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2    **Taxonomic study of Endogonaceae in the Japanese Islands: new species of *Endogone*,**

3    ***Jimgerdemannia*, and *Vinositunica* gen. nov.**

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13   **ABSTRACT**

14   Species of Endogonaceae (Endogonales, Mucoromycotina) are characterized by the formation

15   of relatively large sporocarps and zygosporangia. Numerous species in this family remain

16   undescribed or have unclear phylogenetic positions. In Asia specifically, the species diversity

17   of this family is almost completely unknown. However, many mycobionts of bryophytes

18   belonging to several novel clades in Endogonaceae have recently been identified

19   phylogenetically. Therefore, establishing a robust taxonomic system for this family is

20   essential. We obtained numerous sporocarps of undescribed Endogonaceae-like species from

21   the Japanese Islands. Morphological observation and multi-locus phylogenetic analysis of nuc

22   18S rDNA (18S), nuc 28S rDNA (28S), and portions of two nuclear protein-coding regions –

23   translation elongation factor 1-alpha (*tef1*) and RNA polymerase II large subunit (*rpb1*) –

24   from these species resulted in the description of one new species each of *Endogone* and

25   *Jimgerdemannia* and two new species of *Vinositunica* gen. nov. Because *Vinositunica* is

1 characterized by purplish sporocarps and red-wine colored chlamydospores up to 700 µm in

2 diameter, we emended the definition of Endogonaceae.

3 **KEY WORDS**: chlamydospore, Mucoromycota, mycorrhizal fungi, phylogeny, sporocarp,

4 zygosporangium, 4 new taxa

5 **INTRODUCTION**

6 Fungi in the Endogonales (Mucoromycotina, Mucoromycota) are characterized by the

7 formation of relatively large sporocarps up to 25 mm in diameter (Gerdemann and Trappe

8 1974), in which sexual (zygospores within zygosporangia) or asexual (chlamydospores)

9 structures have been observed (Desirò et al. 2017). *Endogone*, the type genus of the order,

10 was described by Link (1809) based on the zygosporic species *E. pisiformis*. Subsequently,

11 two chlamydospore-forming sporocarpic species, *Glomus macrocarpum* and *G. microcarpum*,

12 were described (Tulasne and Tulasne 1844) and transferred to *Endogone* later (Tulasne and

13 Tulasne 1851). Fries (1849) established "Endogonei" to include *Endogone* and *Glomus*.

14 Although its taxonomic rank was not established at the time, the family Endogonaceae was

15 redefined by Paoletti (1889). In early research into *Endogone* taxonomy, the generic definition

16 was gradually expanded, with non-sporocarpic chlamydosporic and azygosporic species and

17 sporangial asexual species included in the genus (Thaxter 1922; Nicolson and Gerdemann

18 1968). A study by Gerdemann and Trappe (1974) provided a turning point for such research,

19 as Endogonaceae genera were redefined based on their reproductive organs: only zygosporic

20 species were retained in *Endogone* s. str., while chlamydosporic, azygosporic, and

21 sporangiosporic species were excluded. Although the latter species were treated as *Glomus*,

22 *Gigaspora*, and *Modicella*, respectively, these genera remained within Endogonaceae.

23 Pirozynski and Dalpé (1989) established Glomeraceae, which included *Glomus* and

24 *Sclerocystis*. Morton and Benny (1990) raised Glomeraceae to Glomerales (as Glomales),

25 distinguished from Endogonales by the formation of asexual spores and capacity for

1 arbuscular mycorrhizal association. Recent molecular phylogenetic studies have revealed

2 these taxonomic treatments (Gerdemann and Trappe 1974; Pirozynski and Dalpé 1989;

3 Morton and Benny 1990) to be valid. Sporocarpic genera with zygosporangia (Endogonales)

4 are placed in Mucoromycotina (James et al. 2006; Hibbett et al. 2007) in Mucoromycota

5 (Spatafora et al. 2016). However, sporocarpic and non-sporocarpic species with

6 chlamydospores and azygosporangia [referred to collectively as "spores" (Smith and Read

7 2008)] formerly belonging to Endogonales were placed in Glomerales (i.e., *Glomus*,

8 *Funneliformis*, *Sclerocystis*), Diversisporales (i.e., *Diversispora* and *Redeckera*) (Schüßler

9 and Walker 2010; Schüßler et al. 2011; Błaszkowski 2012), and Diversisporales (i.e.,

10 *Acaulospora*; Berch 1985) in Glomeromycotina (Schüßler et al. 2001; Schüßler and Walker

11 2010). Likewise, the sporangiosporic *Modicella* was placed in Mortierellomycotina (Smith et

12 al. 2013) within Mucoromycota.

13    Furthermore, *Sphaerocreas pubescens* (Saccardo 1882) and *Densospora* spp. (McGee

14 1996) have recently been revealed as the *Sphaerocreas pubescens-Densospora* lineage

15 (Yamamoto et al. 2015), of Densosporaceae, a sister clade of Endogonaceae (Desirò et al.

16 2017). *S. pubescens* and *Densospora* spp. were formerly considered as relatives or members

17 of *Glomus* due to chlamydospore formation in sporocarps (Gerdemann and Trappe 1974;

18 Tandy 1975a; Warcup 1985) along with putative saprotrophic or mycoparasitic nutrition (*S.*

19 *pubescens*: Gerdemann and Trappe 1974, Hirose et al. 2014, Yamamoto et al. 2015) and

20 ectomycorrhizal association (*D. tubaeformis* and *D. solicarpa*: Warcup 1985; McGee 1996).

21 Currently, Endogonales is comprised of Endogonaceae (zygosporic) and Densosporaceae

22 (chlamydosporic).

23    To date, five genera including *Endogone* have been classified in Endogonaceae.

24 *Sclerogone* forms minute sporocarps (up to 500 μm in diameter) that contain a small number

25 (1–78) of zygosporangia (Warcup 1975, 1990; Yao et al. 1996). *Peridiospora* is characterized

1   by a unisporic sporocarp in which a brown colored zygosporangium is formed (Wu and Lin

2   1997; Goto and Maia 2006). However, the phylogenetic positions of these two genera are

3   unknown, and the latter may not be in Endogonales (Desirò et al. 2017). *Youngiomyces*, which

4   forms zygosporangia with 2–4 openings to the remnants of gametangia (Yao et al. 1995), was

5   synonymized with *Endogone* because *Y. aggregatus* was phylogenetically nested within

6   *Endogone* (Desirò et al. 2017). Indeed, zygosporangia with two openings have also been

7   observed in *Endogone* spp.; i.e., *E. pisiformis* and *E. incrassata* (Yamamoto et al. 2015). By

8   contrast, *Endogone* species such as *E. lactiflua* and *E. flammicorona* form heterogametic

9   zygosporangia with zygosporangial hyphal mantles after heterogametic conjugation, while

10  other *Endogone* species such as *E. pisiformis* form homogametic zygosporangia without a

11  mantle (Yao et al. 1996). Trappe et al. (2009) suggested that heterogametic species should be

12  treated as a separate genus, which was supported by phylogenetic analysis (Yamamoto et al.

13  2015). Then heterogametic species *E. lactiflua* and *E. flammicorona* were transferred to a new

14  genus, *Jimgerdemannia* (Desirò et al. 2017). The Middle Triassic fossil fungus, *Jimwhitea*

15  *circumtecta*, which forms heterogametic zygosporangia covered by a mantle, was recently

16  placed in Endogonaceae (Krings et al. 2012). This genus appears to be closely related to

17  *Jimgerdemannia* spp. (Krings et al. 2012). At present, *Endogone* and *Jimgerdemannia* are the

18  only genera of Endogonaceae with established phylogenetic positions (Desirò et al. 2017).

19      Diverse lineages of Endogonaceae have been detected from mycorrhiza-like structures

20  of ancestral liverworts (Haplomitriopsida) and hornworts in environmental DNA sequences

21  (Bidartondo et al. 2011; Desirò et al. 2013). However, some of those sequences were placed

22  outside of the *Endogone* clade and the *Jimgerdemannia* clade (Desirò et al. 2017), strongly

23  suggesting that Endogonaceae still contains undescribed taxa and genera. To trace the

24  evolutionary history of plant-fungus associations involving Mucoromycotina during the

25  Paleozoic era (Strullu-Derrien et al. 2014), thorough taxonomic study of extant Endogonales

1    is required.

2        Estimates of species diversity of Endogonaceae in Asia have long been thought to be

3    lower than those in Europe (Yao et al. 1996; Błaszkowski 1997; Błaszkowski et al. 1998),

4    North America (Thaxter 1922; Gerdemann and Trappe 1974; Yao et al. 1996), and Australia

5    (Tandy 1975a; Warcup 1990; Yao et al. 1996) due to a lack of sampling effort. However,

6    recent field sampling and taxonomic studies in Japan have revealed distribution of *E.*

7    *pisiformis*, *E. incrassata*, *E. lactiflua* (= *J. lactiflua*), and *E. flammicorona* (= *J.*

8    *flammicorona*) in Asia (Yamamoto et al. 2015). In addition, a novel *Endogone* species, *E.*

9    *corticioides*, from Japan and China (Yamamoto et al. 2017a), and a novel *Endogone* lineage

10   that forms thick-mantled ectomycorrhiza with oaks from Japan (Yamamoto et al. 2017b) have

11   been reported. Hence, further undescribed lineages of Endogonaceae were expected to be

12   present in the Japanese Islands, which are located at the eastern end of the Eurasian Continent

13   facing the Pacific Ocean, and range 2000 km in latitude from subtropical to subarctic zones.

14       During field surveys of Endogonales in the Japanese Islands, we collected many

15   specimens of unknown sporocarpic taxa that appeared likely to be Mucoromycotina or

16   Glomeromycotina, which were subjected to morphological observation and multi-locus

17   phylogenetic analysis. Here, we describe new species of *Endogone* and *Jimgerdemannia* as

18   well as a new genus *Vinositunica* in the Endoganaceae.

**MATERIALS AND METHODS**

19   

20   ***Field sampling and morphological observation.***–Fresh sporocarps of Endogonaceae were

21   collected from forest sites under litter near ectomycorrhizal trees, on the soil surface, or on the

22   bottom surface of decayed wood through direct observation or using a rake from various

23   geographic regions in the Japanese Islands between 2010 and 2018. Fresh sporocarp samples

24   were observed and described in terms of morphology of tissues and spores, as described

25   previously (Yamamoto et al. 2015) using a dissecting microscope (Stemi 2000C, Carl Zeiss

1 Inc., Göttingen, Germany) and a differential interference contrast microscope (AXIO Imager

2 A1, Carl Zeiss Inc.). Quotients of spore length and width were presented as Q values, and the

3 mean value was presented as $Q_m$. After observation, sporocarps were freeze-dried, oven-dried

4 at 60 C overnight, and deposited with the Kanagawa Prefectural Museum of Natural History

5 (KPM), the Tochigi Prefectural Museum (TPM), and the National Museum of Nature and

6 Science, Tokyo (TNS).

7 ***DNA extraction and PCR amplification.***–DNA extraction from newly obtained sporocarps

8 was performed as described previously (Yamamoto et al. 2015). We also extracted DNA from

9 a relative of Endogonales, *Calcarisporiella thermophila* (Calcarisporiellaceae,

10 Calcarisporiellales, Calcarisporiellomycotina) (Hirose et al. 2014; Tedersoo et al. 2018) and

11 used previously extracted DNA samples from identified sporocarps and ectomycorrhiza

12 (Yamamoto et al. 2015, 2017a, b). PCR amplification of partial sequences of nuc 18S rDNA

13 (18S), D1–D2 domains of nuc 28S rDNA (28S), translation elongation factor 1-alpha (*tef1*),

14 and RNA polymerase II large subunit (*rpb1*), which are stable phylogenetic loci for

15 Endogonales and other zygomycetes (Tanabe et al. 2004; Desirò et al. 2017), was performed

16 using the ProFlex PCR System (Applied Biosystems, Foster City, California) with the

17 KAPA2G Robust Hotstart ReadyMix PCR kit (Kapa Biosystems, Wilmington, Massachusetts)

18 according to the manufacturer's instructions. PCR amplification (including the second round

19 of PCR) of 18S and 28S nrDNA was conducted as described in Yamamoto et al. (2015,

20 2017b). First, *tef1* and *rpb1* were amplified with the primer pairs of 983F (Carbone and Kohn

21 1999) with 2218R (Rehner and Buckley 2005) and Df [Stiller and Hall 1997; also called Dt

22 by Tanabe et al. (2004)] with G2r (5'-GGHGARCCHGCHACHCARATGAC-3':

23 http://faculty.washington.edu/benhall/), respectively. Because DNA amplification from the

24 first PCR was insufficient for sequencing of many samples, nested or semi-nested PCR was

25 conducted as described in Yamamoto et al. (2015). Amplicons from the first PCR were diluted

1 100-fold with sterile distilled water and used as template DNA for the second round of PCR

2 with the primer pairs EF-EnF1 and EF-EnR1 (or 2218R) (Yamamoto et al. 2017a) for *tef1* and

3 Df and Fr (5'-CAYGCHATGGGWGGWMGNGARGG-3': Stiller and Hall 1997) for *rpb1*.

4 PCR of *tef1* and *rpb1* was performed under the following conditions: 95 C for 2 min; 5–10

5 cycles of 95 C for 12 s, annealing at 60–65 C for 12 s (decreasing by 1.0 C per cycle), and 72

6 C for 12 s, followed by 35 cycles of 95 C for 12 s, annealing at 55 C for 12 s, and 72 C for 12

7 s. PCR amplicons were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden,

8 Germany) according to the manufacturer's instructions.

9 ***DNA sequencing.***–Sequencing reactions were performed on both the forward and reverse

10 strands of the PCR amplicons in a 10 μL reaction mixture using the BigDye Terminator v. 3.1

11 Cycle Sequencing Kit (Applied Biosystems). Sequencing of *rpb1* was performed with the

12 same primers used for PCR amplification. Primers for sequencing of 18S and 28S and *tef1* as

13 well as cycling parameters of the sequencing reactions followed the procedure described in

14 Yamamoto et al. (2017a). PCR amplicons obtained from the sequencing reaction were

15 purified with ethanol and sequenced using the ABI PRISM 3130xl genetic analyzer (Applied

16 Biosystems).

17 ***Phylogenetic analysis.***–Specimens used for phylogenetic analysis are listed in

18 SUPPLEMENTARY TABLE 1. The 96 newly obtained sequences were deposited in the DNA

19 Data Bank of Japan (DDBJ; http://www.ddbj.nig.ac.jp) and compared with known sequences

20 using BLAST. All published sequences of mycobionts of liverworts, hornworts, and ferns

21 (Bidartondo et al. 2011; Desirò et al. 2013; Field et al. 2015a, b; Rimington et al. 2015), as

22 well as fine endophytes (Orchard et al. 2017a) that reportedly belong to Endogonaceae, were

23 obtained from DDBJ and UNITE (http://unite.ut.ee) for phylogenetic analysis

24 (SUPPLEMENTARY TABLE 1). *Mortierella chlamydospora* and *M*. *verticillata*

25 (Mortierellaceae) and *C*. *thermophila* and *Echinochlamydosporium variabile*

1  (Calcarisporiellaceae) were selected as outgroup taxa.

2      Sequences of each region were aligned individually using MUSCLE (Edgar 2004) in

3  MEGA 6.06 (Tamura et al. 2013) for multi-alignment. Final alignments were adjusted

4  manually. Alignment gaps were treated as missing data and ambiguously aligned positions

5  were removed. Introns of *tef1* were also excluded from analysis. Finally, four alignments were

6  prepared (18S: 1674 sites, 28S: 811 sites, *tef1*: 983 sites, and *rpb1*: 912 sites). Because only

7  rDNA has been sequenced in many species or mycobionts of Endogonaceae, we created two

8  combined datasets including rDNA: 18S + 28S (dataset 1; 2485 sites), and 28S rDNA + *tef1* +

9  *rpb1* (dataset 2; 2706 sites). Topological conflicts were identified directly through topological

10  comparison of single-gene region trees. Two combined datasets were deposited in TreeBASE

11  (S23452). Phylogenetic analyses were conducted using the maximum likelihood (ML) and

12  maximum parsimony (MP) methods. To evaluate branch support of the resultant trees, 1000

13  replications of bootstrap (BS) analysis were conducted. ML analyses were conducted using

14  RAXMLGUI 1.31 (Silvestro and Michalak 2012) with the general time-reversible (GTR) model

15  of nucleotide substitution with a discrete gamma distribution (+G) selected by MEGA, as

16  described in Yamamoto et al. (2017a). MP analyses were performed using PAUP*, as in

17  Yamamoto et al. (2017a). The tree bisection reconnection model was adapted for MP.

18  **RESULTS**

19  *Morphological identification.*–26 specimens of putative Endogonaceae were collected from

20  various areas of the Japanese Islands. Among them, three and two specimens were identified

21  as *Jimgerdemannia flammicorona* (KPM-NC0026734, KPM-NC0026735, and

22  KPM-NC0026736) and *J. lactiflua* (KPM-NC0026737 and KPM-NC0026738), respectively

23  (SUPPLEMENTARY TABLE 1). One specimen was identified as an undescribed *Endogone*,

24  and three specimens were identified as an undescribed *Jimgerdemannia* sp. based on

25  morphology of zygosporangia. Furthermore, two undescribed chlamydosporic species (with

1    ten and seven specimens) were recognized.

2    ***Multigene phylogeny.***–55 sequences were obtained from sporocarp specimens collected in

3    this study and 41 from specimens and cultures described in previous studies from 13 species

4    of Endogonaceae and Mucoromycotina (SUPPLEMENTARY TABLE 1). Single-gene region

5    trees showed no significant conflicts between strongly supported (MLBS > 70) branches.

6    Finally, we generated two ML trees using ML analysis; i.e., dataset 1: log-likelihood =

7    −13434.566389 (FIG. 1), and dataset 2: log-likelihood = −16631.502160 (FIG. 2).

8    All Endogonaceae species formed a strongly supported clade with many mycobionts of

9    non-vascular and vascular plants (dataset 1: MLBS/MPBS = 73/73, dataset 2: MLBS/MPBS =

10    100/98; FIGS. 1, 2). A clade including the mycobiont of the liverwort genus *Neohodgsonia*

11    (9152.1-D and WR330-B) formed a sister lineage of the Endogonaceae clade (FIG. 1). In this

12    study, the former clade was defined as Endogonaceae s. str., and the taxonomic position of the

13    latter was undefined. The monophyly of Endogonales sensu Desirò et al. (2017) was

14    supported only by dataset 1 (MLBS/MPBS = 95/72).

15    In both datasets, sequences from sporocarps of Endogonaceae belonged to three clades.

16    Species with homogametic and *Youngiomyces*-type zygosporangia belonged to the genus

17    *Endogone*, and those with heterogametic zygosporangium to the genus *Jimgerdemannia*, both

18    defined by Desirò et al. (2017). However, two undescribed chlamydosporic species formed a

19    novel clade at the genus level in Endogonaceae. The sister relationship between this novel

20    clade and that including *Endogone* and mycobionts of the liverwort genus *Allisonia* (clones 2,

21    3, and 4 of MIB 8372) was strongly supported by dataset 2 (MLBS/MPBS = 100/97),

22    although this relationship was not resolved using dataset 1.

23    Environmental sequences reported as Endogonaceae mycobionts of liverworts,

24    hornworts, ferns, and seed plants (Bidartondo et al. 2011; Desirò et al. 2013; Field et al.

25    2015a, b; Rimington et al. 2015; Orchard et al. 2017a) were placed in *Endogone*,

1    *Jimgerdemannia*, and other lineages with unclear taxonomic positions, but not in the novel

2    chlamydosporic clade.

3    **TAXONOMY**

4    Endogonaceae Paol., Sylloge Fungorum 8:905. 1889, emend**.** Koh. Yamam., Degawa, & A.

5    Yamada.

6        *Description*: Sporocarp hypogeous or epigeous on litter, decayed wood, or rarely on

7    old fruiting bodies of polyporoid fungi; globose, irregular, or sometimes resupinate; generally

8    1–10 mm wide, at times composed of an aggregate of numerous zygosporangial clusters.

9    Peridium white, yellow, rarely purple, or absent. Hyphae of sporocarp tissue often aseptate,

10   sometimes secondary septa present. Reproductive structure as zygosporangia, azygosporangia,

11   or chlamydospores, numerous or rarely singly or in small numbers distributed randomly or

12   radially in sporocarps, often less than 200 μm (rarely up to 700 μm) in diameter; contents

13   yellow, uniformly granular. Zygosporangia homogametic or heterogametic, wall composed of

14   outer sporangiothecium with single or 2–4 openings and inner eusporium without openings.

15   Azygosporangia very rare, coexistent with zygosporangia, walls composed of a single layer

16   and separated from the single suspensor by a gametangial septum. Chlamydospore wall

17   continuous with wall of subtending hyphae, septa absent, walls composed of outer and inner

18   layers. Sporangiospores unknown. Includes putative plant saprotrophs, mycobionts of

19   non-vascular plants, fine endophytes, and ectomycorrhizal species.

20   *Zygosporic genera*: *Endogone* (azygosporangium formation also described), *Jimgerdemannia*,

21   *Jimwhitea* (fossil genus), *Peridiospora*, *Sclerogone*. *Chlamydosporic genera*: *Vinositunica*.

22       *Notes*: Since the emendation of Endogonales by Morton and Benny (1990), this order

23   consisted of the family Endogonaceae that included only zygosporic species until recently,

24   when the chlamydosporic Densosporaceae were included (Desirò et al. 2017). A small number

25   of azygosporangia with admixture of zygosporangia were rarely observed in the sporocarp of

1    *E. pisiformis* (Berch and Fortin 1984). In this study, we discovered a novel chlamydosporic

2    genus in the Endogonaceae clade, *Vinositunica* gen. nov., described below. Sporocarps of

3    *Vinositunica* differ morphologically from those of Densosporaceae and Glomeromycotina, as

4    described below. Additionally, numerous environmental sequences suggest that a large

5    number of Endogonales species are associated with vascular plants as fine endophytes

6    (Orchard et al. 2017a, b) or with gametophytes of non-vascular plants as mycorrhiza-like

7    symbionts (FIGS. 1 and 2). However, such environmental sequences have not yet been

8    interpreted in terms of conspecificity with any sporocarpic species.

9    *Endogone* Link, Mag Ges Naturf Freunde Berl 3:33. 1809.

10    *Type species*: *Endogone pisiformis* Link, Mag Ges Naturf Freunde Berl 3:33. 1809.

11    = *Youngiomyces* Y.J. Yao, Kew Bull 50:350. 1995.

12    *Notes*: See Desirò et al. (2017) for a morphological definition of the genus. *Endogone*

13    *botryocarpus*, described below, forms numerous zygosporangial clusters and zygosporangia

14    the sporangiothecia of which have two openings to two gametangial remnants. These are

15    characteristics of the genus *Youngiomyces*. However, *E. botryocarpus* belongs to the *E.*

16    *incrassata*–*E. oregonensis* lineage, in contrast to *Y. aggregatus* (= *E. aggregatus*), which

17    belongs to the Australian lineage along with *E. tuberculosa* (FIG. 2). *Endogone incrassata*, *E.*

18    *oregonensis*, and *E. tuberculosa* all form a typical homogametic zygosporangia (Gerdemann

19    and Trappe 1974; Yao et al. 1996). Therefore, *Youngiomyces*-type species are polyphyletic.

20    This result supports synonymization of *Youngiomyces* with *Endogone* by Desirò et al. (2017).

21    *Endogone* includes putative ectomycorrhizal species, e.g., *E. aggregata*, *E. tuberculosa*

22    (Warcup 1990), *E. oregonensis* (Gerdemann and Trappe 1974), and an undescribed

23    ectomycorrhizal species (Yamamoto et al. 2017b) (FIGS. 1, 2), along with putative

24    saprotrophs, e.g., *E. pisiformis*, that form sporocarps under axenic conditions without a host

25    plant (Berch and Castellano 1986). In addition several environmental sequences from

1    mycobionts of liverworts and hornworts are included in several lineages in *Endogone* (FIGS.

2    1, 2).

3    ***Endogone botryocarpus*** Koh. Yamam., Degawa & A. Yamada, **sp. nov.** FIG. 3

4    MycoBank: MB829983

5        *Typification*: JAPAN. NAGANO: Ueda-shi, Sugadaira Research Station, Montane

6    Science Center (N 36° 31' 15", E 138° 20' 57"), ca. 1330 m a.s.l., on underside of decayed

7    twigs of *Pinus densiflora* on a forest floor dominated by secondary established *P. densiflora*

8    and *Quercus crispula*, 24 Aug 2014, *K. Yamamoto E-14001* (**holotype** KPM-NC0026731;

9    **isotype** TNS-F-70439). DNA sequences ex-holotype: 18S = LC431079; 28S = LC431095;

10   *tef1* = LC431111.

11       *Etymology*: *botryocarpus* (Greek), in reference to sporocarps formed as aggregates of

12   numerous zygosporangial clusters.

13       *Diagnosis*: Zygosporangia much smaller than those of all other species of

14   Endogonaceae and consistently with two openings to gametangial remnants.

15       *Description*: Sporocarp attached to underside of decayed pine twig on the forest floor;

16   flattened, 1.1–4.4 mm long; composed of an aggregate of numerous small globose to

17   subglobose sporocarps; i.e., zygosporangial clusters, 135–144 μm wide; surface smooth,

18   dingy yellow, white in arid conditions; cut surface dingy yellow, without exudation of latex,

19   not containing foreign matter; strongly adhered to substrates with thick-walled hyphae, these

20   up to 16 μm wide, wall up to 2 μm thick, often aseptate. Peridium persistent, well-developed,

21   single-layered, colorless, 13–30 μm thick; composed of tightly woven thick-walled hyphae

22   3–10 μm wide, wall not exceeding 2 μm thick, aseptate. Gleba composed of densely packed

23   zygosporangia and thick- and thin-walled hyphae; thick-walled hyphae colorless, 3–8 μm

24   wide, often aseptate, wall up to 3 μm thick; thin-walled hyphae mostly collapsed in fully

25   developed sporocarps, colorless, about 1–2 μm wide, often aseptate, wall up to 0.5 μm thick.

1 Zygosporangia variable in shape, often broadly ellipsoidal or oblate to oval, sometimes

2 irregular, (15–)18–29(–31) μm long, (12–)14–32 μm wide, mean of 22.5 × 19 μm (Q =

3 0.47–1.83, $Q_m$ = 1.23, n = 36), pale yellow. Sporangiothecia 0.5–1 μm thick, pale yellow,

4 surface smooth; two openings to two gametangial remnants, 1.5–3.5 μm wide, distance

5 between openings 2.5–4.5 μm. Eusporia 2–3 μm thick, thicker than sporangiothecia; dull

6 whitish. Zygosporic contents uniformly granular, up to 2 μm wide, yellowish white.

7 Gametangial remnants two per zygosporangium, discontiguous, equal in size, cylindrical,

8 empty, collapsed after maturation. Suspensors thin-walled, collapsed after maturation. In

9 Melzer's reagent, peridial and glebal thick-walled hyphae orange-brown or reddish-brown

10 (dextrinoid) and partially bluish-purple (amyloid); sporangiothecia weak orange-brown

11 (dextrinoid); eusporia showed almost no staining (inamyloid). Odor not distinctive.

12 *Ecology and distribution*: Known only from the type locality, Japan. Found in summer.

13 *Notes*: Zygosporangial clusters (FIG. 3A, B) and sporangiothecia with two openings to

14 gametangial remnants (FIG. 3H–K) are good characters of *E. botryocarpus*. These characters

15 are also observed in *E. aggregata*, *E. carolinensis*, and *E. multiplex*, formerly classified in

16 *Youngiomyces* (Yao et al. 1995). Zygosporangia of *E. botryocarpus* are much smaller (ca.

17 15–30 μm) than those of the similar species noted above (40–200 μm; Yao et al. 1996).

18 Although *E. oregonensis* forms a zygosporangial cluster, the sporangiothecia have a single

19 opening, and the zygosporangia are large (ca. 50–150 μm) (Gerdemann and Trappe 1974).

20 *Endogone verrucosa* also forms zygosporangial clusters with small zygosporangia (ca. 25–60

21 μm; Gerdemann and Trappe 1974), but the zygosporangia are larger than those of *E.*

22 *botryocarpus*. Zygosporangia of *E. botryocarpus* are the smallest in Endogonaceae.

23 The habitat of *E. botryocarpus* is similar to that of the phylogenetically close *E.*

24 *incrassata* (FIGS. 1, 2). Although the trophic mode of *E. incrassata* is unknown, an

25 environmental DNA sequence of a liverwort mycobiont, *Haplomitrium gibbsiae* (MIB 8360),

was included in a clade with this species (FIG. 1). *Endogone oregonensis* is closely related to *E. botryocarpus* (FIG. 2) and is suggested to be an ectomycorrhizal mycobiont of *Pseudotsuga* (Gerdemann and Trappe 1974). Although we inoculated sporocarp fragments of *E. botryocarpus* on Modified Norkrans's C agar medium (Yamada and Katsuya 1995) and into the rhizosphere of *P. densiflora*, no hyphal growth was observed (data not shown).

*Jimgerdemannia* Trappe, Desirò, M.E. Sm., Bonito & Bidartondo, IMA Fungus 8:249. 2017.

*Type species*: *Jimgerdemannia flammicorona* (Trappe & Gerd.) Trappe, Desirò, M.E. Sm., Bonito & Bidartondo, IMA Fungus 8:251. 2017.

*Notes*: See Desirò et al. (2017) for the generic concept of *Jimgerdemannia*. According to their phylogenetic analysis, *Jimgerdemannia* is composed of two subclades: the *J. flammicorona–J. lactiflua* subclade and a subclade of unidentified sporocarps and mycobionts of bryophytes. Heterogametic zygosporangium formation, a prominent characteristic of the genus, was previously recorded only in *J. lactiflua* and *J. flammicorona*. Desirò et al. (2017) included two unidentified sporocarps from Australia in their phylogenetic analyses identified as *Jimgerdemannia* sp. (T34745-A and T34745-B) without description of zygosporangial morphology. These specimens belong to the latter subclade described above. In the present study, the new species described below as *J. ambigua* was phylogenetically located in the latter subclade using dataset 2 (FIG. 2), and it shared a characteristic with known *Jimgerdemannia* spp.; i.e., heterogametic zygosporangium formation. Therefore, our study supports *Jimgerdemannia* clade sensu Desirò et al. (2017). Additionally, a sequence of *Endogone* sp. (W5994) that was included in the phylogenetic analysis of Desirò et al. (2013) but not that of Desirò et al. (2017) belonged to the *Jimgerdemannia* clade (FIG. 1). Clarification of its zygosporangial morphology is needed.

The *J. flammicorona–J. lactiflua* clade includes ectomycorrhizal mycobionts of Pinaceae. These ectomycorrhizae form a Hartig net but lack a distinct fungal sheath (Fassi

1    1965; Walker 1985) and are clearly distinguishable from those of *Endogone* (Yamamato et al.

2    2017b). Recent research has suggested that *J. flammicorona* and *J. lactiflua* increased their

3    whole genome sizes with numerous transposable elements but reduced the number of

4    enzymes that degrade plant cell walls (Chang et al. 2018), similar to ectomycorrhizal fungi in

5    Dikarya (Martin et al. 2008; Kohler et al. 2015; Peter et al. 2016). The above mentioned latter

6    subclade of *Jimgerdemannia* is composed mostly of mycobionts of liverworts and hornworts

7    (FIGS. 1, 2). Field et al. (2015a) successfully isolated a mycobiont (WR322) from the

8    liverwort *Treubia lacunosa* (Haplomitriopsida) and confirmed the status as a mycorrhiza-like

9    association through inoculation to axenic *Haplomitrium gibbsiae*. This isolate belonged to the

10   latter subclade (FIG. 1).

11   ***Jimgerdemannia ambigua*** Koh. Yamam., Degawa & A. Yamada, **sp. nov.** FIG. 4

12   MycoBank: MB829984

13       *Typification*: JAPAN. SHIGA: Takashima-shi, Kutsuki Ikimono Hureai no Sato (N 35°

14   20' 19", E 135° 55' 11"), ca. 270 m a.s.l., hypogeous in a secondary forest dominated by

15   *Quercus serrata*, with sparse growth of *Cryptomeria japonica* and *Abies firma*, 24 Nov 2014,

16   *K. Yamamoto G-14001* (**holotype** KPM-NC0026732). DNA sequences ex-holotype: 18S =

17   LC431080; 28S = LC431096; *tef1* = LC431112.

18       *Etymology*: From the Latin *ambigua* (= obscure), referring to the nearly undeveloped

19   spore mantle, which is unusual in *Jimgerdemannia*.

20       *Diagnosis*: This species forms a heterogametic zygosporangium, which is a striking

21   character of *Jimgerdemannia,* but lacks the striking spore mantle formation known in other

22   species; i.e., *J. lactiflua* and *J. flammicorona*.

23       *Description*: Sporocarp hypogeous; subglobose; 5–8 mm wide; surface smooth, white

24   or partially pale yellowish-brown, zygosporangia partially visible from the outside; cut

25   surface pale yellow in immature stage, later forming reddish-orange tinge, without exudation

1 of latex, not containing foreign matter. Peridium weakly developed or indistinguishable from

2 glebal tissue, 49–75 µm thick; composed of loosely-woven thin-walled hyphae, 1–4 µm wide,

3 wall not exceeding 1 µm thick, often aseptate. Gleba packed with zygosporangia and thin- or

4 somewhat thick-walled hyphae; hyphae colorless, 1.5–5.5 µm wide, often aseptate, wall

5 0.5–1.5 µm thick. Zygosporangia irregularly distributed, subglobose, (48–)50.5–79(–83.5) µm

6 long, (50.5–)53–71.5(–76) µm wide, mean $67 \times 62.5$ µm ($Q = 0.94$–$1.22$, $Q_m = 1.07$, n = 18),

7 pale reddish-brown. Sporangiothecia 0.5–2.5 µm thick, pale reddish-brown; reticulate sulcus

8 present on surface after detachment of zygosporangial hyphal mantle; single opening to

9 remnant of macrogametangium, 5.5–6.5 µm wide. Zygosporangial hyphal mantle 11.5–26.5

10 µm thick, no specific pattern present, readily detaches from sporangiothecium after

11 maturation; composed of up to four layers of loosely-woven hyphae 6–14 µm wide, often

12 aseptate, thin-walled when immature and thick-walled when mature, wall 0.5–4 µm thick

13 thickness. Eusporia 3.5–6 µm thick, thicker than the sporangiothecia; dull white. Zygosporic

14 contents uniformly granular, 2.5–4.5 µm wide, yellowish-white. Gametangial remnants two

15 per zygosporangium, unequal in size, contiguous, subglobose or irregular, empty, 19.5–36 µm

16 long; wall thickening gradually from the sporangiothecium up to 2 µm thick. Suspensors

17 mostly collapsed. In Melzer's reagent, peridial and glebal hyphae and zygosporangial hyphal

18 mantle stained reddish-brown (dextrinoid) and peridial hyphae partially bluish-purple

19 (amyloid); sporangiothecia stained yellowish-brown (dextrinoid); eusporia showed almost no

20 staining (inamyloid). Odor not distinctive.

21 *Ecology and distribution*: Hypogeous under warm temperate forests dominated by

22 *Quercus* along with *Cryptomeria*, *Carpinus*, and *Abies* in the western region of Honshu Island,

23 Japan. Found in autumn.

24 *Other specimens examined*: JAPAN. SHIGA: Takashima-shi, same locality as holotype

25 specimen, 23 Nov 2015, *K. Yamamoto G-15002* (TNS-F-70440); Nagahama-shi, near

1 Yamakado-shitsugen (N 35° 33' 33", E 136° 07' 11"), ca. 320 m a.s.l., hypogeous under a

2 secondary stand dominated by *Quercus acuta*, *Q. salicina*, *Carpinus tschonoskii*, and *Q.*

3 *serrata* established at the base of a sandy slope, 3 Oct 2015, *K. Yamamoto G-15001*

4 (KPM-NC0026733).

5     *Notes*: This species is indistinguishable from *J. flammicorona* based on sporocarp

6 morphology (FIG. 4A) and size of heterogametic zygosporangium (FIG. 4 G, H). However, the

7 zygosporangial hyphal mantle (FIG. 4E) lacks a specific hyphal arrangement pattern such as

8 the spiral (*J. flammicorona*) or labyrinth-like (*J. lactiflua*) patterns (Yamamoto et al. 2015)

9 and easily detaches from the sporangiothecium after zygosporangium maturation (FIG. 4F, H);

10 the latter finding is in contrast to *J. flammicorona* and *J. lactiflua*. After detachment of the

11 hyphal mantle, a reticulate-labyrinthiform sulcus is observed on the surface of the

12 sporangiothecium (FIG. 4F). Although the phylogenetic position of *J. ambigua* differs

13 between datasets 1 and 2, this species is clearly separated from the *J. flammicorona*–*J.*

14 *lactiflua* clade in both cases (FIGS. 1 and 2). Consequently, *J. ambigua* was regarded as an

15 independent species.

16     Incidentally, *Endogone alba*, which is putatively zygosporic with an uncertain

17 phylogenetic position, is morphologically quite similar to *J. ambigua*. *Endogone alba* was

18 initially described as a species of *Sclerocystis* (Glomerales, Glomeromycotina) based on a

19 specimen from Sri Lanka (Petch 1925). However, several aspects of spore morphology; i.e.,

20 uniformly granular spore contents (Gerdemann and Trappe 1974) and the inner wall of the

21 spore lacking an opening (Yao et al. 1992), strongly suggest that this species has a zygosporic

22 nature despite gametangial conjugation not being observed. Gerdemann and Trappe (1974)

23 transferred this species to *Endogone*. *Endogone alba* produces white sporocarps in which

24 putative zygosporangia are randomly formed: zygosporangia globose to oval, 72–106 ×

25 62–98 μm; sporangiothecia minutely rugulose or reticulate, yellowish to reddish brown, 0.5–2

1 μm thick; eusporia pale yellowish, 3.5–6 μm thick; single opening of sporangiothecium, 5–6

2 μm wide (Petch 1925; Yao et al. 1992). Therefore, although the zygosporangia of *E. alba* are

3 significantly larger than those of *J. ambigua*, these species share similar zygosporangium

4 characteristics. On the other hand, *E. alba* resembles several *Endogone* species formerly

5 placed in *Youngiomyces*, as its sporocarp is composed of zygosporangial cluster (Yao et al.

6 1992). Hence, observation of gametangia is necessary to clarify whether *E. alba* belongs to

7 *Jimgerdemannia*.

8 Species related to *J. ambigua*; i.e., *J. flammicorona* and *J. lactiflua*, are ectomycorrhizal

9 mycobionts specific to Pinaceae (Tandy 1975b; Walker 1985; Warcup 1990). On the other

10 hand, the habitats of *J. ambigua* (KPM-NC0026733, KPM-NC0026732, and TNS-F-70440)

11 were oak-dominated forests. Therefore, *J. ambigua* is suggested to have ectomycorrhizal

12 association with fagaceous trees.

13 ***Vinositunica*** Koh. Yamam., Degawa & A. Yamada, **gen. nov.**

14 MycoBank: MB829985.

15 *Type species*: *Vinositunica radiata* Koh. Yamam., Degawa & A. Yamada, sp. nov.

16 *Etymology*: *Vinositunica* (Latin), in reference to the characteristic wine-colored

17 pigmentation on the peridium and outer wall of the chlamydospores.

18 *Diagnosis*: Describe how it differs from other genera.

19 *Description*: Sporocarp epigeous on soil surface or semi-hypogeous; reniform or

20 irregular, short stipe-like sterile base often present; 2–20 mm wide. Peridium white and

21 partially purple; single layer, sometimes indistinguishable from glebal tissue; composed of

22 thick- or thin-walled aseptate hyphae. Gleba pale yellow or purplish-grey; composed of

23 numerous chlamydospores and thick- or thin-walled aseptate hyphae. Chlamydospore radially

24 or randomly distributed in sporocarp; terminal on single subtending hypha; broadly

25 ellipsoidal; 50–700 μm in diameter; wall composed of brownish-purple or red-wine colored

1    outer layer and colorless inner layer; contents yellow, uniformly granular.

2    *Notes*: *Vinositunica* is the only genus in Endogonaceae that forms chlamydospores but

3    lacks an observation of sexual reproduction. *Sphaerocreas* and *Densospora* (Densosporaceae,

4    Endogonales) resemble *Vinositunica* in the formation of chlamydospores and the lack of a

5    sexual stage. However, these genera have colorless chlamydospore walls (McGee 1996;

6    Yamamoto et al. 2015). Although Glomerales and Diversisporales in Glomeromycotina are

7    also chlamydosporic and include sporocarpic species (Gerdemann and Trappe 1974; Yao et al.

8    1996; Błaszkowski 2012), no species in those taxa shows purplish colored sporocarp tissue,

9    dark red-wine colored spore wall, and yellowish intracellular contents of spores, with two

10   exceptions discussed below. The dark spore wall of *Vinositunica* suggests deposition of

11   sporopollenin, which has been found in the spore walls of Glomeromycotina (Bianciotto and

12   Bonfante 1999) and Mucorales (Gooday et al. 1973) and has the ability to defend against

13   biological degradation and chemical stressors (Gooday et al. 1973; Bianciotto and Bonfante

14   1999).

15   At present this genus includes only two newly described species from Japan. Although

16   *V. radiata* consistently occurs under ectomycorrhizal trees, no mycorrhizae of *Vinositunica*

17   were found, and no plant mycobiont environmental sequences were included in the

18   phylogenetic clade of this genus (FIGS. 1, 2). Therefore, it is necessary to determine whether

19   *Vinositunica* forms ectomycorrhizal associations by field sampling and mycorrhizal synthesis,

20   as do several species of *Endogone* and *Jimgerdemannia* (e.g., Warcup 1990; Yamamoto et al.

21   2017b).

22   ***Vinositunica radiata*** Koh. Yamam., Degawa & A. Yamada, **sp. nov.** FIG. 5

23   MycoBank: MB829986

24   *Typification*: JAPAN. OKINAWA: Okinawa-jima Isl., Kunigami-son, Mt. Nishimedake

25   (N 26° 48' 02", E 128° 16' 32"), ca. 370 m a.s.l., epigeous in a forest of *Castanopsis sieboldii*

1  subsp. *lutchuensis* with sparse *Quercus miyagii*, 14 Oct 2013, *K. Yamamoto B-13004*

2  (**holotype** KPM-NC0026742). DNA sequences ex-holotype: 18S = LC431090; 28S =

3  LC431106; *tef1* = LC431122; *rpb1* = LC431146.

4  *Etymology*: From the Latin *radiata* (= radiate), referring to the radial spore arrangement.

5  *Diagnosis*: The combination of a short sporocarp stipe and radial arrangement of

6  red-wine colored chlamydospores is not observed in other Endogonales species. Although

7  radial arrangement of dark colored chlamydospores is a characteristic of *Glomus cuneatum*

8  sporocarps, the spore size of this species differs from that of *V. radiata*.

9  *Description*: Sporocarp epigeous on the bare soil surface of the forest floor; reniform to

10  pulvinate; 1.3–2.5 mm wide, 0.8–1.8 mm high; short stipe-like sterile base often present,

11  0.7–1.0 mm wide, 0.2–0.5 mm high; surface tomentose, white or pale yellow in immature

12  stage, pale greyish-purple when mature; cut surface white in immature, greyish-purple in

13  mature, not exuding latex, and not containing foreign matter. Peridium persistent,

14  single-layered, white to pale greyish-purple, 78.5–125 µm thick; composed of loosely woven,

15  somewhat fastigiate, trichoderm-like hyphae, 1.5–4 µm wide, somewhat thick-walled up to 1

16  µm thick, often aseptate. Gleba develop on periphery of the central sterile region of sporocarp,

17  densely packed with chlamydospores; hyphae colorless, with radial arrangement from sterile

18  region to surface, 1–5 µm wide, often aseptate, slightly thick-walled, up to 1.5 µm thick.

19  Chlamydospore radially distributed, often broadly ellipsoidal or globose to ellipsoidal,

20  (67–)68–85(–92.5) µm long, (49–)52.5–69(–73) µm wide, mean size of 75.5 × 62 µm (Q =

21  1–1.4, $Q_m$ = 1.23, n = 21), surface smooth, pale yellow when immature, dark red-wine color

22  when mature; wall composed of two layers; i.e., an outer dark red-wine colored layer 1.5–3

23  µm thick, and an inner colorless layer that is thicker than the outer layer, 4.5–5 µm thick;

24  boundary between chlamydospore and subtending hyphae occluded by wall thickening.

25  Chlamydosporic contents uniformly granular, up to 6.5 µm wide, yellow. Subtending hyphae

single, tenacious, same color as outer layer of chlamydospore wall, 4.5–8.5 μm wide. In

Melzer's reagent, peridial and glebal hyphae stained reddish-brown (dextrinoid); outer layer

of chlamydospore stained dark reddish-brown (dextrinoid); inner layer of chlamydospore

showed almost no staining (inamyloid). Odor not distinctive.

*Ecology and distribution*: Epigeous on the ground of clay soil in forests dominated by

*Castanopsis sieboldii* in a warm temperate–subtropical climate on the western part of Honshu

Island and the Nansei Islands, Japan. Found in summer to autumn.

*Other specimens examined*: JAPAN. OKINAWA: Iriomote-jima Isl., Taketomi-cho,

Urauchi-gawa River (N 24° 21' 42", E 123° 47' 53"), ca. 50 m a.s.l., on the ground of a

roadside neighboring a *C. sieboldii* subsp. *lutchuensis* forest, 28 Jun 2018, *K. Yamamoto

B-18001* (KPM-NC0026745); Ishigaki-jima Isl., Ishigaki-shi, at the foot of Mt. Nosokodake

(N 24° 29' 32", E 124° 14' 37"), ca. 15 m a.s.l., on the ground under a young *C. sieboldii*

subsp. *lutchuensis* tree in a secondary forest dominated by non-ectomycorrhizal *Adinandra

yaeyamensis* and *Bischofia javanica*, 11 Oct 2013, *K. Yamamoto B-13002*

(KPM-NC0026740); Okinawa-jima Isl., Kunigami-son, Mt. Yonahadake (N 26° 43' 45", E

128° 12' 48"), ca. 330 m a.s.l., semi-hypogeous in a forest dominated by *C. sieboldii* subsp.

*lutchuensis* with sparse *Quercus miyagii*, 13 Oct 2013, *K. Yamamoto B-13003*

(KPM-NC0026741); same locality, epigeous in a forest dominated by *C. sieboldii* subsp.

*lutchuensis*, with sparse *Q. miyagii*, 13 Nov 2013, *T. Orihara B-13005* (KPM-NC0026743);

same locality as holotype specimen, 31 Aug 2014, *K. Yamamoto B-14002*

(KPM-NC0023961); Okinawa-jima Isl., Kunigami-son, Jashiki (N 26° 46' 05", E 128° 13'

44"), ca. 150 m a.s.l., epigeous in a forest dominated by *Q. miyagii* and *C. sieboldii* subsp.

*lutchuensis*, 31 Aug 2014, *K. Yamamoto B-14003* (KPM-NC0023962; duplicate

TNS-F-70441); Okinawa-jima Isl., Kunigami-son, Mt. Ibudake (N 26° 45' 27", E 128° 17'

41"), ca. 170 m a.s.l., on the ground of a roadside under a young *C. sieboldii* subsp.

1  *lutchuensis* tree, 1 Sep 2014, *K. Yamamoto & H. Masuya B-14004* (KPM-NC0023963;

2  duplicate TNS-F-70442). KAGOSHIMA: Amami-ohshima Isl., Amami-shi, Kinsakubaru (N

3  28° 20' 35", E 129° 27' 17"), ca. 200 m a.s.l., on the eroded ground of a riverside under a *C.*

4  *sieboldii* subsp. *lutchuensis* tree, 27 Jun 2014, *K. Yamamoto B-14001* (KPM-NC0026744).

5  KYOTO: Fukuchiyama-shi, Naiku, Koudai-jinja (N 35° 25' 51", E 135° 09' 16"), ca. 115 m

6  a.s.l., on the ground in a climax forest of *C. sieboldii*, 15 Jul 2013, *K. Yamamoto B-13001*

7  (KPM-NC0026739).

8      *Notes*: Whitish immature sporocarps contained colorless chlamydospores with

9  yellowish oil globules (FIG. 5C). Outer wall of chlamydospores and sporocarp tissue when

10  mature are dark red-wine in color (FIG. 5D, H, I) and pale purple (FIG. 5A, B), respectively. A

11  short stipe-like sterile base was frequently observed (FIG. 5B, C), which is rare in

12  Endogonaceae. Radial arrangement of spores (FIG. 5B) was characteristic of *V. radiata*.

13  *Glomus cuneatum* described from Australia (McGee and Trappe 2002) shares the following

14  characteristics with *Vinositunica*, in particular *V. radiata*: stipe-like sterile base present in

15  sporocarp; spores radially arranged; spore wall blackish at outer surface and hyaline at inner

16  surface; and yellowish viscid fluid is released when sporocarp was sectioned. However, *G.*

17  *cuneatum* clearly differs from the two species of *Vinositunica* described in this study in that

18  the chlamydospores of *G. cuneatum* are ovoid, clavate, or irregular in shape and $70 \times 70\text{–}120$

19  $\times 180$ μm in size, and its sporocarp is fragmented into cuneate segments (McGee and Trappe

20  2002). *Glomus radiatum*, which has an uncertain position within Glomeromycotina (Schüßler

21  and Walker 2010), also forms chlamydospores in a radial arrangement in the sporocarp.

22  However, the spore wall of this species is light yellow, not red-wine colored (Thaxter 1922;

23  Gerdemann and Trappe 1974; Berch and Fortin 1984; Yamamoto et al. 2019). Although

24  *Endogone acrogena* in Endogonaceae also shows radially arranged spores, this species is

25  zygosporic and forms yellowish sporocarps (Gerdemann and Trappe 1974).

1     Sporocarps were often found on the bare ground of clayey soils along trails in forests of

2     *C. sieboldii*. Thus, *V. radiata* is likely an ectomycorrhizal mycobiont of *Castanopsis*.

3     ***Vinositunica ingens*** Koh. Yamam., Degawa & A. Yamada, **sp. nov.** FIG. 6

4     MycoBank: MB829987

5     *Typification*: JAPAN. SHIGA: Nagahama-shi, near Yamakado-shitsugen (N 35° 33' 34",

6     E 136° 07' 12"), ca. 320 m a.s.l., on the ground of a trail in a secondary forest dominated by

7     *Quercus acuta*, *Q. salicina*, *Carpinus tschonoskii*, and *Q. serrata*, 3 Oct 2015, *K. Yamamoto*

8     *F-15002* (**holotype** KPM-NC0026748). DNA sequences ex-holotype: 18S = LC431094; 28S

9     = LC431109; *tef1* = LC431125; *rpb1* = LC431148.

10     *Etymology*: From the Latin *ingens* = huge, referring to the characteristic of quite large

11     chlamydospores.

12     *Diagnosis*: Extremely large, dark red-wine colored chlamydospore differs from those of

13     other sporocarpic Mucoromycotina and Glomeromycotina species.

14     *Description*: Sporocarp semi-hypogeous, subglobose to irregular, 8–11 mm wide;

15     surface smooth, weakly tomentose, sometimes verrucose due to exposure of the

16     chlamydospore surface, dull white when immature, partially stained purple when mature; cut

17     surface dark greyish-purple, greyish-yellow, or yellowish-white, exuding yellowish creamy

18     latex, sometimes containing foreign matter such as leaf litter. Peridium almost absent; hyphae

19     of sporocarp surface colorless, 3–14 μm wide, thin walls up to 1 μm thick, often aseptate.

20     Gleba generally develop in the upper half of the sporocarp, and chlamydospores are absent or

21     present in very small numbers in the lower half; hyphae colorless, almost collapsed when

22     mature, but a few prominently thick hyphae present, 4–32.5 μm wide, often aseptate,

23     sometimes thick-walled, up to 4 μm thick. Chlamydospore randomly distributed, often

24     broadly ellipsoidal or oblate spheroidal to ellipsoidal, (533–)538–713(–747) μm long,

25     (379–)423–599(–619) μm wide, mean size of 623 × 506 μm (Q = 0.94–1.6, $Q_m$ = 1.25, n =

1   20), surface smooth, dark red-wine color when mature; wall composed of two layers; i.e., an

2   outer dark red-wine colored layer, 15–19.5 μm thick, and an inner colorless layer, 13–24 μm

3   thick; boundary between chlamydospore and subtending hyphae occluded by wall thickening.

4   Chlamydosporic contents often uniformly granular up to 20 μm wide, or sometimes a single

5   larger droplet ca. 200 μm wide, yellow. Subtending hyphae single, rarely up to triple,

6   tenacious, elongated from sterile region of sporocarp, same color as outer layer of

7   chlamydospore wall, 26–42 μm wide. In Melzer's reagent, peridial and glebal hyphae stained

8   reddish-brown (dextrinoid); outer layer of chlamydospore stained dark reddish-brown

9   (dextrinoid); inner layer of chlamydospore showed almost no staining (inamyloid). Odor

10   seaweed-like or unpleasant fishy smell present in fully mature sporocarps.

11   *Ecology and distribution*: Epigeous on the ground or hypogeous under ectomycorrhizal

12   trees such as those of *Quercus*, *Carpinus*, and *Pinus* in temperate areas of the western or

13   north-eastern region of Honshu Island, Japan. Found in autumn.

14   *Other specimens examined*: JAPAN. SHIGA: Nagahama-shi, same locality as the

15   holotype specimen, 11 Oct 2010, *K. Yamamoto F-10001* (KPM-NC0026746); Higashiomi-shi,

16   Inokoyama Park (N 35° 10' 31", E 136° 09' 57"), ca. 95 m a.s.l., on the roadside on a slope

17   covered with *Quercus* spp. and planted *Prunus* sp., *Camellia sasanqua*, and *Chamaecyparis*

18   *obtusa*, 12 Oct 2018, *H. Miwa F-18001* (KPM-NC0026749; duplicate TNS-F-70444).

19   MIYAGI: Sendai-shi, Futakuchi-kyokoku (N 36° 16', E 140° 32'), hypogeous under a *Fagus*

20   *crenata* tree, 20 Sep 2014, *S. Wada F-14001* (KPM-NC0026747). SAITAMA:

21   Namegawa-machi, Musashi-Kyuryo National Government Park (N 36° 04' 11", E 139° 21'

22   55"), ca. 60 m a.s.l., hypogeous under a *Pinus densiflora* tree, 26 Sep 2015, *M. Nakajima*

23   *F-15001* (KPM-NC-0024500; duplicate TNS-F-70443). TOCHIGI: Utsunomiya-shi, Nagaoka

24   Park (N 36° 35' 19", E 139° 52' 55"), ca. 150 m a.s.l., semi-hypogeous on the roadside in a

25   forest of *Castanea crenata*, *Carpinus tschonoskii*, *Q. serrata*, *Cryptomeria japonica*, 14 Oct

1  2018, *K. Yamamoto F-18002* (KPM-NC0026750; duplicate TPM-M-9087); Nasu-machi, Iono,

2  Iono-shiroyama Park (N 36° 57' 36", E 140° 09' 29"), ca. 250 m a.s.l., semi-hypogeous under

3  a young *Quercus myrsinifolia* stand grown at the border of a planted *Cryptomeria japonica*

4  forest, 25 Oct 2018, *K. Yamamoto F-18003* (KPM-NC0026751).

5      *Notes*: *Vinositunica ingens* is easily distinguishable from *V. radiata* based on sporocarp

6  morphology and chlamydospore size. In addition, *V. ingens* shows greater variation in the

7  hyphal width of the sporocarp (FIG. 6G, H) than *V. radiata* (FIG. 5F, G), and most hyphae of

8  this species tend to collapse in mature sporocarps. On the other hand, these two species share

9  common characteristics; i.e., purplish sporocarps (FIGS. 5A, B and 6A) and double-layered

10  red-wine colored chlamydospore walls (FIGS. 5H, I and 6I, J), both of which are regarded as

11  diagnostic of this genus. As shown in FIG. 6K, only one chlamydospore was connected to

12  three subtending hyphae. However, this spore was considered to be an abnormal

13  chlamydospore, not a zygosporangium, because the wall of this spore was continuous with the

14  wall of the subtending hyphae. Chlamydospores with multiple subtending hyphae have also

15  been described in Glomeromycotina; e.g., in *Glomus multicaulis*, *Glomus lacteus*,

16  *Funneliformis mosseae*, and *Rhizophagus fasciculatus* (Gerdemann and Trappe 1974;

17  Gerdemann and Bakshi 1976; Rose and Trappe 1980). Multiple subtending hyphae might

18  result from intercalary development of the chlamydospore.

19      *Glomus melanosporum* described from the USA forms large sporocarps (ca. 1 cm wide)

20  containing dark reddish-brown chlamydospores (Gerdemann and Trappe 1974). Although the

21  spore size (166–277 × 129–244 μm) is significantly smaller than that of *V. ingens*, several

22  characteristics of *G. melanosporum* and this species are similar: hypogeous under pinaceous

23  trees; peridium absent; gleba containing foreign matter, exuding creamy latex when cut; spore

24  wall dark reddish-brown at the outer surface and light yellow or sub-hyaline near the inner

25  surface (Gerdemann and Trappe 1974). Therefore, *G. melanosporum* and *G. cuneatum*, as

1  described in the note for *V. radiata* above, are suggested to belong to *Vinositunica*. Many

2  sporocarpic species described in Thaxter (1922) and later studies have been provisionally

3  placed in Glomeromycotina (Schüßler and Walker 2010), but their phylogenetic positions

4  have remained unresolved. As we first confirmed the chlamydosporic species in

5  Endogonaceae, the phylogenetic positions of these sporocarpic species in Glomeromycotina

6  should be clarified in future studies.

7      The habitat of *V. ingens* is mostly in ectomycorrhizal tree forests; i.e., those of Fagaceae,

8  Betulaceae, and Pinaceae, similar to the habitat of *V. radiata* described above. Therefore,

9  *Vinositunica* is a possible ectomycorrhizal taxon.

**DISCUSSION**

11      We provide the first description of a putative asexual chlamydosporic lineage,

12  *Vinositunica*, in Endogonaceae. Thus, both Endogonaceae and Densosporaceae within

13  Endogonales are characterized by chlamydospore formation. Two species of *Vinositunica*; i.e.,

14  *V. radiata* and *V. ingens*, form chlamydospores that exhibit dark cell walls, and this color was

15  stable even when mounted with lactic acid on slides (FIGS. 5H, I, and 6I, J, L, M). By

16  contrast, the chlamydospores of Densosporaceae have colorless cell walls and exhibit frequent

17  degeneration of wall structure under acidic or alkaline conditions on slides (Tandy 1975a;

18  McGee 1996). This difference suggests that ecophysiological differences between the

19  chlamydospores of those two families may exist.

20      Zygosporangia of all described *Jimgerdemannia* spp. are covered with a prominent

21  zygosporangial hyphal mantle, which likely has a protective function (Bonfante-Fasolo and

22  Scannerini 1976; Yamamoto et al. 2015; FIG. 4E, I, J). Sporocarps of *Jimgerdemannia*

23  develop a weak peridium (Gerdemann and Trappe 1974; FIG. 4A, B) in contrast to *Endogone,*

24  which generally has a well-developed peridium (Yao et al. 1996; Yamamoto et al. 2015; FIG.

25  3F), likely to protect the gleba. The sporangiothecia of *Jimgerdemannia* are more pigmented

1   than that of *Endogone* (Yamamoto et al. 2015; FIG. 4F) likely to protect the zygosporangium.

2   Thus, these two genera may have developed different protective features in their sporocarps

3   and zygosporangia.

4   According to Yao et al. (1996), sporocarps of *Youngiomyces* form clusters of numerous

5   zygosporangia. Such zygosporangial clusters enveloped by thick hyphae (Yao et al. 1996)

6   were also observed in a new *Youngiomyces*-type species, *E. botryocarpus* (FIG. 3A, B, F), and

7   in the non-*Youngiomyces*-type species *E. oregonensis* and *E. verrucosa* (Gerdemann and

8   Trappe 1974). Although no zygosporangial clusters were observed in *E. corticioides*

9   (Yamamoto et al. 2017a) or *E.* aff. *pisiformis* collected in the United States (Yamamoto et al.

10  2015), thick-walled hyphal bundles extend throughout the gleba from the base of the

11  sporocarp in these specimens. Their thick-walled hyphae are similar to the hyphae that

12  envelope the zygosporangial cluster in *E. botryocarpus* and *E. carolinensis* (= *Y. carolinensis*)

13  (FIG. 3F, G; Yao et al. 1996). It is inferred that during the primary stage of the evolution of

14  sporocarpic *Endogone* from its non-sporocarpic ancestor, a small zygosporangial mass

15  including tens of zygosporangia developed, which were subsequently gathered into

16  zygosporangial clusters and the hyphal envelope. Finally, those clusters adhered together

17  strongly, and the remnant of the hyphal envelope formed a thick-walled hyphal bundle in the

18  sporocarp, as observed in several extant *Endogone* species.

19  The phylogenetic data collected in the present study suggests that one sporocarp

20  specimen (OSC T14506 from Australia: Desirò et al. 2013) is closely related to mycobionts of

21  a liverwort and hornworts, and does not belong to any described genera of Endogonaceae

22  (FIG. 1). Morphological characterization of the spores and sporocarps of this unclassified

23  phylogroup is necessary to better understand Endogonaceae evolution. In addition,

24  phylogenetic analyses of *Sclerogone* and *Peridiospora* will contribute to this effort.

25  A total of eight Endogonaceae species, including five new species, were identified from

1  the Japanese Islands in this and previous recent studies (Yamamoto et al. 2015, 2017a). This

2  number is comparable to that reported from the North American continent; i.e., 11 species

3  (Gerdemann and Trappe 1974; Yao et al. 1996), or the entire region of Europe; i.e., six species

4  (Yao et al. 1996; Błaszkowski 1997, 1998; Vidal et al. 1997). In addition, the identification of

5  *V. radiata* is the first record of Endogonaceae from a subtropical forest as well as the first that

6  is suggested to have an association with *Castanopsis* in Fagaceae. As *Castanopsis* is highly

7  diverse and common from warm temperate to tropical areas of Asia (Gee et al. 2003), future

8  research in *Castanopsis* forests will likely lead to the discovery of additional lineages of

9  Endogonaceae.

10  Although many sporocarps of Endogonaceae have been collected from forest sites

11  dominated by ectomycorrhizal trees (Gerdemann and Trappe 1974; Warcup 1990; Yao et al.

12  1996; this study), the diversity of sporocarp-forming Endogonaceae in environments

13  dominated by non-ectomycorrhizal plants is largely unknown. Indeed, recent studies have

14  indicated a high diversity of Endogonales mycobionts of bryophytes (Bidartondo et al. 2011;

15  Desirò et al. 2013). In addition, sporocarps of *Endogone* sp. (W5994), belonging to the

16  *Jimgerdemannia* clade (FIG. 1), grew on the root system of a cultivated non-ectomycorrhizal

17  plant, *Streptocarpus venosus*, under greenhouse conditions (Walker 2013). Recent studies

18  have revealed that fine endophytes with arbuscular mycorrhiza-like structures on various

19  non-ectomycorrhizal vascular and non-vascular plants (Orchard et al. 2017b) are

20  phylogenetically placed in Endogonaceae and Densosporaceae (Desirò et al. 2017; Orchard et

21  al. 2017a; FIG. 1). Thus, W5994 may be a fine endophyte that can associate with

22  non-ectomycorrhizal plants. Based on this result, sporocarps of further undescribed species

23  may be collected from environments dominated by liverworts and hornworts or by

24  herbaceous plants associated with fine endophytes.

25  **ACKNOWLEDGMENTS**

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10  **LEGENDS AND FOOTNOTES**

11  **Figure 1.** Maximum likelihood (ML) phylogenetic tree of the combined dataset of 18S and

12  28S nuclear ribosomal DNA (nrDNA; dataset 1). Phylogenetic relationships of four new

13  species (red) of Endogonaceae, and other species including plant mycobionts in Endogonales

14  are shown. Mortierellaceae and Calcarisporiellaceae are used as outgroups. Bootstrap (BS)

15  values (1000 replicates) > 50 % of ML (left) and maximum parsimony (MP) (right),

16  respectively, are shown near the nodes. Branches supported by BS of ML/MP > 70% (black)

17  and ML > 70% (grey) are highlighted with thick lines. Sequence ID is indicated by the

18  voucher no. and locality. Sequences shown in green = mycobionts of ectomycorrhizal root

19  tips or sporocarps of putative ectomycorrhizal species, blue = mycobionts of bryophytes or

20  ferns, pink = fine endophytes. Abbreviations: AUS = Australia; CAN = Canada; FRA =

21  France; ITA = Italy; JPN = Japan; MEX = Mexico; MYS = Malaysia; NZL = New Zealand;

22  PAN = Panama; SAF = South Africa; SCO = Scotland; SPA = Spain.

23  **Figure 2.** ML phylogenetic tree of the combined dataset of the 28S nrDNA gene, *tef1*, and

24  *rpb1* (dataset 2). Phylogenetic relationships of four new species (red) of Endogonaceae and

25  other species including plant mycobionts in Endogonales are shown. Mortierellaceae and

1    Calcarisporiellaceae are used as outgroups. BS values (1000 replicates) > 50% of ML (left)

2    and MP (right) are shown near the nodes. Branches supported by BS of ML/MP > 70%

3    (black) and ML or MP > 70% (grey) are highlighted with thick lines. Sequence ID is indicated

4    by the voucher no. and locality. Sequences shown in green = mycobionts from

5    ectomycorrhizal root tips or sporocarps of putative ectomycorrhizal species, blue =

6    mycobionts of bryophytes. Abbreviations: AUS = Australia; CAN = Canada; ITA = Italy; JPN

7    = Japan; MEX = Mexico; NZL = New Zealand; SCO = Scotland.

8    **Figure 3.** Morphological characteristics of *Endogone botryocarpus* (KPM-NC0026731). A, B.

9    Whole sporocarp (A) and magnified image of aggregate of zygosporangial clusters (B). Thick

10   hyphae on the substrate surface (*th*) are shown. C. Wide, thick-walled aseptate hyphae on the

11   surface of substrate. D. Surface view of peridium composed of thick-walled hyphae. E.

12   Thick-walled aseptate peridial hyphae, stained with Melzer's reagent. Dextrinoid and amyloid

13   reactions are shown. F, G. Cross-section of a zygosporangial cluster (F) and magnified image

14   of thick-walled hyphal mass between zygosporangial clusters (G), stained with Melzer's

15   reagent. H–K. Zygosporangia mounted with lactoglycerol, showing eusporium (*es*) and

16   sporangiothecium (*st*). Arrows indicate two openings to gametangial remnants. Bars: a = 1

17   mm; b = 500 μm; c–e, g = 20 μm; f = 50 μm; h–k = 10 μm.

18   **Figure 4.** Morphological characteristics of *Jimgerdemannia ambigua* (A: KPM-NC0026732;

19   B–D, G, I: KPM-NC0026733; E–F, H, J: TNS-F-70440). A. Surface view (right) and

20   cross-section (left) of a sporocarp. B. Cross-section of surface zone of sporocarp stained with

21   Melzer's reagent, showing zygosporangia and the absence of peridium. C. Narrow hyphae

22   composing the surface zone of sporocarp, stained with Melzer's reagent. D. Wide, thin-walled

23   glebal hyphae, stained with Melzer's reagent. E. Surface view of a mature zygosporangium

24   surrounded by thick-walled zygosporangial hyphal mantle mounted with lactoglycerol. F.

25   Reticulate-labyrinthiform sulcus on the sporangiothecium surface mounted with lactoglycerol.

1 G. Immature zygosporangium (zs) developed from fused macrogametangium (*ag*) and

2 microgametangium (*ig*) accompanied by suspensor (*su*), stained with Melzer's reagent. H.

3 Mature zygosporangium without hyphal mantle, stained with Melzer's reagent. I. Immature

4 zygosporangium surrounded by thin-walled hyphal mantle (*hm*), stained with Melzer's

5 reagent. J. Magnified image of mature zygosporangium showing both sporangiothecium (*st*)

6 and eusporium (*es*), surrounded by thick-walled hyphal mantle (*hm*), stained with Melzer's

7 reagent. Bars: A = 3 mm; B = 100 μm; C–E, I, J = 20 μm; F = 30 μm; G, H = 50 μm.

8 **Figure 5.** Morphological characteristics of *Vinositunica radiata* (A–B: KPM-NC0023963; C:

9 KPM-NC0026739; D, H: KPM-NC0026741; E, G: KPM-NC0023962; F, I:

10 KPM-NC0026742). A, B. A sporocarp on the soil surface (A), and its cross-section (B),

11 showing the radial arrangement of chlamydospores and stipe-like sterile base. C.

12 Cross-section of an immature sporocarp on the soil surface, in which its yellowish contents of

13 chlamydospores are visible due to the colorless outer spore wall. D, E. Cross-section of the

14 surface zone of a sporocarp, showing chlamydospores and peridium mounted with

15 lactoglycerol (E: stained with Melzer's reagent). F. Chlamydospores and glebal hyphae,

16 stained with Melzer's reagent. G. Narrow, thick-walled glebal hyphae, stained with Melzer's

17 reagent. H, I. Chlamydospores. Bars: A–C = 1 mm; D–F = 100 μm; G–I = 50 μm.

18 **Figure 6.** Morphological characteristics of *Vinositunica ingens* (A, E–G, I, J, L, M:

19 KPM-NC0026748; B, H, K: KPM-NC0024500; C: KPM-NC0026746; D: KPM-NC0026751).

20 A. Sporocarp with purplish pigmentation and litter adhered to the surface. B. Cross-section of

21 two immature sporocarps. Arrow indicates an immature chlamydospore with weak

22 pigmentation (photographed by M. Nakajima). C. Surface view of a sporocarp. D, E.

23 Cross-section of a sporocarp showing exudation of yellowish latex (*yl*), a sterile base (*sb*),

24 long subtending hyphae (*sh*), and litter inside the sporocarp (arrow). F. A chlamydospore

25 embedded beneath the surface of a sporocarp without peridium, stained with Melzer's reagent.

G. Glebal tissue consisting of thin-walled hyphae of variable width, with purplish

pigmentation visible in the darker area, mounted with lactoglycerol. H. A wide and

thick-walled glebal hypha mounted with lactoglycerol. I, J. A chlamydospore mounted with

lactoglycerol (J: stained with Melzer's reagent). K. A chlamydospore connected to three

subtending hyphae (*sh*), showing yellowish contents in the central area, mounted with

lactoglycerol. L. Outer and inner wall layers (*ol*, *il*) of a chlamydospore, stained with Melzer's

reagent. M. Boundary between chlamydospore and subtending hyphae (*sh*) where the inner

wall layer (*il*) is occluding the protoplasmic connection, mounted with lactoglycerol. Bars: A,

C–E = 5 mm; B = 1 cm; F, K = 200 μm; G = 50 μm; H, L, M = 20 μm; I, J = 100 μm.

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Fig 1

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Fig 2

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Fig 3

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Fig 4

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Fig 6　Click here to access/download;Figure;Fig.6.jpg
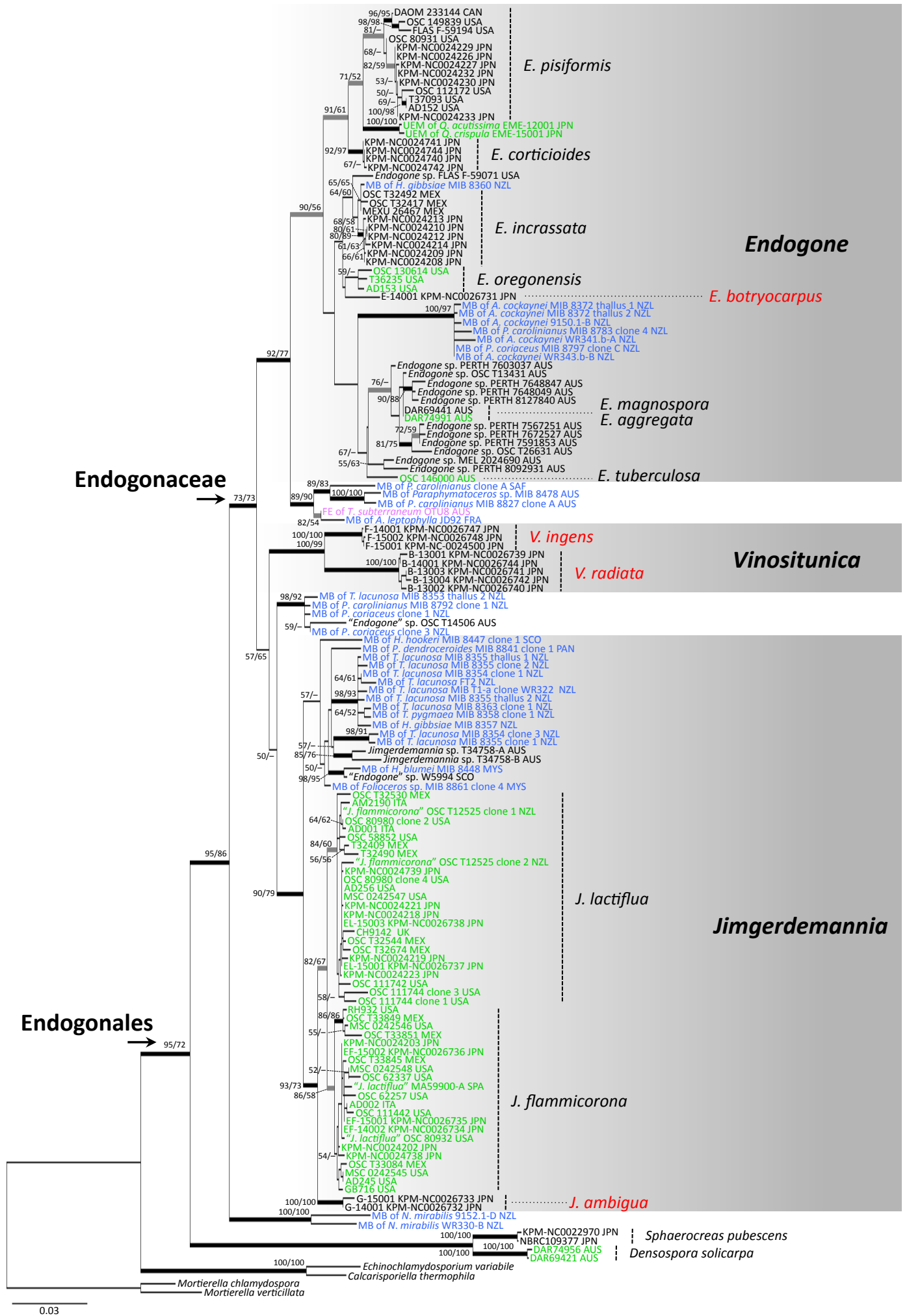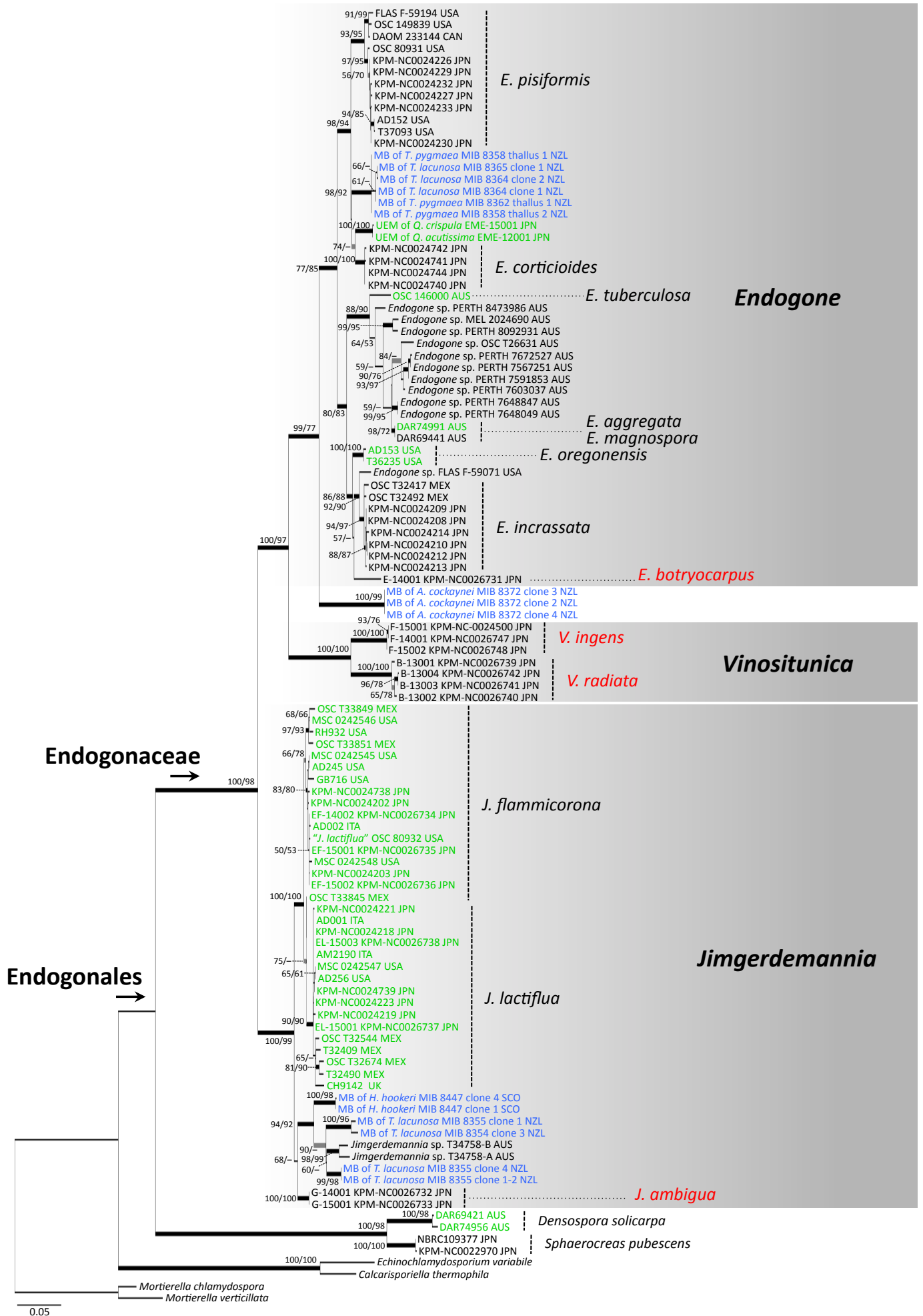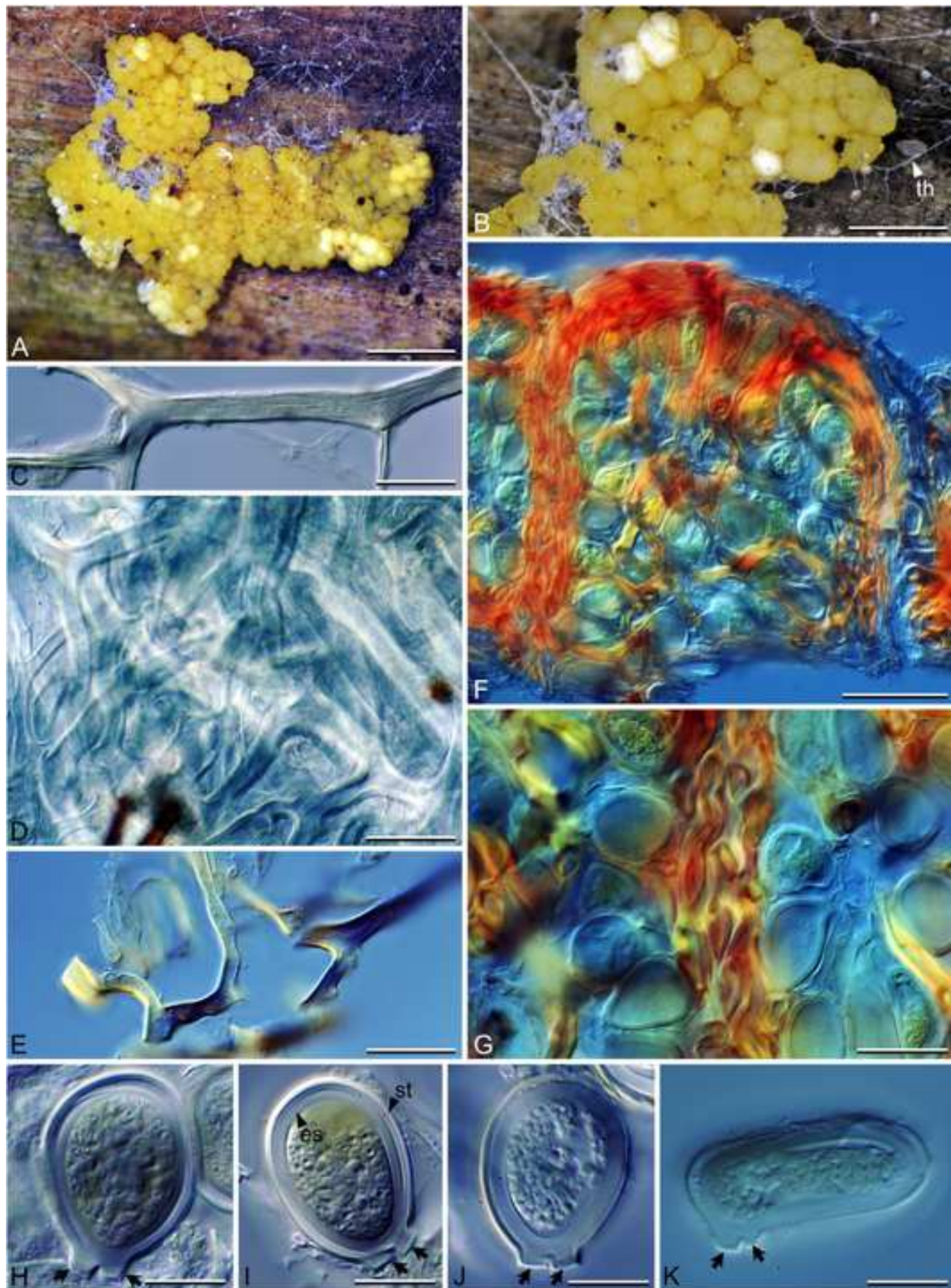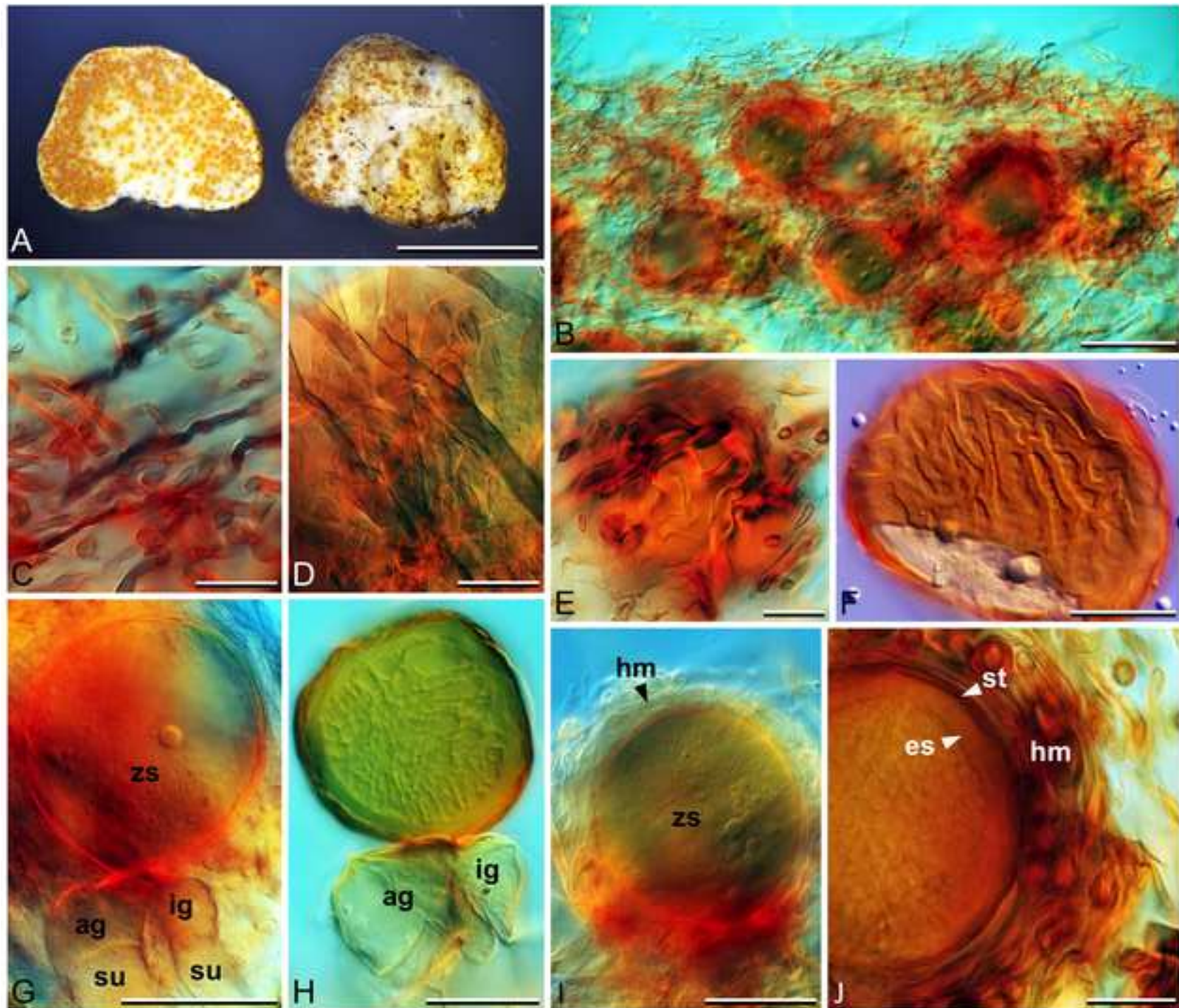
**Supplementary Table S1.** Newly obtained sequences (bold) of Endogonaceae, *Sphaerocreas pubescens*, and *Calcarisporiella thermophila*, and their associated sequences in the DDBJ and UNITE used for phylogenetic analyses.

| Taxon* | Locality | Voucher no. | Accession no. from DDBJ or UNITE | | | |
|---|---|---|---|---|---|---|
| | | | 18S nrDNA | 28S nrDNA | *tef1* | *rpb1* |
| *Endogone aggregata* | Australia, Victoria | DAR74991 | UDB018868 | UDB018867 | – | – |
| *E. botryocarpus* sp. nov. | Japan, Nagano Pref., Ueda-shi | KPM-NC0026731 (E-14001)*** | **LC431079** | **LC431095** | **LC431111** | – |
| *E. corticioides* | Japan, Nagano Pref., Koumi-machi | KPM-NC0024740 (A-11001) | LC107350 | LC107367 | LC107392 | – |
| *E. corticioides* | Japan, Nagano Pref., Koumi-machi | KPM-NC0024741 (A-12001) | LC107351 | LC107368 | LC107393 | – |
| *E. corticioides* | Japan, Nagano Pref., Koumi-machi | KPM-NC0024742 (A-13001-4) | LC107353 | LC107370 | LC107395 | **LC431127** |
| *E. corticioides* | Japan, Nagano Pref., Koumi-machi | KPM-NC0024744 (A-14002)*** | LC107355 | LC107372 | LC107396 | – |
| *E. incrassata* | Japan, Hokkaido, Shikaoi-cho | KPM-NC0024208 (EI-11004) | **LC107333** | **LC107361** | **LC107376** | **LC431128** |
| *E. incrassata* | Japan, Nagano Pref., Saku-shi | KPM-NC0024209 (EI-11005) | **LC107334** | **LC107362** | **LC107377** | – |
| *E. incrassata* | Japan, Nagano Pref., Ueda-shi | KPM-NC0024210 (EI-11006) | **LC107335** | **LC107363** | – | – |
| *E. incrassata* | Japan, Nagano Pref., Matsumoto-shi | KPM-NC0024212 (EI-12001) | **LC107336** | LC002619 | **LC107378** | **LC431129** |
| *E. incrassata* | Japan, Nagano Pref., Tomi-shi | KPM-NC0024213 (EI-12004) | LC107337 | LC002620 | LC107379 | **LC431130** |
| *E. incrassata* | Japan, Hokkaido, Shikaoi-cho | KPM-NC0024214 (EI-12005) | LC107338 | LC002621 | LC107380 | **LC431131** |
| *E. incrassata* | Mexico, Tlaxcala, San Jose' Teacalco | OSC T32492 | JF414199 | – | MF479053 | – |
| *E. incrassata* | Mexico, Veracruz, Cofre de Perote | OSC T32417 | JF414200 | MF479014 | MF479076 | – |

| Species | Location | Voucher | | | | |
|---|---|---|---|---|---|---|
| *E. incrassata* | Mexico, 19.5733 N 103.6275 W | MEXU 26467 (9044-I) | KJ952220 | – | – | – |
| *E. magnospora* nom. nud. | Australia, Tasmania | DAR69441 | UDB018869 | UDB018870 | – | – |
| *E. oregonensis* | USA, Oregon, Polk | OSC 130614 | JF414196 | – | – | – |
| *E. oregonensis* | USA, Oregon, Monmouth | AD153 | MF478989 | MF479015 | MF479073 | – |
| *E. oregonensis* | USA, Oregon, Benton County | T36235 | MF478990 | MF479016 | MF479072 | – |
| *E. pisiformis* | Japan, Hokkaido, Shikaoi-cho | KPM-NC0024226 (EP-11003) | LC107344 | LC002627 | LC107386 | **LC431132** |
| *E. pisiformis* | Japan, Nagano Pref., Matsumoto-shi | KPM-NC0024227 (EP-12001) | LC107345 | LC002628 | LC107387 | – |
| *E. pisiformis* | Japan, Nagano Pref., Matsumoto-shi | KPM-NC0024229 (EP-12003) | LC107346 | LC107365 | LC107388 | **LC431133** |
| *E. pisiformis* | Japan, Nagano Pref., Sakuho-machi | KPM-NC0024230 (EP-12007) | LC107347 | LC002629 | LC107389 | **LC431134** |
| *E. pisiformis* | Japan, Nagano Pref., Ueda-shi | KPM-NC0024232 (EP-12009) | LC107348 | LC107366 | LC107390 | – |
| *E. pisiformis* | Japan, Hokkaido, Shikaoi-cho | KPM-NC0024233 (EP-12010) | LC107349 | LC002630 | LC107391 | **LC431135** |
| *E. pisiformis* | Canada | DAOM 233144 | DQ322628 | DQ273811 | DQ282618 | DQ294601 |
| *E. pisiformis* | USA, Oregon, Benton | OSC 80931 | JF414194 | – | MH449557 | MH449558 |
| *E. pisiformis* | USA, Washington | OSC 112172 (T31477) | KC708389 | – | – | – |
| *E. pisiformis* | USA, Oregon, Corvallis | AD152 | MF478991 | MF479018 | MF479071 | – |
| *E. pisiformis* | USA, New Hampshire, Carroll County | FLAS F-59194 (MES1451) | MF478992 | MF479020 | – | – |
| *E. pisiformis* | USA, New Hampshire, Carroll County | OSC 149839 (T37049) | MF478993 | MF479021 | – | – |
| *E. pisiformis* | USA, Oregon, Lane County | T37093 | MF478994 | MF479019 | MF479070 | – |
| *E. tuberculosa* | Australia, Australian Capital Territory | OSC 146000 (T34145) | – | MF479026 | – | – |

| Species | Location | Voucher | | | | |
|---|---|---|---|---|---|---|
| *Endogone* sp. | Australia, Davies Creek | OSC T13431 | JF414197 | – | – | – |
| *Endogone* sp. | Australia, Bournda National Park | OSC T26631 | JF414198 | MF479025 | JF414136 | – |
| *Endogone* sp. | Australia | OSC T14506 | KC708390 | – | – | – |
| *Endogone* sp. | United Kingdom, Scotland | W5994 | KC708391 | – | – | – |
| *Endogone* sp. | Australia, Western Australia, Dwellingup | PERTH 7648049 | KM594019 | – | MF479074 | – |
| *Endogone* sp. | Australia, Western Australia, Dwellingup | PERTH 7648847 | KM594020 | – | MF479069 | – |
| *Endogone* sp. | USA, Florida, Melrose | FLAS F-59071 (MES866) | MF478995 | MF479017 | MF479075 | – |
| *Endogone* sp. | Australia, Adelaide Hills, Loftia Recreation Park | MEL 2024690 | MF478996 | – | MF479052 | – |
| *Endogone* sp. | Australia, Queensland, Bluewater Park | PERTH 7603037 | MF478997 | – | MF479047 | – |
| *Endogone* sp. | Australia, Queensland, Atherton | PERTH 7567251 | – | MF479022 | MF479048 | – |
| *Endogone* sp. | Australia, Cape York | PERTH 7591853 | – | MF479023 | MF479049 | – |
| *Endogone* sp. | Australia, Queensland, Mount Windsor Tableland | PERTH 7672527 | MF478998 | MF479024 | MF479050 | – |
| *Endogone* sp. | Australia, Leeuwin-Naturaliste National Park | PERTH 8092931 | MF478999 | – | MF479068 | – |
| *Endogone* sp. | Australia, Karakamia Sanctuary | PERTH 8127840 | MF479000 | – | – | – |
| *Endogone* sp. | Australia, Boorabbin National Park | PERTH 8473986 | – | – | MF479051 | – |
| *Jimgerdemannia ambigua* sp. nov. | Japan, Shiga Pref., Takashima-shi | KPM-NC0026732 (G-14001)\*\*\* | **LC431080** | **LC431096** | **LC431112** | – |
| *J. ambigua* sp. nov. | Japan, Shiga Pref., Nagahama-shi | KPM-NC0026733 (G-15001) | **LC431081** | **LC431097** | **LC431113** | **LC431136** |
| *J. flammicorona* | Japan, Nagano Pref., Ueda-shi | KPM-NC0024202 (EF-11001) | **LC107330** | LC002615 | **LC107373** | – |

| | | | | | | |
|---|---|---|---|---|---|---|
| *J. flammicorona* | Japan, Yamanashi Pref., Fujiyoshida-shi | KPM-NC0024203 (EF-11002) | **LC107331** | LC002616 | **LC107374** | **LC431137** |
| *J. flammicorona* | Japan, Hokkaido, Otaru-shi | KPM-NC0024738 (EF-13002) | LC107332 | LC107360 | LC107375 | – |
| *J. flammicorona* | Japan, Nagano Pref., Saku-shi | KPM-NC0026734 (EF-14002) | **LC431082** | **LC431098** | **LC431114** | – |
| *J. flammicorona* | Japan, Nagano Pref., Karuizawa-machi | KPM-NC0026735 (EF-15001) | **LC431083** | **LC431099** | **LC431115** | **LC431138** |
| *J. flammicorona* | Japan, Gifu Pref., Takayama-shi | KPM-NC0026736 (EF-15002) | **LC431084** | **LC431100** | **LC431116** | **LC431139** |
| *J. flammicorona* | Mexico, Jalisco | OSC T33845 | JF414205 | – | JF414138 | – |
| *J. flammicorona* | Mexico, Jalisco, Bosque la Primavera | OSC T33849 | JF414204 | MF479034 | MF479064 | – |
| *J. flammicorona* | USA, Oregon, Benton | OSC 111442 | JF414206 | – | – | – |
| *J. flammicorona* | USA, Idaho | OSC 62257 | KC708378 | – | – | – |
| *J. flammicorona* | USA, West Virginia | OSC 62337 | KC708379 | – | – | – |
| *J. flammicorona* | New Zealand | OSC T12525 clone1 | KC708380 | – | – | – |
| *J. flammicorona* | New Zealand | OSC T12525 clone2 | KC708381 | – | – | – |
| *J. flammicorona* | Mexico | OSC T33084 | KC708382 | – | – | – |
| *J. flammicorona* | Italy, Piemonte, Veglio | AD002 | MF479001 | MF479027 | MF479078 | – |
| *J. flammicorona* | USA, Michigan, Haslett | AD245 | MF479002 | MF479028 | MF479063 | – |
| *J. flammicorona* | USA, Michigan, Haslett | GB716 | MF479003 | MF479029 | MF479061 | – |
| *J. flammicorona* | USA, Michigan, Haslett | MSC 0242545 (AD239) | MF479004 | MF479030 | MF479062 | – |
| *J. flammicorona* | USA, Michigan, Haslett | MSC 0242546 (AD244) | MF479005 | MF479031 | MF479065 | – |
| *J. flammicorona* | USA, Michigan, Haslett | MSC 0242548 (GB737) | MF479006 | MF479032 | MF479067 | – |
| *J. flammicorona* | USA, Iowa, Ledges State Park | RH932 | MF479007 | MF479033 | MF479079 | – |

| Species | Locality | Voucher | | | | |
|---|---|---|---|---|---|---|
| *J. flammicorona* | Mexico, Jalisco, Bosque la Primavera | OSC T33851 | MF479008 | MF479035 | JF414139 | – |
| *J. lactiflua* | Japan, Hokkaido, Biei-cho | KPM-NC0024218 (EL-10001) | **LC107339** | LC002622 | **LC107381** | – |
| *J. lactiflua* | Japan, Gifu Pref., Takayama-shi | KPM-NC0024219 (EL-10002) | **LC107340** | LC002623 | **LC107382** | **LC431140** |
| *J. lactiflua* | Japan, Hokkaido, Asahikawa-shi | KPM-NC0024221 (EL-11001) | **LC107341** | LC002625 | **LC107383** | **LC431141** |
| *J. lactiflua* | Japan, Yamanashi Pref., Fujiyoshida-shi | KPM-NC0024223 (EL-11003) | **LC107342** | LC002626 | **LC107384** | **LC431142** |
| *J. lactiflua* | Japan, Hokkaido, Higashikawa-cho | KPM-NC0024739 (EL-14001) | LC107343 | LC107364 | LC107385 | – |
| *J. lactiflua* | Japan, Nagano Pref., Karuizawa-machi | KPM-NC0026737 (EL-15001) | **LC431085** | **LC431101** | **LC431117** | **LC431143** |
| *J. lactiflua* | Japan, Nagano Pref., Ueda-shi | KPM-NC0026738 (EL-15003) | **LC431086** | **LC431102** | **LC431118** | – |
| *J. lactiflua* | USA | OSC 80932 | DQ536471 | DQ273788 | MH449559 | – |
| *J. lactiflua* | Mexico, Tlaxcala | OSC T32530 | JF414201 | – | – | – |
| *J. lactiflua* | Mexico, Tlaxcala, Huamantla | OSC T32544 | JF414202 | MF479042 | MF479077 | – |
| *J. lactiflua* | Mexico, Tamaulipas | OSC T32674 | JF414203 | MF479043 | MF479056 | – |
| *J. lactiflua* | USA, Oregon | OSC 111742 | KC708383 | – | – | – |
| *J. lactiflua* | USA, Oregon | OSC 111744 clone1 | KC708384 | – | – | – |
| *J. lactiflua* | USA, Oregon | OSC 111744 clone3 | KC708385 | – | – | – |
| *J. lactiflua* | USA, Alaska | OSC 58852 | KC708386 | – | – | – |
| *J. lactiflua* | USA, California | OSC 80980 clone2 | KC708387 | – | – | – |
| *J. lactiflua* | USA, California | OSC 80980 clone4 | KC708388 | – | – | – |
| *J. lactiflua* | Spain, 40.4122 N 3.6911 W | MA59900-A | KJ952221 | – | – | – |

| Species | Locality | Voucher | | | | |
|---|---|---|---|---|---|---|
| *J. lactiflua* | Italy, Piemonte, Veglio | AD001 | KM594016 | MF479036 | MF479054 | – |
| *J. lactiflua* | Italy, Emilia Romagna, Cavola | AM2190 (2190) | KM594017 | – | MF479060 | – |
| *J. lactiflua* | United Kingdom, England, Derbyshire | CH9142 (9142) | KM594018 | MF479038 | – | – |
| *J. lactiflua* | USA, Michigan, Mason | AD256 | MF479009 | MF479037 | MF479059 | – |
| *J. lactiflua* | USA, Michigan, Mason | MSC 0242547 (AD251) | – | MF479039 | MF479058 | – |
| *J. lactiflua* | Mexico, Veracruz, Cofre de Perote | T32409 | MF479010 | MF479040 | MF479057 | – |
| *J. lactiflua* | Mexico, Tlaxcala, San Jose' Teacalco | T32490 | MF479011 | MF479041 | MF479055 | – |
| *Jimgerdemannia* sp. | Australia, Queensland, Main Ranges National Park | T34758-A | MF479012 | MF479044 | MF479066 | – |
| *Jimgerdemannia* sp. | Australia, Queensland, Main Ranges National Park | T34758-B | MF479013 | MF479045 | MF479046 | – |
| *Vinositunica radiata* sp. nov. | Japan, Kyoto Pref., Fukuchiyama-shi | KPM-NC0026739 (B-13001) | **LC431087** | **LC431103** | **LC431119** | **LC431144** |
| *V. radiata* sp. nov. | Japan, Okinawa Pref., Ishigaki-jima Isl., Ishigaki-shi | KPM-NC0026740 (B-13002) | **LC431088** | **LC431104** | **LC431120** | **LC431145** |
| *V. radiata* sp. nov. | Japan, Okinawa Pref., Okinawa-jima Isl., Kunigami-son | KPM-NC0026741 (B-13003) | **LC431089** | **LC431105** | **LC431121** | – |
| *V. radiata* sp. nov. | Japan, Okinawa Pref., Okinawa-jima Isl., Kunigami-son | KPM-NC0026742 (B-13004)*** | **LC431090** | **LC431106** | **LC431122** | **LC431146** |
| *V. radiata* sp. nov. | Japan, Kagoshima Pref. Amami-ohshima Isl., Amami-shi | KPM-NC0026744 (B-14001) | **LC431091** | – | – | – |
| *V. ingens* sp. nov. | Japan, Miyagi Pref., Sendai-shi | KPM-NC0026747 (F-14001) | **LC431092** | **LC431107** | **LC431123** | – |
| *V. ingens* sp. nov. | Japan, Saitama Pref., Namegawa-machi | KPM-NC0024500 (F-15001) | **LC431093** | **LC431108** | **LC431124** | **LC431147** |

| Species | Locality | Voucher | LC431094 | LC431109 | LC431125 | LC431148 |
|---|---|---|---|---|---|---|
| *V. ingens* sp. nov. | Japan, Shiga Pref., Nagahama-shi | KPM-NC0026748 (F-15002)*** | **LC431094** | **LC431109** | **LC431125** | **LC431148** |
| MB of *Allisonia cockaynei* | New Zealand, South Isl., Kelly Creek | MIB 8372 thallus 1 | JF414209 | – | – | – |
| MB of *A. cockaynei* | New Zealand, South Isl., Kelly Creek | MIB 8372 thallus 2 | JF414210 | – | – | – |
| MB of *A. cockaynei* | New Zealand | 9150.1-B | KR779273 | – | – | – |
| MB of *A. cockaynei* | New Zealand | WR341.b-A | KR779275 | – | – | – |
| MB of *A. cockaynei* | New Zealand | WR343.b-B | KR779277 | – | – | – |
| MB of *A. cockaynei* | New Zealand, South Isl., Kelly Creek | MIB 8372 clone 2 | – | – | JF414142 | – |
| MB of *A. cockaynei* | New Zealand, South Isl., Kelly Creek | MIB 8372 clone 3 | – | – | JF414143 | – |
| MB of *A. cockaynei* | New Zealand, South Isl., Kelly Creek | MIB 8372 clone 4 | – | – | JF414144 | – |
| MB of *Anogramma leptophylla* | France, 43.5639 N 7.1244 E | JD92 | KJ952217 | – | – | – |
| MB of *Folioceros* sp. | Malaysia | MIB 8861 clone 4 | KC708427 | – | – | – |
| MB of *Haplomitrium blumei* | Malaysia, Genting Highlands | MIB 8448 | JF414211 | – | – | – |
| MB of *H. gibbsiae* | New Zealand, South Isl., Mt. Arthur | MIB 8360 | JF414208 | – | – | – |
| MB of *H. gibbsiae* | New Zealand, South Isl., Rahu Saddle | MIB 8357 | JF414212 | – | – | – |
| MB of *H. hookeri* | United Kingdom, Scotland, Ben Lawers | MIB 8447 clone 1 | JF414213 | – | – | – |
| MB of *H. hookeri* | United Kingdom, Scotland, Ben Lawers | MIB 8447 clone 1 | – | – | JF414145 | – |
| MB of *H. hookeri* | United Kingdom, Scotland, Ben Lawers | MIB 8447 clone 4 | – | – | JF414146 | – |

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|---|---|---|---|---|---|---|
| MB of *Neohodgsonia mirabilis* | New Zealand | 9152.1-D | KR779279 | – | – | – |
| MB of *N. mirabilis* | New Zealand | WR330-B | KR779282 | – | – | – |
| MB of *Paraphymatoceros* sp. | West Australia | MIB 8478 | JF414207 | – | – | – |
| MB of *Phaeoceros carolinianus* | New Zealand, South Isl. | MIB 8783 clone 4 | KC708411 | – | – | – |
| MB of *P. carolinianus* | New Zealand, South Isl. | MIB 8792 clone 1 | KC708412 | – | – | – |
| MB of *P. carolinianus* | Australia, Victoria | MIB 8827 clone A | KC708419 | – | – | – |
| MB of *P. carolinianus* | South Africa, Drakensberg | Clone A | KC708443 | – | – | – |
| MB of *P. dendroceroides* | Panama | MIB 8841 clone 1 | KC708420 | – | – | – |
| MB of *Phaeomegaceros coriaceus* | New Zealand, South Isl. | MIB 8797 clone C | KC708416 | – | – | – |
| MB of *P. coriaceus* | New Zealand, South Isl. | Clone 3 | KC708433 | – | – | – |
| MB of *P. coriaceus* | New Zealand, South Isl. | Clone 1 | KC708437 | – | – | – |
| MB of *Treubia lacunosa* | New Zealand, South Isl., Kelly Creek | MIB 8353 thallus 2 | JF414214 | – | – | – |
| MB of *T. lacunosa* | New Zealand, South Isl., Ross | MIB 8354 clone 1 | JF414215 | – | – | – |
| MB of *T. lacunosa* | New Zealand, South Isl., Cobb | MIB 8355 thallus 1 | JF414216 | – | – | – |
| MB of *T. lacunosa* | New Zealand, South Isl., Cobb | MIB 8355 thallus 2 | JF414217 | – | – | – |
| MB of *T. lacunosa* | New Zealand, South Isl., Cobb | MIB 8355 clone 2 | JF414218 | – | – | – |
| MB of *T. lacunosa* | New Zealand, North Isl., Piha | MIB 8363 clone 1 | JF414219 | – | – | – |

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|---|---|---|---|---|---|---|
| MB of *T. lacunosa* | New Zealand, 41.2000 S 172.8083 E | MIB T1-a clone WR322 | KJ921770 | – | – | – |
| MB of *T. lacunosa* | New Zealand | FT2 | KM211581 | – | – | – |
| MB of *T. lacunosa* | New Zealand, South Isl., Ross | MIB 8354 clone 3 | – | JF414169 | – | – |
| MB of *T. lacunosa* | New Zealand, South Isl., Cobb | MIB 8355 clone 1 | – | JF414170 | – | – |
| MB of *T. lacunosa* | New Zealand, South Isl., Cobb | MIB 8355 clone 1-2 | – | – | JF414147 | – |
| MB of *T. lacunosa* | New Zealand, South Isl., Cobb | MIB 8355 clone 4 | – | – | JF414148 | – |
| MB of *T. lacunosa* | New Zealand, North Isl., Piha | MIB 8364 clone 1 | – | – | JF414149 | – |
| MB of *T. lacunosa* | New Zealand, North Isl., Piha | MIB 8364 clone 2 | – | – | JF414150 | – |
| MB of *T. lacunosa* | New Zealand, North Isl., Wainau | MIB 8365 clone 1 | – | – | JF414151 | – |
| MB of *T. pygmaea* | New Zealand, South Isl., Mt. Arthur | MIB 8358 clone 1 | JF414220 | – | – | – |
| MB of *T. pygmaea* | New Zealand, South Isl., Mt. Arthur | MIB 8358 thallus 1 | – | – | JF414152 | – |
| MB of *T. pygmaea* | New Zealand, South Isl., Mt. Arthur | MIB 8358 thallus 2 | – | – | JF414153 | – |
| MB of *T. pygmaea* | New Zealand, South Isl., Punchbowl Falls | MIB 8362 thallus 1 | – | – | JF414154 | – |
| FE of *Trifolium subterraneum* | Australia | OTU8 | KX434780 | – | – | – |
| UEM of *Quercus acutissima* | Japan, Nagano Pref. Azumino-shi | EME-12001 | LC159474 | LC159476 | LC159478 | **LC431149** |
| UEM of *Q. crispula* | Japan, Nagano Pref. Ueda-shi | EME-15001 | LC159475 | LC159477 | LC159479 | – |
| *Densospora solicarpa* | Australia, New South Wales, Sydney | DAR69421*** | UDB018865 | UDB018864 | – | – |
| *D. solicarpa* | Australia, New South Wales, Pearl Beach | DAR74956 | UDB018861 | UDB018860 | – | – |

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|---|---|---|---|---|---|---|
| *Sphaerocreas pubescens* | Japan, Kagoshima Pref. Kirishima-shi | NBRC109377 | AB752295 | LC107618 | LC107619 | **LC431150** |
| *S. pubescens* | Japan, Kyoto Pref. Kyoto-shi | KPM-NC0022970 | AB755407 | **LC431110** | **LC431126** | **LC431151** |
| *Calcarisporiella thermophila* | Japan, Okinawa Pref., Iriomote-jima Isl. | NBRC 33279 | AB597204 | AB617739 | – | **LC431152** |
| *Echinochlamydospori um variabile* | China, Northeast region | LN07-7-4*** | EU688964 | EU688963 | – | – |
| *Mortierella chlamydospora* | Unknown** | NRRL 2769 | AF157143 | AF157197 | AF157259 | – |
| *M. verticillata* | Unknown** | NRRL 6337 | AF157145 | DQ273794 | AF157262 | DQ294595 |

\* MB: mycobiont of liverwort, hornwort, or fern; FE: fine endophyte; UEM: uncultured ectomycorrhiza.

\*\* Locarity is not described.

\*\*\* Holotype.

1    **Supplementary Table S1.** Newly obtained sequences of Endogonaceae, *Sphaerocreas*

2    *pubescens*, and *Calcarisporiella thermophila*, and their associated sequences in the

3    DDBJ and UNITE used for phylogenetic analyses.