New genera in the *Calosphaeriales*: *Togniniella* and its anamorph *Phaeocrella,* and *Calosphaeriophora* as anamorph of *Calosphaeria*

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Abstract: During a survey of perithecial ascomycetes in New Zealand, two collections of a *Togninia*-like fungus were made on decayed wood. In culture, colonies produced a *Phaeoacremonium*-like anamorph. In order to reveal the phylogenetic relationships of the unknown fungus and its affinity to *Togninia* and other genera in the *Calosphaeriales*, sequences of nuclear LSU and SSU ribosomal DNA were obtained of several members of this order. These data, supported by morphological and cultural characteristics, confirm that the New Zealand fungus represents a new genus very close to *Calosphaeria*. The genus *Togniniella* is proposed here to accommodate these collections, while *Phaeocrella* is established for their anamorphs. Furthermore, *Calosphaeria pulchella* was found to form a distinct *Acremonium*-like anamorph in culture, for which the genus *Calosphaeriophora* is proposed. *Pleurostoma* with *Pleurostomophora* anamorphs is a sister genus to the above two genera, forming the *Pleurostomataceae*. *Togninia* with its *Phaeoacremonium* anamorphs, together with *Jobellisia*, are closer to the *Diaporthales*, and deserve the rank of family, for which *Togniniaceae* is proposed. The presence of significantly different anamorphs in the *Calosphaeriales*, as well as obvious differences in teleomorph morphology of species accommodated in *Calosphaeria*, suggest that both the *Calosphaeriales* and *Calosphaeria* as presently perceived, are polyphyletic.

Taxonomic novelties: *Calosphaeriophora* Réblová, L. Mostert, W. Gams & Crous gen. nov., *Calosphaeriophora pulchella* Réblová, L. Mostert, W. Gams & Crous sp. nov., *Phaeocrella* Réblová, L. Mostert, W. Gams & Crous gen. nov., *Phaeocrella acerosa* Réblová, L. Mostert, W. Gams & Crous sp. nov., *Pleurostomataceae* Réblová, L. Mostert, W. Gams & Crous fam. nov., *Togniniaceae* Réblová, L. Mostert, W. Gams & Crous fam. nov., *Togniniella* Réblová, L. Mostert, W. Gams & Crous gen. nov., *Togniniella acerosa* Réblová, L. Mostert, W. Gams & Crous sp. nov.
Key words: *Acremonium,* LSU and SSU rDNA phylogeny, *Phaeoacremonium*, *Phialophora*, systematics, *Togninia*.

INTRODUCTION

Traditionally, a number of small pyrenomycetous genera with simple, dark perithecia (occasionally embedded in a stroma), unitunicate asci, hyaline to slightly pigmented, ellipsoid to allantoid ascospores, have been classified in the *Calosphaeriales*. These fungi occupy similar or highly specialized habitats and they have been seen by only a handful of mycologists. Munk (1957) described the *Calosphaeriaceae*, and assigned the family to the broadly perceived *Sphaeriales*. He drew attention to the unique centrum present within ascomata of these fungi, suggesting that it could be used as basis for recognizing a separate order among perithecial ascomycetes. The order *Calosphaeriales* was later recognized by Barr (1983), who outlined the history of the *Calosphaeriaceae* and the respective genera, and published the first modern concept of this family (Barr 1985, Barr *et al*. 1993).

Several researchers have noted that the *Calosphaeriales* represent a polyphyletic group of phenotypically similar taxa that may comprise at least two phylogenetic lineages (Barr *et al*. 1993, Samuels & Candoussau 1996, Barr 1998), *viz.* the putative diatrypaceous lineage (*Diatrypaceae*/*Xylariales*) on the one hand, and the diaporthaceous lineage (apparently the *Gnomoniaceae*/*Diaporthales*) on the other hand. After exclusion of the stromatic calosphaeriaceous family *Graphostromataceae*, associated with *Nodulosporium*-like anamorphs in the *Xylariales* (Barr *et al*. 1993), the *Calosphaeriales* of the *Diaporthales* lineage encompassed six nonstromatic genera, i.e. *Calosphaeria* Tul. & C. Tul., *Jattaea* Berl., *Pleurostoma* Tul. & C. Tul., *Romellia* Berl., *Wegelina* Berl.*,* and *Togninia* Berl., and the stromatic *Pachytrype* Berl. ex M.E. Barr *et al.* (Barr *et al.* 1993). The position of *Enchnoa* Fr. within the *Calosphaeriales* is debatable (Petrak & Sydow 1936, Barr 1985). Apart from *Graphostroma* Piroz. (Pirozynski 1974), we do not have phylogenetic clues to the true relationships of genera within the *Calosphaeriales*.

Current generic concepts in the *Calosphaeriales* are based primarily on the arrangement of ascomata, neck lengths, presence and arrangement of stromatic tissue or subiculum, and arrangement of the asci, *viz.* in

spicate arrangement or in small fascicles (Barr 1985). The perithecia of the *Calosphaeriales* are superficial or immersed in wood, arise separately with separate necks, or are tightly aggregated in circinate groups on wood beneath the periderm, with radially converging beaks that may be united in a disc piercing the periderm. Genera assigned to the *Calosphaeriales* possess true, persistent, apically free paraphyses and hyaline, allantoid to suballantoid, aseptate or delicately 1-septate ascospores arranged 2–3-seriately or in a fascicle within the ascus.

The conidiogenesis of members of the *Calosphaeriales* is reported as either being phialidic or holoblastic. *Pachytrype* Berl. ex M.E. Barr *et al.* has a *Cytospora* Ehrenb. anamorph (Barr *et al.* 1993); *Calosphaeria fagi* Samuels & Candoussau has *Ramichloridium*-like and *Sporothrix*-like synanamorphs (Samuels & Candoussau 1996); *Calosphaeria pulchella* (Pers. : Fr.) J. Schröt. has an *Acremonium*-like anamorph (this study), and *Togninia* has *Phaeoacremonium* W. Gams *et al.* anamorphs (Hausner *et al.* 1992, Mostert *et al.* 2003). Except these few known life histories, the connections to asexual states are little known, nor are there DNA-based phylogenies to reveal likely sexual or asexual relatives.

Phaeoacremonium is a dematiaceous hyphomycete genus of approximately 17 species (Crous *et al.* 1996, Dupont *et al.* 2000, Groenewald *et al.* 2001, Mostert *et al.* 2004), introduced to include fungi that are intermediate between *Acremonium* Link : Fr. and *Phialophora* Medlar, encompassing ecologically important fungi associated with human infections and disease symptoms of woody hosts. The link between *Phaeoacremonium aleophilum* and *Togninia minima* (Tul. & C. Tul.) Berl., the type of the genus, was recently established *in vitro* by Mostert *et al.* (2003). *Togninia* Berl. has historically been classified in the *Calosphaeriaceae* of the *Calosphaeriales* (Berlese 1900, Barr 1985, Eriksson *et al.* 2003, Mostert *et al.* 2003).

During a survey of perithecial ascomycetes in New Zealand in March and February 2003, two collections of a minute, lignicolous, saprobic *Togninia*-like species were encountered. In culture, colonies produced a *Phaeoacremonium*-like anamorph. Herbarium material of a fungus originating in North America, Canada, whose morphological characteristics match well those of the fungus collected in New Zealand, was found in the DAOM herbarium. The unknown fungus resembles *Togninia* in having dark, nonstromatic perithecia with elongate necks; asci arranged in a spicate formation along elongate ascogenous hyphae; a thickened ascal apex lacking any discharge mechanism; true paraphyses; hyaline, aseptate, suballantoid ascospores and a dematiaceous hyphomycete anamorph with phialidic conidiogenesis. The unknown fungus differs from *Togninia* in the shape of the asci, which are apically obtuse, tapering conspicuously towards the base from

the sporiferous portion; the fasciculate arrangement of the ascospores in the upper part of the ascus; the presence of short cells along the ascogenous hyphae, from which each ascus arises as an outgrowth, and the branching pattern of conidiophores of the *Phaeoacremonium*-like anamorph *vs.* asci with obtuse to broadly rounded bases; 2–3-seriately arranged ascospores filling the entire ascus; elongate ascogenous hyphae with attached remnants of the basal parts of the asci after their separation, and *Phaeoacremonium* anamorphs of *Togninia*.

Based on the ecology and the teleomorph and anamorph morphology, the six nonstromatic calosphaeriaceous genera, including the unknown fungus, can be compared with the *Gnomoniaceae* of the *Diaporthales* and the *Magnaporthaceae* (order uncertain).

The *Diaporthales* include either saprobes or endophytes fruiting on moribund tissue, or plant-pathogenic fungi divided into six well-supported phylogenetic lineages, *viz.* the four families *Diaporthaceae*, *Gnomoniaceae*, *Melanconidaceae* and *Valsaceae*, and three less resolved species complexes for which no families are currently available, namely the *Schizoparme* complex, the *Wuestneia* complex, and the *Cryphonectria-Endothia* complex, as evidenced by sequences of the large-subunit nuclear ribosomal DNA (Castlebury *et al.* 2002, Lee *et al.* 2004, this volume). The order is also well-defined morphologically based on dark, beaked perithecia with opaque walls immersed in stromata or freely in host tissue; lack of true paraphyses; unitunicate, short-stipitate asci, rounded at the bottom and often floating free within the centrum at maturity, with a distinct, refractive ring in the ascal apex (Barr 1978, 1990). The known asexual states of members of the *Diaporthales* have been linked to coelomycetous fungi forming pycnidia or acervuli with or without stromata and generally with phialidic, rarely annellidic conidiogenesis. The *Gnomoniaceae* are a phylogenetically (Castlebury *et al.* 2002) and morphologically (Monod 1983) well-defined group within the *Diaporthales*, whose members bear some resemblance to taxa placed in the *Calosphaeriales*, especially in characters of the perithecia, asci and ascospores. The *Gnomoniaceae* include taxa with dark, upright perithecia immersed in herbaceous tissue or wood and erumpent separately, with central, rarely eccentric necks, without stromata or surrounded by a reduced prosenchymatous stroma, with asci basally rounded and hyaline ascospores with variable septation. The anamorphs linked to this family have phialidic conidiogenous cells.

The *Magnaporthaceae* are of uncertain ordinal affiliation within the *Sordariomycetes* (Cannon 1994, Kirk *et al.* 2001, Eriksson *et al.* 2003). The family was erected by Cannon (1994) for six nonstromatic perithe-

Table 1. List of sources, hosts and accession numbers of fungi sequenced in this study.

Fungus	CBS number	Source	Host	GenBank accession number	
				SSU	LSU
Calosphaeria pulchella	115999	France, Pyrénées Atlan-tiques, Ariège	*Prunus avium*	AY761071	AY761075
Togniniella acerosa	113648	New Zealand, South Island, Harihari	Decayed wood	AY761073	AY761076
	113726	New Zealand, South Island, St. Arnaud	*Nothofagus* sp.	AY761072	AY761077
Pleurostomophora richardsiae	270.33	Sweden	Unknown	AY761066	AY761080
Pleurostomophora repens	294.39	United States, Florida, Caryville	Pine lumber	AY761067	AY761078
Pleurostoma ootheca	115329	Thailand, Chiang Mai Province, Mae Taeng, Mokfa	Wood on forest floor	AY761074	AY761079
Togninia minima	213.31	Italy	Unknown	AY761068	AY761082
Togninia fraxinopenn-sylvanica	101585	United States, California	*Vitis vinifera*	AY761070	AY761083
Togninia novae-zealandiae	110156	New Zealand, Auckland, Woodhill State Forest	*Desmoschoenus spiralis*	AY761069	AY761081

cial genera with conspicuous similarities in teleomorph morphology, but whose anamorphs are greatly variable. Members of the family are primarily known as important plant pathogens, specialized as necrotrophic parasites attacking roots and stems, with a preference for *Gramineae* and *Cyperaceae*.

The seven associated anamorph genera form pycnothyrial conidiomata with phialidic conidiogenous cells (*Mycoleptodiscus* Ostazeski, *Pseudotracylla* B. Sutton & Hodges), or they are hyphomycetous, with phialidic (*Harpophora* W. Gams, *Phialophora*-like), or holoblastic, denticulate with rhexolytic secession (*Nakataea* Hara and *Pyricularia* Sacc.), or holoblastic with schizolytic secession (*Clasterosporium* Schwein.). Currently, the *Magnaporthaceae* accommodate nine teleomorph genera (Eriksson *et al.* 2003), including *Gaeumannomyces* Arx & D.L. Olivier (Cannon 1994, Zhang & Blackwell 2001), originally placed in the *Gnomoniaceae* by Monod (1983).

In order to reveal the phylogenetic relationships of the unknown fungus and its *Phaeoacremonium*-like anamorph and its affinity to *Togninia* and *Calosphaeria* and the *Calosphaeriales*, sequences of nuclear LSU and SSU ribosomal DNA of *T. minima*, *T. novaezelandiae* Hausner *et al.*, *T. fraxinopennsylvanica* (Hinds) Hausner *et al.*, *C. pulchella* (the type of *Calosphaeria*), *Pleurostoma ootheca* (Berk. & M.A. Curtis) M.E. Barr, and the unknown fungus were analyzed in two independent sequence data sets using neighbour-joining and maximum parsimony analyses. The phylogenetic relationships of the *Calosphaeriales* to the *Diaporthales* and the *Magnaporthaceae* within the *Sordariomycetes* were tested using homologous LSU and SSU rDNA sequences of representatives of a further 12 ascomycetous orders or families.

MATERIALS AND METHODS

Isolates
Dried herbarium specimens were rehydrated in 3 % (aq.) KOH and studied in water, Melzer's reagent or 90 % lactic acid. All measurements were made in lactic acid. Means ± standard errors (se) based on 30 measurements are given for spore, ascus and conidial dimensions. The length/width ratios (L/W) for asci are given. Images were captured in Melzer's reagent using differential interference microscopy (DIC) and phase contrast (PC) and processed using Adobe Photoshop 6.0 CE.

Single-ascospore isolates were obtained from fresh material with the aid of a single-spore isolator (Meopta, Czech Republic). Cultures were grown on potato-carrot agar (PCA, Gams *et al.* 1998). Colony characters were taken from cultures grown on malt extract agar (MEA; 2 % Oxoid malt extract, 1.5 % Difco agar, 1000 mL water) and oatmeal agar (OA, Gams *et al.* 1998) and placed at 25 °C in the dark. Cardinal temperatures for growth were determined by incubating plates at temperatures ranging from 5 to 40 °C in 5 ° intervals, including 37 °C. Radial growth was determined by calculating the mean of two perpendicular radial measurements of three repeats for every isolate at each temperature after 8 d in the dark. Colony colours were determined after 8 d at 25 °C in the dark using Kornerup & Wanscher (1978). Cultures are maintained at the Centraalbureau voor Schimmelcultures, Utrecht (CBS) and Landcare Research, Auckland (ICMP). The isolates used in this study and their sources are listed in Table 1.

DNA extraction, amplification and sequencing
Genomic DNA was extracted from approximately 200 mg mycelium using the Bio101 FastDNA Kit (Qbiogene, Inc., Carlsblad, U.S.A.) according to the manufacturer's instructions using. Two gene regions were

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amplified. A fragment of approximately 1700 base pairs of the 5' end of the 18S rRNA (SSU) gene was amplified using the primers NS1, NS3, NS4, NS6 (White *et al*. 1990), and NS24 (Gargas & Taylor 1992). Approximately 1400 base pairs of the 5' end of the 28S rRNA (LSU) gene were amplified using primers LR0R (Rehner & Samuels 1994), LR3R, LR5 (Vilgalys & Hester 1990), and LR7. PCR reactions and sequencing were performed as described in Mostert *et al*. (2004).

Phylogeny

Phylogenetic relationships were examined using 58 LSU nrDNA and 57 SSU nrDNA sequences from 13 or 14 different orders or families of the *Sordariomycetes*, respectively, in order to cover the broad spectrum of perithecial ascomycetes and to reveal possible relatives of the taxa under study. Members of the *Dothideomycetes* were used as outgroups in both maximum parsimony analyses. New LSU and SSU nrDNA sequences were obtained for the following: the two ascospore isolates of the unknown fungus, *T. minima*, *T. novaezelandiae*, *T. fraxinopennsylvanica*, *C. pulchella*, *Pleurostoma ootheca* (all ascospore isolates) and *Phaeoacremonium aleophilum*, *Pleurostomophora repens* (R.W. Davidson) L. Mostert, W. Gams & Crous and *Pleurostomophora richardsiae* (Nannf. apud Melin & Nannf.) L. Mostert, W. Gams & Crous (all conidial isolates). Homologous LSU and SSU nrDNA sequences from 97 taxa were retrieved from GenBank; accession numbers are given in Figs 1 and 2.

All sequences were manually aligned in BioEdit 5.0.9 (Hall 1999). Predicted models of the secondary structure of the LSU and SSU rRNA molecules of *Saccharomyces cerevisiae* Meyen ex E.C. Hansen (Gutell 1993, Gutell *et al*. 1993) were used to improve the alignment. The models of the secondary structure of the LSU and SSU rRNA were highly consistent in all taxa. The insertion positions in the SSU rDNA sequences are named for the 5' flanking nucleotide and correspond to the positions of *S. cerevisiae* and *Escherichia coli* (Migula) Castell. & Chalm. (Gutell 1993, Gargas *et al*. 1995). The alignments are available in TreeBASE as M2047.

The phylogenetic analyses were performed with PAUP v. 4.0b10 (Swofford 2002) using maximum parsimony; heuristic search with stepwise-addition option with 1000 random taxon addition replicates and branch swapping (tree bisection-recognition, TBR), and distance analyses; neighbour-joining, Jukes & Cantor evolutionary model. For all analyses, ambiguously aligned positions were excluded. All characters were unordered and given equal weight during the analysis. Gaps were treated as missing data. Branch support for parsimony and distance analyses was estimated by performing 1000 bootstrap replicates with a full heuristic search consisting of 10 random-addition replicates for each bootstrap replicate. Constraint analyses were run using the Kishino-Hasegawa test as implemented in PAUP, with *Calosphaeria*, *Pleurostoma* and *Togninia*, or various interpretations of the *Calosphaeriales*, *Diaporthales* and *Magnaporthaceae* forced to be distinct and monophyletic.

RESULTS

Insertions in the SSU nrDNA of *Pleurostoma ootheca* and the *Togninia*-like fungus

In the SSU we have identified a 375-nt insertion from *P. ootheca* and a 90-nt insertion from the unknown fungus (both isolates). The insertions are located at 1170 (*P. ootheca*) and 1465 (unknown fungus) 5' flanking nucleotide positions of *Saccharomyces cerevisiae* SSU nrDNA and correspond to the insertion positions 943 and 1230 of *Escherichia coli*, respectively, according to Gutell (1993) and Gargas *et al*. (1995). The insertion in *P. ootheca* is the group I intron, the insertion of the unknown fungus represents the group II intron with the 5'- GT and AG- 3' splice sites.

Phylogenetic analysis of the LSU nrDNA sequence data

A maximum parsimony analysis (MP1) was performed using 361 phylogenetically informative characters in an alignment including 1240 nt from 58 taxa. Eight most parsimonious trees (MPT) were obtained [tree length 2086, consistency index (CI) = 0.377, retention index (RI) = 0.630, homoplasy index (HI) = 0.623] (Fig. 1). The trees differed in the arrangement of branches for the *Xylariales* clade, namely between the *Apiospora/Arthrinium* clade and *Amphisphaeria/Lepteutypa* clade. The taxa within the *Togninia/Phaeoacremonium* clade were a source of additional polytomy.
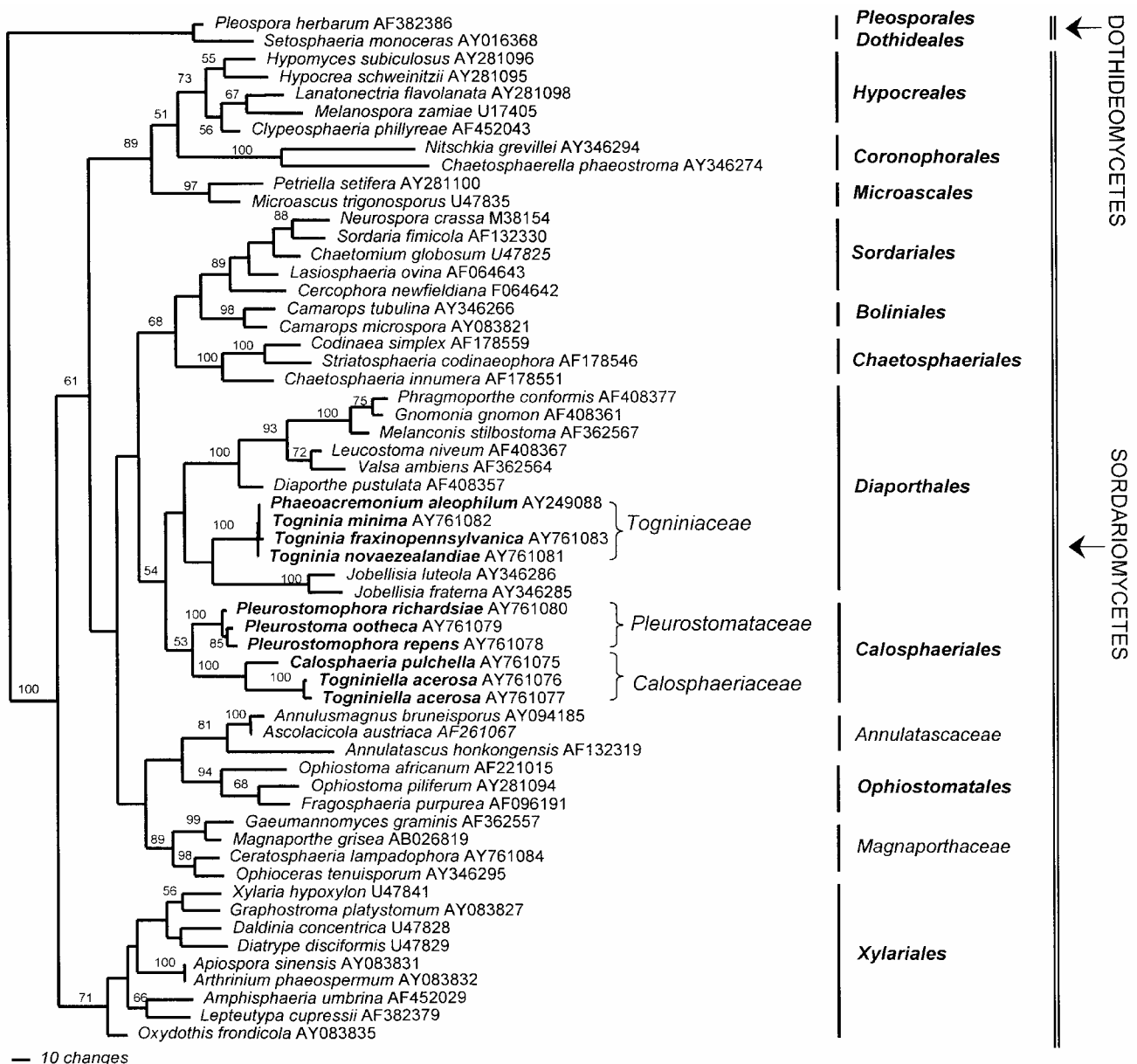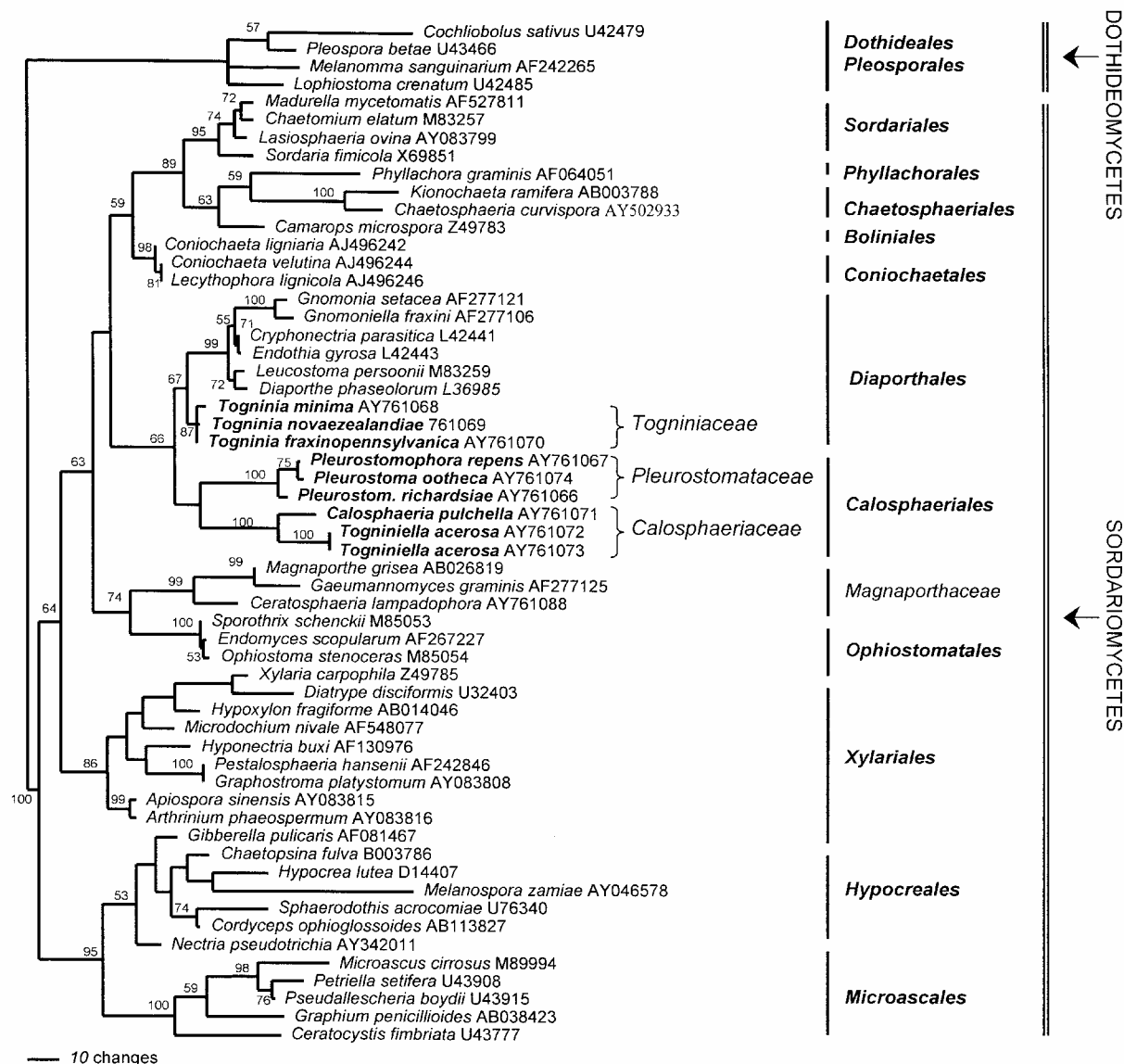
Fig. 1. One of eight equally parsimonious trees from a heuristic analysis of LSU nrDNA sequences. Bootstrap values (> 50 %) from 1000 replicates are included at the nodes. Branch lengths are drawn to scale.

The phylogenetic tree consisted of five major and well-supported phylogenetic lineages of the *Sordariomycetes*, i.e. a lineage (89 % bootstrap support) containing the *Hypocreales*, the *Coronophorales* (100 %) and the *Microascales* (97 %); a lineage (68 %) of the *Sordariales* (89 %), the *Chaetosphaeriales* (100 %) and the *Boliniales* (98 %); a lineage (54 %) of the *Diaporthales* (100 %), the *Jobellisia* clade (100 %) and the putative *Calosphaeriales* (see below); a lineage of the *Annulatascaceae* (81 %), the *Magnaporthaceae* (89 %) and the *Ophiostomatales* (94 %), and a lineage containing members of the *Xylariales* (71 %). The *Gnomoniaceae* represented by *Gnomonia gnomon* (Tode) Grev. in our LSU phylogeny grouped clearly within the *Diaporthales*.

Three calosphaeriaceous genera, *Calosphaeria*, *Togninia* and *Pleurostoma* and the unknown fungus with its *Phaeoacremonium*-like anamorph were divided into three separate lineages with an affinity to the *Diaporthales* within a robust clade, *viz.* *Calosphaeria*/unknown fungus (100 %), *Togninia*/*Phaeoacremonium* (100 %) and *Pleurostoma*/*Pleurostomophora* (100 %). The strongly supported *Togninia*/*Phaeoacremonium* clade grouped together with the *Jobellisia* clade as sister to the *Diaporthales* on one branch. The strongly supported *Pleurostoma*/*Pleurostomophora* and the *Calosphaeria*/unknown fungus clades formed the other branch.

The neighbour-joining (NJ1) analysis produced a tree (not shown) similar to MP1 consisting of two major lineages, i.e. the *Xylariales* (100 %) and a lineage (85 %) containing all other analysed taxa.

Fig. 2. One of three equally parsimonious trees from a heuristic analysis of SSU nrDNA sequences. Bootstrap values (> 50 %) from 1000 replicates are included at the nodes. Branch lengths are drawn to scale

Within the second lineage a large clade with no support was generated with three separate branches discerned, i.e. the *Diaporthales* (100 %) was a sister to the monophyletic clade (83 %) containing the *Pleurostoma*/*Pleurostomophora* (100 %) and *Calosphaeria*/unknown fungus subclades (100%), which are together a sister group to the *Togninia*/ *Phaeoacremonium* clade (100 %). The *Jobellisia* clade (100 %) is shown outside the *Diaporthales* on a basal branch to the *Hypocreales/Microascales* clade. The *Magnaporthaceae* (99 %) grouped as a separate well-supported clade outside *Diaporthales*.

Two constraint analyses (CA) were performed on LSU rDNA data set to assess the inclusion of *Pleurostoma*, *Togninia* and *Jobellisia* in the *Calosphaeriales* and to test the monophyly of the *Calosphaeriales*. The *Calosphaeriales* is represented by a clade of *Calosphaeria pulchella* and the unknown fungus in our phylogenies. When *Calosphaeria*, the unknown fungus, and *Togninia/Phaeoacremonium* were treated

as monophyletic, 24 trees (not shown) were seven steps longer, and the Kishino-Hasegawa (KH) test did not reject them as significantly worse than the MPTs (P* ranged from 0.1938 to 0.3454). The CA forcing *Calosphaeria*, the unknown fungus, *Togninia*, and *Pleurostoma* with two *Phialophora* species to be monophyletic, 16 trees were one step longer than the MPTs and were considered acceptable hypotheses for the phylogeny by the KH test (P* = 0.7964–0.8619). Two other CA were run to assess the inclusion of the *Calosphaeriales* in a) the *Magnaporthaceae*, b) the *Magnaporthaceae* and *Diaporthales* without *Jobellisia*. The CA forcing the *Calosphaeriales* clade including *Togninia*, *Pleurostoma* and two related *Phialophora* species and the *Magnaporthaceae* to be monophyletic, generated 33 trees that were 15 steps longer than the MPTs and the KH test did not reject them as significantly worse than the MPTs (P* = 0.0588–0.1159). When the identical group of taxa from the latter CA was forced to be monophyletic

with the *Diaporthales* 42 trees eight steps longer than the MPTs were obtained and were also accepted by the KH test (P* = 0.3392–0.3940).

Phylogenetic analysis of the SSU rDNA sequence data

A maximum parsimony analysis (MP2) was performed using 316 phylogenetically informative characters in an alignment including 1724 nt from 57 taxa. Three MPTs were obtained (tree length 1128, CI = 0.508, RI = 0.733, HI = 0.492) (Fig. 2). The trees differed in the topology of branches within the *Togninia/Phaeoacremonium* clade.

Two major lineages with branching order slightly different from MP1 were discerned in this analysis. A lineage (95 %) of the *Hypocreales* (53 %) and the *Microascales* (100 %) and a lineage (64 %) consisting of subgroupings of nine orders or families, i.e. the *Sordariales* (95 %), the *Coniochaetales* (98 %), and a group (63 %) of the *Phyllachorales*, the *Chaetosphaeriales* (100 %), and the *Boliniales*; a lineage (66 %) of the *Diaporthales* (99 %), and the calosphaeriaceous taxa; a lineage (74 %) of the *Ophiostomatales* (100 %) and the *Magnaporthaceae* (99 %), and the *Xylariales* lineage (86 %). The topology of branches of the three calosphaeriaceous genera and the unknown fungus within the *Diaporthales* clade was identical to that shown in MP1. These genera formed three strongly supported separate lineages, i.e. the *Calosphaeria*/unknown fungus clade (100 %), the *Pleurostoma/Pleurostomophora* clade (100 %) and the *Togninia/Phaeoacremonium* clade (87 %).

The NJ2 analysis produced a tree (not shown) with similar basic topology as MP2 but with a different branching order of i) the calosphaeriaceous genera, and ii) the *Diaporthales* and the *Magnaporthaceae*. In NJ2, two main lineages were the *Hypocreales* (82 %) and a poorly supported lineage (52 %) with other perithecial ascomycetes. A monophyletic clade with no branch support within the poorly supported lineage contained the three strongly supported calosphaeriaceous lineages, i.e. *Togninia* (99 %) that was a sister to *Calosphaeria*/unknown fungus (100 %) and *Pleurostoma/Pleurostomophora* (100 %). The *Diaporthales* (99 %) and the *Magnaporthaceae* (100 %) grouped within another large unsupported clade containing also the *Ophiostomatales* (100%) as a sister to the *Diaporthales*.

A CA analysis was run on SSU nrDNA sequence data to test the monophyly of the *Calosphaeriales* by inclusion of *Calosphaeria,* the unknown fungus, *Togninia/Phaeoacremonium*, *Pleurostoma* and two related *Phialophora* species. Three trees were generated that were one step longer than the MPTs and were accepted by the KH test (P* = 0.3175). Six trees that were 10 steps longer than the MPTs were obtained in CA, when the *Calosphaeriales* and *Magnaporthaceae* were forced to be monophyletic, all of

which were rejected by the KH test (P* = 0.0330). The CA forcing the *Calosphaeriales*, *Diaporthales* and the *Magnaporthaceae* to be monophyletic resulted in 12 trees that were seven steps longer than the MPTs, all of which were rejected by the KH test (P* = 0.0348).

Taxonomy

Calosphaeria pulchella together with the unknown fungus appeared as a strongly supported monophyletic clade (100 %) in both parsimony and distance analyses. These two taxa share several similarities in teleomorph morphology, i.e. dark, opaque perithecia with a globose venter and an elongate, cylindrical neck; true paraphyses; asci arranged in a palisade along the whole perithecial interior, long-stipitate asci, conspicuously tapering below from the sporiferous portion, floating freely within the centrum, with thickened ascal apex without a visible discharge mechanism; hyaline, suballantoid to allantoid ascospores, arranged in a fascicle in the upper part of the ascus. However, both fungi can be distinguished in the arrangement of perithecia, asci and proliferation of ascogenous hyphae. The perithecia are aggregated in circinate groups with converging necks, but not united in a disc beneath the periderm in *C. pulchella*, while perithecia are separate, superficial to immersed in wood with separately protruding necks in the unknown fungus. Though in both fungi the asci are formed in acropetal succession, in *C. pulchella* the ascogenous hyphae produce terminal and lateral persistent cells, from each of which an ascus arises as an outgrowth, while in the unknown fungus the ascogenous hyphae elongate in the process of ascal formation, producing short, persistent cells along a side, from which the asci then arise.

Both fungi have phialidic conidiogenesis but differ in conidiophore structure and pigmentation. In the unknown fungus the conidiophores branch regularly, both basally and apically, and have prominent constrictions at the septa in comparison with the mostly unbranched, predominantly subcylindrical-shaped conidiophores of *C. pulchella*. The unknown fungus has distinct tuberculate, brown hyphae and subhyaline phialides, that are hyaline towards the tip, with distinct, shallow, flaring collarettes, contrasting with the mostly smooth and hyaline hyphae, perfectly hyaline phialides, with a finely pigmented apical region and deep, flaring collarettes of *C. pulchella*. These two taxa could also be distinguished on cultural characters. The unknown fungus produced brownish grey to olive-brown colonies, compared with the greyish red colonies of *C. pulchella* on 2 % MEA. *Calosphaeria pulchella* is a fast-growing fungus, reaching a colony radius of 18–20 mm after 8 d in the dark, contrasting with the slower-growing unknown fungus reaching 5–6 mm during the same period.

Based on the distinctions between the sequence data, perithecial arrangement, formation of asci on ascogenous hyphae, and conspicuous differences in the anamorphs obtained *in vitro*, the unknown fungus is described as a new genus, *Togniniella*, with a single new species, *Togniniella acerosa*. Two new mono-typic anamorph genera are also erected, namely *Phaeocrella* for the anamorph of *Togniniella*, and *Calosphaeriophora* for the anamorph of *Calosphaeria*.

The parsimony analyses show *Calosphaeria*, *Togninia*, *Pleurostoma* and *Togniniella* as three strongly supported lineages within a large clade containing the *Diaporthales*. *Togninia* with its *Phaeoacremonium* anamorph resides on the basal branch of the *Diaporthales*. The neighbor-joining analyses repeat the separation of the calosphaeri-aceous genera into three lineages but differ from MP analyses in the branching order for *Togninia/Phaeoacremonium*. The *Togninia/Phae-oacremonium* clade is shown either basal to the *Diaporthales* and other calosphaeriaceous taxa in NJ1, or in NJ2 forms a monophyletic group with *Calosphaeria* and *Pleurostoma*.

Although the constraint analyses of LSU and SSU rDNA data sets do not preclude the monophyly of the three putative calosphaeriaceous lineages, their identical grouping on separate strongly supported branches in all analyses and differences in their morphology, life history and ecology led us to introduce two new families, the *Togniniaceae* associated with the *Diaporthales* and the *Pleurostomataceae* of the *Calosphaeriales*. These families can then be distinguished from a refined diagnosis of the ***Calosphaeriaceae***. The *Calosphaeriaceae* based on *Calosphaeria*, the type genus, accommodate fungi with perithecia solitary, superficial or basally immersed on wood, or in ellipsoidal to circinate groups on wood beneath the periderm, nonstromatic, globose to subglobose, dark, opaque, glabrous; necks central, elongate, separate or converging radially; perithecial wall leathery; ostiolar canal periphysate; ascogenous hyphae short-branched with several lateral and terminal cells; asci unitunicate, octosporous, ascal apex thickened, without a discharge mechanism, long-stipitate; ascospores hyaline, allantoid, aseptate. The anamorphs of the *Calosphaeriaceae* are phialidic and have been linked to *Calosphaeriophora* and *Phaeocrella* (this study).

Pleurostomataceae Réblová, L. Mostert, W. Gams & Crous, **fam. nov.** MycoBank MB500153.

Perithecia superficialia vel basi submersa, non stromatica, globosa vel subglobosa, fusca, opaca, glabra, papillata. Paries pericheciorum coriaceus, bistratosus: stratum externum e cellulis pseudoparenchymatosis *textura prismatica* vel *epidermoidea*, stratum internum e cellulis hyalinis applanatis compositum. Canalis ostiolaris periphysatus. Paraphyses haud visae. Hyphae ascogenae breviter proliferentes, hamis praeditae. Asci unitunicati, polyspori, apice inspissato, sporis haud vi expulsis, stipitati, stipite ascis liberatis ad hyphas ascogenas affixo. Ascosporae hyalinae, allantoideae, continuae. Anamorphe (*Pleurostomophora*) hyphomycetosa, hyalina, phialidica.

Perithecia superficial or basally immersed, nonstromatic, globose to subglobose, dark, opaque, glabrous, papillate. *Perithecial wall* leathery, comprising two layers, the outer layer of thin-walled pseudoparenchymatous cells of *textura prismatica* to *textura epidermoidea*, the inner layer of non-pigmented flattened cells. Ostiolar canal periphysate. *Paraphyses* not observed. *Ascogenous hyphae* with short proliferations and formation of croziers. *Asci* unitunicate, polysporous, ascal apex thickened, without a discharge mechanism, stipitate, with stipe attached to the ascogenous hyphae after dehiscence. *Ascospores* hyaline, allantoid, aseptate. *Anamorphs* (*Pleurostomophora*) moniliaceous, hyphomycetous, with phialidic conidiogenesis.

Typus: *Pleurostoma* Tul. & C. Tul., *Selecta Fung. Carpol.* 2: 247. 1863.

Togniniaceae Réblová, L. Mostert, W. Gams & Crous, **fam. nov.** MycoBank MB500154.

Perithecia superficialia vel immersa, non stromatica, globosa vel subglobosa, fusca, opaca, collo lungo, recto vel flexuoso praedita. Paries pericheciorum fragilis vel coriaceus, bistratosus: stratum externum e cellulis tenuitunicatis, brunneis, pseudoparenchymatosis *textura prismatica* vel *angulari*, stratum internum e cellulis hyalinis applanatis compositum. Canalis ostiolaris periphysatus. Paraphyses copiosae, septatae, latae, ad septa modice constricta, ramosae, sursum modice angustatae. Hyphae ascogenae ascis formatis elongascentes, hamis carentes; asci distincte spicati. Asci unitunicati, octospori, apice inspissato, sporis haud vi expulsis, haud stipitati, deorsum rotundati, parte basilari ascis liberatis ad hypham ascogenam affixa. Ascosporae hyalinae, continuae, allantoideae vel suballantoideae vel ellipsoideae. Anamorphe (*Phaeoacremonium*) hyphomycetosa dematiacea phialidica.

Perithecia superficial to immersed, nonstromatic, globose to subglobose, dark, opaque, long-necked; neck straight or flexuous. *Perithecial wall* fragile to leathery, comprising two layers, the outer layer of thin-walled, brown, pseudoparenchymatous cells of *textura prismatica* to *textura angularis*, the inner layer of non-pigmented flattened cells. Ostiolar canal periphysate. *Paraphyses* abundant, broadly cellular, slightly constricted at the septa, branching, slightly tapering apically. *Ascogenous hyphae* elongating during ascus formation, without formation of croziers; asci in distinct spicate arrangement.
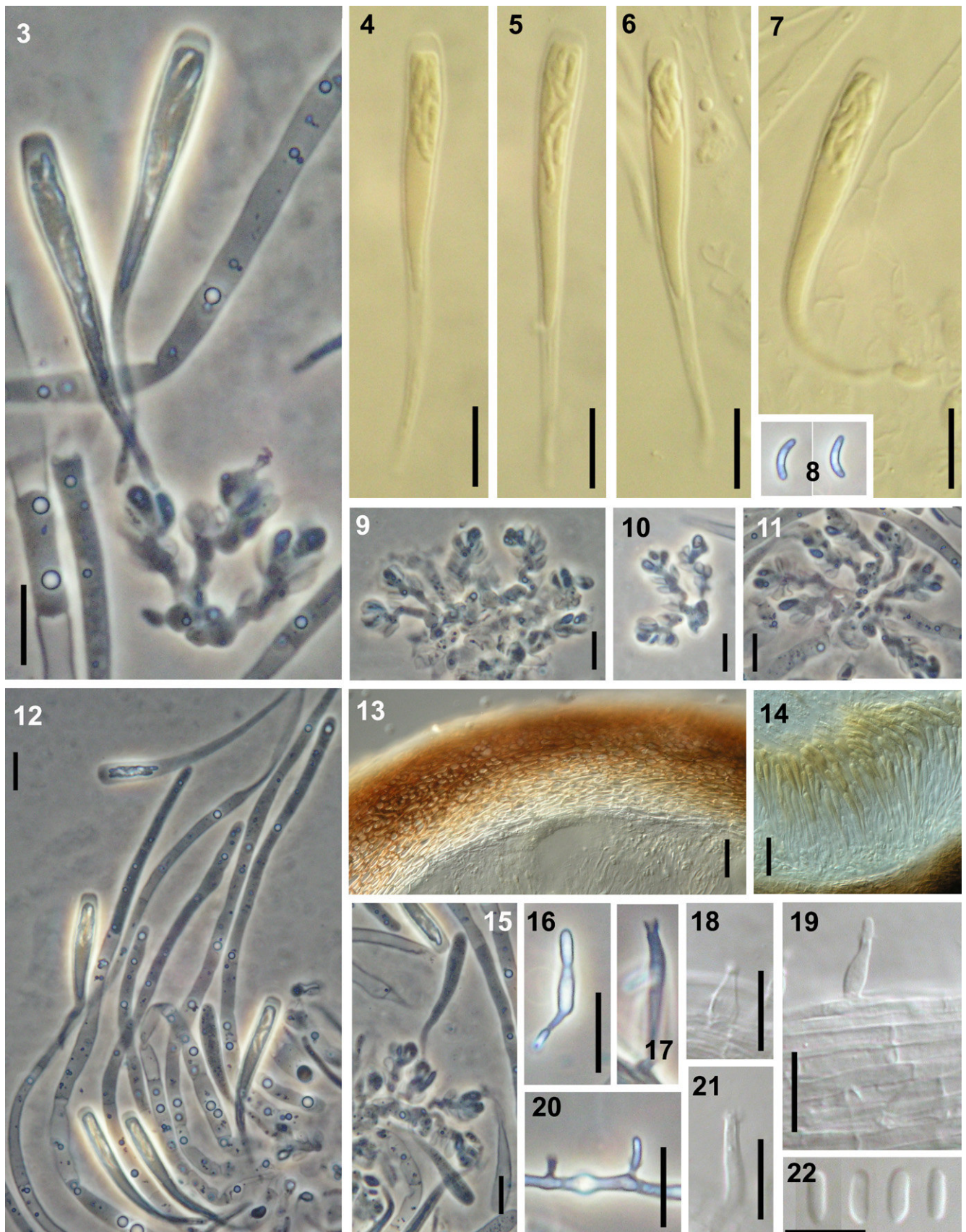
Figs 3–22. *Calosphaeria pulchella.* 3. Asci with ascogenous hyphae. 4–7. Asci. 8. Ascospores. 9–11, 15. Ascogenous hyphae. 12. Paraphyses. 13. Longitudinal section of perithecial wall. 14. Pallisade of asci in a hymenium attached to the inner layer of perithecial wall. Figs 16–22. *Calosphaeriophora pulchella* anamorph of *Calosphaeria pulchella.* 16. Germinating ascospore producing conidia. 17–21. Conidiophores, in culture. 22. Conidia, in culture. Figs 3–22 from PRM; 16–22 from CBS 115999 (holotype) ex PRM 901842 (PCA, 14 d old). DIC: 4–7, 13, 14, 18, 19. 21, 22; PC: 3, 9–12, 15–17, 20. Scale bars = 10 μm.

Asci unitunicate, 8-spored, ascal apex thickened without a discharge mechanism, without stipe, basally rounded, with remnants of basal parts attached to the ascogenous hyphae after dehiscence. *Ascospores* hyaline, aseptate, allantoid to suballantoid to ellipsoid. *Anamorphs* (*Phaeoacremonium*) dematiaceous hyphomycetous, with phialidic conidiogenesis.

Typus: *Togninia* Berl., *Icon. Fung.* 3: 9. 1900.

Calosphaeria pulchella (Pers. : Fr.) J. Schröt., *Pilze Schlesiens* 2: 451. 1897. Figs 3–22, 23A–E.

≡ *Sphaeria pulchella* Pers., *Synop. Meth. Fung.*, p. 43. 1801 : Fries, Syst. Mycol. 2: 406. 1823.
≡ *Valsa pulchella* (Pers. : Fr.) Fr., *Summa Veg. Scand.*, p. 412. 1849.
Anamorph: *Calosphaeriophora pulchella* Réblová, L. Mostert, W. Gams & Crous, sp. nov.

Perithecia nonstromatic, densely aggregated in 2–3 levels in ellipsoidal to circinate groups of 20–40 individuals, 5–7.5 mm long and 3.5–4 mm wide, on wood beneath the periderm, dark brown to black, glabrous, venter globose to subglobose 400–500 µm diam, 400–520 µm high; necks central, elongate, up to 2000 µm long, 150–200 µm wide, straight or slightly flexuous, broadly rounded at the glabrous apex, tightly converging radially, at first decumbent to the substratum then upright, not united in a disc at the top and piercing separately the periderm in a narrow fissure; ostiolum periphysate. *Perithecial wall* leathery, two-layered, 67–80 µm thick, of pale brown to red-brown polyhedral cells of *textura angularis*. *Ascogenous hyphae* persistent, not proliferating, apparently terminated in growth, discrete, short-branched, each branch sequentially and simultaneously producing several lateral and terminal cells, 4–5 × 2.5–3 µm, from each of which an ascus arises as an outgrowth. *Paraphyses* persistent, abundant, unbranched, septate, hyaline, cylindrical, apically free, 3–4.5 µm wide near the base, tapering to 2–3 µm, longer than the asci. *Asci* unitunicate, clavate, (12–)18–24 × (4.5–)5–6 (mean ± se = 18.6 ± 1.4 × 5.4 ± 0.2) µm, L/W 3.5:1 in *pars sporifera*, stipe 27–39 µm long, truncate at the thickened apex, with no distinct discharge mechanism, tapering towards the base from the sporiferous portion, floating freely within the centrum at maturity, 8-spored. *Ascospores* suballantoid, 4.5–5 (mean ± se = 4.8 ± 0.1) × 1 µm, hyaline, aseptate, smooth, arranged in a fascicle in the upper part of the ascus.

Notes: The type or any other authenticated material of *C. pulchella* could not be located in Persoon's herbarium (L).

Calosphaeriophora Réblová, L. Mostert, W. Gams & Crous, **gen. nov.** MycoBank MB500155.
Etymology: Pointing to the teleomorph *Calosphaeria* with suffix from the morphologically similar genus *Phialophora*.

Anamorphe *Calosphaeriae*. Hyphae leves, hyalinae, *Acremonii* similes, sed genus conidiophoris subcylindricis, rarissime ramosis, phialidibus hyalinis, regione apicali indistincte pigmentata et collari profundo expandente distinctum.

Mycelium smooth, hyaline, similar to that of *Acremonium*, but distinct in having subcylindrical, mostly unbranched conidiophores, with hyaline phialides with a finely pigmented apical region and deep, flaring collarettes.

Typus: *Calosphaeriophora pulchella* Réblová, L. Mostert, W. Gams & Crous, sp. nov.

Calosphaeriophora pulchella Réblová, L. Mostert, W. Gams & Crous, **sp. nov.** MycoBank MB500156. Figs 17–22, 23C–E.

Etymology: *pulchellus* (L), small and beautiful, referring to the appearance of perithecial "nests" on natural substratum, chosen to match the epithet of the teleomorph.

Anamorphe *Calosphaeriae pulchellae*. Hyphae ramosae, septatae, singulae vel fasciculatae, plerumque hyalinae, nonnullae dilute brunneae, leves, 2–4(–7) µm latae. Conidiophora micronematosa, ex hyphis aeriis vel submersis oriunda, erecta, simplicia vel prope basim ramosa, plerumque hyalina, recta vel flexuosa, 1–2-septata, longitudine variabilia, (12–)14–29(–31) × 2–3(–4) µm, nonnumquam prope basim angustiora. Phialides terminales vel laterales, saepe ad hyphas fasciculatas dense aggregatae, plerumque monophialidicae, leves, hyaline, elongato-ampulliformes, saepe ad basim angustatae, in parte apicali sub collari pigmentatae, (6–)7–14 × 2–3(–4) µm; adelophialides frequentes, 2–6 × 1–2(–3) µm; collare apicale, infundibuliforme, 1.5–2 µm longum, 1.5–2 µm diam. Conidia in capitulis mucidis aggregata, hyalina, oblonge ellipsoidea vel cylindrica, ad basim angustata, 3–5(–6) × 1.5–2 µm.

Mycelium consisting of branched, septate hyphae that occur singly or in bundles of up to 11; mostly hyaline, with some pale brown hyphae, smooth, 2–4(–7) µm wide. *Conidiophores* micronematous, arising from aerial or submerged hyphae, erect, simple or branched in the basal region, mostly hyaline, straight or flexuous, 1–2-septate, variable in length, (12–)14–29(–31) µm long, 2–3(–4) µm wide, occasionally narrower at the base. *Phialides* terminal or lateral, often aggregated in dense clusters on strands of hyphae, mostly monophialidic, smooth, hyaline. Phialides elongate-ampulliform, often attenuated at the base or subcylindrical, frequently pigmented in the apical region below the collarette, (6–)7–14 × 2–3(–4) µm; adelophialides occurring often, cylindrical or ampulliform, 2–6 × 1–2(–3) µm. Phialides developing a terminal, funnel-shaped collarette, 1.5–2 µm long, 1.5–2 µm wide. *Conidia* aggregated in round, slimy heads at the phialide tips, hyaline, oblong–ellipsoidal or cylindri-

cal, with a tapered base, 3–5(–6) × 1.5–2 (mean ± se = 4.1 ± 0.8 × 1.7 ± 0.2) µm.

Cultural characteristics: Colonies on MEA flat, felty in texture, with entire margins; aerial mycelium medium to sparse; colony surface old rose in the centre (10C5), white (10A1) towards the margin, reverse greyish red (10D5) in the centre, becoming reddish white (10A2) towards the margin. Colony surface on OA hyaline with uneven patches of reddish white (10A2) and grey (10B1). Minimum temperature for growth 15 °C; optimum 30 °C and maximum 37 °C. Colonies reaching a radius of 18–20 mm after 8 d at 25 °C.
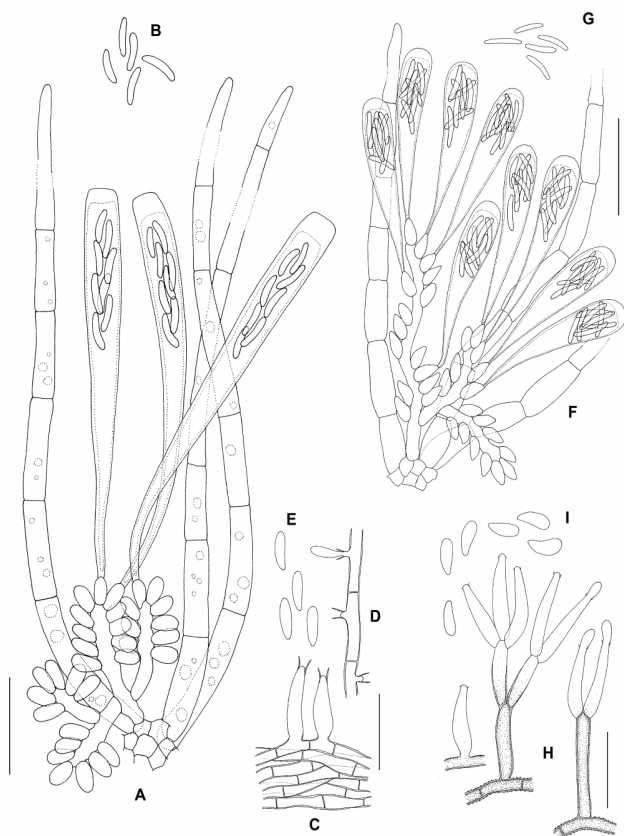
Fig. 23. *Calosphaeria* (A–E) and *Togniniella* (F–I). A–E. *Calosphaeria pulchella*. A. Asci with ascospores, ascogenous hyphae and paraphyses. B. Ascospores. C–E. *Calosphaeriophora pulchella* anamorph of *Calosphaeria pulchella*. C. Mycelial ropes of cohering hyphae of aerial mycelium with phialides, from culture. D. Single hypha with phialidic openings, from culture. E. Conidia, from culture. F–I. *Togniniella acerosa*. F. Asci with ascospores, ascogenous hyphae and paraphyses. G. Ascospores. H, I. *Phaeocrella acerosa* anamorph of *Togniniella acerosa*. H. Conidiophores, in culture. I. Conidia, in culture. A–B from PRM, C–E from CBS 115999 (holotype) ex PRM 901842; F–I from PDD 81431 (holotype); H, I from CBS 113648 ex PDD 81431 (holotype) (PCA, 14 d old). Scale bars = 10 µm.

Habitat: Saprobic on decayed wood.

Specimen examined: **France**, Pyrénées Atlantiques, Ariège, Rimont, Ruisseau de Peyran, under periderm of a branch of *Prunus avium*, 14 Oct. 2003, J. Fournier J.F. 03200 (*Calosphaeria pulchella*, PRM 901842, material from which the **holotype** of *Calosphaeriophora pulchella* was isolated, culture CBS 115999; specimen in herb. CBS).

*Togniniella* Réblová, L. Mostert, W. Gams & Crous, **gen. nov.** MycoBank MB500157.
Anamorph: *Phaeocrella* Réblová, L. Mostert, W. Gams & Crous, gen. nov.

Etymology: Referring to the macroscopically similar *Togninia*.

Genus *Calosphaeriacearum*. Perithecia solitaria, non stromatica, fusca vel atra, glabra; venter globosus vel subglobosus, submersus; collum e substrato protrudens, medianum, elongatum; ostiolum periphysatum. Paries perithecii coriaceus vel fragilis, bistratosus; stratum externum e cellulis brunneis, tenuitunicatis, textura prismatica, collum versus textura prismatica vel porrecta compositum; stratum internum e cellulis tenuitunicatis, subhyalinis vel hyalinis, elongatis compressis compositum. Hyphae ascogenae persistentes, sympodialiter proliferentes, seriem acropetalem cellularum ellipsoidalium, 2.5–3 × 2–2.5 µm proferentes, e quibus asci singuli oriuntur. Paraphyses persistentes, copiosae, simplices, septatae, hyalinae, sursum angustatae et liberae, ascos superantes. Asci unitunicati, clavati, ad apicem inspissatum truncati vel late rotundati, deorsum conspicue gradatim angustatae, maturi liberati in centro ascomatis fluitantes, 8-spori, ascosporis haud vi expulsis. Ascosporae suballantoideae, hyalinae, continuae, leves, in parte superiore asci fasciculatae.

Typus: *Togniniella acerosa* Réblová, L. Mostert, W. Gams & Crous, sp. nov.

Perithecia solitary, nonstromatic, dark brown to black, glabrous; venter globose to subglobose, entirely immersed; neck protruding beyond the substratum, central, elongate; ostiolum periphysate. *Perithecial wall* leathery to fragile, two-layered. Outer wall of brown, thin-walled, brick-like cells with opaque walls of *textura prismatica*, in the neck of *textura prismatica to porrecta*. Inner layer of thinner-walled, subhyaline to hyaline, elongated and compressed cells. *Ascogenous hyphae* persistent, long proliferating sympodially, branched, forming an ascogenous succession of short ellipsoidal cells along a side, *ca.* 2.5–3 µm long, 2–2.5 µm wide. Asci arising singly from these cells and separating from them as they mature. Ascogenous hyphae showing at any moment an apical crown of immature and attached asci and a tail of short lateral cells. *Paraphyses* persistent, abundant, not branching, septate, hyaline, more or less cylindrical, tapering near the tip, apically free, longer than the asci.
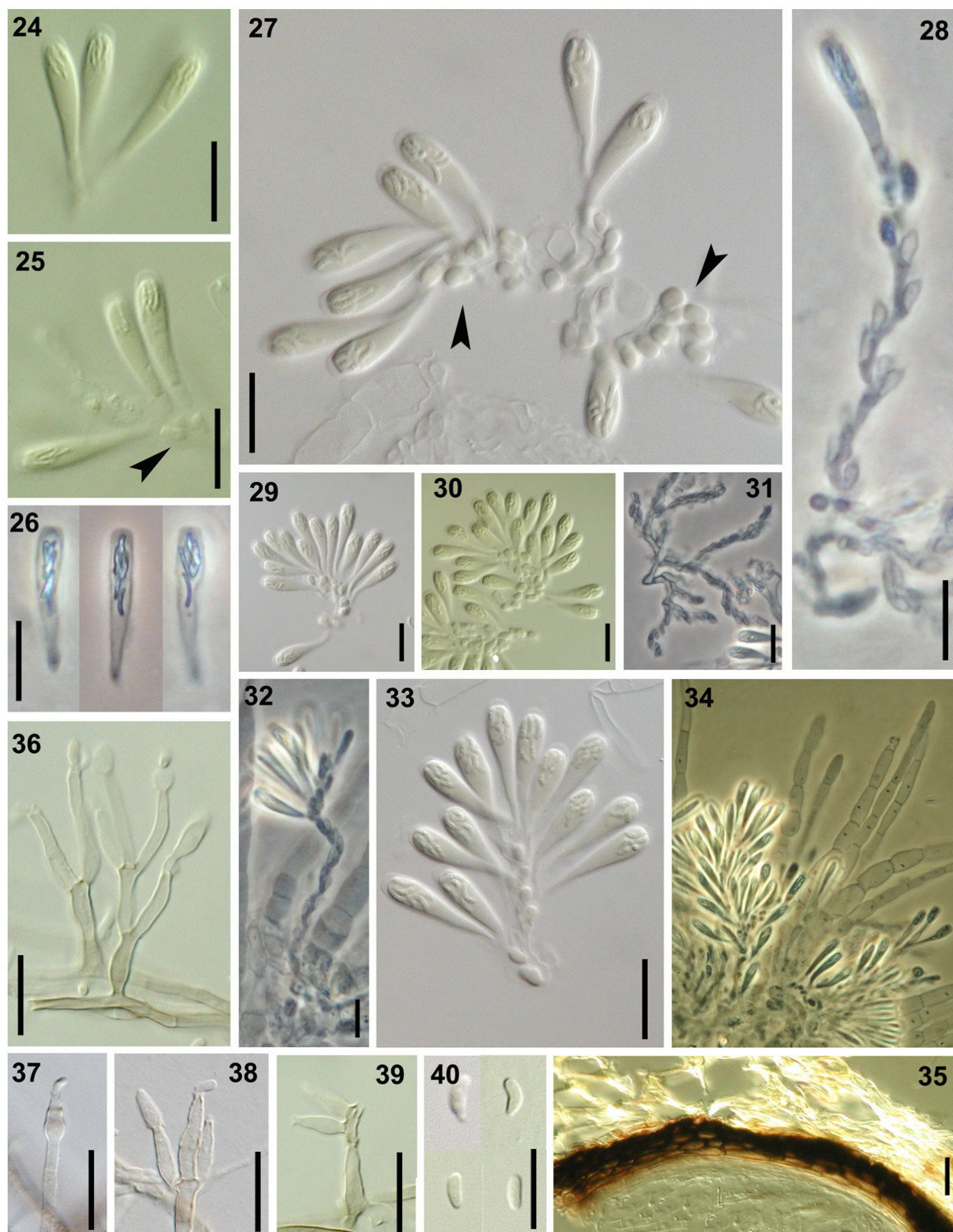
Figs 24–40. *Togniniella acerosa.* 24–26, 29, 30, 33. Asci. 27, 28, 31, 32. Ascogenous hyphae with asci. 34. Paraphyses and asci. 35. Longitudinal section of perithecial wall. Arrows in 25 and 27 indicating cells, which are attached to the ascogenous hyphae and from which single asci arise as outgrowths. Figs 36–40. *Phaeocrella acerosa* anamorph of *Togniniella acerosa.* 36–39. Conidiophores, in culture. 40. Conidia, in culture. Figs 24–26, 28, 35 from PDD 81431 (holotype); 32, 34 from PDD 81432; 27, 29–31, 33 from DAOM 136897; 36, 39 from CBS 113648 ex PDD 81431 (holotype) (PCA, 14 d old); 37, 38, 40 from CBS 113726 ex PDD 81432 (PCA, 14 d old). DIC: 24, 25, 27, 29, 30, 33, 35–40; PC: 26, 28, 31, 32, 34. Scale bars: 3–19 = 10 μm.

Asci unitunicate, clavate, truncate to broadly rounded at the thickened apex, narrowly tapering towards the base from the sporiferous portion, floating freely within the centrum at maturity, with no distinct discharge mechanism, 8-spored. *Ascospores* suballantoid, hyaline, aseptate, smooth, arranged in a fascicle in the upper part of the ascus.

Togniniella acerosa Réblová, L. Mostert, W. Gams & Crous, **sp. nov.** MycoBank MB500158. Figs 23F–I, 24–40.
Anamorph: *Phaeocrella acerosa* Réblová, L. Mostert, W. Gams & Crous

Etymology: *acerosus* (L), acute, acerose, refering to the acute and narrowly tapering ascus stipe.

Perithecia solitaria, fusca vel atra, glabra; venter 250–350 µm diam, 300–360 µm altus; collum e substrato protrudens, cylindricum vel modice flexuosum, 400–600 × 45–60 µm, sursum late rotundatum vel obtusum. Paries perithecii coriaceus vel fragilis, 17–23 µm crassus, bistratosus. Paraphyses septatae, ad septa modice constricta, 7–8 µm latae prope basim, sursum ad *ca.* 3 µm angustatae, ascos superantes. Asci unitunicati, clavati, 18–21(–22) × 3–4 µm, longit.:latit. 6:1, spicati ex hyphis ascogenis oriundi, sub parte sporifera conspicue angustati, sporas haud vi expellentes. Ascosporae suballantoideae, 3–4 × 0.5 µm, longit.:latit. 6.5:1, hyalinae, continuae, leves, in parte superiore asci fasciculatae.

Perithecia solitary, nonstromatic, dark brown to black, glabrous, venter globose to subglobose, semiimmersed to immersed, 250−350 µm diam, 300−360 µm high; neck protruding beyond the substratum, central, elongate, cylindrical or slightly flexuous, 400−600 µm long, 45−60 µm wide, perpendicular to oblique to the substratum, broadly rounded to obtuse at the apex; ostiolum periphysate. *Perithecial wall* leathery to fragile, 17−23 µm thick, two-layered, of *textura prismatica*. *Paraphyses* septate, slightly constricted at the septa, hyaline, 7−8 µm wide near the base, tapering to *ca*. 3 µm, longer than the asci. *Asci* unitunicate, clavate, 18−21(−22) × 3−4 (mean ± se = 19.8 ± 0.2 × 3.3 ± 0.1) µm, L/W 6:1, in spicate arrangement on the ascogenous hyphae, truncate to broadly rounded at the thickened apex, conspicuously tapering towards the base from the sporiferous portion, with no distinct discharge mechanism. *Ascospores* suballantoid, 3−4 (mean ± se = 3.3 ± 0.1) × 0.5 µm, L/W 6.5:1, hyaline, aseptate, smooth, arranged in a fascicle in the upper part of the ascus.

Specimens examined: **New Zealand**, South Island, Tasman Prov., Nelson Lake National Park, St. Arnaud, Lake Rotoiti, Lakehead track *ca.* 1.5 km SE of NP Headquarters, on decayed wood of a trunk of *Nothofagus* sp., 22 Feb. 2003, M. Réblová M.R. 2593/03 (**Holotype** PDD 81431, ex-type cultures deposited as CBS 113726, ICMP 15113; holotype

of *Togniniella acerosa* and *Phaeocrella acerosa*); West Coast Prov., Harihari 73 km SW of Hokitika, Saltwater Forest, Poerua River valley, on decayed wood, 12 Mar. 2003, M. Réblová M.R. 2811/03 (PDD 81432, with isolates preserved as CBS 113648, ICMP 15149). **Canada**, Ontario, University of Toronto Forest near Dorset, Haliburton Co., on decayed wood of *Populus* sp., 1 Nov. 1969, D. Malloch (DAOM 136897).

Habitat: Saprobic on decayed wood.

Phaeocrella Réblová, L. Mostert, W. Gams & Crous, **gen. nov.** MycoBank MB500159.

Etymology: Contraction of the lengthy diminutive "*Phaeoacremoniella*", pointing to the similarity with *Phaeoacremonium*.

Anamorphe *Togniniellae*. *Phaeoacremonio* similis, sed conidiophoris regulariter ramosis, ad septa constrictis, deorsum pigmentatis, sursum pallidioribus distinguenda. Mycelium tuberculatum, brunneum. Phialides praecipue in parte distali hyalinae, collare exiguum, distinctum patens ferentes

Morphologically similar to *Phaeoacremonium*, but distinct in that conidiophores branch regularly, and have prominent constrictions at the septa. Mycelium tuberculate, brown, with subhyaline phialides that are hyaline towards the tip with distinct, shallow, flaring collarettes.

Typus: *Phaeocrella acerosa* Réblová, L. Mostert, W. Gams & Crous, sp. nov.

Phaeocrella acerosa Réblová, L. Mostert, W. Gams & Crous, **sp. nov.** MycoBank MB500160. Figs 23H, I, 36–40.

Etymology: *acerosus* (L), acerose, for the acute stipe of the asci, chosen to match the epithet of the teleomorph.

Anamorphe *Togniniellae acerosae*. Mycelium ex hyphis ramosis, separatis, septatis compositum, medio vel obscure brunneum, in parte conidiogena pallidius, hyphis 1–4.5 µm latis, verrucis ad 2.5 µm diam obtectis. Conidiophora macronematosa vel micronematosa, ex hyphis aeriis oriunda, erecta, simplicia vel ramosa, dilute brunnea, sursum pallidiora, ad basim paucis verrucis praedita, recta vel flexuosa, 1–4-septata, longitudine variabilia, (12–)17–41(–47) × 2–3(–4) µm, ad septa constricta et cellulis inter septa inflatis, nonnumquam ad basim angustioribus; ad basim paucis verrucis obtecta, sed ceterum levia, dilute brunnea vel hyalina. Phialides terminales vel laterales, plerumque monophialidice sed etiam polyphialidicae, subcylindricae, naviculares vel elongato-ampulliformes, nonnumquam irregulariter constrictae, (4–)6–14(–16) × (1.5–)2–2.5(–3) µm; adelophialides nonnumquam visae; collare terminale divergens, 1 µm longum, 2.5–3 µm latum.
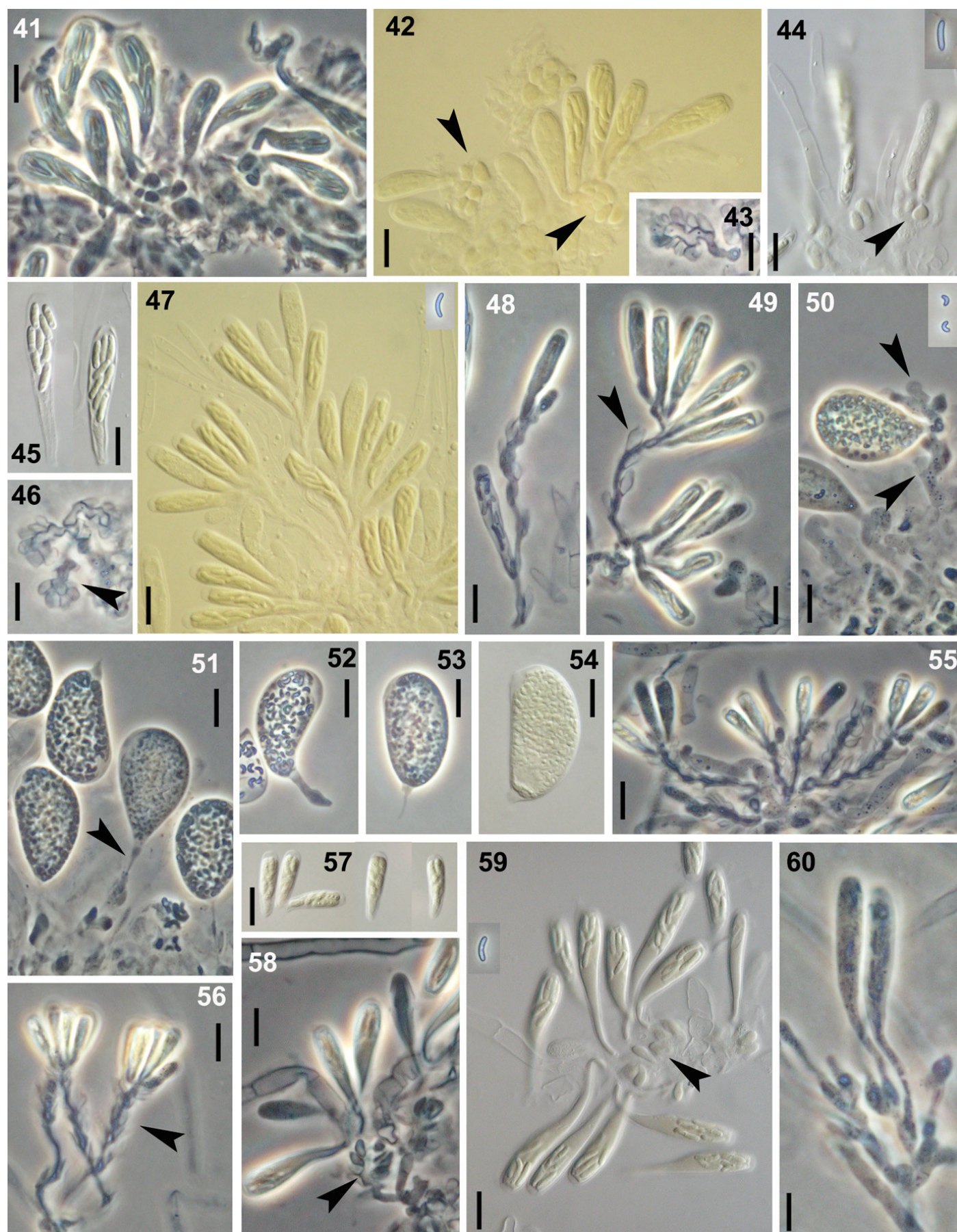
Figs 41–60. Species of the *Calosphaeriaceae*. 41, 42. *Jattaea microtheca*. 41, 42. Asci and ascogenous hyphae (arrows explained below). 43–46. *Jattaea* sp. 43, 46 Ascogenous hyphae with terminally and laterally attached cells. 44. Asci, ascospores and ascogenous hyphae. 45. Asci. 47–49. *Romellia vibratilis*. 47. Asci and ascospore. 48, 49. Asci with ascogenous hyphae. 50–54. *Pleurostoma ootheca*. 50. Asci and ascogenous hypha. 51. Asci detaching from the basal part. 52. Mature ascus with a stipe. 53. Mature ascus after dehiscence with an appendage at the base. 54. Old ascus without appendage. 55–57. *Togninia novaezelandiae*. 55, 56. Asci in a spicate arrangement on ascogenous hyphae. 57. Asci. 58, 59. *Wegelina discreta*. 58, 59. Asci and ascogenous hyphae. 60. *Calosphaeria parasitica*. Asci and branching ascogenous hyphae. Arrows in 42, 44, 46, 58, 59 indicating cells, which are attached to the ascogenous hyphae and from which single asci arise as outgrowths; arrows in 49, 56 indicating hyaline basal parts of the asci remaining attached to the elongated ascogenous hyphae; arrow in 50 indicating short ascogenous hyphae with a crozier system and attached basal parts of the asci; arrow in 51 indicating a thin appendage remaining attached to the base of the ascus after its dehiscence. *Jattaea microtheca*: 41, 42 from NY (isotype); *Jattaea* sp.: 43–46 from J.F. 02068; *R. vibratilis*: 47–49 from K. & L. Holm 3848a (NY); *P. ootheca*: 50–53 from K122385, 54 from K 122386 (isotype); *T. novaezelandiae*: 55–57 from M.R. 2886/03; *W. discreta*: 58, 59 from DAOM 35410; *C. parasitica*: 60 from M.R. 2891/04. DIC: 42, 44, 45, 47, 54, 57, 59; PC: 41, 43, 45, 46, 48–53, 55, 56, 58, 60. Scale bars = 10 µm.

Conidia aggregata in capitulis mucidis, hyalina, plerumque obovoidea, oblongo-ellipsoidea vel reniformia, 3–4(–5) × 1–2 µm.

Mycelium consisting of branched, separate, septate hyphae; medium- to pale brown, becoming paler towards the conidiogenous region, 1–4.5 µm wide; warts on hyphae up to 2.5 µm diam. *Conidiophores* macronematous or micronematous, arising from aerial hyphae, erect, simple or branched in the basal or apical region, pale brown, paler towards the tip, with a few warts, straight or flexuous, 1–4-septate, variable in length, (12–)17–41(–47) µm long, 2–3(4) µm wide, constricted at the septa, inflated between the septa; occasionally narrower at the base. *Phialides* terminal or lateral, mostly monophialidic, but often also polyphialidic, subcylindrical, navicular or elongate–ampulliform, occasionally irregulary constricted or indented, (4–)6–14(–16) × (1.5–)2–2.5(–3) µm; adelophialides occurring rarely, cylindrical, 5–6 × 1–2 µm. Phialides developing a terminal, flaring collarette, 1 µm long, 2.5–3 µm wide. *Conidia* aggregated in round, slimy heads at the apices of the phialides, hyaline, mostly obovoid, oblong–ellipsoid or reniform, 3–4(–5) × 1–2 (mean ± se = 3.7 ± 0.5 × 1.4 ± 0.3) µm.

Cultural characteristics: Colonies on MEA flat, felty to fluffy in texture, dense, with radially striated margins; colony surface brownish grey (4D2) with olive-brown (4F6) undertones, in reverse olive-brown (4F8). Colony surface on OA olive-brown (4F8). Minimum temperature for growth 15 °C; optimum 20 °C and maximum 25 °C. Colonies reaching a radius of 5–6 mm after 8 days at 25 °C.

Notes: Isolate CBS 113648 did not sporulate as abundantly as CBS 113726. CBS 113726 also developed a dark brown colony colour after 22 d, whereas CBS 113648 had more olive-brown colonies. The Canadian specimen (DAOM 136897) of *T. acerosa* is an older herbarium material, chemically conserved, which we did not attempt to sequence.

Specimen examined: For the respective material, from which the type culture was derived, refer to the specimens examined in the teleomorph *T. acerosa*.

DISCUSSION

The six nonstromatic genera traditionally attributed to the *Calosphaeriaceae*, i.e. *Calosphaeria*, *Jattaea*, *Pleurostoma*, *Romellia*, *Togninia*, and *Wegelina* (Barr 1985, Barr *et al.* 1993) have in common a narrow arrangement of asci on ascogenous hyphae; morphology of ascospores; ascal apex morphology (thickness of the ascal apex, presence or absence of an apical ring, apical invagination and a canal), and habitat of perithecia *in vivo*.

The detailed morphology of the centrum of the *Calosphaeriaceae* seems to be of great importance, but has been rather overlooked in the main studies of the group (Barr 1985, Barr *et al*. 1993). In the six genera the asci are formed in one of three patterns: a) ascogenous hyphae proliferating and continuing to elongate during ascus formation, in acropetal succession and separated at maturity with basal parts remaining attached to the ascogenous hyphae, i.e. spicate arrangement with or without formation of croziers [*Togniniaceae*: *Romellia*: Figs 47–49; *Togninia*: Figs 55–57, Hausner *et al.* (1992: 727, Fig. 1), Mostert *et al.* (2003: 651, Figs 1–4, 12, 13)]; b) short ascogenous hyphae proliferating with asci arising from a crozier system; asci in a short spicate formation with a bulbous base that remains attached to the ascogenous hyphae after ascal dehiscence (*Pleurostomataceae*: *Pleurostoma*: Figs 50–54), or c) asci arranged in fascicles; ascogenous hyphae with short branches, both terminated or elongated in growth, producing a sympodial succession of lateral and terminal cells from each of which an ascus arises as an outgrowth [*Calosphaeriaceae*: *Calosphaeria s. str.*: Figs 3, 9–11, 15; *Togniniella*: Figs 23, 27, 28, 32, 33; *Jattaea*: Figs 41–46, Romero & Samuels (1991: 233, Pl. 2J–L; 239, Pl. 4R); *Wegelina*: Figs 58, 59].

The MP1 and MP2 analyses show *Calosphaeria*, *Togninia* and *Pleurostoma*, formerly included in the *Calosphaeriales*, as three separate, strongly supported lineages within a large clade containing the *Di-*

aporthales. The constraint analyses that were run on the LSU rDNA sequence data set forcing the monophyly of the *Calosphaeriales s. l.* and testing the inclusion of the *Calosphaeriales*, *Diaporthales* and *Magnaporthaceae* into a monophyletic clade yielded trees that were all recognized by the KH test as acceptable hypotheses for the phylogeny. However, these results were contradicted in two constraint analyses run on the SSU rDNA sequence data set, with the branching order of the three calosphaeriaceous genera identical to those shown in MP1. Trees generated in these two CAs forcing members of the a) *Calosphaeriales* and *Magnaporthaceae*, or b) *Calosphaeriales*, *Magnaporthaceae* and *Diaporthales*, respectively, to be monophyletic, were all rejected as significantly worse than the MPTs. Although CAs of the LSU rDNA data set do not preclude a relatedness between the *Calosphaeriales* and the *Magnaporthaceae*, and similarities in dark long- or short-beaked perithecia, hyaline ascospores and phialidic conidiogenesis might serve as other arguments for their relationship, we treat them as two distinct, phylogenetic lineages that have evolved similar morphological characteristics in their holomorphs. The unique centrum, asci and ascospore morphology of the *Calosphaeriales* warrant their delimitation from the *Magnaporthaceae.*

Togninia seems to occupy a family on its own, the newly described *Togniniaceae*, which together with *Jobellisia* may be included in the *Diaporthales*. The phenotypically similar *Gnomoniaceae* represented by *G. gnomon* in MP1 and *Gnomonia setacea* (Pers. : Fr.) Ces. & De Not. and *Gnomoniella fraxini* Redlin & Stack in MP2 appears on the top branch of the *Diaporthales* clade. *Togninia* of the *Togniniaceae* shares with the *Diaporthales,* particularly the *Gnomoniaceae*, dark, globose, long-beaked and nonstromatic perithecia; hyaline suballantoid to ellipsoid, smooth ascospores; asci with rounded base, floating freely within the centrum, and a phialidic anamorph with phytopathogenic life style. The *Togniniaceae* occupy an isolated position in the *Diaporthales* and differ from the core taxa of the order by elongating, sympodially proliferating ascogenous hyphae with asci in a distinct spicate arrangement, presence of true paraphyses growing from the tissue at the bottom of the perithecial cavity and being apically freely from the beginning, and absence of any discharge mechanism in the ascal apex. The centrum in the *Diaporthales* is aparaphysate or with paraphysoid tissue in the form of broad elongate cellular strands, soon deliquescent; the asci contain refractive, chitinoid, nonamyloid apical annulus and are not formed on proliferating ascogenous hyphae (Barr 1978).

Togninia of the *Togniniaceae* and *Pleurostoma* of the *Pleurostomataceae* form two separate and well-supported clades in our phylogenies. Both genera are also well-distinguished, *Togninia* having octosporous,

basally rounded asci, ellipsoid to suballantoid to oblong ascospores, long-beaked perithecia and *Phaeoacremonium* anamorphs, and *Pleurostoma* having polysporous, stipitate asci with croziers, strictly allantoid ascospores, short-papillate perithecia and the *Pleurostomophora* Vijaykrishna *et al.* anamorphs (Vijaykrishna *et al.* 2004, this volume). The arrangement of asci on the ascogenous hyphae also shows differences between the two genera. After ascus dehiscence in *Pleurostoma*, the ascal base contains a thin appendage (apparently a remnant of the inner ascus layer of the functionally unitunicate ascus wall) that disappears with age (Figs 52–54), while in *Togninia* the ascus base is smooth without any appendage after dehiscence.

The *Calosphaeriales s. str.* comprise the *Calosphaeriaceae* and the new family *Pleurostomataceae*. The thus characterised *Calosphaeriales* are sister to the *Diaporthales*. The *Diaporthales* and the *Calosphaeriales* share the most recent common ancestry and form two closely related groups among the perithecial ascomycetes. The fungi attributed to the *Calosphaeriales* can be distinguished from the *Diaporthales* by absence of stromatic tissue surrounding the perithecia; presence of true paraphyses; long-stipitate asci with thickened ascal apex without any discharge mechanism; ramifying and proliferating ascogenous hyphae; allantoid to suballantoid ascospores and hyphomycetous phialidic anamorphs.

Based on morphological characters, the new genus *Togniniella* is closely related to *Calosphaeria pulchella,* with which it formed a monophyletic, strongly supported unit. *Togniniella* also resembles *Togninia* in many aspects, i.e. minute, nonstromatic, dark, long-beaked perithecia; spicate arrangement of asci; hyaline ascospores; true paraphyses many times longer than the asci, and phialidic conidiogenesis. However, the details in the shape and arrangement of asci, shape and organization of ascospores within the ascus, and presence of short ellipsoidal cells along the ascogenous hyphae, seem crucial for distinguishing the two genera.

The anamorphs of the *Calosphaeriales* in the broad sense are reported as being either phialidic or holoblastic-denticulate. Phialidic anamorphs have been proven experimentally only for *Togninia* (*Phaeoacremonium* anamorph; Hausner *et al.* 1992, Mostert *et al.* 2003) and *Pachytrype*, *viz.* *P. princeps* (Penz. & Sacc.) M.E. Barr *et al.* and *P. graphidioides* (Syd. & P. Syd.) M.E. Barr *et al.* (*Cytospora* anamorphs; Barr *et al.* 1993). The anamorph of *P. rimosa* F.A. Fern. *et al.*, the third species in the genus, remains unknown (Fernández *et al.* 2004). The stromatic habitat of *Pachytrype*, perithecia with elongate, protruding beaks; short-stipitate asci with a round base floating free within the centrum, formed on a crozier system; diaporthaceous apical annulus; ellipsoid to oblong, hyaline ascospores, and the *Cytospora* anamorph,

suggest affinities to the *Diaporthales*. *Cytospora* anamorphs have already been linked to *Eutypella* (Nitschke) Sacc., *Cryptosphaeria* Ces. & De Not., *Leucostoma* (Nitschke) Höhn., or *Valsa* Fr. of the *Diaporthales* (Grove 1935, Wehmeyer 1941, Shaw 1973, Glawe & Rogers 1986, Farr *et al*. 1989). The anamorph of *Calosphaeria barbirostris* (Dufour : Fr.) Ellis & Everh. is mentioned twice in the literature based on observations *in vivo* (Munk 1957, Barr 1985), but the descriptions of the putative anamorphs differ significantly from each other.

The *Ramichloridium*-like and *Sporothrix*-like synanamorphs of *Calosphaeria fagi* Samuels & Cand. yielded *in vitro* are the only anamorphs within the heterogeneous *Calosphaeriales* representing the holoblastic, denticulate pattern of conidiogenesis. *Graphostroma* Piroz., in the *Graphostromataceae* of the *Xylariales*, formerly included in the *Calosphaeriales*, and some other *Calosphaeria* species, e.g. *C. fagi*, *C. dryina* (Curr.) Nitschke (Samuels & Candoussau 1996), or *C. parasitica* Fuckel (Fig. 60) possess an ascogenous system typical of members of the *Diatrypaceae*; ascogenous hyphae branching, producing croziers and ultimately asci at successively higher levels on each branch. Unfortunately, the type culture of *C. fagi* is no longer viable (Gary J. Samuels, personal comm.) and living cultures of *C. dryina* and *C. parasitica* were not available for this study. The perithecia of these three *Calosphaeria* species arise in sparse circinate groups beneath the periderm with radially converging beaks united in a disc, and asci possessing a distinct, non-amyloid apical ring with an apical invagination and canal. Samuels & Candoussau (1996) noted that *C. fagi* and *C. dryina* should be included in the *Xylariales* representing derivatives from the *Diatrypaceae*. The presence of phialidic and holoblastic (syn)anamorphs of *C. pulchella* and *C. fagi*, respectively, and conspicuous differences in arrangement of perithecia on the natural substratum, organization of the asci on ascogenous hyphae and morphology of ascal apex of *C. fagi*, *C. dryina* and *C. parasitica* and *C. pulchella*, suggest that the three former species are unrelated to *Calosphaeria s. str.*

ACKNOWLEDGEMENTS

REFERENCES

Barr ME (1978). The Diaporthales in North America with emphasis on *Gnomonia* and its segregates. *Mycological Memoirs* **7**: 1–232.

Barr ME (1983). The ascomycete connection. *Mycologia* **75**: 1–13.

Barr ME (1985). Notes on the *Calosphaeriales*. *Mycologia* **77**: 509–565.

Barr ME (1990). Prodromus to nonlichenized, pyrenomycetous members of class Hymenoascomycetes. *Mycotaxon* **39**: 43–184.

Barr ME (1998). *Wegelina*, a reinstated genus in the *Calosphaeriales*. *Cryptogamie Mycologie* **19**: 169–173.

Barr ME, Rogers JD, Yu YM (1993). Revisionary studies in the *Calosphaeriales*. *Mycotaxon* **48**: 529–535.

Berlese AN (1900). *Icones Fungorum ad usum Sylloges Saccardianae adcommodatae. 3. Sphaeriaceae allantosporae*. Padova.

Cannon PF (1994). The newly recognized family *Magnaporthaceae* and its relationships. *Systema Ascomycetum* **13**: 25–42.

Castlebury LA, Rossman AY, Jaklitsch W, Vasilyeva LN (2002). A preliminary overview of the *Diaporthales* based on large subunit nuclear ribosomal DNA sequences. *Mycologia* **94**: 1017–1031.

Crous PW, Gams W, Wingfield MJ, van Wyk PS (1996). *Phaeoacremonium* gen. nov., associated with wilt and decline diseases of woody hosts and human infections. *Mycologia* **88**: 786–796.

Vijaykrishna D, Mostert L, Jeewon R, Gams W, Hyde KD, Crous PW (2004). *Pleurostomophora,* an anamorph of *Pleurostoma* (*Calosphaeriales*), a new anamorph genus morphologically similar to *Phialophora*. *Studies in Mycology* **50**: 387–395.

Dupont J, Laloui W, Magnin S, Larignon P, Roquebert MF (2000). *Phaeoacremonium viticola*, a new species associated with Esca disease of grapevine in France. *Mycologia* **92**: 499–504.

Eriksson OE, Baral HO, Currah RS, Hanser K, Kurtzman CP, Rambold G, Laessøe T. (eds) (2003). Outline of the Ascomycota – 2003. *Myconet* **9**: 1–89.

Farr DF, Bills GF, Chamuris GP, Rossman AY. (1989). *Fungi on plant and plant products in the United States*. APS Press, St. Paul, Minnesota.

Fernández FA, Rogers JD, Ju Y-M, Huhndorf SM, Umana L (2004). *Paramphisphaeria costaricensis* gen. et sp. nov. and *Pachytrype rimosa* sp. nov. from Costa Rica. *Mycologia* **96**: 175–179.

Gams W, Hoekstra ES, Aptroot A (eds) (1998) *CBS course of mycology, 4th ed*. Centraalbureau voor Schimmelcultures, Baarn, Delft.

Gargas A, DePriest PT, Taylor JW (1995). Positions of multiple insertions in SSU rDNA of lichen-forming fungi. *Molecular Biology and Evolution* **12**: 208–218.

Gargas A, Taylor JW (1992). Polymerase chain reaction (PCR) primers for amplifying and sequencing nuclear 18S rDNA from lichenized fungi. *Mycologia* **84**: 589–592.

Glawe DA, Rogers JD (1986). Conidial states of some species of *Diatrypaceae* and Xylariaceae. *Canadian Journal of Botany* **64**: 1493–1498.

Groenewald M, Kang JC, Crous PW, Gams W (2001). ITS and beta-tubulin phylogeny of *Phaeoacremonium*,

Phaeomoniella spp. *Mycological Research* **105**: 651–657.

Grove WB (1935). *British stem- and leaf- fungi (Coelomycetes). I.* Cambridge University Press.

Gutell RR (1993). Collection of small subunit (16S- and 16S-like) ribosomal RNA structures. *Nucleic Acids Research* **21**: 3051–3054.

Gutell RR, Gray MW, Schnare MN (1993). A compilation of large subunit (23S and 23S-like ribosomal RNA structures: *Nucleic Acids Research* **21**: 3055–3074.

Hall TA (1999). BioEdit 5.0.9: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95–98.

Hausner G, Eyjólfsdóttir GG, Reid J, Klassen GR (1992). Two additional species of the genus *Togninia*. *Canadian Journal of Botany* **70**: 724–734.

Kirk PM, Cannon PF, David JC, Stalpers JA eds (2001). *Ainsworth and Bisby's dictionary of the fungi, 9th ed.* CABI Publishing: Wallingford, U.K.

Kornerup, A, Wanscher JH (1978). *Methuen handbook of colour*, 3rd ed. Eyre Methuen, London.

Lee S, Groenewald JZ, Crous PW (2004). Phylogenetic reassessment of the coelomycete genus *Harknessia* and its teleomorph *Wuestneia* (*Diaporthales*), and the introduction of *Apoharknessia* gen. nov. *Studies in Mycology* **50**: 235–252.

Monod M (1983). Monographie taxonomique des *Gnomoniaceae* (Ascomycètes de l´ordre des *Diaporthales* I.). *Beihefte Sydowia* **9**: 1–120.

Mostert L, Crous PW, Groenewald JZ, Gams W, Summerbell RC (2003). *Togninia* (*Calosphaeriales*) is confirmed as teleomorph of *Phaeoacremonium* by means of morphology, sexual compatibility and DNA phylogeny. *Mycologia* **95**: 646–659.

Mostert L, Groenewald JZ, Summerbell RC, Robert V, Sutton DA, Padhye AA, Crous PW (2004). Species of *Phaeoacremonium* associated with human infections and environmental reservoirs in infected woody plants. *Journal of Clinical Microbiology* (in press).

Munk A (1957). Danish Pyrenomycetes. *Dansk Botanisk Arkiv* **17**(1): 1–491.

Pirozynski KA (1974). *Xenotypa* Petrak and *Graphostroma* gen. nov., segregates from *Diatrypaceae*. *Canadian Journal of Botany* **52**: 2129–2135.

Petrak F, Sydow H (1936). Originaluntersuchungen über die Pyrenomyzeten, Sphaeropsideen und Melanconieen. *Annales Mycologici* **36**: 11–52.

Rehner SA, Samuels GJ (1994). Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycological Research* **98**: 625–634

Romero A, Samuels GJ (1991). Studies on xylophilous fungi from Argentina. VI. Ascomycotina on *Eucalyptus viminalis* (Myrtaceae). *Sydowia* **43**: 228–248.

Samuels GJ, Candoussau F (1996). Heterogeneity in the *Calosphaeriales*: a new *Calosphaeria* with *Ramichloridium*- and *Sporothrix*-like synanamorphs. *Nova Hedwigia* **62**: 47–60.

Shaw CG (1973). Host index for the Pacific Northwest II. Fungi. *Washington Agricultural Experiment Station Bulletin* **766** : 1–162.

Swofford DL (2002). *PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods).* Version 4. Sunderland, MA: Sinauer Associates.

Vilgalys R, Hester M (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.

Wehmeyer LE (1941). A revision of *Melanconis*, *Pseudovalsa*, *Prosthecium* and *Titania*. *University of Michigan Studies* **14**: 1–161.

White TJ, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: a guide to methods and applications* (Innis MA, Gelfand DA, Sninsky JJ, White TJ, eds). Academic Press, San Diego, CA, U.S.A.: 315–322.

Zhang N, Blackwell M (2001). Molecular phylogeny of dogwood anthracnose fungus (*Discula destructiva*) and the *Diaporthales*. *Mycologia* **93**: 355–365.