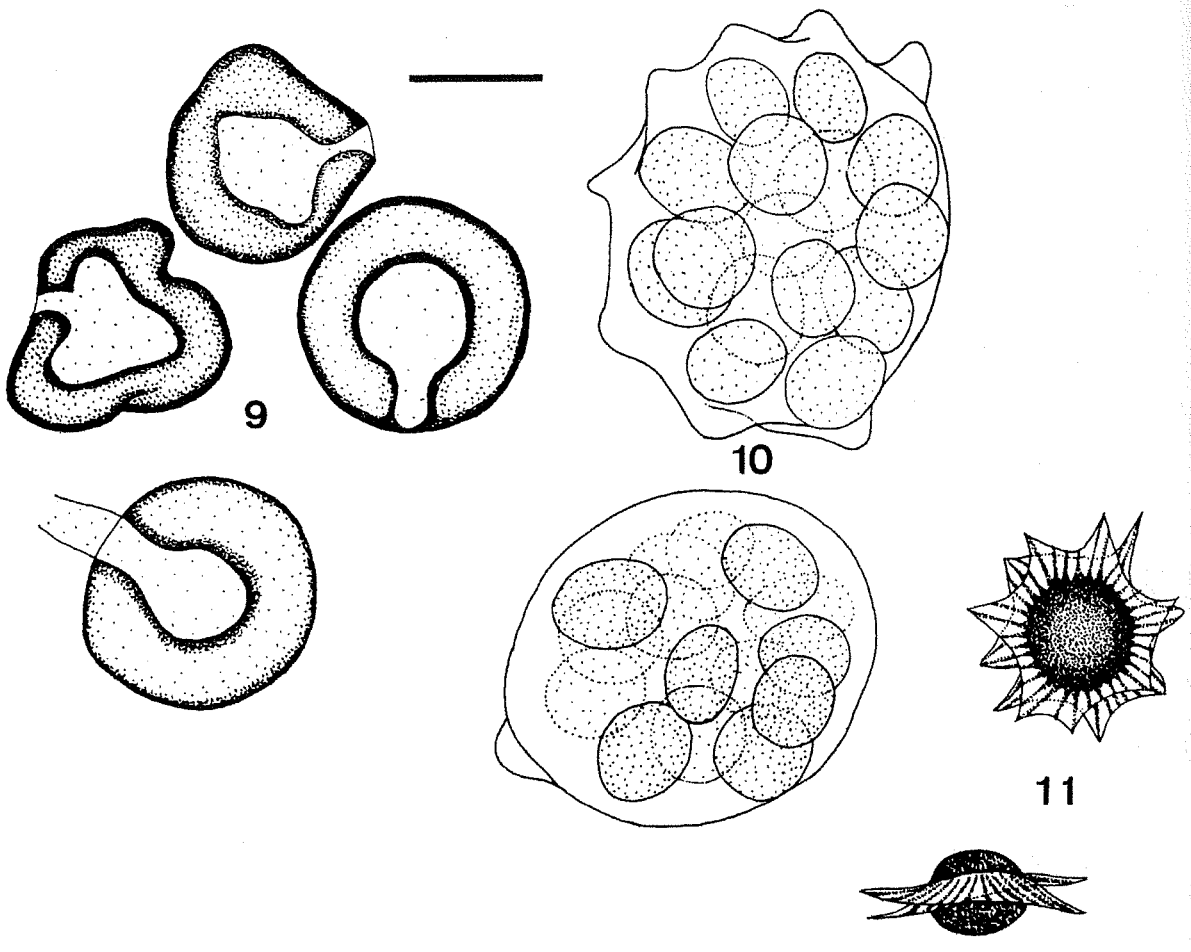

FIGS. 9–11. *Emericella pluriseminata*. 9. Hülle cells. 10. Asci. 11. Ascospores. Scale bar = 10 μm.

35 μm diam, evanescent. Ascospores one-celled, at first hyaline, becoming violet-brown, lenticular, 7–9 × 6–7 μm (crests not included), with two conspicuously pleated, stellate and striate equatorial crests, 4–8 μm wide; convex surfaces tuberculate under SEM. Anamorph absent.

Colonies on OMA growing rapidly at room temperature, attaining 48–51 mm diam in 14 d, similar to those on PCA. Ascomata and conidiophores absent.

Colonies on Czapek-Dox solution agar restricted at room temperature, attaining 18–22 mm diam in 14 d, plane, dendritic, consisting of a submerged poorly-developed mycelium with numerous chlamydospores, greyish-orange (5B5) in the center, white elsewhere; reverse color similar to surface color. Ascomata and conidiophores not produced.

Colonies on CYA growing rapidly at room temperature, attaining 49–53 mm diam in 14 d, plane, powdery in the center, brownish-grey (6B2 and 6C3), coarsely granular at the margins, brown (6D5 and 6E5), radially furrowed; hülle cells abundant; margi of submerged mycelium irregularly lobed, subhy line; reverse dark brown (7E8), with darker brow spots (7F8); carrot red (6B7) exudate; red sunbur (6D5) soluble pigment. Ascomata and conidiophore absent.

Colonies on MEA growing rapidly at room tem perature, attaining 53–55 mm diam in 14 d, consis ing of masses of hülle cells and submerged mycelium coarsely granular, light brown (6D8 and 6E8); margi irregularly lobed, fimbriate, tomato red (8C8); re verse reddish-brown (9E8 and 9F8); exudate hyalin carrot red (6B7) soluble pigment. Ascomata and co nidiophores not produced. At 37 C the growth i more rapid than at 25 C, but the ascomata not ar produced; no growth at 42 C.

Emericella is a genus of Trichocomaceae (Euro tiales, Ascomycota) with approximately 30 species They are present in soils, herbal drugs, dried fruit stored cereals, rhizosphere of cultivated plants, dun and are commonly air-borne. They can colonize d

verse plant debris and occasionally cause opportunistic infections in man and animals (de Hoog and Guarro, 1995; Domsch et al., 1980). *Emericella nidulans* (Eidam) Vuillemin has been used extensively in genetic studies. They have been reported as producers of many phenolic compounds (Cole and Cox, 1980; Domsch et al., 1980; Raper and Fennell, 1965; Turner and Aldridge, 1983). The typical characteristics of the species of *Emericella* are the *Aspergillus* anamorph, cleistothecial ascomata enveloped by a dense layer of hülle cells and orange-red to blue-violet lenticular ascospores, usually showing definite equatorial crests and with convex surfaces ornamented or smooth. This taxon does not form an anamorph, but the rest of its features suggest it should be placed in *Emericella*. *Emericella pluriseminata* is the only species of the genus that has 16-spored asci. Only two other species of *Emericella* lack anamorphs, *E. similis* Horie et al. and *E. desertorum* Samson & Mouchacca. *Emericella similis* also has violet ascospores, but they are smaller (4.0–5.5 × 3–4 μm), and *E. desertorum* has orange-red and nonstellate ascospores. The species of *Emericella* quickly produce abundant ascomata on common media such as Czapek and MEA, but in *E. pluriseminata* the ascomata are only produced on PCA, and then only after several months. Another species of *Emericella* with violet ascospores is *E. violacea* (Fennell & Raper) Malloch & Cain. However, this can be differentiated from *E. pluriseminata* by the small ascospores (5.5–6.5 × 4–5 μm, which are not stellate. *Emericella variecolor* Berk. & Br. has similarly stellate ascospores, but they are orange-red to purple-red and measure 3.6–4.0 × 2.8–3.0 μm (excluding crests). Its asci are 8-spored and the hülle cells are colorless to purple-violet. The lack of anamorph together with the late appearance of the ascomata make the recognition and identification of *E. pluriseminata* difficult.

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1.3. Una nueva especie de *Ascotricha* del suelo de España

1.3.1. A. M. Stchigel & J. Guarro. 1998. A new species of *Ascotricha* from Spanish soil. *Mycological Research* 102, 510-512

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A new species of *Ascotricha* from Spanish soil

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Ascotricha hispanica sp. nov. is described and illustrated. It is characterized by ostiolate ascomata with a translucent peridium and subdichotomously branched setae, cylindrical asci and oblate ascospores with an equatorial germ slit. It is compared with related species.

During the course of a study of soil ascomycetes from Spain, an interesting species of *Ascotricha* Berk. was isolated. Its morphological characteristics differentiate this taxon from all previously described species in this genus (Hawksworth, 1971; Udagawa *et al.*, 1994*a*, *b*) and it is described here as new.

Soil samples were collected near Palencia, Spain. The terrain is basically calcareous, and the vegetation is composed mainly of *Quercus*, *Ilex* and *Pinus* spp., and numerous members of Poaceae. The area is dominated by a temperate continental climate. The average temperature is 0–6 °C in January and 18–24° in July. The total annual precipitation is 300–600 mm. Collections were made mainly from the A_0 soil horizon by using previously sterilized polyethylene bags. These were enclosed by rubber bands and labelled. On return to the laboratory the materials were placed in a refrigerator at 4–7°, where they were held until used. The soil samples were treated with 5 ml of acetone (Sigma) for 10 min. The supernatant was discarded and the solid phase was suspended in 10 ml of water. The suspensions were cultured on potato carrot agar (PCA), at room temperature (22–25°), under 12 h of darkness alternating with 12 h of cool white fluorescent light. Measurement of the fungal structures was performed on material mounted in water or lactophenol.

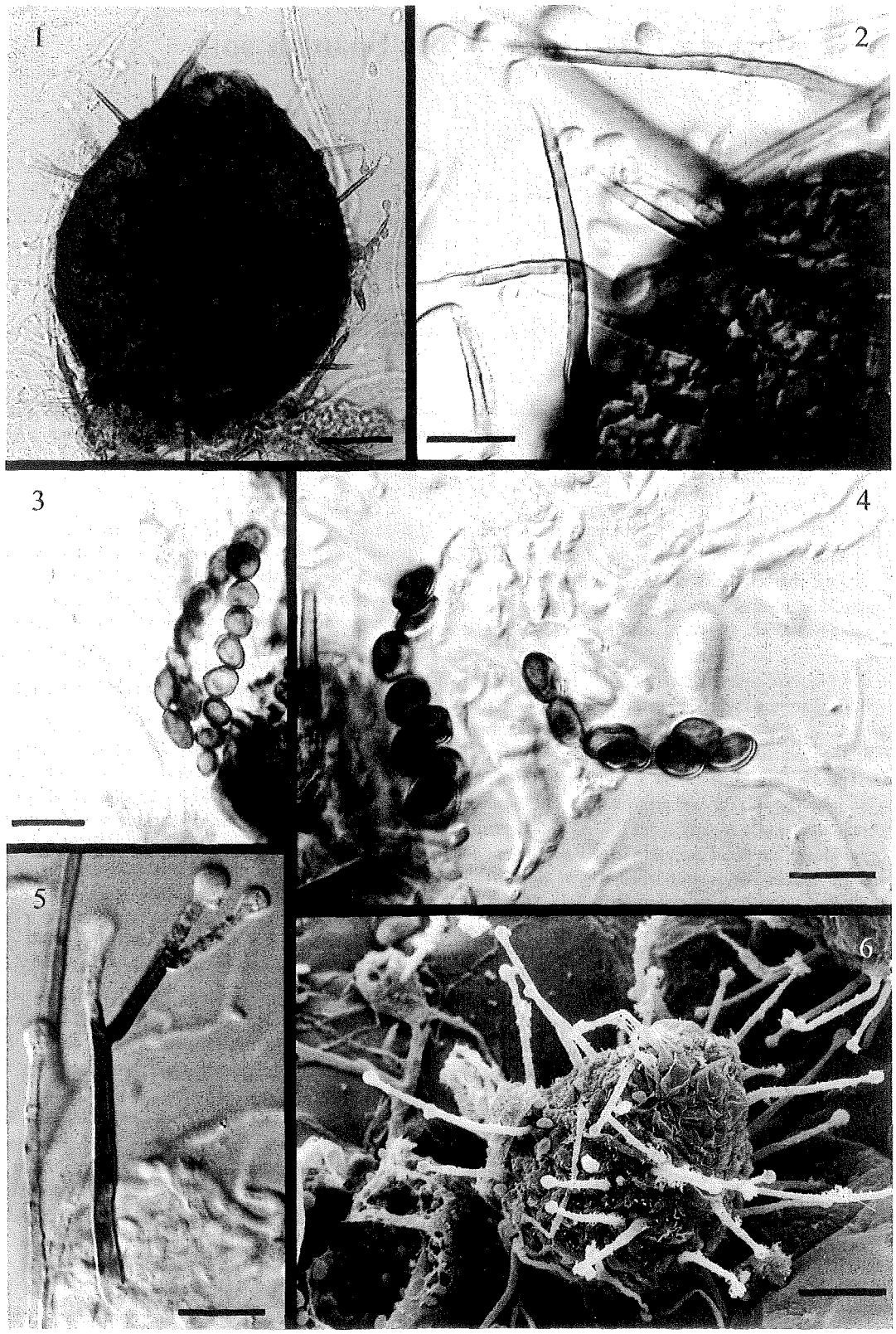
Ascotricha hispanica Stchigel & Guarro, sp. nov. (Figs 1–9)

Coloniae in agaro cum decocto tuberorum et carotarum 'PCA' restrictae, planae, tenues, ex mycelio vegetativo submerso, subhyalinae vel brunneae, ascomata tarde producentes, brunnea vel nigra; mycelio ex hyphis subhyalinis vel brunneis, ramosis, septatis, laevibus vel tuberculatis, 0·5–6 µm diam. composito; reversum brunneum. *Ascomata* superficialia, ostiolata, translucida, brunnea vel atrobrunnea, subglobosa vel piriformia, 90–140 × 60–120 µm, apex cum collo brevi, 10–20 µm alta, 10–30 µm lata, pilosa; *pili* rigidiusculi, 0–2 ramosi, laeves vel subtiliter asperati, septati, atrobrunnei, 30–100 µm longi, 3–5 µm diam. ad basim, cum vesicula globosa vel claviformi et hyalina, laevi vel asperata, 3–6 µm diam. ad apicem formantes; rami hyalini vel brunnei, sterili, aseptati vel septati, rugosi, 8–35 × 1·5–2·5 µm, cum vesicula ad apicem formantes; *peridium* parum brunneum vel brunneum, tenue, ex textura epidermoidea vel intricata compositum, ex 2–3 stratis compositum. *Asci* 8-spori, lineari-cylindrici, cum muris tenuibus, 35–55 × 7–10 µm, stipitati, deliquescentes; *paraphyses* nullae. *Ascosporae* uniseriatae, oblatae, atrobrunneae, 4·5–7 × 3–4 × 3–4 µm, laeves, fissura germinali aequatoria paratae. Status conidialis nullus.

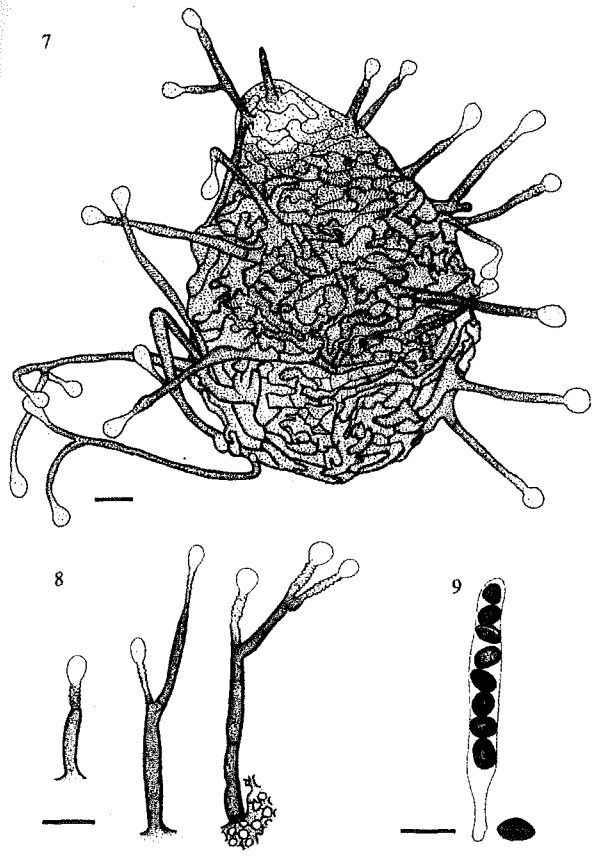
Holotypus IMI 374709 (FMR 5872), ex solo, Palencia, España, 22 Aug. 1996. Leg. O. V. Rocca.

Colonies on PCA growing slowly, 14–20 mm diam. in 14 d at ambient temperature (approx. 25°), flat, thin, consisting of submerged mycelium and sparse aerial hyphae, later producing more or less abundant ascomata; hyphae subhyaline to brown, branched, septate, smooth to tuberculate, 0·5–6 µm thick; reverse brown. Ascoma initially coiled; *ascomata* superficial, scattered to grouped, ostiolate, subglobose to piriform, translucent, brown to dark brown, 90–140 × 60–120 µm, sometimes with a short setose beak 10–20 µm × 10–30 µm (Figs 1, 6, 7). *Setae* stiff, simple to twice branched, with a subdichotomous branching pattern, smooth to slightly tuberculate, septate, dark brown, 30–100 µm long, 3–5 µm diam. at the base, thick-walled, hyaline to pale brown and tuberculate in the upper part, terminated by a vesicle; branches subterminal, hyaline or dark brown, septate or aseptate, tuberculate in the upper part, finishing in a hyaline, smooth to rough, globose to obpyriform vesicle 3–6 µm diam. (Figs 2, 5, 8). *Peridium* 2–3 layered, thin, 4–10 µm thick, pale brown to brown, *textura epidermoidea* to *intricata* in surface view (Figs 2, 7). *Asci* 8-spored, fasciculate, cylindrical, thin-walled, 35–55 × 7–10 µm, stipitate, without apical structures, evanescent (Fig. 9); *paraphyses* not observed. *Ascospores* uniseriate, oblate, ovoid to ellipsoidal in front view, dark brown, 4·5–7 × 3–4 × 3–4 µm, smooth-walled, with an equatorial germ slit (Figs 3, 4, 9). Lageniform branches similar to ascomatal hairs emerged from the mycelium, but conidia were not observed (Fig. 9).

Holotype: IMI 374709 (FMR 5872), from soil, Palencia, Spain, 22 Aug. 1996. Leg. O. V. Rocca.



Figs 1–6. *Ascotricha hispanica*. **Fig. 1.** Setose ascoma. Bar, 25 µm. **Fig. 2.** Setose peridial wall. Bar, 10 µm. **Fig. 3.** Asci and ascospores. Bar, 10 µm. **Fig. 4.** Asci and ascospores. Bar, 10 µm. **Fig. 5.** Typical subdichotomously branched setae. Bar, 10 µm. **Fig. 6.** Ascoma (S.E.M.). Bar, 20 µm.



Figs 7–9. Fig. 7. *Ascotricha hispanica*. Ascoma. Bar, 10 µm. **Fig. 8.** *Ascotricha hispanica*. Setae. Bar, 10 µm. **Fig. 9.** *Ascotricha hispanica*. Ascus with ascospores. Bar, 10 µm.

Ascotricha has been considered a member of the Xylariaceae mainly due to its amyloid plug and type of anamorph (Hawksworth *et al.*, 1995), although some similarities with members of the Coniochaetaceae have been noticed (Hawksworth & Wells, 1973). However, Laessøe (1994) considered it a genus *incertae sedis* due to its non-stromatic nature. *Ascotrichella* Valldos. & Guarro (1988) is a related genus, also non-stromatic and with a *Humicola*-like anamorph, also considered as *incertae sedis* by Laessøe (1994). The relationships between these genera and between the Coniochaetaceae and the Xylariaceae are confusing and need reassessment.

Ascotricha contains 11 species (Ames, 1951; Hawksworth, 1971; Kulshreshtha, Raychardhuri & Khan, 1977; Udagawa, Uchiyama & Kamiya, 1994*a*, *b*). They are characterized by ostiolate or non-ostiolate setose ascomata, a translucent peridial wall, 8-spored asci, brown ellipsoidal ascospores with an equatorial germ slit, and an anamorph in *Dicyma* Boulanger or close to the *Geniculosporium–Nodulisporium* complex. They are isolated mainly from cellulosic substrates (Ames, 1951; Hawksworth, 1971; Calviello, 1978), dung (Hawksworth, 1971; Kahn & Cain, 1977) and soil (Hawksworth, 1971; Horie *et al.*, 1993; Udagawa *et al.*, 1994*a*).

Based on the ascospore arrangement and the shape of the asci we can divide *Ascotricha* into two groups. The first, with cylindrical asci, includes *A. amphitricha* (Corda) S. Hughes, *A. xylina* L. M. Ames, *A. erinacea* Zambett., *A. chartarum* Berk., *A. guamensis* L. M. Ames, *A. amesii* D. Hawksw., *A. bosei* D. Hawksw., *A. lusitanica* Kenn. and *A. delhiana* Kulshr., Raych. & A. M. Khan. The second, with non-ostiolate ascomata and clavate asci, is related to *Coniochaetidium* Malloch & Cain and includes the following species isolated exclusively from soil: *A. distans* Udagawa, Uchiy. & Kamiya and *A. novae-caledoniae* Udagawa, Uchiy. & Kamiya. *Ascotricha hispanica* belongs to the first group. It can be easily differentiated from all previously described species mainly by the setal morphology and by the absence of an anamorph.

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(Accepted 15 July 1997)

1.4. Una nueva especie de *Gelasinospora* del suelo de Argentina

1.4.1. A. M. Stchigel, J. Cano & J. Guarro. 1998. A new species of *Gelasinospora* from Argentinian soil. *Mycological Research* 102, 1405-1408

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A new species of *Gelasinospora* from Argentinian soil

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Gelasinospora bonaerensis sp. nov., isolated from a soil sample collected in Argentina, is described and illustrated and the sequences corresponding to the ITS1/ITS2 regions and the 5·8 S ribosomal RNA are provided. Its taxonomic relationships with other species of the genus are discussed.

During studies on soil Ascomycetes from many regions of the world, we have isolated numerous *Gelasinospora* strains. *Gelasinospora* is characterized by dark, pyriform or globose ascomata, cylindrical, fasciculate, stipitate and unitunicate asci, and one-celled, dark ascospores with ornamented walls. The ornamentation consist of pits or irregular ridges, resulting in a reticulate or punctate pattern in the ascospore surface. It is closely related to *Sordaria* and to *Neurospora*, from which it differs mainly by the ascospores; they are smooth in *Sordaria* and ornamented with longitudinal ridges in *Neurospora*. Arx (1982) reviewed *Gelasinospora*, considering *Anixiella*, its non-ostiolate counterpart, as a synonym. Following this review a number of additional species have been described (Khan & Krug, 1989*a*, *b*; Krug *et al.*, 1994). One of our isolates proved to be sufficiently different from all described species to warrant the creation of a new taxon.

MATERIALS AND METHODS

The soil samples were collected near the Bernal railway station, Quilmes city, Argentina. The terrain is very rich in organic material (humus) and the vegetation is composed mainly of *Araucaria* spp., *Pinus* spp., and members of Poaceae, Asteraceae and Solanaceae. The area has a temperate maritime climate; the average temperature is 5–15 °C in July and 18–34° in January. The total annual precipitation is 500–1000 mm. Collections were made mainly from the A_0 horizon by using previously sterilized polyethylene bags. These were sealed by rubber band and labelled. Materials were placed in a refrigerator at 4–7° until they were used. The soil samples were treated with ethanol according to Warcup & Baker (1963). The suspensions were incubated on potato carrot agar at room temperature, under 12 h of darkness alternating with 12 h of cool white fluorescent light. Measurements were made from slide preparations mounted in water and lactophenol.

DNA extraction

Fungal DNA was extracted as described by Estruch *et al.* (1989) with some modifications (Guillamón *et al.*, 1996).

PCR amplification

The ITS rDNA and 5·8 S rDNA gene were amplified as described by Gené *et al.* (1996), using a Perkin Elmer 2400 thermal cycler (Perking Elmer Cetus corporation, Emeryville, CA). The primers ITS5 and ITS4 (White *et al.*, 1990) were used. The amplification programme consisted of pre-denaturation at 94° for 5 min; 30 cycles at 95° for 30 s, 50° for 1 min and 72° for 1 min; and a final incubation at 72° for 7 min to complete the last extension. The final products were separated by electrophoresis on 2% agarose MP (Boehringer Mannheim), and cleaned following the GENECLEAN II protocol (BIO101). The molecular weights of amplified DNA were estimated by comparison with a 100 bp DNA ladder (Gibco BRL) standard lane.

Sequencing

The protocol 'Taq DyeDeoxy Terminator Cycle Sequencing Kit' (Applied Biosystems, Gouda, The Netherlands) was using for sequencing. The reactions were performed using the primers ITS5 and ITS4 (White *et al.*, 1990). An Applied Biosystems mod. 310 sequencer was used to obtain the DNA sequences.'

RESULTS AND DISCUSSION

Gelasinospora bonaerensis Stchigel & Guarro, sp. nov.
(Figs 1–10)

Mycelium ex hyphis hyalinis vel brunneis, ramosis, anastomosantibus, septatis, laevibus, 2–30 µm diametro compositum. *Coloniae* in agaro cum decocto tuberorum et carotarum expansae, tenues, ex mycelio vegetativo submerso et aereo, griseo-flavescens vel avellaneus;

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Akberto Miguel Stchingel Glikman
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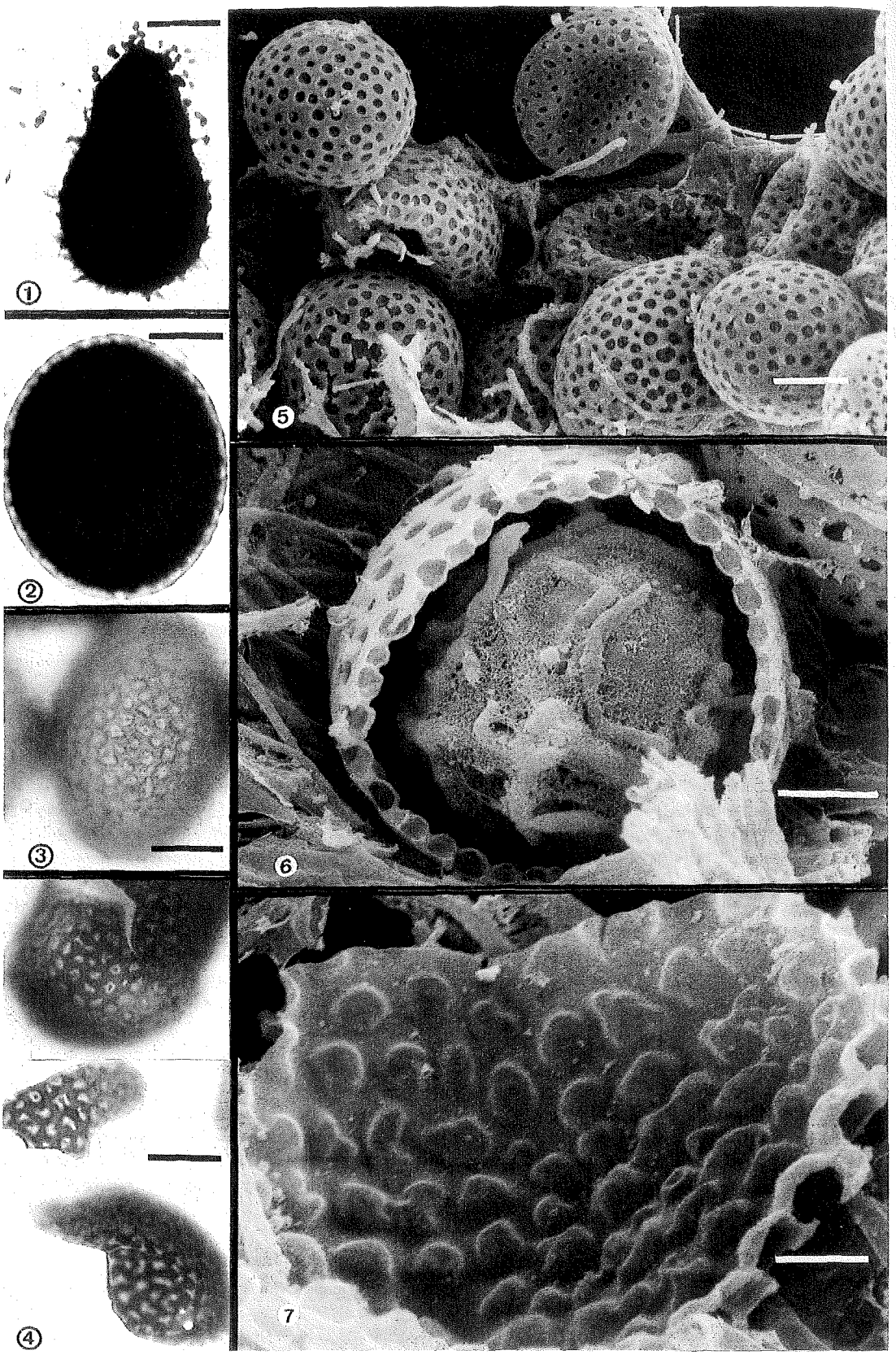
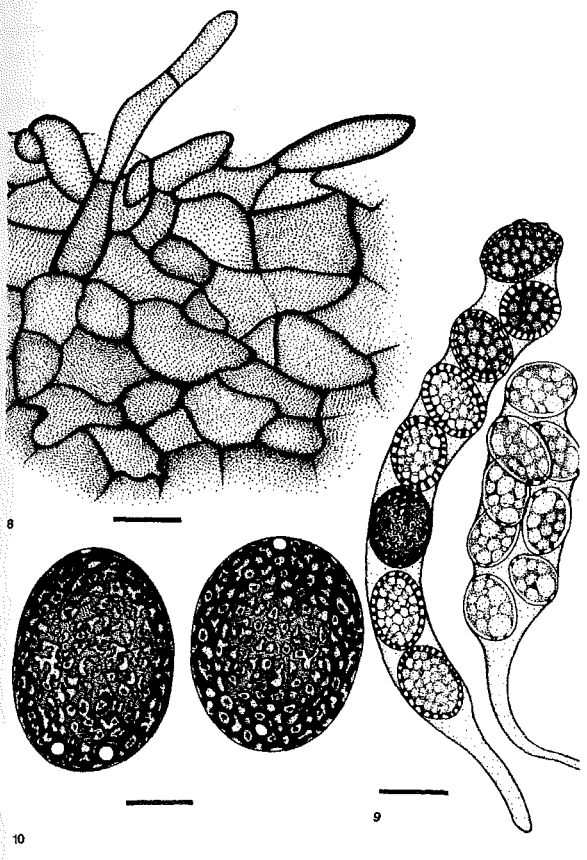


Figs 1–7. *Gelasinospora bonaerensis*. **Fig. 1.** Ascoma. Bar, 100 µm. **Figs 2–4.** Ascospores. Bar, 10 µm. **Fig. 5.** Ascospores (SEM). Bar, 10 µm. **Fig. 6.** Ascospore section (SEM). Bar, 10 µm. **Fig. 7.** Ascospore internal wall view (SEM). Bar, 10 µm.



Figs 8–10. *Gelasinospora bonaerensis*. **Fig. 8.** Peridium detail. Bar, 10 µm. **Fig. 9.** Asci. Bar, 25 µm. **Fig. 10.** Ascospores. Bar, 10 µm.

reversum concolorato. *Ascomata* superficialia, dispersa vel aggregata, pyriformia, ostiolata, fusco-brunnea, 520–650 × 310–420 µm; apex cum collo, 60–170 µm altus, 90–180 µm latus; pili crassitunicati, septati, dilute brunnei vel brunnei, 10–150 µm longi, 2–15 µm diam. ad basim; *peridium* 5–7 stratorum, brunneum, ex textura angularis vel globulosa compositum. *Asci* 8-spori, lineari-cylindrici vel subcylindrici, 250–400 × 30–45 µm, stipitati, cum muris tenuibus; paraphyses nullae. *Ascosporae* uniseriatae, unicellulares, late ellipsoideae vel subglobosae, primo hyalinae deinde atro-brunneae vel nigrae, 35–46 × 29–35 µm magnae, cum foveolis numerosis, irregulariter reticulatis, utrinque cum poris germinalibus hyalinis, circularibus, circa 1·5–2·5 µm diam., totaliter 4–7. Status conidialis ignotus.

Holotypus: IMI 375099, ex solo, Bernal, Quilmes, Buenos Aires, Argentina, 10 Aug. 1996, leg. A. M. Stchigel. Isotypus: FMR 5962.

Colonies on potato carrot agar attaining a diameter of more than 80 mm in 7 d at room temperature (approx. 25°), thin, azonate, consisting of submerged mycelium and sparse aerial hyphae, greyish-yellow to beige (M. 4B3 to 4C3), exudate hyaline; reverse concolorous. *Hyphae* hyaline to brown, branched and anastomosing, septate, smooth, 2–30 µm diam. *Ascomata* superficial, scattered to grouped, pyriform, ostiolate, slightly translucent, dark brown, 520–650 × 310–420 µm; neck cylindrical to conical, 60–170 µm tall and 90–180 µm diam.; setae scarce, stiff, simple, smooth-walled, septate,

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  1 TCCGTTGGTG AACCAGCGGA GGGATCATTA CAGAGTTGCA AAACTTCCCA CAAACCATCG
 61 CGAATCTTAC CCGTACGGTT GCCTCGGCGC TGGCGGTCCG GAAGGCCCTC GGGCCCCCGG
121 ATCCTCGGGT CTCCCGCTCG CGGGAGGCTG CCCGCCGGAG TGCCGAAACT AAACTCTTGA
181 TATTTTATGT CTCTCTGAGT AAACTTTTAA ATAAGTCAAA ACTTTCAACA ACGGATCTCT
241 TGGTTCTGGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT
301 CAGTGAATCA TCGAATCTTT GAACGCACAT TGCGCCCGCC AGTATTCTGG CGGGCATGCC
361 TGTCCGAGCG TCATTTCAAC CATCAAGCTC TGCTTGCGTT GGGGATCCGC GGCTGCCCGC
421 GGTCCCTCAA AAACAGTGGC GGGCTCGCTA GTCACACCGA GCGTAGTAAC TCTACATCGC
481 TATGGTCGTG CGGCGGGTTC TTGCCGTAAA ACCCCCCAAT TTTTAAGGTT GACCTCGGAT
541 CAGGTAGGAA TACCCGCTGA ACTTAAGCAT ATCAATAAGC GG
```

Fig. 11. *Gelasinospora bonaerensis*; sequences of the ITS1/ITS2 and 5·8 S rDNA gene.

ampulliform, digitiform, papilliform or hypha-like, pale to dark brown, 10–150 µm long, 2–15 µm diam. at the base, thin-walled to thick-walled; *peridium* 5–7 layered, 30–55 µm thick, brown, composed of textura angularis to textura globulosa; outer layers 1 to 2, textura angularis to globulosa, consisting of brown and thick-walled cells, 10–25 × 5–20 µm, wall 5–15 µm thick; several inner peridial layers of subhyaline, thick-walled cells, 13–28 × 5–10 µm; ascomatal initials arising as side branches from mycelium, involving neighbouring hyphae, coiled and contorted. *Asci* eight-spored, fasciculate, cylindrical to subcylindrical, thin-walled, 250–400 × 30–45 µm, stipitate, rounded to truncate at the apex, with a broad and distinct apical ring; *paraphyses* not observed. *Ascospores* uniseriate when mature, non-septate, broadly ellipsoidal to subglobose, hyaline when young, becoming dark brown to black, 35–46 × 29–35 µm, with walls ornamented with numerous interconnected pits, variable in size; episporium with depressions and protrusions, only evident with SEM (Figs 5–7); germ pores circular, 4–7, usually concentrated near the ends, 1·5–2·5 µm diam. *Anamorph* unknown. At 37° growth is rapid, but ascomata are not produced; no growth occurs at 42°.

Holotype: IMI 375099, from soil, Quilmes city, Argentina, 10 Aug. 1996, A. M. Stchigel. Isotype: FMR 5962.

The sequences of the ITS1/ITS2 and 5·8 S rDNA gene are shown in Fig. 11. The main features are: 582 bp; 136 A; 160 C; 149 G; 137 T. Location ITS1 from nucleotide 30–218; gene 5·8 S rRNA from nucleotide 219–375; ITS2 from nucleotide 376–528. GeneBank accession number: AJ002029.

Gelasinospora bonaerensis is closely related to *G. varians* Furuya & Udagawa and *G. pseudoreticulata* Matsush., the ascospores of which are also ornamented with an irregular reticulation. *G. bonaerensis* can, however, be easily differentiated from *G. varians*, which has ascospores 22–28 × 16–20 µm and 2 germ pores, and from *G. pseudoreticulata*, which has 2–4 germ pores and no protrusions and depressions in the episporium. Ribosomal DNA sequence analysis has been shown to be useful in taxonomy to complement morphological studies. Sequences of other species of *Gelasinospora* will be useful to investigate phylogenies and to confirm species-level delimitations within the genus.

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1.5. Una nueva especie de *Emericella* y una rara variante morfológica de *E. quadrilineata*

1.5.1. A. M. Stchigel, J. Cano & J. Guarro. 1999. A new species of *Emericella* and a rare morphological variant of *E. quadrilineata*. *Mycological Research* 103, 1057-1064

Mycol. Res. **103** (8): 1057–1064 (1999) *Printed in the United Kingdom*

A new species of *Emericella* and a rare morphological variant of *E. quadrilineata*

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Emericella indica sp. nov. and a peculiar morphological variant of *E. quadrilineata* are described and illustrated. The former is characterized by large, smooth, violet ascospores and the latter by apparently budding ascospores. Analysis of the sequences of the nuclear rDNA ITS region gene of these species and *E. nidulans* and *E. variecolor* support our concepts based on morphological criteria.

During the course of a study of soil ascomycetes an undescribed species of *Emericella* was isolated from soil samples collected in northern India. Its morphological characteristics differentiate this taxon clearly from all previously described species of the genus (Christensen & Raper, 1978; Christensen *et al.*, 1978; Christensen & States, 1982; Horie, 1978, 1980; Horie *et al.*, 1989, 1990, 1996; Horie & Udagawa, 1995; Kong & Qi, 1986; Malloch & Cain, 1972; Mehrotra & Prasad, 1969; Raper & Fennell, 1965; Samson & Mouchacca, 1974, 1975; Stchigel & Guarro, 1997; Udagawa & Horie, 1976; Udagawa & Muroi, 1979). This taxon is proposed here as a new species. Moreover, two interesting strains, one isolated from Indian soil and the other from a sample of white wine, were also studied. They showed some similarities to *E. quadrilineata* (Thom & Raper) C. R. Benj., but the presence of apparently budding ascospores has never before been observed either in *Emericella* or in the Eurotiales. The sequences corresponding to the ITS1 and ITS2 regions and the 5·8 S rRNA gene are identical to those of *E. quadrilineata*.

MATERIALS AND METHODS

Sampling, isolation and morphological characterization of strains

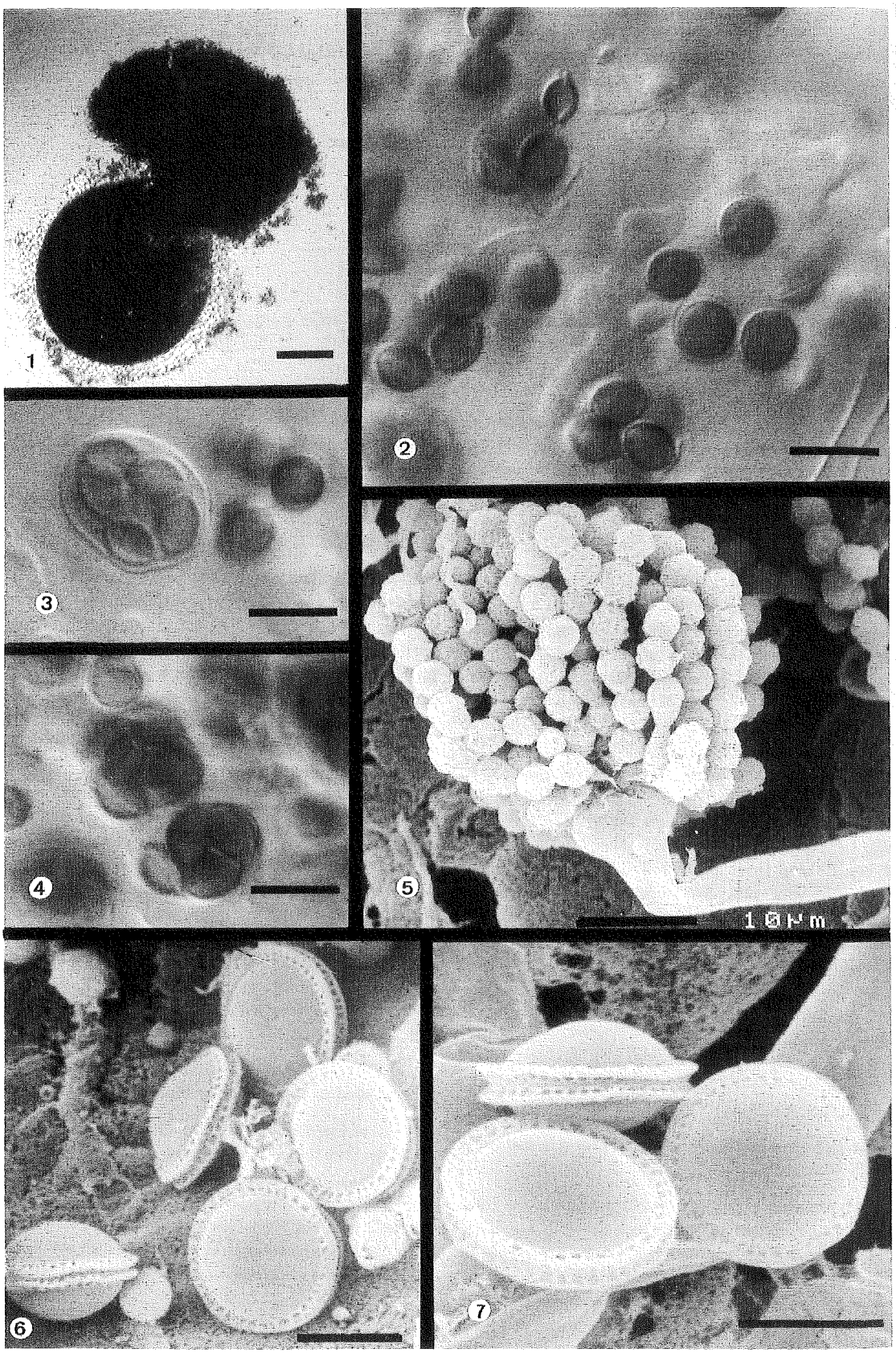
The soil samples were collected in public gardens of the cities Ajmer and Jaipur, Rajasthan, northern India. It is a tropical semiarid region and the vegetation is composed mainly of grasses and shrubs. The area is dominated by a hot climate. The average temperature is 10–35 °C in winter and 25–43° in summer. The total annual precipitation is about 900 mm. Collections were made mainly from the superficial layer of soil by using sterilised polyethylene bags. These were closed by rubber bands and labelled. On returning to the laboratory the materials were kept at 4–7° until used. Approx. 1 g of the soil sample was treated with 65 % (v/v) ethyl alcohol according to Warcup & Baker (1963). The suspensions were cultured on potato carrot agar (PCA; potatoes, 20 g; carrot, 20 g; agar, 20 g; tap water, 1000 ml), at room temperature (approx. 25°), under 12 h of darkness/12 h of cool white fluorescent light. The white wine sample was from a vineyard in the Penedés region, Spain. Approx. 20 ml of wine were filtered through nitro-cellulose filter membranes of 0·45 µm pore size (Millipore) and placed on PCA, at room temperature, under the same conditions described above. The morphological characteristics of the isolated fungi were studied on oat meal agar (OMA; Difco), PCA, Czapek solution agar (Difco), Czapek yeast extract agar (CYA; yeast extract Difco, 5 g; Czapek solution agar Difco, 49g; tap water, 1000 ml) and malt extract agar (MEA; Difco) at room temperature (*ca* 25°), under 12 h of darkness/12 h of cool white fluorescent light. Colour notations in parentheses are from Kornerup & Wanscher (1984). The measurements of the structures were taken in water and lacto-phenol.

Strains used in the molecular biology study

Six taxa of *Emericella*, three from reference culture collections and three from the present study, were sequenced. They were: *Emericella quadrilineata* IMI 370017, *E. nidulans* (Eidam) Vuill. CBS 121.35, *E. variecolor* Berk. & Broome IMI 343522, *Emericella* sp. FMR 5966, *Emericella* sp. FMR 5640 and *E. indica* FMR 6232. *Aspergillus niger* Tiegh. CBS 554·65 was sequenced and used as the outgroup.

DNA extraction

Mycelium for DNA extraction was cultured on OMA for 14 d at room temperature. Fungal DNA was extracted as described



Figs 1–7. *Emericella indica* (holotype). **Fig. 1.** Mature ascoma surrounded by hülle cells, PCM. **Figs 2, 3, 4.** Asci and ascospores, NDICM. **Fig. 5.** Upper part of a conidiophore with conidia, SEM. **Figs 6, 7.** Ascospores, SEM. Scale bars; Fig. 1 = 200 µm; Figs 2–5 = 10 µm; Figs 6, 7 = 5 µm.

by Estruch *et al.* (1989) with some modifications (Guillamón *et al.*, 1996).

PCR amplification of the ITS regions

The rDNA ITS regions containing ITS1 and ITS2 and the intervening 5·8 S rRNA gene were amplified as described by Gené *et al.* (1996), by using a Perkin Elmer 2400 thermal cycler (Perking Elmer Cetus Corporation, Emervyville, CA). The primers ITS5 and ITS4 (White *et al.*, 1990) were used. The amplification program consisted of pre-denaturalization at 94° for 5 min, 30 cycles at 95° for 30 sec, 50° for 1 min and 72° for 1 min, and final incubation at 72° for 7 min to complete the last extension. The final products were resolved by electrophoresis in a 2% agarose MP gel (Boehringer Mannheim), and cleaned following the GENECLEAN II protocol (BIO 101). The mol. wt of amplified DNA were estimated by comparison with 100 bp DNA leader (Gibco BRL) standard lane.

Sequencing and phylogenetic analysis

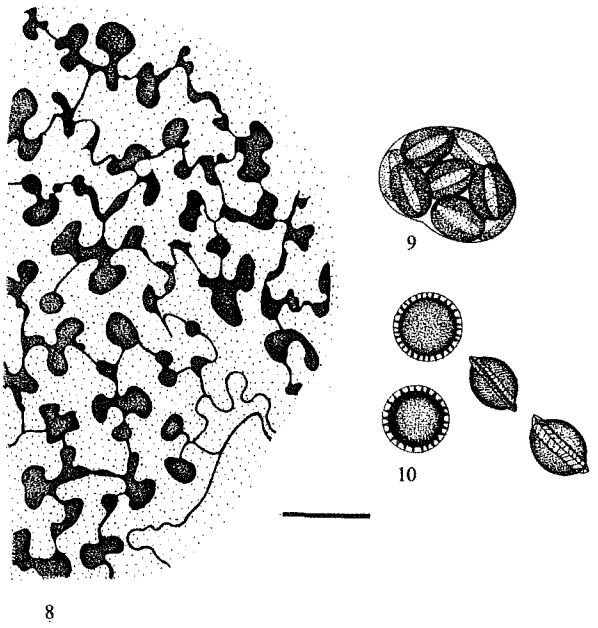
The protocol Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Gouda, The Netherlands) was used for sequencing. Reactions were performed using the primers ITS5 and ITS4 (White *et al.*, 1990) and were run on a 310 DNA sequencer (Applied Biosystems). The sequences published in this paper were aligned using the Clustal W, version 1·5, of multiple sequence alignment computer program (Thompson *et al.*, 1994). The sequences have been deposited in the European Molecular Biology Laboratory (EMBL) under the following numbers: *E. quadrilineata* IMI 370017, AJ000931; *E. variecolor*, AJ000932; *E. nidulans*, AJ000933; *Emericella sp.* FMR 5966, AJ000934; *Emericella* sp. FMR5640, AJ000935; *E. indica*, AJ000936; *Aspergillus niger* AJ223852. Cladistic analyses using the neighbour-joining method (Saiton & Nei, 1987) 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).

RESULTS AND DISCUSSION

Emericella indica Stchigel & Guarro, sp. nov. (Figs 1–10)

Anamorphosis: *Aspergillus* sp.

Coloniae in agaro cum decocto tuberorum et carotarum (PCA) expansae, 44–50 mm ad 14 d, olivaceae, pulverulentae vel granulosae. Ascomata superficialia, non ostiolatae, globosa, 60–170 µm diam., cum hülle cellulis numerosis, globosis, crassitunicatis, hyalinis vel dilute flavis, 14–25 µm diam circumdata. Peridium 6–10 µm crassum, dilute flavo-brunneum vel flavo-brunneum, transluscens, textura intrincata vel epidermoidea. Asci 8-spori, globosi vel ellipsoidei, 12–15 µm diam., evanescentes. Ascosporae unicellulares, primo hyalinae, deinde violaceae, lenticulares, 6–7·5 × 5·5–6 µm, cristis equatorialibus duabus. Capitula conidica viridia vel griseo-viridia, columnaria. Conidiophora pale cinnamomea vel cinnamomea-viridea, sinuosi, exigue septata, exigue tuberculata vel asperata, crassitunicata, ex hyphis aeriis oriunda, 80–170 µm × 4–6 µm; vesiculae pale brunnea vel brunnea, subglobosae vel clavatae, 9–16 µm diam., in summa 1/2 vel 2/3 parte fertiles. Aspergilla biseriata; metulae subhyalinae vel brunneae, 4–6 × 2·5–3·5 µm; phialides sub-hyalinae vel brunneae, 5–7 × 2–3 µm, 3–4 per metula. Conidia fusco-viridiae in massa, globosa vel subglobosa, verrucosa vel tuberculata, 4–5 × 3·5–5·5 µm.



Figs 8–10. *Emericella indica* (holotype). **Fig. 8.** Peridium (detail). **Fig. 9.** Ascus. **Fig. 10.** Ascospores. Scale bar: 10 µm.

Holotypus: IMI 378525, ex solo, Jaipur, India, 29 Oct. 1995. Leg. J. Guarro.

Isotypus: FMR 6232.

Mycelium composed of hyaline to subhyline, branched, anastomosing, smooth-walled, septate hyphae, 1–6 µm diam. Colonies on PCA growing rapidly at room temperature, 44–50 mm diam. in 14 d, flat, powdery to granulate, zonate, olive (M.3D4 to 3F8), consisting of submerged and aerial mycelium with abundant conidiophores and clusters of ascomata surrounded by hyphae and hülle cells; exudate hyaline; reverse colour similar to surface colour. Hyphae 1–10 µm wide, hyaline to pale yellowish-brown, branched, septate.

Ascomata superficial, globose, non-ostiolate, purple-violet, 60–170 µm diam., surrounded by a felt of hyphae and hülle cells; hülle cells globose, thick-walled, smooth, sub-hyaline to pale yellowish, 14–25 µm diam. *Peridium* 6–10 µm thick, pale to yellowish-brown, translucent, *textura intricata* to *epidermoidea*, three-layered, cells of the outer layer 5–20 µm diam. *Asci* 8-spored, globose to broadly ellipsoidal, 12–15 µm diam., evanescent. *Ascospores* one-celled, at first hyaline, becoming violet, lenticular, 6–7·5 × 5·5–6 µm (crest not included), with two conspicuously pleated equatorial crests measuring about 1 µm wide and with smooth convex walls.

Conidial heads green to dull green, columnar. *Conidiophores* light cinnamon to cinnamon green, sinuous, occasionally septate, scarcely tuberculate to verrucose, thick-walled, arising from a basal felt or aerial hyphae, 80–170 µm long, 4–6 µm broad at the middle. Vesicles light brown to mid brown, subglobose to flask-shaped, 9–16 µm diam. Metulae subhyaline

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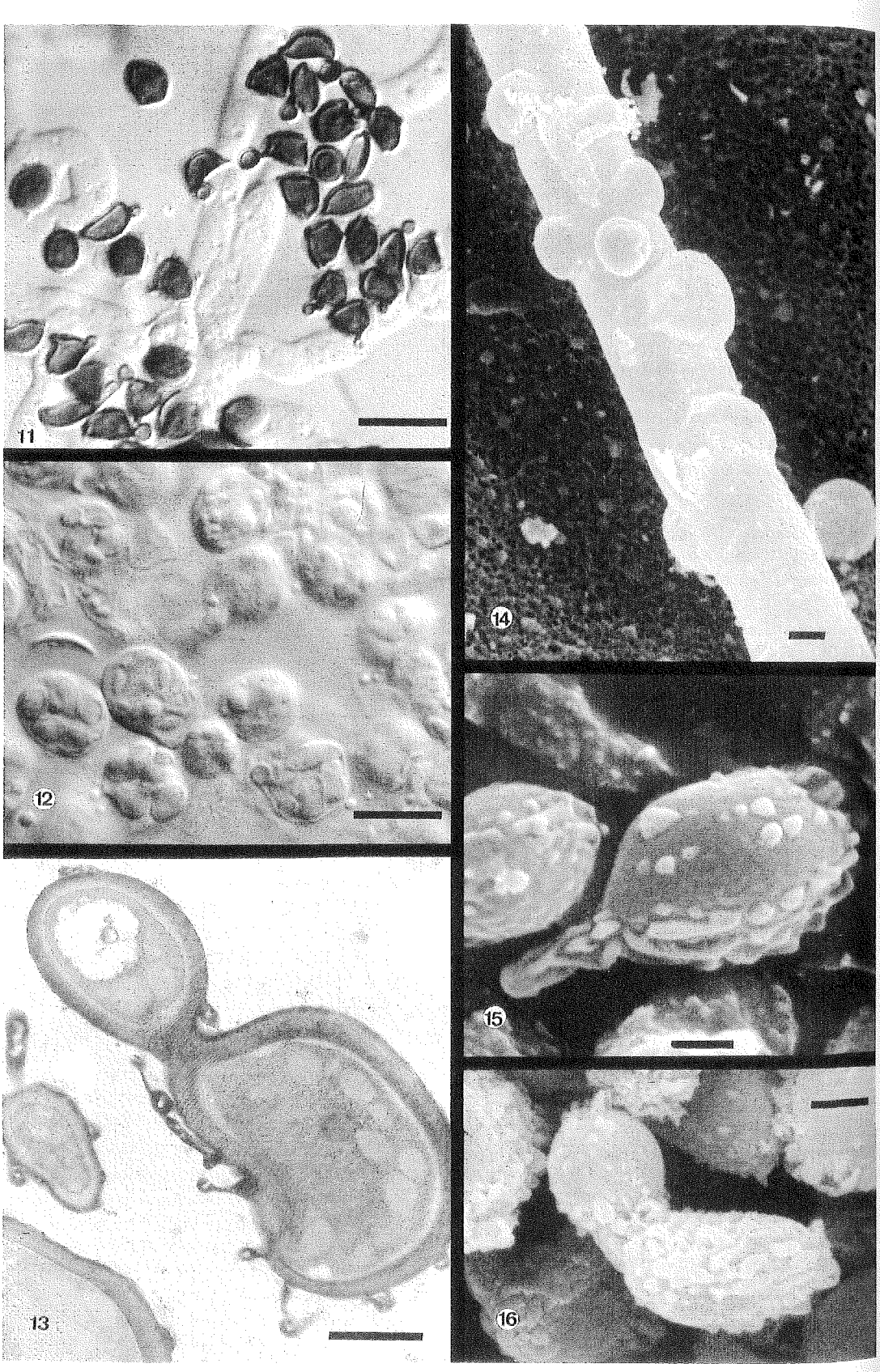


Figs 11–16. *Emericella quadrilineata* FMR 5640. **Fig. 11.** Mature ascospores, PCM. **Fig. 12.** Asci and ascospores. Note the presenc budding ascospores inside the asci, NDICM. **Fig. 13.** Budding ascospore, TEM. **Fig. 14.** Detail of the stipe of a conidiophore, SE **Figs 15, 16.** Asci and ascospores, SEM. Scale bars: Figs 11, 12 = 10 µm; Figs 13–16 = 1 µm.

to brownish, 4–6 × 2·5–3·5 µm; phialides subhyaline to brownish, 5–7 × 2–3 µm, 3–4 per metula. *Conidia* dark green in mass, globose to subglobose, verrucose to tuberculate, 4–5·5 × 3·5–5·5 µm.

Colonies on OMA growing rapidly at room temperature, 55–58 mm diam. in 14 d, floccose to granulose, zonate, olive (M.3D4 to 3F8), consisting of submerged and aerial mycelium with abundant conidiophores and clusters of ascomata surrounded by hyphae and hülle cells; reverse colour similar to surface colour; exudate hyaline.

Colonies on Czapek agar growing restrictedly at room temperature, 20–24 mm diam. in 14 d, cottony, white to yellowish white (M.3A2), consisting of submerged and aerial mycelium; conidiophores and ascomata absent; reverse dark brown (M.7F6 to 7F8); exudate yellowish.

Colonies on MEA growing rapidly at room temperature, 56–60 mm diam. in 14 d, flat, powdery, cream to light yellow (M.4A3 to 4A4), consisting of submerged and aerial mycelium with masses of ascomata surrounded by hyphae and hülle cells; reverse dark brown to violet brown (M.9F8 to 10F8); exudate absent.

Colonies on PDA growing rapidly at room temperature, 55–60 mm diam. in 14 d, granulose, dark ruby (M.12F8) in the centre and olive brown (M.4F8) in the margins, consisting of masses of ascomata surrounded by hyphae and hülle cells; conidiophores scarcely produced; reverse dark brown to violet brown (M.9F8 to 10F8); exudate yellowish.

At 37° and 42° the growth is similar to that at 25°.

Holotype: IMI 378525, garden soil, Jaipur city, India, 29 Oct. 1995. Col. J. Guarro. Isotype: FMR 6232.

Ascospore colour is an important feature in the delimitation of species in *Emericella*. Apart from *E. indica* there are three other species with violet ascospores, *E. violacea* (Fennell & Raper) Malloch & Cain, *E. similis* Y. Horie, Udagawa, Abdullah & Al-Bader and *E. pluriseminata* Stchigel & Guarro. *Emericella indica* differs from *E. pluriseminata* in having 16-spored asci, smaller non-stellate ascospores (7–9 × 6–7 µm in *E. pluriseminata*) and an *Aspergillus* anamorph, which is absent in *E. pluriseminata*. *Emericella indica* can be differentiated from *E. similis* in having larger ascospores (4–5(–5·5) × 3–4 µm in *E. similis*) and an anamorph (absent in *E. similis*). *Emericella violacea* has an anamorph and the ascospore size is also similar (5·5–6·5 × 4·0–5·0 µm), but clearly differs from *E. indica* in having ascospores with an echinulate surface and two very low equatorial crests.

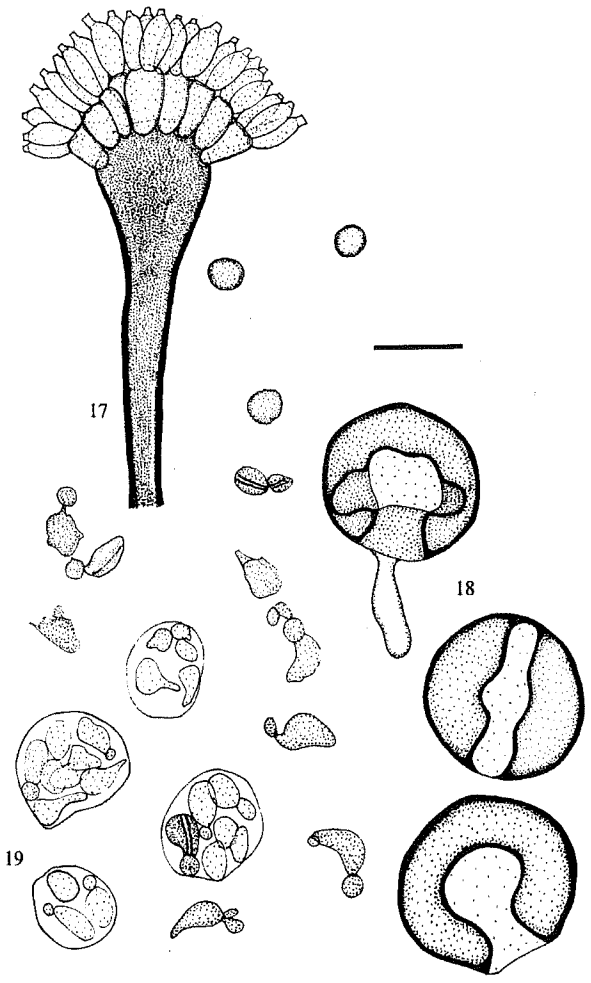
Emericella quadrilineata (Thom & Raper) C. R. Benj., *Mycologia* **47**: 680 (1955). (Figs 11–19)

Synonym: *Aspergillus quadrilineatus* Thom & Raper, *Mycologia* **31**: 660 (1939).

Anamorph: *Aspergillus quadrilineatus* Thom & Raper.

The following description is based on strains FMR 5640 and FMR 5966.

Mycelium composed of hyaline to brownish, branched, anastomosing, smooth-walled, septate hyphae, 2–6 µm diam. Colonies on PCA growing rapidly at room temperature, 54 mm diam. in 14 d; plane, powdery, zonate, deep green (M.29E8); scattered ascomata on a short false stalk and/or



Figs 17–19. *Emericella quadrilineata* FMR 5640. **Fig. 17.** Upper part of a conidiophore. **Fig. 18.** Hülle cells. **Fig. 19.** Ascospores. Scale bar: 10 µm.

surrounded by a mass of hülle cells and mycelium, spherical to irregular, colour champagne (M.4B4) in mass; reverse tomato red (M.8C8) or strawberry (M.10D8); exudate hyaline; soluble pigment ruddy (M.9B5). Ascomata superficial, scattered to gregarious, purple-red to red, non-ostiolate, globose, 70–250 µm diam. (excluding hülle cells), singly elevated on or encased in separable columns composed by masses of hyphae and hülle cells, 160–760 × 110–300 µm. Hülle cells abundant, hyaline to pale brown, globose to subglobose, 7–25 µm diam., thick-walled. Peridium purple-red to red, thin-walled, 5–13 µm thick, *textura intricata*, 2–5 layered, outer layer consisting of long hyphal cells 1–3·5 µm wide. Asci irregularly disposed in the ascoma, borne in clusters, 8-spored, globose to subglobose, 8·5–13 µm diam., evanescent. Ascospores maturing rapidly in less than 7 d, purple-red to red, morphologically variable, triangular, quadrangular, heart-shaped, reniform, ellipsoidal or highly irregular, 5–8 × 3–4 (–5) µm, ornamented with two to many incomplete crests, warts and/or ridges, with single to numerous bud cells 1·5–3 µm diam.

Conidial heads olive green to dull green, columnar. Conidiophores light cinnamon brown, more or less sinuous, occasionally septate, tuberculate, thin- to thick-walled, arising

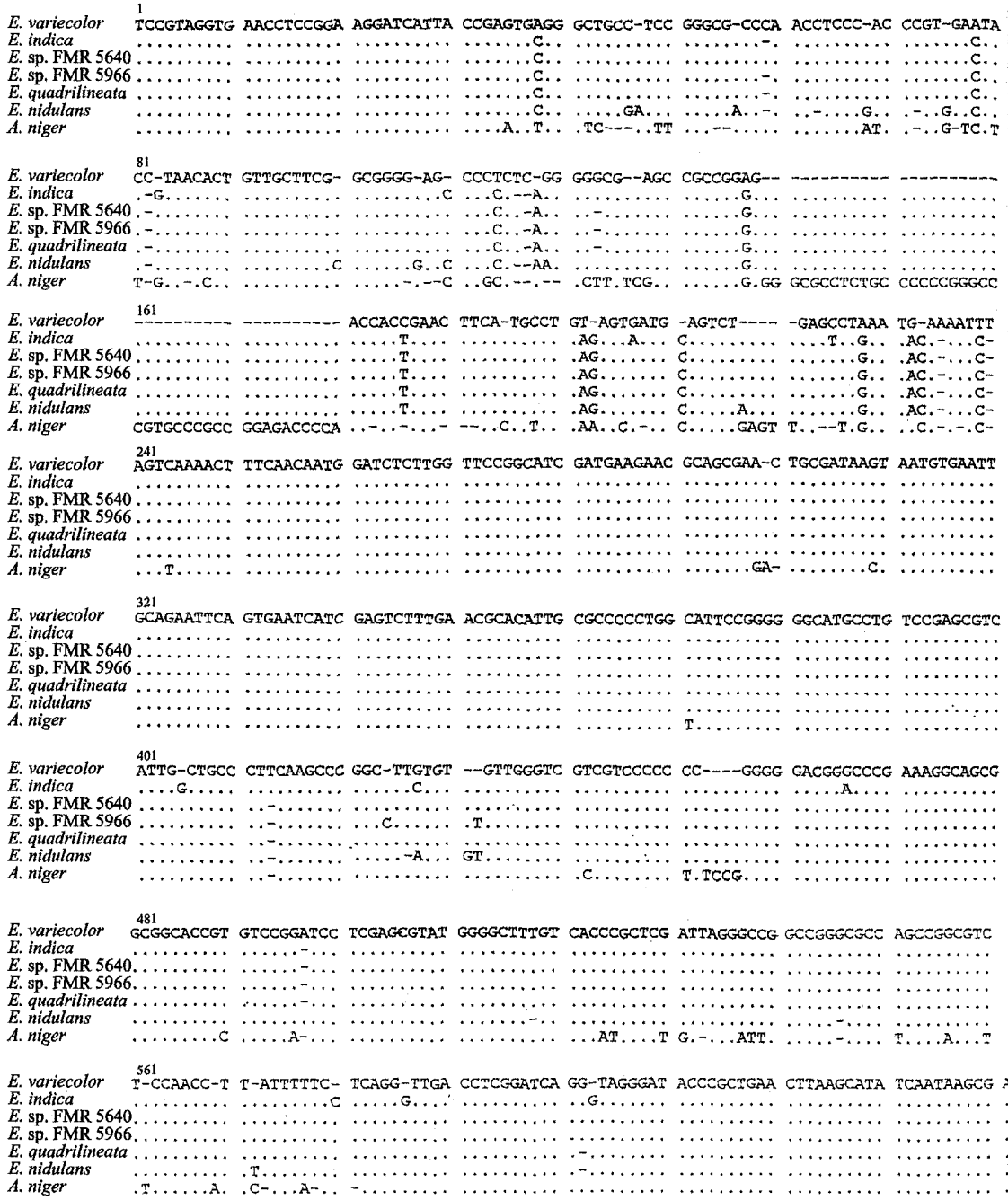


Fig. 20. Differences in ITS1 and ITS2 and rRNA 5·8 S gene sequences among *Emericella* spp. and *Aspergillus niger*. Dot, base identical with *E. variecolor*; dash, indel.

from basal felt or aerial hyphae, developed usually in tandem, 50–150 µm long, 3–6 µm diam. at the middle. Vesicles light brown to mid-brown, hemispherical to flask-shaped, 7–10 µm diam. Metulae hyaline to mid-brown, 3–8 × 2–3·5 µm; phialides hyaline to mid-brown, 3–6 × 2·5–3·5 µm, 3–5 per metula. Conidia olivaceus to dark green in mass, globose to subglobose, echinulate to tuberculate, 2·5–3·5 (–4·5) µm diam.

Colonies on Czapek–Dox solution agar growing rapidly at room temperature (approx. 25°), 74 mm diam. in 14 d, plane, felted, irregular margins, with submerged wine red mycelium extended beyond the area of surface growth in an irregularly lobed to diffused pattern, concentric areas poorly defined, greyish orange (M.5B4) and ash grey (M.1B2); reverse garnet red (M.11E8); exudate present in central area, Persian orange (M.6A7); soluble pigment present, dull red (M.9C4); surface growth consisting of masses of hülle cells and intermixed conidiophores covered with mycelium; ascomata absent.

Colonies on CYA growing rapidly at room temperature, 50 mm diam in 7 d, plane, velvety in the central area, reddish grey (M.10B2), and with ash grey (M1B2), powdery margins, radially furrowed, with submerged mycelium extended beyond the area of surface growth in an irregularly lobed to diffused

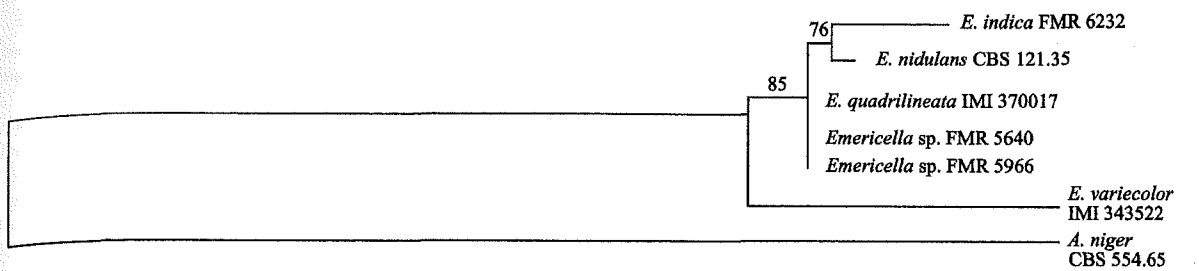


Fig. 21. Neighbour-joining tree based on nucleotide sequences from the ITS regions and 5·8 S gene. Branch lengths are proportional to distance. Bootstrap replications frequences are indicated above the internodes.

pattern; exudate present, violet brown (M.10B2); soluble reddish brown (M.8D5) pigment present; surface growth consisting of masses of hülle cells and conidiophores covered with mycelium; ascomata absent.

Colonies on MEA growing rapidly at room temperature, 48 mm in 14 d, plane, felted to velvety, with entire margins, with submerged reddish golden (M.6C7) mycelium, zonate and radially furrowed, prevailing yellowish white (M.3A2), but with reddish grey (M.10B2), red-haired (M.6C4) and reddish grey (M.8B2) areas; Persian orange exudate present in central area (M.6A7); soluble pigment present, orange (M.5B8); surface growth consisting of masses of hülle cells and conidiophores covered with mycelium; ascomata absent.

Colonies on OMA growing rapidly at room temperature, 40 diam. mm in 7 d, plane, powdery, zonate, grass green (30E7), scattered ascomata surrounded by a mass of hülle cells and mycelium, spherical to irregular in form, yellowish white (M.2A2); reverse greyish green (M.29E5); exudate hyaline; soluble pigment absent.

At 37° and 42° the growth is similar to that at 25°.

Material examined: FMR 5640 (IMI 371927), from garden soil, Ajmer city, India, 2 Nov. 1995, col. J. Guarro; *E. quadrilineata* FMR 5966, from sample of white wine, from a vineyard of the Penedés region, Spain, 6 Feb. 1997, col. J. Cano.

It is surprising that both strains of *Emericella*, from very different origins, have ascospores with budding cells. This is a typical feature in yeasts, primitive ascomycetes, such as *Taphrina deformans* (Mix, 1949), and basidiomycetes (Taylor *et al.*, 1994), but has never been described in Eurotiales. Budding cells seem to be a stable characteristic even in young ascospores within the asci (Fig. 12). It was thought that this feature could be an aberrant morphological structure induced by ethyl alcohol, because one strain was obtained after treatment of the sample with that compound and the other one was isolated from white wine. The treatment of different strains of *Emericella*, however, including *E. quadrilineata*, with different concentrations of ethyl alcohol did not reproduce the phenomenon. Both strains, despite this feature, showed the typical characteristics of *E. quadrilineata* such as spherical cleistothecia surrounded by a layer of hülle cells, red-purple ascospores with 2–4 crests, and an *Aspergillus* anamorph belonging to the Section Nidulantes. Both isolates were, therefore, tentatively identified as belonging to that species. To corroborate this aspect, sequences of nuclear rDNA ITS regions of the two strains and a representative strains of *E. quadrilineata* were compared. To demonstrate the usefulness of this technique for distinguishing species of *Emericella*, two additional very different species of *Emericella* and *E. indica* were included in the study. *Aspergillus niger* was used as an outgroup. The two strains isolated in the present study (FMR 5640 and FMR 5966 and *E. quadrilineata* IMI 370017 showed practically identical sequences, with a maximum of three different bases when FMR 5966 and IMI 370017 were compared (Fig. 20). These three strains together with those of *E. indica* and *E. nidulans* formed a cluster supported by a bootstrap interval of 85% (Fig. 21). The two latter species were related to a bootstrap replication frequency of 76%. The strain of *E. variecolor* was the most phylogenetically distant among the group studied.

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1.6. Una nueva especie de *Melanospora* de Isla de Pascua (Chile)

1.6.1. A. M. Stchigel, J. Cano & J. Guarro. 1999. A new species of *Melanospora* from Easter Island soil. *Mycological Research* 103, 1305-1308

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A new species of *Melanospora* from Easter Island

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Melanospora pascuensis sp. nov., isolated from soil of Easter Island, is described and illustrated. The ITS1, ITS2 and 5·8 S rRNA gene sequences are also provided. The fungus can be recognized by the setose perithecial ascomata and the peculiar ring-like structure surrounding the ascospore germ pores. It is compared with other species of *Melanospora* and related genera.

During the study of soil and herbivorous dung samples from Chile several interesting fungi were isolated (Valldosera & Guarro, 1988). In the present study we describe a new species of *Melanospora* with a peculiar ring-like structure surrounding the germ pores of its ascospores.

MATERIALS AND METHODS

Sampling, isolation and morphological characterization

The soil samples were collected from several places near Hanga-Roa, Easter Island, Chile. It is a triangular, volcanic, semiarid island of 162 km^2 located in the middle of the Pacific Ocean, approx. 3760 km from the coast of South America and 4000 km from Tahiti. The vegetation consisted mainly of grasses, with reduced forest masses made up of a few introduced trees, such as *Eucalyptus* spp. and *Melia azederach* L. The area is dominated by a subtropical maritime climate. The average temperature is 17·8 °C in winter and 23·7° in summer. The total annual precipitation is about 1140 mm, with no dry season. Collections were made mainly from the superficial layer of soil in sterilized polyethylene bags, tied with rubber bands and labelled. On returning to the laboratory the samples were kept at 4–7° until used. Approximately 1 g of the sample was treated with 65% (v/v) ethyl alcohol according to Warcup & Baker (1963). The suspensions were cultured on potato carrot agar (PCA; potato, 20 g; carrot, 20 g; agar, 20 g; tap water, 1 l) at room temperature (approx. 22°), under 12 h dark/12 h of cool white fluorescent light. The morphological characteristics of the fungi grown were studied on oat meal agar (OMA; Difco), PCA, potato dextrose agar (PDA; Difco) and malt extract agar (MEA; Difco) at room temperature, under 12 h dark/12 h of cool white fluorescent light. Colour notations in parentheses are from Kornerup & Wanscher (1984). Measurements of fungal structures were performed in water- and lactophenol-mounted material.

DNA extraction

Mycelium for DNA extraction was cultured on OMA for 14 d at room temperature. Fungal DNA was extracted as described by Estruch *et al.* (1989) with some modifications (Guillamón *et al.*, 1996).

PCR amplification of the ITS regions

The rDNA ITS regions containing ITS1 and ITS2 and the intervening 5·8 S rRNA gene were amplified as described by Gené *et al.* (1996), by using a Perkin Elmer 2400 thermal cycler (Perking Elmer Cetus Corporation, Emervyville, CA). The primers ITS5 and ITS4 (White *et al.*, 1990) were used. The amplification programme consisted of pre-denaturalisation at 94° for 5 min, 30 cycles at 95° for 30 s, 50° for 1 min and 72° for 1 min, and final incubation at 72° for 7 min to complete the last extension. The final products were resolved by electrophoresis in a 2% agarose MP gel (Boehringer Mannheim), and cleaned following the GENECLEAN II protocol (BIO 101). The molecular weights of amplified DNA were estimated by comparison with 100 bp DNA leader (Gibco BRL) standard lane.

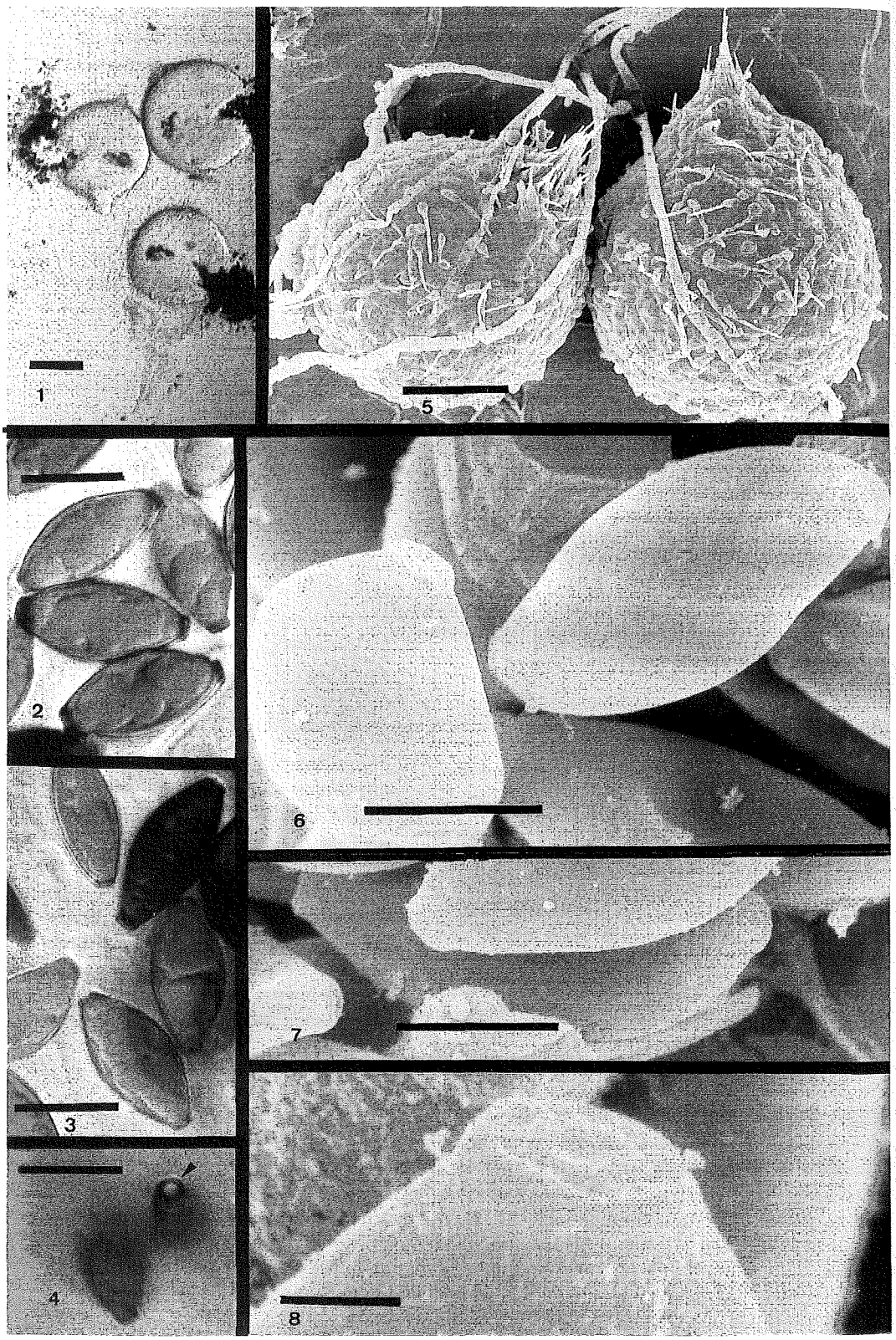
Sequencing

The 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 were run on a 310 DNA sequencer (Applied Biosystems).

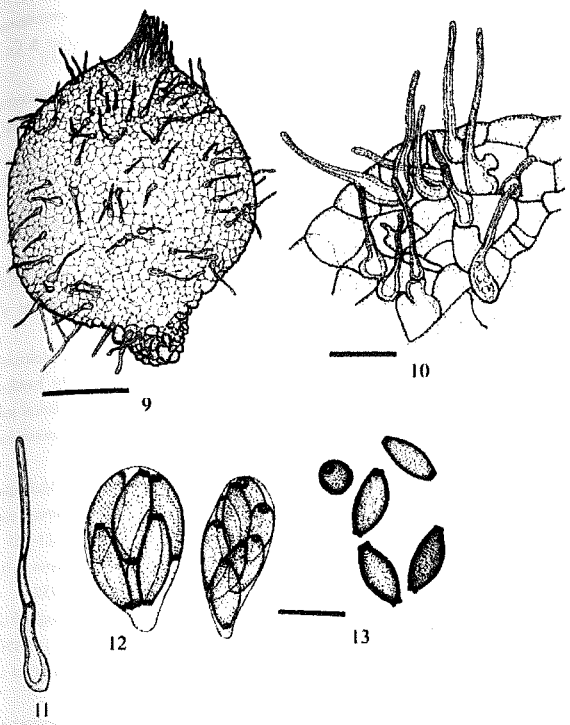
RESULTS

Melanospora pascuensis Stchigel & Guarro, sp. nov. (Figs 1–13)

Coloniae in agaro cum decocto tuberorum et carotarum expansae, 48–52 mm ad 14 d, planae, tenues, hyalinae vel pale flavae, ex mycelio vegetativo submerso et aereo, granulosae; reversum similis



Figs 1–8. *Melanospora pascuensis* FMR 6367. **Fig. 1.** Ascomata, NDICM. **Figs 2–3.** Ascospores, NDICM. **Fig. 4.** Ascospores. Note dark ring-like structure surrounding a prominent germ pore (arrow), NDCIM. **Fig. 5.** Ascomata, SEM. **Figs 6–7.** Ascospores, SEM. **8.** Ring-like structure surrounding the depressed germ pore, SEM. Scale bars: Fig. 1, 200 µm; Figs 2–4, 10 µm; Fig. 5, 50 µm; Figs 6–5 µm; Fig. 8, 1 µm.



Figs 9–13. *Melanospora pascuensis* FMR 6367. **Fig. 9.** Ascoma. **Fig. 10.** Peridium detail. **Fig. 11.** Seta. **Fig. 12.** Asci and ascospores. **Fig. 13.** Mature ascospores. Scale bars: Fig. 9, 100 µm; Figs 10–13, 25 µm.

colorato. Ascomata superficialia vel immersa, gregaria, globosa vel ovoidea, ostiolata (non ostiolata si immersa), 100–350 µm diam. (immersa 80–110 µm diam.), subhyalina vel pallida fulva et fusco-brunnea vel nigra in ascosporis acervulantis, pilosa. Collis nullis vel brevibus, 0–40 µm longis, 20–50 µm latis ad basim, setis coronatis effectissimis; setae rectae vel parum curvae, 30–160 µm longae, 5–10 µm latae, pallide fulvae, non-septatae, crassitunicatae. Peridium membranaceum, pilosum, 6–9 strati, translucido, textura angularis composito. Paraphysibus nullis. Asci 8-spori, late clavati vel obovati, fasciculati, 40–50 × 21–25 µm, superne rotundati, brevistipitati, evanescentes. Ascosporis irregulariter biseriatis, primo hyalinis et gutulatis, deinde atro-brunneis, longis ellipsoideis, 17–23 × 8–10 µm, unicellulati, cum parietibus levibus, crassitunicatis, poris germinationibus terminalibus duobus, 1–1·5 µm diam., cum anuli parietibus circumdati, usque ad 0·5–1 µm acrescentes. Anamorphosis absens.

Holotypus: IMI 378527, ex solo, Hanga-Roa, Easter Island, Chile, 15 Sep. 1995. *Isotypus*: FMR 6367.

Mycelium composed of hyaline to pale yellow, branched, anastomosing, septate, smooth hyphae 1–8 µm diam. Colonies on PCA growing rapidly, attaining a diam. of 48–52 mm in 14 d at room temperature (approx. 22°), flat, hyaline to yellowish-white (M. 4A2), with scarce aerial mycelium, texture granulose due to the production of abundant ascomata; reverse hyaline to yellowish-white (M. 4A2); hyaline exudate present. *Ascomata* superficial to immersed, gregarious, globose to ovoid, inconspicuously ostiolated, 100–350 µm diam., setose, pale yellow, translucent, appearing dark brown to black due the influence of massed ascospores, with a short conical neck; neck 0–40 µm long, 20–50 µm wide at the base, with well developed coronal setae; setae right to slightly

```
  1 TCCGTAGTGT GAACCTGCGG AAGGATCATT ACCGAGTGAG GGCCCTCTGG GTCCAACCTC
 61 CCACCCGTGT TTATCGTACC TTGTTGCTTC GGCGGGCCCG CCGTTCCGGC CGCCGGGGGG
121 CATCCGCCCC CGGGCCCGCG CCCGCCGAAG ACACCATTGA ACGCTGTCTG AAGAATGCAG
181 TCTGAGCGAT TAGCTAAATC AGTTAAAACT TTCAACAACG GATCTCTTGG TTCCGGCATC
241 GATGAAGAAC GCAGCGAAAT GCGATAAGTA ATGTGAATTG CAGAATTCAG TGAATCATCG
301 AGTCTTTGAA CGCACATTGC GCCCCCTGGT ATTCCGGGGG GCATGCCTGT CCGATGCGTC
361 ATTGCTGCCC TCAAGCACGG CTTGTGTGTT GGGCCCCGCC CCCCGGCTAC CGGGGGGCGG
421 GCCCGAAAGG CAGCGGCGGC ACCGCGTCCG GTCCTCGAGC GTATGGGGCT TCGTCACCCG
481 CTCTGTAGGC CCGGCCGGCG CCCGCCGGCG ACCCCCCTCA ATCTTTCTCA GGTTGACCTC
541 GGATCAGGTA GGGGTACCCG CTGAACTTAA GCATATCAAT AAGCGG
```

Fig. 14. *Melanospora pascuensis* FMR 6367; sequences of the ITS1–2 and 5·8 S rRNA gene.

curved or sinuous, 30–160 µm long, 5–10 µm wide at the base, pale yellow, non-septate, thick-walled. *Peridium* membranaceus, 6–9 layered, translucent, *textura angularis*; outer wall 1–2 layered, 4–7 µm thick, composed by thick-walled cells; inner wall 5–7 layered, 20–30 µm thick, composed by thin-walled cells. Paraphyses absent. *Asci* 8-spored, broadly clavate to obovate, fasciculate, 40–50 × 21–25 µm, apex rounded, without any apical structure, short stipitate, evanescent. *Ascospores* irregularly biseriate, at first hyaline and guttulate, becoming dark brown, large ellipsoidal, 17–23 × 8–10 µm, one-celled, smooth and thick-walled, with one germ pore at each end; germ pores 1–1·5 µm diam., surrounded by a dark ring-like structure, 0·5–1 µm high, 3–4 µm external diam. Anamorph absent.

Colonies on OMA growing rapidly, 50–75 mm diam. in 14 d at room temperature, flat, hyaline to yellowish-white (M. 4A2), with scarce aerial mycelium, granulose by production of abundant ascomata; reverse yellowish-brown (M. 5E6); hyaline exudate present.

Colonies on MEA growing rapidly, 51–56 mm diam. in 14 d at room temperature, cottony to granulose by production of abundant ascomata, hyaline, yellowish-brown (M. 5D8 to 5F8) in the middle; reverse greyish-yellow (M. 4B4); hyaline exudate present.

Colonies on PDA growing rapidly, 49–59 mm diam. in 14 d at room temperature, cottony to granulose by production of abundant yellowish-brown ascomata, radiate, raw umber (M. 5F8); reverse greyish-yellow (M. 4B4). At 37° growing slowly and ascomata are not produced; no growth at 42°.

Holotype: IMI 378527, form soil, Hanga-Roa, Easter Island, Chile, 15 Sep. 1995. *Isotype*: FMR 6367.

The sequence of the ITS1–2 and 5·8 S rDNA region is shown in Fig. 14. The main features are: 586 bp; 109 A; 184 C; 173 G and 120 T. Location ITS1 from nucleotide 31 to 205; gene 5·8 S rRNA from nucleotide 206 to 363; ITS2 from nucleotide 364 to 532. It has been deposited in the European Molecular Biology Laboratory (EMBL) as AJ 011312.

Melanospora was erected by Corda (1837) to include numerous species with ostiolate ascomata with thin, translucent walls composed of angular, pale yellow to reddish-brown pseudoparenchymatous cells, glabrous to strongly tomentose, with a short to long ostiolar neck, usually ringed at the apex by hyaline setae; four- to eight-spored evanescent asci; ascospores one-celled, brown, fusiform, ellipsoidal or citriform, with a depressed germ pore at each end, without raised rim and with diverse types of ornamentation; anamorphs belong to a wide range of genera, including *Acremonium*, *Chlamydomyces*, *Harzia*, *Paecilomyces* and *Proteophiala* (Kendrick

& Di Cosmo, 1979). Numerous species have been segregated (Arx & Müller, 1954; Udagawa & Cain, 1969; Hawksworth, 1975; Hawksworth & Udagawa, 1977; Jeng & Cain, 1977; Arx, 1981) and the wide generic concept proposed by Doguet (1955) has been reduced by Cannon & Hawksworth (1982). These authors restricted the genus to species with smooth ascospores and with a depressed germ pore at each end. Several species are very common and have been reported from almost all parts of the world, e.g. *M. fallax* Zukal, *M. zamiae* Corda and *M. zobelii* (Corda) Fuckel. Most species are mycophilous, but some are saprotroph (Cannon & Hawksworth, 1982). The ring-like structures that surround the germ pore of the ascospores are very rare in *Melanospora*, with the exception of *M. collipora* Stchigel & Guarro and, possibly, *M. singaporensis* Morinaga, Minoura & Udagawa, *Microthecium ellipsosporum* Takada and *M. zobelii*. *M. pascuensis* differs clearly from these species by its setose and ostiolate ascomata (glabrous and non-ostiolate in *M. ellipsosporum* and *M. zobelii*, and glabrous and ostiolate in *M. collipora* and *M. singaporensis*). The ascospore size also differentiates the species, e.g. 23–27 × 11–14 µm in *M. collipora*, 15–18 × 9–11·5 µm in *M. singaporensis*, 16–18 × 6–7·5 µm in *M. ellipsosporum*, 21 × 12 µm in *M. zobelii*, and 17–23 × 8–10 µm in *M. pascuensis*. In addition, the ring-like structure that surrounds the germ pores is thick and dark in *M. pascuensis* and thin and hyaline in *M. collipora*. The relationship between these *Melanospora* species and *Pustulipora* was discussed previously by Stchigel *et al.* (1996).

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1.7. Tres nuevas especies termotolerantes de *Corynascus* (Sordariales, Chaetomiaceae) del suelo, con una clave dicotómica de las especies conocidas

1.7.1. A. M. Stchigel, M. Sagués, J. Cano & J. Guarro. 2000. Three new thermotolerant species of *Corynascus* (Sordariales, Chaetomiaceae) from soil, with a key to the known species. *Mycological Research* (en prensa)

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Three new thermotolerant species of *Corynascus* from soil, with a key to the known species

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Three new thermotolerant species of *Corynascus* isolated from Indian and Argentinian soils are described and illustrated: *C. sexualis* sp. nov. characterised by small ascospores and the absence of a *Myceliophthora* anamorph (present in all other species of the genus); *C. similis* sp. nov. characterised by navicular ascospores with two lateral to sub-terminal germ pores; and *C. verrucosus* sp. nov. distinguished by the ellipsoid ascospores with two oblique to sub-terminal germ pores. A study to compare the ITS-region sequences of all species of the genus with representative species of related genera demonstrated that this region is very conserved in this group of fungi and of low taxonomic value. A key to the eight species of the genus now known is provided. The molecular data suggest the genus is best placed in the *Chaetomiaceae* rather than the *Ceratostomataceae*.

INTRODUCTION

The generic name *Corynascus* (*Sordariales*) was introduced by Arx (1973) to accommodate two species described previously as *Thielavia sepedonium* (Emmons 1932) and *T. novoguineensis* (Udagawa & Horie 1972). It is characterised by small, glabrous (occasionally setose), globose, non-ostiolate ascomata, with obovate to globose asci, dark, smooth, one-celled ascospores which have a distinctive germ pore at each pole, and by anamorphs belonging to *Myceliophthora*. *Corynascus* was included by Arx, Figueras & Guarro (1988) in the *Thielaviaceae*, together with *Thielavia*, *Boothiella*, *Chaetomidium*, *Corynascella*, *Melanocarpus*, and *Emilmuelleria*. Hawksworth *et al.* (1995) placed *Corynascus* in *Ceratostomataceae*, but Stchigel, Guarro & Figueras (1996) disagree with this and considered that *Corynascus* was more closely related to *Corynascella*, *Thielavia* and *Melanocarpus* than to the genera of *Ceratostomataceae*. The same point of view was adopted by Eriksson & Hawksworth (1998), who proposed the inclusion of *Corynascus* in *Chaetomiaceae*. Five species are presently accepted in *Corynascus*: *C. heterothallicus*, *C. novoguinensis*, *C. sepedonium*, *C. setosus*, and *C. thermophilus*. Here we describe three new species recently isolated from Argentinian and Indian soils, and provide a key to the known species of the genus.

MATERIALS AND METHODS

Sampling

Soil samples were collected in Argentina and India. Two areas were sampled in Argentina, one near Bernal train station, Quilmes, and the other in the Reserva Ecólogica Costanera Sur, Buenos Aires, both in the province of Buenos Aires. The terrain is rich in humus and clay, and dominated by a temperate maritime climate. The temperatures are 0–15 °C in winter and 18–32° in summer, and the annual precipitation is 500–1000 mm. The vegetation in the first area is mainly *Platanus occidentalis*, *Tilia platyphyllos*, *Melia azederach*, *Agropyron repens*, *Cynodon dactylon*, and *Paspalum* spp.; and in the second mainly different species of *Poaceae*. Indian samples were collected close to Ajmer, Rajasthan, a tropical semiarid region dominated by a hot climate. The temperatures are 10–35° in winter and 25–43° in summer, and the annual precipitation *ca.* 900 mm. Vegetation is mainly grasses and shrubs.

Collections were taken mainly from the superficial layer of soil with sterilised polyethylene bags. These were closed with rubber bands and then labelled. On their arrival at the laboratory the material was refrigerated at 4–7° until used. Approximately 1 g of soil was treated with 5 ml and 2-furfuraldehyde 10^{-3} M and incubated for 30 min at 65°. The suspensions were cultured on potato carrot agar (PCA; potatoes, 20 g; carrot, 20 g; agar, 20 g; tap water, 1000 ml; home-made) and incubated at 42° under 12 h of darkness alternating with 12 h of cool white fluorescent light.

Dried and living cultures have been preserved in the collections indicated in the text.

Morphological study

Strains were grown on oatmeal agar (OA; Difco), PCA (see above), potato dextrose agar (PDA; Difco) and malt extract

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agar (MEA; Difco) at 22–25° and at 42°, under 12 h of darkness alternating with 12 h of cool white fluorescent light. Colour notations in parentheses are from Kornerup & Wanscher (1984). The structures were measured in water and lactophenol.

Molecular study

Strains used. The ITS region of the following strains were sequenced: *Chaetomium nigricolor* FMR 5737, *Gelasinospora bonaerensis* FMR 5962 and *Melanospora pascuensis* FMR 6367, *Corynascus heterothallicus* IEIA 486, *C. novoguineensis* IFO 9556, *C. setosus* IFO 9557, *C. thermophilus* IMI 145136 and *Thielavia terrestris* CBS 492.74. *Emericella nidulans* CBS 121.35 was used as the outgroup.

DNA extraction. Fungal DNA was isolated as described by Estruch *et al.* (1989) with some modifications (Guillamón *et al.* 1996). The strains were grown at 28° in Sabouraud broth contained in Erlenmeyer flasks and shaken at 200 rev min^{-1}. The mycelium was collected by filtration through nytal 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° in 2 ml of extraction buffer 80·2 M Tris/HCl 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 iso-propanol precipitation. The pellet was washed with 70% v/v ethanol, dried under vacuum and resuspended in TE buffer (10 mM Tris/HCl pH 8, EDTA 1 mM).

PCR amplifications. The ITS rDNA and 5·8 S rDNA gene were amplified as described by Gené *et al.* (1996) with a Perkin–Elmer 2400 thermal cycler (Perkin–Elmer Cetus Co., Emeryville, CA). The ITS5 and ITS4 primers (White *et al.* 1990) were used. The amplification programme consisted of pre-denaturalisation at 94°, 5 min; 30 cycles at 95°, 30 s; 50°, 1 min and 72°, 1 min; and final incubation at 72° for 7 min to complete the last extension. The final products were analysed by electrophoresis on 2% agarose MP (Boehringer–Mannheim) and cleaned following the GENECLEAN II protocol (BIO 101). The molecular weights of amplified DNA were estimated by comparison with 100 bp DNA ladder (Gibco–BRL) standard lane.

Sequencing and phylogenetic analysis. The protocol of the Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Gouda) was used for sequencing. Reactions were performed using the primers ITS5 and ITS4 (White *et al.* 1990) and were run on a 310 DNA sequencer (Applied Biosystems). The sequences were aligned using the Clustal W, version 1.5, of multiple sequence alignment computer program (Thompson, Higgins & Gibson 1994). Cladistic analyses using the neighbour-joining method (Saiton & Nei 1987) were performed with the MEGA 1.0 computer programme (Kumar, Tamura & Nei 1993). Confidence values for individual branches were determined by bootstrap analyses (500 pseudoreplicates). The sequences have been deposited in the European Molecular Biology Laboratory (EMBL).

RESULTS AND DISCUSSION

Taxonomy

Corynascus sexualis Stchigel, Cano & Guarro, sp. nov. (Figs 1–6)

Anamorph: unknown.

Ascomata globosa, non-ostiolata, brunnea pallescens vel atrobrunnea, glabra, 40–80 µm diam. Peridium ex *textura epidermoidea* compositum. Asci 19–22 × 15–18 µm, subglobosi vel ellipsoidei, octospori. Ascosporae 9–14 × 8–10 µm, limoniformes, brunneae, cum duobus poris germinalibus et apicalibus.

Typus: **India**: Jaipur, ex solo, 29 Oct. 95, *J. Guarro* [isol. *A. M. Stchigel*] (IMI 378520-holotypus, FMR 5691-isotypus).

Mycelium composed of hyaline to pale yellow, branched, anastomosing, septate, smooth hyphae, 1–5 µm broad. *Colonies* on PCA attaining 28–30 mm diam in 14 d at 22–25°, olive brown (M 4E7 to 4F8), flat, granulose by ascomata production, zonate, exudate golden yellow; reverse olive brown (M 4E7). *Ascomata* superficial, globose, non-ostiolate, pale to dark brown, glabrous, 40–80 µm diam; peridium with *textura epidermoidea*, composed of a layer of irregular, reticulate, golden brown cells. *Paraphyses* absent. *Asci* 19–22 × 15–18 µm, subglobose to broadly ellipsoidal, thin-walled, evanescent, 8-spored. *Ascospores* 9–14 × 8–10 µm, limoniform, hyaline when young and brown when mature, one-celled, smooth-walled, with a distinct germ pore at each end. Anamorph absent.

Colonies on PDA attaining 45–48 mm diam in 14 d at 22–25°, white to greyish yellow (M 3B3), cottony, zonate; exudate absent; reverse yellow to olive (M 3A7 to 3F8). *Ascomata* and conidia absent.

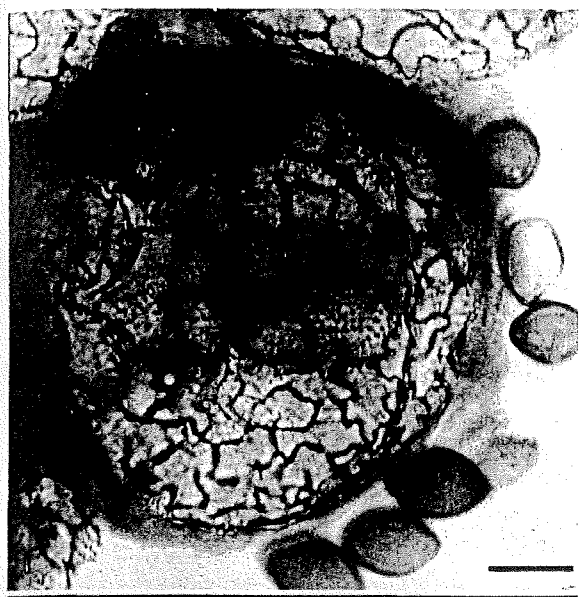
Colonies on OA attaining 42–45 mm diam in 14 d at 22–25°, yellowish white to olive (M 3A2 to 3F8), flat, granulose, zonate, with pale yellow exudate; reverse, yellowish white to olive. *Ascomata* very profusely produced and conidia absent.

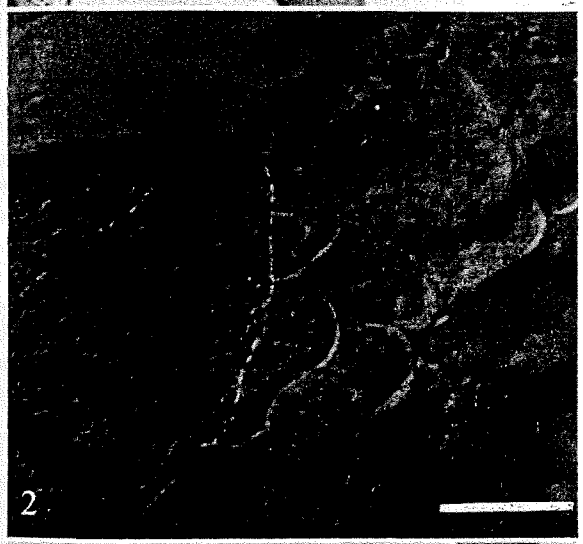
Colonies on MEA attaining 24–27 mm diam in 14 d at 22–25°, greyish orange to brownish orange (M 5B4 to 5C4), flat, slightly floccose, zonate; exudate absent; reverse yellow to olive (M 3A7 to 3F8). *Ascomata* and conidia absent.

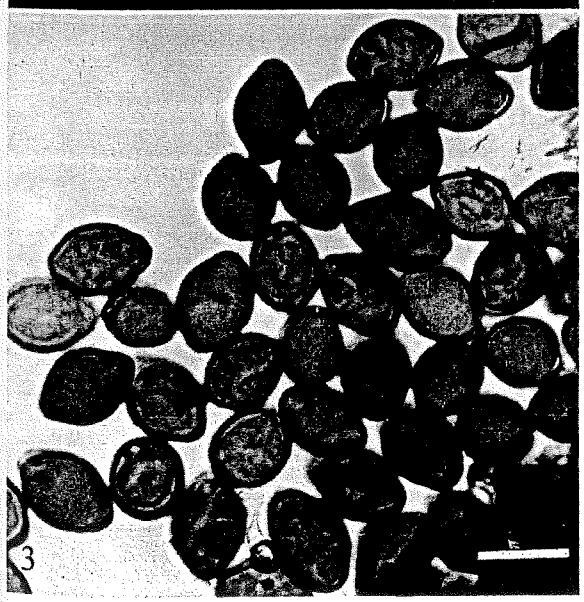
Thermotolerant, growing rapidly at 42°, with profuse production of ascomata in all tested media.

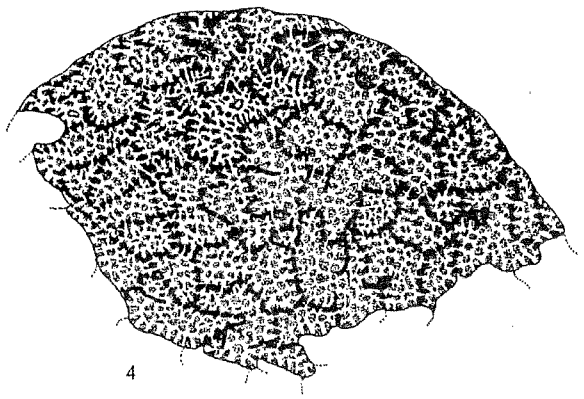
The sequence of the ITS1-2 and 5.8S rDNA regions is shown in Fig. 23. The main features are: 560 bp; 130 A; 163 C; 151 G and 116 T. Location ITS1 nt 29–192; gene 5.8S rRNA nt 193–348; ITS2 nt 349–560. EMBL accession no. AJ224202.

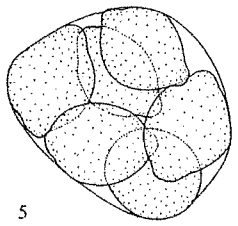
Corynascus sexualis is the only species of the genus that does not produce an anamorph on any of the culture media tested (MEA, OA, PCA, and PDA). Its ascospores are smaller than those of the previously described species with the exception of *C. heterothallus*. The ascospores of *C. sexualis* are, however, wider (8–13 × 5–7 µm in *C. heterothallicus*). The number of germ pores (mostly one in *C. heterothallicus*) and sexual compatibility (*C. heterothallicus* is heterothallic and *C. sexualis* homothallic) also differentiate the species.

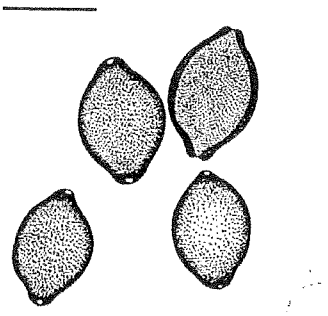












Figs 1–3. *Corynascus sexualis* FMR 5601. **Fig. 1.** Ascoma. **Figs 2.** Detail of the peridium with young ascus and ascospores, NDICM. **Fig. 3.** Ascospores. Bars = 10 µm.

Figs 4–6. *Corynascus sexualis* FMR 5691. **Fig. 4.** Peridium detail. **Fig. 5.** Ascus with ascospores. **Fig. 6.** Ascospores. Bars = 10 µm.

Corynascus similis Stchigel, Cano & Guarro, sp. nov.
(Figs 7–12)

Anamorph: *Myceliophthora* sp.

Ascomata globosa, non-ostiolata, brunnea pallescens vel atro-brunnea, glabra, 50–100 µm diam. Peridium ex *textura epidermoidea* compositum. Asci 26–38 × 20–31 µm, subglobosi vel ellipsoidei, octospori. Ascosporae 11–20 × 6·5–9 µm, navicullariformes, brunneae, cum duobus poris germinalibus lateratis vel sub-apicalibus. Conidia globosa vel piriformia, sub-hyalina vel palide aurantiaca, verrucosa, 7–9 µm diam.

Typus: **India**: Ajmer, ex solo, Nov. 1995, *J. Guarro* [isol. *A. M. Stchigel*] (IMI 378521-holotypus; FMR 5693-isotypus).

Mycelium composed of hyaline to pale yellow, branched, anastomosing, septate, smooth hyphae, 1–3 µm broad. *Colonies* on PCA attaining 45–50 mm diam in 14 d at 22–25°, greyish orange (M 5B3 to 5B4), olive brown (M 4E7 to 4F8) in the central area, flat, floccose to powdery due to the profuse production of conidia and ascomata, zonate, exudate absent; reverse greyish orange (M 5B3). *Ascomata* superficial, globose, non-ostiolate, translucent, pale to dark brown, glabrous, 50–100 µm diam; peridium with *textura epidermoidea*, composed of a layer of irregular, reticulate, golden brown cells. *Paraphyses* absent. *Asci* 26–38 × 20–31 µm, subglobose to

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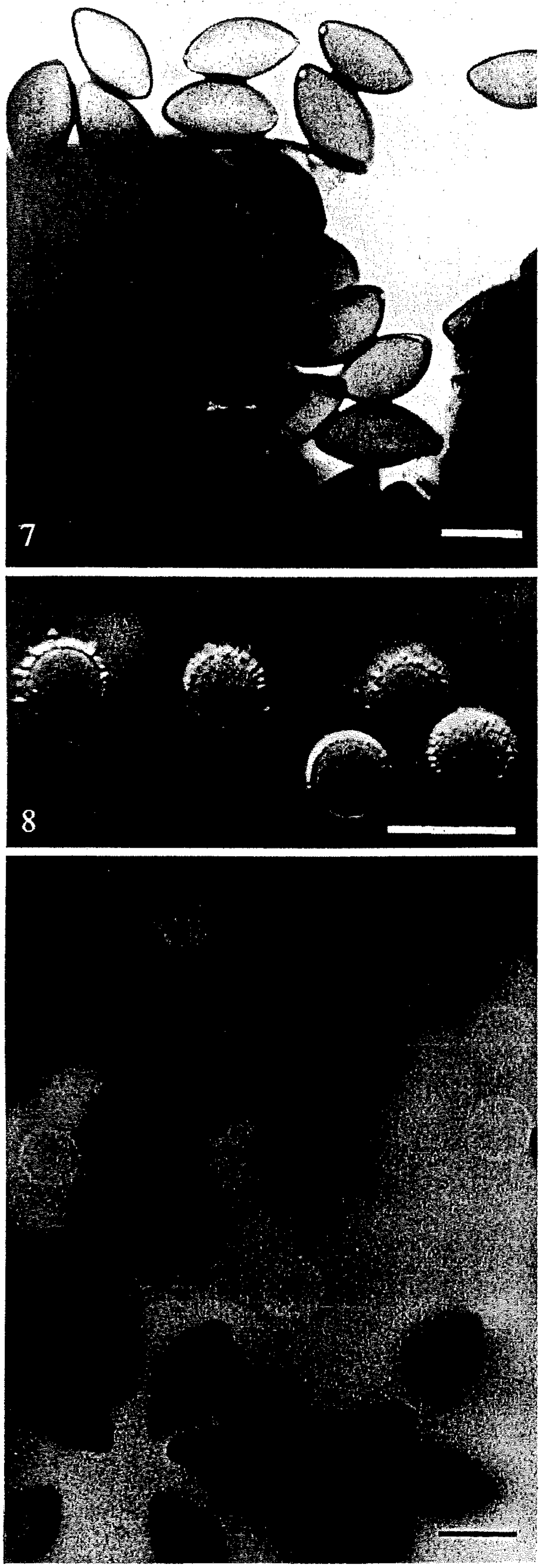
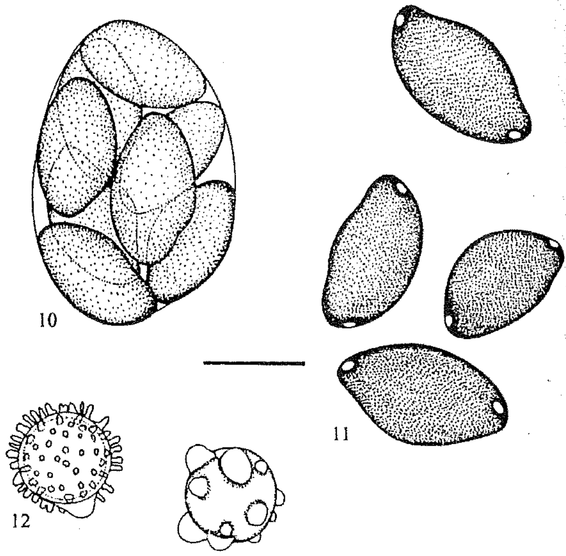


Figs 7–9. *Corynascus similis* FMR 5693. Fig. 7. Ascospores. Fig. 8. *Myceliophthora* anamorph conidia, NDICM. Fig. 9. Ascospores and conidia. Bar = 10 µm.



Figs 10–12. *Corynascus similis* FMR 5693. Fig. 10. Ascus and young ascospores. Fig. 11. Ascospores. Fig. 12. Conidia. Bar = 10 µm.

broadly ellipsoidal, short-stipitate, thin-walled, evanescent, 8-spored. *Ascospores* 11–20 × 6·5–9 µm, navicular in lateral view, hyaline when young and brown when mature, one-celled, smooth-walled, with a distinct lateral 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, 7–9 µm diam.

Colonies on PDA attaining 46–50 mm diam in 14 d at 22–25°, cottony, zonate, irregular margins, yellowish orange (M 4A6), with yellowish orange exudate; reverse yellowish orange (M 4A6).

Colonies on OA attaining 45–50 mm diam in 14 at 22–25°, flat, floccose to powdery, zonate, irregular margins, yellowish orange (M 4A6), with yellow exudate; reverse yellowish orange (M 4A6).

Colonies on MEA attaining 20–25 mm diam in 14 d at 22–25°, cottony, zonate, with irregular margins, light yellow (M 3A5), with yellowish orange exudate; reverse light yellow (M 3A5).

Thermotolerant, growing rapidly at 42°; ascomata and conidia profusely produced on PCA and OA at this temperature.

The sequence of the ITS1–2 and 5.8S rDNA regions is shown in Fig. 23. The main features are: 571 bp; 134 A; 162 C; 149 G and 126 T. Location ITS1 nt 29–193; gene 5.8S rRNA nt 194–350; ITS2 nt 351–571. EMBL accession no. AJ224201.