Phylogeny and morphology of dematiaceous freshwater microfungi from Perú

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Abstract: A survey of freshwater ascomycetes conducted along an elevational gradient in Perú in the Districts of Cusco, Junín, and Madre de Dios yielded specimens of *Cancellidium applanatum*, *Cordana abramovii*, *Sporoschisma juvenile*, *S. uniseptatum*, and *S. saccardoi*. With the exception of *S. saccardoi*, these are new records for Perú. Molecular data was generated for three previously unsequenced species: *Cancellidium applanatum*, *Cordana abramovii* and *Sporoschisma saccardoi*. These taxa are reported herein from the neotropics with an accompanying phylogeny based on partial 28S nuclear ribosomal large-subunit sequence data. The sexual morph of *S. saccardoi* has previously been linked to *Melanochaeta hemipsila* through cultural studies. Molecular data from ascospores and conidia of *M. hemipsila and S. saccardoi*, respectively, were used to demonstrate a genetic connection of the sexual and asexual morphs of these fungi for the first time, resulting in the new combination *Sporoschisma hemipsila* being made.

Key words:

Aquatic fungi Ascomycoyta Cancellidium Cordana Sporoschisma submerged woody debris

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INTRODUCTION

During a study of ascomycetes colonizing submerged, decomposing woody and herbaceous debris in freshwater habitats along an elevational gradient in Perú extending from the Peruvian Amazon to the Peruvian Andes (2010–2012), numerous freshwater mitosporic fungi were encountered. Shearer *et al.* (2007) divided the freshwater mitosporic fungi into three ecological groups: (1) freshwater hyphomycetes; (2) aeroaquatic hyphomycetes; and (3) freshwater miscellaneous mitosporic ascomycetes. This study deals with one aeroaquatic hyphomycete (*Cancellidium applanatum*), and four species of miscellaneous mitosporic ascomycetes (*Cordana abramovii, Sporoschisma saccardoi, S. juvenile*, and *S. uniseptatum*).

Cancellidium is typified by *C. applanatum*, which was originally collected from submerged wood blocks of *Ochroma pyramidale* in Kobe, Japan. *Cancellidium applanatum* has been reported from many Paleotropical localities (Webster & Davey 1980, Shaw 1994, Ho *et al.* 2001, Sivichai *et al.* 2002, Fryar *et al.* 2004, Pinnoi *et al.* 2006, Pinruan *et al.* 2007, Zhao *et al.* 2012). In this study in the Neotropics, multiple collections of *Cancellidium applanatum* (PE0063) were recovered from low and middle altitudes along the elevational gradient, but not from high altitude aquatic habitats. Yeung *et al.* (2006) suggested that the congeneric *C. pinicola* was phylogenetically related to *Hypocreales*. However, they noted that a connection to *Hypocreales* was dubious due to the

questionable nature of the culture from which the DNA was extracted (Yeung *et al.* 2006, Zhao *et al.* 2012). In this study one 28S sequence was generated from a Peruvian specimen and the identity was corroborated with two 28S sequences generated from Thai material.

Another dematiaceous fungus, closely resembling Cordana abramovii, was found in 33 of 86 collections from a range of sites. Cordana is typified by C. pauciseptata. The type is described as acervular, possibly due to the cushiony appearance of the aggregated sporing structures and setae on the substrate (Preuss 1851). The majority of the taxa belonging to the genus are not described as such; rather, conidia simply form on erect conidiophores with surrounding setae. The Peruvian specimens of Cordana abramovii (PE0053) are characterized by pale brown to brown, cylindrical, septate conidiophores with swollen conidiogenous zones; terminal and intercalary polyblastic conidiogenous cells; and golden brown to dark brown, 1-septate, thickwalled, verruculose conidia. Two additional species, C. musae and C. pauciseptata, have previously been reported from Perú (Matsushima 1993). Cordana species are placed in the family Cordanaceae (Cannon & Kirk 2007).

Several species of *Sporoschisma* were collected from multiple sites, including *S. uniseptata* (15 collections), *S. saccardoi* (13), *S. juvenile* (9), and *S. parcicuneatum* (2). *Sporoschisma uniseptata* has 1-septate, rarely 2-septate, reddish brown, verruculose conidia; *S. saccardoi* has brown, 5-septate, doliiform, smooth walled conidia; *S. juvenile*

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has brown 5-septate, cylindrical, verruculose conidia; and S. *parcicuneatum* has brown, 1(–3)-septate, cuneiform, verruculose conidia (Goh *et al.* 1997).

Sexual reproductive structures of *Melanochaeta hemipsila* were found among conidiophores of *S. saccardoi* from substrates collected in Cusco and Junin. *Melanochaeta hemipsila* has been connected to *S. saccardoi* based on studies in which the asexual morph was produced from colonies derived from ascospores (Müller *et al.* 1969, Nag Raj 1975, Sivichai *et al.* 2000). The sexual morph of the Peruvian specimens is characterized by: gregarious, superficial, dark brown to black ascomata with short conical beaks; numerous, septate, capitate setae arising from the external ascomal wall; clavate, unitunicate, 8-spored asci with an I- refractive apical apparatus; biseriate, cylindrical to curved, 5-septate ascospores with olivaceous to brown central cells, hyaline end cells, lacking sheaths or appendages.

The goals of this study were to: (1) describe, illustrate, and provide voucher specimens and sequences for the foregoing species of freshwater mitosporic fungi for which pure cultures were obtained; (2) compare and contrast these fungi with morphologically similar and genetically related taxa; and (3) construct a molecular phylogeny using 28S large subunit (LSU) nrDNA to elucidate the evolutionary relationships of these fungi with other *Ascomycota*.

MATERIALS AND METHODS

Isolates

Submerged woody and herbaceous debris was collected from a variety of freshwater habitats that included rivers, streams, backwaters, swamps, and inundated trails. Approximately 30 pieces of debris were put into a sealable plastic bag along with a wet paper towel at each of 86 sampling sites along an altitudinal gradient stretching from 218-3566 m. Samples were shipped to our laboratory at the University of Illinois at Urbana-Champaign. In the laboratory, substrates 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 mo with an AO stereomicroscope. Digital images of fruiting structures were taken on an Olympus SZX7 stereomicroscope (Olympus Optical Tokyo) fitted with a SPOT RT colour camera using SPOT Advanced software (Diagnostics Instruments, Sterling Hts, MI).

Ascomata were removed from the substrate with a dissecting needle and gently teased apart in a drop of distilled water. Conidiophores and conidia were removed in the same manner and gently placed in a drop of distilled water. Fungal tissue was then sandwiched between 25 × 25 and 18 × 18 mm cover slips in distilled water, and placed on a microscope slide for examination. Glycerin was added after examination in preparation for permanent preservation in our herbarium (ILL) according to the protocol of Volkmann-Kohlmeyer & Kohlmeyer (1996). Examination of fungal structures was performed on an Olympus BHS microscope (Olympus Optical, Tokyo) equipped with Nomarski interference and

phase optics. Digital micrographs were obtained with the SPOT Insight 12 Mp colour camera and Spot Advanced software. Images were processed with Adobe Photoshop and assembled with Adobe InDesign.

For single spore isolation, sterile dissecting needles were used to spread ascospores or conidia on antibiotic water agar (AWA]: 20 g agar (Difco), 0.5 g streptomycin sulfate, 0.5 g penicillin G (Sigma) and 1000 mL deionized H_2O . Single germinated ascospores or conidia were transferred to 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 . They were then grown at ambient temperature with 12/12 hr light/dark conditions.

DNA isolation, amplification and analyses

DNA extraction was performed on mycelium scraped with a sterile spatula from PYG+Ab agar plates. Mycelium was first ground into a fine powder in liquid nitrogen with a sterile mortar and pestle and DNA was extracted with a DNeasy Plant Mini Kit (Qiagen Sciences, Valencia, CA) according to the manufacturer's instructions. PCR of extracted DNA was performed using Illustra Ready-To-Go™ PCR Beads (GE Healthcare) using the primer pair LROR and LR6 (Rehner & Samuels 1994, Vilgalys & Hester 1990) 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 QIAquick PCR Purification Kit (Qiagen Sciences, Valencia, CA) according to the manufacturer's instructions. Sequencing reactions (11 µL) using the primers LROR, LR3, LR3R, and LR6 (Rehner & Samuels 1994, Vilgalys & Hester 1990) were carried out using the BigDye® Sequence Terminator kit 3.1 (Applied Biosystems, Foster City, CA). 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.

In addition to the sequences generated in this study (Table 1), sequences used in a study of Melanochaeta (Mugambi & Huhndorf 2008) were downloaded from GenBank. A taxonomic search of Cordana in GenBank yielded seven LSU sequences as well as a sequence from the sexual morph of Porosphaerella, represented by P. borinquensis. These sequences were added to the gene database. Select Sordariomycetes sequences from Zhang et al. (2006) as well as those of several freshwater ascomycetes were also included. Two members of Magnaporthales and one from Lulworthiales were used as outgroup taxa (Table 2). Sequences were assembled and initially aligned in Sequencher v. 4.9 (Gene Codes, Ann Arbor, MI). Alignment was performed using Muscle v. 3.6 (Edgar 2004) followed by visual correction. Characters at the 5' and 3' ends were excluded due to missing data for some taxa, resulting in a final alignment length of 1062 base pairs.

For Maximum Likelihood and Bayesian analyses, jModeltest v. 0.1.1 (Posada 2008) was used to determine the best-fit model of nucleotide evolution for the data set. The GTR + I + G model was selected (-InL 9963.4715). Base pair frequencies were: freqA = 0.2250, freqC = 0.2513, freqG =

 Table 1. Sequences generated for this study with voucher specimen location, GenBank number, and CBS strain number.

Species	Voucher specimen, Isolate	GenBank Accession Number	CBS no.
Cancellidium applanatum	ILL 41206, <i>TH0063-1a</i>	KF833358	CBS 137654
Cancellidium applanatum	ILL 41206, TH0063-1b	KF833359	CBS 137655
Cancellidium applanatum	ILL 41205, <i>PE0063-1a</i>	KF833360	CBS 137653
Cordana abramovii	ILL 41204, <i>PE0053-24a</i>	KF833361	CBS 137652
Sporoschisma hemipsila	ILL 41207, <i>PE0177-21a</i>	KF833362	CBS 137656
Sporoschisma hemipsila	ILL 41207, PE0177-21b	KF833363	
Sporoschisma hemipsila	ILL 41207, <i>PE0177-21c</i>	KF833364	CBS 138600

Table 2. Sequences retrieved from GenBank for this study.

Species	GenBank Accession Number	Species	GenBank Accession Number
Aniptodera chesapeakensis	U46882	Fusoidispora aquatica	AY780365
Annulatascus triseptatus	AY780049	Gaeumannomyces graminis	AF362557
Annulusmagnus triseptatus	GQ996540	Gnomonia gnomon	AF408361
Apiognomonia errabunda	AF408334	Halosphaeria appendiculata	U46885
Ascitendus austriacus	GQ996539	Lasiosphaeria ovina	AF064643
Bellojisia rhynchostoma	EU999217	Lentomitella cirrhosa	AY761085
Bullimyces aurisporus	JF775590	Lentomitella crinigera	AY761086
Bullimyces communis	JF775585	Lindra thalassiae	DQ470947
Bullimyces cosaricensis	JF775591	Melanochaeta aotearoae	AF466082
Calosphaeria barbirostris	EF577059	Melanochaeta aotearoae	AF466081
Ceratolenta caudata	JX066705	Melanochaeta hemipsila	EU583218
Ceratostomella cuspidata	FJ617558	Melanochaeta hemipsila	EU583217
Ceratostomella pyrenaica	DQ076323	Melanochaeta hemipsila	AF466083
Chaetomidium arxii	FJ666359	Melanochaeta hemipsila	AF466084
Chaetosphaeria innumera	AY017375	Melanopsamella vermiculariodes	AF064644
Chaetosphaeria ovoidea	AF064641	Neurosopra crassa	AF286411
Chaetosphaeria pulviscula	AF466091	Nohea umiumi	U46893
Chaetosphaeria spinosa	AFF466079	Ohiostoma stenoceras	DQ836904
Chaetosphaeria tropicalis	AF466080	Ophioceras tenuisporum	AY346295
Chatosphaeria capitata	AFF466061	Ophiostoma pilferum	DQ470955
Conlarium duplumascospora	JN936993	Papulosa amerospora	DQ470950
Cordana ellipsoidea	HE672156	Porosphaerella borinquensis	EF063573
Cordana ellipsoidea	HE672166	Rhamphoria delicatula	AF261068
Cordana inaequalis	HE672157	Rhodoveronaea varioseptata	FJ617560
Cordana pauciseptata	HE672158	Riomyces rotundus	JF775589
Cordana pauciseptata	HE672159	Sordaria fimicola	AY780079
Cordana pauciseptata	HE672160	Tainosphaeria crassipes	AF466089
Cordana solitaria	HE672161	Thielavia subthermophila	HM448442
Cryptadelphia groenendalensis	EU528007	Thyridium vestitum	AY544671
Cryptadelphia polyseptata	AY281102	Valsa ambiens	AF362564
Diaporthe eres	AF408350	Xylomelasma sordida	AY761087
Fragosphaeria purpurea	AF096191		

0.3204, and freqT = 0.2033. The analysis estimated a rate matrix of transitions and transversions in which r[AC] = 0.8185, r[AG] = 2.3648, r[AT] = 1.8097, r[CG] = 0.5711, r[CT] = 7.3857, and r[GT] = 1. Invariable sites comprised 0.416 of the data set and the gamma shape parameter was 0.427. Maximum likelihood analysis was performed with RAxML

v. 7.0.4 (Stamakis *et al.* 2008) on the LSU dataset on the CIPRES Portal v. 2.0 (Miller *et al.* 2010) using default settings and GTR with 1000 fast bootstrap searches.

Bayesian analysis was conducted using MrBayes v. 3.1.2 with two runs and four chains under default settings (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck **Table 3**. Collection locations of specimens examined in this study. All collections are of submerged woody debris. Taxa present at each site are abbreviated as follows: Ca = *Cancellidium applanatum*, Co = *Cordana abramovii*, Sh = *Sporoschisma hemipsila*, Sj = *Sporoschisma juvenile*, and Su = *Sporoschisma uniseptata*. All Perú collections are made by S.E. Zelski and H. A. Raja, except for C-1797 collected by S.E. Zelski and J. A. Balto. The Thai collections were made by S. E. Zelski.

Collection	Country	State	Site details	Таха
C-1696	Perú	Madre de Dios	Palm swamp off the Interoceanic Highway near Puerto Maldonado, 12°42'48.0954"S, 69°28'11.28"W, 239m, water 23.3 C, pH 5.9, 20 May 2010	Su
C-1697		Madre de Dios	Semi-aquatic habitat on Trail 1, 12°34'06.52"S, 70°06'04.57"W, 263m, 22 May 2010	Са
C-1698		Madre de Dios	Stream at Trail 10, 12°37'48.95"S, 70°05'23.69"W, 287m, water 22.3 C, pH 5.6, 22 May 2010	Ca
C-1699		Madre de Dios	Creek at Trail 23, 12°33'31.03"S, 70°05'56.96"W, 280 m, water 22.2 C, pH 6.4, 22 May 2010	Са
C-1700		Madre de Dios	Stream at Trail 28, 12°34′02.81″S, 70°05′42.96″W, 272 m, water 22.7 C, pH 5.9, 22 May 2010	Ca
C-1702		Madre de Dios	Rio Amigos, 12°34'02.86"S, 70°04'56.26"W, 218m, water 25.3 C, pH 7.9, 22 May 2010	Su
C-1703		Madre de Dios	Pozo Don Pedro, palm swamp at end of Trail 17, 12°33'34.27"S, 70°06'38"W, 243m, 2 May 2010	Са
C-1704		Madre de Dios	Oxbow lake at Trail 14, 12°34'14.74"S, 70°05'23.69"W, 241m, water 23.0 C, pH 6.7, 23 May 2010	Su
C-1705		Madre de Dios	Seasonal lake at Trail 29, 12°34'16.98"S, 70°05'06.70"W, 244m, water 23.2 C, pH 6.4, 23 May 2010	Ca, Sj, Su
C-1708		Madre de Dios	Rio Amigos, 12°33'46.476"S, 70°04'41.808"W, 218 m, water 25.3 C, pH 7.9, 23 May 2010	Co
C-1709		Cusco	River at Quincemil Trail 1, trailhead 13°14'22.5594"S, 70°46'12.6114"W, 688m, water 21.0 C, pH 6.3, 26 May 2010	Са
C-1710		Cusco	Stream at Quincemil Trail 1, 13°13'58.25945"S, 70°46'37.7754"W, 675m, water 22.2C, pH 7.2, 26 May 2010	Sh
C-1711		Cusco	Stream at Quincemil Trail 1, trailhead 13°14'22.5594"S, 70°46'12.6114"W, 688m, water 21.2 C, pH 7.1, 26 May 2010	Co
C-1712		Cusco	Stream at Quincemil Trail 1, trailhead 13°14'22.5594"S, 70°46'12.6114"W, 688m, water 21.2 C, pH 6.8, 26 May 2010	Ca, Co
C-1713		Cusco	Stream at Quincemil Trail 1, trailhead 13°14'22.5594"S, 70°46'12.6114"W, 688m, water 21.0 C, pH 6.0, 26 May 2010	Ca, Co
C-1714		Cusco	Stream at Quincemil Trail 1, trailhead 13°14'22.5594"S, 70°46'12.6114"W, 688m, water 21.2 C, pH 5.5, 26 May 2010	Ca, Co
C-1715		Cusco	Semi-aquatic habitat along Quincemil Trail 1, trailhead 13°14'22.5594"S, 70°46'12.6114"W, 688m, water 21.2 C, pH 6.8, 26 May 2010	Ca, Su
C-1716		Cusco	Stagnant ditch along Quincemil Trail 2, 13°13'40.404"S, 70°45'14.184"W, 659m, water 22.8 C, pH 5.3, 26 May 2010	Ca, Co
C-1717		Cusco	Stream at Quincemil Trail 2, trailhead 13°13' 34.07"S, 70°45'12.67"W, 653m, water 21.8 C, pH 6.2, 26 May 2010	Са
C-1719		Cusco	Stream at Quincemil Trail 2, trailhead 13°13' 34.00"S, 70°45'10.62"W, 653m, water 21.9 C, pH 6.5, 26 May 2010	Ca, Co
C-1720		Cusco	Stream at Quincemil Trail 3, 13°18'27.756"S, 70°48'44.9274"W, 757m, water 20.7 C, pH 6.0, 27 May 2010	Co, Sj
C-1722		Cusco	Stream at Quincemil Trail 3, 13°18'27.756"S, 70°48'44.9274"W, 757m, water 21.3 C, pH 7.5, 27 May 2010	Co, Su
C-1723		Cusco	Stream at Quincemil Trail 3, 13°18'27.76"S, 70°48'44.93"W, 757m, water 22.3 C, pH 7.5, 27 May 2010	Са
C-1725		Cusco	River at Quincemil Trail 3, 13°18'53.128"S, 70°48'44.8194"W, 817m, water 20.3 C, pH 7.6, 27 May 2010	Sj
C-1726		Cusco	Stream crossing Interoceanic Highway, 13°17'7.008"S, 70°47'13.632"W, 653m, water 21.7 C, pH 7.6, 27 May 2010	Sh, Sj
C-1727		Cusco	Stream crossing Interoceanic Highway, 13°27'4.3914"S, 70°54'11.3754"W, 1372m, water 15.0 C, pH 7.6, 28 May 2010	Sh, Sj
C-1728		Cusco	Stream crossing Interoceanic Highway, 13°35'23.3154"S, 70°57'21.888"W, 2562m, water 9.7 C, pH 8.3, 28 May 2010	Ca, Sj
C-1730		Madre de Dios	Stream at Trail 14, 12°34'14.7"S, 70°05'23.69"W, 241m, water 25.1 C, pH 7.3, 30 Sep 2010	Ca, Co
C-1733		Madre de Dios	Stream at Trail 28, 12°34'02.81"S, 70°05'42.96"W, 272 m, water 23.3 C, pH 6.8, 30 Sep 2010	Ca, Co
C-1735		Madre de Dios	Stream at Trail 23, 12°33'31.03"S, 70°05'56.96"W, 280m, water 23.6 C, pH 6.8, 30 Sep 2010	Ca, Co, Su

Table 3. (Continued).

Collection	Country	State	Site details	Таха
C-1736		Madre de Dios	Rio Amigos, 12°33'25.22"S, 70°05'59.89"W, 288 m, water 31.4 C, pH 8.0, 1 Oct 2010	Ca, Co, Su
C-1737		Madre de Dios	CICRA. Rio Amigos, 12°34'13.008"S, 70°41'14.7714"W, 218 m, water 31.4 C, pH 8.0, 1 Oct 2010	Са
C-1739		Cusco	River at end of Quincemil Trail 1, trailhead 13°14'22.5594"S, 70°46'12.6114"W, 688m, water 19.6 C, pH 8.3, 3 Oct 2010	Ca, Co, Sh
C-1740		Cusco	Stream Quincemil Trail 1, trailhead 13°14'22.5594"S, 70°46'12.6114"W, 688m, water 19.0 C, pH 8.3, 3 Oct 2010	Ca, Sh, Su
C-1741		Cusco	Stream at Quincemil Trail 1, trailhead 13°14'22.5594"S, 70°46'12.6114"W, 688m, water 19.2 C, pH 7.7, 3 Oct 2010	Ca, Co
C-1742		Cusco	Stream at Quincemil Trail 1, trailhead 13°14'22.5594"S, 70°46'12.6114"W, 688m, water 19.1 C, pH 6.7, Oct 2010	Са
C-1743		Cusco	Semi-aquatic habitat along Quincemil Trail 1, trailhead 13°14'22.56"S, 70°46'12.61"W, 688m, 26 May 2010	Са
C-1745		Cusco	Stream at Quincemil Trail 1, trailhead 13°14'22.5594"S, 70°46'12.6114"W, 688m, water 19.7 C, pH 5.8, 3 Oct 2010	Ca, Co
C-1746		Cusco	Rio Caliente, 1km south of Quincemil, 13°13'20.87"S, 70°44'30.07"w, 626m, water 25.0 C, pH 7.2, 3 Oct 2010	Co, Su
C-1747		Cusco	Quincemil. Stream at Quincemil Trail 2, 13°13'31.0434"S, 70°45'10.6194"W, 653 m, water 24.0 C, pH 7.4, 4 Oct 2010	Са
C-1748		Cusco	Stream at Quincemil Trail 2, 13°13'31.04"S, 70°45'10.62"W, 653m, water 25.0 C, pH 7.3, 4 Oct 2010	Ca, Co
C-1749		Cusco	Stream at Quincemil Trail 3, trailhead 13°18'22.756"S, 70°48'44.9274"W, 757 m, water 20.5 C, pH 7.18, 4 Oct 2010	Са
C-1750		Cusco	Stream at Quincemil Trail 3, trailhead 13°18'22.756"S, 70°48'44.9274"W, 757 m, water 21.6 C, pH 7.1, 4 Oct 2010	Co, Sh, Su
C-1751		Cusco	Stream at Quincemil Trail 3, trailhead 13°18'22.756"S, 70°48'44.9274"W, 757 m, water 21.4 C, pH 7.5, 4 Oct 2010	Ca, Sj
C-1752		Cusco	Stream at Quincemil Trail 3, trailhead 13°18'27.76"S, 70°48'44.93"W, 757m, water 21.5 C, pH 7.5, 4 Oct 2010	Са
C-1753		Cusco	Stream with red algae along Quincemil trail 3, 13°18'27.756"S, 70°48'44.9274"W, 757m, water 21.8 C, pH 7.2, 4 Oct 2010	Co
C-1754		Cusco	River at end of Quincemil Trail 3, 13°18'23.65"S, 70°48'47.02"W, 772m, water 21.0 C, pH 7.7, 4 Oct 2010	Co
C-1755		Cusco	Stream crossing the Interoceanic Highway, 13°17'7.008"s, 70°47'13.632"W, 737m, water 22.0 C, pH 7.7, 4 Oct 2010	Ca, Co, Sh, Sj, Su
C-1756		Cusco	Stream crossing Interoceanic Highway, 13°27'52.1994"S, 70°53'52.44"W, 1463m, water 15.33 C, pH 8.2, 5 Oct 2010	Sh, Sj
C-1757		Cusco	Stream crossing Interoceanic Highway, 13°32'37.95"S, 70°53'18.95"W, 3421m, water 17.9 C, pH 8.3, 5 Oct 2010	Sh
C-1758		Cusco	Stream along Interoceanic Highway, 13°37'40.3674"S, 71°24'23.9394"W, 3566m, water 17.4 C, pH 8.4, o5 Oct 2010	Su
C-1768		Madre de Dios	Pozo Don Pedro, palm swamp at end of Trail 17, 12°33'34.27"S, 70°06'38"W, 243 m, water 25.4 C, pH 7.9, 9 Apr 2011	Са
C-1769		Madre de Dios	Stream at Trail 20, 12°33'25.22"S, 70°05'59.89"W, 238m, water 23.1 C, pH 8.3, 9 Apr 2011	Ca, Co
C-1770		Madre de Dios	Stream at Trail 23, 12°33'31.03"S, 70°05'56.96"W, 280m, water 23.3 C, pH 7.8, 9 Apr 2011	Ca, Co
C-1772		Madre de Dios	Stream at Trail 19, 12°34'01.04"S, 70°05'43.24"W, 275 m, water 23.7 C, pH 5.1, 9 Apr 2011	Ca, Co
C-1773		Madre de Dios	Stream at Trail 28, 12°34'02.81"S, 70°05'42.96"W, 272 m, water 23.7 C, pH 5.1, 9 Apr 2011	Са
C-1774		Cusco	Rio Frio, 13 °13'20.87"S, 70 °44'30.07"W, 626m, water 25.3 C, pH 8.0, 12 Apr 2011	Ca, Co, Sh
C-1775		Cusco	River at end of Quincemil Trail 1, trailhead 13°14'22.5594"S, 70°46'12.6114"W, 688m, water 21.5 C, pH 7.7, 12 Apr 2011	Ca, Sh
C-1776		Cusco	Stream at Quincemil Trail 1, trailhead 13°14'22.5594"S, 70°46'12.6114"W, 688m, water 21.4 C, pH 7.8, 12 Apr 2011	Са
C-1777		Cusco	Stream at Quincemil Trail 1, trailhead 13°14'22.5594"S, 70°46'12.6114"W, 688m, water 21.3 C, pH 6.0, 12 Apr 2011	Ca, Co
C-1778		Cusco	Stream at Quincemil Trail 1, trailhead 13°14'22.5594"S, 70°46'12.6114"W, 688m, water 21.3 C, pH 6.0, 12 Apr 2011	Ca, Co

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Collection	Country	State	Site details	laxa
C-1779		Cusco	Stream at Quincemil Trail 1, trailhead 13°14'22.5594"S, 70°46'12.6114"W, 688m, water 21.7 C, pH 6.8, 12 Apr 2011	Ca, Co
C-1780		Cusco	Stream at Quincemil Trail 3, trailhead 13°18'27.756"S, 70°48'44.9279"W, 757m, water 21.7 C, pH 6.8, 13 Apr 2011	Co, Su
C-1781		Cusco	Stream at Quincemil Trail 3, trailhead 13°18'27.756"S, 70°48'44.9279"W, 757m, water 21.5 C, pH 7.0, 13 Apr 2011	Co
C-1782		Cusco	Stream at Quincemil Trail 3, 13°18'27.756"S, 70°48'44.9279"W, 757m, water 21.2 C, pH 7.8, 13 Apr 2011	Ca, Co
C-1783		Cusco	River backwater at Quincemil Trail 3, 13°18'27.756"S, 70°48'44.9279"W, 757m, water 22.0 C, pH 7.1, 13 Apr 2011	Ca
C-1784		Cusco	Stream with red algae at Quincemil trail 3, 13°18'27.756"S, 70°48'44.9274"W, 757m, water 21.8 C, pH 7.15, 3 Apr 2011	Su
C-1787		Cusco	Stream crossing Interoceanic Highway, 13°21'2.4114"S, 71°39'21.9954"W, 3327m,	Sh
			water 11.6 C, pH 8.3, 27 May 2010	
C-1797		Junin	River near Satipo, 11°20'1.7154"S, 74°37'36.192"W, 891m, water 22.0 C, pH 9.0, 21 May 2012	Sh
C-1827	Thailand	Chiang Mai	Mushroom Research Center, 19°7'4.512"N, 98°44'2.2194"E, 904m, water 23.5 C, pH 7.6, 9 Jul 2012	Са
C-1832		Chiang Mai	Tham Rusee Nature Trail, 18°40'24.4794"N, 90°54'38.3754"E. 1149m, water 22.4 C, pH 7.2, 18 Jun 2012	Ca
C-1833		Chiang Mai	Sri Lanna National Park. Boa Tong Waterfall. 19°4'10.848"N, 99°4'46.8834"E, 508m, water 22.6 C, pH 7.2, 23 Jun 2012	Co

2003). A total of 10 000 000 generations were run with trees sampled every 1 000 generations, resulting in a total of 10 000 trees. The first 1 000 trees were discarded as burnin, and the remaining 9 000 trees were used to calculate posterior probabilities (PP). The consensus of the trees was viewed in Dendroscope v. 2.7.4 (Huson *et al.* 2007). RAxML analyses of the dataset produced a single most likely tree (In -9231.511787) on which bootstrap support (\geq 75) and PP values (\geq 95) are indicated on the tree. Sequences generated in this study and the alignment used for phylogenetic analysis were deposited respectively in GenBank and in TreeBASE (www.treebase.org, submission 15251).

RESULTS

Field collections

The entire results of field collections will be reported in a separate paper on elevational distribution patterns of freshwater ascomycetes. For this study, five species of dematiaceous hyphomycetes were selected for morphological and molecular phylogenetic study, as noted above (p. 425). *Cancellidium applanatum*, *Cordana abramovii*, *S. juvenile*, and *S. uniseptatum* are reported here as new records for Perú. Specimens examined are listed in the taxonomy portion of this paper with collection numbers whose details are given in Table 3.

Phylogenetic analyses

A single most likely tree from RAxML analysis (Fig. 1) indicated that *Cancellidium applanatum* groups with other freshwater *Sordariomycetidae*, its closest sequenced relative being *Thyridium vestitum*. The three sequences used in this analysis form a strongly supported monophyletic clade, with the Peruvian specimen separated from a clade containing

two specimens from Thailand. Inclusion of the *C. pinicola* sequence from GenBank (DQ144048) places that sequence firmly in *Hypocreales* (results not shown) as Yeung *et al.* (2006) reported. A BLAST search using that sequence produces a 100 % match to *Trichoderma koningiopsis*, suggesting contamination of the *C. pinicola* isolate. The results of this analysis indicate that the taxonomic placement of *C. applanatum* is in *Sordariomycetes incertae sedis* at this time.

Cordana abramovii clusters with other Cordana species in a well-supported monophyletic clade (Fig. 1). Cordana has been linked to Porosphaerella via Porosphaerella cordanophora and was first placed in Trichosphaeriaceae (Müller & Samuels 1982) and later Chaetosphaeriaceae (Réblová et al. 1999). Réblová & Winka (2000) provided molecular evidence that did not support the inclusion of Cordana in Chaetosphaeriaceae, and this study supports their conclusion. Cordanaceae is a separate lineage, widely separated from Chaetosphaeriaceae in our phylogenetic analysis. Porosphaerella borinquensis is closely related, but basal to, Cordanaceae in this analysis, not nesting within the clade. Porosphaerella borinquensis has a Pseudobotrytis terrestris asexual morph, and it has been suggested that the mitosporic morph may be a compound form of basic Cordana features (Fernández & Huhndorf 2004).

Sporoschisma saccardoi has long been linked via cultural studies to Melanochaeta hemipsila and our study supports the sexual-asexual morph connection using LSU sequences from both states. Multiple attempts to sequence the 28S 5' and 3' ends of *M. hemipsila* (KF833362) were made without success. This missing data may account for the long branch for that sequence. The Peruvian specimen is placed in a well-supported clade with *M. hemipsila* and *M. aotearoae* within *Chaetosphaeriaceae*, agreeing with prior molecular studies (Fernandez *et al.* 2006, Mugambi & Huhndorf 2008).





Fig. 1. Most likely tree (In -9231.511787) from LSU nrDNA analysis obtained with RAxML. ML bootstrap support values > 75 are indicated at nodes, BPP support values > 95 indicated by thickened branches.





Fig. 2. *Cancellidium applanatum* (PE0063-1). **A.** Habit view. **B–D.** Conidia. **E.** Base of conidium. **F, G.** Strings of monilioid cells. Bars: A = 200 μm, B–G = 20 μm.

TAXONOMY

Cancellidium applanatum Tubaki, *Trans. Mycol. Soc.* Japan 16: 358 (1975). (Fig. 2)

Description: Colonies on PYG+Ab agar 2 cm diam at 30 days, white to pale yellow, becoming dark grey at the center as conidia are formed, mycelium immersed with scant aerial hyphae, margin entire, discrete, reverse whitish to buff to

pale yellow. *Conidiophores* micronematous, mononematous, arising terminally or laterally from the hyphae, simple, erect, hyaline, smooth walled. *Conidia* bulbils formed as inflated ends of conidiophores, $160-220 \times 51-98$ ($\overline{x} = 183.4 \times 74.9 \mu$ m, n = 30), shiny, silver to black when young, brown with age, obovate to obcordate, composed of parallel rows of septate rectangular cells radiating from point of attachment with conidiophore, outer cells surrounding strings of monilioid cells.

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Specimens examined: C-1709, PE0063-1; C-1715, PE0063-3;
C-1753, PE0063-4; C-1714, PE0063-5; C-1719, PE0063-6; C-1742,
PE0063-7; C-1742, PE0063-8; C-1705, PE0063-10; C-1715,
PE0063-12; C-1698, PE0063-13; C-1717, PE0063-14; C-1723,
PE0063-15; C-1713, PE0063-16; C-1716, PE0063-18; C-1700,
PE0063-19; C-1699, PE0063-20; C-1712, PE0063-21; C-1752,
PE0063-23; C-1745, PE0063-26; C-1734, PE0063-27; C-1744,
PE0063-28; C-1732, PE0063-29; C-1730, PE0063-30; C-1729,
PE0063-31; C-1697, PE0063-36; C-1755, PE0063-38; C-1751,
PE0063-42; C-1736, PE0063-44; C-1735, PE0063-45; C-1747,
PE0063-46; C-1749, PE0063-47; C-1739, PE0063-48; C-1737,
PE0063-50; C-1731, PE0063-52; C-1741, PE0063-70; C-1777,
PE0063-81; C-1769, PE0063-82; C-1772, PE0063-83; C-1832,
TH0063-1; C-1827, TH0063-2.
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Distribution: Known from Australia, Brazil, China, Hong Kong, Japan, Malaysia, Perú, and Thailand.

Notes: This fungus was recovered from a variety of habitats representing a range of environmental conditions. It is saprobic on submerged woody and palm debris in lentic and lotic habitats. The specimens examined in this study are characterized by the production of bulbils on the surface of the substrate that appear silver, brown, or black depending on age, and are composed of parallel rows of cells encapsulating strings of monilioid cells.

Surprisingly, this fungus was not reported by Matsushima (1993, 1995), who studied the fungi colonizing decomposing plant debris along the same river system we sampled. It occurred at water temperatures ranging from 18.7–31.7 °C and pH 5.1–8.3. It was recovered from altitudes ranging from 218–817 m. As the fungus was not recovered from higher elevations and its distribution appears to be mainly tropical (with the exception of the type locality, which has a subtropical climate), it may be that *C. applanatum* is adapted to warmer habitats.

Cordana abramovii Seman & Davydk., *Novosti Sist. Nizsh. Rast.* 20: 115 (1983).

(Fig. 3)

Description: Conidiophores gregarious, erect, straight or flexuous, to 6-septate, smooth, brown, paler towards the apex, 620–990 µm long × 5–6.5 µm wide (between conidiogenous swellings), base to 18 µm diam. Conidiogenous cells polyblastic (to 8), terminal and intercalary, one swelling per cell (8.5–13 µm wide), denticulate. Conidia enteroblastic, verruculose, tan to reddish brown, pyriform to obovate, thick walled (to 3.0 µm), transversely uniseptate with a septal pore, and tapered base bearing the scar of schizolytic abscission, 21–29 µm long × 11.5–16 µm wide ($\overline{x} = 24.6 \times 14.4$, n = 30).

Specimens examined: C-1714, PE0053-1; C-1741, PE0053-3; C-1750, PE0053-4; C-1746, PE0053-5; C-1719, PE0053-9; C-1713, PE0053-11; C-1720, PE0053-12; C-1716, PE0053-13; C-1711, PE0053-14; C-1722, PE0053-15; C-1708, PE0053-16; C-1712, PE0053-17; C-1755, PE0053-18; C-1736, PE0053-20; C-1735,

PE0053-21; C-1753, PE0053-22; C-1739, PE0053-23; C-1782, PE0053-24; C-1779, PE0053-25; C-1748, PE0053-26; C-1770, PE0053-27; C-1730, PE0053-28; PE0053-30; C-1754, PE0053-34; C-1733, PE0053-40; C-1745, PE0053-42; C-1744, PE0053-43; C-1777, PE0053-44; C-1833, TH0053-1.

Distribution: Known from Brunei, Perú, Russia, Seychelles, and Thailand.

Notes: Morphologically, the Peruvian specimens reported and described herein most closely match the description of *C. abramovii*. The conidiophores in the Peruvian specimens are thinner than the type (5–6.5 vs. (8–)10–12.5 µm), as are the swellings of the conidiogenous zones (8.5–13 µm vs. 18 µm). Conidia are thick walled and approximately the same size (21–29 × 11.5–16 µm vs. 27–31 × 15–15.5 µm) as the type. The Peruvian specimens, however, have verruculose wall ornamentation, a feature not noted by Seman & Davydkina (1983).

These morphological differences, as well as the geographic distance between the collection localities, suggest that the Peruvian specimens may represent a variation of C. abramovii s. str. or even a new species. Hyde & Goh (1998) provide evidence of a similar situation in their reports of C. abramovii var. seychellensis, an anatomically similar taxon possessing conidia with a purple, pitted episporium, and C. abramovii var. abramovii, possessing brown conidia and lacking an episporium. These variants were collected in the Old World tropics, while the type was reported from northern Ossetia. The specimens of C. abromovii in this study are restricted to Perú. Further molecular evidence should be gathered to increase our understanding of the phylogenetic affinities of these highly similar taxa as well as other members of Cordanaceae. Information from additional geographically separated specimens as well as additional molecular data, especially ITS, would shed light on whether C. abramovii represents a species complex with geographical variation, or whether these are distinct species.

This fungus was recovered from a variety of habitats with a range of environmental conditions. Its habit is thus far known to be saprobic on submerged woody and palm debris in lentic and lotic habitats. Water temperature ranges from 18.7-31.7 °C and pH ranges from 5.1-8.3. Its altitudinal range is from 218-772 m.

Sporoschisma hemipsila (Berk. & Broome) Zelski, A.N. Mill., & Shearer, comb. nov.

MycoBank MB807636

(Fig. 4)

- Basionym: Sphaeria hemipsila Berk. & Broome, Bot. J. Linn. Soc. **14**: 126 (1873).
- Synonyms: Lasiosphaeria hemipsila (Berk. & Broome) Sacc., Syll. Fung. **2**: 198 (1883).
- Chaetosphaeria hemipsila (Berk. & Broome) Petch., Ann. Roy. Bot. Gard. Peradenija **6**: 336 (1917).
- Melanochaeta hemipsila (Berk. & Broome) E. Müll. et al., Revue Mycol. 33: 377 (1969).
- Chaetosphaeria coelestina Höhn., Sitzungsber. Akad. Wiss. Wein, Math.-Naturwiss. Kl, 1 Abt. **118**: 324 (1909).



Fig. 3. Cordana abramovii (PE0053-24). A. Habit view. B. Conidiophore and conidia. C-H. Conidia. Bars: A = 100, B = 20 µm, C-H = 10 µm.

Sporoschisma saccardoi E. W. Mason & S. Hughes, Mycol. Pap. **31**: 20 (1949).

Description: Colonies on PYG + Ab 2 cm diam at 30 d, effuse, velutinous, with mixed tufts of conidiophores and sterile capitate setae. *Mycelium* immersed, composed of pale to dark brown hyphae. Capitate setae arising from a bulbous stroma 45–60 μ m diam or from ascoma, pale brown, becoming paler towards the apex, straight or slightly flexuous, 5–6 septate,

150–200 μm long, 5–6.5 μm with subhyaline terminal swelling 10–12 μm wide. Ascomata superficial, 284–400 μm high × 280–370 μm wide (\bar{x} = 325 μm × 325 μm, *n* = 10), globose to subglobose, dark brown to black, gregarious, with capitate setae. Paraphyses to 7 μm wide at base, tapering to a rounded apex ~ 3.5 μm wide, as long as asci, free at apices, hyaline, septate, constricted at septa, unbranched. Asci 165–230 × 13.5–22 μm (\bar{x} = 186.6 × 16.8, *n* = 10), cylindrical to cylindro-clavate, 8-spored, biseriate, pedicellate, with an I-



Fig. 4. *Sporoschisma saccardoi* (PE0349-1). **A.** Habit view of sexual and asexual states. **B.** Capitate setae arising from ascoma. **C.** Asci. **D.** Young asci and paraphyses. **E.** Ascus apical rings. **F, G.** Ascospores. **H.** Conidiophore. **I–K.** Conidia. Bars: A = 100 μm, B–K = 20 μm.





Fig. 5. A–E. *Sporoschisma juvenile* (PE0127-7). A. Conidiophore and capitate hypha. B. Young conidiophore. C–E. Conidia. F–M. *Sporoschisma uniseptatum* (PE0172-8). F. Conidiophore. G. Conidiophore and chains of conidia. H. Conidiophores and capitose hyphae. I–M. Conidia. Bars = 20 µm.

refractive apical apparatus 2–2.5 µm high × 4.5–5.5 µm wide ($\overline{x} = 2.3 \times 5.2$, n = 10). Ascospores 44–57 × 7–9.5 µm ($\overline{x} = 51$ × 8 µm, n = 30), cylindrical, bent, 5-septate, not constricted at septa, smooth walled, with lipid droplets in each cell, apices rounded, central cells olivaceous to brown, end cells hyaline, without sheaths or appendages. Conidiophores scattered to gregarious, arising from substrate or directly from ascomata, up to 190 µm long. Conidiogenous cells monophialidic, 9–13 µm wide below venter and 17–20 µm wide above, venter to 22 µm wide, dark brown, paler at the torn apex, simple, erect, dark brown, smooth walled. Conidia formed enteroblastically inside the tubular collarette of the conidiogenous cell and emerging in a chain, doliiform, 48–60 × 11–13.5 µm ($\overline{x} = 55.5 \times 12.5 \mu$ m, n = 30), 5-septate, occasionally constricted at septa, central cells brown, end cells hyaline.

Specimens examined: C-1727, PE0177-1; C-1726, PE0177-2; C-1710, PE0177-3; C-1750, PE0177-4; C-1756, PE0177-5; C-1739, PE0177-6; C-1755, PE0177-7; C-1740, PE0177-10; C-1775, PE0177-15; C-1757, PE0177-12; C-1797, PE0177-21.

Distribution: Known from Australia, Brunei Darussalam, Ecuador, Europe, Hong Kong, Indonesia, Kenya, Malaysia, Perú, South Africa, Taiwan, and Thailand.

Notes: This fungus was recovered from a variety of habitats with a range of environmental conditions. Water temperature ranged from 11.6–22.2 °C and pH ranged from 7.1–9.0. It was recovered from altitudes ranging from 626–3566 m. The new combination is required as the epithet *hemipsila* takes precedence over *saccardoi*. As neither name is widely used, we see no case for not following the rule of priority under the ICN.

Sporoschisma juvenile Boud., *Icones Mycol.***1**: 12 (1904). (Fig. 5A–E)

Description: Setae interspersed among conidiophores, erect, straight or flexuous, to 6-septate, smooth, brown, paler towards apex, 100–150 × 4–6 µm, apex 5–7 µm wide, apex hyaline, capitate, coated with mucilage. Conidiophores scattered to gregarious, arising from dark interwoven hyphae, straight or flexuous, cylindrical, 110–280 µm long, 7–10 µm wide just above substrate, dark brown, smooth. Conidiogenous cells monophialidic, terminal, integrated, lageniform, consisting of a slightly swollen venter 14–20 µm wide and a tubular collarette 80–110 × 9.5–12 µm. Conidia produced in basipetal chains, cylindrical, ends rounded, 34–44 × 10.5–14.5 µm ($\overline{x} = 38 \times 12.93$, n = 30), 3-septate, pale brown, verruculose.

Specimens examined: C-1705, PE0127-1; C-1720, C-1727, PE0127-2; C-1728, PE0127-3; C-1725, PE0127-4; C-1720, PE0127-5; C-1756, PE0127-6; 1751, PE0127-7; C-1726, PE0127-12.

Distribution: Known from Australia, Czechoslovakia, France, Hong Kong, Perú, Seychelles, and the UK.

Notes: This fungus was recovered from a variety of habitats with a range of environmental conditions, and at altitudes

Sporoschisma uniseptatum Bhat & W.B. Kendr., *Mycotaxon* **49**: 71 (1993).

(Fig. 5F-M)

Synonym: Melanochaeta garethjonesii Sivichai & Hywel-Jones, Mycol. Res. **104**: 481 (2000).

Description: Conidiophores dark brown, erect, straight or flexuous, septate, cylindrical, terminating with phialidic conidiogenous cells, 125–190 µm long × 9–11 µm wide, to 22 µm wide at the swollen venter. Capitate setae present among conidiophores, erect, straight or flexuous, 3–6 septate, smooth, pale brown, paler towards the sub-hyaline apex, 120–175 x 8–10 µm, swollen apex 6–13 µm wide, surrounded by mucilage. *Conidia* 25.5–32.5 × 11–14 µm (\bar{x} = 30.8 × 12.6 µm, *n* = 30), formed in chains, cylindrical, truncate at both ends, slightly verruculose, 1-septate, pale brown, uniform in colour.

Specimens examined: C-1704, PE0172-1; C-1696, PE0172-2; C-1702, PE0172-3; C-1722, PE0172-4; C-1715, PE0172-5; C-1705, PE0172-6; C-1746, PE0172-7; C-1755, PE0172-8; C-1735, PE0172-9; C-1758, PE0172-12; C-1750, PE0172-10; C-1740, PE0172-14; C-1736, PE0172-16; C-1784, PE0172-20.

Distribution: Known from Australia, Brunei Darrusalam, Canada, China, Ecuador, French Guiana, Hong Kong, India, Indonesia, Italy, Malaysia, Perú, Seychelles, South Africa, Sri Lanka, Taiwan, and Thailand.

Notes: The fungus was recovered from a variety of habitats with a range of environmental conditions, and altitudes ranging from 218-757 m. Water temperature ranged from 19-31.4 °C and pH ranged from 5.9-8.0.

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