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Two new hypocrealean ascomycetes on bryophytes from North America

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With 16 figures

Abstract: Two bryophilous species of the order Hypocreales (Ascomycetes) are introduced as new. *Bryonectria aphanes* (Bionectriaceae) has hyaline ascomata, four-spored asci, three-celled ascospores and numerous phialids and appressoria on the hyphae. It is recorded on the moss *Platygyrium repens* (Pylaisiadelphaceae, Hypnales) from Maine, U.S.A. *Trichosphaerella goniospora* (Niessliaceae) forms dark ascomata beset with setae deeply rooted in the peridial wall and eight two-celled ascospores. They disarticulate very early during sporogenesis resulting in 16 irregularly cubic part-spores. The species was collected on the liverwort *Ptilidium pulcherrimum* (Ptilidiaceae, Jungermanniales) in New Brunswick, Canada. The Niessliaceae belong to a hitherto unknown line within bryophilous fungi. On the other hand, bryophytes extend the spectrum of substrates for black hypocrealean fungi.

Key words: bryophilous ascomycetes, phialids on ascospores and hyphae, trigonal ascospores.

Introduction

Ascomycetes of the order Hypocreales (Nectriaceae, Bionectriaceae) belong to the most successful fungi colonizing liverworts and mosses. Actually, there are about 50 bryophilous species known in ten genera. They occur on a number of systematically, ecologically and geographically diverse bryophytes. However, North America with a rich bryophyte flora (Tan & Pócs 2000) and therefore a high number of potential hosts is almost *terra incognita* with many fewer records than for the small Costa Rica. The few Hypocreales reported from North America include *Bryonectria hylocomii* on *Hylocomium splendens* from Ontario (Döbbeler 1978) and *B. phyllogena* on *Polytrichum piliferum* and *P. juniperinum* from Prince Edward Island and California (Döbbeler & Hertel 2013). In the following, two hypocrealean, bryophilous ascomycetes collected in North America are described as new.

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Material and methods

Standard techniques were used to analyze dry herbarium material. All measurements apart from ascomatal size were made in lactophenol cotton-blue if not otherwise stated. Apical paraphyses and first stages of sporogenesis in *Trichosphaerella* are best observable in lactophenol cotton-blue with phase contrast optics.

Results

Bryonectria aphanes Döbbeler, P.G.Davison & W.R.Buck, **sp. nov.** (Bionectriaceae, Hypocreales) Figs 1–7

Mycobank no.: 810692

Etymology: *aphanes* (Gr.) = hidden, invisible; because of the hyaline ascomata difficult to detect.

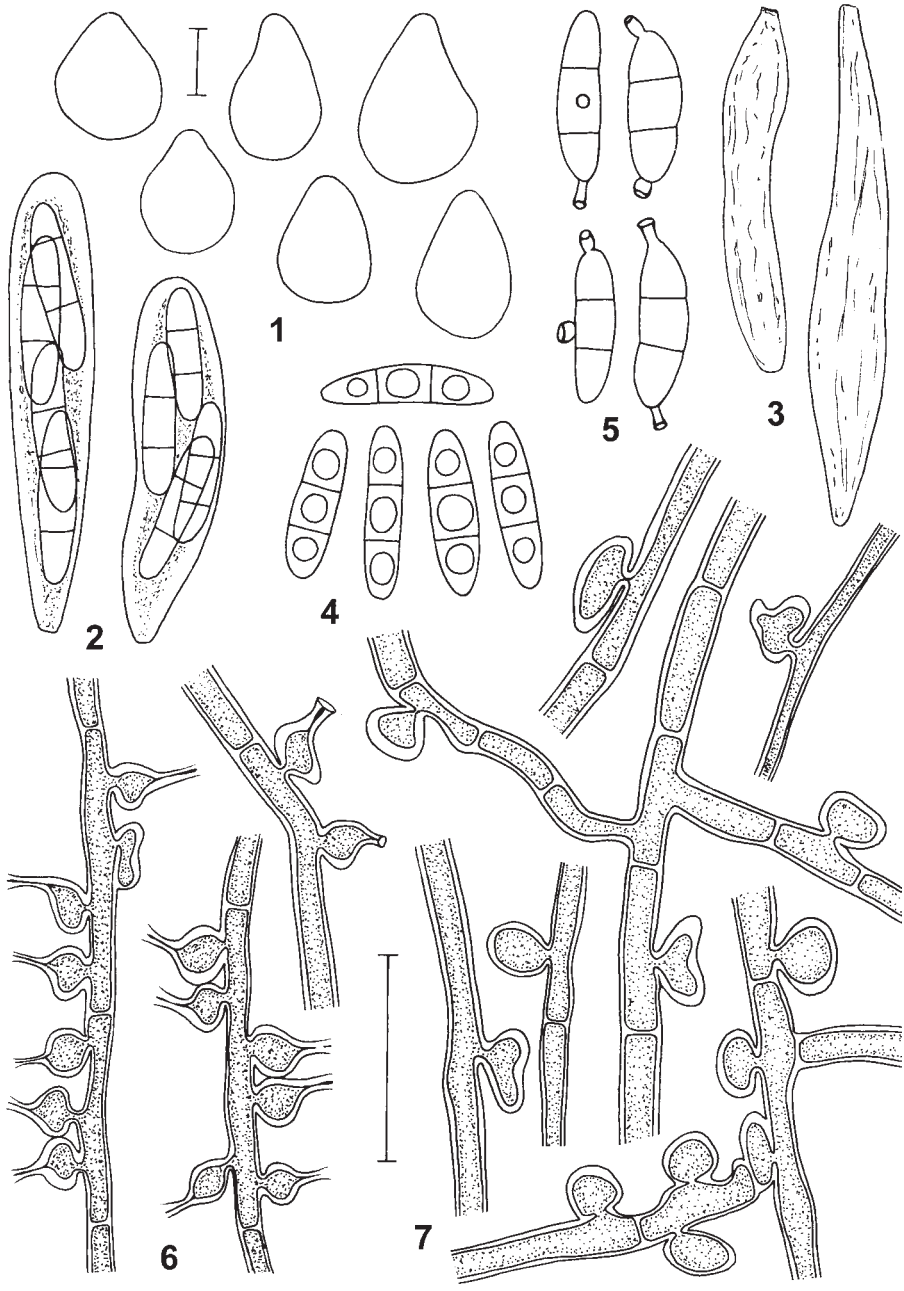
English diagnosis: The species differs from congeneric members by small, thin-walled ascomata to 130 µm long and 100 µm wide, asci with four 3-celled spores, numerous phialids and appressoria on the hyphae and the moss *Platygyrium repens* (Hypnales) as substrate.

Description: Ascomata perithecial, colorless to hyaline, ovoid to conical to almost pyriform, without setae, surface uneven, (90–)100–130(–150) × 70–100 µm, laterally attached to the substrate. Ostiolar canal lined with upwards oriented periphyses. Peridium seen from above with irregularly undulate, thick-walled cells (typical for the genus), cell walls cyanophilous; wall in section laterally 9–14 µm thick, asci visible through the peridial wall by transmitted light. Apical paraphyses not seen. Asci unitunicate, ± cylindrical to subclaviform, 33–46 × 8–10 µm, 4-spored; no degenerating ascospores seen; empty asci longer (to 55 µm) than the mature ones, with an aperture in the often constricted uppermost part. No reaction in iodine (Lugol). Ascospores narrowly ellipsoidal to cylindrical, straight or slightly bent, colorless, with 2 transverse septa, not constricted at the septa, (15–)16.5–19 × 4–5 µm, with 1 oil globule per cell, epispore cyanophilous; ascospores lying on the moss leaves germinating with an appressorium, several times ascospores with 1 or 2 phialids (neck 2–3 µm long) seen, phialids most often at one or both end cells. Hyphae colorless, irregularly growing over the host cells, ramified and anastomosing, with thick walls and often reduced lumina, 3–4 µm wide, sometimes (especially near ascomata) growing side by side forming strands, hyphae within hyphae present. Appressoria laterally formed, numerous, circular to elliptical or slightly undulate in outline, sessile, 5–8(–10) µm in diam. Phialids also lateral on the hyphae, sessile, basal part circular in outline, 5–6 µm in diam., with a filiform, 4 µm long and 2 µm wide, straight or bent neck; conidia very small, globose; surface of hyphae and appendages more or less cyanophilous.

Specimen examined: U.S.A. Maine, Washington County: Town of Steuben, Wilderness Shores, jct of Wilderness Shores Rd N and S, *Thuja* swamp, 44°26'27"N, 67°57'16"W, 17 m, 11 July 2013, Paul G.Davison 8253 (holotype NY, isotypes M, UNAF).

Host: *Platygyrium repens* (Brid.) Schimp. (Pylaisiadelphaceae, Hypnales).

Distribution: Known only from the type collection.



Figs 1–7: *Bryonectria aphanes* (type). 1. Perithecial ascomata in outline. 2. Four-spored asci. 3. Collapsed asci after spore discharge. 4. Ascospores. 5. Ascospores without cytoplasmic content having germinated with phialids. 6. Phialidic conidiogeneous cells on the hyphae. 7. Appressoria on the hyphae. Fig. 1 scale = 50 μm, Figs 2–7 scale = 20 μm.

REMARKS: Ascomata develop between the leaves and especially within the leaf axils. They are irregularly oriented with their apices pointing in different directions. They occur in the middle and upper plant parts and even shortly below the growing points. A 3.5 mm long lateral shoot may yield as many as ten ascomata. The species clearly belongs to the biotrophic parasites that have no visible influence on the hosts.

The fruit-bodies are so inconspicuous that even in heavily infected shoots only some of them are seen by stereomicroscopic magnification. Most of them remain completely invisible. The true number may be two or three times higher when the same shoot is studied by transmitting light in a compound microscope (100×).

Formation of a phialidic anamorph on hyphae is not a rare character within the genus *Bryonectria.* However, *B. aphanes* is the first example of phialids on hyphae and ascospores in a hypocrealean fungus on bryophytes. Phialids on hyphae and ascospores are also known in *Epibryon filiforme* and *E. hepaticola* (Döbbeler 1998).

The new species belongs to *Bryonectria* Döbbeler, a sharply defined genus that occupies an isolated position within the Bionectriaceae. Its ten species grow biotrophically on a variety of mosses and liverworts in many parts of the world. Important characters include small, globose to pyriform, colorless to hyaline perithecial ascomata, *textura irregularis* of the peridial walls, ellipsoidal, one- or two-septate, colorless ascospores, superficially growing mycelia composed of thick-walled hyphae with sessile appressoria and sometimes phialidic anamorphs, and no reactions with KOH or iodine.

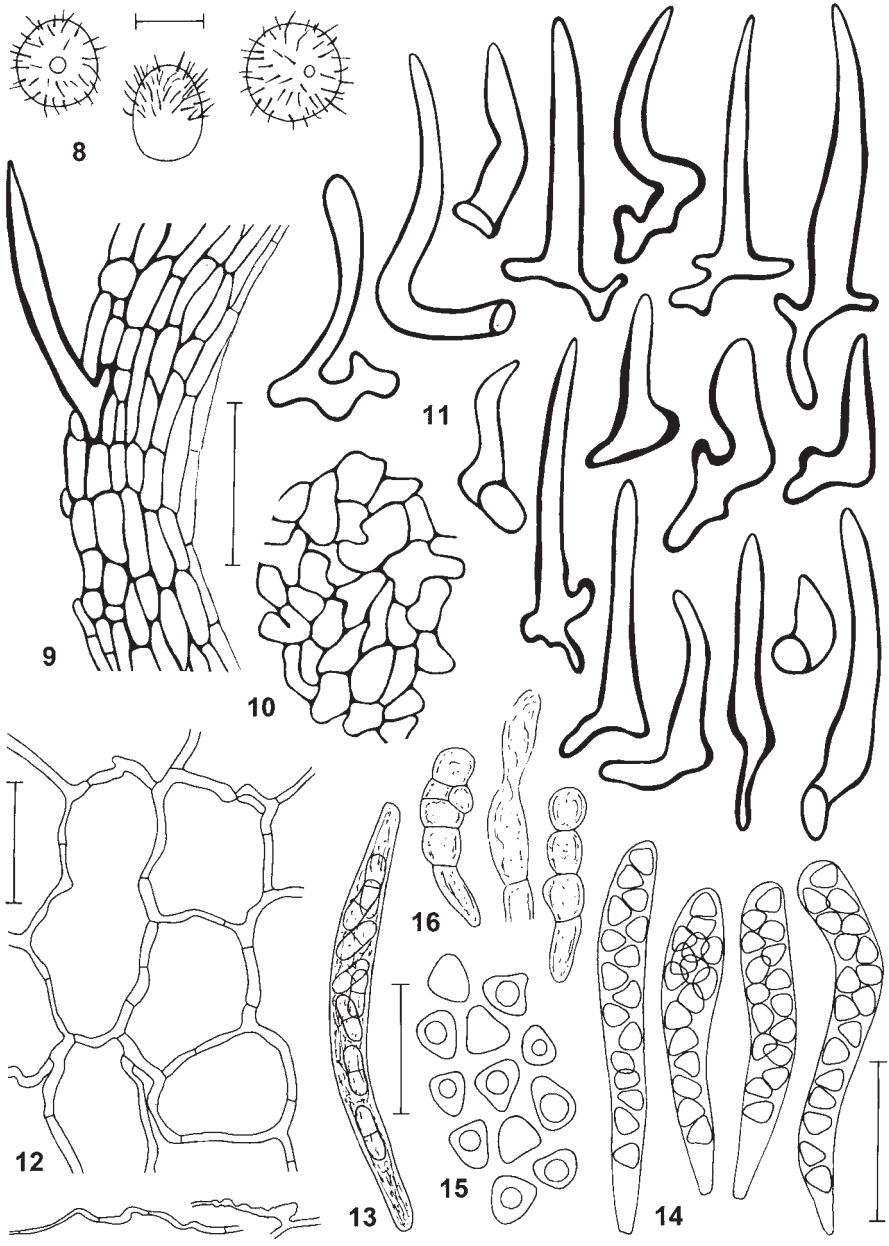
Bryonectria aphanes seems to be most closely related to three species that all have four-spored asci and three-celled spores, namely *B. biseptata* (Döbbeler) Döbbeler, *B. racomitrii* (Döbbeler & Hertel) Döbbeler, and *B. tricellularis* Döbbeler. *Bryonectria biseptata* on *Dawsonia* from Papua New Guinea has larger, almost globose ascomata (to 180 µm in diam.) with an uneven surface and very thick peridia (Döbbeler 1978). *Bryonectria racomitrii* on *Racomitrium lanuginosum* from Marion Island (Subantarctic) deviates by larger ascomata (135–190 × 85–140 µm), longer asci (42–68 µm) and appressoria (8–11 µm in diam.). Phialids, so conspicuous in *B. aphanes*, have not been documented in *B. racomitrii* (Döbbeler & Hertel 1984). *Bryonectria tricellularis* on *Frullania dilatata* from Romania has also larger ascomata (130–170 × 90–120 µm), asci (60–68 × 9–11 µm), and ascospores (18.5–23 × 4.5–5 µm) and especially hemispherical conidiomata with phialids at the inner side (Döbbeler 2005).

Trichosphaerella goniospora Döbbeler, W.R.Buck & P.G.Davison, **sp. nov.** (Niessliaceae, Hypocreales) Figs 8–16

MYCOBANK NO.: 810693

ETYMOLOGY: *gonio* (Gr.) = angle, corner; related to the spore shape.

ENGLISH DIAGNOSIS: The species differs from congeneric members by setose ascomata to 200 µm long and 180 µm wide, setae with an often forked or ramified foot rooted within the peridial wall, asci with 16 irregularly cube-like, in optical section triangular, part-spores that measure 4–4.5 µm in greatest diameter and occurrence on the liverwort *Ptilidium pulcherrimum* (Jungermanniales).



Figs 8–16: *Trichosphaerella goniospora* (type). 8. Setose ascomata in outline. 9. Longitudinal section through peridial wall with emerging seta. 10. Cells of peridium seen from above. 11. Setae. 12. Hyphal net imitating the host cell net and two undulate hyphal sections. 13. Immature ascus with eight two-celled spores. 14. Mature asci with disarticulated spores; the right ascus with 18 part-spores, the others with 16. 15. Ascospores in optical section. 16. Apical paraphyses. Fig. 8 scale = 80 μm, Figs 9–11, 14, 16 scale = 20 μm, Fig. 12 scale = 30 μm, Figs 13, 15 scale = 10 μm.

Description: Ascomata perithecial, dark brown to almost black, globose or ovoid to broadly ellipsoidal, setose, 110–200(–250) × 85–180 µm, not arising from a subiculum, apically or laterally collapsing when drying. Ostiole round, seen from above as a light spot, 15–18 µm in diam.; ostiolar canal outlined by upwards oriented, short, ca 1.5 µm wide periphyses. Setae dark brown, numerous, arising from the whole surface but mostly in the upper half of the ascomata, stiff, straight or less often bent, unbranched, without septa, thick-walled, from very short to as much as 50(–64) µm long (without foot), 3–5.5(–7) µm wide above the foot, basally often forked or with several short ramifications, deeply rooted within the peridial wall (best seen in sections); well-developed setae already present on very small ascomata (50 µm in diam.). Peridium soft-textured, seen from above with *textura epidermoidea*, cells often longer than wide; wall in section laterally 12–18 µm thick, apically becoming thicker, consisting of several (6–8) layers of flattened, 3–10(–15) µm long cells, outer part brown with relatively short cells, surface uneven by projecting cells or group of cells, towards the interior cells becoming longer, more flattened, thinner-walled, and paler. Apical paraphyses extremely delicate, evanescent, consisting of few ± inflated, to 8 µm wide cells forming short rows; inner peridium below the ostiolar canal to the middle of the ascomata beset with thin-walled, septate and ramified hyphae, upper hyphae horizontally oriented and merging into the periphyses, the lower ones oriented downwards to the hymenium. Asci unitunicate, cylindrical or slightly claviform, rarely in the middle or even lower part enlarged due to spore position, apically rounded, sessile or with a sporeless stalk, wall delicate, numerous, (35–)40–45(–55) × 6–9(–10) µm (in H_2O), arising from ramified ascogenous hyphae, without apical refractive ring or disc; even immature asci with 16 part-spores. Ascospores colorless, irregularly cube-like, in outline mostly triangular with rounded angles, part-spores (3.5–)4–4.5(–5) µm in greatest diam. (in H_2O), usually with 1 large oil drop, uni- or biseriate in the asci, in the latter case often alternating in form of a zigzag line; epispore smooth, not reacting with lactophenol cotton-blue. Hyphae light to dark brown, ramifying and forming anastomoses, closely attached to the host surface, sometimes with small undulate sections, 1–2.5(–3.5) µm wide, without any kind of appendages, growing mostly in single strands over the anticlinal host cell walls mirroring the cell areolation, rarely (in old tissue) within the cell walls. No color change or dissolution of peridial wall pigments in KOH; no reaction in iodine (neither Lugol's solution nor Melzer's reagent) of asci, their apices or spores observed. No anamorph detected.

Specimen examined: Canada. New Brunswick, Albert County: Fundy National Park, Dickson Falls Trail, 45°35'12"N, 64°58'20"W, ca 85 m, mixed forest of *Picea rubens*, *Betula* spp. and *Acer rubrum* along rocky brook ravine, 23 September 2013, William R.Buck 61461 (holotype NY, isotype M).

Host: *Ptilidium pulcherrimum* (F.Weber) Hampe (Ptilidiaceae, Jungermanniales).

Distribution: Known only from the type collection.

Remarks: The conspicuous ascomata are formed singly or gregariously on or between the host leaves, occasionally on the outer or even inner side of perianths or perforating leaves. The heavily infected but small host mat is homogeneously light brown-colored, some parts are decaying. It is unclear whether the infection alters the color of the host. The general aspect of the infection, the position of the fruit-bodies and hyphal features do not indicate an incidental occurrence of an otherwise non-bryophilous fungus.

During sporogenesis eight almost rectangular areas measuring 4–4.5 × 2–2.5 µm with a slightly attenuated middle part are cut off the ascus plasma. These very young spores already have a transverse wall, i.e., they are two-celled. One-celled stages have not been observed. Shortly afterwards both cells most probably separate at the septum forming 16 part-spores. They apparently slightly alter their position within the asci hiding their formation by disarticulation.

The alternate position of biseriate-lying ascospores may be advantageous in narrow asci with limited space. A zig-zag line-like position of the spores results. This was already demonstrated for *T. decipiens* by Samuels & Barr (1997, Fig. 31). The spore position in the upper half of the central ascus illustrated is similar to that seen in many asci of *T. goniospora*.

Triangular spores are so far not known in bryophilous fungi but occur sporadically in unrelated ascomycetes. The coprophilous *Chaetomium trigonosporum* (Chaetomiales) for example has eight triangular spores in frontal view (Doveri 2007) and the bark- and wood-inhabiting *Deltopyxis triangulispora* is a polysporous discomycete of unknown systematic position (Baral & Marson 2012) whose spores are similar to those of *T. goniospora*.

Setose perithecial ascomata are not rare in bryophilous fungi and typical, e.g., for the large genus *Epibryon*. The setae arise from the outermost peridial cells and do not have ramifications at the base. Setae with an attenuated base deeply rooted within the peridial wall as in *T. goniospora* are a new character amongst bryophilous fungi. However, they are already reported in the niesslialean monotypic genus *Cryptoniesslia* (Scheuer 1993).

Trichosphaerella goniospora is characterized by small, dark, setose, perithecial ascomata, periphysate ostioles, a soft-textured peridium, unitunicate asci with 16 colorless ascospores (apparently formed by disarticulation), presence of apical paraphyses and no iodine reaction. These characters clearly indicate that the species belongs to the Niessliaceae (Hypocreales), a relatively small family with 12 genera and 54 species (Kirk et al. 2008). Anamorphs produce conidia by phialids. All available characters, especially the stiff, unbranched and tapered setae and the disarticulating ascospores are consistent with the genus *Trichosphaerella* E.Bommer, M.Rousseau & Sacc. typified by *T. decipiens* E.Bommer, M.Rousseau & Sacc. (Samuels & Barr 1997, Rossman et al. 1999). *Trichosphaerella goniospora* deviates from congeneric species especially by angular ascospores and a liverwort as substrate.

With the hepatic genus *Ptilidium*, the Niessliaceae considerably extend their spectrum of substrates. Black hypocrealean fungi were hitherto only known as saprotrophs on herbaceous or woody plants, on fungi (Samuels 1983) and as parasites on lichens (e.g., Tretiach 2002). Actually, twelve ascomycetes are recorded to occur on the widespread and frequent *Ptilidium pulcherrimum* and the closely related *P. ciliare*. *Epibryon polysporum* on *P. ciliare* has also 16-spored asci but is quite different in almost all characters (Döbbeler 1978). Many European *Ptilidium* specimens have been screened during several decades without detecting *Trichosphaerella* or any other hypocrealean fungi.

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