

# A strategy in searching for stress tolerance-correlated characteristics in nematodes while accounting for phylogenetic interdependence

### Nematology

Holterman, M.H.M.; Korthals, G.W.; Doroszuk, A.; van Megen, H.H.B.; Bakker, J. et al <u>https://doi.org/10.1163/138855410X518461</u>

This publication is made publicly available in the institutional repository of Wageningen University and Research, under the terms of article 25fa of the Dutch Copyright Act, also known as the Amendment Taverne.

Article 25fa states that the author of a short scientific work funded either wholly or partially by Dutch public funds is entitled to make that work publicly available for no consideration following a reasonable period of time after the work was first published, provided that clear reference is made to the source of the first publication of the work.

This publication is distributed using the principles as determined in the Association of Universities in the Netherlands (VSNU) 'Article 25fa implementation' project. According to these principles research outputs of researchers employed by Dutch Universities that comply with the legal requirements of Article 25fa of the Dutch Copyright Act are distributed online and free of cost or other barriers in institutional repositories. Research outputs are distributed six months after their first online publication in the original published version and with proper attribution to the source of the original publication.

You are permitted to download and use the publication for personal purposes. All rights remain with the author(s) and / or copyright owner(s) of this work. Any use of the publication or parts of it other than authorised under article 25fa of the Dutch Copyright act is prohibited. Wageningen University & Research and the author(s) of this publication shall not be held responsible or liable for any damages resulting from your (re)use of this publication.

For questions regarding the public availability of this publication please contact  $\underline{openaccess.library@wur.nl}$ 

# A strategy in searching for stress tolerance-correlated characteristics in nematodes while accounting for phylogenetic interdependence

Martijn H.M. HOLTERMAN<sup>1,\*</sup>, Gerard W. KORTHALS<sup>2</sup>, Agnieszka DOROSZUK<sup>3</sup>, Hanny H.B. VAN MEGEN<sup>4</sup>, Jaap BAKKER<sup>4</sup>, Tom BONGERS<sup>4</sup>, Johannes HELDER<sup>4</sup> and Andre VAN DER WURFF<sup>5</sup>

 <sup>1</sup>Agroscope Changins-Waedenswil, Nematologie, Schloss, 8820 Waedenswil, Switzerland
 <sup>2</sup>Praktijkonderzoek Plant & Omgeving B.V. Sector AGV, Edelhertweg 1, Lelystad, The Netherlands
 <sup>3</sup>Evolutionary Biology, Institute of Biology Leiden, Kaiserstraat 63, 2311 GP Leiden, The Netherlands
 <sup>4</sup>Laboratory of Nematology, Department of Plant Sciences, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands
 <sup>5</sup>Wageningen UR Glastuinbouw, Violierenweg 1, 2665 MV Bleiswijk, The Netherlands

> Received: 4 May 2010; revised: 4 June 2010 Accepted for publication: 9 June 2010

Summary – Biological indicators are highly relevant for assessing the condition of a soil as they are integrative; they reflect the overall impact of physical, chemical and biological changes. Indigenous soil organisms are preferable to other test organisms because the diversity and condition of indigenous soil organisms reflect both acute and chronic effects of soil disturbances. Nematodes are ubiquitous, speciose, easily extractable and present in extremely high numbers. Given the ever increasing amount of sequence data, DNA barcode-based community analysis will soon be possible and a next step would be to define objective criteria for the ecological grouping of soil nematodes. Here, we present a framework to ascertain which traits are correlated with a tolerance to stress. For this, a field study on the effects of pH and copper on nematode communities was re-analysed. Changes in abundances of individual genera were correlated with a number of potentially stress tolerance-related characteristics. The generalised least squares (GLS) method was used to account for the phylogenetic dependence of the data. Only the relationship between the ability to enter a survival stage and tolerance to copper at pH 6.1 was found to be significant, but the quantity of missing data probably had a negative impact on the analyses. This study did, however, clearly demonstrate the importance of accounting for the effects of phylogenetic dependence in the data. When the phylogeny was taken into account, we observed an average change in P value of 0.196 (and in some cases as much as 0.6) for the correlations of possible stress-related characteristics and Cu or pH tolerance. This research constitutes a proof-of-principle for a transparent method to relate stress tolerance to (ecological) characteristics. The usefulness of this powerful method should become even clearer when substantially higher numbers of individuals are analysed (as facilitated by using DNA barcodes) and when missing data are filled in.

Keywords - comparative method, interdependence, maturity index, nematodes, phylogenetic stress tolerance.

Because of their abundance and their (trophic) diversity, nematodes occupy important positions in the soil foodweb (De Ruiter *et al.*, 1998). They also display a great variability in sensitivity to environmental stress (Bongers, 1990). As such, the composition of nematode communities is considered to be an informative indicator for soil health. However, little is known about biological characteristics that underlie the very wide variation in stress (in)tolerance. Here, we propose a strategy to investigate what traits are correlated with stress tolerance in nema-todes.

The monitoring of nematode communities as indicators for soil health conditions is widely applied. However, sample size and taxonomic resolution are currently dictated by practical limitations (and are respectively smaller and lower than desired). The morphology of nematodes is

<sup>\*</sup> Corresponding author, e-mail: martijn.holterman@acw.admin.ch

relatively conserved and, therefore, community analysis is time-consuming and requires ample expert knowledge. Recent advances in the use of molecular characteristics for the analysis of nematode communities may remove (in part) these practical obstacles (Holterman *et al.*, 2008).

Several indices can be used to describe community diversity, such as the Shannon-Wiener index (Shannon, 1948), Simpson's diversity index (Simpson, 1949) or the species richness index (S; the number of species present in an ecosystem). An important characteristic of the Maturity Index (MI) as proposed by Bongers (1990) is the inclusion of ecological characteristics, and, over the last decade, this MI has been widely used for nematode community analyses. The Maturity Index assigns each nematode family a so-called cp-value. This 'coloniserpersister' scale ranges from 1 to 5 and corresponds roughly to r-K strategies, with values of 1 and 2 being assigned to the most tolerant r-strategists and values of 4 and 5 to the most sensitive K-strategists. Families assigned to cp-class 1 are enrichment opportunists and, while they can outlast periods of adverse conditions, they are mainly found in food-rich conditions. For this reason they are often excluded from the MI (Bongers, 1999). The MI was demonstrated to be an effective means for assessing and quantifying the impact of soil pollution on nematode communities (Bongers & Ferris, 1999). However, families were assigned to cp-classes mainly on the basis of expert knowledge and some general observations (Bongers, 1999), and there are few clear criteria for each cp-class. In addition, it is known that genera in a single family do not always react similarly to stress (Ettema & Bongers, 1993) and, for some nematode families, a refinement of the MI down to genus, or even species, level would be appropriate. Identification of traits correlated with specific forms of stress tolerance would be helpful for the refinement of the MI or related indices.

When looking for correlations between traits using data from different species, it is important to realise that one of the basic assumptions of the comparative method, *i.e.*, the data are independent, is often violated (Felsenstein, 1985). If a strong phylogenetic correlation exists in the data, it is important to take this into account. Felsenstein designed a method, named phylogenetically independent contrasts, which takes these confounding effects into account (Felsenstein, 1985). However, this method may overestimate the effects of phylogeny (Harvey & Rambaut, 2000). Another (intermediate) approach was proposed by Pagel (1999): the generalised least squares (GLS) method.

To study traits correlated with stress tolerance in nematodes we focused on the effects of copper and pH on nematode communities. The traits involved in this study are body size, reproductive potential, (a)sexual reproduction, feeding type, the ability to form survival stages and cuticle permeability. Body size is an important life history trait which correlates with many processes (Peters, 1983). Reproduction can be expected to play a role in stress tolerance since a high reproduction rate could partially compensate for increased mortality and enhance population recovery from the stress. It is noted that stress tolerant nematodes are often small. have short generation times and produce large numbers of offspring (Bongers, 1999). Unfortunately, generation times and number of offspring are not determinable for most nematodes, since most nematodes cannot be cultured or are barely culturable. Therefore, we looked to the gonad size relative to body size as an alternative measure for fecundity. To the best of our knowledge this has never been done for nematodes, but it has been done in various other animals, such as tardigrades (Guidetti et al., 2007), flat worms (Schärer et al., 2005) and tree frogs (Rodrigues et al., 2005, 2007). Asexual reproduction is often considered a trait for opportunists but this does not necessarily seem to be the case for nematodes (Bongers, 1999). Feeding type could also be related to stress tolerance. In the MI, most bacterial feeders belong to low cp-classes while members of cp-classes 4 and 5 are often predators or omnivores (Bongers, 1999). Entering a survival stage can help to outlast long periods of stress and, finally, changing the cuticle permeability could be an expedient to prevent pollutants from entering the body.

Until now, nematode families have been assigned to cp-classes for the Maturity Index on the basis of expert knowledge. Identifying traits which are correlated with a tolerance to stress is a first step towards defining clear objective criteria for the assigning of nematode taxa to cpclasses. Furthermore, having clear criteria will allow for the refinement of the MI from a family to a genus level index. In this study we set out a framework to identify traits important to stress tolerance. We will demonstrate the importance of taking the effects of phylogeny on the data into account. Finally, the effectiveness of our framework to identify relevant traits will be discussed.

## Materials and methods

# NEMATODE COMMUNITY DATA FOR COPPER AND PH STRESS

To determine the tolerance of several nematode genera to copper and pH stress, data were used from a field study on the long-term effects of copper and pH on nematode communities (Korthals et al., 1996). The study site, known as the Bovenbuurt pastures, is located approximately 3 km NNE of Wageningen, The Netherlands. The field soil is slightly loamy sand. After having been used as a permanent pasture for at least 30 years, in 1978 a crop rotation scheme of silage maize, starch potatoes and oats was applied. In 1982 the field was divided into 128 plots of  $6 \times 11$  m. Four copper levels were created by applying  $CuSO_4 \cdot 5H_2O$  at rates of 0, 250, 500 and 750 kg Cu ha<sup>-1</sup>. Levels of pH were adjusted to 4.0, 4.7, 5.4 or 6.1. This resulted in 16 different treatments with eight replicates per treatment. In March 1992 samples were taken from each plot. A part of the sample was used to determine the pH (pH-KCl) and to determine the total quantity of copper present (Cu-HNO<sub>3</sub>) and the available copper concentration (Cu-CaCl<sub>2</sub>). Nematodes were extracted from another part of the soil sample. Nematodes were counted under a dissecting microscope and, after fixation in formalin, at least 150 nematodes were identified using a light microscope. For full details of field history, soil composition, sampling, pH determination and copper extraction we refer to Korthals et al. (1996).

### REDUNDANCY ANALYSIS

In order to investigate the change in abundance of genera related to pH or copper treatment, a detrended correspondence analysis (DCA) was performed (Ter Braak, 1995). Detrended correspondence analyses by segments revealed a gradient of 1.75 standard deviation units for pH and gradients of 1.53, 1.52, 1.33 and 1.65 for Cu at pH levels of 4.0, 4.7, 5.4 and 6.1, respectively, indicating a strong linear response of taxa. Therefore, a redundancy analysis (RDA) was performed (Ter Braak, 1995). RDA is a constrained version of a principal component analysis (PCA), meaning that it focuses on the part of the variance explained by the environmental variables only.

RDA analysis was performed on a matrix of 128 samples (eight replicates per treatment) and 71 species using CANOCO for Windows 4.5 (Ter Braak & Smilauer, 2002). Nematode abundance data were  $\ln(2x + 1)$  transformed to down-weight high values and approximate a

normal distribution. For the pH and Cu values the true pH (pH-KCl) and the available Cu-concentration (Cu-CaCl<sub>2</sub>) were used. Five RDA were performed. One RDA was to determine the tolerance to pH; for this all the plots with a Cu-treatment were excluded from the analysis. To determine the tolerance to Cu, a separate RDA was performed for each pH-level (4.0, 4.7, 5.3, 6.1), excluding the samples for the other pH levels. The values of the first canonical axis were used as a measure for the tolerance of a genus to the stressor (pH or Cu). A strongly positive or negative value indicated that the variation in abundance was strongly explained by the pH or Cu treatment, *i.e.*, the genus under consideration was strongly affected. A value close to zero indicated that abundance was not well explained by the treatment, *i.e.*, the genus is tolerant.

The redundancy analyses (RDA) were followed by unrestricted Monte Carlo permutation (MC) tests with 499 permutations to assess the significance of the relation between each environmental variable and community composition (Verdonschot & Braak, 1994).

#### NEMATODE TRAITS

Six nematode traits were studied for their possible correlation with tolerance to copper and pH stress. These were adult body size, relative gonad size, (a)sexual reproduction, cuticle permeability, feeding type and the ability to form a survival stage. Wherever possible, only data from species occurring in The Netherlands was used. The cp-values for the maturity index were taken from Bongers (1999).

Adult body size was determined using Andrássy's (1956) formula, using body length and width taken from Bongers (1994). A correction for tail shape was applied as described in Van der Wurff et al. (2007). Body size data was log 10-transformed to approximate a normal distribution of the data. Feeding types were according to Yeates et al. (1993). Survival stages (the ability to form dauer juveniles or resistant stages) were according to Bongers (1999). Reproduction type was based on whether a genus can reproduce asexually or not; no distinction was made between genera that can only reproduce asexually and genera that can reproduce both sexually and asexually. Most data came from Bongers (1994) and Lorenzen (1994), with additional data from the literature where required. When the mode of reproduction was not directly known the presence of males was used as a guideline. If males were unknown or rare, a species was supposed to be able to reproduce asexually. If both sexually and asexually reproducing species were present in a genus, the genus was scored as being able to reproduce asexually.

To assess the permeability of the cuticle, live nematodes were coloured with different staining agents. In a preliminary experiment (results not shown), aqueous solutions of six staining agents - Coomassie R (Merck, Whitehouse Station, NJ, USA), Coomassie G (Merck), Erytrosin B (Kodak, Hemel Hempstead, UK), Trypan blue (Merck), ponceau sodium (Merck) and acid fuchsin (Merck) - were tested with a taxonomically diverse number of species. Three staining agents were selected, one which stained many nematodes (Erytrosin B), one which only stained the nematodes with the most permeable cuticles (Coomassie R) and one which was intermediate between these two (Trypan blue). Living nematodes were transferred to a small well with one drop of staining agent diluted with demineralised water. If only natural openings were stained, such as the mouth cavity or the amphids, the nematode was scored as non-stained. Testing was done on nematodes isolated directly from environmental samples, and, whenever possible, at least five individuals were used for each nematode-stain combination. It is noted that due to the low abundance of some genera, not every combination could be (fully) tested.

To determine the relative gonad size, pictures were taken from slides in the collection at the Laboratory of Nematology (Wageningen University) using a light microscope (Zeiss Axioscope) equipped with differential interference contrast optics and a CCD camera (CoolSnap, RS Photometrics, Tucson, AZ, USA). When available, pictures from multiple species per genus were taken. For each species six individuals were used. The area of the total nematode and the gonad was measured using the program Image-Pro Express 4.0 (Media Cybernetics, Bethesda, MD, USA). Relative gonad size was expressed as the proportion of the total area of the nematode that was occupied by the gonads. Relative gonad size data were normally distributed.

#### PHYLOGENETIC TREE

Based on previous analyses (Holterman *et al.*, 2006, 2008) a user-tree was defined (Fig. 1). Each genus was represented by one full length small subunit ribosomal DNA (SSU rDNA) sequence (GenBank accessions in Appendix A). The Rhabditidae were represented by ten species to represent the large variation present in the family. The Diplogastridae were represented by four sequences. The alignment was created by ClustalW as applied in BioEdit v7.0.1 (Hall, 1999) and improved

manually using arthropod secondary structure information (http://bioinformatics.psb.ugent.be/webtools/rRNA/ secmodel/index.html in accordance with Wuyts *et al.*, 2000). MrBayes 3.1.2 was used to calculate branch lengths. The alignment was divided into a stem and loop partition and the GTR model with invariable sites and gamma correction was used. Parameter values were unlinked between partitions and the rate prior was set to variable. The topology of the user tree was fixed. The analysis was run for 3 million generations using four independent runs and four chains per run. The 'burnin' was 1 200 000 generations. For the Rhabditidae and Diplogastridae the average branch length was calculated.

#### COMPARATIVE ANALYSIS

For the comparative analysis the program Continuous v.1.0d13 (Pagel, 1997, 1999) was used. Dummy variables were used for the feeding types. To maximise the amount of included data (species with missing data in a trait under analysis are excluded), RDA values and traits were analysed pair-wise and a Bonferroni correction was applied to allow for the effects of multiple pair-wise testing. A standard constant variance random walk model of evolution was used. The parameter  $\lambda$  reveals if the data are predicted by the phylogeny and can be estimated by Continuous. When  $\lambda = 0$ , Continuous assumes the trait is evolving independent of phylogeny, if  $\lambda = 1$ , Continuous assumes there is a full correlation between the phylogeny and data (in effect it is using independent contrasts; Felsenstein, 1985). Likelihood ratio tests were performed to test if the value of  $\lambda$  estimated by likelihood was significantly different from 1 or 0 for each pair of RDA values and traits. Next a likelihood ratio test was performed to test if a RDA-value and a trait were significantly correlated by constraining the covariances to zero for the H<sub>0</sub>. This was done using a  $\lambda$  estimated by Continuous and a  $\lambda$  set to zero to study the effect of using a phylogenetic correction.

#### **Results and discussion**

We have set up a framework to investigate if certain nematode traits – body size, reproductive potential, feeding type, survival stage, asexual reproduction and cuticle permeability – are correlated with tolerance for Cu or pH stress. Furthermore, the importance of allowing for the phylogenetic non-independence of the data in the analy-



Fig. 1. Phylogenetic tree of taxa involved in this study. Topology was based on Holterman et al. (2006) and Holterman et al. (2008); branch lengths were calculated with MrBayes 3.1.2. Feeding types and the ability to enter a survival stage are indicated on the tree.

sis was studied. The results will be discussed in the light of improving the MI.

#### NEMATODE RESPONSES

The nematode sensitivity analyses were performed on natural communities, instead of laboratory assays, to mimic a natural response towards stressors. The field site was suitable for this, because the soil was homogenous with respect to other important soil parameters besides pH and copper, such as organic matter and percentage of small ( $<2 \mu m$ ) soil particles (Korthals *et al.*, 1996). Although a multivariate test is not required for testing an (in principle) univariate experimental setup, we choose to use RDA since its use is extremely powerful in natural field conditions, *i.e.*, a soil dominated by multiple (co-)factors. In addition, abundances are patchy by nature and especially disturbance-sensitive groups, such as predators and omnivores typically have low abundances. Furthermore, only a relatively low number of individuals were analysed per sample.

The values of the first canonical axis of the RDA analyses are presented in Table 1 together with the data for the nematode traits. If the RDA values are positive, it means there is a positive correlation between abundance and the stress factor (pH or Cu), whereas a negative value means there is a negative correlation. RDA values close to zero (between -0.5 and 0.5) are considered not to be correlated with the treatment, *i.e.*, the species are tolerant to the stress. RDA values generally display a positive relationship with cp-values in the case of pH (a higher more neutral pH being the less stressed condition) and a negative relationship with copper (Table 1; Fig. 2). This supports the general use of cp-values at the taxonomic level of family. However, differing RDA values for species within the families Cephalobidae and Pratylenchidae show that a family-based cp-value is not always justified (Table 1). This is in accordance with earlier findings that some genera in a family may not always behave as would be expected from their cp-values (Ettema & Bongers, 1993). One of the genera Ettema and Bongers mentioned as being more abundant than expected was Aporcelaimellus, which in our analyses displays RDA values close to zero and therefore is more tolerant than its cp-value of 5 would suggest.

#### PHYLOGENETIC DEPENDENCE

As can be seen from the results (Table 2) many traits are not phylogenetically independent ( $\lambda$  significantly larger

than zero). At the same time, most traits do not evolve entirely according to the tree topology either;  $\lambda$  is significantly smaller than 1 in most cases. This means that the generalised least squares (GLS) method as applied by the program Continuous is ideally suited to investigate the data for correlations between traits. A method such as independent contrasts (Felsenstein, 1985) would overestimate the effect of phylogeny (in effect it assumes that  $\lambda$ is 1), while the GLS method can take an intermediate approach between independent contrasts and not accounting for phylogenetic dependence at all. The importance of using a correct method for accounting for the effects of phylogeny was also demonstrated by other studies (Martins *et al.*, 2002; Stuart-Fox, 2009).

A phylogenetic dependence of the data can easily be visualised. For example, bacterial feeding has a very high  $\lambda$  in all cases. In the tree, almost all bacterial feeders cluster together (Fig. 1). If, by coincidence, the ancestor of these bacterial feeders also happened to be tolerant to stress, it is very possible that its descendants inherited both of these traits even if no actual physical relation between the two traits exists. An analysis of these traits without accounting for their shared ancestry would thus probably show the two traits to be correlated regardless of whether an actual relation between the two exists or not. If, on the other hand, a certain trait is spread over the tree, such as hyphal feeding (Fig. 1;  $\lambda$  is 0 or very close to 0 in all cases), shared ancestry is unlikely to play a role in the correlation of this trait with other traits.

# CORRELATIONS BETWEEN TRAITS AND STRESS TOLERANCE

Correlations between stress tolerance (as represented by RDA values) and nematode traits were tested in a pairwise fashion. To demonstrate the effect of accounting for phylogeny, the same tests were performed using the maximum likelihood estimate for  $\lambda$  in one case and constraining  $\lambda$  to 0 in the other case. After applying a Bonferroni correction only a single significant correlation was found (Table 2). This was between the ability to enter a survival stage and the RDA for copper at pH 6.1 and not using a phylogenetic correction. Given that, in this case,  $\lambda$  was not significantly different from zero (P =  $2.44 \times 10^{-4}$ ;  $\lambda = 0.12$ ), this could be considered a valid result. It certainly seems logical that the ability to enter a survival stage and survive periods of adverse conditions is correlated with a tolerance to stress. Although there are more correlations with a low P value (survival stage

Genus Genus	<i>re atfjerent nematoae t</i> Family	ratts a	na the re loo	Sturs of L Relative	Teeding	Survival	Renro-	Connassie	Ervtrosin	Trvnan	RDA	RDA	RDA	RDA	RDA
	frame a	-T- Violuo	aor Podu	anna	timo	otoco	duction	D	D	hino ould		2	Ż	ł	į
		value	ouuy eize	guilau	rype	stage	nuction	۲	٩	onro	ud	יים		יים	יים
			$(\mu m^3)$	2716							-0.+ 6.1	пч 1.0	пц 7.4	рп 5.4	гц 6.1
Cephalenchus	Tylodoridae	*	5.04	0.15	ЪР	*	Asexual	*	*	*	*	*	*	*	*
Psilenchus	Psilenchidae	*	5.4	0.22	Ъ	*	Sexual	I	Ι	Ι	*	*	*	*	0.091
Panagrolaimus	Panagrolaimidae	-	5.8	0.28	BF	Yes	Asexual	*	*	I	0.363	-0.12	-0.33	-0.12	0.254
Rhabditis	Rhabditidae	-	5.87	0.31	BF	Yes	Asexual	*	Ι	Ι	-0.07	-0.12	0.149	0.278	0.163
Diplogaster	Diplogastridae	-	5.74	0.36	0	Yes	Sexual	*	I	+	-0.24	-0.21	*	*	0.194
Pristionchus	Neodiplogastridae	-	6.66	0.29	Р	*	Sexual	*	I	Ι	-0.01	-0.22	-0.16	0.348	0.407
A crobeloides	Cephalobidae	0	5.17	0.36	BF	No	Asexual	*	I	I	0.256	-0.75	-0.6	-0.6	0.279
Cephalobus	Cephalobidae	0	5.57	0.28	BF	No	Sexual	*	*	Ι	0.524	-0.16	0.104	-0.13	0.345
Heterocephalobus	Cephalobidae	0	5.43	0.26	BF	No	Asexual	*	+	I	-0.2	-0.16	*	0.278	0.203
Eucephalobus	Cephalobidae	0	5.33	0.31	BF	No	Sexual	*	+	Ι	0.381	0.038	-0.13	0.157	0.205
Chiloplacus	Cephalobidae	0	5.86	0.26	BF	No	Asexual	*	I	Ι	-0.33	0.158	0.093	0.513	0.595
Acrobeles	Cephalobidae	0	5.76	0.3	BF	No	Sexual	*	+	Ι	0.743	-0.28	-0.45	-0.8	-0.74
Cervidellus	Cephalobidae	0	5.03	0.29	BF	No	Asexual	*	Ι	+	0.342	-0.61	-0.41	-0.64	-0.55
Drilocephalobus	Osstellidae	0	4.97	0.35	BF	*	Sexual	*	Ι	*	-0.24	-0.56	-0.34	-0.03	0.032
Plectus	Plectidae	0	5.82	0.2	BF	No	Asexual	*	Ι	+	-0.21	-0.64	-0.77	0.183	-0.24
Anaplectus	Plectidae	0	6.19	0.26	BF	No	Sexual	*	Ι	+	0.248	-0.28	-0.09	0.016	-0.34
Eumonhystera	Monhysteridae	0	5.2	0.21	BF	No	Asexual	+	+	+	*	*	*	*	-0.12
Monhystera	Monhysteridae	0	5.86	0.35	BF	No	Sexual	*	*	*	0.367	*	-0.07	-0.06	-0.31
Ditylenchus	Anguinidae	0	5.78	0.31	HF, PP	Yes	Sexual	Ι	I	Ι	0.163	-0.32	-0.2	0.228	0.313
Pseudhalenchus	Anguinidae	0	4.59	0.26	HF	Yes	Sexual	*	*	*	-0.49	-0.25	0.077	0.412	0.231
Nothotylenchus	Anguinidae	0	5.59	0.3	HF	Yes	Sexual	*	*	*	0.13	-0.02	0.04	0.391	0.319
Deladenus	Neotylenchidae	0	5.57	0.42	HF	Yes	Asexual	*	*	*	*	0.355	*	-0.09	*
Aphelenchus	Aphelenchidae	0	5.37	0.25	HF	No	Asexual	*	Ι	Ι	0.771	-0.04	-0.07	-0.24	0.117
Filenchus	Tylenchidae	0	4.54	0.17	HF, PP	No	Asexual	I	Ι	Ι	0.089	-0.5	-0.27	0.187	-0.09
Bursaphelenchus	Parasitaphelenchidae	0	5.51	*	HF, PP	Yes	Sexual	*	*	*	*	*	*	*	*
A phelenchoides	Aphelenchoididae	0	5.2	0.21	HF, PP	Yes	Sexual	I	*	*	-0.62	-0.28	-0.03	0.557	0.645
Seinura	Seinuridae	0	5.12	0.21	Р	Yes	Sexual	*	*	*	*	0.279	0.456	*	*
Paratylenchus	Paratylenchidae	0	4.71	0.23	ЪР	Yes	Asexual	I	I	I	-0.06	*	0.058	-0.18	0.155
Ecphyadophora	Ecphyadophoridae	0	4.15	0.14	ЪР	*	Sexual	*	*	*	-0.09	*	-0.14	0.235	*
Malenchus	Tylenchidae	0	4.75	0.21	ЪР	No	Asexual	I	+	I	*	*	*	*	*
Boleodorus	Tylenchidae	0	4.91	0.17	ЪР	No	Sexual	*	*	*	0.309	*	*	-0.06	-0.35
Lelenchus	Tylenchidae	0	4.45	0.18	ЪР	No	Sexual	*	*	*	-0.19	-0.25	0.163	-0.13	-0.05
Coslenchus	Tylenchidae	0	4.81	0.23	ЪР	No	Asexual	*	I	*	0.155	*	*	*	-0.24
Tylenchus	Tylenchidae	0	5.53	0.16	Ъ	No	Sexual	I	Ι	Ι	0.324	-0.03	-0.23	-0.38	-0.01
A chromadora	Achromadoridae	ς	5.18	0.21	*	No	Asexual	I	+	+	0.008	-0.19	-0.06	*	-0.26
Teratocephalus	Teratocephalidae	ŝ	4.89	0.09	BF	No	Asexual	*	Ι	Ι	0.077	*	*	-0.15	0.091
Euteratocephalus	Metateratocephalidae	ŝ	5.39	0.11	BF	No	Asexual	+	*	*	*	*	*	*	*
Metateratocephalus	Metateratocephalidae	ŝ	4.82	0.19	BF	No	Asexual	I	*	*	-0.07	*	-0.16	*	*
Cylindrolaimus	Cylindrolaimidae	ŝ	5.52	0.24	BF	*	Asexual	I	+	*	0.27	*	-0.1	*	-0.09
Prismatolaimus	Prismatolaimidae	ŝ	5.04	0.12	BF	No	Asexual	+	+	+	0.339	-0.12	-0.24	0.077	0.068
Bastiania	Bastianiidae	3	4.98	0.15	BF	No	Asexual	I	Ι	I	0.215	*	-0.16	-0.07	-0.23

M.H.M.	Holterman	et	al.
--------	-----------	----	-----

Table 1. (Continued)															
Genus	Family	cb	log	Relative	Feeding	Survival	Repro-	Coomassie	Erytrosin	Trypan	RDA	RDA	RDA	RDA	RDA
		value	body	gonad	type	stage	duction	R	В	blue	μd	Cu	Cu	Cu	Cu
			size	size							4.0-	Ηd	Ηd	Hd	Ηd
			$(\mu m^3)$								6.1	4.0	4.7	5.4	6.1
Diphtherophora	Diphtherophoridae	ŝ	5.73	0.27	HF	No	Sexual	+	+	+	0.533	0.168	-0.52	-0.45	-0.34
Tripyla	Tripylidae	с	6.14	0.31	Р	No	Sexual	*	*	*	*	*	-0.01	*	*
Meloidogyne	Meloidogynidae	б	5.56	*	ЪР	Yes	Asexual	Ι	I	Ι	*	*	0.152	0.24	*
Pratylenchoides	Pratylenchidae	З	5.61	*	ΡP	No	Sexual	*	*	*	*	0.244	*	*	*
Pratylenchus	Pratylenchidae	б	5.07	0.14	ΡP	No	Asexual	Ι	I	I	0.254	-0.81	-0.8	-0.3	-0.16
Zygotylenchus	Pratylenchidae	З	5.12	*	РР	No	Sexual	*	*	*	-0.13	*	-0.15	-0.31	*
Helicotylenchus	Hoplolaimidae	б	5.44	0.19	ΡP	No	Asexual	Ι	I	Ι	0.059	-0.19	-0.26	-0.11	-0.27
Rotylenchus	Hoplolaimidae	б	5.68	0.13	ЪР	No	Sexual	Ι	I	I	-0.13	-0.11	-0.14	-0.07	-0.17
Bitylenchus	Telotylenchidae	с	5.6	0.23	ΡP	No	Asexual	Ι	Ι	I	-0.05	-0.32	-0.53	-0.09	0.035
Neodolichorhynchus	Telotylenchidae	ŝ	5.88	0.19	ΡP	No	Sexual	*	*	*	0.203	*	*	*	-0.19
Merlinius	Telotylenchidae	ю	5.21	0.22	ЪР	No	Sexual	I	I	I	0.542	-0.42	-0.32	-0.57	0-
Alaimus	Alaimidae	4	5.56	0.06	BF	No	Asexual	+	+	+	0.389	*	-0.18	-0.29	-0.44
Paramphidelus	Alaimidae	4	5.27	*	BF	No	Asexual	*	+	*	-0.06	*	*	-0.04	-0.08
Tylencholaimus	Tylencholaimidae	4	5.51	0.12	HF	No	Asexual	I	*	+	0.017	-0.3	-0.22	-0.43	-0.16
Mesodorylaimus	Dorylaimidae	4	6.33	0.29	0	No	Sexual	*	*	+	0.39	*	*	-0.24	-0.37
Prodorylaimus	Dorylaimidae	4	6.33	0.16	0	No	Asexual	*	*	+	0.39	*	*	-0.24	-0.37
Eudorylaimus	Qudsianematidae	4	6.42	0.16	0	No	Sexual	+	+	+	0.064	-0.19	0.124	-0.19	-0.11
Thonus	Qudsianematidae	4	6.23	0.19	0	No	Asexual	+	+	I	0.155	-0.21	-0.17	-0.04	-0.22
Enchodelus	Nordiidae	4	6.63	0.16	0	No	Asexual	*	*	*	*	*	*	*	*
Dorylaimoides	Mydonomidae	4	5.83	0.2	0	No	Asexual	*	*	*	0.203	*	*	*	-0.19
Thornia	Thorniidae	4	5.92	*	0	No	Sexual	*	*	*	*	*	*	*	*
Clarkus	Mononchidae	4	6.13	0.2	Р	No	Asexual	+	+	+	0.33	*	*	*	-0.36
Prionchulus	Mononchidae	4	6.81	0.23	Р	No	Asexual	Ι	+	+	*	*	-0.08	*	*
Coomansus	Mononchidae	4	6.35	0.38	Р	No	Asexual	Ι	+	*	-0.03	-0.2	-0.4	-0.31	-0.07
Trichodorus	Trichodoridae	4	5.74	0.16	РР	No	Sexual	*	+	Ι	0.355	-0.11	-0.43	-0.62	-0.35
Paratrichodorus	Trichodoridae	4	5.9	0.27	ΡP	No	Asexual	+	+	Ι	*	*	*	*	*
A porcelaimellus	Aporcelaimidae	S	6.9	0.18	0	No	Asexual	I	+	I	0.182	-0.12	-0.37	-0.24	-0.1
Nygolaimus	Nygolaimidae	5	5.84	0.18	Ρ	No	Asexual	+	+	+	0.155	*	-0.1	*	-0.28
BF = bacterial feede	er; $HF = hyphal fee$	der; Pl	P = pla	nt parasi	te; $0 = 0$	mnivore; I	P = preda	tor. Asexua	l means as	exual rep	roductio	n is pos	sible but	not nec	essarily
the main mode of rep	production.														
* Missing data.															



Fig. 2. RDA values plotted against cp values with trend lines.

with other RDAs, cuticle permeability) these are all insignificant because of the large Bonferroni correction that had to be applied, due to the pair-wise testing.

#### ACCOUNTING FOR THE EFFECTS OF PHYLOGENY

Although no significant correlations were found when accounting for phylogeny, and only one significant corre-

**Table 2.** Results of the comparative analysis with the program Continuous.

RDA	Trait	λ	i	D	Correla	tion	Absolute difference
			$\lambda < 1$	$\lambda > 0$	$\lambda$ estimated	$\lambda = 0$	correlation <i>P</i> -values
pН	log10 body size	0.38	0*	7.43e-5*	0.096	0.0996	0.004
pН	Relative gonad size	0.29	2.65e-14*	0.0482	0.817	0.7	0.117
рH	Bacterial feeder	0.97	0.0419	7.88e-15*	0.7	0.968	0.268
рH	Hyphal feeder	0	2.22e-16*	1	0.295	0.295	0.000
рH	Plant parasite	0.76	4.21e-5*	2.22e-7*	0.888	0.457	0.431
pH	Omnivore	0.56	7.33e-9*	2.85e-6*	0.271	0.175	0.096
pH	Predator	0.95	0.0173	5.06e-7*	0.886	0.731	0.155
pH	Survival stage	0.077	9.44e-7*	0.33	0.0425	0.0283	0.014
pH	Asexual reproduction	0.08	0*	0.186	0.271	0.395	0.124
рН	Coomassie R	0.27	3.88e-9*	0.00351	0.0674	0.0417	0.026
рН	Ervtrosin B	0.48	3.11e-12*	8.70e-17*	0.194	0.25	0.056
pH	Trypan blue	0.16	0*	0.0462	0.699	0.65	0.049
CupH 4.0	log10 body size	0.38	6.40e-13*	0.0124	0.0787	0.176	0.097
CupH 4.0	Relative gonad size	0.46	6.08e-9*	0.0203	0.263	0.708	0.445
CupH 4.0	Bacterial feeder	0.93	0.00443	3.98e-6*	0.423	0.15	0.273
CupH 4.0	Hyphal feeder	0.25	1.08e-13*	1	0.982	0.982	0.000
CupH 4.0	Plant parasite	0 58	9 19e-7*	0.0206	0.279	0.538	0.259
CupH 4.0	Omnivore	0.30	4.05e - 11*	0.00659	0.201	0.165	0.036
CupH 4.0	Predator	0.52	1.03c 11 1.01e $-3*$	0.00052	0.678	0.651	0.030
CupH 4.0	Survival stage	0.07	6.66e - 3*	0.0100	0.177	0.051	0.027
CupH 4.0	A sexual reproduction	0.17	0.000	0.943	0.172	0.173	0.000
CupH 4.0	Coomassie R	0.0004	0 1 44e-6*	0.0135	0.344	0.0412	0.303
Cu pH 4.0	Erstrosin B	0.39	1.440 = 13*	1.019.4*	0.0402	0.0412	0.303
Cu pH 4.0	Trynon blue	0.45	$1.00e - 13^{\circ}$	0.0453	0.0402	0.00324	0.037
Cu pH 4.0	log10 body size	0.23	$1.410 - 14^{\circ}$	0.0433	0.421	0.947	0.520
Cu pH 4.7	Relative gonad size	0.52	$2.786 - 15^{\circ}$	0.00411	0.764	0.78	0.010
Cu pH 4.7	Relative goliau size	0.51	0.0122	1642 8*	0.758	0.887	0.129
Сирп 4.7	Dacterial feeder	0.94	0.0125	1.04e-8*	0.705	0.372	0.191
Сирп 4.7	Diant narrasita	0 62	$1.11e - 10^{-1}$	1	0.449	0.449	0.000
Сирн 4.7	Omniyara	0.02	$7.63e - 7^{+}$	2.586-4*	0.408	0.550	0.128
Cu pH 4.7	Omnivore	0.33	$3.80e - 12^{*}$	0.0128	0.578	0.624	0.040
Cu pH 4.7	Predator	0.88	0.00218	6.9/e-4*	0.294	0.216	0.078
Cu pH 4.7	Survival stage	0.11	1./9e-9*	0.194	0.0253	0.0272	0.002
Cu pH 4.7	Asexual reproduction	0.016	U* 4.00 7*	0.894	0.159	0.161	0.002
Cu pH 4.7	Coomassie R	0.29	4.99e-/*	0.016/	0.572	0.566	0.006
Cu pH 4.7	Erytrosin B	0.44	3.63e-12*	4.48e-5*	0.764	0.494	0.270
Cu pH 4.7	Trypan blue	0.16	7.7/e-16*	0.111	0.701	0.603	0.098
Cu pH 5.4	log10 body size	0.45	8.21e-13*	7.17e-5*	0.942	0.285	0.657
Cu pH 5.4	Relative gonad size	0.43	5.53e-9*	0.0226	0.923	0.734	0.189
Cu pH 5.4	Bacterial feeder	0.97	0.155	1.05e-12*	0.596	0.189	0.407
Cu pH 5.4	Hyphal feeder	0.041	3.53e-13*	0.621	0.299	0.247	0.052
Cu pH 5.4	Plant parasite	0.71	4.44e-5*	2.78e-6*	0.248	0.774	0.526
Cu pH 5.4	Omnivore	0.58	3.89e-5*	6.96e-7*	0.748	0.199	0.549
Cu pH 5.4	Predator	0.97	0.141	1.68e - 6*	0.9	0.938	0.038
Cu pH 5.4	Survival stage	0.13	5.63e-7*	0.183	0.00942	0.00381	0.006
Cu pH 5.4	Asexual reproduction	0.069	0*	0.328	0.533	0.675	0.142
Cu pH 5.4	Coomassie R	0.38	6.72e-6*	0.00362	0.614	0.816	0.202
Cu pH 5.4	Erytrosin B	0.45	2.30e-13*	9.92e-5*	0.627	0.22	0.407
Cu pH 5.4	Trypan blue	0.14	0*	0.0796	0.678	0.462	0.216

RDA	Trait	λ	Ĺ	р	Correl	ation	Absolute difference
			$\lambda < 1$	$\lambda > 0$	$\lambda$ estimated	$\lambda = 0$	correlation <i>P</i> -values
Cu pH 6.1	log10 body size	0.39	1.11e-16*	5.93e-6*	0.947	0.144	0.803
Cu pH 6.1	Relative gonad size	0.34	2.11e-13*	0.00223	0.476	0.808	0.332
Cu pH 6.1	Bacterial feeder	0.99	0.316	5.55e-16*	0.994	0.428	0.566
Cu pH 6.1	Hyphal feeder	0.1	1.78e-15*	0.0867	0.0633	0.04	0.023
Cu pH 6.1	Plant parasite	0.73	1.46e - 4*	1.18e-8*	0.272	0.644	0.372
Cu pH 6.1	Omnivore	0.56	7.87e-8*	3.50e-7*	0.718	0.0776	0.640
Cu pH 6.1	Predator	0.97	0.167	1.89e-8*	0.992	0.613	0.379
Cu pH 6.1	Survival stage	0.12	1.06e - 7*	0.0765	0.00178	2.44e-4*	0.002
Cu pH 6.1	Asexual reproduction	0.16	0*	0.00952	0.677	0.703	0.026
Cu pH 6.1	Coomassie R	0.29	2.47e-08*	0.00182	0.429	0.0483	0.381
Cu pH 6.1	Erytrosin B	0.44	7.22e-14*	7.74e-6*	0.463	0.0325	0.431
Cu pH 6.1	Trypan blue	0.17	0*	0.0166	0.026	0.00195	0.024
Average							0.196

Table 2. (Continued).

Significant *P* values after a Bonferroni correction (0.05/60 = 8.33e-4) are marked with an asterisk (\*).



Fig. 3. The absolute differences of the P values for the correlations tests with and without phylogenetic correction plotted against  $\lambda$ .

lation when phylogeny was not taken into consideration, the data still demonstrate the importance of accounting for phylogeny. The average absolute difference in P values between the tests for correlation with and without accounting for phylogeny is 0.196 (Table 2), and the changes in the P values were both positive and negative. As was to be expected, this difference becomes even larger with

higher  $\lambda$  values (Fig. 3), and testing without phylogenetic correction becomes more inappropriate. The importance of allowing for phylogenetic dependence among the data has been demonstrated by other studies (Holden & Mace, 1997; Pagel, 1999; Espinoza *et al.*, 2004; McKechnie *et al.*, 2006; Swanson & Garland, 2009); in these cases, results contradicting established theory were found because the effects of phylogeny were taken into account in the analysis. Despite its importance, only few authors have allowed for the effect of phylogeny in nematode studies (Morand, 1996; Morand & Sorci, 1998; Poulin & Morand, 2000; and, to a limited extent, Yeates & Boag, 2006). Yet the results presented here in Figure 3 clearly demonstrate the importance of accounting for the effects of phylogenetic dependence in the data when looking for correlations between traits in nematodes.

#### TOWARDS EXPERIMENTALLY DERIVED CP-CLASSES

What relevance do these results have when it comes to defining more objective criteria for assigning nematodes to cp-classes for the MI? First of all, they demonstrate the importance of allowing for the phylogenetic non-independence of data when looking for correlations between traits and stress tolerance. For many traits  $\lambda$  is significantly larger than 0 (Table 2) and, especially if  $\lambda$ becomes larger than 0.3, the effect on *P* values can be substantial (as much as 0.6; Fig. 3). Furthermore, the results also demonstrate the suitability of the GLS method for accounting for the effects of phylogeny on the data. The independent contrast method would, in most cases, overestimate the effect of phylogeny on the data.

Only one significant correlation was found between stress tolerance and nematode traits; the ability to enter a survival stage. This means that for now we cannot design clearer criteria for the cp-classes. However, it seems unlikely that none of these traits, other than the ability to enter a survival stage, is correlated with tolerance to stress. It is known that body size correlates with many other life history traits (Peters, 1983) and most of the nematodes in a low cp-class are fast reproducing bacterial feeders (Bongers & Ferris, 1999). Furthermore, the nematode stains also displayed low *P* values in several cases ( $0.05 > P > 8.33 \times 10^{-4}$ ) and it stands to reason that nematodes with a permeable cuticle are more exposed to pollutants in their body than nematodes with a more or less impermeable cuticle.

Several measures can be taken to improve the chances of finding significant correlations. First of all the amount of data from field studies should be increased by analysing substantially larger numbers of individuals per sample ( $\gg$ 150 individuals per samples) and by repeatedly sampling over a period of time. This would, at least partially, alleviate the problem of genera not being found in all treatments, resulting in RDA values being calculated for more genera. The development of molecular identification methods would be a great help in increasing the number of nematodes analysed per sample by a hundred-fold or more (Holterman *et al.*, 2008). Another possibility would be to identify the nematodes down to species level instead of genus level so that traits such as body size and relative gonad size would no longer have to be averaged over the species in a genus. However, this would require a substantial extra effort when identifying nematodes through light microscopy, and it is unlikely that molecular identification will be developed to the level of species identification of the entire nematode fauna in the near future. Furthermore, certain groups of nematodes, such as the Rhabditidae and members of the Dorylaimida, are notoriously difficult to identify, even at the genus level.

Perhaps most important is the acquisition of the missing data for the species traits, especially for the data on cuticle permeability. These experiments require living, freshly collected and identified nematodes from field samples (most nematodes are considered to be unculturable), and hence it is no surprise that the data were incomplete. Filling in the missing data and perhaps combining the results of the different stains into an index may prove to be more informative. Confirmation of some of the more speculative data - the feeding types and reproductive modes of certain genera - is also important in this regard. More importantly though, filling in the missing data means that the correlations would no longer have be tested pair-wise, but could be tested simultaneously in a single analysis. This would obviate the need for a correction for the effects of multiple pair-wise testing, such as a Bonferroni correction, which in this case study lowered the significance level from 0.05 to 0.00083.

### Conclusion

As a first step towards a more objective cp-classification and to refine the Maturity Index from a family level to genus level, the traits relevant to tolerance for environmental disturbance have to be identified. We have laid out a framework to identify traits correlated with stress tolerance while taking into account the effects of phylogeny on the data. Accounting for the affects of phylogeny was demonstrated to be very important. Unfortunately, our approach is hampered by incomplete data, especially on the permeability of the cuticle and the tolerance for stress (as represented by the RDA values). Improving on the amount of available data may lead to more significant results and the identification of traits relevant to stress tolerance in the future.

#### Acknowledgements

M.H. was supported by the Dutch Technology Foundation (STW) grant WBI 4725. H.v.M. and J.H. acknowledge financial support from SenterNovem grant IS043076 (SenterNovem is an agency of the Dutch Ministry of Economic Affairs).

#### References

- ANDRÁSSY, I. (1956). Die rauminhalts- und gewichtsbestimmung der fadenwürmer (nematoden). Acta Zoologica Academiae Scientiarum Hungaricae 2, 1-15.
- BONGERS, T. (1990). The maturity index an ecological measure of environmental disturbance based on nematode species composition. *Oecologia* 83, 14-19.
- BONGERS, T. (1994). *De nematoden van Nederland*. Utrecht, Koninklijke Nederlandse. Natuurhistorische Vereniging, 408 pp.
- BONGERS, T. (1999). The Maturity Index, the evolution of nematode life history traits, adaptive radiation and cp-scaling. *Plant and Soil* 212, 13-22.
- BONGERS, T. & FERRIS, H. (1999). Nematode community structure as a bioindicator in environmental monitoring. *Trends in Ecology and Evolution* 14, 224-228.
- DE RUITER, P.C., NEUTEL, A.M. & MOORE, J.C. (1998). Biodiversity in soil ecosystems: the role of energy flow and community stability. *Applied Soil Ecology* 10, 217-228.
- ESPINOZA, R.E., WIENS, J.J. & TRACY, C.R. (2004). Recurrent evolution of herbivory in small, cold-climate lizards: breaking the ecophysiological rules of reptilian herbivory. *Proceedings of the National Academy of Sciences of the United States of America* 101, 16819-16824.
- ETTEMA, C.H. & BONGERS, T. (1993). Characterization of nematode colonization and succession in disturbed soil using the maturity index. *Biology and Fertility of Soils* 16, 79-85.
- FELSENSTEIN, J. (1985). Phylogenies and the comparative method. *American Naturalist* 125, 1-15.
- GUIDETTI, R., COLAVITA, C., ALTIERO, T., BERTOLANI, R.
  & REBECCHI, L. (2007). Energy allocation in two species of Eutardigrada. *Journal of Limnology* 66, 111-118.
- HALL, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41, 95-98.
- HARVEY, P.H. & RAMBAUT, A. (2000). Comparative analyses for adaptive radiations. *Philosophical Transactions of the Royal Society of London Series B – Biological Sciences* 355, 1599-1605.
- HOLDEN, C. & MACE, R. (1997). Phylogenetic analysis of the evolution of lactose digestion in adults. <u>*Human Biology* 69</u>, 605-628.

- HOLTERMAN, M., VAN DER WURFF, A., VAN DEN ELSEN, S., VAN MEGEN, H., BONGERS, T., HOLOVACHOV, O., BAKKER, J. & HELDER, J. (2006). Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Molecular Biology and Evolution* 23, 1792-1800.
- HOLTERMAN, M., RYBARCZYK, K., VAN DEN ELSEN, S., VAN MEGEN, H., MOOYMAN, P., PENA-SANTIAGO, R., BONGERS, T., BAKKER, J. & HELDER, J. (2008). A ribosomal DNA-based framework for the detection and quantification of stress-sensitive nematode families in terrestrial habitats. *Molecular Ecology Notes* 8, 23-34.
- KORTHALS, G.W., ALEXIEV, A.D., LEXMOND, T.M., KAM-MENGA, J.E. & BONGERS, T. (1996). Long-term effects of copper and pH on the nematode community in an agroecosystem. *Environmental Toxicology and Chemistry* 15, 979-985.
- LORENZEN, S. (1994). *The phylogenetic systematics of freeliving nematodes*. London, UK, The Ray Society, 383 pp.
- MARTINS, E.P., DINIZ, J.A.F. & HOUSWORTH, E.A. (2002). Adaptive constraints and the phylogenetic comparative method: a computer simulation test. *Evolution* 56, 1-13.
- MCKECHNIE, A.E., FRECKLETON, R.P. & JETZ, W. (2006). Phenotypic plasticity in the scaling of avian basal metabolic rate. *Proceedings of the Royal Society B – Biological Sciences* 273, 931-937.
- MORAND, S. (1996). Life-history traits in parasitic nematodes: a comparative approach for the search of invariants. *Functional Ecology* 10, 210-218.
- MORAND, S. & SORCI, G. (1998). Determinants of life-history evolution in nematodes. *Parasitology Today* 14, 193-196.
- PAGEL, M. (1997). Inferring evolutionary processes from phylogenies. *Zoologica Scripta* 26, 331-348.
- PAGEL, M. (1999). Inferring the historical patterns of biological evolution. *Nature* 401, 877-884.
- PETERS, R.H. (1983). *The ecological implications of body size*. Cambridge, UK, Cambridge University Press, 329 pp.
- POULIN, R. & MORAND, S. (2000). Parasite body size and interspecific variation in levels of aggregation among nematodes. *Journal of Parasitology* 86, 642-647.
- RODRIGUES, D.J., UETANABARO, M. & LOPES, F.S. (2005). Reproductive patterns of *Trachycephalus venulosus* (Laurenti, 1768) and *Scinax fuscovarius* (Lutz, 1925) from the Cerrado, Central Brazil. *Journal of Natural History* 39, 3217-3226.
- RODRIGUES, D.J., UETANABARO, M. & LOPES, F.S. (2007). Breeding biology of *Phyllomedusa azurea* Cope, 1862 and *P. sauvagii* Boulenger, 1882 (Anura) from the Cerrado, Central Brazil. *Journal of Natural History* 41, 1841-1851.
- SCHÄRER, L., SANDNER, P. & MICHIELS, N.K. (2005). Trade-off between male and female allocation in the simultaneously hermaphroditic flatworm *Macrostomum* sp. *Journal* of Evolutionary Biology 18, 396-404.
- SHANNON, C.E. (1948). A mathematical theory of communication. Bell System Technical Journal 27, 379-423.

- SIMPSON, E.H. (1949). Measurement of diversity. *Nature* 163, 688.
- STUART-FOX, D. (2009). A test of Rensch's rule in dwarf chameleons (*Bradypodion* spp.), a group with female-biased sexual size dimorphism. *Evolutionary Ecology* 23, 425-433.
- SWANSON, D.L. & GARLAND, T. (2009). The evolution of high summit metabolism and cold tolerance in birds and its impact on present-day distributions. *Evolution* 63, 184-194.
- TER BRAAK, C.J.F. (1995). Ordination. In: Jongman, R.H.G., Ter Braak, C.J.F. & Van Tongeren, O.F.R. (Eds). Data analysis in community and landscape ecology. Cambridge, UK, Cambridge University Press, pp 91-173.
- TER BRAAK, C.J.F. & SMILAUER, P. (2002). Canoco for Windows Version 4.5., 4.5 edition. Wageningen, The Netherlands, Biometris – Plant Research International.
- VAN DER WURFF, A.W.G., KOOLS, S.A.E., BOIVIN, M.E.Y., VAN DEN BRINK, P.J., VAN MEGEN, H.H.M., RIKSEN, J.A.G., DOROSZUK, A. & KAMMENGA, J.E. (2007). Type of disturbance and ecological history determine structural stability. <u>Ecological Applications</u> 17, 190-202.

- VERDONSCHOT, P.F.M. & BRAAK, C. (1994). An experimental manipulation of Oligochaete communities in mesocosms treated with chlorpyrifos or nutrient additions – multivariate analyses with Monte-Carlo permutation *t* tests. *Hydrobiologia* 278, 251-266.
- WUYTS, J., DE RIJK, P., VAN DE PEER, Y., PISON, G., ROUSSEEUW, P. & DE WACHTER, R. (2000). Comparative analysis of more than 3000 sequences reveals the existence of two pseudoknots in area V4 of eukaryotic small subunit ribosomal RNA. *Nucleic Acids Research* 28, 4698-4708.
- YEATES, G.W. & BOAG, B. (2006). Female size shows similar trends in all clades of the phylum Nematoda. <u>Nematology 8</u>, 111-127.
- YEATES, G.W., BONGERS, T., DE GOEDE, R.G.M., FRECK-MAN, D.W. & GEORGIEVA, S. (1993). Feeding-habits in soil nematode families and genera – an outline for soil ecologists. *Journal of Nematology* 25, 315-331.

Appendix A. GenBank accessions of the SSU rDNA sequences used in this study.

No.         No.           Achromadora ruricola         AY593991         Meloidogyne fallax         AY593895           Acrobeles complexus         AY284673         Mesodrophainus centrocercus         AY284799           Acrobeloides apiculatus         AY284673         Mesodrophainus centrocercus         AY284799           Acrostichus halicti         U61759         Mesorhabditis spiculgera         AY28468           Anaplectus grandepapillatus         AY284684         Monystera riemanni         AY293932           Aphelenchoide bicaudatus         AY284681         Mononchoides striatus         AY284793           Aphelenchoide bicaudatus         AY284681         Mononchoides striatus         AY284793           Aporeciaimellus         AY284681         Mononchoides striatus         AY284792           Aporeciaimellus         AY284610         Nygolainus C1 brachyuris         AY284775           Bolodorus thylactus         AY284684         Paramphidelus hortensis         AY284775           Bolodorus thylactus         AY284684         Paramphidelus hortensis         AY28473           Bursaphelenchus mucronatus         AY284594         Paratricholons anenones         AF036600           Cephalenchus headineatus         AY284512         Pelloditis medirerranea         AF083020           Chohades	Species	Accession	Species	Accession
Achromadora ruricola     AY593941     Meloidogyne fallax     AY593895       Acrobeloides complexus     AY284673     Meroihuis brevidens     AY284795       Acrobeloides apriculatus     AY284673     Mesorhabitis spiculigera     AP083010       Alaimus parvus     AY284673     Mesorhabitis spiculigera     AP083010       Alaimus parvus     AY284697     crassidens     AY284686       Anaplectus grandepapillatus     AY284639     Monnchoides striatus     AY593938       Aphelenchoides biccudatus     AY284639     Monnchoides striatus     AY593936       Aporcelaimellus     AY284639     Monnchoides striatus     AY593930       Aporcelaimellus     AY284611     Neodolichorhynchus     AY284590       Dotusicaudatus     Iamelliferus     Iamegrolainus subelongatus     AY284671       Briytenchus dubius     AY284601     Nygolainus cf. brachyuris     AY284671       Boleodons thylactus     AY284694     Paramphilelus intreisis     AY284671       Bursaphelenchus macronatus     AY284662     Paratylenchus straeleni     AY284680       Cephalenchus hexalineatus     AY284677     Pelodera teres     AF036002       Cerhidolus propinguus     AY284766     Paratylenchus thornei     AY284681       Contodes morgani     AF036539     Pictus acuminatus     AF037532		No.		No.
Acrobeles complexasAY284671Merinius brevidensAY284793Acrobeloides apiculatusAY284673Mesorhabitis spiculigeraAF083016Acrostichus haltictiU61759Mesorhabitis spiculigeraAF083016Alaimus parvusAY284738MetateratocephalusAY284680Anaplectus grandepapillatusAY284639Monnchoides striatusAY593924Aphelenchoides bicaudatusAY284639Monnchoides striatusAY594593AporcelaimellusAY284631Monochoides striatusAY593924AporcelaimellusAY284611Neodolichoritynchus acrisAY593914Bastiania gracilisAY284735Nothotylenchus acrisAY284736Bustainai gracilisAY284601Nygolaimus cf. brachyurisAY284737Boleadorus thylactusAY284594Parampichalus hortensisAY284737Boleadorus thylactusAY284594Paratrichodorus amenonesAF036600Bursaphelenchus hexalineatusAY284594Paratrichodorus anemonesAF036630Cephalobus propinguusAY284572Pelloiditis mediterraneaAF036630Chiloplacus propinguusAY284766Protylenchus straeleniAY284532Condrades morganiAY036459Piectus cuninatusAF03652Carkus papillatusAY284746Pratylenchus thorneiAF03652Carkus papillatusAY284766Protylenchus thorneiAF036262Caracus propinguusAY284786Priatylenchus therriteriAF03620Carlas papillatusAY284766Protylenchus therriteriAF036530 </td <td>Achromadora ruricola</td> <td>AY593941</td> <td>Meloidogyne fallax</td> <td>AY593895</td>	Achromadora ruricola	AY593941	Meloidogyne fallax	AY593895
Acrobeloides opiculatusAY284673Mesodorylainus centrocercusAY284799Acrostichus halictiU01759Mesodorylainus centrocercusAY284686Anajones parvusXY284687CrassidensAY284687Anaplectus grandepapillatusXY284687crassidensAY593938Aphelenchuis svenaeAY284639Mononchoides striatusAY593932Aphelenchus svenaeAY284735Menonchoides striatusAY593924AporcelainellusXY284725Nononchoides striatusAY593924AporcelainellusAY284725Nonhystera rimanniXY284775Bitylenchus dubiusAY284784Paragrolainus cf. brachyurisAY284784Bitylenchus dubiusAY284681Paramphidelus hortensisAY284784Bursaphelenchus mucronatusAY284648Paramphidelus hortensisAY284630Cephalenchus headineatusAY284662Paratrichodorus anemonesAF036000Cephalobus persegnisAY284677Pelodera teresAF083002Chordodes morganiAF036639Plectus acuninatusAF035028Clarkus papillatusAY284766Pratylenchoids ritteriAJ966497Costenchus costatusAY28478Poikitolainus aycercaAF035028Cylindrainus parvusAY28478Pratylenchus thorneiAY2847939309Cylindrainus contunusAY28478Pratylenchus thorneiAY284630Chordodes morganiAF036649Protylenchus thorneiAY284639Chordodes morganiAY284766Pratylenchus thorneiAY284639Deladenus sircidic	Acrobeles complexus	AY284671	Merlinius brevidens	AY284597
Acrostichus halictiU61759Mesorhabditis spiculigeraAF083010Alainus parvusAY284738MetateratocephalusAY284685Anaplectus grandepapillatusAY284663Montochoides stratusAY59392Aphelenchoides bicaudatusAY284643Montochoides stratusAY593924AporcelaimellusAY284613Montochoides stratusAY593924AporcelaimellusAY284598Montochoides stratusAY284598obtusicaudatusMantochoides stratusAY284598Bastiania gracillsAY284725Nothotylenchus acrisAY284739Bitylenchus dubiusAY284601Nygolaimus subelongatusAY284638Bursaphelenchus mucronatusAY284594Parampidelus hortensisAY284739Cephalobus persegnisAY284502Paratrichodorus anemonesAF036600Cephalobus persegnisAY284537Pelodera teresAF036000Chiloplacus propinguusAY284677Pelodera teresAF083002Chiloplacus propinguusAY284786Pratylenchus thoreAF037628Clarkus papillatusAY284766Pratylenchus thorneiAY284636Condustus parvusAY284781Protylenchus strateleniAY284576Conzument parvusAY284581Pratylenchus thorneiAY284630Condustus parvusAY284786Pritanutus oxycercaAF037628Clarkus papillatusAY284784Pritanutus purcutusAY284796Cruzment tripartiaU73449Pritanutos strateniAY2847964Cruzment tripartiaAY284677Priabulus pu	Acrobeloides apiculatus	AY284673	Mesodorylaimus centrocercus	AY284799
Alaimus parvus       AY284738       Metateratocephalus       AY284686         Anaplectus grandepapillatus       AY284643       Monhystera riemanni       AY59393         Aphelenchoides bicaudatus       AY284643       Monhystera riemanni       AY593934         Aphelenchoides bicaudatus       AY284611       Neodolichrhynchus       AY593934         Aporcelaimellus       AY284725       Nothorylenchus acris       AY284598         obusicaudatus       Lamelliferus       Stationa gracilis       AY284725         Bottodatus       AY284601       Nygolaimus subelongatus       AY284681         Bursaphelenchus mucronatus       AY284662       Paranylenchoars anemones       AP304002         Cephalenchus hexalineatus       AY284662       Paranylenchus subelongatus       AY284630         Cervidellus alutus       AY284677       Pelodera teres       AF083002         Cervidellus alutus       AY284748       Poikiloinius oxycerca       AF083002         Chordodes morgani       AY026479       Prelotera teres       AF083002         Coomansus parvus       AY28478       Pratylenchoides ritteri       AJ966497         Costatus       AY28478       Pratylenchoides ritteri       AJ966497         Colarkus papillatus       AY284766       Pratylenchoides ritteri	Acrostichus halicti	U61759	Mesorhabditis spiculigera	AF083016
Anaplecias grandepapillatusAY284697crassidens'Aphelenchoides bicaudatusAY284639Mononchöles striatusAY59938Aphelenchois avenaeAY284639Mononchöles striatusAY59932AporcelaimellusAY284639Mononchöles striatusAY589204AporcelaimellusAY284598lamelliferusAY284598abatiania graciiisXY284707Solahonchus acrisAY284772Boleodorus thylactusAY284601Nygolaimus cl. brachyurisAY284772Boleodorus thylactusAY284648Parangrolaimus subelongatusAY284681Bursaphelenchus nucronatusAY284652Paratrichodorus amemonesAF036600Cephalenchus hexalineatusAY284654Paratrichodorus amemonesAF036600Cephalenchus propinguusAY284677Peliodera teresAF083020Chiloplacus propinguusAY284766Pratylenchoids striteriAJ966497Coomanus parvusAY284766Pratylenchoides tirteriAJ966497Coslenchus costatusAY284766Pratylenchoides tirteriAJ966497Cylindrolaimus communisAY593939Prionchulus punctatusAY284746Diplogaster rivalisAY284838Pristionchus thorneiAY2846364Diplogaster rivalisAY284784Protorylaimus adolichurusAY284746Diplogaster rivalisAY284689Protorhabiditis sp.AF083064Corinchus contatusAY284784Pristionchus theriteriAF0366497Cosleachus costatusAY284689Protorhabiditis sp.AF086600Diplogaster	Alaimus parvus	AY284738	Metateratocephalus	AY284686
Aphelenchoides bicaudatusAY28463Monnystera riemanniAY59393Aphelenchus avenaeAY284639Mononchoides striatusAY593924AporcelainellusAY28411NeodolichorhynchusAY28459obusicaudatusIamellifernsIamellifernsStriatusBastiania gracilisAY284725Nototylenchus acrisAY593914Bitylenchus dubiusAY284601Nygolainus cl. brachyurisAY284681Bursaphelenchus mucronatusAY284648Paramylieldus hortensisAY284632Bursaphelenchus mucronatusAY284648Paratrichodorus anemonesAF036600Cephalenchus hexalineatusAY284659Paratrichodorus anemonesAF036600Cephalobus persegnisAY284667Peltoditis mediterraneaAF083002Chioplacus propinquusAY28477Pelodera teresAF083002Chordodes morganiAF036639Plectus acuminatusAF037628Comansus parvusAY284748Porikolinus oxycercaAF083002ConstatusAY284739Priapulus caudatusZ38009Cytarlorlainus contunuisAY03437Priapulus caudatusZ38009Cytarlorlainus contunuisAY03437Prisandialinus dolichurusAY284746Diplogaster rivalisAY284688Priatonchus parvusAY284739Diplogaster rivalisAY284689Protornabulis parvusAY284430Diplogaster rivalisAY284689Priochulus punctuusAY284593Diplogaster rivalisAY284666Rotylenchus repartiesAY284593Dorylainoides micoletzyi <td>Anaplectus grandepapillatus</td> <td>AY284697</td> <td>crassidens</td> <td></td>	Anaplectus grandepapillatus	AY284697	crassidens	
Aphelenchus avenaeAY284639Mononchoides striatusAY593924AporcelaimellusAY284581NeodolichorbynchusAY28459JanellierusIamellierusIamellierusBastiania gracilisAY284501Nygolaimus cf. horchyurisAY28459Bastiania gracilisAY284001Nygolaimus cf. horchyurisAY284701Birylenchus dubiusAY284001Nygolaimus cf. horchyurisAY284703Boleadorus thylactusAY284594Paratrichodorus anemonesAF036600Cephalobus persegnisAY284594Paratrichodorus anemonesAF036600Cephalobus persegnisAY284574Pelioditis mediterraneaAF083002Chiloplacus propinquusAY284767Pelodera teresAF083002Charlos angeniAF036639Plectux acuminatusAF037528Comansus parvusAY284766Prarylenchuis sviereraAF083002Coslenchus costatusAY284581Pratylenchuidus punctatusAY284612Cruzema tripartitaU73449Priapulus caudatusZ38009Cylindrolainus communisAY28438Pristionchus thorneiAY284593Diplogastrid nematodeAY284689Protorhabditis sp.AF035630Diplogastrid nematodeAY284581PraseudiatusAY284593Diplogastrid nematodeAY284589Protorhabditis sp.AF036630Diplogastrid nematodeAY284581Pratylenchus thorneiAY284594Diplogastrid nematodeAY284581Pratylenchus thorneiAY284593Diplogastrid nematodeAY284581Pristionc	Aphelenchoides bicaudatus	AY284643	Monhvstera riemanni	AY593938
AporcelaimellusAY284811NeodolichorhynchusAY284598obtusicaudatusIamelliferusBastiania gracilisAY284725Nothorylenchus acrisAY284770Bilylenchus dubiusAY284601Nygolaimus cf. brachyurisAY284770Boleodorus thylactusAY284681Paragrolaimus subelongatusAY2844735Borsphelenchus mucronatusAY284648Paragrolaimus subelongatusAY284673Cephalenchus hexalineatusAY284692Paratrichodorus anemonesAF036600Cephalenchus persegnisAY284677Pelodera teresAF083002Chiloplacus propinguusAY284677Pelodera teresAF083002Chordodes morganiAF036639Plectus acuminatusAF083022Coomansus parvusAY284786Pratylenchus striteriAJ966497Costenchus costatusAY284786Pratylenchus striteriAJ966497Costenchus costatusAY284781Priatylenchus thorneiAY284746Delademus stricidicolaAY633447Prismatolaimus dolichurusAY293959Cylindrolaimus communisAY284581Pratylenchus thorneisAY593940Diplogaster rivalisAY284689Protorhabditis sp.AF083002Diplogaster rivalisAY284581Prismatolaimus dolichurusAY2939394Diplogaster rivalisAY284689Pristonchus theritieriAF036649Diplogaster rivalisAY284689Pristonchus theritieriAF083004Diplogaster rivalisAY284680Prishenchus sphapildaAF082997Diplogaster rivalisAY284680 </td <td>Aphelenchus avenae</td> <td>AY284639</td> <td>Mononchoides striatus</td> <td>AY593924</td>	Aphelenchus avenae	AY284639	Mononchoides striatus	AY593924
obusicaudatusIamelliferusBastiania gracilisAY284725Nothorylenchus acrisAY284771Bitylenchus dubiusAY284601Nygolainus cl. brachyurisAY284781Blocodorus thylactusAY284681Paramphidelus horensisAY284681Bursaphelenchus mucronatusAY284594Paratrichodorus anemonesAF036600Cephalenchus hexalineatusAY284592Paratrichodorus anemonesAF036600Cervidellus alutusAF202152Pellioditis mediterraneaAF083002Chiloplacus propinguusAY284677Pelodera teresAF083002Chordodes morganiAF036639Plectus acuminatusAF037628Comansus parvusAY284786Pratylenchus straeleniAJ284617Coslenchus costatusAY284766Pratylenchoides ritteriAJ966497Coslenchus costatusAY284781Pratylenchoides ritteriAJ966497Cruzmena tripartitaU73449Priapulus caudatusZ38009Cylindrolaimus communisAY593939Prionchulus punctatusAY284766Deladenus siricidicolaAY284581Pratylenchus thurseiAY284792Deladenus siricidicolaAY284588Pristionchus lheritieriAF036640Diplogaster rivalisProstylainus masAY593951Dipthetrophora obesaAY284689Protorhabditis sp.AF083001Diylenchus dipsaciAY284680Pristionchus lheritieriAF083002Dirylenchus dipsaciAY284680Protorylainus masAY284638Dorylainnoides micoleirkyiAY284680Protorylainus	Aporcelaimellus	AY284811	Neodolichorhynchus	AY284598
Bastiania gracilisAY284725Nothorylenchus acrisAY593914Bitylenchus dubiusAY284601Nygolainus ci. brachyurisAY284701Boleodorus hylactusAY284601Nygolainus ci. brachyurisAY284703Bursaphelenchus mucronatusAY284684Paramphidelus hortensisAY284739Cephalenchus hexalineatusAY284594Paratrichodorus anemonesAF036600Cephalobus persegnisAY284622Paratrichodorus anemonesAF036000Cervidellus alutusAF202152Pellioditis mediterraneaAF083020Chordodes morganiAF086639Plectus acuminatusAF083023Chordodes morganiAF086639Plectus acuminatusAF083023Comansus parvusAY284766Pratylenchuis riteriAJ966497Coslenchus costatusAY284766Pratylenchuis torneiAY284612Crentem tripartitaU73449Priapulus caudatusZ38009Cylindrolainus communisAY284734Prionchulus punctatusAY284762Deladenus siricidicolaAY633477Prismotalumus dolichurusAY284709Diphterophora obesaAY284689Protorhabditis sp.AF036640Diplogaster rivalisProdorylainus masAY28463AY28463Dirilocephalobus sp.AY284680Priabulenchus minutusAY28463Dirilocephalobus striatusAY284680Priabditis blamiU1395Enchodelus sp.AY284666Rotylenchus robustusAY284653Dirilocephalobus striatusAY284666Rotylenchus robustusAY284658Diriloc	obtusicaudatus		lamelliferus	
BitylenchusAY284601Nygolaimus cf. brachyurisAY284770Boleodorus ihylactusAY284791Panagrolaimus subelongatusAY284730Bursaphelenchus mucronatusAY284684Paramphidelus hortensisAY284733Cephalenchus hexalineatusAY284594Paratrichodorus amemonesAF036600Cephalenchus hexalineatusAY284662Paratylenchus straeleniAY284632Cervidellus alutusAF202152Pellioditis mediterraneaAF083002Chloplacus propinguusAY284677Pelcodera teresAF083002Chordodes morganiAF036639Plectus acuminatusAF036632Coamasus parusAY284748Poikilolaimus oxycercaAF083002Coomasus parusAY284766Pratylenchoides riteriAJ966497Coslenchus costatusAY284766Pratylenchus thorneiAY284762Cruzmena triparitiaU73449Priapulus caudatusZ38009Cylindrolaimus communisAY284581Pratylenchus thorneiAY284746Deladenus sricidicolaAY633447Prismatolaimus dolichurusAY284530Diplogaster rivalisProdorylaimus masAY593957AF083001Diplogaster rivalisProdorylaimus masAY284530Dorylaimoides micoletzkyiAY284680Proiorhabilitis sp.AF083001Diplogaster rivalisAY284666Roylenchus cf. hiarulusAY284565Dorylaimoides micoletzkyiAY284666Roylenchus reginaAF082997Eucephalobus striatusAY284666Roblenchus ripus at AY284651AY284653Euco	Bastiania gracilis	AY284725	Nothotylenchus acris	AY593914
Boleodorus thylactusAY593915Ponagrolaimus subelongatusAY284681Bursaphelenchus mucronatusAY284544Parampinidellus hortensisAY284735Cephalobus persegnisAY284562Paratrichodorus anemonesAF036600Cephalobus persegnisAY284662Paratylenchus straeleniAY284503Cervidellus alutusAF202152Pellioditis mediterraneaAF083002Chirloplacus propinguusAY284677Pelodera teresAF083002Chordodes morganiAF036639Plectus acuminatusAF037628Clarkus papillatusAY284748Poikilolaimus oxycercaAF083002Comansus parvusAY284766Pratylenchus thorneiAY284612Cruznem triparitiaU73449Priapulus caudatusZ38009Cylindrolaimus communisAY284581Prionchulus punctatusAY284746Deladenus sriciticolaAY633447Prisatolenus dolichurusAY593957Diphderophora obesaAY284838Pristoinchus theritieriAF033020Diplogastrid nematodeAY284689Protorhabitis sp.AF083001Diplogastrid nematodeAY284680Rhabditis blumiU1395Drilocephalobus sp.AY284666Rolylenchus mintusAY284583Drilocephalobus sp.AY284666Rolylenchus robustusAY284659Drotoplainus odustinaAY284666Rolylenchus robustusAY284584Derlodus sp.AY284666Rolylenchus robustusAY284583Drilocephalobus sp.AY284666Rolylenchus robustusAY284659Eucophalobus sp.	Bitvlenchus dubius	AY284601	Nygolaimus cf. brachvuris	AY284770
Bursaphelenchus mucronatusAY284648Paramphidelus hortensisAY284739Cephalenchus hexalineatusAY284594Parattrichodorus anemonesAF036600Cephalobus persegnisAY284662Parattrichodorus anemonesAF036600Cervidellus alutusAF20152Pellioditis mediterraneaAF083002Chiloplacus propinquusAY284677Pelodera teresAF083002Chordodes morganiAF036639Plectus acuminatusAF037628Clarkus papillatusAY284748Polikilolaimus oxycercaAF038022Coomansus parvusAY284766Pratylenchoides ritteriAJ966497Coslenchus costatusAY284788Priaylenchoides ritteriAJ966497Curarema tripartitaU73449Priaylenchoides nitteriAY284746Cylindrolaimus communisAY593939Prionchulus punctatusAY284746Deladenus siricidicolaAY633447Prismotohus horneiAY284766Diplogaster rivalisProdorylaimus masAY393935Diplopaster rivalisProdorylaimus masAY384689Diplogaster in ematodeAY284830Psienchus ef. hlarutusAY284583Drilocephalobus sp.AY284680Rhabditella axeiAY284584Cephalobus sp.AY284680Rhabditila daeiAY284584Eucephalobus sp.AY284666Robylenchus reginaAF082997Eucephalobus sp.AY284680Rhabditila axeiAY284585Eucephalobus sp.AY284666Robylenchus spnatulusAY284581Eucephalobus sp.AY284666Robylenchus spnatu	Boleodorus thylactus	AY593915	Panagrolaimus subelongatus	AY284681
Cephalenchus hexalineatusAY284594Paratrichodorus anemonesAF036600Cephalobus persegnisAY284662Paratylenchus straeleniAY284632Cervidellus alutusAF202152Pellioditis mediterraneaAF083002Chiloplacus propinguusAY284677Pelodera teresAF083002Chordodes morganiAF036639Plectus acuminatusAF08302Clarkus papillatusAY284766Pratylenchoides ritteriAJ966497Costenchus costatusAY284581Pratylenchoides ritteriAJ96497Costenchus costatusAY284581Pratylenchus thorneiAY284761Cruznema tripartitaU73449Priapulus caudatusZ38009Cylindrolainus communisAY284388Pristonchus mota dolichurusAY393959Diphterophora obesaAY284838Pristonchus ulteritieriAF036640Diplogaster rivalisProtorhabditis sp.AF036640Diplogastri nematodeAY284689Protorhabditis sp.AF083002Drislenchus dipsaciAY284680Rhabditela axeiAY284585Dorylainoides micoletzkyiAY284680Rhabditis blumiU13935Eucephalobus sp.AY284666Rotylenchus reginaAF082997Eucephalobus sp.AY284666Rotylenchus reginaAF08297Eucephalobus sp.AY284666Rotylenchus reginaAF08297Eucephalobus striatusAY284666Rotylenchus reginaAF08297Eucephalobus striatusAY284666Tortorhabditis synpapillataAF083015Filenchus filifornisAY284680 <t< td=""><td>Bursaphelenchus mucronatus</td><td>AY284648</td><td>Paramphidelus hortensis</td><td>AY284739</td></t<>	Bursaphelenchus mucronatus	AY284648	Paramphidelus hortensis	AY284739
Cephalobus persegnisAY284662Paratylenchus straeleniAY284630Cervidellus alutusAF202152Pelloditis mediterraneaAF083020Chiloplacus propinquusAY284677Pelodera teresAF083020Chordodes morganiAF036639Plectus acuminatusAF037628Clarkus papillatusAY284748Poikilolaimus oxycercaAF083023Comansus parvusAY284766Pratylenchoides ritteriAJ966497Coslenchus costatusAY284581Pratylenchoides ritteriAJ966497Coslenchus costatusAY284581Pratylenchus thorneiAY284746Deladenus stricidicolaAY633447Prianulus punctatusAY593990Cylindrolaimus communisAY284388Pristonchus punctatusAY593946Diphogaster rivalisProdorylaimus masAY593946Diplogastri nematodeAY284689Protorylaimus masAY284583Driglogastri nematodeAY284680Rhabditis sp.AF083001Diytlenchus dipsaciAY284680Rhabditiella axeiAY284584Drilocephalobus sp.AY284666Rotylenchus robustusAY284592Drilocephalobus striatusAY284666Rotylenchus robustusAJ966503Euteratocephalus palustrisAY284592Thonus restrisAY284592Filenchus filiformisAY284592Thonus cetterystiAY284592Gordius aquaticusX80233Thornis steatopygaAY284593Filenchus filiformisAY284666Tylenchus variopapillataAF083015Filenchus filiformisAY284666Ty	Cephalenchus hexalineatus	AY284594	Paratrichodorus anemones	AF036600
Cervidellus alutusAF202152Pelioditis mediterraneaAF083020Chiloplacus propinquusAY284677Pelodera teresAF083020Chordodes morganiAF036639Plectus acuminatusAF037628Clarkus papillatusAY284748Poikilolaimus oxycercaAF083023Coomansus parvusAY284766Pratylenchoides ritteriAJ966497Coslenchus costatusAY284581Pratylenchus thorneiAY284706Cruznema tripartitaU73449Priapulus caudatusZ38009Cylindrolaimus communisAY593939Prionchulus punctatusAY284746Deladenus siricidicolaAY633447Prismatolaimus dolichurusAY593957Diphtherophora obesaAY284888Pristionchus IheritieriAF036404Diplogastri nematodeAY284689Protorhabitis sp.AF083001Dirylenchus dipsaciAY284680Rhabditella axeiAY284638Drilocephalobus sp.AY284830Psilenchus cf. hilarulusAY284534Drilocephalobus sp.AY284792Rhabditila axeiAY284534Euchyadophora tenuissimaAY284792Rhabditis blumiU13935Euchous carteriAJ9666484Seinura sp.AY284635Euteratocephalbus salustrisAY284592Thonus cf. circuliferAY284636Euteratocephalus galustrisAY284666Roylenchus robustusAJ966503Euterotus plugomisAY284592Thonus cf. circuliferAY284635Euterotus plus galustrisAY284684Teratoncephalus terrestrisAY284636Euterotus plus gal	Cephalobus persegnis	AY284662	Paratylenchus straeleni	AY284630
Chiloplacus propinquusAY284677Pelodera teresAF038002Chordodes morganiAF036639Plectus acuminatusAF037628Clarkus papillatusAY284748Poikilolaimus oxycercaAF083002Coomansus parvusAY284766Pratylenchoides ritteriAJ966497Coslenchus costatusAY284581Pratylenchoides ritteriAY284761Cruznema tripartitaU73449Priapulus caudatusZ38009Cylindrolaimus communisAY593939Prionchulus punctatusAY284764Diphterophora obesaAY284383Pristionchuls punctatusAY593957Diphterophora obesaAY284689Protorhabditis sp.AF038640Diplogastri dnematodeAY284581Preudnalenchus minutusAY284583Dorylaimoides micoletzkyiAY284689Protorhabditis sp.AF0883001Dirlocentaciones sp.AY284680Rhabditella axeiAY284593Drilocephalobus sp.AY284680Rhabditiella axeiAY284592Eucophalobus sp.AY284666Rotylenchus robustusAJ966503Eudorylaimus carteriAJ966484Seinura sp.AY284654Euterotephalus striatusAY284592Thonus cf. circuiferAY284593Euterotephalus splaustrisAY284592Thonus cf. circuiferAY284584Euterocephalus aquaturisXY284666Trichodorus variopapillataAF083002Euterocephalus selongatusAY284666Trichodorus variopapillatusAY284733Heicotylenchus alustrisAY284668Tylenchus dipatusAY284733Eutero	Cervidellus alutus	AF202152	Pellioditis mediterranea	AF083020
Chordodes morganiAF036639Plectus acuminatusAF037628Clarkus papillatusAY284748Poikilolaimus oxycercaAF033023Comansus parvusAY284766Pratylenchoides ritteriAJ966497Coslenchus costatusAY284766Pratylenchus thorneiAY284612Cruznema tripartitaU73449Priapulus caudatusZ38009Cylindrolaimus communisAY593939Prionchulus punctatusAY284746Deladenus siriciticolaAY633447Prismatolaimus dolichurusAY593957Diphtherophora obesaAY284838Pristionchus lheritieriAF036640Diplogaster rivalisProdorylaimus masAY284638Diplogaster rivalisProdorylaimus masAY284639Dorylaimoides micoletzkyiAY284689Protorhabditis sp.AF083001Dirlocephalobus sp.AY284680Rhabditella axeiAY284593Drilocephalobus sp.AY284680Rhabditella axeiAY284593Enchodelus sp.AY284792Rhabditoides reginaAF082907Eucephalobus striatusAY284666Rolynehus robustusAJ966503Eudorylaimus carteriAJ966484Seinura sp.AY284792Eudorylainus carteriAJ966484Teratorcephalus terrestrisAY284793Filenchus filjornisAY284592Thonus ct. circuliferAY284787Gordius apuaticusX80233Thorai steatopygaAY284787Filenchus filifornisAY284666Tichodorus variopapillatusAY284787Filenchus leptosomaAY284688Thornia steatopygaA	Chiloplacus propinguus	AY284677	Pelodera teres	AF083002
Clarkus papillausAY284748Poikilolaimus oxycercaAF083023Coomansus parvusAY284766Pratylenchoides ritteriAJ966497Coslenchus costatusAY284581Pratylenchus thorneiAY284612Cruznema tripartitaU73449Priapulus caudatusZ38009Cylindrolaimus communisAY393939Prionchulus punctatusAY284746Deladenus siricidicolaAY633447Prismatolaimus dolichurusAY593957Diphtherophora obesaAY284688Pristionchus lheritieriAF036640Diplogaster rivalisProdorylaimus masAY593964Diplogaster rivalisProdorylaimus masAY284638Dorylaimoides micoletzkyiAY284689Protorhabditis sp.AF083001Diylenchus dipsaciAY284680Rhabditella axeiAY284659Drilocephalobus sp.AY284680Rhabditella axeiAY284654Enchodelus sp.AY284666Rotylenchus robustusAJ966503Eucophalobus striatusAY284666Rotylenchus robustusAJ284651Euwonhystera filiformisAY284684Feratorbalditis synpapillataAF083097Filenchus filiformisAY284684Feratorbalditis synpapillataAF083037Filenchus filiformisAY284684Feratorbalditis synpapillataAF083097Filenchus filiformisAY284666Trichodorus variopapillatusAY284681Euterotocephalus palustrisAY284684Feratorbalditis synpapillataAF083097Filenchus filiformisAY284666Trichodorus variopapillatusAY284787Helico	Chordodes morgani	AF036639	Plectus acuminatus	AF037628
Coomanus parvusAY284766Pratylenchoides riteriAJ966497Coslenchus costatusAY284581Pratylenchuis thorneiAY284612Cruznema tripartitaU73449Priapulus caudatusZ38009Cylindrolaimus communisAY593939Prionchulus punctatusAY284746Deladenus siricidicolaAY633447Prismatolaimus dolichurusAY593957Diphtherophora obesaAY284788Pristionchus lheritieriAF036640Diplogaster rivalisProdorylaimus masAY593946Diplogaster dipasciAY284689Protorhabditis sp.AF083001Diylenchus dipsaciAY284680RhabditelhausAY284638Dorilocephalobus sp.AY284680Rhabditella axeiAY284639Drilocephalobus sp.AY284666Rotylenchus reginaAF082997Eucephalobus striatusAY284792Rhabditioides reginaAF082997Eucephalobus striatusAY284666Rotylenchus robustusAY284651Eutonchystera filiformisAY284684Teratorephalus terrestrisAY284792Gordius aquaticusX80233Thornia steatopygaAY284783HelicotylenchusAY284666Trichodorus variopapillataAF083015Filenchus filformisAY284684Teratorchalus terrestrisAY284792Gordius aquaticusX80233Thornia steatopygaAY284787HelicotylenchusAY284686Tylencholaimus mirabilitsEF207253Letencephalobus elongatusAY284684Teratorhabitis synapillatusAY284783HelicotylenchusAY284686<	Clarkus papillatus	AY284748	Poikilolaimus oxvcerca	AF083023
Coslenchus costatusAY284581Praylenchus thorneiAY284612Cruznema tripartitaU73449Priapulus caudatusZ38009Cylindrolaimus communisAY593939Prionchulus punctatusAY284746Deladenus siricidicolaAY633447Prismatolaimus dolichurusAY284746Diphterophora obesaAY284838Pristionchus lheritieriAF036640Diplogaster rivalisProdorylaimus masAY593957Diplogastri nematodeAY284689Protorhabditis sp.AF083001Ditylenchus dipsaciAY284689Protorhabditis sp.AF083001Ditylenchus dipsaciAY284680Rhabdinella axeiAY284533Drilocephalobus sp.AY284680Rhabditella axeiAY284653Enchodelus sp.AY284792Rhabditioides reginaAF082997Eucephalobus striatusAY284666Rotylenchus robustusAJ966503Eudorylaimus carteriAJ966484Seinura sp.AY284651Eumonhystera filiformisAY284592Thonus cf. circuliferAY284792Gordius aquaticusX80233Thornia steatopygaAY284793HelicotylenchusAY284666Trichodorus variopapillatusAY284793HelicotylenchusAY284684Thornia steatopygaAY284787HelicotylenchusAY284684Thornia steatopygaAY284733Leenchus leptosomaAY284684Tylenchus davaineiAY284733Leenchus leptosomaAY284684Tylenchus davaineiAY284733Leenchus leptosomaAY284684Tylenchus davaineiAY284733	Coomansus parvus	AY284766	Pratylenchoides ritteri	AJ966497
Cruznema tripartitaU73449Priapulus caudatusZ38009Cylindrolaimus communisAY593939Prionchulus punctatusAY284746Deladenus siricidicolaAY633447Prismatolaimus dolichurusAY593957Diphtherophora obesaAY284838Pristionchus IheritieriAF036640Diplogaster rivalisProdorylaimus masAY593946Diplogastri nematodeAY284689Protorhabditis sp.AF083001Ditylenchus dipsaciAY284680Psilenchus minutusAY284593Dorylaimoides micoletzkyiAY284680Rhabditella axeiAY284654Ecphyadophora tenuissimaAY284660Rhabditella axeiAY284654Enchodelus sp.AY284792Rhabditoides reginaAF082097Eucephalobus striatusAY284666Rotylenchus robustusAJ966503Eudorylaimus carteriAJ966484Seinura sp.AY284683Euteratocephalus trissAY284684Teratorphalus terrestrisAY284683Euteratocephalus palustrisAY284684Teratorphalus terrestrisAY284792Filenchus filiformisAY284684Teratorphalus terrestrisAY284683Euteratocephalus palustrisAY284684Teratorphalus terrestrisAY284792Gordius aquaticusX80233Thornia steatopygaAY284787HelicotylenchusAY284666Trichodorus variopapillatusAY284730Heterocephalobus elongatusAY284684Tylenchus davaineiAY284730Heterocephalobus elongatusAY284684Tylenchus davaineiAY284730Heteroceph	Coslenchus costatus	AY284581	Pratylenchus thornei	AY284612
Cylindrolainus communisAY593939Prionchulus punctatusAY284746Deladenus siricidicolaAY633447Prismatolainus dolichurusAY593957Diphtherophora obesaAY284838Pristionchus IheritieriAF036640Diplogaster rivalisProdorylainus masAY593946Diplogastrid nematodeAY284689Protorhabditis sp.AF083001Ditylenchus dipsaciAY284689Protorhabditis sp.AY284583Dorylaimoides micoletzkyiAY284680Rhabditella axeiAY284593Drilocephalobus sp.AY284680Rhabditella axeiAY284654Ecphyadophora tenuissimaRY284792Rhabditiodes reginaAF082997Eucephalobus sp.AY284666Rotylenchus robustusAJ266503Eudorylainus carteriAJ966484Seinura sp.AY284654Eutenotocephalus palustrisAY284684Teratocephalus terrestrisAY284654Eutenchus filiformisAY284592Thonus cf. circuliferAY284795Gordius aquaticusX80233Thornia steatopygaAY284787HelicotylenchusAY284666Trichodarus variopapillatusAY284787Heterocephalobus elongatusAY284684Tylenchus mirabilisEF207253BuedorobustusAY284584Tylenchus davaineiAY284588Malenchus andrassyiAY284587Zygotylenchus guevaraiAY284588	Cruznema tripartita	U73449	Priapulus caudatus	Z38009
Deladenus siricidicolaAY633447Prismatolainus dolichurusAY593957Diphtherophora obesaAY284838Pristionchus IheritieriAF036640Diplogaster rivalisProdorylainus masAY593946Diplogastri nematodeAY284689Protorhabditis sp.AF083001Ditylenchus dipsaciAY284689Protorhabditis sp.AF083001Ditylenchus dipsaciAY284680Psilenchus cf. hilarulusAY284539Dorylaimoides micoletzkyiAY284680Rhabditella axeiAY284593Drilocephalobus sp.AY284680Rhabditella axeiAY284654Ecphyadophora tenuissimaU13935Enchodelus sp.AY284666Eucephalobus striatusAY284666Rotylenchus robustusAJ966503Eudorylaimus carteriAJ966484Seinura sp.AY284651Euteratocephalus palustrisAY284684Teratorephalus terrestrisAY284795Gordius aquaticusX80233Thornia steatopygaAY284795Gordius aquaticusAY284668TylencholatiusAY284792Heterocephalobus elongatusAY284668TylencholatiusAY284795Lelenchus leptosomaAY284684TeratorpapillatusAY284795BeudorobustusAY284668TylencholatiusAY284795Gordius aquaticusAY284668TylencholatiusAY284730Heterocephalobus elongatusAY284684Tripla cf. filicaudataAY284730Heterocephalobus elongatusAY284684Tripla cf. filicaudataAY284730Heterocephalobus elongatusAY284587Zy	Cylindrolaimus communis	AY 593939	Prionchulus punctatus	AY284746
Diphtherophora obesaAY284838Pristionchus IheritieriAF036640Diplogaster rivalisProdorylaimus masAY593946Diplogastrid nematodeAY284689Protorhabditis sp.AF083001Ditylenchus dipsaciAY593911Pseudhalenchus minutusAY284638Dorylaimoides micoletzkyiAY284830Psilenchus cf. hilarulusAY284593Drilocephalobus sp.AY284680Rhabditella axeiAY284654Ecphyadophora tenuissimaU13935Enchodelus sp.AY284666Eucephalobus striatusAY284666Rotylenchus reginaAF082997Eucephalobus striatusAY284666Rotylenchus robustusAY284651Eudorylaimus carteriAJ966484Seinura sp.AY284683Euteratocephalus palustrisAY284684Teratorhabditis synpapillataAF083015Filenchus filiformisAY284684Teratorhabditis synpapillataAF083015Filenchus filiformisAY284606Trichodorus variopapillatusAY284795Gordius aquaticusX80233Thornia steatopygaAY284737Heterocephalobus elongatusAY284668Tylenchus davaineiAY284588Malenchus andrassyiAY284587Zygotylenchus guevaraiAY284588	Deladenus siricidicola	AY633447	Prismatolaimus dolichurus	AY593957
DiplogastriProdorylaimus masAY593946Diplogastrid nematodeAY284689Protorhabditis sp.AF083001Ditylenchus dipsaciAY593911Pseudhalenchus minutusAY284638Dorylaimoides micoletzkyiAY284830Psilenchus cf. hilarulusAY284593Drilocephalobus sp.AY284680Rhabditella axeiAY284654Ecphyadophora tenuissimaU13935Enchodelus sp.AY284666Eucephalobus striatusAY284666Rotylenchus robustusAJ966503Eucephalobus striatusAY284666Rotylenchus robustusAJ284651Eumonhystera filiformisAY284684Teratorhabditis synpapillataAF083015Filenchus filiformisAY284684Teratorhabditis synpapillataAF083015Filenchus filiformisAY284692Thonus cf. circuliferAY284792Gordius aquaticusX80233Thornia steatopygaAY284787HelicotylenchusAY284668Tylencholaimus mirabilisEF207253Lelenchus leptosomaAY284587Zygotylenchus guevaraiAY284588	Diphtherophora obesa	AY284838	Pristionchus lheritieri	AF036640
Diplogastrid nematodeAY284689Protriabditis sp.AF083001Ditylenchus dipsaciAY593911Pseudhalenchus minutusAY284638Dorylaimoides micoletzkyiAY284830Psilenchus cf. hilarulusAY284593Drilocephalobus sp.AY284680Rhabditella axeiAY284654Ecphyadophora tenuissimaU13935Enchodelus sp.AY284666Eucephalobus striatusAY284666Rotylenchus robustusAJ966503Eucephalobus striatusAY284666Rotylenchus robustusAJ284651Eumonhystera filiformisAY284684Teratorhabditis synpapillataAF083015Filenchus filiformisAY284684Teratorhabditis synpapillataAF083015Filenchus filiformisAY284684Teratorhabditis synpapillataAF083015Filenchus filiformisAY284686Trichodorus variopapillatusAY284792Gordius aquaticusX80233Thornia steatopygaAY284730HelicotylenchusAY284668Tylencholaimus mirabilisEF207253Lelenchus leptosomaAY284584Tylencholaimus mirabilisEF207253Malenchus andrassyiAY284587Zygotylenchus guevaraiAF442189	Diplogaster rivalis		Prodorylaimus mas	AY593946
Ditylenchus dipsaciAY593911Pseudhalenchus minutusAY284638Dorylaimoides micoletzkyiAY284830Psilenchus cf. hilarulusAY284593Drilocephalobus sp.AY284680Rhabditella axeiAY284654Ecphyadophora tenuissimaI13935I113935I113935Enchodelus sp.AY284792Rhabditoides reginaAF082997Eucephalobus striatusAY284666Rotylenchus robustusAJ966503Eudorylaimus carteriAJ966484Seinura sp.AY284651Euteratocephalus palustrisAY284684Teratorhabditis synpapillataAF083015Filenchus filiformisAY284684Teratorhabditis synpapillataAF083015Filenchus filiformisAY284692Thonus cf. circuliferAY284795Gordius aquaticusX80233Thornia steatopygaAY284787HelicotylenchusAY284668Tylencholaimus mirabilisEF207253Lelenchus leptosomaAY284584Tylenchus davaineiAY284588Malenchus andrassyiAY284587Zygotylenchus guevaraiAF442189	Diplogastrid nematode	AY284689	Protorhabditis sp.	AF083001
Dorylaimoides micoletzkyiAY284830Psilenchus cf. hilarulusAY284593Drilocephalobus sp.AY284680Rhabditella axeiAY284654Ecphyadophora tenuissimaU13935Enchodelus sp.AY284792Rhabditoides reginaAF082997Eucephalobus striatusAY284666Rotylenchus robustusAJ966503Eudorylaimus carteriAJ966484Seinura sp.AY284683Euteratocephalus palustrisAY284684Teratorephalus terrestrisAY284683Euteratocephalus palustrisAY284684Teratorhabditis synpapillataAF083015Filenchus filiformisAY284692Thonus cf. circuliferAY284795Gordius aquaticusX80233Thornia steatopygaAY284787HelicotylenchusAY284666Trichodorus variopapillatusAY284730Heterocephalobus elongatusAY284684Tylenchus davaineiAY284588Malenchus andrassyiAY284587Zygotylenchus guevaraiAF442189	Ditylenchus dipsaci	AY593911	Pseudhalenchus minutus	AY284638
Drilocephalobus sp.AY284680Rhabditella axeiAY284654Ecphyadophora tenuissimaU13935Enchodelus sp.AY284792Rhabditoides reginaAF082997Eucephalobus striatusAY284666Rotylenchus robustusAJ966503Eudorylaimus carteriAJ966484Seinura sp.AY2846651Eumonhystera filiformisAY284684Teratocephalus terrestrisAY284683Euteratocephalus palustrisAY284684Teratorhabditis synpapillataAF083015Filenchus filiformisAY284592Thonus cf. circuliferAY284795Gordius aquaticusX80233Thornia steatopygaAY284787HelicotylenchusAY284668Tylencholaimus mirabilisEF207253Lelenchus leptosomaAY284587Zygotylenchus guevaraiAF442189	Dorylaimoides micoletzkyi	AY284830	Psilenchus cf. hilarulus	AY284593
Ecphyadophora tenuissimaRhabditis blumiU13935Enchodelus sp.AY284792Rhabditoides reginaAF082997Eucephalobus striatusAY284666Rotylenchus robustusAJ966503Eudorylaimus carteriAJ966484Seinura sp.AY284661Eumonhystera filiformisAY593937Teratocephalus terrestrisAY284683Euteratocephalus palustrisAY284684Teratorhabditis synpapillataAF083015Filenchus filiformisAY284592Thonus cf. circuliferAY284795Gordius aquaticusX80233Thornia steatopygaAY284787HelicotylenchusAY284606Trichodorus variopapillatusAY284730Heterocephalobus elongatusAY284668Tylencholaimus mirabilisEF207253Lelenchus leptosomaAY284587Zygotylenchus guevaraiAF442189	Drilocephalobus sp.	AY284680	Rhabditella axei	AY284654
Encodelus sp.AY284792Rhabditoides reginaAF082997Eucephalobus striatusAY284666Rotylenchus robustusAJ966503Eudorylaimus carteriAJ966484Seinura sp.AY284651Eumonhystera filiformisAY593937Teratocephalus terrestrisAY284683Euteratocephalus palustrisAY284684Teratorhabditis synpapillataAF083015Filenchus filiformisAY284592Thonus cf. circuliferAY284795Gordius aquaticusX80233Thornia steatopygaAY284787HelicotylenchusAY284606Trichodorus variopapillatusAY284730Heterocephalobus elongatusAY284668Tylencholaimus mirabilisEF207253Lelenchus leptosomaAY284587Zygotylenchus guevaraiAF442189	Ecphyadophora tenuissima		Rhabditis blumi	U13935
Eucephalobus striatusAY284666Rotylenchus robustusAJ966503Eudorylaimus carteriAJ966484Seinura sp.AY284651Eumonhystera filiformisAY593937Teratocephalus terrestrisAY284683Euteratocephalus palustrisAY284684Teratorhabditis synpapillataAF083015Filenchus filiformisAY284592Thonus cf. circuliferAY284795Gordius aquaticusX80233Thornia steatopygaAY284787HelicotylenchusAY284606Trichodorus variopapillatusAY284841pseudorobustusTripyla cf. filicaudataAY284730Heterocephalobus elongatusAY284668Tylencholaimus mirabilisEF207253Lelenchus leptosomaAY284584Tylenchus guevaraiAF442189	Enchodelus sp.	AY284792	Rhabditoides regina	AF082997
Eudorylaimus carteriAJ966484Seinura sp.AY284651Eumonhystera filiformisAY593937Teratocephalus terrestrisAY284683Euteratocephalus palustrisAY284684Teratorhabditis synpapillataAF083015Filenchus filiformisAY284592Thonus cf. circuliferAY284795Gordius aquaticusX80233Thornia steatopygaAY284787HelicotylenchusAY284606Trichodorus variopapillatusAY284841pseudorobustusTripyla cf. filicaudataAY284730Heterocephalobus elongatusAY284668Tylencholaimus mirabilisEF207253Lelenchus leptosomaAY284584Tylenchus guevaraiAF284588	Eucephalobus striatus	AY284666	Rotylenchus robustus	AJ966503
Eumonhystera filiformisAY593937Teratocephalus terrestrisAY284683Euteratocephalus palustrisAY284684Teratorhabditis synpapillataAF083015Filenchus filiformisAY284592Thonus cf. circuliferAY284795Gordius aquaticusX80233Thornia steatopygaAY284787HelicotylenchusAY284606Trichodorus variopapillatusAY284841pseudorobustusTripyla cf. filicaudataAY284730Heterocephalobus elongatusAY284668Tylencholaimus mirabilisEF207253Lelenchus leptosomaAY284584Tylenchus davaineiAY284588Malenchus andrassyiAY284587Zygotylenchus guevaraiAF442189	Eudorylaimus carteri	AJ966484	Seinura sp.	AY284651
Euteratocephalus palustrisAY284684Teratorhabditis synpapillataAF083015Filenchus filiformisAY284592Thonus cf. circuliferAY284795Gordius aquaticusX80233Thornia steatopygaAY284787HelicotylenchusAY284606Trichodorus variopapillatusAY284841pseudorobustusTripyla cf. filicaudataAY284730Heterocephalobus elongatusAY284668Tylencholaimus mirabilisEF207253Lelenchus leptosomaAY284584Tylenchus davaineiAY284588Malenchus andrassyiAY284587Zygotylenchus guevaraiAF442189	Eumonhystera filiformis	AY593937	Teratocephalus terrestris	AY284683
Filenchus filiformisAY284592Thonus cf. circuliferAY284795Gordius aquaticusX80233Thornia steatopygaAY284787HelicotylenchusAY284606Trichodorus variopapillatusAY284841pseudorobustusTripyla cf. filicaudataAY284730Heterocephalobus elongatusAY284668Tylencholaimus mirabilisEF207253Lelenchus leptosomaAY284584Tylenchus davaineiAY284588Malenchus andrassyiAY284587Zygotylenchus guevaraiAF442189	Euteratocephalus palustris	AY284684	Teratorhabditis synpapillata	AF083015
Gordius aquaticusX80233Thornia steatopygaAY284787HelicotylenchusAY284606Trichodorus variopapillatusAY284841pseudorobustusTripyla cf. filicaudataAY284730Heterocephalobus elongatusAY284668Tylencholaimus mirabilisEF207253Lelenchus leptosomaAY284584Tylenchus davaineiAY284588Malenchus andrassyiAY284587Zygotylenchus guevaraiAF442189	Filenchus filiformis	AY284592	Thonus cf. circulifer	AY284795
HelicotylenchusAY284606Trichodorus variopapillatusAY284841pseudorobustusTripyla cf. filicaudataAY284730Heterocephalobus elongatusAY284668Tylencholaimus mirabilisEF207253Lelenchus leptosomaAY284584Tylenchus davaineiAY284588Malenchus andrassyiAY284587Zygotylenchus guevaraiAF442189	Gordius aquaticus	X80233	Thornia steatopyga	AY284787
pseudorobustusTripyla cf. filicaudataAY284730Heterocephalobus elongatusAY284668Tylencholaimus mirabilisEF207253Lelenchus leptosomaAY284584Tylenchus davaineiAY284588Malenchus andrassyiAY284587Zygotylenchus guevaraiAF442189	Helicotylenchus	AY284606	Trichodorus variopapillatus	AY284841
Heterocephalobus elongatusAY284668Tylencholaimus mirabilisEF207253Lelenchus leptosomaAY284584Tylenchus davaineiAY284588Malenchus andrassyiAY284587Zygotylenchus guevaraiAF442189	pseudorobustus		Tripyla cf. filicaudata	AY284730
Lelenchus leptosomaAY284584Tylenchus davaineiAY284588Malenchus andrassyiAY284587Zygotylenchus guevaraiAF442189	Heterocephalobus elongatus	AY284668	Tylencholaimus mirabilis	EF207253
Malenchus andrassyi AY284587 Zygotylenchus guevarai AF442189	Lelenchus leptosoma	AY284584	Tylenchus davainei	AY284588
	Malenchus andrassyi	AY284587	Zygotylenchus guevarai	AF442189