Chaetorostrum quincemilensis, gen. et sp. nov., a new freshwater ascomycete and its *Taeniolella*-like anamorph from Peru

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Collections of woody debris from streams in a lower montaine cloud forest in Peru yielded a novel fungus with affinities to the family Annulatascaceae. Characters which place it in the family Annulatascaceae *sensu lato* include ascomata which are brown pigmented; long periphysate necks; long tapering septate paraphyses; unitunicate, pedicellate asci with a prominent bipartite J- apical ring; and ascospores with a gelatinous sheath. Examination of morphological characters provided a diagnosis which did not fit with existing genera and species in this family. The combination of features that distinguish this fungus are a pigmented ascoma with a neck which is hyaline at the apex and has prominent black hairs, fasciculate asci with a spine-like pedicellar extension, and versicolored ascospores which are constricted at the midseptum. The fungus also produces its anamorphic state in culture which is the first record of an asexual state in the Annulatascaceae. The new genus *Chaetorostrum* is erected to accommodate this undescribed fungus. The type species of *Chaetorostrum, C. quincemilensis* is described, illustrated and compared with other morphologically similar taxa in the family.

Key words – Annulatascaceae – fungi – saprobe – Sordariomycetes – stream

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Introduction

Freshwater ascomycetes are thought to play an important role in freshwater ecosystems as decomposers of woody and herbaceous material in lentic and lotic habitats (Shearer 1992, Gessner & Chauvet 1994, Wong M.K.M. et al. 1998, Simonis et al. 2008). Hence it is important to know what species occur in aquatic habitats globally. During a recent survey of freshwater fungi from streams in a lower montaine cloud forest in Peru, a fungus exhibiting morphological characters similar to taxa in the Annulatascaceae was discovered on submerged woody debris collected from a semi-aquatic, intermittent stream habitat.

The family Annulatascaceae was erected by Wong S.W. et al. (1998) to accommodate aquatic ascomycetes found on submerged wood displaying dark ascomata, long tapering septate paraphyses, asci with a prominent apical ring and ascospores with or without sheaths or appendages. The following genera have been included or referred to the Annulatascaceae: *Annulatascus* K.D. Hyde (Hyde 1992), *Annulusmagnus* J. Campb. &

Shearer (Campbell & Shearer 2004), *Aqualignicola* V.M. Ranghoo, K.M. Tsui & K.D. Hyde (Ranghoo et al. 2001), *Aquaticola* W.H. Ho, K.M. Tsui, Hodgkiss & K.D. Hyde (Ho et al. 1999), *Ascitendus* J. Camp. & Shearer (Campbell & Shearer 2004), *Ascolacicola* Ranghoo & K.D. Hyde (Ranghoo & Hyde 1998), *Brunneosporella* V.M. Ranghoo & K.D. Hyde (Ranghoo et al. 2001), *Cataractispora* K.D. Hyde, S.W. Wong & E.B.G. Jones (Hyde et al. 1999), *Clohiesia* K.D. Hyde (Hyde 1995), *Cyanoannulus* Raja, J. Campb. & Shearer (Raja et al. 2003), *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 et al. 1999), *Frondicola* K.D. Hyde (Hyde 1992), *Fusoidispora* D. Vijaykrishna, R. Jeewon & K.D. Hyde (Vijaykrishna et al. 2005), *Pseudoproboscispora* Punith. (Punithalingham 1999), *Rivulicola* K.D. Hyde (Hyde et al. 1997), *Submersisphaeria* K. D. Hyde (Hyde 1996), *Teracosphaeria* Réblová & Seifert (Réblová & Seifert 2007), *Torrentispora* K.D. Hyde et al. (Hyde et al. 2000) and *Vertexicola* K.D. Hyde , V.M. Ranghoo & S.W. Wong (Ranghoo et al. 2000). Our new fungus, while exhibiting characteristics which place it in the family, cannot be accommodated in any of the currently recognized genera. Additionally, this is the first member of the Annulatascaceae that produces its anamorphic state in culture. We therefore establish a new genus, *Chaetorostrum*, for this new fungus.

The goals of this study, therefore, were to (1) analyze the morphological characteristics of the undescribed fungus in relationship to those of other species in the Annulatascaceae, and (2) fully describe and illustrate the morphology of the new genus and species.

Methods

Submerged woody debris was randomly collected from various freshwater habitats in a lower montaine cloud forest in Peru according to the procedures outlined by Shearer et al. (2004). Samples were placed in sealable plastic bags along 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) with 12/12 hr light/dark conditions. Samples were examined for reproductive structures within one week of arrival at the laboratory and periodically thereafter for 6-12 months. Species isolation was performed according to the procedures outlined by Fallah and Shearer (2001) and Shearer et al. (2004). Protocols for morphological examination followed those outlined in Fallah & Shearer (2001). The holotype and additional specimens were deposited at the University of Illinois Herbarium (ILL).

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. Subcultures were grown on CMA + alfalfa [17 g Corn Meal Agar (Becton, Dickenson and Company), sterilized alfalfa and 1000 mL deionized H_20] to stimulate the production of fruiting structures.

Results

Examination of fresh fungal material found on submerged wood samples in moist chambers revealed a novel fungus. The morphological characteristics which set this fungus apart include: immersed to partially immersed, light brown to brown ascomata; a long neck that is hyaline at the apex and bears stiff dark hairs; long, hyaline, tapering, septate paraphyses; cylindrical, unitunicate asci with a large J- bipartite apical ring, pedicel bearing a spine-like appendage, and eight overlapping uniseriate ascospores; 3-septate ascospores with hyaline end cells, pale brown central cells, and a thin gelatinous sheath; and from culture, an anamorph which produces long septate phragmospores.

Chaetorostrum Zelski, Raja, A.N. Mill & Shearer, **gen. nov.** Figs 1-15

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Etymology – chaeto = from Greek for long flowing hair, and rostrum = Latin for beak, referring to the hair-like setae on the neck.

Ascomata dispersa, immersa ad partim immersa, horizontalia, globosa, membranaceis, pallida brunneis ad brunneis, ostiolata. Collum

centrale, longum, cylindricum, cum setae rigidae obscurae. Peridium e textura angularis in facei. Hamathecium paraphysatum. Paraphyses hyalinae, septatae, attenuatae. Asci fasciculati, unitunicati, cylindrici, cum apparatu apicali bipartis, cuneatus basim cum spina tractus, octospori. Ascosporae ellipsoidae, triseptatae, versicolor, hyalinae et pallida brunnae in cellulas centrales, guttulatae, cum vagina muscilagina cum juvenile. Coloniae in culturae floccosae, micronemeae, mononemeae, terminatio en fine hyphae. Conidia elongata, cylindrica, euseptata, brunnea, pallida ad extremum.

Ascomata on submerged wood, scattered, immersed to partially immersed, lying horizontally on the substrate, elongated globose, membranous, light brown to brown, ostiolate, with a long, erumpent, setose neck. Necks central, long, cylindrical, periphysate, bearing long, stiff, dark hairs. Peridium composed of *textura angularis* in surface view. Paraphyses hyaline, long, numerous, septate, broad at the base, tapering at the apex. Asci basal, fasciculate, unitunicate, cylindrical, tapering at the base and having a spine-like extension, containing eight, overlapping uniseriate ascospores, to uniseriate when ascus elongates in water, with a large, bipartite, cylindrical, apical apparatus. Ascospores broadly ellipsoidal, hyaline, one-septate when young, becoming versicolored and 3-septate with brown central cells and hyaline end cells at maturity, guttulate; young ascospores surrounded by a narrow gelatinous sheath. Anamorph conidiophores micronematous, mononematous. Conidia monoblastic on terminal ends of hyaline vegetative hyphae, elongate cylindrical phragmospores, euseptate, brown, paler near apex, dry, schizolytic.
Type species: *Chaetorostrum quincemilensis*

Chaetorostrum quincemilensis Zelski, Raja, A.N. Mill & Shearer, **sp. nov.** Figs 1-15
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Ascomata 800–900 × 200–270 µm, dispersa, immersa ad partim immersa, horizontalia, globosa, membranaceis, pallida brunneis ad brunneis, ostiolata. Collum 600–700 × 64–70 µm, centrale, longum, cylindricum, cum setae rigidae obscurae. Setae 90–150 µm longae × 3–4 µm latae, 8–10 septatae. Peridium e textura angularis in facei. Hamathecium paraphysatum. Paraphyses 137–162 × 5–7 µm, hyalinae, septatae, attenuatae. Asci 180–240 × 12–15 µm, numerosae, fasciculati, unitunicati, cylindrici, cum apparatu apicali bipartis 5–6 µm longum × 7–9 µm latum, cuneatus basim cum spina tractus, octospori. Ascosporae 30–38 × 10–12 µm ellipsoidae, triseptatae, versicolor, hyalinae et pallida brunnae in cellulas centrales, guttulatae interdum multiguttulatae, cum vagina muscilagina cum juvenile. Coloniae in cultura floccosae, micronemeae, mononemeae, terminatio en fine hyphae. Conidia 20-280 × 7-13 µm, elongata, cylindrica, 2-40+ euseptata, brunnea, pallida ad extremum.

Ascomata 800–900 × 200–270 µm, on submerged wood, scattered, immersed to partially immersed, oriented horizontally to the substrate, venter elongated globose, membranous, brown to light brown, ostiolate, with a long, upwardly directed, setose neck. Necks 600–700 × 64–70 µm, central, cylindrical, periphysate, hyaline at the apex, brown towards the base, bearing rigid brown to dark-brown hairs. Hairs light brown and pointed at the apex, dark brown and rounded towards the base, 90–150 µm long, 3–4 µm wide, 8–10 septate (Figs 1-2). Peridium membranous, composed of *textura angularis* in face-view (Fig. 3). Paraphyses 137–162 × 5–7 µm, hyaline, filamentous, numerous, septate, broad at the base, tapering towards the apex (Fig. 4). Asci 180–240 × 12–15 µm, numerous, basal, fasciculate, unitunicate, cylindrical, elongating in water, containing eight overlapping uniseriate ascospores, tapering to a long, narrow, elongate pedicel with a spine-like pedicellar extension, possessing a large, bipartite, cylindrical, apical apparatus 5–6 × 7–9 µm (Figs 5-8). Ascospores 30–38 × 10–12 µm (mean = 33 × 11 µm; n = 30), hyaline, one-septate when young, becoming versicolored and 3-septate with brown central cells and hyaline end cells at maturity; broadly ellipsoidal; equipped with gelatinous apiculate appendages; biguttulate, sometimes multiguttulate, slightly constricted at the midseptum; young ascospores surrounded by a narrow, adpressed gelatinous sheath which

gradually disappears in water (Figs 9-12). Colonies on PYG + Ab agar irregular, raised, grey-brown, dark brown in reverse view. Colonies on CMA + alfalfa light brown to dark brown composed of abundant superficial floccose hyphae, reverse dark brown to black, anamorph present. Anamorph conidiophores micronematous, mononematous. Conidia monoblastic on terminal ends of hyaline vegetative hyphae, elongate cylindrical phragmospores, 20-280 × 7-13 µm, 2-40+ euseptate, brown, paler near apex, dry, schizolytic. Young conidia smooth-walled while older conidia exhibit rough walls that appear to slough off (Figs 13-15).

Etymology: "*quincemilensis*" in reference to the Peruvian town, Quincemil, the town near the collection site.

Habitat: Saprobic on woody debris in a semi-aquatic, intermittent stream.

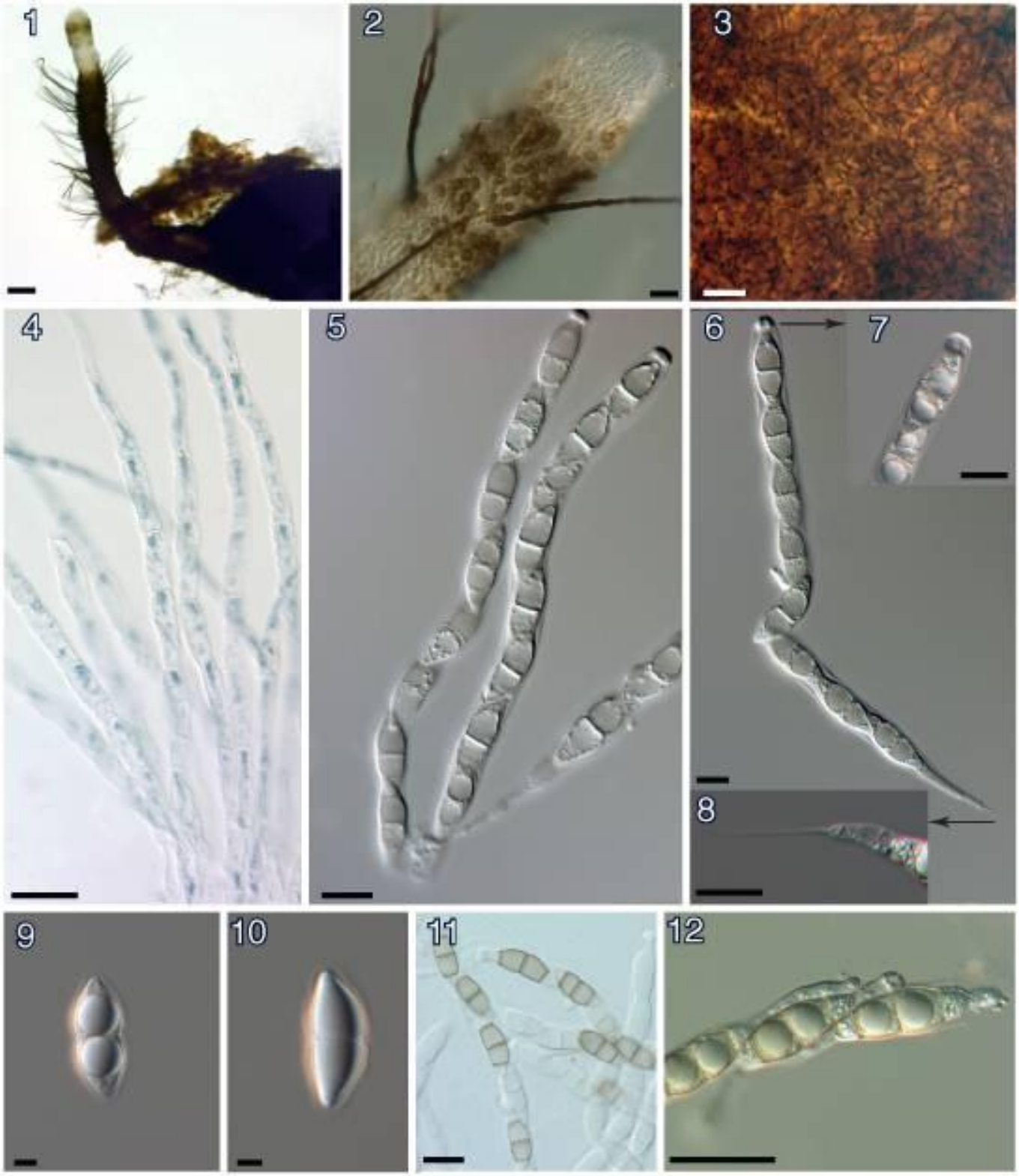
Holotype: PERU, CAMANTI: Stream at Quincemil Trail 1, 13˚14'23"S, 70˚46'13"W, on submerged woody debris, 26 May 2010, *Zelski S.E. and Raja H.A. PE105-1* (HOLOTYPE, ILL 40822).

Discussion

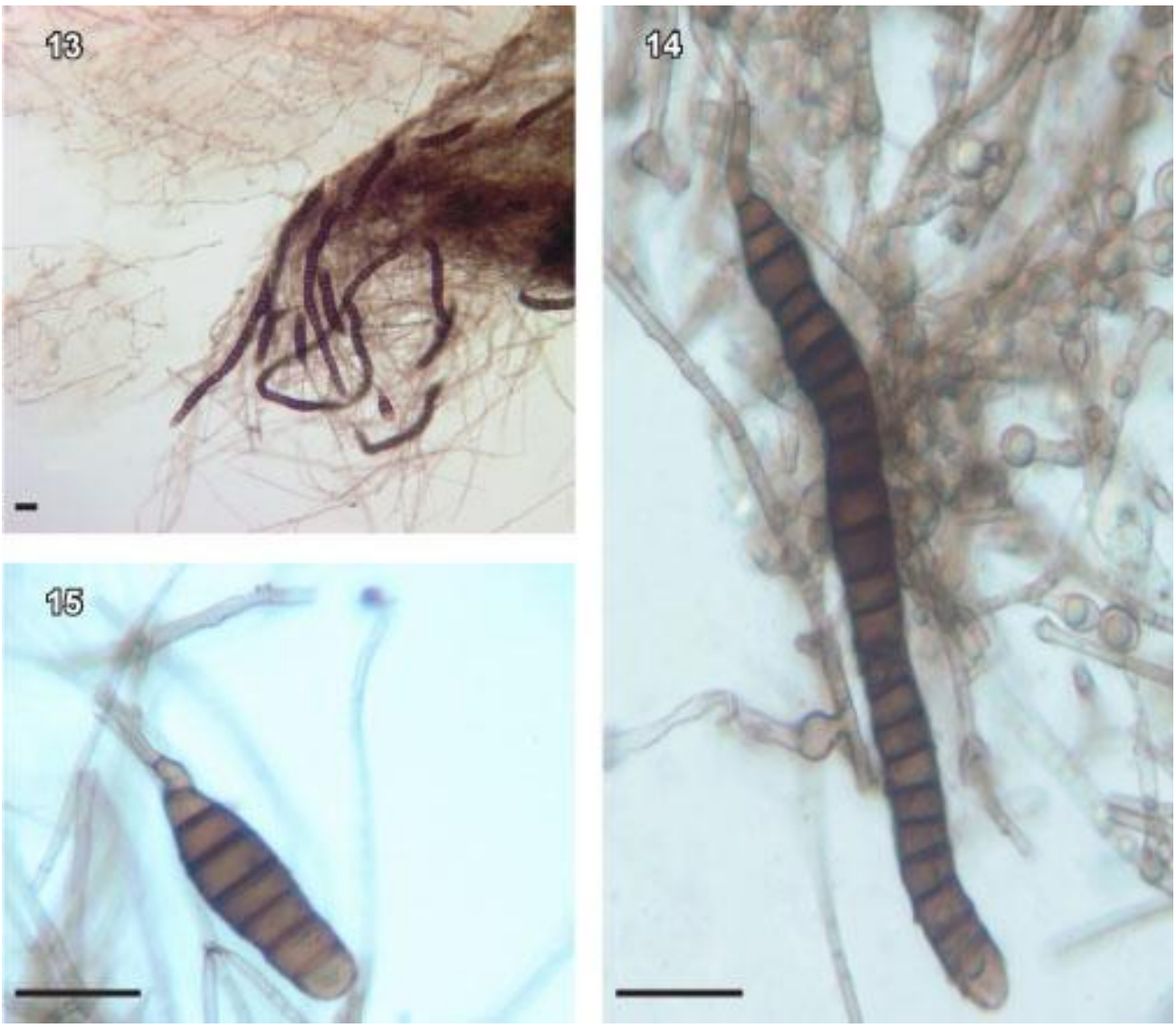
Only a few taxa in the Annulatascaceae have brown pigmented ascospores. These include *Ascitendus*, *Ascolacicola*, *Brunneosporella*, and *Submersisphaeria*. Both *Brunneosporella* and *Submersisphaeria* have ascospores which are completely pigmented and are either aseptate or uniseptate. In addition, the ascospores in these genera are ellipsoidal or fusiform and not constricted at the mid-septum, whereas the ascospores of *C. quincemilensis* are broadly ellipsoidal and constricted at the midseptum. *Ascitendus* and *Ascolacicola*, both monotypic genera, exhibit three septate ascospores with hyaline end cells and brown central cells. *Chaetorostrum quincemilensis* has larger ascospores (30-38 x 10-12 µm) than *Ascit. austriacus* (14-27 x 4-9 µm) and *Ascol. aquatica* (12.5-16.5 x 4-7.5 µm). The shapes of the ascospores also differ among these three taxa; *C. quincemilensis* ascospores are broadly ellipsoidal, while those of *Ascit. autriacus* are fusiform and those of *Ascol. aquatica* are ellipsoidal.

In addition to differences in ascospore shape and size, *C. quincemilensis* has larger ascomata (800-900 x 200-270 µm) compared to those of *Ascit. austriacus* (400-550 x 350-450 µm) and *Ascol. aquatica* (250-375 x 225-275 µm). *Ascitendus austriacus* and *Ascol. aquatica* have completely black necks lacking prominent hairs, which differ from those of *C. quincemilensis* which have a light apex and bear prominent black, septate hairs. The apical ring structure of *C. quincemilensis* is distinctly bipartite whereas the apical ring structure of *Ascit. austriacus* is discoid and that of *Ascol. aquatica* is wedge-shaped. The thin outgrowth of the pedicel of *C. quincemilensis* is long, narrow and tapers to a point while the pedicels of *Ascit. austriacu*s and *Ascol. aquatica* lack such an outgrowth. Narrow outgrowths of the pedicel, however, occur in several species of Annulatascaceae including *Annulatascus apiculatus, A. biatriisporus, A. fusiformis, Aquaticola hyalomura, Cataractispora appendiculata, Fluminicola bipolaris, Pseudoproboscispora caudae-sius,* and *Vertexicola caudatus*. All of these taxa, however, differ from *C. quincemilensis* in ascoma and ascospore morphology.

Chaetorostrum quincemilensis should also be compared to members of the genus *Ascotaiwania*, which were recently placed in the Savoryellales based on molecular analysis (Boonyuen et al. 2011). A key characteristic of *Ascotaiwania* is the presence of ascospores which have central brown pigmented cells and hyaline end cells. The ascospores are 3-7 septate. In addition, taxa in *Ascotaiwania* have cylindrical, pedicellate asci with relatively massive J- apical rings. *Chaetorostrum quincemilensis* has similar asci, but the pedicel is distinctly different in that it has a narrow spine-like extension (Figs 6, 8). *Chaetorostrum quincemilensis* differs from members of *Ascotaiwania* however in that *C. quincemilensis* has ascospores with distinctive guttulation, a constricted midseptum, and are broadly ellipsoidal rather than fusoid or ellipsoidal. *Chaetorostrum quincemilensis* also produces a phragmoconidial anamorph, while reported anamorphs for *Ascotaiwania* include *Monotosporella setosa* (Sivichai et al. 1998) and *Helicoon farinosum* (Fallah et al. 1999, Cai et al. 2006).



Figs 1–12 – *Chaetorostrum quincemilensis* from the holotype (ILL 40822). **1** Squash mount of ascomata. **2** Neck with brown hairs. **3** Peridium showing *textura angularis* pattern in surface view. **4** Paraphyses. **5** Asci. **6** Ascus showing bipartite apical ring and elongated ascus pedicel. **7** Enlarged view of bipartite apical ring. **8** Enlarged view of ascus pedicel. **9** One-septate constricted ascospore showing gelatinous sheath in water. **10** One-septate ascospore showing gelatinous sheath in glycerin. **11–12** Older brown, 3-septate ascospores with hyaline apices. **12** Germinating ascospores. Scale Bars 1 = 100 µm, 2–6, 8–11 = 20 µm, 7, 12 = 40 µm.



Figs 13-15 – *Chaetorostrum quincemilensis* anamorph from single spore isolate of the holotype (ILL 40822). **13** Phragmoconidia arising from vegetative hyphae. **14** Mature phragmoconiduim illustrating rough walls. **15** Young phragmoconidium showing attachment to vegetative hypha and smooth walls. Scale Bars = 20 µm.

The *Taeniolella*-like anamorph which was produced on CMA + alfalfa superficially resembles *Taeniolella plantaginis* (Corda) Hughes but does not produce conidia laterally or in fascicles, and it does not branch.

In addition, the conidia of *T. plantaginis* were originally described as being 1-6-septate (Corda 1839, Hughes 1958). The type species of the genus *Taeniolella, T. exilis*, has not been sequenced, nor has *T. plantaginis*, but recent phylogenetic studies on other taxa have shown that the species *T. alta* and *T. typhoides* belong in the Dothidiomycetes (Crous et al. 2006, Shearer et al. 2009). Our new fungus is placed firmly within the Sordariomycetes based on morphology which suggests that species that have been referred to *Taeniolella* or are *Taeniolella*-like may be polyphyletic as currently circumscribed. The micrographs of the *Taeniolella*-like anamorph presented here should be compared to those of Matsushima (1981).

Based on the morphological differences between *C. quincemilensis* and other members of the Annulatascaceae with pigmented ascospores, we conclude that this undescribed fungus merits a new genus within the family. The phylogenetic placement of *C. quincemilensis* within the family is currently unknown and is an avenue for further investigation. The genus *Taeniolella* also merits further research from a molecular

perspective owing to the simple nature of the fruiting structures found therein and the evidence for polyphyly.

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