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

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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 rp2 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 sequences 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-rp2 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: *Massarinia 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 ascostomal 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 genera with five new species were recognised based on these morphological differences: *Crassiclypeus* (*C. aquaticus*), *Flabellascoma* (*F. cylindroidea* and *F. minimum*), *Leptoparies* (*L. palmarum*), and *Pseudopaucispora* (*Pseudop. brunneospora*). Three new species were added to *Pseudolophiostoma* (*Pseudol. comimsporum*, *Pseudol. obtusispornum*, and *Pseudol. tropicum*), and two new species were added to *Vaginatispora* (*V. amygdali* and *V. scabiosa*). 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 *Parapacispora pseudoaarmatisporum* 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, *Pseudopaucispora* A. Hashim., K. Hiray. & Kaz. Tanaka, *Vaginatispora* A. Hashim., K. Hiray. & Kaz. Tanaka; New species: *Crassiclypeus aquaticus* A. Hashim., K. Hiray. & Kaz. Tanaka, *Flabellascoma cylindroidea* A. Hashim., K. Hiray. & Kaz. Tanaka, *Flabellascoma minimum* A. Hashim., K. Hiray. & Kaz. Tanaka, *Leptoparies palmarum* A. Hashim., K. Hiray. & Kaz. Tanaka, *Pseudolophiostoma comimsporum* A. Hashim., K. Hiray. & Kaz. Tanaka, *Pseudopaucispora obtusispornum* A. Hashim., K. Hiray. & Kaz. Tanaka, *Pseudolophiostoma tropicum* A. Hashim., K. Hiray. & Kaz. Tanaka, *Pseudopaucispora brunneospora* A. Hashim., K. Hiray. & Kaz. Tanaka; New combinations: *Lentistoma bipolare* (K.D. Hyde) A. Hashim., K. Hiray. & Kaz. Tanaka, *Parapacispora pseudoaarmatisporum* (Hay. Takah. et al.) A. Hashim., K. Hiray. & Kaz. Tanaka, *Neovaginatispora fuckelii* (Sacc.) A. Hashim., K. Hiray. & Kaz. Tanaka; Typification: Epitypification (Basionym): *Massarinia 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 silt-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 et al. 1992). The silt-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 et al. 1987, 1992, Yuan & Zhao 1994; Checa 1997, Kirk et al. 2008, Muggami & Huhndorf 2009). A recent generic re-evaluation of *Lophiostomataceae* (Thambugala et al. 2015)
segregated *Lophiostoma* s. lat. into 16 genera according to the multi-focus phylogenies using small subunit rDNA (18S; SSU), large subunit rDNA (28S; LSU), and translation elongation factor 1-α (tef1).

*Lophiostoma bipolare* is recognised by its striking features of the silt-like ostiolar neck surrounded by a well-developed cyphus, 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 silt-like ostiolar neck lacking the cyphus, 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/BI2b (Glass & Donaldson 1995, O’Donnell & Cigelnik 1997), *tef1* = EF1-983F/EF1-2218R (Rehner & Buckley 2005), and *rpb2* = 6RP2-5F/6RP2-7C-R (Liu et al. 1999), respectively. Amplifications were performed in 25 μL volumes consisting of 2 μL DNA, 2.5 μL of 10× TEPase Buffer I, 10 mM dNTP mix, 1 μL of each primer (20 μM), 25 mM MgCl2, 14.5 μL MilliQ water, and 0.5 μL TEMPaste 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 Kukasun4 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 rubristiolata* and *T. trabicola* (*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. | Strain no. | Habitat | GenBank accession no. |
|---------|--------------|--------------|------------|---------|-----------------------|
|         | Old name     | New name     |            |         |                       |
| Lo. bipolare -1 | Crassiclypeus aquaticus | | | | |
| 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* | CBS 143643 | F | =JCM 13087 =MAFF 239597 | LC312472 LC312501 LC312530 LC312559 LC312588 LC312617 |
| Lo. bipolare -2 | Flabellascoma cycadicola | | | | |
| KT 2034 | HHUF 30570* | BCRC FU30901 =CBS 143644 | T | | LC312473 LC312502 LC312531 LC312560 LC312589 LC312618 |
| Lo. bipolare -3 | F. minimum | | | | |
| KT 2013 | HHUF 30571 | BCRC FU30900 =CBS 143645 | T | | LC312474 LC312503 LC312532 LC312561 LC312590 LC312619 |
| KT 2040 | HHUF 30572* | BCRC FU30902 =CBS 143646 | T | | LC312475 LC312504 LC312533 LC312562 LC312591 LC312620 |
| Lo. bipolare -4 | Lentistoma bipolare | | | | |
| HKUCC 10069 | HHUF 30576 | CBS 115370 | U | | LC312476 LC312505 LC312534 LC312563 LC312592 LC312621 |
| HKUCC 10110 | HHUF 30577* | CBS 115375 | U | | LC312477 LC312506 LC312535 LC312564 LC312593 LC312622 |
| HKUCC 8277 | HHUF 30575 | JCM 14139 =CBS 110448 | F | | LC312478 LC312507 LC312536 LC312565 LC312594 LC312623 |
| Lo. bipolare -5 | Leptoparies palmarum | | | | |
| 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 |
| Lo. bipolare -6 | Pseudolophiostoma consiliaporum | | | | |
| KH 322 | HHUF 30582* | CBS 143654 =JCM 32348 | T | | LC312486 LC312515 LC312544 LC312573 LC312602 LC312631 |
| Lo. bipolare -7 | P. obtusisporum | | | | |
| 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* | 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)
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 Bioresource 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, Siganispora, 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 Vaginatispora 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, 1243 from LSU, 900 from ITS, 885 from tef1, and 1017 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 4980 characters included in the alignment, 1387 were variable and 3524 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.

| Species Original no. | SSU ITS LSU tef1 rpb2 TUB |
|----------------------|---------------------------|
| Species New name     | GenBank accession no.     |
| Strain no.           | Habitat 1                |
| Specimen no.         | Specimen no. 2           |
| Hab 2                | Species name             |
| Strain no.           | Habitat 3                |
| Original no.         | Habitat 4                |
| Table 1. (Continued.)|                           |

1. "E": epitype, "H": holotype, "F": freshwater, "M": marine, "T": terrestrial, "U": unknown.
Table 2. Isolates and GenBank accession numbers of species used in the phylogenetic study.

| Species                                | Strain no. | SSU  | ITS  | LSU  | tef1 | rpb2 |
|-----------------------------------------|------------|------|------|------|------|------|
| Apestrisphaeria terricola              | SC-12H     | JX985749 | JN662930 | JX985750 | –    | –    |
| Biappendiculispora japonica            | KT 573H    | AB61866 | LC001728 | AB619005 | LC001744 | –    |
|                                         | KT 666-1H  | AB61867 | LC001729 | AB619006 | LC001745 | –    |
| Capulatispora sagittiformis             | KT 1934H   | AB61893 | AB369286 | AB369267 | LC001756 | –    |
| Coelodictyosporium muniforme           | MFLUCC 13-0351H | KP899127 | KP899136 | KP888841 | KR075163 | –    |
| C. pseudodictyosporum                  | MFLUCC 13-0451H | –      | KR025858 | KR025862 | –    | –    |
| Dimorphiosporis brachystegiae          | CPC 22679H | –      | –    | KF777160 | KF777213 | –    |
| Guttulispora crataegi                  | MFLUCC 13-0442H | KP899125 | KP899134 | KP888639 | KR075161 | –    |
|                                          | MFLUCC 14-0993H | –      | –    | KP899126 | KP899135 | LC001756 | –    |
| Lophiohelichrysum helichrysi           | MFLUCC 15-0701H | KT333437 | KT333435 | KT333436 | KT427535 | –    |
| Lophiopoacea paramacrostoma            | MFLUCC 11-0463H | KP899122 | –    | –    | –    | –    |
|                                          | KT 740     | AB618699 | JN942969 | AB619017 | LC001763 | JN993487 |
|                                          | KT 764     | –      | –    | AB618700 | JN942968 | AB619018 | LC001764 | JN993488 |
| Lophiostoma alpigenum                  | GKM 1091b  | –      | –    | GU385193 | GU327758 | –    |
| L. caulatum                            | CBS 823.86 | GU296163 | –    | GU301833 | –    | GU31791 |
| L. crenatum                            | CBS 629.86 | DQ678017 | –    | DQ678069 | DQ677912 | DQ777965 |
| L. heterosporum                        | CBS 644.86 | AYO16359 | GQ203795 | AYO16369 | DQ497609 | DQ497615 |
| L. macrotomoides                       | CBS 123097 | FJ795482 | –    | FJ795439 | GU456277 | FJ795458 |
| L. macrostomum                         | KT 635     | AB521731 | AB433275 | AB433273 | LC001752 | JN993484 |
| L. quadrinucleatum                     | GKM 1233   | –      | –    | GU385184 | GU327760 | –    |
| L. semiliberum                         | KT 828     | AB618696 | JN942970 | AB619014 | LC001759 | JN993489 |
| Neotrematosphaeria biappendiculata     | KT 1124H   | GU205256 | –    | GU205227 | –    | –    |
| Neovaginatispora fuckelii              | CBS 101952 | FJ795496 | –    | DQ399531 | –    | FJ795472 |
|                                          | KH 161     | AB618689 | LC001731 | AB619008 | LC001749 | –    |
|                                          | KT 634     | AB618690 | LC001732 | AB619009 | LC001750 | –    |
| Parapaucispora pseudobarmatopora       | KT 2237H   | LC100018 | LC100021 | LC100026 | LC100030 | –    |
| Paucispora quadrispora                 | KH 448H    | LC001720 | LC001733 | LC001722 | LC001754 | –    |
| P. quadrispora                         | KT 843H    | AB618692 | LC001734 | AB619011 | LC001755 | –    |
| P. versicolor                          | KH 110H    | LC001721 | AB918731 | AB918732 | LC001760 | –    |
| Platyostomum actiniae                  | KT 521H    | JN491375 | JN942963 | JN491380 | LC001747 | JN993490 |
| P. compressum                          | MFLUCC 13-0343 | KP899129 | KP899134 | KP888643 | KR075165 | –    |
| P. crapegi                            | MFLUCC 14-0925H | KT026113 | KT026117 | KT026109 | KT026121 | –    |
| P. salicola                           | MFLUCC 15-0632H | KT026114 | KT026118 | KT026110 | –    | –    |
| Pseudolophiostoma villegenum           | HH 26930H  | AB618697 | LC001735 | AB619015 | LC001761 | –    |
|                                          | HH 26931H  | AB618698 | LC001736 | AB619016 | LC001762 | –    |
| Pseudoplatystomum scabridisporum       | BCC 22835  | GQ925831 | –    | GQ925844 | GU479857 | GU479830 |
| Sigaritisa purpurea arundinis          | KT 651     | AB618680 | JN942965 | AB618999 | LC001738 | JN993486 |
|                                          | KT 530     | AB618681 | LC001723 | AB619000 | LC001739 | –    |
| S. ononidis                            | MFLUCC 15-267H | KU243126 | KU243128 | KU243125 | KU243127 | –    |
| S. ravenica                           | MFLUCC 14-0005H | KP698415 | KP698413 | KP698414 | –    | –    |
| Teichospora rugiostilatoida            | TR 7H      | –      | KU601590 | KU601590 | KU601609 | KU601599 |
| T. trabicola                          | C 134F     | –      | KU601591 | KU601591 | KU601601 | KU601600 |
| Vaginatispora appendiculata            | MFLUCC 16-0314H | KU743219 | KU743217 | KU743218 | KU743220 | –    |
| V. aquatica                           | MFLUCC 11-0083 | KJ951575 | KJ951577 | KJ951578 | –    | –    |

1: E: ex-epitype, H: ex-holotype, I: ex-isotype, P: ex-paratype.
bipolare complex were scattered to *Pseudolophiostoma*, *Vaginatispora*, and four separate clades from known lophiostomataceous genera. *Lophiostoma bipolare*-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 bipolare*-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 *Leptoparies* was introduced for *Lep. palmarum* (formerly treated as *Lo. bipolare*-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. bipolare*-6, 7, and 8 (*Pseudol. comisporum*, *Pseudol. obtusisporum*, and *Pseudol. tropicum*) – forming a strongly supported clade (98 % ML BS/1.00 Bayesian PP, Fig. 2). *Lophiostoma bipolare*-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. bipolare*-10, 11 received strong support (98 % ML BS/1.00 Bayesian PP, Fig. 2). *Vaginatispora amygdali* and *V. scabrispora* were proposed for *Lo. bipolare*-10, 11, respectively.

**Taxonomy**

Our phylogenetic analyses resolved 11 species that were classified in the *Lo. bipolare* 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. bipolarare* s. str. (basionym: *Massarina* bipolare). Additionally, two new genera and two new combinations are introduced for *Lophostoma pseudoarmatisporum* and *Vaginatispora fuckelii* (See Discussion and Appendix B).

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

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

**Sexual morph:** Ascomata scattered to gregarious, immersed, subglobose. Ostioral neck elongated, laterally compressed, surrounded by a well-developed clypeus. Peridium composed of elongated, brown cells, surrounded by brown hyphae. *Pseudo-paraphyses* numerous, septate, branched and anastomosed. Asci bitunicate, fissitunicate, clavate, 8-spored. Ascospores fusiform, hyaline, 1-septate, with a narrow bipolar sheath. *Asexual morph:* *Conidiotoma* pycnidial, globose to subglobular, up to 165 μm high, 135–180 μm diam, scattered to 3–6 grouped, superficial to immersed. *Peridium* composed of subglobose to rectangular, brown cells. *Conidiophores* absent. *Conidigenous cells* phialidic, ampiliform, hyaline, smooth. *Conidia* subglobose with rounded ends, hyaline, smooth, aseptate.

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

**Notes:** *Crasiclypeus* is established to accommodate *C. aquaticus*, which is characterised by a crest-like, elongated, and laterally compressed ostioral 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 ascomatal wall with 1 zone (Fig. 3D–F) and phialidic conidiogenous cells in the conidiotoma (Fig. 3Y–AA) (vs. an ascomatal peridium composed of 2 zones and holoblastic conidiogenous cells in the conidiotoma; 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 ostioral neck of the ascomata (Thambugala et al. 2015). *Crasiclypeus* has similar morphological features of *Lentistoma*, such as the clypeated ostioral neck, but can be distinguished from *Lentistoma* by the well-developed peridium of the ascomata (up to 70 μm in thickness). *Lentistoma* is characterised by ascomata with less-developed peridium (up to 45 μm in thickness).

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

**Etymology:** Refers to its aquatic habitat.

**Sexual morph:** Ascomata subglobose, 3–5 grouped, immersed, dark brown to black, 400–780 μm high, 600–1000 μm diam. Ostioral 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 paraphyses, 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 (μ = 102.5 × 12.9 μm, n = 96), with a stipe (14–50 μm long, μ = 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 (μ = 25.3 × 6.7 μm, n = 190), l/w 2.9–4.8 (μ = 3.8, n = 190), hyaline, with a septum nearly median (0.42–0.45–0.55, μ = 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:* *Conidiotoma* pycnidial, globose to subglobular, up to 165 μm high, 135–180 μm diam, scattered to 3–6 grouped, superficial to immersed. *Peridium* composed of subglobose to rectangular, brown cells. *Conidiophores* reduced to conidiogenous cells. *Conidigenous cells* phialidic, 6–12 × 2.5–4 μm, ampiliform, hyaline, smooth. *Conidia* subglobose with rounded ends, 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.

*Morphological combinations are introduced for *Massarina* bipolaris, such as the clypeated ostiolar neck, but can be distinguished from *Lentistoma* by the well-developed peridium of the ascomata (Thambugala et al. 2015). *Crasiclypeus* has similar morphological features of *Lentistoma*, such as the clypeated ostioral neck, but can be distinguished from *Lentistoma* by the well-developed peridium of the ascomata (up to 70 μm in thickness). *Lentistoma* is characterised by ascomata with less-developed peridium (up to 45 μm in thickness).

**Notes:** *Crasiclypeus aquaticus* was collected from submerged dead twigs of woody plants during summer, fall, and winter. *Crasiclypeus 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 ostioral neck, which resembles a Japanese fan.

**Sexual morph:** Ascomata scattered, immersed, subglobose to ellipsoidal. Ostioral neck elongated, laterally compressed. *Peridium* composed of elongated, brown cells. *Pseudo-paraphyses* septate, branched and anastomosed. Asci bitunicate, fissitunicate, cylindrical-clavate, 8-spored. Ascospores fusiform, hyaline, 1-septate, with a narrow bipolar sheath. *Asexual morph:* *Conidiotoma* pycnidial, globose to subglobular. *Peridium* composed of subglobose to rectangular, brown cells. *Conidiophores* absent. *Conidigenous cells* holoblastic, cylindrical or ampiliform, 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 ascomatal wall composed of 2 zones (Figs 4D, 5D), and asci with a short stipe. *Flabellascoma* is
morphologically similar to Pseudolophistoma in having ascomata with a well-developed, crest-like ostiolar neck, and a peridium of uniform thickness. However, the ascomatal peridium in Pseudolophistoma 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 5, 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 compressed, 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, thinned-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. Ascii bitunicate, fissitunicate, cylindrical-clavate, 67.5–88 μm × 1.3–2.1 μm. Ascospores with a short stipe, apically rounded with a broad ocular chamber, hyaline, smooth, with a narrow sheath. 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.

Morphology: Ascomata formed within the sheath, up to 1.5 μm wide at side, 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, ampiphycous, hyaline, smooth. Conidia subglobose with rounded ends, 1.5–2.5 × 1.1–1.2 μm (X = 2.0 ± 1.4 μm, n = 60), l/w 1.0–1.8 (X = 1.4, n = 60), hyaline, smooth, aseptate, gutulate 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.

Materials examined: Taiwan. Nantou, Shien, Hui Sun Forest Area, Kuan-Dau river, on petiole of Arenga engleri; 26 Nov. 2005. K. Tanaka, C.Y. Chen & G. Okada, VT 2013 (HHUF 30572: ex-paratype culture BCRC FUS30900 = CBS 143645); Taipei, Wuai, on pods of Banyuna purpurea, 28 Nov. 2005. K. Tanaka, H.S. Chang & G. Okada, VT 2040 (HHUF 30572 holotype designated here; ex-holotype culture BCRC FUS30902 = CBS 143646).

Notes: ITS sequences of ex-holotype and ex-paratype cultures of F. minima isolated from Areceacea (Arecales) and Fabacea (Fabales), respectively, were identical. Although the ascomatal shape was slightly different between the holotype (lageniform, Fig. 5D) and paratype (ellipsoidial, 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 Lophiotomataceae based on the SSU-ITS-LSU-telf-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. Ascii 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:** Lentistoma bipolare was originally described as a species of Massarina (Hyde 1995a). Liew et al. (2002) transferred the species to Lentistoma based on phylogenetic analyses of ITS sequences. This classification was corroborated by subsequent studies (Tanaka & Hosoya 2008, Hirayama & et al. 2015) conducted comprehensive taxonomic revisions in subsequent studies (Tanaka & Hosoya 2008, Hirayama & et al. 2015) conducted comprehensive taxonomic revisions in Lophiostomataceae, but *L. bipolare* was not included. Thus, its generic placement remained unresolved. Our phylogenetic study revealed the distant relationship of this species to *Lentistoma* 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:** Lophistoma bipolaris (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 paraphyses, 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 (X = 119.9 × 10.9 µm, n = 30), with a stipe (7.5–18.5 µm long, 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 (X = 27.0 × 7.2 µm, n = 216), l/w 2.5–4.8 (X = 3.8, n = 216), hyaline, with a septum nearly median (0.46–0.55, 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 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. Inomote, 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); Oromiya 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 Maryu 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 30590; 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 ascocoma.

**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.
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 (X = 93.9 × 11.9 μm, n = 20), with a stipe (8.5–18.5 μm long, 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 (X = 23.1 × 6.1 μm, n = 100), LW 2.8–4.4 (X = 3.5, n = 100), hyaline, with a septum mostly supramedian (0.47–0.55, 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 28883 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 characterized by 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. Ostiole of ascocarp, yellow, with a lateral pad, up to 1.5 mm high, 250–300 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 brown hyphae. Pseudoparaphyses numerous, 1.5–2 μm wide, septate, branched and anastomosed. Asci bitunicate, fissitunicate, cylindrical-clavate, 80–92 × 11–15(–18) μm (X = 86.4 × 13.6 μm, n = 5), with a long stipe (8–15 μm long, 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 (X = 26.6 × 5.3 μm, n = 60), LW (3.9–)4.1–6.3 (X = 5.0, n = 60), hyaline, with a septum nearly median (0.47–0.56, 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, H2 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 MB823140. Figs 8, 14G.

Eymology: Refers to the ascospores with obtuse 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 brown hyphae. Pseudoparaphyses numerous, 1.5–2 μm wide, septate, branched and anastomosed. Asci bitunicate, fissitunicate, cylindrical-clavate, 80–92 × 11–15(–18) μm (X = 86.4 × 13.6 μm, n = 5), with a long stipe (8–15 μm long, 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 (X = 26.6 × 5.3 μm, n = 60), LW (3.9–)4.1–6.3 (X = 5.0, n = 60), hyaline, with a septum nearly median (0.47–0.56, 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, H2 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.

Eymology: 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,
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. *Ascus* bitunicate, fissitunicate, cylindrical-clavate, (81)–103–140 × 8–15 μm (x = 119.9 × 10.9 μm, n = 30), with a long stipe (7.5–18.5 μm long, x = 11.7 μm, n = 11), apically rounded with an ocular chamber, 8-spored. *Ascosporas* fusiform with obtuse ends, (20)–23.5–31.5 × 4–7 μm (x = 27.3 × 5.5 μm, n = 90), lw 3.5–6.9 (x = 5.0, n = 90), hyaline, with a septum nearly median (0.47–0.55, 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 22–27 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. Chichijima, Buta coast, on dead stem of *Bidentis pilosa* var. radiata, 15 Sep. 2012, K. Tanaka, A. Hashimoto & T. Sato, KT 3134 (HHUF 30202 holotype designated here; ex-holotype culture CBS 143680 = MAFF 243989).

**Notes:** In culture, *Pseudol. tropicum* produced ascomata 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 ascomatal wall formed in culture were identical to those on natural specimens.

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

**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. *Ascus* bitunicate, fissitunicate, cylindrical to clavate, 8-spored. *Ascosporas* fusiform, brown, 1-septate, smooth, with a narrow bipolar sheath. Asexual morph: *Conidiofrassata* pseudopycnidial, globose to cylindrical, hyaline, smooth, 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.

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<Fig. 5. Flabelliscoma minimum: A–C. Appearance of ascomata on substrate. D, E. Ascomata in longitudinal section. F. Ostiolar neck of ascoma. G, H. Peridium of ascoma. I. Pseudoparaphyses. J, K. Ascii. L. Ascs apex. M. Ascs stipe. N–T. Ascosporas (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 μm; **H–J** = 50 μm; **K–O** = 10 μm; **P–V** = 5 μm.
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–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 μm; C = 100 μm; D, E = 1 mm; G, H = 50 μm; I–N = 10 μm; O–U = 5 μm.
Notes: *Pseudopaucispora* is introduced to accommodate *Pseudodop. brunnneospora*, which is characterised by small brown ascospores and pseudopycnidioid conidiomata. *Pseudopaucispora* is superficially similar to *Paucispora* (Thambugala et al. 2015). However, *Pseudopaucispora* has an ascomatal 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 brunnneospora** 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, 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 (Wanasingshe 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 ascomatal 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* 35.7–62.5 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, 80–100 × 140–165 μm (× = 115.0 × 18.5 μm, n = 53), with a short stipe (8.5–16 μm long, × = 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 (× = 30.6 × 8.8 μm, n = 120), l/w = 2.6–4.6 (× = 3.5, n = 120), hyaline, with a septum nearly median (0.45–0.58, × = 0.51, 0.58, n = 50), hyaline, 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 conidigenous cells. *Conidigenous cells* holoblastic, 10–15 × 2.5–4.5 μm, ampiform to cylindrical, hyaline, smooth. *Conidia* cylindrical with rounded ends, 2–3(–3.5) × 1–1.3 μm (× = 2.8 × 1.1 μm, n = 50); l/w = 1.8–3.1 (× = 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. bipolarae* 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.

**Notes:** Refers to its brown ascospores.

**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).

**Notes:** Refers to its brown ascospores.

**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).

**Notes:** Refers to its brown ascospores.

**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).

**Notes:** Refers to its brown ascospores.

**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).

**Notes:** Refers to its brown ascospores.

**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).

**Notes:** Refers to its brown ascospores.
Fig. 9. **Pseudolphistoma 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. Ascii. 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 μm; D = 100 μm; G–I = 50 μm; J–M, O = 10 μm; N, P–T = 5 μm.
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, 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.
G. Pseudoparaphyses. longitudinal section (con

J

Sexual morph: 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 amygdaли is morphologically similar to V. armatispora, but ascospores of the latter species are slightly larger (28–39.2 × 7–9.8 μm; Hyde et al. 1992). ITS sequences of V. amygdaли 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. bilopare 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 μm high, 340–360 μm diam. Ostiolar neck crest-like, elongated, laterally compressed, 88–120 μm high, 175–225 μm wide, composed of 3–5 μm diam, globose, brown to black cells, with hyaline periphyses. Peridium 18–28 μm thick at side, composed of 3–5 layers of rectangular, thin-walled, 10–11 × 3–5 μm, brown cells, surrounded by dark brown hyphae (1–1.5 μm wide); 10–17.5 μm thick at base, composed of globose, brown cells. Pseudoparaphyses numerous, 1.5–2 μm wide, septate, branched and anastomosed. Ascii bitunicate, fissitunicate, cylindrical-clavate, (77.5–) 95–115 × 15–20 μm (X = 102.3 × 16.5 μm, n = 10), with a short stipe (7.5–17.5 μm long, X = 13.0 μm, n = 10), apically rounded with a broad ocular chamber, 8-spored. Ascospores fusiform with obtuse ends, 20–23 × 5–6 μm (X = 21.9 × 5.9 μm, n = 50), lw 2.9–3.5 (X = 3.3, n = 50), hyaline, with a septum supramedian (0.42–0.49, X = 0.47, n = 50), slightly constricted at the septum, verrucous, with a narrow sheath. Sheath drawn out 5–7 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: Undetermined.

Culture characteristics: Colonies on PDA attaining 19 mm diam within 25 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. 13P) and mangrove habitat. This species is phylogenetically related to V. amygdaли, but differs from the latter by the smaller sized ascospores (vs. 25–34(−37) × 7–10.5 μm in the latter species).

DISCUSSION

Generic delimitation in Lophiostomataceae

Lophiostoma bilopare 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 (Thambugal et al. 2015). Lophios- toma bilopare 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. bilopare, 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. bilopare 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. bilopare 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 (Thambugal 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. bilopare found on woody plants were flattened at the base, while those on herbaceous plants were ellipsoidal (Figs 5D, 6H–J). Although the length of the ostiolar neck, peridium thickness, and asco-matal 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, Vagi- natispora; Figs 3D–F, 12F, 13E, F), the contexture of the peridium with 1 zone (Crassiclypeus, Lentistoma, Leptoparies, Pseudolophiostoma, Pseudopaucispora, Vagini-

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 condidium 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 μm; C, R = 100 μm; D, S, T = 50 μm; E, F, H, I, U, Y = 10 μm; G, J–O, V–X = 5 μm.
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 μm; C, E = 100 μm; F = 50 μm; K, L = 10 μm; G–J, M–R = 5 μm.
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. 13.** Vaginatispora 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, D, E, 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 μm; E, F = 50 μm; G–J, M = 10 μm; K, L, N–S = 5 μm.
Pseudopaucispora; pycnidial conidiomata and phialidic conidiogenous cells (Crassiclypeus; 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 cyphus. Thus, a new monotypic genus, Parapaucispora, is proposed for Par. pseudoarmatispora (see Appendix B). The validity of the genera Alpestrisphaeria, Coelodictyosporum, Guttulispora, Lophiohelichrysum, Platystomum, and Siganispora remain questionable. Most of these genera were originally generated from Lophiostoma based on insufficient features, such as the form of the ascocoma and the ascospore colour and sepalation, 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 appended 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 appended 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 Vaginatispora (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.

(2008) indicated that the bipolar appendages with the cap-like structures at the tips (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 appended ascospores adapt to small watery environments in terrestrial habitats.

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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 Nomencultural Proposals

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

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

**Basionym:** Lophiotrema fuckelii (Sacc.) A. Hashim. et al.

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

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

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

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

**Basionym:** Lophiotrema fuckelii (Sacc.) A. Hashim. et al.

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

**Diagnosis:** This genus can be distinguished from other lophiotomataceous 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:** Lophiotrema pseudoarmatispora (Hay. Takah. et al.) A. Hashim. et al.

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