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Two new species of *Pseudogymnoascus* with *Geomyces* anamorphs and their phylogenetic relationship with *Gymnostellatospora*

Adrianne V. Rice
Randolph S. Currah[1]

Department of Biological Sciences, University of Alberta, Edmonton, AB, T6G 2E9 Canada

Abstract: Two new psychrophilic *Pseudogymnoascus* species with *Geomyces* anamorphs are described from a *Sphagnum* bog in Alberta, Canada. *Pseudogymnoascus appendiculatus* has long, branched, orange appendages and smooth, fusoid to ellipsoidal ascospores with a faint longitudinal rim. *Pseudogymnoascus verrucosus* has short, subhyaline appendages and warty peridial hyphae and ascospores, and both smooth to asperulate and irregularly warty conidia. Both species produce asci in chains, a feature that supports the distinction between this group and *Myxotrichum*, which produces asci singly. The discovery of species intermediate between *Pseudogymnoascus* and *Gymnostellatospora*, in having both ornamented ascospores and *Geomyces* anamorphs, prompted a re-evaluation of the genera. Sequence analysis of the internal transcribed spacer regions (ITS) of the nuclear ribosomal DNA indicates that the two genera remain distinct and comprise a monophyletic group. *Pseudogymnoascus* species have smooth to warty or lobate-reticulate ascospores while species of *Gymnostellatospora* have walnut-shaped spores with distinct longitudinal crests and striations. Anamorphs assignable to the form genus *Geomyces* are allied with both genera. A key is provided to the four species and varieties of *Pseudogymnoascus*.

Key words: Ascospore ornamentation, *Geomyces*, *Gymnostellatospora*, ITS sequences, Myxotrichaceae, phylogeny, *Pseudogymnoascus*

INTRODUCTION

Pseudogymnoascus Raillo was placed in the Myxotrichaceae (Ascomycota) (Currah 1985) based on the production of ellipsoidal to fusoid, single-celled, hyaline ascospores in globose, evanescent asci, its distinctive yellow to reddish gymnothecial ascomata and cellulolytic abilities. *Pseudogymnoascus roseus* Raillo, the type of the genus (Currah 1985), appears regularly in surveys of fungi associated with soil (e.g. Raillo 1929, Cejp and Milko 1966, Samson 1972, Currah 1985), roots (Currah 1985), and wood (Sigler et al 2000, Lumley et al 2001). *Pseudogymnoascus roseus* var. *ornatus* Udagawa & Uchiyama, the only other taxon in the genus (Udagawa and Uchiyama 1999), is distinguished from the type variety in having lobate reticulate rather than smooth ascospores. Both varieties have dendritic arthroconidial anamorphs in the genus *Geomyces*. Within the Myxotrichaceae, *Gymnostellatospora* Udagawa, Uchiyama & Kamiya is similar to *Pseudogymnoascus* because of its light-colored ascomata but these contain ridged rather than smooth or lobate reticulate ascospores. A *Geomyces* anamorph has been reported for at least one *Gymnostellatospora* species (*Gy. dendroidea* [Locquin-Linard] Udagawa) (Sigler et al 2000). *Myxotrichum*, along with a range of affiliated *Oidiodendron* species (Hambleton et al 1998, Rice and Currah 2005), is distinguished from *Pseudogymnoascus, Gymnostellatospora* and affiliated *Geomyces* anamorphs by having deeply melanized, rather than lightly colored, hyphae associated with sporulating structures (peridial hyphae and conidiophores).

The Myxotrichacaeae was placed in the Onygenales (Currah 1985) but molecular data show it would be better disposed among the inoperculate discomycetes (Leotiomycetes) (Sugiyama et al 1999, Mori et al 2000, Gibas et al 2000). These data also suggest that *Myxotrichum* and *Oidiodendron* form a lineage separate from *Pseudogymnoascus, Gymnostellatospora* and *Geomyces* (Mori et al 2000, Gibas et al 2002).

During a survey of cellulose- and lignin-degrading fungi in a *Sphagnum* bog in the southern boreal forest of western Canada, numerous isolates assignable to *Geomyces* were obtained from bait blocks made of brown-rotted spruce that had been buried in the upper layers of the *Sphagnum* moss for 8–12 mo. Some of these isolates developed ascomata similar to those found in *Pseudogymnoascus* and *Gymnostellatospora* but their characteristics did not match any described species.

Two unique species were discerned: one with orange peridial hyphae, long, concolorous, branched appendages, ascospores with a faint longitudinal rim, and smooth to asperulate conidia; and a second with warty, red to red-brown peridial hyphae, short, unbranched appendages, irregularly ornamented ascospores, and smooth to asperulate and coarsely tuberculate conidia. Because these suites of character states suggested the new species might occupy in-

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[1] Corresponding author. E-mail: r.currah@ualberta.ca

termediate positions between *Pseudogymnoascus* and *Gymnostellatospora*, we compared the nuclear ribosomal region of 24 isolates representing the two new species and 11 similar anamorphic or teleomorphic taxa in the Myxotrichaceae.

MATERIALS AND METHODS

Cultures of *Pseudogymnoascus appendiculatus* and *P. verrucosus* were deposited at the University of Alberta Microfungus Collection and Herbarium (UAMH). Cultures for sequencing were obtained from UAMH (TABLE I). Live cultures of *P. roseus* var. *ornatus*, *Gymnostellatospora parvula* and *Gy. dendroidea* were not available from culture collections.

Fungal isolation and description.—Fungi were isolated from bait blocks that had been buried in nylon mesh litter bags 2 m apart along a transect in a *Sphagnum* bog 5 km east of Perryvale, Alberta (see Rice and Currah 2002, Rice et al 2006). Bait blocks were surface sterilized by flaming and plated onto cornmeal agar amended with benomyl, mycosel agar, or into moist chambers (Rice et al 2006). Plates were incubated at the peat temperatures (5–20 C) and observed 1 y. Fungi were subcultured onto cornmeal agar (CMA; 17 g BBL cornmeal agar [Becton, Dickinson & Co., Sparks, Maryland], 1 L dH_2O) and identified as they appeared. Ascomata were produced on several cultures identified as ''*Geomyces* sp.'' incubated at 5 and 15 C. Single ascospore isolates were plated onto CMA and oatmeal agar (OA; 20 g ground Quaker oatmeal cereal, 20 g BBL agar [Becton, Dickinson Co.], 1 L dH_2O) and incubated at 5 and 15 C. Cultures were described on CMA and OA and ascomata and ascospores examined with light and scanning electron microscopy (SEM). Ascomata were mounted in polyvinyl alcohol mounting media (PVA; 16.6 g polyvinyl alcohol [Sigma Chemical Co., St Louis, Missouri], 100 mL dH_2O, 100 mL lactic acid [Fisher Scientific, Fair Lawn, New Jersey], 10 mL glycerin [Fisher]) and PVA with acid fuschin (APVA, Sigma) and examined with an Olympus BX50 (Olympus Optical Co., Tokyo, Japan) light microscope and photographed with an Olympus DP-12 digital camera (Olympus Optical Co.). Agar plugs bearing mature ascomata were prepared for SEM by air drying, osmium fixation and critical point drying (Tsuneda et al 2001), or freeze drying in liquid nitrogen, and viewed under a Hitachi S-510 (Hitachi Science Systems, Japan) or a JEOL JSM-6301FX7V field emission (JEOL USA Inc., Peabody, Massachusetts) SEM.

DNA sequencing.—Three isolates of *Pseudogymnoascus appendiculatus* and two isolates of *P. verrucosus* were sequenced along with *Geomyces asperulatus*, *G. pannorum*,

TABLE I. Species, strain, and collection information for species of *Pseudogymnoascus*, *Gymnostellatospora* and *Geomyces* where the internal transcribed spacer (ITS) and 5.8S regions of the nuclear ribosomal DNA are newly sequenced

Species	Strain	GenBank number	Collection information, collector
G. asperulatus	UAMH 183[t]	DQ117444	Forest soil, USA, Raymond
G. asperulatus	UAMH 9032	DQ117449	Decayed spruce, Canada, Lumley
G. pannorum	UAMH 714	DQ117446	Human, Netherlands, DeVries
G. pannorum	UAMH 1030	DQ117436	Cold storage food, USA, Kuehn
G. pannorum	UAMH 1088	DQ117442	Frozen food, USA, Kuehn
Geomyces sp.	UAMH 7253	DQ117447	Human sputum, Canada, Rennie
Geomyces sp.	UAMH 9107	DQ117450	Decayed spruce, Canada, Lumley
Gy. alpina	UAMH 9339	DQ117458	Alpine forest soil, Kenya, Udagawa
Gy. alpina	UAMH 9430[t]	DQ117459	*Erica carnea* rhizosphere, Switzerland, Müller
Gy. canadensis	UAMH 8899[t]	DQ117448	Decayed spruce, Canada, Lumley
Gy. canadensis	UAMH 9238[p]	DQ117453	Decayed spruce, Canada, Lumley
Gy. frigida	UAMH 9304[t]	DQ117457	Alpine forest soil, Japan, Udagawa
Gy. japonica	UAMH 9239	DQ117454	Decayed spruce, Canada, Lumley
Gy. japonica	UAMH 9240	DQ117455	Decayed spruce, Canada, Lumley
Gy. subnuda	UAMH 9242[p]	DQ117456	Decayed spruce, Canada, Lumley
P. appendiculatus	UAMH 10510	DQ117437	*Sphagnum* bog, Canada, Rice
P. appendiculatus	UAMH 10511	DQ117438	*Sphagnum* bog, Canada, Rice
P. appendiculatus	UAMH 10512	DQ117439	*Sphagnum* bog, Canada, Rice
P. roseus	UAMH 1658	DQ117443	Forest soil, Ghillini
P. roseus	UAMH 2879	DQ117445	Alpine soil, Canada, Widden
P. roseus	UAMH 9163	DQ117451	Ectomycorrhizal root tip, Canada, Fernando
P. roseus	UAMH 9222	DQ117452	Decayed spruce, Canada, Lumley
P. verrucosus	UAMH 10579[t]	DQ117440	*Sphagnum* bog, Canada, Rice
P. verrucosus	UAMH 10580[p]	DQ117441	*Sphagnum* bog, Canada, Rice

[t] *ex*-type culture
[p] para-type culture

Geomyces sp., *Gymnostellatospora alpina*, *Gy. canadensis*, *Gy. frigida*, *Gy. japonica*, *Gy. subnuda*, and *P. roseus* var. *roseus* (TABLE I). For outgroup comparison a sequence of *Myxotrichum chartarum* (AF062813) was obtained from GenBank.

Cultures were grown on OA overlaid with a cellophane membrane (Carmichael 1962, Gibas et al 2002). DNA extraction followed a modification of Cubero et al (1999) and Gibas et al (2002). Approximately 100 mg of mycelium was scraped from the surface of the membrane and placed in a sterile 2 mL screw-cap microcentrifuge tube, containing acid-washed sand, a ceramic bead, and 750 µL CTAB extraction buffer (2% w/v cetyl-trimethyl ammonium bromide, 1 M NaCl, 100 mM Tris, 20 mM EDTA). The mycelium was ground by centrifuging at least 2 min at maximum speed, then the entire mixture was transferred into a second tube and 1.5 µL β-mercaptoethanol was added before incubating 2 h at 65 C. Seven hundred fifty µL of chloroform : isoamyl alcohol (24 : 1 v/v) was added, and the solution was mixed by inverting the tube about 20 times and centrifuging for 15 min at 10000 *g* at room temperature. The upper aqueous layer, containing crude DNA, was collected and purified with the QIAquick PCR purification kit (QIAGEN Inc, Mississauga, Ontario). Purified DNA was stored at −20 C.

The nuclear ribosomal region that includes the internal transcribed spacer (ITS) 1, 5.8S, and ITS2, was amplified with the primer pair NS1/ITS4 (White et al 1990). PCR reactions were subjected to 30 cycles on a Perkin Elmer GeneAmp 9700 Thermal Cycler (PE Applied Biosystems, Foster City, California). Primers ITS1, ITS2, ITS3 and ITS4 were used to obtain sequence data for both strands using the BigDye™ Terminator Cycle Sequencing Kit (Applied Biosystems) and run on an ABI 377 Automated Sequencer (Amersham Pharmacia Biotech Inc, Piscataway, New Jersey). Consensus sequences were obtained with the Sequencher™ 4.0.2 (Gene Codes Corp., Ann Arbor, Michigan) and aligned manually by eye with Se-Al v 2.0a11 (University of Oxford). Phylogenetic analyses were run with PAUP (phylogenetic analysis using parsimony) v. 4.0b10 (Swofford 2002) and the robustness of the resulting phylogenetic trees and inferred clades was tested with bootstrap analysis (Felsenstein 1985) of 100 resamplings.

TAXONOMY

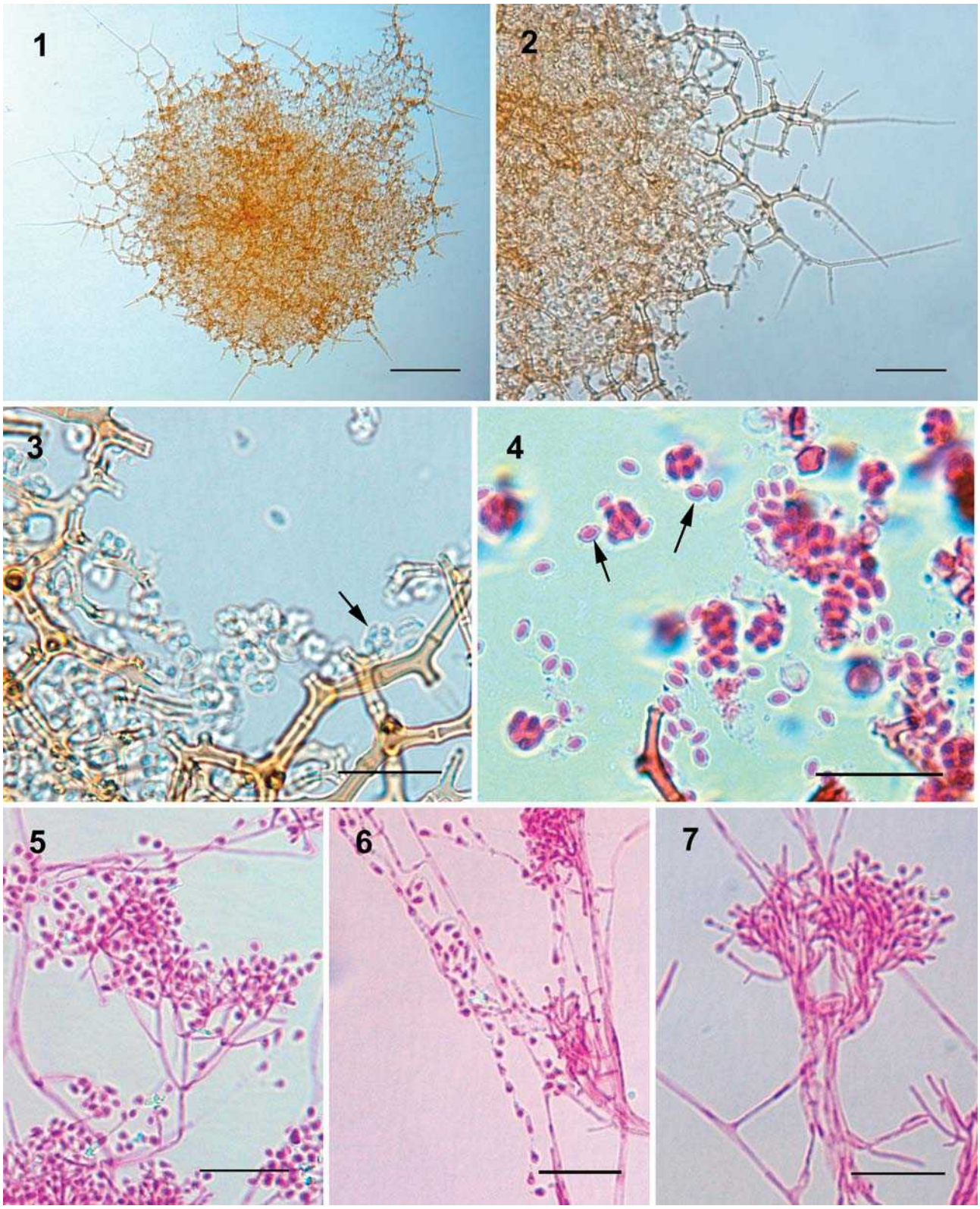
Pseudogymnoascus appendiculatus Rice and Currah sp. nov. FIGS. 1–16

Etymology: Latin, *appendiculatus* = appendage, referring to the prominent, long, branched appendages.

Ascomata fiunt in frigore 2–8 menses post incubationem, vel solitaria vel in globis, globosa ad subglobosa, primum alba, deinde aurantiaco-brunnea in maturitate, appendicibus inclusis 300–650 µm diam. Hyphae peridiales aurantiaco-flavae, leves, septatae, crassiter tunicatae, 2–2.5 µm diam, ramosae, anastomosis reticuloperidium format. Appendices aurantiaco-flavae, crassiter tunicatae, septatae, dichotomose ramosae, leves, cum extremis fastigatis, 40–120 µm longae. Asci octospori, hyalini, globosi ad subglobosi, deliquescentes, 5–7 µm diam. Ascosporae 2.5–5 × 1.5–2.5 µm, hyalinae, fusoideae ad ellipsoideae, crista longitudinalis et indistincta. Status anamorphosis a Geomyci. Conidiophora tenuiter distincta, erecta, hyalina, tenuiter et leviter tunicata, dendritica, verticillate ramosa. Conidia alba ad pallide alba. Conidia terminalia subglobosa ad late pyriformia, cicatrix basalis et prominens, levia ad minute asperulata, 2.5–3.5 × 1.5–2.5 µm. Conidia intercalaria subglobosa ad elongata et dolioformia, extremis vel magis vel minus truncatis, levia ad minute asperulata, 3–5 × 2–2.5 µm. Isolata ex ligno brunneo-putrefacto piceae marianae in sphagno palustro submersae.

Holotypus: Colonia exsiccata ex UAMH 10509 isolato ex ligno brunneo-putrefacto piceae marianae in sphagno palustro submersae.

Colonies on OA 40–45 mm diam at 28 d at 15 C and 38–47 mm diam at 5 C, appressed, white, producing a diffusible yellow pigment and a clear exudate; reverse yellow. Aerial conidia abundant, white. Colonies on CMA 35–40 mm diam at 15 C and 33–40 mm diam at 5 C, appressed, colorless, consisting of immersed, hyaline hyphae; reverse colorless. Aerial conidia sparse, patchy, white. Ascomata produced after 2–8 mo on CMA at 5 C and 15 C and after 3 mo on OA at 5 C. Ascomata solitary or in clusters, globose to subglobose, white at first, peridial hyphae and appendages becoming orange-brown at maturity (FIG. 1), 200–450 µm diam excluding appendages, 300–650 µm diam including appendages (FIGS. 1, 8); centrum white. Peridial hyphae orange, smooth, septate, thick-walled, 2–2.5 µm diam, branched and anastomosed to form a reticuloperidium, giving rise to appendages (FIGS. 2, 9). Appendages orange, thick-walled, septate, thickened at each septum, branched, smooth, tapering toward apices, 40–120 µm long (FIGS. 2, 9). Asci 8-spored, hyaline, globose to subglobose at maturity, deliquescent, 5–7 µm diam (FIGS. 3, 12). Immature asci subglobose to globose (FIGS. 11, 12) or clavate (FIG. 10), stipitate and borne singly (FIGS. 10, 12), or sessile and borne in chains (FIG. 11). Ascospores hyaline, fusoid to ellipsoidal, thick-walled, smooth or with an indistinct longitudinal rim (FIG. 4, 13, 14), 2.5–5 × 1.5–2.5 µm (FIGS. 4, 13, 14). Anamorph: *Geomyces* sp. (FIGS. 5–7, 15, 16). Conidia white to off-white en masse. Aleurioconidia terminal, subglobose to broadly pyriform with prominent truncate basal scars, hyaline, relatively thin-walled, smooth to minutely asperulate, 2.5–3.5 × 1.5–2.5 µm (FIGS. 5, 7, 15, 16). Intercalary arthroconidia subglobose to elongate and barrel-



FIGS. 1–7. Light micrographs of *Pseudogymnoascus appendiculatus* direct mounts from cultures grown on cornmeal agar (CMA) at 5 C in the dark. 1. Ascocarp with smooth, peridial hyphae and long, branched appendages. Bar = 80 µm. 2. Smooth, thick-walled, peridial hyphae with long, branched appendages. Bar = 40 µm. 3. Smooth peridial hyphae with ascospores and asci containing developing ascospores (arrow). Bar = 15 µm. 4. Ellipsoidal ascospores with longitudinal band or rim (arrows).

shaped with more or less truncate ends, hyaline, thin-walled, smooth to minutely asperulate, 3–5 × 2–2.5 μm (FIG. 6). Conidia borne in long chains on undifferentiated hyphae (FIG. 6), or verticillately branched conidiophores that are erect, hyaline, thin- and smooth-walled, 5–40 × 1.5–2.5 μm, with branches fragmenting basipetally into rhexolytically dehiscent arthroconidia or aleurioconidia (FIG. 5). Conidiophores sometimes synnematous (FIGS. 7, 16).

Holotype: Dried culture of UAMH 10509 from brown-rotted black spruce wood under *Sphagnum* peat.

Other specimens: CANADA. ALBERTA: 5 km east of Perryvale (54°28′N, 113°16′W), *Picea mariana-Sphagnum fuscum* bog, *ex* brown-rotted wood bait block, 2002, *A. Rice* (UAMH 10510, UAMH 10511, UAMH 10512).

Pseudogymnoascus verrucosus Rice and Currah sp. nov. FIGS. 17–25

Etymology: Latin, *verrucosus* = covered with warts, referring to the warty ornamentation of the peridial hyphae, ascospores and conidia of this species.

Ascomata fiunt in frigore post 6–8 menses, vel solitaria vel in globis, globosa ad subglobosa, primum alba, deinde rubra in maturitate, 150–400 μm diam. Hyphae peridiales rubro-brunneae, septatae, crassiter tunicatae, 2–2.5 μm diam, crassiter asperulatae, alte ramosae; anastomosis reticuloperidium densum format. Appendices elongatae absunt, hyphae peridiales terminant in appendicibus nonnullis et distinctis, tumidis, subhyalinis, verrucosis, 5–10 × 3–4 μm. Asci octospori, hyalini, globosi ad subglobosi, deliquescentes, 5–8 μm diam. Ascosporae 3–5 × 2–3 μm, late fusoideae ad ellipsoideae, hyalinae, perispora irregulariter asperulata ad verrucosa. Status anamorphosis a Geomyci. Conidiophora tenuiter distincta, erecta, hyalina, tenuiter et leviter tunicata, dendritica, verticillate ramosa. Conidia alba, massiter ad pallide rosea. Conidia terminalia subglobosa ad late pyriformia, cicatrix basalis et prominens, asperulata ad irregulariter verrucosa in maturitate, 2.5–4 × 2–3 μm. Conidia intercalaria subglobosa ad elongata et dolioformia, extremis vel magis vel minus truncatis, asperulata ad irregulariter verrucosa, 2.5–5 × 2–3 μm. Isolata ex ligno brunneo-putrefacto piceae marianae in sphagno palustro submersae.

Holotypus: Colonia exsiccata ex UAMH 10579 isolata ex lingo brunneo-putrefacto piceae marianae in sphagno palustro submersae.

Colonies on OA 40–45 mm diam at 28 d at 15 C, white, floccose, with a pale orange exudate; aerial hyphae and conidia abundant, white to off-white or pale gray; reverse orange. Colonies on CMA 44–48 mm diam at 28 d and 15 C, appressed, colorless, consisting mostly of immersed, hyaline hyphae; reverse colorless. Aerial conidia sparse, concentrated in the center of the colony, initially white, becoming pink with age. Ascomata produced after 6–8 mo on CMA at 15 C. Ascomata solitary or clumped, globose to subglobose, red at maturity, 150–400 μm diam. Peridial hyphae red brown (FIG. 17), septate, thick-walled, 2–2.5 μm diam, coarsely asperulate, highly branched and anastomosing to form a dense reticuloperidium (FIGS. 17, 21). Distinct appendages absent, peridial hyphae with swollen, thin-walled, subhyaline, verruculose apices, 5–10 × 3–4 μm (FIG. 17). Asci 8-spored, hyaline, globose to subglobose, solitary or borne in chains (FIG. 22), deliquescent, 5–8 μm diam (FIGS. 18, 22, 23). Ascospores broadly fusoid to ellipsoidal, hyaline, thick-walled, irregularly asperulate to verruculose at maturity, 3–5 × 2–3 μm (FIGS. 23). Anamorph: *Geomyces* sp. (FIGS. 19, 20, 24, 25). Conidia white to pale pink en masse. Aleurioconidia terminal, subglobose to broadly pyriform with a prominent truncate basal scar, relatively thick-walled, hyaline to lightly pigmented, asperulate (FIGS. 19, 24) or coarsely tuberculate (FIGS. 20, 24, 25) at maturity, 2.5–4 × 2–3 μm. Arthroconidia subglobose to elongate and barrel-shaped with more or less truncate ends, thick–walled, hyaline to lightly pigmented, asperulate or coarsely tuberculate at maturity, 2.5–5 × 2–3 μm (FIG. 25). Conidiophores erect, hyaline, thin- and smooth-walled, dendritic with verticillate branching, 5–25 × 1–1.5 μm (FIG. 19).

Holotype: Dried culture of UAMH 10579 from brown-rotted black spruce wood buried under *Sphagnum* peat.

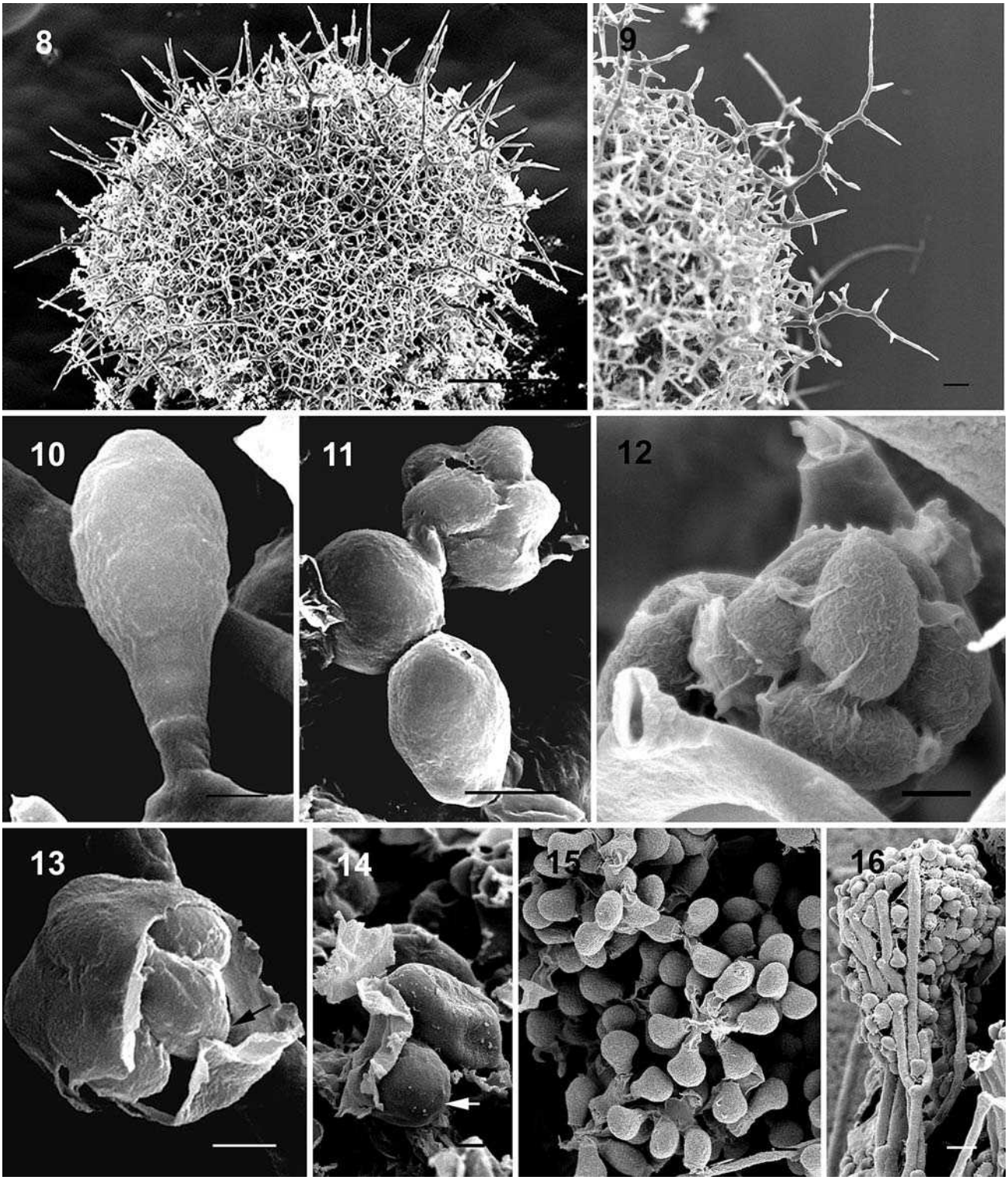
Other specimen: CANADA. ALBERTA: 5 km east of Perryvale (54°28′N, 113°16′W), *Picea mariana-Sphagnum fuscum* bog, ex brown-rotted spruce wood bait, Jul 2002, *A. Rice* (UAMH 10580, paratype).

KEY TO SPECIES OF *PSEUDOGYMNOASCUS*

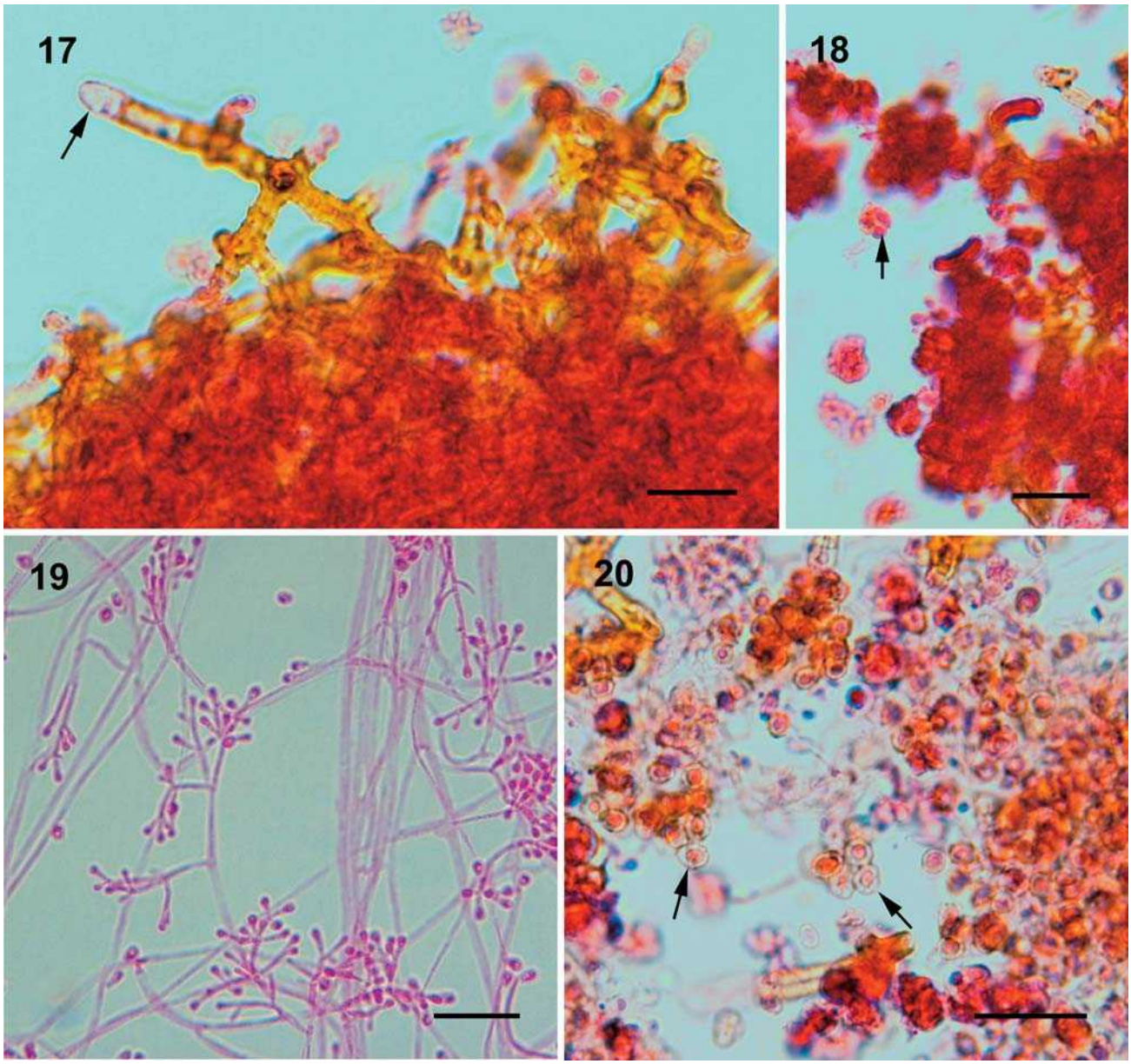
1. Colonies white with yellow reverse. Yellow exudate produced. Peridial hyphae smooth-walled, orange, bearing long (40–120 μm), pigmented, smooth- and thick-walled, branched appendages.

←

Bar = 15 μm. 5. *Geomyces* anamorph with verticillately branched solitary conidiophores bearing small, barrel-shaped or pyriform aleurioconidia and arthroconidia. Bar = 15 μm. 6. Long chains of barrel-shaped to pyriform arthroconidia. Bar = 15 μm. 7. Synnematous bundle of conidiophores bearing pyriform aleurioconidia. Bar = 15 μm.



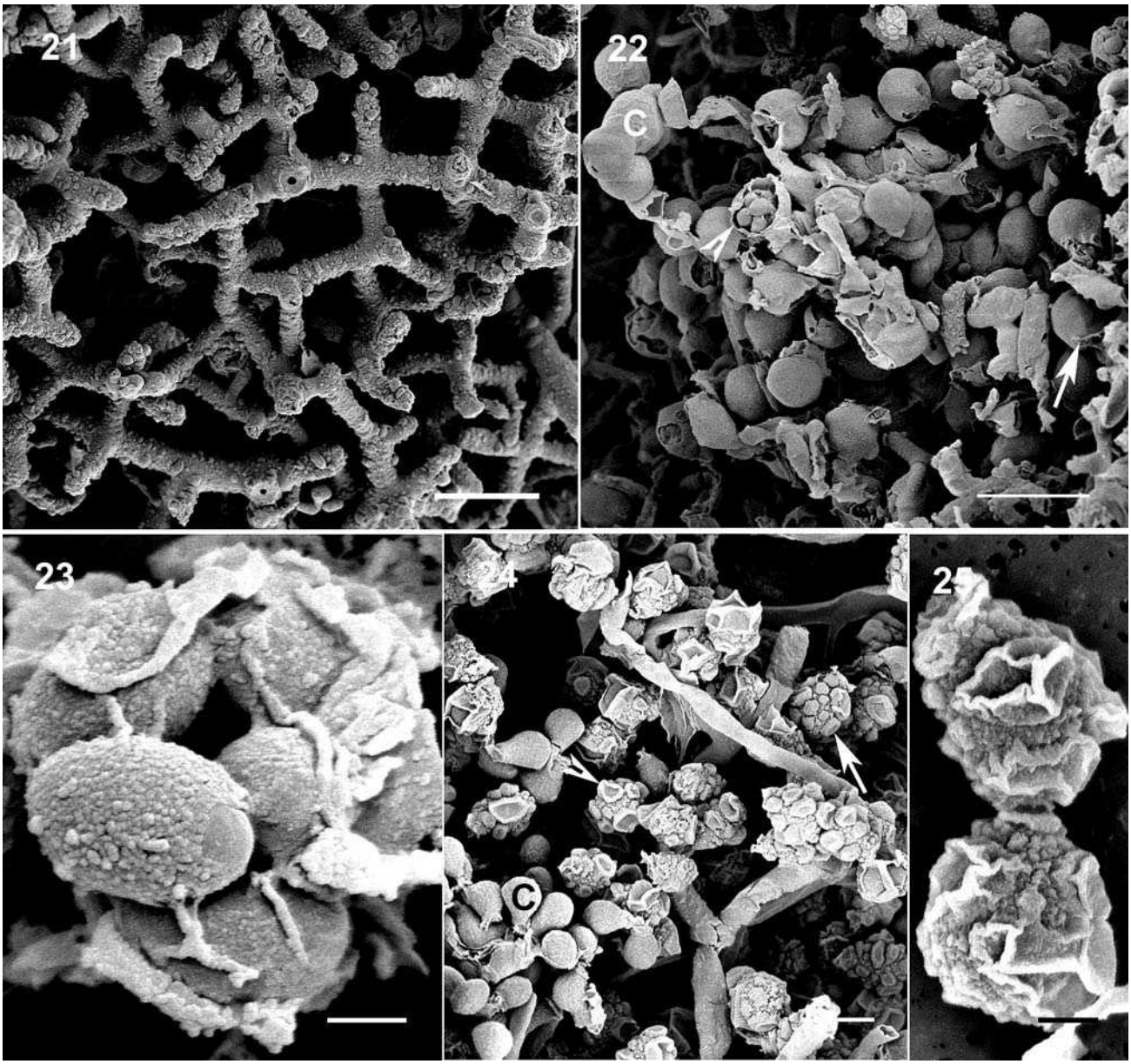
FIGS. 8–16. Scanning electron micrographs of *Pseudogymnoascus appendiculatus* obtained from cultures grown on cornmeal agar (CMA) at 5 C in the dark. 8. Ascocarp with long, smooth, branched, appendages. Bar = 100 µm. 9. Peridial hyphae and long, smooth, branched appendages. Bar = 10 µm. 10. Young ascus. Note clavate shape. Bar = 2 µm. 11. Chain of immature asci at different stages of development. Note the variation in ascus shape. Bar = 2.5 µm. 12. Subglobose to globose, developing ascus containing ascospores close to maturity. Bar = 1 µm. 13. Ascus rupturing to release ascospores. Note the faint longitudinal rim on the ascospores (arrow). Bar = 2.5 µm. 14. Mature ascospores with remnants of ascus. Arrow indicates faint longitudinal band on the ascospore. Bar = 1 µm. 15. *Geomyces* anamorph with pyriform, minutely asperulate aleurioconidia. Bar = 1 µm. 16. Synnematous bundle of conidiophores bearing pyriform, minutely asperulate conidia. Bar = 5 µm.



FIGS. 17–20. Light micrographs of *Pseudogymnoascus verrucosus* direct mounts from cultures grown on cornmeal agar (CMA) at 15 C in the dark. 17. Verrucose peridial hyphae terminating in short, subhyaline apices (arrow). Bar = 40 µm. 18. Ascus containing ascospores (arrow). Bar = 15 µm. 19. *Geomyces* anamorph with short, verticillately branched conidiophores bearing pyriform aleurioconidia. Bar = 15 µm. 20. Thick-walled, tuberculate conidia. Bar = 15 µm.

Ascospores smooth or with a faint longitudinal rim . . . . . *P. appendiculatus*

1. Colonies white to pink or purplish-red; reverse not yellow. Yellow exudate absent. Peridial hyphae rough-walled, red to red-brown, bearing short (typically < 50 µm), subhyaline, thin- and rough-walled, unbranched appendages. Ascospores smooth or irregularly ornamented, lacking a longitudinal rim 2
2. Ascomata abundant. Ascospores smooth-walled . . . . . *P. roseus* var. *roseus*
2. Ascomata rare or abundant. Ascospores ornamented 3
3. Colonies purplish red, vinaceous. Ascomata abundant. Appendages 20–54 µm long. Ascospores lobate-reticulate, 2.5–4 × 2–2.5 µm. Conidia smooth-walled only. . . . . *P. roseus* var. *ornatus*
3. Colonies white to pink. Ascomata rare. Appen-



FIGS. 21–25. Scanning electron micrographs of *Pseudogymnoascus verrucosus* obtained from cultures grown on cornmeal agar (CMA) at 15 C in the dark. 21. Highly branched and anastomosed, coarsely asperulate to verrucose peridial hyphae. Bar = 10 µm. 22. Chains (C) of subglobose to globose asci at different stages of development. Note the ruptured ascus (arrowhead) and scars left by the dehiscence of adjacent asci (arrow). Bar = 10 µm. 23. Asperulate to warty ascospores surrounded by remnants of the ascus. Bar = 1 µm. 24. Two types of conidia: *Geomyces* anamorph with pyriform, minutely asperulate aleurioconidia (C) and pyriform to irregular, tuberculate conidia (arrow and arrowhead). Note that many of the warts are in the process of collapsing (arrowhead). Bar = 1 µm. 25. Two irregularly shaped, tuberculate conidia in a short chain. Note that the warts have collapsed. Bar = 1 µm.

dages 5–10 µm long. Ascospores verrucose, 3–5 × 2–3 µm. Conidia smooth to asperulate or coarsely tuberculate . . . . . *P. verrucosus*

RESULTS

DNA sequencing.—Sequences of the ITS 1, 5.8S subunit, ITS 2, and a short fragment of the 28S subunit ranged from 503 to 545 bases. Manual alignment yielded a total length of 528 characters. Of these, 378 were constant, 85 were variable but parsimony uninformative and 65 were parsimony informative. Three most- parsimonious trees were obtained with the heuristic random sequence addition search option with gaps treated as missing characters. One of these, with a length

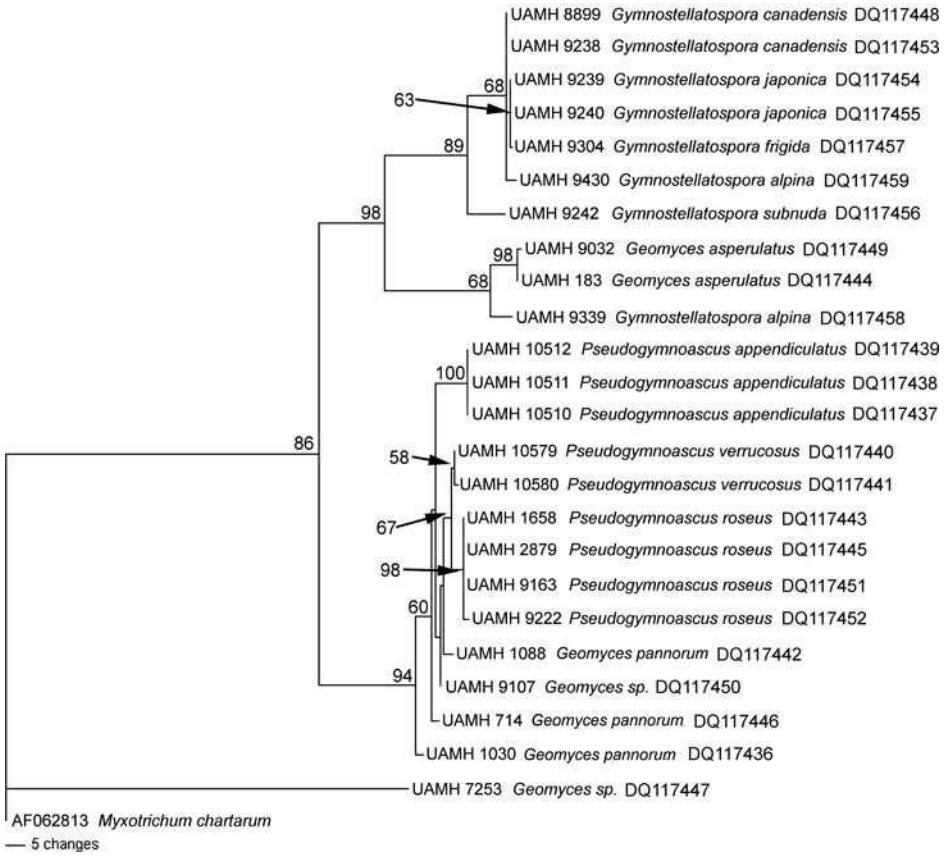


FIG. 26. One of three most parsimonious trees from sequence analysis of the ITS 1, 5.8S, and ITS 2 regions of the nuclear ribosomal DNA of *Pseudogymnoascus, Geomyces*, and *Gymnostellatospora* species and *Myxotrichum chartarum*. Bootstrap values less than 50 are not shown.

of 218, a consistency index of 0.835, a retention index of 0.914, and a homoplasy index of 0.165, is shown (FIG. 26).

Species of *Gymnostellatospora, Pseudogymnoascus* and *Geomyces* formed a well supported clade (bootstrap 86) with one isolate identified as *Geomyces* sp. (UAMH 7253) excluded from the group (FIG. 26). This clade included two groups with strong support. The first (bootstrap 98) included all sequenced species of *Gymnostellatospora* and *Geomyces asperulatus*, with a well supported subclade (bootstrap 89) including all of the *Gymnostellatospora* isolates except UAMH 9339 (*Gy. alpina*), which formed a clade (bootstrap 68) with *G. asperulatus*. The second (bootstrap 94) included all sequenced species of *Pseudogymnoascus*, *G. pannorum* isolates and one isolate of *Geomyces* sp. (UAMH 9107). The three isolates of *P. appendiculatus* formed an extremely well supported clade (bootstrap 100) within this group, as did the four isolates of *P. roseus* (bootstrap 98). The two isolates of *P. verrucosus* formed a clade with relatively low bootstrap support (58) despite differing at less than 1% of the bases (3 bases). *Geomyces* was polyphyletic: *G. asperulatus* was monophyletic (bootstrap 98) and allied with *Gymnostellatospora*, *G. pannorum* was paraphyletic and grouped with *Pseudogymnoascus*, and at least one species was not allied with either teleomorph genus.

DISCUSSION

The presence of small, ellipsoidal to fusiform ascospores, orange to red gymnothecia and *Geomyces* anamorphs indicate the new taxa are best disposed as distinct species within *Pseudogymnoascus*. Species of *Gymnostellatospora* are similar but differ in having ascospores with prominent longitudinal ridges or wing-like crests (Sigler et al 2000). The smooth, orange peridial hyphae and long, concolorous, branched appendages of *P. appendiculatus* contrast markedly with the asperulate to verrucose, red peridial hyphae and short, hyaline, unbranched appendages of other species in the genus, including *P. verrucosus*. Ascospores of the latter species are

verrucose rather than lobate-reticulate as in *P. roseus* var. *ornatus* (Udagawa and Uchiyama 1999) or smooth as in *P. roseus* var. *roseus* (Tsuneda 1982, Sigler et al 2000). In addition the peridial hyphae of *P. verrucosus* are more coarsely ornamented and are warty rather than minutely asperulate under light microscopy. The appendages of *P. roseus* var. *ornatus* are more than twice as long (20–54 μm) (Udagawa and Uchiyama 1999) than those of *P. verrucosus* (5–10 μm) while those of *P. roseus* var. *roseus* are clavate (Sigler et al 2000) rather than slightly swollen as in *P. verrucosus*. Another distinctive feature of *P. verrucosus* is the presence of two types of barrel-shaped to pyriform conidia: one type is minutely asperulate and resembles the anamorphs of other *Pseudogymnoascus* species while the second is coarsely tuberculate.

Raillo (1929) erected *Pseudogymnoascus* (Gymnoascaceae) for two species (*P. roseus* and *P. vinaceus* Raillo) from soil in the Soviet Union. Two additional species were described from soil in the Soviet Union and Canada before 1980: *P. caucasicus* Cejp & Milko (Cejp and Milko 1966) and *P. bhatti* Samson (Samson 1972). All four have smooth ascospores and *Geomyces* anamorphs, characters considered diagnostic for the genus (Samson 1972, Orr 1979, Currah 1985). Currah (1985) considered the four names synonymous and gave priority to *P. roseus*. In 1982, *P. dendroideus* Locquin-Linard from cow dung in Algeria (Locquin-Linard 1982) and *P. alpinus* Müller & von Arx from the rhizosphere of *Erica carnea* L. (Müller and von Arx 1982) were described. Both had ridged ascospores and poorly developed anamorphs. Udagawa et al (1993) erected *Gymnostellatospora* to accommodate species with ornamented ascospores and absent or poorly developed anamorphs. Between 1993 and 2000 five species were described from Japanese and Russian soils and from rotting wood in Canada: *Gy. japonica* Udagawa, Uchiyama & Kamiya (Udagawa et al 1993), *Gy. frigida* Uchiyama, Kamiya & Udagawa (Uchiyama et al 1995), *Gy. canadensis* Lumley, Sigler & Currah, *Gy. submuda* Sigler, Lumley & Currah (Sigler et al 2000), and *Gy. parvula* Udagawa & Uchiyama (Udagawa and Uchiyama 2000). Udagawa (1997) also transferred *P. dendroideus* and *P. alpinus* into *Gymnostellatospora* as *Gy. dendroidea* (Locquin-Linard) Udagawa and *Gy. alpina* (Müller & von Arx) Udagawa. The difference between *Pseudogymnoascus* and *Gymnostellatospora* was less distinct following the description of *P. roseus* var. *ornatus* with ornamented, rather than smooth, ascospores and a *Geomyces* anamorph (Udagawa and Uchiyama 1999). Sigler et al (2000) continued to regard the genera as distinct, despite the presence of ornamented ascospores in *P. roseus* var. *ornatus* and anamorphs in *Gy. alpina, Gy. frigida* and *Gy. canadensis*, noting that none of these anamorphs was a *Geomyces* state characteristic of *Pseudogymnoascus* and that the ascospores of *P. roseus* var. *roseus* lacked the longitudinal ridges and crests characteristic of *Gymnostellatospora*. They added the proviso that the species of *Pseudogymnoascus* and *Gymnostellatospora* represent a gradient of morphological types and recommended that DNA sequences should be compared among the species in both genera (Sigler et al 2000).

DNA sequence analyses of the ITS 1, 5.8S, and ITS 2 regions of the nuclear ribosomal DNA (rDNA) region support *Gymnostellatospora* and *Pseudogymnoascus* as distinct and place most anamorphic *Geomyces* isolates sampled here in the *Pseudogymnoascus* clade although *G. asperulatus* was included among taxa in the *Gymnostellatospora* clade and one was in neither clade. The analyses suggest that presence of the distinct longitudinal ridges on ascospores continues to be a reliable morphological character in the definition of *Gymnostellatospora*.

Species-level relationships are reasonably well supported in *Pseudogymnoascus*, with *P. appendiculatus*, *P. roseus* and *P. verrucosus* appearing distinct and with *P. roseus* and *P. verrucosus* more closely related to each other than to *P. appendiculatus*. Bootstrap support for *P. verrucosus* was lower than that for *P. appendiculatus* and *P. roseus* although the sequences of the two isolates of *P. verrucosus* differed at less than 1% of bases. Consistent morphological differences, including the unique tuberculate conidia, further support conspecificity of the strains designated as *P. verrucosus*. The proximity of the two isolates in the bog also supports their close relationship; the isolates were recovered from bait blocks contained within the same 5 × 10 cm litter bag. The relationship of *P. roseus* var. *ornatus* to the type variety and to *P. verrucosus* cannot be assessed by molecular analyses because cultures are unavailable. Relationships among isolates of *Geomyces* are not well resolved but the group is clearly a polyphyletic assemblage. Resolution of species-level relationships in *Gymnostellatospora* awaits sampling of additional isolates.

Patterns of ascus development are rarely mentioned in descriptions of cleistothecial taxa, and the taxonomic importance of this character is unknown. In both new species asci develop asynchronously and in short chains. Tsuneda (1982) also reported asynchronous ascus development in *P. roseus*, but this is the first report of catenate asci in the genus (Tsuneda 1982, Udagawa and Uchiyama 1999, Sigler et al 2000). Ascus development in both differs when compared to *Myxotrichum deflexum* (Rosing 1985) and *M. arcticum* (Tsuneda and Currah 2004) in which asci arise individually from penultimate cells of

croziers and develop more or less simultaneously. Tsuneda and Currah (2004) suggested this pattern of ascus development in *M. arcticum* is more typical of leotiomycetous than plectomycetous species and supports placement of the *Myxotrichaceae* among the inoperculate discomycetes. The occurrence of catenate asci in both the Leotiomycetes (e.g. as shown here in *Pseudogymnoascus*) and in Euriotiomycetes (e.g. Tzean et al 1992) would suggest this feature is convergent in these lineages and that it confers some ecological or developmental advantage related to the cleistothecial form.

Myxotrichaceous fungi, including *Geomyces* and *Oidiodendron* species, were among the fungi most frequently isolated from Perryvale Bog (Rice and Currah 2002, Rice et al 2006) and their abundance suggests they may be important saprobes in this ecosystem. Most records of Myxotrichaceae are from soil, peat and decaying wood and other organic matter in cool, temperate environments (e.g. Barron 1962, Barron and Booth 1966, Udagawa et al 1994, Hambleton et al 1998, Udagawa and Uchiyama 1999, Sigler et al 2000, Rice and Currah 2006). In vitro physiological studies of these fungi support the assertion that they are important saprobes in soil, decaying wood and peat in temperate and cool environments. Many are cellulolytic and psychrotolerant or psychrophilic (e.g. Currah 1985, Udagawa et al 1993, Uchiyama et al 1995, Udagawa and Uchiyama 1999, Sigler et al 2000, Rice and Currah 2005). Some species also degrade other plant and fungal residues, including polyphenolic compounds, pectin and chitin, that are common in peat, wood and organic soils. In addition to the saprobic habit, some members of the Myxotrichaceae, including *P. roseus* (Dalpé 1989) have been shown to form ericoid mycorrhizal association in vitro and it is possible that at least some occupy a mycorrhizal role in peatlands. The two lineages traditionally included in the Myxotrichaceae are morphologically and ecologically similar, suggesting convergent evolution, possibly in response to a reliance on dispersal by arthropod vectors (Currah 1985, 1994; Greif and Currah 2003).

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