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Lanceispora amphibia gen. et sp. nov., a new amphisphaeriaceous ascomycete inhabiting senescent and fallen leaves of mangrove

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Lanceispora amphibia **gen. et sp. nov. in the Amphisphaeriaceae is described from senescent and fallen leaves of *Bruguiera gymnorrhiza* in mangrove forests in the Southwest Islands, Japan. The fungus produces immersed ascomata in leaf tissue, cylindrical asci with an apical ring staining blue with iodine, and oblanceolate ascospores with a septum above the middle. Studies on the fungal succession on the mangrove leaves revealed that *L. amphibia* infects senescent leaves on the tree and inhabits intertidal fallen leaves, showing the highest frequency of occurrence at the late stage of decomposition. In culture the optimal conditions for hyphal growth were 20 ppt salinity and 30°C, and those for sexual reproduction were 10 ppt salinity and 25°C. Growth at 0 ppt (fresh water) was depressed. The fungus has amphibious habits, growing on the tree and in intertidal water; and it is adapted to the high osmotic conditions in leaf tissues of the mangrove tree and to the subtropical, brackish water environment of mangrove forests.**

Key Words——*Bruguiera gymnorrhiza*; ecology; *Lanceispora amphibia*; mangrove; taxonomy.

In studies on the succession of fungi on mangrove leaves, we found a new ascomycete inhabiting decomposing leaves of *Bruguiera gymnorrhiza* Lamk. on Iriomote Is. and Okinawa Is., Okinawa Pref., Japan. The succession of prevalent fungi occurring on decomposing mangrove leaves has been reported by Fell and Master (1973) and Nakagiri et al. (1989). The latter authors also investigated fungal succession on living leaves on the tree as well as decomposing leaves after leaf fall. In 1989, the fungal succession was examined in more detail on both living and decomposing leaves of *Rhizophora stylosa* Griff. and *B. gymnorrhiza* in the Shiira River, Iriomote Is. (Nakagiri, unpublished data). During the study, an unknown ascomycete was found from *B. gymnorrhiza* leaves. The fungus was isolated from both senescent leaves on the tree and intertidal decomposing fallen leaves. In subsequent surveys of fungal flora on fallen leaves from various mangrove trees, *B. gymnorrhiza, R. stylosa, Kandelia candel* Druce, *Lumnitzera racemosa* Willd., *Avicennia marina* (Forsk.) Vierh. and *Sonneratia alba* J. E. Smith, from Amami Is., Okinawa Is., Iriomote Is. and Minami-Daitou (South Borodino) Is., Japan, the fungus was found only from *B. gymnorrhiza* leaves from Iriomote Is. and Okinawa Is. Morphological characteristics of the fungus indicated it probably belongs to the Amphisphaeriaceae G. Winter (sensu lato), but none of the present genera accommodate this fungus properly. Therefore, we will here describe the new fungus and establish a new genus to accommodate it.

The fungal succession on living and fallen mangrove leaves was studied to clarify the pattern of occurrence of the new fungus and its ecological traits. Cultural studies were carried out to examine the growth and reproductive properties of the fungus in order to better understand its adaptation to natural habitats.

Materials and Methods

Collection and succession study Living and fallen leaves of various mangrove trees were collected from intertidal mangrove forests in Iriomote Is., Okinawa Is., Amami Is. and Minami-Daitou Is. during surveys of mycoflora on mangrove leaves that have been continuing since 1989. Discs were cut with an 8-mm diam cork borer from leaves at different stages of development or decomposition. The leaf discs were washed ten times (ca. 1 min each) with sterilized distilled water or 15 ppt salinity seawater in a test tube by using a flash mixer. Excess water was removed from the discs by blotting with sterilized filter paper, then the discs were placed on cornmeal agar (CMA) plates or 1/5 diluted V-8 juice agar plates prepared with distilled water or artificial seawater (Jamarin S, Jamarin Laboratory, Osaka) of 15 or 30 ppt salinity. The leaf discs on the plates were incubated at 20–25°C. Fungi appearing from the discs were recorded and the frequency of occurrence of each fungus was surveyed, in order to know the successional pattern of fungi on the mangrove leaves.

Isolation Hyphal tips of the new fungus appearing from the edge of the leaf disc were transferred to a new CMA

plate. Ascospores ejected from the ascomata in the leaf disc to the surface of the medium were also isolated with a fine needle and transferred to a new plate. Since these subcultures readily produced ascomata and ascospores on the agar media, single ascospore and single ascus isolates were obtained by using Skerman type micromanipulator. The isolates were used for the following taxonomic and cultural studies.

Observation Ascomata formed in leaf discs on the isolation plates and those formed by single ascospore and ascus isolates in culture media (cornmeal seawater (20 ppt) agar (CMSWA) and V-8 juice seawater (20 ppt) agar (V-8SWA)) were observed under phase-contrast and differential interference light microscopes.

Culture study A single ascospore isolate (AN-1566) and a single ascus isolate (AN-1570) were examined for hyphal growth and reproduction in culture. The effect of salinity was investigated by culturing the isolates at 20°C on CMA prepared with seawater of 0, 10, 20, 30, 40 and 50 ppt salinity. Growth of the isolates was also exmined on CMA supplemented with various concentrations (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 2.0 M) of NaCl, KCl or sucrose, at 25°C. The effect of temperature was examined by culturing the isolates on CMA with 20 ppt salinity at 10, 15, 20, 25, 30 and 37°C. After incubation, colony diam was measured in duplicate experiments. Ascomata and ascospores were examined for maturity under a microscope.

Results

The ascomycete isolated from senescent and fallen leaves of *B. gymnorrhiza* has perithecial ascomata, unitunicate basal asci with a shallow amyloid apical ring, narrow filiform paraphyses, and hyaline 2-celled ascospores of a unique lanceolate shape. The fungus is described here as a new species of a new genus in the family Amphisphaeriaceae (sensu Müller and Arx, 1962) or Hyponectriaceae (sensu Barr, 1990). The morphology of the new fungus suggested its affinity with the genera *Leiosphaerella* Höhnel, *Ceriospora* Niessl, *Pseudomassaria* Jacz. and *Apiospora* Sacc. *Leiosphaerella* is characterized by immersed upright ascomata, unitunicate asci with an amyloid apical ring, paraphysate centrum, and long narrow hyaline ascospores having both ends rounded and a septum at the middle (Müller and Arx, 1962; Samuels and Rossman, 1987). However, the new fungus differs from *Leiosphaerella* in its lanceolate ascospores, which have a rounded upper end and a tapering lower end and are septate above the middle. *Ceriospora* has fusiform hyaline ascospores, which are tapering and somewhat acute at both ends, which appear as filiform appendages (Müller and Arx, 1962). *Pseudomassaria* and *Apiospora* have apiosporous, obovate ascospores. These ascospores have a septum in the lower third or below the middle, resulting a larger upper and a smaller lower cell. The two genera also differ from the new fungus in their paraphyses, which are few and deliquescent at maturity (Barr, 1964, 1976). Thus, no extant genus accommodates the new fungus properly, so that the establishment of the following new genus in the Amphisphaeriaceae is warranted.

Lanceispora Nakagiri, I. Okane, Tad. Ito et Katumoto, gen. nov.

Ascomata immersa, solitaria, papillata, ostiolata. Asci unitunicati, cylindrici, rotundati ad apicem, annulo apicali jodo cyanescenti praediti, paraphysati. Ascosporae hyalinae, oblanceolatae, uniseptatae supra medianum.

Species typica: *Lanceispora amphibia* Nakagiri, I. Okane, Tad. Ito et Katumoto.

Etymology: *Lancea*=lance, *spora*=spore; referring to lance-shaped ascospores.

Ascomata immersed, solitary, papillate, ostiolate. Asci unitunicate, cylindrical, rounded at apex, with an apical ring turning blue with iodine, paraphysate. Ascospores hyaline, oblanceolate, uniseptate above the middle.

Type species: *Lanceispora amphibia* Nakagiri, I. Okane, Tad. Ito et Katumoto.

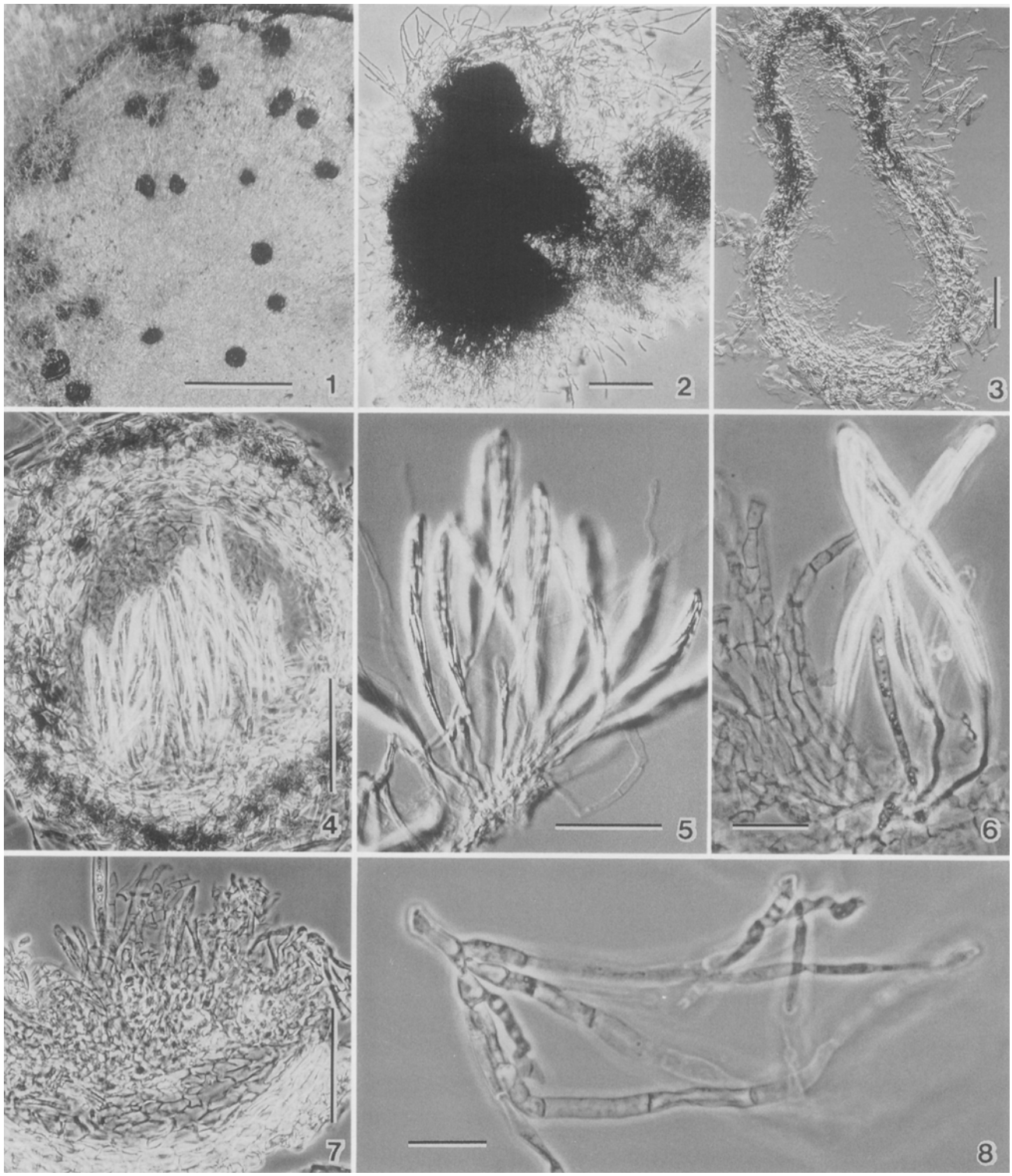
Lanceispora amphibia Nakagiri, I. Okane, Tad. Ito et Katumoto, sp. nov. Figs. 1–13

Ascomata solitaria, immersa, globosa vel subglobosa, 280–400 μm alta, 260–380 μm diam, olivacea vel olivaceo-nigra, papillata. Papillae 65–240 μm altae, 60–180 μm diam, ostiolatae, eperiphysatae. Peridium coriaceum, 20–28 μm crassum, ex cellulis externe brunneis vel brunneolis polygonis 2–3 stratis et interne hyalinis applanatis 2–3 stratis compositum. Paraphyses filiformes, 90–138×4–8 μm, raro ramosae, septatae, hyalinae. Asci unitunicati, octospori, cylindrici, longe pedunculati, 134–176×8–10 μm, apice rotundatis, annulo apicali jodo cyanescenti praeditis, e basi centri oriundi, ordinatim maturescentes. Ascosporae uni- vel biseriales, oblanceolatae, (24–)28–34(–36)×3–4.5 μm ($\bar{x}$=29.6×3.8 μm), hyalinae, rotundatae ad apicem superum, attenuatae versus apicem inferum, uniseptatae super medianum. Anamorphus non visus.

Hospes: frondis senescentes et dejectae *Bruguierae gymnorrhizae* Lamk.

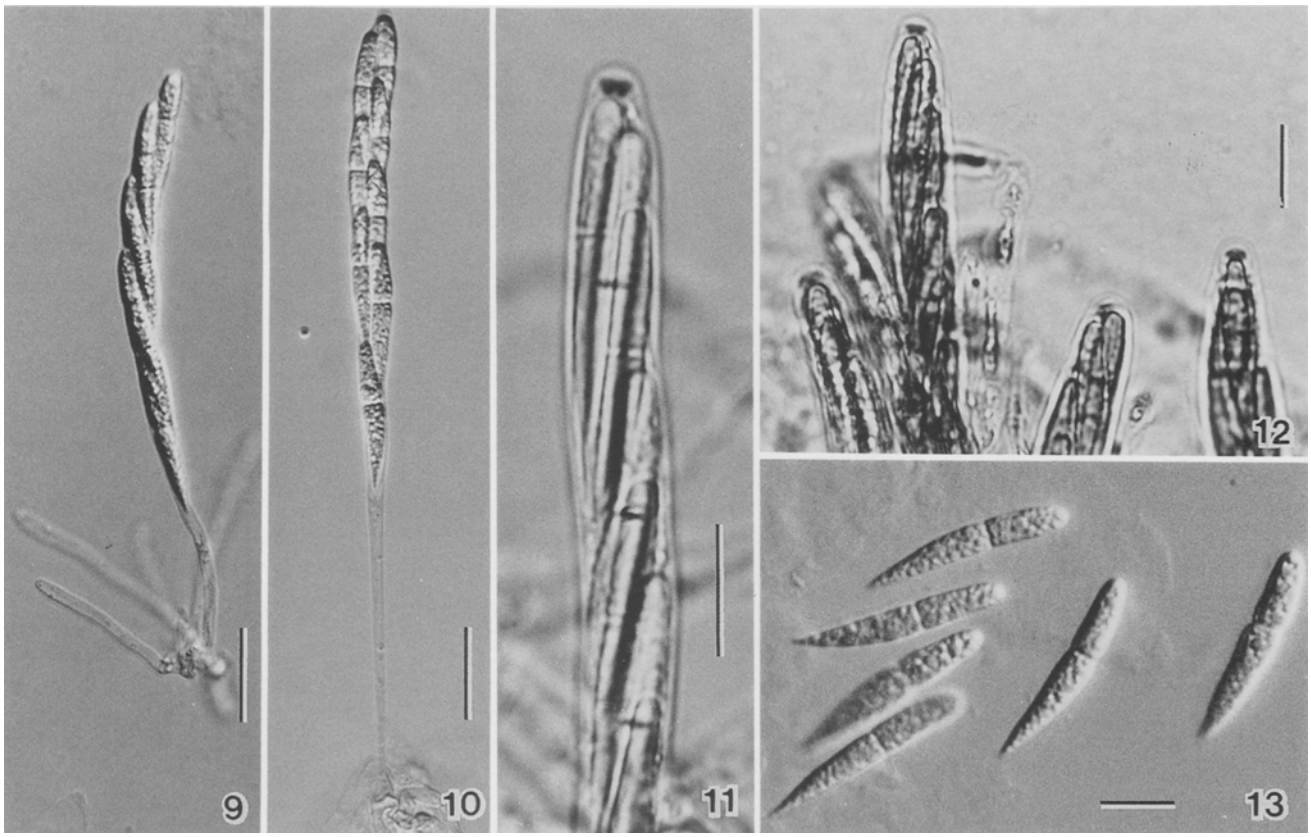
Holotypus: IFO H-12218, colonia exsiccata in cultura ex fronde dejecta submersaque *Bruguierae gymnorrhizae* Lamk., Urauchi Rev., Iriomote Is., Okinawa Pref., Japonia, 26 Oct. 1996, a A. Nakagiri isolata, in Herbario IFO.

Ascomata solitary, immersed, globose to subglobose, 280–400 μm high, 260–380 μm in diam, olivaceous to olivaceous black, dark colored at papilla and upper part of ascoma, papillate. Papillae 65–240 μm high, 60–180 μm in diam, ostiolate, eperiphysate. Peridium coriaceous, 20–28 μm thick, composed of external 2–3 layers of brown to pale brown, polygonal thick-walled cells and inner 2–3 layers of hyaline flattened cells. Paraphyses filiform, 90–138×4–8 μm, rarely branched, septate, hyaline. Asci unitunicate, 8-spored, cylindrical, long pedunculate, 134–176×8–10 μm, rounded at apex, with apical ring staining blue with iodine, arising from the base of centrum, maturing in succession. Ascospores uni- or biseriate,



Figs. 1–8. *Lanceispora amphibia*.
1. Ascomata produced on a leaf disc, with papilla protruding from the leaf surface. 2. A squashed ascoma with short papilla formed in CMSWA. 3. Longitudinal thin section of ascoma through ostiole. 4. Longitudinal section of ascoma, showing asci arising from the bottom of centrum. 5, 6. Asci and paraphyses. 7. A section of the bottom of centrum, from which asci and paraphyses arise. 8. Paraphyses. Bars: 1=1 mm; 2=100 μm; 3–5, 7=50 μm; 6,8=20 μm.

oblanceolate, (24–)28–34(–36)×3–4.5 μm ($\bar{x}$=29.6×3.8 μm), hyaline, rounded at the upper end, tapering toward the lower end, one-septate above the middle. The upper cells 10-13 μm long (=a) and the lower cells



Figs. 9–13. *Lanceispora amphibia*.
9. Successionally developing asci. 10. A mature ascus. 11, 12. Ascus apices with an apical ring stained with iodine. 13. Ascospores. Bars: 9, 10=20 μm; 11–13=10 μm.

17–21 μm long (=b); a/b=0.52–0.76.

Host: Senescent and fallen decomposing leaves of *B. gymnorrhiza*.

Range: Iriomote Is. and Okinawa Is., Okinawa Pref., Japan.

Colonies greenish olivaceous, with sparse aerial hyphae, on CMSWA; olivaceous, with dense mycelia on V-8SWA; forming ascomata abundantly on the media, even from the single-ascospore isolates (homothallic). Ascomata formed on the media mostly nonpapillate. Hyphae 1.5–4 μm in diam, olivaceous buff to greenish olivaceous, branching perpendicularly.

Holotypus: IFO H-12218, dried colony on V-8SWA (20 ppt) of AN-1566 (single ascospore culture)(=IFO 32918) isolated from submerged fallen leaf of *B. gymnorrhiza* in Urauchi River, Iriomote Is., Okinawa Pref., 26 Oct. 1996, collected and isolated by A. Nakagiri; deposited in the herbarium in IFO.

Specimens examined: AN-1567, AN-1568 (single ascospore cultures), AN-1569, AN-1570 (=IFO 32919, IFO H-12219)(single ascus cultures) isolated from submerged fallen leaf of *B. gymnorrhiza* in Urauchi River, Iriomote Is., Okinawa Pref., 26 Oct. 1996; AN-1116 (=IFO 32920, IFO H-12220), AN-1117, AN-1118 (single ascus cultures) isolated from submerged fallen leaf of *B. gymnorrhiza* in Shiira River, Iriomote Is., Okinawa Pref., 21 Sep. 1989.

Etymology: *amphibius*=amphibious, referring to habitats both on leaves on the tree and fallen leaves submerged in brackish water.

Fungal occurrence on mangrove leaves *Lanceispora amphibia* was found to inhabit living leaves on the tree of *B. gymnorrhiza* and its fallen leaves submerged in the intertidal water. The fungal succession observed on living and fallen leaves of *B. gymnorrhiza* in Iriomote Is. is summarized in Table 1, which shows that *L. amphibia* began to appear on senescent yellow leaves on the tree and continued occurring on fallen decomposing leaves. The frequency of occurrence increased up to 60% with the advance of leaf decomposition. The highest frequency of occurrence was observed on the brown colored decomposing leaves at the late stage of decomposition (Table 1). The fungal succession was also investigated on the leaves of *R. stylosa* in Iriomote Is., but *L. amphibia* was not found on them. The fungus has also not been found from other mangrove trees so far examined.

Effect of salinity on growth and reproduction Hyphal growth of *L. amphibia* was good on CMSWA with salinity above 10 ppt and was optimum at 20 ppt (Fig. 14). On the other hand, the freshwater medium remarkably depressed the hyphal growth. Ascoma and ascospore formation occurred on CMSWA with salinity below

Table. 1. Frequency of occurrence of prevalent fungi on living and fallen leaves of *Bruguiera gymnorrhiza* at different stages of development and decomposition.[a]

Fungus	YG[b]	G	SY	leaf fall	Y	Or	Br	Bl
Hormonema sp.	45[c]	10	5			5		
Stenella sp.	50	100	70		25	15	5	5
Phyllosticta sp.		5	10		40	5		
Cladosporium cladosporioides			25		10	10	5	10
Lanceispora amphibia			**10**		**10**	**40**	**60**	**10**
Pestalotiopsis sp.			10		20	15	15	15
Halophytophthora vesicula					45	80	95	50
H. spinosa var. *lobata*					65	100	80	40
Labyrinthula sp.						5	50	75
Acremonium hyalinum						5		30
Fusarium semitectum								30
Humicola alopallonella								30
Trichocladium sp.								30
Lulworthia grandispora								20

a) Leaf samples examined were collected from the Shiira River, Iriomote Is., on 21 Sep. 1989.
b) Developmental and decompositional stages of leaves: **YG**, sprouted young green; **G**, green; **SY**, senescent yellow; **Y**, yellow; **Or**, orange; **Br**, brown; **Bl**, black.
c) Frequency of occurrence (%) is calculated by the following formula: No. of leaf discs on which the fungus appeared / No. of leaf discs examined (20 discs).

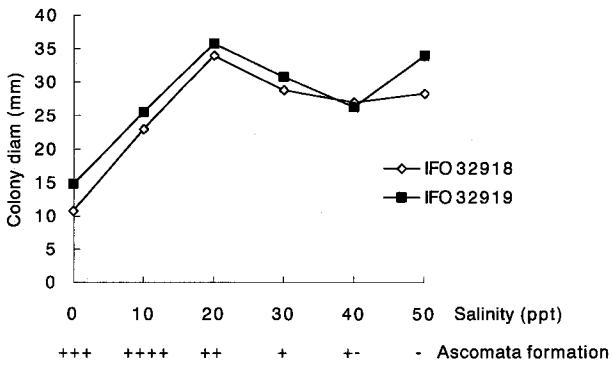


Fig. 14. Hyphal growth and sexual reproduction of IFO 32918 (=AN-1566)(single ascospore isolate) and IFO 32919 (=AN-1570)(single ascus isolate) of *Lanceispora amphibia* after incubation on CMSWA with different salinity levels at 20°C for 16 d.
Relative abundance of ascomata produced is shown as −, +∼++++.

50 ppt after 16 d of incubation at 20°C and was most prevalent at 10 ppt, while ascus maturation was partly retarded at 40 ppt (Fig. 14). On CMA supplemented with NaCl or KCl, almost no hyphal growth was observed (less than 1 mm after 14 d incubation at 25°C). On the other hand, moderate growth was observed on the media containing sucrose, especially at 1.0 M and higher concentration, showing 12–19 mm colony diam after 14 d of incubation at 25°C. The osmotic pressure of 0.8–1.2 M sucrose approximately corresponds to that of 20–35 ppt seawater. The hyphal growth at the high concentration of sucrose, however, is less than that on CMSWA with 10 ppt or more salinity (see Fig. 14). No ascomatal maturation occurred on the media during the incubation period, though ascomatal initials were formed on the media with 1.2 M and lower concentration of sucrose. After prolonged incubation for 30 d, ascomata were produced in the range of 0–1.4 M sucrose, but ascospore formation did not occur above 0.6 M (Fig. 15). The ascomata formed on the media with 0.1–0.4 M sucrose were small and only a few asci matured, while those formed on CMA without sucrose were large and produced ascospores within many asci. Thus, the growth and reproduction of *L. amphibia* on the sucrose media were lower and retarded compared with those on CMSWA and CMA.

Effect of temperature on growth and reproduction

Hyphal growth was good above 20°C and optimum at 30°C, though no growth was seen at 10 and 37°C (Fig. 16). Ascoma and ascospore formation occurred at 20 to 25°C, and it was retarded at 30°C since it did not proceed beyond ascomatal initial formation even after 40 d of incubation. The most prevalent ascomatal formation occurred at 25°C (Fig. 16). Thus, the optimum conditions for hyphal growth differ from those for reproduction, as shown in the salinity experiments.

Discussion

Lanceispora amphibia infects living leaves of *B. gymnorrhiza* as they become senescent. After leaf fall, the fungus continues to inhabit the decomposing leaves submerged in the brackish water and increases its frequency of occurrence as the leaf decomposition proceeds. Thus, as one of the prevalent fungi inhabiting the fallen leaves, it may work for leaf decomposition. This fungus produces ascomata on submerged fallen leaves and forci-

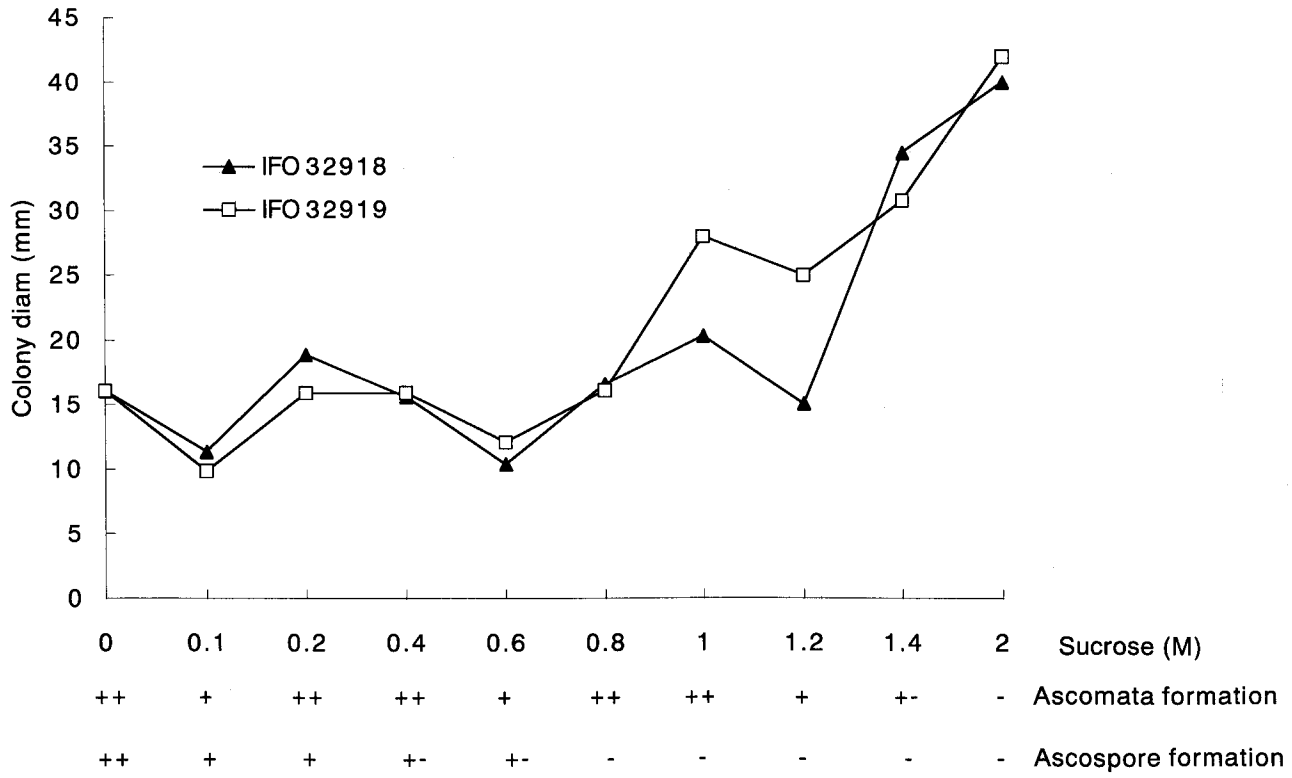


Fig. 15. Hyphal growth and sexual reproduction of the two strains of *Lanceispora amphibia* after incubation on CMA with different concentrations of sucrose at 25°C for 30 d.
Relative abundance of ascomata produced and of asci containing ascospores is shown as −, +, ++.

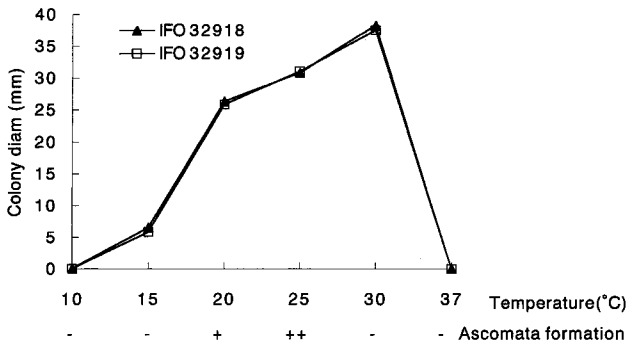


Fig. 16. Hyphal growth and sexual reproduction of the two strains of *Lanceispora amphibia* after incubation on CMSWA (20 ppt) at different temperatures for 13 d.
Relative abundance of ascomata produced is shown as −, +, ++.

bly ejects ascospores into the air when the leaves are exposed to the air, possibly at low tide. Thus, the fungus may have an opportunity to infect living leaves on the mangrove trees.

The fact that *L. amphibia* has been found only from *B. gymnorrhiza* leaves and not from other mangrove species in Japan may indicate its preference for this mangrove species. In a survey on fungi inhabiting mangrove leaves that has been continuing since 1989, we found *L. amphibia* only from samples collected in autumn, in Septmber and October, from Iriomote Is. and in November from Okinawa Is. We also examined samples collected in June and in winter, from December to March, from the islands, and in June and January from Amami Is., but the fungus was not detected from them. This phenomenon may coincide with the physiological propeties of the fungus, which prefers 20–30°C for hyphal growth and 20–25°C for sexual reproduction. The fungus is probably less active in winter (when the temperature is 15–20°C in the Southwest Is.), and it might be more easily detected from autumn samples rather than summer samples, because ascomata are more readily produced or already present on the leaf discs of the former. Further studies by prolonged incubation of leaf discs from the summer and winter samples at appropriate temperatures (20–25°C) may clarify the validity of this inference.

Lanceispora amphibia prefers saline conditions and shows optimum growth and sexual reproduction at 20 and 10 ppt salinity, respectively, which indicates that it is adapted to a brackish water environment. Because *L. amphibia* scarcely grows on CMA supplemented with NaCl or KCl, and shows retarded hyphal growth and sexual reproduction on the media with sucrose, it is not adapted simply to a high osmotic condition. It probably requires mixed salts at a moderate concentration, i.e., seawater, for its vigorous growth and completion of sexual reproduction. It is well known that mangrove trees

have high osmotic potential in their living tissue, to absorb water through the roots from the brackish water in the intertidal region. The osmotic potential of the leaf tissue of mangrove trees is about two times of that of seawater (Scholander et al., 1965), and the leaf tissue contains organic solutes (sugars, amino acids, etc.) and the salts of mostly the same concentration in seawater (Scholander et al., 1966). This may explain why *L. amphibia*, which prefers saline conditions, is able to inhabit living mangrove leaves.

Lanceispora amphibia is peculiar in showing depressed growth at 0 ppt (fresh water) compared with other mangrove-leaf-inhabiting fungi, such as *Pestalotiopsis* sp., *Phyllosticta* sp. and *Cladosporium cladosporioides* (Fresen.) de Vries, which also inhabit both living and fallen leaves (Table 1), but which differ from *L. amphibia* in growing well in freshwater conditions as well as saline conditions (Nakagiri, unpublished data). The physiological property of *L. amphibia* of showing exclusive preference for saline conditions for growth and reproduction is well known in true marine fungi. *Lanceispora amphibia* is probably a fungus specialized in inhabiting mangroves or intertidal plants. It can be postulated that marine fungi have evolved from fungi like *L. amphibia* by adaptation to marine environments.

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