Coronatomyces cubensis gen. et sp. nov., a new ascomycete from Cuban soil

Dania García[1,2], Alberto M. Stchigel[1], José Cano[1] and Josep Guarro[1*]

[1]*Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, C/ Sant Llorenç 21, 43201, Reus, Tarragona, Spain;* [2]*Instituto de Investigaciones Fundamentales en Agricultura Tropical "Alejandro de Humboldt", calle 1 esq. 2, Santiago de las Vegas, Boyeros, C. de La Habana, Cuba*

**Correspondence*: Josep Guarro, umb@fmcs.urv.es

Abstract: The new ascomycete *Coronatomyces cubensis* gen. et sp. nov., isolated from Cuban soil and characterized by ostiolate or non-ostiolate, setose ascomata and one-celled, thick-walled, dark brown, and ellipsoidal ascospores, with a single germ pore at the umbonate base, is described and illustrated. Molecular studies were performed on the internal transcribed spacer (ITS1, ITS2) and the 5.8S gene to investigate the relationships of this new taxon with other members of *Sordariales*.

Taxonomic novelties: *Coronatomyces* D. García, Stchigel & Guarro gen. nov., *Coronatomyces cubensis* D. García, Stchigel & Guarro sp. nov.
Key words: *Ascomycota*, soil-borne fungi, *Sordariales.*

INTRODUCTION

During the course of a survey of soil ascomycetes in Cuba, an undescribed ascomycete was isolated from a soil sample from Ciénaga de Zapata (Matanzas province). This fungus is characterised by ostiolate or non-ostiolate ascomata and thick- and smooth-walled, dark brown, opaque, ellipsoidal ascospores, which have a wide germ pore at the umbonate base. This taxon was easily recognized as belonging to the *Sordariales,* although its inclusion in any particular family was difficult. This fungus is described here as a new genus and compared with morphologically similar taxa.

MATERIAL AND METHODS

Sample origin and fungal isolation

Soil samples were collected in Cuba, Ciénaga de Zapata region, UNESCO Biosphere Reserve. This is a peculiar region due to its high percentage of endemic, rare and endangered organisms. The reserve is located South of Matanzas, at –2 to 10 m above sea level. The annual average temperature is 24–26 ºC. The major ecosystem types are mangrove and swamp forest. Representative species in the mangrove forest are *Avicennia germinans* L., *Conocarpus erectus* L., *Laguncularia racemosa* Gaertn. and *Rhizophora mangle* L. The swamp forest is dominated by *Bucida buceras* L., *Calophyllum antillanum* Britton, *Rauwolfia cubana* A. DC. and *Tabebuia angustata* Britton.

The methods used for sampling, isolation, and for morphological study were previously described (García *et al.* 2002). Colour notations in parentheses are from Kornerup & Wanscher (1984).

Molecular methods

Techniques to isolate genomic DNA were as described by Solé *et al.* (2003). Briefly, DNA was extracted and purified directly from fungal colonies using the Fast DNA kit™ (Bio 101, Joshua Way, Vista California, U.S.A.). Fungal suspensions were vortexed with a FastPrep FP120 Instrument (Thermo Savant) to disrupt the fungal cells. The ITS-regions and 5.8S rDNA gene were amplified as described by Gené *et al.* (1996). The protocol used for sequencing was performed according to Solé *et al.* (2002). The sequence obtained was compared with those in the GenBank DNA database by using the BLAST programme (Altschul *et al.* 1997). A total of 17 strains representing *Chaetomiaceae, Chaetosphaeriaceae, Lasiosphaeriaceae,* and *Sordariaceae* were used in the molecular study. These strains and their EMBL accession numbers are shown in Table 1. *Melanospora pascuensis* Stchigel & Guarro (*Ceratostomataceae, Hypocreales)* was used as out-group. Phylogenetic analyses using the Neighbour-joining (NJ) method (Saitou & Nei 1987) were performed with the MEGA 2.1 computer programme (Kumar *et al.* 2001) with the Kimura two-parameter distance model (Kimura 1980), including transitions and transversions and with pair wise deletion for the treatment of the handling gaps/missing data. Confidence values for individual branches were determined by bootstrap analyses (1000 pseudoreplicates).

Table 1. Strains used in the molecular study.

Strains [1]	EMBL No.
Apiosordaria nigeriensis FMR 6363	AJ458184[3]
Asordaria tenerifae IMI 305078	AJ630460
Cercophora appalachianensis	AF177155[2]
Chaetomium nigricolor FMR 5737	AJ458185[3]
Coronatomyces cubensis FMR 7132	AJ458184
Corynascus sepedonium FMR 5593	AJ458186[3]
Gelasinospora bonaerensis IMI 37 5099	AJ002029[3]
Gelasinospora nigeriensis FMR 5963	AJ002400[3]
Melanocarpus thermophilus FMR 6190	AJ271586[3]
Melanopsammella chloroconia	AF178542[2]
Melanospora pascuensis FMR 6367	AJ011312[3]
Neurospora africana FGSC 1740	AF388913[2]
Neurospora crassa	M13906 [2]
Sordaria alcina IMI 267236	AJ630459
Sordaria macrospora	AF246293[2]
Striatosphaeria codinaeophora	AF178546[2]
Thielavia hyrcaniae CBS 773.85	AJ271581[3]

[1]CBS, Centraalbureau voor Schimmelcultures, Utrecht; GSC, Fungal Genetic Stock Center, Kansas; FMR, Culture Collection Facultat de Medicina, Reus; IMI, CABI Bioscience Genetic Resource Collection, Egham. [2]Obtained from GenBank. [3]Previously sequenced by us.

TAXONOMY

Coronatomyces D. García, Stchigel & Guarro, **gen. nov.** MycoBank MB500037.

Etymology: *coronatus* (Latin) crowned; *mykes* (Greek) fungi, referring to the crown of straight and short setae, which surrounding the neck.

Ascomata pyriformia vel globosa, ostiolata vel non ostiolata, setosa. Asci unitunicati, clavati vel cylindrici, brevistipitati, fasciculati, structura apicalis absens, evanescentes. Paraphyses evanescentes. Ascosporae unicellulares, uniseriatae vel biseriatae, atrobrunneae vel nigrae, glabrotunicatae, obovatae vel late ellipsoideae, cum foramine germinali basilari. Status conidialis ignotus.

Typus: *Coronatomyces cubensis* D. García, Stchigel & Guarro sp. nov.

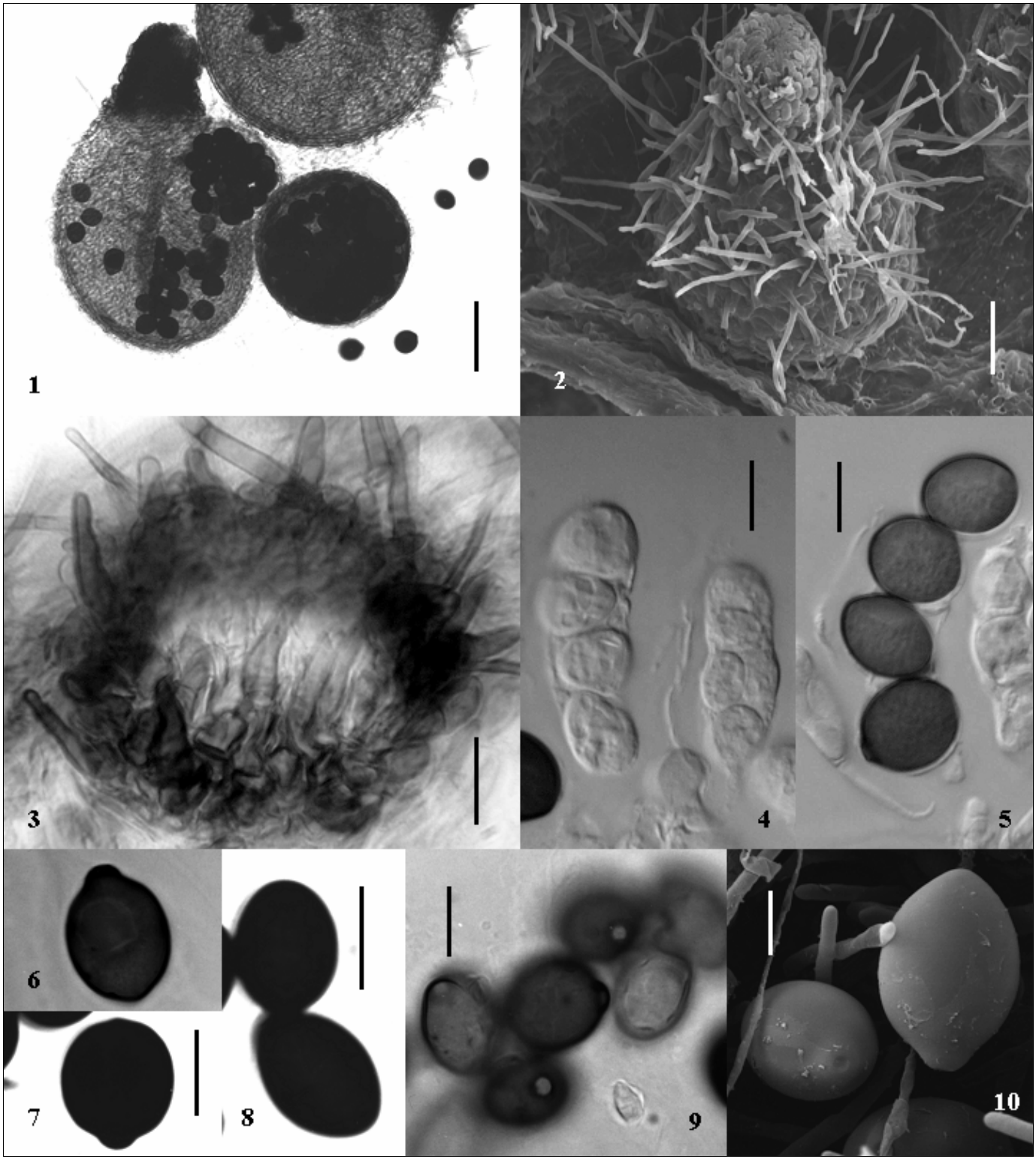
Ascomata pyriform to globose, ostiolate or non-ostiolate, scattered to aggregate, superficial to immersed, non-stromatic, setose. *Peridium* membranaceous, composed of several layers of cells. *Asci* unitunicate, clavate to cylindrical, short-stipitate, fasciculate, without distinct apical structures, evanescent. *Paraphyses* filiform, thin, unbranched, evanescent. *Ascospores* 1-celled, uniseriate to biseriate, dark brown to black, opaque at maturity, smooth-walled, obovate to broadly ellipsoidal, slightly pointed at the apex and with a wide germ pore at the umbonate base. *Anamorph* unknown.

Coronatomyces cubensis D. García, Stchigel & Guarro, **sp. nov**. MycoBank MB500038. Figs 1–15.

Etymology: *cubensis* = from Cuba; referring to the origin of the isolate.

Mycelium ex hyphis hyalinis, simplicibus vel ramosis, septatis, anastomosantibus, 0.5–3 μm diam. Coloniae in agaro cum decocto tuberum et carotarum (PCA) planae, granulosae, radiatae, brunneae. Ascomata dispersa vel aggregata, superficialia vel immersa, pyriformia vel globosa, ostiolata vel non ostiolata, 180–240 × 125–210 μm (non ostiolata 70–80 μm diam), translucida, atrobrunneae vel nigra propter ascosporas acervatas, setosa. Setae atro-brunnea, rectae vel late curvae, septatae, crassitunicatae. Collum atro-brunneum, papillatum, subconicum, 20–45 μm longum, 40–54 μm latum ad basim setis coronatum. Peridium membranaceum, e 3–5 stratis compositum, griseo-brunneum vel brunneum, translucidum, textura intrincata. Asci 2- vel 8-spori, unitunicati, clavati vel cylindrici, brevistipitati, 39–69(–75) × 11–25 μm, fasciculati, superne rotundati, structura apicalis absens, evanescentes. Paraphyses evanescentes. Ascosporae unicellulares, uniseriatae vel biseriatae, primum hyalinae, deinde brunneae vel nigrae, crassitunicatae, glabrotunicatae, obovatae vel late ellipsoideae, 13–17(–19) × (10–)11–13(–15) × (7–)8–11(–13) μm, foramine germinale basilari, 1.5–3 μm diam. Status conidialis ignotus.

Mycelium mainly submerged, composed of hyaline, branched and unbranched, septate, anastomosing, 0.5–3 μm broad hyphae. *Ascomata* scattered or aggregated, superficial to immersed, pyriform to globose, ostiolate with a short conical neck or non-ostiolate, 180–240 × 125–210 μm (70–80 μm diam if non-ostiolate), translucent, appearing dark brown to black due to the mass of ascospores, setose. *Setae* dark brown, straight to slightly curved, septate, thick-walled, 30–83(–104) μm long, 2–4 μm wide at the base. *Neck* dark brown, papillate, sub-conic, 20–45 μm long, 40–54 μm wide at the base, surrounded by a crown of upright setae; setae septate, dark brown, 11–23 μm long, 2–4 μm wide at the base. *Peridium* membranaceous, 3–5-layered, greyish brown to brown, translucent, outer layer of *textura intricata*; inner layer of *textura angularis*, composed by polygonal cells of 5–10 μm diam. *Asci* 2- to 8-spored, unitunicate, clavate to cylindrical, short-stipitate, thin-walled, 39–69(–75) × 11–25 μm, fasciculate, rounded at the tip and without apical structures, evanescent. *Paraphyses* filiform, thin, 35–40 × 0.5–1.5 μm unbranched, evanescent.

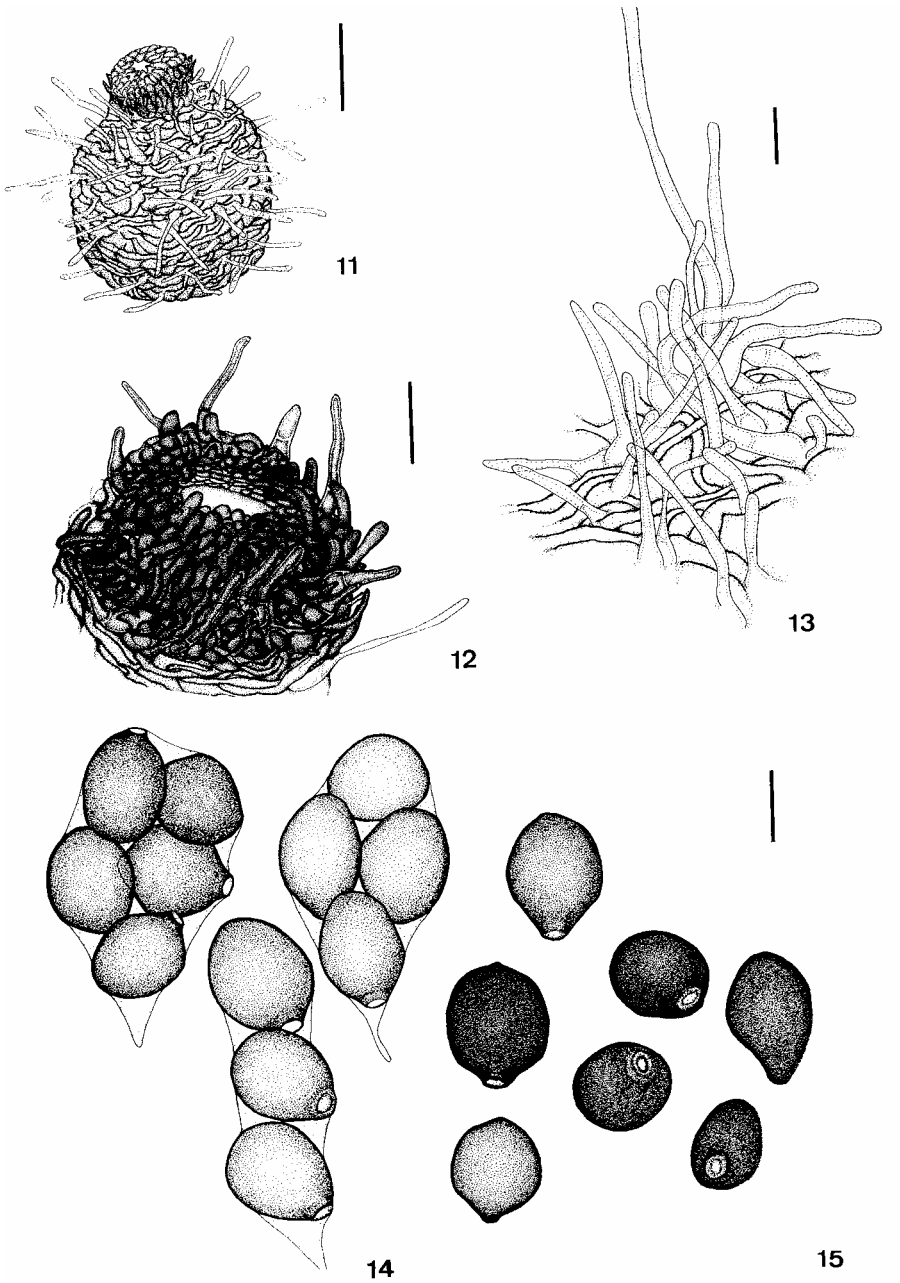


Figs 1–10. *Coronatomyces cubensis* (FMR 7132). 1. Ascomata. 2. Ascoma (SEM). 3. Detail of the neck. 4, 5. Asci and ascospores. 6–9. Ascospores. 10. Ascospores (SEM). Scale bars: 1 = 50 µm, 2 = 20 µm, 3–9 = 10 µm, 10 = 5 µm.

Ascospores 1-celled, uniseriate to biseriate, at first hyaline, becoming brown to black and opaque at maturity, thick-walled, smooth, obovate to broadly ellipsoidal, 13–17(–19) × (10–)11–13(–15) × (7–)8–11(–13) µm, slightly apiculate at the apex and umbonate at the base, with a protruding, basal germ pore; 1.5–3 µm diam.

Anamorph absent.

Cultural characteristics: Colonies at room temperature (22–25 ºC) were incubated under 12 h of darkness alternating with 12 h of cool white fluorescent light. Colonies on PCA attaining a diam of 45–46 mm in 14 d at room temperature, flat, granulose due to production of abundant ascomata, appearing slightly radiate due to the disposition of the ascomata, dark brown (5F5); reverse brown (5E5).



Figs 11–15. *Coronatomyces cubensis* (FMR 7132). 11. Ascoma. 12. Detail of the neck. 13. Detail of the peridium. 14. Asci. 15. Ascospores. Scale bars: 11, 12 = 20 µm, 13–15 = 10 µm.

Colonies on oatmeal agar (OA) attaining a diam of 40–41 mm in 14 d at room temperature, flat, mycelium mainly submerged, brown (5F5), granulose, zonate and slightly radiate because of the uneven production of abundant ascomata; reverse brown (5F4). Colonies on OA attaining a diam of 13 mm in 14 d at 15 ºC, flat, colourless; reverse colourless; ascomata not produced. Colonies on malt-extract agar (MEA) attaining a diam of 20–24 mm in 14 d at room temperature, composed of aerial and submerged mycelium, cottony, yellowish-brown (5D5); ascomata not produced. Colonies on MEA attaining a diam of 5–6 mm in 14 d at 15 ºC; ascomata not produced. Colonies growing slowly at 35 ºC on all media tested; ascomata not produced.

Specimen examined: **Cuba**, Matanzas Province, Ciénaga de Zapata, soil, 7 Dec. 1995, coll. R.F. Castañeda, **holotype** IMI 385316, **isotype** FMR 7132, culture ex-type CBS 109268.

Coronatomyces possesses several outstanding morphological features that make this taxon easy to identify. These are the setose, ostiolate ascomata (although non-ostiolate ascomata are also observed in culture) with a papillate neck, surrounded by a crown of short setae; a membranous peridium with an outer layer formed by closely interwoven hyphae; and opaque ascospores, with a wide, basal, protruding germ pore. These features are typical of members of *Sordariales*, although this combination of features in a single taxon has not previously been described in this order.

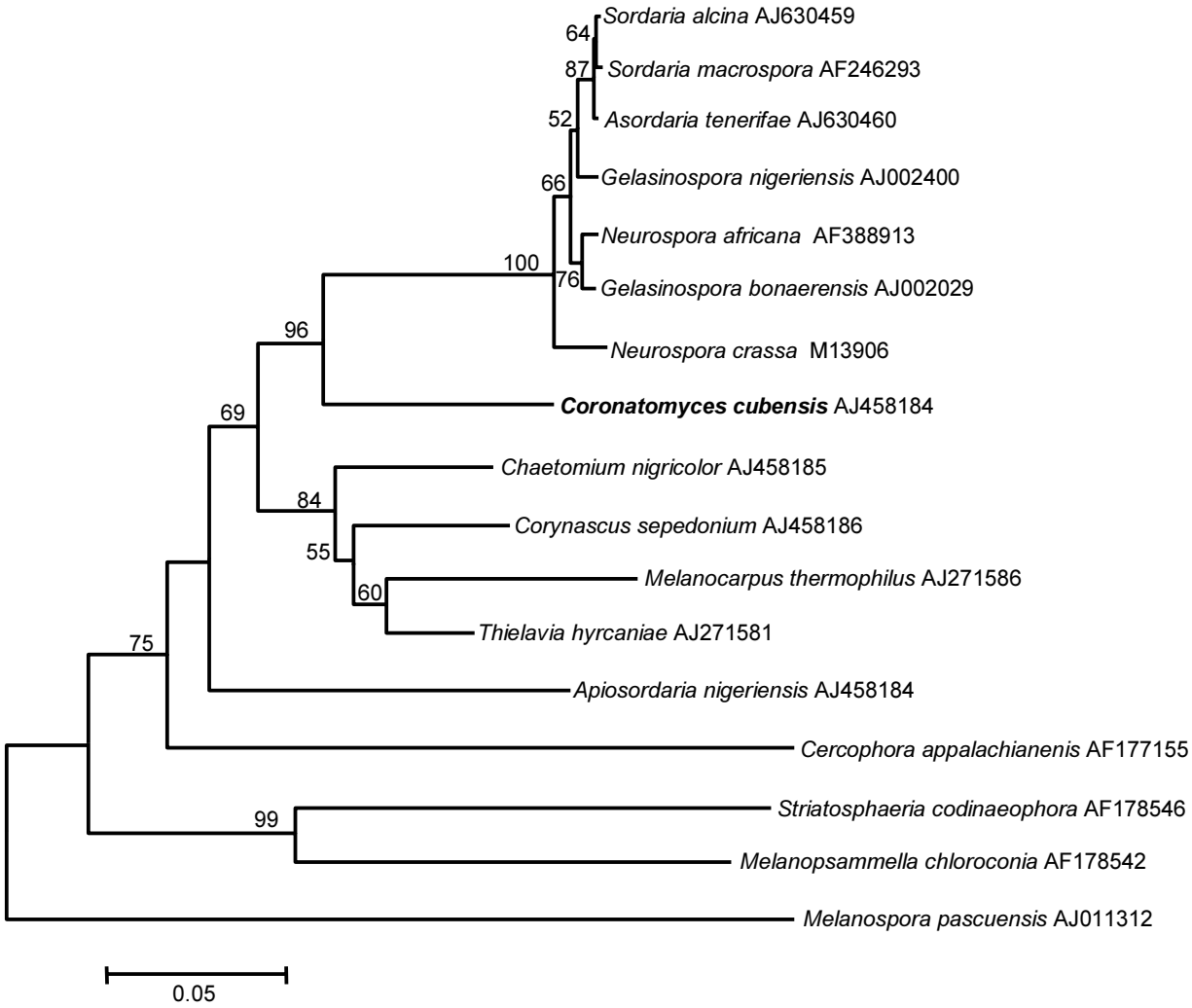


Fig. 16. Neighbour-joining tree based on nucleotide sequences from the ribosomal internal transcribed spacer (ITS) region and 5.8S rDNA gene of the 17 strains listed in Table 1. Branch lengths are proportional to distance. Bootstrap replication frequencies are indicated in the internodes.

A BLAST search comparison of the ITS sequences of the new taxon with the GenBank sequences showed that the closest species was *Strattonia insignis* (E.C. Hansen) Lundq. (*Lasiosphaeriaceae*) (97 % homology), although *Strattonia* Cif. emend. Lundq. is undoubtedly a very different genus characterized by 2-celled ascospores, with a hyaline lower cell. By contrast, *Coronatomyces cubensis* showed more morphological similarities with other members of the *Sordariaceae* and the *Lasiosphaeriaceae*.

The genera of *Sordariaceae* morphologically most similar to *Coronatomyces* are *Apodus* Malloch & Cain, *Asordaria* Arx, Guarro & Aa, and *Sordaria* Ces. & De Not. All three have dark, smooth ascospores with a basal germ pore. However, in the latter three genera the ascomata have an outer layer of *textura angularis*, the asci are cylindrical with an apical ring and the ascospores are violently discharged (Udagawa & Ueda 1981, Guarro & von Arx 1987, von Arx *et al.* 1987, Barr 1990). Moreover, in *Sordaria,* the ascospores are surrounded by a gelatinous sheath.

Some members of the *Lasiosphaeriaceae* also have some morphological features similar to *Coronatomyces.* These are *Apodospora* Cain & Mirza, *Arniella* Jeng & J.C. Krug, *Bombardioidea* C. Moreau ex N. Lundq, *Arnium* Nits., *Emblemospora* Jeng & J.C. Krug, *Fimetariella* Lundq., and *Periamphispora* J.C. Krug. Similar to the new genus, all of them have 1-celled, darkly pigmented and opaque ascospores with one or more germ pores. However, they are differentiated from *Coronatomyces* by the presence of a bombardioid peridial wall or an outer layer formed by angular cells, and ornamented, sheathed or appendaged ascospores (Lundqvist 1972, Jeng & Krug 1976, 1977, Krug 1989, 1995). *Periamphispora* is the most similar to *Coronatomyces*, but the former has a centrum formed by abundant and septate paraphyses, which are longer than the asci and ascospores surrounded by a hyaline gelatinous sheath that can occasionally show irregular striations and rarely 1–2 apical pores (Krug 1989).

To confirm our proposal and in order to clarify the taxonomic position of the new taxon, we performed a phylogenetic study based on sequences of the ITS regions. Fig. 16 shows the tree inferred from the analysis of the sequences of the 16 strains of *Sordariales*. Two main clades were observed. The first, which received a bootstrap support of 99 %, grouped the two members of *Chaetosphaeriaceae* [*Striatosphaeria codineophora* Samuels & E. Müll. and *Melanopsammella chloroconia* (W. Gams & Hol.-Jech.) Réblová, M.E. Barr & Samuels]. This clade was very distant from the other one that comprised members of the three families of *Sordariales* included in the study and the new genus, which received a bootstrap value of 75 %. The four genera representing the *Chaetomiaceae* [*Chaetomium nigricolor* L. M. Ames, *Corynascus sepedonium* (C.W. Emmons) Arx, *Melanocarpus thermophilus* (Abdullah & Al-Bader) Guarro, Abdullah & Al-Bader, and *Thielavia hyrcaniae* Nicot] formed a well-supported subclade (84 % bootstrap support), while the two members of the *Lasiosphaeriaceae* (*Apiosordaria nigeriensis* Stchigel & Guarro and *Cercophora appalachianensis* O. Hilber & R. Hilber) did not cluster together. *Coronatomyces* was closer to the members of the *Sordariaceae* (*Asordaria*, *Gelasinospora* Dowding, *Neurospora* Shear & B.O. Dodge, and *Sordaria*) (96 % bootstrap support) than to the *Chaetomiaceae* or to the two members of the *Lasiosphaeriaceae* included in the study. However, the new genus was placed in a separate branch very distant from members of the *Sordariaceae*. Although these data confirmed our proposed new genus its placement in a given family of the *Sordariales* was not resolved. It is probably due to the fact that the delineation of these families based either on morphological or on molecular criteria remains confused.

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