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Costa Rican gut fungi (Trichomycetes) infecting lotic insect larvae

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Abstract: Trichomycetes infecting freshwater Ephemeroptera and Diptera (Simuliidae, Chironomidae) in various life zones of Costa Rica were studied. Insect populations in 36 streams were sampled, a few repeatedly, at four different periods of time over seven years. Eleven new species of Harpellales are reported, plus six species known to occur also in nontropical parts of the world. Included are new species of *Harpella* and *Pennella*, a new monotypic genus (*Graminelloides)*, and four new species of the previously monotypic genera *Spartiella* and *Genistellospora*. Seven new *Smittium* species, including three that are not named at this time, were cultured axenically. A new *Amoebidium* (Amoebidiales) was found in a stream polluted with organic matter. In the same polluted stream more species of dipteran larvae and a greater number of gut fungi were present than in stretches of unpolluted water upstream. The overall diversity of Harpellales and their aquatic insect hosts in most Costa Rican streams appeared to be lower than what the author has found in many lotic habitats in more northern and southern regions of the world, and thus this order of fungi may be an exception to the 'latitudinal species diversity gradient' concept as it applies to many other tropical organisms. A key to all 23 Trichomycetes now known to be present in Costa Rica is provided.

Key words: Aquatic insects, Diptera, Ephemeroptera, symbiosis, Trichomycetes, tropics.

Costa Rica has numerous types of freshwater insect larvae whose hindguts or midguts are infected with Harpellales, one of several orders of the cosmopolitan arthropod-associated fungal Class Trichomycetes (Zygomycota) (Lichtwardt 1986, 1996). Larval insect hosts of Harpellales are Ephemeroptera (mayflies), Plecoptera (stoneflies), and many families of Nematocera (lower Diptera), including Simuliidae (blackflies), Chironomidae (nonbiting midges), Culicidae (mosquitoes), Ceratopogonidae (biting midges), Tipulidae (craneflies), and other families of dipterans not collected in Costa Rica. A previous publication (Lichtwardt, 1994) described harpellid gut fungi in Costa Rican insects that live in phytotelms, especially bromeliads, and other lentic habitats such as swamps. The current publication is a study of Harpellales that live in stream insects, representing a broader range of hosts and fungal species. This constitutes the first published investigation of the prevalence and distribution of gut fungi in tropical streams.

Harpellales found in Costa Rican streams include one new genus and 11 new species, as well as six species that have been previously reported from nontropical regions of the world. Additionally, three possibly new species of *Smittium* are described and illustrated, but not named at this time. All seven lotic species of *Smittium* were cultured axenically. Also found in Costa Rica were two genera and one new species of Amoebidiales. Amoebidiales, previously classified among Trichomycetes, are protists of unknown phylogenetic affinities, and are believed not to be fungi (Lichtwardt 1986). Rather, they constitute a convergent group of organisms that often live in or on the same kinds of larval hosts inhabited by harpellid gut fungi. A final, future paper in this series on Costa Rican Trichomycetes will deal with the other two orders, Asellariales and Eccrinales, associat-

ed with marine and terrestrial mature arthropods.

Most Trichomycetes appear to be harmless commensals during their life within the arthropod gut, or may at times benefit their hosts by providing some level of organic nutrients when the arthropods are nutritionally impoverished (Horn and Lichtwardt 1981). An exception is *Smittium morbosum* Sweeney that usually kills the mosquito larvae within which it lives (Sweeney 1981; Sato *et al.* 1989; López Lastra 1990). It is now known that some species of Harpellales on occasion grow into the ovaries of maturing insect hosts where fungal cysts develop (Moss and Descals 1986; Labeyrie *et al.* 1996). This results in sterilization of the adult female who then disperses the fungus when she "oviposits" the cysts within other breeding sites. Since all Harpellales live within the nonflying larval stages of aquatic insects, it has been speculated that possibly most or all of these fungi will be found to have this same basic dispersion mechanism (Lichtwardt 1996). Thus, whether Harpellales *infect* or *infest* their insect hosts may depend upon the particular stage of fungal development.

MATERIALS AND METHODS

Collections of Costa Rican lotic insects were made in 1984 from 18 September to 25 November, in 1986 from 3 June to 21 July, in 1988 from 21 May to 28 June, and in 1991 from 13 October to 22 November. During each of those years collections were made at the La Selva Biological Station (Organization for Tropical Studies) and in and near the Monteverde Cloud Forest Reserve (Tropical Science Center). Consequently, most of the streams in those areas were used repeatedly as sources of specimens. Many of the sites in Guanacaste Province were also visited more than once in 1988 and 1991. Streams in other parts of Costa Rica were used in most cases for single sets of collections. Table 1 lists the general regions, life zones, and streams where collections were made. Figure 1 illustrates the distribution of study sites in Costa Rica.

TABLE 1
Regions and streams in Costa Rica where trichomycete-infected lotic insects were collected between 1984 and 1991

Location and general life zone	Stream sites[1]
La Selva Biological Station (low wet forest)	Quebrada El Salto (above waterfall, or at Camino Central, or at Camino Circular Lejano) (**1**); Q. Esquina (**2**); Q. Sábalo (**3**); Q. Surá (**4**); Q. El Taconazo(**5**)
Monteverde (cloud forest, lower montane rain forest)	Q. Cuecha (upstream Río Guacimal) (**6**); R. Guacimal (**7**); Q. Máquina (**8**); within Reserve: headwaters of several streams along Sendero Bosque Nuboso, El Camino, and near campground (**9**)
Orosi valley, SE of Cartago (lower and premontane moist forest)	R. Macho (**10**); R. Grande de Orosi (**11**); R. Chirí (**12**)
NW of Heredia, Rt. 9 (lower montane rain forest)	R. Chorreras (**13**); R. Quizarraces (**14**)
SW of San José (premontane wet forest)	R. Tabarcia (**15**); Q. Chorro del Padre (**16**); unnamed stream S of Q. Chorro del Padre (**17**)
Cordillera de Talamanca, on both sides of the divide (montane wet and moist forest)	S fork R. Humo (**18**); R. Parrita Chiquito (**19**); W branch Q. Macho Mora (**20**); several unnamed streams near Rt. 2 (**21**)
Vicinity of Cahuita and Puerto Viejo (coastal moist forest)	Q. Matarrita (**22**); Q. Hotel (**23**)
SE of Quepos (coastal wet forest)	R. Portalón (**24**); several unnamed streams E of R. Savegre, E of Matapalo, and near road to Portalón (**25**)
Golfito (coastal wet forest)	Catarata in Golfito Reserve (**26**)

(*Continued...*)

South of Las Cruces (premontane wet forest)	E branch R. Jaba, Snake Creek, Lutite Spring, The Spring on Water Trail (Wilson Botanical Garden) (**27**); Q. Cantarrana (**28**); R. Caracol (**29**)
Guanacaste Province (dry forest)	R. Pièdras (**30**); R. Corobicí (**31**); R. Animas (**32**); small stream in Santa Rosa N.P. (**33**); R. Tenorio (**34**)
Cordillera de Guanacaste (premontane wet forest)	R. Bijagua (**35**); R. Naranjo (**36**)

[1] Boldface numbers are site locations referenced in the text. Also refer to Fig. 1.

Fig. 1. Map of Costa Rica showing collection sites. Small dots represent one to three streams; larger dots represent general locations of four or more stream sites. Refer to Table 1 for more specific data.

Insect larvae were obtained from rocks, sticks, vegetation, and various other kinds of substrates in the water to which lotic insects cling. Where appropriate, stream bottoms were disturbed, and the released insects were captured with a net. They were placed in small jars containing minimal amounts of water, and in most instances kept on ice to ensure their live transport to a laboratory, where they were maintained in a refrigerator until dissected. Removal of hindguts and midguts were done under a dissecting microscope using magnifications between 9 and 54X, and living fungal material was studied in slide water mounts with a phase-contrast compound microscope. Photographs of living gut fungi were made on color transparency film with a photomicrographic unit equipped with electronic flash. Specimens were preserved on slides by infiltrating lactophenol cotton-blue under the coverslip, then sealing the coverslip to the slide with clear fingernail polish. Most type specimens of the new taxa published here consist of such mounts; all types are deposited at the Farlow Herbarium (FH), Harvard University. Voucher specimens of insects, either dissected or undissected, were preserved in 70% ethanol for later identification by specialists whose names are given in the Acknowledgment section. Any relevant pupae or adults collected in the field or that developed in the laboratory were also preserved.

Axenic cultures of Harpellales were successfully obtained from some of many isolation attempts, using procedures that have been published (Lichtwardt 1986). For culture attempts, fungal specimens in the removed hindgut of an insect were kept as undisturbed as possible, usually after inspection with a compound microscope, washed at least twice in distilled water with an added penicillin-streptomycin mixture, then transferred to 60-µm petri dishes containing 1/10 BHIv agar medium [dilute Difco brain-heart infusion (0.37 g per 100 ml distilled water) with vitamins (thiamine, biotin)], to which a thin layer of sterile distilled water and antibiotics were added after the 1.5% agar had gelled. Successful cultures were transferred to agar slants of 1/10 BHIv containing a small amount of sterile distilled water, and were refrigerated after growth had occurred. Representative isolates of new species will be deposited with the American Type Culture Collection, Rockville, Maryland, U.S.A.

The isozyme patterns of *Smittium* spp. isolated in Costa Rica used in this report are part of an earlier data matrix that formed the basis of a more comprehensive investigation of cultured Trichomycetes (Grigg 1994; Grigg and Lichtwardt 1996). Isozymic relationships in the current study used numerical taxonomy and multivariate analysis systems (NTSYS-pc ver. 1.80) (Rohlf 1993) with unpaired group mean averages (UPGMA), and were computed from sequential, agglomerative, hierarchical, and nested (SAHN) clustering methods.

DESCRIPTIONS OF NEW FUNGAL SPECIES

Table 2 provides a list of new taxa described in this paper, together with other species of Trichomycetes currently known to infect Costa Rican aquatic insects.

Harpella tica Lichtw., sp. nov.
(Figs. 2-6)

Thalli maturi 180-380 x 6-9 µm, vulgo cellulas genitales circa 8, sed nonnumquam 4-46 gerentes. Basis rotundata, tenaculo ei thalli diametro subaequante. Trichosporae sigmoideae, rarius curvulae vel subconspiratae, (98-)130(-140) x 3-4 µm, appendiculis 2-4. Zygosporae ignotae. In larvarum Simuliidarum membrane peritrophico. Typus: FH (CR-252-2).

Mature thalli 180-380 x 6-9 µm, normally with about 8 generative cells but ranging from 4-46 per thallus. Base rounded with a holdfast about same diameter as the thallus. Trichospores sigmoid, more rarely curved to almost coiled, (98-)130(-140) x 3-4 µm, with 2 or 4 appendages. Zygospores unknown. On peritrophic membrane of Simuliidae larvae.

Holotype: Slide CR-252-2 prepared from a *Simulium* sp. larva collected 16-XI-91 from Quebrada Cuecha (Site **6**, Table 1) in Monteverde.

Other collections: Found in many species of *Simulium*, including *S. samboni* Jennings, *S. ochraceum* Walker, *S. metallicum* group, *S.*

TABLE 2

Costa Rican Trichomycetes (Harpellales and Amoebidiales) and their insect larval hosts[1]

Harpellales	
Harpella tica sp. nov.	Simuliidae
Genistellospora guanacastensis sp. nov.	Simuliidae
Genistellospora homothallica	Simuliidae
Genistellospora nubila sp. nov.	Simuliidae
Genistellospora tepidaria sp. nov.	Simuliidae
Graminelloides biconica gen. et sp. nov.	Simuliidae
Pennella montana sp. nov.	Simuliidae
Pennella simulii	Simuliidae
Simuliomyces microsporus	Simuliidae
Smittium annulatum sp. nov.	Simuliidae
Smittium culisetae	Culicidae
Smittium culicisoides sp. nov.	Chironomidae, Simuliidae
Smittium dipterorum sp. nov.	Chironomidae, Simuliidae
Smittium fasciculatum	Chironomidae
Smittium parvum sp. nov.	Chironomidae
Smittium phytotelmatum	Chironomidae
Spartiella animae sp. nov.	Baetidae (Ephemeroptera)
Stachylina grandispora	Chironomidae
Stachylina nana	Chironomidae
Stachylina paludosa	Chironomidae
Stachylina penetralis	Chironomidae
Amoebidiales	
Amoebidium colluviei sp. nov.	Chironomidae, Simuliidae
Paramoebidium spp.	Simuliidae, Ephemeroptera

[1] Includes taxa described in this paper as well as species of Harpellales reported by Lichtwardt (1994). A key to these fungal species is provided in the text. Chironomidae, Culicidae and Simuliidae are families of Diptera.

panamense Fairchild, *S. chiriquiense* Field, *S. callidium* (Dyar & Shannon), *S. mexicanum* Bellardi, a species of Subg. *Psilopelmia* Enderlein., and other, unidentified species of Simuliidae; from virtually all sites where Simuliidae were found in 1984, 1986, 1988, and 1991.

Etymology: Tica, vernacular name for a Costa Rican.

Harpella tica was the only species of that genus found in Costa Rica. With very few exceptions, all dissected simuliid larvae were infected. The most obvious identifying character of *H. tica* is the sigmoid shape of the trichospores (Figs. 2-6). In a few of the many hundreds of simuliid larvae examined, the trichospores were curved or almost coiled, which is the typical shape in the other three species of *Harpella*. *Harpella melusinae* Léger & Duboscq, has been found in many parts of the world (throughout Europe, North America, Japan, Australasia), has trichospores that are considerably larger (~150 x 6-10 µm) than those of *H. tica*, and can be identified most easily by the tapered holdfast apparatus (Reichle and Lichtwardt, 1972). *Harpella leptosa* Lichtw. & S.T. Moss, currently known only from a few streams in northern Montana, U.S.A., has a base that is slightly tapered above the small holdfast, and has trichospores that measure 110-150 x 4.5 µm. *Harpella meridianalis* Lichtw. & Arenas was recently described from southern Chile (Lichtwardt and Arenas, 1996) as having a rounded base and trichospores measuring 80-100 x 4-8 µm.

Genistellospora guanacastensis Lichtw., sp. nov.
(Figs. 7-10)

Thalli stirps praecipua plerumque 150-250 µm longa, origo ramorum et lateralium et terminalium. Trichosporae elongato-ovales, 50-61 x (8-)10(-13) µm, appendiculas 4(-6) partim conspiratas gignentes. Zygosporae ignotae. In larvarum Simuliidarum proctodaeo. Typus: FH (CR-247-4).

Thallus with a main axis usually 150-250 µm long from which lateral and sometimes terminal branches arise. Trichospores long-oval, 50-61 x (8-)10(-13) µm, bearing 4(-6) partially coiled appendages. Zygospores unknown. In hindgut of Simuliidae larvae.

Holotype: Slide CR-247-4 consisting of hindgut linings of three *Simulium* sp. larvae collected 12-II-91 in Río Animas in northern Guanacaste Province (Site **32**). One hindgut contains some loose sigmoid trichospores of *Harpella tica.*

Other collections: In *Simulium* spp. larvae from R. Pièdras (Site **30**), R. Animas (**32**), R. Bijagua (**35**), and R. Naranjo (**36**), all collected in 1991.

Etymology: After Guanacaste Province, where the fungus was found.

Prior to this description and those that follow, *Genistellospora* was a monotypic genus (based on *G. homothallica* Lichtw.). Though zygospores were not found in *G. guanacastensis* so as to confirm the generic designation, this is clearly a species of *Genistellospora*, which is characterized by a branched thallus consisting of a main axis and a secreted holdfast structure (Figs. 7, 8), and producing ovoid trichospores with multiple fine basal appendages (Fig. 10). Trichospores of *G. guanacastense* are much longer than those of the type species, which measure (24-)30(-41) x (8-)10(-13) µm. The appendages of *Genistellospora* trichospores before release can be seen within the generative cell as straight, parallel lines (Fig. 9) lying adjacent to the cell wall (and outside the plasmalemma) (Moss and Lichtwardt, 1976). However, upon release of the trichospore, the ends of the appendages of *G. guanacastensis* are coiled (Fig. 10).

Genistellospora nubila Lichtw., sp. nov.
(Figs. 11-13)

Thallus 400-1200 µm, tenaculo parco secret. Trichosporae ovoideae, 38-41 x 10-14 µm, inoculator cellulae genital affix, liberate appendiculis 4(-6) praeditae. Zygosporae ignotae. In larvarum Simuliidarum proctodaeo. Typus: FH (CR-198-6).

Thallus 400-1200 µm with a small secreted holdfast. Trichospores ovoid, 38-41 x 10-14 µm, attached eccentrically to generative cell, on release having 4(-6) appendages. Zygospores unknown. In hindgut of Simuliidae larvae.

Holotype: Slide CR-198-6 prepared from a *Simulium* sp. larva collected 25-VI-88 from Q. Máquina (Site **8**) near the cloudforest region of Monteverde.

Other collections: All from the same site and date in *Simulium* spp. larvae. Of 54 larvae

dissected, 25 were infected with *Genistellospora*, in some cases too immature to verify for certain that the fungus was *G. nubila.*

Etymology: L. nubes = cloud, for the cloud forest site.

Trichospores of *G. nubila* have a slightly lesser length to width ratio than *G. homothallica*, giving them a robust appearance. The most obvious characteristic of this species is the eccentric growth of trichospores from their generative cells (Figs. 11, 12).

Genistellospora tepidaria Lichtw., sp. nov. (Figs. 14-18)

Thalli basis tenaculo prominence secret praedia. Thallus ramose paucos vel multos gignens. Trichosporae ovoideae, 21-31 x 9-11 µm, appendiculis 6(-8) tenuibus praeditae. Zygosporae sine conjugationibus productae, 58-70 x 10-15 µm. Zygosporophorae 29-30 x 8-11 µm. In larvarum Simuliidarum proctodaeo. Typus: FH (CR-221-1).

Base of thallus with a prominent secreted holdfast. Thallus with few to numerous branches. Trichospores ovoid, 21-31 x 9-11 µm, with 6(-8) fine appendages. Zygospores produced without conjugations, 58-70 x 10-15 µm. Zygosporophores 29-30 x 8-11 µm. In hindgut of Simuliidae larvae.

Holotype: Slide CR-221-1 prepared from three *Simulium* sp. hindguts, one with a large thallus of *Genistellospora tepidaria* with numerous trichospores, collected 23-X-91 from Río Sábalo, La Selva Biological Station (Site **3**). **Paratype:** Slide CR-5-10 with pieces of many *Simulium* sp. hindguts, one with a thallus with two zygospores and a few trichospores, collected 21-IX-84 from same site as the holotype.

Other collections: *Genistellospora tepidaria* was found frequently in Simuliidae larvae from Sites **1**, **2**, **3**, **4** in 1984, 1986, 1988 and 1991 (not necessarily all sites in the same year). The hosts included, in addition to unidentified species, *S. samboni*, *S. ochraceum*, *S. mexicanum*, and Subg. *Psilopelmia* sp.

The specific epithet (L. tepidus = warm) refers to the relatively warm stream temperatures (24-25.8 C) where the blackfly larvae were living. *Genistellospora tepidaria* is most similar to *G. nubila*, but has smaller trichospores. Trichospores were sometimes attached eccentrically, as they usually are in *G. nubila.*

Graminelloides Lichtw., gen. nov.

Thalli ramis paucis praediti, basi in mucronem attenuato, mucilaginem carente. Trichosporae biconicae, sine collari, appendiculo singulo. Thallus totus, cellulis paucissimis basilibus exceptis, genitalis. In Simuliidarum larvarum proctodaeo.

Thalli with few branches; base tapered to a point, without mucilage. Trichospores biconical, collarless, with a single appendage. Entire thallus may become reproductive except for the very basal cells. In hindgut of Simuliidae larvae.

Basionym: *Graminelloides biconica* Lichtw.

Etymology: Resembles the harpellid genus *Graminella*, which also produces long series of small trichospores.

Graminelloides biconica Lichtw., sp. nov. (Figs. 19-23)

Thalli maturi usque ad 350 µm longi, 5-8 µm diametro, ramis sparsis, immaturi fusiformes. Basis attenuata, sine mucilagine. Thalli saepe fasciculatae, nonnumquam plures quam 24 in circulo. Trichosporae biconicae, 10-16 x 4-5 µm, sine collari, appendiculo singulo brevi. Zygosporae ignotae. In Simuliidarum larvarum proctodaeo. Typus: FH (CR-65-1).

Mature thalli up to 350 µm long by 5-8 µm diameter, with sparse branching. Immature thalli fusiform. Base tapered, without mucilage. Thalli often fascicled, sometimes consisting of more than 24 thalli in a cluster. Trichospores biconical, 10-16 x 4-5 µm, without a collar, and with one short appendage. Zygospores unknown. In hindgut of Simuliidae larvae.

Holotype: Slide CR-65-1 prepared from the hindgut of an unidentified Simuliidae larva collected 25-XI-84 in an unnamed stream W of Portalón (3.3 km E on the road to Portalón from junction of main road) and SE of Quepos in Puntarenas Province (general Site **25**).

Other collections: In *Simulium callidium* and an unidentified species of Simuliidae (same as holotype), from same site and date.

Seven of 26 simuliid larvae were infected with the new fungus. The features that distinguish this new monotypic genus from two other harpellid genera that produce trichospores with

no collar and a single appendage include: (i) the tapered base of the thallus (Figs. 19, 20), (ii) the biconical shape of the trichospore (Figs. 21-23), (iii) a thallus that becomes almost entirely reproductive (Fig. 22), and (iv) the dipteran host. The two other genera with collarless trichospores and a single appendage live in Baetidae (Ephemeroptera): species of *Graminella* ex Manier have bulbous thallic bases, ovoid trichospores, and produce vegetative propagules; and the currently monotypic genus *Spartiella* ex Manier (but see new species below) produces obpyriform trichospores and the thallus has a swollen or lobulate base. The fascicled nature of *Graminelloides biconica* thalli is unusual (Figs. 19, 20), because the origin of each thallus is presumed to be from a separate trichospore that is ingested by the host.

Pennella montana Lichtw., sp. nov.
(Figs. 24-30)

Thallorum maturorum bases semel vel iterum dichotome bifurcatae, mucilaginem secretentes. Trichosporae ovoideae, (39-)60(-72) x 8-10 µm, appendiculis pertenues praeditae. Zygosporae 91-100 x 18-20 µm; zygosporophoris 38-46 x 13-16 µm. In larvarum Simuliidarum proctodaeo. Typus: FH (CR-30-1).

Bases of mature thalli dichotomously bifurcate one or more times, producing a mucilaginous secretion. Trichospores long-ovoid, (39-)60(-72) x 8-10 µm, with multiple very fine appendages. Zygospores 91-100 x 18-20 µm; zygosporophores 38-46 x 13-16 µm. In hindgut of Simuliidae larvae.

Holotype: Slide CR-30-1 prepared from a *Simulium ochraceum* larva collected 16-X-84 from a very small headwater stream crossing Sendero Bosque Nuboso in the Monteverde Cloud Forest Reserve (Site **9**). Consists of several mature thalli with trichospores, one with zygospores. The slide also contains a peritrophic membrane with *Harpella tica* and some loose *H. tica* trichospores.

Other collections: In many species of Simuliidae larvae in the Monteverde area in 1984, 1986, 1988, 1991 (Sites **6, 7, 8, 9**), streams along the Cordillera de Talamanca in 1984 (Sites **18, 19, 20, 21**), and Snake Creek (possibly also Lutite Spring and R. Jaba East Branch, where the fungi were immature) in the Wilson Botanical Garden in 1991 (Site **27**), in *S. ochraceum, S. callidium, S. chiriquiense, S. metallicum* group, and unidentified blackfly species.

Pennella montana (L. montanus = mountain) appears to be a fungal species that is limited to higher altitudes. It was not found in lowland streams, including those in Guanacaste Province. Its larger trichospores and zygospores distinguish it from the other six described species of *Pennella* ex Manier. An unusual feature of *P. montana* is the manner in which zygospores form. A special cell from an adjacent branch, the *conjugation cell* (Fig. 29), produces an extension that brings about a conjugation with the branch that will become the zygosporophore from which the zygospore develops. The conjugation cell subsequently breaks loose from the branch that produced it, but remains attached to the zygosporophore (Fig. 30).

Smittium annulatum Lichtw., sp. nov.
(Figs. 31-33)

Thalli parvi compactique, structura prominence basali cellulis sex in annulo ordinatis constante orti. Trichosporae elongato-ovales, 18-22(-33) x 4-6(-8) µm, collari 3-6 µm longo. Cellulae genitales usque ad 8 vel plures in ramo fertili uno gestae. Zygosporae ignotae. In proctodaeo larvarum Simuliidarum. Typus: FH (CR-143-5).

Thalli small and compact, arising from a prominent basal structure consisting of about six cells arranged in a ring. Trichospores elongate-oval, 18-22(-33) x 4-6(-8) µm, collar 3-6 µm long. Up to 8 or more generative cells per fertile branch. Zygospores unknown. In hindgut of larval Simuliidae.

Holotype: Slide CR-143-5 prepared from a *Simulium* sp. larva (possibly *S. ochraceum* Walker) collected 27-V-88 in Q. Esquina (Site **2**), at the La Selva Biological Station.

Other collections: In *Simulium samboni* Jennings larvae collected at Site **1** in 1984 and Sites **1** and **2** in 1988.

Cultures: CR-143-8 and CR-143-10 (the latter is no longer living) isolated 27-V-88 from Simuliidae larvae (possibly *S. ochraceum*) from the same site as the holotype.

The ring-like structure at the base of the

thallus (Fig. 31) (to which the specific epithet refers), as well as the dimensions of the trichospores, make this species easily identifiable. A study of isozyme patterns in cultured Trichomycetes (Grigg, 1994; Grigg and Lichtwardt, 1996) demonstrated that *S. annulatum* is isozymically the most distinct of the Costa Rican species of *Smittium* (Fig. 79). It was relatively common in Sites **1** and **2**, but, with one exception in 1984, was not found in other simuliid larvae despite a large number of dissections. Nor was *S. annulatum* found in other Costa Rican streams used in this study.

Smittium culicisoides Lichtw., sp. nov.
(Figs. 34-38)

Thalli maturi saepe 200-500 µm longi. Bases attenuatae, nonnumquam prominentiis ex muro minutis ornatae, in hospitibus forte infectis aliquando fasciculatae. Trichosporae ovales, 20-25(-28) x 6-10 µm, collari 5-10 µm. Rami fertiles cellulas genitales 1-4 gerentes. Zygosporae ignotae. In proctodaeo larvarum Chironomidarum et Simuliidarum. Typus: FH (CR-110-19).

Mature thalli often 200-500 µm long. Bases tapered, occasionally with minute projections from the wall. Bases may occur in fascicles in well infected hosts. Trichospores oval, 20-25(-28) x 6-10 µm, collar 5-10 µm. Fertile branches with 1-4 generative cells. Zygospores unknown. In hindgut of larval Chironomidae and Simuliidae.

Holotype: Slide CR-110-19 prepared from a *Cardiocladius* sp. (Chironomidae) larva collected 16-VII-86 from a polluted stretch of Río Guacimal (Site **7**) in Monteverde. The slide also contains a small amount of *S. dipterorum* with a zygospore.

Other collections: All from Site **7**, in 1984, 1986, 1988, and 1991, predominantly found in larvae of *Cricotopus* sp., but also in *Chironomus* sp. and other Chironomidae, and *Simulium* sp. (Simuliidae).

Culture: CR-253-12 isolated from an undetermined species of Chironomidae collected 17-XI-91.

Etymology: Resembles *Smittium culicis.*

Smittium culicisoides was common in many larvae at the type site, often in association with *S. dipterorum* (see below), and other harpellids (*Harpella, Genistellospora, or Pennella* in simuliid larvae). The name of this species alludes to its similarity with *S. culicis* Manier, with which it can be confused. *Smittium culicis*, a common and widely distributed harpellid, is most often found growing in mosquito larvae (Culicidae), but has a broad host range that occasionally includes other dipteran families, including Chironomidae and Simuliidae. The trichospores of *S. culicis* are often formed on arching terminal branchlets, and the branching within the thallus is more divergent than in *S. culicisoides*. The trichospores of both species are very similar in their shape and dimensions, with those of *S. culicisoides* having, on the average, a slightly lesser length to width ratio. That these cryptic species are distinct is supported by differences in their isozymic patterns (Fig. 79).

Smittium dipterorum Lichtw., sp. nov.
(Figs. 39-45)

Thallorum tenaculum simplex secretum, rami crebre verticellati, omnes fertiles usque ad 8 vel plures cellulas genitales gerentes. Trichosporae longe ellipsoidales, subcylindricae, (10-)12-18(-26) x 2-3(-4) µm, appendiculo pertenui, collari 1-3 µm longo. Zygosporae 51-70 x 9-12 µm. In proctodaeo larvarum Simuliidarum et Chironomidarum. Typus: FH (CR-260-2).

Thalli with a simple secreted holdfast, branches often verticillate with up to 8 or more generative cells per fertile branch. Trichospores long-ellipsoidal, almost cylindrical, (10-)12-18(-26) x 2-3(-4) µm, with a very fine appendage; collar 1-3 µm long. Zygospores 51-70 x 9-12 µm. In hindgut of larval Simuliidae and Chironomidae.

Holotype: Slide CR-260-2 (with trichospores) prepared from a *Simulium* sp. (Simuliidae) larva collected 11-XI-91 from an unpolluted stretch of Río Guacimal (Site **7**). Slide also contains *Pennella* sp. and *Genistellospora* sp. **Paratype:** Slide CR-17-3 (includes zygospores) prepared from an *Orthocladius (Euorthocladius)* (Chironomidae) larva collected 9-X-84 from Río Chorreras where it crosses Rt. 9 NW of Heredia (Site **13**). Types deposited at Farlow Herbarium (FH).

Other collections: In many streams, including Sites **1**, **2**, **7**, **8**, **9**, in various Simuliidae (*Simulium samboni, S. callidium,*

and unidentified species), and Chironomidae (*Cardiocladius* sp., *Cricotopus* sp., *Orthocladius* sp., and unidentified species), in 1984, 1986, 1988, and 1991.

Cultures: CR-141-17, isolated 26-V-88 from *Simulium* sp., Site **1**; CR-143-2, 27-V-88 (no longer living) from *Simulium* sp., Site **2**; CR-253-14, 18-XI-91 from *Simulium* sp., Site **7**; CR-254-13, 18-XI-91 from *Chironomus* sp., Site **7**.

Simulium dipterorum was found in more Costa Rican streams and a greater variety of dipteran hosts than any other species of *Smittium*. Based on trichospore dimensions and verticillate branching (Fig. 39), *S. dipterorum* most closely resembles *S. paludis* M.C. Williams & Lichtw., a Tasmanian species found in a swamp midge. However, *S. paludis* has a swollen holdfast cell that is incurved at the point of attachment, whereas in *S. dipterorum* the holdfast structure is a simple secretion and the basal cell is not swollen (Fig. 40). In the Monteverde collections, *S. dipterorum* was often living in some of the same dipteran and simuliid hosts as *S. culicisoides*, as well as sharing the simuliid guts with *Harpella*, *Genistellospora*, or *Pennella*.

Smittium parvum Lichtw., sp. nov.
(Figs. 46-49)

Rami arcte ordinatae, fertiles usque ad 6 vel plures cellulas genitales gerentes. Trichosporae ovales, 9-14 x 3.5 µm, collari 2-6 µm longo. Zygosporae ignotae. In larvarum Chironomidarum proctodaeo. Typus: FH (KU-204-35).

Branches closely arranged, with fertile branches bearing up to 6 or more generative cells. Trichospores oval, 9-14 x 3.5 µm, collar 2-6 µm long. Zygospores unknown. In hindgut of Chironomidae larvae.

Holotype: Kodachrome transparency KU-204-35, consisting of a photomicrograph taken 14-VII-88 of culture CR-184-32 isolated 18-VI-88 from a *Cricotopus* sp. (Chironomidae) larva found living in Río Guacimal (Site **7**) 17-VI-88, showing released trichospores. **Isotype:** Kodachrome transparency KU-204-36, a photomicrograph taken on the same date and from the same culture as the Holotype, showing trichospores attached to fertile branchlets, some released. Deposited at Farlow Herbarium (FH).

Other collections: Culture CR-254-10 (Fig. 49) isolated 18-XI-91 from a *Cricotopus* sp. larva from the same site as the Holotype. Possibly also in a *Cricotopus* sp. larva collected 21-V-88 from Quebrada Sábalo (Site **3**) (Fig. 46).

Cultures: CR-184-32 isolated 18-VI-88 from a *Cricotopus* sp. larva collected in Río Guacimal (Site **7**) (culture no longer living); CR-254-10 isolated 18-XI-91 from a *Cricotopus* sp. larva at Site **7**).

Etymology: L. parvus = small, for the small trichospore size.

Trichospores of *S. parvum* have the shape and long collar of *S. culicis* and *S. culicisoides*, but are smaller in size (compare Figs. 37 and 48), and is isozymically distinct from the other two Smittiums (see Fig. 79). The collection from a *Cricotopus* larva in Q. Sábalo, La Selva Biological Station (Fig. 46), appears to be *S. parvum*, and this may be the only specimen slide prepared directly from a living host and not a culture.

Spartiella animae Lichtw., sp. nov.
(Figs. 50-53)

Thallis valde ramosi, quam 500 µm breviores, cellula basali paulum inflata vel lobulata. Trichosporae obpyriformes, 13-20 x 4-7 µm, sine collari, appendiculum singulum longum tenue gerentes. Zygosporae ignotae. In nympharum Ephemeropteroram proctodaeo. Typus: FH (CR-251-1).

Thalli much branched, less than 500 µm long, with a slightly swollen or lobulate basal cell. Trichospores obpyriform, 13-20 x 4-7 µm, collarless, bearing a single long, fine appendage. Zygospores unknown. In hindgut of Ephemeroptera nymphs.

Holotype: Slide CR-251-1 prepared from a *Baetis* sp. nymph (Ephemeroptera, Baetidae) collected 13-XI-91 in Río Animas in northwestern Costa Rica (Site **32**) near the border with Nicaragua.

Other collections: Nineteen of 42 nymphs of *Baetis* sp. contained *S. animae* from collections of 10-XI-91 and 13-XI-91 in R. Animas. Also found in three of 16 mayfly nymphs of the same species collected 13-VI-88 in R. Pièdras (Site **30**).

Etymology: Named after R. Animas, the

stream where most specimens were found.

Four attempts to culture *S. animae* were unsuccessful. *Spartiella barbata* ex Manier up to now was the only species in the genus. That species has been found in Baetidae nymphs (Ephemeroptera) in southern France, England, and Wales (Lichtwardt, 1986). The genus is characterized by having obpyriform trichospores with no collar and a single appendage, and zygospores attached perpendicularly and medially to the zygosporophore. No zygospores were found in *S. animae*, but the trichospores differ from the type species in being much smaller (those of *S. barbata* are 22-27 x 7.5-10 µm).

Amoebidium colluviei Lichtw., sp. nov.
(Figs. 54-59)

Thalli plerumque minores quam 80 µm longitudine, circa 10 µm diametro, sporangiosporas allantoideis 25-32 x 6-10 µm producentas. Praecipue papillae anali Chironomidarum et Simuliidarum affixi. Typus: FH (CR-254-22).

Thalli usually less than 80 µm long by about 10 µm diameter, producing allantoid sporangiospores 25-32 x 6-10 µm. Attached primarily to anal papillae of Chironomidae and Simuliidae larvae.

Holotype: Slide CR-254-22 consisting of the anal papillae of two large *Chironomus* sp. larvae covered with *Amoebidium colluviei*, collected 18-XI-91 in a highly polluted stretch of Río Guacimal (Site **7**). Deposited in Farlow Herbarium (FH).

Other collections: Common on anal papillae of *Chironomus* sp. and *Simulium* spp. in the same collection site as the Holotype.

Etymology: L. colluvieis = polluted, for the condition of the stream site.

Amoebidiales, as mentioned in the Introduction, are not thought to be Trichomycetes, but are often found living in (*Paramoebidium* spp.) or on (*Amoebidium* spp.) some of the same kinds of hosts that are inhabited by trichomycete gut fungi. The external thalli of *A. colluviei* may have been present but not detected in other years when the author collected in the same polluted section of Río Guacimal. It provides a fuzzy covering to anal papillae of infested chironomid and, to a lesser extent, simuliids. No amoebae were produced by *A. colluviei* specimens (see Discussion). One culture attempt was not successful, though *A. parasiticum* Cienk. has been cultured.

Amoebidium parasiticum has been found in many parts of the world on the external cuticle of small lentic crustaceans such as Cladocera, but also freshwater lentic insects such as Culicidae and bloodworms (Lichtwardt, 1986). Sporangiospores of *A. parasiticum* are usually lunate, and measure 15-50 x 5-10 µm; those of *A. colluviei* are allantoid and much smaller. The fact that the latter species was found in rapidly-flowing waters (*Chironomus* sp. larvae were mostly in sedimentary zones, however), makes this an unusual habitat for an *Amoebidium*. The only other species of *Amoebidium* described until now other than the type species has been *A. recticola* Chatton (1906), an apparently rare species described from the rectal area of *Daphnia* spp. taken from reptile tanks at the Paris Museum. Sporangiospores of *A. recticola* are short-cylindrical to oval, 8-12 µm long.

PROBABLY NEW BUT UNDESCRIBED SPECIES

Several species of *Smittium* were found and cultured, but are not being named at this time because of the unavailability of sufficient numbers of in vivo specimens.

Smittium sp. CR No. 1
(Fig. 60)

This *Smittium* sp. was found in *Tanytarsus* sp. larvae in a rock pool about 0.5 m above the stream water in R. Pièdras (Site **30**), and possibly also in the same host in the river channel. The rock pool was subject to periodic flooding. Trichospores of the gut fungus are cylindrical, 19-21(-30) x 2.3 µm, with a collar about 2-3 µm long. The trichospores are similar to those of *S. phytotelmatum* Lichtw., a species found at La Selva most commonly in bloodworms inhabiting water at the base of bromeliad leaves (Lichtwardt, 1994), but the branching pattern is different. An axenic culture, CR-239-12, was made from one of the *Tanytarsus* larvae collected 9-XI-91. A study of isozyme patterns in cultured Harpellales (Grigg and Lichtwardt, 1996)

showed that *Smittium* sp. CR No. 1 was isozymically distinct from *S. phytotelmatum* isolates and other harpellid species (Fig. 79). It appears to be a new, undescribed gut fungus. Host larvae were more often infected with a species of *Stachylina* than with this *Smittium*.

Smittium sp. CR No. 2
(Fig. 61)

Simulium sp. larvae collected 21-XI-91 from Q. Máquina (Site **8**) were infected with *Smittium* sp. CR No. 2, and an isolate, culture CR-259-4, was obtained (Fig. 61). The culture is isozymically distinct from other Costa Rican Smittiums (Fig. 79) as well as *Smittium* spp. from other geographic areas (Grigg and Lichtwardt 1996). Morphologically, the oval trichospores, measuring 19-27 x 4-8 µm with a collar about 2-4 µm long, have some resemblance to *S. culicisoides* and *S. culicis*, but are somewhat smaller, have a greater length to width ratio, and the collar is generally shorter. In vivo sporulating specimens are required before it is described.

Smittium sp. CR No. 3
(Figs. 62, 63)

The third undescribed—and probably new—species of *Smittium*, though it came from a lentic, rather than a riparian, bloodworm, is illustrated here as culture CR-211-1. It was living in a *Chironomus* sp. larva in a swamp near Sendero Experimental Sur at La Selva. Lichtwardt (1994) originally suggested that it might be similar to *S. phytotelmatum*, but this does not now appear to be the case, either morphologically or isozymically (Fig. 79). Figures 62 and 63 illustrate some aspects of its morphology. Trichospores are elongate elliptical, 15-18 x 3-4 µm, with a very short collar (~1 µm long). The similarity in isozyme pattern with culture CR-253-12, *S. culicisoides*, cannot be explained, because the two are morphologically distinct.

SPECIES WITH WIDER GEOGRAPHIC DISTRIBUTIONS

Genistellospora homothallica Lichtw.
(Figs. 64-68)

Prior to this publication, *G. homothallica* was the only described species of the genus. It is relatively common and widespread in a variety of *Simulium* and *Prosimulium* species in the U.S.A., especially in the Rocky Mountains, and has been reported from southern England (Lichtwardt, 1986). More recently it was found in southern Chile (Lichtwardt and Arenas, 1996) and in Argentina (López Lastra, unpublished). Collections of *G. homothallica* in Costa Rica were in higher altitudes: the Monteverde region (Sites **6, 7, 8, 9**) and the Cordillera de Talamanca (Sites **18, 21**). The Costa Rican specimens had trichospores (measuring 29-36 x 10-11 µm) that were sometimes eccentrically attached and had a somewhat lesser length to width ratio than normally seen in other collections, and the zygospores (measuring 83-90 x 12-17 µm) were a bit narrower than those found in most other collections. However, the Costa Rican specimens generally fit the description of *G. homothallica*, which has the following published measurements: trichospores (24-)30(-41) x (8-)10(-13) µm; zygospores (77-)100(-113) x (15-)20(-22) µm.

Paramoebidium spp.
(Fig. 69)

Though not believed to be phylogenetically related to fungi, as explained in the introduction, species of *Paramoebidium* may be common in Ephemeroptera, Plecoptera, and Simuliidae, and may coinhabit guts with Harpellales. Only six valid species of this unculturable genus have been named. None of the Costa Rican species appeared to be among the currently described species, and none is described in this paper because of a lack of suitable descriptive characters. Nonetheless, *Paramoebidium* spp, were found in most collections of mayflies (Baetidae) in many sites representing different life zones, and somewhat less commonly in many populations of Simuliidae larvae. Thalli of *Paramoebidium* may be quite large and numerous in some hosts. They are more or less cylindrical, straight or curved or bent (Fig. 69), nonseptate, and mature thalli occasionally may be seen releasing large numbers of amoeboid cells when a larva is dissected or is in the process of molting. The various stages of this interesting protist have been described and illustrated (e.g. Lichtwardt 1986; Lichtwardt and Arenas 1996).

Pennella simulii M.C. Williams & Lichtw. (Fig. 70)

On two occasions at La Selva (Sites **1** and **2**), in 1984 and 1991, within *Simulium ochraceum* and possibly another blackfly species, a *Pennella* was found with trichospores resembling the size and shape of *P. simulii*. No zygospores were found on either occasion for substantiation of the identification. *Pennella simulii* has been reported from the Rocky Mountains of Colorado and Wyoming, U.S.A. (Lichtwardt, 1986), but probably has a much broader range of distribution in North America. Measurements of *P. simulii* are: trichospores (30-) 33 (-41) x (6.5-) 8 (-10.5) µm; zygospores (84-) 90 (-96) x (19-) 22 (-24) µm.

Simuliomyces microsporus Lichtw. (Figs. 71, 72)

Simuliomyces microsporus is a widespread Harpellales that is sometimes confused with *Smittium* when zygospores are not present. Trichospores of *Smittium* spp. have a collar and one appendage. The 2-4 appendages of *S. microsporus* often are not clearly visible on released trichospores, and the trichospores are collarless. *Simuliomyces microsporus* is easily recognized, however, because of its common attachment to thalli of other harpellids in simuliid hindguts, such as *Pennella* or *Genistellospora*, but most often it is seen attached to thalli of *Paramoebidium* spp. *Simuliomyces microsporus* was found in the following sites: **7**, **9**, **12**, and **18**. The published measurements of this species are: trichospores 20-30 x 4-6 µm; zygospores 34-45 x 7-9 µm.

Stachylina grandispora Lichtw. (Figs. 73-75)

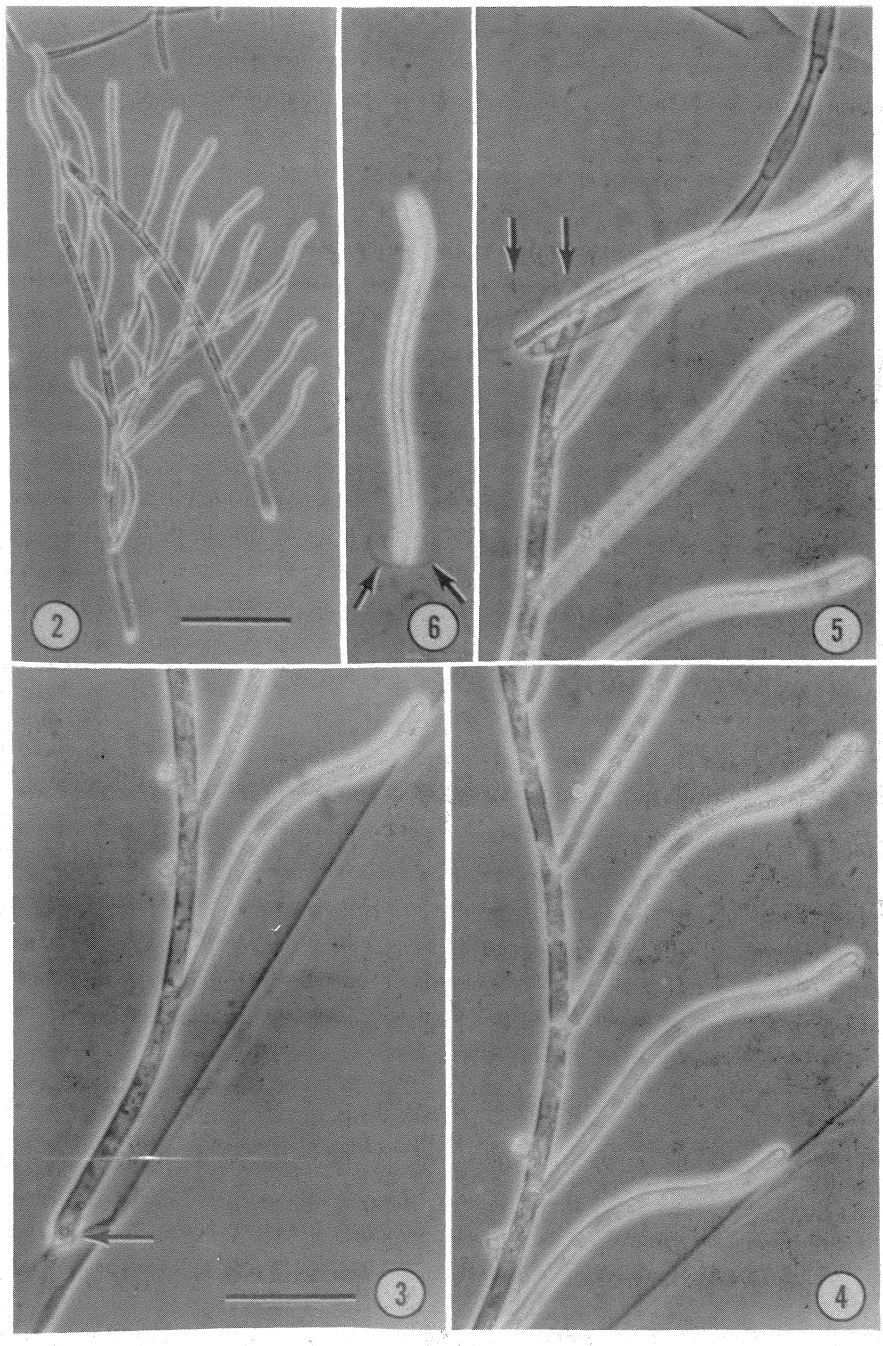
Stachylina grandispora is perhaps the most widespread of all species of the genus, having been found in at least six genera of midge larvae, but especially bloodworms of the genus *Chironomus*, in Europe, U.S.A. (including Hawaii), Japan, Australia, and New Zealand. The species was not common in Costa Rica, however. It was found on the peritrophic membrane of *Chironomus* sp. larvae in polluted stretches of R. Guacimal (Site **7**) in 1986 and 1991, and bloodworms in a barely flowing creek in Santa Rosa National Park (Site **33**). *Stachylina grandispora*, among other characters, produces large trichospores (40-72 x 6-10 µm) that usually have a short but visible collar. The Costa Rican specimens tended to have smaller trichospores than usual and had a slightly greater length to width ratio, but generally fell within the size range for *S. grandispora*. The possibility that those specimens represent a new species cannot be excluded.

Stachylina nana Lichtw. (Figs. 76, 77)

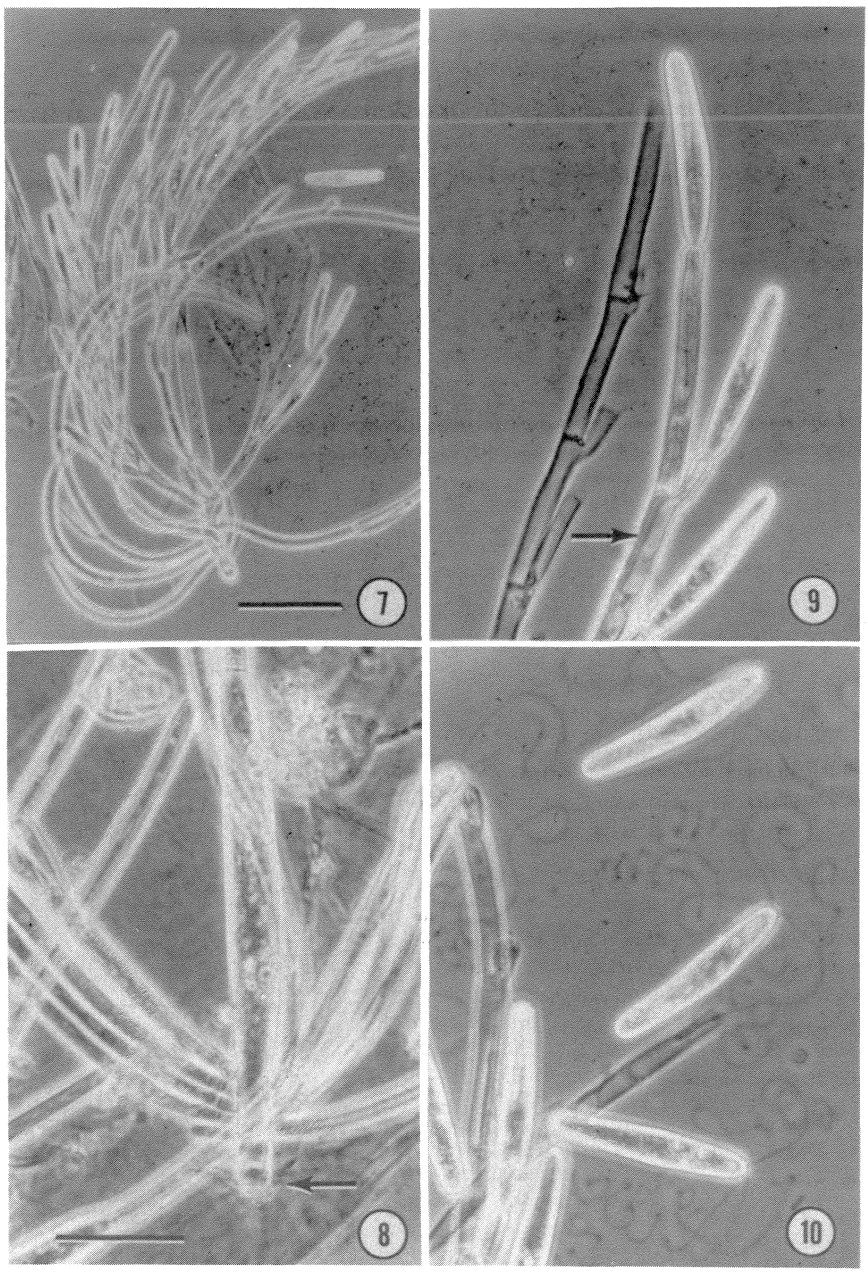
First described from France (Lichtwardt 1984), *Stachylina nana* has been reported from Japan (Lichtwardt *et al.* 1987), New Zealand (Williams and Lichtwardt 1990) , and now from Costa Rica. Trichospore sizes are somewhat variable, ranging from 25-40 x 7-11 µm, but the thallus is distinctively cymbiform to fusiform and produces 2-4(-8) trichospores. In Costa Rica it was found on several occasions in Río Guacimal (Site **7**) predominantly in *Cricotopus* sp. but also in *Chironomus* sp. larvae, and what may be the same species of *Stachylina* was seen once in a larva of *Orthocladius* (*Euorthocladius*) sp. collected in Río Chorreras (Site **17**).

Stachylina penetralis Lichtwardt (Fig. 78)

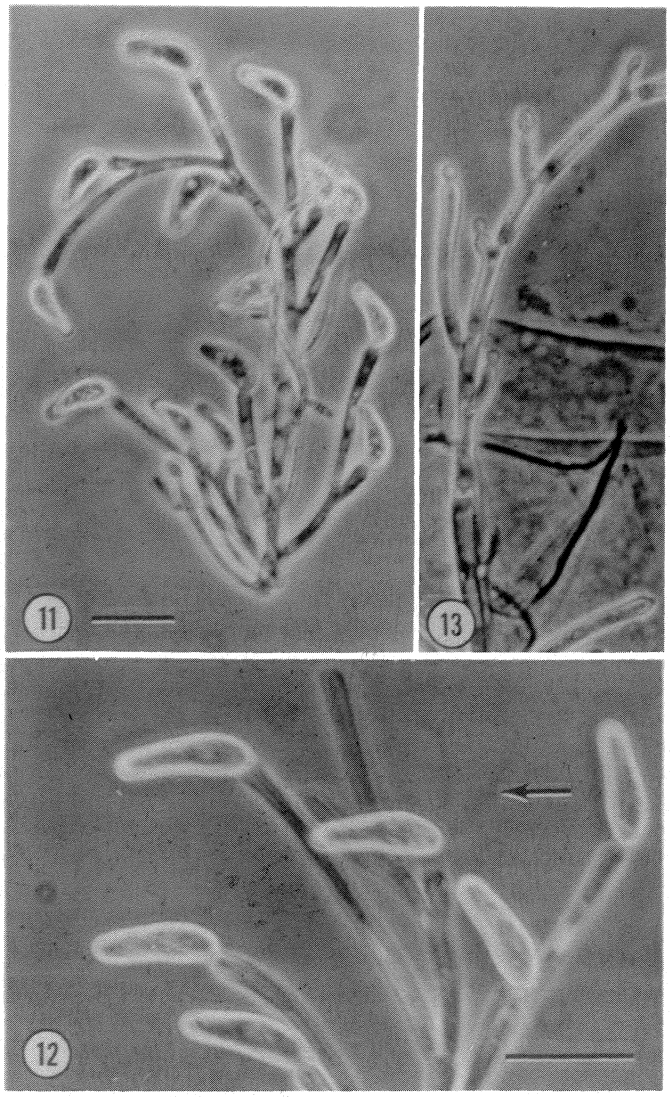
Stachylina penetralis is one of several Stachylinas whose bases penetrate through the loose peritrophic membrane that lines the midgut. In this species the thallus has a bulbous base and produces 2-12 long-ellipsoidal trichospores that range in size from 30-50 x 8-12 µm. Only one specimen was found. It was in a *Cricotopus* sp. larva on the divide of the Cordillera de Talamanca at the initiation of the West Branch of Q. Macho Mora (Site **20**). The species has been found on several continents.



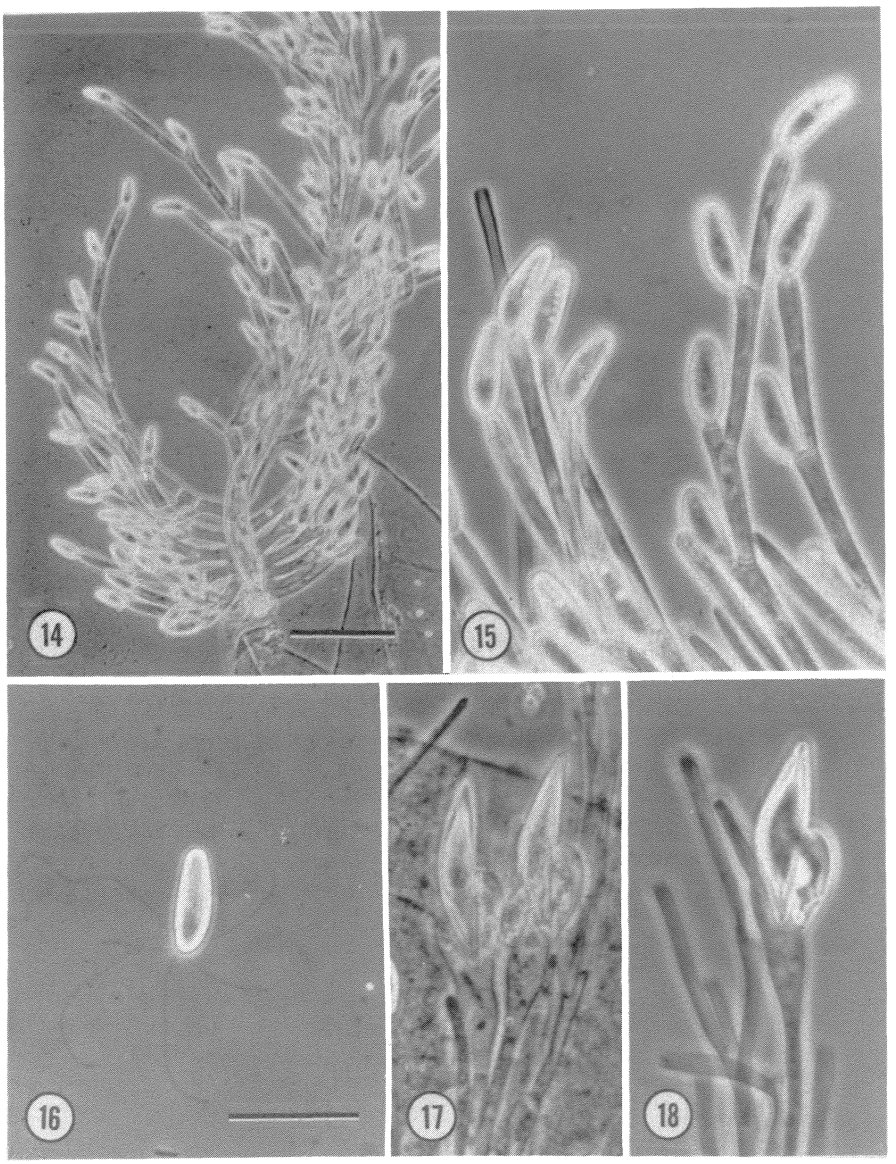
Figs. 2-6. *Harpella tica*. 2. Two sporulating thalli within the transparent peritrophic membrane of a Simuliidae larva. 3-5. Segments of a single thallus attached to a peritrophic membrane by a secreted holdfast (single arrow), the terminal trichospore having just released and with some of the 2-4 very fine basal appendages barely visible (double arrows). 6. Typical sigmoid trichospore with part of two of the basal appendages in focus (arrows). Fig. 2 bar = 80 μm; Fig. 3 bar = 40 μm (same magnification for other Figures).



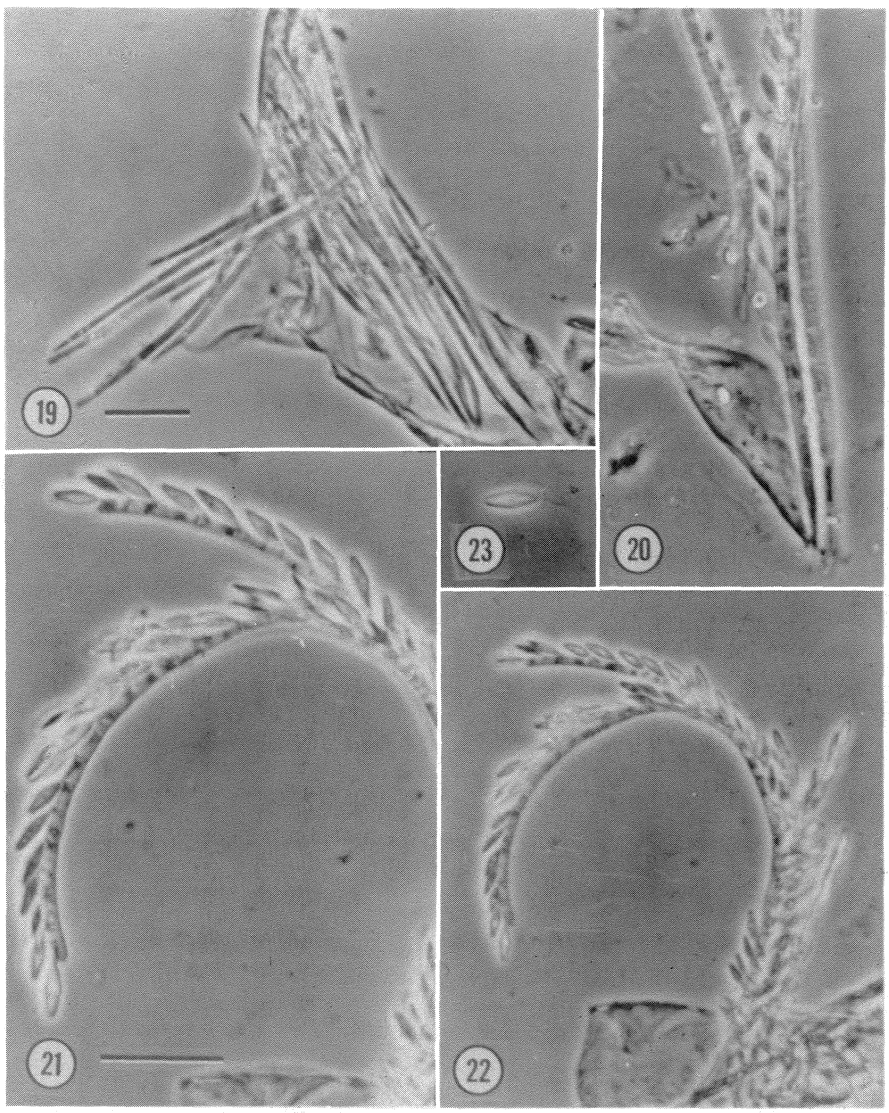
Figs. 7-10. *Genistellospora guanacastensis.* 7. Sporulating thallus removed from the hindgut cuticle of a Simuliidae larva. 8. Base of a thallus attached to the host cuticle by means of a secreted holdfast (arrow). 9. Sporulating branchlet showing appendages within the generative cell (arrow). 10. Released trichospores with multiple coiled basal appendages. Fig. 7 bar = 80 μm; Fig. 8 bar = 40 μm (same magnification for other Figures).



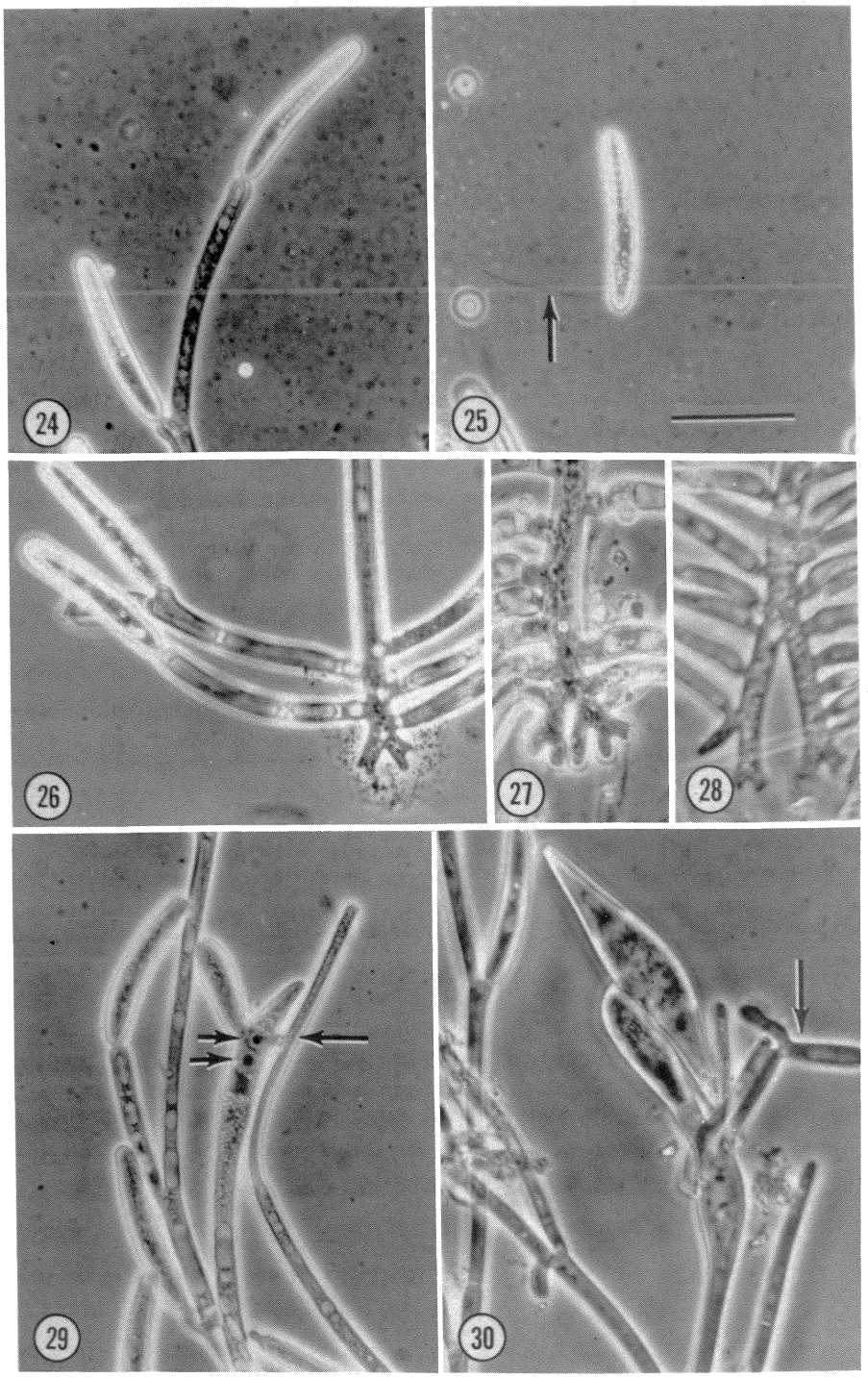
Figs. 11-13. *Genistellospora nubila.* 11. Entire sporulating thallus. 12. Trichospores, one released and showing several shadowy appendages attached to its base (arrow). 13. Initiation of trichospores at the tips of branchlike extensions. Fig. 11 bar = 40 μm (same magnification for Fig. 13); Fig. 12 bar = 40 μm.



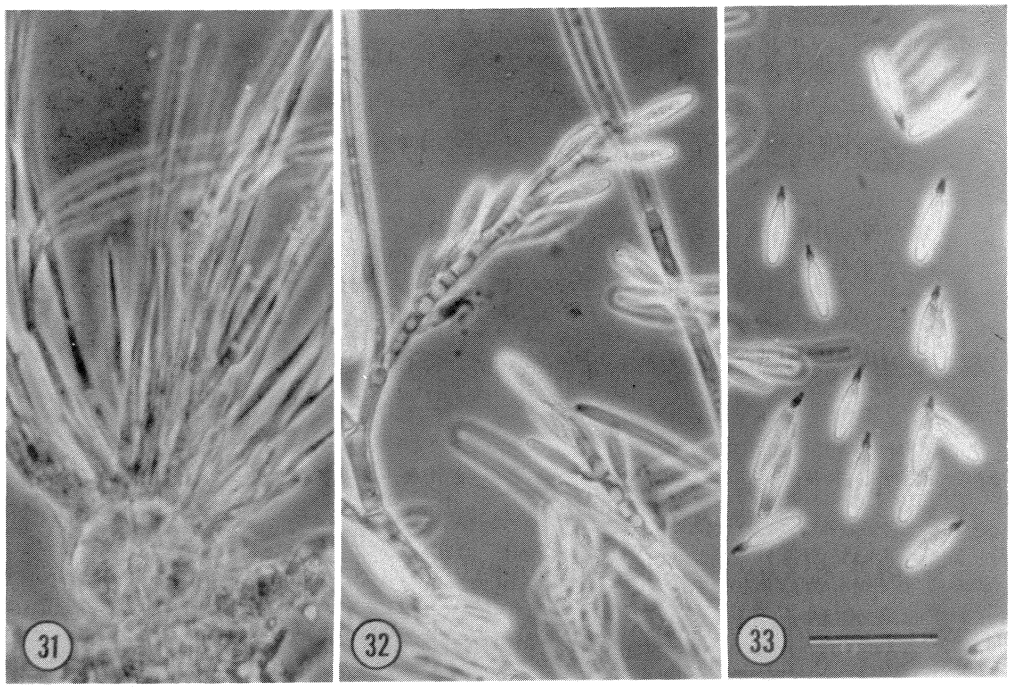
Figs. 14-18. *Genistellospora tepidaria.* 14. Sporulating thallus attached to a piece of hindgut cuticle. 15. Trichospores. 16. Released trichospore with six barely visible appendages. 17, 18. Zygospores formed without conjugation. Fig. 14 bar = 80 µm; Fig. 16 bar = 40 µm (same magnification for other Figures).



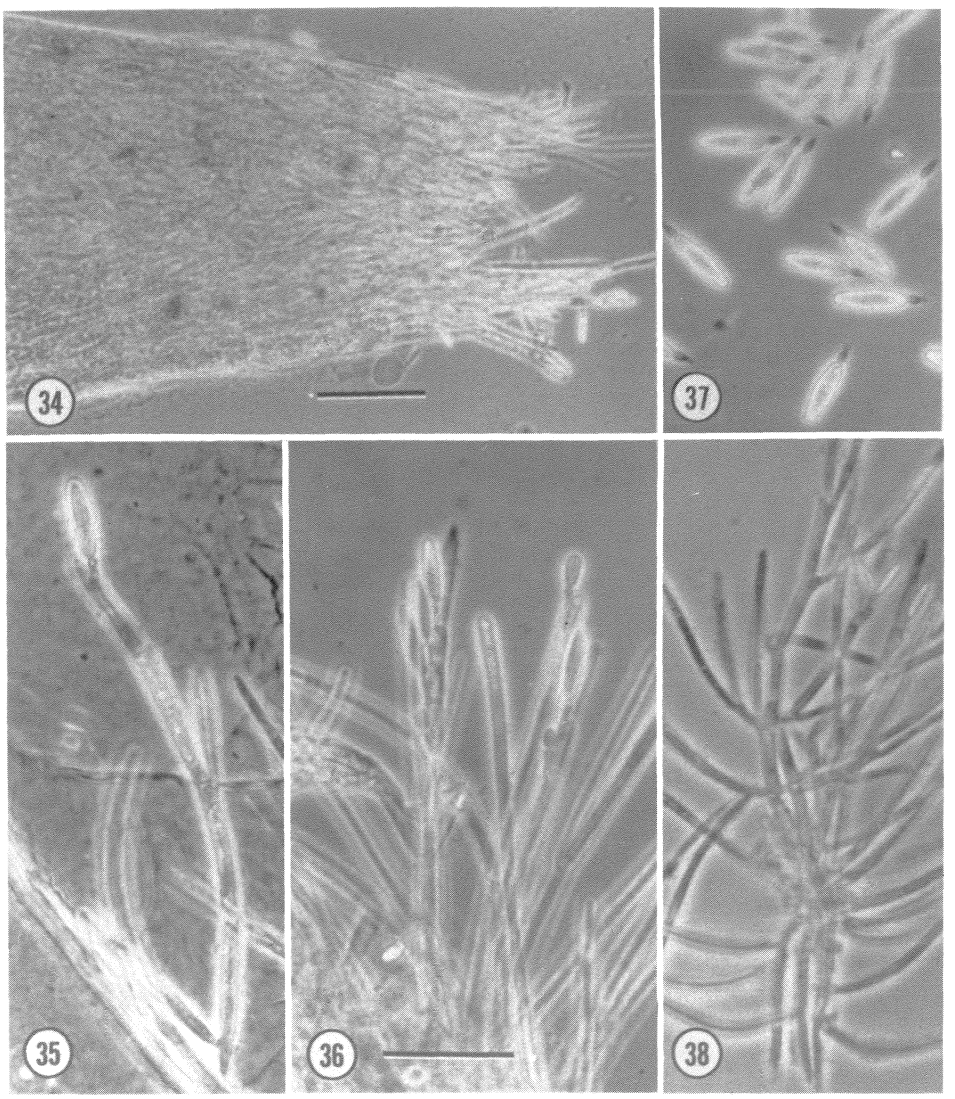
Figs. 19-23. *Graminelloides biconica.* 19. Fascicled (clustered) arrangement of immature thalli removed from the hindgut of a Simuliidae larva. 20. Tapered bases of sporulating thalli attached to a piece of hindgut lining. 21, 22. Trichospores produced by a sparsely branched thallus; note almost complete conversion of thallus to trichospore production. 23. Released trichospore with a short single appendage. Fig. 19 bar = 40 μm (same magnification for Fig. 22); Fig. 21 bar = 40 μm (same magnification for Figs. 20, 23).



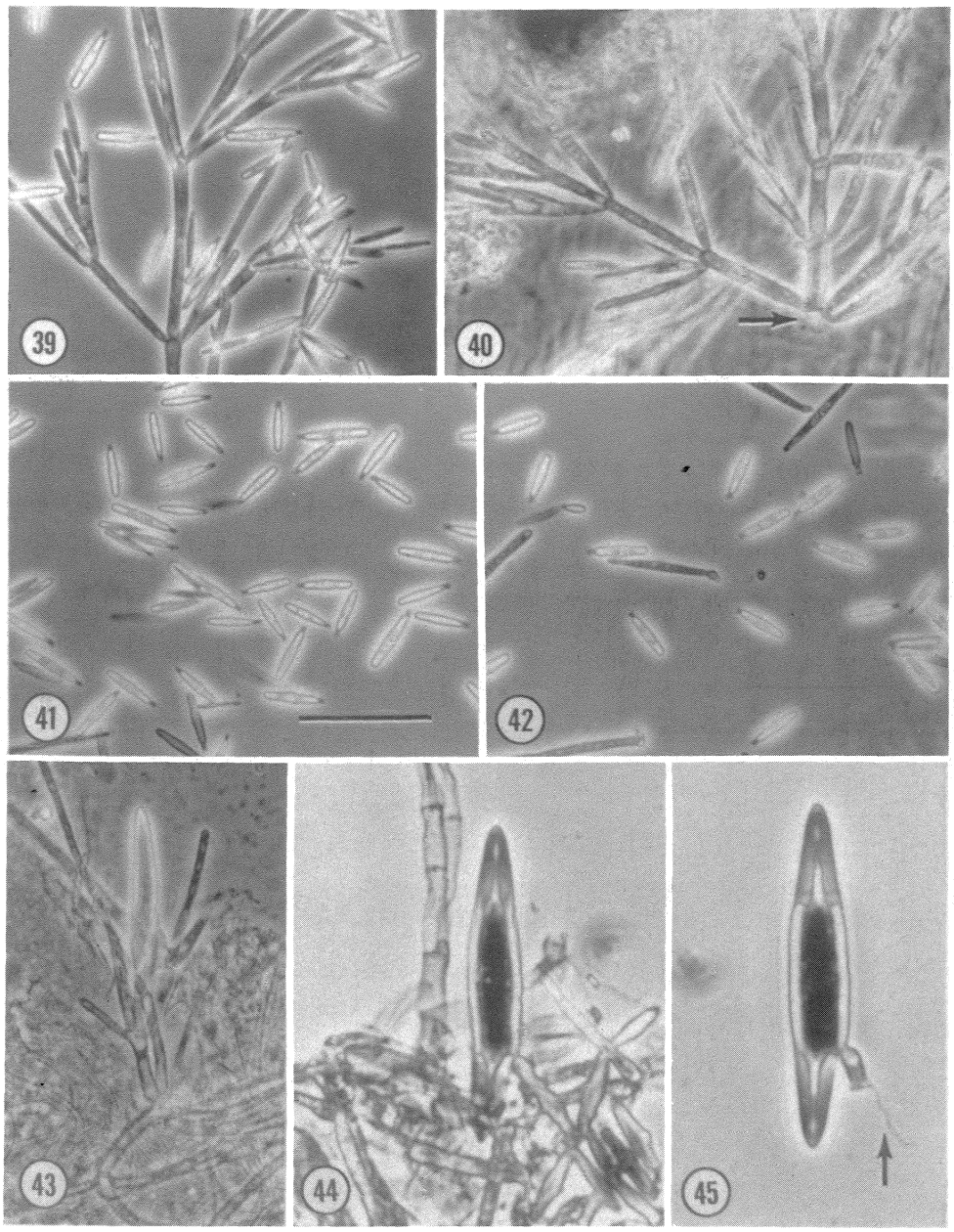
Figs. 24-30. *Pennella montana*. 24. Two attached trichospores. 25. Released trichospore showing one of the multiple appendages in focus (arrow). 26-28. Bases of mature thalli with mucilaginous secretion and with different degrees of bifurcation. 29. Conjugation of two branches (long arrow), a stage that initiates zygospore formation; within the receptive cell that will become the zygosporophore are seen two nuclei (short arrows) prior to their fusion. 30. Zygospore attached to the zygosporophore, with the attached conjugation cell (arrow) now broken loose from the branch that produced it. Fig. 25 bar = 40 µm (applies to all Figures).



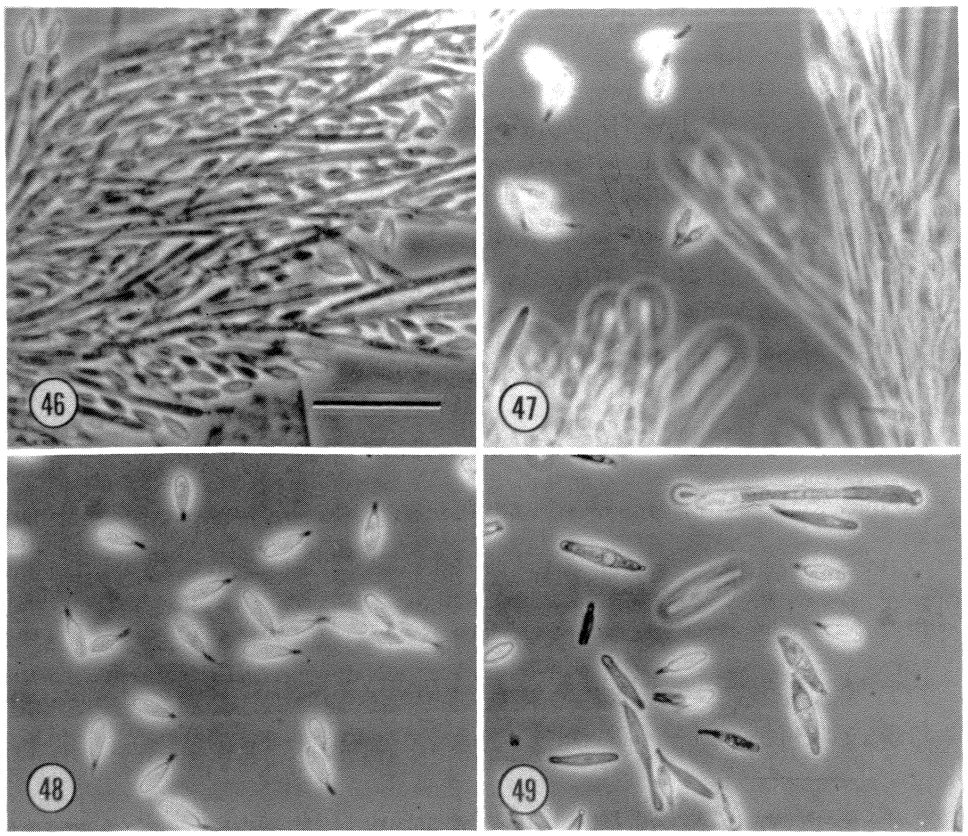
Figs. 31-33. *Smittium annulatum.* 31. Small, compact thallus attached to the hindgut cuticle of a Simuliidae larva by a ring of enlarged cells. 32, 33. Attached and loose trichospores from a pure culture (CR-143-8). Scale bar = 40 μm (applies to all Figures).



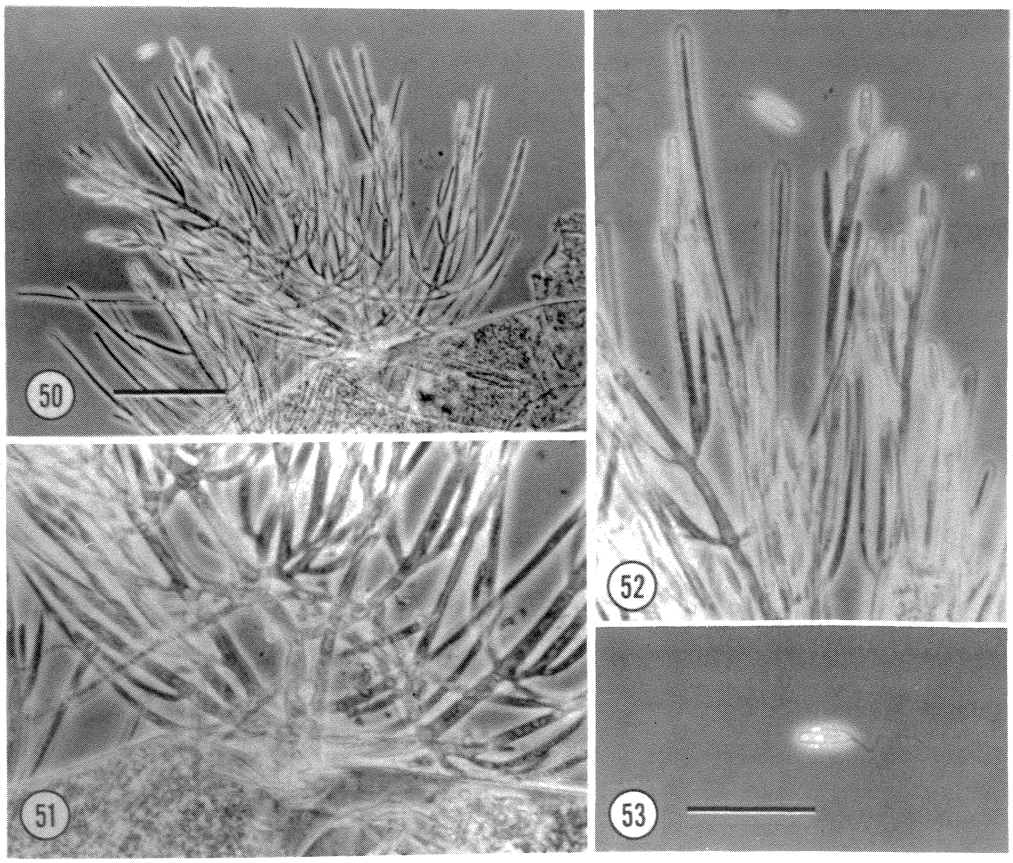
Figs. 34-38. *Smittium culicisoides.* 34. Part of the dissected hindgut of a Chironomidae larva packed with fungal thalli. 35, 36. Sporulating branchlets. 37. Detached trichospores from a pure culture (CR-253-12). 38. An immature thallus showing the tapered basal part. Fig. 34 bar = 80 μm; Fig. 36 bar = 40 μm (same magnification for other Figures).



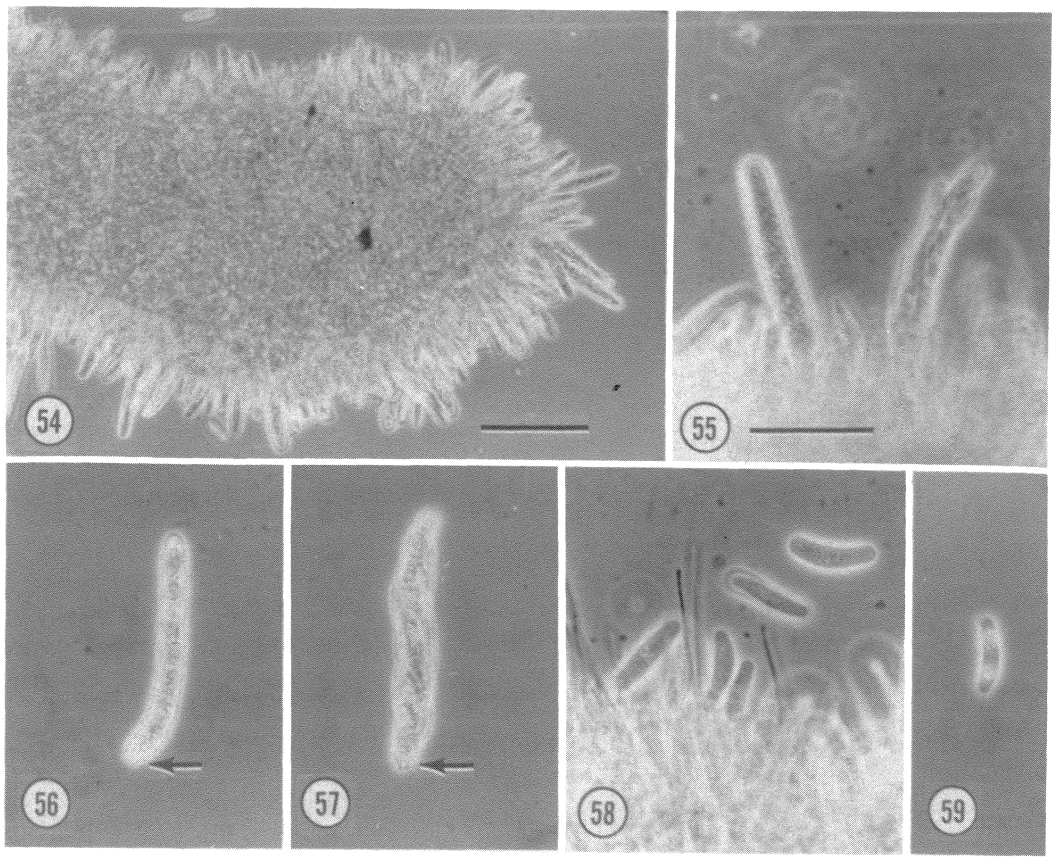
Figs. 39-45. *Smittium dipterorum.* 39, 40. Sporulating thalli showing verticillate branching and a simple holdfast (arrow). 41. Detached trichospores from a pure culture (CR-253-14) isolated from a Simuliidae larva. 42. Culture CR-254-13 isolated from a Chironomidae larva. 43. Maturing zygospore. 44-45. Attached and detached mature zygospores from lactophenol cotton-blue mounts, one showing a collar and one short appendage (arrow). Fig. 41 scale bar = 40 μm (applies to all Figures).



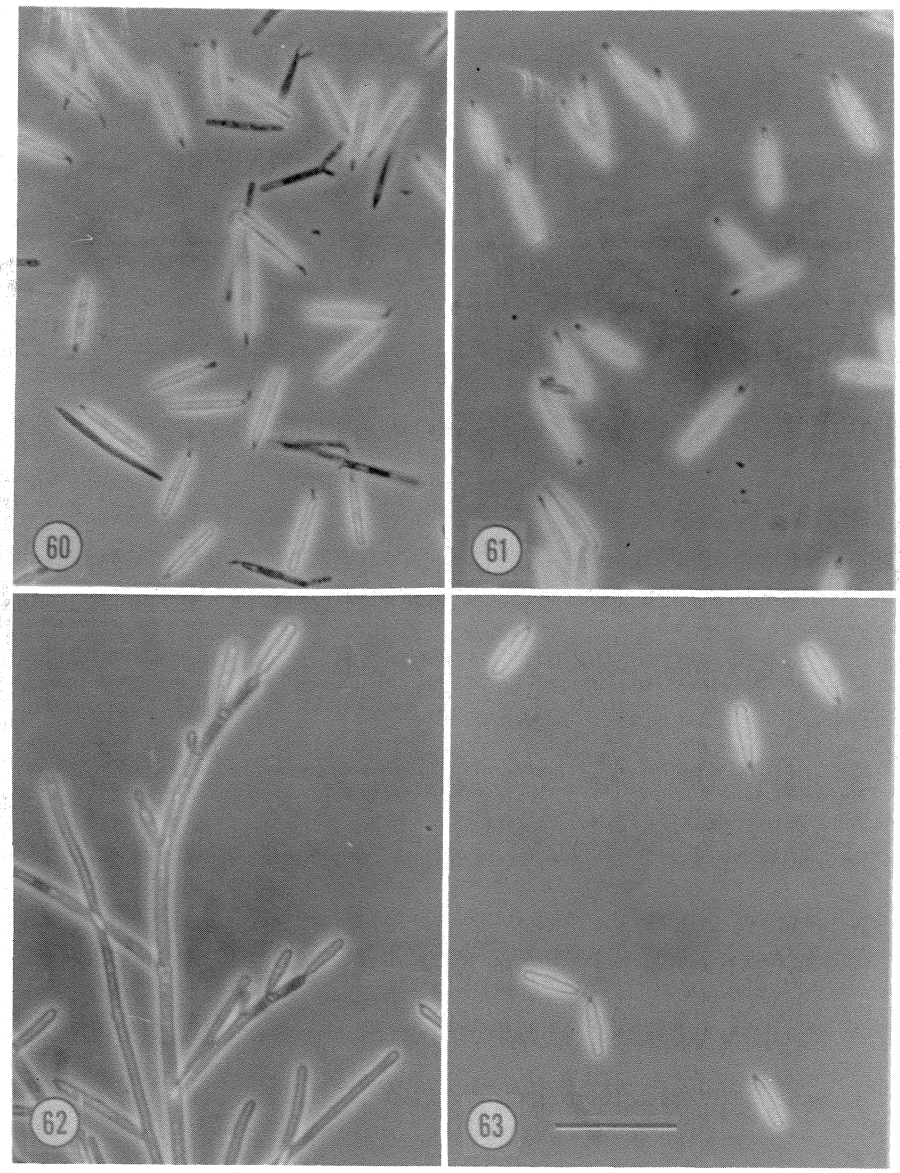
Figs. 46-49. *Smittium parvum.* 46. Part of a thallus with developing trichospores removed from the hindgut of a Chironomidae larva. 47. Attached and detached trichospores reproduced from the Isotype Kodachrome transparency (culture CR-184-32). 48. Trichospores from the Holotype transparency (culture CR-184-32). 49. Trichospores from culture CR-254-10. Fig. 46 bar = 40 μm (same magnification for all Figures).



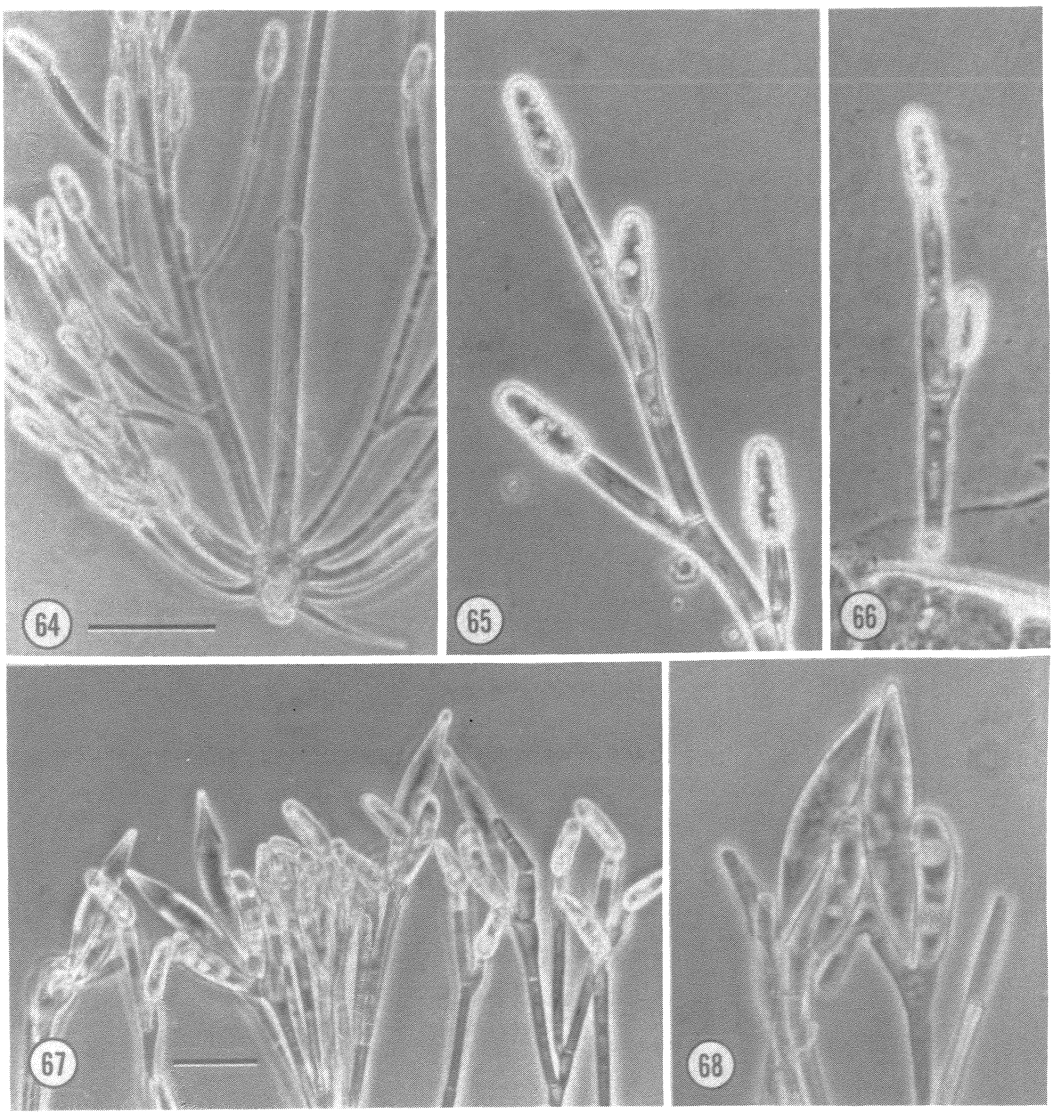
Figs. 50-53. *Spartiella animae*. 50. Highly branched thallus attached to hindgut cuticle of a mayfly nymph. 51. Holdfast region of same thallus. 52, 53. Attached and loose trichospores. Fig. 50 bar = 80 μm ; Fig. 53 bar = 40 μm (same magnification for other Figures).



Figs. 54-59. *Amoebidium colluviei.* Anal tubule of a bloodworm (*Chironomus* sp.) completely covered with thalli of *A. colluviei* in different developmental stages. 55. Attached mature thalli, the one on the right containing sporangiospores. 56, 57. Detached thalli showing small holdfast (arrows), one with sporangiospores. 58, 59. Released allantoid sporangiospores. Fig. 54 bar = 80 μm; Fig. 55 bar = 40 μm (same magnification for other Figures).



Figs. 60-63. Unnamed, possibly new *Smittium* spp. in axenic culture. 60. *Smittium* sp. CR No. 1 (culture CR-239-12). 61. *Smittium* sp. CR No. 2 (culture CR-259-4). 62, 63. *Smittium* sp. CR No. 3 (culture CR-211-1). Bar = 40 µm for all Figures.



Figs. 64-68. *Genistellospora homothallica* from hindgut of Simuliidae larvae. 64. Base of a sporulating thallus with a prominent secreted holdfast. 65. Developing trichospores. 66. Rare 2-celled thallus with immature trichospores. 67. Trichospores and 5 biconical zygospores. 68. Two zygospores which, in this genus, form without prior conjugation of branches. Fig. 64 = 40 μm; same magnification for other Figures except Fig. 67, where bar = 40 μm.

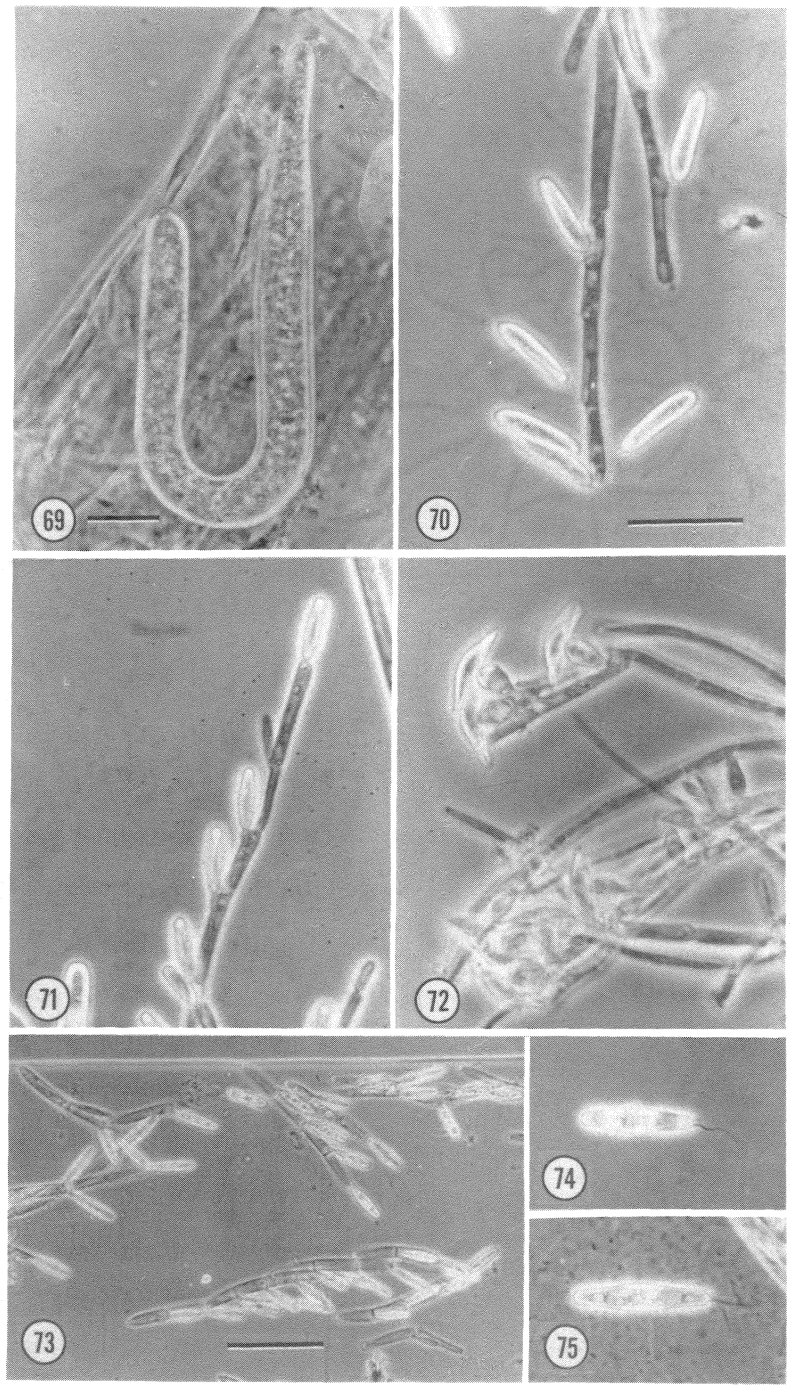


Fig. 69. *Paramoebidium* sp. growing within the hindgut of a mayfly nymph. Fig. 70. Part of a *Pennella simulii* thallus with recently released trichospores with multiple basal appendages. Figs. 71, 72. Trichospores and zygospores of *Simuliomyces microsporus*. Figs. 73-75. Thalli and loose trichospores of *Stachylina grandispora* within the peritrophic membrane of a bloodworm (*Chironomus* sp.). Fig. 69 bar = 40 μm; Fig. 73 bar = 80 μm; Fig. 70 bar = 40 μm (same magnification for other Figures).

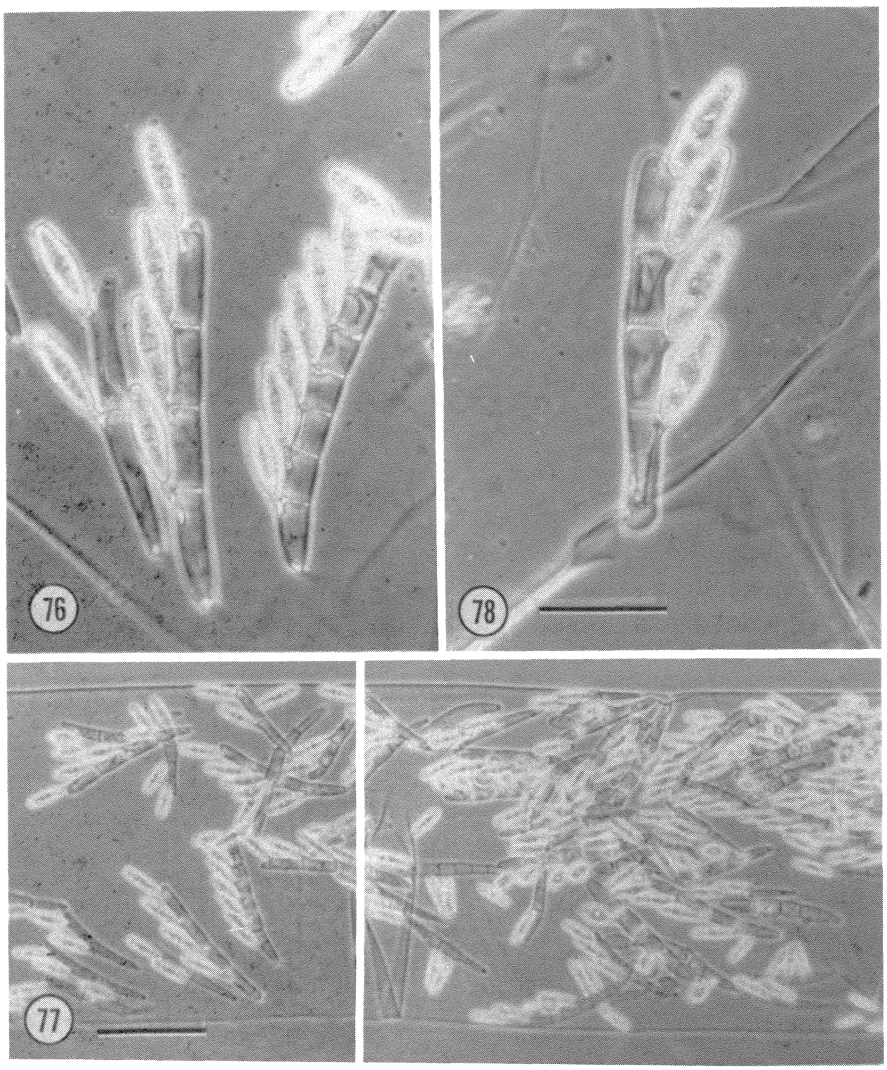


Fig. 76, 77. *Stachylina nana* within the peritrophic membrane of a Chironomidae larva. 76. Three sporulating thalli. 77. Peritrophic membrane with a large number of individual sporulating thalli; each thallus developed from one ingested trichospore. Fig. 78. Sporulating thallus of *Stachylina penetralis* whose slightly bulbous base has penetrated the peritrophic membrane. Fig. 77 bar = 80 μm; Fig. 78 bar = 40 μm (applies also to Fig. 76).

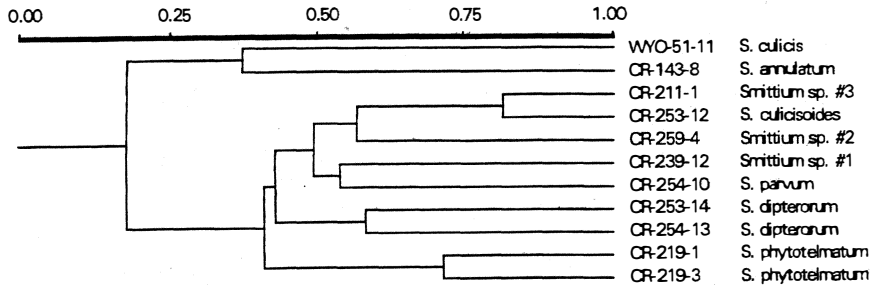


Fig. 79. Phenogram derived from an analysis of isozyme patterns produced by 10 cultured isolates of *Smittium* from Costa Rica (CR) and one from Wyoming, U.S.A. (WYO). Dipteran hosts of the CR species are provided in Table 1 and in the text. *Smittium culicisoides* (CR-253-12), from a Chironomidae larva, produces trichospores that are essentially identical to *S. culicis*, represented here by isolate WYO-51-11 from a Culicidae larva, but they can be seen to be isozymically distinct. The similarity of isozyme patterns between *S. culicisoides* (CR-253-12) and *Smittium* sp. #3 (CR-211-1) is not explainable, because the two species are morphologically quite different. Scale at top represents a numerical measure of similarity of isozyme patterns. Analysis based on isozyme data obtained by Grigg (1994).

KEY TO TRICHOMYCETES ASSOCIATED WITH COSTA RICAN AQUATIC INSECTS

1. Small cylindrical thalli (<100 mm long) attached externally to the anal papillae of Chironomidae or Simuliidae larvae.....*Amoebidium colluviei* (Amoebidiales)
1'. Thalli attached internally to hindgut cuticle or peritrophic membrane.....2
2(1'). Thalli unbranched attached to peritrophic membrane of Diptera larvae.....3
2'(1'). Thalli branched or unbranched, attached to hindgut cuticle of Diptera or Ephemeroptera..... 7
3(2). In Simuliidae larvae; trichospores cylindrical, sinuate or more rarely curved, with 2-4 appendages.....*Harpella tica*
3'(2). In Chironomidae larvae, trichospores ellipsoidal, straight, with 1 appendage.....4
4(3'). Base of thallus bulbous, protruding through the peritrophic membrane.....*Stachylina penetralis*
4'(3'). Base of thallus with a small holdfast, not penetrating the peritrophic membrane.....5
5(4'). Trichospores without a collar, 25-40 x 7-11 µm.....*Stachylina nana*
5'(4'). Trichospores with a very short collar 6
6(5'). Trichospores 40-70 x 6-10 µm.....*Stachylina grandispora*
6(5'). Trichospores 31-40 x 6-8 µm.....*Stachylina paludosa*
7(2'). In Ephemeroptera nymphs..... *Spartiella animae*
7'(2'). In Diptera larvae..... 8
8(7'). Thalli unbranched, nonseptate, cylindrical or sausage-shaped, straight throughout or curved, with a distinct basal holdfast; reproducing by release of amoeboid cells..... *Paramoebidium* spp. (Amoebidiales)
8'(7'). Thalli branched, septate, attached by a secreted holdfast or by a basal mucilaginous secretion; producing trichospores laterally on fertile branches, and sometimes zygospores..... 9

(*Continued...*)

9(8'). Trichospores biconical, without a collar, with 1 basal appendage; in Simuliidae larvae..... *Graminelloides biconica*
9'(8').Trichospores not biconical; either with a collar and 1 basal appendage or without a collar and 2-6 basal appendages..... 10
10(9'). Trichospores without a collar, with more than one basal appendage; in Simuliidae larvae.....11
10'(9'). Trichospores with a collar and a single basal appendage; in Chironomidae or Simuliidae larvae.....17
11(10). Trichospores long-ellipsoidal with 2-4 appendages; zygospores <50 µm long; thalli frequently attached to other gut organisms, especially to thalli of *Paramoebidium* .....*Simuliomyces microsporus*
11'(10). Trichospores ovoid to long-ovoid with 4-8 appendages; zygospores >50 µm long; thalli always attached to hindgut cuticle..... 12
12(11'). Base of mature thalli usually bifurcate, attached to hindgut cuticle by a mucilaginous secretion..... 13
12'(11'). Base of mature thalli not bifurcate, attached to hindgut cuticle by a non-mucilaginous holdfast..... 14
13(12). Trichospores 39-72 x 8-10 µm; zygospores *91-100 x 18-20 µm*.....*Pennella montana*
13'(12). Trichospores 30-41 x 6.5-10.5µm; zygospores 84-96 x 19-24 µm..... *Pennella simulii*
14(12'). Trichospores 50-61 µm long..... *Genistellospora guanacastensis*
14'(12'). Trichospores < 50 µm long..... 15
15(14'). Trichospores 38-41 µm long..... *Genistellospora nubila*
15'(14'). Trichospores almost always <38 µm long (average length 27-30 µm)..... 16
16(15'). Trichospores 24-41 µm long; zygospores 77-113 x 15-22 µm .....*Genistellospora homothallica*
16'(15'). Trichospores 21-31 µm long; zygospores 58-70 x 10-15 µm .....*Genistellospora tepidaria*
17(10'). Trichospores widest below midline (long-ovoid), 11-30 x 3-7 µm, usually (but not always) in Culicidae larvae..... *Smittium culisetae*
17'(10'). Trichospores longitudinally symmetrical (oval to subcylindrical), never in Culicidae larvae..... 18
18(17'). Trichospores more or less cylindrical, 2-4 µm diameter..... 19
18'(17). Trichospores oval to elongate-oval, 3.5-10 µm diameter..... 21
19(18). In Simuliidae or Chironomidae larvae living in streams (lotic) .....*Smittium dipterorum*
19'(18). In Chironomidae larvae living in phytotelms (usually bromeliads) or other lentic habitats.....20
20(19'). Thalli in fascicle-like clusters in hindgut, with some growth into midgut region..... *Smittium fasciculatum*
20'(19'). Thalli not in fascicles and restricted to hindgut region..... *Smittium phytotelmatum*
21(18'). Base of thallus consisting of about 6 cells arranged in a ring.....*Smittium annulatum*
21'(18'). Base of thallus without a ring of cells..... 22
22(21'). Trichospores about 3.5 µm diameter..... *Smittium parvum*
22'(21'). Trichospores about 6-10 µm diameter..... *Smittium culicisoides*

DISCUSSION

Though this report represents the results of 110 collections in a variety of stream habitats over a period of years and at different seasons, conclusions about distributional patterns of Harpellales in Costa Rica are limited by collection biases. Factors such as presence or absence of particular fungal species in similar but disjunct life zones, altitudinal distributions, and temporal influences that affect the prevalence of aquatic insect hosts would have required a differently designed and more lengthy study. Nonetheless, certain conclusions are justified from the data that have been obtained.

First, it is quite clear that Harpellales infect many aquatic insect larvae throughout Costa

Rica, but the degree of diversity and species richness of these fungi has not been thoroughly explored. *Harpella tica* was found in over 95% of Simuliidae larvae and in all populations sampled. This new species is probably not endemic to Costa Rica, but the extent of its distribution in the Neotropics is still to be determined. Studies in southern Chile (Lichtwardt and Arenas 1996) revealed a similar situation, where another new species, *H. meridianalis* Lichtw. & Arenas, was the only *Harpella* encountered, but in high frequency. Surprisingly, the very widespread species, *H. melusinae*, which infects simuliid larvae in many parts of the world, was found neither in Costa Rica nor in southern Chile.

Other Harpellales infecting simuliid larvae were less distributed, and it is possible that some of the fungi that infect such larvae are either restricted to particular stream conditions or to particular species of hosts. Based on studies in other regions of the world, as well as Costa Rica, Trichomycetes in Simuliidae do not in general appear to be species or even genus specific, but it was not possible in these investigations to separate habitat requirements of particular species from host specificity. (It should be noted that the larval stages of many aquatic insects cannot be identified to species because of insufficient studies.) Among the thousands of simuliid larvae dissected, *Genistellospora guanacastensis* was found only in four sites in Guanacaste Province, *G. nubila* in one stream in Monteverde, and *G. tepidaria* in several streams at the La Selva Biological Station. Also apparently restricted in distribution was the new monotypic genus *Graminelloides*, found in two species of simuliid larvae from one stream near Portalón in Puntarenas Province. One expects that each of these fungal species has a greater range than the collections indicate, but it is also evident that many gut fungi have not disseminated to the extent that *H. tica* has. Several other examples of limited distributions are provided in the preceding descriptions of individual fungal species.

Trichomycetes in Costa Rican mayfly nymphs (Ephemeroptera) warrant additional studies. In this paper the only Harpellales reported from mayflies is the new species *Spartiella anima*. The relatively common genus *Paramoebidium* (Amoebidiales) was found in several species of *Baetis*. Other Baetidae were infected with what might be new genera of branched Harpellales, but they are not described here because of insufficient documentation. These were in nymphs of a *Baetis* sp. from S fork R. Humo (Site **18**) and a *Baetodes* sp. from R. Macho (Site **10**). In addition, what is possibly another new genus of Harpellales was found in an unidentified Caenidae nymph from Q. Sábalo (Site **3**).

Twelve successful axenic cultures from dipteran larvae represented seven new species of *Smittium* (three of them not named at this time). *Smittium* is the largest genus of Harpellales, currently containing 47 named species plus the three unnamed ones described and illustrated in this paper (Figs. 60-63). The eight cultured Costa Rican Smittiums can be distinguished from each other not only on a morphological basis, which was given primary taxonomic emphasis, but also by their isozyme patterns (Fig. 79), with the exception of *S. culicisoides* isolate CR-253-12 and *Smittium* sp. #3 isolate CR-211-1. Those two had an 81% measure of isozymic similarity, which normally would indicate species congruence, but they differ morphologically (compare Figs. 35-37 with Figs. 62, 63). *Smittium culicisoides*, on the other hand, produces trichospores that are essentially indistinguishable from the common, widespread *S. culicis*, but the isozyme analysis in Fig. 79 clearly indicates they are distinct.

Water temperature is one of several factors that influence a stream's fauna, and consequently the gut mycota. The warmest streams at the various particular times collections were made were on the Pacific side of the Divide: Río Caracol, 27.5 C (Site **29**), several streams southeast of Quepos, 26-27 C (Site **25**), and R. Pièdras, 26-28 C (Site **30**). At higher altitudes, as might be expected, stream temperatures were considerably cooler: Cordillera de Talamanca, 11-15 C (Sites **18-20**); Monteverde region, 15.5-17.5 C (Sites **6-9**); premontane wet forest southwest of San José, 11-15 C (Sites **15-17**); and Wilson Botanical Garden, 19.5-20 C (Site **27**). Streams in the low wet forest at La Selva (Sites **1-4**) ranged from 24 to 25.8 C.

Another factor affecting stream biota is the content of organic matter. Our collections included pristine streams near the headwaters of drainages with no nearby habitations, as well

as streams obviously polluted by human activity. The most extreme case of pollution by organic supplementation was found in Río Guacimal in Monteverde downstream from where a cheese factory was piping whey into the stream (Site 7). Small pieces of curd from the whey effluent could be filtered in large quantities from the malodorous water, and submerged leaves and rock surfaces were slimy. Some of the particles were found in the guts of Chironomidae larvae. The number of Chironomidae and Simuliidae larvae downstream from the whey discharge was dramatically greater than the number of these insects immediately above the discharge, and the abundance of Trichomycetes in their guts was also greater. In the stream below the effluent during the years of study the following Trichomycetes were found: *Amoebidium colluviei*, *Harpella tica*, *Genistellospora homothallica*, *Paramoebidium sp.*, *Pennella montana*, *Simuliomyces microsporus*, *Smittium culicisoides*, *S. dipterorum*, *S. parvum*, *Stachylina grandispora*, and *S. nana*. In contrast, above the effluent and at various sites along Q. Cuecha (the upper portion of R. Guacimal), only five species were encountered: *H. tica*, *G. homothallica*, *Paramoebidium* sp., *P. montana*, and, once, an undetermined species of *Smittium*. Obviously, the high organic content in the water did not affect the growth and reproduction of many of the gut fungi.

The effect of this organic pollution on composition of the insect fauna was not easy to measure at the species level, because of the difficulty in identifying larvae to species. Among the Chironomidae identified, one or more species of the following genera were present: *Chironomus*, *Cricotopus*, *Cryptochironomus*, *Polypedilum*, *Cardiocladius*, and an unidentified genus of Orthocladiinae. Simuliidae included *Simulium* nr. *callidium* and several unidentified or unidentifiable species. Some time between 17 and 24 June 1988, however, the same stretch of R. Guacimal had undergone a dramatic change, involving a massive kill of almost all insect larvae. This was indicated by the presence of many dozens of dead larvae in some of the leaf packs (115 dead larvae consisting of perhaps four species were counted in one leaf pack, for instance), and with almost no living larvae clinging to their usual substrates, nor within siltation zones where large numbers of *Chironomus* larvae had consistently been found. What caused this phenomenon is not known. On the next visit to that site several years later, on 18 November 1991, both the fauna and gut fungi were present in their usual high numbers.

Streams polluted with certain chemicals may, of course, have a profound negative effect on the fauna and consequently on the presence of trichomycete gut fungi. Such was the case in a few streams visited by the author (not reported in Table 1), most notably in the agricultural area within the general drainage of Río Estrella on the eastern coast north of Cahuita. An almost complete lack of insect fauna, possibly due to the widely reported use of agricultural pesticides in that area, resulted in an absence of trichomycete hosts.

Despite the abundance of larvae and gut fungi in the stretch of R. Guacimal cited above, in most Costa Rican streams the overall density and species richness of aquatic insects with gut fungi were not greater than that found by the author in many nontropical regions of the world. What is often referred to as the latitudinal species diversity gradient remains debatable, both as to the causal factors and whether the concept applies to all groups of organisms (Illies 1969; Rosenzweig 1992). It has been adequately demonstrated that many groups of plants and animals have fewer species the farther one gets from the Equator. Terrestrial insects are one such group, but is this true of insects that have primary aquatic stages? Some investigators have concluded that such a latitudinal gradient possibly does exist among aquatic insects (Stout and Vandermeer 1975; McElravy *et al.* 1981), others that a greater diversity of aquatic insects in the tropics is not demonstrable (Patrick 1966; MacArthur 1972; Allan and Flecker 1993). In this discussion we will focus on the species richness of Harpellales in Costa Rica, which is, at the same time, a reflection of the diversity of their aquatic hosts, namely the larval stages of lower Diptera (Nematocera), Ephemeroptera, and Plecoptera. Excluded from the comments that follow are terrestrial, marine, and freshwater arthropods infected with Eccrinales and Asellariales, only a few of which were collected by the author because of the emphasis in this study on Harpellales.

Seventeen species of Harpellales were

found in 36 Costa Rican streams during more than 25 weeks of collecting from 1984 through 1991. In contrast, and as an example, collections at five sites in two Rocky Mountain streams in Colorado, U.S.A. during a six-week period yielded 20 species of Harpellales (Lichtwardt and Williams 1988). If one considers all species of Harpellales that have now been found during two short investigations in that same region of the Rocky Mountains (a few species are still to be described), they now total several dozen. Both the diversity of stream life and species richness of particular groups of insects in the Rocky Mountains is well documented [Ward and Kondratieff 1992; Peckarsky *et al.* (undated)].

In our Costa Rican studies only nine genera of Chironomidae larvae (species determinations not possible) were found to be infected, and consequently relatively few Costa Rican Harpellales that inhabit such larvae have been reported to date. With one exception, all Ephemeroptera nymphs encountered in Costa Rica were Baetidae, and only one fungal genus (*Spartiella*) is reported. In various nontropical parts of the world, Baetidae are known hosts of seven of nine genera of Harpellales that infect ephemeropterans. The third order of harpellid hosts is Plecoptera, know to harbor six genera of Harpellales in nontropical regions. Plecopterans are species poor in Costa Rica, because these insects are almost completely restricted to cooler running waters (Illies 1969). No Harpellales are reported from Costa Rica plecopterans.

Although quantitative studies have not been carried out by the author, in his experience in temperate North America, Australia, New Zealand, and Patagonia, kicking the substrate in suitable stream habitats and collecting the released insects in a net usually has yielded, as one crude measure, a far greater variety and number of individual larvae that contain gut fungi than in most of the Costa Rican streams that were sampled. (Of course, other more refined collecting methods for particular kinds of insect hosts of trichomycetes were also used.) Stout and Vandermeer (1975) sampled streams in Costa Rica by collecting individuals from rocks in riffles and identifying the OTUs (operational taxonomic units) for nine orders of insects, and they also reported the average number of individuals per rock. They stressed the importance of sampling at least 20-25 rocks (12-30 cm across in riffles of small streams) to obtain an adequate measure of species richness when comparing tropical and mid-latitude streams. We used three of the same Costa Rican streams among those that were sampled by Stout and Vandermeer: Quebrada Sábalo (seven collections over 4 yr), Q. Esquina (six collections over 4 yr), and Río Corobicí (three collections over 2 yr). Though the precise locations of the stream riffles may have been different, our rock samples yielded relatively few insect larvae, even when we once sampled 25 rocks, such that we did not try to tabulate the results. (Río Corobicí yielded almost no insects in selected sites in 1988 and 1991, leading to the suspicion that some detrimental event upstream had reduced the rich insect populations reported by Stout and Vandermeer.)

It would appear that the somewhat low number of Harpellales species found in Costa Rica, considering the temporal and spacial extent of our investigations, might be attributable to a relatively low diversity of the particular kinds of aquatic insect taxa in which these gut fungi have evolved. At the same time it should be noted that this study of tropical lotic Trichomycetes, because of the considerable time it takes to dissect arthropods and prepare their gut fungi, does not represent all kinds of life zones—and certainly not all geographic areas and microhabitats—that might have been explored in Costa Rica. The fact that more than two-thirds of the aquatic Trichomycetes that have been found to date in Costa Rica are new species, suggests that Costa Rica and the Neotropics in general are reservoirs of many yet unknown genera and species of these parasites of arthropods.

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