

## New and interesting species of *Monascus* from soil, with a key to the known species

Alberto M. Stchigel<sup>1\*</sup>, José F. Cano<sup>1</sup>, Samir K. Abdullah<sup>2</sup> and Josep Guarro<sup>1</sup>

<sup>1</sup>Unitat de Microbiologia, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, C/ Sant Llorenç 21, 43201 Reus, Tarragona, Spain; <sup>2</sup>Department of Biology, College of Science, University of Basrah, Iraq

\*Correspondence: Alberto Miguel Stchigel, e-mail: albertomiguel.stchigel@urv.net

**Abstract:** A new species, *Monascus argentinensis*, isolated from soil of Northern Argentina is described and illustrated. It is characterised by its ascomatal wall, which presents dark-coloured patches separated by hyaline areas; a *Basipetospora* anamorph with hyaline conidia; and the production of arthroconidia and sessile conidia. *Monascus lunisporas* Udagawa & Baba, another species with a similar peridial wall, is fully described and reported for first time as a soil-borne fungus. A molecular study based in the nucleotidic sequences of the ITS region support the status of *M. argentinensis* as a new species, and the inclusion of *Xeromyces bisporus* L.R. Fraser as a member of *Monascus*. A key to the accepted species of *Monascus* is provided.

**Taxonomic novelty:** *Monascus argentinensis* Stchigel & Guarro sp. nov.

**Key words:** Ascomycota, Eurotiales, Monascaceae, *Monascus*, soil-borne fungi, *Xeromyces*.

## INTRODUCTION

The genus *Monascus* Tiegh. was erected by van Tieghem (1884) in order to include *M. ruber* Tiegh. and *M. mucoroides* Tiegh. The former species was chosen as lectotype by Clements & Shear (1931), but due to the absence of original material, the latter was excluded from the genus (Hawksworth & Pitt 1983). The species of *Monascus* are characterised by non-ostiolate ascomata arising singly at the tip of stalk-like hyphae scattered on the mycelium, ascomatal wall composed of two distinct layers, the inner wall which results from the swelling of the tips of the stalk-like hyphae forming a vesicle-like structure and the outer layer consisting of hyphal branches growing out from the base and fusing with the inner vesicle; asci evanescent at an early stage; hyaline, 1-celled, ellipsoidal ascospores; and the formation of a *Basipetospora* anamorph. Hawksworth & Pitt (1983) revised the genus and accepted three species viz. *Monascus pilosus* K. Saito ex D. Hawksw. & Pitt, *M. purpureus* Went, and *M. ruber*. However, no mention was made of *M. aurantiacus* Z. Li (1982), a species that has largely remained forgotten. Barnard & Cannon (1987) described *M. floridanus* from pine tissues in U.S.A., and Hocking & Pitt (1988) erected *M. eremophilus*, the unique obligate xerophilic species of the genus from mouldy prunes in Australia. More recently, Cannon *et al.* (1995) added two new species, *M. pallens* P.F. Cannon, Abdullah & B.A. Abbas and *M. sanguineus* P.F. Cannon, Abdullah & B.A. Abbas, isolated from surface sediments of the Shatt al-Arab

river and its creeks in Iraq. These two species showed a non-osmophilic nature. Finally, *Monascus lunisporas* was described by Udagawa & Baba (1998) from mouldy feeds in Japan, and it is characterised by its dark, areolate ascomata, and lunate ascospores. Since the establishment of *Monascus*, its generic position and relationships have been in a continual state of flux. Went (1895) assigned the genus to the family *Thelebolaceae* (Brumm.) Ekblad (*Pezizales*). However, this opinion was rejected by Benny & Kimbrough (1980), who suggested a relationship with the *Ascosphaeriales*. Later, several workers accepted *Monascus* in its own family, *Monascaceae* J. Schröter, and included it in *Pezizales* (Malloch 1981, Hawksworth *et al.* 1983, Eriksson & Hawksworth 1991). More recently it has been placed in the *Eurotiales* (Hawksworth *et al.* 1995).

A similar genus is *Xeromyces* L.R. Fraser, which differs from *Monascus* in having sessile ascomata, 2-spored asci, plano-convex ascospores and a xerophilic habit. Species of *Monascus* have frequently been found in fermented food, foodstuffs rich in starch, mouldy high-moisture fruits, mouldy silages and soil (Domsch *et al.* 1980, Hawksworth & Pitt 1983). Most species are showing osmophilic affinity (Pitt & Hocking 1985). Some strains of *Monascus* are characterised by their economic importance, being involved in the industry of fermented food in the Orient (Hesseltine 1965, Lin 1975), production of food colouring pigments (Wong *et al.* 1981) and for their antibacterial activities (Wong & Bau 1977).

**Table 1.** Strains used in the molecular study.

Species	Strain	GenBank no.
<i>Aphanoascus keratinophilus</i>	IMI 319010, soil, Spain	AJ133436
<i>Byssoschlamys fulva</i>		AY306013
<i>B. nivea</i>		U18361
<i>Fennellia nivea</i>		AY373853
<i>Hamigera avellanea</i>		AF454075
<i>H. striata</i>		AF454074
<i>Monascus anka</i>		AF458473
<i>M. araneosus</i>		AF458471
<i>M. argentinensis</i> (ex-T)	FMR 7393, soil, Argentina	–
<i>M. floridanus</i> (ex-T)	IMI 282587, <i>Pinus clausa</i> , U.S.A.	–
<i>M. kaoliang</i>		AF451859
<i>M. lunisporas</i>	FMR 6679, soil, Brazil	–
<i>M. pallens</i> (ex-T)	IMI 356820, river sediment, Iraq	–
<i>M. pilosus</i> (ex-T)		AF451856
<i>M. purpureus</i>		AF033394
<i>M. ruber</i>		AF451855
<i>M. sanguineus</i> (ex-T)	IMI 356821, river sediment, Iraq	–
<i>Neosartorya fischeri</i>		AF176661
<i>Talaromyces flavus</i>		AF455509
<i>T. rotundus</i>		AF285115
<i>Xeromyces bisporus</i> (ex-T)	CBS 236.71, mouldy stick of liquorice, Australia	–

Ex-T = ex-type strain; CBS= Centraalbureau voor Schimmelcultures; FMR = Facultat de Medicina de Reus; IMI= International Mycological Institute (CABI Bioscience).

During studies on soil ascomycetes from different regions of the world, we have isolated two species of *Monascus* from Brazilian and Argentinian soils, which are characterised by having brownish ascomata. One of them was identified as *Monascus lunisporas* Udagawa & Baba, and is described here as the first report from soil. However, the second strain proved to be different from any currently known species of the genus and, therefore, is described as new.

## MATERIALS AND METHODS

### Sampling and fungal isolation

Argentinian soil samples were collected around Tafi del Valle, Tucumán province, in the Northwest of Argentina. The average temperature is 10–14 °C, with a rainfall less than 180 mm/year. The soil is sandy-stony, and the vegetation is composed mostly by *Asteraceae* (*Aphyllocladus spartioides* Wedd., *Gochnatia glutinosa* D. Don ex Hook. & Arn.), *Bromeliaceae* (*Tillandsia* spp.), *Cactaceae* (*Opuntia soehrensii* Britton & Rose, *O. tilcariensis* Backeb., *Parodia maassii* A. Berger, *P. tilcariensis* (Werdem. & Backeb.) Backeb., *Trichocereus pasacana* Britton & Rose, *T. terscheckii* Britton & Rose), *Papillonaceae* (*Cassia crassiramea* Benth.), and *Poaceae* (*Agrostis nana* Kunth, *Digitaria californica* Henrard, *Eragrostis* spp., *Munroa argentinensis* Griseb., *Stipa leptostachia* Griseb.) (Cabrera 1994). Soil samples from Brazil were collected in the Corcovado Mountain, Rio de Janeiro, a humid tropical forest without a dry season and with rainfall greater than 1300 mm/year. The average temperature is 24–25 °C. The maximum temperature recorded is 38 °C and the minimum is

about 7.4 °C (Mori 1989). The vegetation develops on granite domes and is composed of some members of the *Bromeliaceae* (*Pitcairnia glaziovii* Baker, *Tillandsia reclinata* Pereira & Martinelli), *Poaceae* (*Panicum maximum* Jacq., *Glaziophyton marabile* Franchet), *Orchidaceae* and *Velloziaceae* (Daly & Frank 1989). The soil samples were treated with 5 % (v/v) acetic acid for 10 min following the method of Stchigel *et al.* (2001). The isolation medium was potato carrot agar (PCA) containing chloramphenicol (30 µg/mL) to inhibit bacterial growth.

### Morphology

In order to show accurate comparison of colony characteristics and growth rates between our isolate and the currently accepted *Monascus* species, we followed the scheme described by Hawksworth & Pitt (1983), cultivating the fungi on Czapek yeast autolysate agar (CYA, Difco), malt extract agar (MEA, Difco) and glycerol 25 N agar (G25N, Difco) at 5, 25 and 37 °C, and potato-dextrose agar (PDA, Difco), oatmeal agar (OMA) and cornmeal agar (CMA, Difco) at 25 °C. Colour notations are according to Kornerup & Wanscher (1984). The measurements of the microscopic structures were taken with lactophenol as mounting medium.

### DNA isolation, amplification and phylogeny

Table 1 lists the strains used in the molecular study. Many ITS region nucleotidic sequences of representative species of *Monascus* and other *Eurotiales* were obtained from EMBL database. New sequences were obtained from *Monascus argentinensis* (culture ex-type), *M. floridanus* (culture ex-type), *M. lunisporas*,

*M. pallens* (culture ex-type), *M. sanguineus* (culture ex-type), and *Xeromyces bisporus* (culture ex-type). *Aphanoascus keratinophilus* was used as outgroup. The DNA was isolated as described by Guillamón *et al.* (1996). The strains were grown at 20 °C in Sabouraud broth (Sigma) in Erlenmeyer conical 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 mM 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 re-suspended in TE buffer (10 mM TrisHCl pH 8.0, 1 mM EDTA).

The rDNA ITS region containing ITS1 and ITS 2 and the intervening 5.8S rRNA gene was amplified using a Perkin Elmer 2400 thermal cycler (Perkin Elmer Cetus corporation, Emeryville, CA). Primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.* 1990) were used. The amplification programme consisted of pre-denaturalisation at 94–96 °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 GENECLAN 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 and run on a 310 DNA sequencer (Applied Biosystems). The new sequences were aligned using the Clustal W, version 1.5, computer programme for multiple sequence alignment (Thompson *et al.* 1994). Cladistic analyses using the neighbour-joining method (Saitou & Nei 1987) was performed with the MEGA 1.0 computer programme (Kumar *et al.* 1993) using the Kimura-2-parameter distance model (Kimura 1980). 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 programme.

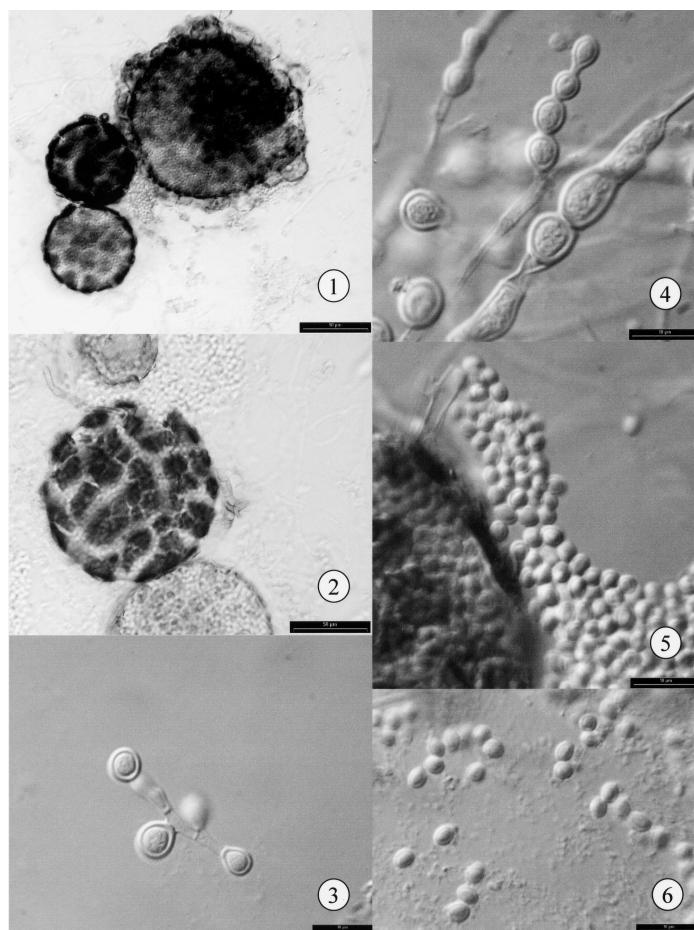
## RESULTS

### Taxonomic part

***Monascus argentinensis* Stchigel & Guarro, sp. nov.** MycoBank MB500076. Figs 1–8.

Similis *Monasco lunisporo* Udagawa & H. Baba sed coloniae 22–25 mm diam in "CYA" 25 °C post 7 dies, neque crescit 37 °C. Ascomata globosa, 20–75 µm diam, stipitata; peridium olivaceo-brunneum, 3–5 µm crassum. Asci octospori (?), evanescentes. Ascospores hyalinae, unicellulares, ellipsoideae vel subglobosae, 3–4 × 2.5–3 µm.

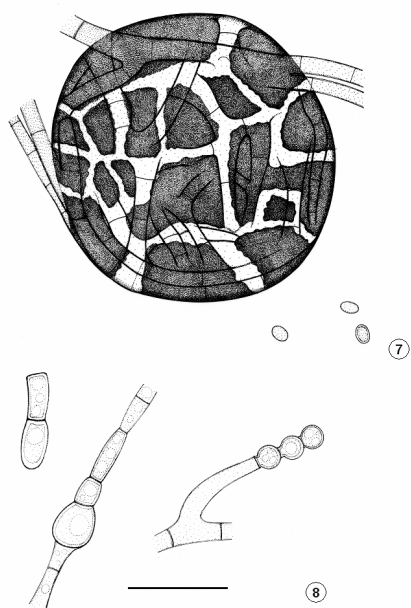
Anamorphosis: *Basipetospora* sp.



**Figs 1–6.** *Monascus argentinensis* (CBS 109402). 1. Ascomata. 2. Detail of peridial wall. 3. Anamorph. 4. Catenate blastoconidia and thallic conidia. 5–6. Ascospores. Scale bars: 1–2 = 50 µm, 3, 5–6 = 10 µm, 4 = 1 mm.

*Mycelium* abundant, hyphae branched, anastomosing, sometimes in fascicles, hyaline to pale brown, septate, smooth-walled, 2–6 µm wide, disarticulated when mature, forming chains of cylindrical, clavate or barrel-shaped arthroconidia, 5–20 × 3–7 µm; chlamydospores present, intercalary or lateral, singly or in chains, spherical to barrel-shaped, 4–15 µm diam. *Anamorph* consisting of erect conidiophores, short to long, 5–150 × 3–6 µm; conidia single or formed in short basipetal chains, usually borne terminally, sometimes borne laterally, globose to obpyriform,

with a flattened base, hyaline to pale olivaceous-brown, thick-walled; globose conidia 5–15 µm diam, obpyriform conidia, 7–15 × 5–9 µm. *Ascomata* non-ostiolate, globose, dark olivaceous-brown in reflected light, 20–75 µm diam, arising singly from a distinct, hyaline to pale olivaceous-brown, stalk-like hypha; *peridium* subhyaline to pale olivaceous-brown from an early stage, 3–5 µm thick; outer wall composed of brown, interwoven hyphae, developing irregularly polygonal plates, surrounded by hyaline areas, 5–25 µm diam; inner wall hyaline to subhyaline, translucent, thin, membranaceous; ascoma cavity filled with released mature ascospores. *Asci* 8-spored (?), early evanescent. *Ascospores* hyaline, 1-celled, broadly ellipsoidal to subglobose, 3–4 × 2.5–3 µm, smooth-walled.



**Figs 7–8.** *Monascus argentinensis* (CBS 109402). 7. Ascoma and ascospores. 8. Anamorph. Scale bar = 20 µm.

**Cultural characteristics:** On CYA at 25 °C after 7 d colonies attain 22–25 mm diam; colonies with sparse, delicately floccose mycelium, hyaline to pale brown (M. 6D5); exudate and soluble pigment absent; reverse of the same colour; ascomata and conidiogenesis abundant. On MEA at 25 °C in 7 d, colonies 20–22 mm diam, velvety, radiate, pale grey (M. 4D1), with white margins, aerial mycelium abundant; exudate and soluble pigment absent; reverse of the same colour; ascomata abundant, conidiogenesis not seen. On PDA at 25 °C in 7 d, colonies 18–21 mm, velvety, radiate, sulcate, dark grey (M. 1F1); exudate and soluble pigments absent; reverse of the same colour; ascomata abundant, conidiogenesis not observed. On CMA at 25 °C in 7 d, colonies 16–19 mm, flat, white, mostly composed of submerged mycelium, with scarce clusters of ascomata; exudate and soluble pigment absent; reverse white, olive-brown (M. 4E4) at the centre; ascomata abundant, conidiogenesis not seen. On OMA at 25 °C in 7 d, colonies 16–19 mm diam, flat, velvety to slightly granulose, greyish

yellow (M. 4C4); ascomata and conidiogenesis abundant.

**Typus:** **Argentina**, Tucumán, from soil sample, 15 May 2000, coll. by A.M. Stchigel, J. Guarro & J. Cano, isol. by A.M. Stchigel, **holotype** FMR 6778; cultures ex-type CBS 109402 = FMR 6778.

No growth was observed at 25 °C on G25N, and at 5 and 37 °C on any culture media tested.

**Notes:** The distinctive characteristics of the new species are the formation of a dark brown outer wall divided in polygonal plates when mature, the subglobose to broadly ellipsoidal ascospores measuring 3–4 × 2.5–3 µm, and hyaline to pale, smooth conidia. In addition, *M. argentinensis* can be distinguished from other *Monascus* species by its lack of growth on G25N at 25 °C. *Monascus argentinensis* is morphological close to *M. lunisporas*. In both species exudate and soluble pigments are absent, and both show the formation of dark ascomatal polygonal plates, but *M. lunisporas* can easily be distinguished by the big size (5–7 × 2.5–3 µm) and the shape (reniform to lunate) of its ascospores.

***Monascus lunisporas*** Udagawa & H. Baba, Cryptogamie Mycol. 19: 270. 1998. Figs 9–15.

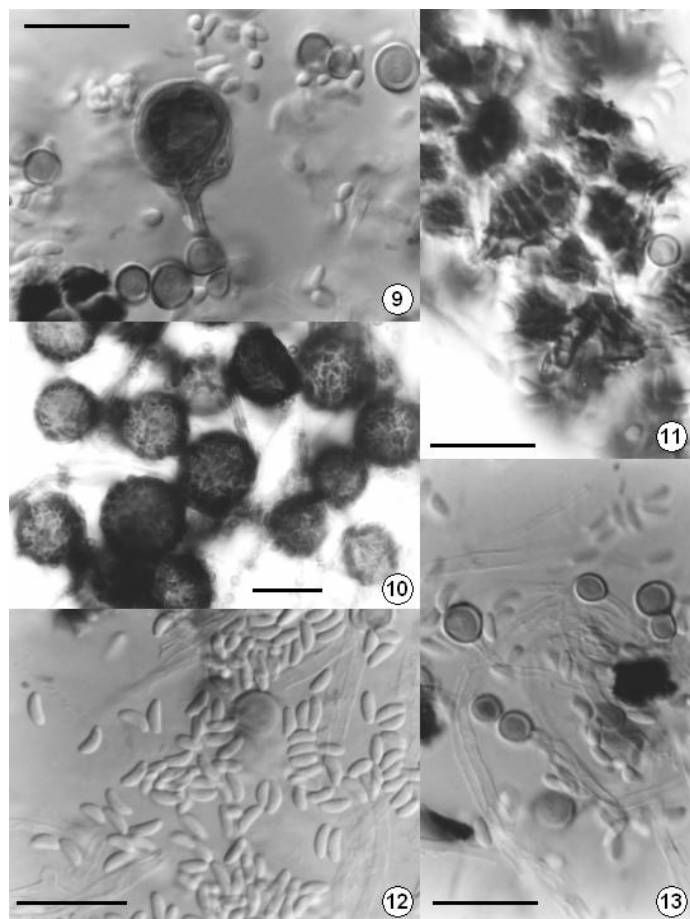
**Mycelium** abundant, hyphae irregularly branched, occasionally anastomosing, sometimes in fascicles, hyaline to brown, septate, smooth-walled, 3–5 µm wide. **Anamorph** consisting of conidiophores that are erect, short to long, 5–500 × 3–5 µm; conidia single or formed in short basipetal chains, usually borne terminally, sometimes borne laterally, globose to obpyriform, at first hyaline, becoming brown, smooth and thick-walled; globose conidia 6–11 µm diam, obpyriform conidia 5–7 × 7–10 µm. **Ascomata** non-ostiolate, globose, 25–60 µm diam, arising singly from a distinct, dark brown, stalk-like hypha; *peridium* brown from an early stage; outer wall composed of brown, interwoven hyphae, divided into dark brown, polygonal plates, surrounded by a hyaline to subhyaline area, 3–17 µm diam; inner wall hyaline to subhyaline, translucent, thin, membranaceous; ascomata cavity filled with released mature ascospores. *Asci* 8-spored, subglobose to globose, 6–8 µm diam, early evanescent. *Ascospores* hyaline, 1-celled, reniform or allantoid, 6–7 × 2–2.5 µm, smooth-walled.

**Cultural characteristics:** On MEA at 25 °C in 7 d, colonies 13–15 mm diam, floccose to lanose; orange-grey (M. 5B2) aerial mycelium abundant; exudates and soluble pigment absent; reverse pale brown (M. 6D5). On G25N at 25 °C in 7 d, colonies 10–12 mm diam, delicately floccose, mycelium raised at the centre, greyish yellow (M. 4FG); exudate and soluble

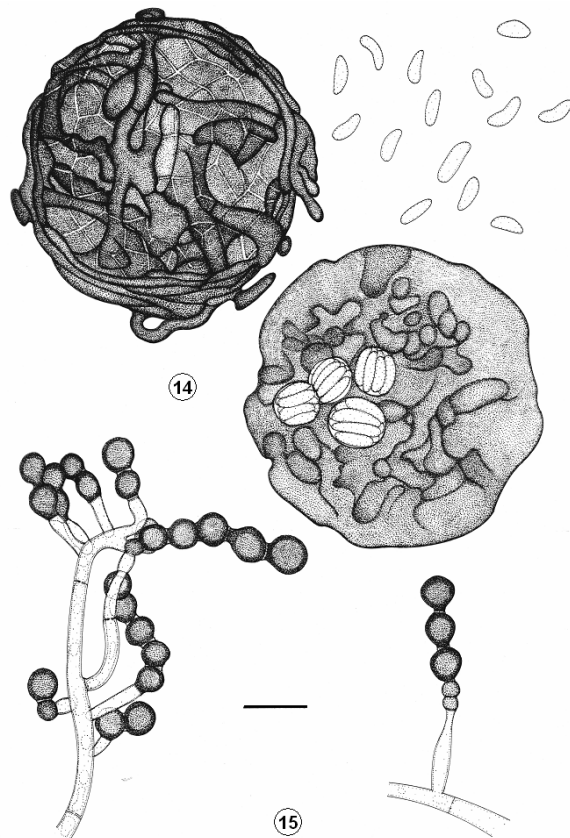
pigment absent; reverse of similar colour. On PDA at 25 °C in 7 d, colonies 15–18 mm diam; mycelium floccose to lanose, white at margin, grey at the centre (M. 4D1); reverse olive-brown (M. 4E3); exudate and soluble pigments absent; ascomata abundantly produced. On CMA at 25 °C in 7 d, colonies 11–13 mm diam, mycelium floccose, white at the margin, becoming greyish brown (M. 5F3); exudate and soluble pigment absent; reverse similar to obverse; ascomata abundantly produced. On OMA at 25 °C in 7 d, colonies 12–15 mm diam, similar characteristics as on CMA; ascomata abundantly produced. No growth at 5 and 37 °C on any culture medium tested.

*Specimen examined:* **Brazil**, Rio de Janeiro, Corcovado Mountain, from forest soil, 10 Feb. 1999, leg. J. Guarro, isol. A.M. Stchigel, CBS 113675 = FMR 6679.

*Notes:* *Monascus lunisporas* was previously described by Udagawa & Baba (1998), being isolated from mouldy feeds for racing horse in Tokyo, Japan. Our strain is morphologically practically identical to that, but is described here as a first report from soil.



**Figs 9–13.** *Monascus lunisporas* (CBS 113675). 9. Young stipitate ascoma. 10. Ascomata. 11. Detail of peridial wall. 12. Ascospores. 13. Anamorph. Scale bars: 9, 11–13 = 20 µm, 10 = 50 µm.



**Figs 14–15.** *Monascus lunisporas* (CBS 113675). 14. Outer and inner view of an ascoma, showing asci and ascospores. 15. Anamorph. Scale bars: 14–15 = 10 µm.

### Phylogeny

The reconstruction of the phylogenetic tree inferred from the analysis of the ITS region (Fig. 16) shows that species of *Monascus* and *Xeromyces bisporus* form a well-supported monophyletic clade (81 % bootstrap support). *Monascus argentinensis*, *M. floridanus*, *M. lunisporas*, *M. pallens* and *Xeromyces bisporus* form unispecific separated branches; whereas *M. pilosus*, *M. purpureus*, *M. ruber*, *M. sanguineus* and synonymized species of *M. purpureus* (*M. anka*, *M. araneosus* and *M. kaoliang*) grouped in the same sub-clade (99 % bootstrap support), with practically no differences among their sequences.

Future studies based on the comparison of sequences of other structural genes (18 rDNA,  $\beta$ -tubulin, etc.) could confirm the possible synonymy of these taxa as *M. ruber* Tiegh.

### DISCUSSION

The phylogenetic analysis of the ITS region of previously accepted species of *Monascus* and related *Eurotiales* has proven to be very useful to support the erection of *Monascus argentinensis* as a new species for this genus, proposal also based on morphological criteria. Moreover, the results showed that *Xeromyces bisporus*, *M. floridanus*, *M. lunisporas* and *M. pallens* are well circumscribed and separated species, whereas the rest of the species included in the study were nested in a unique group. Probably further studies

testing more genes would demonstrate that these species are identical.

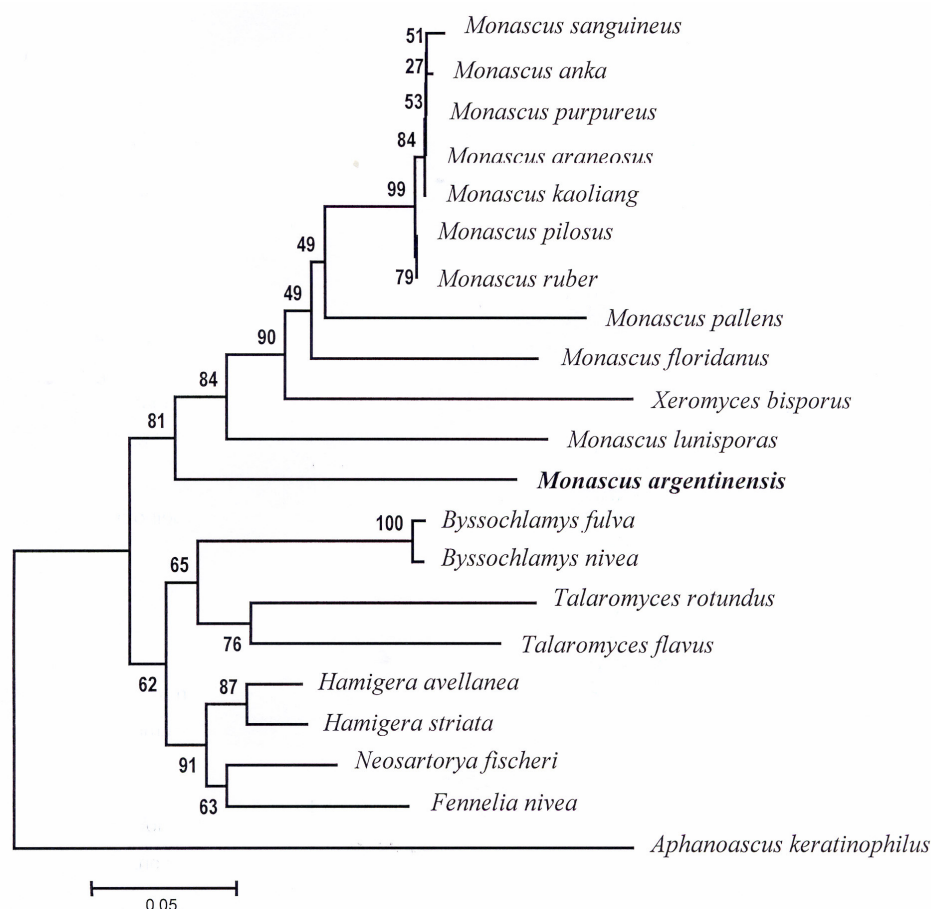
*Xeromyces bisporus* is a unique organism, because it can grow at an  $a_w$  of 0.61. Von Arx (1970) considered that *Monascus* and *Xeromyces* were congeneric, and transferred *X. bisporus* to *Monascus*. However, his proposal was not accepted due to the important differences in some morphological features of *X. bisporus*, such as the nature of its ascomatal initials, the non-stalked ascomata, the 2-spored asci, and the *Fraseriella* Cif. & A.M. Corte anamorph (Domsch *et al.* 1980, Kimbrough 1981, Hawksworth & Pitt 1983, Udagawa & Baba 1998). Recently, in a molecular study using the sequences of the D1/D2 regions of the LSU rRNA gene, Park & Jong (2003) found that *Monascus* and *Xeromyces* form a monophyletic clade,

and that *X. bisporus*, *M. lunisporas*, *M. pallens* and *M. floridanus* are grouped in the same subclade with a 95 % bootstrap value. We agree with these authors, because in our phylogenetic tree *X. bisporus* formed a separated branch between *M. lunisporas* and *M. floridanus*. Consequently, these results, together with morphological data confirm the combination proposed by von Arx in 1970.

***Monascus bisporus*** (L.R. Fraser) Arx, Gen. Fungi Spor. Pure Cult.: 84. 1970.

**Basionym:** *Xeromyces bisporus* L.R. Fraser, Proc. Linn. Soc. N. S. Wales 78: 245. 1953.

**Anamorph:** *Fraseriella bispore* Cif. & A.M. Corte, Atti Ist. bot. Univ. Pavia, ser. 5, 14: 109. 1957.



**Fig. 16.** Phylogenetic tree, using the neighbour-joining method of representatives of *Monascus* spp. and other *Eurotiales* inferred from analysis of the ITS region. Bootstrap values calculated from 1000 replicates are given at the branches.

### Key to the species of *Monascus* (modified from Udagawa & Baba 1998)

1. Ascomata sessile; asci 2-spored ..... ***M. bisporus*** (L.R. Fraser) Arx (1970)
1. Ascomata stalked; asci 8-spored or undetermined ..... 2
2. Colonies not growing on CYA or MEA. .... ***M. eremophilus*** A.D. Hocking & J. Pitt (1988)
2. Colonies growing on CYA or MEA. .... 3
3. Ascomata remaining hyaline or pale coloured. .... 4
3. Ascomata significantly pigmented. .... 7

4. Ascospores  $3.5 \times 2.5\text{--}3\text{ }\mu\text{m}$ .....*M. pallens* P.F. Canon, Abdullah & B.A Abbas (1995)
4. Ascospores  $\geq 5\text{ }\mu\text{m}$  in length.....5
5. Soluble pigment not formed.....*M. aurantiacus* Zhong Q. Li (1982)
5. Soluble pigment formed.....6
6. Ascospores  $5\text{--}7(8.5) \times 3\text{--}3.5(4)\text{ }\mu\text{m}$ . ....*M. pilosus* K. Saitô ex D. Hawksw. & J. Pitt (1983)
6. Ascospores  $(5.5\text{--})6\text{--}7 \times (4\text{--})4.5\text{--}5\text{ }\mu\text{m}$ . ....*M. purpureus* Went (1895)
7. Ascomatal wall with dark-coloured patches.. ....8
7. Ascomatal wall without these patches .....9
8. Ascospores reniform to allantoid,  $6\text{--}7 \times 2\text{--}2.5\text{ }\mu\text{m}$ .....*M. lunisporas* Udagawa & H. Baba (1998)
8. Ascospores broadly ellipsoidal to subglobose,  $3\text{--}4 \times 2.5\text{--}3\text{ }\mu\text{m}$ .....*M. argentinensis*
9. Ascospores  $3.5\text{--}4.5 \times 2\text{--}3\text{ }\mu\text{m}$ .....*M. floridanus* P.F. Canon & E.L. Barnard (1987)
9. Ascospores  $\geq 5\text{ }\mu\text{m}$  in length.....10
10. Red soluble pigment produced... ..*M. sanguineus* P.F. Canon, Abdullah & B.A Abbas (1995)
10. Soluble pigment usually absent, brown when present .....*M. ruber* Tiegh. (1884)

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