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Trichomycete insect symbionts in Great Smoky Mountains National Park and vicinity

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Abstract: Collections of trichomycete symbionts of larval aquatic insects in Great Smoky Mountains National Park and vicinity in the southern Appalachian region of the USA resulted in finding many taxa of Harpellales, including an unusual new monotypic genus, *Barbatospora ambicaudata* in Simuliidae, and five new species in Thaumaleidae or Chironomidae, *Harpellomyces montanus, Smittium lentaquaticum, Sm. minutisporum, Stachylina gravicaudata* and *St. stenospora.* In addition a new species of *Amoebidium* (Amoebidiales), *A. appalachense,* attached to the anal tubules of bloodworms (Chironomidae) is described. Axenic cultures of three of the new taxa were obtained, plus *Sm. culisetae.* Fourteen identified species representing 13 genera of previously known Harpellales are recorded from Plecoptera, Ephemeroptera and Diptera, as well as a new Dipteran host record for an unidentified harpellid that was found in a Blephariceridae. Also identified were *Paramoebidium corpulentum* and many undetermined species of *Paramoebidium* (Amoebidiales) from four orders of aquatic insect larvae. The occurrence of an *Enterobryus* species in Diplopoda and another Eccrinales from an aquatic beetle is noted.

Key words: Amoebidiales, Diptera, Ephemeroptera, gut fungi, Harpellales, Plecoptera, southern Appalachians, Trichoptera

INTRODUCTION

Collections of trichomycetes in winter 1989 and spring and summer 2004 in Great Smoky Mountains National Park and nearby areas in the southern Appalachians in eastern USA resulted in the recovery of many taxa of Harpellales. Harpellales are true fungi with affinities to the Kickxellales (Zygomycota) (O'Donnell et al 1998, Gottlieb and Lichtwardt 2001, White 2002, Cafaro 2005). These gut fungi live obligately in the hindgut or midgut of freshwater insect larvae or more rarely in Isopoda (White 1999). Four new species and a new monotypic genus are reported as well as a new species of an ectozoic arthropod symbiont in the order Amoebidiales. Amoebidiales and Eccrinales, traditionally classified in the Trichomycetes, now are known through molecular studies to be protozoans related to the Mesomycetozoa (Benny and O'Donnell 2000, Ustinova et al 2000, Mendoza et al 2002, Adl et al 2005, Cafaro 2005). Species of the second genus of Amoebidiales, *Paramoebidium,* commonly are encountered in the same hindgut with various true gut fungi (Harpellales). Hosts of *Paramoebidium* spp. include stonefly nymphs (Plecoptera), mayfly nymphs (Ephemeroptera), black fly larvae (Simuliidae, Diptera) and larval caddisflies (Trichoptera), all of which are also hosts of Harpellales. In this paper we use trichomycetes (lowercase ''t'') for arthropod gut organisms, whether or not they belong to the fungal class Trichomycetes, much as the word ''fungi'' has been used for some fungus-like organisms not in that kingdom.

This survey is part of a larger, ongoing effort to inventory the gut fungi in North America. The scope of the current project permitted short-term, intensive sampling of nonpredacious, immature insect stages in one geographic area. Great Smoky Mountains National Park area was selected because of its known diversity of potential hosts. In addition other scientists have been exploring taxonomic diversity in the park in an attempt to inventory the diversity of all taxa (Sharkey 2001). This paper and our findings will be the first survey of gut fungi added to those efforts as a contribution to the Great Smoky Mountains National Park's All Taxa Biodiversity Inventory (ATBI).

MATERIALS AND METHODS

Trichomycete hosts were collected primarily in streams and rivers, occasionally in rock pools above river levels, or ponds, while others were from cliffs with small cascades or waterfalls or other rock faces wetted by water seeps (TABLE I). Depending upon the type of insect and habitat, insects were obtained with aquatic D-nets, strainers, basters, or taken directly from the substrates. Usually they were

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TABLE I. Collection numbers for sites with trichomycetes within and bordering the Great Smoky Mountains National Park[a]

Coll.	Date	Site description	Latitude/Longitude
6	20-XII-89	Walker Camp Prong on Newfound Gap Rd. 2.5 km NW of Newfound Gap	35°37.1′N, 83°25.7′W
7	20-XII-89	Walker Camp Prong on Newfound Gap 5.3 km NW of Newfound Gap	35°37.7′N, 83°27.0′W
8	20-XII-89	Laurel Creek on Laurel Creek Rd. near Schoolhouse Gap Trail	35°37.7′N, 83°43.5′W
9	20-XII-89	West Prong Little Pigeon River at US 441 bridge near Sugarlands Visitor Center	35°41.3′N, 83°32.1′W
10	20-XII-89	West Prong Little Pigeon River crossing Quiet Walkway Trail, 1.6 km SE of Sugarlands Visitor Center	35°40.4′N, 83°31.6′W
13	7-III-04	Two confluent streams at junction of Little Cove, Mill Creek roads.	35°45.91′N, 83°34.18′W
14	7-III-04	Flat Branch of West Prong Little Pigeon River, off US 441 north of Gatlinburg	35°46.07′N, 83°31.80′W
15	7-III-04	Cliff seep on US 321, about 9.5 km NNW of Townsend	35°42.34′N, 83°49.30′W
17	21-VII-04	Rhododendron Creek, a tributary of Little Pigeon River	35°43.20′N, 83°23.86′W
18	22-VII-04	Raven Fork, Big Cove Road side road	35°30.93′N, 83°17.87′W
19	22-VII-04	Raven Fork, upstream of Site 18	35°31.36′N, 83°17.09′W
20	22-VII-04	Oconaluftee River at junction of US 44 and Blue Ridge Parkway.	35°30.14′N, 83°18.25′W
21	22-VII-04	Soco River off US 19 on side road near Soco Road Campground	35°28.39′N, 83°13.09′W
22	22-VII-04	Cliff seep on US 441, 11.2 km south of Sugarlands Park Headquarters	35°38.46′N, 83°27.80′W
23	25-VII-04	Rocky Spur Branch near Grotto Falls Trail parking area	35°40.8′N, 83°27.9′W
24	25-VII-04	Roaring Fork Creek near Baskins Creek trailhead	35°41.06′N, 83°27.94′W
27	25-VII-04	Rock pools above Roaring Fork Creek, just upstream of bridge	35°42.55′N, 83°28.65′W
28	25-VII-04	Seepy cliff above rock pools at Site 27	35°42.55′N, 83°28.65′W
29	25-VII-04	Roaring Fork Creek near Site 27	35°42.55′N, 83°28.65′W
30	25-VII-04	Thousand Drips Falls on Cliff Branch Creek	35°42.73′N, 83°29.03′W
31	26-VII-04	Palmer Creek at Palmer Creek trailhead	35°37.59′N, 83°06.71′W
32	26-VII-04	Mossy Branch of Cataloochee Creek	35°37.82′N, 83°06.72′W
34	26-VII-04	Cataloochee Creek at bridge on National Park Road	35°39.90′N, 83°04.46′W
35	26-VII-04	Small waterfall on side of National Park Road, above Cataloochee Creek	35°39.81′N, 83°04.12′W
38	30-VII-04	Tater Branch of Abrams Creek, at Cades Cove Loop Road	35°36.49′N, 83°49.78′W
40	30-VII-04	Whistling Branch of Abrams Creek on Cades Cove Loop Road	35°35.40′N, 83°49.90′W
41	30-VII-04	Cades Branch of Abrams Creek on Cades Cove Loop Road	35°35.24′N, 83°49.31′W
42	30-VII-04	Stream Perennial across Cades Cove Loop Road	35°35.25′N, 83°48.78′W
43	30-VII-04	Abrams Creek at Sparks Line	35°36.18′N, 83°47.68′W
45	1-VIII-04	Rhododendron Creek, a tributary of Little Pigeon River	35°43.20′N, 83°23.86′W
46	1-VIII-04	Rock pools on side of Little Pigeon River	35°44.1′N, 83°24.8′W
46a	1-VIII-04	Little Pigeon River	35°44.1′N, 83°24.8′W
47	1-VIII-04	Pond at entrance to Tudor Road, just north of US 321	35°44.4′N, 83°25.0′W
48	1-VIII-04	Small stream adjacent to Tudor Road opposite Site 47	35°44.4′N, 83°25.0′W
49	4-VIII-04	Small waterfall on side of National Park Road, above Cataloochee Creek	35°39.81′N, 83°04.12′W

[a] Sites 13–15, 21, 47 and 48 are near the park.

picked in the field and placed in collecting jars, but at many sites larger, supplemental samples of substrates with insects were collected in sealable plastic bags for sorting in the laboratory. Some simuliid (black fly) larvae were placed directly on moist filter paper in disposable, sterile Petri dishes for better survival. All specimens were kept in an ice cooler for transport to a laboratory and transferred to a refrigerator. Some mosquito larvae and bloodworms (Chironomidae) were kept in containers at room temperature, in part to promote their development, where desired.

Each collection at a location per date was given a reference number, which for simplicity we prefaced with a single geographic reference TN (a single state code, although our collections were in both Tennessee and North Carolina). Individual dissection codes include a second number indicating our site code (see TABLE I) and a third with a letter/number combination to correspond to the individual and their specific host dissection (sequentially numbered). Any number in the third position preceded by a letter was a dissection by White (W) or Siri (A) whereas those without a letter were by Lichtwardt.

Insects were dissected under stereo microscopes, and slides of gut symbionts were prepared as water-mount slides and selectively photographed with a phase contrast microscope. Semipermanent slides were prepared by infiltrating with lactophenol cotton-blue (hereafter LCB) and sealing the cover slip to the slide with clear fingernail polish. Some trichomycete specimens, freshly dissected from the digestive tract, were selected for culture attempts with a medium of a 1 : 1 mixture of dilute brain-heart infusion (1/10 BHI) and tryptone-glucose-salts (TG) with vitamins (named BHIGTv) overlayed with a penicillin-streptomycin antibiotic mixture (individual formulae are provided in Lichtwardt et al 2001a, The Trichomycetes, fungal associates of arthropods. Revised. www.nhm.ku.edu/~fungi). Other specimens were selected for preservation in 2× CTAB buffer for extraction of DNA and sequencing. Hosts were preserved in absolute ethanol and deposited with specialists for identification. Types are deposited at the Farlow Herbarium (FH).

NEW TAXA

Barbatospora M.M. White, Siri & Lichtw., gen. nov.

Thalli ramosi, trichosporas cylindricas collare carentes et ad basem appendiculis multiplicibus tenuibus praeditas evolventes; trichosporae in termino apicali interdum tubo vaginanti vel pariete praeditae, is per dehiscentiam interdum filamenta appendiculoidea aperiens.

Branched thalli producing cylindrical trichospores without a collar but with multiple fine appendages at their base; trichospores may bear a cylindrical sleeve or wall at their terminal end that on dehiscence may reveal appendage-like filaments.

Type species. Barbatospora ambicaudata.

Barbatospora ambicaudata M.M. White, Siri & Lichtw., sp. nov. FIGS. 1–15

Thallus cellula basali 8–12 μm diam praeditus, prope basem ramosus, haustorium cupuliforme ferens; ramuli trichosporas evolventes 1–4 cylindraceas 38–42 × 3–4 μm ad basem appendiculis non persistentibus tenuibus multiplicibus praeditas; trichosporae interdum in termino apicali filamentis appendiculoideis 6–13 × 2.5–4 μm primo per tubum vaginantem vel parietem velatis praeditae. Zygosporae ignotae. In proctodaeum larvarum Simuliidarum affixa.

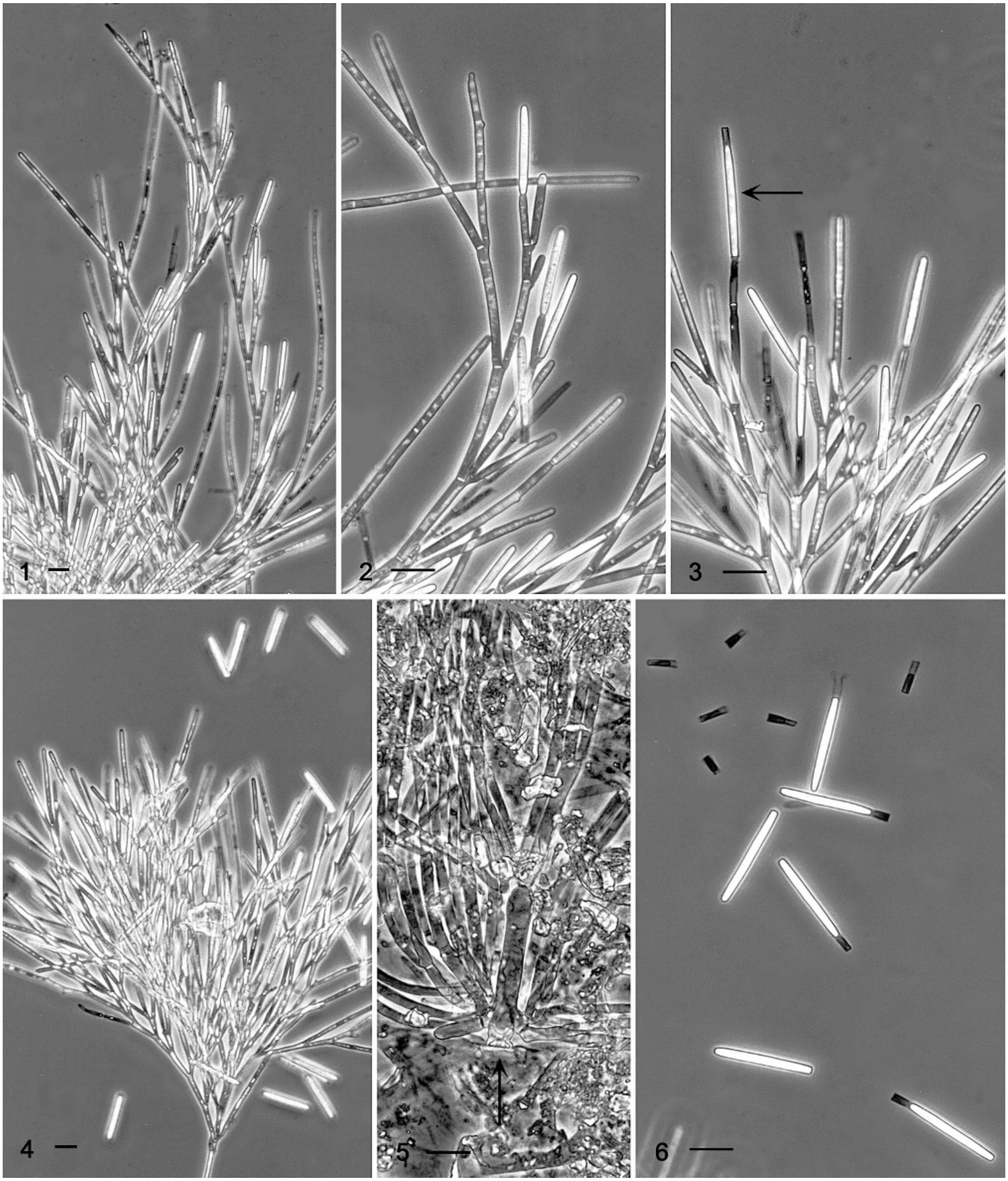
Thallus with a basal cell 8–12 μm diam, branched near the base, and bearing a cup-shaped holdfast. Branchlets producing 1–4 cylindrical trichospores 38–42 × 3–4 μm with nonpersistent fine multiple appendages at their base. Trichospores sometimes may have appendage-like filaments at their terminal end that initially are concealed by a cylindrical sleeve or wall 6–13 × 2.5–4 μm. Zygospores unknown. In hindgut of larval Simuliidae.

Etymology. L. barbatus = bearded, Gr. spora = spore, for the bearded appearance at the ends of the trichospores. L. ambi = both sides, L. cauda = appendage, for the appendage-like structures that develop at both ends of the trichospores.

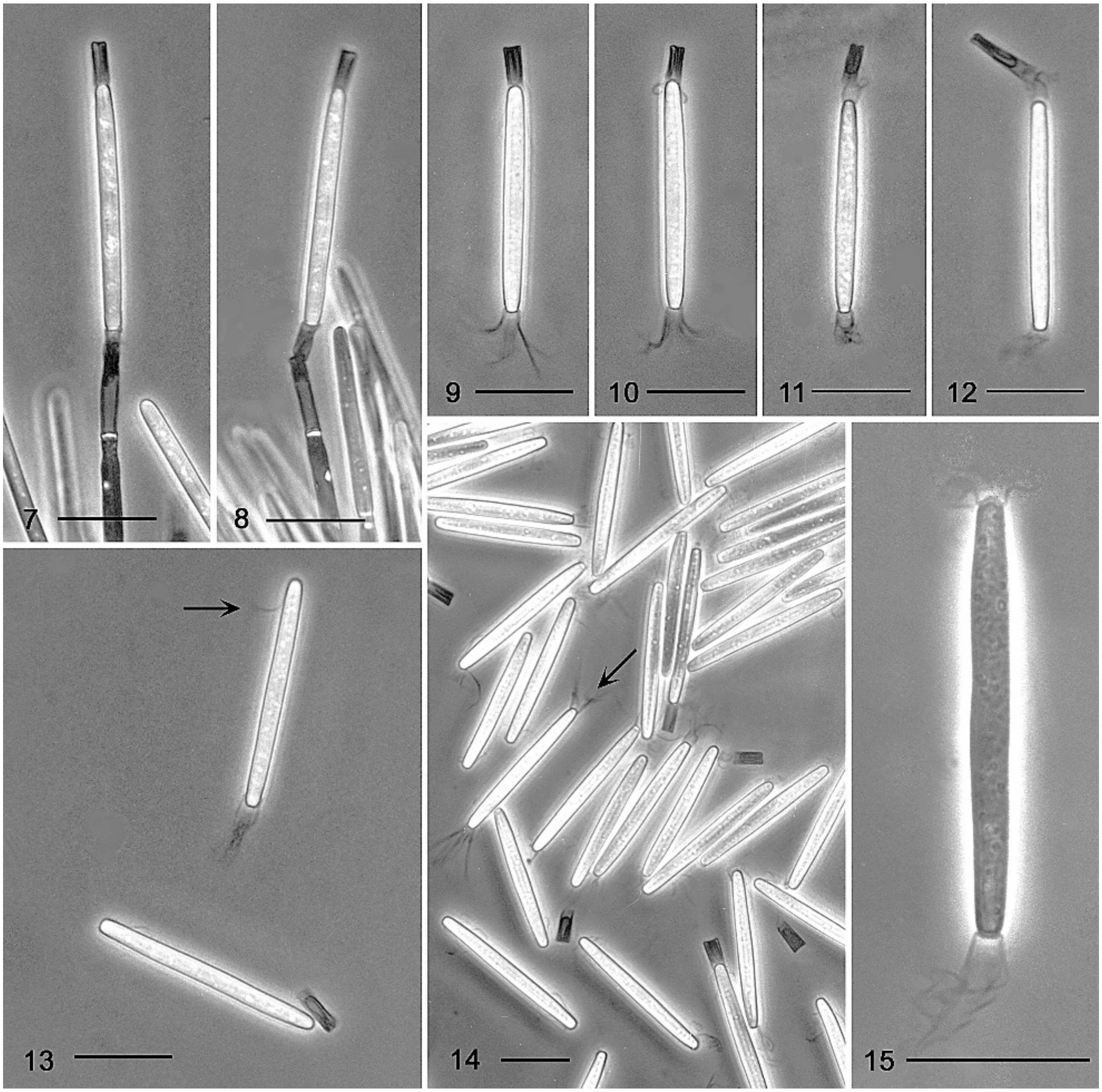
Specimens examined. USA. NORTH CAROLINA: Small, cascading waterfall on side of National Park Road above Cataloochee Creek, 35°39.81′N, 83°04.12′W, (Collection 49, TABLE I), 4-VIII-04. Microscope slide TN-49-2 (HOLOTYPE: FH) showing thallus and trichospores and microscope slide TN-49-W4A (ISOTYPE: FH) from a 5 d axenic culture both from the hindgut of *Simulium vandalicum* Dyar & Shannon larvae (Diptera, Simuliidae). Other specimens from the same site (Collection 35, TABLE I) sampled 26-VII-04.

Cultures. An axenic culture of *B. ambicaudata* was obtained, designated as TN-49-W4A. Deposited in the University of Kansas Mycological Culture Collection.

Trichospores of *B. ambicaudata* bear a superficial resemblance to those drawn by Manier for *Pennella grassei* (Manier 1963, FIG. IV), a species found in Simuliidae larvae in France. The morphology and ontogeny of trichospores in *B. ambicaudata* differ however as do the holdfast structures of the two genera. Stages of formation and release of trichospores from thalli remain somewhat enigmatic under the light microscope. Collarless trichospores are released from generative cells initially with multiple fine appendages at the basal end (FIGS. 7–12). The evacuated generative cell may persist, frequently with a refractive wall adjacent to the subtending cell. As appendages emerge from the generative cell they may appear more or less heavy or dense until they disentangle and unfurl. In other instances the basal appendages either do not always persist or are not resolvable under the light microscope (FIG. 14). The most perplexing aspect of this taxon is that trichospores may bear at their terminal end a cylindrical cap-like sleeve or wall, sometimes even while still attached to the generative cell (FIGS. 7, 8). Indeed we once observed this structure form when the sporangiospore, inside the trichospore wall, suddenly shrank away from the tip. The function of this terminal sleeve or wall is unknown, but it usually breaks off, leaving appendage-like filaments at the terminal end (FIGS. 10–15). The presence of appendage-like filaments at the terminal end of trichospores is unique, and how these develop is not well understood because in all other Harpellales true basal appendages form only within the generative cell, from which the trichospore grows and eventually detaches



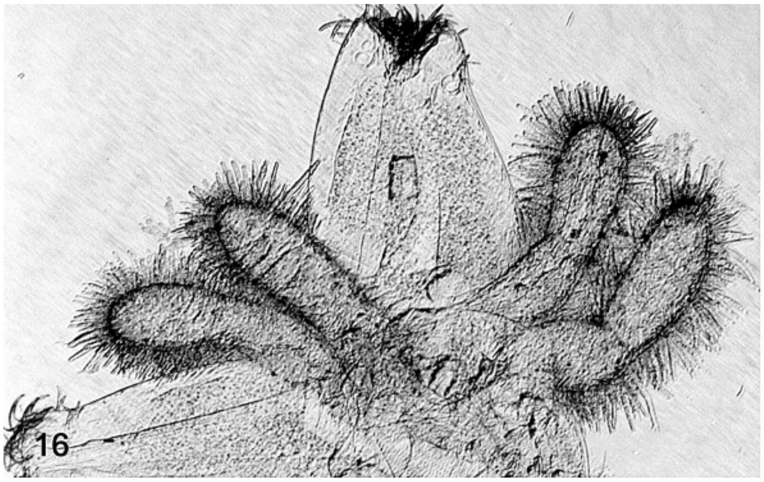
FIGS. 1–6. *Barbatospora ambicaudata*. 1, 2. Sporulating branchlets removed from a simuliid larva (microscope slide TN-35-5). 3. Sporulating branchlets from axenic culture TN-49-W4A. The trichospore on the left (arrow) is in the process of detaching and includes a cylindrical cap-like sleeve or wall at the terminal end. 4. A fan-shaped piece of thallus from same axenic culture, typical of fragments when a dense colony in culture is broken up. 5. Holdfast of a thallus (arrow) attached to the hindgut cuticle of a larva (slide TN-49-1). 6. Released trichospores in culture, both with and without cap-like terminal sleeves or walls (detached remnants of some are scattered in upper left). Bars = 20 μm.



FIGS. 7–15. *Barbatospora ambicaudata*, all from axenic culture TN-49-W4A. 7–8. Two releasing trichospores, one nearly broken away from its generative cell; the nonpersistent, terminal cap-like structure may not be present on all trichospores. 9–12. A reconstructed series of development in released trichospores showing the multiple appendages basally and appendage-like filaments distally, after dehiscence of the cap-like structure at the terminal end. 13. Two released trichospores, one with a barely visible appendage-like filament at the presumptive terminal end (arrow). 14. Mass of trichospores, one with obvious appendages and appendage-like filaments at opposite ends (arrow), while others show variably less dense appendages or filaments sometimes barely visible under the light microscope. Enlarged, stained trichospore with multiple appendage-like filaments (formed terminally) at top and multiple fine appendages (basally) at bottom. Bars = 20 μm.

on maturation. It is possible that only terminal trichospores on fertile branches are capable of forming apical filaments. After release it sometimes is difficult to determine which end of the trichospore is terminal, but where the wall-like cap or sleeve is observed we interpret this to be the distal end. It is evident that electron microscopy will be needed to determine the development of trichospores both before and after release.

Subcultures of *B. ambicaudata* grow rapidly and produce copious trichospores in 3–4 d at room temperature on BHIGTv agar medium with the usual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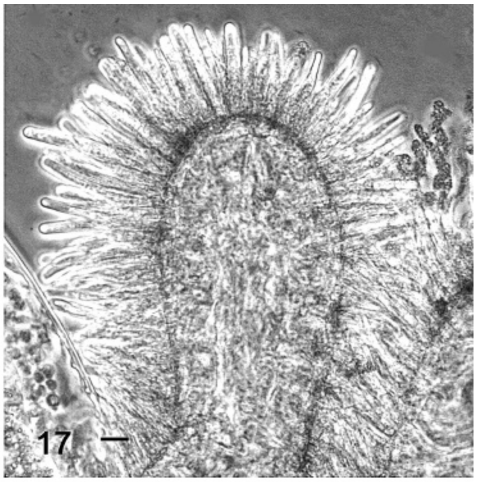
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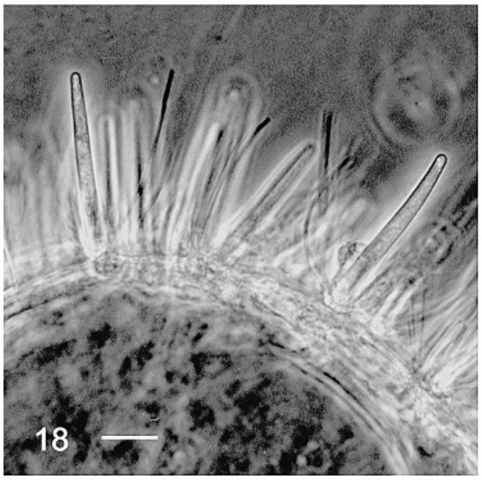
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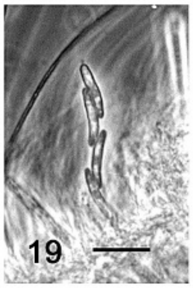
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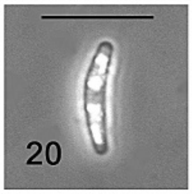
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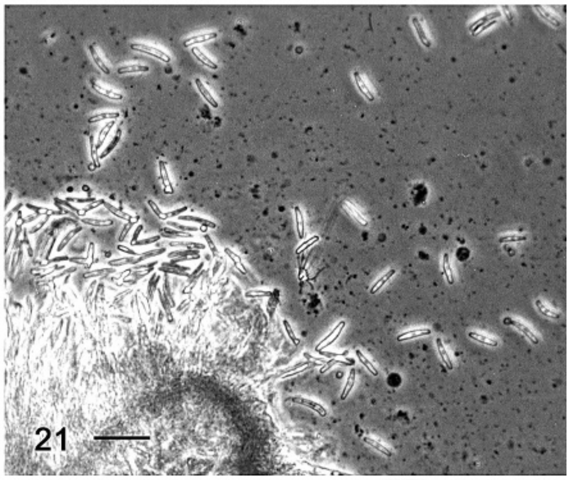
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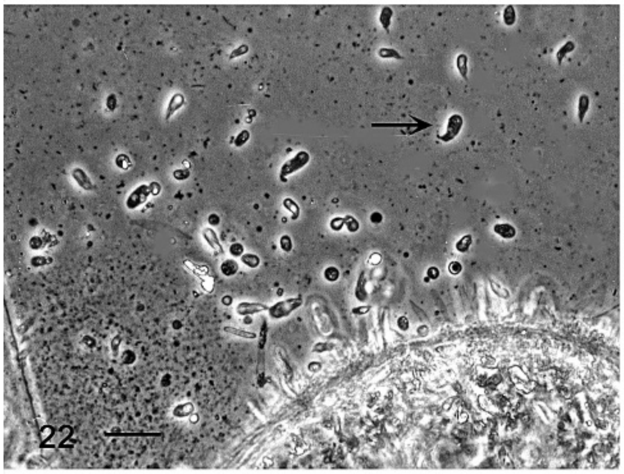
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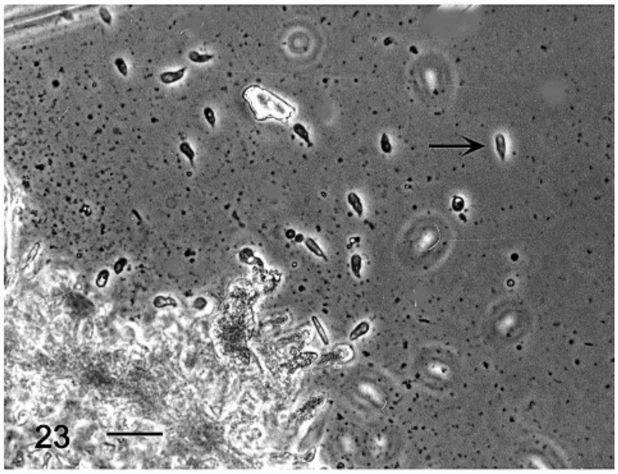
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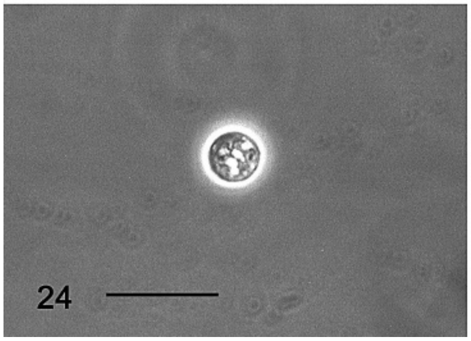
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water overlayer (FIG. 14). Colonies become globose, and when broken up—as is normally done when subculturing—each colony piece consistently has a fan-shaped pattern of branches that radiate from one or a few branches (FIG. 4). These in turn grow into globose colonies.

Amoebidium appalachense Siri, M.M. White & Lichtw., sp. nov. FIGS. 16–25

Thallus cylindricus haustorio brevi discoido praeditus, maturitate circa 55–80 × 4–6(–8) μm, aut sporangiosporas allantoideas (15–)21(–25) × (1.5–)2.8(–4) μm, interdum rectas vel sublunatas producens aut amoebas motiles dacryoideas 20 μm longas, 6–8 μm diam, prope terminum latiorem anteriorem earum cystis sphericis primo ca 6–8 μm diam praeditas. Ad tubulos anales Dipterorum et Chironomidarum affixum.

Thallus cylindrical with a short discoid holdfast, about 55–80 × 4–6(–8) μm at maturity, producing either (i) allantoid sporangiospores (15–)21(–25) × (1.5–)2.8(–4) μm, occasionally straight or almost lunate, or (ii) motile teardrop-shaped amoebae about 20 μm long × 6–8 μm diam near broader anterior end, forming spherical cysts initially about 6–8 μm diam. Attached to anal tubules of bloodworms (Diptera, Chironomidae).

Etymology. Of the Appalachian Mountains.

Specimens examined. USA. TENNESSEE: Rock pools along Little Pigeon River, 35°44.1′N, 83°24.8′W, (Collection 46, TABLE I), 1-VIII-04. Microscope slide TN-46-4 (HOLOTYPE: FH) consisting of anal tubules of a *Chironomus* sp. (Diptera, Chironomidae) larva with attached thalli of *Amoebidium appalachense* and released sporangiospores. Other specimens from same host in rock pools above Roaring Fork Creek, just upstream of bridge, 35°42.55′N, 83°28.65′W (Collection 27, TABLE I), 25-VII-04.

Cultures. Two axenic cultures of *A. appalachense* were obtained from *Chironomus* sp. larvae, labeled TN-27-A3 (from Collection 27) and TN-46-A6 (from Collection 46) (TABLE I). The isolates when grown on BHIGTv medium produce a variety of odd-shaped thalli (FIG. 25), unlike three cultures of *A. parasiticum* Cienkowski in the University of Kansas Culture Collection, A1a, FRA-1-14 and JAP-7-2 (Lichtwardt et al 2001a).

Amoebidium appalachense is similar to *A. colluviei* Lichtw. found attached to the anal tubules of *Chironomus* sp. and black fly larvae in a polluted mountain stream in Monteverde, Costa Rica (Lichtwardt 1997), described (p 1358) as ''Thalli usually less than 80 μm long by about 10 μm diameter, producing allantoid sporangiospores 25–32 × 6–10 μm.'' The main differences in the two species is that *A. appalachense* produces sporangiospores that are shorter and narrower, with essentially no overlap in size with *A. colluviei*, and the thalli are slightly narrower. It is not known whether *A. appalachense* is capable of attaching to the anal tubules of Simuliidae, as does *A. colluviei*, because both collections of *A. appalachense* came from rock pools that are not suitable habitats for black fly larvae.

Another species that attaches to the external surfaces of bloodworms is *A. australiense* Lichtw. & M.C. Williams from Western Australia (Lichtwardt and Williams 1992). However its thalli are much larger (>300 μm long), and produce a much larger holdfast (7–13 × 20 μm). The worldwide species *A. parasiticum* Cienkowski that occurs externally on several kinds of small aquatic crustaceans and some insect larvae such as mosquitoes (Lichtwardt et al 2001a) occasionally has been found on bloodworms. For instance in Japan *A. parasiticum* was found on *Chironomus* sp. primarily on the anal tubules but also attached to other body parts, and a culture was obtained (JAP-7-2) (ATCC 32709) (Lichtwardt et al 1987). The fourth species of *Amoebidium* (*A. recticola*) is morphologically distinct from the other species and was reported by Chatton (1906) from the rectal lining of *Daphnia* spp.

Whether thalli of *A. appalachense* produce sporangiospores or release amoebae depends on the stage of thallial development and other factors. It is known that release of amoebae in *A. parasiticum*, instead of the usual sporangiospores, normally occurs only when a host molts or is injured, such as by dissection (Lichtwardt 1986). We assume that the same conditions pertain to *A. appalachense.* In some cases when anal tubules of bloodworms were cut off and mounted in water on slides, the thalli released large numbers of sporangiospores within 30 min to 2 h (FIG. 21). In

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FIGS. 16–25. *Amoebidium appalachense.* 16. Four anal tubules of a bloodworm with attached thalli (slide TN-46-4). 17. Anal tubule showing density of attached thalli (slide TN-46-4). 18. Enlarged optical section of an anal tubule with nonsporulating thalli; the fine, dark filaments are a type of common prokaryote on bloodworm tubules (slide TN-27-22). 19. Thallus producing sporangiospores (slide TN-27-18). 20. Allantoid sporangiospore (slide TN-27-18). 21. Mass of sporangiospores being released almost simultaneously from their thalli in a slide culture (slide TN-46-2). 22, 23. Release of creeping amoeboid cells (arrows) from thalli in a slide culture (slide TN-46-2). 24. A newly encysted amoeba (slide TN-27-A8). 25. Axenic culture TN-27-W3 showing variation in cells at different developmental stages. Bars = 20 μm.

several other instances amoebae were released (FIGS. 22, 23) within 30 min to 2 h, migrated 30–60 min, then encysted (FIG. 24). The release of amoebae occurred nearly simultaneously in almost all thalli, with only a few thalli releasing sporangiospores. Cysts kept in water mounts did not enlarge significantly, or produce cystospores, as has been seen in species of *Amoebidium* and *Paramoebidium*. *Paramoebidium* spp. have the same amoeba-cyst-cystospore cycle as *Amoebidium* spp. but do not produce sporangiospores (see FIGS. 20–22 in Lichtwardt and Arenas 1996).

Harpellomyces montanus M.M. White, Siri & Lichtw., sp. nov. FIGS. 26–31

Thalli prope basem ramosus, trichosporis ellipsoidalibus 17–21 × 5.5–7 µm in seriebus longis praeditus, eae plerumque sub emissione appendiculis duobus praeditae. Zygosporae biconicae, 35 × 9.5 µm, ad zygosporophorum 20 × 9.5 µm oblique et sub medio affixiae. In proctodaeum larvarum Thaumaleidarum affixum.

Thalli branched near the base, producing long series of ellipsoidal trichospores 17–21 × 5.5–7 µm, usually bearing two appendages on release. Zygospore biconical, 35 × 9.5 µm, attached obliquely and submedially to a zygosporophore 20 × 9.5 µm, both remaining together on release. In hindgut of Thaumaleidae larvae.

Etymology. L. montanum = of mountains.

Specimens examined. USA. TENNESSEE: Cliff seep on US 441, 11.2 km south of Sugarlands Park Headquarters, 35°38.46′N, 83°27.80′W (Collection 22, TABLE I), 22-VII-04. Microscope slide TN-22-W7 (HOLOTYPE: FH) includes thalli with attached trichospores, microscope slide TN-22-W4A (ISOTYPE: FH) with detached spores, both from larvae of *Androprosopa thornburghae* (Vaillant) (Diptera, Thaumaleidae). Other specimens of *H. montanus* from the same species of Thaumaleidae larvae at a small waterfall on side of National Park Road, above Cataloochee Creek, 35°39.81′N, 83°04.12′W (Collection 35, TABLE I), 26-VII-04.

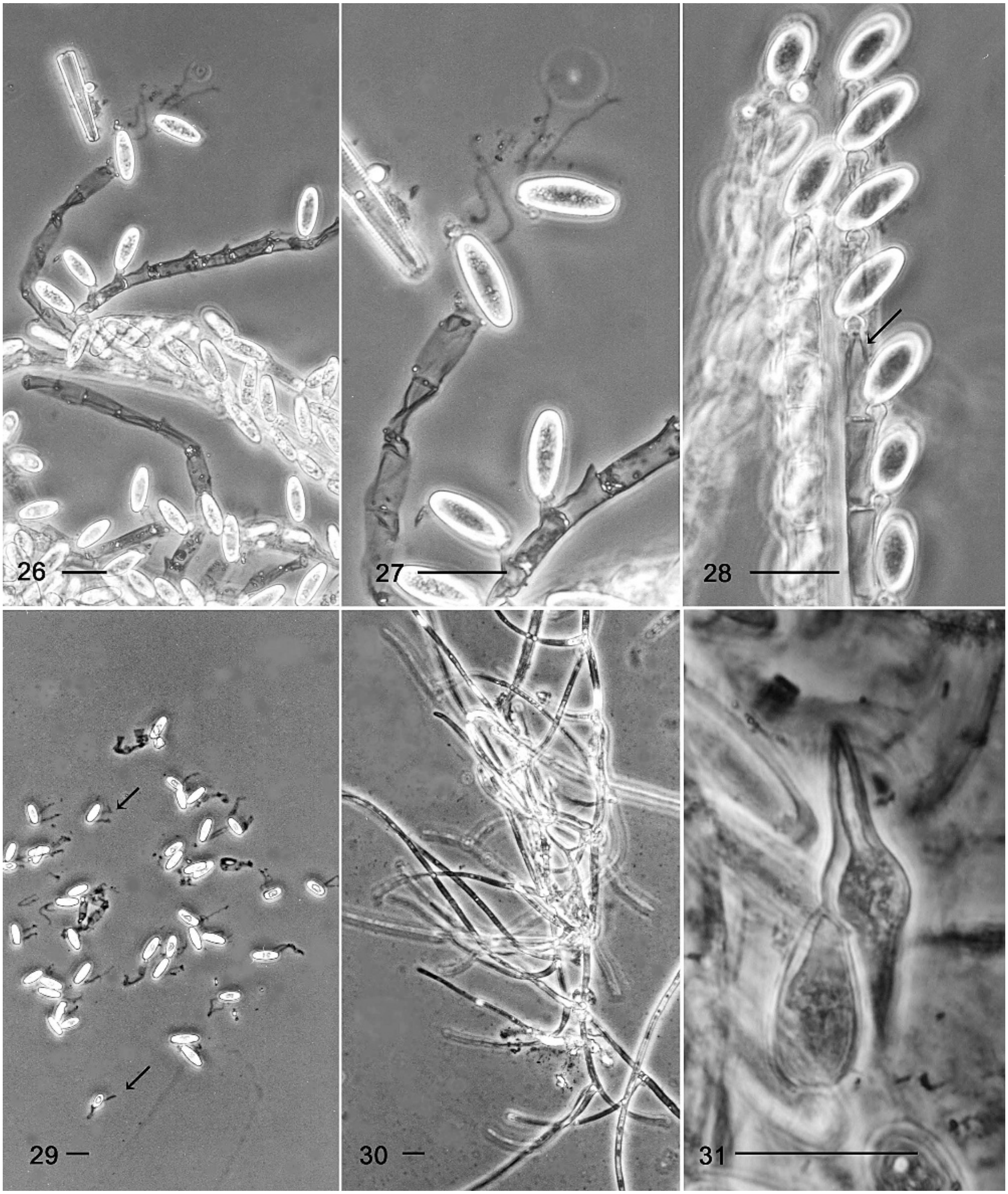
Harpellomyces montanus adds a third species to the genus. The type species, *H. eccentricus* Lichtw. & S.T. Moss has been collected in Sweden, Wales, Norway and Spain in larvae of *Thaumalea* spp. (Lichtwardt and Moss 1984, Valle 2004, White and Lichtwardt 2004). The thalli of the type species are unbranched and produce trichospores slightly larger (20–25 × 6–8 µm) than those of *H. montanus.* Whereas the trichospores of the type usually have three appendages, those of *H. montanus* usually have two. *Harpellomyces eccentricus* is the only other species where zygospores have been found. Only one detached zygospore (FIG. 31) was found for *H. montanus* during review of a LCB slide (TN-22-W8) of remnants kept after transferring the bulk of the fungal thalli to DNA extraction buffer. The zygospore appears to be mature and therefore representative, but it is not possible to list a range for zygospore dimensions at this time. Nonetheless dimensions of both the zygospore (35 × 9.5 µm) and the zygosporophore (20 × 9.5 µm) present a sexual spore for *H. montanus* that is shorter and narrower than in *H. eccentricus*, for both aspects. The third species, *H. abruptus* Lichtw., White & Colbo, described from Newfoundland, Canada, in larvae of *Thaumalea verralli* Edwards (Lichtwardt et al 2001b), has limited branching near the thallial base and produces trichospores with 2–5 appendages that are significantly larger (20–33 × 7–11 µm) than those of *H. montanus.* In *H. montanus* many thalli branched abundantly near the base (FIG. 30) and in our specimens trichospores bore two appendages (FIGS. 26, 27, 29), occasionally noted in the generative cell before detachment (FIG. 28). Two other discoveries of undetermined species of *Harpellomyces* in *Thaumalea* larvae include one in Pennsylvania, USA (Ferrington unpublished), and the other in Japan (Lichtwardt et al 1987).

Not shown for *H. montanus* are images of immature, presumably precocious (nonsporulating) thalli observed on several peritrophic membrane linings of the midgut similar to thalli that were reported earlier in hosts of *H. eccentricus* (Lichtwardt and Moss 1984, Valle 2004). Second, in our collections it was not uncommon to find hosts with obvious infestation based on the presence of thalli and attached trichospores extending beyond the anus of the undissected, intact dipteran host—an observation that is presented here for a genus not known to exhibit this kind of external development. Although in several instances larvae appeared to be active in the chilled jars it remains unclear whether the thallial extension beyond the anus is a natural occurrence or if it might have been caused by postcollection stress on the host.

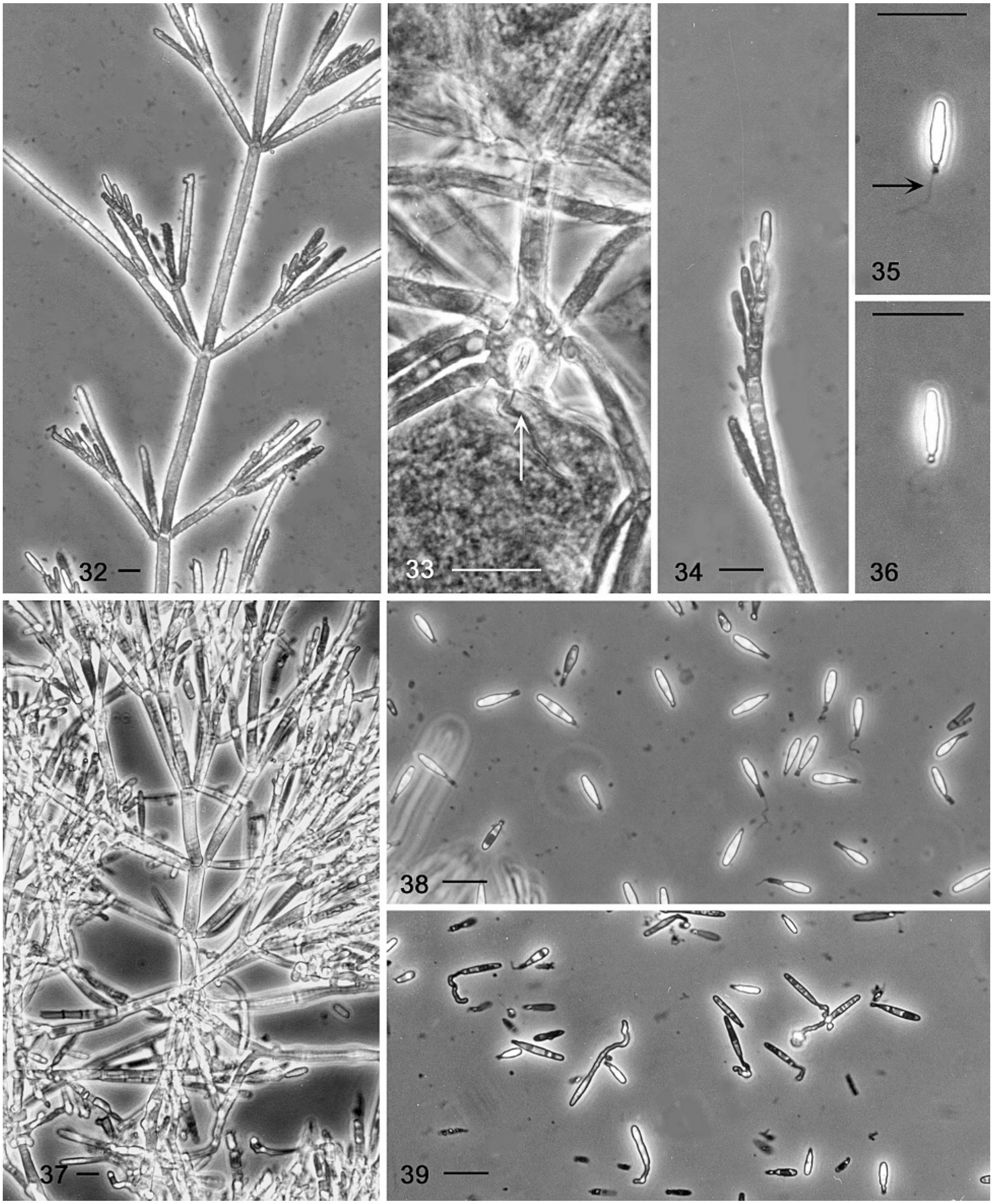
Smittium lentaquaticum Siri, M.M. White & Lichtw., sp. nov. FIGS. 32–39

Thalli usque ad 600 µm longus, verticillatim ramificans, cellula hippocrepiformi basali praeditus; trichosporae 12–19 ×3–5 µm longe ellipsoidales mediale subprotuberantes, collare 1.5–3 µm longo praeditae. Zygosporae ignotae. Ad cuticulam proctodaei Chironomidarum (Dipterorum) affixum.

Thalli up to 600 µm long, with verticillate branching and horseshoe-shaped basal cell. Producing long-ellipsoidal trichospores with a slight median bulge, 12–19 × 3–5 µm, with a collar 1.5–3 µm long. Zygospores unknown. Attached to hindgut cuticle of Chironomidae (Diptera).



FIGS. 26–31. *Harpellomyces montanus.* 26, 27. Attached and released trichospores with two appendages (slide TN-22-W4A). 28. Attached trichospores, one with two appendages visible within its generative cell (arrow) (slide TN-22-W4). 29. Mass of released trichospores, some showing two appendages (arrows) (slide TN-22-W4B). 30. Branching at the base of thalli, typical in this species (slide TN-22-1). 31. Detached zygospore, stained with lactophenol cotton blue (slide TN-22-W8). Bars = 20 μm.



FIGS. 32–39. *Smittium lentaquaticum*. 32. Verticillate branching with fertile branchlets (slide TN-27-A1). 33. Horseshoe-shaped holdfast cell (arrow) attached to hindgut cuticle of a midge larva (slide TN-27-3). 34. Fertile branchlet (slide TN-27-A6). 35, 36. Released trichospores each with a single, very fine appendage (arrow) emanating from within a collar (slide TN-27-A9). 37. Prolific branching in axenic culture TN-27-A5. 38. Released trichospores from culture TN-27-A4. 39. Sporangiospore extrusion after about 6 d in culture TN-27-A5, with limited sporangiospore growth. Bars = 20 μm.

Etymology. Lentic = living in still waters.

Specimens examined. USA. TENNESSEE: Rock pools above Roaring Fork Creek, just upstream of bridge, 35°42.55′N, 83°28.65′W (Collection 27, TABLE I), 25-VII-04. Microscope slide TN-27-A1 (HOLOTYPE: FH) includes a branching thallus and spores, microscope slide TN-27-3 (ISOTYPE: FH) includes holdfast and a few spores; both from bloodworms, *Chironomus* sp. larvae (Diptera, Chironomidae).

Cultures. Three axenic cultures of *Smittium lentaquaticum* were obtained from *Chironomus* sp. larvae, designated as TN-27-A3, TN-27-A4 and TN-27-A5. Many trichospores of this species in culture, especially in isolate TN-27-A5, are capable of extruding their sporangiospores in vitro (FIG. 39), a rare occurrence in isolates of Harpellales. However the extruded sporangiospores in *Sm. lentaquaticum* do not grow into new colonies as happens, for instance, in *Sm. culisetae* isolate COL-18-3. The cultures are deposited in the University of Kansas Mycological Culture Collection.

Many of the bloodworms (Chironomidae) from the pools of Site 27 also had *Amoebidium appalachense* attached to their anal tubules, in addition to *S. lentaquaticum* in their hindguts. The combination of verticillate branching (FIGS. 32, 37), horseshoe-shaped basal cell (FIG. 33), and small trichospores (FIGS. 35, 36) distinguish this species from other Smittiums. *Smittium delicatum* (see below), which we found in a different genus of bloodworm from lentic Site 18, has a horseshoe-shaped basal cell but is not verticillately branched and has longer and narrower trichospores ([18–]25–30 × 2.5 μm). Mosquito larvae from pools of collection Site 27 were hosts to another new species, *S. minutisporum* (see below).

Smittium minutisporum Lichtw., Siri & M.M. White FIGS. 40–45

Thallus non verticillatim ramificans haustorio simplici praeditus; trichosporae ellipticae, 10–15 × 2.5–3.5 μm, medialiter subprotuberantes, collare 1.5–2(–2.5) μm longo praeditae. Zygosporae ignotae. In proctodaeo larvarum Culicidarum (Dipterorum) affixum.

Thallus not verticillately branched with a simple holdfast, producing elliptical trichospores with a slight median bulge, 10–15 × 2.5–3.5 μm, with a collar 1.5–2(–2.5) μm long. Zygospores unknown. In hindgut of larval Culicidae (Diptera).

Etymology. Small-spored.

Specimens examined. USA. TENNESSEE: Rock pools above Roaring Fork Creek, just upstream of bridge, 35°42.55′N, 83°28.65′W (Collection 27, TABLE I), 25-VII-04. Microscope slide TN-27-11 (HOLOTYPE: FH), prepared from hindgut of *Ochlerotatus japonicus* (Theobold) (Culicidae) larva, includes thalli with attached and detached trichospores. Other specimens from same host and site as well as Roaring Fork Creek near Baskins Creek trailhead, 35°41.06′N, 83°27.94′W (Collection 24, TABLE I), 25-VII-04.

Mosquito larvae are known hosts of only a few species of *Smittium.* These include *S. culisetae* Lichtw., *S. culicis* Manier, *S. simulii* Lichtw. and *S. morbosum* Sweeney. We now add another species, *S. minutisporum*, which we found at collections 24 and 27. Both sites had mosquito larvae that were infested also with *S. culisetae* (see below). The trichospore size *S. minutisporum* is closest to that of *S. morbosum*, a mosquito pathogen (Sweeney 1981) whose trichospores measure (10–)15(–18) × (3.5–)4(–4.5) μm. Those of *S. minutisporum* are slightly narrower, and there was no evidence of pathogenicity.

Stachylina gravicaudata Siri, M.M. White & Lichtw., sp. nov. FIGS. 46–48

Thalli ca. 160–250 μm longus × 5–10 μm diam, trichosporis 4–8, 25–31 × 4–5 μm, collare brevi atque appendiculo unico gravi praeditis. Ad membranam peritrophicam larvarum Chironomidarum affixa.

Thalli ca. 160–250 μm long × 5–10 μm diam, bearing 4–8 trichospores, 25–31 × 4–5 μm, with a short collar and one heavy appendage. On peritrophic membrane of Chironomidae larvae.

Etymology. L. gravis = heavy; L. cauda = appendage.

Specimens examined. USA. TENNESSEE: Whistling Branch of Abrams Creek on Cades Cove Loop Road, 35°35.40′N, 83°49.90′W (Collection 40, TABLE I), 30-VII-04. Microscope slide TN-40-A7 (HOLOTYPE: FH), prepared from a *Paralauterborniella* sp. (Diptera, Chironomidae) larva. The thallus and trichospore sizes and the noticeably heavy appendage, distinguish *St. gravicaudata* from other species of *Stachylina.*

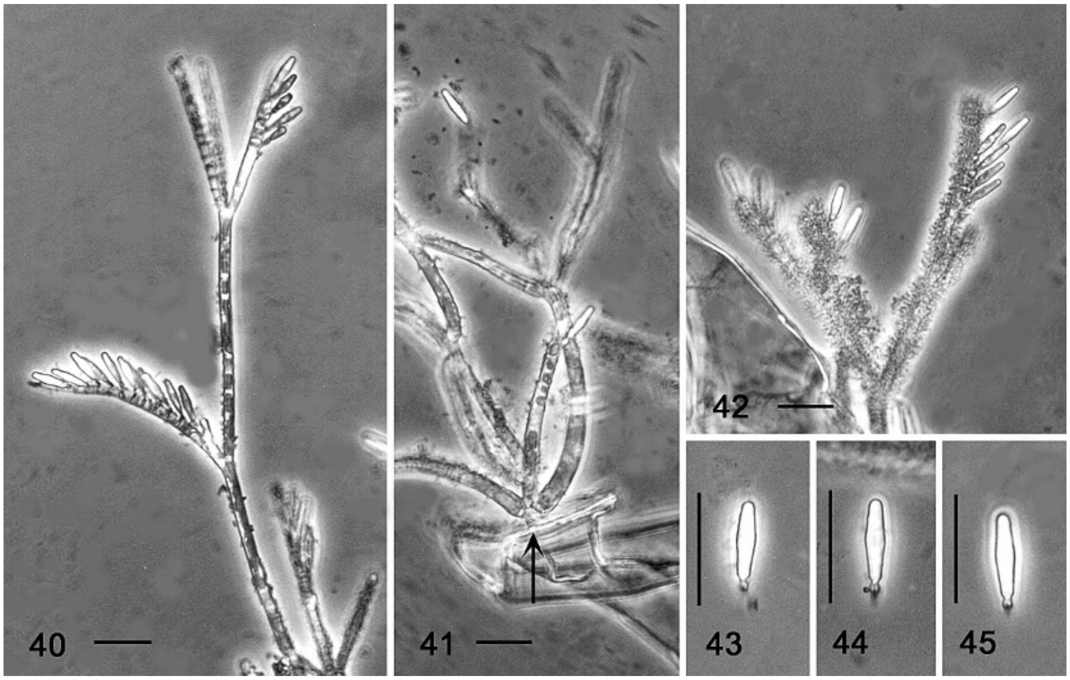
Stachylina stenospora Siri, M.M. White & Lichtw., sp. nov. FIGS. 49–53

Thalli 4.5–7 μm diam, trichosporis subcylindricis 1–4, 42–70 × 4–7 μm, medialiter subprotuberantibus, collare brevi praeditis. Zygosporae ignotae. Ad membranum peritrophicam larvarum chironomidarum affixa.

Thalli 4.5–7 μm diam, producing 1–4 subcylindrical trichospores with a slight median bulge, 42–70 × 4–7 μm, with a short collar. Zygospores unknown. On peritrophic membrane of Chironomidae larvae.

Etymology. Gr. stenos = narrow (spored).

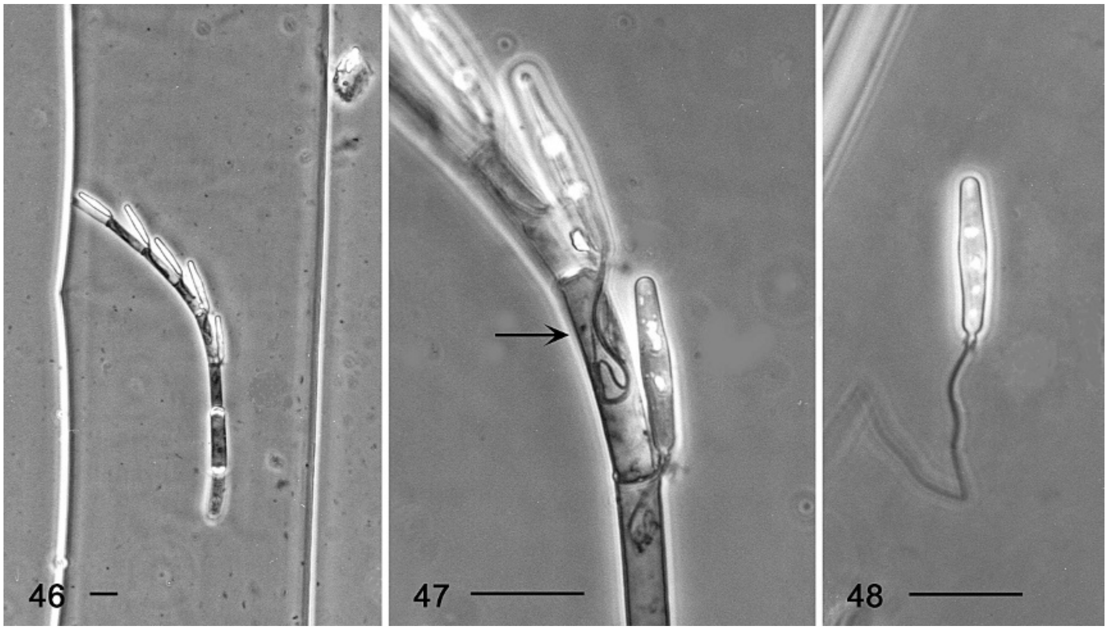
Specimens examined. USA. TENNESSEE: Thousand Drips Falls on Cliff Branch Creek, 35°42.73′N, 83°29.03′W (Collection 30, TABLE I), 25-VII-04. Microscope slide TN-30-6 (HOLOTYPE: FH) consisting of two peritrophic membranes of *Phaenopsectra* sp. (Diptera, Chironomidae) larvae, one with a sporulating thallus (see FIGS. 50, 52). Other



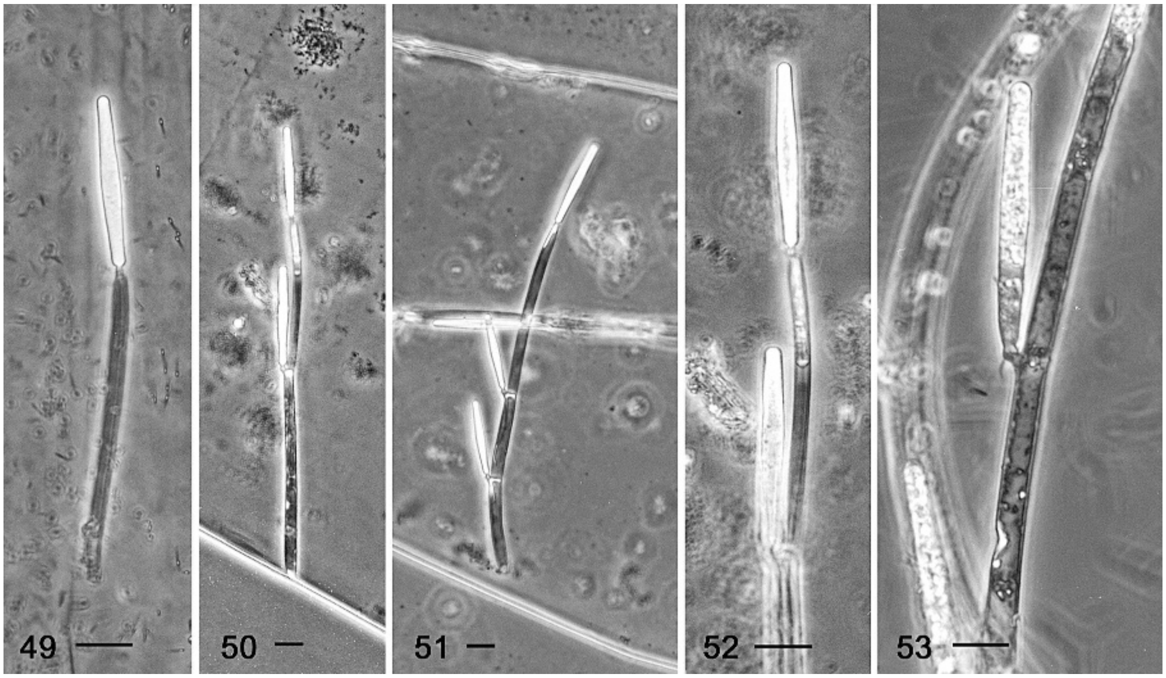
FIGS. 40–45. *Smittium minutisporum*. 40. Pattern of sporulating branchlets (slide TN-27-9). 41. Simple holdfast attaching thallus to a mosquito hindgut cuticle (arrow) (slide TN-27-16). 42. Sporulating branchlets covered with attached bacteria, a common occurrence in some specimens (slide TN-27-14). 43–45. Detached trichospores (slides TN-27-9, TN-27-13, TN-27-11, respectively). Bars = 20 μm.

specimens in same host from same site (TN-30) and same host from Raven Fork, on side road of Big Cove Road, 35°30.93′N, 83°17,87′W (Collection 18, TABLE I), 22-VII-04.

Stachylina stenospora most closely resembles the more common and widespread *St. grandispora* Lichtwardt (see below) frequently found in *Chironomus* spp. bloodworms but also in many other genera of Chironomidae. The thalli of *St. grandispora* are



FIGS. 46–48. *Stachylina gravicaudata* within the peritrophic membrane of a midge larva. 46. Entire thallus. 47. Attached trichospores with heavy appendage seen within the generative cell (arrow). 48. Released trichospore with heavy appendage. All from slide TN-40-A7. Bars = 20 μm.



FIGS. 49–53. *Stachylina stenospora* within the peritrophic membrane of a midge larva. 49–51. One-, two- and four-spored thalli (slides TN-30-8, TN-30-6, TN-30-3, respectively). 52–53. Attached trichospores (slides TN-30-6, TN-18-W5, respectively). Bars = 20 μm.

wider (6–10 μm diam), and the trichospores on the average also are wider (6–10+ μm), resulting in trichospores that are more long-ellipsoidal than subcylindrical than those of *St. stenospora*.

PREVIOUSLY DESCRIBED SPECIES

We report here a number of previously described species but with new geographical distributions and hosts.

Capniomyces stellatus S.W. Peterson & Lichtw.

This species, originally described from the winter stonefly genus *Allocapnia* (Capniidae) (Peterson and Lichtwardt 1983), was found in Collection 13 in a *Strophopterix limata* (Frison) nymphs (Plecoptera, Taeniopterygidae). It is identifiable by the variable number of trichospore appendages (1–6) and Type II zygospores (Moss et al 1975). This monotypic genus previously had been reported from several Midwestern states.

Ejectosporus spica (S.W. Peterson & Lichtw.) Strongman

Another relatively common species in *Allocapnia* spp. nymphs, *E. spica* was found in winter 1989 at collections 7–10, along with *Genistelloides hibernus* (see below). The species originally was described from Missouri and Arkansas under the name *Simuliomyces spica* S.W. Peterson & Lichtw. (Peterson and Lichtwardt 1983), but Strongman (2005) discovered that *S. spica* and *Ejectosporus magnus* S.W. Peterson, Lichtw. & M.C. Williams were conspecific, thus resulting in the name change.

Genistelloides hibernus S.W. Peterson, Lichtw. & B.W. Horn

In winter 1989 we found *G. hibernus* at collections 7–10 in *Allocapnia* spp. nymphs. The species produces obpyriform trichospores with two appendages and small Type I zygospores. The fungus is widespread in this winter stonefly genus within its range in eastern USA (Peterson et al 1981, Lichtwardt et al 1993).

Glotzia ephemeridarum Lichtwardt

Glotzia ephemeridarum was found only at Collection 20 in a Baetidae (Ephemeroptera) nymph but without zygospores. The genus is identified easily by its cylindrical trichospores with two short and one longer basal appendages. The other four species of *Glotzia* live in Baetidae nymphs, except for *G. plecopterorum* Lichtw. found in New Zealand Gripopterygidae (Plecoptera) nymphs (Williams and Lichtwardt 1990).

Graminella microspora S.T. Moss & Lichtw.

A geographically widespread species, *G. microspora* lives in Baetidae (Ephemeroptera) nymphs and has been found in the USA, Chile, Norway and Switzerland. Zygospores and the specialized vegetative propagules produced by some thalli were not found in the *Baetis* sp. nymph from Collection 48, but the characteristic long series of minute trichospores made the species identifiable (Lichtwardt and Moss 1981).

Harpella melusinae Léger & Duboscq

Harpella melusinae—conceivably a species complex—is the most widespread Harpellales, having been found in many genera and species of black flies (Simuliidae) from most continents (Lichtwardt et al 2001a). Up to now it has not been reported from Central or South America where several other species of *Harpella* occur. We found *H. melusinae* in simuliids from collections 17–20, 24, 31, 34, 35, 42, 45, 46 and 49. The unbranched thalli of *H. melusinae* have a characteristic tapered holdfast (Reichle and Lichtwardt 1972) that often has been used to identify immature thalli. Limited specimens from Collection 20 had a more rounded holdfast, making their identification uncertain.

Orphella avalonensis White, Lichtwardt and Colbo

To date *Orphella* Léger & Gauthier has been found only in the northern hemisphere, associated with stonefly families (Plecoptera). It is one of a few genera of Harpellales that at maturity typically may be observed protruding beyond the anus of its host. *Orphella* is the most unusual of the Harpellales, both morphologically and molecularly (White 2002). Specifically, asexual spores are dispersed as a complex of cells called a dissemination unit, quite unlike the more typical trichospore (=monosporous deciduous sporangium) that is unique to the other Harpellales. Forthcoming information of the occurrence of sexuality in the three European species of *Orphella* (Valle and Santamaria 2005) wherein zygospores do not correspond to any of the current four biconical to conical types (designated I–IV) will highlight further the morphological nonconformity of this genus with the other members of this order. In addition phylogenetic analyses of rDNA sequence data indicated that the genus is more closely allied to the Kickxellales (in the other class, Zygomycetes of the Zygomycota) (White 2002).

Orphella avalonensis originally was described from *Leuctra ferruginea* (Walker) nymphs from the Avalon Peninsula of Newfoundland (Lichtwardt et al 2001b). This is one of three species of *Orphella* known from North America, the other two being *O. haysii* Lichtwardt & Williams and *O. hiemalis* Peterson, Lichtwardt & Huss. The latter species have either curved or straight to bent asexual spores, but in *O. avalonensis* they are coiled. Our collections of *O. avalonensis* were from nymphs of *Leuctra* sp. from collections 24, 31, 38, 40–43. At least one *Leuctra* sp. host from Collection 43 had a hindgut with a second species of *Orphella* (with straight spores) coinhabiting the hindgut (see Discussion for further information on the other species). Several of our specimens of *O. avalonensis* were immature and not fully extended, thus not being revealed until we dissected the hindgut. However asexual spore dimensions (diameter of coiled spores by width) and their arrangement on mature sporulating heads match those of the original description of *O. avalonensis*. With occurrences of this species in eastern Canada (White and Strongman unpublished) as well as this extension into the Smoky Mountains it is likely that *O. avalonensis* is more widespread and common than is known currently.

Paramoebidium corpulentum Lichtw. & M.C. Williams and other *Paramoebidium* spp.

The common and worldwide genus *Paramoebidium* Léger & Duboscq (Amoebidiales) has eight described species and 10 other names that are nomina nuda (Lichtwardt et al 2001a). The valid species have been found in the hindgut of various species of Ephemeroptera, Plecoptera and Simuliidae (Diptera) collected from five continents. *Paramoebidium corpulentum* originally was found in the park at collections 6, 8 and 9 in *Allocapnia* spp. (Plecoptera, Capniidae) and other sites in northeastern Tennessee (Lichtwardt et al 1991). Since then *P. corpulentum* has been found in *Allocapnia* spp. in several eastern states and as far west as eastern Kansas (Koontz pers comm).

We also recorded other sightings of *Paramoebidium* spp. Many of these might be new; they lack sufficient morphological characters for us to describe them. The hosts consisted of many genera of Ephemeroptera and Plecoptera, a species of Trichoptera and *Simulium vandalicum* (Simuliidae). We recorded *Paramoebidium* spp. for collections 7–10, 13, 14, 17, 19–24, 29, 31, 32, 35, 38, 40, 41 and 49. In many hosts both *Paramoebidium* and members of the Harpellales were present, sometimes with harpellalean thalli attached to the *Paramoebidium* thalli.

Pennella simulii M.C. Williams & Lichtw.

This species, from Simuliidae (Lichtwardt et al 2001b), which is common in Newfoundland, was found in *Simulium vandalicum* Dyer & Shannon only from stream Collection 46a and one of its tributaries, Collection 17. Although zygospores were not found, the trichospores and other morphological features such as the basal cell conform to those of *P. simulii.*

Simuliomyces microsporus Lichtw.

A widespread and relatively common species in many black flies (Simuliidae) (Lichtwardt et al 2001a), *S. microsporus* was attached to thalli of *Paramoebidium* spp. in *Simulium vandalicum* larvae from collections 35 and 49. The presence of both trichospores and zygospores confirmed the identification. The species has been found in many states of the USA, England, France, Spain, Norway, Sweden and Australia.

Smittium culisetae Lichtw.

Smittium culisetae has been found in dipteran hosts from the USA, Japan, Australia, Europe, and Central and South America. Its most common host is mosquito larvae (Culicidae), but it has an unusually wide host range. Mosquito larvae from collections 24, 27 and 46 contained this fungus. Hosts included *Ochlerotatus japonicus*, from which two cultures were successful (TN-24-A3 and TN-27-A8), and possibly *O. atropalpus* (Coquillet), *Culex territans* (Walker), and *Aedes* sp. from Collection 46. Numerous axenic cultures have been obtained from various parts of the world, and it has been used in several growth, nutritional and electron microscopic studies (Lichtwardt et al 2001a).

Smittium delicatum Lichtw.

In Collection 18 we found *Phaenopsectra* sp. larvae (Chironomidae) with a hindgut fungus that fits the description of *S. delicatum*, a species previously known only from New South Wales, Australia (Lichtwardt and Williams 1990). The Australian specimens were in *Chironomus alternans* and *Cladopelma* sp. The species has a horseshoe-shaped holdfast, relatively few, fine branches and produces subcylindrical trichospores (18–)25–30 × 2.5 μm.

Stachylina grandispora Lichtw.

Often in *Chironomus* spp. bloodworms, as was the case in Collection 46, this widespread species has been found in at least six genera of Chironomidae, with a known distribution in the USA, including Hawaii, and in Europe, Australia and New Zealand. As the specific epithet implies, the trichospores are large and have a short collar from which emanates a long and easily discernable appendage.

Stachylina pedifer M.C. Williams & Lichtw.

Originally described from the peritrophic membrane of chironomid larvae in northwestern Wyoming (Lichtwardt and Williams 1983), the species also has been recorded from Norway (White and Lichtwardt 2004) and Spain (Valle 2004). Both of those collections were from larvae of *Boreoheptagyia lurida* (Garrett) (Chironomidae). Nonetheless our collection of this fungus from a bloodworm at Site 21 appears to fit the description of *S. pedifer*, based on trichospore dimensions and the footlike base of the thallus that penetrates the peritrophic membrane.

Zygopolaris ephemeridarum S.T. Moss, Lichtw. & Manier

Zygopolaris ephemeridarum previously has been reported from Colorado, Montana, Washington and Wyoming in various species of *Baetis* nymphs and less often in *Ephemerella* spp. nymphs (Ephemeroptera) (Lichtwardt et al 2001a). *Zygopolaris* is one of the few genera of Harpellales whose thalli, while attached to the hindgut cuticle, protrude from the anus of the host, with sporulation occurring externally (also see *Orphella* section). *Zygopolaris ephemeridarum* is reported here in nymphs of *Acentrella turbida* (McDunnough) from Collection 13 and in other Baetidae from Collection 21.

DISCUSSION

A summary of the identified trichomycetes reported in this paper is incorporated (TABLE II). It is evident from the brief collections that constitute this survey (TABLE I) that Great Smoky Mountains National Park (GSMNP) has a wealth of insects and other arthropods with associated trichomycetes. Our results do not represent the full range of trichomycetes in the GSMNP because mature larvae of some insects are present only at particular times of the year or are found in microhabitats that we did not explore. More intensive studies during different seasons are necessary to provide a more complete assessment of the prevalence of these fungal symbionts. Nonetheless, in addition to finding new and previously known taxa of trichomycetes, we succeeded in obtaining axenic cultures of some species that will enable future studies on development and host specificity. We also preserved in CTAB buffer many fungal specimens taken directly from the host gut for later DNA amplification and sequencing for phylogenetic studies.

Aside from the new species found in the GSMNP, a few others deserve highlighting for their impact on our understanding of host specificity and biogeography concepts. The occurrence of *Capniomyces stellatus*

TABLE II. Summary of trichomycetes (Harpellales, unless otherwise indicated) found in the Great Smoky Mountains National Park and vicinity

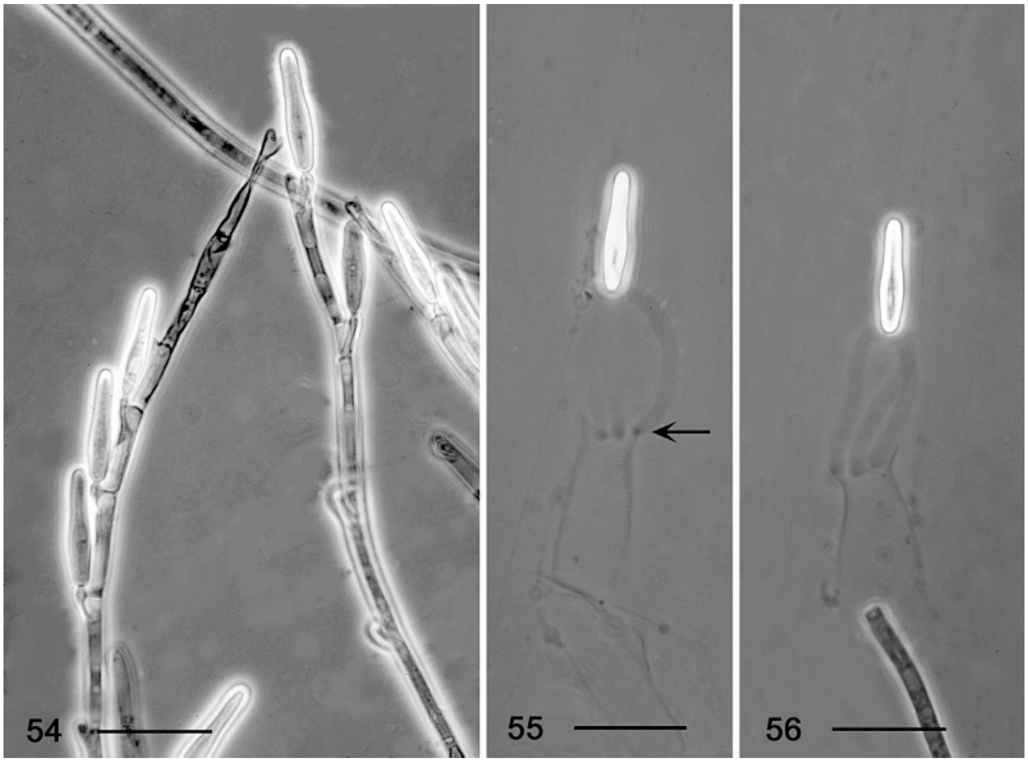
Trichomycete[a]	Collection	Larval host
Barbatospora ambicaudata[b]	35, 49	*Simulium vandalicum* (Simuliidae)
Amoebidium appalachense[b,c]	27, 46	*Chironomus* sp. (Chironomidae)
Capniomyces stellatus	13	*Strophopterix limata* (Taeniopterygidae)
Ejectosporus spica	7, 8, 9, 10	*Allocapnia* spp. (Capniidae)
Enterobryus sp.[d]	17, 28	*Oxidus gracilis* (Diplopoda)
Genistelloides hibernus	7, 8, 9, 10	*Allocapnia* spp. (Capniidae)
Glotzia ephemeridarum	20	*Baetis* sp. (Baetidae)
Graminella microspora	48	*Baetis* sp. (Baetidae)
Harpella melusinae	12 sites	Simuliidae (several species)
Harpellomyces montanus	22, 35	*Androprosopa thornburghae* (Thaumaleidae)
Lancisporomyces sp.	7	Capniidae
Legeriosimilis sp.	6, 7	*Ameletus* sp. (Siphloneuridae)
Orphella avalonensis	7 sites	*Leuctra* spp. (Leuctridae)
Orphella sp.	8 sites	*Leuctra* spp. (Leuctridae)
Paramoebidium corpulentum[c]	6, 8, 9	*Allocapnia* spp. (Capniidae)
Paramoebidium spp.[c]	19 sites	Diptera, Ephemeroptera, Plecoptera, Trichoptera
Pennella simulii	17	*Simulium vandalicum* (Simuliidae)
Simuliomyces microsporus	35, 49	*Simulium vandalicum* (Simuliidae)
Smittium culisetae[b]	24, 27, 46	*Ochlerotatus japonicus* and other Culicidae
Smittium delicatum	18	*Phaenopsectra* sp. (Chironomidae)
Smittium lentaquaticum[b]	27	*Chironomus* sp. (Chironomidae)
Smittium minutisporum	24, 27	*Ochlerotatus japonicus* (Culicidae)
Stachylina pedifer	21	Chironomidae
Stachylina grandispora	46	*Chironomus* sp. (Chironomidae)
Stachylina gravicaudata	40	*Paralauterborniella* sp. (Chironomidae)
Stachylina stenospora	18, 30	*Phaenopsectra* sp. (Chironomidae)
Zygopolaris ephemeridarum	13, 21	*Acentrella turbida* (Baetidae)

[a] Taxa in boldface are new.
[b] Axenic cultures obtained.
[c] Amoebidiales.
[d] Eccrinales.

in Taeniopterygidae establishes, with Capniidae, a second family of the suborder Arctoperlaria for this monotypic genus. Similarly, at two sites in Norway, White and Lichtwardt (2004) observed that nymphs of Taeniopterygidae also could serve as hosts of *Orphella coronata* Léger & Gauthier, a gut fungus previously only known from a Nemouridae stonefly; indeed it was the host family for the remainder of their collections of that fungus at five other sites. Further collections of Taeniopterygidae clearly are warranted as a suitable host group of Harpellales. Meanwhile the occurrence of *Sm. delicatum*, hitherto reported only from Australia, highlights the need for continued collection to better understand such disjunct but widely distributed species. At the same time widespread species such as *Sm. culistae* and *Sm. grandispora* and to some extent *H. melusinae* are better represented globally and ranges are extended for all others.

Some specimens we found are not fully describable because of sparse material and/or incomplete developmental stages but are worthy of note:

1. An undetermined species of the currently monotypic genus *Lancisporomyces* Santamaria was found in Capniidae nymphs from Collection 7. *Lancisporomyces vernalis* was described from Spain in Nemouridae (Plecoptera) nymphs by Santamaria (1997), who noted that another, undetermined species also was present in the USA. *Lancisporomyces* is one of four genera of Harpellales with zygospores designated as Type IV that possess a distally thickened and pointed tip and basal polar attachment to the zygosporophore. In *L. vernalis* the zygospores are lance-shaped with a long and narrow extension below the pointed tip. The genus soon will be expanded to include three new species from eastern Canada (Strongman and White unpublished). Our specimen of *Lancisporomyces* collected in 1989 included a group of thickened and sinuous



FIGS. 54–56. *Legeriosimilis* sp. from a mayfly nymph. 54. Attached trichospores. 55, 56. Detached trichospores with three somewhat diffuse appendages; note the knob-like structures typical of other species in this genus (arrow). All from slide TN-6-1. Bar = 20 µm.

branches that we now interpret as zygospores, and it resembles one of the undescribed species in Nova Scotia from Capniidae nymphs. Thus the species in the park might be more widespread in North America.

2. An undetermined and possibly new species of *Legeriosimilis* was found in *Ameletus* sp. nymphs (Ephemeroptera, Siphloneuridae) in collections 6 and 7. No zygospores were found to compare with the two named species of *Legeriosimilis*, *L. tricaudata,* M.C. Williams, Lichtw., M.M. White & Misra (in USA) and *L. europaeus* M.M. White & Lichtw. (in Norway), both also living in *Ameletus* spp. (Williams and Lichtwardt 1999, White and Lichtwardt 2004). Trichospores of this unnamed *Legeriosimilis*, although clearly belonging to that genus, 30–32 × 6–8 µm (FIGS. 54–56), thus are slightly shorter than those of *L. europaeus* (33–40 × 6–8 µm) and smaller than trichospores of *L. tricaudata* ([33–]47[–52] × [7–]9.5[–11] µm). In a mayfly from a different family found in Collection 38, we found specimens of a fungus with trichospore appendages similar to those of *Legeriosimilis* spp.; however these trichospores were a bit more ellipsoidal than the elongate-obpyriform shape in *Legeriosimilis* spp. Additional specimens will be necessary to determine the genus.
3. A possibly new species of *Orphella* with straight asexual spores, clearly distinguishable from the thalli of the coiled-spored *O. avalonensis*, was found in *Leuctra* sp. nymphs at collections 20, 31, 32, 34, 38, 40, 43 and 45. The identity of this species is not clear based on the combination of characters documented and available to us at this time. This unusual quandary in part is due to having more information than is typical for identifying species of *Orphella.* Based on asexual spores and accompanying cells of the mature sporulating heads where these cells are produced, our straight-spored *Orphella* sp. clearly is most similar to the European species, *Orphella catalaunica* Santamaria & Girbal. In fact the asexual spores (length × width) of this *Orphella* sp. are an excellent match for the sizes of *O. catalaunica* from *Leuctra* nymphs. Our decision to list this as an unnamed species is driven by minimal evidence of the sexual spores for one of our specimens. Based on the timely information on the sexual spores of *O. catalaunica* from Spain (Valle and Santamaria 2005) it is apparent that the zygospore and zygosporophore in our

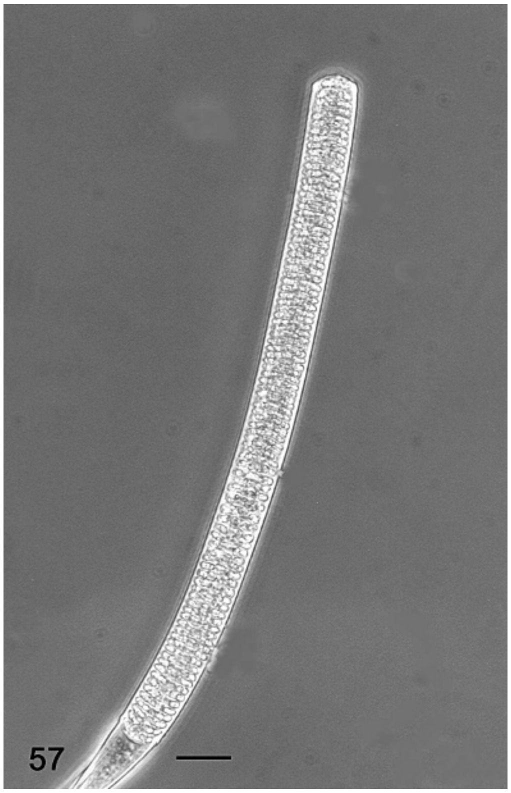


FIG. 57. An Eccrinales from an aquatic beetle adult hindgut with a long series of flat, disk-like sporangiospores somewhat resembling some species of the genus *Arundinula* Léger & Duboscq (slide TN-40-W1). Bar = 20 μm.

collections of this ''*catalaunica*-like'' species are not an exact match for the true European species. However at this time we do not think that we have sufficient morphological data to properly describe this probably new species of *Orphella* with straight asexual spores and coiled sexual spores. Further collections of *Orphella* from Leuctridae and other nonpredacious stoneflies, especially in pursuit of sexual stages, will be warranted to describe this species.

This kind of taxonomic challenge in the Harpellales is not unprecedented, and several species have similar or overlapping trichospore sizes that rely on differences in the sexual spore morphology to distinguish them. This challenge is exacerbated by the frequent absence of sexual stages in collections and in addition for many genera of Harpellales for which we have never observed sexual stages either because they are truly elusive and rare or the process might not occur in some extant species. Valle and Santamaria (2005) have elucidated the sexual process in *Orphella*, which clarifies the morphological variability that might have been observed and even noted as ''unusual features'' in certain earlier descriptions of *Orphella* species. In addition we now routinely voucher excised fungal specimens, including microscopic and unculturable taxa such as *Orphella* for DNA extraction. It is possible that future sequencing analyses will help to delimit species boundaries as well as help construct a molecular-based phylogeny of the Harpellales.

4. A *Stachylinoides*-like fungus was found in collections 20 and 21 in Blephariceridae (Diptera) larvae, an unusual host for Harpellales. *Stachylinoides arctata* Ferrington, Lichtw. & López Lastra is monotypic, and thalli were found filling the peritrophic membranes of minute chironomids, *Thienemaniella* sp., in Argentina (Lichtwardt et al 1999). The *Stachylinoides*-like fungus from the park is interesting because it might represent a new fungus in a new type of host.
5. The Eccrinales genus *Enterobryus*—the first described genus of trichomycetes—is common in millipedes (Diplopoda) worldwide (Lichtwardt et al 2001a). Two collections of *Oxidus gracilis* (Koch), a cosmopolitan temperate species that can build enormous populations, were well infested at collections 17 and 28 but with insufficient developmental stages to characterize the species. At Site 28 the millipedes were crawling about a seepy cliff.
6. An unusual discovery was the presence of Eccrinales thalli in an aquatic beetle. This fungus resembles some species of *Arundinula* Léger & Duboscq (FIG. 57) but all described species of *Arundinula* are symbionts of Crustacea. Since White (1999, FIG. 34) also noted the occurrence of an eccrinid (albeit more immature) in an adult aquatic beetle from New England, there is an obvious need to look at the gut flora of such Coleoptera with greater scrutiny.

It is important to note that we found, on average, one new species every 2–3 d with minimal effort and personnel. Clearly we have completed only a small amount of the necessary work for a complete list of Trichomycetes taxa in the GSMNP. Hopefully this will stimulate future research not only in the southern Appalachians but more broadly in North America toward a global perspective of these intriguing arthropod gut symbionts.

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