Three new polyporicolous species of *Hypomyces* and their *Cladobotryum* anamorphs

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Three new species of Hypomyces that occur on members of the Aphyllophorales are described. The anamorph of H. viridigriseus is Cladobotryum viridigriseum comb. nov. Hypomces favoli and H. puertoricensis have unnamed Cladobotryum anamorphs. Perithecia of H. viridigriseus are known only from the type locality, in Illinois, but its anamorph was described from Ontario and is also known from New York. Hypomyces favoli and H. puertoricensis are known only from Puerto Rico.

Keywords: Hypocreales, Aphyllophorales, fungicolous fungi, systematics.

Hypomyces (Ascomycetes, Hypocreales), with about 50 species recognized in recent studies (Helfer, 1991; Põldmaa, 1996; Rogerson & Samuels, 1985, 1989, 1993, 1994) is the largest genus of almost exclusively fungicolous fungi. Hypomyces species occur mainly on discomycetes, boletes, agarics or polypores. The polyporicolous Hypomyces are more numerous than any other group, with nineteen species accepted by Rogerson & Samuels (1993). Half of the polyporicolous species have been described only within the last twentyfive years. In the present work, three more polyporicolous species with proven or presumed Cladobotryum anamorphs – one temperate and two tropical – are described.

Two of the newly described species, *H. favoli* and *H. puertoricensis*, produce only unicellular ascospores. None of the previously known polyporicolous species of *Hypomyces* has exclusively

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unicellular ascospores, although several (H. albidus Rehm, H. amaurodermatis Rogerson & Samuels, H. semitranslucens G. Arnold, H. sympodiophorus Rogerson & Samuels, H. tegillum Berk. & M. A. Curt.) are known to form at least some unicellular ascospores in addition to bicellular ones. Unicellular ascospores characterize the genus Peckiella (Sacc.) Sacc., which is typified by P. viridis (Alb. & Schw.) Sacc., an agaricicolous species that Rogerson & Samuels (1994) regarded as synonymous with H. luteovirens (Fr.: Fr.) L.-R. Tulasne. This species does not grow in pure culture and no anamorph is known for it. In perithecial anatomy and anamorphs, the two newly described species with unicellular ascospores conform to the overall pattern found in polyporicolous species of Hypomyces, including growing in pure culture and forming a Cladobotryum anamorph. This once more confirms the unreliability of ascospore septation per se as a character for segregating genera from Hypomyces, as has been discussed in the case of boleticolous species (Rogerson & Samuels, 1989).

The anamorphs of the newly described *Hypomyces* species expand the circumscription of *Cladobotryum* beyond what was proposed by Rogerson & Samuels (1993). *Cladobotryum viridigriseum*, originally described in *Sympodiophora* G. Arnold, is the only *Hypomyces* anamorph to have green conidia, a feature which has a restricted distribution in the whole order of Hypocreales (Samuels & Seifert, 1987). Anamorphs of *H. puertoricensis* and *H. viridigriseus* are unusual also because they tend to form very long conidia that may have more than three septa in culture. The tendency to form more variable and longer conidia in vitro has previously been noted also for *Cladobotryum amazonense* Bastos & al. (Bastos & al., 1981).

Materials and methods

Individual ascospores were isolated with the aid of a micromanipulator on cornmeal-dextrose agar (CMD, Difco cornmeal agar + 2% dextrose). Colony characteristics were taken from CMD, incubated at 20–21 C with alternating 12 h darkness and 12 h cool white fluorescent light. Additional characters were described from cultures grown on malt extract agar (MEA, Difco) at 22–24 C in darkness.

Herbarium material was rehydrated briefly in 3% KOH, from which mesurements were made. The conventions KOH+ and KOHindicate whether the original colour of the subiculum or perithecia changes when placed into 3% aq. KOH. Perithecial sections were made from rehydrated herbarium material using a freezing microtome. The optical brightener calcofluor (0.05% w/v in sodium phosphate buffer at pH 8; Sigma Chemical Co.) was used for fluorescence microscopy.

Representative cultures are preserved at CBS.

Descriptions of the species

Hypomyces favoli Samuels, K. Põldmaa et Lodge, sp. nov. – Figs. 1–4, 10–12.

Subiculum album vel luteolum, in KOH immerso color non mutatur. Perithecia ovata vel obpyriformia, (285–)300–400(–425) µm alta, 195–335(–350) µm lata, fere superficialia, caespitosa, aurantiaca, in KOH immersa ad papillam ad roseum vertentia; hyphae aurantiacae vel flavae perithecia investientes. Asci cylindrici, (35–)107–140(–180) × (6.0–)7.5–10.0(–12.0) µm, octospori, apice annulo instructo. Ascospora late fusiformes, (11.5–)14.0–19.5(–21.5) × (6.0–)6.5–9.0(–12.0) µm, unicellulares, verrucosae et apiculatae; apiculi 1.5–3.0 µm longi, obtusi. Anamorphosis Cladobotryum sp. Conidia ellipsoidea vel cylindrica, (10–)25.5–28.5(–35) × (7.5–)9.0–11.5(–13.5) µm, 0-1-septata, catenis brevibus imbricatibus adhaerentia. Chlamy-dosporae abundantes.

Holotypus. – Ad carposomata *Polypori tenuiculi* (Beauv.) Fr., D. J. Lodge PR 1628 (BPI).

Associated anamorph. - Cladobotryum sp. - Figs. 11, 12.

Subiculum restricted to the regions of perithecial production, white to pale yellow; subicular hyphae hyaline, smooth-walled, ca. 6 μ m wide but greatly swollen around perithecia with cells 15–20 μ m diam, KOH-. - Perithecia ovate or obpyriform, (285-)300-400(-425) µm high, 195-335(-350) µm wide, nearly superficial in the subiculum, cespitose, orange, with a furfuraceous coat of pale orange to yellow hyphae, KOH⁺ (roseous) at the papilla, KOH⁻ in the lower part; papilla well developed, 90-100 µm high. - Perithecial wall ca. 25 μ m thick, cells \pm ellipsoidal in section, ca. 15 \times 5 μ m. – Papilla formed of outwardly diverging files of cells, the innermost of which are narrow and brick-like, but becoming more ellipsoidal to \pm circular or clavate at the surface, measuring ca. 5 μ m diam. – Asci cylindrical, $(85-)107-140(-180) \times (6.0-)7.5-10.0(-12.0) \mu m$, apex thickened and with a pore; ascospores uniseriate, partially overlapping. - Ascospores broadly fusiform, (11.5-)14.0-19.5(-21.5) × (6.0–)6.5–9.0(–12.0) µm, unicellular, prominently vertucose and apiculate; apiculi 1.5-3.0 µm long, obtuse.

Characteristics of the associated anamorph. – Anamorph sparse on the natural substratum. – Conidiophores indefinite in length, $4-8 \mu m$ wide, terminating in one to three conidiogenous branches at the tip; terminal branches aseptate or 1–2-septate, $38-125 \mu m$ long, $4-6 \mu m$ wide at base and tapering to 2.5 μm



Figs. 1–4. – Hypomyces favoli. – 1. Habit of perithecia. – 2, 3. Median longitudinal sections of mature perithecia. – 4. Ascus with immature ascospores. – 5–9. Hypomyces puertoricensis. – 5. Habit of perithecia. – 6. Whole mount of three mature perithecia, showing cells at perithecial apex. – 7. Conidia held in short, imbricate chains that appear as radiating heads on CMD. – 8. Conidium held at the tip of a conidiogenous cell. – 9. Periclinal thickening at the conidiogenous locus, fluorescence microscopy. – Figs. 1–3 from Lodge 704; 4 from Lodge 1028; 5, 6 from Lodge 3215; 7–9 from Samuels 96-12. – Scale bars: Fig. 1 = 1 mm, 2, 6 = 100 μ m; 3 = 50 μ m; 4, 8, 9 = 25 μ m; 5 = 0.5 mm; 7 = 200 μ m.



Figs. 10–12. – Hypomyces favoli. – 10. Ascus and ascospores. – 11. Conidia. – 12. Chlamydospore. – Figs. 10, 11 from Lodge 704; 12 from Lodge 1628. – Scale bars = 10 μm.

wide at tip, each bearing a single terminal conidiogenous cell. – Conidia ellipsoidal or cylindrical, (10–)25.5–28.5(–35)×(7.5–)9.0–11.5(–13.5) μm , 0–1-septate, with a protuberant, flat, often laterally displaced basal hilum, held in short imbricate chains. – Chlamy–

dospores abundant in host tissue, arising on short branches of hyphae, the lateral branch sometimes appearing as a specialized 'support' cell, globose, unicellular, $15-45 \mu m$ in diam, yellowish-brown, wall $1.5-4.5 \mu m$ wide, smooth.

Holotype. – PUERTO RICO: Northwest, Guajataca State Forest, on *Polyporus tenuiculus* (Beauv.) Fr. [= *Favolus brasiliensis* (Fr.) Fr.], 15 Oct. 1994, D. J. Lodge PR 1628 (BPI).

Paratype. – PUERTO RICO: Caribbean National Forest, Luquillo Mts., Luquillo Experimental Forest, El Verde Research Area, trail to Río Sonadora, part way up hill, elev. ca. 370 m, on *Polyporus tenuiculus*, 26 Nov. 1991, D. J. Lodge PR 704 (BPD).

Etymology. – From Latin 'favolus', a small honeycomb, also the commonly known, but antedated, name of the host, Favolus brasiliensis, in reference to the honeycomb-like aspect of the host hymenium.

Pure cultures of this species, obtained from *Lodge PR 704*, died before we had a chance to characterize the anamorph beyond noting that it was a *Cladobotryum* species. The *Cladobotryum* described here was taken from the paratype specimen, where it is poorly developed. Chlamydospores (Fig. 12), immersed in host tissue, were found in both specimens.

Because of its orange perithecia, which are KOH^+ at the papilla, and *Cladobotryum* anamorph, *H. favoli* is clearly related to the polyporicolous *Hypomyces* species *H. aurantius* (Pers. : Fr.) L.-R. Tul. and *H. subiculosus* (Berk. & M. A. Curt.) Höhnel. *Hypomyces aurantius* is common at north and south temperate latitudes while *H. subiculosus* is primarily tropical in distribution. Both of these species produce their conidia in long chains. Conidia of *H. aurantius* are held end-to-end whereas conidial chains of *H. subiculosus* are imbricate. In *H. favoli* conidia are held in short, imbricate chains, reminding of radiating heads. The anamorph shows resemblance to *Cladobotryum purpureum* (Morgan-Jones) W. Helfer but the lack of pure cultures in *H. favoli* does not allow their further comparison.

Hypomyces puertoricensis Samuels, K. Põldmaa et Lodge, sp. nov. – Figs. 5–9, 13–17.

Subiculum effusum, album. Perithecia obpyriformia, 230–360 µm alta, 136–270 µm lata, in subiculo immersa vel fere superficialia, dense gregaria, luteola; in KOH immersa colorem non mutant; papilla conspicua, acuta, 70–100 µm alta. Asci cylindracei, (115–)120–134(–138) × 7.0–9.5(–11.5) µm, apice incrassato, poro instructi. Ascosporae fusiformes, (11.0–)12.5–14.5(–16.0) × (4.0–)4.5–5.5(–6.0) µm, unicellulares, prominenter verrucosae, apiculatae; apiculi 3–4 µm longi, obtusi. Anamorphosis *Cladobotryµm* sp. Conidia oblonga, cylindrica vel anguste clavata,

 $29{-}42\times7{-}11~\mu{\rm m},~1{-}3{-}{\rm septata}.$ Chlamydosporae paucae, globosae vel ellipsoideae, unicellulares, 10–20 $\mu{\rm m}$ diam, dilute melleae.

 ${\rm H\,olot\,y\,p\,u\,s.}$ – Ad carposomata $Rigidopori\ lineati$ (Pers.) Ryvarden, ad lignum, G. J. Samuels 8037 (BPI).

Anamorph. - Cladobotryum sp. - Figs. 14-17.

Subiculum effused over old host basidiomata and surrounding rotten wood, white, thin and easily removed from the substratum; subicular hyphae hyaline, smooth-walled, 4-8 µm wide, often forming inflated cells up to 20 µm diam, KOH-. - Perithecia obpyriform, 230-360 µm high, 136-270 µm wide, immersed to halffree in the subiculum, gregarious in large numbers, pale yellow, KOH⁻; papilla prominent, acute, 70-100 μm. - Perithecial wall ca. 25 μ m thick, cells of outer region ellipsoidal, $8-13 \times 5-8 \mu$ m, becoming more fusiform or oblong toward the interior. - Papilla formed of diverging files of cells, those at the surface clavate, 7- $12 \times 4-6 \mu m$. – Asci cylindrical, (115–)120–134(–138) × 7.0–9.5(–11.5) μm, apex thickened and with a pore; ascospores uniseriate, partially overlapping. – Ascospores fusiform, $(11.0-)12.5-14.5(-16.0) \times (4.0-)$ 4.5-5.5(-6.0) µm, unicellular, prominently vertucose and apiculate; apiculi 3–4 µm long, obtuse; many ascospores (probably immature) smooth and nonapiculate.

Characteristics of the associated anamorph. – Anamorph sparse on natural substratum. Conidiophores and conidiogenous cells not observed. – Conidia oblong, cylindrical to narrowly clavate, $29-42 \times 7-11$ µm, 1-3-septate. – Chlamydos-pores scarce among subicular hyphae, globose or ellipsoidal, unicellular, 10-20 µm in diam, pale yellowish-brown, wall 1.5-3 µm wide, roughened.

Characteristics in culture. – Cultures derived from ascospores grown 7 d on CMD >5 cm diam, cottony, white, reverse not coloured. – Conidiation abundant, odour absent. – Conidiophores arising from aerial hyphae, ascending, not differentiated from aerial hyphae, simple or irregularly branched, indefinite in length, 5–6 μ m wide, each terminating in a single conidiogenous cell with a slight periclinal thickening at the tip, forming up to 10 conidia from the single conidiogenous locus. – Conidia oblong, cylindrical to narrowly clavate, straight or bent at the tip, $(20-)32.5-53.5(-59.5) \times (6.5-)8.0-10.5(-12.0) \mu$ m, 1–3-septate (to 75 μ m long and 2–5-septate on MEA), hyaline, with a protuberant, flat, central or slightly lateral, basal hilum, held in short imbricate chains, which appear as radiating heads. – Chlamydospores



Figs. 13–17. – Hypomyces puertoricensis. – 13. Ascus, ascus tip, and ascospores. Tip of ascus on left drawn as seen in phase contrast microscopy using water; tip of ascus on right as seen in phase contrast microscopy using water followed by aqueous phloxine (1%). – 14, 16. Conidia. – 15. Cluster of conidia held together. – 17. Chlamydospore. – Fig. 13 from Lodge 704; 14–17 from Samuels 96–12; 14 from

CMD; 15-17 from MEA. - Scale bars: Figs. 13-15, 17: 10 µm; Fig. 16: 35 µm.

abundant on MEA, arising from aerial hyphae on 10–130 μm long branches, topmost cell globose, 19–38 μm diam., yellow, wall ca. 4 μm thick, unevenly warted, warts to 3 μm high, disappearing in age, usually supported by a specialized, ellipsoidal, basal cell which may have a few warts.

Holotype. – PUERTO RICO: Caribbean National Forest, Luquillo Mts., Luquillo Experimental Area, El Verde Research Area, elev. 350 m, on *Rigidoporus lineatus* and surrounding wood, 19 Feb. 1996, G. J. Samuels 8037 & H.-J. Schroers (BPI, Isotype TAA; cultures: CBS 495.97, Samuels 96-12, TAA 96-29).

Paratype. – PUERTO RICO: Caribbean National Forest, Luquillo Mts., El Verde Research Area, Quebrada Prièta, Vogt wood addition plot, on polypore and rotten wood of standing dead tree, 5 June 1996, D. J. Lodge PR 3215 (BPI).

Etymology. – Refers to Puerto Rico, the geographic origin of the type specimen.

In its pale yellow, KOH^- perithecia and white subiculum (Fig. 5), H. puertoricensis resembles the temperate species H. semitranslucens and H. albidus. Hypomyces puertoricensis is distinguished by its relatively small, unicellular ascospores and – above all – by its Cladobotryum anamorph, which has exceptionally large conidia (Fig. 14–16). According to this feature it is similar to C. amazonense, which is characterized by the verticillate branching pattern of conidiophores and grows on basidiocarps of litter inhabiting agarics (Bastos & al., 1981). Conidiogenesis in H. favoli is presumably retrogressive as hila at the base of conidia seem to become progressively wider in age.

Hypomyces viridigriseus K. Põldmaa et Samuels, sp. nov. – Figs. 18– 30.

Subiculum album, viridescens. Perithecia obpyriformia, 250–400(–500) μ m alta, 165–300(–400) μ m lata, in subiculo semiimmersa vel quasi superficialia, solitaria vel gregaria, aurantio-brunnea, in KOH immersa colorem non mutant; papilla conspicua, 70–135 μ m alta; apex perithecii setosus, setae hyphales, 30–70 × 4–5 μ m, hyalinae, septatae, glabrae. Asci cylindracei, 100–140 × 7–8 μ m, ad apicem leniter incrassati. Ascosporae fusiformes, (14–)17–19(–30) × (4–)4.5–6(–7.5) μ m, bicellulares, septo mediano divisae, in asco aliquae disarticulatae, glabrae vel minute verruco-sae; apiculi nulli vel conspicui, 2(–4) μ m alti. Anamorphosis *Cladobotryum viridi-griseum* (G. Arnold et al.) K. Pôldmaa et Samuels. Conidia in natura ellipsoidea, cylindrica vel clavata, (15–)17.5–28.5(32.5) × 7–10(–11) μ m, 1–3-septata, dilute viridia. Chlamydosporae atrovirides, globosae, unicellulares, 12–18 μ m diam.



Figs. 18–23. – Hypomyces viridigriseus. – 18. Whole mount of a mature perithecium. – 19. Crushed perithecial apex showing hairs. – 20, 21. Asci. – 22. Ascospores disarticulating in an ascus. – 23. Conidiophore with denticulate conidiogenous cells. – All from TAA 169602. – Scale bars: Fig. 18 = 50 μ m; 19, 20, 23 = 25 μ m; 21 = 20 μ m; 22 = 10 μ m.

Holotypus. – Ad carposomata *Phellini laevigati* (Fr.) Bourdot et Galzin, K. Põldmaa (TAA 169602).

Anamorph. – *Cladobotryum viridigriseum* (G. Arnold, Illman & G. P. White) K. Põldmaa et Samuels, comb. nov. – Figs. 23, 26–30.

B a s i o n y m. – *Sympodiophora viridigrisea* G. Arnold, Illman & G. P. White, Mycotaxon 32: 371. 1988.

Subiculum effused over hymenophore of the host, at first white, turning green due to the production of conidia and afterwards



Figs. 24–30. Hypomyces viridigriseus. – 24. Ascospores, showing two that have disarticulated at the septum. – 25. Hairs found around the perithecial apex. – 26–28. Conidia. – 29. Conidiophore with geniculate-denticulate conidiogenous rachis. – 30. Chlamydospore. – Figs. 24–26, 28–30 from TAA 169602; 27 from Samuels 96-271; 26 from MEA; 27–30 from CMD. – Scale bars = 10 µm.

ochraceous at places were perithecia are formed, thin and easily removed from the substratum; subicular hyphae hyaline, smooth-walled, $3.5-5 \mu m$ wide, sometimes becoming swollen, then up to 25 μm diam, KOH-. - Perithecia obpyriform, 250-400(-500) µm high, 165-300(-400) µm wide, semi-immersed, becoming almost superficial in the subiculum, solitary or formed in scattered small groups, orange-brown, KOH⁻; papilla well developed, 70–135 µm high, usually covered with hyphal hairs, $30-70 \times 4-5 \mu m$, hyaline, septate, smoothwalled, with obtuse tips, some cells becoming swollen (up to 15 µm diam.). - Perithecial wall ca. 20 µm thick, pseudoparenchymatous in surface view, with cells 6-15 µm diam. - Papilla formed of outwardly diverging files of cells; the terminal cells clavate, ca. 10 µm diam. – Asci cylindrical, 100-140×7-8 µm, apex slightly thickened; ascospores uniseriate with ends overlapping. -Ascospores fusiform, $(14-)17-19(-30) \times (4-)4.5-6(-7.5) \mu m$, equally two-celled, some disarticulating at the septum while still in the asci, smooth to finely verrucose; nonapiculate or with blunt to acute apiculi up to 2(-4) µm high.

Characteristics of the associated anamorph. – Anamorph abundant on natural substratum. – Conidiophores hyaline, indefinite in length, to 7 μ m wide, irregularly branched, terminal branches by 1–3, septate, 30–260×3.5–5 μ m, producing up to 24 geniculate-denticulate conidiogenous loci over the terminal 1/3 or the whole branch; denticles 2–12×1.5–2.5 μ m. – Conidia mostly ellipsoidal, some cylindrical or clavate, (15–)17.5–28.5(32.5)×7–10(–11) μ m; 1–3-septate, pale green, with a distinct, protuberant hilum at the base. – Chlamydospores dark green, formed in irregular clusters, cells 12–18 μ m diam.

Characteristics in culture. – Colonies on CMD growing very slowly, attaining 12 μ m diam in 7 d, velvety, turning from white to yellow and finally green while conidia are being produced, reverse turning from deep green through yellowish brown to dark brown, sometimes brown pigment diffusing into the agar. – Conidiation abundant, odour absent. – Most of the mycelium submerged, orange-brown, cells often becoming moniliform, up to 18 μ m diam. – Conidiophores arising mostly from submerged hyphae, ascending to suberect, simple or irregularly branched, indefinite in length, 5–7 μ m wide, frequently septate, the upper third or the entire length a geniculate-denticulate rachis, with up to 20 conidiogenous loci, forming one to six conidia from each. – Conidia ellipsoidal to cylindrical, straight or sometimes bent at the tip, (10–)18–34(–47) × (5–)7–9(–11) μ m, (0–)13(4)-septate, green, with a protuberant basal hilum; held singly or in short imbricate chains, which appear as ra-

diating heads. – Chlamydospores forming terminally or laterally on short hyphal branches, composed of 2 to 6 subglobose cells, $14-18 \mu m$ diam, orange-brown.

Holotype. – UNITED STATES. ILLINOIS: Ogle Co., White Pines Forest State Park, Sleepy Hollow, on *Phellinus laevigatus*, 28 Sept. 1996, K. Põldmaa (TAA 169602, Isotype BPI; cultures: CBS 497.97, Samuels 96-234, TAA 96-86).

E tymology. – Refers to the previously described anamorph and to the colour of the conidia.

Additional anamorphic specimens examined in culture. – CANADA. ONTARIO: Temiskaming District, Tarzwell, on *Phellinus punctatus* (Fr.) Pilát, 30 Aug. 1979, G. P. White (DAOM 113666, CBS 630.88, ex type isolate of *Sympodiophora viridigrisea*). UNITED STATES. NEW YORK: Adirondack region, Raquette Lake, Long Point, on *Polyporus varius* Fr., 10 Sept. 1994, collector unknown, comm et det. K. T. Hodge (culture CBS 435.97, Samuels 96–271). ILLI-NOIS: Ogle Co., White Pines Forest State Park, Squirrel Trail, on *Hymenochaete rubiginosa* (Fr.) Lév., 28 Sept. 1996, K. Põldmaa (TAA 169614, cultures: CBS 436.97, Samuels 96–270, TAA 96–87).

Hypomyces viridigriseus is characterized by the formation of perithecia that are nearly superficial on the subiculum, and by the formation of hyphal hairs around the perithecial apex (Fig. 19). The contents of most of the perithecia in the type material were not ripe when collected and ascospores were found only in few asci. Most of the ascospores where not typical of *Hypomyces*, being smooth-walled, nonapiculate and tending to disarticulate while still in the asci (Figs 22, 24). Ascospore formation was not induced by incubating the material in a moist chamber for a week.

The most conspicuous and characteristic feature of the species is the formation of green conidia, which, although found in *Cladobotryum virescens* G. Arnold (Arnold, 1987), has not previously been reported for the proved anamorphs of *Hypomyces*. Most of the characteristics of the anamorph coincide with those given in the original description of *Sympodiophora viridigrisea* (Arnold & al., 1988). However, single ascospore, as well as conidial isolates grown on CMD and MEA produced also 5-celled conidia that were much longer than was given in the original description and that were sometimes bent at the top (Figs 26, 27).

The anamorph of *H. viridigriseus*, *C. viridigriseum*, was originally proposed in the genus *Sympodiophora* G. Arnold. *Sympodiophora* is characterized mainly by the sympodial proliferation of the conidiogenous cells. We follow Rogerson & Samuels (1993) in regarding *Sympodiophora* to be synonymous with *Cladobotryum* and have, accordingly, proposed the new combination.

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