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.

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 Hypocrellaes. However, they noted that a connection to Hypocrellaes 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 conidigenous zones; terminal and intercalary polyblastic conidigenous cells; and golden brown to dark brown, 1-septate, thick-walled, 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 Cordanaeae (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, doliform, smooth walled conidia; S. juvenile

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has brown 5-septate, cylindrical, verruculose conidia; and S. paricinaeum 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 ascoma with short conical beaks; numerous, septate, capitae setae arising from the external ascomal wall; clavate, uniloculate, 8-spored asc 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).

Ascocata 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 Volkman-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 H₂O. 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 H₂O. 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 Illuma 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 Magnaportheales and one from Lulworthiales were used as outgroup taxa (Table 2). Sequences were assembled and initially aligned in Sequecher 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 (~lnL 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, TH0063-1a       | KF833358                 | CBS 137654 |
| Cancellidium applanatum  | ILL 41206, TH0063-1b       | KF833359                 | CBS 137655 |
| Cancellidium applanatum  | ILL 41205, PE0063-1a       | KF833360                 | CBS 137653 |
| Cordana abramovii        | ILL 41204, PE0053-24a      | KF833361                 | CBS 137652 |
| Sporoschisma hemipsila   | ILL 41207, PE0177-21a      | KF833362                 | CBS 137656 |
| Sporoschisma hemipsila   | ILL 41207, PE0177-21b      | KF833363                 | ---------- |
| Sporoschisma hemipsila   | ILL 41207, PE0177-21c      | 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                 |
| Annullus magnus triseptatus| GQ996540                | Gnomonia gnomon          | AF408361                 |
| Apiognomonia errabunda   | AF408334                 | Halosphaeria appendiculata| U46885                  |
| Aschendus austriacus     | GQ996539                 | Lasiosphaeria ovina      | AF064643                 |
| Beltojisia rhynchoistoma | EU999217                 | Lentomitella cirrhosa    | AY761085                 |
| Bullimycetes aurispurors | JF775590                 | Lentomitella crinigera   | AY761086                 |
| Bullimycetes communis    | JF775585                 | Lirnda thalassiae       | DQ470947                 |
| Bullimycetes cosaricensis| JF775591                 | Melanochaeta aoteaeoae   | AF466082                 |
| Calosphaeria barbiorostris| EF577059                | Melanochaeta aoteaeoae   | 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 innumerata| AY017375                 | Melanopsamella vermicullioides | AFO64644               |
| Chaetosphaeria ovoidea   | AF064641                 | Neurosopra crassa        | AF286411                 |
| Chaetosphaeria pulviscula| AF466091                 | Nohea umiumi            | U46893                   |
| Chaetosphaeria spinosa   | AF466079                 | Ohiostoma stenoceras     | DQ836904                 |
| Chaetosphaeria tropicalis| AF466080                 | Ophioceras tenuisporum   | AY346295                 |
| Chatothecastra capitata  | AFF466061                | Ophiostoma pilferum      | DQ470955                 |
| Conlarium duplumascopora | JN936993                 | Papulosa amerospora      | DQ470950                 |
| Cordana ellipsoida       | HE672156                 | Porosphaeraella borniquensis | EFO63573                 |
| Cordana ellipsodea       | 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 solidaria        | HE672161                 | Thielavia subthermophila | HM448442                 |
| Cryptadelphia groenendalensis| EU528007              | Thyridium vestitum       | AY544671                 |
| Cryptadelphia polystepata| AY281102                 | Valsa ambiens           | AF362564                 |
| Diaporthe  eres          | AF408350                 | Xylomelasma sordida      | AY761087                 |

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 2003).
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 | Taxa |
|------------|---------|-------|--------------|------|
| 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 | Ca   |
| 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 | Ca   |
| 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 | Ca   |
| 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 | Co   |
| C-1705     | Madre de Dios | Seasonal lake at Trail 29, 12°34′16.98″S, 70°05′06.70″W, 244m, water 22.2 C, pH 6.4, 23 May 2010 | Ca, Sj, Su |
| 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 | Ca   |
| C-1710     | Cusco    | Stream at Quincemil Trail 1, 13°13′58.25945″S, 70°46′37.7754″W, 675m, water 22.2 C, 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 | Co, Ca |
| 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 | Co, Co |
| C-1715     | Cusco    | Semi-aquatic habitat along Quincemil Trail 1, head 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′14.184″W, 659m, water 21.8 C, pH 6.2, 26 May 2010 | Ca   |
| 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, Sj |
| 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 | Ca   |
| 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                                                                 | Taxa       |
|------------|------------|-----------|-----------------------------------------------------------------------------|------------|
| 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 | Rio Amigos, 12°34'13.008"S, 70°41'14.7714"W, 218 m, water 31.4 C, pH 8.0, 1 Oct 2010 | Ca          |
| C-1739     | Madre de Dios | Rio Amigos, 12°34'13.008"S, 70°41'14.7714"W, 218 m, water 31.4 C, pH 8.0, 1 Oct 2010 | Ca          |
| C-1740     | 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 | Ca          |
| C-1741     | 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 | Ca          |
| C-1742     | 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 | Ca          |
| C-1743     | 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 | Ca          |
| C-1744     | 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 | Ca          |
| C-1745     | 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 | Ca          |
| 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 | Ca          |
| C-1747     | 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 | Ca          |
| C-1748     | 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 | Ca          |
| C-1749     | 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 | Ca          |
| C-1750     | 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 | Ca          |
| C-1751     | 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 | Ca          |
| C-1752     | 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 | Ca          |
| C-1753     | 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 | Ca          |
| C-1754     | 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 | Ca          |
| C-1755     | 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 | Ca          |
| C-1756     | 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 | Ca          |
| C-1757     | 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 | Ca          |
| C-1758     | 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 | Ca          |
| C-1759     | 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 | Ca          |
| C-1760     | 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 | Ca          |
| C-1761     | 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 | Ca          |
| C-1762     | 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 | Ca          |
| C-1763     | 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 | Ca          |
We refer to Table 3 in the paper. The table provides details of the field collections, including the collection number, country, state, and site details, along with the taxa associated with each collection. The taxa are identified using various molecular and morphological methods, including LSU sequences and LSU PCR. The table includes multiple collections from different sites in Peru, each with specific details such as water temperature and pH, which are important for understanding the environmental conditions under which the fungi were found.

RESULTS

Field collections

The results of field collections are reported in a separate paper on elevation 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 Chaetosphaeriaceae (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. Cordanaeaceae is a separate lineage, widely separated from Chaetosphaeriaceae in our phylogenetic analysis. Porosphaerella borinquensis is closely related, but basal to, Cordanaeaceae 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 in our phylogenetic analysis.

In 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 burn-in, 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). 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 burn-in, 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 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.

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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 in our phylogenetic analysis.
Fig. 1. Most likely tree (ln -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 × 51–98 (x̄ = 183.4 × 74.9 µm, 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.*
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-1733, PE0063-52; C-1748, PE0063-56; C-1740, PE0063-63; C-1731, PE0063-66; C-1741, PE0063-70; C-1777, PE0063-81; C-1769, PE0063-82; C-1772, PE0063-83; C-1832, TH0063-1; C-1827, TH0063-2.

Distribution: Known from Australia, Brazil, China, Hong Kong, Japan, Malaysia, Peru, 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. appplanatum is adapted to warmer habitats.

Cordana abramovii Seman & Davyd.k., Novost 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 unisepitate with a septal pore, and tapered base bearing the scar of schizolytic abscission, 21–29 µm long × 11.5–16 µm wide (x = 24.6 × 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, TH0063-1.

Distribution: Known from Brunei, Peru, 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 & Davydkin (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. tr. 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 episporm, and C. abramovii var. abramovii, possessing brown conidia and lacking an episporm. 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 Peru. 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 Cordanaeae. 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 altitudal 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 Höh., Sitzungsber. Akad. Wiss. Wien, Math.-Naturwiss. Kl, 1 Abt. 118: 324 (1909).
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 µm 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 (x̄ = 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 (x̄ = 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 (r = 2.3 ± 5.2, n = 10). Ascospores 44–57 × 7–9.5 µm (r = 51.8 ± 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, doliform, 48–60 × 11–13.5 µm (r = 55.5 ± 12.5 µm, 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, and at altitudes ranging from 244–2562 m. Water temperature ranged from 9.7–22 °C and pH ranged from 6-8.3.

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 × 8–10 µm, swollen apex 6–13 µm wide, surrounded by mucilage. Conidia 25.5–32.5 × 11–14 µm (r = 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 Darussalam, 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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