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Freshwater ascomycetes: Natipusillaceae, a new family of tropical fungi, including *Natipusilla bellaspora* sp. nov. from the Peruvian Amazon

Huzefa A. Raja[1]

Department of Plant Biology, University of Illinois, Room 265 Morrill Hall, 505 South Goodwin Avenue, Urbana, Illinois 61801; Illinois Natural History Survey, University of Illinois, Champaign, Illinois 61820; and Department of Chemistry and Biochemistry, 457 Sullivan Science Building, University of North Carolina, Greensboro, North Carolina 27402-6170

Andrew N. Miller

Illinois Natural History Survey, University of Illinois, Champaign, Illinois 61820

Carol A. Shearer

Department of Plant Biology, University of Illinois, Room 265 Morrill Hall, 505 South Goodwin Avenue, Urbana, Illinois 61801

Abstract: A new ascomycete species, *Natipusilla bellaspora*, collected from submerged woody debris in a freshwater stream at Los Amigos Biological Station, Madre De Dios in the Peruvian Amazon is described and illustrated. This fungus is characterized by small, globose to subglobose, hyaline ascomata; small, globose to subglobose, eight-spored fissitunicate asci; one-septate, multiguttulate ascospores with two different gelatinous sheaths, an outer amorphous sheath that enlarges in water and an inner sheath that has a distinctive persistent shape and is attached to the ascospore apex. Morphologically *N. bellaspora* differs from other *Natipusilla* species in having larger ascospores and two ascospore sheaths. A second *Natipusilla* species, *N. limonensis*, is reported for the first time from Peru. Based on the unique morphological characters of taxa in *Natipusilla* and results of previous molecular phylogenetic analyses with other members of the Dothideomycetes, we establish *Natipusillaceae* fam. nov. for this unique tropical freshwater clade.

Key words: aquatic, lineage, Neotropics, systematics

Introduction

During an altitudinal study of freshwater ascomycetes in southeastern Peru an undescribed species morphologically similar to members of *Natipusilla* A. Ferrer, A.N. Mill. et Shearer (Dothideomycetes) (Ferrer et al. 2011) was collected on submerged wood in a stream located at low elevation (~ 200–300 m) at the Los Amigos Biological Field Station/Centro de Investigación y Capacitación Rio Los Amigos (CICRA) in the Peruvian Amazon. This fungus is best accomodated in *Natipusilla* based on the following morphological characters: small, globose to subglobose, hyaline ascomata; small, globose eight-spored fissitunicate asci; one-septate, multiguttulate ascospores with two gelatinous sheaths.

The type species of *Natipusilla*, *N. limonensis* A. Ferrer, A.N. Mill. et Shearer, was described from material on submerged wood in freshwater habitats in Costa Rica and Ecuador (Ferrer et al. 2011). It is characterized by small, hyaline, globose to subglobose ascomata and fissitunicate globose asci with eight bi- or triseriate, irregularly overlapping, one to many septate, multiguttulate ascospores surrounded by a gelatinous sheath. Three species currently are in the genus: *N. decorospora* A. Ferrer, A.N. Mill. et Shearer, *N. limonensis* and *N. naponensis* A. Ferrer, A.N. Mill. et Shearer. The undescribed species from Peru is distinctive in having larger ascospores than other members of the genus and a unique bipartite gelatinous sheath surrounding the ascospores. Thus it is described and illustrated herein as a new species.

Materials and methods

Sample collection and morphological study.—Sampling was conducted along a defined altitudinal gradient (200–3000 m) in southeastern Peru following procedures outlined in Shearer et al. (2004). During two field expeditions collections were made in diverse habitats such as palm swamps (aguajales), seasonal streams, first order streams, second order streams, rivers, semi-aquatic habitats and small oxbow lakes. Water temperature and pH were recorded in the field with a HI 98219 model (pH/EC/TDS/Temp) probe (Hanna Instruments, Woonsocket, Rhode Island) and latitude and longitude were measured in the field with a Garmin global positioning system device (Garmin, Olathe, Kansas). Field data are presented in *Specimens examined*. Samples were placed in polyethylene bags with zip closures containing moist paper towels and kept in black plastic bags for subsequent transport to the lab at the University of Illinois. Samples were cooled to reduce biological activity. In the lab substrates were gently rinsed with tap water to remove excess mud and plant debris and incubated at ambient temperatures (about 24 C) under 12/12 h light/dark conditions in polystyrene storage boxes lined with moistened paper towels. Samples were examined under a dissecting microscope within 1 mo of collection and periodically over the next 6 mo (Shearer 1993, Shearer

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[1] Corresponding author. E-mail: haraja@uncg.edu

et al. 2004). Cultures were obtained with methods outlined in Shearer (1993) and Shearer et al. (2004) and are maintained in the Department of Plant Biology Fungus Collection, University of Illinois.

RESULTS

TAXONOMY

Natipusillaceae Raja, Shearer & A.N. Mill. fam. nov. MycoBank MB561948

Ascomata in ligno submerso, pusilla, globosa ad subglobosa, erumpentia vel superficiala, primo hyalina demun atrobrunnea. Pseudoparaphyses septate, rarus. Asci fissitunicati, octospori, globosi vel subglobosi. Ascosporae partim uniseptatae, dispositae irregulariter vel regulariter, cylindrici vel fusiforme vel clavati, octospori, multiguttulatae, pallidae brunneae, cum vel sone tunica, mucilagina, vel appendicibus.

Generitypus *Natipusilla* A. Ferrer, A.N. Mill. & Shearer Mycologia 103:417. 2011.

Ascomata small, on submerged wood, globose to subglobose, erumpent to superficial, hyaline to light brown. Peridial wall membranous, composed of pseudoparenchyma cells forming a *textura angularis* in surface view. Pseudoparaphyses septate, sparse. Asci globose, subglobose or obclavate, fissitunicate, eight-spored. Ascospores fusiform to cylindrical, one to several septate, multiguttulate or not, hyaline becoming brown with age, with or without a gelatinous sheath and/or appendages.

Type genus: Natipusilla A. Ferrer, A.N. Mill. & Shearer Mycologia 103:417. 2011.

Natipusilla bellaspora Raja, Shearer & A.N. Mill. sp. nov. FIGS. 1–12

MycoBank MB561924

Ascomata 115–150 × 150–185 μm, in ligno, pusilla, globosa vel subglobosa, hyalina demun pallidae lutea, translucera, membranacea, gregaria, superficiala vel immersa, apapillata. Peridium e textura angularis compositum. Pseudoparaphyses raresceres, e matrice gelatinosa immersa. Asci 50–74 × 40–60 μm, globosum vel subglobosum, interdum oblclavatum, fissitunicatum, ad apicum crassum, octosporum. Ascospores 40–48 × 10–13 μm, fusiformis, ad septatum constrictum, uniseptatum, demum triseptatum, multiguttulatae, hyalinae, ad cinctum duoum tunicatum mucilaginum.

Ascomata 115–150 × 150–185 μm, on wood, small, globose to flattened subglobose, hyaline to cream-colored, translucent, membranous, scattered to gregarious, superficial to partially immersed, non-ostiolate, non-papillate. Peridial wall membranous, thin, composed of hyaline pseudoparenchymatous cells, 9–13 × 5–7 μm, of textura angularis in surface view. Pseudoparaphyses sparse, short, immersed in gel. Asci 50–74 × 40–60 μm (av = 62 × 47 μm, n = 25), globose to subglobose, sometimes broadly obclavate, fissitunicate, thick-walled at the apex, with eight irregularly overlapping ascospores. Ascospores 40–48 × 10–13 μm (av = 44 × 11 μm, n = 50), fusiform, broadest in the middle of the apical cell, slightly curved, multiguttulate, one-septate, slightly constricted at the septum, sometimes breaking at the septum, upper cell wider than lower cell, hyaline when young, becoming three-septate and brown when old, surrounded by two different gelatinous sheaths: an outer amorphous sheath that enlarges in water and an inner sheath that has a distinctive persistent shape and is attached to the ascospore apex, surrounds the entire ascospore and extends ca. 5–8 μm from the ascospore wall. Persistent inner sheath stains blue with aqueous nigrosin.

Colonies on peptone yeast extract glucose agar with antibiotics (PYG, peptone 1.25 g, yeast extract 1.25 g, D-glucose 3.00 g; 1000 mL distilled water, 18 g agar; 250 mg/L streptomycin sulfate, 250 mg/L penicillin G) slow growing, light brown to gray; brown on the reverse; effuse and floccose. Hyphae thin-walled, hyaline to light brown.

Anamorph: Not observed.

Habitat: On submerged, decorticated wood.

Known distribution: Peru.

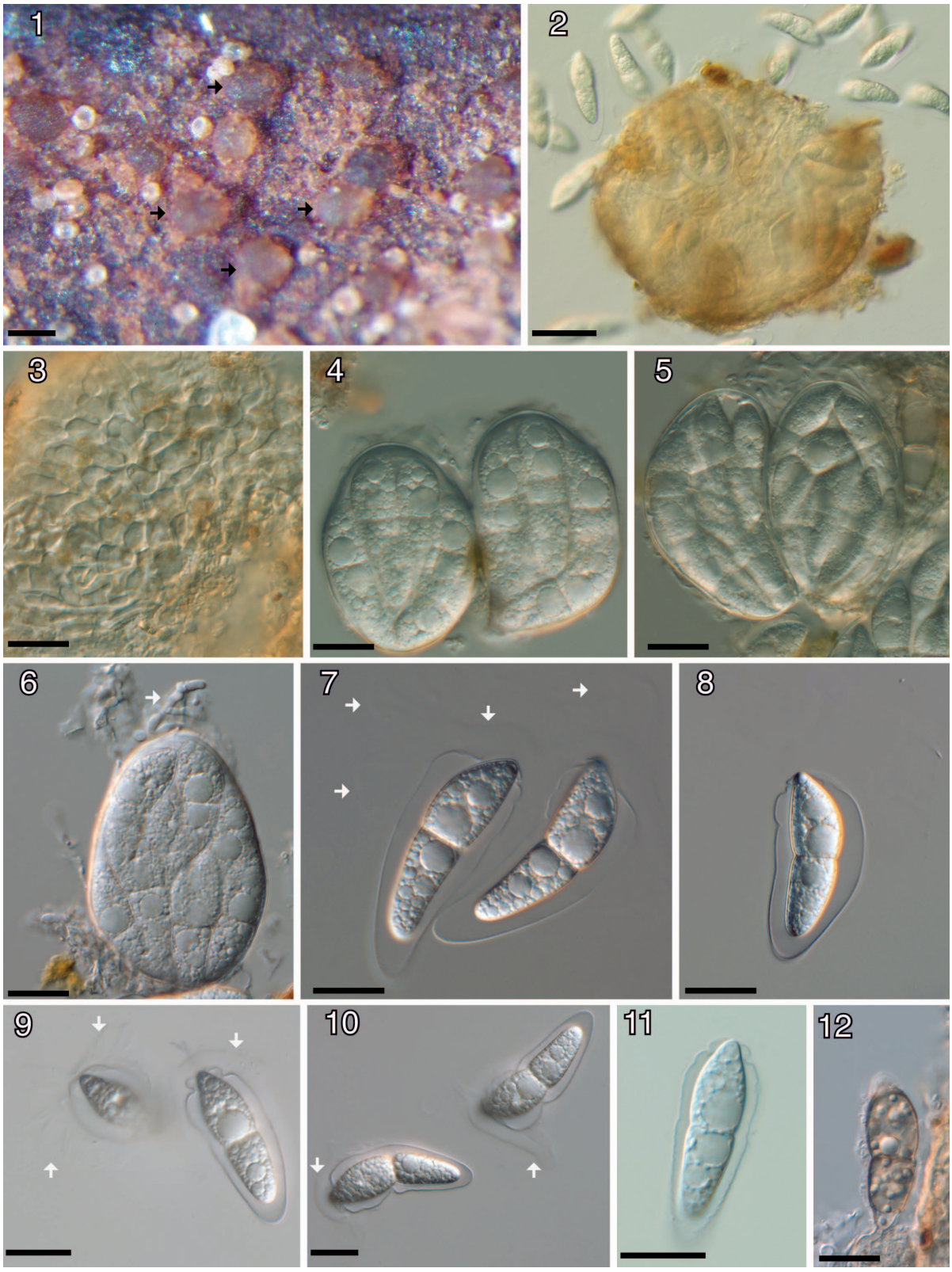
Etymology: bella L. = beautiful, and spora L. = spore referring to the ascospores.

Specimens examined: TYPE. PERU. Madre de Dios. Stream at Trail 10, 12.563597S, 70.096797W, ~ 218 m, Los Amigos Biological Station (CICRA), on submerged decorticated wood, 22 C, pH 5.5, 22 May 2010, *Zelski S. and Raja H.A., PE91-1.* (HOLOTYPE ILL 40795).

Comments: Natipusilla bellaspora can be distinguished easily from previously described species of *Natipusilla* in that it has relatively larger ascospores. In addition the morphology of the ascospore sheath is complex with a diaphanous outer sheath and a rigid inner sheath (FIGS. 7, 9, 10). *Natipusilla decorospora* also has a combination of sheath types, but its amorphous sheath is scythe-shaped and attached apically (Ferrer et al. 2011). The ascospores of *N. limonensis* have a rigid sheath similar to that of *N. bellaspora*, but the sheath in *N. limonensis* is flattened at the apical end of the ascospores. In addition the ascospores of *N. limonensis* are smaller (35–40 × 9–11 μm) compared to those of *N. bellaspora* (40–48 × 10–13 μm).

Natipusilla limonensis A. Ferrer, A.N. Mill et Shearer, Mycologia 103:417–420. 2011.

Specimens examined: PERU. Cusco. Camanti. Stream at Quince Mil Trail 1, 13.23960S, 70.77017W, 688 m, on submerged wood, 21 C, pH 5.5, 26 May 2010, *Zelski S and Raja HA, PE3-1;* stream at Quince Mil Trail 2, 13.22613S, 70.75352W, 668 m, on submerged decorticated wood, 21 C, pH 6, 26 May 2010, *Zelski S and Raja HA, PE3-2;* stagnant ditch at Quince Mil Trail 2, 13.22789S, 70.75394W, 658 m, on submerged wood, 23 C, pH 5, 26 May 2010, *Zelski S and*



FIGS. 1–12. *Natipusilla bellaspora* (From HOLOTYPE. ILL 40795 (PE91-1). 1. Ascomata on wood (shown with black arrows). 2. Squash mount of ascoma. 3. Peridial wall with thickened cell walls. 4–6. Globose to subglobose asci, note arrow showing sparse pseudoparaphyses (6). 7–10. Ascospores in water showing the combination of ascospore sheath types, an outer amorphous sheath spreading in water (shown with white arrows in 7, 9, 10) and an inner rigid sheath. 11. Ascospores with inner sheath mounted in glycerin. 12. Older brown ascospore. Bars: 1 = 200 µm; 2–12 = 20 µm.

Raja HA, PE3-3; Madre de Dios, palm swamp (Aguajale) at Pozo Don Pedro, end of Trail 17 at Los Amigos Biological Station (CICRA), ~ 218 m, on submerged softened, decorticated wood, 22 May 2010, *Zelski S and Raja HA, PE3-4.*

Comments: The specimens collected from Peru agree with the original description of *N. limonensis* by Ferrer et al. (2011). *Natipusilla limonensis* occurred at a low elevation site as well as a high elevation cloud forest site in southeastern Peru. It is a new record for Peru.

DISCUSSION

Recently Schoch et al. (2009) presented a multigene appraisal of the Dothideomycetes that included a comprehensive taxon sampling and phylogenetic analysis of several major orders of bitunicate fungi. In this study on the molecular phylogenetic classification of Dothideomycetes new families of freshwater ascomycetes, such as Amniculicolaceae and Lentitheciaceae, were established in the Pleosporales (Zhang et al. 2009). More recently Hirayama et al. (2010) erected Lindgomycetaceae in the Pleosporales, based on combined SSU + LSU sequence data (also see Shearer et al. 2009). Although members of *Natipusilla* share a few morphological characters with these Pleosporales, such as presence of pseudoparaphyses, globose to subglobose fissitunicate asci and one-septate ascospores with gelatinous sheaths (Luttrell 1973, Kirk et al. 2008, see also Zhang et al. 2009 for an emended description), based on molecular analysis of SSU + LSU data (Ferrer et al. 2011), the *Natipusilla* clade appears to be an independent lineage and shares no molecular phylogenetic relationships within the Pleosporales at familial rank. In addition based on morphology we could not place *Natipusilla* in any of the currently accepted families in the Dothideomycetes (Cannon and Kirk 2007, Schoch et al. 2009, Lumbsch and Huhndorf 2010).

Species in the *Natipusilla* clade also share a number of unique morphological characters with the family Aliquandostipitaceae (Jahnulales), such as a freshwater habit, cells in the outer layer of the peridium having moderately thickened walls, pseudoparaphyses and ascospores with gelatinous sheaths (Inderbitzin et al. 2001, Pang et al. 2002, Campbell et al. 2007, Raja et al. 2006). However members of the Aliquandostipitaceae produce broad, brown, thick-walled hyphae that spread across the substrate and often connect adjacent ascomata; these large, brown hyphae also are produced in cultures derived from single ascospores of species in the Aliquandostipitaceae (Inderbitzin et al. 2001, Campbell et al. 2007, Raja et al. 2006). Thick-walled hyphae that spread across the substrate and connect adjacent ascomata were not observed in *Natipusilla* species and they do not produce large, thick hyphae in culture (Ferrer et al. 2011).

Natipusilla also should be compared with *Ascominuta* V.M. Ranghoo et K.D. Hyde (Ranghoo and Hyde 2000), a bitunicate freshwater ascomycete genus described from submerged wood in Hong Kong. Currently there are two described species in the genus, *A. lignicola* V.M. Ranghoo et K.D. Hyde, the type of the genus, and *A. ovalispora* D.M. Hu et K.D. Hyde, a newly described species on submerged wood from northern Thailand (Hu et al. 2010). Both *Natipusilla* and *Ascominuta* share a number of morphological characters, such as small ascomata occurring on submerged wood, bitunicate globose asci and ascospores with gelatinous sheaths. When Ferrer et al. (2011) established *Natipusilla* as a new Dothideomycete genus they compared it to *Ascominuta* based on morphology. The authors reported that the two genera differed in the color of ascomata, numbers of ascospores per asci and morphology of pseudoparaphyses (Ferrer et al. 2011). In addition Ferrer et al. (2011) were unable to use molecular data to compare *A. lignicola* with sequence data from *Natipusilla* because the sequence of *A. lignicola* deposited in GenBank could not be aligned with sequences of *Natipusilla* species. Further molecular and morphological studies are required to establish whether the two minute ascomycete genera are closely related or even possibly congeneric.

Based on evaluation of morphological data and previous results of molecular analyses (Ferrer et al. 2011), we establish a new family, Natipusillaceae. The ordinal position of Natipusillaceae remains incertae sedis within the Dothideomycetes, pending further molecular work. Future studies with additional taxon sampling and added data on single-copy protein coding genes such as RPB1 and MCM7 might shed light on the ordinal status of Natipusillaceae within the Dothideomycetes.

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