

Fungal symbionts (Harpellales) in Norwegian aquatic insect larvae

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Abstract: Collections of aquatic insect larvae in Norway, over a 40 d period in May and Aug 2002, resulted in finding more than 25 species and one new genus of Harpellales (Trichomycetes). Nine new fungal symbionts are described and named: *Ephemerellomyces aquilonius* (a new monotypic genus), *Glottzia stenospora* and *Legeriosimilis europaeus* in mayfly nymphs (Ephemeroptera); *Genistelloides amplispora* and *G. communis* in stonefly nymphs (Plecoptera); and *Smittium biforme*, *Sm. precipitiorum*, *Stachylina acutibasilaris* and *St. lentica* in midge larvae (Diptera: Chironomidae). Two possibly new species of *Smittium* in Chironomidae larvae are described but not formally named. New hosts and biogeographical distributions are recorded for 14 previously described species, including the rare occurrence of *Smittium simulii* in mosquito larvae.

Key words: biogeography, Diptera, Ephemeroptera, gut fungi, Plecoptera, symbiosis, Thaumaleidae, Trichomycetes

INTRODUCTION

We encountered many new gut fungi in the course of seeking particular living specimens of Harpellales in Norway for preservation and subsequent extraction of their DNA to be used in a comparative molecular study of North American and European species. This paper provides descriptions of nine new species, including one new genus, as well as new host records and other data on geographic distributions of previously described Harpellales. Two possibly new species of *Smittium*, the largest genus of Harpellales, are described but not named. Several previously known species are illustrated in this paper to document their identification and/or to provide photomicrographs not currently available in the literature.

Norway had an excellent diversity of Harpellales in

various kinds of aquatic habitats, considering that collections and laboratory work were conducted within a total of only 40 d and over a restricted range of possible habitats. The months of May and Aug were selected to obtain some of the insects in their more mature larval stages. In particular, we sought two of the least vagile aquatic insect hosts of gut fungi, stonefly nymphs (Plecoptera) (Hynes 1975, 1988, Stewart and Stark 1988) and solitary midge larvae (Thaumaleidae), hosts of *Orphella* and *Harpellomyces*, respectively. We did not find solitary midges in their usual seeping cliff habitats in May, but we did encounter them in Aug, even though it was one of the driest summer seasons in several decades according to local accounts. Undoubtedly, many other Harpellales beyond the taxa we describe here are present in Norway, especially in unexplored regions of the country and in different hosts, habitats and time of year.

MATERIALS AND METHODS

Collecting sites where Harpellales were found are listed in TABLE I. Most insects were collected in flowing streams. Aquatic nets or strainers were used in streams, ponds and rock pools, or insects were picked up with forceps directly from substrate materials in these habitats and from seeping cliffs. Supplemental bulk collections were placed in sealed plastic bags for later sorting. Jars and bags with insects were kept on ice until returned to the laboratory, then placed in refrigerators until dissected.

Laboratory facilities in May were provided first by the University of Oslo's Botany Department and then at the Ekse Field Station (in the mountains west and north of Voss) administered by the University of Bergen. In Aug work was conducted at the University of Trondheim's Lake Snåsavatnet Field Station east of Steinkjer. Collection sites were selected from as wide an area as possible, usually within a half day's drive of the facilities.

Dissections of insects were done under stereo microscopes, and their gut fungi studied and identified in water mounts using a phase-contrast microscope. The fungi selectively were photomicrographed (using Kodak 200 ISO daylight print film) in living condition, then either placed in CTAB DNA extraction buffer (Gottlieb and Lichtwardt 2001) for subsequent sequencing in our own laboratory or prepared as semipermanent slides by infiltrating lactophenol-cotton blue under the cover slip and sealing with clear fingernail polish. Some specimens were selected for culture attempts, using methods described in Lichtwardt et al (2001a). Types of new species are deposited at the Farlow Herbarium (FH).

TABLE I. Collection sites in Norway with Harpellales

Site	Date	Site description, County, Municipality	Latitude/Longitude
2	3-V-2002	Stream near curve on Sørkendalsveien Rd. Oslo. Water temperature 5.5 C.	60°00.72'N, 10°36.74'E
3	3-V-2002	Heggelielva River near Skansebakken parking area. Oslo. 8.5 C.	60°01.19'N, 10°35.20'E
4	3-V-2002	Small stream next to house at old bridge immediately N of newer bridge. Oslo. 5 C.	60°01.14'N, 10°33.88'E
5	5-V-2002	Small unnamed stream off Kampeveien Rd. Oslo. 6.5 C.	59°59.19'N, 10°28.64'E
6	5-V-2002	Østernbekken River, crossing road on Rt. 168 to Haga. Oslo. 10 C.	59°56.52'N, 10°34.70'E
7	5-V-2002	Stream near curve on Sørkendalsveien Rd. Oslo. 7 C.	60°00.72'N, 10°36.74'E
8	8-V-2002	Heggelielva River near Skansebakken parking area. Oslo. 8.5 C.	60°01.19'N, 10°35.20'E
10	8-V-2002	Small stream next to house at old bridge, immediately N of newer bridge. Oslo. 6.5 C.	60°01.14'N, 10°33.88'E
11	9-V-2002	Svenodalen River. Oppland, Jevnaker. 5 C.	60°13.86'N, 10°25.81'E
11A	9-V-2002	Seepy cliff bordering Svenodalen River about 1 km upstream from Site 11. Oppland, Jevnaker.	
15	9-V-2002	Small tributary of River Kverndøla. Akershus, Eidsvoll.	60°15.82'N, 10°57.68'E
16	9-V-2002	Dalbekken stream. Akershus, Eidsvoll. 6 C.	60°29.99'N, 10°08.00'E
17	13-V-2002	Seepy cliff on Rt. 7, 11.5 km from Ørgenvika. Buskerud, Flå.	60°11.98'N, 09°37.59'E
18	13-V-2002	Seepy cliff on Rt. 7 at large parking area. Buskerud, Gol. 9 C.	60°28.31'N, 09°56.68'E
19	14-V-2002	Stream at bottom of Haga foss, a tributary of the Ekso River. Hordaland, Modalen. 2 C.	60°50.11'N, 06°19.48'E
20	15-V-2002	Very small stream on E edge of Ekse field station building. Hordaland, Modalen.	60°49.77'N, 06°14.37'E
21	15-V-2002	Stream at bottom of Haga foss, a tributary of the Ekso River, Hordaland, Modalen. 2 C.	60°50.11'N, 06°19.48'E
22	16-V-2002	Small stream under Rt. E16 W of Voss. Hordaland, Modalen. 6.5 C.	60°37.52'N, 06°23.65'E
23	16-V-2002	Very small stream across road leading to Eksingedal, Teigdal Valley. Hordaland, Modalen. 7 C.	60°40.39'N, 06°05.47'E
24	16-V-2002	Teigdal Elva, main river of Teigdal Valley. Hordaland, Modalen. 5 C.	60°41.92'N, 06°06.66'E
25	16-V-2002	Swampy pool next to Teigdal elva. Hordaland, Modalen. 9 C.	60°41.92'N, 06°06.66'E
26	16-V-2002	Drainage ditch on E side of road near bridge, S of Teigdal Valley pass. Hordaland, Modalen. 1 C.	60°45.27'N, 06°07.90'E
28	18-V-2002	Drainage ditch on E side of road near bridge, S of Teigdal Valley pass. Hordaland, Modalen. 4 C.	60°45.27'N, 06°07.90'E
30	18-V-2002	Stream at bottom of Haga foss, a tributary of the Ekso River. Hordaland, Modalen. 4 C.	60°50.11'N, 06°19.48'E
31	20-V-2002	River Teigdal Elva, main river of Teigdal Valley. Hordaland, Modalen. 6 C.	60°41.92'N, 06°06.66'E
32	20-V-2002	Swampy pool next to River Teigdal Elva. Hordaland, Modalen. 14 C.	60°41.92'N, 06°06.66'E
33	20-V-2002	Drainage ditch on E side of road near bridge, S of Teigdal Valley pass. Hordaland, Modalen. 2.5 C.	60°45.27'N, 06°07.90'E
34	18-VIII-2002	Seep cliff S of Dombås. Oppland, Dovre. 17 C.	61°52.62'N, 09°25.28'E
35	18-VIII-2002	Tributary near junction of Kanten Rd. and Kongsvegen Rd., N of Dovre and E of Rt. E6. Oppland, Dovre. 14.5 C.	61°59.61'N, 09°14.23'E

TABLE I. Continued

Site	Date	Site description, County, Municipality	Latitude/Longitude
36	19-VIII-2002	Small stream crossing Rt. E6 in Dovre mountain area. Oppland, Dovre. 14.5 C.	62°05.70'N, 09°12.87'E
38	21-VIII-2002	Rock pool in Bølareinens park, off Rt. 763. Nord-Trøndelag, Steinkjer. 17 C.	64°08.77'N, 11°56.38'E
40	21-VIII-2002	Geritbekken River, a tributary of Jørstadelva on road S off Hyw. 763. Nord-Trøndelag, Snåsa. 17 C.	64°10.86'N, 12°17.07'E
41	21-VIII-2002	Jørstadelva River SE of Site 40. Nord-Trøndelag, Snåsa. 20 C.	64°09.61'N, 12°25.24'E
42	21-VIII-2002	Grana River, S of Snåsa, downstream from bridge. Nord-Trøndelag, Snåsa. 11.5 C.	64°14.25'N, 12°22.41'E
45	23-VIII-2002	Lake Snåsavatnet rocky bottom near shoreline. Nord-Trøndelag, Snåsa. 21.5 C.	64°07.65'N, 11°45.44'E
45A	23-VIII-2002	Rock pool above shoreline of Site 45. Nord-Trøndelag, Snåsa.	64°07.65'N, 11°45.44'E
46	23-VIII-2002	Seepy cliff on S side of Rt. 74. Nord-Trøndelag, Lierne.	64°28.14'N, 12°54.20'E
51	23-VIII-2002	Djubvasselva stream connecting lake Djupvatnet and lake Laksjøen. Nord-Trøndelag, Lierne. 20 C.	64°26.08'N, 13°36.93'E
52	23-VIII-2002	Stream draining lake 491 at Økstjorna W of Rt. 765. Nord-Trøndelag, Lierne.	64°21.70'N, 13°37.29'E
53	23-VIII-2002	Aunelva stream draining S. Nord-Trøndelag, Steinkjer. 17.5 C.	64°19.66'N, 13°35.52'E
54	28-VIII-2002	Small stream crossing Rt. 759, SSE of Steinkjer. 15.5 C.	63°57.64'N, 11°34.27'E
56	28-VIII-2002	Very small waterfall on E side of Rt. 759. Nord-Trøndelag, Steinkjer. 13 C.	63°48.74'N, 11°35.32'E
57	28-VIII-2002	Large waterfall, Kjaekerfossen, just off Rt. 757. Nord-Trøndelag, Steinkjer. 18.5 C.	63°50.25'N, 12°01.68'E
58	28-VIII-2002	Pools on shore of Verbalselva at Vuku, E of bridge crossing river to Stene at Rt. 758 off of Rt. 757. Nord-Trøndelag, Steinkjer. 20–21.5 C.	63°46.53'N, 11°43.96'E
59	28-VIII-2002	Rock pool in Bølareinens park, off Rt. 763. Nord-Trøndelag, Steinkjer. 17 C.	64°08.77'N, 11°56.38'E
60	28-VIII-2002	Greitbekken River, a tributary of Jørstadelva on road S off Hyw. 763. Nord-Trøndelag, Snåsa.	64°10.86'N, 12°17.07'E
61	29-VIII-2002	Large waterfall, Kjaekerfossen, just off Rt. 757. Nord-Trøndelag, Steinkjer. 18.5 C.	63°50.25'N, 12°01.68'E
62	29-VIII-2002	Pools on shore of Verbalselva at Vuku, E of bridge crossing river to Stene at Rt. 758 off of Rt. 757. Nord-Trøndelag, Steinkjer.	63°46.53'N, 11°43.96'E
63	29-VIII-2002	Lake Snåsavatnet rocky bottom near shoreline. Nord-Trøndelag, Snåsa.	64°07.65'N, 11°45.44'E

NEW TAXA

Ephemerellomyces M.M. White & Lichtw., gen. nov.

Trichosporae longi-ovoideae, appendicibus 2–3 perlongis praeditae, aliquando cum in cuticula proctodaei germinantes cellulam quae trichosporam unicam terminalem gerens producentes. In proctodaeo nympharum Ephemeropterorum affixus.

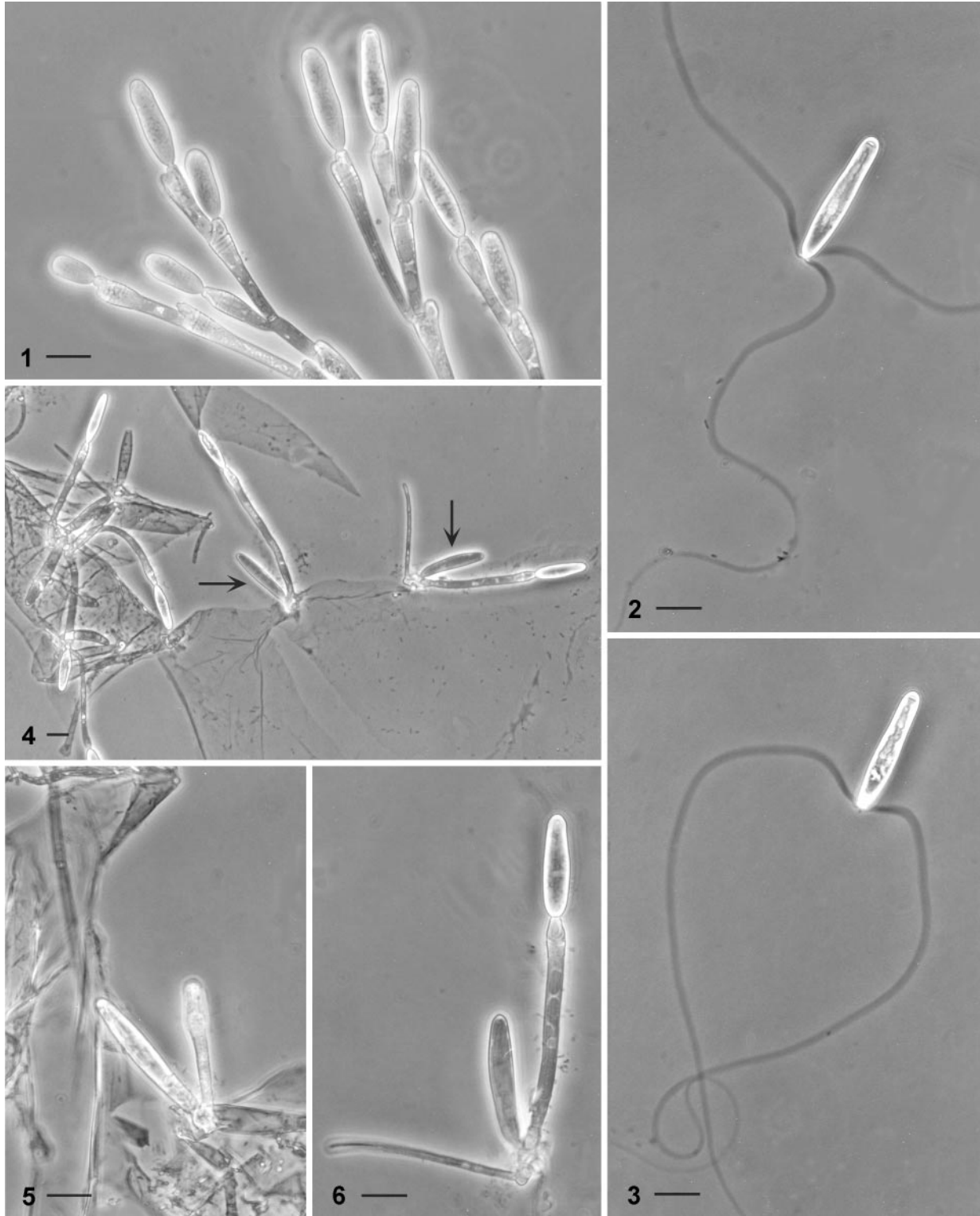
Trichospores long-ovoid with 2–3 long appendages. Trichospores may germinate on hindgut cuticle

to produce a cell bearing one terminal trichospore. Attached to hindgut of Ephemeroptera nymphs.

SPECIES TYPICA: *Ephemerellomyces aquilonius* M.M. White & Lichtw.

Etymology. Fungus in species of the host genus *Ephemerella*.

Ephemerellomyces aquilonius M.M. White & Lichtw., sp. nov. FIGS. 1–6



FIGS. 1–6. *Ephemereatomyces aquilonius* from nymphs of *Ephemereella aurivillii* (Ephemeroptera: Ephemerellidae). 1. Branchlets producing trichospores (from a dissection designated [hereafter = #] NOR-57-W4). 2, 3. Released mature trichospores with three and two appendages, respectively (# NOR-61-W3, # NOR-57-W1). 4. Five germinated trichospores (arrows point to two) attached to the host's hindgut cuticle, each small thallus producing one terminal trichospore. 5. Early stage of trichospore germination; the trichospore is on the left. 6. Small sporulating thallus with a second branch at its base (enlargement of one shown in FIG. 4; FIGS. 4–6 are from # NOR-61-W3). Scale bars = 20 μ m.

Trichosporae longi-ovoideae, (30–)45(–50) × 8–10 μm, appendicibus 2 interdum 3 perlongis praeditae, aliquando in cuticula proctodaei affingentes et germinantes et cellulam 75–110 μm fertilem producentes quae trichosporam unicam terminalem gerens. Zygosporae ignotae. In proctodaeo nympharum Ephemeropterorum affixus.

Trichosporae long-ovoid, (30–)45(–50) × 8–10 μm, with 2, occasionally 3, long appendages. Trichosporae sometime attach and germinate on the hindgut cuticle and produce a fertile cell 75–110 μm long bearing one terminal trichospore. Zygosporae unknown. In hindgut of Ephemeroptera nymphs.

Etymology. *L. aquilonius* = northern.

Specimens examined. NORWAY. Large waterfall, Kjaekerfossen, just off Route 757. 63°50.25'N, 12°01.68'E, 29-VIII-2002. Microscope slide NOR-61-W3 (HOLOTYPE: FH), in hindgut of *Ephemera aurivillii* (Bengtsson) nymph (Ephemeroptera) from Site 61 (TABLE I); microscope slide NOR-57-W1 (PARATYPE: FH) in same host species from Site 57. Other collections from sites 40, 57, 61.

The type of trichospore germination (FIGS. 4–6) by attachment to the hindgut lining and production of a single trichospore has not been found in any other genus. Also, the production of 2 or 3 trichospore appendages (FIGS. 2, 3) is unique to this new genus. Otherwise, the trichosporae resemble those in the 2-appendaged genera *Genistelloides* Peterson, Lichtw. & Horn and *Legeriomyces* Pouzar.

Genistelloides amplispora M.M. White & Lichtw., sp. nov. FIGS. 7–9

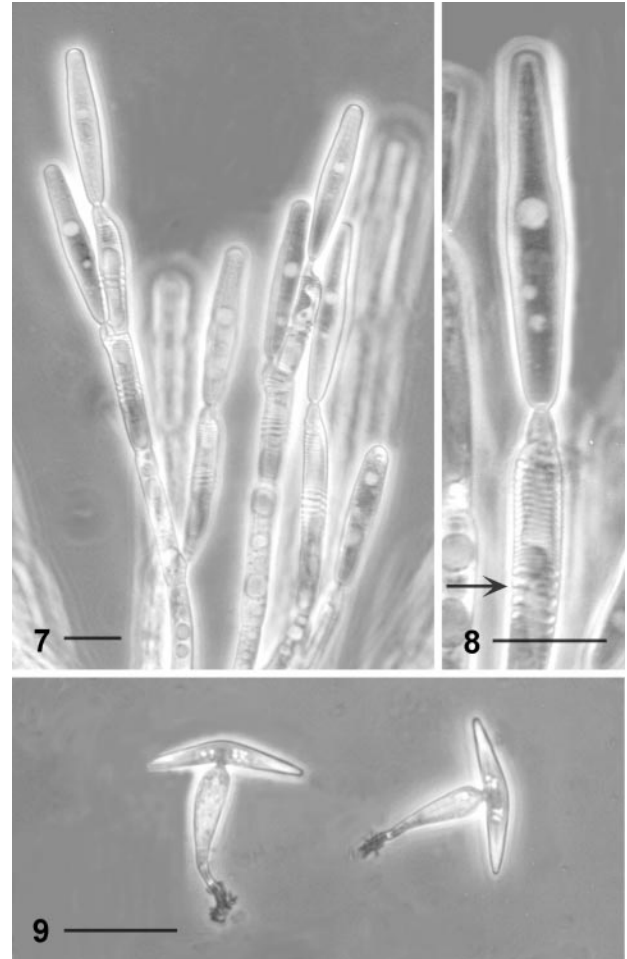
Trichosporae longi-ellipsoidales, 50–60 × 9–10 μm. Zygosporae biconicae (Typus I) 26–28 × 4–4.5 μm; zygosporophora 17–20 × 4–5 μm. In proctodaeo nympharum Nemouridarum (Plecopterorum) affixa.

Trichosporae long-ellipsoidal, 50–60 × 9–10 μm. Biconical zygosporae (Type I) 26–28 × 4–4.5 μm, zygosporophores 17–20 × 4–5 μm. In hindgut of Nemouridae (Plecoptera) nymphs.

Etymology. *L. amplus* = large.

Specimens examined. Stream at bottom of Hagafoss, a tributary of the Ekso River, 60°50.11'N, 06°19.48'E, 15-V-2002. FIG. 9 (HOLOTYPE: FH), a photomicrograph of living zygosporae from microscope slide NOR-19-W3, prepared from the hindgut of an *Amphinemura sulciollis* (Stephens) nymph (Plecoptera: Nemouridae) from Site 19 (TABLE I). FIG. 8 (ISOTYPE: FH), a photomicrograph of a living trichospore from microscope slide NOR-21-W19A, from the same collection locality and host species as the holotype.

The mature trichosporae of *G. amplispora* were not found released, and therefore appendages were not observed. However, within the generative cell the appearance of the appendages is identical to 2-appendaged attached trichosporae of other species (cf. FIGS. 8 and 11). Zygosporae of the new species are of the same type, general appearance and approximate



FIGS. 7–9. *Genistelloides amplispora* from nymphs of *Amphinemura sulciollis* (Plecoptera: Nemouridae). 7, 8. Trichosporae; note appendages within generative cell (arrow) (# NOR-21-W19A). 9. Detached biconical zygosporae with their attached zygosporophores (# NOR-21-W3). Scale bars = 20 μm.

size as the type species, *G. hibernus* Peterson, Lichtw. & Horn (Peterson et al 1981). Despite the similarity in zygosporae (Type I, Moss et al 1975) for both species, *Genistelloides amplispora* is separated from *G. hibernus* on the basis of much larger trichosporae. Trichosporae of *G. amplispora* are only slightly larger on the average (50–60 × 9–10 μm) than those of *G. helicoides* M.C. Williams & Lichtw. (42–)51(–62) × (7.5–)8.7(–10) μm (Williams and Lichtwardt 1987a), but *G. helicoides* presents a slightly larger length to width ratio. Thus, trichosporae of *Genistelloides helicoides* are narrower overall, compared to *G. amplispora*, and trichosporae of the former are slightly wider below the middle. Although we have collected *G. helicoides* on a number of occasions in the Rocky Mountains of Colorado and eastward in the U.S.A., zygosporae have never been seen. In the absence of

zygospores, distinctions between *G. amplispora* and *G. helicoides* are not entirely conclusive. Therefore, we provide two additional criteria for separating the species at this time. First, host association segregates the fungal species inhabiting different stonefly families: *G. hibernus*, *G. helicoides* and *G. amplispora* are associated, respectively, with Capniidae, Nemouridae, and Amphinemouridae. Second, *G. amplispora* has been found in Europe, and the two other species are North American. We predict that zygospores ultimately will provide further support for separation of the two species.

Genistelloides communis M.M. White & Lichtw., sp. nov. FIGS. 10–13

Trichosporae longi-obpyriformes, 31–38 × 5.5–6.5 μm, sub remissione appendicibus 2 longis, primum spiralibus. Zygosporae ignotae. In proctodaeo nympharum Nemouridarum (Plecopterorum) affixa.

Trichosporae long-obpyriform, 31–38 × 5.5–6.5 μm, bearing two long, initially helical appendages upon release. Zygosporae unknown. In hindgut of Nemouridae (Plecoptera) nymphs.

Etymology. *L. communis* = common.

Specimens examined. NORWAY. Very small stream on E edge of Ekse field station building, 60°49.77'N, 06°14.37' E, 15-V-2002. Microscope slide NOR-20-1 (HOLOTYPE: FH), prepared from a nymph of *Nemoura cinerea* (Retzius) from Site 20 (TABLE I); the slide also contains thalli of *Paramoebidium* sp. (Amoebidiales). Microscope slide NOR-2-W1 (PARATYPE: FH) prepared from a Nemouridae nymph from Site 2. Other specimens collected from sites 2, 19, 20, 23 and 33.

The two pronounced appendages of *G. communis* that initially form a tight helix (FIGS. 12, 13) are similar to those of *G. helicoides* (Williams and Lichtwardt 1987a). Trichospore size ranges do not overlap. Zygosporae are unknown for both species, and Type I (Moss et al 1975) zygosporae will be required for both species to confirm their generic placement. See Discussion for further comments on the taxonomic placement of these species.

Glotzia stenospora M.M. White & Lichtw., sp. nov. FIGS. 14–16

Trichosporae cylindratae, 60–68 × 3–5 μm. Zygosporae (Typus II) 61–72 × 11–14 μm; zygosporophora 30–38 × 8–10 μm. In proctodaeo nympharum Ephemeropterorum affixa.

Trichosporae cylindrical, 60–68 × 3–5 μm. Zygosporae (Type II) 61–72 × 11–14 μm. Zygosporophores 30–38 × 8–10 μm. In hindgut of Ephemeroptera nymphs.

Etymology. Gr. *stenos* = narrow.

Specimens examined. NORWAY. Small unnamed stream

off Kampeveien Road, 59°59.19'N, 10° 28.64' E, 5-V-2002. Microscope slide NOR-5-W17A (HOLOTYPE: FH), prepared from a *Centroptilum luteolum* (Müller) nymph (Ephemeroptera: Baetidae) from Site 5 (TABLE I). Other specimen slides: NOR-5-W17B and NOR-5-W19.

Currently five other species of *Glotzia* have been described, four of them in nymphs of the mayfly family Baetidae, the exception being *Glotzia plecopterorum* Lichtwardt found in Gripopterygidae (Plecoptera) nymphs in New Zealand (Williams and Lichtwardt 1990). At Site 5 we collected a few nymphs of *Baetis niger* (L.), but *G. stenospora* was not found in that host species. The mayfly host *C. luteolum* is the same host in which the type species, *G. centroptili* Manier & Lichtw., was discovered, but neither the trichospore nor zygosporae dimensions match the new species (for *G. centroptili*: trichosporae 40 × 4 μm, zygosporae 50–60 × 15 μm). *Glotzia stenospora* has trichosporae that are similar to those of *G. coloradense* Lichtw. & M.C. Williams (in Williams and Lichtwardt 1987b), but zygosporae of the new species are longer and wider and do not overlap in size.

Legeriosimilis europaeus M.M. White & Lichtw., sp. nov. FIGS. 17–19

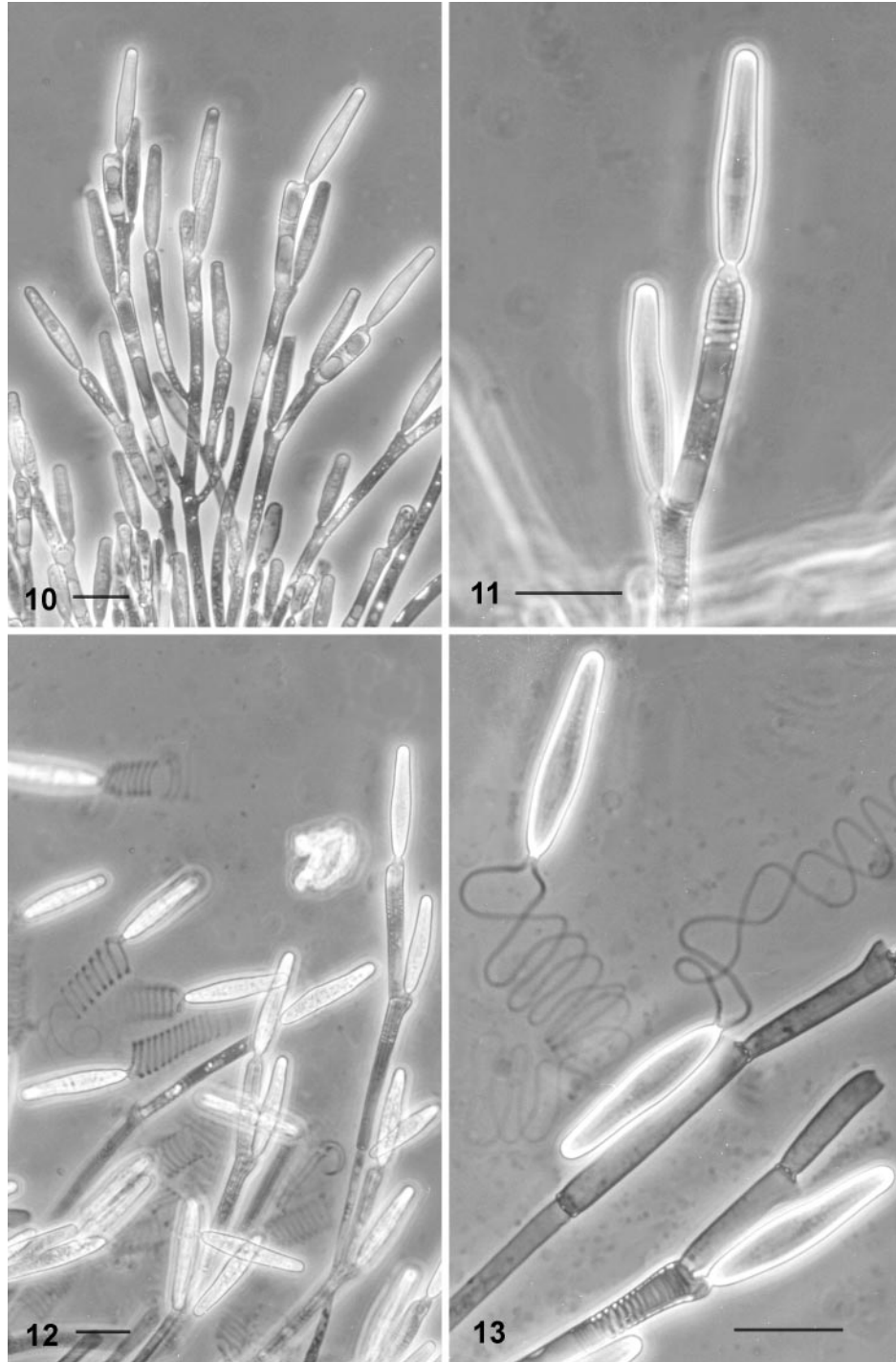
Trichosporae longi obpyriformes, 33–40 × 6–8 μm, appendicibus 3 latis, structura ganglyioidea per appendicem in parte mediana praeditis. Zygosporae (Typus II) 70–83 × 11–13 μm, zygosporophora circa 25–35 × 9 μm. In proctodaeo nympharum Siphonuridarum (Ephemeropterorum) affixa.

Trichosporae long-obpyriform, 33–40 × 6–8 μm, with 3 wide appendages with a knob-like structure part way down each appendage. Zygosporae (Type II) 70–83 × 11–13 μm, zygosporophores about 25–35 × 9 μm. In hindgut of Siphonuridae (Ephemeroptera) nymphs.

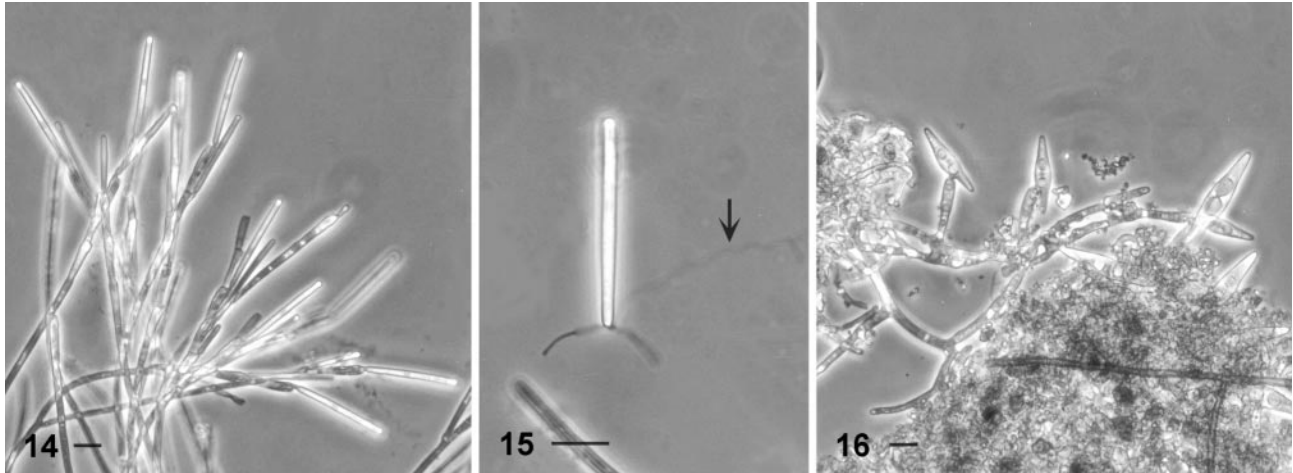
Etymology. *L. europaeus* = of Europe.

Specimens examined. NORWAY. Dalbekken stream, 60°29.99' N, 10°08.00' E, 9-V-2002. Microscope slide NOR-16-W6B (HOLOTYPE: FH), prepared from an *Ameletus inopinatus* Eaton nymph (Ephemeroptera: Siphonuridae) from Site 16 (TABLE I). Additional specimens collected at sites 3, 11, 24, and 31, all in the same host species.

Legeriosimilis tricaudata M.C. Williams & Lichtw., the type species, was described from another *Ameletus* species that was collected in the Colorado Rocky Mountains (Williams and Lichtwardt 1999). The zygosporae of *L. europaeus* are considerably longer (70–83 μm) than the type species (52–58 μm), but there is a slight overlap in the trichospore dimensions (in *L. tricaudata*: [33–]47[–52] × [7–]9.5[–11] μm). The trichospore appendage structure (see FIG. 18) in both species is remarkably similar.



FIGS. 10–13. *Genistelloides communis* from nymphs of *Nemoura cinerea* (Plecoptera: Nemouridae). 10, 11. Attached trichospores (# NOR-19-W7, # NOR-20-1). 12, 13. Newly released trichospores each bearing two appendages initially arranged in a helix (# NOR-33-W11, # NOR-2-W29). Scale bars = 20 μm .



FIGS. 14–16. *Glotzia stenospora* from nymphs of *Centroptilum luteolum* (Ephemeroptera: Baetidae). 14. Attached narrow trichospores (# NOR-5-W17A). 15. Released trichospore with two short appendages and one almost invisible longer appendage (arrow) (# NOR-5-W17A). 16. Biconical zygospores (# NOR-5-W19). Scale bars = 20 μm .

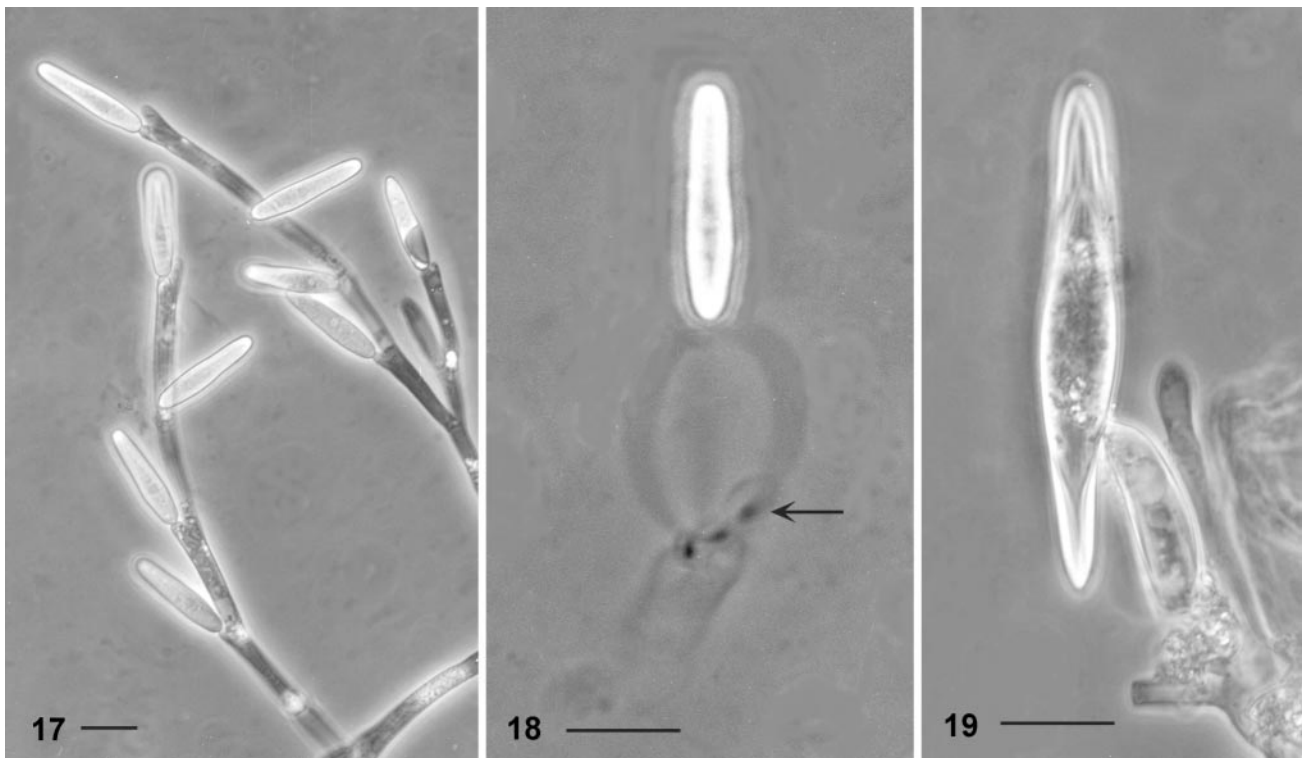
Smittium biforme M.M. White & Lichtw., sp. nov.

FIGS. 20–22

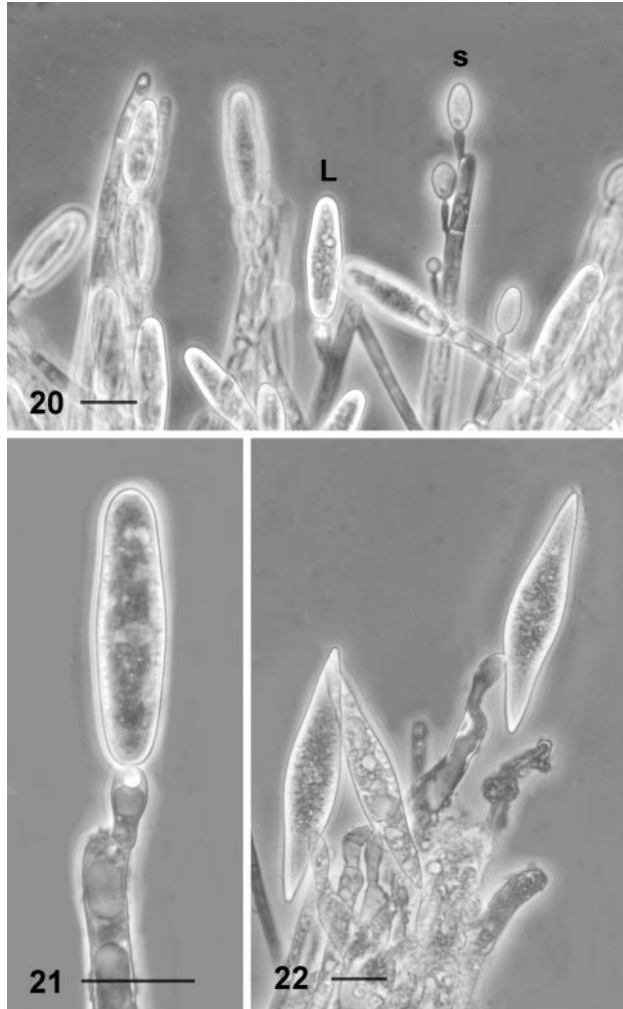
Thalli dimorphi, formas duas trichosporarum producentes, altera longi-ellipsoidalis, 34–42 \times 9–12 μm , collare campanulato, altera ovalis, 13–15 \times 6–7.5 μm , collare cylindra-

ceo. Zygosporeae biconicae (Typus II), 78–82 \times 13–18 μm . In proctodaeo larvarum Chironomidarum affixa.

Thalli dimorphic, producing two trichospore forms: long ellipsoidal trichospores, 34–42 \times 9–12 μm , with a campanulate collar; and oval trichospores, 13–15 \times



FIGS. 17–19. *Legeriosimilis europaeus* from nymphs of *Ameletus inopinatus* (Ephemeroptera: Siphonuridae). 17, 18. Trichospores; upon release trichospores bear three diffuse appendages each with a dark knob-like structure (arrow points to one) part way down the appendage (# NOR-24-2, # NOR-2-W3). 19. Mature biconical zygospore (# NOR-16-W6A). Scale bars = 20 μm .



FIGS. 20–22. *Smittium bifforme* from *Diamesa aberrata* larvae (Chironomidae: Diamesinae). 20. Branches of one thallus producing larger, long-ellipsoidal trichospores (L) and smaller, oval trichospores (s) (# NOR-18-7). 21. A larger trichospore (# NOR-18-W2). 22. Three zygospores (# NOR-18-W2). Scale bars = 20 μm .

6–7.5 μm , with a cylindrical collar. Zygospores biconical (Type II), 78–82 \times 13–18 μm . In hindgut of Chironomidae larvae.

Etymology. L. bis, forma = two shapes

Specimens examined. NORWAY. Seeping cliff on Route 7 at large parking area, 60°28.31'N, 09°56.68'E, 13-V-2002. Microscope slide NOR-18-7 (HOLOTYPE: FH) with two trichospore types; microscope slide NOR-18-W2 (ISOTYPE: FH) with zygospores, from Site 18 (TABLE I) in larvae of *Diamesa aberrata* Lundbeck and possibly *D. bertrami* Edwards (Chironomidae: Diamesinae).

Three other dimorphic *Smittium* spp. currently are described from either Diamesinae or Orthocladinae larvae (*Sm. dimorphum* Lichtw. & Williams, *Sm. esteparum* Lichtw. & López Lastra, *Sm. orthocladii* Manier), but the combined dimensions of trichospores

and zygospores do not match this new species (Lichtwardt et al 2001a). The other species have been found in France, U.S.A. and Argentina.

Smittium precipitorium M.M. White & Lichtw., sp. nov. FIGS. 23–25

Trichosporae ellipsoidales, (13–)17–22 \times 3.5–4.5 μm . Zygosporae 66–75 \times 10–13 μm . In proctodaeo larvarum Chironomidarum.

Trichospores ellipsoidal, (13–)17–22 \times 3.5–4.5 μm . Zygospores 66–75 \times 10–13 μm . In hindgut of Chironomidae larvae.

Etymology. L. precipitium = a cliff (for the seeping cliff where the species was found).

Specimens examined. NORWAY. Seeping cliff on Route 7, 11.5 km from Ørgenvika, Buskerud, Flå, 60°11.98'N, 09°37.59'E, 13-V-2002. Microscope slide NOR-17-W7A (HOLOTYPE: FH) from Chironomidae larvae (both *Orthocladus* [*Eudactylocladius*] *fuscimanus* (Kieffer) and *Diamesa* cf. *aberrata* Lundbeck or *Diamesa* sp. have been identified from among the specimens dissected from this site) at Site 17 (TABLE I). The slide is one of three that include clumps of thalli removed from the guts of five larvae, all of which were infested with sporulating thalli. This slide also contains several clumps of thalli of *Sm. culicis*, which was actually more common at this site than *Sm. precipitorium*, as well as scattered smaller spores, perhaps from a third species (see FIG. 24). However, the slide includes two of five clusters of thalli that are clearly *Sm. precipitorium* on the basis of trichospore size and shape. Combined, these two clumps of thalli bear a total of 18 mature, attached zygospores (with maturity being measured by the wall thickness at the conical tips). The trichospore collar in *Sm. precipitorium*, which is short and exhibits some variability, was nearly campanulate in some instances, a shape that has been noted for the quite variable spores of *Sm. culicis*. This new species description is justified on the basis of the zygospores and trichospores that clearly were evident on the two individual clumps of thalli.

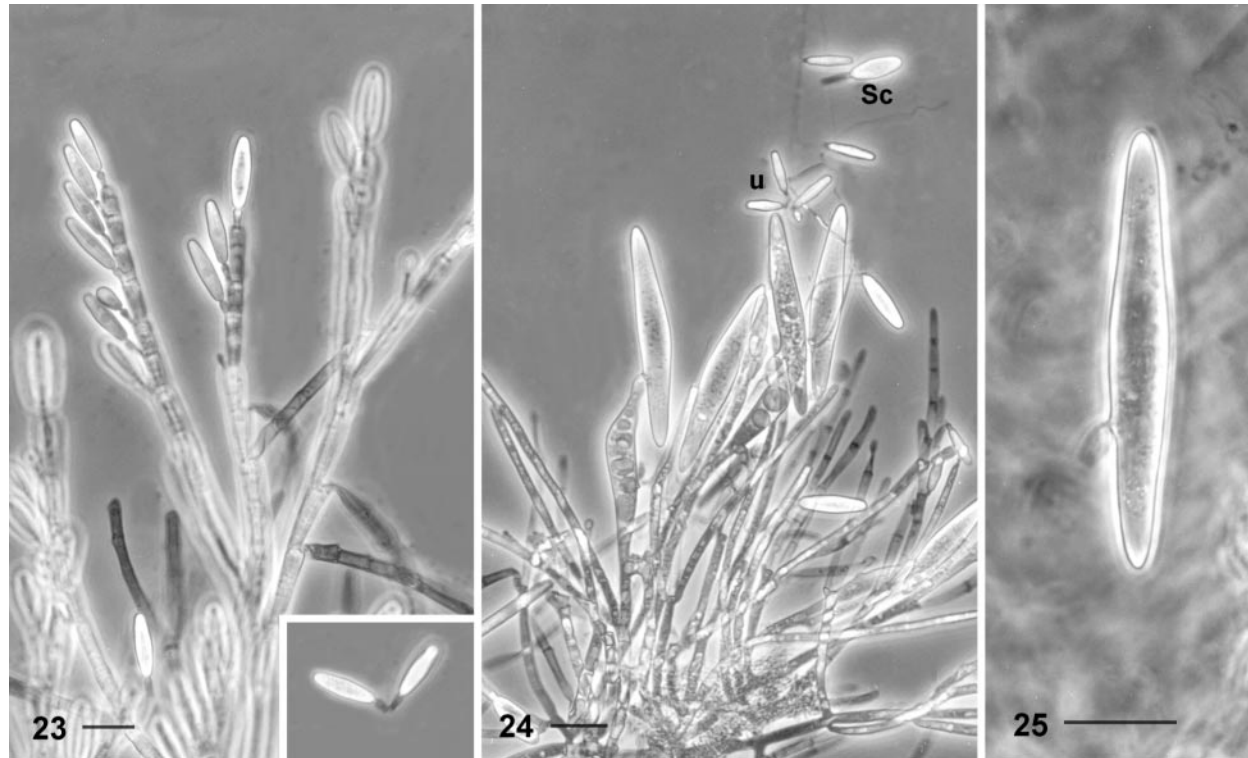
Stachylina acutibasilaris M.M. White & Lichtw., sp. nov. FIGS. 26–28

Thalli 78–155 \times 10–18 μm , cellula basali in haustorio parvo angustata, trichosporas 4 vel plus quam 8 producentes. Trichosporae ellipsoidales, in parte mediana subtumidae, collare carentes, 15–23 \times 5–7 μm . Zygosporae ignotae. In membrana peritrophica larvarum Chironomidarum affixa.

Thalli 78–155 \times 10–18 μm , basal cell tapering to a small holdfast, producing 4–8 or more trichospores. Trichospores ellipsoidal with a slight median swelling, without a collar, 15–23 \times 5–7 μm . Zygospores unknown. Attached to peritrophic membrane of Chironomidae larvae.

Etymology. L. acutus, basilaris = pointed at the base.

Specimens examined. NORWAY. Small stream crossing



FIGS. 23–25. *Smittium precipitiorum* from Chironomidae larvae (Diptera). 23. Attached and (inset) released trichospores (# NOR-17-4, # NOR-17-2). 24. Zygospores and trichospores; this chironomid hindgut also contained *Smittium culicis* (Sc) and another, unidentified *Smittium* sp. (u) with smaller trichospores. (# NOR-17-W7A) 25. Released zygospore with a collar (# NOR-17-W7C). Scale bars = 20 μ m.

Route 759 SSE of Steinkjer, 63°57.64'N, 11°34.27'E, 28-VIII-2002. Microscope slide NOR-54-W14 (HOLOTYPE: FH), prepared from a *Brillia* cf. *longifurca* Kieffer larva (Diptera: Chironomidae) collected from Site 54 (TABLE I).

This species is most similar to *St. ceratopogonidarum* Lichtw. & Arenas, a species from Chilean Ceratopogonidae larvae, but the thalli of *St. acutibasilaris* are wider (10–18 versus 5–8 μ m)

Stachylina lentica M.M. White & Lichtw., sp. nov.

FIGS. 29–33

Thalli 50–150 \times 4–9 μ m, base haustorio rotundato parvo, trichosporas 1–2(–4) producentes. Trichosporae longi-ellipsoideae, (22–)35(–50) \times (4–)5–6(–7) μ m, collare brevissimo, appendice perlonga tenui. Zygosporae ignotae. In membrana peritrophica larvarum Chironomidarum affixa.

Thalli 50–150 \times 4–9 μ m, with a small, rounded holdfast at the base, producing 1–2(–4) trichospores. Trichospores long-ellipsoidal, (22–)35(–50) \times (4–)5–6(–7) μ m, with a very short collar, and a very long and fine appendage. Zygospores unknown. Attached to peritrophic membrane of Chironomidae larvae.

Etymology. L. lentus = slow (for the lentic habitat).

Specimens examined. NORWAY. Lake Snåsavatnet rocky bottom near shoreline, 64°07.65'N, 11°45.44'E, 23-VIII-2002. Microscope slide NOR-45-W1 (HOLOTYPE: FH),

prepared from a *Chironomus* sp. larva (Chironomidae) from Site 45 (TABLE I). Also from Site 45, in a larva of *Brillia* cf. *longifurca* Kieffer (Chironomidae) and in many larvae of *Chironomus* sp. from Site 58.

Twenty-two species of *Stachylina* have been described (Lichtwardt et al 2001a). Of these, *St. lentica* most closely resembles *St. grandispora*, a widespread species reported from Europe, U.S.A. and Australasia in various genera of Chironomidae larvae but most commonly in *Chironomus* spp. Several differences, however, are evident, leading us to describe the Norway species as new. *Stachylina grandispora* on the average has larger trichospores (40–72 \times 6–10 or more μ m), and thalli are generally longer and wider (<100 to >250 μ m long by 6–10 μ m diam). Although *St. grandispora* rarely may have as few as two trichospores per thallus, more commonly it produces 4–16 trichospores. Thalli of *St. lentica* most commonly produce only 1–2 trichospores, and rarely 4.

POSSIBLE NEW SPECIES

The following two species appear to be new but are not named here. We are furnishing descriptions and illustrations together with locations and hosts for future reference.

Smittium sp. 1

FIGS. 34–36

Larvae of *Chironomus* sp. from a rock pool at Site 38 had in their hindgut what appears to be an undescribed species of *Smittium*. Its diagnosis follows. Thallus base horseshoe-shaped, larger thalli may have verticillate branching, smaller thalli sparsely branched, 3–6 trichospores per branchlet. Branches 2–5 μm diam. Trichospores subcylindrical, 15–18 \times 2–2.5 μm .

Smittium sp. 2

FIGS. 37–40

What appears to be another undescribed species of *Smittium* was found in the hindgut of an unusual range of hosts. These included Tipulidae larvae (Site 11), and Chironomidae larvae of *Limnophyes* sp. and *Corynoneura* cf. *lobata* Edwards (both Orthocladinae) (Site 19) and *Corynoneura* sp. from Site 22. Larvae of *Limnophyes* and *Corynoneura* are minute, usually measuring only a few millimeters long. The diagnosis of this species follows. Colonies compact, producing ellipsoidal trichospores 9–13 \times 3.5–4 μm , collar short up to 5 μm long.

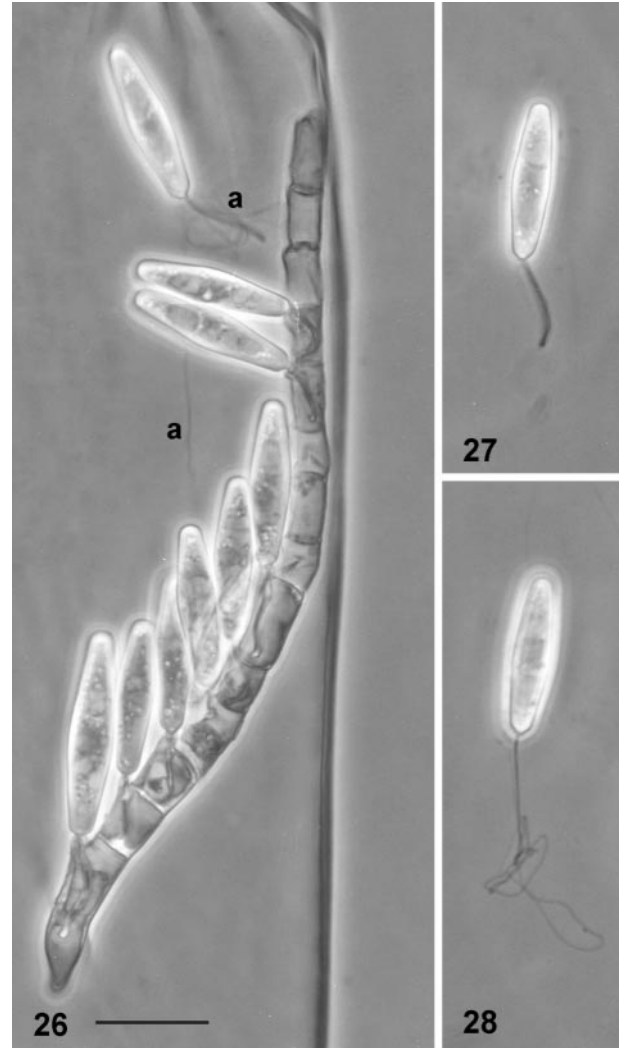
PREVIOUSLY DESCRIBED SPECIES

Graminella microspora S.T. Moss & Lichtw.

Although not particularly common worldwide, *G. microspora* has a widespread distribution in Baetidae (Ephemeroptera), having been reported from the U.S.A., Switzerland, and southern Chile (Lichtwardt 1986, Lichtwardt and Arenas 1996). In Norway, the species was found at Site 35 in *Baetis rhodani* (Pictet). Though the specimens in our collection lacked zygospores, this species is easily identifiable by both the long series of minute collarless trichospores (6–8.5 \times 2–2.5 μm) with a single appendage and the peculiar bulbous outgrowths that detach from the thalli and produce new thalli vegetatively (see FIGS. 27–30 in Lichtwardt and Arenas 1996).

Harpella melusinae Léger & Duboscq

Harpella melusinae is perhaps the most widely distributed species of Harpellales. It has been found attached to the peritrophic membrane (also known as the peritrophic matrix) in most populations of black fly larvae, which have been studied in many parts of the world, although currently it has not been collected in Central or South America where other species of *Harpella* are common. The species was found in almost all streams where Simuliidae were collected in Norway (sites 2, 4, 5, 7, 8, 11, 15, 16, 21, 22, 23, 26, 28, 33, 36, 42, 51, 52, 53, 54). The larval species included *Prosimulium hirtipes* (Fries), *Eusimulium vernum* (Macquart), *Simulium monticola* Friederichs,



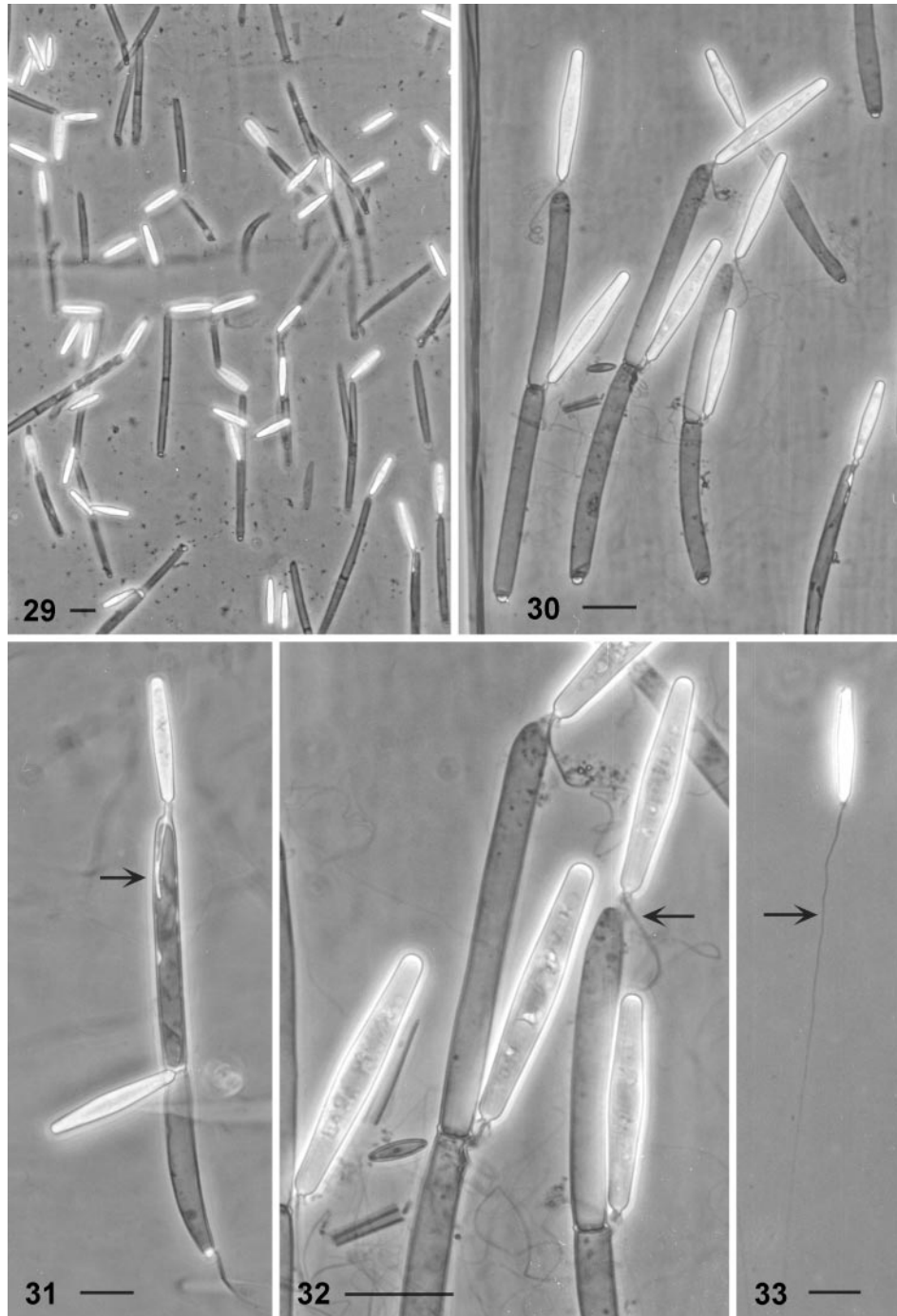
FIGS. 26–28. *Stachylina acutibasilaris* from a *Brillia* cf. *longifurca* larva (Diptera: Chironomidae). 26. Entire thallus (composite of two photomicrographs) with attached and released trichospores as seen within the transparent peritrophic membrane; a = an extended appendage of one trichospore. 27. Trichospore with its single appendage as yet unfurled. 28. Trichospore with unfurling fine appendage. Scale bar = 20 μm (# NOR-54-W14).

S. ornatum Meigen, *S. tuberosum* (Lundström), and *S. rotundatum* (Rubzov).

Harpellomyces eccentricus Lichtw. & S.T. Moss

FIGS. 41–47

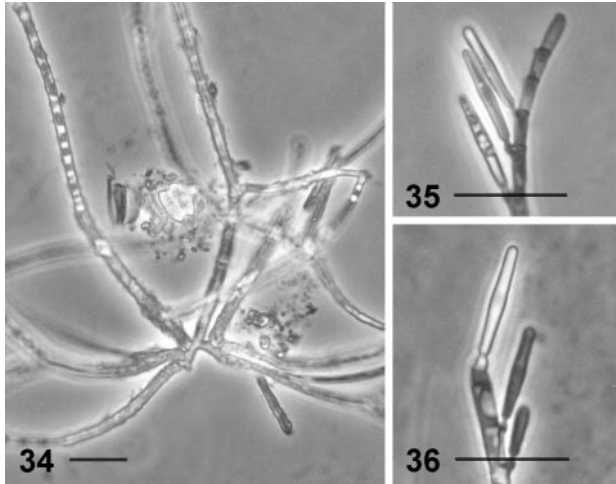
This type species originally was known only from Sweden and Wales in solitary midge larvae, *Thaumalea* spp. (Diptera: Thaumaleidae) (Lichtwardt and Moss 1984). Later, Santamaria and Girbal (1997) reported it from *Thaumalea* sp. in Spain. We obtained



FIGS. 29–33. *Stachylina lentica* from a *Chironomus* sp. larva (Diptera: Chironomidae). 29, 30, 31. Unbranched sporulating thalli within the host's peritrophic membrane; arrow in FIG. 31 points to an appendage within a generative cell (# NOR-58-10, # NOR34-W1, # NOR-58-5). 32. Trichospores in the process of breaking away from the thallus with the appendage of each being released from the generative cell (arrow) (# NOR-45-W1). 33. Trichospore with its very long, fine appendage (arrow) (# NOR-45-W1). Scale bars = 20 μ m.

sporulating specimens of *H. eccentricus* in larval *Thaumalea* sp. living in madicolous habitats (shallow sheets of water running or seeping down steep cliffs) at sites 34 and 56. The fungus probably is distributed more widely in Europe, but thaumaleids often have

been overlooked as potential harpellid hosts. The only other species of *Harpellomyces* currently named is *H. abruptus* Lichtw., M.M. White & Colbo collected from *Thaumalea verralli* Edwards larvae in Newfoundland, Canada (Lichtwardt et al 2001b).



FIGS. 34–36. *Smittium* sp. 1 from *Chironomus* sp. larvae (Diptera: Chironomidae). 34. Thallus with a horseshoe-shaped holdfast cell at its base (# NOR-38-15). 35, 36. Sub-cylindrical trichospores # NOR-38-13, # NOR-38-8). Scale bars = 20 μ m.

Legeriomyces ramosus Pouzar

Legeriomyces ramosus first was discovered in France (Léger and Gauthier 1932), later in France, England, Switzerland, U.S.A. (Lichtwardt 1986) and more recently from Spain (Valle and Santamaria 2002). It is most common in Baetidae but also has been reported from another mayfly family, Ephemerellidae (Lichtwardt 1986). In Norway, both trichospores and zy-

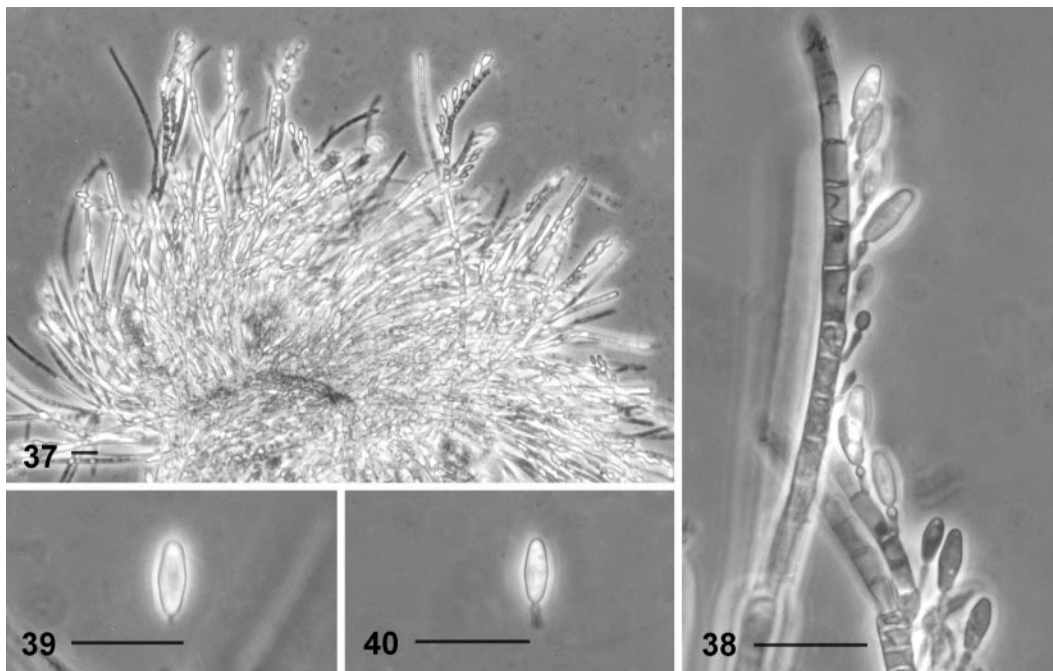
gospores of this species were found. It was no surprise to find this species in *Baetis rhodani* and also in a previously unrecorded mayfly host genus from Site 35, *Siphonurus* (Siphonuridae). Collections were obtained at sites 4, 5, 6, 7 and 35.

Orphella catalaunica Santam. & Girbal FIGS. 48–51

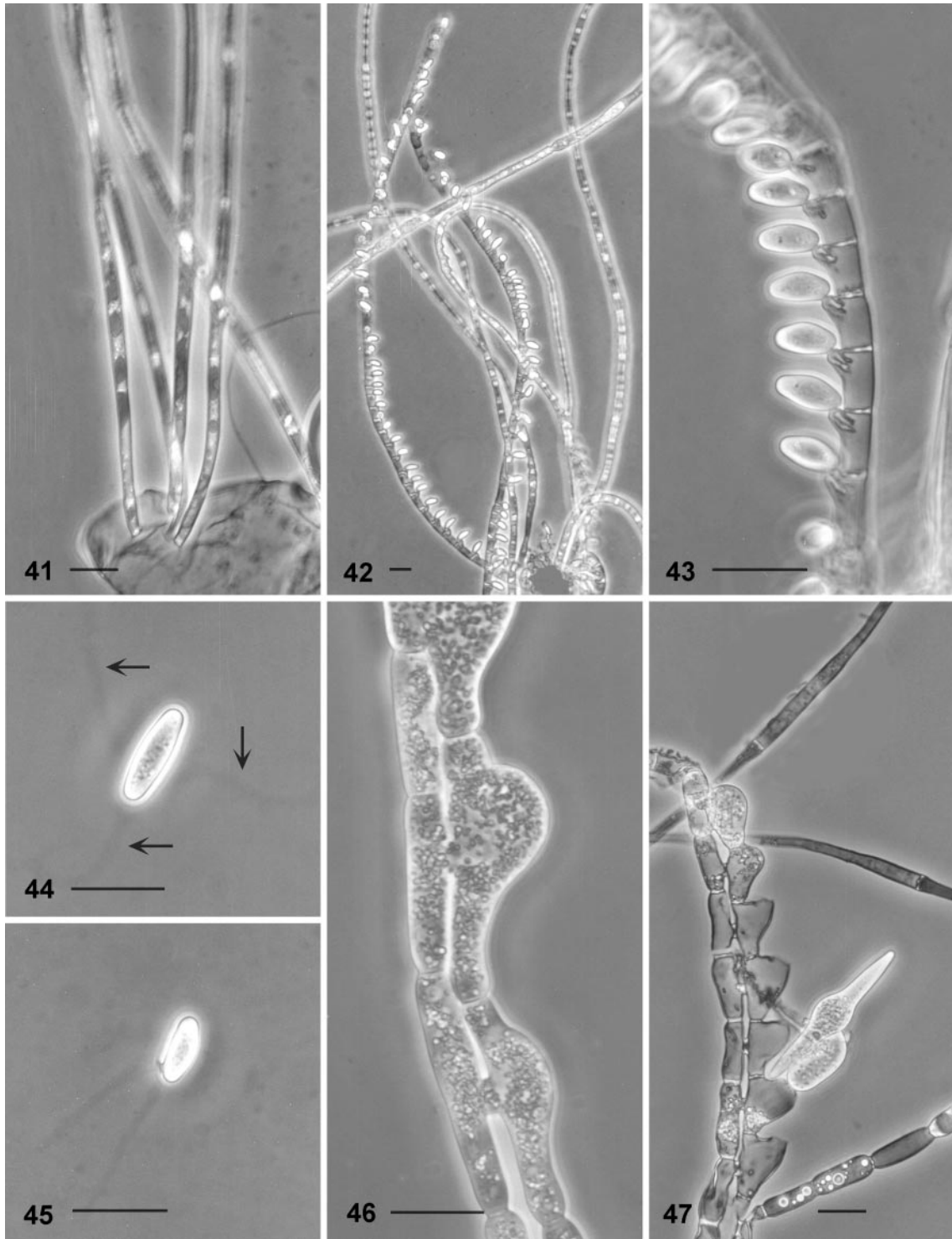
We found *O. catalaunica* at Norway sites 4, 9, 21, 28, 30, 33, 40 and 61 in *Leuctra hippopus* Kempny and probably other *Leuctra* spp. (Plecoptera: Leuctridae). It had been recorded originally from Spain in *Leuctra* sp. nymphs (Santamaria and Girbal 1998). *Orphella* is one of three genera of Harpellales whose mature thalli project from the anus of the host; consequently, often they can be seen with a stereo microscope before dissection. Our specimens conform well to the original description.

Orphella coronata Léger & Gauthier FIGS. 52–55

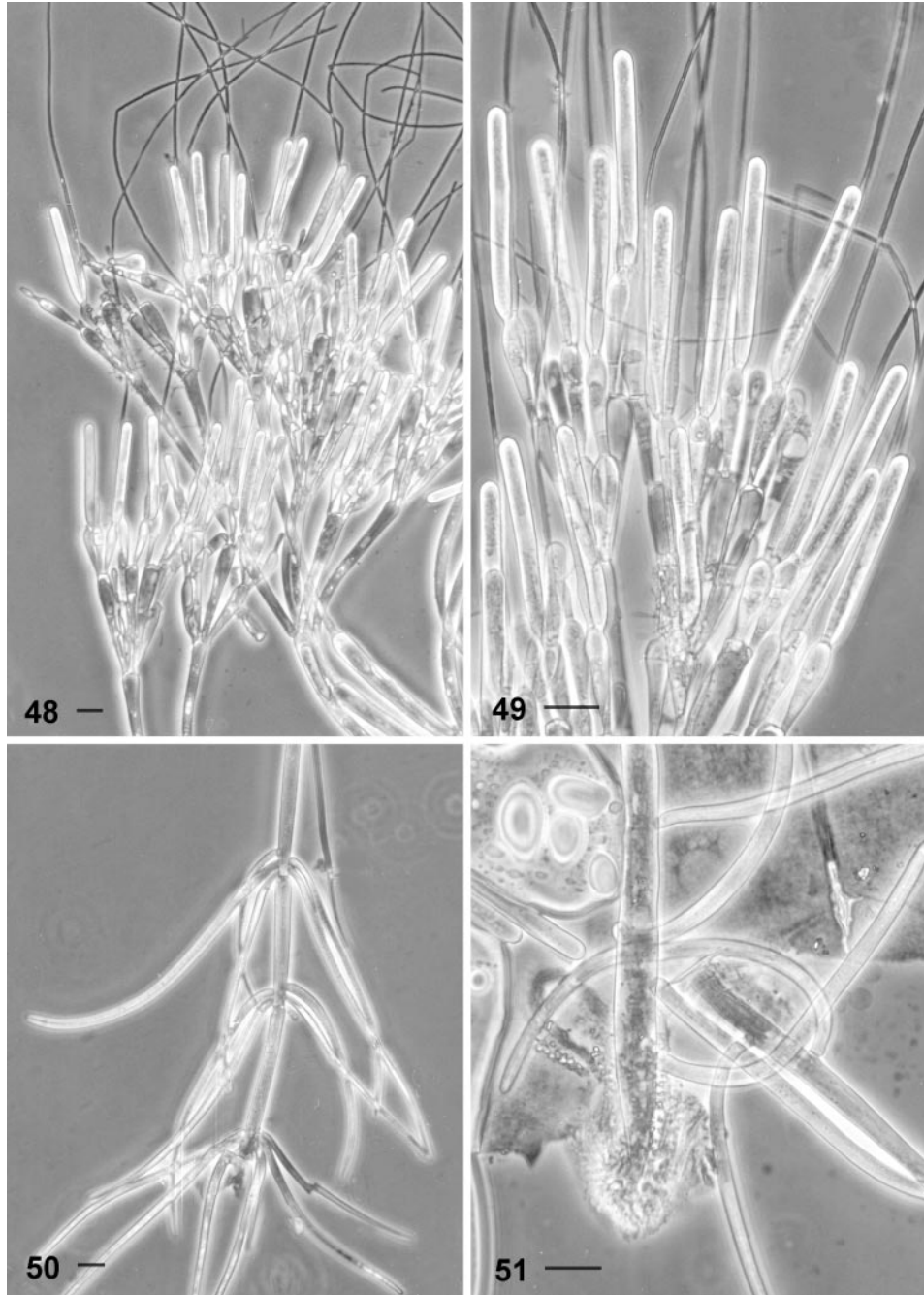
This is the type species of the genus, but it has not been reported since the original publication (Léger and Gauthier 1931). The original description was incomplete, yet our specimens seem to be conspecific with Léger's and Gauthier's species, which they found living in *Protonemura humeralis* Pictet nymphs (Plecoptera: Nemouridae) in mountain streams of southeastern France. Our collections were from sites 4, 5, 10, 19 and 33. Hosts at most sites were *Nemoura*



FIGS. 37–40. *Smittium* sp. 2 from Chironomidae larvae (Diptera). 37. A dense, sporulating colony (# NOR-19-4). 38. Attached trichospores (# NOR-11-W21A). 39, 40. Released trichospores showing variation in overall size and collar lengths (# NOR-22-7, # NOR-11-W21A). Scale bars = 20 μ m.



FIGS. 41–47. *Harpellomyces eccentricus* from *Thaumalea* sp. larvae (Diptera: Thaumaleidae). 41. Unbranched bases of thalli with holdfasts attached to gut lining (# NOR-34-5). 42, 43. Long series of trichospores developing on unbranched thalli (# NOR-56-W2). 44. Released trichospore with three barely visible appendages (arrows) (# NOR-34-W17A). 45. Trichospore with two appendages in focus showing the scar on lower left where it was attached to the generative cell (# NOR-56-W2). 46. Two conjugating thalli with cells of one showing typical swollen structure that precedes zygospore formation (# NOR-34-W2). 47. Zygospore produced from a swollen conjugant cell (# NOR-34-W2). Scale bars = 20 μm.

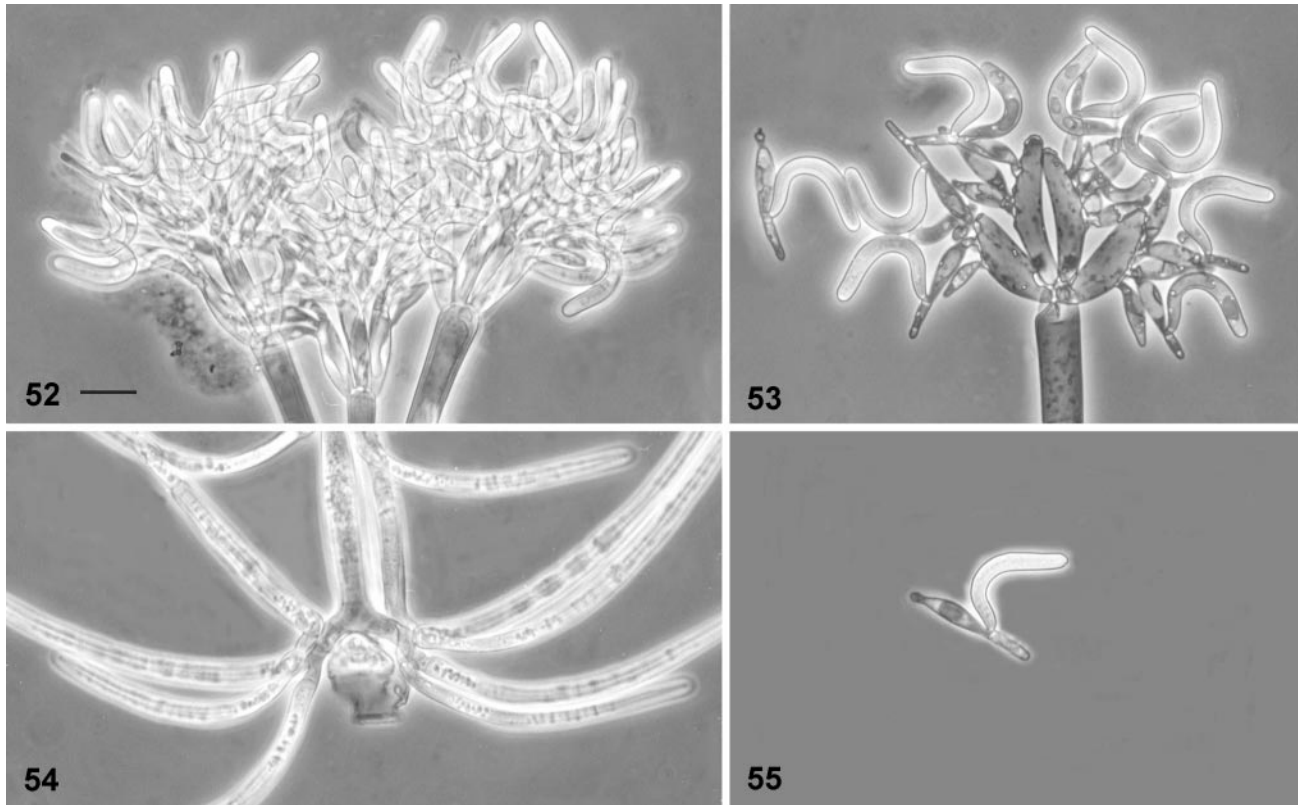


FIGS. 48–51. *Orphella catalaunica* from nymphs of *Leuctra hippopus* (Plecoptera: Leuctridae). 48, 49. Sporulating heads with cylindrical trichospores and long, filiform cells that constitute the terminal cell of the dissemination unit (# NOR-19-W2). 50, 51. Bases of thalli showing variation in the holdfast structure (# NOR-61-W2, # NOR-19-W1). Scale bars = 20 μ m.

cinerea (Retzius), but in two collections (sites 4, 5) we found *O. coronata* in nymphs of *Brachyptera rise* (Morton) that belong to a different family of stoneflies, Taeniopterygidae.

Orphella helicospora Santam. & Girbal FIGS. 56–59
This is the second species of *Orphella* described by

Santamaria and Girbal (1998) from Spain, also in *Leuctra* sp. nymphs. Our collections of this species were found not only in *Leuctra hippopus* (Site 11) but also in *Nemoura cinerea* and *N. nigra* (Olivier) (sites 10, 19). The hindguts of some *Nemoura* nymphs also contained *O. coronata*. The very long, straight terminal cells of the dissemination units shown in FIGS. 56–58 often were shorter in many thalli.



FIGS. 52–55. *Orphella coronata* from nymphs of *Nemoura cinerea* (Plecoptera: Nemouridae). 52. Sporulating head of a thallus with allantoid trichospores (# NOR-33-W8). 53. Dissemination units releasing from a sporulating head (# NOR-19-W7). 54. Base of a thallus with a bulbous holdfast cell (# NOR-19-W3A). 55. A dissemination unit consisting of an allantoid trichospore and two auxiliary cells (# NOR-5-W10). Scale bar = 20 μm .

Pennella arctica Lichtw. & M.C. Williams

Pennella arctica has been reported from northern Sweden and northwestern Montana in several different species of Simuliidae (Lichtwardt 1984). We found this fungus to be common in Norway with records for various species of Simuliidae at sites 2, 7, 22, 26, 28, 33 and 42 (see *Harpella melusinae* above for a list of some of these simuliids). In some instances we pooled (immature) nonsporulating and (mature) sporulating stages of this species of *Pennella* spp. within sites because this is a genus that can be identified by the characteristic simple to dichotomously branched basal cell that attaches to the cuticle by a mucilaginous substance (e.g., see FIGS. 29 and 30 in Lichtwardt et al 2001b).

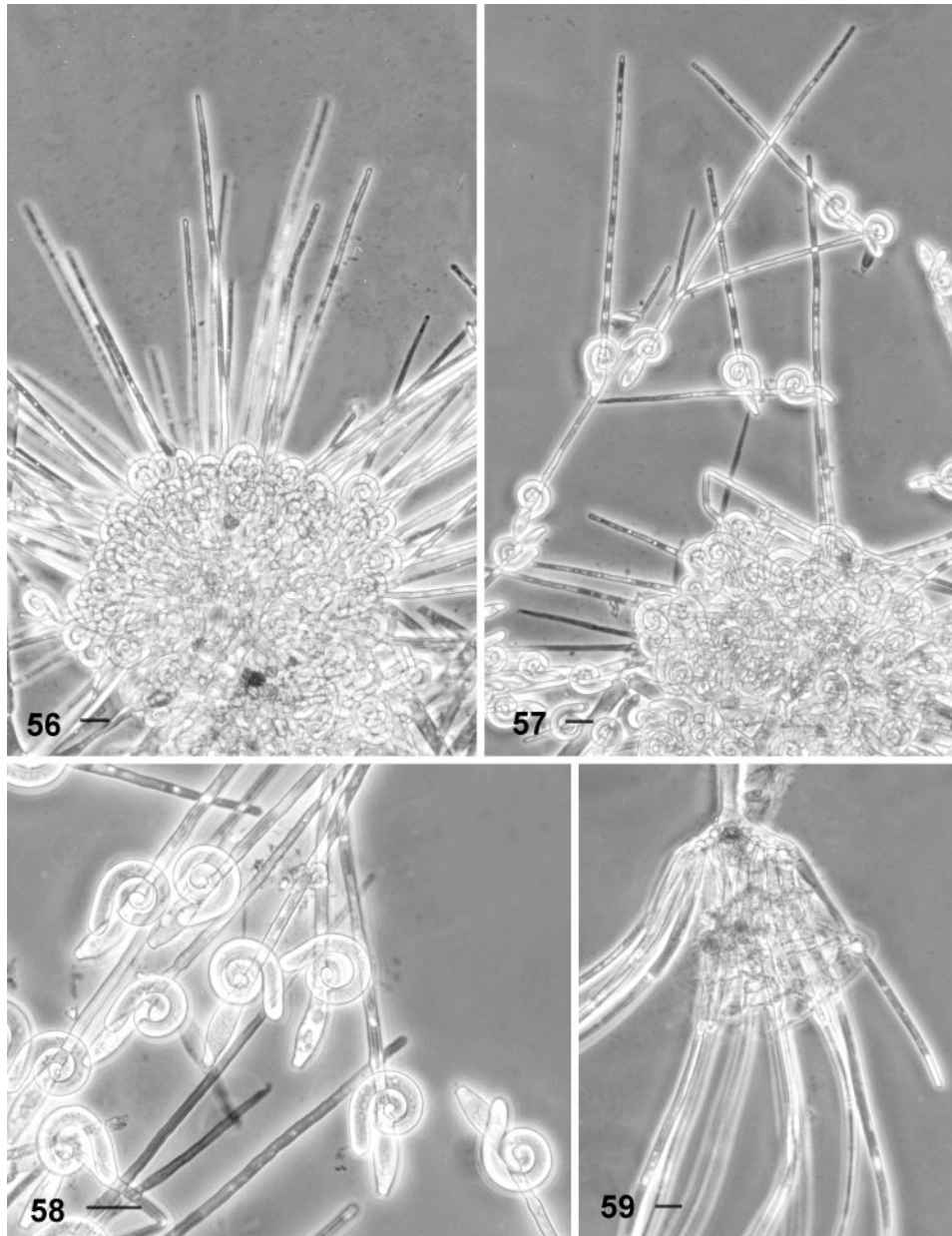
Simuliomyces microsporus Lichtwardt

This species has been found in Simuliidae larvae in Australia, France, Great Britain, Sweden, U.S.A. (Lichtwardt et al 2001a) and now Norway. In most instances this interesting species does not attach directly to the hindgut cuticle but rather to thalli of other gut inhabitants. In Norway we found it attached to *Pennella arctica* or *Paramoebidium* spp.

(Amoebidiales) but it is also known to attach to *Genistellospora homothallica* Lichtwardt. (Species of Amoebidiales are protists that were once considered to be Trichomycetes and may be found in Simuliidae larvae but even more commonly in nymphs of mayflies and stoneflies. We found *Paramoebidium* spp. frequently in Norway [sites 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 15, 16, 19, 20, 21, 22, 30, 33, 35, 40, 42, 43, 50, 52, 53, 54, 61] but these are not reported in detail in this paper.) *Simuliomyces microsporus* was collected at sites 2, 7, 11, 30 and 33.

Smittium coloradense Lichtw. & M.C. Williams

Our specimens of *Sm. coloradense* were obtained from a chironomid midge collected from a seeping cliff in Norway (Site 46). It appears to be the same species originally described in various genera of Chironomidae and *Prosimulium* (Simuliidae) larvae taken from a stream in the Rocky Mountains of Colorado (Williams and Lichtwardt 1987b, Lichtwardt and Williams 1988), based on trichospore morphology and the characteristic tapered basal cell with protrusions. Beard and Adler (2003) subsequently reported *Sm. coloradense* from chironomid larvae cling-



FIGS. 56–59. *Orphella helicospora* in nymphs of *Nemoura cinerea* (Plecoptera: Nemouridae). 56. A dense sporulating head (# NOR-10-W3). 57, 58. Sporulating heads releasing dissemination units with long, terminal filamentous cells; in other specimens the terminal cells may be much shorter (# NOR-10-W3). 59. Bifurcate branching at the base of a thallus (# NOR-11-W17). Scale bars = 20 μm .

ing to a seeping cliff in South Carolina and described zygospores for the first time. A few of the many trichospores in the Norwegian collection were slightly longer ($25\text{--}40 \times 7\text{--}8 \mu\text{m}$) than originally described for the species ($[17\text{--}]26\text{--}[32] \times [7\text{--}]8\text{--}[9.5] \mu\text{m}$), but collar lengths were similar ($3\text{--}3.5 \mu\text{m}$). Zygospores of this fungus were not found in Norway.

Smittium culicis Manier

We found *Sm. culicis*, essentially a worldwide spe-

cies with a wide host range among families of nematoceran Diptera, in mosquito larvae at Site 45A, in chironomid larvae of *Orthocladus* (*Eudactyclus*) *fuscimanus* (Kieffer) (Site 17), *Diamesa aberrata* Lundbeck (Site 18), and *Psectrocladius* (*Psectrocladius*) *limbellatus* group (Sites 38, 59). *Smittium culicis* also was present in larvae from Site 17, living in the same guts with the new species *Smittium precipitorium* described above. The following axenic cultures of *Sm. culicis* (listed with the hosts from which they were

extracted) were isolated and have been deposited in University of Kansas Mycological Culture Collection (KUMYCOL): NOR-25-W3, NOR-25-W10A, NOR-45-W17, NOR-45-W22 (all from mosquitoes) and NOR-59-2 (from *Psectrocladius limbellatus* group).

Smittium mucronatum Manier & Mathiez ex Manier

The discovery of *Sm. mucronatum* in larvae of *Psectrocladius* (*Psectrocladius*) *limbellatus* group from two collections at a small rock pool (sites 58 and 62) in Norway presents an interesting biogeographical distribution. Originally described from southern France in larvae of *P. sordidellus* (Zett.) (Manier and Mathiez 1965), it later was discovered in a high altitude kettle pond in Colorado, within an undetermined species of *Psectrocladius* (Lichtwardt and Williams 1999). Thus, all three disjunct records of *Sm. mucronatum* are in the same chironomid genus. The trichospore has a minute nipple at its tip, and the fungus produces Type II zygospores, characters that confirm the identity of the fungus in all three geographic regions. The trichospores and zygospores from Norway were at the lower range of those published for this species, but the trichospore nipple and host association substantiate the species identification. Axenic cultures of *Sm. mucronatum* were obtained from France (Lichtwardt 1986) and Colorado (Lichtwardt and Williams 1999) but not from Norway. These isolates have been used for several physiological, host specificity and molecular systematic studies (Lichtwardt et al 2001a, Gottlieb and Lichtwardt 2001, White 2002).

Smittium simulii Lichtwardt

Smittium simulii, like *Sm. culicis*, has been found on many continents and in many families of nematoceran Diptera (Lichtwardt et al 2001a). In Norway we found this fungus not only in simuliid larvae at sites 8 and 28, but also in mosquito larvae (Culicidae) at sites 25 and 32. This is apparently only the second finding of *Sm. simulii* in mosquitoes (also recently observed in Spain by Valle and Santamaria pers comm). An axenic isolate, NOR-28-7, was obtained and accessioned in the KUMYCOL.

Stachylina pedifer M.C. Williams & Lichtw.

At Site 21 we found in the peritrophic membrane of an unidentified chironomid larva thalli and trichospores that appear to be morphologically similar to *St. pedifer* found in northwestern Montana, on larvae of *Boreoheptagyia lurida* (Garrett). Thalli of *St. pedifer* are small with a foot-like swelling at the base that penetrates the peritrophic membrane, and produce 2–8 trichospores per thallus (Lichtwardt and

Williams 1983). *Stachylina pedifer* also has been found in South Carolina by Beard and Adler (2003), who induced previously unknown zygospores in vitro.

DISCUSSION

Trichomycetes were common in Norway, and many were found that added new species or extended the hosts and ranges of previously known species, with no major departures from expected morphologies. However, *Ephemerellomyces aquilonius* had a novel stage of trichospore development. The source of the trichospores that attach to and germinate on the hindgut cuticle of this new genus and species is not known (FIGS. 4–6). They might be trichospores that had been ingested by the larvae or ones that were produced by established thalli within the gut. The uniqueness of their development is that the germination process yields a cell that functions as a generative cell to produce a single trichospore. Replication of thalli within the gut by internally produced spores is common in Eccrinales but rare in Harpellales. In the latter order, examples are *Graminella* spp. from Baetidae (Ephemeroptera), where specialized detachable branches break off from thalli, attach to the cuticle and grow into sporulating thalli (see illustrations in Lichtwardt and Moss 1981 or Lichtwardt and Arenas 1996); and in the monotypic Western Australian genus *Allantomyces* from Caenidae (Ephemeroptera), specialized allantoid cells are produced on branchlets, detach and produce new thalli in the same gut (Williams and Lichtwardt 1993). But in none of the species of these two genera do the vegetative propagules resemble the apparent trichospore attachment and germination in *E. aquilonius*.

Generic placement of *Genistelloides communis* from Nemouridae nymphs is based primarily on the nature of the two relatively heavy appendages arranged in a helix when they first emerge from their generative cells. (FIGS. 12, 13). This is similar to *G. helicoides* originally described from Nemouridae nymphs in the Rocky Mountains of Colorado (Williams and Lichtwardt 1987a) but now also known from other streams in eastern North America (unpublished). In neither species are zygospores known, but zygospores (Type I [Moss et al 1975], similar to FIG. 9 in this paper) were described in the type species, *G. hibernus* (Peterson et al 1981), which lives in *Allocapnia* spp. nymphs (Plecoptera: Capniidae) throughout much of eastern U.S.A. (Lichtwardt et al 1993). Zygospores (Type I) are necessary to confirm the generic placement of these two species from nemourids. It is possible that *G. communis* is more closely related to *Lancisporomyces vernalis* Santamaria (Santamaria 1997), a monotypic genus described from *Nemoura* sp. in

Spain. *Lancisporomyces* has unique lance-shaped zygospores (Type IV), discovery of zygospores in *G. communis* consequently would provide confirmation of the correct genus.

The discovery of some previously known, widespread, harpellid species in Norway is not surprising. *Harpella melusinae*, *Simuliomyces microsporus*, *Smittium culicis* and *Sm. simulii* (although the latter's occurrence in mosquitoes is unusual) all have wide geographical distributions in their respective types of hosts. A less common harpellid, however, demonstrates a disjunct distribution: *Smittium mucronatum* first was discovered in France (Manier and Mathiez 1965), later in Colorado (Lichtwardt and Williams 1999) and now in Norway. In all three instances the respective fungi were found in different species of the same chironomid genus, *Psectrocladius*. As another example, *Harpellomyces eccentricus* now is known from Norway (this study), Sweden, Wales (Lichtwardt and Moss 1984) and Spain (Santamaria and Girbal, 1997); *Harpellomyces abruptus* was described from Newfoundland (Lichtwardt et al 2001b) (with possibly another, unpublished species in the U.S.A.); and a species of *Harpellomyces* was recorded from Japan (Lichtwardt et al 1987, but probably not *H. eccentricus* as indicated in that publication). *Harpellomyces* in Europe, North America and Japan all were living in different species of *Thaumalea*. Only *T. verralli* Edwards in Canada was identified to species because of difficulty in identifying larval stages of thaumaleids. In Australia, *Austrothaumalea* spp. hindguts did not contain *Harpellomyces* but were infested with *Smittium culicis* (a species adapted to many nematoceran families) from which five axenic isolates were obtained (Lichtwardt and Williams 1990).

The three *Orphella* spp. we found in Norway are known only from Europe. *Orphella* spp. in the U.S.A. are different from those in Europe, with the exception of *Orphella haysii*, originally described from Colorado (Williams and Lichtwardt 1987a) in nymphs of *Zapada haysi* Ricker, *Z. cintipes* Banks, and *Malenka flexura* Claassen (Nemouridae), which Santamaria and Girbal (1998) subsequently reported from Spain in *Nemoura* sp. Both *O. haysii* and the European *O. coronata* are similar morphologically. It is possible that specimens of *O. haysii* found in Spain and *O. coronata* are conspecific. We expect through DNA sequencing to corroborate their identities and to test the hypothesis that *Orphella* spp., *Harpellomyces* spp. and perhaps disjunct species of other genera such as *Genistelloides* and *Lancisporomyces* have undergone vicariant speciation subsequent to severance of the last land bridges between Europe and North America as they became separated tectonically.

Our survey of Harpellales in Norway emphasizes

the need for continued efforts to find and identify these endosymbionts, not only in unexplored regions of the country (not covered in this survey) but also beyond it to estimate more accurately their biodiversity with a global perspective.

ACKNOWLEDGMENTS

We are indebted to the National Science Foundation for award DEB-0108110, which supported this research. We acknowledge with considerable appreciation a number of biologists in Norway whose assistance materially facilitated our research and made it more successful. Leif Ryvarden provided excellent laboratory space and facilities in the Department of Botany, University of Oslo. John E. Brittain, Trude Vrålstad and Arne Holst-Jensen suggested a number of collecting sites in the vicinity of Oslo. Arne Fjellheim, Stavanger Museum, allowed use of the subalpine Field Station at Ekse administered by the University of Bergen, and arranged, through Ole Kristian Berg, Zoologisk Institutt, University of Trondheim, our use of the Snåsavatnet Field Station in the northern part of Trondheimsfjord. We thank these entomologists for their considerable time and expertise in identifying larval hosts of Harpellales: John E. Brittain (Plecoptera and Ephemeroptera) and Jan Emil Raastad (Simuliidae), The Natural History Museums, University of Oslo; Ole A. Sæther (Chironomidae), Museum of Zoology, University of Bergen; and Brad Sinclair (Thaumaleidae), Museum Koenig. We thank Matías Cafaro who provided valuable comments on a draft of this manuscript. We also are grateful for the thoughtful comments of C. Eddie Beard and an anonymous reviewer that improved the paper.

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