

Mycological Progress

Emendation of the genus *Bactrodesmiastrum* (Sordariomycetes) and description of *Bactrodesmiastrum monilioides* sp. nov. from plant debris in Spain --Manuscript Draft--

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Full Title:	Emendation of the genus <i>Bactrodesmiastrum</i> (Sordariomycetes) and description of <i>Bactrodesmiastrum monilioides</i> sp. nov. from plant debris in Spain	
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Abstract:	<p><i>Bactrodesmiastrum monilioides</i> sp. nov. is described and illustrated from a twig of an unidentified plant collected in Spain. This fungus is characterized by sporodochial conidiomata with moniliform conidiophores, its conidia being solitary, broadly navicular to obovoid, mostly 2-septate, dark brown, with a black band at the septa and basal cell pale brown to brown. Analysis of the ITS region and D1/D2 domains of the LSU of rDNA sequences confirms the placement of this fungus in <i>Bactrodesmiastrum</i> (Sordariomycetes). Because the genus <i>Bactrodesmiastrum</i> was originally characterized by solitary or aggregated conidiophores reduced to a brown, single, monoblastic conidiogenous cell, an emended description of the genus is given to accommodate the novel species. A key to species of <i>Bactrodesmiastrum</i> is provided.</p>	

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2 description of *Bactrodesmiastrum monilioides* sp. nov. from plant debris in
3 Spain

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5 Running title: *Bactrodesmiastrum*: generic emendation and a new species

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13 **Abstract.** *Bactrodesmiastrum monilioides* sp. nov. is described and illustrated
14 from a twig of an unidentified plant collected in Spain. This fungus is
15 characterized by sporodochial conidiomata with moniliform conidiophores, its
16 conidia being solitary, broadly navicular to obovoid, mostly 2-septate, dark
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21 or aggregated conidiophores reduced to a brown, single, monoblastic
22 conidiogenous cell, an emended description of the genus is given to
23 accommodate the novel species. A key to species of *Bactrodesmiastrum* is
24 provided.

25 **Taxonomical novelties.** *Bactrodesmiastrum monilioides* Hern.-Rest., Gené &
26 R.F. Castañeda sp. nov.

27 **Key Words:** Anamorphic fungi, Ascomycota, taxonomy.

28

29 INTRODUCTION

30 During a survey of anamorphic fungi from plant debris, collected in two natural
31 areas in the Eurosiberian region of the north of Spain, two strains of an
32 interesting fungus were collected and isolated in pure culture. The fungus
33 showed some similarity with members of the genus *Bactrosdesmiastrum* Hol.-
34 Jech., but differed mainly by the production of rather long moniliform
35 conidiophores in sporodochial conidiomata. *Bactrosdesmiastrum* was described
36 on the basis of *B. obscurum* Hol.-Jech., the type species, and characterized by
37 solitary or aggregate conidiophores reduced to dark brown conidiogenous cells,
38 which could be conical-cylindrical, lageniform or subulate, and by the production
39 of dark brown, smooth-walled, phragmoconidia (Holubová-Jechová, 1984).
40 Currently, the genus includes two other saprophytic species, viz. *B. obovatum*
41 (M. Calduch et al.) J. Mena et al. and *B. pyriforme* Hern.-Rest. et al.
42 (Hernández-Restrepo et al., 2013). However, none of these fungi showed the
43 type of conidiogenous apparatus observed in the Spanish specimens.

44 Sequence analyses of the internal transcribed spacers (ITS) and the
45 D1/D2 region of the large subunit (LSU) of the rDNA of the two strains
46 confirmed a close phylogenetic relationship with *Bactrosdesmiastrum* species.
47 Therefore, the combination of morphological features supported by molecular
48 evidences allows us to describe a new species of *Bactrosdesmiastrum*. An
49 emended description of the genus is given to accommodate the novel fungus,
50 and a key to the species concerned is also provided.

51 MATERIALS AND METHODS

52 Isolation and identification of fungi

53 Samples of decomposing wood, leaves and bark were collected from the forest
54 floor at the Ordesa y Monte Perdido National Park and at the Picos de Europa
55 National Park, two locations in northern Spain. Samples were treated according
56 to Castañeda-Ruiz (2005) and Hernández-Restrepo et al. (2013). To achieve
57 pure cultures, conidia from the specimens growing on the natural substratum
58 were transferred to potato carrot agar (PCA; 20 g potatoes, 20 g carrots, 20 g
59 agar, 1 L distilled water) and oat-meal agar (OA; 30 g oat flakes, 20 g agar, 1 L
60 distilled water), and incubated at 25 °C in the dark. In the description, colour
61 notations in parentheses are based on Kornerup & Wanscher (1984).

62 Microscopic features were examined in lactic acid wet mounts using an
63 Olympus CH-2 light microscope (Tokyo, Japan). Photomicrographs were
64 obtained with a Zeiss AXIO Imager M1 light microscope (Göttingen, Germany)
65 and a Jeol JSM-6400 scanning electron microscope (Tokyo, Japan).

66 Representative specimens of the fungi examined were deposited in the
67 CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands (CBS), in the
68 Faculty of Medicine, Reus, Spain (FMR), and in the Martin-Luther-University,
69 Halle, Germany (HAL).

70 **DNA extraction, sequencing and phylogenetic analysis**

71 DNA was extracted from pure cultures using the PrepMan Ultra sample
72 preparation reagent (Applied Biosystems, Foster City, CA, USA), according to
73 the manufacturer's protocol. The ITS region and D1/D2 domain of the LSU of
74 the rDNA were amplified with the primer pairs ITS5/ITS4 and NL1/NL4,
75 respectively, following the protocols described by Cano et al. (2004) and
76 Gilgado et al. (2005). PCR products were purified and sequenced at MacroGen
77 Corp. Europe (Amsterdam Zuid-oost, the Netherlands). The program SeqMan

78 7.0 was used to obtain consensus sequences. BLAST sequence identity
79 searches (Altschul et al. 1990) were carried out to compare the investigated
80 strains (CBS 137251 and CBS 137252) with other fungi deposited in GenBank.

81 A phylogenetic analysis using the D1/D2 sequences was performed to
82 assess the relationships of our strains with *Bactrodesmiastrum* species and
83 other members of the Sordariomycetes, and species with some morphological
84 similarity such as those of the genus *Polyschema* Upadhyay in the
85 Dothideomycetes. Sequences used in the analysis were retrieved from
86 GenBank from previous studies (Shenoy et al. 2010, Boonyuen et al. 2011,
87 Réblová & Seifert 2011, Hernández-Restrepo et al. 2013). Sequences of
88 *Morchella esculenta* and *Scutellinia scutellata* (Pezizomycetes) were used as
89 outgroups.

90 The alignments of the sequences were performed first in MEGA5
91 (Tamura et al. 2011) using its Clustal W application, followed by manual
92 adjustments with a text editor. The phylogenetic study was carried out with
93 maximum likelihood analysis (ML), using the software MEGA5. Tamura-Nei with
94 Gamma distribution was the best-fit nucleotide substitution model and we used
95 Nearest-Neighbor-Interchange (NNI) as heuristic model. Confident branch
96 support was defined as bootstrap values (bs) $\geq 70\%$ from a maximum likelihood
97 search with 1000 replicates.

98 Sequences were deposited in GenBank with the following accession
99 numbers: KF771878 (ITS) and KF771879 (D1/D2) for CBS 137251, and
100 KF771876 (ITS) and KF771877 (D1/D2) for CBS 137252. The alignment was
101 deposited in TreeBASE (submission number S16337).

102 RESULTS

103 The BLASTN search using the D1/D2 sequences of the strains CBS 137251
104 and CBS 137252 showed a similarity of 98–99% with *B. pyriforme* (FR870265
105 and HE646637) and *B. obovatum* (FR870266). The ITS sequences of the
106 strains CBS 137251 and CBS 137252 revealed that the closest hits were
107 FR870263 and HE646636 corresponding to *B. pyriforme* with a similarity
108 between 86–87%, and FR870264 corresponding to *B. obovatum* with a
109 similarity of 87%. While the D1/D2 sequences were not useful for separating the
110 investigated strains from the other two *Bactrodesmiastrum* species, the ITS
111 sequences revealed that they can be considered to represent a different
112 species.

113 The D1/D2 domains alignment contained 40 sequences including the two
114 outgroups, and 597 characters were used in the phylogenetic analysis. The
115 groups obtained in the ML tree agreed with several lineages revealed in
116 previous phylogenetic assessments, including 10 orders (i.e.
117 Chaetosphaeriales, Coniochaetales, Coronophorales, Hypocreales,
118 Melanosporales, Microascales, Savoryellales, Sordariales, and Xylariales in
119 Sordariomycetes and Pleosporales) and a family (Annulatasceae). The
120 resulting phylogenetic tree (Fig.1) placed the two investigated isolates in an
121 *incertae sedis* clade (100% bs) of Sordariomycetes, exclusively formed by
122 *Bactrodesmiastrum* species. This appeared as the sister clade of Savoryellales,
123 but both clades appeared separated by considerable branch lengths.

124 TAXONOMY

125 ***Bactrodesmiastrum*** Hol.-Jech., Folia Geobot. Phytotax. 19: 103, 1984.

126 Type species: *Bactrodesmiastrum obscurum* Hol.-Jech., Folia Geobot.

127 Phytotax. 19: 105, 1984.

128 Emended description: *Colonies* effuse, blackish brown to black. *Mycelium* partly
129 immersed in the substratum, composed of branched, septate, pale brown,
130 smooth hyphae. *Conidiophores* semi-macronematous or macronematous,
131 mononematous, simple, usually being reduced to conidiogenous cells arranged
132 singly or aggregated in small groups, or conidiophores arising from pulvinate to
133 subpustulate, scattered and black sporodochial conidiomata; conidiophores
134 from sporodochia simple or branched, mostly moniliform, composed of globose,
135 subglobose or clavate cells centrally or eccentrically connected, pale brown to
136 brown, smooth-walled. *Conidiogenous cells* monoblastic, determinate,
137 integrated, terminal, usually cylindrical to conical with truncate apex and swollen
138 base, mid brown to dark brown, smooth-walled; conidiogenous cells from
139 sporodochial conidiophores globose, subglobose or clavate, pale brown to
140 brown. *Conidial secession* schizolytic. *Conidia* solitary, dry, acrogenous,
141 clavate, obovoid, pyriform or navicular, 2–5-septate, obconico-truncate at the
142 base, brown, with paler proximal cells and darker distal cells, often with black
143 bands around the septa, smooth-walled. *Sexual morph* unknown.

144 ***Bactrodesmiastrum monilioides*** Hern.-Rest., Gené & R. F. Castañeda, sp.
145 nov. (Figs. 2–3)

146 MycoBank: MB 805963

147 Etymology: Latin, *monilioides*- referring to the catenulate swollen cells,
148 constricted at the septa, forming the conidiophores.

149 *Colonies* on the natural substratum effuse, dark brown or black.

150 *Mycelium* superficial and immersed, composed of septate, cylindrical to
151 torulose, branched, pale brown, smooth-walled, 3–5 µm wide hyphae.

152 *Conidiomata* sporodochial, pulvinate to subpustulate, scattered, superficial,
153 black, 85–190 µm diam. *Conidiophores* macronematous, mononematous,
154 compact or loosely fasciculate, erect or prostrate, simple or branched, mostly
155 moniliform, with globose, subglobose, ellipsoidal or clavate cells, 4–6 µm wide,
156 centrically or eccentrically connected, branches sometimes arise from basal
157 racket cells, pale brown to brown, smooth-walled, 15–75 × 3–6 µm;
158 conidiophores reduced to a single conidiogenous cell occasionally present.
159 *Conidiogenous cells* monoblastic, integrated, mostly terminal, globose,
160 subglobose, ellipsoidal or clavate, occasionally subulate or lageniform, pale
161 brown to brown, smooth-walled, 5–8 × 4–6 µm. *Conidia* broadly navicular to
162 obovoid, mostly 2-septate, apical cell bigger than the others, basal cell broadly
163 obconico-subtruncate and pale brown, distal cells dark brown, with a black band
164 around the septa, smooth-walled, 24–35 × 15–18 µm, basal scar 1–1.5 µm
165 wide.

166 *Culture:* Colonies on OA and PCA at 25 °C attaining 24–25 mm after 14
167 days, granulose with black spots at the centre, glabrous or slightly cottony and
168 white towards the periphery; reverse dark blond (5E4). Sporulation after 7 days.
169 Sporodochia similar to those observed on the natural substratum, but producing
170 smaller conidia, 25–26 × 10–12.5 µm.

171 *Holotype:* **SPAIN. Aragón**, Ordesa y Monte Perdido National Park, near
172 Añisclo Canyon , 42°35'17,57"N; 0°01'37,39"O, 1316 masl, on a decaying twig
173 of an unidentified plant, 19 June 2009, *M. Hernández-Restrepo, Julio Mena-*
174 *Portales* and *Josep Cano*. HAL 2613 F (Holotype), FMR H-10756 (Isotype).
175 *Cultures ex-type:* CBS 137251, FMR 10756.

176 Additional specimen examined: **SPAIN. Asturias**, Picos de Europa
177 National Park, Poncebos Municipality, Cares river, AS-264 Road,
178 43°16'34,10"N; 4°50'2,56"O; 199 masl, on a decaying twig of an unidentified
179 plant, 14 July 2010, *M. Hernández-Restrepo, Julio Mena-Portales and Josep*
180 *Guarro* (CBS 137252, FMR 11337).

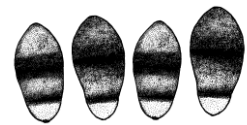
181 **DISCUSSION**

182 The novel species *B. monilioides* is easily distinguishable from all other
183 *Bactrodesmiastrum* species by its moniliform conidiophores arranged in
184 sporodochia, and by its broadly navicular, 2-septate conidia. Diagnostic features
185 to distinguish the currently accepted species of *Bactrodesmiastrum* are keyed
186 out below. The morphology observed in *B. monilioides* can cause some
187 confusion with the identification of this fungus since other genera such as
188 *Polyschema* or *Bactrodesmium* Cooke show similar conidiogenous structures.
189 However, the members of *Polyschema* can be differentiated by their monotretic
190 or polytretic conidiogenous cells (Ellis 1976), and the type species of the genus,
191 *P. terricola* Upadhyay, as well as two other species of *Polyschema*, *P.*
192 *congolensis* Reising & Kiffer and *P. larviformis* (Fairman) M.B. Ellis, have been
193 proved to belong to the order Pleosporales (Dothideomycetes) (Shenoy et al.,
194 2010). By contrast, *Bactrodesmiastrum* has monoblastic conidiogenous cells
195 and phylogenetically its species show an unresolved position within the
196 subclass Hypocreomycetidae (Sordariomycetes), appearing as a sister clade of
197 the Savoryellales (Fig. 1) as previously published (Hernández-Restrepo et al.
198 2013). Most species of *Bactrodesmium* also show monoblastic conidiogenous
199 cells, although none of them have the type of conidiogenous apparatus
200 observed in the novel species and the conidia of *Bactrodesmium* have

201 rhexolytic secession and usually a wider basal scar (3–5 μm wide versus 1–1.5
 202 μm wide in *B. monilioides*). It is noteworthy that the phylogeny of
 203 *Bactrodesmium* remains uncertain; the type species, *B. abruptum* (Berk. &
 204 Broome) E.W. Mason & S. Hughes, has never been sequenced and the few
 205 molecular studies on some *Bactrodesmium* species have suggested that it is a
 206 polyphyletic genus (Hernández-Restrepo et al. 2013). Based on our data, the
 207 D1/D2 sequence of a Spanish specimen morphologically identified as
 208 *Bactrodesmium pallidum* M.B. Ellis fits into the Savoryellales (Hernández-
 209 Restrepo 2013), while *B. abruptum*, according to MycoBank
 210 (www.mycobank.org), is a dothideomycetous fungus.

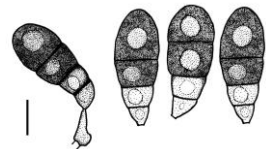
211 **Key to the species of *Bactrodesmiastrum***

212 1. Sporodochial conidiomata present; conidiophores mostly moniliform; conidia
 213 mostly 2-septate, broadly navicular to obovoid, 24–35 \times
 214 15–18 μm , dark brown, with a black band around the
 215 septa and a pale brown basal cell ***B. monilioides***



216 1'. Sporodochial conidiomata absent; conidiophores otherwise, usually reduced
 217 to a single conidiogenous cell 2

218 2. Conidia mostly 3-septate, clavate, obovoid to pyriform,
 219 29–35 \times 11–14 μm , brown, with two darker distal cells
 220 and two paler proximal cells ***B. obscurum***

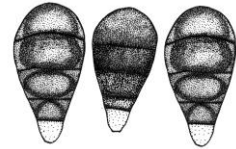


221 2'. Conidia mostly 4-septate 3

222 3. Conidia broadly clavate or obovate, 22.5–33.5 \times 12–15
 223 μm , blackish brown at the apex and progressively paler
 224 towards the base ***B. obovatum***



225 3'. Conidia pyriform or obovoid, 20–50 × 14–28 µm, dark
 226 brown to black, with 1–2 paler proximal cells



227 ***B. pyriforme***

228 (Bars = 10 µm)

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 231 Competitividad, Grant CGL 2011-27185. We are grateful to the Cuban Ministry
 232 of Agriculture for facilities to RFCR.

233 CONFLICT OF INTEREST

234 The authors declare that they have no conflict of interest.

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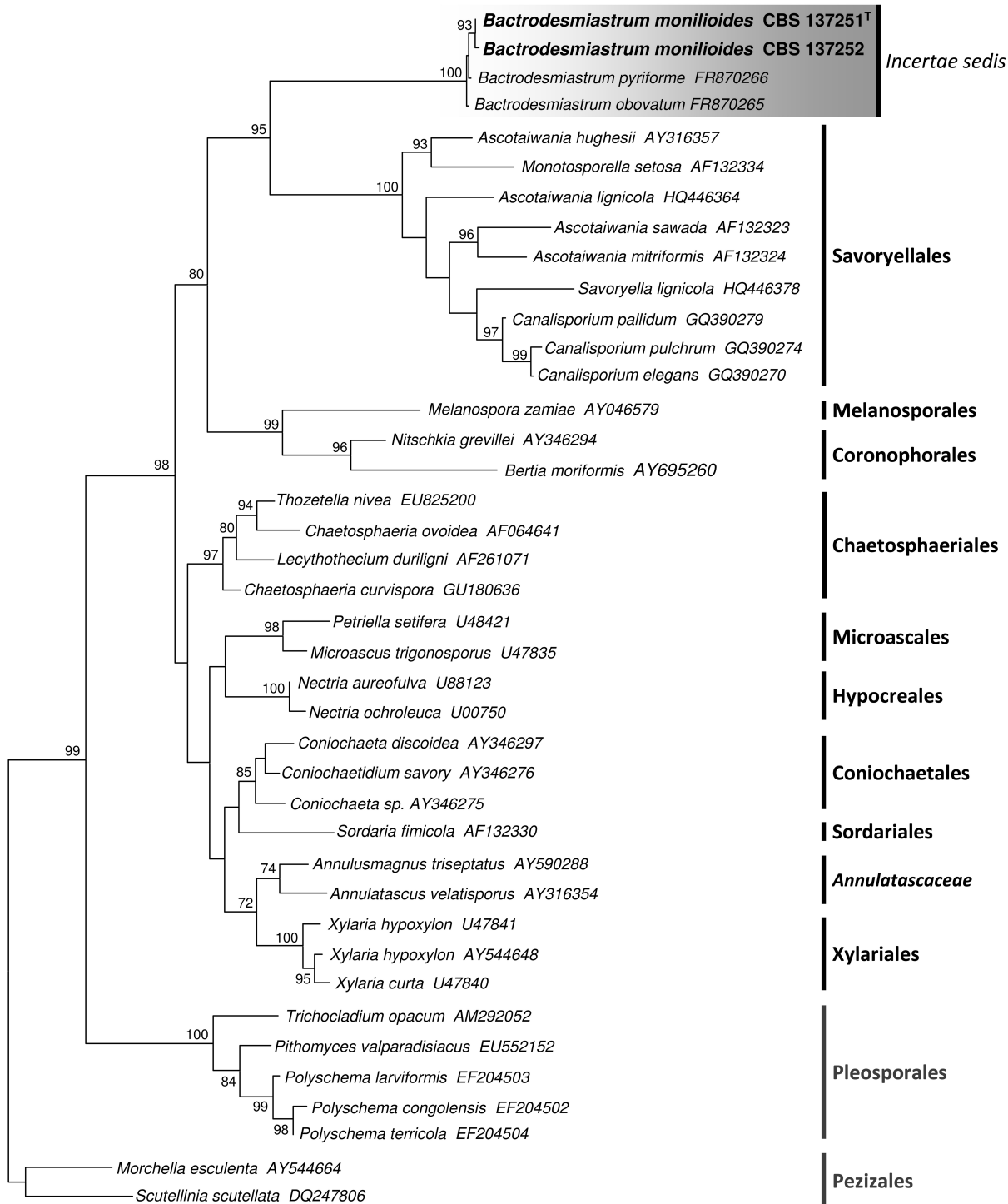
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- 273
- 274

275 Fig. 1. Maximum likelihood tree constructed with the D1/D2 sequences of the
276 LSU rDNA. Branch length is proportional to distance. Bootstrap values >
277 70% are indicated near the internodes. Orders or families of the
278 Sordariomycetes in bold. ^T, type strain.

279 Fig. 2. *Bactrodesmiastrum monilioides* (CBS 137251). a–c. Conidia,
280 sporodochium and moniliform conidiophores from the natural substratum.
281 Bars a = 10 µm; b, c = 20 µm.

282 Fig. 3. *Bactrodesmiastrum monilioides* (CBS 137251). a–d. Conidiogenous
283 apparatus from the natural substratum. e–g. Conidiogenous apparatus
284 from culture. a, e. Sporodochia. b, f, g. Moniliform conidiophores with
285 conidia. c. Detail of a conidiophore showing basal racket cells. d. Conidia.
286 Bars a = 20 µm; b–d, f, g = 10 µm; e = 25 µm.

Figure 1



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Figure 2

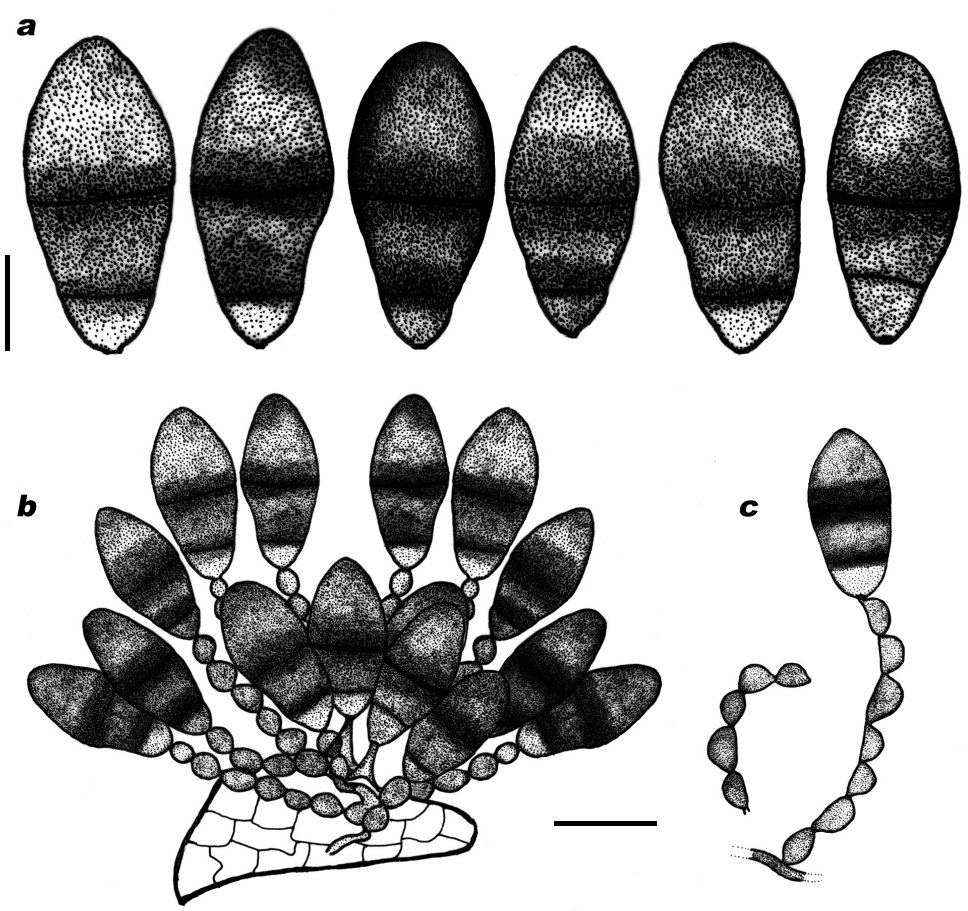
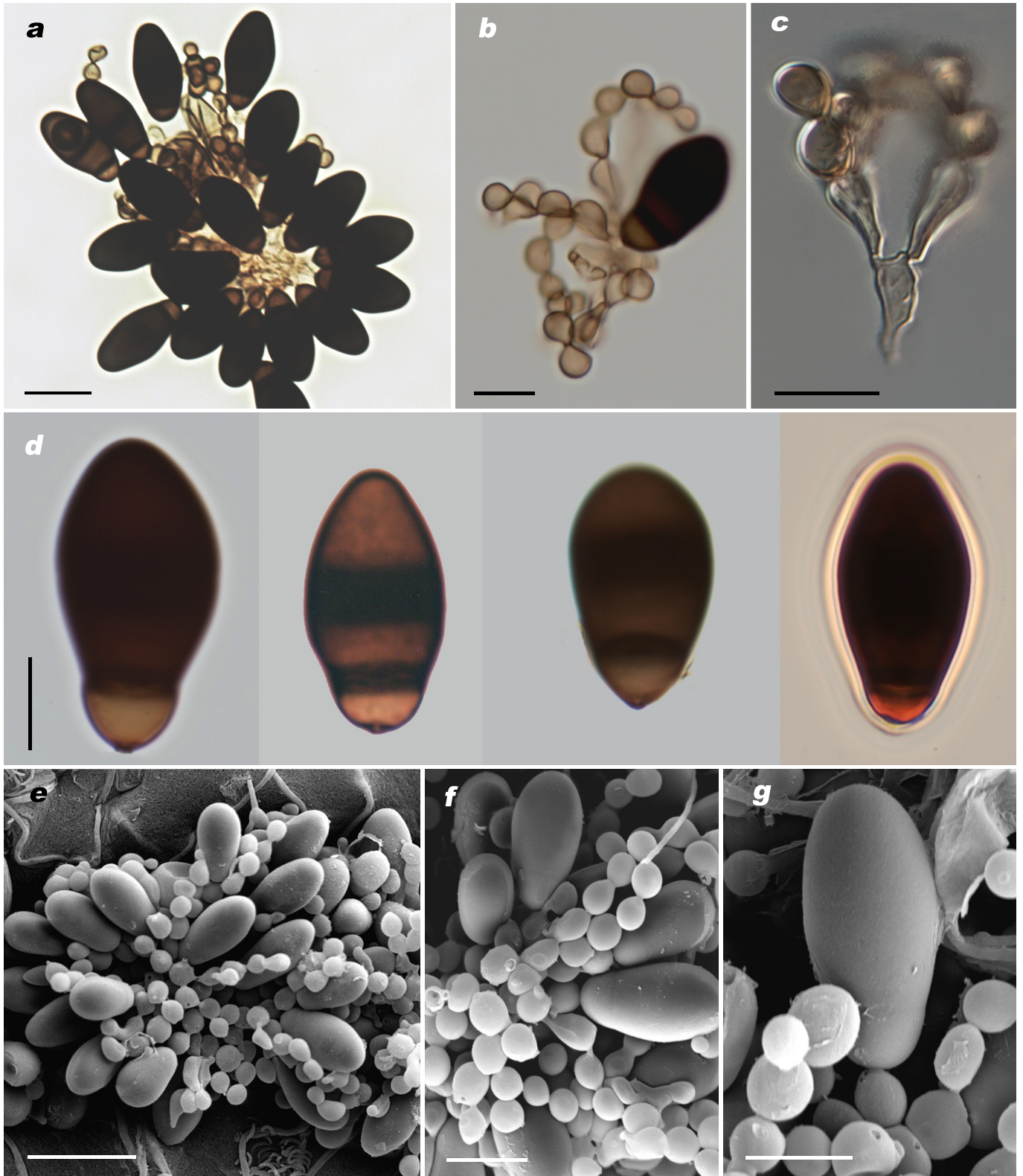
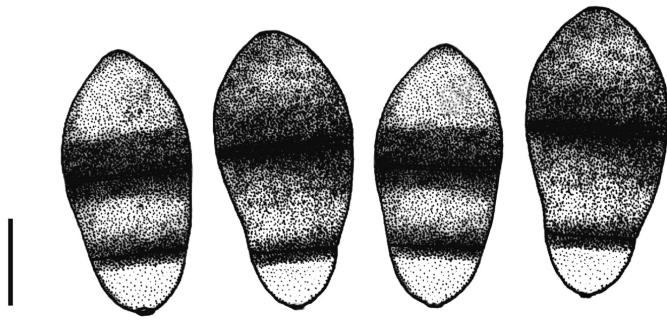
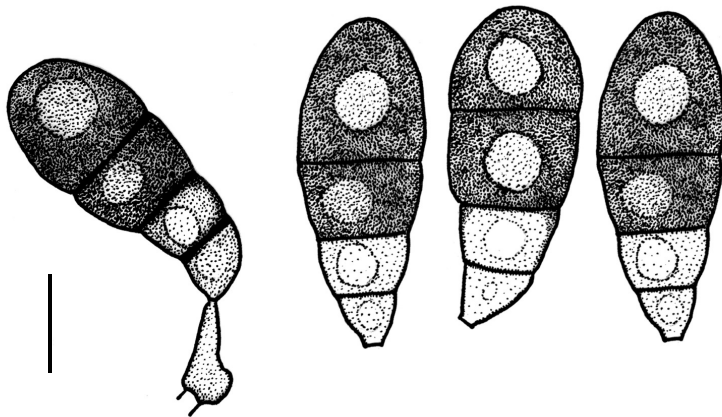


Figure 3

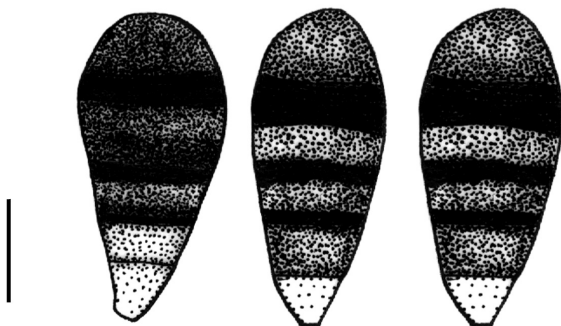




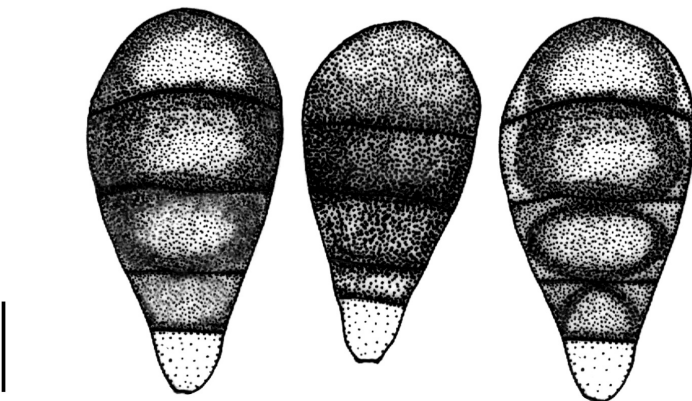
B monilioides



B obscurum



B obovatum



B pyriforme