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Lophiostoma sagittiforme sp. nov., a new ascomycete (Pleosporales, Dothideomycetes) from Island Yakushima in Japan

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Tanaka K. & Hosoya T. (2008) *Lophiostoma sagittiforme* sp. nov., a new ascomycete (Pleosporales, Dothideomycetes) from Island Yakushima in Japan. – Sydowia 60 (1): 131–145.

Lophiostoma sagittiforme sp. nov. was collected from the twigs of *Machilus japonica* in Island Yakushima, southern Japan. It is characterized by the ascospores with an elongated narrow sheath providing arrowhead-like tips. In terms of sheath morphology of ascospores, *L. sagittiforme* is similar to some aquatic species of the genus, e.g., *L. armatisporum*, *L. bipolare*, *L. frondisubmersa*, and *L. proprietunicatum*, but differs form these species in having relatively large-sized ascospores. Phylogenetic analyses based on the internal transcribed region (ITS) –5.8S and partial large subunit of the nuclear ribosomal DNA (LSU nrDNA) also supported the taxonomic classification of *L. sagittiforme* in *Lophiostoma*.

Keywords: appendages, Loculoascomycetes, Lophiostomataceae, *Massarina*, phylogeny.

Lophiostoma Ces. & De Not. is an ascomycetous genus belonging to Lophiostomataceae, Pleosporales, Dothideomycetes (Lumbsch & Huhndorf 2007). Species of *Lophiostoma* occur on the debris of diverse plants, such as deciduous trees, shrubs, reeds, palms, and bamboos, and they might function as saprobes in terrestrial, freshwater, and marine habitats. Due to the characteristic morphology of the ascomatal beak, termed 'crest' with a slit-like ostiole (Holm & Holm 1988), the species of *Lophiostoma* have received considerable attention from mycologists (Lehmann 1886, Chesters & Bell 1970, Holm & Holm 1988, Barr 1992, Yuan & Zhao 1994, Barr & Mathiassen 1998, Hyde *et al*. 2000, Tanaka & Harada 2003a, b). The web site of *Index Fungorum* (Anonymous 2008) currently lists 410 names of *Lophiostoma*. After the study by Holm & Holm (1988) who published a basic monograph of *Lophiostoma* and its allied genera and accepted 19 species in the genus, several species particularly associated with aquatic habitats (Hyde & Aptroot 1998, Hyde & Goh 1998, Tsui

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et al. 1999, Hyde *et al*. 2002, Tanaka *et al*. 2005) have been added to the genus.

In the course of research on fungal diversity in Island Yakushima, southern Japan (Tanaka & Hosoya 2006, Hosoya & Tanaka 2007a, b), an interesting ascomycete belonging to *Lophiostoma* was collected. It has relatively large ascospores surrounded by a characteristic narrow sheath with arrowhead-like tips and can be distinguished from all other members of the genus. The fungus is described here as a new species *Lophiostoma sagittiforme*.

Materials and Methods

The methods of microscopic observation, single spore isolation and induction of fructification are those described in Tanaka & Harada (2003a). Specimens cited in this paper were maintained at the Department of Botany, National Science Museum (TNS) and Herbarium of Hirosaki University (HHUF). Cultures were deposited at the RIKEN BioResource Center (JCM) and MAFF Genebank, National Institute of Agrobiological Sciences (MAFF).

In addition to the new species of *Lophiostoma*, two strains of *L. macrostomum* (Tanaka & Harada 2003a), the type species of the genus, were used for molecular analyses; JCM 13545 (KT 635) isolated from HHUF 27290, and JCM 13546 = MAFF 239447 (KT 709) isolated from HHUF 27293.

Based on the methods provided by Ogata *et al*. (2000), internal transcribed region (ITS) -5.8S and partial large subunit of the nuclear ribosomal DNA (LSU nrDNA) were amplified by polymerase chain reaction (PCR) using the primer pairs ITS1-ITS4 (White *et al*. 1990) and LRO-RLR7 (Rehner & Samuels 1994), respectively. PCR products were sequenced directly at the MACROGEN Co., Ltd., Korea. The sequences newly obtained were deposited in GenBank. To identify the most similar species, a BLAST search of GenBank with the sequences of the new fungus was performed.

The LSU nrDNA sequences of *L. sagittiforme* and *L. macrostomum* determined in this study were aligned manually with published sequence data obtained from GenBank (Table 1) using Clustal W included in the program Molecular Evolutionary Genetic Analysis (MEGA) version 4 (Tamura *et al*. 2007). For LSU analyses, ca. 740 characters of the 5' end of the LSU nrDNA corresponding to bases 54–779 of *L. sagittiforme* were used. Similarly, the obtained ITS-5.8S nrDNA sequences were aligned with those of five *Lophiostoma* species and out group taxa (Sporormiaceae) obtained from GenBank. Alignment data set of the ITS-5.8S region corresponding to bases 21–496 of *L. sagittiforme* was generated. Certain ambiguous regions of

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L. bipolare (113–126, 367–408) and *L. frondisubmersa* (113–130) were removed from the ITS-5.8S alignment. The alignments of the LSU and ITS-5.8S regions were deposited in TreeBASE.

The aligned data set was subjected to two phylogenetic analyses–maximum-parsimony (MP) using close-neighbour-interchange heuristic search with initial tree by random addition sequence (100 replicates) and neighbour-joining (NJ) based on the Kimura 2-parameter model–were performed using MEGA 4. The bootstrap values for nodes were computed from 1000 replicates for both the MP and NJ analyses. Characters were weighted equally, and gaps were ignored.

Tab. 1. – Taxa and GenBank accession numbers used in the phylogenetic analyses of LSU nrDNA.

Species	GenBank accession no.	Classification * Order	Family
Dothideomycetes			
Arthopyrenia salicis A. Massal.	AY538339	Dfis	Arthopyreniaceae
Botryosphaeria ribis Grossenb. & Duggar	AY004336	BO	Botryosphaeriaceae
Byssothecium circinans Fuckel	AY016357	PL	Teichosporaceae
Capnodium citri Berk. & Desm.	DQ678050	CA	Capnodiaceae
Cucurbitaria elongata (Fr.) Grev.	DQ678061	PL	Cucurbitariaceae
Delitschia winteri (W. Phillips & Plowr.) Sacc.	DQ384091	PL	Delitschiaceae
Dothidea insculpta Wallr	DQ247802	DO	Dothideaceae
Elsinoë veneta (Burkh.) Jenkins	DQ678060	MY	Elsinoaceae
Guignardia gaultheriae Aa	DQ678089	BO	Botryosphaeriaceae
Helicomyces roseus Link	DQ678083	–	Anamorphic fungus
Hysteropatella clavispora (Peck) Seaver	AY541493	HY	Hysteriaceae
Lophiostoma arundinis (Pers.) Ces. & De Not.	DQ782384	PL	Lophiostomataceae
Lophiostoma caulium (Fr.) Ces. & De Not.	DQ528763	PL	Lophiostomataceae
Lophiostoma crenatum (Pers.) Fuckel	DQ678069	PL	Lophiostomataceae
Lophiostoma heterosporum (De Not.) M.E. Barr	AY016369	PL	Lophiostomataceae
Lophiostoma macrostomum (Tode) Ces. & De Not. (KT 635)	**AB433273**	PL	Lophiostomataceae
Lophiostoma macrostomum (Tode) Ces. & De Not. (KT 709)	**AB433274**	PL	Lophiostomataceae
Lophiostoma sagittiforme Kaz. Tanaka & Hosoya (KT 1934)	**AB369267**	PL	Lophiostomataceae
Lophiotrema fuckelii Sacc.	DQ399531	PL	Lophiostomataceae
Massaria platani Ces.	DQ678065	PL	Massariaceae
Montagnula opulenta (De Not.) Aptroot	DQ678086	PL	Montagnulaceae
Munkovalsaria appendiculata Aptroot	AY772016	Dfis	Dacampiaceae
Mycosphaerella punctiformis (Pers.) Starbäck	DQ470968	CA	Mycosphaerellaceae
Myriangium duriaei Mont. & Berk.	AY016365	MY	Myriangiaceae
Neotestudina rosatii Segretain & Destombes	DQ384107	PL	Testudinaceae
Phaeosphaeria avenaria (G.F. Weber) O.E. Erikss.	AY544684	PL	Phaeosphaeriaceae
Pleomassaria siparia (Berk. & Broome) Sacc	AY004341	PL	Pleomassariaceae
Pleospora herbarum (Pers.) Rabenh.	DQ678049	PL	Pleosporaceae
Preussia terricola Cain	AY544686	PL	Sporormiaceae
Setomelanomma holmii M. Morelet	AF525678	PL	Phaeosphaeriaceae
Setosphaeria monoceras Alcorn	AY016368	PL	Pleosporaceae
Sporormia lignicola W. Phillips & Plowr.	DQ384098	PL	Sporormiaceae
Sydowia polyspora (Bref. & Tavel) E. Müll.	AY544675	DO	Dothioraceae

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Species	GenBank accession no.	Classification * Order	Family
Trematosphaeria pertusa (Pers.) Fuckel	DQ678072	PL	Melanommataceae
Tubeufia cerea (Berk. & M.A. Curtis) Höhn.	DQ470982	Dfis	Tubeufiaceae
Ulospora bilgramii (D. Hawksw., C. Booth & Morgan-Jones) D. Hawksw., Malloch & Sivan.	DQ384108	PL	Testudinaceae
Verruculina enalia (Kohlm.) Kohlm. & Volkm.-Kohlm.	AY016363	PL	Didymosphaeriaceae
Zopfia rhizophila Rabenh.	DQ384104	PL	Zopfiaceae
Sordariomycetes (out group)			
Sordaria fimicola (Roberge ex Desm.) Ces. & De Not.	AY545728	SO	Sordariaceae
Xylaria hypoxylon (L.) Grev.	AY544648	XY	Xylariaceae

* Clasification follows Lumbsch & Huhndorf (2007). Abbreviations for order are as follows: BO: Botryosphaeriales, CA: Capnodiales, Dfis: Dothideomycetes, families incertae sedis, DO: Dothideales, HY: Hysteriales, MY: Myriangiales, PL: Pleosporales, SO: Sordariales, XY: Xylariales.

Taxonomy

***Lophiostoma* sagittiforme** Kaz. Tanaka & Hosoya, **sp. nov.** – Figs. 1–15.

Anamorph. – Not found.
MycoBank no.: MB 511927
Ascomata 200–250 µm × 500–560 µm, dispersa, immersa, conica in sectione. Rostrum ca. 150–200 µm latum, cristatum, periphysibus, ostiolatum. Paries ascomatis 15–30 µm crassus ad latus, ex cellulis 4–8-stratis angularibus 5–16 µm × 2–10 µm compositus. Pseudoparaphyses copiosae, 1–1.5 µm latae, septatae, ramificantes et anastomosantes, in materia gelatinosa. Asci 125–187.5 µm × 15–22.5 µm, cylindrici, apice rotundati, fissitunicati, camera apicali formantes, stipitati, octospori. Ascosporae (40) 43.5–53 µm × 8–12.5 µm, fusiformes, 1-septatae, hyalinae, leaves, guttulatae, cum vagina gelatinosa obtectae. Vagina gelatinosa 5–32 µm longum, cum apice sagittiformis.
Holotypus. – TNS-F-12453.

Ascomata 200–250 µm × 500–560 µm, scattered, mostly immersed, erumpent at the beak, conical with a flattened base in section view. – Beak ca. 150–200 µm wide, crest-like, composed of globose thick-walled cells of 2.5–10 µm diam., periphysate, with a slit-like ostiole. – Ascomatal wall 15–30 µm thick at sides, composed of 4–8 layers of angular thin-walled cells of 5–16 µm × 2–10 µm, poorly developed at the base. – Pseudoparaphyses numerous, 1–1.5 µm wide, septate, branched and anastomosed, with slime coating. – Asci 125–187.5 µm × 15–22.5 µm ($\overline{x}$ = 162.1 µm × 19.1 µm, n = 20), cyrindrical, rounded at the apex, fissitunicate, with a shallow apical chamber, short-stalked, with 8 overlapping biseriate ascospores. – Ascospores (40) 43.5–53 µm × 8–12.5 µm ($\overline{x}$ = 47.8 µm × 10.3 µm, n = 70), L/W (3.7) 4.0–5.2 (5.9) ($\overline{x}$ = 4.7, n = 70), narrowly fusiform with acute ends, straight to slightly curved, 1-septate, slightly constricted at the septum (median; 0.50), hyaline, smooth,

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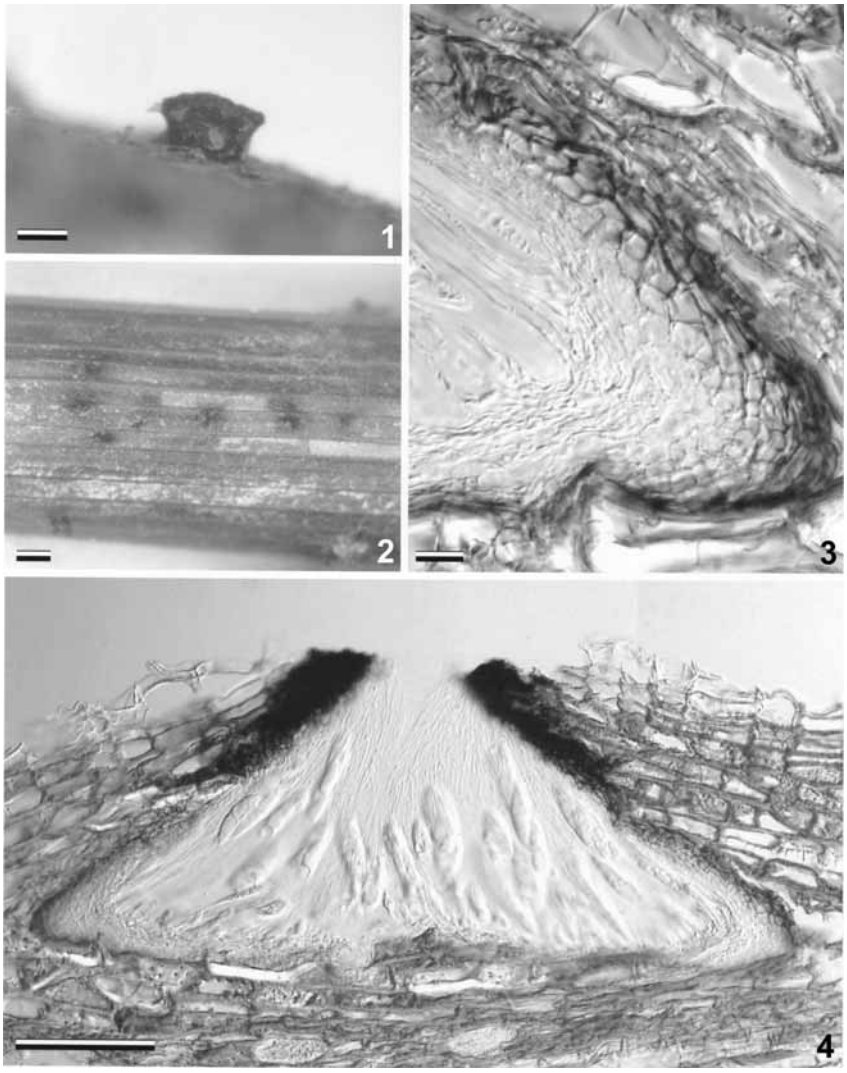

Figs. 1–4. *Lophiostoma sagittiforme*: **1.** Crest-like beak of ascoma. The beak is erumpent above the surface of natural host. **2.** Immersed ascomata on surface of sterilized rice straw in culture condition. **3.** Wall of ascoma composed of angular cells. **4.** Ascoma in longitudinal section. Bars: 1, 4 = 100 µm, 2 = 500 µm, 3 = 10 µm. Figs. 1, 3, 4 from TNS-F-12453, Fig. 2 from HHUF 29754. Figs. 3, 4 from lactophenol mounts.

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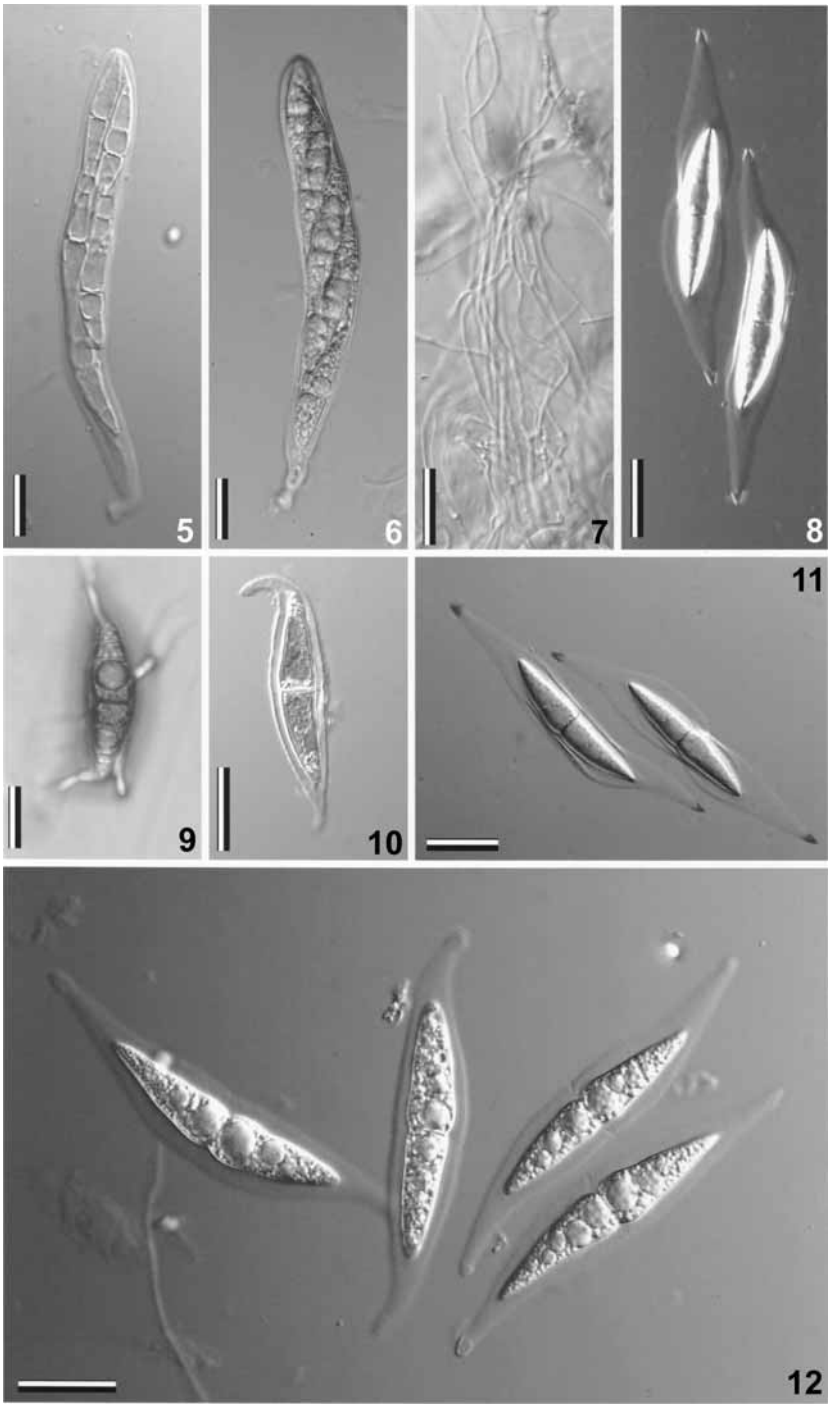

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guttulate when fresh condition, surrounded by a narrow sheath. – Sheath appendage-like, mucilaginous, elongated at both ends, 5–32 µm long (mostly 10–20 µm) from the tip of ascospore to end of the sheath, 2–5 µm wide at sides of the septum, with an internal chamber of 2–5 µm high at the ascospore apex; tips of the sheath arrowhead-like, thickened, 3–6 µm high, staining with blue-ink. – Senescent ascospores brown, echinulate, (1) 3–5-septate.

Ascospores germinate from both poles and/or middle cells on water agar at room temperature. Colonies on potato dextrose agar attaining a diameter of about 16 mm within 3 weeks at 20 °C in the dark, surface velvety in appearance, Greenish-Grey (26E2; Kornerup & Wanscher 1978) with an entire white margin; reverse similar; no pigment produced. On rice straw agar (Tanaka and Harada 2003a), abundant ascomata produced within 2 months. Ascospores resemble those found on the host, measuring (33.5) 37–50 (52) µm × 8.5–11.5 µm ($\overline{x}$ = 44.5 µm × 9.8 µm, n = 100), L/W (3.7) 3.9–5.1 (5.5) ($\overline{x}$ = 4.5, n = 100).

Etymology. – From the Latin *sagittiformis*, meaning arrow-shaped, in reference to the shape of the ascospore sheath provided with arrowhead-like potions at both the ends.

Host plant. – Dead twigs of *Machilus japonica* Siebold & Zucc. (Lauraceae).

Known distribution. – Japan.

Sequences. LSU (AB369267) and ITS1-5.8S-ITS2 (AB369268) ribosomal RNA.

Materials examined. JAPAN, Yakusugi land, Anbou forest path, Isl. Yakushima, Kagoshima Pref., on dead twigs of *Machilus japonica* Siebold & Zucc., 22 Oct 2005, *leg.* K. Tanaka and T. Hosoya, KT 1934 (TNS-F-12453 holotype). Single ascospore culture isolated from TNS-F-12453 (JCM 15100). Dried culture specimens (from JCM 15100) grown on autoclaved culms of *Oryza sativa* L. (HHUF 29754, TNS-F-17603 epitypes designated here).

Figs. 5–12. *Lophiostoma sagittiforme*: **5, 6.** Asci with 8 biseriate ascospores. **7.** Pseudoparaphyses branched and anastomosed. **8.** Ascospores with an elongated sheath. The arrowhead-like tips of the sheath are stained with blue ink (under the phase contrast microscope). **9.** Germinating ascospore. **10.** Senescent brownish ascospore with echinulate ornamentation. **11, 12.** Ascospores surrounded by a narrow sheath. The sheath provides thickened tips staining with blue ink. Bars = 20 µm. Figs. 5, 9 from TNS-F-12453, Figs. 6–8, 10–12 from HHUF 29754. All photos from water mounts.

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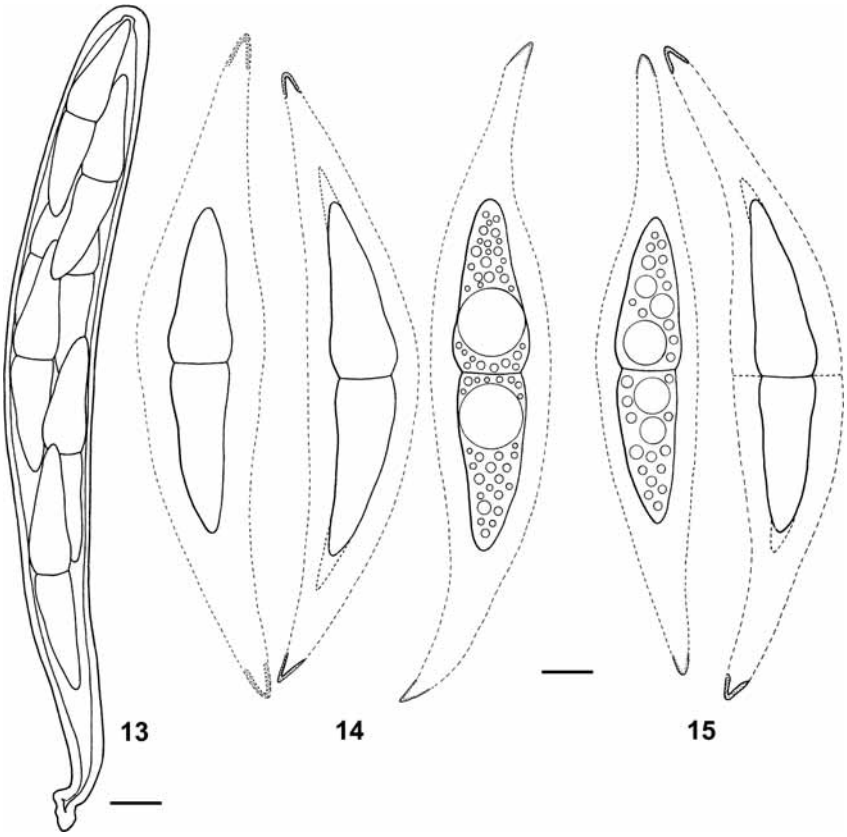

Figs. 13–15. *Lophiostoma sagittiforme*: 13. Ascus with overlapping biseriate ascospores (from HHUF 29754). 14, 15. Ascospores from natural (14: TNS-F-12453) and cultural (15: HHUF 29754) conditions. Note the internal chamber at the ends of ascospores and the thickened tips of the appendage-like sheath. Bars = 10 μm.

Results of phylogenetic analyses

As the sequence of LSU nrDNA, 1292 bp was obtained for *L. sagittiforme* (AB369267). A BLAST search of GenBank using the LSU sequence indicated that *L. sagittiforme* is close to several members of *Lophiostoma*, such as *L. heterosporum* (AY016369; Identities = 1278/1292: 98 %), *L. arundinis* (DQ782384; Identities = 1268/1284: 98 %), and *L. crenatum* (DQ678069; Identities = 1248/1263: 98%). The alignment dataset of LSU nrDNA consisted of 38 taxa with 742 characters (positions 54–779 of *L. sagittiforme*). Of which 287 characters (38.7 %) were variable and 235 characters (31.7 %) were parsimony informative. Maximum parsimony analysis of the dataset resulted in seven equally parsimonious trees with a

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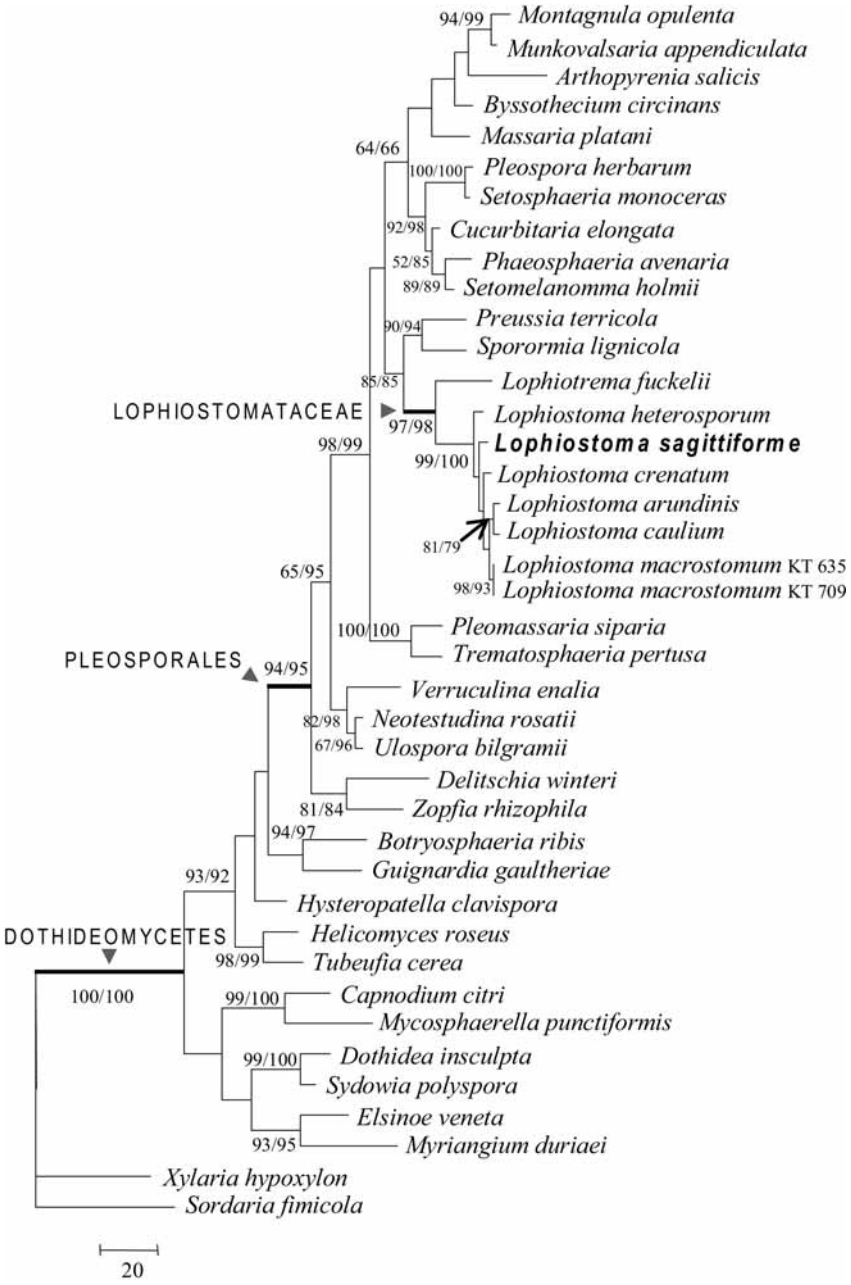

Fig. 16. One of 7 most parsimonious trees based on LSU nrDNA sequences (ca. 740 bp) of loculoascomycetous fungi. Length = 873, CI = 0.462, RI = 0.670. Values above branches are parsimony and neighbor-joining bootstrap supports from 1000 replicates.

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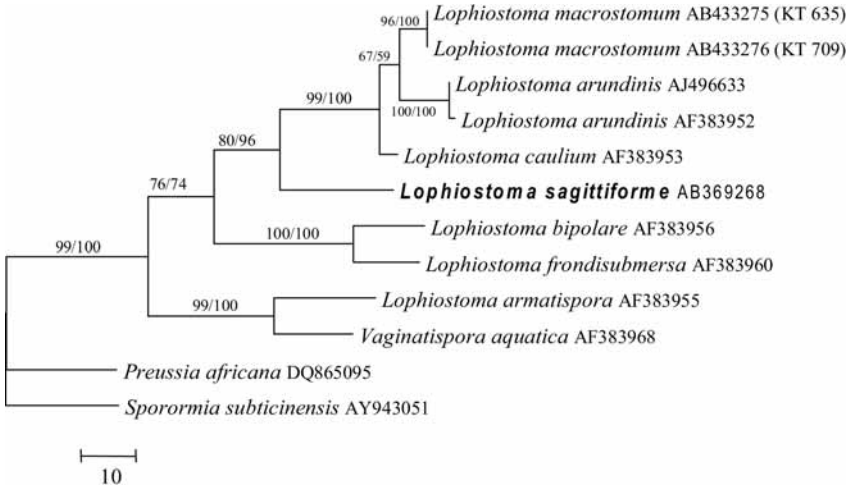

Fig. 17. The most parsimonious tree based on ITS-5.8S nrDNA sequences of *Lophiostoma* species. Length = 257, CI = 0.747, RI = 0.740. Values above branches are parsimony and neighbor-joining bootstrap supports from 1000 replicates.

length of 873 steps (Consistency Index (CI) = 0.462, Retention Index (RI) = 0.670; Fig. 16). The tree obtained from NJ analysis was also similar topology. *Lophiostoma sagittiforme* clustered together with *Lophiostoma* species in GenBank as well as *L. macrostomum* newly obtained in this study (AB433273 and AB433274) with 100 % bootstrap support.

As the sequence of ITS-5.8S nrDNA, 542 bp was obtained for *L. sagittiforme* (AB369268). A BLAST search based on this sequence suggested that *L. sagittiforme* is relate to *Lophiostoma arundinis* (AF383952: Identities = 417/448: 93%) and *L. caulium* (AF383953; Identities = 384/412: 93%). Alignment of the ITS-5.8S sequences comprising of *L. sagittiforme, L. macrostomum* (AB433275 and AB433276) and 8 taxa in Genbank resulted in a 543 character dataset, of which 204 characters (37.6 %) were variable and 130 characters (23.9 %) were parsimony informative. From MP analysis of ITS-5.8S, one tree with a length of 257 steps was obtained (CI = 0.747, RI = 0.740; Fig. 17). The NJ tree was also same branching order. *Lophiostoma sagittiforme* formed a clade with common *Lophiostom* species including *L. macrostomum* with relatively high statistical support (MP 80 %; NJ 96 %).

Discussion

Lophiostoma sagittiforme can be easily identified as a species of *Lophiostoma*, because it has crest-like beak that opens by a long

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thin slit, narrow pseudoparaphyses embedded in gelatinous material, cylindrical asci with fissitunicate dehiscence, and narrowly fusiform ascospores with an appendage-like sheath. The conical ascomata with a flattened base and the trabeculae-like pseudoparaphyses of *L. sagittiforme* superficially resemble those found in some *Astrosphaeriella* species (e.g., *A. barkeriana* (Sacc.) K.D. Hyde & J. Fröhl. 1998; *A. daemonoropsis* J. Fröhl., K.D. Hyde & Aptroot, in Hyde *et al.* 2000). However, *L. sagittiforme* does not have the other characteristic features that define *Astrosphaeriella*, i.e. – large-sized superficial ascomata; a carbonaceous ascomatal wall composed of thick-walled black cells; and a mamilliform or prominent beak with a circular ostiole (Hawksworth 1981, Chen & Hsieh 2004). The phylogenetic analyses of *L. sagittiforme* based on LSU nrDNA support its placement in the genus *Lophiostoma* (Fig. 15). The new species was basal to some *Lophiostoma* species such as *L. crenatum*, *L. arundinis*, *L. caulium*, and also to the type species of the genus i.e. *L. macrostomum*. The sequence data of *L. heterosporum*, which clustered with *Lophiostoma* species in this study, was deposited in Genbank as *Trematosphaeria heterospora* (De Not.) G. Winter (AY016369). However, we considered that this species should be treated as *Lophiostoma*, as proposed by Barr (1992) and Ahn & Sharer (1999), based on its ascomatal morphology. The clade comprising of *L. heterosporum* and other *Lophiostoma* species was supported by a strong bootstrap value of more than 99 %. *Lophiotrema fuckelii* was placed as a sister taxon to *Lophiostoma* species with 97–98 % bootstrap support. Although Tanaka & Harada (2003b) assigned *L. fuckelii* to *Lophiotrema* based on the presence of small ascomata composed of a thin peridial wall, many authors (e.g., Pirozynski & Morgan-Jones 1968, Corlett 1981, Kirk 1984, Holm & Holm 1988, Barr 1992, Hyde *et al.* 2000, Taylor & Crous 2000) have classified *L. fuckelii* in *Lophiostoma*. Furthermore, some authors (e.g., Chesters & Bell 1970) have considered that *Lophiotrema* is a synonym of *Lophiostoma*. It is not clear whether *Lophiotrema* is a valid genus or not, because the sequence data of the type species of *Lophiotrema* (*L. nucula* (Fr.) Sacc.) is currently unavailable. Therefore, based on the morphological and molecular data obtained in our study, the new species *L. sagittiforme* is most suitably classified in *Lophiostoma*.

Several *Lophiostoma* species have bipolar appendages or an elongated sheath in the ascospores similar to that found in *L. sagittiforme*, e.g. *L. armatisporum* (K. D. Hyde, Vrijmoed, Chinnaraj & E. B. G. Jones) E. C. Y. Liew, Aptroot & K. D. Hyde, *L. bipolare* (K. D. Hyde) E. C. Y. Liew, Aptroot & K. D. Hyde, *L. frondisubmersa* (K. D. Hyde) E. C. Y. Liew, Aptroot & K. D. Hyde, *L. macrostomum*, and *L. proprietunicatum* (K. M. Tsui, K. D. Hyde & Hodgkiss) Aptroot &

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K. D. Hyde. In having a narrow sheath provided with arrowhead-like tips, *L. sagittiforme* is most close to *L. proprietunicatum*. However, *L. sagittiforme* has consistently larger ascospores (more than 40 µm vs. 21–28 µm × 5–7 µm; Tsui *et al*. 1999). The range of ascospore length in *L. sagittiforme* is somewhat similar to that in *L. armatisporum* (28–40 µm; Hyde *et al*. 1992) and *L. macrostomum* (28–45.5 µm; Tanaka & Harada 2003a); however, the ascospores of the latter two species have shorter appendages (less than 10 µm long). In terms of sheath morphology, i.e. an inner spine-like structure at both the ends of the ascospores, *L. sagittiforme* resembles *L. bipolare* (Hyde 1995a) and *L. frondisubmersa* (Hyde 1994); however, these two species have more slender ascospores (less than 8 µm wide). A synopsis of these differences is presented in Table 2.

Tab. 2. – Comparison of *Lophiostoma sagittiforme* and similar *Lophiostoma* species having bipolar appendages or an elongated sheath in the ascospores.

Species*	**Ascospores** [µm]	**Sheath morphology of ascospores**			**Habitats**
		Length [µm]	Inner spine	Arrowhead-like tips	
L. sagittiforme	(40) 43.5–53 × 8–12.5	5–32	+	+	terrestrial wood
L. proprietunicatum[a)]	21–28 × 5–7	18–26	–	+	freshwater wood
L. armatisporum[b)]	28–40 × 7–10	6–8	–	–	intertidal mangrove
L. bipolare[c)]	24–32 × 6–8	4–7	+	–	freshwater wood
L. frondisubmersa[d)]	23–28 × 4–7	8–18	+	–	freshwater palm
L. macrostomum[e)]	(28) 30–41.5 (45.5) × 4–7	2–10	–	–	terrestrial wood/grass

* References: [a)] Tsui *et al*. (1999), [b)] Hyde *et al*. (1992), [c)] Hyde (1995a), [d)] Hyde (1994), [e)] Tanaka & Harada (2003a).

In addition to these morphological differences, it was suggested that *L. sagittiforme* is clearly differs from the abovementioned similar taxa in molecular aspects, although the sequence data of *L. proprietunicatum* is unavailable. In the alignment dataset of the ITS region, base differences between *L. sagittiforme* and other *Lophiostoma* species having appendaged ascospores (*L. armatisporum*, *L. bipolare*, and *L. frondisubmersa*) raged from 14.1 % to 14.9 % (77/543 to 81/543), nevertheless some insertions of *L. bipolare* and *L. frondisubmersa* were not included in the calculation. *Lophiostoma sagittiforme* and *L. macrostomum* differed by 9.8 % (53/543).

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Lophiostoma sagittiforme has a unique characteristic with regard to its habitat. Most *Lophiostoma* species with ascospores bearing bipolar appendages or a mucilaginous sheath have been collected from freshwater or mangrove habitats. It is generally considered that the extracellular structures of ascospores may be efficient in causing dispersal and colonization of the species in aquatic habitats (Shearer 1993, Hyde & Goh 2003, Jones 2006). In contrast, *L. sagittiforme* was found on attached dead twigs of *Machilus japonica* in a forest pass at elevations more than 1000 m, it has nevertheless an elongated sheath around the ascospores.

Many terrestrial ascomycetes, particularly species on rainforest palm (Hyde & Aptroot 1997, Fröhlich & Hyde 2000) or bamboo (Tanaka & Harada 2005), also have appendaged ascospores similar to those noted in aquatic environments. Jones (2006) suggested that ascomycetes from terrestrial but high humid conditions like tropical rainforest might be adapted to small watery environment, as was reported for 'terrestrial-aquatic hyphomycetes (Ando 1992)'. Similarly, although *L. sagittiforme* was found in a terrestrial habitat, it might be associated with water films which always exist on the surface of host plant, because Island Yakushima is a region with a large amount of precipitation approximately 5,000–8,000 mm/year. The appendage-like sheath of *L. sagittiforme* may aid spore dispersal in rainwater, and the thickened arrowhead-like tips, may act as anchors for settling the discharged spores to the substratum.

In the phylogenetic analyses based on the ITS regions (Fig. 17), common terrestrial species, namely, *L. macrostomum*, *L. arundinis*, and *L. caulium*, were grouped together in a clade with a high bootstrap support value (99–100%). Other members reported from aquatic habitats including *Vaginatispora aquatica* K.D. Hyde, a species extremely similar to *Lophiostoma* (Hyde 1995b), were located at the basal position of the terrestrial *Lophiostoma* clade. Interestingly, *L. sagittiforme* with appendaged ascospores similar to those observed in freshwater ascomycetes that was found form terrestrial habitats was nested between the terrestrial clade and aquatic species. Phylogenetic trees based on the ITS regions might suggest that *L. sagittiforme* derived from freshwater species and is an ancestral taxon of terrestrial species. However, it would be premature to arrive at this conclusion without additional molecular evidence. It is essential to analyse a greater number of species in the Lophiostomataceae in order to understand the evolutionary speciation of this fungal group.

Acknowledgments

We appreciate the comments provided by the two anonymous reviewers. This work was partially supported by Taxonomic research

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for plants and fungi in areas with remarkable biodiversity in Japan, Research project in the National Museum of Nature and Science.

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(Manuscript accepted 21 April 2008; Corresponding Editor: M. Kirchmair)

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Digitale Literatur/Digital Literature

Zeitschrift/Journal: Sydowia

Jahr/Year: 2008

Band/Volume: 60

Autor(en)/Author(s): Tanaka K., Hosoya Tsuyoshi

Artikel/Article: Lophiostoma sagittiforme sp. nov., a new ascomycete (Pleosporales, Dothideomycetes) from Island Yakushima in Japan. 131-145