



# Progress on the phylogeny of the Omphalotaceae: *Gymnopus* s. str., *Marasmiellus* s. str., *Paragymnopus* gen. nov. and *Pusillomyces* gen. nov.

Jadson J. S. Oliveira<sup>1,2</sup> · Ruby Vargas-Isla<sup>2</sup> · Tiara S. Cabral<sup>2,3</sup> · Doriane P. Rodrigues<sup>4</sup> · Noemia K. Ishikawa<sup>1,2</sup>

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## Abstract

Omphalotaceae is the family of widely distributed and morphologically diverse marasmioid and gymnopoid agaric genera. Phylogenetic studies have included the family in Agaricales, grouping many traditionally and recently described genera of saprotrophic or parasitic mushroom-producing fungi. However, some genera in Omphalotaceae have not reached a stable concept that reflects monophyletic groups with identifiable morphological circumscription. This is the case of *Gymnopus* and *Marasmiellus*, which have been the target of two opposing views: (1) a more inclusive *Gymnopus* encompassing *Marasmiellus*, or (2) a more restricted *Gymnopus* (s. str.) while *Marasmiellus* remains a distinct genus; both genera still await a more conclusive phylogenetic hypothesis coupled with morphological recognition. Furthermore, some new genera or undefined clades need more study. In the present paper, a phylogenetic study was conducted based on nrITS and nrLSU in single and multilocus analyses including members of the Omphalotaceae, more specifically of the genera belonging to the *Letinuloid* clade. The resulting trees support the view of a more restricted *Gymnopus* and a distinct *Marasmiellus* based on monophyletic and strongly supported clades on which their morphological circumscriptions and taxonomic treatments are proposed herein. The results also provide evidence for the description of two new genera: *Paragymnopus* and *Pusillomyces*. *Pusillomyces manuripioides* sp. nov. (type species of the genus) is described with morphological description, taxonomic and ecological remarks, and illustrations.

**Keywords** Agaricales · Amazon forest · Marasmioid · Gymnopoid · Plant pathogen · Taxonomy

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✉ Jadson J. S. Oliveira  
oliveira.j.j.s.86@gmail.com

<sup>1</sup> Divisão do Curso de Pós-graduação em Botânica – DIBOT, Instituto Nacional de Pesquisas da Amazônia – INPA, Av. André Araújo 2936, Manaus, AM 69067-375, Brazil

<sup>2</sup> Coordenação de Biodiversidade – COBIO, Instituto Nacional de Pesquisas da Amazônia – INPA, Av. André Araújo 2936, Manaus, AM 69067-375, Brazil

<sup>3</sup> Divisão do Curso de Pós-graduação em Genética, Conservação e Biologia Evolutiva – DIGEN, Instituto Nacional de Pesquisas da Amazônia – INPA, Av. André Araújo 2936, Manaus, AM 69067-375, Brazil

<sup>4</sup> Laboratório de Evolução Aplicada, BLM, Divisão de Biotecnologia, Instituto de Ciências Biológicas, Universidade Federal do Amazonas, Av. General Rodrigo Otávio Jordão Ramos 3000, Manaus, AM 69077-000, Brazil

## Introduction

Several groups in the euagarics have been the target of important changes in their classification as multiple molecular phylogenetic studies have shaped new systematic understanding in the Agaricales (Moncalvo et al. 2000, 2002; Binder and Hibbett 2002; Matheny et al. 2006; Dentinger et al. 2016). One of the groups of this order that has undergone changes, the family Omphalotaceae was initially proposed within the Boletales (sensu Kühner) by Kämmerer et al. (1985) based on the typical sesquiterpenes and the ability to cause white-rot and including genera such as *Omphalotus* Fayod and *Lampteromyces* Singer. Additionally, many current members of Omphalotaceae were classified in Tricholomataceae in Singer's system (Singer 1986) and in Marasmiaceae in Kühner's system (Kühner 1980). However, based on morphology, Halling (1996) and Antonín et al. (1997) recombined several species or transferred complete sections out of *Collybia* (Fr.) Staude to *Gymnopus* (Pers.) Roussel.

The family, as currently accepted, was classified in the Agaricales based on phylogenetic studies and *Collybia* was shown to be polyphyletic (Moncalvo et al. 2000, 2002, Matheny et al. 2006). *Collybia dryophila* (Bull.) P. Kumm. and *C. polyphylla* (Peck) Singer ex Halling [combined in *Gymnopus* in Murrill (1916) and Antonín et al. (1997), respectively], and *C. maculata* (Alb. & Schwein.) P. Kumm. [combined in *Rhodocollybia* in Singer (1939)] grouped in the “clade A” (Moncalvo et al. 2000) that corresponds to the clade /omphalotaceae in Moncalvo et al. (2002), which is unrelated to the clade /collybia. In Matheny et al. (2006), the clade Omphalotaceae [equivalent to /omphalotaceae in Moncalvo et al. (2002)] grouped within the Marasmioid major clade (IV), but *C. tuberosa* (type of *Collybia*) branched in the subclade Clitocybeae [*Collybia* - *Clitocybe* group including /collybia in Moncalvo et al. (2002)] within the Tricholomatoid major clade (V). *Gymnopus* branched as a member of the clade Omphalotaceae showing no close relatedness with *Collybia* s.str. The clade Omphalotaceae grouped as sister to the clade Marasmiaceae with strong support in the same major clade (Matheny et al. 2006) and in the suborder Marasmiineae (Dentinger et al. 2016).

However, *Gymnopus* branched as multiple, non-monophyletic groups as also did *Marasmiellus* Murrill (Moncalvo et al. 2002; Mata et al. 2004a; Wilson and Desjardin 2005; Hughes et al. 2010; Petersen and Hughes 2017), this latter historically segregated from *Marasmius* Fr. [see Mata et al. (2004a), Wilson and Desjardin (2005) and Antonín and Noordeloos (2010)]. Based on analysis of nrITS data, Mata et al. (2004a) argued that, by placing *Marasmiellus juniperinus* Murrill (type species of the genus) within a clade dominated by accepted *Gymnopus* taxa, either *Gymnopus* would be monophyletic including *Marasmiellus* as a synonym, or the limited clade to which *M. juniperinus* belongs would be a segregated genus (*Marasmiellus* sensu Wilson and Desjardin (2003) or *Collybiopsis* Earle) and *Gymnopus* would be polyphyletic. Mata et al. (2004a) accepted the first scenario, proposing the combination of *M. juniperinus* in a broad concept of *Gymnopus*.

However, Wilson and Desjardin (2005) argued for keeping *Marasmiellus* and *Gymnopus* as distinct genera. Their results based on nrLSU data revealed an unsupported, but discrete clade grouping *M. juniperinus* and *Marasmiellus synodicus* (Kunze) Singer (sect. *Dealbati*), also including many taxa of *Gymnopus* sect. *Vestipedes*. They named the clade /marasmiellus, which was not closely related to their clade /gymnopus, this latter including *Gymnopus fusipes* (Bull.) Gray (type species) along with other taxa of the genus, as well as *Setulipes androsaceus* (L.) Antonín (type of *Setulipes*) and *Micromphale* Gray taxa [among them the type *M. foetidum* (Sowerby) Singer]. They concluded that until further species belonging to *Gymnopus* sect. *Vestipedes* and *Levipedes*, *Marasmiellus* (all sections) and *Setulipes* are added to the

analyses, they do not support recognition of a more inclusive *Gymnopus* [sensu Mata et al. (2004a)]. They suggested that *Marasmiellus* s. str. could accommodate the members of *Gymnopus* sect. *Vestipedes* (i.e., /marasmiellus). Another argument was that by including *Marasmiellus* within a broad *Gymnopus*, then *Rhodocollybia*, *Lentinula* and *Mycetinis* Earle also would have to be accepted as synonyms of *Gymnopus* and then it would be monophyletic (Wilson and Desjardin 2005). Moreover, other *Marasmiellus* representatives grouped in other multiple unrelated positions.

Despite these arguments, the broad concept of *Gymnopus*—encompassing *Marasmiellus*, *Micromphale*, and *Mycetinis*—was kept by Mata et al. (2006), using the most comprehensive sampling of nrITS sequence data for gymnopoid/marasmielloid taxa to date. This broad *Gymnopus* was represented by the clade A–N whereas it is possible to observe two major clades: (1) a superclade grouping the clades A–C, and (2) superclade D consisting of subclades E–N. In comparison with Wilson and Desjardin (2005), the first major clade can correspond to /gymnopus and the second (excluding current *Mycetinis* species) to /marasmiellus. Mata et al. (2006) also observed that their clade D is populated predominantly by members of *Gymnopus* sect. *Vestipedes* and corresponded to such section.

*Micromphale* also had problematic phylogenetic relationships (Moncalvo et al. 2002; Mata et al. 2004a, 2006; Wilson and Desjardin 2005; Petersen and Hughes 2016). See more about the taxonomy of *Micromphale* in Antonín and Noordeloos (2010) and Petersen and Hughes (2016). Petersen and Hughes (2016) focused more on the uncertain phylogenetic position of *Micromphale perforans* (Hoffm.) Gray, analyzing it along with other taxa that could be considered in *Micromphale* sect. *Perforantia* with respect to the traditional taxonomy. They found a distinct and monophyletic clade grouping *M. perforans*, *Gymnopus foliophilus* R.H. Petersen, *G. pinophilus* R.H. Petersen, *G. ponderosae* R.H. Petersen, *G. sequoiae* (Desjardin) R.H. Petersen, and *G. sublaccatus* R.H. Petersen, as sister (with very weak support) to a clade dominated by *Gymnopus* spp. corresponding to /gymnopus in Wilson and Desjardin (2005). According to the broad view of Mata et al. (2004a), using nrLSU for the large tree (Fig. 85 in their study), Petersen and Hughes (2016) combined *Micromphale* sect. *Perforantia* in *Gymnopus* sect. *Perforantia*.

Hughes et al. (2010) proposed the new genus *Connopus* R.H. Petersen to accommodate *Gymnopus acervatus* (Fr.) Murrill, based on nrITS or nrLSU data, finding it somewhat closely related to *Rhodocollybia* taxa likewise in Mata et al. (2006). Dutta et al. (2015) also used nrITS or nrLSU data in singlelocus analyses for the phylogenetic placement of their new species, *Marasmiellus foliophilus* A.K. Dutta, K. Acharya & Antonín, and records of four *Gymnopus* spp. based on collections from India. *Marasmiellus foliophilus* grouped in the moderately supported clade A (Fig. 6a in their study) which

corresponds to the clade /marasmiellus (Wilson and Desjardin 2005). Sandoval-Leiva et al. (2016), based on ITS–5′–28S rDNA, provided a more resolved tree of various marasmioid and gymnopoid genera [Marasmioid clade of Matheny et al. (2006)] and proposed the new and monotypic genus *Gymnopanella* Sandoval-Leiva, J.V. McDonald & Thorn. The pleurotoid, flabelliform to reniform *Gymnopanella nothofagi* Sandoval-Leiva, J.V. McDonald, and Thorn was sister to the clade named “*Gymnopus*, including *Setulipes*” [corresponding to /gymnopus in Wilson and Desjardin (2005)], but with no statistical support. They also found the clade /marasmiellus, corresponding to /marasmiellus in Wilson and Desjardin (2005), but with good statistical support. They did not discuss this finding though.

Petersen and Hughes (2017) conducted an investigation on *Mycetinis*, also segregated from *Marasmius*. The genus was resurrected by Wilson and Desjardin (2005) based on a monophyletic group formed by members of the previous *Marasmius* sect. *Alliacei* (Kühner 1933; Singer 1976, 1986). This clade was distinct and strongly supported, named “clade F” within the “clade A” assigned to Omphalotaceae. In the *Gymnopus* phylogeny with related clades/genera, Petersen and Hughes (2017) also observed two major clades, one with numerous *Gymnopus* taxa, including *G. fusipes*, *G. androsaceus* (L.) Della Magg. & Trassin. (previously *Setulipes*), and *Gymnopus* sect. *Perforantia*, and the second also grouping several *Gymnopus* species, and additional clades treated as *Connopus*, *Lentinula*, *Marasmius pallidocephalus* Gilliam (probably a monotypic genus), and *Rhodocollybia*. They also included *Gymnopanella* as part of this second major clade.

These studies approached groups within Omphalotaceae with single locus phylogenetic analyses using nrLSU or nrITS, except for Sandoval-Leiva et al. (2016) and Petersen and Hughes (2016). Sandoval-Leiva et al. (2016) used the combined ITS–5′–28S rDNA of a broad dataset composed of members of Omphalotaceae (broader sampling), Marasmiaceae, Physalacriaceae, and the outgroup formed by Pleurotaceae, *Panellus* P. Karst., and Entolomataceae strains, recovering a relatively well-resolved tree with many main clades strongly supported. However, their analyses (Bayesian and neighbor-joining) were apparently not partitioned and they only focused on the establishment of *Gymnopanella*, distinguishing it (phylogenetically and morphologically) from other genera. Petersen and Hughes (2016) used combined nrITS and nrLSU only for *Gymnopus* sect. *Perforantia* taxa (Fig. 86 in their study). Their restricted analyses were apparently not partitioned and only dealt with species level comparison within the section.

According to MycoBank (Robert et al. 2013), Omphalotaceae has currently ten associated genera: *Anthracophyllum* Ces., *Caripia* Kuntze, *Gymnopanella*, *Gymnopus*, *Lentinula*, *Marasmiellus*, *Mycetinis*, *Neonothopanus* R.H. Petersen & Krisai, *Omphalotus*, and *Rhodocollybia* (all included in various

phylogenetic studies during these approximately past two decades). We do accept the combination of *Micromphale* in *Gymnopus* (but see the results of this study), and it would be probably the same for *Caripia*, awaiting more study. We also defend *Mycetinis* as a distinct genus, in agreement with Wilson and Desjardin (2005) and Petersen and Hughes (2017).

Initially, this study began with the intent to infer the phylogenetic affiliation of an unusual fungal species (very similar to *Manuripia* Singer) collected in the central Amazonian tropical forest (Amazonas, Brazil), but with distinct micromorphological and ecological characteristics. LSU sequences (also combined with ITS) were used to determine in which marasmioid family this taxon was a member. The results from preliminary analyses not only indicated that this fungus is member of a new genus but also led to a partial revision of the Omphalotaceae, the family of the new fungus. This partial revision addresses the question of which view of *Gymnopus* would reflect more the natural systematics within the family: (i) the more inclusive *Gymnopus* sensu Mata et al. (2004a, 2006), or (ii) a more restricted *Gymnopus* suggested by Wilson and Desjardin (2005). Also, this study allowed the evaluation of the recently proposed genera, sections, and informal clades. Thus, we conducted thorough and broad Bayesian and maximum likelihood analyses to reconstruct phylogenies in Omphalotaceae, with more emphasis on the groups of the clade /lentinuloid (Moncalvo et al. 2002), using combined nrITS and nrLSU (multilocus) in partitioned processes, along with separate single locus analyses as explained in the methodology. The results include the description of the plant pathogen *Pusillomyces manuripioides* sp. nov. et gen. nov., *Paragymnopus* gen. nov. and a solution to the case of *Gymnopus* and *Marasmiellus*. Phylogenetic trees are provided, and morphological description, taxonomic and ecological comments, and illustrations of the new Amazonian species ascribed to Omphalotaceae.

## Material and methods

### Areas sampled

The holotype and 11 additional specimens of *Pusillomyces manuripioides* sp. nov. were collected from the “Reserva Biológica de Campina” (RBC), Km 60 on the road BR-174, between Manaus and Boa Vista districts. The area has 900 ha of typical “Campina” and “Campinarana” (Online Resource – Fig. O6a) vegetation types, the former characterized by sparse shrubby and herbaceous vegetation on sandy white soil in an open and sunny savanna-like area while the latter consists of a wood of arboreal to subarboreal vegetation, with slightly open canopy, also on sandy soil. The specimens were found in the Campinarana area, which forms a matrix

where islands of Campina are found. The RBC is a protected area under the “Instituto Nacional de Pesquisas da Amazônia” (INPA) management.

### Morphological examination

The specimens were photographed both when fresh and dried. Macroscopic description was carried out based on fresh and dried material using a stereo microscope. Color coding follows Küppers (2002). For microscopic examination, samples were rehydrated in 70% ethanol, and thin sections were mounted in 3% KOH or in Melzer's reagent. Basidiospores were described including dimensions as the range of length  $\times$  the range of width, followed by  $x_{\text{m}}$ , the range of the arithmetic means of length  $\times$  the same for width;  $x_{\text{mm}}$ , the mean of the arithmetic means of length ( $\pm$  standard deviation (SD))  $\times$  the same for width ( $\pm$  SD);  $Q_{\text{m}}$ , the range of the means of length/width;  $Q_{\text{mm}}$ , the mean of the means of length/width ( $\pm$  SD);  $n$ , the number of spores measured; and  $s$ , the number of sampled collections. Basidiomata were photographed in the field using a digital camera, and in the laboratory using a Stereoscopic Microscope Leica M205 C with camera Leica MC 190 HD. Microstructures were also photographed using a digital camera coupled to the Leica DM 2500 optical microscope, with an image capture via LEICA EC3 camera and pre-edited in LAS EZ. Line drawings of the microstructures were made with a drawing tube and edited in CorelDRAW X7. The collections were deposited in the Herbarium INPA.

### DNA extraction, amplification, and sequencing

DNA was extracted directly from cultures using a DNeasy Plant Mini Kit (Qiagen). The nrITS and nrLSU regions were amplified using ITS5/ITS4 and LR0R/LR5, respectively (White et al. 1990). The PCR reactions had a final volume of 25  $\mu$ l and included 1 U Platinum® Taq DNA Polymerase, 1 $\times$  PCR Buffer and 1 .5mM MgCl<sub>2</sub> (Invitrogen), 3 mM of each dNTP (Promega Corporation), 2  $\mu$ M of each primer, and 1  $\mu$ l of DNA at 25 ng/ $\mu$ l. The PCR profile for the nrITS and nrLSU amplifications consisted of an initial step of 2 min at 95 °C, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and then a final extension step at 72 °C for 10 min. PCR fragments were visualized in a 1.5% agarose gel stained with GelRED™ (Biotium), under ultraviolet light. The fragments were purified using ExoSAP-IT™ (ThermoFisher Scientific) and then sequenced using BigDye™ Terminator v3.1 Cycle Sequencing (ThermoFisher Scientific). The quality of the electropherograms was analyzed in Geneious R7 (Biomatters Ltd.).

### Molecular data processing and phylogenetic analyses

Corresponding/complementary forward and reverse reads were assembled to obtain clean consensus sequences in Geneious R7, with trimming of extremities. Sequences of nrLSU and nrITS regions were included in datasets according to three approaches: (a) DATASET 1, newly generated nrLSU sequences with those in the GenBank database (NCBI) of closest taxa (BLAST - <http://blast.ncbi.nlm.nih.gov/>) along with representatives of mostly genera of Omphalotaceae to form the ingroup, also including members of other families of the Marasmioid clade and the Mycenaceae clade, and the outgroup with members of the Tricholomatoid clade (Matheny et al. 2006); (b) DATASET 2, only nrITS data using the newly generated sequences with sequences of closest taxa/genus revealed via BLAST searches in the GenBank database (NCBI) along with representatives of genera belonging to Omphalotaceae and representatives of Marasmiaceae as the outgroup (Matheny et al. 2006); (c) DATASET 3, newly generated sequences nrITS + nrLSU of the same collection combined data of representatives of genera belonging to Omphalotaceae in the GenBank database (NCBI) as the ingroup and combined sequences of a few representatives of Marasmiaceae as the outgroup. Sequences in GenBank (NCBI) were downloaded (Online Resource - Table O1), their quality checked, and used in the respective dataset.

DATASET 1 served as a comprehensive analysis to verify the phylogenetic placement of the new species (and new genus) within the Marasmioid major clade of the Agaricales [or suborder Marasmiineae according to Dentinger et al. (2016)], testing the family where it belongs and whether or not the taxon represents a new genus in Omphalotaceae (family and genus level). DATASET 2 grouped representatives of a very comprehensive breadth of species of genera in Omphalotaceae to test the phylogenetic relationship of the new taxon at “species level,” since most of the Omphalotaceae species with publicly available sequence data are represented by nrITS. DATASET 3 tested the phylogenetic placement of the new taxon at genus and species level in a tentatively better tree resolution of the Omphalotaceae. All data used in this study are listed in the Online Resource - Table O1. The alignments were produced via MUSCLE (Edgar 2004). Ambiguously aligned regions were excluded from the nucleotide matrices by visual inspection in Geneious R7 (Kearse et al. 2012). The nucleotide substitution models were inferred via jModeltest 2.1.3 (Darriba et al. 2012) or MrModeltest 2.3. (Nylander 2004). All datasets and alignments can be found in TreeBASE 23780. The model selected per dataset was GTR+I+G for all partitions.

We conducted MC<sup>3</sup> Bayesian analyses (BA) with MrBayes 3.2.1 (Ronquist et al. 2012), using default settings from the model (Nst=6). For all datasets, BA consisted of two independent runs: (a) 10,000,000 generations, sampling frequency

every 1000 generations, six independent chains and two swaps, even for partitioned analysis (LSU and ITS concatenated). All burnin was set at 10%. Final trees were based on 50% majority-rule consensus method. Branch lengths went across the 95% highest posterior density trees. For maximum likelihood (ML), the trees (Online Resource Figs. O2, O4–O5) were reconstructed using the GTR+ $\Gamma$ +I model in RAxML 7.0.4 (Stamatakis 2006) with fast-bootstrapping implementing CAT approximations for 1000 pseudoreplicates and a full ML optimization for the final tree. Pairwise comparisons were performed in Geneious R7 using nrITS data when necessary. The phylogenetic trees were visualized and pre-edited in FigTree 1.3.1, and the main edition was conducted in CorelDRAW X7.

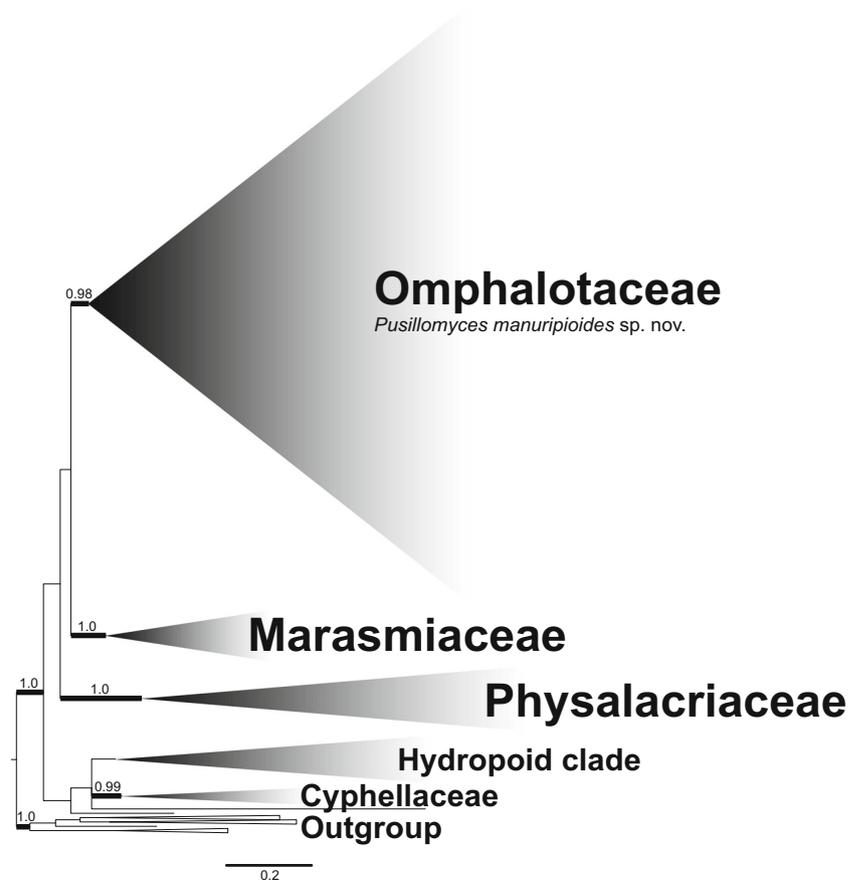
## Results

### Phylogenetic analyses

Three analyses were conducted on three different datasets explained in the “Material and methods.” The analyses on DATASET 1 resulted in the nrLSU trees (50% majority-rule consensus (tree length (TL)) = 23.821670 – Fig. 1, Online

Resource Figs. O3, O4; and ML tree (TL) = 3.961914 in Online Resource Fig. O5). With the estimated marginal likelihood arithmetic mean between BA runs in  $-14951.21$ , model parameters are summarized in Table 1. On an alignment of 737 distinct patterns, the ML analysis and applying GAMMA model parameters estimated up to an accuracy of 0.100000000 log likelihood units, the final ML optimization likelihood was  $-14,090.021877$ , and the parameters for DATASET 1 alignment are in Table 1. The tree ingroup (PP 1.0/BS 65) consisted of five relevant clades: /omphalotaceae (PP 0.95, BS 72), /physalacriaceae (PP 1.0, BS 97), /cyphellaceae (PP 0.99, BS 44), /hydropoid (PP 0.54, BS 18), and /marasmiaceae (PP 1.0, BS 66); the clade /mycenaceae (PP 1.0, BS 99), however, grouped within the outgroup. *Pusillomyces manuripioides* branched within /omphalotaceae. In the same broad tree, the new species grouped with *Gymnopus asetosus* Antonín, R. Ryoo & K. H. Ka and *G. funalis* (Har. Takah.) Antonín, R. Ryoo & K. H. Ka, all these taxa forming a distinct subclade (PP 1.0, BS 100). We also observed these three taxa closely related in the ITS trees (Online Resource Figs. O1, O2) from the analyses on the DATASET 2, forming a strongly supported subclade (PP 1.0, BS 100) in the ingroup (only members of Omphalotaceae).

**Fig. 1** Bayesian 50% majority-rule consensus tree from the single locus (nrLSU) analysis of DATASET 1 [tree length (TL) (mean of the means from two runs), 23.821670;  $-\ln L$  (mean),  $-14,951.21$ ]. Support values at the nodes consist of PP  $\geq 0.95$  and BS  $\geq 70$ ; unsupported nodes under PP 0.5 are collapsed. Thicker stems in black represent highly supported nodes, and those in gray are moderately to weakly supported nodes. Major clades are simplified, representing family level groups as depicted in the figure. Outgroup consists of members of *Clitocybe*, *Collybia*, *Entoloma*, *Lepista*, *Mycena* s. str., and *Tricholoma*



**Table 1** Data from ML and BA analyses of DATASET 1

DATASET 1 alignment		
Taxa	217	
Characters	1340	
Calculations from ML and BA		
	ML (means)	BA (means)
Alpha	0.209886	0.268692
Pinvar		0.440729
Substitution rates		
(A<=>C)	1.027500	0.047838
(A<=>G)	7.738497	0.381134
(A<=>T)	2.201518	0.084752
(C<=>G)	0.482952	0.026203
(C<=>T)	11.054926	0.423704
(G<=>T)	1.000000	0.036369
Bases freq.		
pi(A)	0.270127	0.261056
pi(C)	0.191077	0.179064
pi(G)	0.293142	0.258431
pi(T)	0.245654	0.301449

The BA and ML analyses on DATASET 3 rendered well-resolved trees (50% majority-rule consensus tree (TL) = 36.167380—Fig. 2; and ML tree (TL) = 2.927921 in Online Resource Fig. O5) for the phylogenetic reconstruction of generic groups (included in this study) within the family. With the estimated marginal likelihood arithmetic mean between BA runs in  $-16,947.29$ , model parameters are summarized in Table 2. On a partitioned alignment of 356 distinct patterns for nrITS and 395 distinct patterns for nrLSU, the ML analysis and applying GAMMA model parameters estimated up to the accuracy of 0.1000000000 log likelihood units for the two partitions, the final ML optimization likelihood was  $-16,425.972776$ , and the parameters for DATASET 3 alignment are in Table 2. The tree ingroup (PP 1.0/BS 96) revealed 12 distinct generic representatives: /clade A—*Marasmiellus* s. str. (PP 1.0/BS 57), /clade B—*Pusillomyces* (PP 1.0/BS 100), /clade C—*Connopus* (PP 1.0/BS 100), /clade D—*Pallidocephalus* (PP 1.0/BS 96), /clade E—*Rhodocollybia* (PP 1.0/BS 66), /clade F—*Lentinula* (PP 1.0/BS 99), /clade G—*Gymnopus* s. str. (PP 1.0/BS 88), /clade H—*Gymnopus* sect. *Perforantia* (PP 1.0/BS 100), /clade I—*Gymnopanella* (PP 1.0/BS 77), /clade J—*Mycetinis* (PP 1.0/BS 100), /clade K—*Omphalotus* (PP 1.0/BS 100), and the lineage *Anthracophyllum archeri* (Berk.) Pegler (one strain). The representatives of *Omphalotus* and *Anthracophyllum* form a weakly supported clade (PP 0.87 / BS 50). /Clade B and /clade C are sister with weak support (PP 0.96/BS 47). These clades tend to group with /clade D, /clade E, and /clade F, but in an unsupported major clade (PP 0.66/BS -). This major clade (B, C, D, E, and F) is sister to clade A by an unsupported node (PP 0.76/BS 28).

/Clade G and /clade H are paraphyletic because of the strain “KY026621 *Marasmius* sp.1 TFB3940,” which without support appears as sister to /clade G (PP 0.83/BS 57). Thus, there are two unsupported major clades: (1) clades A, B, C, D, E, and F; (2) clades G and H. /Clade I and /clade J branched individually. An unsupported clade grouping /clade K and lineage L is the most basal in the ingroup. The lineage named as *Gymnopus contrarius* (Peck) Halling branched in an isolated position basal to the whole family, and may represent a distinct genus too, but more information is needed to reach a conclusion. The tree generated from the analyses on DATASET 2 (broad sampling of nrITS data) as well as the parameters data are shown only in the [Online Resource material](#).

Comparing the combined nrITS and nrLSU tree (Fig. 2) with the nrLSU tree (Fig. 1) and the nrITS tree (Online Resource Figs. O1, O2), we observe coherence in the topologies considering that the nrLSU tree and nrITS tree have broader sampling (more taxa included), but limited resolution for the tree at various points. On the other hand, the nrITS + nrLSU tree is better resolved due to the combination of molecular data with high statistical support for all generic representative clades, but various taxa present in the DATASET 1 and 2 are absent in DATASET 3 because of the lack of nrLSU and nrITS data of the correspondent strain. Most clades and subclades found in nrITS + nrLSU tree can also be found in the nrLSU tree and nrITS tree, showing consistency in the topology.

## Taxonomy

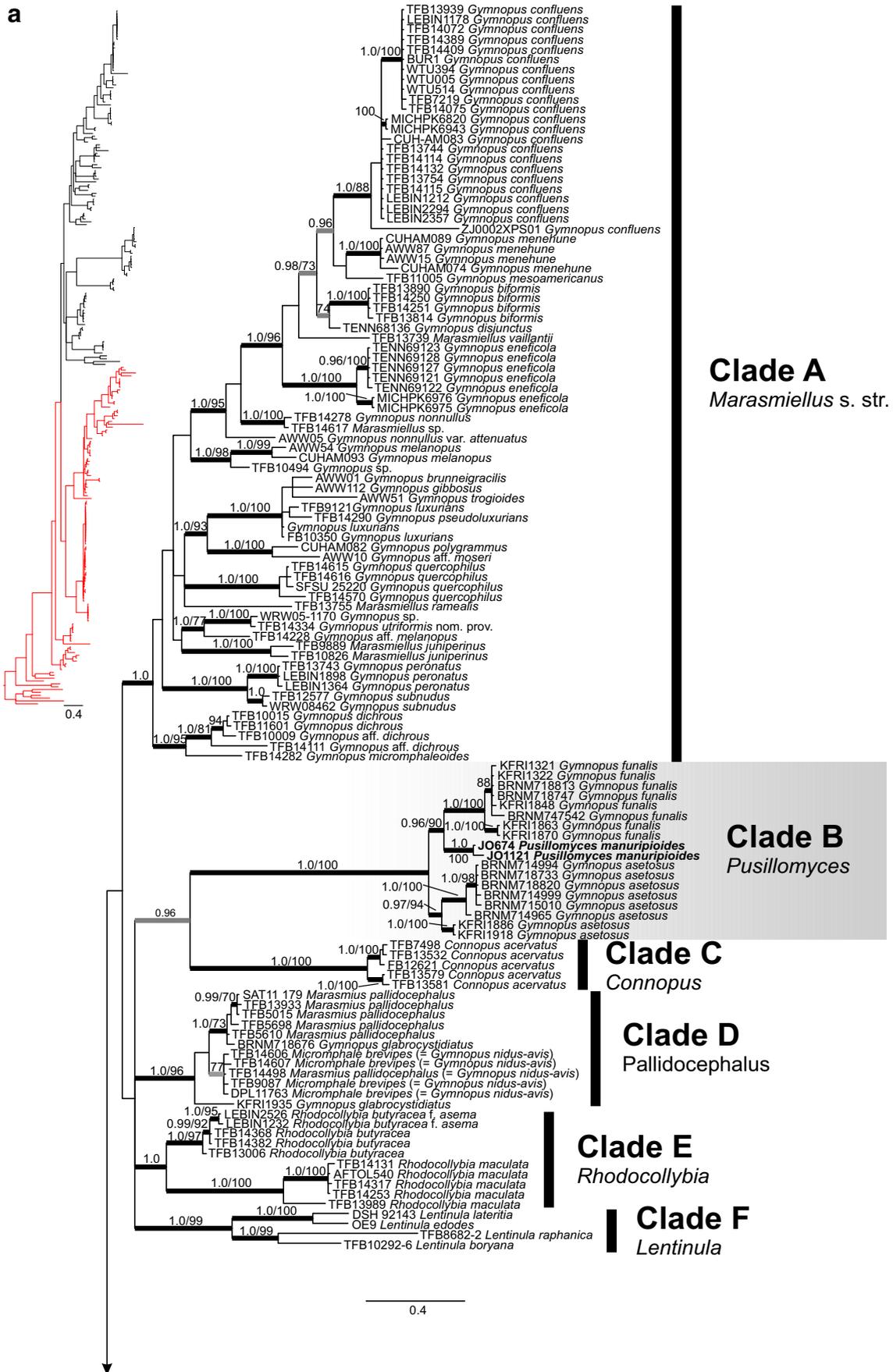
### *Pusillomyces* J.S. Oliveira, **gen. nov.**

Mycobank MB 827357.

Etymology: *Pusillus* (latin) = small; *myces* = mushroom; it refers to the small size of the marasmioid basidiomata.

Diagnose: Basidiomata marasmioid, thin, tiny. *Pileus* hemispheric to convex, or plane, sometimes slightly concave, center plane to depressed, with or without papilla, smooth, or slightly rugulose at the center, and striate-sulcate at the disc and margin, surface finely tomentose, membranous to firmly coriaceous. *Hymenophore* entirely smooth or with well-developed *lamellae*, adnate to occasionally adpressed to a false collarium, distant, whitish to pale cream. *Stipe* filiform, wiry, insititious, rising directly from the substrate or branching from rhizomorphs; chitinous, horny, pliant, hollow; strongly pigmented, mostly brown to dark brown; glabrous or rarely finely pruinose, or even entirely pubescent to hairy. *Rhizomorphs* present, glabrous to pubescent, rare to abundant. *Odor* and *taste* not distinctive. *Basidiospores*

**Fig. 2** Bayesian 50% majority-rule consensus tree from the multilocus (combined nrITS and nrLSU) analysis of DATASET 3 (TL, 35.696274;  $-\ln L, -14,951.21$ ). Support values at the nodes consist of PP  $\geq 0.95$  and BS  $\geq 70$ ; unsupported nodes under PP 0.5 are collapsed. Thicker stems in black represent highly supported nodes, and those in gray are moderately to weakly supported nodes. Outgroup consists of members of the clade Marasmiaceae (Fig. 1)



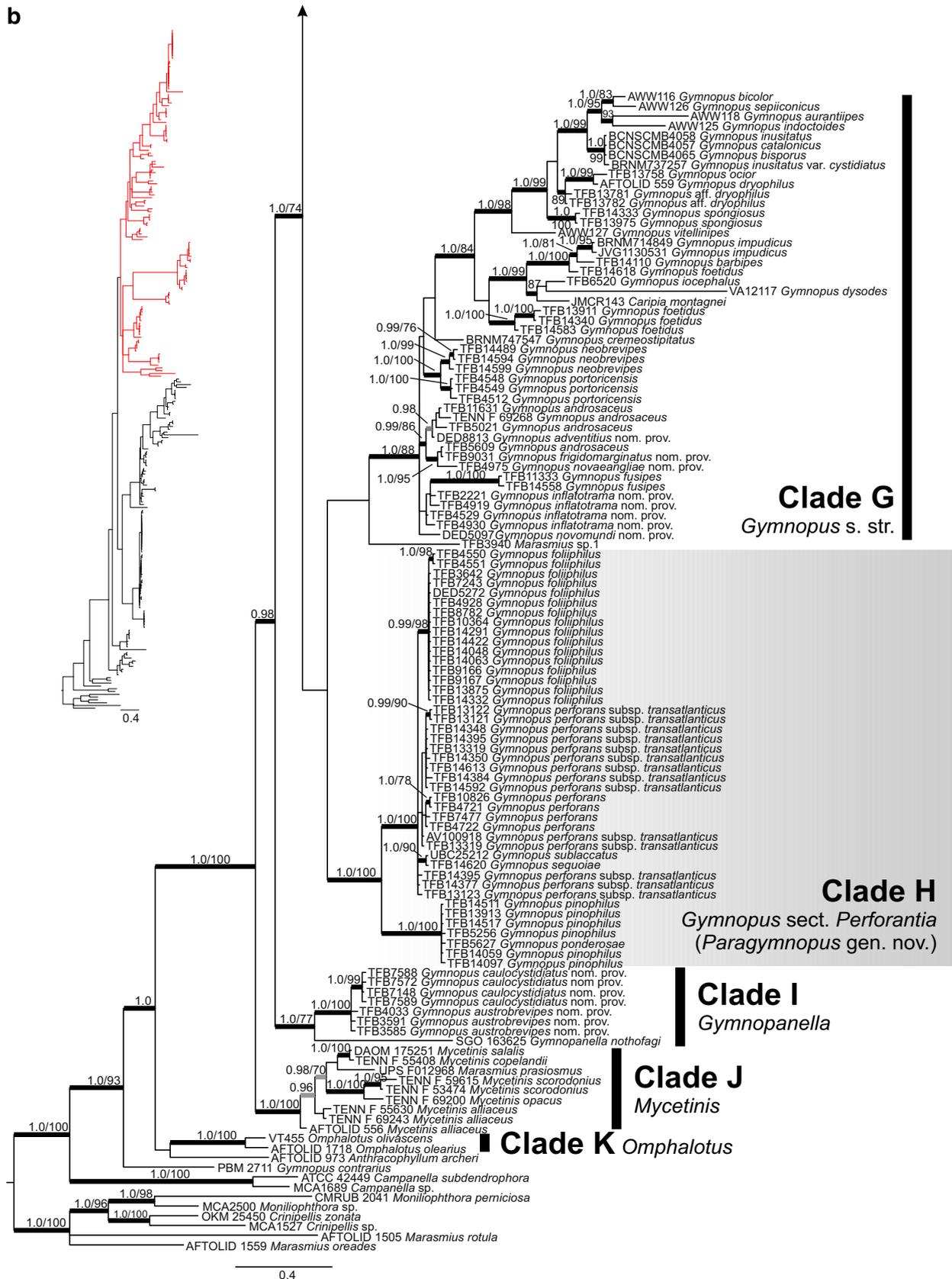


Fig. 2 (Continued)

**Table 2** Data from ML and BA analyses of DATASET 3

DATASET 3 alignment					
Taxa	244				
Characters	545 (ITS) + 955 (LSU)				
Calculations from BA by partition			Calculations from ML by partition		
	ITS (means)	LSU (means)		ITS (means)	LSU (means)
Alpha	0.758406	0.190530	Alpha	0.356165	0.200742
Pinvar	0.302564	0.497144	Pinvar		
Substitution rates			Substitution rates		
(A<=>C)	0.070389	0.036804	(A<=>C)	1.900609	0.485606
(A<=>G)	0.390588	0.207500	(A<=>G)	11.363035	4.003744
(A<=>T)	0.105534	0.085793	(A<=>T)	3.210914	1.300596
(C<=>G)	0.043284	0.037125	(C<=>G)	1.243795	0.492606
(C<=>T)	0.339083	0.601554	(C<=>T)	9.601055	6.098430
(G<=>T)	0.051122	0.031224	(G<=>T)	1.000000	1.000000
Bases freq.			Bases freq.		
pi(A)	0.236077	0.278227	pi(A)	0.235424	0.273905
pi(C)	0.190142	0.171529	pi(C)	0.199045	0.184005
pi(G)	0.204093	0.283168	pi(G)	0.214724	0.292899
pi(T)	0.369687	0.267077	pi(T)	0.350808	0.249191

obovoid to shortly oblong, ellipsoid, ellipsoid-fusoid, lacrymoid to subclavate, smooth, hyaline, thin-walled, inamyloid. *Basidia* clavate, 2- to 4-sterigmate. *Cheilocystidia* not applicable (smooth hymenophore) or when present, in the form of *Siccus*-type broom cells. *Pleurocystidia* absent. *Lamellar* and *pileus trama* irregular, inamyloid, hyphae smooth or incrustated. *Pileipellis* non-gelatinized, non-hymeniform, a trichoderm of disorganized, packed elements, composed of cylindrical, thin- to slightly thick-walled, diverticulate, smooth or incrustated hyphae, mixed with elements similar to *Siccus*-type broom cells, or *Rameales*-structures. *Stipitipellis* and *Stipe trama* dextrinoid. *Caulocystidia* absent or present, dextrinoid. *Clamp connections* absent.

Chemical reactions: In Melzer's reagent, only the stipe trama is dextrinoid; otherwise, inamyloid. No staining in alkaline solution (NH<sub>4</sub>OH or KOH).

Ecology: Saprotrophic or parasitic in plant (phytopathogen), epiphytic or in the forest litter, gregarious, on leafy or woody substrate.

Distribution: Probably worldwide; to date only recorded from the Neotropic (Tropical, Amazon forest, Brazil) and Palearctic regions (Temperate Broadleaf and Mixed Forest, Republic of Korea).

Type species: *Pusillomyces manuripioides* J.S. Oliveira.

*Pusillomyces asetosus* (Antonín, R. Ryoo & K.H. Ka) J.S. Oliveira, comb. nov. (MB 827358)

*Gymnopus asetosus* Antonín, R. Ryoo & K.H. Ka, Mycological Progress 13: 704 (2014)

*Pusillomyces funalis* (Har. Takah.) J.S. Oliveira, comb. nov. (MB 827359)

*Marasmius funalis* Har. Takah., Mycoscience 43 (4): 344 (2002)

*Gymnopus funalis* (Har. Takahashi) Antonín, R. Ryoo & Shin, Mycological Progress 13: 710 (2014)

Notes: To see taxonomic and classification comments on the evidence for the combination of *G. asetosus* and *G. funalis*, and the establishment of *Pusillomyces*, refer to *Phylogeny* and *Morphology* in the Discussion. A synoptic key comparing *Pusillomyces* with the other genera of /letinuloid clade is provided in Online Resource (Chart O1).

*Pusillomyces manuripioides* J.S. Oliveira, sp. nov. (Figs. 3, 4, and 5)

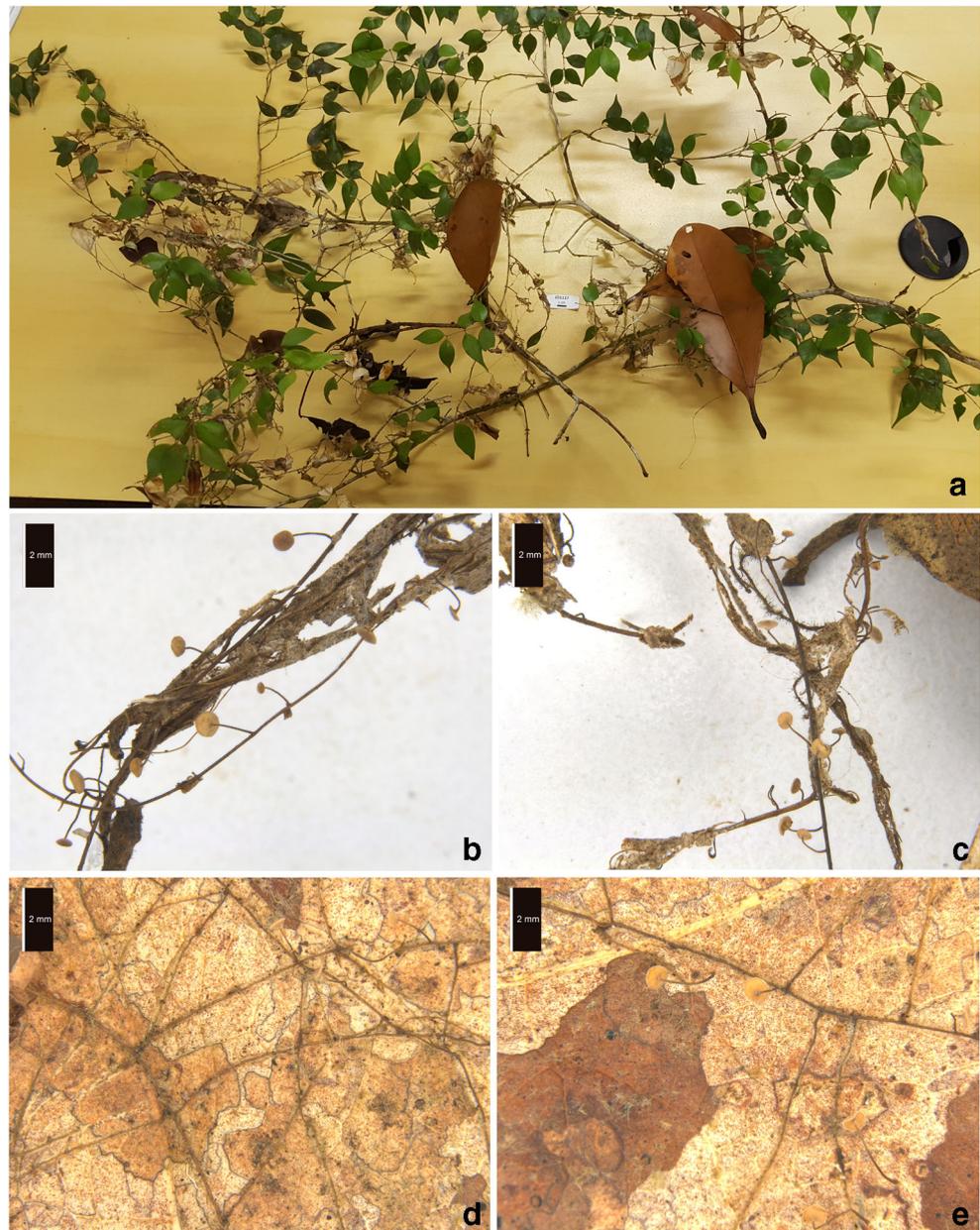
Mycobank MB 827360

Barcode sequences (GenBank accession numbers): nrITS – MK434210 and nrLSU – MK434211 from the holotype J.J.S. Oliveira & L.S. Bento *JO674*; and nrITS – MK434212 and nrLSU – MK434213 from the paratype J.J.S. Oliveira & N.K. Ishikawa *JO1121*.

Etymology: The epithet is based on the strong similarity to *Manuripia bifida* Singer, a monotypic genus. The name *Manuripia* comes from Manuripi (Bolivia), the holotype locality of *M. bifida*.

Diagnose: Basidiomata manuripioid, tiny. Pileus 0.3–1.8 mm diam., plano-convex to subfundibuliform, smooth, brown, chestnut brown to cream. Hymenophore smooth, white to cream. Stipe 1–4 × 0.2–0.3 mm, central, filiform, insititious, glabrous, dark brown, growing mostly from rhizomorphs. Basidiospores 6–9 × 2.8–4.6 μm, usually ellipsoid, inamyloid. Pileus trama inamyloid, irregular,

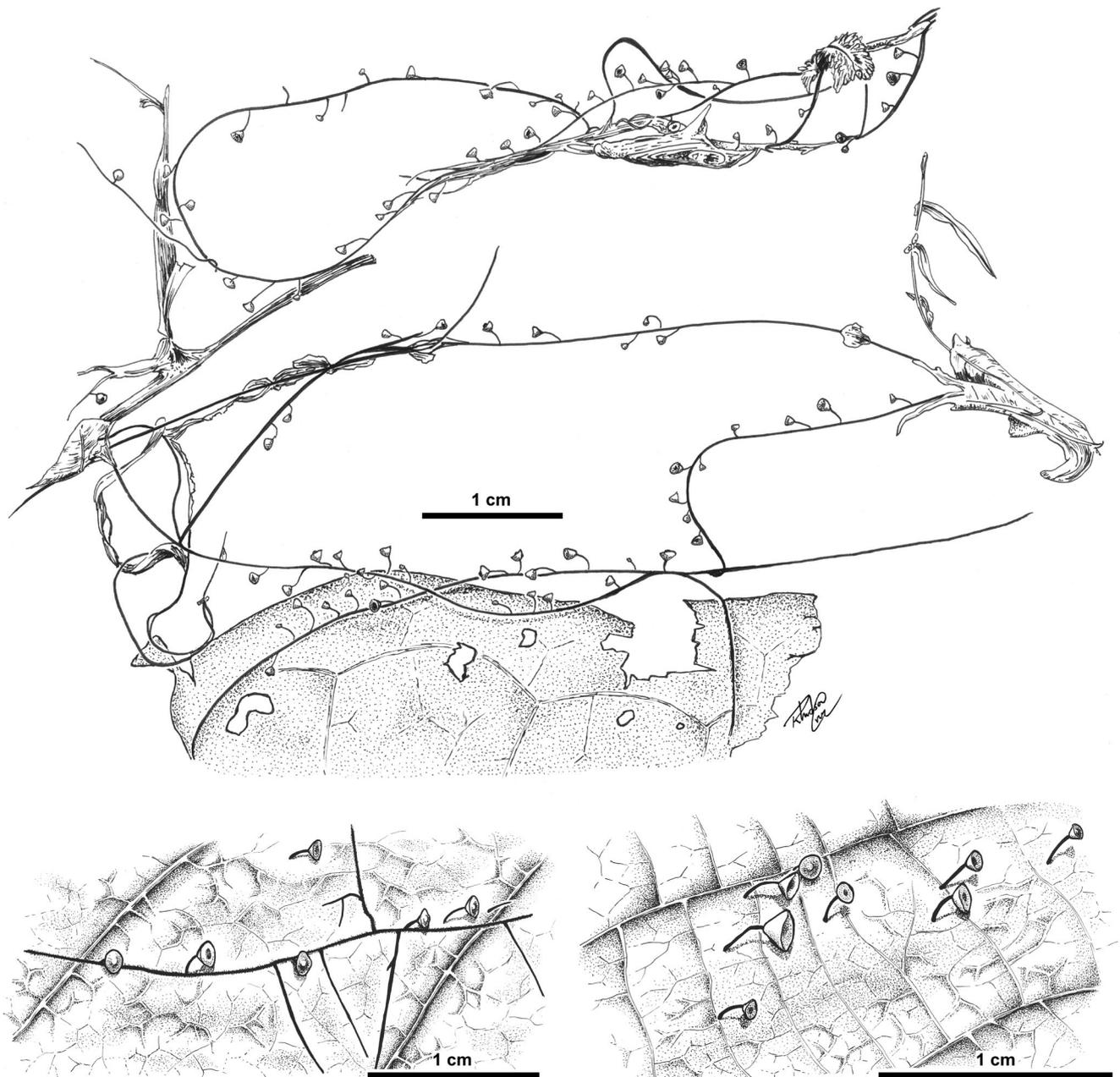
**Fig. 3** *Pusillomyces manuripioides*. **a** Branch of *Eugenia* sp. with net of rhizomorphs and infected parts (JO1117). **b, c** Rhizomorphs bearing basidiomata of *P. manuripioides* (JO674—holotype). **d, e** Rhizomorphs strongly attached to dead foliar face, some producing basidiomata (JO674—holotype)



hyphae smooth or incrusted, clamp-connections absent. Pileipellis a trichoderm of *Rameales*-structures. Rhizomorphs like the stipe, some densely covered by conidia. Epiphytic, forming abundant rhizomorphs in hanging living and dead leaves and juvenile living branches.

*Pileus* 0.3–1.8 mm diam., orbicular, plano-convex, plane, to plano-infundibuliform or subinfundibuliform, center with a shallow papilla especially when young, sometimes depressed, or papilla usually receding in old or larger pileus, disc and margin smooth, margin straight to slightly uplifted, edge entire; surface glabrous, dry, dull, papyraceous or subvelutinous; membranous at the margin,

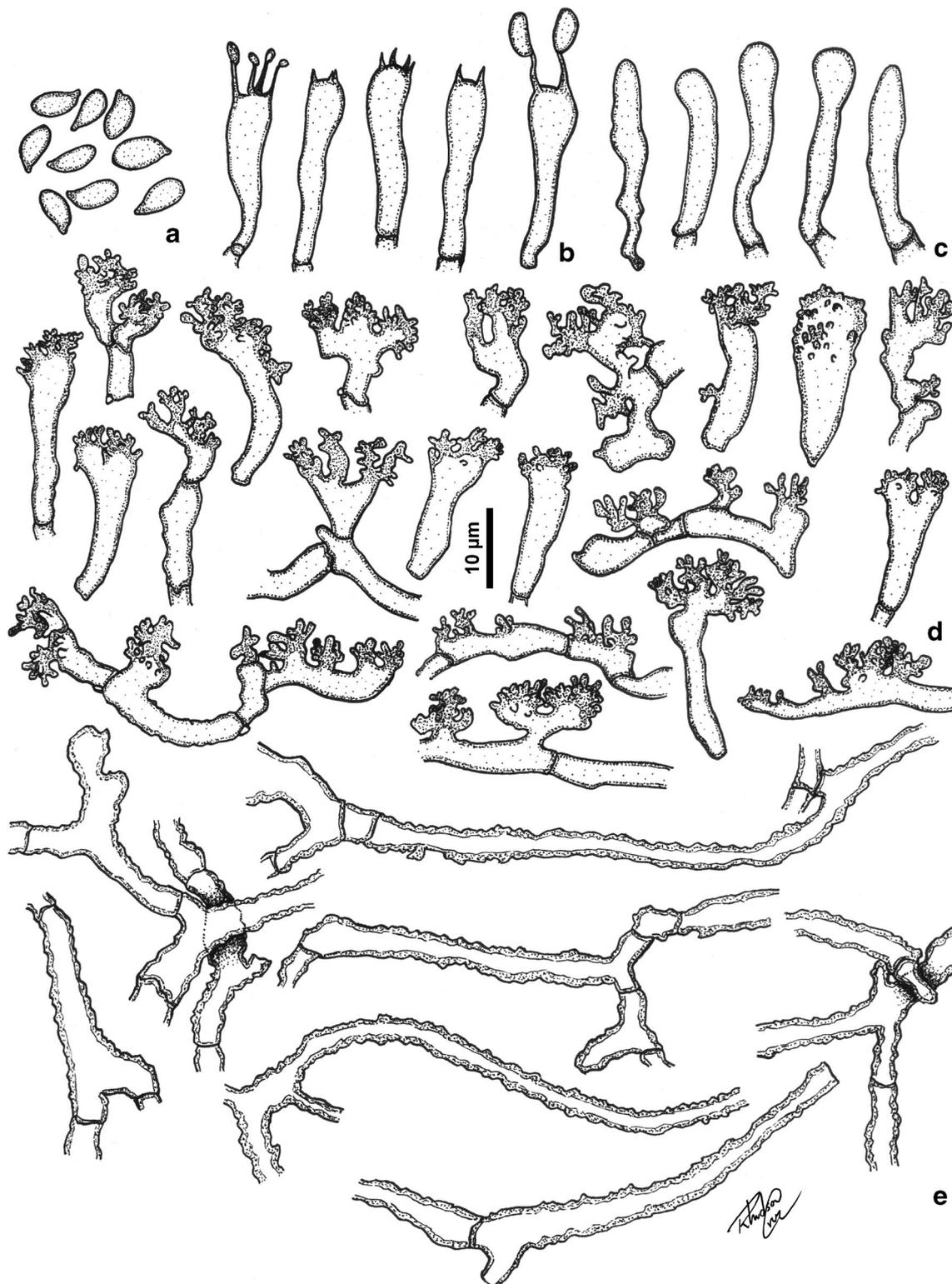
a little thicker, tough or coriaceous at the disc towards the center, context thin (<1 mm), white to whitish cream; with brown to dark brown ( $N_{60}Y_{50}M_{30}$  to  $N_{90}Y_{60}M_{40}$ ) center or papilla, disc chestnut brown ( $N_{50}Y_{60}M_{30}$ ) becoming yellowish brown or buff brown ( $N_{40}Y_{50}M_{30}$ ) towards the margin, or paler ( $N_{20}Y_{30-40}M_{20}$ ), becoming cream ( $N_{00}Y_{10}M_{00}$ ) when very fresh. *Hymenophore* smooth (lamellae absent), white to pale cream ( $N_{00}Y_{10}M_{00}$ ), papyraceous, dull, dry. *Stipe* 1–4 × 0.2–0.3 mm, central, filiform, very thin, equal, cylindrical, insititious, chitinous, hollow, glabrous, smooth, opaque, dark chestnut brown ( $N_{90}Y_{70}M_{30}$ ) or very dark brown ( $N_{99}Y_{50}M_{30}$ ), growing directly from the substrate or more frequently arising



**Fig. 4** *Pusillomyces manuripioides* (JO674—holotype): illustration of macroscopic structures of basidiomata and rhizomorphs, habit and substrate. Scale bar 1 cm

from abundant, very elongate, hair-like rhizomorphs, concolorous with the stipes, part glabrous, part densely pubescent or even hirsute, resembling those found in *Manuripia bifida*, with dark brown to black pubescence. *Basidiospores* (Fig. 5a)  $6\text{--}9 \times (2.2)2.8\text{--}4.6(5) \mu\text{m}$  ( $x_{\text{m}} = 6.9\text{--}7.4 \times 2.8\text{--}3.6 \mu\text{m}$ ,  $x_{\text{mm}} = 7.3 (\pm 0.3) \times 3.3 (\pm 0.4) \mu\text{m}$ ,  $Q_{\text{m}} = 2\text{--}3.3$ ,  $Q_{\text{mm}} = 2.6 (\pm 0.7)$ ,  $n = 30$ ,  $s = 3$ ), obovoid, rarely shortly oblong, usually ellipsoid to subellipsoid, or amygdaliform, lacrymoid to subclavate, smooth, thin-walled, hyaline, inamyloid. *Basidia* (Fig. 5b)  $20\text{--}27.5 \times 5\text{--}7 \mu\text{m}$ , clavate, smooth, hyaline, thin-walled, inamyloid, 2–4 sterigmata. *Basidioles* (Fig. 5c)  $17\text{--}26.8 \times 3.7\text{--}6.4 \mu\text{m}$ , clavate to

broadly clavate, some with tapered apex, smooth, hyaline, thin-walled, inamyloid. *Hymenial cystidia* absent. *Pileus trama* inamyloid, irregular, non-gelatinized, packed, but also lacunose in some parts, especially at the central part of the context, forming a gradient, pale brownish near the pileipellis, fading to more hyaline near the hymenial layer, hyphae interwoven, cylindrical,  $2\text{--}8.2 \mu\text{m}$  diam., regular in outline, branched, smooth, or more frequently incrustated (irregularly ornamented) towards the pileipellis (Fig. 5e), hyaline or some more opaque (wall thickness), thin- to more thick-walled next to the pileipellis, clamp connections absent. *Pileipellis* non-hymeniform, non-gelatinized,



**Fig. 5** *Pusillomyces manuripioides* (JO674—holotype): microscopic structures. **a** Basidiospores. **b** Basidia. **c** Basidioles. **d** Diverticulate elements of the pileipellis. **e** Incrusted hyphae of the pileipellis to the upper layer of the pileus trama. Scale bar **a–e** 10  $\mu$ m

formed by a layer of disorganized elements or trichoderm, pale chestnut brown in 3% KOH with abundant and dominant *Rameales*-structures (Fig. 5d), some in transition to irregular

*Siccus*- or *Rotalis*-type cells (Fig. 4d), usually interconnected with smooth or incrustate hyphae of the pileus trama (Fig. 4e), main body 8.8–29  $\times$  3.3–10.5(–21)  $\mu$ m, hyphoid, branched,

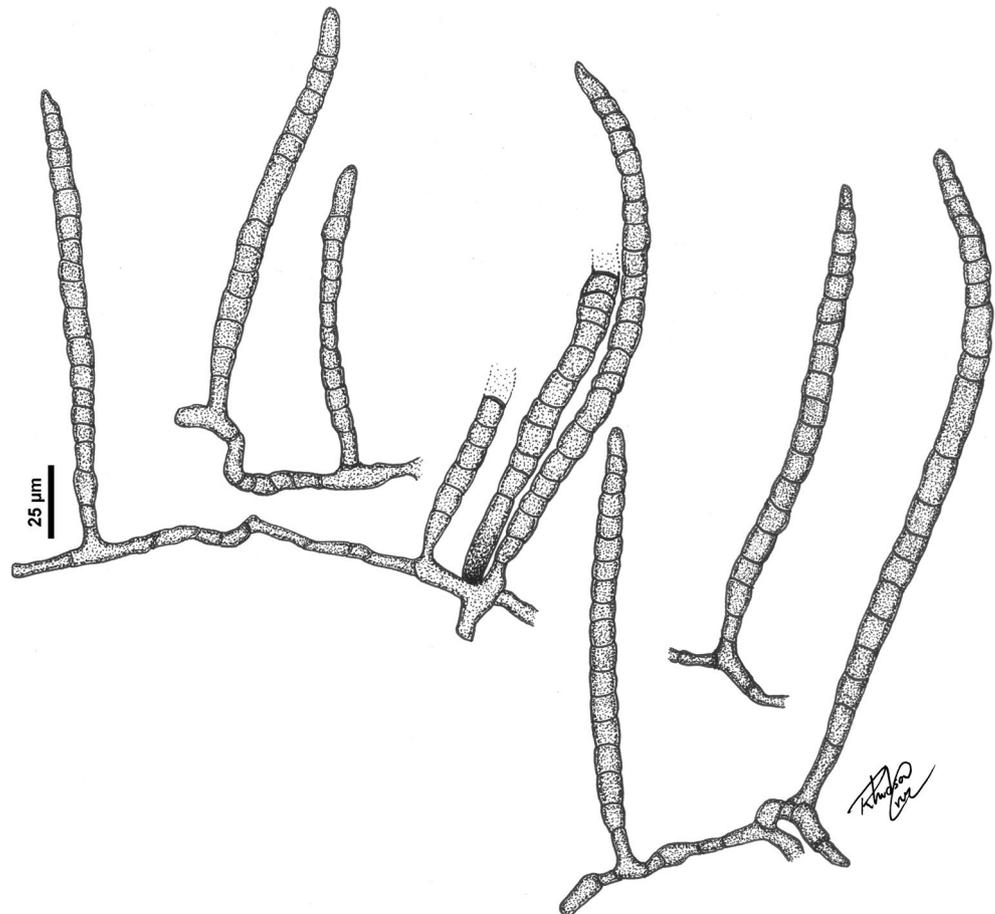
lobed or irregular, but clavate to cylindrical in the *Siccus-/Rotalis*-type cells, hyaline when isolated, smooth, thin-walled, inamyloid; diverticula apical or divergent to the laterals, generally short,  $0.7\text{--}2.2 \times 0.6\text{--}1.2 \mu\text{m}$ , vesiculose, verruciform, to shortly digitiform, sometimes branched, hyaline, solid, with obtuse and rounded apex. *Stipe trama* mostly inamyloid, or cortical hyphae apparently dextrinoid (at least in the stipe apex) or only orangish brown in Melzer's reagent due to pigmentation, parallel, not compact, hyphae easily disassociating, then chestnut brown, losing pigmentation towards the inner trama, cylindrical,  $3\text{--}8.4 \mu\text{m}$  diam., generally regular in outline, septate, thick-walled, smooth or with granular incrustations, sometimes very prominent looking like diverticula, clamp connections absent; internal hyphae  $2.5\text{--}7.4 \mu\text{m}$  diam., with thinner walls, hyaline, usually smooth, sometimes rough, clamp-connections absent. *Stipitipellis* without elements or vestiture on the glabrous stipes and rhizomorphs. *Rhizomorphs* corresponding to the stipe trama for the glabrous segments in micromorphology, but some pubescent segments densely covered by elongate, cylindrical to fusoid (tapered at the apex) conidia (Fig. 6),  $106\text{--}237.2 \times 7.4\text{--}13.2 \mu\text{m}$  diam., presenting an apparent apical orifice, body with numerous septa, each short segment slightly inflated, thick-walled (including septa), dark brown, very distinct from the hyphae of

the trama, non-fragmentary, but breaking at septal point when compressed, interconnected with one another at the base via connecting, thinner, irregular, sometimes branched, repent, similar segments, the whole structure forming the conidiophores which are immersed in the trama, but do not appear to have originated from the hyphae, reaction in Melzer's reagent not distinctive due to the dark pigmentation.

**Habit and Substrate:** Manuripioid (Fig. 3b, c) to gloiocephaloid (those basidiomata growing on the substrate—Fig. 3d, e), tiny, gregarious, epiphytic (about 1.5 m above the ground upward), forming abundant rhizomorphs in hanging living and dead leaves, attaching also on twigs, branches and limbs, specifically or frequently of *Eugenia* cf. *subterminalis* DC (Myrtaceae), in “campinarana”, sometimes in transition to “terra-firme”, Amazon Forest.

**Specimens examined:** Brazil, Amazonas, Manaus, Reserva Biológica de Campina, hanging branches with both living and dead leaves of *Eugenia* cf. *subterminalis* and fallen trapped leaves from the canopy of multiple dicot tree species, 17. 11. 2016, J.J.S. Oliveira & L.S. Bento *JO674*, **holotypus** (INPA 280704!); hanging branches with both living and dead leaves of *Eugenia* cf. *subterminalis* and fallen trapped leaves from the canopy of multiple dicot tree species, 28. 11. 2017, J.J.S. Oliveira & G.S. Sarkis *JO950* (INPA 280705!); hanging

**Fig. 6** *Pusillomyces manuripioides* (*JO674*—holotype): Conidiophore and conidia from the cortex of the pubescent segments of the rhizomorphs. Scale bar  $10 \mu\text{m}$



branches with both living and dead leaves of *Eugenia* cf. *subterminalis* and fallen trapped leaves from the canopy of multiple dicot tree species, 15. 2. 2018, J.J.S. Oliveira & G.S. Sarkis JO986 (INPA 280706!); hanging branches with both living and dead leaves of *Eugenia* cf. *subterminalis* and fallen trapped leaves from the canopy of multiple dicot tree species, 15. 2. 2018, J.J.S. Oliveira & G.S. Sarkis JO987 (INPA 280707!), 23. 10. 2018, J.J.S. Oliveira & N.K. Ishikawa JO1117 (INPA 282046!), J.J.S. Oliveira & N.K. Ishikawa JO1118 (INPA 282047!); J.J.S. Oliveira & N.K. Ishikawa JO1119 (INPA 282048!), J.J.S. Oliveira & N.K. Ishikawa JO1120 (INPA 282049!), J.J.S. Oliveira & N.K. Ishikawa JO1121 (INPA 282050!), J.J.S. Oliveira & N.K. Ishikawa JO1122 (INPA 282051!), J.J.S. Oliveira & N.K. Ishikawa JO1123 (INPA 282052!), J.J.S. Oliveira & N.K. Ishikawa JO1124 (INPA 282053!).

## Discussion

### Phylogeny

We based the analysis of DATASET 1 on the Marasmioid Clade depicted in Matheny et al. (2006), including representatives of the Tricholomatoid Clade (Matheny et al. 2006) as the outgroup. In the present analysis, the /mycenaceae subclade grouped within the outgroup, which does not follow the results in Dentinger et al. (2016). The analysis of DATASET 1 confirmed that, among all families and undefined groups within the Marasmioid Clade or suborder Marasmiineae represented by the major clades in the nrLSU tree (Fig. 1), *P. manuripioides* belongs to Omphalotaceae since it groups with the representatives of the family in the Omphalotaceae clade. This clade is weakly supported if *Gymnopus contrarius* is considered in the family; but disregarding this species, the clade is strongly supported (see Online Resource material). In the same tree (see again the Online Resource material), it is already possible to observe that *P. manuripioides* is part of a very distinct lineage and, therefore a representative of a different genus along with *Gymnopus aetosus* and *G. funalis*.

In the tree reconstructed from the analysis of the combined nrITS and nrLSU data—DATASET 3 (Fig. 2), the ingroup consisted only of representatives of the Omphalotaceae while the outgroup is composed of representatives of the Marasmiaceae. Once again, *P. manuripioides* branched within the very distinct and unique /clade B (*Pusillomyces*). Both trees (Figs. 1 and 2) are strong evidence that *P. manuripioides*, *G. aetosus*, and *G. funalis* do represent a new genus in the family.

Similar to the analysis of the DATASET 3, we also conducted an analysis including only nrITS data with a very large sampling of members of the Omphalotaceae forming the ingroup, and members of the Marasmiaceae as the outgroup. Based on the

trees, it is possible to observe the phylogenetic placement of taxa of the Omphalotaceae in clades that can be correlated to those clades depicted in the nrITS + nrLSU tree (Fig. 2), but such taxa are absent in this multilocus analysis because of the lack of nrLSU data from the same collection. This can restrict and broaden new circumscriptions for genera based on the present phylogeny, strengthening, or redefining boundaries for the groups. Moreover, the resulting nrITS tree (Online Resource Fig. O3) also revealed that *P. manuripioides* is closely related to *G. aetosus* and *G. funalis*, grouping in a strongly supported subclade. This consistently indicates that *G. aetosus* and *G. funalis* are congeneric with *P. manuripioides*, despite all the remarkable morphological divergence between the former two and the latter. We discuss further this relationship in the “Morphology” section (next part of this discussion).

The combined nrITS and nrLSU data tree (Fig. 2) in the present study also brought light to the understanding of the Omphalotaceae family at the genus level. *Gymnopus* and *Marasmiellus* are polyphyletic and have been considered artificial groups in previous studies such as Moncalvo et al. (2002), Mata et al. (2004a, 2006), Wilson and Desjardin (2005), Hughes et al. (2010), and Petersen and Hughes (2016, 2017). In our analyses, however, *Gymnopus* and *Marasmiellus* appear as monophyletic groups corresponding to /clade G and /clade A, respectively. This agrees with the findings of Wilson and Desjardin (2005), who depicted separate clades assigned for these genera (clade B or /marasmiellus and clade D or /gymnopus in their study) and did not accept *Marasmiellus* as a synonym of *Gymnopus*. Similar distinct clades can also be found in Petersen and Hughes (2016, 2017) and Sandoval-Leiva et al. (2016). The clades depicted in the tree are discussed in detail below:

**/Clade A (*Marasmiellus* s. str.):** This clade is strongly supported and contains *Marasmiellus juniperinus*, type species of *Marasmiellus* along with *M. ramealis* (Bull.) Singer and *M. vaillantii* (Pers.) Singer, and all members of *Gymnopus* sect. *Vestipedes* included in the analysis. This is one of three distinct and unrelated lineages grouping species of *Gymnopus* and corresponds to the statistically unsupported clade B or /marasmiellus in Wilson and Desjardin (2005). These authors, while discussing the phylogenetic affiliation between *M. juniperinus* and *Gymnopus* sect. *Vestipedes*, argued that they share characteristics such as the production of basidiomes with insititious to subsititious stipe, acyanophilous, inamyloid, depigmented (and hyaline) basidiospores, and a pileipellis which is a cutis of radially oriented cylindrical hyphae that are non-diverticulate or weakly diverticulate, typically roughened and vested with annular to zebroid, brownish pigmented incrustations. They further indicated that the pileipellis does not have well-developed *Rameales* structures, but rather, that it is composed of strongly diverticulate hyphae

as in *M. ramealis* and as in possibly most *Marasmiellus* spp. In the present paper, we found *M. ramealis* present in the same clade of *M. juniperinus* and members of *Gymnopus* sect. *Vestipedes*, and thus, the later advocated pattern is not consistent. Also, the need to resurrect *Collybiopsis*, considering the discussion presented in Wilson and Desjardin (2005), may be dismissed. Thus, *Marasmiellus* is monophyletic if the genus is restricted based on this clade and a redefinition of the traditional genus is provided in the following section.

**/Clade B (*Pusillomyces*):** *Pusillomyces manuripioides* grouped with *G. asetosus* and *G. funalis*, forming the distinct and highly supported clade B. This clade represents the new genus *Pusillomyces* formally described along with the new species in this paper. Morphological and ecological characteristics shared by these taxa are discussed in the next sections. Also, in /Clade B, *G. asetosus* seems to be rather three different species while *G. funalis* seems to be two and, then, the involved strains should be revised.

**/Clade C (*Connopus*):** This highly supported clade is composed of six specimens of *Connopus acervatus* (Fr.) R.H. Petersen, and recently erected as the monotypic genus *Connopus* R.H. Petersen in Hughes et al. (2010). *Connopus* is sister to *Pusillomyces* gen. nov., but without statistical support. The genus is mainly characterized by producing connate, collybioid or mycenoid basiomata, usually growing in deep polytrichaceous moss juxtaposed to conifer trunks or stumps in temperate to cool forests; hemispheric to convex, hygrophanous, smooth pileus; free to seceding, abundant, close lamellae; central, cylindrical, fistulose, apically glabrous then pruinose stipe, with tomentose base; small, ellipsoid to cylindrical, hyaline, thin-walled, inamyloid basidiospores; lacking pleurocystidia; absent or occasional, slender lecythiform cheilocystidia; conspicuously clamped hyphae in the tramae, and a pileipellis consisting of a lax trichoderm, with occasional, weakly banded, erect terminal cells (Hughes et al. 2010). A synoptic key comparing *Connopus* with the other genera of /letinuloid clade is provided in Online Resource (Chart O1).

**/Clade D (Pallidocephalus):** This strongly supported clade may correspond to the clade “/austrobrevipes and /pallidocephalus” in Petersen and Hughes (2016, 2017). However, the clade contains *Gymnopus pallidocephalus* comb. prov. (officially, *Marasmius pallidocephalus* Gilliam), *Gymnopus glabrocystidiatus* Antonín, R. Ryoo & K.H. Ka and strains named as “*Micromphale brevipes*” (DPL11763, TFB9087, TFB14606, TFB14607, TFB14498). A second lineage previously determined as *Micromphale brevipes* (Berk. & Ravenel) Singer appears again in /clade F, recently proposed as *Gymnopus neobrevipes* Petersen (Petersen and Hughes 2019). Collections of both “*Micromphale brevipes*” lineages were all

obtained from the same geographic region—the US Gulf Coast. Petersen and Hughes (2017) suggest that *Marasmius pallidocephalus* probably forms a monotypic genus while our results point to the inclusion of at least two additional species: *G. glabrocystidiatus* and “*Micromphale brevipes*” (pro parte). Considering this later, a just published paper (César et al. 2018) has revealed that the strains TFB14606, TFB14607, TFB14498, TFB9087, DPL11763, named as “*Micromphale brevipes*” are rather conspecific with the new species *Gymnopus nidus-avis* César, Bandala & Montoya. We agree with their conclusion, although this very recently proposed new species is not *Gymnopus* s. str. according to the present study, but also part of /Clade D (Pallidocephalus). This clade deserves to be more carefully studied to formalize the establishment of another genus in Omphalotaceae. A synoptic key comparing members of this clade with the genera of /letinuloid clade is provided in Online Resource (Chart O1).

**/Clade E (*Rhodocollybia*):** This highly supported clade groups all species of *Rhodocollybia* present in this analysis, including the type species *R. maculata* (Alb. & Schwein.) Singer. The genus is monophyletic in this study and agrees with Wilson and Desjardin (2005) and Mata et al. (2006) but differs from Hughes et al. (2010) and Sandoval-Leiva et al. (2016); however, additional taxa of the genus should be included in future analyses. For more information about *Rhodocollybia* see Mata et al. (2004b). A synoptic key comparing *Rhodocollybia* with the other genera of /letinuloid clade is provided in Online Resource (Chart O1).

**/Clade F (*Lentinula*):** This is a strongly supported clade encompassing all *Lentinula* Earle species included in the analysis: *L. lateritia* (Berk.) Pegler, *L. edodes* (Berk.) Pegler, *L. raphanica* (Murrill) Mata & R.H. Petersen and *L. boryana* (Berk. & Mont.) Pegler. Based on this analysis, the genus is monophyletic. For further information about the genus *Lentinula*, see Pegler (1983) and Mata et al. (2001). A synoptic key comparing *Lentinula* with the other genera of /letinuloid clade is provided in Online Resource (Chart O1).

**/Clade G (*Gymnopus* s. str.):** This strongly supported clade is composed of *Gymnopus fusipes*, the type species of the genus and the only member of *Gymnopus* sect. *Gymnopus*, and members of *Gymnopus* sect. *Androsacei*, sect. *Impudicae*, and sect. *Levipedes*. In this clade, *Gymnopus* s. str. is represented as a monophyletic and less inclusive genus, corresponding to the statistical supported clade D or /gymnopus (excluding *Micromphale peforans* branch) in Wilson and Desjardin (2005). These authors disagreed with the more inclusive genus suggested in Mata et al. (2004a). Mata et al. (2006) maintained a broad view of *Gymnopus* corresponding to a major clade grouping the clades A–N in their tree. In the

trees provided by Hughes et al. (2010) that focused on the establishment of *Connopus*, their ITS tree shows *Gymnopus* as a single monophyletic clade (but not including *G. fusipes* nor *G. androsaceus*, and members of *Gymnopus* sect. *Levipedes*) while the LSU tree presents multiple unrelated clades bearing members of *Gymnopus* (including *G. fusipes*, *G. androsaceus*, and members of *Gymnopus* sect. *Levipedes*). Sandoval-Leiva et al. (2016) provided a tree based on concatenated ITS–5′–28S, but via neighbor-joining method and values of support via Bayesian and BioNJ analyses focused in the establishment of the new genus *Gymnopanella* and presented monophyletic, strongly supported clades “/marasmiellus” and “*Gymnopus*, including *Setulipes*,” unrelated in the tree, where the former is sister to “*Rhodocollybia*, including *Connopus*” and the latter is sister to *Gymnopanella*. Similar results are found in Petersen and Hughes (2016). In the present study, the concept of less inclusive *Gymnopus* (s. str.) as defended by Wilson and Desjardin (2005) is strongly supported, and also observed in Sandoval-Leiva et al. (2016) and corresponds to clades A–C in Mata et al. (2006). In the concatenated nrITS + nrLSU tree (Fig. 2), clade G is distinctly resolved and strongly supported, and includes *G. fusipes* and members of *Gymnopus* sect. *Levipedes*, *Impudicae* and *Androsacei*. Wilson and Desjardin (2005) discussed that *G. fusipes* and members of *Gymnopus* sect. *Levipedes* share a pileipellis composed of relatively short, broad, branched, non-diverticulate hyphae forming a *Dryophila*-structure based on Halling (1983) and Antonín and Noordeloos (1997), but the type species of the genus only differs in developing a rooted stipe and pale pinkish or ochraceous spore print. However, based on Wilson and Desjardin (2005), species of *Micromphale* and those of the old *Setulipes* are quite distinct from members *Gymnopus* sect. *Levipedes* and *G. fusipes*, because the first two groups produce small, marcescent basidiomata with wiry and insititious stipes, usually accompanied by black wiry rhizomorphs and pileipellis with hyphae which are non-diverticulate and (a) gelatinized and encrusted with a brown pigment (*Micromphale*) or (b) diverticulate-knobby and usually non-gelatinized (*Setulipes*). Also, in Wilson and Desjardin (2005), *Micromphale perforans* branched as sister to the clade D and without statistical support. Although being included in /*gymnopus* in the same study, their results regarding *M. perforans* are clarified as part of a separate clade corresponding to *Gymnopus* sect. *Perforantia* (Singer) R.H. Petersen, resulting from the combination of *Micromphale* sect. *Perforantia* Singer in *Gymnopus* by Petersen and Hughes (2016). Our results also show a similar separate clade (clade H in the present study), but instead, support the establishment of a new genus *Paragymnopus*. The clade also bears some informal taxa that need to be confirmed. The strains TFB14489, TFB14594, and TFB14599 represent the species *Micromphale brevipes* (Berk. & Ravenel) Singer ( $\equiv$  *Marasmius brevipes* Berk. & Ravenel), according to

Petersen and Hughes (2019), species now belonging to *Gymnopus*. César et al. (2018) proposed *Marasmius brevipes* to be a synonym of *Marasmius westii* Murrill. This proposal was rejected by Petersen and Hughes (2019), which provided a new name to the species, *Gymnopus neobrevipes* R.H. Petersen, since there is already *Gymnopus brevipes* (Bull.) Gray [now *Melanoleuca brevipes* (Bull.) Pat.].

**/Clade H** (*Gymnopus* sect. *Perforantia*): This clade is composed of *Gymnopus foliophilus* R.H. Petersen, *G. sublaccatus* R.H. Petersen, *G. sequoiae* (Desjardin) R.H. Petersen, *G. perforans* (Hoffm.) Antonín & Noordel., *G. pinophilus* R.H. Petersen and *G. ponderosae* R.H. Petersen. The first evidence of this lineage is found in Wilson and Desjardin (2005), but was further explored in Petersen and Hughes (2016) (see discussion in the previous clade). According to Petersen and Hughes (2016), the species in this clade form *Gymnopus* sect. *Perforantia* and are morphologically characterized by: (1) pileus and lamellar tramae as well as stipe medullary hyphae and pileipellis embedded in slime matrix; (2) pileipellis of two kinds—(i) a layer of repent, incrustated hyphae, conspicuously clamped, usually with thickened walls (through gelatinization) and embedded in slime matrix, or (ii) well-developed *Rameales* structure; (3) cheilocystidia generally absent, if present either clavate to utriform or like *Siccus*-type broom cells; (4) stipe often thread-like (than 1 mm) but usually up to 40 mm long, and often a high ratio of the pileus breadth to stipe length; (5) stipitipellis often with cellular differentiation (excepting *G. glabrosipes* R.H. Petersen), many times minutely barbed under a 30 $\times$  lens; (6) always black, branched or unbranched rhizomorphs almost present in all cases, from 2 to 40  $\times$  0.2–0.7 mm; (7) basidiospores not significantly differing in dimension; (8) basidiomata found on dead conifer needles or rotting deciduous leafy debris, more or less host-specific; (9) fusiform pleurocystidia consistently present, with slight differences at the summit; and (10) clamp connections found in all taxa. More morphological characteristics assigned to the section are detailed in Petersen and Hughes (2016). The stipe in this section is mostly insititious but can also be subinsititious or sometimes non-insititious. Petersen and Hughes (2016) recovered a tree based on nrLSU where the clade assigned to *Gymnopus* sect. *Perforantia* is sister to /*gymnopus*, but with low support (ML BS 44). In the tree from our analysis based on concatenated nrITS and nrLSU data, clade H (*Gymnopus* sect. *Perforantia*) appears paraphyletic relative to clade G (*Gymnopus* s. str.), considering that the taxon labeled “KY026621 *Marasmius* sp.1 TFB3940” is a distinct lineage sister to clade G (then, clade H is sister to “clade G + ‘TFB3940 *Marasmius* sp.1’” without statistical support). Thus, we propose the establishment of a new genus, *Paragymnopus*, instead of recognizing this clade

at a lower rank, *Gymnopus* sect *Perforatia* (see clade G above).

**/Clade I (*Gymnopanella*):** Clade I is formed by *Gymnopanella nothofagi* Sandoval-Leiva, J.V. McDonald & Thorn, plus strains of informal taxa named *Gymnopus caulocystidiatus* nom. prov. and *G. austrobrevipes* nom. prov. in Petersen and Hughes (2016). Unfortunately, a morphological comparison is not possible in order to classify these informal taxa in *Gymnopanella*, since no anatomical data are provided to date. But according to our tree, we suggest that *Gymnopus caulocystidiatus* nom. prov. and *G. austrobrevipes* nom. prov. are better placed in *Gymnopanella*. This genus is mainly characterized by being saprotrophic, producing campanelloid basidiomata that are flabelliform, convex, have a gelatinous pileus; a reticulate-lamellate hymenophore; and a lateral, short, glabrous stipe. Basidiospores are broad ellipsoid to ovate, hyaline, thin-walled, non-amyloid; subhymenium is ramose; gelatinous, dense trama, composed of subparallel to tangled hyphae, with fine ring-like incrustation; and the pileipellis is a cutis of cylindrical, thick-walled hyphae, seldom branched, coarsely externally incrustated with deposits, some hyphae forming fascicles (Sandoval-Leiva et al. 2016). A synoptic key comparing *Gymnopanella* with the other genera of /letinuoid clade is provided in Online Resource (Chart O1).

**/Clade J (*Mycetinis*):** This strongly supported clade represents the genus *Mycetinis* Earle, grouping *M. alliaceus* (Jacq.) Earle (type species of the genus), *M. copelandii* (Peck) A.W. Wilson & Desjardin, *M. opacus* (Berk. & M.A. Curtis) A.W. Wilson & Desjardin, *M. prasioemus* (Fr.) R.H. Petersen, *M. salalis* (Desjardin & Redhead) Redhead and *M. scorodoni* (Fr.) A.W. Wilson & Desjardin. The genus was resurrected in Wilson and Desjardin (2005) based on clade F in the tree shown in their study. Species of this genus were previously included in *Marasmius* sect. *Alliacei*. Based on the same study, *Mycetinis* species share characteristics such as sub- to non-insititious stipe; inamyloid, acyanophilic, white or pale cream spore print, inamyloid trama; and a hymeniform pileipellis made up of smooth, clavate to lobed elements. However, *M. opacus* may have *Rameales*-type structures in the pileipellis at the pileus margin when fully mature along with the usual clavate to lobed, often thick-walled cells (Desjardin et al. 1993) and an insititious stipe accompanied by abundant rhizomorphs (Desjardin et al. 1993; Wilson and Desjardin 2005) and was therefore, previously considered as a *Marasmiellus* species. Interestingly, many taxa in this clade have basidiomata mimicking garlic or cabbage odor and flavor, but the same characteristic can be found in *Gymnopus foetidus*, *G. perforans*, *G. iocephalus* (Berk. & M.A. Curtis) Halling, *G. polyphyllus* (Peck) Halling and several species of the *Androsacei* group (Wilson and Desjardin 2005). A synoptic key comparing

*Mycetinis* with the other genera of /letinuoid clade is provided in Online Resource (Chart O1).

**/Clade K (*Omphalotus*):** Clade K groups together *Omphalotus olearius* (DC.) Singer (type species) and *O. olivascens* H.E. Bigelow, O.K. Mill. & Thiers and represents the genus (type of Omphalotaceae) in the analysis. *Omphalotus* appears as sister to *Anthracophyllum* Ces. (/Lineage L), represented by *A. acheri* (Berk.) Pegler, but this relationship is unsupported.

## Morphology

*Pusillomyces manuripioides* is very similar to *Manuripia bifida* in the macromorphology (Singer 1960, 1976, 1986). In the field, the specimens were immediately associated with the only species of *Manuripia* on account of the very small basidiomata, an orbicular, smooth pileus with smooth hymenophore, and a filiform, dark-colored, insititious stipe growing directly from abundant, both glabrous or densely pubescent rhizomorphs (Fig. 3a–c). Microscopically (Fig. 5), the new species is also like *M. bifida* (Singer 1960, 1976) in having ellipsoid, smooth, hyaline, inamyloid basidiospores, with smaller but still compatible dimensions ( $6\text{--}9 \times 2.8\text{--}4.6 \mu\text{m}$  vs  $8\text{--}9.7 \times 3.8\text{--}4.8 \mu\text{m}$ ), the absence of cystidia in the hymenium, and by the inamyloid pileus trama while the stipe/rhizomorphs trama can be inamyloid or scarcely dextrinoid.

However, *P. manuripioides* differs strikingly from *M. bifida* in having a non-hymeniform pileipellis composed of *Rameales* structures, with some *Siccus*-type broom cells, and also abundant incrustated hyphae in the upper to the mid-trama of the pileus, gradually disappearing near the hymenial layer. On the other hand, *M. bifida* has a hymeniform to subhymeniform pileipellis composed of balloon-shaped or vesiculose-clavate *Rotalis*-type broom cells and no incrustated hyphae in the pileus trama (Singer 1976). The rhizomorphs in *P. manuripioides*, when pubescent (Fig. 3c), are covered by elongate, dark-brown, multiseptated, smooth conidia on multiple conidiophores (Fig. 6) growing along the cortical trama of the stipe. According to Singer (1976), the rhizomorphs of *M. bifida* are covered by setoid, thick-walled, fuliginous to fuliginous-chestnut hairs (Singer 1960), clearly different from the elements found in the cortical layer of the rhizomorphs in *P. manuripioides*. Singer (1976) also described the rhizomorphs in *M. bifida* as thicker than the stipe whereas this distinction is absent or not clear in *P. manuripioides*. The two species also differ in the kind of substrate/habitat, strongly suggesting different niches (see the next section).

Anatomically, the pileipellis arrangement and elements of *P. manuripioides* are compatible with species of *Gymnopus* sect. *Androsacei* (Mata et al. 2004a; Wilson and Desjardin 2005), previously *Marasmius* sect. *Androsacei*. On the other hand, the

arrangement and elements of the pileipellis in *M. bifida* are very similar to the pileipellis of members of *Marasmius* sect. *Marasmius* subsect. *Marasmius* (Singer 1976). In the phylogenetic discussion in the present paper, *P. manuripioides* is confirmed as a member of Omphalotaceae though this lineage does not branch close to *G. androsaceus*. If the hypothesis that *M. bifida* is truly a member of Marasmiaceae, the present study suggests that although *P. manuripioides* and *M. bifida* are strongly similar macroscopically and in some microscopic characteristics, the resemblance possibly represents a case of convergence. The inclusion of *M. bifida* in future analyses is important to have a final conclusion.

In the “Phylogeny” section, *P. manuripioides* (JO674 and JO1121) represents a distinct, new genus and that *G. asetosus* and *G. funalis* (Antonín et al. 2014) are congeneric. *Pusillomyces manuripioides* strongly differs from these two species in producing an even smaller basidioma with a smooth, discoid, up to 1.8 mm diam. pileus, a completely smooth hymenophore, a short and very thin, wiry stipe mostly arising from abundant, hair-like rhizomorphs or directly from the substrate, and the absence of cheilocystidia. In spite of often having some pubescent rhizomorphs, *P. manuripioides* is devoid of true caulocystidia, instead, elongate, dark brown, multiseptated conidia and conidiophores penetrating the cortical layer. Otherwise, both stipe and rhizomorphs are glabrous, which is similar to *G. asetosus* (Antonín et al. 2014). However, these three species share ellipsoid basidiospores, the absence of pleurocystidia, the arrangement and the elements of the pileus trama and the pileipellis, and the stipe trama and stipitipellis (excepting the caulocystidia in *G. funalis*) (Antonín et al. 2014).

Below, a protologue is provided for *Paragymnopus* gen. nov. according to the clade H (Fig. 2), and *Gymnopus* (s. str.) and *Marasmiellus* (s. str.) are redefined based on clade G and clade A (Fig. 2) respectively, with correspondent combinations.

***Paragymnopus* J.S. Oliveira, gen. nov.**

Mycobank MB 827363.

*Micromphale* sect. *Perforantia* Singer, Sydowia 2: 32 (1948)

*Gymnopus* sect. *Perforantia* (Singer) R.H. Petersen, MycoKey 18: 8 (2016)

Etymology: It refers to the phylogenetic relationship of this group with *Gymnopus* s. str.

Diagnose: *Basidiomata* marasmioid, small, thin. *Pileus* convex to plano-convex, occasionally slightly umbonate, sulcate-striate. *Lamellae* adnate, adnexed to decurrent, sometimes collariate to pseudocollariate, pallid or off-white. *Stipe* often up to 40 mm long, ratio of pileus breadth to stipe length often large (see *G. pinophilus*), central, cylindrical, thin, usually less than 1 mm, insititious, usually vested (excepting

*G. glabrosipes*), often minutely barbed under the  $\times 10$  lens, apex pale brown, then darker, dark sooty brown to nearly black towards the base. *Rhizomorphs* usually present, black, thin, filiform, branched or unbranched. *Basidiospores* obovoid, ellipsoid, amygdaliform, hyaline, smooth, thin-walled, non-amyloid. *Pleurocystidia* consistently present, fusiform, somewhat apically versiform. *Cheilocystidia* often absent; if present, either clavate to utriform or similar to *Siccus*-type broom cell. *Pileus* and *lamellar tramae* irregular, usually loosely interwoven, with hyphae embedded in a slime matrix. *Pileipellis* in the form of a gelatinized layer of repent, incrustated hyphae, often thick-walled, conspicuously clamped and embedded in a slime matrix, or as a well-developed *Rameales*-structure. *Stipe medullary* with hyphae embedded in slime matrix. *Clamp connections* present in all tissues.

Chemical reactions: In Melzer’s reagent, dextrinoid only in the stipe cortical trama and caulocystidia of some species; otherwise, all tissues inamyloid. No part staining in alkaline solution (NH<sub>4</sub>OH or KOH).

Ecology: Seemingly host-specific, saprophytic, on dead conifer needles or rotting deciduous leafy debris.

Type species: *Paragymnopus perforans* (Hoffm.) J.S. Oliveira (see new combination below).

Delimitation: According to Petersen and Hughes (2016), *Gymnopus* sect. *Perforantia* (herein as *Paragymnopus*) may be difficult to separate from *Gymnopus* sect. *Androsacei*. Basidiomata of some species in this latter produce a thin slime matrix in the basidiomata, a characteristic traditionally found in *Micromphale* sect. *Perforantia*, the genus/section where *G. perforans* belonged before being combined in *Gymnopus* sect. *Perforantia* (Petersen and Hughes 2016). This characteristic is also relevant for all species in *Gymnopus* sect. *Perforantia*, therefore, also in *Paragymnopus*. However, *Gymnopus* sect. *Androsacei* has a pileipellis consisting of a trichoderm (hymeniform in primordia) made up by diverticulate hyphae together with *Siccus*-type broom cell-like and setulose endings or well-developed *Rameales* structure and lacking pleurocystidia while *Gymnopus* sect. *Perforantia* subsect. *Perforantia* (then, *Paragymnopus*) has a pileipellis formed by a cutis of repent, usually incrustated or varying from diverticulate to non-diverticulate hyphae and having pleurocystidia (Antonín and Noordeloos 2010; Petersen and Hughes 2016). This distinction based on elements of the pileipellis is not observed between *Gymnopus* sect. *Androsacei* and *Gymnopus* sect. *Perforantia* subsect. *Pinophili*, a problem that was also noted by Petersen and Hughes (2016). A solution for this apparent inconsistency is not provided to date based on morphology since it was surprising to see *G. pinophilus* and *G. ponderosae* branching close to *Gymnopus* sect. *Perforantia* subsect. *Perforantia* instead of *Gymnopus* sect. *Androsacei* (Petersen and Hughes 2016). Macroscopically, *Paragymnopus* usually has off-white to dingy pale gray lamellae, never attached to a

pseudocollarium, and almost always a vested stipe (Petersen and Hughes 2016). On the other hand, the lamellae in *Gymnopus* sect. *Androsacei* are often nearly as dark as the pileus, sometimes attached to a pseudocollarium, and the hair-like stipe is usually glabrous and shining (Antonín and Noordeloos 2010; Petersen and Hughes 2016). Finely hairy stipe can be found only in *Gymnopus cremeostipitatus* Antonín, R. Ryoo & K.H. Ka (Antonín and Noordeloos 2010) if the species is considered within the section based on the concept proposed in the present study (see more in the discussion on *Gymnopus* sect. *Androsacei*). Among numerous morphological divergencies, *Paragymnopus* (Clade H) differs from the other sections of *Gymnopus* s. str. (Clade G) in having fusiform pleurocystidia and dextrinoid structures (trama or elements). A synoptic key comparing *Paragymnopus* with the other genera of *Letinuloid* clade is provided in Online Resource (Chart O1).

Selected literature: Antonín and Noordeloos (2010); Petersen and Hughes (2016).

#### ***Paragymnopus* sect. *Paragymnopus***

*Gymnopus* sect. *Perforantia* subsect. *Perforantia*, subsect. auton.

Stipe usually vested; cheilocystidia often absent, when present, clavate to utriform; pileipellis a layer of repent, incrustated or diverticulate hyphae (outgrowths on hyphal segments, not like *Rameales* structure), conspicuously clamped, usually with thickened walls (through gelatinization) and usually embedded in a slime matrix; fruiting on conifer needles or broad-leafed debris [summarized from Petersen and Hughes (2016) as *Gymnopus* sect. *Perforantia*].

Type species: *Paragymnopus perforans* (Hoffm.) J.S. Oliveira.

*Paragymnopus foliiphilus* (R.H. Petersen) J.S. Oliveira, comb. nov. (MB 828406)

*Gymnopus foliiphilus* R.H. Petersen, MycoKeys 18: 17 (2016)

*Paragymnopus foliiphilus* var. *costaricensis* (R.H. Petersen & J.L. Mata) J.S. Oliveira, comb. nov. (MB 828407)

*Gymnopus foliiphilus* var. *costaricensis* R.H. Petersen & J.L. Mata, MycoKeys 18: 28 (2016)

*Paragymnopus perforans* (Hoffm.) J.S. Oliveira, comb. nov. (MB 828408)

*Agaricus perforans* Hoffm., Nomencl. fung.: 215, t.4: 2 (1789)

*Paragymnopus perforans* subsp. *transatlanticus* (R.H. Petersen) J.S. Oliveira, comb. nov. (MB 828409)

*Gymnopus perforans* subsp. *transatlanticus* R.H. Petersen, MycoKeys 18: 52 (2016).

*Paragymnopus sequoiae* (Desjardin) J.S. Oliveira, comb. nov. (MB 828410)

*Micromphale sequoiae* Desjardin, Mycologia 77: 894 (1986)

*Paragymnopus sublaccatus* (R.H. Petersen) J.S. Oliveira, comb. nov. (MB 828411)

*Gymnopus sublaccatus* R.H. Petersen, MycoKeys 18: 97 (2016)

***Paragymnopus* sect. *Pinophili* (R.H. Petersen) J.S. Oliveira, comb. nov.**

*Gymnopus* sect. *Perforantia* subsect. *Pinophili* R.H. Petersen, MycoKeys 18: 9 (2016)

Stipe glabrous-shining; cheilocystidia *Siccus*-type broom cells; pileipellis composed of well-developed *Rameales* structure; fruiting on needles of *Pinus*; discrete clade well-separated from the clade of members of the previous section [summarized from Petersen and Hughes (2016) as *Gymnopus* sect. *Perforantia* subsect. *Pinophili*].

Type species: *Paragymnopus pinophilus* (R.H. Petersen) J.S. Oliveira.

*Paragymnopus pinophilus* (R.H. Petersen) J.S. Oliveira, comb. nov. (MB 828412)

*Gymnopus pinophilus* R.H. Petersen, MycoKeys 18: 62 (2016)

*Paragymnopus ponderosae* (R.H. Petersen) J.S. Oliveira, comb. nov. (MB 828413)

*Gymnopus ponderosae* R.H. Petersen, MycoKeys 18: 70 (2016)

***Gymnopus* (Pers.) Roussel, Flore du Calvados et terrains adjacents, composée suivant la méthode de Jussieu: 62 (1806)**

*Basidiomata* collybioid, rarely tricholomatoid or marasmiod. *Pileus* convex, plano-convex to applanate or slightly concave, with or without umbo or papilla, hygrophanous or not, translucently striate or not, dry or slightly viscid, glabrous or innately radially fibrillose. *Lamellae* free, emarginate or adnate, usually crowded, sometimes fairly distant, regular, segmentiform or ventricose with entire or serrate edge. *Stipe* central, cylindrical fleshy to filiform, wiry, sometimes broadened towards base, glabrous and polished or fibrillose to finely pubescent (mostly when the basidiomata are fetid or dried or when any tissue reacts to alkaline solution), insititious or more often non-insititious, usually with strigose base, some with whitish rhizoids or sometimes deeply rooting or arising from a sclerotium, tough and firm, solid or fistulose. *Rhizomorphs* rarely present. *Odor* indistinct or fetid

(rotten cabbage, sewage, etc.) or onion- or garlic-like. *Spore print* white. *Basidiospores* ellipsoid to short-oblong (never very elongate or long-clavate), not often subglobose to globose or lacrymoid, thin-walled, hyaline, non-amyloid, with confluent or well-delimited hilar appendage. *Basidia* 4-spored, clamped. *Cheilocystidia* usually present, cylindrical, flexuous, clavate or irregularly coralloid, thin-walled, sometimes as broom cells. *Pleurocystidia* absent. *Lamellar trama* regular to subregular. *Pileus trama* irregular. *Pileipellis* a cutis or ixocutis of radially arranged cylindrical hyphae, or interwoven, more like a trichoderm or ixotrichoderm, made up of irregular coralloid terminal elements (*Dryophila*-type structures), sometimes hymeniform in primordial stages only, then soon an irregular trichoderm, composed of irregular, often incrustated, diverticulate hyphal elements, mixed with broom cells and coralloid hyphae. *Clamp connections* present in all tissues (except for *G. bisporiger* Antonín & Noordel.).

Chemical reactions: No reaction to Melzer's reagent or Cresyl Blue, but there are rare cases where incrustations of the hyphae are dextrinoid or in *Gymnopus* sect. *Androsacei* where at least the stipe trama is dextrinoid; some taxa have also tissue of the basidiome turning green, olivaceous or ochraceous in alkaline solution (NH<sub>4</sub>OH or KOH).

Ecology: Saprotrophic, rarely parasitic; in humus, on wood, rarely on roots of dead, not often on living herbaceous or woody plants (Antonín and Noordeloos 2010).

Type species: *Gymnopus fusipes* (Bull.) Gray.

Delimitation: According to the clade G, *Gymnopus* s. str. is composed of members of *Gymnopus* sect. *Gymnopus*, sect. *Androsacei*, sect. *Levipedes* (subsections *Levipedes* and *Alkalivirentes*) and sect. *Impudicae*. With only one exception so far, *G. barbipes* R.H. Petersen & K.W. Hughes that grouped along with members of sect. *Impudicae*, *Gymnopus* sect. *Vestipedes* is segregated and now placed within *Marasmiellus* s. str. In spite of being very distinct genera based on the phylogenetic trees of the present study, *Gymnopus* s. str. and *Marasmiellus* s. str. agree in many features and remain devoid of sharp morphological divergency. As long as members of *Gymnopus* sect. *Vestipedes* can be recognized as such using morphological characteristics, dichotomy between this group (sect. *Vestipedes*) and the other sections of *Gymnopus* s. str. can be warranted. In other words, this is the limit between *Gymnopus* s. str. and *Marasmiellus* s. str. Based on the present study, *Gymnopus* s. str. contains the four cited sections, and they are found either to be monophyletic (with high support) or tending to monophyletic but unsupported. This result is quite concordant with previous phylogenetic studies (Wilson and Desjardin 2005; Mata et al. 2006; Hughes et al. 2010; Coimbra et al. 2015; Petersen and Hughes 2016, 2017). Wilson and Desjardin (2005) were the first to suggest these possible concepts of distinct genera, despite the fact that their /marasmiellus was unsupported and their /gymnopus included *Micromphale perforans* (*Paragymnopus*). Mata et al. (2006), in spite of defending a

broad concept of *Gymnopus* including *Marasmiellus*, found groupings suggested not only two possible genera, but a possible infrageneric relationship of *Gymnopus* s. str. into sections. These possible sections were assimilated by Antonín and Noordeloos (2010), but they did not definitively exclude *Gymnopus* sect. *Vestipedes* from the genus. Hughes et al. (2010) and Petersen and Hughes (2016, 2017) also found this pattern in their tree, but the concept of two genera (*Gymnopus* and *Marasmiellus*) was not established, and only included a new section in the broad *Gymnopus* (Petersen and Hughes 2016). Coimbra et al. (2015) worked on and developed the concept of *Gymnopus* sect. *Impudicae*. The present study not only provides limits between *Gymnopus* and *Marasmiellus*, but also strengthens the concepts of at least four possibly natural sections in *Gymnopus*. The sections are presented below, but the concepts of subsections are not treated in this present paper. Additionally, a synoptic key comparing *Gymnopus* s. str. with the other genera of /letinuloid clade is provided in Online Resource (Chart O1).

Selected literature: Singer (1986), Wilson et al. (2004), Antonín et al. (1997), Antonín and Noordeloos (1997, 2010).

#### *Gymnopus* sect. *Gymnopus*

Basidiomata fleshy; stipe radicate, forming a distinct pseudorrhiza, deeply sulcate-striate; spore print white to pale ochraceous; cheilocystidia present; pileipellis as a transition between a cutis and a trichoderm, often somewhat gelatinized, composed of irregular, inflated, or often coralloid elements, some resembling *Dryophila*-type structures; no dextrinoid or cyanophilous structures; parasitic or saprophytic, in bundles at the base of broad-leaved trees, frequently on roots or stumps [summarized from Antonín and Noordeloos (2010)].

Type species: *Gymnopus fusipes* (Bull.) Gray.

*Gymnopus* sect. *Androsacei* (Kühner) Antonín & Noordeloos, Czech Mycology 60: 25 (2008)

Basidiomata small, marasmoid; pileus dull, dry; lamellae free to adnate, sometimes pseudocollariate; stipe filiform (hair-like), insititious and glabrous (if *G. cremeostipitatus* belongs to this section, then the stipe can be pallid and finely pubescent or hairy), cheilocystidia in the form of *Siccus*-type broom cells or coralloid elements; pileipellis hymeniform in primordia only, then non-hymeniform, an irregular trichoderm of often incrustated, diverticulate hyphal elements, irregular in outline, mixed with *Siccus*-type broom cells and coralloid elements; trama dextrinoid, at least in the stipe apex; saprophytic, on litter of coniferous or broadleaved trees [summarized from Antonín and Noordeloos (2010)].

Type species: *Gymnopus androsaceus* (L.) Della Magg. & Trassin.

Notes: *Gymnopus cremeostipitatus* Antonín, R. Ryoo & K.H. Ka, *G. neobrevipes* R.H. Petersen and *G. portoricensis* R.H. Petersen are additional taxa according to the present circumscription. A small strongly supported clade groups

*G. androsaceus* along with informal taxa named *Gymnopus adventitius* nom. prov., *G. frigidomarginatus* nom. prov., and *G. novaeangliae* nom. prov. Also, perhaps *Gymnopus inflatotrama* nom. prov. and *G. novomundi* nom. prov. are close species, but they await description. These informal taxa seem to belong to *Gymnopus* sect. *Androsacei* along with the four already described species. However, all these species did not form a clade, so a definitive resolution will wait for future studies. Out of these species, only *G. cremeostipitatus* produce non-glabrous and pale stipe and may be a taxon of other section, helping to separate *Gymnopus* sect. *Androsacei* from *Paragymnopus* sect. *Paragymnopus*.

***Gymnopus* sect. *Levipedes*** (Fr.) Halling, Brittonia 48 (4): 487 (1996).

Stipe smooth, polished or pubescent; pileipellis mostly as an entangled (never radially oriented) trichoderm, composed of inflated, often lobed elements or coralloid, *Dryophila*-type structures; trama and elements non-dextrinoid, some species turning green in alkali; saprophytic, usually in coarse humus and forest litter, or on rotten wood [summarized from Antonín and Noordeloos (2010)].

Type species: *Gymnopus dryophilus* (Bull.) Murrill.

Additional species: *Gymnopus alkalivirens* (Singer) Halling; *G. alpinus* (Vilgalys & O.K. Mill.) Antonín & Noordel.; *G. aquosus* (Bull.) Antonín & Noordel.; *G. aurantiipes* (Corner) A.W. Wilson, Desjardin & E. Horak; *G. austrosemihirtipes* A.W. Wilson, Desjardin & E. Horak; *G. bicolor* A.W. Wilson, Desjardin & E. Horak; *G. bisporus* (J. Carbó & Pérez-De-Greg.) J. Carbó & Pérez-De-Greg.; *G. catalanicus* (Vila & Llimona) Vila & Llimona; *G. earleae* Murrill; *G. erythropus* (Pers.) Antonín, Halling & Noordel.; *G. exculptus* (Fr.) J.L. Mata; *G. fagiphilus* (Velen.) Antonín, Halling & Noordel.; *G. hybridus* (Kühner & Romagn.) Antonín & Noordel.; *G. inusitatus* (Vila & Llimona) Vila & Llimona; *G. inusitatus* var. *cystidiatus* Antonín; *G. indoctoides* A.W. Wilson, Desjardin & E. Horak; *G. junquilleus* R.H. Petersen & J.L. Mata; *G. kauffmanii* (Halling) Halling; *G. macropus* Halling; *G. montagnei* (Berk.) Redhead; *G. nubicola* Halling; *G. ocior* (Pers.) Antonín & Noordel.; *G. polyphyllus* (Peck) Halling; *G. salakensis* A.W. Wilson, Desjardin & E. Horak; *G. sepiiconicus* (Corner) A.W. Wilson, Desjardin & E. Horak; *G. spongiosus* (Berk. & M.A. Curtis) Halling; *G. subsulphureus* (Peck) Murrill; *G. vitellinipes* A.W. Wilson, Desjardin & E. Horak.

***Gymnopus* sect. *Impudicae*** (Antonín & Noordel.) Antonín & Noordel.: 222 (2010)

Basidiomata collybioid or marasmioid; smell strong, fetid (rotten cabbage, sewage, etc.) or like onions or garlic; cheilocystidia often inconspicuous; pileipellis composed of diverticulate elements, but never in form of true *Dryophila*-

type structures [summarized from Antonín and Noordeloos (2010)].

Type species: *Gymnopus impudicus* (Fr.) Antonín, Halling & Noordel.

Additional species: *G. barbipes* R.H. Petersen & K.W. Hughes; *G. brassicolens* (Romagn.) Antonín & Noordel.; *G. brassicolens* var. *pallidus* Antonín & Noordel.; *G. dysodes* (Halling) Halling; *G. foetidus* (Sowerby) J.L. Mata & R.H. Petersen; *G. impudicus* var. *graveolens* (G. Poirault ex Boud.) Vila & Llimona; *G. iocephalus* (Berk. & M.A. Curtis) Halling.

***Marasmiellus*** Murrill, North American Flora 9 (4): 243 (1915).

*Marasmius* sect. *Rameales* Kühner, Botaniste 25: 88 (1933).

*Gymnopus* sect. *Vestipedes* (Fr.) Antonín, Halling & Noordel., emed., Mycotaxon 63: 363 (1997).

*Basidiomata* gymnopoid, collybioid or omphalioid, marasmielloid, to pleurotoid. *Pileus* orbicular to semicircular, some reniform, applanate to convex, or campanulate, with or without papilla, flattened or wavy-lobate, white, yellow, pink or brown. *Lamellae* usually well-developed, rarely venose, free, adnate to decurrent. *Stipe* central, eccentric to almost lateral, sometimes reduced and curved, cylindrical, insititious or sub-insititious, rarely with distinct basal mycelium, fistulose to hollow, pale at the apex, often darkening towards base, surface vested, pruinose to pubescent, pulverulent, fibrillose, minutely flocculose to rather distinctly squamulose, but never glabrous or smooth. *Rhizomorphs* usually absent, rarely present. *Odor* indistinct. *Spore print* white to cream. *Basidiospores* ellipsoid to oblong, rarely sub-cylindrical, fusiform, lacrymoid or amygdaliform, usually with confluent hilar appendage, smooth, hyaline, thin-walled. *Basidioles* mostly fusoid. *Lamellar edge* usually sterile. *Cheilocystidia* often present, well-differentiated. *Pleurocystidia* usually absent. *Lamellar* and *Pileus trama* regular, subregular to irregular. *Pileipellis* usually a simple cutis, sometimes in transition to a trichoderm, with weakly to distinctly coralloid or diverticulate terminal elements (*Rameales*-structures), *Dryophila*-type structures never present. *Caulocystidia* present, well-developed, of various shapes. *Clamp connections* usually present.

Chemical reactions: No amyloid, dextrinoid or cyanophilous reactions; usually not metachromatical in Cresyl Blue.

Ecology: Usually gregarious, more rarely solitary, saprotrophic, more rarely parasitic on all kinds of living plants; some are host-specific; many species occur in rather exposed habitats, where moisture may vary considerably (costal dunes, xerophytic grassland); some are salt-tolerant (Antonín and Noordeloos 2010).

Type species: *Marasmiellus juniperinus* Murrill.

Delimitation: Based on Clade A (Fig. 2), *Marasmiellus* s. str. is composed of members of *Marasmiellus*, at least, of sect. *Dealbati*, sect. *Marasmiellus*, sect. *Rameales*, and sect. *Stenophylloides* [see also Wilson and Desjardin (2005)] and *Gymnopus* sect. *Vestipedes* (excepting *G. barbipes*). Based on Wilson and Desjardin (2005), *Marasmiellus* sect. *Candidi* does not belong to this new concept of *Marasmiellus* s. str. As in *Gymnopus* s. str., *Marasmiellus* s. str. is more evidently delimited in cladistic analyses so far. Since the present analysis includes abundant members of *Gymnopus* sect. *Vestipedes*, but the sections of *Marasmiellus* are poorly represented, no infrageneric classifications are tested or newly proposed herein. Based on Wilson and Desjardin (2005) and the present study (Fig. 1), we learn that *Marasmiellus* sect. *Candidi* is polyphyletic and its members are not included in the family Omphalotaceae, but possibly belong to Marasmiaceae within multiple lineages. The presence of pleurocystidia is very rare, observed for instance in *Marasmiellus phaeomarasmioides* G. Moreno, Heykoop, Esteve-Rav. & E. Horak (scarce, filiform, versiform, sometimes with few outgrowths) and *M. maasgeesterani* Robich & E. Campo (sparse, clavate to sphaeropenduculate) (Antonín and Noordeloos 2010). In the same study, *M. trabutii* (Maire) Singer is the only one reported as having pileus trama embedded in gelatinous matrix. Unfortunately, these three species have no sequences included in phylogenetic analyses, and these mentioned characteristics are not confirmed in *Marasmiellus* s. str. A synoptic key comparing *Marasmiellus* s. str. with the other genera of *Letinuloid* clade is provided in Online Resource (Chart O1). Retnowati (2018) has just proposed 16 new species of *Marasmiellus*, two new combinations and eight names are lectotypified in the genus. However, genetic data still need to be provided for phylogenetic evaluation of those taxa.

Selected literature: Singer (1986), Wilson et al. (2004), Wilson and Desjardin (2005), Antonín and Noordeloos (1997, 2010).

*Marasmiellus alnicola* (J.L. Mata & Halling) J.S. Oliveira, comb. nov. (MB 828414)

*Gymnopus alnicola* J.L. Mata & Halling, Fungal Diversity 16: 115 (2004)

*Marasmiellus bififormis* (Peck) J.S. Oliveira, comb. nov. (MB 828415)

*Marasmius bififormis* Peck, Bulletin of the New York State Museum 67: 25 (1903)

*Marasmiellus brunneogracilis* (Corner) J.S. Oliveira, comb. nov. (MB 828416)

*Marasmius brunneigracilis* Corner, Beihefte zur Nova Hedwigia 111: 39 (1996)

Notes: The correct spelling of the epithet is *brunneogracilis* instead of *brunneigracilis*. The name is herein combined

including the correct spelling.

*Marasmiellus collybioides* (Speg.) J.S. Oliveira, comb. nov. (MB 828417)

*Clitocybe collybioides* Speg., Boletín de la Academia Nacional de Ciencias en Córdoba 11 (4): 387 (1889)

*Marasmiellus confluens* (Pers.) J.S. Oliveira, comb. nov. (MB 828475)

*Agaricus confluens* Pers., Annalen der Botanik (Usteri) 15: 8 (1795)

*Marasmiellus cylindricus* (J.L. Mata) J.S. Oliveira, comb. nov. (MB 828477)

*Gymnopus cylindricus* J.L. Mata, Fungal Diversity 16: 118 (2004)

*Marasmiellus dichrous* (Berk. & M.A. Curtis) J.S. Oliveira, comb. nov. (MB 828478)

*Marasmius dichrous* Berk. & M.A. Curtis, Annals and Magazine of Natural History 12: 426 ('326') (1853)

*Marasmiellus disjunctus* (R.H. Petersen & K.W. Hughes) J.S. Oliveira, comb. nov. (MB 828479)

*Gymnopus disjunctus* R.H. Petersen & K.W. Hughes, North American Fungi 9: 2 (2014)

*Marasmiellus eberhardtii* (Pat.) J.S. Oliveira, comb. nov. (MB 828728)

*Laschia eberhardtii* Pat., Bulletin de la Société Mycologique de France 25: 8 (1909)

*Marasmiellus eneficola* (R.H. Petersen) J.S. Oliveira, comb. nov. (MB 828480)

*Gymnopus eneficola* R.H. Petersen, Omphalina 5 (5): 5 (2014)

*Marasmiellus fibrosipes* (Berk. & M.A. Curtis) J.S. Oliveira, comb. nov. (MB 828584)

*Marasmius fibrosipes* Berk. & M.A. Curtis, Journal of the Linnean Society. Botany 10: 293 (1869)

*Marasmiellus fuscotramus* (Mešić, Tkalčec & Chun Y. Deng) J.S. Oliveira, comb. nov. (MB 828481)

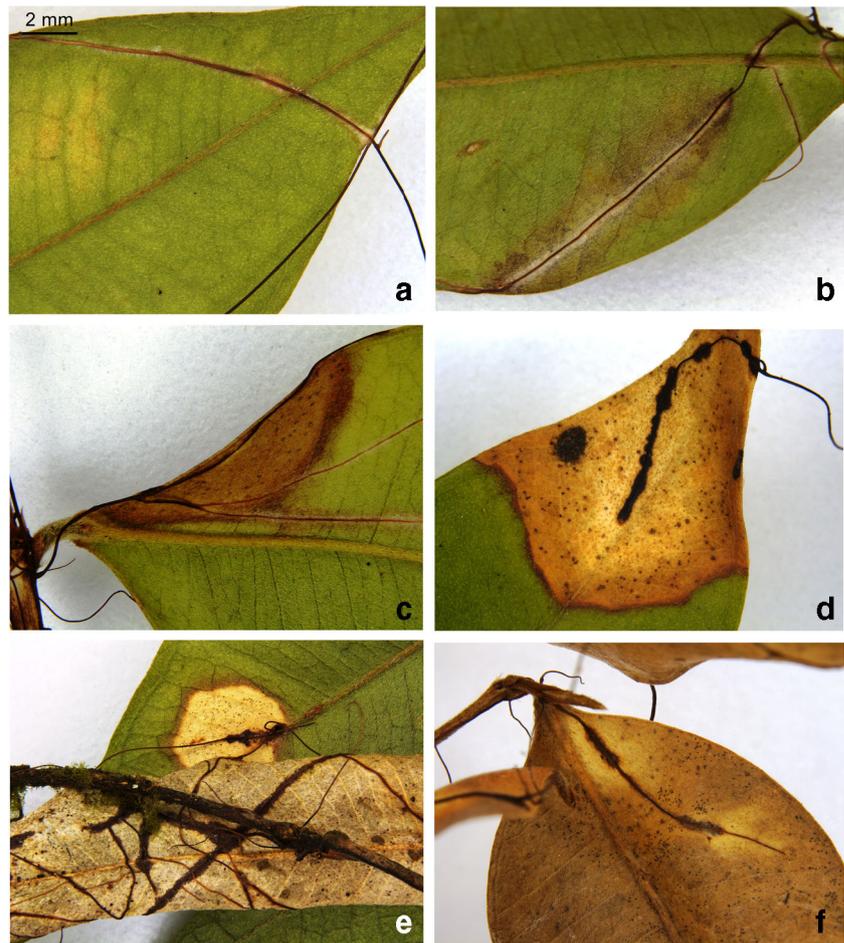
*Gymnopus fuscotramus* Mešić, Tkalčec & Chun Y. Deng, Mycotaxon 117: 324 (2011)

*Marasmiellus gibbosus* (Corner) J.S. Oliveira, comb. nov. (MB 828482)

*Marasmius gibbosus* Corner, Beihefte zur Nova Hedwigia 111: 55 (1996)

- Marasmiellus indoctus* (Corner) J.S. Oliveira, comb. nov. (MB 828483)  
*Marasmius indoctus* Corner, Beihefte zur Nova Hedwigia 111: 60 (1996)
- Marasmiellus luxurians* (Peck) J.S. Oliveira, comb. nov. (MB 828605)  
*Collybia luxurians* Peck, Bulletin of the Torrey Botanical Club 24: 141 (1897)
- Marasmiellus melanopus* (A.W. Wilson, Desjardin & E. Horak) J.S. Oliveira, comb. nov. (MB 828484)  
*Gymnopus melanopus* A.W. Wilson, Desjardin & E. Horak, Sydowia 56 (1): 181 (2004)
- Marasmiellus menehune* (Desjardin, Halling & Hemmes) J.S. Oliveira, comb. nov. (MB 828587)  
*Gymnopus menehune* Desjardin, Halling & Hemmes, Mycologia 91 (1): 173 (1999)
- Marasmiellus mesoamericanus* (J.L. Mata) J.S. Oliveira, comb. nov. (MB 828490)  
*Gymnopus mesoamericanus* J.L. Mata, Sydowia 58 (2): 283 (2006)
- Marasmiellus micromphaloides* (R.H. Petersen & K.W. Hughes) J.S. Oliveira, comb. nov. (MB 828491)  
*Gymnopus micromphaloides* R.H. Petersen and K.W. Hughes, North American Fungi 9: 6 (2014)
- Marasmiellus neotropicus* (Singer) J.S. Oliveira, comb. nov. (MB 828586)  
*Collybia neotropica* Singer, Sydowia 15 (1-6): 54 (1962)
- Marasmiellus nonnullus* (Corner) J.S. Oliveira, comb. nov. (MB 828510)  
*Marasmius nonnullus* Corner, Beihefte zur Nova Hedwigia 111: 76 (1996)
- Marasmiellus nonnullus* var. *attenuatus* (Corner) J.S. Oliveira, comb. nov. (MB 828511)  
*Marasmius nonnullus* var. *attenuatus* Corner, Beihefte zur Nova Hedwigia 111: 77 (1996)
- Marasmiellus parvulus* (J.L. Mata, R.H. Petersen & K.W. Hughes) J.S. Oliveira, comb. nov. (MB 828492)  
*Gymnopus parvulus* J.L. Mata, R.H. Petersen & K.W. Hughes, Sydowia 58 (2): 285 (2006)
- Marasmiellus peronatus* (Bolton) J.S. Oliveira, comb. nov. (MB 828512)  
*Agaricus peronatus* Bolton, An History of Fungusses, Growing about Halifax 2: t. 58 (1788)
- Marasmiellus polygrammus* (Mont.) J.S. Oliveira, comb. nov. (MB 828585)  
*Marasmius polygrammus* Mont., Annales des Sciences Naturelles Botanique 1: 118 (1854)
- Marasmiellus pseudoluxurians* (R.H. Petersen and K.W. Hughes) J.S. Oliveira, comb. nov. (MB 828493)  
*Gymnopus pseudoluxurians* R.H. Petersen and K.W. Hughes, North American Fungi 9: 7 (2014)
- Marasmiellus pseudomphalioides* (Dennis) J.S. Oliveira, comb. nov. (MB 828589)  
*Collybia pseudomphalodes* Dennis, Kew Bulletin 15 (1): 74 (1961)  
 Notes: According to Dennis (1961), the epithet is due to the resemblance to *Collybia omphalodes* (Berk.) Dennis (= *Marasmius omphalodes* Berk.). Berkeley (1856) justified the choice of the name by considering the species between *Marasmius* and *Omphalia*. The correct spelling is *Marasmius omphalioides*, and therefore, *Collybia pseudomphalioides* Dennis. The is combined herein with the correct spelling.
- Marasmiellus quercophilus* (Pouzar) J.S. Oliveira, comb. nov. (MB 828588)  
*Marasmius quercophilus* Pouzar, Česká Mykologie 36 (1): 1 (1982)
- Marasmiellus stevensonii* (E. Horak) J.S. Oliveira, comb. nov. (MB 828726)  
*Collybia stevensoniae* E. Horak, New Zealand Journal of Botany 9 (3): 450 (1971) [nom. nov. to replace the illegitimate = *Crinipellis readii* G. Stev., Kew Bulletin 19 (1): 43 (1964)]
- Marasmiellus subcyathiformis* (Murrill) J.S. Oliveira, comb. nov. (MB 828602)  
*Marasmius subcyathiformis* Murrill, North American Flora 9 (4): 269 (1915)
- Marasmiellus subnudus* (Ellis ex Peck) J.S. Oliveira, comb. nov. (MB 828513)  
*Marasmius subnudus* Ellis ex Peck, Annual Report of the New York State Museum 51: 287. (1898 [as 1897])
- Marasmiellus subpruinus* (Murrill) J.S. Oliveira, comb. nov. (MB 828603)  
*Marasmius subpruinus* Murrill, North American Flora 9 (4): 266 (1915)

**Fig. 7** Infection and necrosis on living leaves until death by *Pusillomyces manuripioides* (JO1117). **a** Rhizomorph attachment to the foliar face. **b** Early stage of the infection. **c** Necrosis of the foliar tissue. **d** Death of the initially infected area. **e** Spot of necrosis in living leaf and decaying of dead leaf. **f** Death of the entire leaf



*Marasmiellus indonesiensis* A.W. Wilson, Desjardin & E. Horak ex J.S. Oliveira, sp. nov. (MB 828712)

Etymology: refers to Indonesia, where the holotype was collected.

Holotype: Indonesia, Bali, Lake Tamblingan, solitary on leaf litter, 15. 01. 2000, Wilson 39, **holotypus** (SFSU).

Diagnosis: The effectively published description in A.W. Wilson, Desjardin & E. Horak, *Sydowia* 56 (1): 193 (2004), as *Gymnopus tamblinganensis* nom. prov., according to the Art. 38.1, 38.11 and 38.13 of the Code.

Notes: The name *Marasmiellus tamblinganensis* is already occupied [*Marasmiellus tamblinganensis* Retn., *Gardens Bulletin Singapore* 70 (1): 227 (2018)].

*Marasmiellus termiticola* (Corner) J.S. Oliveira, comb. nov. (MB 828509)

*Marasmius termiticola* Corner, *Beihefte zur Nova Hedwigia* 111: 101 (1996)

*Marasmiellus trogioides* (A.W. Wilson, Desjardin & E. Horak) J.S. Oliveira, comb. nov. (MB 828494)

*Gymnopus trogioides* A.W. Wilson, Desjardin & E. Horak, *Sydowia* 56 (1): 195 (2004)

*Marasmiellus villosipes* (Cleland) J.S. Oliveira, comb. nov. (MB 828606)

*Marasmius villosipes* Cleland, *Toadstools and mushrooms and other larger fungi of South Australia* 1: 166 (1934)

Additional species: *Marasmiellus ramealis* (Bull.) Singer; *Marasmiellus stenophyllus* (Mont.) Singer; *Marasmiellus synodicus* (Kunze) Singer; *Marasmiellus vaillantii* (Pers.) Singer.

### Ecology of *Pusillomyces manuripioides*

*Pusillomyces manuripioides* is epiphyte, forming a rhizomorph net entangled with hanging living and dead (or dying) leaves, twigs, branches and limbs about 1.5 m and upwards off the ground, seemingly strictly or at least more frequently of *Eugenia* spp. (shrubby to subarboreal trees of the subforest) (Fig. 3a, Online Resource Fig. O6a–b). *Manuripia bifida*, however, was found on dicotyledonous dead fallen woody sticks on the forest floor (Singer 1976). This is a very important distinction in the habit and habitat,

suggesting different niches. Being epiphytic, *P. manuripioides* demonstrates particular adaptations, pattern of reproduction and distribution, and different nutrition mode. It acts as a facultative parasite causing necrosis in living leaves on where the rhizomorphs are attached (Fig. 7), being a kind of Horse Hair Blight disease with true infection of living plant tissues. Field observations suggest that there is host specificity with *Eugenia* spp. for the parasitic mode, whereas there is no substrate specificity for the saprotrophic mode.

To properly understand the ecology of the species, firstly, we observed that the main structural body unit of the hypothetical individuals is represented by an interconnected net of rhizomorphs from where most of the basidiomata grow. Even those basidiomata that seem to grow directly from the leaves were actually arising from rhizomorphs strongly adhered to the foliar face. This adherence of the rhizomorphs is essential since it may serve both for attachment and parasitic nutrition. Therefore, the production of rhizomorphs is the main strategy of the species instead of being an accessory or secondary growth mode. That is, we shall always find the species growing as this interconnected net of rhizomorphs where we possibly can find the tiny basidiomata.

Secondly, the habit of the new species strongly indicates that it can act as both a biotrophic on living leaves and saprotrophic on dead leaves resulting from the parasitic activity. Moreover, dead leaves of various tree species that fall from the canopy can be also captured in the rhizomorphs net, serving as food for the fungus. The infection initiates with the adherence established when a free part of rhizomorph touches the foliar face (Figs. 3, 4, and 7a). Then, generative hyphae grow from the inner part of the rhizomorph out to the substrate (Fig. 7a, b), invading the cortical layer of the leaf. These abundant generative hyphae form a visible cream-colored mycelial tomentum along both sides of the rhizomorph. On living substrate, the rhizomorph of *P. manuripioides* provokes necrosis on previously healthy leaves, starting as a dirty brown diffuse spot around the mycelium tomentum of the rhizomorphs (Fig. 7b). Then, the necrosis progresses damaging the immediate area of the leaf causing it to become pale brown with dark brown border (Fig. 7c). The damage gets more severe, killing that area of the leaf. The killed area becomes pale yellow or brownish yellow with some conspicuous and abundant inconspicuous dark brown dots (Fig. 7d, e). It is possible to see a large dark brown spot around the infecting rhizomorph. The necrosis expands to the entire leaf, causing death of the whole structure (Fig. 7f). Once the leaf is dead, the infecting rhizomorph switches to the saprotrophic mode, decomposing the dead structure. The rhizomorphs can also be found attached along living limbs, branches and twigs bearing infected leaves. These parts do not seem to be damaged and may be used only to conduct the rhizomorphs to the leaves, but this need confirmation.

More evidences of the parasitic activity of *P. manuripioides* such as the morphology of the infection apparatus and the

manner in which the fungus invades the plant, the life cycle, the pathogenicity, the host specificity and a thorough observation on the ecology of the species will be the subject of another paper.

## Conclusions

Significant progress is reached on the phylogeny of Omphalotaceae, retaining the widely used genera, *Gymnopus* and *Marasmiellus*. These genera have been the subject of many efforts firstly (and historically) in morphology and more recently in molecular phylogeny studies to find definitive concepts in the taxonomy and systematics of these groups. The present study offers clear evidences for *Gymnopus* s. str. consisting of *Gymnopus* sections *Androsacei*, *Gymnopus*, *Impudicae* and *Levipedes* while *Marasmiellus* s. str. is composed of *Gymnopus* sect. *Vestipedes* and members of *Marasmiellus* sections *Dealbati*, *Marasmiellus*, *Rameales*, and *Stenophylloides* (so far). Based on Wilson and Desjardin (2005), *Marasmiellus* sect. *Candidi* is excluded from *Marasmiellus* s. str. Therefore, we defend the concept and propose the acceptance of a less inclusive *Gymnopus*; and on the other hand, *Marasmiellus* forming a distinct genus as suggested by Wilson and Desjardin (2005). Two new genera are now added to Omphalotaceae: *Paragymnopus* and *Pusillomyces*. *Paragymnopus* contains species of *Gymnopus* sect. *Perforantia* (Petersen and Hughes 2016). *Pusillomyces* is a new genus composed of *P. manuripioides* and the now combined *Gymnopus aetosus* and *G. funalis*. All genera recognized in this study follow Vellinga et al. (2015). *Pusillomyces manuripioides* is a phytopathogenic fungus found with an epiphytic habit more frequently on *Eugenia* spp. in “Campinarana,” Amazon forest, but specific studies of its pathogenicity ought to be made to understand its biology in depth. Full taxonomic treatment was provided to fulfill nomenclatural requirements for the new taxa.

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