

Multi-locus phylogenies reveal three new truffle-like taxa and the traces of interspecific hybridization in *Octaviania* (*Boletales*)

Takamichi Orihara (✉ t_orihara@nh.kanagawa-museum.jp)

Kanagawa Prefectural Museum of Natural History <https://orcid.org/0000-0002-0409-7810>

Rosanne Healy

University of Florida Department of Plant Pathology

Adriana Corrales

Universidad Del Rosario

Matthew E. Smith

University of Florida Department of Plant Pathology

Research

Keywords: Boletaceae, hypogeous fungi, phylogeography, sequestrate fungi, systematics, 3 new taxa

Posted Date: September 3rd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-71134/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Among many convergently evolved sequestrate fungal genera in *Boletaceae* (*Boletales*, *Basidiomycota*), the genus *Octaviania* is the most diverse. We recently collected many specimens of *Octaviania* subgenus *Octaviania*, including several undescribed taxa, from Japan and the Americas. Here we describe two new species in subgenus *Octaviania*, *O. tenuipes* and *O. tomentosa*, from temperate to subtropical evergreen *Fagaceae* forests in Japan based on morphological observation and robust multi-locus phylogenetic analyses (nrDNA ITS and partial large subunit [LSU], translation elongation factor 1- α gene [*TEF1*] and the largest subunit of RNA polymerase II gene [*RPB1*]). Based on specimens from the Americas as well as studies of the holotype, we also taxonomically re-evaluate the taxon *O. asterosperma* var. *potteri*. Our analysis suggests that *O. asterosperma* var. *potteri* is a distinct taxon within the subgenus *Octaviania* so we recognize this as *O. potteri*. We unexpectedly collected *O. potteri* specimens from geographically widespread sites in the USA, Japan and Colombia. This is the first verified report of *Octaviania* from the South American continent. Our molecular analyses also revealed that the *RPB1* sequence of one *O. tenuipes* specimen was identical to that of a closely related species, *O. japonimontana*, and that one *O. potteri* specimen from Minnesota had an *RPB1* sequence of an unknown species of *O.* subg. *Octaviania*. Additionally, one *O. japonimontana* specimen had an unusually divergent *TEF1* sequence. Gene-tree comparison and phylogenetic network analysis of the multi-locus dataset suggest that these heterogenous sequences are most likely the result of previous inter- and intra-specific hybridization. We hypothesize that frequent hybridization events in *Octaviania* may have promoted the high genetic and species diversity within the genus.

Introduction

The *Boletaceae* (*Boletales*, *Basidiomycota*) is generally recognized as a large family that consists of epigeous, mushroom-forming fungi. However, recent systematic studies have revealed a considerable number of sequestrate (truffle-like and secotioid) fungal lineages in the family that have evolved independently from boletoid mushrooms (e.g., Castellano et al. 2016; Desjardin et al. 2008, 2009; Lebel et al. 2012a, 2012b; Nuhn et al. 2013; Orihara et al. 2010, 2016a, 2016b; Orihara and Smith 2017; Smith et al. 2015, 2018; Sulzbacher et al. 2020; Vadthanarat et al. 2018; Wu et al. 2016). Although many sequestrate genera in *Boletaceae* comprise one or a few species, the genus *Octaviania* Vittad. (orthographic variant: *Octavianina* Kuntze; *Boletaceae*, *Boletales*), which belongs to the subfamily *Leccinoideae* (Wu et al. 2014), is exceptionally diverse and includes more than 25 truffle-like species (Orihara et al. 2012a; Paz et al. 2014, 2016).

The genus *Octaviania* is comprised of sequestrate, truffle-like species that have a marbled gleba and dextrinoid or non-amyloid basidiospores with coarse, conical to pyramidal ornamentation (Orihara et al. 2012a). Historically, the generic concept of *Octaviania* was unsettled and the genus was previously considered by some authors as a synonym of *Arcangeliella* Cavara (*Russulaceae*), *Hydnangium* Wallr (*Hydnangiaceae*). or *Melanogaster* Corda (*Paxillaceae*). Pegler and Young (1979) provided evidence that *Octaviania* is distinct from those morphologically similar sequestrate genera and Orihara et al (2012a)

redefined the current generic concept of the genus. Orihara et al. (2012a) further divided the genus into three subgenera, subg. *Octaviana*, *Fulvoglobus* Orihara and *Parcaea* Orihara, based on multigene phylogenies and the morphology of basidiomata. Paz et al. (2016) reviewed the European species of *Octaviana* and critically examined the type species, *O. asterosperma*. They found that *O. asterosperma* sensu stricto has a pseudoparenchymatous peridium, which is one of the major characteristics of subg. *Fulvoglobus*. Accordingly, they concluded that the subg. *Fulvoglobus* that was introduced by Orihara et al. (2012a) should be synonymized with subg. *Octaviana* sensu Paz et al. (2016), and that the preceding subg. *Octaviana* sensu Orihara et al. (2012a) should be synonymized with subg. *Mutabiles* Paz & J.M. Vidal.

Octaviana subg. *Octaviana* sensu Paz et al. (2016) (hereafter referred to simply as *Octaviana* subg. *Octaviana*) is characterized by cavities in the gleba filled with slightly viscid to dry, brown to blackish brown spore masses, and a peridium composed of inflated hyphae and isodiametric, pseudoparenchymatous cells. So far, the subgenus accommodates eight described species that are known only from the northern hemisphere (Orihara et al. 2012a; Paz et al. 2016). Orihara et al. (2012a) further suggested that there were at least two additional, taxonomically unsettled species (*Octaviana* sp. "E" from Japan and *Octaviana* sp. from North America). Since the publication of Orihara et al. (2012a), we have collected a number of additional specimens of *O.* subg. *Octaviana* from Japan, including the two species mentioned above. In addition, we collected fruitbodies of *O.* subg. *Octaviana* from a *Quercus humboldtii* forest in Colombia, which constitutes the first known record of *Octaviana* sensu stricto from South America. Our primary research objective is to clarify the phylogenetic and systematic positions of those taxonomically unsettled specimens in a robust phylogenetic framework. Here we propose two new species and one new taxonomic status based on morphological observations and nuclear multi-locus phylogenies. Furthermore, we found strong topological conflicts in some species of *O.* subg. *Octaviana* among gene trees. We therefore examined the cause of these conflicts based on gene-tree comparisons and phylogenetic network analyses and we discuss the possibility of inter- and intra-specific hybridization within the subgenus based on their ecology and phylogeography.

Methods

Taxon sampling and morphological observation

Fresh basidiomata were collected throughout Japan, from eastern North America and from Colombia, South America. After morphological observation the fruitbodies were air-dried or freeze-dried and then stored in sterile plastic bags. These specimens are deposited in Kanagawa Prefectural Museum of Natural History, Japan (KPM), Ada Hayden Herbarium, Iowa State University (ISC), Bell Museum of Natural History Herbarium Fungal Collection, University of Minnesota (MIN), Farlow Herbarium, Harvard University (FH), Florida Museum of Natural History Fungal Herbarium, University of Florida (FLAS), and the Oregon State University Herbarium (OSC). Herbarium specimens were obtained from KPM, FLAS, ISC, OSC and the University of Michigan Herbarium (MICH).

For microscopy, hand-cut sections of fresh or dried specimens were mounted in water, 3% KOH or lactoglycerol. To determine amyloidity of basidiospores, dried material was stained with Melzer's reagent. Basidiospore dimensions (range of spore length, from the hilar appendage to the spore tip × spore width), their standard deviations (SD) and the length to width ratio (Q) were determined based on 50 random measurements unless otherwise mentioned. The 95% prediction intervals of basidiospore diameter are shown without parentheses in taxonomic descriptions. Both endpoints of the spore dimensions are shown in parentheses, but when the value is the same as the 95% prediction interval, only the latter is shown. Measurements include the hilar appendage but not the spore ornamentation or pedicel. Basidium sizes are presented as the range of the lengths × the range of the widths. Scanning electron microscopy (SEM) was performed with the HITACHI TM-4000Plus Tabletop Microscope (Hitachi High-Technologies Co. Ltd., Japan). Small fragments of a dried gleba were excised and immersed in 8% ionic liquid (1-ethyl-3-methyl-imidazolium tetrafluoroborate) for conductive treatment (Yanaga et al., 2012) and were observed at 10–15 kV.

DNA extraction, PCR amplification and sequencing

DNA was extracted from fresh or dried fruitbodies using Indicating FTA Cards (Whatman International Ltd, Maidstone, England) based on the protocol by Orihara et al. (2012a, b). We also extracted genomic DNA from some fruitbodies using the protocol of Izumitsu et al. (2012). PCR amplification of the ITS and the large subunit (LSU; 28S) of the nuclear ribosomal DNA (nrDNA), and *TEF1* followed Orihara et al. (2012a). For *RPB1* amplification, we used a newly designed primer set based on sequences of *Boletaceae* deposited in the International Nucleotide Sequence Databases (INSD). The new primers include forward primer RPB1-TO-Bf (5'- AAGGCYGATATYGTGAGTC - 3'), which is located in the intron A between domains A and B of *RPB1*, reverse primer RPB1-TO-Br (5'- GCTTTGATGATRTCYCC - 3'), and reverse primer RPB1-TO-Br2 (5'- ARGCYTTGATRATRTCYCC - 3'). Both of the reverse primers are located in the conserved (exon) domain C. These primer pairs target an 850–1100 bp amplicon which spans the region between primer RPB1-Bf (Nuhn et al, 2013) and primer RPB1-Cr (Matheny et al., 2002). The PCR amplification of *RPB1* was performed using the following procedure: initial incubation at 95 °C for 10 min; subsequent step of 30 cycles at 94 °C for 30 s, 53 °C for 60 s, and 72 °C for 90 s, followed by 13 cycles at 94 °C for 30 s, 52 °C for 60 s, and 72 °C for 90 s; a final elongation step at 72 °C for 7 min. Unidirectional sequencing of the PCR products in the forward and reverse directions were completed according to Orihara et al. (2012a). Sequences were edited and assembled with Sequence Scanner v. 1.0 (Applied Biosystems, Foster City, California, USA), BioEdit version 7.0.9 (Hall 1999) and SeaView version 4 (Galtier et al. 1996). A total of 178 newly obtained sequences were deposited in INSD (Table 1).

Phylogenetic analyses

For the combined ITS-nLSU-*TEF1*-*RPB1* dataset, we retrieved 170 sequences from INSD (Table 1). The sequences were carefully selected so that the dataset could represent all genera and subgenera in the subfamily *Leccinoideae*, which includes the genera *Chamonixia*, *Leccinellum*, *Leccinum*, *Octaviania*, *Rossbeevera* and *Turmalinea* (Orihara et al. 2016a). We selected *Spongispora temasekensis*,

Spongiforma thailandica, *Borofutus dhakanus*, *Tylocinum griseolum*, *Binderoboletus segoi* and *Retiboletus* spp. in subfamily *Leccinoideae* as outgroup taxa based on Henkel et al. (2016) and Wu et al. (2016, 2018). We only included specimens in our analysis whose nucleotide sequences covered more than 50% of the total length of the aligned, full ITS-nLSU-*TEF1*-*RPB1* dataset to reduce a negative effect caused by the lack of sequences in the dataset (i.e., no less than 1802 bp in length in the concatenated 4-gene dataset). Accordingly, we could not include sequences of *Ionosporus*, *Rhodactina*, *Pseudoaustroboletus* and two of the polyphyletic clades of *Leccinum* in the *Leccinoideae* previously shown in Kuo and Ortiz-Santana (2020) (i.e., the *Leccinum talamancae* and *L. longicurvipes* lineages). Similarly, several species of *Octaviania*, including *O. asterosperma* s. str. and *O. arbucaensis*, which belong to subg. *Octaviania* (Yang et al. 2006; Vadthanarat et al. 2017), were not included in the analyses due to insufficient number of DNA loci available from INSD. Instead, we prepared an additional single-gene nLSU dataset that included as many *Octaviania* species as possible available from INSD, including the type species *O. asterosperma* s. str. (Table 1). The ML analyses were conducted with RAxML 8.2.10 (Stamatakis, 2014) under the GTR + I + G model. The BioNJ analyses were conducted with SeaView version 4 (Gouy et al., 2010). Sequence alignment was performed with the online version of MAFFT version 7 (Kato and Standley 2013) under default settings (i.e., the alignment algorithm is automatically selected from FFT-NS-1, FFT-NS-2, FFT-NS-i or L-INS-i). Subsequently, the sites with obvious alignment errors were manually adjusted in SeaView version 4. We referred to the results of the GBlocks option in SeaView (Castresana 2000) to exclude ambiguously aligned sites. Accordingly, the longest part of the insert within the ITS2 region found in all known species of *O.* subg. *Octaviania* (Orihara et al. 2012a) was excluded from our analyses. Prior to the multigene analyses, we compared the BioNJ tree topologies among the ITS, nLSU, *TEF1* and *RPB1* datasets to see if there were any topological conflicts among the gene trees. Sequences that caused considerable topological conflicts (BioNJ bootstrap values $\geq 75\%$; 1000 replicates) among the four single-locus phylogenies were excluded from the multi-locus analyses. Accordingly, *RPB1* sequences of "*Octaviania tenuipes*" nom. prov. KPM-NC 27968 (INSD acc. no.: MT868858) and "*Octaviania potteri*" nom. prov. KPM-NC 17828 (MT868837 & MT868838), that are proposed as new taxa in this study were omitted from the combined multi-locus dataset. We subsequently concatenated the four datasets for the multi-locus analyses. The ITS rDNA region was partitioned by ITS1 + ITS2 and 5.8S, and the *TEF1* and *RPB1* regions were partitioned by codons and introns, and best-fit likelihood models were estimated for each partition and nLSU with MrModeltest 2.3 (Nylander 2004).

Bayesian analyses were conducted with MrBayes 3.2 (Ronquist and Huelsenbeck 2003). Nucleotide substitution models for maximum likelihood (ML) analyses were selected by the Akaike Information Criterion (AIC) in jModeltest2 (Darriba et al., 2012; Guindon and Gascuel 2003). The GTR + I + G model was applied to ITS1 + ITS2, nLSU, the second codon of *RPB1* and the first and third codons of *TEF1*; SYM + I for 5.8S rDNA; F81 + I for the second codon of *TEF1*; HKY + I for the first codon of *RPB1*; HKY + G for the third codon of *RPB1*; and HKY + I + G for the introns of *TEF1* and *RPB1*. Bayesian posterior probabilities (PP) were estimated by the Metropolis-coupled Markov chain Monte Carlo method (Geyer 1991). In the multi-gene (ITS + nLSU + *TEF1* + *RPB1*) analysis, two parallel runs were conducted with one

cold and seven heated chains each for 4M generations. The parameter for temperature of the seven heated chains in both runs was set to 0.10. The 0.10 heating scheme was used instead of the default 0.20 setting, because in previous phylogenetic studies on the *Leccinoideae*, the Markov chains with the 0.10 heating setting converged more smoothly and were less likely to become trapped at local optima (Orihara et al. 2016a; Orihara and Smith 2017). Trees were saved to a file every 1000th generation. We determined that the two runs reached convergence when the average standard deviation of split frequencies (ASDSF) was continuously lower than 0.01. The ASDSF was monitored every 5000 generations. We also verified the convergence by checking that the effective sample size (ESS) of each resulting statistic was sufficiently large (> 200). Trees obtained before reaching convergence were discarded as the burn-in, and the remaining trees were used to calculate a 50% majority consensus topology and to determine PP values for individual branches.

Maximum likelihood (ML) analyses were conducted with RAxML 8.2.10. The same partitioned datasets as those for the Bayesian analyses were used so that different α -shape parameters, GTR rates, and empirical base frequencies could be assigned to each partition. The best-fit ML tree was estimated under the GTRCAT + I model. The rapid bootstrap (BS) analysis was implemented with 1000 replicates.

The single-gene nLSU phylogenies that included all the representative species of *Octaviania* available from INSD were estimated using the ML and BioNJ methods. The ML analysis was conducted using RAxML 8.2.10, setting the substitution model to GTRCAT + I and the number of rapid BS replicates to 1000. The BioNJ analysis was done by SeaView version 4 with the number of BS replicates set to 1000.

To compare tree topologies and examine precise phylogenetic placement of our three target taxa in *Octaviania* subg. *Octaviania*, we further inferred ML gene trees from individual ITS, nLSU, *RPB1*, and *TEF1* datasets of the subgenus using RAxML 8.2.10. The datasets were partitioned by genes for ITS (i.e., ITS1 + ITS2 and 5.8) and by codons for *RPB1* and *TEF1*. The best-fit ML tree was estimated under the GTRCAT + I model. The rapid BS analysis was implemented with 1000 replicates.

Since the comparison of the four gene trees of *Octaviania* subg. *Octaviania* detected several heterogenous sequences in the *RPB1* and *TEF1* regions, we further conducted phylogenetic network analysis based on a smaller multi-locus dataset to find the traces of reticulate evolution among infrageneric taxa in the subgenus *Octaviania*. The dataset for this analysis included 1–2 representative specimens for each species of the subgenus *Octaviania*. We selected specimens for which molecular data were available from all four DNA regions (i.e., ITS, nLSU, *RPB1* and *TEF1*). The *RPB1* sequences of “*Octaviania tenuipes*” *nom. prov.* (MT868858 [KPM-NC 27968]) and “*Octaviania potteri*” *nom. prov.* (seq1: MT868837 [KPM-NC 17828; *RH1181*]), which were omitted in the multi-gene Bayesian and ML analyses discussed above, were included in the combined dataset for this analysis. The analysis was executed with SplitsTree 4 (Huson and Bryant 2006). Networks were constructed by the NeighborNet method using the “distance estimation to uncorrected P value” setting. The resultant networks were displayed with the EqualAngle algorithm (Dress and Huson 2004). Bootstrap analysis was then conducted with 1000 replicates.

Results

Morphological evaluation of the North American *Octaviania* species in subgenus *Octaviania*.

The phylogenetic analyses in Orihara et al. (2012a) explicitly showed that three specimens of *Octaviania* (KPM-NC 17827, KPM-NC 17828 and OSC 13925) from eastern North America (i.e., Iowa, Minnesota and Florida) formed a distinct clade within *Octaviania* subg. *Octaviania* but there was no molecular taxonomic treatment of the unidentified taxon. We critically examined the morphology and habitat of the taxon and we compared it with the previously published literature on North American *Octaviania* species.

The overall macro-morphology, peridial structure and the basidiospore and basidia dimensions matched the original description of *Octaviania asterosperma* var. *potteri* Singer and Smith (Singer and Smith 1960), which was reported from Michigan, USA (see description of *O. potteri* below). We studied the holotype of *O. asterosperma* var. *potteri* (MICH 12376 [*Potter 8898*]) from the University of Michigan Herbarium (MICH). The specimen was well-preserved, but the cells of the peridium were collapsed. The basidiospore morphology matched those of the three North American specimens of *Octaviania* sp. (Table 2). We therefore identify the North American *Octaviania* species as *O. asterosperma* var. *potteri*. Below we propose a new status for this species as *Octaviania potteri* stat. nov.

Phylogenetic placement of new taxa inferred from the multi-locus phylogeny

The multi-locus dataset comprised of ITS and LSU nrDNA, *TEF1* and *RPB1* sequences of the *Leccinoideae* consisted of 94 specimens and 3603 aligned nucleotide positions. The Bayesian inference reached convergence after ca. 1.38M generations. Accordingly, we discarded the first 1400 trees in each parallel run, and the remaining 2601 trees in each run were summarized to approximate Bayesian posterior probabilities (PPs). ESS of all the model parameters were sufficiently large (> 200). The total arithmetic and harmonic mean of estimated marginal log likelihoods (lnL) for runs were - 27576.51 and - 27653.13, respectively. In the RAxML analysis, the final ML optimization of log likelihood was - 27424.085894. The overall topologies between the Bayesian and ML trees were nearly identical.

The resulting phylogenetic trees (Fig. 1) robustly recovered the known generic relationships within the *Leccinoideae*, some with higher statistical support than in previous studies (e.g., / *Spongiforma-Borofutus-Tylocinum* clade; Wu et al 2016, / *Leccinum-Leccinellum-Turmalinea-Rossbeevera* clade: Wu et al. 2018; Orihara et al. 2016a; Kuo and Ortiz-Santana 2020). *Octaviania* sp. "E" (= *O. tenuipes* sp. nov.) from Japan and *O. asterosperma* var. *potteri* from North America (= *O. potteri* stat. nov.) were placed within *Octaviania* subg. *Octaviania*, as was previously shown by Orihara et al. (2012a). A previously unknown species-level clade (= *O. tomentosa* sp. nov.) was also placed within *Octaviania* subg. *Octaviania*.

Specimens of *O. tenuipes* sp. nov. exhibited minimal infraspecific genetic divergence. In contrast, both *O. potteri* stat. nov. and *O. tomentosa* sp. nov. showed considerable genetic divergence among specimens. In the *O. tomentosa* clade, a specimen from Amami-oshima Island in the Ryukyu island chain, was genetically divergent from the other specimens from mainland Japan. In the *O. potteri* stat. nov. clade, the

geographically isolated specimens from Hokkaido, Japan (KPM-NC 18032) and Colombia (HUA 222100) were nested among the North American specimens.

Although the generic type species, *O. asterosperma*, was not included in the multi-locus phylogenies, the nLSU gene tree indicate that *O. potteri* stat. nov. is genetically distant from *O. asterosperma* var. *asterosperma* and it should be treated as a distinct taxon (Fig. S1; the lnL of the ML tree = -3239.444877).

Comparison of single-gene tree topologies within *Octaviana* subgenus *Octaviana*

The four ML gene trees based on ITS nrDNA (ITS1-5.8S-ITS2), LSU nrDNA, *RPB1* and *TEF1* datasets were estimated with the final ML optimization of lnL of -1806.362004, -1975.613722, -2139.994105 and -2417.247141, respectively (Fig. 2). All of the species-level clades in subg. *Octaviana* except *O. potteri* stat. nov. were recovered in each tree with high bootstrap values.

Interestingly, one *O. tenuipes* specimen collected from a *Castanopsis sieboldii* forest in Mt. Tohken, Kagoshima Prefecture, Japan (KPM-NC 27968) had an identical *RPB1* sequence to *O. japonimontana*, which was inferred to be sister to *O. tenuipes* in the *RPB1* tree with moderate BS support. This result was confirmed by sequencing the *RPB1* region of the specimen twice using different primer pairs. Furthermore, one *O. potteri* specimen from Minnesota, USA (*RH1181*; KPM-NC 17828) had at least two heterogeneous *RPB1* sequences, one of which was apparently derived from *O. potteri* but another was remarkably divergent from the other *O. potteri* sequences. The ML phylogeny showed that the divergent sequence from the *RH1181* specimen forms its own clade and is an unknown species-level lineage that is sister to *O. kobayashii* (Fig. 2). It should also be noted that the *TEF1* sequence of a *O. japonimontana* specimen from Mts. Tanzawa, Kanagawa Prefecture (KPM-NC 27623; Fig. 4g) was remarkably divergent from the other *O. japonimontana* sequences (i.e., the *TEF1* identity between the two specimens from Mts. Tanzawa [KPM-NC 27622, 27623] was 98.96% [1051/1060 bp]) despite the high sequence homogeneity of *O. japonimontana* in the other three regions. For comparison, the *TEF1* sequence identity between *O. tenuipes* (INSD acc. no.: MT874813) and *O. durianelloides* (MT874817) was 98.65% (1094/1109 bp.)]

Network analysis based on the multi-locus dataset of subgenus *Octaviana*

The network analysis of the multi-locus dataset supported the *O. tenuipes* specimen with the *RPB1* sequence of *O. japonimontana* (KPM-NC 27968; Fig. 2) in the position of an intermediate lineage between *O. tenuipes* and *O. japonimontana*, showing a high degree of reticulation in the tree (Fig. 3). This relationship was supported with high bootstrap values (86.8–100%). On the other hand, no other clear reticulations suggest recent hybridization among species in subg. *Octaviana*.

Taxonomy

Based on our morphological studies and phylogenetic results (Fig. 1), we describe two new species, *O. tenuipes* and *O. tomentosa*, from Japan. The multi-locus phylogenetic analyses also strongly support *O. potteri* stat. nov. as sister to *O. hesperi* in *O.* subg. *Octaviana* (Fig. 1). Furthermore, our nLSU gene tree

shows that *O. potteri* stat. nov. is phylogenetically distant from *O. asterosperma* var. *asterosperma* (Fig. S1). We conclude that the taxon previously considered as *O. asterosperma* var. *potteri* is a distinct species from *O. asterosperma* s. str., and we propose a new status, *Octaviania potteri*, for the former taxon.

We are aware of the work by Kuo and Ortiz-Santana (2020) that proposed the synonymy of all the sequestrate *Leccinoideae* within a broadly circumscribed genus *Leccinum* s.l.. While the phylogenetic analyses of Kuo and Ortiz-Santana (2020) resolved monophyletic clades for all of the sequestrate genera (e.g., *Octaviania*, *Chamonixia*, *Turmalinea* and *Rossbeevera*), several other major clades of epigeous *Leccinoideae* taxa were otherwise poorly resolved. Based on the lack of resolution in their phylogenetic trees and the uncertainty that remains about the evolutionary relationships within the *Leccinoideae*, we opt to retain the sequestrate genus names *Octaviania*, *Chamonixia*, *Rossbeevera* and *Turmalinea*. We acknowledge that these sequestrate taxa belong to the *Leccinoideae* but feel that synonymy with *Leccinum* is premature, results in the loss of information, and does little to clarify the taxonomy and phylogeny of this group. In our multi-locus phylogeny, some relationships within *Leccinoideae* clades that were unresolved in Kuo and Ortiz-Santana (2020) were resolved with high statistical support (i.e., ML BS $\geq 75\%$ and PP ≥ 0.97 ; Fig. 1). For example, phylogenetic placement of the core clade of *Leccinum* (= *Leccinum* s.str.) was not resolved in Kuo and Ortiz-Santana (2020), whereas our phylogeny supported that *Leccinum* s.str. was sister to the clade comprised of *Leccinellum* s. str., two undefined leccinoid clades, *Rossbeevera* and *Turmalinea* (Fig. 1). Our phylogeny also supported monophyly of the *Leccinellum* s. str. clade, which was not strongly supported in Kuo and Ortiz-Santana (2020). However, we could not include the two independent clades, *Leccinum talamancae* and *L. longicurvipes*, whose phylogenetic positions within the *Leccinoideae* remained uncertain. Our results suggest that we need to further address the phylogeny and systematics of *Leccinoideae* before lumping together all the well-defined epigeous and sequestrate genera into one large and broadly circumscribed genus. We will wait to determine a final taxonomic scheme for the sequestrate genera until a more highly resolved phylogeny becomes available that provides appropriate insight on this group.

Octaviania tenuipes Orihara, **sp. nov.**

Mycobank MB 836874

Figures 4a–f

= *Octaviania* sp. "E", nom. prov., Persoonia 28: 104

Diagnosis: This species is morphologically similar to some species in *Octaviania* subg. *Octaviania*, but is distinguished by the combination of the following characteristics: soft, whitish basidiomata with a more or less developed stipe sometimes exceeding 10 mm long at the base; a thin peridium that is mostly less than 0.5 mm thick; basidiospores $9.3\text{--}(9.5\text{--})13.3\text{--}(15) \times (8.4\text{--})8.5\text{--}11.3\text{--}(11.5) \mu\text{m}$ with acute pyramidal spines that have one simple cavity inside.

Etymology

referring to the slender stipe (sterile base) of the species, which is unique in the genus.

Holotype

JAPAN, Miyazaki Pref., Aya Town, under *Castanopsis sieboldii*, 26 Nov. 2012, leg. T. Orihara & S. Kurogi, KPM-NC 27972.

Description

Basidiomata sequestrate, up to 21 mm in diam., soft, depressed-globose or reniform, surface smooth or slightly floccose, white at first, becoming dirty white to light yellowish brown at maturity, turning immediately wine red when immature specimens are touched, immediately blue when mature specimens are touched, gradually oxidizing further to black, with a more or less developed stipe that sometimes exceeds 10 mm long, becoming conspicuously slender at the bottom. Peridium thin, mostly less than 0.5 mm thick. Gleba whitish in youth, then becoming brown and finally blackish brown, somewhat watery, composed of darker-colored locules filled with basidiospores and whitish mycelial veins. Stipe (sterile base) well developed compared to other typical species of the genus, often with some subhyaline spots inside, occasionally turning red (in immature basidiomata) or blue (in mature basidiomata) when cut. Odor pleasant, sweet.

Basidiospores $9.3\text{--}(9.5\text{--})13.3\text{--}(15) \times (8.4\text{--})8.5\text{--}11.3\text{--}(11.5) \mu\text{m}$, mean $11.3 \times 9.9 \mu\text{m}$ (SD: 1.00 [length], 0.67 [width]), subglobose to broadly ellipsoid ($Q = 1\text{--}1.42$, $Q_m = 1.14$), light yellowish brown to brown, covered with coarse, acute, pyramidal spines $1.1\text{--}3.3 \mu\text{m}$ high and $1\text{--}4.5 \mu\text{m}$ wide with a single, simple cavity inside, spore walls $1\text{--}1.6 \mu\text{m}$ thick. Basidia $21\text{--}32 \times 8\text{--}14 \mu\text{m}$, mean $26 \times 11.1 \mu\text{m}$, clavate, hyaline, 2-, 3- or 4-spored. Hymenium present but poorly developed, comprised of basidia and basidioles. Subhymenium absent, basidia connected to branched filamentous hyphae directly extending from trama. Trama hyaline, of subparallel to loosely interwoven, non-inflated, thin-walled (up to $0.8 \mu\text{m}$) filamentous hyphae $2\text{--}9 \mu\text{m}$ broad. Peridium thin, mostly $100\text{--}400 \mu\text{m}$ and sometimes up to $650 \mu\text{m}$ thick, of densely interwoven, often inflated filamentous hyphae $2\text{--}17 \mu\text{m}$ broad when immature, gradually inflated with age up to $40 \mu\text{m}$ in diam., becoming pseudoparenchymatous cells at maturity, walls $0.5\text{--}1.2 \mu\text{m}$ thick, outermost hyphae pigmented brown to fuscous, somewhat narrower, up to $10 \mu\text{m}$ broad, but not forming a distinct layer. Stipe (sterile base) of compactly interwoven, hyaline, thin-walled, inflated hyphae $3\text{--}22 \mu\text{m}$ broad, partially intermingled with large, irregular-shaped, pseudoparenchymatous cells up to $60 \mu\text{m}$ in diam., walls $0.5\text{--}1.3 \mu\text{m}$ thick. Clamp connections absent in all tissues.

Habitat, distribution and seasonality

hypogeous or subhypogeous under evergreen *Fagaceae*, widely distributed throughout Japan; spring to early summer and autumn to early winter.

Other specimens examined: **JAPAN:** Tokyo Met., Hachioji City, Mt. Takao, M. Nakajima, 7 Sep. 2015, KPM-NC 28187; Hachijo Island, Hachijo Town, along Boh-ei Rd., under *Castanopsis sieboldii*, 31 Oct. 2003, H.

Sasaki 257, KPM-NC 28191; *ibid*, *H. Sasaki 261*, KPM-NC 28192; Hachijo Island, Hachijo Town, Mitsune, Kamogawa Forestry Rd., under *C. sieboldii*, 15 Jul. 2015, *A. Hosono*, KPM-NC 27958; Hachijo Island, Hachijo Town, Mitsune, under *C. sieboldii*, *T. Orihara*, KPM-NC 26008; *ibid*, 29 Jun. 2003, *H. Sasaki 157*, KPM-NC 28190; *ibid*, 2 Jul. 2005, *H. Sasaki 567*, KPM-NC 28193; *Chiba Pref.*, Katsuura City, Okitsu, under *Lithocarpus edulis*, 8 May 2016, *T. Kasuya*, KPM-NC 27956; *Kanagawa Pref.*, Hakone Town, Hakone-yumoto, Soh-un Park, under *C. sieboldii*, 2 Oct. 2016, *leg. T. Orihara*, KPM-NC 27957; Odawara City, Iryuda, near Myoriki-ji Shrine, under *C. sieboldii*, 1 Dec. 2016, *M. Nakajima*, KPM-NC 25370; *Miyazaki Pref.*, Miyazaki City, Tano-cho-otsu, Tano Forest Science Station, Miyazaki Univ., under *C. cuspidata*, 22 Nov. 2012, *T. Orihara*, KPM-NC 27960; Nichinan City, Inohae Valley, 23 Nov. 2012, *T. Orihara*, KPM-NC 27965; *ibid*, under *Q. gilva* and *Q. salicina*, KPM-NC 27964; *Kagoshima Pref.*, Tarumizu City, Mt. Tohken, under *C. sieboldii*, 24 Nov. 2012, *T. Orihara*, KPM-NC 27968; Kimotsuki-gun Minamiosumi Town (the former Sata Town), Nishikata, under *C. sieboldii*, 30 Nov. 2003, *H. Sasaki 306*, KPM-NC 28405; *ibid*, *H. Sasaki 308*, KPM-NC 28406; *ibid*, *H. Sasaki 309*, KPM-NC 28407; *ibid*, *H. Sasaki 310*, KPM-NC 28408; *ibid*, *H. Sasaki 311*, KPM-NC 28409; *ibid*, *H. Sasaki 312*, KPM-NC 28410; Kimotsuki-gun Minamiosumi Town (the former Sata Town), near Kaitaku-iriguchi bus stop, under *C. sieboldii* and *Q. glauca*, *H. Sasaki 317*, KPM-NC 28411; Tanegashima Isl., Nishino-omote City, Anjoh, Ohno Forestry Rd., along Ohkawada River, under *C. sieboldii*, 8 Dec. 2015, *T. Orihara*, KPM-NC 24889; Nishino-omote City, Furuta under *Lithocarpus edulis*, 28 Nov. 2003, *H. Sasaki 301*, KPM-NC 28404; *ibid*, under *C. sieboldii*, 8 Dec. 2015, *T. Orihara*, KPM-NC 24891; Tanegashima Isl., Minamitane Town, Nakanoshita, near Shimonakahachiman Shrine, under *Castanopsis sieboldii*, *Quercus phillyraeoides* and *Lithocarpus edulis*, 28 Nov. 2003, *H. Sasaki 294*, KPM-NC 28401; *ibid*, *H. Sasaki 295*, KPM-NC 28402; Tanegashima Isl., Nakatane Town, Masuda, near Tanegashima Airport, 28 Nov. 2003, *H. Sasaki 298*, KPM-NC 28403; Amami-oshima Isl., Yamato-son, north-eastern foot of Mt Yuwan, under *C. sieboldii* subsp. *lutchuensis*, 17 Nov. 2007, *T. Orihara*, KPM-NC 17813.

Remarks

Orihara et al. (2012a) tentatively described *O. tenuipes* as "*Octaviana* sp. E" because, at that time only one collection of an immature basidioma had been examined and the morphology of this new species was not sufficiently known. This species has now been recorded from subtropical to temperate regions in Japan, associated with *Castanopsis* and evergreen *Quercus* tree species. Morphologically, *O. tenuipes* tends to have a rather slender and well-developed stipe compared to the other species of *Octaviana*. *Octaviana japonimontana*, which is phylogenetically close to *O. tenuipes*, is somewhat similar morphologically, but *O. japonimontana* occurs in deciduous *Fagaceae* forests (with *Q. crispula* and *Fagus* spp.) and tends to have basidiomata with thicker peridia and a more rubbery texture. However, these differences are sometimes inconspicuous so molecular methods are sometimes necessary to confirm the species identification. Another closely related species, *O. durianelloides* Orihara, also resembles *O. tenuipes* when the basidiomata are immature. However, at maturity the basidiomata of *O. durianelloides* have conspicuous brown scales or warts on the surface, which is unique in the genus.

Octaviana tomentosa Orihara, **sp. nov.**

Figure 5

Diagnosis: This species is distinguished from other *Octaviania* species in the combination of the following characteristics: small, soft, felty to tomentose, white to dirty white basidiomata up to 15 mm in diam.; peridium usually very thin (mostly 70–250 μm thick), composed of filamentous hyphae and isodiametric cells with thin cell-walls (up to 0.8 μm thick); Basidiospores 10.2–(11–)14.4(–15) \times 8.6–(8.8–)12.6(–13.2) μm , with acute or sometimes curled, pyramidal spines that have a few slit-like cavities inside.

Etymology

Latin, *tomentosa* (felty or cottony), referring to the tomentose surface of the basidiomata.

Holotype: **JAPAN:** Ibaraki Pref., Kasama City, Mt. Sashiro, under *Q. myrsinifolia*, 9 Sep. 2018, M. Ohmae & T. Orihara, KPM-NC 27954.

Description

Basidiomata sequestrate, up to 15 mm in diam., soft, subglobose, depressed-globose or reniform, surface felty to tomentose, white to dirty white, not becoming yellowish or brownish with age, turning blue or bright red when touched or injured, immature basidiomata tending to turn red rather than blue where touched, after exposure gradually turning black, stipe short, not exceeding 3 mm long, with white rhizomorphs. Peridium usually very thin (less than 0.25 mm) but sometimes variable in thickness, in some cases up to 0.4 mm, context white, showing the same pattern of discoloration as the peridial surface. Gleba beige in youth, becoming brown at maturity, somewhat watery, particularly in young basidiomata, composed of locules filled with yellowish brown to brown basidiospores and whitish mycelial veins, typical of the genus. Stipe (sterile base) context white, sometimes with some subhyaline spots inside. Odor sweet at maturity.

Basidiospores 10.2–(11–)14.4(–15) \times 8.6–(8.8–)12.6(–13.2) μm , mean 12.3 \times 10.6 μm (SD: 1.01 [length], 0.96 [width]), subglobose to broadly ellipsoid ($Q = 1.04\text{--}1.34$, $Q_m = 1.16$), light yellowish brown to ochraceous brown, covered with coarse, acute, sometimes curled, large pyramidal spines 1.6–3.3 μm high and 1.5–4.8 μm wide with a few slit-like cavities inside, spore walls 1.3–3 μm thick, with a long pedicel 6–15.5 \times 1.5–2.2 μm at the base. Basidia 26–39 \times 9–14 μm , mean 32.1 \times 11.1 μm , clavate, colorless, 4-, 2- or more rarely 3-spored. Subhymenium not well developed. Basidia and basidioles randomly extending from hyphae in tramal plates. Tramal plates 15–80 μm thick, of parallel, colorless, thin-walled (up to 0.6 μm) filamentous hyphae 2.5–7 μm broad. Peridium usually 70–250 μm thick, context up to 180 μm thick, colorless, of interwoven, septate, thin-walled (up to 0.6 μm) filamentous hyphae approximately 3–8 μm broad when immature, cells becoming swollen and isodiametric (up to 25 μm in diam.) so that peridial tissue is pseudoparenchyma by maturity, the mature cell walls up to

0.8 µm thick; peridiopellis thin, up to 100 µm across, pigmented yellow-brown, surface turf-like but fragile and easily crushed, of interwoven filamentous hyphae or inflated cells almost the same size as those of inner context (up to 25 µm in diam.). Stipe (sterile base) of compact, interwoven, partially isodiametric, thin-walled (up to 0.8 µm), hyaline hyphae 4–20 µm broad. Clamp connections absent in all tissues.

Habitat, distribution and seasonality

hypogeous or subhypogeous under evergreen *Fagaceae*, found on Amami-oshima Island in the Ryukyu island chain and in eastern Honshu (Kanto region), Japan; summer to autumn.

Specimens examined: **JAPAN**: Kanagawa Prefecture, Minami-ashigara City, Uchiyama, under *Quercus myrsinifolia*, 4 July 2016, T. Orihara, KPM-NC 27945; *ibid*, 3 Sep. 2017, T. Orihara, KPM-NC 27955; *ibid*, 24 Sep. 2018, T. Orihara, KPM-NC 27946; *ibid*, 19 Jul. 2020, Y. Kaneko & T. Orihara, KPM-NC 28415; Ibaraki Pref., Kasama City, Mt. Sashiro, under *Q. myrsinifolia*, 22 Jul. 2017, M. Ohmae, KPM-NC 27953; Tochigi Pref., Sano City, Mt. Karasawa, under *Castanopsis sieboldii*, 24 Jul. 2016, M. Ohmae, KPM-NC 27952; *ibid*, 20 Jul. 2018, M. Ohmae, KPM-NC 27947; Shizuoka Pref., Suntoh District, Oyama Town, Ashigara Pass, 12 Jul. 2020, Y. Kaneko, KPM-NC 28416; *ibid*, 19 Jul. 2020, Y. Kaneko, KPM-NC 28412; *ibid*, Y. Kaneko & T. Orihara, KPM-NC 28413; *ibid*, Y. Kaneko, KPM-NC 28414; Kagoshima Pref., Amami-oshima Isl., Uken-son, Yuwan, under *C. sieboldii* subsp. *lutchuensis*, 29 Jun. 2014, T. Orihara, KPM-NC 23934.

Remarks

This rare species has only been found from four sites in and around the Kanto region in Honshu and from one site in Amami-oshima Island in the Ryukyu island chain despite extensive long-term collecting of *Octaviania* spp. throughout Japan. These two disjunct areas are about 1200 km apart and the climates and vegetation are also quite different between the two areas (temperate evergreen forests on mainland Japan vs. subtropical forests in the Ryukyu Islands). The multi-locus tree (Fig. 1) as well as the single-gene trees (Fig. 2) clearly show generic divergence between the two disjunct lineages. The specimen from Amami-oshima Island had a thicker peridium than the specimens from the Kanto region (ca. 150–400 µm thick in the Amami-oshima specimen vs. 70–250 µm thick in specimens from Kanto). However, we treat these two lineages as infraspecific variations because of the lesser degree of genetic divergence compared to other species-level divergence in both the species tree and individual gene trees (Figs. 1–2). For instance, the sequence similarity of nLSU between the Amami-oshima specimen and the holotype from Honshu is 99.15% (935 bp / 943 bp), whereas the nLSU similarity between holotypes of *O. yaeyamaensis* and *O. etchuensis*, which are genetically the least divergent species within subgenus *Octaviania*, is 98.31% (875 bp / 890 bp). In addition, we cannot infer the potential mating incompatibility between these geographically isolated, but unculturable specimens.

Octaviania tomentosa morphologically resembles *O. hesperi* in the relatively small, whitish basidiomata. However, *O. hesperi* is distinguished from *O. tomentosa* by its slightly larger basidiospores with lower *Q* values (10–15.6(–18.2) × 9.4–(9.9–)14.8(–17.8) µm, mean 12.8 × 12.1 µm; *Q* = 0.96–1.15). *Octaviania hesperi* also has larger pyramidal spore ornamentation (2–(2.1–)3.6(–4) × 1.3–(1.7–)5.4(–5.7) µm) with

irregularly shaped, multiple slits inside (Orihara et al. 2012a). The felty to tomentose surface of the *O. tomentosa* basidiomata is also a distinguishing character that is absent among any of the phylogenetically related species in *O.* subg. *Octaviana*.

Octaviana potteri (Singer & A.H. Sm.) Orihara, Healy, M.E. Sm., **stat. nov.**

MycoBank MB 836876

Figure 6

≡ *Octaviana asterosperma* var. *potteri* Singer & A.H. Sm., *Memoirs of the Torrey Botanical Club* 21 (3): 10 (1959) [MB 353662]

Holotype. **USA**: Michigan, Ithaca, Gratiot, Schovence's Woods on exposed soil along a logging road in rich heavy soil (mud), 17 Sep. 1949, *V. Potter 8898*, MICH 12376.

Description

Basidiomata sequestrate, mostly 8–20 mm in diam., firm, rubbery, subglobose, depressed-globose or reniform, surface smooth or floccose to minutely scaly, white at first then becoming ochraceous at maturity, initially turning red or sometimes greenish blue at the base when touched or injured, gradually turning black, stipe reduced or rudimentary, with white rhizomorphs at the base. Peridium varying in thickness, but mostly not exceeding 0.8 mm thick, context white, showing the same pattern of discoloration as the peridial surface. Gleba brown at maturity, finally becoming blackish brown, composed of locules filled with brown basidiospores and whitish mycelial veins, typical of the genus. Stipe (sterile base) rudimentary to pulvinate, white. Odor sweet to cloyingly sweet at maturity. Taste not recorded.

Basidiospores 9–13.4(–14) × 7.6–12.3(–13.2) μm, mean 11.2 × 9.9 μm (SD: 1.06 [length], 1.14 [width]), subglobose to broadly ellipsoid ($Q = 1–1.42$, $Q_m = 1.13$), dextrinoid, light yellowish brown to ochraceous brown, covered with coarse, large pyramidal spines 1.6–3.4 μm high and 1.6–4.5 μm wide with a single slit-like cavity inside, spore walls 1.4–2.2 μm thick, with a pedicel up to 13 μm long at the base. Basidia 19–35 × 10–15.5 μm, clavate, mostly 2-spored or rarely 3- or 4-spored. Subhymenium not well developed. Tramal plates 30–200 μm thick, hyaline or light yellowish brown, of subparallel to interwoven, inflated, hyaline, filamentous hyphae 3–15 μm broad. Peridium 200–450 μm thick, context hyaline or light yellowish brown, pigmented yellow-brown near surface, of interwoven, inflated filamentous hyphae 3–13 μm broad, pseudoparenchymatous cells up to 45 μm in diam. at maturity, cell walls ca. 1 μm thick; peridiopellis very thin (up to 60 μm thick) or absent in some parts, of partially inflated, septate, filamentous hyphae 3–7 μm broad subparallel to surface. Clamp connections absent in all tissues.

Habitat, known distribution and seasonality

hypogeous or subhypogeous under species of *Fagaceae*; eastern North America (Canada [Quebec], USA [IA, IN, FL, MN, NC, WV]), East Asia (Japan [Hokkaido]), South America (Colombia); summer to autumn.

Other specimens examined: **UNITED STATES OF AMERICA:** Iowa, Boone Co., Ledges State Park, under *Quercus alba*, 20 Sep. 2007, R. Healy RH3, FH 00284316; Emmet Co., Fort Defiance State Park, under *Quercus rubra*, *Ostrya virginiana*, *Tilia americana*, 26 Jul. 2000. R. Healy RH720, ISC-F-0072478; Story Co., Ames, Inis Grove Park, under *Q. alba*, *O. virginiana*, *T. americana*, 18 Aug. 1998, R. Healy RH234, ISC-F-0072476; Ames, YMCA Woods, under *Q. alba*, 21 Sep. 1996, R. Healy, ISC-F-0072477; *ibid*, 25 Jul. 1997, R. Healy RH48 (ISC-F-0072479); *ibid*, 25 Aug. 1999, R. Healy RH555 (ISC-F-0072471); *ibid*, 9 Aug. 2000, R. Healy RH750, ISC-F-0072473; *ibid*, 27 Aug. 2000, R. Healy RH782, ISC-F-0072475; *ibid*, 27 Aug. 2007, R. Healy, FLAS-F-62023; *ibid*, 25 Sep. 2006, E. Braun and Mycology Class, ISC-F-0072472; *ibid*, 6 Sep. 2007, R. Healy RH30, FH 00284311 (duplicate: KPM-NC 17827 & FLAS-F-66562); Hickory Grove Park, under *Quercus macrocarpa* and *T. americana*, on slope by man-made lake, 11 Aug. 2009, R. Healy, FLAS-F-62022; Van Buren Co., Lacey-Keosaqua State Park, under *Q. alba* and *T. americana*, 30 Jul. 2001, L. McCormick, ISC-F-0072474; Indiana, Fort Wayne, 7 Nov. 2014, K. Parker (MES806), FLAS-F-66547; *ibid*, K. Parker (MES807), FLAS-F-66548; Minnesota, Fillmore Co., Forestville State Park, in mixed oak woods, 5 Aug. 2009, E.G. McLaughlin RH973, MIN 912630; *ibid*, 10 Jul. 2010, R. Healy RH1181, KPM-NC 17828 (duplicate: FLAS-F-66563); Rice Co., Nerstrand Big Woods State Park, in mixed oak woods, 8 Aug. 2009, R. Estell RH997, MIN 912622; Washington Co., Afton State Park, in mixed oak woods, 27 Aug. 2009, R. Healy RH1017, MIN912618; *ibid*, D.L. McLaughlin RH1018, MIN912635; North Carolina, McDowell Co., along Blue Ridge Parkway near Mineral Museum, 21 Sep. 2003, T. Elliott (Trappe 32742), FLAS-F-66549; at junction of Jones and Onslow Co., Croatan National Forest, White Oak River, 17 Jul. 2007, T. Elliott (MES801), FLAS-F-66550; Florida, Wakulla Co., Skipper Bay road, St Marks NW refuge, under *Pinus elliottii* and *Q. virginiana*, 29 Dec. 2003, D. Mitchell & W. Roody DMEL04-18 (Trappe 32178), OSC 131925; West Virginia, Randolph Co., Stuarts Park, 19 Sep. 1999, K. St. Louis (Trappe 25908), FLAS-F-66551; McDowell Co., Bervind Wildlife Management Area, 11 Jul. 2002, D. Mitchell (Trappe 27950), FLAS-F-66773; **CANADA:** Quebec, Montreal, 13 Sep. 1991, F. Marzitelli (Trappe 12445), FLAS-F-66546; **JAPAN:** Hokkaido, Tomakomai City, near Kuchinashi-numa Pond, under *Quercus crispula*, 12 Sep. 2011 a, M. Ohmae, KPM-NC 18032; *ibid*, 21 Sep. 2012, K. Yamamoto & T. Orihara, KPM-NC 25043; **COLOMBIA:** Cundinamarca Province, Guacheta, Reserva Natural el Chaute o Robledal, Road from Guacheta to Raquira km 6, under *Quercus humboldtii*, 28 Feb. 2020, A. Corrales 1036, HUA 222100.

Remarks: *Octaviania potteri* was originally described based on a specimen from Michigan, USA (Singer and Smith 1960). The North American specimens have been reported from Quebec province in Eastern Canada and six states in eastern North America (NA). However this taxon has never been collected from western NA despite active ongoing searches for truffles in California and the Pacific Northwest (e.g. Gilkey 1954, Trappe and Castellano 2000, Trappe et al. 2009). Interestingly, this species shows a remarkable disjunct distribution between eastern North America, South America (Colombia) and East Asia (Japan) (Fig. 1). This is the broadest distributional range of any known *Octaviania* species. As far as we know, this is also the first record of *Octaviania* sensu stricto from South America. The mechanism of dispersal of *O. potteri* individuals is worth being investigated from a phylogeographical viewpoint in the

future. Morphologically, it is difficult to characterize this species because most characters of the basidiomata are typical of other taxa in the subgenus *Octaviania*. However, the distinctive dextrinoid reaction of the basidiospores and the very thin peridiopellis (i.e., an outermost filamentous layer of the peridium) that is sometimes absent in patches are two features that are distinct compared to any of the closely related species.

Discussion

Europe was considered the center of biodiversity of the genus *Octaviania* since the first description in 1831 (e.g., Hesse 1891; Pegler & Young 1979; Vittadini 1831) but Orihara et al. (2012a) revealed that this genus is also remarkably diverse in East Asia. Orihara et al. (2012a) also described 11 new species and one species that they provisionally named as "*Octaviania* sp. E". Our study reinforces the high species diversity of *Octaviania* in East Asia by proposing two additional new species, *O. tenuipes* (= "*Octaviania* sp. E") and *O. tomentosa*. Our studies also revealed the new status of *O. potteri*, which was previously known only from eastern North America (Singer & Smith 1960) and is shown here to also occur in East Asia (Japan) and in South America (Colombia). Taking these new results into account, approximately half of the known species of *Octaviania* can be found in Japan (i.e., 14 species). In contrast, only four species are known from North America (Coker and Couch 1928; Orihara et al. 2012a; Singer and Smith 1960; Trappe and Castellano 2000) and approximately six species are currently recognized from Europe (Paz et al. 2016). These results suggest that Japan and the other regions of temperate East Asia are likely the center of diversity for the genus *Octaviania*.

Notably, we also found that the distribution of *O. potteri* extends to a montane dry forest in Colombia dominated by the ectomycorrhizal host tree *Quercus humboldtii*. This is the first record of a true *Octaviania* from South America. Horak (1964) described *Octaviania chilensis* Horak from Chile, but this species was later transferred to *Stephanospora* Pat. in the *Agaricales* (Vidal 2004). Species of *Octaviania* subg. *Octaviania* are always associated with *Fagaceae* trees, and Colombian *Q. humboldtii* is considered to have migrated from Central America via the Isthmus of Panama in the Middle to Late Pleistocene (van der Hammen 1974). Thus, it is most likely that *O. potteri* migrated from North and Central America along with *Q. humboldtii*, the only oak species that is native to South America.

Generic relationships in the *Leccinoideae*, particularly among the genera *Leccinum*, *Leccinellum*, *Chamonixia*, *Octaviania*, *Rossbeevera* and *Turmalinea*, have never been fully resolved with confidence in previous phylogenetic and systematic studies (e.g., Kuo and Ortiz-Santana 2020; Orihara et al. 2012a, 2016a; Wu et al. 2014, 2016). Orihara et al. (2016a) conducted phylogenetic analyses based on three nuclear loci (i.e., ITS, nLSU and *TEF1*), but only the sister relationship between *Rossbeevera* and *Turmalinea* was resolved with strong statistical support (PP \geq 0.98 and ML BS \geq 70%). Kuo and Ortiz-Santana (2020) provided a large-scale, multi-locus phylogeny that focused on epigeous *Leccinum* and *Leccinellum* species based on the nLSU, *TEF1* and *RPB2* regions. The resulting phylogenetic tree showed many polyphyletic clades of *Leccinum* and *Leccinellum* s.l. within *Leccinoideae* and most of their phylogenetic relationships were unresolved. Accordingly, Kuo and Ortiz-Santana (2020) treated all the

epigeous bolete and hypogeous sequestrate taxa in *Leccinoideae* as a broadly circumscribed genus *Leccinum s.l.*. In our study, we incorporated the *RPB1* region as well as ITS, nLSU and *TEF1* into our multi-locus dataset. The resulting trees provided robust phylogenetic support for most of the generic relationships in the *Leccinoideae*. The two exceptions were the lack of resolution in the branching pattern between *Chamonixia* and the other genera as well as the phylogenetic placement of some generic-level clades of epigeous *Leccinoideae* excluded from *Leccinum* and *Leccinellum s.str.* (i.e., *Leccinellum albellum*, *L. quercophilum* and *Leccinum violaceotinctum*; Fig. 1). This multi-locus phylogeny also resolved most of the species-level relationships in *Octaviania*. This exemplifies the usefulness of *RPB1* for phylogenetic studies on *Leccinoideae* and highlights the fact that our multi-locus phylogeny shows promise for resolving the genus-level relationships within the *Leccinoideae* in the near future.

Interestingly, we found that some of the *Octaviania* specimens had heterogeneous *RPB1* sequences compared to the other specimens of the same species. Comparison of the four single-gene tree topologies unexpectedly revealed that one *O. tenuipes* specimen (KPM-NC 27968) had an *RPB1* sequence identical to *O. japonimontana* (Fig. 2). Furthermore, the *RPB1* phylogeny showed that one *O. potteri* specimen from Minnesota (KPM-NC 17828; *RH1181*) was not placed within any of the known species-level clades in *O.* subgenus *Octaviania*. Instead this specimen formed a unique branch that was distant from any species-level clades in the subgenus (Fig. 2). These topological inconsistencies would be most reasonably explained by interspecific hybridization between two closely related species.

Octaviania tenuipes consistently occurs in evergreen *Quercus* and *Castanopsis* forests in subtropical to temperate regions of Japan, whereas *O. japonimontana* occurs in deciduous *Quercus* and *Fagus crenata* forests in mountainous, temperate regions. The possible “hybrid” specimen between *O. tenuipes* and *O. japonimontana* (KPM-NC 27968) was collected in the Takakuma mountain range of Kyushu, Japan, which is known as a southern border of the distribution of *F. crenata*, the potential ectomycorrhizal host of *O. japonimontana*. Although we have not been able to find *O. japonimontana* in that mountain range, it is possible that the two closely related species incidentally inhabited the two adjacent vegetations and hybridized due to minimal putative reproductive barriers between the two species. This potential interspecific hybridization likely occurred very recently because the “hybrid” *O. tenuipes* specimen (KPM-NC 27968) has a conserved *RPB1* sequence of *O. japonimontana* (i.e., 100% identical to the other *O. japonimontana* sequences). The hybrid nature of this specimen was also supported by the network analysis based on the combined dataset of the ITS, nLSU, *RPB1* and *TEF1* regions (Fig. 3). Stukenbrock (2016) summarized that when two allopatric, fungal species come into contact by chance they more readily hybridized than sympatric species, referring to the case of *Neurospora* species (Turner et al. 2011). Leducq et al. (2016) clarified that one North American lineage in *Saccharomyces paradoxus* is an incipient, hybrid species resulting from secondary contact of two geographically isolated, allopatric lineages after the last glaciation. Similarly, Gladioux et al. (2011) showed that hybridization between two closely related European *Microbotryum* species tends to be induced by secondary contact following initial divergence in allopatry. Although genome-level genetic comparisons will be needed to verify a potential hybridization between *O. tenuipes* and *O. japonimontana*, our hypothesis of hybridization is supported by other cases of hybridization in fungi such as those discussed above.

Similarly, the heterogenous *RPB1* sequence in the *O. potteri* specimen from Minnesota (KPM-NC 17828 [RH1181]) is the result of another interspecific hybridization between *O. potteri* and an unknown North American species within subgenus *Octaviania*. So far, no such species closely related to *O. kobayashii* has been described, but our results strongly suggest that there is another undescribed species in this lineage from North America. We assume that this unknown *Octaviania* sp. is sympatric with *O. potteri* or at least they have bordering distributions somewhere in the upper Midwest of eastern North America. Further collecting surveys for truffle-like fungi in this region may confirm the existence of this unknown *Octaviania* species in the future.

In addition, the distinct intraspecific divergence between the *O. japonimontana* KPM-NC 26723 specimen and the other specimens in the *TEF1* phylogeny could be an additional signature of past intraspecific hybridization (Figs. 2 and 4 g). This topological pattern is similar to that of *O. potteri* in the *RPB1* phylogeny, although the genetic distance between the two lineages is less in the case of *O. japonimontana*. Therefore, it is likely that an unknown intraspecific lineage genetically distant to the core *O. japonimontana* clade exists or existed in the recent past around the habitat of the KPM-NC 26723 specimen. Another possible cause of the topological differences is unusually accelerated molecular evolution of the KPM-NC 26723 specimen, but no such clear divergence was recognized in the other three DNA regions and the rate of divergence in the *TEF1* tree seems stable in other species-level clades (Fig. 2). Therefore, accelerated evolutionary rates seems less likely than hybridization. Geographically, the site where this specimen was collected was only about 250 m away from where another *O. japonimontana* specimen (KPM-NC 26723) was collected on the same day, and we did not find any clear morphological differences between these specimens. We will refrain from assigning a new taxonomic status of this intraspecific lineage until specimens that do not show any trace of recent hybridization are collected.

Intra- and inter-specific hybridization in fungi has been frequently documented in plant pathogens (e.g., Brasier 1999; Depotter et al. 2016; Feurtey et al. 2019; Schardl and Craven 2003; Stukenbrock 2016), yeasts (e.g., Gostinčar et al. 2018; Kuehne et al. 2007; Leducq et al. 2016; Marcet-Houben and Gabaldón 2015) and morels (*Morchella* spp.) (Du et al. 2016, 2019, 2020) but has been rarely reported in mushroom-forming basidiomycetes (Anderson et al. 1980; Stenlid and Karlsson 1991). Orihara et al. (2016a) illustrated that the frequent topological incongruences among gene trees of the sequestrate bolete genus, *Rossbeevera*, which is closely related to *Octaviania*, were likely to be derived from intraspecific gene introgression as well as incomplete lineage sorting (ILS). As far as we know, however, the present study is the first case that demonstrated interspecific hybridization in sequestrate basidiomycetes based on molecular evidence. Given the multiple traces of introgression among intraspecific lineages shown by Orihara et al. (2016a) and the interspecific hybridization within *Octaviania* discussed here, it appears that the sequestrate genera in the *Leccinoideae* may be less reproductively isolated when the two lineages have been ecologically isolated (e.g. there is therefore no need for reproductive isolation to reinforce species boundaries). So far, the precise mechanism of these interspecific hybridizations has not been discovered and the ploidy of putative hybrid specimens should be examined based on genome-level comparisons and analyses of chromosomes. We nevertheless

suppose that interspecific hybridization may have promoted high genetic diversity within the sequestrate genera of the subfamily *Leccinoideae*.

Conclusion

The multi-locus phylogeny comprised of ITS and LSU nrDNA, *TEF1* and *RPB1* sequences provided a robust phylogenetic framework of our study and revealed the phylogenetic placement of two new *Octaviania* species, *O. tenuipes* and *O. tomentosa* collected from Japan. In addition to the phylogenetic analyses, critical morphological examination based on recently collected North American specimens and the holotype concluded that *O. asterosperma* var. *potteri* should be treated as an independent species, *O. potteri* stat. nov. Interestingly, *O. potteri* exhibited an unusually broad range of distribution (i.e., North America, Japan and Colombia), and this is the first record of *Octaviania* from South America. Comparison of the four single-gene tree topologies revealed that one *O. tenuipes* specimen had an *RPB1* sequence identical to *O. japonimontana*, and one *O. potteri* specimen from Minnesota had heterogeneous *RPB1* sequences, one of which was proved to be that of an unknown species of *O.* subg. *Octaviania*. The comparison also showed that one *O. japonimontana* specimen had remarkably divergent *TEF1* sequence from the other *O. japonimontana* specimens. Based on these results and potential phylogeography of these species, we consider that these topological inconsistencies are due to inter- and intra-specific hybridization between two phylogenetically closely related lineages. Thus, the high genetic and species diversity of *Octaviania* may partly be due to frequent inter- and intra-specific hybridization. Further genomic comparison among closely related species and precise population genetics will enlighten the speciation and diversification mechanisms within *Octaviania* and other sequestrate genera in the *Leccinoideae*.

Abbreviations

ITS: Nuc rDNA internal transcribed spacer region; nLSU: Nuc rDNA large subunit (28S) region; *TEF1*: translation elongation factor 1- α gene; *RPB1*: the largest subunit of RNA polymerase II gene; INSD: the International Nucleotide Sequence Databases.

Declarations

Acknowledgement

We sincerely appreciate Michael A. Castellano, Shuichi Kurogi, Yumiko Ohba, Joseph W. Spatafora, Kohei Yamamoto, Hiroaki Yamashita and Tatiana Sanjuan for supporting our field trip or facilitating our access to herbarium specimens. We also thank to Redge Estell, Yoshinori Kaneko, Taiga Kasuya, Minoru Nakajima, Laura McCormick, David and Esther McLaughlin, Muneyuki Ohmae, Hiromi Sasaki, Hikaru and Hitomi Yamashita for providing us valuable specimens used for this study. We thank Patricia Rogers for her assistance with a loan of the holotype of *Octaviania asterosperma* var. *potteri* from MICH. Collecting in Colombia was done under the ANLA permit 0530 de 2014.

Authors' contributions

Takamichi Orihara designed the study, implemented the molecular analyses and wrote the manuscript of this paper with supports by Matthew E. Smith and Rosanne Healy. Rosanne Healy collected *O. potteri* specimens from NA and examined holotype of the species, and took part in the molecular work. Adriana Corrales collected the *O. potteri* specimen from Colombia and provided its data to the study. Matthew E. Smith helped to obtain a loan of the *O. potteri* holotype, designed the fieldtrip to Colombia, contributed molecular data for North American specimens of *O. potteri* and thoroughly edited the manuscript.

Funding

This study was financially supported by JSPS KAKENHI Grant-in-Aid for Young Scientists (B) (nos. 17K15184 and 25840149) and the Grant-in-Aid from Institute for Fermentation, Osaka (IFO). Matthew Smith's participation in this work was supported in part by the University of Florida's Institute for Food and Agricultural Sciences (IFAS) and by the NIFA-USDA award FLA-PLP-005289 and McIntire-Stennis project 1011527. Rosanne Healy's participation was supported in part by an award from the Iowa Academy of Sciences, grants from the Iowa Department of Natural Resources, and The Minnesota Department of Natural Resources, and IFAS.

Availability of data and materials

Nucleotide sequences generated for this study were deposited in INSD via NCBI GenBank website (Table 1). The full alignments of datasets for phylogenetic analyses were submitted to TreeBASE and they are available under the following URL: <http://purl.org/phylo/treebase/phylows/study/TB2:S26821>.

Ethics approval and consent to participate

Not applicable

Adherence to national and international regulations

All the experiments and surveys undertaken in this study comply with the current laws of the country where they were performed. The authors declare that they have no conflict with Nagoya Protocol compliances.

Consent for publication

Not applicable

Competing interests

The authors declare no conflicts of interest.

References

1. Anderson JB, Korhonen K, Ullrich RC (1980) Relationships between European and North American biological species of *Armillaria mellea*. *Exp Mycol* 4:87–95
2. Brasier CM, Cook DEL, Duncan JM (1999) Origin of a new *Phytophthora* pathogen through interspecific hybridization. *Proc Natl Acad Sci USA* 96:5878–5883. doi:10.1073/pnas.96.10.5878
3. Castellano MA, Elliott TF, Truong C, Séné O, Dentinger BTM, Henkel TW (2016) *Kombocles bakaiana* gen. sp. nov. (*Boletaceae*), a new sequestrate fungus from Cameroon. *IMA Fungus* 7:239–245. doi:10.5598/imafungus.2016.07.02.03
4. Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 17:540–552
5. Coker WC, Couch JN (1928) *The Gasteromycetes of the Eastern United States and Canada*. University of North Carolina Press, Chapel Hill, North Carolina
6. Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 9:772. doi:10.1038/nmeth.2109
7. Depotter JRL, Seidl MF, Wood TA, Thomma BPHJ (2016) Interspecific hybridization impacts host range and pathogenicity of filamentous microbes. *Curr Opin Microbiol* 32:7–13. doi:10.1016/j.mib.2016.04.005
8. Desjardin DE, Wilson AW, Binder M (2008) *Durianella*, a new gasteroid genus of boletes from Malaysia. *Mycologia* 100:956–961. doi:10.3852/08-062
9. Desjardin DE, Binder M, Roekring S, Flegel T (2009) *Spongiforma*, a new genus of gasteroid boletes from Thailand. *Fungal Divers* 37:1–8
10. Dress AWM, Huson DH (2004) Constructing splits graphs. *IEEE/ACM Transactions in Computational Biology Bioinformatics* 1:109–115. doi:10.1109/TCBB.2004.27
11. Du XH, Wang HC, Sun JJ, Xiong LY, Yu JJ (2019) Hybridization, characterization and transferability of SSRs in the genus *Morchella*. *Fungal Biology* 123:528–538. doi:10.1016/j.funbio.2019.05.005
12. Du XH, Wu DM, Kang H, Wang H, Xu N, Li T, Chen K (2020) Heterothallism and potential hybridization events inferred for twenty-two yellow morel species. *IMA Fungus* 11:4. doi:10.1186/s43008-020-0027-1
13. Du XH, Zhao Q, Xu J, Yang ZL (2016) High inbreeding, limited recombination and divergent evolutionary patterns between two sympatric morel species in China. *Sci Rep* 6:22434. doi:10.1038/srep22434
14. Feurtey A, Stevens DM, Stephan W, Stukenbrock EH (2019) Interspecific Gene Exchange Introduces High Genetic Variability in Crop Pathogen. *Genome Biology Evolution* 11:3095–3105. doi:10.1093/gbe/evz224
15. Galtier N, Gouy M, Gautier C (1996) SEAVIEW and PHYLO_WIN: Two graphic tools for sequence alignment and molecular phylogeny. *Comput Appl Biosci* 12:543–548
16. Geyer CJ (1991) Markov chain Monte Carlo maximum likelihood. In: Keramidas EM (ed) *Computing science and statistics. Proceedings of the 23rd Symposium on the Interface*. Interface Foundation,

Fairfax, VA, pp 156–163

17. Gilkey HM (1954) *Tuberales*. North American Flora Series 2. 1:1–36
18. Gladieux P, Vercken E, Fontaine MC, Hood ME, Jonot O, Couloux A, Giraud T (2011) Maintenance of fungal pathogen species that are specialized to different hosts: allopatric divergence and introgression through secondary contact. *Mol Biol Evol* 28:459–471. doi:10.1093/molbev/msq235. Epub 2010 Sep 13
19. Gostinčar C, Stajich JE, Zupančič J, Zalar P, Gunde-Cimerman N (2018) Genomic evidence for intraspecific hybridization in a clonal and extremely halotolerant yeast. *BMC Genom* 19:364. doi:10.1186/s12864-018-4751-5
20. Gouy M, Guindon S, Gascuel O (2010) SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol* 27:221–224. doi:10.1093/molbev/msp259
21. Guindon S, Gascuel O (2003) A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood. *Syst Biol* 52:696–704
22. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 96/98/NT. *Nucleic Acids Symposium Series* 41:95–98
23. Henkel TW, Obase K, Husbands D, Uehling JK, Bonito G, Aime MC, Smith ME (2016) New *Boletaceae* taxa from Guyana: *Binderoboletus segoi* gen. and sp. nov.
24. *Guyanaporus albipodus* gen. and sp. nov., *Singerocomus rubriflavus* gen. and sp. nov.
25. and a new combination for *Xerocomus inundabilis*. *Mycologia* 108:157–173. doi:10.3852/15-075
26. Hesse R (1891) *Die Hypogaeen Deutschlands: Band I. Die Hymenogastreen*. Verlag L. Hopstetter, Halle
27. Horak E (1964) *Fungi austroamerici IX. Beitrag zur Kenntnis der Gattungen Gautieria Vittad., Martellia Matt. und Octavianina Kuntze in Südamerika (Chile)*. *Sydowia* 17:308–313
28. Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. *Mol Biol Evol* 23:254–267. doi:10.1093/molbev/msj030
29. Izumitsu K, Hatoh K, Sumita T, Kitade Y, Morita A, Tanaka C, Gafur A, Ohta A, Kawai M, Yamanaka T, Neda H, Ota Y (2012) Rapid and simple preparation of mushroom DNA directly from colonies and fruiting bodies for PCR. *Mycoscience* 53:396–401. doi:10.1007/S10267-012-0182-3
30. Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Mol Biol Evol* 30:772–780. doi:10.1093/molbev/mst010
31. Kuehne HA, Murphy HA, Francis CA, Sniegowski PD (2007) Allopatric divergence, secondary contact, and genetic isolation in wild yeast populations. *Curr Biol* 17:407–411. doi:10.1016/j.cub.2006.12.047
32. Kuo M, Ortiz-Santana B (2020) Revision of leccinoid fungi, with emphasis on North American taxa, based on molecular and morphological data. *Mycologia* 112:197–211.

doi:10.1080/00275514.2019.1685351

33. Lebel T, Orihara T, Maekawa N (2012a) The sequestrate genus *Rosbeeva* T. Lebel & Orihara gen. nov. (*Boletaceae*) from Australasia and Japan: new species and new combinations. *Fungal Divers* 52:49–71
34. Lebel T, Orihara T, Maekawa N (2012b) Erratum to: the sequestrate genus *Rosbeevera* T. Lebel & Orihara gen. nov. (*Boletaceae*) from Australasia and Japan: new species and new combinations. *Fungal Divers* 52:73
35. Leducq JB, Nielly-Thibault L, Charron G, Eberlein C, Verta JP et al. (2016)
36. Speciation driven by hybridization and chromosomal plasticity in a wild yeast. *Nature Microbiology* 1:15003. doi:10.1038/NMICROBIOL.2015.3
37. Matheny PB, Liu YJ, Ammirati JF, Hall BD (2002) Using RPB1 sequences to improve phylogenetic inference among mushrooms (*Inocybe*, *Agaricales*). *Am J Bot* 89:688–698
38. Marcet-Houben M, Gabaldón T (2015) Beyond the whole-genome duplication: phylogenetic evidence for an ancient interspecies hybridization in the Baker's yeast lineage. *PLOS Biology* 13:e1002220. doi:10.1371/journal.pbio.1002220
39. Nuhn ME, Binder M, Taylor AF, Halling RE, Hibbett DS (2013) Phylogenetic overview of the Boletineae. *Fungal Biology* 117:479–511. doi:10.1016/j.funbio.2013.04.008
40. Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre. Uppsala University, Uppsala
41. Orihara T, Sawada F, Ikeda S, Yamato M, Tanaka C, Shimomura N, Hashiya M, Iwase K (2010) Taxonomic reconsideration of a sequestrate fungus, *Octaviania columellifera*, with the proposal of a new genus, *Heliogaster*, and its phylogenetic relationships in the *Boletales*. *Mycologia* 102:108–121. doi:10.3852/08-168
42. Orihara T, Smith ME, Shimomura N, Iwase K, Maekawa N (2012a) Diversity and systematics of the sequestrate genus *Octaviania* in Japan: two new subgenera and eleven new species. *Persoonia* 28:85–112. doi:10.3767/003158512X650121
43. Orihara T, Smith ME, Ge Z-W, Maekawa N (2012b) *Rosbeevera yunnanensis* (*Boletaceae*, *Boletales*), a new sequestrate species from southern China. *Mycotaxon* 120:139–147. doi:10.5248/120.139
44. Orihara T, Lebel T, Ge Z-W, Smith ME, Maekawa N (2016a) Evolutionary history of the sequestrate genus *Rosbeevera* (*Boletaceae*) reveals a new genus *Turmalinea* and highlights the utility of ITS minisatellite-like insertions for molecular identification. *Persoonia* 37:173–198. doi:10.3767/003158516X691212
45. Orihara T, Ohmae M, Yamamoto K (2016b) First report of *Chamonixia caespitosa* (*Boletaceae*, *Boletales*) from Japan and its phylogeographic significance. *Mycoscience* 57:58–63. doi:10.1016/j.myc.2015.08.005
46. Orihara T, Smith ME (2017) Unique phylogenetic position of the African truffle-like fungus, *Octaviania ivoryana* (*Boletaceae*, *Boletales*) and the proposal of a new genus, *Afrocastellanoa* *Mycologia* 109:323–332. doi:10.1080/00275514.2017.1301750

47. Paz A, Vidal JM, Lavoise C, Moreau P-A (2014) Primeros datos para una revisión del género *Octaviania* en Europa: *O. depauperata* comb. & stat. nov., *O. depauperata* var. *laurarum* var. nov. y *O. vacekii* sp. nov. Boletín Micológico de FAMCAL 9:77–97
48. Paz A, Vidal JM, Lavoise C, Moreau P-A (2016) Revisión taxonómica del género *Octaviania* (*Boletales*) en Europa. Boletín Micológico de FAMCAL 9:101–138
49. Pegler DN, Young TWK (1979) The gasteroid *Russulales*. Transactions of the British Mycological Society 72:353–388
50. Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference
51. under mixed models. Bioinformatics 19:1572–1574. doi: 10.1093/bioinformatics/btg180
52. Schardl CL, Craven KD (2003) Interspecific hybridization in plant-associated fungi and
53. oomycetes: a review. Molecular Ecology 12:2861–2873. doi:10.1046/j.1365-294x.2003.01965.x
54. Singer R, Smith AH (1960) Studies on secotiaceous fungi IX. The astrogaceous
55. series. Memoirs of the Torrey Botanical Club. 21:1–112
56. 10.5598/ima fungus.2015.06.02.03
Smith ME, Amses KR, Elliott TF, Obase K, Aime MC, Henkel TW (2015) New sequestrate fungi from Guyana: *Jimtrappea guyanensis* gen. sp. nov., *Castellanea pakaraimophila* gen. sp. nov., and *Costatisporus cyanescens* gen. sp. nov. (*Boletaceae*, *Boletales*). IMA Fungus 6:297–317. doi:10.5598/ima fungus.2015.06.02.03
57. Smith ME, Castellano MA, Frank JL (2018) *Hymenogaster macmurphyi* and *Splanchnomyces behrii* are sequestrate species of *Xerocomellus* from the western United
58. States. Mycologia 110:605–617. doi:10.1080/00275514.2018.1465299
59. Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690. doi:10.1093/bioinformatics/btl446
60. Stenlid J, Karlsson J-O (1991) Partial intersterility in *Heterobasidion annosum*. Mycol Res 95:1153–1159
61. Stukenbrock EH (2016) The role of hybridization in the evolution and emergence of new fungal plant pathogens. Phytopathology 106:104–112. doi:10.1094/PHYTO-08-15-0184-RVW
62. Sulzbacher MA, Orihara T, Grebenc T, Wartchow F, Smith ME, Martin MP, Giachini AJ, Baseia IG (2020) *Longistriata flava* (*Boletaceae*, *Basidiomycota*) - a new monotypic sequestrate genus and species from Brazilian Atlantic Forest. Mycokeys 62:53–73. doi:10.3897/mycokeys.62.39699
63. Trappe JM, Castellano MA (2000) New sequestrate Ascomycota and Basidiomycota covered by the Northwest Forest Plan. Mycotaxon 75:153–179
64. Trappe JM, Molina R, Luoma DL, Cázares E, Pilz D, Smith JE, Castellano MA, Miller SL, Trappe MJ (2009) Diversity, ecology, and conservation of truffle fungi in forests of the Pacific Northwest. Gen. Tech. Rep. PNW-GTR-772. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. 194 p. http://www.fs.fed.us/pnw/pubs/pnw_gtr772.pdf

65. Turner E, Jacobson DJ, Taylor JW (2011) Genetic architecture of a reinforced, postmating, reproductive isolation barrier between *Neurospora* species indicates evolution via natural selection. *PLoS Genet* 7:e1002204. doi:10.1371/journal.pgen.1002204
66. Vadthanarat S, Raspé O, Lumyong S (2018) Phylogenetic affinities of the sequestrate genus *Rhodactina* (*Boletaceae*), with a new species. *R rostratispora* from Thailand *Myckeys* 29:63–80. doi:10.3897/mycokeys.29.22572
67. van der Hammen T (1974) The Pleistocene changes of vegetation and climate in tropical South America. *J Biogeogr* 1:3–26
68. Vidal JM (2004) The Genus *Stephanospora* Pat., two new combinations. *Revista Catalana de Micologia* 26:97–111
69. Vittadini C (1831) *Monographia Tubercearum*. Mediolani, Milano
70. Wu G, Feng B, Xu J, Zhu XT, Li YC, Zeng NK, Hosen MI, Yang ZL (2014) Molecular phylogenetic analyses redefine seven major clades and reveal 22 new generic clades in the fungal family *Boletaceae*. *Fungal Divers* 69:93–115. doi:10.1007/s13225-014-0283-8
71. Wu G, Li YC, Zhu XT, Zhao K, Han LH, Cui YY, Li F, Xu JP, Yang ZL (2016) One hundred noteworthy boletes from China. *Fungal Divers* 81:25–188. doi:10.1007/s13225-016-0375-8
72. Wu G, Lee SML, Horak E, Yang ZL (2018) *Spongispora temasekensis*, a new boletoid genus and species from Singapore. *Mycologia* 110:919–929. doi:10.1080/00275514.2018.1496387
73. Yanaga K, Maekawa N, Shimomura N, Ishigaki Y, Nakamura Y, Takegami T, Tomosugi N, Miyazawa S, Kuwabata S (2012) Use of ionic liquid in fungal taxonomic study of ultrastructure of basidiospore ornamentation. *Mycological Progress* 11:343–347. doi:10.1007/s11557-011-0794-6
74. Yang ZL, Trappe JM, Binder M, Sanmee R, Lumyong P, Lumyong S (2006) The sequestrate genus *Rhodactina* (*Basidiomycota*, *Boletales*) in northern Thailand. *Mycotaxon* 96:133–140

Tables

Due to technical limitations, table 1 and 2 are only available as a download in the Supplemental Files section.

Figures

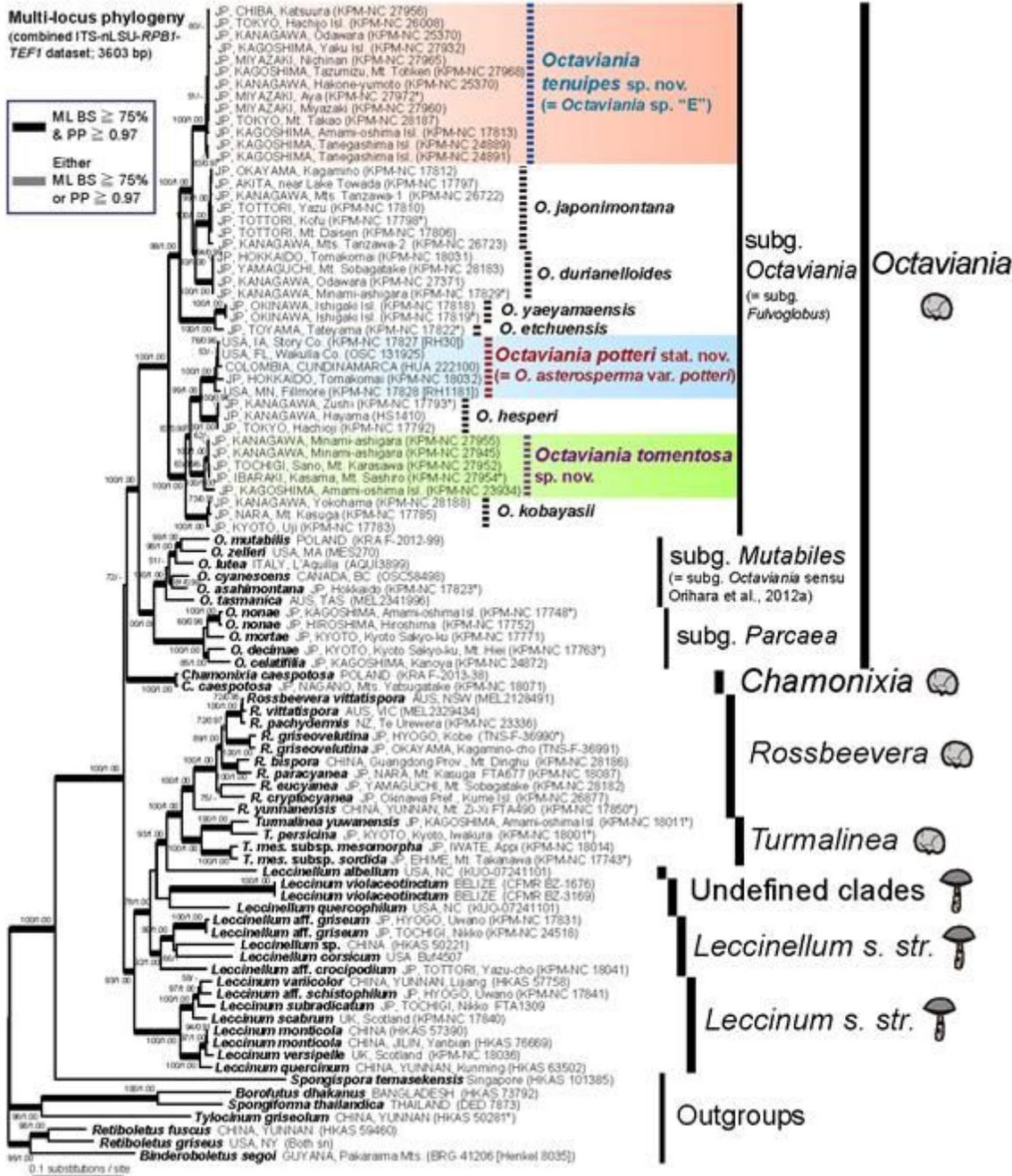


Figure 1

Maximum likelihood (ML) tree of the Leccinoideae inferred from the multi-locus (ITS and LSU nrDNA, RPB1 and TEF1) dataset. Branches supported by both BS \geq 75% and PP \geq 0.97 and are depicted as thickened black lines. Branches supported by either BS \geq 75% or PP \geq 0.97 are shown as thickened gray lines. Statistical values below BS < 50% or PP < 0.90 are not shown. Holotype materials are designated with asterisks (*). Taxa outside of the core clade of Leccinoideae are used as outgroups.

Phylogenetic network of the combined multi-locus (ITS and LSU nrDNA, RPB1 and TEF1) dataset of *O.* subg. *Octaviana* constructed by the NeighborNet method and displayed with the EqualAngle algorithm (Dress and Huson 2004). BS values below 50% are not shown. Holotype materials are designated with asterisks (*).

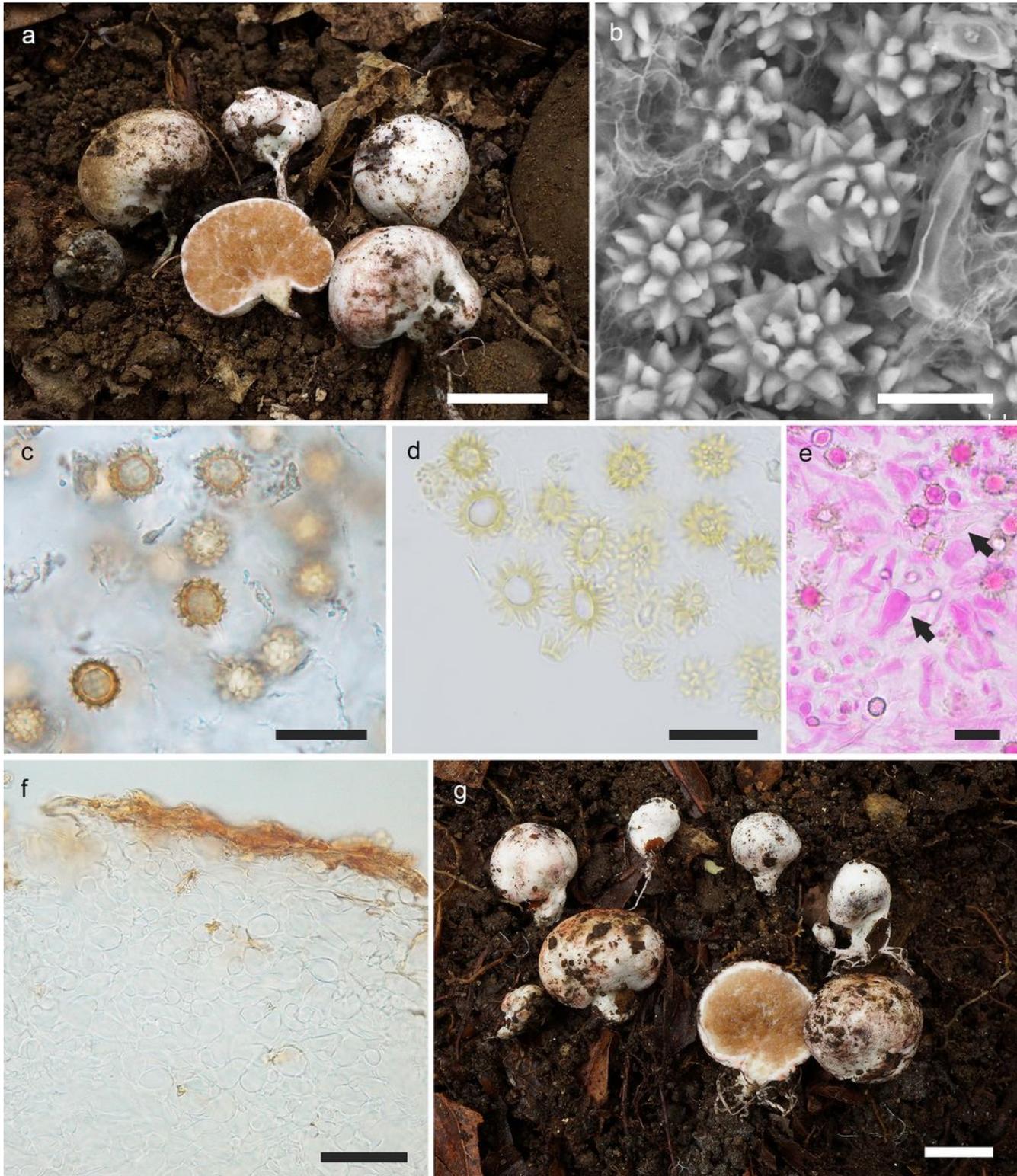


Figure 4

a–f *Octaviania tenuipes*. a Basidiomata (holotype [KPM-NC 27972]). b Basidiospores under SEM (holotype). c Basidiospores mounted in water (KPM-NC 27957). d Basidiospores with elongated ornamentation mounted in lactic acid after pre-soaking in 3% KOH (holotype). e Basidia (arrows) and basidiospores mounted in 3% KOH after staining with 1% phloxine (holotype). f Peridium (holotype). g Basidiomata of *O. japonimontana* (KPM-NC 27623). Topological comparison among gene trees reveals that this specimen has a remarkably divergent TEF1 sequence from those of other *O. japonimontana* specimens (Fig. 2). Scale bars: a, g = 1 cm, b = 10 μ m, c–e = 20 μ m, f = 50 μ m.

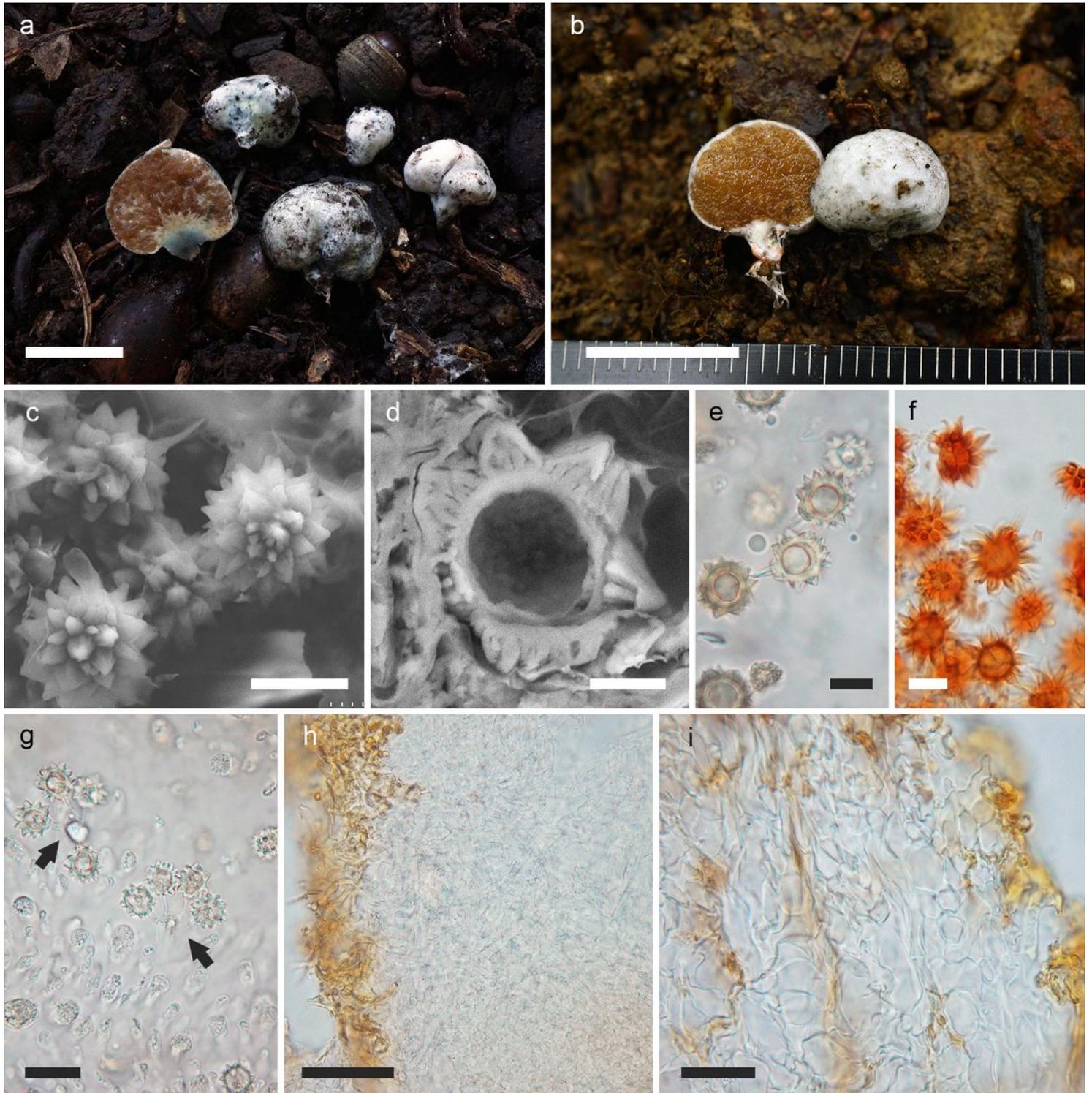


Figure 5

Octaviania tomentosa. a–b Basidiomata (a holotype from central Honshu, Japan [KPM-NC 27954]; b specimen from Amami-oshima Island, Japan [KPM-NC 23934]). c Basidiospores under SEM (holotype). d section of a basidiospore showing multiple slit-like cavities inside the spiny ornaments. e Basidiospores mounted in water (KPM-NC 27945). f Dextrinoid basidiospores with elongated ornamentation mounted in lactic acid after pre-soaking in Melzer’s reagent (KPM-NC 23934). g Basidiospores connected to 4-spored basidia (arrows; KPM-NC 27955). h Peridium of an immature basidiome (KPM-NC 27955). i Peridium of a mature basidiome (KPM-NC 27953). Scale bars: a–b = 1 cm, c, e–f = 10 μ m, d = 5 μ m, g, i = 20 μ m, h = 50 μ m.

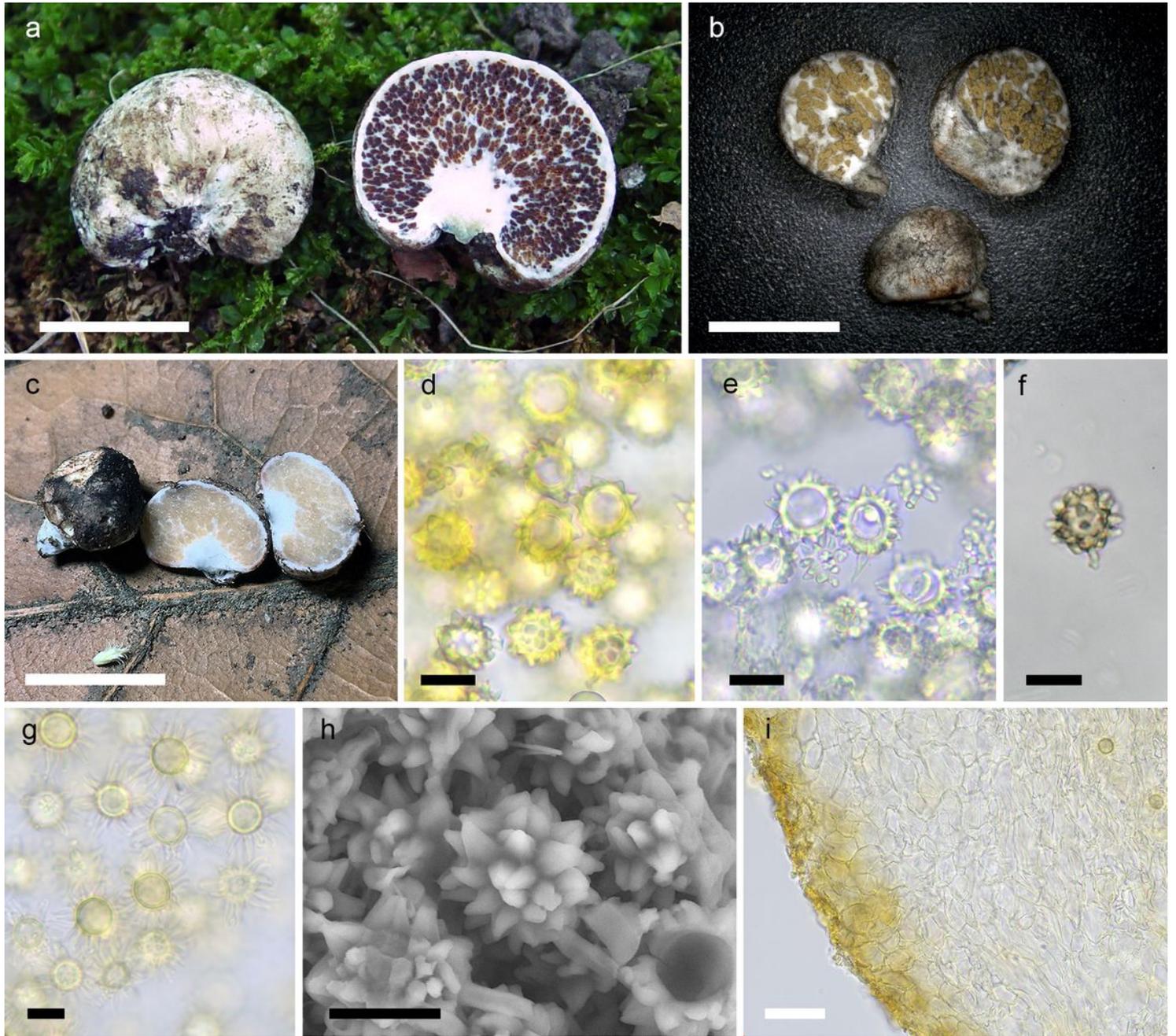


Figure 6

Octaviania potteri. a–c Basidiomata (a FH 00284311 [RH30; duplicate: KPM-NC 17827] from Iowa, USA; b KPM-NC 18032 from Hokkaido, Japan; c HUA 222100 from Cundinamarca Province, Colombia). d–f

Basidiospores mounted in water (d KPM-NC 17827 [RH30]; e KPM-NC 17828 [RH1181; a potential hybrid specimen between *O. potteri* and a closely related unknown species]; f holotype [MICH 12376]). g Basidiospores mounted in lacto-glycerol after pre-soaking in 3% KOH and water (MES807 [FLAS-F-66548]). h Basidiospores under SEM (FLAS-F-62022). i Peridium (MES807 [FLAS-F-66548]). Scale bars: a, c = 1 cm, b = 5 mm, d–h = 10 μ m, i = 50 μ m.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1Specimensforphylogeneticanalyses.xlsx](#)
- [SupplFigS1OctavianaRaxMLLSUOriharaetal..pdf](#)
- [Table2ComparisonofsporesofOctavianiapotteri.xlsx](#)