4-12-2010

First Documentation of the European Gut Fungus, *Ephemerellomyces*, and Other Insect Associated Endosymbionts in the Dry Creek Drainage, Boise, Idaho

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Abstract

Trichomycetes, a former class of obligate endosymbiotic fungi, are now recognized as an ecological group that inhabits the gut(s) of immature insects. Though the biodiversity and geographical distribution of trichomycetes are worldwide, our knowledge of the group in the Pacific Northwest is limited due to the few researchers conducting studies on them. Dry Creek drainage in Boise, Idaho was selected in the winter of 2009-10 as a potential site to find gut fungi. This initial survey provides the first account of Ephemerellomyces aquilonius, a species previously documented and studied only in Norway. Ephemerellomyces aquilonius (a monotypic genus) is a member of the Harpellales, an order of trichomycetes, and was dissected from the digestive tracts of mayflies (Ephemeroptera) collected from the creek. Amongst the various aquatic habitats surveyed and hosts recovered, gut fungi were also documented in stoneflies (Plecoptera) and black flies (Diptera: Simuliidae). Our investigation provides a significant new record of the very rare and unusual taxon, E. aquilonius, previously only known in Western Europe. The success of this brief survey demonstrates promise for further discoveries of gut fungi at this site, in Idaho and the Pacific Northwest. This disjunct distribution bridges an enormous geographical divide, although the species actually may be more widespread than earlier anticipated. Future collections and research on specimens from Dry Creek, with sequence data to be generated in our laboratory will be eagerly awaited as we place this unusual species in our expanding molecular-based phylogenies. We highlight here the morphology of E. aquilonius and some of the other endosymbionts found there.

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Boise State University, Department of Biological Sciences, Undergraduate Conference 2010

Dry Creek Drainage, Boise, ID

Some of the Trichomycetes Team at Collection Site, near Bogus Basin Rd, showing the foothills during winter collecting event (left to right):

Eric Tretter, Daleesa Cole, Emma Wilson, Prasanna Kandel and Yan Wang.

Overview

Trichomycetes, a former class of obligate endosymbiotic fungi, are now recognized as an ecological group that inhabits the gut(s) of immature insects. Though the biodiversity and geographical distribution of trichomycetes are worldwide, our knowledge of the group in the Pacific Northwest is limited due to the few researchers conducting studies on them. Dry Creek drainage in Boise, Idaho was selected in the winter of 2009-10 as a potential site to find gut fungi. This research contributes to the broader goals of our lab's REVSYS program, for Phylogenetic and Revisionary Systematics. The study of trichomycetes by interested students of these fungi is greatly aided by online resources found at http://www.nhm.ku.edu/~fungi which includes world literature, interactive Lucid Keys, a Trichomycetes Database and the online monograph: The Trichomycetes: Fungal Associates of Arthropods. It provided an interactive backdrop as the following fungi were being documented from Dry Creek.

Materials and Methods

Several methods such as the collection of hosts, the maintenance and preservation of hosts, dissection techniques, and the preparation of specimens for microscopy were used to observe for the presence of Trichomycetes in insects collected from Dry Creek.

Aquatic sampling was with equipment such as wading boots, nets, pans, and sieves. Insects were collected from different parts of the creek, including fast flowing, slow moving or stagnant water near banks, leaflet zones and twigs at high flow. Stream emperature ranged from 0.5 C during the first trip to 2 C during the second trip. A useful technique to collect aquatic insects was using a net to catch the insects flowing downstream to the net while dredging up the creek bed. Insects were found under rocks, clinging to submerged branches, and leaf packs. Creek debris and contents from the sieves or nets was placed in shallow pans with water for examination. Collected specimens were then placed in petri dishes or zip lock bags for return to the lab.

In the lab, all insects were stored in a standard refrigerator (4-6 ^{o}C) in stream water except that black flies were placed in petri dishes with a circular Whatman filter paper moistened with distilled water. Insects were dissected within 48-72 hours, using a pair of mounted needles and fine forceps. The head was carefully removed and then both the hindgut and midgut retrieved for examination. The peritrophic matrix lining the midgut sometimes contained masses of algae and other ingested materials, so it was important to dissect further in order to detect any presence of fungal taxa. Hindgut linings required opening the gut to reveal the thalli attached inside. For the peritrophic matrix, once emptied, the transparent sleeve could be mounted directly on a slide for microscopic examination. Detached thalli or thalli still attached to gut linings were placed on a clean slide with 1-2 drops of distilled water and then a cover slip placed over the specimen and pictures were taken using a Nikon research microscope (magnifications from 10X to 60x). Slide-mounted specimens were then preserved with Lactophenol Cotton Blue. Hosts were preserved in 70% ethanol for use as vouchers or for subsequent identification. *Ephemerellomyces aquilonius* (a monotypic genus), a member of the Harpellales, an order of trichomycetes, and was dissected from the digestive tracts of mayflies (Ephemeroptera) collected from the creek. Amongst the various aquatic habitats surveyed and hosts recovered, gut fungi were also documented in stoneflies (Plecoptera) and black flies (Diptera, Simuliidae).

Mayfly (Ephemeroptera) Fungal Taxa

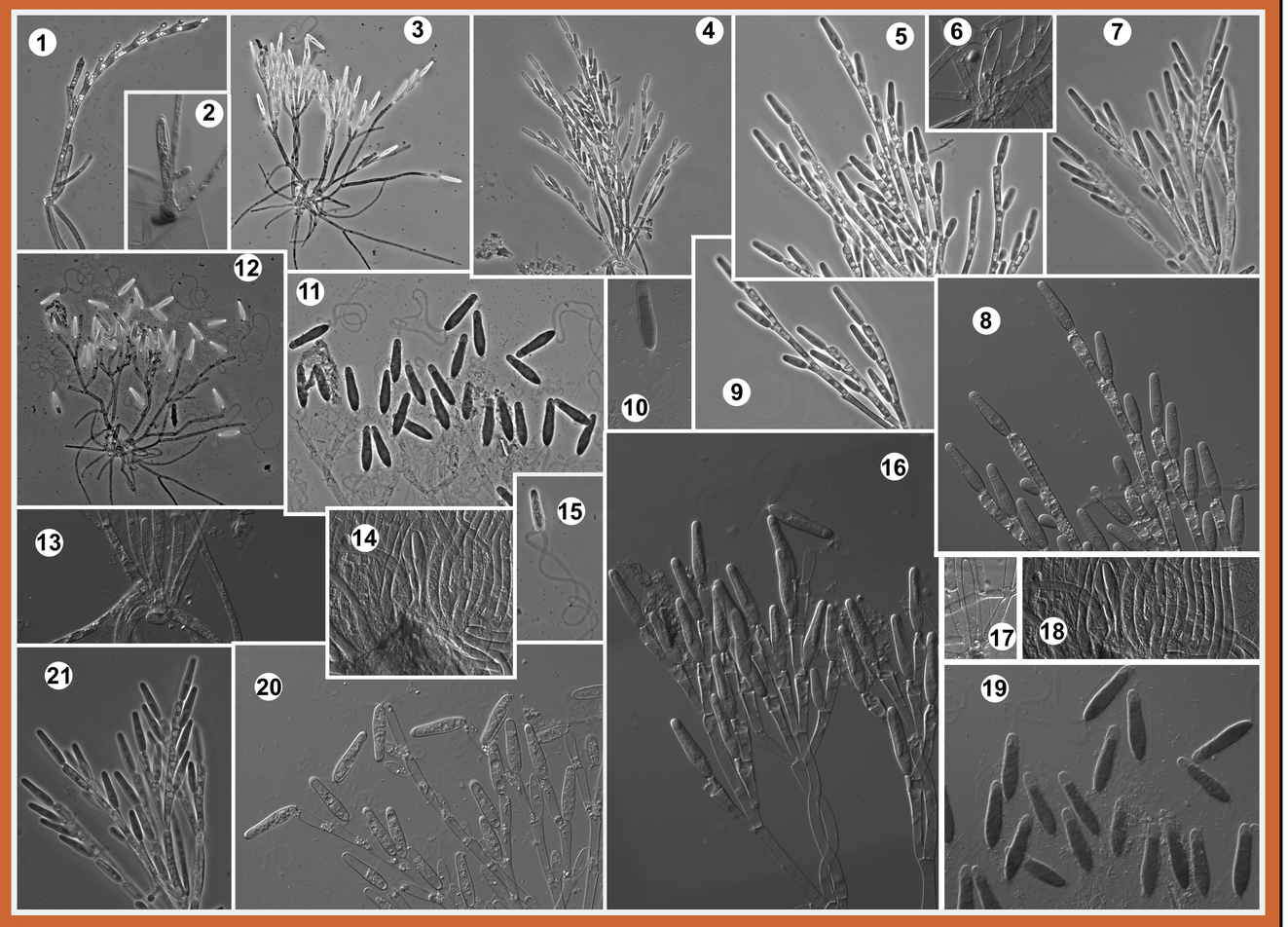

Plate A. *Ephemerellomyces aquilonius* highlighting key morphological features. Thalli with attached and detached trichospores, and the unique basal cell development of this genus (Figs. 2, 4, 6, 12-14, 17, 18). Loose spores revealed 2 appendages (Figs. 1, 3, 10-12, 15, 16, 19, 20). Spores measurements (35.30 µm x 7.62 µm), to (36.26 µm x 9.52 µm) when incubated on slides in a moist chamber over a 20 hour period.

Fungal Taxa associated with Stoneflies (Plecoptera)

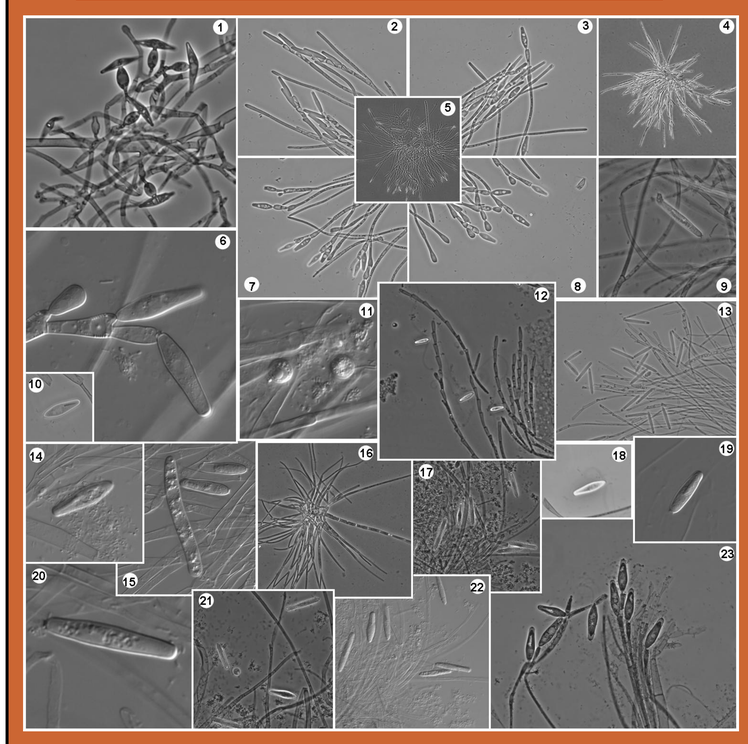

Plate B. Collage of hindgut associates: *Genistelloides* (Figs. 1-8, 10, 12, 14, 17-19, 21-23) *Lancisporomyces* (Figs. 9, 13, 17, 20-22) and *Paramoebidium* (Figs. 11, 15). Trichospores of different species, attached and detached (all Figs. except 1, 11, 16), sexual, biconical zygospore (Fig. 1), cyst (Fig. 11) and thallus (Fig. 15) of the protistan *Paramoebidium*.

Fungal Taxon associated with Black Flies (Diptera, Simuliidae)

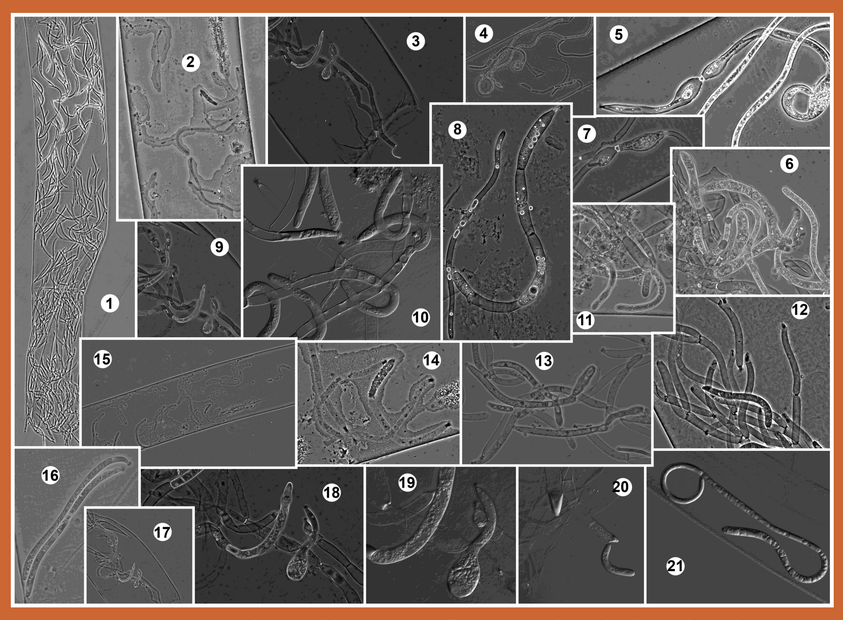

Plate C. Various features of a possibly new *Harpella* sp., an unbranched member of the Harpellales. Demonstrating the holdfast (Figs. 3, 4, 5, 8-10, 12-19), trichospores (Figs. 1, 3, 10, 12, 13, 15, 18, 20), unusual thallial development (Figs. 3-9, 17-19, 21).

Protistan and fungal endosymbionts from several host types

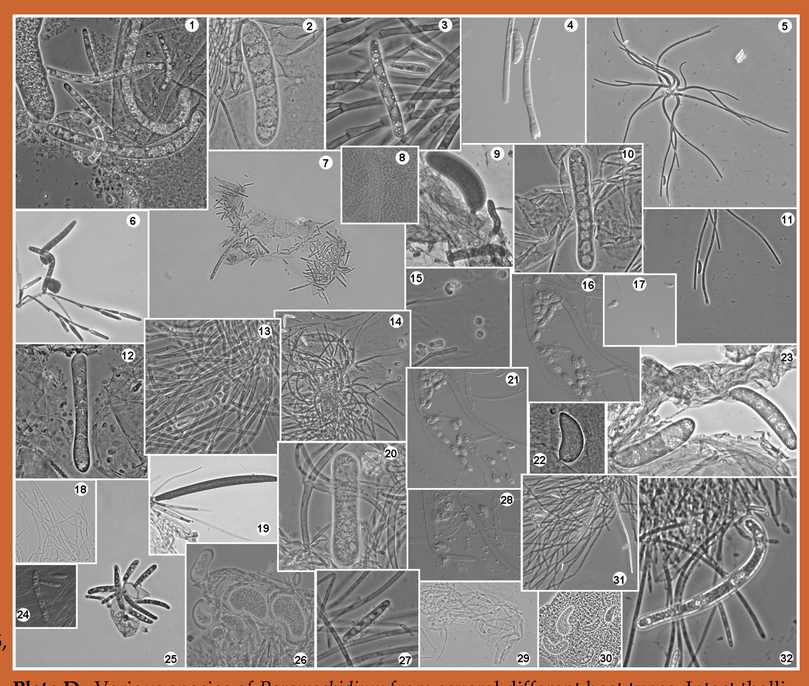

Plate D . Various species of *Paramoebidium* from several different host types. Intact thalli attached to gut linings (Figs. 1, 2, 6-10, 12, 18, 20, 22-26), intact thalli attached to gut fungi (Figs. 3-6, 11, 19,27,31-32), amoebae released from thalli (Figs. 13-16, 21, 28), amoebae forming cysts (Figs. 13-15).

Results

The wide geographic distribution of many trichomycete species and the frequency with which they infest arthropods attest to their successful dissemination. Distribution of trichomycetes is determined by their ability to transmit spores from host to host over short or long distances as well as by the motility and migratory activities of the hosts themselves. Insects were dissected and gut observed for the presence of Trichomycetes. Both the midgut and hindgut was observed for the black flies and Tipulidae, whereas only the hindgut was observed in the Mayflies, Stoneflies, Trichoptera. There was remarkable host and fungal diversity in Dry Creek and based on morpohotaxonomy seven different genera are reported: *Ephemerellomyces*, *Harpella*, *Genistelloides*, *Lancisporosmyces*, *Legeriomyces*, *Zygopolaris* and *Paramoebidium*.

The initial survey from Dry Creek provided the first account of E. *aquilonius*, a species previously documented and studied only in Norway. The success of this brief survey demonstrates promise for further discoveries of gut fungi at this site, in Idaho and the Pacific Northwest. The specimens we found seem a bit ambiguous, compared to the original description from Norway, for *E. aquilonius*. The genus is recognizable based on the development of the basal cell, with one particular branch that stops growing and helps to distinguish it from another mayfly genus, *Legeriomyces*. The Norwegian *E. aquilonius* trichospores previously reported to have only 2 or 3 appendages, yet we were fortunate enough only to see the trichospores with 2 appendages (measuring 35.30 µm x 7.62 µm when taken directly from the gut and 36.62 µm x 9.52 µm after wet-mounting for a 20 hour period at RT. It will be interesting to collect further specimens, for trichospores showing 3 appendages as well, and to search for the zygospore, which has been elusive to date for this species.

Conclusion

Contributions to our understanding of trichomycetes biodiversity and biology have come from a large number of individuals locally and worldwide. Explorations have included not only new regions of Europe and North America, but also Costa Rica and Puerto Rico in the Neotropics, Hawaii, Japan, Taiwan, and many countries in the Southern Hemisphere, including Australia, New Zealand, Chile, Argentina, and South Africa. Research has advanced our understanding of trichomycete distribution, host types and specificity, and biogeography. A preliminary survey of Dry Creek drainage in Boise, Idaho has produced the first report of the *E. aquilonius* in North America since it was described from Norway. The subtle morphological differences can be tested in our molecular systematics lab and specimens have been preserved in 2X CTAB DNA extraction buffer for possible future study. This disjunct distribution bridges an enormous geographical divide, although the species actually may be more widespread than earlier anticipated. Future collections and research on specimens from Dry Creek, with sequence data to be generated in our laboratory will be eagerly awaited as we place this unusual species in our expanding molecular-based phylogenies. We highlight here the morphology of *E. aquilonius* and some of the other endosymbionts found there.

Acknowledgments: Financial support from NSF Award 0918182, "REVSYS: RUI: Phylogenetic and Revisionary Systematics of early-diverging, ecologically unique clades of fungi, Harpellales and Asellariales" supported this contribution.