Longicollum biappendiculatum gen. et sp. nov., a new freshwater ascomycete from the Neotropics

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Longicollum biappendiculatum gen. et sp. nov. is described from submerged woody debris from a river in Peru. Additional material on submerged wood from Brazil, Costa Rica, Florida and Peru was also examined. The fungus is morphologically similar to members of the family Annulatascaceae. Traits which are shared include dark ascomata with a cylindrical neck, a hamathecium of long septate tapering paraphyses, unitunicate asci with a relatively large non-amyloid ascus apical ring, and hyaline ascospores. The lack of morphometric overlap with existing genera in the Annulatascaceae prompted the erection of a new genus. The new fungus is described, illustrated and compared to morphologically similar taxa.

Key words – Annulatascaceae – aquatic – fungi – saprobe – Sordariomycetes – submerged wood

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Introduction

Freshwater ascomycetes play an important role in the decomposition of submerged woody debris occurring in aquatic habitats (Shearer 1992, Gessner & Chauvet 1994, Wong et al. 1998a, Gessner & Van Ryckegem 2002). In our recent investigations of filamentous fungi in tropical streams in Peru and Costa Rica we encountered a previously undescribed freshwater ascomycete with characteristics that place it in the class Sordariomycetes. Simultaneously, a morphologically similar fungus was also collected by colleagues in a stream in the Brazilian semi-arid region. Members of the class Sordariomycetes typically possess perithecial ascomata, unitunicate asci, paraphyses, and a saprobic habit (Eriksson & Winka 1997, Kirk et al. 2008). The presence of dark ascomata with a long hyaline periphysate neck, pedicellate asci, a relatively large, non-amyloid bipartite ascus apical ring, long septate tapering paraphyses, and hyaline ascospores further refines the classification to the family Annulatascaceae. This combination of morphological characters also places this novel fungus close to the genus *Annulatascus*.

The goals of this study, therefore, were to (1) analyze the morphology of the undescribed fungus in comparison to species in the Annulatascaceae, and (2) describe and illustrate this new fungus.

Methods

Submerged woody debris was collected at random from various freshwater habitats along an altitudinal gradient in Peru according

to the procedures outlined by Shearer et al. (2004). Samples were placed in double seal plastic bags (Presto Products Company) lined with moist paper towels and then shipped to our laboratory at the University of Illinois. In the laboratory, samples were placed in moist chambers (sealable plastic boxes lined with moist paper towels) and incubated at room temperature (~25°C) and 12/12 hr light/dark conditions (Shearer et al. 2004). Within one week of arrival at the laboratory and periodically thereafter for 6–12 months samples were examined for fungal reproductive structures. Species isolation was performed according to the procedures outlined by Fallah & Shearer (2001) and Shearer et al. (2004). Protocols for morphological examination and embedding and sectioning followed those outlined in Fallah & Shearer (2001). Brazilian samples were processed according to the procedures outlined in Barbosa et al. (2008). The holotype and additional specimens were deposited at the University of Illinois Herbarium (ILL), a paratype was deposited at the herbarium at the Universidad de San Marcos (USM) and the Brazilian specimens at the Herbario Universidade Estadual de Feira de Santana (HUEFS).

Single spore isolates were grown on PYG+Ab agar plates [1.25 g peptone, 1.25 g yeast extract, 18 g agar (Difco), 5 g D-glucose (Acros), 0.5 g streptomycin sulfate, 0.5 g penicillin G (Sigma) and 1000 mL deionized H_2O] at ambient temperature with 12/12 hr light/dark conditions.

Results

Among the many fungi found in our collections was a novel fungus. Examination of fresh material revealed a unique combination of morphological characteristics including: immersed, light brown ascomata with a long, hyaline, periphysate neck; long, septate, hyaline paraphyses tapering at the apex; cylindrical, unitunicate asci with a relatively large J- bipartite apical ring and eight overlapping uniseriate ascospores; and multiguttulate, non-septate, hyaline ascospores with ephemeral, bipolar mucilaginous appendages.

Longicollum Zelski, F.R. Barbosa, Raja, A.N. Mill & Shearer **gen. nov.** Figs 1–13
MycoBank 653241

Etymology – longi = Latin for long, and collum = Latin for neck, referring to the long neck.

Ascomata dispersa, immersa, ostiolata; venteribus subglobosis, membranaceis, brunneis; collum longum, hyalinum, cylindricum, periphysatum; asci unitunicati, cylindrici, pedicellati, octospori, cum apparatu apicali bipartis; ascosporae ellipsoidae, aseptatae, hyalinae, multiguttulatae, appendiculatae.

Type species – *Longicollum biappendiculatum*

Ascomata scattered, immersed in the substrate, with long prominent hyaline, erumpent necks. Venter membranous, subglobose, light brown. Necks hyaline, long, cylindrical, periphysate. Peridium membranous, pseudoparenchymatous in surface view, in longitudinal section composed of 4–5 cell layers of brown cells towards the outside, and laterally compressed hyaline cells towards the inside. Paraphyses hyaline, septate, broad at the base, slightly tapering towards the apex. Asci unitunicate, cylindrical, persistent, with a large bipartite apical ring, tapering towards the base; base bearing a narrow tapering pedicel; with 8, overlapping uniseriate ascospores. Ascospores broadly ellipsoidal, hyaline, multiguttulate, non-septate; with short, hyaline, bipolar, ephemeral, mucilaginous appendages.

Type species – *Longicollum biappendiculatum*

Longicollum biappendiculatum Zelski, F.R. Barbosa, Raja, A.N. Mill & Shearer **sp. nov**. Figs 1–13
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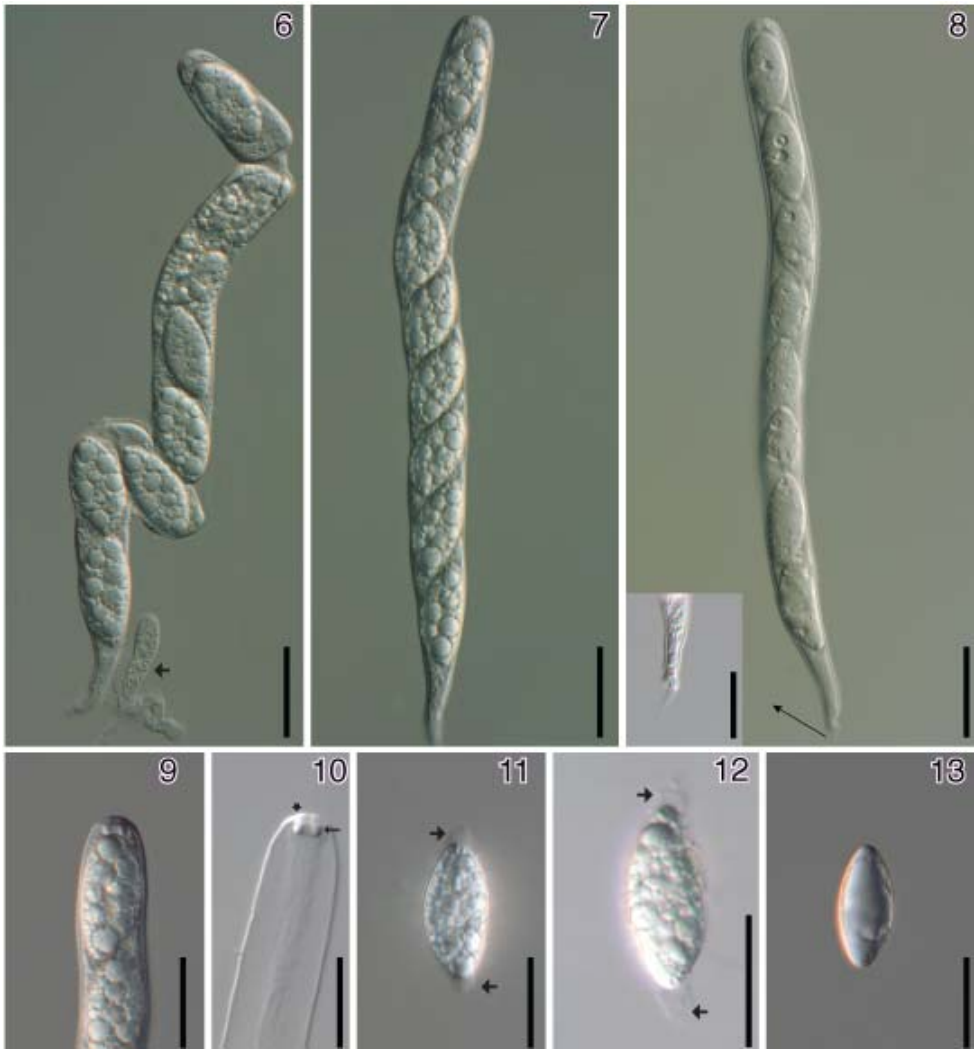
Etymology – Latin bi + appendix, referring to the two ephemeral apical appendages of the ascospores.

Ascomata dispersa, immersa, 545–585 µm alta, 155–160 µm diam, pallide brunnea, subglobosa, ostiolata; paries ascomatis duostratus, ca. 15–20 µm latum. Stratum exterior cellulis brunneis, 4–6 cellularum compositum. Stratum interior hyalinum compressum. Collum 315–370 × 74–82 µm, longum, hyalinum, cylindricum, periphysatum. Hamathecium paraphysatum. Paraphyses 140–170 × 5–7 µm, hyalinae, septatae, attenuatae; Asci 180–226 × 15–17 µm, unitunicati, cylindrici, cum apparatu apicali bipartis, grandis, pedicellati, octospori.

Figs 1–5 – *Longicollum biappendiculatum* from the holotype (ILL 40794). **1** Whole mount of ascoma showing long hyaline neck and light brown venter. **2** Longitudinal section through ascoma. **3** Periphysate neck. **4** Longitudinal section through peridium. **5** Paraphyses. Scale Bars 1–2 = 100 µm, 3–5 = 20 µm.

Ascosporae 26–35 × 12–14 µm, ellipsoidae, aseptatae, hyalinae, multiguttulatae, appendiculatae.

Ascomata 545–585 × 155–160 µm, scattered, venter immersed in wood, with a hyaline, erumpent, central neck; venter 205–220 × 155–160 µm, membranous, subglobose, light brown. Neck long, 315–370 × 74–82 µm, cylindrical, hyaline, periphysate. Peridium 15–20 µm thick, composed of ca. 4–6 layers of pseudoparenchymatic cells; cells brown towards the outside, subhyaline and laterally compressed towards the inside. Paraphyses 140–170 µm long × 5–7 µm wide at the base, simple, septate, hyaline, broader at the base, tapering towards the apex. Asci 180–225 × 15–17 µm, (mean = 205 × 16 µm, n = 30), cylindrical, with a prominent J–, bipartite apical ring, 2–4 × 3–5 µm wide, staining blue with aqueous nigrosin, with 8, overlapping uniseriate ascospores, tapering towards the base, with a thin, elongate, tapering pedicel ca. 5 µm long, sometimes extending in length in water, separating from the hymenial layer, persistent. Ascospores 26–35 × 12–14 µm, (mean = 28 × 13 µm, n = 50), broadly ellipsoidal, hyaline, multiguttulate, non-septate, with short hyaline, bipolar, ephemeral, mucilaginous appendages, ca. 3–6 × 6–9



Figs 6–13 – *Longicollum biappendiculatum* from the holotype (ILL 40794). **6** Mature and young asci (arrow). **7** Mature ascus showing overlapping uniseriate hyaline ascospores. **8** Mature expanded ascus showing pedicel with tiny extension (inset). **9** Mature ascus apex with apical ring. **10** Bipartite apical ring (arrows). **11–13** Mature ascospores showing bipolar mucilaginous appendages (arrows). Scale Bars 6–13 = 20 µm.

µm. Colonies on PYG + Ab agar immersed, brown, comprised of hyaline to light brown septate, branching hyphae. Anamorphic state not observed.

Known distribution – Brazil (Bahia), Costa Rica (Heredia), Peru (Camanti), USA (Florida).

Holotype – Peru. Camanti: Stream at Quincemil Trail 1, 13°23960S, 70°77017W, on submerged woody debris, pH 6, water temp 20°C, 3 October 2010, *Zelski S*. and *Raja H.A*,. *PE17-2* (Holotype, ILL 40794)

Additional material examined – Brazil, Bahia, stream at Serra da Jibóia, 12°51'00"S, 39°28'46"W, on submerged bark debris, pH 4.3, water temp 21.8°C, 21 January 2009, F.R. Barbosa and L.F.P. Gusmão (HUEFS 158104). Costa Rica, Heredia, La Selva Biological Station, La Selva stream, 10°25'7"N, 84°01'27"W, on submerged woody debris, pH 5, water temp 25°C, 10 January 2006, M. Salazar and A. Ferrer, AF267-1. Peru, Camanti, stream at Quincemil Trail 1, 13°14'23"S, 70°46'13"W, on submerged woody debris, pH 6, water temp 21°C, 26 May 2010, S.E. Zelski and H.A. Raja PE17-1. USA, Florida, Wildcat Lake, Ocala National Forest, 29°10'14"N, 84°37'40"W, on submerged woody debris, pH 5, water temp 14°C, 2 February 2006, J.L. Crane and H.A. Raja F107-1; Dorr Lake, Ocala National Forest, 29°00'24"N, 81°38'13"W, on submerged woody debris, pH 5, water temp 33°C, 16 July 2006, J.L. Crane and H.A. Raja F107-2.

Notes – *Longicollum biappendiculatum* appears to be widely distributed in the Neotropics. Thus far it has been found in freshwater habitats in Brazil, Costa Rica, Peru, and USA, where it has been reported from Florida (as undescribed sp. F107) during a latitudinal survey of freshwater ascomycetes in north, central and south Florida (Raja et al. 2009). So far, *L. biappendiculatum* has been reported from water temperatures ranging from 14–33°C, therefore, it might be a warm water, as opposed to a strictly tropical species. Although we found considerable variation in the ascospore and asci measurements from different collections of *L. biappendiculatum*, at this time we consider these size differences to be intraspecific variations. Molecular sequences from the internal transcribed spacer (ITS) region of the ribosomal RNA gene might help shed light on this aspect in the future. Further, it was not clear whether the ascospore appendages were formed from a tightly adpressed mucilaginous sheath surrounding the entire ascospore or as direct outgrowths from the ascospore apices.

Discussion

The family Annulatascaceae was erected to accommodate aquatic species of filamentous fungi which have dark perithecia, long tapering paraphyses, long cylindrical unitunicate asci with prominent non-amyloid apical rings, and hyaline, or occasionally brown spores that often have sheaths or appendages (Wong et al. 1998b, Ho & Hyde 2000). The type species of the family, *A. velatisporus* K.D. Hyde (Hyde 1992), exhibits all of these characteristics, and in addition has ascospores with verruculose wall ornamentation.

Longicollum biappendiculatum shows some similarities to many of the currently accepted genera in the family *sensu lato* such as *Annulatascus* K.D. Hyde (Hyde 1992), *Annulusmagnus* J. Campb. & Shearer (Campbell & Shearer 2004), *Aquaticola* W.H. Ho, K.M. Tsui, Hodgkiss & K.D. Hyde (Ho et al. 1999), *Ascitendus* J. Campb. & Shearer (Campbell et al. 2003), *Cataractispora* K.D. Hyde, S.W. Wong & E.B.G. Jones (Wong et al. 1999), *Clohiesia* K.D. Hyde (Hyde 1995), *Diluviocola* S. W. Wong, K.D. Hyde & E. B. G. Jones (Hyde et al. 1998), *Fluminicola* S.W. Wong, K.D. Hyde & E.B.G. Jones (Wong SW et al. 1999), and *Submersisphaeria* K.D. Hyde (Hyde 1996). The main feature which sets *L. biappendiculatum* apart from all other species of Annulatascaceae is the presence of a long hyaline neck and light brown ascomata in combination with aseptate, multiguttulate, hyaline ascospores with small irregular bipolar appendages. We consider this combination of characteristics to be significant at the generic level.

Longicollum biappendiculatum shares many characteristics with *Submersisphaeria aquatica* (Hyde 1996). The ascomata of *L. biappendiculatum* are similar to those of *S. aquatica* in that they are immersed, possess ostiolate, central necks, and have subglobose venters composed of pseudoparenchymatic tissue. They differ in that the venter of *L. biappendiculatum* is smaller (205–222 × 155–160 µm vs. 300–520 × 240–530 µm), the peridium is thinner (15–20 µm vs. 31–43 µm), and the neck is completely hyaline as opposed to black with a lighter apex as in *S. aquatica*. The lengths of the asci fall within a similar range (180–225 µm in *L. biappendiculatum* and 175–210 µm in *S. aquatica*), but the asci in *L. biappendiculatum* are wider (15–17 µm) than those of *S. aquatica* (11–12.5 µm). Other members of *Submersisphaeria* have ascus dimensions well outside of this range. The ascospores of *L. biappendiculatum* are broadly ellipsoidal and multiguttulate with dimensions of 26–35 × 12–14 µm, similar to the ascospores of *S. aquatica* which are ellipsoidal to fusiform, contain granular material and have dimensions of 23–27 × 7.5–10 µm. The two taxa differ, however, in that the ascospores of *S. aquatica* are 1-septate, lack apical appendages, possess hyaline germ pores, and are brown, while the ascospores of *L. biappendiculatum* are aseptate, possess ephemeral bipolar apical appendages, lack germ pores, and are hyaline. The remaining species of *Submersisphaeria* also exhibit pigmented spores (Hyde 1996, Fröhlich & Hyde 2000, Zhou & Hyde 2000, Pinnoi et al. 2004, Wang et al. 2004).

Members of the Annulatascaceae occur in similar habitats in both the paleotropics and the neotropics, with many showing a pantropical distribution. To date, *L. biappendiculatum* has been recorded only from the neotropics and subtropical Florida.

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