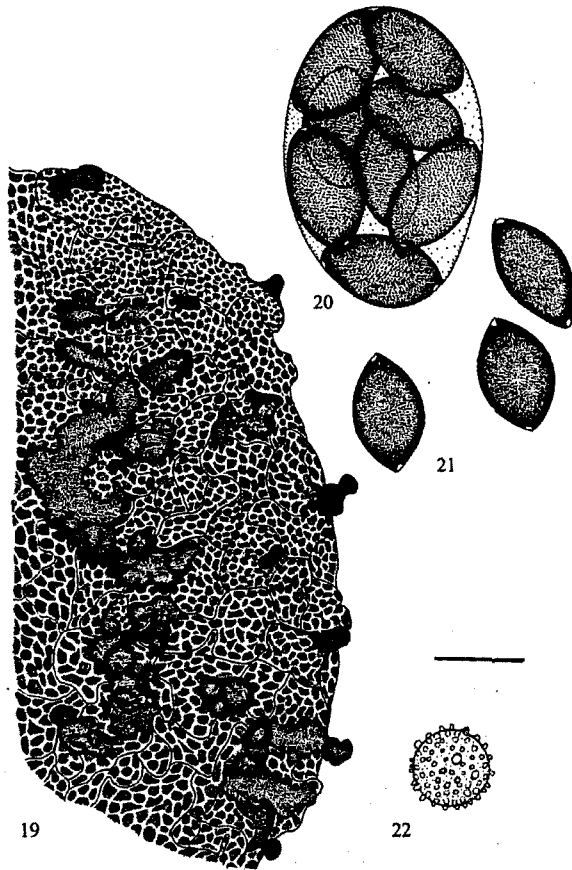


Figs 13–18. *Corynascus verrucosus* FMR 5904. Fig. 13. Peridial wall detail. Fig. 14. Ascoma, SEM. Figs 15–16. Peridium detail, showing the *textura epidermoidea* and their verruciform projections, SEM. Figs 17–18. Ascospores showing oblique to sub-terminal germ pores, and *Myceliophthora* anamorph conidia. Bars = 10  $\mu$ m.

Three new thermotolerant species of *Corynascus*



Figs 19–22. *Corynascus verrucosus* FMR 5904. Fig. 19. Peridium detail, showing the *textura epidermoidea* and their verruciform projections. Fig. 20. Ascus and ascospores. Fig. 21. Ascospores. Fig. 22. *Myceliophthora* anamorph conidia. Bar = 10 µm.

*Corynascus similis* differs from the other species of the genus in the navicular ascospores with two lateral to subterminal germ pores. The ascospore size, although slightly narrower, is similar to that of *C. sepedonium* (12–19 × 8–11 µm). In the latter species, however, the ascospores have terminal germ pores. The anamorphs of both species are very similar.

**Key to *Corynascus* species**

This key is updated from that of Arx *et al.* (1988).

1	Conidia clavate to obovate or absent; heterothallic . . . . .	2
	Conidia more or less spherical, always present; homothallic . . . . .	3
2(1)	Ascospores mostly with one germ pore, 8–13 × 5–7 µm . . . . .	heterothallicus
	Ascospores all with two germ pores, 22–32 × 17–23 µm . . . . .	thermophilus
3(1)	Ascomata setose or with a verrucose peridium . . . . .	4
	Ascomata glabrous . . . . .	5
4(3)	Ascomata with brown setae; ascospores with a germ pore at each end, 17–23 × 11–13 µm . . . . .	setosus
	Ascomata covered with verruciform projections; ascospores with two oblique to subterminal germ pores, 11–18 × 6.5–9 µm . . . . .	verrucosus
5(3)	Ascospores with two lateral to subterminal germ pores; ascospores navicular in lateral view, 11–20 × 6.5–9 µm . . . . .	similis
	Ascospores with two terminal germ pores . . . . .	6
6(5)	Anamorph absent; ascospores limoniform, 9–14 × 8–10 µm . . . . .	sexualis
	Anamorph present . . . . .	7
7(6)	Conidia finely echinulate to tuberculate; ascospores 12–19 × 8–11 µm . . . . .	sepedonium
	Conidia smooth; ascospores 18–24 × 8–10 µm . . . . .	novoguineensis

***Corynascus verrucosus* Stchigel, Cano & Guarro, sp. nov.**  
 (Figs 13–22)

Anamorph: *Myceliophthora* sp.

Ascomata globosa, non ostiolata, brunnea pallescens vel atrobrunnea, glabra, 50–140 µm diam. Peridium *ex textura epidermoidea* compositum cum processis verrucaeformibus. Asci 25–37 × 21–32 µm, sub-globosi vel ellipsoidei, octospori. Ascospores 11–18 × 6.5–9 µm, ellipsoideae cum apibus acutiformibus, brunneae, cum duobus germinalibus obliquis vel sub-apicalibus. Conidia globosa vel piriformia, sub-hyalina vel palide aurantiaca, verrucosa, 7–10 µm diam.

Typus: Argentina: Buenos Aires, Quilmes, Bernal, ex solo, 2 Nov. 1995, A. M. Stchigel (IMI 378522-holotypus; FMR 5904-isotypus).

*Mycelium* composed of hyaline to pale yellow, branched, anastomosing, septate, smooth hyphae, 1–3 µm broad. Colonies on PCA attaining 59–63 mm diam in 14 d on PCA at 22–25°, orange grey to greyish/orange (M 5B2 to 5B3), olive brown (M 4E7 to 4F8) in the central area, flat, floccose to powdery by conidia and ascomata production, zonate; reverse orange grey (M 5B2); exudate yellow. Ascomata superficial, globose, non-ostiolate, dark brown to very dark brown, 40–70 µm diam; peridium with *textura epidermoidea*, composed of a layer of irregular, reticulate, golden/brown cells, with dark brown, verruciform projections. Paraphyses absent. Asci 25–38 × 21–32 µm, subglobose to broadly ellipsoidal, short-stipitate, thin-walled, evanescent, 8-spored. Ascospores 11–18 × 6.5–9 µm, ellipsoidal with acute ends, hyaline when young and brown when mature, one-celled, smooth-walled, with a distinct oblique to subterminal germ pore at each end.

Anamorph: *Conidiophores* short, not sharply differentiated from the vegetative hyphae. Conidia blastic, terminal or lateral, sessile or on short protrusions, globose to pyriform, subhyaline to pale golden, thick-walled, verrucose, 7–10 µm diam.

Colonies on PDA attaining 43–47 mm diam in 14 d at 22–25°, yellowish/orange (M 4A6 to 4B7), cottony, zonate, with yellow exudate; reverse yellowish/orange to olive yellow (M 4A7 to 3C6). Ascomata absent and conidia profused produced.

18/ #



Three new thermotolerant species of *Corynascus*

*Colonies* on PA attaining 65–75 mm diam in 14 d at 22–25°, white to pale yellow (M 3A3), flat, floccose to powdery, zonate, with golden yellow exudate; reverse light yellow (M 3A5). *Ascomata* and conidia present.

*Colonies* on MEA attaining 24–26 mm diam in 14 d at 22–25°, light yellow to yellow (M 3A4 to 3A7), cottony; zonate, with yellow exudate; reverse light yellow (M 3A5). *Ascomata* absent and conidia profusely produced.

Thermotolerant, growing rapidly at 42°; ascomata and conidia profusely produced in all tested media.

The sequence of the ITS1–2 and 5.8S rDNA regions is shown in Fig. 23. The main features are: 560 bp; 132 A; 161 C; 146 G and 121 T. Location ITS1 nt 29–191; gene 5.8S rRNA nt 192–348; ITS2 nt 349–560. EMBL accession no. AJ224203.

The ascospore size of *Corynascus verrucosus* is very similar to that of *C. similis*, but the species differ in their spore morphology (irregularly limoniform in *C. similis*; ellipsoidal with acute ends in *C. verrucosus*), and because a peridium with verruciform projections is present in *C. verrucosus*. The anamorph of *C. verrucosus* is very similar to those of *C. sepedonium* and *C. similis*.

DISCUSSION

The alignment of the ITS-region sequences of all known *Corynascus* species and of several species of morphologically similar genera are shown in Fig. 23. Fig. 24 shows the phylogram obtained when these sequences were analysed with the neighbour-joining method (Saitou & Nei 1987). The sordariaceous *Chaetomium nigricolor*, *Corynascus* spp., *Gelasinospora bonaerensis* and *Thielavia terrestris* group together

in a clearly defined clade, supported by a bootstrap value of 100%. The *Corynascus* species form a subclade supported by a bootstrap of 99%. The sequences of the species of *Corynascus* studied were very similar and the greatest difference was between *C. heterothallicus* and *C. sepedonium* (34 nucleotide substitutions). The three new species, *Corynascus sexualis*, *C. similis* and *C. verrucosus*, although clearly having specific morphological features, differ only in 16, 10 and 16 bp from *C. sepedonium*, respectively (Fig. 23). *C. sexualis* differs in 16 bp from *C. similis* and in 12 bp from *C. verrucosus*, and *C. similis* differs from *C. verrucosus* in 6 bp. The differences in the sequences of the *Corynascus* species are not discriminatory alone. This was already noticed in a previous study of sordariaceous fungi (Stchigel, Cano & Guarro 1998). The only exception is the heterothallic *C. heterothallicus*, which is considered to be a hypothetical ancestor of the others. This is in agreement with Yun *et al.* (1998), who studied the organisation of the *MAT* gene in homothallic and heterothallic species of *Cochliobolus* (*Pleosporaceae*), and suggested that the heterothallic condition probably came first. In the phylogenetic tree, *Melanospora pascuensis* (*Ceratostomataceae*) and *Emericella nidulans* (*Trichocomaceae*) are clearly distant from the sordariaceous fungi. These findings demonstrate that *Corynascus* is closer to *Chaetomiaceae* than to *Ceratostomataceae*.

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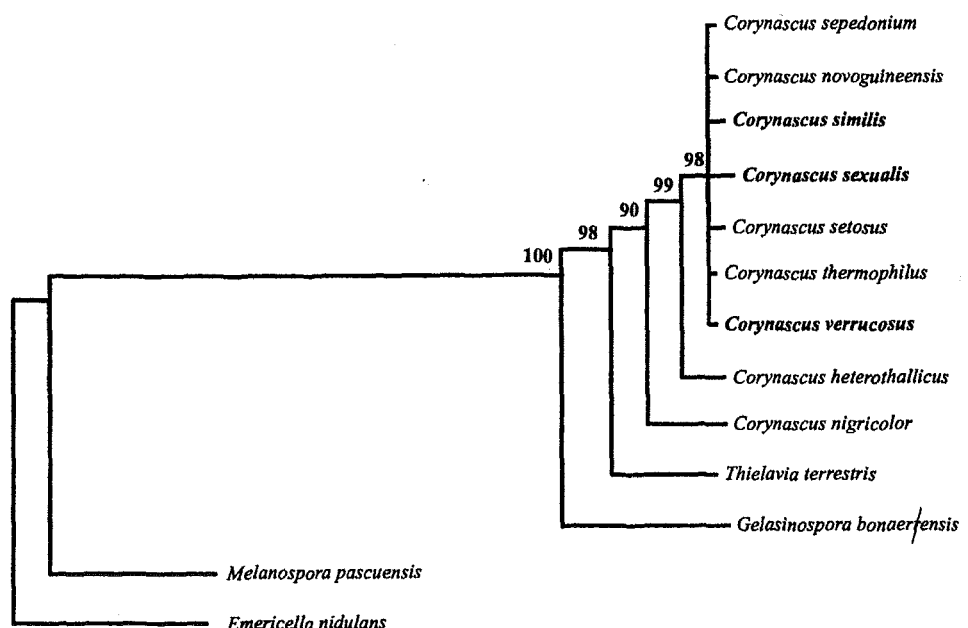


Fig. 24. Neighbour-joining phylogenetic tree of the aligned sequences of the strains studied. Confidence limits of branches (indicated in % along the branches) were created in a bootstrap analysis using 500 trials. The scale bar represents 0.1% sequence divergence.

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**1.8. Ascomicetos del suelo de España. XII. *Ascotricha canariensis* sp. nov.**

1.8.1. A. M. Stchigel, J. Guarro, D. García & B. Acosta. Soil ascomycetes from Spain. XII. *Ascotricha canariensis* sp. nov. *Mycologia* (en prensa)

Short title: New *Ascotricha* species

**Soil ascomycetes from Spain. XII. *Ascotricha canariensis* sp. nov.**

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**Abstract:** *Ascotricha canariensis* sp. nov. (Xylariaceae, Ascomycetes) isolated from soil of the Canary Islands is described and illustrated. It belongs to the group of *Ascotricha* species with cylindrical asci and is differentiated from the other species by bearing ascomata with short, single or once-branched setae and by the presumed absence of an anamorph.

**Key Words:** Ascomycota, soil fungi, Xylariales

1 During the course of a study of soil ascomycetes from Spain, an interesting species of  
2 *Ascotricha* Berk. was isolated from Gran Canaria, Canary Islands. Gran Canaria is an  
3 island located near the African continent, in the Atlantic Ocean. Its origin is volcanic  
4 and it has a surface area of 100.55 km<sup>2</sup>. The terrain is basically basaltic, and the  
5 vegetation is mainly composed of *Phoenix* spp., some Cactaceae, and numerous  
6 members of the Poaceae. The area is dominated by a Mediterranean climate, with a  
7 hot summer. The average temperature is 22 C, with a minimum of 17C and a  
8 maximum of 25 C. The main annual precipitation is 150-200 mm. The morphological  
9 characteristics differentiate this taxon from all previously described species in the  
10 genus (Hawksworth 1971, Udagawa et al 1994 a, b, Stchingel and Guarro 1998,  
11 Udagawa and Uchiyama 1999) and is thus described here as new.

12

### 13 *Materials and methods*

14 Soil was mainly collected from the A<sub>0</sub> horizon with sterilized polyethylene bags. These  
15 were sealed with a rubber band and labelled. On returning to the laboratory soil was  
16 stored at 4--7 C until used. Approx 1 g of the sample was treated with 60% (v/v) ethyl  
17 alcohol during 10 min (after Warcup and Baker 1963). The supernatant was  
18 discarded and the solid phase was suspended in 10 ml of distilled water. The  
19 suspensions were cultured on potato carrot agar (PCA) with chloramphenicol (50  
20 mg/L) at room temperature (22--25 C) under 12 h of darkness alternating with 12 h of  
21 cool white fluorescent light. The morphological characteristics of the colonies were  
22 studied on malt extract agar (MEA; Difco), oat meal agar (OMA; Difco), PCA, and  
23 potato dextrose agar (PDA, Difco) at 10, 15, room temperature (22--25), 37 and 42 C,  
24 under 12 h of darkness alternating with 12 h of cool white fluorescent light. Color  
25 notations in parentheses are from Kornerup and Wanscher (1984). The



measurements of the fungal structures were taken in water or lactophenol. Photomicrographs were obtained with a Leitz Dialux 20 EB microscope. Scanning electron microscopy techniques were described previously by Figueras and Guarro (1988).

**Ascotricha canariensis** Stchingel, D. García et Guarro, sp. nov. Figs. 1-9

*Mycelium* ex hyphis subhyalinis vel brunneis, ramosis, septatis, levibus vel tuberculatis, 1--6  $\mu\text{m}$  diam composito. *Coloniae* in agaro cum decocto tuberorum et carotarum "PCA" restrictae, planae, tenues, ex mycelio vegetativo submerso, subhyalinae vel brunneae; reversum olivaceo-brunneo vel nigrum. *Ascomata* superficialia vel immersa, ostiolata, translucida, olivaceo-brunnea vel atro-brunnea, subglobosa vel globosa, 160--180 x 140--160  $\mu\text{m}$ , apex cum collo brevi, 12--25  $\mu\text{m}$  alta, 25--40  $\mu\text{m}$  lata, pilosa. *Pili* rigidiusculi, non ramosi vel 1-ramosi, verrucosi, septati, atrobrunnei, 15--45  $\mu\text{m}$  longi, 3--5  $\mu\text{m}$  diam ad basim, cum vesicula hyalina et globosa vel piriformia, laevia, 3--6  $\mu\text{m}$  diam, ad apicem formanti. *Peridium* parum olivaceo-brunneum vel brunneum, tenue, ex textura epidermoidea vel intricata, 4--6 stratorum compositum, 10--15  $\mu\text{m}$  crassitunicatum. *Asci* 47--54 x 5--7  $\mu\text{m}$ , lineari-cilindrici, stipitati, cum muris tenuibus, deliquescenti, octospori. *Paraphyses* nullae. *Ascosporae* 6--8 x 4.5--6 x 2--4  $\mu\text{m}$ , ovoideae vel ellipsoideae, oblatae, atro-brunneae, laeves, fissura germinali aequatoriale paratae, uniseriatae. *Status conidialis* nullis.

*Mycelial hyphae* subhyaline to brown, branched, septate, smooth to tuberculate, 0.5--6  $\mu\text{m}$  diam. *Colonies* on PCA growing slowly, attaining 17-20 mm

1 diam in 14 d at room temperature, olive brown (M. 4F5) to blackish, flat, thin, powdery  
2 to granulose, consisting of submerged mycelium and sparse aerial hyphae, producing  
3 abundant ascomata; reverse olive brown (M. 4F5) to blackish; ascomal initials coiled.  
4 *Ascomata* superficial to immersed, scattered to grouped, ostiolate, subglobose to  
5 globose, translucent, olive-brown to dark brown, 160--180 x 140--160  $\mu\text{m}$ , sometimes  
6 with a short beak 12--25 x 25--40  $\mu\text{m}$ . *Setae* stiff, simple or bifurcate at the middle, 2--  
7 3 septate, verrucose and dark brown at the base, becoming smooth and hyaline at  
8 the apex, 15--45  $\mu\text{m}$  long, 3--5  $\mu\text{m}$  diam at the base, thin-walled, terminated by a  
9 hyaline, globose to pyriform, smooth-walled vesicle, 2.4--4  $\mu\text{m}$  diameter. *Peridium* 4--  
10 6 layered, 10--15  $\mu\text{m}$  thick, pale olivaceous-brown to brown, *textura epidermoidea* to  
11 *intricata* in surface view. *Asci* 47--54 x 5--7  $\mu\text{m}$ , spore-bearing part 35--48  $\mu\text{m}$  long,  
12 cylindrical, short stipitate, thin-walled, evanescent, fasciculate, 8-spored, without  
13 apical structures. *Paraphyses* not observed. *Ascospores* 6--8 x 4.5--6 x 2--4  $\mu\text{m}$ ,  
14 ovoid to ellipsoidal in front view, oblate, dark brown, smooth-walled, with an equatorial  
15 germ slit on the narrow side, uniseriate. *Anamorph* not observed.

16 On OMA at room temperature, colonies attaining 20--22 mm diam in 14 d,  
17 similar to those on PCA but strongly zonate.

18 On MEA at room temperature, colonies attaining 15-16 mm diam in 14 d,  
19 raised, cottony, white; exudate absent; soluble pigment orange; reverse orange  
20 (4B6). *Ascomata* not formed.

21 On PDA at room temperature, colonies attaining 19-21 mm diam in 14 d,  
22 similar to those on MEA but without a soluble pigment and reverse whitish. *Ascomata*  
23 not formed.

On PCA, OMA, MEA and PDA at 15 and 37 C, growing very slowly, attaining 3-4 mm diam in 14 d, raised, hairy, white. Ascomata not produced. No growth was observed at 10 and 42 C.

Specimens examined: SPAIN. GRAN CANARIA: La Laguna (15° 25' GM, 28° 9' N), from soil, 22-VIII-1998, col. B. Acosta, isol. A. M. Stchigel (IMI 381334-HOLOTYPE, FMR 6738:ISOTYPE). Living cultures ex type: CBS 102197, FMR 6738, IMI 381334.

### Discussion

The genus *Ascotricha* encompasses 13 species (Ames 1951, Hawksworth 1971, Kulshreshtha et al 1977, Stchigel and Guarro 1998, Udagawa et al 1994 a, b, Udagawa and Uchiyama 1999), characterized by ostiolate or nonostiolate setose ascomata with translucent peridial wall, 8-spored asci, brown ellipsoidal ascospores with an equatorial germ slit, and anamorphs belonging to *Dicyma* Boulanger or to the *Geniculosporium-Nodulisporium* complex. *Ascotricha* spp. are generally found on cellulosic substrates (Ames 1951, Hawksworth 1971, Calviello 1978), dung (Hawksworth 1971, Kahn and Cain 1977) and soil (Hawksworth 1971, Horie et al 1993; Stchigel and Guarro 1998, Udagawa et al 1994 a, b). The genus *Ascotricha*, morphologically very close to *Chaetomium* Kuntze, was erected by Berkeley (1838) for *A. chartarum* Berk. Hawksworth (1971), in an extensive review of the genus, considered it included in the Chaetomiaceae of the order Sphaeriales although some similarities with members of the Coniochaetaceae were later noticed (Hawksworth and Wells 1973). Khan and Cain (1977) considered *Ascotricha* as phylogenetically distant from *Chaetomium* and related genera, and placed it in the Xylariaceae. Laessøe (1994) considered *Ascotricha* and the closely placed *Ascotrichella*

1 Valldosera & Guarro (Valldosera and Guarro 1988) as genera *incertae sedis* due  
2 mainly to the nonstromatic nature of their ascomata, which differentiated them from  
3 the other genera of the Xylariaceae. *Ascotricha* was recently included in the  
4 Xylariaceae (Hawksworth et al 1995, Eriksson and Hawksworth 1998), and this view  
5 has been substantiated by Lee and Hanlin (1999) on the basis of 18S rDNA  
6 sequence analyses. *Ascotricha canariensis* belongs to the group of species with  
7 cylindrical asci which includes *A. amesii* Hawksworth, *A. amphitricha* (Corda) S. J.  
8 Hughes, *A. bosei* Hawksworth, *A. chartarum* Berk., *A. delhiana* Kulshreshtha,  
9 Raychardhuri & Khan, *A. erinacea* Zambett., *A. guamensis* Ames, *A. hispanica*  
10 Stchigel & Guarro, *A. lusitanica* Kenn. and *A. xylina* Ames. It can be easily  
11 differentiated from all previously described species (with the exception of *A.*  
12 *hispanica*) mainly by the setal morphology and by the presumed absence of an  
13 anamorph. *Ascotricha hispanica* also has short and simple ascomal setae and lacks  
14 an anamorph, but differs from *A. canariensis* by its longer (30--100 x 3--5  $\mu\text{m}$ ) setae  
15 with a more elaborate branching pattern (sub-dichotomous and once- to twice-  
16 branched) and by its smaller ascospores (4.5--7 x 3--4 x 3-4-  $\mu\text{m}$ ).

17

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23

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25 <sup>1</sup> E-mail: [ams@astor.urv.es](mailto:ams@astor.urv.es)

1 Figs. 1-6. *Ascotricha canariensis*. 1. Ascoma. 2. Detail of the peridium. 3  
2 Simple and branched setae. 4. Asci and ascospores. 5. Asci and ascospores  
3 ascospore showing the germ slit (arrow). 6. Ascospores (SEM). Scale bars: 1 = 50  
4  $\mu\text{m}$ ; 2-6 = 10  $\mu\text{m}$ .

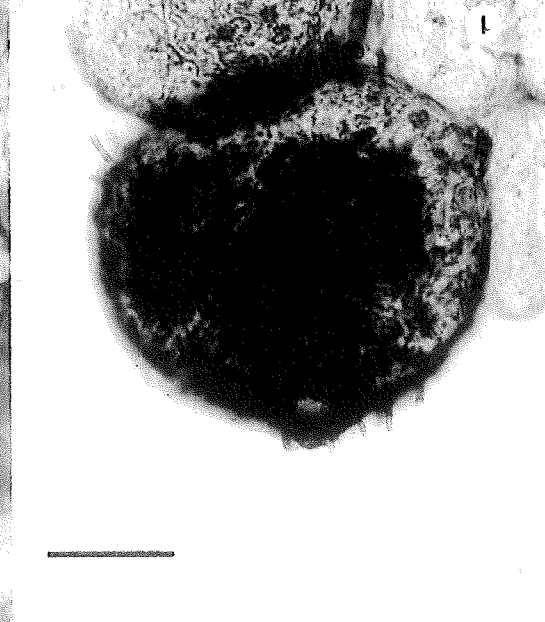
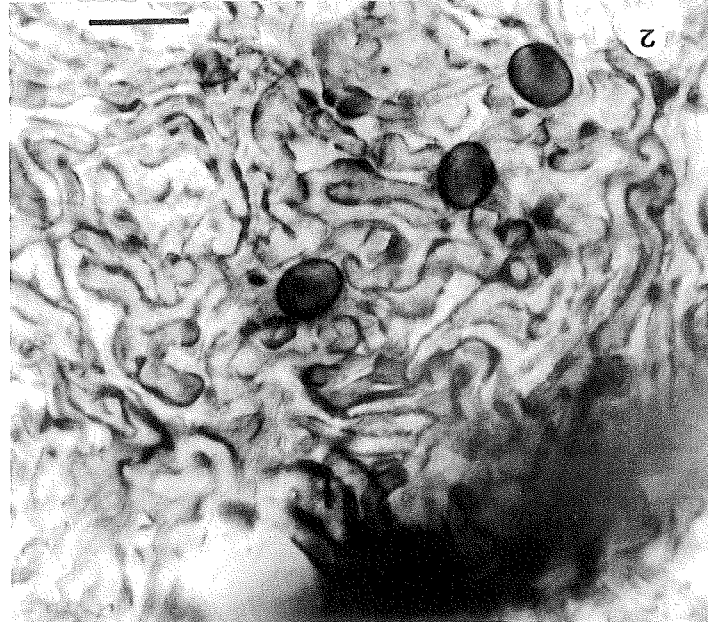
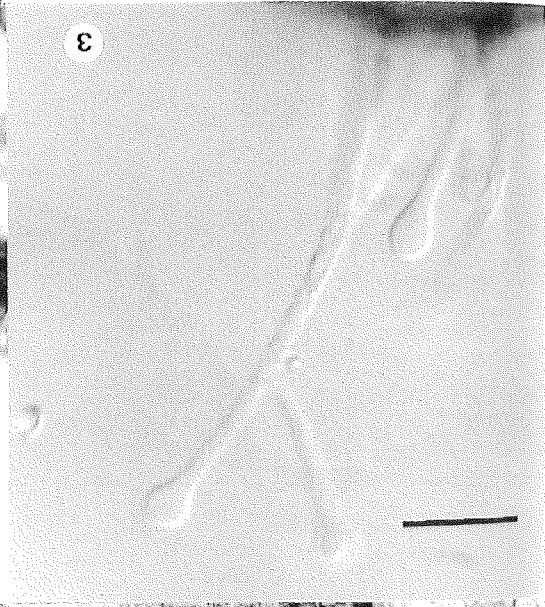
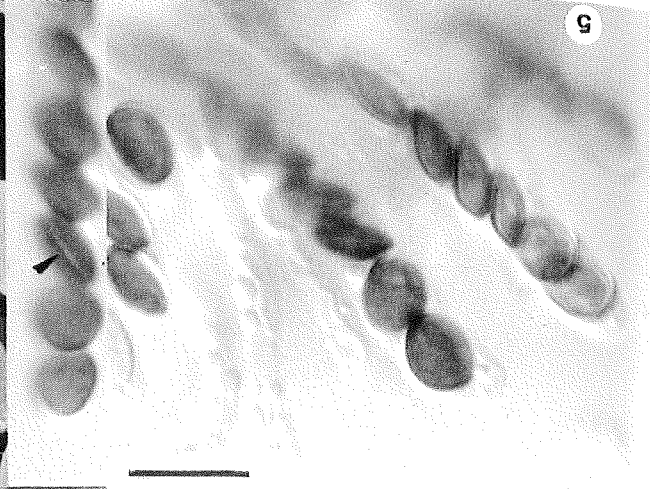
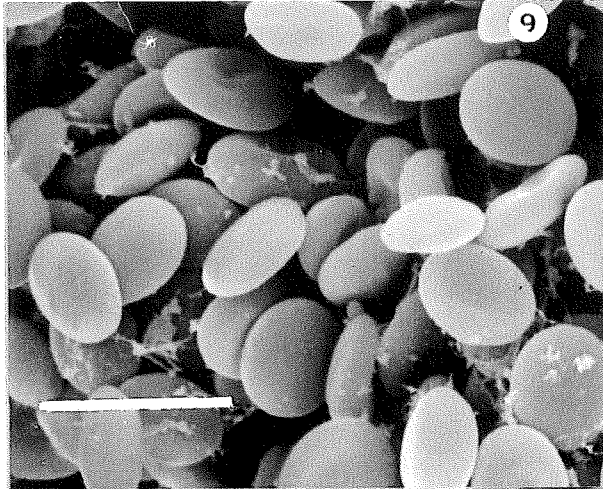
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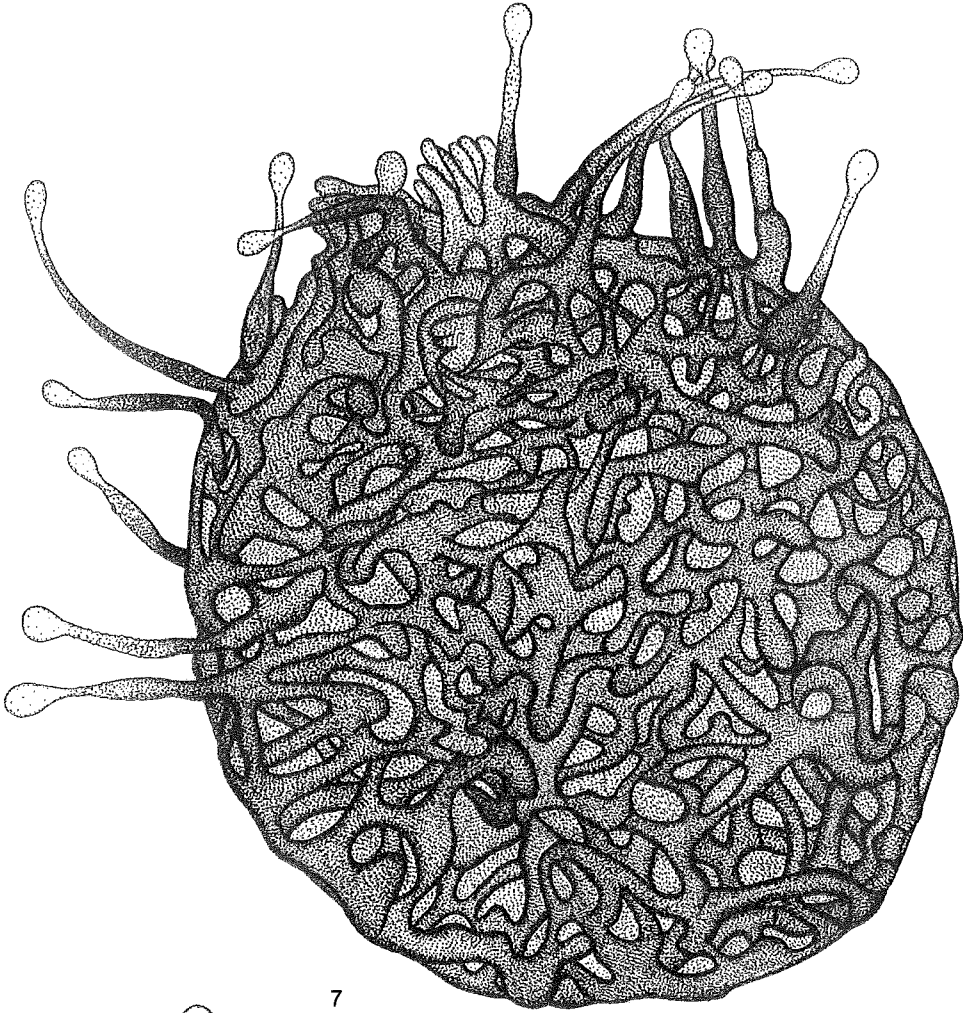
6 Figs. 7-9. *Ascotricha canariensis*. 7. Ascoma. 8. Ascus with ascospores. 9  
7 Ascospores. Scale bars: 7 = 25  $\mu\text{m}$ ; 8 - 9 = 10  $\mu\text{m}$ .

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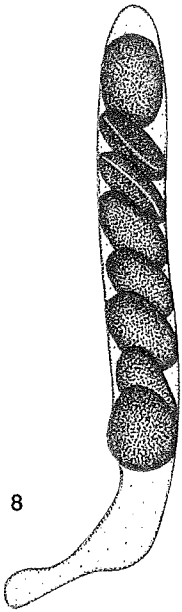
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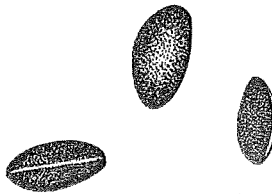




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## 1.9. Una nueva *Apiosordaria* de Nigeria, con una clave dicotómica de las especies del suelo

1.9.1. A. M. Stchigel, J. Cano, J. Guarro & C. Gugnani. A new *Apiosordaria* from Nigeria, with a key of the soil-borne species. *Mycologia* (en prensa)

Short title: *Apiosordaria nigeriensis* sp. nov.

**A new *Apiosordaria* from Nigeria, with a key to the soil-borne species**

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**Abstract:** A new species, *Apiosordaria nigeriensis*, isolated from soil of Nigeria is described and illustrated. This fungus is characterised by its two-celled ascospores with spinulose walls and an apical germ pore surrounded by a dark area. Reexamination of *Apiosordaria angulispora* resulted in a new combination, *Cercophora angulispora* based in the presence of cephalothecoid peridium, cylindrical asci with thickened apical ring and curved to sigmoid ascospores with long apical and caudal appendages. A key to the soil-borne species of *Apiosordaria* is provided.

**Key Words:** Africa, Ascomycota, soil fungi, Lasiosphaeriaceae, Sordariales

1 During an expedition to the rainforests in southeast Nigeria in May-Jun 1997,  
2 numerous litter and soil samples were collected in Anambra and Cross River states.  
3 An ascomycete isolated from a soil sample of Nsukka was sufficiently different from  
4 all described Sordariales to warrant the proposal of a new species of the genus  
5 *Apiosordaria* Arx & Gams. In the present report the species is fully described and  
6 illustrated.

7  
8 The soil samples were collected on the campus of the Nsukka University, Anambra  
9 state, Nigeria. The altitude is below 300 m. The soil is of the lateritic type and the  
10 vegetation is mostly composed of lowland forest trees, consisting of *Piptadeniastrum*  
11 *africanum* (Hok.) Brenan, *Uapaca* spp., *Pycanthus* sp., *Lophira alata* Baks & Gaertn.  
12 and *Khaya ivorensis* A. Chev. The area is dominated by a tropical interland climate  
13 with an average temperature of greater than 27 C. The total annual precipitation is  
14 2000-3000 mm. The methods used for sampling, soil activation, culture and  
15 measurement of the structures were described previously (Stchigel et al 1998). Color  
16 notations in parentheses are from Kornerup and Wanscher (1984).

17  
18 ***Apiosordaria nigeriensis* Stchigel et Guarro, sp. nov.** Figs. 1-9

19 *Mycelio* ex hyphis hyalinis vel dilute brunneis, septatis, ramosis,  
20 anastomosans, levibus vel tuberculatis, 1--6  $\mu\text{m}$  diametro composito. *Coloniae* in  
21 PCA expansae, planae, tenues, sub-granulosae, ex mycelio vegetativo submerso et  
22 aereo, olivaceo-brunnea; reversum olivaceo-brunneum. *Ascomata* immersa vel  
23 superficialia, non-ostiolata, translucida, sub-hyalina vel pallide brunnea, globosa vel  
24 subglobosa, 300--600  $\mu\text{m}$  diam compositae, pilosae. *Peridium* pallide brunneum, ex  
25 textura angularis compositum, 3-6 stratorum compositum. *Asci* 8-spori, clavati, cum

1 muris tenuibus, 160--180 x 29--36  $\mu\text{m}$ , longistipitati, 60--90  $\mu\text{m}$ . *Paraphysibus*  
2 hyalinis, filiformibus, septatis, 3--4  $\mu\text{m}$  diam. *Ascospores* biseriatae, primum  
3 unicellulares, clavatae, hyalinae, deinde transverse septatae ad bicellularis; cellula  
4 superiore ovoidea cum apice acuminato, brunnea, crassitunicata, 24--32 x 20--23  $\mu\text{m}$ ,  
5 spinis ad 1--7  $\mu\text{m}$  longis, cum foramine germinali singulari, apicali praedita, 1.5--2  $\mu\text{m}$   
6 diam; cellula inferiore subhyalinae vel brunnea, cylindrico-conica vel conica, cum  
7 spinis formantes, 7--10 x 4--5  $\mu\text{m}$ , sine foramine germinali. *Ascospores* sine  
8 appendicibus gelatinosis et sine strato mucosa. *Status conidialis* nullis.

9  
10 *Mycelium* composed of hyaline to pale brown hyphae, septate, branched,  
11 smooth to tuberculate, 1--6  $\mu\text{m}$  diam. *Colonies* on PCA growing rapidly, attaining 45--  
12 47 mm diam in 7 d at room temperature (approx 25 C), flat, thin, consisting of  
13 submerged mycelium and sparse aerial hyphae, olive brown (M. 4D8-4E8), producing  
14 abundant ascomata; reverse olive brown; ascomatal initials originating as coiled  
15 hyphae of vegetative mycelium soon interwoven by neighbouring hyphae. *Ascomata*  
16 immersed to superficial, scattered, non-ostiolate, globose, translucent, appearing dark  
17 brown to black due to mass of ascospores, 300--600  $\mu\text{m}$  diam, covered by hyaline to  
18 subhyaline hyphae. *Peridium* 3-6 layered, 7--15  $\mu\text{m}$  thick, translucent, subhyaline to  
19 pale ochraceous brown, *textura angularis*, cells 6--25  $\mu\text{m}$  diam. *Asci* 8-spored,  
20 fasciculate, clavate, thick-walled when young, 160--180 x 29--36  $\mu\text{m}$ , broadly rounded  
21 at the apex, stipe 60--90  $\mu\text{m}$  long, without conspicuous apical structures, evanescent.  
22 *Paraphyses* hyaline, filiform, septate, 3-4  $\mu\text{m}$  diam *Ascospores* biseriate, clavate,  
23 hyaline and one-celled when young, becoming two-celled by the formation of a  
24 transverse septum; upper cell ovoid, truncated at the base and with an acuminate

1 apex, brown, thick-walled, 24--32 x 20--23  $\mu\text{m}$ , uniformly ornamented with numerous  
2 spines (1--7  $\mu\text{m}$  long), darker coloured around the germ pore; germ pore single,  
3 apical, conspicuous, 1.5--2  $\mu\text{m}$  diam; lower cell subhyaline to medium brown,  
4 cylindrical-conical to conical, spinulose, 7--10 x 4--5  $\mu\text{m}$ . *Anamorph* unknown. At 37 C  
5 growing more rapidly than at room temperature, but ascomata are not produced. No  
6 growth 42 C.

7

8 SPECIMENS EXAMINED: NIGERIA. Enugu, Nsukka University campus, from  
9 garden soil, 26-V-1997, col. M. Caldusch, A. M. Stchigel and J. Guarro, isol. A. M.  
10 Stchigel (HOLOTYPE:IMI 378909, ISOTYPE:FMR 6363). Living cultures: CBS  
11 100895, FMR 6363, IMI 378910, UAMH 9419.

12

13 The genus *Apiosordaria* Arx & Gams (Lasiosphaeriaceae) is characterised by  
14 dark ascomata, non-amyloid asci with no distinct apical structures, and two-celled  
15 ascospores, composed of a dark and ornamented upper cell and a lower hyaline cell  
16 (Arx and Gams 1967). In the most recent revision of the genus 16 species and 2  
17 varieties were accepted, with *Echinopodospora* Robinson and *Lacunospora* Cailleux  
18 considered as synonyms (Krug et al 1983). Subsequently, five more species and a  
19 new variety have been reported (Guarro and Cano 1988, Mukerji et al 1995,  
20 Udagawa 1990), four of them were transferred from *Triangularia* Boedijn (Guarro and  
21 Cano 1988).

22 *Apiosordaria nigeriensis* together with *A. jamaicensis* (Robinson) Krug,  
23 Udagawa & Jeng, *A. sacchari* (Robinson) Krug, Udagawa & Jeng and *A. spinosa*  
24 (Cailleux) Krug, Udagawa & Jeng share spherical, translucent and non-ostiolate  
25 ascomata with spinulose to spiny ascospores which in part correspond to the main

1 features of *Echinopodospora* B. M. Robinson. *Apiosordaria nigeriensis* can be easily  
2 differentiated from the other mentioned species by the characteristic darker surface  
3 region around the germ pore and the spinulose lower cell of the ascospore. Other  
4 species with spinulose upper cell of the ascospores are *A. effusa* (Morinaga et al)  
5 Krug, Udagawa & Jeng, *A. microcarpa* Udagawa & Muroi, *A. terrestris* Jong & Davis,  
6 *A. vermicularis* (Morinaga et al) Krug, Udagawa & Jeng, *A. tuberculata* Krug,  
7 Udagawa & Jeng and *A. verruculosa* (Jensen) Arx & Gams. *Apiosordaria nigeriensis*  
8 can be distinguished from these taxa by the presence of non-ostiolate ascomata  
9 (ostiolate in *A. microcarpa* and *A. verruculosa*), clavate asci (cylindrical in *A. effusa*,  
10 *A. microcarpa*, *A. vermicularis* and *A. verruculosa*), ascospores with a relatively large  
11 upper cell (24--32 x 20--23  $\mu\text{m}$  in *A. nigeriensis* and 18--22 x 11--17  $\mu\text{m}$  in *A. effusa*,  
12 15--19 x 10--12.5  $\mu\text{m}$  in *A. microcarpa* and 16--22 x 12--15  $\mu\text{m}$  in *A. vermicularis*),  
13 which are ornamented with long aculeate spines (up to 7  $\mu\text{m}$  long in *A. nigeriensis*  
14 and 3--5  $\mu\text{m}$  in the other species), and with a spinulose lower cell (smooth or near so  
15 in the other species).

17 ***Cercophora angulispora*** (Cain & Farrow) Stchigel et Guarro, comb. nov.

18  $\equiv$  *Triangularia angulispora* Cain & Farrow, *Can J Bot* 34, 690 (1956).

19 *Apiosordaria angulispora* (Cain & Farrow) Guarro formerly considered a  
20 species of *Triangularia* Boedijn, was placed in *Apiosordaria* by Guarro and Cano  
21 (1988) on the basis of the verruculose cell wall of the upper cell of the ascospore.  
22 However, after a careful reexamination of the strain ATCC 32380 (=CBS 265.77) we  
23 noticed that some of its features such as the cephalothecoid peridium, cylindrical asci  
24 with a distinct, thickened apical ring, curved to sigmoid ascospores with both long  
25 apical and caudal appendages, long lower cell of the ascospore and subapical to



1 lateral pore are very rare or absent in *Apiosordaria* spp. By contrast all these  
 2 characteristics are typical of *Cercophora* Fuckel (Lundqvist, 1972). Therefore, the  
 3 new combination is proposed.

4 Considering the new taxon proposed here and the exclusion of *A. angulispora*, there are  
 5 currently 16 soil-borne species of *Apiosordaria*. For their identification, the following key  
 6 is proposed:

7

8 1.- Upper cell of the ascospore polygonal, five-angled in side view, ornamented with  
 9 longitudinal ribs, measuring 10--12 x 8--9  $\mu\text{m}$ .....*A. striatospora*

10 Upper cell of the ascospore more or less ellipsoidal.....2

11

12 2.- Asci 4-spored.....3

13 Asci 8-spored.....6

14

15 3.- Ascomata globose, non-ostiolate; upper cell of the ascospore verrucose,  
 16 measuring 18--22 x 14--17  $\mu\text{m}$ ; lower cell of the ascospore measuring 4--6 x  
 17 5--6  $\mu\text{m}$ .....*A. effusa*

18 Ascomata pyriform, ostiolate.....4

19

20 4.- Upper cell of the ascospore spinulose or warty, measuring 16--21 x 13--15  $\mu\text{m}$ ;  
 21 lower cell of the ascospore measuring 6--10 x 9--19  $\mu\text{m}$ .....*A. verruculosa*

22 Upper cell of the ascospore pitted.....5

23

24 5. Lower cell of the ascospore measuring 10--14 x 7.5--9  $\mu\text{m}$ .....*A. longicaudata*

25 Lower cell of the ascospore measuring 4--7.5 (10) x 6--8 (11)  $\mu\text{m}$ .....*A. tetraspora*

6. Upper cell of the ascospore longer than 25  $\mu\text{m}$ .....7  
 Upper cell of the ascospore measuring 25  $\mu\text{m}$  or less.....11
7. Upper cell of the ascospore measuring 33.2--40.5 x 24.8--31.3  $\mu\text{m}$ ,  
 spinulose.....*A. sacchari*  
 Upper cell of the ascospore smaller.....8
8. Ascomata ostiolate, pyriform; upper cell of the ascospore pitted, measuring 27--34  
 x 18--23  $\mu\text{m}$ .....*A. backusii*  
 Ascomata non-ostiolate, globose; upper cell of the ascospore more or less  
 spinulose.....9
9. Upper cell of ascospore ornamented with fat-topped ridges, forming an incomplete  
 reticulum.....*A. terrestris*  
 Upper cell of the ascospore ornamented with spines.....10
10. Upper cell 24--32 x 20--23  $\mu\text{m}$ , with a dark area surrounding the germ pore and  
 ornamented with spines up to 7  $\mu\text{m}$  long; lower cell 7--10  $\mu\text{m}$   
 long.....*A. nigeriensis*  
 Upper cell 24--30 x 17--26  $\mu\text{m}$  and ornamented with spines up to 3  $\mu\text{m}$  long;  
 lower cell 11--18  $\mu\text{m}$  long.....*A. jamaicensis*
11. Ascomata non-ostiolate, globose; upper cell of ascospore spinulose, measuring  
 16--20 (22) x 12--15  $\mu\text{m}$ ; lower cell 12--20 x 8--9 (16)  $\mu\text{m}$ .....*A. vermicularis*  
 Ascomata pyriform, ostiolate.....12

1	12. Upper cell of the ascospore more or less spinulose.....	13
2	Upper cell of the ascospore pitted.....	15
3		
4	13. Upper cell of the ascospore measuring 20--24 x 15--18 $\mu\text{m}$ , warty.....	<i>A. otanii</i>
5	Upper cell of the ascospore smaller and spinose.....	14
6		
7	14. Upper cell of the ascospore measuring 15--19 x 10--18 $\mu\text{m}$ ; lower cell measuring	
8	5--6.5 x 7.5--9 $\mu\text{m}$ .....	<i>A. microcarpa</i>
9	Upper cell of the ascospore measuring 10--12.5 x 6--8 $\mu\text{m}$ ; lower cell measuring	
10	4--6 x 4.5--6.5 $\mu\text{m}$ .....	<i>A. yaeyamensis</i>
11		
12	15. Upper cell of the ascospore measuring 20--25 x 12--15 $\mu\text{m}$ , regularly pitted; lower	
13	cell measuring 4--5 x 3--4 $\mu\text{m}$ .....	<i>A. vestita</i>
14	Upper cell of the ascospore measuring 14--18 x 10--12 $\mu\text{m}$ , ornamented with	
15	numerous longitudinal ridges and pitted; lower cell measuring 2.5--5 x	
16	3--4 $\mu\text{m}$ .....	<i>A. rugosa</i>

## 18 ACKNOWLEDGMENTS

19  
20 This work was supported by grant PM95-0160 from CICYT (Ministerio de Educación y  
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22 grateful for the fellowship grant from Instituto de Cooperación Iberoamericana (I.C.I.),  
23 Spain.

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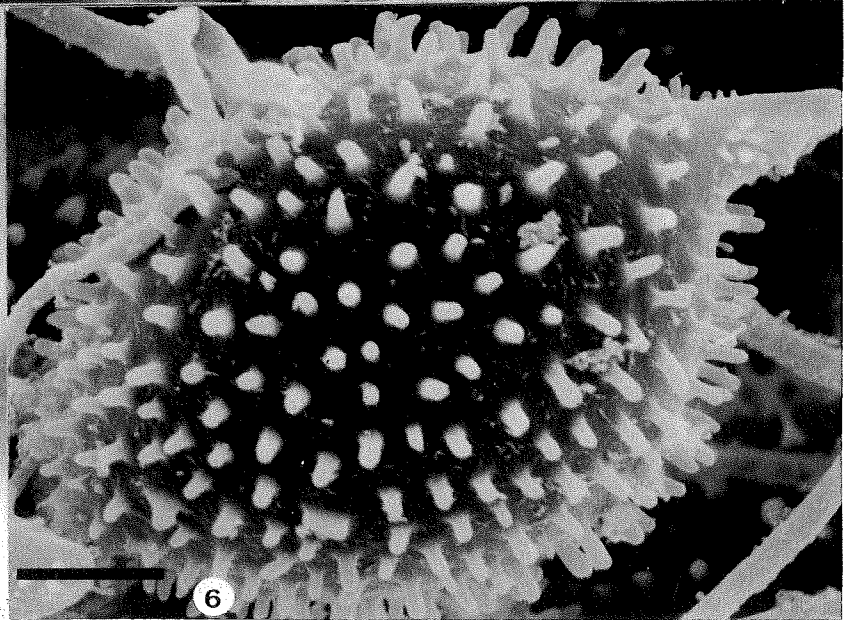
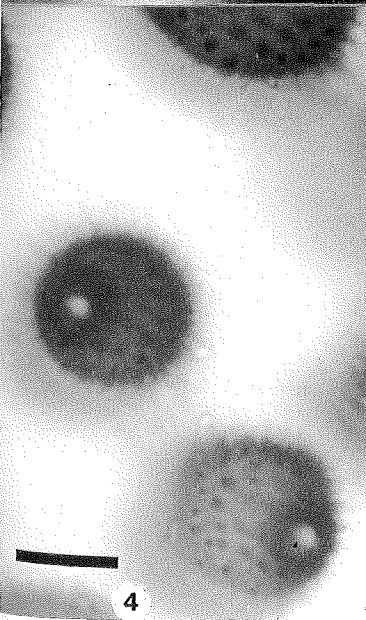
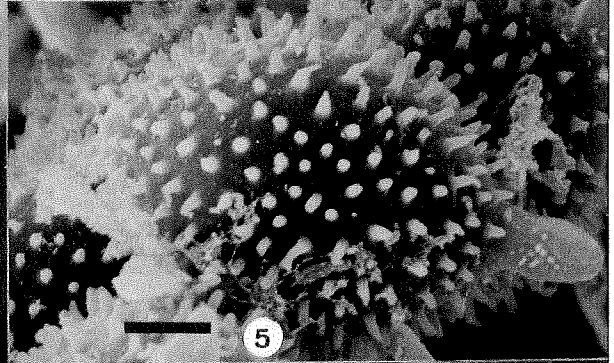
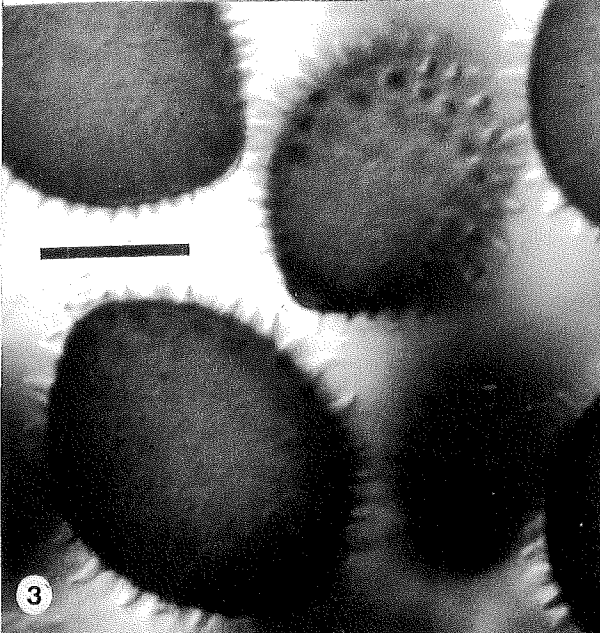
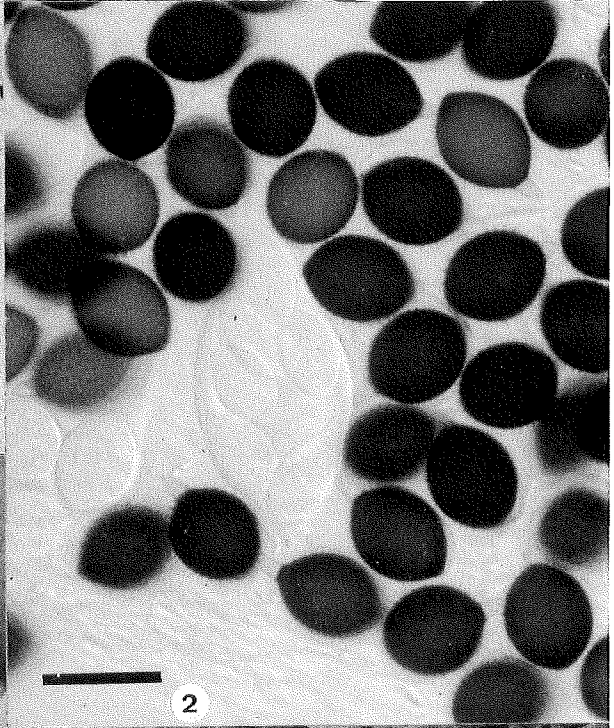
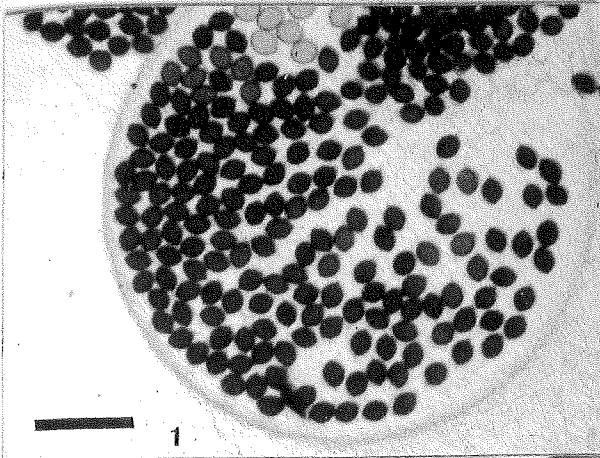
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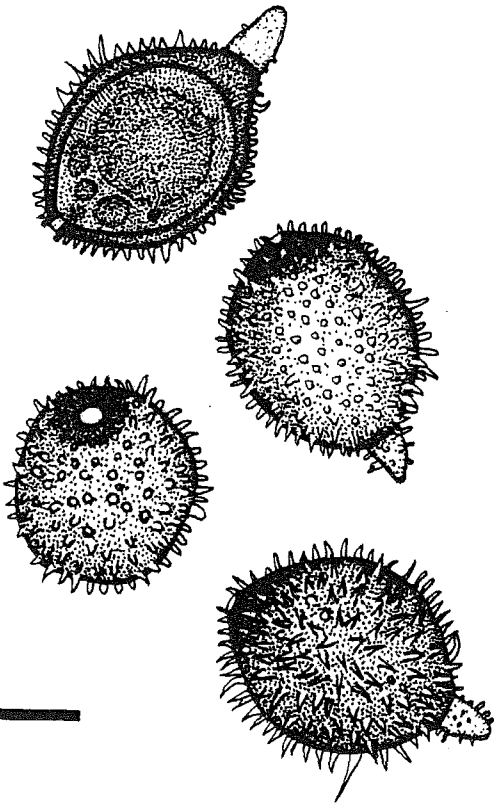
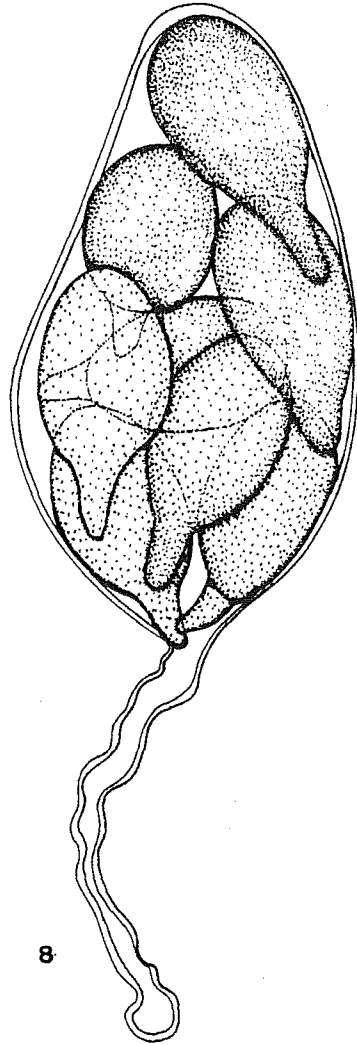
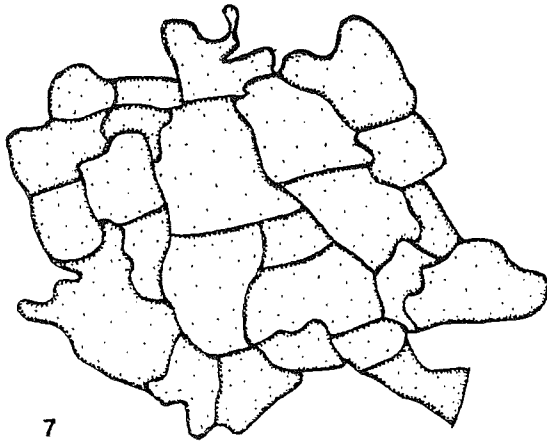
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- 1 Figs. 1-6. *Apiosordaria nigeriensis*. 1. Ascoma. 2. Ascus and ascospores. 3.  
2 Ascospores showing the spines. 4. Dark colored ascospore wall around the germ  
3 pore. 5. Ascospore (SEM). 6. Ascospore (SEM). Scale bars: 1 = 100  $\mu\text{m}$ , 2 = 30  $\mu\text{m}$ ,  
4 3, 4 = 10  $\mu\text{m}$ , 5, 6 = 5  $\mu\text{m}$ .
- 5
- 6 Figs. 7-9. *Apiosordaria nigeriensis*. 7. Peridium detail. 8. Ascus. 9. Ascospores. Scale  
7 bars: 7-9 = 25  $\mu\text{m}$ .
- 8
- 9 E-mail: [umb@astor.urv.es](mailto:umb@astor.urv.es)





**1.10. *Antarctomyces psychrotrophicus* gen. et sp. nov., un nuevo ascomiceto de la Antártida**

1.10.1. A. M. Stchigel, J. Cano, W. Mac Cormack & J. Guarro. *Antarctomyces psychrotrophicus* gen. et sp. nov., a new ascomycete from Antarctica. *Mycological Research* (aceptado)



***Antarctomyces psychrotrophicus* gen. et sp. nov., a new ascomycete  
from Antarctica.**

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We describe and illustrate a new ascomycete, *Antarctomyces psychrotrophicus* gen. et sp. nov., characterised by naked asci, hyaline, thick-walled, ellipsoidal to fusiform, echinulate ascospores, and blastoconidia, isolated from Antarctic soil samples. Analysis of the nuclear rDNA ITS region sequences showed that this taxon is related with members of Thelebolaceae.

Antarctic region, Ascomycota, *Calyptrozyma arxii*, *Monascella botryosa*, South Shetland  
Islands

During the summer expedition of the "Instituto Antártico Argentino" to the Antarctica, one of our party (W. M. C.) collected a number of soil samples from near the "Jubany" Argentinian base (King George Island, South Shetland Islands). Two strains of an undescribed ascomycete were also isolated in axenic culture. These were characterised by rudimentary ascomata, composed of a cluster of a small number of asci without peridium, and hyaline, ellipsoidal to fusiform, spinulose ascospores. It was difficult to determine its taxonomic placement from morphological characteristics alone, so we compared the sequence of its ITS region with those of other morphologically similar fungi of uncertain taxonomic position and others belonging to Eurotiales, Onygenales, Pezizales and Sordariales.

## MATERIAL AND METHODS

### Fungal isolation

Soil samples were collected near the "Jubany" Argentinian base (62° 14'S, 58° 40'W) is located on King George Island, South Shetland Islands, Antarctica. The terrain is basically basaltic and meteorised rocks and penguin dung is very common. The vegetation is mainly algae as *Prasiola crispa*, lichens as *Acarospora molybdina*, *Lecidea auriculata*, *Placodium spp.*, mosses (*Andreaea depressinervis*, *A. regularis*, *Brachytecium antarcticum*, *Bryum dichotomum*, *Grimmia antarctici*, *Hypnum sarmentosum*, *Pogonatum alpinum*, *Tortula excelsa*, etc.), *Colobanthus quitensis*, *Deschampsia antarctica* and *Poa pratensis* (Cabrera, 1994; Lindsay, 1971; Möller and Dreyfuss, 1996). The area is dominated by a cold climate. The following data were reported for 1995: average temperature was -1.5 °C, with a minimum of -19.9 °C and a maximum of 10.4 °C; total annual precipitation was 273 mm and total annual snow was 1257 cm;

minimum, average and maximum atmospheric pressure were 946 mb, 989.9 mb and 1025 mb, respectively; average humidity was 88 %. Material was collected mainly from the A horizon, placed into sterilised polyethylene bags closed by rubber band, and stored in a refrigerator at -20 °C.

Fungal isolation was done using the soil plate method described by Warcup (1950) in which suspensions were cultured on potato carrot agar with 30 mg/l chloramphenicol (PCA; potatoes, 20 g; carrot, 20 g; agar, 20 g; tap water, 1,000 ml; prepared by ourselves). We also performed a modification of Furuya and Naito's method (1979). Approx. 1 g of soil was suspended in 5 ml of 5 % v/v acetic acid, shaken vigorously for 5 min and left for a further 5 min. The layer of acetic acid was removed by decantation, the residual soil was resuspended with 9 ml of sterilised water, and the suspensions were plated in a Petri dish. PCA with chloramphenicol was placed on top of the soil suspension and mixed. All cultures were incubated at 11-12 °C under 12 h of darkness, alternating with 12 h of cool white fluorescent light.

The strains were grown on oatmeal agar (OA; Difco), PCA, potato dextrose agar (PDA; Difco) and malt extract agar (MEA; Difco) at room temperature (22-25 °C), at 11-12 °C and at 4-6 °C under 12 h of darkness, alternating with 12 h of cool white fluorescent light. Colour notations in parentheses are from Kornerup and Wanscher (1984). The structures were measured in lactophenol.

### **Molecular study**

Table 1 lists the strains used in the study. The sequences obtained from Momol & Kimbrough (1994) are not available in any DNA sequences database checked.

*Monascus purpureus*, *Neurospora cassa* and *Talaromyces flavus* var. *macrosporus* were obtained from EMBL. New sequences were obtained from *Amauroascus niger*,

*Amauroascus volatilis-patellus*, *Antarctomyces psychrotrophicus*, *Aphanoascus keratinophilus*, *Calypotryma arxii* and *Monascella botryosa*. The DNA was isolated as described by Estruch et al. (1989) with some modifications (Guillamón et al., 1996). The strains were grown at 20 °C in Sabouraud broth in ehrlenmeyer flasks and shaken at 200 rpm. The mycelium was collected by filtration through nylal mesh (42 µm pore size), washed with distilled water, blotted with paper towels, frozen with liquid nitrogen and ground to a fine powder with a mortar and pestle. The powder was incubated for 1 h at 65 °C in 2 ml of extraction buffer 80.2 M TrisHCl pH 8.0, 0.25 M NaCl, 25 mM EDTA, 0.5 % SDS). The lysate was extracted with phenol-chloroform-isoamyl alcohol solution (25:24:1) and DNA was recovered by isopropanol precipitation. The pellet was washed with 70 % v/v ethanol, dried under vacuum and resuspended in TE buffer (10 mM TrisHCl pH 8.0, EDTA 1mM).

The rDNA ITS region containing ITS1 and ITS 2 and the intervening 5.8 S rRNA gene were amplified as described by Gené et al. (1996), using a Perkin Elmer 2400 thermal cycler (Perking Elmer Cetus corporation, Emeryville, CA). Primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990) were used. The amplification program consisted of pre-denaturalisation at 94-6 °C for 5 min, 30 cycles at 95 °C for 30 sec, 50 °C for 1 min and 72 °C for 1 min, and final incubation at 72 °C for 7 min to complete the final extension. The final products were resolved by electrophoresis in a 2 % agarose MP gel (Boehringer Mannheim), and cleaned following the GENE CLEAN II protocol (BIO 101). The molecular weights of amplified DNA were estimated by comparing them with 100 bp DNA leader (Gibco BRL) standard lane.

The protocol "Taq DyeDeoxy Terminator Cycle Sequencing Kit" (Applied Biosystems, Gouda, The Netherlands) was used for sequencing. Reactions were performed using the primers ITS5 and ITS4 (White et al., 1990) and run on a 310 DNA sequencer (Applied Biosystems). The new sequences were aligned using the Clustal W, version 1.5, computer program for multiple sequence alignment (Thompson et al., 1994). Cladistic analyses using the neighbor-joining method (Saitou and Nei, 1987) and parsimony were performed with the MEGA 1.0 computer program (Kumar et al., 1993). Confidence values for individual branches were determined by bootstrap analyses (1000 pseudoreplicates). Nucleotide composition, frequencies from pairwise comparisons and alignment gap sequences were performed with the MEGA 1.0 computer program.

## RESULTS AND DISCUSSION

### Taxonomy

#### **Antarctomyces** Stchigel & Guarro, gen. nov

Hyphis septatis, ramosis vel simples, anamostosantes, hyalinis. Ascوماتа per asci nudi compositum, sine excipulos. Asci ellipsoidei vel subglobosi, unitunicati, non-catenati, octospori. Paraphyses nullae. Ascospорae ellipsoideae vel fusiformis, hyalinae, spinulosae, sine poro germinali, unicellulares. Species typica: *Antarctomyces psychrotrophicus* Stchigel & Guarro.

Mycelium mainly submerged, composed of septate, branched and unbranched, anastomosing, hyaline hyphae. Ascوماتа composed of naked asci, without excipulum. Asci ellipsoidal to subglobose, unitunicate, non-catenate, 8-spored. Paraphyses absent.

Ascospores ellipsoidal to fusiform, hyaline, spinulose, without germ pores, 1-celled. Type species: *Antarctomyces psychrotrophicus* Stchigel & Guarro.

***Antarctomyces psychrotrophicus* Stchigel & Guarro, sp. nov.** (Figs 1-15)

Anamorph: blastoconidia.

Hyphis hyalinis, ramosis vel simples anastomosantes, septatis, (1-)4-7  $\mu\text{m}$  diam composito, tenuitunicati vel crassitunicati. Coloniae in agarum cum decocto tuberorum et carotarum (PCA) planae, tenues, hyalinae. Ascomata e hyphis initialibus duabus involutis formantes. Ascomata per asci nudi compositum, 2-7 in numero, sine excipulos. Asci 15-19 x 12-13  $\mu\text{m}$ , ellipsoidei vel subglobosi, non-estipitati, unitunicati, crassitunicati, non-catenati, octospori. Paraphyses nullis. Ascosporae 7-10 x 4-5.5  $\mu\text{m}$ , ellipsoideae vel fusiformis, hyalinae, spinulosae, sine poro germinali, unicellulares.

Typus: Antarctica, South Shetland Islands, King George Island, ex solo, 10 Nov 1996, leg. W. Mac Cormack, isol. A. M. Stchigel (IMI 378528- holotypus, FMR 6368-isotypus).

Mycelium mainly submerged, composed of hyaline, branched and unbranched, anastomosing, septate hyphae; hyphae (1-)4-7  $\mu\text{m}$  broad, thin to thick-walled. Colonies on PCA attaining a diam of 33-47 mm in 14 d at 22-25  $^{\circ}\text{C}$ , plane, thin, white, with irregular margins; reverse uncolored. Ascomatal initials begin to develop from the coiling of two side branches, occasionally disposed in tandem. Ascomata composed of naked asci, single or grouped in number of 2-7, arising directly from the fertile hyphae, without excipulum. Asci 15-19 x 12-13  $\mu\text{m}$ , subglobose to ellipsoidal, non-stipitate, unitunicate, thick-walled, non-catenate, 8-spored, developed form croziers. Paraphyses absent.

Ascospores 7-10 x 4-5.5  $\mu\text{m}$ , ellipsoidal to fusiform, hyaline, spinulose, thick-walled, without germinal pores, one-celled; spines approx. 0.5  $\mu\text{m}$  long. Anamorph:  
Conidiophores 4-7  $\mu\text{m}$  thick, hyaline, with lateral cylindrical protuberances measuring 2-5 x 1-2  $\mu\text{m}$ . Conidiogenous cells enteroblastic, integrated, intercalary, determinate. Conidia 3-20 x 2-5  $\mu\text{m}$ , subglobose to irregularly cylindrical, hyaline, smooth, thick-walled, aggregated in slimy masses, one-celled. Chlamydozoospores 10-15 x 5-8  $\mu\text{m}$ , irregular, single or forming long chains, one or two-celled.

Colonies on PCA after 14 d attaining a diam of 32-43 mm at 11-12 °C and 26-28 mm at 4-6 °C, plane, thin, zonate, vegetative mycelium mainly submerged, uncolored; reverse uncolored. Asci and chlamydozoospores abundant; conidia absent.

Colonies PDA attaining a diam of 65-71 mm in 14 d at 22-25 °C, plane, thin, irregular margins, vegetative mycelium mainly submerged, uncolored; reverse uncolored. Chlamydozoospores present; in long chains, asci absent; conidia present. At 11-12 °C the colonies attain a diam of 62-68 mm in 14 d and 35-37 mm at 4-6 °C, plane, thin, irregular margins, vegetative mycelium mainly submerged, dull blue (M 23D4), light blue funicles in the central area, composed of sterile hyphae; reverse with the same colour. Asci abundant in the marginal area; chlamydozoospores in long chains; conidia absent.

Colonies on OMA attaining a diam of 50-55 mm in 14 d at 22-25 °C, plane, thin, margins fimbriate, vegetative mycelium mainly submerged, uncolored; reverse uncolored. Chlamydozoospores very abundant, in long chains; asci absent; conidia present. At 12 °C the colonies attain a diam of 35-45 mm and at 4-6 °C a diam of 26-29 mm in 14 d, plane, thin, vegetative mycelium mainly submerged, uncolored; reverse uncolored. Asci and chlamydozoospores abundant; conidia absent.

Colonies on MEA attaining a diam of 54-58 mm in 14 d at 22-25 °C, with the same cultural characteristics as in OMA. Moniliform mycelium present; asci and

chlamydospores absent; conidia absent. At 12 °C the colonies attaining a diam of 45-49 mm in 14 d and 20-22 mm at 4-6 °C, plane, thin, with vegetative mycelium mainly submerged, uncolored; reverse uncolored. Moniliform mycelium present; asci and chlamydospores absent; conidia absent.

The main features of the *A. psychrotrophicus* ITS1-2 and 5.8 S rDNA region sequence are: 547 bp; 130 A; 131 C; 130 G and 156 T. The location ITS1 from nucleotide 33 to 192, the gene 5.8S rRNA from nucleotide 193 to 364 and the location ITS2 from nucleotide 365 to 499.

Two other ascomycetes with simple sexual structures consisting of clusters of a few asci with no type of peridium or excipulum and with more or less ellipsoidal and hyaline ascospores are *Calypetrozyma arxii* Boekhout & Spaay and *Monascella botryosa* Guarro & Arx each of which is monotypic. The latter was isolated from Spanish soil (Guarro and Arx, 1986) and accommodated in the Onygenaceae (Hawksworth et al., 1995). No anamorph has been observed in this species. *Calypetrozyma* was isolated from human oesophagus in the U.S.A. (Boekhout et al., 1995) and provisionally placed in Eurotiales, though not in any family (Eriksson and Hawksworth, 1996). The three taxa are distinguished mainly by their anamorphs, which are blastoconidia in *Antarctomyces*, aleurio-, arthro- and blastoconidia in *Calypetrozyma* and absent in *Monascella*; the ascomata initials which are erect ascogonia surrounded by coiled anteridia in *M. botryosa*, aggregations of generative hyphae in *C. arxii* and clustered antheridia and ascogonia in *A. psychrotrophicus*; the asci which are clavate to obovate in *M. botryosa*, cylindrical in *C. arxii*, and spherical to subspherical in *A. psychrotrophicus*; and the ornamentation of the ascospores which are smooth walled in *C. arxii* and in *M. botryosa*,



and spinulose in *A. psychrotrophicus*. Differences in the ITS-rRNA gene sequences of these three species confirmed their placement in different genera.

To infer the phylogenetic relationships of these taxa with other morphologically similar ones and to establish a more precise taxonomic position, we have compared their ITS sequences with those of 13 other species, some of which were obtained from the EMBL. We chose representatives of Thelebolaceae (*Thelebolus* sp), Eurotiales (*Monascus ruber* and *Talaromyces flavus*), Onygenales (*Amauroascus niger*, *A. volatilis-patellis* and *Aphanoascus keratinophylum*), Pezizales (*Ascodesmis nigricans*, *A. sphaerospora*, *Eleutherascus lectardii*, *Lamprospora* sp., *Pyronema domesticum* and *Saccobolus depauperatus*), and Sordariales (*Neurospora crassa*). The phylogenetic tree, based on analyses of the ITS-5.8 rRNA gene sequences of all taxa studied with the neighbor-joining method, demonstrated that *Antarctomyces psychrotrophicus*, *Calyptrozyma arxii* and *Monascella botryosa* were not closely related phylogenetically. These analyses showed the existence of two well-supported clades (Fig. 16). The first clade, supported by a bootstrap value of 56 % encompasses the Onygenales, Eurotiales and a sister subclade composed of *Antarctomyces psychrotrophicus*, *Calyptrozyma arxii* and *Thelebolus* sp. (with a bootstrap value of 100 %). In this case *C. arxii* may be the ancestor of the other two taxa. However, it is very difficult to establish any morphological relationship among these tree taxa. *Thelebolus* and *Antarctomyces* share only thick-walled asci and ellipsoidal to fusiform, thick-walled ascospores, which do not have germ pores. The second clade, supported by a bootstrap value of 100 %, is formed by the pezizalean fungi. *Monascella botryosa* was also included in this group. It did not cluster surprisingly with those species that have simpler ascomatal structures, such as *Ascodesmis* spp., *Eleutherascus lectardii* and *Saccobolus depauperatus*, but with *Lamprospora* sp. and *Pyronema domesticum* (Pyronemataceae) whose ascomata are

more developed. Kimbrough (1989) pointed out a close relationship of some members of Onygenales with simple structures and naked asci such as *Amauroascus* with the Pezizales. He included this genus, together with *Ascodesmis* and *Eleutherascus*, in the family Ascodesmidaceae (Pezizales). Considering the asci structure, we disagreed that there was such a relationship between Pezizales and Onygenales (Guarro *et al.*, 1992). In this study the two species of *Amauroascus* were placed in a different clade from the Pezizales, which confirms our previous opinion.

## ACKNOWLEDGEMENTS

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Table 1. List of strains, sources and sequences used in the analysis

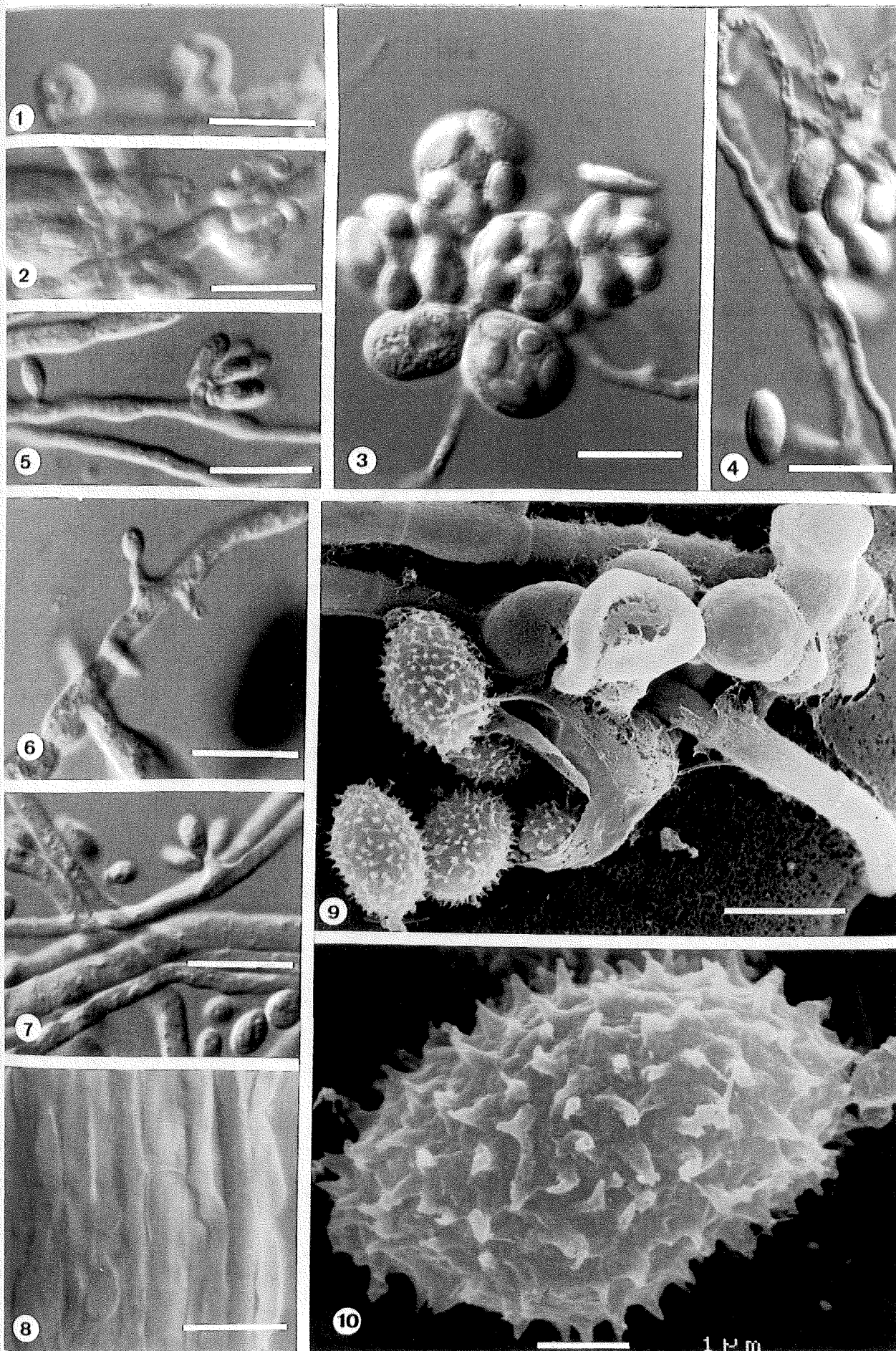
Species	Strain	Origin	EMBL accession numbers
<i>Amauroascus niger</i> Schroeter	IFO 32599	soil	AJ 133434
<i>Amauroascus volatilis-patellus</i> (Orr & Kuehn) Currah	UAMH 3406	soil	AJ 133435
<i>Antarctomyces psychrotrophicus</i> gen. et sp. nov	FMR 6368	soil	AJ 133431
<i>Aphanoascus keratinophilus</i> Punsola & Cano	IMI 319010	soil	AJ 133436
<i>Ascodesmis nigricans</i> V. Thieghem*	FLAS 122	soil	-
<i>Ascodesmis sphaerospora</i> Obrist*	FLAS 260	rat dung	-
<i>Calyptrozyma arxii</i> Boekhout et al.	CBS 354.92	human oesophagous	AJ 133432
<i>Eleutherascus lectardii</i> (Nicot) Arx*	FLAS 300	salty soil	-
<i>Lamprospora</i> sp.*	FLAS 346	soil	-
<i>Monascella botryosa</i> Guarro & Arx	CBS 233.85	soil	AJ 133433
<i>Monascus purpureus</i> Went.	ATCC 16365	-	U18356
<i>Neurospora cassa</i> Shear & B. O. Dodge	-	-	M13906
<i>Pyronema domesticum</i> (Sowerby ex Gray)	ATCC 14881	steamed soil	-
Saccardo*			
<i>Saccobolus depauperatus</i> (Berk. & Br.) Kimbrough*	FLAS 106	cow dung	-
<i>Talaromyces flavus</i> var. <i>macrosporus</i> Stolk &	FRR 2386	-	U18354
Samson			
<i>Thelebolus</i> sp.*	IMI 67944	dung	-

\*The sequences of these strains were obtained directly from Momol & Kimbrough (1994)  
 ATCC= American Type Culture Collection; CBS= Centralbureau voor Schimmelcultures; FLAS= Florida Agricultural Experiment Station culture collection; FMR= Facultat de Medicina de Reus culture collection; FRR= CSIRO Food Research Laboratory; IFO= Institute of Fermentation of Osaka; IMI= International Mycological Institute, CABI; UAMH=University of Alberta Microfungus Collection and Herbarium

**Figs 1-10.** *Antarctomyces psychrotrophicus*. **Fig. 1.** Ascomata initials. **Fig. 2.** Ascomata initials forming a crozier. **Fig. 3.** Cluster of asci containing the ascospores. **Fig. 4.** Free ascospores. Note the spinulose surface and the thick ascospore wall. **Figs 5-7.** *Sporothrix*-like anamorph. **Fig. 8.** View of a funicle formed at 12 °C on PDA. **Fig. 9.** Ascus and ascospores. **Fig. 10.** Ascospore showing the spinose outer wall. **Figs. 1, 2, 5-8,** Bar 20 µm. **Figs. 3,4,** Bar 12.5 µm. **Fig. 9,** Bar 10 µm. **Fig. 10,** Bar 1 µm.

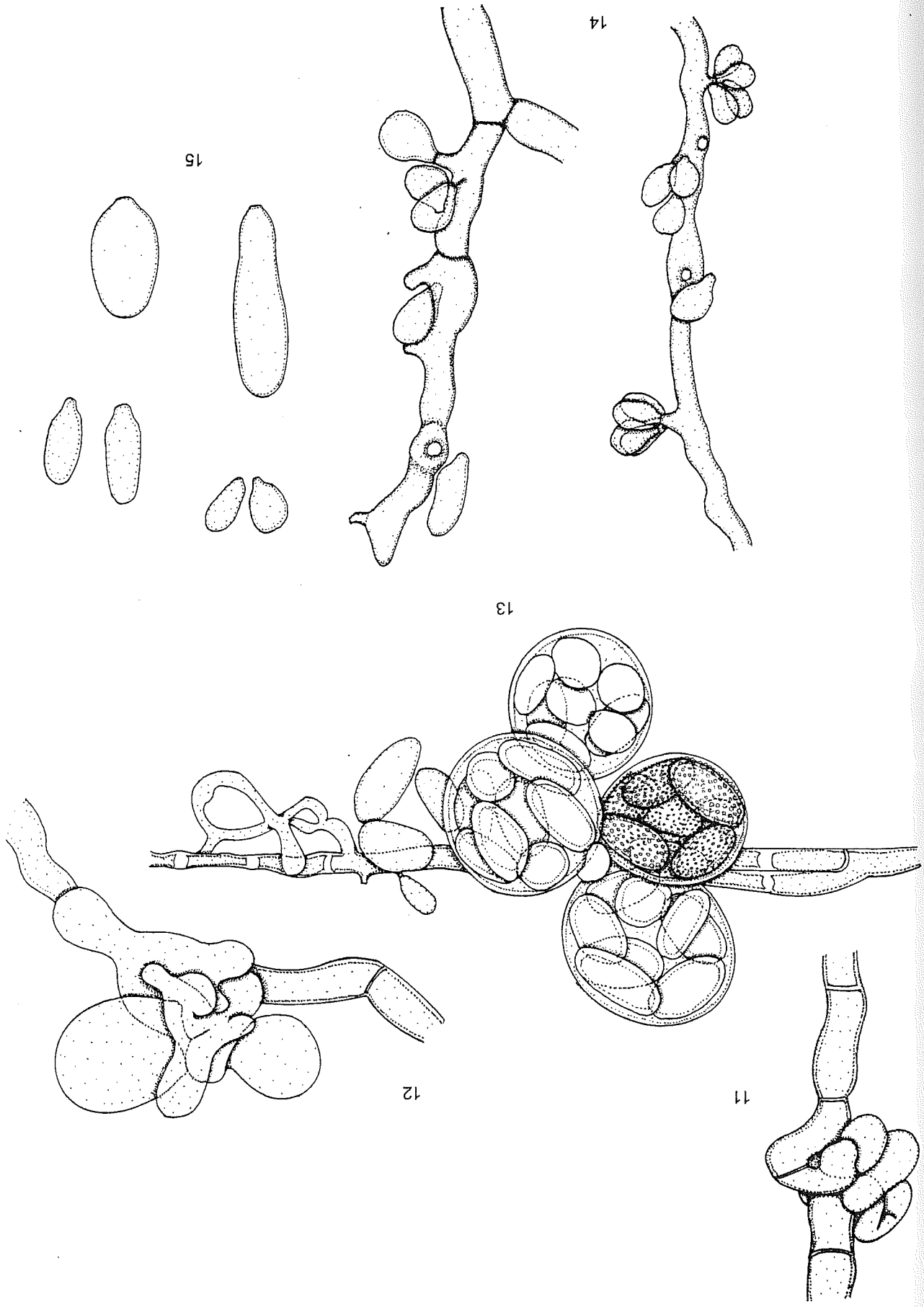
**Figs 11-15.** *Antarctomyces psychrotrophicus*. **Figs 11-12.** Ascomatal initials forms. **Fig. 13.** Cluster of thick-walled asci, showing young and mature ascospores inside. **Fig. 14.** Anamorph. **Fig. 15.** Conidia of different size. **Figs 11-15,** Bar 15 µm.

**Fig. 16.** Neighbor-joining phylogenetic tree of the aligned sequences of the studied strains. Confidence limits of branches (indicated in % along the branches) were created in a bootstrap analysis using 500 trials. The scale barr represents 0.68 % sequence divergence.





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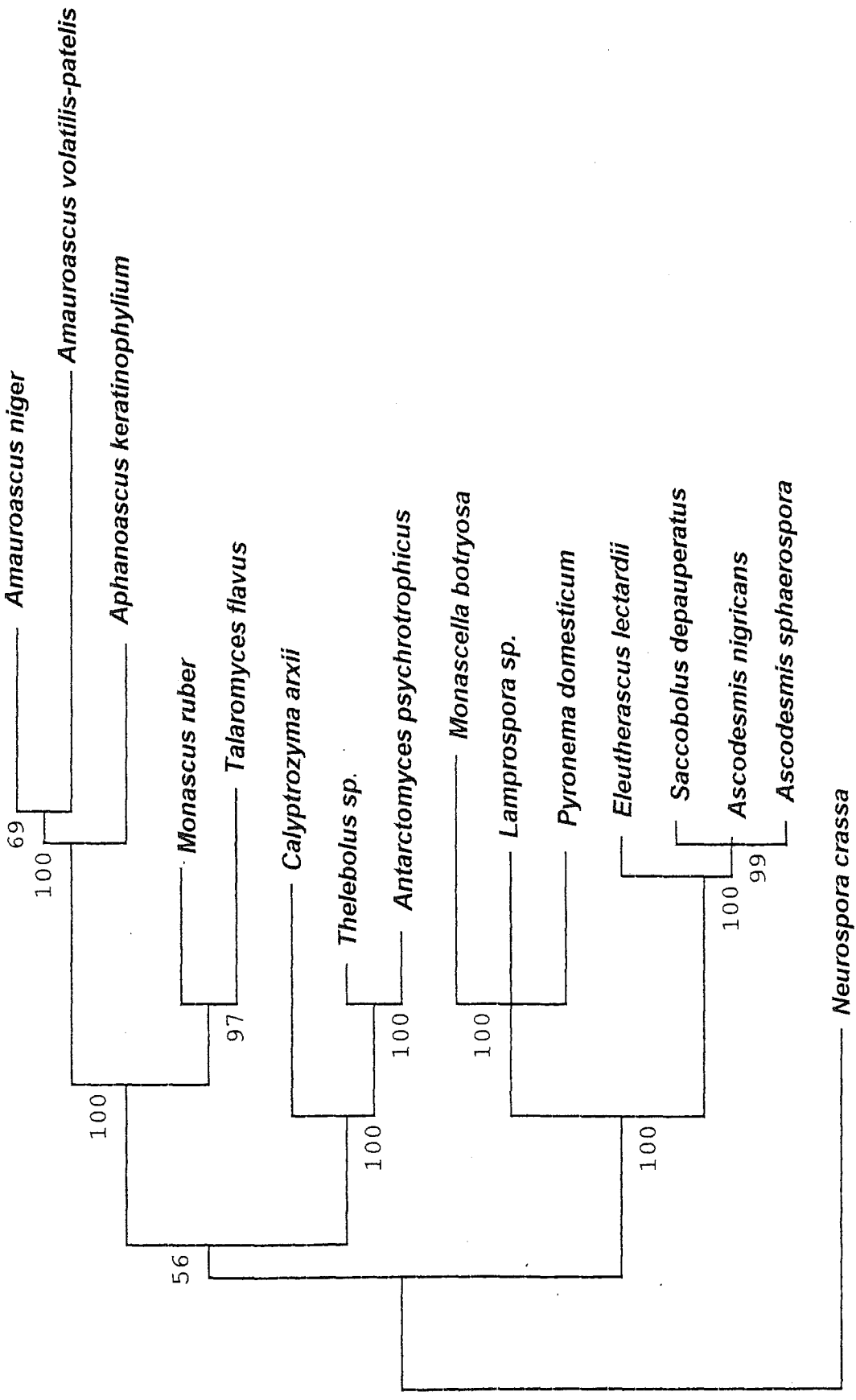


Fig. 16. Scale: each — is approximately equal to the distance of 0.0068

## 1.11. Dos nuevas especies termotolerantes de *Thielavia* del suelo, y estudio molecular de la región ITS1-2 de especies representativas del género

1.11.1. A. M. Stchigel, L. Figuera, J. Cano & J. Guarro. Two new soil-borne thermotolerant species of *Thielavia* and a molecular study (ITS region) of representative species of the genus. *Mycologia* (sometido)

1 Short title: New *Thielavia* species

2 **Two new soil-borne thermotolerant species of *Thielavia* and a molecular**  
3 **study (ITS region) of representative species of the genus**

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11  
12 **Abstract:** Two new thermotolerant species of *Thielavia* isolated from Easter Island  
13 and Indian soils, respectively, are described and illustrated. *Thielavia intermedia* is  
14 characterized by large, obovate, dark brown ascospores with a terminal germ pore.  
15 *Thielavia rapa-nuiensis* is distinguished by their large, ellipsoidal to fusiform, brown  
16 ascospores with a prominent, lateral to medial germ pore. A phylogenetic study of  
17 the ITS 1-2 sequences of 18 species of *Thielavia* and *Melanocarpus thermophilus*  
18 was performed. This analysis revealed that *T. intermedia* could be the ancestor of  
19 the remaining species and that all the species tested are genetically closely related.

20  
21 **Key Words:** Ascomycota, Chaetomiaceae, soil fungi, Sordariales,  
22 Thielaviaceae