Bicornispora exophiala, a new genus and species of the Coryneliales and its black yeast anamorph

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Bicornispora, gen. nov. based on *B. exophiala* sp. nov., is described and tentatively included in the Coryneliales (Ascomycotina). The most noteworthy features of this taxon are its bicornute ascospores, massive unitunicate asci, and non-ostiolate ascomata. An *Exophiala* anamorph obtained from monoascosporic cultures provides evidence for the occurrence of black-yeast anamorphs in ascomycetes outside of the loculoascomycetes. Relationships of the new genus to other members of Coryneliales and to some members of the bitunicate ascomycetes are discussed.

In the course of a survey of fungi in the mountains of Central Spain, a member of an undescribed genus was found on dead branches of shrubs, near melting snow banks. This fungus is placed in the order Coryneliales based on its non-ostiolate ascomata, aparaphysate centrum, and deliquescent and unitunicate asci. The proposed genus *Bicornispora* somewhat resembles *Coryneliopsis* Butin in its unitunicate asci and non-ostiolate ascomata. However, these genera differ in ascospore morphology, anamorphs, and ecology.

Ascomycete systematics have been mainly based in the correlation of the species with unitunicate asci and ascohymenial development, and butinicate asci with ascolocular development (Luttrell, 1951; Ainsworth, 1973; Wehmeyer, 1975; Barr, 1983). Members of Coryneliales have been reported traditionally to be incongruous in possessing both unitunicate asci and ascolocular development (McCormack, 1936; Funk, 1963). Our new taxon is tentatively placed in the order with uncertain affinity to the Coryneliaceae, but its *Exophiala* J. W. Carmich. anamorph seems to implicate a relationship also with the bitunicate ascomycetes.

In the present paper, the morphological, ecological and geographical features of *Bicornispora* are evaluated and compared with other members of the Coryneliaceae. Implications relative to the taxonomic position of the new genus within Coryneliales and relations with some other members of the bitunicate ascomycetes are also considered.

MATERIALS AND METHODS

Ascoma, ascus and ascospore characteristics were described from a collection containing nearly one hundred specimens fruiting on the natural substrate.

Pure cultures were obtained from single or mass ascospores plated on PGA (20% potato, 2% glucose, 1·5% agar), MEA (2% malt extract, 2% agar) and OGYE (glucose–yeast extract–agar, with 50 ppm of oxytetracycline hydrochloride). Cultures were incubated at room temperature in natural daylight.

Light microscopy (LM) was carried out with an Olympus BX50 using bright field and phase contrast. Ascomata were rehydrated in 10% KOH.

Scanning electron microscopy (SEM) was performed on fixed (3% glutaraldehyde) and dehydrated (critical point dried) samples coated with gold–palladium under a Zeiss DMS 950 at 25 kV.

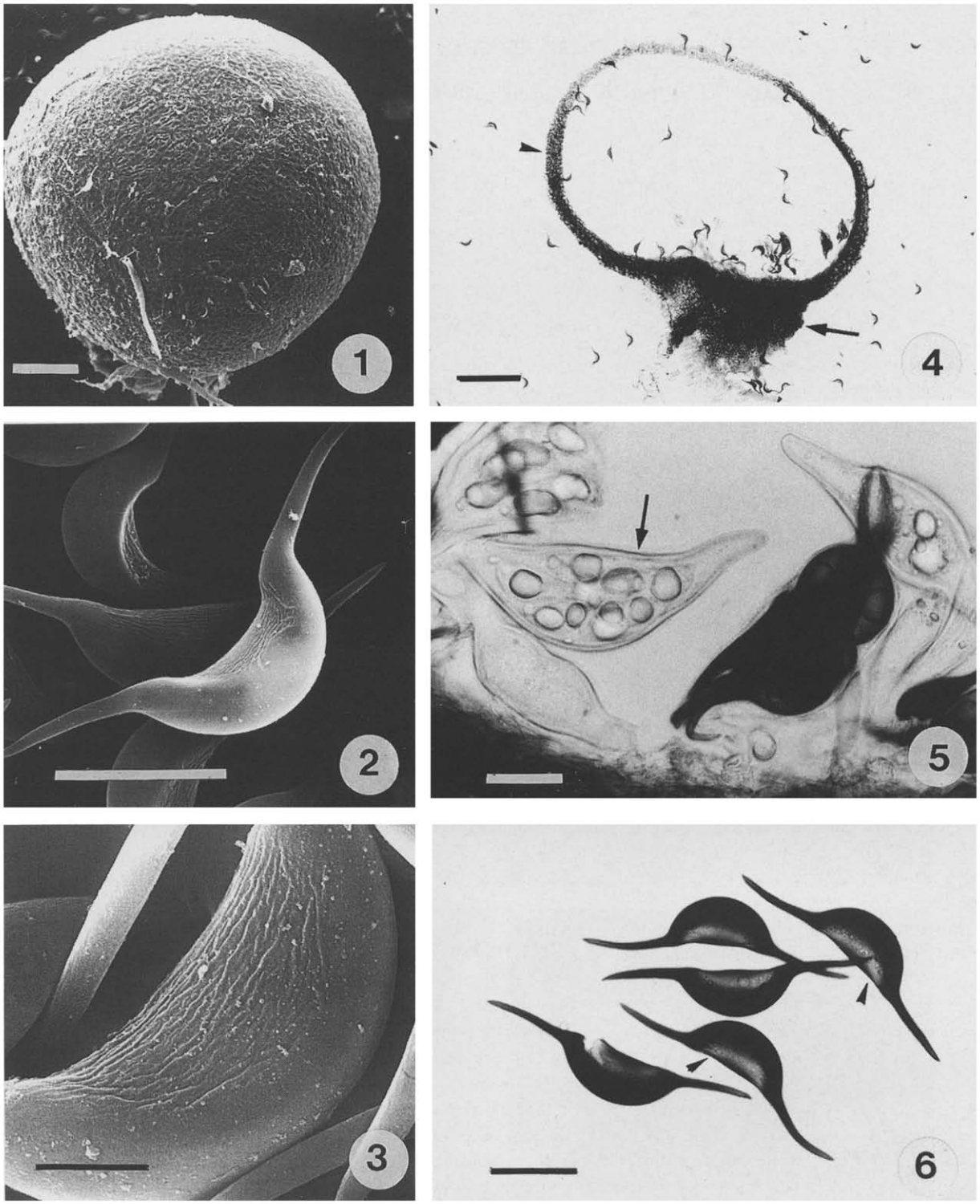
Material is deposited in the Herbaria of Real Jardín Botánico de Madrid, Spain (MA-Fungi) and in the Departamento de Botánica, Universidad de Alcalá de Henares, Spain (AH). A living culture of the *Exophiala* anamorphic state is deposited in the culture collection of the Instituto de Microbiología Jaime Ferrán, Centro de Investigaciones Biológicas, C.S.I.C., Madrid, Spain (IJFM).

DESCRIPTIONS

Bicornispora Checa, Barrasa, M. N. Blanco & A. T. Martínez gen. nov.

Etym.: From the Latin *bicornis*, *-e* (two-horned) and *spora*, *-ae* (spore), referring to the horn-like expansions of the spore.

Ascocarpi sparsi, globosi, glabri, saturate brunnei, non ostiolati. Paraphyses nullae. Asci ovales vel ellipsoidales, evanescentes, non

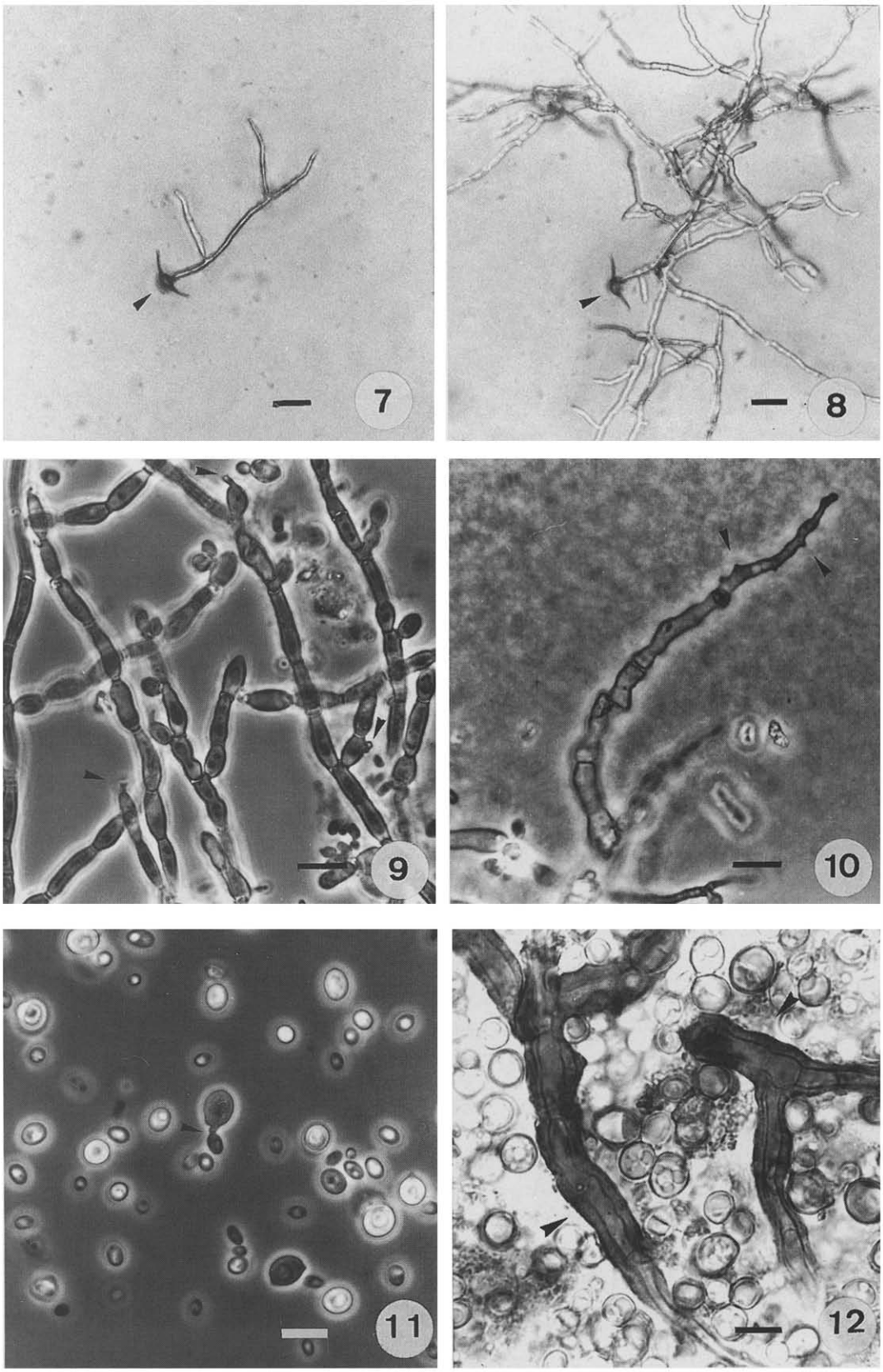


Figs 1–6. *Bicornispora exophiala*. **Figs 1–3.** SEM. **Figs 4–6.** LM. **Fig. 1.** Subglobose ascoma. **Fig. 2.** Ascospores. **Fig. 3.** Ridged surface of ascospore. **Fig. 4.** Ascoma showing basal stalk (arrow) and pseudoparenchymatic peridium (arrowhead). **Fig. 5.** Asci showing single-layered wall (arrow) with attenuate ends and irregularly biseriate ascospores. **Fig. 6.** Ascospores showing light-brown germ zone (arrowheads). Bars, 100 μm in Figs 1 and 4; 20 μm in Figs 2, 5 and 6; 5 μm in Fig. 3.

amyloidei, unitunicati, octosporati. Ascosporae unicellulares, reniformes, brunneae, laeves; in extremo utroque in distinctam appendicem acute productae, poris et lineis germinalibus nullis.

Species typica: *Bicornispora exophiala* Checa, Barrasa, M. N. Blanco & A. T. Martínez sp. nov.

Ascomata scattered, globose, glabrous, dark-brown, non-ostiolate, without a preformed aperture or zone of weakness. Paraphyses lacking. Asci unitunicate, eight-spored, ovoid to ellipsoidal, non-amyloid, evanescent. Ascospores one-celled, reniform with an acute, horn-like process at each end, dark-brown, with a clear brown zone on the concave side, smooth-walled under LM, faintly ridged under SEM.



Figs 7–12. *Bicornispora exophiala. Exophiala* anamorph. **Figs 7, 8.** Germinating ascospore on PGA (arrowheads) showing germ-tube emergence from the concave side. **Fig. 9.** Lateral and terminal conidiogenous cells with short annellated zones (arrowheads). **Fig. 10.** Conidiogenous hyphae with scars (arrowheads). **Fig. 11.** Budding cells (arrowhead) with short, annellated zones. **Fig. 12.** Dark hyphae with mucilaginous substance (arrowheads), and inflated older, yeast-like cells. Bars, 50 µm in Figs 7 and 8; 10 µm in Figs 9–12.

Type species: *Bicornispora exophiala* Checa, Barrasa, M. N. Blanco & A. T. Martínez sp. nov.

Bicornispora exophiala Checa, Barrasa, M. N. Blanco & A. T. Martínez sp. nov. (Figs 1–12)

Etym.: From *Exophiala*, referring to the anamorphic state.

Ascocarpi sparsi, globosi, 500–600 μm diam., breviter (150 × 300 μm) stipitati, saturate brunnei, irisati, glabri, non ostiolati; peridio pseudoparenchymatico, brunneo. Paraphyses nullae. Asci ovales vel ellipsoidales, ventricosi, 90–120 × 25–30 μm, unitunicati, evanescentes, octosporati. Ascosporae biseriatae, unicellulares, 60–65 × 8–10 μm longae lataeque, laeves, brunneae, reniformes – in parte cava, germinali, colore dilutae, in extremo utroque longe (15–20 × 2–2·5 μm) atque acute productae.

Anamorphosis: *Exophiala* J. W. Carmich. Cellulae conidiogenae intercalares, laterales vel terminales. Conidia subglobosa vel late ellipsoidea, 5–8 × 4–5 μm. Chlamidosporae absunt.

Holotypus. Madrid: Manzanares el Real, Ventisquero de la Condesa, 2240 m, 30TVL1715, in ramis dejectis *Cytisus purgans*, 31 May 1991, leg. C. Lado. MA-Fungi 32825 (isotypus in AH sub no. 15779). Cultura pura: IJFM A633.

Ascomata scattered, globose, 500–600 μm diam., short stalked (150 × 300 μm), dark-brown, iridescent, glabrous, non-ostiolate, without preformed apertures or zones of weakness. Peridium pseudoparenchymatous, brownish. Paraphyses lacking. Asci unitunicate, eight-spored, ovoid to ellipsoidal, *p. sp.* 90–120 × 25–30 μm, stipe 30–35 μm in length, without an apparent apical apparatus, non-amyloid, soon evanescent, arising from a basal hymenium. Ascospores irregularly biseriate, one-celled/60–65 × 8–10 μm, dark-brown, smooth, reniform with an acute, horn-like process at each end (15–20 × 2–2·5 μm), smooth walled by LM, faintly ridged by SEM, concave side with a clear-brown zone observed under LM from which the germ-tube emerges.

Cultures from single and mass ascospores on PGA and OGYE producing an *Exophiala* anamorph. Colony slow growing, 4 cm in diam. on OGYE after 1 wk at room temperature; surface black velvety, mucous at the centre; reverse dark-brown to black. Conidiogenous cells lateral or terminal, intercalary pegs or bottle-shaped cells, 11 × 6 μm. Conidiogenesis blastic, with short annellated zones (1 μm long). Conidia, one-celled, smooth, thin-walled, subglobose to broadly elliptical, 5–8 × 4–5 μm, sometimes with an inconspicuous basal scar. Chlamydospores absent. Old cultures becoming coriaceous, showing thick-walled pigmented hyphae covered by mucilage, and some hyphae with numerous condiogenous scars. Yeast-like development abundant on OGYE. Mother-cells globose to subglobose, 5–6·5 μm diam., some of them showing short annellated zones (daughter-cells like conidia of *Exophiala*).

Anamorph: *Exophiala* sp.

DISCUSSION

Several monographic studies on the genera of Coryneliales have been carried out by Benny *et al.* (1985 *a, b, c, d*). Johnston & Minter (1989) studied the structure of the asci of most of the members of the Coryneliales and demonstrated the presence of more than a single, functional wall layer except in *Coryneliopsis antarctica* Butin, in which only one wall layer was observed. Moreover, differences in ascus structure and function, with respect to other types of multilayered asci recognized by Eriksson (1981), indicate that the Coryneliales must be considered as a separate order with the single family Coryneliaceae.

Several morphological features observed in *B. exophiala*, such as a lysigenous aparaphysate centrum and evanescent, unitunicate asci that lack an apical structure, justify its inclusion in the Coryneliales (pseudoprototunicate ascomycetes) (Eriksson, 1981). However, the ascospore morphology of *B. exophiala* has no parallel among known genera. Although species with ascospores possessing relatively large protuberances have been described in *Tripospora* Sacc., they are tetraradiate or triangular (Benny *et al.*, 1985 *c*). The presence of a single-layered ascus wall, not differentiated at the apex, observed under LM, somewhat resembles *Coryneliopsis*, which Johnston & Minter (1989) suggested may be excluded from the Coryneliaceae. However, *Coryneliopsis* and *Bicornispora* differ in their world distribution, ecology and anamorphs. *Coryneliopsis*, restricted to southern Chile, contains two species, *C. antarctica* and *C. cupulifera* Butin (Butin, 1971), both of which are known to have *Anthracoderma* Speg. anamorphs in the Coelomycetes. These species are hyperparasites of *Cyttaria* Berk. species occurring on *Nothofagus* Blume. *B. exophiala* is unusual in the group since Coryneliales are widespread in the southern hemisphere, and only species belonging to *Caliciopsis* Peck occur in the northern hemisphere. In addition, most of the non-tropical species of *Caliciopsis* (*C. calicioides, C. brevipes* Fitzp., *C. cochlearis* Fitzp. and *C. nigra* Fitzp.) have been found growing on plant remains at different altitudes (Benny *et al.*, 1985 *d*) showing ecological characteristics similar to those of *B. exophiala*.

The anamorph of *B. exophiala* is placed in *Exophiala* due to the black yeast development and annellidic conidiogenesis (de Hoog & Hermanides-Nijhof, 1977). Species of *Exophiala* are pathogens of vertebrates and occur widely as saprobes on decaying plant materials, wood products and soil (de Hoog, 1983, 1993). *Exophiala* anamorphs are known in Herpotrichiellaceae (e.g. in some *Capronia* Sacc. species), and other black yeasts have been described as anamorphs of Dothideaceae and Venturiaceae species (Sivanesan, 1984; Müller *et al.*, 1987). The mono ascosporic cultures of *B. exophiala* provide the first evidence for the presence of a black yeast anamorph in an ascomycete not belonging to the loculoascomycetes. The similarities between this *Exophiala* anamorph and those found in some *Capronia* species (Müller *et al.*, 1987) support a taxonomic connexion between this new member of the Coryneliales and these bitunicate ascomycetes. Although *Bicornispora* can be included in the Coryneliales, its relationship to the other members of the Coryneliaceae and possibly to the bitunicate ascomycetes requires further investigation. Additional studies, for example 18S ribosomal DNA sequencing in different genera of the group and some species of *Exophiala*, should be made to better understand the taxonomic relationships of the new fungus here proposed.

We are grateful to Dr O. Eriksson (University of Umeå, Sweden) for taxonomic comments, to Dr J. A. Leal (CIB, CSIC, Madrid, Spain) for help in monoascosporic culture, to Dr E. Descals (Instituto Estudios Avanzados, CSIC, Palma de Mallorca, Spain) for critical reading of the manuscript and linguistic assistance and to Dr M. Laínz, S.J. for the Latin diagnosis. This research has been supported by the Environmental Programme (C104/91 Project) of the Comunidad Autónoma de Madrid and the DGICYT (PB92-0012, "Flora Micológica Ibérica Project").

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(*Accepted 22 September 1995*)