# *Varicosporella*, a new aquatic genus in the *Nectriaceae* from France

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**Summary:** *Varicosporella aquatica* gen. and sp. nov. is described and illustrated based on six collections on submerged wood in South of France. A fusarium-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 species in 22 genera of *Hypocreales* including 16 genera in the *Nectriaceae*. It is primarily characterized by obpyriform, greyish yellow to pale orange nonstromatic ascomata, short-ribbed ascospores and freshwater habitat.
**Keywords:** Ascomycota, freshwater habitat, *Hypocreales*, ribosomal DNA, taxonomy.

**Résumé :** *Varicosporella aquatica* gen. et sp. nov. est décrite et illustrée d'après six récoltes, sur bois immergé, effectuées dans le sud de la France. Un stade asexué de type fusarium 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 22 genres d'Hypocréales incluant 16 genres de Nectriacées. Il est principalement caractérisé par des ascomes obpiriformes, non stromatiques, de couleur jaune grisâtre à orange pâle, des ascospores ornées de courtes crêtes leur donnant un aspect variqueux et un habitat aquatique.
**Mots-clés :** Ascomycota, habitat aquatique, Hypocréales, ADN ribosomal, taxinomie.

## Introduction

In the course of an ongoing survey of freshwater pyrenomycetes in southwestern France several hypocrealean fungi have been repeatedly collected, which proved different from known terrestrial species and different from species reported in the literature and listed at http://fungi.life.uiuc.edu/world_records (Shearer & Raja, 2010). One of them, *Lasionectria fournieri* Lechat (Lechat, 2008), was described as new but most of them are still under investigation. These fungi frequently occur in very small colonies and their isolation often proves unsuccessful, rendering their characterization unsatisfying. Aquatic hypocrealean fungi are poorly represented in the literature, usually reported under the genus name *Nectria* (Fr.) Fr. in a very broad sense. Often only limited information on morphological data accompanies the records, which rarely allows a generic placement, let alone species delimitation. Moreover the available information often suggests that terrestrial fungi fortuitously present in water are more likely involved rather than true aquatic fungi. It is noteworthy that the aquatic hypocrealean fungi we encountered appear erumpent from the wood and gradually become almost superficial with the base remaining slightly immersed and are never stromatic. These features, especially the erumpent habit, are usually not encountered in terrestrial species and might relate to their aquatic lifestyle.

A distinctive taxon characterized by obpyriform greyish yellow to pale orange ascomata and pale yellow brown ascospores with short-ribbed ornamentation was repeatedly collected on submerged wood; the ascomata did not change colour in KOH. This species may be accommodated in the *Bionectriaceae* (Rossman *et al.*, 1999). Unexpectedly, its ascomatal wall appeared to turn pale yellow in lactic acid, suggesting possible affinities with the Nectriaceae, which was confirmed by a fusarium-like asexual morph obtained in artificial culture, which is unknown in the *Bionectriaceae*. Further molecular analyses of ITS and LSU sequences compared with those of 22 genera of *Hypocreales* including 16 genera in the *Nectriaceae* and six in the *Bionectriaceae* (Table 1) supported its placement in the *Nectriaceae*, in a clade composed of morphologically unrelated terrestrial taxa.

For these reasons we conclude that this fungus represents a previously undescribed genus in the *Nectriaceae* and we propose the new genus *Varicosporella* to accommodate the new species *Varicosporella aquatica*.

## Materials and Methods

Specimens were examined using the method described by Rossman *et al.* (1999). Microscopic observations and measurements were made in water. The ascospore ornamentation was observed in lactic Cotton Blue not heated. The holotype specimen and paratypes 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 5 mg/l of streptomycin in Petri dishes 9 cm 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. A 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 65ºC. A similar volume of chloroform: isoamylalcohol (24:1) was added and carefully mixed with the samples until their emulsion.

It was then centrifuged 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 ethanol 70%, centrifuged 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 under a program consisting of a hot start at 95ºC for 5 min, followed by 35 cycles at 94ºC, 54ºC and 72ºC (45, 30 and 45 s respectively) and a final 72ºC step 10 min. PCR products were checked in 1% agarose gels, and positive reactions were sequenced with primer ITS4. Chromatograms were checked 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

***Varicosporella*** Lechat & J. Fourn. *gen. nov.* — MB 810690

**Diagnosis:** Differs from other genera of the *Nectriaceae* with fusarium-like asexual morph by pale orange nonstromatic ascomata not changing colour in KOH, aquatic habitat and ascospores conspicuously ornamented with short sinuous ribs.

**Type species:** *Varicosporella aquatica* Lechat & J. Fourn.

**Etymology:** "*Varicosporella*" refers to the ribbed ornamentation of ascospores, from Latin *varix* = varicose vein.

***Varicosporella aquatica*** Lechat & J. Fourn. *sp. nov.* — MB 810691, Plates 1-2.

**Diagnosis:** Ascomata on submerged wood, superficial, nonstromatic, obpyriform, pale orange, not turning red or purple in 3% KOH, turning pale yellow in lactic acid, wall 28–35 µm thick composed of thick-walled angular to flattened cells; hamathecium of fugacious moniliform paraphyses, asci 8-spored, cylindric-clavate with a J- flattened apical apparatus; ascospores 21–24 × 8.5–10 µm, equally two-celled, pale yellow brown, ornamented with short sinuous ribs.

**Holotype:** France, Ariège, Vernajoul, Vernajoul brook, ca. 350 m asl, on submerged wood of *Populus* sp., associated with *Bactrodesmium obovatum* and *Cosmospora* sp., 6 Jul. 2009, JF 09197 (LIP). Ex-type culture CBS126103, ITS and LSU GenBank sequences KP192669 and KP192671.

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

**Known distribution:** South of France: Ariège, Lozère.

**Ascomata** nonstromatic, solitary, superficial with base slightly immersed in substratum, soft-textured, greyish yellow to pale orange, becoming pale yellow in 3% KOH and in lactic acid, obpyriform, 340–390 µm high × 250–300 µm diam. (Me = 370 × 285 µm, n = 10), laterally collapsing when dry, uniloculate, translucent, with a broadly conical to rounded apex 100–170 µm long, 80–110 µm diam, composed of cylindrical yellow cells 15–40 µm long, 3.5–4.5 µm diam, thick-walled, septate, clavate at top.

**Ascomatal wall** in vertical section 28–35 µm thick, composed of subglobose to angular thick-walled cells, wall 1.5–2.5 µm thick, becoming more flattened inwardly. Perithecial surface cells forming a textura angularis in surface view. Basal hyphae sparse and short, thick-walled, hyaline. **Asci** unitunicate, cylindrical, short–stipitate, with eight obliquely uniseriate ascospores, 140–165 × 17–20 µm, apically truncate to slightly rounded with a conspicuous, refractive apical apparatus 1.5–2 µm high × 5–6 µm wide, discoid to wedge-shaped, slightly stained in blue ink, interspersed with slightly moniliform, thin-walled, early deliquescing paraphyses 8–14 µm wide at base. **Periphyses** copious, embedded in gel matrix, simple or branched, septate, 15–40 × 1.5–2 µm. **Ascospores** 21–24 × 8.5–10 (–11) µm (Me = 22.5 × 9.5 µm, n = 30), ellipsoid with narrowly to broadly rounded ends, equally two-celled, slightly constricted at septum, hyaline to pale yellowish brown, with two large guttules in each cell, wall roughened by short, sinuous, brown, thick ribs, sometimes anastomosed. Hyaline ascospores germinate more often than pigmented ones.

**Cultural characteristics:** After 20 days at 25°C on Difco PDA containing 5 mg/L streptomycin, colony 3.5–4 cm diam., producing fast-growing fusarium-like, culture slimy, lacking aerial mycelium, white to pale yellowish, becoming pale pinkish and cottony with aerial mycelium. No microconidia produced; macroconidia cylindrical, slightly curved, acute at tip, truncate to rounded at base, 3–4 (–5)-septate: 3-septate (62–) 67–75 (–78) × 7–8.5 µm (Me = 72 × 8 µm, n = 30), 4–5-septate (72–) 75–77 (–85) × 7–8.5 µm (Me = 77.4 × 8 µm, n = 30). Pale yellow fertile ascomata produced on cultures after five weeks, containing asci and ascospores identical to the type.

**Additional specimens examined:** France: Ariège, Clermont, Le Pujol brook along road D 119, ca. 360 m asl, on submerged twig of *Buxus sempervirens*, 31 Jul. 2009, JF 09213 (LIP), (Cultured, CBS125538); Ariège, Vernajoul, Vernajoul brook, Pont Fagé, ca. 370 m asl, on submerged wood of *Salix* sp., soc. *Jahnula aquatica*, *Lentithecium aquaticum* and *Pseudohalonectria lutea*, 18 Jul. 2013, JF13150 (LIP); Ariège, Vernajoul, Vernajoul brook, Pont Fagé, on submerged wood of *Alnus glutinosa*, soc. *Jahnula aquatica*, *Pseudohalonectria lutea*, 18 Jul. 2013, JF13152 (LIP); Ariège, Castelnau-Durban, L'Artillac, on submerged wood of *Fraxinus* soc. *Jahnula aquatica*, *Lindgomyces griseosporus*, *Pleurotheciella rivularia* and *Trematosphaeria hydrela*, 24 Jul. 2014, JF14074 (LIP), (Cultured, CBS138883, ITS and LSU GenBank sequences KP192668 & KP192670); Lozère, Saint-Germain-du-Teil, Malbousquet brook, 3°12'00" E, 44°28'03" N, 575 m asl, on submerged wood, 23 Oct. 2014, *leg*. A. Gardiennet, pers. herb. AG14191.

## Discussion

*Varicosporella aquatica* is characterized by superficial, nonstromatic obpyriform ascomata with a soft pale orange peridium that does not turn red or purple in 3% KOH, unitunicate cylindrical asci with a discoid refractive inamyloid apical apparatus, deliquescent paraphyses and equally two-celled, pale brown ascospores ornamented with short sinuous ridges; its asexual morph obtained in culture is fusarium-like.

Based on the characters of its sexual and asexual morphs, this taxon clearly belongs to the *Hypocreales*, either in the *Bionectriaceae* or in the *Nectriaceae* as defined by Rossman *et al.* (1999) and Schroers (2001). The pale orange ascomata not changing color in 3% KOH or lactic acid are typical of the *Bionectriaceae* but a fusarium-like asexual morph is unknown in this family and is only represented in the *Nectriaceae*. The delimitation of the two families is largely based on the type of asexual morph and strongly supported by phylogenetic studies (Rossman, 2000; Rossman *et al.*, 2013). Therefore, our new taxon having a fusarium-like asexual morph is best placed in the *Nectriaceae*, which is well supported by our phylogenetic analysis (Fig. I).

Unlike *Varicosporella*, most genera of the *Nectriaceae* typically have dark orange to red ascomata that turn darker red or purple in KOH, and yellow in lactic acid (Rossman *et al.*, 1999). However two genera currently accommodated in the *Nectriaceae* likewise lack the typical KOH reaction, viz. *Albonectria* Rossman & Samuels and *Pseudonectria* Seaver (Rossman *et al.*, 1999). *Albonectria* differs from *Varicosporella* in having warted, thick-walled, white ascomata on a sparse to well-developed pseudoparenchymatous stroma and 3-to multiseptate ascospores, while *Pseudonectria* has thin-walled ascomata (wall less than 20 µm thick), usually nonseptate smooth ascospores and a volutella-like asexual morph.

Molecular analysis carried out in the present study, comparing 16 genera in the *Nectriaceae* including the type species of *Albonectria* and *Pseudonectria* (Fig. 1), shows that *Varicosporella* is nested within the *Nectriaceae* but placed on a basal branch distant from other genera clustering in the same subclade, viz. *Viridispora* Samuels & Rossman, and *Neocosmospora* E.F. Sm.

*Viridispora* differs from *Varicosporella* in having red strongly warted or roughened ascomata, penicillifer-like asexual morph and green to yellow brown, smooth ascospores. *Neocosmospora* resembles *Varicosporella* in having often pigmented and coarsely ornamented ascospores but is readily distinguished in having smooth or roughened to coarsely warted, orange brown to red ascomata, turning reddish brown to dark purple in 3% KOH.

Moreover, none of the genera discussed above was reported to occur on submerged wood in freshwater habitats. The aquatic lifestyle of *V. aquatica* is supported by six collections on submerged

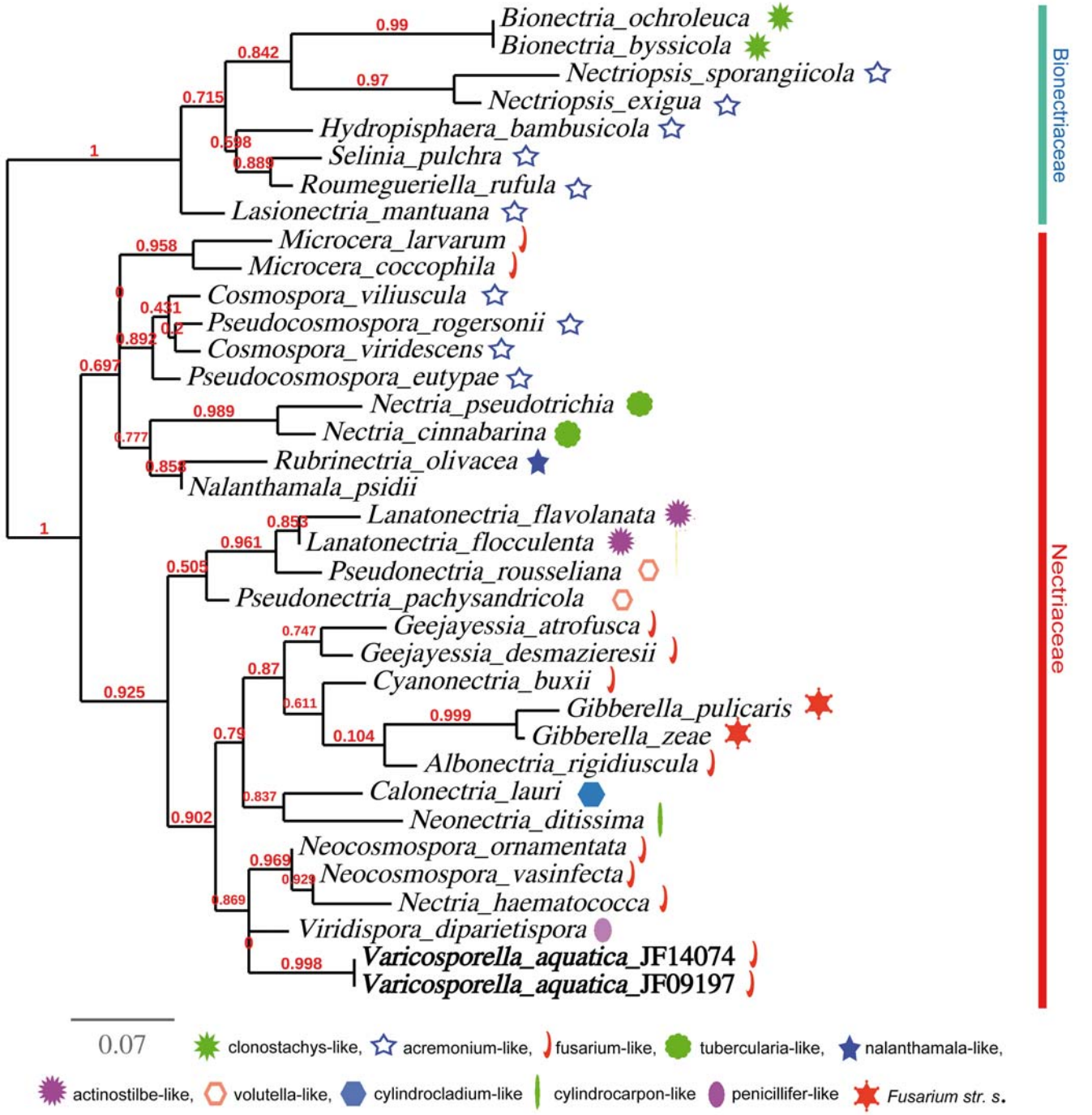


**Fig. 1** — Maximum likelihood phylogeny of *Varicosporella* based on combined ITS1-5.8S-ITS2 and LSU sequences.

wood, often associated with known aquatic ascomycetes (Plate 2, Fig. a), and no occurrence on terrestrial substrates despite extensive collecting and investigations on pyrenomycetes over the last 15 years in the same area (Ariège). A thorough comparison with the descriptions of hypocrealean ascomycetes recorded in freshwater habitats by Shearer (1993), Cai *et al.* (2003), Cai *et al.* (2006) and Shearer & Raja (2010) at http://fungi.life.uiuc.edu/world_records did not show any genus or species conforming to the peculiar combination of characters displayed by *V. aquatica*. Thus, based on morphological, cultural, molecular and ecological data, a new nectriaceous genus *Varicosporella* is introduced to accommodate *V. aquatica*.

The new rules imposed by the ICN at Melbourne Congress in 2011 (McNeill *et al*., 2012) specify that in pleomorphic fungi a new genus name cannot be introduced when an older name is available for the asexual or the sexual morph. Following these rules one might consider the genus name *Fusarium* Link for our new taxon which has a fusarium-like asexual morph. The status of *Fusarium* as a genus name is controversial since its origin, having been split into various "sections". The genus in the broad sense was recently delimited into "a" *Fusarium* terminal clade, distant from other "basal fusarium-like clades" by Gräfenhan *et al.* (2011). The terminal clade proposed by these authors includes the type species *F. sambucinum* Fuckel as its sexual morph *Gibberella pulicaris* (Fr.) Sacc., along with sexual morphs described in *Albonectria*, *Cyanonectria*, *Haematonectria* and *Neocosmospora*, but "without significant statistical support" (Gräfenhan *et al.*, 2011). On the one hand phytopathologists strongly advocate for *Fusarium* to represent the whole terminal clade (Geiser *et al.*, 2013) while on the other hand taxonomists reasonably assign *Fusarium* to the monophyletic group of species clustering around *F. sambucinum* (Schroers *et al*., 2011; Rossman, 2013). Our new species

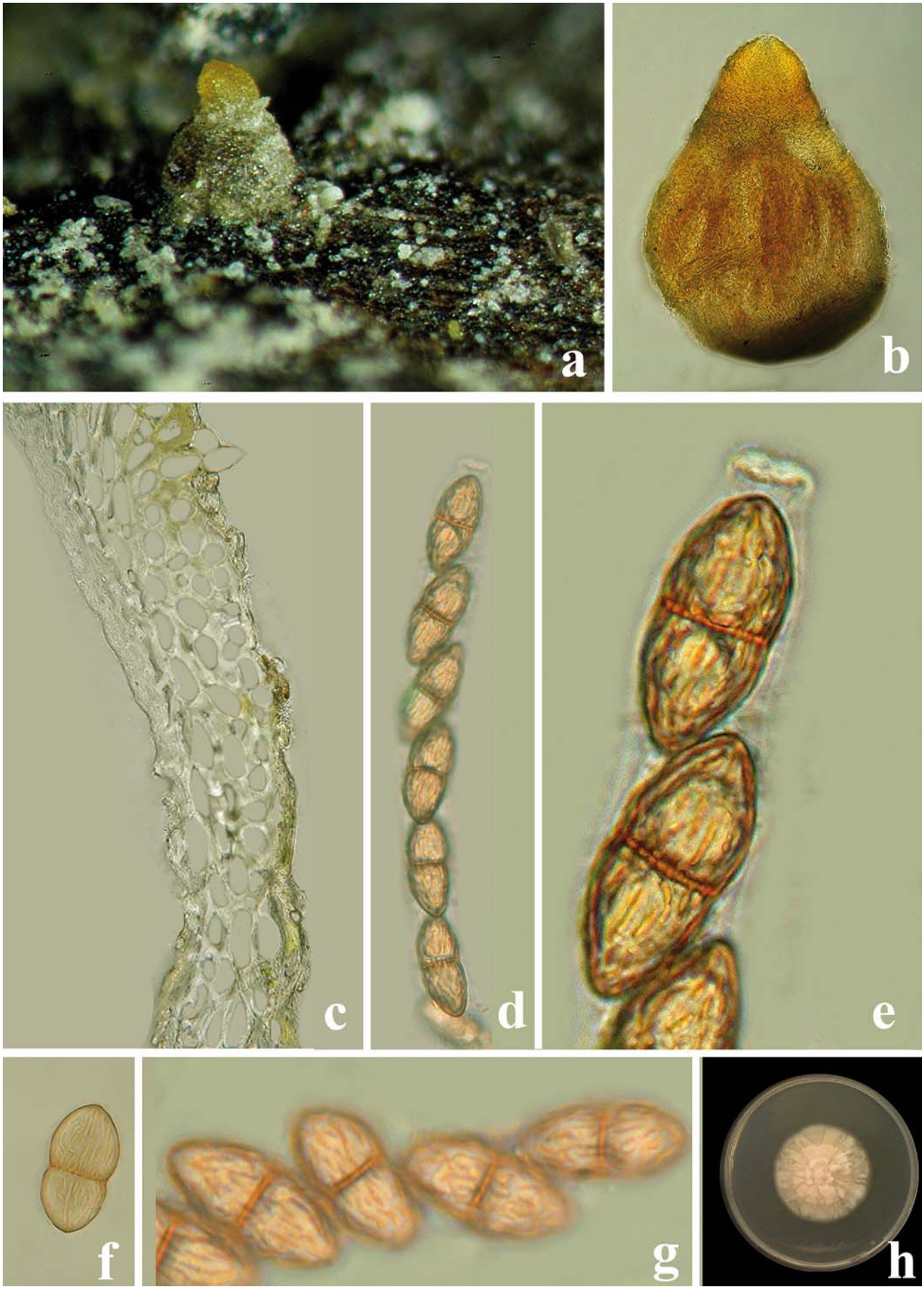


**Plate 1.** a–e: *Varicosporella aquatica* (Holotype JF09197). a: Perithecium on natural substratum. b: Close-up of perithecium in water. c: Section through the ascomatal wall. d: Ascus and ascospores (in water). e: Close-up of ascospores in water showing ornamentation.

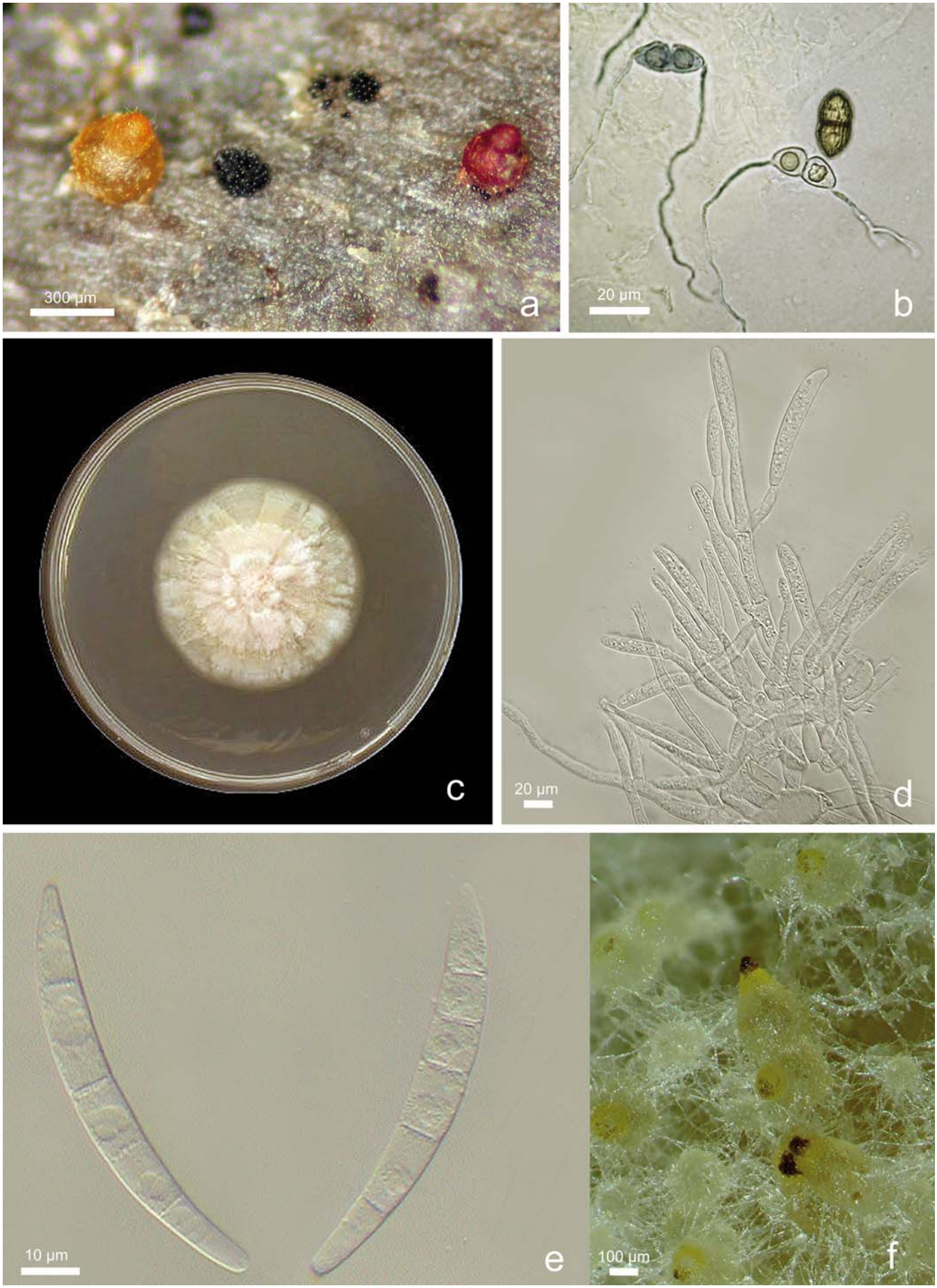


**Plate 2.** a–e: *Varicosporella aquatica* (Holotype JF09197). a: Perithecium on natural substratum mixed with *Cosmospora* sp. and *Bactrodesmium obovatum*. b: Germinating ascospores. c: Culture in Petri dish. d: Conidiophores and conidia. e: Conidia. f: Ascomata appearing after five weeks in culture.

**Table 1 —** Genera, species and GenBank accession numbers of sequences used in the phylogenetic analyses. The taxon names are from GenBank.

| | | GenBank accession numbers | |
|---|---|---|---|
| **Species** | **Asexual morph** | **ITS** | **LSU** |
| *Albonectria rigidiuscula* (Berk. & Broome) Rossman & Samuels | fusarium-like | HM054158 | HM042403 |
| *Bionectria ochroleuca* (Schwein.) Schroers & Samuels | clonostachys-like | KF055399 | GQ50600 |
| *Bionectria byssicola* (Berk. & Broome) Schroers & Samuels | clonostachys-like | AF358252 | GQ506011 |
| *Calonectria lauri* (Vanderw.) Lechat & Crous | cylindrocladium-like | GQ280584 | GQ280706 |
| *Cosmospora viliuscula* (Samuels, Yoshim. Doi & Rogerson) Rossman & Samuels | acremonium-like | KC291732 | KC291777 |
| *Cosmospora viridescens* (C. Booth) Gräfenhan & Seifert | acremonium-like | KC291731 | KC291765 |
| *Cyanonectria buxi* (Fuckel) Schroers, Gräfenhan & Seifert | fusarium-like | HQ728144 | HM626673 |
| *Gibberella pulicaris* (Kunze) Sacc. | fusarium-like | KC445242 | U85523 |
| *Gibberella zeae* (Schwein.) Petch | fusarium-like | HQ651168 | HQ147601 |
| *Hydropisphaera bambusicola* Lechat | acremonium-like | GU059594 | GU059595 |
| *Hypomyces armeniacus* Tul. & C. Tul. | cladobotryum-like | FN859424 | AF160239 |
| *Lanatonectria flavolanata* (Berk. & Broome) Samuels & Rossman | actinostilbe-like | EF121860 | HQ232157 |
| *Lanatonectria flocculenta* (Henn. & E. Nyman) Samuels & Rossman | actinostilbe-like | JF832657 | JF832714 |
| *Lasionectria mantuana* (Sacc.) Cooke | acremonium-like | HM484858 | GQ505994 |
| *Microcera larvarum* (Fuckel) Gräfenhan, Seifert & Schroers | fusarium-like | KC354705 | KC338992 |
| *Microcera coccophila* Desm. | fusarium-like | KC338994 | KC338993 |
| *Nalanthamala psidii* (Sawada & Kuros.) Schroers & M.J. Wingf. | nalanthamala-like | AY554208 | AY554255 |
| *Nectria cinnabarina* (Tode : Fr.) Fr. | tubercularia-like | HM484712 | HM484756 |

**Table 1** — (continued)

| | | GenBank accession numbers | |
|---|---|---|---|
| **Species** | **Asexual morph** | **ITS** | **LSU** |
| *Nectria haematococca* Berk. & Broome | fusarium-like | AY354252 | AY489729 |
| *Nectria pseudotrichia* (Schwein.) Berk. & M. A. Curtis | tubercularia-like | KF611683 | JF832704 |
| *Nectriopsis sporangiicola* (Samuels) Samuels | acremonium-like | AF210661 | AF210662 |
| *Nectriopsis exigua* (Pat.) W. Gams | acremonium-like | HM484865 | GQ505986 |
| *Neocosmospora vasinfecta* E.F. Sm. | fusarium-like | AY381155 | AY381155 |
| *Neocosmospora ornamentata* M.A.F. Barbosa | fusarium-like | AF178413 | AF178382 |
| *Neonectria ditissima* (Tul. & C. Tul.) Samuels & Rossman | cylindrocarpon-like | HM364298 | HM364311 |
| *Pseudocosmospora eutypae* C. Herrera & P. Chaverri | acremonium-like | KC291735 | KC291766 |
| *Pseudocosmospora rogersonii* C. Herrera & P. Chaverri | acremonium-like | KC291729 | KC291780 |
| *Pseudonectria pachysandricola* B.O. Dodge | volutella-like | JF832658 | JF832715 |
| *Pseudonectria rousseliana* (Mont.) Wollenw. | volutella-like | JF937565 | JF937575 |
| *Roumegueriella rufula* (Berk. & Broome) Malloch & Cain | gliocladium-like | - | GQ505999 |
| *Rubrinectria olivacea* (Seaver) Rossman & Samuels | nalanthalama-like | AY554219 | AY554244 |
| *Selinia pulchra* (G. Winter) P. Karst. | acremonium-like | HM484859 | GQ505992 |
| *Viridispora diparietispora* (J.H. Mill., Giddens & A.A. Foster) Samuels & Rossman | penicillifer-like | HM484859 | AY489735 |
| *Varicosporella aquatica* Lechat & J. Fourn. JF09197 | fusarium-like | KP192669 | KP192671 |
| *Varicosporella aquatica* Lechat & J. Fourn. JF14074 | fusarium-like | KP192668 | KP192670 |

belongs to this "terminal clade" but is clearly distant from *Fusarium sensu Gibberella* and stands apart on a basal branch.

Based on phylogenetic, morphological and ecological divergences, our new taxon cannot be accommodated in any of the lineages given in Gräfenhan *et al.* (2011) nor in any known sexual morph, thus we propose the new genus *Varicosporella*.

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