Rev. Biol. Trop., 42 (1/2): 31-48, 1994

Trichomycete fungi living in the guts of Costa Rican phytotelm larvae and other lentic dipterans

Robert W. Lichtwardt
Department of Botany, University of Kansas, Lawrence, KS 66045-2106, U.S.A.

(Rec. 27-X-1993. Acep. 17-II-1994)

Abstract: Species of Harpellales (Trichomycetes: Zygomycotina) were found living in the guts of Chironomidae, Culicidae and Ceratopogonidae larvae inhabiting reservoirs of water retained by the leaves of epiphytic bromeliads and a few other plants, including pineapple and banana, as well as from a swamp. Periodic collections in Costa Rica between 1984 and 1991 included a low wet forest (La Selva Biological Station), a cloud forest (Monteverde Cloud Forest Reserve), and a zone of premontane rain forest (Las Cruces Biological Station). A new trichomycete, *Smittium phytotelmatum*, that inhabits bloodworms (Chironomidae) was found primarily but not exclusively in bromeliads, and several axenic cultures of the fungus were obtained. A second, rarer, new species from a bromeliad bloodworm, *S. fasciculatum*, is also described, as is the new species *Stachylina paludosa* from swamp bloodworms. Two possibly new but unnamed species of *Smittium* are described from lentic dipteran larvae. Mosquito, midge and ceratopogonid larvae were hosts to a geographically widespread Harpellales, *Smittium culisetae*. Bracts of *Heliconia* inflorescences, though often populated by mosquito larvae and other insects, contained no larvae with gut fungi. Water in *Heliconia* bracts averaged 2.36 pH units above that in bromeliads (average pH 5.02). It was demonstrated in the laboratory that *Heliconia*-inhabiting mosquito larvae maintained in their native water could be artificially infested with axenic cultures of *S. culisetae*. The apparent lack of natural fungal infestation of mosquito larvae in heliconias may be due to the ephemeral nature of the plants' water-holding bracts and extrinsic factors such as recruitment of fungal inoculum.

Key words: Aquatic insects, Bromeliaceae, Ceratopogonidae, Chironomidae, Culicidae, gut fungi, phytotelmata, *Smittium*, *Stachylina*, Trichomycetes.

Terrestrial plants that impound water in their leaf axils or other exposed anatomical structures, known as phytotelmata or tank plants, provide a suitable microhabitat for the development of many kinds of invertebrates and some vertebrates. In the moist tropics these reservoirs are breeding grounds for a variety of species of Diptera with aquatic immature stages, including Culicidae (mosquitoes), Chironomidae (midges) and Ceratopogonidae (biting midges). Particularly significant phytotelm habitats in the Neotropics are the epiphytic Bromeliaceae, whose leaf axils can provide veritable microcosms of interacting biota. Costa Rica has one of the richest bromeliad floras in Central America (Burt-Utley & Utley 1975), and in several parts of the country where precipitation is sufficiently constant the broader-leaved bromeliads may store enough water in their axils to support continual small communities of organisms. Clodomiro Picado Twight, the first Costa Rican biologist with academic training (Gómez & Savage 1983), made substantial and pioneering contributions to understanding the complex biology of Bromeliaceae (Picado 1913). It is of interest that he reported finding a septate fungus, though he did not describe it, in the anterior part of the gut of a common bromeliad-inhabiting species of oligochaete (Picado 1913, p. 321). However, based on our current knowledge it may not have been a

trichomycete because of the type of host and location of the fungus in the gut.

Reported here is the occurrence of commensalistic Harpellales gut fungi (Zygomycotina; Trichomycetes) in some of the dipteran larvae that live in bromeliads, and to a lesser extent that breed in other phytotelmata and nonphytotelm lentic habitats. This study was part of a more comprehensive investigation, to be reported in other publications, of Trichomycetes found living in the guts of various other Costa Rican arthropods, including blackfly and lotic midge larvae, mayfly nymphs, beetles, millipedes, and a variety of terrestrial and marine crustaceans.

Trichomycetes have been found in all regions of the world where they have been sought, and are common in some families of arthropods (Lichtwardt 1986). Virtually all studies on Harpellales, whose species are associated with immature aquatic Diptera, Ephemeroptera and Plecoptera, have been carried out in temperate or arctic biomes, with the exception of a few species reported from Hawaii and tropical Queensland in Australia (Lichtwardt 1986, Lichtwardt & Williams 1990). Neotropical Harpellales and those in other tropical areas of the world remain essentially unknown. This article is not a comprehensive study of Trichomycetes in Costa Rican phytotelm insects, but it does present data that add to bromeliad biology and to our knowledge of the association of gut fungi with tropical insects.

MATERIAL AND METHODS

Collections of larvae in phytotelmata and other lentic habitats were made in three general zones of Costa Rica between the dates indicated: 1. La Selva Biological Station, Puerto Viejo de Sarapiquí (low wet forest, 35+ m alt.), 22-28 September 1984, 6-27 June 1986, 22 May to 4 June 1988, and 12-21 October 1991. 2. Monteverde Cloud Forest Reserve (~1500 m alt.), 13 October 1984, 20-22 July 1986, 16-22 June 1988, 22 November 1991. 3. The Robert and Catherine Wilson Botanical Garden, Las Cruces Biological Station (premontane rain forest, ~1300 m alt.), 28 October to 2 November 1991.

Larvae were removed from water reservoirs in plants in a nondestructive manner by using a large kitchen baster made of translucent plastic and a rubber bulb with a 7 mm tube opening and capacity of 60 ml. By forcefully sucking in and ejecting phytotelm water and debris several times in rapid succession, most of the larvae could be drawn out of the reservoir and deposited in a capped collecting jar. This technique did not necessarily remove all larvae, especially some of the tube-building bloodworms, but it did permit, if desired, making collections from the same plant after a few days or even in subsequent years. Extracted contents from each leaf base or flower bract were placed into an individual container when the volume was sufficient. If less than about 50 ml was drawn up, water and associated materials from two or more leaf bases or bracts were combined in one container.

Impounded waters from some bromeliads and most Heliconias that were studied could be reached from ground level. Higher phytotelmata were sampled using a ladder, sometimes extended to 7 m. One set of collections from bromeliads ~15 m above ground level required the use of a climbing rig. Collections in a swamp were made by wading into the water with a net and a 12 cm diam. metal food strainer to obtain larvae in the muddy substrate and at several water levels and among different types of aquatic vegetation.

Measurements of pH were made either in the field after placement of the water and debris into a container, or immediately after returning to the laboratory. A portable Cole-Parmer Digital pH Wand, model 5985-50, with an accuracy of ±0.01 pH units was used. The electrode was standardized between each series of measurements.

Living larvae of Culicidae and Chironomidae were dissected by removing the hindgut, as well as the peritrophic membrane in the case of Chironomidae, using procedures described by Lichtwardt (1986). Other insects in the water samples that were less likely to contain gut fungi were occasionally selectively dissected, including Ceratopogonidae, Psychodidae, Tipulidae, and Coleoptera larvae. Some species of these dipteran families are predacious, and predacious ones were not expected to and did not contain Harpellales in their guts.

For microscopic examination of the living fungi, water mounts were prepared under a

dissecting microscope by stripping off the hindgut epithelium and then exposing the fungal thalli attached to the cuticle using fine jeweler's forceps and minuten needles. Peritrophic membranes of the midgut, being transparent, were cleaned of loose food and other particles and mounted in water intact. Observations were made with a phase-contrast microscope (Leitz or Zeiss), and selected specimens were photographed with Kodachrome 64 film using an electronic flash lighting system.

Attempts were made to culture representatives of most species of *Smittium* Poisson (but not species of *Stachylina* Léger & Gauthier, none of which has proved to be culturable to date) by rinsing the hindgut at least twice in a Penicillin-Streptomycin antibiotic solution, then placing the infested gut in a 60-mm petri dish containing Brain-heart Infusion Agar (Difco) diluted to 1/10th the recommended rehydration concentration. The medium contained added thiamine and biotin and a shallow overlayer of sterile distilled water which was added after the agar had gelled. The formulae for the 1/10 BHIv medium and the antibiotic concentrations added to the water have been provided elsewhere (Lichtwardt 1986). The dishes of medium contained the same antibiotic solution as the rinse water. If growth of the fungus became evident and appeared to be free of bacteria and other contaminants, it was transferred to dishes of 1/10 BHIv medium without antibiotics in the water layer, and subsequently to test tube slants of 1/10 BHIv with a small amount of sterile distilled water added to the bottom of the slant. Successfully grown cultures were later stored in a refrigerator. Upon return to the author's laboratory, the cultures were periodically transferred to fresh medium, regrown at room temperature, then kept at 5 C. Most of the cultures were also stored in liquid nitrogen.

Voucher specimens of intact larvae were preserved in 70% ethanol. Where it was desirable to associate a dissected larva with a particular fungus, the dissected specimen was preserved for identification. Pupae or adults were available in some cases, either when the collection was first made or following ecdysis in the laboratory. Specimens for identification were sent to specialists, and those persons are mentioned with gratitude in the Acknowledgments section of this paper. Because Chironomidae of Costa Rica are poorly known and larval stages have seldom been associated with adults, identifications were made at the generic level, where possible.

DESCRIPTIONS OF NEW FUNGAL SPECIES

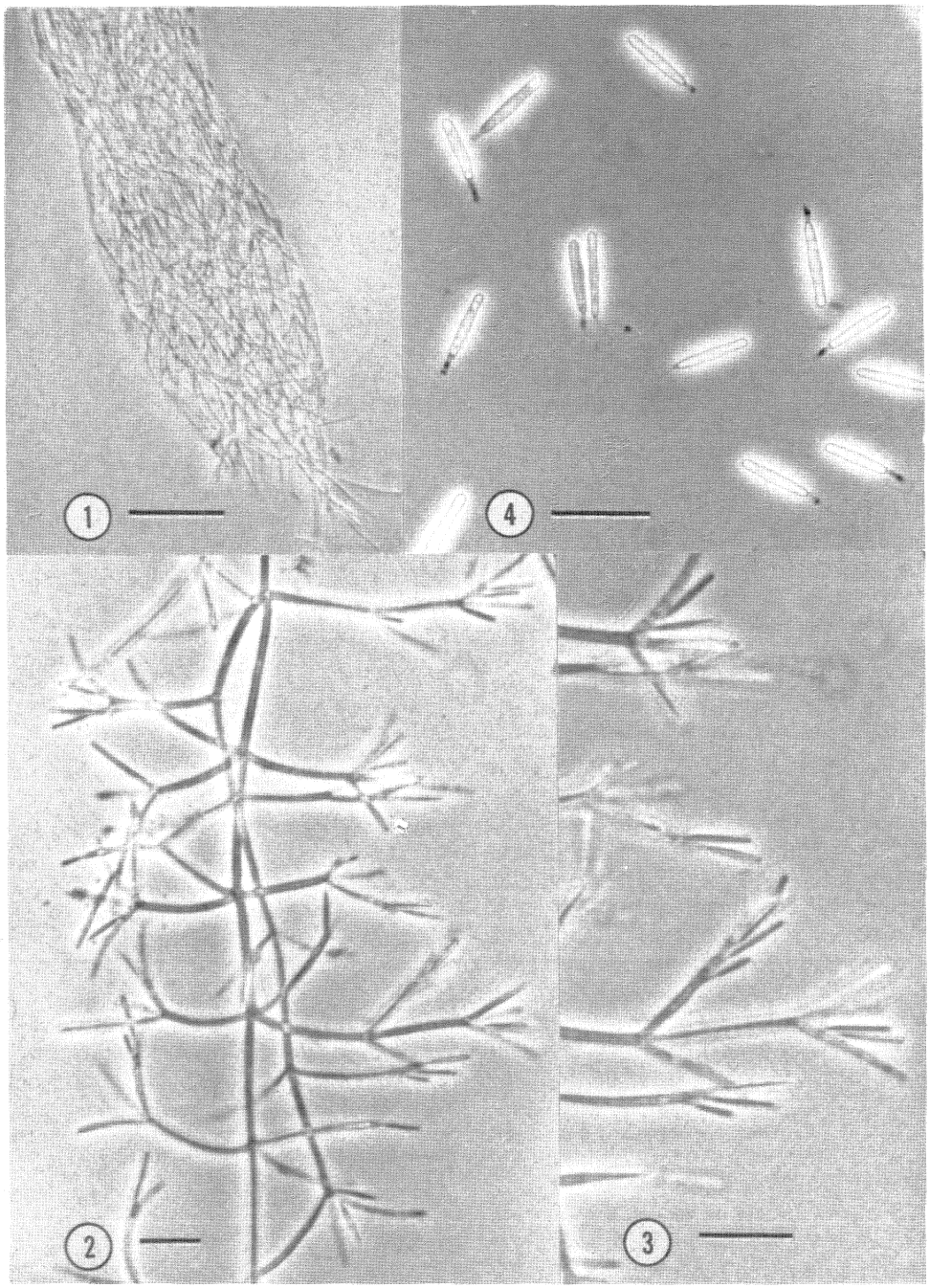
Smittium phytotelmatum Lichtwardt, sp. nov. (Figs. 1-6)

Thalli sparse ramosi, ramis praecipuis manipulos ramulorum brevium singulorum vel crebre verticillatorum gerentium, cellularum series brevium genitalium producentium. Trichosporae cylindricae, (14-)17-25(-30) x 2-3 µm, collari vulgo 2-3 µm longo. Zygosporae ignotae. In proctodaeo larvarum Chironomidarum.

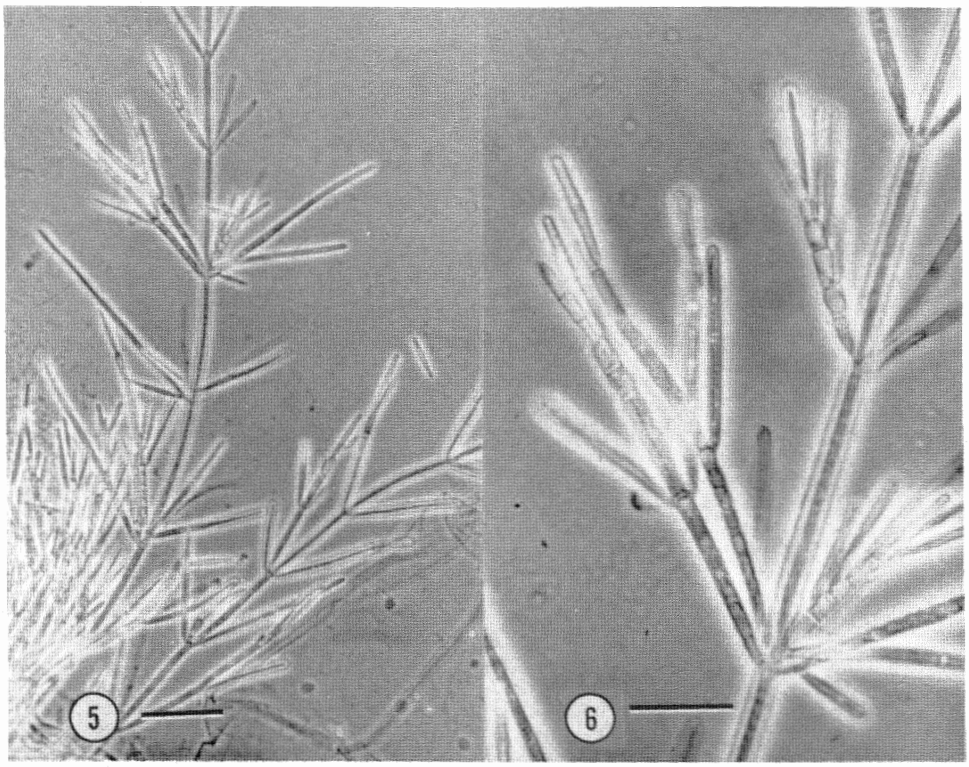
Thalli sparsely branched, main branches bearing sets of single to often verticillate short branchlets with series of short generative cells. Trichospores subcylindrical, (14-)17-25(-30) x 2-3 µm, collar usually 2-3 µm long. Zygospores unknown. In hindgut of larval Chironomidae.

Holotypes: Slide CR-133-17 prepared from the larval hindgut of a species of *Chironomus* Meigen (Chironomidae; Chironomini) living in water in the leaf base of a large *Aechmea mariae-reginae* Wendl. (Bromeliaceae) on a pejibaye stump (*Bactris gasipaes* H. B. K.) near Cabina 1, La Selva Biological Station, Puerto Viejo de Sarapiquí, in northeastern Costa Rica. Deposited with R. W. Lichtwardt, University of Kansas.

Other collections: In small bloodworms that included *Chironomus* sp. and several species of *Polypedilum* Kieffer (Chironomini) from many phytotelm bromeliads, including species of *Aechmea* R. & P. and *Vresia* Lindl., and *Guzmania monostachya* (L.) Rusby ex Mez. In *Polypedilum* sp. larvae from leaf base of a banana plant (*Musa* sp.). In other small bloodworms that included species of *Tanytarsus* and *Polypedilum*, and larvae belonging to two unknown genera of Chironominae and Tanitarsini, from a swamp near Camino Experimental Sur 200 m marker. Possibly same species of fungus from lotic *Polypedilum* sp. bloodworms taken from a submerged log above the Quebrada El Salto waterfalls (Figs. 5 & 6). All at La Selva



Figs. 1-4. *Smittium phytotelmatum:* phase-contrast photomicrographs of living specimens. Fig. 1. Fungus which grows within the hindgut cuticle of a dissected bloodworm (*Polypedilum* sp.) collected from water in a bromeliad leaf axil. Figs. 2, 3. Typical branching pattern with sporulating branchlets. Fig. 4. Trichospores from an axenic culture (isolate CR-133-2 from a *Chironomus* sp. larva). Fig. 1 bar = 100 µm; other bars = 25 µm.



Figs. 5, 6. *Smittium phytotelmatum* from a lotic bloodworm (*Polypedilum* sp.) inhabiting a submerged log. Fig. 5 bar = 50 µm; Fig. 6 bar = 25 µm.

Biological Station. What may be a variant of *Smittium phytotelmatum* with slightly smaller trichospores was found in bloodworms living in a bromeliad in the Wilson Botanical Garden (Las Cruces Biological Station), San Vito de Java in southern Costa Rica.

Cultures: Many attempts were made to culture *S. phytotelmatum* axenically, and nine were successful. Nonetheless, some cultures died out after 1-3 yr, despite repeated transfers and before they were preserved in liquid nitrogen. Since there are photographic and other records of five of the now extinct cultures, which were obtained from bromeliad-inhabiting *Polypedilum* spp. or *Chironomus* sp. larvae, a list of these follows with their isolation dates: CR-62-2 and CR-62-4 (6-VI-86), CR-83-3 (16-VI-86), CR-83-10 (17-VI-86), CR-133-16 (23-V-88). Extant cultures obtained from bromeliad bloodworms (*Chironomus* spp.) and maintained either in liquid nitrogen storage or in refrigerated test tube slants are: CR-133-2 (22-V-88) (Fig. 4), CR-219-1 and CR-219-3 (21-X-91). Another isolate, CR-211-1 (17-X-91), originated from a swamp-inhabiting bloodworm (*Polypedilum* sp.); the fungus differs slightly from the bromeliad strains in its morphology and pattern of isozyme banding (Roger Grigg, University of Kansas, unpublished), but may be the same species. In culture, *S. phytotelmatum* often produces trichospores with a greater range of size than *in vivo*, and the growth form of the colonies tends to show a more compact type of branching instead of the loose, verticillate branching usually seen in the host (Figs. 2, 3).

Smittium phytotelmatum differs morphologically from the other 38 currently described species of *Smittium* in the form of its branching and the dimensions of its trichospores. Especially notable is the narrowness of the trichospores in relation to their length.

Smittium fasciculatum Lichtwardt, sp. nov.
(Figs. 7-11)

Thalli in globos fasciculatos usque ad 750 µm longos aggregati, proctodaeo anteriori applicati, in partem utramque crescentes. Rami in mesenteron extensi attenuati ad cacumina tenuia sterilia arcuata vel circinata producenda. Rami in proctodaeo in seriem cellilarum 2-6 genitalium desinentes producentium trichosporas subcylindricas 18-24(-29) x 2-3.5 µm collari perbrevi (< 1 µm) et appendiculo brevi ornatas. Zygosporae ignotae. In Chironomidarum larvis.

Thalli aggregated into fascicled clumps up to 750 µm long, attached to anterior hindgut but growing in both directions. Branches extending into midgut tapering to produce fine sterile curved to circinate tips. Branches in hindgut terminating in series of 2-6 generative cells that produce subcylindrical trichospores 18-24(-29) x 2-3.5 µm with a very short (< 1 µm) collar and short appendage. Zygospores unknown. In larval Chironomidae.

Holotype: Slide CR-102-10 prepared from the gut of a bloodworm, *Chironomus* sp. (Chironomidae; Chironomini), collected 27-VI-86 from water in the leaf base of a bromeliad attached to trunk of a *Bactris gassipes* about 3.2 m above ground level, south of the main laboratory, La Selva Biological Station. Deposited with R. W. Lichtwardt, University of Kansas.

Other collections: *Smittium fasciculatum* found in 18 other bloodworms of the same species taken from several leaf bases of the same bromeliad, which is the only site recorded for the fungus.

This is the only known Harpellales that grows from the hindgut backward into the midgut, with the exception of the species *Smittium morbosum* Sweeney (1981) which is pathogenic to mosquitoes because it prevents successful molting of the larvae. Unlike *S. morbosum*, however, *S. fasciculatum* does not penetrate the midgut epithelium, nor does it kill its host. The reason for the anteriad growth of the thalli is not known, though the midgut could possibly contain more undigested food than the hindgut. The bloodworms were unusually small (2-3 mm long), such that in some cases with proper lighting it was possible to see the fungus within the undissected larva. Upon the next visit to the site, in 1988, the pejibaye that had supported the bromeliad had fallen to the ground. Several other bromeliads sampled in 1986, 1988 and 1991 in the general vicinity of that site contained bloodworms with *S. phytotelmatum* but not *S. fasciculatum*. Several attempts to culture *S. fasciculatum* were unsuccessful.

The trichospores of *S. fasciculatum* (Fig. 11) are similar in size and shape to those of *S. phytotelmatum* (Fig. 4), though the collar of the former is normally much shorter. Thallial development and branching patterns of the two species are so different, however, that even immature thalli can be distinguished.

Stachylina paludosa Lichtwardt, sp. nov.
(Figs. 12-15)

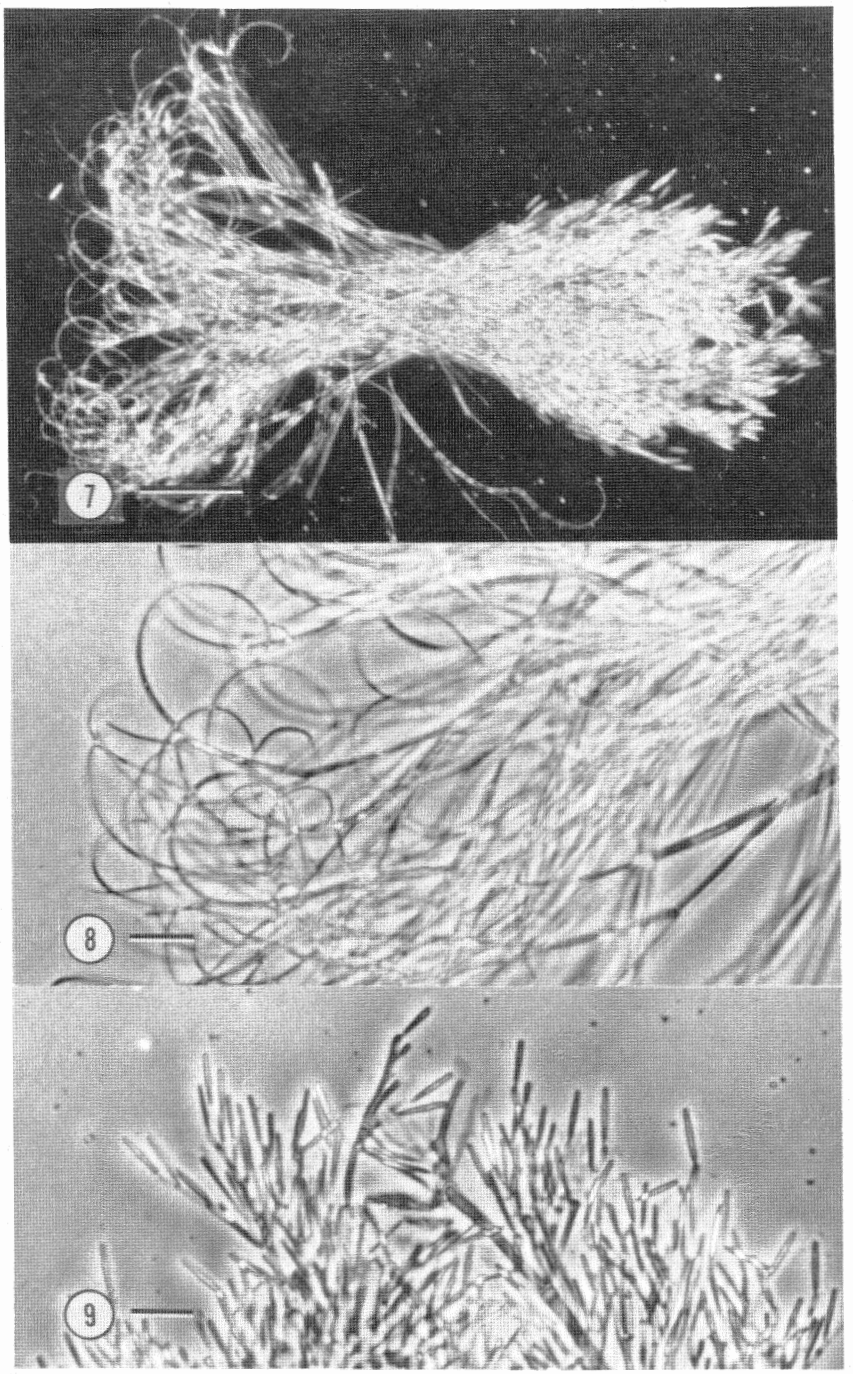
Thalli 80-150 x 7-9 µm, cellulas ± 8 genitales producentes, tenaculo 1 x 2 µm. Trichosporae elongato-ellipsoidales, in medio nonnihil tumentes, 31-40 x 6-8 µm, collari fere 1 µm longo. Zygosporae ignotae. In membrano peritrophico larvarum Chironomidarum.

Thalli 80-150 x 7-9 µm, producing ± 8 generative cells, holdfast 1 x 2 µm. Trichospores long-ellipsoidal with a slight median bulge, 31-40 x 6-8 µm, with a collar about 1 µm long. Zygospores unknown. On peritrophic membrane of Chironomidae larvae.

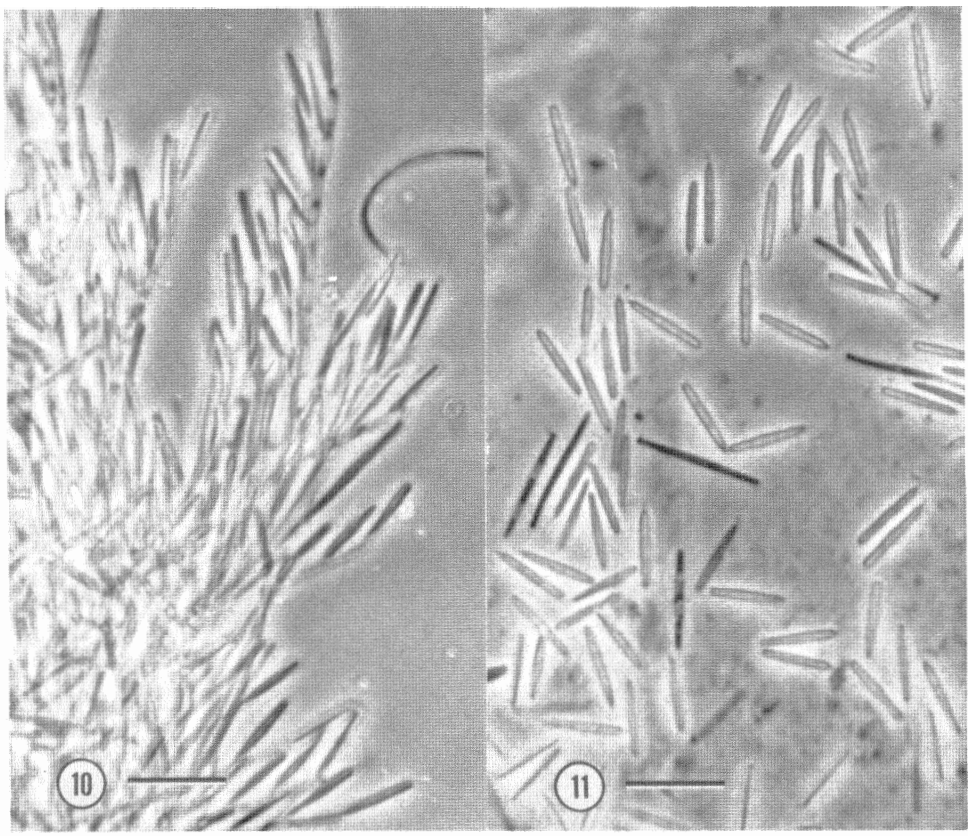
Holotype: On slide CR-211-5 containing the peritrophic membrane of a small bloodworm, *Polypedilum* sp. (Chironomidae; Chironomini), collected 17-X-91 in a swamp near the 200 m marker on Sendero Experimental Sur, La Selva Biological Station, Costa Rica. Deposited with R. W. Lichtwardt, University of Kansas.

Other collections: In larvae of *Chironomus* sp. and *Polypedilum* sp. from the same site as the holotype.

The shape of *Stachylina paludosa* trichospores most closely resembles those of *St. grandispora* Lichtwardt, a widely distributed species common in larger bloodworms of the genus *Chironomus*. Both species have a small



Figs. 7-9, *Smittium fasciculatum*. Fig. 7. Fascicled clump of thalli removed intact from the gut of a minute bromeliad bloodworm (*Chironomus* sp.); the left half of the clump was growing from the hindgut backwards into the midgut. Fig. 8. Curved to circinate hyphal tips from the midgut region. Fig. 9. Sporulating branchlets from the hindgut region. Fig. 7 bar = 100 µm; other bars = 25 µm.



Figs. 10, 11. *Smittium fasciculatum:* attached and released trichospores. Bars = 25 µm.

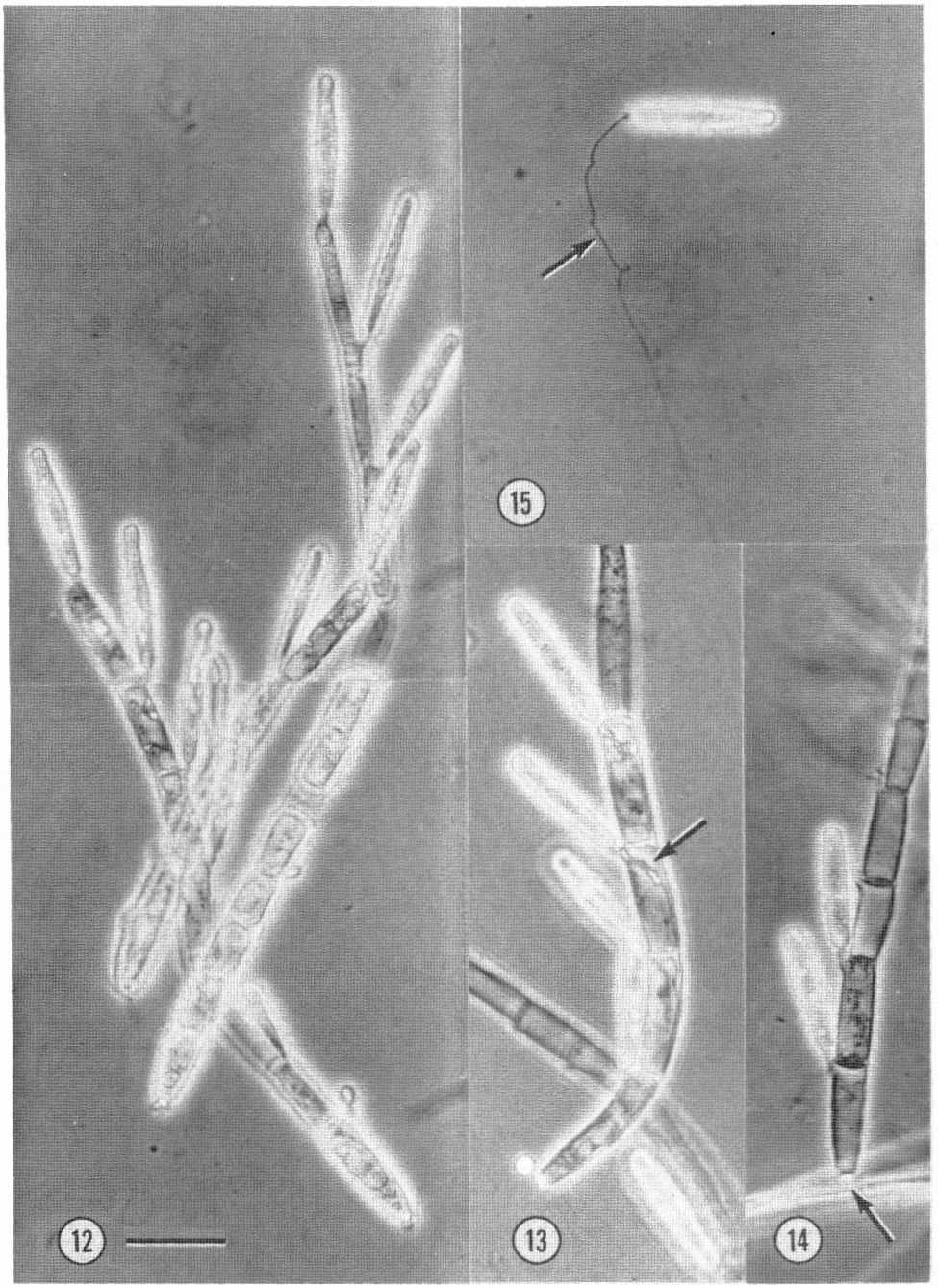
collar at the base of the trichospore, a feature shared with only three of the other 16 species of described Stachylinas. Trichospores of *St. grandispora* are on the average considerably larger, usually measuring 40-72 x 6-10 µm (Lichtwardt 1986)

In 1988 the author also found in five bloodworms (*Polypedilum* sp.) from the same swamp another species of *Stachylina* which will not be described here due to what is considered insufficient sporulating thalli. A few mature trichospores of that species measured 26-27 x 4-6 µm, and were clearly not *St. paludosa*. Likewise not described due to insufficient information, are collections in 1986 of a *Stachylina* sp. found in larvae of *Chironomus* sp. collected from an epiphytic bromeliad located ~15 m above ground level at La Selva, and perhaps a different species of *Stachylina* in a larva of *Metriocnemus* v. d. Wulp (Chironomidae; Orthocladiinae) found in an epiphytic bromeliad on Sendero Brillante in Monteverde. Species of *Stachylina* were also found in lotic Chironomidae larvae, and some of these will be described in another publication.

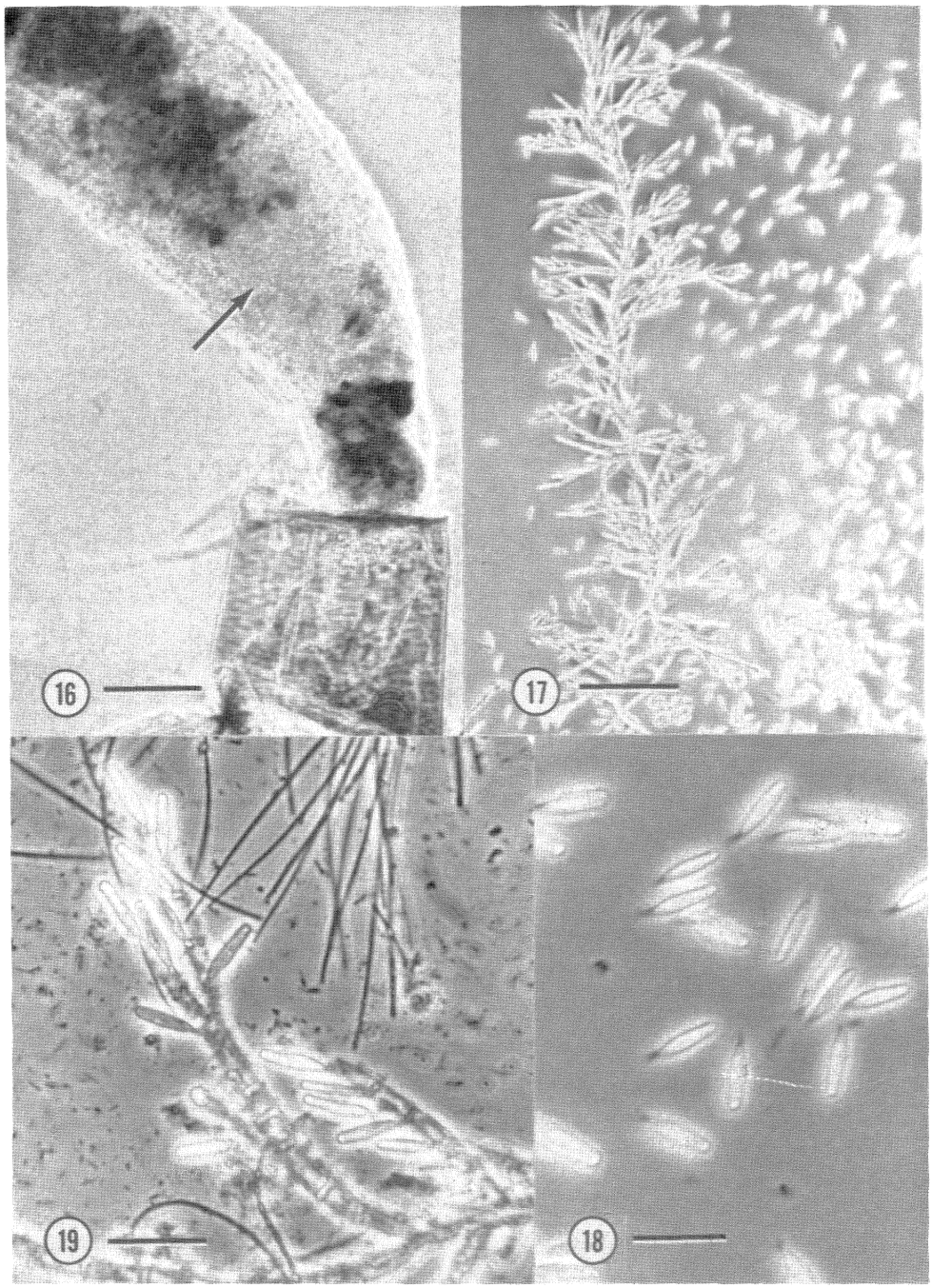
OTHER SPECIES OF HARPELLALES

Smittium culisetae Lichtwardt (Figs. 16-19)

Two of the most cosmopolitan Harpellales are normally found in mosquito larvae: *Smittium culisetae* and *S. culicis* Manier. They are also unusual among species of Harpellales in having a wide range of hosts. These include larvae belonging to six families of nematoceran Diptera (Lichtwardt & Williams 1990). Oddly, despite abundant mosquito populations in some parts of Costa Rica, *S. culicis* has not yet been found in their larvae. *Smittium culisetae*, on the other hand, is reported here from several lentic habitats:



Figs. 12-15. *Stachylina paludosa* from swamp bloodworms. Fig. 12. Three unbranched sporulating thalli as seen through the transparent peritrophic membrane of the midgut of a *Polypedilum* sp. larva. Fig. 13. Sporulating thallus in the peritrophic membrane of a *Chironomus* sp. larva with a folded appendage visible within the generative cell which produced the trichospore (arrow). Fig. 14. Thallus with all but two trichospores released, and a small, secreted holdfast (arrow) that is attached to the peritrophic membrane. Fig. 15. Released trichospore with its single unfolded basal appendage (arrow). Bar = 25 m for all figures.



Figs. 16-19. Fig. 16. Dissected hindgut of a mosquito larva taken from a *Heliconia* flower bract and experimentally infested with a culture of *Smittium culisetae;* the arrow points to masses of thalli within the hindgut. Figs. 17, 18. Axenic culture (CR-73-10) of *S. culisetae* isolated from a mosquito larva living in a pineapple leaf axil, and used to infest *Heliconia* mosquito larvae as in Fig. 16. Fig. 19. Naturally-occurring *S. culisetae* from the hindgut of a mosquito larva living in a bird bath; note the many bacteria and filamentous prokaryotes that also were living in the hindgut. Figs. 16 & 17 bars = 100 µm, other bars = 25 µm.

1. *Ananas comosus* (L.) (pineapple). *Smittium culisetae* was present in larvae collected from the leaf bases of pineapples growing in the La Selva station clearing. On 23-IX-84 the fungus was found in one of two *Wyeomyia* sp. mosquito larvae. On 9-VI-86 and 11-VI-86 bigger harvests of dipteran larvae with *S. culisetae* from pineapple plants in the same general area included: (i) *Wyeomyia (Dendromyia) pertinans* group, *Wyeomyia (Dendromyia)* sp., and *Culex (Microculex) jenningsii* Dyar & Knab, (ii) *Polypedilum* spp. bloodworms, and (iii) an unidentified Ceratopogonidae larva. Two axenic cultures of *S. culisetae* were obtained, one (CR-73-10, Figs. 17 & 18) from *Culex jenningsii,* the other (CR-73-13) from a Ceratopogonidae larva. Both cultures were lost in 1987, but prior to that, in 1986, the two isolates were used in experimental infestations of mosquito larvae at La Selva, as described in the next section of this paper.

2. Epiphytic bromeliads. *S. culisetae* was found but twice in epiphytic bromeliads at La Selva. On 28-V-88 in larvae of *Wyeomyia (Dendromyia)* sp. from a bromeliad 6 m above ground level on a pejibaye at the beginning of Sendero Surá, and on 12-X-91 in an unidentified white midge larva from a bromeliad on a dead tree near Cabina 1. Some of the *Wyeomyia* sp. larvae in the 1988 collection contained a second and probably new species of *Smittium,* living together with *S. culisetae,* which remains unnamed even though a culture (CR-146-3, Figs. 21 & 22) was obtained.

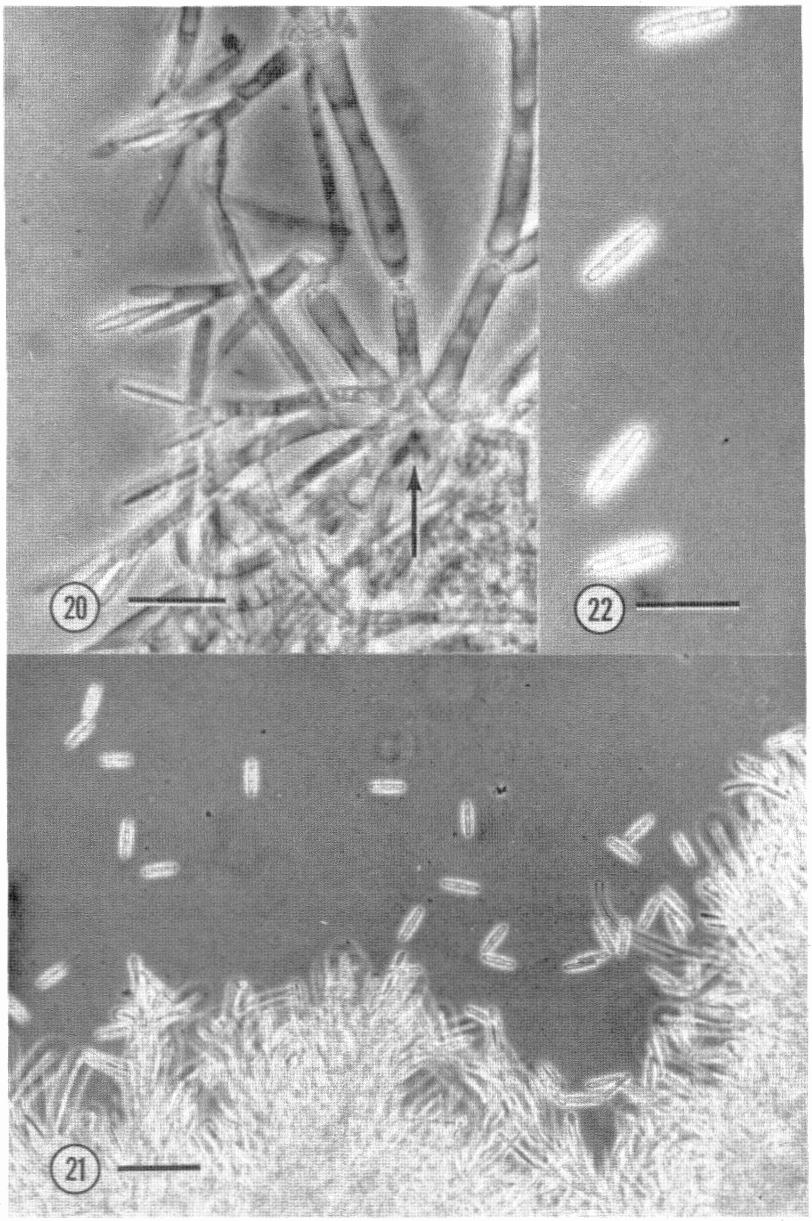
3. Swamp. Mosquito larvae *[Aedes (Ochlerotatus) dupreei* (Coquillett) group, *Aedes (Ochlerotatus)* sp. (but not *A. dupreei*), and *Culex (Melanoconion) pilosus* (Dyar & Knab)] collected 3-VI and 4-VI-88 in the swamp near Camino Experimental Sur, where *S. phytotelmatum* was also found, contained thalli of *S. culisetae.* Species of another harpellid genus, *Stachylina,* was also found at the site (see below). That remarkably rich swamp is also the locality where a new fungal pathogen of mosquitoes was found in 1988, *Coelomomyces neotropicus* Lichtwardt & Gómez (1993).

4. Bird bath. A limited number of unidentified mosquitoes collected 22-XI-91 from a stone bird bath in the garden of Pension Quetzal in Monteverde had *S. culisetae* living in their hindguts (Fig. 19). Mosquito larvae collected from this same bird bath in 1988 were infested with another *Smittium;* see below.

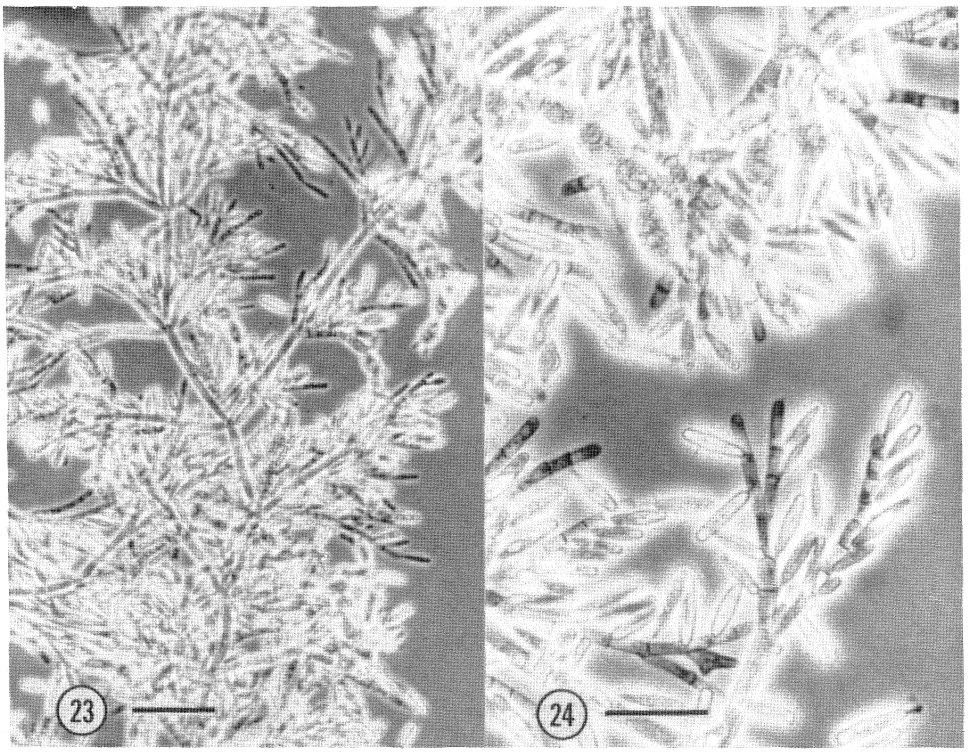
Smittium sp. no. 1
(Figs. 20-24)

Among several unidentifiable species of *Smittium* found in phytotelm and other lentic dipterans, one resembling *S. simulii* Lichtwardt was found on several occasions in chironomid and mosquito larvae, and records of collections and isolations and a few illustrations are provided here for future reference. It is not certain that all of these specimens represent the same species of fungus, but they have a basic common morphology. *Smittium simulii* has a wide geographic distribution, and is usually found in the hindgut of blackfly (Simuliidae) and lotic Chironomidae larvae, but the fungus has been found as well rarely in Culicidae and Tipulidae (Lichtwardt 1986). Sixteen axenic cultures have been made of *S. simulii* from larvae of three of those dipteran families collected in the U.S.A., Japan, France, and Sweden. As a consequence, *S. simulii* is a rather well-known fungus. It has a characteristic horseshoe-shaped thallial basal cell from which arise a number of branches (Lichtwardt 1986, Fig. 11.22A). The trichospores of *S. simulii* are almost cylindrical but swollen in the middle, and measure (16-) 23(-30) x (3-)5(-7) µm. Zygospores have never been seen in that species. The Costa Rican specimens had a somewhat different basal structure (Fig. 20) and trichospore shape (Figs. 22 & 24), but the trichospores were approximately 18-20 x 3-4 m. Although this falls within the lower range of *S. simulii* trichospore measurements, their consistently smaller size and general form of branching suggest a different and perhaps undescribed species.

The records of this possibly new species are as follows: 1. Midge larvae (*Metriocnemus* sp.) in various bromeliads in the Monteverde Cloud Forest Reserve, 1986 and 1988. 2. Larva of *Wyeomyia* sp. from a bromeliad (culture isolate no. CR-146-3, Figs. 21 & 22) and bloodworms (*Polypedilum* sp.) from a swamp (isolate no. CR-169-22, Figs. 23 & 24), La Selva, 1988. 3. Larva of *Culex (Culex)* sp. in bird bath at Pension Quetzal, Monteverde, 1988 (Fig. 20). 4. Possibly also in a Culex sp. larva from a pineapple plant, La Selva, 1984.



Figs. 20-22. *Smittium* sp. no 1 from a mosquito larva (*Culex* sp.) collected in a bird bath. Fig. 20. Thallus showing its branching base with a holdfast (arrow) attached to the hindgut cuticle. Figs. 21, 22. Sporulating branchlets and loose trichospores from an axenic culture (isolate CR-146-3 from a *Wyeomyia* sp. larva). Fig. 21 bar = 50 µm; other bars = 25 µm.



Figs. 23, 24. *Smittium* sp. no. 1 from a swamp bloodworm (*Polypedilum* sp.). Branching pattern and prolific sporulation in an axenic culture (CR-169-22). Fig. 23 bar = 50 µm; Fig. 24 bar = 25 µm.

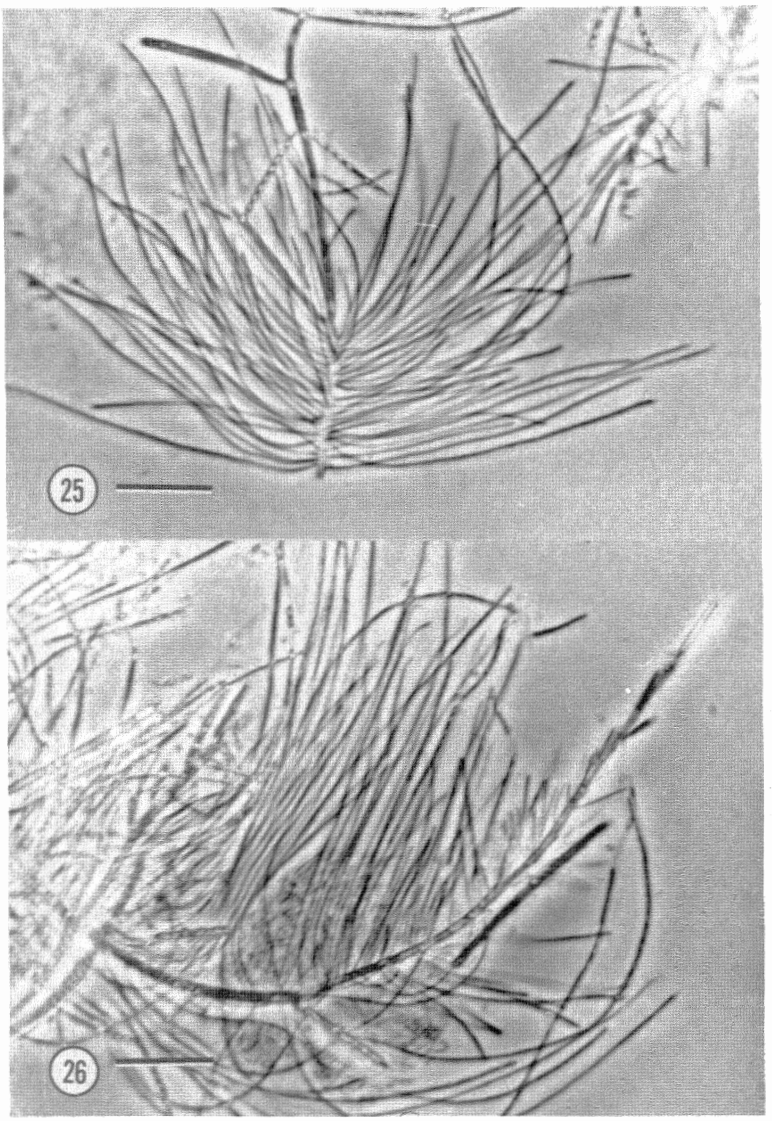
Smittium sp. no. 2
(Figs. 25 & 26)

Two small bloodworms (*Polypedilum* sp.) from the La Selva swamp near Sendero Experimental Sur collected 4-VI-88 contained thalli of a possibly new species of *Smittium* with distinctive and unusual plumose branching. Thalli consisted of a main axis from which a multitude of side branches arose in a more or less two-dimensional pattern. Specimens with trichospores were too few to adequately describe this distinctive species. Attached trichospores, possibly not completely mature, measured 12-13 x 1.5-2 µm.

pH OF PHYTOTELMATA WATERS AND LARVAL INFESTATION BY FUNGI

Species of *Heliconia* whose flower bracts are erect and retain a sufficient volume of water during the life of the inflorescence often support communities of insects. These communities have been studied extensively (Seifert & Seifert 1977, Seifert 1982, Frank & Lounibos, 1983). Among common insect inhabitants of Heliconias are mosquitoes and midges whose larvae might be expected to host trichomycetes, such as those species of fungi described above. Consequently, mosquito larvae, and less frequently midge larvae, from the flowers of many *Heliconia* inflorescences were removed and dissected. However, none of the larvae was found to be infested with trichomycetes. Most of the mosquito species were *Wyeomyia (Dendromyia) pseudopecten* Dyar & Knab group and *Wyeomyia* sp. The majority of flowers sampled were from *Heliconia imbricata* (Kuntze) Baker, but other Heliconias in flower at the time of collecting were investigated, including several hybrids in the former La Selva *Heliconia* garden.

It was found during the sampling that there was a consistent difference in the pH values of the water reservoirs of Heliconias and bromeliads.



Figs. 25, 26. *Smittium* sp. no. 2 from a swamp bloodworm (*Polypedilum* sp.). Thalli with a central axis from which a plumose pattern of side branches arises. Bars = 25 µm.

The mean average in Heliconias was pH 7.38 (range = 6.95-7.77; N = 18), whereas the bromeliad waters had an average pH of 5.02 (range = 3.76-6.18; N = 54). The bromeliad pH values agree in general with Laessle's (1961), who obtained with a colorimetric pocket comparator an overall mean pH value of 4.93 for the outer axils of Jamaican bromeliads (my calculation based on his data). Likewise, Machado-Allison *et al.* (1983) reported a mean pH value of 7.23 for *Heliconia caribaea* Lamarck in Venezuela.

In view of the apparent lack of fungal infestation in *Heliconia*-inhabiting mosquito and midge larvae, an experiment was set up in the laboratory to see if mosquitoes from Heliconias maintained in their natural aquatic milieu were suitable hosts for *Smittium culisetae*. A collection of *Wyeomyia pseudopecten* and *Wyeomyia* sp. larvae from a nearby *Heliconia imbricata* plant

was brought to the laboratory in their native water, and divided into two containers with 36 mosquito larvae in each. The larvae in one of two containers were fed young sporulating thalli of a mixture of two "neutral" axenic cultures of *S. culisetae* [CR-73-10 (Figs. 17 & 18) and CR-73-13] which had been isolated from a *Culex jenningsii* and a Ceratopogonidae larva, respectively, living in a pineapple plant (see previous section). The inoculum consisted of two fungal cultures to ensure a sufficient supply of trichospores, because the *S. culisetae* isolations had been initiated only seven days prior to use and were just beginning to sporulate. Periodic dissections of larvae in the uninoculated control container remained uninfested for the 4-day duration of the experiment. In the other set with added *S. culisetae*, sample dissections revealed fungal infestation of larvae beginning on the 3rd day (although infestation in the larval population probably began earlier), and on the 4th day most of the larvae selected for dissection were infested with *S. culisetae* (Fig. 16). It was not expected that all larvae in the inoculated container would contain gut fungi, because during ecdysis the hindgut cuticle, to which the fungal thalli are attached, is shed with the exoskeleton and the hindgut may as a consequence be devoid of fungi until reinfestation occurs. Larval reinfestation depends upon ingestion of external trichospores lying in the aquatic medium, followed by extrusion of the inner spore and attachment of the newly developing thallus to the hindgut cuticle (Horn 1989). Under favorable conditions, mosquito larvae can produce new sporulating thalli of *S. culisetae* within 24 hr (Williams & Lichtwardt 1972), and these trichospores, after passage through the gut, can supply new inoculum for a population of larvae.

Using the same experimental methods, it was also demonstrated that larvae of *Wyeomyia pseudopecten* taken from *H. imbricata* bracts and transferred to water collected from bromeliad reservoirs could be infested with *S. culisetae* (isolate CR-73-13). These experiments indicate that *Heliconia*-inhabiting mosquito larvae can indeed become infested with *S. culisetae* if supplied with fungal inoculum, and that the apparent lack of natural infestation is probably due to causes other than pH of the water or inhospitable factors for the fungus within the bracts of Heliconias.

DISCUSSION

How species of Harpellales spread their propagules to disjunct habitats is not well understood, considering that thalli grow and sporulate in nonflying larval stages of their hosts. Harpellales are basically aquatic fungi, and it is not believed that their trichospores normally become airborne, as do the spores of most terrestrial fungi. Phoretic dispersal from one water reservoir to another is a distinct possibility. Birds and other animals are known to visit phytotelmata such as bromeliads and Heliconias, and conceivably could occasionally carry infested larvae or trichospores from one plant to another. A more direct and efficient means of propagule dispersal, one which targets the fungus to breeding sites, has been found in blackflies (Simuliidae) in Canada, Great Britain, and the U.S.A., but the developmental aspects of the process are not understood at present [Yeboah et al. 1983, Moss & Descals 1986, Lichtwardt & Molloy (unpublished)]. It now seems evident that several species of simuliid Harpellales occasionally are capable of invading the ovaries of blackflies where the fungi form numerous small cysts that suppress egg development. The cysts are "oviposited" by the sterile female, and they germinate to produce one or more modified trichospores, and these are presumed to be capable of infesting the gut of blackfly larvae in the stream. Although the ovarian cyst cycle still needs elucidation, it raises the question of whether dispersal of Harpellales in other families and orders of insect hosts involves a similar type of mechanism.

It is possible that the ovarian cyst stage in blackflies is somewhat seasonal in its occurrence, at least in temperate zones. But even a sporadic mechanism for dispersal, whatever it may be, could provide an initial source of inoculum which may allow a gut fungus in a relatively stable microhabitat to grow and reproduce more or less continuously provided the appropriate hosts were present. Bromeliads in general are thought to provide relatively stable habitats for phytotelm insects. All bromeliads are perennial. Some monocarpous species may live for a couple of decades, and polycarpous species have been know to live for over one century (Frank 1983).

Smittium phytotelmatum at La Selva was found infesting bloodworms in one bromeliad *(A. mariae-reginae)* in 1984, 1986, and 1988 (but not in 1991), and it is reasonable to assume that the fungus was resident for many years in the plant's leaf axils and had infested many generations of larvae.

The same cannot be true for Heliconias, whose ephemeral flowers persist long enough to allow eclosion to adult stages of insects that are adapted to that habitat (Seifert & Seifert 1976) but whose relatively short existence may reduce the chances for fungal recruitment into the bracts. An interesting feature of Heliconias is that much of the water in their bracts is supplied by transportation of fluid from the inflorescence itself (Seifert & Seifert 1976), leading to the possibility that some substance inimical to gut fungi could be present in that fluid, or that the pH of the bract water may be unsatisfactory. Neither of these possibilities is likely, since it was demonstrated that mosquito larvae removed from *Heliconia* bracts and kept in their native water could be infested with *S. culisetae*, and it is known from other experiments that thalli and trichospores of that fungal species in culture have a wide pH tolerance (El-Buni & Lichtwardt 1976). A more likely explanation for the apparent lack of gut fungi in insects living in *Heliconia* bracts is that, within the time frame of Heliconia flower bud opening and subsequent rotting, the establishment of the fungi is less probable than in the more permanent bromeliad leaf bases. A more thorough survey of *Heliconia*-inhabiting larvae than has been provided here might well reveal the occasional presence of Harpellales. Longevity of habitat does not in itself assure larval infestation, because in this study more than half of the bromeliads supporting types of larvae that might be expected to contain gut fungi were apparently devoid of Harpellales.

Other less permanent phytotelmata with trichomycete-infested larvae included banana and pineapple plants, as reported above. Of several *Xanthosoma* sp. leaf axils sampled in Monteverde, one contained a midge larva with sporulating thalli of an unknown species of *Smittium*. Collections from many *Colocasia esculenta* (L.) Schott leaf axils at La Selva yielded a number of mosquito larvae [*Johnbelkinialulopus* (Dyar & Knab)], but none was infested.

Saprobic fungi have been found –and would certainly be expected to be active–in the decomposing organic matter present in phytotelmata (Frank & Lounibos 1983). In addition to the commensalistic partnership of fungi and insects described here, there have been a few tropical or subtropical entomopathogenic fungi found in phytotelmata. Hall and Anthony (1979) reported a species of the lethal zoosporic genus *Coelomomyces* (Blastocladiales) in larvae of *Wyeomyia vanduzeei* Dyar & Knab inhabiting leaf axils of *Tillandsia utriculata* L. in the Everglades National Park, Florida; Couch and Bland (1985) believed the fungus to be *Coelomyces stegomyiae* var. *stegomyiae* Keilin. The hyphomycete *Culicinomyces bisporalis* Sigler, Frances & Panter (1987) was described from larvae of *Aedes kochi* (Donitz) from the leaf axils of a taro, *Colocasia macrorrhiza* Schott, in Queensland, Australia. This same plant species was the habitat for ceratopogonid larvae *(Forcipomyia marksae* Tokunaga*)* later found to be infected with a new zoosporic genus/species, *Crypticola clavulifera* Humber, Frances & Sweeney (Lagenidiales) (Frances et al. 1989), as well as another oomycete, *Lagenidium giganteum* Couch.

Habitats provided by phytotelmata have been the basis for many studies of intriguing interactions among a wide range of organisms. This paper is a contribution to the microorganismal dimension of the complex biology of phytotelmata, and emphasizes that there are still many facets of intimate organismal interrelationships in such habitats that have not been adequately explored.

ACKNOWLEDGEMENTS

The author acknowledges with appreciation support for this research by the National Science Foundation, Washington, DC, grants BSR-8019724, BSR-8516581, BSR-9006368, and DEB-9220518. Laboratory space and field facilities were provided through the Organization for Tropical Studies at the La Selva Biological Station and the Las Cruces Biological Station, and at the Monteverde Cloud Forest Reserve by the Tropical Science Center. Special thanks are given to Leonard C. Ferrington, Jr., State Biological Survey of

Kansas (Chironomidae), and Thomas J. Zavortink, University of San Francisco (Culicidae), for the time-consuming identification of insect specimens. Donald P. Rogers, Auburn, WA, kindly translated the diagnoses of new species into Latin. The author also thanks Donald Perry for providing a tree-climbing rig and instructions on its use.

RESUMEN

Se encontró varias especies de hongos Harpellales (Trichomycetes: Zygomycotina) en los tubos digestivos de insectos larvales (Chronomidae, Culicidae y Ceratopogonidae) de tanques de agua en bromelias epífitas y algunas otras plantas (como piña y banano), y en un pantano. Se hicieron recolecciones periódicas en Costa Rica entre 1984 y 1991 en bosque pluvial de bajura (La Selva), nuboso (Monteverde) y pluvial premontano (Jardín de Las Cruces). Se halló un nuevo tricomiceto, *Smittium phytotelmatum*, que habita en quironómidos y se encontró básicamente en bromelias, obteniéndose varios cultivos axénicos. Una segunda especie, menos común, *S. fasciculatum* (de un quironómido bromelícola) y otra bautizada como *Stachylina paludosa* (de quironómidos de pantano), se describen como nuevas para la ciencia. Se describe también, sin darles nombre, dos posibles especies nuevas de *Smittium* de quironómidos de pantano. Larvas de mosquito (zancudo), midges y ceratopogónidos sirven de hospederos al *Smittium culisetae* (Harpellales), de amplia distribución geográfica. Aunque las brácteas de inflorescencias de *Heliconia* suelen tener larvas de mosquito y otros insectos, ninguna tenía hongos en el tubo digestivo. El agua en estas brácteas es de un pH promedio 2.36 unidades mayor que en las bromelias. En laboratorio se comprobó la posibilidad (usando el agua original) de infestar artificialmente las larvas con cultivos axénicos de *S. culisitae*. La ausencia aparente de estos hongos en las larvas de mosquito de las heliconias podría deberse tanto a la corta vida de estos depósitos de agua como a factores extrínsecos (*e.g.* dificultad de llegada para el inóculo fúngico).

REFERENCES

Burt-Utley, K. & J. F. Utley. 1975. Supplementary notes: phytogeography, physiological ecology and the Costa Rican genera of Bromeliaceae, p. 9-29. *In* L. D. Gómez (ed.). Biología de las Bromeliaceae. Hist. Nat. Costa Rica, Vol. 1. Museo Nacional de Costa Rica, San José.

Couch, J. N. & C. E. Bland. 1985. The genus *Coelomomyces*. Academic, New York. 399 p.

El-Buni, A. & R. W. Lichtwardt. 1976. Asexual sporulation and mycelial growth in axenic cultures of *Smittium* spp. (Trichomycetes). Mycologia 68: 559-572.

Frances, S. P., A. W. Sweeney & R. A. Humber. 1989. *Crypticola clavulifera* gen. et sp. nov. and *Lagenidium giganteum:* oomycetes pathogenic for dipterans infesting leaf axils in an Australian Rain Forest. J. Invert. Path. 54: 103-111.

Frank, J. H. 1983. Bromeliad phytotelmata and their biota, especially mosquitoes, p. 101-128. *In* J. H. Frank & L. P. Lounibos (eds.). Phytotelmata: terrestrial plants as hosts for aquatic insect communities. Plexus, Medford, New Jersey.

Frank, J. H. & L. P. Lounibos (eds.). 1983. Phytotelmata: terrestrial plants as hosts for aquatic insect communities. Plexus, Medford, New Jersey. 293 p.

Gómez, L. D. & J. M. Savage. 1983. Searchers on that rich coast: Costa Rican field biology, 1400-1980, p. 1-11. In D. H. Jansen (ed.) Costa Rican Natural History. University of Chicago, Chicago. 816 p.

Hall, D. W. & D. W. Anthony. 1979. *Coelomomyces* sp. (Phycomycetes: Blastocladiales) from the mosquito *Wyeomyia vanduzeei* (Diptera: Culicidae). J. Med. Entomol. 16: 84.

Hom, B. W. 1989. Requirement for potassium and pH shift in host-mediated sporangiospore extrusion from trichospores of *Smittium culisetae* and other *Smittium* species. Mycol. Res. 93: 303-313.

Laessle, A. M. 1961. A micro-limnological study of Jamaican bromeliads. Ecology 42: 499-517.

Lichtwardt, R. W. 1986. The Trichomycetes, fungal associates of arthropods. Springer-Verlag, New York. 343 p.

Lichtwardt, R. W. & L. D. Gómez. 1993. A new Coelomomyces pathogenic to mosquitoes in Costa Rica. Rev. Biol. Trop. 41: 407-410

Lichtwardt, R. W. & M. C. Williams. 1990. Trichomycete gut fungi in Australian aquatic insect larvae. Can. J. Bot. 68: 1057-1074.

Machado-Allison, C. E., D. J. Rodriguez, R. Barrera R. & C. G. Cova. 1983. The insect community associated with inflorescences of *Heliconia caribaea* Lamarck in Venezuela, p. 247-270. *In* J. H. Frank & L. P. Lounibos

(eds.). Phytotelmata: terrestrial plants as hosts for aquatic insect communities. Plexus, Medford, New Jersey.

Moss, S. T. & E. Descals. 1986. A previously undescribed stage in the life cycle of Harpellales (Trichomycetes). Mycologia 78: 213-222.

Picado, C. 1913. Les Broméliacées épiphytes considérées comme milieu biologique. Bull. Sci. France Belgique 67: 16-360 + 24 plates. *Reprinted in* L. D. Gómez (ed.). Biología de las Bromeliaceas. Hist. Nat. Costa Rica, Vol. 1. Museo Nacional de Costa Rica, San José.

Seifert, R. P. 1982. Neotropical *Heliconia* insect communities. Quart. Rev. Biol. 57: 1-28.

Seifert, R. P. & F. H. Seifert. 1976. Natural history of insects living in inflorescences of two species of *Heliconia*. J. New York Entomol. Soc. 84: 233-242.

Sweeney, A. W. 1981. An undescribed species of *Smittium* (Trichomycetes) pathogenic to mosquito larvae in Australia. Trans. Br. Mycol. Soc. 77: 55-60.

Sigler, L., S. P. Frances & C. Panter. 1987. *Culicinomyces bisporalis*, a new entomopathogenic hyphomycete from larvae of the mosquito *Aedes kochi*. Mycologia 79: 493-500.

Williams, M. C. & R. W. Lichtwardt. 1972. Infection of *Aedes aegypti* larvae by axenic cultures of the fungal genus *Smittium* (Trichomycetes). Amer. J. Bot. 59: 189-193.

Yeboah, D. O., A. H. Undeen & M. H. Colbo. 1983. Phycomycetes parasitizing the ovaries of blackflies (Simuliidae). J. Invert. Path. 43: 363-373.