STUDIES IN MYCOLOGY 50: 77–82. 2004.

A new species of *Achaetomium* from Indian soil

Kendra Rodríguez[1,2], Alberto M. Stchigel[1], José F. Cano[1] and Josep Guarro[1*]

[1]*Unitat de Microbiologia, Facultat de Medicina i Ciències de la Salut, and Institut d' Estudis Avançats, Universitat Rovira i Virgili, C/Sant Llorenç 21, 43201 Reus, Spain;* [2]*Instituto de Ecología y Sistemática, Ministerio de Ciencias, Tecnología y Medio Ambiente, Carretera de Varona Km 3.5, Capdevila Boyeros, AP 8029, CP 10800, Ciudad de la Habana, Cuba*

**Correspondence*: Josep Guarro, josep.guarro@urv.net

Abstract: The new species *Achaetomium umbonatum* (*Chaetomiaceae*, *Ascomycota*), isolated from soil in Northern India, is described, illustrated and compared with morphologically similar taxa. It is characterised by its thermotolerance, and the opaque, big, limoniform ascospores. Analysis of the D1 and D2 regions of the LSU rRNA gene supports the proposal that this new species.

Taxonomic novelties: *Achaetomium irregulare* (Sörgel) Kendra Rodríguez, Stchigel & Guarro comb. nov., *A. umbonatum* Kendra Rodríguez, Stchigel & Guarro sp. nov.
Key words: *Achaetomium, Ascomycota, Chaetomiaceae, Chaetomium,* soil.

INTRODUCTION

The genus *Achaetomium* J.N. Rai & J.P. Tewari was established by Rai *et al.* (1964) to include three species, *Achaetomium globosum* J.N. Rai & J.P. Tewari (type species), *Achaetomium luteum* J.N. Rai & J.P. Tewari and *Achaetomium strumarium* J.P. Rai, J.P. Tewari & Mukerji. These species are characterised by ostiolate, tomentose (covered with yellowish hypha-like hairs), globose to pyriform ascomata; a rather thick peridium with *textura intricata*; cylindrical asci; and opaque, dark brown ascospores with an apical germ pore. Moreover, their colonies usually show a reddish brown exudate (von Arx 1985, von Arx *et al.* 1988). The species of *Achaetomium* are usually isolated from soil (von Arx 1985), but they have occasionally been isolated from human infection (Abbot *et al.* 1995), and cytotoxic metabolites were described from some strains (Udagawa *et al.* 1979). The ability to grow at high temperatures (Udagawa 1982, de Hoog *et al.* 2000) and high osmotic pressure (Chowdhery & Rai 1980) are two typical physiological features of *Achaetomium*. *Chaetomium* Kunze 1817 is its morphologically closest genus. However, *Chaetomium* has paler ascospores, setiform hairs, and a thinner and paler peridium with *textura angularis* to *epidermoidea*. In addition, *Chaetomium* species are usually mesophilic (growing between 15 to 35 °C) (von Arx *et al.* 1986), although some of them can also be thermotolerant (Millner 1977, Mouchacca 1997, 2000). Cannon (1986) reviewed the previous works on the taxonomy of *Achaetomium,* and only accepted *A. globosum* in this genus. However, von Arx *et al.* (1988) did not agree with this opinion, and reconsidered the three original species.

During a survey of soil ascomycetes from different regions of the world, we have isolated numerous strains belonging to *Achaetomium*. One of them, isolated from India, showed a combination of morphological features that did not fit with any other species of the genus. In this study, it is fully described and illustrated, and proposed as a new species. To determine the molecular relationships of this taxon with the other species of the genus and related species of *Chaetomiaceae* and *Sordariaceae*, we have studied the nucleotidic sequences of the D1 and D2 regions of the LSU rDNA gene.

MATERIALS AND METHODS

Sampling and fungal isolation

Soil samples were collected from a public garden in Delhi, 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, with an average temperature of 10 to 35 °C in winter and 25 to 43 °C in summer. The total annual precipitation is about 900 mm. Samples were collected from the A_o horizon using sterilised polyethylene bags, which were sealed with a rubber band and labelled. On return to the laboratory the samples were stored at 4–7 °C. The soil samples were treated with ethanol according to Warcup & Baker (1963) for the selective isolation of ascomycetes, and cultured on potato-carrot agar (PCA; potato 20 g, carrot 20 g, agar 15 g, water 1 L) with chloramphenicol (50 mg/L) at room temperature (22 to 25 °C) for 14 d under 12 h darkness alternating with 12 h of cool white fluorescent light. Cultures were examined under a stereomicroscope.

Table 1. Strains and sequences used in the molecular study.

Species	Collection no.	Substrate	Country	EMBL no
A. globosum	FMR 7205	Soil	India	AJ312096*
A. globosum	FMR 7206	Soil	India	AJ312097*
A. luteum	FMR 7207	Soil	India	AJ312105*
A. strumarium	IMI 082624 (T)	Soil	India	AJ312098*
A. umbonatum	IMI 381871 (T)	Soil	India	AJ312099*
C. irregulare	IFO 32979	Soil	Spain	AJ312100*
C. hexagonosporum	CBS 171.84	Dung	U.S.A.	AJ312103*
C. bostrychodes	FMR 7196	Soil	India	AJ312101*
C. robustum	FMR 7201	Soil	Cuba	AJ312102*
C. quadrangulatum	FMR 7202	Soil	Cuba	AJ312104*
C. globosum	ATCC 44699	Soil	Japan	U47825
S. fimicola	HKUCC 3714	_	_	AF132330
N. crassa	MUCL 19026	_	_	AF286411
G. bonaerensis	IMI 375099	Soil	Argentina	AJ 002029*

(T) = type strain; *A.* = *Achaetomium*; *C.* = *Chaetomium*; *G.* = Gelasinospora; *N.* = *Neurospora; S.* = *Sordaria*; CBS = Centraalbureau voor Schimmelcultures; FMR = Facultad de Medicina de Reus; IFO = Institute for Fermentation, Osaka; IMI = CABI Bioscience International Mycological Institute; MUCL= Mycotheque de l' Universite Catholique de Louvain; HKUCC = University of Hong Kong Culture Collection; ATCC= American Type Culture Collection. * = Sequences that were newly generated.

Mature ascomata were dissected using sterile needles, and single ascospores were transferred to oatmeal agar (OA; oatmeal 30 g, agar 15 g, water 1 L), potato dextrose agar (PDA, Difco) and PCA to obtain pure cultures.

Morphological study

The cultural features were studied on OA, PDA and PCA in Petri dishes of (90 mm diam) at 15, 25, 35, 40 and 45 °C. Colour notations in parentheses are from Kornerup & Wanscher (1984). The measurements of the microscopic structures were taken in lactophenol. Photomicrographs were obtained with a Leitz Dialux 20 EB microscope. Dried and living cultures have been preserved in the collections indicated in the text.

Molecular study

The strains used in the study are listed in Table 1. The DNA was isolated as described by Cano *et al*. (2002). The domains D1 and D2 of the 28 S rRNA gene were amplified as described by O'Donnell (1993) using a Perkin Elmer 2400 thermal cycler (Perkin Elmer Cetus Co., Emervyville, CA) and NL1 and NL4 primers. The amplification programme consisted of pre-denaturation at 94 °C, 5 min; 30 cycles at 94 °C, 45 s; 51 °C, 1 min and 72 °C, 3 min; and final incubation at 72 °C for 10 min to complete the final extension. The final products were purified by GFX™ PCR DNA purification kit (Pharmacia Biotech) and stored at -20 °C until used in sequencing. The molecular weight of the amplified DNA was estimated by comparison with 100 bp DNA ladder (Gibco BRL) standard lane. The protocol of the Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Gouda) was used for sequencing. Reactions were performed using the primers NL1 and NL4, 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) were performed with the MEGA 1.0 computer program (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). The sequences have been deposited in the European Molecular Biology Laboratory (EMBL).

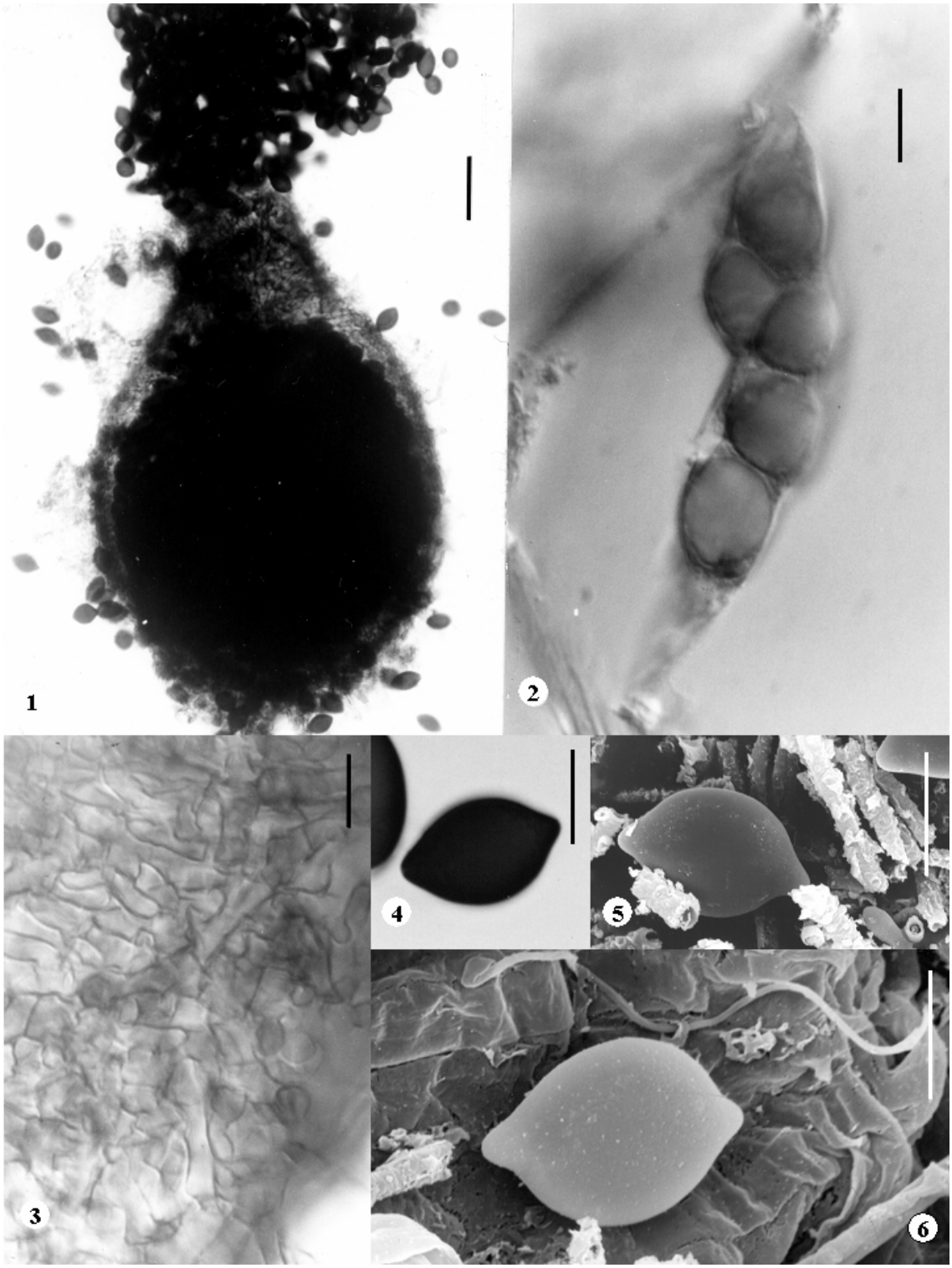
RESULTS

Taxonomy

Achaetomium umbonatum Kendra Rodríguez, Stchigel & Guarro, **sp. nov.** MycoBank MB500019. Figs 1–9.

Etymology: Latin *umbonatus*, referring to the umbonate ends of the ascospores.

Hyphae subhyalinae, septatae, laeves, 1–5 µm latae. *Coloniae* in agaro farina avenacea confecto celeriter crescentes, planae, ex mycelio vegetativo diffuso et submerso constantes, numerosa ascomata formantes, superficie et reverso flavo-albis. *Ascomata* superficialia, aurantio-brunnea, ovoidea vel piriformia, gregaria, 162–280(–310) µm alta, 160–210 µm diam, celeriter maturantia, ostiolum latum, usque ad 90 µm diam. *Peridium* brunneum, ostiolum versus pallide, e 4–6 stratis cellularum compositum, 10–12 µm crassum, textura epidermoidea vel textura intricata. Pili delicati hyphis similis, flexuosi vel undulati, pallido brunnei vel aurantio-brunnei, septati, 2–3.5 µm lati, simplices, verrucosi. *Asci* fasciculati, lineari-cylindrici vel subcylindrici, 8-spori, evanescentes, 45–50 × 7.5–16.5 µm, brevistipitati. *Paraphyses* et *periphyses* nullis.

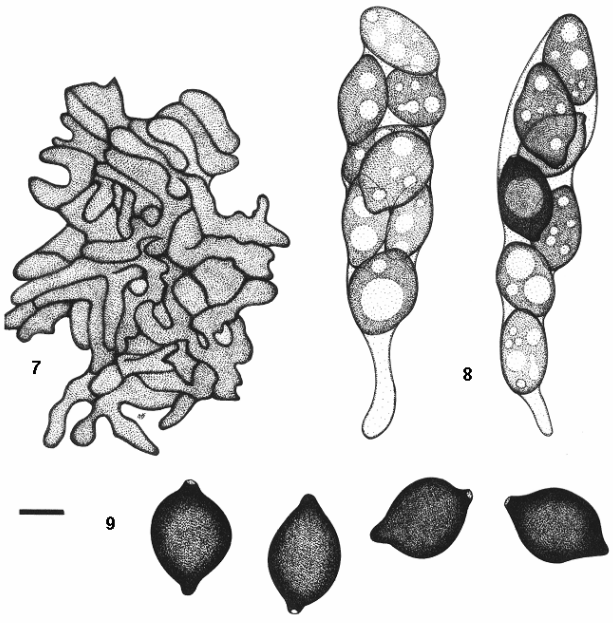


Figs 1–6. *Achaetomium umbonatum* (IMI 38289). 1. Ascoma and ascospores. 2. Asci and ascospores. 3. Peridium wall. 4. Ascospores (LTM). 5, 6. Ascospores (SEM). Scale bars:1 = 40 µm, 2–5= 10 µm, 6 = 5 µm.

Ascosporae unicellulares, atrobrunneae, limoniformes, 13.5–17(–19) × 9.5–11.5 × 7–9.5 µm, laeves, poro germinali apicali visibili. *Status conidialis* nullus.

Mycelium composed of subhyaline, septate, smooth-walled, anastomosing, 1–5 µm wide hyphae. *Colonies* on OA growing rapidly, covering the Petri dishes completely after 14 d at 25 °C, flat, felty, consisting of spreading, submerged vegetative mycelium, producing abundant ascomata which are covered by a loose network of aerial hyphae, yellowish white (M. 3A2), with a reddish brown exudate; reverse similar in colour to the surface. *Ascomata* superficial, clustered, tomentose, golden-brown, ovoid to pyriform, 162–280(–310) high, 160–210 µm diam, maturing within 14 d, with a broad apical ostiole, up to 90 µm diam, non-papillate. *Peridium* brown, becoming paler towards the ostiole, 4–6 layered, 10–12 µm thick,

textura epidermoidea to *textura intricata*. *Ascomatal hairs* delicate, hypha-like, flexuous or undulate, brown to reddish brown, septate, 2–3.5 µm wide at the base, unbranched, verrucose, often covered with acicular crystals of various size. *Asci* fasciculate, cylindrical to subcylindrical, irregularly biseriate, 8-spored, soon evanescent, 45–50 × 7.5–16.5 µm, short-stipitate, without apical structures. *Paraphyses* and *periphyses* not observed. *Ascospores* 1-celled, dextrinoid when young, opaque, dark brown, limoniform, dorsiventrally slightly flattened, 13.5–17(–19) × 9.5–11.5 × 7–9.5 µm, smooth-walled, with an apical germ pore. *Anamorph* not observed. On PCA at 25 °C, colonies similar to those on OA at 25 °C, with profuse sporulation. On PDA at 25 °C, colonies growing rapidly, attaining a diameter of 80–85 mm in 14 d, cottony, strongly zonate, olive (M. 3E4), and honey yellow (M. 5D6) at the centre, consisting of submerged and aerial mycelium with abundant ascomata; reverse brownish orange (M. 5C4) to pale orange (M. 5C3); exudate red. On OA and PCA at 45 °C colonies growing very slowly, attaining a diameter of 20–25 mm in 14 d, flat, felty, yellowish white (M. 2A2); reverse similar in colour to the surface; ascomata not produced. No growth observed at 45 °C. On OA, PCA and PDA at 35 and 40 °C, colonies growing rapidly, covering the whole Petri dish surface after 14 d, rather similar to those on OA at 25 °C. No growth was observed at 15 °C.



Figs 7–9. *Achaetomium umbonatum* (IMI 38289). 7. Peridium wall. 8. Asci with ascospores. 9. Ascospores. Scale bars: 7–9 = 10 µm.

Typus: **India**, Delhi, from garden soil, 11 Jul. 1996, coll. J. Guarro, isol. A.M. Stchigel, IMI 38289 **holotype**, cultures ex-type IMI 381871 = CBS 102436 = MUCL 43150 = FMR 6778.

Notes: The ascospores of *A. umbonatum* are similar to those of *A. luteum* and *A. strumarium*, but in the latter two species spores are smaller (8–11.5 × 5.5–7.5 µm and 10–13 × 6–8 µm, respectively). Furthermore, both species have narrowly cylindrical asci with uniseriate ascospores, while asci are broadly cylindrical to subcylindrical, with irregularly biseriate spores in *A. umbonatum*. *Achaetomium strumarium* also differs from *A. umbonatum* by the presence of a *Lecythophora*-like anamorph (Abbott *et al.* 1995), and *A. luteum* by the presence of chlamydospores.

Phylogeny

The phylogram obtained from the analysis of the sequences of the D1 and D2 regions of the 28S rRNA gene using the neighbour-joining method (Fig. 10) shows two well-delimited clades. The first clade encompasses the members of *Chaetomiaceae*, and the second the members of *Sordariaceae*, both supported by a bootstrap value of 100 %. These results do not confirm Cannon's (1986) hypothesis, based on the dark colour of the ascospores, which indicate that *Achaetomium* might have been derived from *Sordariaceae* ancestors rather than *Chaetomiaceae*. In this study, the species of *Chaetomium* and *Achaetomium* were grouped together sharing a common ancestor, while *Chaetomium globosum* Kunze 1817 appears as the basal taxon in the *Chaetomiaceae*. The cluster formed by the species of *Achaetomium* received a bootstrap value of 64 %, and also included *Chaetomium irregulare* Sörgel 1966. *Achaetomium umbonatum* showed a high degree of nucleotide sequence similarity, greater than 98%, with the other three species of the genus. The maximum difference between nucleotide sequences was observed between *A. luteum* and *A. strumarium*, with a similarity of 97.2 %.

DISCUSSION

In this study we have analysed the partial LSU of rDNA nucleotide sequences because in previous studies (Stchigel *et al*. 2000) we noticed that the ITS region, other very informative region of the fungal genome for taxonomic purposes frequently used in similar studies, was not useful for establishing phylogenetic relationships in *Sordariales*. Lee & Hanlin (1999), in a phylogenetic study of *Chaetomium* and similar genera based on 18S rDNA gene sequence analysis, included two species of *Achaetomium* in an attempt to confirm the distinctness of *Achaetomium* from *Chaetomium* on a molecular basis. The species chosen were *A. strumarium* and *A. macrosporum* J.N. Rai, Wadhwani & J.P. Tewari (1970), but the latter is currently considered as a synonym of *Chaetomium vitellinum* A. Carter (von Arx *et al*. 1986) and consequently such approach did not allow taking any firm conclusion.

In our study, *Achaetomium* arises from *Chaetomium*, which resulted to be paraphyletic if the former is recognized as a separate genus. *Chaetomium irregulare* was also nested among the *Achaetomium* species. This is not surprising because it shows a similar thick ascomatal wall, peridial hairs and cultural characteristics to those of *Achaetomium*, and its ascospores are similar in shape to those of *A. luteum.* Consequently, based on molecular and morphological data we propose the transference of *C. irregulare* to the genus *Achaetomium*.

Achaetomium irregulare *(*Sörgel) Kendra Rodríguez, Stchigel & Guarro, **comb. nov.**
MycoBank MB500020.
Basionym: *Chaetomium irregulare* Sörgel ex W. Gams, *Nova Hedwigia* **12**: 386. 1966.

Notes: This work should be considered only a preliminary phylogenetic study of *Achaetomium*, but which has been useful to confirm the proposal of the new species. It demonstrated, however, that the D1 and D2 nucleotide region is also very conserved in the members of *Chaetomiaceae*, which highlights the need to analyse other genes in order to obtain more conclusive results.

Key to species of *Achaetomium*

1	Ascospores nearly spherical, 10–13 × 8–11 µm	***A. globosum***
	Ascospores ellipsoidal, fusiform or limoniform	2
2(1)	Ascospores, 13.5–17(–19) × 9.5–11.5 × 7–9.5 µm, limoniform	***A. umbonatum***
	Ascospores shorter than 13 µm	3
3(2)	Ascospores 8–10 × 5–7 µm; chlamydospores present; ascomata usually separated	***A. luteum***
	Ascospores 9–12 × 6–7 µm; ascomata clustered or aggregated	***A. strumarium***

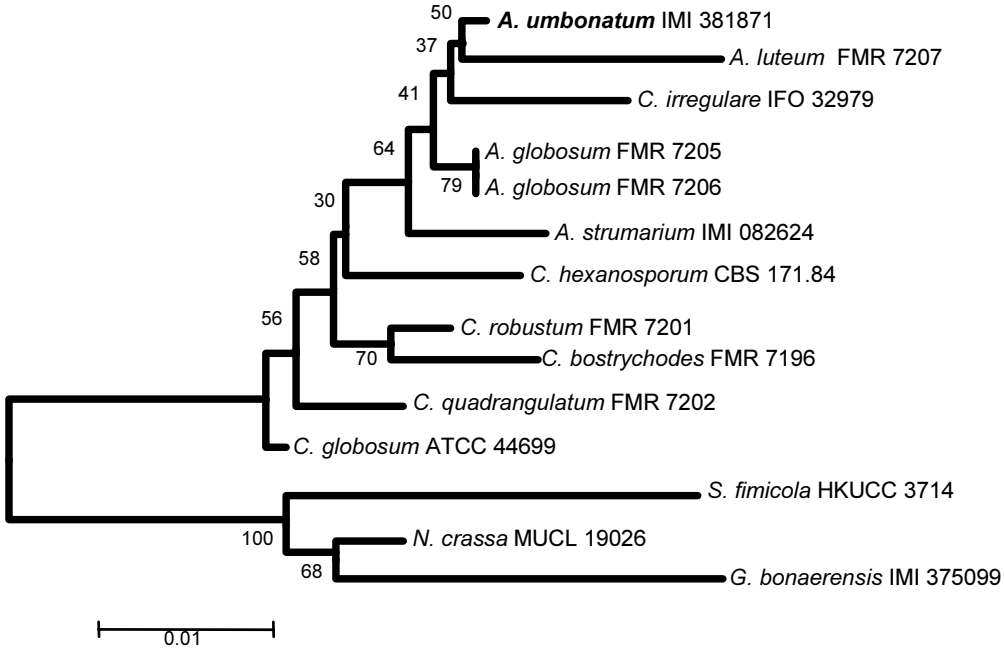


Fig. 10. Phylogenetic tree, using the neighbour-joining method, of representatives of *Chaetomiaceae* and *Sordariaceae* inferred from analysis of the D1 and D2 regions of partial 28S rDNA sequences data. Bootstrap values calculated from 1000 replicates are included at the branches.

ACKNOWLEDGEMENTS

The first author acknowledges funds from Agencia Española de Cooperación Internacional (AECI). We thank L. Pitarch and D. García for kindly providing cultures, and to Susana de Haro, for technical assistance. This study was supported by the Spanish Ministerio de Ciencia y Tecnología, grant CGL 2004-00425/BOS.

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