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New *Bactrodesmiastrum* and *Bactrodesmium* from decaying wood in Spain

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Abstract: Several new species belonging to the anamorph genera *Bactrodesmiastrum* and *Bactrodesmium*, collected from plant debris in Spain, are described and illustrated. *Bactrodesmiastrum pyriforme* sp. nov. is characterized by large, pyriform conidia. *Bactrodesmiastrum obovatum* comb. nov. is proposed to accommodate *Janetia obovata*, and the Spanish specimen of *B. obscurum*, the type species of the genus, represents the second known collection of the species worldwide. Analyses of rDNA sequences confirm the distinction between *B. obovatum* and *B. pyriforme* and reveal their relationships with the Savoryellales (Sordariomycetes). *Bactrodesmium diversum* sp. nov. is distinguished from the other species of the genus by its large, pale brown conidia with a conspicuous pore at each septum.

Key words: plant debris, Sordariomycetes, taxonomy

Introduction

During a continuous survey of the mycobiota of the Iberian Peninsula, several species of the anamorph genera *Bactrodesmiastrum* Hol.-Jech. and *Bactrodesmium* Cooke were found on decaying wood. Both genera have similar conidiogenesis and conidial morphology. While *Bactrodesmiastrum* is characterized by solitary or aggregated conidiophores reduced to a brown, single, monoblastic conidiogenous cell (Holubová-Jechová 1984), *Bactrodesmium* has sporodochial conidiomata and differentiated, hyaline or brown, simple or branched conidiophores supporting mono- or polyblastic conidiogenous cells (Ellis 1971, Holubová-Jechová 1972).

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The specimens had a combination of morphological features that did not match previously described species. Therefore new species of *Bactrodesmiastrum* and *Bactrodesmium* are described and illustrated here. In addition, a new combination in *Bactrodesmiastrum* is proposed based on *Janetia obovata* Calduch et al. (2002). Sequence analyses of internal transcribed spacers (ITS) and the D1/D2 region of nuclear large subunit (LSU) ribosomal DNA (rDNA) confirmed the identity and the taxonomic position of cultured specimens.

Materials and methods

Sampling area.—Plant debris was collected in these Spanish regions: Aragon, Castilla-La Mancha and the Comunidad de Valencia (Fig. 1). Samples from Aragon were from the Ordesa y Monte Perdido National Park (Huesca), which has a mountain climate with vegetation dominated by *Fagus sylvatica* L., *Pinus* spp. and *Quercus* spp., and from Nogueruelas (Teruel), which is in the south of Aragon, practically on the border of the Comunidad de Valencia. The area of Nogueruelas is characterized, like the other two areas sampled from the Comunidad de Valencia (Loriguilla and Los Herreros), by a Mediterranean climate and forests of mainly *Pinus* spp., *Quercus ilex* L., *Q. rotundifolia* Lam. and *Q. pyrenaica* Willd. Samples from Castilla-La Mancha were collected in the protected natural area of Monumento Natural del Río Cuervo, characterized by a Mediterranean climate, from humid to subhumid, where the predominantly tree species is *Pinus sylvestris* L.

Isolation and identification of fungi.—Plant material was put into polyethylene bags and kept at 4–7 C until processed. Samples were placed in moist chambers, incubated at room temperature and examined periodically under the stereomicroscope over a 2 mo period. Semipermanent and permanent slides of the microscopic fungi growing on the natural substratum were mounted in 85% lactic acid and polyvinyl alcohol and examined under a light microscope. To get pure cultures, conidia were transferred from the natural substratum to potato carrot agar (PCA; 20 g potatoes, 20 g carrots, 20 g agar [Pronadisa, Spain], 1 L distilled water) and oatmeal agar (OA; 30 g flakes, 20 g agar, 1 L distilled water) and incubated at 25 C in the dark. Photomicrographs were obtained with a Zeiss AXIO Imager M1 microscope (Göttingen, Germany) and a Jeol JSM-6400 scanning electron microscope.

DNA extraction, amplification and sequencing.—Isolates were grown on PCA 2–4 wk at 25 C, and DNA was extracted with a PrepMan Ultra sample preparation reagent (Applied Biosystems, Foster City, California), according to the manufacturer's protocol. The DNA was quantified with GeneQuantpro (Amersham Pharmacia Biotech, Cambridge, England). The ITS region and the D1/D2 domains

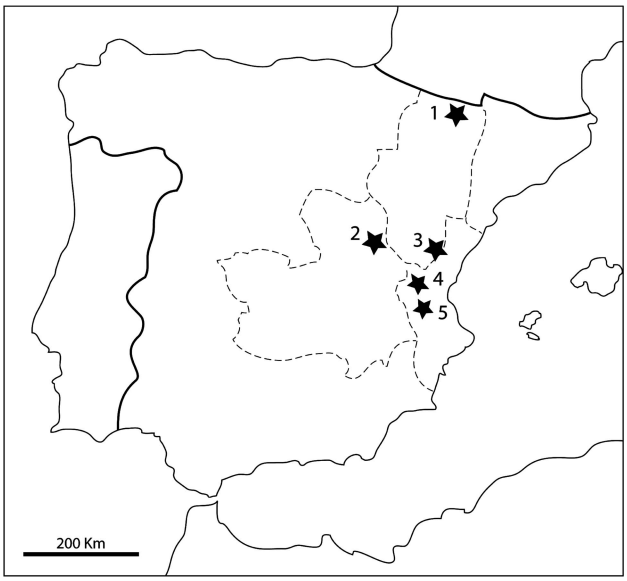

FIG. 1. Collection sites in Spain. 1. Ordesa y Monte Perdido National Park (Huesca, Aragón). 2. Monumento Natural del Rio Cuervo (Cuenca, Castilla-La Mancha). 3. Nogueruelas (Teruel, Aragon). 4, 5. Loriguilla and Los Herreros respectively (Valencia, Comunidad Valenciana).

of the LSU rDNA were amplified following Cano et al. (2002) and sequenced with the primer pair ITS5 and NL4b in both directions at Macrogen (Seoul, Korea). The SeqMan program (Lasergene, Madison, Wisconsin) was used to obtain consensus sequences. A BLAST sequence identity query (Altschul et al. 1990) was carried out to compare data of the strains isolated in this study with those of other fungi deposited in the GenBank database. Nucleotide sequence alignments were made with Clustal X 2.0 (Thompson et al. 1997), followed by manual adjustments with a text editor. The D1/D2 region sequence, instead of the ITS region, was chosen for the phylogenetic study because the latter one was excessively variable and generated numerous regions with ambiguous alignments. We conducted a phylogenetic analysis of our isolates and 24 sequences retrieved from GenBank, corresponding to morphologically similar fungi or genera representing different orders or families of ascomycetes. We used MEGA5 (Tamura et al. 2011) with the maximum likelihood (ML) algorithm and the Tamura Nei substitution model with gamma distribution. The robustness of branches was assessed by bootstrap analysis of 1000 replicates.

Sequences generated in this study were deposited in GenBank with these accession numbers: FR870264 (ITS) and FR870266 (D1/D2) for CBS 127867, HE646636 (ITS) and HE646637 (D1/D2) for FMR 11931, and FR870263 (ITS) and FR870265 (D1/D2) for CBS 101300. The alignment was deposited in TreeBASE (www.treebase.org, accession URL: http://purl.org/phylo/treebase/phylows/study/TB2: S12649).

RESULTS

DNA sequence analyses.—With the primers used we were able to amplify and sequence 632 bp of the ITS region and 535 bp of the D1/D2 domains of three strains, one corresponding to the type strain of *J. obovata* (CBS 101300) and two strains of *Bactrodesmiastrum* (CBS 127867, FMR 11931). The genetic similarity between the ITS sequences of *J. obovata* and the *Bactrodesmiastrum* strains was 94–95.28%, while between the two *Bactrodesmiastrum* strains the similarity was 98.8%. Although the D1/D2 sequences were not useful for separating the three strains, a BLAST query with this region of the two *Bactrodesmiastrum* strains and of *J. obovata* showed a similarity of 93% and 94% respectively, with *Ascotaiwania persoonii* Fallah, J.L. Crane & Shearer (GenBank accession number AY094190). The ITS sequences did not reveal any close hits for any strain of either species, therefore the cladistic analysis was based on sequences of the D1/D2 domains. After removing ambiguously aligned regions we obtained a D1/D2 dataset of 395 characters. The groupings obtained in the ML phylogenetic tree (FIG. 2) agree with the main lineages shown in previous phylogenetic assessments of the Sordariomycetes (Campbell and Shearer 2004, Boonyuen et al. 2011). Two *Bactrodesmiastrum* strains, *J. obovata* and *A. persoonii*, formed a clade with 99% bootstrap support (bs) (FIG. 2), appearing as a sister clade of the Savoryellales (Sordariomycetes).

Based on the results of the sequence analyses and phenotypic characteristics, a new species of *Bactrodesmiastrum* is proposed to accommodate strains CBS 127867 and FMR 11931, and *J. obovata* is transferred to this genus.

TAXONOMY

Bactrodesmiastrum pyriforme M. Hern.-Rest., J. Mena, Gené & Guarro, sp. nov. FIGS. 3, 4a–c. MycoBank MB518552

Etymology: Latin ''*pyriforme*'', referring to the conidial shape.

Colonies on the natural substratum effuse and blackish brown to black. Mycelium partly superficial, partly immersed in the substratum, composed of branched, septate, pale brown, smooth, 1.5–2.5 µm wide hyphae. Conidiophores semi-macronematous, reduced to a single conidiogenous cell, solitary first, later aggregated in small groups. Conidiogenous cells borne on basal hyphae, monoblastic, cylindrical or lageniform, medium brown to dark brown, thick-walled, 8–13 × 4–7 µm, 2.5–4 µm wide at the truncate apex. Conidia solitary, dry, acrogenous, straight or slightly curved, pyriform or obovoid, (2–)3–4-septate, smooth, dark brown to black, with 1–2 proximal cells paler, basal cell conico-truncate, 20–50 × 14–28 µm, basal scar 2.5–3 µm wide. Conidial secession schizolytic. Teleomorph unknown.

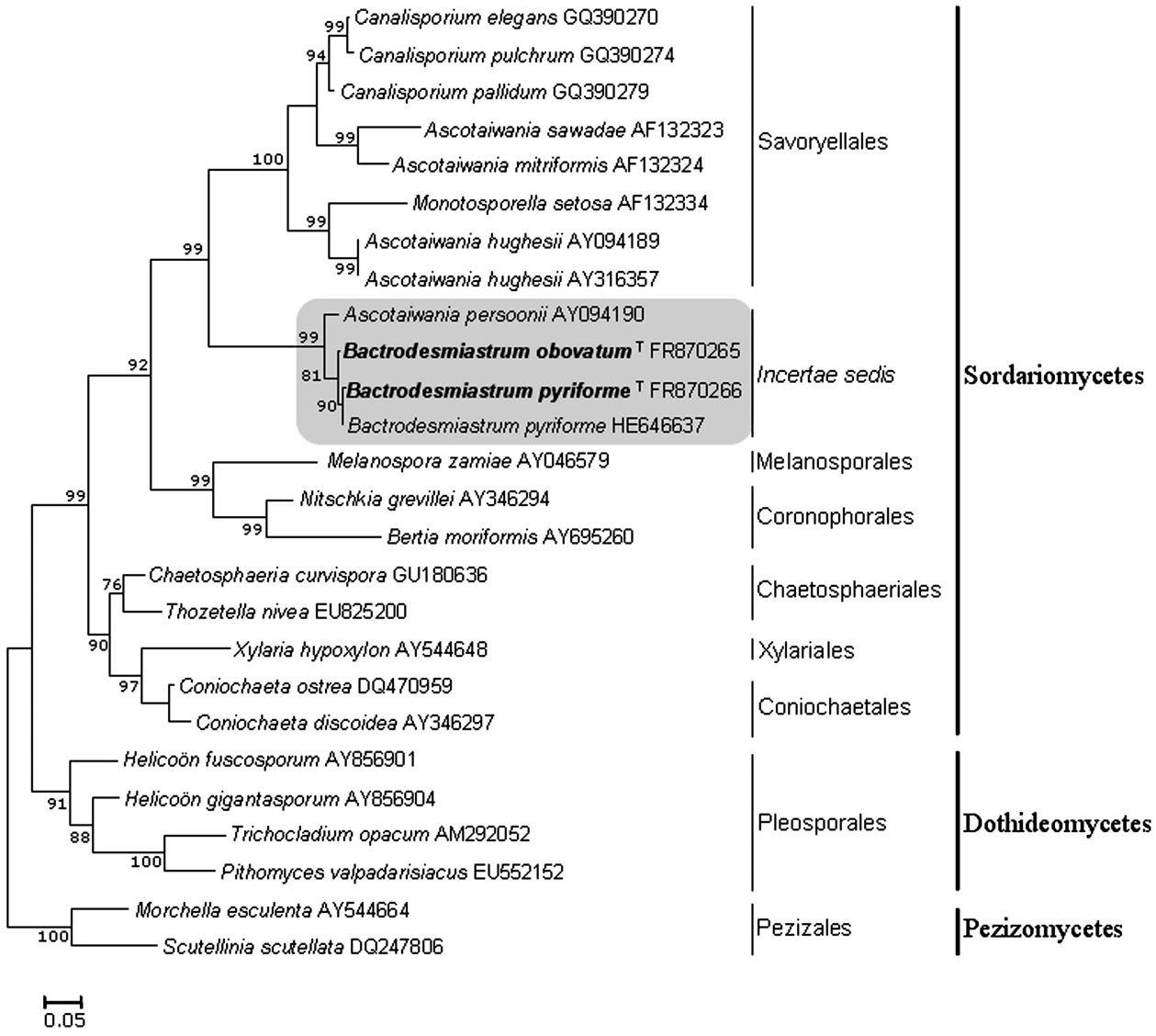

FIG. 2. Maximum likelihood tree constructed with the D1/D2 sequence segments of the 28S rRNA gene. Branch length is proportional to distance. Bootstrap values > 70% are indicated near the internodes. T Type strain.

Culture: Colonies on PCA and OA at 25 C were very similar, growing slowly, attaining 8–10 mm diam in 2 wk, powdery in the center, golden gray, white and glabrous at the periphery, margin regular; reverse black. Sporulation after 4 wk. Conidiogenous cells similar to those observed in vivo, mostly lageniform, 7–12 × 5–7 µm, with truncate apex, 2–2.5 µm wide. Conidia mostly pyriform, 1–4-septate, 20–52 × 11–21 µm.

Specimens examined: SPAIN, ARAGÓN: Huesca, Ordesa y Monte Perdido National Park, Añisclo Canyon, 42°33′29.02″N; 0°02′59.21″E, 1006 m, on deadwood, Jun 2009, *M. Hernández-Restrepo, J Mena-Portales & J. Cano* (HOLOTYPE IMI 398789; ex-type cultures: CBS 127867, MUCL 52960, FMR 10747); CASTILLA-LA MANCHA: Cuenca, protected natural area Monumento Natural del Rio Cuervo, 40°26′04.07″N, 1°57′02.19″W, 1327 m, on deadwood, May 2011, *M. Hernández-Restrepo, J Mena-Portales & J. Guarro* (FMR 11931).

Commentary: The genus *Bactrodesmiastrum* was established by Holubová-Jechová (1984) with *B. obscurum* Hol.-Jech. as the type species. This monotypic genus was characterized by short, aseptate, cylindrical to conical conidiophores arising on repent basal hyphae, and solitary, clavate, obovoid to pyriform, multiseptate conidia originating holoblastically at the apex of conidiophores. *Bactrodesmiastrum pyriforme* can be differentiated from *B. obscurum* by its larger, pyriform conidia (20–50 × 14–28 µm vs. 24–35 × 11–15 µm).

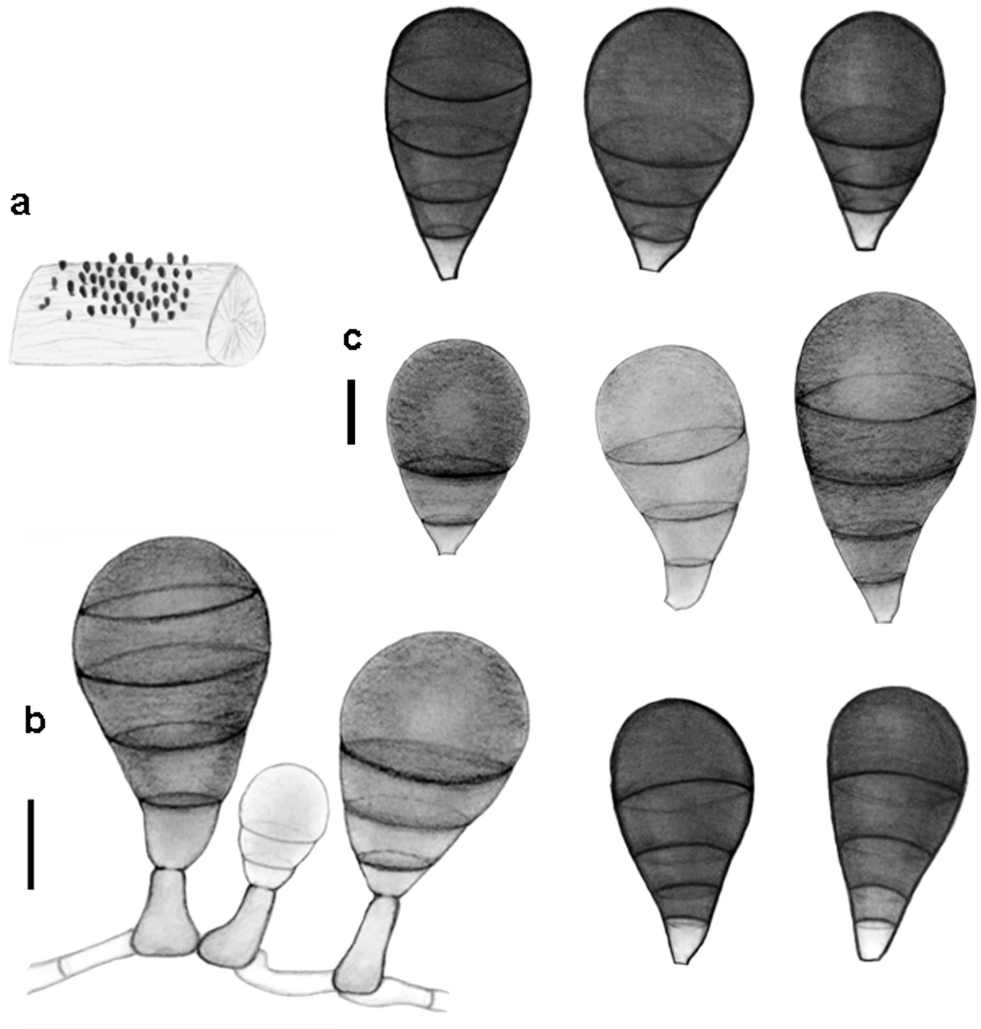

FIG. 3. *Bactrodesmiastrum pyriforme* (IMI 398789). a. Habit. b. Conidiogenous cells producing conidia. c. Pyriform to obovoid conidia. Bars: a–c = 10 μm.

Bactrodesmiastrum obovatum (Calduch, Gené, Abdullah & Guarro) J. Mena, M. Hern.-Rest., Gené & Guarro, comb. nov. FIG. 4d
MycoBank MB518557

Basionym: Janetia obovata Calduch, Gené, Abdullah & Guarro, Mycologia 94:355, 2002.

Specimens examined: SPAIN, BALEARIC ISLANDS: Mallorca, Tramuntana Mountains, Escorca, Gorg Blau Reservoir, 39.88N, 2.78E, on unidentified submerged wood, Mar 1997, *S. K. Abdullah* and *M. Calduch* (ISOTYPE: FMR 6482; ex-type culture: CBS 101300); CATALONIA: Tarragona, Ports de Tortosa Besseit, El Parrisal, 40.78 N, 0.38 E, on unidentified submerged wood, Apr 1999, *M. Calduch* (FMR 7274).

Commentary: On the basis of two specimens growing on litter originating from Spain, Calduch et al. (2002) described *J. obovata*, a fungus morphologically similar to *B. obscurum*. They interpreted the narrow neck of the ampulliform or lageniform conidiogenous cells as denticulate and consequently placed the species in *Janetia* M.B. Ellis. After a reexamination of its herbarium material (FMR 6482, FMR 7274) and the ex-type culture (CBS 101300), we concluded that the conidiogenesis of *J. obovata* is identical to that of *B. obscurum* and the morphological features of *J. obovata* agree more with *Bactrodesmiastrum*. Species of *Janetia* can be differentiated mainly by their polyblastic, denticulate conidiogenous cells (Ellis 1976). *Bactrodesmiastrum obovatum* has monoblastic conidiogenous cells and can be differentiated from the other two species of the genus, *B. obscurum* and *B. pyriforme*, mainly by producing broadly clavate or obovoid conidia up to five septa and paler basal cells.

Bactrodesmiastrum obscurum Hol.-Jech., Folia geobot. phytotax. 19:105 (1984). FIG. 4e
MycoBank MB106624

Colony effuse, blackish brown to black. Conidiogenous cells cylindrical to conical, pale brown to brown, thick-

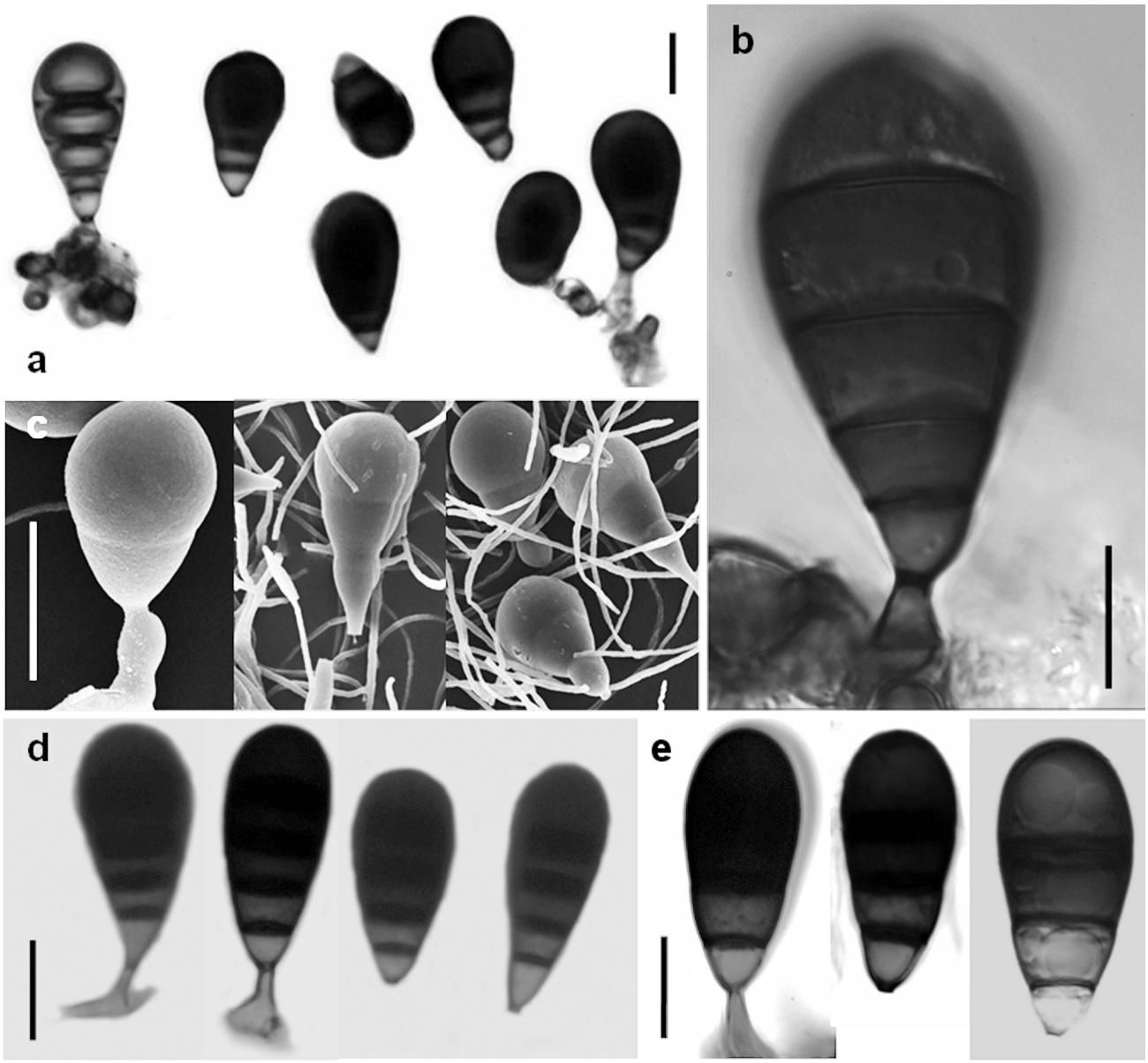

FIG. 4a–e. *Bactrodesmiastrum* spp. a–c. *B. pyriforme* (IMI 398789), conidiogenous cells and 3–4-septate conidia from the natural substratum (a, b) and from culture (c). d. *B. obovatum* (CBS 101300), conidiogenous cells and 2–4-septate conidia from culture. e. *B. obscurum* (FMR 10958), conidiogenous cells and three-septate conidia from the natural substratum. Bars: a, b, d, e = 10 µm; c = 40 µm.

walled, 7.5–10 × 5–7.5 µm, 2–2.5 µm wide at the truncate apex. Conidia acrogenous, clavate or obovoid, three-septate, smooth, brown to dark brown, with two distal darker cells and two proximal paler cells, (21.5–)24–33 × 12–15 µm. Conidial secession schyzolytic.

Specimen examined: SPAIN, ARAGÓN: Huesca, Ordesa y Monte Perdido National Park, Añisclo Canyon, 42°33′29.02″N, 0°02′59.21″E, 1006 m, on deadwood, Jun 2009, *M. Hernández-Restrepo, J Mena-Portales & J. Cano* (FMR 10958).

Commentary: Bactrodesmiastrum obscurum is known only from the type specimen originating from rotten trunk of *F. sylvatica* in Czechoslovakia (Holubová-Jechová 1984). The present specimen seems to be the second known collection of this species worldwide. The characteristics of the Spanish specimen agree in general with the original description, although the conidiogenous cells and conidia are slightly shorter (7–15 × 4–8 µm and 29–35 × 11–14 µm respectively in the type, fide Holubová-Jechová 1984).

Only sequences of *B. obovatum* and *B. pyriforme* were included in our molecular study of *Bactrodes-*

TABLE I. Conidial features to distinguish *Bactrodesmiastrum* species

Species	Shape	Size (μm)	Number of septa
B. obovatum	broadly clavate or obovoid	22.5–33.5 × 12–15	2–4(–5)
B. obscurum	clavate or obovoid	(21.5–)24–35 × 11–15	3
B. pyriforme	pyriform	20–50 × 14–28	(2–)3–4

miastrum species. We could not sequence the type species of the genus because there is no ex-type culture and we were unable to get cultures from the Spanish specimen. In the analysis (FIG. 2), the two *Bactrodesmiastrum* species seem to form a new lineage with *A. persoonii* that falls into a well supported clade (99%) with other *Ascotaiwania* species and some other member of Savoryellales studied by Boonyuen et al. (2011). That phylogenetic study demonstrated that the genus *Ascotaiwania* Sivan. & H.S. Chang was polyphyletic and that *A. lignicola* Sivan. & H.S. Chang, the type species of the genus, *A. hughesii* Fallah, J.L. Crane & Shearer, *A. mitriformis* Ranghoo & K.D. Hyde and *A. sawada* H.S. Chang & S.Y. Hsieh formed a monophyletic clade in Savoryellales. The taxonomic position of *A. persoonii* was uncertain because it was not included in Boonyuen et al. (2011). Although our study reveals a close relationship of *Bactrodesmiastrum* and *A. persoonii* with the Savoryellales, more studies are required to determine the proper taxonomic position of these fungi.

Ascotaiwania is a saprophytic ascomycete observed in both freshwater and terrestrial habitats. Although no anamorph has been described for *A. persoonii*, conidial states are known for other *Ascotaiwania* species (Fallah et al. 1999, Rangoo and Hyde 1998, Sivichai et al. 1998). The anamorph of *A. hughesii*, *Helicoön farinosum* Linder, clearly differs from *Bactrodesmiastrum* by the hyaline helicoidal conidia, while the *Monotosporella* anamorphs of *A. mitriformis* and *A. sawadae* differ from *Bactrodesmiastrum* by the presence of differentiated conidiophores with rhizoid cells that grow out from the conidiogenous cells and elongate downward.

To our knowledge *Bactrodesmiastrum* seems to be an uncommon genus so far known only in Europe. The morphological features that distinguish the three species of *Bactrodesmiastrum* are summarized (TABLE I).

Bactrodesmium diversum J. Mena, Hern.-Rest. M, Gené & Guarro, sp. nov. FIGS. 5, 6
Mycobank MB563321

Etymology: Latin ''*diversum*'', referring to the diverse conidial shape.

Colonies on the natural substratum sporodochial. Mycelium partly immersed in the substratum, composed of smooth, branched, septate, hyaline or subhyaline, thin-walled hyphae 2–3.5 μm wide. Sporodochia scattered, punctiform, black, up to 300 μm

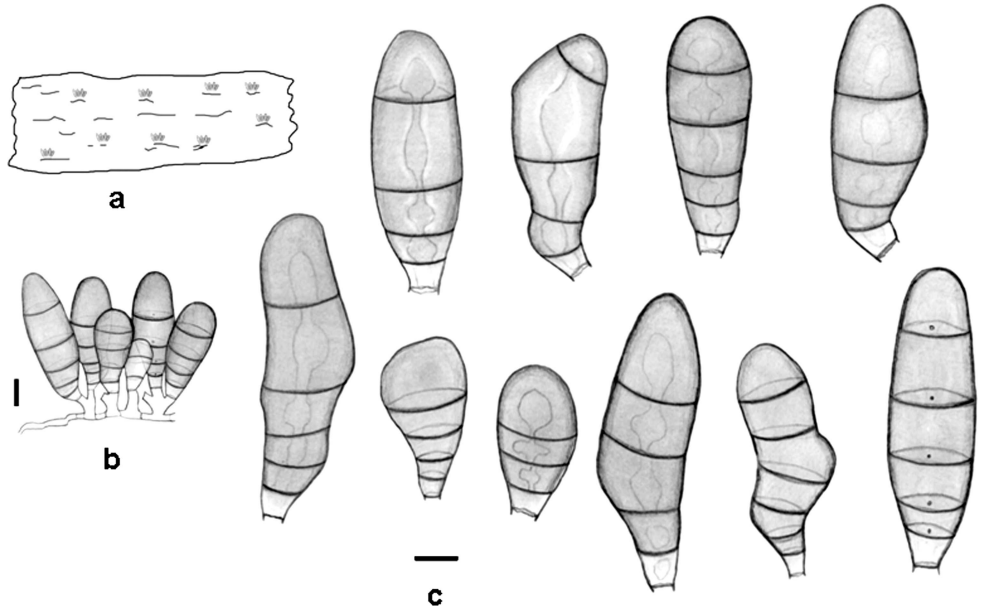

FIG. 5. *Bactrodesmium diversum* (CBS H-20747). a. Habit. b. Sporodochium with mono- and polyblastic conidiogenous cells producing conidia. c. Rhexolytic conidia of diverse shape. Bars: a–c = 10 μm.

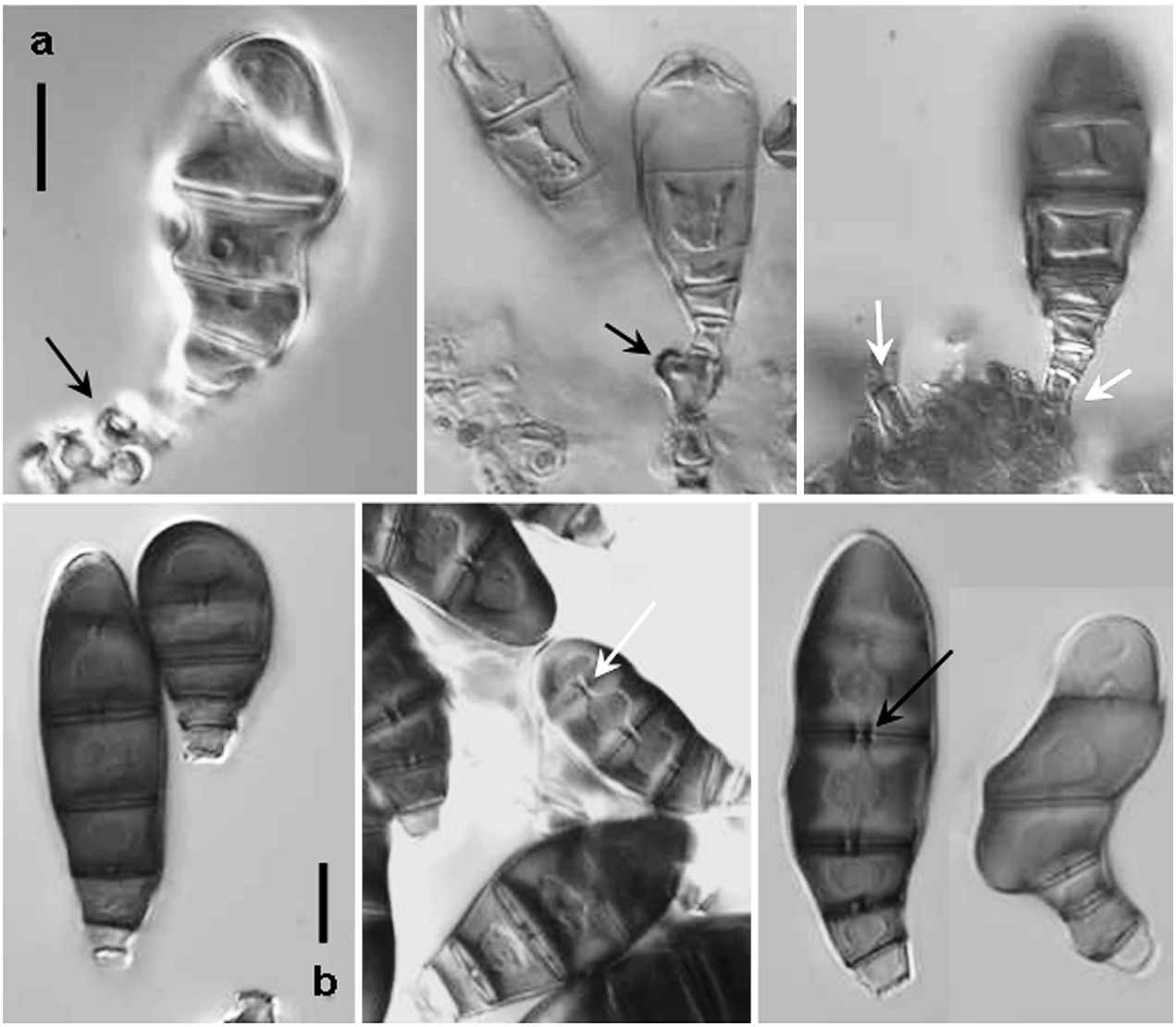

FIG. 6. *Bactrodesmium diversum* (CBS H-20747). a. Poly- and monoblastic conidiogenous cells (arrows) with attached conidium. b. Conidia of diverse shape, with pore at the septa (arrows). Bars: a–b = 10 µm.

diam. Conidiophores micronematous to semi-macronematous, simple, composed of a single conidiogenous cell. Conidiogenous cells commonly polyblastic, cylindrical, smooth, hyaline to pale brown, 6–10 × 4–5 µm. Conidia solitary, dry, acrogenous, straight or curved, mostly ellipsoidal or clavate, occasionally obovoid, pyriform or sigmoid, 2–5-septate, with a conspicuous central pore at each septum, smooth, pale brown to brown, basal cell paler, 18–64 × 10–18 µm. Conidial secession rhexolytic. Teleomorph unknown.

Specimens examined: SPAIN, ARAGON: Huesca, Ordesa y Monte Perdido National Park, Bujaruelo Valley, 2°39′58.02″N, 0°07′10.93″W, Jun 2009, on deadwood, *M. Hernández-Restrepo M., J. Mena-Portales & J. Cano* (HOLOTYPE CBS H-20747, ISOTYPE FMR 11853); Pineta Valley 42°38′53.79″N, 0°09′05.70″E, Jun 2009, on deadwood, *M. Hernández-Restrepo, J. Mena-Portales & J Cano* (FMR 11852); Teruel, Nogueruelas 40°16′11.74″N, 0°36′44.53″W, Oct 2009, on deadwood, *M. Hernández-Restrepo* (FMR 11854); VALENCIAN COMMUNITY: Loriguilla, 39°37′49″N, 1°0′19″W, on deadwood, Mar. 2010, *M. Hernández-Restrepo & K. Rodríguez* (FMR 10964); Los Herreros, 39°18′26″N, 0°54′52″W, Mar. 2010, on deadwood, *M. Hernández-Restrepo & K. Rodríguez* (FMR 11856).

Commentary: Bactrodesmium was segregated from *Sporidesmium* Link ex. Fr., with *B. abruptum* (Berk. & Broome) E.W. Mason & S. Hughes as lectotype, and currently the genus encompasses 42 species, most growing on rotten wood of various plants.

In addition to the presence of sporodochia, most species of *Bactrodesmium* have monoblastic conidiogenous cells that produce brown to dark brown conidia

of various shapes (from ellipsoidal to cylindrical) with numerous transverse septa, sometimes with a dark brown to black band at one or more septa (Ellis 1959, 1971, 1976; Holubová-Jechová 1972). Only a few species have polyblastic conidiogenous cells that produce pale brown conidia without bands, as in *B. diversum*. These species are *B. biformatum* S. Hughes (Hughes 1983, 1989), *B. pithoideum* (Dearn. & House) B. Sutton (Sutton 1975), *B. pluriseptatum* Révay (Révay 1993) and *B. spilomeum* (Berk. & Broome) E.W. Mason & S. Hughes (Ellis 1959, Holubová-Jechová 1972). However, the species mainly differs from *B. diversum* by the number of septa and/or by the smaller conidial size (*B. biformatum*: 4–9-septate, 18–42 × 7–10 µm; *B. pithoideum*: 4–6-septate, 17–34 × 9–14.5 µm; *B. pluriseptatum*: 6–9-septate, 30–44 × 11–14 µm; *B. spilomeum*: 2–6-septate, 22–45 × 6–14 µm).

Based on known data (Funk and Shoemaker 1983, Raja et al. 2008, Koukol and Kolářová 2010) *Bactrodesmium* appeared polyphyletic. Funk and Shoemaker (1983) considered *B. obliquum* Sutton var. *suttonii* Hughes & White as the anamorph of *Stuartella suttonii* A. Funk & Shoemaker, an incertae sedis genus within the Dothidiomycetes. More recently, based on LSU and ITS sequences, Koukol and Kolářová (2010) demonstrated that *B. gabretae* Koukol & Kolářová is closely related to *Aquapoterium pinicola* Raja & Shearer, a freshwater member of the Helotiales (Leotiomycetes). In our case, no anamorph-teleomorph connection was observed on the natural substratum and molecular studies were not carried out because we failed to isolate *B. diversum* in pure culture. Therefore, the taxonomic position of this species is undetermined.

New molecular studies are required to assess the boundaries of the *Bactrodesmium* species and their phylogenetic relationships. However, the difficulty in growing species of this genus in culture and the limited availability of specimens present a challenge to molecular studies of *Bactrodesmium*.

ACKNOWLEDGMENTS

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