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Revisiting *Salisapiliaceae*

R.M. Bennett[1,2], M. Thines[1,2,3*]

[1]Senckenberg Biodiversity and Climate Research Centre (SBik-F), Senckenberganlage 25, 60325 Frankfurt am Main, Germany
[2]Department of Biological Sciences, Institute of Ecology, Evolution and Diversity, Goethe University Frankfurt am Main, Max-von-Laue-Str. 9, 60438, Frankfurt am Main, Germany
[3]Integrative Fungal Research Cluster (IPF), Georg-Voight-Str. 14-16, 60325 Frankfurt am Main, Germany

*Corresponding author: m.thines@thines-lab.eu

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Salisapilia

Abstract: Of the diverse lineages of the Phylum *Oomycota*, saprotrophic oomycetes from the salt marsh and mangrove habitats are still understudied, despite their ecological importance. *Salisapiliaceae*, a monophyletic and monogeneric taxon of the marine and estuarine oomycetes, was introduced to accommodate species with a protruding hyaline apical plug, small hyphal diameter and lack of vesicle formation during zoospore release. At the time of description of *Salisapilia*, only few species of *Halophytophthora*, an ecologically similar, phylogenetically heterogeneous genus from which *Salisapilia* was segregated, were included. In this study, a revision of the genus *Salisapilia* is presented, and five new combinations (*S. bahamensis*, *S. elongata*, *S. epistomia*, *S. masteri*, and *S. mycoparasitica*) and one new species (*S. coffeyi*) are proposed. Further, the species description of *S. nakagirii* is emended for some exceptional morphological and developmental characteristics. A key to the genus *Salisapilia* is provided and its generic circumscription and character evolution in cultivable *Peronosporales* are discussed.

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INTRODUCTION

The Phylum *Oomycota* is a monophyletic group of fungal-like eukaryotes of the Kingdom *Straminipila* (Beakes & Thines 2017). Members of this group are saprotrophs, pathogens, or parasites of various plant and animal species in both aquatic and terrestrial environments. Habitats in which oomycetes seem to play a major role are the mangrove and salt marshes (Marano *et al.* 2016). Fallen senescent leaves of mangrove and salt marsh plants have proven to be rich in oomycete decomposers, which were originally subsumed as estuarine or marine *Phytophthora* (Fell & Master 1975, Pegg & Alcorn 1982, Nakagiri *et al.* 1989). Based on their environmental preference, they were later assigned to a morphologically diverse genus of their own, *Halophytophthora* (Ho & Jong 1990).

Halophytophthora was found to be polyphyletic on the basis of phylogenetic studies (Hulvey *et al.* 2010, Lara & Belbahri 2011). Based on recent phylogenetic analyses, there are only five known species of the *Halophytophthora s. str.*, namely *H. vesicula* (the type species of the genus), *H. avicenniae*, *H. batemanensis*, *H. polymorphica* (Hulvey *et al.* 2010, Lara & Belbahri 2011, Nigrelli & Thines 2013, Marano *et al.* 2014, Thines 2014), and the freshwater isolate, *H. fluviatilis* (Yang & Hong 2014) – the only known congener to date which was isolated from a freshwater biome. A few species of *Halophytophthora* were transferred to *Phytopythium* (*Phytopythium kandeliae*, basionym: *H. kandeliae*) (Thines 2014), and *Salispina* (*Salispina lobata*, basionym: *Phytophthora spinosa* var. *lobata*, and *Salispina spinosa*, basionym: *Phytophthora spinosa* var. *spinosa*) (Li *et al.* 2016); whereas some species were either associated with *Phytophthora* or *Salisapilia*, or are forming separate lineages (Li *et al.* 2016, Marano *et al.* 2014, Jung *et al*. 2017).

The genus *Salisapilia*, which type species, *Salisapilia sapeloensis*, was isolated from *Spartina alterniflora*, was described based on the following features contrasting to *Halophytophthora*: a small hyphal diameter, the formation of an apical or protruding hyaline plug, the absence of an evanescent or persistent vesicle during zoospore release, and homothallism. However, *Salisapilia nakagirii*, a homothallic species described by Hulvey *et al.* (2010), did not develop sporangia under the cultivation conditions applied, so the description of this species was based only on the morphology of gametangia and its phylogenetic placement within *Salisapiliaceae*. However, Hulvey *et al*. (2010) included only a small fraction of the species described in *Halophytophthora* in their dataset. Thus, it cannot be ruled out that several lineages not strongly supported as nested within *Halophytophthora* (Lara & Belbahri 2011) represent members of the genus *Salisapilia*. It was the aim of this study to close this knowledge gap by detailed phylogenetic and morphological analyses.

MATERIALS AND METHODS

Acquisition of strains and sporulation

Ex-type strains of *Halophytophthora* and *Salisapilia* were either acquired from NBRC in Japan or the Westerdijk Fungal Biodiversity Institute (formerly CBS-KNAW) in the Netherlands. Strains were

cultivated and maintained on clarified-vegetable juice agar (VJA) (Medium No. 15 NBRC, using Alnatura Gemüsesaft or Campbell V8 Juice) (http://www.nite.go.jp/en/nbrc/cultures/media/culture-list-e.html) with or without antibiotics: Nystatin (500 mg/mL), as well as Rifampicin (30 mg/mL) or Streptomycin (0.5 mg/mL).

All strains used in this study were tested for sporulation in saline solution at 0, 10, 20 and 30 promille (w/v) from 3–7-d-old cultures in 60 mm Petri plates. Plates were incubated in the dark at room temperature for 18–24 h or until sporangia were formed. Morphological characteristics were observed using a Motic AE31 trinocular inverted microscope (Motic, Wetzlar, Germany) and photos were taken using a Canon Digital Camera EOS 500D (Canon, Tokyo, Japan). Isolates were also grown on agarised media: Potato Carrot Agar (PCA), Peptone Yeast Glucose Agar (PYGA) and Potato Dextrose Agar (PDA) at room temperature (~20–25 °C) (Crous *et al.* 2009).

DNA extraction, PCR, and phylogenetic reconstruction

Cultures were grown on VJA plates at room temperature in a dark compartment. After 7–10 d, mycelia were harvested and subjected to DNA extraction following the method outlined in Bennett *et al*. (2017a). Extracted genomic DNA for all samples was amplified by PCR for the internal transcribed spacers (ITS), and the large nuclear ribosomal subunit (LSU). The primers ITS1-O (Bachofer 2004) and LR0 (Moncalvo *et al.* 1995) were used for the ITS region, while LR0R (Moncalvo *et al*. 1995) and LR6-O (Riethmüller *et al*. 2002) were used for the LSU region.

The 25 µL PCR reaction mixes contained 1× PCR Buffer, 0.2 mM dNTPs, 2.0 mM $MgCl_2$, 0.8 µg bovine serum albumin, 0.4 µM of each primer, 0.5 U *Taq* polymerase and 10–50 ng of DNA. Cycling conditions for the ITS included an initial denaturation at 94 °C for 4 min, followed by 36 cycles of denaturation at 94 °C for 40 s, annealing at 55 °C for 20 s, and elongation at 72 °C for 60 s; and a final elongation at 72 °C for 4 min. For the LSU region, initial denaturation was set at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 20 s, annealing at 53 °C for 20 s, and elongation at 72 °C for 2 min; and a final elongation at 72 °C for 7 min. All amplification reactions were carried out in an Eppendorf Mastercycler Pro equipped with a vapoprotect lid (Eppendorf AG, Hamburg, Germany).

PCR amplicons were sequenced by the laboratory centre of the Senckenberg Biodiversity and Climate Research Centre (SBiK-F, Frankfurt am Main, Germany) using the primer used in PCR. Sequences were analysed, assembled into contigs, and edited using Geneious v. 5.0.4 (Biomatters Ltd., USA). Edited contigs in FASTA format and ex-type sequences from the NCBI (https://www.ncbi.nlm.nih.gov/nuccore) and the *Phytophthora* database (http://www.phytophthoradb.org/) (Table S1) were uploaded to the TrEase webserver (http://www.thines-lab.senckenberg.de/trease/) for multiple sequence alignment using MAFFT, version 7 (Katoh *et al.* 2002). A primary phylogenetic tree computation using Minimum Evolution (ME) was generated using FastTree, version 1 (Price *et al.* 2009) as implemented on the TrEase webserver following the Generalized Time-Reversible (GTR) algorithm and 1 000 bootstrap replicates. Maximum Likelihood (ML) inference was done as the secondary tree using the FastTree, version 2 (Price *et al.* 2010) with the GTR algorithm model and 1 000 bootstrap replicates. A third phylogenetic reconstruction was done using Bayesian Inference (BI) as implemented in the TrEase webserver using MrBayes, version 3.2 (Ronquist *et al.* 2012). For Bayesian analysis the 6-GTR substitution model was used and 1 M generations were run, with trees sampled at every 10 000th generation, discarding the first 30 % of the sampled trees to ensure sampling always reached the stationary phase. After checking that there were no supported conflicts between the datasets, alignments of each locus were concatenated into a single alignment file using SequenceMatrix (Vaidya *et al.* 2010) and phylogenetic trees of concatenated alignments were generated following the above-mentioned protocols. Phylogenetic trees were viewed using MEGA v. 6 or 7 (Tamura *et al*. 2013).

Ancestral state reconstruction for papilla and hyaline apical plug

The ancestral state reconstruction of the papilla and the hyaline apical plug was done using observed or recorded characteristics for *Halophytophthora* (Anastasiou & Churchland 1969, Gerrettson-Cornel & Simpson 1984), *Salisapilia* (Table 1), and other members of *Peronosporaceae* (e.g. *Phytophthora*, *Phytopythium*, and *Pythium*) (van der Plaats-Niterink 1981, de Cock *et al.* 1987, Paul 1987, Erwin & Ribeiro 1996, Paul *et al*. 1999, Paul 2000, Nechwatal & Oßwald 2003, Uzuhashi *et al*. 2010, Kroon *et al.* 2012, de Cock *et al.* 2015). The traits were mapped on the Bayesian phylogeny of the concatenated dataset using Mesquite v. 3.2, and the likelihood ancestral reconstruction algorithm (Maddison & Maddison 2018) was run using the following the character data: (0) Papilla (P) forming an apical plug (AP); (1) P not forming an AP; (2) Semi-papilla (SP) forming an AP; (3) SP not forming an AP; (4) Non-papillate; and, "?" when sporangial germination was neither reported nor observed.

RESULTS

Phylogenetic reconstructions

According to the phylogeny based on concatenated sequences of ITS and LSU in this study (Fig. 1), strains *Halophytophthora bahamensis* NBRC 32556 (Fig. 2), the strain NBRC 32557 (Fig. 4), which was named as *H. bahamensis*, but is not conspecific with the ex-type strain, *H. elongata* NBRC 100786 (Fig. 3), *H. epistomia* NBRC 32617 (Fig. 5), *H. masteri* NBRC 32604 (Fig. 6), and *H. mycoparasitica* NBRC 32966 (= NBRC 32967) (Fig. 7) clustered with other members of the *Salisapiliaceae* with strong to maximum support (Fig. 1). Further, these strains were distinct from *S. nakagirii* LT6456 (= CBS 127947) (Fig. 8), *S. sapeloensis* LT6440 (= CBS 127946) (Fig. 9), and *S. tartarea* CBS 208.95 (Fig. 10).

Morphology

Halophytophthora bahamensis NBRC 32556 (Fig. 2E–F), *H. elongata* NBRC 100786 (Fig. 4C–D), *H. epistomia* NBRC 32617 (Fig. 5E–F), *H. masteri* NBRC 32604 (Fig. 6C–D), and *H. mycoparasitica* NBRC 32966 (= NBRC 32967) (Fig. 7E–F), were all forming a distinct hyaline apical plug at the apex of the discharge tube similar to *S. sapeloensis* CBS 127946 (Fig. 9E) and *S. tartarea* CBS 208.95 (Fig. 10E–F). The apical hyaline plug was indistinct in *S. nakagirii* CBS 127947 (Fig. 8F–G). The shape of sporangia varied among species. The mode of zoospore release was either directly through a discharge tube or by the formation

Table 1. Morphological comparison of *Salisapilia* spp. Measurements for sporangia are given as (min.–)average_minus_SD–SD–average_plus_SD(–max.).

Structure	***S. sapeloensis*** (Hulvey *et al*. 2010)	***S. coffeyi*** (This study)	***S. bahamensis*** (Fell & Master 1975)	***S. elongata*** (Ho *et al*. 2003)	***S. epistomia*** (Fell & Master 1975, Ho *et al*. 1990[a])	***S. nakagirii*** (Hulvey *et al*. 2010, This study[b])	***S. masteri*** (Nakagiri *et al*. 1994)	***S. mycoparasitica*** (Fell & Master 1975)	***S. tartarea*** (Nakagiri *et al*. 1994)
Hyphal diam (µm)	1–2	1–3	1–3	3–9	2–4	1–2	2–10	2–9	1–3(–9)
Septa	Occurs at maturity	Occurs at maturity	Occurs at maturity	Occurs at maturity	Occurs at maturity	Occurs at maturity	Occurs at maturity	Develop numerous septa with age	Non-septate, or septate with age
Branching pattern	Branched or unbranched	Branched or unbranched	Branching, rare	Unbranched	Branching, rare	Branched or unbranched	Branched or unbranched	Branched or unbranched	Unbranched or branched
Sporangiogenic hyphae	Undifferentiated to vegetative hyphae	Undifferentiated to vegetative hyphae	Undifferentiated to vegetative hyphae	Undifferentiated to vegetative hyphae	Undifferentiated to vegetative hyphae	Undifferentiated to vegetative hyphae	Undifferentiated to vegetative hyphae	Undifferentiated to vegetative hyphae	Undifferentiated to vegetative hyphae
Sporangia									
Size (µm)	34–97 (av. 59)	44.05–107.33 × 6.51–16.92 (av. 74.01 × 10.32)	26–119 × 19–43 (av. 61 × 28) 39–97 × 14–31 (av. 68 × 23)	115–530 × 32–64	43–184 × 56–107 (av. 127.6 × 63.3)	81.5–205.25 × 32.25–113 (av. 136.88 × 66.43) [b]	26–92 × 18–91 (av. 64 × 62.6)	26–131 × 14–111 (av. 82 × 61)	20–104 × 18–96 (av. 55.6 × 47.6)
Discharge tube size (µm)	6–18	4.81–13 × 2.58–3.94 (av. 9.07 × 3.12)	3–7	-	10–51 × 9–10	6.18–18.02 × 4.3–8.7 (av. 11.95 × 6.63) [b]	5–28 × 6–10	Av. 22, tapering	10–22 × 4–8
Apical plug size (µm)	3–8, protruding	~1–3	1–2 (width)	10 × 5.6	14–90 × 9–10	Indistinct [b]	5–24 × 5–14	5–15 × 3–10	11–29 × 5–8
Surface	Smooth, partly rough	Smooth	Smooth	Smooth	Smooth	Non-smooth [b]	Smooth	Denticulate, few spines	Smooth
Vacuole	Absent	Present	Present	Absent	Absent	Absent [b]	Absent	Absent	Absent
Basal plug	Present in some, hyaline	Present, hyaline	Present, hyaline	Present, hyaline	Present, hyaline	Present, hyaline [b]	Present, hyaline	Present, hyaline	Present, hyaline
Detachment	Non-caducous	Non-caducous	Non-caducous	Non-caducous	Non-caducous	Non-caducous [b]	Non-caducous	Non-caducous	Non-caducous
Shape	Ovoid, obpyriform	Bursiform to often narrowly bursiform, obpyriform to narrowly-elongate and obclavate; Setiform appendages, absent	Highly variable, bursiform, multi-lobed, obclavate, obpyriform; Setiform appendages, present, aseptate to septate	Obovoid, obclavate, bursiform, cylindrical, elongated	Lageniform, obpyriform	Ovoid, globose, obpyriform [b]	Spherical, ovoid, obpyriform	Obnapiform	Spherical, ovoid to obpyriform

Table 1. (Continued).

Structure	***S. sapeloensis*** (Hulvey *et al*. 2010)	***S. coffeyi*** (This study)	***S. bahamensis*** (Fell & Master 1975)	***S. elongata*** (Ho *et al*. 2003)	***S. epistomia*** (Fell & Master 1975, Ho *et al*. 1990[a])	***S. nakagirii*** (Hulvey *et al*. 2010, This study[b])	***S. masteri*** (Nakagiri *et al*. 1994)	***S. mycoparasitica*** (Fell & Master 1975)	***S. tartarea*** (Nakagiri *et al*. 1994)
Zoospore release	Zoospores exit through the discharge tube after ejection of the apical plug	Zoospores exit through the discharge tube after ejection of the apical plug	Zoospores exit through the discharge tube after ejection of the apical plug	Zoospores exit through the discharge tube after ejection of the apical plug	Zoospores exit through the discharge tube after ejection of the apical plug	Zoospores are released in a vase-like discharge vesicle [b]	The apical plug is extruded, and a tubular vesicle is ejected. Zoospores exit through the opening	Zoospores exit through the discharge tube after ejection of the apical plug. Plug evanesces rapidly.	Zoospores exit through the discharge tube after ejection of the apical plug
Vesicle	Absent	Absent	Absent	Present, tubular	Absent	Present, vase-like [b]	Present, tubular	Absent	Absent
Oogonia									
Size (µm)	35–60, 49	Not observed	Not observed	Not observed	34–40, 37 [a]	33–48, 39	Not observed	Not observed	33–66
Surface	Smooth				Smooth [a]	Smooth			Smooth
Shape	Spherical, ovoid				Spherical [a]	Spherical			Spherical, tapered base
Oospore	Plerotic	Not observed	Not observed	Not observed	Plerotic [a]	Plerotic	Not observed	Not observed	Aplerotic
Size (µm)	28–56, 48				- [a]	28–44			24–62
Wall (µm)	2–9				4–5 [a]	1–7			3–10
Antheridia	Paragynous	Not observed	Not observed	Not observed	Paragynous [a]	Paragynous	Not observed	Not observed	Diclinous, paragynous
Size (µm)	2–9				6–24 × 2–8, 12–6 [a]	3–10			4–10
Shape	Simple, lobed or branched				- [a]	Club-shaped, lobed			Partly enwraps oogonia

- no data provided.

[a] Data from Ho *et al.* (1990).

[b] Characteristics of *S. nakagirii* observed in this study.

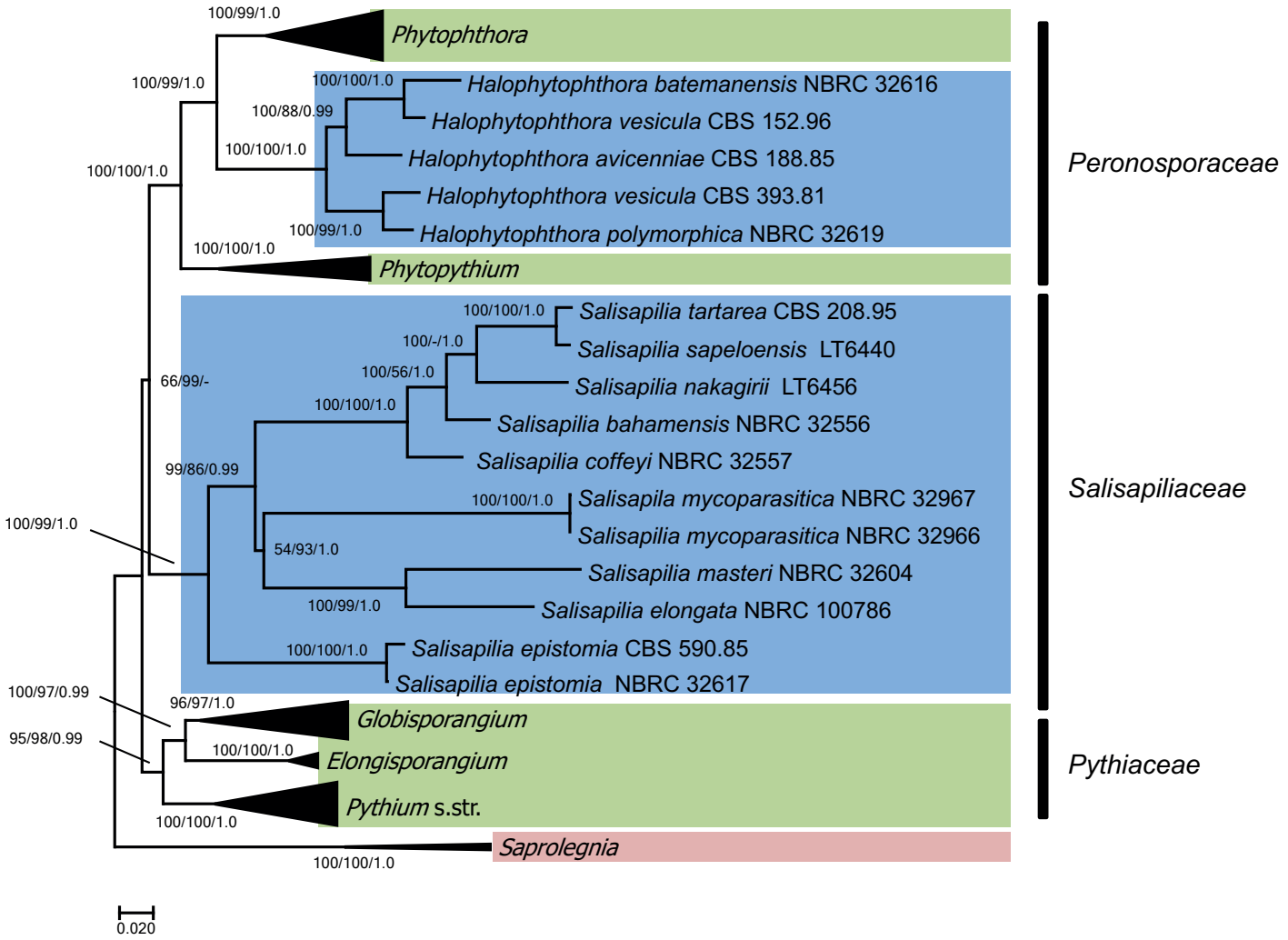


Fig. 1. Phylogenetic tree based on concatenated ITS and LSU alignments based on Minimum Evolution (ME) inference, with bootstrap support values from ME and Maximum Likelihood, as well as posterior probabilities from Bayesian Inference, in the respective order. (-) indicates support below 50 % (bootstrap) or 0.8 (posterior probability), or alternating but not strongly supported topology (support below 70 % bootstrap or 0.9 posterior probability). The scale bar indicates the number of nucleotide substitutions per site.

of an evanescent vesicle. A summary of the morphology of *Salisapilia* spp. is presented in Table 1.

The shape of sporangia of NBRC 32557 (Fig. 3E–H) was different from the ex-type culture of *H. bahamensis* (NBRC 32556) to which it had been assigned. The sporangia of the ex-type culture were bursiform, obclavate, obpyriform to highly variable and multi-lobed; whereas strain NBRC 32557 has narrowly bursiform, obpyriform to narrowly-elongated and obclavate sporangia. Variation of the shape of the sporangium was pronounced for *H. bahamensis* NBRC 32556, and some sporangia bore two discharge tubes. In contrast, NBRC 32557 always formed a single discharge tube and its sporangial shape was more stable. The strain NBRC 32557 releases its zoospore after extrusion of the small hyaline apical plug from the discharge tube. Zoospores are released directly out from the discharge pore and a vesicle was absent. After the sporangia had released zoospores, an umbonate or elevated basal plug was observed. Gametangia and chlamydospores were not observed for the strain NBRC 32557.

The ancestral trait reconstruction (Fig. 11) of the papilla and hyaline apical plug suggested that papillate and semi-papillate sporangia were putatively derived from non-papillate sporangia. Further, a sporangium with papilla forming a discharge tube and a hyaline apical plug appeared to be a synapomorphic trait for *Salisapilia*.

Taxonomy

Based on the presented phylogenetic and morphological analyses of the different taxa included in this study, the genus *Salisapilia* contains several additional species previously treated as members of *Halophytophthora*. As a consequence, five new combinations (i.e. *S. bahamensis*, *S. elongata*, *S. epistomia*, *S. masteri*, and *S. mycoparasitica*) and a new species (*S. coffeyi*) for the genus *Salisapilia* are introduced here. Measurements for sporangia are given as (min.–)average_minus_SD–SD–average_plus_SD(–max.).

Salisapilia Hulvey *et al.*, *Persoonia* **25**: 112 (2010), ***emend.*** MycoBank MB517465.

Colonies on VJ agar stellate, indistinct, petalloid; *aerial hyphae* limited; *vegetative hyphae* with regular branching, septae occur at maturity; *hyphal swellings* present, shape variable; *sporangia* produced in saline water, shape obpyriform, ovate, obovate, elongate to irregular; *proliferation* often external; *dehiscence or*

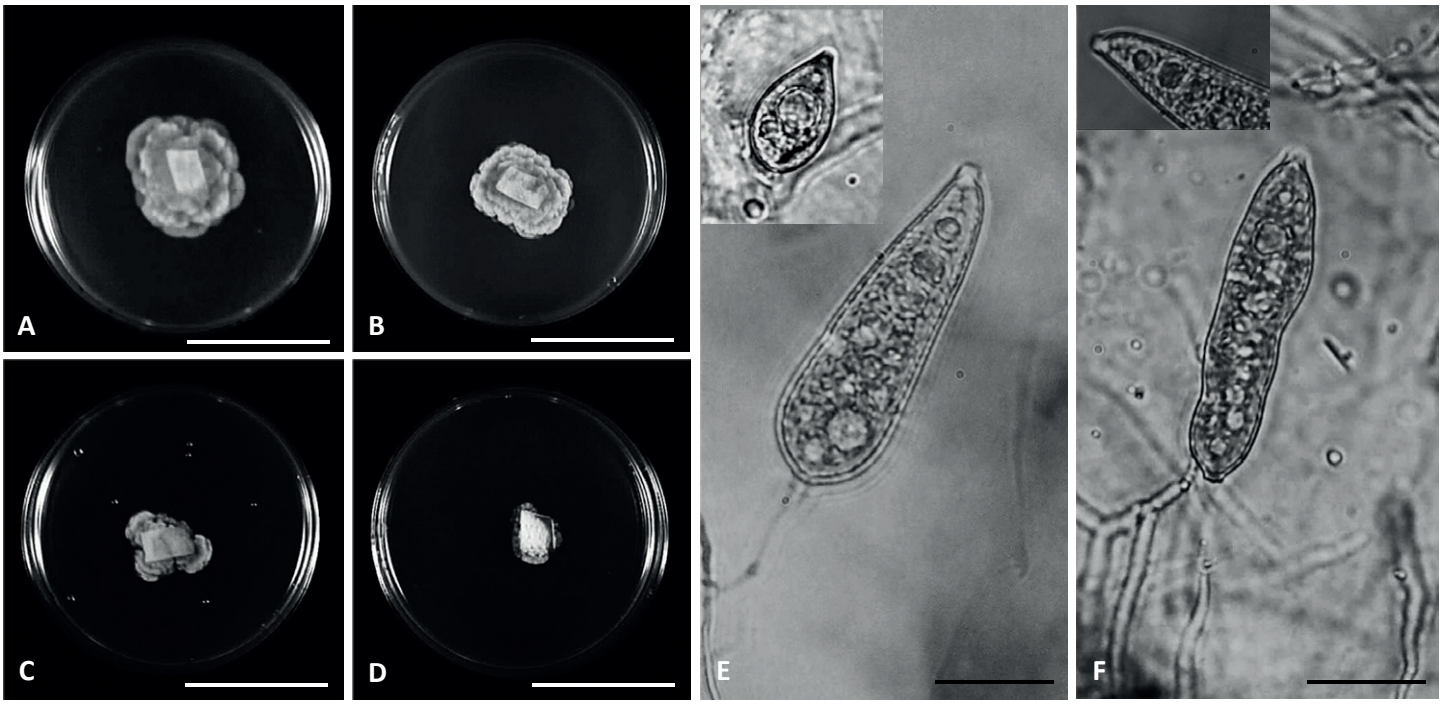


Fig. 2. *Salisapilia bahamensis* NBRC 32256. Colony patterns on **A.** Vegetable juice agar. **B.** Potato carrot agar. **C.** Peptone yeast glucose agar. **D.** Potato dextrose agar. **E, F.** Mature, vacuolated sporangia, (inset figure, sporangium showing hyaline apical plug). Scale bars: A–D. = 30 mm, E, F. = 20 µm.

discharge tube present, usually with a hyaline plug at the apex; *zoospore release* occurs after dehiscence of the hyaline plug at the apex of the discharge tube, or zoospores exit directly from the discharge pore or through an evanescent tubular to vase-like vesicle; *gametangia* observed for some species; *antheridial attachment* paragynous, diclinous; *oogonia* smooth-walled; *oospores* spherical to ovoid, terminal or intercalary.

Type species: *Salisapilia sapeloenesis* Hulvey *et al*.

Synopsis of species included in *Salisapilia*

Salisapilia bahamensis (Fell & Master) R. Bennett & Thines, ***comb. nov*.** MycoBank MB823448. Fig. 2.

Basionym: *Phytophthora bahamensis* Fell & Master, *Canad. J. Bot*. **53**: 2913. 1975. MB320472.
Synonym: *Halophytophthora bahamensis* (Fell & Master) Ho & Jong, *Mycotaxon* **36**: 381. 1990. MB126014.

Typus: **Holotype** ATCC 28296, cultures ex-type = CBS 586.85 = IMI 330182 = NBRC 32556 , voucher ex ex-type strain NBRC3256 = USTH 014147, University of Santo Tomas Herbarium, Manila, Philippines.

Distribution: Bahamas, Philippines.

Salisapilia coffeyi R. Bennett & Thines, ***sp. nov*.** MycoBank MB823342. Fig. 3.

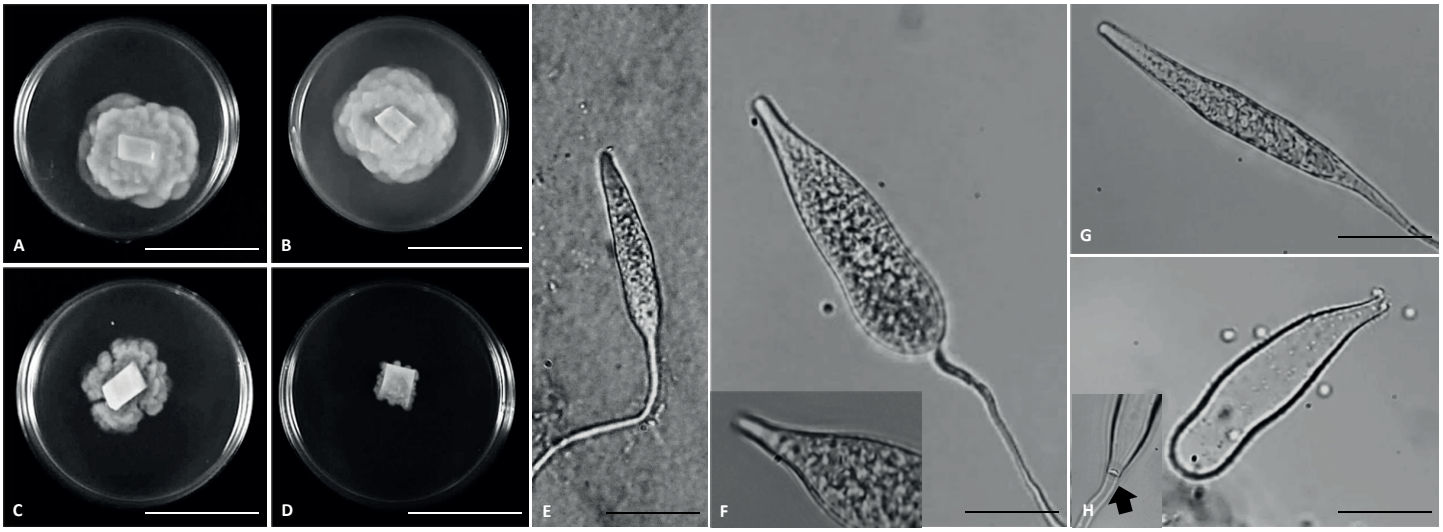


Fig. 3. *Salisapilia coffeyi* NBRC 32557. Colony patterns on **A.** Vegetable juice agar. **B.** Potato carrot agar. **C.** Peptone yeast glucose agar. **D.** Potato dextrose agar. **E.** Immature sporangium. **F, G.** Mature sporangia, (inset figure) sporangium showing hyaline apical plug. **H.** Empty sporangium; inset, elevated or umbonate basal plug. Scale bars: A–D = 30 mm, E–H = 20 µm.

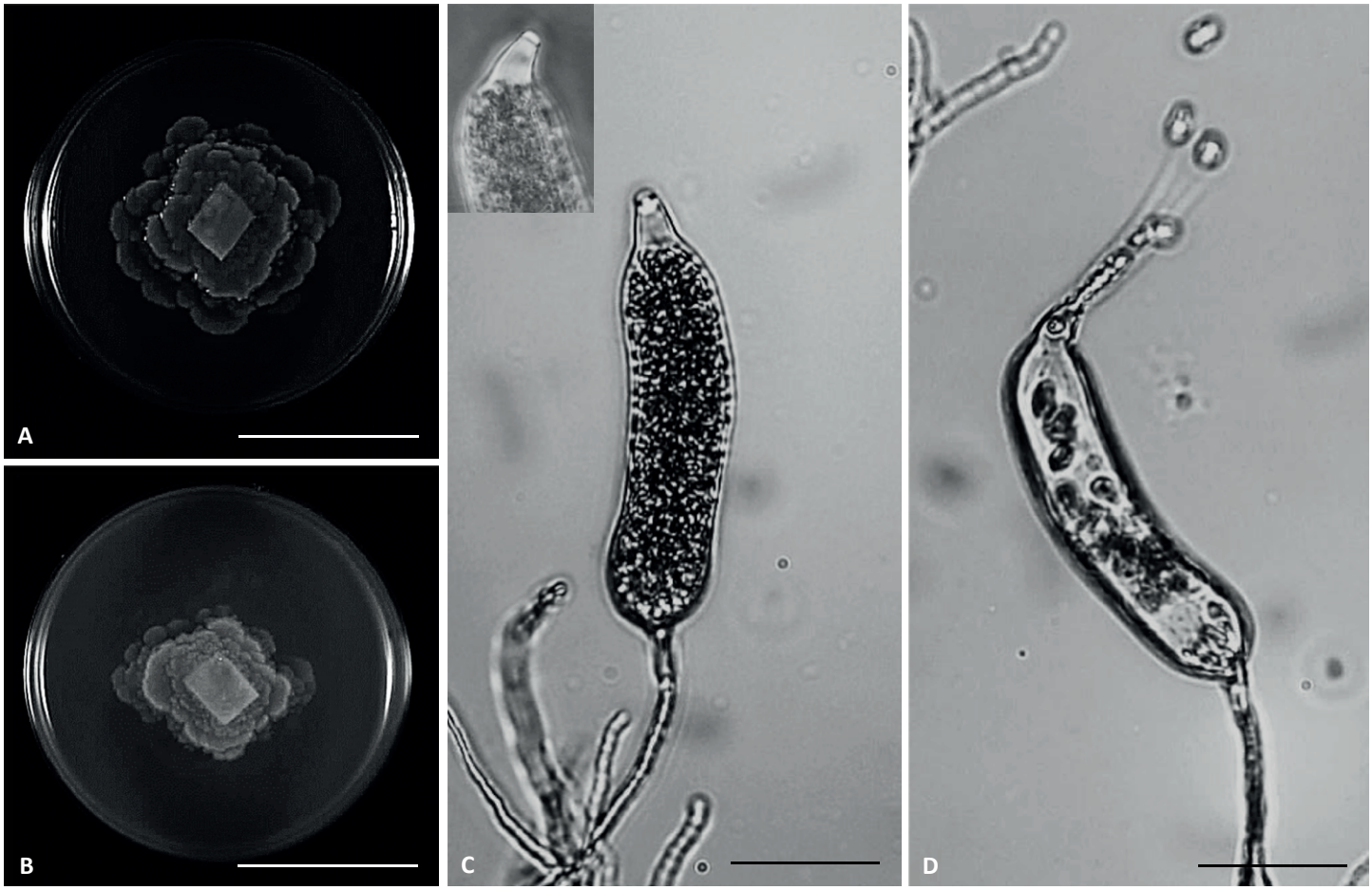


Fig. 4. *Salisapilia elongata* NBRC 100786. Colony patterns on **A.** Vegetable juice agar. **B.** Potato carrot agar. **C.** Mature sporangium; hyaline apical plug (inset). **D.** Mature sporangium releasing zoospores through a tubular vesicle. Scale bars: A, B = 30 mm, C, D. = 20 µm.

Etymology: Dedicated to Michael Coffey for his contributions to the study of cultivable oomycetes.

Colony pattern on vegetable juice agar and potato carrot agar petaloid to rosette-like; *vegetative hyphae* highly branched, with septae at maturity; *sporangiogenic hyphae* undifferentiated from vegetative hyphae, bearing a single sporangium; *sporangia* smooth and thin-walled, with vacuoles, non-deciduous, (25.5–) 44–74–107(–126) × (4–)6.5–10.5–17(–19.5) µm, bursiform, narrowly bursiform, obpyriform to narrowly-elongate and obclavate, mostly with a tapering apex; *dehiscence tube* present; 5–13 × 2.5–4 µm; *dehiscence plug* present, hyaline, 1–3 µm in diameter; *basal plug* present, hyaline, raised to umbonate; *proliferation* external; *zoospore release* directly through the dehiscence tube after ejection of the dehiscence plug; *vesicle* not observed; *chlamydospores* not observed; *gametangia* not observed.

Typus: **Bahamas**, Conception Island, isolated from decaying leaf of *Rhizophora mangle*, Oct. 1972, *J.W. Fell & I.M. Master* (**holotype** USTH 014149, ex-type culture NBRC 32557, GenBank: ITS, MF979510; LSU, MF979503).

Salisapilia elongata (Ho & Chang) R. Bennett & Thines, ***comb. nov.*** MycoBank MB823450. Fig. 4.
Basionym: *Halophytophthora elongata* Ho & Chang, *Mycotaxon* **85**: 417. 2003. MB372647.

Typus: **Holotype** 17II2001, Y.M. Ju, Institute of Botany, Academica Sinica, Taipei, Taiwan, cultures ex-type BCRC 33983 = NBRC 100786.

Distribution: Taiwan, Philippines.

Salisapilia epistomia (Fell & Master) R. Bennett & Thines, ***comb. nov.*** MycoBank MB823449. Fig. 5.
Basionym: *Phytophthora epistomium* Fell & Master, *Canad. J. Bot*. **53**: 2913. 1975. MB320475.
Synonym: *Halophytophthora epistomia* (Fell & Master) Ho & Jong, *Abstracts IMC-4*, Regensburg, 1990. MB126016.

Typus: **Holotype** ATCC 28293, cultures ex-type IMI 330183 = CBS 590.85 = NBRC 32617, voucher ex ex-type strain NBRC32617 = USTH 014147, University of Santo Tomas Herbarium, Manila, Philippines.

Distribution: USA.

Salisapilia masteri (Nakagiri & Newell) R. Bennett & Thines, ***comb. nov.*** MycoBank MB823447. Fig. 6.
Basionym: *Halophytophthora masteri* Nakagiri & Newell, *Mycoscience* **35**: 227. 1994. MB363473.

Typus: **Holotype** NBRC H-12169, NITE Biological Resource Center, Japan, cultures ex-type IFO 32604 = ATCC 96906 = CBS 207.95 = NBRC 32604.

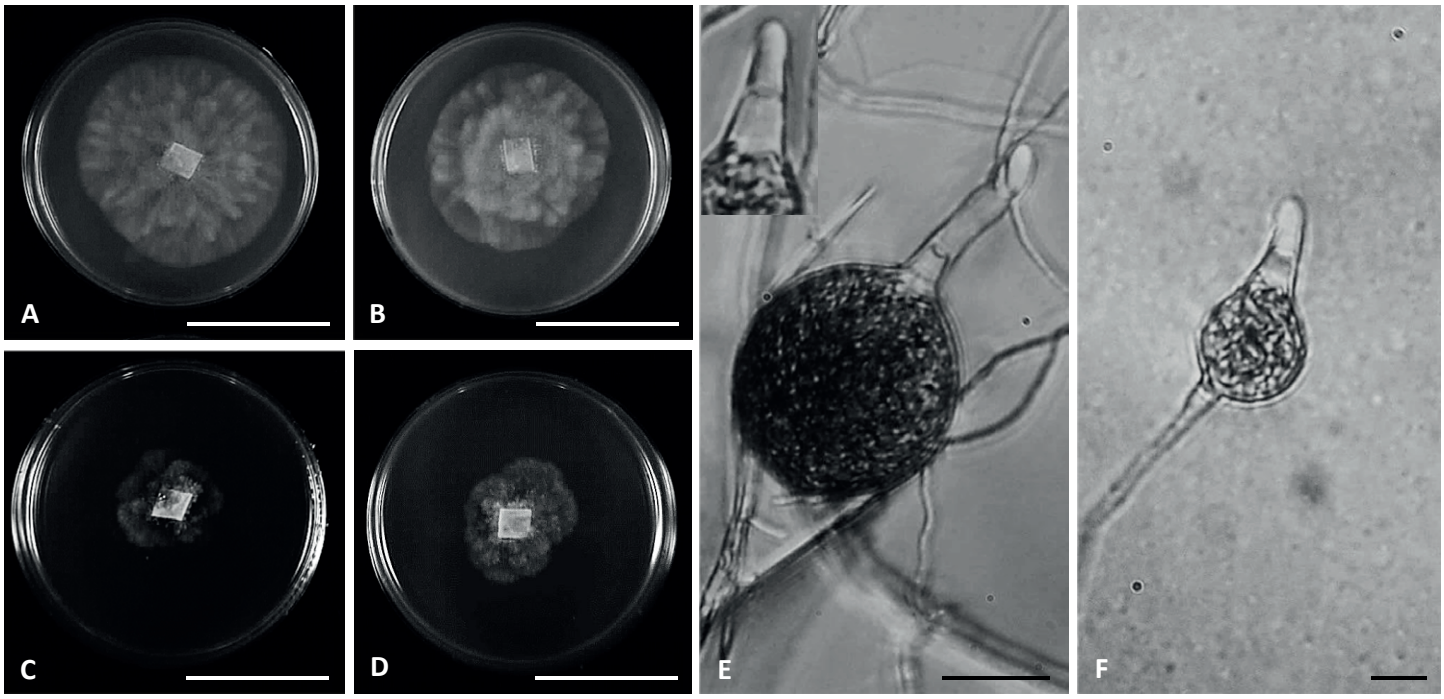


Fig. 5. *Salisapilia epistomia* NBRC 32617. Colony patterns on **A.** Vegetable juice agar. **B.** Potato carrot agar. **C.** Peptone yeast glucose agar. **D.** Potato dextrose agar. **E–F.** Mature sporangia; hyaline apical plug (inset, Fig. 4E). Scale bars: A–D. = 30 mm, E, F. = 20 µm.

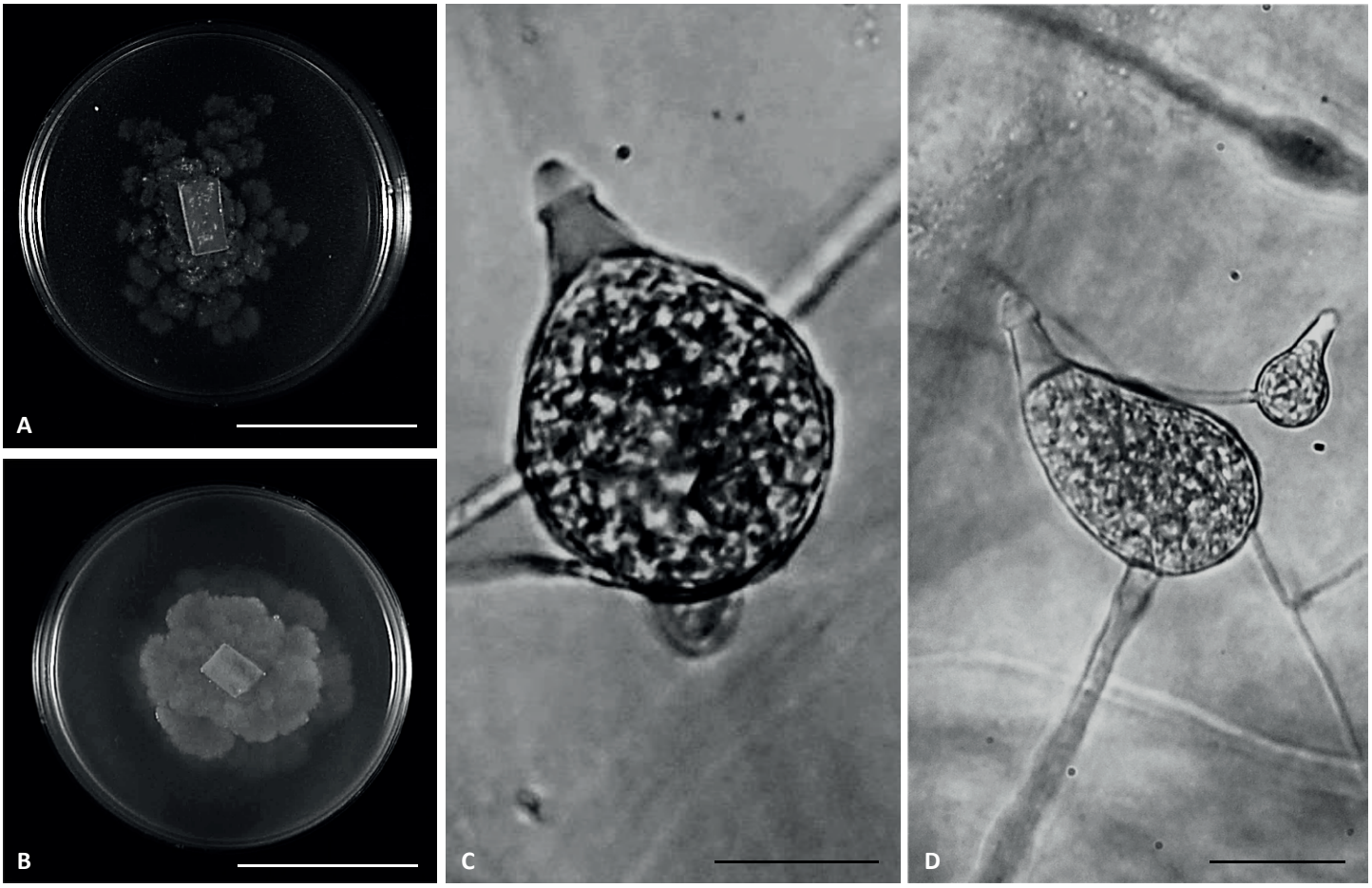


Fig. 6. *Salisapilia masteri* NBRC 32604. Colony patterns on **A.** Vegetable juice agar. **B.** Potato carrot agar. **C, D.** Mature sporangia. Scale bars: A, B = 30 mm, C, D = 20 µm.

Distribution: Bahamas.

Salisapilia mycoparasitica (Fell & Master) R. Bennett & Thines, ***comb. nov.*** MycoBank MB824539. Fig. 7.

Basionym: *Phytophthora mycoparasitica* Fell & Master, *Canad. J. Bot.* **53**: 2916. 1975. MB320485.
Synonym: *Halophytophthora mycoparasitica* (Fell & Master) Ho & Jong, *Mycotaxon* **36**: 381. 1990. MB126017.

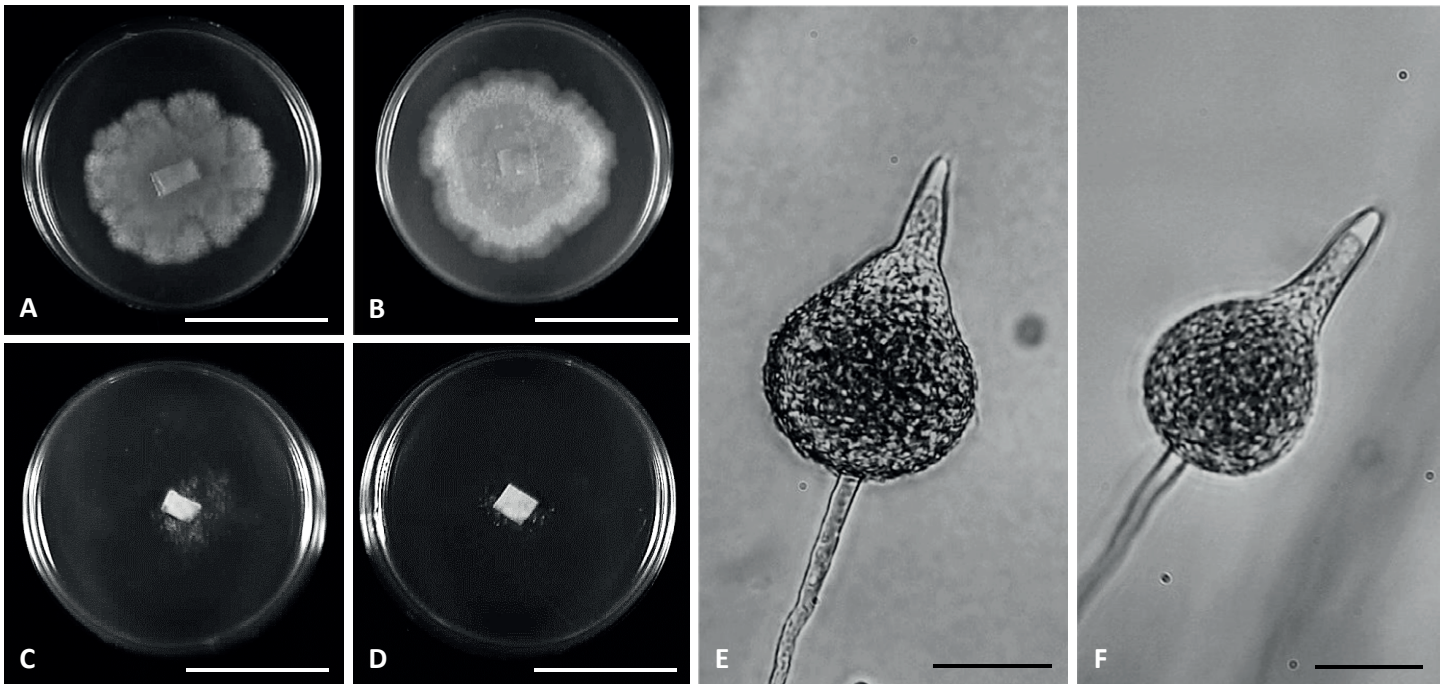


Fig. 7. *Salisapilia mycoparasitica* NBRC 32966. Colony patterns on **A.** Vegetable juice agar. **B.** Potato carrot agar. **C.** Peptone yeast glucose agar. **D.** Potato dextrose agar. **E, F.** Mature sporangia. Scale bars: A–D = 30 mm, E–F = 20 µm.

Typus: **Holotype** ATCC 28292 (discarded), (**lectotype** designated here fig. 16, *Canad. J. Bot.* **53**: 2918 (1975), MBT386266; **epitype** designated here NBRC H-12221, MBT386249, ex-epitype culture NBRC 32966, NITE Bioresource Centre, Japan).

Other materials examined: NBRC 32967, NITE Bioresource Centre, Tokyo Japan.

Distribution: Malaysia, Japan.

Notes: The designated type, ATCC 28292, is no longer available, and no additional specimen was deposited in any recognised fungarium at the time *Phytophthora mycoparasitica* was proposed. Since neither inactive nor living material appears to remain from the collection of Fell & Master (1975), fig. 16 from that publication is designated as the **lectotype**, the specimen NBRC H-12221 is designated as the **epitype** and NBRC 32996 (NBRC, Japan) as the **ex-epitype culture**.

Salisapilia nakagirii Hulvey *et al.*, *Persoonia* **25**: 113. 2010, ***emend.*** MycoBank MB517466. Fig. 8.

Typus: **Holotype** CBS H-20478, Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands, ex-type cultures CBS 127947 = NBRC 108757 = LT6456.

Distribution: USA.

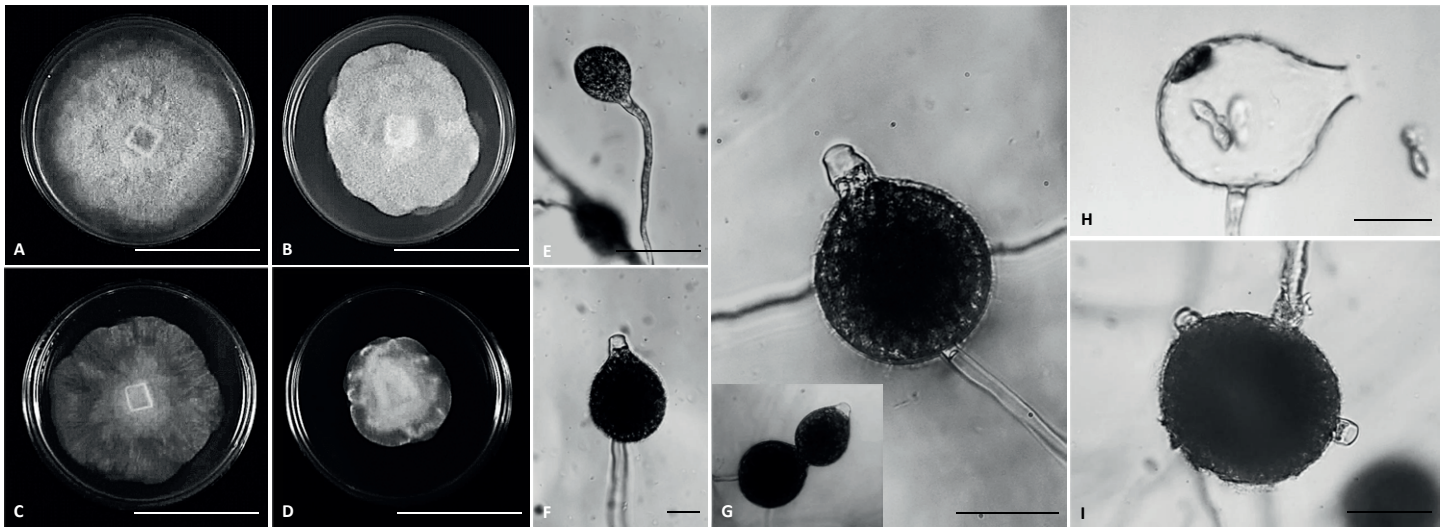


Fig. 8. *Salisapilia nakagirii* CBS 127947. Colony patterns on **A.** Vegetable juice agar. **B.** Potato carrot agar. **C.** Peptone yeast glucose agar. **D.** Potato dextrose agar. **E.** Immature sporangium. **F–I.** Mature sporangia, (inset figure) modified shape of a sporangium. **H.** Empty sporangium. **I.** Mature sporangium with two discharge tubes. Scale bars: A–D = 30 mm, E–I = 20 µm.

Colony pattern on Vegetable juice agar and potato carrot agar indistinct; stellate to rosette-like on peptone yeast agar; *hyphae* branched with *septae* at maturity; *sporangia* ovoid, globose to obpyriform, (26–)81.5–137–205(–231) × (11.5–)32–66.5–113(–133.5) µm; *dehiscence tube* present, filled with non-sporogenous protoplasmic mass, size 6–18.0 × 4.5–8.5 µm; *hyaline apical plug* indistinct; *sporangial wall* wrinkled in some sporangia; *basal plug* present in few sporangia; *proliferation* external; *zoospore release* through an evanescent vesicle; *vesicle* vase-shaped; *gametangia* present; *antheridia* diclinous, paragynous, club-shaped or lobed, 3–10 µm in length; *oogonia* hyaline, spherical, 33–48 µm; *oospores* 28–44 µm, hyaline, with a uniformly refractile ooplast vacuole; wall 1–7 µm.

Salisapilia sapeloenesis Hulvey *et al*., *Persoonia* **25**: 113. 2010. MycoBank MB517467. Fig. 9.

Typus: **Holotype** CBS H-20477, Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands, ex-type cultures NBRC 108756 = LT6440 = CBS 127946.

Distribution: USA.

Salisapilia tartarea (Nakagiri & Newell) Hulvey, Nigrelli, Telle, Lamour & Thines, ***comb. nov.*** MycoBank MB517468. Fig. 10.
Basionym: *Halophytophthora tartarea* Nakagiri & Newell, *Mycoscience* **35**: 224. 1994. MB363474.
Synonym: Salisapilia tartarea (Nakagiri & S.Y. Newell) Hulvey *et al*., *Persoonia* **25**: 114. 2010. Nom. inval., Art. 41.5 (Melbourne).

Typus: **Holotype** NBRC H-12168, NITE Biological Resource Center, Japan, ex-type cultures NBRC 32606 = ATCC 96905 = CBS 208.95.

Distribution: USA.

Note: Invalidly proposed in *Persoonia* **25**: 114 (2010), as the date of publication of the basionym was omitted.

DISCUSSION

Estuarine and saltmarsh oomycetes are a diverse group of heterokonts that recently received much attention. Members of this ecological group are in the genera *Halophytophthora* (Ho & Jong 1990), *Phytopythium* (Bala *et al.* 2010), *Salisapilia* (Hulvey *et al*. 2010), *Salispina* (Li *et al*. 2016), and *Calycofera* (Bennett *et al.* 2017b). Of these taxa, *Halophytophthora* and *Salisapilia* were regarded to be in need of taxonomic revision (Nigrelli & Thines 2013, Marano *et al.* 2014, Beakes & Thines 2017), and the latter genus was resolved in this study.

Members of the monogeneric *Salisapiliaceae* are characterised by a small hyphal diameter, a protruding hyaline apical plug, and the absence of a vesicle during zoospore release (Hulvey *et al*. 2010). However, the sporangia of *S. nakagirii* CBS 127947 were reported to release zoospores into a semi-persistent vesicle and that the typical hyaline apical plug was absent (Marano *et al.* 2014). These observations are largely confirmed in this study, demonstrating that *S. nakagirii* has an exceptional mode of sporulation, even though we classify the vesicle as evanescent, as the structure is not readily observable sometime after zoospore release. Marano *et al.* (2014) reached the conclusion that *S. nakagirii* is papillate; however, it appears that the discharge tube is rather filled with some non-sporogenous mass, which is protoplasmic of origin, and its distalmost part is probably homologous to the apical plug observed in other species of *Salisapilia*, giving the impression of a papilla (Gerrettson-Cornell & Simpson 1984).

Hulvey *et al*. (2010) suggested that the intricacies of zoospore release might be of phylogenetic relevance and, thus, useful for resolving some systematic complexities of saprotrophic oomycetes. However, the example of *S. nakagirii*,

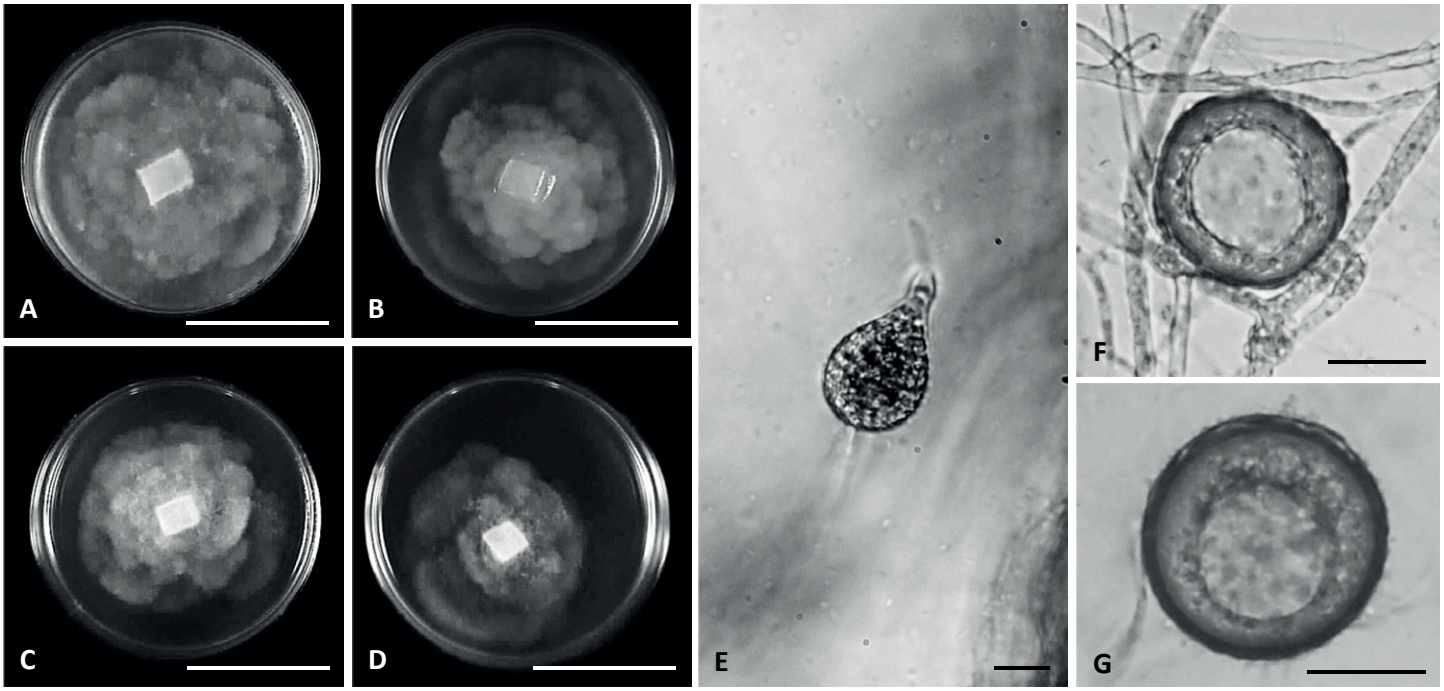


Fig. 9. *Salisapilia sapeloensis* CBS 127946. Colony patterns on **A.** Vegetable juice agar. **B.** Potato carrot agar. **C.** Peptone yeast glucose agar. **D.** Potato dextrose agar. **E.** Mature sporangium. **F, G.** Oogonia. Scale bars: A–D = 30 mm, E–G = 20 µm.

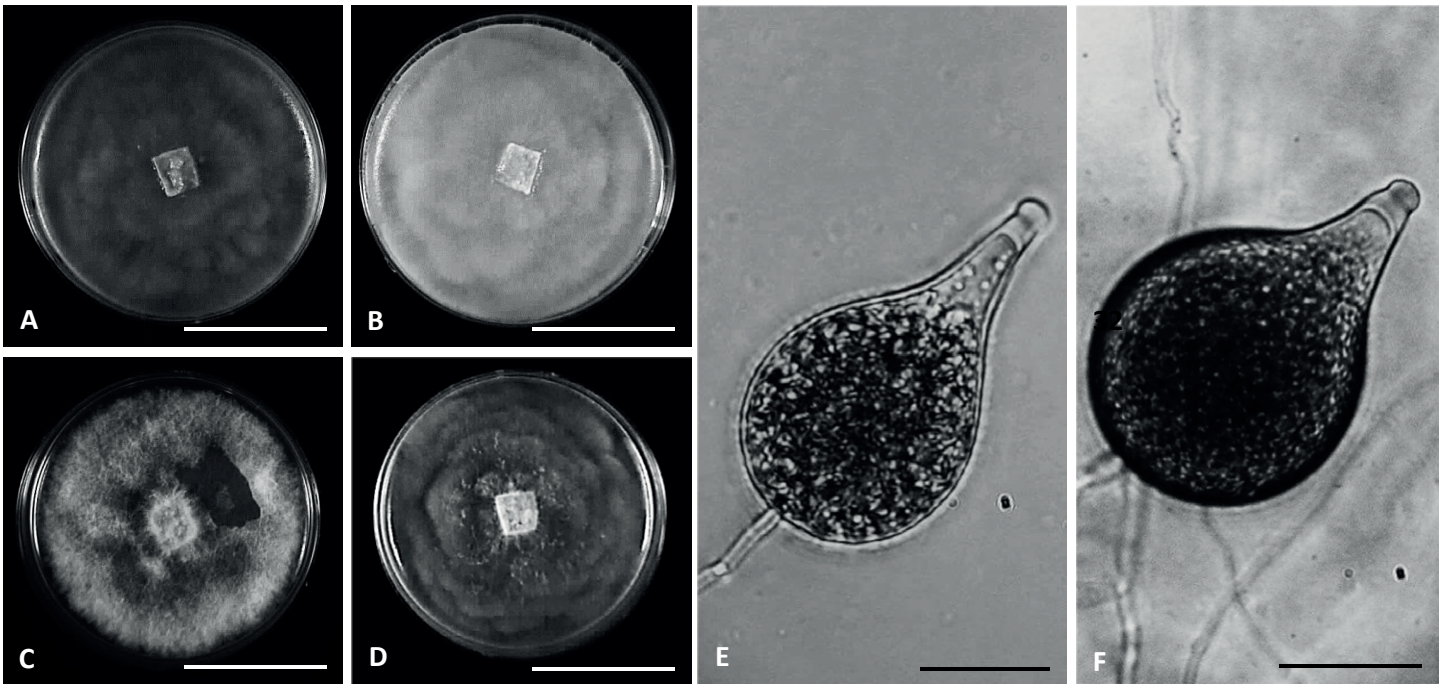


Fig. 10. *Salisapilia tartarea* CBS 208.95. Colony patterns on **A.** Vegetable juice agar. **B.** Potato carrot agar. **C.** Peptone yeast glucose agar. **D.** Potato dextrose agar. **E, F.** Mature sporangia. Scale bars: A–D = 30 mm, E, F = 20 µm.

in line with observations on other species of saprotrophic or hemibiotrophic *Peronosporales*, demonstrates the necessity to combine morphological and ontogenetic data with molecular phylogenetics, as the process of zoospore release might be variable within genera (Gisi *et al*. 1979, Gerrettson-Cornell & Simpson 1984, Bala *et al.* 2010, de Cock *et al*. 2015). Difficulty in finding clade specific-synapomorphies is common in saprotrophic and hemibiotrophic oomycetes. A good example of this is the paraphyletic genus *Phytophthora*, where the classification by Waterhouse (1963) or Stamps *et al*. (1990) does not reflect natural groupings resolved by multigene-phylogenies (Cooke *et al.* 2000, Kroon *et al*. 2004, Blair *et al.* 2008, Runge *et al*. 2011). *Halophytophthora elongata* (Ho *et al.* 2003) and *H. masteri* (Nakagiri *et al*. 1994) formed elongated to tubular-shaped, discharge-tube-like vesicles, similar to the vase-like vesicle of *S. nakagirii* prior to zoospore release.

While the absence of a vesicle does not seem to be a characteristic useful for delineating *Salisapilia*, the hyaline apical plug is a feature that seems to be of more diagnostic value. It is a usually readily observable cone-like structure nested at and eventually protruding from the apex of the discharge tube. Prior to zoospore release, the hyaline plug is ejected or detached from the discharge tube (Nakagiri *et al.* 1994, Ho *et al*. 2003) giving way for the release of zoospores. The only species in which this feature does not manifest is *S. nakagirii*. Haowever, variation in size of the hyaline apical plug is present among members of *Salisapilia* (Table 1). Based on the ancestral trait reconstruction analysis, it was observed that non-papillate sporangia appear as an ancestral trait to papillate and semi-papillate sporangia (Yang *et al*. 2017). In the present ancestral state reconstruction (Fig. 11), the absence of the hyaline apical plug seems to be a derived feature in *S. nakagirii,* and otherwise appears to be an exclusive synapomorphy for the genus *Salisapilia*.

Phylogenetically, *Salisapiliaceae* is a well-supported clade that appears to be a sister group to *Peronosporaceae* and *Pythiaceae* (Hulvey *et al*. 2010, this study). Hulvey *et al*. (2010) suggested that *H. bahamensis*, *H. epistomia*, *H. exoprolfiera,* and *H. operculata* might belong to the genus *Salisapilia*, but as no sequence data were available at that time to support this, Hulvey *et al*. (2010) refrained from proposing new combinations for any of these taxa. Of these species, *Halophytophthora operculata* was recently transferred to the genus *Calycofera* (Bennett *et. al*. 2017b), which was inferred to be the sister taxon to *Phytopythium*. Jung *et al*. (2017) suggested that *H. epistomia* might need to be accommodated in a genus of its own, but in the present study, it could be demonstrated that the morphology of *H. epistomia* fits well to the emended diagnosis of *Salisapilia*. Thus, it was combined into that genus instead of erecting a new one.

Key to the species of *Salisapilia*

1. Sporangia non-papillate; hyaline plug absent ..... ***S. nakagirii***
1. Sporangia papillate; hyaline plug present 2

2. Zoospore release through an evanescent vesicle 3
2. Zoospore release directly through the discharge tube 4

3. Dehiscence tube ragged appearance, with collar-like folds;
   sporangium shape ovoid, obpyriform, spherical ..... ***S. masteri***
3. Dehiscence tube smooth with cone-like plug; sporangium shape, mainly elongated, bursiform,
   cylindrical-elongated ..... ***S. elongata***

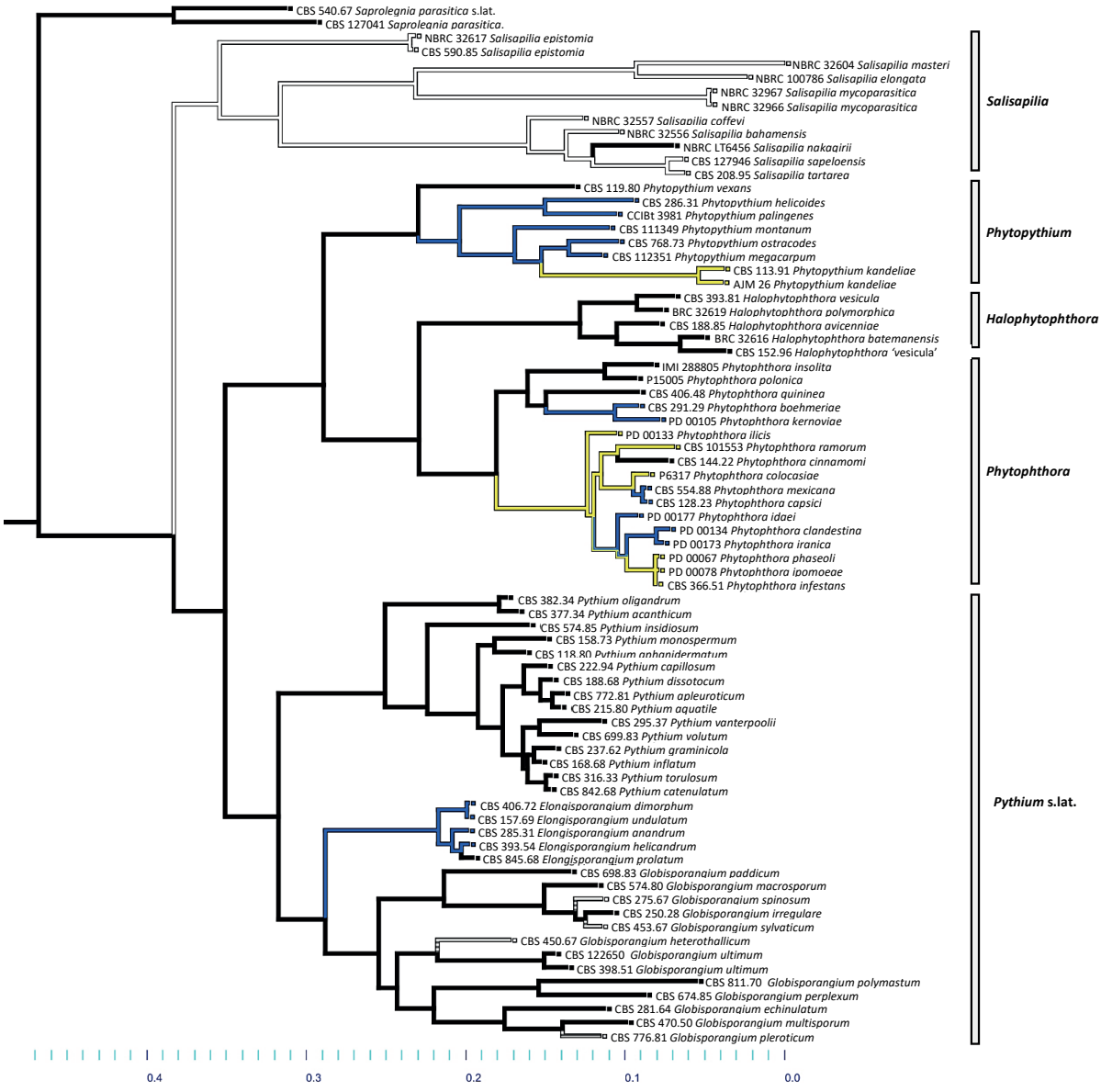
4. Sporangia vacuolated 5
4. Sporangia non-vacuolated 6

5. Sporangium shape bursiform; multi-lobed with aseptate or septate
   setiform appendages ..... ***S. bahamensis***
5. Sporangium shape narrowly bursiform, obpyriform, elongate to obclavate;
   single-lobed, setiform appendages absent ..... ***S. coffeyi***

6. Sexual structures absent; sporangium surface denticulate with few spines ..... ***S. mycoparasitica***
6. Sexual structures present, homothallic; sporangium surface smooth, spines absent 7

7. Oospores aplerotic ..... ***S. tartarea***
7. Oospores plerotic 8

8. Hyaline apical plug protruding through the discharge tube, 3–8 µm long;
   sporangium shape ovoid to obpyriform ..... ***S. sapeloensis***
8. Hyaline apical plug nested at the discharge tube, 14–90 µm long;
   sporangium shape langeniform to obpyriform ..... ***S. epistomia***



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REFERENCES

Anastasiou CJ, Churchland LM (1969). Fungi on decaying leaves in marine habitats. *Canadian Journal of Botany* **47**: 251–257.

Bachofer M (2004). *Molekularbiologische Populationsstudien an* Plasmopara halstedii*, dem Falschen Mehltau der Sonnenblume*. PhD thesis, University of Hohenheim, Germany.

Bala K, Robideau GP, Lévesque CA, *et al.* (2010). *Phytopythium* Abad, de Cock, Bala, Robideau, Lodhi and Lévesque, *gen. nov*. and *Phytopythium sindhum* Lodhi, Shahzad and Lévesque, *sp. nov*. *Persoonia* **24**: 136–137.

Beakes GW, Thines M (2017). *Hyphochytriomycota* and *Oomycota*. In: *Handbook of the Protists* (JM Archibald, AGB Simpson, C Slamovits, eds). Springer International Publishing, Germany: 435–505.

Bennett RM, Dedeles GR, Thines M (2017a). *Phytophthora elongata* (*Peronosporaceae*) is present as an estuarine species in Philippine mangroves. *Mycosphere* **8**: 959–967.

Bennett RM, de Cock AWAM, Lévesque CA, *et al.* (2017b). *Calycofera* gen. nov., an estuarine sister taxon to *Phytopythium, Peronosporaceae*. *Mycological Progress* **16**: 947–954.

Blair JE, Coffey MD, Park SY, *et al*. (2008). A multi-locus phylogeny for *Phytophthora* utilizing markers derived from complete genome sequences. *Fungal Genetics and Biology* **45**: 266–277.

Cooke DEL, Drenth A, Duncan JM, *et al*. (2000). A molecular phylogeny of *Phytophthora* and related Oomycetes. *Fungal Genetics and Biology* **30**: 17–32.

Crous PW, Verkley GJM, Groenewald JZ, *et al.* (2009). *Fungal Biodiversity*. *CBS Laboratory Manual* **1**: 1–269. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

de Cock AW, Mendoza L, Padhye AA, *et al.* (1987). *Pythium insidiosum* sp. nov., the etiologic agent of pythiosis. *Journal of Clinical Microbiology* **25**: 344–349.

de Cock AWAM, Lodhi AM, Rintoul TL, *et al.* (2015). *Phytopythium*: molecular phylogeny and systematics. *Persoonia* **34**: 25–39.

Erwin DC, Ribeiro OK (1996). Phytophthora *Diseases Worldwide*. American Phytopathological Society Press. Minnesota, USA.

Fell JW, Master IM (1975). Phycomycetes (*Phytophthora* spp. nov. and *Pythium* sp. nov.) associated with degrading mangrove (*Rhizophora mangle*) leaves. *Canadian Journal of Botany* **53**: 2908–2922.

Gerrettson-Cornell L, Simpson J (1984). Three new marine *Phytophthora* species from New South Wales. *Mycotaxon* **19**: 453–470.

Gisi U, Hemmes DE, Zentmyer GA (1979). Origin and significance of the discharge vesicle in *Phytophthora*. *Experimental Mycology* **3**: 321–339.

Ho HH, Chang HS, Huang SH (2003). *Halophytophthora elongata*, a new marine species from Taiwan. *Mycotaxon* **85**: 417–422.

Ho HH, Hsieh SY, Chang HS (1990). *Halophytophthora epistomium* from mangrove habitats in Taiwan. *Mycologia* **82**: 659–662.

Ho HH, Jong SC (1990). *Halophytophthora* gen. nov., a new member of the family *Pythiaceae*. *Mycotaxon* **36**: 377–382.

Hulvey J, Telle S, Nigrelli L, *et al.* (2010). *Salisapiliaceae* – a new family of oomycetes from marsh grass litter of southeastern North America. *Persoonia* **25**: 109–116.

Jung T, Scanu B, Bakonyi J, *et al.* (2017). *Nothophytophthora* gen. nov., a new sister genus of *Phytophthora* from natural and semi-natural ecosystems. *Persoonia* **39**: 143–174.

Katoh K, Misawa K, Kuma K, *et al.* (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast fourier transform. *Nucleic Acid Research* **30**: 3059–3066.

Kroon LPNM, Bakker FT, van den Bosch GB, *et al.* (2004). Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. *Fungal Genetics and Biology* **41**: 766–782.

Kroon LP, Brouwer H, de Cock AW, *et al.* (2012). The genus *Phytophthora* anno 2012. *Phytopathology* **102**: 348–364.

Lara E, Belbahri L (2011). SSU rRNA reveals major trends in oomycete evolution. *Fungal Diversity* **49**: 93–100.

Li GJ, Hyde KD, Zhao RL, *et al.* (2016). Fungal diversity notes 253–366: taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity* **78**: 1–237.

Maddison WP, Maddison DR (2018). *Mesquite: a modular system for evolutionary analysis*. http://mesquiteproject.org.

Marano AV, Jesus AL, de Souza JI, *et al.* (2016). Ecological roles of saprotrophic *Peronosporales* (*Oomycetes, Straminipila*) in natural environments. *Fungal Ecology* **19**: 77–88.

Marano AV, Jesus AL, Pires-Zottarelli CLA, *et al.* (2014). Phylogenetic relationships of *Pythiales* and *Peronosporales* (*Oomycetes, Straminipila*) within the "peronosporalean galaxy". In: *Freshwater fungi and fungal-like organisms*. (EBG Jones, KD Hyde, KL Pang, eds). Walter de Gruyter, Germany: 177–199.

Moncalvo JM, Wang HH, Hseu RS (1995). Phylogenetic relationships in *Ganoderma* inferred from the internal transcribed spacers and 25S ribosomal DNA sequences. *Mycologia* **87**: 223–238.

Nakagiri A, Newell SY, Ito T (1994). Two new *Halophytophthora* species, *H. tartarea* and *H. masteri*, from intertidal decomposing leaves in saltmarsh and mangrove regions. *Mycoscience* **35**: 223–232.

Nakagiri A, Tokumasu S, Araki H, *et al.* (1989). Succession of fungi in decaying mangrove leaves in Japan. In: *Recent Advances in Microbial Ecology* (T Hattori, Y Ishida, Y Moruyama, *et al*., eds). Japan Scientific Society Press, Japan: 297–301.

Nechwatal J, Oßwald WF (2003). *Pythium montanum* sp. nov., a new species from a spruce stand in the Bavarian Alps. *Mycological Progress* **2**: 73–80.

Nigrelli L, Thines M (2013). Tropical oomycetes in the German Bight – climate warming or overlooked diversity? *Fungal Ecology* **6**: 152–160.

Paul B (1987). A new species of *Pythium* with filamentous sporangia from Algeria. *Transactions of the British Mycological Society* **89**: 195–198.

Fig. 11. Ancestral trait reconstruction of the papilla and hyaline apical plug for *Elongisporangium*, *Globisporangium*, *Halophytophthora*, *Phytophthora*, *Phytopythium*, *Pythium*, and *Salisapilia*. White-coloured branches represent lineages with papillate sporangia bearing a hyaline apical plug; blue – papillate sporangia with no hyaline apical plug; yellow – semi-papillate sporangia with no hyaline apical plug; black – non-papillate sporangia. The scale corresponds to species divergence relative to nucleotide substitution rates based on the Bayesian phylogenetic inference.

Paul B (2000). ITS1 region of the rDNA of *Pythium megacarpum* sp. nov., its taxonomy and its comparison with selected species. *FEMS Microbiology Letters* **186**: 229–233.

Paul B, Galland D, Masih I (1999). *Pythium prolatum* isolated from soil in the Burgundy region: a new record for Europe. *FEMS Microbiology Letters* **173**: 69–75.

Pegg KG, Alcorn JL (1982). *Phytophthora operculata* sp. nov., a new marine fungus. *Mycotaxon* **15**: 99–102.

Price MN, Dehal PS, Arkin AP (2009). FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. *Molecular Biology and Evolution* **26**: 1641–1650.

Price MN, Dehal PS, Arkin AP (2010). FastTree 2 – Approximately Maximum-Likelihood trees for large alignments. *PLoS ONE* **5**: e9490.

Riethmüller A, Voglmayr H, Göker M, *et al.* (2002). Phylogenetic relationships of the downy mildews (*Peronosporales*) and related groups based on nuclear large ribosomal DNA sequences. *Mycologia* **84**: 834–849.

Ronquist F, Teslenko M, van der Mark P, *et al.* (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model spaces. *Systematic Biology* **61**: 539–542.

Runge F, Telle S, Ploch S, *et al.* (2011). The inclusion of downy mildews in a multi-locus-dataset and its reanalysis reveals a high degree of paraphyly in *Phytophthora*. *IMA Fungus* **2**: 163–171.

Stamps SJ, Waterhouse GM, Newhook FJ, *et al.* (1990). Revised tabular key to the species of *Phytophthora*. *CMI Mycological Papers* **162**: 1–28.

Tamura K, Stecher G, Peterson D, *et al.* (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**: 2725–2729.

Thines M (2014). Phylogeny and evolution of plant pathogenic oomycetes – a global overview. *European Journal of Plant Pathology* **138**: 431–447.

Uzuhashi S, Tojo M, Kakishima M (2010). Phylogeny of the genus *Pythium* and description of new genera. *Mycoscience* **51**: 337–365.

Vaidya G, Lohman DJ, Meier R (2010). SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* **27**: 171–180.

van der Plaats-Niterink AJ (1981). Monograph of the genus *Pythium*. *Studies in Mycology* **21**: 1–244.

Waterhouse GM (1963). Key to the species *Phytophthora* de Bary. *CMI Mycological Papers* **92**: 1–22.

Yang X, Hong C (2014). *Halophytophthora fluviatis* sp. nov. from freshwater in Virginia. *FEMS Microbiology Letters* **352**: 230–237.

Yang X, Tyler BM, Hong C (2017). An expanded phylogeny for the genus *Phytophthora*. *IMA Fungus* **8**: 355–384.

Supplementary Material: http://fuse-journal.org/

Table S1. GenBank numbers of sequences used in this study.

Fig. S1. Phylogenetic tree based on ITS sequences. The primary phylogenetic tree was inferred using Minimum Evolution (ME), with bootstrap support values from ME and Maximum Likelihood, and posterior probabilities from Bayesian Inference, in the respective order. (-) indicates unsupported alternating topology or bootstrap value and posterior probability of ≤ 50 / 0.8, respectively. The scale bar indicates the number of nucleotide substitutions per site.

Fig. S2. Phylogenetic tree based on LSU sequences. The primary phylogenetic tree was inferred using Minimum Evolution (ME), with bootstrap support values from ME and Maximum Likelihood, and posterior probabilities from Bayesian Inference, in the respective order. (-) indicates unsupported alternating topology or bootstrap value and posterior probability of ≤ 50 / 0.8, respectively. The scale bar indicates the number of nucleotide substitutions per site.