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ARTICLE

A revision of *Salispina*, its placement in a new family, *Salispinaceae* (*Rhipidiales*), and description of a fourth species, *S. hoi* sp. nov.

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Abstract: The genus *Salispina* was recently described for saprotrophic estuarine oomycetes with aculeolate or spiny sporangia. The genus currently contains three species, *S. intermedia*, *S. lobata*, and *S. spinosa*, the latter two previously included in *Halophytophthora*. During a survey of mangrove-inhabiting oomycetes in the Philippines, an isolate of *Salispina* (USTCMS 1611), was obtained from a decaying mangrove leaf. This isolate differed from other species in the genus in a unique combination of morphological and biological characters. Phylogenetic analysis revealed it to be the sister lineage of *S. lobata.* Consequently, the new species name *S. hoi* is introduced for the isolate. In addition, *Salispina* spp. grouped with *Sapromyces* of *Rhipidiales* with strong support, but differs from all other known genera of the order in the weak formation of hyphal constrictions, and absence of basal thalli and a holdfast network. The new family *Salispinaceae* is, therefore, described to accommodate *Salispina* in the order *Rhipidiales*.

Key words:
Mangrove
new taxa
Oomycota
phylogenetics
Sapromyces
taxonomy

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INTRODUCTION

Mangroves are inhabited by saprotrophic oomycetes, fungal-like eukaryotes in the kingdom *Straminipila* (Fell & Master 1975, Leaño *et al.* 2000, Leaño 2001, Thines 2014, Marano *et al*. 2016, Bennett *et al*. 2017a). These organisms are the first colonisers of fallen senescent mangrove leaves and, thus, have an important role in the nutrient cycling in estuarine ecosystems (Newell *et al.* 1987, Nakagiri *et al.* 1989, Leaño *et al*. 2000). Of the diverse mangrove oomycetes, *Salispina* is a genus currently comprising three described species (Li *et al*. 2016): *S. intermedia* (type species), *S. spinosa* (syn. *Phytophthora spinosa* var. *spinosa*, *Halophytophthora spinosa* var. *spinosa*), and *S. lobata* (syn. *Phytophthora spinosa* var. *lobata*, *Halophytophthora spinosa* var. *lobata*). This genus was erected to accommodate saprotrophic mangrove oomycetes with aculeolate or spiny, variously shaped sporangia, and direct zoospore release through an apical discharge tube. However, the higher taxonomic affinity of *Salispina* remained uncertain, and the genus was not assigned to a family or order (Li *et al.* 2016).

In the Philippines, Leaño (2001) recognized *S. lobata* (as *H. spinosa* var. *lobata*) as the first record of *Salispina* for the Philippines, and we did not find any other report of these organisms in the Philippines. It was the aim of this study to investigate the presence of additional species of *Salispina* in Philippine mangroves, and to resolve the family and order classification.

MATERIALS AND METHODS

Isolation, morphological investigation, and sporulation

The isolation and purification of the isolate used in this study, which came from decaying leaves collected from mangroves at Davao del Sur, Philippines, followed the method of Bennett & Thines (2017). For morphological investigations, samples were processed as described in Bennett & Thines (2017), but values were rounded to the nearest half micron, except for mean values. For sporulation, the development of sporangia from agarised media plugs was observed in saline concentrations of 0–3 % incubated at room temperature (~20–25 °C) in a dark compartment. Zoospore release was induced by placing mycelia with mature sporangia in a saline solution (≥ 3.5 %) and at 35 °C without light. Colony radial growth at 20, 25, 30, and 35 °C was tested in vegetable juice agar (VJA, commercial V8 Juice, Campbell or Alnatura Gemüsesaft, Alnatura; NBRC, medium no. 15), with or without seawater (http://www.nite.go.jp/en/nbrc/cultures/media/culture-list-e.html); and potato carrot agar (PCA; Crous *et al.* 2009), based on Alnatura Demeter Karotten mit Kartoffeln,

Alnatura). Mean colony radial growth was measured for five days and expressed as mm/day following the method of Solis *et al*. (2010).

Salispina sp. USTCMS 1611, *S. spinosa* CBS 591.85, and *S. lobata* CBS 588.85 were tested for growth in VJA at room temperature (~20–25 °C) for 5 d using a candle jar incubation method as described below and mean colony radial growth was measured according to Solis *et al.* (2010). For sporangium development under depleted oxygen conditions, mycelium in VJA from a 7 d-old culture plate (three per strain) was cut and the resulting pieces of ~1-2 cm² were placed in 60 mm Petri dishes containing 3 % saline solution. The Petri dishes were placed in a desiccator with a burning candle instead of silica gel. Then the desiccator was closed, allowing the candle to consume the oxygen until the flame could not be supported anymore. Subsequently, the desiccator was incubated at room temperature (~20–25°C). Another set-up was incubated in ambient air on a work-bench at room temperature (~20–25 °C). For zoospore release, the same settings were used, except for incubation at 35 °C and a saline solution of 3.5 %.

DNA Extraction and PCR amplification

For DNA extraction, a phenol-isoamyl-chloroform method was used (Bennett *et al.* 2017b). Subsequently, PCR amplification of *cytochrome oxidase* 1 (*cox*1), *cytochrome oxidase* 2 (*cox*2), and large nuclear ribosomal subunit (nrLSU) was done using the PCR primers listed in Table 1. The PCR reaction mix contained 1× PCR buffer, 0.2 mM dNTPs, 2.0 mM $MgCl_2$, 0.8 µg BSA, 0.4 µM of each primer, 0.5 U *Taq* polymerase and 10–50 ng DNA. PCR amplification of the *cox*1 region was done with an initial denaturation at 95 °C for 4 min, followed by 36 cycles of denaturation at 95 °C for 40 s, annealing at 51 °C for 40 s, and elongation at 72 °C for 60 s. A final elongation was done at 72 °C for 5 min. The cycling conditions for the *cox*2 region included an initial denaturation at 94 °C for 4 min, followed by 36 cycles of denaturation at 94 °C for 40 s, annealing at 51 °C for 40 s, and elongation at 72 °C for 40 s. A final elongation was carried out at 72 °C for 4 min.

For the LSU region, the cycling conditions were as follows: – initial denaturation 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 120 s. Subsequently, a final extension was carried out at 72 °C for 7 min.

PCR amplicons were sent to the SBiK-F Central Laboratory for sequencing with the primers used for PCR amplification. Sequences were assembled, converted into contigs and edited using Geneious version 5.0.4 (Biomatters, New Zealand). The resulting contigs were exported in fasta file format along with reference sequences selected from NCBI (https://www.ncbi.nlm.nih.gov/nucleotide) (Table 2). The resulting sequences were uploaded to the TrEase phylogeny webserver (http://www.thines-lab.senckenberg.de/trease/) for sequence alignment and phylogenetic tree reconstruction. The program MAFFT (Katoh *et al.* 2002) was used for multiple sequence alignment of *cox*1, *cox*2, and nrLSU sequences. Specifically, the FFT-NS-1 (fast) model was the chosen algorithm for *cox*1 and *cox*2 due to the absence of gaps and because taxa used in the multiple sequence alignments were closely related species. The G-INS-i was the algorithm used for nrLSU sequences. The primary phylogenetic tree, Minimum Evolution (ME), was generated using FastTree (Price *et al.* 2009), with 1000 bootstrap replicates and following the Generalized Time-Reversible (GTR) algorithm. Maximum Likelihood (ML) was generated using RAxML (Stamatakis 2014) where GTR-GAMMA was the chosen algorithm supported by 1000 bootstrap replications. Bayesian Inference was done using MrBayes (Ronquist *et al.* 2012) with the GTR model of substitutions and running four incrementally heated chains for 1 000 000 generations, discarding the first 30 % of the resulting trees to ensure sampling of trees and posterior probability calculations from the stationary phase. After making sure no supported incongruences were present for the different loci, alignments of *cox*1, *cox*2, and nrLSU sequences were concatenated using SequenceMatrix (Vaidya *et al*. 2010) and phylogenetic trees were computed as outlined above. Phylogenetic trees were viewed and annotated using MEGA, version 6 and 7 (Tamura *et al.* 2013).

RESULTS

Morphology

Salispina sp. USTCMS 1611 was isolated from decaying leaves collected from mangroves at Davao del Sur. Colony morphology of the isolate was appressed on both VJA and PCA (Fig. 1A–B). The strain developed aculeolate sporangia similar to known taxa of *Salispina* (Fig. 1) (Table 3). Hyphae were 2–9 µm wide, with retraction septae forming in some hyphae in old cultures submerged in 2–3 % saline solution incubated at room temperature (~20–25 °C). The branching pattern was irregular. Sporulation was achieved when plugs with mycelia were placed in 2–3 % saline solution and incubated at room temperature (~20–25 °C) in the dark. Sporangiogenic hyphae are not differentiated

Table 1. PCR Primers used in this study.

Loci	Primer pair	Sequence (5' – 3')	Reference
*cox*1	Oom*cox*1_Levup	GCT TAA GTT CAG CGG GT	Robideau *et al.* (2011)
	Oom*cox*1_Levlo	CYT CHG GRT GWC CRA AAA ACC AAA	Robideau *et al.* (2011)
*cox*2	*cox*2-F	GGC AAA TGG GTT TTC AAG ATC C	Hudspeth *et al.* (2000)
	*cox*2-RC4	TGA TTW AYN CCA CAA ATT TCR CTA CAT TG	Choi *et al.* (2015)
nrLSU	LR0R	ACC CGC TGA ACT TAA GC	Moncalvo *et al.* (1995)
	LR6-O	CGC CAG ACG AGC TTA CC	Riethmüller *et al.* (2002)

Table 2. GenBank* sequences (accession numbers) used in this study.

Species	Strain information	*cox*1	*cox*2	LSU
Halophytophthora				
H. vesicula ex-type	NBRC 32216• (= CBS 393.81 = IFO 32216)	MG019397	MF991427	KT455418
Phytopythium				
P. helicoides	CBS 286.31•	MF397921	MF397926	HQ665186
P. kandeliae	CBS 111.91•	HQ708207	MF397928	HQ665065
P. megacarpum	CBS 112351•	HQ708435	AB690665	HQ665067
P. montanum	CBS 111349•	HQ708436	AB690667	HQ665064
P. ostracodes	CBS 768.73•	EF408874	AB690668	HQ665295
P. vexans	CBS 119.80•	EF426548	EF426547	HQ665090
Phytophthora				
Ph. boehmeriae	CBS 291.29• (= PD_00181 = P6950)	HQ261251	PD_00181	HQ665190
Ph. insolita	IMI 288805• (= PD_00175 = P6195)	PD_00175	PD_00175	EU080180
Ph. kernoviae	P10958•	PD_00105	PD_00105	PD_00105
Ph. quininea	CBS 406.48 (= P3247)•	PD_00126	PD_00126	PD_00126
Ph. ramorum	CBS 101553• (= PD_00065 = P10103)	HQ708387	PD_00065	HQ665053
Pythium				
Py. aquatile	CBS 215.80•	HQ708492	KJ595355	HQ665153
Py. capillosum	CBS 222.94•	HQ708529	KJ595360	HQ665164
Py. torulosum	CBS 316.33•	HQ708900	KJ595374	HQ665206
Py. inflatum	CBS 168.68•	HQ708610	KJ595352	HQ665140
Salisapilia				
S. sapeloensis ex-type	LT6440• (= CBS 127946 = NBRC 108756)	KT897704	KJ654178	HQ232457
Salispina				
S. intermedia ex-type	CCIBt 4155	KT886053	NS	KT920432
S. intermedia	CCIBt 4153	KT886052	NS	KT920431
S. intermedia	CCIBt 4156	KT886054	NS	KT920433
S. intermedia	CCIBt 4115	KT886055	NS	NS
S. hoi ex-type	USTCMS 1611•	MG019399	MF991430	MG385863
S. lobata ex-type	CBS 588.85 (= NBRC 32592 = IFO 32592 = ATCC 28291)	KT886056	MF991429	NS
S. spinosa ex-type	CBS 591.85• (= NBRC 32593 = IFO 32593 = ATCC 28294)	KT886057	MF991428	KT920434
Sapromyces				
S. elongatus	CBS 213.82•	MG019398	KT257452	AF235950
Saprolegnia				
S. parasitica	CBS 223.65•	NW012157837	NW012157837	HQ665165
S. ferax	P1.5.14	KP965743	KP965749	NS

NS: No sequence was used for the respective loci.

*Some sequences of *Phytophthora* spp. were downloaded from the *Phytophthora* database (http://www.phytophthoradb.org/).

•Strains used in multigene analyses.

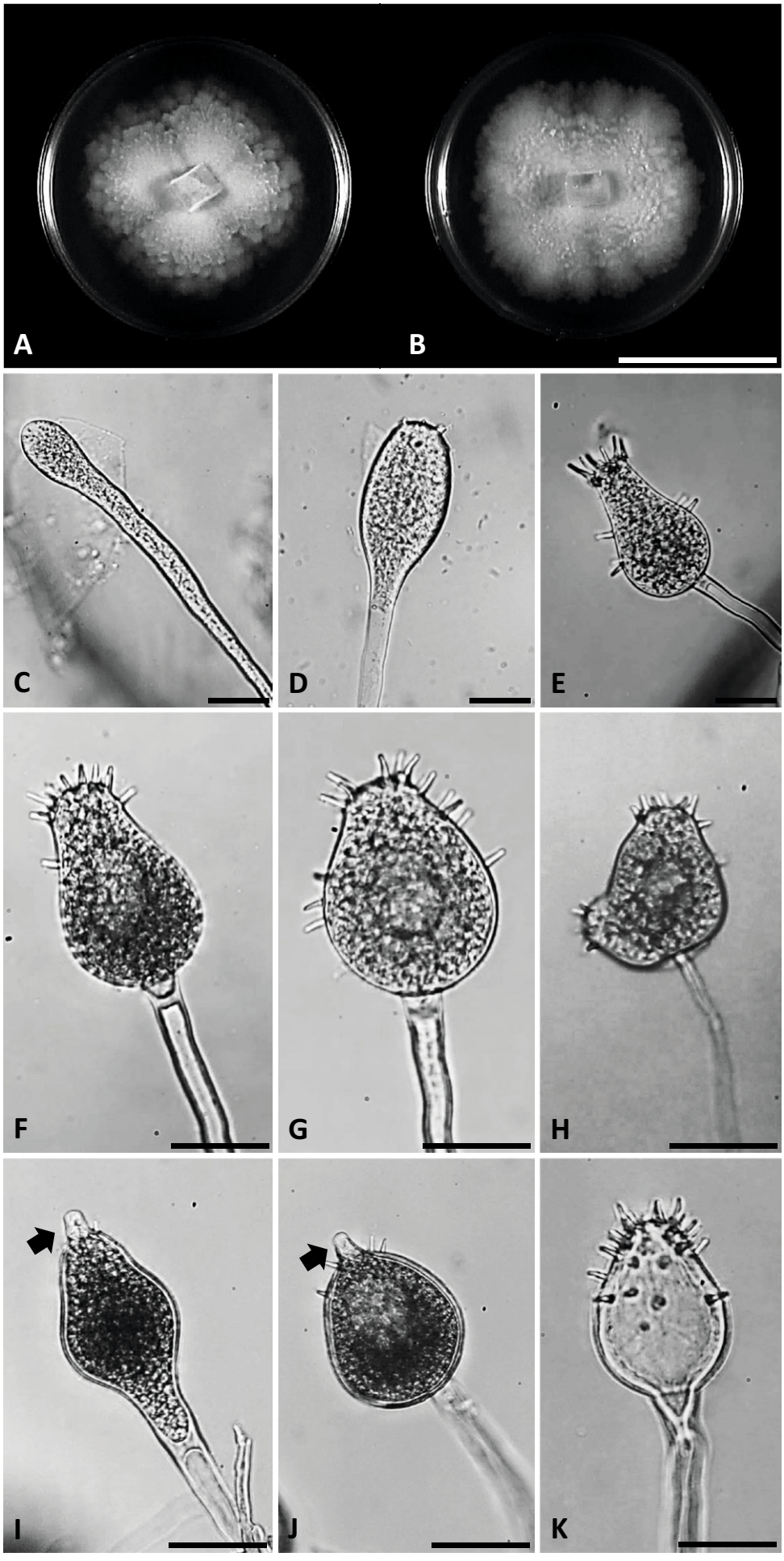

Fig. 1. Morphology of *Salispina hoi* (USTCMS 1611). Colony pattern on: **A.** Potato carrot agar (PCA), and **B.** Vegetable juice agar (VJA). **C.** Protosporangium. **D.** Immature or young sporangium. **E**–**H.** Mature sporangia; spines are forming at the apex of sporangia, while others are either having scattered spines on the surface of the sporangia or a smooth surface. **H.** Irregularly-shaped aculeolate sporangium. **I–J.** Sporangia with dehiscence tube (arrow), zoospores differentiate inside the sporangia. **K.** Empty sporangium. Bars: **A–B** = 30 mm, **C–K** = 20 µm.

Table 3. Morphology of *Salispina* species.

Structure	***Salispina hoi* sp. nov.** **USTCMS 1611**	***S. intermedia*** **(Li *et al.* 2016)**	***S. lobata*** **(Fell & Master 1975)**	***S. spinosa*** **(Fell & Master 1975)**
Colony pattern	Appressed and petaloid on VJA	Petaloid on PYGA	Appressed and petaloid on VJA	Appressed and rosette on VJA
Septa	Few, present at maturity	Few, present at maturity	Few, present at maturity	Non-septate at all ages
Hyphal diam (µm)	2–9	2.5–10	3–12	3–9
Sporangiogenic hypha	Undifferentiated from vegetative hypha, bears 1 terminal sporangium	Undifferentiated from vegetative hypha, bears 1 terminal sporangium	Undifferentiated from vegetative hypha, bears 1 terminal sporangium	Undifferentiated from vegetative hypha, bears 1 terminal sporangium
Sporangia				
Shape	Ovoid, clavate, globose, obpyriform, variable	Obovate, obpyriform, globose, elongate, variable	Obypriform to auriculate, botryose-like, similar to fused globose sporangia	Globose, ovate, obovate
Papilla	Non-papillate	ND	Inconspicuous, unipapillate	Inconspicuous, unipapillate
Size (µm)	(33.5–)43–57.6–77.5(–87) × (10.5–)20–36.6–66(–75.5)	33–197 × 25–183 (av. 82 × 62)	51–75 × 56–150 (av. 67 × 97)	60–107 (av. 80) diam.
Surface spines	Most spines at the apex of the sporangia, forming a crown-like appearance, Some sporangia have scattered spines or non-aculeolate	Smooth to spiny, variable degree of coverage from one at the tip to entirely aculeolate	Entirely, partially or non-aculeolate	Entirely, partially or non-aculeolate
Vacuole	Present	Present	Present	Present
Basal plug	Present, hyaline	Present, hyaline	Present, hyaline	Present, hyaline
Zoospore discharge	Through a thin-walled dehiscence tube, often inconspicuous after full release of zoospores	Through a persistent tube	Through a thin-walled, flask-shaped dehiscence tube	Through a thin-walled, flask-shaped dehiscence tube
Vesicle	Absent	Absent	Absent	Absent
Chlamydospores	Not observed	Not observed	Not observed	Not observed
Sexual structures	Not observed	Not observed	Not observed	Not observed

ND: No data provided.

from vegetative hyphae until the hyphal apex swells to form a protosporangium (Fig. 1C–D). The sporangia are ovoid, clavate, globose to obpyriform (Fig. 1E–J) but some were irregularly shaped (Fig. 1H); they measured (33.5–)43–57.5–77.5(–87) × (10.5–)20–36.5–66(–75.5) (*n* = 100). Spines were predominantly forming at the apex of the sporangia, resulting in a crown-like appearance (Fig. 1 D–E, J), while some sporangia were partially covered in spines, rarely entirely aculeolate (Fig. 1 F–H, J), or smooth-walled sporangia were observed (not depicted). The sporangia were non-caducous and non-papillate. The sporangial content was vacuolated. The inner base of the sporangia, where the basal plug is located, was concave (Fig. 1 I, K). The basal plug was observed to be hyaline, separating the sporangiogenic hypha from the sporangium. Zoospore release occurred only when mycelium with mature sporangia was placed in a saline solution with ≥ 3.5 % and incubated at 35 °C. The apex of the dehiscence tube (Fig. 1 I–J) deliquesces and zoospores swim directly out from the tube, i.e. no vesicle was observed. No chlamydospores and gametangia were observed. A summary of morphological features of known *Salispina* spp. is given in Table 3.

The mean colony radial growth of *Salispina* sp. USTCMS 1611 in VJA and PCA at different temperatures is given in Fig. 2A. The growth and sporulation of the three *Salispina* spp. in VJA in candle jar incubation at room temperature (~ 20–25 °C) are presented in Fig. 2B.

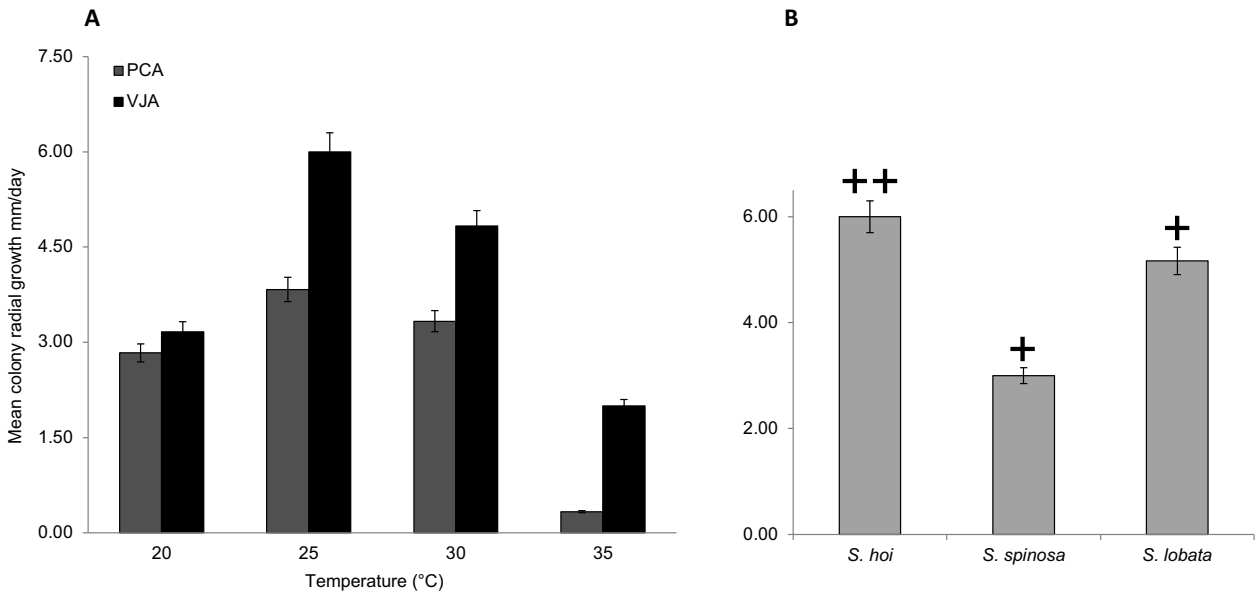

Fig. 2. Mean colony radial growth. **A.** Mean colony radial growth of *Salispina hoi* (USTCMS 1611) on VJA and PCA at different temperatures. **B.** Mean colony radial growth of the three *Salispina* species on VJA at room temperature in a candle jar. (++) = sporulation both under candle jar and ambient air conditions; (+) = sporulation under ambient air condition.

Phylogeny

The multigene phylogenetic analysis (Fig. 3) and the single-gene phylogenetic trees (Figs S1–S3) showed that USTCMS 1611 is a distinct member of the *Salispina* clade, with maximum support in all analyses. *Salispina* sp. USTCMS 1611 was not conspecific with any known species of *Salispina* (Figs S1, S3), and grouped as sister to *S. lobata* (Figs S1–S2). In addition, the genus *Salispina* was found to be sister to *Sapromyces elongatus* (*Rhipidiaceae*, *Rhipidiales*) with strong to maximum support in the phylogenetic reconstruction based on the concatenated dataset with nuclear and mitochondrial loci (Fig. 3).

DISCUSSION

The genus *Salispina* was proposed based on phylogenetics and sporangial characteristics with *Salispina intermedia* as the type species (Li *et al*. 2016). The two additional species, *S. spinosa* and *S. lobata*, were first considered to be members of *Phytophthora* (Fell & Master 1975; as *Ph. spinosa* var. *spinosa*, and *Ph. spinosa* var. *lobata*, respectively), and later transferred to *Halophytophthora* (Ho & Jong 1990; as *H. spinosa* var. *spinosa*, and *H. spinosa* var. *lobata*) based on their occurrence in estuarine environments. However, Nakagiri (2002) reported in a conference note that *S. spinosa* (referred to as *H. spinosa*) has close affinities to *Sapromyces* of *Rhipidiales*. Phylogenetic analyses in the present study revealed a strongly supported sister-group relationship between *Sapromyces elongatus*, which is the only species of *Rhipidiales* with sequences deposited at NCBI, and *Salispina*.

The family *Rhipidiaceae* includes *Araiospora* (Thaxter 1896), *Aqualinderella* (Emerson & Weston 1967), *Mindeniella* (Kanouse 1927), *Nellymyces* (Batko 1971), *Rhipidium* (Cornu 1871), and *Sapromyces* (Fritsch 1893). These taxa occur in freshwater habitats anchored to submerged twigs and fruits (Sparrow 1960, Beakes & Thines 2017). Most members of the family have arborescent thalli (except *Mindeniella* and *Sapromyces*) with a more or less distinct basal cell derived from a germinated zoospore (Minden 1916), a holdfast network, and all known members feature jointed or constricted hyphae, as well as stalked sporangia and gametangia (Sparrow 1960, Blackwell *et al*. 2015). Sporangia of members of the family are either aculeolate or smooth-walled. Examples with aculeolate sporangia include *Araiospora spinosa* (syn. *Rhipidium spinosum*) (Thaxter 1896), *A. coronata* (Linder 1926), *A. pulchra* (Kevorkian 1934), *A. streptandra* (Kevorkian 1934, Shanor & Olive 1942), *M. spinospora* (Kanouse 1927, Sparrow & Cutter 1941), *M. asymmetria* (Johnson 1951), and *N. megaceros* (Batko 1971). The formation of spines was believed to be influenced by the availability of nutrients in the substrate as outlined below. *Mindeniella* has the tendency to form aculeolate sporangia after colonies are well established in the substrate (Kanouse 1927, Sparrow & Cutter 1941). However, Sparrow (1960) mentioned that Ralph Emerson had informed him that there was a correlation between the formation of spines and the near absence of oxygen in axenic cultures. Zoospore release in the family is either directly through a discharge tube (e.g. *Aqualinderella fermentans*, *M. asymmetria*) or a vesicle (e.g. *Araiospora coronata*, *M. spinospora*, *R. americanum*). The discharge tube is generally formed at the sporangial apex, but its length varies in different species. Gametangia of *Rhipidiaceae* are often pedicellate, and some species apparently produce oospores parthenogenically (e.g. *Aqualinderella fermentans*, *M. spinospora*, *N. megaceros*, *R. parthenosporum*), similar to *Phytophthora insolita* (Ann & Ko 1980). Several members of *Rhipidiaceae* were reported to grow in low oxygen concentrations (e.g. *Aqualinderella*, *Mindeniella*, *Rhipidium*) (Emerson & Weston 1967, Gleason 1968, Dogma 1975, Natvig 1981) and, hence, can be considered as facultative anaerobes. Dick (2001) suggested in his diagnosis of the order *Rhipidiales* that members had either a facultative or an obligate fermentative metabolism.

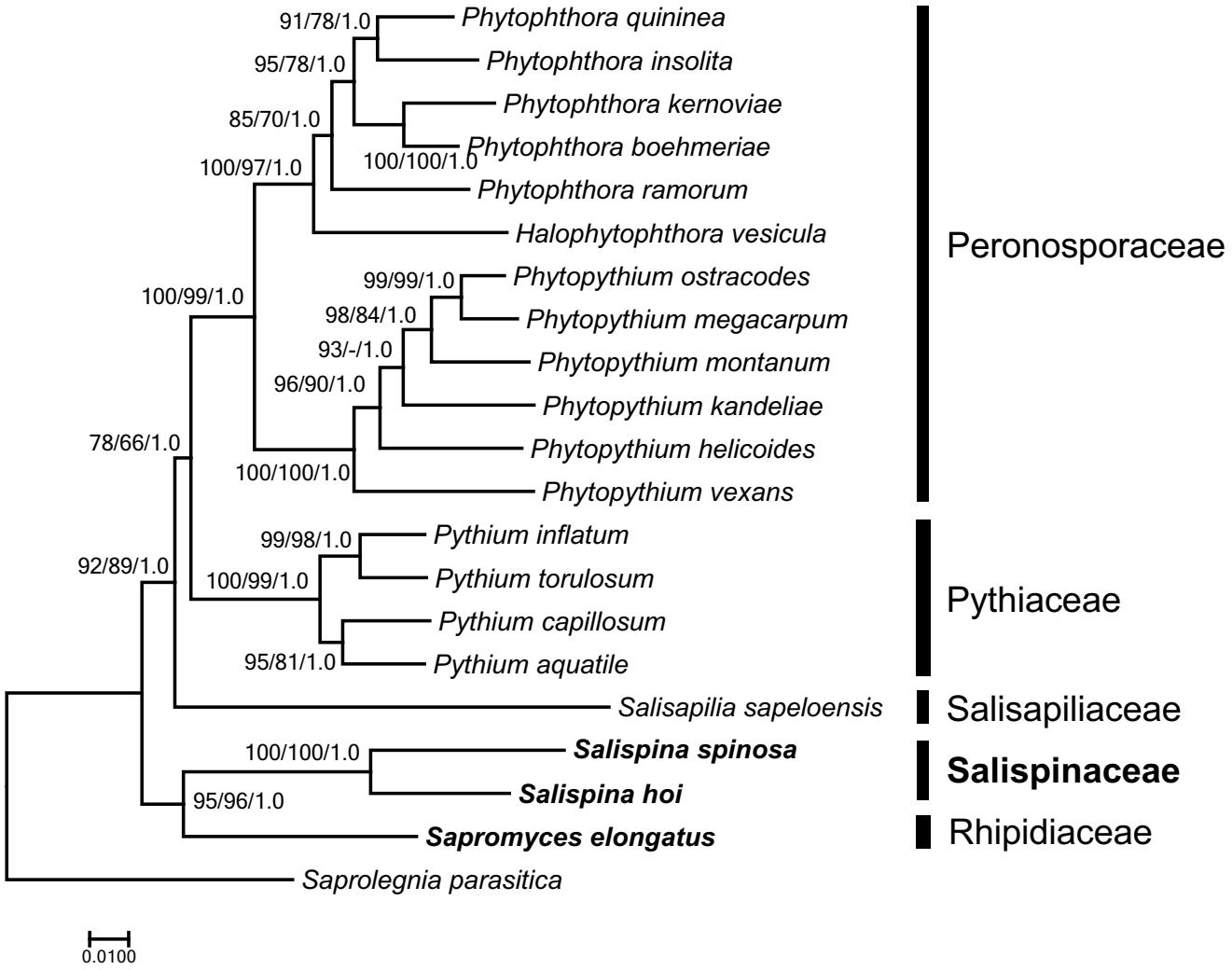

Fig. 3. Phylogenetic tree based on concatenated sequences of *cox*1, *cox*2, and LSU. Minimum Evolution (ME) was used as the primary tree with bootstrap support values from ME, and Maximum Likelihood (ML), and Bayesian posterior probability. (-) indicates bootstrap support values lower than 50 % or unsupported alternating topology from the corresponding primary tree. Scale bar indicates the number of substitutions per site.

In not displaying hyphal constrictions or stalked sporangia, *Salispina* is morphologically divergent from the accepted genera of *Rhipidiaceae*. Interestingly, Fell & Master (1975) inferred that nutrition plays an important role in the development of spines in *S. spinosa* (as *Phytophthora spinosa* var. *spinosa*), similar to the conclusions presented before by Kanouse (1927), Sparrow & Cutter (1941), and Sparrow (1960) for *Rhipidiaceae*. The three strains of *Salispina* (*S. lobata* CBS 588.85, *S. spinosa* CBS 591.85, and *Salispina* sp. USTCMS 1611) tested in this study were able to grow in a candle jar arrangement, where atmospheric oxygen is around 10–14 % and carbon dioxide about 2–5 % (Luechtefeld *et al*. 1982, El-Sherbeeny 1996). In a mangrove environment, abiotic factors (i.e. salinity, temperature, and oxygen concentration) constantly fluctuate (Leaño *et al.* 2000, Kathiresan 2004, Krauss *et al.* 2008). In particular, the oxygen concentration is often depleted during low tide, and gas production (e.g. CH_4, NH_3, H_2S) by anaerobic bacteria can be observed (Kathiresan 2004). This provides suitable conditions for both obligate or facultative anaerobes and microaerophiles. In line with the fermentative or microaerophilic habit observed for various members of *Rhipidiales*, *Salispina* sp. USTCMS 1611 showed normal vegetative growth in candle jars, but sporulation of members of *Salispina* was triggered by normal oxygen levels, and increased salinity and temperature, conditions that probably correspond to the early rise of the sea level after a low tide. While the physiological properties of *Salispina* support placement in *Rhipidiales*, the high morphological and phylogenetic divergence between *Salispina* and members of the *Rhipidiaceae* does not support a placement of *Salispina* in that family. Such a taxonomic classification would render the morphologically well-delineated family highly heterogenous. We therefore introduce the new family name *Salispinaceae* to accommodate the genus *Salispina*.

Salispina sp. USTCMS 1611 is a sister taxon to *S. lobata*, which has sporangia with a peculiar shape. Initially obpyriform, the sporangia of *S. lobata* subsequently develop lateral lobes until the sporangium looks botryose (Fell & Master 1975). However, USTCMS 1611 has ovoid, clavate, globose, to obpyriform sporangia, with some sporangia showing variations in shape, but not becoming botryose. In addition, the formation of spines appears to be different

between the two species, with most spines of USTCMS 1611 formed at the apex of the sporangium, while some sporangia have scattered spines or are even smooth-walled. In contrast, sporangia of *S. lobata* are either entirely or partially aculeolate (with no distinct pattern), or non-aculeolate (Table 3). Based on morphology and phylogenetic relationships, this strain cannot be assigned to any known taxon in *Salispina*, and so is described here as a new species.

This raises the number of known species in *Salispina* to four, but, given the still fragmentary knowledge regarding estuarine oomycetes in general and *Salispina* in particular, it seems likely that additional species of this genus will be discovered. In contrast to other orders of *Oomycota*, such as *Albuginales* (Choi *et al*. 2007, Thines *et al*. 2009, Ploch *et al*. 2010, Ploch & Thines 2011, Mirzaee *et al*. 2013), *Peronosporales* (Riethmüller *et al*. 2002, Voglmayr 2003, Voglmayr *et al.* 2004, Thines *et al*. 2006, 2007, Göker *et al*. 2007, Thines *et al*. 2008, 2015, Choi & Thines 2015), and *Saprolegniales* (Dick *et al*. 1999, Riethmüller *et al*. 1999, Leclerc *et al.* 2000, Spencer *et al.* 2002, Diéguez-Uribeondo *et al*. 2007, Hulvey *et al*. 2007, Steciow *et al.* 2013, Sandoval-Sierra *et al.* 2014, Steciow *et al.* 2014, Rocha *et al.* 2018), the *Rhipidiales* has received relatively little attention, probably owing to a lower degree of cultivation success from environmental samples due to their often microaerophilic to anaerobic nature. Thus, it seems promising to undertake targeted sampling in oxygen-depleted limnic environments in order to gain further insights into these understudied organisms which might play an important role in nutrient cycling.

TAXONOMY

Rhipidiales M. W. Dick, *Straminipilous Fungi:* 305 (2001).

Salispinaceae R. Bennett & Thines, **fam**. **nov**.
MycoBank MB824253

Diagnosis: Differs from *Rhipidiaceae* in the absence of conspicuous hyphal constrictions.

Type: *Salispina* Marano *et al.*, *Fungal Diversity* **78**: 198 (2016).

Salispina hoi R. Bennett & Thines, **sp. nov.**
MycoBank MB823076

Etymology: Dedicated to Hon Ho, for his pioneering studies into mangrove oomycetes.

Diagnosis: Differ from its sister taxon, *S. lobata* in sporangia that do not become botryose at maturity and from all species of the genus by a pronounced preference of spine formation at the apex and a quickly evanescing discharge tube.

Type: **Philippines**: Davao del Sur, 6.579667°N 125.453667°E, isolated from decaying mangrove leaf litter, 6 Sep. 2015, *R.M. Bennett*, *M.K. Devanadera*, *& G.R. Dedeles* (USTH 014145 – holotype; USTCMS 1611 – ex-type culture).

Description: *Mycelium* appressed on VJA and PCA. *Hyphae* 2–9 µm wide; septae forming at maturity, branching irregular; sporangiogenic hyphae not differentiated from vegetative hyphae, bearing a single terminal sporangium. *Sporangia*, shape ovoid, globose, obpyriform to variable; size (33.5–) 43–57.6–77.5(–87) × (10.5–)20–36.6–66(–75.5) µm; papilla absent, basal plug concave and hyaline; sporangial content vacuolate; surface aculeolate, with spines mostly forming at the apex of sporangia resulting in a crown-like appearance, some sporangia are smooth or with very few scattered spines. *Zoospores* discharge directly through a dehiscence tube; the apex of the tube deliquescent, allowing zoospores to escape from sporangia; vesicle absent. *Chlamydospores* not observed. *Gametangia* not observed.

Sequences: *cox*1 MG019399, *cox*2 MF991430, and LSU MG385863.

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RMB and MT conceived the study. RMB, MKD, and GRD arranged legal documents for collection, and conducted field sampling and isolation. RMB conducted laboratory work. RMB and MT analysed and interpreted the data. RMB and MT wrote the manuscript with contributions from the co-authors.

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Supplementary files can be found on the IMA Fungus website, http://www.imafungus.org/:

Fig. S1. Phylogenetic tree based on *cox*1 sequence data. Minimum Evolution (ME) was used as the primary tree with bootstrap support values from ME, and Maximum Likelihood (ML), and Bayesian posterior probability. (-) indicates bootstrap support values lower than 50% or unsupported alternating topology from the corresponding primary tree. Scale bar indicates the number of substitutions per site.

Fig. S2. Phylogenetic tree based on *cox*2 sequence data. Minimum Evolution (ME) was used as the primary tree with bootstrap support values from ME, and Maximum Likelihood (ML), and Bayesian posterior probability. (-) indicates bootstrap support values lower than 50 % or unsupported alternating topology from the corresponding primary tree. Scale bar indicates the number of substitutions per site.

Fig. S3. Phylogenetic tree based on LSU sequence data. Minimum Evolution (ME) was used as the primary tree with bootstrap support values from ME, and Maximum Likelihood (ML), and Bayesian posterior probability. (-) indicates bootstrap support values lower than 50 % or unsupported alternating topology from the corresponding primary tree. Scale bar indicates the number of substitutions per site.