

# Resolving the *Lophiostoma bipolare* complex: Generic delimitations within *Lophiostomataceae*

A. Hashimoto<sup>1,2</sup>, K. Hirayama<sup>3</sup>, H. Takahashi<sup>1</sup>, M. Matsumura<sup>1,4</sup>, G. Okada<sup>5</sup>, C.Y. Chen<sup>6</sup>, J.W. Huang<sup>7</sup>, M. Kakishima<sup>8,9</sup>, T. Ono<sup>10</sup>, and K. Tanaka<sup>1,4\*</sup>

<sup>1</sup>Faculty of Agriculture and Life Sciences, Hirosaki University, 3 Bunkyo-cho, Hirosaki, Aomori 036-8561, Japan; <sup>2</sup>Research Fellow of the Japan Society for the Promotion of Science, 5-3-1 Kojimachi, Chiyoda-ku, Tokyo 102-0083, Japan; <sup>3</sup>Apple Experiment Station, Aomori Prefectural Agriculture and Forestry Research Center, 24 Fukutami, Botandaira, Kuroishi, Aomori 036-0332, Japan; <sup>4</sup>The United Graduate School of Agricultural Sciences, Iwate University, 18–8 Ueda 3 chome, Morioka, Iwate 020-8550, Japan; <sup>5</sup>Japan Collection of Microorganisms, RIKEN BioResource Center, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan; <sup>6</sup>National Chung Hsing University, 145 Xingda Rd., South Dis., Taichung 402, Taiwan, ROC; <sup>7</sup>College of Agriculture and Natural Resources, National Chung Hsing University, 250 Kuo-kuang Rd., Taichung 402, Taiwan, ROC; <sup>8</sup>Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, Changchun, Jilin Province 130118, China; <sup>9</sup>Faculty of Life and Environmental Sciences, University of Tsukuba, Ibaraki 305-8572, Japan; <sup>10</sup>Ogasawara Subtropical Branch of Tokyo Metropolitan Agriculture and Forestry Research Center, Chichijima, Ogasawara-mura, Tokyo 100-2101, Japan

\*Correspondence: K. Tanaka, [k-tanaka@hirosaki-u.ac.jp](mailto:k-tanaka@hirosaki-u.ac.jp)

**Abstract:** *Lophiostoma bipolare* was taxonomically revised based on the morphological observations and phylogenetic analyses of molecular data from nuclear rDNA SSU-ITS-LSU, *TUB*, *tef1*, and *rpb2* genes. Twenty-nine strains were morphologically similar to *Lo. bipolare*. A total of 174 sequences were generated from the *Lo. bipolare* complex. Phylogenetic analyses based on *TUB* sequence revealed 11 distinct species within the *Lo. bipolare* complex. Morphological features of the ascospores and the anatomical structure of the ascomata from both field collections as well as axenic culture, which have been reported previously as variable features at intraspecific levels, were compared to evaluate the taxonomic reliability of these features. To clarify the generic position of the 11 species, phylogenetic analyses were done on SSU-ITS-LSU-*tef1-rpb2* gene sequences. The *Lo. bipolare* complex shared phylogenetic relationships with *Pseudolophiostoma* and *Vaginatispora*, and formed an additional five distinct clades from other members of *Lophiostomataceae*. According to its phylogenetic position, *Lo. bipolare sensu stricto* was distantly related to *Lophiostoma s. str.*, and formed an independent clade within *Lophiostomataceae*. *Lophiostoma bipolare s. str.* could be distinguished from the other lophiostomataceous genera by the clypeus around the ostiolar neck and by the thin and uniformly thick peridium. A novel genus described as *Lentistoma* was established to accommodate this species, and the epitypification of *Lentistoma bipolare* (basionym: *Massarina bipolaris*) was proposed. Other lineages of the *Lo. bipolare* complex could not be separated on the basis of the ascospore size and sheath variations, but were distinguished based on ascomatal features, such as the existence of the clypeus, brown hyphae surrounding the peridium, and the contexture of the peridium, which were stable indicators of generic boundaries in *Lophiostomataceae*. Four additional new genera with five new species were recognised based on these morphological differences: *Crassiclypeus* (*C. aquaticus*), *Flabellascoma* (*F. cycadicola* and *F. minimum*), *Leptoparies* (*Lep. palmarum*), and *Pseudopaucispora* (*Pseudop. brunneospora*). Three new species were added to *Pseudolophiostoma* (*Pseudol. cornisporum*, *Pseudol. obtusisporum*, and *Pseudol. tropicum*) and two new species were added to *Vaginatispora* (*V. amygdali* and *V. scabrispora*). The re-evaluation of the validity of several previously recognised genera resulted in the introduction of two new genera with new combinations for *Lophiostoma pseudoarmatisporum* as *Parapaucispora pseudoarmatispora* and *Vaginatispora fuckelii* as *Neovaginatispora fuckelii*.

**Key words:** Freshwater fungi, Pleosporales, Species complex, Systematics, Taxonomy, 21 new taxa, 1 new typification.

**Taxonomic novelties:** **New genera:** *Crassiclypeus* A. Hashim., K. Hiray. & Kaz. Tanaka, *Flabellascoma* A. Hashim., K. Hiray. & Kaz. Tanaka, *Lentistoma* A. Hashim., K. Hiray. & Kaz. Tanaka, *Leptoparies* A. Hashim., K. Hiray. & Kaz. Tanaka, *Neovaginatispora* A. Hashim., K. Hiray. & Kaz. Tanaka, *Parapaucispora* A. Hashim., K. Hiray. & Kaz. Tanaka, *Pseudopaucispora* A. Hashim., K. Hiray. & Kaz. Tanaka; **New species:** *Crassiclypeus aquaticus* A. Hashim., K. Hiray. & Kaz. Tanaka, *Flabellascoma cycadicola* A. Hashim., K. Hiray. & Kaz. Tanaka, *Flabellascoma minimum* A. Hashim., K. Hiray. & Kaz. Tanaka, *Leptoparies palmarum* A. Hashim., K. Hiray. & Kaz. Tanaka, *Pseudolophiostoma cornisporum* A. Hashim., K. Hiray. & Kaz. Tanaka, *Pseudolophiostoma obtusisporum* A. Hashim., K. Hiray. & Kaz. Tanaka, *Pseudolophiostoma tropicum* A. Hashim., K. Hiray. & Kaz. Tanaka, *Pseudopaucispora brunneospora* A. Hashim., K. Hiray. & Kaz. Tanaka, *Vaginatispora amygdali* A. Hashim., K. Hiray. & Kaz. Tanaka, *Vaginatispora scabrispora* A. Hashim., K. Hiray. & Kaz. Tanaka; **New combinations:** *Lentistoma bipolare* (K.D. Hyde) A. Hashim., K. Hiray. & Kaz. Tanaka, *Parapaucispora pseudoarmatispora* (Hay. Takah. et al.) A. Hashim., K. Hiray. & Kaz. Tanaka, *Neovaginatispora fuckelii* (Sacc.) A. Hashim., K. Hiray. & Kaz. Tanaka; **Typification:** **Epitypification (Basionym):** *Massarina bipolaris* K.D. Hyde.

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## INTRODUCTION

*Lophiostomataceae* was established by Saccardo (1883) based on the subfamily *Lophiostomeae*. Members of the family are readily recognised by their carbonaceous ascomata with the slit-like ostiolar neck. They are saprophytes that grow on herbaceous and woody plants from terrestrial, freshwater, and marine environments (Chesters & Bell 1970, Holm & Holm 1988, Barr 1987, 1992). The slit-like ostiolar neck and peridium of the ascomata are regarded as variable structures within a single

specimen. Chesters & Bell (1970) adapted ascospore features including colour and longitudinal or transverse septation for generic circumscription. However, Holm & Holm (1988) considered ascospore septation as an unimportant characteristic at the generic level, but useful at the species level, and therefore used broad generic concepts for *Lophiostomataceae*. These broad generic concepts of *Lophiostoma* have been used by several authors (Barr 1987, 1992, Yuan & Zhao 1994, Checa 1997, Kirk et al. 2008, Mugambi & Huhndorf 2009). A recent generic re-evaluation of *Lophiostomataceae* (Thambugala et al. 2015)

segregated *Lophiostoma* s. lat. into 16 genera according to the multi-locus phylogenies using small subunit nrDNA (18S; SSU), large subunit nrDNA (28S; LSU), and translation elongation factor 1- $\alpha$  (*tef1*).

*Lophiostoma bipolare* is recognised by its striking features of the slit-like ostiolar neck surrounded by a well-developed clypeus, an ascus with a broad ocular chamber, and ascospores bearing an appendage-like sheath (hereafter referred to as the bipolar sheath) (Hyde 1995a). *Lophiostoma bipolare* has been reported in freshwater (Shearer & Raja 2010) and marine habitats (Hyde et al. 2002). The species was originally described as a member of *Massarina* (Hyde 1995a). Although *Lo. bipolare* slightly differs in morphology from the generic type *Lo. macrostomum* – which is characterised by a well-developed carbonaceous ascoma, a slit-like ostiolar neck lacking the clypeus, and an ascus with a small ocular chamber (Zhang et al. 2009) – this species was transferred to the genus *Lophiostoma* based on the results of the phylogenetic analyses using internal transcribed spacer (ITS) sequences (Liew et al. 2002). *Lophiostoma bipolare* was not included in the recent comprehensive study on *Lophiostomataceae* by Thambugala et al. (2015). Thus, its generic placement remains unresolved.

During our studies of ascomycetous fungi in Japan (Hirayama & Tanaka 2011, Tanaka et al. 2015, Hashimoto et al. 2017a, b), we obtained strains that were morphologically similar to *Lo. bipolare*. The main objectives of the present study were to clarify the generic placement of the *Lo. bipolare* complex and to establish a taxonomic framework of genera in *Lophiostomataceae* based on the morphological observations and molecular phylogenetic analyses of the sequences of SSU, ITS, LSU, *tef1*, and *rpb2* (the second largest subunit of the DNA-directed RNA polymerase II).

## MATERIALS AND METHODS

### DNA isolation and amplification

DNA extraction was carried out with an ISOPLANT II kit (Nippon Gene, Japan) based on the manufacturer's protocol. Sequences of SSU, ITS, LSU, *TUB*, *tef1*, and *rpb2* were amplified by PCR with the following primer pairs: SSU = NS1/NS4, ITS = ITS1/ITS4 (White et al. 1990), LSU = LR0R/LR7 (Rehner & Samuels 1994, Vilgalys & Hester 1990), *TUB* = T1/Bt2b (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997), *tef1* = EF1-983F/EF1-2218R (Rehner & Buckley 2005), and *rpb2* = fRPB2-5F/fRPB2-7cR (Liu et al. 1999), respectively. Amplifications were performed in 25  $\mu$ L volumes consisting of 2  $\mu$ L DNA, 2.5  $\mu$ L of 10 $\times$  TEMPase Buffer I, 10 mM dNTP mix, 1  $\mu$ L of each primer (20 pM), 25 mM MgCl<sub>2</sub>, 14.5  $\mu$ L MilliQ water, and 0.5  $\mu$ L TEMPase Hot Start DNA polymerase (Ampliqon, Denmark). PCR was carried out on a PC 320 thermocycler (ASTEC, Japan) as follows: 95 °C for 15 min, 35 cycles of 1 min at 94 °C, 1 min at the designated annealing temperature (42.2 °C for SSU, 61.5 °C for ITS, 46 °C for LSU, 50 °C for *TUB*, 60 °C for *tef1*, and 58 °C for *rpb2*), and 1 min at 72 °C, with a final denaturation step of 7 min at 72 °C. The PCR products were sequenced directly at SolGent (South Korea).

### Phylogenetic analyses

Newly generated sequences were deposited in GenBank (Table 1). The primary analyses of *TUB* sequences were applied

to 29 strains of *Lo. bipolare* complex to assess species diversity (Table 1). Secondary analyses were conducted on SSU-ITS-LSU-*tef1-rpb2* sequences from 73 taxa of *Lophiostomataceae* to clarify the generic placement (Table 1, 2). *Vaginatispora armatispora* was excluded from the analyses because the sequences were limited to ITS data only. All sequences were aligned using the MUSCLE algorithm as implemented in MEGA v. 5 (Tamura et al. 2011). Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian methods. The optimum substitution models for each dataset were estimated using the Kakusan4 program (Tanabe 2011) based on the Akaike information Criterion (AIC; Akaike 1974) for the ML analysis, and the Bayesian Information Criterion (BIC; Schwarz 1978) for the Bayesian analysis. The ML analysis was performed using the TreeFinder Mar 2011 program (Jobb 2011) based on the models selected with the AICc4 parameter (a proportional model among genes and codons). TN93+G was used for *TUB* in the first dataset. The second dataset used HKY85+G for SSU, TN93+G for LSU, J2ef+G for ITS, F81+G for *tef1* first codon position, J1ef+G for *tef1* second codon position, J2+G for *tef1* third codon position, J2+G for *rpb2* first codon position, F81+G for *rpb2* second codon position, and J2+G for *rpb2* third codon position. Bootstrap proportions (BPs) were obtained via 1 000 bootstrap replicates. Bayesian analysis was performed with MrBayes v. 3.2.2 (Ronquist et al. 2012) using substitution models containing the BIC4 parameter (i.e., proportional model among loci and among codons). HKY85+G was used for *TUB* in the first dataset. The second dataset used K80+G for SSU, K80+G for LSU, SYM+G for ITS, F81+G for *tef1* first codon position, GTR+G for *tef1* second codon position, GTR+G for *tef1* third codon position, GTR+G for *rpb2* first codon position, F81+G for *rpb2* second codon position, and HKY85+G for *rpb2* third codon position. Two simultaneous and independent Metropolis-coupled Markov chain Monte Carlo (MCMC) runs were performed for 1 M and 2 M generations with the trees sampled every 1 000 generations for the first and second analyses, respectively. Convergence of the MCMC procedure was assessed from the average standard deviation of split frequencies (< 0.01) and effective sample size scores (all > 100) using MrBayes and Tracer v. 1.6 (Rambaut et al. 2014), respectively. The first 25 % of the trees were discarded as burn-in, and the remainder were used to calculate the 50 % majority rule trees and to determine the posterior probabilities (PPs) for individual branches. *Teichospora rubriostiolata* and *T. trubicola* (*Teichosporaceae*; Jaklitsch et al. 2016) were used as outgroups in the secondary analyses. The alignments were submitted to TreeBASE under study number S21190.

### Morphology and isolation

All fungal structures were observed in preparations mounted in distilled water. Morphological characters were observed by differential interference and phase contrast microscopy (Olympus BX53) using images captured with an Olympus digital camera (DP21). A total of 29 single-spore isolates were used for morphological observations and phylogenetic analyses (Table 1). The colony characteristics of the cultures grown on potato dextrose agar (PDA; Difco) were observed after 3 wk of growth at 20 °C in the dark. Colours were noted as described by Rayner (1970). To induce sexual or asexual fructification in culture, 5 mm squares of mycelial agar were placed on water agar containing sterilised natural substrates including rice straw and

**Table 1.** Specimens, isolates and new sequence accessions used in this study.

Species		Original no.	Specimen no. <sup>1</sup>	Strain no.	Habitat <sup>2</sup>	GenBank accession no.					
Old name	New name					SSU	ITS	LSU	<i>tef1</i>	<i>rpb2</i>	<i>TUB</i>
<i>Lo. bipolare</i> -1	<i>Crassiclypeus aquaticus</i>	KH 56	HHUF 30566	CBS 143639	F	LC312468	LC312497	LC312526	LC312555	LC312584	LC312613
		KH 91	HHUF 30567	CBS 143640	F	LC312469	LC312498	LC312527	LC312556	LC312585	LC312614
		KH 104	HHUF 30568	CBS 143641	F	LC312470	LC312499	LC312528	LC312557	LC312586	LC312615
		KH 185	HHUF 30569	CBS 143642	F	LC312471	LC312500	LC312529	LC312558	LC312587	LC312616
		KT 970	HHUF 27985 <sup>H</sup>	CBS 143643 =JCM 13087 =MAFF 239597	F	LC312472	LC312501	LC312530	LC312559	LC312588	LC312617
<i>Lo. bipolare</i> -2	<i>Flabellascoma cycadicola</i>	KT 2034	HHUF 30570 <sup>H</sup>	BCRC FU30901 =CBS 143644	T	LC312473	LC312502	LC312531	LC312560	LC312589	LC312618
<i>Lo. bipolare</i> -3	<i>F. minimum</i>	KT 2013	HHUF 30571	BCRC FU30900 =CBS 143645	T	LC312474	LC312503	LC312532	LC312561	LC312590	LC312619
		KT 2040	HHUF 30572 <sup>H</sup>	BCRC FU30902 =CBS 143646	T	LC312475	LC312504	LC312533	LC312562	LC312591	LC312620
<i>Lo. bipolare</i> -4	<i>Lentistoma bipolare</i>	HKUCC 10069	HHUF 30576	CBS 115370	U	LC312476	LC312505	LC312534	LC312563	LC312592	LC312621
		HKUCC 10110	HHUF 30577 <sup>E</sup>	CBS 115375	U	LC312477	LC312506	LC312535	LC312564	LC312593	LC312622
		HKUCC 8277	HHUF 30575	JCM 14139 =CBS 110448	F	LC312478	LC312507	LC312536	LC312565	LC312594	LC312623
		KH 214	HHUF 30578	CBS 143647	F	LC312479	LC312508	LC312537	LC312566	LC312595	LC312624
		KH 216	HHUF 30579	CBS 143648	T	LC312480	LC312509	LC312538	LC312567	LC312596	LC312625
		KH 222	HHUF 30580	CBS 143649	F	LC312481	LC312510	LC312539	LC312568	LC312597	LC312626
		KH 311	HHUF 30581	CBS 143650	F	LC312482	LC312511	LC312540	LC312569	LC312598	LC312627
		KT 2415	HHUF 30573	CBS 143651	T	LC312483	LC312512	LC312541	LC312570	LC312599	LC312628
		KT 3056	HHUF 30574	CBS 143652	T	LC312484	LC312513	LC312542	LC312571	LC312600	LC312629
<i>Lo. bipolare</i> -5	<i>Leptoparies palmarum</i>	KT 1653	HHUF 28983 <sup>H</sup>	CBS 143653 =JCM 13089 =MAFF 239599	T	LC312485	LC312514	LC312543	LC312572	LC312601	LC312630
<i>Lo. bipolare</i> -6	<i>Pseudolophiostoma cornisporum</i>	KH 322	HHUF 30582 <sup>H</sup>	CBS 143654 =JCM 32348	T	LC312486	LC312515	LC312544	LC312573	LC312602	LC312631
<i>Lo. bipolare</i> -7	<i>P. obtusisporum</i>	KH 228	HHUF 30584	CBS 143655	T	LC312487	LC312516	LC312545	LC312574	LC312603	LC312632
		KH 336	HHUF 30585	CBS 143656	T	LC312488	LC312517	LC312546	LC312575	LC312604	LC312633
		KT 2838	HHUF 30583 <sup>H</sup>	CBS 143657 =JCM 32349	T	LC312489	LC312518	LC312547	LC312576	LC312605	LC312634
		KT 3098	HHUF 30171	CBS 143941 =MAFF 243969	T	LC312490	LC312519	LC312548	LC312577	LC312606	LC312635

(continued on next page)

Table 1. (Continued).

Old name	Species New name	Original no.	Specimen no. <sup>1</sup>	Strain no.	Habitat <sup>2</sup>	GenBank accession no.					
						SSU	ITS	LSU	tef1	rpb2	TUB
<i>Lo. bipolare</i> -8	<i>P. tropicum</i>	KT 3119	HHUF 30189	CBS 143658 =MAFF 243983	T	LC312491	LC312520	LC312549	LC312578	LC312607	LC312636
		KH 352	HHUF 30586	CBS 143659	T	LC312492	LC312521	LC312550	LC312579	LC312608	LC312637
		KT 3134	HHUF 30202 <sup>H</sup>	CBS 143660 =MAFF 243989	T	LC312493	LC312522	LC312551	LC312580	LC312609	LC312638
<i>Lo. bipolare</i> -9	<i>Pseudopaucispora brunneospora</i>	KH 227	HHUF 30587 <sup>H</sup>	CBS 143661 =JCM 32350	T	LC312494	LC312523	LC312552	LC312581	LC312610	LC312639
<i>Lo. bipolare</i> -10	<i>Vaginatipora amygdali</i>	KT 2248	HHUF 30588 <sup>H</sup>	CBS 143662 =JCM 32351	T	LC312495	LC312524	LC312553	LC312582	LC312611	LC312640
<i>Lo. bipolare</i> -11	<i>V. scabrisspora</i>	KT 2443	HHUF 30589 <sup>H</sup>	CBS 143663 =JCM 32352	M	LC312496	LC312525	LC312554	LC312583	LC312612	LC312641

<sup>1</sup> "E": epitype, "H": holotype.

<sup>2</sup> "F": freshwater, "M": marine, "T": terrestrial, "U": unknown.

banana leaves. The plates were incubated at 20 °C for 2 wk in the dark. When the substrate was colonised, the plates were incubated at 20 °C under black light blue illumination for 2 mo to observe for sporulation. Cultures were deposited in the Bio-resource Collection and Research Center of Food Industry Research and Development Institute, Hsinchu, Taiwan (BCRC); the Japan Collection of Microorganisms (JCM); the Genebank Project NARO, Japan (MAFF); and the Westerdijk Fungal Biodiversity Institute (CBS). Specimens were deposited in the Herbarium of Hirosaki University, Fungi (HHUF).

## RESULTS

### Phylogeny

Alignment of the first analyses was based on *TUB*, and consisted of 29 strains with 628 nucleotide positions. Of these positions, 256 were variable and 357 were conserved. Both ML and Bayesian analyses showed 11 distinct operational taxonomic units for the *Lo. bipolare* complex (Fig. 1).

SSU-LSU phylogenies displayed low resolution at the generic and species levels. SSU-LSU phylogeny also failed to distinguish between *Guttulispora*, *Sigarispora*, and *Platystomum* (Fig. S1A). ITS phylogeny was able to distinguish at the generic and species levels with good resolution, except for *Platystomum* (Fig. S1B). *tef1* phylogeny showed highly supported clades at the species level, while the monophyletic status of genera *Lophiostoma*, *Platystomum*, and *Vaginatipora* were weakly supported (< 70 % ML BS/ < 0.95 Bayesian PP) and *Pseudolophiostoma* was not reconstructed (Fig. S1C). *rpb2* phylogeny was able to distinguish all 12 genera in both analyses, although the dataset included several missing taxa (Fig. S1D).

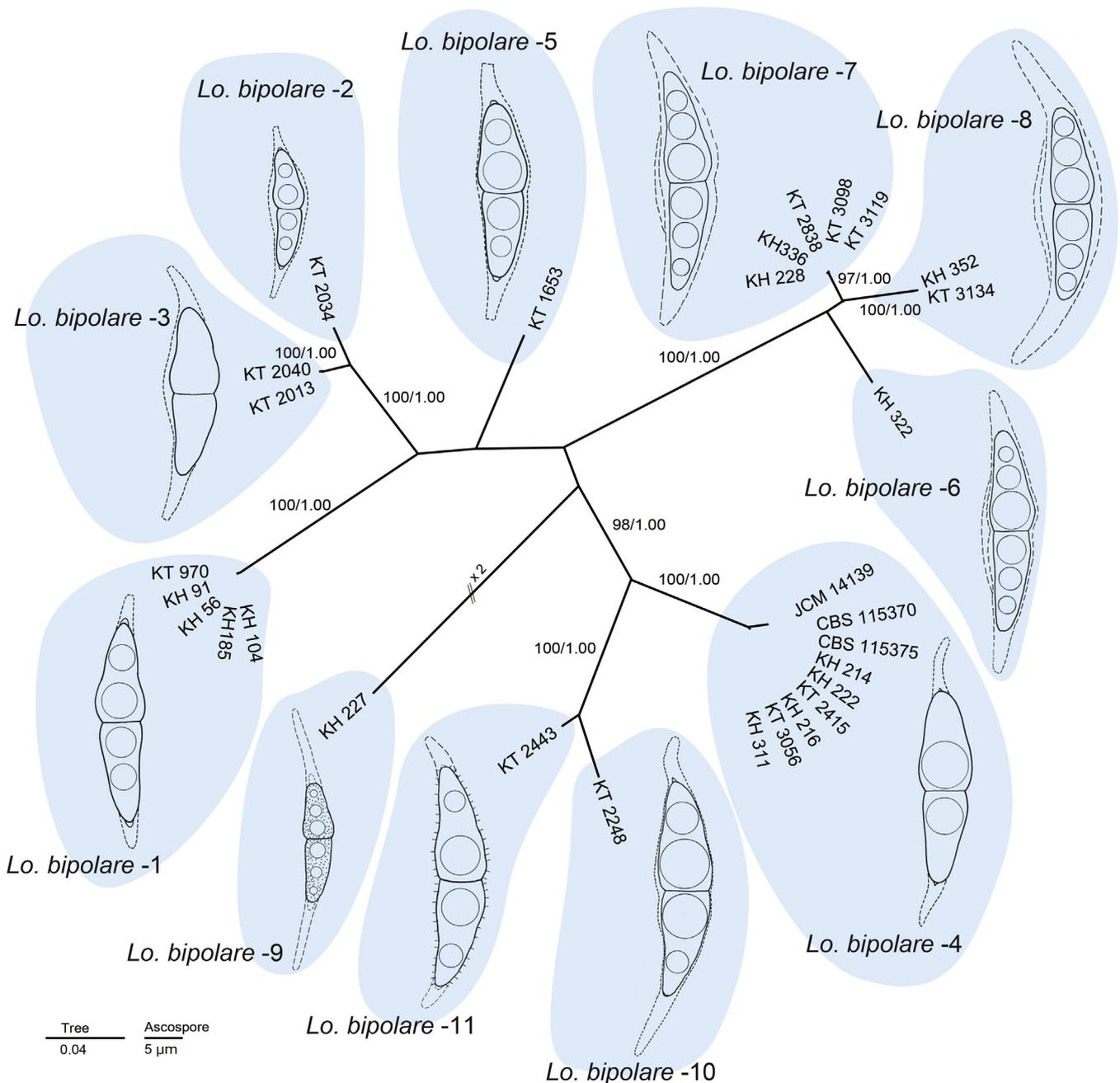
For the second analyses, ML and Bayesian phylogenetic analyses were conducted using an aligned sequence dataset comprising 935 nucleotide positions from SSU, 1 243 from LSU, 900 from ITS, 885 from *tef1*, and 1 017 from *rpb2*. The alignment contained a total of 75 taxa, which consisted of 69 taxa (92 %) in SSU, 75 (100 %) in LSU, 63 (84 %) in ITS, 64 (85.3 %) in *tef1*, and 44 (58.7 %) in *rpb2* (Tables 1, 2). This combined dataset provided higher confidence values for the generic and species levels than those of the individual gene trees, and a total of 23 genera were reconstructed (Fig. 2, S1). Of the 4 980 characters included in the alignment, 1 387 were variable and 3 524 were conserved. The ML tree with the highest log likelihood (-26083.925) is shown in Fig. 2. The Bayesian likelihood score was -26185.401. The topology recovered by the Bayesian analysis was almost identical to that of the ML tree, except for the positions of *Alpestrisphaeria*, *Coelodictyosporium*, and *Lophiohelichrysum*.

The phylogenetic analyses showed that 11 of the *Lo. bipolare* complex appeared polyphyletic (Fig. 1), and were scattered within *Lophiostomataceae* (Figs 2, S1). The phylogenetic positions of *Lo. bipolare* (*Lo. bipolare*-4), including an ex-epitype strain (CBS 115375), was distantly related to *Lophiostoma* s. str. and was located in a clade separate from other members of *Lophiostomataceae* (Fig. 2). The results of the phylogenetic analyses suggested that the species should be excluded from *Lophiostoma* s. str. *Lophiostoma bipolare* was transferred to a novel, individual genus *Lentistoma*, and a new combination *Lentistoma bipolare* was proposed. Other members of the *Lo.*

**Table 2.** Isolates and GenBank accession numbers of species used in the phylogenetic study.

Species	Strain no.	GenBank Accession no. <sup>1</sup>				
		SSU	ITS	LSU	<i>tef1</i>	<i>rpb2</i>
<i>Alpestrisphaeria terricola</i>	SC-12 <sup>H</sup>	JX985749	JN662930	JX985750	–	–
<i>Biappendiculispora japonica</i>	KT 573 <sup>H</sup>	AB618686	LC001728	AB619005	LC001744	–
	KT 686-1 <sup>P</sup>	AB618687	LC001729	AB619006	LC001745	–
<i>Capulatispora sagittiformis</i>	KT 1934 <sup>H</sup>	AB618693	AB369268	AB369267	LC001756	–
<i>Coelodictyosporium muriforme</i>	MFLUCC 13-0351 <sup>H</sup>	KP899127	KP899136	KP888641	KR075163	–
<i>C. pseudodictyosporium</i>	MFLUCC 13-0451 <sup>H</sup>	–	KR025858	KR025862	–	–
<i>Dimorphiopsis brachystegiae</i>	CPC 22679 <sup>H</sup>	–	KF777160	KF777213	–	–
<i>Guttulispora crataegi</i>	MFLUCC 13-0442 <sup>H</sup>	KP899125	KP899134	KP888639	KR075161	–
	MFLUCC 14-0993 <sup>P</sup>	KP899126	KP899135	KP888640	KR075162	–
<i>Lophiohelichrysum helichrysi</i>	MFLUCC 15-0701 <sup>H</sup>	KT333437	KT333435	KT333436	KT427535	–
<i>Lophiopoacea paramacrostoma</i>	MFLUCC 11-0463 <sup>H</sup>	KP899122	–	KP888636	–	–
<i>L. winteri</i>	KT 740	AB618699	JN942969	AB619017	LC001763	JN993487
	KT 764	AB618700	JN942968	AB619018	LC001764	JN993488
<i>Lophiostoma alpigenum</i>	GKM 1091b	–	–	GU385193	GU327758	–
<i>L. caulium</i>	CBS 623.86	GU296163	–	GU301833	–	GU371791
<i>L. crenatum</i>	CBS 629.86	DQ678017	–	DQ678069	DQ677912	DQ677965
<i>L. heterosporum</i>	CBS 644.86	AY016354	GQ203795	AY016369	DQ497609	DQ497615
<i>L. macrostomoides</i>	CBS 123097	FJ795482	–	FJ795439	GU456277	FJ795458
<i>L. macrostomum</i>	KT 635	AB521731	AB433275	AB433273	LC001752	JN993484
<i>L. quadrinucleatum</i>	GKM 1233	–	–	GU385184	GU327760	–
<i>L. semiliberum</i>	KT 828	AB618696	JN942970	AB619014	LC001759	JN993489
<i>Neotrematosphaeria biappendiculata</i>	KT 1124 <sup>H</sup>	GU205256	–	GU205227	–	–
	KT 975 <sup>P</sup>	GU205254	–	GU205228	–	–
<i>Neovaginatisspora fuckelii</i>	CBS 101952	FJ795496	–	DQ399531	–	FJ795472
	KH 161	AB618689	LC001731	AB619008	LC001749	–
	KT 634	AB618690	LC001732	AB619009	LC001750	–
<i>Parapaucispora pseudoarmatispora</i>	KT 2237 <sup>H</sup>	LC100018	LC100021	LC100026	LC100030	–
<i>Paucispora quadrispora</i>	KH 448 <sup>P</sup>	LC001720	LC001733	LC001722	LC001754	–
<i>P. quadrispora</i>	KT 843 <sup>H</sup>	AB618692	LC001734	AB619011	LC001755	–
<i>P. versicolor</i>	KH 110 <sup>H</sup>	LC001721	AB918731	AB918732	LC001760	–
<i>Platystomum actinidiae</i>	KT 521 <sup>H</sup>	JN941375	JN942963	JN941380	LC001747	JN993490
<i>P. compressum</i>	MFLUCC 13-0343	KP899129	–	KP888643	KR075165	–
<i>P. crataegi</i>	MFLUCC 14-0925 <sup>H</sup>	KT026113	KT026117	KT026109	KT026121	–
<i>P. salicicola</i>	MFLUCC 15-0632 <sup>H</sup>	KT026114	KT026118	KT026110	–	–
<i>Pseudolophiostoma vitigenum</i>	HH 26930 <sup>H</sup>	AB618697	LC001735	AB619015	LC001761	–
	HH 26931 <sup>1</sup>	AB618698	LC001736	AB619016	LC001762	–
<i>Pseudoplatystomum scabridisporum</i>	BCC 22835	GQ925831	–	GQ925844	GU479857	GU479830
	BCC 22836	GQ925832	–	GQ925845	GU479856	GU479829
<i>Sigarispora arundinis</i>	KT 651	AB618680	JN942965	AB618999	LC001738	JN993486
<i>S. caudata</i>	KT 530	AB618681	LC001723	AB619000	LC001739	–
<i>S. ononidis</i>	MFLUCC 15-2667 <sup>H</sup>	KU243126	KU243128	KU243125	KU243127	–
<i>S. ravennica</i>	MFLUCC 14-0005 <sup>H</sup>	KP698415	KP698413	KP698414	–	–
<i>Teichospora rubriostiolata</i>	TR 7 <sup>H</sup>	–	KU601590	KU601590	KU601609	KU601599
<i>T. tricolora</i>	C 134 <sup>E</sup>	–	KU601591	KU601591	KU601601	KU601600
<i>Vaginatisspora appendiculata</i>	MFLUCC 16-0314 <sup>H</sup>	KU743219	KU743217	KU743218	KU743220	–
<i>V. aquatica</i>	MFLUCC 11-0083	KJ591575	KJ591577	KJ591576	–	–

<sup>1</sup> "E": ex-epitype, "H": ex-holotype, "I": ex-isotype, "P": ex-paratype.



**Fig. 1.** Maximum-likelihood (ML) tree of the *Lophiostoma bipolarare* complex based on *TUB* sequences. An ML bootstrap proportion (BP) greater than 90 % and Bayesian posterior probabilities (PP) above 0.95 are presented at the nodes as ML BS/Bayesian PP. The scale bars represent nucleotide substitutions per site and ascospore size (5 μm).

*bipolare* complex were scattered to *Pseudolophiostoma*, *Vaginatispora*, and four separate clades from known lophiostomataceous genera. *Lophiostoma bipolarare*-1 was resolved as a strongly supported clade (100 % ML BS/ 1.00 Bayesian PP, Fig. 2) and a new generic name *Crassiclypeus* was proposed for a single novel species, *C. aquaticus*. *Lophiostoma bipolarare*-2, 3 formed a robust clade (100 % ML BS/ 1.00 Bayesian PP, Fig. 2). A new genus *Flabellascoma* was introduced for these two species (*F. cycadicola* and *F. minimum*). The monotypic genus *Leptopariis* was introduced for *Lep. palmarum* (formerly treated as *Lo. bipolarare*-5), which separated from the other lophiostomataceous genera in the phylogenetic tree (Fig. 2). *Pseudolophiostoma* comprised four species – its type species *Pseudol. vitigenum* (Thambugala et al. 2015), as well as *Lo. bipolarare*-6, 7, and 8 (*Pseudol. cornisporum*, *Pseudol. obtusisporum*, and *Pseudol. tropicum*) – forming a strongly supported clade (98 % ML BS/ 1.00 Bayesian PP, Fig. 2). *Lophiostoma bipolarare*-9

represented a basal clade among *Lophiostomataceae*, for which a new genus and species *Pseudopaucispora brunneospora* was introduced. A clade containing *V. appendiculata*, *V. aquatica*, and *Lo. bipolarare*-10, 11 received strong support (98 % ML BS/ 1.00 Bayesian PP, Fig. 2). *Vaginatispora amygdali* and *V. scabrispora* were proposed for *Lo. bipolarare*-10, 11, respectively.

## Taxonomy

Our phylogenetic analyses resolved 11 species that were classified in the *Lo. bipolarare* complex (Figs 1, 2). These 11 species could not be distinguished solely based on the ascospore morphology due to their close resemblance (Fig. 1). Detailed morphological observations of the ascospores as well as other morphological features (Figs 3–13), culture characteristics (Fig. 14) and multi-locus phylogeny differentiated the complex. Seven genera (including five new genera), 11 species (including

10 new species), and one new combination are proposed below. An epitype is designated for *Lo. bipolare* s. str. (basonym: *Massarina bipolaris*). Additionally, two new genera and two new combinations are introduced for *Lophiostoma pseudoarmatisporum* and *Vaginatipora fuckelii* (See Discussion and Appendix B).

**Crassiclypeus** A. Hashim., K. Hiray. & Kaz. Tanaka, **gen. nov.** MycoBank MB823131.

*Etymology*: Refers to its well-developed clypeus.

*Sexual morph*: Ascumata scattered to gregarious, immersed, subglobose. Ostiolar neck elongated, laterally compressed, surrounded by a well-developed clypeus. Peridium composed of elongated, brown cells, surrounded by brown hyphae. Pseudoparaphyses numerous, septate, branched and anastomosed. Asci bitunicate, fissitunicate, clavate, 8-spored. Ascospores fusiform, hyaline, 1-septate, with a narrow bipolar sheath. *Asexual morph*: Conidiomata pycnidial, globose to subglobose, superficial to immersed. Peridium composed of subglobose to rectangular, brown cells. Conidiophores absent. Conidiogenous cells phialidic, ampliform, hyaline, smooth. Conidia subglobose with rounded ends, hyaline, smooth, aseptate.

*Type species*: *Crassiclypeus aquaticus* A. Hashim., K. Hiray. & Kaz. Tanaka.

*Notes*: *Crassiclypeus* is established to accommodate *C. aquaticus*, which is characterised by a crest-like, elongated, and laterally compressed ostiolar neck, well-developed peridium surrounded by brown hyphae (Fig. 3D–F), and an ascus with a long stipe (up to 50 µm, Fig. 3K, L).

The genus is superficially similar to *Flabellascoma*, but differs from the latter by having an ascumatal wall with 1 zone (Fig. 3D–F) and phialidic conidiogenous cells in the conidiomata (Fig. 3Y–AA) (vs. an ascumatal peridium composed of 2 zones and holoblastic conidiogenous cells in the conidiomata; Fig. 4D, W, X). It is also similar to *Neotrematosphaeria*, but the latter genus has a poorly developed peridium at the base and lacks the clypeated ostiolar neck of the ascumata (Thambugala *et al.* 2015). *Crassiclypeus* has similar morphological features of *Lentistoma*, such as the clypeated ostiolar neck, but can be distinguished from *Lentistoma* by the well-developed peridium of the ascumata (up to 70 µm in thickness). *Lentistoma* is characterised by ascumata with less-developed peridium (up to 45 µm in thickness).

**Crassiclypeus aquaticus** A. Hashim., K. Hiray. & Kaz. Tanaka, **sp. nov.** MycoBank MB823132. Figs 3, 14A.

*Etymology*: Refers to its aquatic habitat.

*Sexual morph*: Ascumata subglobose, 3–5 grouped, immersed, dark brown to black, 400–780 µm high, 600–1000 µm diam. Ostiolar neck crest-like, elongated and laterally compressed, 80–100 µm high, 160–300 µm wide, composed of 2.5–7 µm diam, globose, thick-walled, brown to black cells, with hyaline periphyses, surrounded by a well-developed clypeus (up to 390 µm wide). Peridium uniform, (30–)45–70 µm thick at side, composed of 8–9(–15) layers of elongated, thin-walled, 5–18 × 2.5–5 µm, brown cells, surrounded by brown hyphae. Pseudoparaphyses numerous, 1.5–2 µm wide, septate, branched and anastomosed. Asci bitunicate, fissitunicate, clavate, 85–125 × 11–17.5 µm ( $\bar{x}$  = 102.5 × 12.9 µm, n = 96),

with a stipe (14–50 µm long,  $\bar{x}$  = 26.3 µm, n = 24), apically rounded with a broad ocular chamber, 8-spored. Ascospores fusiform with obtuse ends, 20–32.5 × 5–8 µm ( $\bar{x}$  = 25.3 × 6.7 µm, n = 190), l/w 2.9–4.8 ( $\bar{x}$  = 3.8, n = 190), hyaline, with a septum nearly median ((0.42–)0.45–0.55,  $\bar{x}$  = 0.49, n = 167), slightly constricted at the septum, smooth, with a narrow sheath. Sheath drawn out 2–5 µm long at both ends, with an internal chamber at both ends of ascospores. *Asexual morph*: Conidiomata pycnidial, globose to subglobose, up to 165 µm high, 135–180 µm diam, scattered to 3–6 grouped, superficial to immersed. Peridium 11.5–18.5 µm thick, composed of 2–4 layers of 8–13.5 × 3–4 µm, subglobose to rectangular, brown cells. Conidiophores reduced to conidiogenous cells. Conidiogenous cells phialidic, 6–12 × 2.5–4 µm, ampliform, hyaline, smooth. Conidia subglobose with rounded ends, 2–3(–4) × 1.2–1.8 µm ( $\bar{x}$  = 2.4 × 1.5 µm, n = 60), l/w 1.2–2.2 ( $\bar{x}$  = 1.6, n = 60), hyaline, smooth, aseptate, guttulate when young.

*Culture characteristics*: Colonies on PDA attaining 19 mm diam within 21 d at 20 °C in the dark, velvety, centrally raised, greenish grey (110; Rayner 1970); reverse dull green (70) (Fig. 14A); red pigment produced in water agar media (Fig. 3T); asexual morph formed.

*Materials examined* (all on submerged dead twigs of woody plant): **Japan**, Aomori, Hirosaki, Aoki, near Mohei-pond, 7 Dec. 2002, K. Tanaka & N. Asama, KT 970 (HHUF 27985 **holotype** designated here; ex-holotype culture CBS 143643 = JCM 13087 = MAFF 239597); *ibid.*, 21 Jul. 2007, K. Hirayama & K. Tanaka, KH 56 (HHUF 30566; ex-paratype culture CBS 143639); *ibid.*, 23 Sep. 2007, K. Hirayama & K. Tanaka, KH 91 (HHUF 30567; ex-paratype culture CBS 143640); *ibid.*, 29 Oct. 2007, K. Hirayama & K. Tanaka, KH 104 (HHUF 30568; ex-paratype culture CBS 143641); *ibid.*, 30 Aug. 2008, K. Hirayama & K. Tanaka, KH 185 (HHUF 30569; ex-paratype culture CBS 143642).

*Notes*: *Crassiclypeus aquaticus* was collected from submerged dead twigs of woody plants during summer, fall, and winter. *Crassiclypeus aquaticus* strains produced a red pigment in water agar medium.

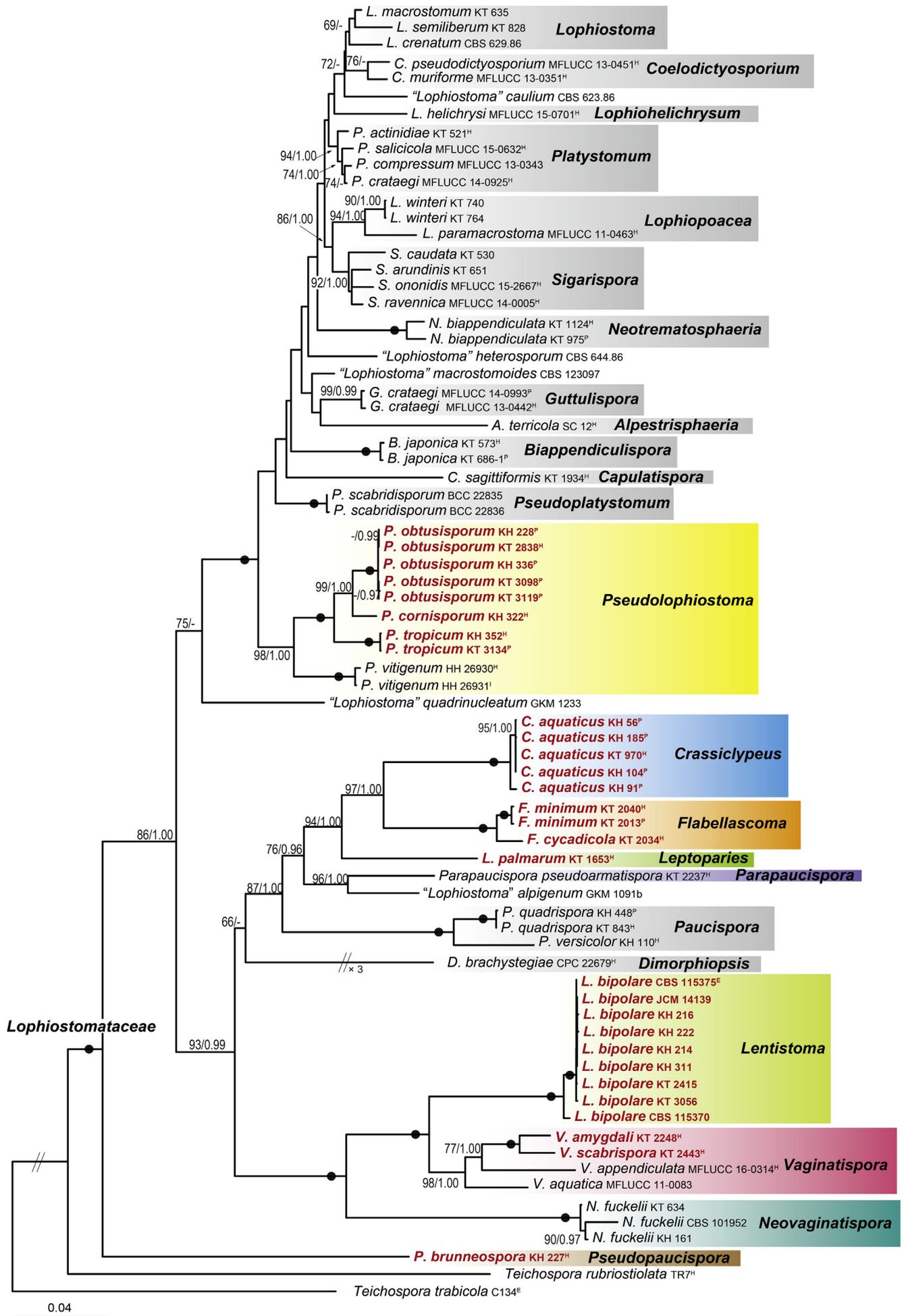
**Flabellascoma** A. Hashim., K. Hiray. & Kaz. Tanaka, **gen. nov.** MycoBank MB823133.

*Etymology*: Refers to its ostiolar neck, which resembles a Japanese fan.

*Sexual morph*: Ascumata scattered, immersed, subglobose to ellipsoidal. Ostiolar neck elongated, laterally compressed. Peridium composed of elongated, brown cells. Pseudoparaphyses septate, branched and anastomosed. Asci bitunicate, fissitunicate, cylindrical-clavate, 8-spored. Ascospores fusiform, hyaline, 1-septate, with a narrow bipolar sheath. *Asexual morph*: Conidiomata pycnidial, globose to subglobose. Peridium composed of subglobose to rectangular, brown cells. Conidiophores absent. Conidiogenous cells holoblastic, cylindrical or ampliform, hyaline, smooth. Conidia subglobose with rounded ends, hyaline, smooth, aseptate.

*Type species*: *Flabellascoma minimum* A. Hashim., K. Hiray. & Kaz. Tanaka.

*Notes*: The genus *Flabellascoma* is proposed to include *F. cycadicola* and *F. minimum*. These two species have well-developed, crest-like ostiolar necks (Figs 4C, 5C) and a uniformly thickened ascumatal wall composed of 2 zones (Figs 4D, 5D), and asci with a short stipe. *Flabellascoma* is



morphologically similar to *Pseudolophiostoma* in having ascomata with a well-developed, crest-like ostiolar neck, and a peridium of uniform thickness. However, the ascomatal peridium in *Pseudolophiostoma* is composed of 1 zone (Thambugala *et al.* 2015, this study Figs 8G, 9G, 10G) rather than the 2 zones in *Flabellascoma*.

***Flabellascoma cycadicola*** A. Hashim., K. Hiray. & Kaz. Tanaka, **sp. nov.** MycoBank MB823134. Figs 4, 14B.

*Etymology*: Refers to the generic name of the host plant.

*Sexual morph*: Ascomata subglobose, scattered, immersed, dark brown to black, 490–530 µm high, 600–620 µm diam. *Ostiolar neck* crest-like, elongated, laterally compressed, 190–210 µm high, 320–380 µm wide, composed of 3–6 µm diam, globose, brown to black cells, with hyaline periphyses. *Peridium* uniform, 45–50 µm thick at side, composed of 2 zones; outer zone 28–38 µm thick, composed of 5–8 layers of rectangular, thin-walled, 10–17 × 3–4 µm, brown cells; inner zone 12–25 µm thick, composed of globose, 1.5–2.5 µm diam, hyaline cells. *Pseudoparaphyses* numerous, 1–3 µm wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical-clavate, 67.5–88 × 9–12 µm ( $\bar{x}$  = 77.2 × 10.2 µm, n = 20), with a short stipe, apically rounded with a broad ocular chamber, 8-spored. *Ascospores* fusiform with obtuse ends, 17–23 × 4.5–7 µm ( $\bar{x}$  = 20.4 × 5.4 µm, n = 70), l/w 3.0–4.5 ( $\bar{x}$  = 3.8, n = 70), hyaline, with a septum nearly median (0.47–0.55,  $\bar{x}$  = 0.50, n = 70), slightly constricted at the septum, smooth, with a narrow sheath. *Sheath* drawn out 7–10 µm long at both ends, with a lateral pad-like structure within the sheath, with an internal chamber at both ends of ascospores. *Asexual morph*: *Conidiomata* pycnidial, globose to subglobose, up to 90 µm high, 50–85 µm diam, 4–10 grouped, superficial. *Peridium* 7–13 µm thick, composed of 2–4 layers of 6.5–10.5 × 3.5–4 µm, subglobose to rectangular, brown cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, 6–8 × 1.5–2.5 µm, ampliform, hyaline, smooth. *Conidia* subglobose with rounded ends, 1.5–2.5 × 1.1–2 µm ( $\bar{x}$  = 2.0 × 1.4 µm, n = 60), l/w 1.0–1.8 ( $\bar{x}$  = 1.4, n = 60), hyaline, smooth, aseptate, guttulate when young.

*Culture characteristics*: Colonies on PDA attaining 20 mm diam within 21 d at 20 °C in the dark, velvety, centrally raised, greenish grey (110); reverse grey olivaceous (107) (Fig. 14B); asexual morph formed.

*Material examined*: Taiwan, Taipei, Wulai, on petiole of *Cycas revoluta*, 28 Nov. 2005, K. Tanaka, H.S. Chang & G. Okada, KT 2034 (HHUF 30570 **holotype** designated here; ex-holotype culture BCRC FU30901 = CBS 143644).

*Notes*: *Flabellascoma cycadicola* superficially resembles *F. minimum*, but can be distinguished from the latter by its larger ascospores (17–23 × 4.5–7 µm vs. 12–17.5 × 3.5–5 µm). The two species differ at 15 positions with one gap in their ITS regions.

***Flabellascoma minimum*** A. Hashim., K. Hiray. & Kaz. Tanaka, **sp. nov.** MycoBank MB823135. Figs 5, 14C.

*Etymology*: Refers to its small-sized ascospore.

*Sexual morph*: Ascomata ellipsoidal to lageniform, scattered, immersed, dark brown to black, 250–320 µm high, 350–500 µm diam. *Ostiolar neck* crest-like, elongated, laterally compressed, 160–310 µm high, 210–280 µm wide, composed of 3–5 µm diam, globose, brown to black cells, with hyaline periphyses. *Peridium* uniform, 43–50(–62) µm thick at side, composed of 2 zones; outer zone 18–27(–42) µm thick, composed of 3–5 layers of rectangular, thin-walled, 5–18 × 5–8 µm, brown cells; inner layer 20–25 µm thick, composed of globose, 1–2 µm diam, hyaline cells. *Pseudoparaphyses* numerous, 1.5–3 µm wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical-clavate, 45–77.5 × 7.5–12 µm ( $\bar{x}$  = 61.9 × 9.3 µm, n = 123), with a stipe, apically rounded with a broad ocular chamber, 8-spored. *Ascospores* fusiform with obtuse ends, 12–17.5 × 3.5–5 µm ( $\bar{x}$  = 14.8 × 4.3 µm, n = 17), l/w 2.6–4.3 ( $\bar{x}$  = 3.4, n = 170), hyaline, with a septum nearly median (0.46–0.54,  $\bar{x}$  = 0.50, n = 170), slightly constricted at the septum, smooth, with a narrow sheath. *Sheath* drawn out 5.5–8 µm long at both ends, with a lateral pad-like structure within the sheath, up to 1.5 µm wide at side, with an internal chamber at both ends of ascospores. *Asexual morph*: *Conidiomata* pycnidial, globose to subglobose, up to 125 µm high, 85–110 µm diam, 3–5 grouped, superficial. *Peridium* 12–17 µm thick, composed of 2–4 layers of 6.5–10 × 3.5–5 µm, subglobose to rectangular, brown cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, 7.5–9 × 2.5–4 µm, cylindrical, hyaline, smooth. *Conidia* subglobose with rounded ends, 1.9–2.4(–3.2) × 1.3–1.8 µm ( $\bar{x}$  = 2.1 × 1.5 µm, n = 50), l/w 1.1–1.6(–2.1) ( $\bar{x}$  = 1.3, n = 50), hyaline, smooth, aseptate, guttulate when young.

*Culture characteristics*: Colonies on PDA attaining 17 mm diam within 21 d at 20 °C in the dark, velvety, centrally raised, smoke grey (105); reverse sienna (8) (Fig. 14C); asexual morph formed.

*Materials examined*: Taiwan, Nantou Hsien, Hui Sun Forest Area, Kuan-Dau river, on petioles of *Arenga engleri*, 26 Nov. 2005, K. Tanaka, C.Y. Chen & G. Okada, KT 2013 (HHUF 30571; ex-paratype culture BCRC FU30900 = CBS 143645); Taipei, Wulai, on pods of *Bauhinia purpurea*, 28 Nov. 2005, K. Tanaka, H.S. Chang & G. Okada, KT 2040 (HHUF 30572 **holotype** designated here; ex-holotype culture BCRC FU30902 = CBS 143646).

*Notes*: ITS sequences of ex-holotype and ex-paratype cultures of *F. minima* isolated from *Areaceae* (*Arecales*) and *Fabaceae* (*Fabales*), respectively, were identical. Although the ascomatal shape was slightly different between the holotype (lageniform, Fig. 5D) and paratype (ellipsoidal, Fig. 5E) of *F. minima*, the peridial structure of their ascomata and the ascospore size were almost identical. The differences in the ascomatal shape appeared to vary depending on the condition of the substrates. We, therefore, regard these specimens as conspecific.

***Lentistoma*** A. Hashim., K. Hiray. & Kaz. Tanaka, **gen. nov.** MycoBank MB823136.

*Etymology*: Refers to its lenticular ascomata.

**Fig. 2.** Maximum-likelihood (ML) tree of *Lophiostomataceae* based on the SSU-ITS-LSU-*tef1-rpb2* sequences. An ML bootstrap proportion (BP) greater than 60 % and Bayesian posterior probabilities (PP) above 0.95 are presented at the nodes as ML BS/Bayesian PP. The circle (●) indicates nodes with 100 % ML BS/ 1.00 Bayesian PP. A hyphen (“-”) indicates values lower than 60 % BP or 0.95 PP. Ex-holotype, isotype, paratype, and epitype strains are indicated with superscripts H, I, P, and E, respectively. The newly obtained sequences are shown in bold and red. The scale bar represents nucleotide substitutions per site.



*Sexual morph*: Ascomata scattered, immersed, subglobose. *Ostiolar neck* elongated, laterally compressed, surrounded by a well-developed clypeus. *Peridium* composed of globose, brown cells. *Pseudoparaphyses* numerous, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical-clavate, 8-spored. *Ascospores* fusiform, hyaline, 1-septate, with a narrow bipolar sheath. *Asexual morph*: Undetermined.

*Type species*: *Lentistoma bipolare* (K.D. Hyde) A. Hashim., K. Hiray. & Kaz. Tanaka

*Notes*: *Lophiostoma bipolare* was originally described as a species of *Massarina* (Hyde 1995a). Liew *et al.* (2002) transferred the species to *Lophiostoma* based on phylogenetic analyses of ITS sequences. This classification was corroborated by subsequent studies (Tanaka & Hosoya 2008, Hirayama & Tanaka 2011). Thambugala *et al.* (2015) conducted comprehensive taxonomic revisions in *Lophiostomataceae*, but *Lo. bipolare* was not included. Thus, its generic placement remained unresolved. Our phylogenetic study revealed the distant relationship of this species to *Lophiostoma* s. str. (Fig. 2). *Lentistoma* is well-characterised and is differentiated from *Lophiostoma* by its clypeus around the ostiolar neck and by its thinner and uniformly thickened peridium (up to 45 µm in thickness, Fig. 6H–J).

***Lentistoma bipolare*** (K.D. Hyde) A. Hashim., K. Hiray. & Kaz. Tanaka, **comb. nov.** MycoBank MB823137. Figs 6, 14D, E.

*Basionym*: *Massarina bipolaris* K.D. Hyde, Nova Hedwigia 61: 131. 1995.

*Synonym*: *Lophiostoma bipolare* (K.D. Hyde) E.C.Y. Liew *et al.*, Mycologia 94: 812. 2002.

*Sexual morph*: Ascomata subglobose, scattered, immersed, dark brown to black, 160–200 µm high, 470–540 µm diam. *Ostiolar neck* crest-like, elongated, laterally compressed, 100–125 µm high, 210–225 µm wide, composed of globose, brown to black cells, with hyaline periphyses, surrounded by a well-developed clypeus (up to 500 µm wide). *Peridium* uniform, 25–45 µm thick at side, composed of 5–7 layers of rectangular, thin-walled, 12.5–15 × 5 µm, brown cells. *Pseudoparaphyses* numerous, 1–2 µm wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical-clavate, (82–) 105–140 × 8–15 µm ( $\bar{x}$  = 119.9 × 10.9 µm, n = 30), with a stipe (7.5–18.5 µm long,  $\bar{x}$  = 11.7 µm, n = 11), apically rounded with a broad ocular chamber, 8-spored. *Ascospores* fusiform with obtuse ends, 20–33 × 5.5–9(–11) µm ( $\bar{x}$  = 27.0 × 7.2 µm, n = 216), l/w 2.5–4.8 ( $\bar{x}$  = 3.8, n = 216), hyaline, with a septum nearly median (0.46–0.55,  $\bar{x}$  = 0.50, n = 216), slightly constricted at the septum, smooth, with a narrow sheath. *Sheath* drawn out 5–10 µm long at both ends, with a cap-like structure at tips of the sheath, with an internal chamber at both ends of ascospores. *Asexual morph*: Undetermined.

*Culture characteristics*: Colonies on PDA attaining 16 mm diam within 21 d at 20 °C in the dark, velvety, plane, dull green (110); reverse grey olivaceous (107) (Fig. 14D, E); asexual morph formed.

*Materials examined*. Australia, Queensland, Kauri Creek, on woody plant, 23 May 2003 (HHUF 30576, dried culture specimen made from culture CBS

115370). China, Hong Kong, Tai Po Country Park, on submerged wood, Aug. 1993, K.D. Hyde (BRIP 21489, **holotype**); Sai Kung, Highland Reservoir, on submerged wood, 3 May 2003 (HHUF 30575, dried culture specimen made from culture JCM 14139 = CBS 110448); Mt. Nicholson, on woody plant, 9 Sep. 2003 (HHUF 30577, dried culture specimen made from culture CBS 115375, **epitype** designated here; MBT379010). Japan, Okinawa, Isl. Iriomote, near Kampire waterfall, on dead herbaceous plant, 27 Sep. 2007, K. Tanaka & H. Yonezawa, KT 2415 (HHUF 30573; culture CBS 143651); *ibid.*, on dead twigs of woody plant, 5 Aug. 2012, K. Tanaka, KT 3056 (HHUF 30574; culture CBS 143652); Oomijya river, on submerged dead twigs of woody plant, 22 Nov. 2008, K. Hirayama & K. Tanaka, KH 214 (HHUF 30578; culture CBS 143647); *ibid.*, on submerged dead twigs of woody plant, 12 Jul. 2011, K. Hirayama & K. Tanaka, KH 311 (HHUF 30581; culture CBS 143650); near Maryudu water falls, on herbaceous plant, 21 Nov. 2008, K. Hirayama & K. Tanaka, KH 216 (HHUF 30579; culture CBS 143648); *ibid.*, on submerged dead twigs of woody plant, 21 Nov. 2008, K. Hirayama & K. Tanaka, KH 222 (HHUF 30580; culture CBS 143649).

*Notes*: Our phylogenetic and morphological studies revealed 11 species scattered among *Lophiostomataceae* (Figs 1, 2). They were originally misidentified as *Lo. bipolare* based on the morphological resemblance of their ascospores, but a precise morphological observation of the *Lo. bipolare* complex including its holotype (BRIP 21489) distinguished the *Lo. bipolare* s. str. from other species of the *Lo. bipolare* complex on the basis of a clypeus around the ostiolar neck (Fig. 6H–J); an internal chamber at both ends of the ascospores (Fig. 6U); and a bipolar sheath with a cap-like structure at the tips (Fig. 6T). Here, we designated an epitype specimen (HHUF 30577) that was collected from the same country as the holotype specimen. Although the species was previously reported to have been collected from either freshwater or marine habitats (Hyde *et al.* 2002, Shearer & Raja 2010), this is the first report of the species from a terrestrial habitat.

***Leptoparies*** A. Hashim., K. Hiray. & Kaz. Tanaka, **gen. nov.** MycoBank MB823138.

*Etymology*: Refers to the thin peridium of the ascomata.

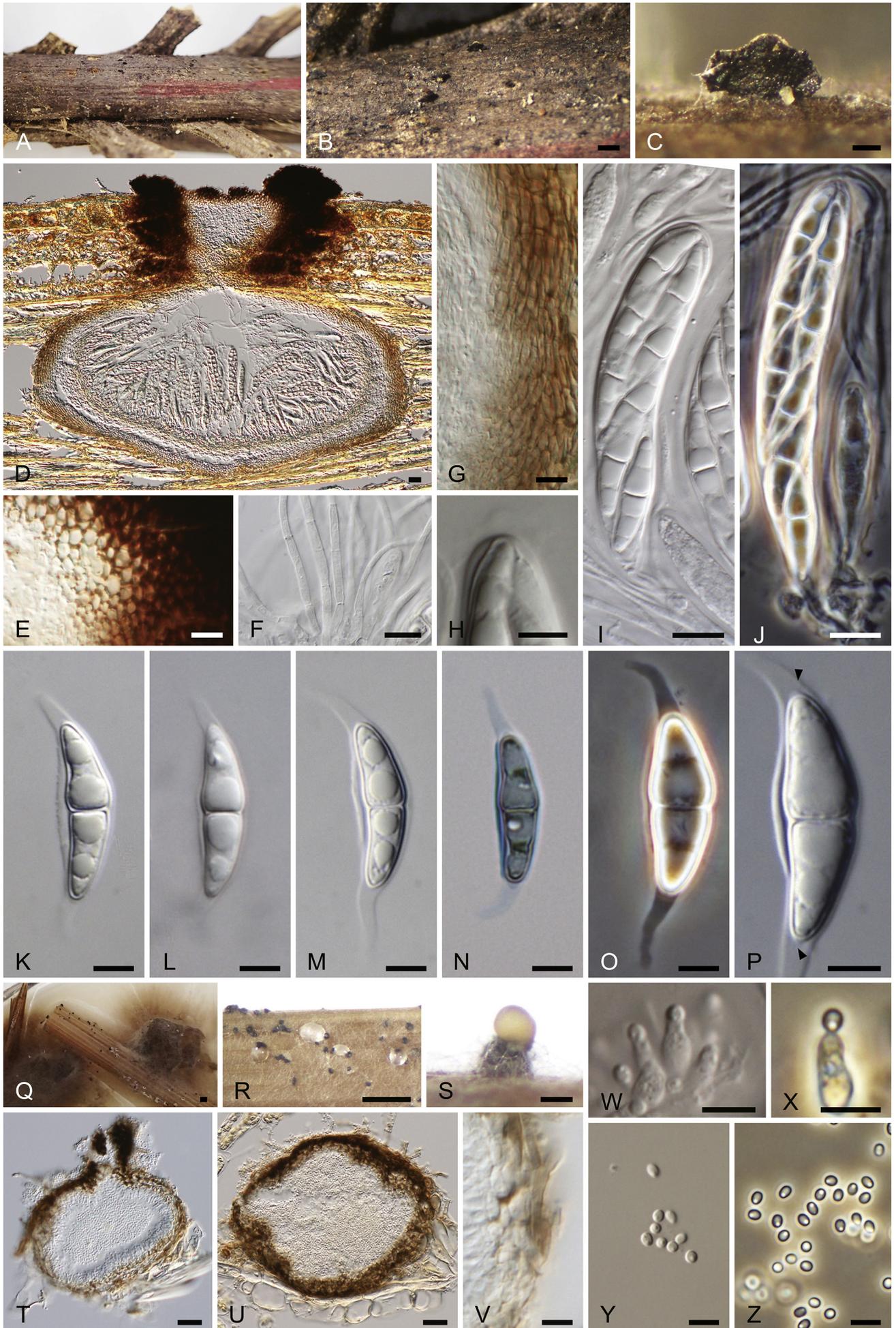
*Sexual morph*: Ascomata scattered, immersed, subglobose. *Ostiolar neck* elongated, laterally compressed. *Peridium* relatively thin, composed rectangular, brown cells. *Pseudoparaphyses* numerous, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical-clavate, 8-spored. *Ascospores* fusiform, hyaline, 1-septate, with a narrow bipolar sheath. *Asexual morph*: Undetermined.

*Type species*: *Leptoparies palmarum* A. Hashim., K. Hiray. & Kaz. Tanaka.

*Notes*: *Leptoparies* is a new monotypic genus characterised by a relatively thinner and non-carbonised peridium, which represents an atypical character for *Lophiostomataceae*. *Leptoparies* can be easily distinguished from other genera by the thin peridium composed of rectangular cells and the absence of the surrounding brown hyphae (Fig. 7J). The genus is similar to *Capulatispora* due to the thin peridium and the ascospores with the drawn-out sheaths; however, *Capulatispora* differs from *Leptoparies* due to its short ascus stipe (Tanaka & Hosoya 2008, Thambugala *et al.* 2015).

***Leptoparies palmarum*** A. Hashim., K. Hiray. & Kaz. Tanaka, **sp. nov.** MycoBank MB823139. Figs 7, 14F.

**Fig. 3.** *Crassiclypeus aquaticus*. A–C. Appearance of ascomata on substrate. D–F. Ascomata in longitudinal section. G. Peridium of ascoma. H. Ostiolar neck of ascoma. I. Ascus apex. J. Ascus stipe. K, L. Asci. M. Pseudoparaphyses. N–S. Ascospores (arrowheads indicate an internal chamber in S). T–V. Conidiomata in culture. W. Conidioma in longitudinal section. X. Peridium of conidioma. Y–AA. Conidiogenous cells. AB, AC. Conidia. A, G–L, O–S from HHUF 30569. B–F from HHUF 27985 (holotype); M, N from HHUF 30567; T–AC from culture CBS 143640. Scale bars: A, T = 1 mm; B = 300 µm; C, U, V = 100 µm; D–F = 50 µm; K–M, W = 10 µm; G–J, N–S, X–AC = 5 µm.



**Etymology:** Refers to the host plant.

**Sexual morph:** *Ascomata* subglobose, scattered, immersed, dark brown to black, 210–320 µm high, 490–650 µm diam. *Ostiolar neck* crest-like, elongated, laterally compressed, 90–140 µm high, 200–300 µm wide, composed of 6–8 × 3–4 µm diam, globose, brown to black cells, with hyaline periphyses. *Peridium* uniform, 25–32 µm thick at side, composed of 3–5 layers of rectangular, thin-walled, 8–10 × 3–7 µm, brown cells, surrounded by brown hyphae. *Pseudoparaphyses* numerous, 1.5–2 µm wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical-clavate, (67–) 77–118 × 10–14 µm ( $\bar{x}$  = 93.9 × 11.9 µm, n = 20), with a stipe (8.5–18.5 µm long,  $\bar{x}$  = 13.5 µm, n = 20), apically rounded with a broad ocular chamber, 8-spored. *Ascospores* fusiform with obtuse ends, 20–25 × 5–7 µm ( $\bar{x}$  = 23.1 × 6.1 µm, n = 100), l/w 2.8–4.4 ( $\bar{x}$  = 3.5, n = 100), hyaline, with a septum mostly supramedian (0.47–0.55,  $\bar{x}$  = 0.49, n = 100), slightly constricted at the septum, smooth, with a narrow sheath. *Sheath* drawn out 6–8 µm long at both ends, with a lateral pad, up to 1.5 µm wide at side. **Asexual morph:** Undetermined.

**Culture characteristics:** Colonies on PDA attaining 21 mm diam within 21 d at 20 °C in the dark, velvety, centrally raised, smoke grey (105); reverse grey olivaceous (107) (Fig. 14F); sexual morph formed.

**Material examined:** Japan, Kanagawa, Yokohama, Nakaku, near Sankei-garden, on petioles of *Trachycarpus fortunei*, 9 Mar. 2004, K. Tanaka & Y. Harada, KT 1653 (HHUF 28983 **holotype** designated here; ex-holotype culture CBS 143653 = JCM 13089 = MAFF 239599).

**Notes:** *Leptoparies palmarum* and *Flabellascoma minimum* can be found on the petioles of palms. The former species is characterised by the larger ascospores (20–25 × 5–7 µm) distinguishing it from the latter species, which has smaller ascospores (12–17.5 × 3.5–5 µm).

***Pseudolophiostoma*** Thambug. *et al.*, Fungal Diversity 74: 235. 2015.

**Sexual morph:** *Ascomata* scattered, immersed, globose to subglobose. *Ostiolar neck* elongated, laterally compressed. *Peridium* composed of rectangular, dark brown to black cells. *Pseudoparaphyses* numerous, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical-clavate, 8-spored. *Ascospores* fusiform, hyaline, 1-septate, with a narrow bipolar sheath. **Asexual morph:** Undetermined.

**Type species:** *Pseudolophiostoma vitigenum* (Kaz. Tanaka & Y. Harada) Thambug. *et al.*

**Notes:** Thambugala *et al.* (2015) established the monotypic genus *Pseudolophiostoma* to accommodate its generic type *Pseudol. vitigenum*, which was found on *Vitis coignetiae* (Vitaceae), and was collected from a boreal region in Japan. Although this species was considered a member of *Lophiotrema* (Tanaka & Harada 2003), phylogenetic analyses based on SSU and LSU sequences revealed that this species belonged to *Lophiostoma* (Hirayama & Tanaka 2011). Later, a monotypic genus *Pseudolophiostoma* was introduced to segregate *L. vitigenum* from

*Lophiostoma* s. str. based on phylogenetic analyses (Thambugala *et al.* 2015). In our study, three new species (*Pseudol. cornisporum*, *Pseudol. obtusisporum*, and *Pseudol. tropicum*), collected from a subtropical region in Japan, were introduced. These species are also characterised by an ascotal wall having uniform thickness (Figs 8G, H, 9G–I, 10G–I) and asci with a relatively long stipe (up to 18.5 µm) (Figs 8L, M, 9L, M, 10L, M). Although *Pseudolophiostoma* possess morphologically similar ascospores to those of *Lentistoma*, the latter genus is easily differentiated from *Pseudolophiostoma* by having a clypeated ostiolar neck.

***Pseudolophiostoma cornisporum*** A. Hashim., K. Hiray. & Kaz. Tanaka, **sp. nov.** MycoBank MB823140. Figs 8, 14G.

**Etymology:** Refers to the ascospores with acute ends.

**Sexual morph:** *Ascomata* subglobose, scattered, immersed, dark brown to black, 650–700 µm high, 580–650 µm diam. *Ostiolar neck* crest-like, elongated, laterally compressed, 100–190 µm high, 120–140 µm wide, composed of 2–3 µm diam, globose, brown to black cells, with hyaline periphyses. *Peridium* uniform, 12–17 µm thick at side, composed of 4–5 layers of rectangular, thin-walled, 8–12 × 3–4 µm, brown cells, surrounded by dark brown hyphae. *Pseudoparaphyses* numerous, 1.5–2 µm wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical-clavate, 80–92 × 11–15(–18) µm ( $\bar{x}$  = 86.4 × 13.6 µm, n = 5), with a long stipe (8–15 µm long,  $\bar{x}$  = 11.6 µm, n = 7), apically rounded with an ocular chamber, 8-spored. *Ascospores* fusiform with acute ends, 21–32 × 4.5–6 µm ( $\bar{x}$  = 26.6 × 5.3 µm, n = 60), l/w (3.9–)4.1–6.3 ( $\bar{x}$  = 5.0, n = 60), hyaline, with a septum nearly median (0.47–0.56,  $\bar{x}$  = 0.51, n = 60), slightly constricted at the septum, smooth, with a narrow sheath. *Sheath* drawn out 2–11 µm long at both ends, with a lateral pad-like structure within the sheath, up to 2.5 µm wide at side. **Asexual morph:** Undetermined.

**Culture characteristics:** Colonies on PDA attaining 14–18 mm diam within 21 d at 20 °C in the dark, velvety, centrally raised, olivaceous buff (89); reverse olivaceous (48) (Fig. 14G); sexual morph formed.

**Material examined:** Japan, Okinawa, Isl. Iriomote, near Sonai trail, on dead stem of herbaceous plant, 13 Jul. 2011, K. Hirayama & K. Tanaka, KH 322 (HHUF 30582 **holotype** designated here; ex-holotype culture CBS 143654 = JCM 32348).

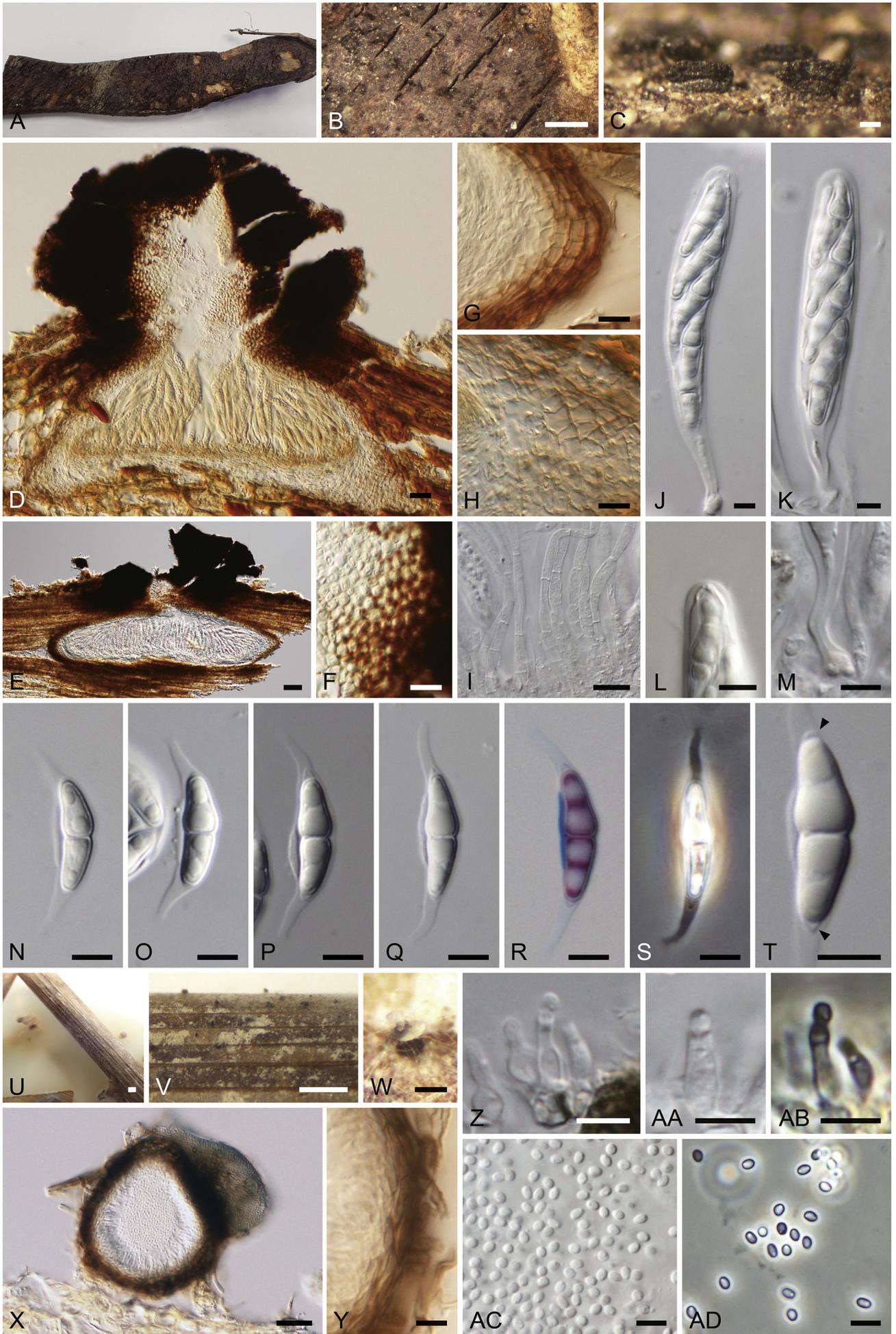
**Notes:** This species resembles *Pseudol. vitigenum* by having ascospores with acute ends. Ascospores of the new species are smaller (21–32 × 4.5–6 µm), while those of *Pseudol. vitigenum* are larger ((30.5–)34–44(–51) × (8–)9–11.5(–13) µm; Thambugala *et al.* 2015).

***Pseudolophiostoma obtusisporum*** A. Hashim., K. Hiray. & Kaz. Tanaka, **sp. nov.** MycoBank MB823141. Figs 9, 14H.

**Etymology:** Refers to the ascospores with obtuse ends.

**Sexual morph:** *Ascomata* subglobose, scattered, immersed, dark brown to black, 350–400 µm high, 250–350 µm diam. *Ostiolar neck* crest-like, elongated, laterally compressed, 110–200 µm high, 150–250 µm wide, composed of 2–4 µm diam, globose,

**Fig. 4.** *Flabellascoma cycadicola*. A–C. Appearance of ascomata on substrate. D. Ascoma in longitudinal section. E. Ostiolar neck of ascoma. F. Pseudoparaphyses. G. Peridium of ascoma. H. Ascus apex. I, J. Asci. K–P. Ascospores (arrowheads indicate an internal chamber in P). Q–S. Conidiomata in culture. T, U. Conidiomata in longitudinal section. V. Peridium of conidioma. W, X. Conidiogenous cells. Y, Z. Conidia. A–P from HHUF 30570 (holotype). Q–Z from culture BCRC FU30901 = CBS 143644 (ex-holotype). Scale bars: B = 500 µm; C, S = 100 µm; D, T, U = 20 µm; G, I, J = 10 µm; E, F, H, K–P, V–Z = 5 µm; Q, R = 1 mm.



brown to black cells, with hyaline periphyses. *Peridium* uniform, 10–17 µm thick at side and base, composed of 3–4 layers of rectangular, thin-walled, 8–13 × 3–4 µm, brown cells, surrounded by dark brown hyphae. *Pseudoparaphyses* numerous, 1.5–2 µm wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical-clavate, (81–)103–140 × 8–15 µm ( $\bar{x}$  = 119.9 × 10.9 µm,  $n$  = 30), with a long stipe (7.5–18.5 µm long,  $\bar{x}$  = 11.7 µm,  $n$  = 11), apically rounded with an ocular chamber, 8-spored. *Ascospores* fusiform with obtuse ends, (20–)23.5–31.5 × 4–7 µm ( $\bar{x}$  = 27.3 × 5.5 µm,  $n$  = 90), l/w 3.5–6.9 ( $\bar{x}$  = 5.0,  $n$  = 90), hyaline, with a septum nearly median (0.47–0.55,  $\bar{x}$  = 0.51,  $n$  = 90), slightly constricted at the septum, smooth, with a narrow sheath. *Sheath* drawn out 5–11 µm long at both ends, with a lateral pad-like structure within the sheath, up to 3 µm wide at side. *Asexual morph*: Undetermined.

**Culture characteristics:** Colonies on PDA attaining 22–29 mm diam within 21 d at 20 °C in the dark, velvety, centrally raised, lavender grey (125); reverse smoke grey (105) (Fig. 14H); sexual morph formed.

**Materials examined:** **Japan**, Okinawa, Isl. Iriomote, near Midara river, on dead stem of herbaceous plant, 22 Nov. 2008, K. Hirayama & K. Tanaka, KH 228 (HHUF 30584; ex-paratype culture CBS 143655); Isl. Ishigaki, Mt. Banna, near small stream, on dead stem of herbaceous plant, 14 Jul. 2011, K. Hirayama & K. Tanaka, KH 336 (HHUF 30585; ex-paratype culture CBS 143656); *ibid.*, on dead stem of herbaceous plant, 14 Jul. 2011, K. Tanaka & K. Hirayama, KT 2838 (HHUF 30583 **holotype** designated here; ex-holotype culture CBS 143657 = JCM 32349); Tokyo, Ogasawara Islands, Isl. Hahajima, Shizukasawa, on dead stem of *Livistona boninensis*, 14 Sep. 2012, K. Tanaka, A. Hashimoto, T. Ono & T. Sato, KT 3098 (HHUF 30171; ex-paratype culture CBS 143941 = MAFF 243969); Isl. Chichijima, near Mt. Yoake, on dead stem of *Stachytarpheta jamaicensis*, 9 Sep. 2012, K. Tanaka, A. Hashimoto, T. Ono & T. Sato, KT 3119 (HHUF 30189; ex-paratype culture CBS 143658 = MAFF 243983).

**Notes:** *Pseudolophiostoma obtusisporum* is commonly distributed among subtropical islands in Japan and is found on various herbaceous plants or palm trees. The wall of the ascumata is composed of equal thickness among specimens on herbaceous plants (Fig. 9G) and palm trees (Fig. 9H). Other morphological features, such as the ascospore size, were identical and ITS sequences among these isolates also completely matched. Thus, we regard these isolates as conspecific. *Pseudolophiostoma obtusisporum* can be distinguished from other *Pseudolophiostoma* species by its obtuse-ended ascospores (Fig. 9P–T).

***Pseudolophiostoma tropicum*** A. Hashim., K. Hiray. & Kaz. Tanaka, **sp. nov.** MycoBank MB823142. Figs 10, 14I.

**Etymology:** Refers to the species occurring in the tropical regions.

**Sexual morph:** *Ascomata* subglobose, scattered, immersed, dark brown to black, 530–880 µm high, 550–840 µm diam. *Ostiolar neck* crest-like, elongated, laterally compressed, 100–160 µm high, 100–200 µm wide, composed of 2–4(–7) µm diam, globose, brown to black cells, with hyaline periphyses. *Peridium* uniform, 15–22 µm thick at side and base, composed of 3–4 layers of rectangular, thin-walled, 9–13(–18) × 3–5 µm, brown cells, surrounded by dark brown hyphae. *Pseudoparaphyses*

numerous, 1–1.5 µm wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical-clavate, (75–) 85–120 × 10–15 µm ( $\bar{x}$  = 104.4 × 12.3 µm,  $n$  = 14), with a long stipe (8–18.5 µm long,  $\bar{x}$  = 11.7 µm,  $n$  = 10), apically rounded with an ocular chamber, 8-spored. *Ascospores* fusiform with acute ends, 23.5–29.5 × 4–6 µm ( $\bar{x}$  = 26.3 × 5.4 µm,  $n$  = 70), l/w 4.0–6.3 ( $\bar{x}$  = 4.9,  $n$  = 70), hyaline, with a septum nearly median (0.46–0.56,  $\bar{x}$  = 0.51,  $n$  = 70), slightly constricted at the septum, smooth, with a narrow sheath. *Sheath* drawn out 6–9 µm long at both ends, with a lateral pad-like structure within the sheath, up to 2 µm wide at side. *Asexual morph*: Undetermined.

**Culture characteristics:** Colonies on PDA attaining 24–27 mm diam within 21 d at 20 °C in the dark, velvety, centrally raised, greyish blue (97); reverse grey olivaceous (107) (Fig. 14I); sexual morph formed.

**Materials examined:** **Japan**, Okinawa, Isl. Iriomote, Takana, on dead stem of herbaceous plant, 13 Jul. 2011, K. Hirayama & K. Tanaka, KH 352 (HHUF 30586 ex-paratype culture CBS 143659); Tokyo, Ogasawara Islands, Isl. Chichijima, Buta coast, on dead stem of *Bidens pilosa* var. *radiata*, 15 Sep. 2012, K. Tanaka, A. Hashimoto & T. Sato, KT 3134 (HHUF 30202 **holotype** designated here; ex-holotype culture CBS 143660 = MAFF 243989).

**Notes:** In culture, *Pseudol. tropicum* produced ascumata that were slightly different from those on natural substrates, with a slightly thicker peridium and a well-developed ostiolar neck (Fig. 10F, H, I). Although these differences were observed, the anatomical structure of the ascumatal wall formed in culture were identical to those on natural specimens.

Both *Pseudol. tropicum* and *Pseudol. obtusisporum* have ascospores overlapping in size, but can be distinguished by the ascospore shape. Obtuse-ended ascospores were identified as *Pseudol. obtusisporum* (Fig. 9P–T) and acute-ended ascospores as *Pseudol. tropicum* (Fig. 10P–U). ITS sequences between these species differed in nine nucleotide positions with three gaps.

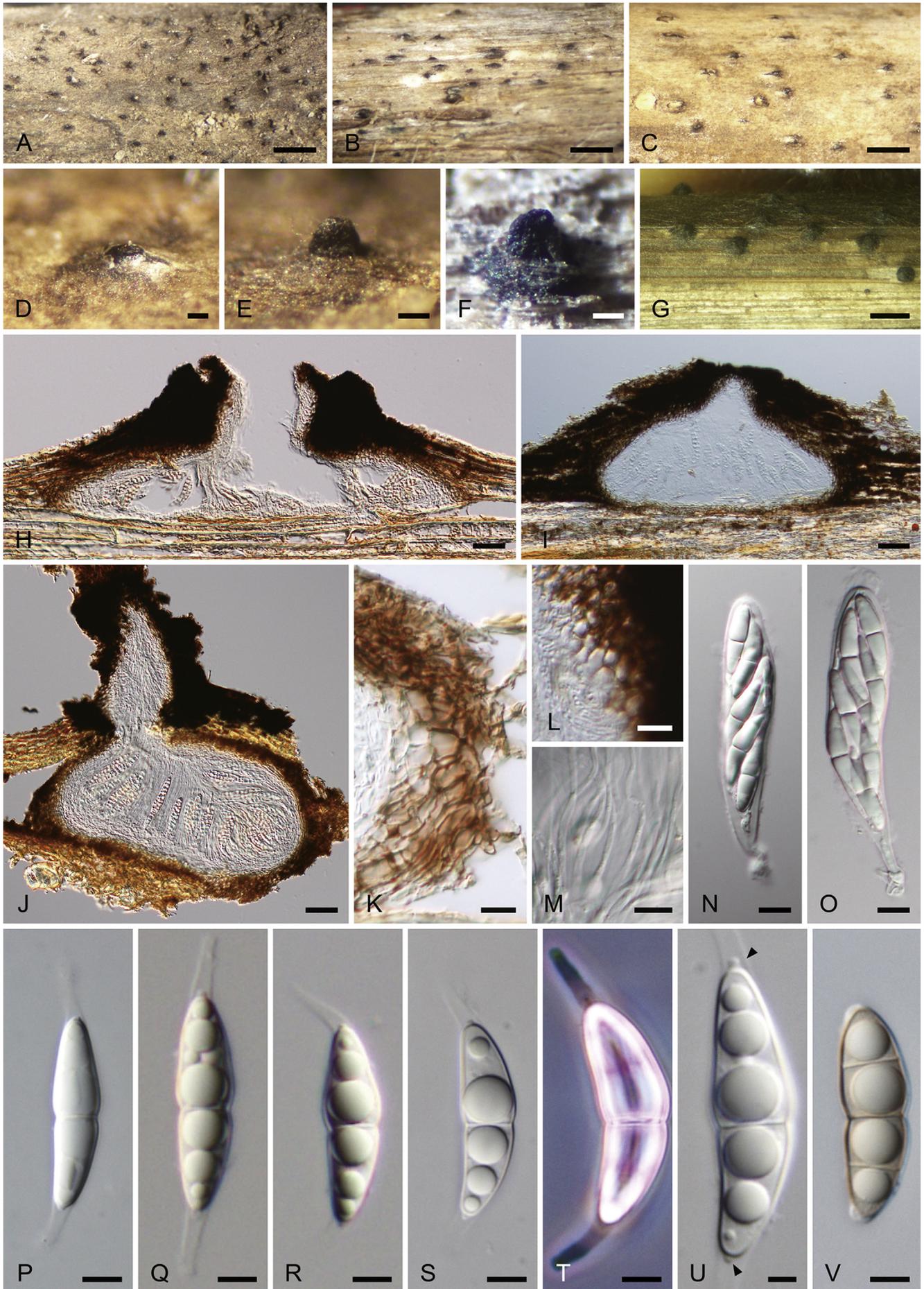
***Pseudopaucispora*** A. Hashim., K. Hiray. & Kaz. Tanaka, **gen. nov.** MycoBank MB823143.

**Etymology:** Refers to its morphological resemblance to *Paucispora*.

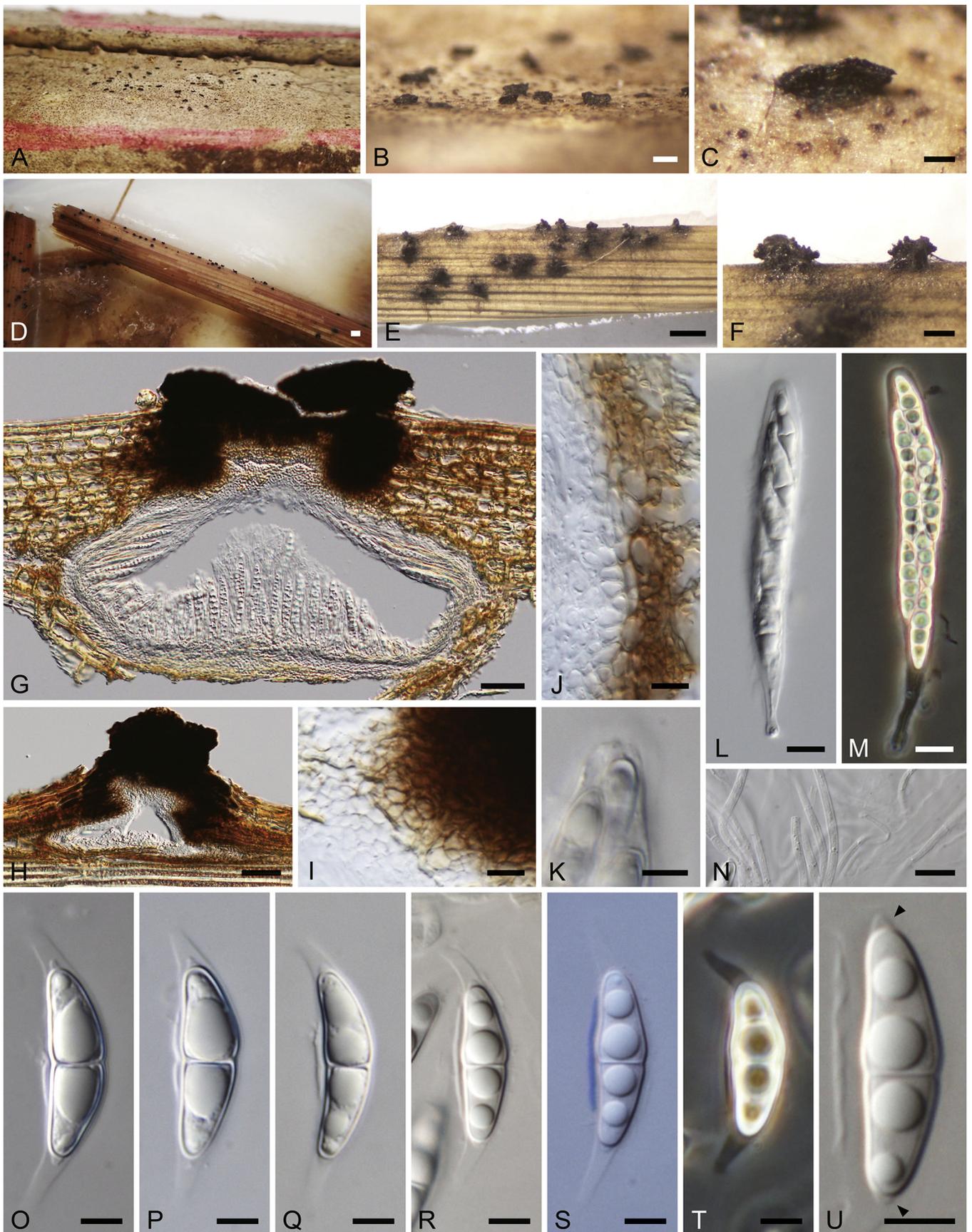
**Sexual morph:** *Ascomata* scattered, immersed, subglobose. *Ostiolar neck* elongated, laterally compressed. *Peridium* composed of rectangular, brown cells. *Pseudoparaphyses* numerous, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical to clavate, 8-spored. *Ascospores* fusiform, brown, 1-septate, smooth, with a narrow bipolar sheath. *Asexual morph:* *Conidiomata* pseudopycnidial, globose to cylindrical, sometimes deformed, confluent, multiloculate, scattered, semi-immersed, black. *Ostiolar neck* papillate. *Peridium* composed of rectangular, brown cells. *Conidiophores* absent. *Conidiogenous cells* holoblastic, ampliform to cylindrical, hyaline, smooth. *Conidia* cylindrical with rounded ends, hyaline, smooth, aseptate, guttulate when young.

**Type species:** *Pseudopaucispora brunneospora* A. Hashim., K. Hiray. & Kaz. Tanaka.

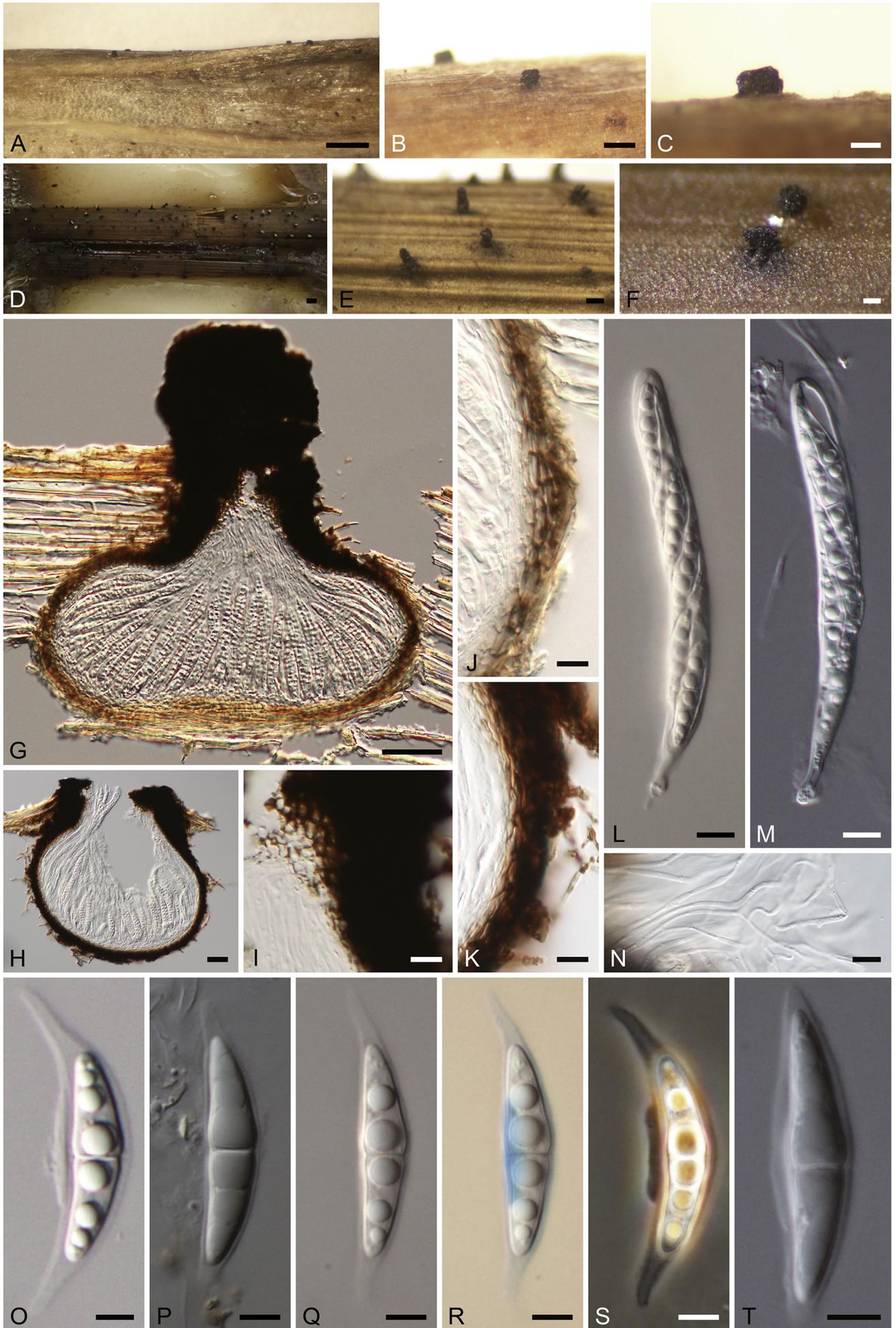
**Fig. 5.** *Flabellascoma minimum*. **A–C.** Appearance of ascumata on substrate. **D, E.** Ascumata in longitudinal section. **F.** Ostiolar neck of ascoma. **G, H.** Peridium of ascoma. **I.** Pseudoparaphyses. **J, K.** Asci. **L.** Ascus apex. **M.** Ascus stipe. **N–T.** Ascospores (arrowheads indicate an internal chamber in T). **U–W.** Conidiomata in culture. **X.** Conidioma in longitudinal section. **Y.** Peridium of conidioma. **Z–AB.** Conidiogenous cells. **AC, AD.** Conidia. **A, B, D, F, H–K, N, O** from HHUF 30572 (holotype); **C, E, G, L, M, P–T** from HHUF 30571; **U–AD** from culture BCRC FU30902 = CBS 143646 (ex-holotype). Scale bars: B, U, V = 1 mm; C, W = 100 µm; D, E, X = 20 µm; F–K = 10 µm; L–T, Y–AD = 5 µm.



**Fig. 6.** *Lentistoma bipolare*. **A–F.** Appearance of ascomata on substrate. **G.** Ascomata in culture. **H–J.** Ascomata in longitudinal section. **K.** Peridium. **L.** Ostiolar neck of ascoma. **M.** Pseudoparaphyses. **N, O.** Asci. **P–U.** Ascospores (arrowheads indicate an internal chamber in **U**). **V.** Senescent ascospore. **A, E, I, T, U** from HHUF 30578; **B** from HHUF 30574; **C, D, O** from HHUF 30573; **F, M, N, P** from BRIP 21489 (holotype); **G** from culture CBS 115370; **H, K, L, S, V** from HHUF 30579; **J, R** from culture CBS 115375 (ex-epitype); **Q** from culture CBS 143652. Scale bars: **A–C, G** = 1 mm; **D–F** = 100  $\mu$ m; **H–J** = 50  $\mu$ m; **K–O** = 10  $\mu$ m; **P–V** = 5  $\mu$ m.



**Fig. 7.** *Leptoparies palmarum*. **A–C.** Appearance of ascomata on substrate. **D–F.** Ascomata in culture. **G, H.** Ascomata in longitudinal section. **I.** Ostiolar neck of ascoma. **J.** Peridium of ascoma. **K.** Ascus apex. **L, M.** Asci. **N.** Pseudoparaphyses. **O–U.** Ascospores (arrowheads indicate an internal chamber in U). **A–C, G, I–L, N–Q** from HHUF 28983 (holotype); **D–F, H, M, R–U** from culture CBS 143653 = JCM 13089 = MAFF 239599 (ex-holotype). Scale bars: B, F = 300  $\mu$ m; C = 100  $\mu$ m; D, E = 1 mm; G, H = 50  $\mu$ m; I–N = 10  $\mu$ m; O–U = 5  $\mu$ m.



*Notes:* *Pseudopaucispora* is introduced to accommodate *Pseudop. brunneospora*, which is characterised by small brown ascospores and pseudopycnidial conidiomata. *Pseudopaucispora* is superficially similar to *Paucispora* (Thambugala *et al.* 2015). However, *Pseudopaucispora* has an ascotal peridium composed of 1 zone and an ascus with a short stipe, while *Paucispora* is characterised by a peridium composed of 2 zones and an ascus with a relatively long stipe (up to 34 µm in length; Thambugala *et al.* 2015).

***Pseudopaucispora brunneospora*** A. Hashim., K. Hiray. & Kaz. Tanaka, **sp. nov.** MycoBank MB823144. Figs 11, 14J.

*Etymology:* Refers to its brown ascospores.

*Sexual morph:* *Ascomata* subglobose, scattered, immersed, dark brown to black, 210–300 µm high, 215–355 µm diam. *Ostiolar neck* crest-like, elongated, laterally compressed, 145–175 µm high, 95–190 µm wide, composed of 2–4 µm diam, globose, brown to black cells, with hyaline periphyses. *Peridium* uniform, 15–18 µm thick at side and base, composed of rectangular, thin-walled, 6–16 × 3–4 µm, brown cells. *Pseudoparaphyses* numerous, 1–1.5 µm wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical-clavate, 67–89 × 7–10.5 µm ( $\bar{x}$  = 75.0 × 8.7 µm, n = 20), with a short stipe (6.5–15 µm long,  $\bar{x}$  = 11.9 µm, n = 20), apically rounded with an ocular chamber, 8-spored. *Ascospores* fusiform with obtuse ends, 14–17 × 3.5–4.5 µm ( $\bar{x}$  = 15.3 × 4.0 µm, n = 50), l/w 3.0–4.7 ( $\bar{x}$  = 3.8, n = 50), brown, with a septum usually supra-median (0.44–0.56,  $\bar{x}$  = 0.48, n = 50), slightly constricted at the septum, smooth, with a narrow sheath. *Sheath* drawn out 6–10 µm long at both ends. *Asexual morph:* *Conidiomata* pseudopycnidial, globose to cylindrical, up to 230 µm high, 150–190 µm diam, sometimes deformed, confluent, multiloculate, scattered, semi-immersed, black. *Ostiolar neck* mainly single, occasionally three, papillate. *Peridium* 10–18 µm wide, composed of 7.5–16.5 × 3–4 µm, rectangular, brown cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous* cells holoblastic, 10–15 × 2.5–4.5 µm, ampliform to cylindrical, hyaline, smooth. *Conidia* cylindrical with rounded ends, 2–3(–3.5) × 1–1.3 µm ( $\bar{x}$  = 2.8 × 1.1 µm, n = 50), l/w 1.8–3.1 ( $\bar{x}$  = 2.6, n = 50), hyaline, smooth, aseptate, guttulate when young.

*Culture characteristics:* Colonies on PDA attaining 9–15 mm diam within 21 d at 20 °C in the dark, velvety, centrally raised, pale luteous (11); reverse sienna (8) (Fig. 14J); asexual and sexual morph formed.

*Material examined:* Japan, Okinawa, Isl. Yonaguni, near Kubura pond, on dead stem of *Asteraceae* sp., 23 Nov. 2008, K. Hirayama & K. Tanaka, KH 227 (HHUF 30587 **holotype** designated here; ex-holotype culture CBS 143661 = JCM 32350).

*Note:* *Pseudopaucispora* can be easily distinguished from the other *Lo. bipolare* complex by the brown ascospores, which possesses a sheath without a lateral pad-like structure (Fig. 11J–O).

***Vaginatispora*** K.D. Hyde, *Nova Hedwigia* 61: 234. 1995.

*Sexual morph:* *Ascomata* scattered, immersed, subglobose. *Ostiolar neck* elongated, laterally compressed, with hyaline

periphyses. *Peridium* composed of rectangular, brown cells, surrounded by dark brown hyphae. *Pseudoparaphyses* numerous, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical-clavate, 8-spored. *Ascospores* fusiform, hyaline, 1-septate, with a bipolar or entire sheath. *Asexual morph:* Undetermined.

Type species: *Vaginatispora aquatica* K.D. Hyde.

*Notes:* The genus *Vaginatispora* was established to accommodate *V. aquatica*, which was found on submerged twigs of woody plants and was originally characterised by *Massarina*-like ascomata with much longer ostiolar necks and ascospores bearing an entire sheath (Hyde 1995b). Liew *et al.* (2002) suggested that this genus was related to *Lophiostoma*, according to the phylogenetic analyses using ITS sequences. However, the authors could not determine the fundamental differences between these two genera. Thus, no taxonomic conclusion regarding whether *Vaginatispora* was synonymous with *Lophiostoma* was drawn. Subsequently, the genus was considered to be synonymous with *Lophiostoma* due to its phylogenetic affinities to the latter genus (Zhang *et al.* 2014). Thambugala *et al.* (2015) recently retained *Vaginatispora*, emphasising the structures of the peridium and asci, as well as based on results of their multi-locus phylogenetic analyses. They accepted *V. fuckelii* (formerly *Lo. fuckelii*) as a member of the genus. Although this species was morphologically atypical in the genus because of the 2 zoned peridium, this proposal was accepted by subsequent studies (Wanasinghe *et al.* 2016, Tibpromma *et al.* 2017). Our phylogenetic analyses showed a paraphyletic nature of *Vaginatispora sensu* Thambugala *et al.* (2015) (Fig. 2). We re-circumscribed the genus to include five species with well-developed ascotal peridium at the sides, while poorly-developed at the base, with numerous brown hyphae around the ascomata, and asci with a broad ocular chamber. *Vaginatispora fuckelii* is excluded from *Vaginatispora* and transferred to its own new genus, *Neovaginatispora* (see Appendix B).

***Vaginatispora amygdali*** A. Hashim., K. Hiray. & Kaz. Tanaka, **sp. nov.** MycoBank MB823145. Figs 12, 14K.

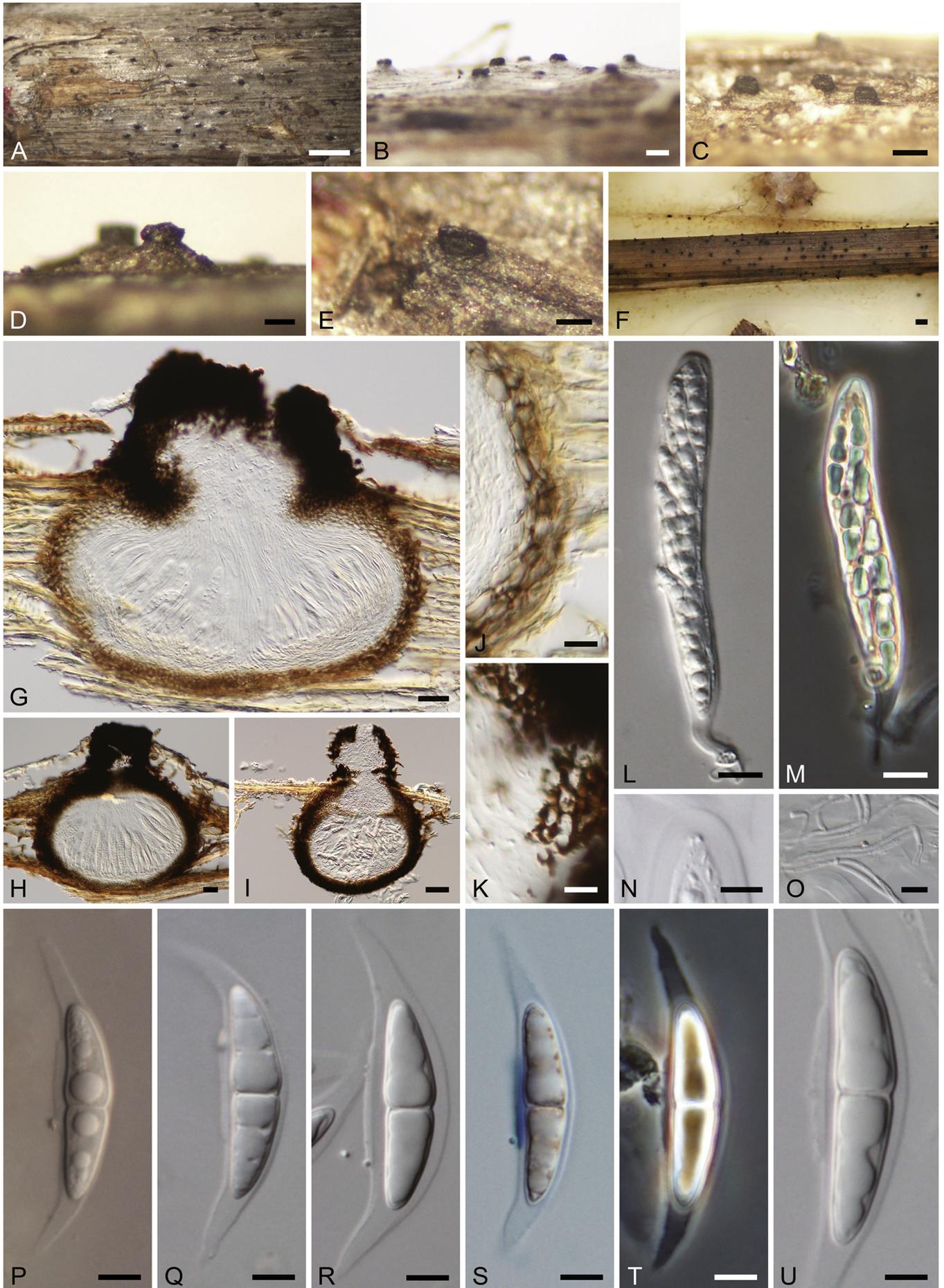
*Etymology:* Refers to the generic name of the host plant.

*Sexual morph:* *Ascomata* subglobose, scattered, immersed, dark brown to black, 330–360 µm high, 480–500 µm diam. *Ostiolar neck* crest-like, elongated, laterally compressed, 150–225 µm high, 275–445 µm wide, composed of 3–7 µm diam, globose to elongated, brown to black cells, with hyaline periphyses. *Peridium* 37.5–62.5 µm thick at side, composed of 9–15 layers of rectangular, thin-walled, 11–13 × 4–5 µm, brown cells, surrounded by dark brown hyphae; 10–17.5 µm thick at base, composed of globose, 6–10 µm diam, pale brown cells. *Pseudoparaphyses* numerous, 1.5–2.5 µm wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical-clavate, (81–) 100–140 × (11.5–)16.5–22.5 µm ( $\bar{x}$  = 115.0 × 18.5 µm, n = 53), with a short stipe (8.5–16 µm long,  $\bar{x}$  = 12.0 µm, n = 10), apically rounded with a broad ocular chamber, 8-spored. *Ascospores* fusiform with obtuse ends, 25–34(–37) × 7–10.5 µm ( $\bar{x}$  = 30.6 × 8.8 µm, n = 120), l/w 2.6–4.6 ( $\bar{x}$  = 3.5, n = 120), hyaline, with a septum nearly median (0.45–0.58,  $\bar{x}$  = 0.51,

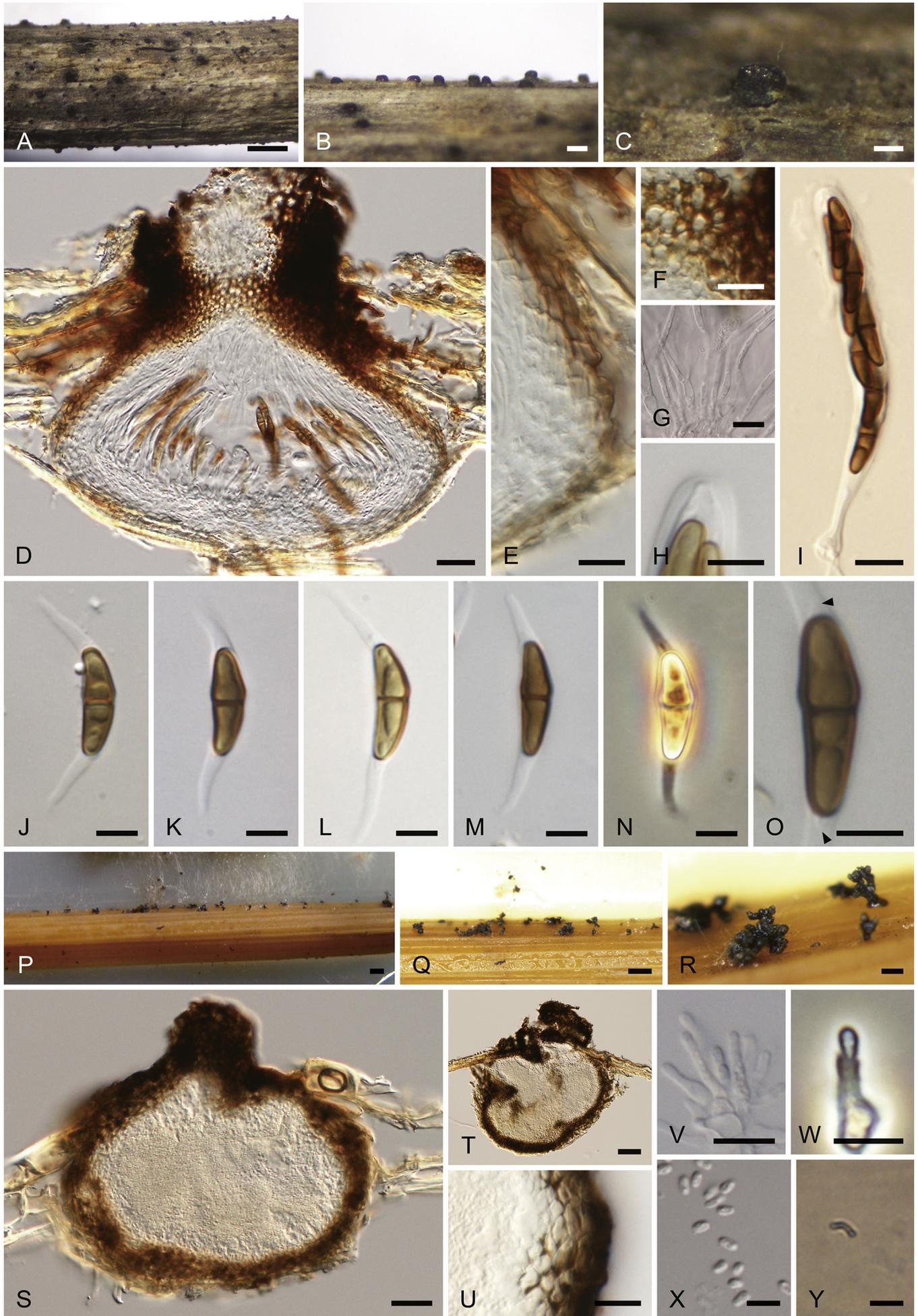
**Fig. 8.** *Pseudolophiostoma cornisporum*. **A–C.** Appearance of ascomata on substrate. **D–F.** Ascomata in culture. **G, H.** Ascomata in longitudinal section. **I.** Ostiolar neck of ascoma. **J, K.** Peridium of ascoma. **L, M.** Asci. **N.** Pseudoparaphyses. **O–T.** Ascospores. **A–C, G, I, J, P, T** from HHUF 30582 (holotype). **D–F, H, K–O, Q–S** from culture CBS 143654 = JCM 32348 (ex-holotype). Scale bars: A, D = 1000 µm; B, E = 200 µm; C, F = 100 µm; G, H = 50 µm; I–N = 10 µm; O–T = 5 µm.



**Fig. 9.** *Pseudolophiostoma obtusisporum*. **A–D.** Appearance of ascomata on substrate. **E, F.** Ascomata in culture. **G–I.** Ascomata in longitudinal section. **J.** Peridium of ascoma. **K.** Ostiolar neck of ascoma. **L, M.** Asci. **N.** Ascus apex. **O.** Pseudoparaphyses. **P–T.** Ascospores. **A, M, R** from HHUF 30189; **B, G, J–L** from HHUF 30583 (holotype); **C, D, H, N** from HHUF 30171; **E, F, I, O, S** from culture CBS 143658 = MAFF 243983; **P, T** from culture CBS 143941 = MAFF 243969; **Q** from HHUF 30584. Scale bars: **A, E, F** = 1 mm; **B, C** = 200  $\mu$ m; **D** = 100  $\mu$ m; **G–I** = 50  $\mu$ m; **J–M, O** = 10  $\mu$ m; **N, P–T** = 5  $\mu$ m.



**Fig. 10.** *Pseudolophiostoma tropicum*. **A–E.** Appearance of ascomata on substrate. **F.** Ascomata in culture. **G–I.** Ascomata in longitudinal section. **J.** Peridium of ascoma. **K.** Ostiolar neck of ascoma. **L, M.** Asci. **N.** Ascus apex. **O.** Pseudoparaphyses. **P–U.** Ascospores. **A, B, H, K, Q** from HHUF 30586; **C–E, G, J, M, N, R–U** from HHUF 30202 (holotype); **F, I, L, O, P** from culture CBS 143659. Scale bars: **A, F** = 1 mm; **B, C** = 200 µm; **D, E** = 100 µm; **G–I** = 50 µm; **J–M, O** = 10 µm; **N, P–U** = 5 µm.



$n = 120$ ), slightly constricted at the septum, smooth, with a narrow sheath. *Sheath* drawn out 6–8  $\mu\text{m}$  long at both ends, with a lateral pad-like structure within the sheath, with an internal chamber at both ends of ascospores. *Asexual morph*: Undetermined.

*Culture characteristics*: Colonies on PDA attaining 18 mm diam within 21 d at 20 °C in the dark, velvety, centrally raised, greenish grey (110); reverse dull green (70) (Fig. 14K); sexual morph formed.

*Material examined*: Japan, Wakayama, Kinokawa, Kishigawa, Kita, on endocarp of *Amygdalus persica*, 9 May 2007, S. Hatakeyama, KT 2248 (HHUF 30588 holotype designated here; ex-holotype culture CBS 143662 = JCM 32351).

*Notes*: *Vaginatispora amygdali* is morphologically similar to *V. armatispora*, but ascospores of the latter species are slightly larger (28–39.2  $\times$  7–9.8  $\mu\text{m}$ ; Hyde *et al.* 1992). ITS sequences of *V. amygdali* and *V. armatispora* (AF383955), which were derived from an authentic specimen of the species, differed in 17 positions with five gaps.

This species is difficult to distinguish from other *Lo. bipolare* complexes based on ascospore features, but detailed features of the ascomata and asci are well-matched to the characteristics present in *Vaginatispora*.

***Vaginatispora scabrispora*** A. Hashim., K. Hiray. & Kaz. Tanaka, **sp. nov.** MycoBank MB823146. Figs 13, 14L.

*Etymology*: Refers to its verruciform ascospores.

*Sexual morph*: *Ascomata* subglobose, scattered, immersed, dark brown to black, 220–340  $\mu\text{m}$  high, 340–360  $\mu\text{m}$  diam. *Ostiolar neck* crest-like, elongated, laterally compressed, 88–120  $\mu\text{m}$  high, 175–225  $\mu\text{m}$  wide, composed of 3–5  $\mu\text{m}$  diam, globose, brown to black cells, with hyaline periphyses. *Peridium* 18–28  $\mu\text{m}$  thick at side, composed of 3–5 layers of rectangular, thin-walled, 10–11  $\times$  3–5  $\mu\text{m}$ , brown cells, surrounded by dark brown hyphae (1–1.5  $\mu\text{m}$  wide); 10–17.5  $\mu\text{m}$  thick at base, composed of globose, brown cells. *Pseudoparaphyses* numerous, 1.5–2  $\mu\text{m}$  wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical-clavate, (77.5–) 95–115  $\times$  15–20  $\mu\text{m}$  ( $\bar{x} = 102.3 \times 16.5 \mu\text{m}$ ,  $n = 10$ ), with a short stipe (7.5–17.5  $\mu\text{m}$  long,  $\bar{x} = 13.0 \mu\text{m}$ ,  $n = 10$ ), apically rounded with a broad ocular chamber, 8-spored. *Ascospores* fusiform with obtuse ends, 20–23  $\times$  5–6  $\mu\text{m}$  ( $\bar{x} = 21.9 \times 5.9 \mu\text{m}$ ,  $n = 50$ ), l/w 2.9–3.5 ( $\bar{x} = 3.3$ ,  $n = 50$ ), hyaline, with a septum suprmedian (0.42–0.49,  $\bar{x} = 0.47$ ,  $n = 50$ ), slightly constricted at the septum, verrucous, with a narrow sheath. *Sheath* drawn out 5–7  $\mu\text{m}$  long at both ends, with a lateral pad-like structure within the sheath, with an internal chamber at both ends of ascospores. *Asexual morph*: Undetermined.

*Culture characteristics*: Colonies on PDA attaining 19 mm diam within 21 d at 20 °C in the dark, velvety, centrally raised, smoke grey (105); reverse smoke grey (105) (Fig. 14L); sexual morph formed.

*Material examined*: Japan, Okinawa, Isl. Iriomote, near Shiira river (intertidal region), on submerged dead twigs of *Rhizophora mucronata*, 25 Sep. 2007, K. Tanaka & H. Yonezawa, KT 2443 (HHUF 30589 holotype designated here; ex-holotype culture CBS 143663 = JCM 32352).

*Notes*: *Vaginatispora scabrispora* is easily distinguished from other species of *Vaginatispora* due to its verrucous ascospores (Fig. 13R) and mangrove habitat. This species is phylogenetically related to *V. amygdali*, but differs from the latter by the smaller sized ascospores (vs. 25–34(–37)  $\times$  7–10.5  $\mu\text{m}$  in the latter species).

## DISCUSSION

### Generic delimitation in *Lophiostomataceae*

*Lophiostoma bipolare* has a worldwide distribution in freshwater and marine habitats, and is characterised by ascomata with a slit-like ostiolar neck surrounded by a clypeus and ascospores with a bipolar sheath (e.g. Hyde 1995a, Hyde *et al.* 2002, Liew *et al.* 2002). This species was originally treated as a member of *Massarina* (Hyde 1995a). Later, Liew *et al.* (2002) transferred this species to *Lophiostoma* based on the results of molecular phylogenetic analyses using ITS region. The genus *Lophiostoma* was taxonomically revised on the basis of the phylogenetic analyses of multi-locus genes (Thambugala *et al.* 2015). *Lophiostoma bipolare* was not included in the analyses and thus the taxonomic position of this species has remained unclear. Our phylogenetic analyses, which included 29 strains provisionally identified as *Lo. bipolare*, indicate that the species is not monophyletic (Fig. 1) and is scattered into seven genera and 11 species within *Lophiostomataceae* (Fig. 2). The present data also indicate that *Lo. bipolare* s. str. is phylogenetically distinct from *Lophiostoma* s. str. and should be separately placed in the novel genus *Lentistoma* (Fig. 2). *Lentistoma* is clearly different from other lophiostomataceous genera owing to its well-developed clypeus around the ostiolar neck (Fig. 6H–J). Other *Lo. bipolare* complexes are scattered among six distinct genera that are morphologically defined and whose monophyly is strongly supported (Fig. 2). As mentioned in previous studies (Chesters & Bell 1970, Holm & Holm 1988, Hyde 1995b), the length of the ostiolar neck and the peridium thickness varies both on natural substrate and in culture (Figs 6H–J, 7G, H, 8G, H, 9G–I, 10G–I, 13E, F). The differences in the ascomatal shape are used to differentiate between several lophiostomataceous genera (Thambugala *et al.* 2015). However, our results suggest that it may also vary depending on the condition of the substrates (herbaceous or woody plants) within the same species. For example, the ascomata of *F. minimum* and *Len. bipolare* found on woody plants were flattened at the base, while those on herbaceous plants were ellipsoidal (Figs 5D, E, 6H–J). Although the length of the ostiolar neck, peridium thickness, and ascomatal forms were unstable characteristics depending on different conditions, their peridial features, such as the existence of the clypeus (*Crassiclypeus*, *Lentistoma*; Figs 3D–F, 6H–J), the brown hyphae surrounding the peridium (*Crassiclypeus*, *Vaginatispora*; Figs 3D–F, 12F, 13E, F), the contexture of the peridium with 1 zone (*Crassiclypeus*, *Lentistoma*, *Lep-toparies*, *Pseudolophiostoma*, *Pseudopaucispora*, *Vaginatispora*;

**Fig. 11.** *Pseudopaucispora brunneospora*. **A–C.** Appearance of ascomata on substrate. **D.** Ascoma in longitudinal section. **E.** Peridium of ascoma. **F.** Ostiolar neck of ascoma. **G.** Pseudoparaphyses. **H.** Ascus apex. **I.** Ascus. **J–O.** Ascospores (arrowheads indicate an internal chamber in O). **P–R.** Conidiomata in culture. **S, T.** Conidiomata in longitudinal section (confluent conidioma in T). **U.** Peridium of conidioma. **V, W.** Conidiogenous cells. **X.** Conidia. **Y.** Germinating conidium. **A–F, H–O** from HHUF 30587 (holotype); **G, P–Y** from culture CBS 143661 = JCM 32350 (ex-holotype). Scale bars: A, P, Q = 1 mm; B = 200  $\mu\text{m}$ ; C, R = 100  $\mu\text{m}$ ; D, S, T = 50  $\mu\text{m}$ ; E, F, H, I, U, Y = 10  $\mu\text{m}$ ; G, J–O, V–X = 5  $\mu\text{m}$ .

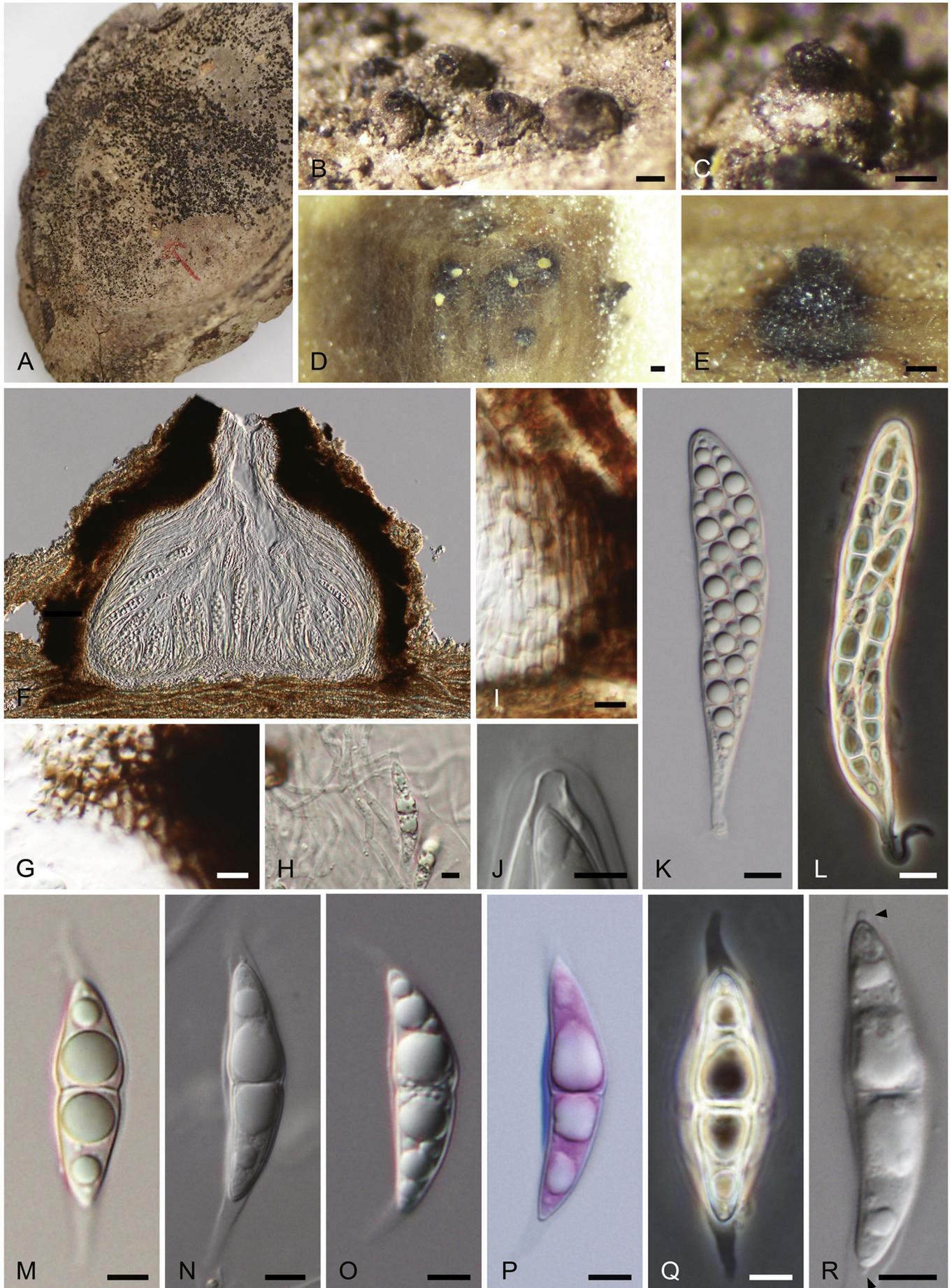
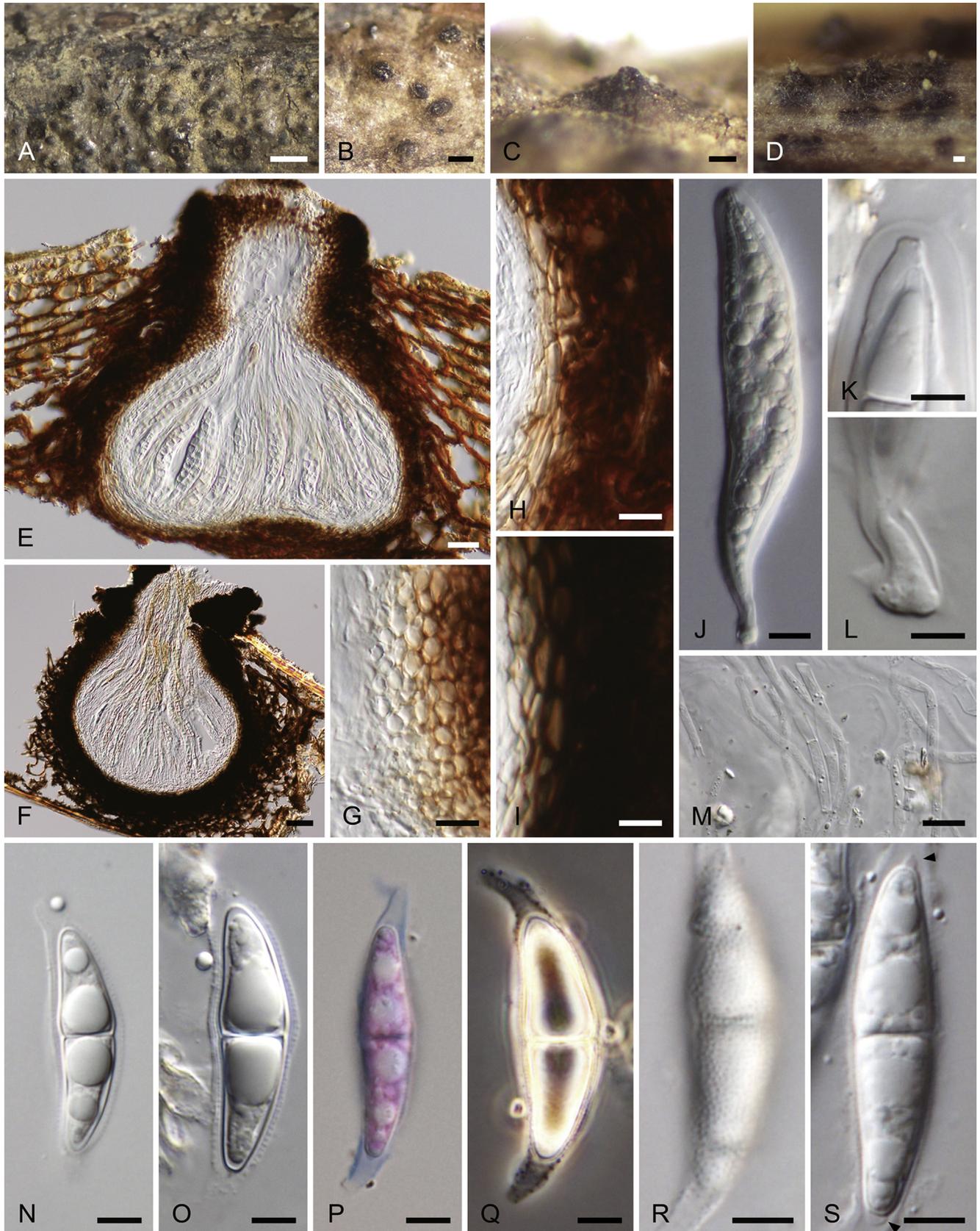


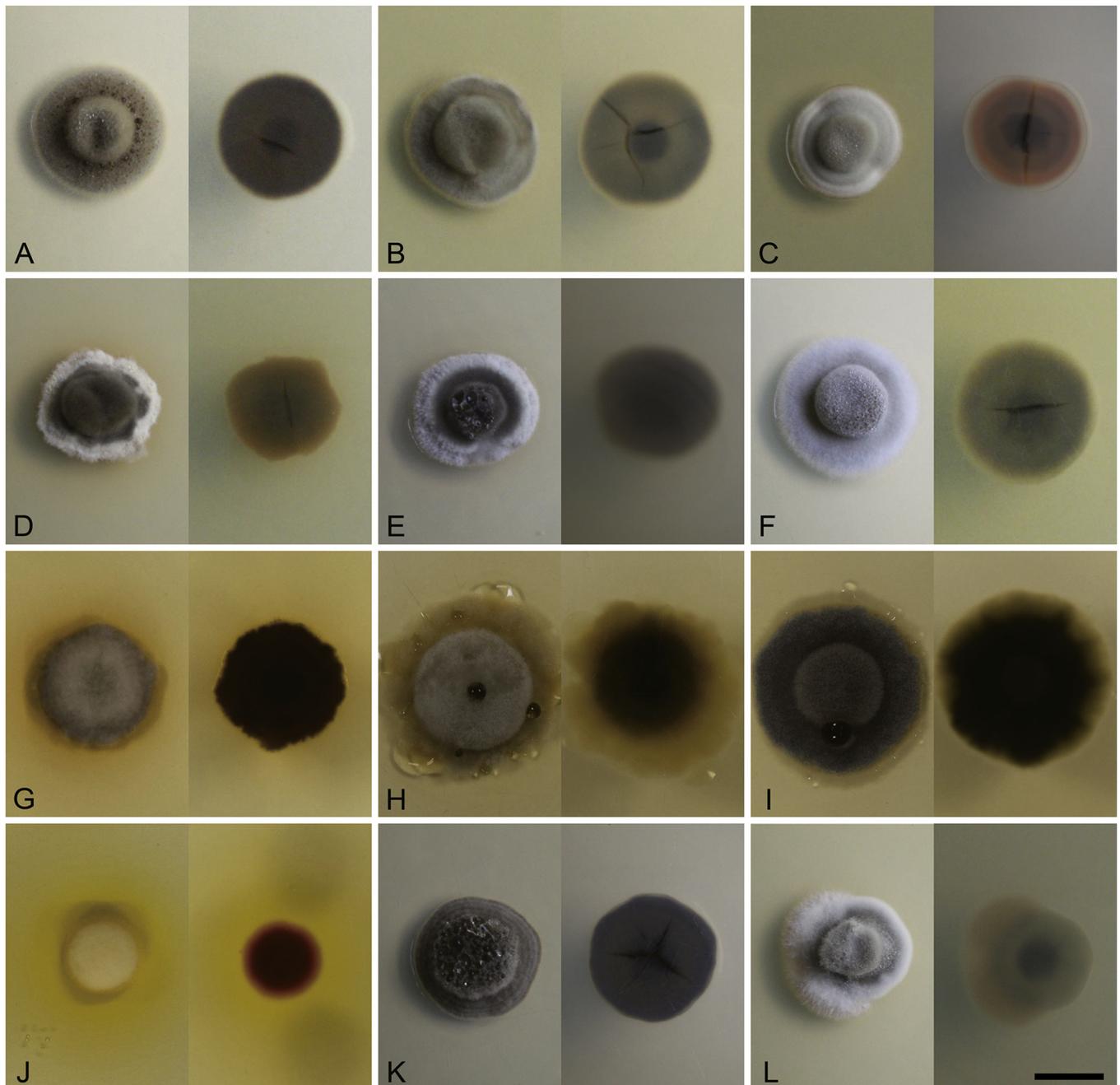
Fig. 12. *Vaginatispora amygdali*. A–C. Appearance of ascomata on substrate. D, E. Ascomata in culture. F. Ascoma in longitudinal section. G. Ostiolar neck of ascoma. H. Pseudoparaphyses. I. Peridium of ascoma. J. Ascus apex. K, L. Asci. M–R. Ascospores (arrowheads indicate an internal chamber in R). A–C, F–J, L–N, P–R from HHUF 30588 (holotype); D, E, K, O from culture CBS 143662 = JCM 32351 (ex-holotype). Scale bars: B, D = 200  $\mu$ m; C, E = 100  $\mu$ m; F = 50  $\mu$ m; K, L = 10  $\mu$ m; G–J, M–R = 5  $\mu$ m.



**Fig. 13.** *Vaginatipora scabrispora*. **A–C.** Appearance of ascomata on substrate. **D.** Ascomata in culture. **E, F.** Ascomata in longitudinal section. **G.** Ostiolar neck of ascoma. **H, I.** Peridium of ascomata. **J.** Ascus. **K.** Ascus apex. **L.** Ascus stipe. **M.** Pseudoparaphyses. **N–S.** Ascospores (arrowheads indicate an internal chamber in **S**). **A–C, E, F, G, H, K–M, O, P, S** from HHUF 30589 (holotype); **D, F, I, J, N, Q, R** from culture CBS 143663 = JCM 32352 (ex-holotype). Scale bars: **A** = 1 mm; **B–D** = 100  $\mu$ m; **E, F** = 50  $\mu$ m; **G–J, M** = 10  $\mu$ m; **K, L, N–S** = 5  $\mu$ m.

**Figs 3D–F, 6H, I, 7G, 8G, 9G, 10G, 11D, 12F, 13E)** or with 2 zones (*Flabellascoma*; **Figs 4D, 5D**), were always stable even on different hosts or culture conditions (**Figs 5E, 6J, 7H, 8H, 9H, I, 10H, I, 13F**). These anatomical differences could be useful for

generic circumscriptions. Thus, we treated these seven genera as distinct, their monophyly being strongly supported (**Fig. 2**). Additionally, clear morphological differences were observed in the asexual morphs of *Crassiclypeus*, *Flabellascoma*, and



**Fig. 14.** Colony characters of *Lophiostoma bipolare* complex used in this study on PDA within 3 wk at 20 °C in the dark (left: upper, right: reverse). **A.** *Crassiclepeus aquaticus* (CBS 143643 = JCM 13087 = MAFF 239597, ex-holotype). **B.** *Flabellascoma cycadicola* (BCRC FU30901 = CBS 143644, ex-holotype). **C.** *F. minimum* (BCRC FU30902 = CBS 143646, ex-holotype). **D, E.** *Lentistoma bipolare* (D. CBS 115375, ex-epitype, E. CBS 143647). **F.** *Leptoparies palmarum* (CBS 143653 = JCM 13089 = MAFF 239599, ex-holotype). **G.** *Pseudolophiostoma cornisporum* (CBS 143654 = JCM 32348, ex-holotype). **H.** *Pseudol. obtusisporum* (CBS 143657 = JCM 32349, ex-holotype). **I.** *Pseudol. tropicum* (CBS 143659, ex-paratype). **J.** *Pseudopaucispora brunneospora* (CBS 143661 = JCM 32350, ex-holotype). **K.** *Vaginatispora amygdali* (CBS 143662 = JCM 32351, ex-holotype). **L.** *V. scabrispora* (CBS 143663 = JCM 32352, ex-holotype). Scale bar: A–L = 1 cm.

*Pseudopaucispora*; pycnidial conidiomata and phialidic conidiogenous cells (*Crassiclepeus*; Fig. 3W, Y–AA); pycnidial conidiomata and holoblastic conidiogenous cells (*Flabellascoma*; Figs 4T, U, W, X, 5X, Z–AB); and pseudopycnidial conidiomata and holoblastic conidiogenous cells (*Pseudopaucispora*; Fig. 11S, T, V, W).

Resolution of the *Lo. bipolare* complex led us to reconsider the generic placement of unresolved species and the generic delimitations of the broadly defined genera from previous studies. Thambugala *et al.* (2015) retained *Vaginatispora* as a natural genus and subsequent studies accepted four species in this genus according to the results of phylogenetic analyses (Wanasinghe *et al.* 2016, Tibpromma *et al.* 2017). On the other

hand, multi-locus phylogenetic analyses revealed the paraphyletic nature of this genus in the present study (Fig. 2). The morphological observations suggested that the genus was restricted to *V. amygdali*, *V. appendiculata*, *V. armatispora*, *V. aquatica*, and *V. scabrispora*, although *V. armatispora* was not included in our phylogenetic analyses due to the limited availability of the sequence data. *Vaginatispora fuckelii* is atypical for this genus, because this species possesses a thinner peridium (up to 25 µm in thickness) that is uniformly thick and composed of 2 zones (Thambugala *et al.* 2015). Therefore, we propose a new genus, *Neovaginatispora*, to accommodate this species (see Appendix B). *Lophiostoma pseudoarmatisporum* was introduced as a species of *Lophiostoma* s. lat. *Lophiostoma*

*pseudoarmatisporum* is characterised by fusiform, hyaline ascospores with thin mucilaginous appendages (Li *et al.* 2016). The authors did not resolve the generic placement of the species (Hyde *et al.* 2016). The species is phylogenetically related to *Crassiclypeus*, *Flabellascoma*, *Leptoparies*, and *Paucispora* in our phylogenetic trees (Figs 1, S1A–C), but can be distinguished from these genera by the peridium that is composed of 1 zone and an ostiolar neck without the clypeus. Thus, a new monotypic genus, *Parapaucispora*, is proposed for *Par. pseudoarmatispora* (see Appendix B). The validity of the genera *Alpestrisphaeria*, *Coelodictyosporium*, *Guttulispora*, *Lophiohelichrysum*, *Platystomum*, and *Sigarispora* remain questionable. Most of these genera were originally divided from *Lophiostoma* based on insufficient features, such as the form of the ascomata and the ascospore colour and septation, and comprised single species and strain (Thambugala *et al.* 2015). Further discovery of more specimens along with additional morphological and molecular data will help to fully elucidate the taxonomic validity of these problematic genera in *Lophiostomataceae*.

### Form and function of the ascospore sheath

Ascospores of the *Lo. bipolare* complex possess a gelatinous sheath that may help these organisms to attach to plant substrates in aquatic or marine habitats (Shearer 1993, Hyde & Goh 2003, Jones 2006). Several terrestrial ascomycetes with appendaged ascospores have been reported from moist environments near a waterfall (Wanasinghe *et al.* 2016), a humid subtropical mountain (Tanaka & Hosoya 2008), and bamboo (Hashimoto *et al.* 2017b). It is interesting to note that most of the *Lo. bipolare* complexes were also collected from terrestrial habitats (Table 1). Jones (2006) suggested that these appendaged ascospores adapt to small watery environments in terrestrial habitats.

Ascospore characteristics are particularly useful in species identification of freshwater or marine fungi. Several morphological variations of the ascospore sheath were observed in *Lophiostomataceae* (Read *et al.* 1994, 1997, Tsui *et al.* 1999, Au *et al.* 1999, Hyde *et al.* 2002). *Capulatispora sagittiforme* and *Lentistoma bipolare* have ascospores with bipolar sheaths, providing cap-like structures at the tips (Tanaka & Hosoya 2008, Thambugala *et al.* 2015, this study Fig. 6T). Tanaka & Hosoya (2008) indicated that the bipolar appendages with the cap-like structures of the ascospores may contribute to the settlement of the discharged ascospore on the substrate. The lateral, gelatinous, pad-like structure was observed in most *Lo. bipolare* complexes (Figs 4N, 5R, 7S, 8R, 9R, 10S, 12P, 13P), which is suspected to contribute to their attachment to plant substrates in aquatic or marine habitats, as evident in other freshwater fungi (Jones 2006, Shearer *et al.* 2009). The presence of the internal chamber or inner spine structure at both ends of the ascospore sheath was observed in *Capulatispora*, *Crassiclypeus*, *Flabellascoma*, *Lentistoma*, *Leptoparies*, *Pseudopaucispora*, and *Vaginatisspora* (Read *et al.* 1997, Hyde *et al.* 2002, Tanaka & Hosoya 2008, this study, Figs 3S, 4P, 5T, 6U, 7U, 11O, 12R, 13S). Ultrastructural examination of *Len. bipolare* ascospores suggested that the chamber is comprised of concentrated fibrillar material (Read *et al.* 1997, Hyde *et al.* 2002). Although these morphological variations are considered a result of the adaptation to their habitats, their taxonomic importance remains unclear (Hyde *et al.* 2002). Our phylogeny showed that these structures may have evolved several times within *Lophiostomataceae*

(Fig. 2). From these results, it appears that the ascospore sheath has less importance to the understanding of the phylogenetic relationships as already reported (Shearer *et al.* 2009). Identification with an emphasis on these ascospore features alone may lead to misidentification of morphologically similar genera. A typical example of morphological convergence was reported in the study of “*Massarina*” *ingoldiana* s. lat. (Hirayama *et al.* 2010), in which the unrelated species complex was comprised of two distinct lineages at the familial level. Precise re-identification of the *Lo. bipolare* complex based on detailed morphological characteristics (not only ascospores, but also ascomatal features) and molecular evidences will be needed to reveal their generic placements, accurate geographical distributions, ecological traits, and species diversity.

Intraspecific variability of morphological features has traditionally been reported in lophiostomataceous species, even within a single specimen. This complicates their generic and species delimitations (Chesters & Bell 1970, Holm & Holm 1988). Although recent studies on *Lophiostomataceae* using multiple genes revealed new lineages (Thambugala *et al.* 2015, Li *et al.* 2016, Wanasinghe *et al.* 2016, Tibpromma *et al.* 2017), 16 genera were separated from *Lophiostoma* based on insufficient morphological features or unclear generic circumscriptions that relied mainly on the molecular phylogenetic topologies. The present data provide insight into the complicated delimitations within the *Lophiostomataceae* genera and revealed the taxonomic importance of anatomical characteristics of the ascomata, which were previously unclear. To build a stable and comprehensive taxonomic framework, detailed morphological observations based on multiple specimens and comparisons of morphological variability both on natural substrate and in culture, as well as molecular data with high resolution, such as *rpb2*, will be needed. The data will be helpful to resolve the taxonomic placement of genera within this family and to validate the various problematic genera that currently exist in *Lophiostomataceae*.

### ACKNOWLEDGEMENTS

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### APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.simyco.2018.03.001>.

### APPENDIX B. OTHER NOMENCLATURAL PROPOSALS

While resolving the *Lo. bipolare* complex, two new genera and two new combinations for *Lophiostoma pseudoarmatisporum* and *Vaginatisspora fuckelii* were required. They are introduced as follows:

**Neovaginatispora** A. Hashim., K. Hiray. & Kaz. Tanaka, **gen. nov.** MycoBank MB823147.

*Etymology:* Refers to its morphological similarity to *Vaginatispora*.

*Diagnosis:* Differs from *Vaginatispora* via the thinner peridium (composed of 2 zones) having uniform thickness.

Type species: *Neovaginatispora fuckelii* (Sacc.) A. Hashim. *et al.*

**Neovaginatispora fuckelii** (Sacc.) A. Hashim., K. Hiray. & Kaz. Tanaka, **comb. nov.** MycoBank MB823148.

*Basionym:* *Lophiostoma fuckelii* Sacc., *Michelia* 1: 336. 1878.

*Synonym:* *Vaginatispora fuckelii* (Sacc.) Thambug. *et al.*, *Fungal Diversity* 74: 243. 2015.

For other synonyms, see Holm & Holm (1988), Barr (1992), and Tanaka & Harada (2003).

**Parapaucispora** A. Hashim., K. Hiray. & Kaz. Tanaka, **gen. nov.** MycoBank MB815297.

*Etymology:* Refers to its morphological similarity to *Paucispora*.

*Diagnosis:* This genus can be distinguished from other lophiostomataceous genera by the single-zoned peridium that is wider at the sides and thinner at the base in the ascomata without a clypeus near the ostiolar neck.

Type species: *Parapaucispora pseudoarmatispora* (Hay. Takah. *et al.*) A. Hashim. *et al.*

**Parapaucispora pseudoarmatispora** (Hay. Takah. *et al.*) A. Hashim., K. Hiray. & Kaz. Tanaka, **comb. nov.** MycoBank MB824639.

*Basionym:* *Lophiostoma pseudoarmatisporum* Hay. Takah. *et al.*, *Fungal Diversity* 78: 35. 2016.

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