The diversity and ecology of Trichomycetes from lentic and lotic habitats in Nova Scotia, Canada

by

Robert T. William

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Approved:	Dr. Doug Strongman Supervisor Department of Biology Saint Mary's University
Approved:	Dr. David Cone Supervisory Committee Department of Biology Saint Mary's University
Approved:	Dr. Michelle Patriquin Supervisory Committee Department of Anthropology Saint Mary's University
Approved:	Dr. R.W. Lichtwardt External examiner Department of Ecology and Evolution University of Kansas
Approved:	Dr. Jeremy Lundholm Graduate Studies Representative Saint Mary's University
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Abstract

Trichomycetes are endosymbiotic fungi and protists found in the guts of arthropods. Aquatic insects were collected from one lotic and one lentic site in each of three watersheds around the Halifax Regional Municipality and the trichomycetes present were documented. Weekly sampling over a 17 month period resulted in identification of 64 taxa of trichomycetes including 19 new species, five new continental and five new geographic records for Nova Scotia. Ecological results indicate that species richness and prevalence was higher at lotic sites than at lentic sites, primarily due to the presence of additional host types found only at lotic sites. Most trichomycete taxa occurred at relatively low prevalence at all sites. Watershed analyses suggested that differences in species richness and prevalence occur between watersheds. Several species exhibited seasonality and host preference over the course of the study.

To my mother Patricia Ann William (13 June 1937- 17 November 1992)

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Introduction

Trichomycetes are an ecological group of fungi and protists that live within the guts of insects and other arthropods found in marine, freshwater, and terrestrial habitats (Lichtwardt, 1986). The name trichomycetes comes from the Greek word 'tricho' which translates to hair, alluding to thalli which appear hair-like inside the insect gut and 'mycetes', also Greek for fungi. Endosymbiotic in nature, trichomycetes have eluded concrete classification since they were originally described as colorless algae by Joseph Leidy, who in 1849 isolated them from beetles and millipedes (Nelder et al., 2006). Later classification conducted by R.W. Lichtwardt and S.T. Moss led to the construction of the class Trichomycetes within the Phylum Zygomycota (Zygomycetes) and was divided into four fungal orders consisting of the Amoebidiales; Asellariales; Eccrinales and Harpellales (Lichtwardt, 1986). A subsequent reassessment of the Kingdom Fungi conducted by Hibbett et al. (2007) separates the protistan orders (Amoebidiales and Eccrinales) from the fungal orders (Asellariales and Harpellales) assigned tentatively to the Kickxellomycotina and using *trichomycetes* (lower case –t) to describe this ecological group living within the guts of arthropods. Despite the deconstruction of the class Trichomycetes, the four orders remain intact to refer to previously and newly described species.

Under the four order system, the Harpellales are the most speciose order of trichomycetes and are divided into two distinct families, the Harpellaceae and Legeriomycetaceae (Lichtwardt et al., 2001a). The Harpellaceae possess unbranched thalli and is currently comprised of only six genera and 56 species contrasting with the

Legeriomycetaceae containing trichomycetes with branched thalli comprised of 38 genera and 206 species. The second fungal order, the Asellariales, is much less speciose having only one family (Asellariaceae), two genera and 13 species. The two protistan orders beginning with the Amoebidiales are split into one family (Amoebidiaceae), two genera and 18 species while the Eccrinales consist of three families (Eccrinaeceae, Palavasciaceae and Parataeniellaceae) subdivided into 17 genera and 65 species.

Worldwide, trichomycetes are reported from six of seven continents throughout the world (Misra 1998; Lichtwardt et al. 2001a) and as new species continue to be reported it is becoming clear that they are virtually everywhere. By the end of 2011, there were approximately 66 genera containing 326 species of trichomycetes described in the literature (Lichtwardt, 2004). With a slowly increasing number of taxonomists studying trichomycetes, 10 new genera and 78 new species have been described between 2000 and the end of 2011 which should continue to expand as new geographic regions are explored.

Trichomycetes have been described mostly from insect hosts collected from freshwater lotic systems including streams, rivers, and springs (Lichtwardt et al., 2001a) although they have also been seen in crayfish, crabs, millipedes, isopods and amphipods (Lichtwardt, 2004). Despite limited, sporadic sampling there are numerous species of trichomycetes described from lentic habitats (standing waters such as ponds, lakes, swamps) and the diversity is high. Perhaps accounting in part for investigators preference for lotic investigations is the fact that a larger group of herbivorus insects are found in lotic sysytems that are prolific hosts of diverse species of trichomycetes (Lichtwardt et al.

2001a).

According to Lichtwardt et al. (2001a) there are several species of *Stachylina* reported from lentic habitats such as ponds, including *Stachylina chironomidarum* Lichtw., *Stachylina euthena* Manier & Coste, *Stachylina macrospora* L. Leger & M. Gauthier, as well as *Smittium mucronatum* Manier & Mathiez ex Manier, *Smittium phytotelmatum* Lichtw. and *Smittium typhellum* Manier & F. Coste, all from chironomid (non-biting midge) larvae. *Smittium culicis* Manier and *Zancudomyces culisetae* Y. Wang, Tretter, Lichtw. & M.M. White (as *Smittium culisetae* Lichtw.) are both widely distributed in mosquito larvae from lentic environments (Lichtwardt et al., 2001a). Strongman and White (2008) described three new species from lentic systems in Algonquin Park, Ontario, *Legeriomyces algonquinensis* Strongman & M.M. White from a mayfly nymph, *Glotzia incilis* Strongman & M.M. White from a dipteran (Dixidae) and *Arundinula opeongoensis* M.M. White & Strongman from a crayfish. Lichtwardt (1994) reported trichomycetes in larvae extracted from the phytotelm of plants and other lentic habitats in Costa Rica. However, freshwater lentic systems are understudied and they clearly represent a potentially abundant source of trichomycetes yet to be discovered. Lentic systems such as lakes and ponds present limitations in sampling not found with lotic sampling. Often lentic sampling is restricted to edges of the body of water and standing water in temperate regions are subject to ice cover making these habitats difficult to access for significant periods of time (winter months) although insects are present under the ice (Merritt and Cummins, 1996).

The dipteran order, or true flies, is known to host up to 80% of the currently

described Harpellales (Valle et al., 2011) and in particular, midge larvae of the family Chironomidae and black flies (Simuliidae) are replete with these harpellid fungi. Two genera account for a large proportion of the described Harpellales: *Smittium* spp. with over 90 described species and *Stachylina* spp. with over 40 described species each of which are largely found in the hindguts and midguts, respectively, of midge larvae. Conversely, Plecoptera (stoneflies) and Ephemeroptera (mayflies) nymphs host a wider variety of much less speciose genera of harpellids (Lichtwardt et al., 2001a).

The life cycle of Harpellales is common for the two families (Harpellaceae and Legeriomycetaceae) differing in subtle ways. The unbranched thalli of the Harpellaceae are septate and each cell functions as a generative cell producing exogenously an asexual, membrane bound, trichospore with a single appendage. The branched Legeriomycetaceae in contrast commonly produce trichospores at the terminal end of a fertile tip allowing in some cases for vegetative growth simultaneous with trichospore production. In both groups, trichospores break free from the thallus and are released to the exterior aquatic habitat where they are assumed to remain dormant until ingested by an appropriate host and attach themselves via a holdfast or hyphal surface to either the peritrophic matrix or hindgut. The trichospore then extrudes its contents, attaches to the lining and a new thallus grows. Zygospores, like trichospores perhaps extrude their contents which develop into individual thalli; however, to date there is no existing evidence supporting this hypothesis.

Within the Harpellales, there is variation in thallus types beginning with the simple unbranched thalli of the Harpellaceae that range in size from as short as 30 µm as

in the case of *Stachylina minuta* M. Gauthier ex Lichtw. or can extend up to 900 µm as described for *Stachylina magna* Indoh, Lichtw. & Kobayasi. The diameter of the Harpellaceae thalli have a much narrower range from 3-4 µm seen in *Harpella amazonica* Ríos-Velásquez, Lichtw., Hamada & Alencar and up to a diameter of 14-25 µm exhibited by *St. magna*.

The more complex branching patterns exhibited in the Legeriomycetaceae are more difficult to describe in general, but different branching patterns are recognized. In some instances, thalli take on a distinct verticilliate pattern of branching as observed in species such as *Smittium brasiliense* Alencar, Lichtw., Ríos-Velásquez & Hamada, *Smittium culisetae* and *Smittium dipterorum* Lichtw.; however, in the latter two species they are described as 'often verticilliate' in regards to their branching pattern suggesting it is not absolute. Other branching patterns include profusely branched, as in *Smittium bisporum* Manier & F. Coste (Manier and Coste, 1971), and sparsely branched as described for *Smittium bulbosporophorus* L.G. Valle & Santam (Valle and Santamaria, 2004). Additionally dimorphic thalli, as seen with *Smittium biforme* M.M. White & Lichtw. (White and Lichtwardt, 2004) have been observed resulting in the production of two trichospore sizes and *Graminelloides biconica* Lichtw. in which individual sparsely branched thalli aggregate together in a cluster (Lichtwardt, 1997).

Within the insect host, trichomycete thalli are found anchored on the peritrophic matrix (in dipterans) as well as the hindgut lining of most herbivorous insects via a holdfast structure or system (Lichtwardt, 2001). In certain species such as *Pteromaktron protrudens* Whisler and *Pteromaktron timberleaense* R.T. William & Strongman the

thallus is anchored to the gut via surface area by multiple short sterile branched hyphae, whereas in other species much more complex holdfasts are observed. In the vast majority of the over 40 species of *Stachylina*, the holdfast is either a rounded or tapered basal cell while only a few have holdfasts that penetrate the peritrophic matrix in dipterans. Mature or free trichospores have been observed with several spherical 'apical spore bodies' terminal to the trichospore full of adhesive used to anchor the newly extruded thalli (Horn 1989a; 1989b; Moss and Lichtwardt 1976; Williams, 1983).

Trichomycetes have been documented to reproduce via sexual and asexual means of reproduction; however, it is much more common to observe production of asexual trichospores more so than zygospores resulting from sexual reproduction. Most trichospores are produced directly within the gut lumen of the host; although there are cases where the trichospores actually protrude from the anus of their insect hosts. Both *Pteromaktron protrudens* and *Pteromaktron timberleaense* produce trichospores projecting from the anus of mayfly nymphs (Whistler, 1963; William and Strongman, 2012) as do *Zygopolaris ephemeridarum* Moss, Lichtw. & Manier, *Zygopolaris borealis* Lichtw. & M.C. Williams (Moss et al., 1975; Lichtwardt and Williams, 1984), *Stipella vigilans* L. Léger & M. Gauthier (Moss, 1970) and all *Orphella* species for instance.

Associated with the trichospores of most observable species of the Harpellales is an appendage or appendages that are generally thin and much longer than the trichospore such as those observed in almost all *Smittium* trichomycetes, yet there are instances of shorter, thicker appendages such as those observed in *Pennella hovassi* Manier ex Manier and *Legeriomyces algonquinensis*. The number of appendages can range from one to

seven depending on the species and there are instances within the harpellids including species of *Carouxella, Bojamyces, Caudomyces, Orphella*, and *Zygopolaris* in which no appendages have been observed (Lichtwardt, 2004).

Sexual reproduction produces thick-walled zygospores and has been described for many species of trichomycetes within the Harpellales (Lichtwardt, 2004). The Harpellales were long considered the only order to produce zygospores from a truly sexual process that includes conjugation, thickened walls and storage materials within the spore; however, *Asellaria jatibonicua* L.G. Valle & Cafaro is the first species from the Asellariales known to produce zygospores not directly from conjugation tubes as with Harpellales, but on contiguous cells, always next to a septum (Valle and Cafaro, 2008). Of the over 200 species of harpellid trichomycetes, there is an almost even split between those with documented zygospores and those without (Lichtwardt, 2004). Interestingly, there are many examples where some species in a genus have zygospores described while other species in the same genus do not, including; *Austrosmittium*, *Genistelloides*, *Genistellospora*, *Glotzia*, *Legeriomyces*, *Pennella*, *Smittium*, and *Stachylina* (Lichtwardt, 2004). Based on how infrequently many species have been encountered, it is not surprising that zygospores are not observed for many species. Also, the production of zygospores often is limited to a short period just before the insect molts (Lichtwardt et al, 2001a). In some scenarios such as *Orphella dalhousienesis* Strongman & M.M. White and *Capniomyces celatus* L.G. Valle, zygospores are produced at the same time as trichospores, whereas in other instances trichospore production is reduced or absent when zygospores are formed (Strongman and White, 2006).

Zygospores are mostly thick-walled structures compared to trichospores, possibly designed for surviving harsher environments and have also been found to have an associated appendage. Zygospores are produced from the zygosporophore with four distinct types described: Type I with zygospores that lie perpendicular to the zygosporophore; Type II with zygospores lying oblique to the zygosporophore; Type III where zygospores lay parallel to the main axis of the zygosporophore and Type IV where the zygospores is attached at one pole so that the zygospores and zygosporophore are considered to have a coaxial attachment (Moss and Lichtwardt, 1977). Recently, a fifth type of zygospore has been added when Valle and Santamaria (2005) found them in three species of *Orphella* (*Orphella catalaunica,* Santam. & Girbal *Orphella coronata* L. Léger & M.Gauthier and *Orphella. helicospora* Santam. & Girbal) that are produced laterally from a generative cell, but have shapes that are curved (*O. catalaunica*) or helicoidal (*O. coronata* and *O. helicospora*).

Trichomycetes are classified largely based on morphology by taking microscopic measurements of trichospores (asexual) and zygospores (sexual); finding an average, maximum and minimum range, for length and width of the spores and also describing trichospore shape, appendage number and arrangement. To a lesser extent the holdfast structure and thallus characteristics are used to distinguish species, but most cannot be accurately identified to species without detailed trichospore morphology (Lichtwardt, 1986). Complicating the precise identification of trichomycetes is that most are unable to be cultured on artificial media (Lichtwardt 1986). Molecular techniques are currently being employed to elucidate phylogeny and relationships among taxa (White 2002; 2006;

White et al., 2006*a*); however, these efforts are limited due to the inability to grow the majority of trichomycetes in axenic culture. As a consequence, the importance of employing accurate descriptions in conjunction with quantitative analysis becomes paramount to accurate trichomycete taxonomy.

An investigation of trichomycetes in Nova Scotia has been conducted by Dr. Doug Strongman and Dr. Merlin M. White beginning in the early 2000's. These investigations have yielded an inventory of 42 species of trichomycetes, as well as two unidentified species (*Parataeniella* sp. and *Orchesellaria* sp.) reported from various locations within Nova Scotia. Their work describes 22 new species including three new genera *Trifoliellum, Bactromyces* and *Laculus* as well as 20 species of trichomycetes that have been previously reported from different locations outside of Nova Scotia (Strongman 2005; Strongman and White 2006; 2008; 2011; William and Strongman 2012; White and Strongman 2012*a*; 2012*b*). In all cases, the hosts were aquatic insects from the Orders Plecoptera, Ephemeroptera or Diptera with the exception of *Parataeniella* sp. from a pill bug (Isopoda) (Strongman and White 2011) and *Orchesellaria* sp. recorded in springtails (Collembola) (White and Strongman 2012*b*).

This thesis contains an inventory of trichomycetes from a lotic and a lentic site from each of three separate watersheds within the Halifax Regional Municipality in Nova Scotia, Canada. The Harpellales were the central focus of this study with a few *Paramoebidium* spp. (Amoebidiales) also recorded. The study was conducted over a 17 month period beginning in August 2010 and ending in late December 2011 resulting in duplicate seasonal data collected from August to December of both 2010 and 2011.

Repeated collections at two sites in each of three separate watersheds over the study period provided data on seasonality and site specificity in trichomycetes. The prevalence of each taxon documented in this study is reported and comparison between lentic and lotic habitats are given for common (ephemeropteran and chironomid) hosts found in both site types. These two hosts were also used to compare prevalence, and species richness among watersheds. This is one of very few studies detailing new species recorded over a long term, multi-seasonal collection of trichomycetes and will supplement a larger project attempting to catalogue trichomycetes occurring in Canada and assist in developing an ecological understanding of these cryptic fungi.

Materials and Methods

Study Design

In undertaking a project of this nature it was imperative to find appropriate sites from which to draw samples and to standardize key components of the study in order to be able to make appropriate comparisons among sites. Initially, potential sites were examined for similar physical characteristics and for the presence of paired lotic/lentic sites within a watershed. All preliminary sites were assessed for trichomycete populations by collecting timed samples of aquatic insects rather than covering a standardized area which could be complicated by the logistics of sites that might not allow for an area of equal size to be covered. The stream or lake bottoms were disturbed by kicking stones and muddy areas (kick sampling) then sweeping a dip net into the disturbed substrate catching dislodged insects (Lichtwardt et al., 2001a). Initial timings used for preliminary samples were 20 minutes, and were stepwise reduced down to four- minute samples after it was demonstrated that more insects were collected after four minutes than could be dissected before the insects would begin to die and decompose, thus obscuring any trichomycetes. Similarly, when sorting the insects from each sample into target host groups for subsequent dissections, the amount of time spent sorting was standardized. Beginning with one hour sort times, this too was stepwise reduced to a duration of 30 minutes as again, more insects than necessary for dissections were recovered in that time frame.

All families of herbivorous Ephemeroptera and Plecoptera were selected while two families of Diptera, Simuliidae and Chironomidae, were target hosts for dissections.

It was determined that nine specimens each of the four target hosts were to be dissected from each sample in order to again ensure dissections were completed before insect death and decomposition began.

Methodology

Three lotic sites and three lentic sites, one pair from each of three watersheds located in the Halifax Regional Municipality, Nova Scotia (NS), Canada were selected as the permanent study sites. The three lotic sites were: a stream feeding Long Lake, Halifax, NS (LLLO, 44 37' 45N; 063 39' 31W; elevation 60 m); a stream draining Lake Mill Pond in Timberlea, NS (TLLO, 44 39' 44N; 063 44' 41W; 59 m) and a stream feeding Lake Micmac in Shubie Park, Dartmouth, NS (SPLO, 44 44'46N; 063 33 51W; 27 m). Two of the lentic sites were Governor's Lake in Timberlea, NS (GLLE, 44 38' 36N; 063 42 19W; 54m) and Lake Micmac in Shubie Park, Dartmouth, NS (SPLE). The third lentic site was located at Long Lake, NS (LLLE, 44 41' 52N; 063 33 13W; 28 m) and was situated approximately 30 m from the mouth of a stream that emptied into the lake. It became evident after several months that this site was influenced by outflow from the stream when large rain events happened. Therefore, the LLLE site is considered an interphase site between the stream and lake habitats that was different from the other two lake sites not directly influenced by stream inputs. The types of insects collected from this interphase site for a part of the sampling period reflected both lentic and lotic communities. The stream substrates were quite similar in composition including largely rocky bottoms interspersed with sandy sections. The lake sites had rocky and sandy to

muddy substrates largely devoid of aquatic vegetation. There were 22 collections made from each stream site but only 19 from the lake and interphase sites due to ice cover at these sites from Jan-Mar 2011 (Appendix I).

Three timed (four minute) samples were collected from the substrate at each lentic and lotic site by kicking stones and muddy areas (kick sampling) then sweeping a dip net into the disturbed substrate catching dislodged insects. Samples were taken from a pair of lentic/lotic sites within a specific watershed on a collection date such that sampling occurred approximately every three weeks at each watershed. The sampling dates are given in Appendix I and, except for GLLE on 3 Jan 2011, ice cover prevented collections at this site and Lake Micmac from January to March 2011.

The target hosts (Ephemeroptera and Chironomidae as well as Plecoptera and Simuliidae when present) were sorted, counted, and then up to nine individuals from each host type were selected from each subsample (maximum 27 of each type per sample/collection date) for dissection. The numbers of insects in each of the target groups collected and dissected on each collection date are given in Appendix I. Guts of target insects were dissected in a drop of water on a glass Petri dish on the stage of a stereomicroscope with a transmitted light source. Guts were transferred to a drop of water on a microscope slide and thalli were separated from insect tissue using fine tipped forceps and insect pins mounted in pin vises (Grobet, Carlstadt, N.J., USA). Slides (wet mounts) were examined with a compound microscope equipped with phase and differential interference contrast (Nomarski) optics, then examined for taxonomically relevant characteristics including trichospores and zygospores, if present. Dimensions of

spores as well as holdfast structure and thallus branching patterns were recorded from digital micrographs. Semi-permanent voucher slides were made by infiltrating the specimen on the slide with a drop of lactophenol cotton blue stain placed on the edge of the coverslip. After 18-24 hours excess stain was wiped away with a moistened tissue before sealing the edge of the cover slip with fingernail polish (Lichtwardt, 1986). Identifications were made from digital micrographs taken from both live (before staining) and stained material, using the Lucid keys accessible on the University of Kansas website (Lichtwardt, 2004) which are updated frequently as new species are described.

Type specimens for all new species are deposited at the National Mycological Herbarium, Agriculture & Agri-Food Canada, Ottawa, Ontario (DAOM). Some preserved specimens are available from D. B. Strongman, Biology Department, Saint Mary's University.

Ecological Methodology

Several aspects of trichomycete ecology were analyzed beginning with trichomycete species richness where all identified taxa from each site (three lentic sites, two lotic sites and one interphase site) were totaled and numerically compared to each other. Next, seasons were compared that included dividing collection dates into four discrete periods (August to December 2010, January to April 2011, May to August 2011, and September to December 2011), totaling the taxa identified and comparing each period. Collection periods from six sites were typically conducted over three week intervals (one watershed per week) such that seasonal periods often overlapped from the

end of one month to the start of another. The actual dates of these collection periods are: 17-August 2010 to 15 December 2010 (Aug to Dec 2010), 17 December 2010 to 24-April 2011 (Jan to Apr 2011), 27 April 2011 to 17 August 2011 (May to Aug 2011), and 21 August 2011 to 22 December 2011 (Sep to Dec 2011).

Prevalence of each trichomycete taxon was examined for the four target host groups. For each of these host groups, the ratio of larvae colonized by each taxon to the number of hosts examined was used to generate trichomycete prevalence (%). An arbitrary number of 2% was used to separate common taxa (> 2%) from rare taxa (< 2%) with prevalence of each trichomycete taxon at each site recorded. Habitat preference based on prevalence was analyzed where the three lotic sites were compared to each other, two lotic sites compared to each other to assess prevalence at each site, before comparing prevalence at lotic sites to those from lentic. The one interphase site was excluded from lotic and lentic comparisons, but was considered separately in the analysis.

Trichomycete species composition within Ephemeroptera and Chironomidae hosts were generated by compiling a mean from the three lotic sites and mean from the two lentic sites. There was only one interphase site so actual values were given for this site. Assessments of each taxon were used to characterize all taxa as occurring in lotic, lentic, both lentic and lotic or interphase habitats.

Finally, for each of the three watersheds (Long Lake, Woodens River and Shubenacadie) mean number of taxa from the lentic and lotic site in each watershed were combined to compare trichomycete overall community structure among the watersheds. The Long Lake watershed was largely excluded from the comparison due to the nature

of the interphase site not being a true lentic site like those in the other two watersheds.

Results (Taxonomy)

There were 64 taxa of trichomycetes (61Harpellales, 3 Amoebidales) collected from the insect groups (Ephemeroptera, Plecoptera and Diptera (Simuliidae and Chironomidae) targeted in this study (Appendix II). Nineteen of these species presented morphological features sufficiently distinct from existing taxa to justify descriptions of new species including two new genera. The remaining 37 species have been previously described from different locations around the globe including five species establishing new continental records for North America and five species establishing new regional records for Nova Scotia. A complete list of trichomycete species recovered from all the collection sites can be seen in Appendix II including the insect host, and collection dates. All new species described herein have been validly published (William and Strongman, 2012; 2013a; 2013b; 2013c).

New Species

Bactromyces gen.nov. R. T. William & Strongman

Mycobank MB 561721

Thallus compact with thick central axes branching profusely distally; the holdfast is a long, winding hypha with a few branches and small papillae scattered on the surface. Fertile terminal branches bear long, cylindrical trichospores swollen slightly above the mid-line and tapered at the base, with a short collar and no appendage. Zygospores are unknown. Attached to the hindgut lining of stonefly nymphs (Plecoptera).

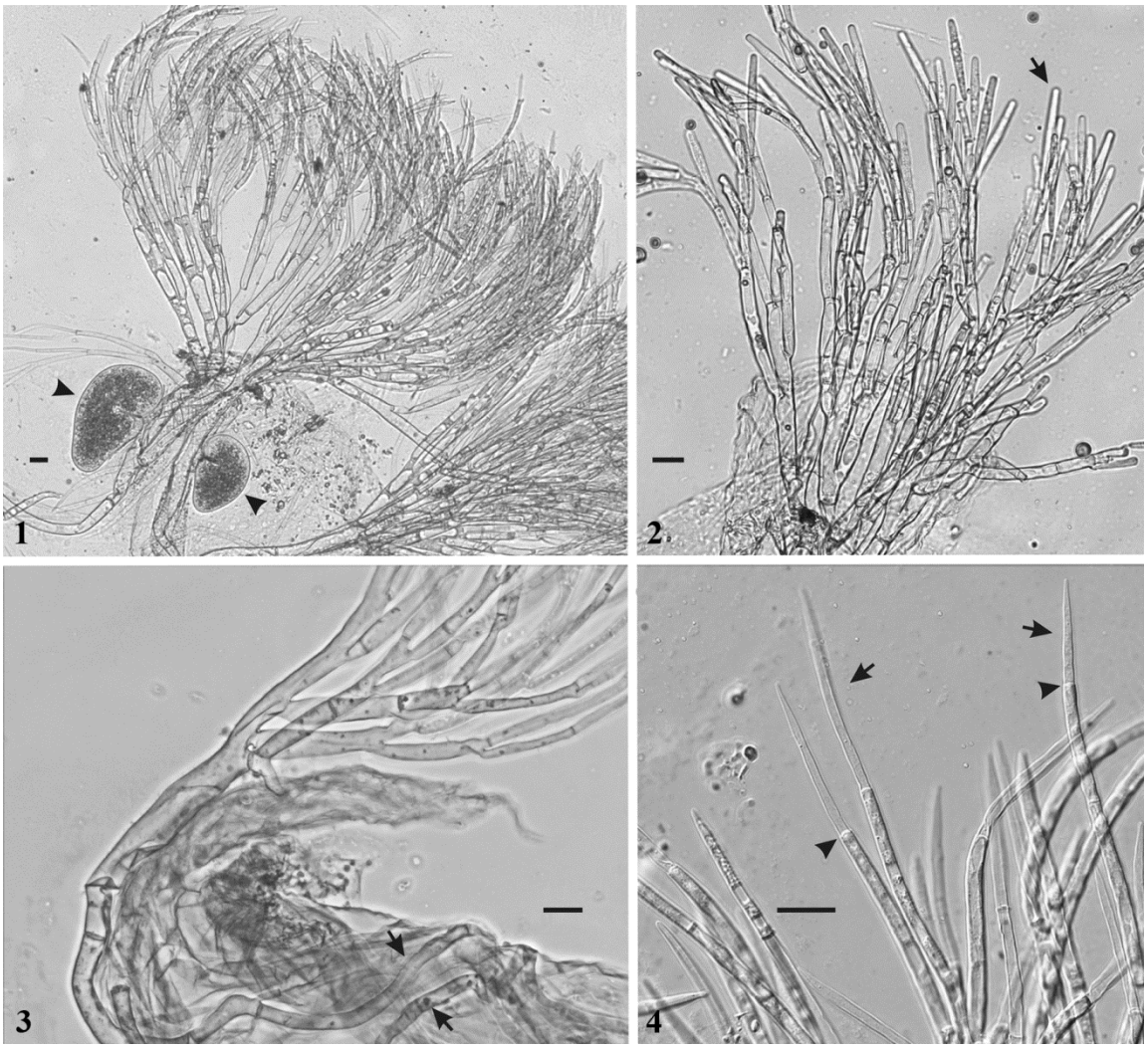
Bactromyces fluminalis sp. nov. R. T. William & Strongman (Figs. 1–8)

Mycobank MB 561722

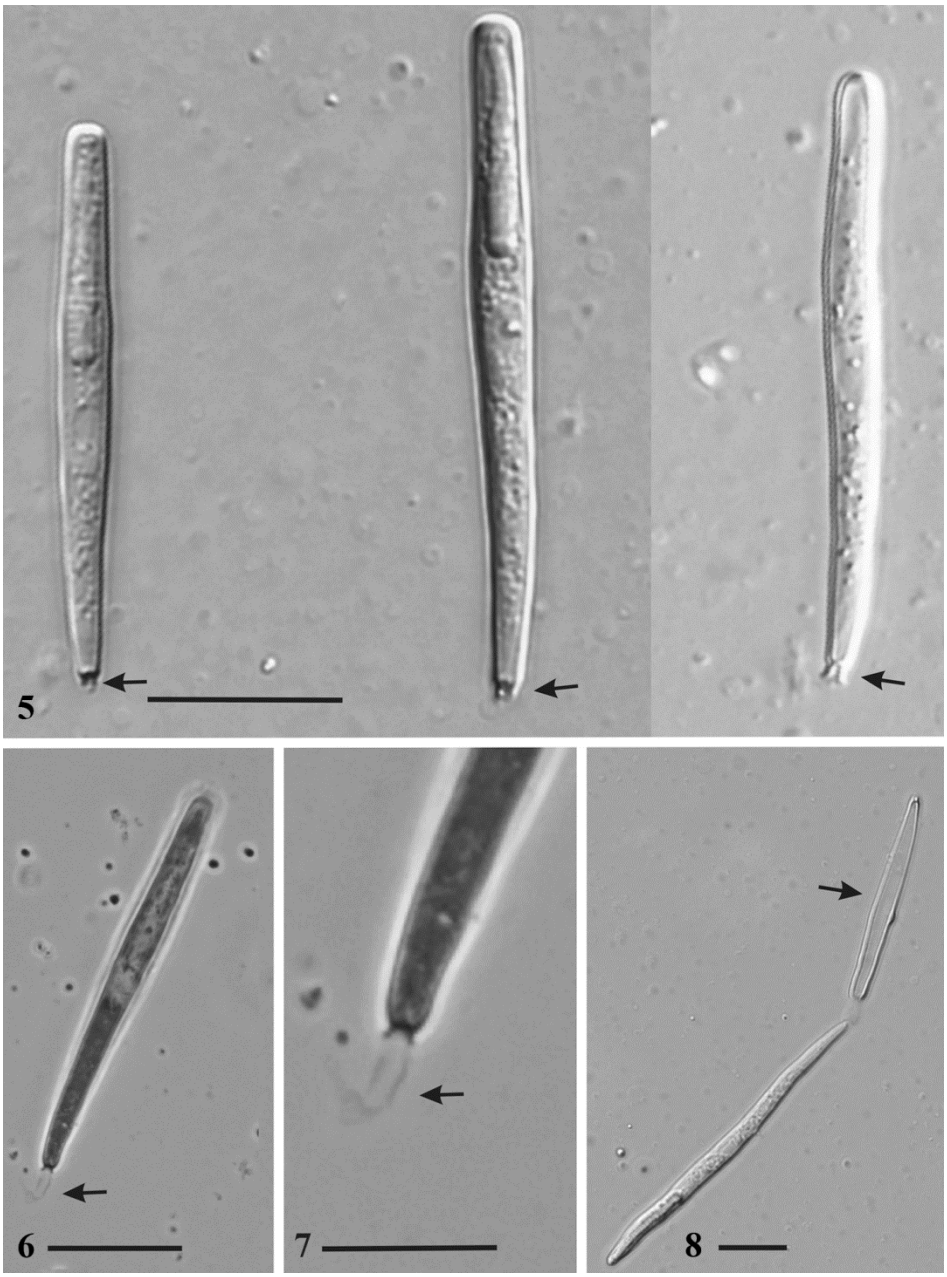
Thallus arborescent, spreading at the ends by profuse branching (Fig. 1), thick central hyphae about 20 µm thick (Figs. 1 and 2), secondary branches (12–16 µm wide) narrowing to 4.5–7 µm at the sporulating terminal branches (Fig. 2), holdfast winding, sparsely branched hypha with scattered papillae on the surface (Fig. 3). Immature thalli with branches terminating in an elongated cell tapering to a point (Fig. 4). Trichospores, typically 4 per terminal branch, cylindrical, 62–81 µm long, 3.5–5.5 µm wide, swollen slightly above the midline tapering to 2–4 µm at the base, with a short collar (1–4 µm wide x 2–3 µm long), no appendage (Fig. 5). No zygospores seen. Attached to the hindgut lining of stonefly nymphs (Capniidae).

ETYMOLOGY**:** From the Greek for stick or rod *baktron* alluding to the long rod-like trichospores in this species and the Latin, *fluminalis*, meaning *river* or *stream* referring to the aquatic habitat where it was discovered.

HOLOTYPE: Lactophenol cotton blue stained thalli and trichospores on microscope slide LL-4 (DAOM 241339) made from the hindgut of a stonefly nymph (*Paracapnia angulata* Hanson) collected on 2 Jan 2011 at Long Lake Provincial Park, off Route 333, within the Halifax Regional Municipality, NS. The holotype slide also has thalli and trichospores of *Lancisporomyces falcatus* Strongman & M.M. White and these two species are often found together in the same host gut. Another slide, LL-5 (DAOM 241340), collected from the same site on 2 Jan 2011 (PARATYPE) has immature thalli



Figs. 1–4. *Bactromyces fluminalis*. Fig. 1. Thallus branching. Arrowheads show two thalli of *Paramoebidium cassidula* Strongman & M.M. White. Fig. 2. Thallus with trichospores attached (arrow). Fig. 3. Basal part of thallus showing winding holdfast with papillae (arrows). Fig. 4. Immature thallus with tapered, empty terminal cells (arrows) delineated by a septum (arrowheads). All images from live, unstained material. Scale bars = 20 μm.



Figs. 5–8. *Bactromyces fluminalis*. Fig. 5. Released trichospores with a short flared collar (arrows). Figs. 6 and 7. Released trichospore with amorphous material attached to the collar (arrow). Fig. 8. Trichospore wall (arrow) after extruding the sporangiospore. Figs. 5 and 8 from unstained material. Figs. 6 and 7 taken from a lactophenol cotton blue stained specimen. Scale bars = 20 μm, except 10 μm in Fig. 7.

of *B. fluminalis* only and shows the holdfast characteristics and the thallus branching pattern that are typical for the species.

HABITAT: Dissected from the hindgut of stonefly (*Paracapnia angulata*) nymphs collected from a flowing stream in Long Lake Provincial Park on 28 Dec 2009, 6 Jan 2010, 20 Jan 2010, 15 Mar 2010, 22 Mar 2010, 2 Jan 2011 and 23 Jan 2011.

COMMENTARY: *Sinotrichium chironomidarum* J. Wang, S.Q. Xu & Strongman has long, cylindrical trichospores with a collar in the same size range but the spores are not tapered as in *B. fluminalis*. *S. chironomidarum* has one long thin appendage and the collar is longer, also, *S. chironomidarum* is found in chironomids (Wang et al., 2010) while *B. fluminalis* comes from the gut of capniid stoneflies. *Bojamyces* spp. have long trichospores with a collar, with or without an appendage, but the size and shape of the spores is different and the thallus branching structure is different from *B. fluminalis*. Species of *Tectimyces* have clavate trichospores like *B. fluminalis* with a short collar and no appendage, but the thallus in *Tectimyces* is pinnate or umbelliferous (Valle and Santamaria, 2002). Both *Bojamyces* and *Tectimyces* spp. inhabit guts of mayflies (Lichtwardt, 2004) while *B. fluminalis* colonizes the guts of stoneflies. *Genistelloides* spp. and *Lancisporomyces* spp. have been described from stonefly hosts and have cylindrical trichospores like *B. fluminalis*, but none of these species have a collar and both have two appendages on the trichospores (Lichtwardt, 2004).

Glotzia gemina sp. nov. R.T.William and Strongman (Figs. 9–13)

Mycobank MB 80375

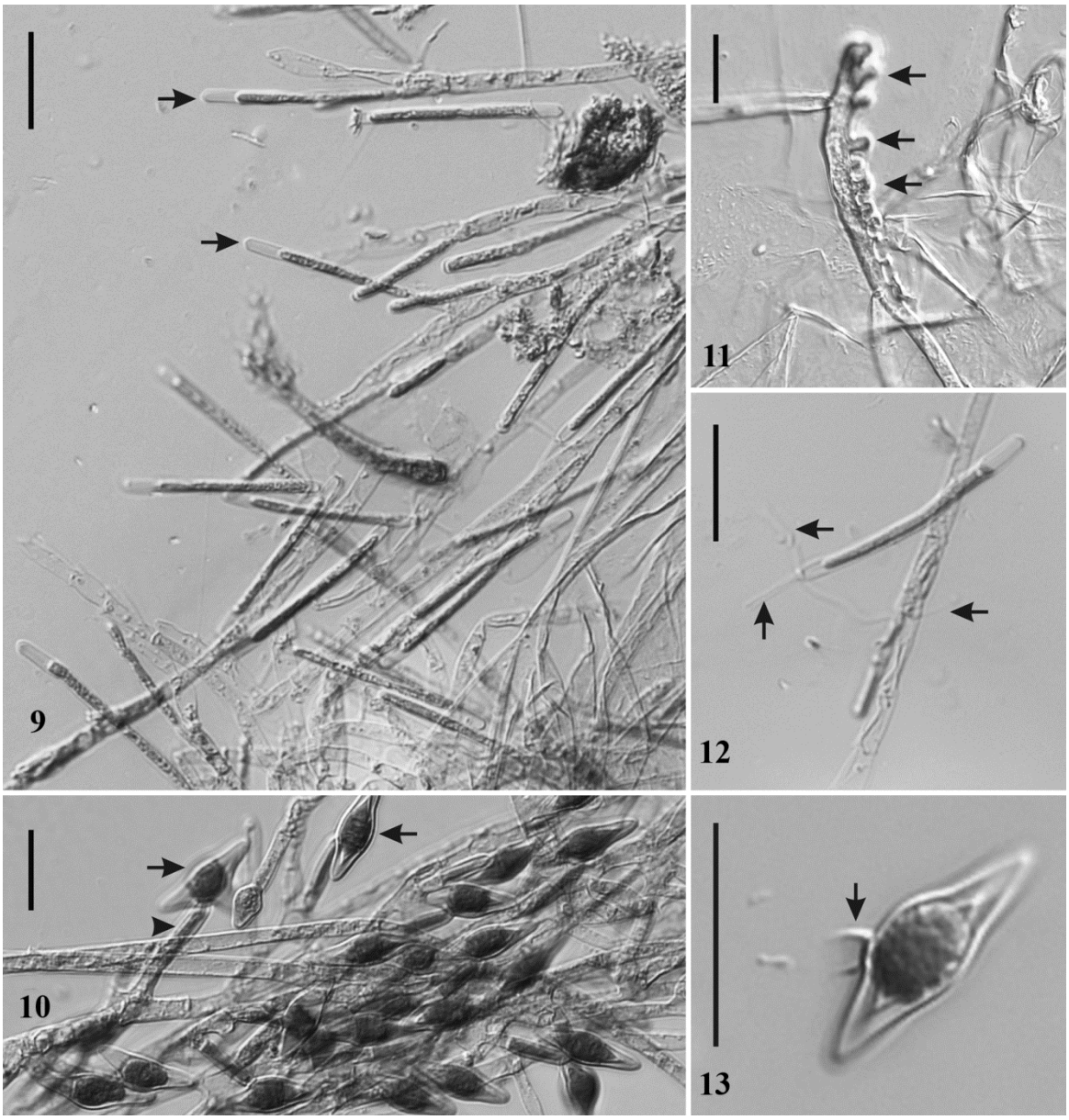
Sparse, irregularly branched thallus (Fig. 9) emanating from basal cell with peg-like projections (Fig. 10), producing trichospores at the end of fertile tips (Fig. 9). Trichospores (Fig. 11) cylindrical 40–55 µm × 2–5 µm with three appendages (2 short and 1 long), projecting in different directions from the base of the trichospore. Zygospores 25–29 µm × 8–11 µm (Fig. 13) often flattened to incurved, with a sub-medial, oblique attachment to the zygosporophore 19–26 µm × 4.5–5.5 µm (Fig. 12). Detached zygospores with a short collar (Fig. 13). Attached to the hindgut lining of mayfly (Baetidae) nymphs.

ETYMOLOGY**:** Latin *geminus twin* referring to the similarity between this species and *Glotzia ephemeridarum* Lichtw.

HOLOTYPE: Microscope slide SPLO-1 (4 Sept 2010) with thalli bearing trichospores, attached to hindgut lining (DAOM 242389). PARATYPE: Slide SPLO-1 (12 Oct 2010) (DAOM 242390) containing thalli with zygospores. The baetid host containing the holotype specimen was collected at Shubie Park stream (SPLO) Dartmouth, NS on 4 Sept 2010 and the paratype came from a baetid collected at the same site on 12 Oct 2010. Both the holotype and paratype specimens were stained with lactophenol cotton blue.

HABITAT: Collected from hindgut lining of mayfly (Baetidae) nymphs at SPLO on 30 Aug 2010, 19 Sep 2010, 12 Oct 2010, 13 Jul 2011, and 4 Sep 2011 (Appendix II).

COMMENTARY: The unique feature exhibited by *Glotzia gemina* that places it in the *Glotzia* genus is the long cylindrical trichospores with three appendages diverging from



Figs. 9–13. *Glotzia gemina*. Fig. 9. Thallus with developing immature trichospores (arrows). Fig. 10. Swollen basal cell of holdfast structure with peg-like projections (arrows) anchoring the fungus to the hindgut lining. Fig. 11. Released trichospore displaying three distinct appendages (arrow). Fig. 12. Developing zygospores (arrows) affixed to zygosporophore (arrowhead). Fig. 13. Released zygospore with small collar (arrow). All Figs. from lactophenol cotton blue stained specimens. Scale bars = 20 µm.

one another (Lichtwardt et al. 2001a). Of the seven species in this genus only four have similar morphological features to *G. gemina***.** *Glotzia incilis* trichospores (21.5–26.5 µm × 3.5–4.5 µm) are smaller and a different shape while *Glotzia tasmaniensis* Lichtw. & M.C. Williams has trichospore measurements [(30–)46(–78) µm × (3.2–)4.0(–6.0) µm] with a broader range than those in *G. gemina.* Additionally, both *G. incilis* and *G. tasmaniensis* have a simple or bulbous basal cell holdfast (Lichtwardt, 2004). The arrangement of trichospores on the thallus is different in *Glotzia plecopterorum* Lichtw. and the trichospore dimensions (39–50 µm × 6–8 µm) are much wider than *G. gemina* (Williams and Lichtwardt, 1990). No zygospores are reported for these three described species so comparison with the zygospores of *G. gemina* is not possible.

At first glance, *G. gemina* is strikingly similar to *G. ephemeridarum* Lichtw., but upon further inspection both the trichospores and zygospores differ. *Glotzia ephemeridarum* trichospore length range (45–70 µm × 4.5–7 µm) overlaps with those of *G. gemina* (40–55 µm × 2–5 µm) but *G. ephemeridarum* trichospores are 2 to 3 times wider (Lichtwardt et al., 2001a). The average trichospore length and width for *G. gemina* (n=38 spores) was 45.5 µm × 3.3 µm which falls into the lowest end of the range for *G. ephemeridarum*. Additionally the length of mature zygospores of *G. gemina* (25–29 µm × 8–11 µm) was consistently below the range described for *G. ephemeridarum* (28–37 µm × 7.5–10 µm) which can help to differentiate between the two species (Lichtwardt et al., 2001a).

Laculus gen. nov. R.T. William & Strongman

Mycobank MB 561718

Thallus branching sparse, verticilliate with trichospores produced on terminal branches. Trichospores elliptical with a slight sub-medial swelling, a conspicuous collar and two appendages. Biconical zygospores, attached to zygosporophores obliquely (Type II) and submedially, with a collar and one appendage. Attached to hindgut lining of mayfly nymphs (Ephemeroptera).

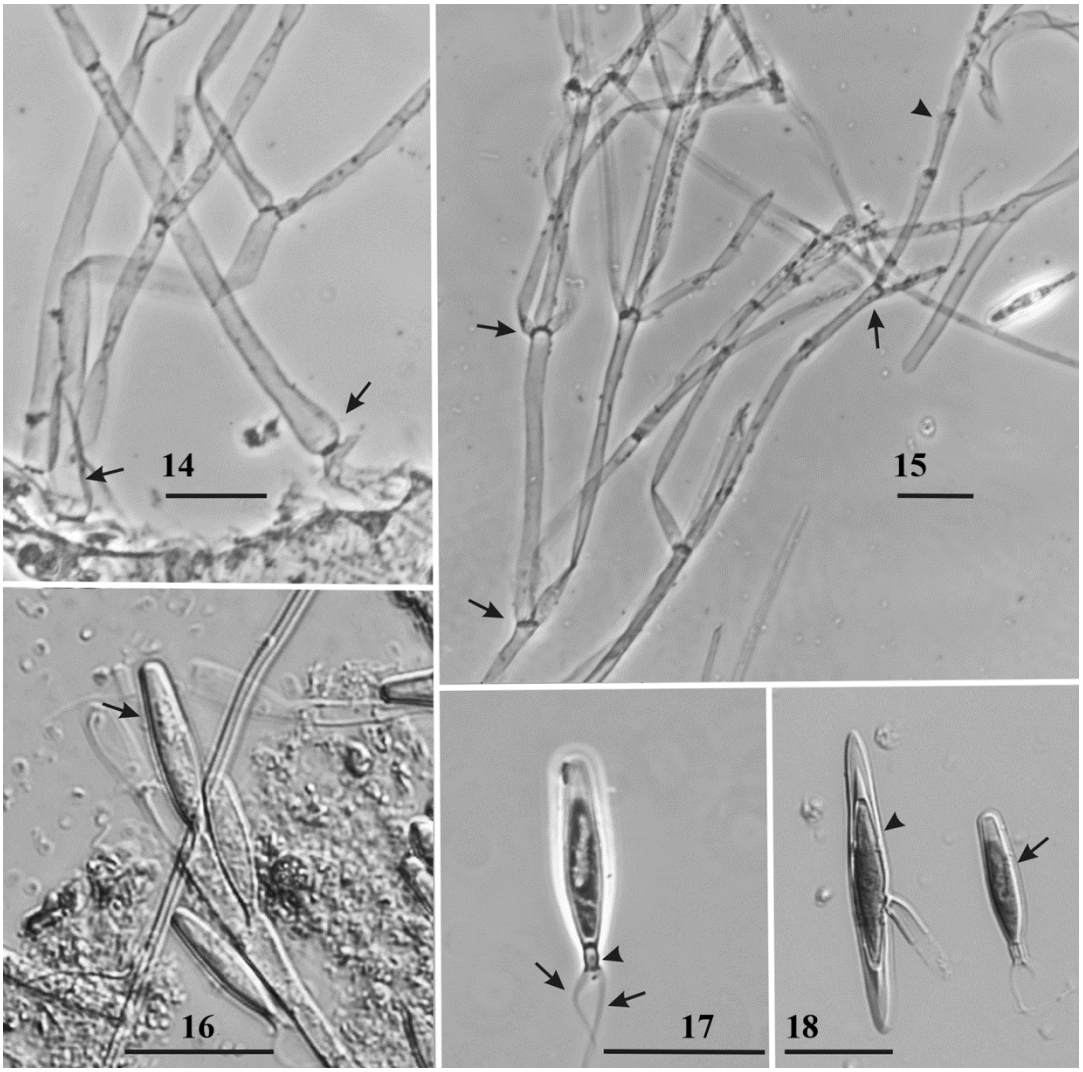
Laculus insecticola sp. nov. R. William & Strongman (Figs. 14–23)

Mycobank MB 561719

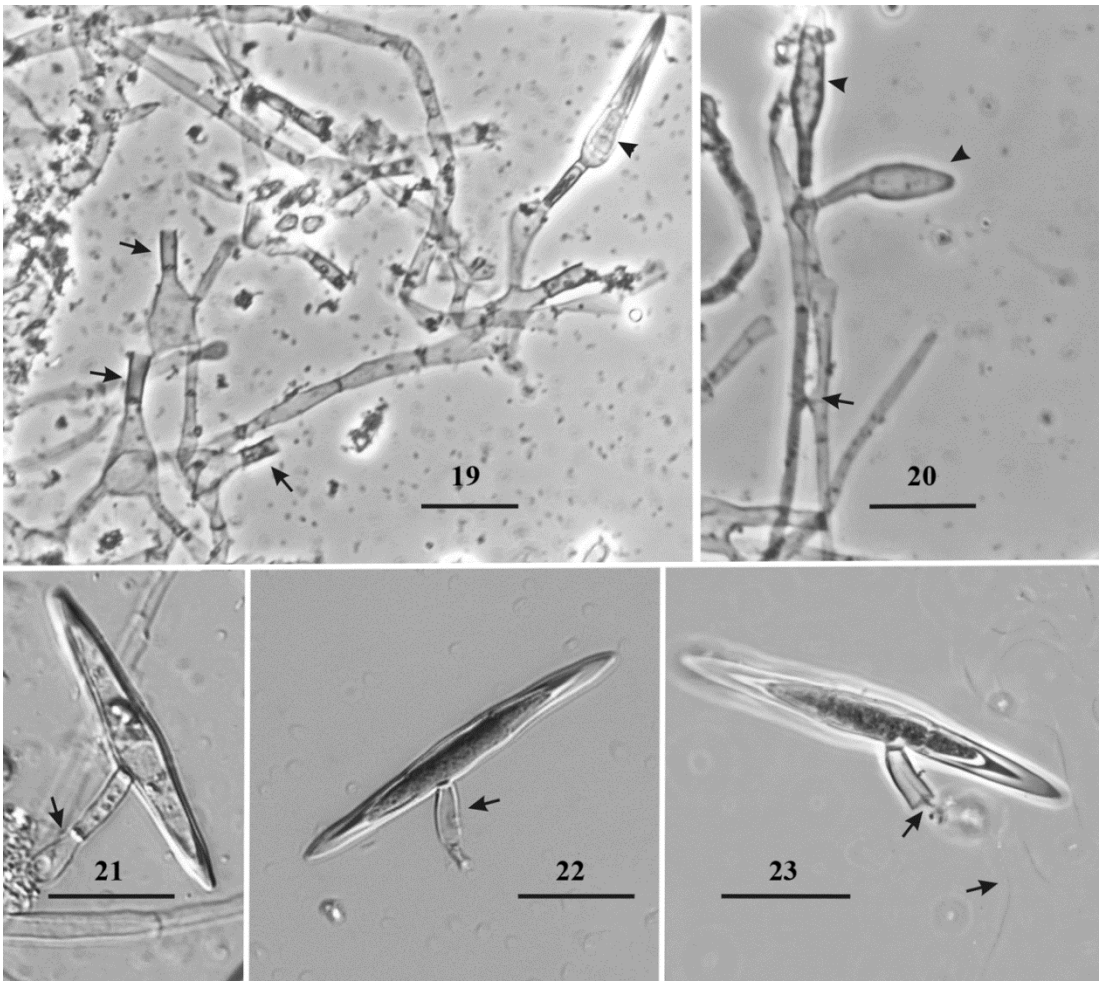
Thallus attached to hindgut lining by a slightly bulbous holdfast, 7.5–8.5 µm wide, on a pad of secreted material (Fig. 14). Sparse verticilliate branching (Fig. 15) with whorls of 2–3 long branches. Trichospores produced on terminal branches (Fig. 16), elliptical, 19–28.5 x 3.7–7

µm with a slight sub-medial swelling and a conspicuous, straight collar 2–5 µm long, sometimes with a distal flare, with two appendages (Figs. 17 and 18) that often appear to be appressed to the collar edge where they emerge. Short zygosporophores 11.5–18 µm attached to a swollen basal cell arising from conjugated hyphae (Figs. 14–16), biconical zygospores 47–66.5 x 6.5–9.5 µm borne on the zygosporophore obliquely (Type II) and submedially, (Figs. 13 and 6–18), with a collar 6–15 x 3–5.5 µm and one appendage (Figs. 17 and 18). Attached to the hindgut lining of mayfly nymphs (Caenidae).

ETYMOLOGY**:** from the Latin *lacus* meaning lake, in reference to the lentic habitat



Figs. 14–18. *Laculus insecticola.* Fig. 14. Swollen basal holdfast cells (arrows) with dark adhesive pad attaching thallus to the hindgut lining. Fig. 15. Verticilliate branching pattern in the thallus at multiple nodes (arrows). Arrowhead indicates a generative cell that has shed a trichospore. Fig. 16. Terminal branch with immature trichospores attached (arrow). Fig. 17. Released trichospore with 2 appendages (arrows) and a prominent collar (arrowhead). Fig. 18. A released trichospore (arrow) and zygospore (arrowhead). All figures are from lactophenol cotton blue stained material except for Fig. 16, which was from unstained material. Scale bars = 20 μm.



Figs. 19–23. *Laculus insecticola* zygospore features. Fig. 19. Conjugation showing remains of the zygosporophore (arrow) and an immature zygospore (arrowhead). Fig. 20. Conjugation (arrow) of adjacent hyphae and developing zygospores attached to the thallus (arrowheads). Figs. 21. Fully formed zygospore attached to the thallus by the zygosporophore (arrow). Fig. 22. Released zygospore with a submedial collar. Fig. 23. Released zygospores showing long single appendage (arrows). Material in Figs. 19, 20, 22, and 23 stained with lactophenol cotton blue. Material in Fig. 21 unstained. Scale bars = 20 μm.

where this fungus was found and *insecticola* which translates into "insect inhabiting" reflecting the obligate association between this fungus and its insect host.

HOLOTYPE: The holotype for *Laculus insecticola* is lactophenol cotton blue stained thalli, trichospores and zygospores on microscope slide (GL-4) made from the hindgut of a mayfly nymph (Caenidae) collected at Governor Lake on 9 July 2010 (DAOM 241341).

HABITAT: Caenid mayfly nymph exuviae collected on 9 July 2010 had prolific zygospore production with some trichospores and spent thalli within the shed hindgut lining of the skins (exuviae). Living mayfly specimens collected from Governor Lake on 16 Sept 2010, 27 Oct 2010, 19 Nov 2010, 8 Dec 2010 and 3 Jan 2011 had immature thalli in the gut resembling thallus of *L. insecticola*, lacking trichospores or zygospores so positive identification was not possible.

COMMENTARY: *Laculus insecticola* most closely resembles species of *Legeriomyces*, with all these species having two-appendaged trichospores and inhabiting the hindguts of mayfly nymphs. However, *Laculus insecticola* has a very prominent collar which is absent in all species of *Legeriomyces*. *Laculus insecticola* can be separated from species of *Legeriosimilis*, which resemble *Legeriomyces* spp., by the absence of a collar and trichospores with three broad appendages in *Legeriosimilis* spp. (Lichtwardt, 2004). *Lancisporomyces vernalis* Santam., described from stonefly nymphs (Santamaria, 1997), and *Legeriomyces algonquinensis* (Strongman and White, 2008) have trichospores in the same size range as *L. insecticola* but neither have a collar. *Legeriomyces rarus* Lichtw. & M.C. Williams has trichospore (25–31 x 5.5–8 µm) and zygospore (42–51 x 6–9 µm) (Lichtwardt, 2004) sizes which overlap with *L. insecticola* (trichospores 19–28 x 3.7–7

μm and zygospores 47–66.5 x 6.9–9.5 μm). This species is also described from Caenid mayflies and has been collected in Canada by Strongman and White (2008) but *L. rarus* trichospores have no collar, distinguishing them from *L. insecticola*. *Legeriomyces ramosus* Pouzar has Type II zygospores in the same size range and is known from mayfly hosts, like *L insecticola*; but the trichospores in *L. ramosus* are larger and have a sub-medial swelling and no collar (Lichtwardt, 2004).

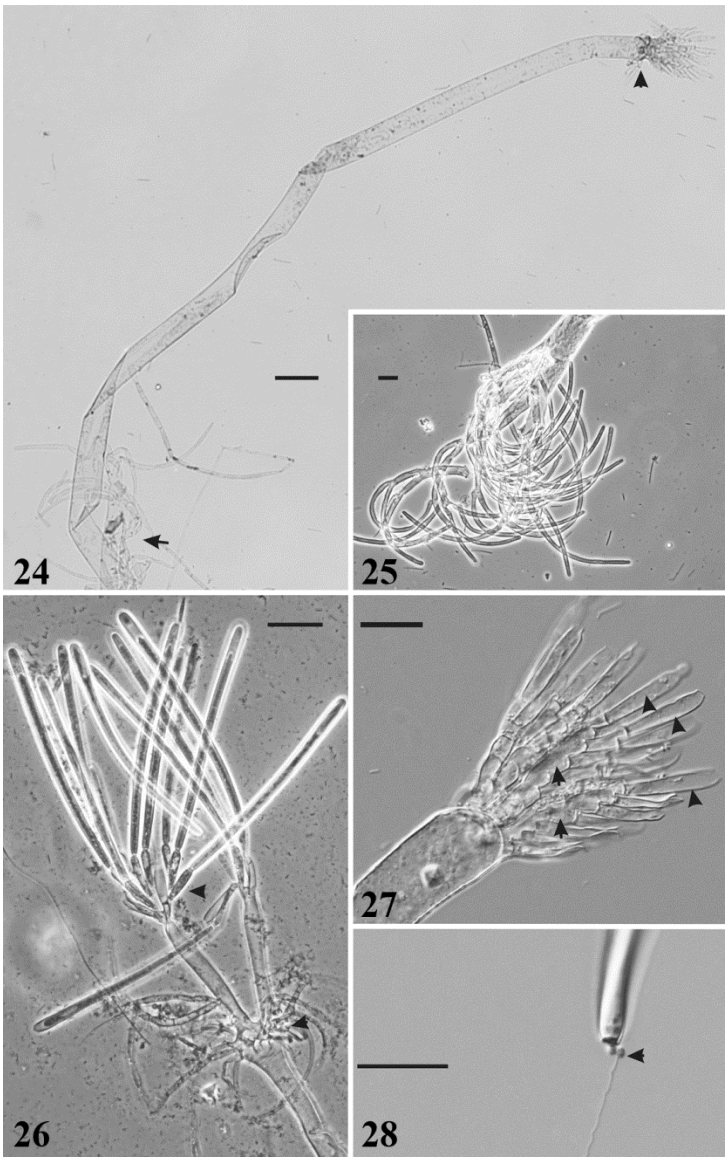
Pteromaktron timberleaense sp. nov. R.T. William & Strongman (Figs 24–30)

Mycobank MB 561720

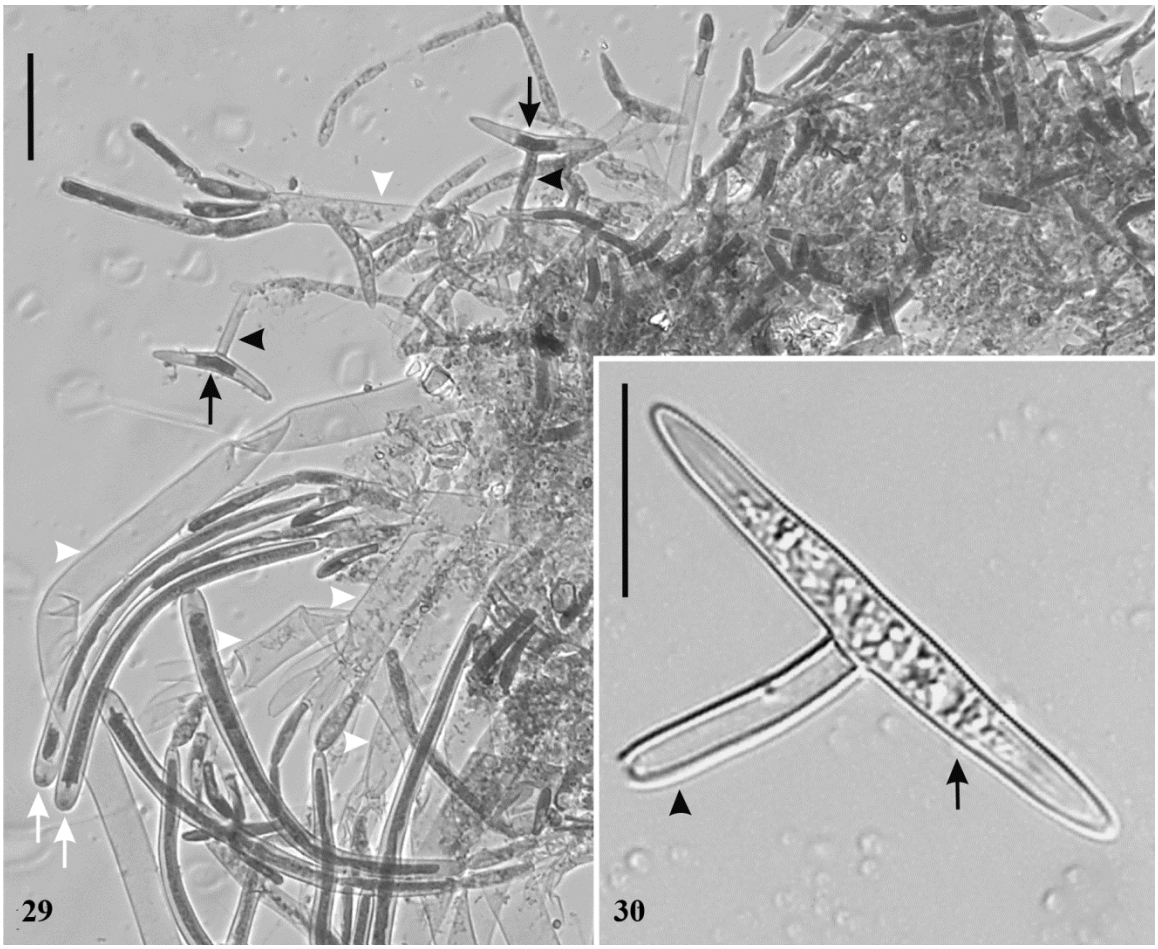
Pteromaktron timberleaense R.T. William and Strongman *in* William and Strongman, Botany 90:101-111. 2012 (Figs. 24–28). **Emend.** R.T. William and Strongman. *in* William and Strongman, Botany 91:368-381. 2013 (Figs. 24–28) Figs. 29 and 30.

Thalli aseptate, up to 1100 μm long × 19–33 μm wide, attached basally to hindgut lining by multiple short sterile branched hyphae (holdfast). Thallus terminating apically in a sporulating head composed of basal cells (37–49 μm × 7–9.5 μm), each producing 4–6 clavate generative cells (28–42 μm × 5–7 μm), each with a cylindrical trichospore (145–180 μm × 6–9 μm); released trichospores with a fine basal appendage much longer than trichospore. Zygospores (Type I) produced heterothallically, perpendicular to the zygosporophores 25–31 μm × 4–6 μm, arising from densely branched hyphae. Detached zygospores 46–58 μm × 6–7 μm with a long narrow collar 17–23 μm × 4 μm. Attached to hindgut lining of mayfly nymphs (Heptageniidae).

ETYMOLOGY**:** Named for the type location; a stream in Timberlea, NS.



Figs. 24–28. Pteromaktron timberleaense. Fig. 24. Whole aseptate thallus showing holdfast at the base (arrow) and trichospore-producing terminal head (arrowhead) with all trichospores shed. Fig. 25. Enlarged view of a branched basal holdfast. Fig. 26. Trichospores attached to generative cells (arrowhead) arising from a basal cell on a terminal sporulating head. Note the spent branches (arrow) at the base of the sporulating heads. Fig. 27. Compact trichospore-producing head with basal cells (arrows) producing generative cells (arrowheads). All trichospores are released. Fig. 28. Basal end of a trichospore showing the "knot-like" structure near where the long single appendage emerges from the trichospore. All images made from slides stained with lactophenol cotton blue. Scale bars = 20 μm, except 50 μm in Fig. 24.



Figs. 29–30. ***Pteromaktron timberleaense***. Fig. 29. Thalli (white arrowheads) and attached zygospores (black arrows) atop zygosporophores (black arrowheads). Fig. 30. Zygospore (arrow) and long collar (arrowhead), which remains attached to the zygospore after release from the zygosporophore. Fig. 29 from lactophenol cotton blue stained material. Fig. 30 is unstained. Scale bars = 20 µm.

HOLOTYPE: Thalli and trichospores of *Pteromaktron timberleaense* stained with lactophenol cotton blue on microscope slide TLLO-1 (DAOM 241342) made from the hindgut of a mayfly nymph (Heptageniidae) collected at the outflow of Mill Pond, Timberlea, NS on 3 Oct 2010. Zygospores were discovered from the same host and same site on 8 Oct 11. Slides with zygospores were submitted to national herbarium (DAOM).
HABITAT: *Pteromaktron timberleaense* was common at one site sampled, the outflow from Mill Pond in Timberlea, NS, where it was collected from mayfly (Heptageniidae) nymphs on 17 Aug 2010, 13 Sep 2010, 3 Oct 2010, 24 Oct 2010, 6 Jul 2011, 27 Jul 2011, 28 Aug 2011, 17 Sep 2011, 8 Oct 2011.
COMMENTARY: Originally described without zygospores (William and Strongman, 2012), these sexual spores were described in William and Strongman, 2012b.The trichospores of *P. timberleaense* are much longer, 145–180 x 6–9 um, than those in the only other described species, *Pteromaktron protrudens* (85–97 x 4–6 um) (Whisler 1963). Otherwise, the characteristics of the holdfast and thallus are very similar in both species. The long straight trichospores in *P. timberleaense* resemble those in *Orphella catalaunica*, which are shorter, and the arrangement and structure of the cells forming the sporulating head in *Orphella* spp. is different compared to *Pteromaktron*.spp. (Lichtwardt, 2004).

Smittium adaiosporum sp. nov. R.T.William and Strongman (Figs. 31–35)
Mycobank MB 801379

Thalli with profuse branching (Fig. 31) emerging from a simple inconspicuous

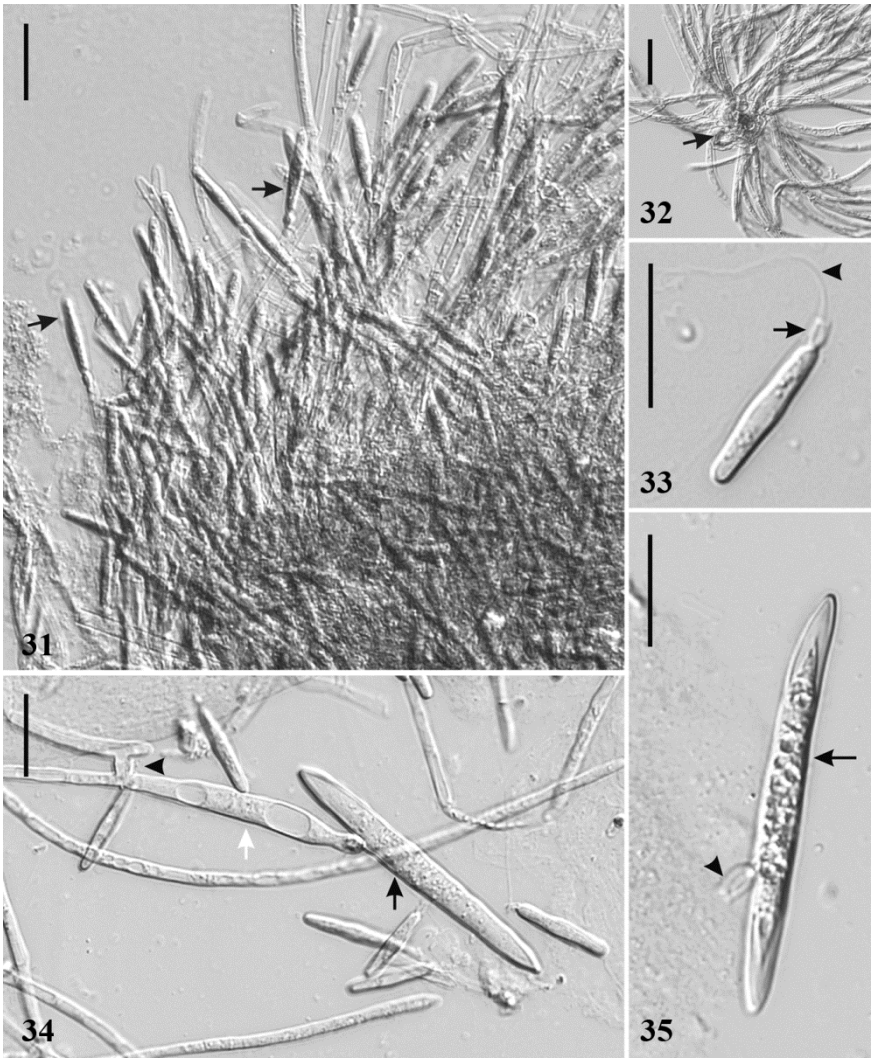
holdfast (Fig. 35), trichospores developing on the tips of long hyphae, up to 4 trichospores per fertile tip. Trichospores ellipsoidal with slight medial swelling 20–27 µm × 3–4 µm, with a conspicuous collar 3–6 µm × 1–3 µm and a single long appendage after detachment from the thallus (Fig. 33). Zygospores (Type II), 70–90 µm × 7–10 µm, attached submedially and obliquely to the zygosporophore (Fig. 34). Zygospores with a short collar 8–9 µm × 4–5 µm after release from the zygosporophore (Fig. 35). Attached to the hindgut lining of midge (Chironomidae) larvae.

ETYMOLOGY**:** From the Greek, *adaio* (abundant) and *spora* (spore), as this species produces an abundance of trichospores.

HOLOTYPE: Microscope slide LLLE-1 (DAOM 242391) with lactophenol cotton blue stained thalli and spores attached to hindgut lining. The midge host was collected at Long Lake (LLLE), Halifax, NS on 28 Sep 2010.

HABITAT: Collected at the Long Lake-stream interface site (LLLE), from the hindgut lining of midge (Chironomidae) larvae on 28 Sep 2010 (Appendix II).

COMMENTARY: The trichospore dimensions of *Smittium adaiosporum* (20–27 µm × 3–4 µm) overlap with over 15 different *Smittium* spp. (Lichtwardt, 2004).but none of these share zygospore characteristics with *Sm. adaiosporum*. For instance, *Smittium rarum* Lichtw. trichospores are 20–26 µm × 3–4 µm and have a collar comparable to that in *Sm. adaiosporum*, but the zygospores (100 µm × 10 µm) are outside the range for those described for *Sm. adaiosporum* (70–90 µm × 7–10 µm). *Smittium angustum* M.C. Williams & Lichtw. has trichospores that are smaller (17–26 µm × 2.3–2.8 µm) with a slightly shorter collar (Lichtwardt, 2004). Also, *Sm. angustum* thalli exhibit verticilliate



Figs. 31–35. *Smittium adaiosporum*. Fig. 31. Densely branching thalli with attached and released trichospores (arrows). Fig. 32. Multiple branches arising from holdfast (arrow). Fig. 33. Released trichospore with a collar (arrow) and single appendage (arrowhead). Fig. 34. Zygospore (black arrow) attached to zygosporophore (white arrow) arising from a conjugation tube (black arrowhead). Released trichospores also present. Fig. 35. Mature detached zygospore (arrow) with a short collar (arrowhead). Figures 31, 32, and 35 are from lactophenol cotton blue stained material; Figs. 33 and 34 are images from unstained specimens. Scale bars = 20 µm.

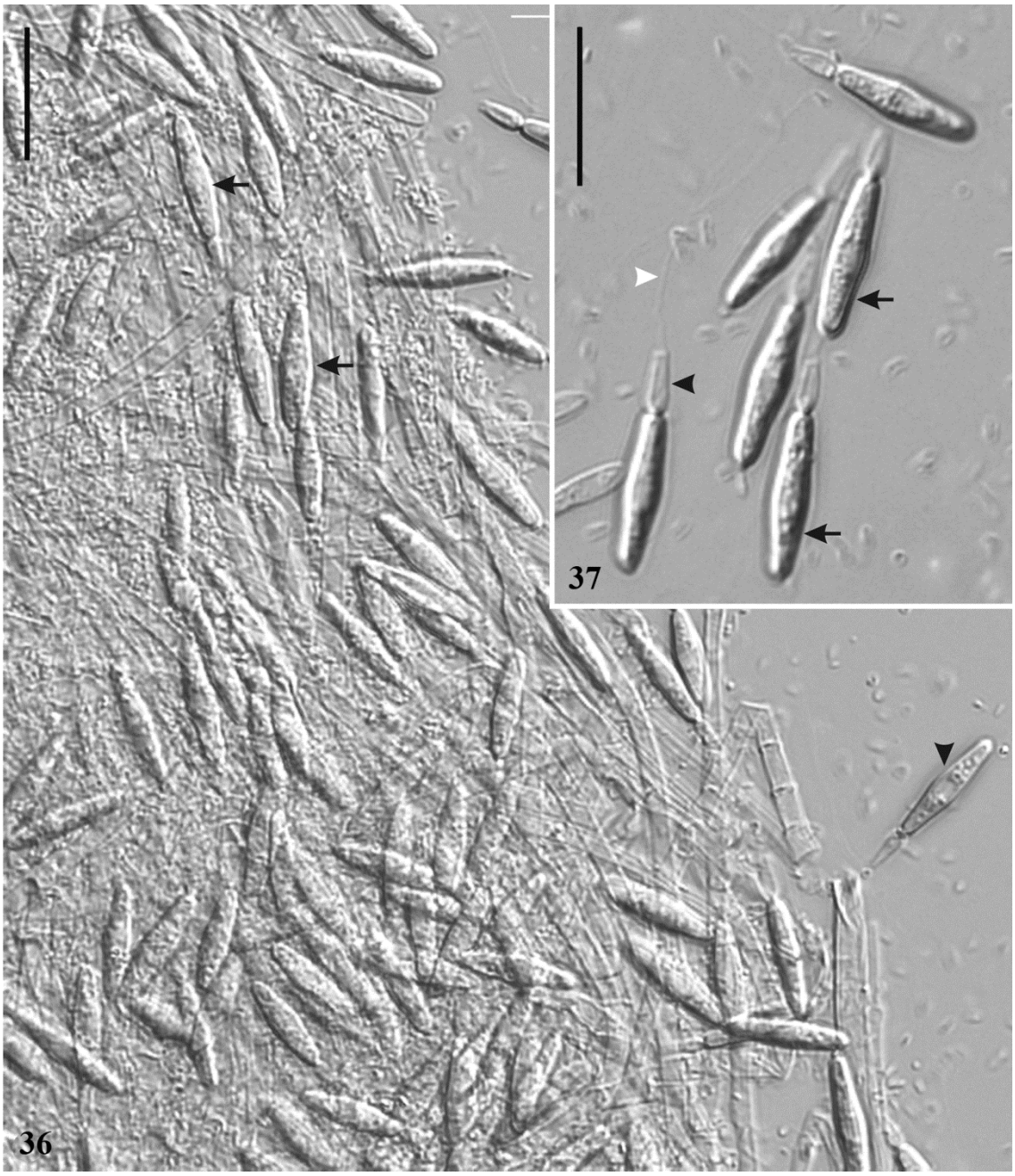
branching patterns not observed in *Sm. adaiosporum*. *Smittium gronthidium* Strongman & M.M. White tends to have a slightly broader trichospore range [15–31 (–40) µm × 3–5µm] while also exhibiting shorter and wider zygospore dimensions (61–74.5 µm × 7.5–8 collar 9–12) than *Sm. adaiosporum* (White and Strongman, 2012a).

Smittium gracilis L.G. Valle & Santam. and *Smittium urbanum* López Lastra, Mazzucchelli & Lichtw. trichospore measurements [(18–)20–26(–29) µm × 2–3.5 µm and 19–27 µm × 3–4 µm respectively] are well within the range described for *Sm. adaiosporum*, however, in both cases zygospores have not been described (Lichtwardt, 2004) so comparison of the sexual spore features with *Sm. adaiosporum* is not possible. The thallus branching patterns and holdfast structure in *Sm. gracilis* differ from *Sm. adaiosporum* and *Sm. gracilis* also has been reported to produce 2–8 generative cells on a fertile branch (Valle and Santamaria, 2004) in contrast to the 2–4 generative cells produced in *Sm. adaiosporum*. *Smittium urbanum* has a much less prominent collar and also exhibits compact, verticilliate branched thalli (Lichtwardt et al., 2000) not observed in *Sm. adaiosporum*.

Smittium ampliboja sp. nov. R.T.William and Strongman (Figs. 36–37)

Mycobank MB 801376

Densely branched thalli (Fig. 36), attached to hindgut lining of the host by an inconspicuous holdfast. Trichospores long ellipsoidal with a medial swelling, 16–23 µm × 3.5–5.5µm, a long campanulate collar 5–8 µm × 2–3 µm and single appendage (Fig. 37). No zygospores observed. Attached to the hindgut lining of midge (Chironomidae)



Figs. 36–37. *Smittium ampliboja*. Fig. 36. Heavily sporulating thallus with trichospores (arrows) and released trichospore (arrowhead). Fig. 37. Detached trichospores (arrows) each with a long campanulate collar (arrowhead) and single fine appendage (white arrowhead). All Figs. from unstained material. Scale bars = 20 µm.

and black fly (Simuliidae) larvae.

ETYMOLOGY**:** A combination of the Latin, *amplus* (large) and *boja* (collar) referring to the large collar on the trichospores.

HOLOTYPE: Thalli and trichospores, stained with lactophenol cotton blue, attached to the hindgut lining on microscope slide TLLO-3 (DAOM 242392). The midge host was collected at Mill Pond stream, Timberlea, NS (TLLO) on 3 Oct 2010.

HABITAT: Collected at Mill Pond Stream (TLLO) Timberlea, NS from the hindgut lining of midge (Chironomidae) and black fly (Simuliidae) larvae on 3 Oct 2010 (Appendix II).

COMMENTARY: *Smittium ampliboja* has an inconspicuous holdfast and trichospore dimensions (16–23 µm × 3.5–5.5 µm) that overlap to varying degrees with 17 other *Smittium* spp. but only three, *Smittium tipulidarum* M.C. Williams & Lichtw., *Smittium culicis* and *Smittium papillum* Strongman & M.M. White, have very similar characteristics overall (Lichtwardt et al., 2001a). The short collar length of *Sm. tipulidarum* (2–3.2 µm) distinguishes it from *Sm. ampliboja* (collar 5–8 µm) otherwise, the trichospores [(15–)17.5(–20) µm × (3.5–)4.5(–5) µm] are within the range. The broad trichospore size range of *Sm. culicis* (15–)20(–32) µm × (4–)6(–8) µm extends through the range for *Sm. ampliboja* but the ovoid shape of *Sm. culicis* trichospores is different from the long ellipsoidal shape described for trichospores of *Sm. ampliboja*. Another species, *Sm. papillum*, has similar trichospore (20–28 µm × 3–4.5 µm) and collar (6–12.5 µm) dimensions but it has a small papilla on the tip of the trichospore (White and Strongman, 2012*a*) not observed in *Sm. ampliboja*.

Smittium cryptancora sp. nov. R.T.William and Strongman (Figs. 38–40)

Mycobank MB 801380

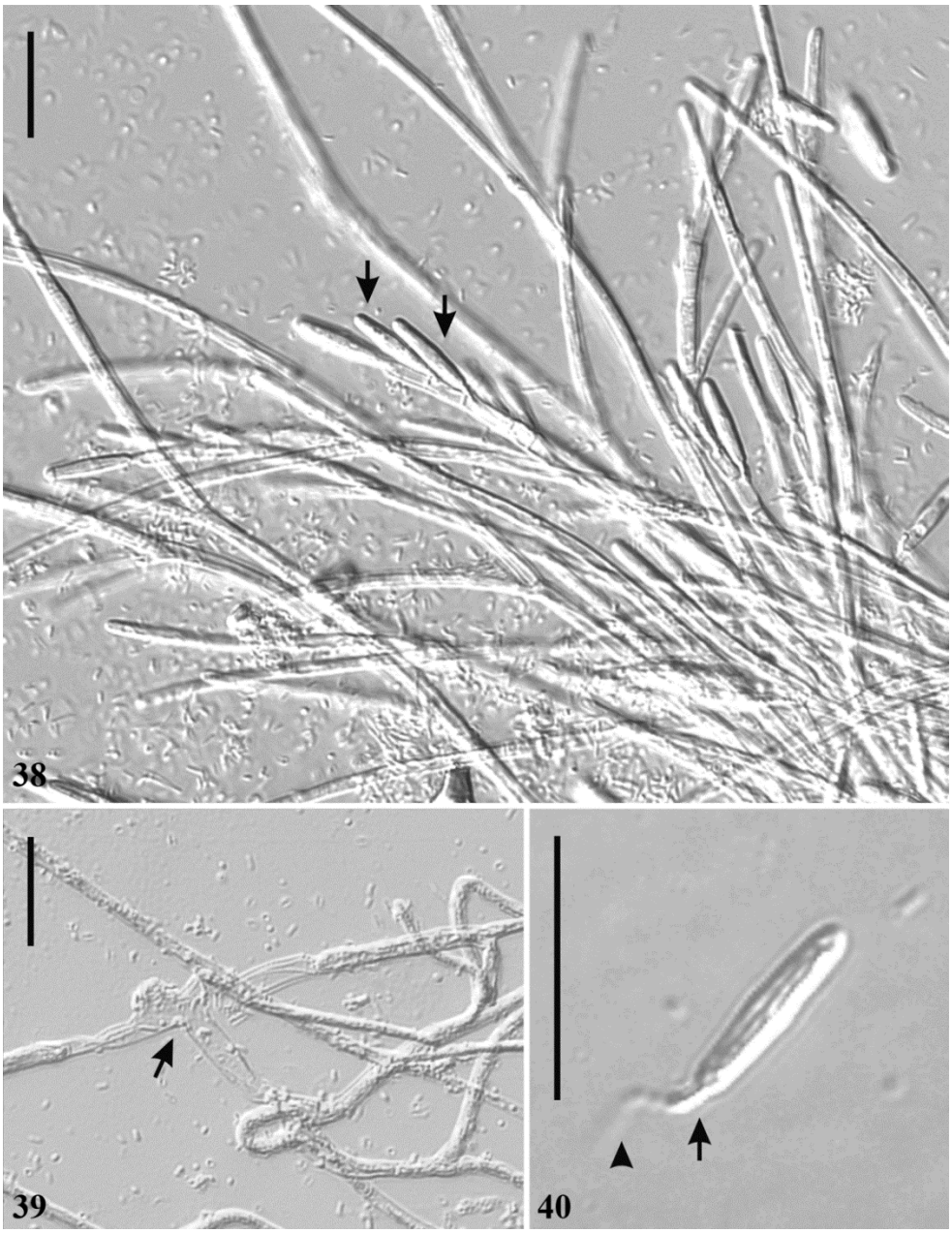
Thalli with sparse divergent branching (Fig. 38), attached to hindgut by a simple swollen basal holdfast cell (Fig. 7), producing 1–8 (typically 4) trichospores at the end of long fertile branches (Fig. 38). Trichospores ellipsoidal 15–19 µm × 2.5–4.5 µm, with a tightly appressed sleeve-like collar 2–4 µm × 1–2 µm (Fig. 40). No zygospores observed. Attached to the hindgut lining of midge (Chironomidae) larvae.

ETYMOLOGY**:** From the combination of the Greek, *crypto* (hidden) and *ancoralis* (anchor), in reference to the inconspicuous holdfast attaching the thalli to the host gut.

HOLOTYPE: Microscope slide GLLE-7 (DAOM 242393) with thalli and spores, stained with lactophenol cotton blue, attached to the hindgut lining. The midge host was collected at GLLE on 21 September 2011.

HABITAT: Collected at GLLE from the hindgut lining of midge (Chironomidae) larvae on 21 September 2011 (Table 4).

COMMENTARY: Based upon trichospore dimensions, *Smittium cryptancora* (15–19 _m × 2.5–4.5 µm; collar 2–4 µm × 1–2 µm) bears close resemblance to *Smittium insulare* Strongman (15–20 µm × 3–4 µm collar 2.5–4 µm), however, zygospores have not been observed in *Sm. cryptancora* preventing comparison to those of *Sm. insulare*. The simple, swollen holdfast structure described for *Sm. cryptancora* is absent in *Sm. insulare*. Trichospore arrangement on the thalli also separates the two as *Sm. insulare* typically produces few trichospores, generally one or two per fertile branch (Strongman, 2007),



Figs. 38–40. *Smittium cryptancora*. Fig. 38. Branched thalli with attached trichospores (arrows) at the end of a fertile branchlet. Fig. 39. Holdfast comprised of slightly swollen basal cell (arrow). Fig. 40. Trichospore with a sleeve-like collar (arrow) and appendage (arrowhead). Fig. 38 is from an unstained preparation. Specimens in Figs. 39 and 40 were stained with lactophenol cotton blue. Scale bars = 20 µm.

whereas *Sm. cryptancora* produces up to eight trichospores per fertile branch.

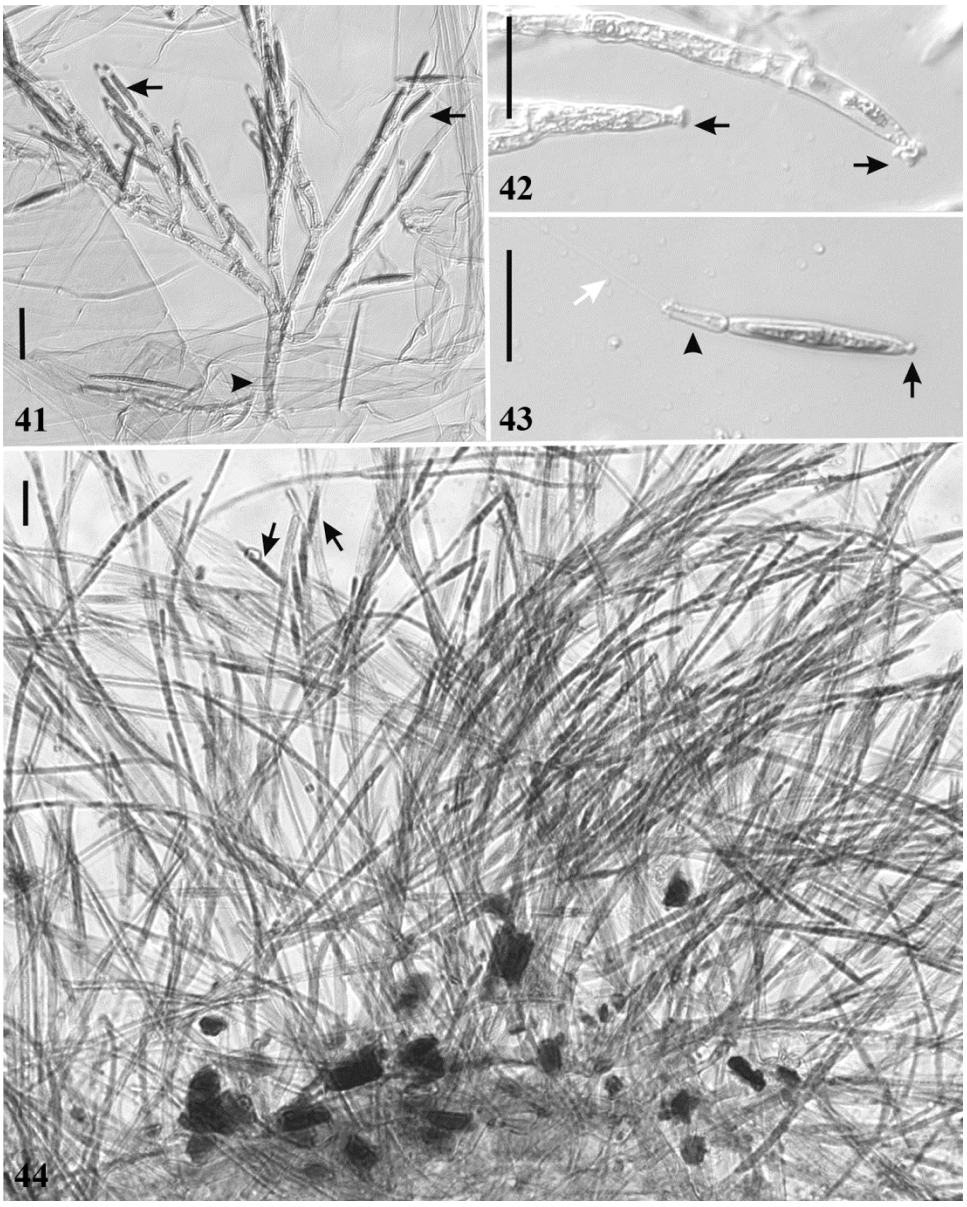
Smittium tipulidarum trichospores ((15–) 17.5(–20) µm × (3.5–)4.5(–5) µm with a collar 2–3.2 µm × ca. 2 µm) are also in the range for *Sm. cryptancora*, but tend to be wider and *Sm. tipulidarum* has up to 14 or more trichospores per fertile branch compared to a maximum of eight observed in *Sm. cryptancora*. The tipulid host of *Sm. tipulidarum* (Williams and Lichtwardt, 1987) differs from the chironomid host of *Sm. cryptancora* further distinguishing these two species.

Trichospore dimensions of *Smittium imitatum* Lichtw. & Arenas ((16–)19(–21) µm× (3–)5(–6) µm and a collar ~2 µm) resemble those described for *Sm. cryptancora*, but are wider, with a shorter collar. Similarly, *Smittium precipitiorum* M.M. White & Lichtw. trichospores (14–22 × 2.5–3 µm and collar 2–3.5 µm × 1.2–1.5 µm) match *Sm. cryptancora*, but are consistently narrower (Lichtwardt , 2004). The thallus branching is more compact in *Sm. precipitiorum* contrasting with the long sparsely branched thallus seen in *Sm. cryptancora*.

Smittium guttisporum sp. nov. R.T.William and Strongman (Figs. 41–44)

Mycobank MB 801372

A tapering basal cell (Fig. 41) anchors the immature thallus to the hindgut lining, atop a small amorphous globule (Fig. 42). Immature thalli branch from a central axis, developing trichospores on the ends of short terminal branches (Fig. 41). Mature thalli profusely branched (Fig. 44), developing trichospores on the tips of long fertile branches. Trichospores elongate-ellipsoidal, 26–34 µm × 4–6 µm, swollen medially, with a minute



Figs. 41– 44. *Smittium guttisporum*. Fig. 41. Thallus with trichospores (arrows) attached to hindgut via tapering basal cell (arrowhead). Fig. 42. Immature holdfast with small globule of adhesive (arrows). Fig. 43. A detached trichospore with a papilla at tip (arrow), collar (arrowhead), and fine appendage (white arrow). Fig. 44. Mature, profusely branching thallus with trichospores (arrows) developing on fertile tips. All figures are from fungi stained with lactophenol cotton blue. Scale bars = 20 µm

apical papilla, long, cylindrical to campanulate collar, 8–13 µm × 2–4 µm and a single fine appendage (Fig. 43). No zygospores observed. Attached to the hindgut lining of midge (Chironomidae) larvae.

ETYMOLOGY: Derived from the combination of the Latin, *gutta* (drop) and *sporum* (spore), in reference to the small papilla associated with the tip of the trichospores.

HOLOTYPE: Microscope slide GLLE–1 (DAOM 242394) containing thalli with trichospores, stained with lactophenol cotton blue, attached to hindgut linings. The midge host was collected at Governor's Lake, Timberlea, NS (GLLE) on 5 Oct 2010.

HABITAT: Collected at Governor's Lake (GLLE) from the hindgut lining of midge (Chironomidae) larvae on 5 Oct 2010 and 8 May 2011 (Appendix II).

COMMENTARY: The central defining feature of *Smittium guttisporum* is an apical papilla on the tip of the trichospores. This feature is found in only two other *Smittium* spp., *Smittium mucronatum* (Lichtwardt et al., 2001a) and *Smittium papillum* (White and Strongman, 2012a). *Smittium mucronatum* trichospores (33–37 µm × 6.5–7 µm) are both longer and wider than *Sm. guttisporum* (26–34 µm × 4–6 µm) and have a shorter collar (7.5– 9 µm). In contrast, *Sm. papillum* trichospores (20–28 µm × 3–4.5 µm) are shorter and thinner than *Sm. guttisporum*, but the collar length (6–12.5 µm) is about the same. *Smittium prostratum* L.G.Valle & Santam. has trichospores (26–30 µm × 3.5–4.5 µm) within the range for *Sm. guttisporum*, but lacks the distinctive apical papilla and has a shorter collar 4.5–5.5 µm (Valle and Santamaria, 2004).

Smittium insolitum sp. nov. R.T.William and Strongman (Figs. 45 –47)

Mycobank MB 801377

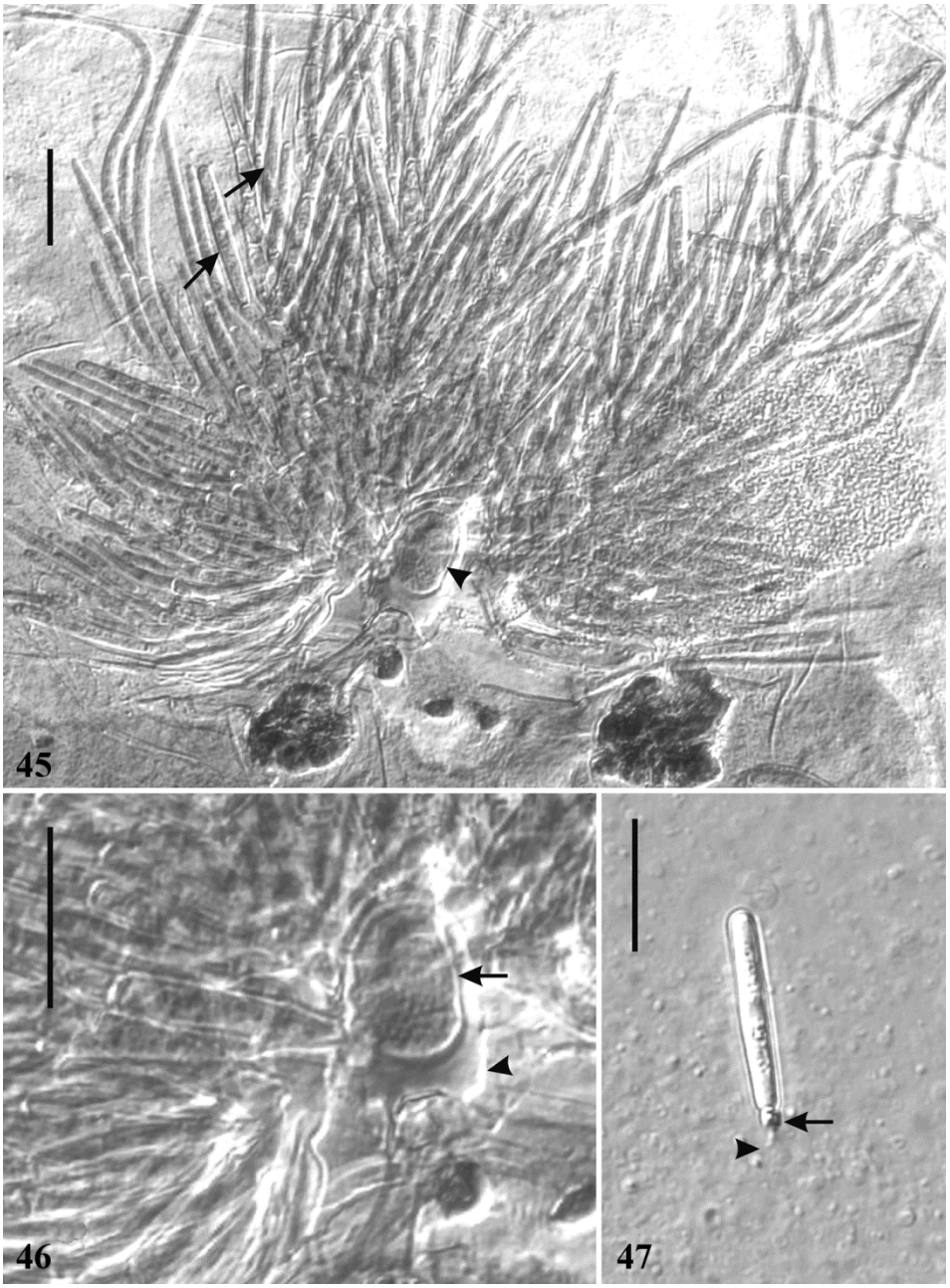
Compact, densely branched thallus (Fig. 45) emanating from a large swollen basal cell (16.5 × 11 µm) attached to the hindgut lining by a disk-like holdfast (Fig. 46) and secreted material. Trichospores cylindrical to slightly clavate 27–30 µm × 3–6 µm with a short wide collar 2–3 µm × 2–3 µm (Fig. 47). No zygospores observed. Attached to the hindgut lining of midge (Chironomidae) larvae.

ETYMOLOGY: From the Latin *insolitus* (unusual), referring to this species unusual holdfast structure.

HOLOTYPE: Microscope slide TLLO-15 (DAOM 242395) with thalli and spores attached to hindgut lining, stained with lactophenol cotton blue. The midge host was collected at Mill Pond Stream (TLLO) Timberlea, NS on 10 Mar 2011.

HABITAT: Collected at TLLO from the hindgut lining of midge (Chironomidae) larvae on 10 March 2010 midge (Chironomidae) larvae on 10 Mar 2010 (Appendix II).

COMMENTARY: Despite the narrow range in trichospore dimensions found in *Smittium insolitum* (27–30 µm × 3–6 µm), there are 14 species with trichospore morphology that are similar (Lichtwardt, 2004). The combination of trichospore measurements, collar dimensions, and holdfast characteristics sets *Sm. insolitum* apart from all other *Smittium* spp. Trichospores in *Smittium acutum* Lichtw. & Grigg [(21–)28–30 µm × 4–6 µm] and the collar length (Lichtwardt and Grigg 1998) are a close match but *Sm. acutum* lacks the large disk-like holdfast structure in *Sm. insolitum*. Trichospores of *Smittium fastigatum* Lichtw. & M.C. Williams are similar in length (23–32 µm × 3.5–4 µm) to those of *Sm.*



Figs.45–47. *Smittium insolitum*. Fig. 45. Overview of thallus including attached trichospores (arrows) and conspicuous holdfast (arrowhead). Fig. 46. Holdfast with swollen basal cell (arrow) and mucilage (arrowhead). Fig. 47. Released trichospore with short collar (arrow) and single fine appendage (arrowhead). Figures 45 and 46 are stained with lactophenol cotton blue. Figure 47 unstained specimen. Scale bars = 20 µm

insolitum, but they are typically thinner and are anchored to the host with a pointed basal cell (Lichtwardt et al., 2001a) much different than that seen in *Sm. insolitum*. The undifferentiated holdfast and trichospore collar length described for *Smittium prostratum* separate it from *Sm. insolitum* despite overlap in trichospore dimensions (26–30 µm × 3.5–4.5 µm) (Valle and Santamaria, 2004).

Smittium peculiare sp. nov. R.T.William and Strongman Figs. 48–52.

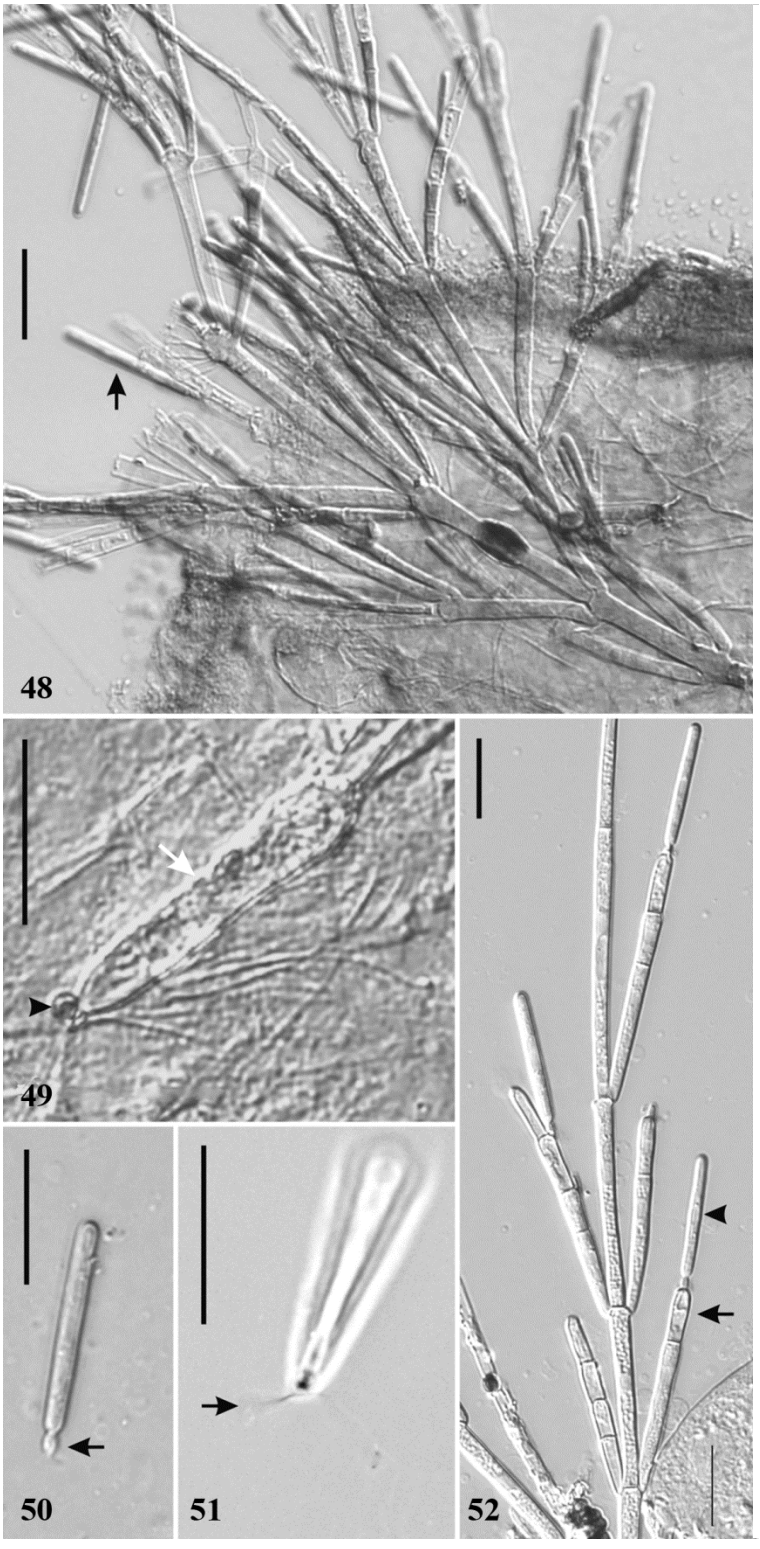
Mycobank MB 801382

Thalli verticilliate with a thick main axis and thick branches (Fig. 48). Distal hyphal branches producing 2–6 generative cells per fertile branch, shorter than the trichospores (Fig. 52). Holdfast structure of immature thalli (Fig. 49) is a thickened basal cell affixed to the hindgut lining by a small blob of amorphous material. Mature thalli have a thickened blunt basal cell. Trichospores cylindrical (26–)31(–37) µm × 2–4 µm with a conspicuous collar 2–3 µm × 1–2 µm and a single fine appendage (Figs. 50 and 51). No zygospores observed. Attached to the hindgut lining of midge (Chironomidae) larvae.

ETYMOLOGY: From the Latin *peculiaris* (peculiar) in reference to the perfectly cylindrical shape of the trichospores which is unusual for *Smittium* spp.

HOLOTYPE: Microscope slide LLLE-2 (DAOM 242396) with lactophenol cotton blue stained thalli and spores attached to the hindgut lining. The midge host was collected from Long Lake (LLLE) in Long Lake Provincial Park, Halifax, NS on 12 Jun 2011.

HABITAT: Collected from the hindgut lining of midge (Chironomidae) larvae at the



Figs. 48–52. ***Smittium peculiare*.** Fig. 48. Verticilliately branched thalli with thick main axis, thick branches and attached trichospore (arrow). Fig. 49. Thick basal cell (white arrow) affixed to hindgut by small blob of amorphous material (arrowhead). Fig. 50. Detached trichospore with a collar (arrow). Fig. 51. Released trichospore (unfocussed) showing its single appendage (arrow). Fig. 52. Developing trichospore (arrowhead) on the apex of the generative cell (arrow). Figs. 48 and 49 are from stained material, Figs. 50–52 are from unstained specimens. Scale bars = 20 µm.

Long Lake interface site (LLLE) on 12 Jun 2011 (Appendix II).

COMMENTARY: The distinctly cylindrical trichospores of *Smittium peculiare* distinguishes this species from most other *Smittium* spp., but it is compared to six species that have trichospore size and shape in the same range as *Sm. peculiare* [(26–)31(–37) µm × 2–4 µm]. Trichospore dimensions of *Smittium kansense* Lichtw. & Grigg, [(17–)23(–32) µm × (1.5–)2.7(–3.5) µm**],** *Smittium phytotelmatum* [(14–)17–25(–30) µm × 2–3 µm] and *Smittium simulii* Lichtw. [(16–)23(–30) µm × (3–)5(–7) µm] are all described as sub-cylindrical or cylindrical with a slight median swelling in the case of *Sm. simulii*, but also have average trichospore sizes much shorter than those described for ***Sm.*** *peculiare* (Lichtwardt, 2004). The trichospore length range for *Smittium gravimetallum* Lichtw., Ferrington & Hayford is broader [(20–)27–30(–45) µm × 2.5–3.5(–4.5) µm] but spans the range for *Smittium peculiare* (Ferrington et al., 2000). However, the trichospores of *Sm. gravimetallum* are described as ellipsoidal with a median bulge, not observed in *Sm. peculiare*. *Smittium georgense* Strongman has sub-cylindrical trichospores [34–45 µm × 5–7 µm] (Strongman, 2010) that are longer and wider than *Sm. peculiare* and the thallus of *Sm. georgense* features a knobby holdfast unlike the blunt holdfast of *Sm. peculiare*. Finally, *Smittium typhellum* possesses cylindrical trichospores (25–30 µm × 3–3.5 µm) with a slight median swelling produced on short generative cells (Manier and F. Coste, 1971). Both the trichospores and generative cells are shorter than those in *Sm. peculiare*.

Furculomyces, with two species described from Australia *Furculomyces*

boomerangus M.C. Williams & Lichtw., *Furculomyces westraliensis* M.C. Williams & Lichtw. and one from the Rocky Mountains in Colorado, USA, *Furculomyces septentrionalis* Misra, M.M. White & Lichtw., have strongly cylindrical trichospores and a single appendage like those described for *Sm. peculiare* (Lichtwardt, 2004). *Furculomyces boomerangus* thalli are anchored to the host gut via a horseshoe shaped holdfast not seen in *Sm. peculiare* and have trichospores [(20–)25(–30) µm × (3.0–)3.6(–4.2) µm] shorter and thicker than *Sm. peculiare* while the trichospores of *F. westraliensis* [36–40 µm × 2.3–3.0 µm] are much longer than *Sm. peculiare*. *Furculomyces septentrionalis* trichospores are described as subcylindrical, [(26–)33(–39) µm × (4.6–)4.9(–5.5) µm] that are the same length as those in *Sm. peculiare* but are almost twice as wide. Wishbone-shaped conjugation cells and characteristically bent zygospores are also found in *Furculomyces* spp. and are a defining feature of the genus (Lichtwardt, 2004) but no zygospores were seen in my collections of *Sm. peculiare* so no comparison of this feature was possible. Overall, the trichospores of *Sm. peculiare* are more like those of *Furculomyces* spp. than *Smittium* so the discovery of zygospores for *Sm. peculiare* is necessary to confidently assign it to a genus.

Smittium petilum sp. nov. R.T.William and Strongman (Figs. 53–56)

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Long sparsely branched thalli (Fig. 53) with weakly verticilliate (Fig. 54) branching from thickened central axes, attached to the hindgut lining by a conspicuously swollen, basal cell (Figs. 53 and 55). Subcylindrical to ellipsoidal trichospores (Fig. 56)

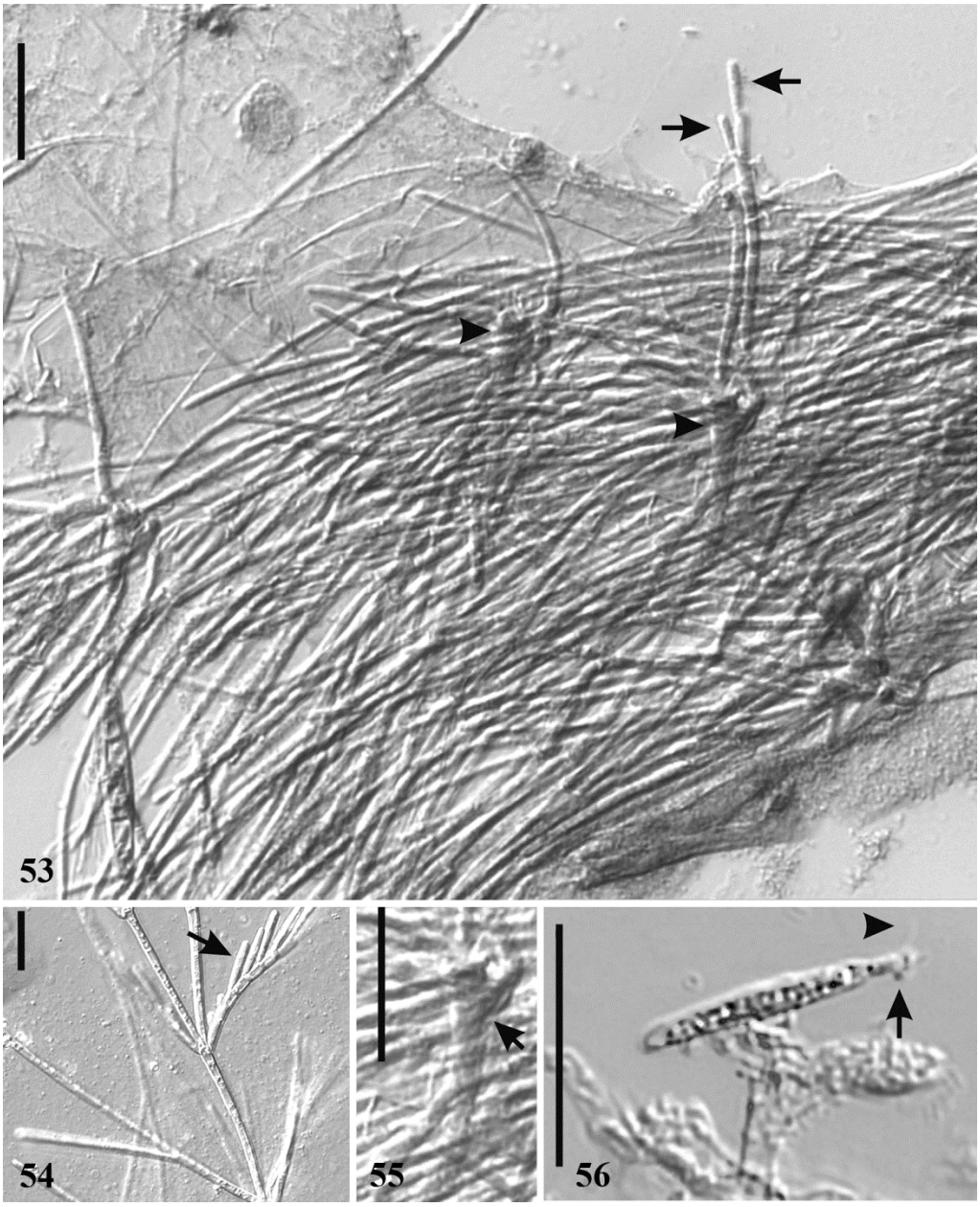
17–22 µm × 2–4 µm, with a small cylindrical collar 2–4 µm, produced in clusters of up to 8 on the end of fertile branchlets (Fig. 54). No zygospores observed. Attached to the hindgut lining of midge (Chironomidae) larvae.

ETYMOLOGY: From the Latin, *petilus* (thin), alluding to the narrow trichospores described for this species.

HOLOTYPE: Microscope slide LLLE-2 (DAOM 242397) with thalli and spores, stained with lactophenol cotton blue, attached to the hindgut lining. The midge host was collected at Long Lake (LLLE), Halifax, NS on 12 Sep 2010.

HABITAT: Collected at LLLE on 12 Sep 2010, TLLO on 3 May 2011 and GLLE on 30 Aug 2011 from the hindgut lining of midge (Chironomidae) and black fly (Simuliidae) larvae (Appendix II).

COMMENTARY *Smittium petilum* has trichospore dimensions (17–22 µm × 2–4 µm, collar 2–4 µm) similar to five other species from this genus (*Smittium angustum*, *Smittium fasciculatum* Lichtw., *Smittium hecatei* L.G. Valle & Santam., *Smittium radiculans* Strongman & M.M. White, and *Smittium insulare*) but is distinguished from four of the five based on the conspicuous holdfast in *Sm. petilum* comparable only to one species, *Sm. radiculans* (Lichtwardt, 2004). Trichospores (15–23 µm × 2–4 µm; collar 1–3 µm) of *Sm. radiculans* are indistinguishable from *Sm. petilum*, but *Sm. radiculans* has a unique mature holdfast constructed of three or more fingerlike projections surrounded by mucilage (White and Strongman, 2012) while *Sm. petilum* has a swollen, basal cell holdfast structure. *Smittium angustum* produces trichospores (17–26 µm × 2.3–2.8µm) Trichospore with a collar (arrow) and appendage (arrowhead). Figs 53, 55, and 56 are



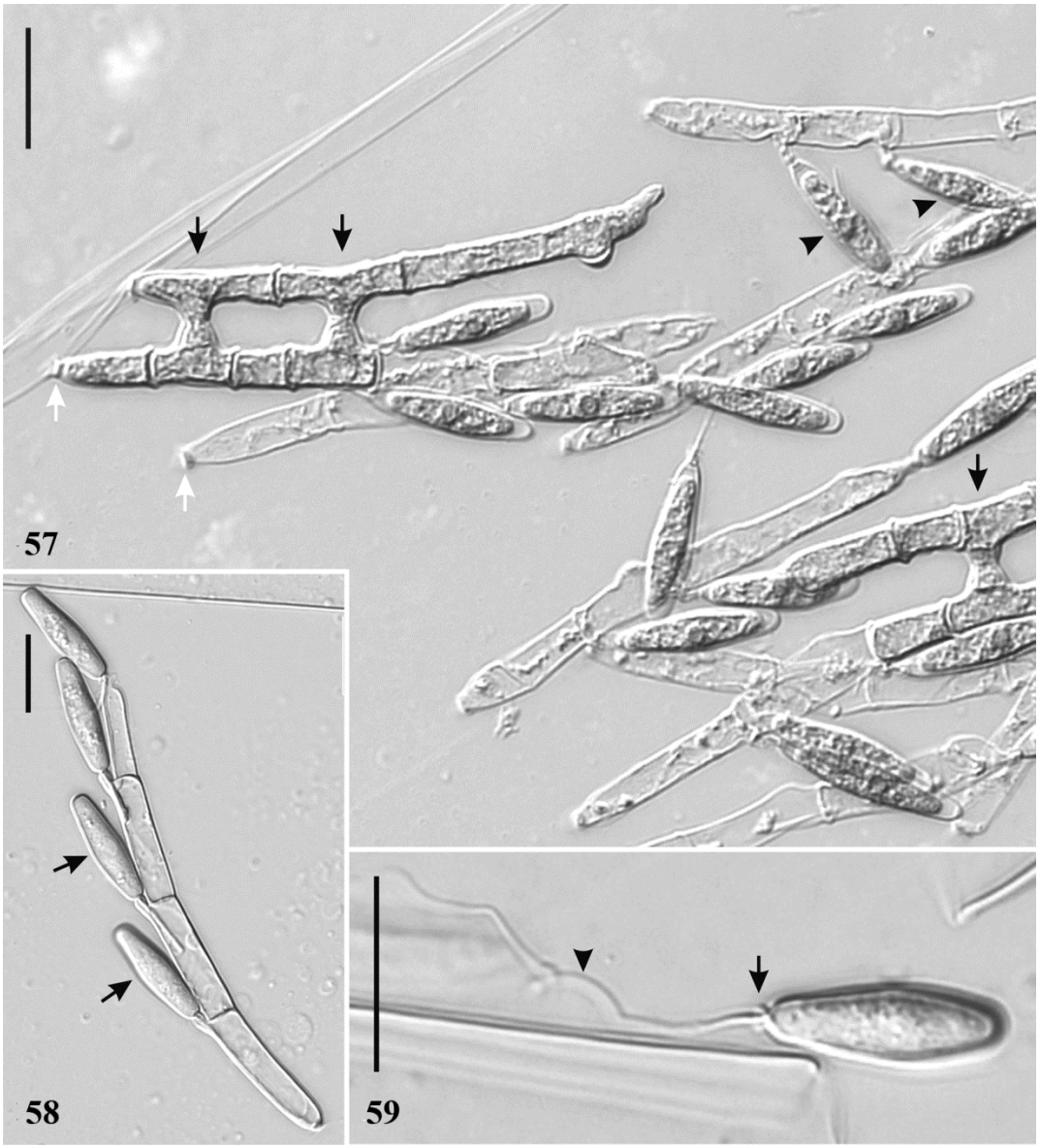
Figs. 53–56. *Smittium petilum*. Fig. 53. Profusely branched thalli with developing trichospores (arrows) and holdfasts with swollen basal cells (arrowheads). Fig. 54. Thallus fragment demonstrating weakly verticilliate branching with attached trichospores (arrow**).** Fig. 55. Holdfast (arrow) structure comprised of swollen basal cell. Fig. 56. from stained specimens and Fig. 54 is unstained. Scale bars = 20 µm.

that are both slightly longer and narrower than *Sm. petilum* resulting in a much more elongated trichospore shape. *Smittium angustum* also displays profuse, radiating, strongly verticilliate thalli (Lichtwardt et al. 2001a) not observed in *Sm. petilum*. Trichospore dimensions of *Sm. fasciculatum* are much longer [18–24(–29) µm × 2–3.5 µm] (Lichtwardt, 2004) resulting in a more elongated trichospore shape with a much less prominent collar (<1µm) than that described for *Sm. petilum* (2–4 µm). The dimorphic species *Sm. hecatei* produces small trichospores (14.5–24 µm × 2.5–3.5 µm) with a collar (1.5–2.5 µm) (Valle and Santamaria, 2004) that have a broader range, and with a less conspicuous collar than that described for *Sm. petilum*. No evidence of dimorphism was observed in *Sm. petilum* and the holdfast is different than the "leg-like" mature basal cell holdfast in *Sm. hecatai* (Valle and Santamaria, 2004). *Smittium insulare* has trichospore dimensions (15–20 µm × 3–4 µm) and a collar (2.5–4µm) that is very close to *Sm. petilum*, but has a holdfast described as inconspicuous and undifferentiated from the thallus (Strongman, 2007), contrasting with the prominently swollen, basal cell of *Sm. petilum*.

Stachylina abundans sp. nov. R.T.William and Strongman (Figs. 57–59)

Mycobank MB 801383

Thalli short, 84–156(–197) µm × 5–10 µm (Fig. 57), producing 2–4 (rarely 8) trichospores (Fig. 58) attached to peritrophic matrix by a tapering basal cell atop a small amount of amorphous adhesive material (Fig. 57). Trichospores ellipsoidal 20–27 µm × 3–4 µm with a collar 3–6 µm × 1–3 µm (Fig. 59). No zygospores observed. Attached to



Figs. 57–59. *Stachylina abundans*. Fig. 57. Conjugation tubes (arrows) forming between thalli anchored to peritrophic matrix by tapering basal cell atop small amount of amorphous material (white arrow) and a released trichospore (arrowhead). Fig. 58. Thallus with four attached trichospores (arrows). Fig. 59. Released trichospore with short collar (arrow) and single appendage (arrowhead). Fig. 57 is from a stained specimen. Figs. 58 and 59 are unstained. Scale bars = 20 µm.

the peritrophic matrix of midge (Chironomidae) larvae.

ETYMOLOGY: This species name derives from the Latin, *abundatus* (abundance), in reference to the high abundance of this species in collections.

HOLOTYPE: Microscope slide LLLO-2 (DAOM 242398) with thalli and spores, stained with lactophenol cotton blue, attached to peritrophic matrix. The midge host was collected at Long Lake stream (LLLO), Halifax, NS on 11 Aug 2010.

HABITAT: Collected from peritrophic matrix of chironomid larvae at LLLO 11 Aug, 7 Sep, 2010, 21 Aug 2011; TLLO 17 Aug, 21 Sep, 24 Oct, and 15 Nov 2010, 17 Sept, 15 Jun and 11 Dec 2011; GLLE 24 Aug 2010, 10 Aug, 30 Aug, 21 Sep, and 11 Nov 2011; LLLE 12 Sep 2010; SPLO 13 Jul 2011 (Appendix II).

COMMENTARY: The small trichospore size of *Stachylina abundans* (20–27 µm × 3–4 µm) is shared by only *Stachylina queenslandiae* Lichtw. (20–30 µm × 8–9 µm), *Stachylina gravicaudata* Siri, M.M. White & Lichtw., (25–31 µm × 4–5 µm) and *Stachylina manicata* M.C. Williams & Lichtw. (17.5–20 µm × 4.5 µm). *Stachylina queenslandiae* trichospores are wider and lack a collar whereas *St. gravicaudata* bears trichospores that are slightly longer and are borne on much longer thalli and *St. manicata* produces shorter trichospores that have an inconspicuous collar (Lichtwardt, 2004). *Stachylina nana* Lichtw. thalli typically produce 2-4 trichospores [(25-)30(-40) x (7-)8.5(-10) µm] so are much larger and lack a collar, distinguishing it from *St. abundans*.

Stachylina extensiva sp. nov. R.T.William and Strongman (Figs. 60–62)

Mycobank MB 801384

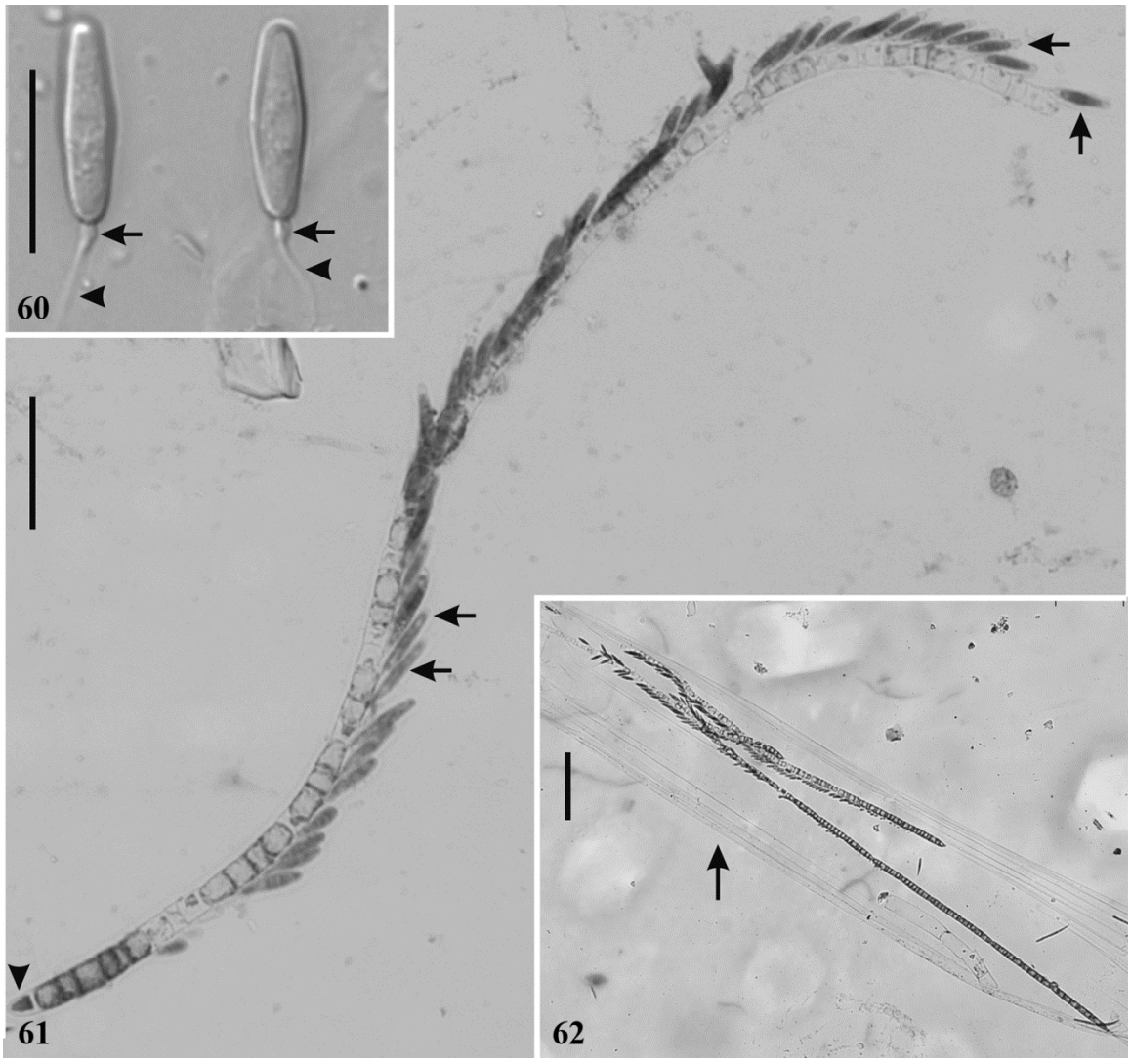
Thalli long, 500–800 µm × 8–11 µm, with a basal cell tapering to a point, producing as many as 60 trichospores (Fig. 61 and 62). Basal cell attached to peritrophic matrix by a small amount of amorphous material. Trichospores ellipsoidal 20–25 µm × 4–5.5 µm, with small collar 2–3 µm × 1.5–2 µm and one thin appendage (Fig. 60). Generative cells on thallus less than half as long as trichospore length (Fig. 61). No zygospores found. Attached to the peritrophic matrix of midge (Chironomidae) larvae.

ETYMOLOGY: From the Latin, *extensivus* (long), in reference to the long thalli of this species.

HOLOTYPE: Thalli and trichospores stained with lactophenol cotton blue attached to peritrophic matrix, on slide LLLE-8 (DAOM 242399). The midge host was collected at Long Lake (LLLE), Halifax, NS on 3 July 2011.

HABITAT: Collected from peritrophic matrix of chironomid larvae at the Long lake interface site (LLLE) on one date, 3 July 2011 (Appendix II).

COMMENTARY: *Stachylina extensiva* is distinguished from other species in the genus by virtue of the very long thalli (500–800 µm) typical for the species. Based solely on trichospore dimensions, 9–10 other species overlap with *St. extensiva*, however, there are no other described species in the genus with this combination of extreme thallus length, and trichospore shape, size, and collar length. *Stachylina magna* has thalli (400–900 µm) as long as *St. extensiva*, but the trichospores are longer and wider [(30–)56(–80) µm × (6.5–)11(–15) µm] and are without a collar. *Stachylina prolifica* Lichtw., Kobayasi &



Figs. 60–62. *Stachylina extensiva.* Fig. 60. Trichospores with collar (arrows) and single appendage (arrowheads). Fig. 61. Fertile thallus with many attached trichospores (arrows) and tapering basal cell (arrowhead) holdfast. Fig. 62. Typical long thalli inside peritrophic matrix (arrow) dissected from the midge host. Figs. 61 and 62 are from lactophenol cotton blue stained material, Fig. 60 unstained. Fig. 60, scale bar = 20 µm; Figs. 61 and 62, scale bars = 50 µm.

Indoh thalli measure up to 450 µm but the trichospores are considerably larger (22–36 µm × 5–5.5 µm) and also lack a collar (Lichtwardt et al., 1987). *Stachylina tianensis* J. Wang, S. Q. Xu & Strongman thalli can reach 350 um, but the trichospores are much longer and wider and the holdfast penetrates the peritrophic matrix (Wang et al., 2010), while the holdfast in *St. extensiva* does not.

Stachylina infrequens sp. nov. R.T.William and Strongman (Figs. 63–64)

Mycobank MB 801385

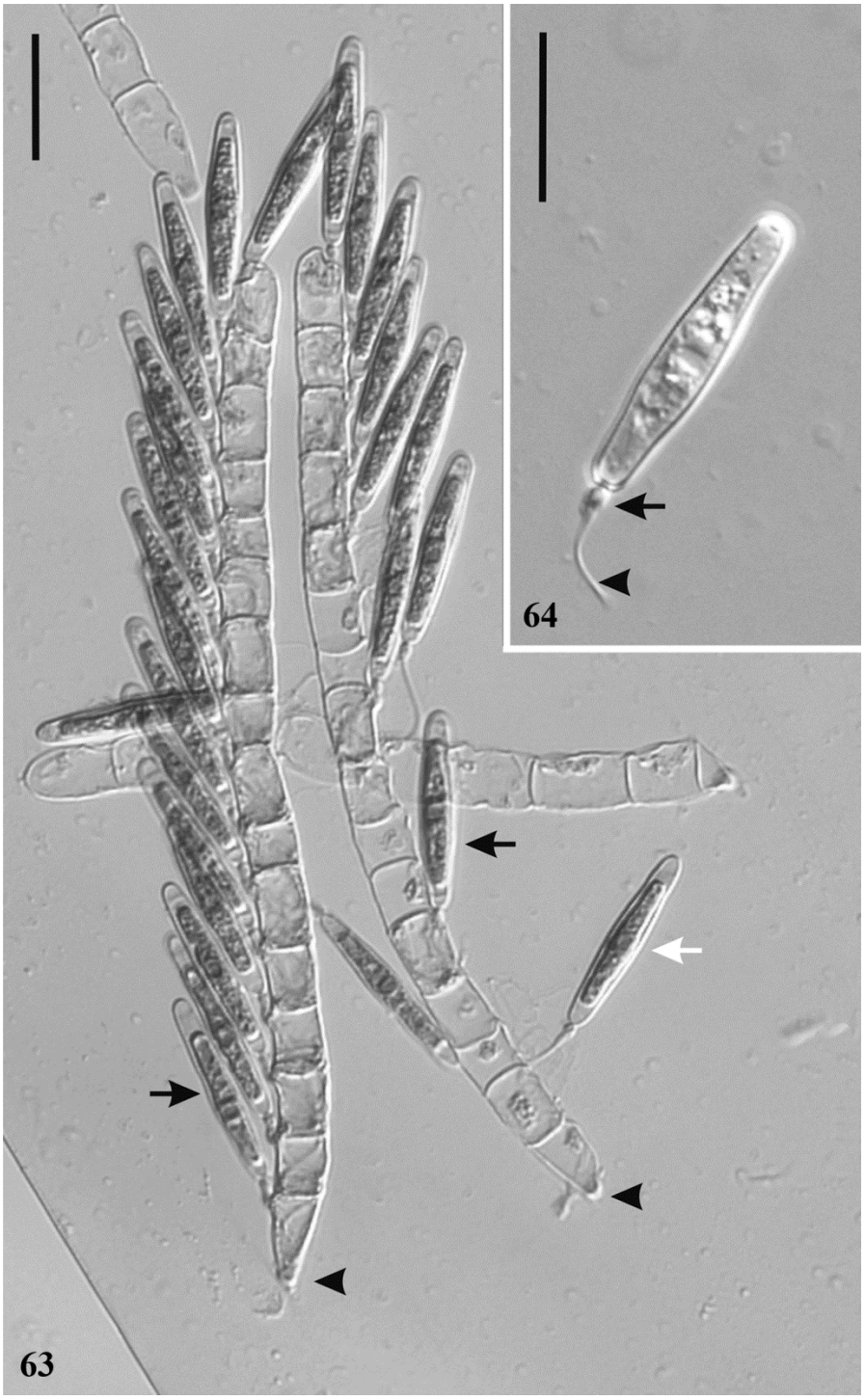
Thalli producing up to 16 trichospores, tapered basal cell attached to peritrophic matrix by a small amount of adhesive (Fig. 63). Trichospores elongate-ellipsoidal 30–36 µm × 5–7 µm with small knot-like collar 3–4 µm × 2 µm (Fig. 64). No zygospores observed. Attached to the peritrophic matrix of midge (Chironomidae) larvae.

ETYMOLOGY: From the Latin, *infrequens* (not frequent) referring to the scarcity of this species in the collections.

HOLOTYPE: Microscope slide LLLE-4 (DAOM 242400) with thalli and spores, attached to the peritrophic matrix. The specimen is stained with lactophenol cotton blue. The midge host was collected at Long Lake (LLLE), Halifax, NS on 1 May 2011.

HABITAT: Collected on the peritrophic matrix of chironomid larvae at the Long Lake interface site (LLLE) on 1 May 2011 and 24 Jul 2011 (Appendix II).

COMMENTARY: Three other *Stachylina* species exhibit similar trichospore features like *Stachylina infrequens*. *Stachylina platensis* López Lastra, Lichtw. & Ferrington (30–37 µm × 8–9 µm) and *Stachylina robusta* Lichtw. & M.C. Williams (30–37 µm × 8–9 µm)



Figs. 63 and 64. *Stachylina infrequens*. Fig. 63. Thalli with trichospores (arrows) on short generative cells, anchored to the peritrophic matrix via a tapered basal cell holdfast (arrowheads). One trichospore is released (white arrow). Fig. 64. Detached trichospore with short, sleeve-like collar (arrow) and single appendage (arrowhead). Fig. 63 from stained material, Fig. 64 is unstained. Scale bars = 20µm

both produce trichospores similar in length to *St. infrequens* (30–36 µm × 5–7 µm), but are wider and lack collars (Lichtwardt, 2004). *Stachylina paludosa* Lichtw. trichospores (31–40 µm × 6–8 µm) are on average wider than *St. infrequens* and also have a shorter collar. To date, *St. paludosa* has only been described from hosts in water trapped in tropical plants (phytotelm) in Costa Rica (Lichtwardt, 1994), which is a unique habitat.

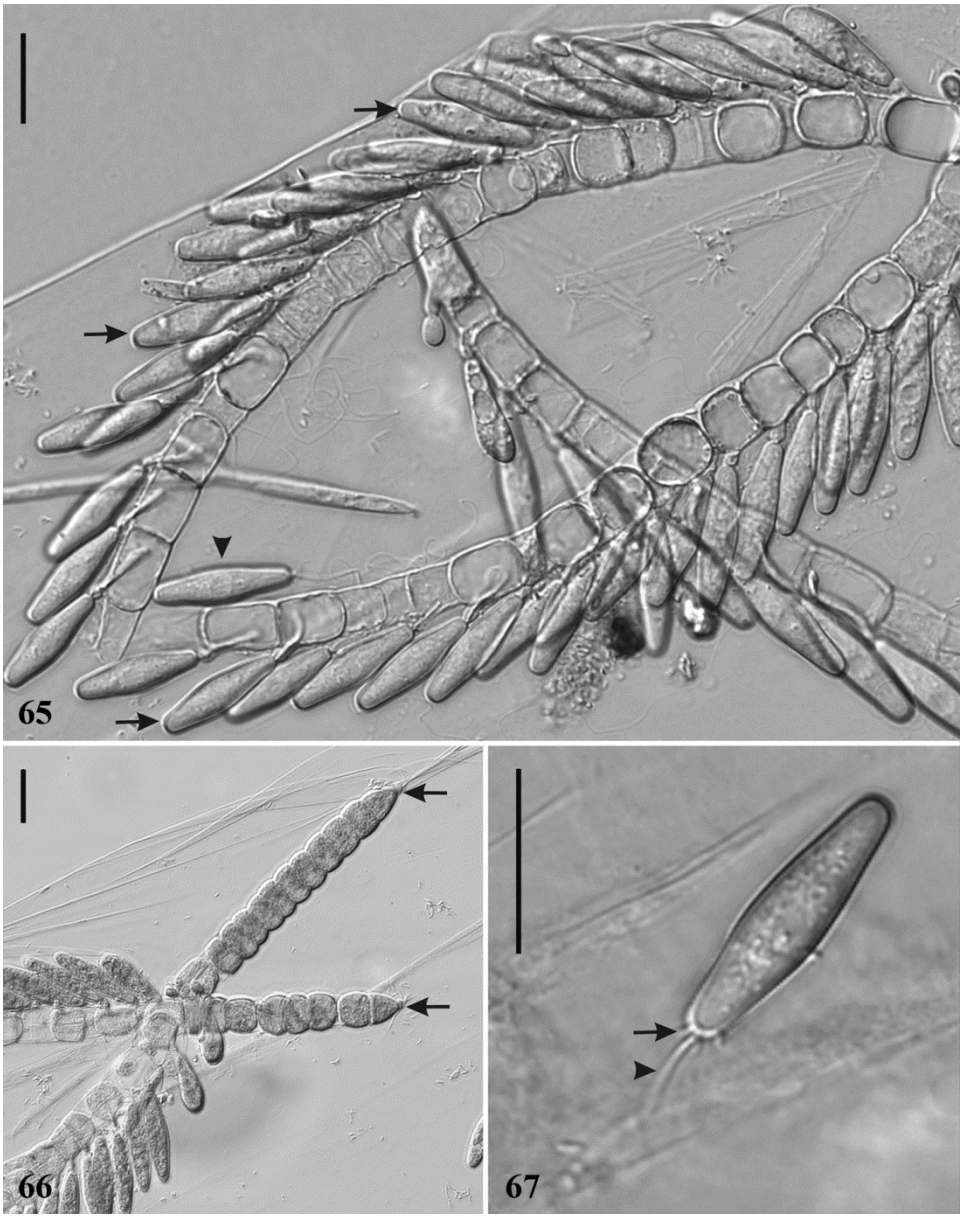
The collar of *St. infrequens* is described as "knot-like" because it looks rounded and somewhat amorphous (Fig. 25). *Spartiella barbata* Tuzet & Manier ex Manier and *Pteromaktron protrudens* both are described as having a "knob" on the appendage near the base of the trichospore and some *Legeriosimilis* spp. have a knob near the end of the appendages (Lichtwardt, 2004). The morphology and the consistent tight adherence of the knot-like collar to the base of the trichospore in *St. infrequens* differentiates this collar from the knobs on the appendages of these other species.

Stachylina serpula sp. nov. R.T.William and Strongman (Figs. 65 – 67)

Mycobank MB 801373

Thalli long, 300–450 µm × 8–16 µm (Fig. 65), often with more than 20 flattened generative cells with some swollen and rounded (Figs. 65 and 66). Basal cell tapering to a small blunt holdfast (Fig. 66). Trichospores (Fig. 67) tapered at both ends with sub-medial swelling, 29–38 µm × 6–9 µm, very short collar 1.5–2.5 × 1.5–2.5 µm, and one fine appendage. No zygospores observed. Attached to the peritrophic matrix of midge (Chironomidae) larvae.

ETYMOLOGY: From the Latin, *serpula* (little snake), in reference to the snake-like



Figs. 65–67. *Stachylina serpula*. Fig. 65. Prolific sporulating thalli, each producing more than 24 trichospores (arrows). One trichospore (arrowhead) is detached. Fig. 66. Tapering holdfast attached to chironomid peritrophic matrix by a small globule of adhesive (arrows). Fig. 67. Trichospore with short collar (arrow) and single fine appendage (arrowhead). Figs. 65 and 67 are from lactophenol cotton blue stained material. Fig. 66 is from an unstained specimen. Scale bars = 20 µm.

appearance of the thallus.

HOLOTYPE: Microscope slide GLLE-2 (DAOM 242401) with thalli bearing trichospores, attached to the peritrophic matrix. The holotype is stained with lactophenol cotton blue. The midge host was collected at Governor's Lake, Timberlea, NS (GLLE) on 30 Aug 2011.

HABITAT: Collected from the midgut lining of chironomid larvae at Governor's Lake (GLLE) on 30 Aug 2011 and 21 Sep 2011 (Appendix II).

COMMENTARY: *Stachylina serpula* is characterized by having long thalli (300–450 µm × 8–16 µm) which is a feature seen in only five other species of *Stachylina* (Lichtwardt et al., 2001a; William and Strongman, 2013a; 2013b). *Stachylina prolifica* trichospores are generally smaller (22–36 µm × 5–5.5 µm) and lack a collar. *Stachylina magna* has trichospores [(30–)56(–80) µm × (6.5–)11(–15) µm] that are larger than *St. serpula* and lack a collar. *Stachylina tianensis* possesses thalli about the same length but with a penetrating holdfast and much larger trichospores (55–90 µm × 7–10 µm) than *St. serpula*. Two other species described in my NS collections, *Stachylina tanysoma* with trichospores 23–30 µm × 4.5–6 µm and *Stachylina extensiva* (20–25 µm × 4–5.5 µm) both possess long thalli but produce smaller trichospores (William and Strongman, 2013*a*; 2013*b*).

Based on trichospore size alone, *St. serpula* overlaps with five other *Stachylina* species (Lichtwardt, 2004), but none of these species have thalli as long as *St. serpula*. *Stachylina platensis* has trichospores similar in size 25–36 µm × 5–7.8 µm, but are generally smaller and thinner than those in *St. serpula***.** *Stachylina paucispora* Lichtw.

and *Stachylina robusta* have trichospore dimensions in the same range (29–40 µm × 6–8 µm and 30–37 × 8–9 µm respectively) as *St. serpula*, but both lack collars on the trichospores (Lichtwardt, 2004). *Stachylina euthena* trichospores measure 25–35 µm × 7–8 µm, but are fusiform with a more prominent collar (Lichtwardt, 2004) than *St. serpula*. *Stachylina paludosa* also has similarly sized trichospores (31–40 µm × 6–8 µm) but the thallus typically has only 8 generative cells (Lichtwardt, 2004), whereas *St. serpula* usually has more than 20 cells per thallus.

Stachylina somnisimilis sp. nov. R.T.William and Strongman Figs. 68–69.

Mycobank MB 801386

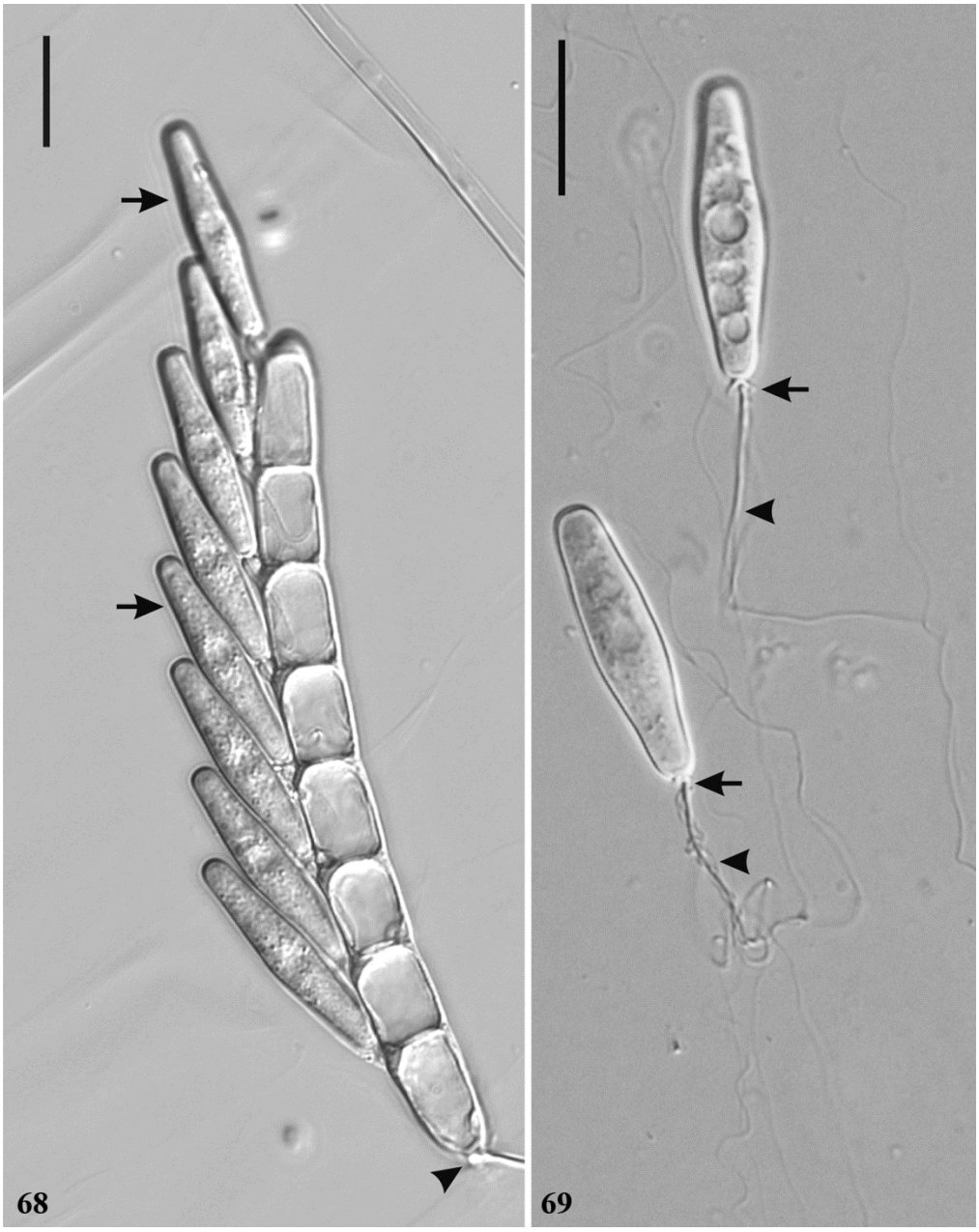
Thalli 90–140 µm × 6–11 µm, attached to peritrophic matrix via rounded basal cell and secreted material (Fig. 68). Trichospores ellipsoidal with a slight median bulge 29–49 µm × 6–11µm, bearing a short collar 2–3 µm × 3–4 µm and single long appendage when detached from the thallus (Fig. 69). No zygospores observed. Attached to the peritrophic matrix of midge (Chironomidae) larvae.

ETYMOLOGY: From the Latin, *somnium* (dream) and *similis* (like). Name chosen because of how it sounded when spoken; it had a dream-like quality.

HOLOTYPE: Microscope slide GLLE-4 (DAOM 242402) with lactophenol cotton blue stained thalli and spores attached to peritrophic matrix. The midge host was collected at Governor's Lake (GLLE), Timberlea, NS on 30 Aug 2011.

HABITAT: Collected from the peritrophic matrix of chironomid larvae at GLLE on 19 Jun 2011, 30 Aug 2011, 13 Oct 2011 and from LLLO on 22 Oct 2011 (Appendix II).

COMMENTARY: There are six *Stachylina* spp., *Stachylina penetralis* Lichtw.,



Figs. 68 and 69. *Stachylina somnisimilis***.** Fig. 68. Thallus comprised of eight generative cells and attached trichospores (arrows), anchored to the peritrophic matrix by a blunt holdfast (arrowhead). Fig. 69. Released trichospores showing collar (arrows) and single unfurling appendage on each one (arrowheads). Figs. from unstained material. Scale bars = 20µm.

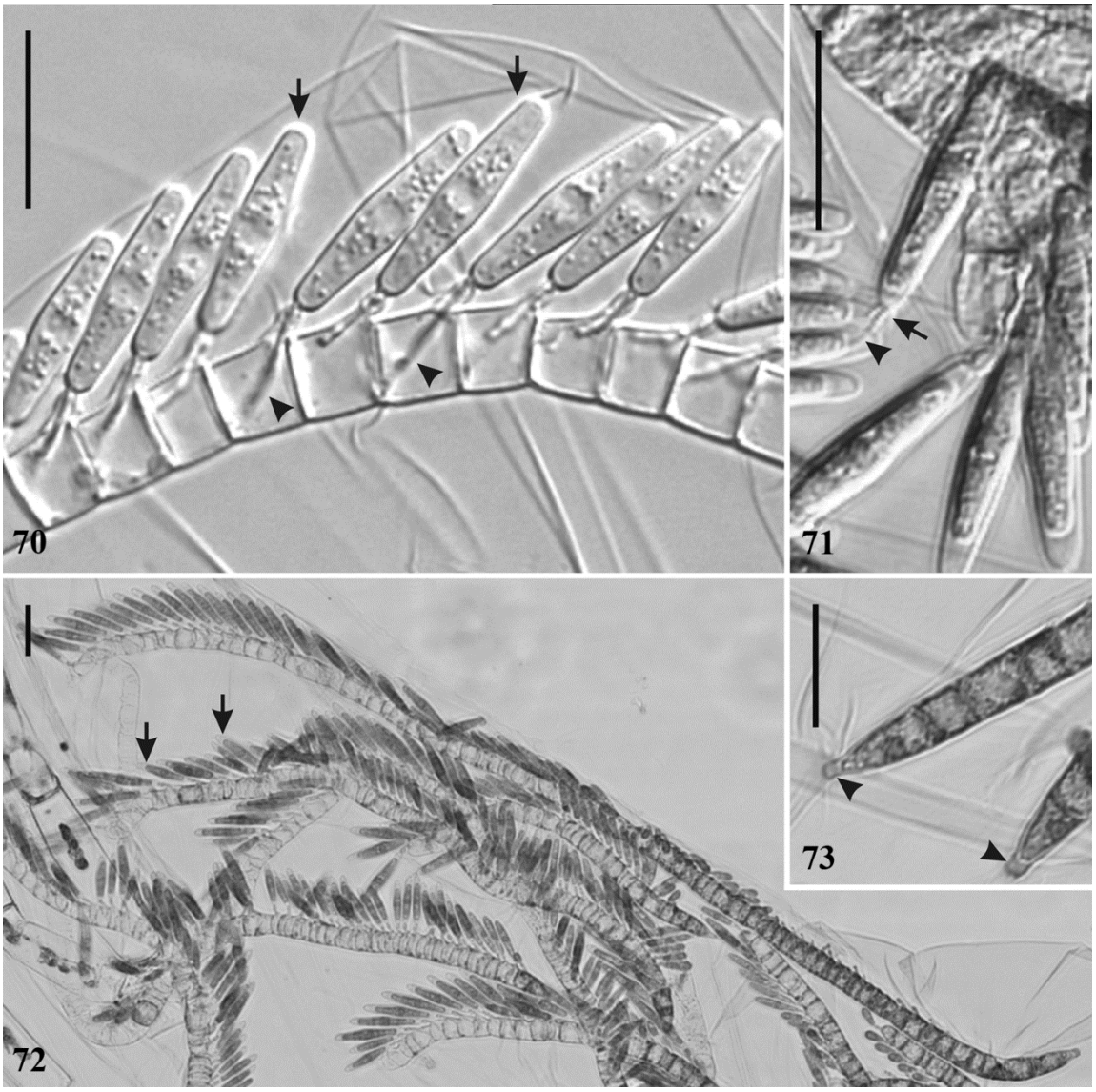
Stachylina jujuyensis Mazzucchelli, López Lastra & Lichtw., *Stachylina litoralis* Lichtw., M.M. White & Colbo , *St. robusta, St. paludosa,* and *St. platensis,* that produce similarly sized trichospores (Lichtwardt, 2004) as in *St. somnisimilis*. *Stachylina penetralis* has an almost identical trichospore range (30–50 µm × 8–12 µm) as *St. somnisimilis* (29–49 µm × 6–11µm) but *St. penetralis* trichospores lack a collar and it has a holdfast that penetrates the peritrophic matrix (Lichtwardt, 2004) which is not the case in *St. somnisimilis*. *St. jujuyensis*, *St*. *litoralis*, and *St*. *robusta* all have trichospores (30–37 µm × 8–9 µm, 39–47 µm ×10–12 µm, and 30–37 µm × 8–9 µm, respectively) that overlap somewhat with the size range for *St*. *somnisimilis* trichospores, but all lack collars (Lichtwardt, 2004). *Stachylina paludosa* and *St. platensis* both have short collars, but also produce trichospores (31–40 µm × 6–8 µm and 25–36 µm × 5–7.8 µm, respectfully) which are generally shorter and narrower than *St. somnisimilis* (Lichtwardt, 2004; Lichtwardt et al., 2000).

Stachylina tanysoma sp. nov. R.T.William and Strongman (Figs. 70–73)

Mycobank MB 801427

Thalli 200–450 µm × 8–12 µm, elongated tapering basal cell attached to peritrophic matrix by a small bulbous holdfast (Fig. 73), producing 40–50 trichospores per thallus (Fig. 72).

Generative cells short, 3–10 µm × 9–11 µm (Fig. 70). Trichospores ellipsoidal, tapered at both ends, 23–30 µm × 4.5–6 µm with small flared collar (Fig. 71). No zygospores observed. Attached to the midgut lining of midge (Chironomidae) larvae.



Figs. 70–73 *Stachylina tanysoma*. Fig. 70. Trichospores (arrows) attached to septate thallus with developing appendages (arrowheads) inside generative cells. Fig. 71. Trichospore detached from thallus, revealing a collar (arrow) and a single fine appendage (arrowhead). Fig. 72. Whole, long thalli, with attached trichospores (arrows). Fig. 73. Enlarged view of holdfast showing strongly tapered basal cell (arrowheads) attached to the midgut lining. Fig. 70 unstained; Figs. 71–73 stained with lactophenol cotton blue. Scale bars = 20 µm.

ETYMOLOGY: From the Greek, *tany* (long) and *soma* (body), referring to the long thalli of this species.

HOLOTYPE: Slide TLLO-4 (DAOM 242403) with thalli and spores, stained with lactophenol cotton blue, attached to host midgut lining. The midge host was collected at Mill Pond stream (TLLO) Timberlea, NS on 17 Sep 2011.

HABITAT: Collected from midgut lining of chironomid larvae at TLLO on 17 Sep 2011 (Appendix II).

COMMENTARY: *Stachylina tanysoma* is another species in this genus demonstrating thallus length in excess of 300 µm. There are three known species (*Stachylina prolifica* , *Stachylina magna***,** and *Stachylina tianensis*) and two new species, *Stachylina extensiva* and *Stachylina serpula* R.T. William & Strongman, described elsewhere in this issue (William and Strongman, 2013*b*), that have particularly long thalli like *St. tanysoma*. The closest match to *St. tanysoma* is *Stachylina prolifica* but the trichospores lack a collar (Lichtwardt, 2004) while *St. tanysoma* has a short collar. *Stachylina magna* trichospores [(30–)56(–80) µm × (6.5–)11(–15) µm] are larger than *St. tanysoma* (23–30 µm × 4.5–6 µm) and lack a collar (Lichtwardt, 2004). *Stachylina tianensis* trichospores are much larger (55–90 µm × 7–10 µm) and the thallus holdfast penetrates the peritrophic matrix (Wang et al., 2010) differentiating it from *St. tanysoma*. *Stachylina extensiva* and *St. serpula* both possess long thalli, but trichospores of *St. extensiva* are smaller (20–25 µm × 4–5.5 µm) and are a slightly different shape while *St. serpula* trichospores (29–38 µm × 6–9 µm) are longer and thicker (William and Strongman, 2013*b*).

Trichospore dimensions for *St. tanysoma* overlap with three other species: *St.*

euthena, *Stachylina longa* L. Léger & M.Gauthier and *St. gravicaudata*. *Stachylina euthena* trichospores (25–35 µm × 7–8 µm) are generally wider and longer than *St. tanysoma*, *St. gravicaudata* trichospores are 25–31 µm × 4–5 µm but only 4–8 trichospores are typically produced on a thallus and *St. longa* trichospores are 25 µm × 5–6 µm but lack a collar and thalli are shorter.

Stachylina uranus sp. nov. R.T.William and Strongman (Figs. 74–76)

Mycobank MB 801387

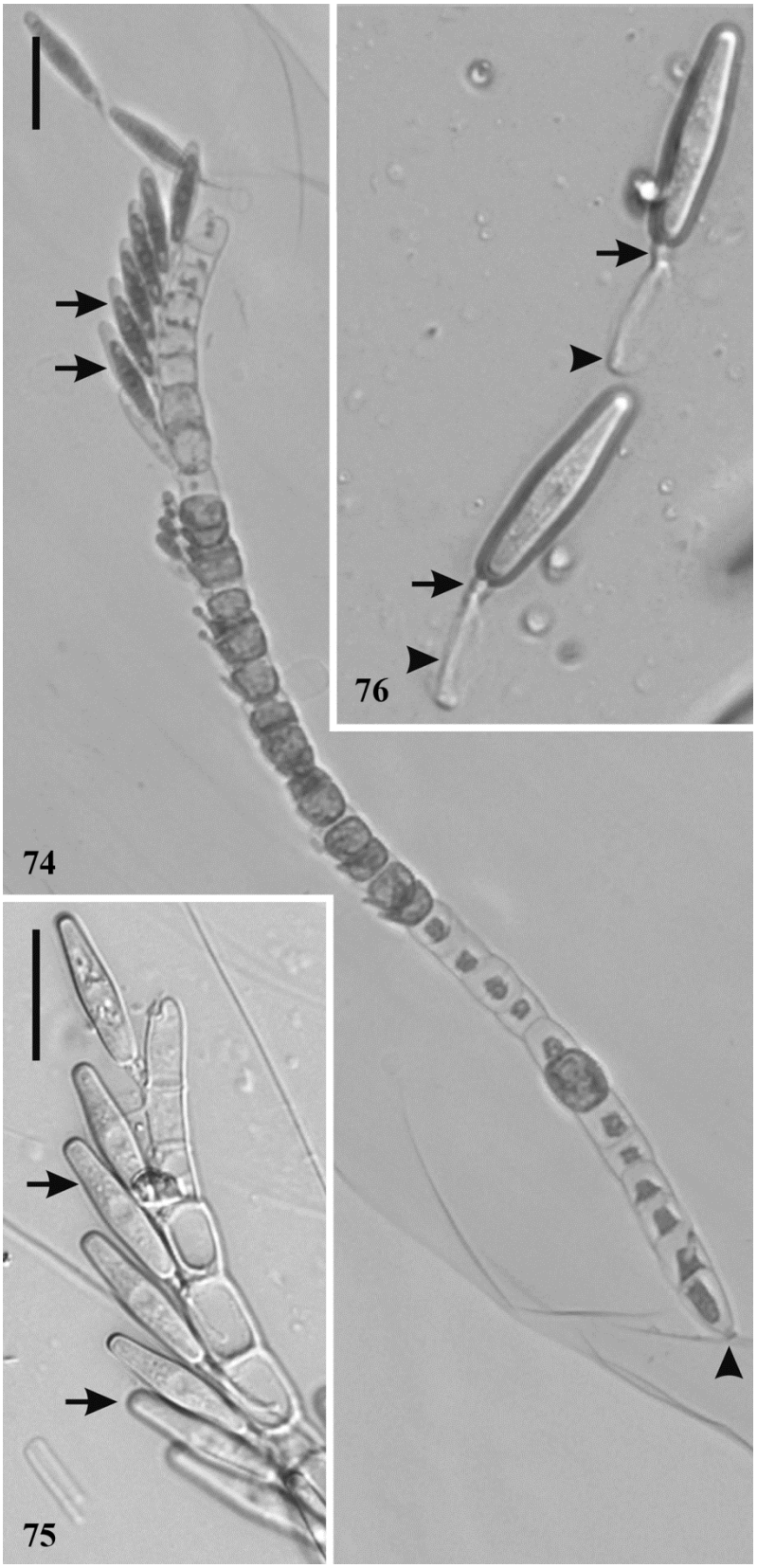
Thalli 150– 210 µm × 6–12 µm, basal cell rounded and attached to the peritrophic matrix by a thin pad of secreted material (Fig. 74). Trichospores ellipsoidal with a slight medial bulge, 22–29 µm × 5–7 µm, with small knot-like collar closely adhering to the base of the trichospore and with a single appendage (Figs. 75 and 76). No zygospores observed. Attached to the peritrophic matrix of midge (Chironomidae) larvae.

ETYMOLOGY: From Uranus in reference to the sixth planet in our solar system and the first planet discovered that was not known in ancient times.

HOLOTYPE: Thalli and spores stained with lactophenol cotton blue, attached to peritrophic matrix, on slide LLLE-3 (DAOM 242404). The midge host was collected at Long Lake (LLLE), Halifax, NS on 3 Jul 2011.

HABITAT: Collected from peritrophic matrix of chironomid larvae at LLLE 10 Apr and 3 Jul 2011; SPLO 13 Jul 2011 and GLLE 21 on Sept 2011 (Appendix II).

COMMENTARY: There are trichospore characteristics of *Stachylina uranus* that overlap with five other *Stachylina* spp. *Stachylina tanysoma* has trichospores (23–30 µm ×



Figs. 74–76. *Stachylina uranus*. Fig. 74. Thalli and developing trichospores (arrows), attached to the peritrophic matrix via tapering basal cell holdfast (arrowhead). Fig. 75. Thallus with attached trichospores (arrows). Fig. 76. Released trichospores with knot-like collar (arrows) and single appendage (arrowheads). Specimen in Fig. 74 is stained, Figs. 75 and 76 are unstained. Scale bars = 20 µm.

4.5–6 µm) that are ellipsoidal with a small conical collar and are almost the same length and width (William and Strongman, 2013*b*), however, *St. uranus* trichospores (22–29 µm × 5–7 µm) differ in shape and the collar is knot-like. *Stachylina platensis* trichospore length range (25– 36 µm × 5–7.8 µm) is longer (Lichtwardt et al., 2000) than *St. uranus*, whereas *Stachylina lotica* M.C. Williams & Lichtw. and *St. longa* have trichospore dimensions (24–32 µm × 8–10 µm and 25 µm × 5–6 µm, respectively) comparable to *St. uranus*, but both lack collars (Lichtwardt, 2004). Trichospores of *St. gravicaudata* tend to be slightly longer and thinner than *St. uranus* and have been reported to produce only 4–8 trichospores per thallus (White et al. 2005).

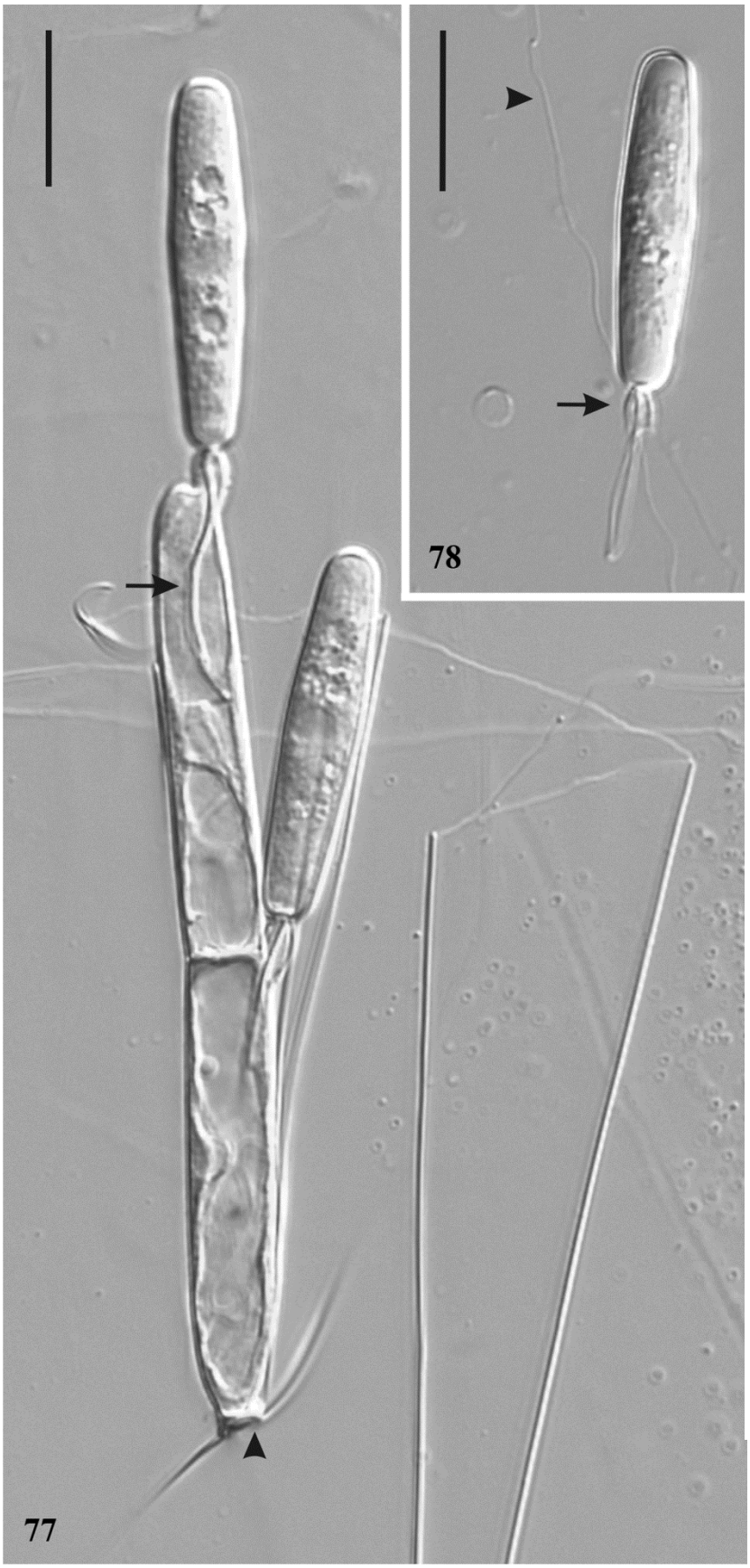
Stachylina zeppelin sp. nov. R.T.William and Strongman (Figs. 77 and 78)

Mycobank MB 801374

Short thalli attached to peritrophic matrix by a thin flat pad at the base (Fig. 77). Generative cells 40–60 µm long producing 2–4 trichospores (Fig. 77) and 10–25 µm when producing 8 trichospores per thallus. Trichospores large, ellipsoidal 42–52 µm × 9–11 µm with a conspicuous collar 3–6 µm × 3.5–4 µm (Fig. 78). No zygospores observed. Attached to the peritrophic matrix of midge (Chironomidae) larvae.

ETYMOLOGY: This species name is in reference to the trichospores resembling the zeppelin airships of old.

HOLOTYPE: Microscope slide GLLE-5 (DAOM 242405) containing thalli and trichospores stained with lactophenol cotton blue, attached to peritrophic matrix. The midge host was collected at Governor's Lake, Timberlea, NS (GLLE) on 21 Sep 2011.



Figs.77 and 78. *Stachylina zeppelin*. Fig. 77. Short thallus affixed to chironomid peritrophic matrix by a small disk (arrowhead) with mature, trichospores attached but showing a single appendage (arrow) inside the long generative cells. Fig. 78. Released trichospore with conspicuous collar (arrow) and long unfolding appendage (arrowhead). All Figs. taken from unstained preparations. Scale bars = 20 µm.

HABITAT: Collected from the midgut lining of chironomid larvae at GLLE on 21 Sep 2011 and 13 October 2011 (Appendix II).

COMMENTARY: The relatively large trichospores of *Stachylina zeppelin* is a feature shared by six other species in this genus. However, *St. litoralis* **,** *St. macrospora, St. magna* and *St. penetralis* all lack the conspicuous collar seen in *St. zeppelin* (Lichtwardt, 2004). *Stachylina stenospora* Siri, M.M. White & Lichtw. trichospores (42–70 µm × 4.5–7 µm) can be longer and thinner than *St. zeppelin* (42–52 µm × 9–11 µm) with a less prominent collar (White et al., 2006b). *Stachylina grandispora* Lichtw. also has trichospores [(40–72 µm × 6–10 µm (or more)] that are generally longer, thinner and have a smaller collar than *St. zeppelin* (Lichtwardt, 2004).

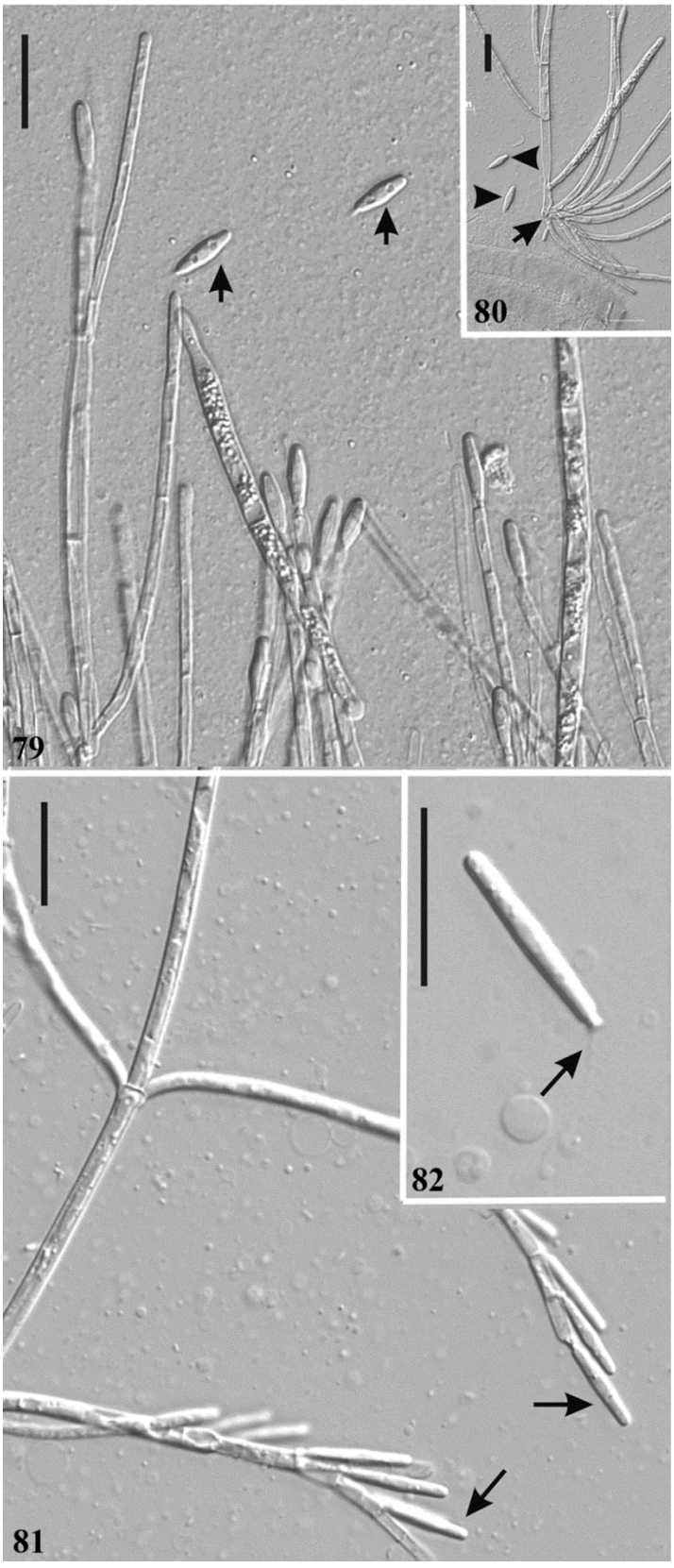
Other Species

There were 45 taxa of trichomycetes in addition to the 19 new species described in this section and a detailed list of taxa including host, collection dates and sites are given in Appendix II. Also presented here are five species representing new continental records for North America and five species as new geographic records for Nova Scotia.

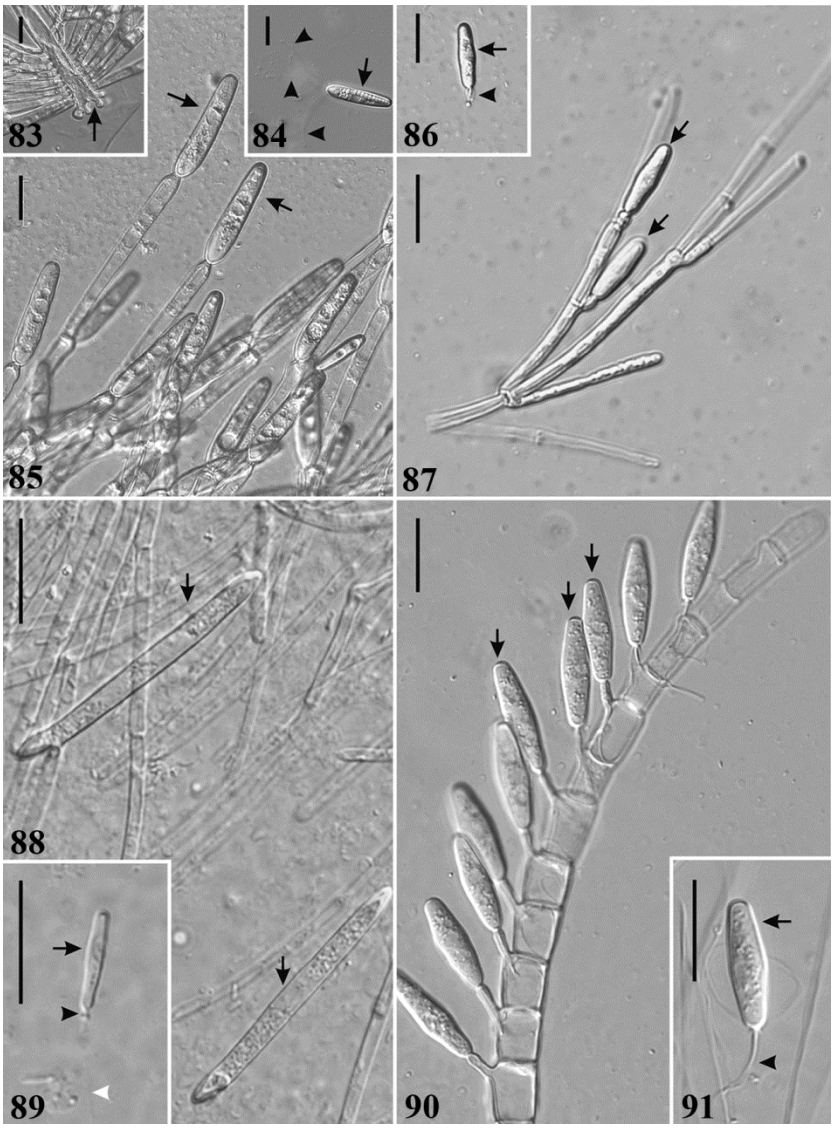
The five species occurring as new continental records include: *Smittium bulbosporophorus* Valle and Santam. (Figs. 79–80); *Smittium hecatei* Valle and Santam. (Fig. 95); *Smittium pusillum* Manier and F. Coste (Figs. 88 and 89); *St. euthena* Manier and F. Coste (Figs. 90 and 91) and *Sm. nodifixum* Strongman and Xu (Figs. 86 and 87). These five species were reported from chironomid hosts by others (Valle and Santamaria,

2004; Manier and Coste, 1971; Strongman and Xu, 2006), and were reported from the same hosts in my study as well.

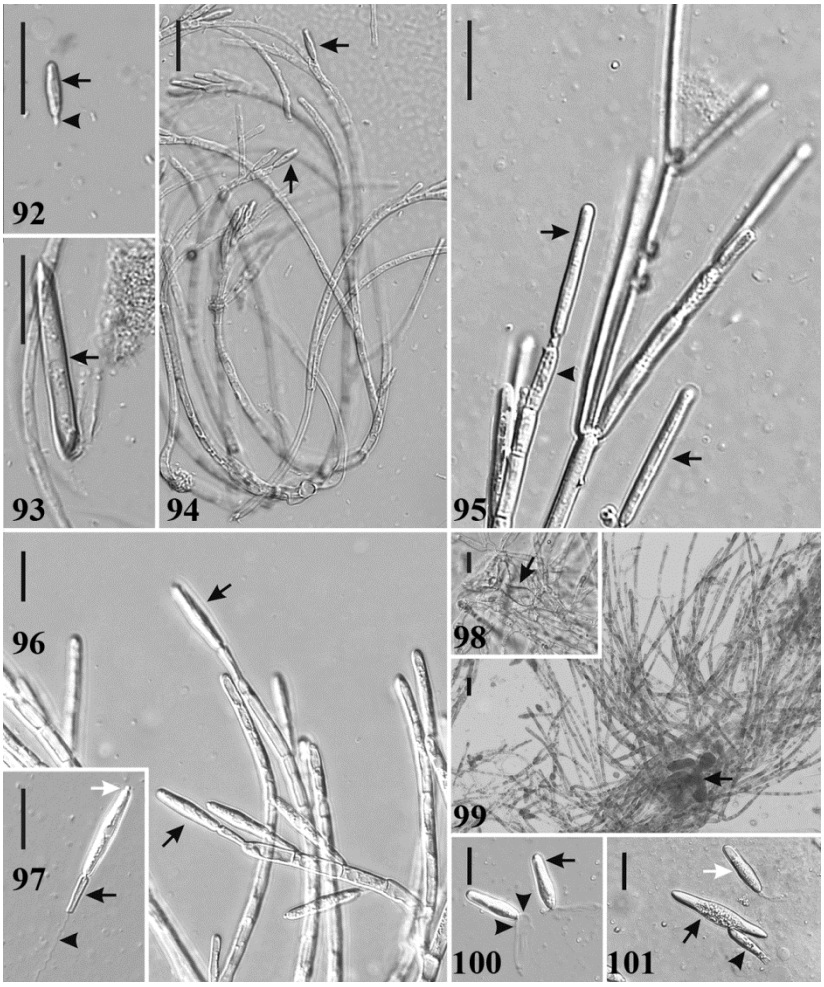
Three of the five species occurring as new geographic records were isolated from chironomid hosts including: *Smittium dipterorum* (Figs. 81 and 82); *Smittium minutisporum* Lichtw., Siri and M.M.White (Figs. 92–94) and *Smittium mucronatum* (Figs. 96 and 97) (Lichtwardt, 1997; Valle and Cafaro, 2010; Valle et al., 2011; White, Siri and Lichtwardt, 2006b; Lichtwardt, 2004). *Capniomyces sasquachoides* M.M. White & Bench (Figs. 98-101) was isolated from plecopteran hosts (Bench and White, 2012) while *Pennella arctica* Lichtwardt & Williams (Figs. 83–85) isolated from simuliid hosts (Lichtwardt, 1984).



Figs. 79–82. Other species. Figs. 79 and 80. *Smittium bulbosporophorus*. Fig. 79. Mature sporulating thalli with attached and two detached (arrows) trichospores. Fig. 80. Hyphae emanating from central point of attachment forming an inconspicuous holdfast (arrow). Two detached trichospores (arrowheads). Figs. 81 and 82. *Smittium dipterorum*. Fig. 81. Thallus demonstrating sparse verticilliate branching with developing trichospores (arrows) on the ends of fertile branchlet. Fig. 82. Detached trichospore with short collar (arrow). All figures are from unstained specimens. Scale bars = 20 µm.



Figs. 83–91. Other species. Figs. 83–85. *Pennella **a**rctica*. Fig. 83. Holdfast (arrow) with short bifurcated basal cell. Fig. 84. Released mature trichospore (arrow) with multiple appendages (arrowheads). Fig. 85. Trichospores (arrows) attached to the thallus. Figs. 86 and 87. *Smittium nodifixum*. Fig. 86. Released trichospore (arrow) with campanulate collar (arrowhead). Fig. 87. Trichospores (arrows) attached to fertile tip of thallus. Figs. 88 and 89. *Smittium pusillum*. Fig 88. Elongated, biconical zygospores (arrows). Fig. 89. Released trichospore (arrow) with short collar (arrowhead) and single appendage (white arrowhead). Figs. 90 and 91. *Stachylina euthena*. Fig. 90. Thallus with attached trichospores (arrows). Fig. 91. Released trichospore (arrow) with single long appendage (arrowhead). All figures are from unstained specimens. Scale bars = 20 µm.



Figs. 92–101. Other species. Figs. 92–94. *Smittium minutisporum*. Fig. 92. Released trichospore (arrow) with collar (arrowhead). Fig. 93. Zygospore (arrow). Fig. 94. Thalli with attached trichospores (arrows). Fig. 95. *Smittium hecatei*. Verticilliately branched thallus with attached trichospores (arrows) on the apex of a terminal branch (arrowhead). Figs. 96 and 97. *Smittium mucronatum*. Fig. 96. Branching thalli with attached trichospores (arrows**).** Fig. 97. Detached trichospore displaying apical papilla (white arrow), long collar (black arrow) and single appendage (arrowhead). Figs 98–101. *Capniomyces sasquachoides*. Fig. 98. Holdfast basal cell (arrow). Fig. 99. Thallus anchored to host via holdfast cell (arrow). Fig. 100. Released trichospores (arrow) with two appendages (arrowheads). Fig. 101. Released zygospore (black arrow) with prominent collar (arrowhead). Free trichospore (white arrow). All Figs. from unstained material except Fig. 99, which was stained. Scale bars = 20 µm.

Discussion (Taxonomy)

After 17 months of data collection at six sites within three watersheds, a total of 64 taxa of trichomycetes were documented including 19 new species described in the Results (Taxonomy) section. Aside from these new species, 45 previously described taxa were observed during this study (Appendix II). Of particular interest were reports of five species appearing in Nova Scotia as new continental records including: *Smittium bulbosporophorus* and *Sm. hecatei* originally reported from Spain (Valle and Sanatamaria, 2004), *Sm. nodifixum* from China (Strongman and Xu, 2006) and *Sm. pusillum* and *Stachylina euthena* both described previously from France (Lichtwardt, 2004).

Additionally, another five species (*Capniomyces sasquachoides*, *Pennella arctica*, *Smittium dipterorum*, *Sm. minutisporum* and *Sm. mucronatum*) collected during this study established new regional records for Nova Scotia. Recently described from Idaho, USA, (Bench and White, 2012) *Capniomyces sasquachoides* was also collected in this study now extending its range to Nova Scotia while *Sm. dipterorum* was previously collected from tropical locations in Costa Rica, the Dominican Republic and Mexico (Lichtwardt, 1997; Valle and Cafaro, 2010; Valle et al., 2011). *Pennella arctica* has been collected in Sweden and Montana, USA (Lichtwardt, 1984), *Sm. minutisporum* has only been described from the Great Smoky Mountains National Park, USA (White et al., 2006b) and *Sm. mucronatum* is known from France, Norway, Colorado, USA, and Ontario, Canada (Lichtwardt, 2004).

The collection of these new regional and continental records along with other

studies from Nova Scotia, indicate that at least certain species of trichomycetes have a widespread distribution. These large extensions in geographic distribution for some species and 19 new species reported from this study make it exceedingly difficult to address the biogeography, speciation and evolutionary aspects of trichomycetes that would allow us to understand the mechanisms that drive present day geographical distributions. Future documentation of these symbionts from around the world will continue to shed light on their distribution so that a clearer understanding might emerge.

The accumulated taxa were predominantly harpellid trichomycetes with the exception of three taxa (*Paramoebidium* spp., *Paramoebidium cassidula* and *Paramoebidium curvum* Lichtw.) that are from the Amoebidiales. Currently there are 40 species of trichomycetes derived from ephemeropteran hosts, 26 from plecopteran, 25 from simuliid and the vast majority of taxa (149) are from chironomid hosts (Lichtwardt, 2004). The abundance of chironomid-associated trichomycetes in the published literature was reflected in this study as well. *Smittium ampliboja* and *Sm. culicis* occurred in both chironomid and simuliid hosts and *Paramoebidium* sp. was recorded from three host types. There were 37 taxa collected from chironomids, 8 from ephemeropterans, 13 from plecopteran and 10 taxa from simuliid hosts (Appendix II).

Chironomid trichomycetes include nine genera and 149 species with *Smittium* by far the most speciose with over 94 described species (Lichtwardt et al., 2001a), many of which are only distinguishable based on precise measurements of trichospores and, if present, zygospores. The emphasis on measurements arises from difficulties in growing trichomycetes outside of their host and a general inability to obtain enough material,

when encountered, for DNA extraction and sequencing. Fortunately, a substantial number (approximately 40%) of *Smittium* species are culturable and Wang et al. (2013) recently published a review of the genus identifying several clades within it. Consequently, they removed *Sm. culisetae* and constructed a new genus, *Zancudomyces*, to accommodate this species that was the only *Smittium* species with trichospores that have a submedial swelling. *Zancudomyces culisetae* also was separated from other *Smittium* species based on molecular analysis (Wang et al., 2013). Another complicating factor when using spore morphology alone to discriminate among similar *Smittium* species is the wide range of trichospore lengths and widths and, to a lesser extent, these dimensions for zygospores. There are examples within *Smittium* (Lichtwardt, 2004) of trichospore lengths spanning 20 µm or more in one species. Dimorphism, where asexual trichospores in some species have two discrete size ranges on the same thallus, sometimes with only one size present, also presents further difficulties when attempting to identify members of this confounding genus.

The addition of *Sm. guttisporum* to the genus indicates how narrow the morphological differences can be between certain species. There are now three species with papillate trichospores and long collars, *Sm. mucronatum*, *Sm. papillum*, and *Sm. guttisporum* with remarkably similar thallus morphology. Zygospores have been described for *Sm. mucronatum*, but not observed in either *Sm. papillum* or *Sm. guttisporum*. The inability to compare the morphology of sexual spores emphasizes the importance of differences in the trichospore dimensions, which are distinct, but overlap: *Sm. guttisporum* (26–34 µm × 4–6 µm; collar 8–13 µm); *Sm. mucronatum* (33–37 µm ×

6.5–7 µm; collar: 7.5–9 µm); and *Sm. papillum* (20–28 µm × 3–4.5 µm; collar: 6–12.5 µm). The addition of *Sm. guttisporum* to the genus based on trichospore size ranges between those of the two other species, *Sm. mucronatum* and *Sm. papillum*, demonstrates how crucial precise measurements are for making accurate identifications. Future efforts to culture and obtain sufficient material for DNA extraction and sequencing might provide more clarity on the distinctiveness of these species. The description of another species with papillate trichospores is timely since Lichtwardt and White (2011) have designated *Sm. mucronatum* a lectotype for the genus. It would be interesting to determine if *Sm. mucronatum*, *Sm. guttisporum*, and *Sm. papillum* are all phylogenetically related.

A feature of *Sm. guttisporum*, that is not uncommon in other species of *Smittium*, is the small amount of adhesive anchoring immature thalli to the host gut. The adhesive is clearly documented in the early stages of thallus development, but it becomes largely obscured in mature thalli by the profuse branching that occurs in later stages of *Sm. guttisporum* growth. Similar developmental changes in the holdfast were illustrated by White and Strongman (2012a) for other *Smittium* spp. It appears that key morphological features could go undetected, depending on the developmental phase observed when the trichomycete is encountered. This also applies to the development of zygospores that in many species develop generally after there has been sufficient growth of the fungus, or in the latter stages of host development.

As investigations into trichomycetes continue, *Smittium* and other genera with many species (*Stachylina*, *Enterobryus*) will likely add more to their accumulating lists.

Accurate measurements will continue to be vital to identifications of trichomycetes; however with advances in molecular techniques and with continuing attempts to culture newly or previously encountered taxa, elucidation of these cryptic organisms should progress.

Introduction (Ecological)

There have been few ecological studies on trichomycetes in part due to the immense effort required to inventory trichomycetes from sites around the globe and the relatively few people working in the field. The accumulation of these studies in different parts of the world is beginning to shed light on the worldwide distribution of trichomycetes indicating that many species are not limited to certain geographic areas. Reports from this study alone include five new continental records of species previously reported only from France (*Smittium pusillum*, *Stachylina euthena*), Spain (*Smittium hecatei*, *Smittium bulbosporophorus*) and China (*Smittium nodifixum*) (William and Strongman, 2013a; 2013b; 2013c).

Smaller scale distribution studies have been conducted in Cambridge, New York (Labeyrie et al., 1996) with trichomycetes from black flies, and distribution of *Smittium culisetae* within pitcher plants has been reported (Reeves, 2004). Other ecological aspects of trichomycetes have been explored such as seasonality (Beard and Adler, 2002; Nelder et al., 2010; Hernandez Roa and Cafaro, 2012) and host specificity (Nelder, 2005). The relationships between black fly larvae and symbiotic organisms including distribution, diversity and scale have been discussed by McCreadie et al., 2011. Also, Beard and Adler et al. (2003), Nelder et al. (2010) and Labeyrie et al. (1996) have presented some data on the prevalence of trichomycetes found associated with black fly larvae.

The majority of ecological studies has been conducted with trichomycetes occurring in black fly larvae from streams, and thus is comprised of sampling disproportionally from lotic habitats. Trichomycetes have largely been reported from

lotic habitats (moving waters such as streams, rivers, and brooks), but as investigations from lentic habitats (still waters such as lakes, ponds, seeps) increase, they are proving to be a rich source of trichomycetes including new species and warrant further study (Lichtwardt et al., 2001a).

Despite the distinction of lentic from lotic habitats as separate and distinct it is understood that habitats do not exist in isolation from one another as there is significant recognition of cross habitat flow of nutrients, matter and energy and the importance of this for recipient species and ecosystems (Polis et al., 1997). For example, terrestrial carbon has been implicated in playing a central role in support of lake food webs (Pace et al., 2004) and aquatic insects found in freshwater streams and lakes often emerge into terrestrial environments creating a cross habitat linkage between aquatic and surrounding terrestrial ecosystems (Power et al., 2004). The mosaic flow of nutrients, energy, and populations of vertebrate and invertebrate communities throughout ecosystems is difficult to assess in terms of overall effect on an ecosystem; however, the fact that there is an influence is undeniable.

Within watersheds streams can receive high amounts of leaf detritus from the terrestrial or riparian zones that get carried downstream and may be deposited into lakes. Addition or elimination of detritus has been implicated in causing quick changes in freshwater invertebrate community structure and density of functional feeding groups (Wallace et al., 1999; Rowe and Richardson, 2001).

Most fresh water insects are adapted to either lentic or lotic habitats but overlap of invertebrate communities in these habitats is common, such as in the floodplain of large

rivers (Merritt and Cummins, 1996). Extreme rain events or snow melt can often create torrential stream flow within watershed systems that can potentially carry lotic insects from the stream, depositing them into a lotic-lentic interphase for which their oxygen and other resource requirements may or may not be sufficient. A recent study from the University of British Columbia suggests that there is a littoral zone where streams flow into lakes that has resident invertebrate populations not found in either the lake or stream habitats, but only in this littoral zone (Klemmer, 2011).

The majority of stonefly species are primarily associated with clean, cool running waters (Merritt and Cummins, 1996). Oxygen diffuses rapidly through the air but the rate can be slowed down 324,000 times in water such that availability of oxygen is heavily dependent on the velocity of the stream (Merritt and Cummins, 1996) and this factor can strongly affect the species composition of insect communities in aquatic habitats.

This study compares the community structure of trichomycetes occurring in lentic and lotic habitats within three separate watersheds. Comparisons between these two habitats are examined for species richness (number of taxa at each site) and prevalence (percentage occurrence of each species within host type). Months into the study, an extreme rain event occurred flushing normally stream dwelling plecopteran insects into the lentic site chosen at the Long Lake watershed. This site was eliminated from lotic/lentic comparisons and is designated as an interphase site since it was comprised of both lotic and lentic insects on nine of 22 separate collection dates.

There were four target hosts examined for trichomycetes including mayflies (Ephemeroptera), stoneflies (Plecoptera), and both chironomids and simuliids (Diptera).

Since lentic sites generally only draw from ephemeropteran and chironomid hosts, closer examination of trichomycetes occurring in only these hosts is examined more in depth. To a lesser extent, both seasonality and host specificity were observed in several species and are investigated further. Finally, the community structure occurring in three watersheds (Long Lake, Woodens River and Shubenacadie) were examined, but comparisons are limited to two (Woodens River and Shubenacadie) due to the loss of a true lentic site at Long Lake.

Results (Ecological)

Hosts Collected and Dissected

In this section, the aim is to consider several ecological aspects of trichomycetes including species richness, seasonality, and prevalence; however, before addressing the ecological aspects both the number of insects collected and dissected needs to be examined. The insect data are important to understand trichomycete ecology because they are obligate colonizers of insect guts in all aquatic habitats (Lichtwardt, 1986).

The total number of insect hosts collected at each site (Table 1) demonstrates that there was an abundance of insects available at each of the six sites providing substantial numbers for dissection. Insect collection numbers were similar from all sites with the exception of TLLO which had nearly twice the number of insects than any of the other five sites (Table 1). Lotic sites drew from ephemeropteran, plecopteran, and dipteran (both chironomids and simuliids) hosts whereas the lentic sites had only ephemeropterans and chironomids, and yet total insect collection numbers were similar. The interphase site (LLLE) contained mostly ephemeropterans and chironomids, but also had plecopterans on occasion due to these otherwise lotic-dwelling hosts being flushed from an adjoined stream after heavy rain events.

The largest number of insects was collected from TLLO, (6839), while LLLO and SPLO reported fewer yet substantial numbers, 3284 and 3728 respectively. Insects dissected were a subset of the insects collected at each of the three sites and similar numbers of insects were dissected from each site ranging from a low of 1222 at SPLO, 1404 at LLLO to a high of 1487 at TLLO (Table 1). Insect collections from the two lentic

sites were similar to lotic sites with 3231 collected at GLLE and 2844 collected at SPLE leading to comparable numbers dissected (806 and 577 at GLLE and SPLE respectively) at each lentic site. Similar numbers of insects (3685) were collected from the interphase site (LLLE) and 855 were dissected (Table 1). The insect collections and dissections for each site have been broken down into four time periods roughly representing seasons in Nova Scotia and are shown in Figs.102-104.

Table 1. Total Mayflies (Ephem.), Stoneflies (Plec.), Midges [Dip. (Ch)], and Black flies [Dip. (Si)] collected and dissected at lotic, lentic and interphase sites from watersheds around the Halifax Regional Municipality, NS.

Site	Total number collected	Ephem. collected	Plec. collected	Dip. (Ch) collected	Dip. (Si) collected	Total number dissected
Lotic sites						
[1]LLLO	3284	825	671	1054	734	1404
TLLO	6839	4423	919	352	1145	1487
SPLO	3728	1585	338	446	1359	1222
Lentic sites						
GLLE	3231	2646	1	584	0	806
SPLE	2844	2600	1	242	1	577
Interphase site						
LLLE	3685	2881	247	557	0	855

[1] Site abbreviations are Long Lake Provincial Park stream (LLLO), Timberlea stream (TLLO), Shubie Park stream (SPLO), Governor's Lake, Timberlea (GLLE), Shubie Park lake (SPLE) and Long Lake Provincial Park lake (LLLE)

The insect data show that fewer insects were collected and dissected from the lentic sites compared to the lotic sites among the time periods (seasons), with the exception of Sep to Dec 2011. There were 2412 insects collected from GLLE during this time period, higher than any other period at any site and 1681 insects collected from SPLO, higher than all other time periods or sites aside from TLLO and SPLO during the

Figure 102. Insects collected and dissected by date range at lotic sites.

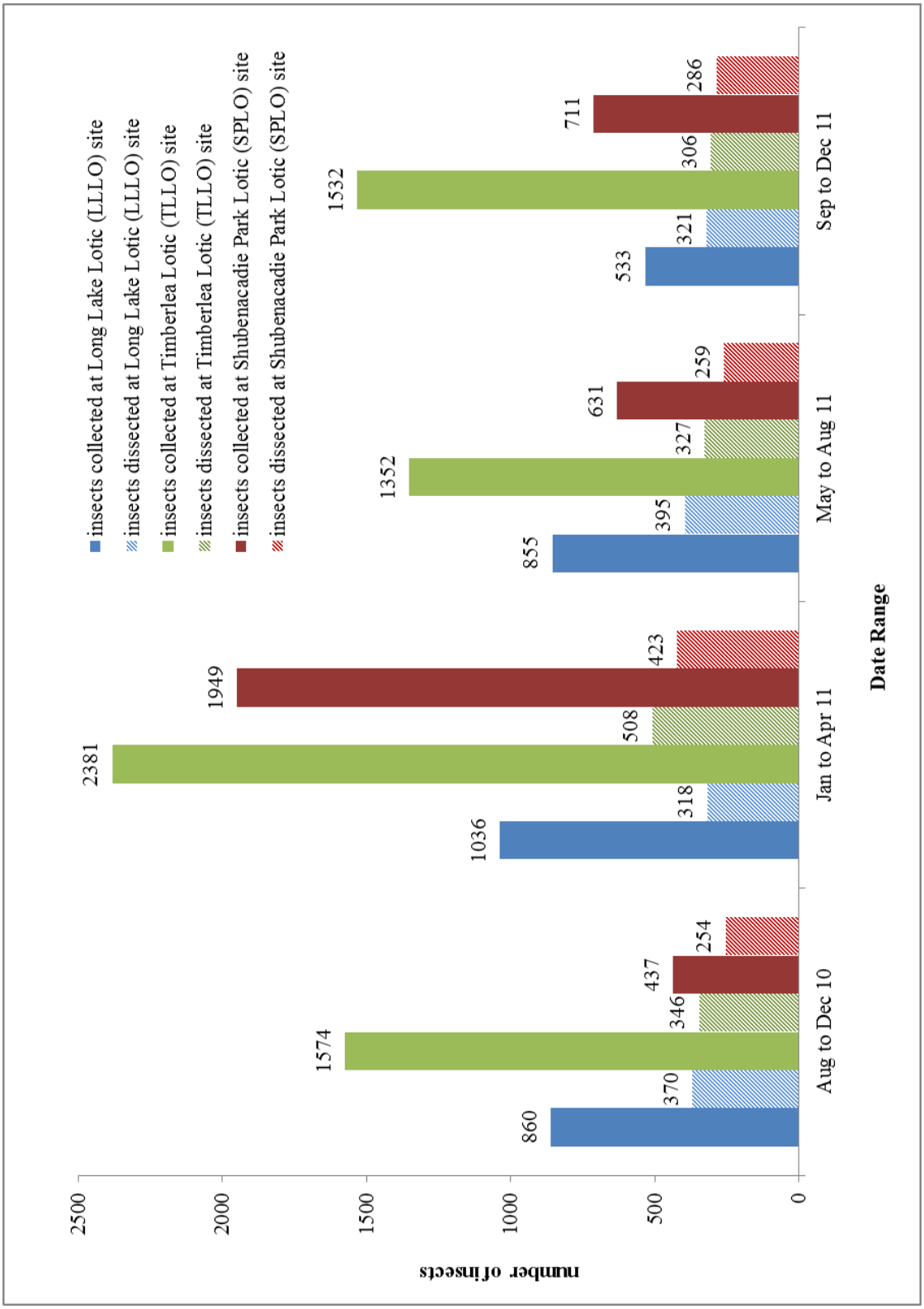


Figure 103. Insects collected and dissected by date range at lentic sites.

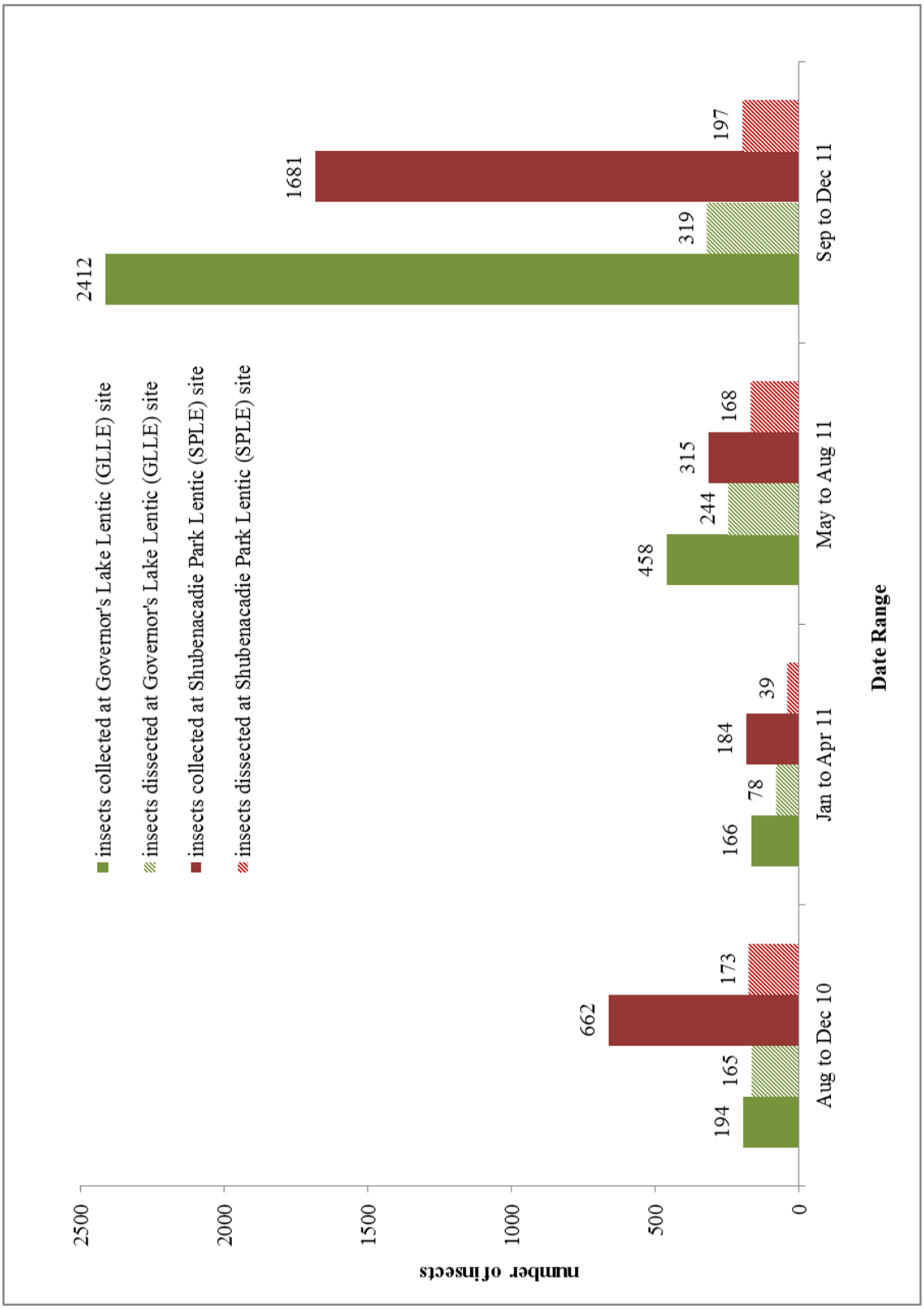
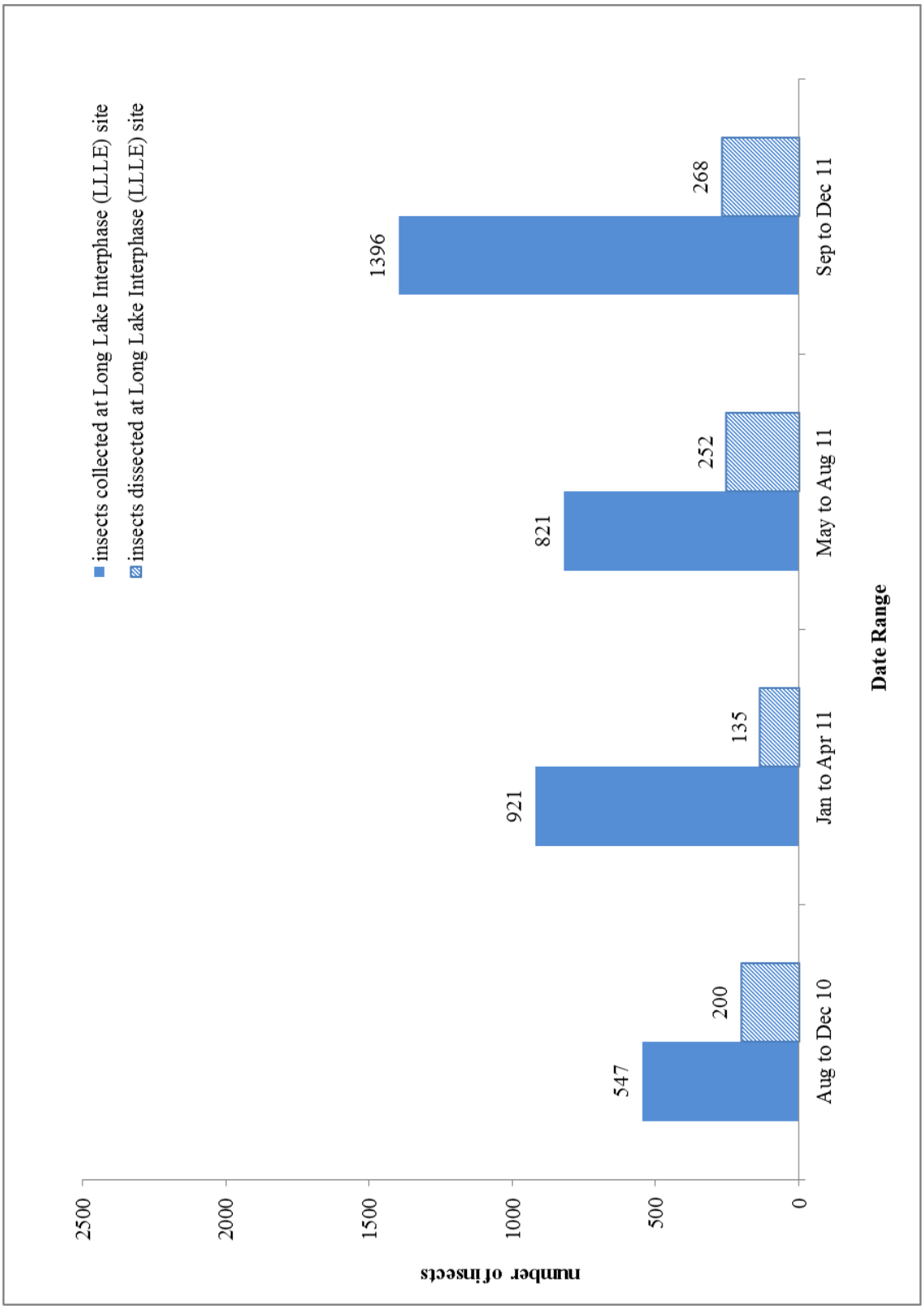


Figure 104. Insects collected and dissected by date range at the interphase site.



Jan to Apr 2011 period (Figs. 102 and 103). Despite this spike in insects collected from lentic sites for this period, dissection numbers were only slightly higher compared to dissections of lentic insects from the other three periods (Figs. 102 and 103). Insect collections and dissections for each of the two lentic sites were similar for the other three date ranges (Fig 103).

The interphase site (LLLE) was intermediate in terms of insects collected in comparison to the lotic and lentic sites (Fig. 104). Insect density at this interphase site was higher than from lentic sites, with the exception of the Sep to Dec 2011 period where again a spike in collection numbers (1396 specimens) was observed, but not to the degree in either lentic site over this time period (Fig. 104). The intermediate numbers of insects collected and dissected at this interphase site is due to capniid stoneflies as well as ephemeropterans and chironomids likely being flushed from the stream into this site. The introduction of capniid stoneflies into the interphase was not an isolated incident as there were nine occasions when stoneflies were retrieved ranging in numbers from 1 to over 80 per collection.

A maximum of 27 insects of each of the four host types; ephemeropterans, plecopterans and dipterans (simuliids and chironomids) were dissected on each collection date. This was an arbitrary number set at the outset of the study (See Materials and Methods) in order to process the maximum number of each host type before decomposition began to obscure the contents of the insect guts. This translated to a somewhat uniform number of host dissections at the lotic sites (1404, 1487, and 1222 at LLLO, TLLO, and SPLO respectively), lower numbers at the lentic sites (806 and 577 at

GLLE and SPLE) largely due to the fact that two of the four groups of insects did not occur in this habitat type (Table 1) and 855 dissections at LLLE, the interphase site drawing from three of four host types, excluding simuliids. Additionally, mayfly and stonefly dissections could include multiple families which occurred at different densities at different times at each site. If certain host types (or Families) typically are colonized by more trichomycetes, the trichomycete species richness could reflect, in part, the specific host available (e.g. stonefly vs. mayfly).

The number of insects collected (density) may also affect the types and densities of trichomycetes simply because larger numbers of insects should translate to a higher spore load in aquatic habitats increasing the potential to recover trichomycetes. The insect densities and dissections processed during this study are high compared to other studies where this type of information is provided (Beard and Adler 2002; Beard et al., 2003; Nelder et al., 2010) and thus should make the data on trichomycete ecology representative of the trichomycete communities present.

Trichomycete Species Richness and Seasonality

This section examines the trichomycete species richness (number of species recovered) at each of three lotic, two lentic and the one interphase site over four different periods, broadly representing the different seasons in Nova Scotia. Replicate data are presented for Sep to Dec in 2010 and Sept to Dec 2011 (Figs. 105-107).

Examining total trichomycete taxa (species richness) recovered at each of the six sites, TLLO was found to have the greatest number of trichomycetes with 34 taxa,

Figure 105. Trichomycete species from lotic sites (LLLO, TLLO, and SPLO).

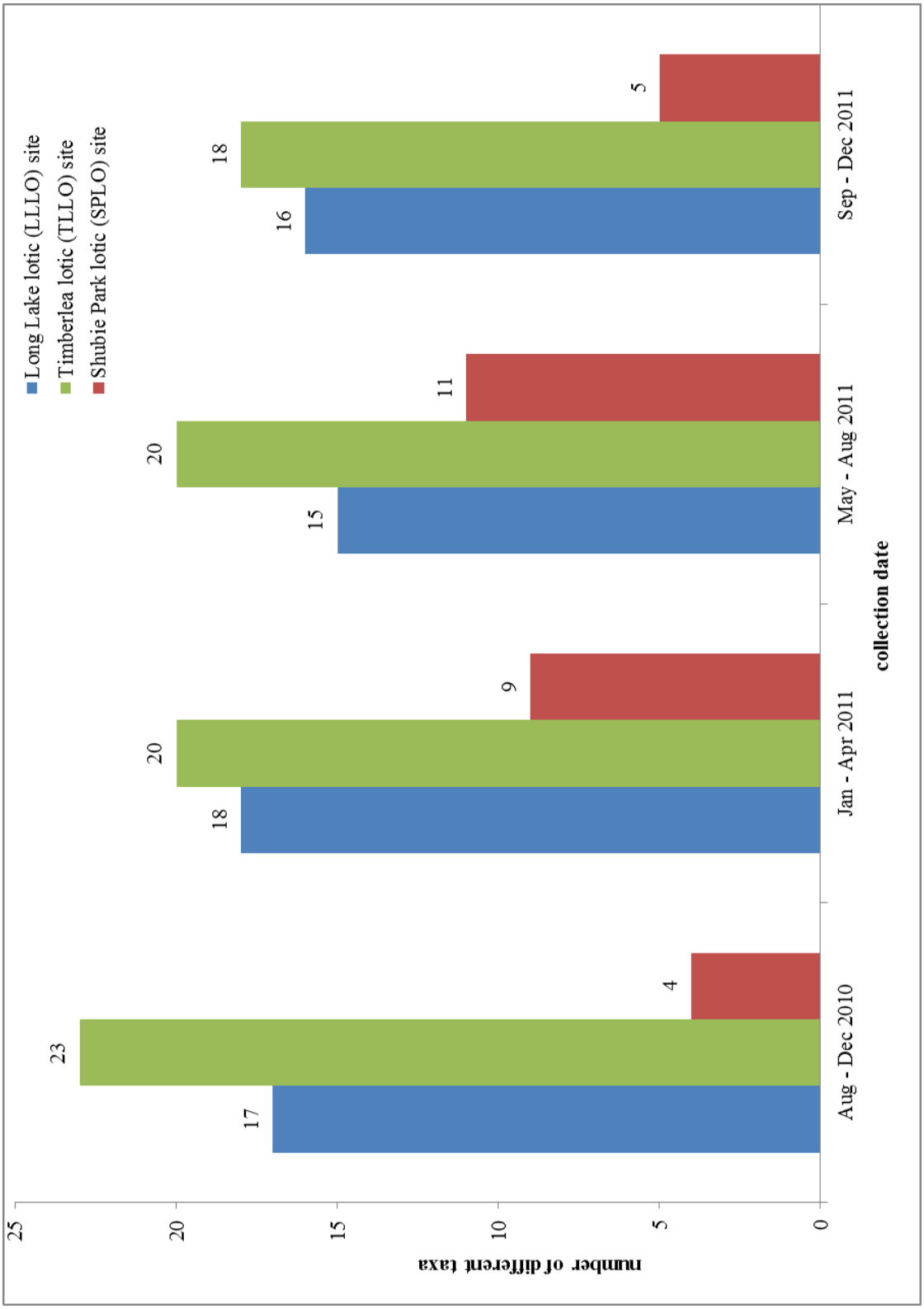


Figure 106. Trichomycete taxa from lentic sites (GLLE and SPLE).

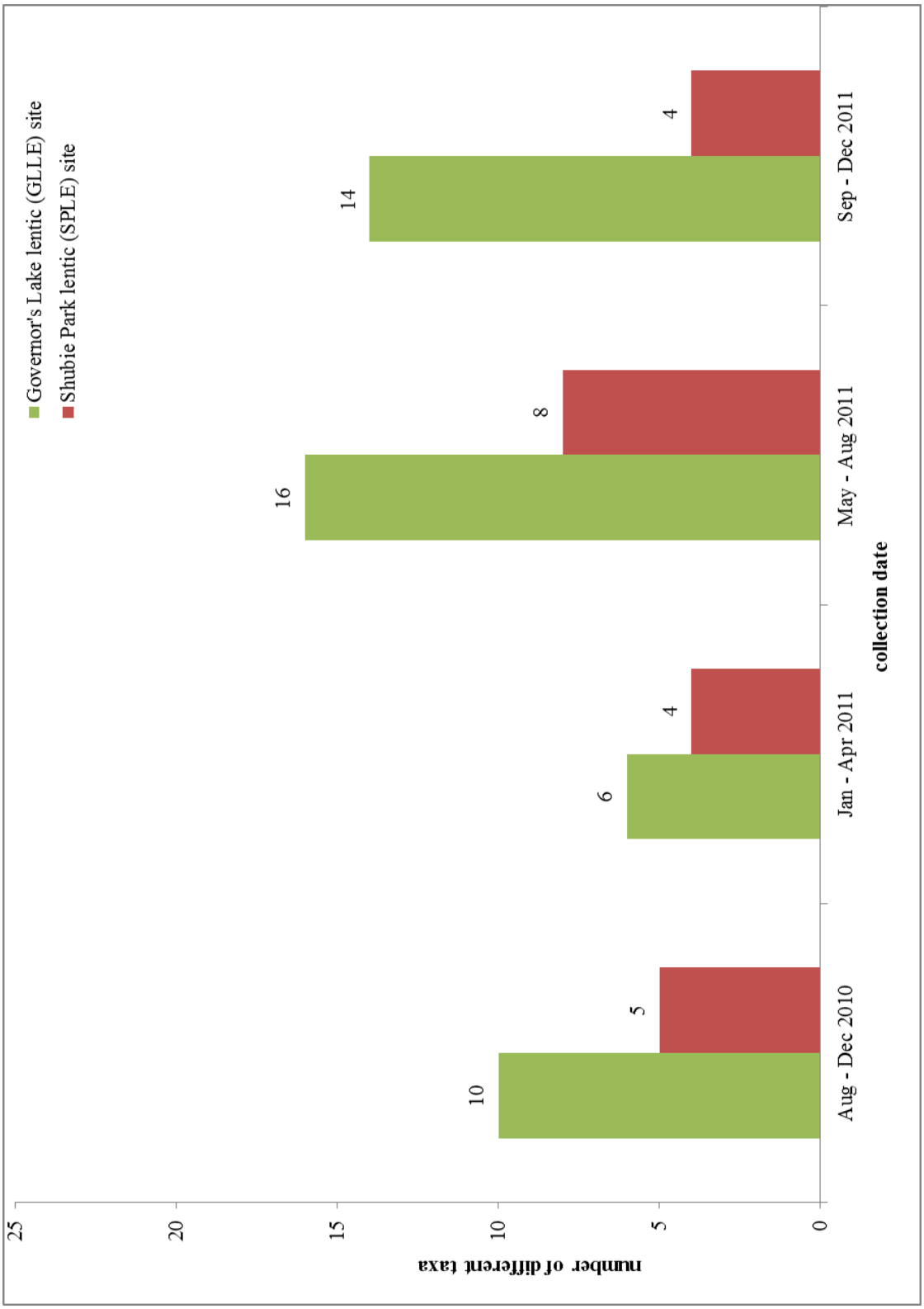
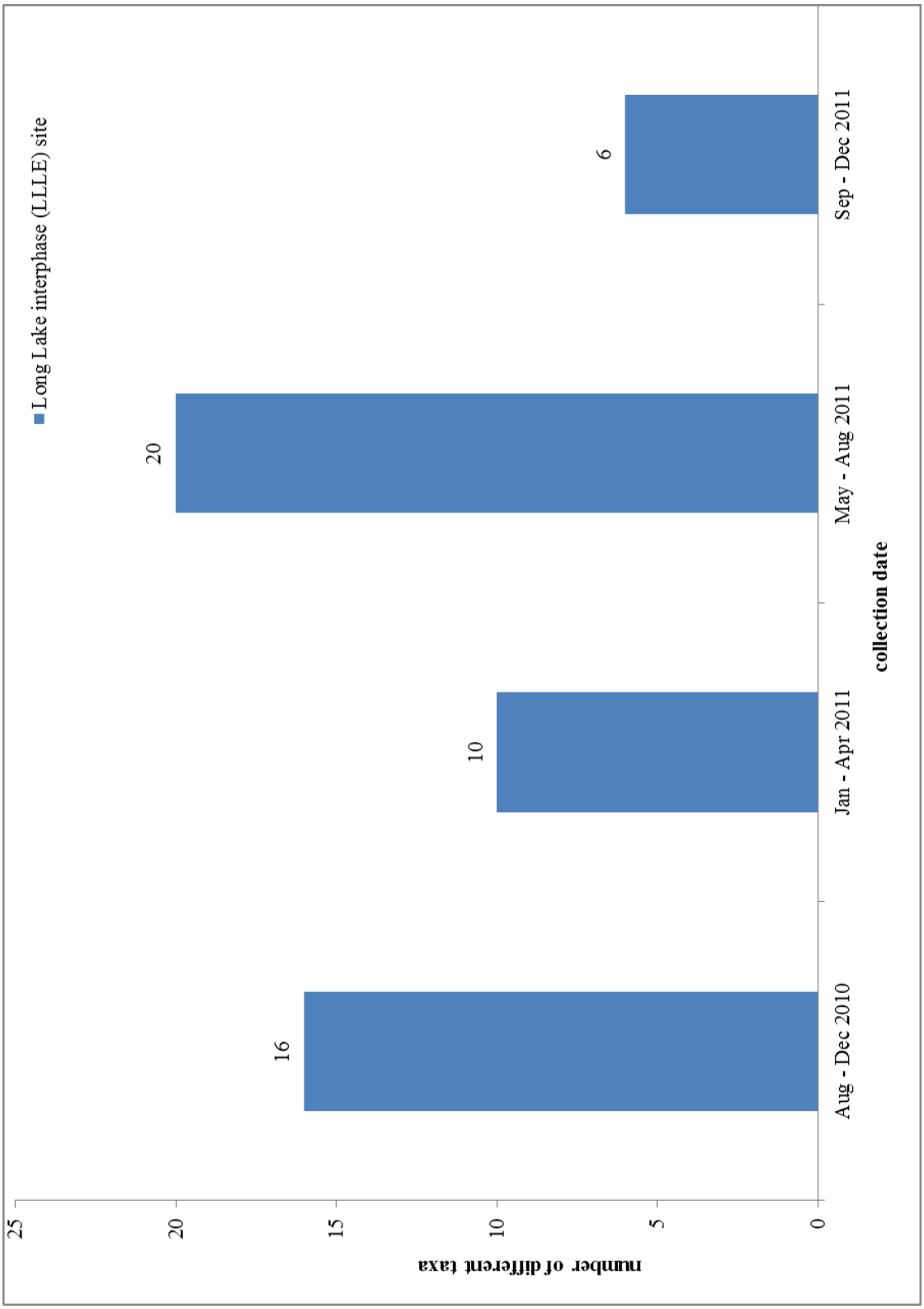


Figure 107. Trichomycete taxa from the interphase site (LLLE).



followed by LLLO (29), LLLE (28), GLLE (21), SPLO (16) and SPLE (12) (Figs. 108-113). These data indicate a higher number of taxa reported from the three lotic sites with a mean of 26.3 ± 9.3 taxa per site, than the two lentic sites (16.5 ± 6.4). The 28 taxa reported from the interphase site LLLE was higher than either of the two lentic sites as well as one lotic site (SPLO). Included in these data was a group, unidentifiable spp., which was treated as one taxon. Clearly this represents many different species and it occurred in all insect types but was included only to record the presence of trichomycetes, otherwise the occurrence of gut symbionts would be substantially underestimated.

The species richness data were considered over four time periods to detail any evidence of seasonality in trichomycete abundance. Species richness per site was considerably higher at the lotic sites than at the lentic sites for each of the time periods (Figs 105-107). Overall there were 44 species from (three) lotic sites and 15 from (two) lentic sites for the period of Aug to Dec 2010 followed by ratios of 47:10; 46:24 and 39:18 over the periods of Jan to Apr, May to Aug and Sep to Dec 2011 respectively. Considering the distribution of taxa over the four time periods, there was an average of 14.7 ± 6.1 trichomycete species reported from each of the three lotic sites over the four time periods compared to 8.4 ± 4.6 per lentic site/time period and 13 ± 6.2 from the single interphase site/time period.

The stream site in Timberlea (TLLO) was the most speciose of the three lotic sites with 18 to 23 different taxa of trichomycetes from the four seasonal time periods (Fig. 105). LLLO was the next richest source of trichomycetes ranging from 15 to 18 taxa

while SPLO reported the fewest taxa, 4 to 11 (Fig. 105). Of the two lentic sites, a range of 6 to 16 taxa were reported from GLLE during each of the four seasonal categories while 4 to 8 taxa were reported from SPLE during the same time period (Fig. 106). Trichomycetes collected from the interphase site within the Long Lake watershed (LLLE) ranged from 6 to 20 taxa (Fig. 107) where the high for any period occurred during the May to Aug 2011 period.

The insect data (Figs. 102-104) show that the numbers of insects dissected in each time period (season) was similar within each habitat type (lotic, lentic and interphase), but generally there were fewer insects dissected from the lentic and interphase sites than from the lotic sites except for Sep to Dec 2011 (Figs. 102-104).

In summary, there are more taxa of trichomycetes found within lotic sites compared to either lentic or the interphase sites. The initial date ranges (Aug to Dec 2010 and Jan to Apr 2011) saw a clear distinction between lentic and lotic taxa numbers; however became less distinct for the latter date ranges (May to Aug and Sep to Dec 2011). The interphase site had its highest recovery of trichomycetes during the May to Aug 2011 date range and the second highest for Aug to Dec 2010.

Prevalence

Prevalence of trichomycetes (percentage occurrence of each species within host type) was examined as a way to measure the abundance of trichomycete taxa among the site types (lentic/lotic and interphase). A trichomycete prevalence value greater than 2 % of the hosts dissected at a site was arbitrarily chosen to differentiate between frequently

Figure 108. Prevalence of Trichomycete species at LLLO

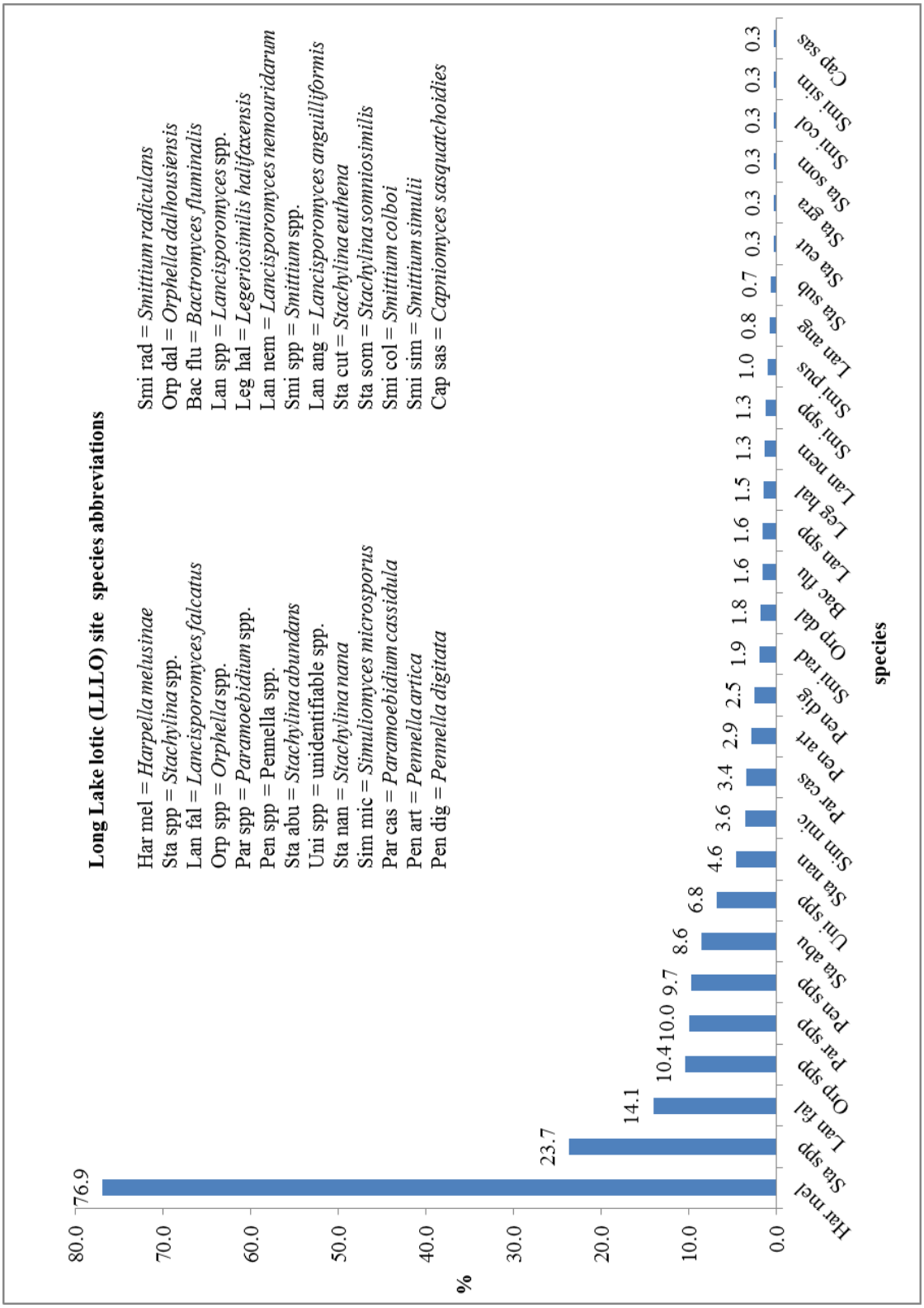


Figure 109. Prevalence of Trichomycete species at TLLO

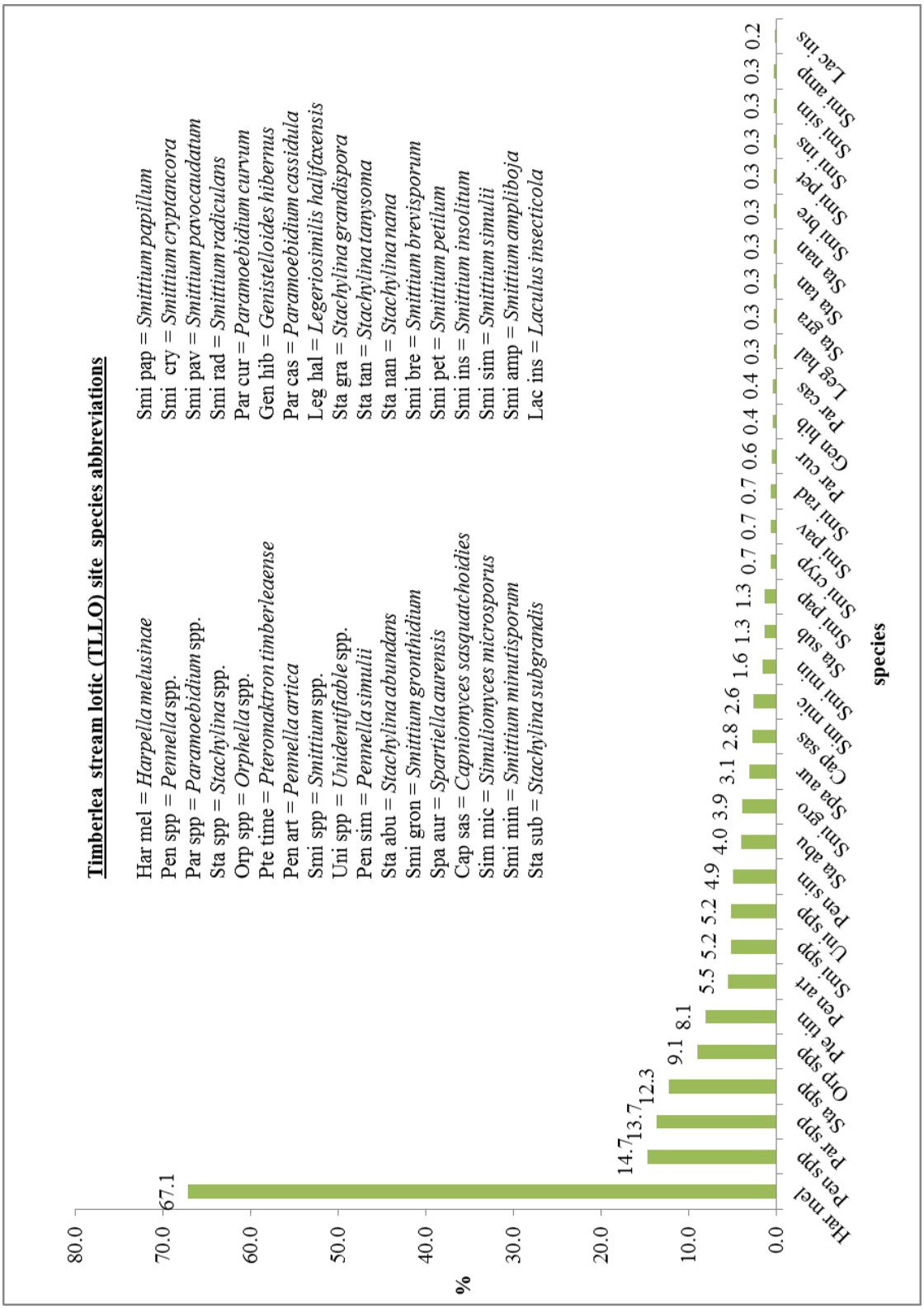
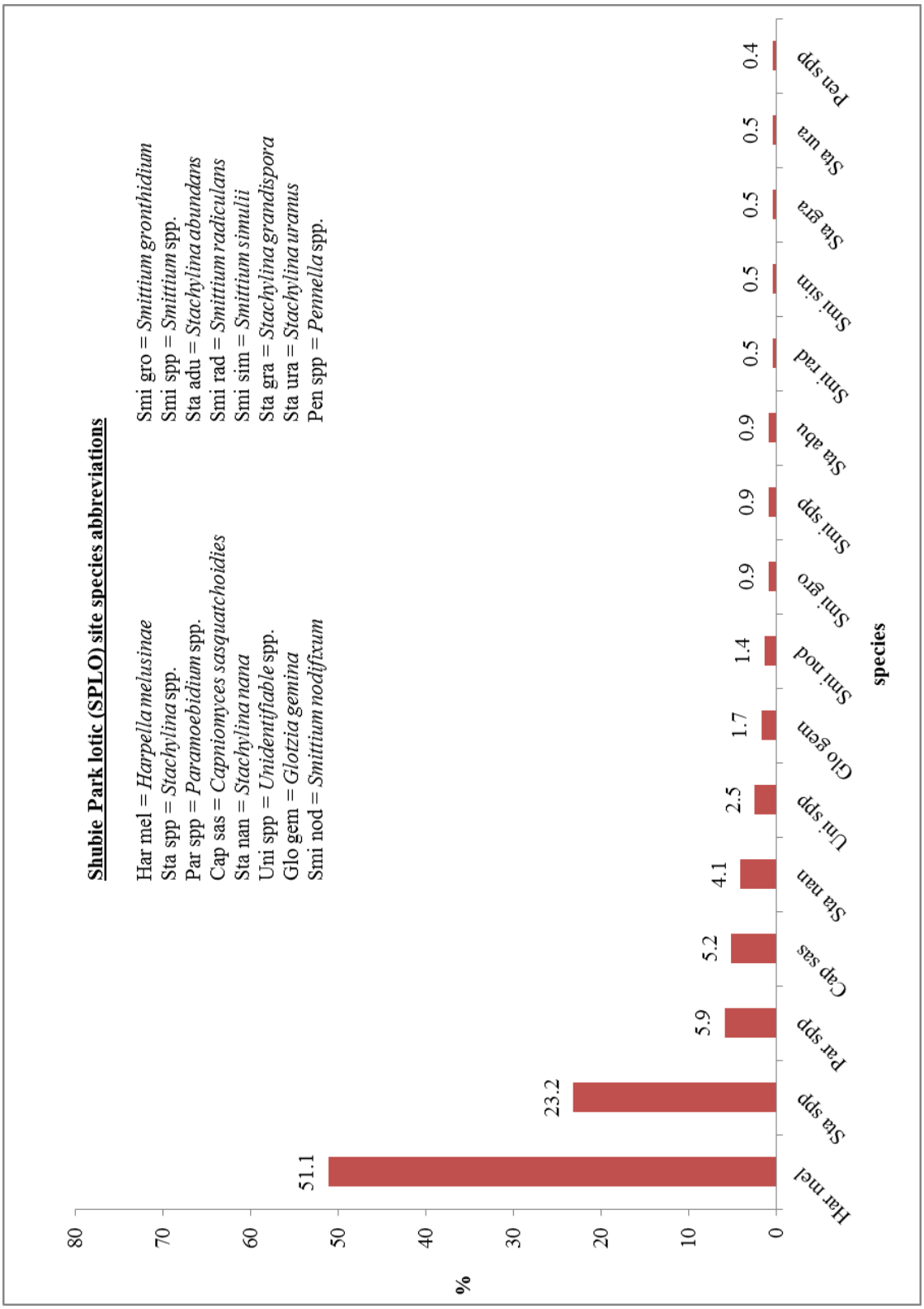


Figure 110. Prevalence of Trichomycete species at SPLO



occurring species and those considered rare. The rationale for this is that species occurring in 2% or less of the hosts dissected were so rare that they possibly were at other sites, but could have been missed.

At all three lotic sites (Fig. 108-110), *Harpella melusinae* L. Léger & Duboscq was by far the most common trichomycete encountered with prevalence values of 76.9% (LLLO), 67.2% (TLLO), and 51.1% (SPLO). These values eclipse all other species of trichomycetes by a considerable margin at each site.

Apart from the high prevalence of *H. melusinae* at lotic sites, the next most common taxa were *Stachylina* spp., *Pennella* spp. and *Paramoebidium* spp. Unable to be identified beyond the genus level, these taxa undoubtedly represent a number of species, and probably include species already recorded from the site, explaining their high prevalence. *Stachylina* spp. had prevalence values of 23.7% (LLLO), 23.2% (SPLO) and 12.3%, at TLLO (Fig 108-110). Interestingly *Stachylina* spp. and *Harpella melusinae* were by far the most common at all three sites and both colonize the peritrophic matrix of chironomid and simuliid insect hosts respectively. The peritrophic membrane is a unique environment in the insect gut and the species found here are likely specifically adapted to this environment. *Pennella* spp. was quite common at TLLO (14.7%) and LLLO (9.7%), but was rarely encountered at SPLO (0.4%) while *Paramoebidium* spp. was common at all sites, TLLO (13.7%), LLLO (10.0%) and SPLO (5.9%) (Figs. 108-110). *Paramoebidium* spp. differ from most other trichomycetes as they are found in three host orders; Ephemeroptera, Plecoptera and Diptera (Simuliidae).

Capniomyces sasquachoides and *Stachylina nana* were species collected from all three lotic sites. *Capniomyces sasquachoides* was common at TLLO (2.8%) and SPLO (5.2%), but rare at LLLO (0.3%). *St. nana* had a prevalence of 4.6% at LLLO and 4.1% at SPLO, but was rare at TLLO (0.3%) (Figs. 108-110). Taxa occurring at all three lotic sites and with prevalence values lower than 2% include *Sm. radiculans*, *Sm. simulii*, and *St. grandispora* (Figs. 108-110).

The lotic site SPLO had many fewer trichomycetes overall than either TLLO or LLLO (Figs. 108-110) and thus shared fewer common species with the other sites. Twelve of the 16 taxa from SPLO were also reported at both LLLO and TLLO (Figs. 108-110). Prevalent taxa shared by LLLO and TLLO only (Figs. 108 and 109), were *Orphella* spp. (LLLO 10.4%; TLLO 9.1%), *Pennella arctica* (LLLO 2.9%; TLLLO 5.5%) and *Simuliomyces microsporus* Lichtw. (LLLO 3.6%; TLLO 2.6%). *Paramoebidium cassidula* (LLLO 3.4%; TLLO 0.4%), and *Legeriosimilis halifaxensis* Strongman & M.M.White (LLLO 1.5%; TLLO 0.3%) were found at both these sites less frequently. TLLO and SPLO had only *Sm. gronthidium* in common at 3.9% and 0.9% respectively from chironomids dissected at these sites (Figs. 108 and 110).

There were 12 taxa at LLLO, 12 at TLLO, and 3 at SPLO that were unique to one of these three lotic sites with varying prevalence (Figs. 108-110). Of these taxa, seven were newly described species with *Pteromaktron timberleaense* at 8.1%, but *Sm. insolitum*, *St. tanysoma*, and *Sm. ampliboja* all occurred at < 2% and were collected only from TLLO. *Bactromyces fluminalis* and *Glotzia gemina* were found only at LLLO and SPLO respectively, but were rare (<2%). The most common taxa at both GLLE and

Figure 111. Prevalence of Trichomycete species at GLLE

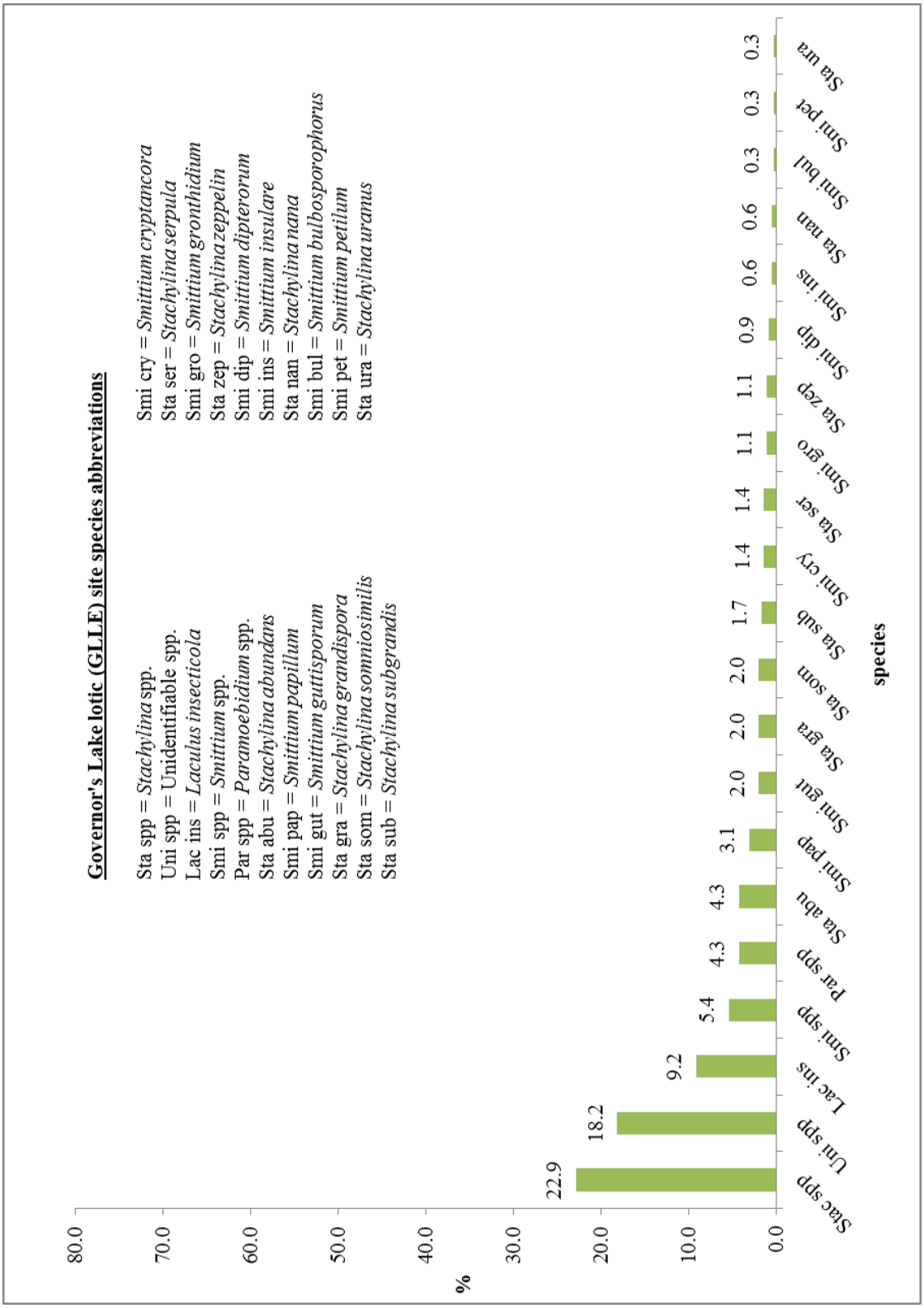


Figure 112. Prevalence of Trichomycete species at SPLE

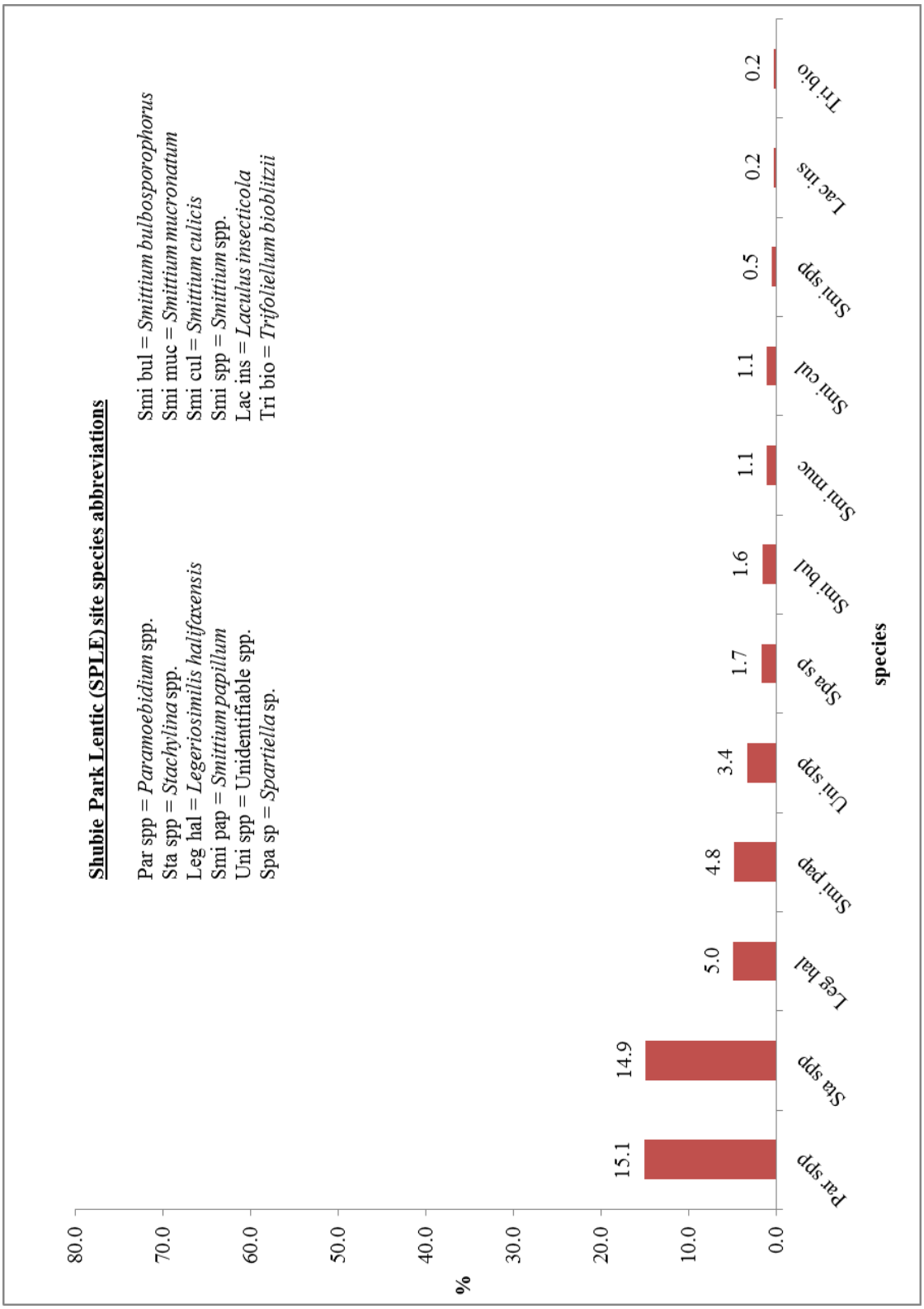
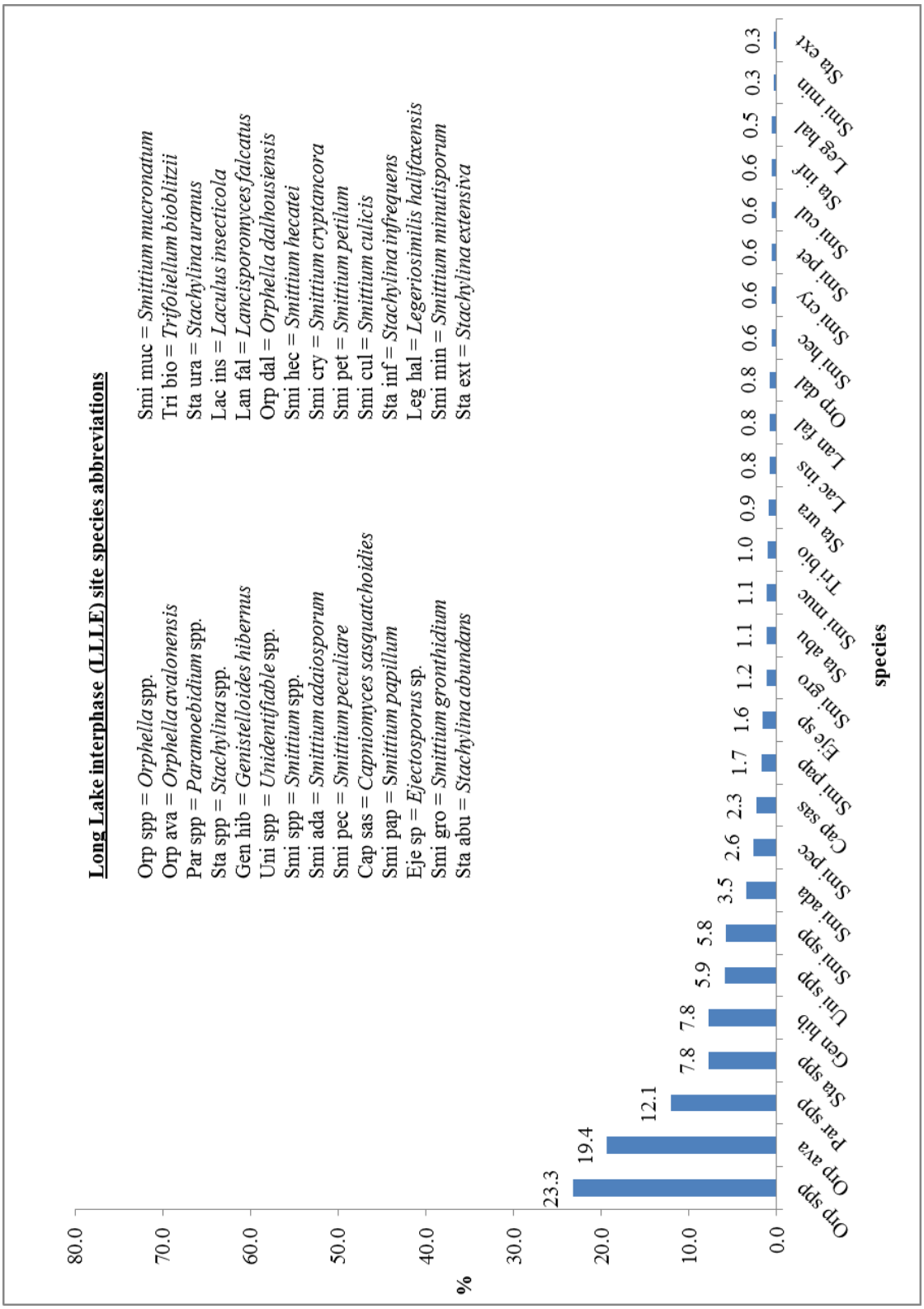


Figure 113. Prevalence of Trichomycete species at LLLE



SPLE were *Stachylina* spp. and *Paramoebidium* spp. (Figs. 111 and 112). The prevalence of *Stachylina* spp. was 22.9% at GLLE and 14.9% at SPLE while *Paramoebidium* spp. was 4.3% at GLLE and 15.1% at SPLE (Figs. 111 and 112).

The next most common species at both sites was *Sm. papillum* with prevalence values of 9.2% at GLLE and 3.1% at SPLE. *Laculus insecticola* was prevalent (9.2%) at GLLE which is the type locality (William and Strongman, 2011), but was also found at SPLE at a much lower prevalence (0.2%). Interestingly, this species is also reported from one lotic site (TLLO) and the interphase site (LLLE) (Fig. 109 and 113). *Smittium* spp. from chironomids was described from both sites with a prevalence of 5.4% and 0.5% at GLLE and SPLE respectively (Figs. 111 and 112). *Sm. bulbosporophorus* was rare (> 2%), but found at both GLLE (0.3%) and SPLE (1.6%) (Figs.111 and 112).

There were five taxa unique to GLLE (Fig. 111) all collected with rare prevalences including three newly described species *Sm. guttisporum* (2.0%), *St. serpula* (1.4%) and *St. zeppelin* (1.1%). *Sm. dipterorum* (0.9%); and *Sm. insulare* (0.6%) were the other two taxa collected only at this site. There were no new species reported only from SPLE, but seven of the twelve taxa collected here had frequencies < 2% (Fig. 112).

The interphase site (LLLE) is distinctive because it contained a mixture of insect hosts from both lentic and lotic environments. There were 28 trichomycetes reported from this site (Fig. 113); eight of which were recovered from stoneflies, likely originating from the stream flowing into the lake at this site. *Orphella* spp. (22.3%), *O. avalonensis* (19.4%) *Genistelloides hibernus* Peterson, Lichtw. & Horn (7.8%) and *C. saquatchoides* (2.3%) were prevalent in these hosts, while *Ejectosporus* sp. (1.6%), *L. falcatus* (0.8%)

and *O. dalhousiensis* (0.8%) were rare. Two taxa (*Ejectosporus* sp. and *O. avalonensis*) were collected only from this interphase site. Five other taxa were found only at this site including four newly described species from chironomid larvae: *Sm. adaiosporum* (3.5%), *Sm. peculiare* (2.6%), *St. obviorarus* (0.3%), *St. extensiva* (0.3%) and *Sm. hecatei* (0.6%) reported previously only from Spain (Valle and Santamaria, 2004) (Fig. 113). The remaining 15 taxa reported from LLLE were also reported from at least one or more lotic or lentic sites with *Paramoebidium* spp. (12.1%) and *Stachylina* spp. (7.8%) encountered frequently while the rest were collected rarely (Figs.111-113).

Habitat Preference

There were a total of 57 taxa reported from the combined investigations of three lotic and two lentic sites with 7 taxa isolated from the interphase site only. Based on data from this study, these taxa can be separated into three categories, species only reported from lotic sites, species reported from only lentic sites and finally species that were collected from at least one lotic and one or more lentic sites. *Stachylina* spp., *Smittium* spp. and *Paramoebidium* spp. were all prevalent at each of the lotic and lentic sites (Figs 108-112). These three genera contain the most common trichomycete species known (Lichtwardt et al., 2001a).

There were 34 taxa collected from lotic sites only (Figs. 108-110) including the new genus *Bactromyces fluminalis* and five new species (*G. gemina*, *P. timberleaense*, *Sm. ampliboja*, *Sm. insolitum*, and *St. tanysoma*) as well as four species *Sm. nodifixum*, *St. euthena*, *Sm. pusillum* and *Sm. hecatei* previously known from Asia or Europe

(Lichtwardt et al., 2001a). *Pennella arctica*, and *C. sasquachoides* from the USA were also collected at lotic sites. Six species were reported from lentic habitats only (Figs. 111-112) consisting of a new genus for *Laculus insecticola*, three new species (*Sm. guttisporum*; *St. serpula*; and *St. zeppelin*) as well as *Smittium bulbosporophorus* previously described from Spain (Valle, 2004) and *Sm. dipterorum* with reports from Costa Rica, Dominican Republic and Mexico (Lichtwardt, 1997; Valle and Cafaro, 2010; Valle et al., 2011).

There were 18 taxa reported from what were considered 'lentic/lotic species' as they were collected from at least one lotic and one lentic site (Figs 108-112). These taxa included five new species: *Sm. cryptancora*, *Sm. petilum, St. abundans*, *St. somnisimilis* and *St. uranus*. Additionally, S*m. mucronatum* reported from Nova Scotia for the first time was also collected at both site types.

Finally, a group of five species could not definitely be assigned to any of the previous three categories and thus habitat preference does not exist or is unknown. These include four new species *Sm. adaiosporum*, *Sm. peculiare*, *St. extensiva* and *St. infrequens* and the fifth species, *Sm. minutisporum*.

Species composition in Ephemeroptera and Chironomidae

This section deals with trichomycete species composition and prevalence in mayflies (Ephemeroptera) and chironomids (Diptera). These hosts were selected because of their ubiquitous distribution at all site types (lotic, lentic, and interphase) whereas the Plecoptera and simuliids (Diptera) were not found at any lentic habitats during this study.

Seven taxa of trichomycetes (*Paramoebidium* spp., *L. insecticola*, *L. halifaxensis, T. bioblitzii*, *G. gemina*, *P. timberleaense,* and *Sm. aurensis*) were found in ephemeropteran hosts from one or more habitat (lotic, lentic or interphase). A *Spartiella* sp. was also recorded that is likely *S. aurensis,* but lacked mature spores so species identification could not be confirmed (Table 2). *Laculus insecticola*, *Paramoebidium* spp. and *L. halifaxensis* are the only species found in all three site types. Three species *Glotzia gemina*, *Pteromaktron timberleaense,* and *Spartiella aurensis* were only reported from lotic sites. *Spartiella* sp. was the only taxon reported solely from lentic sites and *T. bioblitzii* was the only species collected at both a lotic and the interphase site (Table 2).

Table 2. Prevalence of trichomycetes in ephemeropteran hosts at lotic, lentic and interphase sites within Halifax and Dartmouth, NS.

Species	Lotic	Lentic	Interphase
Paramoebidium spp.	9.5 ±12.9	9.5 ± 7.3	5.2
Laculus insecticola	0.1 ± 0.1	4.7 ± 6.4	0.8
Legeriosimilis halifaxensis	0.6 ± 0.8	2.5 ± 3.5	0.5
Trifoliellum bioblitzii	0.0	0.1± 0.1	1.0
Glotzia gemina	0.6 ± 1.0	0.0	0.0
Pteromaktron timberleaense	2.7 ± 4.7	0.0	0.0
Spartiella aurensis	1.0 ± 1.8	0.0	0.0
Spartiella sp.	0.0	0.9 ± 1.2	0.0

[1] Site abbreviations are Long Lake stream (LLLO), Long Lake (LLLE), Timberlea stream (TLLO), Governor's Lake (GLLE), Shubie stream (SPLO) and Shubie Park lake (SPLE). See Appendix I for details.

The second group of insect hosts examined in this section was midge (Chironomidae) larvae also found in lentic, lotic and interphase habitats. They contained 37 different taxa (Table 3). All were fungal trichomycetes belonging either to the genus *Smittium* (24 taxa) or *Stachylina* (13 taxa) with some taxa isolated from all three site

types (lotic, lentic and interphase) while others were recorded from a single type or a combination of two. Seven of the 24 *Smittium* spp. and 8 of the 13 *Stachylina* spp. were newly described species from Nova Scotia (William and Strongman, 2013a; 2013b; 2013c).

There were a total of eight taxa (*Stachylina* spp., *St. abundans*, *St. uranus*, *Smittium* spp., *Sm. cryptancora*, *Sm. gronthidium*, *Sm. papillum*, and *Sm. petilum*) of trichomycetes reported from all three habitat types (lotic, lentic, and interphase) and four species (*St. nana*, *St. subgrandis*, *St. grandispora*, and *St. somnisimilis*) reported from both lentic and lotic habitats (Table 3). Eleven species (*Sm. ampliboja*, *Sm. brevisporum*, *Sm. colboi*, *Sm. insolitum*, *Sm. nodifixum*, *Sm. pavocaudatum*, *Sm. pusillum*, *Sm. radiculans*, *Sm. simulii*, *St. euthena* and *St. tanysoma*) were collected from lotic sites only while there were seven species (*Sm. bulbosporophorus*, *Sm. culicis*, *Sm. dipterorum*, *Sm. guttisporum*, *Sm. insulare*, *Stachylina serpula*, and *St. zeppelin*) reported from lentic habitats only (Table 3).

Trichomycetes reported from only the interphase habitat consisted of five species *Sm. adaiosporum*, *Sm. hecatei*, *Sm. peculiare*, *St. extensiva* and *St. infrequens*. *Smittium mucronatum* and *Sm. microsporum* were both collected from the interphase site, but were also found in one other site type: lentic in the case of *Sm. mucronatum* and lotic for *Sm. microsporum* (Table 3). All species found in chironomids occurred at relatively low frequency (< 2%) except for unidentified *Stachylina* and *Smittium* spp., *St. abundans*,

Table 3. Prevalence of harpellids in chironomids from lotic, lentic and interphase sites in Halifax and Dartmouth, NS.

Species	Lotic	Lentic	Interphase
In 3 Habitats			
Stachylina spp.	19.7 ± 6.4	18.9 ± 5.7	7.8
Smittium spp.	2.5 ± 2.4	3.0 ± 3.4	5.8
Stachylina abundans	4.5 ± 3.8	2.2 ± 3.0	1.1
Smittium papillum	0.4 ± 0.8	3.9 ± 1.2	1.7
Smittium gronthidium	1.6 ± 2.0	0.6 ± 0.8	1.2
Smittium cryptancora	0.2 ± 0.4	0.7 ± 1.0	0.6
Stachylina uranus	0.2 ± 0.3	0.2 ± 0.2	0.9
Smittium petilum	0.1 ± 0.2	0.2 ± 0.2	0.6
Both lotic and lentic habitats			
Stachylina nana	3.0 ± 2.4	0.3 ± 0.4	0
Stachylina subgrandis	0.7 ± 0.7	0.9 ± 1.2	0
Stachylina grandispora	0.4 ± 0.1	1.0 ± 1.4	0
Stachylina somnisimilis	0.1 ± 0.2	1.0 ± 1.4	0
Lotic only			
Smittium radiculans	1.0 ± 0.8	0	0
Smittium nodifixum	0.5 ± 0.8	0	0
Smittium simulii	0.4 ± 0.1	0	0
Smittium pusillum	0.3 ± 0.6	0	0
Smittium pavocaudatum	0.2 ± 0.4	0	0
Smittium ampliboja	0.1 ± 0.2	0	0
Smittium brevisporum	0.1 ± 0.2	0	0
Smittium colboi	0.1 ± 0.2	0	0
Smittium insolitum	0.1 ± 0.2	0	0
Stachylina euthena	0.1 ± 0.2	0	0
Stachylina tanysoma	0.1 ± 0.2	0	0
Lentic only			
Smittium guttisporum	0	1.0 ± 1.4	0
Smittium bulbosporophorus	0	0.9 ± 0.9	0
Stachylina serpula	0	0.7 ± 1.0	0
Stachylina zeppelin	0	0.6 ± 0.8	0
Smittium culicis	0	0.6 ± 0.8	0
Smittium dipterorum	0	0.5 ± 0.6	0
Smittium insulare	0	0.3 ± 0.4	0
Interphase			
Smittium mucronatum	0	0.5 ± 0.8	1.1

Smittium minutisporum	0.5 ± 0.9	0	0.3
Smittium adaiosporum	0	0	3.5
Smittium peculiare	0	0	2.6
Stachylina infrequens	0	0	0.6
Smittium hecatei	0	0	0.6
Stachylina extensiva	0	0	0.3

[1] Site abbreviations are Long Lake stream (LLLO), Long Lake (LLLE), Timberlea stream (TLLO), Governor's Lake, Timberlea (GLLE), Shubie Park stream (SPLO) and Shubie Park lake (SPLE).

St. nana and *Sm. papillum* (Table 3).

In summary, trichomycetes from ephemeropteran and chironomid hosts were collected from each habitat type (lotic, lentic, and interphase) with some species found in only a single site type, whereas others were found in two or three site types. The interphase habitat, considered different than either the lotic or lentic habitats due to the presence of lotic insects found in an otherwise lentic habitat, had a mixture of trichomycetes including some reported only from this site, but also species found in either a lotic, lentic, or both habitats as well.

Watershed Comparisons

The study was designed to sample in equivalent lentic and lotic sites within three replicate watersheds, two in Halifax (Woodens River and Long Lake) and the other in Dartmouth (Shubenacadie). Since these taxa occurred in all site types, comparison of trichomycetes in Ephemeroptera and Chironomidae from combined habitat types should shed light on the nature of trichomycete distribution at the watershed level. Overall, Long

Lake with 57 taxa and Woodens River (55) were numerically similar and had nearly twice the species richness than that seen in the Shubenacadie watershed, with 28 taxa.

Watershed distribution of trichomycete taxa from ephemeropteran hosts consisted of five taxa reported from Shubenacadie, four taxa from Woodens River and three taxa from Long Lake (Table 4). *Laculus insecticola* and *L. halifaxensis* were the only two species collected from all three watersheds. *Trifoliellum bioblitzii* was isolated from both Long Lake and Shubenacadie while the remaining two taxa, *G. gemina* and *Spartiella* sp. occurred at only one watershed (Table 4).

In the chironomid hosts dissected, the distribution of trichomycete taxa among watersheds revealed 26 taxa isolated from Woodens River that included ten taxa common to all three watersheds and ten collected only at Woodens River (Table 4). There were five species shared between Woodens River and Long Lake and one species shared with

Table 4. Prevalence by watershed of ephemeropteran harpellids

Species	Long Lake	Woodens River	Shubenacadie
Laculus insecticola	0.4 ± 0.6	4.7 ± 6.4	0.1 ± 0.1
Legeriosimilis halifaxensis	1.0 ± 0.7	0.2 ± 0.2	2.5 ± 3.5
Paramoebidium spp.	14.9 ± 0.6	4.3 ± 0.1	7.3 ± 10.3
Trifoliellum bioblitzii	0.5 ± 0.7	0.0	0.1 ± 0.1
Glotzia gemina	0.0	0.0	0.9 ± 1.2
Pteromaktron timberleaense	0.0	4.1 ± 5.8	0.0
Spartiella aurensis	0.0	1.6 ± 2.2	0.0
Spartiella sp.	0.0	0.0	0.9 ± 1.2

[1] Site abbreviations are Long Lake stream (LLLO), Long Lake (LLLE), Timberlea stream (TLLO), Governor's Lake (GLLE), Shubie Park stream (SPLO) and Shubie Park lake (SPLE). See Appendix I for details.

the Shubenacadie watershed (Table 5). Twenty-four taxa were recovered from the Long lake watershed including eight species only from that system, and one species (*Sm. mucronatum*) found at both

Shubenacadie and Woodens River (Table 5). The 14 taxa reported from Shubenacadie represented the fewest reported from any of the three watersheds. There were only two species (*Sm. culicis* and *Sm. nodifixum*) reported exclusively from Shubenacadie.

To summarize the watershed distribution of trichomycetes, ephemeropteran derived trichomycetes displayed a somewhat consistent degree of taxa found in each of the three watersheds with four taxa collected from Long Lake, five taxa from Woodens River and six from Shubenacadie. In contrast, analysis of trichomycetes from chironomid hosts in each of the watersheds suggests a close association between Woodens River (26 species) and Long lake (24 species) watersheds in terms of overall taxa, with 15 shared species but only five in common exclusively at these two watersheds. The Shubenacadie watershed reported close to 50 % fewer taxa collected from chironomids (14 species) but ten of these were also found in both the Woodens River and Long Lake watersheds.

Table 5. Prevalence by watershed of chironomid harpellids

Species	Long Lake	Woodens River	Shubenacadie
Stachylina spp.	15.7 ± 11.3	17.6 ± 7.5	19.0 ± 5.9
Smittium spp.	3.5 ± 3.2	5.3 ± 0.1	0.7 ± 0.3
Stachylina abundans	4.9 ± 5.2	4.2 ± 0.2	0.5 ± 0.6
Stachylina nana	2.3 ± 3.3	0.5 ± 0.2	2.0 ± 2.9
Smittium radiculans	1.0 ± 1.4	0.4 ± 0.5	0.2 ± 0.3
Smittium papillum	0.9 ± 1.2	2.2 ± 1.3	2.4 ± 3.4
Smittium gronthidium	0.6 ± 0.8	2.5 ± 2.0	0.5 ± 0.6
Stachylina uranus	0.4 ± 0.6	0.2 ± 0.2	0.2 ± 0.3
Stachylina grandispora	0.2 ± 0.2	1.2 ± 1.2	0.2 ± 0.3
Smittium simulii	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.3
Smittium cryptancora	0.3 ± 0.4	1.1 ± 0.5	0.0
Smittium petilum	0.3 ± 0.4	0.3 ± 0.0	0.0
Stachylina subgrandis	0.3 ± 0.5	1.5 ± 0.3	0.0
Smittium minutisporum	0.1 ± 0.2	0.8 ± 1.1	0.0
Stachylina somnisimilis	0.2 ± 0.2	1.0 ± 1.4	0.0
Smittium mucronatum	0.6 ± 0.8	0.0	0.5 ± 0.8
Smittium bulbosporophorus	0.0	0.2 ± 0.2	0.8 ± 1.1
Smittium colboi	0.2 ± 0.2	0.0	0.0
Smittium pusillum	0.5 ± 0.7	0.0	0.0
Stachylina euthena	0.2 ± 0.2	0.0	0.0
Smittium adaiosporum	1.7 ± 2.4	0.0	0.0
Smittium hecatei	0.3 ± 0.4	0.0	0.0
Smittium peculiare	1.3 ± 1.8	0.0	0.0
Stachylina extensiva	0.1 ± 0.2	0.0	0.0
Stachylina infrequens	0.3 ± 0.4	0.0	0.0
Smittium pavocaudatum	0.0	0.4 ± 0.5	0.0
Smittium insolitum	0.0	0.2 ± 0.2	0.0
Stachylina tanysoma	0.0	0.2 ± 0.2	0.0
Smittium ampliboja	0.0	0.2 ± 0.2	0.0
Smittium brevisporum	0.0	0.2 ± 0.2	0.0
Smittium dipterorum	0.0	0.5 ± 0.6	0.0
Smittium insulare	0.0	0.3 ± 0.4	0.0
Smittium guttisporum	0.0	1.0 ± 1.4	0.0
Stachylina serpula	0.0	0.7 ± 1.0	0.0
Stachylina zeppelin	0.0	0.6 ± 0.8	0.0
Smittium nodifixum	0.0	0.0	0.7 ± 1.0
Smittium culicis	0.0	0.0	0.6 ± 0.8

[1] Site abbreviations are Long Lake stream (LLLO), Long Lake (LLLE), Timberlea stream (TLLO), Governor's Lake, Timberlea (GLLE), Shubie Park stream (SPLO) and Shubie Park lake (SPLE).

Discussion (Ecological)

The initial intent of this study was to survey the trichomycete population existing within three watersheds, comparing trichomycete presence and community structure in lentic and lotic habitats in three replicate watersheds. Several months into data collection the lentic site (LLLE) within the Long Lake watershed was sampled and found to have plecopteran (stoneflies) hosts not normally associated with lentic habitats; therefore, this site was treated as unique in the data analysis and designated an 'interphase' site due to the flushing of stream dwelling insects into this otherwise lentic site.

The insect abundance collected from all six sites was comparable with the exception of TLLO where roughly twice the numbers of insects were collected. The discrepancy between this site and the other six is due largely to vastly more ephemeropteran hosts collected at this site (Table 1). The insect dissections from all six sites were relatively uniform so an overabundance of hosts from any particular site would not skew the trichomycete numbers of taxa at any given site.

The trichomycetes reported from each site over time periods (seasons) are a conservative estimate of taxa based on the incidence of unidentifiable species and the taxa only identified to genus. These unidentifiable species and taxa were detected in 0.4 to 23.7 percent of examined hosts (Figs. 108-113).

The lotic taxa reported within seasons (Fig. 105) clearly indicate that both LLLO and TLLO were considerably richer in trichomycete taxa than SPLO, which at times had five times fewer taxa. Considering that there were actually more insects collected at SPLO than at LLLO and with dissection numbers at SPLO only slightly lower (Table 1)

than either LLLO or TLLO, the discrepancy is likely not related to host availability. The physical make-up of all three sites was similar and there were 19 collections taken at all three sites, so the difference in richness in LLLO and TLLO compared to SPLO is obvious, but unexplained by these parameters.

In terms of consistency, both LLLO and TLLO maintained somewhat consistent numbers of taxa over each time period of the study with 14-17 taxa at LLLO and 16-21 taxa at TLLO whereas SPLO displayed a wider range with fewer taxa (4-11). The slight variability in taxa numbers at both LLLO and TLLO was not enough to suggest a level of seasonality over the course of the four seasons with one duplicate season; however, taxa numbers at SPLO exhibited a steady increase of four to eight, then 11 taxa over the course of the first three seasons before returning to five taxa for the duplicate season (Figs. 105-107). Seasonality has been demonstrated in trichomycetes (Hapsari et al., 2009; Siri et al., 200; López Lastra et al., 2003; Beard and Adler, 2002) but in this study, with only one of the sites demonstrating fluctuations in taxa numbers over four seasons, there is not enough data to suggest there is seasonal distribution of trichomycete taxa at the three watersheds studied.

The consistent numbers of trichomycetes in all hosts at the TLLO and LLLO sites over the seasons was unexpected because both plecopteran and simuliid hosts were much more prevalent in the fall, winter and spring time periods. The absence of these insect species during summer months did not influence the overall number of taxa at these times perhaps indicating an increase in chironomid or ephemeropteran borne taxa over this period.

Comparison of the two lentic sites (GLLE and SPLE) clearly shows that GLLE had significantly more taxa than SPLE during all time periods. Total insect dissections were slightly higher at GLLE (806) compared to SPLE (577), but this is not considered to be enough to account for the discrepancy between the sites. Three of the four seasonal time periods reported twice the trichomycete taxa at GLLE than at SPLE, with one season (Jan-Apr 2011) where taxa numbers were closer (six and four taxa at GLLE and SPLE respectively). This time period also happened to have the fewest taxa at both lentic sites, most likely due to ice cover reducing lentic collections for most of that season. For both GLLE and LLLE, May to Aug 2011 was the period of highest trichomycete richness. The data suggest differences in total trichomycete taxa, but no evidence exists that demonstrates seasonality in the occurrence of trichomycetes in general.

The interphase site, also subject to ice cover followed a similar pattern of taxa accumulation during the course of the study. The highest number of taxa was reported from May to Aug 2011 as with the lentic sites while the second least amount of taxa were collected during Jan to Apr 2011 corresponding to the lowest taxa numbers at the lentic sites. This result could be due to restricted insect sampling over the winter months.

Direct comparison of species richness at individual sites indicated a much higher number of trichomycete taxa recovered from lotic sites compared to the lentic sites. Three lotic sites (LLLO, TLLO, and SPLO) produced an average of 26.3 ± 9.3 taxa per site where the two lentic sites (GLLE and SPLE) averaged 16.5 ± 6.4 over the course of the 17 month study.

The overall picture of trichomycete distribution among individual sites over four

time periods, suggests lotic sites are considerably richer than lentic sites; however, further examination indicates that the discrepancy between lotic and lentic may not be as pronounced as initially suggested by taxa numbers. Both the LLLO and TLLO sites were far more replete with trichomycetes than either of the two lentic sites (GLLE and SPLE) despite the lotic site at SPLO reporting far fewer taxa that either LLLO or TLLO. In addition, the lotic sites displayed a much more consistent level of taxa occurring during each time period with LLLO and TLLO maintaining a minimum of 15 taxa over all periods, while SPLO demonstrated a much higher degree of inconsistency much like the lentic sites. The lowest taxa reported from the lentic sites (Jan-Apr-2011) at least in part could be due to reduced sampling effort in winter months. A contributing factor to lower numbers of trichomycetes reported from lentic sites may be that lotic sites drew from four groups of hosts (Ephemeroptera, Plecoptera, Simuliidae and Chironomidae) whereas lentic sites only drew from two groups (Ephemeroptera and Chironomidae). Chironomids were by far the richest source of trichomycetes contributing 37 taxa while ephemeropterans were quite low containing only eight taxa. Plecopteran and Simuliidae derived trichomycetes contributed a total of 12 and eight taxa respectively to the lotic sites that are excluded from the number of lentic trichomycetes (Figs. 108-113).

The average number of trichomycete taxa reported from the three lotic sites over the four time periods was 14.7 ± 1.1 compared to the average from two lentic sites of 8.4 ± 2.9. Since the site at LLLE is considered an interphase site, comparisons between lentic and lotic sites only considered data from the Woodens River and Shubenacadie watersheds. The taxa numbers from these two watershed sites lowers the ratio of lotic

versus lentic taxa to slightly closer numbers, 13.8 ± 1.7 vs. 8.4 ± 2.9 for lotic and lentic sites respectively. Additionally, subtracting the 20 taxa reported from plecopteran and simuliid hosts closes the gap between lotic and lentic ratios significantly to a much more similar 9.7 ± 1.8 from lotic sites vs. 8.4 ± 2.9 from lentic.

Lentic trichomycete taxa have been collected from many sites worldwide (Lichtwardt, 2004); however many more taxa have been reported from lotic sites which is largely due to the fact that lotic sites have been explored more than lentic habitats (Strongman and White, 2007). The appeal of lotic habitats to investigators can be attributed to the presence of plecopteran and simuliid hosts that are not normally found in lentic habitats (Merritt and Cummins, 1996) and also because it is generally easier to sample from streams rather than lakes where sampling is often relegated to collections close to shore. Lotic dwelling plecopteran and simuliid hosts have contributed 26 and 25 species respectively to harpellid trichomycetes described to date (Lichtwardt, 2004). Ephemeropteran trichomycetes have contributed 40 species (Lichtwardt, 2004), but do not seem to colonize their hosts nearly as frequently as those in plecopterans and simuliids. Chironomid hosts have contributed 136 species (Lichtwardt, 2004) of trichomycetes and are by far the most prolific of the four hosts targeted in this study. It seems clear that assessment of trichomycete diversity and inventory should include as many host types as possible over extended periods of time to capture all the hosts exploiting the habitat.

In terms of prevalence, the sites were originally chosen to compare lotic to lentic systems, but introduction of non-resident insects into the LLLE site slightly skews the

study by eliminating one of the original lentic sites. Despite losing a direct comparison from this site, it is apparent from taxa collected at the two true lentic sites that both lentic and lotic habitats have a resident flora of trichomycetes present at varying levels of prevalence. The data also suggest that there are taxa of trichomycetes with an ability to populate all three site types (lentic, lotic and interphase) while other taxa were found specifically in either a lotic or a lentic or the interphase habitat only, with other taxa still found in some combination of two different site types. For the sake of this discussion, taxa are assigned to one of three categories, those only observed in lotic habitats (including those from plecopteran hosts found in the interphase site), those only from lentic, or those observed in both habitat types. In total, 32 of the 64 taxa of trichomycetes were reported from lotic habitats only, six from lentic habitats only and 26 reported as taxa found in both lotic and lentic habitats.

Beginning with taxa considered as 'lotic only' it is apparent that these trichomycetes existed in these habitats with varied prevalence values. In some cases, taxa occurred with a very high prevalence such as the case of *Harpella melusinae* encountered at all three lotic sites in 50 to 75% of all simuliid hosts dissected at a particular site. On the other end of the spectrum, *Smittium simulii* was also recovered at all three lotic sites, but with much lower prevalence ranging between 0.3 - 0.5% at each of the three lotic sites. Intermediate to these two extremes of prevalence, the bulk of taxa were recovered in this study were below 2% of dissected hosts. Specifically, 16 of 29 taxa recovered at LLLO, 19 of 34 at TLLO and 10 of 16 at SPLO were considered rare.

The collection and prevalence of the new species *Stachylina abundans* serves as

an indication that trichomycete prevalence can fluctuate from one site to the next despite an abundance of hosts. The prevalence of *St. abundans* ranged from a high of 8.6% at LLLO to a low of 0.9% at SPLO (Figs.108-113) This species is particularly useful as it was the only species to be collected from five of the six sites aside from taxa identified only to genus. At LLLO and TLLO there were an almost identical number of chironomid dissections conducted (304 and 302 respectively) with *St. abundans* reported in 26 hindguts at LLLO and only 12 at TLLO. At SPLO, 202 chironomid dissections were conducted with only two hindguts containing *St. abundans*. Finally, this species was also collected at LLLE where it only appeared in four of 384 chironomid hindguts and also at GLLE in 15 of 350 dissections.

The collection of *Stachylina abundans* at these five sites represents one of only seven species collected from all habitat types (lotic, lentic and interphase) while also suggesting a varied rate of prevalence across all site types. Of particular interest is that the prevalence at the interphase site was quite similar to the low observed at SPLO, while both GLLE and TLLO representing a lentic and a lotic site exhibited an intermediate prevalence between the high and low ratios. In this case, implications suggest that *St. abundans* is capable of existence in either
lotic or lentic habitats including the interphase habitat and exists with varied prevalence seemingly irrespective of the nature of the habitat.

Harpella melusinae was reported with an extremely high prevalence and this confirms published records of high prevalence of this species in simuliid hosts (Labeyrie et al., 1996; Hapsari et al., 2009). Considered to have a worldwide temperate distribution

(Lichtwardt, 2004), *H. melusinae* has been previously documented in Nova Scotia (White and Strongman, 2012a) and from my study where it was reported from 558 simuliid hosts of 844 examined, so strengthens the notion that it is widespread and frequently encountered. Further support comes from a study from Northern Thailand (Hapsari et al., 2009) that documented *H. melusinae* in 80 to 100% of 1260 simuliid hosts examined.

Aside from the high incidence of *H. melusinae* at the three lotic sites the prevalence of any particular species occurring at any site was markedly lower. The next highest prevalence at lotic sites was for *Orphella avalonensis* and *Lancisporomyces falcatus* at 19.4 and 14.1 % respectively. Both of these taxa were isolated from plecopteran hosts and are considered to be species found only in lotic habitats despite the fact that *O. avalonensis* was reported only from the interphase site (LLLE).

Unlike *H. melusinae* with worldwide distribution, *O. avalonensis* has been described previously from streams in Newfoundland (Lichtwardt et al., 2001b) Ontario (Strongman and White, 2008) and Nova Scotia (Strongman and White, 2006) in Canada and from the Great Smoky Mountains in Tennessee, USA (White, et al., 2006) while *L. falcatus* has only been reported previously from streams in Nova Scotia (Strongman and White, 2006). In this study, *O. avalonensis* was reported from only one site (LLLE) and *L. falcatus* was collected from two (LLLO and LLLE). Interestingly, the prevalence of *L. falcatus* at both sites was extremely different, reported frequently from LLLO at 14.1% and at only 0.9% from LLLE. The incidence at LLLE was likely due to overflow from a connected stream, where appropriate hosts were flushed into the system. Upon further inspection, it was recorded that the plecopteran hosts of *L. falcatus* were flushed into

LLLE on at least nine occasions that led to 129 dissections, and yet it was recovered only once contrasting with the incidence at LLLO where 384 hosts were dissected with 54 positive for the presence of *L. falcatus*. There were no collections made from the stream inundating LLLE so the incidence of trichomycetes in stoneflies there is unknown.

Despite numerous reports of taxa from lentic sites (21 from GLLE and 12 from SPLE), it is difficult to assign any particular species as strictly occurring in lentic habitats. From the combined reports of trichomycetes at the two lentic sites (including records of the same taxa at each site) there were 26 individual taxa to consider. Sixteen of these taxa were also reported from lotic sites in this study (Figs. 108-112), while five taxa (*Sm. bulbosporophorus, Sm. culicis Sm. dipterorum Sm. insulare*, and *Sm. mucronatum*,) although not seen at the lotic sites sampled, have previously been reported from lotic habitats in other studies (Valle and Santamaria, 2004; Valle and Santamaria, 2010; Strongman, 2007; Strongman and White, 2008). These 16 species include *Laculus insecticola* which is a special case discussed later. Three species (*St. serpula*, *St. zeppelin* and *Sm. guttisporum*) with prevalence ranging between 0.3 to 2.0% were described as new from these lentic sites so it is unknown if they are exclusively found in lentic habitats.

The only taxa found exclusively in lentic sites was *Spartiella* sp., a genus which currently has three described species, all collected previously in lotic systems (Lichtwardt, 1997; Strongman, 2010; White and Strongman, 2012b), and *Trifoliellum bioblitzii*, originally described from Kearney Lake in Nova Scotia (Strongman and White, 2011). This study presents a second record confirming *T. bioblitzii* is a lentic species.

To truly assess species for their preference for lotic, lentic or both habitat types taxa that occurred only at the interphase were examined. The introduction of stream dwelling stoneflies recovered from the interphase site at LLLE contributed seven taxa at this site considered to be lotic based solely on the fact that their plecopteran hosts are almost exclusively found only in lotic systems. Since these taxa (*Ejectosporus* sp., *Capniomyces sasquachoides*, *Genistelloides hibernus*, *Lancisporomyces falcatus*, *Orphella* spp., *O. avalonensis*, and *O. dalhousiensis*) were not found in either of the two lentic sites, it is probable that collection at this interphase site was a direct result of their hosts being flushed from the adjoining stream. It is also likely that taxa documented from some ephemeropteran and chironomid hosts at this site may also have originated from the stream. Fifteen taxa (*Paramoebidium* spp., unidentifiable spp., *Smittium* spp., *Sm. cryptancora*, *Sm. culicis*, *Sm. gronthidium*, *Sm. hecatei*, *Sm. minutisporum Sm. mucronatum*, *Sm. papillum*, *Sm. petilum*, *Stachylina* spp. *St. abundans*, *St. uranus* and *Legeriosimilis halifaxensis*) have all been either described from a lotic site in this study or have been reported from different lotic sites (Lichtwardt, 2004), and four taxa (*Sm. adaiosporum*, *Sm. peculiare*, *St. infrequens*, and *St. extensiva*) are newly described species lacking sufficient information to confidently assign them exclusively to one habitat. The final two species found at the interphase site were *T. bioblitzii* which is reported only from a lake (Strongman and White, 2011) and *L. insecticola*, also known only from lakes in Nova Scotia (William and Strongman, 2012).

Laculus insecticola, reported from both lentic sites (GLLE and SPLE) and the interphase site (LLLE) had a high prevalence at GLLE (collected in 41 of 441 hindguts

examined) which was not observed in any of the other two sites where it was found. *Laculus insecticola* was also collected at one lotic site (TLLO), but only from one hindgut (a Caenid) of 590 mayflies (several families) so might indicate a rare drift from a connected lake into the stream given the host (a caenid mayfly) was not collected from this site again.

As well as having a high prevalence at GLLE, *L. insecticola* was found exclusively in caenid mayflies and was collected year round at this site. The apparent persistence of *L. insecticola* at GLLE throughout all sampled months is potentially connected to the persistence of caenid mayflies at this site. The species might be host specific since other ephemeropteran hosts (Baetidae, Heptageniidae, Leptophlebiidae and Ephemerellidae) were collected without any reports of *L. insecticola*. Most trichomycetes have only ordinal host specificity according to Lichtwardt (2004) so perhaps this species shows host specificity at the family level. Reports of trichomycetes exhibiting host specificity must be prefaced with the fact that in general, insufficient collections and lack of studies of related arthropod species may be responsible for the presumed restricted host range (Lichtwardt, 2004).

Some insects can be multivoltine which could create a constant habitat for particular species of trichomycetes. Stable year-round populations of trichomycetes have been observed in *Harpella melusinae* and *Stipella vigilans* both from black flies (Taylor, 1992). Hosts with this life cycle feature may house trichomycetes adapted more specifically to the host genus or species.

The potential of taxa being present at any particular site and simply not

encountered from hosts selected for dissection is a distinct possibility. This phenomena becomes more tangible upon review of the majority of taxa reported having prevalence values below 2% of hosts dissected. Many more hosts were collected than were able to be dissected and in some cases less than 5% of one of the four target hosts from any one collection date were examined. Compounding this low prevalence of taxa reported with the often low percentage of hosts actually dissected could easily result in some taxa simply being missed.

For example, *Smittium simulii* was documented at all three lotic sites; however, at a very low prevalence ranging from 0.3-0.5%. At each site, *Sm. simulii* was isolated from only one hindgut accounting for only three reports from over 836 chironomid hindguts examined from the three lotic sites. Previously described worldwide from lentic and lotic systems (Lichtwardt et al., 2001), *Sm. simulii* was not found in any lentic collections in this study. With such a low prevalence exhibited from lotic sites, it is possible this species and others were present in lentic habitats and missed. *Smittium simulii* has been observed infrequently from chironomid hosts (Lichtwardt, 2004), displaying ordinal specificity and indicating a stronger host preference for simuliid guts perhaps. This ordinal specificity with a perceived preference may account for the particular low prevalence of *S. simulii* in my collections.

Pteromaktron timberleaense was collected at only one lotic site (TLLO) and is representative of a large number of trichomycete species (32) that were only found at one particular site (Appendix II). In contrast to *L. insecticola*, *P. timberleaense* displayed a seasonal trend rather than the persistent presence observed for *L. insecticola* despite also

having a steady supply of heptageniid hosts available year round. Collections of *P. timberleaense* from TLLO were reported on only five dates during mid-summer to early fall (July to October) at TLLO in both 2010 and 2011, but were observed in many heptageniid guts at these times. Despite the lack of seasonality in terms of overall taxa at each site of this study, individual trichomycete species can display seasonality in their presence at particular sites. Beard and Adler (2002) described the changes in abundance in three harpellid trichomycetes from black fly larvae collected at streams in South Carolina, and Nelder et al. (2010) showed that several harpellids exhibited seasonality and some degree of species preference in the black fly hosts they colonized. Seasonality was also seen in an eccrinid endobiont (*Enterobryus halophilus* Cronin & Johnson) of mole crabs (Hernández Roa and Cafaro, 2012).

The prevalence of all trichomycete taxa at the six individual sites sampled during this study was calculated. The taxa from ephemeropteran and chironomid hosts were selected because these hosts were present at all sites. Only collected at lotic sites normally, trichomycetes from plecopteran and simuliid hosts were excluded from the analysis.

There were 45 taxa recovered from chironomid and ephemeropteran hosts and these were used to compare trichomycete community structure in these two habitat types. The data collected from this study (Tables 2 and 3) in tandem with habitat reports from the current literature (Lichtwardt, 2004) show these taxa separate into 22 species now documented from both habitat types, ten from lotic only, three from lentic only and ten newly described species isolated from only one site type. It is likely the majority of

harpellid trichomycetes from non-predaceous dipteran and ephemeropteran hosts may have the ability to live in both habitats but often species may be better adapted to either a lotic or lentic system. It is also possible that some trichomycetes might show host specificity such that they are restricted to a habitat type based on restrictions in host distribution.

Ephemeropteran hosts contributed eight taxa to the inventory. Three taxa were collected from all three site types (lentic, lotic, and interphase) with *Paramoebidium* spp. the most prevalent of any taxon and is likely a collection of species contributing to this high incidence. Of particular interest is the almost identical prevalence observed from both lotic and lentic environments 9.5 ±12.9 and 9.5 ± 7.3 respectively, suggesting no habitat preference, but the data were variable. *Legeriosimilis halifaxensis*, also reported from all three site types, was collected commonly at lentic sites, but rarely from lotic sites. Originally reported from a lake in Nova Scotia (Strongman and White, 2011), this species was detected in 20 different mayfly hindguts at the Shubie Park stream (SPLO) expanding the range for *L. halifaxensis* into lotic systems. *Laculus insecticola*, as discussed previously, is considered a lentic only species based on its host, despite it being reported from one hindgut at a lotic site and from the interphase site (Figs. 109 and 113).

Three other trichomycetes from ephemeropteran hosts were collected only in lotic sites, two considered rare *Glotzia gemina* (0.6 ± 1.0) and *Spartiella aurensis* (1.0 ± 1.8) while the third; *Pteromaktron timberleaense* at 2.7 ± 4.7 was more common. In each case, these taxa were collected from only one of the three lotic sites perhaps indicating a level of site specificity within lotic systems.

The remaining two taxa, *Spartiella* sp. and *Trifolielum bioblitzii* were both rarely encountered in lentic sites. *Trifolielum bioblitzii* was also collected at the interphase site more frequently than in lentic sites suggesting that this otherwise lentic species might be part of the lentic flora of trichomycetes at LLLE and not necessarily flushed from the adjoining stream. *Spartiella* sp. was collected rarely and only at lentic sites. This may in fact be *Spartiella aurensis* which was found at lotic sites and this suggests that it can exist in both lentic and lotic habitats. *Spartiella aurensis* was described from Nova Scotia in Baetidae and Ephemerellidae hosts (White and Strongman, 2012b) from both streams and lakes respectively. In my study, *S. aurensis* from Baetidae hosts was collected from streams, while *Spartiella* sp. from Ephemerellidae hosts was collected only in lakes. Since Baetidae hosts are usually collected only from streams, and Ephemerellidae are found in both, *S. aurensis* displays an ability to select hosts perhaps related to what type of system (lotic or lentic) they are found in.

There were many more taxa of trichomycetes (37) collected from chironomids hosts, with varying levels of prevalence, that were subdivided into five different categories (Table 3). The predominant observation was that these taxa were rare. There were 11 species collected from lotic sites only and seven species from lentic only that all had prevalence values < 2%. The seven trichomycetes from the interphase site only had two species considered common (*Smittium adaiosporum* and *Smittium peculiare*), but the uncertainty of whether or not they were lentic or lotic in nature is compounded by the fact they are new species without previous information.

Of the 37 taxa in chironomids, 24 were observed from lotic habitats and an

additional six species, independent of this study, have been reported from lotic habitats in other parts of the world (Lichtwardt, 2004). The remaining seven species not reported from lotic sites in this study are all new species that were largely infrequently encountered and thus there is limited ecological information. These new species in some cases were observed from only one hindgut. With such low prevalence, these species might have been present in lotic waters as well but missed. The three new species reported from lentic sites only (*Smittium guttisporum*, *Stachylina serpula* and *Stachylina zeppelin*) ranged in prevalence from 0.6-1.0% while the other four were observed only at the interphase site and thus their habitat preference is unknown.

Three of the four species collected from both lentic and lotic sites were rare with only *Stachylina nana* considered common, but only from lotic collections while rarely seen in lentic collections. *Stachylina nana* has been reported worldwide from France, Thailand, Ontario, Prince Edward Island and Nova Scotia (Lichtwardt, 1984; Hapsari et al., 2009; Strongman and White, 2008; Strongman, 2007; Strongman, 2010; White and Strongman, 2012a) but data on prevalence was not reported.

Lentic sites had 20 taxa and an additional two more have been observed from lentic habitats from around the globe (*Stachylina euthena* and *Smittium minutisporum*) for a total of 22 of 37 with an additional three new species with identical 0.1% prevalence values observed only in lotic environments. The 22 taxa reported from lentic sites are only drawn from two sites whereas the 30 taxa from lotic drew from three sites. Simple division in this case suggests that the lentic sites actually might have slightly more taxa.

Finally, eight taxa were reported from all three site types (lotic, lentic and

interphase) with only four collected frequently. *Stachylina* spp. and *Smittium* spp. were common at all three site types, but likely represent a collection of species leaving only *St. abundans* and *Sm. papillum* as common species from this category. *Stachylina abundans*, a new species known only from this study was found in five of the six sites and may be the most common trichomycete species from chironomids regardless of habitat type. *Smittium papillum*, recently described from streams in Nova Scotia (White and Strongman, 2012a) was collected commonly at lentic sites, but was rarely encountered from lotic hosts in my study.

Reports of trichomycetes from chironomid and ephemeropteran hosts were used to compare the taxa distribution among the three watersheds. Like the comparison of prevalence in ephemeropteran and chironomid hosts only, plecopteran and simuliid hosts were excluded from consideration since they normally are not associated with lentic sites. The flushing of plecopteran hosts was observed at the interphase site from the Long Lake watershed thus making direct comparison between this watershed and either Shubenacadie or Woodens River difficult.

The eight taxa of trichomycetes reported from ephemeropteran hosts displayed a distribution of taxa with five from Woodens River and six from Shubenacadie. The even distribution of these few taxa suggests that ephemeropteran trichomycetes are evenly distributed in both watersheds, but there were relatively few taxa so it is difficult to draw any firm conclusions on their distribution.

The 37 trichomycetes taxa from chironomid hosts (less the eight species collected only from the Long lake watershed) suggest that Woodens River was a much richer

source of taxa than Shubenacadie. A direct comparison between these two watersheds reported 26 taxa from Woodens River and only 14 from Shubenacadie (Table 5). Since the substrates at each watershed were similar, the sampling effort identical and available hosts (Table 1) were comparable; the observation that Shubenacadie was less species rich than the Woodens River watershed seems obvious, but is difficult to explain. The two groups (Ephemeroptera and Chironomidae) examined had many species of trichomycetes with more similarity in ephemeropteran numbers as well as species composition than chironomids, but perhaps the fact that many species were rare explains the differences. In general, the two watersheds shared similar species, but also reported species found in only one or the other suggesting that trichomycete community structure and distribution varies among watersheds.

The identification of so many different taxa of trichomycetes from this study expands the previously known distribution of these organisms and adds to the accumulating data from Nova Scotia. Currently there are 262 harpellid trichomycetes (Lichtward, 2004) reported worldwide and since 2006 when initial reports from Nova Scotia began, 67 species have been reported from this small province in Canada, 37 of which are new species. The identification of so many new species within a short time span in Nova Scotia suggests that the current number of worldwide trichomycete taxa may only be a small proportion of what is actually there.

Conclusion

As a consequence of intensive sampling over 17 months, 19 new species of trichomycetes have been added to the growing inventory of taxa along with identification of an additional 45 previously described taxa. The distribution of taxa within lotic and lentic systems indicates that there are more trichomycete taxa from lotic sites based largely on the fact that these systems draw from more families of hosts. Though the species composition may differ, taxa drawn only from hosts found in both systems suggest that the number of trichomycete species found in lotic and lentic systems is the same.

The prevalence of the documented taxa varied, but in most cases trichomycetes should be considered rare with many species in < 2 % of hosts dissected and very few species eclipsed

10 %. Seasonality and host specificity was observed in several species and could contribute to the low prevalence of most species. Varied prevalence among sites was also observed for trichomycetes found in more than one site, often occurring commonly in one site and rarely in others.

Trichomycetes were present year round in all three watersheds sampled, but indications are that species richness within different watersheds can fluctuate.

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Appendix I. Sampling dates, numbers of insects collected and number dissected (brackets) from the lotic sites (LLLO, TLLO, SPLO) lentic sites (GLLE, SPLE) and interphase site (LLLE) from the Halifax Regional Municipality.

Long Lake lotic site (LLLO)				
Date	Ephemeroptera	Plecoptera	Chironomidae	Simuliidae
11 Aug 2010	0 (0)	0 (0)	150 (27)	19 (17)
7 Sep 2010	46 (27)	11 (11)	75 (27)	11 (11)
27 Sep 2010	92 (27)	12 (12)	23 (15)	1 (1)
17 Oct 2010	82 (27)	7 (7)	79 (27)	0 (0)
12 Nov 2010	73 (27)	15 (15)	12 (11)	7 (7)
28 Nov 2010	75 (27)	46 (27)	3 (3)	13 (13)
17 Dec 2010	43 (27)	16 (16)	0 (0)	16 (16)
23 Jan 2011	3 (3)	36 (27)	1 (1)	123 (27)
21 Feb 2011	49 (27)	53 (27)	4 (4)	144 (27)
2 Mar 2011	7 (7)	18 (18)	0 (0)	22 (18)
7 Apr 2011	20 (20)	45 (27)	0 (0)	117 (27)
27 Apr 2011	66 (27)	28 (24)	7 (7)	36 (25)
18 May 2011	26 (23)	28 (26)	19 (19)	3 (3)
8 Jun 2011	25 (25)	62 (27)	151 (27)	45 (26)
29 Jul 2011	20 (19)	35 (27)	123 (27)	5 (5)
20 Jul 2011	0 (0)	4 (4)	131 (27)	41 (27)
21 Aug 2011	1 (1)	0 (0)	166 (27)	4 (4)
10 Sep 2011	25 (23)	7 (7)	9 (9)	3 (3)
1 Oct 2011	37 (27)	52 (27)	2 (2)	2 (2)
22 Oct 2011	6 (6)	6 (6)	11 (11)	0 (0)
10 Nov 2011	16 (16)	57 (27)	24 (24)	4 (4)
7 Dec 2011	24 (23)	22 (18)	32 (23)	23 (15)
Totals	**736 (408)**	**568 (384)**	**1030 (318)**	**639 (278)**
Timberlea lotic site (TLLO)				
Date	Ephemeroptera	Plecoptera	Chironomidae	Simuliidae
17 Aug 2010	21 (21)	0 (0)	11 (11)	71 (27)
13 Sep 2010	109 (27)	0 (0)	7 (7)	40 (27)
3 Oct 2010	199 (27)	1 (1)	3 (3)	5 (5)
24 Oct 2010	295 (27)	0 (0)	16 (16)	2 (2)
15 Nov 2010	361 (27)	2 (2)	29 (22)	1 (1)
5 Dec 2010	326 (27)	25 (25)	18 (17)	32 (26)
28 Dec 2010	272 (27)	98 (27)	73 (27)	52 (27)

30 Jan 2011	192 (27)	133 (27)	15 (15)	167 (27)
16 Feb 2011	138 (27)	156 (27)	36 (27)	239 (27)
10 Mar 2011	122 (27)	177 (27)	25 (22)	182 (27)
13 Apr 2011	66 (27)	243 (27)	11 (11)	301 (27)
3 May 2011	251 (27)	37 (25)	20 (20)	24 (23)
25 May 2011	231 (27)	2 (2)	15 (15)	29 (25)
15 Jun 2011	371 (27)	1 (1)	20 (19)	0 (0)
6 Jul 2011	171 (27)	4 (4)	35 (26)	24 (20)
27 Jul 2011	105 (27)	1 (1)	10 (10)	0 (0)
28 Aug 2011	64 (27)	0 (0)	7 (7)	4 (4)
17 Sep 2011	98 (27)	0 (0)	15 (15)	7 (7)
8 Oct 2011	191 (27)	0 (0)	2 (2)	1 (1)
29 Oct 2011	298 (27)	4 (4)	1 (1)	6 (6)
19 Nov 2011	288 (27)	56 (27)	9 (9)	21 (18)
11 Dec 2011	343 (27)	81 (27)	4 (4)	32 (23)
Totals	**4512 (590)**	**1021 (254)**	**382 (306)**	**1240 (347)**

Shubie Park lotic site (SPLO)

Date	Ephemeroptera	Plecoptera	Chironomidae	Simuliidae
30 Aug 2010	30 (24)	0 (0)	55 (27)	0 (0)
19 Sep 2010	53 (27)	0 (0)	10 (10)	1(1)
12 Oct 2010	59 (27)	1(1)	0 (0)	0 (0)
31 Oct 2010	45 (27)	0 (0)	2 (2)	0 (0)
21 Nov 2010	125 (27)	12 (12)	4 (4)	4 (4)
12 Dec 2010	77 (27)	18 (18)	4 (4)	12 (12)
11 Jan 2011	79 (27)	50 (26)	7 (6)	116 (27)
11 Feb 2011	79 (27)	72 (26)	11 (11)	244 (27)
25 Feb 2011	71 (27)	61 (27)	13 (13)	469 (27)
23 Mar 2011	74 (27)	38 (26)	4 (4)	356 (27)
20 Apr 2011	156 (27)	8 (8)	14 (14)	27 (19)
11 May 2011	57 (27)	6 (6)	5 (5)	1 (1)
1 Jun 2011	50 (27)	7 (7)	54 (27)	57 (27)
22 Jun 2011	40 (26)	0 (0)	8 (8)	7 (7)
13 Jul 2011	9 (9)	0 (0)	184 (27)	1 (1)
14 Aug 2011	59 (27)	0 (0)	86 (27)	0 (0)
4 Sep 2011	74 (27)	0 (0)	7 (7)	0 (0)
24 Sep 2011	127 (27)	0 (0)	15 (15)	2 (2)
14 Oct 2011	85 (27)	0 (0)	0 (0)	1 (1)
5 Nov 2011	81 (27)	1 (1)	0 (0)	5 (5)
26 Nov 2011	231 (27)	11 (11)	2 (2)	18 (18)

19 Dec 2011	110 (27)	53 (25)	7 (7)	30 (20)
Totals	**1771 (574)**	**338 (194)**	**492 (220)**	**1353 (226)**
Long Lake interphase site (LLLE)				
Date	Ephemeroptera	Plecoptera	Chironomidae	Simuliidae
11 Aug 2010	0 (0)	0 (0)	23(19)	0 (0)
12 Sep 2010	2 (2)	0 (0)	9 (9)	0 (0)
28 Sep 2010	10 (10)	1 (1)	31 (25)	0 (0)
20 Oct 2010	35 (26)	2 (2)	19 (19)	0 (0)
14 Nov 2010	9 (9)	4 (4)	0 (0)	0 (0)
1 Dec 2010	353 (27)	26 (26)	23 (22)	0 (0)
22 Dec 2010	539 (27)	71 (27)	4 (4)	0 (0)
Jan 2011	NC	NC	NC	NC
Feb 2011	NC	NC	NC	NC
Mar 2011	NC	NC	NC	NC
10 Apr 2011	197 (27)	80 (27)	30 (23)	0 (0)
1 May 2010	316 (27)	47 (27)	10 (10)	0 (0)
22 May 2011	142 (27)	0 (0)	48 (27)	0 (0)
12 Jun 2011	69 (27)	0 (0)	86 (27)	0 (0)
3 Jul 2011	27 (26)	0 (0)	45 (27)	0 (0)
24 Jul 2011	19 (15)	0 (0)	11 (11)	0 (0)
23 Aug 2011	3 (3)	0 (0)	10 (10)	0 (0)
13 Sep 2011	30 (23)	0 (0)	7 (7)	0 (0)
4 Oct 2011	41 (27)	0 (0)	57 (27)	0 (0)
25 Oct 2011	186 (27)	0 (0)	32 (25)	0 (0)
16 Nov 2011	364 (27)	4 (4)	76 (27)	0 (0)
9 Dec 2011	539 (27)	12 (12)	35 (27)	0 (0)
Totals	**2881(384)**	**247 (130)**	**556(347)**	**0**
Governor's Lake lentic site (GLLE)				
Date	Ephemeroptera	Plecoptera	Chironomidae	Simuliidae
24 Aug 2010	14 (14)	n/a	31 (24)	n/a
16 Sep 2010	11 (11)	n/a	6 (6)	n/a
5 Oct 2010	5 (5)	n/a	6 (6)	n/a
27 Oct 2010	21 (21)	n/a	1 (1)	n/a
19 Nov 2010	38 (23)	n/a	15 (14)	n/a
8 Dec 2010	31 (25)	n/a	15 (15)	n/a
3 Jan 2011	31 (25)	n/a	19 (19)	n/a
Jan 2011	NC	NC	NC	NC
Feb 2011	NC	NC	NC	NC
Mar 2011	NC	NC	NC	NC

17 Apr 2011	109 (27)	n/a	8 (8)	n/a
8 May 2011	69 (27)	n/a	27 (25)	n/a
29 May 2011	62 (27)	n/a	14 (14)	n/a
19 Jun 2011	68 (27)	n/a	37 (23)	n/a
10 Jul 2011	39 (27)	n/a	47 (25)	n/a
10 Aug 2011	71 (26)	n/a	24 (23)	n/a
30 Aug 2011	71 (27)	n/a	67 (27)	n/a
21 Sep 2011	344 (27)	n/a	69 (27)	n/a
13 Oct 2011	506 (27)	n/a	36 (27)	n/a
1 Nov 2011	440 (27)	n/a	55 (27)	n/a
22 Nov 2011	380 (27)	n/a	44 (27)	n/a
15 Dec 2011	336 (27)	n/a	64 (27)	n/a
Totals	**2646 (447)**	**n/a**	**584 (365)**	**n/a**
Shubie Park lentic site (SPLE)				
Date	Ephemeroptera	Plecoptera	Chironomidae	Simuliidae
27 Aug 2010	1 (1)	n/a	9 (9)	n/a
22 Sep 2010	26 (25)	n/a	6 (5)	n/a
13 Oct 2010	72 (27)	n/a	6 (5)	n/a
3 Nov 2010	102 (27)	n/a	3 (3)	n/a
24 Nov 2010	240 (27)	n/a	12 (12)	n/a
15 Dec 2010	115 (27)	n/a	5 (5)	n/a
15 Jan 2011	NC	NC	NC	NC
Jan 2011	NC	NC	NC	NC
Feb 2011	NC	NC	NC	NC
Mar 2011	NC	NC	NC	NC
24 Apr 2011	172 (27)	n/a	12 (12)	n/a
15 May 2011	106 (27)	n/a	14 (14)	n/a
5 Jun 2011	64 (27)	n/a	12 (10)	n/a
26 Jun 2011	33 (25)	n/a	41 (27)	n/a
17 Jul 2011	1 (1)	n/a	32 (25)	n/a
17 Aug 2011	1 (1)	n/a	11 (11)	n/a
7 Sep 2011	53 (27)	n/a	5 (5)	n/a
28 Sep 2011	116 (27)	n/a	0 (0)	n/a
15 Oct 2011	145 (27)	n/a	8 (8)	n/a
9 Nov 2011	209 (27)	n/a	3 (3)	n/a
1 Dec 2011	565 (27)	n/a	19 (19)	n/a
22 Dec 2011	560 (27)	n/a	15 (15)	n/a
Totals	**2581 (404)**	**n/a**	**213 (188)**	**n/a**

NC = no collections due to ice cover. n/a = no hosts collected

Appendix II. Harpellales and Amoebidales collected from lentic, lotic and interphase habitats.

Species	Host	Location	Collection dates
Bactromyces fluminalis	Plecoptera	LLLO [1]	23 Jan 11; 10 Nov 11
Capniomyces sasquatchoides	Plecoptera	TLLO	16 Feb 11; 13 Apr 11
		SPLO	23 Mar 11
		LLLO	7 Apr 11
		LLLE	1 Dec 10
Ejectosporus sp.	Plecoptera	LLLE	1 Dec 10
Genistelloides hibernus	Plecoptera	LLLE	1 Dec 10; 22 Dec 10
		TLLO	16 Feb 11
Glotzia gemina	Plecoptera	SPLO	30 Aug 10; 19 Sep 10; 12 Oct 10; 13 Jul 11; 4 Sep 11
Harpella melusinae	Diptera: Simuliidae	LLLO	12 Nov 10; 28 Nov 10; 17 Dec 10; 23 Jan 11; 21 Feb 11; 2 Mar 11; 7 Apr 11; 27 Apr 11; 18 May 11; 8 Jun 11; 29 Jun 11; 20 Jul 11; 21 Aug 11; 10 Sep 11; 7 Dec 11
		TLLO	17 Aug 10; 3 Oct 10; 5 Dec 10; 28 Dec 10; 30 Jan 11; 16 Feb 11; 10 Mar 11; 13 Apr 11; 25 May 11; 6 Jul 11; 17 Sep 11; 19 Nov 11; 11 Dec 11
		SPLO	11 Jan 11; 11 Feb 11; 25 Feb 11; 23 Mar 11; 22 Jun 11; 13 July 11; 5 Nov 11; 26 Nov 11; 19 Dec 11
Laculus insecticola	Ephemeroptera	GLLE	9 Jul 10; 24 Aug 10; 8 Dec 10; 17 Apr 11; 8 May 11; 29 May 11; 19 Jun 11; 10 Jul 11; 10 Aug 11; 30 Aug 11; 21 Sep 11; 13 Oct 11

		LLLE	3 Jul 11; 24 Jul 11
		SPLE	5 Jun 11
		TLLO	13 Sep 10
Lancisporomyces anguilliformis	Plecoptera	LLLO	22 Oct 11
Lancisporomyces falcatus	Plecoptera	LLLO	17 Oct 10; 12 Nov 10; 28 Nov 10; 17 Dec 10; 23 Jan 11; 2 Mar 11; 7 Apr 11; 10 Nov 11; 7 Dec 11
		LLLE	9 Dec 11
Lancisporomyces nemouridarum	Plecoptera	LLLO	18 May 11; 8 Jun 11
Lancisporomyces sp.	Plecoptera	LLLO	7 Sep 10; 27 Sep 10; 1 Oct 11; 7 Dec 11
Legeriosimilis halifaxensis	Ephemeroptera	LLLO	7 Sep 10; 17 Dec 10; 7 Apr 11; 7 Dec 11
		LLLE	10 Apr 11; 12 Jun 11
		TLLO	5 Dec 10; 13 Apr 11
		SPLE	22 Sep 10; 15 Dec 10; 24 Apr 11; 15 May 11; 9 Nov 11; 3 Nov 10; 26 Jun 11
Orphella avalonensis	Plecoptera	LLLE	1 Dec 10; 22 Dec 10; 10 Apr 11; 1 May 11
Orphella dalhousiensis	Plecoptera	LLLO	17 Oct 10; 23 Jan 11; 1 Oct 11; 10 Nov 11
		LLLE	1 Dec 10
Orphella sp.	Plecoptera	LLLO	7 Sep 10; 27 Sep 10; 17 Oct 10; 12 Nov 10; 28 Nov 10; 21 Feb 11; 2 Mar 11; 10 Sep 11; 1 Oct 11; 22 Oct 11; 10 Nov 11; 7 Dec 11
		LLLE	20 Oct 10; 1 Dec 10; 22 Dec 10; 10 Apr 11; 1 May 11; 9 Dec 11
		TLLO	5 Dec 10; 28 Dec 10; 30 Jan 11; 16 Feb 11; 10 Mar 11; 6 Jul 11; 19 Nov 11; 11 Dec 11

Paramoebidium cassidula	Plecoptera	LLLO	7 Sep 10; 1 Oct 11; 10 Nov 11; 7 Dec 11
		TLLO	19 Nov 11
Paramoebidium curvum	Diptera: Simuliidae	TLLO	25 May 11
Paramoebidium spp.	Ephemeroptera, Plecoptera,	LLLO	7 Sep 10; 27 Sep 10; 17 Oct 10; 12 Nov 10; 28 Nov 10; 17 Dec 10; 23 Jan 11; 21 Feb 11; 2 Mar 11; 7 Apr 11; 27 Apr 11; 8 Jun 11; 29 Jun 11; 10 Sep 11; 1 Oct 11; 22 Oct 11; 10 Nov 11; 7 Dec 11
	Diptera: Simuliidae	LLLE	1 Dec 10; 22 Dec 10; 10 Apr 11; 1 May 11; 12 Jun 11; 3 Jul 11; 9 Dec 11
		TLLO	3 Oct 10; 24 Oct 10; 15 Nov 10; 5 Dec 10; 28 Dec 10; 30 Jan 11; 16 Feb 11; 10 Mar 11; 13 Apr 11; 3 May 11; 25 May 11; 15 Jun 11; 6 Jul 11; 27 Jul 11; 29 Oct 11; 19 Nov 11; 11 Dec 11
		GLLE	5 Oct 10; 27 Oct 10; 19 Nov 10; 8 Dec 10; 3 Jan 11; 17 Apr 11; 22 Nov 11; 15 Dec 11
		SPLO	21 Nov 10; 12 Dec 10; 11 Jan 11; 11 Feb 11; 25 Feb 11; 23 Mar 11; 5 Nov 11; 26 Nov 11; 19 Dec 11
		SPLE	13 Oct 10; 3 Nov 10; 24 Nov 10; 15 Dec 10; 24 Apr 11; 5 Jun 11; 26 Jun 11; 9 Nov 11; 1 Dec 11; 22 Dec 11
***Pennella arctica*	Diptera: Simuliidae	TLLO	5 Dec 10; 28 Dec 10; 30 Jan 11; 10 Mar 11
		LLLO	21 Feb 11; 7 Apr 11; 27 Apr 11
Pennella digitata	Diptera: Simuliidae	LLLO	7 Apr 11; 27 Apr 11
Pennella simulii	Diptera: Simuliidae	TLLO	5 Dec 10; 16 Feb 11; 25 May 11; 19 Nov 11; 11 Dec 11

Pennella sp.	Diptera: Simuliidae	LLLO	21 Feb 11; 7 Apr 11; 27 Apr 11; 21 Aug 11; 10 Nov 11; 7 Dec 11
	Diptera: Simuliidae	TLLO	17 Aug 10; 13 Sep 10; 3 Oct 10; 5 Dec 10; 28 Dec 10; 30 Jan 11; 16 Feb 11; 10 Mar 11; 13 Apr 11; 25 May 11; 6 Jul 11; 28 Aug 11; 17 Sep 11; 19 Nov 11; 11 Dec 11
	Diptera: Simuliidae	SPLO	25 Feb 11
Pteromaktron timberleaense	Ephemeroptera	TLLO	17 Aug 10; 13 Sep 10; 3 Oct 10; 24 Oct 10; 6 Jul 11; 27 Jul 11; 28 Aug 11; 17 Sep 11; 8 Oct 11
Simuliomyces microsporus	Diptera: Simuliidae	LLLO	16 Feb 11; 7 Apr 11; 27 Apr 11
		TLLO	30 Jan 11; 10 Mar 11; 11 Dec 11
Smittium adaiosporum	Diptera: Chironomidae	LLLE	28 Sep 10
Smittium ampliboja	Diptera: Simuliidae; Chironimidae	TLLO	3 Oct 10
Smittium brevisporum	Diptera: Chironomidae	TLLO	17 Aug 10
**Smittium bulbosporophorus*	Diptera: Chironimidae	SPLE	22 Sep 10
	Diptera: Chironomidae	GLLE	8 May 11
Smittium colboi	Diptera: Chironomidae	LLLO	10 Sep 11

Smittium cryptancora	Diptera: Chironomidae	LLLE	11 Aug 10
		TLLO	25 May 11
		GLLE	30 Aug 11; 21 Sep 11
Smittium culicis	Diptera: Simuliidae; Chironomidae	LLLE	3 Jul 11
		SPLO	1 Jun 11
***Smittium dipterorum*	Diptera: Chironomidae	GLLE	29 May 11
Smittium gronthidium	Diptera: Chironomidae	LLLE	3 Jul 11; 24 Jul 11
		TLLO	5 Dec 10; 28 Dec 10; 30 Jan 11; 16 Feb 11; 13 Apr 11; 3 May 11
		GLLE	19 Jun 11
		SPLO	1 Jun 11
Smittium guttisporum	Diptera: Chironomidae	GLLE	5 Oct 10; 8 May 11
**Smittium hecatei*	Diptera: Chironomidae	LLLE	10 Apr 11
Smittium insolitum	Diptera: Chironomidae	TLLO	10 Mar 11
Smittium insulare	Diptera: Chironomidae	GLLE	24 Aug 10
***Smittium minutisporum*	Diptera: Chironomidae	LLLE	12 Jun 11

		TLLO	15 Jun 11; 27 Jul 11
***Smittium mucronatum*	Diptera: Chironomidae	SPLE	24 Apr 11
		LLLE	1 May 11
**Smittium nodifixum*	Diptera: Chironomidae	SPLO	23 Mar 11
Smittium papillum	Diptera: Chironomidae	LLLE	11 Aug 10; 23 Aug 11
		SPLE	15 May 11; 5 Jun 11; 26 Jun 11
		GLLE	10 Jul 11; 10 Aug 11; 30 Aug 11; 21 Sep 11
		TLLO	27 Jul 11; 17 Sep 11
Smittium pavocaudatum	Diptera: Chironomidae	TLLO	13 Sep 10
Smittium peculiare	Diptera: Chironomidae	LLLE	12 Jun 11; 16 Nov 11; 9 Dec 11
Smittium petilum	Diptera: Chironomidae	LLLE	12 Sep 10
		TLLO	3 May 11
		GLLE	30 Aug 11
**Smittium pusillum*	Diptera: Chironomidae	LLLO	7 Sep 10
Smittium radiculans	Diptera: Chironimidae	TLLO	15 Nov 10; 30 Jan 11
		LLLO	2 Mar 11; 27 Apr 11; 7 Apr 11
		SPLO	1 Jun 11

Smittium simulii	Diptera: Chironomidae	LLLO	23 Jan 11
		SPLO	20 Apr 11
		TLLO	3 May 11
Smittium spp.	Diptera: Chironomidae	LLLO	7 Apr 11; 27 Apr 11
		LLLE	11 Aug 10; 28 Sep 10; 10 Apr 11; 1 May 11; 12 Jun 11; 3 Jul 11
		TLLO	13 Sep 10; 28 Dec 10; 16 Feb 11; 10 Mar 11; 13 Apr 11; 3 May 11; 17 Sep 11
		GLLE	19 Nov 10; 3 Jan 11; 8 May 11; 19 Jun 11; 10 Jul 11; 10 Aug 11; 30 Aug 11
		SPLO	11 Feb 11; 25 Feb 11
		SPLE	26 Jun 11
Spartiella aurensis	Ephemeroptera	TLLO	17 Aug 10; 13 Sep 10; 3 Oct 10; 28 Aug 11; 17 Sep 11
Spartiella sp.	Ephemeroptera	SPLE	22 Dec 11
Stachylina abundans	Diptera: Chironomidae	LLLO	11 Aug 10; 7 Sep 10; 21 Aug 11
		LLLE	12 Sep 10
		TLLO	17 Aug 10; 13 Sep 10; 24 Oct 10; 15 Nov 10; 15 Jun 11; 17 Sep 11; 11 Dec 11
		GLLE	24 Aug 10; 10 Aug 11; 30 Aug 11; 21 Sep 11; 1 Nov 11
		SPLO	13 Jul 11
**Stachylina euthena*	Diptera: Chironomidae	LLLO	7 Sep 10
Stachylina extensiva	Diptera: Chironomidae	LLLE	3 Jul 11

Stachylina grandispora	Diptera: Chironomidae	LLLO	17 Oct 10
		TLLO	30 Jan 11
		GLLE	3 Jan 11; 29 May 11; 19 Jun 11; 10 Aug 11; 1 Nov 11; 15 Dec 11
		SPLO	13 Jul 11
Stachylina infrequens	Diptera: Chironomidae	LLLE	1 May 11; 24 Jul 11
Stachylina nana	Diptera: Chironomidae	LLLO	11 Aug 10; 20 Jul 11; 21 Aug 11
		TLLO	24 Oct 10
		GLLE	21 Sep 11
		SPLO	19 Sep 10; 13 Jul 11; 24 Sep 11
Stachylina serpula	Diptera: Chironomidae	GLLE	30 Aug 11; 21 Sep 11
Stachylina somnisimilis	Diptera: Chironomidae	GLLE	19 Jun 11; 30 Aug 11; 13 Oct 11
		LLLO	22 Oct 11
Stachylina subgrandis	Diptera: Chironomidae	GLLE	16 Sep 10; 10 Jul 11; 10 Aug 11
		LLLO	11 Aug 10; 8 Jun 11
		TLLO	13 Sep 10; 13 Sep 10; 3 May 11; 27 Jul 11
Stachylina tanysoma	Diptera: Chironomidae	TLLO	17 Sep 11
Stachylina uranus	Diptera: Chironomidae	LLLE	10 Apr 11; 3 Jul 11

		GLLE	21-Sep-11
		SPLO	13 Jul 11
Stachylina zeppelin	Diptera: Chironomidae	GLLE	21 Sep 11; 13 Oct 11
Stachylina spp.	Diptera: Chironomidae	LLLO	11 Aug 10; 7 Sep 10; 27 Sep 10; 17 Oct 10; 21 Feb 11; 18 May 11; 8 Jun 11; 29 Jun 11; 20 Jul 11; 21 Aug 11; 10 Sep 11; 22 Oct 11; 10 Nov 11
		LLLE	11 Aug 10; 28 Sep 10; 20 Oct 10; 1 Dec 10; 10 Apr 11; 1 May 11; 22 May 11; 12 Jun 11; 3 Jul 11; 24 Jul 11; 13 Sep 11; 25 Oct 11
		TLLO	17 Aug 10; 13 Sep 10; 3 Oct 10; 24 Oct 10; 15 Nov 10; 28 Dec 10; 30 Jan 11; 16 Feb 11; 10 Mar 11; 13 Apr 11; 3 May 11; 25 May 11; 15 Jun 11; 27 Jul 11; 28 Aug 11; 19 Nov 11
		GLLE	24 Aug 10; 5 Oct 10; 8 Dec 10; 3 Jan 11; 8 May 11; 29 May 11; 19 Jun 11; 10 Jul 11; 10 Aug 11; 30 Aug 11; 21 Sep 11; 13 Oct 11; 1 Nov 11; 22 Nov 11; 15 Dec 11
		SPLO	30 Aug 10; 19 Sep 10; 11 Jan 11; 11 Feb 11; 25 Feb 11; 23 Mar 11; 11 May 11; 1 Jun 11; 22 Jun 11; 13 Jul 11; 14 Aug 11; 4 Sep 11; 24 Sep 11; 5 Nov 11; 26 Nov 11; 19 Dec 11
		SPLE	22 Sep 10; 26 Jun 11; 17 Jul 11
Trifoliellum bioblitzii	Ephemeroptera	LLLE	12 Jun 11; 3 Jul 11
		SPLE	26 Jun 11
unidentifiable spp.[2]	Ephemeroptera, Chironomidae, Plecoptera, Simuliidae	LLLO	7 Sep 10; 27 Sep 10; 17 Oct 10; 21 Feb 11; 2 Mar 11; 7 Apr 11; 27 Apr 11; 18 May 11; 8 Jun 11; 29 Jun 11; 1 Oct 11; 10 Nov 11; 7 Dec 11
		LLLE	11 Aug 10; 28 Sep 10; 20 Oct 10; 1 Dec 10; 22 Dec 10; 10 Apr 11; 1 May 11; 22 May 11; 12 Jun 11; 3 Jul 11; 24 Jul 11; 23 Aug 11;

	13 Sep 11; 25 Oct 11; 16 Nov 11; 9 Dec 11
TLLO	17 Aug 10; 13 Sep 10; 3 Oct 10; 24 Oct 10; 15 Nov 10; 5 Dec 10; 28 Dec 10; 30 Jan 11; 16 Feb 11; 10 Mar 11; 13 Apr 11; 3 May 11; 25 May 11; 6 Jul 11; 28 Aug 11; 17 Sep 11; 11 Dec 11
GLLE	24 Aug 10; 16 Sep 10; 27 Oct 10; 19 Nov 10; 8 Dec 10; 3 Jan 11; 17 Apr 11; 8 May 11; 29 May 11; 19 Jun 11; 10 Jul 11; 10 Aug 11; 30 Aug 11; 21 Sep 11; 13 Oct 11; 1 Nov 11; 22 Nov 11; 15 Dec 11
SPLO	19 Sep 11; 11 Jan 11; 11 Feb 11; 25 Feb 11; 23 Mar 11; 20 Apr 11; 1 Jun 11; 22 Jun 11; 24 Sep 11
SPLE	13 Oct 10; 24 Nov 10; 24 Apr 11; 15 May 11; 5 Jun 11; 17 Jul 11; 15 Oct 11

[1] Site abbreviations are Long Lake Provincial Park stream (LLLO), Long Lake Provincial Park lake (LLLE), Timberlea stream (TLLO), Governor's Lake, Timberlea (GLLE), Shubie Park stream (SPLO) and Shubie Park lake (SPLE). See Appendix I for details.

[2] unidentifiable spp. were fungi that could not be identified due to lack of spore morphological characteristics.

Bolded taxa names are new species described from this study.

[*] new continental record

[**] new geographical record