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FULL PAPER

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Two new *Corollospora* species and one new anamorph based on morphological and molecular data

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Abstract Two new species of the genus *Corollospora*, namely, *C. anglusa* sp. nov. with its anamorph *Varicosporina anglusa* sp. nov. and *C. portsaidica* sp. nov., which were isolated from the coast of the Mediterranean Sea in Egypt, are described in this article based on morphological and molecular evidence. The two new species have one-septate ascospores. *Corollospora anglusa* resembles *C. gracilis* by having narrow one-septate hyaline ascospores; however, they differ in ascomata and ascospore dimensions and in pure culture characteristics. Single-ascospore culture of *C. anglusa* produces the conidia of its anamorph, whereas an anamorph has not been reported for *C. gracilis*. *Varicosporina anglusa* differs from the other two known *Varicosporina* species by having conidial branches that are filamentous, rectangularly branched, hypha like, and disarticulated into two- or one-celled fragments. *Corollospora portsaidica* is morphologically similar to *C. cinnamomea*, but the two species differ in the dimensions, shape, and ornamentation of the ascospores. The new *Corollospora* species were confirmed to be divergent from other similar *Corollospora* species based on phylogenetic analyses of partial sequences of the LSU rDNA region.

Key words Halosphaeriaceae · Marine fungi · Mediterranean Sea · Molecular systematics · Subtropical fungi

Introduction

The genus *Corollospora*, typified by *C. maritima* Werdermann, was first described by Werdermann (1922) as a coelomycete. Barghoorn and Linder (1944) described the same fungus as a new genus, *Peritrichospora*, under the name *P. integra* Linder, which was synonymized by Kohlmeyer (1962) to *C. maritima*. Jones et al. (1983) made a revision of the genus *Corollospora* based on appendage ontogeny at the electron microscope level. They updated the genus concept by retaining 5 species and assigning 4 other species to other or new genera. Koch (1986) described *C. cinnamomea* Koch from Thailand. This fungus was the first member of the genus that has brown ascospores with an ornamented surface. Nakagiri and Tokura (1987) described 7 new *Corollospora* species from the sandy beaches of Japan. Subsequently, 5 species were described (Kohlmeyer and Volkmann-Kohlmeyer 1989, 1991, 1997; Sundari et al. 1996; Prasannarai et al. 2000), bringing the total number of *Corollospora* species to 18. Campbell et al. (2002) studied the phylogeny of *Corollospora* species and related taxa based on the sequences of large subunit ribosomal DNA and found that *Corollospora* species are monophyletic.

Corollospora is a morphologically diverse genus in which species vary from uniseptate (*C. cinnamomea*, *C. gracilis* Nakagiri & Tokura, *C. maritima*) to phragmoseptate (*C. angusta* Nakagiri & Tokura, *C. besarispora* Sundari, *C. californica* Kohlm. & Volkm.-Kohlm., *C. colossa* Nakagiri & Tokura, *C. filiformis* Nakagiri, *C. pseudopulchella* Nakagiri & Tokura, *C. pulchella* Kohlm., Schmidt & Nair, *C. quinqueseptata* Nakagiri), and muriform (*C. fusca* Nakagiri & Tokura and *C. novofusca* Kohlm. & Volkm.-Kohlm.). All *Corollospora* species are hyaline except *C. cinnamomea*, *C. fusca*, and *C. novofusca*, which have brown to dark brown ascospores with an ornamented surface.

The most distinctive feature of *Corollospora* species is their apical and equatorial ribbon-shaped secondary appendages, which are formed by the fragmentation and peeling off of the exospore layer. The equatorial appendages are stiffened and their margins thickened at a later stage of ascospore maturity so that they stand out from the equator as a double frill (Lutley and Wilson 1972; Jones et al. 1983). Other characteristic features of *Corollospora* species are the following: (1) carbonaceous ascomata that are seated with a subiculum on sand grains, shells of marine animals, and other hard substrates; (2) a two-layered peridial wall composed of thick-walled flat cells inside and round

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cells to the outside, although a few species have either a third layer on the outside (*C. colossa*, *C. filiformis*, *C. fusca*) or a thin one-layered peridium (*C. cinnamomea*) (Koch 1986; Nakagiri and Tokura 1987); (3) papillae that are formed near the ascomatal subiculum and filled with thick-walled cells which are separated from the ascomatal centrum by a layer of melanized brown cells (Kohlmeyer and Volkmann-Kohlmeyer 1987; Nakagiri and Tokura 1987); (4) the venter of the young ascomata is filled with thin-walled pseudoparenchymatous cells with pitted walls to connect plasmatic strands of neighboring cells (Hsieh et al. 2007); and (5) asci with thin walls, without an apical apparatus, that deliquesce before or at ascospore maturity.

Teleomorph–anamorph relationships of three *Corollospora* species have been discovered: *C. pulchella–Clavatospora bulbosa* (Anast.) Nakagiri & Tubaki, *C. luteola* Nakagiri & Tubaki–*Sigmoidea luteola* Nakagiri & Tubaki and *C. intermedia* I. Schmidt–*Varicosporina prolifera* Nakagiri. Anamorphs of *Corollospora* species produce conidia sympodially (Shearer and Crane 1971; Nakagiri and Tubaki 1982; Nakagiri 1986).

During our examination of driftwood, decayed seagrasses, and algae for marine fungi from the coast of the Mediterranean Sea in Egypt, we recorded two unknown *Corollospora* species with one-septate ascospores. One of the two new *Corollospora* species produced a *Varicosporina* anamorph in culture. In this study, the large subunit ribosomal DNA (LSU rDNA) D1–D3 region was sequenced for those two fungi and closely similar *Corollospora* species. Their morphology and phylogenetic placement showed that they are different from the 18 species in the genus, so they are described here as new. With the description of these 2 new species, the number of *Corollospora* species becomes 20, making it one of the largest genera in marine habitats.

Materials and methods

Collection of the fungi

Driftwood, decayed seagrasses, and algae were collected from the intertidal zone of the Mediterranean Sea in Egypt. The samples were placed in clean plastic bags and examined with a dissecting microscope for marine fungi upon return to the laboratory, incubated in sterile humid plastic boxes, and examined periodically for 2 months. To obtain single-ascospore cultures of the recorded fungi, ascomata were cut open with a sterile razor blade, and the centrum tissue containing ascospores was removed with sterile forceps and placed in sterile seawater. Small drops of this ascospore suspension were placed on GYA (10 g glucose, 1 g yeast extract, 18 g agar in 1 l seawater) in petri dishes and incubated at 25°C in the dark. Germinated ascospores were transferred to new GYA petri dishes with sterile forceps and incubated at 25°C in the dark. Ascomatal squash were mounted in seawater for all measurements and photography. Materials for scanning electron microscopy (SEM) were prepared as described by Wong et al. (2003). Voucher slides and type material of the new fungi were deposited at International Mycological Institute (IMI). Pure cultures of the new fungi and fungal strains used in this study were deposited at the Extremobiosphere Research Center, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Japan and National Institute of Technology and Evaluation, Biological Resource Center (NBRC), Japan.

DNA extraction, sequencing, and phylogenetic analysis

Single-spore isolates of the fungi (Table 1) were grown in YMG broth (4 g yeast extract, 10 g glucose, 10 g malt extract, 30 g sea salts in 1 l distilled water) until sufficient mycelium had formed to allow DNA to be extracted. DNA extraction for polymerase chain reaction (PCR) was performed using the Microbial DNA Extraction Kit (MOBIO; Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The D1–D3 region of LSU ribosomal DNA was amplified using primers LR0R and LR7 (Bunyard et al. 1994). PCR reactions were carried out in 0.2-ml microcentrifuge tubes in 25 µl containing 20 ng template DNA, 2.5 µl 10× Fast buffer (containing 0.75 mM Mg), 2.5 mM deoxyribonucleotide triphosphate (dNTP), 0.25 µM of each primer, and 0.625 unit Speedstar HS DNA polymerase (Takara Bio, Shiga, Japan). Cycling parameters were as follows: an initial step of 98°C for 2 min, followed by 30 cycles of 98°C for 5 s, 52°C for 15 s, and 72°C for 20 s, and completed with a final step of 72°C for 1 min. PCR products were purified by Exo-SAP digestion with Exonuclease I (USB Corp., Cleveland, OH, USA) and shrimp alkaline phosphatase (SAP; Promega, Madison, WI, USA) for 20 min at 37°C, and 30 min at 80°C to inactivate the

Table 1. Fungal isolates used in this study

Species	Source	Country of collection	GenBank accession no.
Corollospora species			
C. anglusa Abdel-Wahab & Nagahama	MF 827[a]	Egypt	AB361008
C. cinnamomea J. Koch.	NBRC 32125	Singapore	AB361017
C. gracilis Nakagiri & Tokura	NBRC 32110	Japan	AB361018
C. gracilis	MF 828[a]	Egypt	AB361019
C. maritima Werderm.	MF 812[a]	Egypt	AB361011
C. portsaidica Abdel-Wahab & Nagahama	MF 832[a]	Egypt	AB361016
Anamorph			
Varicosporina ramulosa Meyer & Kohlm.	NBRC 31325	USA	AB361020

[a] Japan Agency for Marine-Earth Science and Technology (JAMSTEC) culture collection

enzymes. PCR products were sequenced using primers LR0R, LR3, LR5, and LR7 (Vilgalys and Hester 1990) and using DYEnamic ET terminator reagent on a MegaBACE 1000 (Amersham Biosciences) automatic sequencer. Sequences were assembled using Sequencher 4.2.2 (Gene Codes Corporation).

Sequences were aligned with others retrieved from GenBank using ClustalX (Thompson et al. 1997) and optimized manually. The positions where one or more species contained a length mutation and ambiguously aligned regions were not included in the subsequent phylogenetic analysis. The alignment was deposited in TreeBASE (http://www.treebase.org) under the accession numbers S2325 and M4414. Nucleotide sequence phylogenies were constructed using PAUP* 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. The optimal model of nucleotide substitution for the ML analyses was determined using hierarchical likelihood ratio tests as implemented in Modeltest 3.7 (Posada and Crandall 1998). The model selected as the best fit for LSU rDNA data set was TrNef + I + G. For the bootstrap analyses (Felsenstein 1985), 250 replicates were generated with 5 random additions and TBR. Maximum-parsimony (MP) trees were obtained by 100 random addition heuristic search replicates using PAUP, and 1000 bootstrap replicates were performed employing 5 random addition heuristic searches. A posteriori probabilities were obtained by using the Bayesian phylogenetic inference on the program MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) with the SYM + G model that was determined using MrModeltest 2.2 (Nylander 2004). Five million generations 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 numbers on the branches are estimates of a posteriori probabilities. The sequences of fungi were deposited at GenBank/DDBJ; their accession numbers are given in Table 1.

Results and discussion

Taxonomy

Corollospora anglusa Abdel-Wahab & Nagahama, sp. nov. Figs. 1–5

Ascomata 60–110 µm diametro, globosa vel subglobosa, superficiala, nigra, coriacea, solitaria vel gregaria, ostiolata, papillata, subiculata. Peridium 10–16 µm crassum, brunneum, extracellulis angularis, intus applanatis. Papilla 20–40 µm diametro, 20–30 µm alta, conica, basalis; canalis ostioli cellulis pseudoparenchymaticis, pachydermis, hyalinis, impletus. Subiculum e cellulis crasso-tunicatis, polygonus compositum. Centrum ascomatum immaturorum cellulis pseudoparenchymaticis, hyalinis, leptodermis, foveolatis, deliquescentibus. Paraphyses nullae. Asci 35–65 × 14–20 µm, octospori, ellipsoidei, unitunicati, mox deliquescentes. Ascosporae 18–30 × 3–4(–5) µm, fusiformes, 1-septatae, ad septa leviter constrictae, hyalinae. Appendices bigeneae: (i) appendices primariae polares, spiniformes, 4–7 µm longae, 1–2 µm diametro; (ii) appendices secundariae circa septum centrale 10–12 µm longae, peritrichiatae, per fragmenta exosporarum effectae et appendices apicales 4–6 µm longae.

Anamorphosis: *Varicosporina anglusa* Abdel-Wahab & Nagahama.

Holotypus: In *Zostera marina*, Egypt, Alexandria, Mediterranean Sea, June 2006; coll. M.A. Abdel-Wahab (holotype, IMI 395681). Ex-type strain: MF 827 (= NBRC 104919).

Ascomata (Fig. 1) 60–110 µm in diameter, globose to subglobose, superficial, black, coriaceous, solitary or gregarious, ostiolate, papillate, subiculate, attached to calcareous material that is precipitated on decayed leaves of the seagrass *Zostera marina*. Peridium (Fig. 2) 10–16 µm thick, composed of brown, thick-walled cells that are polygonal or irregularly rounded, large cells on the outside, flattened on the inside. Papilla 20–40 µm in diameter, 20–30 µm high, conical, basal, close to the subiculum; Ostiolar canal filled with thick-walled, pseudoparenchymatous, hyaline cells that are separated from the ascomatal centrum by a layer of brown cells. Subiculum composed of polygonal thick-walled cells. Centrum of immature ascomata is filled with thin-walled, rounded, deliquescing pseudoparenchyma cells with pit-connections in their walls. Paraphyses absent. Asci (Fig. 3) 35–65 × 14–20 µm, eight-spored, ellipsoidal, unitunicate, early deliquescing. Ascospores (Figs. 4, 5) 18–30 × 3–4(–5) µm ($\bar{x} = 26 \times 3.9$ µm, $n = 50$) (excluding polar spines and appendages), fusiform, 1-septate, slightly constricted at the septum, hyaline. Appendages of two kinds: (i) a single terminal primary appendage at each end of the spore, 4–7 µm long, 1–2 µm in diameter, spine- or thorn-like, attenuate; (ii) equatorial double frill of ribbon-like secondary appendages, 10–12 µm long, developing by fragmentation and peeling off of the exospore and fragments of the exospore remain attached to the polar spines forming a tube or sheets 4–6 µm long (Fig. 5).

Type material: Egypt, Alexandria, Mediterranean Sea, on decayed leaves of *Zostera marina* covered with calcareous material in the intertidal zone, June 2006, coll. M.A. Abdel-Wahab (holotype, IMI 395681). Ex-type strain: MF 827 (= NBRC 104919).

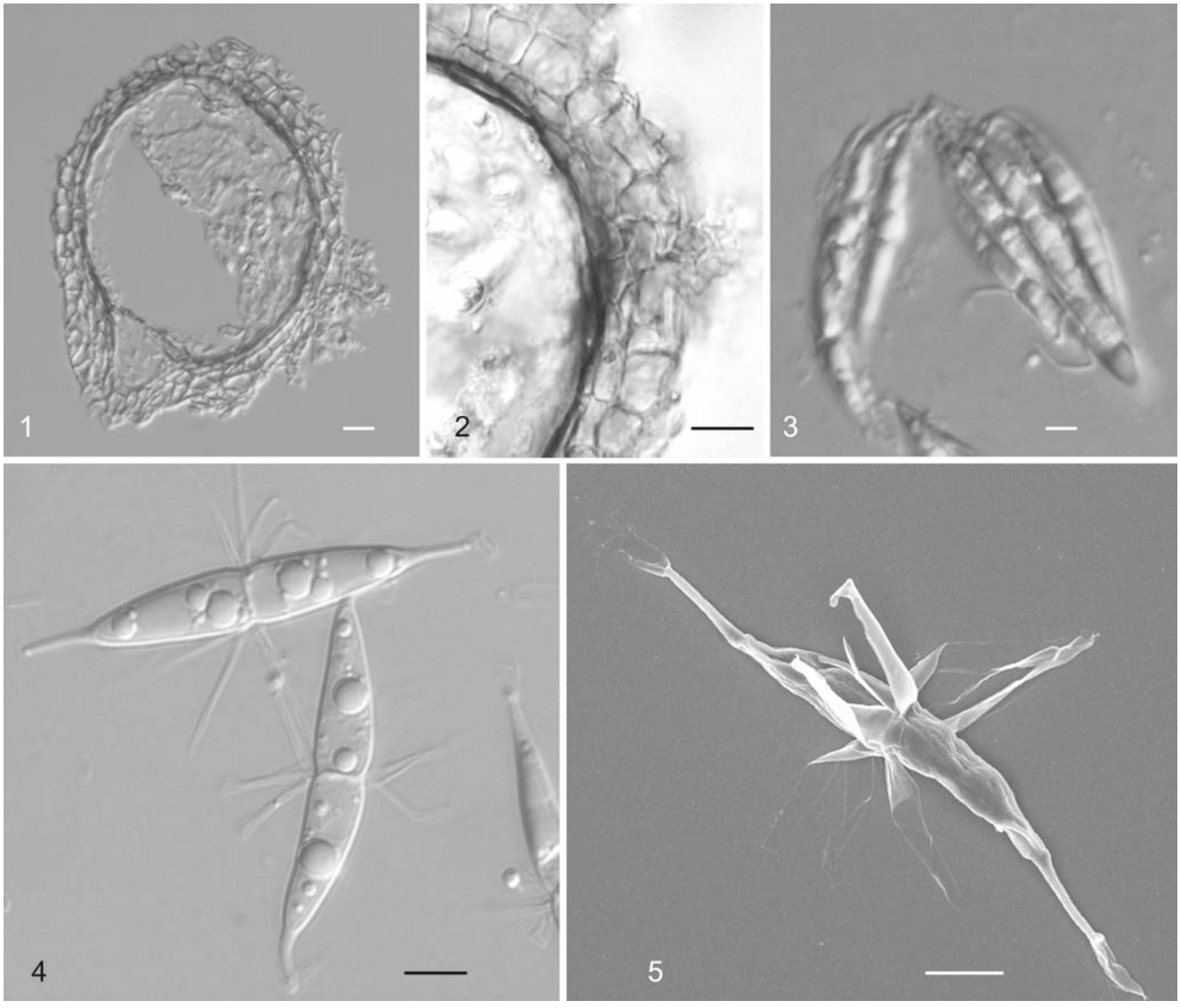
Habitat: On calcareous material associated with *Zostera marina* in the intertidal zone.

Distribution: Egypt.

Anamorph: *Varicosporina anglusa* Abdel-Wahab & Nagahama.

Etymology: In reference to the Arabic name "*Anglus*" for *Zostera marina* on which the holotype material of the fungus was recorded.

A single-ascospore colony on GYA (10 g glucose, 1 g yeast extract, 18 g agar in 1 l artificial seawater) is white at first, then turns to olive brown to black. Colonies produce the anamorphic stage after 1 month incubation, on GYA medium, as white tufts of aerial conidiophores that bear the



Figs. 1–5. *Corollospora anglusa* (holotype). **1** Vertical section of ascomata. **2** Vertical section through part of the ascomata showing peridium structure. **3** Asci. **4** Ascospores. **5** Scanning electron micrograph of the ascospore. **1–4** Differential interference contrast. *Bars* **1** 10 µm; **2–5** 5 µm

conidia (Fig. 6) but do not produce ascomata. The fungus produces chlamydospores in culture that are olive brown, globose, and in irregular large masses.

This species is similar to *Corollospora gracilis* by having narrow, 1-septate, hyaline ascospores; however, *C. anglusa* is characterized by having shorter ascospores [18–30 × 3–4(–5) vs. 26–45 × 3–5.5(–7) µm for *C. anglusa* and *C. gracilis*, respectively]; shorter polar spines (4–7 vs. 6.5–12 µm long for *C. anglusa* and *C. gracilis*, respectively); smaller ascomata (60–110 vs. 88–195 µm in diameter for *C. anglusa* and *C. gracilis*, respectively); and producing the anamorph *Varicosporina anglusa* in culture. The *C. gracilis* (ex-type culture, NBRC 32110) colony is white and produces ascomata abundantly in culture both on agar and on glass whereas that of *C. anglusa* is white and turns to olive brown to black and does not produce ascomata in culture but does produce chlamydospores abundantly. *Corollospora anglusa* is phylogeneticaly divergent from *C. gracilis* based on molecular sequence data (as described below; see Fig. 22).

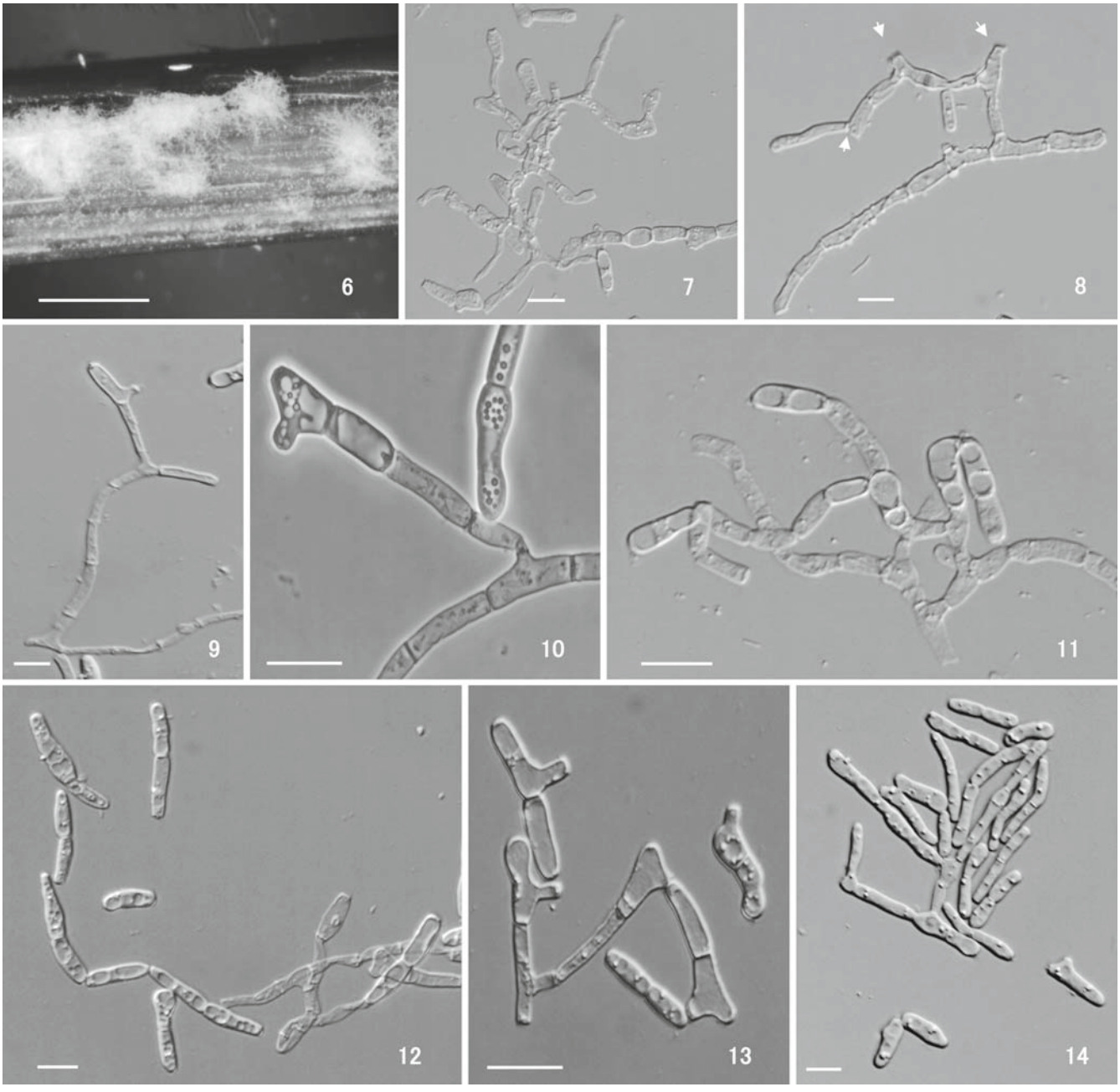
Varicosporina anglusa Abdel-Wahab & Nagahama, anam. sp. nov. Figs. 6–14

Etymology: In reference to the name of the teleomorph: *Corollospora anglusa*.

Teleomorph: *Corollospora anglusa* Abdel-Wahab & Nagahama.

Hyphae 2.5–4.5 µm diametro, septatae, ramosae, hyalinae. Conidiophora micronematosa, 18–65 × 3–4 µm, 0–5 septata, simplicia vel ramosa. Cellulae conidiogenae ad apicem sympodialiter proliferantia monoblasticae. Conidia filamentosa, ramosa, septata.

Teleomorphosis: *Corollospora anglusa* Abdel-Wahab & Nagahama.



Figs. 6–14. *Varicosporina anglusa* (holotype). **6** Micrograph of white aerial tufts of conidiophores and conidia on balsa wood incubated with culture of *Corollospora anglusa*. **7, 8, 14** Sympodially proliferating conidiophores bearing conidia. Note the sympodial branching of the conidiophore (*arrows*) in **8. 9, 10** Monobalstic formation of the conidium. **11–13** Conidia disarticulating into cells. **6** Stereomicroscope; **7–9, 11–14** differential interference contrast; **10** phase contrast. *Bars* **6** 1 mm; **7–14** 15 µm

This anamorphic state is known only from single-ascospore cultures of *C. anglusa* after 1 month incubation and growing as white tufts of conidiophores and conidia (Fig. 6). Hyphae 2.5–4.5 µm in diameter, septate, branched, hyaline. Conidiophores micronematous, 18–65 × 3–4 µm, 0–5 septate, cylindrical, septate, simple or branched, hyaline, arising laterally or terminally from the vegetative hyphae. Conidiogenous cells proliferating sympodially at the apex (Figs. 7, 8, 14) or monoblastic (Figs. 9, 10). Conidia are composed of rectangularly branching filaments that are up to 300 µm long and disarticulate to give bi-celled (rarely one-celled) segments 16–42 × 2–5 µm ($\bar{x}$ = 26.5 × 3.5 µm, n = 50), hyaline, similar to hyphal cells (except they have slightly thicker walls), cylindrical, constricted at the septa (Figs. 11–14).

Teleomorph: *Corollospora anglusa* Abdel-Wahab & Nagahama.

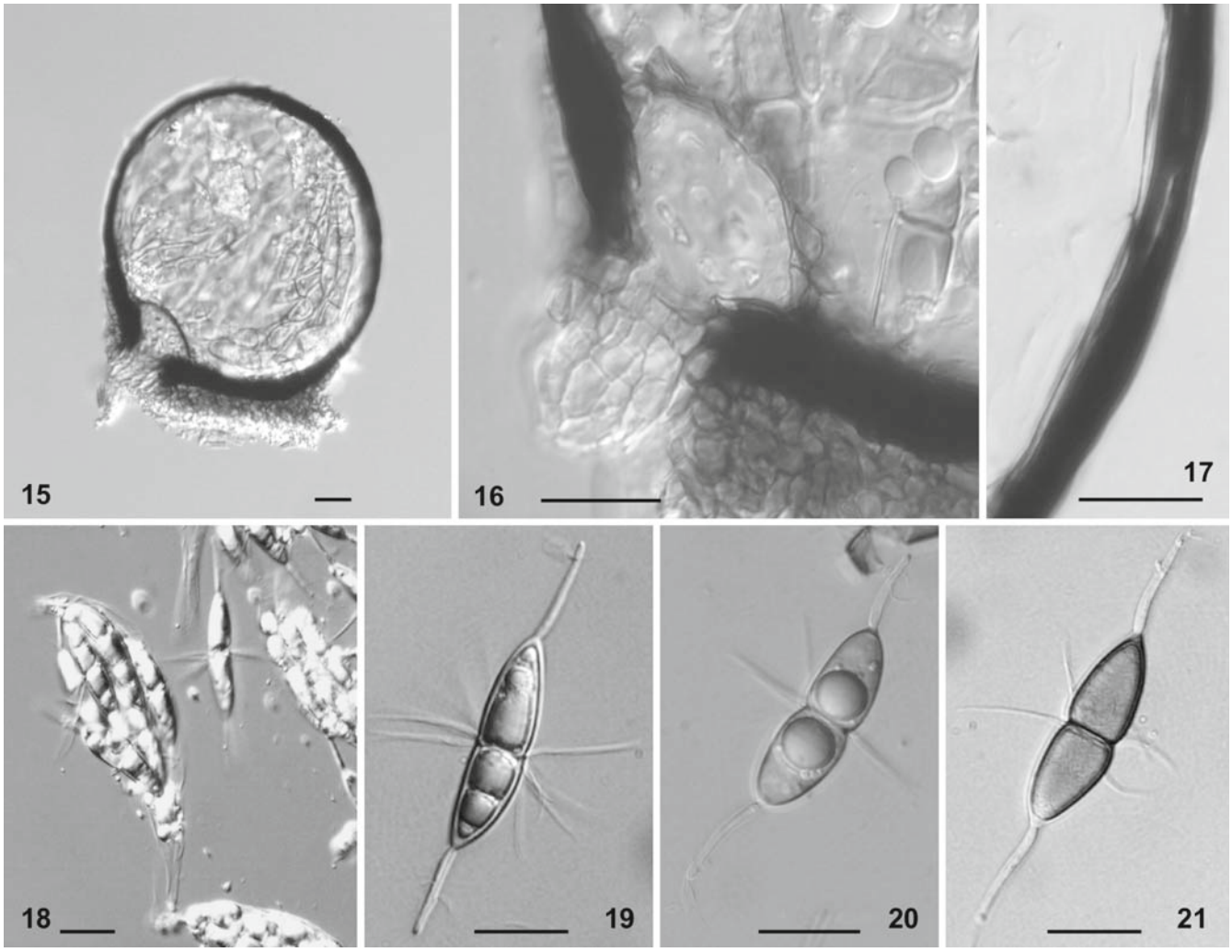
Type material: Produced from 1-month-old culture of *Corollospora anglusa*, on balsa wood incubated with the culture (holotype, IMI 395682).

Etymology: In reference to the name of the teleomorph: *Corollospora anglusa*.

Varicosporina anglusa differs from the other two *Varicosporina* species by having conidial branches that are filamentous, hypha like, and disarticulate into two- or one-celled fragments (Figs. 11–14). Conidia of *V. prolifera* and *V. ramulosa* Meyers & Kohlm. consist of a system of axes: a main axis consistently with two, and less frequently with a third, side branches that does not disarticulate into individual cells (Meyers and Kohlmeyer 1965; Kohlmeyer and Kohlmeyer 1979; Nakagiri 1986). Therefore, we amend the generic concept of the genus *Varicosporina* to include species that are producing branched filamentous conidia which disarticulate into smaller segments. *Varicosporina prolifera* was proved to be the anamorphic stage of *Corollospora intermedia* (Nakagiri 1986). *Corollospora intermedia* has three septate ascospores that are longer and wider than those of *C. anglusa*. The teleomorph stage of *V. ramulosa* is not known; however, it produces sclerocarps in nature and in culture that have a *Corollospora*-like peridial wall, an ostiole-like structure, and pseudoparenchymatous cells with pit-like thickenings (Kohlmeyer and Charles 1981; Nakagiri and Tubaki 1985; Nakagiri 1986). Phylogenetic analyses of LSU rDNA support the placement of *V. anglusa* in the genus *Varicosporina*, showing that it is divergent from *V. ramulosa* and warrants separation at the species level (see following, and Fig. 22).

Corollospora portsaidica Abdel-Wahab & Nagahama, sp. nov. Figs. 15–21

Ascomata 80–130 µm diametro, globosa, superficiala, ostiolata, papillata, nitida, nigra, carbonacea, solitaria, subiculata. Papilla 30–40 µm diametro, 25–40 µm alta, conica, basalis, prope subiculo; canalis ostioli cellulis pseudoparenchymaticis, pachydermis, dilute brunnis, impletus. Peridium 4–6 µm crassum. Centrum ascomatis immaturi cellulis pseudoparenchymaticis, hyalinis, leptodermis, foveolatis, deli-



Figs. 15–21. *Corollospora portsaidica* (holotype). **15** Vertical section of the ascomata. **16** Vertical section through part of the ascomata showing papilla close to subiculum; ostiolar canal with thick-walled pseudoparenchymatous cells and brown separation layer. **17** Vertical section of the ascomata through part of the ascomata showing peridium structure. **18** Ascus. Note the hyaline immature ascospores. **19–21** Ascospores showing frills of exospore fragments characteristic of the genus *Corollospora*. **15–21** Differential interference contrast. *Bars* **15–21** 15 µm

quescentibus. Paraphyses nullae. Asci 80–105 × 18–27 µm, octospori, late fusiformes, unitunicati, mox deliquescentes. Ascosporae 27–32 × 8–9 µm, fusiformes, 1-septatae, ad septum constrictae, brunneae. Appendices bigeneae: (i) appendices primariae polares, spiniformes, 14–18 µm longae, 1.5–2 µm diametro; (ii) appendices secundariae circa septum centrale 10–12 µm longae, peritrichiatae, per fragmenta exosporarum effectae et appendices apicales 7–10 µm longae.

Holotypus: In ligno indet., Egypt, Port Said, Mediterranean Sea coast, June 2006, coll. M.A. Abdel-Wahab (holotype, IMI 395684). Ex-type strain: MF 832 (= NBRC 105265).

Ascomata (Fig. 15) 80–130 µm in diameter, globose, superficial, ostiolate, papillate, shiny black, carbonaceous, solitary, subiculate, attached to the shell of dead shipworm that had penetrated decayed driftwood. Papilla (Fig. 16) 30–40 µm in diameter, 25–40 µm long, conical, basal, close to or surrounded by the subiculum; ostiolar canal filled with thick-walled, pseudoparenchymatous, light brown cells that are separated from the ascomatal centrum by a layer of brown cells. Peridium (Fig. 17) 4–6 µm thick, fragile, thin, black, consists of three- to four-celled layer of elongated, flattened, heavily melanized, thick-walled cells of which the inner one is lighter in color and thinner. Subiculum composed of thick-walled dark brown cells that form a textura epidermoidea. Centrum of immature ascomata is filled with thin-walled, polygonal to rounded, deliquescing pseudoparenchyma cells with pit-connections in their walls. Paraphyses absent. Asci (Fig. 18) 80–105 × 18–27 µm, eight-spored, broadly fusoid, unitunicate, early deliquescing. Ascospores (Figs. 19–21) 27–32 × 8–9 µm ($\bar{x}$ = 29.7 × 8.2 µm, n = 31) (excluding polar spines and appendages), fusiform, 1-septate, constricted at the septum, brown, the two cells of the ascospores either similar in size and shape or slightly different. Immature ascospores are hyaline. Appendages of two kinds: (i) a single terminal primary appendage at each end of the spore, 14–18 µm long, 1.5–2 µm in diameter, constricted at its base, spine- or thorn-like, attenuate; (ii) equatorial double frill of ribbon-like secondary appendages, 10–12 µm long, developing by fragmentation and peeling off of the exospore, and fragments of the exospore remain attached to the polar spines forming a tube or sheets 7–10 µm long.

Habitat: on shell of the shipworm in decayed driftwood.

Distribution: Egypt.

Anamorph: Unknown.

Etymology: In reference to the city name, Port Said, where the type material was collected.

Type material: Egypt, Port Said, Mediterranean Sea coast, on shell of shipworm in decayed driftwood in the intertidal zone, June 2006, coll. M.A. Abdel-Wahab (holotype, IMI 395684). Ex-type strain: MF 832 (= NBRC 105265).

Corollospora portsaidica is closely similar to *C. cinnamomea* by having small shiny black ascomata with a thin, one-layered peridial wall and one-septate brown ascospores. Ascospores of *C. portsaidica*, however, are fusiform, smooth walled, and longer and narrower in size [27–32 × 8–9 ($\bar{x}$ = 29.7 × 8.2) vs. 16.4–24.5 × 8–12.1 ($\bar{x}$ = 20.5 × 9.5) µm for *C. portsaidica* and *C. cinnamomea*, respectively] than those of *C. cinnamomea*, which are broadly ellipsoidal with a rugose surface (Koch 1986; Kohlmeyer and Volkmann-Kohlmeyer 1987). *Corollospora portsaidica* differs from *C. maritima* by having smaller ascomata with a thin, one-layered peridial wall and brown, longer ascospores. *Corollospora portsaidica* is phylogeneticaly divergent from both *C. cinnamomea* and *C. maritima* based on molecular sequence data (Fig. 22).

Phylogenetic analyses

We sequenced D1, D2, and D3 domains of the 5′-end of LSU rDNA for 6 *Corollospora* strains (including the 2 new species) and 1 strain of *Varicosporina ramulosa* (Table 1) to verify the taxonomy of the new *Corollospora* species. These regions were used to test phylogenetic relationships at the species and genus levels (Hillis and Dixon 1991; Wang et al. 2005). The obtained sequences were aligned with some *Corollospora* species sequences from the GenBank along with other representatives of the order Halosphaeriales. Representatives of the orders Microascales and Xylariales were used as outgroups (Fig. 22). Sequences of 26 taxa, of which 6 were outgroup taxa, were used in the analyses. Of 759 unambiguously aligned nucleotides, we excluded 63 gapped nucleotides. Of the remaining 696 nucleotides, 431 were constant, 98 were variable parsimony uninformative, and 167 were parsimony informative. Characters were weighted equally. Eight MP trees resulted with a tree length of 578 steps, a consistency index of 0.6090, and a retention index of 0.7280. Maximum likelihood analysis produced two trees with –ln likelihood = 3842.84425, of which tree number one is shown in Fig. 22. The difference between the two trees was in the position of *Cucullosporella mangrovei*. Maximum-likelihood (ML), MRBAYES, most parsimonious (MP), and neighbor-joining (NJ) produced similar trees to the one shown in Fig. 22. However, they differed in the position of *Corollospora portsaidica* and *C. luteola*. The 2 taxa formed a basal clade to the other *Corollospora* species (as shown in Fig. 22) in ML and MRBAYES analyses, whereas they formed a basal clade to clade A (see Fig. 22) in NJ and MP analyses (data not shown).

The two new species are well placed in the *Corollospora* clade with 100% support in Baysian analyses (Fig. 22). The relationship between *Corollospora portsaidica* and the other *Corollospora* species included in the analyses is not well resolved; however, the species is distantly related to the morphologically similar species *C. cinnamomea* and *C. maritima*. Therefore, *C. portsaidica* is proposed for a new taxon.

Corollospora anglusa grouped consistently within a clade (clade A) that contains *C. gracilis*, *C. cinnamomea*, and *Varicosporina ramulosa* with 99% and 60% support in Bayesian and likelihood analyses, respectively. Ascomata of these three *Corollospora* species and sclerocarps of *Varicosporina ramulosa* are small in size, and the three

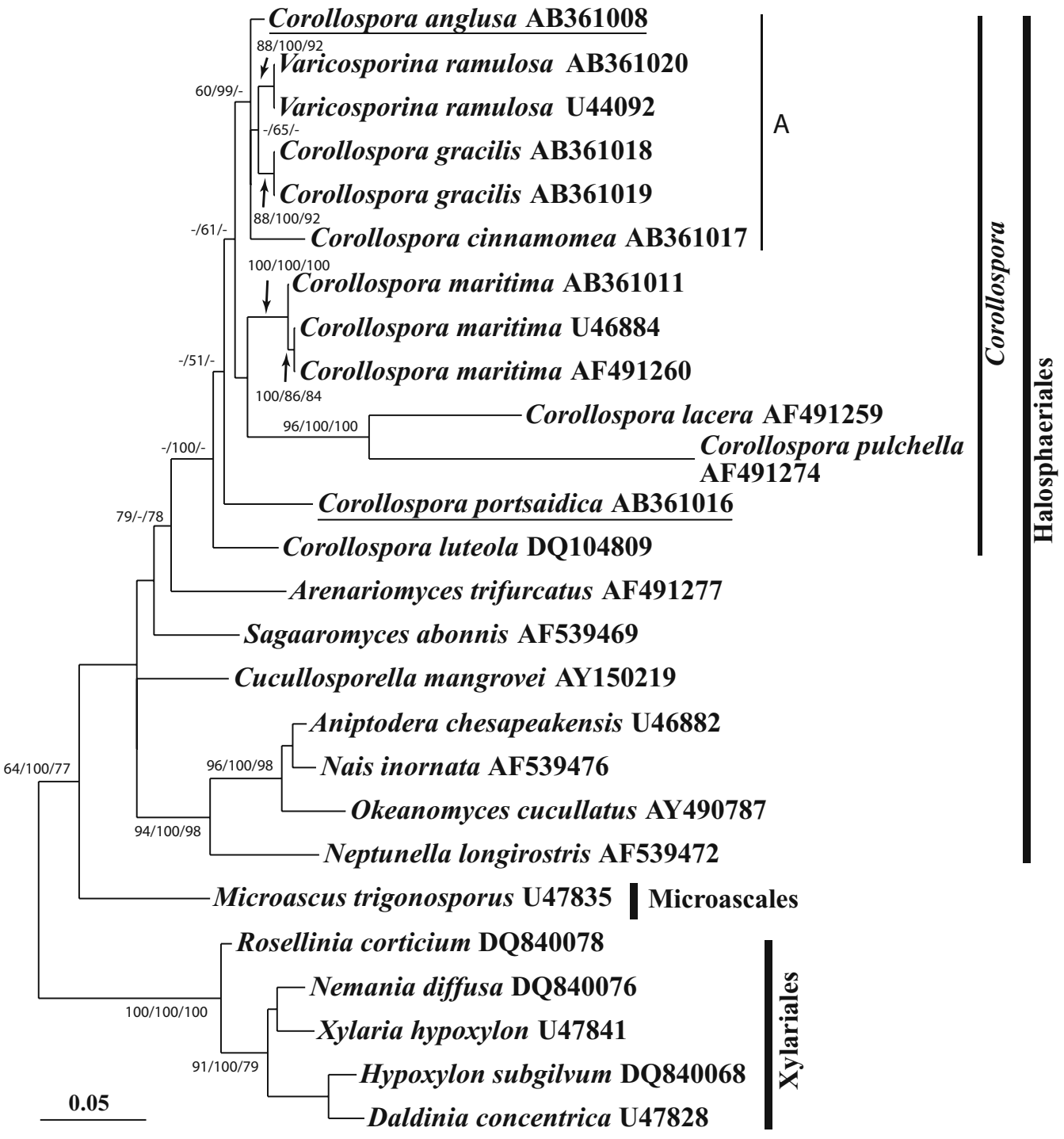


Fig. 22. Phylogenetic relationships of the two new *Corollospora* species and closely similar fungi, based on the nucleotide sequences of the large subunit (LSU) rDNA. The maximum likelihood tree (ML) (–ln likelihood = 3842.84425) was constructed as described in the text. The numbers are the bootstrap values for the nodes supported by >50% (250 replicates, ML/posteriori probabilities, Bayesian/1000 replicates, MP). New species are *underlined*

Corollospora species produce one-septate ascospores. *Corollospora cinnamomea* have brown rugose ascospores whereas *C. gracilis* have larger ascomata and ascospores than those produced by *C. anglusa*. *Corollospora anglusa* produce an anamorphic state that is different from *V. ramulosa*, so we describe the taxon as new to science.

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