

Salisapiliaceae – a new family of oomycetes from marsh grass litter of south-eastern North America

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Introduction

During recent surveys of oomycetes from leaf litter of estuarine marsh grasses of southeastern GA several unique isolates were recovered which appeared similar to some of the previously described species of *Halophytophthora* (Fig. 1, 2, Table 1).

BLAST searches of the internal transcribed spacer (ITS) and portion of the nuclear ribosomal large subunit (nrLSU) sequences showed some isolates to be not only highly divergent from *Halophytophthora*, but to all other oomycete sequences in GenBank (as of early 2010) as well.

The objective of this work was to characterize these taxa using DNA sequence phylogeny and microscopy of morphological features to determine if they represented a new lineage of oomycetes warranting taxonomic recognition.



Fig. 1. Marsh site at Sapelo Island, GA. *Spartina alterniflora* is the predominant marsh grass.

Table 1. Collection and strain details for the oomycete isolates investigated in this study.

Taxa recovered	Collector	Location	GPS coordinates
<i>S. sapeloensis</i> (LT6440)	J. Hulvey	USA, GA, Sapelo I., Cabretta I.	N31 43888, W81 23909
<i>S. nakagirii</i> (LT6456)	J. Hulvey	USA, GA, Sapelo I., Cabretta I.	N31 43888, W81 23909
<i>Salisapilia</i> sp. (LT6466)	J. Hulvey	USA, GA, Sapelo I., Cabretta I.	N31 43888, W81 23909
<i>Salisapilia</i> sp. (LT6400)	J. Hulvey	USA, GA, Sapelo I., Teal Boardwalk	N31 39509, W81 27936
<i>Salisapilia</i> sp. (LT6471)	J. Hulvey	USA, GA, Sapelo I., Teal Boardwalk	N31 39509, W81 27936
<i>Halophytophthora</i> sp. 1 (LT6430)	J. Hulvey	USA, GA, Sapelo I., Teal Boardwalk	N31 39509, W81 27936
<i>Halophytophthora</i> sp. 2 (LT6455)	J. Hulvey	USA, GA, Sapelo I., Teal Boardwalk	N31 39509, W81 27936
<i>Halophytophthora</i> sp. 1 (LT6430)	J. Hulvey	USA, GA, Saint Simons Island	N31 15289, W81 41602

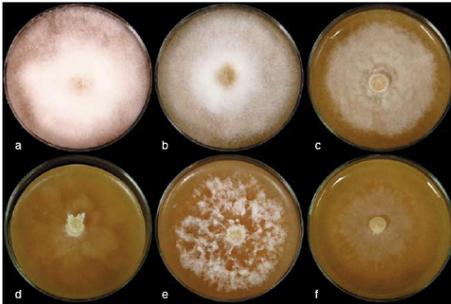


Fig. 2. Photographs of colonies of: a–c. *Halophytophthora* s.str. and d–f. *Salisapilia* spp. isolates. a. LT6430; b. LT6455; c. *H. vesiculata* CBS 152.96; d. *S. tartarea* CBS 208.95; e. *S. sapeloensis* LT6440; f. *S. nakagirii* LT6456.

Methods

Partial ITS and nrLSU sequences were aligned to a sequence dataset of Pythiaceae and Peronosporaceae (Villa et al. 2006), including several species of *Halophytophthora*, some of which were not previously available in GenBank. Phylogenies were inferred using Maximum Likelihood, Minimum Evolution, and Bayesian posterior probabilities methods.

The colony morphologies and microscopic features of the isolates were observed with differential interference contrast and phase contrast light microscopy. One hundred each of sporangia, gametangia, and oospores were measured and the values averaged, and light micrographs were taken.

Results

Results of the phylogenetic analyses show maximum support for the monophyly of this group of taxa. As a consequence, a new family, the Salisapiliaceae, and genus, *Salisapilia*, were created and are shown below to diverge basal to the Pythiaceae and Peronosporaceae (Fig. 3).

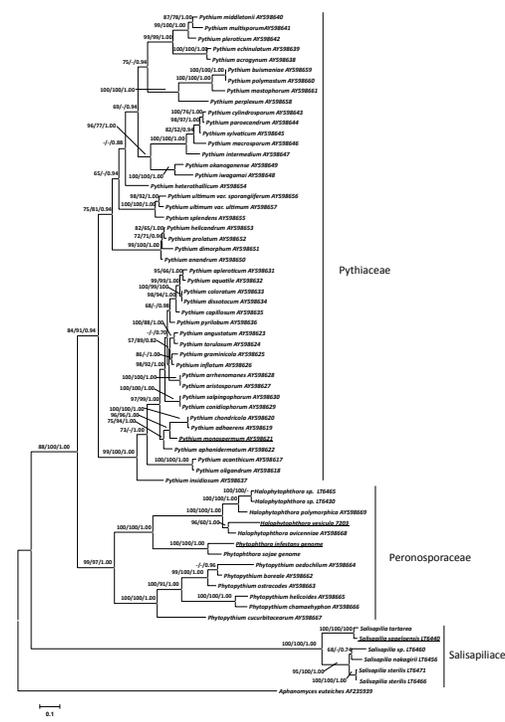


Fig. 3. Best tree from the Maximum Likelihood Analysis based on concatenated ITS and nrLSU sequences with bootstrap support values in Maximum Likelihood and Minimum Evolution analyses and Bayesian posterior probabilities in the respective order on the branches. Type species are underlined.

Salisapilia spp. are evolutionarily distant from all other described oomycete families, which can be observed in the sequence similarities of partial nrLSU sequences to other type species from other families of oomycetes (Table 2).

Table 2. Homology of *Salisapilia sapeloensis* (HQ232457) nrLSU sequence to selected oomycetes.

Family	Species	(GenBank accession no.)	Maximum identity
Peronosporaceae	<i>Phytophthora infestans</i>	FJ869987.1	76 %
Peronosporaceae	<i>Bremia lactucae</i>	EF553478.1	74 %
Peronosporaceae	<i>Halophytophthora vesiculata</i>	HQ232463.1	76 %
Peronosporaceae	<i>Phytophthora oedochilum</i>	AY598664.1	78 %
Pythiaceae	<i>Pythium monospermum</i>	AY598621.1	76 %
Pythiaceae	<i>Leguminidium chittamalophilum</i>	AF235946.1	77 %
Albuginaceae	<i>Albugo candida</i>	AF235938.1	76 %
Rhizophidaceae	<i>Sapromyces elongatus</i>	AF235950.1	74 %
Saprolegniaceae	<i>Saprolegnia ferax</i>	AF235953.1	77 %
Leptodermiaceae	<i>Aphanomyces piscicida</i>	AF235941.1	77 %

* Query coverage 99 %.

Morphological analyses show that *Salisapilia* spp. differ from *Halophytophthora* s. str. by hyphal diameter, method of zoospore release, and the production of gametangia and oospores (Table 3).

Table 3. Summary of some morphological features for species of *Salisapilia* and *Halophytophthora* s.str.

Species (strain number)	Culture collection no.	Plugged discharge tube	Zoospores discharged into vesicle	Oogonium diam (µm)	Antheridial origin	Hyphal diam (µm)
<i>Salisapilia tartarea</i> *	CBS 208.95	Yes	No	33–66	diclinous	1–3
<i>Salisapilia sapeloensis</i> (LT6440)	CBS 127946	Yes	No	35–60	paragynous	1–3
<i>Salisapilia nakagirii</i> (LT6456)	CBS 127947	NA	NA	33–48	diclinous	1–3
<i>Salisapilia</i> sp. (LT6400)	CBS 127949	NA	NA	NA	NA	1–3
<i>Salisapilia</i> sp. (LT6471)	CBS 127949	NA	NA	NA	NA	1–3
<i>Halophytophthora vesiculata</i>	CBS 152.96	No	Yes	NA	NA	1–6
<i>Halophytophthora</i> sp. 1 (LT6430)	NA	No	Yes	NA	NA	1–5
<i>Halophytophthora</i> sp. 2 (LT6455)	NA	No	Yes	NA	NA	1–6

* syn. *Halophytophthora tartarea*.

Two new species are described, *S. sapeloensis* and *S. nakagirii*, supported by sequence divergence and morphology of antheridia (Fig. 3, 4, Table 3). One species of *Halophytophthora*, *H. tartarea*, is now placed within *Salisapilia*, due to both phylogenetic and morphological affinity with *Salisapilia*, rather than *Halophytophthora* s. str. (Fig. 3, Table 3).

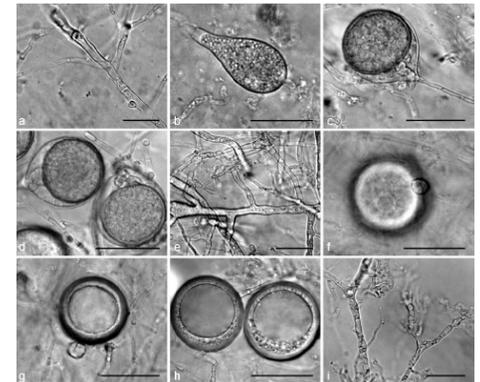


Fig. 4. Micrographs of *Salisapilia*. a–d. Micrographs of *Salisapilia sapeloensis* LT6440. a. Branching hyphae with septae; b. ripe sporangium, note plug of material at tip of discharge tube; c. maturing oospore with simple paragynous antheridium; d. two fertilised oospores (on the left, a lobed paragonous antheridium is seen, and on the right, a branching paragynous antheridium is present). — e–h. Micrographs of *Salisapilia nakagirii* LT6456. e. Branching hyphae with septations; f. oogonium with antheridial cell attached; g. maturing oospore with diclinous antheridium; h. two fertilised oospores. — i. Micrographs of hyphae of *Salisapilia* sp. LT6456. — Scale bars: a, e = 10 µm; b, d, f, h = 5 µm.

Concluding Remarks/Future Directions

The Salisapiliaceae represent a highly diverged monophyletic lineage basal to the Pythiaceae and Peronosporaceae.

The genus *Halophytophthora* is likely in need of further taxonomic revision, to better reflect evolutionary relationships of species.

Genome annotation of *Salisapilia* is underway with the research group of Sophien Kamoun, of the Sainsbury Labs. Preliminary data suggest the genome to be compact at ~24 MB, and with few putative secreted effector genes, which are found in abundance in genomes of pathogenic oomycetes. Comparative genomics with *Salisapilia* may help shed light on the evolutionary origins of pathogenicity among oomycete lineages.

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