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Morphology and phylogenetic placement of *Endoconidioma*, a new endoconidial genus from trembling aspen

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Abstract: *Endoconidioma populi* gen. et sp. nov. is described from black subicula on twigs of trembling aspen, *Populus tremuloides,* in Alberta, Canada. Pycnidium-like conidiomata are produced on twigs and in culture, but, unlike pycnidia, conidiomata of *E. populi* have a closed peridium and a locule filled with conidiogenous cells that form conidia endogenously. These endoconidia are hyaline, unicellular and released by the dissolution of the peridial cell wall. In addition to endoconidia, mostly two-celled conidia that form blastically from undifferentiated hyphae occur often in culture but are observed only occasionally on *Populus* twigs. No coelomycetous taxa have been reported to produce endoconidia, and both the morphological features and DNA sequence data demonstrate that *Endoconidioma* is distinct from the previously established endoconidial genera. Parsimony analyses of portions of the nuclear ribosomal RNA gene (SSU and ITS) suggest that *Endoconidioma* is closely related phylogenetically to members of the Dothideales and allied anamorphs in *Hormonema* and *Kabatina*.

Key words: black yeasts, Dothideomycetes, meristematic fungi, SSU and ITS rDNA, subiculum, taxonomy

INTRODUCTION

In our survey of filamentous fungi occurring on trembling aspen, *Populus tremuloides* Michx., in Alberta, Canada, we found on apparently dying or dead twigs, peculiar, black subicula bearing pycnidium-like conidiomata and scattered, hyaline unicellular conidia. Also, brown, two-celled conidia were found occasionally on some subicula. Axenic cultures obtained from the subicula formed both pycnidium-like conidiomata and undifferentiated hyphae bearing brown, two-celled conidia. Unlike pycnidia, the peridium was closed entirely and contained a locule filled with conidiogenous cells that produced hyaline, unicellular conidia endogenously (i.e., endoconidia). No endoconidial coelomycetous taxa have been reported previously and the conidiomatal morphology and the presence of blastic conidia excluded this fungus from previously established endoconidial hyphomycetous genera *Hyphospora* Ramaley and *Phaeotheca* Sigler et al.

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MATERIALS AND METHODS

Isolation of fungus.—Pieces of bark bearing fungal subicula were removed from the twigs of *P. tremuloides* under a dissecting microscope, washed thoroughly with sterile distilled water, immersed in 0.5% NaOCl for 0.5–1 min, rinsed with sterile distilled water, and air-dried about 30 min on sterile filter paper in a biohazard hood. The subicula were crushed with forceps in a small amount of sterile distilled water and spread evenly on agar media. The media used for isolation were cornmeal agar (CMA; Difco, Detroit, Michigan), cornmeal agar with dextrose (CMAD, Difco), 2% malt-extract agar (MEA, Difco), potato-dextrose agar (PDA, Difco) and water agar (WA, 3% Bactoagar, Difco). Streptomycin sulphate (Pfizer, Montreal, Quebec) was added, 50 mg per L, to all media. Inoculated plates were incubated at 16–20 C in the dark.

Microscopy.—Cultural and morphological characteristics were examined periodically for up to 3 mo after inoculation. Microscopic observations were made from cultures grown in Petri dishes or in slide cultures (Malloch 1981). For light microscopy of endoconidiogenesis, sporulating conidiomata were removed from 2–3 mo old CMAD or MEA cultures and embedded in Araldite. Thin sections (about 1 μm) of the embedded material were stained with a slightly alkaline solution of toluidine blue (1%) in borax (1%) (Meek 1970).

DNA sequencing and phylogenetic analysis.—Genomic DNA was extracted from mycelium grown on CMAD using an UltraClean™ Microbial DNA Isolation Kit (MO BIO Laboratories Inc., Solana Beach, California). The small subunit (SSU) and internal transcribed spacer region (ITS) for UAMH 10297 and the ITS for UAMH 10298 were amplified

and sequenced as described in Hambleton et al (2003), except that an UltraClean™ PCR Purification Kit (MO BIO Laboratories Inc.) was used to clean PCR products.

BLAST searches of GenBank and preliminary analyses of the SSU data indicated that the closest relatives of *Endoconidioma populi* included the taxa sampled previously to examine the phylogenetic placement of *Scleroconidioma sphagnicola* Tsuneda et al (Hambleton et al 2003). Therefore, the sequences for *E. populi* were added to the SSU and ITS data matrices constructed for that study and aligned by eye. *Leotia lubrica* (Scop.) Pers. L37536, *L. viscosa* Fr. AF113715, *Microglossum viride* (Schrad.) Gillet U46031, and *Cudonia confusa* Bres. Z30240 (Leotiomycetes), and *Peziza badia* Pers. L37539, *Morchella elata* Fr. L37537 and *Urnula hiemalis* Nannf. Z49754 (Pezizomycetes) served as outgroup for the SSU data matrix. The ITS data matrix was adjusted with the addition of two sequences for *E. populi* determined in this study and a GenBank sequence (AF182375) derived from an unnamed species of *Hormonema* Lagerb. & Melin. Two sequences (AF013230 and AF182376) used by Hambleton et al (2003) since have been updated and the new data substituted in the ITS data matrix analyzed here. *Sarcinomyces crustaceus* Lindner AJ244258 was chosen as outgroup based on the SSU results and the degree of ITS sequence divergence observed. The SSU and ITS alignments have been deposited in TreeBASE (accession No. S1122).

Parsimony analyses were performed with PAUP* version 4.0b10 (Swofford 1999) using random step-wise addition of taxa and tree bisection-reconnection (TBR) branch swapping (SSU data matrix) or branch and bound analysis with simple step-wise addition of taxa and TBR branch swapping (ITS data matrix). Gaps were treated as missing data. Bootstrap percentages used to assess support for the branching topologies were determined from 500 (SSU) or 1000 (ITS) resamplings of the data set employing the full heuristic search option.

RESULTS

Endoconidioma Tsuneda, Hambleton & Currah, gen. nov.

Fungus mitosporicus et dematiaceus. Conidiomata subglobosa ad ampulliformia cum peridio clauso et fusco-pigmentato, continentia cellulas conidiogenosas quae parunt endoconidia hyalina et monocellularia; conidia exsoluta dissolutione tunicarum cellularum conidiogenosarum cellularumque peridialium conidiomae. Conidia blastica, pallide ad fusco-brunnea, formantur etiam modo holoblastico ex hyphis pigmentatis et indiscretis.

Species typica. *Endoconidioma populi* Tsuneda, Hambleton & Currah.

Mitosporic dematiaceous fungus. Conidiomata, forming on a black subiculum, subglobose to flask-shaped, consisting of an entirely closed, darkly pigmented peridium and a locule filled with conidiogenous cells. Endoconidia formed endogenously, hyaline, unicellular, released by dissolution of the conidiogenous and the peridial cells of the conidioma. Blastic conidia, mostly two-celled, light to dark brown, produced holoblastically from pigmented, undifferentiated hyphae.

Teleomorph. Unknown.

Type species. *Endoconidioma populi* Tsuneda, Hambleton & Currah

Etymology. Conidioma producing endoconidia.

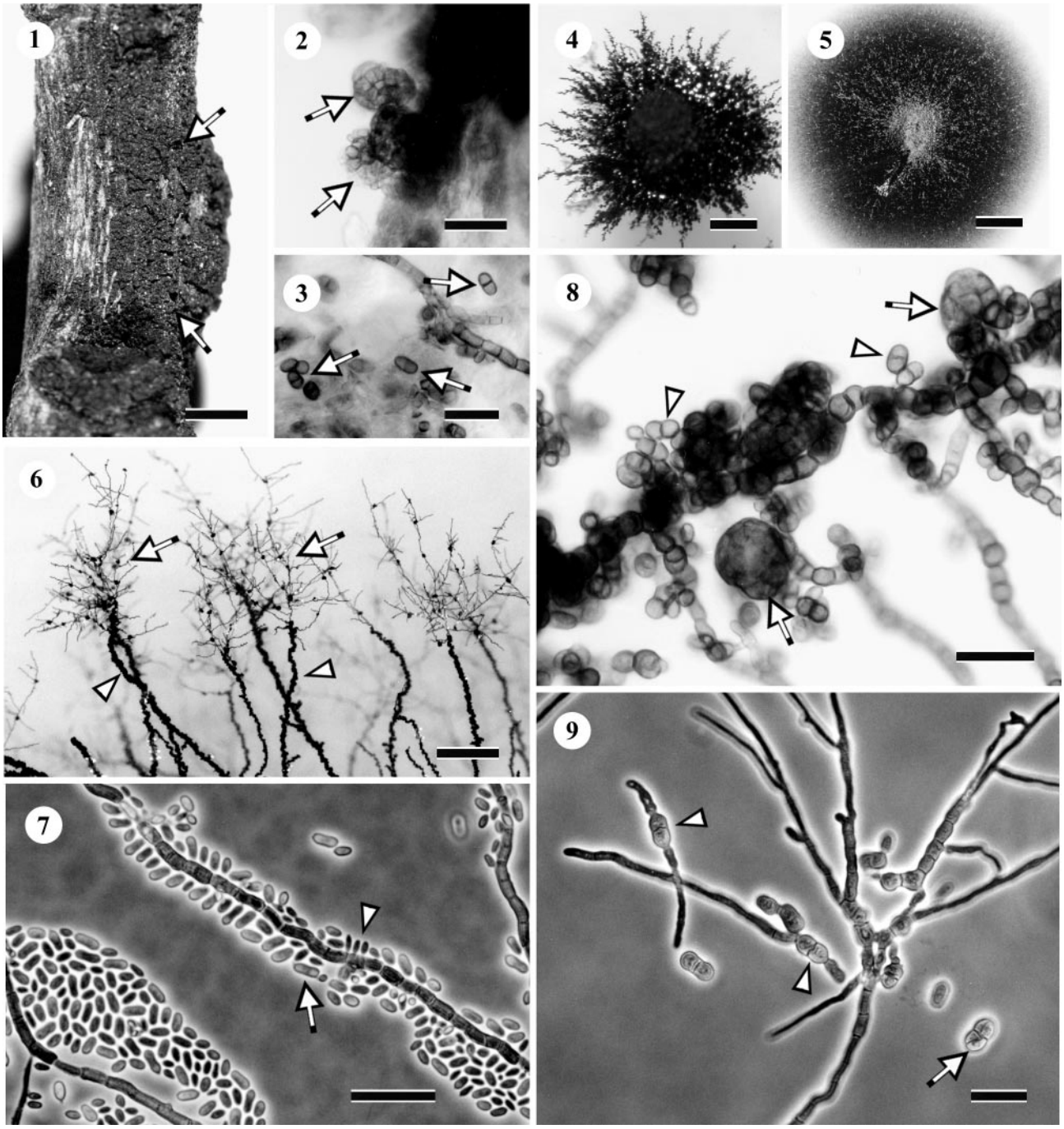
Endoconidioma populi Tsuneda, Hambleton & Currah, sp. nov. FIGS. 1–19

In ramis populi tremuloidis (FIG. 1–3): conidiomata subglobosa, brunnea, orientia ex subiculis nigris cellularum scleroticarum. Endoconidia levia, hyalina, monocellularia, plerumque oblongata, obtusa, 3.4–4.5 × 1.7–2 μm. In cultura, colonia lente crescens, primo mucoidea et similis fermenti, deinde massa hemispherica et elevata et carbonacea cellular scleroticarum, conidiorum, et conidiomatorum. Hyphae leves, dematiaceae, septatae. Conida ex hyphis, plerumque duocellularia, pallide ad fusco-brunnea, ellipsoidea, 7.5–12.5 × 5–10 μm. Teleomorphosis ignota.

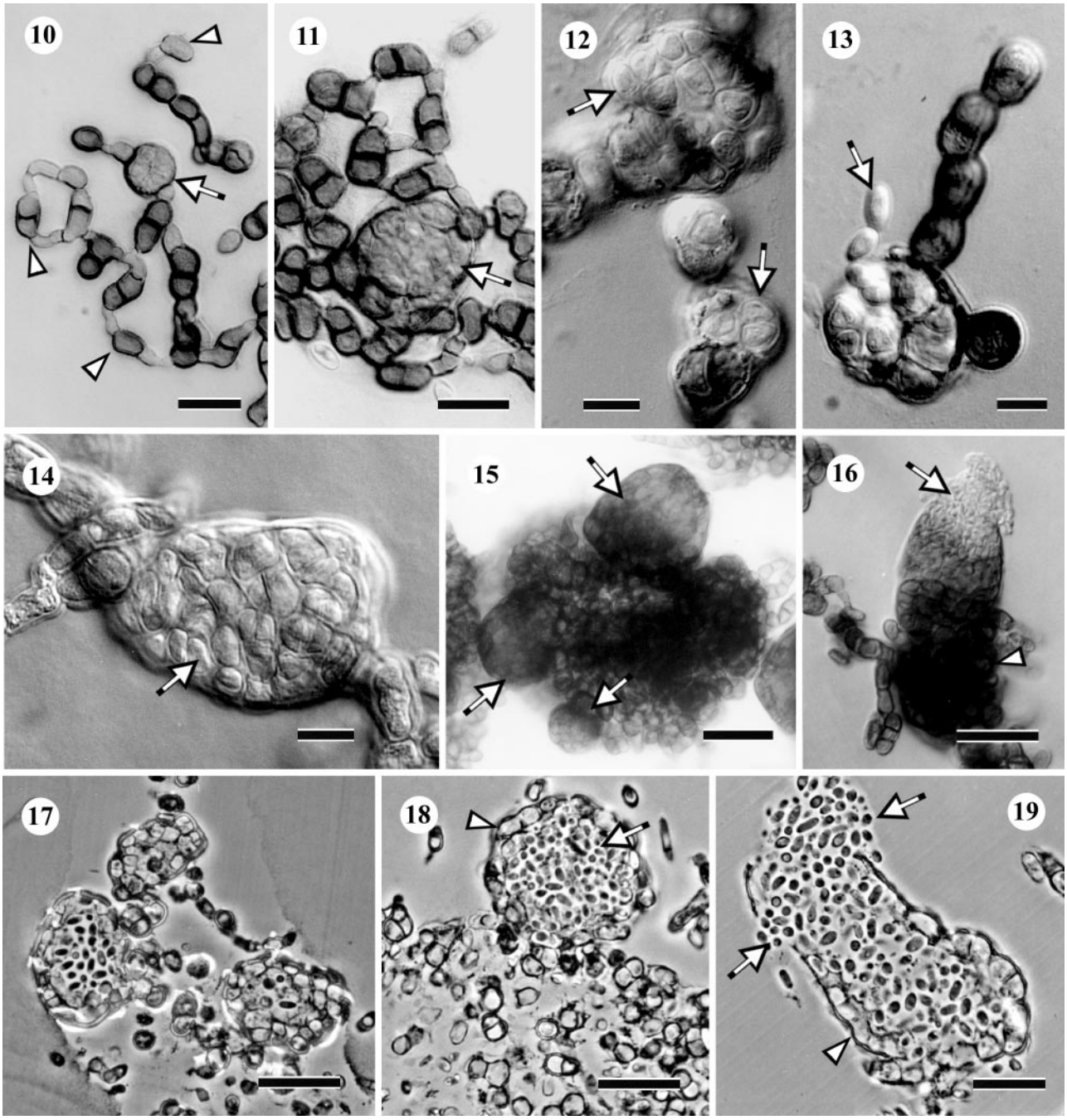
HOLOTYPE: A dried culture (UAMH 10297) established from conidia of *Endoconidioma populi* growing on a twig of *Populus tremuloides* collected at Whitemud Creek, ca 1 km west of the Northern Forestry Centre, Lansdowne, Edmonton, Alberta, 7 Aug 2001 by *A. Tsuneda*. A living culture is deposited at the University of Alberta Microfungus Collection and Herbarium (UAMH), Edmonton.

On twigs of *Populus tremuloides* (FIGS. 1–3): Conidiomata forming on black subicula, subglobose, non-ostiolate, brown, mostly 39–45 × 27–36 μm, containing conidiogenous cells 5–10 × 5–7.5 μm. Endoconidia formed endogenously, smooth, hyaline, unicellular, mostly oblong, obtuse, 3.4–4.5 × 1.7–2 μm. Hyphae smooth, dematiaceous, septate. Conidia from hyphae, mostly two-celled, light to dark brown, ellipsoidal, 7.5–12.5 × 5–10 μm, observed only occasionally.

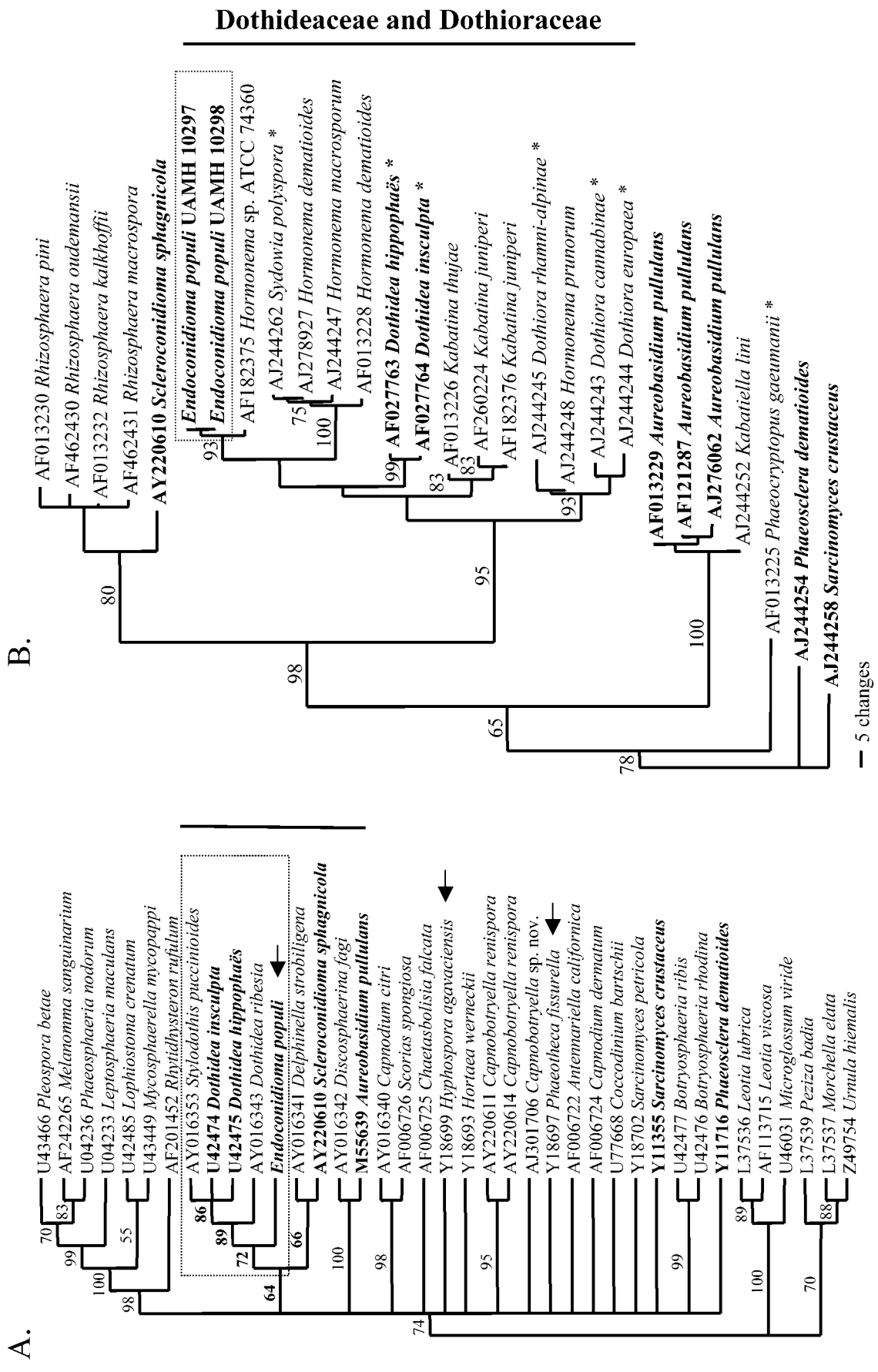
On MEA: Colonies slow growing, attaining a diam of 9–14 mm in 7 d at 16–18 C, initially mucoid and yeast-like, becoming a hemispherical, raised, carbonaceous mass of thick-walled cells, with a thin peripheral area, consisting mostly of submerged hyphae. Conidiomata, developing on masses of thick-walled cells or directly from hyphal cells, brown to black, subglobose to flask-shaped, non-ostiolate, 37.5–87.5(–200) × 30–55(–105) μm, with a peridium consisting of a single layer of darkly pigmented, somewhat flattened, thick-walled cells. Locule filled with subglobose to oval conidiogenous cells, mostly 4–10 × 5–8 μm. Endoconidia mostly oblong, obtuse, hyaline, unicellular, 3.7–7.5 × 2–3 μm, forming by septation of conidiogenous cells and schizolysis of the septa, released by dissolution of walls of the conidiogenous cells and the peridial cells. Hyphae smooth,



FIGS. 1–9. *Endoconidioma populi* on a twig of *Populus tremuloides* (FIGS. 1–3) and in culture (FIGS. 4–9, UAMH 10297, except FIG. 5, UAMH 10298). 1. Black subiculum (arrows) formed on a small twig. 2. Developing conidiomata from subiculum (arrows). 3. Dematiaceous hypha and mostly two-celled, blastic conidia (arrows). 4. Colony on MEA. 7 wk. 5. Colony on CMAD. 7 wk. 6. Hyphae bearing conidiomatal initials at the colony periphery (arrows). Numerous blastic conidia and conidiomatal initials occur below the periphery along the length of the hyphae (arrowheads). 4 wk on CMAD. 7. Unicellular, hyaline conidia (arrowhead) and mostly two-celled, pigmented conidia (arrow) formed blastically from the hyphae. 4 wk on CMAD. 8. Two-celled blastic conidia (arrowheads) and conidiomatal initials (arrows). 4 wk on MEA. 9. Bipolar germ tubes emerging from two-celled conidia (arrowheads). In some conidia, cell enlargement and subdivision by septation indicate germination (arrow). 4 d on CMAD. Scale bars: 1 = 2 mm; 2, 3, 8, 9 = 15 μm; 4, 5 = 5 mm; 6 = 200 μm; 7 = 30 μm.



FIGS. 10–19. Formation of endoconidia by *Endoconidioma populi* in slide culture (FIGS. 10–13, MEA) or on CMAD (FIGS 14–19). UAMH 10297 except in FIG. 14, UAMH 10298. 10. Two-celled conidia germinating to form secondary conidia (arrowheads). The arrow indicates a multicellular body initial developed from a conidial cell. 2 d. 11, 12. Enlarging multicellular bodies (arrows). The arrows in FIG. 12 indicate subdividing cells. 4 d. 13. Multicellular body releasing endoconidia (arrow). 7 d. 14. Multicellular body developed from hyphal cells (arrow). 4 wk. 15. Conidiomata (arrows) arising from darkly pigmented, thick-walled cells. 4 wk. 16. Conidioma releasing endoconidia (arrow). The arrowhead indicates a mass of thick-walled cells. 2 mo. 17–19. Thin sections of conidiomata. 2 mo. 17. Conidiomata of different developmental stages. 18. Conidioma containing abundant endoconidia (arrow). Peridium is intact (arrowhead). 19. Conidioma releasing endoconidia (arrows). Peridial cells remain intact (arrowhead), except in the areas where conidial release takes place (arrows). Scale bars: 10 = 10 μm; 11 = 15 μm; 12–14 = 5 μm; 15, 19 = 20 μm; 16 = 40 μm; 17, 18 = 30 μm.


A.
U43466 Pleospora betae
AF242265 Melanomma sanguinarium
U04236 Phaeosphaeria nodorum
U04233 Leptosphaeria maculans
U42485 Lophiostoma crenatum
U43449 Mycosphaerella mycopappi
AF201452 Rhytidhysteron rufulum
AY016353 Stylodothis puccinioides
U42474 Dothidea insculpta
U42475 Dothidea hippophaës
AY016343 Dothidea ribesia
Endoconidioma populi
AY016341 Delphinella strobiligena
AY220610 Sclerococonidioma sphagnicola
AY016342 Discosphaerina fagi
M55639 Aureobasidium pullulans
AY016340 Capnodium citri
AF006726 Scorias spongiosa
AF006725 Chaetasbolisia falcata
Y18699 Hyphospora agavaciensis
Y18693 Hortaea werneckii
AY220611 Capnobotryella remispora
AY220614 Capnobotryella remispora
AJ301706 Capnobotryella sp. nov.
Y18697 Phaeotheca fissurella
AF006722 Antennariella californica
AF006724 Capnodium dermatum
U77668 Coccodinium bartschii
Y18702 Sarcinomyces petricola
Y11355 Sarcinomyces crustaceus
U42477 Botryosphaeria ribis
U42476 Botryosphaeria rhodina
Y11716 Phaeoscleroa dematioides
L37536 Leotia lubrica
AF113715 Leotia viscosa
U46031 Microglossum viride
L37539 Peziza badia
L37537 Morchella elata
Z49754 Urnula hiemalis
B.
Dothideaceae and Dothioraceae
AF013230 Rhizosphaera pini
AF462430 Rhizosphaera oudemansii
AF013232 Rhizosphaera kalkhoffii
AF462431 Rhizosphaera macrospora
AY220610 Sclerococonidioma sphagnicola
Endoconidioma populi UAMH 10297
Endoconidioma populi UAMH 10298
AF182375 Hormonema sp. ATCC 74360
AJ244262 Sydowia polyspora *
AJ278927 Hormonema dematioides
AJ244247 Hormonema macrosporum
AF013228 Hormonema dematioides
AF027763 Dothidea hippophaës *
AF027764 Dothidea insculpta *
AF013226 Kabatina thujae
AF260224 Kabatina juniperi
AF182376 Kabatina juniperi
AJ244245 Dothiora rhamni-alpinae *
AJ244248 Hormonema prunorum
AJ244243 Dothiora cannabinae *
AJ244244 Dothiora europaea *
AF013229 Aureobasidium pullulans
AF121287 Aureobasidium pullulans
AJ276062 Aureobasidium pullulans
AJ244252 Kabatiella lini
AF013225 Phaeocryptopus gaeumanii *
AJ244254 Phaeosclera dematioides
AJ244258 Sarcinomyces crustaceus
5 changes

subhyaline to brown, septate, 3–12 μm wide, cylindrical, becoming moniliform with age, forming conidia holoblastically. Conidia arising from sides of hyphae, either hyaline or pigmented; hyaline conidia unicellular, cylindrical, mostly 4–8.5 × 3.5–5 μm; pigmented conidia light to dark brown, mostly 2-celled (1- to 4-celled), ellipsoidal, occasionally constricted at the septum, 8.7–16 × 5.5–7.5 μm, often multiplying blastically to form aggregated masses of thick-walled cells.

Teleomorph. Unknown.

Etymology. Occurring on *Populus*.

Additional material examined. CANADA. ALBERTA: Near Lansdowne, Edmonton, on a twig of *P. tremuloides,* 3 Nov 2001, *A. Tsuneda* (UAMH 10298, living culture).

Comments.—Colony morphology of *E. populi* varies with the culture medium and strain. Unlike the rough, carbonaceous and crustose colonies on MEA (FIG. 4), colonies on PDA are initially creamy white and mucoid, becoming shiny black, wrinkled and rubbery with age, while those on CMAD (FIG. 5) are olivaceous brown and with the mycelium mostly submerged in agar. On PDA the production of blastic conidia (FIGS. 6–8) is most prevalent and both uni- and two-celled conidia often exhibit yeast-like budding. UAMH 10298 forms much fewer blastic conidia than UAMH 10297, and its hyphae are mostly superficial on agar (FIG. 5). Regardless of the medium or strain, however, hyphae at the colony periphery usually lack blastic conidia and bear darkly pigmented conidiomatal initials (FIG. 6, arrows).

Two-celled, blastic conidia show three different forms of germination on agar media: (i) by cylindrical germ tubes; (ii) by forming another conidium (secondary conidiation); and (iii) by continuous divisions of both or one of the two cells resulting in the formation of enlarged, multicellular bodies (FIGS. 9–12). In slide culture, component cells of the multicellular bodies often separate from each other to form endoconidia that subsequently are released by the breakdown of the mother cell wall (i.e., cell wall of the two-celled conidium from which the multicellular body originated) (FIG. 13). Multicellular bodies also develop from hyphal cells and are particularly abundant in UAMH 10298 (FIG. 14). Mature conidiomata usually occur after incubation for 2–3 mo at 18 C (FIGS. 15–19). Release of endo conidia from the conidiomata follows dissolution of peridial cells (FIGS. 16, 19).

DNA sequencing and phylogenetic analysis.—The SSU sequence determined for *E. populi* UAMH 10297 was 1762 bp in length, and was complete at the 3′ end, finishing with the CATTA box that precedes the first internal transcribed spacer. The ITS sequences for UAMH 10297 and 10298 were identical and 499 bp in length. GenBank accession numbers for the newly determined sequences are AY604526 (UAMH 10297, SSU-ITS) and AY604527 (UAMH 10298, ITS).

The alignment for the SSU data matrix comprised 39 taxa and 1652 aligned characters. Of these, 1285 were constant, 210 were parsimony uninformative and 157 were parsimony informative. A bootstrap analysis of the informative characters only was performed and the resulting tree (FIG. 20A) indicated that there was support for the grouping of *E. populi* with the Dothideaceae (*Dothidea* Fr. and *Stylodothis* Arx & E. Müll.; 72% bootstrap) but not with either of the other two endoconidial genera, *Hyphospora* and *Phaeotheca* (arrows). In general, relationships among the dothideomycetous taxa included, other than the Pleosporales (with *Rhytidhysteron rufulum* [Spreng.] Speg. AF201452), were mostly unsupported.

The data matrix used to compare ITS sequences comprised 28 taxa and 567 aligned characters. Of these 369 were constant, 78 were parsimony-uninformative and 120 were parsimony-informative. A branch and bound analysis of the informative characters only resulted in 10 most parsimonious trees (MPTs) of 314 steps and with CI = 0.573, RI = 0.788 and RC = 0.452. Differences among the trees resulted from rearrangements of taxa within the clades corresponding to *Dothiora* Fr. and *Rhizosphaera* L. Mangin & Har. Results of a full bootstrap analysis are given on one MPT (FIG. 20B). The ITS-based phylogenetic hypothesis places *E. populi* in the Dothideaceae/Dothioraceae clade with strong bootstrap support (95%) but relationships among subclades were unresolved. Groups within the clade supported by

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FIG. 20. Results of parsimony analyses of rDNA data matrices constructed to examine the phylogenetic affinity of *Endoconidioma populi*. Names in bold indicate the taxa that are present in both trees. A. The 50% consensus tree based on a bootstrap analysis of the small subunit region resulting from 500 replicates of the full heuristic bootstrap search option with random sequence addition. Arrows indicate endoconidial fungi. The bar at the side of the tree corresponds to the Dothideaceae and Dothioraceae. B. One of 10 equally parsimonious trees based on a Branch and Bound analysis of the internal transcribed spacer region of *Endoconidioma populi* and related fungi (314 steps, CI = 0.573, RI = 0.788, RC = 0.452). Parsimony bootstrap values, 65% and greater, are given above the branches. An asterisk indicates a teleomorphic species.

bootstrap values corresponded to the acervular genus *Kabatina* R. Schneid. & Arx and the loculoascomycete genera *Dothidea, Dothiora* Fr. and *Sydowia* Bres., with associated cultural states of *Hormonema*. A sequence attributed to the genus *Hormonema* (AF182375; Peláez et al 2000) clustered with *E. populi* with strong bootstrap support.

DISCUSSION

Endoconidioma populi is unique in producing nonostiolate conidiomata filled with conidiogenous cells that subdivide to form endoconidia. Conidia are released from conidiomata by the dissolution of the peridial walls. With regard to endoconidiogenesis, *E. populi* is similar to the endoconidial hyphomycete *Phaeotheca*. In species of *Phaeotheca,* conidiogenous cells subdivide by septation and form two to several daughter cells. Schizolysis of the cross walls results in separation of the daughter cells to form endoconidia that subsequently are released by rupture of the mother cell walls (Tsuneda and Murakami 1985, DesRochers and Ouellette 1994). Results of our comparative ultrastructural study on endoconidiogenesis in *E. populi* and *P. fissurella* Sigler et al are reported in a separate paper in this issue (Tsuneda et al 2004). Likewise, in the *Hyphospora* state of *Comminutispora agavaciensis* Ramaley, numerous endoconidia develop from multicellular bodies (like the one shown in FIG. 14) and the endoconidia are released by breakdown of mother cell walls (Ramaley 1996). However, ultrastructural details of the developmental process have not been documented for *Hyphospora*. *Endoconidioma* also resembles *Phaeotheca* and *Hyphospora* in producing slowly expanding, dematiaceous colonies and in reproduction by nearly isodiametric enlargement with subdividing cells, i.e., meristematic growth (de Hoog et al 1999) (FIGS. 11–14). *Endoconidioma,* however, is distinct from these endoconidial genera in the conidiomatal and endoconidial morphology and the presence of darkly pigmented, two-celled, blastic conidia. Both *Phaeotheca* and *Hyphospora* lack conidiomata with peridia. Endoconidia in *Phaeotheca* are pigmented (Sigler et al 1981, Tsuneda and Murakami 1985, de Hoog et al 1997, Zalar et al 1999a) and are the only form of spore produced, except for the secondary ameroconidia that develop between the endoconidial masses in *P. dimorphospora* DesRochers & Ouellette (DesRochers and Ouellette 1994). *Hyphospora* lacks blastic conidia (Ramaley 1996, Zalar et al 1999a). *Endoconidioma populi* produces pycnidium-like conidiomata but unlike typical pycnidia, the peridium is closed with no predefined ostiole and, rather than lining the inner surface, the conidiogenous cells fill the locule and form conidia endogenously. No coelomycetous taxa have been reported to produce endoconidial conidiomata.

Analyses of 5.8S and partial ITS2 rDNA presented by de Hoog et al (1999) indicated that among the melanized, meristematic fungi and black yeasts with an affinity to the Dothideomycetes, *Phaeotheca* and *Hyphospora* were not closely related and both were distant from the Dothioraceae. The SSU phylogram of Hambleton et al (2003, FIG. 27) showed that *Hyphospora* clustered in a large clade comprising the Capnodiales that also included two other dematiaceous hyphomycete genera, *Capnobotryella* Sugiy. and *Hortaea* Nishim. & Miyaji, while *P. fissurella* was on its own branch in a basal position to the Capnodiales and Dothideales. Based on the same sampling of dothideomycetous taxa with *E. populi* included, our SSU bootstrap tree indicates that the new species is most closely related to members of the genus *Dothidea* and is phylogenetically distinct from both endoconidial genera *Phaeotheca* and *Hyphospora*. The ITS analysis presented here suggests a close relationship for *E. populi* to the Dothideales sensu stricto, restricted to one family (Dothideaceae) in the classification of Eriksson et al (2003), to genera in the Dothioraceae and to species in *Hormonema* and *Kabatina*. A common feature of ingroup taxa is the tendency to produce *Hormonema*-like cultural synanamorphs. The spermatial state of *Dothidea insculpta* Wallr. was described in the stromatal coelomycetous genus *Asteromellopsis* Hess & Müller (Sutton 1980). The genus *Kabatina* produces acervuli on a range of coniferous hosts and has a *Hormonema*-like synanamorph (Schneider and von Arx 1966, Butin and Schneider 1976, Hermanides-Nijhof 1977, Sutton 1980, Ramaley 1992). The genus *Hormonema,* which includes black yeasts characterized by basipetal conidial development from undifferentiated conidiogenous cells on hyphae (Schneider and von Arx 1966, Hermanides-Nijhof 1977), is used for species known to be cultural anamorphs of the ascomycetous genera *Sydowia, Dothiora, Pringsheimia* Schulzer and *Guignardia* Viala & Ravaz. *Hormonema* and some other black yeast genera form endoconidia, but the formation is only occasional and occurs in undifferentiated hyphal cells (Hermanides-Nijhof 1977, de Leo et al 1999, Wollenzien et al 1997, Zalar et al 1999b).

In *E. populi,* the endoconidial coelomycetous state predominates both on natural substrate and in culture and the *Hormonema*-like synanamorph (FIG. 7) lacks basipetal conidiation. Peláez et al (2000) detected a potent antifungal compound, Enfumafungin, in a culture of endophytic *Hormonema* sp. (ATCC 74360, AF182375) and suggested that the fungus could be a cultural synanamorph of an undetermined *Kabatina* species based on the analysis of the

ITS1-5.8S-ITS2 region. However, given the results of our ITS analysis, it is more likely that the antibiotic-producing fungus belongs to *Endoconidioma* (FIG. 20). Species of *Rhizosphaera* and *Scleroconidioma sphagnicola* formed a well-supported sister group to the large clade that included *Endoconidioma*. However, *Rhizosphaera* and *S. sphagnicola* differ from *Endoconidioma* by the production of pycnidial conidiomata and microsclerotial conidiomata bearing abundant, phialidic conidiogenous cells on the surface, respectively (Tsuneda et al 2000, 2001).

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