

Two new dothideomycetous endoconidial genera from declining larch

A. Tsuneda, M.L. Davey, I. Tsuneda, and R.S. Currah

Abstract: Two endoconidial, black meristematic fungi, *Celosporium larixicolum* gen. et sp. nov. (Dothideales) and *Hispidococonidioma alpina* gen. et sp. nov. (Capnodiales) are described from black subicula on twigs of declining larch (*Larix lyallii* Parl) trees in Alberta, Canada. Conidioma morphology and phylogenetic analysis of LSU and ITS regions indicate that these taxa are both distinct from each other and from previously described endoconidial genera. Conidiomata of *C. larixicolum* consist of black cellular clumps (aggregated conidiogenous cells) that are either naked or enveloped by scant to dense mycelium that sometimes organizes into a cupulate peridium. Endoconidia are 1–3 celled, hyaline when released but become pigmented as they age, and very variable in size and shape, e.g., globose, pear-shaped, osteoid, or discoid with an irregular flange. In *H. alpina*, colonies are three-layered, consisting of a central pseudoparenchymatous layer sandwiched between an upper and a basal hyphal layers, and conidiogenesis occurs in sporadic areas of the central layer. Endoconidia are unicellular, hyaline, and subglobose to ellipsoid. The strong phylogenetic affinities between these newly described taxa and slow-growing, melanized fungi isolated from rocks suggest individual black meristematic fungus lineages may have broad habitat ranges.

Key words: black yeasts, conidiogenesis, Dothideomycetes, *Larix*, LSU, ITS rDNA.

Résumé : Les auteurs décrivent deux champignons noirs conidiens méristématiques, les *Celosporium larixicolum* gen. et sp. nov. (Dothideales) et *Hispidococonidioma alpina* gen. et sp. nov. (Capnodiales) obtenus de subicules noirs venant sur des ramilles de mélèzes dépérissants (*Larix lyallii* Parl), en Alberta au Canada. La morphologie des conidiomes et l'analyse phylogénétique des régions LSU et ITS indiquent que ces taxons sont tous les deux distincts l'un de l'autre, ainsi que d'autres genres endoconidiens déjà décrits. Les conidiomes du *C. larixicolum* comporte des amas cellulaires noirs (agrégation de cellules conidiogènes) qui sont soit nues ou soit enveloppées par un mycélium clair à dense, s'organisant quelquefois en un périidium cupulé. Les endoconidies comportent 1–3 cellules, hyalines à leur relâchement, mais qui deviennent pigmentées avec l'âge, et sont très variables en forme et en dimension, globulaires à piriformes, ostéoïdes ou discoïdes avec une collerette irrégulière. Chez le *H. alpina*, les colonies ont trois couches, une couche pseudoparenchymatique centrale située entre des couches d'hyphes supérieure et basale; la conidiogenèse s'effectue sporadiquement dans des endroits de la couche centrale. Les endoconidies sont unicellulaires, hyalines, de subglobulaires à ellipsoïdes. Les fortes affinités phylogénétiques entre ces deux taxons nouvellement décrits avec des champignons mélanisés à croissance lente isolés à partir de rochers suggèrent que les lignées individuelles des champignons méristématiques pourraient posséder un vaste ensemble d'habitats.

Mots-clés : levures noires, conidiogenèse, Dothideomycetes, *Larix*, LSU, ITS rADN.

Introduction

Endoconidia are defined as asexual spores that are produced endogenously in a sporogenous cell and have no wall layers in continuum with the sporogenous cell (Hennebert and Sutton 1994; Tsuneda and Currah 2006). Endoconidium-forming dematiaceous fungi are phylogenetically allied to Dothideomycetidae, Pleosporomycetidae (Dothideomycetes), and Chaetothyriomycetidae (Eurotiomycetes) (sensu Hibbett et al. 2007) (Tsuneda and Currah 2006), and most of them are informally considered to be members of a polyphyletic assemblage of dematiaceous hyphomycetes called

black yeasts or black meristematic fungi (BMFs) (de Hoog et al. 1999). Five BMF genera are known to form endoconidial conidiomata: *Phaeotheca* (Sigler et al. 1981; Tsuneda and Murakami 1985), *Hyphospora* (Ramaley 1996), *Scleroconidioma* (Hambleton et al. 2003), *Endoconidioma* (Tsuneda et al. 2004a, 2004b), and *Endosporium* (Tsuneda et al. 2008), differing from each other primarily in conidioma structure. A teleomorph is known only for *Hyphospora*, viz., *Comminutispora agavacearum* Ramaley (Ramaley 1996). BMFs have been reported from a variety of substrates, including plant material (Sigler et al. 1981; DesRochers and Ouellette 1994; Ramaley 1996; Tsuneda et al.

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A. Tsuneda¹ and R.S. Currah. Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada.

M. Davey. Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, Box 5003, Ås 1432, Norway; Microbial Evolution Research Group, Department of Biology, University of Oslo, PO Box 1066 Blindern, Oslo N-0316, Norway.

I. Tsuneda. Medicine/Dentistry Electron Microscopy Unit, University of Alberta, Edmonton, AB T6G 2N8, Canada.

¹Corresponding author (e-mail: atsuneda@ualberta.ca).

2000, 2008; Crous et al. 2007b), rock (Wollenzien et al. 1997; Ruibal 2004; Ruibal et al. 2008), water from a saltern (Zalar et al. 1999), and human tissues (Matsumoto et al. 1990). Although little is known about their ecology, BMFs are known to be resistant to harsh environments (Onofri et al. 2007), and endoconidial BMFs include pathogens of humans and plants (Tsuneda et al. 2001; Chabasse 2002).

In our survey of tree-associated BMFs in the Larch Valley, Banff National Park, Alberta, Canada, in August 2002, we noticed black subicula of unknown BMFs on twigs and trunks of many larch trees (*Larix lyallii* Parl), but their occurrence at that time was mostly confined along the walking trail. In August 2008, however, the BMFs had extended over a large area in the valley: young trees were dead and many mature trees showed signs of decline. We isolated three BMFs of which one is still unidentified and the other two developed endoconidial conidiomata in culture from black subicula on twigs of declining larch trees. Because these endoconidial BMFs differed both morphologically and phylogenetically from each other, and from all previously described endoconidium-forming fungi, we propose herein two new endoconidial BMF genera and species, *Celosporium larixicolum* and *Hispidoconidioma alpina*.

Materials and methods

Isolation and microscopy

Cultures of both *Celosporium larixicolum* and *Hispidoconidioma alpina* were obtained from black subicula on twigs of *Larix lyallii* in Larch Valley, Banff National Park, Canada (51°19'N, 116°12'W), using the method described in Tsuneda et al. (2000). Isolates were deposited at the University of Alberta Microfungus Collection and Herbarium (UAMH).

Colony morphology, growth rates, and microscopic descriptions of both genera/species were made using subcultures grown on 2% malt extract agar (MEA; Difco Laboratories, Detroit, Michigan) or potato dextrose agar (PDA; Difco Laboratories) at 20 °C in the dark. To induce conidioma formation, cultures were first incubated at 20 °C for 20–30 d and then kept at 10 °C in the dark for 1–2 months. Both fungi produced conidiomata by this method when freshly isolated, but ceased production after subculturing several times. Specimens for thin-section observations by light microscopy (LM) were prepared according to Tsuneda et al. (2008), and those for transmission and scanning electron microscopy (TEM and SEM, respectively) were prepared according to Tsuneda et al. (2000). SEM and TEM specimens were examined and photographed using a Hitachi S-510 electron microscope at 10 or 15 kV and a Hitachi H-7000 at 75 kV, respectively.

DNA sequencing and phylogenetic analysis

Fungal isolates were grown on PDA overlaid with a Cellophane™ membrane (UCB Films, Somerset, UK) for 25 d at ambient light and temperature. Genomic DNA was extracted using a CTAB extraction buffer, as described by Davey and Currah (2007) and purified using a QIAquick PCR Purification kit (QIAGEN, Mississauga, Ontario). The ITS and LSU regions were amplified using the forward and reverse primer sets ITS5 and LR1 (Vilgalys and Hester 1990;

White et al. 1990), and LROR and LR7 (Vilgalys and Hester 1990), respectively. Amplicons were purified as described by Davey and Currah (2007) and sequenced with an ABI 3100 automated sequencer (Applied Biosystems, Foster, California) and the primers ITS1, ITS 4, ITS 5 and LR1 (Vilgalys and Hester 1990; White et al. 1990) for the ITS region, or LROR, LR3R, LR5, LR7, and LR16 (Vilgalys and Hester 1990) for the LSU region.

A data matrix was assembled from LSU sequences of *C. larixicolum*, *H. alpina*, and members of the Dothideales, Capnodiales, and Myriangiales (Dothideomycetidae) retrieved from GenBank. Additional matrices were assembled from ITS sequence data for *C. larixicolum* and members of the Dothideales, and for *H. alpina* and members of the Capnodiales. Sequences of each matrix were aligned using MAFFT version 5.8 (Katoh et al. 2005) and the subsequent alignments were manually verified. These matrices were subjected to maximum parsimony analysis using PAUP* version 4.0b10 (Swofford 2003) with Fitch parsimony, random simple step-wise addition of taxa, tree bisection–reconnection (TBR), branch swapping, and gaps treated as missing data. Support for branching topologies was evaluated using 1000 resamplings of the data by bootstrapping analysis using the same criterion described above (Felsenstein 1985). All trees were scored for length in steps, consistency index (CI), retention index (RI), and homoplasy index (HI). Bayesian analysis was conducted on all matrices using MrBayes version 3.1 (Ronquist and Huelsenbeck 2003) with a general time reversible model of DNA substitution (GTR) with gamma distributed rate variation across sites (invariance, partitioning across genes) for two independent Markov Chain Monte Carlo runs with between 1×10^6 and 1×10^7 generations each, sampling trees every 100th cycle. A final standard deviation of < 0.01 for the split frequency was taken as an indication that convergence had been achieved. The first 25% of sampled trees were discarded as burn-in and posterior probabilities for each node of the 50% majority rule consensus tree were recorded.

Results

Taxonomy

Celosporium Tsuneda & Davey, gen. nov.

TYPUS GENERIS: *Celosporium larixicolum*

ETYMOLOGY: *Celosporium* — “hidden spore” in reference to the production of endoconidia.

Hyphae dematiaceae, formantes globos cellularios terminales vel intercalarios, nigros et irregulariter-formatos, subdivisae a septis multiplicibus et temere orientatis. Conidiomata ex globis cellulariis aggregatis, nuda vel mycelio tecta. Conidia e 1–3 cellulis, hyalina, forma et magnitudine varia, formantia in cellulis conidiogenosis intra globos cellularios et dissolutione tunicae cellulariae exsoluta.

Hyphae dematiaceous, forming terminal or intercalary, black, irregular-shaped cellular clumps, subdividing by multiple, randomly oriented septations to form daughter cells inside. Conidiomata composed of aggregated cellular clumps, naked or with a mycelial envelope. Endoconidia forming within cellular clumps (conidiogenous cells), 1–3 celled, hyaline, variable in shape and size, and released by cell-wall dissolution of the conidiogenous cells.

Celosporium larixicolum Tsuneda & Davey, sp. nov.
Figs. 1–24

ETYMOLOGY: Larixicolum — in reference to the fungus' host.

Formans subicula cerebriformia in ramis Laricis lyallii et conidiomata sustinentia et magnitudine varia, e paucis vel multis cellulis aggregatis et conidiogenesis, peridium cupulatum et brunneum adest vel abest. Endoconidia e 1–2 cellulis, hyalina, subglobosa ad fusiformia. Conida et in superficie subiculi blastice formantia. In cultura, in agaro extracti malti, coloniae nigrae, formantes conidiomata globorum fusce-pigmentatorum ex cellulis aggregatis et conidiogenesis, interdum peridium cupulatum adest. Endoconidia e 1–3 cellulis, hyalina, forma et magnitudine varia.

ON TWIGS OF *LARIX LYALLII*: Subicula black, carbonaceous, cerebriform, consisting of numerous cellular clumps of meristematically growing cells that develop into conidiogenous cells when mature. Conidiomata variable in size, composed of a few to many aggregated conidiogenous cells, with or without a peridium. Peridium cupulate, consisting of brown, subglobose, thick-walled cells, 4–6 µm in diameter. Endoconidia 1–2 celled, hyaline, subglobose to fusiform, 5–6.5 µm × 6–8 µm. Blastocidia on the surface of subicula, hyaline, ellipsoidal or obovoid, 1.5–2 µm × 2–4 µm.

ON 2% MEA: Colonies attaining a diameter of 9–15 mm in 10 d at 20 °C in the dark, consisting of a black (initially creamy white), moist, raised central area containing numerous subdividing cells and blastocidia, and a peripheral area of radiating brown hyphae, producing pigments that turn the agar light yellow to orange. Hyphae pale to dark brown, cylindrical or torulose, regularly septate. Conidiomata consisting of darkly pigmented clumps (aggregated endoconidiogenous cells), hemispherical to irregular in shape, up to 600 µm in long axis, naked or with a scant to dense mycelial envelope that is sometimes organized into a cupulate peridium. Endoconidiogenous cells subspherical, 8.5–13.5 µm × 6.5–10 µm. Endoconidia, 1–3 celled, hyaline, variable in size and shape, globose, pear-shaped, ostoid, or discoid with an irregular flange, (3)4–6 µm × 6–9(13) µm. Blastocidia hyaline, subglobose to ellipsoidal, 2–3 µm × 3–7 µm.

HOLOTYPE: Dried culture prepared from an isolate (UAMH 11008) obtained from a black subiculum formed on a twig of *Larix lyallii* collected at the Larch Valley, Banff National Park, Alberta, Canada, 5 August 2008 by A. Tsuneda.

ADDITIONAL MATERIAL EXAMINED: Living culture (UAMH 11009) isolated from a black subiculum formed on a twig of *Larix lyallii*, collected at the Larch Valley, Banff National Park, Alberta, Canada, 23 September 2008 by A. Tsuneda.

Hispidoconidioma Tsuneda & Davey, gen. nov.

TYPUS GENERIS: *Hispidoconidioma alpina*

ETYMOLOGY: Hispidoconidioma — “bristled conidioma” in reference to the bristle-like hyphal layers at the surface of the conidioma.

Hyphae pallide ad fusce brunneae, rectae vel moniliformes, constanter septatae. Conidiomata fusce pigmentata, cum strato mediali et pseudoparenchymatoso, et hyphas rigidas et erectas in superficie superiore ferente. Endoconidia

unicellularia, hyalina, subglobosa ad ellipsoidea, formantia in cellulis strati medialis.

Colonies slow-growing, black when mature, three-layered, consisting of a pseudoparenchymatous central layer sandwiched between two hyphal layers. Hyphae light to dark brown, straight or moniliform, regularly septate. Conidiogenesis occurring in sporadic areas of the pseudoparenchymatous central layer. Endoconidia, unicellular, hyaline, subglobose to ellipsoid.

Hispidoconidioma alpina Tsuneda & Davey, sp. nov. Figs. 25–37

ETYMOLOGY: Alpina — in reference to the high elevation habitat of the type collection.

Globos nigros cellularum in ramis Laricis lyallii formantia. In cultura, in agaro dextrosi solani tuberosi, coloniae fuscae, cerebriformes, a hyphis brunneis et determinatis tegatae. Conidiomata fusce pigmentata, cum strato mediali et pseudoparenchymatoso, cum strato superiore hypharum rigidarum, erectarum, determinatarum, et filamentosarum, infra hyphae temere dispositae et indeterminatae. Endoconidia unicellularia, hyalina, subglobosa ad ellipsoidea, formantia in cellulis strati medialis et pseudoparenchymatosi.

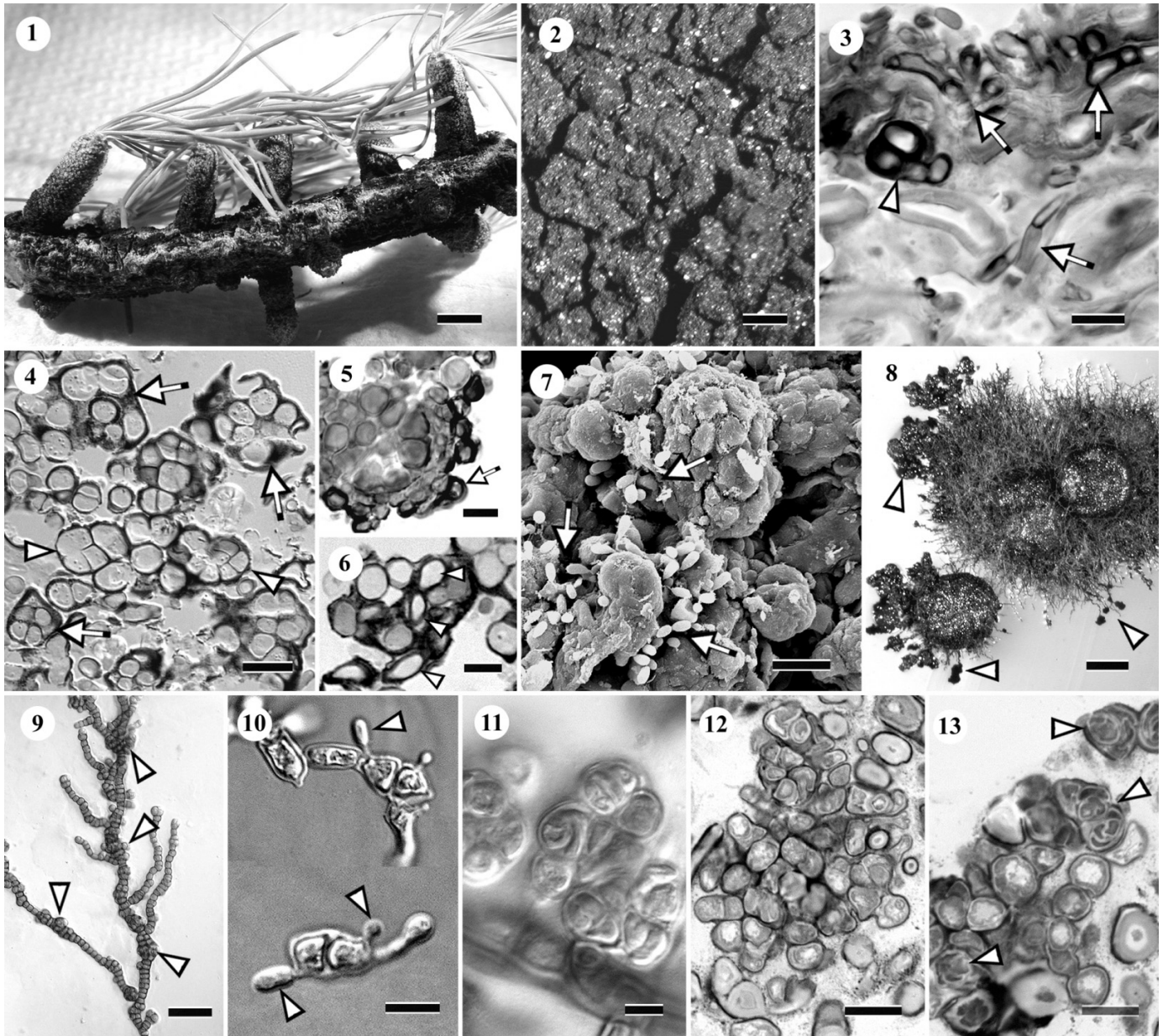
Colonies slow-growing, subspherical to irregular in shape, attaining 2–4 mm in diameter in 3 weeks on PDA at 20 °C in the dark, initially creamy white, soon becoming black, raised, often cerebriform, three-layered, consisting of a pseudoparenchymatous central layer (60–100 µm in height) sandwiched between two hyphal layers. Conidiogenesis occurring in sporadic areas of the pseudoparenchymatous central layer. Hyphae dematiaceous, straight or irregularly moniliform, septate, occasionally forming terminal or intercalary, endoconidiogenous cellular clumps. Endoconidia unicellular, hyaline, subglobose to ellipsoid, 2–3(3.5) µm × 3–5(8) µm.

HOLOTYPE: Dried culture prepared from an isolate (UAMH 11010) obtained from a black colony formed on a twig of *Larix lyallii* Parl. collected at Larch Valley, Banff National Park (collection permit: LL-2008–1753), Alberta, Canada, 5 August 2008 by A. Tsuneda.

Microscopic observations

On twigs of larch, *Celosporium larixicolum* formed black subicula (Figs. 1 and 2) consisting mostly of meristematic cells and scant thick-walled hyphae. Hyphae and meristematic cells were also present in the peridermal region of the twigs (Fig. 3). LM (thin sections) and SEM observations of naturally formed subicula revealed that the meristematic cells were organized into cellular clumps that varied in size and developmental stage (Figs. 4–7); i.e., some were in early stages of meristematic growth (Fig. 4, arrowheads) and others were in the process of endoconium development and release (Fig. 4, arrows). Conidiomata (mature cellular clumps) were either naked (Figs. 4 and 6) or provided with a peridium consisting of a single layer of cells that were brown to black, globose to subglobose, and thick-walled (Fig. 5, arrow). Occasionally, 1–2 layers of hyaline, thin-walled hyphal layers occurred between the peridium and conidiogenous cells. Endoconidia were hyaline and varied in size and shape (Figs. 4 and 6). When subicula were hy-

Figs. 1–13. *Celosporium larixicolum*. Fig. 1. Twig of larch bearing black subicula; scale bar = 5 mm. Fig. 2. Black subiculum viewed under a dissecting microscope; scale bar = 20 μ m. Fig. 3. Thin section showing dematiaceous hyphae (arrows) and meristematic cells (arrowhead) in a peridermal region of a twig; scale bar = 10 μ m. Fig. 4. Thin section of a portion of a subiculum showing cellular clumps in early stage of meristematic growth (arrowheads) and in the process of endoconidium development (arrows); scale bar = 10 μ m. Figs. 5 and 6. Thin sections of conidiomata formed on bark, showing a peridium (arrow) and endoconidia (arrowheads); scale bars = 5 μ m. Fig. 7. SEM micrograph of a naturally formed subiculum giving rise to abundant unicellular, blastic conidia (arrows) after hydrated with distilled water; scale bar = 10 μ m. Figs. 8–13. On 2% malt extract agar. Fig. 8. Colonies after 2 months. Arrowheads indicate aggregated cellular clumps; scale bar = 0.5 mm. Fig. 9. Early stages of cellular clump formation by meristematic growth of hyphal cells (arrowheads); scale bar = 30 μ m. Fig. 10. Free cells giving rise to blastic conidia (arrowheads); scale bar = 10 μ m. Fig. 11. Merging young cellular clumps; scale bar = 5 μ m. Figs. 12 and 13. Thin sections showing cellular clumps enlarging by meristematic growth. Arrowheads indicate conidiogenous cells actively dividing to form daughter cells; scale bars = 10 μ m.



drated with distilled water and kept for 48 h at room temperature (20–22 °C) in the dark, they gave rise to numerous, unicellular, blastic conidia (Fig. 7).

On MEA, colonies of *C. larixicolum* initially resembled those of white yeasts, forming subspherical masses of hyaline yeast-like cells, but these cellular masses soon became

darkly pigmented and gave rise to dematiaceous hyphae from their peripheries (Figs. 8 and 9). Meristematic cell divisions often occurred in terminal and intercalary hyphal cells, forming masses of thick-walled cells (cellular clumps) (Figs. 8 and 9; arrowheads). Cellular clumps also originated from free cells that sometimes formed blastic conidia while

undergoing meristematic divisions (Fig. 10). Adjacent cellular clumps often merged (Figs. 11 and 12) to form irregular-shaped black aggregates (Fig. 14) that developed into conidiomata when kept at 10 °C for 1–2 months in the dark. At the onset of endoconidiogenesis, active cell divisions took place within individual cells of cellular clumps to form daughter cells (Fig. 13; arrowheads). One-to-several daughter cells developed in each mother cell and they were separated from one another to become endoconidia. Mature conidiomata were black (Fig. 14), and either naked or partially enclosed by vegetative hyphae (Fig. 15) that sometimes were organized into a cupulate peridium (Fig. 16, arrowhead). Release of endoconidia was by the degeneration of the conidiogenous (mother) cell walls (Figs. 17 and 18). Endoconidia were 1- to 3-celled, extremely variable in shape and size (Figs. 17–24), hyaline and thin-walled when released but became light brown and thick-walled with age (Fig. 24).

Hispidoconidioma alpina also formed black cellular clumps on larch twigs but unlike *Celosporium*, its cellular clumps exclusively gave rise to germ tubes (hyphae) after hydration and incubation at 20 °C in the dark (Fig. 25). Sporulating colonies were not found on larch twigs. Colonies on PDA were shiny olivaceous and covered with abundant aerial hyphae that were determinate in growth and hyphae at the colony periphery were mostly submerged (Figs. 26, 27). As in *C. larixicolum*, hyphal cells often developed into cellular clumps through meristematic growth (Fig. 28) and occasionally formed endoconidia within them (Fig. 29, arrows). However, *H. alpina* differed from *C. larixicolum* in that its colonies were three-layered (Figs. 30 and 31) and conidiogenesis occurred in the central, pseudoparenchymatous layer. Endoconidiogenesis first began at the surface areas of the central (now conidiogenous) layer (Figs. 32 and 33) and subsequently spread to the inner portion of the layer (Fig. 34). Multiple mounts of various portions of colonies were needed to locate the discontinuous areas of conidiogenesis in the central layer that were concealed by the hyphae of the surface layer. *Hispidoconidioma alpina* also differed from *C. larixicolum* in endoconidium morphology. Endoconidia of *H. alpina* (Figs. 34–37) were unicellular, hyaline, and much less variable in size and shape than those of *C. larixicolum*. In both species, endoconidiogenesis involved (i) formation of daughter cells in conidiogenous mother cells by septum formation; (ii) accumulation of an electron-dense material between the daughter and mother cell walls; and (iii) separation of daughter cells by septum schizolysis (separated daughter cells = endoconidia) (Fig. 35). Both *C. larixicolum* and *H. alpina* ceased to form endoconidia after a few transfers under our cultural conditions.

Phylogenetic analyses

The aligned LSU matrix included 42 taxa distributed among the Dothideales, Myriangiales, and Capnodiales, and consisted of 1248 characters, of which 867 were constant, 128 were parsimony uninformative, and 253 were parsimony informative. Three equally parsimonious trees were generated by maximum parsimony analysis, each with 1200 steps (CI = 0.463, RI = 0.683, HI = 0.537). Results of both the bootstrap analysis and Bayesian inference are shown on a single most parsimonious tree (Fig. 38). *Celosporium larixi-*

colum demonstrates affinities to the Dothideales [72% Bootstrap (BS), 98 Bayesian Posterior Probability (BPP)] and is supported (72% BP, 98BPP) as sister to all other dothidealean taxa, including *Endoconidioma populi* Tsuneda et al., another endoconidium-producing taxon. *Hispidoconidioma alpina* is allied with the Capnodiales (72% BP, 100 BPP) and is included in a weakly to moderately supported (55% BP, 86BPP) sub-clade of 7 taxa that also contains two other endoconidium forming taxa [*Phaeothecoidea eucalypti* Crous & Summerell and *Catenulostroma abietis* (Butin & Pehl) Crous & U. Braun].

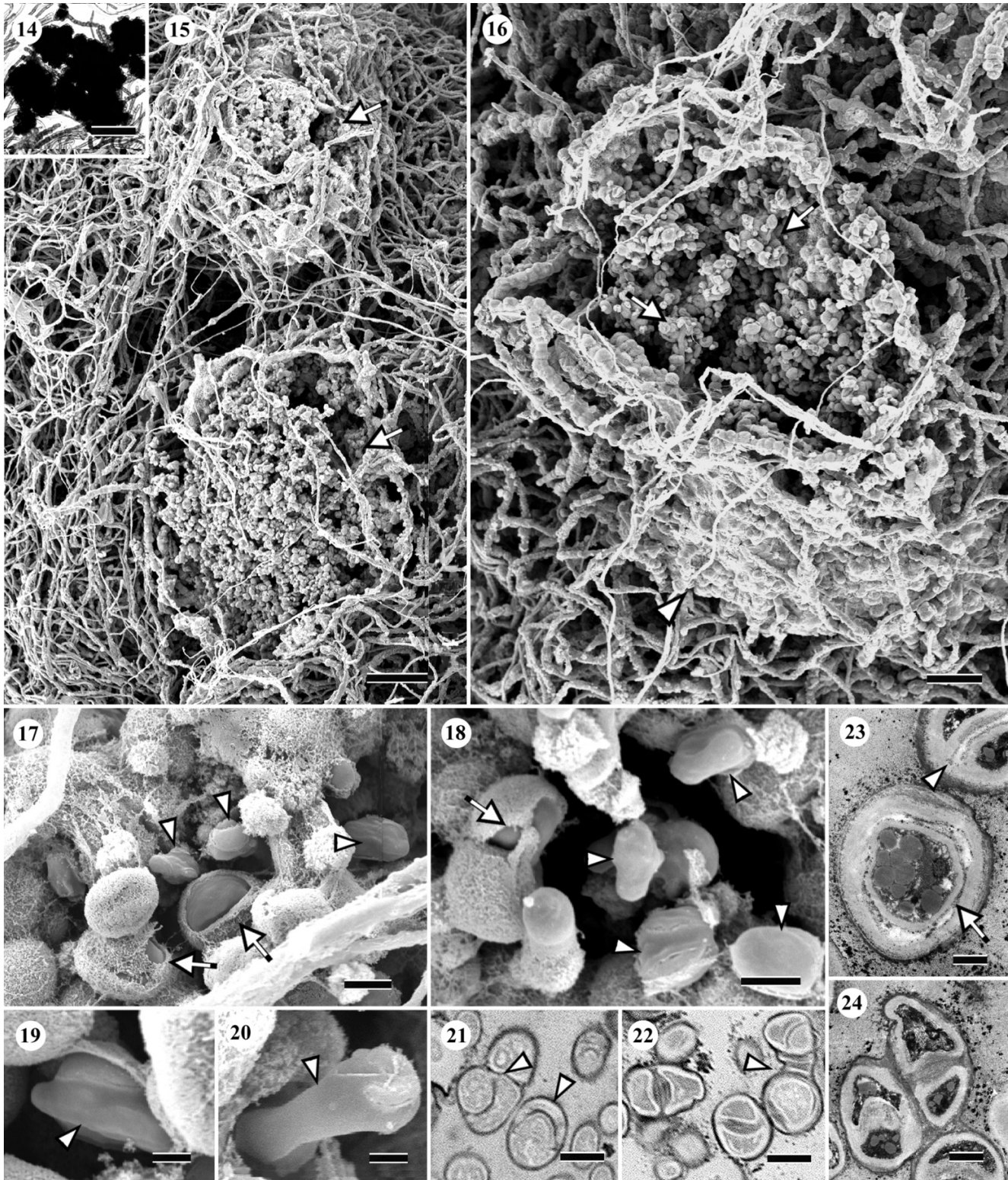
The ITS region of *C. larixicolum*, 28 members of the Dothideales, and the outgroup taxon *Elsinoë eucalyptorum* Crous & Summerell was aligned in a matrix consisting of 569 characters, of which 336 were constant, 90 were parsimony uninformative, and 143 were parsimony informative. Maximum parsimony analysis generated 1041 equally parsimonious trees that were consistent in their placement of *C. larixicolum*, each with 481 steps (CI = 0.663, RI = 0.827, HI = 0.337). Results of both the bootstrap analysis and Bayesian inference are shown on a single most parsimonious tree (Fig. 39). *Celosporium larixicolum* forms a strongly supported clade (100%BP, 100BPP) with six endophytic taxa and four unidentified taxa from rock, two of which produce endoconidia (Ruibal et al. 2008). This clade is nested within the Dothideales, and remote from other described endoconidial genera, including *Scleroconidioma* and *Endoconidioma*.

The aligned ITS matrix of *H. alpina* and members of the Capnodiales included 41 taxa, and consisted of 561 characters, of which 230 were constant, 78 were parsimony uninformative, and 253 were parsimony informative. Three equally parsimonious trees were generated by maximum parsimony analysis, each with 1581 steps (CI = 0.393, RI = 0.503, HI = 0.607). Results of both the bootstrap analysis and Bayesian inference are shown on a single most parsimonious tree (Fig. 40). *Hispidoconidioma alpina* forms a strongly supported clade (90% BS) with three unidentified endolithic fungi from the Central Mountain System in Spain. This clade is sister to *Catenulostroma elginense* (95% BS, 100 BPP) and does not group with other capnodialean endoconidium-forming taxa (i.e., *Phaeothecoidea*, *Capnobotryella*, *Catenulostroma*, *Comminutispora*).

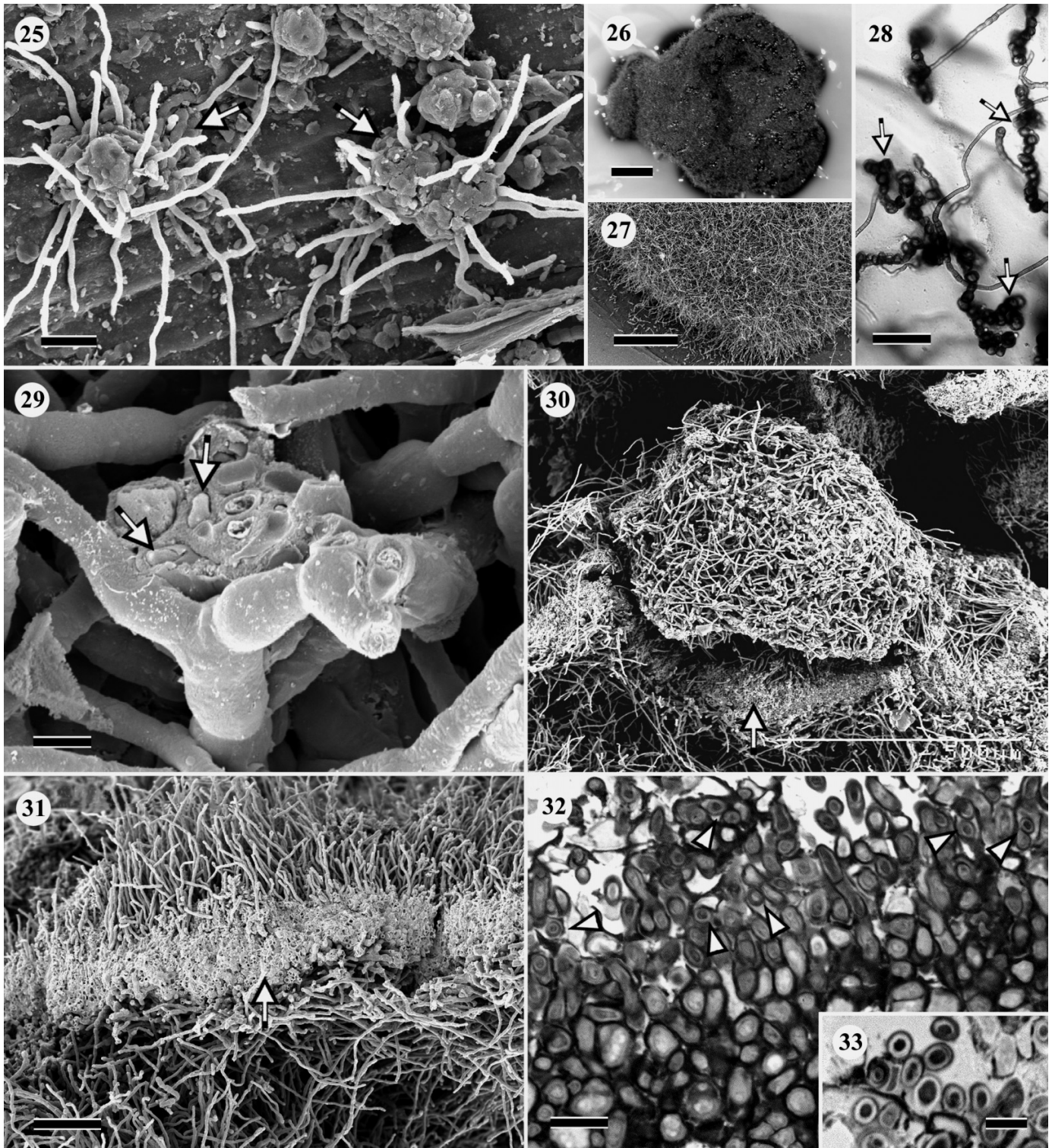
Discussion

The conidioma and endoconidium morphology of *Celosporium larixicolum* and *Hispidoconidioma alpina* are distinct both from one another and from the other five BMF genera that are known to develop endoconidial conidiomata, i.e., *Endoconidioma*, *Endosporium*, *Hyphospora* (anamorph of *Comminutispora*), *Phaeotheca*, and *Scleroconidioma*. *Celosporium larixicolum* is unique in that (i) endoconidial cellular clumps often bear a mycelial envelope that sometimes is organized into a cupulate peridium, and (ii) endoconidia are 1–3 celled and extremely variable in size and shape, from globose to discoid with an irregular flange. Conidiomata of both *Endoconidioma* and *Scleroconidioma* also possess a peridium but those of the former are cleistopycnidial, having a closed peridium and a locule filled with conidiogenous mother cells (Tsuneda et al. 2004b), while those of the

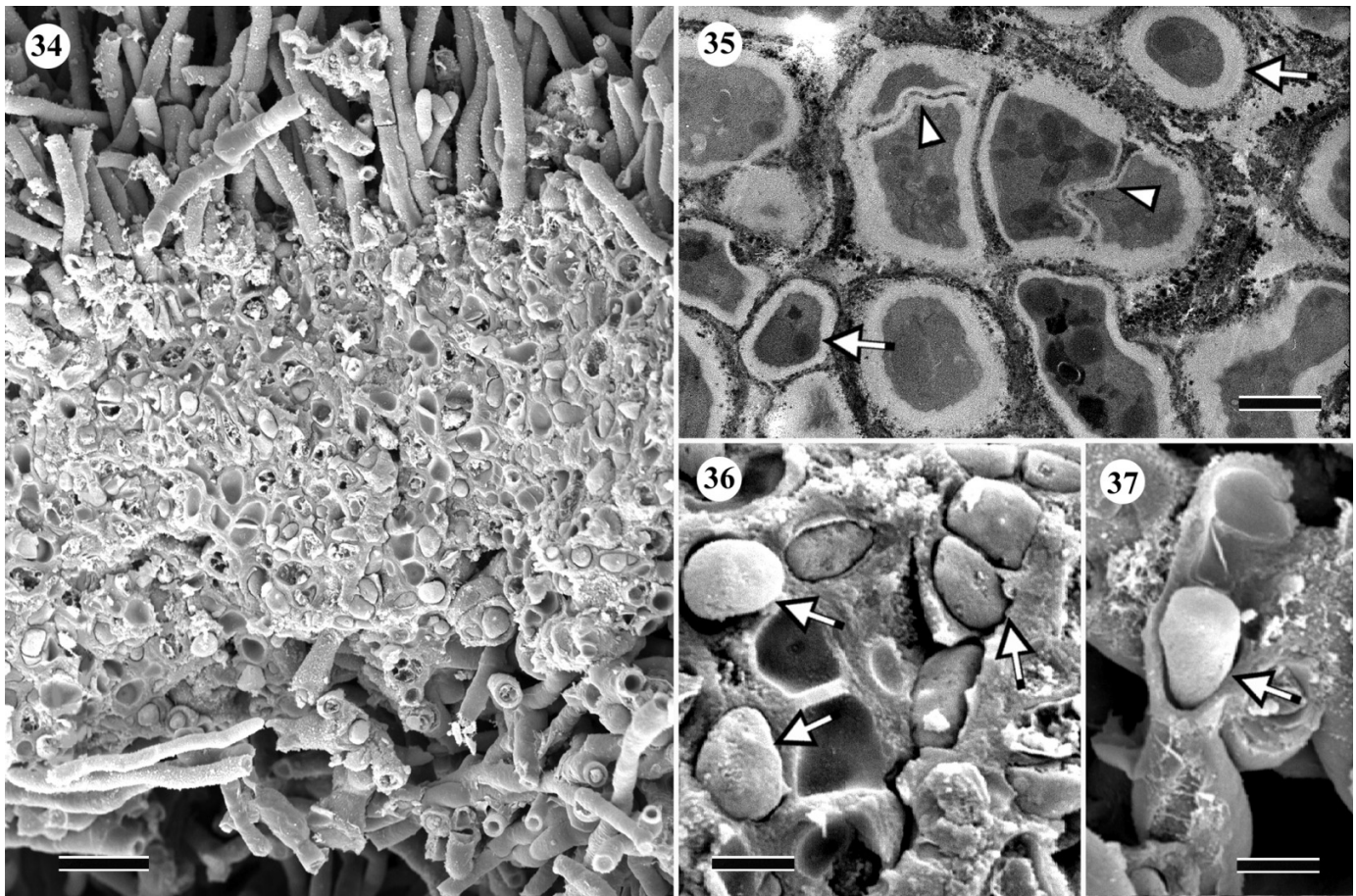
Figs. 14–24. Conidiomata and endoconidia of *Celosporium larixicolum*. Figs. 14, 21, and 22, LM; Figs. 15–20, SEM; Figs. 23 and 24, TEM. Fig. 14. Black conidiomata; scale bar = 0.5 mm. Fig. 15. Conidiomata (arrows) consisting of aggregated cellular clumps and excipular mycelial envelope; scale bar = 100 μ m. Fig. 16. Conidioma with a cupulate peridium (arrowhead). Numerous endoconidia have been released (arrows); scale bar = 50 μ m. Figs. 17–20. Enlarged views of endoconidia (arrowheads) and degenerating walls of mother (conidiogenous) cells (arrows). Note that endoconidia are extremely variable in size and shape. Figs. 17 and 19, scale bar = 5 μ m; Figs. 18 and 20, scale bar = 2 μ m. Figs. 21 and 22. Thin-sectional views of endoconidia. Arrowheads indicate variation in their shape. Endoconidia are thin-walled when released; scale bars = 4 μ m. Fig. 23. Dividing mother cell by septation (arrowhead) and a developing endoconidium (arrow) within its mother cell. Cell walls of mother cells are still intact; scale bar = 2 μ m. Fig. 24. Two-celled endoconidia. Endoconidia become thick-walled with age; scale bar = 4 μ m.



Figs. 25–33. *Hispidiconidioma alpina*. Figs. 25, 27, 29–31, SEM; Figs. 26, 28, 32, 33, LM. Fig. 25. Cellular clumps (arrows) formed on a larch twig, giving rise to hyphae (germ tubes) after hydrated with distilled water; scale bar = 40 μ m. Fig. 26. Colony on PDA (1 month); scale bar = 2 mm. Fig. 27. Colony surface covered with numerous hyphae, determinate in growth; scale bar = 0.5 mm. Fig. 28. Cellular clumps (arrows) developing from hyphal cells; scale bar = 10 μ m. Fig. 29. Fractured cellular clump showing endoconidia (arrows); scale bar = 5 μ m. Figs. 30 and 31. Horizontally and vertically sectioned young colonies showing a pseudoparenchymatous central layer (arrows) sandwiched between (upper and basal) hyphal layers. Endoconidiogenesis is not evident in these colonies; scale bars = 50 μ m (Fig. 30) and 80 μ m (Fig. 31). Fig. 32. Sectional view of a pseudoparenchymatous layer. Endoconidiogenesis begins at the surface area (arrowheads); scale bar = 10 μ m. Fig. 33. Thin section showing released, unicellular endoconidia; scale bar = 5 μ m.



Figs. 34–37. Endoconidiogenesis in *Hispidoconidioma alpina*. Figs. 34, 36, 37, SEM; Fig. 35, TEM. Fig. 34. Numerous endoconidia formed in pseudoparenchymatous (now conidiogenous) layer; scale bar = 15 μ m. Fig. 35. Dividing conidiogenous mother cells showing septa (arrowheads) and endoconidia (arrows); scale bar = 4 μ m. Figs. 36 and 37. Enlarged views of endoconidia (arrows); scale bars = 4 μ m.

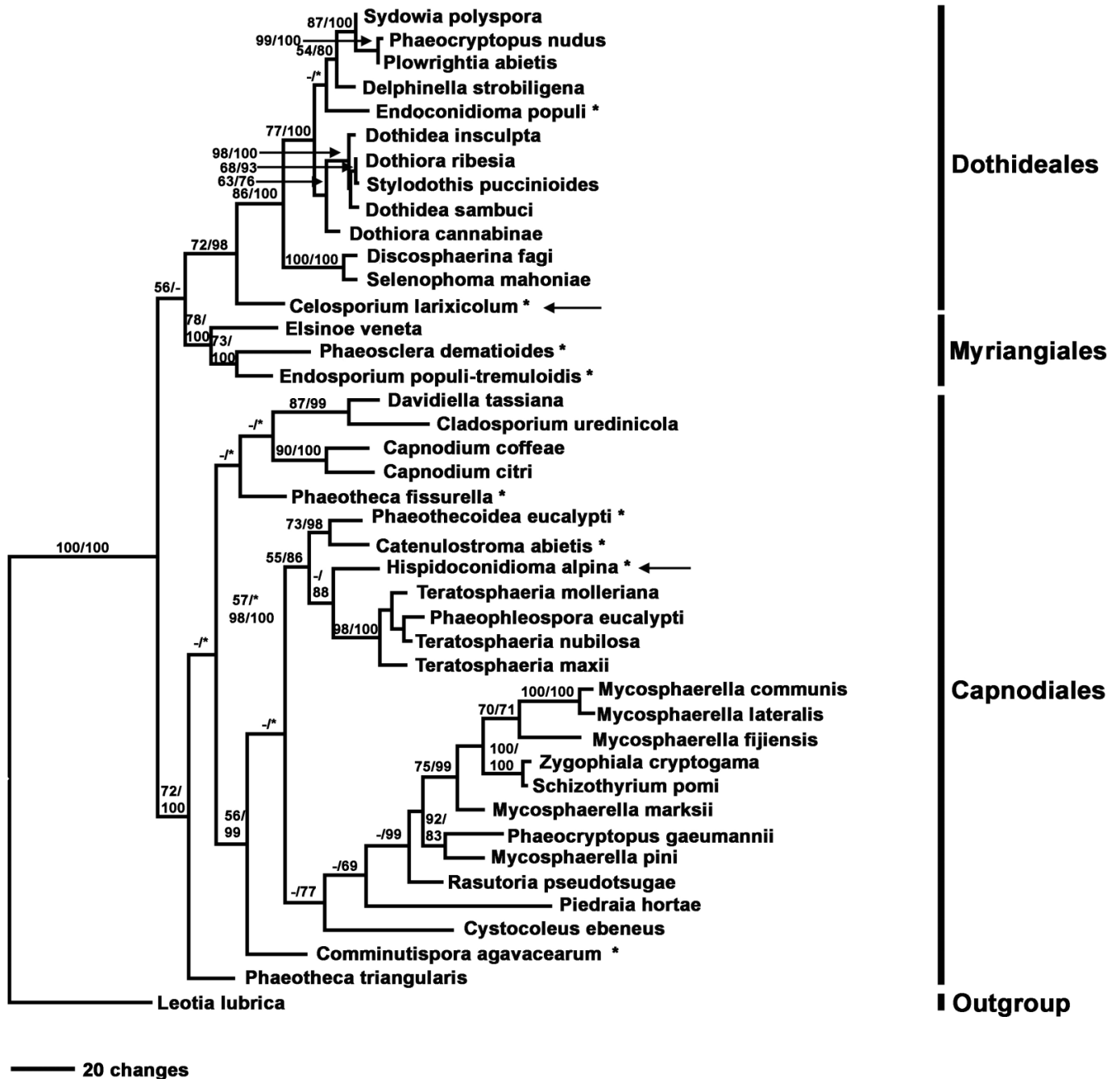


latter are microsclerotial (Hambleton et al. 2003). Conidiomata in other endoconidial genera lack a peridium. Furthermore, the conidiomata in *Endosporium* disintegrate upon maturation to release a mixture of conidiogenous cellular clumps and endoconidia in slimy liquid (Tsuneda et al. 2008). Endoconidia in *Endosporium* and *Hyphospora* are exclusively unicellular (Ramaley 1996; Tsuneda et al. 2008), and those of *Phaeotheca* are pigmented (Sigler et al. 1981; de Hoog et al. 1997). *Hispidoconidioma alpina* differs from all other endoconidial genera in that colonies are three-layered and endoconidiogenesis occurs sporadically in the central pseudoparenchymatous layer. Conidiogenous cells in *Endoconidioma* and *Scleroconidioma* also develop from pseudoparenchymatous cells, but conidiomata of these genera are cleistopycnidial and microsclerotial, respectively. With regard to ultrastructural process of endoconidiogenesis, however, *C. larixicolum* and *H. alpina* are similar to each other and resemble *Phaeotheca*, *Endoconidioma*, and *Endosporium* (Tsuneda and Murakami 1985; Tsuneda et al. 2004b, 2008).

Phylogenetically, *C. larixicolum* and *H. alpina* are distinct from one another, and from other previously described endoconidial genera. Phylogenetic analyses of the LSU and ITS regions are consistent in their placement of *C. larixicolum* among the Dothideales, and *H. alpina* with the Capnodiales.

In ITS analyses, *C. larixicolum* forms a strongly supported clade with endophytes of spruce (Stefani and Bérubé 2006; Higgins et al. 2007) and slow-growing dematiaceous endoliths (Ruibal 2004), but is remote from the other endoconidial dothidealean taxa, including *Scleroconidioma sphagnicola* Tsuneda et al. and *Endoconidioma populi*. Among those endoliths allied with *C. larixicolum* are Fungal sp. TRN272 and Fungal sp. TRN275, two unidentified endoconidium-forming isolates described as *Endoconidioma*-like in Ruibal et al. (2008). Given their close affinity to *C. larixicolum* and their phylogenetic remoteness from *E. populi* (the type species of *Endoconidioma*), further morphological characterization of these isolates is needed to determine if they are instead congeneric with *Celosporium*. In ITS analyses, *H. alpina* does not exhibit affinities to other capnodialean endoconidial taxa (i.e., *Catenulostroma abietis*, *Phaeothecoidea eucalypti*, *Capnobotryella renispora* Sugiyama, the *Hyphospora* state of *Comminutispora agavacearum*) and instead forms a strongly supported clade with three sterile, slow growing dematiaceous fungi isolated from rock (Ruibal et al. 2008), supporting morphological data that indicate it is a new endoconidial genus. Given that *H. alpina* is nested within a clade including *Teratosphaeria encephalarti*, it is expected that any teleomorph of *H. alpina* would be *Teratosphaeria*-like.

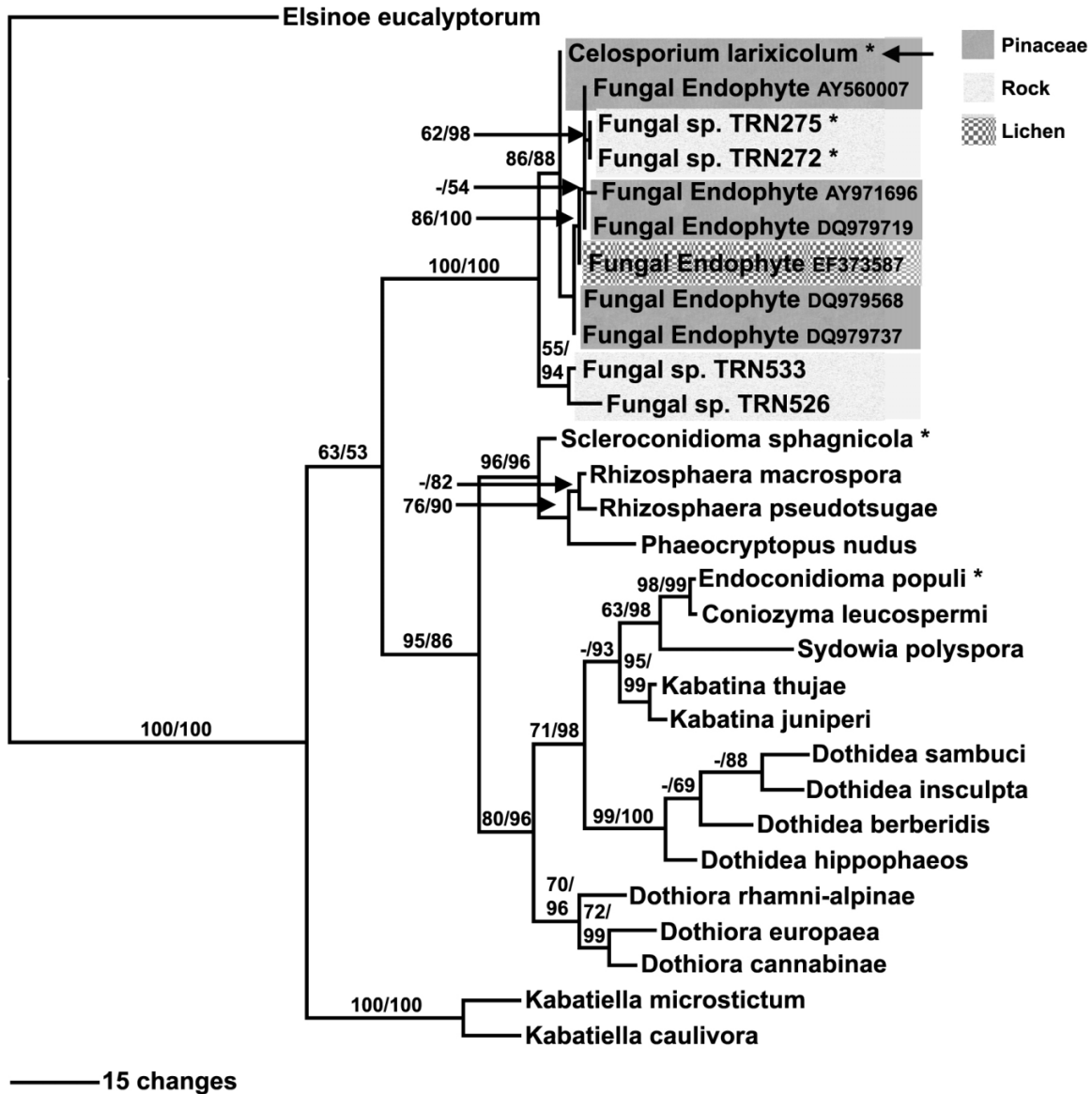
Fig. 38. One of three equally parsimonious trees (1200 steps, CI = 0.463, RI = 0.683, HI = 0.537) inferred from a maximum parsimony analysis of large subunit rDNA sequences showing the placement of *Celosporium*, *Hispidoconidioma* and other endoconidial taxa among the Dothideales, Myriangiales, and Capnodiales. Support values are given above the branches as bootstrap proportion/Bayesian posterior probability. Gaps (–) indicate a collapsed node, and asterisks (*) indicate a differently resolved node in the Bayesian analysis. *Leotia lubrica* serves as the outgroup taxon. *Celosporium* and *Hispidoconidioma* are indicated by arrows. Taxa producing endoconidia are indicated by an asterisk (*) following the name. Accession numbers for sequences retrieved from GenBank can be found in Table 1.



In general, black meristematic growth forms are associated with tolerance of extreme environmental conditions, including temperature, drought, and UV stresses (Onofri et al. 2007) and as such, this phylogenetically diverse group contains many examples of morphologically convergent taxa (Tsuneda and Currah 2006; Ruibal et al. 2008). Endoconidium-forming BMFs have been isolated from a wide variety of substrata, including plant material (DesRochers and Ouel-

lette 1994; Ramaley 1996; Tsuneda et al. 2000, 2008; Tsuneda and Currah 2006), rock (Wollenzien et al. 1997; Ruibal 2004; Ruibal et al. 2008), water from a saltern (Zalar et al. 1999), and human tissues (Matsumoto et al. 1990), but little is known about their ecology. Given the close phylogenetic affinities of *C. larixicolum* and *H. alpina* to black meristematic fungal taxa isolated from rocks, it would appear that this growth form is well adapted for a variety of niches,

Fig. 39. One of 1041 equally parsimonious trees that were consistent in their placement of *C. larixicum* within the Dothideales (481 steps, CI = 0.663, RI = 0.827, HI = 0.337). Tree is inferred from a maximum parsimony analysis of the internal transcribed spacer region of rDNA. Support values are given above the branches as bootstrap proportion/Bayesian posterior probability. Gaps (–) indicate a collapsed node in one of the analyses, and asterisks (*) indicate a differently resolved node in the Bayesian analysis. *Elsinoe eucalyptorum* serves as the outgroup taxon. *Celosporium larixicum* is indicated by an arrow. Taxa producing endoconidia are indicated by an asterisk (*) following the name. The substrates colonized by members of the clade that includes *C. larixicum* are indicated by shaded boxes surrounding the taxa. Fungal endophyte sequences are named by GenBank accession number. Accession numbers for other sequences retrieved from GenBank can be found in Table 1.

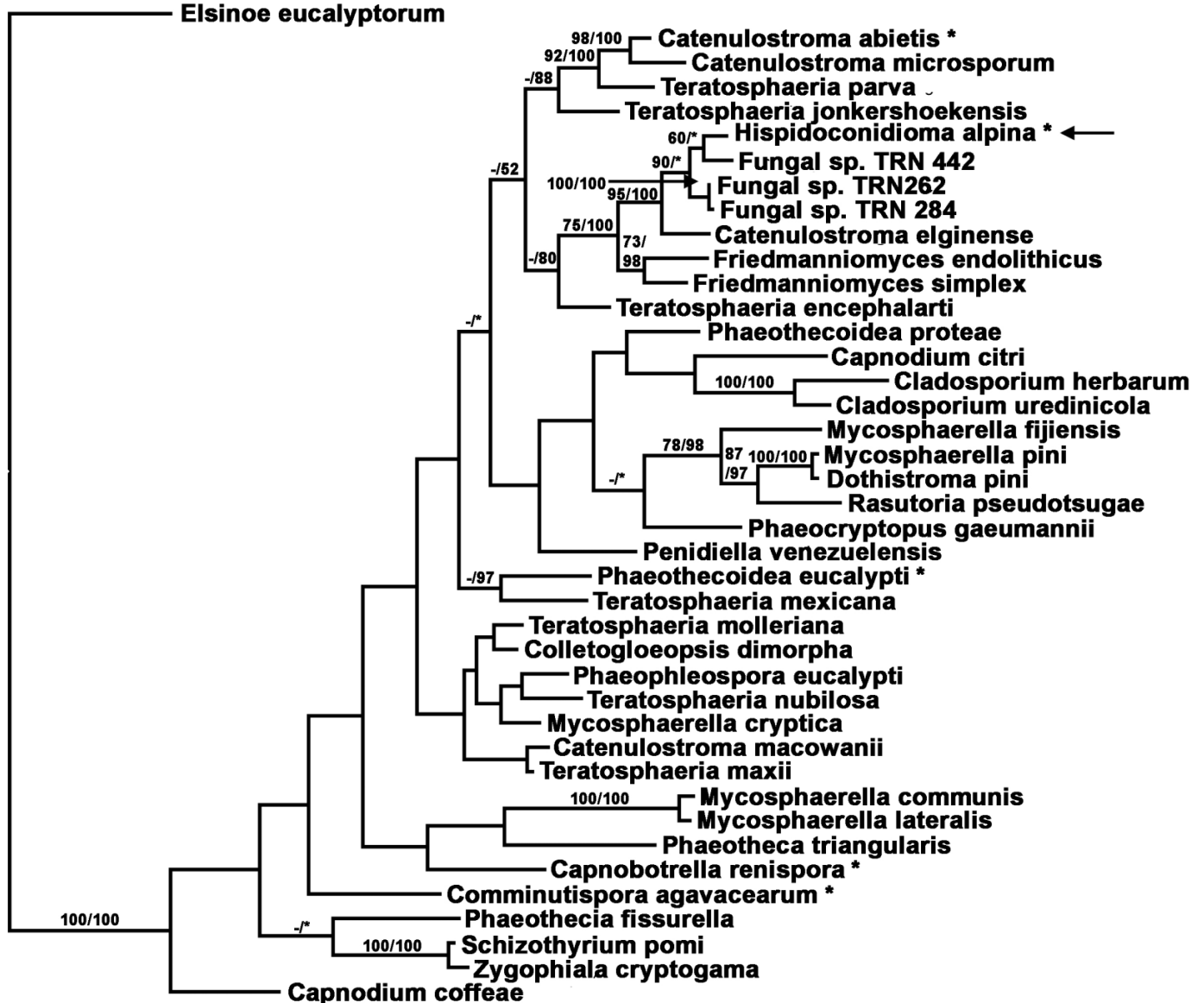


allowing separate BMF evolutionary lineages to colonize diverse substrates, such as rock and bark, that present similar ecological challenges (e.g., drought and UV stresses).

Some species of endoconidial BMFs are pathogens, for example, *Phaeotheca fissurella* causes mycotic diseases in humans (Chabasse 2002) and *S. sphagnicola* is a necrotrophic pathogen of peat moss (Tsuneda et al. 2001). *Endosporium populi-tremuloidis* Tsuneda was suggested to be a potential pathogen of trembling aspen because it colonized young buds, replacing them with a mass of black meristematic fungal cells, and its hyphae were abundant in the peridermal area of infected twigs (Tsuneda et al. 2008).

Likewise, *C. larixicum* was associated with declining and dead larch trees, and its hyphae and meristematic cells were present in the peridermal layers of infected twigs, suggesting that it is either a pathogen or an endophyte. Members of the Dothideales are widely known as saprobes, pathogens, and endophytes of plants, and the order includes several pathogens that specifically attack members of the Pinaceae [e.g., *Phaeocryptopus nudus* (Peck) Petrak and *Plowrightia abietis* (Barr) Barr]. As such, *C. larixicum* should be investigated as a potential causal agent of the larch decline observed in Banff National Park. In parsimony analyses of the ITS region, *C. larixicum* is contained within a strongly supported

Fig. 40. One of three equally parsimonious trees (1581 steps, CI = 0.0.393, RI = 0.503, HI = 0.607) inferred from a maximum parsimony analysis of the internal transcribed spacer region of rDNA showing the placement of *Hispidoconidioma alpina* among members of the Capnodiales. Support values are given above the branches as bootstrap proportion/Bayesian posterior probability. Gaps (–) indicate a collapsed node, and asterisks (*) indicate a differently resolved node in the Bayesian analysis. *Elsinoe eucalyptorum* serves as the outgroup taxon. *Hispidoconidioma alpina* is indicated by an arrow. Taxa producing endoconidia are indicated by an asterisk (*) following the name. Accession numbers for sequences retrieved from GenBank can be found in Table 1.



clade that includes endophytes reported from other members of the Pinaceae [*Picea glauca* (Moench) Voss, *P. mariana* (Mill.) Britton], suggesting that it may also function as an endophyte during part of its life cycle. If *C. larixicola* is indeed endophytic, this would be a pattern inconsistent with previous research that suggests particular endophyte lineages are associated with specific host species (Hoffman and Arnold 2008), as those endophytes that are closely allied to *C. larixicola* have been isolated from a variety of hosts belonging to the Pinaceae. Like the Dothideales, the Capnodiales are known to encompass a variety of plant pathogens, parasites, and endophytes, including several pathogens of the

Pinaceae [e.g., *Mycosphaerella pini* E. Rostrup and *Phaeocryptopus gaemannii* (Rhode) Petrak]. Furthermore, phylogenetic analyses of the ITS region show *H. alpina* has affinities to *Catenulostroma elginense* (Joanne E. Taylor & Crous) Crous & U. Braun, a leaf spot pathogen of *Protea* L. (Taylor and Crous 2000), suggesting that, like *C. larixicola*, *H. alpina* should be investigated as a potential causal agent of the larch decline observed in Banff National Park. Additional inoculation tests and disease etiology studies are needed to determine if the larch decline observed in Banff National Park is caused by either one or both of *C. larixicola* and *H. alpina*.

Table 1. Sources and accession numbers of the isolates and sequences used in this study.

Taxon	Source ^{a,b}	Substrate and Locality	GenBank Accession No.		References
			LSU	ITS	
<i>Capnobotryella renispora</i>	UAMH 9870	<i>Sphagnum</i> sp., Canada	—	AY220611	Hambleton et al. 2003
<i>Capnodium citri</i>	CBS 451.66	<i>Citrus sinensis</i> , Spain	—	AJ244240	Lumbsch et al. 2000
	CBS 131.34	<i>Bursaria spinosa</i> , Indonesia	AY004337	—	de Hoog et al. 1999
<i>Capnodium coffeae</i>	CBS 147.52	<i>Coffea robusta</i> , Zaire	DQ247800	DQ491515	Schoch et al. 2006
<i>Catenulostroma abietis</i>	CBS 459.93	<i>Abies</i> sp., Germany	—	AY128699	Schoch et al. 2006
	CBS 145.97	Sandstone, Germany	DQ678092	—	De Leo et al. 2003
<i>Catenulostroma elginense</i>	CBS 111030	<i>Protea grandiceps</i> , South Africa	—	AY260093	Taylor et al. 2003
<i>Catenulostroma macowanii</i>	CPC 13966	<i>Protea nitida</i> , South Africa	—	EU707985	Crous et al. 2008a
<i>Catenulostroma microsporum</i>	CBS 101951	<i>Protea cynaroides</i> , South Africa	—	EU707872	Crous et al. 2008a
<i>Celosporium larixicolum</i>	UAMH 11008	<i>Larix lyallii</i> , Canada	FJ997288	FJ997287	This study
<i>Cladosporium herbarum</i>	CBS 108.85	<i>Diospyros kaki</i> , Morocco	—	AF222829	Crous et al. 2000
<i>Cladosporium uredinicola</i>	ATCC 46649	<i>Cronartium fusiforme</i> , USA	EU019264	AY362001	Crous et al. 2007a, Park et al. 2004
<i>Colletogloeopsis dimorpha</i>	CBS 120085	<i>Eucalyptus nitens</i> , Australia	—	DQ923529	Summerell et al. 2006
<i>Comminutispora agavacearum</i>	CBS 619.95	<i>Dasyllirion leiophyllum</i> , USA	EU981286	AJ244250	Tsuneda et al. 2008, de Hoog et al. 1999
<i>Coniozyma leucospermi</i>	CBS 111289	<i>Leucospermum conocarpodendron</i> , South Africa	—	EU552113	Marcinowitz et al. 2008
<i>Cystocoleus ebeneus</i>	NA	Not known, Austria	EU048578	—	Muggia et al. 2008
<i>Davidiella tassiana</i>	CBS 399.80	Human foot, The Netherlands	DQ678074	—	Spatafora et al. 2006
<i>Delphinella strobiligena</i>	CBS 735.71	<i>Pinus halepensis</i> , Greece	AY016358	—	Lumbsch and Lindemuth 2001
<i>Discosphaerina fagi</i>	CBS 171.93	<i>Populus</i> sp., UK	AY016359	—	Lumbsch and Lindemuth 2001
<i>Dothidea berberidis</i>	CBS 186.58	<i>Berberis vulgaris</i> , Switzerland	—	EU167601	Simon et al. 2009
<i>Dothidea hippophaës</i>	DAOM 231303	Not known	—	AF027763	Jacobs and Rehner 1998
<i>Dothidea insculpta</i>	CBS 189.58	<i>Clematis vitalba</i> , France	DQ247802	AF027764	Schoch et al. 2006, Jacobs and Rehner 1998
<i>Dothidea sambuci</i>	CBS 198.58	<i>Acer pseudoplatanus</i> , Switzerland	—	DQ491505	Jeewon et al. 2002
	DAOM 231303	Not known	AF382387	—	Unpublished
<i>Dothiora cannabinae</i>	CBS 737.71	<i>Daphne cannabina</i> , India	DQ470984	AJ244243	Spatafora et al. 2006, de Hoog et al. 1999
<i>Dothiora europaea</i>	CBS 739.71	<i>Salix helvetica</i> , Switzerland	—	AJ244244	de Hoog et al. 1999
<i>Dothiora rhamni-alpinae</i>	CBS 745.71	<i>Rhamnus alpina</i> , Italy	—	AJ244245	de Hoog et al. 1999
<i>Dothiora ribesia</i>	CBS 195.58	<i>Ribes</i> sp., Switzerland	AY016360	—	Lumbsch and Lindemuth 2001
<i>Dothistroma pini</i>	CMW 23769	<i>Pinus palassiana</i> , Ukraine	—	DQ926966	Barnes et al. 2008
<i>Elsinoe eucalyptorum</i>	CBS 120084	<i>Eucalyptus propinqua</i> , Australia	—	DQ923530	Summerell et al. 2006
<i>Elsinoe veneta</i>	CBS 164.29	<i>Rubus</i> sp., not known	DQ678060	—	Spatafora et al. 2006
<i>Endoconidioma populi</i>	UAMH 10297	<i>Populus tremuloides</i> , Canada	EU981287	AY604526	Tsuneda et al. 2004a, 2008
<i>Endosporium populi-tremuloidis</i>	UAMH 10529	<i>Populus tremuloides</i> , Canada	EU304348	—	Tsuneda et al. 2008
<i>Friedmanniomyces endolithicus</i>	NA	Sandstone, Antarctica	—	DQ028272	Selbmann et al. 2005
<i>Friedmanniomyces simplex</i>	CBS 116775	Sandstone, Antarctica	—	DQ028271	Selbmann et al. 2005
Fungal Endophyte	NA	<i>Picea glauca</i> , Canada	—	AY560007	Stefani and Bérubé 2006
Fungal Endophyte	NA	<i>Picea mariana</i> , Canada	—	AY971696	Sokolski et al. 2007

Table 1 (continued).

Taxon	Source ^{a,b}	Substrate and Locality	GenBank Accession No.		References
			LSU	ITS	
Fungal Endophyte	NA	<i>Picea mariana</i> , Canada	—	DQ979719	Higgins et al. 2007
Fungal Endophyte	NA	<i>Cetraria islandica</i> , Not known	—	EF373587	Unpublished
Fungal Endophyte	NA	<i>Picea mariana</i> , Canada	—	DQ979568	Higgins et al. 2007
Fungal Endophyte	NA	<i>Picea mariana</i> , Canada	—	DQ979737	Higgins et al. 2007
Fungal sp. TR526	NA	Rock surface, Spain	—	AY843197	Ruibal et al. 2008
Fungal sp. TRN 272	NA	Rock surface, Spain	—	AY843116	Ruibal et al. 2008
Fungal sp. TRN 275	NA	Rock surface, Spain	—	AY843117	Ruibal et al. 2008
Fungal sp. TRN262	NA	Rock surface, Spain	—	AY843109	Ruibal et al. 2008
Fungal sp. TRN284	NA	Rock surface, Spain	—	AY843123	Ruibal et al. 2008
Fungal sp. TRN442	NA	Rock surface, Spain	—	AY843140	Ruibal et al. 2008
Fungal sp. TRN533	NA	Rock surface, Spain	—	AY843202	Ruibal et al. 2008
<i>Hispidoconidioma alpina</i>	UAMH 11010	<i>Larix lyallii</i> , Canada	FJ997286	FJ997285	This study
<i>Kabatiella microsticta</i>	CBS 342.66	<i>Convallaria majalis</i> , Germany	—	EU167608	Simon et al. 2009
<i>Kabatiella caulivora</i>	CBS 242.64	<i>Trifolium incarnatum</i> , USA	—	EU167576	Simon et al. 2009
<i>Kabatina juniperi</i>	NA	<i>Juniper chinensis</i>	—	AY183367	Unpublished
<i>Kabatina thujae</i>	CBS 238.66	<i>Thuja occidentalis</i> , Germany	—	AF013226	Unpublished
<i>Leotia lubrica</i>	NA	Not known	AY544644	—	Spatafora et al. 2006
<i>Mycosphaerella communis</i>	CBS 110976	<i>Eucalyptus cladocalyx</i> , South Africa	—	DQ302950	Hunter et al. 2006
	CPC 11792	<i>Eucalyptus</i> sp., Portugal	DQ246261	—	Crous et al. 2006
<i>Mycosphaerella cryptica</i>	MUCC 274	<i>Eucalyptus camaldulensis</i>	—	EU301085	Unpublished
<i>Mycosphaerella fijiensis</i>	NA	Not known	—	AF297225	Schoch et al. 2006
	NA	<i>Musa</i> sp., Not known	DQ678098	—	Goodwin et al. 2001
<i>Mycosphaerella lateralis</i>	CBS 111169	<i>Eucalyptus globulus</i>	—	DQ302975	Hunter et al. 2006
	CPC 11789	<i>Eucalyptus</i> sp., Portugal	DQ246260	—	Crous et al. 2006
<i>Mycosphaerella marksii</i>	CBS 110920	<i>Eucalyptus botryoides</i> , Australia	DQ246250	—	Hunter et al. 2006
<i>Mycosphaerella pini</i>	ATCC 28973	<i>Pinus ponderosa</i> , USA	EF114697	EU330228	Winton et al. 2007, Hanso and Drenkhan 2008
<i>Penidiella venezuelensis</i>	CBS 106.75	Man with <i>Tinea nigra</i> , Venezuela	—	EU019278	Crous et al. 2007a
<i>Phaeocryptopus gauemanni</i>	CBS 267.37	<i>Pseudotsuga menziesii</i> , Germany	EF114698	EU700365	Winton et al. 2007
<i>Phaeocryptopus nudus</i>	CBS 268.37	<i>Abies balsamea</i> , Germany	EF114700	EU700371	Winton et al. 2007
<i>Phaeophleospora eucalypti</i>	CBS 113992	<i>Eucalyptus nitens</i> , New Zealand	DQ246225	DQ267598	Hunter et al. 2006
<i>Phaeosclera dematioides</i>	UAMH 4265	<i>Pinus contorta</i> , Canada	EU981288	—	Tsuneda et al. 2008
<i>Phaeotheca fissurella</i>	UAMH 4245	<i>Cronartium cladosporioides</i> canker, Canada	—	AJ244255	Tsuneda et al. 2008
	CBS 520.89	<i>Cronartium coleosporioides</i> canker, Canada	EU981289	—	de Hoog et al. 1999
<i>Phaeotheca triangularis</i>	CBS 471.90	Wet surface of humidifier, Belgium	EU019279	AJ244256	Crous et al. 2007a, de Hoog et al. 1999
<i>Phaeothecoidea eucalypti</i>	CPC 12918	<i>Eucalyptus botryoides</i> , Australia	—	EU019280	Crous et al. 2007b
	CPC 13010	<i>Corymbia henryi</i> , Australia	EU019281	—	Crous et al. 2007b
<i>Phaeothecoidea proteae</i>	CBS 114129	<i>Protea repens</i> , South Africa	—	EU707898	Crous et al. 2008a
<i>Piedraia hortae</i>	CBS 480.64	Human hair, Brazil	AY016366	—	Lumbsch and Lindemuth 2001
<i>Plowrightia abietis</i>	ATCC 24339	<i>Pseudotsuga menziesii</i> , Canada	EF114703	—	Winton et al. 2007
<i>Rasutoria pseudotsugae</i>	NA	<i>Pseudotsuga menziesii</i> , USA	EF114704	EF114687	Winton et al. 2007

Table 1 (concluded).

Taxon	Source ^{a,b}	Substrate and Locality	GenBank Accession No.		References
			LSU	ITS	
<i>Rhizosphaera macrospora</i>	ATCC 4636	<i>Abies alba</i> , France	—	AF462431	Unpublished
<i>Rhizosphaera pseudotsugae</i>	CBS 101222	<i>Pseudotsuga menziesii</i>	—	EU700369	Unpublished
<i>Schizothyrium pomi</i>	CBS 406.61	<i>Rubus idaeus</i> , The Netherlands	EF134949	EF134949	Batzer et al. 2008
<i>Scleroconidioma sphagnicola</i>	UAMH 9731	<i>Sphagnum fuscum</i> , Canada	—	AY220610	Hambleton et al. 2003
<i>Selenophoma mahoniae</i>	CBS 388.92	<i>Mahonia repens</i> , USA	FJ150943	—	Zalar et al. 2008
<i>Stylothis puccinioides</i>	CBS 193.58	<i>Viburnum lantana</i> , Switzerland	AY004342	—	Lumbsch et al. 2000
<i>Sydowia polyspora</i>	NA	Not known	—	AY293068	Unpublished
	CBS 544.95	<i>Larix decidua</i> , The Netherlands	AY544675	—	Crous et al. 2003
<i>Teratosphaeria encephelarti</i>	CPC 15465	<i>Encephalartos lebomboensis</i> , South Africa	—	FJ372400	Crous et al. 2008b
<i>Teratosphaeria jonkershoensis</i>	CBS 122897	<i>Protea</i> sp., South Africa	—	EU707864	Crous et al. 2008a
<i>Teratosphaeria maxii</i>	CBS 120137	<i>Protea repens</i> , South Africa	DQ885899	DQ885899	Crous et al. 2007a
<i>Teratosphaeria mexicana</i>	CBS 110502	<i>Eucalyptus globulus</i> , Australia	—	AY725558	Crous et al. 2004
<i>Teratosphaeria molleriana</i>	CMW 4940	<i>Eucalyptus globulus</i> , Portugal	—	DQ302991	Hunter et al. 2006
	CPC 12056	<i>Eucalyptus</i> sp., Uruguay	DQ246220	—	Crous et al. 2006
<i>Teratosphaeria nubilosa</i>	CBS 114708	<i>Eucalyptus nitens</i> , South Africa	—	AY725572	Hunter et al. 2006
	CBS 116005	<i>Eucalyptus globulus</i> , Australia	DQ246229	—	Crous et al. 2004
<i>Teratosphaeria parva</i>	CBS 122893	<i>Protea repens</i> , South Africa	—	EU707876	Crous et al. 2008a
<i>Zygothiala cryptogama</i>	NA	Not known	FJ147157	FJ425208	Unpublished

^aNA indicates the strain is not available.

^bCultures are deposited in the following collections: ATCC, American Type Culture Collection, Manassas; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CMW, Tree Pathology Co-operative Programme, and Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa; CPC, culture collection of Pedro Crous, housed at CBS, Utrecht, The Netherlands; DAOM, National Mycological Herbarium, Ottawa, Canada; MUCC, Murdoch University Culture Collection, Perth, Australia; UAMH University of Alberta Microfungus and Herbarium, Edmonton, Alberta..

Acknowledgments

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