip region continuous with body contour, rounded, lips separate. First annulus appears 2-3 µm from anterior end, cephalic capsule absent. Inner labial sensilla indistinct. Outer labial sensilla papilliform, located on anterior surface of lips. Cephalic sensilla setiform, directed forward; their bases located anterior to amphid, at base of lip region. Subcephalic setae absent. Amphid 4.5-5.5 µm wide, unispiral, located at level of stegostom. Ocelli and deirid absent. Secretory-excretory system present; renette cell large, located opposite to ventral side of cardia and anterior part of intestine, extends anteriorly

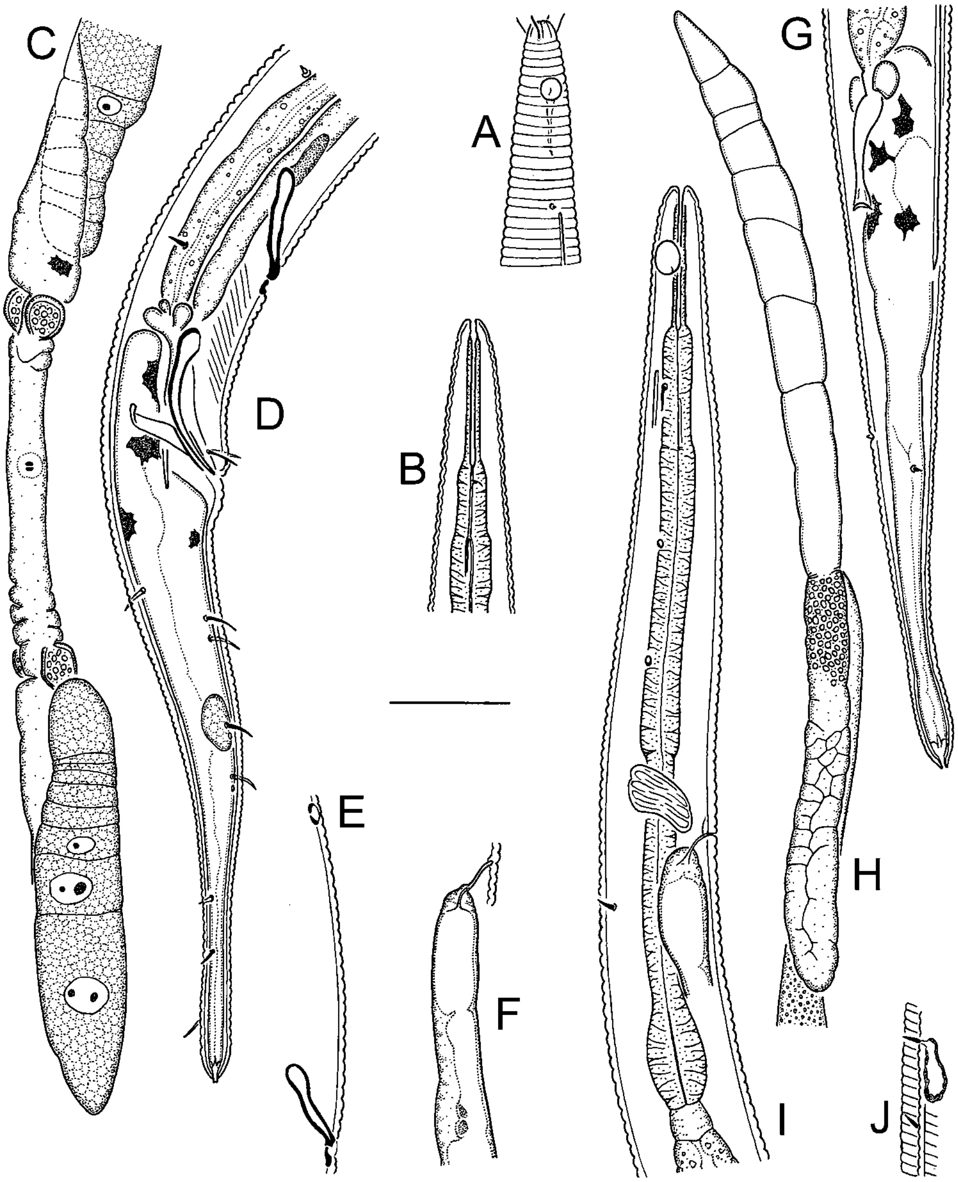

Figure 3.5. *Leptolaimus mixtus* Lorenzen, 1982. A: Female anterior end, surface view; B: Female anterior end, median section; C: Female gonalds, ventral view; D: Male caudal region; E: Supplements; F: Excretory ampulla; G: Female tail; H: Testes; I: Male pharyngeal region; J: Hypodermal glands. Scale bar 20 µm.

Table 2.2. Measurements (in µm) of *Leptolaimus mixtus* Lorenzen, 1972, *Paraplectonema loofi* Holovachov & Boström, 2004 and *Rhadinema flexile* Cobb, 1920.

Species	*Leptolaimus mixtus*		*Paraplectonema loofi*				*Rhadinema flexile*	
			La Amistad Caribe		Tortuguero			
	Male	Female	Males	Holotype	Males	Females	Male	Female
n	1	1	6	1	3	3	1	1
Body length	784	784	776±42.1 (740-859)	841	751-825	823-881	1548	2157
Body diameter	23.0	25.5	26.7±2.0 (23.0-29.0)	28.0	24.5-27.0	27.0-30.0	22.0	32.0
Pharyngeal region length	150	166	112±6.1 (106-120)	118	102-116	115-122	151	168
Tail length	100	95	145±21.9 (129-189)	187	147-167	189-200	113	144.5
Anal or cloacal body diameter	21.0	18.0	19.8±0.8 (19.0-21.0)	17.0	19.0	17.0-19.0	22.0	20.0
a	33.6	30.7	29.2±2.5 (26.5-33.0)	30.3	30.7-32.5	28.9-33.0	69.7	66.9
b	5.2	4.7	7.0±0.3 (6.6-7.2)	7.1	6.9-7.3	7.1-7.7	10.2	12.9
c	7.8	8.3	5.4±0.5 (4.5-5.9)	4.5	4.8-5.1	4.4-4.5	13.7	14.9
c’	4.7	5.3	7.3±0.9 (6.4-8.9)	11.2	7.8-8.8	10.1-12.0	5.1	7.2
Labial region diameter	5.5	5.5	9.3±0.6 (9.0-10.0)	9.0	9.0-11.0	10.0-11.0	10.0	13.0
Cephalic setae length	4.5	3.0	2.0	2.0	2.0	2.0	5.5	5.5
Amphid location	9.0	9.0	2.0 (1.0-2.0)	2.0	2.0	2.0-3.0	10.0	11.0
1st body pore location	32.0	32.0	46.1±5.1 (42.0-54.5)	50.0	47.0-53.0	50.0-52.0	12.0	13.0
Stoma length	22.0	24.5	13.9±1.2 (12.0-15.5)	14.5	14.5-17.0	14.5-17.0	5.5	5.5
Dorsal gland orifice location	25.5	28.0	13.9±1.2 (12.0-15.5)	14.5	14.5-17.0	14.5-17.0	5.5	5.5
Cardia length	7.0	7.0	18.5±1.8 (17.0-21.0)	19.0	13.0-20.0	13.0-17.0	2.0	2.0
Nerve ring location	100.0	106.0	68.9±5.2 (64.5-78.0)	74.5	67.0-75.5	73.0-74.5	89.0	97.0
Nerve ring (% of phar. region)	66.7	63.8	61.8±1.9 (59.8-64.8)	63.2	61.2-65.4	60.9-65.0	49.5	57.6
Excretory pore location	106	118	-	-	-	-	98	113
Excretory pore (% of phar. region)	70.4	71.1	-	-	-	-	58.8	67.5

Table 2.2. Continued.

Species	*Leptolaimus mixtus*		*Paraplectonema loofi*				*Rhadinema flexile*	
			La Amistad Caribe		Tortuguero			
	Male	Female	Males	Holotype	Males	Females	Male	Female
n	1	1	6	1	3	3	1	1
Spinneret length	3.0	3.0	1.0	?	1.0-2.0	2.0	2.0	2.0
Supplement number	3alv+ 4tub	abs.	9-10 tub	abs	10-12 tub	absent	10 tub	abs.
V (%) or T (%)	51.7	51.7	48.0±1.5 (45.1-49.8)	42.8	46.4-52.4	41.7-42.6	49.5	58.9
G1 (%)	-	13.0	-	10.2	-	9.0-12.9	-	15.0
G2 (%)	-	13.6	-	9.9	-	7.8-10.6	-	15.6
Vagina or Spicule length	24.5	?	24.4±1.0 (23.0-25.5)	5.5	25.5-27.0	5.5-8.0	25.5	12.0
Rectum or gubernaculum length	9.0	20.0	7.0±0.6 (7.0-8.0)	23.0	7.0-9.0	24.5-31.0	9.0	22.0

along ventral side of pharynx and forms an excretory ampulla just posterior to nerve ring level. Excretory canal weakly cuticularised, extends from excretory ampulla for a short distance anteriorly and opens to the exterior via a pore. Stoma uniformly tubular. Cheilostom and gymnostom short, undifferentiated; stegostom tubular, with uniformly thickened lumen. Pharynx muscular, cylindrical anteriorly, with distinct oval cardial bulb, lacking radial tubules and valvular apparatus. Anterior cylindrical part of pharynx subdivided by breaks in muscular pharyngeal tissue into a narrow procorpus, a somewhat wider metacorpus and a narrow isthmus. Dorsal gland orifice penetrates pharyngeal lumen posterior to stoma base, subventral gland orifices not seen. Pharyngeal glands indistinct. Cardia cylindrical, free. Tail conoid, straight. Three caudal glands present, their nuclei located at rectum level and in tail. Spinneret functional, weakly cuticularised.

Male: Reproductive system diorchic, testes opposed; anterior testis 92 µm long; posterior testis 69 µm long. Spicules symmetrical, with oval manubrium and gradually narrowing, arcuate shaft. Gubernaculum plate-like, with dorsal apophysis. Four similar midventral tubular plus three alveolar supplements and a single midventral precloacal sensillum. Each tubule is connected with a single gland-like cell, both lying on the right-hand side of intestine. There is a small sclerotised rod in the cuticle, caudally at distal end of each tubule. Posteriormost tubule located 37 µm anterior to cloacal opening. Alveolar supplements are circular invaginations, each consists of two bar-shaped sclerotisations in median section. Precloacal sensillum is papilliform in shape; it is located on anterior cloacal lip. One subventral pair of precloacal setae are located at level with spicules. Two subventral pairs and one subdorsal pair of setae plus two unpaired subdorsal setae located on tail.

Female: Reproductive system didelphic, amphidelphic; ovary branches reflexed antidromously. Anterior ovary 66 µm long, posterior ovary 72 µm long. Oviduct a broad tube extending from about middle of ovary. Two offset, sac-like spermathecae located on each (right and left) side of each (anterior and posterior) gonoduct in oviduct-uterus junction. Spermathecae filled with oval spermatozoa. Vagina straight; vaginal sclerotisations present, arcuate in ventral view. Intrauterine egg not seen. Rectum 1.1 times ABD long; its proximal part surrounded by three gland-like cells: one dorsal and two ventrosublateral. One subventral pair of caudal setae located at middle of tail.

Material examined: The Netherlands, Oosterschelde, August 1991, *legit* R. Alkemade.

Relationships: The male of the recent population agrees fairly well with the original description in general morphology and measurements, except being somewhat larger (L= 784 *vs* 695-735 µm) and having more alveolar supplements (3 *vs* 2) than the type specimens.

Paraplectonema loofi Holovachov & Boström, 2004

(Fig. 3.6, Table 3.2)

Adult: Body slender, cylindrical, gradually narrowing anteriorly in pharyngeal region and posteriorly on tail; straight in the anterior half, curved ventrad only on tail. Cuticle annulated; annuli 1-2 µm wide at midbody, without ornamentation. Lateral alae a narrow and smooth band of cuticle demarcated by two straight lines, originating at level of posterior part of pharynx, terminating at middle of tail. Body pores present, arising from oval sublateral hypodermal gland cells, distributed as follows: 2-3 pairs in pharyngeal region and a few scattered along the body. Sensilla connected with all hypodermal glands and extending to the exterior through body pores. Lip region truncate, continuous with body contour, lips fused. Inner labial sensilla indistinct. First annulus appears 2-3 µm from anterior end, cephalic capsule absent. Outer labial sensilla papilliform, located on anterior surface of lips. Cephalic sensilla setiform, directed outward; their bases located anterior to amphids, at the base of labial region. Subcephalic setae absent. Amphid 3-4 µm wide, unispiral, located at level of gymnostom. Ocelli and deirid absent. Secretory-excretory system present; renette cell 122-153 µm long, large, located opposite to ventral side of cardia and anterior part of intestine. Cuticularised excretory duct originates from renette cell, extends forward along ventral side of pharynx, and opens to the exterior via a pore on anterior surface of labial region. Stoma uniformly tubular. Cheilostom short, supported by six fine longitudinal ribs. Gymnostom short, with plate-like rhabdia. Stegostom tubular, with uniformly thickened lumen. Pharynx muscular, cylindrical anteriorly, with distinct oval cardiac bulb, lacking radial tubules and valvular apparatus. Anterior cylindrical part of pharynx subdivided by breaks in muscular pharyngeal tissue into a narrow procorpus, a somewhat wider metacorpus and a narrow isthmus. Cardiac bulb with thick and strongly cuticularised lumen. Dorsal gland orifice penetrates pharyngeal lumen at stoma base, subventral gland orifices not seen. Pharyngeal glands indistinct. Cardia cylindrical, free, with posterior part surrounded by intestinal tissue. Three caudal glands present, their nuclei are incaudal. Swollen, non-annulated tail terminus. Spinneret functional, weakly cuticularised.

Male: Reproductive system diorchic, testes opposed; anterior testis 33-50 µm long; posterior testis 30-65 µm long. Spicules symmetrical, with oval manubrium and gradually

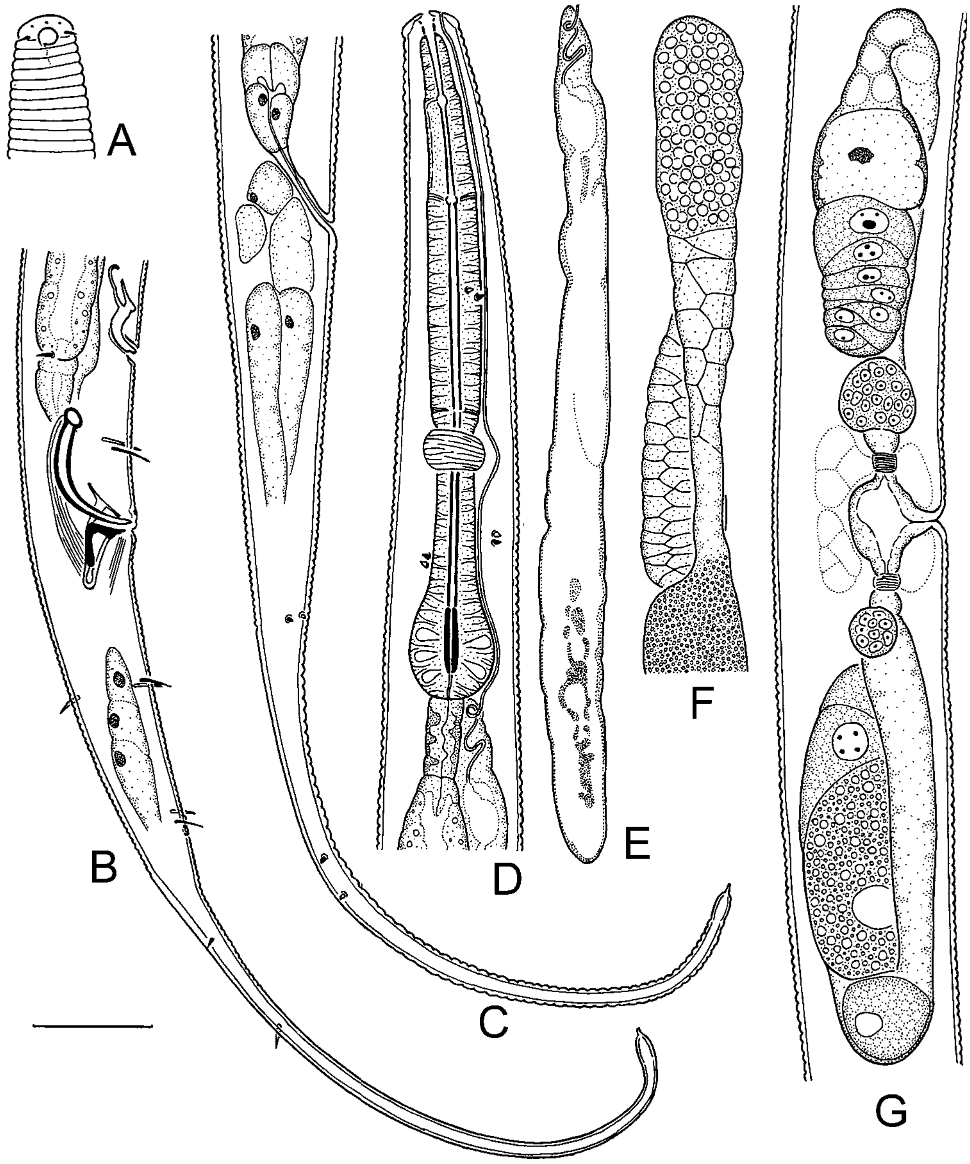

Figure 3.6. *Paraplectonema loofi* Holovachov & Boström, 2004. A: Male anterior end, surface view; B: Male caudal region; C: Female tail; D: Pharyngeal region; E: Renette cell; F: Testes; G: Female gonads. Scale bar 20 µm.

narrowing, arcuate shaft. Gubernaculum plate-like with strong caudal apophysis. Nine to eleven similar midventral tubular supplements, and a single midventral precloacal sensillum on anterior cloacal lip. Tubular supplements of peculiar sigmoid shape, able to protrude. Posteriormost tubule located 19-30 μm anterior to cloacal opening. Precloacal sensillum papilliform in shape, located on anterior cloacal lip. One subventral pair of precloacal setae located at level of spicules. Two subventral pairs and one subdorsal pair of setae and one subventral pair of papillae located on tail. One single subdorsal seta located on the left body side posterior to cloaca.

Female: Reproductive system didelphic, amphidelphic, ovary branches reflexed antidromously. Anterior ovary 42-90 μm long, located on the right-hand side of intestine; posterior ovary 40-68 μm long, located on the left-hand side of intestine. Oviduct a broad tube extending from ovary tip. Two offset, sac-like spermathecae located on each (right and left) side of each (anterior and posterior) gonoduct, in oviduct-uterus junction. Spermathecae filled with oval spermatozoa. Uterus short, an undifferentiated cylindrical tube. Vagina straight, 0.2-0.3 times vulval body diameter long, without sclerotisations. Rectum 1.4-1.6 times ABD long; its proximal part surrounded by three gland-like cells: one dorsal and two ventrosublateral. One subventral and one subdorsal pair of caudal setae present.

Type locality and habitat: Costa Rica: La Amistad Caribe Conservation Area, Barbilla National Park, secondary forest, sediment and rotten leaves under still water, altitude 300-400 m a.s.l., June 2002, *legit* L. Acosta.

Other locality and habitat: Costa Rica: Tortuguero Conservation Area, Wild Life Refuge Barra del Colorado, soil and roots, altitude 0-100 m a.s.l., August 2002, *legit* T. Bongers.

Type material: Holotype female and six paratype males deposited in the nematode collection of INBio, Santo Domingo de Heredia, Costa Rica. Three paratype females and three paratype males deposited in the nematode collection of the Wageningen University, the Netherlands.

Differential diagnosis: The new species differs from all previously described species of *Paraplectonema* Strand, 1934 by the position of excretory pore on lip region (*vs* at level of middle part of pharynx when present at all). A comparison of the the new species with other populations of the genus *Paraplectonema* is also made in Table 3.3.

Etymology: The new species is named after Dr. P.A.A. Loof in recognition of his many contributions to the taxonomy of nematodes and for his kind assistance during the preparation of this manuscript.

Remarks: Gagarin (2001) described *Leptolaimus primitivus* from a freshwater lake in Japan. The species is characterised particularly by the far anterior location of amphid and the sigmoid shape of tubular supplements, thus resembling the genus *Paraplectonema*. The combination: *Paraplectonema primitivum* (Gagarin, 2001) Holovachov & Boström, 2004 is proposed.

Table 3.3. Comparison of different populations of the genus *Paraplectonema* Strand, 1934.

	Body length, µm	Tail, µm	c'	Excretory pore	Somatic sensilla	Tubuli number	Spicula, µm
pedunculatum							
Coomans & Raski, 1991b	990-1100	173-197	8.5-10.3	post. to nerve ring	present	-	-
Alekseev, 1992	?	?	?	?	?	9	21-22
Eyualem, 1996	830-1186	164-234	9.2-14.6	post. to nerve ring	present	-	-
multitubiferum							
Imamura, 1931	940-1117	?	?	?	absent?	13	?
Alekseev, 1992	910-1120	110-173	6-9	post. to nerve ring	?	13	31-32
canadianum							
Hopper, 1968	1120-1190	273-360	?	post. to nerve ring	present	9	34-38
primitivum							
Gagarin, 2001	1138-1178	172-214	6.2-7.0	level with bulb	?	12	38
americanum							
Zullini et al., 2002	850-1070	137-225	6.9-12.5	indistinct*	absent?	10-11	28-30
vietnamicum							
Gagarin et al., 2003	630-920	98-158	4.7-7.5	post. to nerve ring	present	11-13	29-35
loofi							
recent data	740-881	129-200	6.4-12.0	at labial region	present	9-12	23-27

*after re-examination of the type material by P.A.A. Loof

***Rhadinema flexile* Cobb, 1920**

(Fig. 3.7, Table 3.2)

Adult: Body slender, cylindrical, gradually narrowing anteriorly in pharyngeal region and posteriorly on tail; ventrally curved upon fixation in posterior part. Cuticle annulated; annuli without additional ornamentation. Lateral alae absent. Hypodermal glands and body pores present, distributed in two sublateral rows on each side of the body. Somatic setae present only on tail. Lip region rounded, continuous with body contour, lips fused. Inner labial sensilla indistinct. Outer labial sensilla papilliform, located on anterior surface of lips. Cephalic sensilla setiform, directed outward, their bases located anterior to amphids, 4-5 µm from anterior end. Subcephalic setae absent. Amphid 5-7 µm wide, unispiral. Ocelli and deirid absent. Secretory-excretory system present; renette cell indistinct, located posterior to cardia, along ventral side of anterior part of intestine; extends

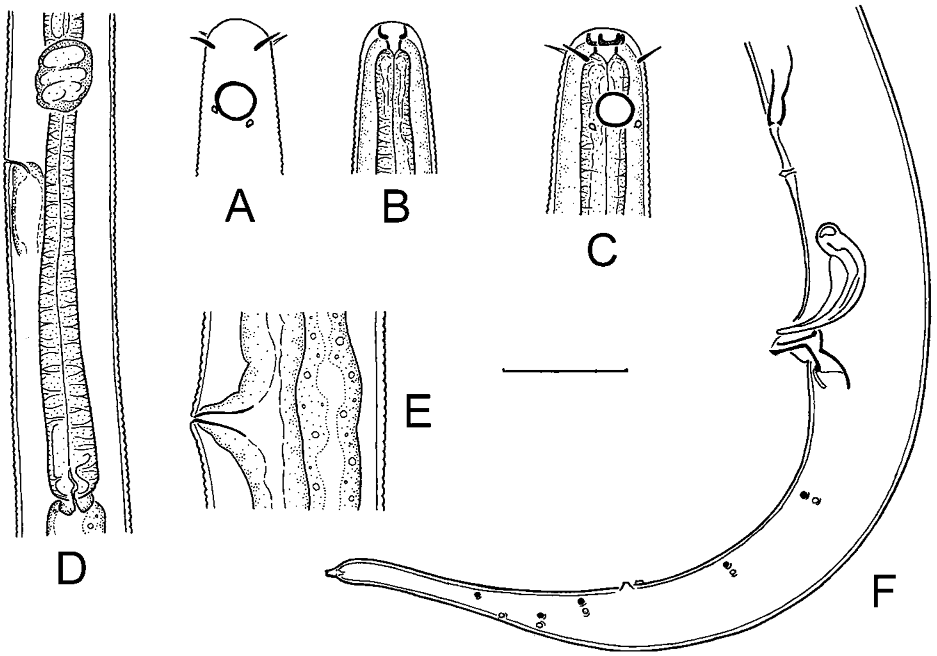

Figure 3.7. *Rhadinema flexile* Cobb, 1920. A: Male anterior end, surface view; B: Male anterior end, median section; C: Female anterior end, combined view; D: Posterior part of pharynx; E: Vulval region; F: Male caudal region. Scale bar 20 µm.

anteriorly along ventral side of pharynx and forms an excretory ampulla just posterior to level of nerve ring. Excretory canal weakly cuticularised, extends from excretory ampulla for a short distance anteriorly and opens to the exterior via a pore. Stoma broad, 4-6 µm wide, funnel-shaped. Cheilostom broad, consists of a sclerotised ring with six forwardly directed projections: one dorsal, one ventral and four sublateral in position. Gymnostom short, gymnorhabdia plate-like. Stegostom undifferentiated, conoid. Pharynx cylindrical anteriorly, gradually widening posteriorly; heavily muscularized; with uniformly thickened lumen; without valves and bulbs. Dorsal and subventral (one pair) gland orifices penetrate pharyngeal lumen somewhat posterior to stoma base. Nuclei of pharyngeal glands indistinct. Cardia short, embedded in intestine. Tail gradually narrowing, arcuate ventrad. Three caudal glands present. Spinneret functional, weakly cuticularised.

Male: Reproductive system diorchic. Spicules symmetrical, with oval manubrium and arcuate conoid shaft. Gubernaculum complex, with caudal apophysis. Ten similar midventral tubular supplements, a single midventral precloacal sensillum and two subventral postcloacal sensilla. Posteriormost tubule located 34 µm anterior to cloaca. Precloacal sensillum setiform in shape and consists of a nerve ending, located 25 µm anterior to cloacal opening. Postcloacal sensilla papilliform, located subventrally, 65 µm from cloacal opening. Ten caudal setae arranged in three subventral and two dorsosublateral pairs.

Female: Reproductive system didelphic, amphidelphic; ovary branches reflexed antidromously. Anterior ovary 241 µm long, located on the left-hand side of intestine, posterior ovary 219 µm long, located on the right-hand side of intestine. Oviduct and uterus indistinct. Spermatheca axial. Vagina straight, 0.4 times vulval body diameter long, without sclerotisations. Intrauterine egg not seen. Rectum 1.1 times ABD long. One subventral pair of caudal setae present.

Material examined: USA, North Atlantic Ocean, South Carolina, 4 miles south-east of Edisto river, depth 10 m, May 1963, *legit* N. Chamberlain.

Relationships: The male described agrees with the short original description given by Cobb (1920) except for a longer tail (113 *vs* 87 µm). Cobb did not give any information on spicula and tubular supplements. These specimens also agree in morphology and measurements with the population described by Lorenzen (1972a), except for the body ratios, longer spicula (25 *vs* 18 µm) and larger number of tubular supplements (10 *vs* 7-8).

Stephanolaimus elegans Ditlevsen, 1918

(Figs 3.8 & 3.9, Table 3.1)

Adult: Body slender, cylindrical, gradually narrowing anteriorly in pharyngeal region and posteriorly on tail; ventrally curved upon fixation, more strongly curved ventrad in posterior part. Cuticle annulated; annuli 1.7-2.4 µm wide at midbody, without ornamentation. Lateral alae absent. Hypodermal glands and body pores present, distributed in two sublateral rows on each side of the body, 139-276 in total. Somatic setae present, not connected to hypodermal glands; distributed as follows: two pairs of long “cervical” setae located at level of anterior one-fourth of pharynx (one female with four setae on the right-hand side and three setae on the left-hand side of the body) and one pair of setae just posterior to nerve ring. Lip region strongly offset, lips separate. First annulus appears 7-10 µm from anterior end, posterior to cephalic setae bases and amphids, forming the base of the cephalic capsule. Inner labial sensilla indistinct. Outer labial sensilla papilliform, located on anterior surface of lips. Cephalic sensilla setiform, directed outward; their bases located anterior to amphids, 5-9 µm from anterior end. Subcephalic setae absent. Amphid 3-5 µm wide, in shape of a transverse oval slit. Ocelli and deirid absent. Secretory-excretory system present; renette cell 113-171 µm long, elongated, located posterior to cardia, along ventral side of anterior intestine, extends anteriorly along ventral side of pharynx and forms an excretory ampulla just posterior to level of nerve ring. Excretory canal weakly cuticularised, extends from excretory ampulla for a short distance anteriorly and opens to the exterior via a pore. Stoma funnel-shaped, probably triangular in median section. Cheilostom undifferentiated. Gymnostom short, with three “odontia-like” sclerotisations (one dorsal and two ventrosublateral) pointing forward. Stegostom funnel-shaped, undifferentiated, with strongly sclerotised walls. Pharynx cylindrical anteriorly, gradually widening posteriorly; heavily muscularized; with uniformly thickened lumen;

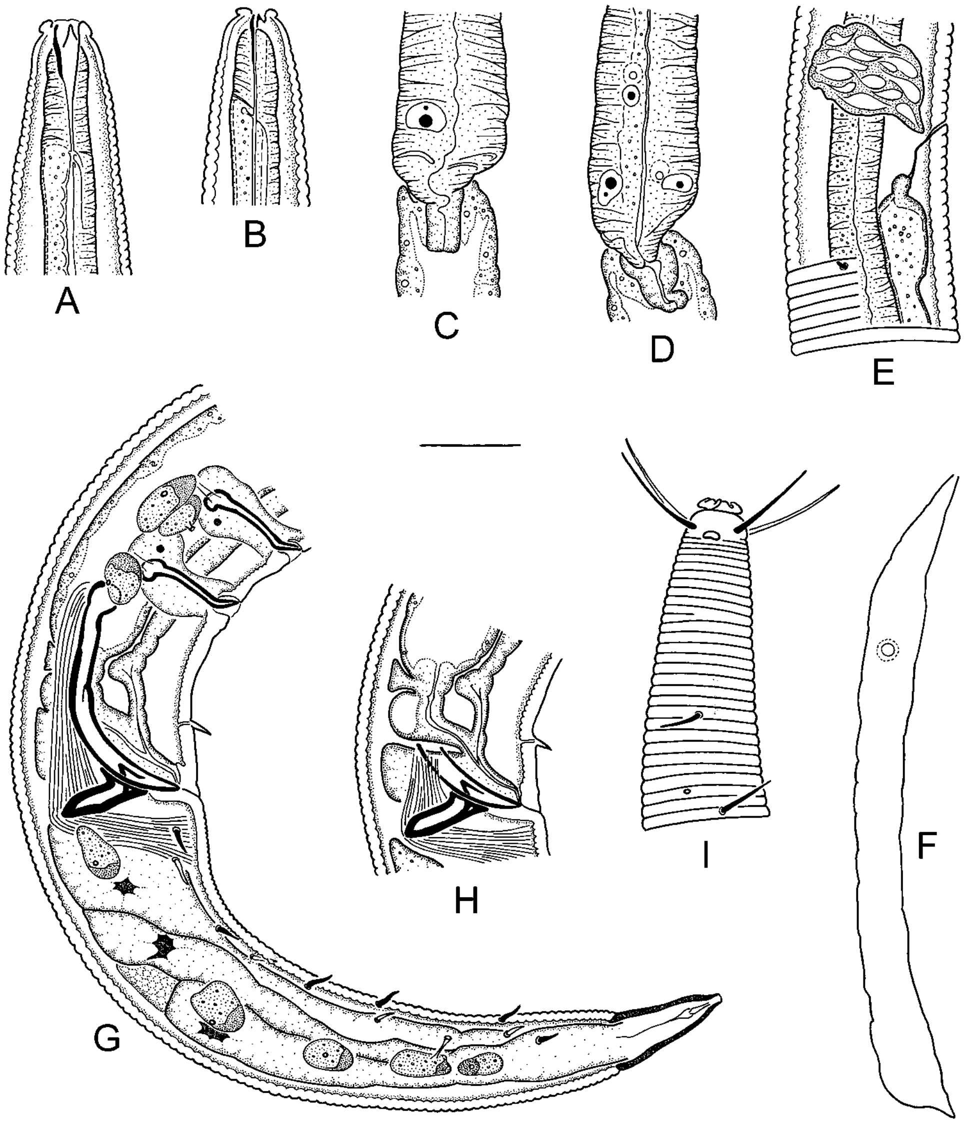

Figure 3.8. *Stephanolaimus elegans* Ditlevsen, 1918. A-B: Female anterior end, median section; C-D: Posterior part of pharynx; E: Nerve ring and excretory ampulla; F: Renette cell; G: Male caudal region; H: Male cloacal region, median section; I: Male anterior end, surface view. Scale bar 20 µm.

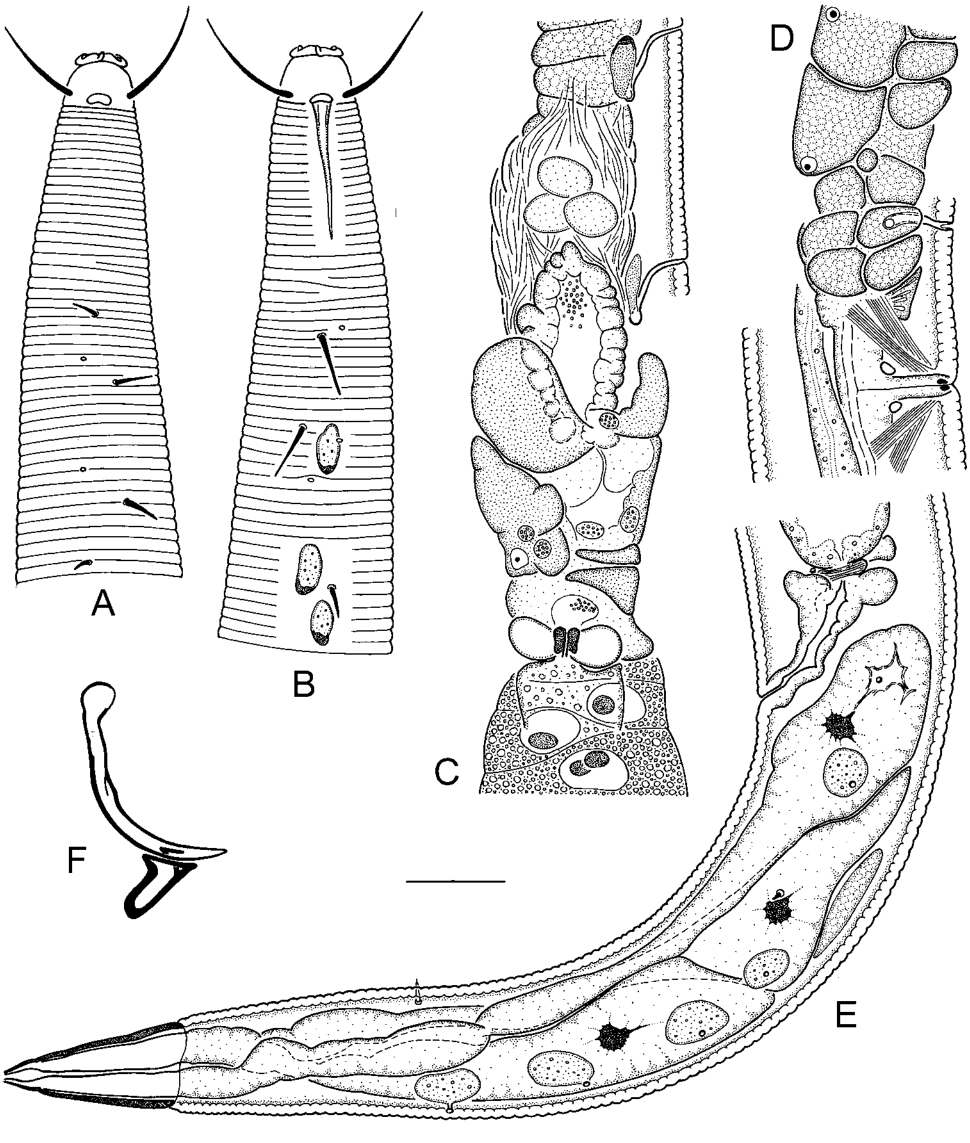

Figure 3.9. *Stephanolaimus elegans* Ditlevsen, 1918. A-B: Female anterior end, surface view; C: Posterior spermatheca, oviduct and ovary; D: Vulval region; E: Female tail; F: Spicule and gubernaculum. Scale bar 20 µm.

without valves and bulbs. Dorsal and subventral (one pair) gland orifices penetrate pharyngeal lumen somewhat posterior to stoma base. Usually nuclei of only three pharyngeal glands distinct, but in one specimen nuclei of five pharyngeal glands visible. Dorsal gland nucleus located posterior to nuclei of all subventral glands. Cardia cylindrical, embedded in intestine. Tail conoid, arcuate ventrad. Three caudal glands present, their nuclei are incaudal. Spinneret functional, large and strongly cuticularised.

Male: Reproductive system diorchic, testes opposed; anterior testis 600-890 µm long, outstretched, on the right-hand side of intestine; posterior testis 202-245 µm long, reflexed, on the left-hand side of intestine. Spicules symmetrical, with oval manubrium and arcuate subcylindrical shaft. Gubernaculum plate-like, with dorsal apophysis. 32 to 43 similar midventral tubular supplements and a single midventral precloacal sensillum. Each tubular supplement is connected with a single gland-like cell, both lying on the right-hand side of intestine. Supplement glands attach on one side to cuticle on ventral body side. Posteriormost tubule located 47-61 µm anterior to cloacal opening. Precloacal sensillum setiform in shape and consists of a nerve ending, located 15-22 µm anterior to cloacal opening. 8-14 caudal setae arranged in two sublateral rows.

Female: Reproductive system didelphic, amphidelphic; ovary branches reflexed antidromously. Anterior ovary 155-262 µm long, located on the right-hand side of intestine; posterior ovary 198-311 µm long, located on the left-hand side of intestine. Oviduct short, separated from uterus by a strong sphincter. Spermatheca axial, in proximal part of uterus. Vagina straight, 0.4-0.6 times vulval body diameter long; encircled by a single sphincter muscle, with rod-like sclerotisations. Intrauterine egg measuring 183×44 (178-189×42-46) µm, 4.2 times its own diameter long. Tubular supplements present along the ventral line: 9-18 anterior to vulva, extending to cardia level; and 10-18 posterior to vulva, extending almost to rectum. Intestine-rectum junction is surrounded by the rectal sphincter muscle, its cell is located dorsally from the junction. Rectum 0.7-0.8 times ABD long; its proximal part surrounded by tree gland-like cells: one dorsal and two ventrosublateral. Two caudal setae present, one on the left-hand body side at anterior one-fourth and another on the right-hand body side at posterior two-fifths of tail length.

Material examined: The Netherlands, Oostershelde, August 1991, *legit* R. Alkemade.

Relationships: *Stephanolaimus elegans* Ditlevsen, 1918 was described from the Danish Little Belt Sea on the basis of a single immature female, which however is similar morphologically to our specimens. The specimens described by Platt & Warwick (1988) agree fairly well with the recent material except for having a larger number of supplements in both males (42-45 *vs* 32-43) and females (33-40 *vs* 19-34). Platt & Warwick (1988) also stated: "Buccal cavity small and flask-shaped with six small pointed teeth at its anterior end.", which may be due to a misinterpretation of the gymnostom structure, or that their specimens represent a different species.

Review of the morphological characters used in the analyis

Cuticle and hypoderm

The cuticle is usually finely to coarsely annulated, smooth under LM only in species of *Onchium* Cobb, 1920, *Ionema* Cobb, 1920 and *Smithsoninema* Hope & Tchesunov, 1999 (Cobb, 1920; Hope & Tchesunov, 1999), probably due to a decrease in the size of the annulation. Fine longitudinal striations are present in *Onchiolistia tubifera* (Furstenberg & Vincx, 1988) Blome, 2002, *O. multipapillata* Blome, 2002 and species of the genus *Alaimella* Cobb, 1920, but without any additional ornamentation (Blome, 2002; Cobb, 1920; Furstenberg & Vincx, 1988). Both characters are here considered as of secondary origin from the more common annulated cuticle without ornamentation in the taxon.

Lateral differentiations of the cuticle (lateral alae) are present in members of the genera *Anguinoides*, *Anomonema* Hopper, 1963, *Antomicron*, *Camacolaimus* de Man, 1889, *Deontolaimus* de Man, 1880, *Leptolaimoides*, *Leptolaimus* and *Paraplectonema* (Coomans & Raski, 1991b; Holovachov, 2003a, 2003b; Hopper, 1963, 1968; Platt & Warwick, 1988; Vitiello, 1974; recent data), and also in *Diodontolaimus parasabulosus* Keppner, 1992 and *Manunema pectenophora* Stewart & Nicholas, 1995 (Keppner, 1992; Stewart & Nicholas, 1995). In all mentioned taxa the lateral alae are a single smooth band of cuticle with straight or crenate margins on the lateral sides of the body, never consisting of two or more bands, thus differing morphologically from the lateral alae in *Plectus* Bastian, 1865 and other Plectidae (see Maggenti, 1961a, 1961b). In some species where lateral alae are absent, the cuticular annulation on the lateral sides of the body is interrupted irregularly (e.g. *Leptoplectonema* Coomans & Raski, 1991). Presence of lateral alae is considered as a derived character, but probably reversible in nature. Due to the many differences in the structure of the lateral alae in Leptolaimoidea and Plectoidea, we consider them to have evolved independently on the same “base”, namely the lateral hypodermal chord.

A few taxa have strongly developed hypodermal glands opening to the exterior via pores and arranged in sublateral rows along the body: *Antomicron, Leptolaimus, Leptoplectonema, Manunema* Gerlach, 1957*, Paraplectonema, Rhadinema* Cobb, 1920 and *Stephanolaimus elegans* (Coomans & Raski, 1991b; Hopper, 1968; Platt & Warwick, 1988; recent data). Hypodermal glands are characteristic for most aquatic nematodes and their presence is here considered as a primitive feature.

Somatic sensilla

Somatic sensilla are present in all leptolaimoid taxa except in *Ionema*, *Onchium* and *Smithsoninema* (Cobb, 1920; Hope & Tchesunov, 1999). Their presence or absence was not mentioned in the descriptions of *Onchiolistia tubifera, O. africana* (Furstenberg & Vincx, 1988) Blome, 2002 and *Listia* (Blome, 1982; Furstenberg & Vincx, 1988) but somatic sensilla were found in *Onchiolistia multipapillata* (Blome, 2002).

Species of *Antomicron*, *Leptolaimus*, *Manunema* and *Paraplectonema* have setae arising from the hypodermal glands (Hopper, 1968; recent data) in the same way as in the family Aphanolaimidae (Chapter 2), similar to the “somatic pore” on Fig. 49Б in

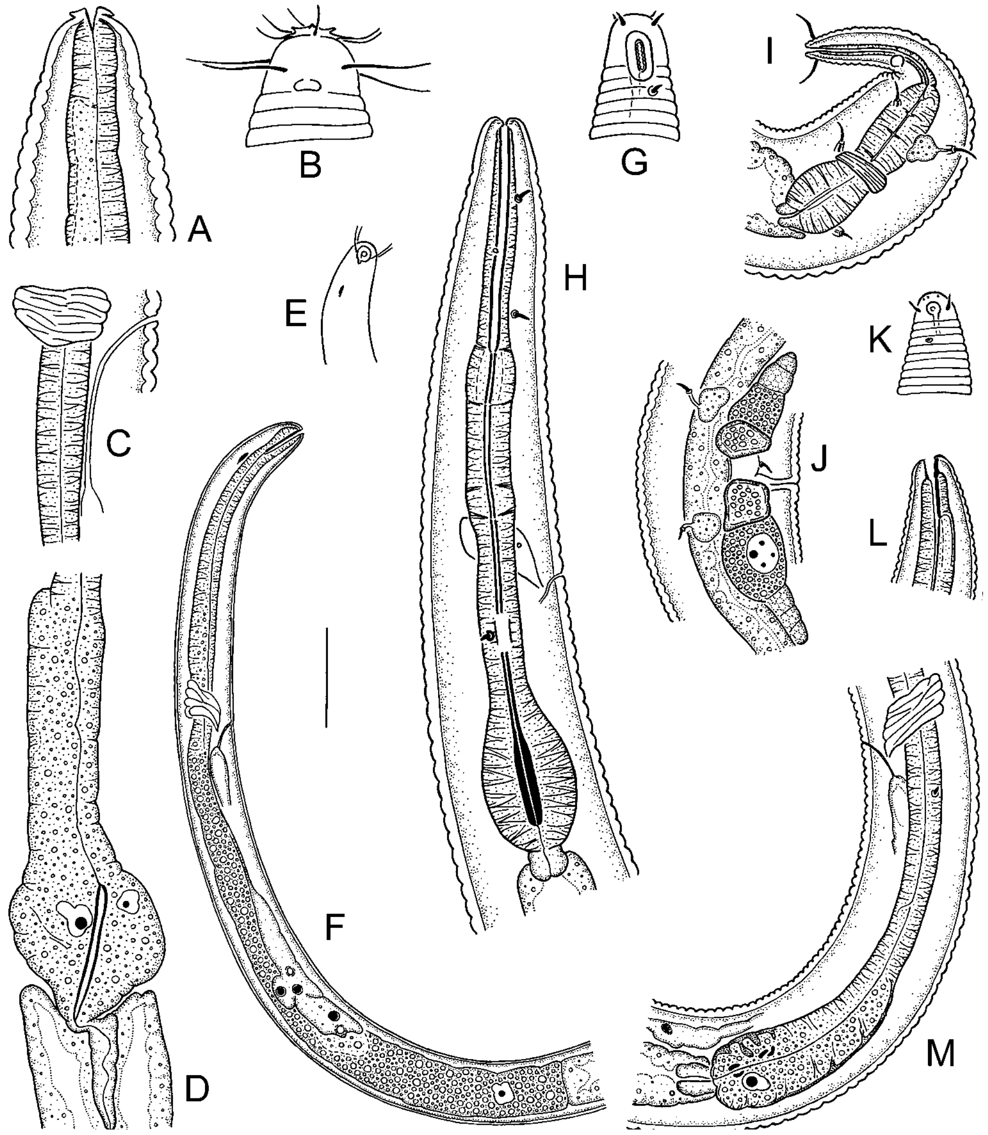

Figure 3.10. Morphology of *Setostephanolaimus* sp. (A-D), *Onchium* sp. (E-F). *Antomicron elegans* (de Man, 1922) De Coninck, 1965 (G-H), *Manunema* sp. (I-J). *Camacolaimus* sp. (K-M). A, L: Anterior end, median section; B, E, G, K: Anterior end, surface view; C: Nerve ring and excretory duct; D, M: Posterior part of pharynx; F, H, I: Pharyngeal region; J: Female gonads. Scale bar 20 µm.

Malakhov (1986). In *Manunema* these setae are moreover located on sockets (Riemann *et al.*, 1971; Stewart & Nicholas, 1995; recent data), which is considered as an advanced character state. However, pre- and postcloacal setae in males of *Antomicron*, *Leptolaimus*, *Leptolaimoides* and *Paraplectonema* belong to the "somatic setae" type discussed below.

In many taxa, e.g. *Anguinoides, Camacolaimus*, *Cricolaimus* Southern, 1914, *Dagda, Deontolaimus*, *Diodontolaimus* Southern, 1914*, Hemiplectus* Zell, 1991, *Leptoplectonema, Procamacolaimus* Gerlach, 1954, *Rhadinema*, *Setostephanolaimus* Tchesunov, 1994 and *Stephanolaimus*, the somatic setae are independent from the hypodermal glands (Coomans & Raski, 1991b; Holovachov, 2003a, b; Keppner, 1992; Platt & Warwick, 1988; Tchesunov, 1994; Vitiello, 1974; Zell, 1991; recent data), similar to the "somatic seta" on Fig. 49Г in Malakhov (1986), although hypodermal glands are often present or even numerous (at least in *Leptoplectonema* and *Stephanolaimus*). They are distributed along the whole body or may be restricted to the caudal region only, like in *Rhadinema*.

According to Malakhov (1986) the first type, "somatic pore", is more primitive while "somatic setae" are derived forms. Therefore, we consider the presence of "somatic pores" as a primitive feature and the presence of "somatic setae" as a derived one. However, the absence of both types is most probably due to a secondary reduction.

Deirids are primarily absent in all members, although a pair of somatic setae are often present in the region of the nerve ring or somewhat posteriorly in *Leptoplectonema* and *Stephanolaimus* (Coomans & Raski, 1991b; recent data).

Anterior sensilla

Inner labial sensilla have not been described for many of leptolaimoid taxa, although Jensen (1976) and Keppner (1992) described papilliform inner labial sensilla located on the anterior surface of the lips for *Anomonema deconincki* Jensen, 1976 and *Diodontolaimus parasabulosus*, respectively. We found papilliform inner labial sensilla on the anterior surface of the labial region in *Dagda bipapillata, Stephanolaimus* spp. and *Hemiplectus muscorum*, whereas in Plectidae they seem to be located anteriorly in the cheilostom (unpublished observations).

Outer labial sensilla are papilliform except for *Anomonema deconincki* Jensen, 1976*, Diodontolaimus* and *Setostephanolaimus*-species (Keppner, 1992; Jensen, 1976; Vitiello, 1974; recent data), which have setiform outer labial sensilla. When papilliform, they are usually located on the outer surface of the lips as for example in *Anguinoides, Deontolaimus, Hemiplectus* (Fig. 3.12)*, Leptolaimus, Procamacolaimus* and *Stephanolaimus* (Holovachov, 2003a, 2003b). SEM data on the morphology of the lip region and labial sensilla are available only for *Dagda bipapillata, Leptoplectonema fuegoensis*, *Manunema pectenophora* and *Paraplectonema pedunculatum* (Coomans & Raski, 1991b; Stewart & Nicholas, 1995; recent data) showing papilliform outer labial sensilla on the lips. It is generally accepted that setiform labial sensilla are primitive, while papilliform labial sensilla represent a derived state.

Cephalic sensilla are always setiform, usually located posterior to the lip region, anterior to amphids as in *Antomicron* (Fig. 3.10 G)*, Cricolaimus, Hemiplectus* (Fig. 3.12)*, Leptolaimoides, Leptolaimus, Leptoplectonema, Listia, Manunema* (Fig. 3.10 I)*,* *Paraplectonema, Rhadinema, Setostephanolaimus* and *Stephanolaimus*, or posterior to amphids as in *Anguinoides, Camacolaimus* (Fig. 3.10 K)*, Deontolaimus, Diodontolaimus, Ionema, Onchium* (Fig. 3.10 E)*, Onchiolistia, Procamacolaimus* and *Smithsoninema* (various sources). The location of cephalic setae posterior to amphids is here considered as derived in relation to the opposite position, probably due to migration of the amphids anteriorly.

Cervical sensilla are present in species of *Setostephanolaimus*, located somewhat posterior to the circle of cephalic setae but anterior to amphids and usually setiform (Jensen, 1976). *Diodontolaimus parasabulosus* and *Stephanolaimus elegans* have several pairs of cervical setae located along the anterior part of the pharyngeal region (Keppner, 1992; Platt & Warwick, 1988; recent data).

Amphids and ocelli

Amphids are in most cases ventrally unispiral with a more or less developed central elevation. A derivation from the simpler ventrally unispiral-type is the loop-shaped amphid in *Antomicron* and *Cricolaimus* (Boucher & Helléouët, 1977; Ditlevsen, 1930; Keppner, 1992; recent data) and the longitudinally oval amphid in *Leptolaimoides* (Vitiello, 1971). Transverse oval amphids with an elongated pocket-like pouch are characteristic for the genera *Anomonema, Setostephanolaimus* and *Stephanolaimus* (Hopper, 1963; Platt & Warwick, 1988; recent data). Compared with the outgroup taxa (Monhysterida and Araeolaimida), the unispiral type of amphid should be considered as primitive, while the transverse oval and loop-shaped types are both derived forms.

Ocelli are present in the lateral sectors of the body, at level with the anterior part of pharynx in the genera *Onchium* and *Ionema* as areas of pigment accumulations (Blome, 1982; Cobb, 1920; Gerlach, 1962, 1965). They are present in representatives of limnic and marine nematode genera of the families Araeolaimidae, Chromadoridae, Enoplidae, Leptosomatidae, Monhysteridae, Oncholaimidae, Phanodermatidae, Enchelidiidae and some other taxa.

Stoma

Stoma in the members of Leptolaimoidea is fairly divergent in structure and function. The simplest (but not obligatory primitive) stoma is characteristic for several different genera. A narrow and short cylindrical stoma without differentiations, developed rhabdia or armament was described for *Setostephanolaimus gandavensis* Jensen, 1976 and *Stephanolaimus graciosus* Tchesunov, 1994 (Jensen, 1976; Tchesunov, 1994). Both *Anomonema haplostoma* Hopper, 1963 and *A. deconincki* have a “shallow” cylindrical stoma (probably gymnostom) with weakly cuticularised rhabdia (Hopper, 1963; Jensen, 1976). Species of the genera *Listia* and *Hemiplectus* (Fig. 3.12) have an uniformly cylindrical gymnostom with weakly cuticularised rhabdia and a short stegostom without rhabdia (Blome, 1982; Furstenberg & Vincx, 1988; Zell, 1991). *Ionema* has a simple narrow stoma where the onchiostyle has probably been lost secondarily (Hope & Tchesunov, 1999).

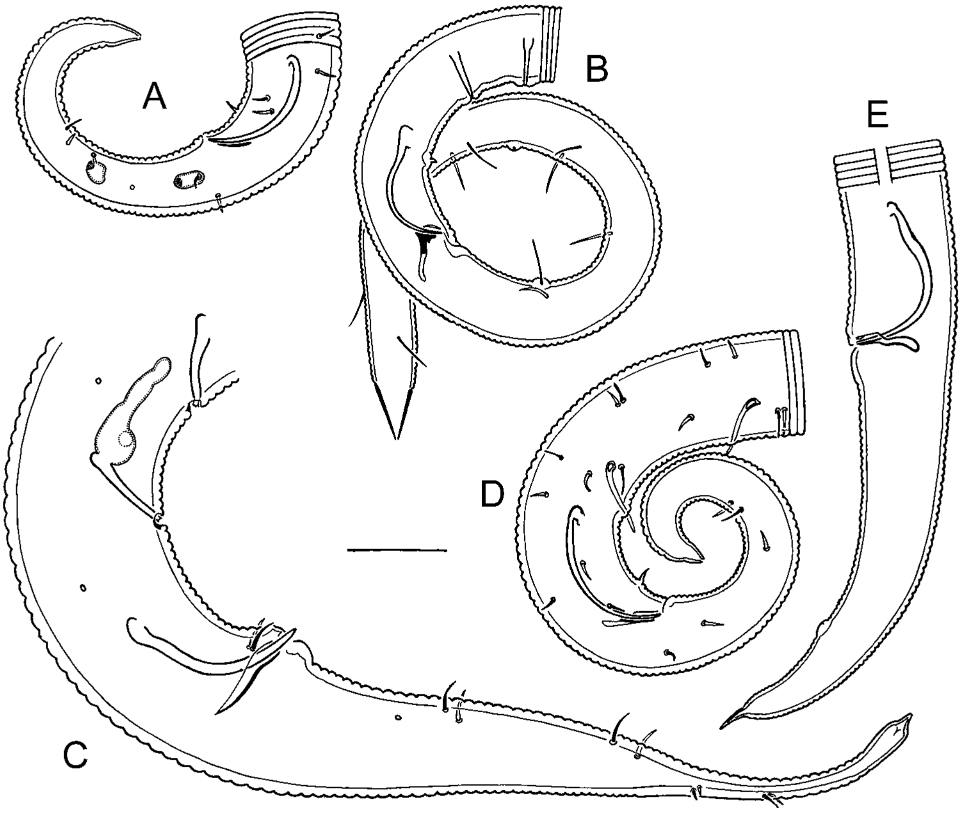

Figure 3.11. Caudal region of males of “Leptolaimidae”. A: *Manunema annulatum* (Vitiello & De Coninck, 1968) Riemann, von Thun & Lorenzen, 1971; B: *Setostephanolaimus bicoronatus* Boucher & Helléouët, 1977; C: *Antomicron elegans* (de Man, 1922) De Coninck, 1965; D: *Manunema proboscidis* Gerlach, 1957; E: *Camacolaimus* sp. Scale bar: 20 µm.

Stoma is a narrow and long cylinder in *Antomicron* (Fig. 3.10 H), *Leptolaimoides, Leptolaimus, Leptoplectonema, Manunema* (Fig. 3.10 I) and *Paraplectonema*: with short cheilostom, short and weakly cuticularised gymnostom and long uniformly cuticularised stegostom, often gradually extending into the pharyngeal lumen; at least in *Paraplectonema* its base is marked by the dorsal gland orifice and herewith named “leptolaimoid” (Hopper, 1968).

The genus *Rhadinema* has a funnel-shaped stoma, with cheilostom consisting of a sclerotised ring bearing six forwardly directed projections: one in dorsal, one in ventral and four in sublateral position; while both gymnostom and stegostom are short without developed rhabdia (Cobb, 1920; recent data). A similar stoma is characteristic for *Cricolaimus,* where *C. coronatus* Ditlevsen, 1930 has six but *C. elongatus* Southern, 1914 only two projections, one dorsal and one ventral (Boucher & Helléouët, 1977; Keppner, 1992; Southern, 1914).

Another group of genera usually classified within the family Camacolaimidae has so-called stoma armament. *Onchium robustum* Gerlach, 1965 and both species of *Anguinoides* have a strong solid dorsal onchiostyle almost completely surrounded by muscular pharyngeal tissue (=dorsal stegorhabida) and it is probably able to protrude anteriorly; gymnostom in these species is distinct only in the subventral sector and has no rhabdia (Gerlach, 1965; Holovachov, 2003a, 2003b; Vitiello, 1974). *Procamacolaimus dorylaimus* Holovachov, 2003 has an even more complex tubular onchiostyle partly surrounded by muscular tissue (Holovachov, 2003b). Other species of *Onchium* as well as *Smithsoninema inaequale* have a small and narrow dorsal onchiostyle (Cobb, 1920; Gerlach, 1962; Hope & Tchesunov, 1999).

Odontia in *Dagda* and *Diodontolaimus* are derivations of the gymnostom. They show superficial similarities to the anteriorly pointing gymnorhabdia in *Stephanolaimus*. The stegostom is cylindrical and undifferentiated in these species (Keppner, 1992; Vitiello, 1974; recent data).

The dorsal onchiostyle of *Onchiolistia* is a thickening of the gymnostom's dorsal sector with or without a central hollow (Blome, 2002; Furstenberg & Vincx, 1988). Stomatal armament in *Camacolaimus* and *Deontolaimus*, probably also in *Procamacolaimus phinney* (Murphy, 1964) Hope & Tchesunov, 1999, consists of two distinct but closely attached parts: the anterior one is a derivative of the gymnostom and similar in shape to the odontia of *Dagda* and *Diodontolaimus*, while the posterior one is a thickening of the dorsal wall of the stegostom and may be homologous to the typical onchiostyle of *Onchium, Anguinoides* and *Smithsoninema* (Hope & Tchesunov, 1999; recent data).

Pharynx and pharyngeal glands

There are three different types of pharynx structure in the studied group: **1)** equally muscular along most of its length, uniformly cylindrical, without differentiation into corpus, isthmus and bulbus, in some cases the dorsal glandular sector of the post-pharynx being more strongly developed than the subventral ones ("muscular type"); **2)** muscular only in the anterior cylindrical part, posterior portion with reduced "musculature", but with strongly developed gland cells and thus having irregularly cylindrical or fusiform shape ("glandular type"); **3)** equally muscular along most of its length, with a distinct division into procorpus, metacorpus, isthmus and basal bulb, the latter often with strongly cuticularised lining ("leptolaimoid type").

The "muscular type" of pharynx is characteristic for the genera *Camacolaimus*, *Dagda*, *Deontolaimus*, *Diodontolaimus*, *Hemiplectus* (Figs. 3.12)*, Listia, Onchiolistia, Procamacolaimus*, *Rhadinema* and *Stephanolaimus* (Furstenberg & Vincx, 1988; Tchesunov, 1994; Vitiello 1974; Zell, 1991), although in *Camacolaimus* (Fig. 3.10 M) and *Deontolaimus* the glandular tissue is strongly developed in the dorsal sector of the basal part of the pharynx. *Cricolaimus*-species have a cylindrical pharynx with oval basal bulb (Boucher & Helléouët, 1977; Keppner, 1992).

The "glandular type" is found in species of *Anguinoides*, *Ionema*, *Onchium* (Fig. 3.10 F), *Setostephanolaimus* (Fig. 3.10 D) and *Smithsoninema* (Blome, 1982; Cobb, 1920; Holovachov, 2003a, 2003b; Hope & Tchesunov, 1999). In *Setostephanolaimus* the pharyngeal musculature is reduced, but the glandular tissue is equally developed in dorsal and subventral sectors. In at least *Onchium* and *Smithsoninema* the granulated glandular tissue in the dorsal sector is strongly developed, occupying more than half of the pharyngeal region, while the subventral pharyngeal glands are small and not granular.

The "leptolaimoid type" is characteristic for *Antomicron*, *Leptolaimoides*, *Leptolaimus*, *Leptoplectonema* and *Paraplectonema* (Coomans & Raski, 1991b; Hopper, 1968; recent data), and with some modifications (pharyngeal parts more slender and elongated) for *Anomonema* (Hopper, 1963). Pharynx in *Manunema* also belongs to this type but with some modifications: corpus and isthmus are shorter and thicker (Fig. 3.10 I).

In our opinion, the "muscular type" is the simplest type of pharynx, widely distributed in closely and distantly related taxa (Monhysterida, Enoplida, Araeolaimida), and thus considered as primitive. The other two types of pharynx structure, as well as the "plectoid" pharynx with radial tubules, valvular apparatus, corpus, isthmus and bulbus, are derived forms.

The number and location of pharyngeal glands are known only for a few representatives, among them *Hemiplectus muscorum* (Fig. 3.12), *Leptoplectonema fuegoense, Onchium* sp. (Fig. 3.10 F), *Paraplectonema pedunculatum*, *Procamacolaimus dorylaimus*, *Stephanolaimus elegans*, *Smithsoninema inaequale* and *Setostephanolaimus* sp. (Fig. 3.10 D) (Coomans & Raski, 1991b; Holovachov, 2003b; Hope & Tchesunov, 1999). The dorsal gland orifice always opens at or somewhat posterior to stoma base. The orifices of the subventral glands are usually indistinct, but at least in *Dagda, Hemiplectus, Rhadinema* and *Stephanolaimus* they open at the same level as the dorsal gland orifice or slightly anterior to it (recent data). Worth mentioning is that in Plectidae the dorsal gland orifice opens inside the stegostom, anterior to the radial tubules, while the anterior pair of subventral gland orifices are located in the corpus-isthmus junction, representing an advanced state.

Secretory-excretory system

The secretory-excretory system is present in most leptolaimoid taxa except *Anguinoides, Anomonema*, *Leptoplectonema*, *Listia*, *Manunema* (Fig. 3.10 I), *Onchiolistia* (two out of three known species), *Procamacolaimus* and *Smithsoninema* (Coomans & Raski, 1991b; Furstenberg & Vincx, 1988; Holovachov, 2003a, 2003b; Hope & Tchesunov, 1999; Hopper, 1963; Vitiello, 1974). For other taxa the secretory-excretory system was mentioned in at least some of the existing descriptions. Although the secretory-excretory system can be primarily absent in certain nematode taxa (some Leptosomatidae (Malakhov, 1986) with the exception of *Leptosomatum* (Bongers, 1983)), we consider that in the above mentioned taxa the renette was reduced secondarily.

The renette cell is round to elongate, located posterior to pharynx, ventral to cardia or at anterior part of intestine. It extends anteriorly along the ventral side of pharynx and forms an excretory ampulla just posterior to level of nerve ring. Excretory canal short and weakly cuticularised; its anterior end opens to the exterior while its posterior end is connected with the renette ampulla (Alekseev & Rassadnikova, 1977; Cobb, 1920; Jensen, 1976; Keppner, 1992; Vitiello, 1974; recent data).

Paraplectonema is an exception as it has a cuticularised canal which extends directly from the ventral gland and runs along the ventral side of pharynx to the level of nerve ring or stoma and opens to the exterior via a pore somewhat posterior to nerve ring (Coomans & Raski, 1991b; Hopper, 1968) or on the labial region (recent data). This is also the case in the family Aphanolaimidae, but here the renette opens into the cheilostom (Chapter 2). The elongation of the excretory canal is here considered as a derived character, while the presence of an excretory ampulla close to nerve ring and a short duct are considered as a primitive trait.

An even more complicated secretory-excretory system is characteristic for *Hemiplectus muscorum* (Fig. 3.12). The renette cell is located at level with the posterior part of pharynx and followed anteriorly usually by two coelomocytes. The cytoplasm of the renette cell surrounds the proximal coiled part of the excretory canal like in many Plectidae (Maggenti, 1961a; Allen & Noffsinger, 1968) and such a structure is considered secondary.

Female reproductive system

Female gonads are paired in most leptolaimoid taxa except for *Alaimella*, *Camacolaimus monhystera* Gerlach, 1967, *Listia* and *Onchiolistia* (Cobb, 1920; Gerlach, 1967; Furstenberg & Vincx, 1988), where the anterior gonad probably is secondarily reduced. No females have ever been described for *Anomonema*. Reflexed ovaries are present in all taxa except for *Manunema* (Fig. 3.10 J) (Riemann et al., 1971; Stewart & Nicholas, 1995). In the latter case the outstretched ovaries probably appeared secondarily due to a strong decrease in body size.

Spermatheca is axial in *Dagda, Deontolaimus, Hemiplectus, Procamacolaimus, Rhadinema, Setostephanolaimus* and *Stephanolaimus* (Holovachov, 2003b; recent data), while *Listia* and *Onchiolistia* have spermatozoa in the rudiment of the anterior gonad (Furstenberg & Vincx, 1988). Species of *Antomicron*, *Leptolaimus*, *Manunema* and *Paraplectonema* have four offset sac-like spermathecae connected on each right and left side of the ovary-oviduct junction of each anterior and posterior gonad (Alekseev & Rassadnikova, 1977; recent data) like in Aphanolaimidae (Chapter 2). This is probably a derivation from the more common and presumably more primitive axial type.

The vagina is encircled by a single sphincter muscle and in many cases the *pars refringens vaginae* is developed. Supplements in females are present only in some species of *Leptolaimus* (see below), in *Stephanolaimus elegans* (numerous tubular in pre- and postvulval regions, see Platt & Warwick, 1988; recent data), *Onchiolistia africana* (single alveolar in front of vulva, see Furstenberg & Vincx, 1988), and *Aphanonchus* (Coomans & Raski, 1991a; Holovachov & Sturhan, 2004b).

Male reproductive system and copulatory apparatus

Testes usually two, either both directed anteriorly as in *Camacolaimus,Dagda, Deontolaimus, Listia, Onchiolistia*, *Rhadinema* and *Smithsoninema* (Furstenberg & Vincx, 1988; Lorenzen, 1981; Tchesunov *et al.*, 2000; recent data) or opposed with posterior one reflexed as in *Antomicron, Hemiplectus*, *Leptolaimus*, *Paraplectonema*, *Setostephano-*

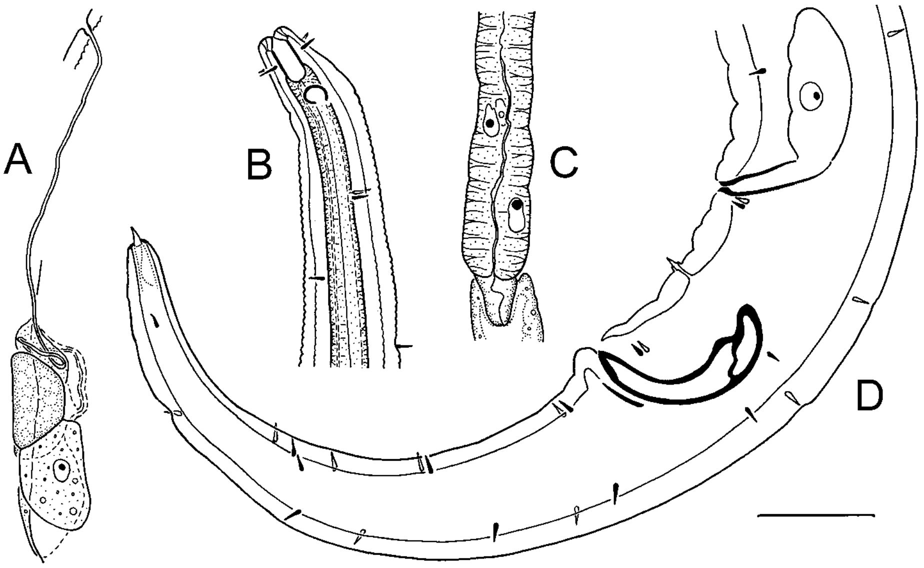

Figure 3.12. *Hemiplectus muscorum* Zell, 1991, male. A: Secretory-excretory system; B: Anterior end; C: Posterior part of pharynx; D: Caudal region. Scale bar 20 µm.

laimus and *Stephanolaimus* (Jensen, 1976; Tchesunov, 1994; Zell, 1991; recent data). The former character state is here considered as derived from the latter, mainly because it is rarely found in nematode taxa (see Lorenzen, 1981 for details). A single anterior or posterior testis is present in *Anomonema*, *Paraplectonema canadianum* and *Manunema* (Hopper, 1963, 1968; Jensen, 1976; Stewart & Nicholas, 1995) and is most probably the result of the reduction of one testis. Spicules and gubernaculum are highly variable in structure and we were unable to use them in the phylogenetic analysis.

Both alveolar and tubular supplements are found in some species of *Antomicron, Leptolaimus, Listia* and *Onchiolistia* (Blome, 2002; Lorenzen, 1966; Furstenberg & Vincx, 1988) and this is considered as the most primitive state, which is also characteristic for *Aphanonchus* Coomans & Raski, 1991. Only tubular supplements are found in species of *Anguinoides*, *Anomonema*, *Dagda*, *Diodontolaimus*, *Hemiplectus*, *Paraplectonema*, *Procamacolaimus, Setostephanolaimus* and *Stephanolaimus*, and in some of the species of *Antomicron* and *Leptolaimus* (Holovachov, 2003a, 2003b; Hopper, 1963, 1968; Jensen, 1976; Keppner, 1992; Platt & Warwick, 1988; Tchesunov, 1994; Vitiello, 1974; Zell, 1991; recent data). Only alveolar supplements are present in *Deontolaimus papillatus* and a few species of *Leptolaimus*. Males of *Camacolaimus, Ionema, Onchium* and *Smithsoninema* have neither tubular nor alveolar supplements.

Alveolar supplements were sometimes described as circular concave invaginations in the cuticle with a small papilla (sensillum?) located at the bottom of the invagination and connected with the duct leading inside the body (for *Onchiolistia tubifera* by Furstenberg

& Vincx, 1988; *Leptolaimus longispiculus* by Alekseev & Rassadnikova, 1977). Tubular supplements are always connected with a gland-like cell. Males of *Manunema pectenophora* has tubular supplements with a strongly widened distal part bearing about eleven prongs (Stewart & Nicholas, 1995). Similar supplement shape was described for *Leptolaimus scotlandicus* Jayasree & Warwick, 1977. Although precloacal tubules were noted in Cyatholaimidae, namely in *Paracanthonchus longicaudatus* Warwick, 1971, it is not clear if they are homologous to the tubules of Leptolaimoidea.

A midventral precloacal sensillum was found in *Anguinoides*, *Cricolaimus*, *Dagda*, *Diodontolaimus*, *Hemiplectus* (Fig. 3.12), *Onchiolistia, Procamacolaimus, Stephanolaimus elegans* and *Setostephanolaimus* (Fig. 3.11 B) (Boucher & Helléouët, 1977; Furstenberg & Vincx, 1988; Holovachov, 2003a, 2003b; Keppner, 1992; Platt & Warwick, 1988; Southern, 1914; Vitiello, 1974). It was described as a "prominent papilla with duct" for *Onchiolistia tubifera, O. africana* and *Listia capensis* (Furstenberg & Vincx, 1988). Jensen (1976) described "a fine ventral duct is present, 43 µm anterior to the cloaca; the duct is associated with a small gland" for *Setostephanolaimus gandavensis*. This structure was not found in *Stephanolaimus graciosus* Tchesunov, 1994, but is present in *S. elegans* (recent data). The midventral precloacal sensillum in *Leptolaimus* was first described by Alekseev and Rassadnikova (1977) as papilliform in shape and located on the anterior cloacal lip, which is also the case in *Antomicron* and *Paraplectonema* (recent data) as well as in Aphanolaimidae (Chapter 2). A precloacal sensillum is absent in *Onchium* and *Smithsoninema* (Blome, 1982; Hope & Tchesunov, 1999). A setiform precloacal sensillum located far anteriorly from cloaca was found in a variety of taxa and is most probably plesiomorphic, whereas the papilliform precloacal sensilla located on the anterior cloacal lip should be considered as apomorphic.

Postcloacal sensilla are rarely described in Leptolaimoidea but they are much more widely distributed as was thought before. A single postcloacal sensillum is present in *Anguinoides profundus* (Vitiello, 1974) Holovachov, 2003*, Camacolaimus, Listia, Onchiolistia, Setostephanolaimus longispiculus* (= *S. jayasreei* Platt, 1983) and *Stephanolaimus graciosus* (Boucher & Helléouët, 1977; Blome, 2002; Furstenberg & Vincx, 1988; Platt & Warwick, 1988; Tchesunov, 1994; Vitiello, 1974). Two postcloacal sensilla are present in *Anguinoides stylosum*, *Camacolaimus* (Fig. 3.11 E)*, Dagda, Deontolaimus, Diodontolaimus, Procamacolaimus dorylaimus, Rhadinema* and *Setostephanolaimus bicoronatus* (Fig. 3.11 B) (Holovachov, 2003a, 2003b; Keppner, 1992; Southern, 1914; Vitiello, 1974).

Males of *Onchiolistia tubifera, O. africana* and *Listia capensis* have a single "papillae-like structure with no gland connection or ducts" (Furstenberg & Vincx, 1988). In *Stephanolaimus graciosus* Tchesunov, 1994 there is "a small midventral supplement present like a sucker on the posterior third of the tail". Keppner (1992) described the postcloacal sensilla in *Diodontolaimus* as "cup-shaped". "Three minute, papilliform, ventromedian supplements" are present on the tail of *Smithsoninema inaequale* (Hope & Tchesunov, 1999). We consider the presence of postcloacal sensilla in males as an advanced character.

Postcloacal sensilla are absent in *Anomonema, Antomicron* (Fig. 3.11 C)*, Cricolaimus, Hemiplectus, Leptolaimoides, Leptolaimus, Manunema* (Fig. 3.11 A, D)*, Paraplectonema* and *Onchium* (Alekseev & Rassadnikova, 1977; Blome, 1982; Boucher &

Helléouët, 1977; Hopper, 1963, 1968; Keppner, 1992; Lorenzen, 1972b). The absence of the postcloacal sensilla in *Stephanolaimus elegans* is considered as a secondary state (reduction), since it is present in the other species of the genus, *S. graciosus*.

Caudal setae (sensilla) are usually present in males except for *Ionema, Onchium* and *Smithsoninema* (Blome, 1982; Cobb, 1920; Hope & Tchesunov, 1999). They are arranged in two subventral or four (two subventral and two subdorsal) rows, the latter seems to be a plesiomorphic condition. Setae in the caudal region in males of the genera *Leptolaimus, Antomicron* and *Paraplectonema* are arranged in the same way as in members of the family Aphanolaimidae (Chapter 2): one pair of precloacal setae is located subventrally, in *Leptolaimus* and *Antomicron* they are usually located just in front of the cloacal opening, while in *Paraplectonema* and Aphanolaimidae they are located somewhat more anteriorly at level with the spicules, and a few pairs of setae are located subventrally and subdorsally on tail. We consider this scheme as apomorphic.

Phylogenetic analysis

Characters and their polarisation

A data matrix for the characters and their states used for the phylogenetic analysis is presented in Table 3.4. The character states are as follows: **1)** Cuticular annulation: conspicuous [0]; inconspicuous [1]. **2)** Longitudinal striation of the cuticle: absent [0]; present [1]. **3)** Lateral alae: absent [00]; present, single midlateral band [01]; present, two sublateral bands [10]. **4)** Hypodermal glands: present [0]; absent [1]. **5)** Somatic sensilla structure: “somatic pore” type[0]; “somatic setae” type [1]. **6)** “Somatic pore” shape: simple [0]; located on sockets [1]. **7)** Somatic sensilla (“somatic setae”) in females: present along the whole body [00]; present on tail only [01]; absent [11]. **8)** Somatic sensilla (“somatic setae”) in males: present along the whole body [00]; present on tail only [01]; absent [11]. **9)** Inner labial sensilla location: on the outer surface of lips [0]; inside the cheilostom [1]. **10)** Outer labial sensilla shape: setiform [0]; papilliform [1]. **11)** Cephalic sensilla location: anterior to amphids [0], posterior to amphids [1]. **12)** Amphid shape: unispiral [00]; loop shaped [01]; transverse oval [10]. **13)** Ocelli: absent [0]; present [1]. **14)** Deirid: absent [0]; present [1]. **15)** Cheilostom structure: undifferentiated [00]; with six projections [01]; with peristomatal vestibulum [10]. **16)** Gymnostom structure: uniformly cylindrical or conoid, undifferentiated [00]; with two or three odontia [01]; with a single dorsal odontium [10]. **17)** Stegostom structure: funnel-shaped, undifferentiated [000]; uniformly tubular, “leptolaimoid” [001]; funnel-shaped, subdivided into two sections, “plectoid” [010]; with dorsal onchiostyle [100]. **18)** Dorsal gland orifice location: posterior to or at stoma base [0]; inside the stegostom [1]. **19)** Subventral gland orifices (anterior pair) location: posterior to or at stoma base [0]; at middle of pharynx, in the corpus-ithmus junction [1]. **20)** Pharynx structure: “muscular type” [000]; “glandular type” [001]; “leptolaimoid type” [010]; “plectoid type”, with radial tubules and valvular apparatus [100]. **21)** Secretory-excretory system: present [0]; absent [1]. **22)** Renette cell location: at level of cardia and intestine [0]; at level of pharynx [1]. **23)** Excretory ampulla: present, located at level of nerve ring [0]; absent [1]. **24)** Excretory duct length: short [00]; long, free-standing [01]; long, with

Table 3.4. Data matrix for some genera and species of Leptolaimoidea Örley, 1880, Camacolaimoidea Micoletzky, 1924 and allien taxa. See text for explanation of characters and character states.

Character number, dataset	`000000000000111111111111111222222222222223333333333333` `12334567788901223455667778900012344556789001234567889`
Hypothetical ancestor	`00000000000000000000000000000000000000000000000000000`
Anguinoides stylosum	`000111-0000?1100000000100??0011------00??00?100100010`
Antomicron elegans	`0001000--01?1001000000001??0100000000000001000100101001`
Aphanonchus intermedius	`0001000--01?10000010000000?0000010111000101000100 1001`
Camacolaimus spp. *	`000111-0000?11000000111000?000000000000000000111-11-010`
Dagda bipapillata	`000011-00000110000000100000000000000000000001100100010`
Deontolaimus papillatus	`000111-0000?11000000111000?000000000000000000111-01-010`
Diodontolaimus sabulosus	`000011-0000?110000000001000??0000000000000????100100010`
Hemiplectus muscorum	`000011-00000100000000000000000011110000000000100100??0`
Leptolaimus mixtus	`0001000--01?10000000000010?010000000000001000100001001`
Listia capensis	`000011-?????1000000000000??0001------1000001100000010`
Manunema proboscidis	`0000001----?1000000000001??0101------0101100100100000`
Onchiolistia tubifera	`010011-?????1100000011000??0001------1000001100000010`
Onchium spp. **	`100011-1111?1100100000001000?0010000000000????11-11-??0`
Paraplectonema loofi	`0001000--01?10000000000010?0100010101000100010 0101001`
Plectus parietinus	`001001-000011000010000010111000111100001-000100100010`
Procamacolaimus dorylaimus	`000011-0000?110000000001000?0001------0000000100100010`
Rhadinema flexile	`000001-0101?10000001000000000000000000000??1100100010`
Setostephanolaimus spp. ***	`000011-0000000100000000000?001000000000000000100100010`
Smithsoninema inaequale	`100011-1111?1100000000100??0011------00??00111-11-010`
Stephanolaimus elegans	`000001-0000?1010000000000000000000000000000000000100110`

* – data for *Camacolaimus tardus, C. barbatus, Camacolaimus* sp.
** – data for *Onchium ocellatum, Onchium* sp.
*** – data for *Setostephanolaimus bicoronatus, Setostephanolaimus* sp.

proximal part enveloped by the renette cell [11]. **25)** Excretory pore location: at level of nerve ring [00]; on the labial region [01]; in the cheilostom [11]. **26)** Ovary number: paired [0]; single, posterior [1]. **27)** Ovary shape: reflexed [0]; outstretched [1]. **28)** Spermatheca: present [0]; absent [1]. **29)** Spermatheca shape: axial [0]; paired and offset [1]. **30)** Testis number: paired [00]; paired, but anterior non-functional, glandular [01]; single [10]. **31)** Testis shape: opposed, with posterior one reflexed [0]; both directed anteriorly [1]. **32)** Advulval supplements: present [0]; absent [1]. **33)** Precloacal tubular supplements: present [0]; absent [1]. **34)** Precloacal tubular supplement shape: uniform in shape and size [0]; heterogeneous, second from cloaca elevated [1]. **35)** Precloacal alveolar supplements: present [0]; absent [1]. **36)** Midventral precloacal sensillum: present [0]; absent [1].

37) Midventral precloacal sensillum shape: setiform, precloacal [0]; papilliform, on the anterior cloacal lip [1]. **38)** Postcloacal sensilla: absent [00]; present [01]; reduced secondarily [11]. **39)** Precloacal pair of sublateral setae: absent [0]; present [1].

Entire dataset

A phylogenetic analysis of the entire dataset resulted in 40 most parsimonious trees. A strict consensus tree of the 40 most parsimonious trees is given in Fig. 3.13. All analysed taxa split into two distinct clades, but the relationships within these clades are not clearly resolved. Therefore, we made further comparative-morphological analyses of the two clades separately.

The monophyly of Clade 1, which includes *Manunema, Leptolaimus, Antomicron, Aphanonchus* and *Paraplectonema,* is supported by at least one synapomorphy, i. e. presence of two sack-like offset spermathecae connected with each reproductive tube in the oviduct-uterus junction (character 29, advanced state). Such a spermatheca structure was not found in other closely related taxa, and is considered to be unique and characteristic for the "monophylum" including the five above mentioned genera which were treated in the analysis, but also the genera *Leptolaimoides, Leptoplectonema* and *Anomonema*, and members of the family Aphanolaimidae (for further details see below). Some of these genera have a very consistent arrangement of setae in the caudal region of the males: one subventral precloacal pair and several caudal pairs, arranged subventrally and subdorsally (character 39, advanced state) and a precloacal midventral sensillum, which is papilliform in shape and located on the anterior cloacal lip (character 37, advanced state), but this needs additional analysis. All taxa of this clade also have the "somatic pore" type of somatic sensilla (character 5, primitive state).

The other clade (Clade 2) is supported by at least one synapomorphy, i.e. the presence of postcloacal sensillae in males (character 38, advanced state), although it is evident that this structure could be secondarily reduced in some species/genera. However, no postcloacal sensilla were found in existing descriptions of the members of any superfamilies other than Leptolaimoidea and Plectoidea. Certain species of Desmodoridae possess a group of postcloacal thorns arranged latero-ventrally in several longitudinal rows with a few thorns in each row. This was described at least for *Pseudochromadora interdigitatum* Muthumbi, Verschelde & Vincx, 1995 and *Desmodorella spineacaudata* Verschelde, Gourbault & Vincx, 1998. Their function and origin remain unknown (Muthumbi et al., 1995; Verschelde et al., 1998) and they are most probably not homologous to the post-cloacal papilliform sensilla found in the genera *Anguinoides, Camacolaimus, Dagda, Deontolaimus, Diodontolaimus, Listia, Onchiolistia, Procamacolaimus, Rhadinema, Setostephanolaimus* and *Stephanolaimus,* and in many Plectidae.

Clade 1

The phylogenetic analysis was repeated for the five genera in clade 1 (*Manunema, Leptolaimus, Antomicron, Aphanonchus* and *Paraplectonema*) resulting in three equally parsimonious trees. The characters used for this analysis are presented in the legends to Fig. 3.14. Their strict consensus tree gave *Aphanonchus* as the sister taxon for the other four

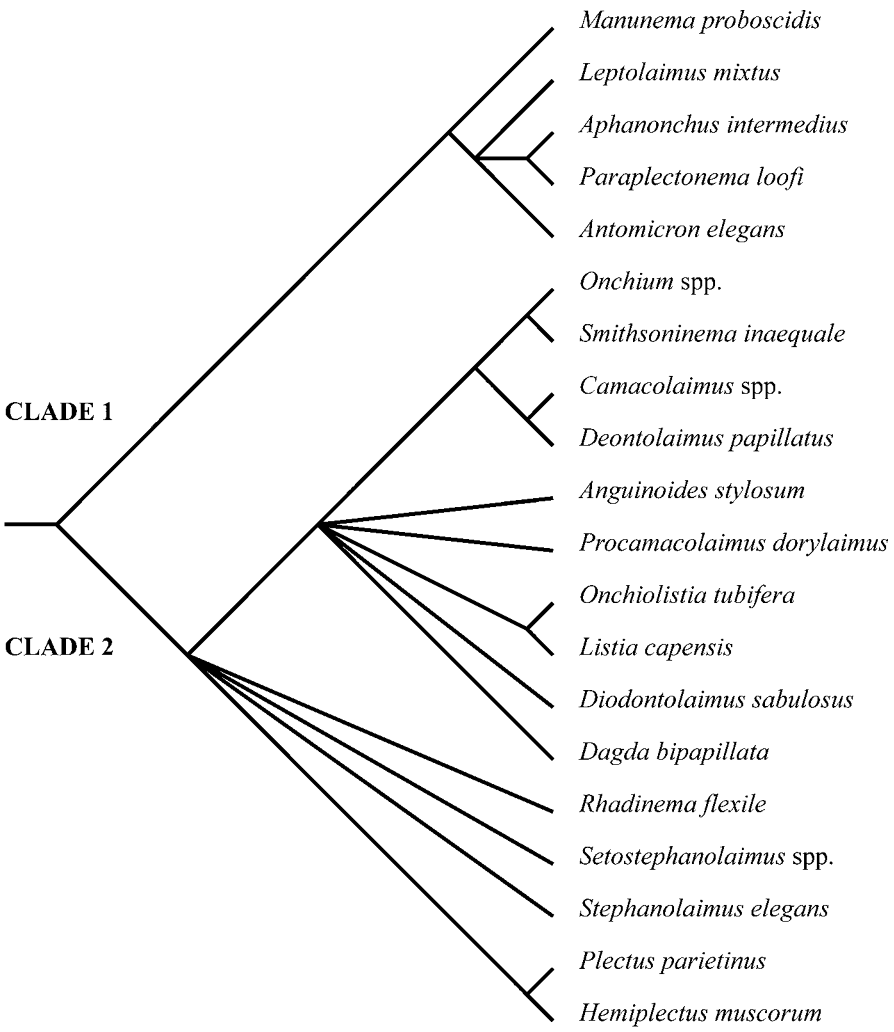

Figure 3.13. Strict consensus tree showing the phylogenetic relationships of 19 species of the superfamily Leptolaimoidea and *Plectus parietinus* Bastian, 1865 based on the analysis of entire dataset presented in Table 3.4.

genera, but the relationships of the latter could not be resolved. We may propose two hypotheses for the relationships of this group, also considering some other taxa which were not included in the previous analysis due to lack of relevent information. The first tree resulted from the cladogram of the five genera of Clade 1 with addition of further possible representatives of the clade (Fig 3.14, upper cladogram). Several additional features

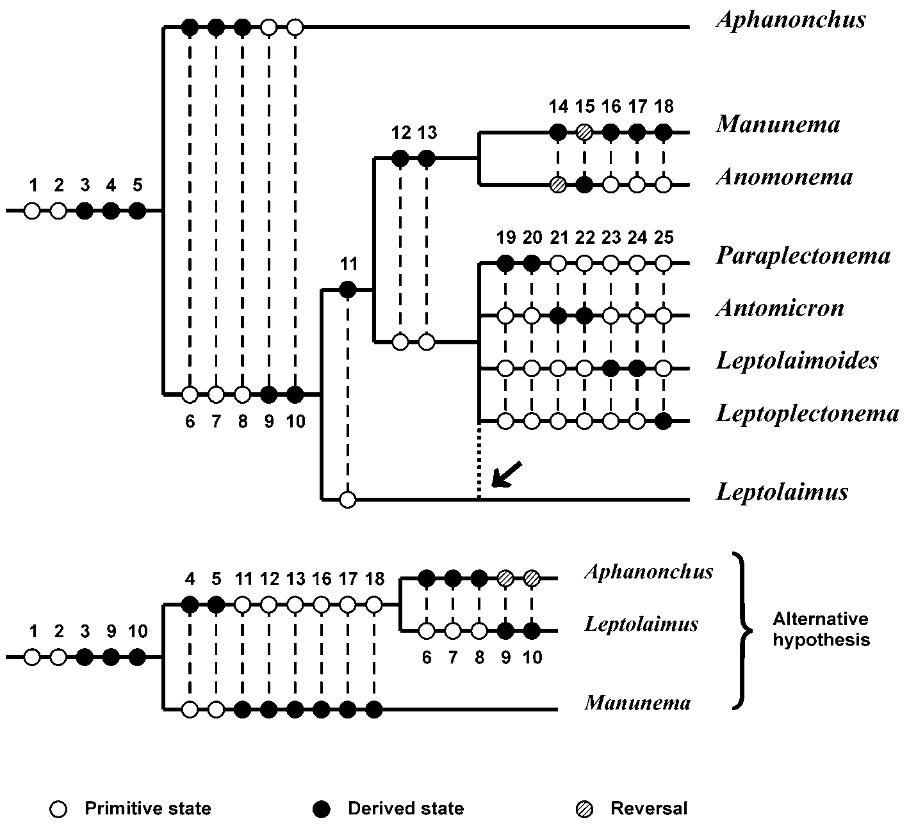

Figure 3.14. Cladogram showing the phylogenetic relationships of the genera *Aphanonchus* Coomans & Raski, 1991, *Manunema* Gerlach, 1957, *Anomonema* Hopper, 1963, *Paraplectonema* Strand, 1936, *Antomicron* Cobb, 1920, *Leptolaimoides* Vitiello, 1971, *Leptoplectonema* Coomans & Raski, 1991 and *Leptolaimus* de Man, 1880 (Clade 1 in Fig. 13). **Characters uniting all taxa:** (1) "somatic pore" type of somatic sensilla. (2) postcloacal sensilla absent. (3) "double" offset spermathecae present. (4) setae in male arranged in one precloacal and few caudal pairs. (5) precloacal midventral sensilla papilliform in shape and located on anterior cloacal lip. **Derived states:** (6) excretory canal opens inside the cheilostom. (7) anterior testis glandular. (8) second supplement on the cuticular elevation. (9) stegostom long cylindrical. (10) pharynx subdivided into corpus, isthmus and bulbus. (11) supplements in female absent (unknown in *Anomonema*). (12) only "somatic pore" type of sensilla on male tail, "somatic setae" type, which is characteristic for other taxa, reduced. (13) single testis present. (16) pharynx short and plump, metacorpus, isthmus and bulbus almost fused. (17) ovaries outstretched. (18) "somatic pore" type of somatic sensilla inserted on sockets. (19) supplements of characteristic "S"-shape. (20) excretory canal long, excretory ampulla reduced. (21) amphid loop-shaped. (22) cephalic capsule. (23) caudal glands absent. (24) amphid in the shape of a longitudinal slit. (25) "organellum ovale" present. **Reversals:** (14) stegostom short, reversal from character 9. (15) precloacal midventral sensilla setiform in shape and located anteriorly to cloaca, reversal from character 5. Arrow points at another possible rooting of the branch leading to *Leptolaimus* (see text for details). The alternative hypothesis shows another possibility of the phylogenetic relationships of three main genera (see text for details).

characteristic only for the added genera were included. One character (absence of the secretory excretory system) was excluded from the analysis, since it may occur convergently in closely related genera and does not resolve the phylogeny. The secretory-excretory system has not been found in several species of genera in which other species do have this character: *Paraplectonema primitivum* by Gagarin (2001) and personal observations of some *Leptolaimus*.

The resulting cladogram needs reversals of two characters: **1)** reduction of the stegostom in *Anomonema* (character 14), and **2)** reversal in the structure and position of the midventral precloacal papilla in *Manunema* (character 15). We can also suggest an alternative hypothesis, in which *Manunema* is the sister taxon of both *Aphanonchus* and *Leptolaimus*. This requires a secondary reduction from the complex "leptolaimoid" stoma and pharynx to the simpler stoma and cylindrical "muscular" type of pharynx. We prefer the first hypothesis since it is supported by the cladistic analysis, and requires "less constructive" reversals. Furthermore personal observations of the juvenile morphology of the family Aphanolaimidae do not reveal any evidence of such a morphological reversal in the structure of the digestive system during post-embryonic development.

The phylogenetic system of De Ley & Blaxter (2002) places the family Peresianidae Vitiello & De Coninck, 1968 in the superfamily Haliplectoidea, but in our phylogenetic analysis this family tends to have a close alliance with *Leptolaimus*. The main differences between Peresianidae and Haliplectidae are in the structure of the male supplementary apparatus (one precloacal setiform sensillum plus tubular supplements in *Manunema vs* several precloacal papillae and a midventral cuticular ridge in *Haliplectus*) and lip region (twelve finger-like projections are present in *Haliplectus*) (Swart et al., 1993). The structure of the digestive system, and female and male reproductive systems are very similar in both *Manunema* and *Leptolaimus*. Another character that may support a close relationship between these two genera is the peculiar structure of tubular supplements in *M. pectenophora*, which somewhat resemble those of *L. scotlandicus*.

The genus *Manunema* and the family Peresianidae were also considered as an intermediate link between leptolaimids and desmoscolecids (Freudenhammer, 1975; Riemann et al., 1971; Vitiello & De Coninck, 1968) because of the similarities in body shape, structure and pattern of somatic setae (inserted on sockets, tricomoid pattern) and female reproductive system (with outstretched ovaries). Decraemer (1975) pointed out that somatic setae in *Desmoscolex* Claparède, 1863 are connected with unicellular glands similar to *Manunema* and *Leptolaimus*. Nevertheless, the differences in structure of amphid and pharynx (number and arrangement of pharyngeal glands, level of muscularity of different parts of the pharynx, etc.) between the two mentioned groups are strong (see Decraemer, 1975, 1978).

Taxonomy of the genus Leptolaimus

We must also consider the validity and phylogenetic relationships of another three taxa which were separated from the genus *Leptolaimus*. The heterogeneity of this taxon was first noted by de Bovee (1974) and a subgeneric division was suggested by Alekseev & Rassadnikova (1977), who proposed three new subgenera: *Tubulaimus* (later considered synonymous with *Dermatolaimus*), *Alveolaimus* and *Boveelaimus* in addition to the type

Table 3.5. Combinations of the alveolar and tubular supplements in females and males of the genus *Leptolaimus* de Man, 1880.

		Females				
	Supplements	no alveoli and no tubules	alveoli in postcardial region	tubules in postcardial region	tubules in preanal region	tubules in postcardial and preanal regions
Males	no alveoli and no tubules	*Boveelaimus*	?	?	?	?
	alveoli	*Alveolaimus*	?	?	?	?
	tubules in precloacal region	*Tubulaimus*	?	?	*venustus*	?
	tubules in postcardial & precloacal regions	?	?	sp. 1	?	*elegans, luridus*
	alveoli and tubules	*Leptolaimus*	sp. 2	?	?	?

subgenus *Leptolaimus*. The diagnostics of the subgenera were based solely on the presence or absence of alveolar and/or tubular supplements. The authors also suggested a transformation series from *Alveolaimus* through *Leptolaimus* towards *Tubulaimus*. The position of the subgenus *Boveelaimus* was not resolved. Moreover, Southern (1914) proposed the new genus *Halaphanolaimus*, which was characterised by both males and females having tubules arranged in two groups, the anterior group along the pharynx and the posterior group in the precloacal region. Although Southern (1914) compared his new genus with *Aphanolaimus*, later descriptions showed it to be quite similar to *Leptolaimus*, differing only in the presence of supplements in females and their distribution in males (see also Platt & Warwick, 1988).

However, the presence and arrangement of tubular and alveolar supplements in males and females are highly variable and could not be easily explained from a phylogenetic point of view. For instance, *Leptolaimus luridus* Timm, 1963 is characterised by having one post-cardial and one preanal tubule in females and one post-cardial and four precloacal tubules in males, thus fitting the diagnosis of *Halaphanolaimus*. On the other hand, *L. venustus* Lorenzen, 1972, by having one preanal tubule in females and three precloacal tubules in males but no post-cardiac tubules in either sex, is intermediate between *Halaphanolaimus* and *Dermatolaimus* (for species descriptions see Lorenzen, 1972b). Moreover, we found two other *Leptolaimus* species, adding more complications to the taxonomy of the genus. *Leptolaimus* sp. 1. from the Dutch coast is characterised by having only one post-cardiac tubule in females and one post-cardiac and four precloacal tubules in males; while *Leptolaimus* sp. 2. from the southern Atlantic is characterised by having one post-cardiac alveolar supplement in females and males having four tubules and eight alveoli (males fit within the diagnosis of the subgenus *Leptolaimus*). All available information on the alveolar and tubular supplements in females and males of the genus *Leptolaimus* is summarised in Table 3.5.

We agree with Alekseev (1979) that tubular supplements are derived from alveolar ones; however, this process took part much earlier than the stem species of *Leptolaimus* appeared. But we do not agree with Alekseev & Rassadnikova (1977) that *Boveelaimus* represents the most primitive group. When considering the evolution of the supplementary structures in males of *Leptolaimus* s. l., we assume *Leptolaimus* s. str. to represent the most primitive (stem) group for the following reasons: males of at least one species of the closely related genus *Antomicron* (*A. pratense* Lorenzen, 1966) have both tubular and alveolar supplements, and members of two genera in the sister taxon Aphanolaimidae also have both tubular and alveolar supplements in males (*Aphanonchus* and *Anonchus*). On the other hand, the state of this character in *Dermatolaimus, Alveolaimus* and *Halaphanolaimus* has probably evolved from *Leptolaimus* s. str. connected with the reduction process. Moreover, the origin of *Boveelaimus* may have ocurred in three or even four possible ways: from either *Dermatolaimus, Alveolaimus* or *Halaphanolaimus*, or *Leptolaimus* s. str., in all cases due to the reduction and thus making the genus *Boveelaimus* polyphyletic. Worth mentioning is that the possibility of a reversal from a more simple (without any supplements) to a more complex (with one or two kinds of supplements) organisation of the supplementary apparatus seems less likely. Until other characters can be included in the phylogenetic analysis, we prefer to unite all five generic groups (*Dermatolaimus, Alveolaimus, Halaphanolaimus, Leptolaimus* and *Boveelaimus*) in the genus *Leptolaimus* without making any division into subgenera. When considering the evolution of the supplementary structures in both sexes (females and males), the situation is even more complex and at present clearly irresolvable. We may only suppose a possible hypothetical ancestral taxon with both types of supplements in females and males (tubular supplements are present in *Stephanolaimus elegans*, while alveolar ones are present in *Aphanonchus intermedius* Coomans & Raski, 1991a), which gave origin to all extant and known, and possibly extinct or unknown (non-existence is non-provable), taxa of *Leptolaimus* sensu lato However, the subdivision of the genus *Leptolaimus* into a few morphological groups (which correspond to the five above mentioned “subgenera”) without taxonomic status will make identification easier.

Clade 2

A computerised cladisitic analysis restricted to the members of Clade 2 gave no clear results, putting many of the included genera in polytomy and also rendering many characters homoplastic. This could be due to difficulties in interpreting and coding the stoma structure in many “camacolaimid” genera. A manual experimentation with the characters presented in the legends to Figure 3.15 also resulted in a polytomic divergence of the basal branches, but the phylogeny of the taxa within these three branches could be interpreted in detail (Fig. 3.15). We excluded *Procamacolaimus dorylaimus* from the manual experimentation due to the difficulties in interpreting the homology of its stomatal armament and added *Cricolaimus, Ionema* and *Procamacolaimus phinney*, whose descriptions do not include enough information needed for the computer analysis.

The clade uniting *Plectus* and *Hemiplectus* is supported by only one character, i.e. the structure of the renette cell and excretory canal (character 4). However, the morphological differences in the structure of the sensory organs and digestive system between these genera are ample, questioning the reliability of this clade. At present we find it premature to consider *Plectus* and *Hemiplectus* as sister taxa, mainly because other representatives currently assigned to the superfamily Plectoidea and the genera *Caribplectus*, *Cynura* and *Pakira* were not analysed.

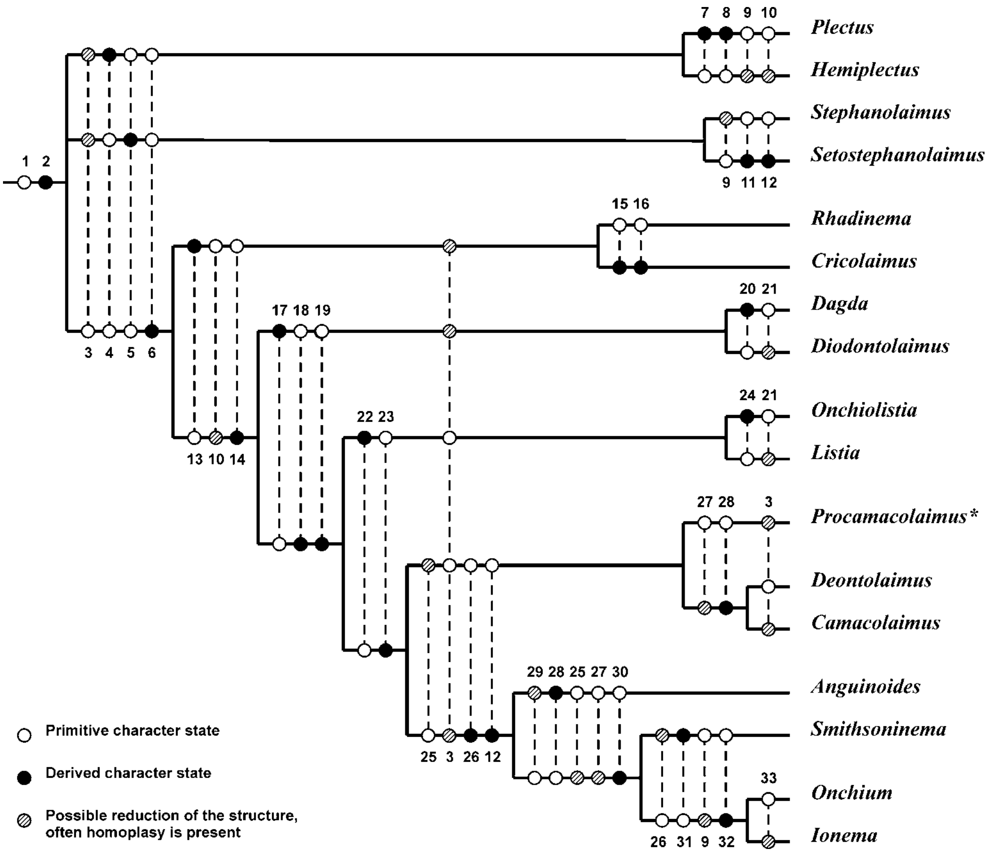

Figure 3.15. Cladogram showing the phylogenetic relationships of the genera *Anguinoides* Chitwood, 1936, *Camacolaimus* de Man, 1889, *Cricolaimus* Southern, 1914, *Dagda* Southern, 1914, *Deontolaimus* de Man, 1880, *Diodontolaimus* Southern, 1914, *Hemiplectus* Zell, 1991, *Ionema* Cobb, 1920, *Listia* Blome, 1982, *Onchiolistia* Blome, 2002, *Plectus* Bastian, 1865, *Procamacolaimus* Gerlach, 1954, *Rhadinema* Cobb, 1920, *Setostephanolaimus* Tchesunov, 1994 and *Smithsoninema* Hope & Tchesunov, 1999 (Clade 2 on Fig. 3.13). In this dataset *Procamacolaimus phinney* (Murphy, 1964) Hope & Tchesunov, 1999 was used instead of *P. dorylaimus* Holovachov, 2003, see text for details. **Characters uniting all taxa:** (1) presence of hypodermal glands; inner and outer labial sensilla papilliform, on lips; amphid unispiral; renette cell with excretory ampulla; subventral gland orifices same level as dorsal gland orifice; pharynx cylindrical and uniformly muscular; ovaries reflexed; spermatheca axial; testes opposed (posterior reflexed); precloacal sensilla setiform; tubular and alveolar supplements present; caudal glands open through spinneret. (2) postcloacal sensilla present (single known synapomorphy). **Derived states** (reductions are also considered as derived, but homoplasy is often present): (3) alveolar supplements absent (reduced). (4) excretory canal long; excretory ampulla reduced; renette complex, multicellular. (5) amphid transverse oval. (6) both testes directed anterior, parallel. (7) pharynx "plectoid", i.e. with radial tubules, corpus, isthmus, bulbus and valvular apparatus (grinder). (8) inner labial sensilla open inside cheilostom. (9) postcloacal sensilla absent (reduced). (10) hypodermal glands absent (reduced). (11) outer labial sensilla setiform.

The two genera *Stephanolaimus* and *Setostephanolaimus* were previously treated as a single taxon until Tchesunov (1994) erected the latter for species possessing setiform outer labial sensilla. In the cladogram (Fig. 3.15), these taxa are united by a single feature: transversely oval amphids (character 5), although a similar amphid shape is present in *Anomonema* (Clade 1) and in a number of other nematode species (see Lorenzen, 1981). Furthermore, species of the two genera share a similar stoma morphology, which is undifferentiated, narrow or conoid. They, however, differ considerably in one more respect: species of *Setostephanolaimus* have a pharynx with a glandular posterior part since the pharyngeal musculature is probably reduced (character 12). A similar condition of the pharynx is characteristic also for some "camacolaimid" genera, but with a somewhat different morphology (see below). The two genera are here considered as sister taxa, but since their systematic position and relations to other taxa is not yet clear, we prefer not to place them in any existing family or propose a new family taxon for them.

The last clade unites the remaining genera and is supported by a single character: both testes directed anteriorly, outstretched (character 6). This clade further splits into two: one containing *Rhadinema* and *Cricolaimus*, and another uniting the genera that are usually included in the (sub-)family Camacolaimi(-nae)dae (see for example Hope and Tchesunov, 1999). The clade uniting the genera *Rhadinema* and *Cricolaimus* is supported by one feature (character 13): the structure of the stoma with six forwardly directed projections (in *Cricolaimus coronatus*). Unfortunately females have never been described for *Cricolaimus* and therefore no detailed analysis could be performed. Both genera are here united in the family Rhadinematidae.

The monophyly of the family Camacolaimidae (as it is accepted in the present paper) is supported by a single character: presence of stomatal armament (character 14). It further splits into two clades: one containing *Dagda* and *Diodontolaimus* (two or three odontia present), and another united by two characters: presence of a single odontium and strong development of the glandular tissue in the basal part of the pharynx. Further branching of the cladogram is usually supported by only one synapomorphy for each clade, and also by several character states caused by a probable reduction of the feature which may occur several times independently (see Fig. 3.15). Homoplasy is present also in character 28 (presence of lateral alae), but the probable multiple origin of this feature seems to be common in nematodes.

Figure 3.15. (Continued). (12) pharynx "glandular", i.e. glandular tissue of the pharynx strongly developed, while muscular part is probably reduced (homoplasy present). (13) cheilostom with sclerotized ring and six forwardly directed projections. (14) gymnostomal armament present. (15) amphid loop-shaped. (16) tubular supplements arranged in two groups. (17) several (two or three) odontia arranged dorsally and ventrosublaterally. (18) a single dorsal odontium present. (19) dorsal sector in the posterior part of pharynx glandular. (20) obliquely directed musculature present in pharyngeal region. (21) dorsal odontium absent (reduced). (22) anterior ovary absent (reduced), anterior uterus functioning as spermatheca. (23) onchiostyle present. (24) cuticle longitudinally striated. (25) precloacal sensillum absent (reduced). (26) odontium reduced or fused with onchiostyle. (27) tubular supplements absent (reduced). (28) lateral alae present (homoplasy). (29) excretory system absent (reduced). (30) cuticular annulation inconspicuous. (31) sexual dimorphism. (32) ocelli present. (33) onchiostyle absent (reduced).

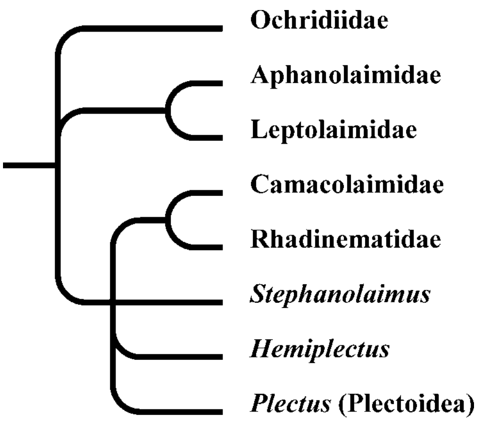

Figure 3.16. Summarised phylogeny of the suborder Leptolaimina based on the analysis presented in Figs 3.14 and 3.15.

The genera *Anguinoides, Smithsoninema, Onchium* and *Ionema* are united particularly by the presence of a glandular pharynx. A similar condition was found in *Setostephanolaimus* in the present study, but is also common in many aquatic and terrestrial nematode taxa. Several morphological differences do exist between pharynges of *Setostephanolaimus* and *Onchium*. In the former the musculature is reduced, but the glandular tissue is equally developed in dorsal and subventral sectors. The latter genus, as well as *Smithsoninema*, are characterised by the strong development of the granulated glandular tissue in the dorsal sector, occupying more than half of the pharyngeal region, while the subventral pharyngeal glands are small and not granular. Therefore, we suggest an independent appearance of the "glandular" type of the pharynx in *Setostephanolaimus* and the four camacolaimid genera listed above.

We were unable to clarify the position of *Procamacolaimus dorylaimus*. This species appeared either as a sister taxon of the "*Onchiolistia-Ionema*" clade (homoplastic origin of onchiostyle required), or as a sister taxon of the "*Anguinoides-Ionema*" clade (two reversals in characters 6 and 12 required). Instead, we analysed the data on *P. phinney*, which tends to be a sister taxon of *Deontolaimus* and *Camacolaimus*, showing similarities in the structure of the stomatal armament and absence of a precloacal sensillum. As a consequence, the genus *Procamacolaimus* is most probably polyphyletic in origin, but not possible to revise until new information on the morphology of a large part of the species in this genus is available.

Systematic position of selected genera and families

The following section presents a discussion of the systematic position of several genera and families assigned to the superfamily Leptolaimoidea by De Ley & Blaxter (2002). First of all, the genera *Cynura*, *Pakira* and *Caribplectus*, which were included in the family Leptolaimidae by Lorenzen (1981), are characterised by the peculiar structure of pharynx and excretory system, showing strong affinities to members of the Plectoidea, and therefore provisionally placed within this superfamily. Their morphology and systematics will be discussed in a separate paper (Holovachov, unpublished). The systematic position of the following genera is not clear: *Alaimella* Cobb, 1920, *Digitonchus* Cobb, 1920, *Domorganus* Goodey, 1947, *Neurella* Cobb, 1920 and *Prodomorganus* Gagarin, 1993. They could not at this point be placed with confidence in the proposed phylogeny, although they most probably belong to the clade discussed in this paper.

The genus *Alaimella* includes two species which are characterised particularly by a tiled cuticle, undifferentiated stoma, one posterior ovary, and absence of supplements in the male (Cobb, 1920; Vitiello, 1974; Blome, 1982). The presence of two anteriorly directed testes supports its close relations to some camacolaimid genera. However, the absence of stomatal armament and absence of some important information about the structure of the pharynx and reproductive system, hamper our attempts to elucidate the systematic position of this genus.

The type species of the genus *Digitonchus*, i.e. *D. uniformis* Cobb, 1920, is known on the basis of a single female. Its buccal armament was described as "onchium spear-like, solid". It has no inner canal and does not appear to be composed from two parts, like the buccal armament in *Camacolaimus* [see for example the description by Cobb (1920) of a typically *Camacolaimus*-like buccal armament in *Ypsilon exile* Cobb, 1920, transferred to *Camacolaimus* by Wieser (1956)]. In this respect *Digitonchus* shows strong similarities to *Anguinoides* (see Holovachov, 2003a, 2003b), but since no males of the type species are known its taxonomic status cannot be clarified at present. The other species that was assigned originally to the genus *Digitonchus*, *D. cylindricaudatus* Chitwood, 1951 is more similar to the genus *Camacolaimus* in the structure of the stoma and male copulatory apparatus. Although *Digitonchus* was considered synonymous with *Acontiolaimus* Filipjev, 1918 by Wieser (1956), we treat it as *genus inquirendum* until more information about its morphology is available.

Domorganus is a member of the family Ohridiidae Andrássy, 1976, and is characterised by a very characteristic structure of the pharynx and excretory system, absence of somatic sensilla, hypodermal glands, presence of unispiral amphids, paired and reflexed ovaries, presence of just a single precloacal papilliform sensillum in the male, thus causing difficulties to find its close relatives (see also Tchesunov & Sturhan, 2004). The family Ohridiidae is provisionally included here in an unresolved polytomy as a sister taxon of the superfamilies Leptolaimoidea, Camacolaimoidea and Plectoidea.

The monotypic genus *Prodomorganus* Gagarin, 1993 was originally treated close to *Domorganus* (Gagarin, 1993), differing in the structure of the pharynx and excretory pore position. The original description does not contain many important characters and we were unable to locate the type. Therefore, the systematic position of this genus cannot be resolved. *Neurella* is another monotypic genus included in Leptolaimidae and known only from the original description (Cobb, 1920). It shows superficial similarities to the genus *Domorganus* in the following characters: lateral alae starting near amphid, renette cell located near the middle of the body and males with a single large papilliform precloacal sensillum. However, the systematic position of the genus remains unsettled until the type material can be studied.

The systematic position of a number of other genera and families currently placed in the superfamily Leptolaimoidea by De Ley & Blaxter (2002), needs to be discussed in the context of a revision of the systematics of the superfamilies Leptolaimoidea and Plectoidea. These are the genera *Aegialoalaimus* de Man, 1907, *Bastiania* de Man, 1880, *Odontolaimus* and *Tobrilia,* and the family Diplopeltoididae.

Aegialoalaimus is the type and only genus of the family Aegialoalaimidae, which was placed in the superfamily Leptolaimoidea by De Ley & Blaxter (2002). It is characterised by a very specialised structure of the pharynx (Tchesunov, 1990). Presence of several precloacal papilliform supplements in the male and separate outlets of the caudal glands places this genus outside the superfamily Leptolaimoidea.

The genus *Bastiania*, type genus of the family Bastianiidae, has often been placed near to the genus *Prismatolaimus* de Man, 1880 in different classification systems, in spite of strong differences in stoma structure. Lorenzen (1981) and Ryss (1988) placed both genera within the Leptolaimina or Araeolaimida, respectively, on the basis of amphid structure. However, the most recent data (morphological and SSU rDNA sequences) showed Prismatolaimidae to be close to the family Tobrilidae (De Ley & Blaxter, 2002). Coomans & Raski (1988) showed that the copulatory musculature in males of *Prismatolaimus* is similar in structure to that of *Tobrilus* Andrássy, 1959 and *Tripyla* Bastian, 1865. They also pointed out the numerous similarities between *Prismatolaimus* and *Bastiania* in the structure of sensilla, amphid, cardia, supplements, spicules and gubernaculum (Coomans & Raski, 1988). *Prismatolaimus, Bastiania* and *Dintheria* de Man, 1921 have a similar structure of the male copulatory apparatus: spicula and gubernaculum shape, arrangement of spicule protractors and supplement shape (Fig. 3.17 F-G) (see Brzeski, 1997; Coomans & Raski, 1988; Tchesunov & Sturhan, 2001; Winiszewska et al., 2001). Coomans & Raski (1988) described a peculiar glandular organ with a prominent dorsal pore slightly behind the base of the pharynx in *Prismatolaimus novoporus* Coomans & Raski, 1988. The same structure was recorded in *P. dolichurus* de

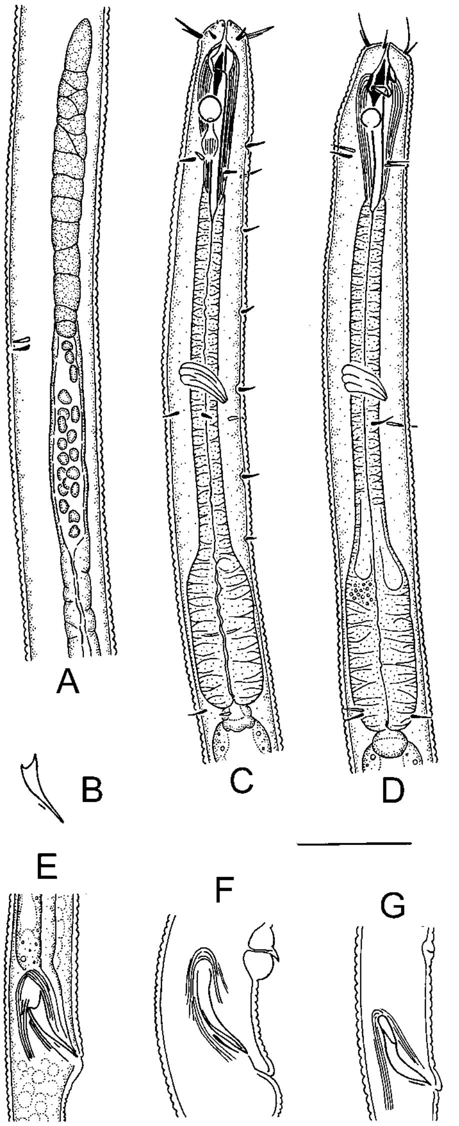

Figure 3.17. *Odontolaimus chlorurus* de Man, 1880: A-E. A: Testis; B: Spicule and gubernaculum; C: Male pharyngeal region; D: Female pharyngeal region; E: Male cloacal region; F: Male cloacal region of *Dintheria tenuissima* de Man, 1921; G: Male cloacal region of *Bastiania acarayensis* Andrássy, 1968. Scale bar 20 µm.

Man, 1880 and *P. mulcoomus* Brzeski, 1997 (Brzeski, 1997) and named "*organellum ovale*" by Andrássy (2001a, 2001b). Andrássy (2001a) described the same character for *Onchulus dorsalis* Andrássy, 2001, while Tchesunov & Sturhan (2001) found it in at least one species of *Bastiania*. However, a similar *organellum ovale* was found in *Leptoplectonema fuegoense* by Coomans & Raski (1991b).

Another family of uncertain position is Odontolaimidae. A peculiar feature of the male of *Odontolaimus* is the presence of five setiform sensilla along the ventral side of the pharyngeal region and no precloacal supplementary sensilla (see Fig. 3.17 C). Within Leptolaimoidea only *Deontolaimus* has a similar distribution of male sensilla (alveolar supplements present only along the ventral side of the pharyngeal region), but the morphology of the sensory structures are different. Nonetheless, several unrelated taxa within Enoplia posses sensory structures ventrally along the pharyngeal region and these differ morphologically from the precloacal ones. Among them members of the family Trichodoridae and Trefusiidae (see Vincx & Vanreusel, 1989), *Kinonchulus sattleri* Riemann, 1972 and *Isolaimium collare* Andrássy, 2001.

Although we agree with Eyualem & Coomans (1996) about the position of the family Rhabdolaimidae within Microlaimoidea, the taxonomic position of the genus *Tobrilia* still remains unclear. The shape and location of the supplements and spicula shape in the single available male of *Tobrilia* (Fig. 3.18) show strong similarities to genera of the family Tripylidae de Man, 1876 (Brzeski & Winiszewska-Šlipinska, 1993; Hernández & Jordana, 1988). Particularly the males of *Tobrilia imberbis* and *Tripylella intermedia* (Bütschli, 1873) Brzeski & Winiszewska-Šlipinska, 1993 are similar in the number and shape of all supplements, and the far anterior position of the posteriormost supplement in relation to cloaca. On the other hand males of *Tobrilia* differ considerably from the male of *Rhabdolaimus terrestris* in the shape of spicules and absence of supplements in the latter species as described by Ocaña (1991).

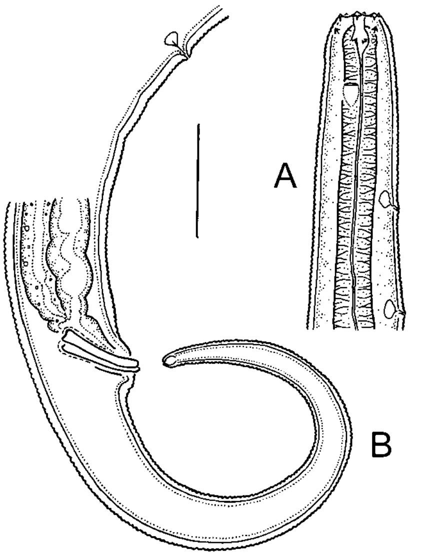

Figure 3.18. Male of *Tobrilia imberbis* (Andrássy, 1953) Andrássy, 1967. A: Anterior end; B: Caudal region. (Scale bar: 20 µm).

De Ley & Blaxter (2002) listed in the superfamily Ceramonematoidea Cobb, 1933 three families, viz. Ceramonematidae Cobb, 1933, Tarvaiidae Lorenzen, 1981 and Tubolaimoididae Lorenzen, 1981. This agrees fairly well with the opinion of Lorenzen (1981) about the relations of Ceramonematidae to Tubolaimoididae and of Tchesunov & Miljutina (2002) about the relations of

Table 3.6. Compendium of the superfamily Ceramonematoidea Cobb, 1933 based on literature data.

Family	Ceramonematidae	Tarvaiidae	Tubolaimoididae	Diplopeltoididae
Cuticle	annulated; complex ornamentation (zygapophyses); lateral alae absent	annulated; no ornamentation; lateral alae absent	smooth or annulated; no ornamentation; lateral alae absent	annulated; no ornamentation or longitudinal striae; lateral alae absent
Anterior end	cephalic capsule present; lips three	cephalic capsule present	cephalic capsule absent; lips three	cephalic capsule present
Anterior sensilla	outer labial sensilla papilliform or setiform; cephalic – setiform, in two or one circle	outer labial sensilla papilliform; cephalic – setiform	inner labial sensilla grouped in pairs; outer labial and cephalic sensilla setiform, in separate circles	outer labial sensilla papilliform; cephalic – setiform
Amphid	ventrally spiral type (inverted U)	ventrally spiral type (loop-shaped with 1.5 turn), loops run from inside to outside	ventrally spiral type (unispiral or inverted U)	ventrally spiral type (inverted U)
Digestive system	stoma undifferentiated; posterior part of pharynx muscular or glandular	stoma undifferentiated, pharynx weakly muscularized	stoma undifferentiated; dorsal and sub-ventral pharyngeal glands open into stoma	stoma undifferentiated; anterior 1/3 of pharynx cylindrical, muscular, middle – narrow, posterior – glandular
Female reproductive system	didelphic, ovaries reflexed antidromously	didelphic, ovaries reflexed antidromously	didelphic, ovaries reflexed antidromously	didelphic, ovaries reflexed antidromously
Male reproductive system	diorchic, testes opposed; supplements absent	diorchic, testes opposed; supplements absent	diorchic; testes outstretched; supplements absent or papilliform	diorchic, testes outstretched; supplements absent
Tail	caudal glands present; spinneret present; caudal setae in males	caudal glands present; spinneret present	caudal glands present; spinneret present	caudal glands present, their outlets separate; caudal setae in males
References	Lorenzen, 1981; Tchesunov & Miljutina, 2002	Lorenzen, 1981	Lorenzen, 1981; Tchesunov, 1993	Tchesunov, 1990

Ceramonematidae to Tarvaiidae. Moreover, the latter authors suggested a similarity between Ceramonematidae and Diplopeltoididae in the structure of amphids (loop-shaped), digestive system (undifferentiated stoma, glandular posterior part of the pharynx), cephalic capsule, absence of tubular supplements etc. We therefore propose to treat all four families within the superfamily Ceramonematoidea (See also Table 3.6).

Features characteristic for the clade that unites superfamilies Leptolaimoidea, Camacolaimoidea and Plectoidea

It is difficult to find synapomorphies for the clade which includes superfamilies Leptolaimoidea, Camacolaimoidea and Plectoidea, but it may be characterised by the sum of the following symplesiomorphies (most of which have passed through modifications): cuticle annulated; hypodermal glands and somatic sensilla present; inner and outer labial sensilla papilliform, located on lips; cephalic sensilla setiform, located posterior to lip region; amphid unispiral; renette cell forming an excretory ampulla near nerve ring; excretory duct short; stoma funnel-shaped, undifferentiated; pharynx uniformly cylindrical, muscular; dorsal and two subventral gland orifices located at stoma base; ovaries paired and reflexed antidromously; spermatheca axial; supplementary structures similar to those in males may be present in females; testes paired and opposed (posterior reflexed); copulatory apparatus composed of a single precloacal sensillum and several tubular and alveolar supplements; tubular supplements always connected with unicellular glands; caudal glands empty through a common duct, forming a spinneret. A summarised phylogeny of the superfamilies Leptolaimoidea, Camacolaimoidea and Plectoidea as well as the genera *Domorganus, Stephanolaimus, Setostephanolaimus* and *Hemiplectus* is given in Fig. 3.16.

Appendix: an updated classification of the superfamily Leptolaimoidea

Superfamily Leptolaimoidea Örley, 1880

Key diagnostic features: "Somatic pore" type of sensilla present. Somatic setae present only in males. Female reproductive system didelphic, amphidelphic, each ovary branch with two lateral sack-like spermathecae. Male reproductive system usually diorchic, with opposed testes, or with anterior testis nonfunctional, glandular. Precloacal sensilla usually papilliform, on the anterior cloacal lip. Caudal setae arranged in one subventral precloacal pair and few subventral and subdorsal caudal pairs.

Type family: Leptolaimidae Örley, 1880 (Syn.: Halaphanolaimidae De Coninck & Schuurmans Stekhoven, 1933; Peresianidae Vitiello & De Coninck, 1968)

Other family: Aphanolaimidae Chitwood, 1936.[*]

Family Leptolaimidae Örley, 1880

Diagnosis: Leptolaimoidea. Medium sized nematodes. Cuticle annulated, with or without narrow ridge-like lateral alae. Lateral hypodermal glands present, opening through prominent pores. "Somatic pore" type of sensilla present. Somatic setae present only in males. Outer labial sensilla papilliform. Four cephalic setiform sensilla. Amphid unispiral or derived therefrom. Stoma tubular, with strongly developed stegostom (except in *Anomonema*). Pharynx equally muscular, with a distinct division into procorpus, metacorpus, isthmus and basal bulb, the latter often with strongly cuticularised lining. Only dorsal gland orifice located at stoma base. Secretory-excretory system present, renette cell located at level with intestine, forming an excretory ampulla close to nerve ring, duct opens to the exterior. Female reproductive system didelphic, amphidelphic. Ovary branches reflexed antidromously (except *Manunema*), each branch with two lateral sack-like spermathecae. Vagina encircled by a single sphincter muscle. Male reproductive system usually diorchic, with opposed testes (posterior one reflexed). Spicules paired and symmetrical. Gubernaculum present. Tubular supplements present or absent. Alveolar supplements present or absent. Precloacal sensilla usually papilliform, on the anterior cloacal lip. Setae arranged in one subventral precloacal pair and few subventral and subdorsal caudal pairs. Caudal glands present, opening via a spinneret on tail tip.

Type genus: *Leptolaimus* de Man, 1876 (Syn.: *Aplectus* Cobb, 1914; *Halaphanolaimus* Southern, 1914; *Dermatolaimus* Steiner, 1916; *Polylaimium* Cobb, 1920; *Boveelaimus* Alekseev & Rassadnikova, 1977 (Alekseev, 1979); *Leptolaimus (Alveolaimus)* Alekseev & Rassadnikova, 1977; *Leptolaimus (Tubulaimus)* Alekseev & Rassadnikova, 1977).

Other genera: *Antomicron* Cobb, 1920 (Syn.: *Eutelolaimus* de Man, 1922); *Paraplectonema* Strand, 1934 (Syn.: *Paraplectus* Filipjev, 1930); *Manunema* Gerlach, 1957 (Syn.: *Peresiana* Vitiello & De Coninck, 1968); *Anomonema* Hopper, 1963; *Leptolaimoides* Vitiello, 1971; *Leptoplectonema* Coomans & Raski, 1991.

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[*] For the classification of the family Aphanolaimidae see Chapter 2.

Appendix: an updated classification of the superfamily Camacolaimoidea

Superfamily Camacolaimoidea Micoletzky, 1924

Key diagnostic features: "Somatic pore" type of sensilla absent. Somatic setae present in both sexes or in male only. Cheilostom with sclerotized ring and six or two digitate projections or stoma with strongly developed gymnostomatal and/or stegostomatal armament. One dorsal and often two subventral gland orifices located at stoma base. Spermatheca axial. Male reproductive system diorchic, with usually outstretched testes. Precloacal sensilla setiform, anterior to cloaca, or absent. Postcloacal sensilla papilliform or absent. Caudal setae arranged in few subventral and subdorsal pairs.

Type family: Camacolaimidae Micoletzky, 1924 (Syn.: Procamacolaiminae De Coninck, 1965)

Other family: Rhadinematidae Lorenzen, 1981.

Family Camacolaimidae Micoletzky, 1924

Diagnosis: Leptolaimoidea. Medium to large sized nematodes. Cuticle annulated or smooth, with or without narrow ridge-like lateral alae. Lateral hypodermal glands and "somatic pore" type of sensilla absent. Somatic setae present in both sexes or in male only. Inner and outer labial sensilla papilliform. Four cephalic setiform sensilla. Amphid unispiral, located anterior to or at the same level as cephalic sensilla. Stoma funnel-shaped, with strongly developed gymnostomatal and/or stegostomatal armament (except in *Listia* and *Ionema*). Pharynx equally muscular or with more developed glandular tissue in dorsal sector or glandular in posterior part. One dorsal and often two subventral gland orifices located at stoma base. Secretory-excretory system present, renette cell located at level with intestine, forming an excretory ampulla close to nerve ring, duct opens to the exterior. Female reproductive system didelphic, amphidelphic or monodelphic, opisthodelphic. Ovary branches reflexed antidromously. Spermatheca axial. Vagina encircled by a single sphincter muscle. Male reproductive system diorchic, usually with outstretched (both directed anteriorly) testes. Spicules paired and symmetrical. Spicule knobs usually turned ventrally. Gubernaculum present. Tubular supplements present or absent. Alveolar supplements present or absent. Precloacal sensilla setiform, anterior to cloaca, or absent. Postcloacal sensilla papilliform, one to three in number or absent. Caudal setae arranged in few subventral and subdorsal pairs. Caudal glands present, opening via a spinneret on tail tip.

Type genus: *Camacolaimus* de Man, 1889 (Syn.: *Acontiolaimus* Filipjev, 1918; *Ypsilon* Cobb, 1920)

Other genera: *Deontolaimus* de Man, 1880; *Dagda* Southern, 1914; *Diodontolaimus* Southern, 1914; *Onchium* Cobb, 1920 (Syn.: *Onchulella* Cobb, 1920); *Ionema* Cobb, 1920 (Syn.: *Nemella* Cobb, 1920); *Anguinoides* Chitwood, 1936; *Procamacolaimus* Gerlach, 1954; *Listia* Blome, 1982 (Syn.: *Eontolaimus* Furstenberg & Vincx, 1988); *Smithsoninema* Hope & Tchesunov, 1999; *Onchiolistia* Blome, 2002

Genera inquirendae: *Camacolaimoides* De Coninck & Schuurmans Stekhoven, 1933; *Digitonchus* Cobb, 1920

Family Rhadinematidae Lorenzen, 1981

Diagnosis: Leptolaimoidea. Medium to large sized nematodes. Cuticle annulated or smooth, without narrow ridge-like lateral alae. Lateral epidermal glands present. "Somatic pore" type of sensilla absent. Somatic setae present in both sexes. Inner and outer labial sensilla papilliform. Four cephalic setiform sensilla. Amphid unispiral or loop-shaped. Stoma funnel-shaped, cheilostom with sclerotized ring and six or two digitate anteriorly directed projections. Pharynx equally muscular. One dorsal and two subventral gland orifices located at stoma base. Secretory-excretory system present, renette cell located at level with intestine, forming an excretory ampulla close to nerve ring, duct opens to the exterior. Female reproductive system didelphic, amphidelphic. Ovary branches reflexed antidromously. Spermatheca axial. Male reproductive system diorchic, with outstretched (both directed anteriorly) testes. Spicules paired and symmetrical. Gubernaculum present. Tubular supplements present. Alveolar supplements absent. Precloacal sensilla setiform. Postcloacal sensilla papilliform, two in number or absent. Caudal setae arranged in few subventral and subdorsal pairs. Caudal glands present, opening via a spinneret on tail tip.

Type genus: *Rhadinema* Cobb, 1920

Other genus: *Cricolaimus* Southern, 1914

Chapter 4

Morphology and systematics of the genera *Wilsonema* Cobb, 1913, *Ereptonema* Anderson, 1966 and *Neotylocephalus* Ali, Farooqui & Tejpal, 1969

Published in a modified form as:

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Abstract

Descriptions of populations of *Wilsonema otophorum*, *W. schuurmansstekhoveni*, *Ereptonema arcticum*, *Neotylocephalus annonae* and *N. inflatus* are given on the basis of studies with light and scanning electron microscope. The processes of the complex labial region of the genera within the subfamily Wilsonematinae are homologized. Emended diagnoses as well as revised classifications of the genera *Wilsonema*, *Ereptonema* and *Neotylocephalus* are given. Phylogenetic analyses were made to elucidate the relationships between the genera of Wilsonematinae.

Nomenclatorial remarks

Folloving nomenclatorial changes were proposed in the original publication: **1)** Species *Wilsonema andersoni* Zell, 1985 and *Plectus (Wilsonema) bolivianus* Zell, 1993 were considered junior synonyms of *Wilsonema otophorum* (de Man, 1880) Cobb, 1913. **2)** *Paraereptonema ciliatum* Eroshenko, 1977 was considered a junior synonym of *Ereptonema fimbriatum* Anderson, 1966. **3)** *Wilsonema cheliferum* Andrássy, 1986 and *Wilsonema agrarum* Nesterov, 1973 were transferred to *Ereptonema* Anderson, 1966, resulting in new combinations *Ereptonema cheliferum* (Andrássy, 1986) Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003 and *Ereptonema agrarum* (Nesterov, 1973) Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003. **4)** The genera *Spatiocephalus* Patil & Khan, 1982 and *Coronacephalus* Ganguly & Khan, 1986 were considered junior synonyms of *Neotylocephalus* Ali, Farooqui & Tejpal, 1969. **5)** *Spatiocephalus venustrus* Patil & Khan, 1982 was considered a junior synonym of *Neotylocephalus annonae* Ali, Farooqui & Tejpal, 1969. **6)** *Ereptonema inflatum* Yeates, 1967 was transferred to the genus *Neotylocephalus* Ali, Farooqui & Tejpal, 1969, resulting in new combination *Neotylocephalus inflatus* (Yeates, 1967) Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003. **7)** *Coronacephalus indicus* Ganguly & Khan, 1986 and *Plectus (Wilsonema) gangulykhani* Zell, 1993 were considered junior synonyms of *Neotylocephalus inflatus* (Yeates, 1967) Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003. **8)** New name *"Neotylocephalus haryanensis* Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003" was proposed for *Wilsereptus indicus* Walia & Bajaj, 1999, which becomes a junior homonym of *N. indicus* (Ganguly & Khan, 1986) Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003; when transferred to *Neotylocephalus* Ali, Farooqui e& Tejpal, 1969. The date of publication of new names and new combinations, which were introduced in the original article, is 20 April 2003.

Introduction

Members of the subfamily Wilsonematinae are found in many different habitats worldwide. A basic morphological character of the subfamily is the laterally “flattened” labial region with numerous cuticular processes. Due to the difficulties to appreciate the complexity of the anterior end with the light microscope, several genera of wilsonematids have been described over the years. Cobb (1913) established the genus *Wilsonema*, which he differentiated from the related genus *Plectus* by having “empty chitinous expansions” of the head. Subsequently two more monotypic genera were described: *Pycnolaimus* Cobb, 1920 and *Bitholinema* De Coninck, 1931. Crossman (1933) proposed the genus *Tylocephalus* to accommodate those species with two dorsosubmedian and two ventrosubmedian cornua. These genera were not properly illustrated and placement of species remained doubtful. Their positions became clarified when Anderson (1966) studied several species and revised the taxonomy of Wilsonematinae. He emended the descriptions of *Wilsonema otophorum* and *Tylocephalus auriculatus*, proposed the new genus *Ereptonema* Anderson, 1966, and gave new combinations and synonyms. Later five more genera, viz. *Neotylocephalus* Ali, Farooqui & Tejpal, 1969, *Wilsotylus* Chawla, Khan & Prasad, 1969, *Wilsereptus* Chawla, Khan & Saha, 1977, *Spatiocephalus* Patil & Khan, 1982 and *Coronacephalus* Ganguly & Khan, 1986 were added to the subfamily Wilsonematinae, which was raised to family rank by Eroshenko (1973) and Ganguly & Khan (1986).

SEM studies of *Tylocephalus auriculatus* were first published by Sauer & Annells (1982) and Sauer (1985) and later supplemented by De Ley & Coomans (1997). SEM observations on three more species (*Ereptonema arcticum*, *Tylocephalus palmatus* Tahseen, Ahmad & Jairajpuri, 1995 and *Tylocephalus nimius* De Ley & Coomans, 1997) were published by Boström (1988), Tahseen et al. (1995) and De Ley & Coomans (1997)*. Ever since the first description of a wilsonematid by Bütschli (1873), the taxonomy of this group has been hampered by the limitations of light microscopy for observation and interpretation of their lip region. The discrepancy between recent data and former descriptions of the structure of the labial region illustrates the great importance of SEM in morphological as well as taxonomic studies of the subfamily Wilsonematinae. The aim of this paper is to provide a detailed description of species of the genera *Wilsonema*, *Ereptonema* and *Neotylocephalus* on the basis of light and scanning electron microscopy, describe the morphology of the anterior end and homologize its elements, give emended diagnoses of all three genera, and revise their classifications.

Material and methods

Table 4.1 lists available data on all the samples from which specimens were used for measurements and descriptions. These specimens were collected by the authors, their

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* A description of new species *Tylocephalus primitivus* Holovachov, Boström & Mundo-Ocampo, 2004 includes also information from SEM studies (Holovachov et al., 2004). Postembryonic development of *Ereptonema arcticum* was also studied on the base of SEM data (De Ley et al., 2002).

Table 4.1. Origin of samples used in the study.

Pop.	Country	Locality
Wilsonema otophorum		
I	Ukraine	Lviv, “Znesinnja” Landscape Park, Khomets
II	Ukraine	Lviv, “Znesinnja” Landscape Park, Khomets
III	Russia	Altai Mountains, Akkem valley, altitude 1400 m a.s.l
IV	New Caledonia	Mont Mou, altitude 800-1000 m.a.s.l.
V	Mexico	Mazatlan, 65 km East, Sierra Madre Occidental, altitude 1500-1800 m a.s.l.
VI	Israel	Haifa, the Lower Nahal Oren, Mount Carmel
VII	Ecuador	Galápagos, Isla Isabela, Cerro Gavilan at 850 m a.s.l.
Wilsonema schuurmansstekhoveni		
VIII	Sweden	Gästrikland, Ivantjärnsheden
IX	Ukraine	Volynska Prov., Shutsk National Natural Park
X	Costa Rica	Las Amistad Pacifico Conservation Area, Chirripó, altitude 2450 m a.s.l.
XI	Poland	Warszawa, Dziekonow Lešny, Kampinosky National Park
XII	Malaysia	Borneo, Sabah, Mt. Kinabalu, altitude 2700 m a.s.l.
XIII	Australia	Queensland, Palm Croove National Park
Ereptonema arcticum		
XIV	Norway	Spitzbergen
XV	Norway	Spitzbergen, Kongsfjorden, Blomstrandhalvöya, alt. 50 m a.s.l
XVI	Ukraine	As II
XVII	Ukraine	Lvivska prov., Roztochya Natural Reserve
XVIII	Russia	Altai Mountains, Akkem valley, altitude 1500 m a.s.l
XIX	USA	San Bernardino National Forest off highway 18, altitude 2000 m a.s.l.
XIX'	USA	Southern California, Mojave desert, Barstow
XX	Mexico	No data available
Neotylocephalus annonae		
XXI	Australia	Great Dividing Range
XXII	Reunion	
XXIII	India	Bangalore, Narayanaswamy
XXIV	Yemen	Jum’ at Sâri’
Neotylocephalus inflatus		
XXV	Mexico	Cotem Veracruz
XXVI	Mexico	Rosario Izopa, Celeba bordio
XXVII	Mexico	Union Juarez, Chis, Muxbal valley
XXVIII	Mexico	Zacatepec
XXIX	Ecuador	Galápagos, Isla Santa Cruz, N slope at 16.4 km from Itabaca along road at 500 m a.s.l.
XXX	Ecuador	Galápagos, Isla Floreana, at 350 m a.s.l.
XXXI	Ecuador	Galápagos, Isla Fernandina, foot of Cerro Verde on SW slope at 200 m a.s.l.
XXXII	Costa Rica	Tempsique Conservation Area, Nacional Diria, Diria river
XXXIII	USA	Virgin Islands, St. John, Trunk Bay Beach

Table 4.1. Continued.

Biotope	Habitat	Date	Collector	Kept at
steppe cereals	soil	September 2000	O. Holovachov	DZL, NRS
steppe cereals	soil	September 1998	A. Susulovsky	MNH
	moss	September 2000	I. Dykyy	DZL
rain forest, ferns	soil	1989		MIZ
	sandy soil		L. Jankiewicz	MIZ
Pistaceo calliprini typicum	soil	February 1999		MNH
fern-sedge zone	soil	May 1991	K. Desender	INB
coniferous forest (*Pinus sylvestris*)	humus	September 1999	B. Sohlenius	DZL, NRS
coniferous forest (*Pinus sylvestris*)	moss	June 2000	O. Holovachov	DZL, NRS
	moss and rotting wood	May 1999	A. Esquivel	INB
Carpinus betulus	rotting wood	February 2000	O. Holovachov	DZL
rain forest	soil, org. material, moss	November 1994	B. Gustafsson	DZL, NRS
	moss	September 1993	S. Šlipinski	MIZ
			H. van Rossen	LNW
moorland	moss	July 1985	G. Rudbäck	NRS
steppe cereals	soil	September 1998	O. Holovachov	DZL
decidious forest, (*Fagus silvatica*)	moss	April 2000	O. Holovachov	DZL
decidious forest, (*Betula* sp.)	litter	September 2000	I. Dykyy	DZL
pines and *Yucca brevifolia*	soil and litter	July 2001	W. Bert	DZL
Rhizosphere of *Larea tridentata*	cultured on agar	May 2002	I.T. De Ley	UCR
			L. Jankiewicz	MIZ
	red dry soil	July 1998	M. Drygant	DZL
grassland				LNW
				LNW
grassland		January 1990	D. Sturhan	IFN
			L. Jankiewicz	MIZ
	litter	November 1985	L. Jankiewicz	MIZ
		November 1985	L. Jankiewicz	MIZ
			L. Jankiewicz	DZL
Pisonia forest	soil	February 1988	A. Coomans	MVD
secondary forest with guava trees	soil	February 1988	A. Coomans	MVD
moister patch in arid zone	soil	February 1988	A. Coomans	MVD
secondary forest	litter	May 2000	R. Gómez	INB
	sand and litter	October 2000	C. Erséus	NRS

colleagues, or came from and are now kept in the nematode collections of the following institutions: Laboratorium voor Nematologie, Wageningen Universiteit, the Netherlands (LNW in the Table 4.1); Naturhistoriska riksmuseet, Stockholm, Sweden (NRS); Museum i Instytut Zoologii, Warszawa, Poland (MIZ); Instituto Nacional de Biodiversidad, Santo Domingo de Heredia, Costa Rica (INB), Museum voor Dierkunde, Universiteit Gent, Belgium (MVD), Institut für Nematologie, Biologische Bundesanstalt, Münster, Germany (IFN); State Museum of Natural History, Lviv, Ukraine (MNH); Department of Zoology, Ivan Franko National University of Lviv, Ukraine (DZL); Department of Nematology, University of California, Riverside, USA (UCR). Specimens were extracted by a range of methods: Baermann funnel extraction, decanting and sieving method with final separation on extraction sieves with filters, and centrifugal flotation. Nematodes were relaxed by gentle heat, fixed in cold TAF or 4% formaldehyde, processed to pure glycerin by a slow evaporation method or a rapid method according to Seinhorst (1959) and mounted on permanent slides in glycerin with paraffin wax as support for the coverslip.

Coiled and curved structures were measured along the median line. Stoma length was measured from the mouth opening to the pharyngeal radial tubuli. Amphid location was measured from the mouth opening to the anterior margin of the amphidial fovea. Neck length was measured from the tip of labial structures to the base of cardial bulb (excluding cardia). Measurements are given in the format mean ± standard deviation and range where appropriate.

Specimens of *Ereptonema arcticum* from the USA (population XIX') were cultured on 1% water agar with 5 µL /ml cholesterol in petri dishes at 17°C and fed with drops of Escherichia coli OP50.

For SEM studies specimens of *Wilsonema otophorum* (Ukraine = populations I, II), *W. schuurmansstekhoveni* (Sweden = population VIII, Ukraine = population from the Roztochya Natural Reserve), and *Ereptonema arcticum* (Ukraine = population XVII, Russia = population XVIII) were processed as described in Holovachov et al. (2001) and examined in an Hitachi S-4300 SEM at an accelerating voltage of 10 kV. Specimens of *E. arcticum* from the USA (population XIX') were processed for SEM as described in De Ley et al. (1999) and observed with a JEOL 35 Scanning Electron Microscope at 5 kV or an XL30-FEG Phillips 35 Scanning Electron Microscope at 10 kV. Specimens of *Neotylocephalus inflatus* (Galápagos = populations XXIX, XXXI and one specimen from the sample # 2 in De Ley, Loof & Coomans, 1993) were prepared for SEM following the method described in De Ley & Coomans (1997) and observed with a JEOL LSM-840 at 10kV.

A phylogenetic analysis was initially attempted by manual experimentation with character sets and cladogram construction. A preferred character matrix was then selected and entered in MacClade 4.02 (Maddison & Maddison, 2000) for further editing: all multistate characters were recoded as multiple binary characters, and we added two species of Plectus presumed to be morphologically similar to the exclusive common ancestor of wilsonematids (Table 4.7). Next, we analysed the dataset with exhaustive search of the maximum parsimony algorithms implemented in PHYLIP 3.2 (Felsenstein, 1989) and PAUP*4,0b10 (Swofford, 2002), assigning *P. cryptoptychus* De Ley & Coomans, 1994 as outgroup taxon. The resulting trees were imported into MacClade 4.02 for character tracing, and the single best tree was exported as a graphics file (Fig. 4.10). We also analysed a slight modification of the matrix, in which character 12 was interpreted slightly differently and encoded as an autapomorphy for *T. auriculatus*. We furthermore investigated alternative codings of character 16, respectively by considering only long flabella apomorphic, or by splitting this character into two separate ones (flabella absent *vs* present as well as flabella absent-short vs long).

Type specimens of the following species were studied: *Ereptonema arcticum* Loof, 1971 (slides # WT-1145, WT-1149, WT-1157 deposited in the Laboratorium voor Nematologie, Wageningen Universiteit, the Netherlands = population XIV), *Ereptonema inflatum* Yeates, 1967 (slides # 667 and 668 deposited in the National Nematode Collection of New Zealand, Auckland, New Zealand), *Spatiocephalus venustrus* Patil & Khan, 1982 (slide # 1279 deposited in the National Nematode Collection, Division of Nematology, Indian Agricultural Research Institute, New Delhi, India), and *Coronacephalus indicus* Ganguly & Khan, 1986 (slide # 1673 deposited in the National Nematode Collection, Division of Nematology, Indian Agricultural Research Institute, New Delhi, India).

Terminology

Since the lip region of these nematodes is rather complex and includes several taxonomically important structures, we should clarify our usage of the following terminology (see also Fig. 4.1):

"Cervical expansion" (Anderson, 1966) = middorsal and midventral expansions of the cervical peripheral cuticle. Cervical expansions are annulated when transverse striae are prominent, visible under the LM, continue to the body annulation on the lateral sides of the anterior end (cf. *Tylocephalus, Ereptonema*). Cervical expansions are non-annulated (smooth) when transverse striations are absent or very fine (discernible only under SEM), do not continue to the body annulation on the lateral sides of the anterior end.

"Cornu" (Anderson, 1966) = strongly modified cephalic seta with leaf-shaped appearance and smooth or incised sublateral edge, extending forward and inward, towards the mouth opening. Syn.: "appendage" (Andrássy, 1986), "Lappen" (Zell, 1985), "perioral flap" (Sauer & Annells, 1982; Boström, 1988).

"Flabellum" (Anderson, 1966) = fan- or feather-shaped dorsal or ventral extension of the peripheral cuticle. The term "cornu" was erroneously used by Boström (1988) for the short flabella found in *Ereptonema*.

"Lateral rim" (Anderson, 1966) = anterior edge of the peripheral cuticle on either lateral side.

"Median ridge" (De Ley & Coomans, 1997) = each pair of subdorsal and subventral lips strongly modified and fused to form a ridge.

"Midlateral projection" = modified lateral lip, elongate oval to digitate in shape, supported by the ridge and carrying outer labial sensillum apically.

"Quadrant" (De Ley & Coomans, 1997) = one of two annulated or smooth sublateral extensions of the surface of each lateral lip, i.e. one half of each subdivided lateral rim in *Tylocephalus*.

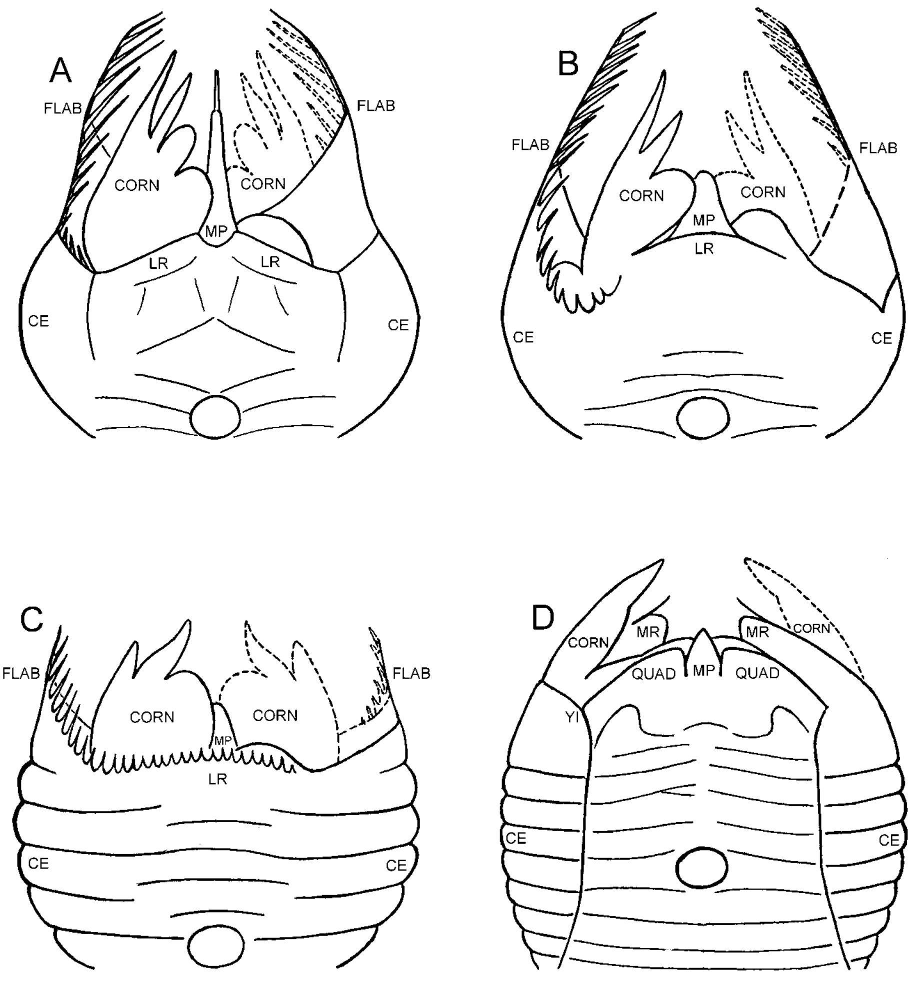

Figure 4.1. Scheme of the labial region structure of *Wilsonema* Cobb, 1913 (A), *Neotylocephalus* Ali, Farooqui & Tejpal, 1969 (B), *Ereptonema* Anderson, 1966 (C) and *Tylocephalus* Crossman, 1933 (D). Cornua and flabellae drawn on the right half of each figure are those on the body side not facing the viewer. Abbreviations: CE – cervical expansion; CORN – cornu; flab – flabellum; LR – lateral rim; MP – midlateral projection; MR – median ridge; QUAD – quadrant; RR – radial ridge; YI – Y-shaped incisure. Not to scale.

"Perioral cuticle" = cuticle of the lip region that immediately surrounds the mouth opening; it usually faces anteriad and is often demarcated from the surrounding cuticle by prominent rims, ridges, incisures and processes such as flabella and cornua.

"Peripheral cuticle" = cuticle of the lip region that is flush with rest of the body cuticle and is not separated from the latter by particular ridges, rims or incisures.

"Radial ridge" = ridge extending over the perioral cuticle from the inner side of each flabellum to the mouth opening (cf. analogous structures in Cephalobidae, e.g. Rashid et al., 1989).

"Y-shaped incisure" (De Ley & Coomans, 1997) = short incisure originating in the axils at the base of the cornu and separating each quadrant from the adjacent cervical expansion.

Species descriptions

Wilsonema otophorum (de Man, 1880) Cobb, 1913

(Figs 4.2 & 4.3,Tables 4.1 & 4.2)

Female: Body small, fusiform, ventrally curved upon fixation. Cuticle thin, annulated. Lateral field 2.5-4.0 µm wide at midbody, consisting of two separate wings divided by striated cuticle, fading anteriorly at level of pharyngeal corpus and ending as a single wing near tail terminus. Deirid setiform, close to excretory pore, inside the lateral field. Pharyngeal region with six (three pairs) somatic setae distributed as follows (n=28): one subventral pair and one subdorsal pair just posterior to the cervical expansions, and one ventrosublateral pair slightly ventral to the lateral field at the level of the middle of corpus. Body furthermore with four-six somatic setae (n=19): one dorsosublateral pair at the level of or posterior to cardia and three-four setae distributed between vulva and anus – one left ventrosublateral (sometimes missing), one right dorsosublateral, one left dorsosublateral and one right ventrosublateral (sometimes missing). Anterior end with pronounced bilateral and dorsoventral symmetry. Cervical cuticular expansions bulbiform, without annulation. Each expansion extends forward into one long median fimbriate flabellum. Each flabellum is supported by the arcuate radial ridge of the perioral cuticle that extends from the oral aperture towards the middle of the flabellum or somewhat anteriorly. Each flabellum carrying long and slender fimbriae, which are symmetrically located on both sides of the flabellum and directed inward and somewhat forward. Fimbriae only faintly visible under LM. Each lateral sector carrying a pair of sublateral cornua and a midlateral projection set between the lateral rim and mouth opening. Cornua "triangular", having a wide base, with their facing rims divided into four or rarely five tines by deep incisures. The innermost two-three tines are broadly triangular, while the outermost two tines are elongate. Midlateral projection of lateral lip 4-6 µm long and slender, finger-like, extending well forward and reaching the tips of cornua, with a papillar terminus and two small flaps (discernible under SEM) supported by a fine adoral ridge. Each midlateral projection has a small terminal opening and contains a nerve ending. Subventral and subdorsal lips obscure under SEM. They apparently have a nerve ending each, probably corresponding to the outer labial sensilla. Lateral rims somewhat expanded, without fimbriae. Peripheral cuticle on the lateral sides of the anterior end with only few incomplete transverse striae, its posterior part ending near amphid. Amphidial aperture 2.0-3.5 µm wide, circular, located about halfway

Table 4.2. Measurements (in µm) of different populations of *Wilsonema otophorum* (de Man, 1880) Cobb, 1913

Country	Ukraine			Russia	N. Caledonia	Mexico	Israel	Ecuador
Population #	I		II	III	IV	V	VI	VII
	Females	Males	Females	Females	Females	Females	Females	Female
n	12	8	15	12	2	2	2	1
Body length	324±14.8 (303-360)	279±14.6 (251-302)	300±12.6 (282-311)	332±26.8 (284-357)	250; 265	269; 252	305; 264	250
Body diameter	18.1±0.9 (17-19.5)	15.1±0.4 (14.5-15.5)	18.5±1.5 (16-20.5)	23.4±2.1 (21-25.5)	14.5; 15	15; 16	17.7; 15.2	18
Neck length	92.4±3.8 (87.5-102)	82.3±6.5 (69-90)	82.3±2.1 (78-86.5)	94.4±8.3 (81-106)	75; 81	86; 88	87.7; 77.7	75
Tail length	39.1±1.5 (37-41.5)	38.6±2.2 (36-41.5)	38.7±3.4 (32-44)	32.9±2.7 (26.5-37)	29; 33	29; 30	39.6; 37.5	29
ABD	9.9±0.6 (9-10.5)	12.9±0.7 (12-14)	9.9±0.6 (9-11)	10.2±0.7 (9-11.5)	8; 9	8; 10	10.0; 8.8	8.5
a	18.0±1.3 (15.7-20.8)	18.5±1.3 (17.8-20.9)	16.3±1.5 (14.2-19.4)	14.3±1.1 (12.8-16.9)	17.2; 17.4	18.3; 18.1	17.2; 17.4	14.0
b	3.5±0.1 (3.3-3.7)	3.4±0.2 (3.1-3.6)	3.7±0.2 (3.4-3.9)	3.5±0.1 (3.3-3.8)	3.3; 3.3	3.1; 3.3	3.5; 3.4	3.3
c	8.3±0.3 (7.7-8.7)	7.3±0.4 (6.4-7.7)	7.8±0.5 (7.1-8.8)	10.1±0.7 (8.8-11.0)	8.6; 8.1	9.4; 9.8	7.7; 7.0	8.6
c’	4.0±0.3 (3.7-4.5)	3.0±0.2 (2.6-3.2)	3.9±0.4 (3.5-4.5)	3.2±0.4 (2.6-3.9)	3.7; 3.7	3.5; 3.0	4.0; 4.3	3.4
Flabella length	8.0±0.8 (7-9)	8.2±1.1 (6-9.5)	7.7±0.6 (6.5-8.5)	8.3±0.8 (7-9)	7.5; 7.5	9; 9	8; 9	10
Expansion length	8.0±0.6 (7-9)	7.2±0.3 (6.5-8)	7.3±0.9 (6-9)	9.6±1.3 (7-11.5)	7; 7	6.5; 7.5	6.5; 7	5.5
Expansion width	18.1±0.8 (17-20)	17.4±0.5 (16.5-18)	17.4±0.9 (15.5-19)	18.5±0.7 (17-19.5)	15; 15.5	15.5; 14.5	16.4; 17.8	18
Stoma length	12.3±0.8 (11.5-14)	11.4±1.2 (10-14)	11.5±0.8 (10-13)	13.6±1.1 (11.5-15)	13.5; 13	13; 13	11; 11	9
Amphid location	7.4±0.6 (7-8)	6.3±0.9 (5-7)	7.9±0.8 (6.5-9)	7.3±0.9 (6-8)	7; 7	8; 8	6; 5.5	6.5
Rectum or spicula	10.6±1.6 (8-13)	14.1±1.6 (12-16.5)	10.3±1.0 (8.5-12)	11.8±1.1 (10.5-14)	10; 9	11.5; 10	11.5; 10	9
R/ABD	1.1±0.2 (0.8-1.4)	—	0.9±0.1 (0.8-1.1)	1.2±0.2 (0.9-1.4)	1.2; 1.0	1.4; 1.0	1.2; 1.1	1.1
Vagina	5.6±0.5 (4.5-6)	—	5.4±0.5 (5.0-6.5)	5.5±0.7 (4.5-7)	7; 6	7.5; 5	7; 6	5
V, % or T, %	50.2±1.2 (49.1-53.6)	50.3±3.1 (47.0-54.3)	49.3±1.2 (47.7-51.3)	52.9±1.1 (50.4-55.0)	52.3; 52.8	52.1; 51.4	48.9; 49.9	50.0
G1, %	9.0±1.5 (6.7-11.8)	—	11.1±2.3 (8.5-14.9)	14.1±1.6 (10.5-16.2)	?	?	?; 6.4	18.0
G2, %	12.0±1.8 (8.4-15.0)	—	12.6±4.0 (8.0-17.7)	12.0±1.6 (9.0-14.2)	?	?	?; 10.9	14.0

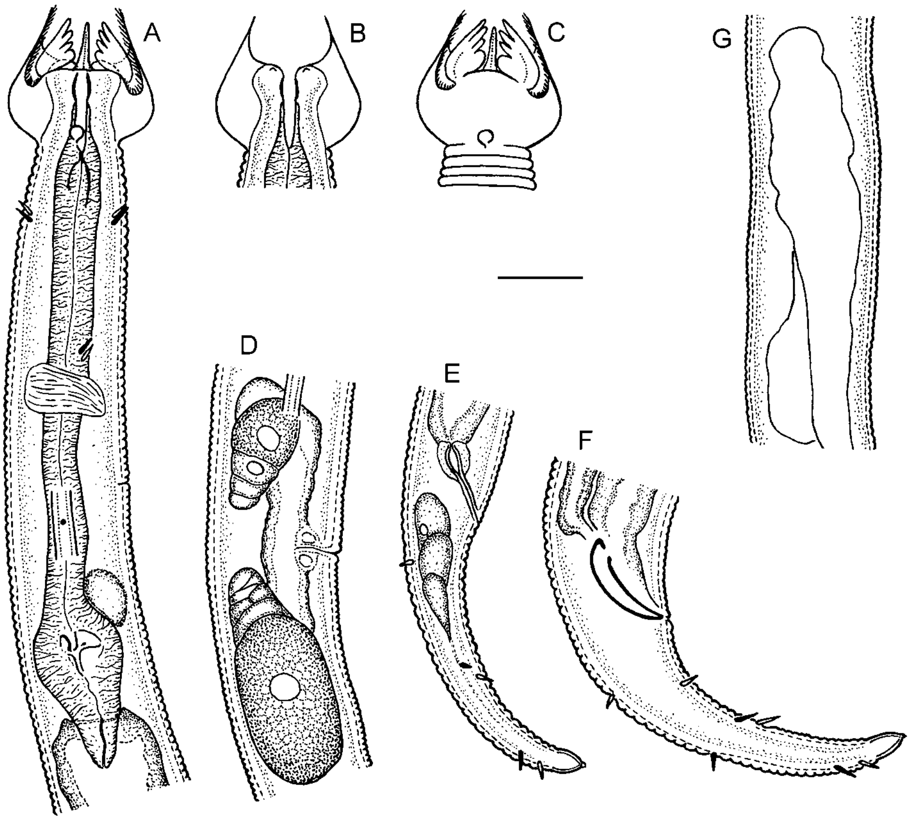

Figure 4.2. *Wilsonema otophorum* (de Man, 1880) Cobb, 1913. Population from Ukraine (I). A-E: Female. A: Pharyngeal region; B: Labial region, lateral median view; C: Labial region, lateral surface view; D: Reproductive system; E: Tail. F-G: Male. F: Caudal region; G: Proximal part of reproductive system. Scale bar 10 µm. Black setae are located on the side of the body facing the viewer and white setae are located on the opposite side.

of stoma. Stoma plectoid. Pharyngeal corpus cylindrical, plump, radial tubules present. Isthmus plump, surrounded by the nerve ring at its anterior end and by the secretory-excretory gland cell at its posterior end. Basal pharyngeal bulb oval, with valves. Cardia embedded by intestinal tissue. Excretory gland duct weakly cuticularized. Excretory pore posterior to the nerve ring. Female reproductive system didelphic, amphidelphic, ovary branches reflexed. Vulva equatorial, transverse. Vagina short, encircled by sphincter muscle. Epiptygmata absent. Among 30 females, the anterior genital branch located on the right side of the body in 27 and on the left side of the body in three specimens, the posterior genital branch located on the right side of the body in 11 and on the left side of the body in

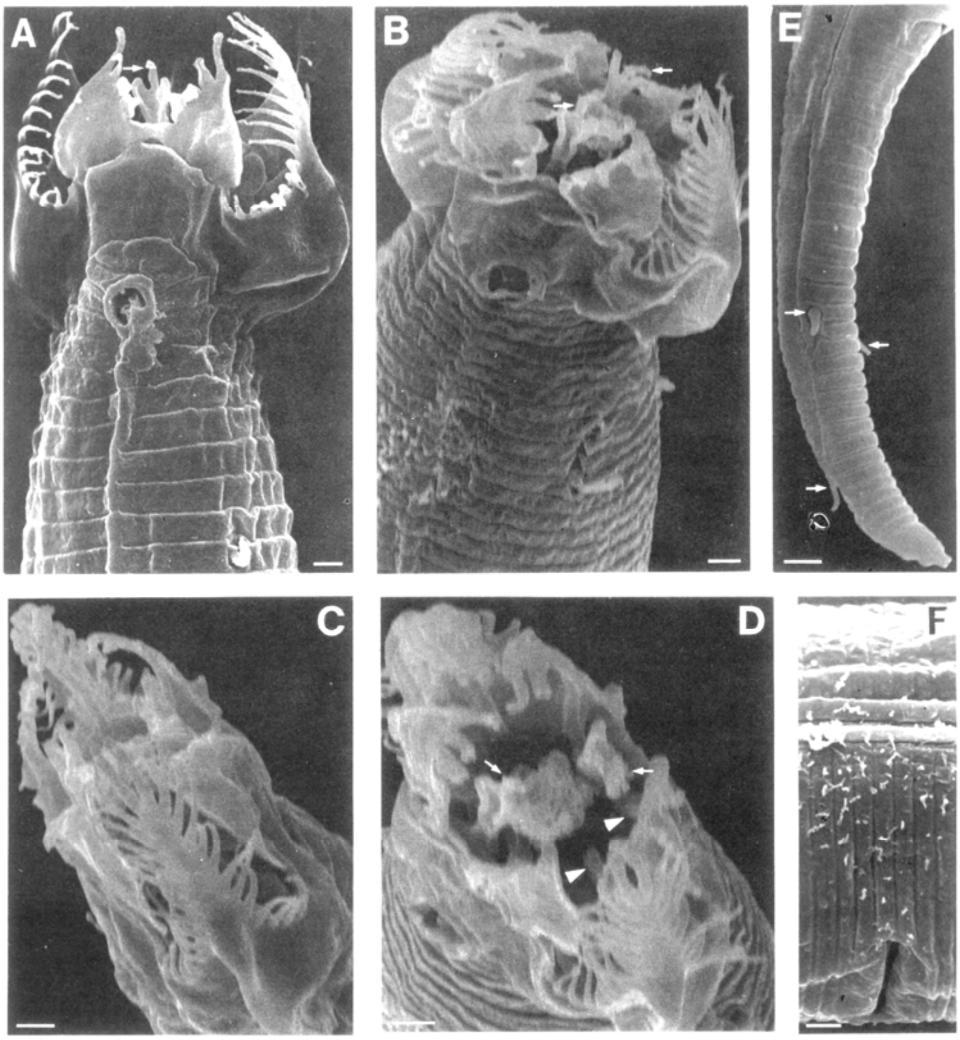

Figure 4.3. *Wilsonema otophorum* (de Man, 1880) Cobb, 1913. Females only. Population from Ukraine (I). A: Labial region, lateral view (arrow points at midlateral projection); B: Labial region, oblique view (arrows point at midlateral projections); C: Labial region, dorsal view; D: Labial region, apical view (arrows point at midlateral projection, arrowheads point at radial ridges); E: Tail (arrows point at caudal setae); F: Vulva and lateral field. Scale bars: A-D, F = 1 µm; E = 2 µm.

19 specimens. All combinations present except anterior genital branch on the left and posterior genital branch on the right side in the same female. Intrauterine egg (n=11) measuring 46-60×13-21 µm, each egg 2.4-4.0 times longer than its own diameter. Rectum

short and stout, 0.8-1.4 times anal body diameter long. Tail gradually narrowing, arcuate ventrad, with five caudal setae (n=26) distributed as follows: a single subdorsal seta on the left side of the tail at anterior one-fifth of the tail length, one subventral pair at anterior one-third of the tail (at ca 12 annuli from anus), and one subdorsal pair (including a spur at 4-9 µm from tail tip on the left side of the body) located at 14-25% of the tail length from tail terminus. Caudal glands present.

Male: General morphology similar to female except sexual characters. Body strongly curved ventrad, more so in posterior part. Setae pattern on the neck equal to female. Testes two, opposed, poorly developed: anterior one straight, 23-35 µm long, posterior one reflexed, 29 µm long. Spermatocytes not discernible. Numerous rounded spermatozoa were found in the proximal part of the testes of a single male. Spicules slightly asymmetrical, arcuate ventrad. Gubernaculum absent. Midventral precloacal tubular supplements or precloacal setiform sensillum absent. Posterior body portion with 4-7 subventral setae (n=5) arranged in two rows: 2-4 setae on the left and 2-4 setae on the right side of the body. Two males also had two subdorsal setae on each side of the body anterior to cloaca. Tail with 4-8 caudal setae: 2-4 subventral setae and 2-4 subdorsal setae, variously arranged, but the posteriormost pair always subdorsal.

Diagnosis: Cervical expansions non-annulated, each extends into a single long median fimbriate flabellum. Cornua each with four tines. Midlateral projection long, fingerlike. Lateral rims not fimbriate. Pharyngeal region with six somatic setae. Tail with five caudal setae. Rectum about one anal body diameter long.

Remarks: A single male was infested by two *Nannomermis*-like parasites. Males of *W. otophorum* were previously described by Šlepetene (1962), Anderson (1966), and Mateo & Campoy (1983).

Wilsonema schuurmansstekhoveni (De Coninck, 1931) Zell, 1985

(Figs 4.4 & 4.5, Tables 4.1 & 4.3)

Female: Body small, fusiform, ventrally curved upon fixation. Cuticle thin, annulated. Lateral field 3-4 µm wide at midbody, consisting of two separate wings divided by striated cuticle, starting on 10th-15th annulus from base of cervical expansions (in one female one wing starts on 11th and the other on 15th annulus) and ending as a single wing near tail terminus. Deirid setiform, close to excretory pore, inside the lateral field. Pharyngeal region with four (two pairs) somatic setae distributed as follows (n=17): one subdorsal pair 5-11 annuli from the base of cervical expansions, and one ventrosublateral pair 25-30 annuli from base of cervical expansion, slightly ventral to lateral field, at the level of the middle of corpus. Body furthermore with three-four somatic setae (n=14): one dorsosublateral pair (in one female only one setae on the right side) at the level of or posterior to the cardia and one dorsosublateral seta on each side of the body between vulva and anus (in one specimen only one seta on the right side). Anterior end with pronounced bilateral and dorsoventral symmetry. Cervical cuticular expansions bulbiform, without annulation. Each expansion extends forward into one long median fimbriate flabellum.

Table 4.3. Measurements (in µm) of different populations of *Wilsonema schuurmansstekhoveni* (De Coninck, 1931) Zell, 1985

Country	Sweden	Ukraine	Costa Rica	Poland	Malaysia	Australia
Population #	VIII	IX	X	XI	XII	XIII
	Females	Females	Females	Females	Females	Females
n	9	10	5	6	4	5
Body length	308±10.7 (294-324)	320±20.3 (292-354)	286±10.4 (272-296)	316±12.6 (308-341)	280 (246-314)	295±17.1 (268-312)
Body diameter	17.9±1.6 (15.5-21)	21.8±4.0 (18.5-32)	17.5±1.0 (16-18.5)	19.4±1.4 (17-21)	15.0 (13-17)	19.8±1.1 (18.5-21)
Neck length	90.7±2.5 (86.5-94.5)	89.2±7.1 (81-101.5)	80.3±5.1 (72.5-85)	86.9±5.2 (81-96)	91.1 (84-97)	86.0±5.0 (78-91)
Tail length	24.0±1.5 (21-25.5)	24.1±2.4 (18.5-26.5)	24.2±1.2 (23-25.5)	24.4±2.4 (22-28)	20.2 (14-25.5)	24.4±3.0 (20-27.5)
ABD	7.3±0.6 (7-8)	7.4±0.8 (5.5-8)	7.8±1.0 (7-9)	7.1±0.5 (7-8)	6.3 (6-6.5)	7.8±1.0 (6.5-9)
a	17.3±1.1 (15.6-18.9)	15.0±1.8 (10.5-16.9)	16.3±0.8 (15.7-17.8)	16.3±1.0 (14.9-17.8)	18.8 (17.5-20.2)	15.0±1.0 (14.0-16.2)
b	3.4±0.1 (3.2-3.7)	3.6±0.1 (3.4-3.8)	3.6±0.1 (3.4-3.7)	3.6±0.1 (3.5-3.8)	3.1 (2.9-3.3)	3.4±0.1 (3.4-3.6)
c	12.9±1.0 (11.6-14.4)	13.4±1.8 (11.5-17.9)	11.8±1.0 (10.7-12.9)	13.0±1.2 (11.4-14.2)	14.3 (12.4-18.5)	12.2±0.8 (11.2-13.3)
c’	3.3±0.4 (2.9-3.7)	3.3±0.5 (2.7-4.4)	3.1±0.4 (2.8-3.7)	3.4±0.4 (3.0-4.0)	3.2 (2.0-4.0)	3.2±0.5 (2.5-3.6)
Flabella length	11.8±0.8 (10.5-13)	10.5±0.9 (9-11.5)	10.8±0.6 (10.5-11.5)	9.2±0.7 (8-10.5)	9.8 (8-11.5)	10.6±1.3 (9-12)
Expansion length	8.8±1.0 (8-10.5)	8.5±0.6 (8-9)	8.1±0.8 (7-9)	7.3±0.6 (7-8)	7.5 (7-8)	7.4±1.3 (6-9)
Expansion width	18.8±0.8 (17-19.5)	19.1±0.8 (17-19.5)	18.5	18.0±0.8 (17-18.5)	16.1 (15-17)	17.6±1.0 (16.5-19)
Stoma length	11.7±0.7 (10.5-13)	12.0±1.0 (10.5-14)	10.8±0.6 (10.5-11.5)	11.9±0.6 (11.5-13)	11.5 (10.5-13)	11.4±1.1 (10-13)
Amphid location	8.3±0.8 (7-9)	7.8±0.7 (7-9)	7.6±0.6 (7-8)	7.5±0.6 (7-8)	7.2 (7-8)	6.5±1.4 (5-8)
Rectum	26.5±1.2 (24-28)	24.3±1.9 (22-28)	21.9±2.6 (18.5-25.5)	24.0±1.7 (22-26.5)	21.6 (18.5-25.5)	22.5±3.3 (18-26.5)
R/ABD	3.7±0.2 (3.3-4.0)	3.4±0.6 (2.9-4.6)	2.8±0.6 (2.3-3.7)	3.4±0.3 (3.0-3.8)	3.4 (3.0-4.4)	2.9±0.5 (2.2-3.6)
Vagina	6.2±0.6 (6-7)	4.3±0.8 (3.5-6)	5.5±0.5 (4.5-6)	5.7±0.9 (4.5-7)	5.2 (4.5-6)	5.6±0.3 (5.5-6)
V, %	51.4±0.8 (50.2-52.3)	52.3±1.1 (50.8-54.2)	50.4±0.7 (49.6-51.4)	51.8±0.4 (51.4-52.4)	53.5 (52.5-54.5)	51.5±1.0 (50.1-52.9)
G1, %	9.1±0.8 (8.3-10.6)	13.3±2.2 (10.6-17.5)	9.0±0.7 (8.3-10.0)	12.2±1.9 (10.1-14.3)	11.0 (9.5-12.5)	11.8 (11.0-13.1)
G2, %	7.5±1.4 (4.9-9.6)	9.0±1.6 (6.7-11.9)	8.0±0.6 (7.2-8.6)	11.3±1.5 (9.9-13.5)	8.6 (8.0-9.2)	11.3 (10.3-12.6)

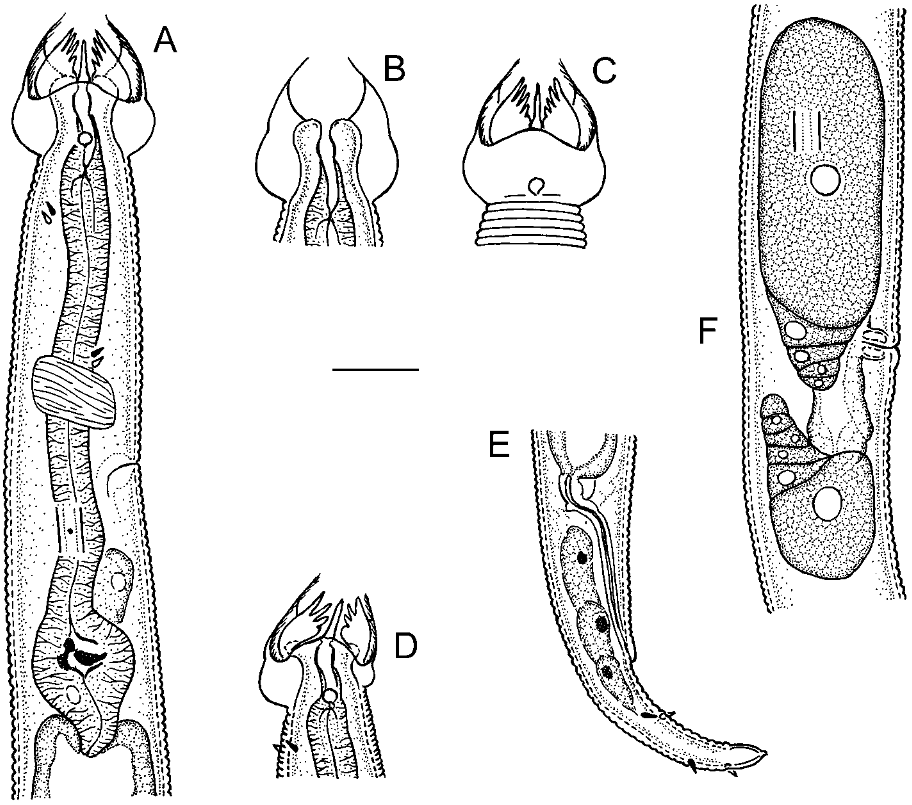

Figure 4.4. *Wilsonema schuurmansstekhoveni* (De Coninck, 1937) Zell, 1985. Females only. A-C, E-F: Population from Ukraine (IX). A: Pharyngeal region; B: Labial region, lateral median view; C: Labial region, lateral surface view; E: Tail; F: Reproductive system. D: Specimen from Korea with an aberrant structure of the anterior end. Scale bar 10 µm. Setae as in Fig. 4.2.

Each flabellum is supported by the arcuate radial ridge of the perioral cuticle that extends from the oral aperture towards the middle of the flabellum or somewhat anteriorly. Each flabellum carrying long and slender fimbriae, which are symmetrically located on both sides of the flabellum and directed inward and somewhat forward or with tips curved outward as in the Swedish (VIII) population. Fimbriae only faintly visible under LM. Each lateral sector carrying a pair of sublateral cornua and a midlateral projection set between the lateral rim and mouth opening. Cornua "triangular", having a wide base, with their facing rims divided into four tines by deep incisures. The innermost two tines are broadly triangular, while the outermost two tines are elongate to filiform (SEM). Midlateral projection of lateral lip 5-8 µm long and slender, finger-like, extending well forward and

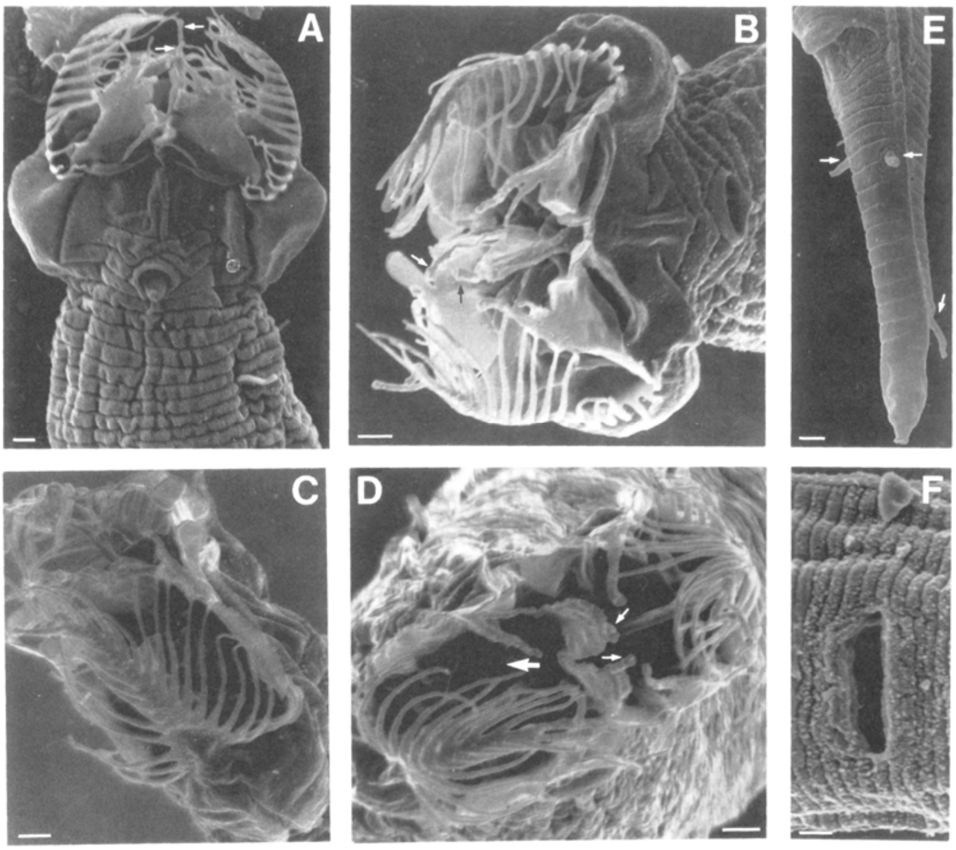

Figure 4.5. *Wilsonema schuurmansstekhoveni* (De Coninck, 1937) Zell, 1985. Females only. Population from Sweden (VIII). A: Labial region, lateral view (arrows point at midlateral projections); B: Labial region, oblique view (arrows point at midlateral projections); C: Labial region, ventral view; D: Labial region, apical view (small arrows point at midlateral projections, large arrow points at fused subventral lips); E: Tail (arrows point at caudal setae); F: Vulva. Scale bars: A-D, F = 1 µm; E = 2 µm.

reaching tips of cornua, with a papillar terminus (discernible under SEM and seen under LM in Swedish (VIII) and Costa Rican (X) specimens) supported by a fine adoral ridge. Each midlateral projection has a small terminal opening and contains a nerve ending. Subventral and subdorsal lips strongly reduced (SEM), they are probably the small flaplet-like expansions near the bases of each midlateral projection. They apparently have a nerve ending each, probably corresponding with the outer labial sensilla. Large flap-like expansions of the perioral cuticle in the axils between perioral flaps and median ridge of flabella provide an additional support to the median ridge and flabella. Lateral rims somewhat expanded, without fimbriae (smooth). Peripheral cuticle on the lateral sides of the anterior end with only few incomplete transverse striae, its posterior part ending near amphid. Amphidial aperture 2 µm wide, circular, located about halfway of stoma. Stoma plectoid. Pharyngeal corpus cylindrical, plump, radial tubules present. Isthmus plump, surrounded by the nerve ring at its anterior end and by the secretory-excretory gland cell at its posterior end. Basal pharyngeal bulb oval, with valves. Cardia embedded by intestinal tissue. Excretory gland duct weakly cuticularized, excretory pore posterior to the nerve ring. Female reproductive system didelphic, amphidelphic, ovary branches reflexed. Vulva equatorial, transverse. Vagina short, with oval lumen in the apical view, encircled by sphincter muscle. Epiptygmata absent. Among 29 females, the anterior genital branch located on the right side of the body in 27 and on the left side in two specimens, the posterior genital branch located on the right side of the body in 19 and on the left side in ten specimens. Intrauterine egg (n=3) measuring 52-55 x 17-20 µm, each egg 2.7-3.2 times longer than its own diameter. Rectum long, 2.2-4.6 times anal body diameter long. Tail gradually narrowing to almost cylindrical, straight or arcuate ventrad, with four paired and opposed caudal setae distributed as follows (n=18): one subventral pair located at anterior one-third of the tail (at ca 6 annuli from anus) and one subdorsal pair (including a spur at 6-8 µm from tail tip on the left side of the body) located at 20-25% of the tail length from tail terminus. Caudal glands present.

Male: Not found.

Diagnosis: Cervical expansions non-annulated, each extends into a single long median fimbriate flabellum. Cornua each with four tines. Midlateral projection long, fingerlike. Lateral rims not fimbriate. Pharyngeal region with four somatic setae. Tail with four caudal setae. Rectum two to four times longer than anal body diameter.

Remarks: Distribution of the ovaries in relation to the intestine and body axis is highly variable in different populations. Among nine females from the Swedish (VIII) population, the anterior ovary was always located on the right side, and the posterior on the left side in eight and on the right side in one female. Among six females from the Polish (XI) population, the anterior ovary was always located on the right side, the posterior on the left side in one and on the right side in five females. Among nine females from the Ukrainian (IX) population, the anterior ovary was located on the right side in seven and on the left side in two females, the posterior on the left side in five and on the right side in four females. Among five females from the Costa Rican (X) population, the anterior ovary was always located on the right side and the posterior always on the left side. Specimens from Sweden (VIII) had more prominent fimbriae, probably due to the quality of the material.

Ereptonema arcticum Loof, 1971

(Figs 4.6 & 4.7, Tables 4.1 & 4.4)

Female: Body small, fusiform, ventrally curved upon fixation. Cuticle thin, annulated. Lateral field 2.5-4.5 µm wide at midbody, consisting of two separate wings divided by striated cuticle, fading anteriorly at level of pharyngeal corpus and posteriorly on tail. Deirid setiform, close to excretory pore, inside the lateral field. Pharyngeal region

Table 4.4. Measurements (in µm) of different populations of *Ereptonema arcticum* Loof, 1971

Country	Spitzbergen		Ukraine		Russia	USA		Mexico
Population #	XIV	XV	XVI	XVII	XVIII	XIX	XIX’	XX
	Females	Female	Females	Females	Females	Females	Females	Females
n	8	1	8	6	6	6	20	4
Body length	301±15.5 (273-317)	346	306±17.1 (278-329)	282±20.2 (256-314)	357±15.9 (340-376)	305±9.6 (291-314)	368±20.5 (327-405)	249 (243-255)
Body diameter	17.4±1.0 (16.0-18.5)	21	21.0±1.0 (19-23)	18.7±2.1 (16.5-21.5)	21.7±1.9 (18.5-23)	19.6±1.1 (18-21)	22.4±1.7 (20-26)	15.9 (15-17)
Neck length	85.8±3.3 (81-90)	103	84.5±5.4 (74-89)	84.3±4.1 (79-90)	99.4±3.0 (96-104)	86.5±4.2 (81-92)	96.4±4.0 (88-104)	79.0 (77-83)
Tail length	36.7±1.7 (33.5-38)	43	39.9±3.9 (35-46)	29.2±2.5 (26-32)	39.2±2.4 (34.5-41.5)	34.4±1.2 (33-37)	39.1±2.3 (35-44)	28.8 (28-30)
ABD	8.8±0.9 (8-10.5)	11.5	10.3±1.0 (9-12)	8.6±0.5 (8-9.5)	10.2±0.5 (9-10.5)	10	11.6±0.9 (10-14)	8.4 (8-9)
a	17.2±0.9 (15.8-18.2)	16.5	14.6±0.6 (13.4-15.9)	15.1±1.0 (14.0-16.9)	16.5±1.3 (14.8-18.4)	15.6±0.8 (14.8-16.5)	16.5±1.4 (14.0-19.5)	15.8 (14.7-16.4)
b	3.5±0.1 (3.3-3.6)	3.4	3.6±0.3 (3.3-4.1)	3.3±0.1 (3.2-3.5)	3.6±0.1 (3.5-3.8)	3.5±0.2 (3.2-3.8)	3.8±0.1 (3.6-4.0)	3.2 (3.1-3.2)
c	8.2±0.3 (7.6-8.8)	8.1	7.7±0.5 (7.1-8.4)	9.7±0.6 (9.2-10.9)	9.1±0.5 (8.6-9.9)	8.9±0.5 (8.3-9.4)	9.4±0.5 (8.2-10.5)	8.7 (8.4-9.2)
c’	4.2±0.5 (3.6-4.7)	3.7	3.7±0.2 (3.6-4.2)	3.4±0.4 (3.0-3.9)	3.9±0.3 (3.3-4.4)	3.4±0.1 (3.3-3.7)	3.4±0.3 (2.9-4.0)	3.5 (3.1-3.7)
Flabella length	3.6±0.4 (3.5-4.5)	3.5	3.1 (2-3.5)	3.2±0.1 (3-3.5)	4.0±0.6 (3.5-4.5)	3.5±0.5 (3-4.5)	4.1±0.5 (3-5)	3.8 (3.5-4.5)
Expansion length	7.3±0.6 (7-8)	10.5	8.1 (8-9)	9.1±0.6 (8.5-10)	8.7±0.6 (8-9)	8.5±0.9 (7-9)	10.2±1.1 (9-13)	8.4 (8-9)
Expansion width	14.7±0.9 (14-16)	16	17.2±0.9 (16-18)	15.4±0.2 (15-15.5)	16.9±0.6 (16-17)	17.4±0.6 (17-18)	16.0±0.8 (14-17)	15
Stoma length	12.3±0.6 (11.5-13)	13	12.2±1.0 (10-13.5)	12.4±1.2 (11-14)	13.1±0.6 (13-14)	11.8±1.3 (10-13)	14.0±1.2 (12-16)	11.3 (10.5-13)
Amphid location	5.6±0.4 (4.5-6)	6	6.1±0.9 (5-7.5)	7.0±0.8 (6-8)	6.3±0.6 (6-7)	6.7±0.7 (5.5-8)	6.3±1.0 (4-8)	5.1 (4.5-6)
Rectum	10.2±0.8 (9-11.5)	11.5	10.9±0.5 (10-12)	9.0±1.5 (8-12)	9.6±0.9 (9-11.5)	10.4±0.6 (10-11)	10.2±1.4 (8-13)	8.9 (8-9)
R/ABD	1.2±0.2 (0.9-1.4)	1	1.1±0.1 (0.9-1.2)	1.1±0.2 (0.9-1.4)	0.9±0.1 (0.9-1.1)	1.0±0.1 (1.0-1.1)	0.9±0.1 (0.6-1.1)	1.1 (1.0-1.1)
Vagina	6.1±0.9 (4.5-7)	8	5.9±0.7 (5-7)	4.2±0.8 (3-5)	5.0±1.3 (3.5-6)	6.9±0.5 (6.5-8)	7.5±0.9 (6-9)	5.2 (4.5-6)
V, %	51.5±0.8 (50.2-52.4)	51.3	51.0±2.4 (46.2-54.1)	54.0±0.4 (53.5-54.7)	54.0±1.0 (52.6-55.1)	53.7±2.6 (50.4-58.4)	51.7±1.1 (48.7-53.1)	53.8 (53.2-54.7)
G1, %	9.7±0.7 (8.7-10.7)	7.3	11.9±0.2 (8.4-16.0)	13.0±2.6 (10.4-17.4)	11.9±2.0 (8.5-13.9)	7.7±1.2 (5.6-8.8)	17.6±4.4 (10.4-27.0)	8.9 (7.6-10.0)
G2, %	8.6±0.9 (6.7-9.4)	8.0	11.6±1.5 (9.2-13.7)	12.1±1.1 (10.8-13.5)	11.1±1.2 (9.8-12.8)	8.1±1.6 (6.0-10.0)	19.2±3.4 (12.3-26.8)	8.6 (7.7-9.5)

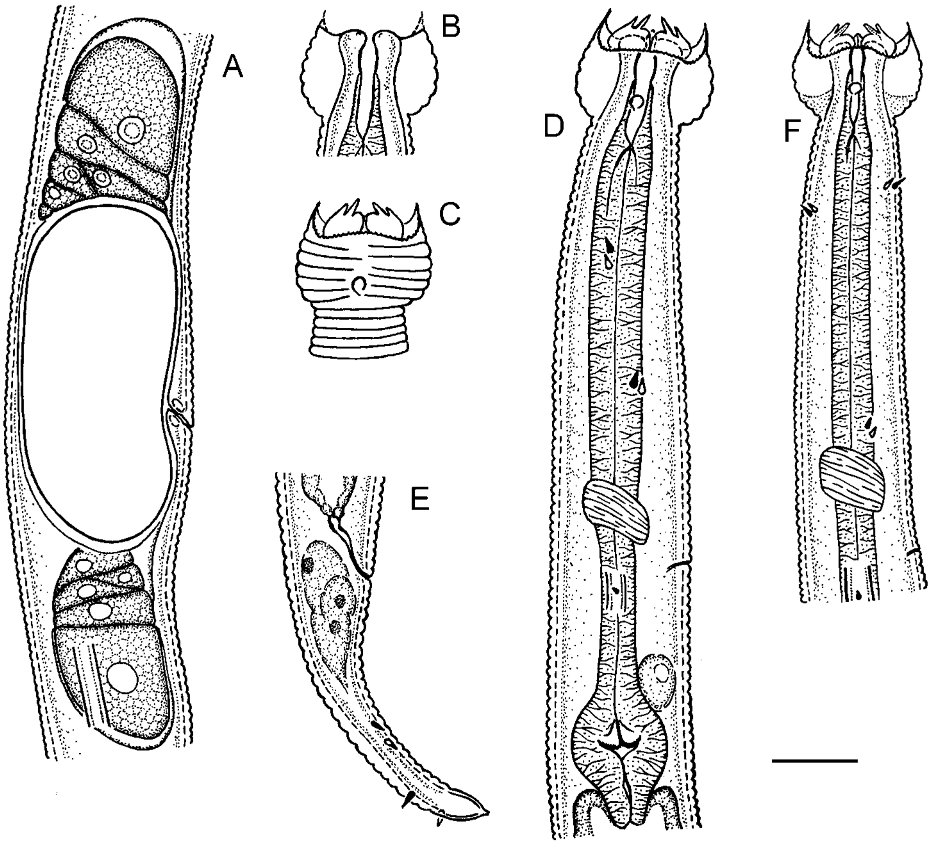

Figure 4.6. *Ereptonema arcticum* Loof, 1971. Females only. A-D: Population from Russia (XVIII). A: Reproductive system; B: Labial region, lateral median view; C: Labial region, lateral surface view; D: Pharyngeal region; E: Tail; F: Population from Ukraine (XVII). Anterior body region. Scale bar 10 μm. Setae as in Fig. 4.2.

with four (two pairs) somatic setae distributed as follows (n=14): one subdorsal pair just posterior to the cervical expansions, and one ventrosublateral pair slightly ventral to the lateral field at the level of the middle of corpus in nine females (USA-XIX, and Norway-XIV and XV), or one subdorsal pair plus a single right subventral setae just posterior to the cervical expansions and one ventrosublateral pair slightly ventral to the lateral field at the level of the middle of corpus in one female (Mexico-XX), or one subdorsal pair and one subventral pair just posterior to the cervical expansions, and one ventrosublateral pair slightly ventral to the lateral field at the level of the middle of corpus in four females (Ukraine-XVI). Other somatic setae present but obscure. Anterior end with pronounced

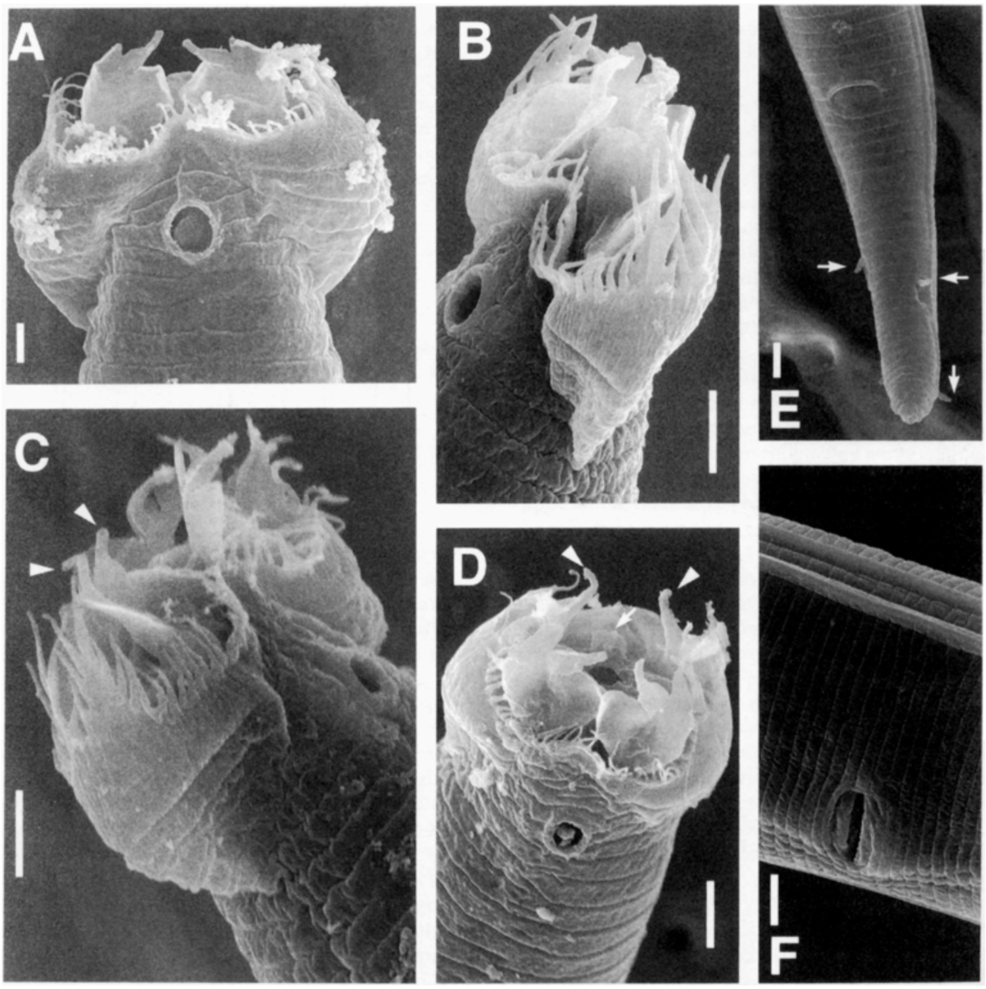

Figure 4.7. *Ereptonema arcticum* Loof, 1971. Females only. A: Population from Ukraine (XVII). Labial region, lateral view. B-F: Population from USA (XIX'). B: Labial region, oblique view; C: Labial region, subventral view (arrow heads point at nerves in cornua); D: Labial region, apical view (arrow points at midlateral projection; arrow heads point at nerves in cornua); E: Tail (arrows point at caudal setae); F: Vulva and lateral field. Scale bars: A = 1 µm; B-F = 2 µm.

bilateral and dorsoventral symmetry. Cervical cuticular expansions bulbiform, with 4-6 transverse striations. Each expansion extends forward into two sagittal small flabella, which are hardly discernible from and slightly longer than the adjacent fimbriae. Each flabellum is supported by the arcuate radial ridge of the perioral cuticle that extends from the oral aperture towards the middle of the flabellum. Each flabellum carrying long and slender fimbriae, which are symmetrically located on both sides of the flabellum and directed forward. Anterior margin of each cervical expansion as well as the lateral rims carrying fimbriae, which are somewhat shorter than fimbriae on flabella and also directed forward. They are only faintly visible under the LM. Each lateral sector carrying a pair of cornua and midlateral projection set between the lateral rim and mouth opening. Cornua "ovoid", having a narrow base, with their facing rims divided into three tines by deep incisures. The middle tine is the longest and contains a nerve ending. Midlateral projection of lateral lips 1-2 µm long, small and rounded, extending forward and reaching at least half the length of cornua. Each midlateral projection has a small terminal aperture and contains a nerve ending. Subventral and subdorsal lips obscure. They apparently have a nerve ending each, probably corresponding with the outer labial sensilla. Peripheral cuticle on the lateral sides of the anterior end with prominent transverse striations continuous with the expansions' striations. Amphid aperture 2.0-3.5 µm wide, circular, located halfway of stoma. Stoma plectoid. Pharyngeal corpus cylindrical, plump, radial tubules present. Isthmus plump, surrounded by the nerve ring at its anterior end and by the secretory-excretory gland cell at its posterior end. Basal pharyngeal bulb oval, with valves. Cardia embedded by intestinal tissue. Excretory gland duct weakly cuticularized, excretory pore posterior to the nerve ring. Female reproductive system didelphic, amphidelphic, ovary branches reflexed. Vulva equatorial, transverse. Vagina short, encircled by sphincter muscle. Epiptygmata absent. Among 31 females the anterior genital branch located on the right side of the body in all specimens and posterior genital branch located on the left side of the body in 29 and on the right side in two specimens. Intrauterine egg (n=6) measuring 37-42×16-20 µm, each egg 2.1-2.7 times longer than its own diameter. Rectum short and stout, about one anal body diameter long. Tail gradually narrowing, arcuate ventrad, with four paired and opposed caudal setae distributed as follows (n=19): one subventral pair located about halfway of the tail and one subdorsal pair (including a spur at 3.5-6.0 µm from tail tip on the left side of the body) located close to tail terminus. Caudal glands present.

Male: Not found.

Diagnosis: Cervical cuticular expansions annulated, each extends into two short submedian fimbriate flabella. Cornua each with three tines, middle one clearly longer. Midlateral projection short, oval. Lateral rims fimbriate. Pharyngeal region with four-six somatic setae. Tail with four caudal setae. Rectum about one anal body diameter long.

Neotylocephalus annonae Ali, Farooqui & Tejpal, 1969

(Figs 4.8 A, B, E-G, I, K, Tables 4.1 & 4.5)

Female: Body small, fusiform, ventrally curved upon fixation. Cuticle thin, annulated. Lateral field 2.5-4.5 µm wide at midbody, consisting of two separate wings divided by striated cuticle, fading anteriorly at level of pharyngeal corpus and posteriorly on tail. Deirid setiform, close to level of excretory pore, inside the lateral field. Pharyngeal region with four-six (two-three pairs) somatic setae distributed as follows (n=3): one subdorsal pair just posterior to cervical expansion, one subventral pair just posterior to cervical expansion (absent in one female), and one ventrosublateral pair around middle of

Table 4.5. Measurements (in µm) of different populations of *Neotylocephalus annonae* Ali, Farooqui & Tejpal, 1969

Country Population #	Australia XXI Female	Reunion XXII Female	India XXIII Female	Yemen XXIV Females
n	1	1	1	6
Body length	300	281	315	232±15.2 (214-250)
Body diameter	25	17	23	15.7±2.2 (11.5-17.5)
Neck length	81	84	87.5	71.5±6.5 (60-74)
Tail length	33	31	41.5	24.3±2.0 (21.5-27)
ABD	11	10	10.5	8.7±0.6 (8-9.5)
a	12.2	16.2	13.6	15.0±2.0 (13.1-18.7)
b	3.7	3.3	3.6	3.3±0.2 (3.1-3.6)
c	9.1	9.0	7.6	9.6±0.8 (9.0-10.5)
c’	3.1	3.0	4.0	2.8±0.2 (2.5-3.0)
Flabella length	3.5	3.5	?	3.1±0.8 (2.5-4.5)
Expansion length	8	7	?	7.0±1.0 (6-8)
Expansion width	16.5	21	?	14.1±1.0 (13-15.5)
Stoma length	12.5	14.5	14	10.8±0.8 (10-12)
Amphid location	7	8	8	5.6±0.7 (4.5-6)
Rectum	9	11.5	?	10.2±1.0 (8.5-11.5)
R/ABD	0.8	1.1	?	1.2±0.2 (0.9-1.3)
Vagina	4.5	7	?	5.1±0.5 (4.5-6)
V, %	52.5	53.3	52.3	55.3±1.8 (52.1-57.0)
G1, %	17.6	9.9	?	10.2 (7.7-13.4)
G2, %	12.7	6.7	?	9.7 (7.0-11.7)

corpus. Body furthermore with six-seven somatic setae (n=2): one dorsosublateral pair posterior to cardia, two setae on the right side and 2-3 on the left side of the body between vulva and anus. Anterior end with pronounced bilateral and dorsoventral symmetry. Cervical cuticular expansions bulbiform, smooth under LM. Each expansion extends forward into two short submedian fimbriate flabella. Each flabellum is supported by the arcuate radial ridges of the perioral cuticle that extend from the oral aperture towards the middle of the flabellum. Each flabellum carrying slender fimbriae, which are symmetrically located on both sides of the flabellum and directed forward. Fimbriae only faintly visible under LM. Each lateral sector carrying a pair of sublateral cornua and a midlateral projection set between lateral rim and mouth opening. Cornua “ovoid”, having a narrow base, with their facing rims divided by deep incisures into three tines, of which the middle one is the longest and contains a nerve ending. Midlateral projection of lateral lip short, 2.5-3.0 µm long, extending forward to near the middle of perioral flaps; it contains a nerve ending. Lateral rims without fimbriae. Perioral cuticle on the lateral sides of the anterior end with only few incomplete transverse striations, its posterior part ending near amphid.

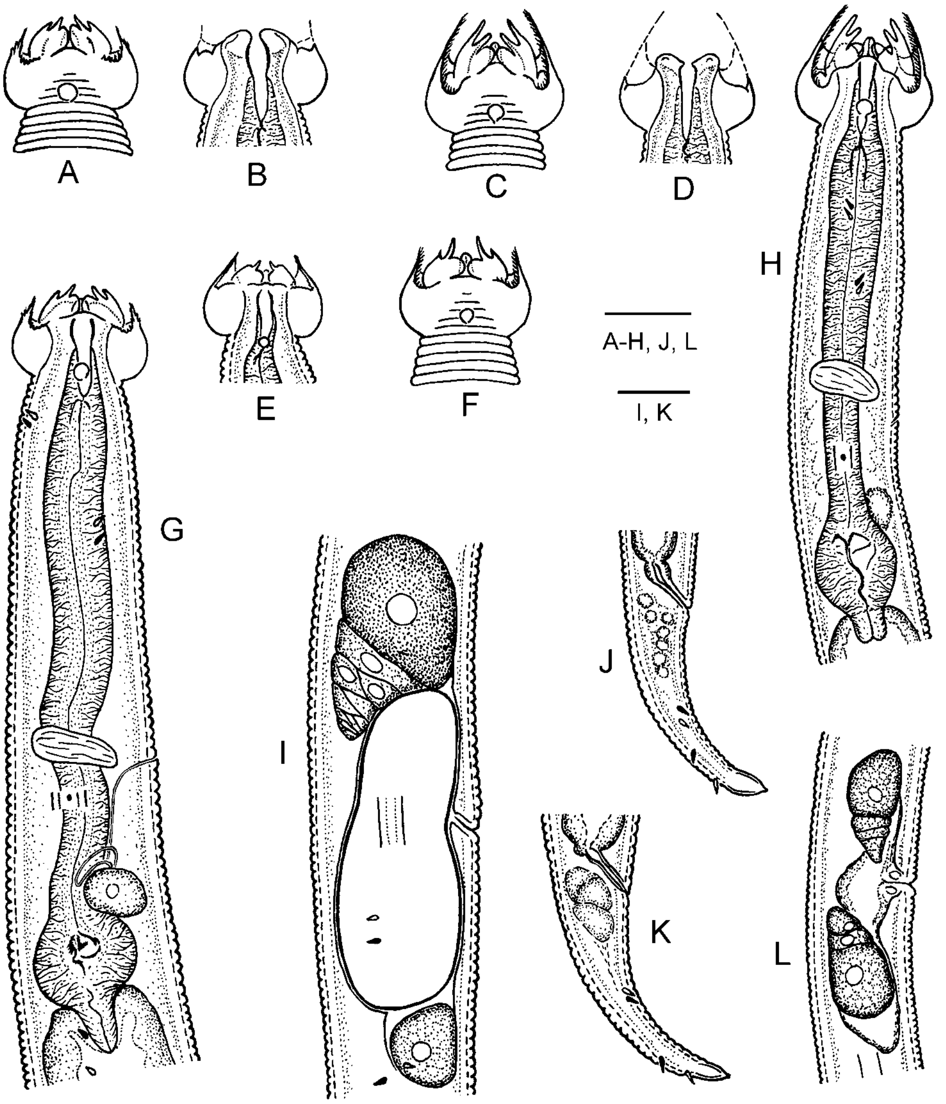

Figure 4.8. *Neotylocephalus annonae* Ali, Farooqui & Tejpal, 1963. Females only. A-B, G, I, K: Population from Australia (XXI). A. Labial region, lateral surface view; B: Labial region, lateral median view; G: Pharyngeal region; I: Reproductive system; K: Tail; E: Population from Yemen (XXIV). Labial region of juvenile. F: Population from Reunion (XXII). Labial region, lateral surface view. *Neotylocephalus inflatus* (Yeates, 1967) Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003. Females only. C-D, H, J, L: Population from Mexico (XXVI). C: Labial region, lateral surface view; D: Labial region, lateral median view; H: Pharyngeal region; J: Tail; L: Reproductive system. Scale bars 10 µm. Setae as in Fig. 4.2.

Amphidial aperture 3 µm wide, circular, located at the level of posterior part of stoma. Stoma plectoid. Pharyngeal corpus cylindrical, plump, radial tubules present. Isthmus plump, surrounded by the nerve ring at its anterior end and by the secretory-excretory gland cell at its posterior end. Basal pharyngeal bulb oval, with simple valves. Cardia embedded by intestinal tissue. Excretory gland duct weakly cuticularized, excretory pore posterior to the nerve ring. Female reproductive system didelphic, amphidelphic, ovary branches reflexed. Vulva equatorial, transverse. Vagina short, encircled by sphincter muscle. Epiptygmata absent. Anterior genital branch located on the right side of the body, posterior genital branch on the left side of the body in all studied females. Intrauterine egg measuring 48×17 µm, 2.8 times longer than its own diameter. Rectum short and stout, shorter than anal body diameter. Tail gradually narrowing, arcuate ventrad, with four paired and opposed caudal setae (n=5): one subventral pair located at middle of tail and one subdorsal pair (including a spur at 4.0-6.5 µm from tail tip on the left side of the body) close to tail terminus. Caudal glands present.

Male: Not found.

Diagnosis: Cervical cuticular expansions smooth, each extends into two short submedian fimbriate flabella. Cornua each with three tines, middle one clearly longer. Midlateral digitate projection short, oval. Lateral rims not fimbriate. Pharyngeal region with four somatic setae. Tail with four setae. Rectum about one anal body diameter long.

Remark: Single available juvenile has relatively longer flabella and less developed cornua than the adults.

Justification of identification: Since deposition of types of *N. annonae* was not mentioned in the original description and the quality of type specimens of *Spatiocephalus venustrus* was not satisfactory for any morphological observations, our taxonomic conclusions are based on the original descriptions and illustrations by Ali et al. (1969) and Patil & Khan (1982), respectively. Type populations of both species are similar to each other as well as to the recent specimens by the following characters: cervical expansions non-annulated extending forward into short ("rudimentary") flabella, and midlateral projection short. Neither perioral flaps nor fimbriae were sufficiently described or depicted. The small morphometric differences present are probably due to intraspecific variability or different ways of measuring and are not considered significant.

Neotylocephalus inflatus **(Yeates, 1967) Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003**

(Figs 48 C, D, H, J, L & 4.9, Tables 4.1 & 6.1)

Female: Body small, fusiform, ventrally curved upon fixation. Cuticle thin, annulated. Lateral field 2.5-3.5 µm wide at midbody, consisting of two separate wings divided by striated cuticle, fading anteriorly at level of pharyngeal corpus and posteriorly on tail. Deirid setiform, close to excretory pore, inside the lateral field. Pharyngeal region with four (two pairs) somatic setae distributed as follows (n=16): one dorsosublateral pair

Table 4.6. Measurements (in µm) of different populations of *Neotylocephalus inflatus* (Yeates, 1967) Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003.

Country	Mexico				Ecuador			Costa Rica	USA
Population #	XXV Female	XXVI Females	XXVII Females	XXVIII Females	XXIX Females	XXX Females	XXXI Females	XXXII Female	XXXIII Female
n	1	2	2	3	2	2	9	1	1
Body length	215	233; 222	235; 257	211-229	215-225	187-244	247±13 (233-277)	273	226
Body diameter	14.5	15; 14	13.5; 14	14-14.5	15.5-16	15-16	14-16	21	12
Neck length	61.5	67; 65.5	70; 79	68-69	77-82	67-78	78±4 (74-86)	80	67
Tail length	26	30; 29	31; 32	25.8-31	27-28	22-26	29±2 (27-32)	26.5	24.5
ABD	8	7.5; 8	7; 8	8.5-9	7.5-8.5	8-9	7-9	9	7
a	14.8	15.6; 16.1	17.0; 19.0	14.9-15.8	14	12-15	15-18	13	18.5
b	3.5	3.5; 3.4	3.3; 3.4	3.1-3.4	2.8	2.8-3.1	3.2±0.2 (2.7-3.5)	3.4	3.4
c	8.2	7.7	7.6; 8.0	7.3-8.4	7.7-8.2	8.3-9.6	8.6±0.4 (8.1-9.2)	10.3	9.2
c’	3.3	3.5; 4.0	4.0; 4.4	3.0-3.7	3.3-3.7	2.5-3.2	3.5±0.2 (3.3-3.9)	2.9	3.6
Flabella length	5.5	5.5; 6	4.5; 6	5-5	5-8	5-7.5	6-8.5	6	4.5
Expansion length	6.5	6; 8	6; 6.5	6.5-7	6	5-6	5-7.5	7	7
Expansion width	15.5	15; 15.5	15.5; 17	14.5-16	15-16	15.5	15-16	?	11
Stoma length	11	11; 11.5	10.5; 11.5	9-10	11-12	10	11-13	13	10
Amphid location	8	7; 7	6; 8	5-6.5	?	?	?	7	7
Rectum	6	7; 9	8	7.5-9.5	7-8	7.5-9	7-10	8	7
R/ABD	0.7	0.9; 1.1	1.0; 1.2	0.9-1.0	?	?	?	0.9	1.0
Vagina	5	4; 5	5	5-6	4	4-5	3-5	3.5	4.5
V, %	51.2	50.5; 52.0	51.9; 53.7	52.1-55.4	51-52	50-52	51±1 (49-53)	52.4	52.2
G1, %	9.2	8.8	8.9	7.9-9.3	6-7	9	11±3 (6-15)	10.3	?
G2, %	12.3	8.6	10.1	7.8-8.5	7-9	6-11	10±2 (6-15)	14.8	?

near anterior end of corpus and one ventrosublateral pair opposite posterior part of corpus. Body furthermore with six somatic setae (n=15): one dorsosublateral pair at level of cardia or somewhat more posteriorly, two single setae on the right and two single ones on the left side of the body between vulva and anus. Anterior end with pronounced bilateral and dorsoventral symmetry. Cervical cuticular expansions bulbiform, smooth under LM but with very fine transverse striations posteriorly under SEM. Each expansion extends forward into two long submedian fimbriate flabella. Each flabellum is supported by the arcuate radial ridge of the perioral cuticle that extends from the oral aperture towards the middle of the flabellum or somewhat anteriorly. Each flabellum carrying long and slender fimbriae, which are symmetrically located on both sides of the flabellum and directed forward. Fimbriae only faintly visible under LM. Each lateral sector carrying a pair of sublateral cornua and a midlateral projection set between lateral rim and oral aperture. Cornua “triangular”, having a wide base, with their facing rims divided by deep incisures into three tines of comparable length, while the middle one contains a nerve ending and is usually more distinct under light microscope. Midlateral projection of lateral lip short, 2-3 µm long,

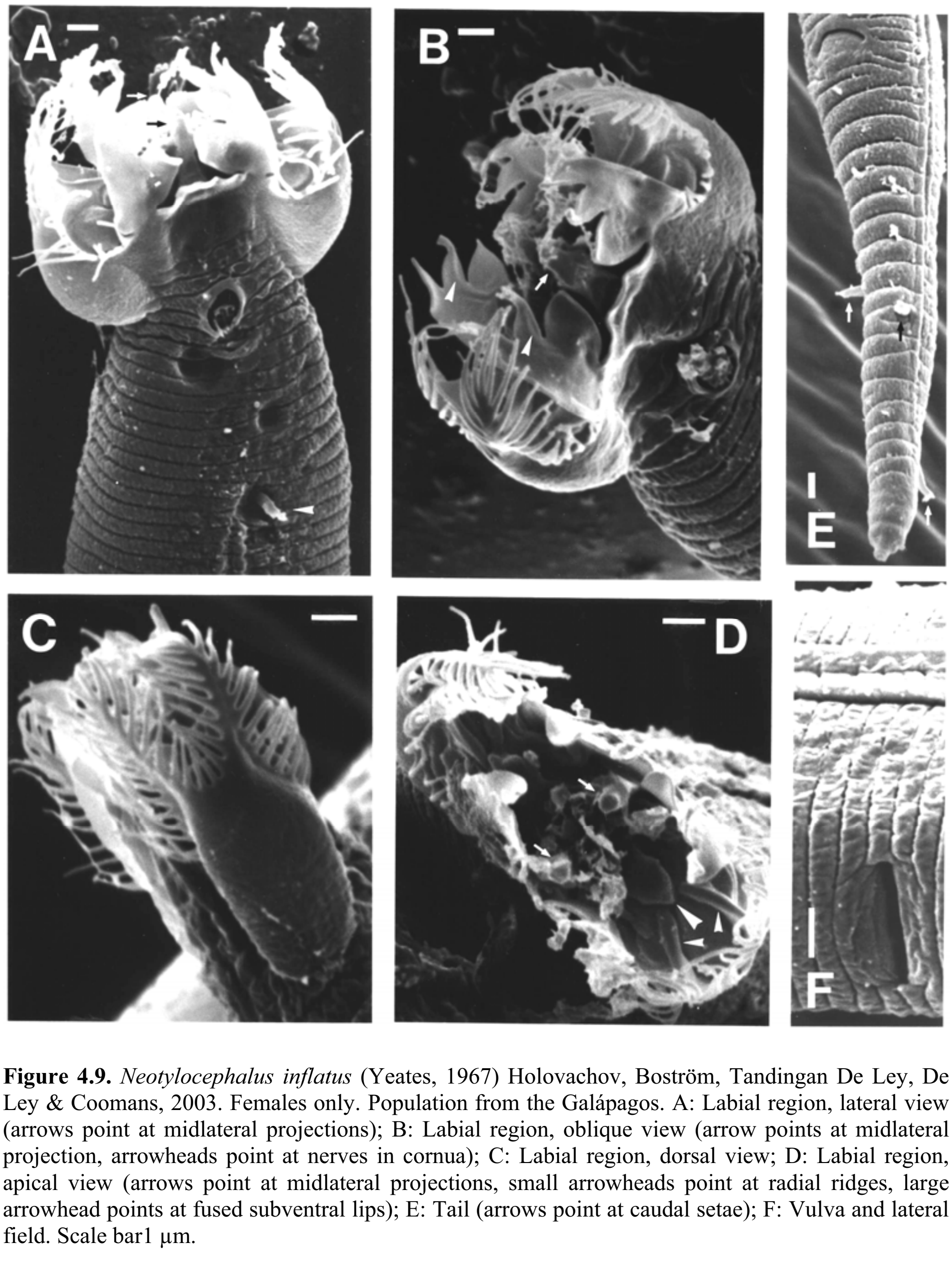

Figure 4.9. *Neotylocephalus inflatus* (Yeates, 1967) Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003. Females only. Population from the Galápagos. A: Labial region, lateral view (arrows point at midlateral projections); B: Labial region, oblique view (arrow points at midlateral projection, arrowheads point at nerves in cornua); C: Labial region, dorsal view; D: Labial region, apical view (arrows point at midlateral projections, small arrowheads point at radial ridges, large arrowhead points at fused subventral lips); E: Tail (arrows point at caudal setae); F: Vulva and lateral field. Scale bar1 µm.

extending forward to near the middle of cornua; contains a nerve ending, probably of lateral outer labial sensillum. Lateral rims without fimbriae. Perioral cuticle on the lateral sides of the anterior end with only few incomplete transverse striations, its posterior part ending near amphid. Amphidial aperture 1.5-3.0 µm wide, circular, located at the level of posterior part of stoma. Stoma plectoid. Pharyngeal corpus cylindrical, plump, radial tubules present. Isthmus plump, surrounded by the nerve ring at its anterior end and by the secretory-excretory gland cell at its posterior end. Basal pharyngeal bulb oval, with simple valves. Cardia embedded by intestinal tissue. Excretory gland duct weakly cuticularized, excretory pore posterior to the nerve ring. Female reproductive system didelphic, amphidelphic, ovary branches reflexed. Vulva equatorial, transverse. Vagina short, encircled by sphincter muscle. Epiptygmata absent. Anterior genital branch on the right side of the body in 21 females and on the left side of the body in the female from Costa Rica, posterior genital branch on the left side of the body in all 22 studied females. Intrauterine eggs not observed. Rectum short and stout, almost equal to anal body diameter. Tail gradually narrowing, arcuate ventrad, with four paired and opposed caudal setae (n=16): one ventrosublateral pair located at middle of tail and one slightly more spaced subdorsal pair (including a spur at 4-7 µm from tail tip on the left side of the body) at posterior third of tail length. Caudal glands present.

Male: Not found.

Diagnosis: Cervical cuticular expansions non-annulated, each extends into two long submedian fimbriate flabella. Cornua each with three tines of similar length. Midlateral projection short, oval. Lateral rims not fimbriate. Pharyngeal region with four somatic setae. Tail with four caudal setae. Rectum about one anal body diameter long.

Justification of identification: Our material turned out to be very similar to the type material of two nominal species, originally described with rather different labial structures and placed in different genera: *Ereptonema inflatum* Yeates, 1967 and *Coronacephalus indicus* Ganguly & Khan, 1986. One paratype of each species was in sufficiently good condition to reveal the presence of two pairs of long flabella, and in both cases the fimbriae on these flabella were apparently misinterpreted as annulations in the original descriptions. Both paratypes also had two pairs of setae on the neck, arranged as in our specimens, and the paratype of *C. indicus* also matched our material in number and placement of caudal setae. Body setae could not be distinguished clearly from cuticular debris in either paratype, while the paratype of *E. inflatum* was too poorly preserved to see caudal setae. Overall, we conclude that both nominal species are conspecific with our specimens. Subsequent to the original description of *E. inflatum* by Yeates (1967), this species was considered to be a junior synonym of W. *otophorum* (cf. Yeates 1969, 1988). As the chronologically senior epithet, we now reinstate E. inflatum on the basis of its unique character combination (see diagnosis above), and consider it sufficiently similar to *Neotylocephalus annonae* to warrant transfer to *Neotylocephalus*. The range of localities from which *N. inflatus* (Yeates, 1967) Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003. is now known, suggests that its geographic range extends across the Indo-Pacific, and that it is confined to tropical and subtropical soils.

On the identification of *W. otophorum* and *W. schuurmansstekhoveni*

W. schuurmansstekhoveni was first synonymized with *W. otophorum* by Anderson (1966) and then reinstated by Zell (1985), who considered it to be distinct from *W. otophorum* by its elongated rectum and absence of a subventral pair of setae just posterior to the ventral cervical expansion. While Fig. 34b in de Man (1876) clearly shows a short rectum for *W. otophorum* (then described as *Plectus auriculatus*), De Coninck (1931) did not mention either of the relevant features in *W. schuurmansstekhoveni*. Apart from speculations on the shape and size of flabella and cervical expansions and the number of fimbriae on flabella (none of these characters were clearly specified in the original descriptions of the two species), Zell (1985) used the c-ratio to justify the correctness of the identification. The type population of *W. schuurmansstekhoveni* has c = 13 and the type specimen of *W. otophorum* has c = 9.1, while the specimens of Zell (1985) have c=8.4-14. Although the difference is rather small, recently studied populations support the opinion of Zell and we consider it justified to use the name *W. schuurmansstekhoveni* for the specimens described in this paper.

The differences between *W. otophorum* and *W. schuurmansstekhoveni* are as follows: rectum length (7-14 *vs* 18-28 μm respectively), R/ABD-ratio (0.8-1.4 *vs* 2.2-4.6), number of setae in pharyngeal region (6 *vs* 4), and number of caudal setae (5 *vs* 4). Recently studied populations of both species also differ slightly by some morphometric characters, i.e. tail length (24-44 *vs* 14-28 μm), c-ratio (6-11 *vs* 11-19), and flabella length (6-10 *vs* 8-13 μm).

Several populations of *Wilsonema* studied by Anderson (1966) undoubtedly belong to the two different species as considered in the present paper. The distribution of setae in the pharyngeal region given in the description and the structure of the caudal region shown in Anderson's Fig. 2G (short rectum, five caudal setae) fit well with the morphology of *W. otophorum* as considered here. However, the distribution of caudal setae given in the description and the structure of the caudal region shown in Anderson's Fig. 2I (long rectum, four caudal setae) fit well with the morphology of *W. schuurmansstekhoveni*. Nevertheless, the caudal region in Anderson's Fig. 2H shows an intermediate character state (short rectum, four caudal setae). This remains to be explained.

Ultrastructure, homology and evolution of the labial structures in Wilsonematinae

All representatives of the subfamily Wilsonematinae are characterised by the pronounced bilateral and dorsoventral symmetry of the labial region carrying numerous cuticular processes and appendages, which differ in structure, arrangement and origin between genera. As noted above, the limitations of light microscopy preclude appropriate observations and interpretations of labial structures in species of the subfamily and consequently hamper a phylogenetic classification of the group. It is obvious from the differences between e. g. our findings and the description of *Neotylocephalus inflatus* by Yeates (1967) and Ganguly & Khan (1986) that SEM is a basic necessity for the study of these animals. *Tylocephalus* was until now the best studied genus in this respect. Among nine valid species of the genus, three species (*T. auriculatus*, *T. palmatus* and *T. nimius*) and one unidentified population were described on the basis of SEM-studies (De Ley & Coomans, 1997; Sauer, 1985; Sauer & Annells, 1982; Tahseen et al., 1995)[*]. The only representative of another genus studied with SEM was *Ereptonema arcticum*, but the limited number of available specimens made it impossible to carry out a detailed study (Boström, 1988). Our descriptions of four species in three of the four genera (presently considered to be valid) of the subfamily Wilsonematinae give grounds for a first phylogenetic analysis of the systematics of the group.

The morphology of *Tylocephalus becki* Zell, 1985 and *T. cornutus* Zell, 1985[**], and the postembryonic development of *T. auriculatus* (De Ley & Coomans, 1997) suggest that *Tylocephalus* represents the least derived morphology within the subfamily and that its ancestor was similar in many respects to species of the subgenus *Ceratoplectus* Andrássy, 1984. On the other hand, the genera *Wilsonema*, *Ereptonema* and *Neotylocephalus* possibly represent a separate lineage as they differ from *Tylocephalus* by different and more complex structures in the labial region. All three genera are characterised by the presence of flabella, fimbriae, more elevated peripheral cervical cuticle, and leaf-shaped cornua, that are subdivided into several tines by deep incisures.

Within this group, *Neotylocephalus* and *E. arcticum* possess four submedian flabella, while *Wilsonema* and *E. fimbriatum* have two median flabella. The fimbriate flabella of *Wilsonema*, *Ereptonema* and *Neotylocephalus* are not innervated and therefore not homologous to the median ridges in *Tylocephalus*. The submedian labial sensilla in all genera of Wilsonematinae are shifted towards the mouth opening. We therefore assume that the flabella are cuticular formations without a sensory function. They are probably derived from the lateral transverse flanges of the median ridges in *Tylocephalus* (see De Ley & Coomans, 1997: Figs 4B, 5A, 5B). The flabella seem to be useful for gathering or scratching the food sources (bacteria) from the substrate. Each flabellum is always supported by the radial ridge that extends from the inner side of the flabellum towards the mouth opening. Submedian lips have formed median ridges in *Tylocephalus*, but other genera of Wilsonematinae do not possess these structures. At first glance, the presence of relatively short flabella in *Ereptonema* seems to represent a probably plesiomorphic condition when compared only to the longer flabella in *Wilsonema* and *Neotylocephalus inflatus*. However, the presence of short flabella in *N. annonae* may be a secondary condition, in view of the fact that the single available juvenile of this species has relatively longer flabella than the adult. We therefore remain cautious for the time being about this character. Phylogenetic analyses result in greater ambiguity when including flabella length as an additional or alternate character (see below).

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[*] According to the latest revision, the genus *Tylocephalus* includes 11 species (Holovachov et al., 2004), while one more species, e. g. *T. primitivus* Holovachov, Boström & Mundo-Ocampo, 2004 was studied with SEM.

[**] Also of *T. primitivus* and *T. longicornis* Holovachov, Boström & Mundo-Ocampo, 2004, see Holovachov et al. (2004).

Table 4.7. Character state matrix for *Plectus cryptoptychus* de Ley & Coomans, 1994, *P. (Ceratoplectus) armatus* (Btschli, 1873) Andrássy, 1984, *Tylocephalus auriculatus* (Bütschli, 1873) Anderson, 1966, *Ereptonema arcticum* Loof, 1971, *E. fimbriatum* Anderson, 1966, *Neotylocephalus annonae* Ali, Farooqui & Tejpal, 1969, *N. inflatus* (Yeates, 1967) Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003, *Wilsonema otophorum* (de Man, 1880) Cobb, 1913 and *W. schuurmansstekhoveni* (De Coninck, 1931) Zell, 1985 used in the phylogenetic analyses.

#	Character	Primitive state	Derived state	*P. cryptoptychus*	*P. (C.) armatus*	*T. auriculatus*	*E. arcticum*	*E. fimbriatum*	*N. annonae*	*N. inflatus*	*W. otophorum*	*W. schuurmansstekhoveni*
1	labial symmetry	hexaradiate	bilateral	0	0	1	1	1	1	1	1	1
2	cervical expansions	absent	present	0	0	1	1	1	1	1	1	1
3	cervical expansions	absent or annulated	smooth	0	0	0	0	0	1	1	1	1
4	cervical exp. join neck at	shallow angle	straight angle	0	0	0	0	0	1	1	1	1
5	midlateral projection	absent	present	0	0	1	1	1	1	1	1	1
6	midlateral projection	absent or short	long	0	0	0	0	0	0	0	1	1
7	midlateral projection	absent or smooth	2 tines	0	0	0	0	0	0	0	1	0
8	cephalic sensilla	round	flattened	0	1	1	1	1	1	1	1	1
9	cephalic sensilla	setae	cornua	0	0	1	1	1	1	1	1	1
10	edge of cornua	smooth	with tines	0	0	0	1	1	1	1	1	1
11	cornua tines	0-3	4-5	0	0	0	0	0	0	0	1	1
12	lateral rim	absent	present	0	0	1	1	1	1	1	1	1
13	lateral rim	divided	continuous	0	0	0	1	1	1	1	1	1
14	lateral rim	smooth	fimbriate	0	0	0	1	1	0	0	0	0
15	median ridge	absent	present	0	0	1	0	0	0	0	0	0
16	flabella	absent	present	0	0	0	1	1	0	1	1	1
17	flabella number	0-2	4	0	0	0	1	0	1	1	0	0
18	rectum	short	long	0	0	0	0	0	0	0	0	1

Each flabellum is carrying long and slender fimbriae, which are symmetrically located on both sides of the flabellum. Similar analogous structures are present in other, unrelated groups of bacterivorous nematodes like e.g. in Chambersiellidae and Cephalobidae and probably take active part in feeding. Fimbriae are undoubtedly cuticular neoformations and are present in all studied genera. The genus *Ereptonema* is characterised particularly by the fimbriate lateral rims. Fimbriae on the flabella and on the lateral rims are morphologically similar and appear to have the same function. It is not clear if the fimbriae on lateral rims and flabella developed simultaneously in *Ereptonema* and were later reduced in *Neotylocephalus* and *Wilsonema*. The other possibility is that the fimbriae on the lateral rims have been formed by the extension of fimbriae from the flabella. The latter assumption seems to be more probable. Therefore, this character (fimbriated lateral rims) is considered synapomorphic for *Ereptonema*. Some species (e.g. *W. andersoni* – now in *Ereptonema*) were described as having non-fimbriate lateral rims but this could not be stated confidently.

The lateral lips of *Ceratoplectus*-like wilsonematid ancestors were modified into midlateral projections containing a nerve ending and sensory pore of the outer labial sensilla and adjacent quadrants in *Tylocephalus*. This is also indirectly suggested by the fact that the lateral lips of *Plectus (Ceratoplectus) armatus* are as wide as both submedians together resulting in the bilateral and dorsoventral symmetry (vs hexaradial in other species of *Plectus* studied under SEM, cf. De Ley & Coomans, 1994). The anterior elevation of lateral outer labial sensilla is a synapomorphy of Wilsonematinae relative to the "regular" structure of lateral lips in *Plectus*. However, the state of this character in *Tylocephalus* represents only the beginning of the transitional series towards the elongated, digitate midlateral projection in Wilsonema through the intermediate state in *Ereptonema* and *Neotylocephalus*.

All three genera are characterized by the presence of the lateral rim – the anterior edge of the peripheral cuticle on each lateral side. On the basis of the positional relationships to other parts of the body, we assume that the lateral rim is derived by fusion of two submedian quadrants in *Tylocephalus*.

The cornua of *Tylocephalus* are clearly derived cephalic setae. This was apparent from the conditions in two Tylocephalus species described by Zell (1985) and recent data (Holovachov et al., 2004): *T. cornutus* and *T. becki* both have cornua that are plump and less reclining than in other species, resembling the cephalic setae of some species of the subgenus *Ceratoplectus* Andrássy, 1984 in Plectinae. The homology is also consistent with the morphology of first- and second-stage juveniles of *T. auriculatus* (De Ley & Coomans, 1997). Recent observations of nerve endings in the cornua of *Wilsonema*, *Ereptonema* and *Neotylocephalus* suggest that these flaps are homologous with the cornua of *Tylocephalus* and derived from cephalic setae. The morphological differences between cornua of the *Wilsonema-Ereptonema-Neotylocephalus* group and *Tylocephalus* are more relevant than the differences between *Tylocephalus* and *Plectus*, especially when the most primitive forms (cf. *T. cornutus, T. becki*) and juveniles are considered. However, the shape of the cornua in more derived species (cf. *T. auriculatus*) are more flattened, leaf-shaped, with additional basal flap (Fig. 4E, F in De Ley & Coomans, 1997).

One of the characters unique for Wilsonematinae as a whole is the presence of cervical expansions. Annulated cervical expansions in *Tylocephalus* and *Ereptonema* is undoubtedly a primitive state, while non-annulated cervical expansions in *Neotylocephalus* and *Wilsonema* is a derived state.

Phylogenetic analysis

Figure 4.10 presents a phylogram of the single best tree obtained with parsimony analysis of the morphological characters described above and listed in Table 4.7. Our dataset was found to be highly structured: PAUP* calculated the tree distribution skewness statistic g1 = -1.25, indicating that the data were non-random with probability in excess of

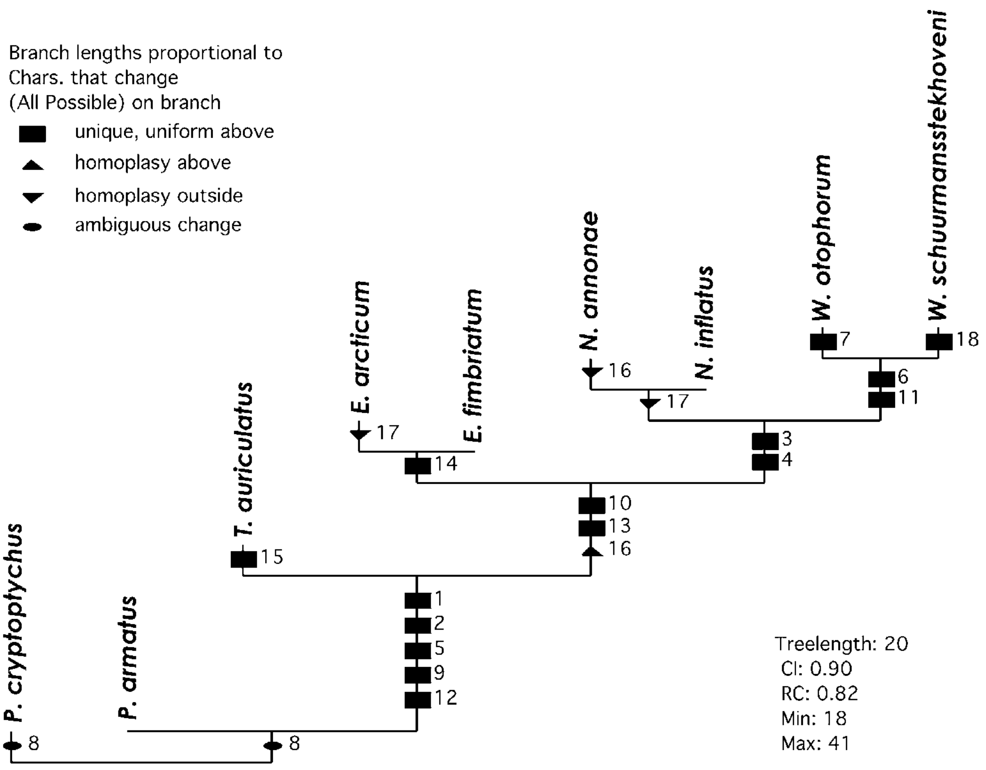

Figure 4.10. Phylogram showing the phylogenetic relationships of *Plectus* Bastian, 1865, *Tylocephalus* Crossman, 1933, *Ereptonema* Anderson, 1966, *Neotylocephalus* Ali, farooqui & Tejpal, 1969 and *Wilsonema* Cobb, 1913 (for explanation see text).

0.99 (Hillis & Huelsenbeck, 1992). The best tree was 20 steps long, while six trees were only one step longer, and sixteen trees were two steps longer. Fourteen of these twenty-two trees with a length of 21-22 steps had at least one unresolved polytomy, and in eight trees among these twenty-two lack of full resolution was entirely due to “soft” polytomies, i.e. absence of synapomorphies on at least one internal branch. This suggests that phylogenetic signal in our matrix was rather unevenly distributed among internal branches. For example, all trees with length < 23 had five synapomorphies supporting monophyly of Wilsonematinae, but only two of these trees had any synapomorphies supporting monophyly of *Neotylocephalus*. Clearly, the dataset would still benefit substantially from the addition of further characters, if such could be found in future. Parsimony analysis of the data matrix with alternate scoring of character 12 yielded very similar results. Alternate scorings of character 16 (in which only long flabella are considered apomorphic, or in which flabella length is added separately to absence vs presence) resulted in greater differences: both alternatives yielded three shortest trees instead of one, and only one of the three supported monophyly of both *Ereptonema* and *Neotylocephalus*. Because of uncertainty about the evolutionary polarity of flabella length (see above), we consider these alternative analyses more precarious, at least until more is known about the changes of flabella length during successive moults in the relevant species.

On the basis of our matrix, a monophyletic origin of the subfamily Wilsonematinae is strongly supported. By contrast, one of the two included Plectus species was in all respects plesiomorphic, while the other shared a single synapomorphy with wilsonematids. It is tempting to conclude that *Plectus*, and by extension the entire subfamily Plectinae, is paraphyletic compared to Wilsonematinae (see Zell, 1993). Despite the absence of obviously complex structures such as the labial appendages of Wilsonematinae, Plectus is nevertheless an extremely diverse genus, and many of its species characters are not only difficult to score (mostly because of lack of information in published descriptions) but even harder to polarise with respect to other Plectidae. Zell (1993) attempted such an analysis, applying a hybrid method combining cladistics with other principles, and concluded that wilsonematids should be relegated to subgenus level within Plectus. However, he did not address the rooting of Plectidae among other Leptolaimina/Plectida, and it is quite likely that at least some of his character polarity hypotheses should actually be reversed.

At present, it seems quite possible that some species of *Plectus* such as *P. (C.) armatus* share exclusive common ancestry with Wilsonematinae, while other *Plectus* species might constitute one or more truly monophyletic taxa in their own right. However, the taxonomic consequences of such a relationship will depend greatly on the exact position of the root for the entire family. In the absence of a well-supported root, we prefer to practice caution in taxonomic rearrangements of Wilsonematinae, maintaining previously proposed genera as valid (until proven otherwise by study of type material) and maintaining the subfamily Wilsonematinae as valid until relationships within *Plectus* and with other genera of Plectinae can be adressed with greater confidence.

Appendix: an updated classification of the genus *Wilsonema*

Genus *Wilsonema* Cobb, 1913

Synonimy: *Bitholinema* De Coninck, 1931; *Pycnolaimus* Cobb, 1920[1]; *Wilsotylus* Chawla, Khan & Prasad, 1969.

Diagnosis: Wilsonematinae. Body length between 0.2 and 0.4 mm. Anterior end with bilateral and dorsoventral symmetry. Cervical expansions bulbiform, non-annulated under LM and SEM, each extends forward into one median fimbriate flabellum. Four cornua with four tines each. Midlateral projection reaching anterior end of perioral flaps. Lateral rims not-fimbriate. Amphid aperture circular, halfway of the stoma. Stoma plectoid. Pharynx plectoid, basal bulb with valves. Vulva equatorial. Female reproductive system didelphic, amphidelphic. Male reproductive system diorchic, testes opposed. Spicules paired, curved ventrad. Midventral precloacal tubular supplements, precloacal setiform sensillum and gubernaculum absent. Tail conoid at first, then cylindrical, ventrally curved. Caudal glands present, opening through a terminal spinneret.

Type species:

W. otophorum (de Man, 1880) Cobb, 1913 (Syn.: *Plectus otophorus* de Man, 1880; *Plectus auriculatus* apud de Man, 1876; *W. capitatum* Cobb, 1913[2]; *Plectus tentaculatus* Fuchs, 1930; *W. andersoni* Zell, 1985[3]; *Plectus (Wilsonema) bolivianus* Zell, 1993[4].

Other species:

W. schuurmansstekhoveni (De Coninck, 1931) Zell, 1985 (Syn.: *Bitholinema schuurmansstekhoveni* De Coninck, 1931; *W. otophorum* apud Anderson, 1966 partim; *W. otophorum* apud Zell, 1982);

W. bangaloreiensis (Chawla, Khan & Prasad, 1969) Zell, 1993[5] (Syn.: *Wilsotylus bangaloreiensis* Chawla, Khan & Prasad, 1969);

W. longicaudatum Holovachov & Háněl, 2004.

Species inquirendae vel incertae sedis:

W. pygmaea (Cobb, 1920) Andrássy, 1984[1] (Syn.: *Pycnolaimus pygmaeus* Cobb, 1920);

W. fausti (Kreis, 1930) Goodey, 1951[6] (Syn.: *Plectus (Wilsonema) fausti* Kreis, 1930);

W. promissum Khan, Seema & Khan, 1990[7].

Appendix: an updated classification of the genus *Ereptonema*

Genus *Ereptonema* Anderson, 1966

Synonimy: *Wilsereptus* Chawla, Khan & Saha, 1977; *Paraereptonema* Eroshenko, 1977[8].

Diagnosis: Wilsonematinae. Body length between 0.2 and 0.4 mm. Anterior end with bilateral and dorsoventral symmetry. Cervical expansions bulbiform, annulated (under LM and SEM), each extends forward into one median or two submedian fimbriate flabella. Four cornua with two or three tines each. Midlateral projection reaching middle of perioral flaps. Lateral rims fimbriate. Amphid aperture circular, halfway the stoma. Stoma plectoid. Pharynx plectoid, basal bulb with valves. Vulva equatorial. Female reproductive system didelphic, amphidelphic. Male reproductive system diorchic, testes opposed. Spicules paired, curved ventrad. Midventral precloacal tubular supplements, precloacal setiform sensillum and gubernaculum absent (See Chapter 5). Tail conoid at first then cylindrical, ventrally curved. Caudal glands present, opening through a terminal spinneret.

Type species:

E. fimbriatum Anderson, 1966 (Syn.: *Plectus otophorus* apud Hofmänner & Menzel, 1915; *Paraereptonema ciliatum* Eroshenko, 1977[9]; *E. ciliatum* (Eroshenko, 1977) Andrássy, 1984).

Other species:

E. arcticum Loof, 1971 (Syn.: *Wilsonema agrarum* apud Bussau, 1990[10]);

E. andersoni (Chawla, Khan & Saha, 1977) Andrássy, 1984 (Syn.: *Wilsereptus andersoni* Chawla, Khan & Saha, 1977);

E. cheliferum (Andrássy, 1986) Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003[11] (Syn.: *Wilsonema cheliferum* Andrássy, 1986).

Species inquirendae vel incertae sedis:

E. agrarum (Nesterov, 1973) Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003[12] (Syn.: *Wilsonema agrarum* Nesterov, 1973; *Paraereptonema agrarum* (Nesterov, 1973) Eroshenko, 1977).

Appendix: an updated classification of the genus

Genus *Neotylocephalus* Ali, Farooqui & Tejpal, 1969

Synonimy: *Spatiocephalus* Patil & Khan, 1982; *Coronacephalus* Ganguly & Khan, 1986.

Diagnosis: Wilsonematinae. Body length between 0.2 and 0.3 mm. Anterior end with bilateral and dorsoventral symmetry. Cervical expansions bulbiform, appearing non-annulated under LM, but with faint transverse annulation under SEM, each expansion extends forward into two submedian fimbriate flabella. Four cornua with three tines each. Midlateral projection reaching middle of perioral flaps. Lateral rims smooth. Amphid aperture circular, plectoid, halfway of the stoma. Stoma plectoid. Pharynx plectoid, basal bulb with valves. Vulva equatorial. Female reproductive system didelphic, amphidelphic. Males unknown. Tail conoid to completely cylindrical, ventrally curved. Caudal glands present, opening in a terminal spinneret.

Type species:

N. annonae Ali, Farooqui & Tejpal, 1969 (Syn.: *Ereptonema annonae* (Ali, Farooqui & Tejpal, 1969) Andrássy, 1984; *Spatiocephalus venustrus* Patil & Khan, 1982; *N. venustrus* (Patil & Khan, 1982) Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003).

Other species:

N. inflatus (Yeates, 1967) Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003 (Syn.: *Ereptonema inflatum* Yeates, 1967; *Coronacephalus indicus* Ganguly & Khan, 1986; *N. indicus* (Ganguly & Khan, 1986) Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003; *Plectus (Wilsonema) gangulykhani* Zell, 1993[13]);

N. haryanensis Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003[14] (Syn.: *Wilsereptus indicus* Walia & Bajaj, 1999; *N. indicus* (Walia & Bajaj, 1999) Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003).

Notes to the updated classification

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[1] *Pycnolaimus pygmaeus* Cobb, 1920, type species of the genus *Pycnolaimus*. By the morphology of the anterior end, i.e. presence of long median flabella, long midlateral projection, and non-annulated cervical expansions, the specimen described by Cobb (1920) resembles Wilsonema, and by having a short rectum it comes close to *W. otophorum*. However, *P. pygmaeus* was described on the basis of a single juvenile specimen, and its taxonomic position could therefore not be stated with confidence. We regard it as species inquirenda.

[2] *Wilsonema capitatum* Cobb, 1913 (p. 435) = *Wilsonema cephalatum* in Cobb, 1913 (p. 443) nec *Plectus cephalatus* Cobb, 1893 = *Tylocephalus cephalatus* (Cobb, 1893) Anderson, 1966

[3] *Wilsonema andersoni* Zell, 1985 was originally described from a single female and differentiated from *W. otophorum* by the 4 µm wide amphid (2.0-3.5 µm in recent specimens), more rounded cornua, and smaller number of somatic setae. The difference (0.5) µm in amphid diameter is not considered significant since it is a common error of the measuring tools. Shape of the cornua (more rounded or more angular) as appeared under the LM strongly depends on the position of the specimen on the slide, and is rather variable as seen under SEM. The number and distribution of body setae given for *W. andersoni* fit within the variability in recent specimens (one pair posterior to cardia, one seta at anterior third and one seta at posterior third of vulva-anus distance). The number and distribution of setae in pharyngeal region and tail (one side is shown in Zell, 1985: Abb. 7) as well as the length of the rectum also agree with recent observations. Therefore, we consider *W. andersoni* identical with *W. otophorum*.

[4] *Plectus (Wilsonema) bolivianus* Zell, 1993 = nomen novum by Zell, 1993 pro *W. andersoni* Zell, 1985 = *Plectus (Wilsonema) andersoni* Zell, 1985 (Zell, 1993) – secondary homonym of *Plectus (Wilsonema) andersoni* Chawla, Khan & Saha, 1977 (Zell, 1993) = *Wilsereptus andersoni* Chawla, Khan & Saha, 1977. Since these species are retained here in different genera the homonymy is absent and the original name is reinstated.

[5] The genus *Wilsotylus* with type species *Wilsotylus bangaloreiensis* was originally distinguished from *Wilsonema* by having non-fimbriate flabella and dorsoventrally oblong amphidial aperture. By the morphology of the anterior end, i.e. presence of long submedian flabella and long midlateral projection(s), and non-annulated cervical expansions, the specimens described by Chawla et al. (1969) resemble *Wilsonema*, and by having a short rectum they come close to *W. otophorum*. Flabella were described as lacking fimbriae but being finely annulated, and their structure could therefore have been misinterpreted. Number and distribution of caudal setae (four in two pairs) are identical with those of *W. schuurmansstekhoveni*, while R/ABD=1.4 (vs R/ABD=2.2-4.6 in the latter). The pharyngeal region was described as bearing eight setae arranged in two lateral rows in four opposed pairs. Absence of some important taxonomic characters in the description, makes it impossible to be confident about the status of this species without re-examination of type material.

[6] *Plectus (Wilsonema) fausti* Kreis, 1930 resembles genera *Wilsonema* and *Neotylocephalus* by its non-annulated cervical expansions. No other useful information is evident from the original description and schematic drawings. The taxonomic position of the species cannot be clarified without re-examination of the type material or new specimens from the type locality. Andrássy (1984) synonymized *P. (W.) fausti* with *W. otophorum*. We regard it as *species inquirenda and incertae sedis*.

[7] *Wilsonema promissum* Khan, Seema & Khan, 1990 resembles genera *Ereptonema* and *Tylocephalus* by its annulated cervical expansions. No other useful information could be taken from the original description and schematic drawings. The length of the body (0.46-0.54 mm) is above the ranges known for any Ereptonema species (< 0.4 mm) but fits within the limits of *Tylocephalus* (0.30-0.52 mm). The taxonomic position of the species cannot be clarified without re-examination of the type material. We regard it *species incertae sedis*.

[8] Khan et al. (1990: p. 439) erroneously used "*Paraereptocephalus*" and assigned this generic name to Eroshenko (1977) for *Paraereptonema – lapsus calami*.

[9] The specific epithet has been emended from *ciliata* to *ciliatum* to follow the gender of the genus name. Andrássy (1984) considereded *Paraereptonema ciliatum* synonymous with *E. arcticum*. However, *P. ciliata* differs from *E. arcticum* and is similar to *E. fimbriatum* by having two comparatively short median flabella (vs four comparatively long submedian flabella). Eroshenko (1977) stated that *Paraereptonema* differs from *Ereptonema* by the small number of thin cephalic setae (=fimbriae) and presence of four labial receptors around the oral opening (=cornua). Not the cornua but nerve endings of the cornua are shown on the figure of anterior end of *P. ciliatum* (Eroshenko, 1977: Fig. 1B). In the original description of *E. fimbriatum* Anderson (1966: p. 929) wrote "flabella rudimentary, margins with or without fimbriae, when present, few, short", although the character is difficult to interpret correctly under the light microscope without SEM confirmation. Therefore, we agree with Andrássy (1984) about the synonymization of *Paraereptonema* and *Ereptonema*, but consider *E. ciliatum* synonymous with *E. fimbriatum* and not with *E. arcticum* as proposed by Andrássy (1984).

[10] The specimen described by Bussau (1990) as *W. agrarum* agrees completely with *E. arcticum* as described herewith on the basis of type and new material. Regarding taxonomic status of *W. agrarum* see below.

[11] The morphology of the anterior end of *Wilsonema cheliferum* Andrássy, 1986 undoubtedly places it in the genus *Ereptonema* and it is thus transferred to the latter. The characters are: annulated cervical expansions, fimbriate lateral rim and short midlateral projection. It comes close to *E. arcticum* by comparatively long flabellae, but differs from the latter by the structure of the cornua (with two vs three tines respectively). It is unclear whether *E. cheliferum* has two median or four submedian flabellae.

[12] *Wilsonema agrarum* Nesterov, 1973 resembles *Ereptonema arcticum* by annulated cervical expansions and comparatively long flabella. No other useful information could be taken from the original description and schematic drawings. The taxonomic position of the species could not be stated confidently without re-examination of the type material. We regard it as species inquirenda. Khan et al. (1990: p. 439-440) erroneously used "*Wilsonema agasatum* Nesterov" and "*W. agastum* Nesterov" for *W. agrarum* (now in *Ereptonema*) – *lapsus calami*.

[13] *Plectus (Wilsonema) gangulykhani* Zell, 1993 = nomen novum by Zell (1993) pro *Coronacephalus indicus* Ganguly & Khan, 1986 = *Plectus (Wilsonema) indicus* Ganguly & Khan, 1986 (Zell, 1993) – secondary homonym of *Plectus (Plectus) indicus* Khera, 1972 (Zell,1993) = *Plectus indicus* Khera, 1972. Since these species are placed in different genera the homonymy is absent and the original name should be reinstated.

[14] *Neotylocephalus haryanensis* Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003 nomen novum by Holovachov et al. (2003) pro *Neotylocephalus indicus* (Walia & Bajaj, 1999) = *Wilsereptus indicus* Walia & Bajaj, 1999, a secondary homonym of *Neotylocephalus indicus* (Ganguly & Khan, 1986) Holovachov, Boström, Tandingan De Ley, De Ley & Coomans, 2003 = *Coronacephalus indicus* Ganguly & Khan, 1986. By the morphology of the anterior end, i.e. presence of short midlateral projection(s), and non-annulated cervical expansions, smooth lateral rims and (probably) four submedian flabella (judging from schematic drawings), the specimens described by Walia & Bajaj (1999) resemble *Neotylocephalus*. By the relatively short flabella they come close to N. annonae from which they differ by the absence of setae in pharyngeal region and presence of three caudal setae (vs four). Absence of some important taxonomic characters in the description, makes it impossible to be confident about the status of this species without re-examination of type material.

Chapter 5

Morphology, phylogeny and evolution of the superfamily Plectoidea Örley, 1880

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Abstract

The phylogeny and classification of the superfamily Plectoidea is revised on the basis of published and updated morphological data for 35 ingroup and 2 outgroup species. The following features are here considered to support the monophyletic origin of the superfamily: **1)** stegostom developed and differentiated into two sections; **2)** dorsal gland orifice opening into the second stegostom section; **3)** pharynx cylindrical, with distinct subdivision into corpus and postcorpus by the orifices of the subventral pharyngeal glands and a discontinuity in the muscular pharyngeal tissue; **4)** corpus cylindrical, with subdivision into procorpus and metacorpus homologues; **5)** pharyngeal radii of the corpus with prominent pharyngeal tubes along the procorpus; **6)** cuticular lumen of the basal part of postcorpus (within basal bulb if latter is present) is modified to form a valvular apparatus. In addition the inner labial sensilla open inside the cheilostom. New data on postembryonic development of *Anaplectus grandepapillatus, Plectus parietinus, P. decens* and *P. communis* are given and supplemented with a discussion of the phylogenetic significance of the ontogeny in Plectoidea. Following the proposal of a phylogeny, some key events in the evolution of Plectidae are discussed. It is suggested that the superfamily Plectoidea includes four families: Pakiridae (*Pakira*), Chronogastridae (*Caribplectus, Chronogaster* and *Cynura*), Metateratocephalidae (*Euteratocephalus* and *Metateratocephalus*) and Plectidae (*Anaplectus, Arctiplectus, Perioplectus, Plectus, Ceratoplectus, Tylocephalus, Ereptonema, Neotylocephalus* and *Wilsonema*).

Nomenclatorial notes

Folloving nomenclatorial changes are proposed in the original publication: **1)** the genus *Chiloplectus* Andrássy, 1984 was considered a junior synonim of the genus *Plectus* Bastian, 1865; **2)** the genus *Keralanema* Siddiqi, 2003 was considered synosymous with the genus *Chronogaster* Cobb, 1913; **3)** the family Anaplectidae Zell, 1993 was downgraded to the rank of a subfamily; **4)** *Plectolaimus supplementatus* Keppner, 1988 was transferred to the genus *Caribplectus* Andrássy, 1973 resulting in a new combination *Caribplectus supplementatus* (Keppner, 1988) Holovachov, 2004. The date of publication of new names and new combinations, which were introduced in the original article, is 23 December 2004.

Introduction

The genus *Plectus* Bastian, 1865 is one of the most widely distributed and common nematode taxa in freshwater and terrestrial habitats, occurring all over the world (Zell, 1993). It often attracts the attention of nematode taxonomists, thus resulting in a rather large number of nominal species described (about 70 species, see Holovachov & De Ley (2005) for detailes) and taxonomic papers published. The first detailed morphological and taxonomic studies of this genus were performed by Maggenti (1961a, 1961b), later supplemented by papers of Andrássy (1985, 1998), Zell (1993) and De Ley & Coomans (1994). The relationships of *Plectus* with other nematode taxa have been considered several times based on different approaches and still remains a matter of discussion. The group is of particular interest for the origin of the Rhabditida, which includes several scientifically and economically important groups of nematodes such as plant and animal parasites of the suborders Tylenchina and Spirurina, and the model species for molecular, developmental and genetic studies (*Caenorhabditis elegans*).

The similarities between plectids and rhabditids were noted already by Bütschli (1873) and Örley (1880), but only Chitwood & Chitwood (1974) and Maggenti (1963) discussed the relationships between Plectidae and secernentean nematodes in more detail. The latter author suggested that "on gross examination the Plectidae and Rhabditidae seem to exhibit a resemblance of morphologic structures. Close examination reveals, however, that their similarity of structure is the result not of homologous development, but of convergent development" (Maggenti, 1963). Contrary to this, Paramonov (1964) listed several characters to prove the hypothesis of the plectid origin of Secernentea: presence of a putative deirid, cylindrical stoma with joint rhabdions, pharynx with corpus, isthmus and basal bulb holding a valvular apparatus, complex renette with long and cuticularised excretory duct, rectal glands and meromyarian somatic musculature. All these characters were considered by Paramonov (1964) to be homologous in plectids and rhabditids and he argued that rhabditids originated from primitive plectids under saprobiotic conditions. However, all views mentioned above were based strictly on morphological data and were often criticised, because of the particular difficulties with the use of morphological similarity for distinguishing between homology and convergence (homoplasy).

On the other hand, the parsimony analysis by Litvaitis et al. (2000) based on nucleotide sequences suggested a link between *Plectus* and Rhabditida. Blaxter et al. (1998) found *Plectus* to be the sister taxon of Secernentea or of the ascarid-spirurid clade within Secernentea, depending on the analytical criteria used in the phylogenetic analysis of SSU rRNA sequences. De Ley & Blaxter (2002) downgraded the entire class Secernentea to order Rhabditida and placed family Plectidae Örley, 1880 in the centre of the order Plectida Malakhov, 1982, but they pointed out that the taxonomic position and relationships of many families currently placed in the Plectida are not yet known.

Tahseen et al. (1992) when describing the new species *P. zelli* Tahseen, Ahmad & Jairajpuri, 1992 gave the following additional information: there are four juvenile stages; the cleavage pattern is similar to that in *Hypodontolaimus inaequalis* (Bastian, 1865) de Man, 1886*, Caenorhabditis elegans* and *Teratorhabditis andrassyi* Tahseen & Jairajpuri, 1988 with the first three divisions all along precisely similar planes resulting in the formation of a rhomboid four cell stage, but postembryonic development is unlike that of

Secernentea because development of the female gonads starts from two separate primordia. Embryological studies of several species of *Plectus*, *Tylocephalus auriculatus* and *Ereptonema arcticum* by Lahl et al. (2003) showed that Plectidae share many basic features of early development with the order Rhabditida but not with the other investigated Adenophorea, although the early embryogenesis of Plectidae differs from Rhabditida in particularly two aspects, i.e. formation of bilateral symmetry and gastrulation. Finally, Fürst von Lieven (2003) described and compared the morphology and functioning of the three-part pharynx in Cephalobidae, Rhabditidae and Plectida, and suggested a common origin of the "grinder" (=valvular apparatus) in the basal bulb of Plectidae and Secernentea.

I therefore made an attempt to provide a phylogenetic analysis of the origin and evolution of the genus *Plectus* and its relatives, grouped in the superfamily Plectoidea. This analysis is based on updated morphological data, uses the comparative morphological approach, and combines the principles of cladistic analysis and evolutionary taxonomy, which may further be used for the evaluation of the relationships between plectids and rhabditids.

History

Although the first members of the superfamily Plectoidea (in its present understanding) were described already in 1865 by Bastian, detailed taxonomic analyses of the group appeared about one hundred years later (see Table 5.1). Gagarin (1975) provided the first detailed analysis of the composition and phylogeny of the superfamily Plectoidea, based on the morphology of the stoma, pharynx and excretory system, and proposed a hypothesis of the phylogeny of the group. He also proposed the new family Chronogasteridae Gagarin, 1975 (emended to "Chronogastridae" by Siddiqi, 2003) and discussed the morphological peculiarities of the family Teratocephalidae Andrássy, 1958.

Andrássy (1976) characterised the superfamily Plectoidea as having: "anterior part of stoma somewhat widened; terminal bulb with valvular apparatus; female gonads paired; male genital supplements tubular, cuticularised and protrusible, or, rarely, papilliform". He listed a single family, Plectidae, containing two subfamilies and eight genera. *Chronogaster* was considered to belong to the family Leptolaimidae, whereas *Euteratocephalus* Andrássy, 1958 and *Metateratocephalus* Eroshenko, 1973 were placed within the Rhabditida (Table 5.1).

Lorenzen (1981) made no groupings of families to higher taxa in the suborder Leptolaimina, since he was unable to find criteria to establish holophyletic superfamilies. He included the families Chronogastridae, Plectidae and Teratocephalidae side by side with 14 other families in the suborder Leptolaimina (Table 5.1). The holophyly of the family Chronogastridae was established by the holapomorphy that females always have monoprodelphic gonads; and this family was also characterised by the following features: the pharynx has one ventral and two subdorsal pharyngeal tubes just posterior to the buccal cavity; the cervical gland outlet is cuticularised only a short distance; not coiled up. Lorenzen (1981) found no holapomorphy to establish the holophyly of Plectidae, but described the family as having: cuticle with lateral field; deirids present; three tubes in the pharynx, one ventral and two subdorsal; pharyngeal muscular bulb with a valvular

Table 5.1. Composition of the superfamily Plectoidea Örley, 1880 – opinions by different authors.

System of Plectoidea after Gagarin (1975)

- Chronogasteridae Gagarin, 1975
    - *Chronogaster* Cobb, 1913
- Plectidae Örley, 1880
    - Plectinae Örley, 1880
        - *Anaplectus* De Coninck & Schuurmans Stekhoven, 1933; *Periplectus* Sanwal, 1968; *Plectus* Bastian, 1865
    - Wilsonematinae Chitwood, 1951
        - *Ereptonema* Anderson, 1966; *Tylocephalus* Crossman, 1933; *Wilsonema* Cobb, 1913
- Teratocephalidae Andrássy, 1958
    - *Teratocephalus* de Man, 1876; *Euteratocephalus* Andrássy, 1958

System of Plectoidea after Andrássy (1976)

- Plectidae Örley, 1880
    - Plectinae Örley, 1880
        - *Anaplectus* De Coninck & Schuurmans Stekhoven, 1933 (Syn.: *Marinoplectus* Kreis, 1963); *Oligoplectus* Taylor, 1935*; *Perioplectus* Sanwal in Gerlach & Riemann, 1973 (Syn.: *Periplectus* Sanwal, 1968); *Plectus* Bastian, 1865 (Syn.: *Plectoides* de Man, 1904; *Pycnolaimus* Cobb, 1920); *Proteroplectus* Paramonov, 1964
    - Wilsonematinae Chitwood, 1951
        - *Ereptonema* Anderson, 1966; *Tylocephalus* Crossman, 1933; *Wilsonema* Cobb, 1913 (Syn.: *Bitholinema* De Coninck, 1931); *Wilsotylus* Chawla, Khan & Prasad, 1970 (Syn.: *Neotylocephalus* Ali, Farooqui & Tejpal, 1969)

System of Lorenzen (1981): families of Leptolaimina considered in the present paper

- Chronogasteridae Gagarin, 1975
    - *Chronogaster* Cobb, 1913 (Syn.: *Walcherenia* de Man, 1921)
- Plectidae Örley, 1880
    - Plectinae Örley, 1880
        - *Anaplectus* De Coninck & Schuurmans Stekhoven, 1933 (Syn.: *Marinoplectus* Kreis, 1963); *Oligoplectus* Taylor, 1935*; *Perioplectus* Sanwal in Gerlach & Riemann, 1973 (Syn.: *Periplectus* Sanwal, 1968); *Plectus* Bastian, 1865 (Syn.: *Plectoides* de Man, 1904; *Proteroplectus* Paramonov, 1964)
    - Wilsonematinae Chitwood, 1951
        - *Anthonema* Cobb, 1906; *Ereptonema* Anderson, 1966; *Pycnolaimus* Cobb, 1920; *Tylocephalus* Crossman, 1933; *Wilsereptus* Chawla, Khan & Saha, 1975; *Wilsonema* Cobb, 1913 (Syn.: *Bitholinema* De Coninck, 1931); *Wilsotylus* Chawla, Khan & Prasad, 1970 (Syn.: *Neotylocephalus* Ali, Farooqui & Tejpal, 1969)
- Teratocephalidae Andrássy, 1958
    - Teratocephalinae Andrássy, 1958
        - *Teratocephalus* de Man, 1876 (Syn.: *Mitrephoros* Linstow, 1877)
    - Metateratocephalinae Eroshenko, 1973
        - *Euteratocephalus* Andrássy, 1958 (Syn.: *Metateratocephalus* Eroshenko, 1973)

* - problematic fossil taxon

apparatus; and the cuticularised outlet of the cervical gland twisted in the region of the pharyngeal bulb. Finally, the holophyly of the Teratocephalidae was established by the holapomorphy that the labial region has six deep cuticularised incisions and six extensions.

Material

Most of the material studied here was collected by the author in the Ukraine or came from the following collections and institutions: Laboratorium voor Nematologie, Wageningen Universiteit, Nederland (*Euteratocephalus palustris, Chronogaster multispinatoides, Cynura* sp, *Hemiplectus muscorum*), Institut für Nematologie und Wirbeltierkunde, Biologische Bundesanstalt für Land- und Forstwirtschaft, Münster, Deutschland (*Pakira orae, Chronogaster* sp 1, *Chronogaster* sp 2), UC Davis Nematode Collection, Department of Nematology, University of California, Davis, CA, USA (*Chronogaster spinicorpus*), Escuela de Ciencias Agrarias, Universidad Nacional, Heredia, Costa Rica (*Caribplectus magdalenae*), Muzeum i Instytut Zoologii PAN, Warszawa, Poland (*Chronogaster boettgeri*). Additional specimens were extracted from samples collected in Russia and Antarctica by colleagues. Label data for the recent material examined in this study are given below. To clarify the structure of the digestive system in the genus *Cynura* I used seven unidentified juveniles of this genus from Cata, Venezuela.

The species listed below were included in the morphological comparison, analysis of postembryonic development and phylogenetic investigation. For ***Arctiplectus alaskanus*** Andrássy, 2003, ***Chronogaster andrassyi*** Loof & Jairajpuri, 1965, ***Cynura cerambus*** Andrássy, 1973, ***C. klunderi*** Murphy, 1965, ***Perioplectus labiosus*** (Sanwal, 1968) Sanwal in Gerlach & Riemann, 1973 relevant data were extracted from the original descriptions. New material includes the 24 species listed below.

Anaplectus grandepapillatus (Ditlevsen, 1928) Andrássy, 1973: 6♀♀, 6♂♂, 26 juv, Russia, Altai Mountains, Tiungur village, Akkem river, *Larix* litter, September 2000, legit I. Dykyy, Fig. 5.8.

Caribplectus magdalenae (Riemann, 1970) Andrássy, 1973[*]: 3♂♂, Costa Rica, Puerto Vargas, brackish environment, Figs 5.2 A-E; additional data taken from Andrássy (1973).

Ceratoplectus assimilis (Bütschli, 1873) Andrássy, 1984: 20♀♀, 1♂, Russia, Altai Mountains, Tiungur village, Akkem river, moss and litter, 1400 m a.s.l., September 2000, legit I. Dykyy, Figs 5.5 D, E, J, W, 5.7 D.

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[*] Recent specimens identified as *C. magdalenae* are morphologically intermediate (1.86-2.29 mm long body, 38-40.5 µm long spicules, 18-20 tubular supplements) between two male specimens of this species described in literature by Riemann (1970) from Colombia (1.58 mm long body, 27 µm long spicules, 16 tubular supplements) and by Andrássy (1973) from Cuba (1.63 mm long body, 33 µm long spicules, 19 tubular supplements) and between males of *Caribplectus supplementatus* (Keppner, 1988) Holovachov, 2004 (= *Plectolaimus supplementatus* Keppner, 1988) from Florida, USA (1.95-2.08 mm long body, 41 µm long spicules, 21-23 tubular supplements). However, study of type material of both species is necessary to confirm or reject the conspecific status of *C. magdalenae* and *C. supplementatus*.

Chronogaster boettgeri Kischke, 1956: 10♀♀, Ukraine, Volynska province, Shutsk National Natural Park, Pisotchne lake, sandy soil and rhizosphaere of semiaquatic plants on the shore of the lake, June 2000, legit O. Holovachov; additional data taken from Winiszewska (1998); the male described by Winiszewska (1998) was re-studied Fig. 5.3 G. A SEM photograph of the labial region is depicted on Fig. 11 D in Holovachov & De Ley (2005).

C. multispinatoides Heyns & Coomans, 1983: 3♀♀, 1♂, USA, Georgia, Tifton, wet meadow, Fig. 5 F in Holovachov & De Ley (2005); additional data taken from Heyns & Coomans (1984).

C. spinicorpus Maggenti, Raski, Koshy & Sosamma, 1983 = *Keralanema spinicorpus* sensu Siddiqi (2003): 1♀, 6♂♂ paratypes, India, Kerala, Kayangulam, soil around roots of palm, June 1979 and April 1980, legit V.K.Sosamma, Fig. 5.3 D.

C. typica (de Man, 1921) De Coninck, 1935: 27♀♀, 1♂, Ukraine, Volynska province, Shutsk National Natural Park, Pisotchne lake, sandy soil and rhizosphere of semiaquatic plants on the shore, June 2000, legit O. Holovachov, Fig. 5.3 A-C, F.

***Chronogaster* sp 1**[*]: 1♀, 2♂♂, Cameroon, Mbode settlement, about 30 km south of Kribi, in northern part of Campo Reserve, virgin rainforest about 3 km east of coastal line, with almost no understory vegetation and sandy soil covered by fallen leaves, March 1994, legit D. Sturhan, Fig. 5.3 E.

***Chronogaster* sp 2**[**]: 1♀, Cameroon, Mbode settlement, about 30 km south of Kribi, in northern part of Campo Reserve, virgin rainforest about 3 km east of coastal line, with almost no understory vegetation and sandy soil covered by fallen leaves, March 1994, legit D. Sturhan, Fig. 5.15.

Ereptonema arcticum Loof, 1971: 1♂, Ukraine, Lvivska province, Roztochya Natural Reserve, rotting wood, May 2003, legit A. Zatushevsky, Fig. 5.7 E; additional data taken from Chapter 4.

Euteratocephalus palustris (de Man, 1880) Andrássy, 1958: 20♀♀, 1♂, Ukraine, Volynska province, Shutsk National Natural Park, Pisotchne lake, sandy soil and rhizosphere of semiaquatic plants on the shore of the lake, June 2000, legit O. Holovachov, Fig. 5.4 A-C, E; 1♂, the Netherlands, Fig. 5.4 D; additional data taken from Karegar et al. (1997).

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[*] This species is particularly characterised by the structure of its cuticle (ornamented with fourteen rectangular ridges arranged in longitudinal rows along the body), gonochoristic mode of reproduction and measurements, thus differing from all other species of the genus. The complete description and diagnosis of this species will be published in a separate paper.

[**] The single female of *Chronogaster* sp 2 shows a very peculiar structure of stoma (long and broad anterior stegostom section), pharynx (valvular apparatus in the shape of longitudinal denticulate plates, without rounded thickenings in its posterior part) and vagina (with vaginal sclerotizations), never found in any other species of this genus. It also has the amphid shape typical for the genus *Chronogaster* (stirrup-shaped with paired projections on the posterior rim of the amphidial fovea), reproductive system (monoprodelphic) and tail (caudal glands absent). Although the availability of only a single specimen prevents us from ascertaining the taxonomic position of this nematode, its morphology is very valuable from a phylogenetic point of view and is further discussed in this chapter. See also Fig. 5.15.

Metateratocephalus gracilicaudatus Andrássy, 1985: 9♀♀, 1♂, Ukraine, Volynska province, Shutsk National Natural Park, Pisochne lake, pine-birch forest, *Betula* sp., litter and soil, 29.05.2000, legit O. Holovachov, Fig. 5.4 F-H.

Pakira orae Yeates, 1967: 1♂, 1♀, New Zealand, Putara, native forest, December 1996, legit D. Sturhan, Fig. 5.1; additional data taken from Heyns & Coomans (1990).

Plectus aquatilis Andrássy, 1985: 1♀, 1♂, Ukraine, Volynska Province, Shutsk National Natural Park, wet meadow, June 2000, legit O. Holovachov; additional data taken from Zell (1993)

P. cancellatus Zullini, 1978 = *Chiloplectus cancellatus* sensu Holovachov et al. (2000): 20♀♀, 1♂, Russia, Altai Mountains, Tiungur village, Akkem river, *Larix* litter, 1000 m a.s.l., September 2000, legit I. Dykyy, Fig. 5.7 F; additional data taken from Holovachov et al. (2000).

P. communis Bütschli, 1873: 20♀♀, 22♂♂, Ukraine, Volynska province, Shutsk National Natural Park, pine forest, moss, June 2000, legit O. Holovachov, Figs 5.5 A, Q, V, 5.6 B-D; 9♀♀, 1♂, Ukraine, Lviv province, National Park "Skolivski Beskydy", moss on a rock, July 1998, legit. O. Holovachov; 20♂♂, Ukraine, Lviv province, Roztochya Natural Reserve, moss, April, 1998, legit O. Holovachov; 10♀♀, 36 juv, Ukraine, Volynska province, Shutsk National Natural Park, pine forest, litter, October 2000, legit O. Holovachov, additional data taken from Zell (1993).

P. decens Andrássy, 1985: 2♀♀, 1♂ Ukraine, Volynska province, Shutsk National Natural Park, Pisotchne lake, sandy soil and rhizosphaere of semiaquatic plants on the shore, June 2000, legit O. Holovachov, Figs 5.5 I, O, X, 5.7 G; 14♀♀, 1♂, Ukraine, Ivano-Frankivsk province, Carpathian National Natural Park, Zaroslyak, soil, legit I. Kaprus; 10♀♀, 46 juv, Ukraine, Lviv province, Yavoriv district, Nemyriv village, sphagnum, September 2002, legit A. Zatushevskyy, Fig. 10; additional data taken from Zell (1993).

P. geophilus de Man, 1880 (= *P. minor* Novikova & Gagarin, 1971): 6♀♀, 3♂♂, Ukraine, Crimea, Jaltynsky district, Simeiz town, Kishka mountain, *Homalothecium lutescens* moss, July 1998, legit O. Holovachov, Figs 5.5 F, K, R, 5.7 B; 4♀♀, 1♂, Ukraine, Sumy province, Lebedyn district, Ukrainian Steppe Reserve, "Mykhailivska Tsilyna", pine litter, August 1997, legit O.Holovachov; additional data taken from Novikova & Gagarin (1971).

P. minimus Cobb, 1893: 9♀♀, 21♂♂, Ukraine, Volynska province, Liubeshiv district, Khotsun village, meadow, grassland, June 2002, legit O. Holovachov, Figs 5.5 H, L, U, 5.7 C; 1♀, 1♂, Ukraine, Zakarpatska province, Rakhiv district, Kvasy village, Karpathian Biosphaere Reserve, Petros mountain, soil around roots of *Saxifraga aizoon,* July 2002, legit O. Holovachov; 10♀♀, 2♂♂, Ukraine, Crimea, Jaltynsky district, Simeiz town, Kishka mountain, *Frullania dilatata* moss, July 1998, legit O. Holovachov; additional data taken from De Ley & Coomans (1994), Hernandez et al. (1998).

P. murrayi Yeates, 1970: 8♀♀, 1♂, Antarctic peninsula, moss, 2003, Fig. 5.6 A.

P. parietinus Bastian, 1865: 10♀♀, 1♂, Ukraine, Crimea, Jaltynsky district, Simeiz town, Kishka mountain, *Frullania dilatata* moss, July 1998, legit O. Holovachov, Figs 5.5 C, N, 5.6 E; 1♂, Ukraine, Crimea, Jaltynsky district, Partenit town, Au-Dag mountain, reserve, July 1996, legit O. Holovachov; 20♀♀, 3♂, 76 juv, Russia, Altai Mountains, Tiungur village, Akkem river, moss and *Betula* litter, September 2000, legit I. Dykyy, Figs 5.7 I-J, 5.9; additional data taken from Zell (1993).

P. parvus Bastian, 1865: 16♀♀, 10♂♂, Ukraine, Crimea, Jaltynsky district, Simeiz town, Kishka mountain, *Frullania dilatata* moss, July 1998, legit O. Holovachov, Figs 5.5 G, P, S, 5.7 A; 1♀, 1♂, Ukraine, Lugansk province, Lugansky Natural Reserve, "Provalsky step", steppe, soil, November 2002, legit O. Kondratenko; 4♀♀, 4♂♂, Ukraine, Volynska province, Shutsk National Natural Park, Pisotchne lake, sandy soil and rhizosphaere of semiaquatic plants on the shore of the lake, July 2004, legit V. Merlavskyy, additional data taken from Maggenti (1961b), Zell (1993).

P. velox Bastian, 1865 = *P. parietinus* sensu Maggenti (1961b) partim: 20♀♀, 2♂♂, Ukraine, Lviv, Khomets, dry meadow, soil, September 2000, Figs 5.5 B, M, T, 5.6 F, 5.7 H; 1♂, Ukraine, Volynska Province, Shutsk National Natural Park, wet meadow, June 2001, legit O. Holovachov; additional data taken from Maggenti (1961b).

Wilsonema otophorum (de Man, 1880) Cobb, 1913: 6♀♀, 2♂♂, Ukraine, Volynska province, Liubeshiv district, Khotsun village, meadow, grassland, June 2002, legit O. Holovachov; additional data taken from Chapter 4.

Morphology of the following ten species was taken from subsequent redescriptions: ***Anaplectus atubulatus*** Andrássy, 1987 and ***A. granulosus*** (Bastian, 1865) De Coninck & Schuurmans Stekhoven, 1933 (from Holovachov et al., 2004); ***Ceratoplectus armatus*** (Bütschli, 1873) Andrássy, 1984 (from De Ley & Coomans, 1994; Mulk & Coomans, 1978; Novikova, 1971); ***Hemiplectus muscorum*** Zell, 1991 (from Zell, 1991; Chapter 3); ***Plectus andrassyi*** Timm, 1961 = *Chiloplectus andrassyi* sensu Andrássy (1984, 1985) (from Holovachov et al., 2000; Mulk & Coomans, 1978); ***P. longicaudatus*** Bütschli, 1873 (from Hernandez et al., 1998; Zell, 1993); ***P. paracuminatus*** Zell, 1993 (from Holovachov et al., 2001; Zell, 1993); ***P. pusteri*** Fuchs, 1930 (from Holovachov, 2001; Zell, 1993); ***Stephanolaimus elegans*** Ditlevsen, 1919 (from Chapter 3); ***Tylocephalus auriculatus*** (Bütschli, 1873) Crossman, 1933 (from De Ley & Coomans, 1997; Holovachov et al., 2004).

Methods

Specimens were extracted by a range of methods: Baermann funnel extraction, decanting and sieving method with final separation on extraction sieves with filters, and centrifugal flotation. Nematodes were relaxed by gentle heat, fixed in cold TAF or 4% formaldehyde, processed to pure glycerine by a slow evaporation method or a rapid method according to Seinhorst (1959) and mounted on permanent glass or Cobb slides in glycerine with paraffin wax as support for the coverslip.

The outgroup comparison is thought to be the weakest point of the cladistic analysis (Iwan, 2002), because there are no guarantees that any particular outgroup will ensure a correct inference. Since previous phylogenetic studies did not reveal the unequivocal sister-group relationships of Plectoidea with its closest relatives, the direct outgroup comparison as postulated in cladistic literature (Forey et al., 1996; Wiley et al., 1991) was not used. All characters were polarised before the tree-building procedures, by using several phylogenetic criteria, which were discussed particularly by Riemann (1977) and Rasnitsyn (2002).

For the phylogenetic analysis, multistate characters were re-coded into binary characters using the FACTOR programme of PHYLIP (Phylogeny Inference Package) Version 3.61 (Felsenstein, 1989) in the following way: i) multistate characters “A” and “B”, that developed independently from the same primitive state “P” (A←P→B) were coded as: “P” = 00, “A” = 01, “B” = 10; ii) multistate characters that developed sequentially (ordered), “A” from “P” and then “B” from “A” (P→A→B) were coded as: “P” = 00, “A” = 01, “B” = 11. Characters, that were not seen, but may be present, or unavailable from the literature were coded as “?”; not applicable data were coded as “-“

Tree-building procedures of the entire dataset were performed using the MIX programme, based on WAGNER (Kluge & Farris, 1969) and CAMIN-SOKAL (Camin & Sokal, 1965) optimality criteria, of the PHYLIP 3.61 package. A strict consensus tree was obtained using the CONSENSE programme of the same package. Some of the trees were plotted and edited using TreeView 1.6.6 (Page, 2001). The entire dataset was also analysed with the aid of NONA 2.0 (Goloboff, 1993) and WINCLADA (Nixon, 2002).

Body volume was calculated according to the formula proposed by Tsalolikhin (1980): $V = \left[\pi L\left(d^2 + dD + D^2\right) + \pi L D^2\right]/24$ with L – body length, d – labial region diameter, D – body diameter. Egg volume was calculated according to Zullini & Pagani (1989): $V_o = (D_o/2)^2 \pi (L_o - D_o) + 4/3 \pi (D_o/2)^2$ where L_o – egg length and D_o – egg diameter.

The most recent nematode classification by De Ley & Blaxter (2002) is followed throughout the paper. The terminology of male copulatory sensilla is given in Holovachov et al. (2001) and terminology of the labial region structures is given in Chapter 4. The following terms are used in the present paper: **1)** “renette cell” is used instead of “ventral gland” or “ventral secretory-excretory gland” (Turpeenniemi & Hyvärinen, 1996); **2)** “valvular apparatus” or “valves” instead of “grinder”, since the term “grinder” implies a restricted function that was apparently not observed directly to occur; **3)** “cardia” instead of “oesophago-intestinal valve”; **4)** “pharyngeal tubes” instead of “radial tubules”; **5)** “basal bulb” instead of “cardiac bulb”; **6)** “lateral alae” is used here for the external longitudinal bands of smooth cuticle, while “lateral chord” is used throughout the paper for the internal longitudinal bands of hypodermal cells on the lateral sectors of the body.

Ingroup: a search for the common ancestry

Following the diagnosis of Gagarin (1975), the superfamily Plectoidea is particularly characterised by the structure of pharynx, which is subdivided into corpus, isthmus and basal bulb, the latter including the valvular apparatus. He also listed a number of other characters, such as number and structure of anterior sensilla, shape of amphid, structure of excretory system, female and male reproductive systems and tail. However, later researchers (Lorenzen, 1981) were unable to establish a monophyletic origin of the superfamily on the basis of morphological data, since most of the characters listed by Gagarin are plesiomorphic.

In the following paper I consider the structure of the digestive system as a major feature on which the monophyletic origin (holophyly) of the superfamily Plectoidea could be established using the principle of morphological complexity: **1)** stegostom developed and differentiated into two sections; **2)** dorsal gland orifice opens into the second stegostom section; **3)** pharynx cylindrical, with distinct subdivision into corpus and postcorpus by the orifices of the subventral pharyngeal glands and discontinuity in the muscular pharyngeal tissue; **4)** corpus cylindrical, with subdivision into procorpus and metacorpus homologues; **5)** pharyngeal radii of the corpus ending in prominent pharyngeal tubes along the procorpus; and **6)** cuticular lumen of the basal part of postcorpus (within basal bulb if latter is present) is modified forming a valvular apparatus. In addition, the inner labial sensilla open inside the cheilostom.[*]

The following taxa of Chromadorea or Plectida fit within the above proposed "diagnosis": the genera *Pakira* Yeates, 1967, *Cynura* Cobb, 1920, *Caribplectus* Andrássy, 1973, *Chronogaster* Cobb, 1913, *Euteratocephalus* Andrássy, 1958 and *Metateratocephalus* Eroshenko, 1973 and the family Plectidae (sensu Lorenzen, 1981).

Variability and phylogenetic interpretation of the adult morphology[**]

Cuticle and hypodermis

In most species, the cuticle is finely to coarsely annulated, usually without additional outgrowths. Annules are very fine and visible only under SEM in *Euteratocephalus* and *Metateratocephalus*. On the other hand these two genera have prominent intracuticular ornamentation composed of transverse rows of refractive granules, which are located in the median cuticular layer. Similar intracuticular ornamentation has been found in some Chromadorida, but since its origin, function and ultrastructure are not yet clear, one is not able to prove or disprove a common origin of the intracuticular ornamentation in at least the families Metateratocephalidae and Achromadoridae (Eyualem & Coomans, 1995). Due to the relative rarity of intracuticular ornamentation in nematodes, and absence of it in other Plectoidea, I consider it to be apomorphic for the two above mentioned genera and derived from a simpler annulated cuticle.

Out of the 35 analysed species, only three have an annulated cuticle, with additional ornamentation besides the annulation itself. Among them *Plectus cancellatus* has an annulation interrupted by deep longitudinal incisures, starting somewhat posterior to the anterior end and continuing on the tail. Longitudinal striation of the annulated cuticle occurs frequently in many unrelated groups of nematodes (in Xyalidae, Cephalobidae and Tylenchidae, etc.) and is considered to be derived from the regularly annulated cuticle. The appearance of longitudinal striation may be connected with increased thickness of the

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[*] At the first glance most of the features could be plesiomorphic or homoplastic throughout the phylum Nematoda. However, ultrastructural and developmental studies are needed to clarify this point. For instance pharyngeal tubes are present in the post-stomatal region of the pharynx in some Comesomatidae (pers. obs.) and Axonolaimidae (Maggenti, 1963), while seemingly the same structure of the pharynx is present in Rhabditida.

[**] Most of the references are not given in this section of the chapter in order to make it easy to read; they are all summarised in the "Material" section.

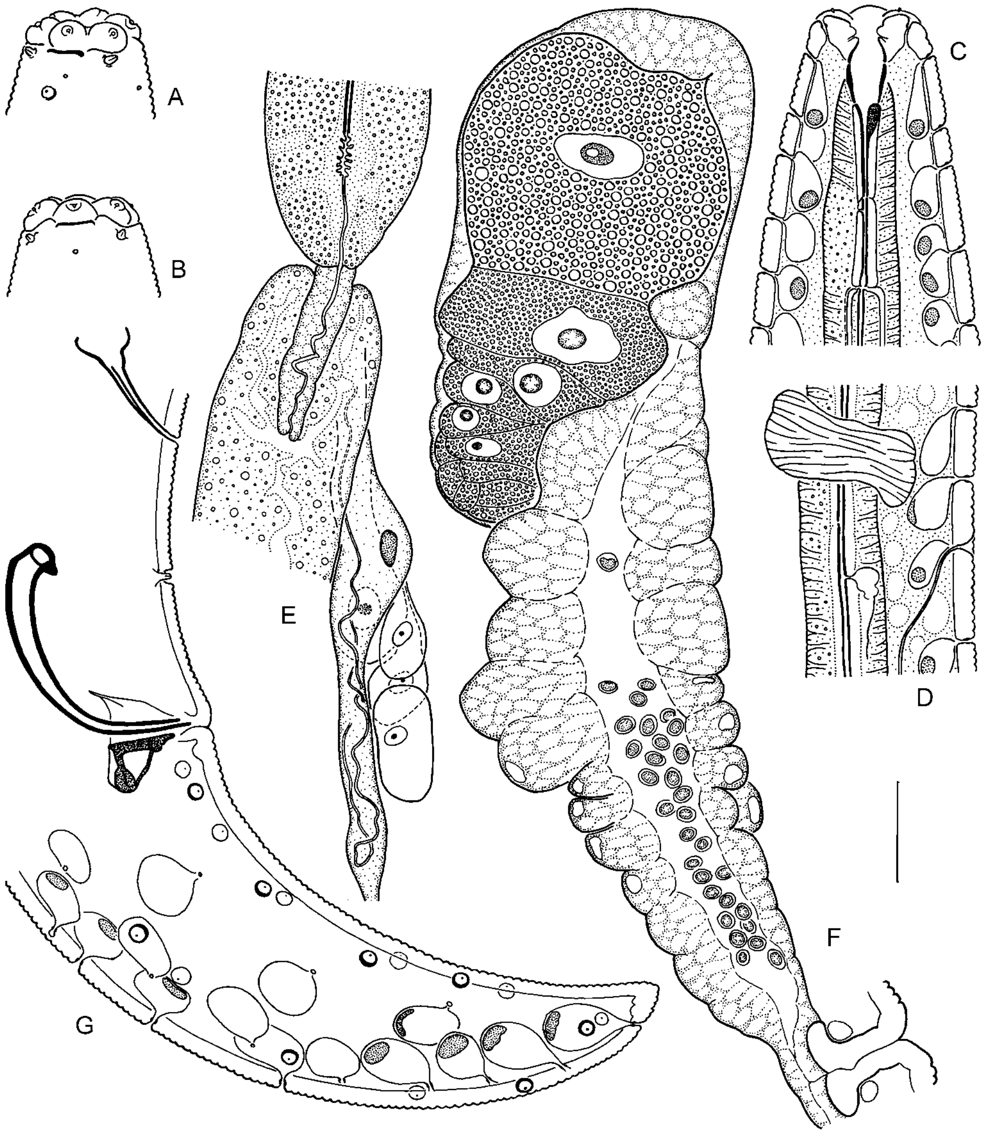

Figure 5.1. Morphology of the family Pakiridae Inglis, 1983: *Pakira orae* Yeates, 1967. A: Male labial region, surface view; B: Female labial region, surface view; C: Anterior end, median section; D: Nerve ring, excretory pore and subventral gland orifices; E: Posterior part of pharynx, renette and coelomocytes; F: Anterior female genital branch; G: Male posterior end. Scale bar 20 µm.

cuticle: compare cuticle thickness in *Plectus cancellatus* (3.3-3.7 µm) with *P. andrassyi* (1.9-3.3 µm), where the latter species does not have longitudinal incisures (Holovachov et al., 2000). The increased thickness of the cuticle may be connected with an increased protective function. At the same time a thick cuticle is less flexible, constraining locomotion and expansion of body diameter. The longitudinal incisures may thus preserve regular flexibility in the relatively thicker cuticle.

Two other studied species with modified cuticle belong to the genus *Chronogaster*. Among them an undescribed species, here named *Chronogaster* sp 1, has each annule "armed" with fourteen rectangular ridges, arranged in longitudinal rows along the body (Fig. 5.3 E). They start on the first body annule and extend to the posterior part of the tail. The other species, *C. spinicorpus*, has a cuticle ornamented with up to twelve longitudinal rows of hook-like spines attached to the middle of each annule and overlap the preceding and succeeding annules (Fig. 5.3 D). Several similarities are present between these two species: a cup-shaped labial region, amphids with a strong posterior projections, a long postuterine branch, filiform tail terminus and presence of males (bisexuality). These suggest a close relationship between the two species, and it is likely that the cuticular ornamentation of *Chronogaster* sp 1 is an intermediate state between a relatively simple annulated cuticle and the complex cuticle of *C. spinicorpus*.

To understand the function of the above mentioned types of cuticular ornamentation is not easy without direct observation on living specimens. I suppose that cuticular outgrowths make rubbing of the cuticle against solid particles of the soil more effective, thus improving the locomotion of the nematode in small cavities of the soil. Therefore, I suggest that presence of cuticular ornamentation is a derived character, adapted to certain substrate structures. Finally, the regularly annulated cuticle is thought to be the most primitive character state within the group (also the most common case in nematodes).

Another modification of the cuticle called lateral differentiation appears in Plectida as one or several longitudinal alae of generally smooth cuticle located within the lateral sectors of the body. It is thought that lateral differentiation originates at the base of the lateral chords and functions in two possible ways: as antagonists to ventral or dorsal muscle sets (rigid ribs), or as a "sliding surface". In any case, presence of lateral differentiation may influence movement and expansion of diameter of the nematode, and is more common in terrestrial nematodes (Rhabditida) than aquatic ones (Monhysterida, Enoplida etc). The presence of lateral differentiations may be connected with the snake-like movements of terrestrial nematodes against surface tension of surrounding water films or between densely packed small soil particles when they push their bodies away from the substrate. In such conditions the presence of rigid ribs increases the efficiency of locomotion. On the other hand, many aquatic nematodes use caudal glands, somatic setae or even the renette as adhesive structures for movement (Turpeenniemi & Hyvärinen, 1996).

Lateral differentiation of the cuticle (lateral alae) was not found in *Pakira orae, Cynura cerambus, Cynura* sp, *Caribplectus magdalenae,* or any *Chronogaster*-species. Murphy (1965) described *Cynura klunderi* as having lateral alae "manifested as two rows of punctations", which is not the same morphologically as the lateral differentiation in for example *Plectus* (see below). Lateral differentiation is only present in species of the families Metateratocephalidae and Plectidae. *Euteratocephalus palustris* and *Metateratocephalus gracilicaudatus* have no distinct lateral alae, but the intracuticular ornamentation is much larger in the lateral sectors of the cuticle, than in other body regions (also in chromadorids). Studied species of the genera *Anaplectus, Ceratoplectus* and *Plectus,* as well as *Perioplectus labiosus, Tylocephalus auriculatus, Ereptonema arcticum* and *Wilsonema otophorum* have lateral alae composed of two wings separated by regularly annulated cuticle, which usually start at the same level at the anterior one-third of the pharyngeal region, and fuse on the tail. This is probably the case also for *Arctiplectus alaskanus*. Here I consider presence of lateral alae to be a derived character.

One more character that may give an important phylogenetic information is the hypodermal glands and their associated body pores. They are usually located in sublateral rows and are characteristic for many aquatic nematodes. Presence of large numbers of hypodermal glands was thought to be a primitive feature, whereas their absence is caused by a reduction process and thus is probably irreversible. They may occur distributed over the whole body or aggregated in certain body regions. Hypodermal glands arranged in four sublateral rows along the whole body is the most common case and characteristic in *Cynura klunderi, Cynura* sp., *Caribplectus magdalenae, Chronogaster typica* (the so-called "vacuolated lateral glandular bodies"), *Perioplectus labiosus, Arctiplectus alaskanus,* all studied species of *Anaplectus* and in some *Plectus* species, viz. *P. communis, P. paracuminatus, P. murrayi, P. parietinus, P. pusteri* and *P. velox*. In *Metateratocephalus gracilicaudatus* and *Euteratocephalus palustris* hypodermal glands are arranged "irregularly" along the whole body, whereas in some *Plectus*-species they are restricted to the posterior region as in *P. paracuminatus, P. pusteri* and *P. murrayi.* One pair of hypodermal glands is present at about mid-distance between the posterior ovary and rectum in *P. communis*. In *Pakira orae* hypodermal glands are arranged in eight longitudinal rows: four sublateral, two subventral and two subdorsal; moreover, of the two anteriormost hypodermal glands, one is located middorsally and the other midventrally at the base of respectively the subdorsal and subventral lips and opening via pores between the lips.

Hypodermal glands are absent in *Chronogaster andrassyi, C. boettgeri, C. spinicorpus, Chronogaster* sp 1, *Tylocephalus auriculatus, Ereptonema arcticum, Wilsonema otophorum* and the remaining species of *Plectus*: *P. andrassyi, P. aquatilis, P. armatus, P. assimilis, P. cancellatus, P. decens, P. geophilus, P. longicaudatus* and *P. minimus*, although in one specimen each of *P. decens* and *P. parvus* I found one hypodermal gland in the posterior body region.

Here I suggest the following transition series of the arrangement of hypodermal glands in Plectoidea. The most primitive case is when hypodermal glands are arranged in four sublateral rows along the whole body, since this is the most common condition and since this feature is present in the closely related superfamilies Camacolaimoidea and Leptolaimoidea (criterion of systematic character precedence). The gradual reduction of hypodermal glands within the genus *Plectus* is suggested also by their occasional presence in *P. decens* and *P. parvus* (teratological criterion – singular anomalies represent ancestral condition) will be considered as multistate characters that developed sequentially and are thought to be irreversible. Arrangement of hypodermal glands in eight longitudinal rows in *Pakira orae* and location of the anteriormost pair at base of lips in this species are treated as autapomorphic features. Since the function of the hypodermal glands is not clear (at least not in Plectoidea), the evolutionary meaning of their reduction is also unknown.

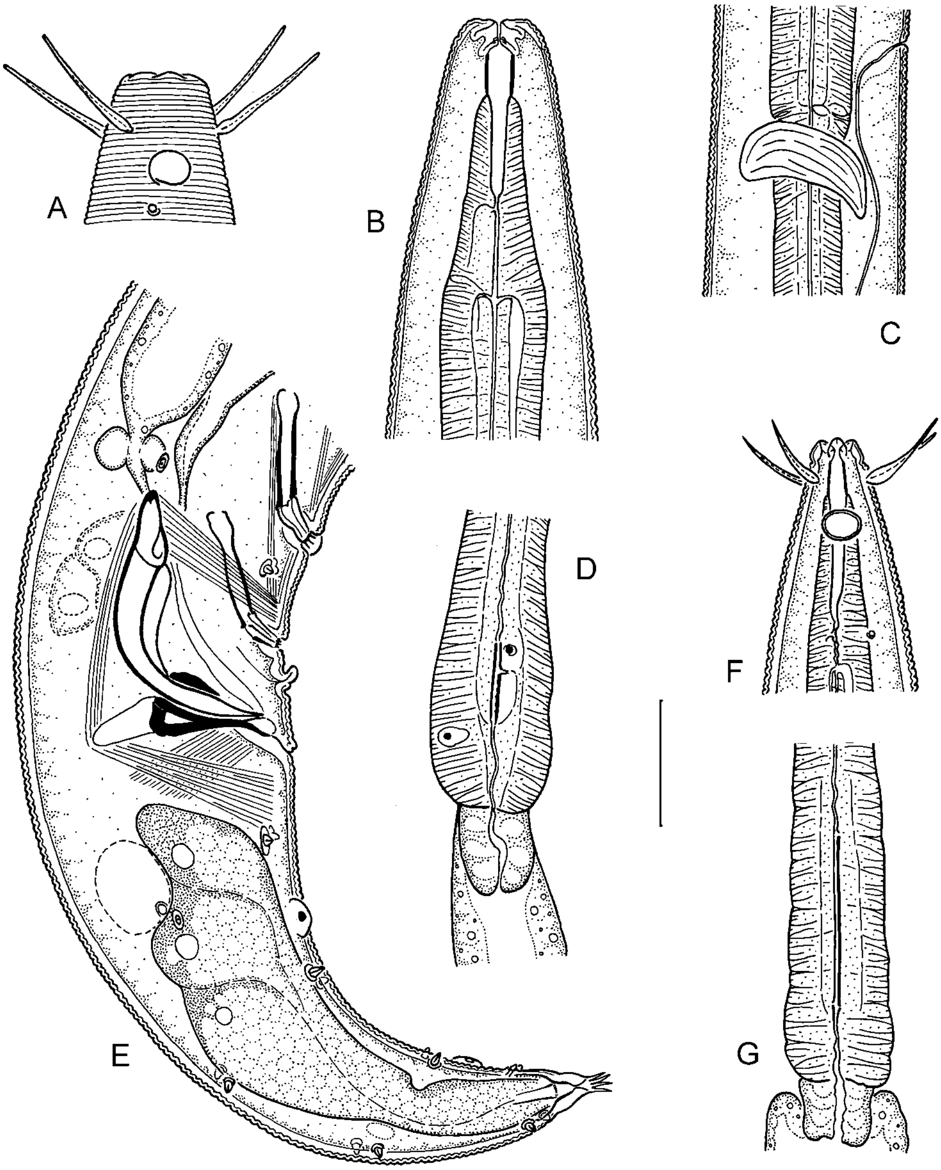

Figure 5.2. Morphology of the family Chronogastridae Gagarin, 1975: *Caribplectus magdalenae* (Riemann, 1971) Andrássy, 1973 male (A-E), and *Cynura* sp. juvenile (F-G). A: Labial region, surface view; B: Anterior end, median section; C: Nerve ring, excretory pore and subventral gland orifices; D and G: Posterior part of pharynx; E: Male posterior end; F. Labial region, combined view. Scale bar 20 µm.

Anterior sensilla

Somatic sensilla are phylogenetically the most simple type of sensory structures in nematodes (Malakhov, 1986). In the most primitive case they are arranged along the whole body, which is the case in *Caribplectus magdalenae, Euteratocephalus palustris, Metateratocephalus gracilicaudatus, Tylocephalus auriculatus, Ereptonema arcticum, Wilsonema otophorum* and all studied species of *Cynura, Ceratoplectus* and *Plectus*. They are papilliform in *Caribplectus* and *Cynura* and setiform in other studied taxa (if present).

Absence of somatic sensilla in certain body regions or on the whole body is a derived feature caused by reduction processes. Only two pairs of somatic setae are present on the female tail in *Anaplectus* spp*.*, *Arctiplectus alaskanus* and *Perioplectus labiosus*. Somatic sensilla are absent in *Pakira orae* and all *Chronogaster* species studied[*] – a condition that is considered irreversible.

The distribution of somatic sensilla is rather variable in most species of the family Plectidae (except Anaplectinae) and shows peculiar patterns along the anteriormost part of the body (at level of anterior part of corpus). A ventrosublateral pair of setae level with the middle of the corpus, just subventral to the lateral alae, is present in all studied species of the genera *Ceratoplectus, Plectus, Tylocephalus, Ereptonema* and *Wilsonema*, and seen already in juveniles of the second developing stage (see below). Its presence and position is very constant within the group encompassing the above mentioned genera and may represent a synapomorphy of it. Further, I would like to consider only those somatic sensilla that are located anterior to this pair of setae.

In five species (*Ceratoplectus assimilis, C. armatus, Tylocephalus auriculatus, Wilsonema otophorum* and some populations of *Ereptonema arcticum*) the anteriormost position is occupied by four setae, which are arranged in one subdorsal and one subventral pair, located at the level of the stoma (stegostom). On the other hand, one dorsosublateral pair of setae is located most anteriorly on the body (but posterior to the stoma) in all species of the genus *Plectus*. It is also present in *C. assimilis* in a similar position, posteriorly to the stoma base, but also posterior to the anteriormost subdorsal and subventral pairs of setae.

Other somatic setae in *Plectus* are usually arranged in subdorsal and/or subventral pairs. There are usually two (one subdorsal and one subventral) pairs of somatic setae in *Plectus parietinus, P. andrassyi, P. aquatilis, P. paracuminatus* and *P. pusteri* at level of the anterior part of the corpus. Only the subventral pair was found in *P. cancellatus, P. communis, P. murrayi, P. parvus* and *P. velox* in the same body region. On the other hand neither subventral nor subdorsal pairs of setae are present in *P. decens, P. geophilus, P. longicaudatus* and *P. minimus*. Comparing the whole variability of the above described feature, I suggest the transformation series represented in Table 5.2, on the basis of the following assumptions: **1)** presence of numerous setae is a plesiomorphic feature, while the reduction of them is a derivation; **2)** presence of a single dorsosublateral pair of setae in the anteriormost position is a primitive feature according to the criterion of systematic character precedence, since it is present in *Hemiplectus, Caribplectus* and *Cynura*.

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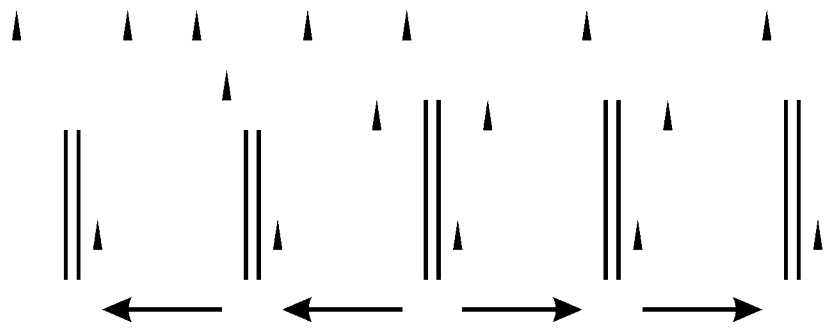
[*] Cervical sensilla and sensilla of male copulatory apparatus are treated separately, even if they are the derivatives from somatic sensilla.

Table 5.2. Transformation series of the distribution of somatic setae along the anteriormost body part in different species of the genera *Plectus* Bastian, 1865*, Ceratoplectus* Andrássy, 1984*, Tylocephalus* Crossman, 1933*, Ereptonema* Anderson, 1966 and *Wilsonema* Cobb, 1913.

Species	*C. armatus* *T. auriculatus* *E. arcticum* *W. otophorum*	*C. assimilis*	*P. parietinus* *P. paracuminatus* *P. pusteri* *P. andrassyi* *P. aqautilis*	*P. velox* *P. cancellatus* *P. murrayi* *P. communis* *P. parvus*	*P. decens* *P. geophilus* *P. longicaudatus* *P. minimus*
Setae pattern					
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It is assumed that labial and cephalic sensilla are derived from somatic ones by a process of migration and differentiation. Here I consider the structure and location of the inner and outer labial and cephalic sensilla. Amphids, cervical sensilla and deirids are sometimes also considered to have their origin from somatic sensilla.

Unfortunately, information about the location of inner labial sensilla is often lacking in descriptions. They are not mentioned in the descriptions of *Pakira orae, Arctiplectus alaskanus, Perioplectus labiosus, Chronogaster andrassyi, Euteratocephalus palustris* and *Metateratocephalus gracilicaudatus*. I was also unable to find them in specimens of the latter two species, but in all other studied taxa they are located on the inner surface of the lips, inside the cheilostom[*] (Figs 5.1 A-B, 5.2 B, 5.5 A-C, E), sometimes very close to its anterior rim. In the description of *Cynura clunderi*, Murphy (1965) mentioned both circles of inner and outer labial sensilla (called "labial and cephalic papillae") located on the anterior surface of lips, while Andrássy (1973) found only six outer labial sensilla on the

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[*] The term "cheilostom" describes "the anterior main region (of stoma), which has the labial cuticle as lining and the membrane wedge ring as posterior edge" according to the latest revision of the stoma ultrastructure and terminology in Rhabditida (De Ley et al., 1995), where authors clearly defined the posterior end of cheilostom, while its anterior end was not fixed. In this chapter we propose to define the anterior margin of cheilostom by the plane that is perpendicular to body axis and touches the anterior tip the of lips. Delineation of the anterior end of cheilostom in Wilsonematinae (as well as in other groups with elaborated labial appendages, e.g. Cephalobidae) is problematic.

lips of *C. cerambus*. Juveniles studied of *Cynura* sp. have inner labial sensilla inside the cheilostom and outer labial sensilla on the lips (Fig. 5.2 F). I therefore suggest the same character condition for the other two species of the genus. Based on the criterion of systematic character precedence and data about the postembryonic development and evolution of cephalic sensilla published by Malakhov (1986), I think that the location of outer labial sensilla on the outer surface of lips is a primitive trait, while their moving inside the cheilostom is a derived feature.

Outer labial sensilla are always located on the anterior surface of the lips in the taxa considered in the present paper, and are papilliform in all taxa except for *E. palustris* and *M. gracilicaudatus*, which have setiform outer labial sensilla. Although it is generally considered that setiform sensilla are primitive and papilliform are derived, I think that setiform outer labial sensilla in members of the family Metateratocephalidae appeared secondarily in the process of evolution unless these genera are basal to Plectoidea in the nematode tree.

The four cephalic sensilla are usually setiform (cephalic setae), located posterior to the labial region and anterior to amphids in most of the studied species. However, there are two exceptions: *Pakira orae*, which has cephalic papilliform sensilla located posterior to amphids (Fig. 5.1 A-B) and members of the subfamily Wilsonematinae, which have cephalic sensilla surrounded by cuticular outgrowths (lateral rim) and modified into flat cornua that are elongate in *Tylocephalus auriculatus*, oval, incised into three tines in *Ereptonema arcticum* and triangular, incised into four tines in *Wilsonema otophorum*. As already stated in Chapter 4, the cornua are derived from seta-like cephalic sensilla. The elongate cornua of *Tylocephalus* are intermediate between the setiform cephalic sensilla of other plectids and the indented cornua of *Ereptonema* and *Wilsonema*, which is also supported by the criterion of the ontogenetic character precedence (presence of typical setiform cephalic sensilla in 1st stage juveniles of Wilsonematinae).

Cervical sensilla can be separated from the somatic ones only in two cases: when they differ morphologically, or when somatic sensilla are totally absent (e.g. *Pakira orae*). Cervical sensilla are present in males of *Pakira orae, Perioplectus labiosus* and in both sexes of all studied species of *Anaplectus*, in which they are papilliform and located dorsosublaterally somewhat posterior to the amphids. In females of *Anaplectus* and *Perioplectus*, they differ morphologically from somatic sensilla, which are setiform and restricted to the tail (caudal setae), and resemble the sensilla of the male copulatory apparatus. The presence of cervical sensilla is here considered to be a derived feature.

It is generally assumed that the ventrally unispiral amphid is the primitive condition in Plectida, Monhysterida and Araeolaimida (Lorenzen, 1981), whereas all other amphid shapes are derived. Among all species included in the present analysis, most have ventrally unispiral amphids with a more or less developed central elevation. However, there are exceptions: e.g. the amphidial aperture is pore-like in *Euteratocephalus* and short tubular in *Metateratocephalus*, while the amphidial fovea is large unispiral in both genera. Species of two other genera are particularly characterised by an amphid in the shape of a transverse slit, viz. *Pakira orae* (Figs 5.1 A-B) and species of the genus *Anaplectus*. The most variable amphid shape is seen in different species of *Chronogaster*. Among them *C. boettgeri* has a unispiral amphid, while others are stirrup-shaped to horseshoe-shaped with more or less

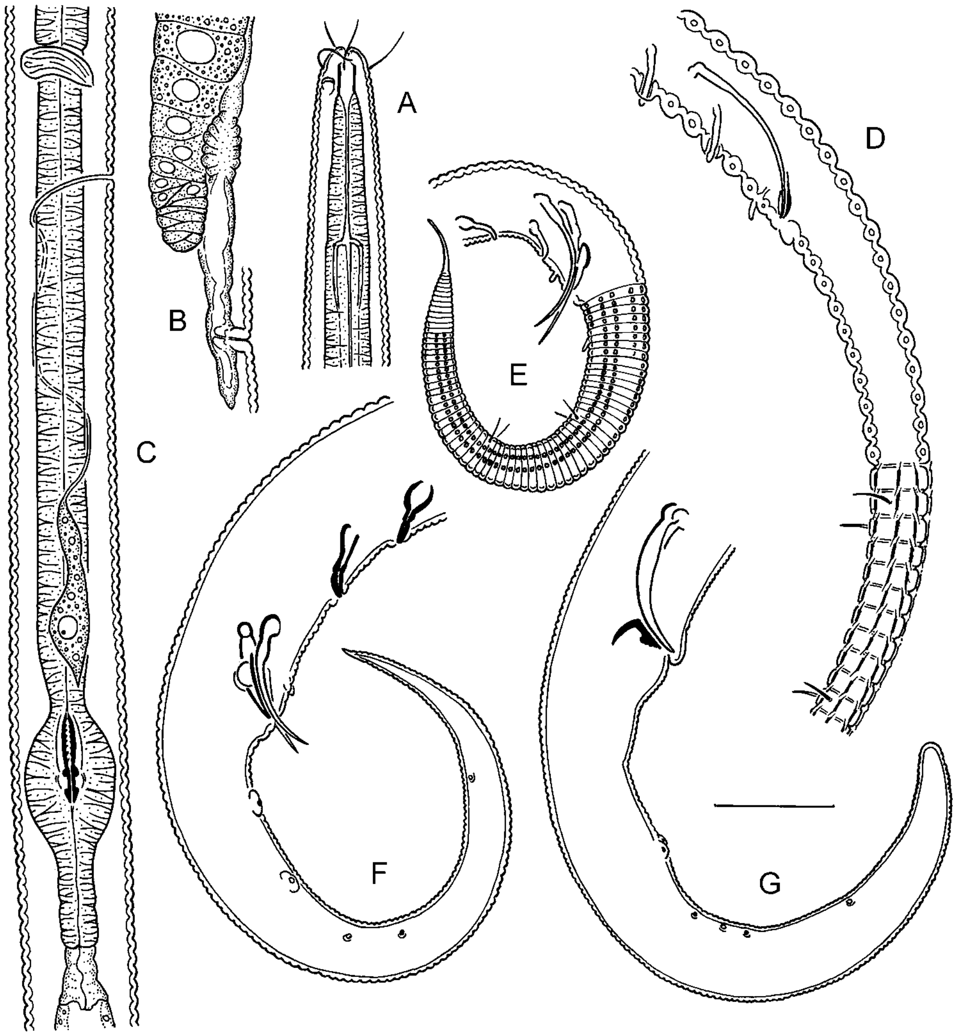

Figure 5.3. Morphology of the family Chronogastridae Gagarin, 1975: *Chronogaster typica* (de Man, 1921) De Coninck, 1935 (A-C, F), *C. spinicorpus* Maggenti, Raski, Koshy & Sosamma, 1983 (D), *Chronogaster* sp 1 (E), *C. boettgeri* Kischke, 1956 (G). A: Anterior end, combined view; B: Female reproductive system; C: Posterior part of pharynx; D-G: Male posterior end. Scale bar 20 µm.

developed paired and forwardly directed projections located sublaterally on the posterior rim of the amphid. Although the entire set of functions of the amphid is not yet known and may differ between different groups, its function as a chemoreceptor is unquestionable. Since it plays an important role in the nematode life, the amphid is under strong evolutionary pressure. The latter presumably causes fast and various modifications in amphid shape, which in this particular group probably diverged repeatedly from a unispiral ground plan.

The deirid was found only in members of the family Plectidae, e.g. in the genera *Anaplectus, Ceratoplectus* and *Plectus*, and in *Arctiplectus alaskanus, Perioplectus labiosus, Tylocephalus auriculatus, Ereptonema arcticum* and *Wilsonema otophorum*. It is characteristically setiform in all Plectidae and usually papilliform or subcuticular in the order Rhabditida. Its presence is here considered a derived feature, suggesting close phylogenetic affinities between Plectidae and Rhabditida.

Labial region

Six lips surround the oral aperture and take part in sensing and capturing of food. They exhibit various morphologic features depending not only on the type of food but also on the method of collecting food particles. The simplest and probably the most primitive labial region is composed of six more or less separated rounded lips of equal size and structure, without additional outgrowths. This type is characteristic for most of the species included in the present analysis. Due to its broad occurence among nematode taxa and based on comparison with closely related taxa (Leptolaimoidea, Camacolaimoidea, Monhysterida and Araeolaimida), I consider this shape of the labial region to be the ancestral state, while all other shapes discussed below have probably evolved from it.

The simplest derivatives from the ancestral ground plan are the labial regions of *Plectus andrassyi* and *P. cancellatus*, which have the anterior one-third of each lip abruptly narrowed and sharply pointed; of *Chronogaster spinicorpus* and *Chronogaster* sp 1, which have lips fused into a cap-like, smooth structure; and of *Ceratoplectus armatus* which has lateral lips broader than the subventral and subdorsal ones (it is not clear if *C. assimilis* has a similar labial region judging from the lateral view). Further modifications of the labial region occurr in *Perioplectus labiosus*. It has the most strongly offset labial region, with three lobes on the inner surface of each lip and the anteriormost lobe secondarily bifurcated. Lips in representatives of the family Metateratocephalidae are crown-shaped and strongly sclerotized, with refringent U-shaped rims, continuous with body contour in *Euteratocephalus palustris* and strongly offset in *Metateratocephalus gracilicaudatus*.

The most complex labial region is characteristic for the members of the subfamily Wilsonematinae. In the previous chapter I discussed the morphology and evolution of the labial region in much more detail (Chapter 4). Here I will focus on three species of particular interest for the present study: *Tylocephalus auriculatus, Ereptonema arcticum* and *Wilsonema otophorum*. Like other representatives of the subfamily they are characterised by the pronounced bilateral and dorsoventral symmetry of the labial region. These species possess midlateral projections: the anterior elevation of the outer lateral labial sensilla, which is considered to represent a synapomorphy of Wilsonematinae relative to the “regular” structure of lateral lips in *Plectus* (Chapter 4). However, the state of this character in *T. auriculatus* and *E. arcticum* represents only the beginning of a transitional series towards the elongate, digitate midlateral projection in *W. otophorum*. The submedian outer labial sensilla in both genera are shifted towards the mouth opening. The cornua of Wilsonematinae are assumed to be derived cephalic setae (see above).

One character unique for Wilsonematinae as a whole is the presence of cervical expansions. Annulated cervical expansions in *T. auriculatus* represent a primitive state, while non-annulated cervical expansions in *W. otophorum* are a derived feature. Thus, *T. auriculatus* was found to be the morphologically least derived within the subfamily; it is particularly characterised by the submedian lips forming median ridges in this species. On the other hand, *E. arcticum* and *W. otophorum* do not possess such structures. However, the latter two species were shown to be representatives of a separate lineage as they differ from the former species by different and more complex structures in the labial region: presence of flabella, fimbriae, more elevated peripheral cervical cuticle, and leaf-shaped cornua subdivided into several tines by deep incisures. The fimbriate flabella of *W. otophorum* are not innervated and probably not homologous to the median ridges in *T. auriculatus*. They seem to be useful for gathering or sweeping food sources from the substrate. Furthermore, presence of fimbriate lateral rims in *E. arcticum* was found to be a synapomorphy of this genus (Chapter 4).

Secretory-excretory system

The secretory-excretory system is clearly present in most of the studied taxa except for *Chronogaster spinicorpus* and *Chronogaster* sp 1 where its absence is considered secondary and irreversible. I was unable to find the renette cell in the studied specimens of *Cynura* sp., while it was not described in enough detail in the literature for *Arctiplectus alaskanus, Perioplectus labiosus* and other *Cynura* species. It was also impossible to discern the renette cell in the specimens of *Caribplectus magdalenae* available to me.

The excretory pore is usually located just posterior to the level of nerve ring, except for *Caribplectus magdalenae*. An excretory ampulla is totally absent in all "ingroup" taxa. The excretory canal is long and cuticularised in all representatives, extending from the excretory pore backward. It is uniformly cuticularised except for *Anaplectus* spp., in which it is surrounded by a strongly cuticularised ring at the point where the canal penetrates the hypoderm.

The renette cell is usually located at the level of the posterior part of isthmus and anterior part of basal bulb. Only in *Pakira orae* is the renette located posteriorly, subventral to the intestine (Fig. 5.1 E). In all *Chronogaster* species in which the excretory system is present, there is just one small cell connected with the excretory duct and thus representing the renette (Fig. 5.3 C). In all other species, for which the quality of the specimens allowed clear observations and interpretation, the renette cell is usually followed by two coelomocytes. Another pair of coelomocytes lies one to two corresponding body diameters posterior to cardia, subventral to intestine. A similar arrangement of coelomocytes (two pairs, one level with renette cell and one pair posterior to it) was described for *Sphaerolaimus gracilis* de Man, 1876 (Turpeenniemi, 1993), and was thought to be involved in the metabolism of body cavity fluid. The renette itself envelops the coiled proximal part of the excretory duct. The latter makes two loops around the isthmus in Plectidae (Figs 5.5 R-T, V-X) or is coiled into a dense spiral in Metateratocephalidae (Fig. 5.4 C).

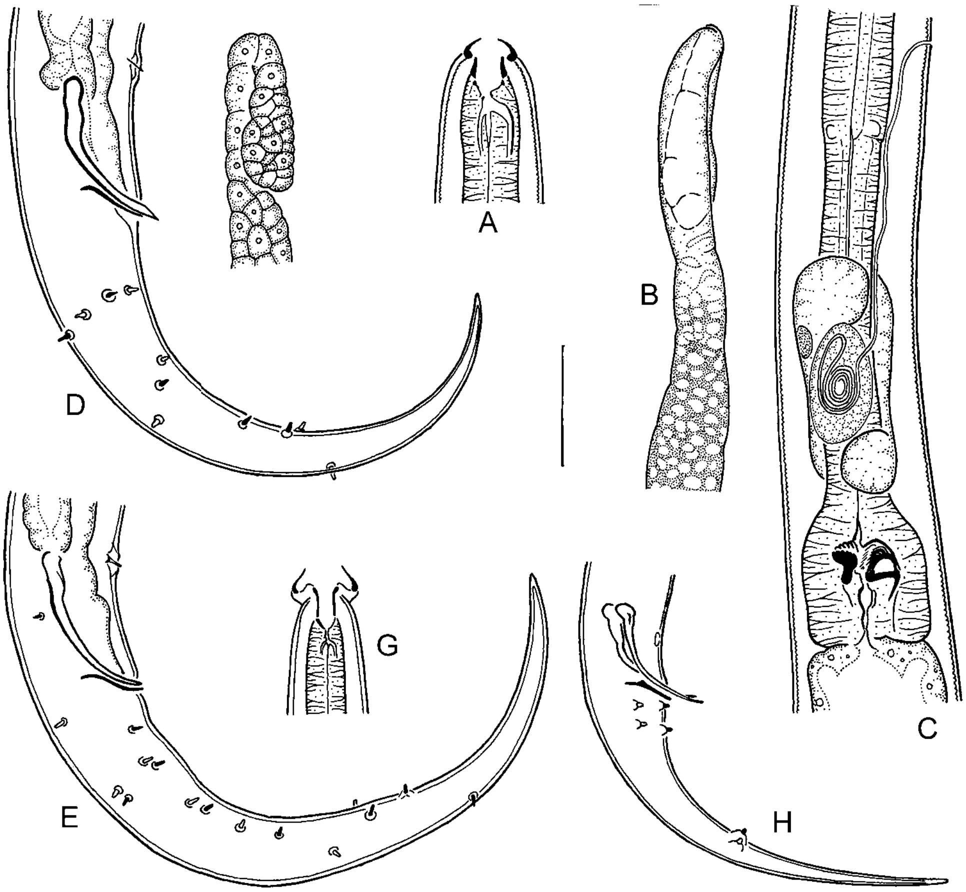

Figure 5.4. Morphology of the family Metateratocephalidae Eroshenko, 1973: *Euteratocephalus palustris* (de Man, 1880) Andrássy, 1968 (A-E) and *Metateratocephalus gracilicaudatus* Andrássy, 1985 (F-H). A and G: Labial region, median section; B and F: Testis; C: Posterior part of pharynx, renette and coelomocytes; D, E and H: Male posterior end. Scale bar 20 µm.

Comparing with different outgroup taxa of Camacolaimoidea (Chapter 3) and Monhysterida (Van de Velde & Coomans, 1987; Turpeenniemi & Hyvärinen, 1996), I may suggest the following features to represent primitive conditions: **1)** renette cell located opposite to intestine; **2)** excretory ampulla present, excretory canal short; **3)** excretory canal not enveloped by a renette cell; **4)** excretory canal coiled but not bent into a spiral. Thus, the opposite traits should be considered apomorphic: **1)** renette cell located opposite to pharynx; **2)** excretory ampulla absent, excretory canal long; **3)** excretory canal enveloped; **4)** excretory canal bent into a spiral.

Digestive system

Fürst von Lieven (2002) was the first to apply the tripartite stoma terminology of De Ley et al. (1995) for plectids, particularly for *Ceratoplectus armatus*. Following this idea, we found that the stoma in most Plectida consists of three distinct sections: a cheilostom marked by labial cuticle, a gymnostom enveloped by non-pharyngeal epithelial tissue, and a stegostom enveloped by the so called "pharyngeal sleeve" (muscular cells of pharynx). The posterior end of stoma is marked by the appearance of pharyngeal radii and pharyngeal tubes. Although pharyngeal tubes were not described for *Pakira orae* and misinterpreted as openings of pharyngeal glands in *Cynura klunderi* (Murphy, 1965), they are actually present in all ingroup species. Furthermore, in all studied species of the genus *Anaplectus* I observed "posterior pharyngeal tubes", which originate somewhat posterior to corpus-isthmus junction and extend posteriorly for some distance. On transverse sections they look like widenings of the lumen of pharyngeal radii and not like tubes at tips of radii[*]. Such features were not found in other representatives of the superfamily Plectoidea, and are thus considered to be apomorphic for the genus *Anaplectus*.

As the entire structure for capturing of food, the stoma has undergone profound evolutionary modifications in nematodes (see for example Chapter 3 for stoma variability in Leptolaimoidea and Camacolaimoidea) and differs considerably among genera. The cheilostom is generally undifferentiated and does not possess sclerotizations except in *Pakira, Metateratocephalus* and some *Plectus* species. It is probably flexible in most cases, allowing for opening or closing of the mouth. The broad cylindrical cheilostom in *Pakira* is strongly sclerotized in its proximal part (Fig. 5.1 C). *M. gracilicaudatus* has a broad cheilostom surrounded by leaf-shaped lips with strongly sclerotized rounded cheilorhabdia located in the proximal part of the cheilostom (Fig. 5.4 G). Among the *Plectus*-species, *P. andrassyi, P. aquatilis, P. cancellatus, P. communis, P. decens, P. geophilus, P. longicaudatus, P minimus, P. murrayi, P. paracuminatus, P. parietinus, P. parvus, P. pusteri* and *P. velox* have small bar- or granule-shaped cheilorhabdia (Figs 5.5 A-C, F-I). Presence of cheilorhabdia may be interpreted as an additional support which function is uncertain.

The gymnostom in all species of Plectoidea is a cylindrical, conical or barrel-shaped structure with strongly sclerotized walls, with the exception of *Anaplectus*, which has bar-shaped cheilorhabdia, and *Ceratoplectus assimilis*, which has small and oval cheilorhabdia (Fig. 5.5 E). Both the latter cases are probably derived conditions. The stegostom is visibly subdivided into two subsections, of which the posterior one is narrow and penetrated by the dorsal gland orifice in all species. The anterior stegostom section is narrow in *Pakira orae* (Fig. 5.1 C) and *Perioplectus labiosus*; funnel-shaped in *Arctiplectus alaskanus, Caribplectus magdalenae* (Fig. 5.2 B), *Tylocephalus auriculatus, Wilsonema otophorum* and the studied species of *Chronogaster, Cynura* and *Plectus*; cylindrical in *Anaplectus*; and conoid with strong rhabdia in *Euteratocephalus palustris* and *Metateratocephalus gracilicaudatus* (Figs 5.4 A, C).

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[*] Rather similar structures were described by Anderson & Sudhaus (1985) for *Rhabditis (Pellioditis) dolichuroides* Anderson & Sudhaus, 1985 as "corpus at junction with isthmus marked by expansion of the triradiate lumen and appearance of marginal tubes".

Proximally the stoma continues into the pharynx, whose morphology includes several phylogenetically important characters. If stoma morphology is variable between species or genera, pharynx structure is rather constant and characteristic for the higher taxa. Within the studied group several different morphological types of pharynx were found, although the differences among them are rather small. The most distinct pharynx is characteristic for *Pakira orae*: there is no subdivision into corpus and isthmus; the anterior part of the pharynx is cylindrical and strongly muscularized; the posterior part is somewhat broader and has more glandular tissue; the cuticular lining in the basal part is folded, probably functioning as a very simple valve (Fig. 5.1 E); no pharyngeal gland nuclei were seen, while subventral gland orifices were distinct somewhat posterior to the level of nerve ring (Fig. 5.1 D). The uniformly cylindrical and muscularized pharynx of *Cynura* spp. and *Caribplectus magdalenae* is subdivided into corpus and isthmus by a discontinuity in the muscular tissue and subventral gland orifices (Fig. 5.2 C). Its posterior part carries a weak valvular apparatus in *Caribplectus magdalenae* (Fig. 5.2 D), or a simple thicker lumen in *Cynura* sp (Fig. 5.2 G). Species of *Chronogaster* also have a uniformly muscular pharynx: an anterior cylindrical part distinctly subdivided into corpus and isthmus by a discontinuity in the muscular tissue and subventral gland orifices, and a posterior part expanding into a subterminal bulb (post-bulbal prolongation present) which carries a valvular apparatus (Fig. 5.3 C) with three longitudinal denticulate ridges, followed by two sets of oval thickenings (here named “chronogasteroid valve”).

Other members of the superfamily Plectoidea are characterised by rather similar pharynges. It is subdivided into corpus and postcorpus by a discontinuity in the muscular tissue and subventral gland orifices, the postcorpus is composed of a cylindrical isthmus and an oval or pear-shaped basal bulb, which is terminal or subterminal in position, e.g. a postbulbal prolongation that is half as long as the bulb itself is present in *Plectus decens, P. geophilus, P. longicaudatus* and *P. minimus* (Figs 5.5 R, U, X). Furthermore, the basal bulb holds a valvular apparatus with transverse denticulate plates and a posterior sclerotized haustrulum-like structure (here named “plectoid valve”). Two types of valvular apparatuses were found by Zell (1993) and Fürst von Lieven (2003) in members of the genus *Plectus*: “*parietinus*”- type and rhabditoid type, however, ultrastructural studies are needed to clarify the morphological differences between them.

Taking into consideration the level of complexity of different pharynges and data from postembryonic development discussed below I suggest the following character polarities (plesiomorphic *vs* apomorphic): **1)** pharynx uniformly cylindrical *vs* subdivided into corpus and isthmus; **2)** pharynx muscular *vs* with posterior glandular part; **3)** basal bulb absent *vs* present; **4)** valves absent *vs* present; **5)** valves in the shape of longitudinal denticulate ridges *vs* longitudinal denticulate ridges with two sets of oval thickenings *vs* transverse denticulate plates (independent origin, see below). Thus the most primitive but also specialised (in development of glandular tissue in the posterior part) pharynx was found in *Pakira orae*. On the other hand, the pharynx type characteristic for *Caribplectus* and *Cynura* may give rise to two independent lineages leading to chronogasteroid and plectoid valves. Trends in the evolution of location of pharyngeal gland orifices were discussed in Chapter 3. Here I should state only that the position of dorsal (DGO) and subventral (SvGO) gland orifices at stoma base is a primitive trait, while the positions of DGO inside stoma and SvGO at midpharynx are derived features.

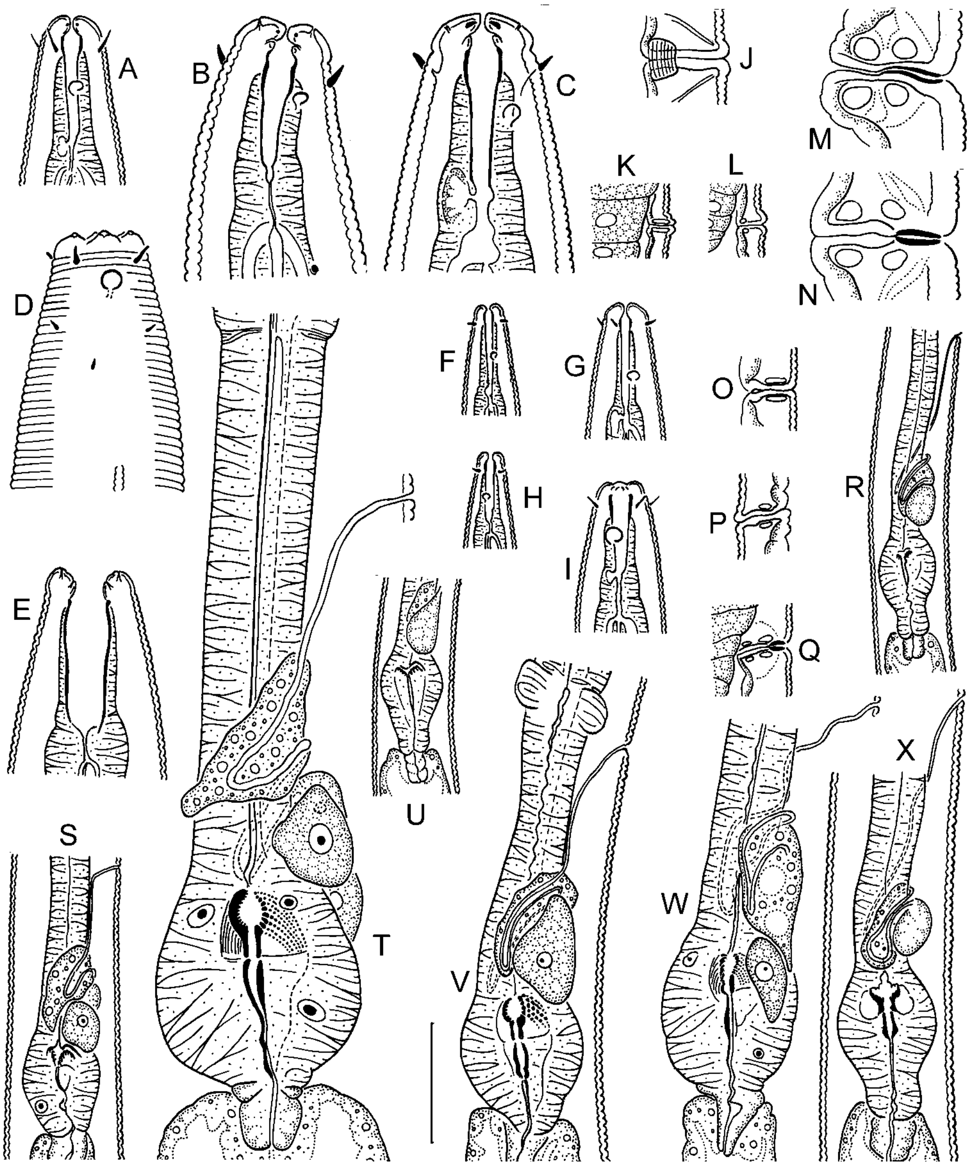

Figure 5.5. Morphology of the family Plectidae Örley, 1880: *Plectus communis* Bütschli, 1873 (A, Q, V), *P. velox* Bastian, 1865 (B, M, T), *P. parietinus* Bastian, 1865 (C, N), *Ceratoplectus assimilis* (Bütschli, 1873) Andrássy, 1984 (D, E, J, W), *P. geophilus* de Man, 1880 (F, K, R), *P. parvus* Bastian, 1865 (G, P, S), *P. minimus* Cobb, 1893 (H, L, U), *P. decens* Andrássy, 1985 (I, O, X). A-C and F-I: Anterior end, combined view; D: Labial region, surface view; E. Anterior end, median section; J-Q: Vagina region; R-X: Posterior part of pharynx, renette, coelomocytes. Scale bar 20 μm.

Female reproductive system

The female reproductive system consists of two branches except for members of the genus *Chronogaster* which have only an anterior ovary. The posterior ovary is reduced to a postvulval sac that is shorter than the vulval body diameter in *C. andrassyi, C. boettgeri, C. multispinatoides* and *C. typica* (Fig. 5.3 B) and longer than the vulval body diameter in *C. spinicorpus* and *Chronogaster* sp 1. The uterus in other genera is often differentiated into a spermatheca and a crustaformeria[*], whose presence and morphology may give some interesting features for phylogenetic speculations. I also found two specimens of *Plectus parietinus* with a double sets of completely developed and functional (egg producing) female reproductive systems (Figs 5.7 I-J).

If present, the spermatheca is always axial and located in the proximal part of the uterus. Its presence was mentioned in the descriptions of *Cynura klunderi, Arctiplectus alaskanus* and *Perioplectus labiosus,* it is also characteristic for the genus *Anaplectus*. The bisexual *Pakira orae* (Fig. 5.1 F), *Chronogaster spinicorpus* and *Chronogaster* sp 1, as well as thelytokous species of the genera *Chronogaster* and *Plectus,* and *Euteratocephalus palustris, Metateratocephalus gracilicaudatus, Tylocephalus auriculatus, Ereptonema arcticum* and *Wilsonema otophorum* have no developed spermatheca. It is difficult to state whether presence of a spermatheca is primitive or derived since it may be either present or absent in outgroup taxa as well. Here I propose to treat presence of a spermatheca as primitive, which may be secondarily reduced in parthenogenetic taxa.

In species of *Anaplectus* and *Perioplectus*, the distal part of the uterus is further modified into a crustaformeria. It is composed of cells with granular content, coloured in dark-yellow or brown *in vivo* and in totomounts in glycerine. I consider presence of a crustaformeria as a derived feature, playing a role in producing the thorny egg shell (pers. obs.), which may be a possible additional protection for eggs and developing embryos.

The vagina and vulval area also include several phylogenetically important characters, for example a vagina with strongly sclerotized lips was found in *Cynura klunderi* and *C. cerambus*. On the other hand the *pars distalis vaginae* in several species of *Plectus* is modified into a pair of sclerotized folds, the so-called epiptygmata. Among studied species, epiptygmata were found in *Plectus andrassyi, P. cancellatus, P. communis, P. murrayi, P. paracuminatus, P. parietinus, P. pusteri* and *P. velox* (Figs 5.5 M-N, Q). Although, the function of epiptygmata is unclear, I consider its presence to be a derived trait. The number of vaginal sphincters varies from one to two in different species. One sphincter is most common and here considered as ancestral, characteristic for all but some species of *Plectus*, viz. *P. aquatilis, P. cancellatus, P. andrassyi, P. paracuminatus, P. parietinus, P. murrayi, P. pusteri* and *P. velox* (Figs 5.5 M-N, Q). Both the presence of epiptygmata and/or two vaginal sphincters are treated here as derived features. Finally, *Cynura klunderi* is the onlyconsidered here species in which females have ventral alveoli in the postvulval area and this is thought to represent a primitive feature.

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[*] Crustaformeria – the part of uterus taking part in formation of the external thorny egg layer

Male reproductive system and copulatory apparatus

The male reproductive system and copulatory apparatus include several characters which could be treated effectively in the phylogenetic analysis. The first feature is the number and location of testes. There are two testes in most representatives of the superfamily Plectoidea, the anterior one is outstretched (directed anteriorly) and the posterior one is reflexed towards caudal region and located subdorsally. Both testes in *Cynura klunderi* were described to be directed anteriad. The genera *Euteratocephalus* and *Metateratocephalus* have only one anterior testis, with the germinative zone reflexed and located subventrally (Figs 5.4 B, F). Spicules and gubernaculum are rather variable and I was unable to resolve the evolutionary polarities of these structures (which is why they are not included in the data matrix).

The copulatory apparatus includes structures that play an important role in the process of mating. The most intriguing of these are the midventral precloacal tubular supplements, which are located in a single row along the midventral body line. The strongly cuticularised tube is connected with a more or less distinct glandular cell that is always located on the right-hand side of the intestine. The number of tubular supplements varies from one in *Plectus murrayi* (Fig. 5.6 A) and *P. communis* (Fig. 5.6 C) to 18-20 in *Caribplectus magdalenae*. Species of *Chronogaster* usually have a higher number of tubules: 16-19 in *Chronogaster typica*, 13 in *C. multispinatoides*, six to nine in *Chronogaster* sp 1, four to seven in *C. spinicorpus*, and five in *C. andrassyi*. Only the male of *C. boettgeri* has none (Fig. 5.3 G). Two supplements are characteristic for *Pakira orae* (Fig. 5.1 G), *Arctiplectus alaskanus, Perioplectus labiosus* and species of *Cynura.* The anteriormost of the two tubular supplements in *Cynura klunderi* is provided with a strong guiding piece, while in *C. cerambus* it has a rasp-like terminal part (derived feature). *Anaplectus granulosus* and *A. atubulatus* usually have three tubules, of which the posteriormost is slightly longer than the others (apomorphy). Moreover its gland is larger and more developed, which may indicate a special function or greater activity of the posteriormost supplement, perhaps producing a copulatory plug or adhesive.

The most variable number of tubular supplements was observed in species of *Plectus*. Males of *P. velox* have four, five or six tubuli, *P. paracuminatus* and *P. pusteri* have one to three tubuli, while *P. communis* usually has one, but in a few cases two or none (Figs 5.6 B-D). Two or three tubuli are present in males of *P. aquatilis,* three in *P. parietinus*. *P. cancellatus* usually has two tubuli, whereas *P. andrassyi* has one to four tubular supplements. Male specimens of *P. andrassyi* described by Mulk & Coomans (1978) have a rather aberrant structure of the copulatory apparatus: in addition to three sclerotised and well developed tubular supplements, there is a weakly developed tubule instead of a precloacal setiform sensillum. Except for *Chronogaster boettgeri,* tubular supplements are absent also in *Ceratoplectus armatus, C. assimilis, Plectus decens, P. geophilus, P. longicaudatus, P. minimus, P. parvus, Tylocephalus auriculatus, Ereptonema arcticum* and *Wilsonema otophorum* (Figs 5.7 A-E, G).

A midventral precloacal sensillum has been found in a wide range of chromadorian nematode taxa and is present in many species of plectids as well. Setiform precloacal sensilla were found in *Cynura cerambus, C. klunderi, Chronogaster andrassyi, C. multispinatoides, C. typica*, *C. spinicorpus, Chronogaster* sp 1 (Figs 5.3 D-F), *Plectus*

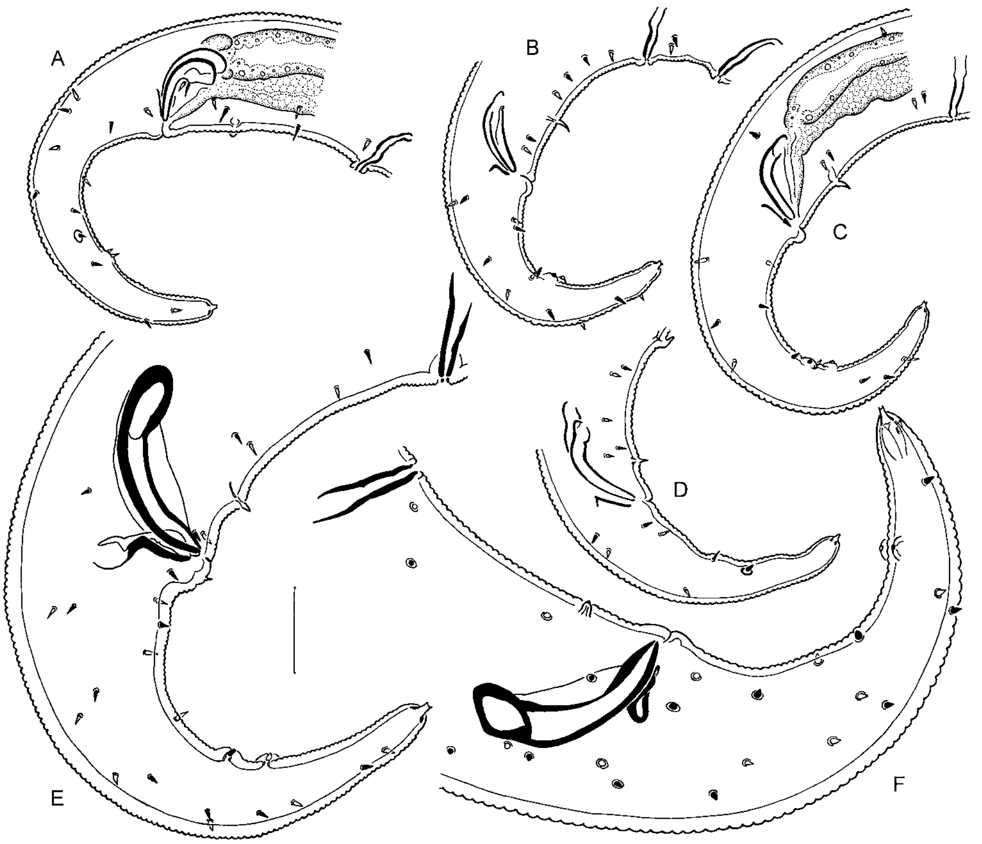

Figure 5.6. Morphology of the family Plectidae Örley, 1880: *Plectus murrayi* Yeates, 1970 (A), *P. communis* Bütschli, 1873 (B-D), *P. parietinus* Bastian, 1865 (E), *P. velox* Bastian, 1865 (F). A-F: Male posterior end. Scale bar 20 μm.

aquatilis, P. murrayi, P. communis, P. paracuminatus, P. parietinus, P. pusteri, P. velox etc. (Figs 5.6 A-F); a papilliform precloacal sensillum is present in *Caribplectus magdalenae* (Fig. 5.2 E), *Arctiplectus alaskanus, Anaplectus atubulatus, A. granulosus, Perioplectus labiosus, Pakira orae* (Fig. 5.1 G), *Metateratocephalus gracilicaudatus* (Fig. 5.4 H) and *Euteratocephalus palustris* (Figs 5.4 D-E), although the difference between "papilliform" and "setiform" is rather unclear. The following species have no precloacal sensillum at all: *Chronogaster boettgeri* (Fig. 5.3 G), *Ceratoplectus armatus, C. assimilis, Plectus decens, P. geophilus, P. longicaudatus, P. minimus, P. parvus, Ereptonema arcticum* (Figs 5.7 A-E, G), *Tylocephalus auriculatus* and *Wilsonema otophorum.*

Two subventrally located postcloacal sensilla are present in *Cynura cerambus, C. klunderi* ("postanal supplements", in Murphy, 1965), *Caribplectus magdalenae* (Fig. 5.2 E), *Chronogaster andrassyi*, *C. boettgeri* (Fig. 5.3 G), *C. multispinatoides,* *C. typica* (Fig. 5.3 F), *Arctiplectus alaskanus* ("especially the two posterior subventral pairs are well discernible", Andrássy, 2003), *Anaplectus atubulatus, A. granulosus, Plectus andrassyi* ("the posteriormost of subventral setae situated on a papilliform elevation", in Mulk & Coomans, 1978), *P. aquatilis, P. murrayi, P. cancellatus, P. communis, P. palustris, P. paracuminatus, P. parietinus, P. pusteri* and *P. velox* (Figs 5.6 A-F, 5.7 F). A single postcloacal sensillum was observed in *Chronogaster* sp 1 (Fig. 5.3 E). No clear information about the postcloacal sensilla is available from the original descriptions of *Perioplectus labiosus*, but they may be present in this species as well. Postcloacal sensilla are absent in *Ceratoplectus armatus, C. assimilis, Plectus decens, P. geophilus, P. longicaudatus, P. minimus, P. parvus, Tylocephalus auriculatus, Ereptonema arcticum* (Figs 5.7 A-E, G), *Wilsonema otophorum, Chronogaster spinicorpus* (Fig. 5.3 D), *Pakira orae* (Fig. 5.1 G), *Euteratocephalus palustris* (Figs 5.4 D-E) and *Metateratocephalus gracilicaudatus* (Fig. 5.4 H). In Chapter 3 I stated that postcloacal sensilla are present in the ancestral relatives of the superfamily Plectoidea and therefore absence of this structure is here considered to be derived due to secondary reduction.

Caudal sensilla (caudal setae, morphologically similar to somatic sensilla if the latter are present) are present and usually arranged in subventral and subdorsal, or only in subventral rows. Setiform sensilla in males of *Euteratocephalus palustris* (Figs 5.4 D-E) are distributed irregularly, while in *Metateratocephalus gracilicaudatus* they are arranged in three subventral pairs (Fig. 5.4 H). In both analysed species of *Anaplectus* and also in *Perioplectus labiosus* caudal sensilla are papilliform and arranged in the following way: two to four subventral precloacal pairs, one subventral pair at level of cloaca, and several pairs arranged subventrally, subdorsally and sublaterally on the tail. Two subventral pairs of caudal sensilla are characteristic for the genus *Chronogaster*, they are setiform in *Chronogaster* sp 1 (Fig. 5.3 E) and *C. spinicorpus* (Fig. 5.3 D), and papilliform in *C. andrassyi, C. boettgeri* (Fig. 5.3 G), *C. multispinatoides* and *C. typica* (Fig. 5.3 F). The unusual pattern of the arrangement of caudal sensilla in *Anaplectus* and *Perioplectus* (few precloacal pairs) is considered here to represent a derived feature.

Caudal glands and spinneret

Caudal glands and spinneret constitute a complex structure, which functions mainly for locomotion and attaching (anchoring) to the substrate. When considering all nematode taxa, it becomes clear that caudal glands and spinneret are characteristic mainly for primary aquatic nematodes, and totally absent in most free-living terrestrial and all parasitic taxa. Caudal glands produce a glue-like substance that is secreted through the spinneret and attaches the nematode tail to solid substrate particles. Thus, the anterior end of the nematode may either move around searching for prey or attach to another point of substrate with the secretion of the renette (see Turpeenniemi & Hyvärinen, 1996). Spinneret and caudal glands are more useful for aquatic nematodes that live in relatively large volumes of moving water, on aquatic plants etc., than for terrestrial nematodes that are moving between densely packed soil particles. The reduction of spinneret and caudal glands, as well as various modifications of the spinneret, often occurs in unrelated nematode taxa.

Modification of the spinneret is seen in species of the genus *Cynura*, which all have a large and strongly sclerotized capsule surrounding the orifice of the caudal glands.

Anaplectus atubulatus has no capsule of the spinneret, while functional caudal glands open to the exterior via a pore on the tail tip. Females of *Plectus minimus* have a ventrally curved spinneret, while males of this species have a spinneret of typical structure; caudal glands in this species are indistinct, probably absent. The most curious spinneret is present in *P. decens*, which has functional caudal glands and a spinneret with a terminal tube provided with two small thorns located subventral and perpendicular to the main body axis (Fig. 5.7 G). Finally, caudal glands and spinneret are absent in *Pakira orae*, also in *Plectus amorphotelus* Ebsary, 1985 and *P. spicacaudatus* Ebsary, 1985, and all studied species of *Chronogaster*, except females of *C. boettgeri*.

The tail terminus in some species of *Chronogaster* is further modified and armed with mucro and spines. Among the studied species of this genus, *C. andrassyi* and *C. multispinatoides* have a tail terminus provided with one large terminal mucro and a circle of four subterminal spines. The tail terminus in *C. spinicorpus* and *Chronogaster* sp 1 is elongate filiform (Figs 5.3 E). I also noted sexual dimorphism in the tail terminus structure. In *C. andrassyi* and *C. multispinatoides* the male tail tip is bluntly rounded, while female tails carry mucro and spines. In *C. boettgeri* the male tail tip is bluntly rounded (Fig. 5.3 G) and the female tail is provided with a spinneret. All modifications, mentioned above, and derivations from the ground plan (= caudal glands and spinneret present) are treated in the present paper as apomorphic features.

Anomalies

Riemann (1977) following Zimmerman (1967) suggested that “singular anomalies demonstrate, as a rule, an ancestral condition”. Thus, occasional findings of hypodermal glands in *Plectus decens* and *P. parvus* (in which they are normally absent) may suggest the presence of hypodermal glands in ancestors of these species and agree with the hypothesis of gradual reduction of these glands in species of the genus *Plectus*. On the other hand, some anomalies (of genetic nature) may reflect potential directions of future evolution of this particular character. Among the whole variety of known aberrations published in literature and observed personally only two kinds may be interpreted as representing possible future character states.

First of all a high variability of the number of tubular supplements in males of certain species of the genus *Plectus* may suggest future trends towards the oligomerisation and complete reduction of tubules. For example males of *P. pusteri* and *P. paracuminatus* usually have two or three tubules and only exceptionally just one tubule (four out of 72 males of *P. pusteri* and one out of 18 males of *P. paracuminatus*). On the other hand, the morphologically close *P. communis* usually has just one tubule and rarely two or none (two males with two tubules and three males with a weakly cuticularized tube out of 22 males from the same population, while all 20 males from another population have a single tubule each). I may suggest a trend toward the gradual reduction of tubular supplements in this group of nematodes, resulting in their complete absence in some species of this taxon.

Secondly, different authors (Zell, 1993, Holovachov & Susulovsky, 2000) found specimens of the genus *Plectus* without caudal glands and spinneret in populations of species which normally have these structures. Appearance of such abnormalities may be caused by at least two different reasons like mechanical injuries and possible genetic

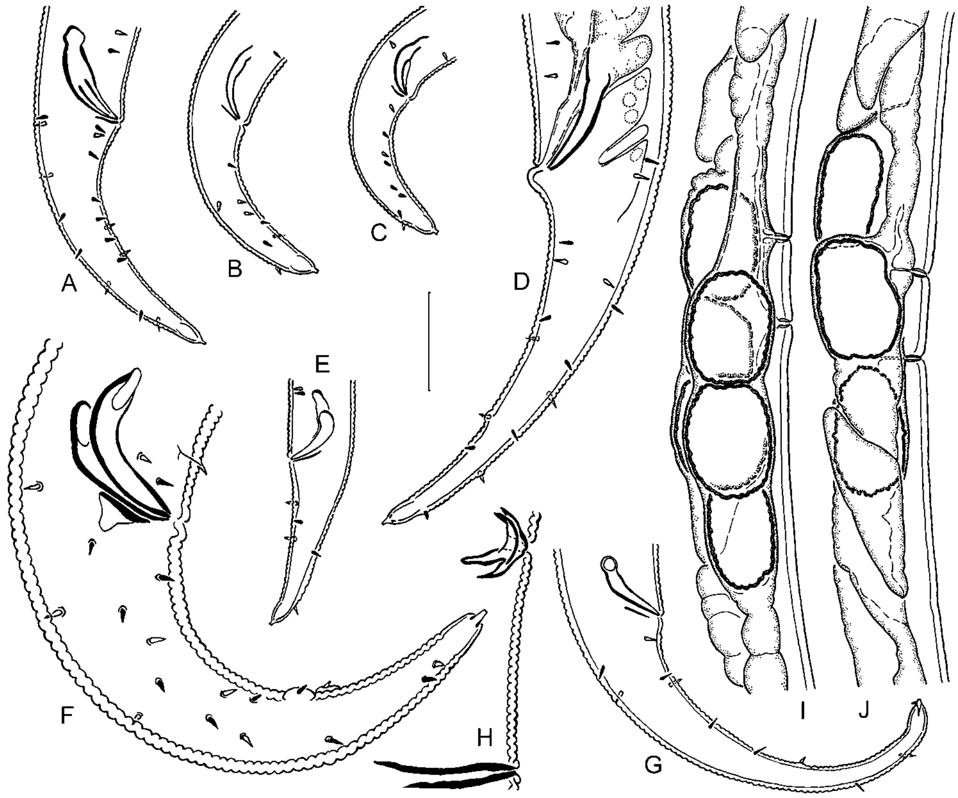

Figure 5.7. Morphology of the family Plectidae Örley, 1880: *Plectus parvus* Bastian, 1865 (A), *P. geophilus* de Man, 1880 (B), *P. minimus* Cobb, 1893 (C), *Ceratoplectus assimilis* (Bütschli, 1873) Andrássy, 1985 (D), *Ereptonema arcticum* Loof, 1971 (E), *P. cancellatus* Zullini, 1978 (F), *P. decens* Andrássy, 1985 (G), *P. velox* Bastian, 1865 (H), *P. parietinus* Bastian, 1865 (I-J). A-F and G: Male posterior end; H: Normal and aberrant tubular supplements in male; I and J: Double female reproductive system. Scale bars: A-H = 20 μm, I-J = 50 μm.

anomalies. The aberrations of the second type in species with a presumably parthenogenetic mode of reproduction may serve as material for speciation (although this suggestion should be verified experimentally), or may depict possible future evolutionary trends (reduction of caudal glands and spinneret), which were already realized in the past in lineages leading to present living *Plectus spicacaudatus* and *P. amorphotelus* (Ebsary, 1985; Zell, 1993; Holovachov & Susulovsky, 2000; Andrássy, 2003).

Some anomalies do not carry any phylogenetic meanings, such as intersexuality, bivulvarity and presence of double female reproductive system (Figs 5.7 I-J). They may point at the organs, whose abnormal structure do not considerably reduce the surviving viability of an aberrant individual.

Phylogenetic significance of the postembryonic development

Postembryonic development in nematodes

Studies of the postembryonic development of nematodes on the basis of populations, collected in nature, are very often complicated by the following three phenomena: **1)** Co-occurence in one sample of more than one species of a genus (e.g. genus *Plectus*), which are hard to differentiate at early developmental stages. In the case of the genus *Plectus* the overall body shape and structure of the labial region are the best features for the primary separation of different species (personal observations). **2)** Difficulty of finding and recognizing early (1st) stages in e.g. a bulk sample, which may be masked by juveniles of other species re genera, for example 1st stage juvenile of *P. parietinus vs* 1st-2nd stages of *P. geophilus*. The greatest difficulties occur when trying to separate 1st stage juveniles of Wilsonematinae from those of *Plectus*, since their general morphology is very similar. **3)** Difficulties of finding moulting specimens, which may help to separate different stages on the basis of morphometrics, because in many cases measurements overlap (see for example Brzeski & Háněl, 1999).

Analysis of the postembryonic development in natural populations is sometimes possible only when measurements of successive stages do not overlap, or when the frequency distributions of certain morphometric characters are ascertainable (large number of specimens required). For instance, one such analysis was made by Halbrendt & Brown (1992) to reveal three juvenile stages in some species of the genus *Xiphinema* Cobb, 1913, and was based on measurements of stylet (odontostyle, odontophore, total stylet and replacement odontophore length). The clear cut separation of juvenile stages on the basis of measurements was possible because the length of the functional odontostyle (solid and strong structure) does not seem to change in a particular specimen during the time between moults, and because there was good agreement between the replacement and functional odontostyle lengths of successive stages. On the other hand, body measurements "alone did not readily separate nematodes into identifiable groups corresponding to life stages" (Halbrendt & Brown, 1992).

When studying postembryonic development in Plectidae I used the following suggestions and facts, which may help during separation of diverse juvenile stages: **1)** 1st stage juveniles in Plectidae are particularly characterised by the valvular apparatus being composed of three longitudinal denticulate ridges, while succeeding stages have valves with transverse denticulate plates (Maggenti, 1961a; De Ley & Coomans, 1997; De Ley et al., 2002)*. **2)** Specimens of the 4th stage usually have strongly developed genital primordia and even primordia of vagina or copulatory musculature and spicules at a later stage of development. **3)** successive stages, only features that do not change in structure or number between moults may be used as markers of a particular stage. It seems that somatic

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* To be sure about the identification of the 1st stage juveniles I also compared the body volume of the suggested earliest stage with the egg volume for theree *Plectus* species respectively. Calculations show that the body volume (V) in 1st stage juveniles is smaller than the egg volume (V_o) in each compared species, e.g. 30,000 μm^3 (V) *vs* 40,000 μm^3 (V_o) in *P. parietinus*, 10,000 μm^3 (V) *vs* 9,000-11,000 μm^3 (V_o) in *P. decens* and 12,000 μm^3 (V) *vs* 14,000-16,000 μm^3 (V_o) in *P. communis*.

sensilla[**] (setae) and/or somatic pores are useful, because they are associated with the cuticular lining, the morphology of which is apparently constant between moults. The arrangement of somatic setae on the retained outer cuticle of moulting specimens was found to correspond to the state of this character in preceding juvenile stage, whereas somatic setae are usually indistinct on the newly formed cuticle.

Postembryonic development in Plectidae

The complete cycle of postembryonic development was studied in *Anaplectus grandepapillatus, Plectus parietinus* (both from Altai mountains, Russia), *P. decens* (L'viv Province, Ukraine) and *P. communis* (Shutsk National Natural Park, Ukraine). The gross morphology of all juvenile stages is similar to the adults in many respects, except for the undeveloped reproductive system and details in structure of the digestive system, glands, number and arrangement of somatic sensilla, and also in measurements.

Four morphologically distinct juvenile stages were observed in *Anaplectus grandepapillatus* (Table 5.3, Fig. 5.8). The cuticle is devoid of somatic sensilla in the 1st stage juvenile, only a deirid inside narrow lateral alae and a pair of subventral caudal setae are present. Three caudal setae (two subventral and one left subdorsal) are present on the tail in the 2nd stage; number and arrangement of caudal setae (two subventral and two subdorsal setae) typical for adults were found in 3rd and 4th stage juveniles. Hypodermal glands in this species appear first in the 2nd stage; they are few in number and distributed in a zig-zag manner; the anteriormost of them is located level with middle of corpus. At later stages (3rd and 4th) the number of hypodermal glands increases considerably, they are arranged in almost opposite pairs along the most part of body, while the anteriormost of them is located at level of stegostom – features also characteristic for adults. Coelomocytes (including those associated with the renette cell) were found in the 2nd-4th juvenile stages. Structure of the labial region, anterior sensilla and amphids are similar to adults in all developmental stages, except the cervical sensilla, which are clearly distinct only in the 4th stage (but may be present in preceding stages). The digestive system (stoma and pharynx) has similar morphology over the whole period of post-embryonic development except for the following: "posterior pharyngeal tubes" are seen in 2nd and later stages; valvular apparatus with longitudinal denticulate ridges is present in the 1st stage while valve with transverse denticulate plates was found in the 2nd-4th stages and in adults. Genital primordia are two separate oval structures lying subventral to intestine in the 1st-3rd juvenile stages. A narrow junction between primordia is seen only in some 3rd stage juveniles. Only in the 4th stage, cells of genital primordia start to grow and differentiate. Although no moulting specimens were found, four juvenile stages could be identified in *A. grandepapillatus* on the basis of pharynx morphology, number and arrangement of somatic sensilla and pores, and development of reproductive system.

Morphological and morphometric analyses of postembryonic development of *Plectus parietinus* revealed four juvenile stages at least by the presence of moulting specimens (Table 5.4, Fig. 5.9), but also taking into consideration the arrangement and

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[**] Somatic setae in Plectidae were studied only along the pharyngeal region and tail.

Table 5.3. Morphometrics of juveniles and adults of *Anaplectus grandepapillatus* (Ditlevsen, 1928) Andrássy, 1973 (all measurements in µm).

Stage	1st	2nd	3rd	4th	females	males
Number	2	7	11	6	6	6
Body length	299; 339	424 (353-476)	598 (490-689)	866 (742-1009)	1337 (1242-1450)	1281 (1172-1487)
Body diameter	12; 13	16.0 (12-18)	20.0 (17-24.5)	28.0 (24.5-33)	42.2 (39-50)	38.3 (37-41)
Pharynx length	112; 113	137 (130-144)	167 (158-178)	205 (191-218)	258 (247-278)	249 (237-277)
Tail length	33; 37	41 (36-49)	49 (47-60)	60 (57-66)	80 (74-89)	83 (77-89)
Anal body diameter	9; 10	11.9 (9-13)	16.0 (14.5-18)	21.3 (19-25.5)	28.3 (25.5-31)	37.8 (34.5-41)
a	24.5; 25.4	26.6 (24.9-28.9)	30.2 (28-31.4)	31.1 (30.3-33.7)	31.9 (29-34.6)	33.4 (32.1-37.2)
b	2.7; 3.0	3.1 (2.7-3.5)	3.6 (3.1-3.9)	4.2 (3.9-4.6)	5.2 (4.9-5.5)	5.2 (4.8-5.4)
c	9.0; 9.2	10.5 (9.7-12.1)	12.3 (10.9-14.7)	14.6 (13.1-16.5)	16.7 (15.3-17.6)	15.4 (14.2-16.7)
c’	3.8; 3.7	3.4 (3.0-4.0)	3.1 (2.9-3.5)	2.8 (2.4-3.1)	2.8 (2.7-3.2)	2.2 (2.0-2.5)
Labial region diameter	5.5; 5.5	6.5 (5.5-7)	7.9 (7-9)	9.1 (9-10)	12.0	11.1 (10-12)
Amphid location	9; 8	8.4 (8-9)	9.1 (8-10)	9.3 (8-10)	9.1 (8-11)	9.3 (9-10)
Anteriormost body pore	-	41.3 (38-44.5)	13.0 (11-14.5)	13.5 (13-14.5)	12.6 (10-15.5)	12.5 (11-13)
Stoma length	18; 18	19.7 (19-21)	21.8 (20-23)	24.1 (22-25.5)	28.2 (27-30)	27.8 (25.5-32)
Setae on tail (right/left side)	1/1	1/2	2/2	2/2	2/2	-
Hypodermal glands (one side)	0	12-18	52-70	131-165	151-164	125-160

number of somatic sensilla. Just like in *A. grandepapillatus*, 1st stage juveniles have only deirids, lateral alae and two ventrosublateral setae on the tail; the 2nd stage is characterised by the presence of two ventrosublateral setae (one pair) level with middle of corpus and three setae on tail (two subventral setae and spur – subterminal seta in left subdorsal position). In the 3rd stage five pairs of setae are arranged along pharyngeal region and two-three pairs of setae on the tail. In the 4th stage there are 7-10 pairs of setae in pharyngeal

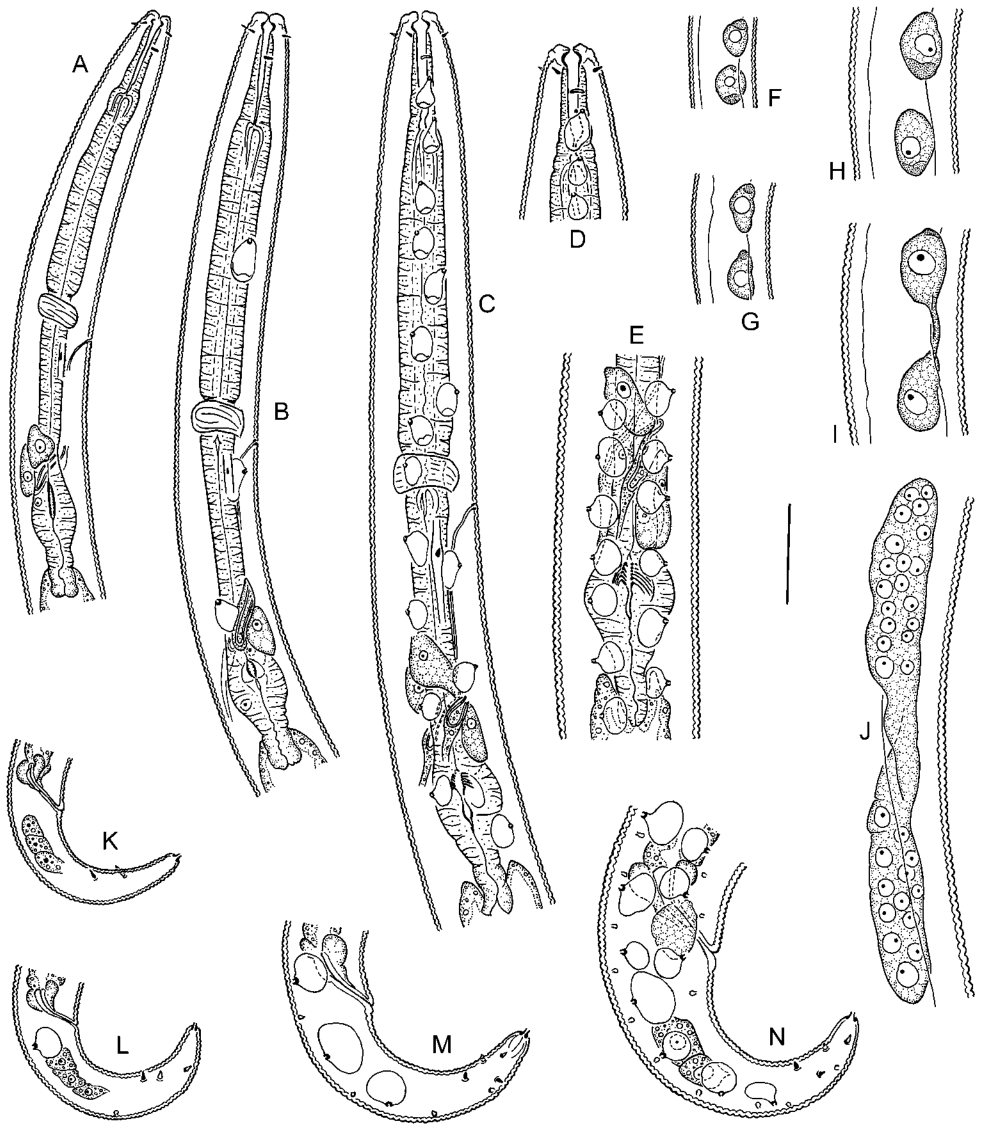

Figure 5.8. Postembryonic development of *Anaplectus grandepapillatus* (Ditlevsen, 1928) Andrássy, 1973: Juveniles of the 1st (A, F, K), 2nd (B, G, L), 3rd (C, H-I, M) and 4th (D-E, J, N) stages. A-C: Pharyngeal region; D: Labial region, combined view; E: Posterior part of pharynx, renette and coelomocytes; F-J: Female gonad primordia; K-N: Tail. Scale bar 20 µm.

Table 5.4. Morphometrics of juveniles and adults of *Plectus parietinus* Bastian, 1865 (all measurements in µm).

Stage	1st	1st-2nd*	2nd	2nd-3rd*	3rd	3rd-4th*	4th	females
Number	11	5	12	1	21	5	21	10
Body length	361 (284-427)	404 (373-452)	488 (421-559)	578	640 (541-811)	738 (674-766)	963 (797-1233)	1262 (1046-1545)
Body diameter	15.6 (11-18)	18.2 (17-21)	20.9 (18-23)	23	28.3 (22-33)	31.3 (29-34.5)	45.9 (33-61)	65 (47-79)
Pharynx length	119 (99-132)	130 (119-148)	150 (129-162)	170	187 (169-206)	207 (192-211)	244 (214-283)	301 (268-351)
Tail length	47.0 (34.5-60)	53.3 (49-59)	61.3 (58-71)	63	74.0 (63-83)	78.2 (69-87)	97.8 (81-116)	120 (111-133)
Anal body diameter	11.1 (8-12)	13.1 (12-14.5)	14.2 (11-17)	19	18.3 (15.5-23)	20.9 (19-23)	26.7 (20-34.5)	32.8 (26.5-38)
a	23.2 (20.1-26.0)	22.2 (21.0-23.8)	23.4 (20.1-27.6)	25.1	22.9 (18.8-25.9)	23.7 (20.9-26.5)	21.3 (17.7-25.7)	19.7 (16.8-25.5)
b	3.0 (2.9-3.2)	3.1 (3.0-3.1)	3.2 (3.1-3.4)	3.4	3.4 (3.1-3.9)	3.6 (3.5-3.7)	3.9 (3.6-4.5)	4.2 (3.9-4.5)
c	7.8 (7.1-9.1)	7.6 (7.1-7.9)	8.0 (6.9-8.7)	9.2	8.7 (8.0-10.5)	9.5 (8.8-11.0)	9.9 (8.5-10.9)	10.5 (9.4-11.7)
c’	4.2 (3.7-4.7)	4.1 (3.7-4.3)	4.4 (3.7-5.5)	3.3	4.1 (3.0-4.7)	3.8 (3.4-4.3)	3.7 (3.0-4.3)	3.7 (3.3-4.2)
Labial region diameter	6.5 (5.5-7)	6.9 (6.5-8.0)	8.2 (8-9)	9	9.8 (9-10)	10.2 (10-11)	12.9 (12-14.5)	15.8 (14.5-17)
Amphid location	11.6 (10-12)	11.8 (10-13)	12.5 (11-13)	14.5	13.4 (12-14.5)	14.0 (13-15.5)	14.4 (12-17)	14 (11-17)
Stoma length	22.3 (19-25.5)	22.9 (22-24.5)	24.6 (22-27)	28	28.9 (25.5-32)	31.6 (29-35.5)	35.1 (31-40)	41.9 (38-49)
Setae in pharyngeal region (right/left side)	0	NA	1/1	NA	(3,4)5/(3)5	NA	7-10/7/10	12-17/13
Setae on tail (right/left side)	1/1	NA	1/2	NA	(2)3/(2)3	NA	3-4/3-4	3-5/3-5
Hypodermal glands (right/left side)	0	NA	0	NA	2-5/3-5	NA	6-9/6-10	10-12/8

* - moulting juveniles, measurements were taken along the inner cuticle

region and three-four pairs of setae on tail. Hypodermal glands are present in *P. parietinus* starting from the 3rd juvenile stage (two-five on each body side). Their number only increases in succeeding stages (6-10 on each body side in the 4th stage and 8-12 in adults). Structure of the labial region, anterior sensilla and amphids is similar in all stages. The

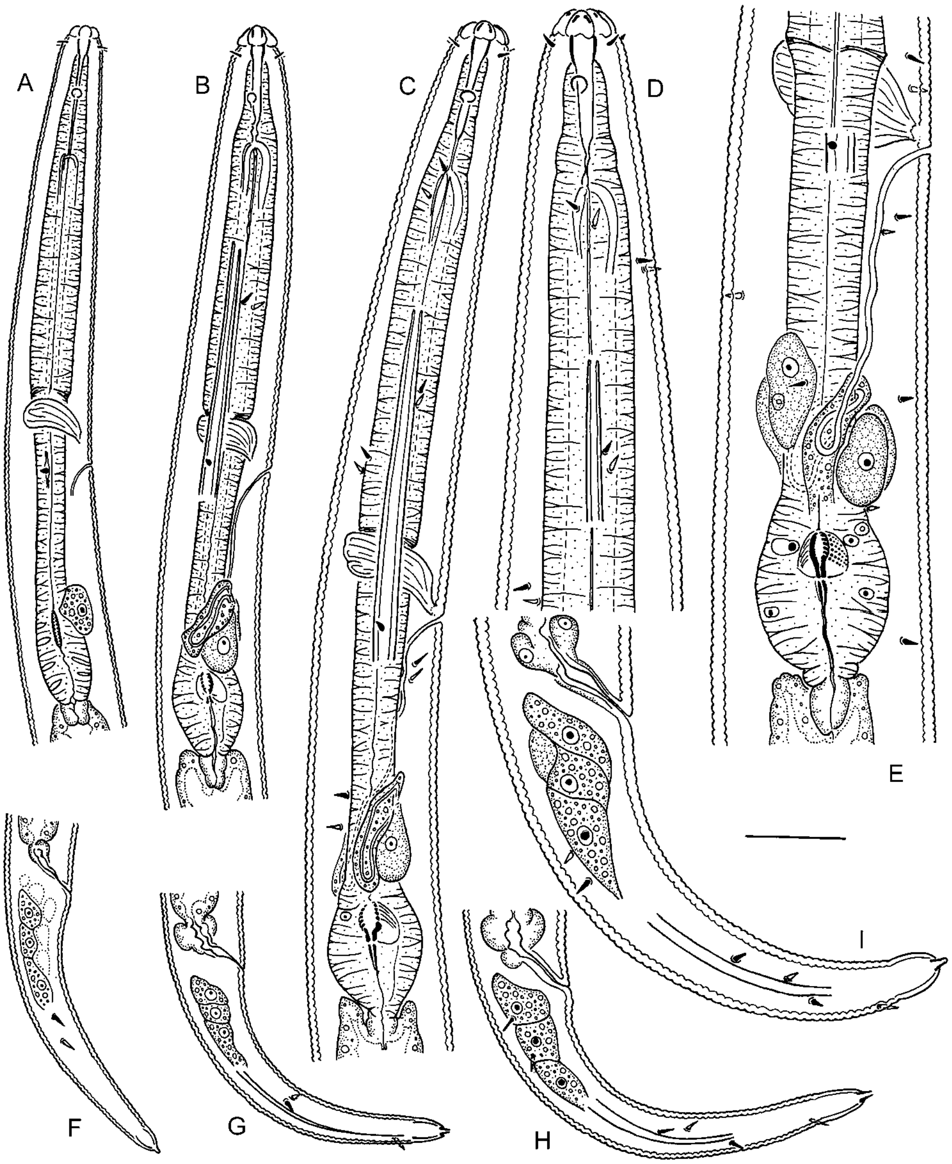

Figure 5.9. Postembryonic development of *Plectus parietinus* Bastian, 1865: Juveniles of the 1st (A, F), 2nd (B, G), 3rd (C, H) and 4th (D, E, I) stages. A-D: Pharyngeal region; E: Posterior part of pharynx, renette and coelomocytes; F-I: Tail. Scale bar 20 µm.

Table 5.5. Morphometrics of juveniles and adults of *Plectus decens* Andrássy, 1985 (all measurements in µm).

Stage	1st	1st-2nd	2nd	2nd-3rd	3rd	3rd-adults	females
Number	10	6	10	5	10	5	10
Body length	326 (308-340)	394 (379-408)	442 (416-470)	488 (458-512)	557 (500-655)	629 (552-667)	700 (611-824)
Body diameter	9.1 (8-10)	10.7 (10-11)	12.3 (11-13)	13.3 (11-14.5)	16.9 (14.5-20)	18 (14.5-20)	22.4 (18-27)
Pharynx length	116 (110-118)	130 (128-134)	140 (135-147)	148 (142-158)	159 (152-170)	172 (157-189)	184 (173-198)
Tail length	39 (35.5-42)	47.4 (43-49)	51.9 (48-55.5)	57.8 (55.5-60)	60.2 (54.5-65.5)	68.9 (65.5-72)	75 (67-84.5)
Anal body diameter	6.2 (5.5-7)	7.0 (5.5-9)	8 (7-9)	8 (8-9)	9.9 (9-11)	10.2 (8-11)	11.4 (9-13)
a	36.0 (32.3-42.1)	36.8 (34.6-39.4)	36.0 (33.8-39)	36.8 (33.9-41.2)	33.1 (30.2-35.9)	35.1 (33.1-38.2)	31.4 (28.3-34.4)
b	2.8 (2.7-2.9)	3.0 (2.9-3.2)	3.2 (3.0-3.3)	3.3 (3.2-3.4)	3.5 (3.2-3.8)	3.7 (3.5-3.8)	3.8 (3.4-4.2)
c	8.4 (8.0-8.7)	8.3 (7.9-9.1)	8.6 (8.0-9.4)	8.5 (8.1-8.7)	9.3 (8.5-10.0)	9.2 (8.1-10.2)	9.3 (8.5-9.9)
c’	6.3 (5.3-7.2)	6.9 (5.4-8.6)	6.5 (5.4-7.2)	7.2 (6.6-7.7)	6.1 (5.7-6.9)	6.9 (5.9-8.7)	6.6 (5.2-8.3)
Labial region diameter	5.0 (4.5-5.5)	5.5	6.2 (5.5-7)	6.2 (5.5-7)	7.8 (7-9)	7.6 (7-8)	9.3 (9-10)
Amphid location	8.8 (8-9)	9.1 (9-10)	9.6 (9-10)	9.6 (9-11)	10.2 (10-11)	11.3 (10-12)	11 (10-11)
Stoma length	19 (18-20)	19.8 (18-22)	20.4 (19-23)	21.1 (19-22)	21.4 (20-23)	23.6 (21-24.5)	23.8 (21-25.5)
Setae in pharyngeal region (right/left side)	0	NA	1/1	NA	2/2	NA	2/2
Setae on tail (right/left side)	1/1	NA	1/2	NA	2/3	NA	2/3

renette is present in all developmental stages, while coelomocytes associated with the renette appear first in the 2nd juvenile stage. A distinct excretory duct is seen along its distal part in all developmental stages; its proximal coiled part embedded in the renette cell was not observed in the 1st stage, but it is distinct in succeeding juvenile stages and in adults. The stoma and basal bulb undergo morphological changes during the first moult. The cheilostom is not sclerotized in the 1st stage of this species, distinct cheilorhabdia appear first in the 2nd stage. The second stegostom section is relatively longer in the 1st stage than in "older" juveniles and adults, thus showing similarities to the stoma of *Caribplectus magdalenae*. The basal bulb contains valves with three longitudinal denticulate ridges in the 1st stage juveniles and with transverse denticulate plates in 2nd-4th stages and in adults. The development of the female reproductive system in this species does not differ considerably from the description by Tahseen et al. (1992) for *P. zelli* and recent data for *A. grandepapillatus* and is not described here.

Three juvenile stages were distinguished in *P. decens*, particularly by the presence of moulting specimens and considering the arrangement and number of somatic sensilla (Table 5.5, Fig. 5.10). Specimens of the 1st juvenile stage have deirids, lateral alae and two subventral setae on the tail; the 2nd stage is characterised by the presence of two ventrosublateral setae (one pair) at level of the middle of corpus and three setae on tail (two subventral setae and spur). In the 3rd stage two pairs of setae (one dorsosublateral and one ventrosublateral) are arranged along pharyngeal region and five setae on the tail, similar to adults. Hypodermal glands are absent in all developmental stages. Coelomocytes are seen in all but the 1st juvenile stages. Structure of the labial region, anterior sensilla and amphids is similar in all stages. The renette is present in all developmental stages, but its detailed morphology and proximal

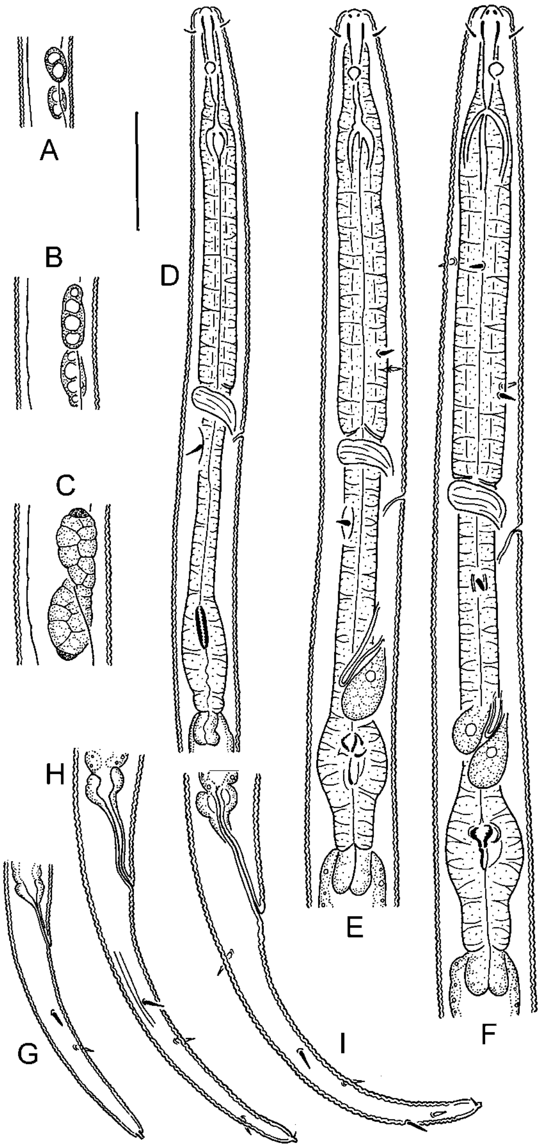

Figure 5.10. Postembryonic development of *Plectus decens* Andrássy, 1985: Juveniles of the 1st (A, D, G), 2nd (B, E, H) and 3rd (C, F, I) stages. A-C: Female gonad primordia; D-F: Pharyngeal region; G-I: Tail. Scale bar 20 μm.

Table 5.6. Morphometrics of juveniles and adults of *Plectus communis* Bütschli, 1873 (all measurements in µm).

Stage	1st	2nd	3rd	females
Number	12	12	12	10
Body length	245 (225-271)	313 (287-342)	396 (373-444)	548 (474-622)
Body diameter	11.7 (11-13)	14.5 (12-15.5)	19.3 (15.5-22)	28.0 (25.5-31)
Pharynx length	89 (83-95)	107 (98-119)	126 (116-140)	150 (141-161)
Tail length	32 (29-35)	39 (37-43)	46 (43-51)	61 (58-68)
Anal body diameter	8.3 (8-9)	9.6 (9-10)	11.4 (11-12)	14.2 (12-17)
a	21.0 (19.2-23.3)	21.6 (20.2-23.5)	20.7 (17.6-24.1)	19.6 (18.6-21.5)
b	2.8 (2.6-2.9)	2.9 (2.7-3.2)	3.2 (3.0-3.4)	3.7 (3.4-3.9)
c	7.7 (7.3-8.3)	8.1 (7.3-9.1)	8.7 (8.1-9.5)	9.0 (7.9-10.0)
c’	3.8 (3.5-4.4)	4.0 (3.7-4.3)	4.0 (3.7-4.6)	4.3 (3.7-4.9)
Labial region diam.	5.6	7.5 (7-10)	8.9 (8-10)	11.2 (11-12)
Amphid location	9.0 (8-10)	9.9 (9-11)	10.3 (10-11)	10.1 (9-11)
Stoma length	16.9 (15.5-18)	18.3 (17-20)	19.7 (18-21)	22.9 (21-27)
Setae in phar. Region (right/left side)	0	1/1	3/3	(3)4/4
Setae on tail (right/left side)	1/1	1/2	2/3	(2)3/3
Hypodermal glands (right/left side)	0	0	0-1/0-1	1(2)/1(2)

part of excretory duct embedded into renette cell are seen only in the 3rd stage and in adults. The stoma and basal bulb undergo morphological changes exactly similar to those described for *P. parietinus*. Genital primordia are two separate oval structures lying subventral to intestine in 1st-2nd juvenile stages; they fuse and start to grow and differentiate during the 3rd stage. Ventral thorns on the spinneret appear in the 2nd stage, and are visible in subsequent stages (3rd and adults).

The study of about 150 juvenile specimens of *Plectus communis* also revealed only three morphologically separate “units”, which are assumed to represent three juvenile stages in this species (Table 5.6, Fig. 5.11). Unfortunately not a single moulting specimen was found which could support or reject the presence of only three juvenile stages. Specimens of the 1st juvenile stage have deirids, lateral alae and two subventral setae on the tail; the 2nd stage is characterised by the presence of two ventrosublateral setae (one pair) level with middle of corpus and three setae on tail (two subventral setae and spur). In the 3rd stage two pairs of setae (one dorsosublateral and one ventrosublateral) are arranged along pharyngeal region and five setae on the tail. In adult specimens one more pair of somatic setae appears in the pharyngeal region and six setae are present on tail. In all but the 1st stage one dorsosublateral pair of setae is present near level of cardia. Hypodermal glands

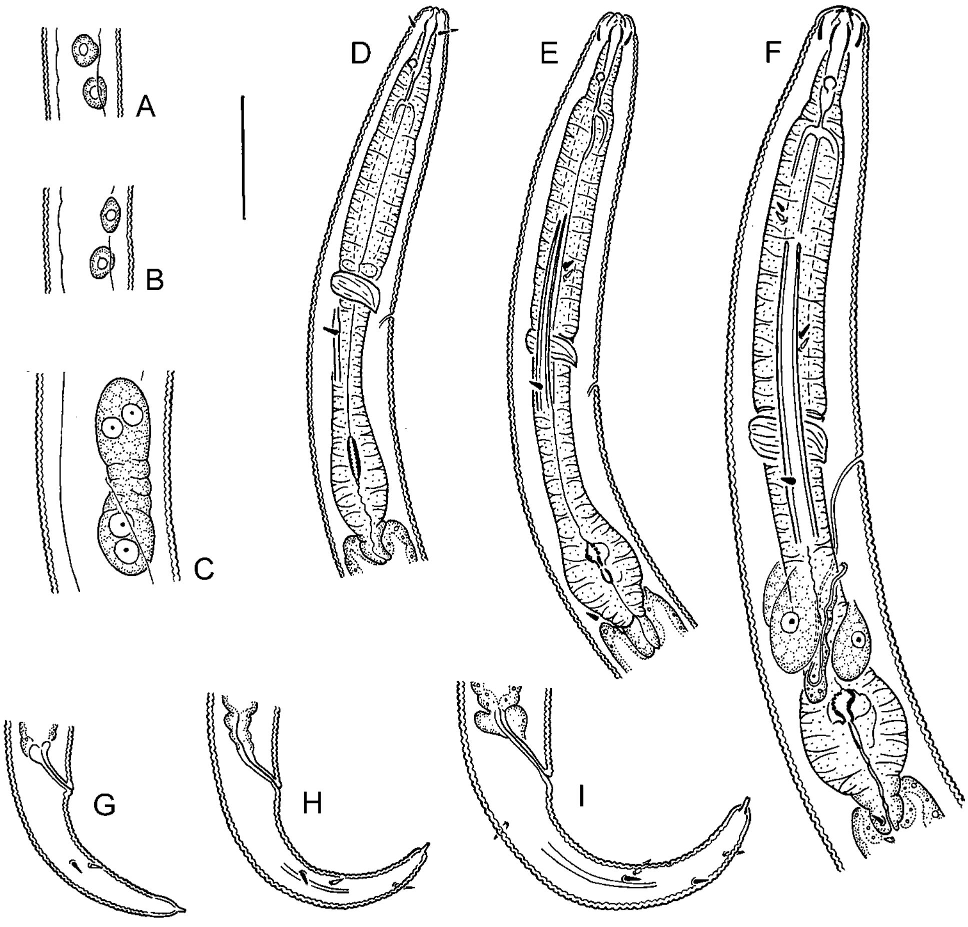

Figure 5.11. Postembryonic development of *Plectus communis* Bütschli, 1873: Juveniles of the 1st (A, D, G), 2nd (B, E, H) and 3rd (C, F, I) stages. A-C: Female gonad primordia; D-F: Pharyngeal region; G-I: Tail. Scale bar 20 µm.

are present only in the 3rd stage (one single cell or one pair) and in adults (one or two on each body side) between the flexure of posterior ovary and anus. Coelomocytes are seen in all but the 1st juvenile stages. Structure of the labial region, anterior sensilla and amphids is similar in all stages. The renette is present in all developmental stages, but its detailed morphology and proximal part of excretory duct embedded into renette cell are seen only in the 3rd stage and in adults. The stoma and basal bulb undergo morphological changes exactly similar to those described for *P. parietinus*. Genital primordia are two separate oval structures lying subventral to intestine in 1st-2nd juvenile stages; they fuse and start to grow and differentiate during the 3rd stage.

In summarising recently described and already published data about the process of postembryonic development I may point at the following major “events” and regularities in Plectidae: **1)** Deirid and lateral alae are present in all stages, the latter are composed of single band of smooth cuticle in the 1st stage and of two sublateral bands in later stages. **2)** Two caudal setae are present in 1st and three in 2nd juvenile stages; in later stages their number grows in a genus specific manner (four in *Anaplectus* and five-six in *Plectus* and *Tylocephalus auriculatus*). **3)** The pharyngeal region is devoid of any somatic sensilla in the 1st stage juveniles of the genus *Plectus* and of *Ereptonema arcticum* and *T. auriculatus*; one ventrosublateral pair of setae appears in the 2nd stage; in subsequent stages their number and arrangement are more variable and characteristic for each particular species and genus. **4)** Hypodermal glands are absent in 1st and 2nd stages in *Plectus* but appear later, after the respective moult. **5)** In *Plectus* species, the stoma undergoes morphological changes during the first moult, while the stoma in 1st stage juveniles of *Anaplectus* is similar to adults. The cheilostom is not sclerotized in the 1st stage of *Plectus*, distinct cheilorhabdia appear first in the 2nd stage. The second stegostom section of all three studied *Plectus* species is relatively longer in the 1st stage than in “older” juveniles and adults, thus showing similarities to the stoma of *Caribplectus magdalenae*. **6)** In all developmental stages the pharynx is similar morphologically, bearing pharyngeal tubes and subdivided into corpus and isthmus. Only the basal bulb and valvular apparatus undergo pronounced morphological changes during development. The basal bulb is weakly developed in the 1st juvenile stage. The valvular apparatus is built like three straight denticulate ridges located in the anterior half of the basal bulb. They are not associated with any further sclerotizations, which are characteristic for adults of *Chronogaster* and Plectidae, but very similar to the simple valvular apparatus in *Caribplectus*. The typical transverse valves with sclerotized haustrulum and oval basal bulb appears first in the 2nd stage juveniles of *Plectus* and *Anaplectus*.

It is obvious that not only the reproductive system but also some other organs and characters undergo changes during postembryonic development in Plectidae. Among them the morphological changes of stoma, valvular apparatus and renette presumably recapitulate the evolution of these organs, while development of hypodermal glands and somatic sensilla represent only age variability. Thus, according to the biogenetic convergence rule (Lorenzen, 2000) I may suggest the following character polarities (plesiomorphic *vs* apomorphic): **1)** cheilostom undifferentiated *vs* with sclerotized rhabdia; **2)** posterior stegostom section long *vs* short; **3)** valvular apparatus with longitudinal denticulate ridges *vs* transverse denticulate plates; **4)** renette cell without neighbouring coelomocytes *vs* followed by coelomocytes.

Number of juvenile stages in Plectidae

Four juvenile stages were described by Tahseen et al. (1992) in *P. zelli*, but only three stages were suggested to occur in *Tylocephalus auriculatus* by De Ley & Coomans (1997) and *Ereptonema arcticum* by De Ley et al. (2002). It is generally accepted that the members of the phylum Nematoda are particularly characterised by four juvenile stages during postembryonic development. The rare cases of three or even two “post-hatching” juvenile stages in saprobiotic and parasitic Rhabditida appear when the first (and sometimes second) moult takes place inside the egg before hatching (Malakhov, 1986).

In the present analysis I found four juvenile stages in *Anaplectus grandepapillatus* and *Plectus parietinus* but only three stages (or at most three morphological types of juveniles) in *P. decens* and *P. communis*. Taking into consideration the great morphological similarity of the 1st stage juveniles of all Plectidae, for which postembryonic development was described in detail (*A. grandepapillatus, P. parietinus, P. decens, P. communis, T. auriculatus, E. arcticum*)*, I assume that there is no moult inside the egg before hatching in species that have only three morphological types of juveniles, although this should be verified by direct observations. Since 1st stage juveniles of all six studied species have similar morphology (structure of labial region, lateral field, basal bulb, number and arrangement of caudal setae), and since 2nd stage juveniles of *Plectus* and species of Wilsonematinae have constant numbers and distributions of somatic setae in pharyngeal region and on tail, it appers that developmental pathways have changed specifically with the 3rd and 4th stage juveniles. If this is really the case, then I can suggest two contradictory hypotheses in order to explain the presence of three morphological types of juveniles in Plectidae (particularly in Plectinae and Wilsonematinae). **1)** The moult between thr 3rd and 4th stages was totally lost, or **2)** the moult between the 3rd and 4th stages is present, but the juveniles of these stages (within particular species) are morphologically identical, the growth and developmental process are restricted only to reproductive system. Anyway, both hypotheses can be proved or disproved only on the basis of observations on live material.

Phylogenetic analysis

Justification of the outgroup

As outgroups I selected two species: *Hemiplectus muscorum* and *Stephanolaimus elegans* which were included in my phylogenetic analysis of the superfamily Leptolaimoidea (Chapter 3) and are described in detail. *Stephanolaimus elegans* group in polytomy with *Plectus* according to the previous analysis. *Hemiplectus muscorum* on the other hand was shown to be the sister taxon of *Plectus* and is the only genus of Leptolaimoidea which has a “plectoid” structure of the renette cell. To root the tree and polarize character states in computer tree-building procedures I also used a “hypothetical ancestor” with the primitive character state in all characters.

Characters and their polarisation

A data matrix for the characters and their states used for the cladistic analysis is presented in Table 5.7. In one case the CAMIN-SOKAL optimality criterion was assigned to characters № 6, 9, 10, 34, 37, 58, 59, 60, 63, 64, 66 and 67. The character states are as follows: **1)** Cuticular annulation: conspicuous [0]; inconspicuous [1]. **2)** Intracuticular ornamentation: absent [0]; present [1]. **3)** Longitudinal incisures on annules: absent [0]; present [1]. **4)** Plate-like projections on annules: absent [00]; present, simple [01]; present, carry anterior and posterior thorn-like projections [11]. **5)** Lateral alae: absent [0]; present

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* Also personal observation (unpublished) for *P. geophilus, P. minimus, P. exinocaudatus* etc.

Table 5.7. Character state matrix for species of Plectoidea and two outgroup species (*Hemiplectus muscorum* and *Stephanolaimus elegans*) used in the phylogenetic analyses. See text for explanation of characters and character states.

Character number, dataset	0000000000000111111111111 1234456667899012222345678
Optimality criteria, MIX	wwwwwwssswwwwswwwwwwwwwww
1 Hypothetical ancestor	0000000000000000000000000
2 Hemiplectus muscorum	000000111--00000000000000
3 Stephanolaimus elegans	000000000000000----000000
4 Pakira orae	00000000011111-----000000
5 Cynura cerambus	000000???--00000000000000
6 Cynura klunderi	0000000000000000000000000
7 Caribplectus magdalenae	0000000000000000000000000
8 Chronogaster boettgeri	000000111--111-----000000
9 Chronogaster typica	00000000000111-----000000
10 Chronogaster sp 1	000010111--111-----010000
11 Chronogaster spinicorpus	000110111--111-----010000
12 Chronogaster andrassyi	000000111--111-----000000
13 Chronogaster multispinatoides	000000111--111-----000000
14 Euteratocephalus palustris	110000000000000----001000
15 Metateratocephalus gracilicaudatus	110000000000000----001000
16 Anaplectus granulosus	00000100000011-----000000
17 Anaplectus atubulatus	00000100000011-----000000
18 Perioplectus labiosus	00000100000011-----000100
19 Arctiplectus alaskanus	00000100000011?----000000
20 Plectus velox	0000010000000011000000000
21 Plectus parietinus	0000010000000010000000000
22 Plectus pusteri	0000010010000010000000000
23 Plectus paracuminatus	0000010010000010000000000
24 Plectus murrayi	0000010010000011000000000
25 Plectus aquatilis	000001111--00010000000000
26 Plectus communis	000001011--00011000000000
27 Plectus parvus	000001111--00011000000000
28 Plectus longicaudatus	000001111--00011100000000
29 Plectus decens	000001111--00011100000000
30 Plectus geophilus	000001111--00011100000000
31 Plectus minimus	000001111--00011100000000
32 Plectus andrassyi	000001111--00010000100000
33 Plectus cancellatus	001001111--00011000100000
34 Ceratoplectus armatus	000001111--00010011000000
35 Ceratoplectus assimilis	000001111--00010010000000
36 Tylocephalus auriculatus	000001111--00010011000010
37 Ereptonema arcticum	000001111--00010011000010
38 Wilsonema otophorum	000001111--00010011000011

Table 5.7. (Continued).

1122222222222222223333333333444444444444444555555555556666666667
8900111234567889012345678901112345678889012234567890123456789 0
wwwwwwwwwwwwwwwwwwwwswwswwwwwwwwwwwwwwwwwwwwwwwwwwwwwssswwsswsswww
0000000000-00000000000000-00000-00000000-00000000000000000000
0000000000-000000000111100010-00000000-00000000100000100000 0
0000000000-0000010000000000-00000-00000000-0000000000000001000000
0000000010-10010010000111-1001101010000 0-1010000010000001011--0
0000000000-1000000000??1?-0001100110001 0-11100?001000100000000
0000000000-1000000000??1?-0001100110001 0-11100?000000100000000
0000000000-1000000000??10-0001100110001 0-1110??001000000000000
0000000000-1000000001010-00011001100111111111000101--10000000
0000000000-1000000100101 0-0001100110011111111110001000000011--0
0000000000-1000000101-----00011001100111111111100010000000 11--0
0000000000-1000000101-----000110011001111111111000100000 1011--0
0000000000-1000000100101 0-0001100110011111111110001000000011--1
0000000000-1000000100101 0-0001100110011111111110001000000011--1
0000000000-?10010000011111100111011010110111010001 11--01011--0
0000000000-?10010000011111100111011010110111010001 11--01011--0
0000000000-1011001010111100011110111101101110010010010001 00000
0000000000-10110010101111000111101111011011100100100100 0101--0
0000000000-?0010000101?11?000111011?1011011100100100000?100000
0000000000-?0??0000101?11?000111011?1011011100000100000 0?00000
0000000000-1000000010111101001110110101101110101110000000000 00
0000000000-1000000010111101001110110101101110101110000000000 00
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0000000000-1000000010111101001110110101101110101110000000000 00
0000000000-1000000010111101001110110101101110100010 1--11000000
0000000000-1000000010111101001110110101111110100010 1--11000000
0000000000-1000000010111101001110110101111110100010 1--11000010
0000000000-1000000010111101001110110101111110100010 1--11000000
0000000000-1000000010111101001110110101111110100010 1--11000100
0000000000-1000000010111101001110110101101110101110000000000 00
0000000000-1000000010111101001110110101101110101110000000000 00
0000000000-1000000010111100001110110101101110100010 1--11000000
0000000000-1000000010111100101110110101101110100010 1--11000000
110100010101000000001011110000111011010110111010001 01--11000000
100101110111000000001011110000111011010110111010001 01--11000000
101111010111000000001011110000111011010110111010001 01--11000000

[1]. **6)** Hypodermal glands and pores: present over entire body [000]; present, few in posterior region [001]; present, single in posterior region [011]; absent [111]. **7)** Hypodermal glands and pores arranged in: four sublateral rows [0]; eight rows [1]. **8)** First body pore location: posterior to amphid [0]; between lips [1]. **9)** Somatic sensilla in females: present over the whole body [00]; present on tail only [01]; absent [11]. **10)** Somatic sensilla in males: present [0]; absent [1]. **11)** Ventrosublateral pair of setae at level of the middle of corpus: absent [0]; present [1]. **12)** Arrangement of somatic setae at level of anterior part of the corpus: three pairs of setae with dorsosublateral pair in anteriormost position [0000]; two pairs of setae with dorsosublateral pair in anteriormost position [1000]; one dorsosublateral pair of setae [1100]; three pairs of setae with subdorsal and subventral pairs in anteriormost position [0010]; only subventral and subdorsal pairs of setae [0011]. **13)** Labial region structure: lips rounded, simple or different [0]; lips with one setiform projection on its inner side [1]. **14)** Labial region structure: lips round, simple or different [0]; lips fused, labial region cap-like [1]. **15)** Labial region structure: lips rounded, simple or different [0]; lips crown-shaped, sclerotized [1]. **16)** Labial region structure: lips rounded, simple or different [0]; lips with three projections on its inner side, characteristic for *Perioplectus* [1]. **17)** Labial region structure: lips rounded, simple or different [0]; labial region with complex outgrowths, biradial [1]. **18)** Cervical expansions: absent [00]; present, annulated [01]; present, smooth [11]. **19)** Median ridge: absent [0]; present [1]. **20)** Midlateral projection: absent [00]; short oval [01]; long digitate [11]. **21)** Flabella: absent [00]; present, four submedian [01]; present, two median [11]. **22)** Lateral rim: absent [00]; present, smooth [01]; present, fimbriate [11]. **23)** Cephalic sensilla: setiform or different [0]; papilliform [1]. **24)** Cephalic sensilla: setiform or different [0]; developed into cornua [1]. **25)** Cornua: elongate oval [0]; incised [1]. **26)** Inner labial sensilla: on lips [0]; in cheilostom [1]. **27)** Outer labial sensilla: papilliform [0]; setiform [1]. **28)** Cervical sensilla near amphid: absent [00]; in male only [01]; in both sexes [11]. **29)** Amphid: circular or different [0]; with tubular orifice and subcuticular fovea [1]. **30)** Amphid: circular or different [0]; pocket-shaped [1]. **31)** Amphid: circular or different [0]; transverse slit [1]. **32)** Amphid: circular or different [0]; stirrup-shaped or horseshoe-shaped [1]. **33)** Deirid: absent [0]; present [1]. **34)** Renette: present [0]; absent [1]. **35)** Renette location: at intestine [0]; at postcorpus [1]. **36)** Renette: single [0]; followed by coelomocytes [1]. **37)** Excretory ampulla: present [0]; absent [1]. **38)** Excretory duct: free [0]; embedded [1]. **39)** Excretory duct shape: making two loops around pharynx [0]; making spiral on right-hand side [1]. **40)** Cheilostom structure: undifferentiated [0]; with sclerotized rhabdia [1]. **41)** Gymnostom structure: undifferentiated, conoid [000]; with plate-like rhabdia [001]; with bar-shaped rhabdia [011]; with small oval rhabdia [101]. **42)** Stegostom structure: undifferentiated [0]; developed, with two sections [1]. **43)** Posterior stegostom section: long [0]; short [1]. **44)** Pharynx: cylindrical, uniformly muscularized or different [0]; cylindrical with posterior glandular part [1]. **45)** Pharynx: cylindrical, uniformly muscularized or different [0]; subdivided into corpus and postcorpus (isthmus and bulbus) [1]. **46)** Pharyngeal tubes: absent [0]; present [1]. **47)** "Posterior pharyngeal tubes": absent [0]; present [1]. **48)** Valvular apparatus: absent [000]; with thickened lumen or simple longitudinal denticulate ridges [001]; with longitudinal denticulate ridges and two sets of round thickenings [011]; with transverse denticulate plates [101]. **49)** Basal bulb: absent [0]; present [1]. **50)** Basal bulb location: terminal [0]; subterminal [1]. **51)** Dorsal gland

orifice location: just posterior to stoma [0]; in stegostom [1]. **52)** Subventral gland orifices location: just posterior to the stoma [00]; at middle of the pharynx [01]; within the corpus-isthmus junction [11]. **53)** Female reproductive system: paired [0]; monoprodelphic [1]. **54)** Spermatheca: present [0]; absent [1]. **55)** Crustaformeria: absent [0]; present [1]. **56)** Vaginal sphincter number: single [0]; two [1]. **57)** Epiptygmata: absent [0]; present [1]. **58)** Advulval sensitive structures: present [0]; absent [1]. **59)** Testes: two [0]; single anterior [1]. **60)** Tubular supplements: present [0]; absent [1]. **61)** Tubular supplement shapes: equal or different [0]; posteriormost with more developed gland [1]. **62)** Tubular supplement shapes: equal or different [0]; anteriormost with rasp-like structure or guiding piece at distal part [1]. **63)** Precloacal sensillum: present [0]; absent [1]. **64)** Postcloacal sensilla: present [0]; absent [1]. **65)** Precloacal pairs of subventral sensilla in male: absent [0]; present [1]. **66)** Caudal glands: present [0]; absent [1]. **67)** Spinneret: present [0]; absent [1]. **68)** Spinneret shape: typical or different [0]; ventrally bent [1]. **69)** Spinneret shape: typical or different [0]; with two lateral thorns [1]. **70)** Mucro on tail tip: absent [0]; present [1].

Computer tree-building

A cladistic analysis of the entire dataset with the aid of NONA under WAGNER optimality criterion for all characters resulted in 115 most parsimonious trees and a strict consensus cladogram of these is shown in Fig. 5.12. In the first step it branches basally as a trichotomy between the hypothetical ancestor, the outgroup *Stephanolaimus elegans* and the clade leading to Plectoidea and *Hemiplectus muscorum*. The latter clade is supported by the following characters: renette followed by coelomocytes, excretory ampulla absent, plate-like gymnorhabdia present. The structure of the renette cell and associated coelomocytes has already been discussed in Chapter 3. Such a complex renette probably reflects an adaptation to a fresh-water environment (See Chapter 6). If we consider the presence of coelomocytes in the renette cell vicinity as a synapomorphy for the clade uniting *Hemiplectus* with Plectoidea, the secondary loss of coelomocytes in *Chronogaster* is obvious.

A monophyletic origin of the superfamily Plectoidea is supported by the location of inner labial sensilla inside the cheilostom, appearance of stegostom and pharyngeal tubes, location of dorsal gland orifice inside the stegostom, and subventral gland orifices at midpharynx. Although each of these characters is not unique among nematodes, in combination they represent a step towards a further modification of the entire pharynx. The genus *Pakira* is considered here to be the least derived member of Plectoidea. The monophyly of its sister taxon is supported by the division of the pharynx into corpus and isthmus, appearance of valvular apparatus and location of the subventral gland orifices at corpus-isthmus junction.

The branching order between *Cynura, Caribplectus* and other plectids is left unresolved as a trichotomy. Presence of basal bulb and reduction of spermatheca unite the clade leading to *Chronogaster*, Plectidae and Metateratocephalidae, although both features are equivocal. Shortening of the stegostom and complexity of the valvular apparatus structure both support the monophyly of the clade that leads to Metateratocephalidae and Plectidae. Uniqueness of the family Metateratocephalidae is suggested by the characteristic

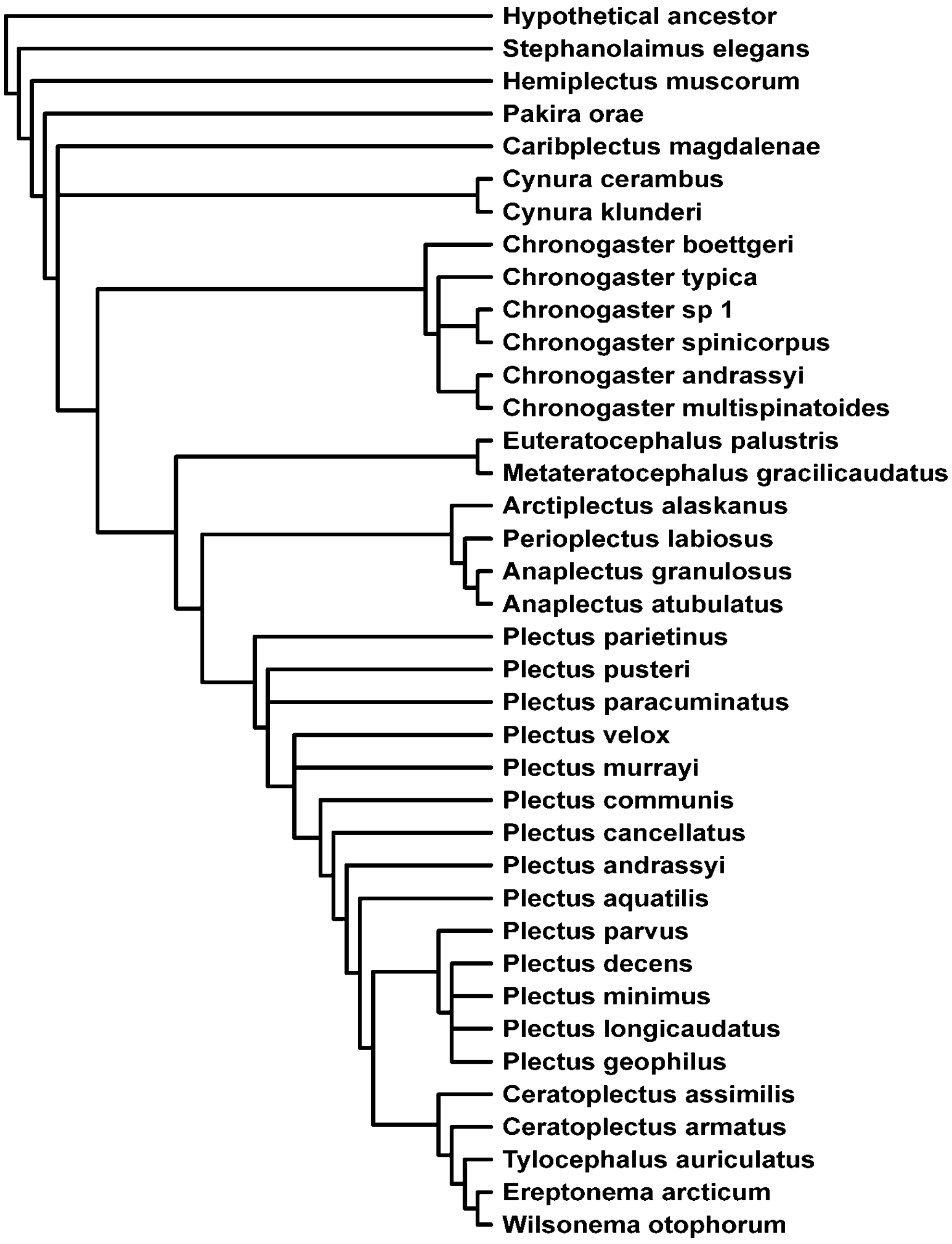


Figure 5.12. Strict consensus tree of 115 maximum parsimonious trees obtained with the aid of NONA (WAGNER optimality criterion for all characters, tree length is 131 steps).

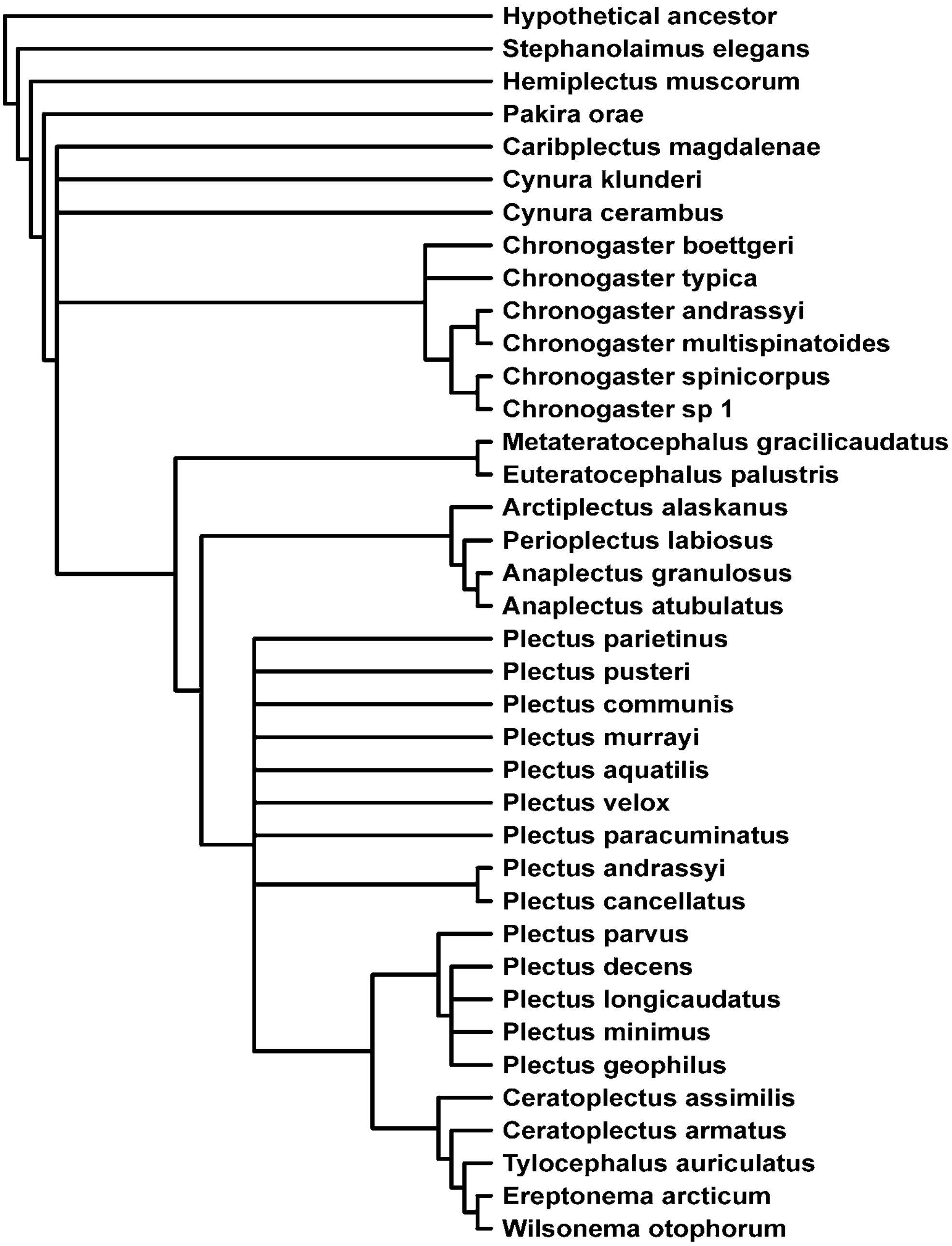


Figure 5.13. Strict consensus tree of 100 maximum parsimonious trees obtained with the aid of MIX (CAMIN-SOKAL optimality criterion for characters № 6, 9, 10, 34, 37, 58, 59, 60, 63, 64, 66 and 67, tree length is 135 steps).

cuticle with intracuticular ornamentation, crown-shaped lips, setiform labial sensilla, amphid shape, spirally coiled excretory duct and monorchic male gonad. The monophyletic origin of the Plectidae is supported by two features: presence of lateral alae and deirids.

The relationships within the family Plectidae are unclear and difficult to resolve unequivocally. The reduction of somatic sensilla in females and in males and presence of spermatheca unite the genera *Arctiplectus, Perioplectus* and *Anaplectus* into a single clade here treated as subfamily Anaplectinae. The remaining taxa are grouped together by the presence of two vaginal sphincters and epiptygmata. Such a grouping necessitates a reversal of both these characters in some *Plectus*-species, and is not accepted here. I would rather unite *Plectus, Tylocephalus* and *Wilsonema* by the presence of a parthenogenetic mode of reproduction and thus simple structure of the female gonads. The only clearly supported monophyletic taxon is the subfamily Wilsonematinae, which possesses a complex labial region. The remaining species represent a paraphyletic assemblage awaiting further studies.

Analysing the morphology of the group in question, several characters seem to have evolved by reductions that may be irreversible. These are: the absence (reduction) of hypodermal glands, somatic sensilla, renette, excretory ampulla, advulval sensitive structures, one of the testes, tubular supplements, precloacal and postcloacal sensilla, caudal glands and spinneret. The assignment of CAMIN-SOKAL optimality criterion to these characters and further analysis of the entire dataset by using the MIX programme of the PHYLIP-3.61 package (other characters under WAGNER optimality criterion) resulted in an even more poorly resolved phylogeny. Fig. 5.13 shows a strict consensus tree of 100 maximum parsimonious trees. It agrees with previous results regarding the position of *Stephanolaimus elegans, Hemiplectus muscorum* and *Pakira orae*, and supports the monophyly of the genus *Chronogaster*, families Metateratocephalidae and Plectidae, and subfamilies Anaplectinae and Wilsonematinae. However, the relations of the genera *Cynura, Caribplectus, Chronogaster* and the clade Metateratocephalidae+Plectidae are left unresolved. This is also the case for the genus *Plectus*, of which most species are grouped in a polytomy.

Suggested phylogeny and system of the superfamily Plectoidea

Manual tree-building and consideration of different character weights resulted in the phylogenetic hypothesis given in Fig. 5.14. I added some features, which were not treated during the computer-based procedures. The suggested phylogeny agrees with the computer-based analysis regarding the monophyly of the genus *Chronogaster*, families Metateratocephalidae and Plectidae, subfamilies Anaplectinae and Wilsonematinae.

The grouping of the genera *Caribplectus, Cynura* and *Chronogaster* is based on plesiomorphies and thus does not merit the definition of a monophyletic group. However, I unite them in a single family Chronogastridae since this family name already exists and species species of all three genera are characterised by a common morphology of the pharynx (long second stegostom section and valvular apparatus with longitudinal ridges), thus differing from the closely related families Pakiridae, Metateratocephalidae and Plectidae. On the other hand, the allocation of the genera *Cynura* and *Caribplectus* within a separate new family on the basis of unresolved cladograms (Figs 5.12-5.13) seems unjustified.

The suggested phylogeny of the family Chronogastridae is presented on the Fig. 5.14. All three genera are placed in trichotomy. For the genus *Chronogaster*, *C. boettgeri* is the most basal taxon. On the other hand *C. spinicorpus*, for which the genus name *Keralanema* Siddiqi, 2003 was currently proposed, is deeply nested between other *Chronogaster*-species, close to *Chronogaster* sp 1. Siddiqi (2003) stated that *Keralanema* differs from *Chronogaster* "in having cuticular annules divided into plate-like structures bearing 12 hook-like spines attached at the middle of annules and overlapping preceding and succeeding annules, and amphid aperture appearing bilobed and inverted U-shaped in lateral view." Already in the original description of *C. spinicorpus* the authors (Maggenti et al., 1983) stated that "if one insists that the external ornamentation is sufficient to establish new genera, than *Chronogaster* could be split into several genera…" Moreover, the structure of the cuticle in *Chronogaster* sp 1 is intermediate between the highly modified cuticle of *C. spinicorpus* and other species of this genus with simply annulated or "tiled" cuticle (e. g. *C. magnifica* Andrássy, 1958). Furthermore, the shape of the amphid in *C. spinicorpus* and *Chronogaster* sp 1 does not differ qualitatively from amphids in other species of the genus – in all but one species the posterior rim of the amphidial aperture bears two sublateral anteriorly directed projections. Thus, a separate genus for *C. spinicorpus* is not accepted in the present paper.

Morphological peculiarities of *Chronogaster* sp 2 (Fig. 5.15) add more complications to the hypothesis about the phylogeny of the family Chronogastridae. In addition to its own unique features (vaginal sclerotization, long postvulval uterine sac, long tail and large anterior uterus), this species is also characterised by the features characteristic for "typical" *Chronogaster*-species (stirrup-shaped amphid with paired and forwardly directed projections located sublaterally on the posterior rim of the amphid, monoprodelphic female reproductive system, subterminal basal bulb and reduced caudal glands) and for Caribplectus-*Cynura* representatives (long cylindrical anterior stegostom section and valvular apparatus with three longitudinal denticulate ridges but without two sets of oval thickenings). This species was not included in the cladistic analysis for the following reasons: **1)** only female characters are known, no male was found; **2)** since just one specimen is available one cannot exclude the possibility that this animal was abnormal in one or more characters.

A fully resolved phylogeny of the genus *Plectus* is impossible to propose at present. The number of qualitative characters, which were included in the cladistic analysis, is insufficient to resolve the relationships completely, also because many species of the genus *Plectus* are differentiated on the basis of measurements and quantitative features. The proposed cladogram of the genus *Plectus* is based largely on the gradual reduction of somatic sensilla, hypodermal glands and tubular supplements.

My hypothesis about the phylogenetic relationships in the family Plectidae is not congruent with the cladogram developed by Zell (1993) in two major respects: **1)** I suggest *Ceratoplectus*+Wilsonematinae to represent a sister group of the genus *Plectus*, and **2)** the former genus *Chiloplectus* is deeply nested amoung species of the genus *Plectus*, close to the *P. parietinus*-group. Similar to Zell (1993), the previous analysis (Chapter 4) and other observations (Holovachov et al., 2004) showed that *Ceratoplectus* represents the closest sister group to the members of the subfamily Wilsonematinae, having similar biradial

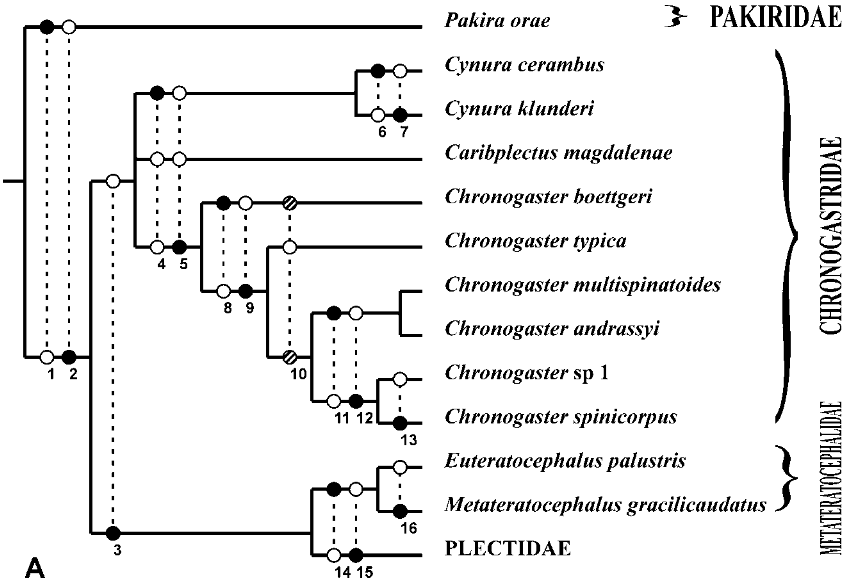
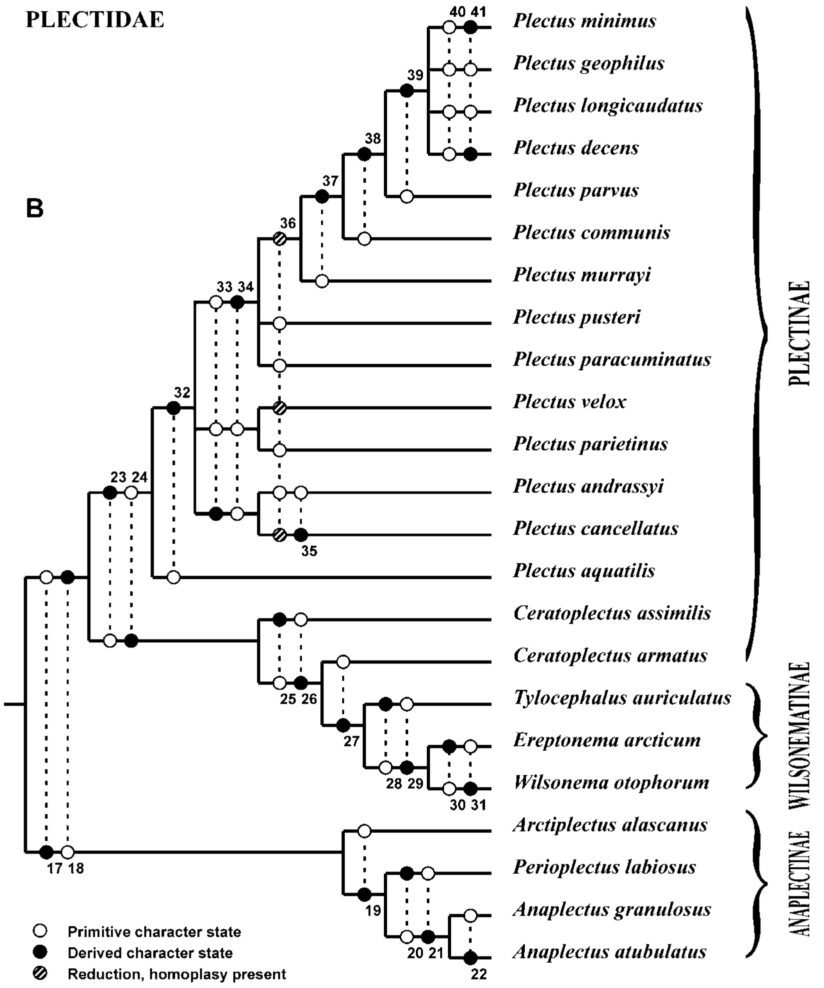


Figure 5.14. Suggested phylogeny and classification of the superfamily Plectoidea Örley, 1880 in general (A) and family Plectidae Örley, 1880 in particular (B). Derived charactes states (including reductions) are as follows: (1) Eight rows of hypodermal glands and pores (*vs* four); anteriormost body pores located at base of lips (*vs* posterior to amphid); cephalic sensilla papilliform (*vs* setiform); cervical sensilla present; amphid in shape of transverse slit; basal part of pharynx glandular (*vs* muscular); somatic sensilla, caudal glands and spinneret reduced. (2) Pharynx subdivided into corpus and isthmus; subventral gland orifices located within corpus-isthmus junction (*vs* at midpharynx); valves present. (3) Second stegostom section short (*vs* long); valves as transverse denticulate plates (*vs* as longitudinal denticulate ridges); excretory duct embedded into renette cell. (4) Spinneret enlarged, strongly cuticularised. (5) Monoprodelphic female gonad (*vs* didelphic); first stegostom section short (*vs* long); subterminal basal bulb; valves with thickenings. (6) Tubule with rasp-like structure. (7) Tubule with guiding piece. (8) Tubules and precloacal sensilla reduced. (9) Stirrup- or horseshoe-shaped amphid; caudal glands reduced. (10) Hypodermal glands reduced. (11) Female tail terminus with mucro, blunt in male. (12) Cuticle with longitudinal ridges; tail terminus filiform. (13) Cuticle ridges with spines; postcloacal sensilla reduced. (14) Finely annulated cuticle with intracuticular ornamentation; crown-shaped sclerotized lips; amphid with subcuticular fovea; spirally coiled excretory duct; single anterior testis; caudal glands and spinneret reduced. (15) Deirid and lateral alae present; excretory duct making two loops around isthmus. (16) Male caudal sensilla in opposite pairs (*vs* irregular); lips offset (*vs* continuous); cheilostom sclerotized. (17) Spermatheca present; somatic sensilla in females on tail only, reduced in males. (18) Ventrosublateral pair of setae at level of midcorpus present, just subventral to lateral alae. (19) Crustaformeria present; several subventral precloacal pairs of papilla present. (20) Characteristic labial region and stoma structure; cervical sensilla in male only. (21) Amphid a transverse slit; cervical sensilla in both sexes; posterior pharyngeal tubes present; posteriormost tubular supplement with enlarged gland (*vs* equal to others). (22) Spinneret reduced. (23) Two vaginal sphincters (*vs* single); cheilostom sclerotized. (24) Subventral and subdorsal pairs of setae at level of stoma, anterior to dorsosublateral pair (*vs*



opposite); tubular supplements and pre- and postcloacal sensilla reduced. (25) Gymnorhabdia ovoid (*vs* plate-like). (26) Dorsosublateral pair of setae at anterior part of corpus reduced. (27) Cervical expansions, cornua, midlateral projection and lateral rim present. (28) Median ridge present. (29) Cornua incised (*vs* ovoid). (30) Lateral rim fimbriate (*vs* smooth). (31) Midlateral projection digitate (*vs* rounded); two flabellae (*vs* four); cervical expansions smooth (*vs* annulated). (32) Epiptygmata present. (33) Hypodermal glands reduced; lips with inner setiform projections. (34) Hypodermal glands present only between vulva and anus, few (*vs* over entire body, numerous). (35) Cuticle tiled. (36) Subdorsal pair of setae at anterior part of corpus reduced. (37) One pair of hypodermal glands between vulva and anus (*vs* few); tubular supplements reduced to one. (38) Tubular supplements, pre- and postcloacal sensilla, epiptygmata, hypodermal glands reduced; one vaginal sphincter (*vs* two). (39) Basal bulb subterminal (*vs* terminal); subventral pair of setae at anterior part of corpus reduced. (40) Spinneret with lateral thorns. (41) Spinneret bent ventrad.

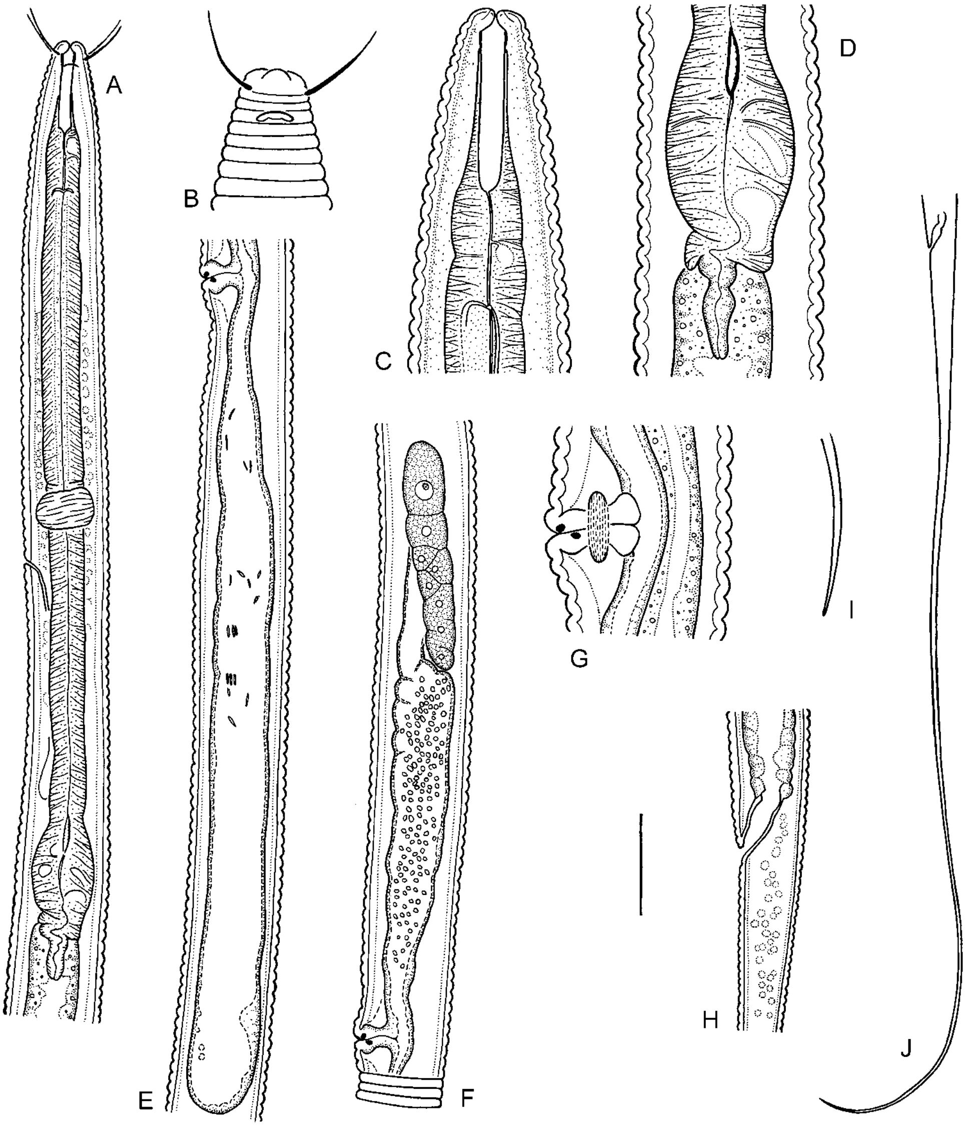


Figure 5.15. Morphology of the female of *Chronogaster* sp 2. A: Pharyngeal region; B: Labial region, surface view; C: Anterior end, median section; D: Posterior part of pharynx; E: Posterior part of reproductive system; F: Anterior part of reproductive system; G: Vagina region; H: Rectum region. I: Tail terminus; J: Tail. Scale bars: A, E, F, H = 20 μm, B, C, D, G, I = 10 μm, J = 50 μm.

symmetry of labial region, arrangement of somatic sensilla along the anterior part of the pharynx, absence of tubular supplements, as well as pre- and postcloacal sensilla in males. The morphology of the labial region in the recently described *Tylocephalus primitivus* Holovachov, Boström & Mundo-Ocampo, 2004 (Holovachov et al., 2004) further bridges the gap between *Ceratoplectus* and Wilsonematinae. Thus, maintaining the subfamily Wilsonematinae in a separate taxonomic category implies that the subfamily Plectinae may well be paraphyletic, but for the time being I do not alter its current taxonomic status. The subfamily Wilsonematinae is thus equal to the crown-group, while subfamily Plectinae is equal to the stem group of the same monophyletic taxon.

Morphological evidence of parthenogenesis in Plectidae

The parthenogenetic mode of reproduction in the genus *Plectus* and its relatives (subfamily Wilsonematinae) is suggested by the rarity of males. However, this is also the case when hermaphroditism is the main mode of reproduction. Lahl at al. (2003) found experimentally that parthenogenesis (thelytoky) is the only way of reproduction in some species of the genus *Plectus*. The first morphological evidence of parthenogenesis is the absence of a spermatheca and intrauterine spermatozoa in egg-producing females of the genus *Plectus* and wilsonematids. I may further point at several features in the male morphology that may suggest the existence of thelytoky and atavism of males. For this I need to present a brief overview of the variability of the male copulatory apparatus in several *Plectus* species.

Males of *P. pusteri* were studied by Holovachov (2001), who described 72 specimens: four had a single tubule, 37 had two and 31 had three tubules respectively. Moreover, considerable variability was noted in the position and length of different supplements. In eighteen males of *P. paracuminatus* from five habitats described in Holovachov et al. (2001), the number of tubules varied from one to three, and also tubule length and arrangement varied. Three populations of *P. communis* were considered in the present study, one population included 20 males and the other 22 males, the third one had only one male. All males of the first population had a single tubule, whereas among 22 males from the second population, 17 had one tubule, two specimens two tubules and in the three remaining males the cuticularised duct of the supplement was reduced. All 13 studied males of *P. parvus* were devoid of supplements and pre- and postcloacal sensilla, just like all studied males of *P. decens* (two in number), *P. geophilus* (four specimens) and *P. minimus* (23 males).

A comparison with a bisexual species is necessary to assess the intraspecific variability of supplement numbers. The 147 studied males of *Anaplectus granulosus* from different localities showed the following variability: 139 specimens (94.6%) had three supplements typical for this species, four males (2.7%) had two tubules, three specimens (2.0%) had four tubules and one male (0.7%) had one tubule (see also Figs 2 B-G in Holovachov et al. (2004)). It is clear that in bisexual species the number of tubular supplements is under strong selection and depends on the method of copulation.

Not only the number, length and relative position of tubular supplements in *Plectus* are highly variable, but also their shape is an unstable feature, unlike in the genus *Anaplectus*. The irregular thickness of the cuticularised supplementary tubule and poor development of the supplementary gland cell suggest dysfunction of the tubular supplements in those species of *Plectus* where these structures are present. Two hypotheses may be proposed to explain the above described peculiarities: **1)** the possible parthenogenetic mode of reproduction has reduced selective pressure on the functionality of tubular supplements, or **2)** dysfunction of tubular supplements has increased the preponderance of parthenogenesis.

Remarks

Based on the obtained cladogram of the family Plectidae (Fig. 5.14), two possible "key events" in the evolution of this group may be proposed on the basis of the number of known species in a certain genus (genera), morphological diversity, ecological plasticity etc. The first event is the change of the mode of reproduction from amphimixis to parthenogenesis (thelytoky) and the morphological diversification in the clade uniting the genus *Plectus sensu lato* and subfamily Wilsonematinae, no one member of which was ever reported to be bisexual. The development of the complex labial region in the four genera currently united in the subfamily Wilsonematinae is considered here as a second key event in the evolution of Plectidae, mainly because of their distinct feeding behaviour.

Morphological differences among species of the genus *Plectus* (*sensu lato*) are restricted mainly to measurements, qualitative characters and fine details in the structure of labial region, stoma, vagina and spinneret. *Plectus* diverged into a relatively large number of known species, occupying (almost) all possible fresh-water and terrestrial habitats all over the world (see Zell, 1993) and developed mechanisms allowing several species to coexist in the same locality. The number of valid *Plectus* species is about 70 (Holovachov & De Ley, 2005). Amoung them at least nine *Plectus* species have adapted to exist on the Antarctic continent (Andrássy, 1998). Moreover, four or five species of *Plectus* together with some Wilsonematinae may regularly inhabit the same locality.

Possible morphological clues for understanding the mechanisms of niche partitioning of species in the genus *Plectus* are given below. The genus *Plectus* is particularly characterised by a relatively uniform labial region, but broad morphometric variability, ranging in length from 0.3 to about 1.7 mm. Niche partitioning may occur in two different ways: **1)** differences in body size (length and diameter) allow different species to use soil pore space (microspaces) of various diameter, thus avoiding competition; and **2)** different reproduction rates, which could be indirectly studied by the number of simultaneous intrauterine eggs, egg length/width ratio and relation of the egg volume to body volume in different species, thereby presumably reducing competition.

Smaller species have relatively larger and on the average more elongated eggs. The result of large "egg length/egg width" ratio and relative egg volume in smaller species is that only one egg at a time could be present in the uterus. In species with the highest "egg length/egg width" ratio the temporary asymmetry of gonads was also noted, which may influence the egg production, i.e. a new egg could not be formed before the previous egg was layed. On the other hand more than two intrauterine eggs (up to four-six eggs) were found in larger species. This suggests that larger species may produce eggs faster than the smaller ones, having an advantage by a higher reproduction rate. On the other hand, smaller species may exploit smaller (narrower) spaces, which are unavailable for larger ones.

De Ley & Coomans (1997) pointed out that *Tylocephalus auriculatus* sweep up bacteria attached to more or less flat surfaces in the soil (from the solid surfaces) by moving the anterior body region in a dorso-ventral direction, thus differing from the feeding behaviour of other Plectidae. Other Wilsonematinae often behave in the same manner (observations on living specimens in suspensions of nematodes). Thus, the different shape and size of cuticular outgrowths of the labial region in these species may reflect selectivity in food collection by these nematodes, allowing them to avoid competition for food and allowing them to coexist in the same locality.

Appendix: an updated classification of the superfamily Plectoidea

Superfamily Plectoidea Örley, 1880

Diagnosis (emended): Plectida, Leptolaimina. Medium sized nematodes. Cuticle annulated or smooth, with or without lateral differentiation. Hypodermal glands and somatic sensilla usually present. Inner labial sensilla open inside cheilostom. Outer labial sensilla papilliform or rarely setiform. Four cephalic setiform sensilla. Amphid unispiral or derived therefrom. Stoma with cylindrical gymnostom and developed stegostom, the latter subdivided into two sections. Pharynx usually muscular. Pharyngeal tubes present. Dorsal gland orifice opens inside stegostom. Subventral gland orifices open into pharyngeal lumen at middle of pharynx. Secretory-excretory system present, renette cell usually located at level of pharynx. Excretory ampulla absent. Excretory duct long, coiled and often enveloped by the renette cell, opens to the exterior at level of nerve ring. Female reproductive system didelphic, amphidelphic or monodelphic, prodelphic. Ovary branches reflexed antidromously. Spermatheca axial when present. Vagina encircled by one or two sphincter muscles. Male reproductive system diorchic with opposed testes (posterior one reflexed) or monorchic. Spicules paired and symmetrical. Gubernaculum usually present. Alveolar supplements absent. Tubular supplements present or absent. Precloacal sensillum setiform or papilliform, anterior to cloaca. Postcloacal sensilla present or absent. Caudal setae arranged in few subventral, subdorsal and sublateral pairs. Caudal glands usually present, opening via a spinneret on the tail tip.

Type family: Plectidae Örley, 1880.

Other families: Pakiridae Inglis, 1983; Chronogastridae Gagarin, 1975; Metateratocephalidae Eroshenko, 1973.

Family Pakiridae Inglis, 1983

Diagnosis (emended): Plectoidea. Cuticle annulated, without lateral differentiation. Hypodermal glands present. Somatic sensilla absent. Four papilliform cephalic sensilla. Amphid a transverse slit. Pharynx cylindrical, muscular anteriorly, with basal glandular part. Subventral gland orifices open into pharyngeal lumen at midpharynx. Renette cell located at level of intestine. Excretory duct long, coiled and enveloped by the renette cell. Female reproductive system didelphic, amphidelphic. Spermatheca absent. Vagina encircled by one sphincter muscle. Male reproductive system diorchic with opposed testes. Spicules paired and symmetrical. Gubernaculum present. Tubular supplements present. Precloacal sensillum papilliform, anterior to cloaca. Postcloacal sensilla absent. Caudal setae arranged in few subventral and subdorsal pairs. Caudal glands and spinneret absent.

Type and only genus: *Pakira* Yeates, 1967.

Family Chronogastridae Gagarin, 1975

Diagnosis (emended): Plectoidea. Cuticle annulated, without lateral differentiation. Hypodermal glands present or absent. Somatic sensilla present or absent. Four setiform cephalic sensilla. Amphid unispiral or horseshoe-shaped. Pharynx muscular, divided into corpus, isthmus and basal bulb. Subventral gland orifices open into pharyngeal lumen at corpus-isthmus junction. Valvular apparatus in shape of three longitudinal denticulate ridges. Renette cell located at level of isthmus. Excretory duct long and free, coiled around isthmus. Female reproductive system didelphic, amphidelphic or monodelphic, prodelphic. Spermatheca axial when present. Vagina encircled by one sphincter muscle. Male reproductive system diorchic with opposed testes. Spicules paired and symmetrical. Gubernaculum present. Tubular supplements present or absent. Precloacal sensillum setiform or papilliform, anterior to cloaca or absent. Postcloacal sensilla present. Caudal setae arranged in few subventral and subdorsal or only in two subventral pairs. Caudal glands present, opening via a spinneret on tail tip, or absent.

Type genus: *Chronogaster* Cobb, 1913 (Syn.: *Walcherenia* de Man, 1921; *Keralanema* Siddiqi, 2003).

Other genera: *Cynura* Cobb, 1920 (Syn.: *Plectolaimus* Inglis, 1966); *Caribplectus* Andrássy, 1973.

Family Metateratocephalidae Eroshenko, 1973

Diagnosis (emended): Plectoidea. Cuticle smooth, with intracuticular ornamentation. Hypodermal glands present. Somatic sensilla present. Outer labial sensilla setiform. Four setiform cephalic sensilla. Amphid with subcuticular fovea and pore-like aperture. Pharynx muscular, divided into corpus, isthmus and basal bulb. Valvular apparatus with transverse denticulate plates and a posterior sclerotized haustrulum-like structure. Subventral gland orifices open into pharyngeal lumen at corpus-isthmus junction. Renette cell located at level of isthmus. Excretory duct long, coiled into a spiral on right side of isthmus and enveloped by the renette cell. Female reproductive system didelphic, amphidelphic. Spermatheca absent. Vagina encircled by one sphincter muscle. Male reproductive system monorchic with anteriorly reflexed testis. Spicules paired and symmetrical. Gubernaculum present. Tubular supplements absent. Precloacal sensillum papilliform, anterior to cloaca. Postcloacal sensilla absent. Caudal setae arranged in few subventral and subdorsal or only in few subventral pairs. Caudal glands and spinneret absent.

Type genus: *Metateratocephalus* Eroshenko, 1973.

Other genus: *Euteratocephalus* Andrássy, 1958.

Family Plectidae Örley, 1880

Diagnosis (emended): Plectoidea. Cuticle annulated, with lateral differentiation. Hypodermal glands present or absent. Somatic sensilla present. Outer labial sensilla papilliform. Four setiform cephalic sensilla. Amphid unispiral or derived therefrom. Pharynx muscular, divided into corpus, isthmus and basal bulb. Valvular apparatus with transverse denticulate plates and a posterior sclerotized haustrulum-like structure. Pharyngeal tubes present. Dorsal gland orifice opens inside stegostom. Subventral gland orifices open into pharyngeal lumen at corpus-isthmus junction. Secretory-excretory system present, renette cell located at level of isthmus. Excretory duct long, coiled around isthmus and enveloped by the renette cell. Female reproductive system didelphic, amphidelphic. Spermatheca axial when present. Vagina encircled by one or two sphincter muscles. Male reproductive system diorchic with opposed testes. Spicules paired and symmetrical. Gubernaculum present. Tubular supplements present or absent. Precloacal sensillum setiform or papilliform, anterior to cloaca. Postcloacal sensilla present or absent. Caudal setae arranged in few subventral, subdorsal and sublateral pairs. Caudal glands usually present, opening via a spinneret on tail tip.

Three subfamilies: Anaplectinae Zell 1993; Plectinae Örley, 1880; Wilsonematinae Chitwood, 1951.

Subfamily Anaplectinae Zell, 1993

Diagnosis (emended): Plectidae. Hypodermal glands present. Somatic sensilla restricted to tail. Four setiform cephalic sensilla. Amphid unispiral or a transverse slit. Spermatheca axial. Crustaformeria present or absent. Vagina encircled by one sphincter muscle. Male reproductive system diorchic, anterior testis outstretched, posterior testis reflexed. Spicules paired and symmetrical. Tubular supplements present. Precloacal sensillum papilliform. Postcloacal sensilla present. Caudal setae arranged in few subventral, subdorsal and sublateral pairs. Two to three precloacal subventral pairs of sensilla may be present. Caudal glands present, opening via a spinneret on tail tip.

Type genus: *Anaplectus* De Coninck & Schuurmans Stekhoven, 1933 (Syn.: *Marinoplectus* Kreis, 1963).

Other genera: *Perioplectus* Sanwal in Gerlach & Riemann, 1973 (Syn.: *Periplectus* Sanwal, 1968); *Arctiplectus* Andrássy, 2003.

Subfamily Plectinae Örley, 1880

Diagnosis (emended): Plectidae. Hypodermal glands present or absent. Somatic sensilla present. Four setiform cephalic sensilla. Amphid unispiral. Spermatheca absent. Vagina encircled by one or two sphincter muscles, epiptygmata may be present. Male reproductive system diorchic with opposed testes. Spicules paired and symmetrical. Gubernaculum present. Tubular supplements present or absent. Precloacal sensilla setiform, anterior to cloaca. Postcloacal sensilla present or absent. Caudal setae arranged in few subventral and subdorsal pairs. Caudal glands usually present, opening via a spinneret on tail tip.

Type and only genus: *Plectus* Bastian, 1865 (Syn.: *Plectoides* de Man, 1904; *Proteroplectus* Paramonov, 1964; *Chiloplectus* Andrássy, 1984).

Other genus: *Ceratoplectus* Andrássy, 1984.

Subfamily Wilsonematinae Chitwood, 1951

Diagnosis (emended): Plectidae. Hypodermal glands absent. Somatic sensilla present. Labial region biradial, cervical expansions present, median ridges and flabellae present or absent. Lateral outer labial sensilla modified into midlateral projections. Four cephalic sensilla modified into cornua. Amphid unispiral. Spermatheca absent. Vagina encircled by one sphincter muscles. Male reproductive system diorchic with opposed testes. Spicules paired and symmetrical. Gubernaculum present. Tubular supplements, precloacal and postcloacal sensilla absent. Caudal setae arranged in few subventral and subdorsal pairs. Caudal glands present, opening via a spinneret on tail tip.

Type genus: *Wilsonema* Cobb, 1913 (Syn.: *Bitholinema* De Coninck, 1931; *Pycnolaimus* Cobb, 1920; *Wilsotylus* Chawla, Khan & Prasad, 1969).

Other genera: *Ereptonema* Anderson, 1966 (Syn.: *Wilsereptus* Chawla, Khan & Saha, 1977; *Paraereptonema* Eroshenko, 1977); *Neotylocephalus* Ali, Farooqui & Tejpal, 1969 (Syn.: *Spatiocephalus* Patil & Khan, 1982; *Coronacephalus* Ganguly & Khan, 1986); *Tylocephalus* Crossman, 1933.

Chapter 6

General discussion

> *Phylogeny cannot be observed. It is necessarily on inference from observative that bear on it, sometimes rather distantly and that can usually be interpreted in more than one way*
>
> G.G.Simpson
> The principles of classification and
> classification of mammals

Towards a better understanding of the systematics of Plectida

New interpretation of morphology

Careful examination with light microscopy (LM) and scanning electron microscopy (SEM) allowed uncovering a number of morphological characters, to which little attention has been paid by other researchers. The careful examination also allowed reconsideration of the phylogenetic significance of some morphological traits including SEM and LM features of the labial region (especially in wilsonematids), as well characters of the stoma and male copulatory apparatus. These insights into morphology together with review of the literature all contributed to a substantial source for the phylogenetic analysis, with special attention to freshwater and soil-inhabiting representatives. More specifically, the new interpretation of morphology and homology of the labial region, stoma, pharynx, as well as female and male reproductive systems has provided a basis for the newly proposed phylogeny and classification of the order Plectida.

Omitted taxa

Inclusion of a few marine representatives in the current analysis was necessary and important for the following reasons. The primary radiation of nematodes occurred in the marine environment, whereas their invasion to terrestrial habitats, via freshwater bodies, took place several times independently. It is difficult to conclude that all terrestrial representatives of the morphologically and ecologically diverse order Plectida have a common origin. Phylogenetic analysis, including marine representatives, allowed not only meaningful scoring of morphological characters, but also the approach revealed plectid nematodes to have at least three independent lineages to the terrestrial environment. However, a large portion of marine species and genera, currently included in Plectida, still await future detailed analysis. Thus, the currently proposed classification of many marine taxa is provisional (e.g. superfamily Ceramonematoidea), or it is unchanged from classification schemes proposed by my predecessors (e.g. superfamily Haliplectoidea).

Just one terrestrial taxon, genus *Aulolaimus* de Man, 1880, was omitted from the current analysis, thus leaving the area for future research. The unique morphology of *Aulolaimus*, together with absence of shared characters and doubtful homology with other Plectida, made impossible its correct placement on the cladogram, regardless of the availability of the unpublished new morphological information and data from SEM studies.

Updated classification of Plectida

During the course of the present study, the computer based-cladistic analysis proved to be a suitable and effective tool to produce a preliminary phylogenetic hypothesis, which is a beginning point for further testing and improvement using independent data. That is, the suggested or chosen phylogenetic hypothesis provides a set of positive statements that can then be contradicted, confirmed or amended. Subjective weighting of characters, based on consideration of function and possible impact on the life of an organism, may give clues to understanding how/why homoplasy appeared, and thus provide a rationale to improve the phylogeny.

Because of our findings, some changes and improvements in the classification scheme of the order Plectida were made. The most important changes relative to classifications of Lorenzen (1981) and De Ley & Blaxter (2002, 2004) are the restoration and rearrangement of the families Aphanolaimidae and Pakiridae, as well as the superfamily Camacolaimoidea. Furthermore, families Bastianiidae and Odontolaimidae were relocated from Plectida to Triplonchida and family Paramicrolaimidae from Plectoidea to Ceramonematoidea. The genera *Manunema*, *Cricolaimus*, *Caribplectus*, *Cynura* are newly positioned in the classification scheme. The updated arrangement of diverse genera in the family-level categories is given in Figure 6.1.

There are no objective criteria for ranking taxonomic categories above the species level, and therefore the classification scheme, in many cases, does not reflect the phylogeny. I disagree with those who argue to reject all categories in systematics. New morphological data and new species will change the phylogenetic hypothesis, which will result in changes either in a “Linnaean type” of classification or in a classification based on the principles of PhyloCode.

When building the classification scheme, I tried to maintain monophyletic taxa to the extent possible. However, there are still several paraphyletic groups in the current classification (e.g. see placement of the genus *Plectus* or the family Chronogastridae in the computer-generated cladistic analysis). The main reason to maintain such paraphyletic groups is that future possible analyses, including additional taxa, may further improve the resolution of the phylogenetic hypothesis and lead to better-grounded taxonomic conclusions. On the other hand, in the classification of the present study, all paraphyletic taxa were split and rearranged into more “natural” monophyletic or, at least, morphologically uniform paraphyletic units.

What comparative morphology tells about the evolution of nematodes?

Absence of historic data and direct observations

When discussing the evolution of nematodes in general, the researcher meets with difficulties, caused by the almost complete absence of historical (paleontological) information and insufficient knowledge about the taxonomic and morphological diversity of free-living and especially marine representatives of the phylum. Another problem is that direct observation of nematode behaviour and biology under natural conditions is

Order Plectida Malakhov, 1982

- Incertae sedis
  - Genera *Domorganus, Aulolaimus, Alaimella, Aegialoalaimus*
- Superfamily Leptolaimoidea Orley, 1880
  - Family Leptolaimidae Orley, 1880
    - Genera *Anomonema, Antomicron, Leptolaimoides, Leptolaimus, Leptoplectonema, Manunema, Paraplectonema*
  - Family Aphanolaimidae Chitwood, 1936
    - Subfamily Aphanolaiminae Chitwood, 1936
      - Genera *Aphanolaimus, Aphanonchus, Paraphanolaimus*
    - Subfamily Anonchinae Andrassy, 1973
      - Genus *Anonchus*
- Superfamily Camacolaimoidea Micoletzky, 1924
  - Incertae sedis
    - Genera *Hemiplectus, Setostephanolaimus, Stephanolaimus*
  - Family Camacolaimidae Micoletzky, 1924
    - Genera *Anguinoides, Camacolaimus, Dagda, Deontolaimus, Diodontolaimus, Ionema, Listia, Onchiolistia, Onchium, Procamacolaimus, Smithsoninema*
  - Family Rhadinematidae Lorenzen, 1981
    - Genera *Cricolaimus, Rhadinema*
- Superfamily Plectoidea Orley, 1880
  - Family Pakiridae Inglis 1983
    - Genus *Pakira*
  - Family Chronogastridae Gagarin, 1975
    - Genera *Caribplectus, Chronogaster, Cynura*
  - Family Metateratocephalidae Eroshenko, 1973
    - Genera *Euteratocephalus, Metateratocephalus*
  - Family Plectidae Orley, 1880
    - Subfamily Anaplectinae Zell, 1993
      - Genera *Anaplectus, Arctiplectus, Perioplectus*
    - Subfamily Plectinae Orley, 1880
      - Genus *Plectus, Ceratoplectus*
    - Subfamily Wilsonematinae Chitwood, 1951
      - Genera *Ereptonema, Neotylocephalus, Tylocephalus, Wilsonema*
- Superfamily Ceramonematoidea Cobb, 1913
  - Family Ceramonematidae Cobb 1913
    - Genera *Ceramonema, Dasynemella, Dasynemoides, Metadasynemella Metadasynemoides, Pselionema, Pterygonema*
  - Family Tubolaimoididae Lorenzen, 1981
    - Genera *Chitwoodia, Tubolaimoides*
  - Family Tarvaiidae Lorenzen, 1981
    - Genus *Tarvaia*
  - Family Paramicrolaimidae Lorenzen, 1981
    - Genus *Paramicrolaimus*
  - Family Diplopeltoididae Tchesunov, 1990
    - Genus *Diplopeltoides*
- Superfamily Haliplectoidea Chitwood, 1951
  - Family Haliplectidae Chitwood, 1951
    - Genera *Haliplectus, Setoplectus*

Fig. 6.1. Proposed classification of the order Plectida.

impossible presently. Therefore, we must often rely upon indirect observations, experimental data and disputable statements and speculations, based on morphology.

Our results allow discussing possible patterns of the evolution of freshwater and terrestrial members of the order Plectida and their morphological adaptations associated with radiation including newly exploited environments. The proposed phylogeny of terrestrial Plectida is shown in Figure 6.2. The Plectida considered most primitive from a morphological and phylogenetic point of view, are found within the taxa Leptolaimidae, Camacolaimidae, Rhadinematidae, as well as the genera *Stephanolaimus* and *Setostephanolaimus*; these occur in marine environments, as are their close relatives (orders Araeolaimida, Monhysterida, Chromadorida). I suppose that the hypothetical ancestor of Plectida originated from the marine environment while its descendents radiated to freshwater and soil. This radiation was accompanied by certain morphological, physiological and behavioural changes; some are briefly discussed below.

Possible morphological adaptations for the existence in freshwater

"Colonisation" of the brackish and freshwater habitats within the order Plectida occurred independently in several clades (Fig. 6.2). Both superfamilies Leptolaimoidea and Plectoidea include inland representatives, whereas members of the families Camacolaimidae and Rhadinematidae as well as genera *Stephanolaimus* and *Setostephanolaimus* are typically found in marine habitats. The only member of the monotypic genus, *Hemiplectus,* is known from mosses, but its phylogenetic relations are not yet clearly resolved. However, the morphological peculiarities of *Hemiplectus* support our opinion about the structural adaptations needed for existence in a freshwater environment.

Within the family Leptolaimidae species of the genus *Paraplectonema* were recorded in the continental seas, river estuaries, and freshwater bodies and even soil, while members of the other genera of this family are representatives of typical marine fauna. On the other hand, the family Aphanolaimidae unites four genera; these are adapted for brackish and freshwater as well as soil. Among ten known species of the genus *Anonchus* (Chapter 2), populations of four species are known from the seashore (*A. millelacunatus, A. venezolanus, A. laureatus, A. mangrovi*), and one is from a river lagoon (*A. coomansi*). The remaining species occur in freshwater. Among the eight valid species of the genus *Aphanonchus* (Holovachov & Sturhan, 2004b), two were found in river estuaries (*A. bayensis* (Keppner, 1988) Holovachov & Sturhan, 2004 and *A. africanus* Holovachov & Sturhan, 2004); this habitat is particularly characterised by unstable salt concentration. The remaining six *Aphanonchus*-species were found in freshwater, whereas species of the genera *Paraphanolaimus* and *Aphanolaimus* occur in both freshwater and soil. We suppose that the hypothetical ancestor of the family Aphanolaimidae was a marine representative, which was able to live in brackish habitats, while its descendants adapted to a freshwater and terrestrial environment. This is supported by the fact that the most morphologically primitive representatives, *Anonchus coomansi* and *Aphanonchus* spp (Holovachov, unpublished), were found in river estuaries, whereas the most derived members of the genus *Aphanolaimus* are specialized for soil and litter.

Within the whole superfamily, Plectoidea, only the genus *Cynura* contains marine representatives. Some populations of the genus *Chronogaster* are recorded from brackish sites, whereas the distribution of the genus *Caribplectus* is restricted to the sea coast of Central America and the Caribbean. On the other hand, the most basal species (according to our cladogram), *Pakira orae,* was found only is sand dunes along the marine shore of New Zealand and South Africa. The early evolution of the superfamily Plectoidea took place in the marine habitats, while ancestors of *Pakira* populated dunes, as also is the case for the genera *Haliplectus* Cobb, 1913 and *Synonchium* Cobb, 1920.

The main difference between marine and freshwater is salt content. The salinity is usually 35-40 ‰ in marine habitats and less than 0.5‰ in freshwater, with brackish intermediate. Under marine conditions, the osmotic value of the environment is rather stable, in soils it is not. Therefore, functional changes of the secretory-excretory system are necessary to successfully occupy an environment with low osmotic pressure and variable salt content. These functional changes may be expressed morphologically. Comparing the renette cell morphology in marine and freshwater inhabitants of the order Plectida, I noted several morphological differences in its structure. Only typically freshwater or soil-inhabiting genera, *Paraplectonema*, *Hemiplectus*, Aphanolaimidae and Plectoidea, have a long excretory duct and no ampulla (feature №1, Fig. 6.2). The renette cell in Aphanolaimidae probably includes functions in addition to excretion (of ammonia and urea) and these may include involvement in feeding or digestion processes (Coomans & De Waele, 1983). The proximal part of the excretory duct is embedded in the renette cell in *Hemiplectus muscorum*, *Pakira orae* and families Plectidae and Metateratocephalidae (feature №2, Fig. 6.2); in Metateratocephalidae, the duct forms loops or spirals and the renette cell is closely associated with a few coelomocytes. Probably, such elaborations of the secretory-excretory system ensure maintaining the necessary salt concentration in the schizocoel of the nematode in an otherwise hypo-osmotic environment. Detailed physiological studies are needed to clarify how the nematode renette system works under different environmental conditions.

Morphological changes while populating the soil

One of the differences between soil and aquatic habitats is the water volume in which the nematode moves, and in relation to the size of hard particles in the substrate as well as to the overall configuration of the nematode body. After the invasion of soil by nematodes, further adaptations might include the ability to withstand total desiccation or the development of mechanisms to regulate cuticle permeability. Unfortunately, the morphological evidence of such adaptations has not yet been detected with confidence.

Morphological adaptations could appear during the process of colonisation of soil, which may increase the efficiency of locomotion in such an environment. One such adaptation is development of lateral alae and/or cuticular ornamentation; these are primarily characteristic of Chromadoria. Since free-living nematodes generally move in a serpentine-like manner (except Desmoscolecida, Epsilonematidae, Criconematidae, Bunonematidae) by curving the body in a dorso-ventral plane, the lateral sides of the body, modified like rigid ribs, are the point of movement.

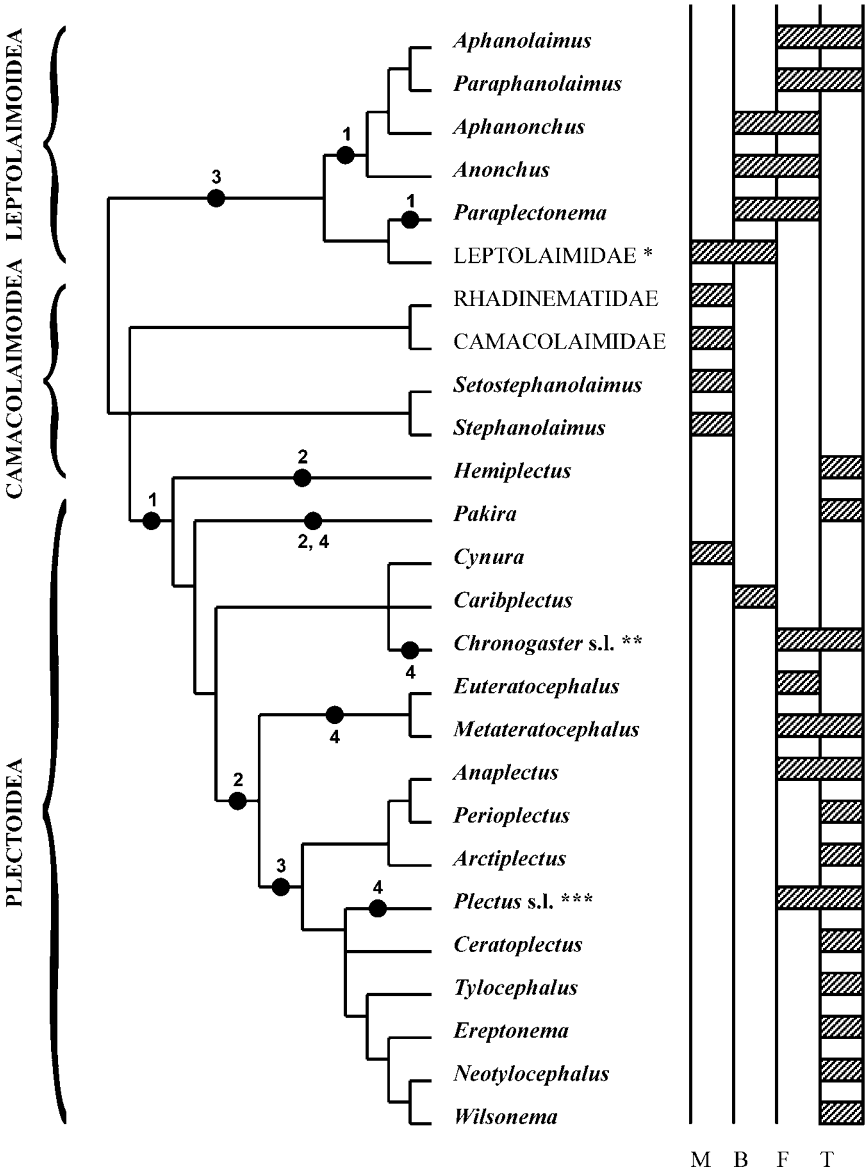


Fig. 6.2. Phylogeny of the order Plectida with special attention to its freshwater and terrestrial representatives and indicating their preferable habitat: M – marine, B – brackish, F – freshwater, T – soil. Numbers indicate the appearance of some morphological features, which are discussed in the text. (* *Paraplectonema* is an ingroup of Leptolaimidae; ** *Chronogaster* s.l. includes its ingroup genus *Keralanema*; *** *Plectus* s.l. includes its ingroup genus *Chiloplectus*).

The lateral alae independently appear three times in members of Plectida, namely in the superfamily Leptolaimoidea, the genus *Deontolaimus* and the family Plectidae (Feature №3, Fig. 6.2). The origin of the lateral differentiation of the cuticle is probably connected with nematodes adapting to dense sediments. Members of the family Plectidae typically are terrestrial inhabitants, and species that occur in freshwater are descendants of terrestrial ancestors. The Plectidae have lateral alae that consist of two sublateral parallel cuticular ridges. Since its sister taxa are typically aquatic (Chronogastridae, *Euteratocephalus*), the lateral differentiation of the cuticle in the family Plectidae appeared before or during colonisation of soil by its ancestors.

Changes in environmental conditions are not only accompanied by the appearance of new structures but also by the reduction or loss of the non-functional features. It is evident that most terrestrial nematodes do not have caudal glands or a spinneret. Exceptions are members of the orders Plectida, Monhysterida, Chromadorida, Triplonchida and Mononchida, most species of which are aquatic and only a small minority of these taxa occur in soil. Caudal glands play an important role in locomotion in an aquatic environment, producing products that fix the nematode body to a solid surface. Moreover, some aquatic species, including *Sphaerolaimus* (Turpeenniemi & Hyvarinen, 1996), use both caudal glands and renette cell products to aid movement.

Within the studied order Plectida, caudal glands are absent in typically terrestrial species, *Pakira orae, Plectus spicacaudatus, P. amorphotelus,* and freshwater genera, *Chronogaster* (except *C. boettgeri*), *Euteratocephalus* and *Metateratocephalus* (feature №4, Fig. 6.2). I suggest that the role of caudal glands during movement between densely packed soil particles is insignificant and leads to their reduction or loss. However, the reduction of caudal glands in *Chronogaster* may have been associated with selection of mucrons and spines of different shape and size, present on the tail terminus in many *Chronogaster*-species and perhaps functioning as an anchor for fixing the animal to substrate.

Conclusion

Thus, the transition to exploiting the freshwater environment required changes in morphology and functioning of the secretory-excretory system, while successfully populating soil led to adaptations of structures and mechanisms associated with locomotion. Specifically, the evolution of Plectidae in freshwater and soil, and associated morphological changes, allowed this group to successfully populate most of the terrestrial habitats and thus gave rise to another, even more successful order of primarily terrestrial nematodes, Rhabditida.

Searching for the hypothetical ancestor of Rhabditida

Fürst von Lieven (2003) suggested in his cladogram that the valvular apparatus (“grinder”, *loc. cit.*) in *Ceratoplectus, Plectus parvus* and *Wilsonema* is homologous with the grinder in Secernentea and that Rhabditida possibly originated “somewhere” within the genus *Plectus* (subfamily Plectinae in other and recent classifications). Although the morphological features discussed by Fürst von Lieven support this assumption, there is one important feature in the biology of Plectinae (and Wilsonematinae) that weakens such a hypothesis. That is, all currently known species of Plectinae and Wilsonematinae reproduce by obligate parthenogenesis, which is associated with extensive reduction in morphology and function of the male copulatory apparatus in these two subfamilies. It is hard to accept that there occurred a reversal from obligate parthenogenesis (thelytoky) in the ancestral group to bisexual reproduction in its descendants.

Another candidate in Plectoidea with greater potential as a hypothetical rhabditid relative is the bisexual genus *Anaplectus*, which possesses several morphological similarities to Rhabditida, but with its own autapomorphic features. Comparing the main morphological characters of *Anaplectus* and Rhabditida, I found the following five similarities, which may either be homologous and support a common ancestry of both taxa, or homoplasious (analogous) and show a remarkable case of parallel evolution in terrestrial nematodes.

1) Stoma distinctly divided into cheilo-, gymno- and stegostom, its posterior end is marked by the anterior end of pharyngeal tubes; the dorsal gland opens inside the stegostom. **2)** Pharynx subdivided into procorpus and postcorpus by a break in muscular tissue and orifices of the subventral pharyngeal glands; corpus includes a distinct procorpus (with pharyngeal tubes) and metacorpus; postcorpus consists of isthmus and basal bulb with valvular apparatus. **3)** The renette cell envelops the proximally coiled anterior end of the excretory duct; the cell also extends posterior along the lateral chord, at least on the left lateral side. **4)** Deirids present, while somatic sensilla are reduced throughout the body, except for the female tail. **5)** Arrangement of male caudal papillae shows similarities to that characteristic for different taxa within Rhabditida, particularly by the presence of several precloacal subventral pairs, and the distribution of papillae on the tail.

Regardless, I cannot state that *Anaplectus* is a direct ancestor of Rhabditida, nor the direct descendant from a common ancestor with the rhabditid clade. I rather suppose that the morphology of *Anaplectus* may show certain features similar with the “Ur-Rhabditida” (Arche-Rhabditida), and suggest that the hypotheses of the shared characters reflecting a common origin is a consideration for future research of the origin and early evolution of Rhabditida.

Literature cited

Alekseev V.M. 1979. Evolution of supplementary organs in nematodes *Leptolaimus* and establishment of the genus *Boveelaimus* (Nematoda, Leptolaimidae). *Zoologicheskii Zhurnal* 58: 1296-1301 (in Russian).

Alekseev V.M. 1981. Nematodes of the family Leptolaimidae (Nematoda, Araeolaimidae) from the brackish waters of the South Sakhalin. *Svobodnozhivushchie i fitopatogennye nematody fauny Dalnego Vostoka* 3-7: 64-67 (in Russian).

Alekseev V.M. 1990. The morphology of *Anonchus mirabilis* (Nematoda: Araeolaimida), rare and new for the USSR fauna from Lake Khanka. *Zoologicheskii Zhurnal* 69: 139-141 (in Russian).

Alekseev V.M. 1992. Morphology of new and rare nematode species from waters in the south of the Far East of Russia. *Zoologicheskii Zhurnal* 71: 9-18 (in Russian).

Alekseev V.M. & Naumova A.D. 1977. New species of nematodes from the Khasan Lake. *Zoologicheskii Zhurnal* 56: 292-295 (in Russian).

Alekseev V.M. & Rassadnikova I.V. 1977. A new species and taxonomic analysis of the genus *Leptolaimus* (Nematoda, Araeolaimida) *Zoologicheskii Zhurnal* 56: 1766-1774 (in Russian).

Ali S.M., Farooqui M.N. & Tejpal S. 1969. *Neotylocephalus annonae* n. gen. n. sp. (Nematoda: Wilsonematinae) from Maratwada, India. *Rivista di Parassitologia* 30: 287-290.

Allen M.W. & Noffsinger E.M. 1968. Revision of the genus *Anaplectus* (Nematoda: Plectidae). *Proceedings of the Helminthological Society of Washington* 35: 77-91.

Anderson R.V. 1966. An emendation of the diagnosis of both the subfamily and two genera of Wilsonematinae and a new genus, *Ereptonema* n. g. (Plectidae: Nematoda). *Canadian Journal of Zoology* 44: 923-935.

Anderson R.V. & Sudhaus W. 1985. Description of *Rhabditis (Pellioditis) dolichuroides* n. sp. (Nematoda: Rhabditidae) from Kenya. *Canadian Journal of Zoology* 63:1711-1715.

Andrássy I. 1953. Eine neue Art der Gattung *Trilobus* Bastian. *Annales historico-naturales Musei nationalis hungarici* 4: 71-73.

Andrássy I. 1958. Erd- und Süsswassernematoden aus Bulgarien. *Acta Zoologica Hungarica* 4: 1-88.

Andrássy I. 1958. Noch einmal über die Gattung *Chronogaster* Cobb, 1913. *Opuscula Zoologica, Budapest* 2: 7-11.

Andrássy I. 1959. Neubenennungen einiger homonymen Nematoden-Gattungen. *Nematologica* 4: 223-226

Andrássy I. 1967. Ergebnisse der zoologischen Forschungen von Dr. Z. Kaszab in der Mongolei. 92. Weitere Bodennematoden aus den Jahren 1964 und 1965. *Opuscula Zoologica Budapest* 6: 203-233.

Andrássy I. 1968. Fauna Paraguayensis. 2. Nematoden aus den galeriewäldern des Acaray-Flusses. *Opuscula Zoologica Budapestinensis* 8: 167-315.

Andrássy I. 1973. Nematoden aus Strand- und Höhlenbiotopen von Kuba. *Acta Zoologica Hungarica* 19: 233-270.

Andrássy I. 1973. *Plectus granulosus* var. *grandepapillatus* Ditlevsen – ein älteres Synonym von *Anaplectus submersus* (Hirschmann) (Nematoda: Plectidae). *Opuscula Zoologica, Budapest* 12: 105-106.

Andrássy I. 1976. *Evolution as a basis for the systematization of nematodes.* Budapest, Akadémiai Kiado, 288 pp.

Andrássy I. 1984. *Klasse Nematoda (Ordnungen Monhysterida, Desmoscolecida, Araeolaimida, Chromadorida, Rhabditida).* Stuttgart, Gustav Fischer Verlag, 509 pp.

Andrássy I. 1985a. The genus Plectus Bastian, 1865 and its nearest relatives (Nematoda: Plectidae). *Acta Zoologica Hungarica* 31: 1-52.

Andrássy I. 1985b. A dozen new nematode species from Hungary. *Opuscula Zoologica Budapestinensis* 19/20: 3-39.

Andrássy I. 1986. Fifteen new nematode species from the Southern Hemisphere. *Acta Zoologica Hungarica* 32: 1-33.

Andrássy I. 1989. Six new nematode species from South America. *Acta Zoologica Hungarica* 35: 1-16.

Andrássy I. 1998. Nematodes in the Sixth Continent. *Journal of Nematode Morphology and Systematics*. 1: 107-186.

Andrássy I. 2001a. Some species of curious genera of the Class Penetrantia (Nematoda). *International Journal of Nematology* 11: 43-57.

Andrássy I. 2001b. On two uncommon structures in nematodes. *Opuscula Zoologica Budapest* 33: 133-137.

Andrássy I. 2003. New and rare nematodes from Alaska. I. Three species of the family Plectidae // *Journal of Nematode Morphology and Systematics* 5: 33-48.

Armendáriz G.I. & Hernández M.A. 1994. Especies del Orden Araeolaimida (Nematoda) encontrados en pinares de *Pinus nigra* Arnold, 1785 en Navarra (Norte de España). *Boletín de la Real. Sociedad Española de Historia Natural (Sec. Biol)* 91: 135-141.

Bastian H.C. 1865. Monograph on the Anguillulidae, or free Nematoids, marine, land, and freshwater; with descriptions of 100 new species. *Transactions of the Linnean Society of London – Zoology* 25: 73-184.

Blaxter M., De Ley P., Garey J.R., Liu L.X., Scheldeman P., Vierstraete A., Vanfleteren J.R., Mackey L.Y., Dorris M., Friesse L.M., Vida J.T. & Thomas W.K. 1998. A molecular evolutionary framework for the phylum Nematoda. *Nature* 392: 71-75.

Blaxter M., Dorris M. & De Ley P. 2000. Patterns and processes in the evolution of animal parasitic nematodes. *Nematology* 2: 43-55.

Blome D. 1982. Systematik der Nematoda eines Sandstrandes der Nordseeinsel Sylt. *Mikrofauna Meeresbodens* 86: 1-194.

Blome D. 2002. Five new genera of free-living marine nematodes from sandy beaches of eastern Australia. *Memoirs of the Queensland Museum* 48: 29-43.

Bongers T. 1983. Revision of the genus *Leptosomatum* Bastian, 1865. *Proceedings of the Biological Society of Washington* 96: 807-855.

Boström S. 1988. A scanning electron microscope study of some species of terrestrial nematodes from Spitzbergen. *Nematologica* 33 (1987): 366-374.

Boucher G. & Helléouët M.-N. 1977. Nématodes des sables fins infralitoraux de la Pierre Noire (Manche Occidentale). III. Araeolaimida et Monhysterida. *Bulletin du Muséum National d'Histoire Naturelle* 427: 87-122.

de Bovee F. 1974. *Leptolaimus pocillus* n. sp. espéce nouvelle de Leptolaimidae (Nematoda). *Vie et Milieu* 24: 73-81.

Brzeski M.W. 1997. Studies on some species of the genus *Prismatolaimus* de Man, 1880 with description of *P. mulcoomus* sp. n. (Nematoda: Prismatolaimidae). *Annales Zoologici* 46: 153-166.

Brzeski M.W. & Winiszewska-Šlipinska G. 1993. Taxonomy of Tripylidae (Nematoda: Enoplia). *Nematologica* 39: 12-52.

Brzeski M.W. & Háněl L. 1999. Paratylenchinae: postembryonic developmental stages of *Paratylenchus straeleni* (De Coninck, 1931) and *P. Steineri* Golden, 1961 (Nematoda: Tylenchulidae). *Nematology* 1: 673-680.

Bussau C. 1990. Freilebende Nematoden aus Küstendünen und angrenzenden Biotopen der deutschen und dänischen Küsten. I. Gesamtüberblick und Chromadorida (Nematoda). *Zoologischer Anzeiger* 225: 161-188.

Bütschli O. 1873. Beiträge zur Kenntnis der freilebende Nematoden. *Nova Acta der Ksl. Leopold-Carol. Deutschen Akademie der Naturforscher* 36: 1-124.

Camin J.H. & Socal R.R. 1965. A method for deducing branching sequences in phylogeny. *Evolution* 19: 311-326.

Chawla M.L., Khan E. & Prasad S.K. 1969. *Wilsotylus bangaloreiensis* gen. nov., sp. nov. (Nematoda, Plectidae) from soil around roots of mango, (*Mangifera indica*) from Bangalore, India. *Bulletin of Entomology* 10: 146-149.

Chawla M.L., Khan E. & Saha M. 1977. *Wilsereptus andersoni* gen. n., sp. n. (Wilsonematinae: Plectidae, Nematoda) from soil around roots of grass at Dalhousi, India with a key to the genera of the subfamily. *Indian Journal of Nematology* 5 (1975): 176-179.

Chitwood B.G. 1936. Some marine nematodes from North Carolina. *Proceedings of the Helminthological Society of Washington* 3: 1-16.

Chitwood B.G. 1951. North American marine nematodes. *Texas Journal of Science* 3: 617-672.

Chitwood B.G. & Chitwood M.B. 1936. The histology of nemic esophagi. VI. The esophagus of members of the Chromadoria. *Journal of the Washington Academy of Sciences* 26: 331-346.

Chitwood B.G. & Chitwood M.B. 1950. *Introduction to Nematology. Section 1. Anatomy*. Baltimore, 213 pp.

Chitwood B.G. & Chitwood M.B. 1974. *Introduction to Nematology*. University Park Press, 336 pp.

Claparède A.R.E. 1863. *Beobachtungen über Anatomie und Entwicklungsgeschichte wirbelloser Thiere an der Küste der Normandie angestellt*. Leipzig, Verlag von Wilhelm Engelmann, 120 p.

Cobb N.A. 1893. Nematode worms found attacking sugar cane. *Agricultural Gazette of New South Wales* 4: 808-833.

Cobb N.A. 1906. Free living nematodes inhabiting the soil around roots of cane, and their relation to root deseases. *Bulletin of Hawaii Sugar Planters' Association experimental Station*: 163-195.

Cobb N.A. 1913. New nematode genera found inhabiting fresh water and non-brackish soils. *Journal of the Washington Academy of Sciences* 3: 432-444.

Cobb N.A. 1914a. The North American free-living fresh-water nematodes. *Transactions of the American microscopical Society* 33: 69-119.

Cobb N.A. 1914b. Antarctic marine free-living nematodes of the Shackleton expedition. *Contributions to a Science of Nematology* 1: 1-33.

Cobb N.A. 1920. One hundred new nemas (type species of 100 new genera). *Contribution to a science of Nematology* 9: 217-343.

Cobb N.A. 1933. New nemic genera and species with taxonomic notes. *Journal of Parasitology* 20: 81-94.

Coomans A. 1979. The anterior sensilla of nematodes. *Revue de Nématologie* 2: 259-283.

Coomans A. 2000. Nematode systematics: past, present and future. *Nematology* 2: 3-7.

Coomans A. & De Waele D. 1979. Species of *Aphanolaimus* (Nematoda, Araeolaimida) from Africa. *Zoologica Scripta* 8: 171-180.

Coomans A. & Raski D.J. 1988. Two new species of *Prismatolaimus* de Man, 1880 (Nemata: Prismatolaimidae) in southern Chile. *Journal of Nematology* 20: 288-303.

Coomans A. & Raski D.J. 1991a. *Aphanonchus intermedius* n. g., n. sp. (Nemata: Araeolaimida). *Nematologica* 37: 8-19.

Coomans A. & Raski D.J. 1991b. Observations on *Paraplectonema* Strand, 1934 and description of *Leptoplectonema fuegoense* n. g., n. sp. (Nematoda: Leptolaimidae). *Revue de Nématologie* 14: 197-205.

Crossman L. 1933. Preliminary observations on the life history and morphology of *Tylocephalus bacillivorus* n. g., n. sp., a nematode related to the genus *Wilsonema*. *Journal of Parasitology* 20: 106-107.

von Daday E. 1905. Untersuchungen über die Süßwasser-Mikrofauna Paraguayas. *Zoologica* 18: 1-374.

De Coninck L. 1931. Sur trois espèces nouvelles de nématodes libres trouvés en Belgique. *Bulletin du Musée royal d'Histoire naturelle de Belgique* 7: 1-15.

De Coninck L.A. 1935. Contribution à la Connaissance des Nématodes libres du Congo Belge. I. – Les Nématodes libres des marais de la Nyamuamba (Ruwenzori) et des sources chaudes du Mont Banze (Lac Kivu). *Revue de Zoologique et Botanique Africaine* 26: 211-232, 245-326.

De Coninck L.A. 1965. Classe des Nématodes – Systématique des Nématodes et sous-classe des Adenophorea. *Traité de Zoologie* 4: 586-681.

De Coninck L.A. & Schuurmans Stekhoven J.H. 1933. The freeliving marine nemas of the Belgian coast. II. *Mémoires du Musée Royal d'Histoire Naturelle de Belgique* 58: 1-163.

De Ley P. & Blaxter M. 2002. Systematic position and phylogeny. In: Lee D.L. (Ed.) *The biology of nematodes*. London and New York, Taylor & Francis, 1-30.

De Ley P. & Blaxter M. 2004. A new system for Nematoda: combining morphological characters with molecular trees, and translating clades into ranks and taxa. *Nematology Monographs & Perspectives* 2: 633-653.

De Ley P. & Coomans A. 1994. Terrestrial nematodes from the Galápagos Archipelago IV: The genus *Plectus* Bastian, 1865, with description of three new species (Leptolaimina: Plectidae). *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Biologie* 64: 43-70.

De Ley P. & Coomans A. 1997. Terrestrial nematodes from the Galapagos Archipelago. 7. Description of *Tylocephalus nimius* sp. n. and new data on the morphology, development and behaviour of *T. auriculatus* (Bütschli, 1873) Anderson, 1966 (Leptolaimina: Plectidae). *Fundamental and Applied Nematology* 20: 213-228.

De Ley I., Holovachov O. & De Ley P. 2002. Scanning electron microscopy of the juvenile stages of some Wilsonematinae (Plectidae). *Nematology* 4: 244 (abstract).

De Ley P., Loof P.A. A. & Coomans A. 1993. Terrestrial nematodes from the Galápagos Archipelago II: Redescription of *Aporcelaimellus obtusicaudatus* (Bastian, 1865) Altherr, 1968, with review of similar species and a nomenclature for the vagina in Dorylaimida (Nematoda). *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Biologie* 63: 13-34.

De Ley P., Van de Velde M.C., Mounport D., Baujard P. & Coomans, A. 1995. Ultrastructure of the stoma in Cephalobidae, Panagrolaimidae and Rhabditidae, with a proposal for a revised stoma terminology in Rhabditida (Nematoda). *Nematologica* 41: 153-182.

De Waele D. & Coomans A.V. 1993. Four new species of *Aphanolaimus* de Man, 1880 (Nematoda: Araeolaimida) from South America. *Fundamental and applied Nematology* 16: 87-95.

Decraemer W. 1975. Scientific report on the Belgian expedition to the Great Barrier Reef in 1967. Nematodes I: *Desmoscolex*-species (Nematoda – Desmoscolecida) from Yonge Reef, Lizard Island and Nymphe Island with general characteristics of the genus *Desmoscolex*. *Annales de la Société Royale Zoologique de Belgique* 104: 103-130.

Decraemer W. 1978. Morphological and taxonomic study of the genus *Tricoma* Cobb (Nematoda: Desmoscolecida), with the description of new species from the Great Barrier Reef of Australia. *Australian Journal of Zoology, Supplementary Series* 55: 1-121.

Ditlevsen H. 1919. Marine freeliving nematodes from Danish waters. *Videnskabelige Meddelelser fra Naturhistorik Forening* 70: 147-214.

Ditlevsen H. 1928. Land- and freshwater nematodes. *Zoology of the Faroes* 1(2), 13: 1-28.

Ditlevsen H. 1930. Papers from Dr. Th. Mortensen's Pacific Expedition 1914-16. 52. Marine free-living nematodes from New Zealand. *Videnskabelige Meddelelser fra Naturhistorisk Forening* 87: 201-242.

Dougherty E.C. 1955. The genera and species of the subfamily Rhabditinae Mikoletzky, 1922 (Nematoda): a nomenclatorial analysis, including an addendum on the composition of the family Rhabditidae Örley, 1880. *Journal of Helminthology* 29: 105-152.

Drozdovsky E.M. 1978. Characteristics of the structure and formation of preblastula as a index of phylogenetic relationships and taxonomic position of different nematode groups. *Fitogelmintologicheskie Issledovania*: 14-31 (in Russian).

Ebsary B.A. 1985. Two new species of *Plectus* with redescription of *Plectus cancellatus* Zullini, 1978 (Nematoda: Plectidae) from Canada. *Canadian Journal of Zoology* 63: 2395-2400.

Eroshenko A.S. 1973. New data on taxonomy of the family Teratocephalidae Andrássy (Nematoda). *Zoologischesky Zhurnal* 52: 1768-1776 (in Russian).

Eroshenko A.S. 1977. Nematodes of the ordines Araeolaimida and Dorylaimida of the soil fauna of the forests of the Primorskii Krai. *Paraziticheskie i svobodnozhyvushchie chervi fauny Dalnego Vostoka. Trudy Biologo-pochvennogo instituta* 47: 3-20 (in Russian).

Ettema C.H., Rathbun S.L. & Coleman D.C. 2000.On spatiotemporal patchiness and the coexistence of five species of *Chronogaster* (Nematoda: Chronogasteridae) in a riparian wetland. *Oecologia* 125: 444-452.

Eyualem A. 1996. Aquatic nematodes from Ethiopia IV. The family Leptolaimidae örley, 1880 sensu Lorenzen, 1981 (Chromadorida: Nematoda) with the description of *Anonchus coomansi* n. sp. *Hydrobiologia* 332: 1-26.

Eyualem A. & Coomans A. 1995. Freshwater nematodes of the Galápagos. *Hydrobiologia* 299: 1-51.

Eyualem A. & Coomans A. 1996. Aquatic nematodes from Ethiopia. VII. The family Rhabdolaimidae Chitwood, 1951 sensu Lorenzen, 1981 (Chromadorida: Nematoda) with the description of *Udonchus merhatibebi* n. sp. *Hydrobiologia* 341: 197-214.

Felsenstein J. 1989. PHYLIP – Phylogeny Inference Package. *Cladistics* 5: 164-166.

Fischer M. 1894. Über eine Clematis-Krankheit. *Ber. Phzysiol. Lab., Landwirthsch. Inst., Univ. Halle* 11: 1-11.

Filipjev I. 1918. Free-living marine Nematodes of the Sevastopol area. *Trudy osoboi zoologicheskoi laboratorii I sevastopol'skoi biologicheskoi stantsii Rossiiskoi Akademii Nauk* 2: 1-350 (in Russian).

Filipjev I.N. 1930. Les Nématodes de la baie de la Néva et de l'extrémité orientale du Golfe de Finlande. Deuxième partie. *Archiv für Hydrobiologie* 21: 1-64.

Freudenhammer I. 1975. Desmoscolecida aus der Iberishen Tiefsee, zugleich eine Revision dieser Nematoden-Ordnung. *"Meteor" Forschung-Ergebnisse* 20: 1-65.

Forey P.L., Humphries C.J., Kitching I.J., Scotland R.W., Siebert D.J. & Williams D.M. 1996. *Cladistics: a practical course in Systematics*. New York, Oxford University Press, 191 pp.

Fuchs G. 1930. Neue an Borken- und Rüsselkäfer gebundene Nematoden, halbparasitische und Wohnungseinmieter. Freilebende Nematoden aus Moos und Walderde in Borken- und Rüsselkäfergängen. *Zoologische Jahrbücher, Abteilung für Systematik* 59: 505-646.

Furstenberg J.P. & Vincx M. 1988. *Procamacolaimus tubifer* Gerlach, 1953, *Procamacolaimus africanus* sp. nov. and *Eontholaimus capensis* gen. nov., sp. nov. (Nematoda, Leptolaimidae) from the South Africa. *Suis-Afrikaanse Tydskrif vir Dierkunde* 23: 208-214.

Fürst von Lieven A. 2002. The sister group of the Diplogasterina (Nematoda). *Russian Journal of Nematology* 10: 127-137.

Fürst von Lieven A. 2003. Functional morphology and evolutionary origin of the three-part pharynx in nematodes. *Zoology* 106: 183-201.

Gagarin V.G. 1975. A contribution to the taxonomy and phylogeny of the superfamily Plectoidea (Nematoda). *Zoologicheskii Zhurnal* 56: 503-509 (in Russian)

Gagarin V.G. 1993. *Freeliving nematodes of the fresh waters of Russia and neighbouring countries.* St. Petersburg, Gidrometeoizdat, 352 pp. (In Russian).

Gagarin V.G. 2001. New species of free-living freshwater nematodes from Eurasia. *Zoosystematica Rossica* 9 (2000): 11-17.

Gagarin V.G., Nguyen Vu Thanh & Nguyen Dinh Tu. 2003. Three new species of free-living nematodes from freshwater bodies of Vietnam. *Zoosystematica Rossica* 12: 7-14.

Ganguly S. & Khan E. 1986. A new genus of the family Wilsonematidae rank. n. (Plectoidea: Nematoda). *Indian Journal of Nematology* 16: 226-230.

Gaugler R. (ed.) 2002. *Entomopathogenic Nematology.* CABI Publishing, 400 pp.

Gerlach S.A. 1953. Recherches sur la faune des eaux interstitielles de Madagascar. III. Sur quelques Nématodes libres des eaux souterraines littorales de Madagascar. *Mémoires de l'Institut scientifuque de Madagascar* 8: 73-86.

Gerlach S.A. 1954. Les Nématodes marins libres des eaux souterraines littorales d'Esponde (Portugal). *Vie Milieu* 4: 83-94.

Gerlach S.A. 1957a. Die nematoden des Sandstrandes an der Küste von Mittelbrasilien. *Mitteilungen aus dem Zoologischen Museum in Berlin* 33: 411-460.

Gerlach S.A. 1957b. Marine nematoden aus dem mangrovegebiet von Cananéia (Brazilianische meeres-nematoden III). *Abhandelungen der Mathematisch-naturwissenschaftlichen Klasse der Akademie der Wissenschaften Mainz* 5: 1-48.

Gerlach S.A. 1962. Freilebende Meeresnematoden von den Malediven*. Kieler Meeresforschungen* 18: 81-108.

Gerlach S.A. 1965. Freilebende Meeresnematoden aus der Gezeitenzone von Spitzbergen. *Veröffentlichungen des Instituts für Meeresforschung in Bremerhaven* 9: 109-172.

Gerlach S.A. 1966. Bemerkungen zur Phylogenie der Nematoden. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem* 118: 25-39

Gerlach S.A. 1967. Zwei neue freilebende marine Nematoden vergesellschaftet mit Crustaceen des Supralitoralis. *Veröffentlichungen des Instituts für Meeresforschung in Bremerhaven* 10: 209-215.

Gerlach S.A. & Riemann F. 1973. The Bremerhaven checklist of aquatic nematodes. A catalogue of Nematoda Adenophorea excluding the Dorylaimida. Part 1. *Veröffentlichungen des Instituts für Meeresforschung in Bremerhaven, Supplement* 4: 1-404.

Goloboff P. 1993. NONA. Noname, version 2.0. Program and documentation.

Goodey T. 1947. *Domorganus macronephriticus* n.g., n.sp., a new cylindrolaimid free-living soil nematode. *Journal of Helminthology* 21: 175-180.

Goodey T. 1951. *Soil and freshwater nematodes.* London, Methuen, 390 pp.

Guiral D., Gourbault N. & Helleouet M.N. 1995. Étude sédimentologique et méiobenthos d'un écosystème lagunaire modifie par un récif artificiel à vocation aquacole: l'acadja. *Oceanologica Acta* 18: 543-555.

Halbrendt J.M. & Brown D.J.F. 1992. Morphometric evidence for three juvenile stages in some species of *Xiphinema americanum* sensu lato. *Journal of Nematology* 24: 305-309.

Hernández M.A. & Jordana R. 1988. Description of the male of *Paratrypila intermedia* (Bütschli, 1873) (Nematoda, Enoplida). *Miscellanea Zoologica* 12: 23-26.

Hernandez M.A., Imaz A & Armendariz I. 1998. Description of a new species of *Plectus* Bastian, 1865 (Nematoda: Plectidae) and the males of two other species from Northern Spain. *Nematologica* 44: 631-642.

Heyns J. & Coomans A. 1980. Freshwater nematodes from South Africa. 5. *Chronogaster* Cobb, 1913. *Nematologica* 26: 187-208.

Heyns J. & Coomans A. 1983. New and known species of *Chronogaster* Cobb, 1913 (Nematoda: Leptolaimidae). *Nematologica* 29: 245-265.

Heyns J. & Coomans A. 1990. *Pakira orae* Yeates, 1967 from Transkei (Nematoda: Leptolaimidae). *Sud-Afrikaans Tydskrift Dierkunde* 25: 46-53.

Hillis D.M. & Huelsenbeck J.P. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. *Journal of Heredity* 83: 189-195.

Hofmänner B. 1926. Beiträge zur Kenntnis freilebender Nematoden, *Dadayia* (nec *Bathylaimus*) *mirabilis* Hofm. *Zoologischer Anzeiger* 67: 273-276.

Hofmänner B. & Menzel R. 1914. Neue Arten freilebender Nematoden aus der Schweiz. *Zoologischer Anzeiger* 44: 80-90.

Hofmänner B. & Menzel R. 1915. Die freilebenden Nematoden der Schweiz. *Revue Suisse de Zoologie* 23: 109-243.

Holovachov O. 2001. Study of Plectidae (Nematoda) from Ukraine. Variability of male sexual characters in *Plectus pusteri* Fuchs, 1930. *International Journal of Nematology* 11: 209-214.

Holovachov O. 2003a. Redescription of *Anguinoides stylosum* Chitwood, 1936 (Nematoda: Leptolaimidae). *Russian Journal of Nematology* 11: 23-25.

Holovachov O. 2003b. *Procamacolaimus dorylaimus* sp. nov. (Nematoda: Leptolaimidae) from the Southern Atlantic. *Annales Zoologici* 53: 551-557.

Holovachov O.V. 2004. Two new species of the genus *Aphanolaimus* de Man, 1880 (Nematoda: Aphanolaimidae) from Cameroon. *Vestnik Zoologii* 38 (5): 19-25.

Holovachov O. & Sturhan D. 2004. Two new species of the genus *Aphanonchus* Coomans & Raski, 1991 (Nematoda: Aphanolaimidae) and a revised taxonomy of the genus *Nematology* 6: 357-373.

Holovachov O., Boström S. & Mundo-Ocampo M. 2004. Description of three known and two new species of the genus *Tylocephalus* Crossman, 1933 with a revised taxonomy of the genus and key to species of the subfamily Wilsonematinae (Plectida). *Russian Journal of Nematology* 12: 115-130.

Holovachov O., Boström S. & Susulovsky A. 2001. Study of Plectidae (Nematoda) from Ukraine. Description of *Plectus paracuminatus* Zell, 1993 with observations of male sexual characters. *Journal of Nematode Morphology and Systematics* 4: 11-20.

Holovachov O., Boström S., Winiszewska G. & Háněl L. 2004. Description of two known and one new species of the genus *Anaplectus* De Coninck & Schuurmans Stekhoven, 1933 from Europe, and a revised taxonomy of the genus (Nematoda: Plectida). *Russian Journal of Nematology* 12: 45-58.

Holovachov O. & De Ley P. 2005. Order Plectida. In: Eyualem A. (ed) *Freshwater nematodes: ecology and taxonomy*, **in press**.

Holovachov O. & Háněl L. 2004. *Wilsonema longicaudatum* sp. nov. (Nematoda: Plectidae) from Poland. *Annales Zoologici* 54: 471-473.

Holovachov O. & Sturhan D. 2004a. Studies of the genus *Paraphanolaimus* Micoletzky, 1923 (Nematoda: Aphanolaimidae) with description of *P. paraguayensis* sp. n. *Nematology* 5 (2003): 793-807.

Holovachov O. & Sturhan D. 2004b. Two new species of the genus *Aphanonchus* Coomans & Raski, 1991 (Nematoda: Aphanolaimidae) and a revised taxonomy of the genus. *Nematology* 6: 357-373.

Holovachov O. & Susulovsky A. 2000. Study of Plectidae (Nematoda) from Ukraine. Description of two *Plectus* species with a discussion of some tail abnormalities. *Annales Zoologici* 50: 233-238.

Holovachov O., Susulovsky A. & Boström S. 2000. Description of two species of *Chiloplectus* Andrássy, 1984 (*Nematoda*: *Plectidae*) from Ukraine and a revised taxonomy of the genus. *Nematology* 2: 381-394.

Hope W.D. & Tchesunov A.V. 1999. *Smithsoninema inaequale* n. g. and n. sp. (Nematoda, Leptolaimidae) inhabiting the test of a foraminiferan. *Invertebrate Biology* 118: 95-118.

Hopper B.E. 1963. Marine nematodes from the coast line of the Gulf of Mexico. III. Additional species from gulf shores, Alabama. *Canadian Journal of Zoology* 41: 841-863.

Hopper B.E. 1968. *Paraplectonema canadianum* n. sp. (Nematoda: Leptolaimidae), a fresh-water nematodes from the Ottawa River. *Canadian Journal of Zoology* 46: 655-661.

Imamura S. 1931. Nematodes in the paddy field, with notes on their population before and after irrigation. *Journal of the College of Agriculture, Imperial University of Tokyo* 11: 193-240.

Inglis W.G. 1966. Marine nematodes from Durban, South Africa. *Bulletin of the British Museum (Natural History), Zoology* 14: 81-106.

Inglis W.G. 1983. An outline of classification of the phylum Nematoda. *Australian Journal of Zoology* 31: 243-255.

Iwan D. 2002. Generic classification of the tribe Platynotini (Coleoptera: Tenebrionidae), with notes on phylogeny. *Annales Zoologici* 52: 1-149.

Jayasree K. & Warwick R.M. 1977. Free-living marine nematodes of a polluted sandy beach in the Firth of Clyde, Scotland. Description of seven new species. *Journal of Natural History* 11: 289-302.

Jensen P. 1976. Free-living marine nematodes from a sublittoral station in the North Sea of the Belgian coast. *Biologisch Jaarboek Dodonaea* 44: 231-255.

Karegar A., De Ley P. & Geraert E. 1997. Three teratocephalid nematodes from Iran. *Fundamental and Applied Nematology* 20: 459-471.

Keppner E.J. 1988. Six new species of free-living marine nematodes (Nematoda: Araeolaimida: Enoplida) from two estuaries in Northwest Florida, U.S.A. *Transactions of the American Microscopical Society* 107: 79-95.

Keppner E.J. 1992. Some free-living marine nematodes from Northwest Florida, U.S.A. with description of three new species (Nematoda: Chromadorida, Trefusiida). *Transactions of the American Microscopical Society* 111: 199-210.

Khan H.A., Seema N. & Khan S.A. 1990. Preliminary studies on free living nematodes from Indus delta. *Pakistan Journal of Scientific and Industrial Research* 33: 439-443.

Khera S. 1972. Nematodes from the banks of still and running waters.12. Order Araeolaimida. *Proceedings of the Zoological Society of Calcutta* 25: 49-58.

Kischke U. 1956. Die Nematoden aus der Torf-Zone der Hochmoore des Oberharzes. *Archiv für Hydrobiologie* 52: 210-277.

Klekowski R.Z., Schiemer F. & Duncan A. 1979. A bioenergetic study of a bentic nematode, *Plectus palustris* de Man, 1880, throughout its life cycle. I. The respiratory metabolism as different densities of bacterial food. *Oecologia* 44: 119-124.

Kluge A.G. & Farris J.S. 1969. Quantitative phyletics and the evolution of animals. *Systematic Zoology* 18: 1-32.

Kreis H.A. 1930. Freilebende terrestrische Nematoden aus der Umgebung von Peking (China). II. *Zoologischer Anzeiger* 87: 67-87.

Kreis H. 1936. Süßwasser-Nematoden aus der Umgebung von Madras (Indien). *Revue Suisse de Zoologique* 43: 641-645.

Kreis H.A. 1963. Marine Nematoda. *Zoology of Iceland* 2: 1-68.

Lahl V., Halama C. & Schierenberg E. 2003. Comparative and experimental embryogenesis of Plectidae (Nematoda). *Developmental Genes and Evolution* 213: 18-27.-

Laybourn J. 1979. The effects of temperature on the respiration and production of the freshwater nematode *Anonchus* sp. *Oecologia* 41: 329-337.

Litvaitis M.K., Bates J.W., Hope W.D. & Moens T. 2000.Inferring a classification of the Adenophorea (Nematoda) from nucleotide sequences of the D3 expansion segment (26/28S rDNA). *Canadian Journal of Zoology* 78: 911-922.

Loof P.A.A. 1961. The nematode collection of Dr. J.G. de Man. *Beaufortia* 93: 169-254.

Loof P.A.A. 1971. Freeliving and plant parasitic nematodes from Spitzbergen, collected by Mr. H. van Rossen. *Mededelingen Landbouwhogeschool Wageningen* 71: 1-86.

Loof P.A.A. & Jairajpuri M.S. 1965. Two new species of *Chronogaster* Cobb, 1913 (Nematoda: Plectidae). *Proceedings of The Helminthological Society of Washington* 32: 181-186.

Lorenzen S. 1966. Diagnosen einiger freilebender Nematoden von der schleswig-holsteinischen Westküste. *Veröffentlichungen des Instituts für Meeresforschung Bremerhaven* 10: 31-48.

Lorenzen S. 1969. Freilebende Meeresnematoden aus dem Schlickwatt und den Salzwiesen der Nordseeküste. *Veröffentlichungen des Instituts für Meeresforschung in Bremerhaven* 11: 195-238.

Lorenzen, S. 1972a. Die Nematodenfauna im Verklappungsgebiet für Industrieabwasser nordwestlich von Helgoland. I. Araeolaimida und Monhysterida. *Zoologischer Anzeiger,* 187: 223-248.

Lorenzen, S. 1972b. *Leptolaimus*-Arten (freilebende Nematoden) aus der Nord- und Ostsee. *Kieler Meeresforschungen,* 28: 92-97.

Lorenzen S. 1981. Entwurf eines phylogenetischen Systems der freilebenden Nematoden. *Veröffentlichungen des Instituts für Meeresforschung Bremerhaven, Supplement* 7: 1-472.

Lorenzen S. 2000. The role of the biogenetic convergence rule in polarising transformation series – arguments from nematology, chaos science, and phylogenetic systematics. *Annales Zoologici* 50: 267-275.

Maddison W.P. & Maddison D.R. 2000. *MacClade 4: analysis of phylogeny and character evolution*. Sinauer, Sunderlands. Massachusetts.

Maggenti A.R. 1961a. Morphology and biology of the genus *Plectus* (Nematoda: Plectidae). *Proceedings of the Helminthological Society of Washington* 28: 118-130.

Maggenti A.R. 1961b. Revision of the genus *Plectus* (Nematoda: Plectidae). *Proceedings of the Helminthological Society of Washington* 28: 139-166.

Maggenti A.R. 1963. Comparative morphology in nemic phylogeny. In: Dougherty et al. *The lower Metazoa, comparative biology and phylogeny*. University of California Press, 273-282.

Maggenti A.R. 1981. *General nematology*. Springer Verlag, 384 pp.

Maggenti A.R., Raski D.J., Koshy P.K. & Sosamma V.K. 1983. A new species of *Chronogaster* Cobb, 1913 (Nemata: Plectidae) with an amended diagnosis of the genus and discussion of cuticular ornamentation. *Revue de Nématologie* 6: 257-263.

Malakhov V.V. 1986. *Nematodes. Structure, development, system and phylogeny.* Moskva, Nauka, 216 pp. (In Russian.)

Malakhov V.V., Ryzhykov K.M. & Sonin M.D. 1982. [System of higher taxa of nematodes]. *Zoologicheskii Zhurnal* 64: 1125-1134 (in Russian).

de Man J.G. 1876. Onderzoekingen over vrij in de aarde levende Nematoden. *Tijdschrift Nederlandsche dierkundige Vereeniging* 2: 78-196.

de Man J.G. 1880. Die einheimischen, frei in der reinen Erde und im süβen Wasser lebenden Nematoden. Vorläufige Bericht und descriptiv-systematischer Theil. *Tijdschrift Nederlandsche dierkundige Vereeniging* 5: 1-104.

de Man J.G. 1886. *Anatomische Untersuchungen über freilebende Nordsee-Nematoden*. Leipzig, 82 pp.

de Man J.G. 1889. Espèces et genres nouveaux de Nématodes libres de la mer du Nord et de la Manche. *Mémoires de la Société zoolgique de France* 2: 1-10.

de Man J.G. 1904. Nématodes libres (Expédit. Antarctique Belge). *Résults of the Voyage S. Y. Belgica*: 1-51.

de Man J.G. 1907. Sur quelques espèces nouvelles ou peu connues de Nématodes libres vivant sur les côtes de la Zélande. *Tijdschrift Nederlandsche dierkundige Vereeniging* 10: 227-244.

de Man J.G. 1921. Nouvelles recherches sur les Nématodes libres terricoles de la Hollande. *Capita Zoologica* 1: 1-62.

de Man J.G. 1922. Vrijlevende nematoden. In: *Flora en Fauna der Zuiderzee*, 214-261.

Mateo M.D. & Campoy A. 1983. Estudio de los Nematodos libres de las Peñas de Echauri (Navarra). *Publícaciones de Biología de la Universidad de Navarra*, *Série Zoológica* 9: 1-64.

Maupas E. 1900. Modes et formes de reproduction chez les nématodes. *Archives Zool. Exp. Gén.* 8: 463-624.

Meyl A.H. 1954. Die Fadenwürmer (Nematoda) einiger Salzstellen südöstlich von Braunschweig. *Abhandlungen der Braunschweigischen wissenschaftlichen Gesellschaft* 6: 84-106.

Micoletzky H. 1922. Die freilebenden Erdnematoden. *Archivum für Naturgeschichte* 87: 1-650.

Micoletzky H. 1923. Freilebende Nematoden der Wolga mit besonderer Berücksichtigung der Umgebung von Saratow. *Raboty volzhskoi biologicheskoi stantsii* 7: 1-29.

Micoletzky H. 1924. Weitere Beiträge zur Kenntnis freilebenden Nematoden aus Suez. *Sitzungsberichte der Akademie der Wissenschaften in Wien* 132: 225-262.

Mulk M.M. & Coomans A. 1978. Freeliving nematodes from Mount Kenya. II. Scientific report of the Belgian Mt. Kenya bio-expedition. *Revue Zoologique Africane* 92: 593-608.

Murphy D.G. 1964. Free-living marine nematodes, I. *Southerniella youngi*, *Dagda phinneyi*, and *Gammanema smithi*, new species. *Proceedings of the Helminthological Society of Washington* 31: 190-198.

Murphy D.G. 1965. *Cynura Klunderi* (Leptolaimidae), a new species of marine nematode. *Zoologischer Anzeiger* 175: 217-222.

Muthumbi A., Vershelde D. & Vincx M. 1995. New Desmodoridae (Nematoda: Desmodoroidea): three new species from *Ceriops* mangrove sediments (Kenya) and one related new species from the North Sea. *Cahiers de Biologie Marine* 36: 181-195.

Nesterov P.I. 1973. New species of plant nematodes from the rhizosphere of cultivated and wild plants in Moldavia. *Parazity zhyvotnykh i rastenii* 9: 239-246 (in Russian).

Nixon K.C. 2002. *Winclada (BETA) ver. 0.9.9.* NY.

Novikova S.I. 1971. Description of males of *Plectus armatus* Buetschlii and *Teratocephalus costatus* Andrassy (Nematoda, Plectidae, Teratocephalidae). *Zoologicheskii Zhurnal* 50 : 1400-1402 (in Russian).

Novikova S.I. & Gagarin V.G. 1971. New species of the genus *Plectus* (Nematoda, Plectidae). *Zoologicheskii Zhurnal* 50: 1097-1098 (in Russian).

Ocaña A. 1991. A redescription of two nematode species found in hot springs. *Nematologia mediterranea* 19: 173-175.

Örley L. 1880. Az anguillulidák magánrajza. *Természetrajzi Füzetek* 4: 1-165.

Ott J.A., Novak R., Schiemer F., Hentschel U., Nebelsich M. & Polz M. 1991. Tackling the sulfide gradient: a novel strategy involving marine nematodes and chemoautotrophic ectosymbionts. *Pubblicazioni della Stazione Zoologia di Napoli I, Marine Ecology* 12: 261-279.

Page R.D.M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12: 357-358.

Paramonov A.A. 1964. *Principles of Phytonematology. 2. Taxonomy of phytonematodes*. Moskwa, 446 pp (in Russian).

Patil K.J. & Khan E. 1982. Taxonomic studies of nematodes of Vidarbha region of Maharashtra, India. VI. *Spatiocephalus venustrus* gen. n. sp. n. (Nematoda: Wilsonematinae). *Indian Journal of Nematology* 12: 254-257.

Platt H.M. 1983. New species of *Metadesmolaimus* and *Stephanolaimus* (Nematoda: Chromadoria) from the Northern Ireland with reviews of the genera. *Zoological Journal of the Linnean Society* 78: 363-373.

Platt H.M. & Warwick R.M. 1988. *Free living marine nematodes. Part II. British chromadorids.* In: Kermack, D.M. & Barnes, R.S.K. (eds) Synopses of the British Fauna 38, 502 pp.

Poinar G.O., Jr 1976. Description and biology of a new insect parasitic rhabditoid, *Heterorhabditis* bacteriophora n. gen. n. sp. (Rhabditida; Heterorhabditidae n. fam.). *Nematologica* 21: 463-470.

Prejs K. 1977a. The nematodes of the root region of aquatic macrophytes with special consideration of nematode groupings penetrating the tissues of roots and rhizomes. *Ekologia Polska* 25: 5-20.

Prejs K. 1977b. The littoral and profundal benthic nematodes of lakes with different trophy. *Ekologia Polska* 25: 21-30.

Prejs K. 1977c. The species diversity, numbers and biomass of benthic nematodes in central part of lakes with different trophy. *Ekologia Polska* 25: 31-44.

Rashid F., Geraert E., Coomans A. & Suatmadji W. 1989. Cephalobidae from the Krakatau Region (Nematoda: Rhabditida). *Nematologica* 34 (1988):125-143.

Rasnitsyn A.P. 2002. [Process of evolution and the methodology of systematics]. *Proceedings of the Russian Enthomological Society* 73: 1-108 (in Russian).

Raski D.J. & Coomans A.V. 1990. Five new species of *Aphanolaimus* (Nemata: Araeolaimida) with a key to species. *Nematologica* 36: 22-54.

Raski D.J. & Coomans A.V. 1991. Three new species of *Paraphanolaimus* (Nemata: Araeolaimida) with key to species. *Nematologica* 37: 44-62.

Riemann F. 1970. Freilebende Nematoden aus dem Grenzbereich Meer-Süß-Wasser in Kolumbien, Südamerika. *Veröffentlichungen des Instituts für Meeresforschung Bremerhaven* 12: 365-412.

Riemann F. 1972. *Kinonchulus sattleri* n. g. n. sp. (Enoplida, Tripyloidea), an aberrant freeliving nematode from the lower Amazonas. *Veröffentlichungen des Instituts für Meeresforschung in Bremerhaven* 13: 317-326.

Riemann F. 1977. Causal aspects of nematode evolution: relations between structure, function, habitat and evolution. *Mikrofauna Meeresboden* 61: 217-230.

Riemann F., von Thun W. & Lorenzen S. 1971. über den phylogenetischen Zusammenhang zwischen Desmoscolecidae und Leptolaimidae (freilebende Nematoden). *Veröffentlichungen des Instituts für Meeresforschung in Bremerhaven* 13: 147-152.

Ryss A.Yu. 1988. Review of the genus *Prismatolaimus* (Araeolaimida). *Proceedings of the Zoological Institute Leningrad* 180: 82-95 (in Russian).

S'Jacob J.J. & van Bezooijen J. 1984. *A manual for practical work in nematology.* Wageningen, The Netherlands, Agricultural University Wageningen, 77 pp.

Sanwal K.C. 1968. Morphology and relationships of *Periplectus labiosus* n. gen., n. sp. (Nematoda: Plectinae) with remarks on its evolutionary significance. *Canadian Journal of Zoology* 46: 991-1003.

Sauer M.R. 1985. *A scanning electron microscope study of plant and soil nematodes*. CSIRO, 64 pp.

Sauer M.R. & Annells C.M. 1982. An SEM study of *Tylocephalus auriculatus* (Nematoda: Plectidae). *Nematologica* 28: 123-125.

Schiemer F., Duncan A. & Klekowski R.Z. 1980. A bioenergetic study of a bentic nematode, *Plectus palustris* de Man, 1880, throughout its life cycle. II. Growth, fecundity and energy budgets at different densities of bacterial food and general ecological considerations. *Oecologia* 44: 205-212.

Seinhorst J.W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* 4: 67-69.

Siddiqi M.R. 2003. *Keralanema spinicorpus* (Maggenti et al., 1983) gen. n., comb. n. (Nematoda: Chronogastridae). *International Journal of Nematology* 13: 236.

Šlepetene J.A. 1962. The description of some males and *Aphelenchoides* sp. (intersex). *Lietuvos TSR Mokslų Akademijos Darbai, Serija C* 1: 135-140 (in Russian).

Southern R. 1914. Clare Island Survey – Nemathelmia, Kinorhyncha and Chaetognatha. *Proceedings of the Royal Irish Academy* 31: 1-80.

Steiner G. 1916. Freilebende Nematoden aus der Barentssee. *Zoologische Jahrbücher, Abteilung für Systematik* 39: 511-676.

Stewart A.C. & Nicholas W.L. 1995. *Manunema pectenophora* sp. nov. (Peresianiidae, Leptolaimina), a nematode possessing unusual male supplementary organs. *Transactions of the Royal Society of South Australia* 119: 163-169.

Strand E. 1934. Miscellanea nomenclatorica zoologica et palaeontologica VI. *Folia Zoologica et Hydrobiologica* 6: 271.

Swart A., Heyns J. & Coomans A. 1993. Studies on *Haliplectus* Cobb, 1913. Introduction and redescription of *H. bickneri* Chitwood, 1956 (Nematoda: Haliplectidae). *Fundamental and applied Nematology* 16: 129-135.

Swofford D. 2002. *PAUP* 4.0 beta version - Phylogenetic Analysis Using Parsimony (and Other Methods)*. Sinauer Associates, Sunderland, Massachusetts.

Tahseen Q. & Jairajpuri M.S. 1988. Description and developmental biology of *Teratorhabditis andrassyi* n. sp. (Nematoda: Rhabditida). *Revue de Nématologie* 4: 333-342.

Tahseen Q., Ahmad I. & Jairajpuri M.S. 1992. Description and developmental biology of *Plectus zelli* n. sp. (Nematoda: Araeolaimida). *Fundamental and Applied Nematology* 15: 503-510.

Tahseen Q., Ahmad I. & Jairajpuri M.S. 1995. Two new species of Plectidae from India (Nematoda: Araeolaimida). *Fundamental and Applied Nematology* 18: 471-477.

Taylor A.L. 1935. A review of the fossil nematodes. *Proceedings of the Helminthological Society of Washington* 2: 47-49.

Tchesunov A.V. 1990. A critical analysis of the family Aegialoalaimidae (Nematoda, Chromadoria), trends in evolutionary development of marine nematode pharynx and a proposal of two new families. *Zoologicheskii Zhurnal* 69(8): 5-18 (in Russian).

Tchesunov A.V. 1993. Notes on the family Tubolaimoididae Lorenzen, 1981 (Nematoda: Chromadoria) with a description of *Chitwoodia tenuipharyngealis* sp. n. *Russian Journal of Nematology* 1: 121-128.

Tchesunov A.V. 1994. Description of a marine free-living nematode *Stephanolaimus graciosus* sp. n. and erection of *Setostephanolaimus* gen. n. (Chromadoria: Leptolaimidae). *Russian Journal of Nematology* 2: 79-82.

Tchesunov A.V., Miljutin D.M. & Evseev A.V. 2000. Multicellular parasites of protozoans. *Priroda* 3: 6-12 (in Russian).

Tchesunov A.V. & Miljutina M.A. 2002. A review of the family Ceramonematidae (marine free-living nematodes), with descriptions of nine species from the White Sea. *Zoosystematica Rossica* 11: 3-39.

Tchesunov A.V. & Riemann F. 1995. Cryonema. Arctic sea ice nematodes (Monhysteroidea), with descriptions of *Cryonema crassum* gen. n., sp. n. and *C. tenue* sp. n. *Nematologica* 41: 35-50.

Tchesunov A.V. & Sturhan D. 2001. On the taxonomy of Bastianidae (Chromadorida, Leptolaimina). *Russian Journal of Nematology* 9: 164 (abstract).

Tchesunov A.V. & Sturhan D. 2002. Redescription of *Dintheria tenuissima* de Man, 1921 (Nematoda: Bastianidae). *Russian Journal of Nematology* 10: 37-42.

Tchesunov A.V. & Sturhan D. 2004. Studies on *Domorganus macronephriticus* Goodey, 1947 (Nematoda: Ohridiidaae. *Journal of Nematode Morphology and Systematics* 7: 139-150.

Timm R. 1961. Antarctic soil and freshwater nematodes from the McMurdo Sound Region. *Proceedings of the Helminthological Society of Washington* 38: 42-52.

Timm R.W. 1963. Marine nematoden of the Suborder Monhysterina from the Arabian Sea at Karachi. *Proceedings of the Helminthological Society of Washington* 30: 34-49.

Travassos L. 1927. Sobre o genera *Oxysomatium*. Boletim Biologico 5:20-21.

Tsalolikhin S.Ya. 1980. Wieght estimation of freshwater nematodes. *Evolution, taxonomy, morphology and ecology of freeliving* nematodes: 80-85 (in Ukrainian).

Tsalolikhin S.Ya. 1989. New nematode species in Tanganyika Lake. *Zoologicheskii Zhurnal* 68: 6-16 (in Russian).

Turpeenniemi T. 1993. Ultrastructure of coelomocytes in *Sphaerolaimus gracilis* de Man, 1876 (Nematoda). *Journal of Nematology* 25: 616-624.

Turpeenniemi T.A. & Hyvärinen H. 1996. Structure and role of the renette cell and caudal glands in the nematode *Sphaerolaimus gracilis* (Monhysterida). *Journal of Nematology* 28: 318-327.

Van de Velde M.C. & Coomans A. 1987. Ultrastructure of the excretory system of the marine nematode *Monhystera disjuncta. Tissue and Cell* 19: 713-725.

Verschelde D., Gourbault N. & Vincx M. 1998. Revision of *Desmodora* with descriptions of new desmodorids (Nematoda) from hydrothermal vents of the Pacific. *Journal of the Marine Biological Association* 78: 75-112.

Vincx M. & Vanreusel A. 1989. Free-living marine nematodes from the Southern Bight of the North Sea. II. Notes on species of the Trefusiidae Gerlach, 1966. *Hydrobiologia* 175: 213-221.

Vitiello P. 1971. Espèces nouvelles de Leptolaimidae (Nematoda) et description du genre *Leptolaimoides* n. gen. *Cahiers de Biologie Marine* 12: 419-432.

Vitiello P. 1974. Considérations sur la systématique des nématodes Araeolaimida et description d'espèces nouvelles ou peu connues. *Archives de Zoologie expérimentale & générale* 115: 651-669.

Vitiello P. & De Coninck L.A. 1968. *Peresiana annulata* n. gen., n. sp., type interessant de Desmoscolecida. *Rapport et Procès-Verbaux des Réunions de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée* 19: 201-204.

Walia K.K. & Bajaj H.K. 1999. *Wilsereptus indicus* sp. n. (Wilsonematidae) from the rhizosphere of cotton from Haryana, India. *Haryana Agricultural University Journal of Research* 29: 35-38.

Warwick R.M. 1971. The Cyatholaimidae (Nematoda, Chromadoroidea) off the coast of Northumberland. *Cahiers de Biologie Marine* 12: 95-110.

Wieser W. 1956. Freeliving marine nematodes III. Axonolaimoidea and Monhysteroidea. *Acta Universitatis Lundensis* 52: 1-115.

Wiley E.O., Siegel-Casey D., Brooks D.R. & Funk V.A. 1991. *The Compleat Cladist: a primer of phylogenetic procedures.* The University of Cansas Museum of Natural History, 159 pp.

Winiszewska 1998. Redescription of *Chronogaster boettgeri* Kischke, 1956 (Nematoda: Leptolaimidae). *Annales Zoologici* 48: 333-335.

Winiszewska G., Holovachov O. & Susulovsky A. 2001. *Dintheria tenuissima* de Man, 1921 and *Stenonchulus troglodytes* Schneider, 1940 (Nematoda: Bastianiidae and Onchulidae) – two nematode species new for the fauna of Ukraine and Poland. *Fragmenta Faunistica* 44: 169-177.

Wu J. & Liang Y. 2000. Inland free-living nematodes in China: a historical review of taxonomic studies with descriptions of *Aphanonchus orientalis* sp. nov. (Chromadorida: Leptolaimidae) and *Daptonema limnobia* sp. nov. (Monhysterida: Xyalidae). *Annales Zoologici* 50: 307-319.

Yeates G.W. 1967. Studies on nematodes from dune sands. 2. Araeolaimida. *New Zealand Journal of Science* 10: 287-298.

Yeates G.W. 1967. Studies on nematodes from dune sands. 8. *Hemicycliophora halophila* n. sp. and *Ereptonema inflatum* n. sp. *New Zealand Journal of Science* 10: 802-807.

Yeates G.W. 1969. The status of *Trichodorus clarki* Yeates, 1967, *Ereptonema inflatum* Yeates,1967 and *Mononchoides potohikus* Yeates, 1969. *Nematologica* 15: 430.

Yeates G.W. 1970. Two terrestrial nematodes from the McMurdo Sound region, Antarctica, with a note on *Anaplectus arenicola* Killick, 1964. *Journal of Helminthology* 44: 27-34.

Yeates G.W. 1988. Distribution of Plectidae (Nematoda: Araeolaimida) in pasture soils. *New Zealand Journal of Zoology* 15: 191-202.

Zakhidov M.T. 1974. Nematodes from sand and ooze biotopes in the Kurskiy Zaliv (Baltic Sea). *Zoologicheskii Zhurnal* 53: 280-282 (in Russian).

Zell H. 1982. Nematoden eines Buchenwaldbodens. 1. *Wilsonema tentaculatum* (Fuchs, 1930) (Nematoda, Araeolaimida). *Carolinea* 40: 99-100.

Zell H. 1985. Nematoden eines Buchenwaldbodens. 5. Die Wilsonematinae (Nematoda, Araeolaimida). *Carolinea* 43: 77-92.

Zell H. 1991. *Hemiplectus muscorum* n. gen., n. spec. (Nematoda, Leptolaimidae). *Zoologischer Anzeiger* 226: 298-306.

Zell H. 1993. Die Gattung *Plectus* Bastian, 1865 *sensu lato* (Nematoda, Plectidae) – Ein Beitrag zur Ökologie, Biogeographie, Phylogenie und Taxonomie der Plectidae. *Andrias* 11: 1-173.

Zimmerman W. 1967. Methoden der Evolutionswissenschaft. In: G. Heberer (ed.), Die Evolution der Organismen. Stuttgart, G. Fisher Verlag, 61-160.

Zullini A. 1978. Nematodi liberi dell'Isola di Tavolara (Sardegna). *Rendiconti (V)* 3: 67-76.

Zullini A. 1982. *Guide per il riconoscimento delle specie animali delle acque interne italiane. 17. Nematodi*. Verona, 120 p.

Zullini A. 1988. A new genus and five new species of nematodes from Ethiopian lakes. *Revue de Nématologie* 11: 279-288.

Zullini A., Loof P.A.A. & Bongers T. 2002. Free-living nematodes from nature reserves in Costa Rica. 3. Araeolaimida. *Nematology* 4: 709-724.

Zullini A. & Pagani M. 1989. The ecological meaning of relative egg size in soil and freshwater nematodes. *Nematologica* 35: 90-96.

Summary

Nematodes constitute one of the largest animal phyla. Hitherto about 20.000 species have been described but there are estimates that this number is less than 2% of the total number of existing species. Although the larger animal and human parasites were known since ancient times the description of free living nematodes and plant parasites started around 1850. Although nematodes can be found in almost every habitat, they are still one of the least known groups of extant metazoans. In a hierarchical organisation (classification) of nematodes, as for some other groups of multicellular animals, the higher the level, the greater the confusion and discrepancy among different opinions. It is clear, that the applied, prognostic and educational value of such artificial classification is low and requires further improvement.

This thesis represents a small brick in the castle of humans' knowledge about nematode morphology, diversity and phylogeny. The author brings to light some new data and ideas about the form of some nematodes, how they develop and how they apparently evolved. Four main chapters deal with one of the smallest but morphologically and ecologically diverse orders, Plectida, whose members are found in marine, terrestrial and fresh water habitats.

At the first stage of studies, just one genus, *Anonchus,* is analysed. Among the six known species included in the analysis, however, *Anonchus* shows considerable variability in morphology of reproductive organs, of both females and males. It is worth mentioning that morphological peculiarities of female gonads and the male copulatory apparatus are often used to separate populations into different species and to create higher taxonomic categories. The discovery of two new species, *A. venezolanus* and *A. winiszewskae,* adds even more to understanding the structural diversity of this genus. The analysis of the morphology of *Anonchus* species results in considerable rearrangement of the classification of this genus and its allies, which are placed in the newly reinstated family Aphanolaimidae.

The main objective of the third chapter was to uncover the morphological diversity of representatives of mostly marine and presumably primitive groups of Plectida, and to propose a phylogenetic hypothesis of their relationships with each other and with their terrestrial allies (descendants ?). Additional descriptions are given for five species. Morphological and taxonomic analysis has been followed by the discovery of new taxa; the description of one, *Paraplectonema loofi,* is given in this chapter. Another, *Procamacolaimus dorylaimus*, is described in a separate article, but is also included in the phylogenetic reconstructions, which, on the whole, include 20 in-group taxa representing all distinct genera. A thorough systematic study required at least a brief discussion of phylogenetic affinities of the families Bastianiidae and Odontolaimidae and the genus *Tobrilia* which were previously included in the order Plectida. The taxonomic position of these taxa has become clearer in light of the new morphological data, particularly in the structure of male copulatory apparatus.

The subsequent part of the thesis deals with the few species of the subfamily Wilsonematinae, whose members are one of the smallest terrestrial nematodes. The taxonomy of the Wilsonematinae has been severely hampered by the limits of light

microscopy for correct observation and interpretation of their highly elaborate, complex labial region. The discrepancy between recent data and previous published descriptions of the structure of the labial region illustrates the great importance of scanning electron microscopy (SEM) for morphological and taxonomic studies of the group in question. Keeping this in mind, the author provides detailed redescriptions of five species of the genera *Wilsonema, Ereptonema* and *Neotylocephalus* on the basis of both type and new material from diverse habitats and localities throughout the world. Reappraisal of the earlier morphological descriptions of these taxa resulted in substantial changes in their classification, and critical revision of all allied species and genera. Ultrastructural studies (SEM) made it possible to propose a phylogenetic hypothesis for the subfamily Wilsonematinae.

The fifth chapter of this thesis includes a phylogenetic analysis of the largest terrestrial taxon of the order Plectida, superfamily Plectoidea. This analysis is based on published and updated morphological data for 35 in-group species. One of these species will be described as new in a separate publication. To better understand the character polarity, the postembryonic development of four species is described in detail. It was found that not only the general growth and development of reproductive system occurs during postembryonic development but that somatic sensilla, digestive and excretory systems also change morphologically during successive stages. Possible historical relationships of the superfamily Plectoidea are analysed using different methods and principles; one of the phylogenetic hypotheses was selected for thorough discussion, and as a basis for the revised classification of this taxon. Morphological data, which are elucidated and summarised in this chapter, provide the basis to further speculate on structural evidence for parthenogenetic reproduction in one of the studied genera, and the data also suggest several probable ways of niche partitioning in the superfamily Plectoidea.

The general discussion summarises all taxonomic changes and suggestions proposed in preceding chapters, and gives a revised and updated classification of the order Plectida. Possible morphological adaptations to the fresh water habitat and structural changes associated with the soil habitat are briefly discussed based on the proposed phylogenetic hypothesis. Since family Plectidae sometimes has been suggested to be closely related to the scientifically and economically important order Rhabditida (=Secernentea), the author proposed that morphology of plectid genus, *Anaplectus,* may be a source for future research on the origin and evolution of rhabditids.

Резюме

Нематоди складають один із найбільших типів тваринного царства, чиє справжнє різноманіття було відкрите лише після винайдення мікроскопа. Вони залишаються однією із найменш досліджених груп сучасних багатоклітинних тварин, які, тим не менше, населяють майже всі відомі біотопи. В ієрархічній класифікації нематод, як і деяких інших Metazoa, чим вищий рівень – тим більше неузгодженостей і суперечностей в поглядах. Зрозуміло, що прикладне, прогностичне та навчальне значення таких класифікацій мале, і вони потребують подальшого вдосконалення.

Ця робота представляє собою невеликий внесок до людського знання про морфологію, різноманіття та філогенію нематод. Автор намагався висвітлити деякі нові дані про те, як нематоди побудовані тепер, як відбувається їхній розвиток, і як вони імовірно еволюціонували в минулому. В чотирьох основних розділах розглядається один із найменших, але морфологічно та екологічно різноманітний ряд Plectida, представників якого можна виявити як у водних так і в наземних біотопах, та роль яких у функціонуванні екосистем залишається не до кінця зрозумілою.

На першому етапі досліджень автор піддав аналізу лише один рід, який проте, характеризується значною мінливістю в будові статевої системи самиць і самців, шести включених в аналіз видів – рис, які часто використовуються для розділення популяцій на види та створення таксономічних категорій надвидового рангу. Більш того, знахідка двох нових видів цієї групи, а саме: *Anonchus venezolanus* та *A. winiszewskae*, ще більше розширила межі мінливості роду. Аналіз морфології видів роду Anonchus призвів до значних змін в його класифікації, та класифікації близьких таксонів, що згруповані зараз в межі родини Aphanolaimidae.

Основним завданням третього розділу було описати різноманіття будови в основному морських і, ймовірно, примітивних груп роду Plectida, а також запропонувати гіпотезу про їх зв'язки між собою та з близькими наземними таксонами (? нащадками). П'ять видів було детально переописано. Морфологічний та таксономічний аналі, звичайно, супроводжувався відкриттям нових видів, опис одного з яких, а саме *Paraplectonema loofi*, поданий в розділі. Інший новий вид *Procamacolaimus dorylaimus* був описаний в окремій статті, проте теж був включений у філогенетичні побудови, які загалом, об'єднують 20 внутрішньогрупових таксонів, що представляють всі основні роди. Детальні систематичні дослідження були не можливі без короткого обговорення філогенетичних зв'язків таксонів, які часом об'єднувалися в межі роду Plectida, але чиє реальне таксономічне положення стало яснішим зараз, з огляду на нові дані з їх будови, зокрема морфології копулятивного апарату. Це родини Bastianiidae та Odontolaimidae, а також рід *Tobrilia*.

Наступна частина роботи присвячена досить дрібній (таксономічно та морфологічно) групі плектидних нематод – підродині Wilsonematinae, таксономія якої була обмежена недостатністю світлової мікроскопії для коректного опису та інтерпретації будови їх складно побудованої губної ділянки. Розбіжності між власними даними та попередніми опублікованими описами в будові губної ділянки вказують на важливість растрової електронної мікроскопії для морфологічних та таксономічних досліджень цієї групи нематод. Зважаючи на вище сказане, автор подав детальний переопис п’яти видів з родів *Wilsonema, Ereptonema* та *Neotylocephalus* на основі типового та нового матеріалу з різних біотопів та місцезнаходжень з усього світу. Перегляд морфології цих таксонів спонукав до значних змін в їх класифікації, та до критичного огляду (ревізії) усіх близьких видів та родів. Ультраструктурні дослідження також дали змогу запропонувати гіпотезу про філогенетичні зв’язки в межах підродини Wilsonematinae.

Пердостанній (п’ятий) розділ містить аналіз філогенії найбільшого наземного таксону ряду Plectida, а саме – надродини Plectidea, що грунтується на опублікованих та нових морфологічних відомостях для 35 видів. Один з них буде описаний як новий для науки в окремій статті. Для кращого розуміння напрямків трансформації ознак, автор описав пост-ембріональний розвиток у чотирьох видів. Виявилось, що під час пост-ембріонального розвитку відбуваються не лише загальний ріст частин тіла: розвиток статевої системи, а й значні зміни в будові органів чуттів, травної та видільної систем.

Імовірні історичні зв’язки в межах групи були проаналізовані, із застосуванням різних підходів та принципів, в результаті чого була обрана та обговорена єдина гіпотетична філогенія, яка послужила основою для змін в класифікації надродини Plectoidea. Морфологічні дані, що були згромаджені та розглянуті в цьому розділі, дозволили висловити припущення про морфологічні ознаки партеногенезу та запропонувати кілька можливих шляхів послаблення конкурентних відносин у видів цієї групи.

В загальному обговорені об’єднано разом всі таксономічні зміни і пропозиції попередніх розділів та подана узагальнена філогенія і класифікація ряду Plectida. На основі запропонованої філогенетичної гіпотези, коротко обговорені імовірні морфологічні пристосування для колонізації прісних водойм та структурні зміни під час освоєння грунту.

Оскільки родину Plectidea іноді зближають із представниками теоретично та екологічно важливого ряду Plectida (= Secernentea), автор пропонує, що морфологія роду *Anaplectus* може бути джерелом для подальшого дослідження походження та еволюції рабдитид.

Samenvatting

Nematoden vormen een van de omvangrijkste phyla van de dieren. Tot nu toe zijn er ongeveer 20.000 soorten beschreven maar er zijn schattingen dat dit aantal minder dan 2% is van het totale aantal bestaande soorten. Hoewel de grotere mens- en dierparasieten al in de oudheid bekend waren begonnen de vrijlevende nematoden en plantenparasieten vanaf 1850 meer aandacht te krijgen. Hoewel nematoden in vrijwel elk habitat aangetroffen worden vormen ze nog steeds een van de minst bekende groepen van de meercellige dieren. Zoals voor veel andere meercellige dieren geldt, geldt ook voor nematoden dat de indeling op hoger niveau verwarrend is en dat er veel verschillende opvattingen bestaan. Het is duidelijk dat de toegepaste, voorspellende en educatieve waarde van een artificiële classificatie laag is en voor verbetering vatbaar.

Dit proefschrift draagt een steentje bij aan de kennis over de nematodenmorfologie, de diversiteit en de fylogenie. De auteur heeft getracht nieuw licht te werpen op de bouw van nematoden, hoe ze zich ontwikkelen en hoe ze zich in het verleden mogelijk geëvolueerd hebben. Vier hoofdstukken hebben betrekking op de kleinste maar morfologisch en ecologisch diverse orde van de Plectida waarvan de leden zowel onder mariene omstandigheden als in het binnenland voorkomen

In eerste instantie werd aandacht geschonken aan het geslacht *Anonchus* waarvan zes soorten bestudeerd werden. Dit geslacht waarvan zowel mannetjes als wijfjes bestudeerd werden, vertoont een aanzienlijke morfologische variatie in het geslachtsapparaat. Juist dit geslachtsapparaat wordt vaak gebruikt om populaties in verschillende soorten te splitsen en voor indelingen op hoger niveau. De ontdekking van twee nieuwe soorten, *A. venezolanus* en *A. winiszewskae*, droegen nog aanzienlijk bij aan de structurele diversiteit van dit geslacht. De morfologische analyse van de *Anonchus*-soorten resulteerde in een aanzienlijke herziening van de classificatie van dit geslacht en naaste verwanten die nu weer in de herstelde familie Aphanolaimidae geplaatst worden.

Het belangrijkste doel van het derde hoofdstuk was het ontrafelen van de morfologische diversiteit van de overwegend mariene en vermoedelijk meest primitieve groepen van de Plectida en om een fylogenetische hypothese op te stellen met betrekking tot hun onderlinge verwantschap en met de in het binnenland voorkomende verwanten. Aanvullende beschrijvingen werden gegeven van vijf soorten. De morfologische en taxonomische analyse resulteerde in de ontdekking van nieuwe taxa; de beschrijving van een van deze, *Paraplectonema loofi*, wordt in dit hoofdstuk gegeven. Een ander nieuw taxon, *Procamacolaimus dorylaimus*, werd beschreven in een apart artikel en werd ook opgenomen in de fylogenetische analyse van vertegenwoordigers van alle 20 afzonderlijke geslachten.

Een diepgaande systematische studie was niet mogelijk zonder een korte discussie met betrekking tot de fylogenetische verwantschap van taxa die voorheen in de orde Plectida geplaatst werden en waarvan de verwantschappen duidelijk werden in het licht van nieuwe morfologische kenmerken, in het bijzonder die van de structuur van het mannelijke geslachtsapparaat. Dit betrof de families Bastianiidae en Odontolaimidae en het geslacht *Tobrilia*.

Het daaropvolgende deel van het proefschrift heeft betrekking op de relatief kleine en soortenarme groep van de plectide nematoden en name de Wilsonematinae waarbij de beperkingen van de lichtmicroscoop correcte observatie en interpretatie van de complexe lipstreek sterk bemoeilijken. De tegenstelling tussen recente waarnemingen en eerdere beschrijvingen van de structuur van de lipstreek illustreert het grote belang van de scanning elektronen microscoop voor morfologische en taxonomische studies van de desbetreffende groep. Met deze beperkingen in gedachte heeft de auteur een gedetailleerde herbeschrijving gegeven van vijf soorten behorende tot de geslachten *Wilsonema*, *Ereptonema* en *Neotylocephalus* op basis van zowel typemateriaal en nieuw materiaal van mondiaal diverse herkomsten. Herwaardering van de morfologische waarnemingen aan deze taxa resulteerde in aanzienlijke veranderingen in hun classificatie en kritische revisie van verwante geslachten en soorten. De studies van de ultrastructuur, met behulp van scanning elektronen microscopie maakte het mogelijk om een fylogenetische hypothese op te stellen voor de subfamilie Wilsonematidae.

Het vijfde hoofdstuk van dit proefschrift bevat een fylogenetische analyse van het grootste terrestrische taxon van de orde Plectida, de superfamilie Plectoidea, waarbij 35 soorten betrokken werden.

Een van deze soorten zal nog als nieuwe soort in een aparte publicatie beschreven worden. Om beter inzicht te krijgen in de polariteit van de kenmerken is de postembryonale ontwikkeling van vier soorten in detail beschreven. Waargenomen werd dat er niet alleen veranderingen optraden in de grootte en ontwikkeling van het reproductiesysteem gedurende de postembryonale ontwikkeling maar ook werden successievelijke veranderingen waargenomen in tastorgaantjes, het spijsverteringsapparaat en het excretiesysteem. Mogelijke historische verwantschappen van de desbetreffende groep werden geanalyseerd met behulp van verschillende methoden en principes; er werd een fylogenetische hypothese gekozen en uitvoerig bediscussieerd. De analyse werd ook gebruikt als basis voor een herziene classificatie van de superfamilie Plectoidea. Morfologische kenmerken, die in dit hoofdstuk belicht en samengevat werden, maakten het mogelijk om veronderstellingen te doen over het structurele bewijs van parthenogenese en suggereert verschillende mechanismen van nichedifferentiatie in de superfamilie Plectoidea.

In de algemene discussie werden alle voorgestelde veranderingen en suggesties samengevoegd voor zover die in eerdere hoofdstukken voorgesteld werden en wordt een nieuwe fylogenie en classificatie van de orde Plectida gegeven. Mogelijke morfologische aanpassingen voor het overleven in zoetwater en structurele veranderingen die met het koloniseren van grond te maken hebben worden beknopt besproken op basis van de fylogenetische hypothese. Daar de familie Plectidae in het verleden als nauw verwant beschouwd werd aan de wetenschappelijk en economische belangrijke orde Rhabditida (=Secernentea) suggereert de auteur dat de morfologie van het plectide genus *Anaplectus* een bron zou kunnen zijn voor toekomstig onderzoek naar de oorsprong en evolutie van de rhabditiden.

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Curriculum Vitae

Oleksandr Holovachov was born on 15 July 1978 in the city of Lviv, Ukraine. During the study in "53 Lviv secondary school with profound study of English language", he participated in the biological and ecological olympiads at different levels, and won 2nd award in the Ukrainian Biological Olympiad in 1994, 1st award in the Ukrainian Ecological Olympiad in 1994, 3rd award in the Ukrainian Biological Olympiad in 1995, 2nd award in the Russian Biological Olympiad in 1995, and, finally, Silver medal in the 6th International Biological Olympiad 1995. Years of studies at the Department of Zoology, Biological faculty, Ivan Franko National University of Lviv coincided with the first nematological studies under the supervision of Dr. Andriy Susulovsky (State Museum of Natural History, Lviv). They were partly supported by the grants from the International Science Education Programme of the International Renaissance Foundation in 1995-1999.

In 2000 he was graduated from the university and received the diploma with distinction of "Master of Science in Biology/Zoology, teacher of biology and chemistry". In the same year he became employed as PhD student, and in 2002 – as an assistant professor at the Department of Zoology, Biological faculty, Ivan Franko National University of Lviv. A fellowship from the International Agricultural Centre (Wageningen) and the Netherlands Ministry of Agriculture, Nature Management and Fisheries provided the basis for initial collaboration with the Laboratory of Nematology, Wageningen University, where O. Holovachov passed successfully the Nematode Identification Course in 2001 and prepared the PhD thesis entitled "Morphology and Systematics of the Order Plectida Malakhov, 1982 (Nematoda)".

Currently he occupies a fulltime position of assistant professor at the Department of Zoology, Biological faculty, Ivan Franko National University of Lviv, Ukraine; he gives practicals in protist and invertebrate zoology and field practice on local invertebrate fauna. From 2005 he was appointed as a managing editor of the "Visnyk of Lviv University, Biology Series" journal, which is published by the Biological faculty of the Ivan Franko National University of Lviv; and as an associate editor of the "Russian Journal of Nematology", which is published by the Russian Society of Nematology.

Publication list

Journal articles

Nadler S.A., De Ley P., Mundo-Ocampo M., Smythe A.B., Stock S.P., Bumbarger D., Adams B.J., Tandingan De Ley I., Holovachov O. & Baldwin J.G. 2006. Phylogeny of Cephalobina (Nematoda): molecular evidence for substantial phenotypic homoplasy and incongruence with traditional classifications. *Molecular Phylogenetics and Evolution* **in press**.

Holovachov O. & Boström S. 2006. Description of *Acrobeloides arctowskii* sp. n. (Rhabditida: Cephalobidae) from King George Island, Antarctica. *Russian Journal of Nematology* **in press**.

Holovachov O., Esquivel A. & Bongers T. 2005. The genus *Deficephalobus* De Ley & Coomans, 1990 (Cephalobina, Ostellidae) from nature reserves in Costa Rica. *Nematology* 7: 469-477.

Holovachov O. & Boström S. 2005. Description of *Tricirronema tamdaoensis* sp. n. (Rhabditida: Bicirronematidae) from Vietnam. *Journal of Nematode Morphology and Systematics* 8: 51-56.

Zagorodniuk I. & Holovachov O. 2005. Cladogenesis in chordate animals (Chordozoa). Part 1. Concept and features of the phylum, relations with other groups. *Scientific Bulletin of the Uzhgorod University. Series Biology* 16: 5-21 (in Ukrainian).

Holovachov O. 2004. Morphology, phylogeny and evolution of the superfamily Plectoidea Örley, 1880 (Nematoda: Plectida). *Annales Zoologici* 54: 631-672.

Holovachov O. & Boström S. 2004. Morphology and systematics of the superfamilies Leptolaimoidea Örley, 1880 and Camacolaimoidea Micoletzky, 1924 (Nematoda: Plectida). *Journal of Nematode Morphology and Systematics* 7: 1-49.

Holovachov O., Boström S. & Mundo-Ocampo M. 2004. Description of three known and two new species of the genus *Tylocephalus* Crossman, 1933 with a revised taxonomy of the genus and key to species of the subfamily Wilsonematinae (Plectida). *Russian Journal of Nematology* 12: 115-130.

Holovachov O., Boström S., Winiszewska G. & Háněl L. 2004. Description of two known and one new species of the genus *Anaplectus* De Coninck & Schuurmans Stekhoven, 1933 from Europe, and a revised taxonomy of the genus (Nematoda: Plectida). *Russian Journal of Nematology* 12: 45-58.

Holovachov O.V. 2004. Description of *Metateratocephalus bialowieziensis* sp. n. (Nematoda, Metateratocephalidae). *Vestnik Zoologii* 38 (1): 71-73.

Holovachov O. & Háněl L. 2004. *Wilsonema longicaudatum* sp. nov. (Nematoda: Plectidae) from Poland. *Annales Zoologici* 54: 471-473.

Holovachov O. & Sturhan D. 2004. Two new species of the genus *Aphanonchus* Coomans & Raski, 1991 (Nematoda: Aphanolaimidae) and a revised taxonomy of the genus *Nematology* 6: 357-373.

Holovachov O. & Sturhan D. 2004. Studies of the genus *Paraphanolaimus* Micoletzky, 1923 (Nematoda: Aphanolaimidae) with description of *P. paraguayensis* sp. n. *Nematology* 5 (2003): 793-807.

Holovachov O.V. 2004. Two new species of the genus *Aphanolaimus* de Man, 1880 (Nematoda: Aphanolaimidae) from Cameroon. *Vestnik Zoologii* 38 (5): 19-25.

Abolafia J., Holovachov O., Boström S. & Peña-Santiago R. 2004. Nematodes of the order Rhabditida from Andalucía Oriental, Spain. The genus *Cervidellus* Thorne, 1937 with additional data on *C. hamatus* Thorne, 1937. *Nematology* 5 (2003): 753-776.

Holovachov O., Esquivel A. & Bongers T. 2004. Freeliving nematodes from the nature reserves in Costa Rica. 5. Bunonematoidea. *Nematology* 5 (2003): 665-676.

Holovachov O., Esquivel A. & Bongers T. 2003. Freeliving nematodes from the nature reserves in Costa Rica. 4. Cephalobina. *Nematology* 5: 1-16.

Holovachov O. 2003. Redescription of *Anguinoides stylosum* Chitwood, 1936 (Nematoda: Leptolaimidae). *Russian Journal of Nematology* 11: 23-25.

Holovachov O. 2003. *Procamacolaimus dorylaimus* sp. nov. (Nematoda: Leptolaimidae) from the Southern Atlantic. *Annales Zoologici* 53: 551-557.

Holovachov O. 2003. Morphometric variability of species of the genus *Metateratocephalus* Eroshenko, 1973 (Nematoda). *Visnyk of Lviv University, Biology Series* 33: 110-116 (in Ukrainian).

Holovachov O. & Sturhan D. 2003. *Adelonema camerunense* gen. et sp. n. (Araeolaimida: Diplopeltidae) from rain forest in Cameroon. *Russian Journal of Nematology* 11: 63-66.

Holovachov O., Boström S., Tandingan De Ley I., De Ley P. & Coomans A. 2003. Morphology and systematics of the genera *Wilsonema* Cobb, 1913, *Ereptonema* Anderson, 1966 and *Neotylocephalus* Ali, Farooqui & Tejpal, 1969 (Leptolaimina: Wilsonematinae). *Journal of Nematode Morphology and Systematics* 5 (2002): 73-106.

Holovachov O., Zullini A., Loof P.A.A., Bongers T. 2002. Morphology and systematics of the genus *Anonchus* Cobb, 1913 (Nematoda: Leptolaimina) and reappraisal of the family Aphanolaimidae Chitwood, 1936 n. rank. *Nematology* 4: 725-757.

Gagarin V.G. & Holovachov O.V. 2002. Redescription of *Theristus pannonicus* Andrássy, 1985 (Nematoda, Monhysterida) from the Ukraine. *Zoosystematica Rossica* 11: 85-89.

Tsaryk J., Gorban I., Holovachov O., Shydlovskyy I., Gorban L., Dykyy I., Lesnik V., Matejchyk V., Pudlyk J. & Senyk M. 2002. Ecological factors influencing biodiversity preservation in the Shatsk National Natural Park. *Acta Agrophysica* 67: 275-285.

Holovachov O. 2001. Study of Plectidae (Nematoda) from Ukraine. Variability of male sexual characters in *Plectus pusteri* Fuchs, 1930. *International Journal of Nematology* 11: 209-214.

Holovachov O., Boström S. & Susulovsky A. 2001. Study of Plectidae (Nematoda) from Ukraine. Decription of *Plectus paracuminatus* Zell, 1993 with observation of male sexual characters. *Journal of Nematode Morphology and Systematics* 4: 11-20.

Holovachov O., Boström S. & Susulovsky A. 2001. Description of *Acroukrainicus sagittiferus* gen. et sp. n. (Nematoda: Cephalobidae) from the area of an old sulfur industry in Ukraine. *Russian Journal of Nematology* 9: 51-56

Holovachov O. & De Ley P. 2001. Description of *Pseudacrobeles (Bunobus) bostromi* sp. n. (Rhabditida: Cephalobidae) from rotting wood in Roztochya, Ukraine. *Journal of Nematode Morphology and Systematics* 4: 21-29.

Holovachov O. 2001. Description of *Plectus (Ceratoplectus) brzeskii* sp. nov. (Nematoda: Plectidae) from New Caledonia. *Annales Zoologici* 51: 1-4.

Holovachov O., Boström S., Susulovsky A. & Nevo E. 2001. Description of *Cervidellus capricornis* sp. n. (Nematoda: Cephalobidae) from Israel. *Nematologia Mediterranea* 29: 223-230.

Susulovsky A., Boström S. & Holovachov O. 2001. Description of *Acromoldavicus skrjabini* (Nesterov & Lisetskaya, 1965) Nesterov, 1970 from Israel and the Ukraine, and redescription of *Kirjanovia discoidea* Ivanova, 1969 (Cephalobina, Elaphonematidae). *Journal of Nematode Morphology and Systematics* 3 (2000): 151-163.

Holovachov O. & Bongers T. 2001. Description of *Aspidonema scheucherae* (Sachs, 1949) Andrássy, 1958 (Nematoda: Bunonematidae) from Turkey. *Annales Zoologici* 51: 395-401.

Winiszewska G., Holovachov O. & Susulovsky A. 2001. *Dintheria tenuissima* de Man, 1921 and *Stenonchulus troglodytes* Schneider, 1940 (Nematoda: Bastianiidae and Onchulidae) – two nematode species new for the fauna of Ukraine and Poland. *Fragmenta Faunistica* 44: 169-177.

Gagarin V.G. & Holovachov O.V. 2001. The genus *Tridentulus* Eyualem & Coomans, 1995 with description of *Tridentulus palustris* sp. n. from the Ukraine (Nematoda: Monhysterida) and a key to the species. *Russian Journal of Nematology* 9: 113-117.

Boström S. & Holovachov O. 2001. Study of Teratocephalidae (Nematoda) from Ukraine. II. Description of a population of *Teratocephalus tenuis* Andrassy, 1958 with a discussion of male sexual characters. *Russian Journal of Nematology* 9: 69-76

Boström S., Holovachov O. & Susulovsky A. 2000. Study of Teratocephalidae (Nematoda) from Ukraine. Decription of a population of *Teratocephalus* de Man, 1876 with a compendium on species from the ”*lirellus*-group”. *Russian Journal of Nematology* 8: 139-145.

Holovachov O. & Susulovsky A. 2000. Study of Plectidae (Nematoda) from Ukraine. Description of two *Plectus* species with a discussion of some tail abnormalities. *Annales Zoologici* 50: 233-238.

Holovachov O., Susulovsky A. & Boström S. 2000. Description of two species of *Chiloplectus* Andrássy, 1984 (*Nematoda*: *Plectidae*) from Ukraine and a revised taxonomy of the genus. *Nematology* 2: 381-394.

Holovachov O.V. & Susulovsky A.S. 1999. Description of *Plectus cladinosus* sp. n. from Ukraine with notes on *P. longicaudatus* Butschli, 1873 and *P. decens* Andrassy, 1985 (Nematoda, Araeolaimida: Plectidae). *Zoosystematica Rossica* 8: 11-15.

Books and book chapters

Holovachov O. 2006. Nematodes. In: Tsaryk Y. (ed.) *Shatsk lakes: characterization of abiotic and biotic compounds of ecosystems*. Ivan Franko National University of Lviv, Lviv: **in press**. (In Ukrainian).

Holovachov O. & De Ley P. 2005. Order Plectida. In: Eyualem A., Andrássy, I. & Traunspurger, W. (ed.) *Freshwater nematodes: ecology and taxonomy.* CABI Publishing: **in press**.

Holovachov O. 2004. Nematodes (Nematoda). In: Zagorodniuk I. (ed.) *Cave fauna of Ukraine.* Kyiv: 42-44. (In Ukrainian).

Shydlovskyy I., Lysachuk T. & Holovachov O. (editors). 2002. *Western-Ukrainian ornithological station: fields and results of activity*. Lviv: 90 pp. (In Ukrainian).

Abstracts and short communications

Holovachov O.V. 2005. Posembryonic development in nematode family Plectidae (ordo Plectida). *Abstracts of the Sixth English Language International Symposium of the Russian Society of Nematologists, Russian Journal of Nematology* 13: **in press.**

Holovachov O.V. 2005. Fauna of soil and freshwater nematodes of Shatsk national natural park. *State and biodiversity of ecosystems of Shatsk national natural park*: 11-13 (in Ukrainian).

De Ley I.T., Holovachov O. & De Ley P. 2002. Scanning electron microscopy of the juvenile stages of some Wilsonematinae (Plectidae). *Symposium abstract, Nematology* 4: 244.

Holovachov O.V. & Susulovsky A.S. 1999. On Study of Cephalobids from Ukraine. *Problems of Nematology (Abstracts of the Third International Nematology Symposium), Proceedings of the Zoological Institute RAS* 280: 49.

Pavliuk R.S. & Holovachov O.V. 1999. First record of *Orthetrum anceps* (Odonata, Libellulidae) in Ukraine. *Vestnik Zoologii* 4-5: 44 (in Ukrainian).

Holovachov O.V. 1998. First record of *Plectus elegans* Zell, 1993 (Nematoda: Plectoidea) in Ukraine on the territory of Ukrainian Steppe Reserve. *Role of Protected Areas in Saving Biodiversity.,* Kaniv: 168-169 (in Ukrainian).

Holovachov O.V., Susulovsky A.S. 1997. The status of study of free-living soil nematodes of the family Plectidae in Ukraine. *Conservation and Biodiversity in Ukraine, ed. D.W.Minter.,* Egham-Kiev: 70.

Susulovsky A.S. & Holovachov A.V. 1997. *Chiloplectus* Andrassy, 1984 (*Nematoda*: *Plectida*) – new genus of nematodes for the fauna of Ukraine. *Naukovi Zapysky Derzhavnogo Pryrodoznavchogo muzeju NAN Ukrainy* 13: 87 (in Ukrainian).

Catalogues and reports

Shydlovskyy I.V. & Holovachov O.V. 2005. *Homopteran insects from the collection of E.-F.Germar in the Zoological museum of LNU (Catalogue).* Ivan Franko National University of Lviv, Lviv: VI+80 pp. (In Ukrainian).

Shydlovskyy I.V., Holovachov O.V., Lysachuk T.I. & Romanova Ch.Y. 2001. *Catalogue of the eggs and nests of birds of the Benedykt Dybowsky Zoological museum.* Ivan Franko National University of Lviv, Lviv: 66 pp. (In Ukrainian).

Tsaryk Y.V., Shydlovskyy I.V., Holovachov O.V., Lysachuk T.I., Romanova Ch.Y., Paslavska T.M., Yedynak G.Z., Pavluk R.S. & Vozniuk M.N. 2000. *Catalogue of rare and Red Data Book species of animals of the Zoological museum collections. Insecta, Pisces, Amphibia, Reptilia, Aves, Theria.* Ivan Franko National University of Lviv, Lviv: 60 pp. (In Ukrainian).

Shydlovskyy I.V., Lysachuk T.I. & Holovachov O.V. 1999. *Western-Ukrainian ornithological station. Report on the results of five-year activity: 1995-1999.* “Spolom”, L’viv: 32 pp.

PE&RC PhD Education Statement Form

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 22 credits (= 32 ECTS = 22 weeks of activities)

Review of Literature (4 credits)
- Present state of knowledge on the biology of the order Plectida Malakhov, 1982 (2001-2005)

Writing of Project Proposal (3 credits)
- Towards a better understanding of the sytematics of Plectida (Morphology and systematics of the order Plectida Malakhov, 1982) (2001)

Post-Graduate Courses (5 credits)
- Nematode identification course (2001)
- Post-graduate course in computer science (2003)

Deficiency, Refresh, Brush-up and General Courses (4 credits)
- General techniques in nematology (2001)
- Advanced course in English language (2002)

PhD Discussion Groups (3 credits)
- Seminars at the Department of Fauna and Systematics of Invertebrates, I.Shmalhausen Institute of Zoology, Kyiv, Ukraine (2005)

PE&RC Annual Meetings, Seminars and Introduction Days (3 credits)
- Annual meetings of the Ivan Franko University of Lviv, Zoology section (2001-2004)
- Seminars of the Department of Zoology, Ivan Franko University of Lviv (2001-2004)
- Stanislav Lem Workshop on Evolution (2005)

International Symposia, Workshops and Conferences (4.2 credits)
- IX International Theriological Workshop “Methods of study of mammal fauna”, Ukrainian Theriol. Soc. (2002)
- Fourth International Nematology congress, Tenerife. Eur. Soc. Nem. (2004)
- 6th English language international symposium of the Russian society of Nematologists, Russ. Soc. Nem. (2005)
- State and biodiversity of ecosystems of Shatsk national natural park Lviv Univ. (2005)

Laboratory Training and Working Visits (3.2 credits)
- Laboratory of Nematology, Gent University, Belgium (discussion) (2001)
- Museum and Institute of Zoology, Warszawa, Poland (discussion and study of type material) (2001, 2002, 2004)
- Institut für Nematologie und Wirbeltierkunde, Münster, Germany (discussion and study of type material) (2001, 2004)

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Bibliographic abstract:

This thesis includes a detailed light and electron microscopic study of morphology and postembryonic development; taxonomic revision; analysis of phylogenetic relationships of selected marine, freshwater and soil-inhabiting representatives of the nematode order Plectida. The new interpretation of morphology and homology of the labial region, stoma, pharynx, female and male reproductive systems has provided a basis for the newly proposed phylogeny and classification of the taxon in question. The discussion of possible patterns of the evolution of freshwater and terrestrial members of the order Plectida and their morphological adaptations associated with radiation including newly exploited environments is discussed.

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Anterior end of a nematode *Plectus* sp. as seen under the light microscope, courtesy of P. De Ley and M. Yoder (University of California Riverside, USA), design by O. Holovachov

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