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Two new *Halophytophthora* species, *H. tartarea* and *H. masteri*, from intertidal decomposing leaves in saltmarsh and mangrove regions

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Two new pythiaceous fungi were obtained from decaying leaves submerged in saltmarsh environments of the east coast of the U.S.A., or fringing mangroves in the Bahama Islands, and are described here as *Halophytophthora tartarea* and *H. masteri*. The two species have superficially similar zoosporangia whose dehiscence tubes have ragged-looking apices. However, differences in fine structures and development of the dehiscence tube and plug, characteristics of the dehiscence plugs, and presence or absence of vesicles clearly distinguish the two species. *Halophytophthora masteri* is the only species of *Halophytophthora* that has a zoospore release mechanism involving both an extruded plug and a vesicle. Cultural properties concerning growth and asexual reproduction at various salinities and temperatures are also different between the two, probably reflecting adaptation to their respective habitats. Though zoospore release in *H. masteri* occurs spontaneously from mature zoosporangia, it is remarkably enhanced in *H. tartarea* and also *H. masteri* by mildly dehydrating mature zoosporangia followed by rewetting with seawater, which suggests a possible relation between the asexual reproduction of these oomycetes and the tidal rhythm in their natural habitats.

Key Words——*Halophytophthora masteri*; *Halophytophthora tartarea*; mangrove; oomycetes; saltmarsh.

Introduction

In the course of ecological study of pythiaceous oomycetes on decaying leaves of a mangrove-saltmarsh boundary along the south-eastern coast of the U.S.A. (Newell, 1992) and mangrove ecosystems in the Bahama Islands (Newell and Fell, 1994), two previously unknown pythiaceous fungi were isolated by the second author (SYN). One species (represented by strain SAP 69) was found on decaying leaves of live oak (*Quercus virginiana* Mill.), loblolly pine (*Pinus taeda* L.), and smooth cordgrass (*Spartina alterniflora* Loisel.) submerged in coastal seawater in saltmarshes and was (mistakenly) tentatively assigned to '*Pythium* sp. 3' by Newell (1992). The other species (strains SAP 82, 83 and 84) was found on submerged decaying leaves of black mangrove [*Avicennia germinans* (L.) L.] within mangrove stands and previously referred to '*Halophytophthora* sp. B' by Newell and Fell (1994). Investigation on their morphology and cultural properties revealed both species to be members of *Halophytophthora* Ho & Jong. Comparison of the characteristics of the new strains with those of the 11 known species and 2 varieties of *Halophytophthora* warranted their description as two different new species in the genus. Because the two species have similar papillate zoosporangia with a dehiscence plug that extends beyond the exit pore and appears to be dissolving outside the dehiscence tube, and because this resemblance may lead to misidentification and confusion between the two, we here describe the two new species together and clarify their distinctive characteristics in morphology, ecology and physiology. Autoecological studies were carried out to learn how the new oomycetes have adapted to their own habitats. Effects of temperature and salinity on vegetative growth and asexual reproduction were investigated. The conditions permitting zoospore release from zoosporangia were also examined.

Materials and Methods

Isolation The strain SAP 69 (IFO 32606) was isolated from a decaying leaf of smooth cordgrass (*Spartina alterniflora*) submerged in seawater [23°C, 20‰ salinity (S)], which was collected from Ochlockonee Bay (30°N, 84°W), Florida, in November 1991 (Newell, 1992). The strains SAP 82-84 (IFO 32603-32605) were isolated from submerged decaying leaves of black mangrove (*Avicennia germinans*), which were collected from mangrove stands on the western or southern border of Exuma Sound (24°N, 76°W) in the Bahama Islands in July 1993 (seawater: 31-37°C, 37-42‰ S) (Newell and Fell, 1994). The strains were isolated by removing disks from leaf samples and plating drained disks on agar medium selective for oomycetes. SAP 69 was isolated on dilute (2% v/v) V-8 agar with pimaricin (10 mg/l) (see Newell, 1992) and SAP 82, 83 and 84 were isolated

respectively on media No. 2, No. 7 and No. 6 (V-8 agar media containing various antibiotics; Newell and Fell, 1994). Isolates were observed on vegetable juice seawater agar [VJSWA-20: 20% (v/v) non-salted vegetable juice (Kikkoman, Noda, Japan), 0.3% (w/v) calcium carbonate, 1.5% agar in 20‰ S artificial seawater (Jamarin S; Jamarin Lab., Osaka)] plates or corn meal seawater agar [CMSWA-20: Corn Meal Agar (Nissui, Tokyo) dissolved in 20‰ S seawater] plates.

Hyphal growth tests Vegetative growth at different salinities [0, 10, 20, 30, 40, 50, 60‰ S] or temperatures (10, 15, 20, 25, 30, 37°C) was examined by Nakagiri's (1993) method with some modifications. For SAP 69 (*H. tartarea*), CMSWA dissolved in seawater of different salinities was used. VJSWA was employed for SAP 82-84 (*H. masteri*) because growth of these strains on CMSWA was highly restricted. For subculturing the strains to obtain inocula for growth tests, CMSWA or VJSWA with 20‰ S seawater (CMSWA-20 or VJSWA-20) was employed. Agar discs (8 mm diam) cut from the edge of the subcultured colony were inoculated on the test agar plates. For the growth test at different salinities, cultures were incubated at 20°C. Growth at different temperatures was examined on CMSWA-20 or VJSWA-20 plates. After 2-4 days of incubation in the dark, colony diam was measured in duplicate experiments.

Zoosporangia formation Though the two new species produce zoosporangia on and in VJSWA plates, zoosporangia formation is enhanced by submerging mycelium in water. Zoosporangia formation was examined under different conditions of salinity and temperature (see Nakagiri, 1993). After subculturing strains on VJSWA-20 at 25°C for 7 days, agar discs with mycelium were submerged in water of different salinities (0-40‰ S) and incubated at 25°C for 1-2 days under a fluorescent lamp (300-500 lux) or in the dark. To test suitability of temperatures for zoosporangia formation, agar discs containing mycelium were soaked in 20‰ S (for *H. tartarea*) or 30‰ S (for *H. masteri*) seawater and incubated at different temperatures (10-37°C) for 1-2 days.

Induction of zoospore release To find effective conditions for inducing zoospore release, sudden increase or decrease in salinity or temperature and a combination of both factors of water surrounding mature zoosporangia were arranged in the manner employed by Nakagiri (1993). In addition, the effect of desiccation on zoospore release was examined. Agar discs with mature zoosporangia that had been produced by submersion in seawater were mildly dehydrated by transferring the agar discs onto a filter paper and keeping the filter in a petri dish for 1-24 h at room temperature (22-25°C). The dehydrated and shrunken agar discs were transferred into water (0-40‰ S) to observe the occurrence of zoospore release (if any).

Results

Taxonomy

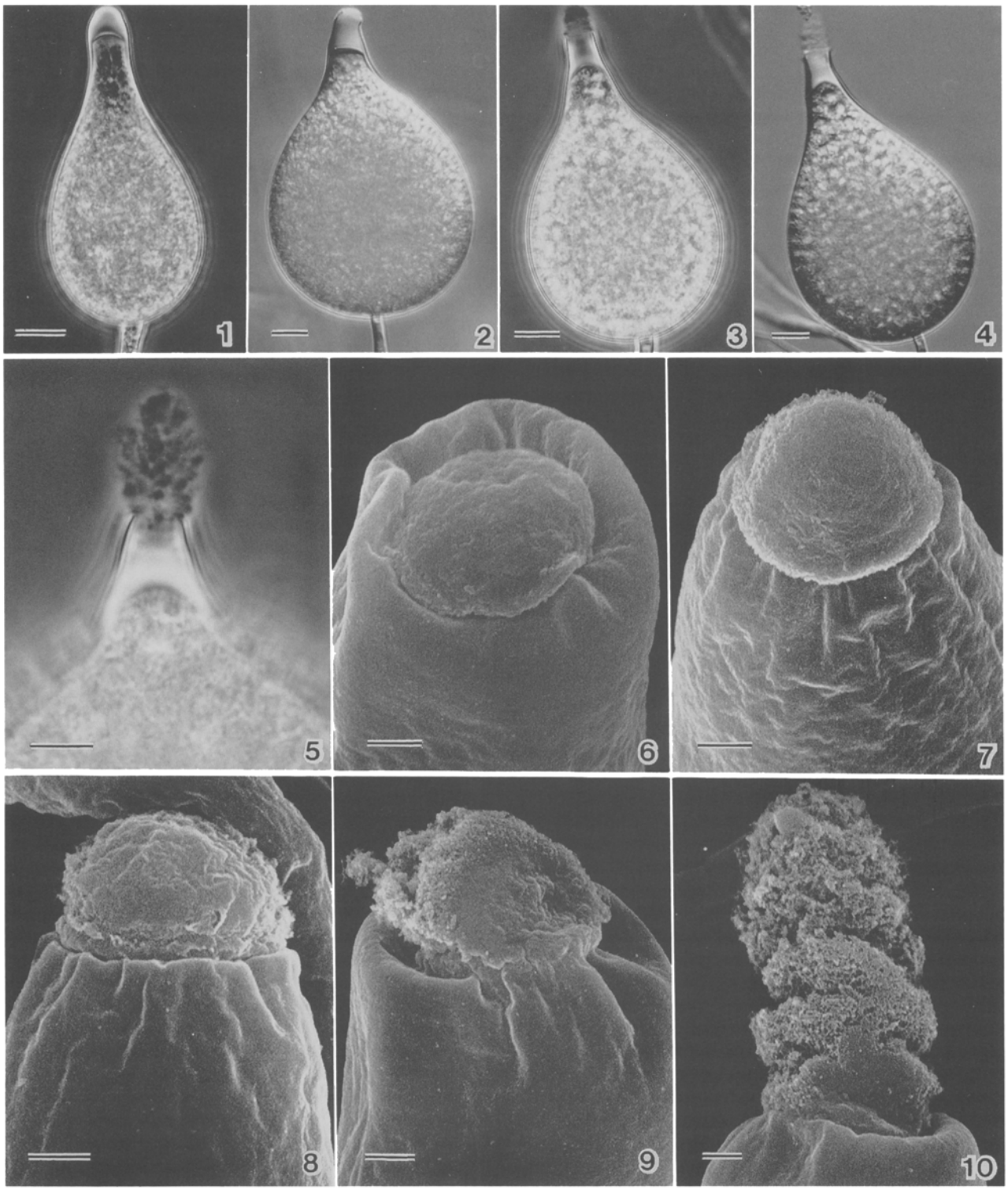
Halophytophthora tartarea Nakagiri & Newell, sp. nov. Figs. 1-21

Hyphae hyalinae, ramosae, glabrotunicatae, septatae in aetate, 1-3(-9) μm diam. Sporangiophora haud dissimilia, 140-580 μm longa, 1.5-4 μm diam, interdum ramosa sed haud sympodialia. Zoosporangia hyalina, terminalia, sphaerica ad ovata vel obpyriformia, (20-)55.6(-104) × (18-)47.6(-96) μm, sine prolificatione. Tubus dehiscens (10-)16.5(-22) × (4-)6.1(-8) μm. Obturamentum dehiscens incolor, (11-)19.5(-29) × (5-)6.5(-8) μm, ad maturitatem tartareum et per porum [(4-)6.1(-8) μm diam] extensum (usque ad 24 μm). Obturamentum extrusum, prolatum (usque ad 80 μm), prompte dissolvens. Zoosporae in sporangiis factae, (10-)12.3(-14) × (5-)5.3(-6) μm, ovoideae ad reniformes, lateraliter biflagellatae, post incystem sphaericae (5-)6.9(-9) μm diam, tubis germinationis 2-4 pullulantes. Antheridia paragyna, diclina, 4-10 μm diam ad maturitatem. Oogonia sphaerica (33-)53(-66) μm diam, ad basim angustata. Oosporae singulares, apleroticae praebentes spatium basale, hyalinae ad flavo-brunneae, sphaericae, (24-)48.2 (-62) μm diam, pariete laevi 3-10 μm crasso. Chlamydosporae non visae.

Holotypus, IFO H-12168.

Etymology. From the Latin *tartareus*=with a rough, crumbling surface, in reference to the dehiscence plug of eroding appearance (see Figs. 5, 10).

Hyphae hyaline, branched, smooth-walled, nonseptate or septate with age, 1-3(-9) μm diam. Colony on VJSWA-20 without rosette or stellate pattern (see Stamps et al., 1990). Sporangiophores 140-580 μm long, 1.5-4 μm wide, undifferentiated, unbranched or branched (not sympodial). Zoosporangia hyaline, spherical, ovoid to obpyriform, papillate, 20-104 μm long ($\bar{x}$=55.6 μm) (excluding dehiscence tube), 18-96 μm wide ($\bar{x}$=47.6 μm), length/breadth (L/B) ratio 1.0-1.9(-2.7) (Fig. 16). On the surfaces of VJSWA-20 plates, ellipsoidal zoosporangia tend to be produced. No internal or external proliferation observed. Subsporangial plug 1-10 μm thick, inconspicuous. Dehiscence tube 10-22 μm long ($\bar{x}$=16.5 μm), 4-8 μm wide ($\bar{x}$=6.1 μm), with dehiscence plug, 11-29 μm long ($\bar{x}$=19.5 μm), 5-8 μm wide ($\bar{x}$=6.5 μm). In immature zoosporangia, the dehiscence tube has a rounded apex with hyaline plug material inside (Figs. 1, 2). SEM photos show that the plug becomes swollen into a dome-like structure at the exit pore of the dehiscence tube (Figs. 6, 7). When zoospores are differentiated, the plug apex becomes loose (Figs. 3, 4). Plug material outside the exit pore extends up to 24 μm long, and presents a "cigarette-ash" shape (Figs. 5, 8-10). At zoospore release, the plug is extruded and elongated up to 80 μm (Figs. 11-15). The released plug dissolves in water, evanescing in 1-5 min. Exit pore 4-8 μm diam ($\bar{x}$=6.1 μm). Zoospores ovoid to reniform, laterally biflagellate, 10-14 × 5-6 μm ($\bar{x}$=12.3 × 5.3 μm) (Fig. 17), encysting by withdrawing flagella into spores (Fig. 18). Encysted spores 5-9 μm diam ($\bar{x}$=6.9 μm), germinating via 2-4 hyphae (Fig. 19). Oogonia spherical with tapered base, 33-66 μm diam ($\bar{x}$=53 μm). Oospores globose, single, hyaline to yellow brown, 24-62 μm diam ($\bar{x}$=48.2 μm), with smooth wall 3-10 μm thick, aplerotic with a slight space at the basal part of oo-

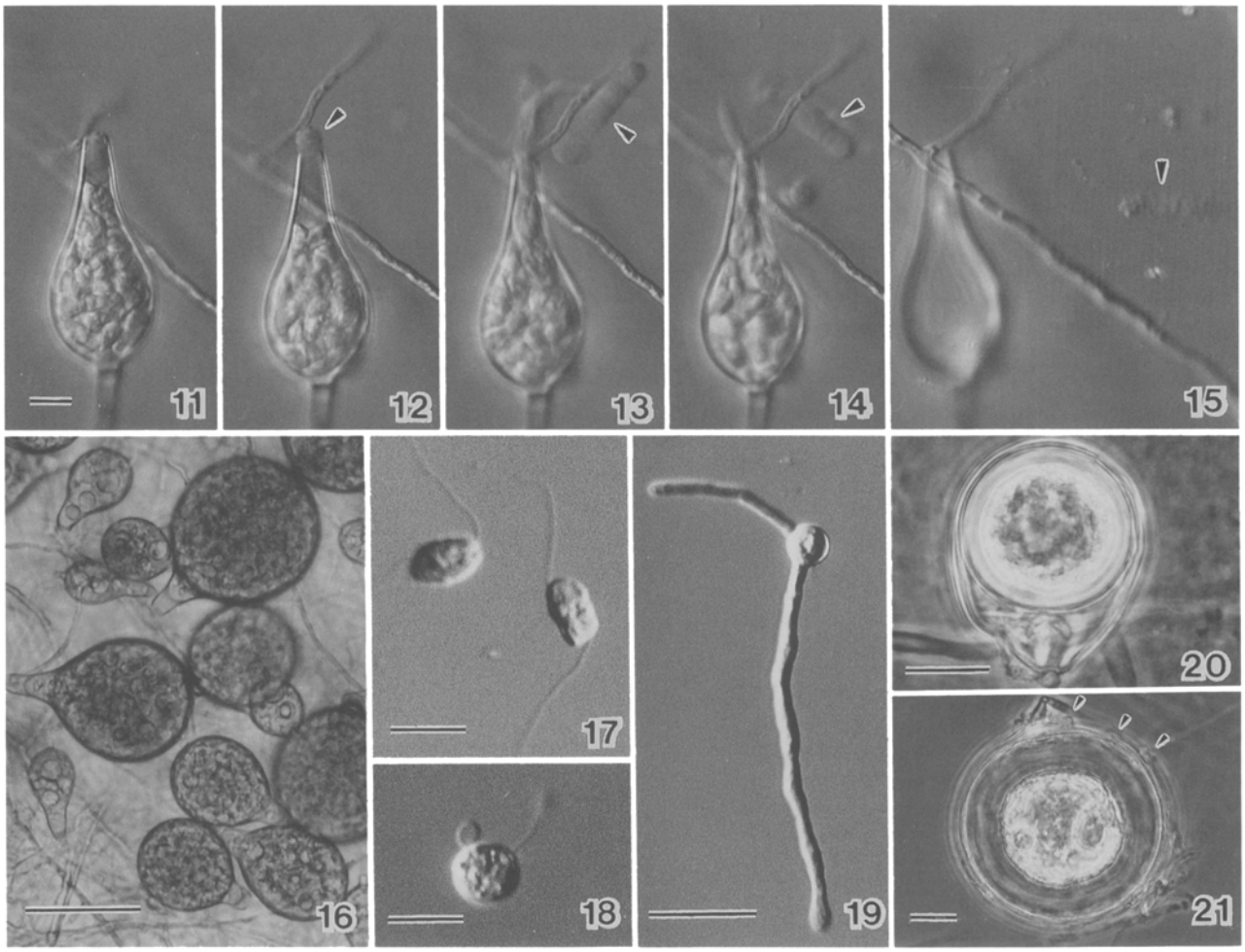


Figs. 1–10. Light and scanning electron micrographs of *Halophytophthora tartarea*. 1, 2. Immature zoosporangium with rounded apex of dehiscence tube. 3, 4. Mature zoosporangium with dissolving dehiscence plug. 5. Dissolving dehiscence plug outside the exit pore, resembling cigarette-ash. 6–10. Dissolving process of dehiscence plug. 6. Intact plug in young zoosporangium. 7. Plug apex swollen into dome-like shape in immature zoosporangium (see Fig. 1). 8, 9. Extruding and dissolving plugs in immature zoosporangia (see Fig. 2). 10. Extruding plug dissolved into cigarette-ash shape in mature zoosporangium (see Fig. 3–5). Bars: 1–5 = 10 μm; 6–10 = 1 μm.

gonia (Figs. 20, 21). Antheridia diclinous, paragynous, at first 1.5-2 μm wide, at maturity 4-10 μm wide, partly enwrapping oogonium (Fig. 21). Homothallic. Sexual reproduction was decreased by repetitive subculturing on V-8SWA and VJSWA, though soon after isolation from smooth cordgrass leaf, oospores had been produced abundantly on sitosterol-containing V-8SWA. Chlamydospores absent.

Holotype, IFO H-12168, voucher slides of the strain SAP 69 (IFO 32606) isolated from submerged decaying leaf of *Spartina alterniflora*, from Ochlockonee Bay, Florida, Nov. 1991, deposited in the herbarium of the Institute for Fermentation, Osaka (IFO).

The closest species to *H. tartarea* among the known halophytophthoras is *H. bahamensis* (Fell & Master) Ho & Jong whose dehiscence plug protrudes slightly from the exit pore and can be ragged in appearance (Fell and Master, 1975). For comparison with the new species, three strains of *H. bahamensis* including a culture derived from the holotype [ATCC 28296 (=IFO 32556), ATCC 28297 (=IFO 32557) and SAP 38 (=ATCC 64761, IFO 32607)] were examined in their morphology and culture properties. Consequently, we conclude that *H. bahamensis* differs from the new species in the shape of zoosporangium (highly variable, bursiform to obclavate for *H. bahamensis*), the appearance of the ragged dehiscence plug (not as extended and "cigarette-ash" shaped as in *H. tartarea*), the lower growth rates on CMSWA-20 at 25°C (1-4.3 mm/day vs. 22 mm/day for *H. tartarea*) and the absence of sexual reproduction. Comparison with the original description of *H. bahamensis* by Fell and Master (1975) also clearly separates *H. bahamensis* from *H. tartarea* in the smaller diam of encysted zoospores (1-6 μm, $\bar{x}=2.6$ μm vs. 5-9 μm, $\bar{x}=6.9$ μm), shorter dehiscence tubes (3-7 μm, $\bar{x}=5.1$ μm vs. 10-22 μm, $\bar{x}=16.5$ μm) and the nature of dehiscence plug (semi-persistent and dissolving in water less than 1 h after ejection in the former species, but evanescent and dissolving in 1-5 min in the latter). *Halophytophthora tartarea* can be inserted in Ho et al.'s (1991) key to species of *Halophytophthora* by adding a third choice at step 4: Dehiscence tube intermediate, 10-22 μm; plug extending



Figs. 11-21. Light micrographs of *Halophytophthora tartarea*. 11-15. Serial photos of zoospore release from a zoosporangium. Note dehiscence plug (arrows) extruded from exit pore, and elongated and dissolving in water. 16. Zoosporangia produced on VJSWA agar disc after submersion. 17. Zoospores. 18. Encysting zoospore retracting flagella. 19. Germinating zoospore. 20, 21. Oospores and antheridia (arrows). Bars: 11 (=12-15), 17-21=10 μm; 16=50 μm.

(up to 24 μm) through exit pore and taking-on a disintegrating or "cigarette-ash" shape.

Halophytophthora masteri Nakagiri & Newell, sp. nov. Figs. 22-45

Hyphae hyalinae, ramosae, glabrotunicatae, septatae in aetate, 2-10 μm diam. Tumores in hyphis clavati ad sphaerici, 12-36 μm longi, 8-30 μm lati, terminales vel intercalares, singulares vel catenati, interdum ramosi. Sporangiophora haud dissimilia, 80-1200 μm longa, 2.5-4 μm diam, interdum ramosa (raro sympodialia). Zoosporangia hyalina, terminalia, sphaerica ad ovata vel obpyriformia vel elongato-ellipsoidea (nonnunquam irregularia), (26-) 64 (-92 [nonnunquam > 200]) × (18-) 62.6 (-91) μm, sine prolificatione. Tubus dehiscens (5-) 12.1 (-28) × (6-) 8.5 (-10) μm. Obturamentum dehiscens hyalinum, (5-) 13.6 (-24) × (5-) 10.8 (-14) μm, extensum ad maturitatem, extrorsum laceratum, inflatum, laxe affixum in aetate. Vesicula tubularis 20-90 × 12-20 μm, extrinsecus expansa. Obturamentum dehiscens extrusum, prolatum (ad 18-40 μm longum), persistens, interdum affixum ad apicem vesiculae. Vesicula mox retracta in sporangium vel separata. Zoosporae in sporangiis factae, (13-) 13.8 (-15) × (5-) 6.3 (-8) μm, elabens per vesiculam. Zoosporae post incystem sphaericum, (8-) 10.1 (-12) μm diam, tubo germinationis singulari. Chlamydosporae non visae.

Holotypus, IFO H-12169.

Etymology. In honour of I. M. Master, co-author of a keystone paper on mangrove-oomycete ecology (Fell and Master, 1975).

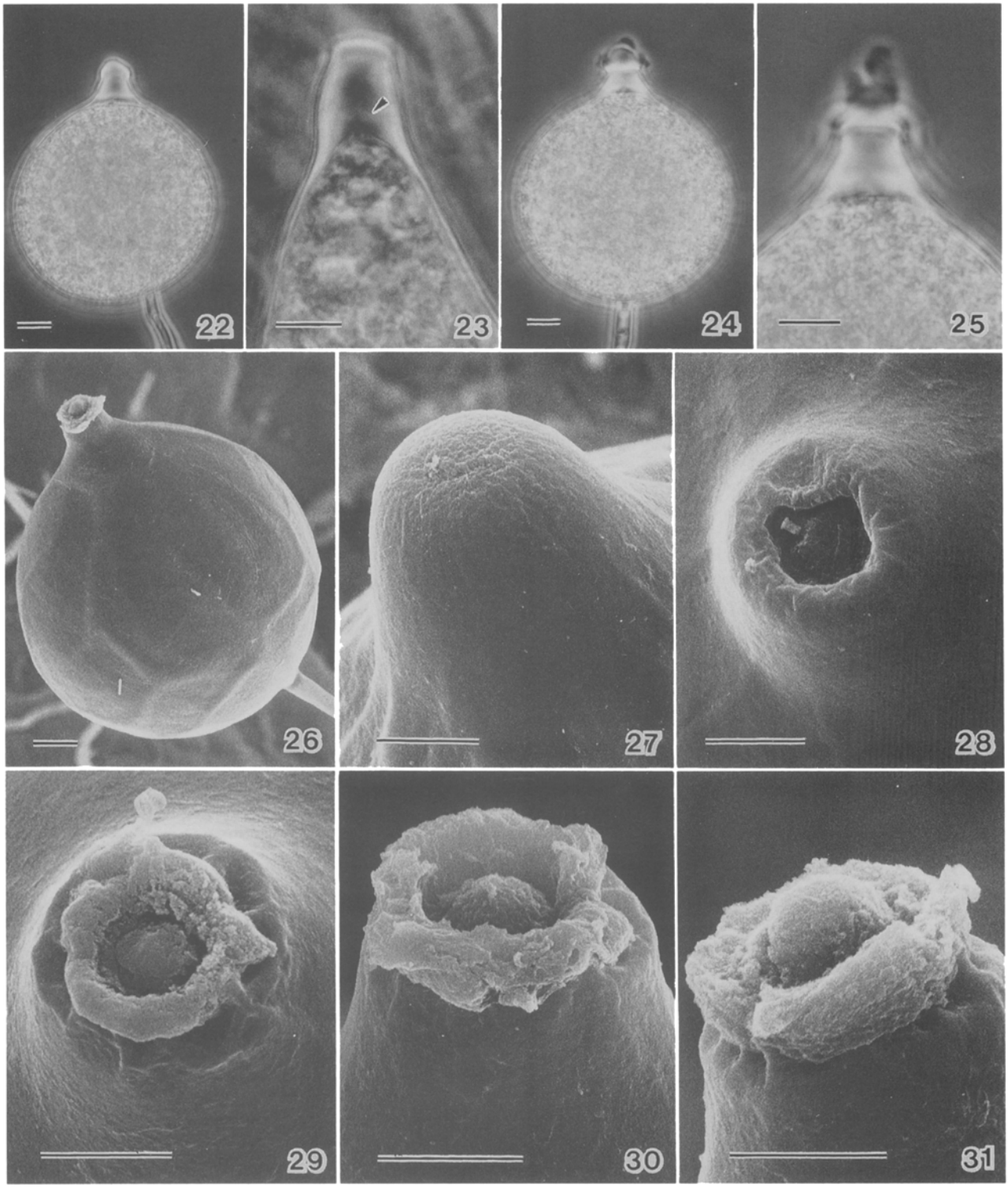
Hyphae hyaline, branched, smooth-walled, nonseptate or septate with age, 2-10 μm diam. Hyphal swellings clavate to spherical 12-36 μm long, 8-30 μm wide, intercalary or terminal, solitary or catenulate sometimes branched. Colony on VJSWA-20 with rosette pattern (see Stamps et al., 1990). Sporangiophores 80-1200 μm long, 2.5-4 μm wide, undifferentiated, unbranched or branched (rarely sympodial). Zoosporangia hyaline, spherical, ovoid to obpyriform, papillate, 26-92 μm long ($\bar{x}$ = 64 μm) (excluding dehiscence tube), 18-91 μm wide ($\bar{x}$ = 62.6 μm), L/B ratio 0.8-1.9(-2.5) (Fig. 26). On the surfaces of VJSWA-20 plates, ellipsoid to elongated [longer than 200 μm (including dehiscence tube)] and distorted zoosporangia tend to be produced. No internal or external proliferation observed. Subsporangial plug 1.5-8 μm thick. Dehiscence tube 5-28 μm long ($\bar{x}$ = 12.1 μm), 6-10 μm wide ($\bar{x}$ = 8.5 μm), with dehiscence plug, 5-24 μm long ($\bar{x}$ = 13.6 μm), 5-14 μm wide ($\bar{x}$ = 10.8 μm). In immature zoosporangia, the dehiscence tube has a rounded apex with hyaline plug material inside (Figs. 22, 27). Before and during zoospore differentiation, a small peak is sometimes seen at the top of the cytoplasm in zoosporangia (Fig. 23). In mature zoosporangia, the wall of the dehiscence tube tears away from the center of the apex (Figs. 24, 28, 29). The torn wall curls back and plug material is exposed (Figs. 30, 31), which then takes on irregular shapes (Fig. 25). Just before zoospore release, the plug material is extruded from the dehiscence tube (Figs. 32, 33). Then, a tubular vesicle (20-90 × 12-20 μm) is pushed out and zoospores swim out through an opening at the apex of the vesicle (Figs. 34-36). The extruded plug material becomes long (18-40 × 2-6 μm) in water (Figs. 35, 40, 41) and persists longer than 3 h. Since the plug material sometimes remains attached to the depressed apex of the vesicle (Fig. 42), the vesicle may be derived from a membrane surrounding the plug material. Vesicles usually shrank and were retracted completely into zoosporangia 7-10 min after zoospore release (Figs. 37-39), but sometimes they were detached from the exit pore of the dehiscence tube. Exit pore 6-10 μm diam ($\bar{x}$ = 8.5 μm). Zoospores ovoid to reniform, laterally biflagellate, 13-15 × 5-8 μm ($\bar{x}$ = 13.8 × 6.3 μm) (Fig. 43), encysting by withdrawing flagella into spores (Fig. 44). Encysted spores 8-12 μm ($\bar{x}$ = 10.1 μm), germinating via one hypha (Fig. 45). Sexual reproduction not observed. Chlamydospores absent.

Holotype, IFO H-12169, voucher slides of the strain SAP 83 (IFO 32604) isolated from submerged decaying leaf of *Avicennia germinans*, from Sampson Cay, Exuma Sound, the Bahama Islands, July 1993, deposited in the herbarium of the Institute for Fermentation, Osaka (IFO).

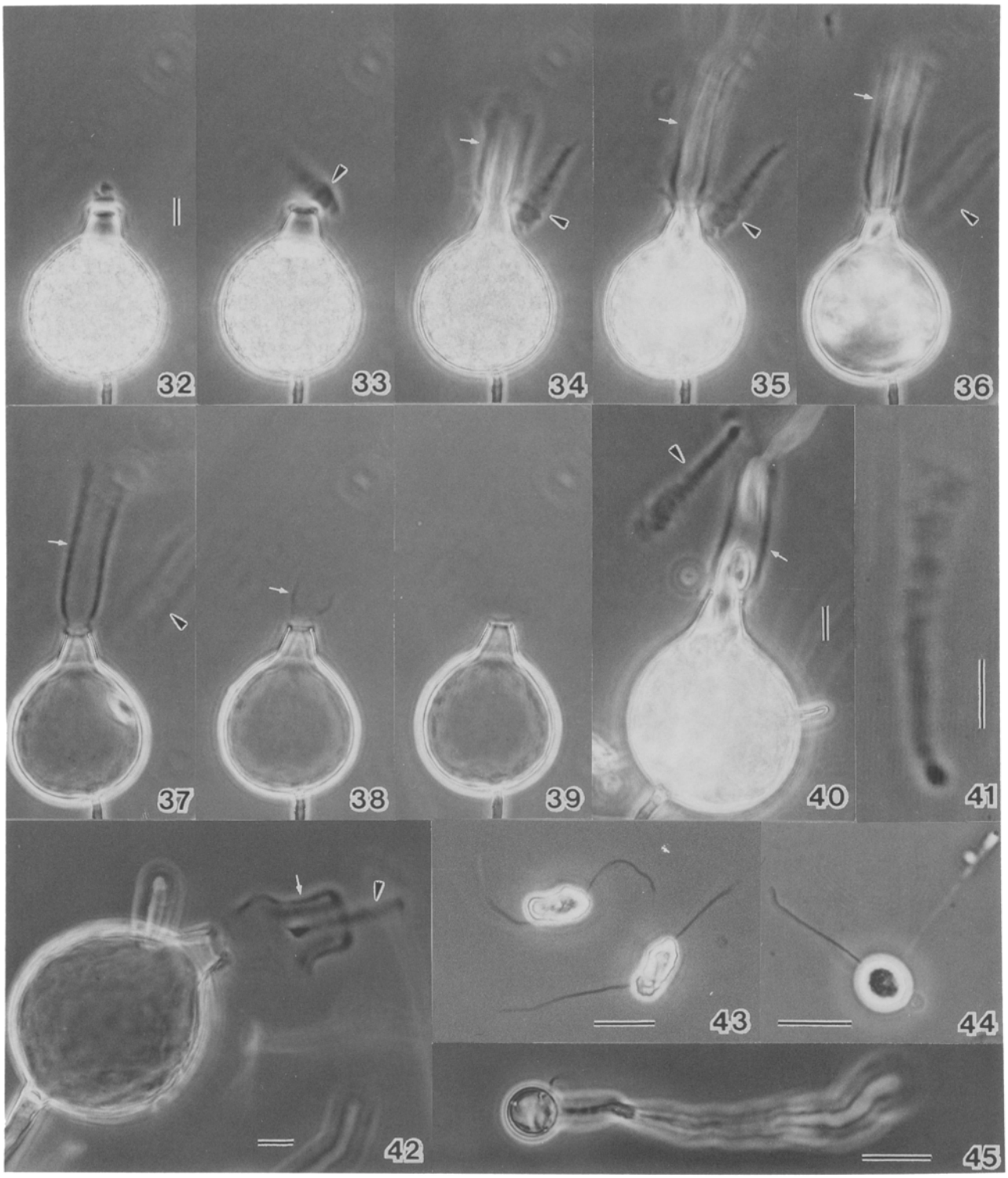
Other specimens examined, IFO H-12170 and H-12171, voucher slides of SAP 82 (IFO 32603) and SAP 84 (IFO 32605) isolated from submerged decaying leaf of *Avicennia germinans*, from Shroud Cay (SAP 82) and Norman's Pond Cay (SAP 84), Exuma Sound, the Bahama Islands, July 1993.

Halophytophthora masteri is roughly similar to *H. vesicula* (Anastasiou & Churchland) Ho & Jong in the shape of zoosporangia, having a vesicle and releasing zoospores spontaneously from mature sporangia. However, *H. masteri* is distinctly different from *H. vesicula* in that a dehiscence plug exposed at the apex of the dehiscence tube becomes loose and ragged-looking and that a semi-persistent plug (absent for *H. vesicula*) is extruded just prior to vesicle formation at zoospore release. Also, for *H. masteri*, the vesicle does not clearly result from eversion of an inverted-cone-shaped membrane as for *H. vesicula* (see Nakagiri, 1993). In culture, *H. masteri* shows almost no hyphal growth on CMSWA, whereas *H. vesicula* grows well on CMSWA (Fell and Master, 1975; Nakagiri, 1993). *Halophytophthora masteri* shares the characteristic of weak growth on cornmeal agar (rare among halophytophthoras: Fell and Master, 1975; Nakagiri, 1993) with another halophytophthoran species reported from decaying leaves of a mangrove in the genus *Avicennia, H. avicennae* (Gerrettson-Cornell & Simpson) Ho & Jong, but it shares no other distinctive characteristics with *H. avicennae*, which has no dehiscence tube, forms a spheroid to ovoid vesicle at zoospore release, and has smaller ($\bar{x}$ = 8 × 7 μm) zoospores (Gerrettson-Cornell and Simpson, 1984). *Halophytophthora masteri* can be placed in the key of Ho et al. (1991) by adding another alternative at step 4: Dehiscence tube yielding first an extruded plug, then a tubular vesicle.

Growth and reproduction Hyphal growth of *H. tartarea* (SAP 69) was seen over the entire salinity range (0-60‰ S) and optimal at 40‰ S (Fig. 46 A). The optimum tem-



Figs. 22–31. Light and scanning electron micrographs of *Halophytophthora masteri*. 22. Immature zoosporangium with a rounded apex of dehiscence tube. 23. Small peak of cytoplasm (arrow) in immature zoosporangia. 24. Mature zoosporangium with ragged-looking apex of dehiscence tube. 25. Ragged apex of dehiscence tube. 26. Mature zoosporangium. 27–31. Development process of the apex of dehiscence tube. 27. Intact apex of immature zoosporangium (see Fig. 22). 28. Pore formation in the wall of the dehiscence tube by tearing from the center of the apex. 29–31. Exposure of dehiscence plug by curling back of the torn wall of the dehiscence tube apex in mature zoosporangia (see Figs. 24, 25). Bars: 22–26 = 10 μm; 27–31 = 5 μm.



Figs. 32–45. Light micrographs of *Halophytophthora masteri*. 32–39. Serial photos of zoospore release from a zoosporangium. Note dehiscence plug (black arrows) extruded from exit pore and vesicle (white arrows) through which zoospores are released. The vesicle is retracted into zoosporangia after zoospore release (Figs. 37–39). 40. Elongated dehiscence plug in water (black arrow) and vesicle (white arrow) of a zoosporangium releasing zoospores. 41. Persistent dehiscence plug. 42. A dehiscence plug (black arrow) attaching to the depressed apex of the vesicle (white arrow). 43. Zoospores. 44. Encysting zoospore retracting flagella. 45. Germinating zoospore. Bars: 32 (=33–39), 40–45=10 μm.

perature for hyphal growth was 25°C, and this fungus showed slow growth even at 10°C but no growth at 37°C (Fig. 46 B). Zoosporangia were produced in water of 10-40‰ S at 25°C and most abundantly at 20‰ S (Table 1). In 20‰ S water, zoosporangia formation occurred at 15-30°C and was most abundant at 25°C (Table 1). The formation began within 5 h of submergence of agar discs in 20‰ S water, with or without light. Zoospore release did not occur if the agar discs were kept in seawater with no salinity change. If the zoosporangia were kept submerged in seawater of constant salinity for more than 4 days, they produced many vacuoles inside and zoospores became inconspicuous. Zoospore release was moderately induced by changing salinity (more effectively by decreasing salinity) of water surrounding mature zoosporangia; e.g., when zoosporangia were transferred from 20‰ S water (25°C) to 10‰ S water (25°C), the zoospore release occurred 80 min after the transfer. Change of temperature of seawater did not show any effect on the release, and combination with change in salinity did not enhance the effect. The most remarkable enhancement of zoospore release was achieved by the mild desiccation treatment. Dehydrated agar discs with zoosporangia were returned into 0-40‰ S water (25°C) after 1, 2, 4 and 24 h of desiccation. Zoospore release was induced in 10-30‰ S seawater in all cases, and most effectively (quickly and abundantly) when the zoosporangia dehydrated for 2 h were transferred into 10‰ S water. With this treatment, the release began 30 min after rewetting. In 20 or 30‰ S water, the induction time was retarded to 40 min or more than 60 min, respectively. In distilled water and 40‰ S water, no release occurred. When the agar discs were rewetted after 1 h of desiccation, the induction time for release in 10‰ S water was 80 min. Even after 24 h of desiccation, rewetted zoosporangia began to release zoospores after 40 min in 10‰ S water. This fungus produces zoosporangia on the surface of VJSWA plates without submersion. When zoosporangia produced on the agar surface were immersed in seawater (10-30‰ S), they began to release zoospores within 20-60 min.

Halophytophthora masteri showed strong hyphal growth on VJSWA (10-11 mm/day; 20‰ S, 30°C) but not on CMSWA (0.4-0.8 mm/day; 20‰ S, 30°C). The three strains (SAP 82-84) of this species grew over the entire salinity range (0-60‰ S), and showed strong growth at the higher salinities (more than 20‰ S) and through the temperature range from 15 to 30°C (Fig. 47). The optimum temperature was 30°C, but growth was abruptly suppressed at 37°C. At 10°C, no growth was observed in this species. Zoosporangia formation occurred in 10-40‰ S seawater at 25°C and most abundantly at 30‰ S (Table 2). The temperature for maximum zoosporangia formation was from 20 to 30°C in 30‰ S water (Table 2), especially at 30°C at which temperature the zoosporangia began to develop within 8 h af-

Table 1. Zoosporangia formation of *Halophytophthora tartarea* induced by submersion in water of different salinities and temperatures.

Strain	Salinity (‰)[1]					Temperature (°C)[2]					
	0	10	20	30	40	10	15	20	25	30	37
SAP 69	−[3]	++	+++	++	+	−	++	++	+++	+	−

[1] Incubated at 25°C for 1-2 days.
[2] Incubated in 20‰ S water for 1-2 days.
[3] Relative abundance of produced zoosporangia is shown as −, +, ++, +++.

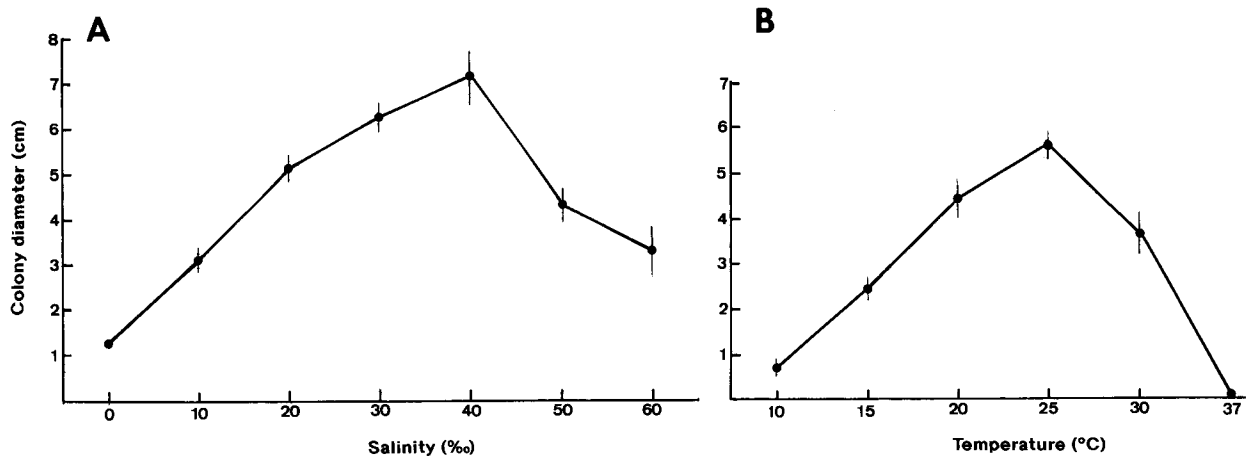


Fig. 46. Hyphal growth of *Halophytophthora tartarea* (SAP 69).
A. Colony diam after incubation on CMSWA plates with different salinities at 20°C for 50 h.
B. Colony diam after incubation on CMSWA-20 plates at different temperatures for 72 h.
(Mean values of duplicate examinations are plotted.)

ter submergence and were abundantly produced in 40 h. Light was unnecessary for zoosporangia formation. Zoospores were spontaneously released from mature zoosporangia in 10-40‰ S seawater. The release began at 16-22 h after submerging agar disks in seawater. Light was unnecessary for this process. In 10‰ S water, zoospores were released most abundantly, but no release occurred in distilled water. When the zoosporangia produced in 30‰ S water were transferred to 10‰ S water, the time before release was reduced to 15-20 min after the transfer. Increase or decrease of temperature of the water (from 25°C to 30°C, or from 25°C to 15 or 20°C) and combination with change in salinity showed minor enhancement of release. The most remarkable enhancement of release was achieved by the desiccation treatment, as in the case of *H. tartarea*. When agar disks with zoosporangia were returned into seawater (10-30‰ S) after 17 h of mild desiccation, zoospores were released abundantly and simultaneously from many zoosporangia within 30 min. Rewetting with 20‰ S water was the most effective, but no release was observed in distilled water. This species also produces zoosporangia on the surface of VJSWA without submersion. When the zoosporangia produced on the agar surface were immersed in seawater (10-40‰ S, most effectively in 10‰ S water), zoospores were released after 60-85 min. No release occurred in distilled water.

Discussion

Eleven species and two varieties of halophytophthoras have been described so far (Anastasiou and Churchland, 1969; Fell and Master, 1975; Pegg and Alcorn, 1982; Gerrettson-Cornell and Simpson, 1984; Ho et al., 1991, 1992). The two new species described here are members of the group having a dehiscence plug which is ejected from an exit pore of a dehiscence tube at zoospore release (Stamps et al., 1990) and are unique, as is *H. bahamensis*, in having the ragged dehiscence plug. *Halophytophthora masteri* is characterized by having both the dehiscence plug and vesicle. The two new species resemble each other in the appearance of zoosporangia, especially under a low-power microscope. However, the fine structure of the apex of the dehiscence tube and the development of the ragged plug are different between the two species as described above and shown in Figs. 6-10 and 27-31. Evanescent (*H. tartarea*) or persistent (*H. masteri*) dehiscence plugs are also different be-

Table 2. Zoosporangia formation of *Halophytophthora masteri* induced by submersion in water of different salinities and temperatures.

Strain	Salinity (‰)[1]					Temperature (°C)[2]					
	0	10	20	30	40	10	15	20	25	30	37
SAP 82	−[3]	+	++	++++	+++	−	−	+	++	++	+
SAP 83	−	+−	++	+++	+++	−	−	+	+	+++	+

[1] Incubated at 25°C for 1-2 days.
[2] Incubated in 30‰ S water for 1-2 days.
[3] Relative abundance of produced zoosporangia is shown as −, +−, +, ++, +++, ++++.

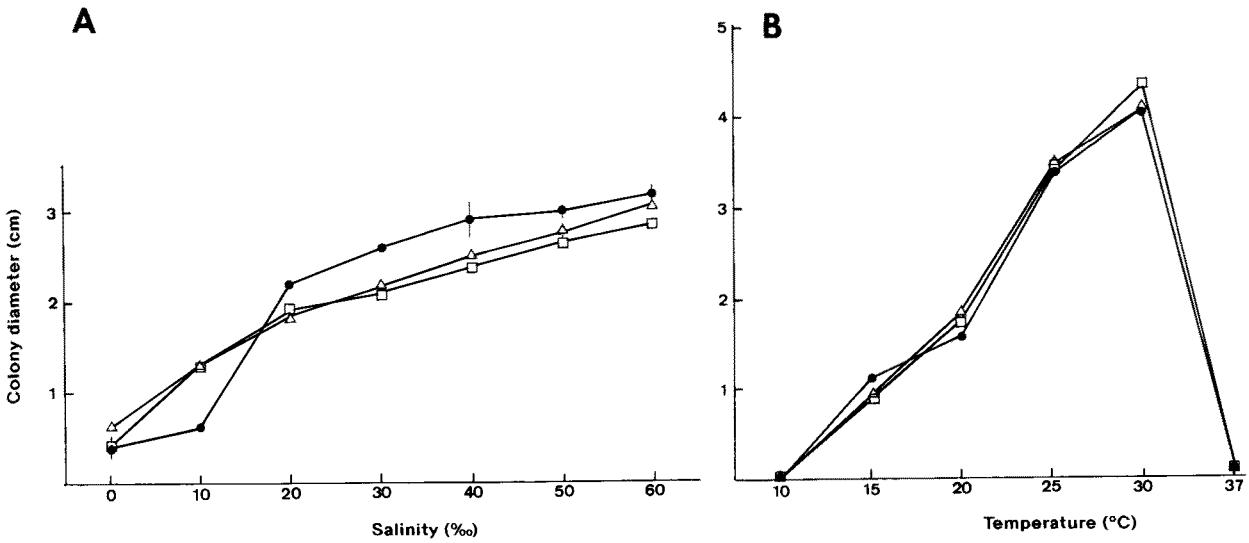


Fig. 47. Hyphal growth of *Halophytophthora masteri* (SAP 82-84).
A. Colony diam after incubation on VJSWA plates with different salinities at 20°C for 94 h.
B. Colony diam after incubation on VJSWA-20 plates at different temperatures for 94 h.
(●: SAP 82, △: SAP 83, □: SAP 84; mean values of duplicate examinations are plotted.)

tween the two. Zoospore release through a vesicle in *H. masteri* is the most obvious characteristic distinguishing it from *H. tartarea*.

In culture properties, the two species are distinguished by the fact that the colony of *H. masteri* on VJS-WA shows a rosette pattern, but no pattern is shown by *H. tartarea*. The optimal growth conditions for *H. tartarea* and *H. masteri* are 25°C, 40‰ S and 30°C, 60‰ S (more than 20‰ S), respectively. Hyphal growth at 10°C of *H. tartarea* distinguishes it from *H. masteri*. Zoosporangia formation in *H. tartarea* occurs at 15-30°C (opt. 25°C), but at (15-)20-37°C (opt. 30°C) in *H. masteri*. The preference for lower temperature by *H. tartarea* for growth and reproduction may reflect the fact that this species has been found only on decaying leaves collected from temperate-subtropical boundary regions along the coast from Georgia to Florida (28.2-31.4°N) in November (seawater: 14-23°C, 18-23‰ S) (Newell, 1992). Contrarily, the preference for higher temperature of *H. masteri* may correspond to its subtropical distribution and frequent occurrence on submerged leaf samples collected in July (seawater: 31-37°C, 37-42‰ S) (Newell and Fell, 1994). This species has been found only from submerged leaves of black mangrove. Further ecological research focusing on geographical and seasonal distributions of these fungi may clarify the above inference. At any rate, though there are some differences between *H. tartarea* and *H. masteri* in required conditions, their growth and reproductive properties revealed here indicate that the two species are well adapted to the coastal and brackish environments of their own habitats.

Induction of zoospore release of *H. tartarea* by dehydration followed by rewetting zoosporangia is reported here for the first time for halophytophthoras. Though *H. masteri* releases zoospores spontaneously from mature zoosporangia, the desiccation treatment enhances the release. Zoospore release in the two species occurs only in seawater (10-30‰ S), not in distilled water. These phenomena suggest the following natural sequence. Zoosporangia formed on submerged leaves in seawater will be exposed to the atmosphere for several hours in the intertidal zone of saltmarshes and estuaries at low tide, and when they are submerged again in seawater at high tide, zoospores will be released and swim away from the zoosporangia for new substrates. Induction of zoospore release by changing the temperature and/or salinity of water surrounding zoosporangia was reported for *H. spinosa* var. *lobata* (Fell & Master) Ho & Jong and *H. exoprolifera* Ho, Nakagiri & Newell, and their adaptation to the fluctuating enviroment of mangrove ecosystems was proposed (Nakagiri, 1993). The two new species, *H. tartarea* and *H. masteri*, are also considered to be well adapted to coastal and mangrove environments, utilizing the tidal rhythm in their life cycles.

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