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Short title: New *Cordana* species

New species of *Cordana* and epitypification of the genus

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Abstract: Two interesting fungi belonging to the genus *Cordana* have been isolated recently in Spain from plant debris. Both are proposed here as new species, described and illustrated. *Cordana mercadiana* sp. nov. produces 0–1-septate conidia, with a prominent basal scar. *Cordana verruculosa* sp. nov. differs from the other species of the genus by its unique combination of aseptate, verruculose and small conidia. Both species are compared

morphologically with other species of *Cordana* and their identities supported by the analysis of rDNA sequences. LSU sequence analysis revealed the congeneric relationship of *Cordana* and *Pseudobotrytis*; the members of both genera are in a well supported monophyletic linage that appears to be related to the Coniochaetales but remains incertae sedis within the Sordariomycetes. To establish nomenclatural stability of the genus *Cordana*, an isolate of *C. pauciseptat*a is designed here as epitype and the two species of *Pseudobotrytis* are transferred to *Cordana*. A dichotomous key is provided to identify the currently accepted species of *Cordana*.

Key words: fungal diversity, plant debris, *Porosphaerella*, *Pseudobotrytis*, Sordariomycetes, taxonomy

INTRODUCTION

In an ongoing survey of microfungi from plant debris in Spain several specimens were recovered in pure culture. They are morphologically consistent with the genus *Cordana* but with unique features. The genus *Cordana* Preuss (1851), with *C. pauciseptata* Preuss as lectotype species (Hughes 1955), is morphologically characterized by its differentiated, solitary, unbranched conidiophores which proliferate percurrently and bear inflated, terminal or intercalary polyblastic conidiogenous cells which leave small cylindrical denticles. The conidia are acropleurogenous, simple, ovoid, obovoid, ellipsoidal, pyriform or obpyriform, 0–1-septate, brown to pale brown, and seceding schizolytically (Hughes 1955, Ellis 1971). Although more than 25 species have been included in *Cordana* (www. mycobank.org), only 13 species were accepted in the last revision (Markovskaja 2003), which is followed in the present study. Two additional species later were reported (i.e. *C. uniseptata* L. Cai, McKenzie & K.D. Hyde [Cai et al. 2004] and *C. versicolor* D.J. Soares & R.W. Barreto [Soares et al. 2005]).

The members of *Cordana* are distributed worldwide and frequently found on different substrates, such as soil, plant debris and other fungi. They occasionally cause pale brown spots on leaves of *Musa* spp. and *Canna denudata* Roscoe (Ellis 1976, de Hoog et al. 1983, Rao and de Hoog 1986, Markovskaja 2003, Cai et al. 2004, Soares et al. 2005).

There are few data on anamorph-teleomorph connections and on the taxonomic position of *Cordana* species among the ascomycetes, and what little is available only refers to *Porosphaerella* species.

The genus *Porosphaerella* (*Po*.) E. Müll. & Samuels is based on *Po*. *cordanophora* E. Müll. & Samuels, the sexual state of *C*. *pausiceptata* (Müller and Samuels 1982). Two other species, *Po*. *setosa* A.I. Romero & Samuels (1991) and *Po*. *borinquensis* F.A. Fernández & Huhndorf (2004), were added later; while *Po*. *setosa* has no known anamorph, *Po*. *borinquensis* is the teleomorph of *Pseudobotrytis terrestris* (Timonin) Subram. (Fernández and Huhndorf 2004). Based mainly on ascomata morphology, Müller and Samuels (1982) suggested that *Po*. *cordanophora* should be placed in the family Trichosphaeriaceae (Trichosphaeriales) while Réblová et al. (1999) suggested Chaetosphaeriaceae (Chaetosphaeriales). However, neither of these taxonomic decisions is supported phylogenetically. LSU sequence analyses related *Po*. *cordanophora* and *Po*. *borinquensis* with the Coniochaetales (Réblová and Winka 2000, Huhndorf et al. 2004; Réblová and Seifert 2007). *Poronosphaerella setosa* tentatively was placed in the Trichosphaeriaceae (Romero and Samuels 1991), however the taxonomic position of this species never has been confirmed molecularly.

The genus *Pseudobotrytis* (*Ps*.) Krzemien. & Badura is characterized by erect brown conidiophores, bearing a terminal world of discrete polyblastic conidiogenous cells, which produce single one-septate conidia from small denticles. It was described on the bases of *Ps*. *fusca* Krzemien. & Badura (Krzemieniewska and Badura 1954), but later this species was

considered conspecific of *Spicaria terrestris* Timonin (1940), therefore being transferred to *Pseudobotrytis* (Subramanian 1956). Unfortunately no original material of *Ps. fusca* is preserved to study and confirm this synonymy. *Pseudobotrytis terrestris*, the type species of the genus, is a cosmopolitan fungus usually found on plant debris and soil from temperate and tropical areas (Subramanian 1956, Fernández and Huhndorf 2004). Despite the differences, several authors recognized morphological similarities between *Ps. terrestris* and *C. pausiceptata* (Müller and Samuels 1982, Fernández and Huhndorf 2004). LSU analysis, which placed *Po. borinquensis* and *Po. cordanophora* in a well supported monophyletic clade, and the morphological affinities of the respective anamorphic and teleomorphic states of both fungi suggested they could be congeneric (Fernández and Huhndorf 2004, Réblová and Seifert 2007, Shenoy et al. 2011). The other species of the genus, *Pseudobotrytis*, is *Ps. bisbyi* Timonin (1961), a fungus that lacks the teleomorphic state and differs from *Ps. terrestris* mainly by the conidial morphology (nonseptate vs. one-septate respectively). It is noteworthy that the phylogenetic relationship between both *Pseudobotrytis* species has never been studied.

Unfortunately, there are no ex-type cultures of *Porosphaerella* species or of *C. pausiceptata*, and the above-mentioned phylogenetic studies including *Po. cordanophora* and *Po. borinquensis* were based on single sequences from the same fresh isolates, which are not available for study. Therefore, the rDNA sequences of our Spanish isolates could be compared only with those of ex-type or reference strains of *Cordana* species available in culture collections (i.e. *C. ellipsoidea* de Hoog, *C. inaequalis* S. Hughes, *C. musae* [Zimm.] Höhn., *C. pauciseptata*, *C. solitaria* V. Rao & de How) and other related fungi, such as *Ps. terrestris* and probably *Ps. bisbyi*.

In the present study we attempt to clarify the taxonomy of *Cordana* and *Pseudobotrytis* and to assess its phylogenetic relationship within the ascomycetes. Following

the new rules on fungal nomenclature that give priority to the oldest generic name irrespective of whether it is originally based on the anamorph or teleomorph (Hawkswoth 2011), the name *Cordana* (Preuss 1851) takes priority over *Porosphaerella* (Müller and Samuels 1982) and *Pseudobotrytis* (Krzemieniewska and Badura 1954), and for the purpose of stability an isolate of *C. pauciseptata* is selected as epitype.

MATERIALS AND METHODS

Sampling area and specimen examination.—Plant debris was collected in the Aragon and Castilla y León regions in northern Spain, previously described in Hernández-Restrepo et al. (2012).

Plant material (twigs, leaves, seeds, bark, submerged wood) was collected and treated according to Mena-Portales et al. (2011). Monoconidial cultures were obtained on oatmeal agar (OA; 30 g filtered oat flakes, 20 g agar [Pronadisa, Spain], distilled water 1 L) and potato carrot agar (PCA; potatoes, 20 g; carrot, 20 g; agar 20 g; distilled water 1 L) and incubated at room temperature (25 ± 2 C) in the dark. The characterization of the new species was made after Hernández-Restrepo et al. (2012). Drawings initially were done with a drawing tube, then scanned and the images treated with the AdobeIllustrator software and colored with AdobePhotoshop (Barber and Keane 2007).

Fungal isolates.—In addition to the *Cordana* isolates collected in Spain, we examined 11 type or reference strains of different species of *Cordana* and *Pseudobotrytis* from culture collections (TABLE I). For morphological characterization, the strains were subcultured on OA, PCA and on water agar with pieces of wood, and incubated at 25 C in the dark.

DNA extraction, *sequencing and phylogenetic analysis*.—Isolates were grown on PCA for 2–4 wk at 25 C, and DNA was extracted with a PrepMan Ultra sample preparation reagent (Applied Biosystems, Foster City, California), following the manufacturer's protocol. The DNA was quantified with GeneQuantpro (Amersham Pharmacia Biotech, Cambridge, UK). The ITS region and the D1/D2 domains of the rDNA were amplified with the primer pair ITS5 and NL4b, following protocol of Cano et al. 2004). PCR products were purified and sequenced at Macrogen Corp. Europe (Amsterdam Zuid-oost, the Netherlands) with an Abi prism 3730XL DNA analyzer (Applied Biosystems). The program SeqMan 7.0 (Lasergene, Madison, Wisconsin) was used to obtain consensus sequences. A BLAST sequence identity search (Altschul et al. 1997) was carried out to compare data from our isolates with those of other fungi deposited in the GenBank database. The D1/D2 and ITS sequences

were aligned with the Clustal application in MEGA 5 (Tamura et al. 2011), followed by manual adjustments with a text editor.

A phylogenetic analysis of D1/D2 sequences was performed to assess the relationships of *Cordana* spp. and *Pseudobotrytis* species within the Sordariomycetes. Apart from the above 15 strains of *Cordana* and *Pseudobotrytis* species, additional sequences of other fungi belonging to different orders and families of the Sordariomycetes (i.e. Cephalothecaceae, Chaetosphaeriales, Coniochaetales, Diaporthales, Hypocreales, Magnaporthales, Ophiostomatales, Sordariaceae, Xylariales) retrieved from the GenBank also were included in the study. These sequences were mainly published by Réblová and Winka (2000), Huhndorf et al. (2004), Réblová and Seifert (2007) and Shenoy et al. (2010). Sequences of *Pleospora herbarum* (AF 382386) and *Scutellinia scutellata* (DQ247806) were used as outgroups.

To confirm the genetic distinction between the putative new species and species of *Cordana* and *Pseudobotrytis* available in culture, a phylogenetic analysis based on ITS sequences was carried out. With exception of *C. musae* CBS 151.34, unrelated to other *Cordana* species with the D1/D2 phylogenetic analysis, ITS dataset included 12 sequences of *Cordana* isolates (one retrieved from GenBank as *Po. cordanophora*) and three sequences of *Pseudobotrytis* isolates. *Coniochaeta ligniaria* (JQ619824) and *Coniochaeta velutina* (GQ154544) were used as outgroups taxa.were used as outgroup taxa.

Phylogenetic analyses were made with maximum likelihood (ML), maximum parsimony (MP) criteria and Bayesian inference. ML and MP analyses were carried out woth the software program MEGA 5 (Tamura et al. 2011). Tamura-Nei-parameter was the best-fit model of nucleotide substitution for ML tree determined with MEGA 5 and we used nearest-neighbor-interchange (NNI) as the heuristic method (Saitou and Nei 1987). The robustness of the branches was assessed by bootstrap analysis of 1000 replicates for both analyses (BML and BMP respectively). Bayesian analysis was carried out with MrBayes 3.1 (Huelsenbeck and Ronquist 2001) for two replicates by running 2 466 000 times for the D1/D2 dataset in four chains every 100 generations. The last 24 661 trees were used to construct a 50% majority rule, for Bayesian posterior probabilities (BPP). Confident branch support is defined as bootstrap values BML and BMP ≥70% and BPP ≥ 0.95. Novel sequences were deposited in GenBank (TABLE I) and the alignments used were deposited in TreeBASE (submission number 13364).

RESULTS

Morphological study.—One of the isolates (FMR 11828), referred to as *Cordana* sp. I, was characterized by its 0–1-septate conidia with a prominent basal scar. Three other isolates

collected from different Spanish regions (FMR 10754, FMR 11594, FMR 9490), which were named *Cordana* sp. II, had pale brown and nonseptate conidia with a slightly verrucose wall.

The morphological characteristics of the type or references strains of *C. ellipsoidea*, *C. inaequalis*, *C. pauciseptata* and *C. solitaria* and also those of *Ps. bisbyi* and *Ps. terrestris* were consistent with published descriptions (Timinin 1940, 1961; de Hoog 1983; Müller and Samuels 1982; Rao and de Hoog 1986; Fernández and Huhndorf 2004). The reference strain of *C. musae* (CBS 151.34) bore cylindrical conidophores with polyblastic conidiogenus cells, the conidia leaving denticles with dark-pigmented scars, features more typical of *Pyricularia* species. None of the strains studied developed the teleomorph.

DNA sequences analysis.—The length of sequences obtained from *Cordana* strains were 455–546 bp for the ITS and 534–560 bp for the D1/D2 domains. A BLASTn query of the ITS sequence of *Cordana* sp. I revealed the highest percentage of identity with *C. pauciseptata* (EU139244, 92%) and *C. ellipsoidea* (EF029210, 90%), while the closest D1/D2 sequences were those of *Po. borinquensis* (EF063573, 97%) and *Coniochaeta* sp. (AY346275, 96%). The ITS sequences of the three isolates of *Cordana* sp. II had the highest identity with *Po. cordanophora* (AF178563, 89–91%; EU139244, 89–92%) and the D1/D2 sequences with those of *Po. borinquensis* (EF063573, 95–96%).

The alignment of the D1/D2 sequences resulted in a 576-character dataset. The topology of the phylogenetic trees inferred in the ML, MP and Bayesian analyses were congruent (FIG. 1). The well supported clades obtained in the tree agreed with several lineages of the Sordariomycetes (i.e. Cephalothecaceae, Chaetosphaeriales, Coniochaetales, Diaporthales, Hypocreales, Magnaporthales, Sordariaceae, Xylariales). With the exception of the *C. musae* (CBS 151.34) sequence, those of *Cordana* spp., *Porosphaerella* spp. and *Pseudobotrytis* spp. included in the analysis constituted a strongly supported monophyletic lineage (*Cordana* clade), which appeared as a sister group of the Coniochaetales but remained

incertae sedis among the Sordariomycetes. The strain of *C. musae* fell into the Magnaporthales clade together with sequences of other *Pyricularia/Magnaporthe* species. The *Cordana* clade included eight lineages, each of which represented the morphological species included in the study (i.e. the GenBank sequences of *Po. cordanophora* and *Po. borinquensis* were grouped in two well supported clades with the respective sequences of the isolates morphologically identified as *C. pauciseptata* and *Ps. terrestris*, representing two of the phylogenetic species. The rest of the species corresponded to *C. ellipsoidea*, *C. inaequalis*, *C. solitaria*, *Ps. bisbyi*, and the two putative new *Cordana* species (I, II).

The ITS phylogenetic analysis of the *Cordana* clade comprised a dataset of 556 bp. Kiamura-2-parameter was the best-fit model of nucleotide substitution. The topology of the ML tree (FIG. 2) was similar to that obtained with the D1/D2 analysis. The sequences of *Cordana* and *Pseudobotrytis* species formed a clade with 100% bootstrap support. While *Cordana* sp. I was placed in a branch clearly separated from the other taxa included in the analysis, isolates of *Cordana* sp. II formed a basal subclade with 100% BML close to *C. inaequalis*. The sequences of the three isolates of *Cordana* sp. II had 97.6–99.8% of pairwise identity (492–503 bp of 504 characters). Similarity among sequences of both *Pseudobotrytis* species was 96.3–96.6% (488–490 bp of 507 characters).

Although in both analyses the two *Pseudobotrytis* species grouped together, the phylogenetic distance between the closest subclades of *Cordana* species and the absence of distinctive morphological features among these latter subclades did not support *Pseudobotrytis* as a distinct genus.

TAXONOMY

According to the above results, we conclude that the species investigated in this study constitute a monophyletic clade that represents only one genus. Considering the recent roles on fungal nomenclature, the name *Cordana* takes priority. Therefore below we provide an

emended description of the genus *Cordana* on the basis of the holomorphic concept the type species and to accommodate the fungi previously included in *Pseudobotrytis*. Likewise phenotypic differences and supported by molecular data let us propose *Cordana* sp. I and *Cordana* sp. II as new species respectively named *C. mercadiana* and *C. verruculosa*.

Cordana Preuss, Linnaea 24:129, 1851

= *Pseudobotrytis* Krzemien. & Badura, Acta Soc. Bot. Pol. 23:761, 1954

= *Umbellula* E.F. Morris, Mycologia 47:602, 1955

= *Porosphaerella* E. Müll. & Samuels, Sydowia 35:151, 1982

Emended description

Colonies effuse, velvety, brown, grayish brown or black. Mycelium mostly immersed. Conidiophores differentiated, mononematous, straight or flexuous, simple or branched at the apex, pale to dark brown, smooth. Conidiogenous cells polyblastic, terminal and intercalary, integrate or discrete, in some species umbellately arranged over the swollen apex of the stipe, sympodial, doliiform, spherical or subspherical, clavate, denticulate; denticles small, cylindrical. Conidia solitary, acropleurogenous, simple, ellipsoidal, oblong, obovoid, ovoid or pyriform, pale to dark brown, smooth, 0–1-septate, in some species with a thick dark band at the septum, often with a protuberant hilum. Chlamydospores sometimes formed in culture. Ascomata perithecial, solitary or gregarious, usually superficial and globose with a short papilla at the apex, dark brown to black. Paraphyses unbranched, filiform, septate and hyaline. Asci unitunicate, cylindrical, apical ring inconspicuous, eight-spored. Ascospores one-septate, ellipsoid to fusiform, light brown to brown, smooth-walled.

Type species: Cordana pauciseptata Preuss

Cordana bisbyi (Timonin) M. Hern.-Rest., Gené & Guarro, comb. nov.

MycoBank MB807980

Basionym: Pseudobotrytis bisbyi Timonin, Ceiba 9:28, 1961

Cordana mercadiana M. Hern.-Rest., J. Mena, Gené & Guarro, sp. nov. FIGS. 3–4

MycoBank MB800253

Etymology: Latin, *mercadiana*, after the Cuban mycologist Angel Mercado-Sierra for his outstanding contribution to mycology.

Diagnosis: Differing from *C. ellipsoidea*, *C. inaequalis*, *C. pauciseptata* and *C. uniseptata* by its shorter conidia lacking a dark septal band and the absence of chlamydospores.

Colonies on the natural substratum effuse, brown. Mycelium partly superficial, partly immersed in the substratum, hyphae 1–2.5 µm wide, septate, pale brown to brown, smooth-walled. Conidiophores macronematous, mononematous, straight to flexuous, unbranched, with intercalary nodes 4–6 µm wide, brown, paler toward the apex, smooth, 57–150 µm long, 2.5–3 µm wide, 3.5–4 µm wide at the swollen base. Conidiogenous cells integrated, polyblastic, terminal and intercalary, with subhyaline small denticles; proliferations percurrent, cylindrical to lageniform, inflated at the conidiogenous loci, 4–6 µm wide. Conidia solitary, dry, acropleurogenous, 0–1-septate, often slightly constricted at the septa, oblong, obovoid or cylindrical, with a prominent basal scar, brown, smooth-walled, 6–10 × 3–4 µm, basal cell sometimes darker. Conidial secession schizolytic. Teleomorph not observed.

Cultural characters: Colonies on OA and PCA at 25 C growing slowly, attaining 10–15 mm diam in 14 d, lanose to velvety, dark brown, margin slightly fimbriate; reverse medium gray. Sporulation was obtained after 2 wk incubation. Conidiophores unbranched, 137–350 × 3–4 µm. Conidia solitary, 0–1-septate, oblong, obovoid or cylindrical, brown, smooth-walled, 7–11 × 3–4.5 µm, basal cell sometimes darker.

Material examined: SPAIN, ARAGÓN, Ordesa y Monte Perdido National Park, Torla, Viu de Linares, on dead twig, Mar 2011, Col. *M. Hernández-Restrepo & J. Capilla* (HOLOTYPE: CBS H-20902; cultures ex-type: CBS 131866, FMR 11828).

Cordana pauciseptata Preuss, Linnaea 24:129, 1851

MycoBank MBT177296

≡ *Acrothecium pauciseptatum* (Preuss) Sacc., Michelia 1:74, 1877

≡ *Preussiaster pauciseptatus* (Preuss) O. Kuntze, Revis. Gen. Pl. 2:867, 1891

= *Porosphaerella cordanophora* E. Müll. & Samuels, Sydowia 35:151, 1982

Material examined: UNITED KINGDOM, DEVON, on wood of *Quercus robur*, 1978, Col. *P.M. Kirk* (epitype: CBS H-21148; cultures ex-epitype: IMI 232041a, CBS 135070, FMR 12074). CANADA, ONTARIO, from woodland soil, 1963, Col. *G.L. Barron* (OAC 10079, IMI 102120, FMR 12073). SPAIN, NAVARRA, Roncal Valley, on dead wood, Oct 2005, Col. *C. Silvera & J. Cano* (CBS 121804, FMR 9491).

This species is a common saprophyte with a worldwide distribution and extensively described by different authors (Hughes 1955, 1974; Ellis 1971; Matsushima 1975; Müller and Samuels 1982). Following the recent rule of fungal nomenclature (Hawksworth 2011), the name of the type species of *Cordana*, *C. pauciseptata*, described earlier (Hughes 1955) takes priority over the teleomorph name *Po. cordanophora* (Muller and Samuels 1982), which must be considered a synonym. However, because type material of *C. pauciseptata* is lost (de Hoog 1973) and there are no ex-type cultures, an epitype must be chosen for the taxonomic stability of the genus. Although no strain of *C. pauciseptata* included in the study produced the teleomorph, we chose the strain IMI 232041a as epitype because it is the most morphologically similar to the fungus described by Hughes (1955) and furthermore it is genetically the closest (D1/D2 99.1%, ITS 99.8%) to the reference sequence deposited in GenBank by Réblová and Winka (2000) under the name *Po. cordanophora* (AF 178563-CBS 101318). In addition, other authors also have used that sequence for phylogenetic studies on these fungi (Huhndorf et al. 2004, Reblova and Seifert 2007, Shenoy et al. 2010).

Cordana terrestris (Timonin) M. Hern.-Rest., Gené & Guarro, comb. nov.

MycoBank MB807979

Basionym: *Spicularia terrestris* Timonin, Can. J. Res., 18:315, 1940

≡ *Pseudobotrytis terrestris* (Timonin) Subram., Proc. Indian Natn. Sci. Acad. Biol. Sci. 43:277, 1956

≡ *Umbellula terrestris* (Timonin) E.F. Morris, Mycologia 47:603, 1955

= *Pseudobotrytis fusca* Krzemien. & Badura, 1954, Acta Soc. Bot. Pol. 23:762

= *Porosphaerella borinquensis* F.A. Fernández & Huhndorf, Fungal Diver. 17:12, 2004

Cordana verruculosa M. Hern.-Rest., J. Mena, Gené & Guarro, sp.nov. FIGS. 5–6

MycoBank MB518553

Etymology: Latin for "*verruculose*", referring to the conidial ornamentation.

Diagnosis: Differing from *C. solitaria* and *C. semaniae* by its smaller, verruculose and pale brown conidia.

Colonies on the natural substratum effuse, brown to mid-brown. Mycelium partly superficial, partly immersed in the substratum, hyphae 2.5–4 µm wide, septate, pale brown, smooth-walled. Conidiophores macronematous, mononematous, straight to flexuous, unbranched, with intercalary nodes 3–4.5 µm wide, brown, paler toward the apex, smooth-walled, up to 187 µm long, 2.5–3 µm wide, 4.5–6 µm at the swollen base. Conidiogenous cells integrated, polyblastic, with subhyaline and slightly prominent scars, terminal and intercalary; proliferations percurrent, cylindrical to lageniform, usually inflated at the conidiogenous loci, 2.5–4.5 µm wide. Conidia dry, solitary, acropleurogenous, 0-septate, ellipsoidal to obovoid, with a subapiculate and dark basal scar, pale brown, verruculose, 3–5.5 × 2–3.5 µm. Conidial secession schizolytic. Teleomorph not observed.

Cultural characters: Colonies on OA and PCA at 25 C growing slowly, attaining 9–16 mm diam in 14 d, velvety, grayish brown, margin irregular; reverse black. Sporulation was obtained after 3 wk incubation. Conidiophores unbranched, 75–170 × 3–4 µm. Conidia solitary, 0-septate, ellipsoidal to obovoid, pale brown, verruculose, 4–5.5 × 3–3.5 µm, occasionally arranged in short chains (of up to three conidia).

Material examined: SPAIN, ARAGON, Ordesa y Monte Perdido National Park, Torla, Viu de Linares, on dead wood, Jun 2009, Col. *M. Hernández-Restrepo*, *J. Mena-Portales & J. Cano* (HOLOTYPE: IMI 398792; cultures ex-type: CBS 127868, MUCL 52964, FMR 10754). CASTILLA Y LEÓN, BURGOS, Monasterio de Arlanza, on dead wood, Nov 2010, Col. *M. Hernández-Restrepo & J. Gené* (CBS 130364, IMI 500764, FMR

11594). SPAIN, ASTURIAS, Somiedo Natural Park, El Peral, on plant debris, Oct 2006, Col. *A. Mercado & C. Silvera* (CBS 121870, FMR 9490).

DISCUSSION

The genus *Cordana* currently comprises 19 species, including those investigated here. Diagnostic morphological features are keyed out below. In spite of the number of *Cordana* species, only *C. pauciseptata* has been included in phylogenetic analyses (Réblová and Winka 2000, Huhndorf et al. 2004, Reblova and Seifert 2007, Shenoy et al. 2010). In our study, with the exception of the isolate of *C. musae* (CBS 151.34), which probably belongs to *Pyricularia* (Magnaporthales), the *Cordana* species selected were grouped in a well supported clade within the Sordariomycetes but are unrelated to the known fungal orders or families. In agreement with Huhndorf et al. (2004), Reblova and Seifert (2007) and Shenoy et al. (2010), *Cordana* species appear related to the Coniochaetales but without clear statistical support to be considered members of this order.

In our D1/D2 analysis, the GenBank sequence of *Po. borinquensis* (EF063573), a fresh isolate morphologically identified as *Ps. terrestris* and a CBS strain labeled as an authentic strain of *Spicularia terrestris*, formed a well supported clade within the *Cordana* clade, clearly separated from the other species included in the analysis. Similar results were obtained with the analysis of the ITS region, this being the first genetic evidence of the anamorph-teleomorph connection of *Po. borinquensis* and *Ps. terrestris*. *Porosphaerella borinquensis* was described by Fernández and Huhndorf (2004) from a leaf of *Cecropia* from Puerto Rico; they proved the biological connection by culture of the ascospores in vitro. The teleomorphs of *Ps. terrestris* and *C. pauciseptata* have similar ascomata and ascospore morphologies (Müller and Samuels 1982, Fernández and Huhndorf 2004), and their anamorphic structures share morphological affinities (i.e. both have polyblastic, swollen and denticulate conidiogenous cells producing pigmented, one-septate, ellipsoid to oblong conidia). According to our molecular study, those morphological affinities are correlated with

the genetic relationship not only of *Ps*. *terrestris* but also of *Ps*. *bisbyi* with the *Cordana* species investigated. Therefore morphological and genetic evidences support that *Pseudobotrytis* and *Cordana* are congeneric, being subsequently transferred from the only two *Pseudobotrytis* species to the latter genus.

Porosphaerella setosa was not included in the study because of the lack of available strains to investigate. Nevertheless, we think the identification of this species is doubtful because morphologically it differs from the other two species included previously in *Porosphaerella* in several important aspects (i.e. absence of anamorph, presence of stiff setae on the acomata that lack parafisis and the asci are claviforms). These morphological features do not fit with the holomorphic concept of the genus *Cordana* presented here and therefore the species is not included in the genus.

While the taxonomic position of *Cordana* remains unresolved, our rDNA sequence analysis supports the proposal of two new species, *C*. *mercadiana* and *C*. *verruculosa*. The relevant morphological features that distinguish *C*. *mercadiana* from the other species of the genus are the presence of 0–1-septate, obovoid or oblong conidia with prominent basal scars and absence of chlamydospores. Conidia of *C*. *ellipsoidea*, *C*. *inaequalis* and *C*. *uniseptata* are similar to those of *C*. *mercadiana*. However, *C*. *ellipsoidea* differs in having a dark band at the conidial septum, conidiophores are verrucose toward the apex and chlamydospores are present in culture (de Hoog 1973). *Cordana inaequalis* has larger conidia. (9–)10–12.5(–13.5) × (3.6–)4–5(5.4) µm, with the basal cell larger than the apical one and a dark band at the septum (Hughes 1983); whereas *C*. *uniseptata* has larger conidia (13.5–23 × 8.5–11.5 µm) with a paler apical cell (Cai et al. 2004).

The other new species, *C*. *verruculosa*, has smaller (3–5.5 × 2–3.5 µm) unicellular, verruculose, and light brown conidia. Other *Cordana* species with aseptate conidia are *C*. *solitaria* (Rao and de Hoog 1986) and *C*. *semaniae* Davydkina, Mel'nik & Novozh.

(Davydkina and Mel'nik 1989), but their conidia are smooth-walled and larger (6.5–9.5 × 4.5–6.5 µm in *C. solitaria* and 21–27 × 9–15 µm in *C. semaniae*). In addition, those of *C. semaniae* are black and *C. solitaria* has a *Bispora*-like synanamorph (Rao and de Hoog 1986). Because *C. verruculosa* forms short conidial chains in culture, it resembles *Gonatobotryum* Sacc. However, members of this genus are characterized by their differentiated, unbranched and percurrently proliferating conidiophores and terminal or intercalary inflated conidiogenous cells synchronously producing simple or branched chains 0–(1)-septate conidia seceding rhexolytically (Walker and Minter 1981).

KEY DIFFERENTIATING *CORDANA* SPECIES (ADAPTED FROM MARKOVSKAJA 2003)

1a. Conidiogenous cells discrete, arranged in a world at the apex of the conidiophores 2

1b. Conidiogenous cells integrated in the conidiophores, intercalary or terminal 3

2a. Conidia 1-septate, 6–11 × 3–4 µm; teleomorph, when present, with ascospores ellipsoidal, one-septate, constricted at the setum, 7–9 × 3–4 µm ..... *C. terrestris*

2b. Conidia 0-septate, 5–7 × 2.5–4.5 µm; teleomorph absent ..... *C. bisbyi*

3a. Conidia only zero-septate 4

3b. Conidia only one-septate 6

3c. Conidia zero–one-septate ..... *C. mercadiana*

4a. Conidia verruculose ..... *C. verruculosa*

4b. Conidia smooth-walled 5

5a. Conidia black, 21–27 × 9–15 µm ..... *C. semaniae*

5b. Conidia pale brown, 6.5–9.5 × 4.5–6.5 µm ..... *C. solitaria*

6a. Conidial septum markedly thickened, brownish black 7

6b. Conidia septum otherwise 9

7a. Conidia with an acute apex; teleomorph, when present, with ascospores ellipsoidal to elliptic-fusiform, one-septate, constricted at the setum, 9–12 × 3.5–5 µm ..... *C. pauciseptata*

7b. Conidia with a rounded apex; teleomorph absent 8

8a. Conidia broadly ellipsoidal or obovate, both cells concolorous, pale brown to brown ..... *C. ellipsoidea*

8b. Conidia ellipsoidal or ovoid, the basal cell brown and larger than the distal cell ..... *C. inaequalis*

9a. Conidia obpyriform, distal cell consistently narrower than the basal cell 10

9b. Conidia ovoid or pyriform, distal cell usually wider than the basal cell 11

9c. Conidia ellipsoidal, oval or oblong, both cells of nearly equal width 12

10a. Basal cell dark brown, distal cell light brown with an acute apex, 18.5–27 × 9.5–15 µm ..... *C. crassa*

10b. Both cells concolorous, brown, distal cell with rounded apex, 13–17.5 × 8–10 µm ..... *C. lithuanica*

11a. Conidia 11–18 × 7–9 µm, ovoid or pyriform, light brown ..... *C. musae*

11b. Conidia 18–31 × 12.5–15.5 µm, ovoid, at first light brown, later dark brown, with a pigmented septal pore ..... *C. abramovii*

12a. Conidia broadly ellipsoidal to obovoid, more than 8 µm wide 13

12b. Conidia ellipsoidal, oval or oblong, up to 8 µm wide 16

13a. Conidia concolorous, subhyaline to pale brown 14

13b. Conidia discolorous 15

14a. Conidia 20–30 × 12–18 µm, broadly ellipsoidal, ... *C. johnstonii*

14b. Conidia 17–19 × 10–13 µm, obovoid ..... *C. andinopatagonica*

15a. Basal cell brown, apical cell paler, 13.5–23 × 8.5–11.5 µm ..... *C. uniseptata*

15b. Basal cell subhyaline, apical cell pale brown, 15–25 × 10–15 µm ..... *C. versicolor*

16a. Conidia ellipsoidal or oval, 11–20 × 4.5–7 µm ..... *C. indica*

16b. Conidia oblong, 6–10 × 3–5 µm ..... *C. parasitica*

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LITERATURE CITED

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402.

Barber PA, Keane PJ. 2007. A novel method of illustrating microfungi. Fungal Divers 27:1–10.

Cai L, McKenzie EHC, Hyde KD. 2004. New species of *Cordana* and *Spadicoides* from decaying bamboo culms in China. Sydowia 56:222–228.

Cano J, Guarro J, Gené J. 2004. Molecular and morphological identification of *Colletotrichum* species of clinical interest. J Clinic Microbiol 42:2450–2454.

Davydkina TA, Mel'nik VA. 1989. Two new hyphomycetes from the genera *Cordana* and *Pyriculariopsis*. Mikol Fitopatol 23:110-113.

de Hoog GS. 1973. A new species of *Cordana* (Dematiaceae, Hyphomycetes). Acta Bot Neerland 22:209–212.

———, van Oorschot CAN, Hijwegen T. 1983. Taxonomy of the *Dactylaria* complex II. *Dissoconium* gen. nov. and *Cordana* Preuss. Proceedings of the Koninklijke Nederlandse Akademie von Wetenschappen. Series C 86:197–206.

Ellis MB. 1971. Dematiaceous Hyphomycetes. Kew, UK: Commonwealth Mycological Institute. 608 p.

———. 1976. More dematiaceous hyphomycetes. Kew, UK: Commonwealth Mycological Institute. 507 p.

Fernández FA, Huhndorf SM. 2004. Neotropical pyrenomycetes: *Porosphaerella borinquensis* sp. nov. and its *Pseudobotrytis terrestris* anamorph. Fungal Divers 17:11–16.

Hernández-Restrepo M, Silvera-Simón C, Mena-Portales J, Mercado-Sierra A, Guarro J, Gené J. 2012. Three new species and a new record of *Diplococcium* from plant debris in Spain. Mycol Prog 11:191–199.

Hawksworth DL. 2011. A new dawn for the naming of fungi: impacts of decisions made in Melbourne in July 2011 on the future publication and regulation of fungal names. MycoKeys 1:7–20.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics. 17:754–755.

Hughes SJ. 1955. Microfungi I. *Cordana*, *Brachysporium*, *Phragmocephala*. Can J Bot 33:259–268.

———. 1974. *Cordana pauciseptata*. Fungi Canadenses 34:1–2.

———. 1983. *Cordana inaequalis*. Fungi Canadenses 246:1–2.

Huhndorf SM, Miller AN, Fernández FA. 2004. Molecular systematics of the Sordariales: the order and the family *Lasiosphaeriaceae* redefined. Mycologia 96:368–387.

Krzemieniewska H, Badura L. 1954. Przyczynek do znajomosci micro-organismow sciolki i gleby lasu bukowego (A contribution to the knowledge of the micro-organims from the litter and soil of beechwood). Acta Soc Bot Pol 23:727–781.

Markovskaja S. 2003. A new species of *Cordana* from Lithuania. Mycotaxon 87:179–185.

Matsushima T. 1975. Icones microfungorum a Matsushima lectorum. Kobe, Japan: Published by the author. 209 p, 415 pl.

Mena-Portales J, Hernández-Restrepo M, Minter DW, Gené J. 2011. A new species of *Ceratocladium* from Spain. Mycol Prog 10:493–496.

Müller E, Samuels GJ. 1982. Anamorphs of pyrenomycetous ascomycetes II. *Porosphaerella* gen. nov. and its *Cordana* anamorph. Sydowia 35:150–155.

Preuss GT 1851. Synopsis fungorum cognitorum praesertim prope Hoyerswerda. Linnaea 24:101–153.

Rao V, de Hoog GS. 1986. New or critical Hyphomycetes from India. Stud Mycol 28:1–84.

Réblová M, Barr ME, Samuels GJ. 1999. *Chaetosphaeriaceae*, a new family for *Chaetosphaeria* and its relatives. Sydowia 51:49–70.

———, Seifert KA. 2007. A new fungal genus, *Teracosphaeria*, with a phialophora-like anamorph *(Sordariomycetes*, *Ascomycota)*. Mycol Res 3:287–298.

———, Winka K. 2000. Phylogeny of *Chaetosphaeria* and its anamorphs based on morphological and molecular data. Mycologia 92:939–954.

Romero AI, Samuels GJ. 1991. Studies on xylophilous fungi from Argentina VI. Ascomycotina on *Eucalyptus viminalis* (Myrtaceae). Sydowia 43:228-248.

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425.

Shenoy BD, Jeewon R, Wang H, Amandeep K, Ho WH, Bhat DJ, Crous PW, Hyde KD. 2010. Sequence data reveal phylogenetic affinities of fungal anamorphs *Bahusutrabeeja*, *Diplococcium*, *Natarajania*, *Paliphora*, *Polyschema*, *Rattania* and *Spadicoides*. Fungal Divers 44:161–169.

Soares DJ, Nechet KL, Barreto RW. 2005. *Cordana versicolor* sp. nov. (dematiaceous hyphomycete) causing leaf-spot on *Canna denudata* (*Cannanceae*) in Brazil with observations on *Cordana musae*. Fungal Divers 18:147–155.

Subramanian CV. 1956. On *Spicularia terrestris* Timotin. Proceedings of the Indian National Science Academy, Part B. Biol Sci 43:276–278.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA 5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol Biol Evol 28:2731–2739.

Timonin MI. 1940. The interaction of higher plants and soil microorganims I. Microbial population of rhizosphere of seedlings of certain cultivated plants. Can J Res 18:307–317.

———. 1961. New species of Pseudobotrytis. Ceiba 9:27–29.

Walker JC, Minter DW. 1981. Taxonomy of *Nematogonum*, *Gonatobotrys*, *Gonatobotryum* and *Gonatorrhodiella*. Trans Br Mycol Soc 77:299–319.

LEGENDS

FIG. 1. Phylogram inferred from the D1/D2 sequences from maximum likelihood analysis. Support values (maximum likelihood bootstrap MLBS/maximum parsimony bootstrap MPBS/Bayesian posterior probability BPP) are indicated at the nodes. Only support values BS > 50 and PP ≥ 0.95 are indicated. ^{T} = ex-type strain. ^{ET} = ex-epitype strain. ^{IT} = ex-isotype strain.

FIG. 2. Phylogram inferred from the ITS sequences of *Cordana* spp. from maximum likelihood analysis. Bootstrap support ≥ 50 indicated at the nodes. ^{T} = ex-type strain. ^{ET} = ex-epitype strain. ^{IT} = ex-isotype strain. *GenBank sequence of *Porosphaerella cordanophora*.

FIG. 3. *Cordana mercadiana* sp. nov. FMR 11828. a. Habit. b. Conidiophores. c. Conidia. Bars: a = 200 µm; b, c = 10 µm.

FIG. 4. *Cordana mercadiana* sp. nov. FMR 11828. a, b. From the natural substratum. c–e. SEM microphotographs from culture. a, d, e. Conidiophores and swollen denticulate conidiogenous cells with attached conidia. b, f. Conidia. Bars a, b, d, e = 10 µm; c = 50 µm

FIG. 5. *Cordana verruculosa* sp. nov. FMR 10754. a. Habit. b. Conidiophores. c. Conidia. Bars : a = 200 µm; b, c = 10 µm.

FIG. 6. *Cordana verruculosa* sp. nov. FMR 10754. a–f. Specimen from the natural substratum. a, d. Conidiophores. b, e. Conidiogenous cells. c, f. Conidia. Bars: a–f = 10 µm.

FOOTNOTES

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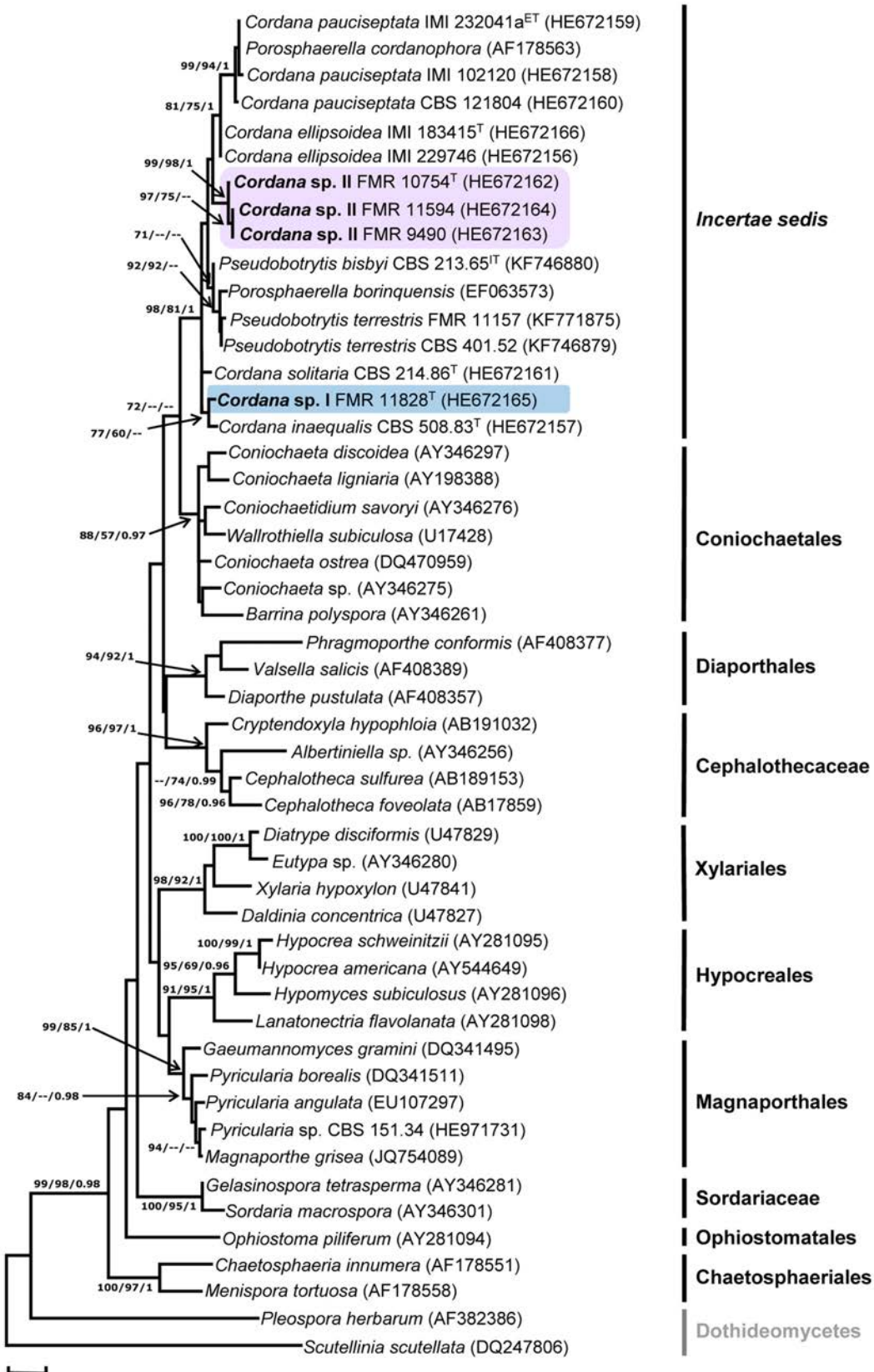
TABLE I. Culture numbers, accession numbers, origin and substrata of the *Cordana* spp. sequenced in this study

Species	Strain	Origin	GenBank numbers	
			ITS	D1/D2
C. bisbyi (received as *Pseudobotrytis terrestris)*	CBS 213.65[IT]	Soil under *Musa sapientum*, Honduras	KF733464	KF746880
C. ellipsoidea	IMI 183415[T]	Forest soil *Quercus* sp., Nepal	HE672155	HE672166
	IMI 229746	Soil, Japan	HE672145	HE672156
C. inaequalis	CBS 508.83[T]	Decaying wood, Canada	HE672146	HE672157
C. mercadiana	FMR 11828[T]	Dead twig, Spain	HE672154	HE672165
C. pauciseptata	IMI 102120	Soil, mixed with wood; Canada	HE672147	HE672158
	IMI 232041a[ET]	Wood *Quercus robur*, Great Britain	HE672148	HE672159
	CBS 121804	Decaying wood, Spain	HE672149	HE672160
C. solitaria	CBS 214.86[T]	Decaying wood, India	HE672150	HE672161
C. verruculosa	FMR 10754[T]	Dead twig, Spain	HE672151	HE672162
	FMR 11594	Dead twig, Spain	HE672153	HE672164
	FMR 9490	Decaying wood, Spain	HE672152	HE672163
C. terrestris (received as *Pseudobotrytis terrestris)*	CBS 401.52[a]	Soil, Canada	HE733463	KF746879
	FMR 11157	Dead twig, Spain	HE677173	KF771875
Pyricularia sp. (received as *C. musae)*	CBS 151.34	*Musa sapientum*	HE971730	HE971731

CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; FMR: Facultad de Medicina, Reus, Spain; IMI, International Mycological Institute, Surrey, England; [T] Culture ex-type; [ET] Culture ex-epitype; [IT] Culture ex-isotype. [a]According to CBS collection it is an authentic strain of *Spicularia terrestis*.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Porosphaerella cordanophora (AF178563)
Cordana pauciseptata IMI 102120 (HE672158)
Cordana pauciseptata CBS 121804 (HE672160)
Cordana ellipsoidea IMI 183415^T (HE672166)
Cordana ellipsoidea IMI 229746 (HE672156)
Cordana sp. II FMR 10754^T (HE672162)
Cordana sp. II FMR 11594 (HE672164)
Cordana sp. II FMR 9490 (HE672163)
Pseudobotrytis bisbyi CBS 213.65^IT (KF746880)
Porosphaerella borinquensis (EF063573)
Pseudobotrytis terrestris FMR 11157 (KF771875)
Pseudobotrytis terrestris CBS 401.52 (KF746879)
Cordana solitaria CBS 214.86^T (HE672161)
Cordana sp. I FMR 11828^T (HE672165)
Cordana inaequalis CBS 508.83^T (HE672157)
Coniochaeta discoidea (AY346297)
Coniochaeta ligniaria (AY198388)
Coniochaetidium savoryi (AY346276)
Wallrothiella subiculosa (U17428)
Coniochaeta ostrea (DQ470959)
Coniochaeta sp. (AY346275)
Barrina polyspora (AY346261)
Phragmoporthe conformis (AF408377)
Valsella salicis (AF408389)
Diaporthe pustulata (AF408357)
Cryptendoxyla hypophloia (AB191032)
Albertiniella sp. (AY346256)
Cephalotheca sulfurea (AB189153)
Cephalotheca foveolata (AB17859)
Diatrype disciformis (U47829)
Eutypa sp. (AY346280)
Xylaria hypoxylon (U47841)
Daldinia concentrica (U47827)
Hypocrea schweinitzii (AY281095)
Hypocrea americana (AY544649)
Hypomyces subiculosus (AY281096)
Lanatonectria flavolanata (AY281098)
Gaeumannomyces gramini (DQ341495)
Pyricularia borealis (DQ341511)
Pyricularia angulata (EU107297)
Pyricularia sp. CBS 151.34 (HE971731)
Magnaporthe grisea (JQ754089)
Gelasinospora tetrasperma (AY346281)
Sordaria macrospora (AY346301)
Ophiostoma piliferum (AY281094)
Chaetosphaeria innumera (AF178551)
Menispora tortuosa (AF178558)
Pleospora herbarum (AF382386)
Scutellinia scutellata (DQ247806)
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81/75/1
99/98/1
97/75/--
71/--/--
92/92/--
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72/--/--
77/60/--
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94/92/1
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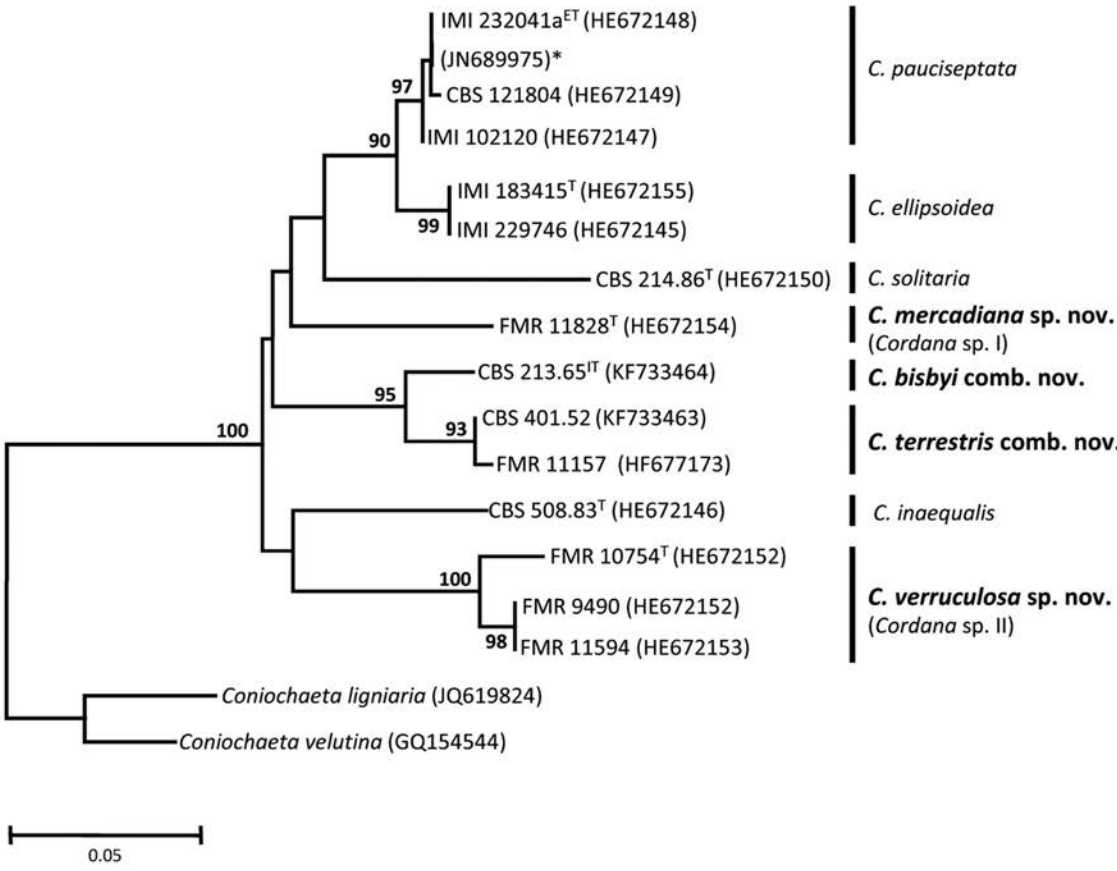
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FMR 9490 (HE672152)
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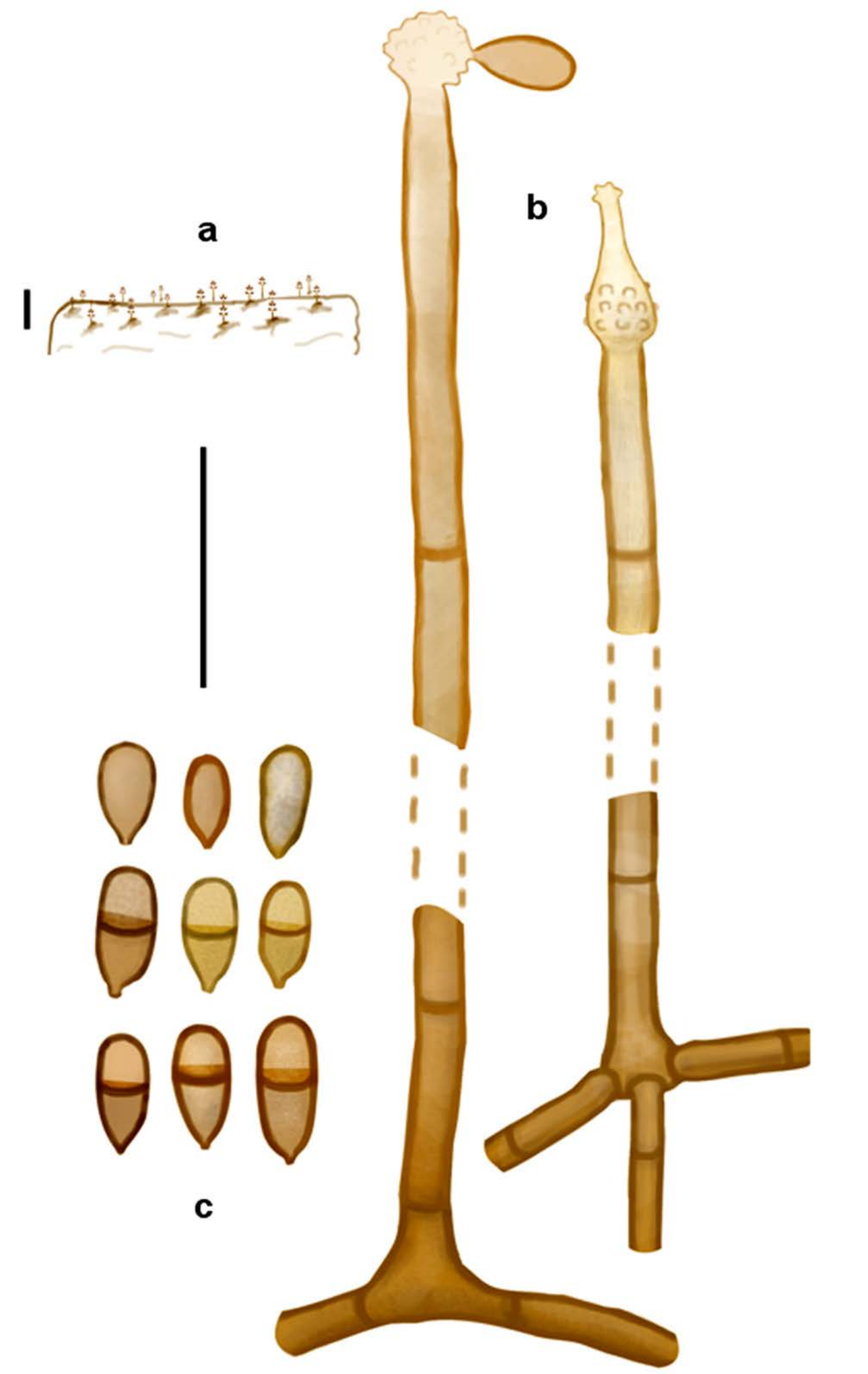
Coniochaeta ligniaria (JQ619824)
Coniochaeta velutina (GQ154544)
97
90
99
95
93
100
100
98
0.05
C. pauciseptata
C. ellipsoidea
C. solitaria
C. mercadiana sp. nov.
(Cordana sp. I)
C. bisbyi comb. nov.
C. terrestris comb. nov.
C. inaequalis
C. verruculosa sp. nov.
(Cordana sp. II)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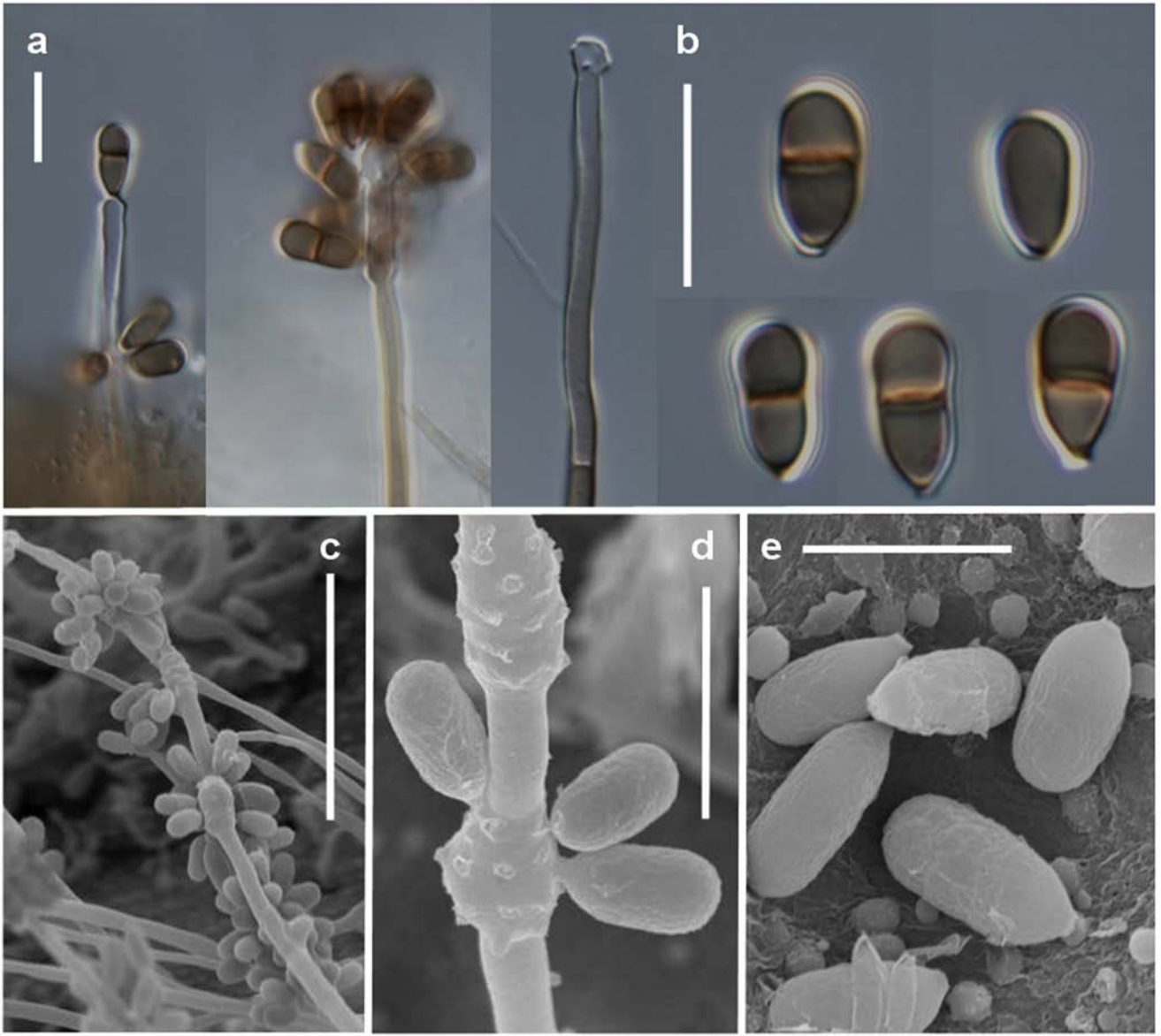
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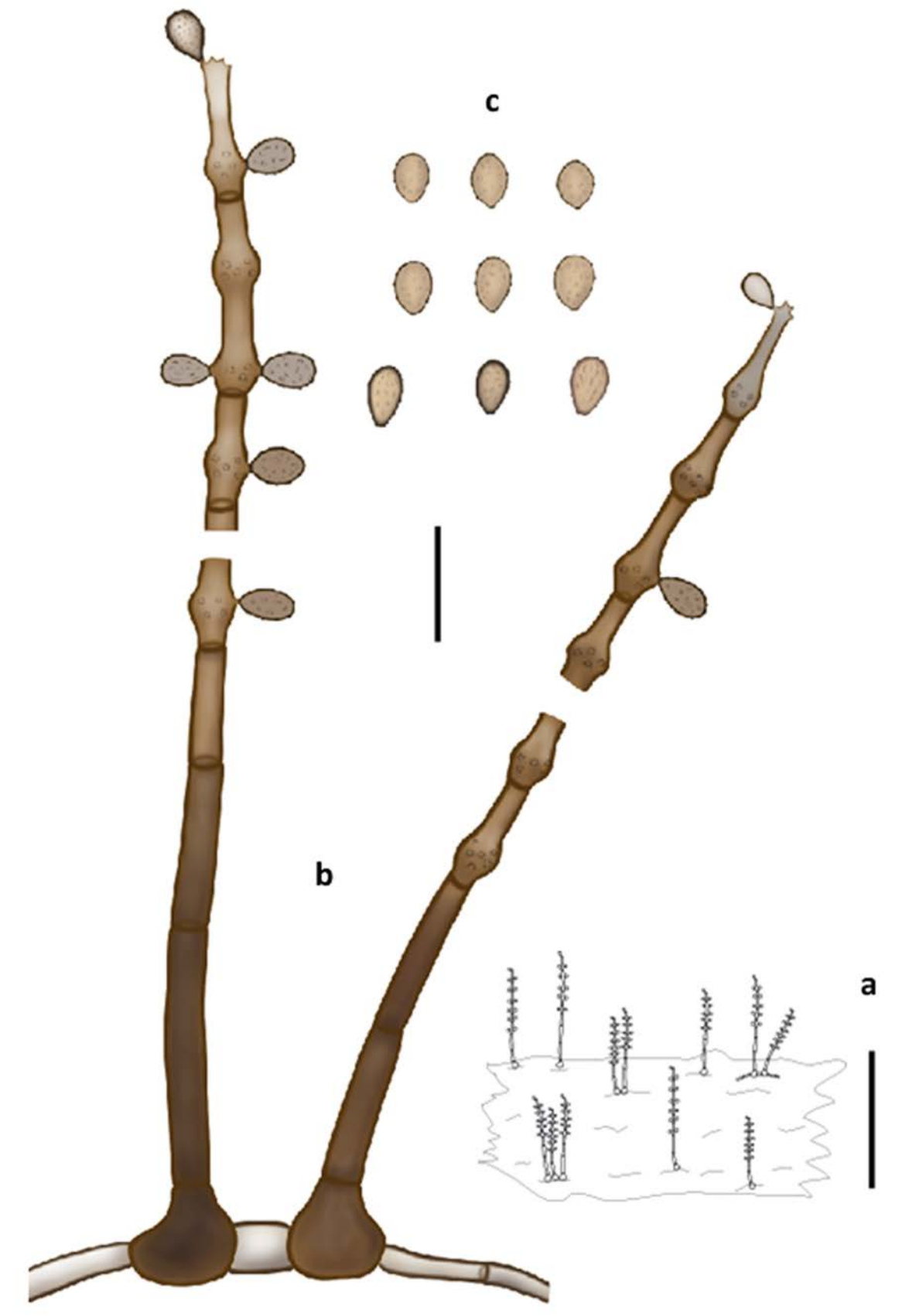
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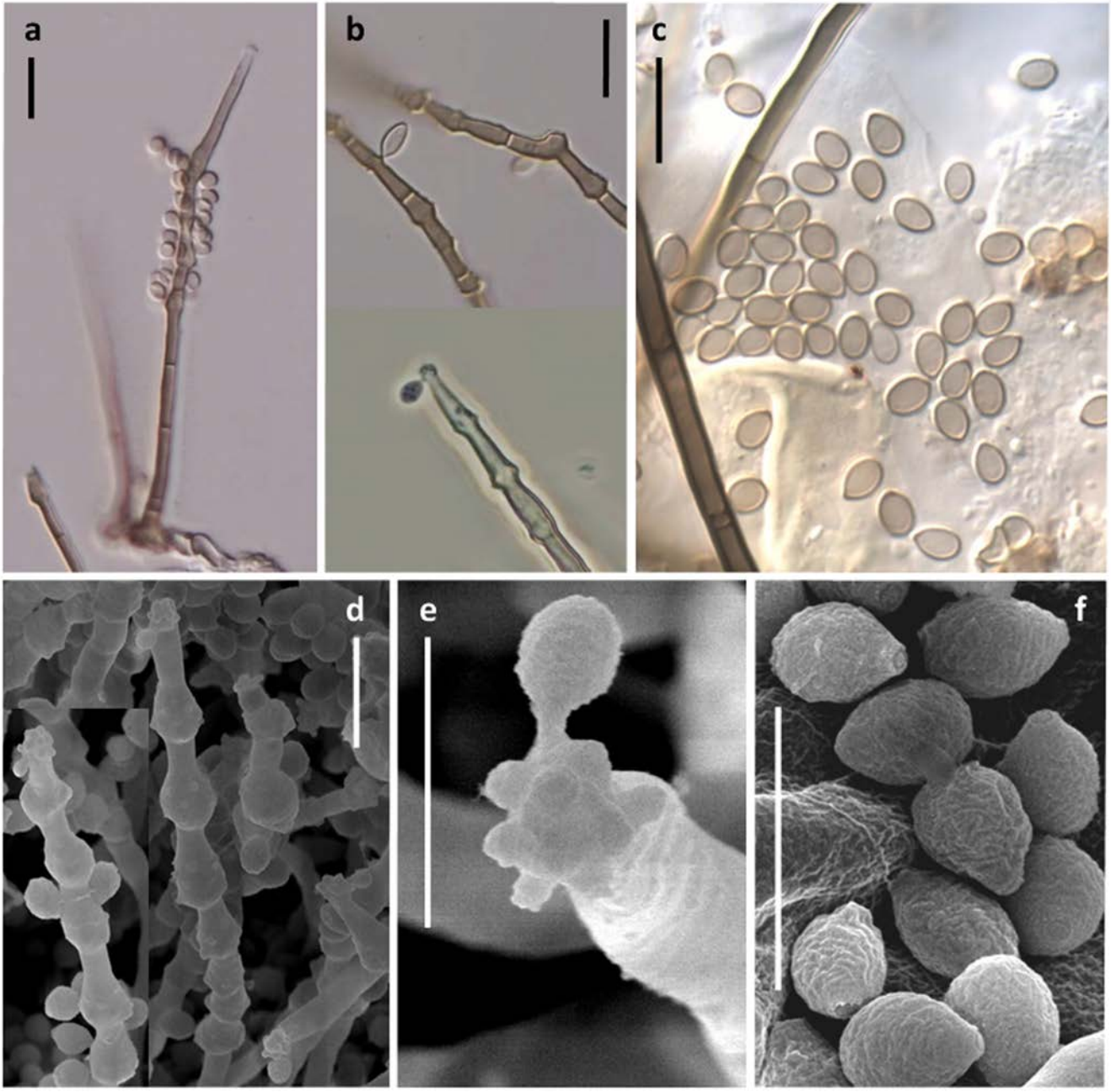
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