

Annulatascus nilensis sp. nov., a new freshwater ascomycete from the River Nile, Egypt

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Abstract: *Annulatascus nilensis* sp. nov., from freshwater habitats in Egypt, is described, illustrated and compared to other species in the genus. Phylogenetic analyses of its LSU rDNA sequence with similar fungi placed the new species in the genus *Annulatascus* (*Annulatascaceae*, *Sordariomycetidae incertae sedis*). *Annulatascus nilensis* is characterized by immersed ascomata with an ascomatal neck oriented horizontally to the substrate surface, asci with a long, narrow stalk and massive bipartite apical ring, and 5–11-septate, hyaline ascospores surrounded by a large irregular, granular sheath that is not seen in water.

Key words:

Ascomycota
molecular phylogeny
Phragmites
Sordariomycetes
subtropics

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INTRODUCTION

The generic name *Annulatascus* was introduced by Hyde (1992) to accommodate two freshwater fungi, *A. velatissporus* (the type species) and *A. bipolaris*, which were collected from submerged wood in Australia. Fifteen species are currently included in the genus (Barbosa *et al.* 2008), most of which were described from freshwater habitats in the tropics (Barbosa *et al.* 2008, Shearer *et al.* 2010, <www.fungi.life.uiuc.edu>). However, two of the species were discovered on palm rachides on land (Fröhlich & Hyde 2000). *Annulatascus* species are characterized by dark, immersed or superficial perithecioid ascomata; wide, tapering paraphyses; long cylindrical asci with a relatively massive refractive bipartite apical ring; and hyaline, fusiform, sheathed or appendaged ascospores. During an ongoing investigation of freshwater fungi on wood submerged in the River Nile in Egypt, we discovered a previously unknown species of *Annulatascus* which is described and illustrated here, and compared to other species in the genus using morphological and molecular data. Phylogenetic analyses of the LSU rDNA sequence of the new fungus placed the new fungus in the same clade as the type species of the genus (*Annulatascus velatissporus*) and *A. hongkongensis*.

plastic bags, and taken to the laboratory where they were examined immediately under a stereomicroscope for fungal spore-producing structures. Samples were then incubated on moist filter paper in sterile plastic boxes, and examined periodically over a 6 mo incubation period. Single-ascospore cultures of the new fungus were obtained by cutting open ascomata with a sterile razor-blade, and the centrum tissue containing ascospores was removed with sterile forceps and placed in sterile freshwater. Small drops of the ascospore suspension were placed on Czapek's medium in Petri dishes and incubated at 22 °C in the dark. Germinated ascospores were transferred to new Czapek's medium Petri dishes with sterile forceps and incubated at 22 °C in the dark. Photographs were taken using an Olympus B×51 differential interference contrast light microscope and Olympus DP12 digital imaging system (Olympus Corporation, Tokyo). Reference specimens were prepared by drying at 60 °C for 24 h, and deposited along with fungal cultures in the collections of the Department of Botany, Faculty of Science, Sohag University, Egypt. Representative slides and a portion of the material selected to serve as the holotype were deposited in the Biosystematics Reference Collection of CAB International (IMI). Pure cultures of the new fungus used in this study, were deposited at the Extremobiosphere Research Center, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), under accession number "MF808".

MATERIALS AND METHODS

Collection

Submerged decayed wood was recovered from the River Nile in the Sohag governorate. Samples were placed in clean

DNA extraction, sequencing, and phylogenetic analysis

DNA was extracted from pure fungal mycelium using the Microbial DNA Extraction Kit (MOBIO; Mo Bio Laboratories,

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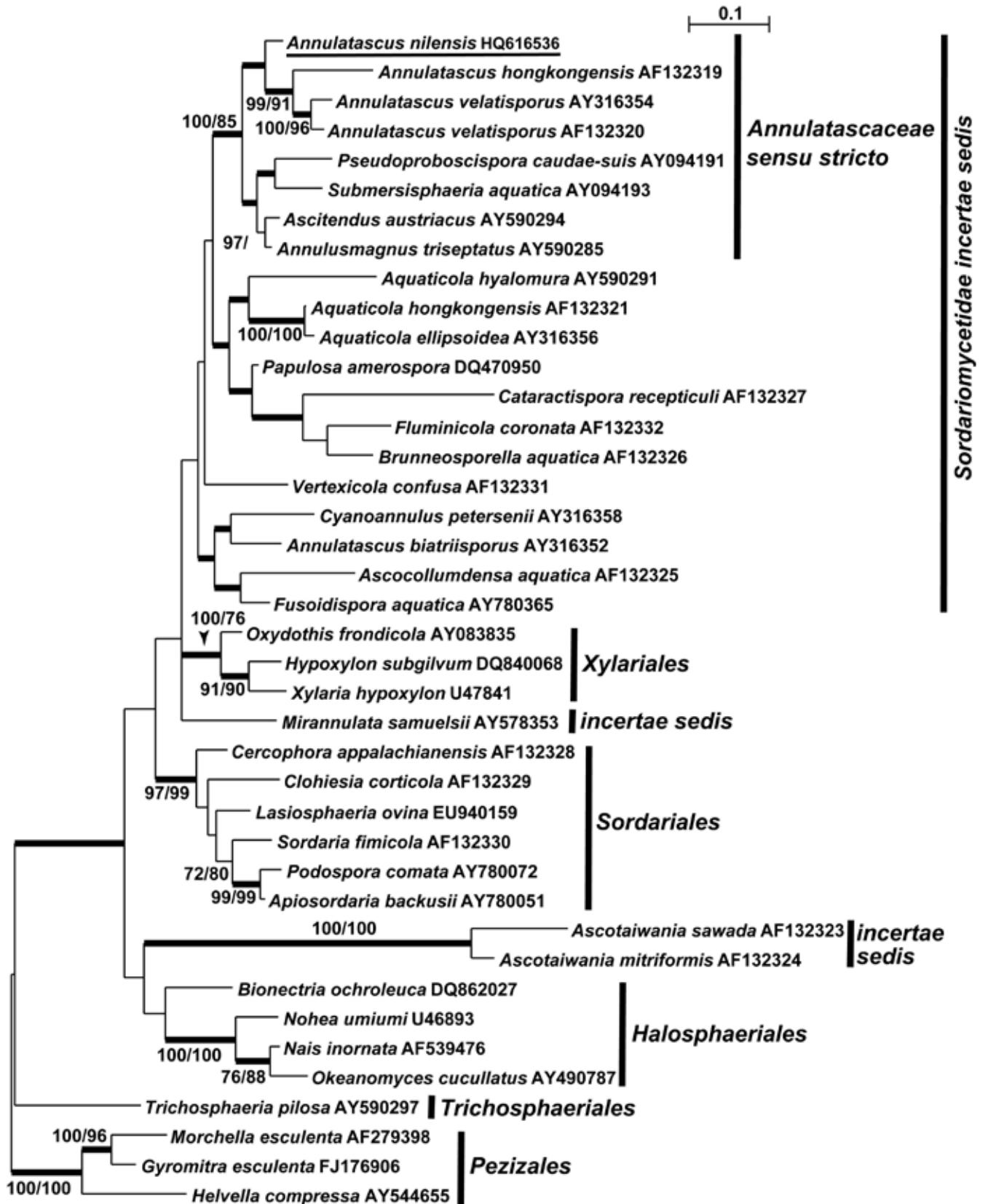


Fig. 1. Phylogenetic relationships of *Annulatasacus nilensis* and similar fungi, based on the nucleotide sequences of LSU rDNA. The Bayesian phylogenetic tree (MrB) was constructed as described in the text. The numbers indicate pp values $\geq 95\%$ (in bold), MP, ML bootstrap values ≥ 70 . The new species, *Annulatasacus nilensis*, is underlined.

Carlsbad, CA) according to the manufacturer's instructions. Partial LSU ribosomal DNA was amplified using primers LR0R and LR7 (Bunyard *et al.* 1994). PCR reactions, cycling parameters and sequencing were carried out as described by Abdel-Wahab *et al.* (2009). Sequences were assembled using Sequencher v. 4.2.2 (Gene Codes Corporation). Sequences were aligned with pertinent ones retrieved from GenBank using Clustal X (Thompson *et al.* 1997) and optimized manually. The positions where one or more species contained a length mutation or ambiguously aligned regions were not included in the phylogenetic analysis. Nucleotide sequence phylogenies were constructed using PAUP v. 4.0b10 (Swofford 2002). Maximum-likelihood (ML) analyses (Felsenstein 1981) were performed using heuristic searches with the random stepwise addition of 100 replicates and tree bisection-reconnection (TBR) rearrangements in effect. The optimal model of nucleotide substitution for the ML analyses was determined using hierarchical likelihood ratio tests as implemented in Modeltest v. 3.7 (Posada & Crandall 1998). The model selected as the best fit for LSU rDNA data set was TrN+G. For the bootstrap analyses (Felsenstein 1985), 100 replicates were generated with five random additions and TBR. Maximum-parsimony (MP) trees were obtained by 100 random addition heuristic search replicates using PAUP, and 1 000 bootstrap replicates were performed employing five random addition heuristic searches. Posterior probability values were obtained using MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) with the GTR+G model that was determined using MrModeltest v. 2.2 (Nylander 2004). Nodal supports in the Bayesian analyses were examined by posterior probabilities from five million generations that were run in four chains with sampling every 100 generations, yielding 50 000 trees, of which the first 12 500 were discarded as "burn in". The LSU sequence of the ex-type isolate used in this study was deposited at GenBank under the accession number "HQ616536" (MF 808, JAMSTEC). The alignment was deposited in TreeBASE (<treebase.org>) under accession number S10747, and the description of the new species was deposited in MycoBank (<mycobank.org>; Crous *et al.* 2004).

RESULTS

Phylogenetic analyses

The partial LSU rDNA sequence of *Annulatascus nilensis* aligned with representatives of the *Annulatascaceae*, along with representatives of *Halosphaeriales*, *Sordariales*, *Trichosphaeriales*, and *Xylariales*. In total, the LSU rDNA dataset included 40 taxa of which three belonged to *Pezizales* were used as outgroup. The dataset consisted of 879 characters, of which 329 were constant, 183 were variable but parsimony-uninformative, and 367 were parsimony informative. The ten most parsimonious trees produced using heuristic search parameters were of equal length with 1 308 steps, and have a CI of 0.4602, an RI of 0.5864, and a Rescaled CI of 0.2699. The maximum likelihood analysis

produced one tree with a likelihood score of 6995.34. Bayesian analyses yielded two trees, of which one is shown here as Fig. 1. The maximum parsimony and likelihood analyses both produced trees with similar topologies (data not shown).

Annulatascus nilensis was found to be phylogenetically close to *A. velatisporus* (the type species of *Annulatascus*) and *A. hongkongensis*, and was nested within a highly supported clade (100/100/85 for Bayesian/MP/ML respectively) which included the genera *Annulatascus*, *Annulusmagnus*, *Ascitendus*, *Pseudoproboscispora*, and *Submersisphaeria*. This clade, was phylogenetically distant from clades of other *Annulatascaceae* genera, and represents the family *Annulatascaceae* s. str. (Fig. 1). Phylogenetic analyses of LSU rDNA showed *Annulatascus* as currently circumscribed to be polyphyletic, with *A. biatriisporus* phylogenetically distant from *A. velatisporus* (Raja *et al.* 2003, Campbell & Shearer 2004).

Taxonomy

Annulatascus nilensis Abdel-Wahab and Abdel-Aziz, sp. nov.

MycoBank MB517837

(Figs 2, 3)

Etymology: From the Latin word *Nilus*, the River Nile, where the fungus was collected.

Ascomatibus immersis, obyriformibus, axe principali horizontali, nigris, coriaceis, ostiolatis, papillatis, solitariis vel gregariis. Collis assurgentibus, periphysatis. Paraphysibus raro septatis, hyalines, apice attenuato. Ascis 260–400 × 12–14 µm, octosporis, cylindricis, unitunicatis, cum apparatus apicali. Ascosporis uniseriatis vel imbricate uniseriatis, 32–52 × 7–10 µm, fusiformibus, 5–9(–11)-septatis, ad septa constrictus, hyalinis, laevibus, cum vagina ampla irregulari granulose.

Typus: EGYPT: Sohag, in the River Nile, on decayed submerged stems of *Phragmites australis*, Feb. 2006, A.E. Abdel-Aziz (IMI 397966 – holotypus; culture ex-type MF 808; GenBank accession no. HQ616536).

Ascomata 400–600 µm long, 220–280 µm wide, immersed, obyriform, oriented horizontally to the substrate surface, black, coriaceous, ostiolate, papillate, solitary or gregarious (Fig. 2A). *Peridium* 20–26 µm thick, forming a *textura angularis* of thick-walled flattened cells, the cells dark brown and encrusted with pigmented particles in the outer parts, and hyaline and unencrusted towards the inside (Fig. 2A, B). *Ostiolar necks* curved upward, 240–360 µm long, 96–112 µm wide. *Hamathecium* of periphyses lining the ostiolar canal, to 20 µm long and 1.5–2 µm wide, and paraphyses in the centrum which are 3–12 µm wide at the widest part, rarely septate, unbranched, hyaline, and taper distally (Fig. 2C, D). *Ascis* 260–400 × 12–14 µm (av. = 322.5 × 12.2 µm, *n* = 20), cylindrical, unitunicate, deciduous in water mount, persistent, with a tapered elongated base when mounted in

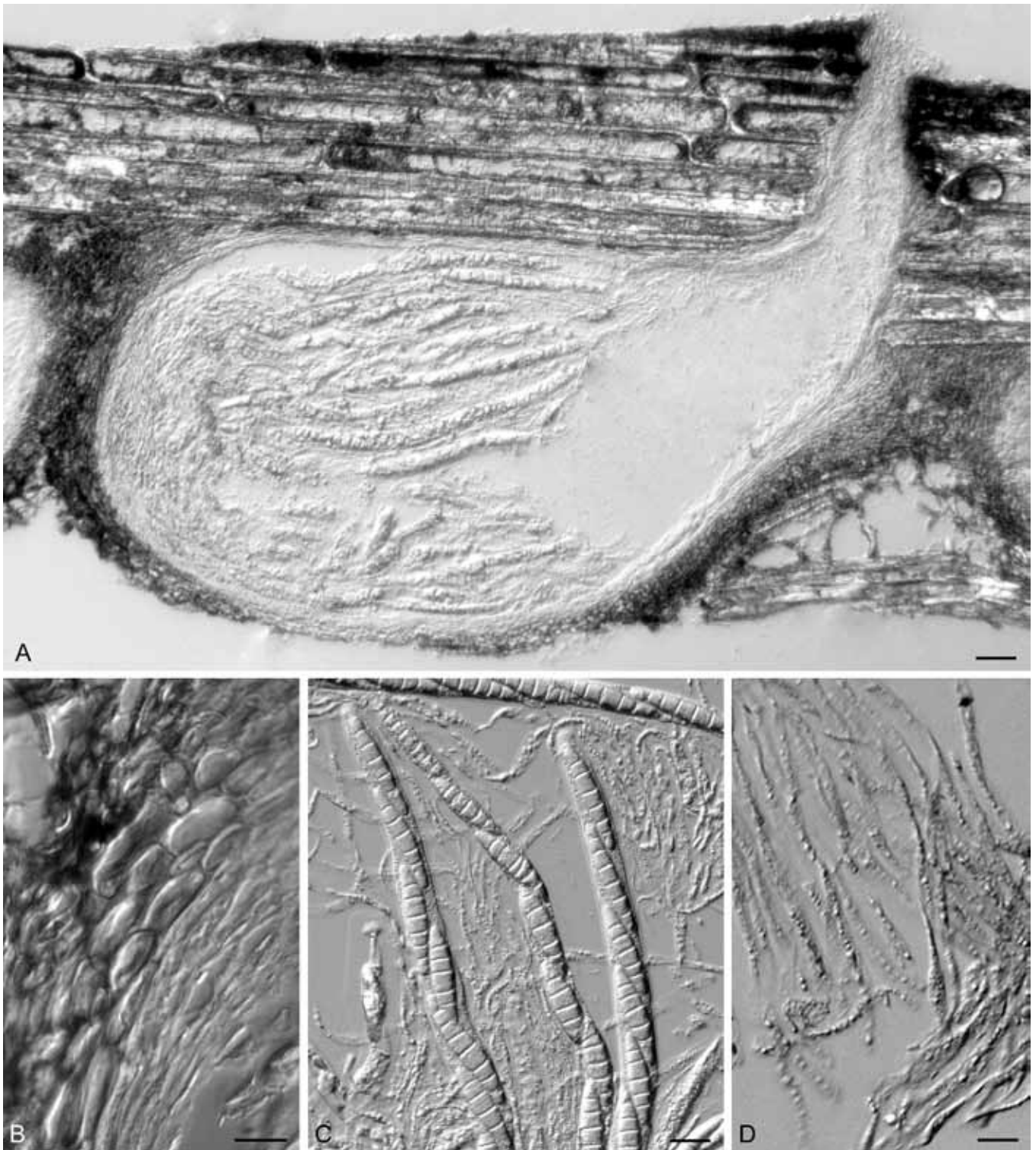


Fig. 2. *Annulatasacus nilensis* (holotype). **A.** Vertical section through ascomata showing the hymenium at the base of the ascomatal venter and periphysate neck. **B.** Section through the peridial wall. **C.** Squash of the ascoma venter showing asci and paraphyses. **D.** Paraphyses. Bars: A = 20 μm , B = 5 μm , C, D = 10 μm .

water. and a large non-amyloid, refractive, bipartite apical ring 3–4 μm high \times 5–6 μm wide (Fig. 3A–C), discharge by deliquescence, 8-spored. Ascospores 32–52 \times 7–10 μm (av. = 40.8 \times 8 μm , $n = 41$), uniseriate to overlapping uniseriate, fusoid, 5–9(–11)-septate, constricted at the septa, hyaline, smooth-walled, surrounded by a large, irregular, granular sheath (Fig. 3D–F) that is not evident in water mounts.

Notes: *Annulatasacus nilensis* has ascospore dimensions that

overlap with those of *A. biatriisporus*, *A. velatisporus*, and *A. tropicalis*. However, the first two species have aseptate ascospores, while *A. tropicalis* has 1–3-septate ascospores that also lack a mucilaginous sheath or appendages (Hyde 1992, Tsui et al. 2002). *Annulatasacus nilensis* differs from *A. fusiformis* in that the later has ascospores that are smaller, 1–5-septate, thick-walled, straight or slightly curved, with a warty ornamentation in the apical and subapical regions

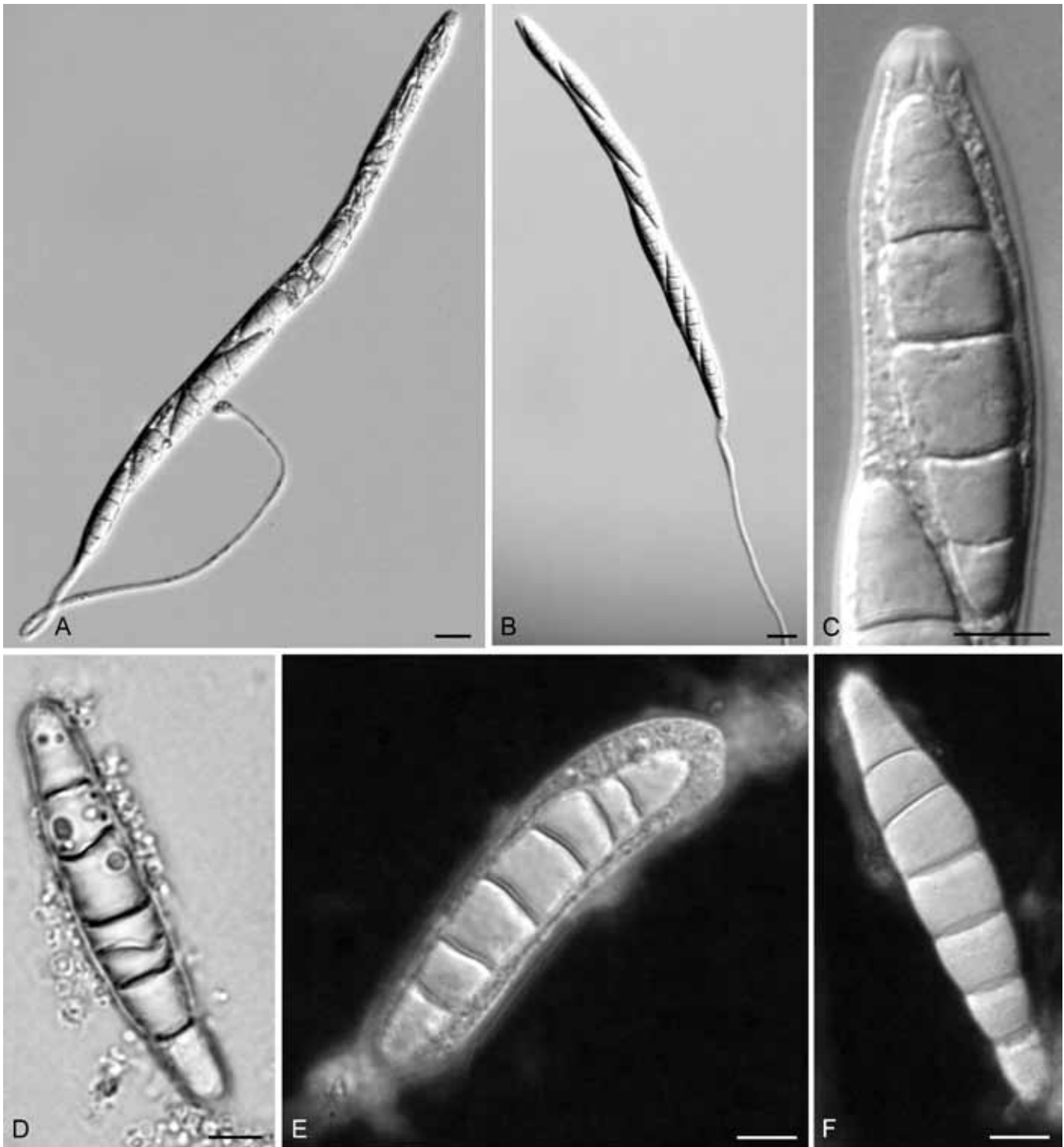


Fig. 3. *Annulatascus nilensis* (holotype). **A, B.** Asci at different stages of maturity; note the long narrow stalk. **C.** Ascus with apical mechanism. **D–F.** Ascospores surrounded by an irregular, granular sheath that peels off at a later stage. Bars: A, B = 10 μm , C–F = 5 μm .

(TEM), bipolar mucilaginous pad-like apical appendages, and ascomata with a longer neck (Hyde & Wong 2000). *Annulatascus nilensis* differs from all the described *Annulatascus* species in having asci with an elongated narrow stalk (when mounted in water) and 5–11-septate ascospores surrounded by a large, irregular, granular sheath. An elongating basal stalk is also found in *Cataractispora receptaculorum*, but the ascospores of that species are smaller, more tapered, with polar pads that unfurl in water into

long thin filamentous appendages, and are not surrounded by a gelatinous sheath (Ho *et al.* 2004).

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