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 Jobellisias, 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.


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 & Candous-sau 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 Nodosporium-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., Wegelinia 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 *Cytopspora* 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 (Hauser *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 ascus 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 calosphaeraceous 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 *Schizoparmaceae* complex, the *Wuestneia* complex, and the *Cryphonectriaceae*-*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 anellidic 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 herbaric 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-
ceral 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 Cypereaceae.

The seven associated anamorph genera form pycnothryl conidiomata with phialidic conidigenous cells (Mycopliptidiscus Ostaszek, Pseudotracryla B. Sutton & Hodges), or they are hyphomycteous, with phialidic (Harpendora 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 Gaecumomiomyces 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 Phaeacremonomium-like anamorph and its affinity to Togninia and Calosphaeria and the Calosphaeriales, sequences of nuclear LSU and SSU ribosomal DNA of T. minima, T. novezealandiae Hauser et al., T. fraxinopensylvanica (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

<table>
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<th>CBS number</th>
<th>Source</th>
<th>Host</th>
<th>GenBank accession number</th>
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<td>Decayed wood</td>
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<td></td>
<td>113726</td>
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<td>Wood on forest floor</td>
<td>AY761074 AY761079</td>
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<td>Unknown</td>
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<td>New Zealand, Auckland, Woodhill State Forest</td>
<td>Desmoschoenus spiralis</td>
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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 (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. 1995) 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 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.

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 ApiosporalArthrinium clade and Amplesphaeria/ 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 Jobellia clade (100 %) and the putative Calosphaeriales (see below); a lineage of the Annulatacaceae (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. Calosphaeriales/unknown fungus (100 %), Togninia/Phaeoacremonium (100 %) and Pleurostoma/Pleurostomophora (100 %). The strongly supported Togninia/Phaeoacremonium clade grouped together with the Jobellia clade as sister to the Diaporthales on one branch. The strongly supported Pleurostoma/Pleurostomophora and the Calosphaeriales/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 Pleurostomata/Pleurostomophora (100 %) and Calosphaeriales/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 %) are a separate well-supported clade outside Diaporthales.

Two constraint analyses (CA) were performed on LSU rDNA data set to assess the inclusion of Pleurostomata, 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 Pleurostomata 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 *Magnaporthaceae* (100 %), 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 *Pleurostomal/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 *Pleurostomal/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 ascapex 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 ascosporogenous 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 asc 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.

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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 monotypic 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 calosphaeriaceous genera into three lineages but differ from MP analyses in the branching order for Togninia/Phaeoacremonium. The Togninia/Phaeoacremonium 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 Calosphaeriales. The Calosphaeriales 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, stipitate, with stipe attached to the ascogenous hyphae after dehiscence. Ascospores hyaline, allantoid, aseptate. Anamorph (Phaeostomomorpha) moniliaceous, hyphomycetous, with phialidic conidiogenesis.


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


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


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 ellipsoid. Anamorphs (Phaeoacremonium) dematiaceous hyphomycetous, with phialidic conidiogenesis.


Perithecia nonstromatic, densely aggregated in 2–3 levels in ellipsoid to cinctate 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 peripherosphate. Perithecial wall leathery, twolayered, 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, spire 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 & Crouse, gen. nov. MycoBank MB500155. Etymology: Pointing to the teleomorph *Calosphaeria* with suffix from the morphologically similar genus *Phialophora*.


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

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

**Calosphaeriophora pulchella** Réblová, L. Mostert, W. Gams & Crouse, 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.

Anamorph *Calosphaeriae pulchella*. Hyphae ramosae, septateae, singulae vel fasciculatae, perumque hyalinae, nonnullae dilute brunneae, leves, 2–4(–7) µm latae. Conidiophora micronematosa, ex hyphis aeris 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, perumque monophialideae, leves, hyalinae, 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, oblongo elliptoideae 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 subcylin- drical, 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. 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.


Habitat: Saprobic on decayed wood.

Specimen examined: France, Pyrénées Atlantiques, Ariège, Rimont, Ruissau 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 solitary, nonstromatic, fusca vel atra, glabra; venter globosus vel subglobosus, submersus; collum e substrato protrudens, medianum, elongatum; ostiolum periphysatum. Paries peritheci 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 ascogena persistentes, sympleidialiter proliferantes, seriem acropetalem cellulararum 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 hau 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 leafy 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.

Anamorph: Phaeoacremonia Rěbllová, L. Mostert, W. Gams & Crous

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

Perithecium solitary, fusca vel altr, glabra; venter 250−350 µm diam, 300−360 µm alta; collum e substrato protrudens, cylindricum vel modicé flexuosum, 400−600 × 45−60 µm, sursum late rotundatum vel obtusum. Paries perithecii coriaceus vel fragilis, 17−23 µm crassus, bistratosus.

Perithecia solitaria, fusca vel atra, glabra; venter 250−350 µm diam, 300−360 µm alta; collum e substrato protrudens, cylindricum vel modicé 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 modicé constricta, 7−8 µm latae prope basim, sursum ad ca. 3 µm angustatae, ascos superantes. Ascii unitunicati, clavati, 18−21 (−22) × 3−4 µm, longit.:latit. 6:1, spicati ex hyphis ascogenis oriundi, sub acute and narrowly tapering ascus stipe.

Superiore asci fasciculatae. Expellentes Ascosporae suballantoidae, 3–4 µm superantes. Asci unitunicati, clavati, 18−21 (−22) × 3−4 µm, longit.:latit. 6:1, spicati ex hyphis ascogenis oriundi, sub acute and narrowly tapering ascus stipe.


Habitat: Saprobic on decayed wood.

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

Etymology: Contraction of the lengthy diminutive “Phaeoacremoniella”, pointing to the similarity with Phaeoacremonium.

Anamorph *Togniniales*. *Phaeocrella* similis, sed conidiophoris regulariter ramosis, ad septa constrictis, deorsum pigmentatis, sursum pallidoribus distinguenda. Mycelium tuberculatum, brunneum. Phialides praeципue 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 tuberculatum, brown, with subhyaline phialides that are hyaline towards the tip with distinct, shallow, flaring collarettes.

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


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

Anamorph *Togniniales* 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 oriundi, sub acute and narrowly tapering ascus stipe.


Habitat: Saprobic on decayed wood.
Conidia aggregata in capitulis mucidis, hyalina, plerumque obovoida, oblongo-ellipsoida 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–amphilliform, occasionally irregularly 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 ascis 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 ascis 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 ascis arising from a crozier system; ascis 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) ascis 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; Togniniellae: 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 *Calosphaeriaceae*, 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 Magnaportaceae 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 calosphaeriacous genera identical to those shown in MP1. Trees generated in these two CAs forcing members of the a) Calosphaeriales and Magnaportaceae, or b) Calosphaeriales, Magnaportaceae 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 Magnaportaceae, and similarities in dark long- or short-beaked perithecia, hyaline ascospores and phialidic conidio genesis 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 Magnaportaceae.

_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 subballantoid to ellipsoidal, 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 a paraphysate 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, ellipsoidal to subballantoid 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 Calosphaeriales 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 perithelial ascomycetes. The fungi attributed to the Calosphaeriales can be distinguished from the Diaporthales by absence of stromatic tissue surrounding the perithecium; presence of true paraphyses; long-stipitate asci with thickened ascus apex without any discharge mechanism; ramifying and proliferating ascogenous hyphae; allantoid to subballantoid 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 conidio genesis. 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 crozoner system; diaporthaceous apical annulus; ellipsoidal to oblong, hyaline ascospores, and the _Cytospora_ anamorph,
suggest affinities to the *Diaporthales*. *Cytospora* anamorphs have already been linked to *Euptypella* (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* (Curt.) Nitschke (Samuels & Candoussau 1996), or *C. parasitica* Fückel (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.*

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**REFERENCES**


