

Available online at www.sciencedirect.com**MYCOSCIENCE**

ISSN 1340-3540 (print), 1618-2545 (online)

journal homepage: www.elsevier.com/locate/myc**Full paper**

Conioscypha peruviana sp. nov., its phylogenetic placement based on 28S rRNA gene, and a report of Conioscypha gracilis comb. nov. from Peru



Steven E. Zelski ^{a,*}, Huzefa A. Raja ^{a,b}, Andrew N. Miller ^c,
Carol A. Shearer ^a

^a Department of Plant Biology, University of Illinois at Urbana-Champaign, Room 265 Morrill Hall,
505 South Goodwin Avenue, Urbana, IL 61801, USA

^b Department of Chemistry and Biochemistry, 457 Sullivan Science Building, University of North Carolina,
Greensboro, NC 27402-6170, USA

^c Illinois Natural History Survey, University of Illinois at Urbana-Champaign, Champaign, IL 61820, USA

ARTICLE INFO**Article history:**

Received 29 April 2014

Received in revised form

16 September 2014

Accepted 29 September 2014

Available online 4 December 2014

Keywords:

28S rRNA gene

Altitudinal gradient

Freshwater ascomycetes

Submerged wood

ABSTRACT

A new species, *Conioscypha peruviana* was isolated from submerged woody debris collected in streams, rivers and a swamp in Peru. The anamorph formed in culture and morphological and molecular data support the inclusion in the genus. A phylogeny generated with maximum likelihood and Bayesian approaches placed the fungus in a well-supported clade with other *Conioscypha* and *Conioscyphascus* species. Nomenclature used herein reflects changes made in the Melbourne Code, with *Conioscyphascus* being considered a later synonym of *Conioscypha*. The fungus is described, illustrated and compared to morphologically similar taxa. In addition, *Conioscypha gracilis* comb. nov. is identified from both lentic and lotic habitats in Peru.

Published by Elsevier B.V. on behalf of the Mycological Society of Japan.

1. Introduction

A survey of freshwater ascomycetes decomposing woody and herbaceous debris along an altitudinal gradient stretching from the Peruvian Andes into the Peruvian Amazon yielded a new fungus from lower montane cloud forest and lowland Amazonian sites. It is characterized by having hyaline ascospores that are scattered to gregarious, immersed to superficial;

long, cylindrical, periphysate, hyaline necks; cylindrical to sigmoidal, unitunicate, 8-spored asci with a small thimble-shaped, J-refractive apical apparatus; and cylindrical to navicular, hyaline ascospores lacking sheaths or appendages. An anamorph superficially resembling *Conioscypha fabiformis* Matsush. was produced in culture, however it differs from *C. fabiformis* in having larger conidia.

The teleomorph of the fungus fits well within the genus *Conioscyphascus* Réblová & Seifert (Réblová and Seifert 2004;

* Corresponding author. Tel.: +1 847 312 2910.

E-mail address: zelski@illinois.edu (S.E. Zelski).

<http://dx.doi.org/10.1016/j.myc.2014.09.002>

1340-3540/Published by Elsevier B.V. on behalf of the Mycological Society of Japan.

abbreviated here as Cs.). Recently, *Conioscyphascus* was connected to *Conioscypha* Höhn. by Réblová and Seifert (2004). According to recent changes in the ICN (McNeill et al. 2012), specifically the new article 59.1, as of January 2013, there can only be one name for one fungus. Réblová et al. (2012) have already reverted to the older anamorph-based name for fungi in this genus. Thus, herein we describe the holomorph of the new *Conioscypha* species and provide cultural and molecular evidence for the link between the two morphological forms. The second species of *Conioscyphascus*, *Cs. gracilis* Réblová & Seifert, which was described without an anamorph name, is transferred to *Conioscypha*.

The goals of this study were to: 1) describe and illustrate the new species *Conioscypha peruviana*, 2) compare and contrast the fungus with other members in the genus and other morphologically similar taxa, and 3) construct a molecular phylogeny using partial 28S large subunit rRNA genes to illustrate evolutionary relationships within *Conioscypha* and among morphologically similar taxa.

2. Materials and methods

2.1. Sample collection and morphological examination

Submerged woody and herbaceous debris was collected at random along an altitudinal gradient from 218 to 3566 m from a variety of freshwater habitats that included rivers, streams, backwaters, swamps, and a few inundated trails. Approximately 30 pieces of debris were put into a sealable plastic bag along with a wet paper towel at each sampling site and the material was shipped to our laboratory at the University of Illinois at Urbana-Champaign. 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 h light/dark conditions. Samples were examined for reproductive structures within one week of arrival and periodically thereafter for 12 months with a stereomicroscope. Digital images of reproductive structures were taken using an Olympus SZX7 stereomicroscope (Olympus Optical Co. Ltd, Tokyo, Japan) fitted with a SPOT RT color camera using SPOT Advanced software (Diagnostics instruments Inc., Sterling Hts., MI).

Reproductive structures were removed from the substrate with a dissecting needle, gently teased apart in a drop of distilled water, sandwiched between 25 × 25 and 18 × 18 mm cover slips, and placed on microscope slides for examination. These slides were then preserved according to the protocol of Volkmann-Kohlmeyer and Kohlmeyer (1996). Ascomata were fixed and embedded for thin sectioning using a modified procedure by Huhndorf (1991). The osmium tetroxide step was deleted, and acetone was used instead of ethanol for dehydration and infiltration of the ascomata. Sections were obtained using a Reichert One U2 ultramicrotome (Reichert, Austria) fitted with a glass knife made using an LKB 7800A KnifeMaker (LKB Instruments Inc., Stockholm, Sweden) at thicknesses of 3.5–6 µm. Examination of ascomal architecture was performed on an Olympus BHS microscope equipped with Nomarski interference and phase optics. Digital micrographs were obtained with a SPOT Insight 12 Mp color camera

and Spot Advanced software. Images were processed with Adobe Photoshop v. 9.0.2 and assembled with Adobe InDesign v. 4.0.5.

2.2. DNA extraction and PCR amplification

For spore isolation, sterile dissecting needles were used to gently tease apart the contents of ascomata on antibiotic water agar [AWA, 20 g agar (Difco Laboratories Inc., Detroit MI), 0.5 g streptomycin sulfate (Sigma), 0.5 g penicillin G (Sigma–Aldrich Co. LLC, St. Louis MO) (added immediately after autoclaving) and 1000 mL deionized H₂O]. Single germinated ascospores were transferred to plates containing peptone yeast glucose agar with antibiotics [PYG + Ab, 1.25 g peptone, 1.25 g yeast extract, 18 g agar, 5 g D-glucose (Acros), 0.5 g streptomycin sulfate and 0.5 g penicillin G, and 1000 mL deionized H₂O] and grown at ambient temperature with 12/12 h light/dark conditions.

Single ascospores were transferred to PYG + Ab agar plates. DNA was extracted from mycelium of single ascospore colonies scraped with a sterile spatula from PYG + Ab plates. Mycelium was ground into a fine powder in liquid nitrogen with a mortar and pestle and DNA was extracted with a DNeasy Plant Mini Kit (Qiagen Sciences Inc., Valencia CA) according to the manufacturer's instructions. PCR amplification of extracted DNA was performed using Illustra Ready-To-Go™ PCR Beads (GE Healthcare) using the primer pair LROR and LR6 (Vilgalys and Hester 1990; Rehner and Samuels 1994) on an MJ Research PTC-200 thermocycler using the following parameters: initial denaturation at 95 °C for 5 min, followed by 40 cycles at 95 °C for 30 s, 50 °C for 15 s, 72 °C for 10 s with a final extension step of 72 °C for 10 min. PCR products were purified using a QIAquick PCR Purification Kit (Qiagen Sciences Inc., Valencia CA) according to the manufacturer's instructions. Sequencing reactions were carried out using the BigDye® Sequence Terminator Kit 3.1 (Applied Biosystems, Foster City, CA) using the primers LROR, LR3, LR3R, and LR6 (Vilgalys and Hester 1990; Rehner and Samuels 1994). Sanger DNA sequencing was performed on an AB 3730xl DNA Analyzer at the W. M. Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign.

2.3. Taxon sampling and phylogenetic analysis

In addition to the 28S sequence from the new Peruvian fungus, select sequences used in a study of *Conioscyphascus* (Réblová and Seifert 2004) were obtained from GenBank. Morphologically similar species with available 28S sequences used in a recent study of Magnaporthaceae (Thongkantha et al. 2009) were also obtained from GenBank and three Dothideomycetes were used as outgroup taxa (Supplementary Table S1). Sequences were assembled and initially aligned using Sequencher 4.9 (Gene Codes Corp., Ann Arbor, MI). Further alignment was performed using Muscle 3.6 (Edgar 2004) followed by visual correction. Sequences generated in this study and the alignment used for phylogenetic analysis were deposited respectively in GenBank and in TreeBASE (www.treebase.org, submission ID 15250).

Maximum likelihood analysis was performed with RAxML7.0.4 (Stamakis et al. 2008) on the 28S dataset on the CIPRES Portal 2.0 (Miller et al. 2010) using default settings and GTR model with 1000 fast bootstrap searches. Bayesian analysis was conducted using MrBayes 3.1.2 with two runs and four chains under default settings (Huelsenbeck et al. 2001; Ronquist and Huelsenbeck 2003). A total of 10,000,000 generations were run with trees sampled every 1000th generation, resulting in a total of 10,000 trees. The first 1000 trees were discarded as burn-in. The analysis had reached stationarity before that point, and the remaining 9000 trees were used to calculate posterior probabilities (PP). The consensus of the trees was viewed in PAUP* 4.0b10 (Swofford 2002).

3. Results


3.1. Phylogenetic analysis of 28S dataset

The 28S alignment consisted of 51 taxa and a length of 922 bp. Two ambiguous regions consisting of a total of 25 bp were excluded from further analysis. Characters at the 5' and 3' ends were also excluded because of missing data in most taxa. For Maximum Likelihood and Bayesian analyses, jModelTest 0.1.1 (Posada 2008) was used to determine the best-fit model of nucleotide evolution for the 28S data set. The GTR + I + G model was selected (AIC = 17673.8506, $-\ln(L)$ 8726.9253). Base pair frequencies were: freqA = 0.2177, freqC = 0.2563, freqG = 0.3219, and freqT = 0.2041. The analysis also estimated a rate matrix of transitions and transversions in which $r[AC] = 0.9334$, $r[AG] = 2.1286$, $r[AT] = 1.3051$, $r[CG] = 0.7264$, $r[CT] = 6.5521$, and $r[GT] = 1$. Invariable sites comprised 36.4% of the data set and the gamma shape parameter was 0.57. RAxML (Stamakis et al. 2008) analysis of the dataset produced a single most likely tree (Fig. 1) on which 1000 replicates of bootstrap support (BS) values and posterior probability (PP) values are shown for well-supported nodes. The new *Conioscypha* species belongs to a well-supported monophyletic group comprised of other *Conioscypha* taxa that is sister to the well-supported branch including Savoryellales and *Carpoligna pleurothecii*, the “CCA” clade sensu Réblová and Seifert (2004).

The 28S rRNA gene derived from pure culture of the Peruvian specimen (CBS 137657) most closely matched deposited sequences for the genera *Conioscyphascus* and *Conioscypha* in GenBank. The new fungus morphologically resembles the holomorph description of *Conioscyphascus* (Réblová and Seifert 2004), but differs from both described species of *Conioscyphascus*, *Cs. gracilis* Réblová & Seifert and *Cs. varius* (Shearer) Réblová & Seifert, in both anamorph and teleomorph morphologies.

The new Peruvian fungus and three other species, *Conioscypha japonica* Udagawa & Toyaz., *C. lignicola* Höhn, and *C. varia* (= *Cs. varius*), formed a well-supported monophyletic clade, sister to the Savoryellales in this analysis. PE0283 is best placed in *Conioscypha*.

3.2. Taxonomy

Conioscypha peruviana Zelski, Raja, A.N. Mill & Shearer, sp. nov. 

MycoBank no.: MB 805993.

Type: Peru. Cusco. Camanti. First stream along Quincemil Trail 1, on submerged woody debris, trailhead, 13°14'22.5594" S, 70°46'12.6114" W, 688 m, water 19.7° C, pH 5.83, 3 October 2010, S.E. Zelski and H.A. Raja (holotype ILL 41202).

Etymology: *peruviana*, in reference to Peru, the country of origin.

Ascomata immersed to partially immersed, or superficial, venter 280–400 µm high, 280–370 µm diam. (mean = 324 µm high, 324 µm diam., $n = 10$), globose to subglobose, hyaline, scattered to gregarious, occasionally fusing (Fig. 2A). Necks 390–510 µm long × 70–140 µm wide, cylindrical, hyaline, comprised of interwoven hyphae, of *textura intricata* in surface view (Fig. 2B), periphysate (Fig. 2C). Peridium coriaceous, glabrous, hyaline, 19–28 µm wide and 5–6 cell layers thick, composed of large thick-walled, polyhedral cells toward the exterior and thinner-walled, flattened cells toward the interior, of *textura angularis* in surface view. Paraphyses up to 7 µm wide at base, tapering to a rounded apex up to 3.5 µm wide, as long as asci, free at apices, hyaline, septate, constricted at septa, unbranched. Asci 136–184 × 6–8 µm (mean = 160.4 × 7.2 µm, $n = 10$), cylindrical to clavate, straight to sigmoidal (Fig. 2D), 8-spored, biseriolate at apex to uniseriate at ascus base, attached by a triangular pedicel to ascogenous hyphae when immature (Fig. 2E), floating freely in the centrum with a rounded ascus base when mature, with a thimble-shaped, Meltzer's reagent negative, refractive apical apparatus 1.5–2.5 µm high, 1.5–2 µm wide (mean = 1.8 × 1.9 µm, $n = 10$) (Fig. 2F). Ascospores 31.5–42 × 3–4 µm (mean = 35.4 × 3.5 µm, $n = 30$), cylindrical to navicular (ascospore apices bent in one plane), apices rounded, hyaline, 5–7-septate, not constricted at septa, smooth-walled, with lipid droplets in each cell, without sheaths or appendages (Fig. 2G–I).

Ex-type culture (ILL 41202, CBS 137657) forming colonies on PYG + Ab agar at 12/12 h light/dark at 25 °C. Colonies 2 cm diam. at 30 days, white to pale yellow, becoming dark gray at the center as conidia are formed, silky, mycelium immersed with scant aerial hyphae, margin entire, discrete, reverse whitish to buff to pale yellow.

Conidiation occurred in vitro. Conidiophores micro-nematous, mononematous, arising terminally or laterally from the hyphae (Fig. 2M), simple, erect, hyaline, smooth-walled. Conidiogenous cells sessile or on short stalks, with hyaline multilayered, cyathiform cup-like collarettes 14.5–18.5 × 7–11.5 µm (mean = 17.3 × 9.42 µm, $n = 10$) (Fig. 2J). Conidia 13.5–18 × 5–8.5 µm (mean = 16.3 × 6.5 µm, $n = 30$), l/w ratio 2.5:1, aseptate, formed singly, released successively by rupture of an outer transparent wall layer at the cell apex, percurrent. Conidia at first hyaline to pale gray, becoming brown, aseptate, ellipsoidal to allantoid or fabiform, containing lipid droplets (Fig. 2K–L).

Additional specimens examined: Peru. Cusco. Camanti. Backwater of river along Quincemil Trail 3, on submerged decorticated wood, 13°18'27.756" S, 70°48'44.9279" W, 757 m, water 22.0 °C, pH 7.1, 14 Apr 2011, S.E. Zelski and H.A. Raja, ILL 41208; 3rd stream along Quincemil Trail 1, on submerged woody debris, trailhead 13°14'22.5594" S, 70°46'12.6114" W, 688 m, water 19.0 °C, pH 8, 3 Oct 2010, S.E. Zelski and H.A. Raja, ILL 41209; 3rd stream along Quincemil Trail 1, on submerged woody debris, trailhead 13°14'22.5594" S, 70°46'12.6114" W, 688 m, water 21.4 °C, pH 7.8, 12 Apr 2011, S.E. Zelski and

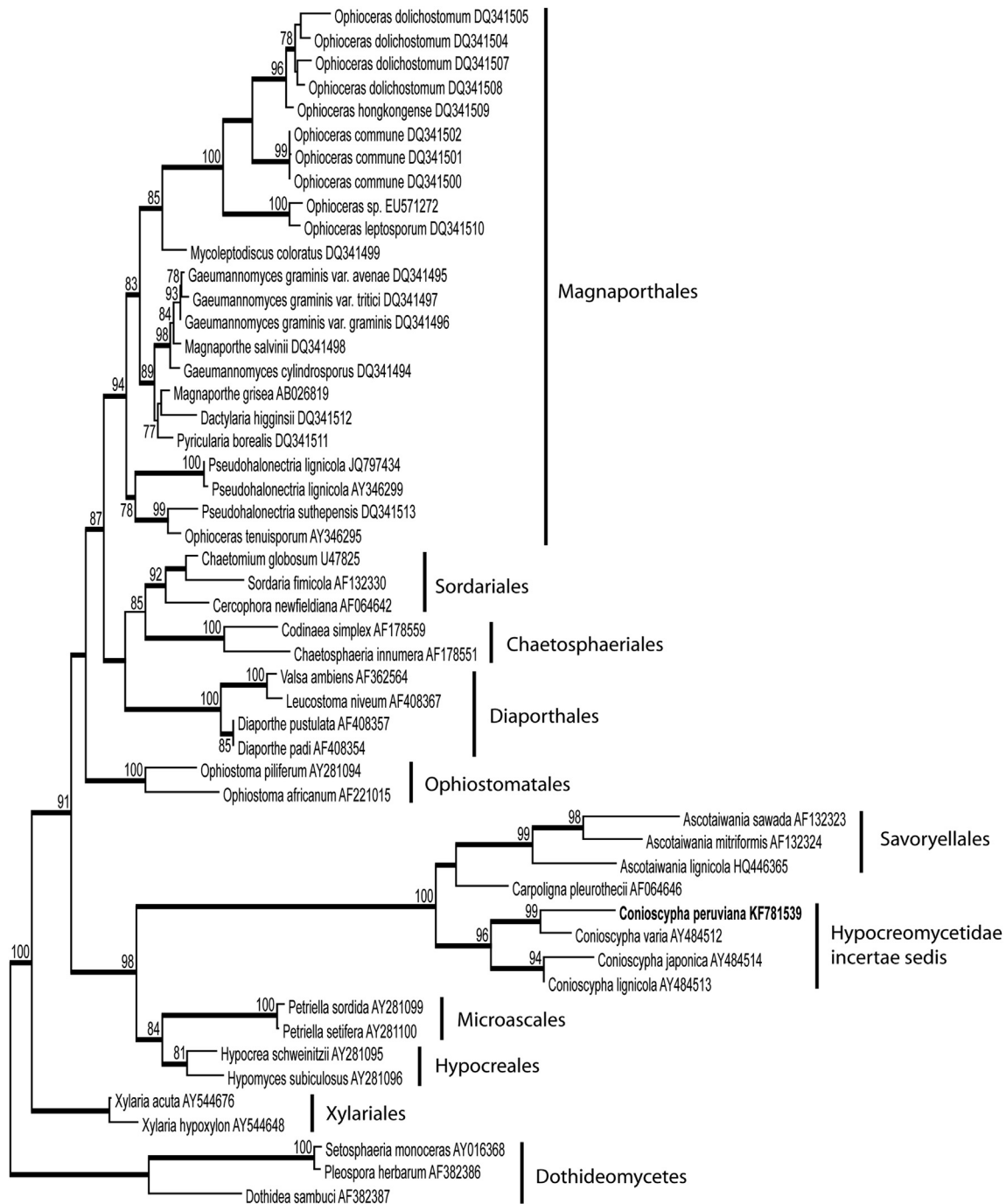


Fig. 1 – Phylogram of the most likely tree from 28S rRNA gene analysis obtained with RAxML (–ln = 8731.4922). Numbers above or below branches indicate ML bootstrap support values $\geq 75\%$, Posterior probability support values $\geq 95\%$ are indicated by thickened branches.

H. A. Raja, ILL 41210; River at end of Quincemil Trail 1, on submerged woody debris, trailhead 13°14'22.5594" S, 70°46'12.6114" W, 688 m, water 21.5 °C, pH 7.7, 12 Apr 2011, on submerged woody debris, S.E. Zelski and H.A. Raja, ILL 41213; 4th stream along Quincemil Trail 1, on submerged woody debris, trailhead 13°14'22.5594" S, 70°46'12.6114" W, 688 m, water 21.3 °C, pH 6.0, on submerged woody debris, 12 Apr 2011, S.E. Zelski and H.A. Raja, ILL 41214; Stream with red algae

along Quincemil trail 3, 13°18'27.756" S, 70°48'44.9274" W, 757 m, water 21.8 °C, pH 7.15, on submerged woody debris, 13 Apr 2011, S.E. Zelski and H.A. Raja, ILL 41211. Madre De Dios. Los Amigos Biological Field Station/Centro de Investigación y Capacitación Rio Los Amigos (CICRA). Pozo Don Pedro, palm swamp (aguajale) at end of Trail 17, 12°33'34.27" S, 70°06'38" W, 243 m, water 25.4 °C, pH 7.9, on submerged woody debris, S.E. Zelski and H.A. Raja, ILL 41211.



Fig. 2 – *Conioscypha peruviana* (holotype ILL 41202). A: Habit view. B: Surface view of neck composed of interwoven hyphae. C: Section of neck illustrating periphyses. D: Long sigmoidal unitunicate ascus. E: Young asci with triangular bases attached to hamothecium. F: J-thimble-shaped apical apparatus. G–I: Ascospores. J: Conidiogenous cells. K, L: Conidia. Bars: A 100 μm , B–D 20 μm ; E, F, L 5 μm ; G–K 10 μm .

Habitat: Saprobic on submerged woody debris in lentic and lotic habitats.

Known distribution: Peru (Cusco, Madre de Dios).

Conioscypha gracilis (Munk) Zelski, Raja, A.N. Mill. & Shearer, comb. nov.

Mycobank no.: MB 810095.

\equiv *Debaryella gracilis* Munk, Bot. Tidsskr. 51: 226. 1957 (Basionym).

\equiv *Cryptoleptosphaeria gracilis* (Munk) Rossman & Samuels, Stud. Mycol. 42: 185. 1999.

\equiv *Conioscyphascus gracilis* (Munk) R blov  & Seifert, Stud. Mycol. 50: 104. 2004.

Conidiogenous cells readily separable from mycelium, hyaline, lamellate, ovoid, 12–15 μm long, 8.5–10 μm wide. Conidia ellipsoidal to flammiform, truncate at the base, slightly tapering towards apex, 8.5–9.5 μm \times 5.5–7 μm , (mean = 9.1 \times 5.9 μm , $n = 30$), L/W 1.6:1, reddish brown, germinating from the base. The conidial dimensions of the Peruvian specimens closely match those of *C. gracilis* and are not similar to any of the other known species of *Conioscypha*. Teleomorph not found.

Specimens examined: Peru. Cusco. Camanti, Quincemil, stream with red algae along Quincemil trail 3, 13°18'27.756" S, 70°48'44.9274" W, 757 m, water 21.8 °C, pH 7.15, on submerged woody debris, 4 Oct 2010, S.E. Zelski and H.A. Raja, ILL 41215. Madre De Dios. Los Amigos Biological Field Station/Centro de Investigación y Capacitación Rio Los Amigos (CICRA), semi-aquatic habitat on Trail 1, 12°34'06.52" S, 70°06'04.57" W, 263 m, on submerged woody debris, 22 May 2010, S.E. Zelski and H.A. Raja, ILL 41203.

4. Discussion

Conioscyphascus was erected to accommodate teleomorph species having *Conioscypha* anamorphs, and included the type species of the genus, *Cs. varius*, and *Cs. gracilis* (Réblová and Seifert 2004). Keeping with tradition and following the ICBN at the time, the authors used a teleomorphic name for the genus. Réblová and Seifert's molecular analyses of 18S and 28S rRNA genes placed *C. varia* Shearer (= *Cs. varius*) in a well-supported clade with the anamorphic *C. japonica* and *C. lignicola*. Five other species lacking molecular data are currently included in *Conioscypha*: *C. bambusicola* Matsush., *C. dimorpha* Matsush., *C. fabiformis*, *C. hoehnelii* P.M. Kirk, and *C. taiwania* J.L. Chen & Tzean.

Prior to the anamorph-teleomorph connection made by Réblová and Seifert (2004), species of *Conioscypha* were characterized by enteroblastic percurrent conidiogenesis in distinct conidiogenous cells that retain successive wall layers as each conidium ruptures through the apex and by dematiaceous aseptate conidia of various shapes. The addition of teleomorph data in this study has expanded the concept of *Conioscypha*, and analyses of molecular data of *C. peruviana* and *C. varia* support the monophyly of *C. peruviana*, *C. japonica*, *C. lignicola*, and *C. varia*.

The *Conioscypha peruviana* teleomorph agrees well with the description of *Conioscyphascus*. It has ascomata that are larger than those of *C. gracilis* and smaller than those of *C. varia* and has longer, thinner asci than both. Evagination of the apical apparatus was not observed as in *Conioscyphascus varius* (Réblová and Seifert 2004). *Conioscypha peruviana* mean ascospore measurements (35.5 × 3.5 μm) fall in between those of the two other species. Although the conidiogenous cell of *C. gracilis* was not observed by Réblová and Seifert (2004), those of *C. varia* and *C. peruviana* both exhibit terminal or lateral, hyaline, lamellate, conidiogenous cells with cup-like collarettes (Shearer and Motta 1973; Shearer 1973). Conidia in *Conioscypha peruviana* are bean-shaped at maturity, similar to *C. fabiformis*, while conidia in other *Conioscypha* spp. are variously shaped, but not fabiform. *Conioscypha peruviana* conidia do not possess arms as seen in *C. varia* by Réblová and Seifert (2004). A table comparing *Conioscypha* anamorph characters can be found in Goh and Hyde (1998).

Conioscypha peruviana, described herein, is similar to *C. fabiformis*, but differs in some respects. Conidiogenesis is identical in the two taxa, but the conidiogenous cells are larger in our specimen (on average 17.3 × 9.4 μm) than in the *C. fabiformis* (13 × 5.8 μm). Conidia in *C. peruviana* are light gray to light brown to brown while those in the type specimen were reported as olivaceous. Conidia in both specimens have the same shape and possess prominent lipid droplets. Conidial

dimensions of *C. peruviana* (13.5–18 × 5–8.5 μm, mean = 17.3 × 9.4 μm, n = 30) are larger than those of the *C. fabiformis* (10–16 × 4.5–6.6 μm, mean = 13 × 5.6 μm). The conidial length to width ratio is also slightly larger in our specimen (2.5 vs. 2.3 μm). The specimens in this study came from an elevational range of 250–750 m, within the range reported in the original diagnosis of *C. fabiformis* (106–1880 m). Both the type of *C. fabiformis* (found near Iquitos) and the new species reported here are from the eastern slopes of the Andes Mountains in Peru. All attempts to obtain the type specimen of *C. fabiformis* (MFC 1P-405) were unsuccessful. Therefore it was necessary to compare the Peruvian specimen to the protologue of the holotype (Matsushima 1993).

The *C. peruviana* teleomorph should also be compared to *Ophioceras* Sacc. and *Pseudohalonestria* Minoura & T. Muroi based on its morphology. These taxa share similarities in centrum, ascus and ascospore morphologies. Long tapering septate hyaline paraphyses are found in all three genera as well as asci that float freely in the centrum at maturity. Asci are unitunicate, cylindrical to clavate, 8-spored, and possess a J-refractive apical ring that is taller than wide. Ascospores are hyaline, multiseptate, cylindrical to long-cylindrical and curved. *Conioscypha* species differ from those of *Ophioceras* in that they have light-colored coriaceous ascomata and significantly shorter ascospores, while *Ophioceras* species have dark-colored carbonaceous ascomata and long-cylindrical to filiform ascospores (Shearer et al. 1999). The coloration of ascomata in species of *Conioscypha* (hyaline, subhyaline to pale-colored) resembles species of *Pseudohalonestria* (yellow, orange to brown) to a degree, but *Pseudohalonestria* species produce a yellow pigment while *Conioscypha* species do not (Shearer 1988). *Conioscypha* species mean ascospore lengths (30.4–38.4 μm) are in line with *P. miscanthicola* B.D. Shenoy, R. Jeewon, & K.D. Hyde, *P. fuxiani* L. Cai, C.K.M. Tsui, K. Zhang & K.D. Hyde, *P. halophila* Kohlm. & Volkm.-Kohlm., and *P. adversaria* Shearer (29–41.9 μm), but the mean widths of *Conioscypha* ascospores are smaller (3.5–4.4 vs. 5.9–9 μm). Inclusion of sequences of *Ophioceras* and *Pseudohalonestria* spp. in the molecular analyses shows that despite morphological similarities, *Conioscypha* is not phylogenetically closely related to these taxa. *Conioscypha* forms a monophyletic sister group with *Ascotaiwania* Sivan. & H.S. Chang and *Carpoligna* F.A. Fern. & Huhndorf. For a detailed discussion of the similarities and differences within the *Conioscypha*-*Carpoligna*-*Ascotaiwania*, or “CCA clade,” and potential higher level taxonomic placement, see Réblová and Seifert (2004).

Acknowledgments

The authors would like to thank John Paul Janovec, Jason Dean Wells, and Pedro Centeno Checalla of the Andes to the Amazon Biodiversity Project (AABP) for providing logistical support and aid in collecting. Renán Valega Rosas, also of the AABP, is thanked for assistance in obtaining collecting and export permits. The authors would like to thank the staff of the Los Amigos Biological Station and the Amazon Conservation Association (ACA) and especially Adrian Tejador, Sarah Federman, and Sarah Carbonel for assistance on successful

collection trips. The authors also thank the Peruvian government and the agencies DGFFS, MINAG and MINAM for permission to perform research in Peru. Financial support from the National Science Foundation (NSF Grant DEB 08-44722) is greatly appreciated.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.myc.2014.09.002>.

REFERENCES

- Edgar RC, 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797; <http://dx.doi.org/10.1093/nar/gkh340>.
- Goh TK, Hyde KD, 1998. A new hyphomycete genus, *Conioscyphopsis*, from wood submerged in a freshwater stream and a review of *Conioscypha*. *Mycological Research* 102: 308–312. Published online: 08 September 2000.
- Huelsenbeck JP, Ronquist FR, 2001. Mr. Bayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755; <http://dx.doi.org/10.1093/bioinformatics/17.8.754>.
- Huhndorf SM, 1991. A method of sectioning ascomycete herbarium specimens for light microscopy. *Mycologia* 83: 520–524; <http://dx.doi.org/10.2307/3760366>.
- Matsushima T, 1993. *Matsushima mycological memoirs no. 7*. Published by the author, Kobe.
- McNeill J, Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhold K, Prado J, Prud'homme van Reine WF, Smith GF, Wiersema JH, Turland NJ, 2012. *International Code of Nomenclature for algae, fungi and plants (Melbourne Code)*. Regnum Vegetabile 154. Koeltz Scientific Books.
- Miller MA, Pfeiffer W, Schwartz T, 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments workshop (GCE)*, 2010; <http://dx.doi.org/10.1109/GCE.2010.5676129>.
- Posada D, 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253–1256; <http://dx.doi.org/10.1093/molbev/msn083>.
- Réblová M, Seifert KA, 2004. *Conioscyphascus*, a new ascomycetous genus for holomorphs with *Conioscypha* anamorphs. *Studies in Mycology* 50: 95–108.
- Réblová M, Seifert KA, Fournier J, Štěpánek V, 2012. Phylogenetic classification of *Pleurothecium* and *Pleurotheciella* gen. nov. and its *dactylaria*-like anamorph (Sordariomycetes) based on nuclear ribosomal and protein-coding genes. *Mycologia* 104: 1299–1314; <http://dx.doi.org/10.3852/12-035>.
- Rehner SA, Samuels GJ, 1994. Taxonomy and phylogeny of *Gliocladium* analyzed from nuclear large subunit DNA sequences. *Mycological Research* 98: 625–634; [http://dx.doi.org/10.1016/S0953-7562\(09\)80409-7](http://dx.doi.org/10.1016/S0953-7562(09)80409-7).
- Ronquist F, Huelsenbeck JP, 2003. MRBAYES 3.1.2: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574; <http://dx.doi.org/10.1093/bioinformatics/btg180>.
- Shearer CA, 1973. Fungi of the Chesapeake Bay and its tributaries II. The genus *Conioscypha*. *Mycologia* 65: 128–136; <http://dx.doi.org/10.2307/3757793>.
- Shearer CA, 1988. *Pseudohalonectria* (Lasiosphaeriaceae), an antagonistic genus from wood in freshwater. *Canadian Journal of Botany* 67: 1944–1955; <http://dx.doi.org/10.1139/b89-247>.
- Shearer CA, Crane JL, Chen W, 1999. Freshwater ascomycetes: *Ophioceras* species. *Mycologia* 91: 145–156; <http://dx.doi.org/10.2307/3761204>.
- Shearer CA, Motta JJ, 1973. Ultrastructure and conidiogenesis in *Conioscypha* (Hyphomycetes). *Canadian Journal of Botany* 51: 1747–1751; <http://dx.doi.org/10.1139/b73-226>.
- Stamakis A, Hoover P, Rougemont J, 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* 57: 758–771; <http://dx.doi.org/10.1080/10635150802429642>.
- Swofford DL, 2002. *PAUP*. phylogenetic analysis using parsimony (*and other methods) Version 4.0b10*. Sinauer Associates, Sunderland, Massachusetts.
- Thongkantha S, Jeewon R, Vijaykrishna D, Lumyong S, McKenzie EHC, Hyde KD, 2009. Molecular phylogeny of Magnaporthaceae (Sordariomycetes) with a new species *Ophioceras chiangdaoense* from *Dracaena loureiroi* in Thailand. *Fungal Diversity* 34: 157–173.
- Vilgalys R, Hester M, 1990. Rapid identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238–4246; <http://dx.doi.org/10.1128/jb.172.11.4238-4246.1990>.
- Volkman-Kohlmeier B, Kohlmeier J, 1996. How to prepare truly permanent microscope slides. *Mycologist* 10: 107–108; [http://dx.doi.org/10.1016/S0269-915X\(96\)80068-1](http://dx.doi.org/10.1016/S0269-915X(96)80068-1).