Varicosporellopsis, a new aquatic genus from southern France

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Abstract: *Varicosporellopsis aquatilis* gen. and sp. nov. is described and illustrated based on two collections on submerged wood in southern France. An acremonium-like asexual morph was obtained in culture and sequenced. The genus is placed in the *Nectriaceae* based on its asexual morph and phylogenetic comparison of ITS and LSU sequences with 21 genera of *Hypocreales* including 14 genera in the *Nectriaceae* and 7 genera in the *Bionectriaceae*. *Varicosporellopsis* is primarily characterized by obpyriform, pale orange, non-stromatic ascomata not changing colour in 3% KOH, becoming very pale yellow to nearly hyaline in lactic acid, short-ribbed ascospores, freshwater habitat and an acremonium-like asexual morph.
Keywords: Ascomycota, freshwater habitat, *Hypocreales*, *Nectriaceae*, ribosomal DNA, taxonomy.

Résumé : *Varicosporellopsis aquatilis* gen. et sp. nov. est décrite et illustrée d'après deux récoltes effectuées sur bois immergé dans le sud de la France. Un stade asexué de type acremonium a été obtenu en culture et séquencé. Le placement du nouveau genre dans les Nectriacées repose sur le stade asexué et sur la comparaison phylogénétique de ses séquences ITS et LSU avec celles de 21 genres d'*Hypocreales* incluant 14 genres de Nectriacées et 7 genres de Bionectriacées (fig. 2, table 1). *Varicosporellopsis* est caractérisée par des ascomes orange pâle, non stromatiques, ne changeant pas de couleur dans KOH à 3%, devenant jaune très pâle à presque hyalins dans l'acide lactique, des ascospores variqueuses, un habitat aquatique et un stade asexué acremonium-morphe.
Mots-clés : ADN ribosomal, Ascomycota, habitat aquatique, Hypocréales, Nectriacées, taxinomie.

Introduction

In the continuity of a survey of freshwater pyrenomycetes in southwestern France, a hypocrealean fungus was twice collected on submerged wood, which proved different from known terrestrial species and different from species reported in the literature listed in Lechat & Fournier (2015). Two species of *Hypocreales* occurring on submerged wood were recently described: *Lasionectria fournieri* Lechat (Lechat, 2008) and *Varicosporella aquatica* Lechat & J. Fourn. (Lechat & Fournier, 2015), but these genera are different from *Varicosporellopsis*. *Varicosporella* differs primarily from *Varicosporellopsis* and *Lasionectria* in having a fusarium-like asexual morph, while *Lasionectria* differs from *Varicosporellopsis* in having flexuous hyphae or long, stiff, erect, thick-walled setae on the ascomatal surface.

Materials and methods

Specimens were examined using the method described by Rossman *et al.* (1999). Microscopic observations and measurements were made in water and the ascospore ornamentation was observed in lactic cotton blue without heating. The holotype specimen and the paratype are deposited in LIP herbarium (University of Lille) and cultures at CBS. Cultures of the living specimen were made on PDA (Potato Dextrose Agar) with 5mg/l of streptomycin in Petri dishes 55 mm diam. A mass of ascospores and asci was removed from a perithecium with a fine needle and placed in a drop of sterile water that was stirred with a needle to distribute the elements on the slide. Part of the drop containing ascospores was placed on PDA using a sterile micropipette, then the Petri dish was incubated at 25°C.

DNA extraction, amplification, and sequencing were performed by ALVALAB (Santander, Spain). Total DNA was extracted from dry specimens blending a portion of them using a micropestle in 600 µl CTAB buffer (CTAB 2%, NaCl 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min at 65°C. A similar volume of chloroform: isoamylalcohol (24:1) was added and carefully mixed with the samples until their emulsion.

It was then centrifugated for 10 min at 13.000 g, and the DNA in the supernatant was precipitated with a volume of isopropanol. After a new centrifugation of 15 min at the same speed, the pellet was washed in cold 70% ethanol, centrifugated again for 2 min and dried. It was finally resuspended in 200 µl ddH2O. PCR amplification was performed with the primers ITS1F and ITS4 (White *et al.*, 1990; Gardes & Bruns, 1993) for ITS, while LR0R and LR5 (Vilgalys & Hester, 1990) were used to amplify the 28S nLSU region. PCR reactions were performed using a program consisting of a hot start at 95°C for 5 min, followed by 35 cycles at 94°C, 54°C and 72°C (45, 30 and 45 s respectively) and a final 72°C step 10 min. PCR products were checked in 1% agarose gels, and positive reactions were sequenced with primer ITS4. Chromatograms were reviewed searching for putative reading errors, and these were corrected.

Analyses were performed online at www.phylogeny.lirmm.fr (Dereeper *et al.*, 2008). Maximum likelihood phylogenetic analyses were performed with PhyML 3.0 aLRT (Zwickl, 2006), using the GTR + I + Γ model of evolution. Branch support was assessed using the non-parametric version of the approximate likelihood-ratio test, implemented in PhyML (SH-aLRT; Anisimova & Gascuel, 2006).

Taxonomy

Varicosporellopsis Lechat & J. Fourn., *gen. nov.* – MB 815311

Diagnosis: Differs from the genus *Varicosporella* in having smaller ascomata and ascospores, and an acremonium-like asexual morph.

Type species: *Varicosporellopsis aquatilis* Lechat & J. Fourn.

Etymology: *Varicosporellopsis* refers to the morphological similarity with *Varicosporella* Lechat & J. Fourn.

Varicosporellopsis aquatilis Lechat & J. Fourn., *sp. nov.* – MB 815312 – Fig. 1-2.

Diagnosis: Ascomata on submerged wood, superficial, non-stromatic, obpyriform, pale orange, not changing colour in 3% KOH, becoming very pale yellow to nearly hyaline in lactic acid. Differs from *Varicosporella aquatica* in having smaller ascospores and an acremonium-like asexual morph.

Holotype: France, Ariège: Rimont, La Maille, La Maille brook, 550 m, 6 May 2015, on submerged wood of *Sambucus nigra*, coll. Jacques Fournier JF15039 (LIP). Ex-type culture CBS 140158, ITS and LSU GenBank sequences KU233187 and KU233189.

Additional material examined: France, Ariège: Clermont, Le Pujol brook along road D 119, ca. 360 m, 31 Jul. 2009, on submerged twig of *Buxus sempervirens*, coll. Jacques Fournier JF 09213 (LIP).

Etymology: The epithet refers to the freshwater lifestyle of the fungus.

Known distribution: Southern France (Ariège).

Ascomata non-stromatic, solitary, superficial with base slightly immersed in substratum, soft-textured, pale orange, not changing colour in 3% KOH, becoming very pale yellow to nearly hyaline in lactic acid, subglobose, laterally collapsing on drying, 240–280 µm high, 170–230 µm diam, with a translucent, broadly conical to rounded apex 50–80 µm high, 80–100 µm diam., composed of cylindrical hyaline to pale yellow cells 15–35 µm long, 3–3.5 µm diam, thick-walled, septate, clavate at top. **Ascomatal wall** in vertical section 30–35 µm thick, composed of two regions; outer region 18–22 µm thick, composed of globose, subglobose to ellipsoidal, pale yellow, thick-walled cells 6–14 × 4–7.5 µm with wall 1.5–2 µm thick; inner region 10–15 µm thick, composed of hyaline and more flattened cells 6–10 × 3–5 µm. Ascomatal surface made of subglobose, subangular cells, covered by smooth, tan or hyaline hyphae 2.5–3 µm diam. Ostiolar canal periphysate. **Asci** unitunicate, cylindrical, short–stipitate, with eight obliquely uniseriate ascospores, 65–80 × 6.5–7.5 µm, apically truncate to slightly rounded with a faint apical ring-like thickening, interspersed with moniliform early deliquescing paraphyses up to 15 µm wide at base. **Ascospores** (9.5–)10–11(–12) × 4.5–6(–6.5) µm (Me = 10.5 × 5.5 µm, n = 30), ellipsoid with narrowly to broadly rounded ends, equally two–celled, slightly

Table 1 – Genera, species and GenBank accession numbers of sequences used in the phylogenetic analyses

Species	Asexual morph	ITS	LSU
Albonectria rigidiuscula	fusarium-like	HM054158	HM042403
Bionectria ochroleuca	clonostachys-like	KC460538	GQ50600
Calonectria lauri	cylindrocladium-like	GQ280584	GQ280706
Cosmospora viliuscula	acremonium-like	KC291732	KC291777
Cosmospora viridescens	acremonium-like	KC291731	KC291765
Hydropisphaera fusigera	acremonium-like	GU059594	GU059595
Hypomyces aurantius	cladobotryum-like	KM509060	KC009213
Lanatonectria flocculenta	sarcopodium-like	JF832657	JF832714
Lasionectria mantuana	acremonium-like	HM484858	GQ505994
Lasionectria sp.	acremonium-like	JX306092	–
Microcera larvarum	fusarium-like	KC354705	KC338992
Microcera coccophila	fusarium-like	KC338994	KC338993
Myrothecium inundatum	gliocladium-like	AJ302005	–
Myrothecium inundatum	gliocladium-like	FJ797514	–
Myrothecium roridum	gliocladium-like	JF724154	–
Myrothecium inundatum	gliocladium-like	HQ165763	–
Nalanthamala olivacea	acremonium-like	AY554219	AY554255
Nalanthamala psidii	acremonium-like	AY554208	AY554258
Nectria cinnabarina	tubercularia-like	HM484712	HM484756
Nectria pseudotrichia	tubercularia-like	KF611683	JF832704
Nectriopsis exigua	acremonium-like	HM484865	GQ505986
Neocosmospora vasinfecta	fusarium-like	KF612306	AY381155
Neocosmospora ornamentata	fusarium-like	JX868635	AF178382
Neonectria ditissima	cylindrocarpon-like	JF735309	KM515936
Pseudocosmospora eutypae	acremonium-like	KC291736	KC291786
Pseudocosmospora rogersonii	acremonium-like	KC291728	KC291780
Pseudonectria pachysandricola	volutella-like	JF832658	JF832715
Pseudonectria rousseliana	volutella-like	JF937563	JF937574
Roumegueriella rufula	acremonium-like	–	DQ518776
Selinia pulchra	acremonium-like	HM484859	GQ505992
Stachybotrys chlorohalonata	acremonium-like	AY489712	JN938870
Stachybotrys chartarum	acremonium-like	AF081468	AY489714
Stachybotrys echinata	acremonium-like	KF626506	AY489736
Stachybotrys sp.	acremonium-like	–	JF14101
Stachybotrys sp.	acremonium-like	–	JF14099
Varicosporella aquatica	fusarium-like	KP192669	KP192671
Varicosporella aquatica	fusarium-like	KP192668	KP192670
Xenoacremonium recifei	acremonium-like	KM231834	KM231714
Viridispora diparietispora	penicilifer-like	JN049838	AY489735

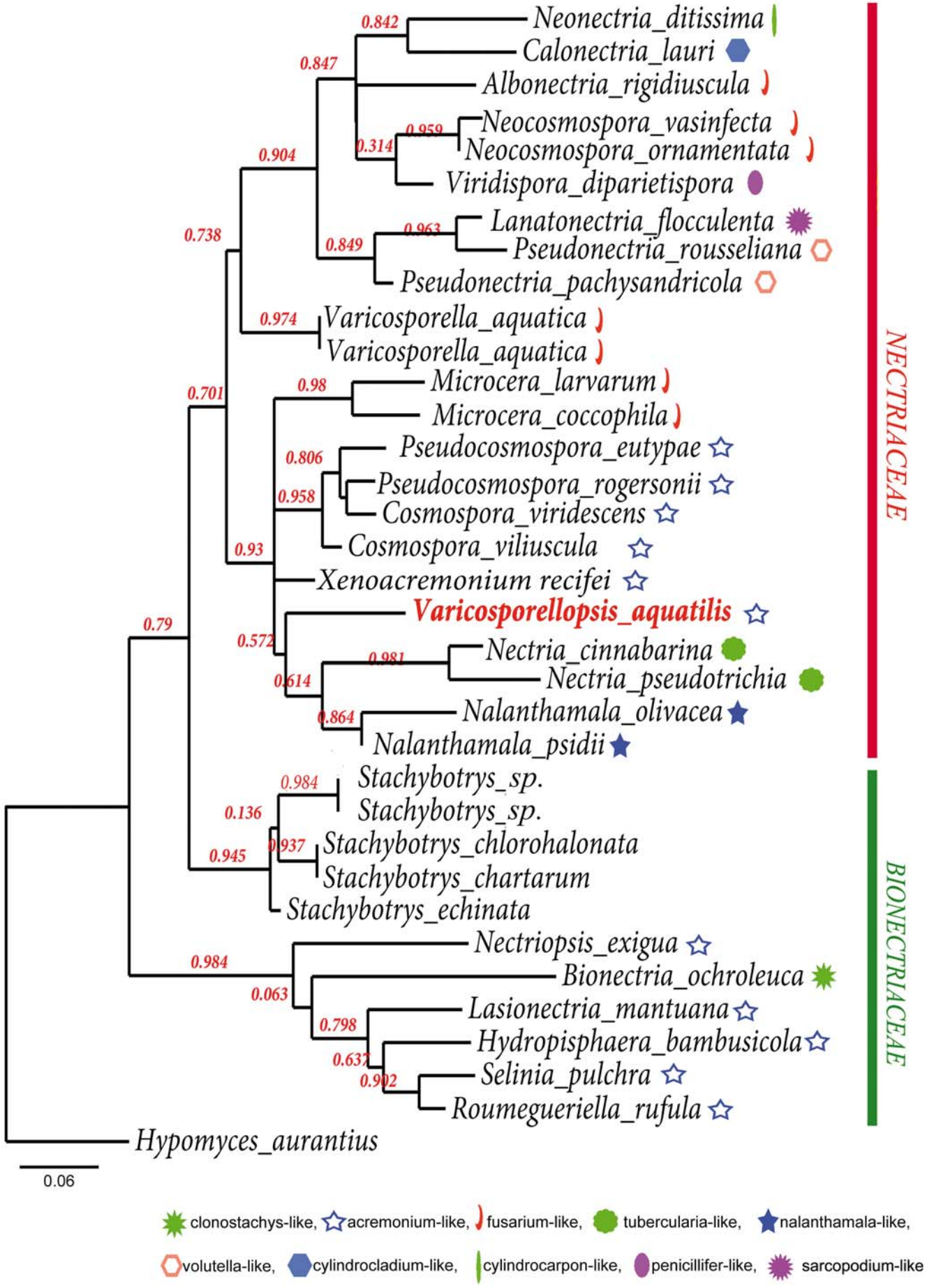

Fig. 1 – Maximum likelihood phylogeny of *Varicosporellopsis aquatilis* based on combined ITS and LSU sequences, rooted with *Hypomyces aurantius*.

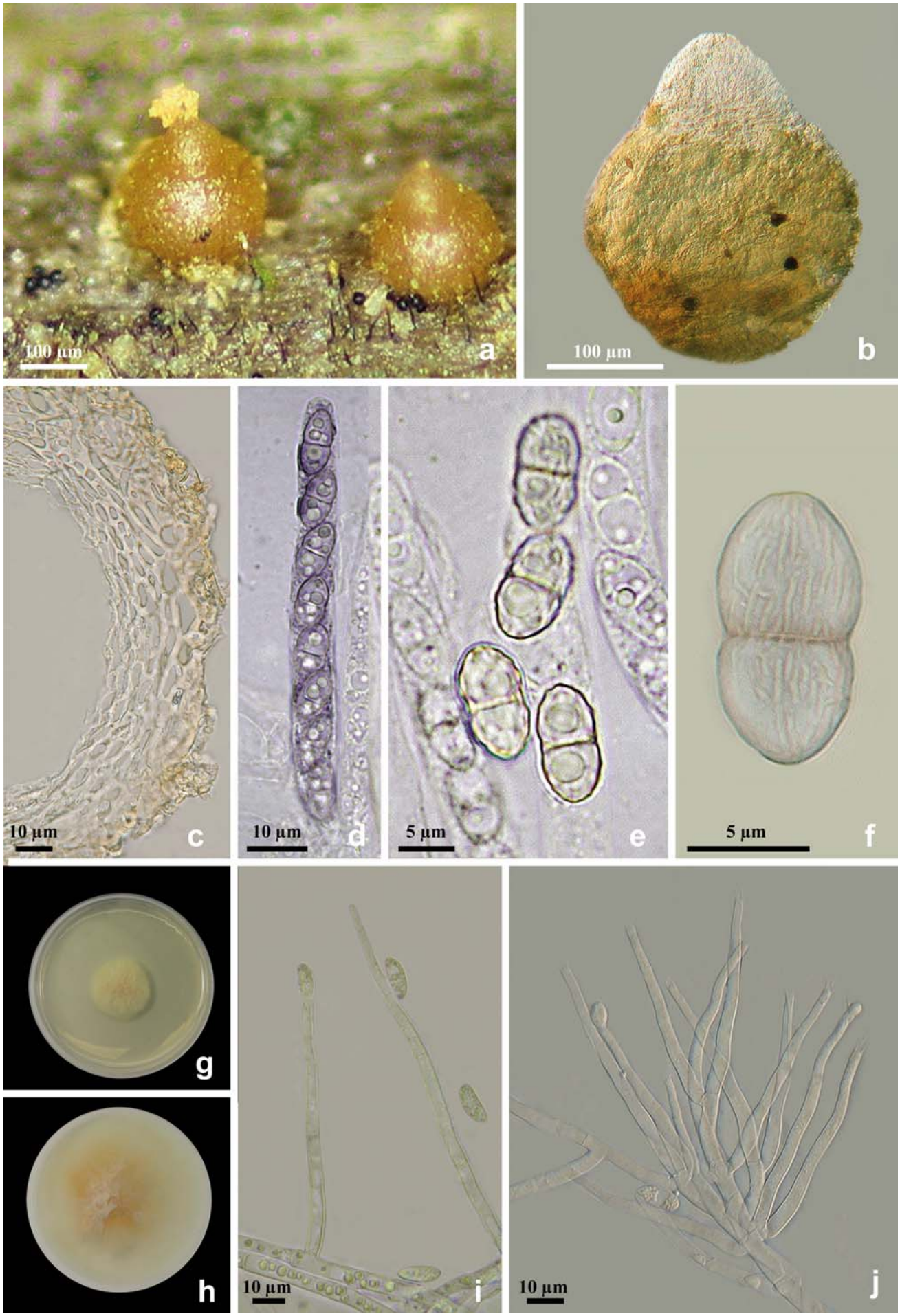

Fig. 2 – a-e: *Varicosporellopsis aquatilis*, Holotype. a: Ascomata on the substratum; b: Close-up of perithecium in water; c: Lateral ascomatal wall in vertical section; d-f: Ascus and ascospores; g: Culture after two weeks; h: Culture after three weeks; i-j: Conidiophores and conidia.

constricted at septum, hyaline to pale yellowish brown, with 2–3 large guttules in each cell, wall roughened by short, sinuous, brown, thick ribs, sometimes anastomosed.

Cultural characteristics: Colony after two weeks on PDA measuring 45–50 mm diam, aerial mycelium white in center, salmon in median area and pale yellowish at margin, abundantly sporulating; reverse pale yellow to pale yellowish brown. Conidiophores macronematous, mononematous, unbranched, flexuous, hyaline, smooth; conidiogenous cells monophialidic, 1.5–2 µm wide at apex with a slightly flared collarette, producing narrowly ellipsoidal to subcylindrical conidia 6–11 × 2.8–3.2 µm. After three weeks verticillate conidiophores appear, arising laterally from submerged hyphae. Phialides cylindrical, subulate, smooth, hyaline to pale salmon producing widely ellipsoidal conidia, attenuated at base without a flat abscission scar, 5–8 × 3–4.5 µm, smooth, hyaline.

Discussion

Typically, the ascomata of *Nectriaceae* are brightly coloured, changing colour in 3% KOH or lactic acid, as defined by Rossman *et al.* (1999) and Schroers (2011). However, some genera are known to have ascomata not changing colour in 3% KOH but turning yellow in lactic acid: *Albonectria* Rossman & Samuels, *Pseudonectria* Seaver (Rossman *et al.*, 1999) and *Varicosporella* (Lechat & Fournier, 2015). *Varicosporellopsis aquatilis* is primarily characterized by obpyriform, pale orange, non-stromatic ascomata, not changing colour in 3% KOH, becoming very pale yellow to nearly hyaline in lactic acid, short-ribbed ascospores and an acremonium-like asexual morph. Based on the characters of its sexual and asexual morphs as well as phylogenetic analysis (Fig. 1), this taxon clearly belongs to the *Nectriaceae*. Morphologically, *Varicosporellopsis* is similar to *Variscoporella* in having pale orange, non-stromatic ascomata, not changing colour in 3% KOH and freshwater habitat, but differs from it in having smaller ascomata and ascospores, as well as an acremonium-like asexual morph while *Varicosporella* possesses a fusarium-like asexual morph. Molecular analysis carried out in the present study, comparing 14 genera in the *Nectriaceae* including the type species of *Albonectria*, *Pseudonectria* and *Varicosporella* (Fig. 1), shows that *Varicosporellopsis* belongs to the *Nectriaceae* but is placed on a branch distant from these three genera. In the phylogenetic tree, the closest genera to *Varicosporellopsis* are *Nalanthamala* Subram., *Nectria* (Fr.) Fr. and *Xenoacremonium* L. Lombard & Crous.

Only the asexual morph of *Xenoacremonium* is known and although it has acremonium-like morphology, like *Varicosporellopsis*, it differs from the latter in having unbranched or rarely branched conidiophores, and conidia forming slimy heads at tip of the phialide, as defined by Lombard *et al.* (2015). *Nalanthamala* and *Nectria* are very different from *Varicosporellopsis* in having brightly coloured ascomata, seated on a pseudoparenchymatous stroma, changing colour in 3% KOH and lactic acid, ascomatal wall over 50 µm thick and a sprodochial asexual morph as defined respectively by Lombard *et al.* (2015) and Hirooka *et al.* (2012). Based on phylogenetic, morphological and ecological divergences, the new genus *Varicosporellopsis* is proposed.

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