Salisapiliaceae – a new family of oomycetes from marsh grass litter of southeastern North America

J. Hulvey¹, S. Telle², L. Nigrelli², K. Lamour¹*, M. Thines²,³*

Key words
internal transcribed spacer
nuclear ribosomal large subunit (nrLSU)
Peronosporales
phylogeny
Pythiaceae

Abstract  Several filamentous oomycete species of the genus Halophytophthora have recently been described from marine environments, mostly from subtropical and tropical ecosystems. During a survey of oomycetes from leaf litter of Spartina alterniflora in salt marshes of southeastern Georgia, isolates of four taxa were recovered that bore similarity to some members of Halophytophthora but were highly divergent from isolates of Halophytophthora s.str. based on a combined sequence analysis of two nuclear loci. In phylogenetic analyses, these isolates were placed basal to a monophyletic group comprised of Pythium of the Pythiaceae and the Peronosporales. Sequence and morphology of these taxa diverged from the type species Halophytophthora vesicula, which was placed within the Peronosporales with maximum support. As a consequence a new family, the Salisapiliaceae, and a new genus, Salisapilia, are described to accommodate the newly discovered species, along with one species previously classified within Halophytophthora. Morphological features that separate these taxa from Halophytophthora are a smaller hyphal diameter, oospore production, lack of vesicle formation during sporulation, and a plug of hyaline material at the sporangial apex that is displaced during zoospore release. Our findings offer a first glance at the presumably much higher diversity of oomycetes in estuarine environments, of which ecological significance requires further exploration.

Article info  Received: 23 September 2010; Accepted: 26 October 2010; Published: 14 December 2010.

INTRODUCTION

The described species of marine oomycetes are diverse and include pathogens of algae, marine nematodes and crustaceans, as well as decomposers of leaf litter (Dick 2001, Sekimoto et al. 2007, Beakes & Sekimoto 2009). Species of the genera Pythium and Halophytophthora (commonly placed in the Pythiaceae) are among the few oomycetes reported from marine leaf litter from all over the world (Newell 1992, Nakagiri et al. 2001), with an assumed centre of diversity in the subtropics and tropics. The genus Halophytophthora was originally erected to accommodate pythiaceous taxa that were formerly referred to as Phytophthora but which all originated from marine leaf litter (Ho & Jong 1990), thus representing a heterogeneous genus defined by its ecological preference. Members of the genus exhibit zoospore release with or without the presence of a vesicle or with a semi persistent vesicle. These features, along with other assexual characters, were used to initially segregate the first nine species of the genus from Phytophthora and for delineation among them. Subsequently, additional members of this genus have also been described on the basis of morphological characters, with the most recent in 2003 (Ho et al. 2003). However, there is so far no comprehensive phylogeny for this genus, and although some conference abstracts report some phylogenetic investigation in this group (Nakagiri & Okane 2005, Nakagiri et al. 2008) only a single species of Halophytophthora s.str. has been included in multigene phylogenetic investigations in the Peronosporales (Göker et al. 2007). Given the range of zoospore release patterns exhibited, many of which are present in either Phytophthora or Pythium, it is reasonable to question whether the genus is indeed a monophyletic group, or whether the inclusion of all marine oomycetes from leaf litter in a single genus is synthetic and not reflecting evolutionary relationships. Phylogenetic analyses in several groups of oomycetes have revealed morphological characters suitable for the delineation of phylogenetic groups that had not been previously considered valuable for taxonomic studies, e.g. in Albuginaceae (white blister rusts, Voglmayr & Riethmüller 2006, Choi et al. 2007, 2008, Thines et al. 2009a, Ploch et al. 2010), Peronosporaceae (downy mildews and Phytophthora, Göker et al. 2003, Voglmayr et al. 2004, Thines et al. 2006, 2007, Voglmayr & Constantinescu 2008), and water moulds (Saprolegniales, Riethmüller et al. 1999, Hulvey et al. 2007, Sekimoto et al. 2009). It was thus the aim of this study to evaluate with molecular phylogenetic tools, whether the morphologically divergent isolates recently sampled from salt marshes in southeastern North America and the type species of Halophytophthora, form a monophyletic assemblage or are polyphyletic in origin.

MATERIALS AND METHODS

Isolates
Marsh grass (Spartina alterniflora) leaf litter was collected from three salt marsh sites on Sapelo Island and adjacent islands (Georgia, USA) during the summer of 2009. Leaf litter and leaf fragments from the mud surface were collected, rinsed in ambient brackish water, and plated onto dilute V8 seawater agar (40 ml V8 juice, 3 g CaCO₃, 16 g Bacto agar and 960 ml seawater) amended with 25 ppm pimarin, 100 ppm ampicillin, 25 ppm rifampicin, 25 ppm pentachloronitrobenzene (PARP). Mycelium was observed growing from leaf material into agar after 1–3 d

¹ University of Tennessee, Genome Science and Technology Graduate Program, Knoxville, TN, USA.
² Biodiversity and Climate Research Centre (BiK-F), Senckenberganlage 25, D-60325 Frankfurt (Main), Germany; corresponding author e-mail: Marco.Thines@senckenberg.de.
³ Johann Wolfgang Goethe University, Department of Biological Sciences, Institute of Ecology, Evolution and Diversity, Siesmayerstrasse 70, D-60323 Frankfurt am Main, Germany.
* joined senior authors, listed alphabetically.

© 2010 Nationaal Herbarium Nederland & Centraalbureau voor Schimmelcultures
You are free to share - to copy, distribute and transmit the work, under the following conditions:
Attribution: You must attribute the work in the manner specified by the author or licensor (but not in any way that suggests that they endorse you or your use of the work).
Non-commercial: You may not alter, transform, or build upon this work.
No derivative works: You may not alter, transform, or build upon this work.
For any reuse or distribution, you must make clear to others the license terms of this work, which can be found at http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode. Any of the above conditions can be waived if you get permission from the copyright holder. Nothing in this license impairs or restricts the author’s moral rights.
Gene amplification and sequencing

Genomic DNA was extracted from cultures of the isolates and two of culture collection specimens using methods described previously (Lamour & Finley 2006). Subsequent PCR amplification of the rDNA ITS region (comprising partial ITS1, 5.8S, and partial ITS2 sequences), and partial nrLSU, from the nuclear genome was done for phylogenetic analyses. Amplification of the ITS gene was done using the primers ITS4 and ITS5 (White et al. 1990). Primers for amplification of the D1 and D2 regions of the rDNA large subunit were LROR (Moncalvo et al. 1995) and L96R (Riethmüller et al. 2002). The PCR temperature regime is as follows for all loci amplified: an initial denaturation at 96 °C for 2 min was followed by 35 cycles consisting of denaturation at 96 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min. A final extension step at 72 °C for 10 min was added for the completion of only partially amplified strands (Lee & Taylor 1992). All PCR reactions were done on a thermal cycler (Mastercycler, Eppendorf, Hamburg, Germany). The amplicons were sent to the University of Tennessee Knoxville Molecular Biology Resource Center for sequencing, with the primers used in PCR. Forward and reverse sequence electropherograms were manually trimmed of poor sequence data, and assembled using the CodonCode Aligner v3.0.3 sequence alignment software (CodonCode, Dedham, MA). The sequences obtained were submitted to GenBank (for accession numbers see Table 2).

Phylogenetic analyses

The set of sequences used in this analysis was combined from the dataset of Lévesque & de Cock (2004) and the sequences of the species Aphanomyces euteiches ATCC 201684 (AY683887.1, AF235939.1), Phytophthora infestans (genome, WGS-AATU-cont1-5907, WGS-AATU-cont1-5932) and P. sojae (genome, AAQY01000172-1, AAQY01000172) with the addition of LT6440, LT6456, LT6460, LT6465, LT6466, and LT6471, which were isolated from Sapelo Island and LT6430 isolated from Saint Simon’s Island (Table 1). Also included in the analysis were H. vesicula (CBS 152.96) and H. tartarea (CBS 208.95). Sequences were aligned using MAFFT (Katoh et al. 2005), v6 (Katoh & Toh 2008a) using the Q-INS-i option (Katoh & Toh 2008b). Afterwards sequences were concatenated for phylogenetic inference. To ensure reproducibility and to avoid subjective biases, no manual editing except for the removal of leading and trailing gaps was done. Minimum evolution trees were computed using MEGA v4.0 phylogenetic analysis software (Tamura et al. 2007), with factory settings, except for using the Tamura-Nei model of nucleotide substitution. For inferring the robustness of

Table 2  Summary of some morphological features for species of Salisapilia and Halophytophthora s.str. – NA = not available.

| Species (strain number) | Culture collection no. | Plugged discharge tube | Zoospores discharged into a vesicle | Oogonial diam (µm) | Antheridial origin | Hyphal diam (µm) | GenBank accession no. |
|-------------------------|------------------------|------------------------|-----------------------------------|-------------------|-------------------|------------------|----------------------|
| Salisapilia tartarea*   | CBS 208.95             | Yes                    | No                                | 33–66             | diclinous         | 1–3              | HQ232472 HQ232464   |
| Salisapilia sapeloensis | CBS 127948             | Yes                    | No                                | 35–60             | paragynous        | 1–3              | HQ232466 HQ232457   |
| Salisapilia nakagiri   | CBS 127947             | NA                     | NA                                | 33–48             | diclinous         | 1–3              | HQ232467 HQ232458   |
| Salisapilia sp. (LT6465) | CBS 127948             | NA                     | NA                                | NA                | NA                | 1–3              | HQ232468 HQ232459   |
| Salisapilia sp. (LT6466) | CBS 127947             | NA                     | NA                                | NA                | NA                | 1–3              | HQ232470 HQ232461   |
| Salisapilia sp. (LT6471) | CBS 127949             | NA                     | NA                                | NA                | NA                | 1–3              | HQ232471 HQ232462   |
| Halophytophthora vesicula | CBS 152.96             | No                     | Yes                               | NA                | NA                | 1–6              | HQ232473 HQ232463   |
| Halophytophthora sp. 1 (LT6430) | NA                   | No                     | Yes                               | NA                | NA                | 1–5              | HQ232465 HQ232456   |
| Halophytophthora sp. 2 (LT6465) | NA                   | No                     | Yes                               | NA                | NA                | 1–6              | HQ232469 HQ232460   |

* syn. Halophytophthora tartarea.

Table 3  Homology of Salisapilia sapeloensis nrLSU (HQ232457) compared to selected oomycetes.

| Family | Species (GenBank accession no.) | Maximum identity |
|--------|---------------------------------|------------------|
| Peronosporaceae | Phytophthora infestans FJ896987.1 | 76 % |
| Peronosporaceae | Bremia lactuca EF533478.1 | 74 % |
| Peronosporaceae | Halophytophthora vesicula HQ232463.1 | 78 % |
| Peronosporaceae | Pythophyllum oedechouli AY598664.1 | 77 % |
| Phytieae | Pythium monomorum AY598621.1 | 76 % |
| Phytieae | Lagendidium chthamalophilum AF235946.1 | 77 % |
| Albuginaceae | Albugo candida AF235938.1 | 76 % |
| Rhizophiae | Sapromyces elongatus AF235950.1 | 74% |
| Saprolegniaceae | Saprolegnia ferox AF235953.1 | 77 % |
| Leptogniaceae | Aphanomyces piscicida AF235941.1 | 77 % |

* Query coverage 99 %.

and was aseptically transferred to water agar plates, resulting in diffuse colonies. Single hyphal tips were transferred to dilute V8 PARP agar Petri dishes and these cultures were utilised for genetic and morphological characterisation. The oomycete isolates used in this study are listed in Table 1 and 2.

Morphology

Colony morphology was documented from cultures growing on V8 PARP plates. For light microscopy of sporangia and gametangia, agar cubes were aseptically removed from the leading edge of agar colonies and incubated in 15 ml of half-strength seawater (13 ‰ salinity) or distilled water in Petri dishes for 5–10 d. Sporangia and oospores were photographed and measured using a Nikon Eclipse 80i microscope, and the Nikon NIS-Elements v2.2 digital imaging software. One hundred measurements were taken for all morphological features, from which mean values were calculated.

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

| Taxa recovered | Collector | Location | GPS coordinates |
|----------------|-----------|----------|-----------------|
| Salisapilia sapeloensis (LT6440) | J. Hulvey | USA, GA, Sapelo Island, Cabretta Island | N31.43888, W81.23908 |
| Salisapilia nakagiri (LT6456) | J. Hulvey | USA, GA, Sapelo Island, Cabretta Island | N31.43888, W81.23908 |
| Salisapilia sp. (LT6466) | J. Hulvey | USA, GA, Sapelo Island, Cabretta Island | N31.43888, W81.23908 |
| Salisapilia sp. (LT6460) | J. Hulvey | USA, GA, Sapelo Island, Teal Boardwalk | N31.43888, W81.23908 |
| Salisapilia sp. (LT6471) | J. Hulvey | USA, GA, Sapelo Island, Teal Boardwalk | N31.43888, W81.23908 |
| Halophytophthora sp. 2 (LT6465) | J. Hulvey | USA, GA, Sapelo Island, Teal Boardwalk | N31.43888, W81.23908 |
| Halophytophthora sp. 1 (LT6430) | J. Hulvey | USA, GA, Saint Simon's Island | N31.15288, W81.41602 |

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

Phylogenetic analyses

The set of sequences used in this analysis was combined from the dataset of Lévesque & de Cock (2004) and the sequences of the species Aphanomyces euteiches ATCC 201684 (AY683887.1, AF235939.1), Phytophthora infestans (genome, WGS-AATU-cont1-5907, WGS-AATU-cont1-5932) and P. sojae (genome, AAQY01000172-1, AAQY01000172) with the addition of LT6440, LT6456, LT6460, LT6465, LT6466, and LT6471, which were isolated from Sapelo Island and LT6430 isolated from Saint Simon’s Island (Table 1). Also included in the analysis were H. vesicula (CBS 152.96) and H. tartarea (CBS 208.95). Sequences were aligned using MAFFT (Katoh et al. 2005), v6 (Katoh & Toh 2008a) using the Q-INS-i option (Katoh & Toh 2008b). Afterwards sequences were concatenated for phylogenetic inference. To ensure reproducibility and to avoid subjective biases, no manual editing except for the removal of leading and trailing gaps was done. Minimum evolution trees were computed using MEGA v4.0 phylogenetic analysis software (Tamura et al. 2007), with factory settings, except for using the Tamura-Nei model of nucleotide substitution. For inferring the robustness of
Fig. 1 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.
Phylogenetic analyses

Several oomycete isolates from marsh grass litter (LT6440, LT6456, LT6460, LT6466, LT6471), and *H. tartarea* formed a monophyletic clade with maximum support values in all analyses (Fig. 1). This clade contained five phylogenetically distinct lineages and is sister to a monophyletic subtree that contains all members of the genus *Pythium*, as well as *Phytophthora* species, which was moderately (88 % bs) supported in Maximum Likelihood, but received maximum support in both Minimum Evolution and Bayesian analyses. Within this subtree, *Pythium* was placed sister to the *Peronosporaceae*, which contain *Phytophthora* and *Pythoophthora*, but also *Halophytophthora* s.str. (the type of the genus, *H. vesicula*, as well as *H. poly-morpha*, and *H. avicenniae*). The isolates LT6430 and LT6465 also cluster together with *Halophytophthora* s.str., which was a monophyletic assemblage that received maximum support in all analyses. The sequence divergence of the oomycetes from marsh grass litter was similar to other oomycete families (Table 3), and ranged from 74 to 78 % homology, highlighting the isolated position of this group.

**Taxonomy**

The fact that the newly discovered phylogenetic lineage is the sister group to all other *Peronosporales* (*Peronosporaceae* s.l. and *Pythiaceae*) included in this study requires the recognition of the new family *Salisapiliaceae*, as the *Pythiaceae* would become paraphyletic through the inclusion of the phylogenetic lineage revealed here. This necessitates both the description of a new genus and a new family for accommodating the species of the new phylogenetic lineage.

**Salisapiliaceae** Hulvey, Nigrelli, Telle, Lamour & Thines, fam. nov. — MycoBank MB517464

*Salisapilia* Hulvey, Nigrelli, Telle, Lamour & Thines, gen. nov.

*Straminipila*, Oomycota, Peronosporales. Mycelium saepe hyphae, hyphae regularis 1–3 µm in diametro, nonnumquam inflata et septata. Zoosporangia hyalina, obovata vel obovata, cum materia lentiformi in aqua marina siniseminalis vel tota salina, materia lentiformis absens in aqua destillata, tubus emittens perspicuus, cum materia hyalina lentiformi eminente ex apice qui inter egressionem zoosporarum absolutus est. Vesicula emittens perpetua non adest.

Type. *Salisapilia* Hulvey, Nigrelli, Telle, Lamour & Thines, gen. nov.

*Straminipila*, Oomycota, Peronosporales. Mycelium frequently branched, regular vegetative hyphae 1–3 µm diam, with occasional septations and hyphal swellings. Zoosporangia hyaline, obovate, with ovoid to oblate, with plugs in half strength or full strength seawater, absent in distilled water, discharge tube conspicuous, with hyaline plug protruding from apex, which is displaced during zoospore discharge. No persistent discharge vesicle present.

**Salisapilia** Hulvey, Nigrelli, Telle, Lamour & Thines, gen. nov. — MycoBank MB517465; Fig. 2

Colonie in agaro V8 stellate vel non-stellate vel petalatea, nonnumquam cum hyphis aeris. Hyphae regularis 1–3 µm in diametro, glabra vel irregularis, interdum septatae, saepe ramosae. Hyphae nonnumquam cum tumoris. Zoosporangia hyalina abundantis in fragmentis agari V8 in aqua marina semiseminalis vel tota sahina, in aqua destillata absoluta. Zoosporangia obovata vel obovata, cum materia lentiformi, cum materia hyalina lentiformi eminente ex apice. Boylaria hyalina lentiformis 1–9 µm. Liberatio zoosporarum detractione materiae lentiformis et exitu zoosporarum mobilium per foramen emittens. Species oosporae facentes homothallicae sunt. Oosporeae 33–66 µm in longitudine, cum materia hyalina lentiformi eminente ex apice. Materia hyalina lentiformis 1–9 µm. Liberatio zoosporarum detractione materiae lentiformis et exitu zoosporarum mobilium per foramen emittens. Species oosporae facentes homothallicae sunt. Oosporeae 33–66 µm in diametro, globosae vel ovatae, crescentes terminales vel intercalares inter hyphas. Anthedera paraguay vel diclina. Cellula antheridii glabra et clavata in speciebus diclinis vel simplici, lobula vel ramosa in speciebus paragynis.

Type. *Salisapilia* sapeloensis Hulvey, Nigrelli, Telle, Lamour & Thines, sp. nov.

**Etymology**

From Latin *sal* = salt and *-sapilis* = of muck or detritus.

Colonies on V8 agar stellate, or non-stellate, or petalate with occasional aerial hyphae (Fig. 2d–h). Regular vegetative hyphae 1–3 µm diam, smooth to irregular, with occasional...
septations, frequently branching. Hyphal swellings occasional. Abundant hyaline zoosporangia produced on V8 agar plugs in half strength or full strength seawater, absent in distilled water. Zoosporangia obovate, ovoid, to obovate. Discharge tube conspicuous, 33–97 µm in length, with hyaline plug protruding from apex. Hyaline plug 1–9 µm. Zoospore release occurs first by displacement of the plug, followed by exit of motile zoospores from the discharge pore. Species with known sexual reproduction homothallic. Oospores 33–66 µm diam, spherical to ovoid, arising terminal or intercalary along hyphae. Antheridia paragynous or diclinous. Antheridial cell smooth and club-like in diclinous species, or simple, lobed or branching in paragynous species.

**Salisapilia nakagirii** Hulvey, Nigrelli, Telle, Lamour & Thines, *sp. nov.* — MycoBank MB517466; Fig. 2f, 3e–h
Coloniae in agaro V8 stellatae. Hyphae glabrae vel irregulares, ramosae nonnumquamque septatae. Zoosporangia in aqua marina semisalina vel tota salina vel destillata absentia. Oogonia hyalina, globosa, 33–48 µm (medio 39 µm), pariete glabra, 2–7 µm in crassitudine. Antheridia diclinia. Cellula antheridii forma clavae, 3–10 µm in longitudine. Oosporae hyalinae, 28–44 µm in diametro (medio 36 µm), pariete glabra 1–7 µm in crassitudine.

*Etymology.* Dedicated to Dr. Akira Nakagiri, who characterised several marine filamentous oomycetes.

Colony on V8 agar stellate (Fig. 2f). Hyphae smooth to irregular, branching and occasionally septate (Fig. 3e). Zoosporangia absent in half or full strength seawater, or distilled water. Oogonia hyaline, spherical, 33–48 µm (mean = 39 µm), with a smooth wall, 2–7 µm thick. Antheridia diconoid. Antheridial cell club shaped, 3–10 µm in length (Fig. 3f, g). Oosporae hyalinae, with a uniformly refractile ooplast vacuole, surrounded by cytoplasm with uniformly dispersed small lipid droplets, 28–44 µm diam (mean = 36 µm), with a smooth wall 1–7 µm thick (Fig. 3h).

**Salisapilia sapeloensis** Hulvey, Nigrelli, Telle, Lamour & Thines, *sp. nov.* — MycoBank MB517467; Fig. 2e, 3a–d
Coloniae in agaro V8 irregularae, plerumque hyphis coloniarum submersis in agaro, nonnumquam cum hyphis aeris. Hyphae glabrae vel irregulares, ramosae et nonnumquam septatae. Zoosporangia abundantia in aqua marina semisalina vel tota salina. Sporangiophori ramosi vel non ramosi, 1–2 µm in latitudine. Oosporae hyalinae, ovata vel obpyriformia et papillata. 34–97 µm in longitudine (medio 59 µm) sine tubo emittente. Zoosporangia non aucta. Materia lentiformis sporangii in sporangii maturi 3–8 µm. Tubus emittens oblongus, 6–18 µm in longitudine, pauciulare ab basi ad apicem angustior. In maturitate sporangiorum materia lentiformis secedit ab pariete sporangii et elongat, ergo eminens ex tubo emittente. Zoosporae emissis materia lentiformis liberatur et crebro comprimit et extendit in longitudine. Sporangiophora ovatae vel reniformes, latere bilacliaetatae, 5–6 µm in diametro, 12–20 sporangia in sporangio singularia (medio 15). Sporangiophora disgressione flagellarii stadium quietis inquietat. Zoosporae in studio quietis 5–7 µm in diametro. Oogonia hyalina, globosa vel ovata, 35–60 µm (medio 49 µm). Oosporae hyalinae, 28–56 µm (medio 48 µm), pariete glabra, 2–9 µm in crassitudine. Antheridia paragyna. Cellula antheridii simplex, lobula vel ramosa, pariete glabra, 2–9 µm in longitudine.

*Etymology.* Sapeloensis = of Sapelo Island, the location where the species was first isolated from.

Colony on V8 agar irregular, with colony hyphae mostly submerged in agar, with aerial hyphae (Fig. 2e). Hyphae smooth to irregular, branching and occasionally septate (Fig. 3a). Zoosporangia abundant in half or full strength seawater (Fig. 3a). Sporangiophores branched or unbranched, 1–2 µm wide. Zoosporangia hyaline, ovoid to obpyriform, and papillate. 34–97 µm in length (mean = 59 µm), excluding discharge tube. Zoo-
sporangia non-proliferating. Sporangial plug 3–8 µm in mature sporangia (Fig. 3b). Discharge tube elongate, 6–18 µm in length, slightly tapering from base to tip (Fig. 3b). During ripening of sporangia, the plug appears to become separate from the sporangial wall and elongates so that it is partially protruding from the discharge tube (Fig. 3b). At zoospore discharge, the plug is released, at which time it decompresses and expands several times its initial length. Zoospores ovoid to reniform, laterally biflagellate, 5–6 µm diam, each sporangium containing 12–20 zoospores (mean = 15). Zoospores encyst by withdrawal of flagella. Encysted zoospores 5–7 µm diam. Oogonia hyaline, spherical to ovoid, 35–60 µm (mean = 49). Oospores hyaline, with a uniformly refractile ooplast vacuole, surrounded by cytoplasm with uniformly dispersed small lipid droplets, 28–56 µm (mean = 48 µm), with a smooth wall, 2–9 µm thick. Antheridia paragynous. Antheridial cell may be simple, lobed or branched (Fig. 3c, d) with a smooth wall, 2–9 µm in length.

Substratum — Decaying litter of Spartina alterniflora. Known distribution — Southeastern North America.

Specimens examined. USA, Georgia, Sapelo Island, isolated from leaf litter of Spartina alterniflora at Sapelo Island, July 2009, Jon Hulvey, holotype CBS H-20477, culture ex-type CBS 127946 = LT6440.

**Salisapilia tartarea** (Nakagiri & S.Y. Newell) Hulvey, Nigrelli, Telle, Lamour & Thines, comb. nov. — MycoBank MB517468

*Basionym*. *Halophytophthora tartarea* Nakagiri & S.Y. Newell, Mycologia 35: 224.

**DISCUSSION**

Originally, all species of *Halophytophthora* were united by their lack of production of oospores, until the description of *H. tartarea* from leaf litter from Florida (Ho & Jong 1990, Nakagiri et al. 1994). Here we show that *H. tartarea* is highly divergent from *Halophytophthora* s.str. (the monophyletic group which includes the type species), and belongs to the newly described genus *Salisapilia*. Species of *Salisapilia* are united by the absence of a vesicle during spore discharge, and the presence of a protruding plug of material at the discharge tube apex that is displaced during zoospore release. *Salisapilia tartarea* is closely related to *S. sapeloensis* and is morphologically similar to this species with regards to size of hyphae, zoospores, sporangia, as well as homothallic reproduction, but differs markedly from it by antheridial origin, which is mostly diclinous in *S. tartarea* and *S. nakagiri*, but paragynous (sensu Nakagiri et al. 1994) in *S. sapeloensis*. The exact mode of oosporic production in
Salisapilia will require future detailed studies, as the antheridal origin might be variable (Nakagiri et al. 1994). Several other species of Halophytophthora, H. bahamensis, H. epistomium, H. exoprolifera, and H. operculata exhibit zoospore release without the presence of a vesicle (Nakagiri 2000). These species also possess a plug of material at the discharge pore apex which is displaced during zoospore release, which is considered typical for Salisapilia. None of these species seems to be conspecific with either S. nakagiri or S. sapeloensis, based on morphological and biological characteristics. However, in the absence of phylogenetic data, we refrain from transferring these species to Salisapilia, because of their partly deviating morphology, and because it cannot be ruled out at present that the operculate sporangia represent an ancestral trait of the Peronosporales s.l.

Subtleties in the zoospore release, including the presence of a persistent vs a semi persistent vesicle during zoospore release (H. masten), or the presence of an operculum that is displaced during zoospore release (H. operculata) are features that may be phylogenetically informative, and future investigations will reveal if species sharing these features may subsequently deserve separate generic status from Halophytophthora or Salisapilia (Ho et al. 1990, 1991, 1992, Pegg & Alcorn 1982, Nakagiri et al. 1994). This seems possible, since subtleties in zoospore release have been revealed to be phylogenetically informative for other oomycete genera, such as the genera Saprolegnia, Protoachyla, and Pythiosis of the Saprolegniales (Riethmüller et al. 1999). For H. spinosa, a phylogenetic position outside Halophytophthora s.str. has been reported in conference abstracts (Nakagiri & Okane 2005, Nakagiri et al. 2008), but it is unclear if this species belongs to the Salisapiliaceae, because of its divergent oospore morphology.

Oospores of Salisapilia exhibit a uniformly refractive, centric to subcentric ooplastic vacuole, surrounded by cyttoplasm with uniformly dispersed small lipid droplets. This is similar to other oomycetes in the peronosporalean lineage. The rather thin, smooth, and uniform oospore wall is similar to many species of Phythium and Phytophthym. but is much different from the more complex, multilayered resting spore walls reported from members of the Rhipidiales and the Albuginales. Also mycelium growth and sporangium formation provide further evidence for the inclusion of the Salisapiliaceae within the Peronosporales, rather than the description of a new order, which might be justified on the basis of the basal phylogenetic position, but seems superfluous at the moment.

It has been suggested that oomycetes originated from marine environments and migrated to land with host organisms early in the evolution of eukaryotes (Beakes & Sekimoto 2009). However, as no pathogenic growth could be associated with Salisapilia, it could well be possible, that several marine lineages were either non pathogenic or have reverted to a saprophytic lifestyle. Whether the plant pathogenic Peronosporaceae arose from a saprophytic ancestor, which gradually evolved to becoming pathogens in an intertidal environment, which is one plausible evolutionary scenario revealed by this study, or not, has to be revealed by future studies encompassing a broader sampling of oomycetes from marshes and mangroves. It is not finally resolved, whether several lineages of the Peronosporales independently managed the transition from marine to terrestrial and limnic environments, or whether multiple reversal events to a marine lifestyle have taken place. Considering the results from Thines et al. (2009b), who found that the Rhipidiales and Albuginales were the most basal groups of the Peronosporaceae, a reversal to adaptation to the marine environment is the more parsimonious explanation over the theory that Halophytophthora, and possibly also Salisapilia, represent phylogenetic lineages that are originally marine and have not made the transition to a terrestrial or limnic environment (Nakagiri 2000). The fact that Halophytophthora is the sister group of Phytophthora points at the possibility that Phytophthora might have directly arisen from a marine Halophytophthora-like ancestor. However, as the genus Phytophthora is the sister group to Halophytophthora and Phytophthora, and is represented by terrestrial plant pathogens, it is equally parsimonious to assume that Halophytophthora species have colonised marsh and mangrove habitats from terrestrial or limnic environments.

The Salisapiliaceae appear to represent an ancient lineage of the Peronosporales, which is sister to a monophyletic group containing both the Pythiaceae, represented by Phythium, and the Peronosporaceae, represented by the genera Phytophthora, Phytophthym, and Halophytophthora. This phylogenetic placement not only favours the broad circumscription of the Peronosporaceae to include the closely related groups of Phytophthora and the downy mildews (Göker et al. 2007, Thines 2009) as supposed in Thines et al. (2009b), but also the inclusion of the genera Halophytophthora s.str. and Phytophthym, which are morphologically similar to Phytophthora, for avoiding an inflationary introduction of family names within the Peronosporales. All species of Phythium included in the analysis were placed in a monophyletic group sister to the Peronosporales, thus demonstrating that the family status of the Pythiaceae is well-deserved. Whether additional genera now classified within the Pythiaceae will associate with this group or occupy distinct phylogenetic positions will have to be clarified by future studies. The current study represents a first step towards a taxonomy of the Peronosporomyceses mirroring the evolutionary relationships of these organisms. However, phylogenetic data for several rarely sampled phytaceous genera is lacking, and it seems likely that additional taxonomic revision will be necessary in the Peronosporales.

Acknowledgements LN, MT and ST have been supported by the research funding programme ‘LOEWE – Landes-Offensive zur Entwicklung Wissenschaftlich-ökonomischer Excellenz’ of Hesse’s Ministry of Higher Education, Research, and the Arts. Mark Windham (University of Tennessee) is gratefully acknowledged for allowing access to microscopic facilities. JH acknowledges the Genome Science and Technology Graduate Program (University of Tennessee) for financial support. Thanks are due to affiliates of the University of Georgia Marine Institute (UGAMI) of Sapelo Island, GA, including Research Scientist Dr. Melissa Booth, for use of facilities, and Christoph Rost, University of Hohenheim, for help with Latin descriptions. This work is dedicated to Professor Emeritus Dr. David Porter of the University of Georgia and retired Senior Research Scientist Dr. Steve Newell from UGAMI for supporting JH’s interest in the biodiversity of marine zoospore fungi during his undergraduate studies.

Author contributions – JH, KL, MT study design; JH strain isolation, microscopy, and morphological analyses; JH, ST initial sequence analysis; ST phylogenetic analyses; JH, LN, MT taxonomic analyses; JH, KL, LN , MT, ST manuscript preparation.

REFERENCES

Beakes GW, Sekimoto S. 2009. The evolutionary phylogeny of oomycetes - insights gained from studies of holocaric parasites of algae and invertebrates. In Lamour K, Kamoun S (eds), Oomycete genetics and genomics: 165–177. Wiley-Blackwell, New Jersey, USA.

Choi Y-J, Shin H-D, Hong S-B, Thines M 2007. Morphological and molecular discrimination among Albugo candida materials infecting Capsella bursapastoris worldwide. Fungal Diversity 27: 11–34.

Choi Y-J, Shin H-D, Ploch S, Thines M 2008. Evidence for uncharted biodiversity in the Albugo candida complex, with description of a new species. Mycological Research 112: 1327–1334.

Dick MW. 2001. Straminipilous fungi. Kluwer Academic Publishers, Dordrecht, Netherlands.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791.

Göker M, Voglmayr H, Riethmüller A, Oberwinkler F. 2007. How do obligate parasites evolve? A multi-gene phylogenetic analysis of downy mildews. Fungal Genetics and Biology 44: 105–122.
Göker M, Voglmayr H, Riemthüler A, Weiß M, Oberwinkler F. 2003. Taxonomic aspects of Peronosporaceae inferred from Bayesian molecular phylogenetics. Canadian Journal of Botany 81: 672–683.

Ho HH, Chang HS, Hsieh SY. 1991. Halophytophthora kandeliae: a new marine fungus from Taiwan. Mycologia 83: 419–424.

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

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

Hö Ha, Jong SC. 1990. Halophytophthora gen. nov., a new member of the family Pythiaceae. Mycotoxan 36: 377–382.

Lévesque CA, Cock WAM de. 2004. Molecular phylogeny and taxonomy of oomycetes. Mycologia 94: 834–849.

Ho HH, Chien YL. 1982. Phytophthora operculata sp. nov., a new marine fungus of Taiwan. Mycologia 74: 640–642.

Lee SB, Taylor JW. 1992. Phylogeny of five fungus-like protoctistan genera. Mycological Research 108: 951–962.

Lamour KH, Finley SL. 2006. A strategy for recovering high quality genomic DNA from a large number of Phytophthora isolates. Mycologia 98: 514–517.

Katoh K, Kuma K, Toh H, Miyata K. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Research 33: 511–518.

Katoh K, Toh H. 2008a. Recent developments in the MAFFT multiple sequence alignment program. Briefings in Bioinformatics 9: 286–298.

Katoh K, Toh H. 2008b. Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. BMC Bioinformatics 9: 212.

Lamour KH, Finley SL. 2006. A strategy for recovering high quality genomic DNA from a large number of Phytophthora isolates. Mycologia 98: 514–517.

Lee SB, Taylor JW. 1992. Phylogeny of five fungus-like protoctistan genera. Mycological Research 108: 951–962.

Lévesque CA, Cock WAM de. 2004. Molecular phylogeny and taxonomy of genus Pythium. Mycological Research 108: 1363–1383.

Moncalvo J, Wang H, Hseu R. 1995. Phylogenetic relationships in Gano-

Nakagiri A, Okane I. 2005. Phylogeny, taxonomy and ecology of Halophytophthora. Mycoscience 46: 333–339.

Nakagiri A, Newell SY. 1992. Halophytophthora species, inferred from the internal transcribed spacers of ribosomal DNA. Molecular Biology and Evolution 94: 636–653.

Nakagiri A, Inaba S, Toyama K, Hsieh S-Y. 2008. Diversity of marine oomycetes, Halophytophthora. Abstracts of papers presented at the meeting of the Mycological Society of Japan 52: 11.

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.

Kato K, Kuma K, Toh H. 2002. Phylogenetic studies of Sap-

Ploch S, Choi Y-J, Rost C, Shin H-D, Schilling E, Thines M. 2010. Evolution of diversity in Albugo is driven by high host specificity and multiple speciation events on closely related Brassicaceae. Molecular Biology and Evolution 57: 812–820.

Riemthüler A, Voglmayr H, Göker M, Weiß M, Oberwinkler F. 2002. Phylogenetic relationships of the downy mildews (Peronosporales) and related groups based on nuclear large subunit ribosomal DNA sequences. Mycologia 94: 834–849.

Riemthüler A, Weiß M, Oberwinkler F. 1999. Phylogenetic studies of Sap-

Sekimoto S, Hatai K, Honda D. 2007. Molecular phylogeny of an unidentified Haliphthoros-like marine oomycete and Haliphthoros milfordensis inferred from nuclear-encoded small- and large-subunit rRNA genes and mitochondri-encoded cox2 gene. Mycologia 48: 212–221.

Sekimoto S, Klokochova TA, West JA, Beakes GW, Honda D. 2009. Olpidiopsis bostrychiae sp. nov.: an endoparasitic oomycete that infects Bostrychia and other red algae (Rhodophyta). Phycologia 48: 460–472.

Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web servers. Systematic Biology 57: 758–771.

Stamatakis S. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24: 1596–1599.

Thines M. 2009. Bridging the gulf: Phytophthora and downy mildews are connected by rare grass parasites. PLoS ONE 4: e4790.

Thines M, Choi Y-J, Kemen E, Ploch S, Holub EB, Shin H-D, Jones JDG. 2009a. A new species of Albugo parasitic to Arabidopsis thaliana reveals new evolutionary patterns in white blister rusts (Albuginaceae). Persoonia 22: 123–128.

Thines M, Göker M, Oberwinkler F. Spring O. 2007. A revision of Plasmopara pennisetii, with implications for the host range of the downy mildews with pyriform haustoria. Mycological Research 111: 1377–1385.

Thines M, Göker M, Spring O, Oberwinkler F. 2006. A revision of Bremia graminicola. Mycological Research 110: 646–656.

Stamatakis S, Voglmayr H, Göker M. 2009b. Taxonomy and phylogeny of the downy mildews (Peronosporales). In: Lamour K, Kamoun S (eds). Oomycete genetics and genomics: 165–177. Wiley-Blackwell, New Jersey, USA. Voglmayr H, Constantinescu O. 2008. Revision and reclassification of three Plasmopara species based on morphological and molecular phylogenetic data. Mycological Research 112: 487–501.

Voglmayr H, Riemthüler A. 2006. Phylogenetic relationships of Albugo species (white blister rusts) based on LSU rDNA sequence and oospore data. Mycological Research 110: 75–86.

Voglmayr H, Riemthüler A, Göker M, Weiss M, Oberwinkler F. 2004. Phylo-

genic relationships of Plasmopara, Bremia and other genera of downy mildew pathogens with pyriform haustoria based on Bayesian analysis of partial LSU rDNA sequence data. Mycological Research 108: 1011–1024.

White TJ, Bruns SL, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), PCR protocols: a guide to methods: 315–322. Academic Press Inc., New York, USA.