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Corylomyces: a new genus of *Sordariales* from plant debris in France

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ABSTRACT

The new genus *Corylomyces*, isolated from the surface of a hazelnut (*Corylus avellana*) in the French Pyrenees, is described, illustrated and compared with morphologically similar taxa. It is characterised by tomentose, ostiolate ascomata possessing long necks composed of erect to sinuose hairs, and one- or two-celled, opaque, lunate to reniform ascospores. Analyses of the SSU and LSU fragments rDNA gene sequences support its placement in the *Lasiosphaeriaceae* (*Sordariales*).

Introduction

During a survey of ascomycetes from soil and plant debris along the French Pyrenees, a rare fungus was found that was morphologically similar to members of *Sordariales*. It possesses a combination of features that do not fit any other genus in the order; it is therefore, described as a new genus. The taxonomic placement of this new taxon has been determined through phylogenetic analyses of the 18 S and 28 S rDNA gene sequences, which included several morphologically related species in *Sordariales*.

Materials and methods

Sampling and fungal isolation

Plant debris samples were collected in the 'forêt communale' of Saint Pé de Bigorre, Hautes Pyrénées, France (*ca* 43° 07′N, 0° 09′W; altitude *ca* 1100 m, average annual temperature 5 °C; average annual rainfall 1600 mm). The vegetation is mainly composed of *Buxus sempervirens*, *Castanea sativa*, *Corylus avellana*, and *Fagus sylvatica*.

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Plant debris, consisting of leaves, small branches, pieces of wood, and fruits, were placed into sterilised polyethylene bags, labelled, and stored in a refrigerator at 4–7 °C until they were processed. The material was placed into humid chambers and incubated at 25 ± 1 °C. Chambers were examined every day under a stereomicroscope. In order to obtain pure cultures from the developing fungi, fungal propagules were transferred from the natural substrate to oatmeal agar (OA; 30 g oat flakes, 15 g agar–agar, 1 l tap water) with chloramphenicol (100 mg l^{-1}), and incubated at 15 ± 1 and 25 ± 1 °C.

Morphological study

Colony morphology was studied on OA, potato carrot agar (PCA; 20 g potato, 20 g carrot, 15 g agar–agar, 1 l tap water) and potato dextrose agar (PDA; Difco Laboratories, Detroit, MI), incubated at 5, 15, 25 and 35 ± 1 °C in 12 h darkness, alternating with 12 h of cool fluorescent light. Colour notations in parentheses are from Kornerup & Wanscher (1984). The measurements of the fungal structures were made from material mounted in lactic acid. Transmission electron microscopy (TEM) techniques were described by Figueras & Guarro (1988).

Molecular study

The strains included in the present study along with their respective sequences and EMBL accession numbers are listed in Table 1. Procedures for DNA isolation, amplification and sequencing, alignment of the sequences, and NJ analyses follow those previously described (Cano *et al.* 2002; Rodríguez *et al.* 2004). MP analyses was performed using PAUP* 4.0b10 (Swofford 2002). One hundred heuristic searches were conducted with random sequence addition and tree bisection–reconnection branch-swapping algorithms, collapsing zero-length branches and saving all minimal-length trees (MulTrees) on different data sets. *Aphanoascus fulvescens and Chaetosphaeria chalarioides* were chosen as out-group in the SSU and LSU analysis, respectively. Support for internal branches was assessed using a heuristic parsimony search of 1000 bootstrapped data sets.

Results

The length of the fragments of the LSU and SSU genes, used in the phylogenetic analyses was 943 bp and 773 bp, respectively. NJ trees, based on the SSU (Fig 3) and LSU (Fig 4) sequences of different members of *Sordariomycetes* retrieved from the EMBL, show that *Corylomyces selenospora* belongs to *Sordariales*. The clade formed by members of this order received statistical support of 73% and 86% in the SSU and LSU trees, respectively (Figs 3–4). In the LSU tree (Fig 4), *Corylomyces selenospora* was grouped together with some *Cercophora* spp. (*C. sulphurella, C. sparsa* and *C. areolata*) with a BS of 88%. Parsimonic analysis of the LSU fragment yielded the 7 most parsimonious trees (MPT) (Fig 5). Of the 943 nucleotides sequenced, 761 were constant, 125 parsimony-informative and 57 variable parsimony-uninformative. All seven MPT had a consistency index (CI) of 0.3847, a retention index (RI) of 0.5837, and a homoplasy index (HI) of 0.6153. In this tree (Fig 5), *Corylomyces selenospara* was grouped with the same species as in the NJ tree, but without significant BS.

Taxonomy

Corylomyces Stchigel, Calduch & Guarro, **gen. nov.**
MycoBank No.: MB 510040

Etym.: *Corylo-* refers to hazel tree, *-myces* to *fungus*.

Ascomata superficialia, ostiolata, longicollea. Peridium cum textura angularis compositum. Pili delicati hyphis similis. Paraphysis et periphysis nullis. Asci cylindrici, 8-spori, evanescentibus, brevistipitati. Ascosporae unicellulares vel bicellulares, atrobrunneae, seleniformis, cum porus germinalibus apicalis.

Typus: Corylomyces selenospora

Ascomata superficial, ostiolate, tomentose, with a long neck composed of hairs. Peridium of textura angularis. Hairs hypha-like. Paraphyses and periphyses absent. Asci cylindrical, 8-spored, evanescent, short stipitate. Ascospores one- or two-celled, dark brown, lunate, bilaterally compressed, with an apical germ pore.

Corylomyces selenospora Stchigel, Calduch & Guarro, **sp. nov.** Figs 1–2
MycoBank No.: MB 510041

Etym.: *selenospora* refers to the lunate morphology of the ascospore.

Coloniae in substractum naturale ex mycelio vegetativo aereo et diffuso compositum, cum numerosis ascomatibus formantes, flavo-olivaceis. Ascomata superficialia, pilosa, flavo-olivacea vel olivacea, piriformis, gregaria, ostiolata, longicollea. Peridium olivaceum, textura angularis. Pili delicati, flexuosi vel undulati, olivacei vel flavo-olivacei, septati, simplices, laevi. Asci fasciculati, evanescentibus, brevistipitati, 8-spori. Ascosporae unicellulares et bicellulares, atrobrunneae, seleniformis vel reniformis, laeves, cum porum germinalibus apicalis visibilis.

Typus: **France**: *Hautes Pyrénées*: Saint Pé de Bigorre, from a hazelnut (*Corylus avellana*) decomposing on soil, 19 Sep. 2002, *M. Calduch, A. M. Stchigel & A. N. Miller*, isol *A. M. Stchigel* (ILLS 57506 – holotypus; FMR 8279 cultura viva).

Mycelium composed of olivaceous–yellow to olivaceous–brown, septate, smooth-walled, anastomosing hyphae, 1–5 μm diam. *Colonies* on natural substratum cottony, yellowish olive to olive, composed of aerial mycelium with numerous ascomata partially immersed; colonies on PCA growing slowly, 25–27 mm after 14 d at 25 °C, zoned, felty, consisting of aerial and submerged vegetative mycelium, producing few ascomata after three to four months, vivid yellow to olive (M. 3A8 to 3E8), without exudate; reverse similar in colour to the surface. *Ascomata* perithecia, superficial, tomentose, olivaceous–yellow to olive, pyriform, gregarious, 1100–1500 × 300–400 μm, maturing slowly, ostiolate, with a long neck; neck more or less cylindrical, funnel-shaped at the apex, 800–1000 μm long, 130–180 μm wide, nearly black, composed of parallel hairs, straight at first, becoming sinuous and sometimes branched at the tip, septate, olive; ascomatal wall olive, 10–17 μm thick, *textura angularis*, composed of 8–10 translucent cells, 5–20 μm diam; ascomatal hairs delicate, hypha-like, flexuous to undulate, olive to olivaceous–yellow, septate, 3 μm broad, unbranched,

Table 1 – List of strains, sources and sequences used in the analyses

Taxon	Strain	Origin	EMBL accession numbers
Aphanoascus fulvescens	CBS 111.58	Dung of bear, Canada	AJ315172 (SSU)
Apiosordaria backusii	ATCC 34568	Sandy soil, Japan	AY780051 (LSU)
A. verruculosa	F-152,365	Soil, Spain	AY346258 (LSU)
Bombaria bombarda	SMH 4821	Log, USA	AY780053 (LSU)
Bombardioidea anartia	HHB 99-1	Moose dung, USA	AY346264 (LSU)
Cercophora areolata	UAMH 7495	Porcupine dung, Canada	AY587936 (LSU)
C. macrocarpa	SMH 2000	Wood, Puerto Rico	AY780060 (LSU)
C. mirabilis	SMH 4002	Cow dung, Costa Rica	AY346271 (LSU)
C. scortea	GJS L556	Wood, USA	AY780063 (LSU)
C. septentrionalis	D. Malloch	N/A	U32400 (SSU)
C. sordarioides	UAMH 9301	Cow dung, France	AY780064 (LSU)
C. sparsa	JF 00229	Plant debris, France	AY587937 (LSU)
C. striata	SMH 4036	Wood, Costa Rica	AY780066 (LSU)
C. sulphurella	SMH 2531	Wood, USA	AY587938 (LSU)
C. terricola	ATCC 200395	River sediment, Japan	AY780067 (LSU)
Chaetomidium cephalothecoides (as *Thielavia cephalothecoides*)	MUCL 40270	Dung mouse, USA	AF286413 (LSU)
Chaetomium elatum	UCB 81-063	N/A	M83257 (SSU)
C. floriforme	CBS 815.97	Fallen leaves, Thailand	AF286402 (LSU)
C. globosum	SMH 4214b	Cow dung, Jamaica	AY346272 (LSU)
C. longicolleum (as *Farrowia longicollea*)	CBS 366.84	Soil, Madagascar	AF286408 (LSU)
	ATCC 24603	Forest soil, USA	AF207685 (SSU)
C. microascoides	F-153395 (A-12898)	Ethanol pasteurised soil, Spain	AY346273 (LSU)
C. seminudum (as *Farrowia seminuda*)	ATCC 28155	Balsa wood blocks, USA	AF207686 (SSU)
Chaetosphaeria curvispora	CBS 113.644	Decorticated wood, New Zealand	AY502933 (SSU)
C. chalaroides	SMH 2018	Plant decay, Panama	AY017372 (LSU)
Coniochaeta ligniaria	SMH 2569	Log, USA	AY346275 (LSU)
Corylomyces selenospora	ILLS 57506	Hazelnut, France	**AM268483 (SSU)** **DQ327607 (LSU)**
Guanomyces polythrix	MEXU 24486	Bat dung, México	AF207683 (SSU)
Immersiella immersa	SMH 4104	Branch in stream, USA	AY436409 (LSU)
Jugulospora rotula	ATCC 38359	Ant hill soil, USA	AY346287(LSU)
Lasiosphaeria glabrata	SMH 4617	Beech log, Denmark	AY436411 (LSU)
L. lanuginosa	SMH 3819	Plant decay, USA	AY436412 (LSU)
L. lanuginosa	SMH 4594	Birch log, England	AY587942 (LSU)
L. ovina	CBS 958.72	Decaying wood of *Fagus sylvatica*, Germany	AY587946 (LSU) AY083799 (SSU)
L. ovina	SMH 2670	Log, USA	AY587947 (LSU)
L. ovina	SMH 4124	Wood, USA	AY587948 (LSU)
L. rugulosa	SMH 1518	Wood, USA	AY436414 (LSU)
L. sorbina	CBS 885.85	Dead twig of *Prunus avium*, Germany	AY436416 (LSU)
Lasiosphaeris hispida	TL 6019	Elm, Denmark	AY436420 (LSU)
L. hispida	SMH 3336	Wood, USA	AY436419 (LSU)
Neurospora crassa	NRRL 13141	N/A	AY046271 (SSU) AY681158 (LSU)
N. pannonica	TRTC 51327	N/A	AY780070 (LSU)
N. tetrasperma (as *Gelasinospora tetrasperma*)	ATCC 96230	Soil, Canada	AY346281 (LSU)
Petriella setifera	CBS 385.87	Toenail of man, Finland	U43908 (SSU)
Pseudallescheria boydii (as *Scedosporium apiospermum*)	IP-1907.90	Human Sinus, France	U43915 (SSU)
P. ellipsoidea	CBS 418.73	Soil, Tajikistan	U43911 (SSU)
Poroconiochaeta discoidea	SANK 12878	Soil, Japan	AY346297 (LSU)
Schizothecium vesticola	SMH 3187	Dung, USA	AY780076 (LSU)
Sordaria fimicola	SMH 4106	Dung, USA	AY780079 (LSU)
S. fimicola	CBS 508.50	Dung, Canada	AY681160 (LSU)
S. humana	ATCC 22796	Peanut pod, USA	AY780078 (LSU)
S. lappae	SMH 4107	USA	AY780080 (LSU)
S. macrospora	Buck s.n.	Caribou dung, Canada	AY346301 (LSU)
Triangularia mangenotii	ATCC 39947	Soil, Japan	AY346303 (LSU)
Zopfiella ebriosa	CBS 111.75	Wine cork, The Netherlands	AY346305 (LSU)

Abbreviations: ATCC = American Type Culture Collection; CBS = Centralbureau voor Schimmelcultures; F = Field museum of Natural History; FLAS = Florida Agricultural Experiment Station culture collection; GJS = Gary J. Samuels; HHB = H. H. Burdsall; JF = Jacques Fournier; ILLS = Illinois Natural History Survey Herbarium; IP = Institut Pasteur; LSU = Large subunit of the rRNA; MEXU = Universidad Autónoma de México; MUCL = Mycothèque de l'Universite Catholique de Louvain; N/A = Not Available; NRRL = National Center for Agricultural Utilization Research; SANK = Fermentation Research Laboratories, San Kyo Co.; SMH = Sabine M. Huhndorf; s.n. = *sine numero*; SSU = small subunit of the rRNA gene; TL = Thomas Læssøe; UAMH = University of Alberta Microfungus Collection and Herbarium; UCB = University of California, Berkeley Collection. Newly obtained sequences are in bold face.

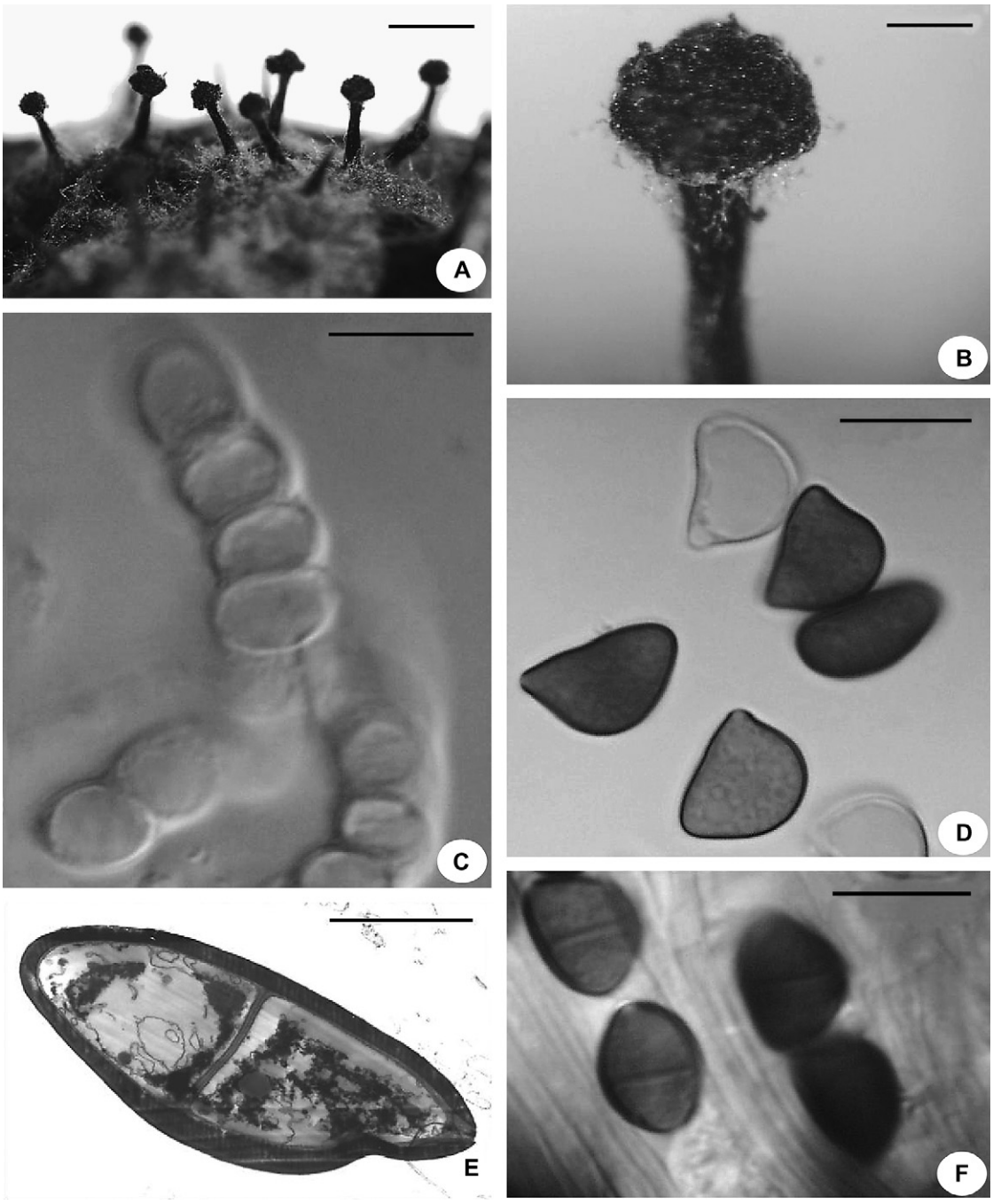

Fig 1 – *Corylomyces selenospora*. A. Ascomata; bar = 100 μm. B. Detail of the neck; bar = 50 μm. C. Asci; bar = 10 μm. D, F. Ascospores; bar = 10 μm. E. Section of an ascospore showing the septum (TEM); bar = 3 μm.

smooth-walled. *Hamathecium* not observed. *Asci* fasciculate, cylindrical, soon evanescent, 50–70 × 7–10 μm, short-stipitate (5–10 μm long), without apical structures, 8-spored. *Ascospores* uniseriate in the ascus, 1- or 2-celled, dark brown, opaque, lunate to reniform, dorsiventrally slightly flattened, 10–12 × 8–10 × 5–6 μm, smooth-walled, with an apical, umbonate germ pore. Anamorph not observed.

On OA at 25 °C, colonies 20–22 mm in 14 d, flat, whitish; reverse similar in colour to the surface. On PDA at 25 °C, colonies 25–27 mm in 14 d, flat to felty, yellowish grey to olive grey (M. 3B2 to 3E2) at the centre, and vivid yellow (M. 3A8 to 3B8) at the periphery; reverse similar in colour to the surface. On PCA, OA and PDA at 15 °C colonies growing very slowly, similar at 25 °C; ascomata produced after three to four months on PCA and OA. No growth was observed at 5 and 35 °C.

Discussion

The order *Sordariales* was recently redefined to include the families *Chaetomiaceae*, *Lasiosphaeriaceae*, and *Sordariaceae* (Huhndorf *et al.* 2004). Although *Corylomyces selenospora* is

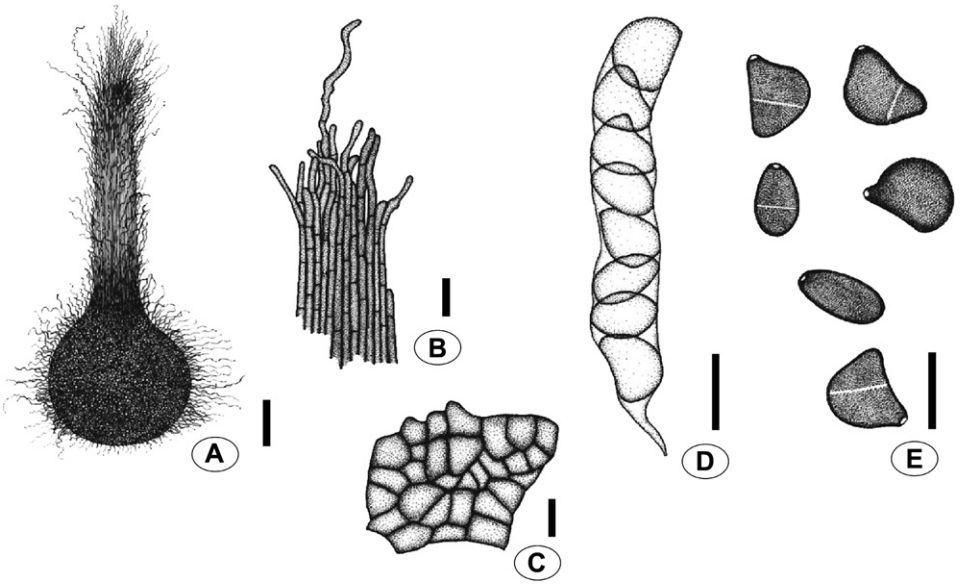

Fig 2 – *Corylomyces selenospora*. A. Ascoma; bar = 100 μm. B. Detail of the neck; bar = 10 μm. C. Detail of the ascomal wall; bar = 10 μm. D. Ascus; bar = 10 μm. E. Ascospores; bar = 10 μm.

morphologically similar to some members of *Chaetomiaceae*, with the exception of its bicelled ascospores, it did not group with taxa in this family in the phylogenetic analyses (Figs 3–5). Surprisingly, *Corylomyces* clustered with certain species of *Cercophora*, a genus pertaining to *Lasiosphaeriaceae*. With the exception of the presence of a tomentose peridial wall in *Corylomyces selenospora*, *Cercophora sulphurella* and *C. sparsa*, no other remarkable characteristic is shared by the taxa of this group. However, it should be taken into account that the relationships between *Chaetomiaceae* and *Lasiosphaeriaceae* are still unclear (Huhndorf *et al.* 2004), and further multi-locus analyses should clarify their evolutionary relationships.

Corylomyces possesses the same type of ascomata as *Farrowia*, a genus erected to accommodate *Chaetomium*-like fungi with a neck composed of fused hyphae, a distinct base to the perithecia, citriform and bilaterally flattened ascospores, and with a *Botryotrichum* anamorph (Hawksworth 1975). However, *Corylomyces* differs from *Farrowia* in the morphology of the ascospores, and in the absence of any known anamorph. Other morphologically related genera are *Chaetomium* and *Guanomyces* (González *et al.* 2000). In *Chaetomium*, the neck, when it is produced, is shorter than that of *Corylomyces* and composed of angular cells. Two species of *Chaetomium*, *C. cupreum* and *C. subcurvisporum* (Abdullah & Al-Bader 1989)

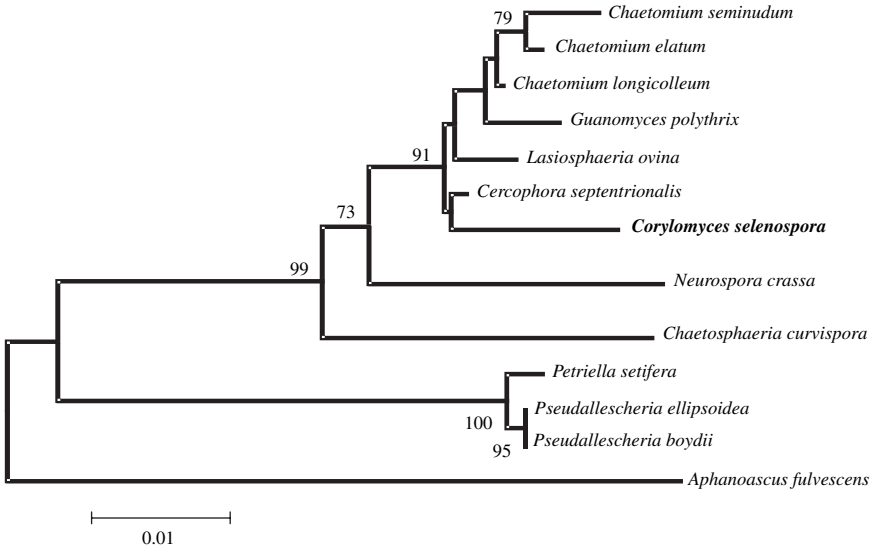

Fig 3 – NJ tree of the LSU fragment sequences of the studied strains. BS values above 70 % determined from an analysis of 1000 replicates, are shown at the nodes.

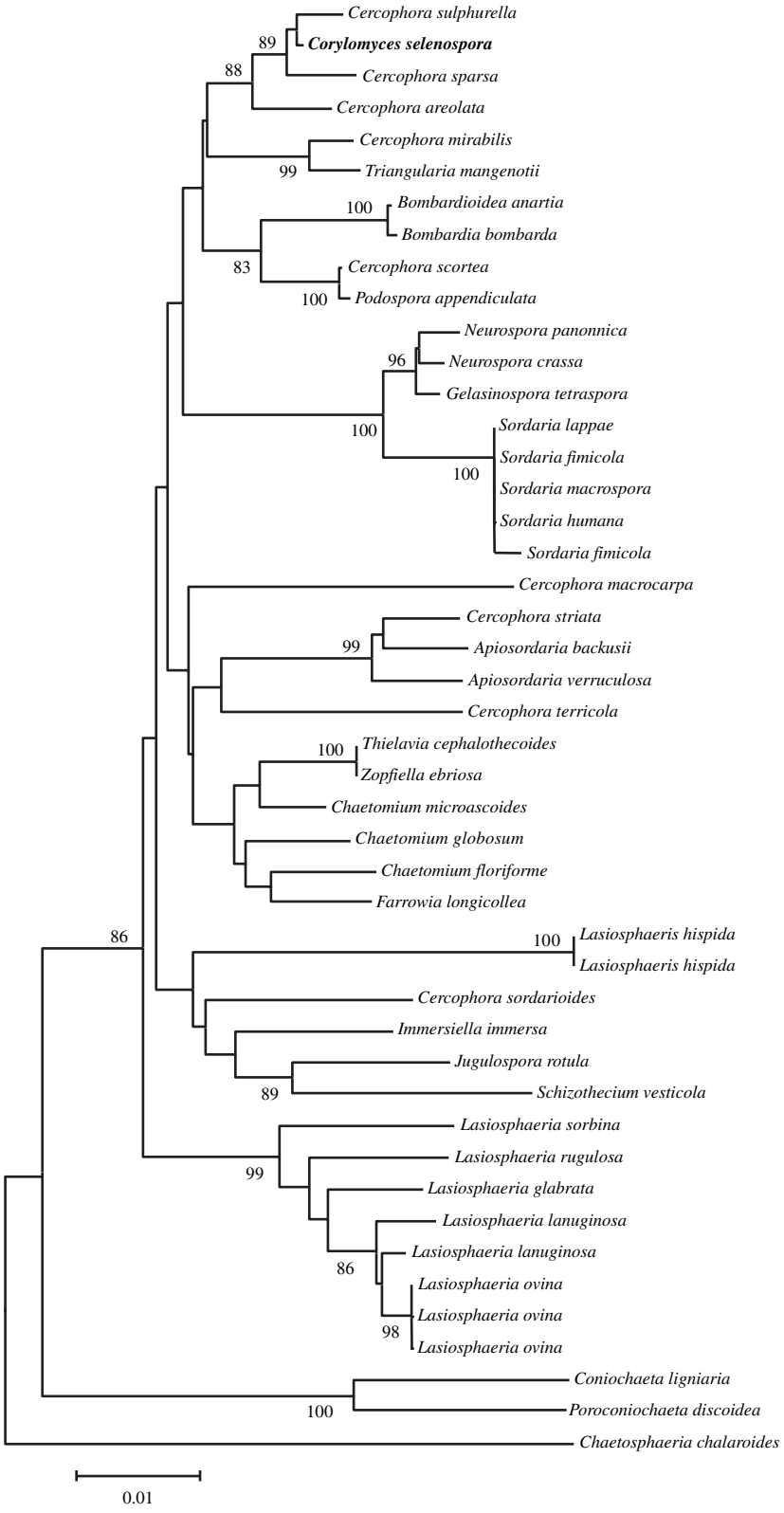

Fig 4 – NJ tree of the LSU fragment sequences of the studied strains. BS values as in Fig 3.

Fig 5 – One of the 7 most parsimonious trees obtained from heuristic searches, based on LSU fragment sequences of the studied strains. BS values as in Fig 3.

also have more or less lunate to reniform ascospores. However, these are exclusively one-celled and slightly smaller than in *C. selenospora* (7–10 × 4.5–6 μm in *C. cupreum versus* 8–13 × 4.5–6 μm in *C. subcurvisporum*). The only known species of *Guanomyces*, *G. polythrix*, also has morphologically similar ascomata to *Corylomyces*, but it has hyaline ascomatal hairs, a neck composed of polyhedral, translucent cells (formed by setae-like hairs in *Corylomyces*), and ellipsoidal, hyaline, one-celled ascospores with a finely aculeate wall (one to two-celled and smooth-walled in *Corylomyces*).

A few species of *Lasiosphaeriaceae* also have two-celled and lunate ascospores (e.g. *Podospora curvispora*, *P. selenospora*; Stchigel *et al.* 2003). However, they are easily distinguished from *Corylomyces* by their papillate necks, which are composed of prismatic cells, and by ascospores composed of an apical dark cell with a germ pore, and a long, hyaline basal cell.

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