UNIVERSITAS INDONESIA

TAXONOMIC STUDY OF THE GENUS *MARASMIELLUS* MURRILL IN JAVA AND BALI

DISERTASI

ATIK RETNOWATI
0706220934

FAKULTAS MATEMATIKA DAN ILMU PENGETAHUAN ALAM
PROGRAM STUDI BIOLOGI
PROGRAM PASCASARJANA
DEPOK
JANUARI 2012

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Diajukan sebagai salah satu syarat untuk memperoleh gelar Doktor dalam bidang Biologi

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HALAMAN PERNYATAAN ORISINALITAS

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Nama	:	Atik Retnowati, S.P., M.Sc.
NPM	:	0706220934
Tanda Tangan	:	.....
Tanggal	:	Januari 2012

HALAMAN PENGESAHAN

Disertasi ini diajukan oleh	:	
Nama	:	Atik Retnowati
NPM	:	0706220934
Program Studi	:	Biologi
Judul Disertasi	:	Taxonomic Study of The Genus *Marasmiellus* Murrill in Java and Bali

Telah berhasil dipertahankan di hadapan Dewan Penguji dan diterima sebagai **bagian** persyaratan yang diperlukan untuk memperoleh gelar Doktor pada **Program** Studi Biologi Program Pascasarjana Fakultas Matematika dan Ilmu **Pengetahuan** Alam, Universitas Indonesia.

DEWAN PENGUJI

Promotor	: Prof. Emeritus Dr. Indrawati Gandjar	(.....)
Ko-Promotor	: Prof. Mien A. Rifai, B.Sc., M.Sc., Ph.D.	(.....)
	Wellyzar Sjamsuridzal, Ph.D.	(.....)
Tim Penguji	: Dr. Kartini Kramadibrata (Ketua)	(.....)
	Ariyanti Oetari, Ph.D. (Anggota)	(.....)
	Dr. Nampiah Sukarno (Anggota)	(.....)

HALAMAN PENGESAHAN

JUDUL : **TAXONOMIC STUDY OF THE GENUS *MARASMIELLUS* MURRILL IN JAVA AND BALI**
NAMA : **ATIK RETNOWATI**
NPM : **0706220934**

MENYETUJUI:

1. Komisi pembimbing

Prof. Emeritus Dr. Indrawati Gandjar
Promotor

Prof. Mien A. Rifai, B.Sc., M.Sc., Ph.D.
Ko-Promotor

Wellyzar Sjamsuridzal, Ph.D.
Ko-Promotor

2. Penguji

Dr. Kartini Kramadibrata
Penguji 1

Ariyanti Oetari, Ph.D.
Penguji 2

Dr. Nampiah Sukarno

Ketua Program Studi Biologi
Program Pascasarjana FMIPA UI

Dr. Luthfiralda Sjahfirdi, M. Biomed.
NIP: 19650405-199103-2001

Ketua Program Pascasarjana
FMIPA UI

Dr. Adi Basukriadi, M.Sc.
NIP: 19580423-198503-100

Name : Atik Retnowati (0706220934) Date : Januari 2012
Title : Taxonomic Study of the Genus *Marasmiellus* Murrill in Java and Bali
Promoter : Prof. Emeritus Dr. Indrawati Gandjar
Co-Promoters : Prof. Mien A. Rifai, B.Sc., M.Sc., Ph.D., Wellyzar Sjamsuridzal, Ph.D.

SUMMARY

Marasmiellus is a tropical and subtropical genus which consists of more than 400 species. The genus plays important roles in ecosystem as a decomposer, and not many information on its economic value. Several monographs of the genus have been published based on morphological data from several tropical forests (neotropics, Africa, Srilanka), and no report on the Indonesian *Marasmiellus*. At present, taxonomic problem occurs in the genus related to the position of *Marasmiellus juniperinus* as a type genus in the phylogenetic tree based on Internal Transcribed Spacer (ITS) region. *Marasmiellus juniperinus* nested into different clade from other *Marasmiellus* species, and it was clustered in *Gymnopus* clade. Since *Marasmiellus* has never been reported from Indonesia and the taxonomic problem occurs at the genus, thus the objectives of this study are 1) To describe the species of *Marasmiellus* found in Java and Bali; 2) To provide an identification key to sections and species; 3) To analyse the phylogenetic relationship within the genus *Marasmiellus* in Java and Bali based on morphological and molecular characters performed by Maximum Parsimony method; 4) To clarify the relationship of the genus *Marasmiellus* and *M. juniperinus* to its closely related genera with inclusion of more *Marasmiellus* spp. based on ITS region of rDNA sequence data; and 5) To describe novel species of *Marasmiellus* based on morphological and molecular data.

Java and Bali were chosen as research sites in this study due to several reasons. First, historically most of Indonesian reported agarics were collected from Java, particularly from Mount Gede-Pangrango, Cibodas and Bogor Botanical Gardens. Second, forest degradation is going rapidly in Java and Bali. As a result Indonesia is loosing habitats of *Marasmiellus*.

This study was divided into three topics according to the purposes of this

study. The first topic entitled Species of *Marasmiellus* in Java and Bali. The study was carried out in Herbarium Bogoriense, The Botany Division, Research Center for Biology, The Indonesian Institute of Sciences (LIPI). Herbarium materials are kept in Herbarium Bogoriense (BO) and Harry D. Thiers Herbarium, San Francisco State University (SFSU), SF, CA, USA. Materials used in this research were collected in 1998-2010 from 10 different locations in Java and Bali which consisted of 22 sites. A representative material of *Marasmiellus juniperinus*, as a type genus, was borrowed from the Herbarium of the University of Tennessee, Knoxville, Tennessee, USA (TENN). The identification results revealed that there were 35 known species; one species as a new combination; 17 undescribed species. Those 35 species consist of 25 species of *Marasmiellus* found in Java, 7 species found in Bali, and 3 species found both in Java and Bali. All described species were treated based on infrageneric classification of Singer (1973) which divided the genus into 10 sections based on morphological characters. Based on Singer's infrageneric classification *Marasmiellus* in Java and Bali belonged to 5 sections, i.e sect. *Marasmiellus*, sect. *Rameales*, sect. *Dealbati*, sect. *Candidi*, and sect. *Stenophylloides*.

The second topic entitled Phylogenetic study of the genus *Marasmiellus* based on morphological and molecular analysis. Thirty five morphological characters of 37 taxa were scored manually for phylogenetic analysis based on morphological characters. Those taxa consisted of 35 species of *Marasmiellus* in Java and Bali found (topic 1), a type species of *Marasmiellus* (*M. juniperinus*), and *Crinipellis brunneipurpurea* as an outgroup. Matrix data was analyzed by Phylogenetic Analysis Using Parsimony (PAUP) program. The topology of phylogenetic tree based on morphological data was compared to topology of phylogenetic tree based on molecular data. The phylogenetic tree based on morphological and molecular characters showed that *Marasmiellus* spp. were divided into four major clades. Each clade consisted of several Singer's sections. This result indicated that the sections within Singer's infrageneric classification were polyphyletic. Morphological and molecular phylogenies resulted in this study did not support traditional classification, i.e. Singer's classification of *Marasmiellus*.

The phylogenetic relationship of *Marasmiellus* taxa, *Gymnopus* and its closely related genera were analyzed using molecular data of ITS rDNA. Forty one sequences based on ITS region representing of 36 species of *Marasmiellus* were used for phylogenetic analysis. Nine sequences of *Marasmiellus* were generated from this study, and 32 sequences were obtained from the international DNA database (GenBank). The phylogenetic tree showed that *Marasmiellus* taxa were divided into 4 clades. The taxa of *Marasmiellus* and *Gymnopus* were clustered together in three of four clades. The type species, *Marasmiellus juniperinus* was located within the same clade as *Gymnopus fusipes*. This result indicated that the genus *Marasmiellus* was polyphyletic.

The third topic entitled Description of a novel species, *Marasmiellus javanicus*, based on morphological and molecular analysis. *Marasmiellus javanicus* is one of the 17 new species in this study, and it was described by using the integrated morphological and molecular data.

Marasmiellus javanicus was described as a new species based on 5 specimens found in Bogor Botanical Garden, West Java. Morphologically it was similar to *M. hondurensis* (Murrill) Singer which was collected from Belice in 1906 by Peck (Singer 1973). The characters similarities of *M. javanicus* and *M. hondurensis* were off-white, same size of width, convex with flattened disc of pileus; subdistant lamellae; tomentose and insititious stipe; cutis with diverticulate of pileipellis, and presence of stipe vesture. *Marasmiellus hondurensis* was different from *M. javanicus* in having longer stipe (6–22 mm diam), fusoid basidiospores, *Rotalis*-type of cheilocystidia, and *M. hondurensis* was found on dicotyledon plant.

At present, there is no sequence based on ITS rDNA of *M. hondurensis*. Thus, genetically *M. hondurensis* was unable to be compared to *M. javanicus*. To know the closest species of *M. javanicus*, phylogenetic tree of *Marasmiellus* was constructed. Phylogenetic tree showed that the most closely related species to *M. javanicus* was *M. mesosporus*. *Marasmiellus javanicus* and *M. mesosporus* have ITS rDNA 94% sequence similarity. They differ in 27 nucleotides which were 7 insertion and deletion, and 24 of substitution (A↔G=11, C↔T=13). This study showed that integrated morphological and molecular data are required to identify the *Marasmiellus*.

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I am indebted to Prof. Emeritus Dr. Indrawati Gandjar, Prof. Mien A. Rifai, and Wellyzar Sjamsuridzal, Ph.D. who gave me guidance, support, and encouragement to finish my study. I thank Prof. Dr. Dennis E. Desjardin (San Francisco State University, California, USA) for helping me to understand the taxonomic problem of the genus *Marasmiellus*, and providing answer to my questions. I am thankful to Dr. Kartini Kramadibrata, Ariyanti Oetari, Ph.D., and Dr. Nampiah Sukarno for critical comments that helped improve the dissertation.

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Depok, January 2012

Author

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Nama	: Atik Retnowati
NPM	: 0706220934
Program Studi	: Biologi
Departemen	: Biologi
Fakultas	: Matematika dan Ilmu Pengetahuan Alam
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CONTENT

Pages

LIST OF FIGURES

LIST OF TABLES

GENERAL INTRODUCTION

Marasmiellus is a tropical and subtropical genus which consists of more than 400 species (Wilson & Desjardin 2005). Due to its significant ecological role as a decomposer, the genus is a very important element in the tropical and subtropical forests. Most of its species are saprophytic, degrading leafy or woody debris of monocotyledonous or dicotyledonous plants. A few species are parasitizing economically important cultivated plants, such as bananas, sugar cane, maize, and coconut palms (Singer 1973). *Marasmiellus cocophilus* was first described by Pegler (1977) from Kenya, and was found growing on root of *Cocos nucifera* and it is thought to be the cause of serious bole rot of young coconut palms in East Africa (Pegler 1977). Meanwhile, *Marasmiellus* was also used as a bioremediation agent. Nemergut *et al.* (2000) used litter rot of *M. troyanus*, and it is currently used in several researches to reduce Benzo[a]pyrene (B[a]p) in the environment. This B[a]p is a carcinogenic polyaromatic hydrocarbon resulting from an incomplete combustion production of fossil fuels.

Marasmiellus is a member of Agaricales (macro fungi), and limited research on Indonesian Agaricales was already undertaken by Levéillé (1844), Henning (1900), Overeem (1922), and Boedijn (1940). Continuing their previous research,"The Agaricales of Java and Bali" Project (1998-2000) was carried out in order to explore the species of agarics in Java and Bali by Dennis E Desjardin and Egon Horak. So far it has yielded several monographs on a number of genera (Desjardin *et al*. 2000, Verbeken *et al*. 2001, Desjardin & Horak. 2002, Wilson *et al*. 2004, Horak & Desjardin 2006). In line with the previous research, the present study was carried out by focusing on the occurrence of *Marasmiellus* species in Java and Bali.

The genus was first established by Murrill (1915) for a group of diminutive agarics with few distinguishing morphological characters, so that it was simply diagnose as pileus small, irregular in shape, membranous, reviving, lamellae adnate or adnexed, basidiospores hyaline, partial veil absent, stipe filiform, tubular, short, lateral or eccentric.

In emending the generic description, Singer (1973) compared

Marasmiellus to its closely related genera (*Marasmius* Fr., *Gymnopus* (Pers.) Roussel, *Collybia* (Fr.) Staude, *Rhodocollybia* Singer, and *Lentinula* Earle), especially *Marasmius* section *Androsacei*. The genus was considered also closely related to *Campanella*, *Collybia*, and *Neoclitocybe* Singer. *Campanella* and *Collybia* possess a gelatinous trama which distinguishes them from *Marasmiellus*. Whereas, *Collybia* differ from *Marasmiellus* by their macroscopic characters. The stipe base of *Collybia* is tomentose whereas it is not tomentose in *Marasmiellus*. Lamellar attachment distinguishes *Neoclitocybe* from *Marasmiellus*. The former genus has decurrent lamellae, whereas the latter genus has subdecurrent or adnate lamellae.

Singer (1986) classified *Marasmiellus* as a member of family Tricholomataceae tribus Collybieae together with *Trogia* Fr., *Pleurocybella* Singer, *Cheimonophyllum* Singer, *Anthracophyllum* Ces., *Collybia* (Fr.) Staude, *Neoclytocybe* Singer, *Micromphale* Gray, *Campanella* Henn., and some series of genera (*Cymatella* Pat., *Skepperiella* Pilát, *Mniopetalum* Donk & Singer, *Cyphella* Fr., *Phaeodepas* D.A. Reid, and *Calyptella* Quél.). Meanwhile *Marasmius* was placed in tribus *Marasmieae* together with *Oudemansiella* Speg., *Mycenella* (J.E. Lange) Singer, *Physocystidum, Strobilurus* Singer, *Rimbachia* Pat., *Physalacria* Peck, *Deigloria* Agerer, *Gloiocephala* Massee, *Palaeocephala* Singer, *Manuripia* Singer, *Epicnaphus* Singer, *Hymenogloea* Pat., *Crinipellis* Pat., *Chaetocalathus* Singer, *Amyflagellula*, *Lacnella*, and *Flagelloschypha*.

Furthermore Singer (1986) wrote about basidioles, and he mentioned that "this immature basidia, often called basidioles, are usually of approximately the same shape, as the mature basidia, often slightly too considerably smaller, or narrower or rather more fusiform than clavate. It is clear that there was no significant taxonomic value to basidioles in delimiting genera of marasmioid fungi.

Fusiform basidioles are rather characteristic for certain genera, such as *Marasmius* Fr., *Marasmiellus* Murrill, *Crinipellis* Pat., and *Collybia* (Fr.) Staude, also smaller tricholomataceous genera, related to these four genera".

Corner (1996) considered that *Marasmiellus*, *Collybia*, and *Marasmius* have no distinction, so that in his book all species of white-spored agarics, with

rather tough and skinny fruit-bodies, and having fusoid or subacerose basidium (basidioles) in the hymenium were included in the genus *Marasmius*. Due to this broad generic concept, many *Marasmiellus* which he described are hidden under the generic name of *Marasmius*. Corner pointed out that the presence of acerose basidioles become the character which united them into one genus, *Marasmius*. In different part of his book, however, he described some acerose basidioles which expand to become clavate basidioles.

At present, taxonomic delimitation of *Marasmiellus* and *Marasmius* have been investigated in which acerose basidioles can not be the main character to unite them into one genus. The type of sterile cells which are present at the pileus layer (pileipellis) and the Melzer's reaction are important characters to separate them as different genera.

Several monographs of the genus *Marasmiellus* have been published based on morphological data. Singer (1973) described 134 species of Neotropical *Marasmiellus*. This monograph is the most complete published paper on *Marasmiellus*, and it becomes a basic reference for taxonomic work of the genus *Marasmiellus*. Pegler (1977, 1983, and 1986) made a significant contribution by publishing several monographs from several different regions, by reporting 14 described species of *Marasmiellus* of East Africa (Pegler 1977), describing 23 species from Lesser Antilles (1983), and 17 species from Srilanka (Pegler 1986). Twenty one species of *Maramiellus* were described by Antonín & Noordeloos (1993) from Europe, most of them are widespread, cosmopolitan, with numerous species in the subtropic. There was no report of the genus in Indonesia.

Compared to other marasmioid fungi (*Marasmius* Fr., *Mycena* (Pers. ex Fr.) S.F. Gray, *Campanella* Henn.), *Marasmiellus* has a smaller number of variation in morphological characters resulting in difficulty in identifying into species level. Therefore, genetic data has to be employed in order to solve the problem, and the molecular data based on ITS regions of rDNA were used in this research. The ITS rDNA has been widely used in taxonomy and molecular phylogeny. Internal Transcribed Spacer (ITS) has higher degree of variation than other regions of rDNA (White *et al*. 1990). With high variation of nucleotides, ITS has proven especially useful for elucidating relationships among species level,

infrageneric level, and closely related genera. A combination of morphological and molecular data will help to delimit the *Marasmiellus* to its species rank.

Previous research performed by Mata *et al*. (2004) showed the phylogenetic position of type species of the genus, *Marasmiellus juniperinus*, within a phylogenetic tree of gymnopoid and marasmioid groups has illustrated a taxonomic problem. Analysis of molecular data based on ribosomal large subunit (LSU) and internal transcribed spacer (ITS) sequences showed that *M. juniperinus* nested among species in the genus *Gymnopus*. Based on this result, Mata *et al.* (2004) proposed that *Marasmiellus* is synonym of *Gymnopus*. This analysis needs to be evaluated by using more molecular data. The limited samples of *Marasmiellus* used in Mata's analysis may be one of the reasons why *M. juniperinus* nested in the *Gymnopus* clade. Wilson & Desjardin (2005) provided molecular data (nLSU sequences) which indicated that most taxa of *Marasmiellus* formed a distinct clade sister to most species of *Gymnopus*. By including more *Marasmiellus* taxa and its closely related taxa (*Gymnopus*, *Collybia*, *Rhodocollybia*, *Lentinula*, and *Marasmius*) in the analysis, it was expected that *M. juniperinus* was nested in a *Marasmiellus* taxa clade. In fact, the result of this study was in agreement to previous research (Mata *et al*. 2004).

On the basis of the above facts, this study was aimed 1) To describe the species of *Marasmiellus* found in Java and Bali; 2) To provide an identification key to sections and species; 3) To analyse the phylogenetic relationship within the genus *Marasmiellus* in Java and Bali based on morphological and molecular characters performed by Maximum Parsimony method; 4) To clarify the relationship of the genus *Marasmiellus* to its closely related genera with inclusion of more *Marasmiellus* spp. based on ITS region of rDNA sequence data; and 5) To describe novel species of *Marasmiellus* based on morphological and molecular data.

There were several reasons in choosing Java and Bali as the major sites for research. First, historically most of Indonesian reported agarics were collected from Java, particularly from Mount Gede-Pangrango, Cibodas and Bogor Botanical Gardens. Second, forest degradation is going rapidly in Java and Bali. As a result Indonesia is loosing habitats of *Marasmiellus*. On the other hand,

there is still no report on the species of *Marasmiellus* in Java and Bali on all published papers of Agaricales from Java. Consequently, the study on species of *Marasmiellus* is urgently needed.

Specimens used in this research were collected in 1998-2010 from different 10 different locations in Java and Bali which consisted of 22 sites. The sites located in Java were Bogor Botanical Garden, Cibodas Botanical Garden, Mount Halimun-Salak National Park, Bodogol (southern part of Mount Gede Pangrango National Park), Haurbentes, Karimunjawa National Park, Mount Slamet, Baturraden Botanical Garden, and Ujung Kulon National Park. The sites located in Bali were Mount Pohen, Ekakarya Botanical Garden, Forest around Lake Tamblingan, and a Forest around Lake Bratan. The encountered species will be published as a monograph. All species were described, illustrated, and compared to morphologically similar species of the previously described *Marasmiellus* from many regions.

A provisional dichotomous key to aid in determining *Marasmiellus* species in Java and Bali was constructed.

TOPIC 1

THE SPECIES OF *MARASMIELLUS* IN JAVA AND BALI

Atik Retnowati
Department of Biology, Faculty of Mathematics and Natural Sciences
University of Indonesia

ABSTRACT

In the framework of taxonomic study of the genus *Marasmiellus* in Indonesia, 70 specimens were collected in 1998-2010 from 10 locations which consisted of 22 sampling sites in Java and Bali. Macro and micromorphological characters of those specimens were observed and grouped based on Singer's infrageneric classification. The morphological characters used for grouping consisted of type of pileipellis (*Rameales*-structure, cutis with or without diverticulate); presence or absence of cheilocystidia; basidiospores sizes; stipe attachment (central, eccentric); and pileus color (pigment or non-pigmented). A total of 35 species of *Marasmiellus* belonging to 5 sections (*Dealbati*, *Rameales*, *Marasmiellus*, *Stenophylloides*, and *Candidi*) were discovered from Java and Bali. This study detected the presence of 17 new species, and 1 species as a new combination in Java and Bali. There were 25 species found in Java and 7 species in Bali. There were 3 species found both in Java and Bali. The number of encountered *Marasmiellus* species in Java was higher than in Bali. Comparison of morphologically similar taxa was presented. Comprehensive descriptions of 35 species, key to sections and species of *Marasmiellus* were presented.

Keywords: Bali, Java, *Marasmiellus*, morphology, Singer's infrageneric classification.

1. INTRODUCTION

The genus *Marasmiellus* was first established by Murrill (1915) with a very simple descriptions: " Pileus small, irregular in shape, membranous, reviving, lamellae adnate or adnexed, basidiospores hyaline, partial veil absent, stipe filiform, tubular, short, lateral or eccentric". Later Singer (1973) wrote more complete descriptions which defined the genus concept more clearly: "Basidiocarp small to medium. Pileus 0.3–3 cm, marasmioid-collybioid, collybioid-mycenoid, or sometimes pleurotoid, white, greyish white, reddish brown or dark brown. Lamellae attached, slightly decurrent, intervenose. Stipe

central, eccentric, lateral, or no stipe, insititious or subinsititious, rarely base tomentose. Basidiospores white to off-white, ellipsoid, oblong, smooth, inamyloid. Pileipellis or stipipellis composed of *Rameales*-structures".

According to Singer (1973), the genus *Marasmiellus* was divided into 10 sections; i.e section *Defibulati*, *Nigripedes*, *Tetrachroi*, *Stenophylloides*, *Candidi*, *Dealbati*, *Tricholores*, *Rameales*, *Distantifolii*, and *Marasmiellus*. Separation of these infrageneric classifications of *Marasmiellus* taxa depends on recognition of a combination of macromorphological and micromorphological characters. The arrangement of cells forming the pileus layer is the most important taxonomic character at the sectional level in *Marasmiellus*. If pileipellis type elements are non-hymeniform, repent hyphae with *Siccus*-type of terminal cells, they are termed *Rameales*-structures. *Rameales*-structures distinguish section *Rameales* and *Marasmiellus* from other sections; i.e section *Stenophylloides*, *Dealbati*, and *Candidi*. Additionally, section *Rameales* is distinguished from sections *Marasmiellus* by having collybioid basidiomes, well developed and central stipe, while section *Marasmiellus* has a pleurotoid basidiomes, not well-developed and eccentric stipe. The remaining sections of *Marasmiellus*, i.e section *Stenophylloides*, *Dealbati*, and *Candidi*, have cutis pileipellis with or without diverticulate. Section *Stenophylloides* is characterized by the presence of seta-like elements in pileus. Section *Candidi* is distinguished by having basidiospores size more than 10.4 µm and stipe tends to grey, and section *Dealbati* is characterized by basidiospores size less than 10.4 µm, and stipe does not tend to grey.

The genus *Marasmiellus* is widely distributed throughout tropical and subtropical areas around the world. Based on Wilson and Desjardin (2005) *Marasmiellus* consists of more than 400 species. It is a neglected genus compared to other marasmioid genera (*Marasmius*, *Mycena*, *Micromphale*, etc.), due to its small basidiocarp, uncolorful pileus, and a low variation of morphological characters. A low variation of morphological characters caused problem to delimit the species of *Marasmiellus*.

As tropical and subtropical basidiomycetous fungi, the genus *Marasmiellus* is playing a significant ecological role in forests. Its species are saprophytic, degrading leafy or woody debris of monocotyledonous or

dicotyledonous plants. A few species are parasitic, and attack various economically important plants i.e bananas, sugar cane, maize, and coco palms (Singer 1973).

The Singer's infrageneric classification of the genus *Marasmiellus* (1973) was applied by several mycologists who published monographs from several regions. Singer (1973) described 134 species of Neotropical *Marasmiellus*. This monograph is the most complete published paper on *Marasmiellus*, and it becomes a basic reference for taxonomic work of the genus *Marasmiellus*. Pegler (1977, 1983, and 1986) made a significant contribution by publishing several monographs from several different regions. He reported 14 described species of *Marasmiellus* of East Africa (Pegler 1977), 23 species from Lesser Antilles (1983), and 17 species from Srilanka (Pegler 1986). Twenty one species of *Maramiellus* were described by Antonín & Noordeloos (1993) from Europe, most of them are widespread, cosmopolitan, with numerous species in the subtropic. Desjardin (1997) reported 7 species of *Marasmiellus* from North America. There was no report on the genus *Marasmiellus* in Indonesia.

Prior to this taxonomic study of the genus *Marasmiellus*, Desjardin *et al*. (2000) have reported species of *Marasmius*, a member of marasmioid fungi, in Java and Bali. The 37 species of *Marasmius* were reported, and 12 of them were new to science. According to this result, there is a possibility to discover other marasmioid fungi in Java and Bali. Additionally, based on Lodge & Cantrell (1995), the species number of saprobic fungi which include marasmioid fungi is higher in area with low altitudes, medium to low altitudes, moderately to high rainfall, and high habitat diversity. These characteristics are widely present in Indonesia, especially in Java and Bali, and support to expectation of high number of maramioid species in Indonesia.

Because *Marasmiellus* from Java and Bali has never been studied intensively, this research has two objectives: 1) To describe the species of *Marasmiellus* found in Java and Bali; and 2) To provide an identification key to sections and species.

2. MATERIALS AND METHODS

2.1 Fungal Materials

Specimens were used in this study are kept in Herbarium Bogoriense (BO) and Harry D. Thiers Herbarium, San Francisco State University (SFSU), SF, USA. Specimens used in this research were collected in 1998-2010 from different 10 different locations which consisted of 22 sites in Java and Bali (Figure 1.1). One hundred forty five specimens were collected from 7 locations in Java and 3 locations in Bali (Figure 1.1).

A representative specimen of the type species of *Marasmiellus*, *M. juniperinus*, was borrowed from the Herbarium of the University of Tennessee, Knoxville, Tennessee, USA (TENN). These specimens were examined for their micro-macro morphological characters, and grouped based on Singer's infrageneric classification (1973).

2.2 Methods

2.2.1 Sampling methods

The specimens of *Marasmiellus* were collected in Java and Bali by Purposive Random Sampling with particular finding on *Marasmiellus* specimens (Figure 1.1)

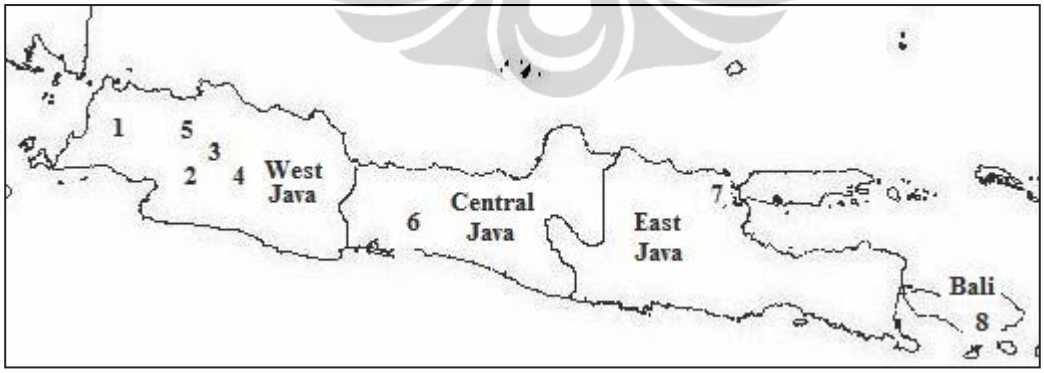

Figure 1.1. Sampling locations in Java and Bali (7 locations in Java: 1=Ujung Kulon National Park; 2= Haurbentes; 3= Bogor Botanical Garden; 4=Cibodas Botanical Garden; 5=Mount Halimun-Salak National Park; 6=Baturraden Botanical Garden and Mount Slamet; 7= Surabaya; 3 locations in Bali: 8= Eka Karya Botanical Garden and Mount Pohen; Mount Catur and Lake Bratan; and Tamblingan Lake).

Basidiocarps were collected representing as many growth stages as were available in order to examine variability within the species. When needed, photographs were taken of suitable basidiocarps. Each specimen was wrapped in aluminium foil or put in plastic boxes. Then the specimens were placed in plastic boxes with moist tissue paper, dried on portable oven, and identified tentatively in the field station.

2.2.2 Morphological observation

Many taxonomically important characters were observed only in the field, hence notes were taken in the field on habit of growth, odor, taste, substrate type, and plant associates. Odor was checked by smelling the fresh basidiome, and taste was checked by taking a small tissue of basidiome and taste it. Notes on macromorphological features were obtained in the field soon after collecting, paying particular attention to macromorphological features: a). size, shape, surface, color, and margin characteristics of different parts of the pileus; b). thickness, texture, color, taste, and odor of the flesh; c). attachment, spacing, color and edge color of lamella; d). size, shape, color, and surface features of stipe; the attachment of the stipe to the substrate; and f). type of substrate. Brief notes were completed on fungus habit and habitat. Color notation was based on Kornerup & Wancher (1983). Micromorphological data were obtained from dried specimens and carried out in the laboratory. All micro characters were studied from dried specimens rehydrated in distilled water and 3% of KOH solution, with the use of Melzer's reagent or Congo Red dye. Spore sizes were based on measurements of 25 basidiospores per specimen.

3. RESULTS

3.1 Sampling result

After examining all specimens of *Marasmiellus* based on macro-micromorphological characters, many of them have not been verified as *Marasmiellus*. They were mostly single specimens with a low morphological variation. Thus more specimens were needed to confirm that they belong to

Marasmiellus. The number of specimens which was approved as *Marasmiellus* was 70 specimens (Table 1.1).

Tabel 1.1. Number of specimens collected from each sampling site.

Island	Locations	Number of sampling sites	Number of specimens
Java	Bogor Botanical Garden, West Java	1	10
	Cibodas Botanical Garden, West Java	1	17
	Haurbentes, Jasinga, West Java	1	1
	Mount Halimun-Salak National Park, West Java Mount Halimun: Cikaniki, Mount Botol, Mount Telaga, and Loop trail, Perth Zoo trail. Mount Salak: Cidahu, Curug Nangka	6	15
	Mount Slamet, East Java: Block 3-6, block 55 via pancuran 7, block 58 via Pancuran 3, Ambangan sector, and Baturraden Botanical Garden	5	1
	Ujung Kulon National Park, West Java: Mount Honje via Cibiuk, Cimenteng, Curug Kikacang, and Jago Besar (Southern part of Mount Honje).	4	6
	Surabaya, East Java	1	2
Bali	Mount Pohen, Eka Karya Botanical Garden	1	10
	Forest surrounding Lake Tamblingan	1	3
	Forest surrounding Lake Bratan and Mount Catur	1	5
	TOTAL	22	70

3.2 Determination key to sections of *Marasmiellus*

Morphological characterization succesfully identified 70 specimens into 35 species of *Marasmiellus* in Java and Bali into 5 sections, i.e 8 species of sect. *Rameales*, 1 species of sect. *Stenophylloides*, 16 species of sect. *Marasmiellus*, 3 species of sect. *Candidi*, and 7 species of sect. *Dealbati*.

According to the descriptions of 35 *Marasmiellus* found in Java and Bali, determination key was constructed. The arrangement of cells forming the pileus layer and other macro-micro morphological characters of *Marasmiellus* species found in Java and Bali were used to construct key to 5 sections and species which occurred of in Java and Bali.

3.2.1 Key to sections of *Marasmiellus* in Java and Bali

Key to sections of *Marasmiellus* in Java and Bali were provided below.

1. Pileipellis composed of *Rameales*-structure, interwoven, with *Siccus*-type at the apex cells, diverticulate……..……...…….2
1*. Pileipellis composed of cutis-structure, non-interwoven, no terminal cells, diverticulate or smooth……..…….…..3
2. Basidiomes pleurotoid. Stipe not well developed, absent or reduced, lateral or strongly eccentric……...…….sect. *Marasmiellus*
2*. Basidiomes collybioid. Stipe well developed, present, central…… ……..sect. *Rameales*
3. Sclerocystidia present. ……...sect. *Stenophylloides*: 35. *M. bisporus*
3*. Sclerocystidia absent. …… ……....4
4. Cheilocystidia consist of clavate to cylindrical or lageniform cells, smooth or with a few diverticulates at the half apex……sect. *Candidi*
4*. Cheilocytidia consist of *Siccus*-type broom cells, with diverticulate at the apex……...……..……..…...……sect. *Dealbati*

3.2.2 Key to species of section *Marasmiellus*

1. Pileus pigmented, ranging from orange, pale brown pinkish orange, or greyish purple. Lamellae composed of 3–4 series of lamellulae…...…….2
1*. Pileus not pigmented, ranging from white, off white, cream, buff, beige. Lamellae composed of less than 3 series of lamellulae……...……...6
2. Stipe present and strongly eccentric…….....…….……3
2*. Stipe absent……..……5
3. Pileus up to 15 mm diam, greyish purple. Lamellae intervenose absent. Host specificity on banana stem. Cell-walled incrustation on pileipellis… ……..……1. *M. purpureoalbus*
3*. Pileus up to 22 mm diam, not grayish purple. Lamellae intervenose present. Host on dicot. No incrustation in pileipellis…...…….4
4. Basidiospores subglobose to globosewith length up to 10.4 µm. Fusoid basidioles present……...2. *M. globosus*
4*. Basidiospores fusoid, with length up to 8.8 µm. Fusoid basidioles absent

……..……..……..3. *M. inodermatoides*

5. Strongly garlic odor. Intervenose lamellae present…….....4. *M. ignobilis*

5*. Indistinctive odor. Intervenose lamellae absent…….....5. *M.* aff *concolor*

6. Pileipellis composed of *Rameales*-structures, interwoven hyphae…….…7

6*. Pileipellis cutis, parallel hyphae……...…..…10

7. Basidiocarps reniform to convex. Lamellae intervenose. Hyphae of stipe no diverticulate, coralloid stipe vesture present……..…6. *M. epochnous*

7*. Basidiocarps convex with flattened disc to broadly convex. Lamellae well developed. Hyphae of stipe diverticulate, stipe vesture absent……..……8

8. Pileus small up to 10 mm diam. Lamellae distant with 1–2 series of Lamellulae. Stipe length up to 5 mm…….....9

8*. Pileus large up to 39 mm diam. Lamellae subdistant with 2–3 series of lamellulae. Stipe length up to 15 mm, ……..……7. *M. zingiberius*

9. Stipe glabrous. Basidiospores large (12–15.2 µm). Hymenial cystidia absent. Stipe vesture absent ..……...……...8. *M. rifaii*

9*. Stipe pruinose. Basidiospores small (7.2–8.8 µm). Hymenial cystidia present. Stipe vesture present……..…….……..9. *M. nugatorius*

10. Lamellae well developed without anastomose or interveined, lamellulae 2–5 series……...……...……..11

10. Lamellae not well developed with anastomose or interveined, lamellulae 1–2 series……...……...……..12

11. Pileus pure white to off-white or cream. Lamellae cream to off white or white. Stipe glabrous, stipe vesture present composed of clavate to cylindrical…….……10. *M. dimidiatus*

11. Pileus sometimes staining bright yellow. Lamellae with pinkish tint. Stipe pruinose, stipe vesture absent…...…….……...11. *M. reniformis*

12. Basidiospores > 8.8 µm long,…..……..…12. *M. haurbentesis*

12*. Basidiospores < 8.8 µm long……..……13

13. Stipe vesture absent..……..……14

13*. Stipe vesture common, composed of clavate to cylindrical cells……...15

14. *Siccus*-type broom cells with a few setulae present at the edge of lamellae. Pileipellis cutis without diverticulate and incrustation….13. *M. longisiccus*

14*. *Siccus*-type broom cells absent at the edge of lamellae. Pileipellis cutis with diverticulate and incrustation…….……14. *M. bolivarianus*

15. Lamellae white with staining reddish brown in age. Pileus surface suede-like, terminal apex of pileipellis unseen clearly or absent……
…….15. *M. pernambucensis*

15*. Lamellae white without staining reddish brown. Pileus surface smooth, terminal apex of pileipellis composed of *Siccus*-type with a very short setulae……..……16. *M. idroboi*

3.2.3 Key to species of section *Rameales*

1. Basidiocarp small. Pileus up to 20 mm diam, convex to plano convex, or broadly convex often with or without flattened shallowly depressed disc……...…2

1*. Basidiocarp large. Pileus up to 50 mm diam, convex to plano with depressed disc……...……..…….7

2. Pileus not pigmented, ranging from pure white, light yellow, light brown, beige, or soda brown……..……..……....3

2*. Pileus pigmented, ranging from pale grayish orange to white orange disc and margin……..……6

3. Lamellae not well developed, anastomosing or interveines lamellae present, with closed to crowded lamellae spacing……..17. *M. clavatus*

3*. Lamellae well developed, anastomosing or interveines lamellae absent, with subdistant or distant lamellae spacing……..……4

4. Cheilocystidia present..…….....18. *M. tamblingensis*

4*. Cheilocystidia absent…….……...5

5. Lamellae not serrate margin. Stipe length up to 15 mm, non-insititious attachment, with a thin subiculum on substrate……..……..19. *M. delicius*

5*. Lamellae serrate margin. Stipe length up to 6 mm, insititious attachment, without a subiculum on substrate……..……20. *M. diverticulatus*

6. Lamellae forked, nearly poroid with 0 series of lamellulae. Fusoid basidioles present. Stipe vesture absent..…….……21. *M. piperus*

6*. Lamellae subdistant to distant, with 1–2 series of lamellulae. Fusoid basidioles absent. Stipe vesture present……..…22. *M. pruinosus*

7. A small bulb present at the base of stipe. Edge of lamellae composed of *Rotalis*-type. Pileipellis incrusted, inamyloid on hyphae, thin to thick-walled up to 0.8 µm hyphae…….....23. *M. umbilicatus*

7*. A small bulb absent at the base of stipe. Edge of lamellae absent. Pileipellis incrusted, dextrinoid on cell-walled only, thin-walled…..... ……..…24. *M. nanus*

3.2.4 Key to species of section *Candidi*

1. Pileus pigemented, width up to 45 mm diam. Lamellae subdistant to distant. Basidiospore large to 17.6 µm.....25. *M. subnigricans*

1*. Pileus non pigmented, width up to 10 mm diam. Lamellae distant. Basidiospores small up to 13.6 µm.....2

2. Cheilocystidia present with a few diverticulate. Pleurocystidia present. Pileipellis composed of *Rameales*-structures.....26. *M. setulosipes*

2*. Cheilocystidia present without diverticulates. Pleurocystidia absent. Pileipellis cutis.....27. *M. albofuscus*

3.2.5 Key to species of section *Dealbati*

1. Cheilocystidia absent……...2

1*. Cheilocystidia present. ……..……..4

2. Pileus more than 10 mm diam. Lamellae distant. A small bulb absent at the stipe base…...…….....28. *M. cikanikiensis*

2*. Pileus less than 10 mm diam. Lamellae subdistant. A small bulb present at the stipe base……...…….3

3. Pileus white to pale orange/flesh, turning yellow…….….29.*M. desjardinii*

3*. Pileus light brown at first, becoming dark brown….....30. *M. cibodasensis*

4. Caulocystidia composed of cells same as cheilocystidia (*Siccus*-type)…… ……..……...…….31. *M.* cf. *stenophyllus*

4*. Caulocystidia composed of cells different type from cheilocystidia (cheilocystidia= *Siccus*-type cells; caulocystidia= smooth cells)……....5

5. Grow on dicotyledone leaves. Stipe up to 7 mm long…....32. *M. hirtellus*

5*. Grow on monocotyledon leaves ……..……..…..…….6

6. Pileipellis cutis with diverticulate…….....…..33. *M. javanicus*

6*. Pileipellis cutis without diverticulate.....34. *Marasmiellus* aff. *hirtellus*

3.3 Species of *Marasmiellus* found in Java and Bali

Seventy specimens of *Marasmiellus* used in this study were collected from 22 sampling sites Java and Bali. The ecological difference, particularly on the degree of temperature and humadity (Table 1.2), of Java and Bali resulted in the different number of encountered *Marasmiellus* species. There were 25 species found in Java, 7 species in Bali, 3 species both in Java and Bali. The number of encountered *Marasmiellus* species in Java was higher than in Bali (Table 1.3).

Based on Table 1.2, the highest number of species was at Mount Halimun-Salak National Park, followed by Cibodas Botanical Garden, and Eka Karya Botanical Garden. Low land forest produced more species of *Marasmiellus*. Mount Halimun is an example. This national park comprises lowland forests (<1000 asl), such as Cikaniki, Mount Botol, Mount Telaga, and loop trail, Perth Zoo trail, where many specimens of *Marasmiellus* were collected, and the lowland forest is a favorable habitat for *Marasmiellus*.

Table 1.2 Number of species found each sampling site.

Island	Locations	Tempe-rature (°C)	Huma-Dity (%)	Number of species
Java	Bogor Botanical Garden, West Java	26	89	6
	Cibodas Botanical Garden, West Java	18	90	13
	Haurbentes, Jasinga, West Java	-	-	1
	Mount Halimun-Salak National Park, West Java	18	98	15
	Mount Slamet, East Java	17-20	90	1
	Ujung Kulon National Park, West Java	15.5-31.2	85	3
	Surabaya, East Java	28	99	1
Bali	Mount Pohen, Eka Karya Botanical Garden	17-20	80-90	8
	Forest surrounding Lake Tamblingan	17-20	80-90	3
	Forest surrounding Lake Bratan and Mount Catur	17-20	80-90	4

Table 1.3 List of *Marasmiellus* species found in Java, Bali, or Java and Bali.

No.	List of species	Java	Bali
1.	*M. purpureoalbus*	+	–
2	*M. globosus*	+	–
3	*M. ignobilis*	+	–
4	*M.* aff. *concolor*	+	–
5	*M. epochnous*	+	–
6	*M. zingiberius*	+	–
7	*M. rifaii*	+	–
8	*M. nugatorius*	+	–
9	*M. haurbentesis*	+	–
10	*M. pernambucensis*	+	–
11	*M. idroboi*	+	–
12	*M. clavatus*	+	–
13	*M. diverticulatus*	+	–
14	*M. piperus*	+	–
15	*M. pruinosus*	+	–
16	*M. nanus*	+	–
17	*M. setulosipes*	+	–
18	*M. albofuscus*	+	–
19	*M. cikanikiensis*	+	–
20	*M. javanicus*	+	–
21	*M.* cf. *stenophyllus*	+	–
22	*M. costusus*	+	–
23	*M.* aff. *hirtellus*	+	–
24	*M. bisporus*	+	–
25	*M. cibodasensis*	+	–
26	*M. inodermatoides*	–	+
27	*M. reniformis*	–	+
28	*M. longisiccus*	–	+
29	*M. bolivarianus*	–	+
30	*M. tamblingensis*	–	+
31	*M. umbilicatus*	–	+
32	*M. hirtellus*	–	+
33	*M. dimidiatus*	+	+
34	*M. delicius*	+	+
35	*M. subnigricans*	+	+

3.4 Descriptions of *Marasmiellus* species in Java and Bali

Section *Marasmiellus*

Type species of the section: *Marasmiellus juniperinus* Murr.

Sixteen species of *Marasmiellus* in Java and Bali were members of section *Marasmiellus*, namely *M. purpureoalbus, M. globosus, M. inodermatoides, M. ignobilis, M.* aff. *concolor, M. epochnous, M. zingiberius, M. rifaii, M. nugatorius, M. dimidiatus, M. reniformis, M. haurbentesis, M. longisiccus, M. bolivarinus, M. pernambucensis*, and *M. idroboi*. Descriptions of species were presented below:

1. *Marasmiellus purpureoalbus* (Petch) Singer, Sydowia 15: 57. 1961. Figure 1.2 and 1.5.

Syn. : *Marasmius purpureoalbus* Petch, Trans. Brit. Mycol. Soc. 31: 43. 1948.

Type : Sri Lanka. Peradeniya, on decaying banana stem, Aug. 1917, Petch 5303 (K. Holotype).

Pileus 10–15 mm diam, convex to plano-convex, rugulo-striate, dry, glabrous; greyish purple (12E5–6; 12 F5) to paler greyish reddish purple (12D4–6). Context thick, concolorous. Lamellae adnate, distant (7–9) with 3–4 series of lamellulae, broad, pale yellowish white (1–4A2) to buff, non-marginate. Stipe 3–5 x 1–1.5 mm, terete, strongly eccentric, solid, dry, pruinose, insititious, white to dingy buff. Odor and taste indistinctive.

Basidiospores (10.4)11.2–12.8(13.6) x (5.6)6.4–8.0 µm ($\bar{x}$ = 12.06 ± 0.69 x 7.17 ± 0.63, Q = 1.40–2.14, $\bar{q}$ = 1.70 ± 0.18) n = 25 spores per 1 specimen, ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 40–44 x 8.0–10.4 µm, clavate, 4-spored. Basidioles clavate. Cheilocystidia common, composed of *Siccus*-type broom cells; main body 15.2–24.8 x 8.0–11.2 µm, clavate to subglobose, with a few diverticulate, thin-walled, hyaline; diverticulate 1.6–4.0 x

0.8–2.4 µm, obtuse to conical, thin-walled, hyaline. Pleurocystidia absent. Pileipellis cutis; hyphae 5.6–8.0 µm, cell walled incrusted, thin-walled, hyaline, other tissues inamyloid. Pileal trama cutis; hyphae 3.2–7.2 µm, thin-walled, hyaline. Stipe tissue monomitic; hyphae 4.0–12 µm, cylindrical, parallel, thin-walled, hyaline, weakly incrusted, inamyloid. Stipe vesture common; main body

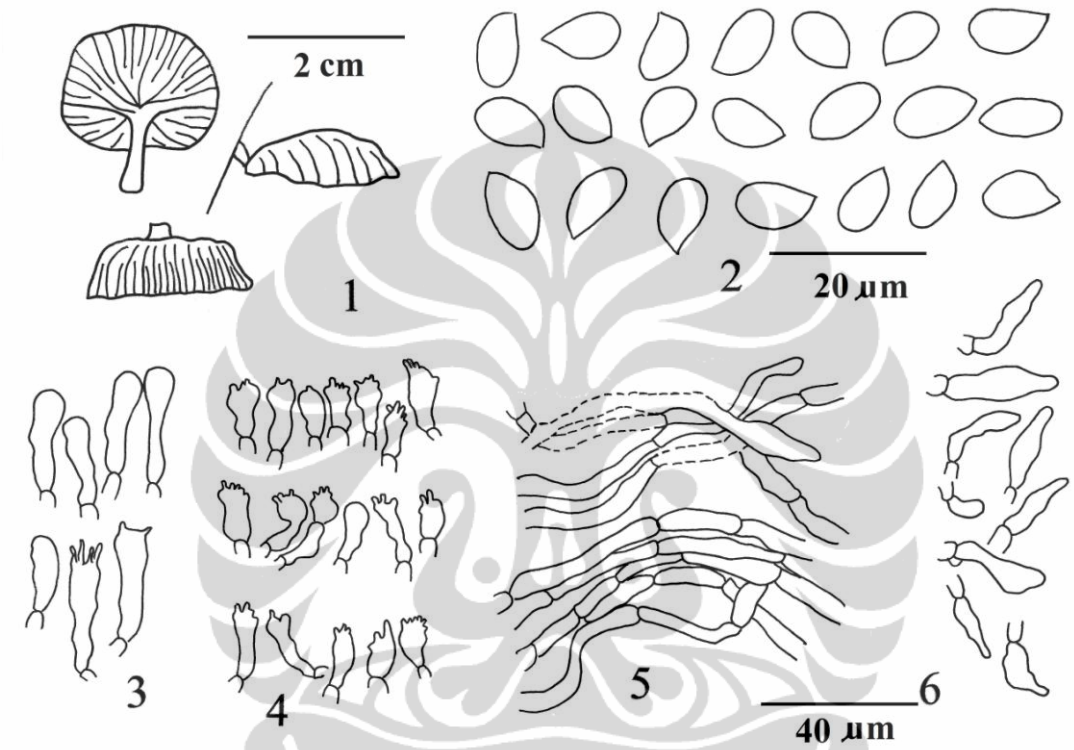

Figure 1.2. *Marasmiellus purpureoalbus* (Petch) Singer (D.E. Desjardin 7102); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis; 6. Stipe vestures (number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

12–32 x 4.8–8.0 µm, fusoid to clavate, thin-walled, hyaline. Clamp connections present.

Habit, habitat, and distribution. Solitary on stems of banana in montane rain forest. Indonesia (Java), Srilanka (Peradeniya), Kenya, and Tanzania.

Specimen examined. Indonesia: Java, slope of Mount Salak, on stems of banana in montane rain forest, 11 January 2000, D.E. Desjardin 7102.

Notes. The easy field character of this species is greyish purple pileus. It has host specifity on musa stems or bark of *Lagerstrumia*, and this Javanese species grows on musa stem. Another species which is similar to *M. purpureoalbus* is *M. purpureus* (Berk. & Curt.) Murrill (Pegler 1983). *Marasmiellus purpureus* (Berk. & Curt.) Murrill is northern hemisphere species, and it differs from *M. purpureoalbus* in having bigger pileus, 2 series of lamellulae only, and shorter basidiospores (6–9 x 3.2–4.5 µm).

2. ***Marasmiellus globosus*** Retnowati sp. nov. Figure 1.3 and 1.5.
Type : Indonesia, Java, Cibodas Botanical Garden, elev. 1423–1500 m, on wood, 11 January 2000, A.W. Wilson 27 (Holotype: BO).

Pileus 2–22 mm diam, plano-convex to plane, round to reniform, uneven of undulate, rugulo-striate to rugulose-lumpy, dull, dry, glabrous to minutely felted; margin entire, wavy, straight to greatly reflexed color, ranges from buff, to cream (4A3) to grayish orange (6B3) or beige/flesh (5–6A3). Context unobserved. Lamellae horny, shallowly adnate to adnexed, distant (7–11), with 3–4 series of lamellulae, forked to anastomosing to interveinose, narrow (0.1–0.5 mm), buff to pale orange, white/5A, or white beige (5A1–2). Stipe strongly eccentric to nearly lateral, 0.5–3 x 0.5–1.5 mm, cylindrical, dry, glabrous to pruinose, insititious, solid, white to light yellow (1–2A1) to cream (4A3). Odor and taste indistinctive.

Basidiospores (7.2)8.0–10.4(12) x 5.6–8.0 µm ($\overline{x}$ = 9.30±0.7 x 6.71± 0.2, Q = 1.1–1.9, $\overline{q}$ = 1.39 ± 0.1, n = 25 spores per 1 specimen), subglobose to globose, smooth, hyaline, inamyloid, thin-walled. Basidia 32–48 x 5.6–11.04 µm, clavate, 4-spored. Basidioles clavate. Cheilocystidia absent. Pleurocystidia absent. Pileipellis cutis with diverticulate; hyphae 2.4–8.8 µm, thin to thick walled up to 1.6 µm, weakly incrusted, inamyloid. Pileal trama interwoven; hyphae 3.2–7.2 µm, thin to thick-walled up to 0.8 µm, inamyloid. Stipe tissue monomitic; hyphae 1.6–7.2 µm, parallel, cylindrical, thin-walled to thick-walled up to 1.6 µm, with diverticulate, inamyloid to weakly dextrinoid, hyaline. Stipe vesture uncommon to common; main body 10.4–36 x 4.0–5.6 µm, fusoid to

clavate or irregular shaped, thin to thick-walled up to 0.8 µm, hyaline, inamyloid to very weakly dextrinoid. Clamp connections present.

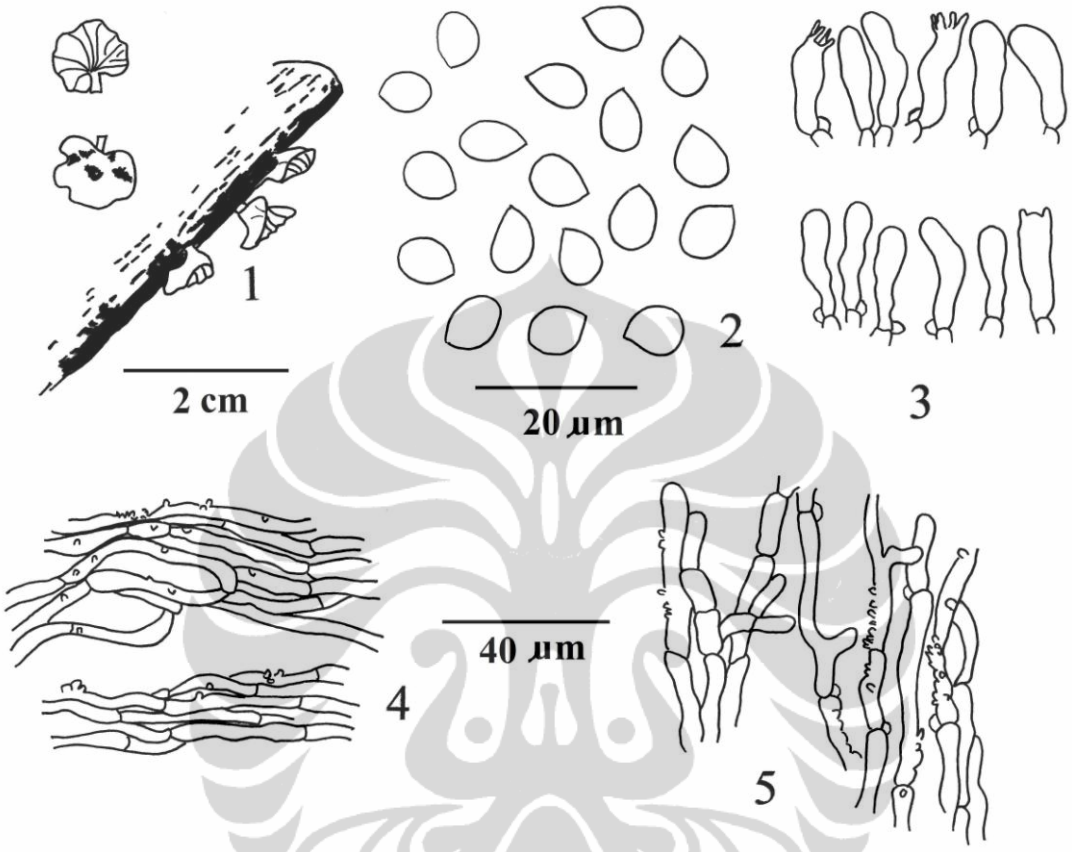

Figure 1.3. *Marasmiellus globosus* Retnowati (A.W. Wilson 27); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Pileipellis; 5. Stipe vesture; number 3, 4, and 5 use the same scale (scale bar = 40 µm).

Habit, habitat, and distribution. Gregarious on bark of *Macropiper* or on wood. Indonesia (Java).

Specimens examined. Indonesia: Java, Mount Halimun National Park, loop trail from Cikaniki, elev. ca.1000 m asl, on bark of *Macropiper*, 8 January 1999, D.E Desjardin 6888; Java, Cibodas Botanical Garden, elev. 1423–1500 m, on wood, 11 January 2000, A.W. Wilson 27; same location, on wood, 22 April 2000, A. Retnowati 250; Java, Central of Java, Purwokerto, Mount Slamet, Baturraden, Block 58, on rotten wood, 22 April 2005, A. Retnowati 542.

Etymology: *globosus* refers to the shape of basidiospores which are sub-globose to globose.

Notes. The species is characterized by small, plano-convex to plane, round to reniform pileus, shallowly adnate with 3–4 lamellulae, forked to anastomosing to intervenose lamellae, eccentric to narrowly lateral stipe, small basidiospores, and common stipe vesture. This species is similar to *M. stenophyllus* (Mont.) Singer (Singer 1973). *Marasmiellus stenophyllus* (Mont.) Singer differs from the Indonesian species in having central and longer stipe (6–15 x 0.5–1.7 mm) and smaller basidiospores (6.8–8 x 2.7–3.5 µm) (Singer 1973).

3. ***Marasmiellus inodermatoides*** Singer, Beih. Nova Hedwig. 44: 328. 1973. Figure 1.4 and 1.5.

Type : Bolivia, Beni, Vaca diez, Guayaramerin, on fruits, branches, and logs of dicotyledon trees in tropical rain forest 9–3–1956, R. Singer B 1720 (F).

Pileus 8–20 mm diam, irregular convex to irregular plano-convex to leaf-shaped in side view, oval/one dept in top view, smooth or minutely rugulose, to rugulo-striate in age, dull, dry, suede-like; disc pale brownish orange (6C4), grading outward to pale peachy orange (6A3), to pinkish buff or orangish white (5A2–3) at the margin, in age sometimes washing out to newly white. Context unobserved. Lamellae adnate, horny, subdistant to distant with 3–4 series of lamellulae, weakly intervenose in age, narrow, convex to straight or concave, white. Stipe 1–3 x 0.5–1 mm, curved, lateral arising from the cleft, terete, cylindrical, equal, pruinose overall, white. Odor and taste indistinctive.

Basidiospores (7.2)8.0–8.8(9.6) x 4.0-4.8 µm ($\overline{x}$ = 8.32±0.57 x 4.26± 0.36, Q = 1.67–2.20, $\overline{q}$ = 1.97 ± 0.17; n = 25 spores per 1 specimen), cylindrical, smooth, hyaline, inamyloid, thin-walled. Basidia 17.6 x 6.4 µm, clavate, 4-spored. Basidioles fusoid to clavate. Cheilocystidia common, 13.6–24 x 4.8–8.8 µm, fusoid to clavate, subglobose or irregular in shaped, thin-walled, hyaline, with diverticulate; diverticulate 5.6 x 1.6–3.2 µm, conical, to clavate or irregular in shaped, thin-walled. Pileipellis cutis, some diverticulate difficult to be observed;

hyphae 4.0–8.8 µm, not incrusted, thin-walled, thin-walled, hyaline, inamyloid. Pileal trama interwoven; hyphae 1.6–4.0 µm, thin-walled, inamyloid. Stipe tissue

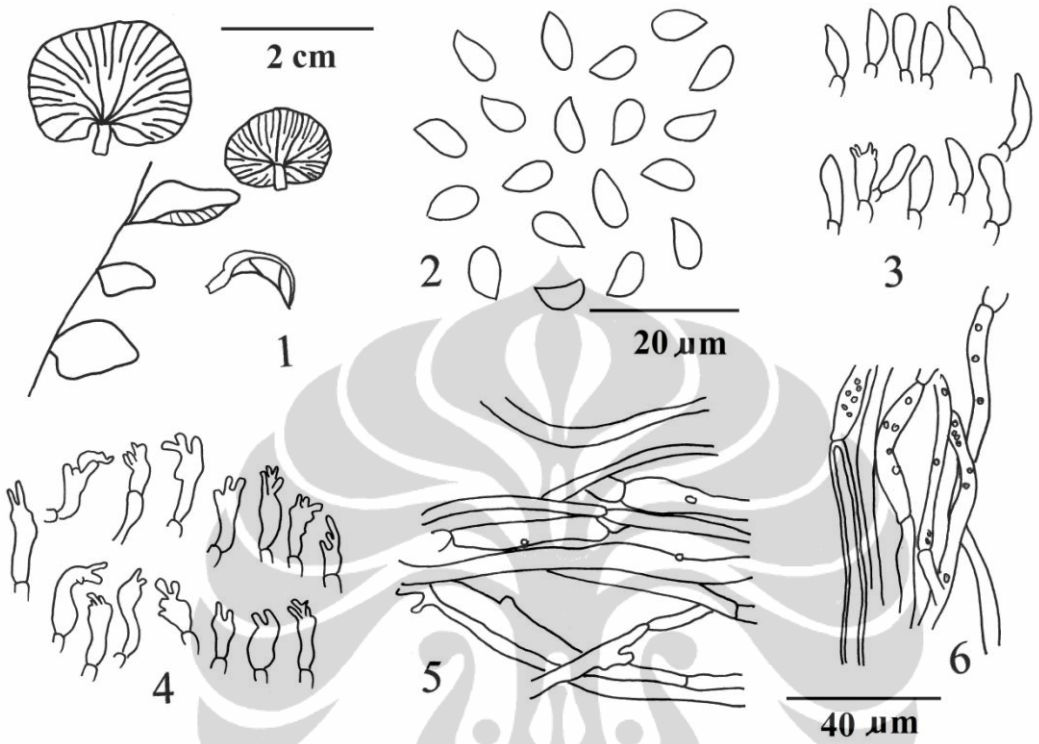

Figure 1.4. *Marasmiellus inodermatoides* Singer (D.E. Desjardin 6808); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis; 6. Hyphae of stipe with diverticulate; number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

monomitic; hyphae 3.2–8.0 µm, equal, with some diverticulates, cylindrical, parallel, thin to thick-walled up to 1.6 µm, hyaline, inamyloid to weakly dextrinoid. Stipe vesture absent. Clamp connections present.

Habit, habitat, and distribution. Densely gregarious on undetermined dicot branches. Indonesia (Bali), Colombia (Valle, Buenaventura), Bolivia (Beni), Martinique, and Guadeloupe.

Specimen examined. Indonesia: Bali, Bedugul, Ekakarya Botanical Garden trail to Mount Pohen, primary forest area, on branches of undetermined dicot, 17 January 1998, D.E. Desjardin 6808.

Notes. *Marasmiellus inodermatoides* Singer described from Bolivia (Singer 1973), and it was common in neotropic, such as Colombia (Singer 1973), Martinique and Guadeloupe (Pegler 1983). Characters of Indonesian specimen are slightly different from Bolivian specimen. The presence of stipe vesture, bigger pileus (3–33 mm diam), longer stipe (2–10 mm), and bigger basidiospores (7.5–10 x 4.5–6.5 µm) are Bolivian specimen's character, which distinguished Bolivian specimen from Indonesian specimens. Those differences are character variations.

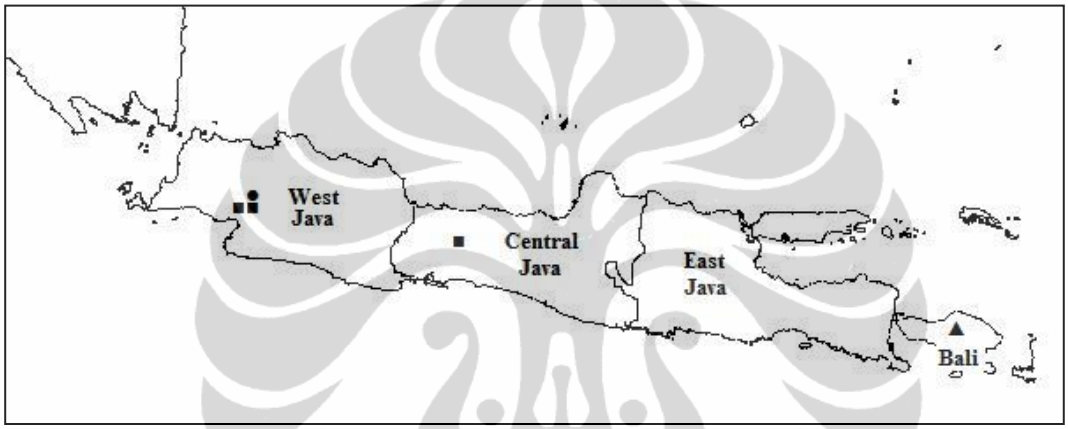

Figure 1.5. Distribution of *M. purpureoalbus* (●), *M. globosus* (■), and *M. inodermatoides* (▲) in Java and Bali.

4. ***Marasmiellus ignobilis*** (Berk. & Br.) Singer, Beih. Nova Hedwig. 44: 264. 1973. Figure 1.6 and 1.9.

Syn. : *Marasmius ignobilis* Berk. & Br., Journ. Linn. Soc. Bot. 14: 40. 1973.

Type : Srilanka, Peradeniya, on wood, Thwaites 100 (K, holotype).

Pileus 5–12 mm diam, plano-convex in side view, flabelliform to semiorbicular in face view; margin wavy to irregular, decurved; surface rugulose, sometimes rugulo-striatulate or with smooth margin, felted to suede-like, dull, dry, pale brownish orange (6C3) to pale brown pinkish orange (7C3). Context unobserved. Lamellae adnate, distant (4–6) with 3–4 series of lamellulae, wavy, intervenose in age, narrow, pale cream-buff. Stipe absent or only a small bulb at the edge of pileus. Odor strongly of garlic; taste indistinctive.

Basidiospores 8.0–8.8(9.6) x 3.2–4.8 µm (the observed spores 9 only), ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia unobserved. Basidioles fusoid to clavate. Cheilocystidia common; main body 13.6–24 x 7.2–9.6 µm, fusoid, to clavate, or irregular shaped, with diverticulate, thin-walled, hyaline; diverticulate 8.0–9.6 x 1.6–2.4 µm, clavate to cylindrical, or irregular in shaped, thin-walled. Pleurocystidia absent. Pileipellis composed of *Rameales*-structure, diverticulate; hyphae 2.4–4.0 µm, slightly incrusted, thin-walled, hyaline, inamyloid. Pileal trama interwoven; hyphae 3.2–4.0 µm, thin-walled, slightly incrusted, clearly incrusted on cell walled, inamyloid. Stipe tissue monomitic; hyphae 4.8–5.6 µm, hyaline to yellowish white, thick-walled up to 0.8 µm, no diverticulate. Stipe vesture common, present at the outer layer, pileipellis like, incrusted. Clamp connections present.

Habit, habitat, and distribution. Gregarious on *Philodendron* liana bark. Indonesia (Java), Srilanka, Kenya, Tanzania, Uganda, and Mexico.

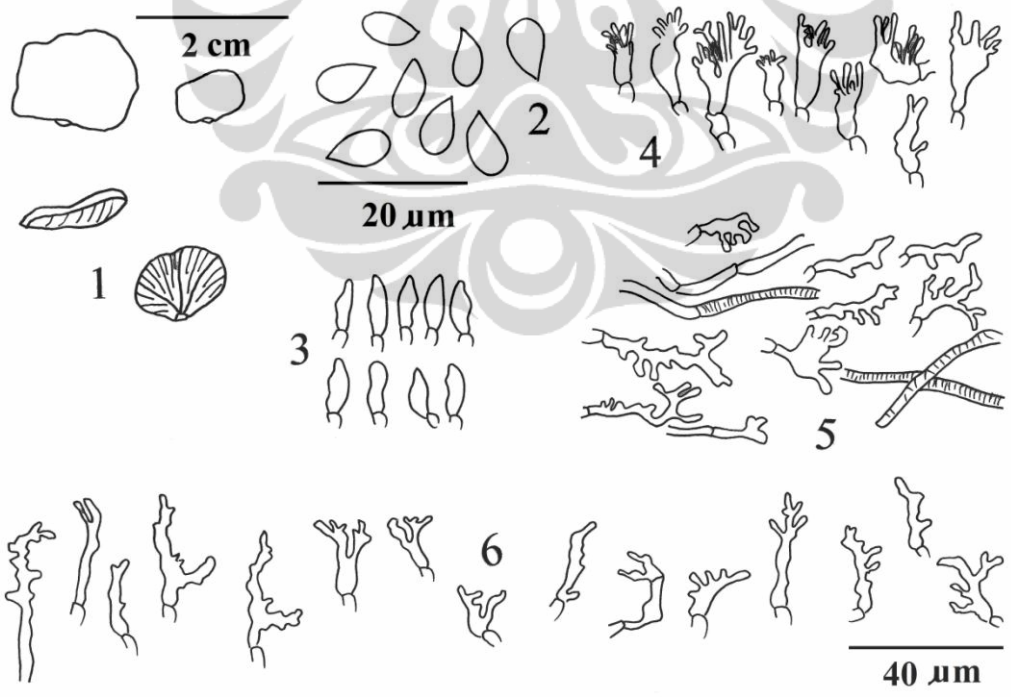

Figure 1.6. *Marasmiellus ignobilis* (Berk. & Br.) Singer (D.E. Desjardin 6808); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis; 6. Hyphae of stipe with diverticulate; number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

Specimen examined. Indonesia: Java, Bogor Botanical Garden, on *Philodendron* liana bark, 8 January 2000, D.E. Desjardin 7063.

Notes. *Marasmiellus ignobilis* (Berk. & Broome) Singer is recognized by having strong odor of garlic. *Marasmiellus ignobilis* widely distributed from East Africa (Kenya, Tanzania, and Uganda) (Pegler 1977), Neotropic (Mexico) (Singer 1973), and Asia (Srilanka (Pegler 1986), and Indonesia), but no report from Lesser Antilles (Pegler 1983). It is very interesting that characters of this species are highly varies (Table 1.2).

Garlic odor, as a key character of *M. ignobilis* is absent from East African specimen, and Pegler (1977) described it as *M. ignobilis* without any information. The very short stipe on the Indonesian specimen added the Pegler'note (1977) "It is often found in large numbers with somewhat variable habit so that individual basidiocarp occur with lateral, eccentric, or almost central stipe".

Table 1.4. Character variations of *M. ignobilis* from different regions.

Area	Characters					
	Pileus (mm)	**The number of lamellu-lae**	**Stipe (mm)**	**Basidiospores (µm)**	**Cheilocys-tidia (µm)**	**Garlic odor**
Srilanka	2–15	2	1–1.5 x 0.3–0.5	7.5–10 x 4.5–5.5	28–37 x 5–10	present
Indonesia	3–12	3–4	Very reduced	8.0–8.8(9.6)x3.2–4.8	13.6–24 x 7.2–9.6	present
East Africa	5–12(–20)	2	3–7x1–2	6.5–8.5 x 4.3–6	25–37 x 4–12	Absent? (not mentioned)
Neotropic	10–37	–	1–6 x 0.5–2	9.5–12.2x4.5–6.5(7)	12–22 x 8–11	present

Others species of *Marasmiellus* with garlic odor are *M. alliioodorus* (Bertero ex. Mont.) Singer (Singer 1973), *M. subingratus* (Dennis) Singer (Pegler

1983), and *M. osmophorus* Dennis (Singer 1973). The last two species belong to section *Rameales*.

5. ***Marasmiellus*** aff. ***concolor*** (Berk. & Curt.) Singer, Sydowia 9: 392. 1955. Figure 1.7 and 1.9.

Syn. : *Marasmius concolor* Berk. & Curt., Journ. Linn. Soc., Bot. 10: 299. 1869.

Type : Cuba, on dead branches of dicotyledon, Wright 74, (FH, K).

Pileus 10–15(–35) mm diam, sessile, chordate in face view, convex in side view, striate, glabrous, dull, dry; at first light yellowish (4A4) to light orange (5A4) fading with age to white. Context unobserved. Lamellae adnexed to point of attachment, distant, with 3–4 series of lamellulae, convex, broad (1–3 mm), non-marginate, white. Stipe absent. Odor and taste indistinctive.

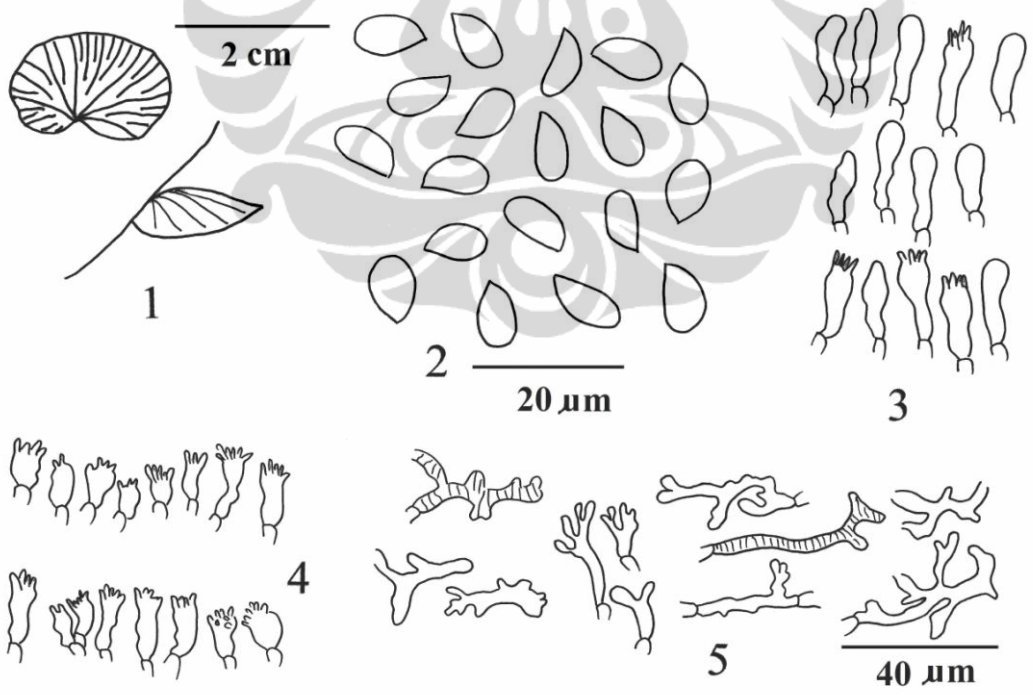

Figure 1.7. *Marasmiellus* aff. *concolor* (Berk. & Curt.) Singer (D.E. Desjardin 6730); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis; number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

Basidiospores (9.6)10.4–11.2(12) x 5.6–6.4(7.2) µm ($\bar{x}$ = 9.6±0.61 x 5.6± 0.45, Q = 1.50–2.14, $\bar{q}$ = 1.77 ± 0.17; n = 25 spores per 1 specimen), ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 25.6–29.4 x 7.2–10.4 µm, clavate, 4-spored. Basidioles fusoid to clavate. Cheilocystidia composed of *Siccus*-type, main body 12–24 x 5.6–8.8 µm, fusoid to clavate, or subglobose, thin-walled, hyaline; setulae 1.6–4.8 x 0.8–1.6 µm, conical, some with pointed apex or some not, thin-walled, hyaline. Pleurocystidia absent. Pileipellis cutis with *Rameales*-structure; hyphae 3.2–4.0 µm, diverticulate, not incrusted to very weakly incrusted, thin-walled, hyaline, inamyloid; diverticulate 3.2–5.6 x 2.4–3.2 µm, conical to clavate or irregular in shaped, thin-walled, hyaline. Pileal trama interwoven; hyphae 2.4–5.6 µm, thin-walled, hyaline, inamyloid. Clamp connections present.

Habit, habitat, and distribution. Gregarious on sticks of undetermined dicot in *Castanopsis javanica* forest. Indonesia (Java) and Cuba.

Specimen examined. Indonesia: Java, West, Cibodas Botanical Garden, trail to Mount Gede, on sticks of undetermined dicot, 9 January 1998, D.E. Desjardin 6730.

Notes. This species is closely related to *M. concolor* (Berk. & Curt.) which was described from Cuba (Singer 1973). *Marasmiellus concolor* has smaller basidiocarp and basidiospores (5–7 x 2.5–3.3 µm).

6. *Marasmiellus epochnous* (Berk. & Curt.) Singer, Sydowia 15: 57. 1961. Figure 1.8 and 1.9.

Syn. : *Marasmius epochnous* Berk. & Curt., Journ. Linn. Soc., Bot. 14: 41. 1873.

Type : Ceylon, on branches and fallen twigs, (K).

Pileus <1–5 mm diam, reniform, convex; surface dry, dull, smooth, powdery, suede-like; margin straight, eroded, torn, especially with age, beige

colored, sometimes margin lighter than disc. Contex thin, same color as cap. Lamellae free to adnate, closed, with 1–2 series of lamellulae, furcated, anastomosing, narrow, near stipe subporoid, centrally to lamellae, same color as cap. Stipe < 1–2 x 0.5–1 mm, terete, curved, equal, solid, dull, dry, insititious, lateral insertion, much reduced, same color as cap. Odor and taste indistinctive.

Basidiospores 8.0–8.8(9.6) x 4.0–4.8 µm, ($\bar{x}$ = 8.58 ± 0.54 x 4.45 ± 0.41, Q = 1.67–2.20, $\bar{q}$ = 1.94 ± 0.19, n = 25 spores per 1 specimen), ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 20–26.4 x 6.4–7.2 µm, clavate, 4-spored. Basidioles fusoid to clavate. Cheilocystidia common, composed of *Siccus*-type broom cells; main body 12–24 x 5.6–11.2 µm, cylindrical to clavate, broadly clavate, or irregular shape, thin-walled, hyaline; setulae 1.6–11.2 x 2.4-5.6 µm,

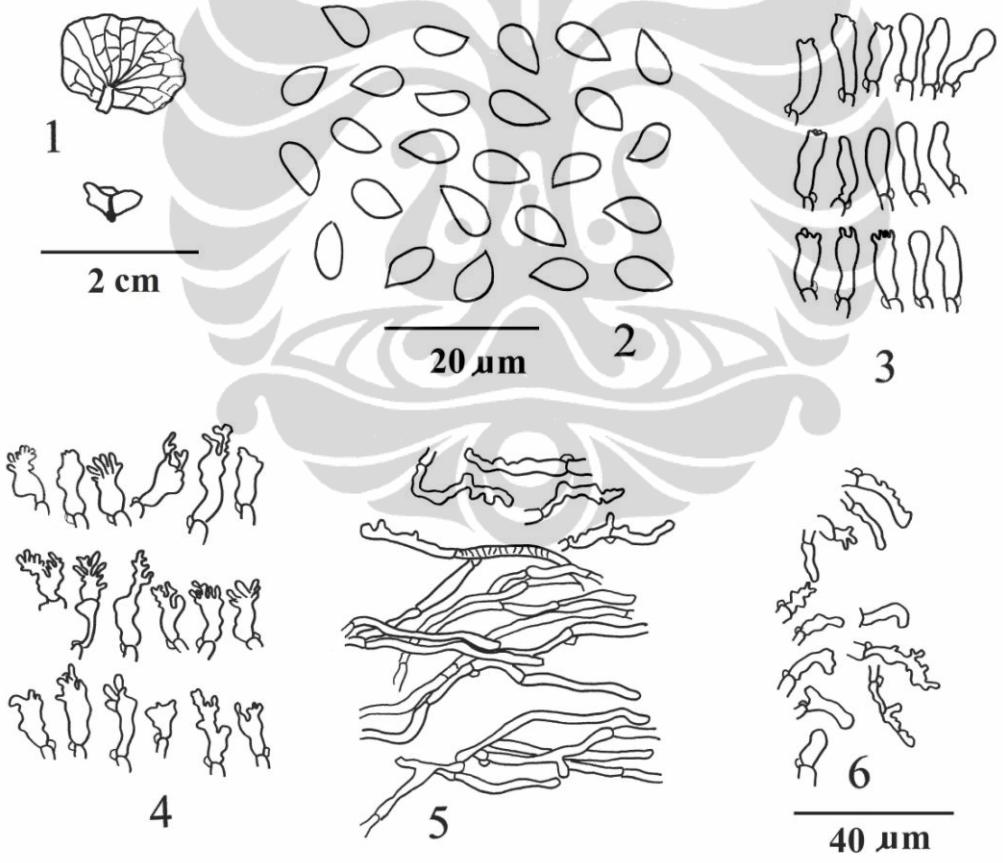

Figure 1.8. *Marasmiellus epochnous* (Berk. & Curtis) Singer (K.P. Collins 98–24); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis; 6. Stipe vesture; number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

conical to cylindrical, clavate, or irregular shape, thin-walled, hyaline. Pleurocystidia absent. Pileipellis composed of *Rameales*-structures, hyphae 3.2–6.4 µm, some incrusted either hyphae or cell walls only, thin to thick-walled up to 0.8 µm, with irregular hyphae tips, inamyloid. Pileal trama interwoven, 3.2–6.4 µm, hyaline, thick-walled, inamyloid. Stipe tissue monomitic; hyphae 1.16–9.6 µm, parallel, cylindrical, equal, thick-walled up to 2.4 µm, inamyloid. Stipe vesture common, main body 4.0–40 x 3.2–10.4 µm, fusoid to clavate or irregular shape, thin to thick-walled up to 0.8 µm, inamyloid. Clamp connections present.

Habit, habitat, and distribution. Scattered to gregarious on undetermined dicot and fallen twigs. Indonesia (Java), Cuba, Brazil, and Ceylon.

Specimens examined. Indonesia: West Java, Halimun National Park, Cikaniki trail, on fallen twigs 13 January 1998, K.P. Collins 98–24; same location, loop trail from Cikaniki, elev ca. 1000 m, on undetermined dicot twigs 9 January 1999, D.E. Desjardin 6904.

Notes. Singer (1973) mentioned that one of his examined specimens of *M. epochnous* was from Java collected by Zollinger. Unfortunately the information was very limited "….Java: Zollinger, Iter. Jav. Sec. 3641 RI (G)…"

The species is closely related to *M. ignobilis* (Berk. & Broome) Singer (Pegler 1977) and *M. inconspicuous* Murrill (Pegler 1983). The former species was described from Sri Lanka (Pegler 1977), and it differs from *M. epochnous* in having strongly garlic odor and the absence of stipe vesture. However, by reading carefully the macro descriptions of Sri Lankan specimen, the stipe is densely hispid (Pegler 1977). In fact, stipe vesture has to be recorded from *M. ignobilis*, but Pegler missed this character. The latter species differs from *M. epochnous* by having slightly bigger spores (6.5–10 x 4–5.5 µm) and pointed setulae toward apex (Pegler 1983). The presence of stipe vesture did not occur on the Srilankan specimen, but it happened in neotropics specimens (Singer 1973).

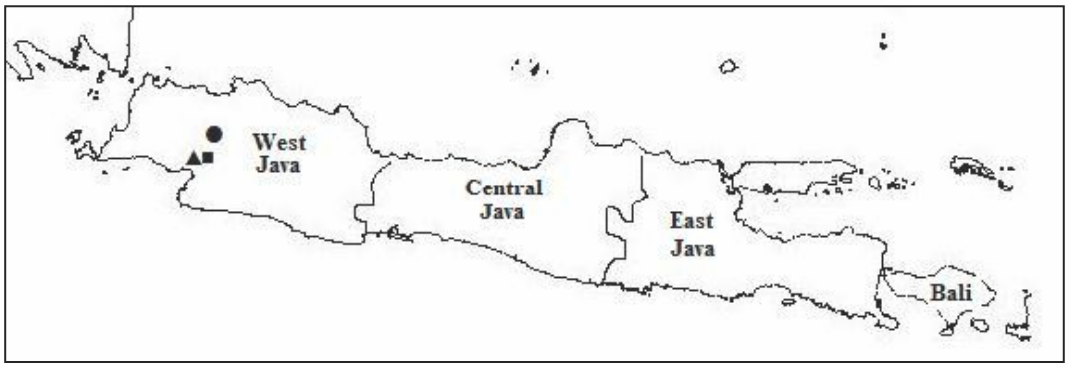

Figure 1.9. Distribution of *M. ignobilis* (●), *M.* aff. *concolor* (■), and *M. epochnous* (▲) in Java and Bali.

7. ***Marasmiellus zingiberius*** Retnowati sp. nov. Figure 1.10 and 1.13.

Type : Indonesia, West, Java, Ujung Kulon National Park, Mount Honje northern part, via Cilimus, on dicot leaves, 15 June 2008, A. Retnowati 597, (Holotype: BO).

Pileus 3–39 mm diam, circular, to convex, broadly convex, sulcate, striate, strongly hygrophanous; margin incurved to wavy, surface smooth to slightly wrinkled, glabrous, off-white. Context thick, off-white (concolorous with pileus). Lamellae adnate to adnexed, subdistant, with 2–3 series of lamellulae, narrow to moderately broad, white to off-white. Stipe 2–15 x 1–4 mm, cylindrical, hollow to solid, eccentric, smooth, glabrous to slightly venose, non-insititious; off-white, with white base tomentose. Odor and taste indistinctive.

Basidiospores 8.0–12.8(13.6) x 4.0–7.2 µm ($\overline{x}$ = 9.94±1.5 x 5.20± 0.8, Q = 1.4–2.8, $\overline{q}$ = 1.92 ± 0.2, n = 25 spores per 1 specimen), ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 16.8–32 x 5.6–8.0 µm, clavate, 4-spored. Basidioles clavate. Cheilocystidia common, composed of *Siccus*-type broom cells, main body 9.6–23 x 4.8–11.2 µm, clavate to broadly clavate, thin-walled, hyaline; diverticulate 0.8–6.4 x 0.8–1.6 µm, conic to clavate or irregular shaped. Pleurocystidia absent. Pileipellis composed of weakly *Rameales*-structures, with or without diverticulate, some incrusted; hyphae 3.2–8.8 µm, thin-walled, hyaline, inamyloid. Pileal trama interwoven; hyphae 4.0–12 µm, thin-walled, hyaline. Stipe tissue monomitic; hyphae 2.4–10.4 µm, parallel, cylindrical, thin-walled,

inamyloid to very weakly dextrinoid, diverticulate, smooth, hyaline to yellowish brown. Stipe vesture absent. Clamp connections present.

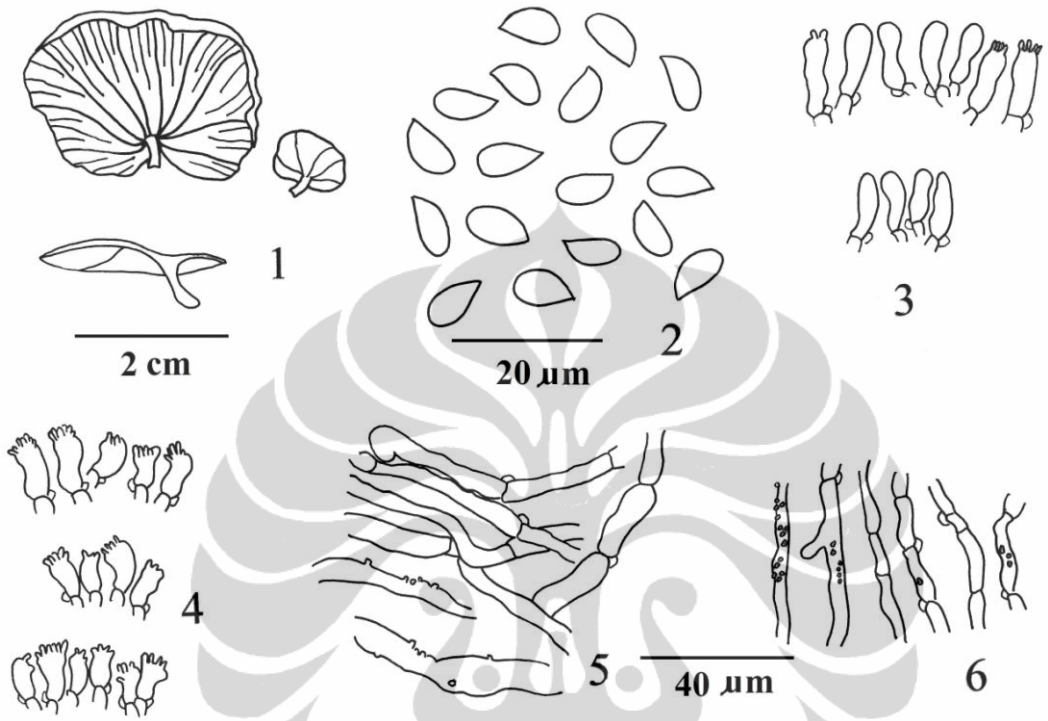

Figure 1.10. *Marasmiellus zingiberius* Retnowati (A. Retnowati 597); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis; 6. Hyphae of stipe with diverticulate, number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

Habit, habitat, and distribution. Solitary to gregarious on dicot leaves and wood, and on *Zingiber* leaves. Indonesia (Java).

Specimens examined. Indonesia: Java, West, Ujung Kulon, Mount Honje, East-South part, via Cibiuk, on dicot wood, 14 June 2008, A. Retnowati 595; same location, Mount Honje northern part, via Cilimus, on dicot leaves 15 June 2008, A. Retnowati 597; same location, on Zingiber stem 15 June 2008, A. Retnowati 599; same location, on dicot wood, 15 June 2008, A. Retnowati 604; same location, on dicot wood or twig, 17 June 2008, A. Retnowati 626.

Etymology: *zingiberius* refers to the substrate of the species which grows on Zingiber.

Notes. This species is similar to *M. troyanus* (Murr.) Dennis (Pegler 1983) and *M. semiustus* (Berk. & Broome) Singer (Pegler 1983). Both are growing on different host of monocotyledon. The first species grows on palm, and the latter species grows on banana leaves. The Indonesian species grows on Zingiber leaves (monocot) and dicot twig and wood.

8. ***Marasmiellus rifaii*** Retnowati sp. nov. Figure 1.11 and 1.13.
Type : Indonesia: Java, East, Surabaya, Darmo street, on living trunk of *Lagerstrumia indica*, 7 March 2008, Mien A. Rifai s.n. (BO).

Pileus 4–10 mm diam, convex with flattened disc, strongly hygrophanous; margin straight to slightly incurved in age; surface dry, dull, glabrous; white to dirty white, slightly darker below to brownish white. Context unobserved. Lamellae adnate, distant, with 1–2 series of lamellulae, moderatley broad, white to dirty white. Stipe 3–5 x 1–2 mm, eccentric, equal to slightly tappered at the base, cylindrical, insititious, dull, dry, glabrous, white to dirty white. Odor and taste not observed.

Basidiospores 12.0–15.2 x 5.6–6.4 µm ($\bar{x}$ = 13.57 ± 0.97 x 6.30 ± 0.27, Q = 1.88–2.57, $\bar{q}$ = 2.15 ± 0.16, n = 25 spores per 1 specimen), fusoid, smooth, hyaline, inamyloid, thin-walled. Basidia 32 x 6.4 µm, clavate, 4-spored. Basidioles clavate. Cheilocystidia absent. Pleurocystidia absent. Pileipellis composed of *Rameales*-structures, the presence of diverticulate unseen; hyphae 5.6–9.6 µm, thin to thick-walled up to 0.8 µm, inamyloid. Pileal trama interwoven; hyphae 4.8–13 µm, thick-walled up to 2.4 µm, hyaline. Stipe tissue monomitic; hyphae 3.2–6.4 µm, cylindrical, parallel, thin to thick-walled up to 2.4 µm, diverticulate, inamyloid, hyaline. Stipe vesture absent. Clamp connections present.

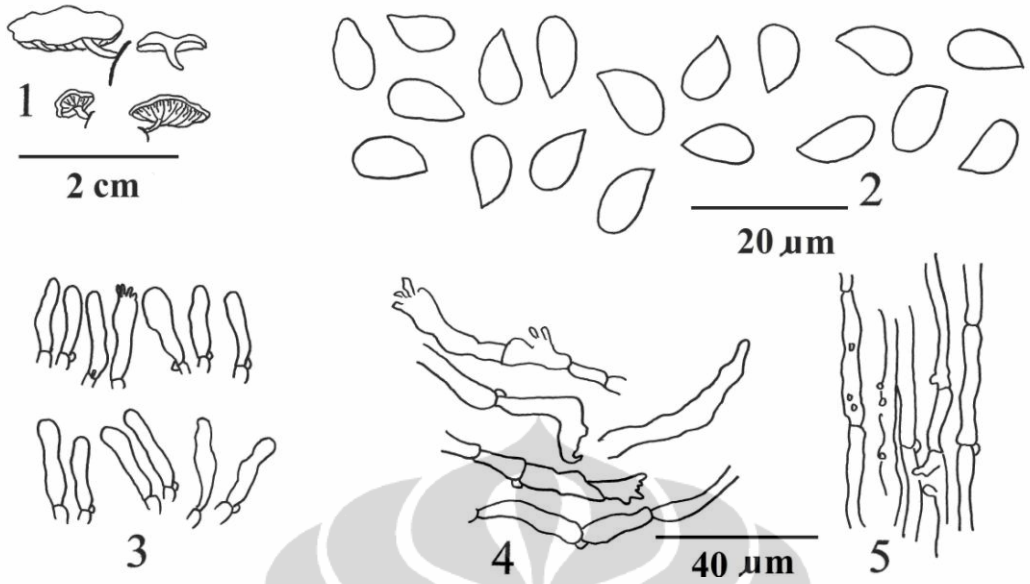

Figure 1.11. *Marasmiellus rifaii* Retnowati (Mien A. Rifai s.n.); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Pileipellis; 5. Hyphae of stipe with diverticulate; number 3, 4, and 5 use the same scale (scale bar = 40 µm).

Habit, habitat, and distribution. Gregarious to caespitose on living trunk of *Lagerstrumia indica*. Indonesia (Java).

Specimens examined. Indonesia: Java, East, Surabaya, Darmo street, on living trunk of *Lagerstrumia indica*, 7 March 2008, Mien A. Rifai s.n.; same location, on living trunk of *Lagerstrumia indica*, 14 April 2011, Mien A. Rifai s.n.

Etymology: *rifaii* is honour to Prof. Dr. Mien A. Rifai who has contributed to the development of mycology in Indonesia.

Notes. This species is characterized by an eccentric stipe, large basidiospores up to 15.2 µm, and host specificity on living bark of *Lagerstrumia*. Since these characters can not be matched with any described taxa in section *Marasmiellus*, this is proposed as a new species. The species is similar to *M. sanctaemarthae* from Colombia and it has a very large basidiospores (9–18 µm long) and host specificity on *Eugenia confusa* (Singer 1973).

9. ***Marasmiellus nugatorius*** (Corner) Retnowati com. nov. Figure 1.12 and 1.13.

Bas. : *Marasmius nugatorius* Corner, Beih. Nova Hedwigia. 111: 80.

Type : Singapore, Bukit Timah, 28 July 1940, on dead leaves of trees in forest.

Pileus 3–8 mm diam, plano-convex or convex in side view, semiorbiculum in surface view, margin often cleft to stipe; dull, dry, glabrous, even (not striate) to striatulate; white. Context unobserved. Lamellae shallowly adnate, distant with 1 series of lamellulae, narrow, white, non-marginate. Stipe 1–2 x 0.5 mm, eccentric to nearly lateral, terete, curved, solid, pruinose, insititious, white. Odor and taste indistinctive.

Basidiospores 7.2–8.8(10.4) x 3.2–4.8 µm (only 15 spores observed), ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia unobserved. Basidioles 24–29.6 x 4.0–5.6 µm, clavate. Hymenial cystidia common; main body 18.4–25.6 x 8.0–11.2 µm, clavate to subglobose, smooth, no diverticulate, thin-walled, hyaline. Pileipellis composed of weakly *Rameales*-structure; hyphae 4.0–12 µm, diverticulate, incrusted, thin-walled, hyaline, inamyloid. Pileus trama cutis; hyphae 2.4–12 µm, incrusted, thin-walled, inamyloid. Stipe tissue monomitic; hyphae 1.6–4.6 µm, some slightly incrusted, thin-walled, hyaline, inamyloid. Stipe vesture common; main body 11.2–48 x 4.0–10.4 µm, fusoid to clavate, subglobose or irregular shape, with or without diverticulate, thin-walled, hyaline, inamyloid. Clamp connections present.

Habit, habitat, and distribution. Gregarious on ascorted dicot twigs in montane rain forest. Indonesia (Java) and Singapore (Bukit Timah).

Specimen examined. Indonesia: Java, Cibodas Botanical Garden, on ascorted dicot twigs, ca. 1550 m asl, 10 January 2000, D.E. Desjardin 7100.

Notes. *Marasmius nugatorius* described by Corner (1996) based on specimen collected from Bukit Timah, Singapore, and it is treated in this study as *Marasmiellus nugatorius*. Examination of species protologue, the characters of

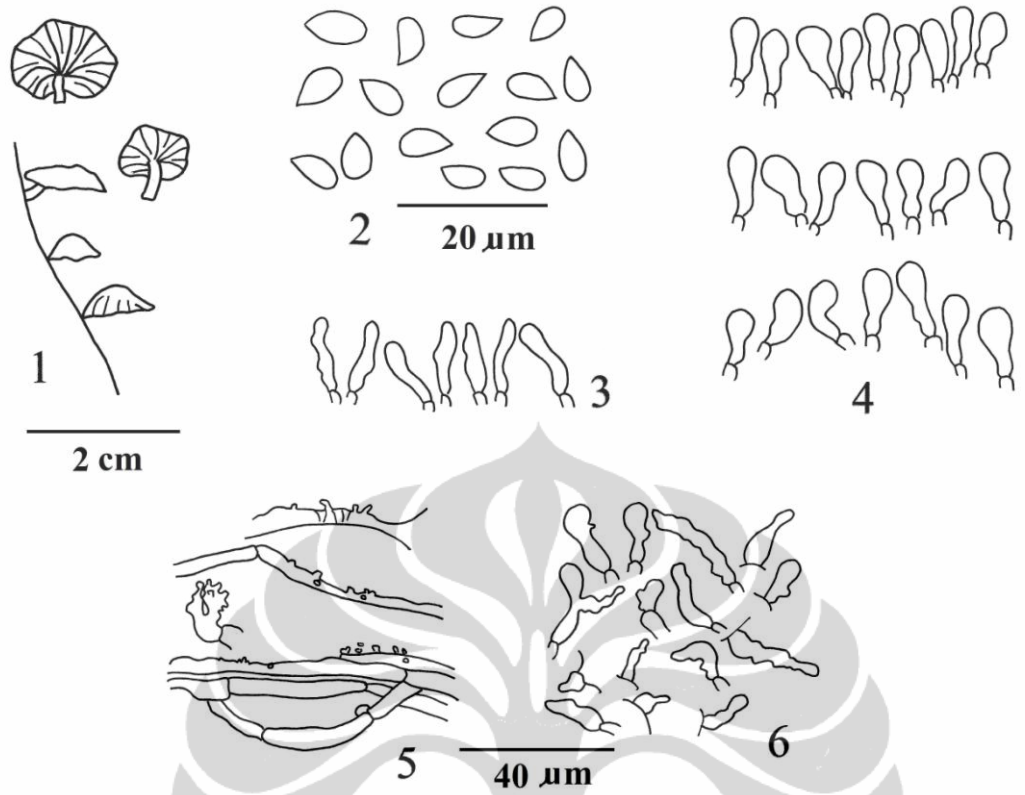

Figure 1.12. *Marasmiellus nugatorius* (Corner) Retnowati (D.E. Desjardin 7100); 1. Basidiomes; 2. Basidiospores; 3. Basidioles; 4. Hymenial cystidia; 5. Pileipellis; 6. Stipe vesture; number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

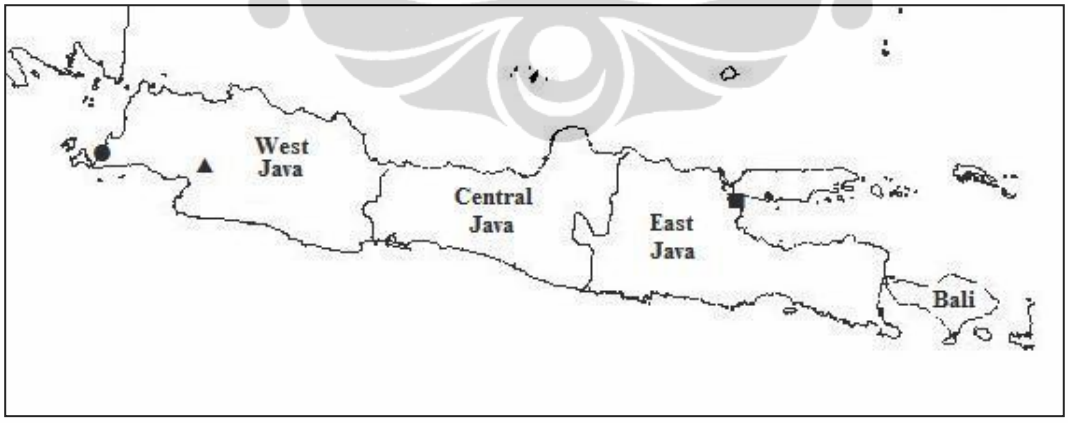

Figure 1.13. Distribution of *M. zingiberius* (●), *M. rifaii* (■), and *M. nugatorius* (▲) in Java and Bali.

this species are considered to represent a species of *Marasmiellus* section *Rameales*. The character which transfers the species from genus *Marasmius* into

genus *Marasmiellus* is *Rameales*-structures of pileipellis with finely terminal and subterminal cells and densely verrucose (Corner 1996).

10. ***Marasmiellus dimidiatus*** Retnowati sp. nov. Figure 1.14 and 1.17.
Type : Indonesia, Java, Mount Halimun-Salak National Park, Plot Ecology, Trail to Pameungpeuk, elev. ca. 1200–1240 m. asl, on monocot, 8 May 2010, A. Retnowati 748, (Holotype: BO).

Pileus 4–15 mm diam, hemispherical to asymmetrically convex or plano-convex, old expanded, ear shaped or dimidiate, membranaceous, tough, rugulose-sulcate to rugulo-striate; margin decurrent, entire; surface irregularly grooved or venose, suede-like, dull, dry, opaque; cream to off white, or pure white overall, often with apricot tinge. Context unobserved. Lamellae shallowly adnate to adnate, distant (4-7), with 2–3 series of lamellulae, forked, anastomosing, intervenose, narrow to moderately broad, cream to off white or white, even edges concolorous. Stipe 1–3 x 0.5–1 mm, cylindrical, base often slightly swollen, strongly eccentric to nearly lateral, curved, equal, terete, solid, dry, insititious, glabrous to suede-like, base with inconspicuous fibrils or scurfy, white. Odor and taste indistictive.

Basidiospores (8.8)9.6–11.2(13.4) x (5.6)6.4–7.2(8.0) µm ($\bar{x}$ = 10.2±0.4 x 6.72± 0.3, Q = 1.3–1.9, $\bar{q}$ = 1.56 ± 0.1; n = 25 spores per 1 specimen), subglobose, smooth, hyaline, inamyloid, thin-walled. Basidia 35.2–42.4 x 7.2–9.6 µm, clavate, 4-spored. Basidioles fusoid to clavate or broadly clavate. Cheilocystidia absent. Pleurocystidia absent. Pileipellis cutis, hyphae 3.2–8.0 µm, with or without diverticulate, slightly incrusted, thick-walled up to 3.2 µm, hyaline to yellowish brown, inamyloid. Pileal trama interwoven; hyphae 3.2–4.0 µm, thick-walled up to 1.6 µm, yellowish brown, inamyloid. Stipe tissue monomitic; hyphae 2.4–7.2 µm, thin to thick-walled up to 2.4 µm, hyaline, diverticulate up to 7.2 µm long, incrusted particularly at cell-walled, inamyloid. Stipe vesture uncommon or absent; main body 12–32 x 4.0–5.6 µm, clavate to cylindrical, thin-walled. Clamp connections present.

Habit, habitat, and distribution. Solitary in groups or gregarious on rotting bark of undetermined dicot sticks in deciduous forest dominated by *Castanopsis-Quercus*, ca. 950 m, or imbricate on rotten dicot twigs. Indonesia (Java and Bali).

Specimens examined. Indonesia: Java, Mount Halimun National Park, Cikaniki Research Station, loop trail, on rotting bark of twigs (of shrub) in deciduous forest dominated by *Castanopsis-Quercus,* 13 January 1998, ZT 7052; Java, Cibodas Botanical Garden, on bark of undetermined dicot stalks, 11 January 1999, D.E. Desjardin 6913; Bali, Eka Karya Botanical Garden, trail to Mount Pohen, elev. ca. 1500 m, on rotten dicot twigs, 14 January 1999, D.E. Desjardin 6932; Java, Mount Halimun-Salak National Park, Ecology Plot, trail to Pameungpeuk, elev. ca. 1200–1240 m. asl, on monocot, 8 May 2010, A. Retnowati 748.

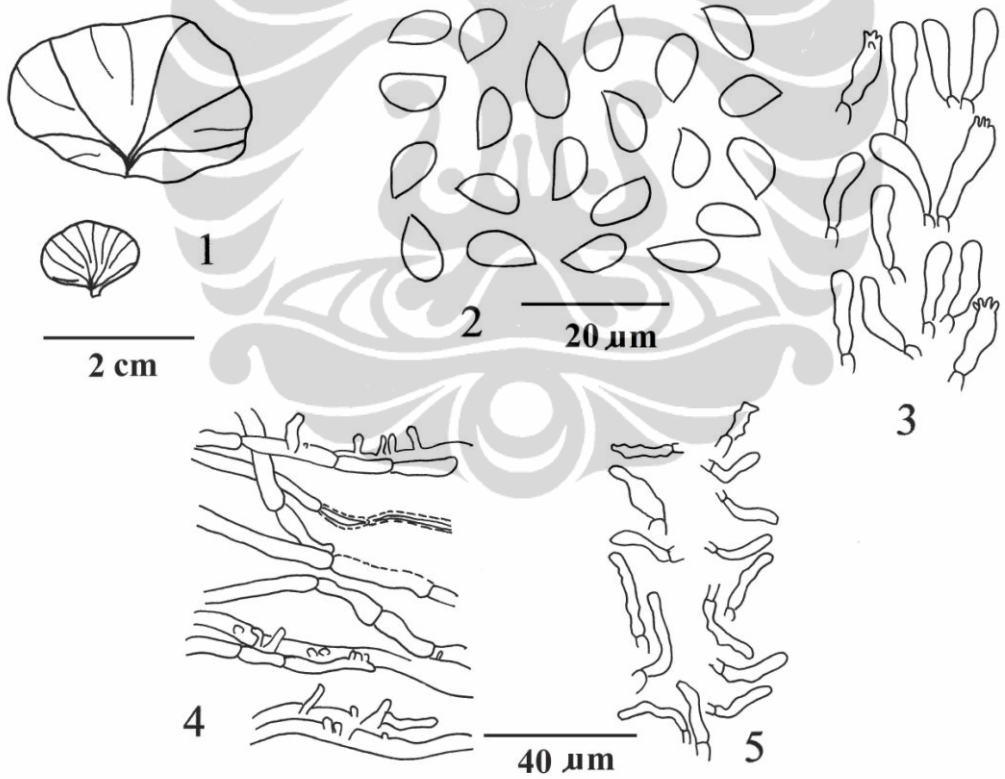

Figure 1.14. *Marasmiellus dimidiatus* Retnowati (A. Retnowati 748); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Pileipellis; 5. Stipe vestures; number 3, 4, and 5 use the same scale (scale bar = 40 µm).

Etymology: *dimidiatus* refers to the shape of pileus of the species which is dimidiate.

Notes. The species is similar to *M. inodermatoides* Singer (Singer 1973). *Marasmiellus inodermatoides* is distinguished by having bigger basidiocarp (<33 mm), smaller basidiospores (7.5–10 x 4.5–6.5 µm) and the presence of cheilocystidia.

11. ***Marasmiellus reniformis*** Retnowati sp. nov. Figure 1.15 and 1.17.
Type : Indonesia, Bali, Eka Karya Botanical Garden, trail to Mount Pohen, primary forest area, on decaying wood, 16 January 1998, K.P. Collins 98–37 (Holotype: BO).

Pileus 3–20 mm diam, convex to plano convex, hemispherical, or reniform in side/face view, rounded chordate in top view, with a dept where stipe attaches, dull, dry, rugulo-striate to rugulo-ridged or plicate, smooth to wrinkled, suede-like; margin straight; pure white to buff, sometimes staining bright yellow in age. Context unobserved. Lamellae adnate, distant to remote (7–9), with 3–5 series of lamellulae; forked and anastomosing, intervenose, often strongly so, narrow to medium broad; edge smooth, white with a pinkish tint. Stipe lateral to strongly eccentric, 2–3 x 1 mm, terete, cylindrical, equal, insititious, pruinose, white overall with basal tomentum. Odor and taste indistinctive.

Basidiospores (8.0)8.8–9.6(10.4) x 5.6–7.2(8.0) µm ($\overline{x}$ = 9.52 ± 0.3 x 0.66 ± 0.1, Q = 1.2–1.8, $\overline{q}$ = 1.43 ± 0.1, n = 25 spores per 1 specimen), ovoid, smooth, hyaline, inamyloid, thin-walled. Basidia 28–36 x 8.0–9.6 µm, clavate, 4-spored. Basidioles clavate. Cheilocystidia absent. Pleurocystidia absent. Pileipellis cutis, constituent hyphae 3.2–5.6 µm, parallel, cylindrical, inamyloid, hyaline, thin to thick walled up to 0.8 µm. Pileal trama 3.2–6.4 µm, interwoven, inamyloid, hyaline, thick-walled up to 0.8 µm. Stipe tissue monomitic, 2.4–5.6 µm, parallel, cylindrical, hyaline, inamyloid, thick-walled up to 1.6 µm. Stipe vesture absent. Clamp connections present.

Habit, habitat, and distribution. Densely gregarious on dead branch of undetermined dicot. Indonesia (Bali).

Specimens examined. Indonesia: Bali, Eka Karya Botanical Garden, trail to Mount Pohen, primary forest area, on decaying wood, 16 January 1998, K.P. Collins 98–37; same location, on dead branch of undetermined dicot, 17 January 1998, D.E. Desjardin 6807.

Etymology: *reniformis* refers to the shape of pileus of the species which is reniform.

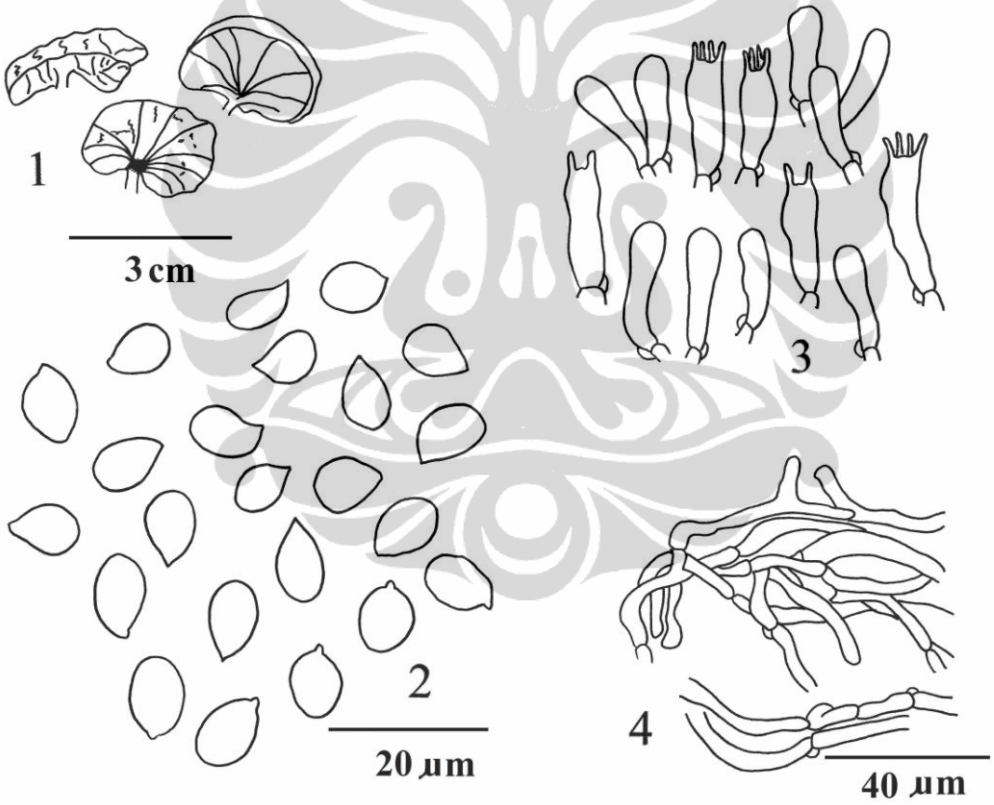

Figure 1.15. *Marasmiellus reniformis* Retnowati (K.P. Collins 98–37); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Pileipellis; number 3 and 4 use the same scale (scale bar = 40 µm).

Notes. This species is characterized by having white with pinkish tints of lamellae, lateral or eccentric stipe, ovoid basidiospores. *Marasmiellus* species

which has pinkish lamellae is *M. rhodophyllus*. It was described by Singer from Colombia (1973), and no other characters match with *M. reniformis*.

12. ***Marasmiellus haurbentesis*** Retnowati sp. nov. Figure 1.16 and 1.17. Type : Indonesia, Java, West, Artificial *Dipterocarpaceae* forest "Haurbentes", Forestry Department, 4 on dicot wood, June 2009, A. Retnowati 649, (Holotype: BO).

Pileus 7–23 mm diam, convex with depressed center, slightly hygrophanous, wavy margin, flower-like, sulcate, smooth, glabrous, white. Context thin, white. Lamellae adnate, subdistant with 2 series of lamellulae, broad, white. Stipe 4–7 x 1 mm, solid, eccentric, cylindrical, non-insititious, smooth, pruinose to glabrous, white, bulb at the base. Odor and taste indistinctive.

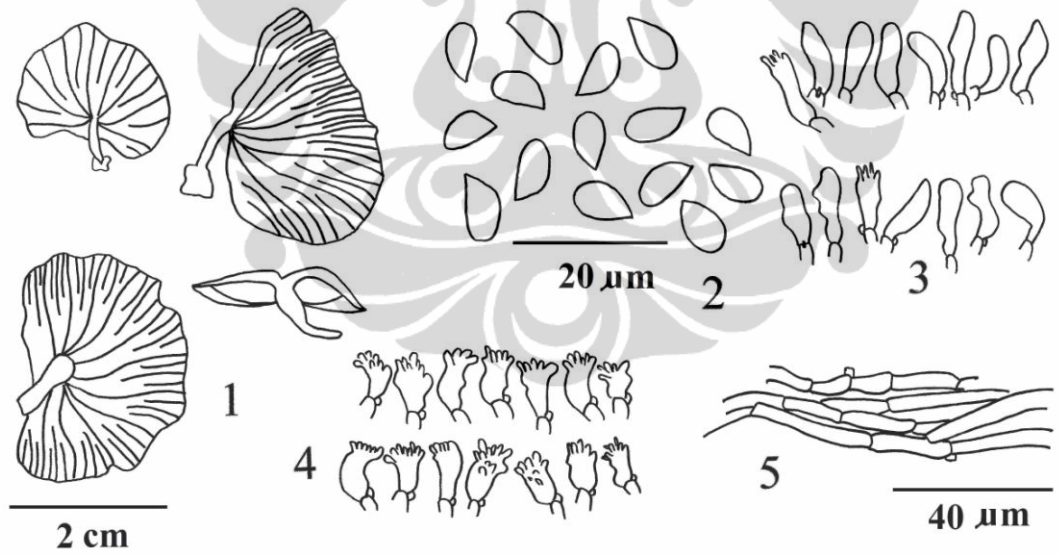

Figure 1.16. *Marasmiellus haurbentesis* Retnowati (A. Retnowati 649); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis; number 3, 4, and 5 use the same scale (scale bar = 40 µm).

Basidiospores 8.8–11.2 x (4.0)4.8–5.6 µm ($\bar{x}$ = 9.95 ± 0.70 x 4.96 ± 0.40, Q = 1.57–2.40, $\bar{q}$ = 2.02 ± 0.19) n = 25 spores per 1 specimen, ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 24–26.4 x 8.4 µm, clavate, 4-spored.

Basidioles fusoid to clavate. Cheilocystidia common; composed of *Siccus*-type broom cells, main body 9.6–19.2 x 7.2–10.4 µm, clavate to broadly clavate, thin-walled, hyaline; diverticulate 1.6 x 0.8–1.6 µm, obtuse conical, thin-walled, hyaline. Pleurocystidia absent. Pileipellis cutis without diverticulate; hyphae 4.8–6.4 µm, thin-walled, hyaline, inamyloid. Pileal trama interwoven; hyphae 3.2–7.2 µm, thin-walled, hyaline, inamyloid. Stipe tissue monomitic; hyphae 2.4–10.4 µm, cylindrical, parallel, thick-walled up to 1.6 µm, some inflated, inamyloid. Stipe vesture absent. Clamp connections present.

Habit, habitat, and distribution. Scattered to gregarious on dicot wood. Indonesia (Java).

Specimen examined. Indonesia: Java, West, Jasinga, Artificial *Dipterocarpaceae* forest "Haurbentes", Forestry Department, on dicot wood , 4 June 2009, A. Retnowati 649.

Etymology: *haurbentesis* refers to the type locality where the species was collected.

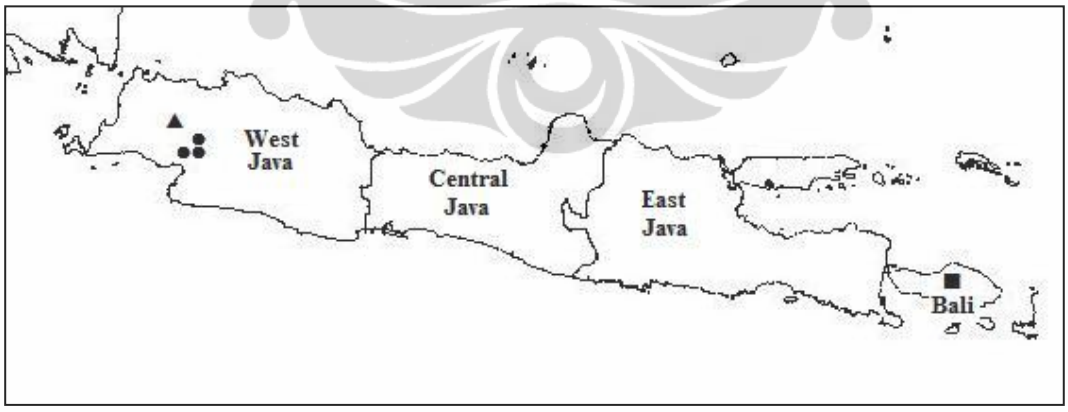

Figure 1.17 Distribution of *M. dimidiatus* (●), *M. reniformis* (■), and *M. haurbentesis* (▲) in Java and Bali.

Notes. This species characterized by relatively small pileus (7–23 mm diam), subdistant lamellae, with 2 series of lamellulae, eccentric stipe, ellipsoid basidiospores, presence of cheilocystidia, and cutis pileipellis. Many species of

Marasmiellus have those all characters, but the most similar species to *M. haurbentesis* is *M. troyanus* (Murrill) Dennis (Singer 1973). *Marasmiellus troyanus* differs from this new species by having caulocystidia and grows on monocotyledon.

13. ***Marasmiellus longisiccus*** Retnowati sp. nov. Figure 1.18 and 1.21.
Type : Indonesia, Bali, south ridge of Mount Catur, East of Lake Bratan, 1200–1800 m asl, on a liana in primary forest, 17 January 1999, D.E. Desjardin 6953, (Holotype: BO).

Pileus 3–8 mm diam, obtusely conic in face view, round to oval in lower lamellar view, rugulose overall, not striate, suede-like to minutely felted, glabrous, dull, dry, appressed-silky opaque, white, yellowish white (4A2) to orangish white (5A2) in age. Context unobserved. Lamellae adnexed to central point of attachment, closed to subdistant, with 1–2 series of lamellulae, medium broad, convex, white, discoloring like pileus. Stipe absent; pileus attached to center of disc. Context thin, fisible, membranous but delicate. Odor and taste indistinctive.

Basidiospores 8.0–8.8(9.6) x (4.8)5.6–6.4(7.2) µm ($\overline{x}$ = 8.74±0.18 x 5.87± 0.11, Q = 1.2–1.7, $\overline{q}$ = 1.49 ± 0; n = 25 spores per 1 specimen), ovoid, smooth, hyaline, inamyloid, thin-walled. Basidia 20–26.4 x 5.6–7.2 µm, clavate, 4–spored. Basidioles clavate. Cheilocystidia common, composed of *Siccus*-type broom cells with 2–3 long setulae, main body 28–34.4 x 8.0–9.6 µm, clavate to irregular shaped, thin-walled, hyaline; setulae 6.4–17.6 x 2.4–3.2 µm, conic, to clavate, fusoid, or irregular shaped, thin-walled, hyaline. Pleurocystidia absent. Pileipellis cutis; hyphae 2.4–9.6 µm, thin-walled, hyaline, not incrusted, inamyloid to very weakly dextrinoid. Pileal trama interwoven, 1.2–7.2 µm, thin-walled, hyaline, inamyloid. Stipe tissue not examined due to sessile. Clamp connections present.

Habit, habitat, and distribution. Densely gregarious on a liana in primary forest and on undetermined dicot twigs. Indonesia (Bali).

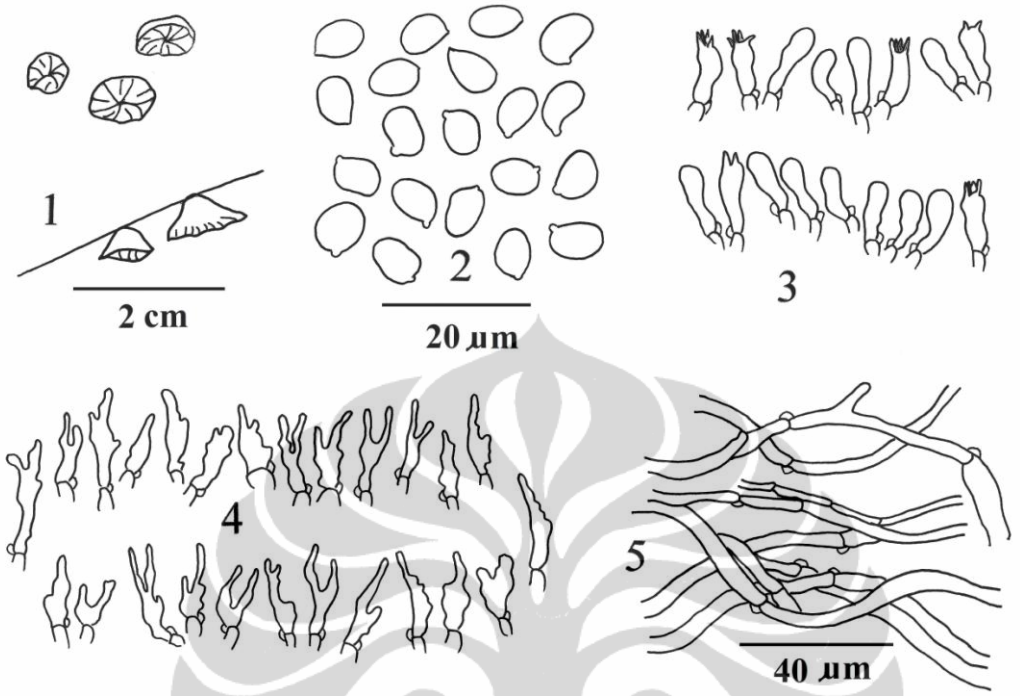

Figure 1.18. *Marasmiellus longisiccus* Retnowati (D.E. Desjardin 6953); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis; number 3, 4, and 5 use the same scale (scale bar = 40 µm).

Specimens examined. Indonesia: Bali, south ridge of Mount Catur, East of Lake Bratan, 1200–1800 m asl, on a liana in primary forest, 17 January 1999, D.E. Desjardin 6953; same location, Lake Bratan, trail to Mount Catur, ca. 1300–1500 m asl, on undetermined dicot twigs, 21 January 2001, D.E. Desjardin 7291.

Etymology: *longisiccus* refers to the presence of long setulae (*sicci*) at the pileipellis.

Notes. The species is characterized by long setulae up to 17.6 µm at the the tip of cheilocystidia. The number of setulae are 2–3, and it becomes an important character to distinguish the species from others.

14. ***Marasmiellus*** aff. ***bolivarianus*** Singer, Beih. Nova Hedwig. 44: 257–258. 1973. Figure 1.19 and 1.24.

Type : Venezuela, Bolívar, Chimantá Massif, Toronó-tepuí, 1555–2090 m alt., on wooden litter, rotten wood in tropical forest, 23 Nov. 1955, J.A. Steyermark & J. J. Wurdack 117 (NY).

Pileus 4–8 mm diam, broadly convex to plano-convex to plane with straight margin, striate to sulcate, faintly translucent, glabrous to silky-felted, dry, white. Context very thin, soft and fragile, white. Lamellae narrowly adnate, distant, with 1 series of lamellulae, narrow white. Stipe 1–2 x 0.5 mm, eccentric, terete, equal, curved, solid, pruinose overall, insititious, white. Odor alliaceum to rotten cabbage; taste indistinctive.

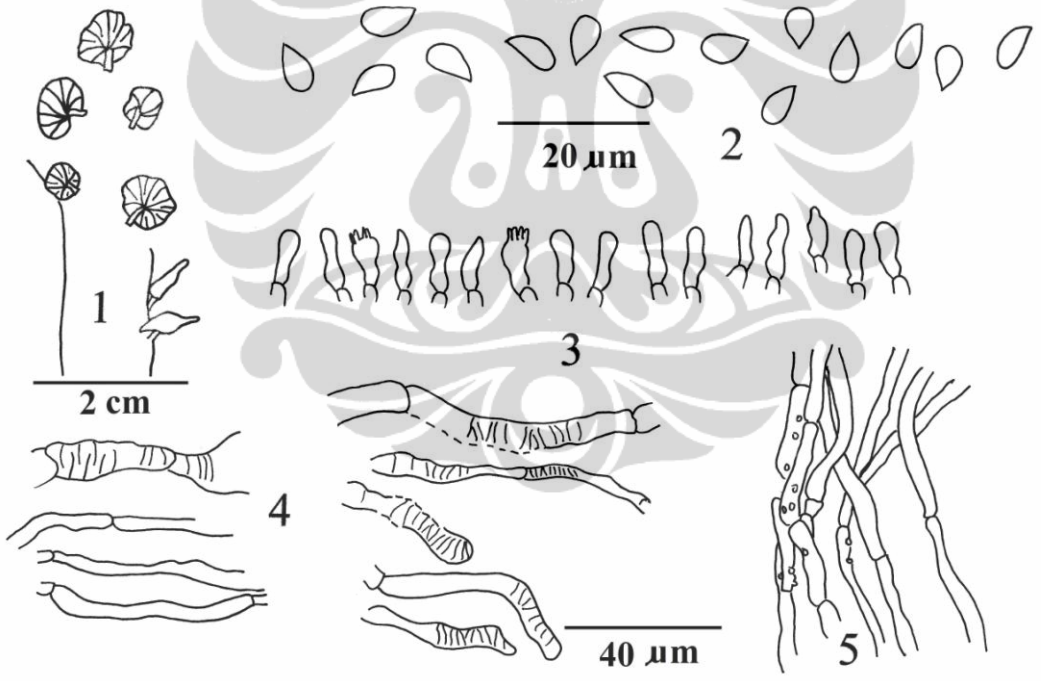

Figure 1.19. *Marasmiellus* aff. *bolivarianus* Singer (D.E. Desjardin 7304); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Pileipellis; 5. Hyphae of stipe with diverticulate; number 3, 4, and 5 use the same scale (scale bar = 40 µm).

Basidiospores 6.4–8.8 x 3.2–4.0 µm (only 15 spores observed), cylindrical, smooth, hyaline, inamyloid, thin-walled. Basidia 15.2–20.8 x 5.6–6.4

µm, clavate, 4-spored. Basidioles fusoid to clavate. Cheilocystidia absent. Pleurocystidia absent. Pileipellis cutis; hyphae 4.0–8.0 µm, incrusted, a few diverticulate, thin-walled, hyaline to yellowish white, inamyloid. Pileal trama interwoven; hyphae 4.0–9.6 µm, incrusted, thin-walled, hyaline, inamyloid. Stipe tissue monomitic; hyphae 4.0–11.2 µm, thin-walled to thick-walled up to 1.6 µm, diverticulate, hyaline, inamyloid. Stipe vesture absent. Clamp connections present

Habit, habitat, and distribution. Densely gregarious on sticks of undetermined dicot in botanical garden area. Indonesia (Bali) and Venezuela (Bolìvar).

Specimen examined. Indonesia: Bali, Eka Karya Botanical Garden, loop trail road at North East corner of garden, on sticks of undetermined dicot in botanical garden area 22 January 2001, D.E. Desjardin 7304.

Notes. Reading carefully the description of *M. bolivarianus* (Singer 1973), it is noted that the Indonesian specimen is very similar to this species. The only difference is presence of cheilocystidia in *M. bolivarianus*, and it is absence in Indonesian specimen. At present, this specimen was described as *M.* aff. *bolivarianus*, till more specimens are collected.

15. ***Marasmiellus pernambucensis*** Singer, Beih. Nova Hedwig. 44: 233. 1973. Figure 1.20 and 1.21.

Type : Brazil, Pernambuco, Caramagibe, on small sticks and vines, 14–7–1960, R. Singer B 3408 (F).

Pileus 2–7 mm diam, convex, soon plano-convex, often with a shallow depression; margin decurrent to straight, short-striate, hygrophanous; surface dull, dry to moist, opaque, glabrous, suede-like; white to buff overall, staining reddish brown in age. Context unobserved. Lamellae adnate to short decurrent, subdistant, with 1–2 series of lamellulae, narrow, thick, non-marginate, white, staining reddish brown in age. Stipe 1–4 x 0.5–0.75 mm, eccentric, often curved,

terete, cylindrical, equal, dry, insititious, pruinose to granulose overall, white to cream buff (4A2). Odor indistinctive or sweet; taste indistinctive.

Basidiospores (6.4)7.2–8.0 x 3.2–4.0 µm ($\overline{x}$ = 7.50±0.07 x 3.58± 0.32, Q = 1.6–2.5, $\overline{q}$ = 2.12 ± 0.17; n = 25 spores per 1 specimen), ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 20–24 x 4.8–8.8 µm, clavate, 4-spored. Basidioles fusoid to clavate. Cheilocystidia 13.6–29.4 x 3.2–9.6 µm, fusoid to clavate or irregular in shaped, with diverticulate, thin-walled, hyaline, hyaline; diverticulate 1.6–4.0 x 0.8 µm, conic, clavate, obtuse, thin-walled, hyaline.

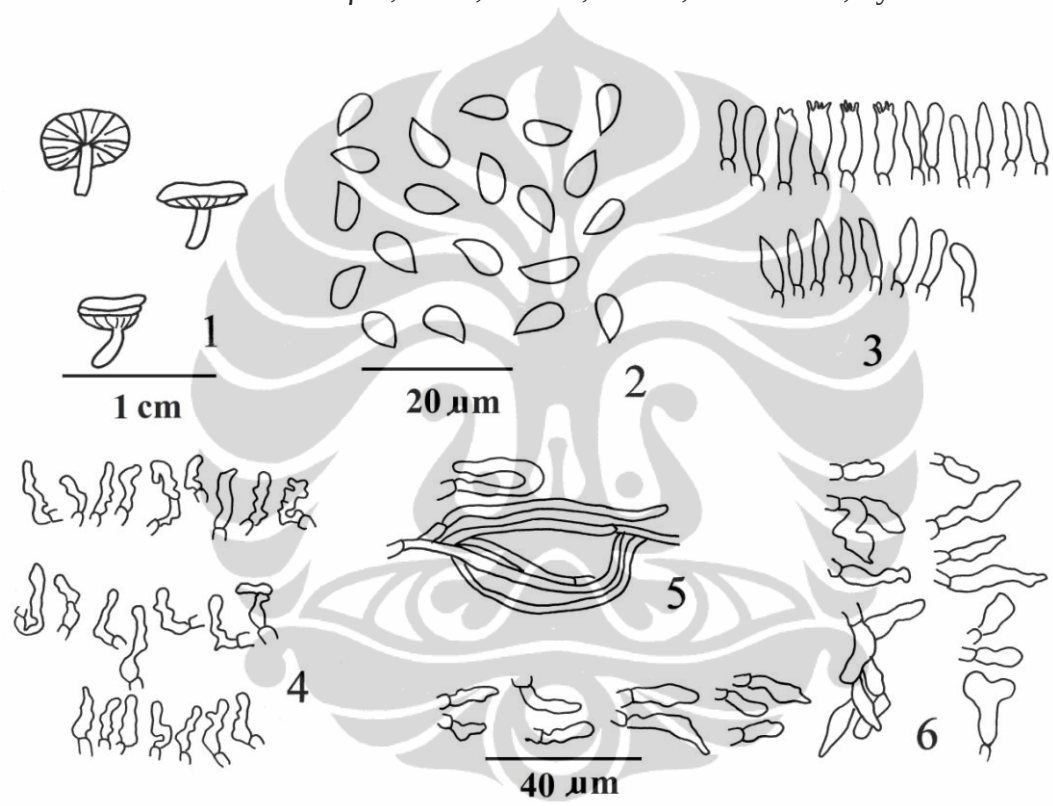

Figure 1.20. *Marasmiellus pernambucensis* Singer (D.E. Desjardin 7057); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis; 6. Stipe vestures; number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

Pleurocystidia absent. Pileipellis cutis; hyphae 1.6–5.6 µm, with diverticulate, not incrusted, thin-walled, hyaline, inamyloid. Pileus trama interwoven; hyphae 3.2–5.6 µm, thin-walled, hyaline. Stipe tissue monomitic; hyphae 3.2–12 µm, cylindrical, parallel, some slightly incrusted, thick-walled up to 2.4 µm, inamyloid. Stipe vesture common; main body 16–48 x 4.0–8.0 µm, cylindrical to

clavate or irregular in shaped, thin-walled, hyaline, inamyloid. Clamp connections present.

Habit, habitat, and distribution. Gregarious on twigs of undetermined dicot or fern rachis. Indonesia (Java).

Specimens examined. Indonesia: Java, Cibodas Botanical Garden, trail to Mount Gede, on twigs of undetermined dicot, 9 January 1998, D.E. Desjardin 6742; Java, north slope of Mount Salak, Curug Nangka, on fern rachis, 7 January 2000, D.E. Desjardin 7057.

Notes. *Marasmiellus pernambucensis* was described by Singer from Brazil (Singer 1973). It is similar to *M. paspali* (Petch) Singer (Pegler 1986), and *M. ramealis* (Bull. Ex Fr.) Singer (Pegler 1983). *Marasmiellus paspali* is firstly collected by Petch in 12 August 1922 from Central Province, Kandy District, Old Peradeniya (Pegler 1986) differs from *M. pernambucensis* in having bigger basidiospores (8.5–12 x 4–6.5 µm), absence of cheilocystidia, and diverticulate hyphae of pileipellis. *Marasmiellus ramealis* is a member of section *Rameales*, but it was compared to *M. pernambucensis* due to a very few differences. *Marasmiellus ramealis* has longer stipe, and the central attachment of stipe.

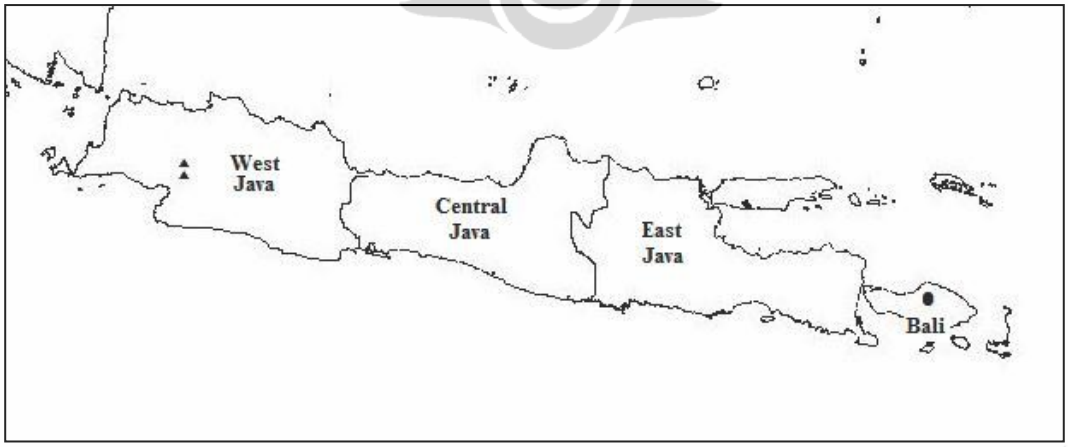

Figure 1.21. Distribution of *M. longisiccus* (●) and *M. pernambucensis* (▲) in Java and Bali.

16. ***Marasmiellus idroboi*** Singer, Beih. Nova Hedwig. 44: 233. 1973. Figure 1.22 and 1.24.

Type : Colombia, Valle, Buenaventura, Calima, on woody stick, 23 April 1968, R. Singer B 6333 (F).

Pileus 2–5 mm diam, convex to plano-convex, dry, hygrophanous, translucent striate at the margin, smooth, glabrous, pure white overall. Context broad, concolorous to pileus. Lamellae adnate, distant (9–12), with 1 series of lamellulae, broad, smooth, non-marginate, pure white. Stipe 1–1.5 x 0.5 mm, eccentric, equal, typically short, insititious, smooth, glabrous; pure white. Odor and taste indistinctive.

Basidiospores 7.2–8.0 x 3.2–4.8 µm ($\bar{x}$ = 7.52 ± 0.40 x 2.56 ± 0.33, Q = 1.75–3.00, $\bar{q}$ = 2.61 ± 0.42) n = 25 spores per 1 specimen, ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 15.2–20 x 6.4–7.2 µm, clavate, 4-spored. Basidioles fusoid to clavate. Cheilocystidia common; main body 12–24 x 4.0–7.2

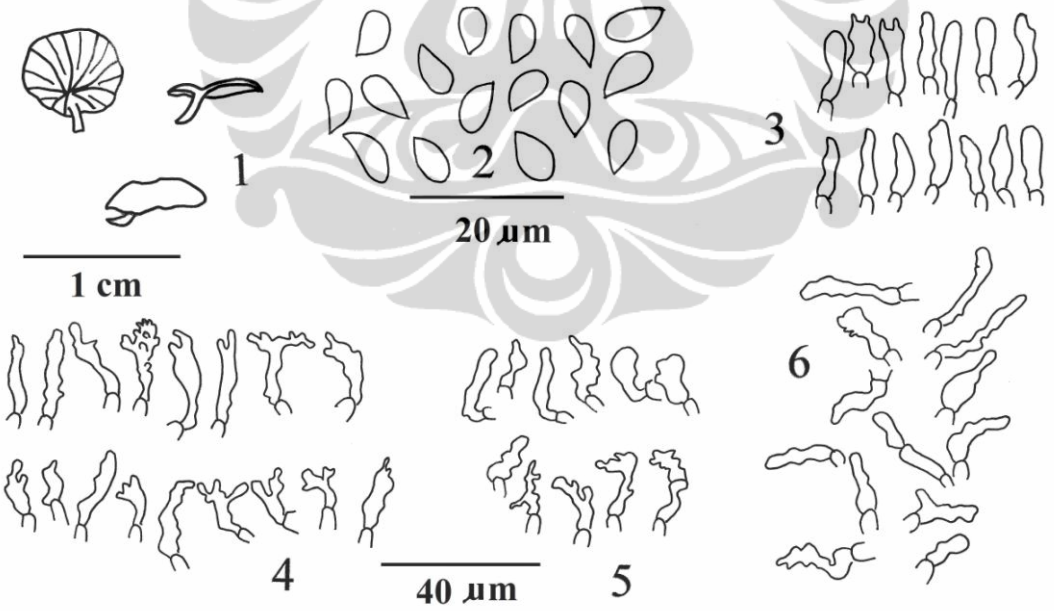

Figure 1.22. *Marasmiellus idroboi* Singer (A. Retnowati 152); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis; 6. Stipe vestures; number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

µm, fusoid to clavate or irregular in shaped or coralloid with one or more broad finger-like, thin-walled, hyaline. Pleurocystidia absent. Pileipellis hardly seen, composed of *Rameales*-structure; main body 16.56–29.44 µm, clavate to sublobose with smooth or wavy edges, thin-walled; hyphae 4–8 µm, thin-walled, slightly incrusted, inamyloid. Pileal trama interwoven; hyphae 4.8–12 µm, weakly incrusted, thin-walled, hyaline, inamyloid. Stipe tissue monomitic; hyphae 2.4–5.6 µm, cylindrical, parallel, thin-walled, hyaline, inamyloid. Stipe vesture common; main body 11.2–34.4 x 4.0–8.0 µm, fusoid to clavate or irregular in shaped, thin-walled, hyaline, inamyloid. Clamp connections present.

Habit, habitat, and distribution. Gregarious on wood. Indonesia (Java) and Colombia (Valle).

Specimen examined. Indonesia: Java, Cibodas Botanical Garden, trail to Mount Gede, on wood, 22 January 1999, A. Retnowati 152.

Notes. The type of pileipellis of this species was hardly seen. By looking the apex of hyphae at the pileipellis, it can be estimated that pileipellis type is *Rameales*-structures. Due to the type of pileipellis, this species was placed in section *Marasmiellus*. The protologue of *M. idroboi* mentioned that it has a cup-like disc at the base and absence of stipe vestures.

Section Rameales (J. Lange) Singer, Lilloa 22: 229. 1951.

Eight species of *Marasmiellus* in Java and Bali were members of section *Rameales*, namely *M. clavatus*, *M. tamblingensis*, *M. delicius*, *M. diverticulatus*, *M. piperus*, *M. pruinosus*, *M. umbilicatus*, and *M. nanus*. Descriptions of species were presented below:

17. ***Marasmiellus clavatus*** Retnowati sp. nov. Figure 1.23 and 1.24.
Type : Indonesia, Mount Halimun National Park, trail from Cikaniki, on dicot wood, 10 January 2001, A.W. Wilson 73 (Holotype: BO).

Pileus 5.5–19 mm diam, plano convex to concave, undulating, moist, glabrous, smooth, margin entire, lobed, circular, translucent striate, finely rugulose, slightly wavy, at some sub-umbonate, color of center of soda brown (6C6) to dark beige (5D5) becoming light beige at margin (4–5A2), strongly hygrophanous. Context 2 mm at stipe, dark beige (5D5). Lamellae adnexed to free, closed-crowded, multiple series of lamellulae, segment form-arcuale, strait to anastomosing rarely, 1 mm deep. Stipe 5–16 mm long x 1 mm diameter, cylindrical, central, fistulose, pliant, pruinose, dark brown at base (6–7EF–8) to at least 0.5 way up stipe, then lightening to beige/white (3–5 A 1–2), inserted into wood and leaves. Odor and taste indistinctive.

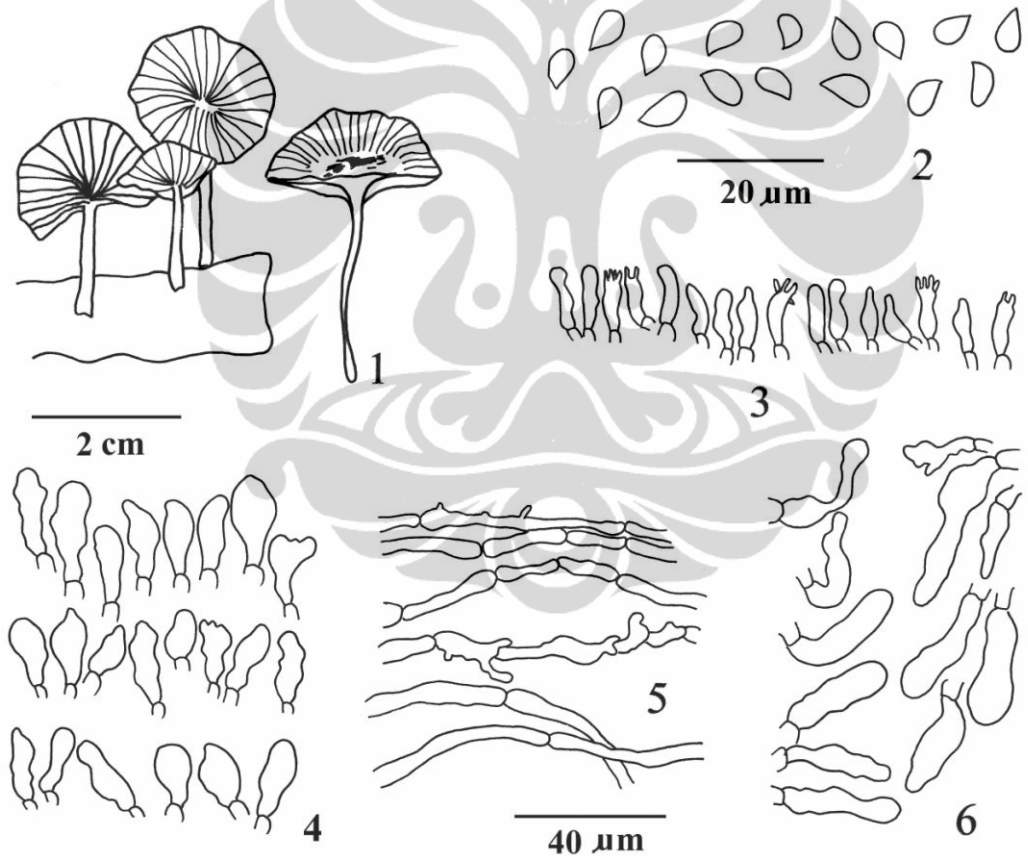

Figure 1.23. *Marasmiellus clavatus* Retnowati (A.W. Wilson 73); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis; 6. Stipe vesture; number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

Basidiospores (5.6)6.4–7.2(8.0) x 3.2–4.0 µm ($\bar{x}$ = 6.59±0.74 x 3.94 ± 0.32, Q = 1.40–2.00, $\bar{q}$ = 1.68 ± 0.15; n = 25 spores per 1 specimen), ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 20–20.8 x 4.8–5.6 µm, clavate, 4-spored. Basidioles fusoid to clavate. Cheilocystidia common, main body 24–34.4 x 10.4–18.4 µm, fusoid to clavate, or subglobose, smooth, without diverticulate, thin-walled, hyaline. Pleurocystidia absent. Pileipellis composed of *Rameales*-type; hyphae 4.0–7.2 µm, some with cylindrical to clavate tips, without diverticulate, not incrusted, thin-walled, inamyloid, hyaline. Pileal trama interwoven; hyphae 4.8–12 µm, incrusted, thin to thick-walled up to 0.8 µm, hyaline, inamyloid. Stipe tissue monomitic; hyphae 4.8–10.4 µm, thick-walled up to 1.6 µm, hyaline, inamyloid. Stipe vesture common; main body 27.2–60 x 6.4–14.4 µm, fusoid to clavate or cylindrical, thick-walled up to 0.8 µm, inamyloid. Clamp connections present.

Habit, habitat, and distribution. Scattered on dicot wood. Indonesia (Java).

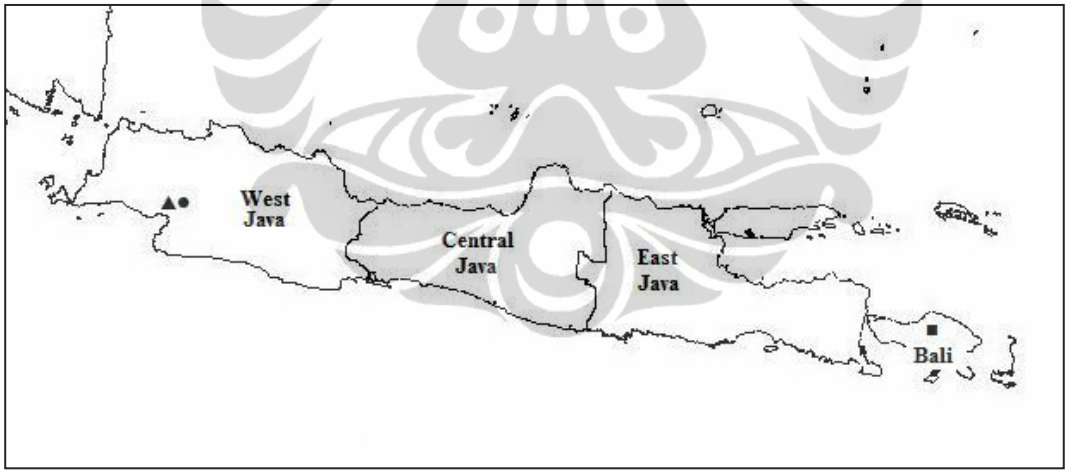

Figure 1.24. Distribution of *M.* aff. *bolivarianus*, *M. pernambucensis* (■), *M. idroboi* (●) and *M. clavatus* (▲) in Java and Bali.

Specimen examined. Indonesia: Java, West, Mount Halimun National Park, trail from Cikaniki, on dicot wood, 10 January 2001, A.W. Wilson 73 (Holotype: BO).

Etymology: *clavatus* refers to the shape of cheilocystidia which is clavate.

Notes. *Marasmiellus clavatus* is similar to *M. corticigenus* (Berk. & Broome) Pegler (Pegler 1986) which was collected from Central Province, Kandy District, Peradeniya by Thwaites in 1868. It has clavate to cylindrical caulocystidia with nodulose apex, and the absence of stipe vestures.

18. ***Marasmiellus tamblingensis*** Retnowati sp. nov. Figure 1.25 and 1.28.
Type : Indonesia, Bali, Lake Tamblingan, on dicot twigs, 20 January 2001, A. Retnowati 344 (Holotype: BO).

Pileus 2–15 mm diam, convex with umbo disc to flattened disc at first, then becoming plano convex in age, hygrophanous, slightly sulcate; margin incurved when young, becoming outcurved when mature, crenate, strong striation; surface smooth, glabrous; light yellow (4–A4) with paler edge, pinkish white in age. Context thin up to 0.5 mm, concolorous to the pileus. Lamellae adnexed, closed (15–17), with 1–2 series of lamellulae, moderately broad up to1 mm, non-marginate; concolorous to the pileus. Stipe 4–14 x 0.5–1 mm, cylindrical, equal, hollow, central, insititious, smooth, white granulose; light brown; a small disc at the base. Odor and taste indistinctive.

Basidiospores have two sizes: the small one: (7.2)8.0–9.6 x 3.2–4.0 µm, $\bar{x}$ = 8.74 ± 0.2 x 3.49 ± 0.2, Q = 1.8–3.0, $\bar{q}$ = 2.53 ± 0.2, n = 25 spores per 1 specimen), the large one: 8.8–12 x 2.4–3.2 µm, $\bar{x}$ = 10.34 ± 0.86 x 3.17 ± 0.16, Q = 2.75–3.75, $\bar{q}$ = 3.27 ± 0.26, n = 25 spores per 1 specimen), ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 19.2–22.4 x 5.6–7.2 µm, 4-spored. Basidioles fusoid to clavate. Cheilocystidia common, tip of the hyphae composed of *Siccus*-type broom cells; main body 9.6–41.6 x 8.0–16 µm, fusoid to clavate, subglobose or irregular shaped, hyaline, thin-walled; setulae 1.6–10.4 x 0.8–3.2 µm, conical to fusoid, or clavate, hyaline, thin-walled. Pleurocystidia absent. Pileipellis composed of *Rameales*-structures, with diverticulae; hyphae 3.2–9.6 µm, hyaline to greenish white, thin to thick-walled up to 0.8 µm, incrusted particularly on cell walls, inamyloid; diverticulate 2.4–10.4 x 0.6–0.8 µm, conic to clavate, thin-walled. Pileus trama interwoven; hyphae 3.2–10.4 µm, thin-walled, hyaline to yellowish white, inamyloid. Stipe tissue monomitic; hyphae 1.6–10.4

µm, parallel, cylindrical, thin to thick-walled up 0.8 µm, inamyloid to weakly dextrinoid. Stipe vesture common, hyphae 7.2–40 x 3.2–8.8 µm, fusoid to clavate or irregular shaped, often forked at the tips, thin to thick-walled up to 0.8 µm, inamyloid. Clamp-connections present.

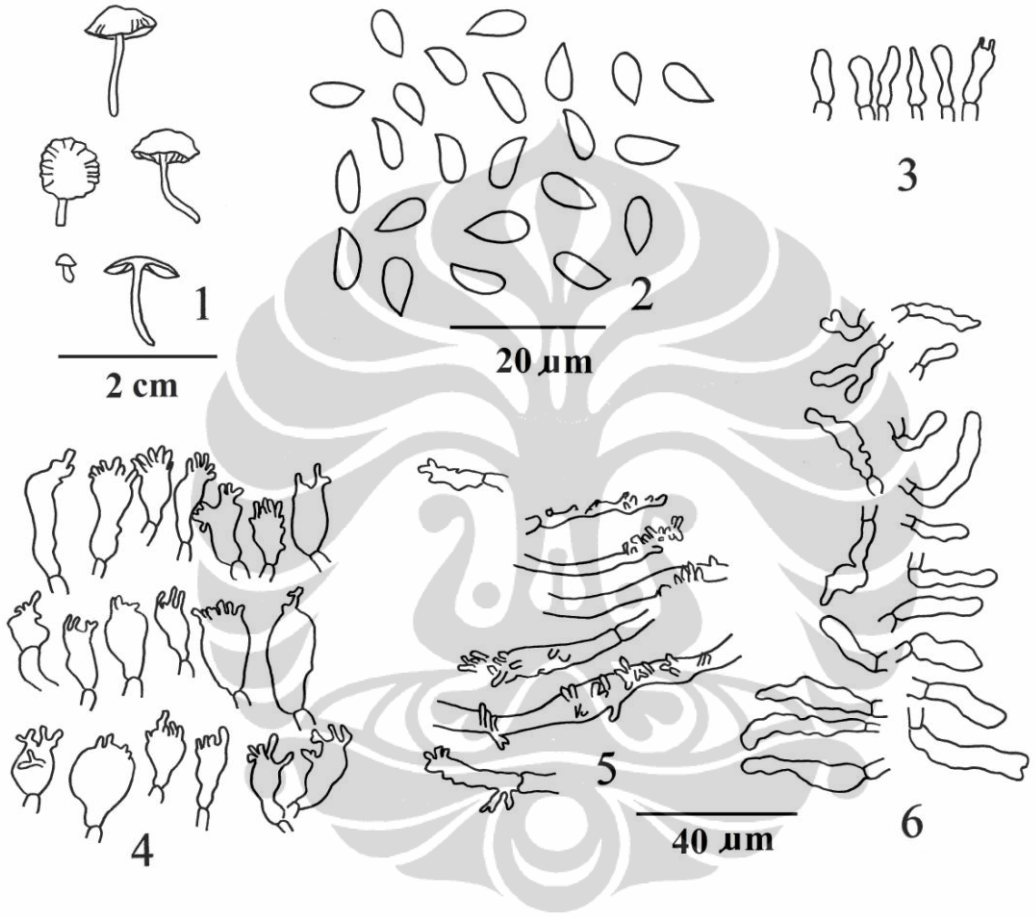

Figure 1.25. *Marasmiellus tamblingensis* Retnowati (A. Retnowati 344); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis; 6. Stipe vesture; number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

Habit, habitat, and distribution. Gregarious on dicot twigs. Indonesia (Bali).

Specimens examined. Indonesia: Bali, Eka Karya Botanical Garden, on dicot twigs, 19 January 2001, A. Retnowati 341; same location, Lake Tamblingan, on dicot twigs, 20 January 2001, A. Retnowati 344; same location, forest along Lake Bratan, 21 January 2001, A. Retnowati 349.

Etymology: *tamblingensis* refers to the type locality where the species was collected in Tamblingan (Bali).

Notes. Four specimens were examined in this study and it was found that basidiospores of 3 specimens were 7.2–8.8 x 3.2–4.0 µm, and another was (8.8)9.6–11.1 x 2.4–3.2 µm. To identify this specimen, the large spores were used. The smaller basidiospores were assumed that they have been not matured yet.

19. ***Marasmiellus delicius*** (Berk. & Broome) Pegler, Kew Bull. Add. Ser. 12: 114. 1986. Figure 1.26 and 1.30.

Syn. : *Agaricus delicia* Berk. & Broome in Journ. Linn. Soc., Bot. 11: 527. 1871.

Type : Kandy District, Peradeniya, July 1869, Thwaites 398, (K).

Pileus 1–8 mm diam, convex, soon plano-convex to plane, sometimes with a shallow central depression, not striate to short-striate, hygrophanous; margin inrolled when young, later becoming straight; surface dull, dry, opaque, glabrous, suede like to pruinose, smooth; pure white overall, but discoloring reddish brown (8D4–5) or pale pinkish in age. Contex extremely thin to moderately thick up to 0.5 mm, soft, white. Lamellae adnate to subdecurrent, subdistant (14–19), with 1–3 series of lamellulae, narrow to moderately broad, convex, non-marginate, white. Stipe 2–6 x 0.5–0.75 mm, central to slightly eccentric, or eccentric, curved, terete, equal above and enlarged base, hollow, tough, pliant, apex pruinose to pruinose overall, base hirsute, dull, dry, non-insititious to sub-insititious; pure white but often discoloring in age to pinkish brown, a pale reddish brown, base becoming yellow white (4A2) in age, with coarse, white rhizomorph or substrate, often a thin subiculum on substrate present. Odor indistinctive or mild; taste indistinctive or mild.

Basidiospores 6.4–8.8 x 3.2–4.0(4.8) µm, ($\overline{x}$ = 7.67 ± 0.4 x 3.36 ± 0.4, Q = 1.7–2.8, $\overline{q}$ = 2.27 ± 0.2, n = 25 spores per 1 specimen), ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 17.6–27.2 x 4.8–6.4 µm, clavate, 4-

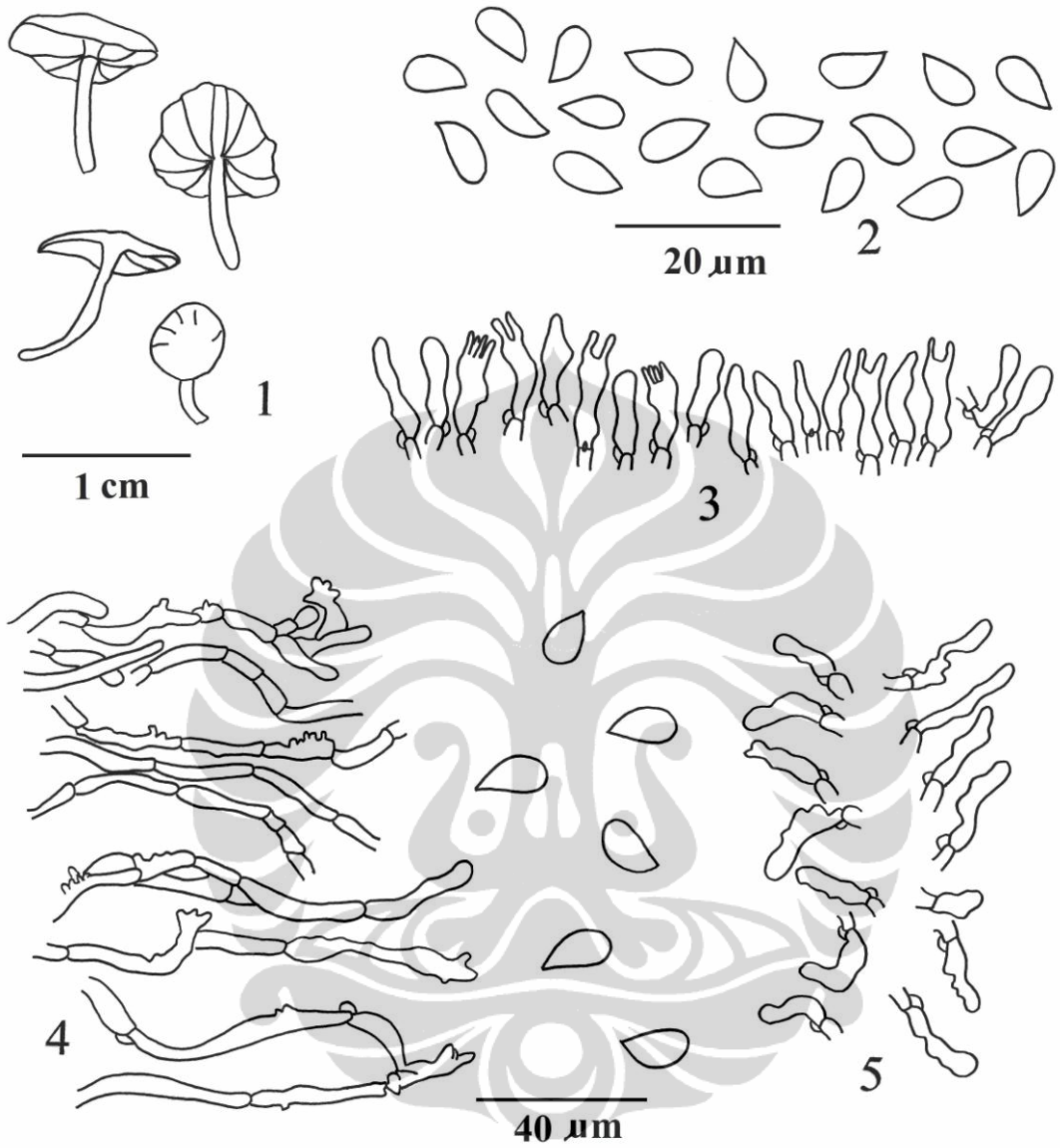

Figure 1.26. *Marasmiellus delicius* (Berk. & Broome) Pegler (A. Retnowati 339); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Pileipellis; 5. Stipe vesture; number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

spored. Basidioles fusoid to clavate. Cheilocystidia absent. Pleurocystidia absent. Pileipellis cutis with *Rameales*-structures; hyphae 2.4–5.6 µm, parallel, cylindrical, with diverticulate, smooth to weakly incrusted, hyaline, inamyloid, thin to thick walled up to 0.8 µm. Pileus trama 2.4–5.6 µm, interwoven, slightly incrusted, inamyloid, hyaline, thin-walled. Stipe tissue monomitic; hyphae 2.4–10.4 µm, parallel, cylindrical, incrusted, with or without diverticulate, hyaline,

inamyloid, thin to thick-walled up to 0.8 µm. Stipe vesture common, 11.2–49.8 x 3.2–6.4 µm, clavate to broadly clavate, cylindrical or fusoid, hyaline, inamyloid, thin-walled. Clamp connections present.Habit, habitat and distribution. Densely gregarious on undetermined dicot twigs or rattan stem in botanical garden. Indonesia (Bali and Java) and Sri Lanka.

Specimens examined. Indonesia: Bali, Eka Karya Botanical Garden, trail to Mount Pohen in primary forest area, on stem of thorny vine, 17 January 1998, D.E. Desjardin 6802; same location, by Lake Bratan along trail to Mount Catur, on twigs, 1 January 2000, AW. Wilson 124; same location, Eka Karya Botanical Garden, on dicot twigs and rattan stem, 19 January 2001, A. Retnowati 339; same location, loop trail at North East of Garden, on undetermined dicot sticks, 22 January 2001, D.E. Desjardin 7306; Java, West Java, Cibodas Botanical Garden, on dicot twigs, 27 October 2000, A. Retnowati 309.

Notes. *Marasmiellus delicius* is similar to *M. ugandensis* (Pegler 1977), and *M. nanus* (Pegler 1983). The first species was reported from Lesser Antilles (Pegler 1983), and the latter species was first described by Pegler (1977). Both differ from the Indonesian specimen only by having cheilocystidia.

20. ***Marasmiellus diverticulatus*** Retnowati sp. nov. Figure 1.27 and 1.28.
Type : Indonesia, Java, Mount Halimun National Park, trail from Cikaniki to Mount Halimun, on undetermined hardwood twigs, 13 January 1998, D.E. Desjardin 6771 (Holotype: BO).

Pileus 2–10 mm diam, conical with a very pointed disc at first, then becoming convex to plano-convex, rugulo-striate to rugulose disc, with or without a small rounded papillae, suede-like, strongly hygrophanous; margin inrolled in young specimens, becoming straight to slightly decurved in age; surface dull, dry, wrinkle overall, glabrous; opaque, pure white with a pale orangish white (5A2) disc. Context thick, white. Lamellae shallowly adnate to adnate, horny, subdistant (13–14 reaching stipe) to distant, with 1–2 series of lamellulae, narrow to

moderately broad, serrate margin, convex to straight, non-marginate, white. Stipe 4–15 x 0.5–1 mm, central, terete, cylindrical, curved, tough, solid, pruinose overall, insititious, pure white. Odor and taste indistinctive.

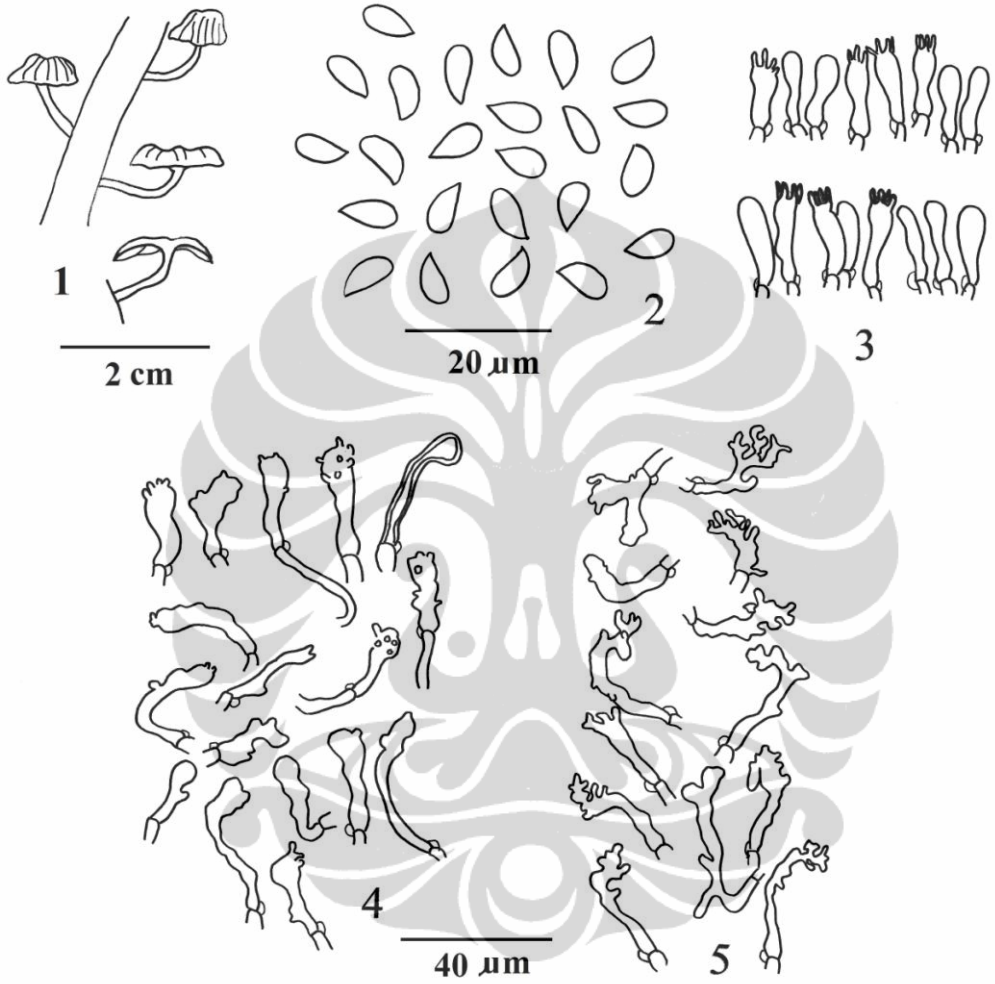

Figure 1.27. *Marasmiellus diverticulatus* Retnowati (D.E. Desjardin 6771) 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Pileipellis; 5. Stipe vesture; number 3, 4, and 5 use the same scale (scale bar = 40 µm).

Basidiospores 6.4–8.0(8.8) x 3.2–4.0 µm, ($\overline{x}$ = 8.0 ± 0.68 x 3.63 ± 0.29, Q = 1.4–2.8, $\overline{q}$ = 2.22 ± 0.01, n = 25 spores per 1 specimen), ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 21.6–35.2 x 6.4–7.2 µm, clavate, 4-spore. Basidioles fusoid to clavate. Cheilocystidia absent. Pleurocystidia absent. Pileipellis composed of *Rameales*-structures with diverticulate elements, main

body of terminal cells 12–24 x 5.6–8.8 µm, clavate to broadly clavate, not incrusted, thin to thick-walled, inamyloid, hyaline to yellowish brown; hyphae2.4–5.6 µm, not diverticulate, thin-walled; diverticulate 0.8–1.6 x 0.8 µm, conical, thin-walled. Pileus trama interwoven, 4.0–5.6 µm, thin to thick-walled up to 0.8 µm, inamyloid, hyaline. Stipe tissue monimitic; hyphae 2.4–3.2 x 4.0–8.8 µm, cylindrical, parallel, thin to thick-walled, hyaline, incrusted, inamyloid. Stipe vesture like pileipellis, composed of *Rameales*-structures with diverticulate elements; main body 24–49.6 x 4.0–6.4 µm, clavate to irregular shape, thin-walled to thick-walled, hyaline, slightly incrusted, inamyloid. Clamp connections present.

Habit, habitat, and distribution. Scattered to gregarious on undetermined hardwood twigs. Indonesia (Java).

Specimens examined. Indonesia: Java, Mount Halimun National Park, trail from Cikaniki to Mount Halimun, on undetermined hardwood twigs, 13 January 1998, D.E. Desjardin 6771; same location, on dicot twigs, 10 January 2001, A. Retnowati 337.

Etymology: *diverticulatus* refers to the presence of diverticulate at the pileipellis and stipe vestures.

Notes. The distinctive features of this species are the absence of cheilocystidia, and the shape of diverticulate on pileipellis is the same as stipe vesture. It is similar to *M. bermudensis* (Berk.) Singer, which was first described from specimen collected in Paynter's Vale, Bermuda and Mexico. Unfortunately, the information of type specimen was not written completely, so that collector and collector number are not mentioned in this monograph. Based on description of *M. bermudensis* (Singer 1973), the size of Indonesian specimen is bigger (2–10 mm).

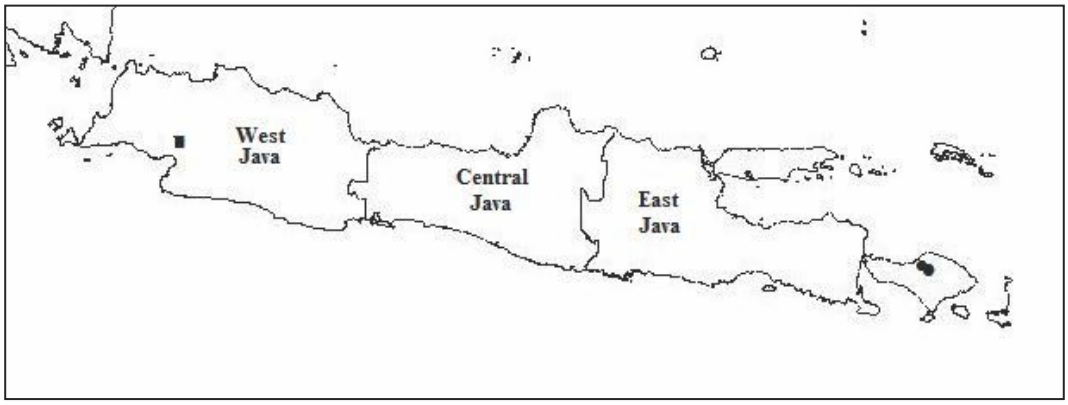

Figure 1.28. Distribution of *M. tamblingensis* (●) and *M. diverticulatus* (■) in Java and Bali.

21. ***Marasmiellus piperus*** Retnowati sp. nov. Figure 1.29 and 1.30.

Type : Indonesia: Java, Mount Halimun National Park, loop trail from Cikaniki, ca. 1000 m asl, on bark of *Macropiper*, 8 January 1999, D.E. Desjardin 6896 (Holotype: BO).

Pileus 2–3 mm diam, plano-convex to plane-depressed, non-striate, suede-like, dull, dry, pale greyish orange (5B3) overall. Context unobserved. Lamellae horny, adnate, nearly poroid, forked, anastomosing, with 0 series of lamellulae, intervenose in age, narrow, buff. Stipe 1–2 x 0.1–0.2 mm, eccentric to central, terete, equal, insititious, glabrous above, pruinose, tough; apex buff, base reddish brown (8E4) to brown (7E). Odor and taste indistinctive.

Basidiospores 7.2–8.8 x 2.4 µm, ($\bar{x}$ = 8.29 ± 0.51 x 2.40 ± 0, Q = 3.00–3.67, $\bar{q}$ = 3.45 ± 0.21, n = 25 spores per 1 specimen), ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 12 x 4.8–5.6 µm, clavate, 4-spored. Basidioles fusoid to clavate. Cheilocystidia common, composed of *Siccus*-type broom cells; main body 12 x 7.2–8.0 µm, with densely diverticulate, fusoid to clavate, subglobose, or irregular in shaped, thin-walled, hyaline. Pleurocystidia absent. Pileipellis composed of *Rameales*-structures; hyphae 4.0–8.0 µm, densely diverticulate, thin-walled, hyaline, weakly dextrinoid. Pileal trama interoven; hyphae 4.0-4.8 µm, inamyloid, hyaline. Stipe tissue monomitic; hyphae 4.0–8.0 µm, parallel, cylindrical, with densely diverticulate, thin-walled, hyaline, weakly dextrinoid. Stipe vesture absent. Clamp connections present.

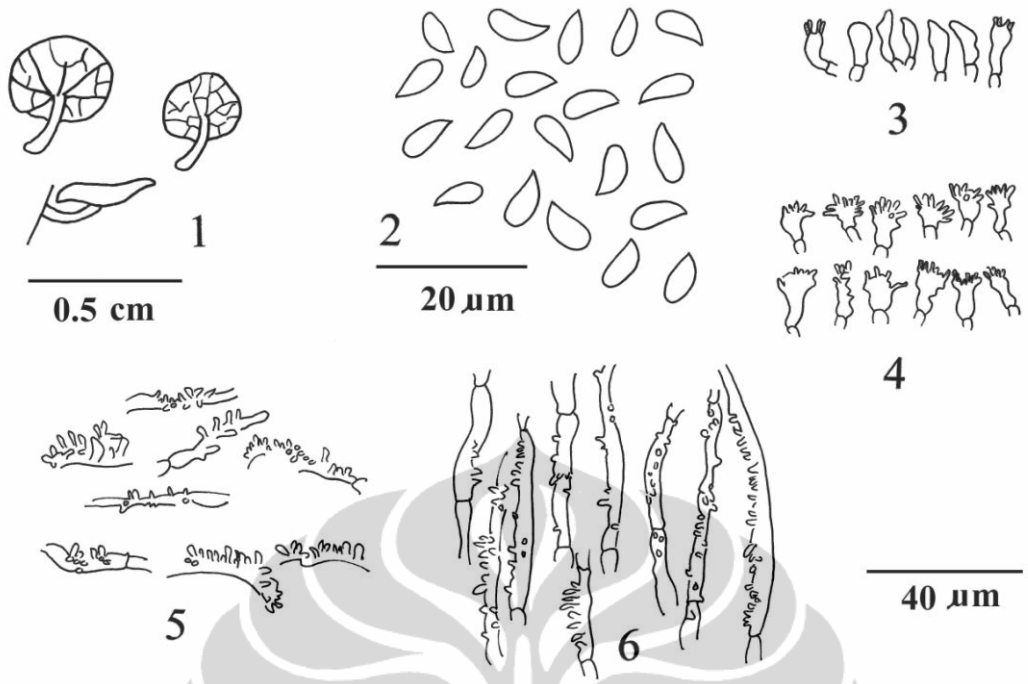

Figure 1.29. *Marasmiellus piperus* Retnowati (D.E. Desjardin 6896); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis; 6. Hyphae of stipe with diverticulate; number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

Habit, habitat, and distribution. Densely gregarious on bark of *Macropiper*. Indonesia (Java).

Specimen examined. Indonesia: Java, Mount Halimun National Park, loop trail from Cikaniki, ca. 1000 m asl, on bark of *Macropiper*, 8 January 1999, D.E. Desjardin 6896.

Etymology: *piperus* refers to the substrate of the species which grows on *Macropiper* sp. (*Piperaceae*).

Notes. This species is similar to *M. stypinus* (Berk. & Broome) Pegler which was first described from Kandy District, Peradeniya on dead stick (Pegler 1986). It differs from *M. piperus* in having well developed lamellae, serrate lamellae edge, and longer stipe (up to 9 mm).

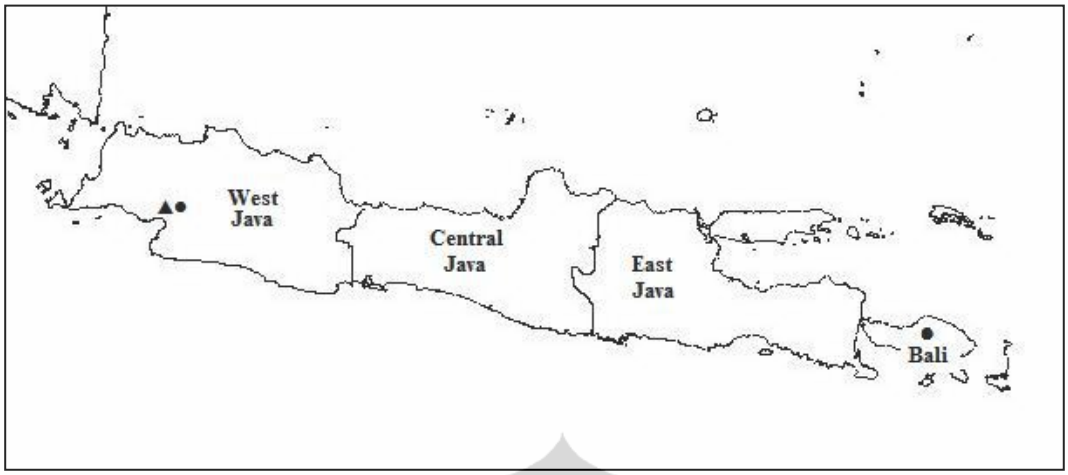

Figure 1.30. Distribution of *M. delicius* (●) and *M. piperus* (■) in Java and Bali.

22. ***Marasmiellus pruinosus*** Retnowati sp. nov. Figure 1.31 and 1.34.

Type : Indonesia, Java, Mount Halimun National Park, loop trail from Cikaniki, ca. 1000 m asl, on undetermined dicot leaves, 8 January 1999, DED 6889 (Holotype: BO).

Pileus 2–4 mm diam, convex to obtusely conic or plano-convex, often shallowly depressed, striatulate, non-striate, even at first, later striate, decurved margin; surface suede-like, dull, dry, disc light orange (5A3; pinkish buff"), margin pale orangish white (5A2), white to buff overall or white to very pale yellowish white (<4A2). Context unobserved. Lamellae adnate, horny, subdistant to distant (8–11), with 1–2 series of lamellulae, convex, moderately broad, edges granulose, white to buff. Stipe 1–4 x 0.2–0.4 mm, central, terete, equal above an enlarged base, curved, insititious, pruinose to granulose overall, tough, dull, dry; glabrous above, purpuraceus to scattered pruinose base, tough, apex buff to pale orangish white (5A2), base becoming reddish brown (8D4-5) tones in age, white overall at first, white to pale yellowish white (4A2) seldom turning reddish brown in age. Odor and taste indistinctive.

Basidiospores 5.6–7.2 x 2.4–3.2 µm ($\bar{x}$ = 6.56 ± 0.57 x 2.56 ± 0.33, Q = 1.75–3.00, $\bar{q}$ = 2.61 ± 0.42) n = 25 spores per 1 specimen, ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia unobserved. Basidioles fusoid to clavate. Cheilocystidia common, composed of *Siccus*-type broom cells; main body 12–17.6 x 8.0–12.8 µm, clavate to broadly clavate, subglobose or irregular

in shaped, with diverticulate, thin-walled, hyaline; diverticulate 2.4–16 x 0.8–1.6 µm, obtuse, conical to cylindrical, thin-walled, hyaline. Pleurocystidia absent. Pileipellis composed of *Rameales*-structure; hyphae 4.0–6.4 µm, thin-walled, hyaline, inamyloid, with diverticulate, no incrustation; diverticulate 3.2–8.0 x 0.8 µm, numerous, obtuse, conical to cylindrical, thin-walled, hyaline, inamyloid. Pileus trama interwoven; hyphae 2.4–8.8 µm, thin-walled, hyaline, inamyloid. Stipe tissue monomitic; hyphae 2.4–4.0 µm, with diverticulate, cylindrical, parallel, thin-walled, hyaline, weakly dextrinoid to dextrinoid. Stipe vesture uncommon; main body 16–20 x 4.8–5.6 µm, fusoid to clavate, thin-walled, hyaline. Clamp connections present.

Habit, habitat, and distribution. Scattered to solitary on various veins that cling to buttres roots of *Ficus* tree, to densely gregarious on hardwood leaves, under *Castanopsis* or undetermined dicot leaves. Indonesia (Java).

Specimens examined. Indonesia: Java, Mount Halimun National Park, loop trail from Cikaniki, on hardwood leaves, 14 January 1998, D.E. Desjardin 6787; same location, ca. 1000 m asl, on undetermined dicot leaves, 8 January 1999, D.E. Desjardin 6889; same location, on various veins that cling to buttres roots of *Ficus* tree, 8 January 1999, D.E. Desjardin 6895.

Etymology: *pruinosus* refers to the pruinose stipe of the species.

Notes. *Marasmiellus pruinosus* is similar to *M. ugandensis* (Pegler 1983), *M. stypinoides* (Petch) Pegler (Pegler 1986), and *M. humillimus* (Quél.) Singer (Antonín & Noordeloos 1993). The first species is different from *M. pruinosus* by having the bigger basidiocarp (pileus 10–20 mm diam, stipe 5–15 x 0.3–0.7 mm), and the latter species differs from *M. pruinosus* in having longer stipe (4–8 mm). *Marasmiellus humillimus*, the European species, differs in having longer basidiospores (7.0–9.5(–10.5)) µm and this species is found on grasses and open vegetation.

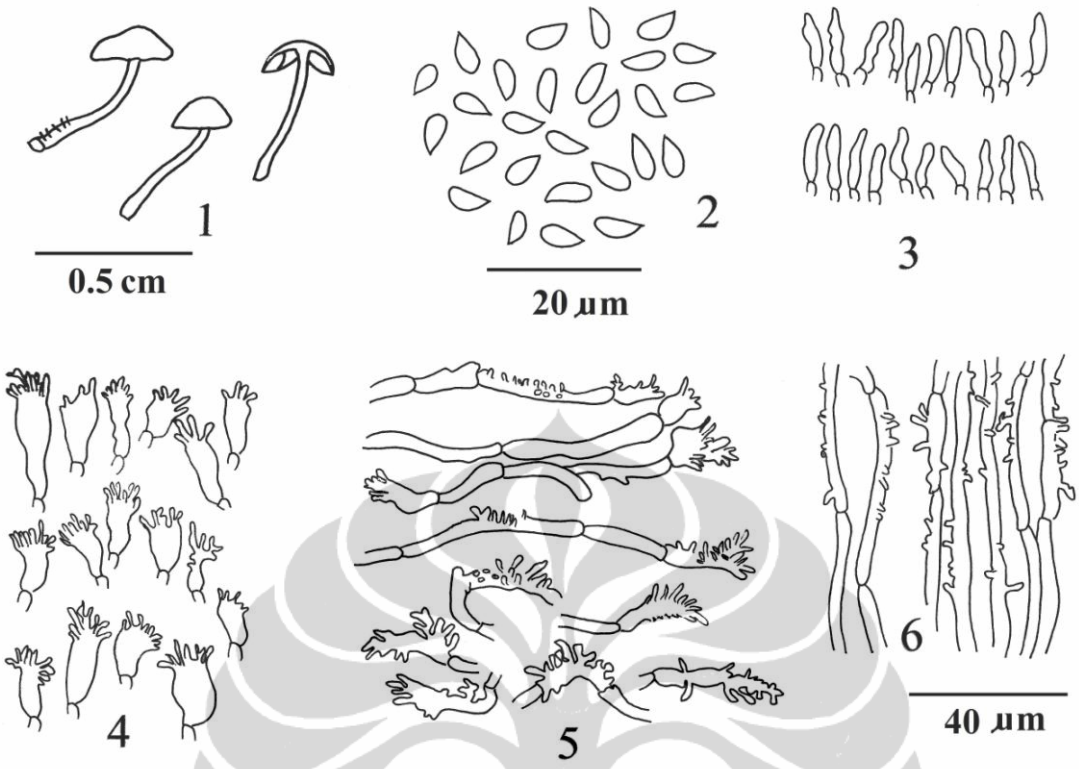

Figure 1.31. *Marasmiellus pruinosus* Retnowati (D.E. Desjardin 6889); 1. Basidiomes; 2. Basidiospores; 3. Basidioles; 4. Cheilocystidia; 5. Pileipellis; 6. Hyphae of stipe with diverticulate; number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

23. ***Marasmiellus umbilicatus*** Singer, Beih. Nova Hedwig. 44: 129. 1973. Figure 1.32 and 1.39.

Type : Colombia, Valle, Buenaventura, Juanchaco, on fallen leaves and leaf petioles, 21 April 1968, R. Singer B 6270 (F).

Pileus 5–49 mm diam, plano-convex, irregularly circular, becoming depressed in age, translucent striate, strongly hygrophanous mostly in depression; margin straight, crenate in a few specimens to wavy; surface dull, dry, pruinose to tomentose; off white with orangish brown in the disc, darker disc than margin, turning dark red-brown in age. Context thin up to 0.5 mm to broad, off-white to reddish brown. Lamellae adnate to subdecurrent or decurrent, subdistant (13–20) to distant, with 2–4 series of lamellulae; narrow to moderately broad; non-marginate, edge smooth; off-white to white. Stipe 7.0–25 x 1.0–3.0 mm, equal to

cylindrical with slightly tappered at the base, central to eccentric, hollow, shiny, glabrous to pruinose or fibrillose, non-insititious; beige at the apex, becoming reddish brown starting ¼ way down from apex to base, off-white to pale brown; a small bulb at the disc; basal tomentum present. Taste and odor indistinctive.

Basidiospores 4.0–6.4(7.2) x 2.4–3.2(4.0) µm, ($\bar{x}$ = 5.41 ± 0.4 x 2.86 ± 0.3, Q = 1.4–3.0, $\bar{q}$ = 1.92 ± 0.2, n = 25 spores per 1 specimen), ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 16–24 x 4.0–5.6 µm, clavate, 4-spored. Basidioles fusoid to clavate. Cheilocystidia 16.8–40 x 6.4–16 µm,

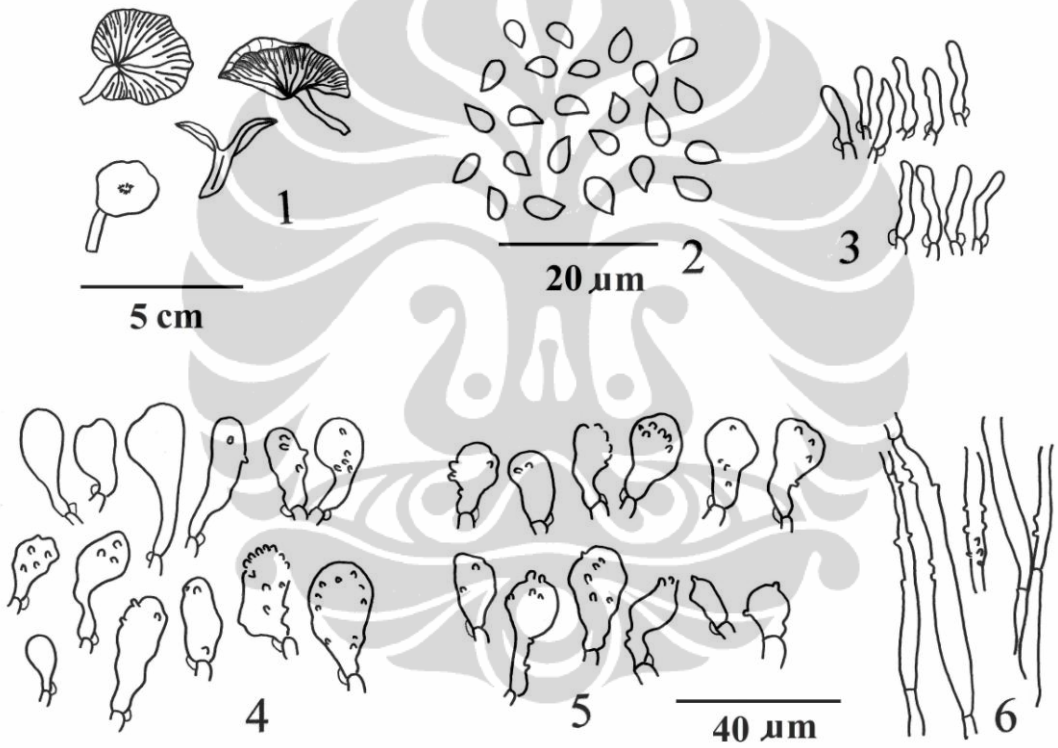

Figure 1.32. *Marasmiellus umbilicatus* Singer (A. Retnowati 743) 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis; 6. Hyphae of stipe with diverticulates; number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

polymorphic, ranging from cylindrical to clavate, or fusoid, covered by numerous diverticulate, thin-walled, hyaline. Pleurocystidia absent. Pileipellis composed of *Rameales*-structure with clavate to fusoid tips, main body 12–45.6 x 8.0–19.2 µm, with diverticulate, slightly incrusted to incrusted, thin to thick-walled up to

0.8 µm, hyaline, inamyloid, dextrinoid on incrusted part only. Pileal trama interwoven; hyphae 1.2–13.6 µm, thin to thick-walled up to 1.6 µm, hyaline. Stipe tissue monomitic; hyphae 2.4–10.4 µm, with or without diverticulate, hyaline, thin to thick-walled up to 2.4 µm, inamyloid to weakly dextrinoid. Stipe vesture absent. Clamp connections present.

Habit, habitat, and distribution. Scattered to gregarious on decaying wood. Indonesia (Java and Bali) and Colombia (Valle).

Specimens examined. Indonesia: Bali, Eka Karya Botanical Garden, on rotten wood, 16 January 1998, K.P. Collins 98–35; same location, on wood, 14 January 2000, A. Retnowati 180; Tamblingan trail, south side of Lake Tamblingan, near Bedugul, on decaying wood, 18 January 1998, K.P. Collins 98–44; Java, West Java, Cibodas Botanical Garden, trail to Mount Gede, on wood, 22 January 1999, A. Retnowati 150; same location, montane rain forest, 1430–1500 m asl, on decaying bark of *Castanopsis*, 10 January 2000, E. Horak 8386; Java, West, Mount Halimun-Salak National Park, Cidahu, Pameungpeuk trail, Plot Ecology-LIPI, on dicot wood, 8 May 2010, A. Retnowati 743.

Notes. The species is similar to *M. iguazuensis* which was described by Singer from Argentina (Singer 1973) and *M. eburneus* (Theissen) Singer described from Rio Grande, Brazil (Singer 1973). *Marasmiellus iguazuensis* Singer differs in having stipe vestures, and the latter species differs from the Indonesian species in having smaller basidiome. Another species which is comparable to the Indonesian specimen is *M. stenosporus* Singer (Singer 1973). Both are having *Rotalis*-type of cheilocystidia.

24. ***Marasmiellus nanus*** (Massee) Dennis, Kew Bull, Addit. Ser. 3: 33. 1970. Figure 1.33 and 1.34.

Syn. : *Marasmius nanus* Masse, Journ. Bot. 30: 161. 1892.

Type : St. Vincent, Morne Cochon, on bark of fallen trees, 7 May 1892, Elliot (K).

Pileus 9–50 mm diam, convex firstly, broadly convex in age to infundibuliform, shiny, especially in age, translucent striate to umbilicate, hygrophanous, glabrous, margin uplifted, crisped; surface moist to dry, dull, smooth to wrinkled, light brown overall, sometimes off-white at the edged to cream with brownish/pink to reddish brown (8–E4) at center when young, turning brownish pink entirely upon bruising. Context moderately thick to thick up to 1.5 mm, concolorous with pileus. Lamellae adnate to adnexed or subdecurrent, subdistant (16–19), with 2–3 series of lamellulae, moderately broad, about 5 mm wide, widest toward apex, smooth, non-marginate to slightly orangish brown margin, color same as cap and with same bruising reaction. Stipe 10–39 x 1–4 mm, equal but swollen a little at base, central, solid in young specimen, become

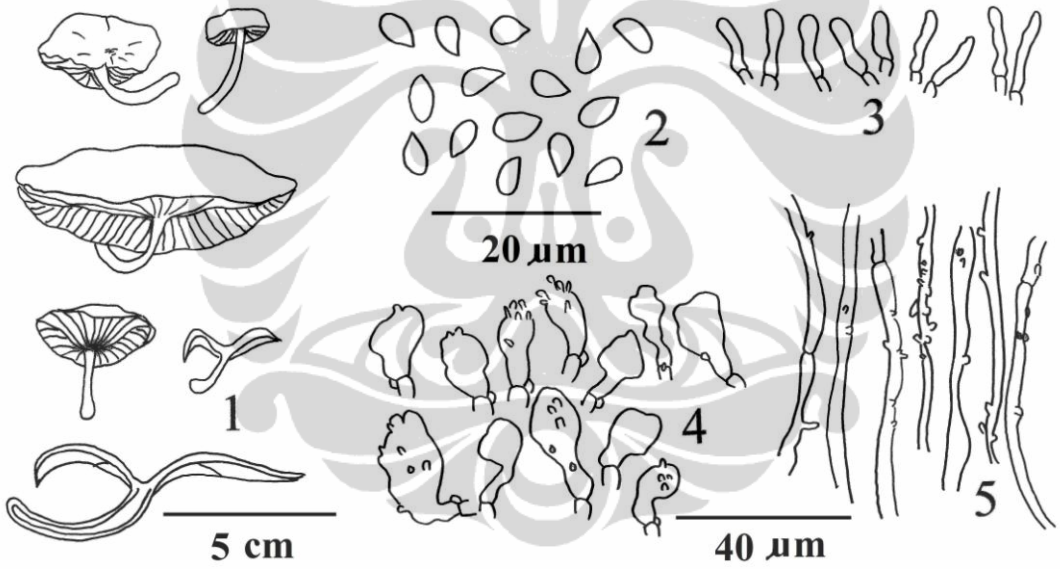

Figure 1.33. *Marasmiellus nanus* (Massee) Dennis (A. Retnowati 268); 1. Basidiomes; 2. Basidiospores; 3. Basidioles; 4. Pileipellis; 5. Hyphae of stipe with diverticulate; number 3, 4, and 5 use the same scale (scale bar = 40 µm).

hollow in old specimen, non-insititious, usually curved; shiny, striated longitudinally, off-white to light brown overall, orangish brown to reddish brown; white base tomentose. Odor and taste indistinctive. Basidiospores 4.8–6.4 x 2.4–4.0 µm ($\overline{x}$ = 5.49±0.5 x 2.86± 0.4, Q = 1.5–2.05, q = 1.95 ± 0.1; n = 25 spores per 1 specimen), ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 11.2–

20 x 4.0–5.6 µm, clavate, 4-spored. Basidioles fusoid to clavate. Cheilocystidia absent. Pleurocystidia absent. Pileipellis composed of *Rameales*-structures; tips of the hyphae composed of fusoid to clavate or irregular shaped, 16.8–32 x 8.8–13.6 µm, diverticulate, thin-walled, hyaline to yellowish brown, some incrusted, particular on cell-walled, inamyloid; diverticulate 1.6–5.6 x 1.6 µm, conical, thin-walled, hyaline. Pileus trama interwoven; hyphae 3.2–11.2 µm, thin-walled, hyaline, incrusted, inamyloid. Stipe tissue monomitic; hyphae 2.4–11.2 µm, with diverticulate, thin to thick-walled up to 1.6 µm, hyaline, inamyloid. Stipe vesture absent. Clamp connections present.

Habit, habitat, and distribution. Scattered on litter or gregarious on living tree (bark of living tree). Indonesia (Java) and St. Vincent.

Specimens examined. Indonesia: Java, West, Cibodas Botanical Garden, on litter, 9 January 1998, K.P. Collins 98–8; same location, on wood, 13 February 2000, A. Retnowati 207; Bogor Botanical Garden, on bark of living tree, 5 May 2000, A. Retnowati 268.

Notes. This species is similar to *M. incarnatipallens* (Singer 1973), and it differs in having smaller basidiocarp (2 mm broad) and presence of stipe vesture.

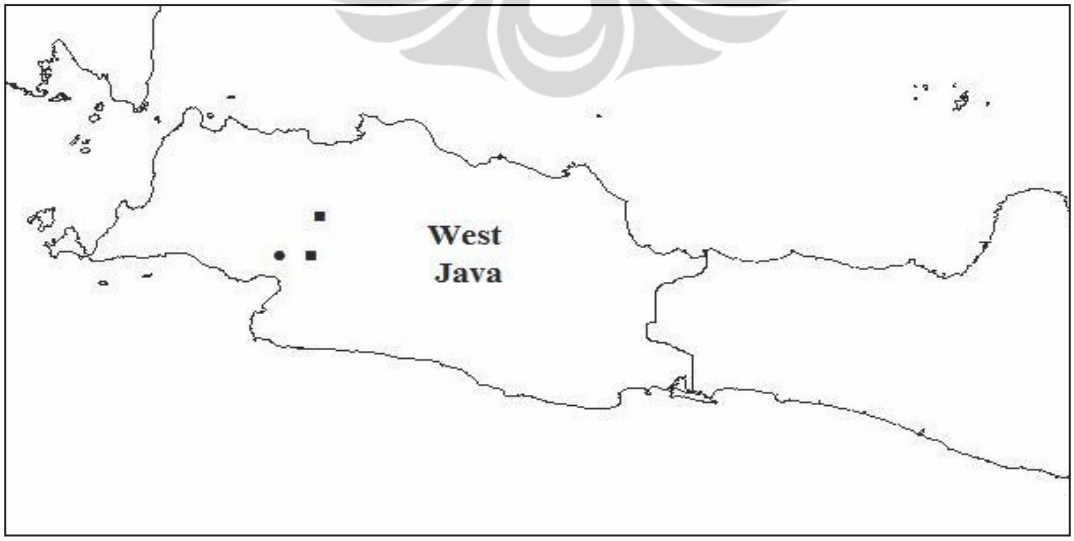

Figure 1.34. Distribution of *M. pruinosus* (●) and *M. nanus* (■) in Java.

Section *Candidi* (Bat.) Singer, Sydowia 15: 58. 1961.

Three species of *Marasmiellus* in Java and Bali were members of section *Candidi*, namely *M. subnigricans, M. setulosipes*, and *M. albofuscus*. Descriptions of species were presented below:

25. ***Marasmiellus subnigricans*** (Murrill) Singer, Beih. Nova Hedwig. 44: 26. 1973. Figure 1.35 and 1.37.

Syn. : *Marasmius subnigricans* Murrill, Bull. Torr. Bot. Cl. 67: 152. 1940.

Type : USA, Florida, Alachua Co., Gainesville, on small stems and twigs, 1–7–1938, Murrill (FLAS).

Pileus 11–45 mm diam, convex-umbonate to plano-convex, with or without a small umbo, often deeply with uplifted margin in age, wavy, sulcate to disc, moist, hygrophanous, subtranslucent to strong translucent, rubbery-membranous, dull, glabrous, wrinkle; disc brown (6–7E6–8), elsewhere dingy cream (4A2–3) to beige, staining in spots brown to reddish brown (7–8E7–8) or pure white to off white. Context thin to thick, light brown. Lamellae adnate to adnexed, subdistant (7–14) to distant (10–15) with 1–2 series of lamellulae, broad (–6 mm), convex, dingy cream to pinkish buff (5A3), non-marginate, spotted reddish brown as in pileus, white. Stipe 13–50 x 1–3 mm, equal, central to slightly eccentric, tough, hollow, striate, pruinose overall, smooth, dry, subinsititious to not insititious, off white to brown (7E4–6) above, base darker brown (7F4–6), white base tomentose present. Odor and taste indistinctive.

Basidiospores (12.8)13.6–17.6(18.40) x 4.0–5.6 µm ($\overline{x}$ = 15.08±1.0 x 4.86±0.6, Q = 2.4–4.5, $\overline{q}$ = 3.15 ± 0.3; n = 25 spores per 1 specimen), cylindrical, smooth, hyaline, inamyloid, thin-walled. Basidia 24–32 x 8.0–8.8 µm, long, clavate, 4-spored. Basidioles clavate. Cheilocystidia common, main body 36.8–84 x 4.0–15.2 µm, clavate to cylindrical cells, thin-walled, hyaline, raise clearly at the edge of lamellae. Pleurocystidia absent. Pileipellis cutis; hyphae 5.6–8.0 µm, main body of tips of hyphae 1.6–5.6x 6.4–11.2 µm, clavate to subglobose or

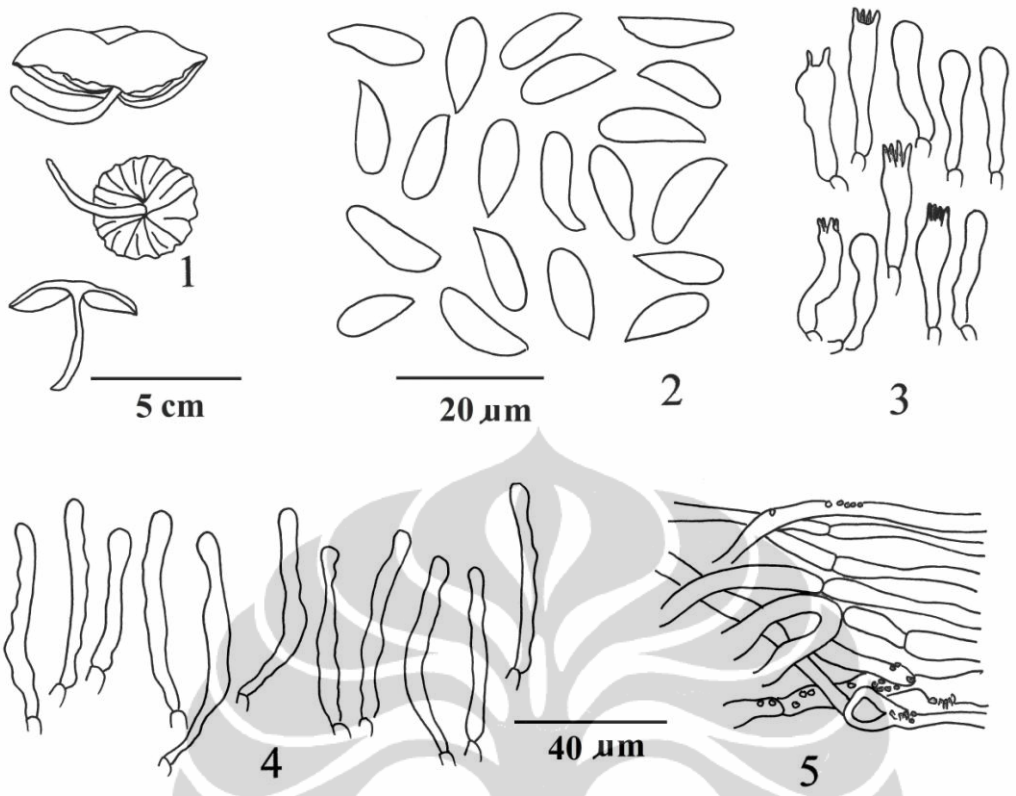

Figure 1.35. *Marasmiellus subnigricans* (Murrill) Singer (A. Retnowati 333); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis; number 3, 4, and 5 use the same scale (scale bar = 40 µm).

irregular shaped, with or without diverticulate, incrusted or not incrusted, thin-walled, hyaline, inamyloid. Pileus trama interwoven, hyphae 3.2–16 µm, thin-walled, some incrusted, hyaline, inamyloid. Stipe tissue monomitic; hyphae 1.6–9.6 µm, parallel, cylindrical, thin-walled, with diverticulate, hyaline to yellowish brown, inamyloid. Stipe vesture absent. Clamp connections present.

Habit, habitat, and distribution. Gregarious on rotten dicot wood or twigs in Botanical Garden. Indonesia (Java and Bali), USA, and Argentina.

Specimens examined. Indonesia: Bali, east of Lake Bratan, south ridge of Mountain Catur, on wood, 17 January 1999, A. Retnowati 142; Java, Bogor Botanical Garden, on rotten undetermined wood, 8 January 2000, D.E. Desjardin 7074; Java, Cikaniki, loop trail Perth Zoo, Mount Halimun National Park, on dicot

twigs, 9 January 2001, A. Retnowati 333; Java, West, Ujung Kulon National Park, southern part of Mount Honje, trail to Jago Besar (ca. 100 m asl), dicot wood, 16 June 2008, A. Retnowati 609.

Notes. *Marasmiellus subnigricans* is characterized by large basidiospores and the presence of clavate cheilocystidia. This species was also reported from Argentina (Singer 1973).

26. ***Marasmiellus setulosipes*** (Murrill) Dennis, Kew Bull. Ser. 3: 33. 1970. Figure 1.36 and 1.37.

Syn. : *Marasmius setulosipes* Murrill

Type : -

Pileus 5–10 mm diam, plano-convex with umbilicate disc, dry, hygrophanous, strongly striate, glabrous, smooth; greyish white, with dark grey at disc. Context narrow, white to greyish white. Lamellae adnate to slightly subdecurrent; distant (8–9), with 1 series of lamellulae, pure white to greyish white; narrow up to 1.5 mm. Stipe 4–8 x 0.5 mm, mostly with short stipe, central to eccentric, equal above to base, smooth, glabrous. Odor and taste indistinctive. Basidiospores have 2 sizes: 4.8–5.6 x 2.4–3.2 µm ($\bar{x}$ = 4.83 ± 0.16 x 3.04 ± 0.33, Q = 1.5–2.00, $\bar{q}$ = 1.61 ± 0.21) and and 6.4–7.2 x 3.2–4.0 µm ($\bar{x}$ = 6.69 ± 0.39 x 3.62 ± 0.41, Q = 1.60–2.25, $\bar{q}$ = 1.87 ± 0.25), n = 25 spores per 1 specimen, ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 16–20 x 3.2–4.8 µm, clavate, 4-spored. Basidioles clavate. Hymenial cystidia common; main body 28–68 x 8–14.4 µm, fusoid to clavate, or broadly clavate, with a very few and short diverticulate, thin-walled, hyaline. Pileipellis composed of *Rameales*-structure; hyphae 2.4–6.4 µm, thin-walled, hyaline to yellowish brown, with a very thin incrusted, inamyloid. Stipe tissue monomitic; hyphae 2.4–3.2 µm, cylindrical, parallel, thin-walled, hyaline, inamyloid. Stipe vesture absent. Clamp connections rare.

Habit, habitat, and distribution. Gregarious on wood. Indonesia (Java).

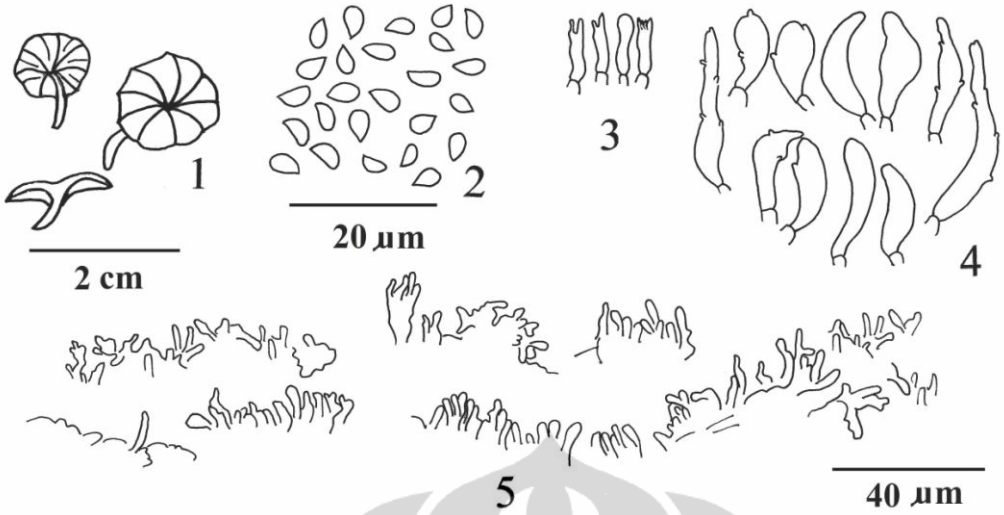

Figure 1.36. *Marasmiellus setulosipes* (Murrill) Singer (A. Retnowati 065); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Hymenial cystidia; 5. Pileipellis; number 3, 4, and 5 use the same scale (scale bar = 40 µm).

Specimen examined. Indonesia: Java, Cibodas Botanical Garden, on wood, 29 December 1998, A. Retnowati 065.

Notes. The presence of fusoid to clavate, or broadly clavate hymenial cystidia, with a very few and short diverticulate shape is one of particular character of *M. setulosipes*. Other characters to identify this species are small size of basidiospores and slightly incrusted tissue of pileipellis.

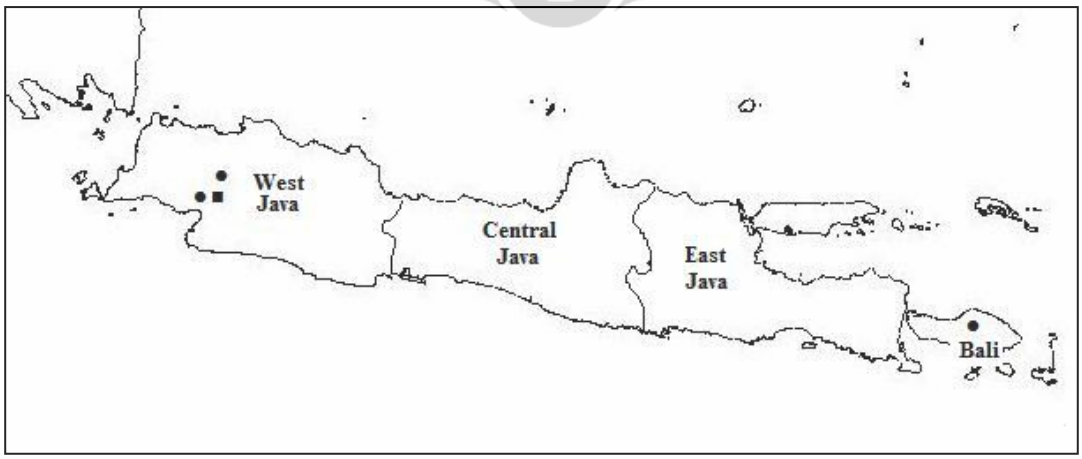

Figure 1.37. Distibution of *M. subnigricans* (●) and *M. setulosipes* (■) in Java and Bali.

27. *Marasmiellus albofuscus* (Berk. & Curt.) Singer Beih. Nova. Hedwig. 44: 24. 1973. Figure 1.38 and 1.39.

Syn. : *Marasmius albofuscus* Berk. & Curt.

Type : Cuba, on logs or woods, June, Wright 87 (K).

Pileus 4–8 mm diam, campanulate to convex, with slightly depressed disc, hygrophanous, sulcate; margin incurved; surface glabrous, dry, dull; off white. Context moderately thick, cream. Lamellae adnate, subdistant, with 0 series of lamellulae, moderately broad, off white. Stipe 4–8 x 1 mm, central, insititious, cylindrical, dull, dry, glabrous, off-white. Odor and taste indistinctive. Basidiospores (12)12.8–13.6(14.4) x 4.8–5.6(6.4) µm ($\overline{x}$ = 13.12 ± 0.65 x 4.99 ± 0.53, Q = 1.88–3.40, $\overline{q}$= 2.66 ± 0.31) n = 25 spores per 1 specimen, cylindrical, smooth, hyaline, inamyloid, thin-walled. Basidia unobserved.

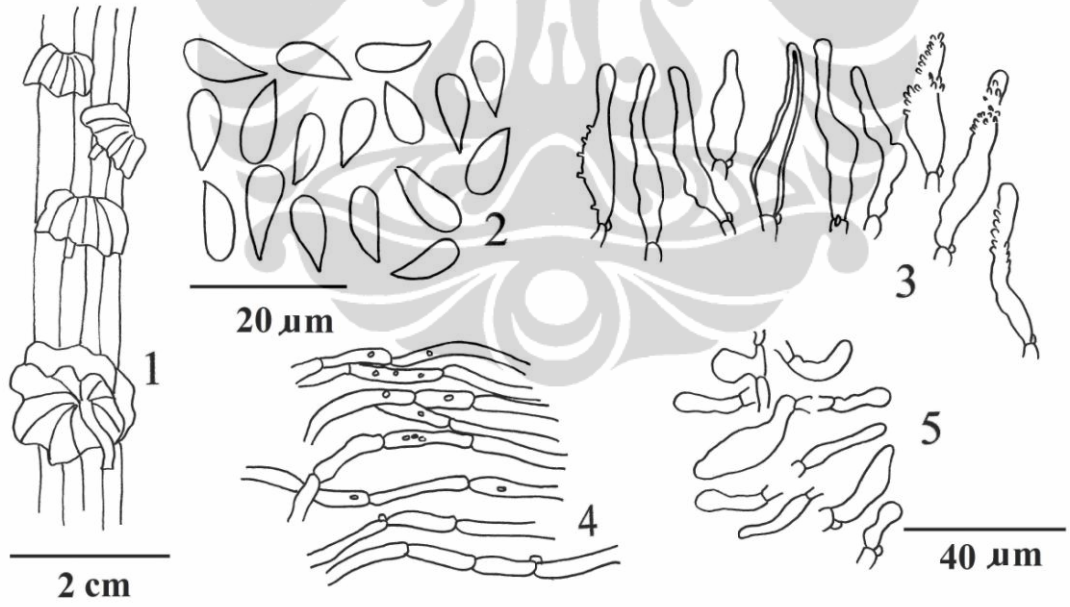

Figure 1.38. *Marasmiellus albofuscus* (Berk. & Curt.) Singer (A. Retnowati 817); 1. Basidiomes; 2. Basidiospores; 3. Cheilocystidia; 4. Pileipellis; 5. Stipe vesture; number 3, 4, and 5 use the same scale (scale bar = 40 µm).

Basidioles clavate. Cheilocystidia common, composed of clavate to cylindrical or lageniform cells, with diverticulate at the half apex; main body 37.6–68.8 x 7.2–12 µm, thin to thick-walled up to 0.8 µm, arise from deep of hymenium, hyaline. Pleurocystidia absent. Pileipellis cutis, with diverticulate; hyphae 3.2–8.8 µm, thin to thick walled, hyaline, not incrusted, inamyloid. Pileal trama interwoven; hyphae 3.2–9.6 µm, thin walled, inamyloid. Stipe tissue monomitic; hyphae 4.0–6.4 µm, thin-walled, hyaline, inamyloid. Stipe vesture uncommon; main body 20–44 x 6.4–12 µm, thin-walled, hyaline, inamyloid. Clamp connections present.

Habit, habitat, and distribution. Gregarious on monocotyledon. Indonesia (Java) and Cuba.Specimen examined. Indonesia: Java,West, Bogor Botanical Garden, on monocotyledon, 1 December 2010, A. Retnowati 817.

Notes. *Marasmiellus albofuscus* is characterized by large basidiospores ((12)12.8–13.6(14.4) x 4.8–5.6(6.4) µm) and the presence of clavate to cylindrical or lageniform cheilocystidia. The species is similar to *M. coilobasis* (Berk.) Singer (Singer 1973), and it differs in having larger basidiospores (11.5–19 x 4–7.8(8.2) µm or (17)19–25(27.5) x 5.5–6.2 µm) and longer cheilocystidia (44–100 µm) (Singer 1973).

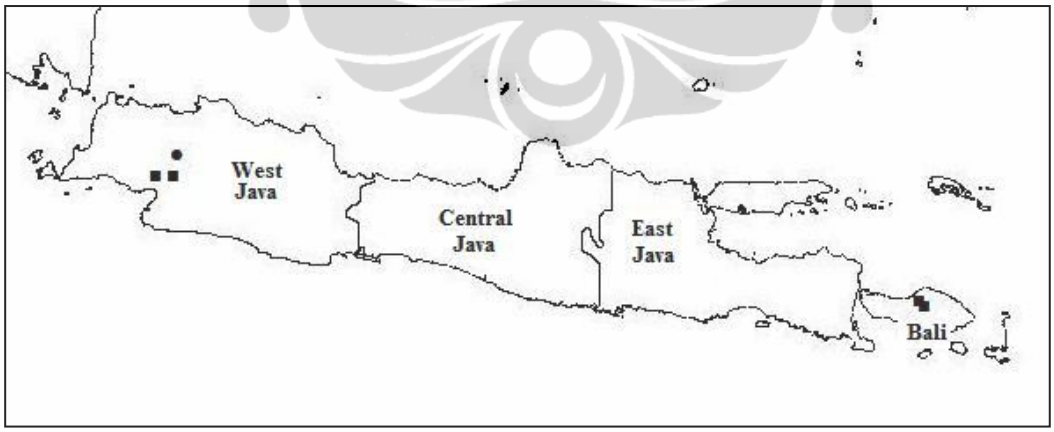

Figure 1.39. Distribution of *M. umbilicatus* (■) and *M. albofuscus* (●) in Java and Bali.

Section *Dealbati* Singer, Beih. Nova Hedwig. 44: 33. 1973.

Seven species of *Marasmiellus* in Java and Bali were members of section *Dealbati*, namely *M. cikanikiensis, M. desjardinii, M. cibodasensis, M*. cf. *stenophyllus, M. hirtellus, M. javanicus*, and *M*. aff. *hirtellus*. The descriptions of species were presented below:

28. *Marasmiellus cikanikiensis* Retnowati sp. nov. Figure 1.40 and 1.44.
Type : Indonesia, Java, West, Mount Halimun National Park, road from Cikaniki to Bogor, on dicot wood, 11 January 2001, A.W. Wilson 79 (Holotype: BO).

Pileus 19 mm diam, circular, applanate to plano convex depressed, glabrous, smooth, moist, margin translucent striate, hygrophanous forming white, entire to undulate-crenate, creamy white in color (3–4 A 3–3) to white in younger bodies. Context thick, off-white. Lamellae adnate to slightly decurrent, 1.5–2 mm deep, segmentiform, distant, rugulose, forked, several series of lamellulae, white to some color as pileus. Stipe 3–13 x 0.5–1 mm, cylindrical, pliant, floccose, solid, white to some color as pileus, inserted into twigs, elevated above growing. Odor and taste indistinctive.

Basidiospores 8.0–8.8 x (3.2)4.0(4.8) µm (only 16 basidiospores observed), ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 20.8–24 x 5.6–6.4 µm, clavate, 4-spored. Basidioles clavate. Cheilocystidia absent. Pleurocystidia absent. Pileipellis cutis; hyphae 4.0–10.4 µm, with diverticulate, not-incrusted, thin-walled, hyaline to yellowish white, inamyloid. Pileus trama interwoven; hyphae 4.0–12 µm, thin-walled, inamyloid. Stipe tissue monomitic; hyphae 4.0–16 µm, cylindrical, parallel, thin-walled, with diverticulate, inamyloid. Stipe vesture common at the above part of stipe, main body 16–40 x 4.0–8.8 µm, clavate with capitate or irregular shaped, thin-walled, hyaline. Clamp connections present.

Habit, habitat, and distribution. Gregarious on dicot wood. Indonesia (Java).

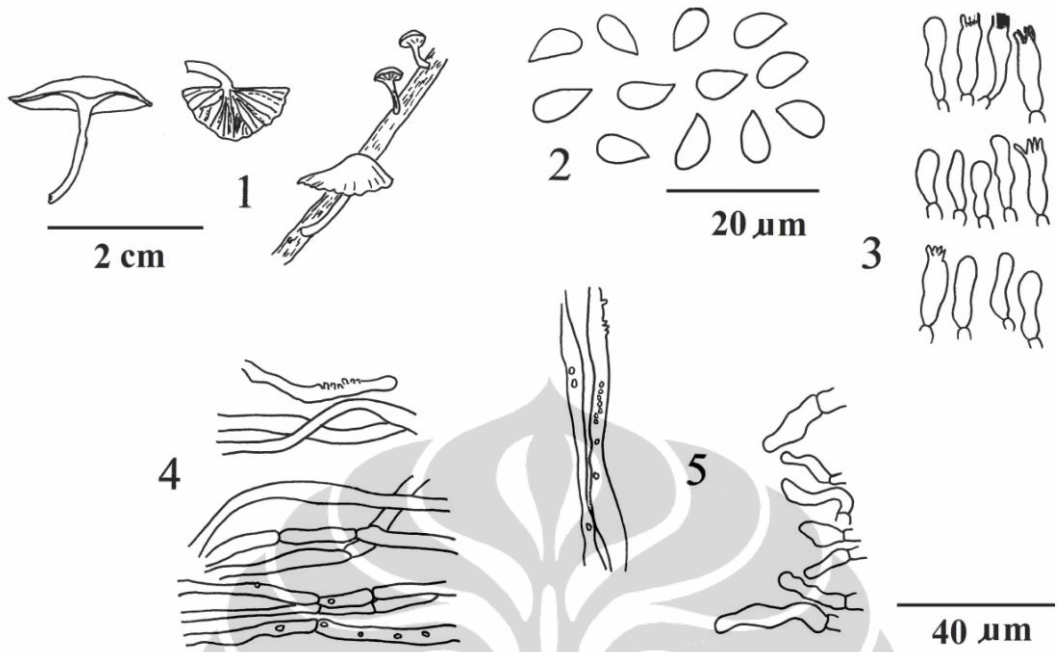

Figure 1.40. *Marasmiellus cikanikiensis* Retnowati (A.W. Wilson 79); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Pileipellis; 5. Hyphae of stipe with diverticulates and stipe vesture; number 3, 4, and 5 use the same scale (scale bar = 40 µm).

Specimen examined. Indonesia: Java, West, Mount Halimun National Park, road from Cikaniki to Bogor, on dicot wood, 11 January 2001, A.W. Wilson 79.

Etymology: *cikanikiensis* refers to the type locality of the species.

Notes. *Marasmiellus cikanikiensis* is similar to *M. dealbatus* (Berk. & Curtis) Singer (Singer 1973) and *M. stenophyllus* (Mont.) Singer (Singer 1973). *Marasmiellus dealbatus* which was described from Cuba is graminicolous agarics. It is principally grow on dead leaves, culms, and roots of gramineae (Singer 1973). The later species is lignicolous agarics which grow on wood or twigs of dicotyledonae, and it differs with *M. cikanikiensis* having cylindrical cheilocystidia.

29. ***Marasmiellus desjardinii*** Retnowati sp. nov. Figure 1.41 and 1.44. Type : Indonesia, Java, West, north slope of Mount Salak, Curug Nangka, on sticks, 11 January 2000, A.W. Wilson 28.

Pileus 1–10 mm diam, circular to convex when young, becoming plano-convex in age with depressed center, hygrophanous; margin straight to incurved, then upturned in age, crenate, occasionally umbilicate, crenate to wavy, translucent striate; surface smooth, moist, radially rivulose, glabrous; white (1A1) to pale orange/flesh (4–5 A2–4), turning yellow (2A2). Context thin, concolorous with pileus. Lamellae adnate, arcuate, subdistant (15) with 1–3 series of lamellulae, narrow up to 1 mm, entire; white (1A1) to salmon (6A4–5) (concolorous with pileus). Stipe 2–5 x 0.3–1 mm, equal, reduced, central to slightly eccentric, terete, curved, hollow to solid, subinsititious to insititious with a small pad at the base, smooth, glabrous, mostly with short stalk, pure white (1A1) to pale orange/flesh (4–5A2–4). Odor indistinctive. Taste slightly sweet.

Basidiospores 6.4–8.0(8.8) x 3.2–4.0 µm ($\bar{x}$ = 7.6±0.6 x 3.28± 0, Q = 1.8–2.8, $\bar{q}$ = 2.28 ± 0.2, n = 25 spores per 1 specimen), ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 13.6–24 x 4.6–6.4 µm, clavate, 4-spored. Basidioles fusoid to clavate. Cheilocysidia absent. Pleurocystidia absent. Pileipellis cutis with diverticulate; hyphae 2.4–4.8 µm, not incrusted to slightly incrusted, thin-walled, hyaline, inamyloid. Pileal trama interwoven; hyphae 4.0–7.2 µm, thin-walled, inamyloid. Stipe tissue monomitic; hyphae 1.6–13.6 µm, thin-walled, parallel, cylindrical, hyaline, a few with incrusted wall, diverticulate absent to a few, weakly dextrinoid to inamyloid. Stipe vesture common; 9.6–40 x 4.0–9.6 µm, clavate to fusoid, cylindrical, or irregular shaped, thin-walled, hyaline, inamyloid. Clamp connections present.

Habit, habitat, and distribution. Gregarious on twigs, leaves, or sticks of undetermined dicot leaves under *Castanopsis javanica*. Indonesia (Java).

Specimens examined. Indonesia: Java, West, Cibodas Botanical Garden, trail to Mount Gede, on twigs of undetermined dicot, 8 January 1998, D.E. Desjardin

6722; same location, on twigs or dicot leaves, 2 January 1999, A. Retnowati 076; Java, West, north slope of Mount Salak, Curug Nangka, on sticks, 11 January 2000, A.W. Wilson 28.

Etymology: *desjardinii* is an honour to the Prof. Dr. Dennis E. Desjardin who has given much supports for the author to study Agaricales.

Notes. *Marasmiellus desjardinii* is closely related to *M. dealbatus* (Berk. & Curtis) Singer described from Cuba (Singer 19873). *Marasmiellus dealbatus* differs in forming not orange pileus and longer stipe (up to 20 mm) and the presence of cheilocystidia. *Marasmiellus dealbatus* (Berk. & Curtis) Singer is recognized as pathogen species which attacks *Saccharum officinale* (Singer 1973).

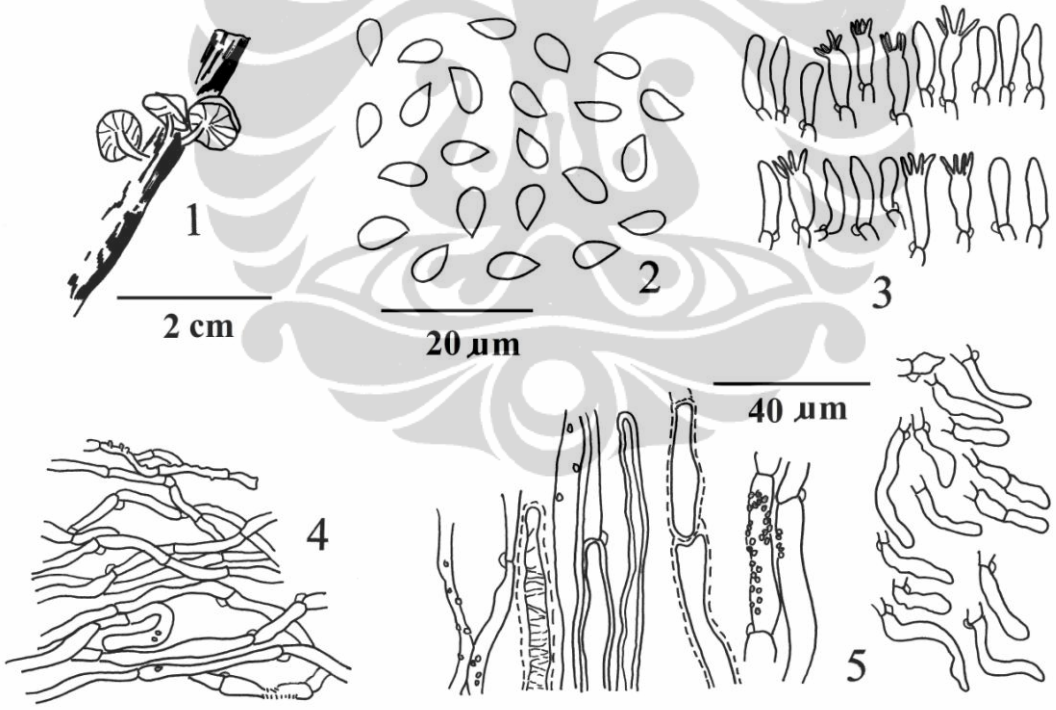

Figure 1.41. *Marasmiellus desjardinii* Retnowati (A.W. Wilson 28); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Pileipellis; 5. Hyphae of stipe with diverticulate and stipe vesture; number 3, 4, and 5 use the same scale (scale bar = 40 µm).

30. ***Marasmiellus cibodasensis*** Retnowati sp. nov. Figure 1.42 and 1.47.
Type : Indonesia, Java, Cibodas Botanical Garden, on wood, 16 April 2000, A. Retnowati 246 (Holotype: BO).

Pileus 4–10 mm diam, convex with flattened center or slightly umbilicus, sulcate, non-hygrophanous, translucent-striate; margin crenate, incurved; surface pruinose, smooth to slightly wrinkled, dry; light brown (6–DA) when young, then become dark brown (6–F8). Context thick up to 1 mm, off-white. Lamellae adnate, subdistant (12–16), with 1 series of lamellulae, narrow, non-marginate; light brown (6–D4). Stipe 2.5–8 x 0.5–1 mm, cylindrical, slightly tappered at the apex, central, insititious, a small bulb at the base, smooth, white granulose mostly at the upper part; no-rhizomorph association. Odor and taste indistinctive.

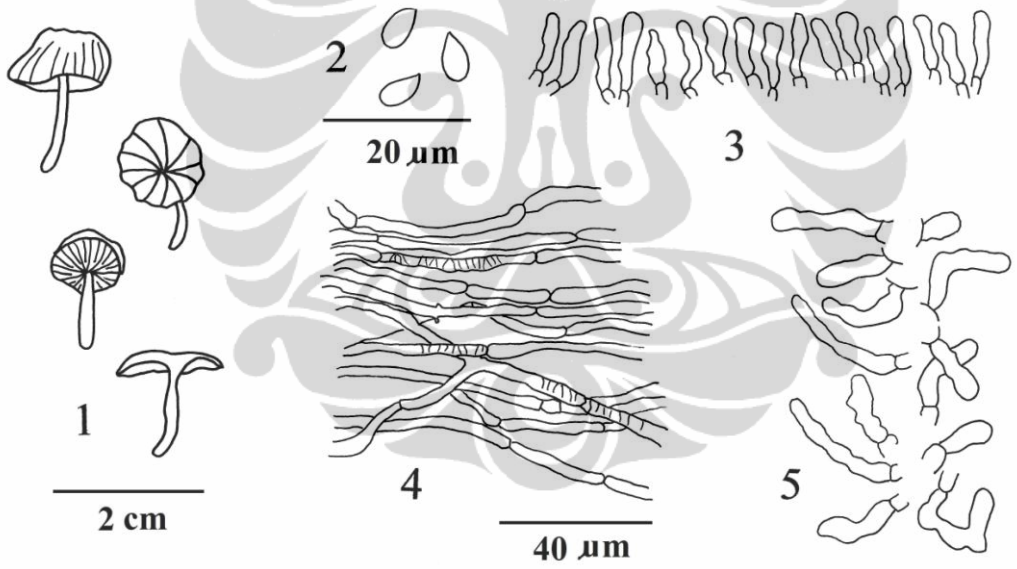

Figure 1.42. *Marasmiellus cibodasensis* Retnowati (A. Retnowati 246); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Pileipellis; 5. Stipe vesture; number 3, 4, and 5 use the same scale (scale bar = 40 µm).

Basidiospores 7.2–8.0 x 4.0 µm (3 basidiospores observed), ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia unobserved. Basidioles fusoid to clavate. Cheilocystidia absent. Pleurocystidia absent. Pileipellis cutis; hyphae 3.2–9.6 µm, incrusted, with a few diverticulate, thin to thick-walled, hyaline,

inamyloid. Pileal trama cutis; hyphae 2.4–5.6 µm, parallel, incrusted, with a few diverticulate, thin to thick-walled up to 0.8 µm, inamyloid. Stipe tissue monomitic; hyphae 2.4–11.2 µm, parallel, thin to thick-walled up to 1.6 µm, hyaline, some incrusted particularly on cell walled, inamyloid. Stipe vesture common; main body 18.4–62.4 x 4.8–8.0 µm, fusoid to clavate, thin-walled, hyaline. Clamp connections present.

Habit, habitat, and distribution. Gregarious on wood. Indonesia (Java).

Specimen examined. Indonesia: Java, Cibodas Botanical Garden, on wood, 16 April 2000, A. Retnowati 246.

Etymology: *cibodasensis* refers to the type locality of the species (Cibodas, West Java).

Notes. This species is similar to *M. hirtellus* (Berk. & Br.) Pegler which was described from Sri Lanka (Pegler 1977). *Marasmiellus hirtellus* differs from *M. cibodasensis* in having diverticulate cheilocystidia.

31. ***Marasmiellus*** cf. ***stenophyllus*** (Mont.) Singer, Sydowia 15: 58. 1961. Figure 1.43 and 1.44.

Syn. : *Marasmius stenophyllus* Mont., Ann. Sc. Nat. IV 1: 116. 1854.

Type : Frech Guyana, on bark, fallen twigs, Leprieur 1027, 1029 (PC, FH).

Pileus 4–9 mm diam, circular, rounded flabelliform, applanate/plano-convex to infundibuliform/deeply infundibuliform, rugulo-striate to smooth disc, glabrous, moist to dry, dull, suede-like, hygrophanous; margin entire, translucent-striate, straight; color dark brown (6F7–8) to brown (6E7–8) at center lightening gradually toward margin, outer millimeter of pileus turning light fleshy peach (5A2–3), upon drying color becoming more peach pink overall (6A2–3), off white with a small central to pinkish brown region or greyish brown (6B-C3) (DED). Context moderately thick, cream. Lamellae narrowly adnate to adnate, hairy,

arcuate, entire, densely crowded, 2–3 series of lamellulae, seldom forked, 0.2–0.5 mm deep; non-marginate; color white (1A1) to buff. Stipe 8–17 mm x 0.1–1.5 cm, cylindrical, central, round to sub-compressed, terete, equal, dry, solid, inserted into substrate, glabrous to minutely scaborous at top or pruinose overall; color milk chocolate brown (7D5–6) to fleshy white (4A1–2), apex white, base brownish grey (6C3). Odor and taste indistinctive.

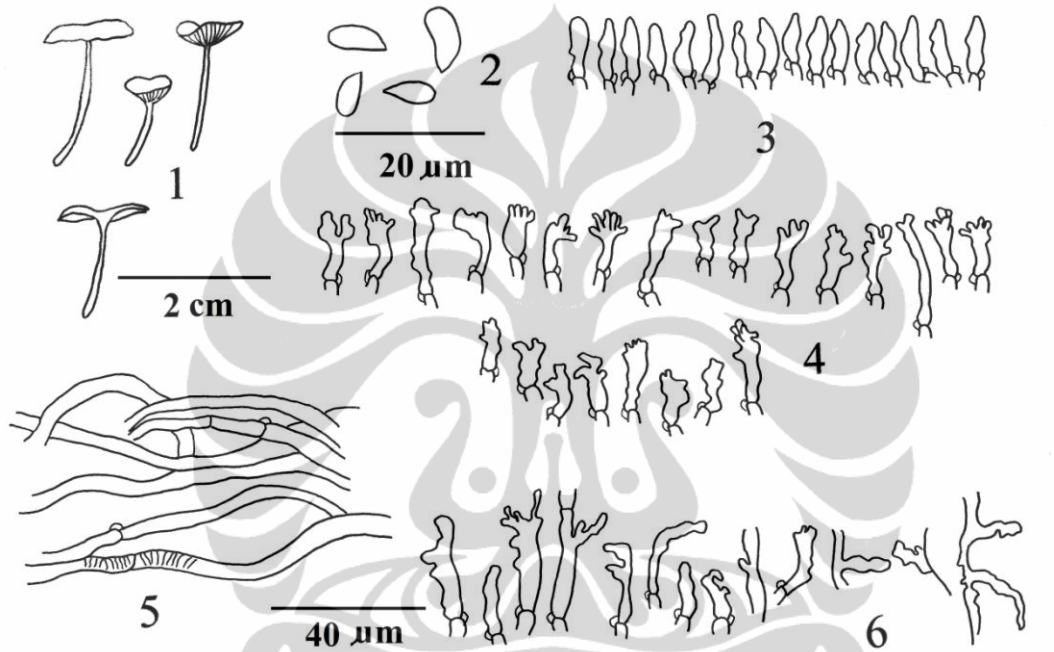

Figure 1.43. *Marasmiellus* cf. *stenophyllus* (Mont.) Singer (D.E. Desjardin 7065); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis; 6. Stipe vesture; number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

Basidiospores 6.4–9.6 x 3.2 µm (only 4 basidiospores observed), ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 12–17.6 x 4.8–6.4 µm, clavate, 4 spored. Basidioles fusoid to clavate. Cheilocystidia common, composed of *Siccus*-type broom cells; main body 12–34.4 x 4.8–8.0 µm, fusoid to clavate, or irregular shaped, with diverticulate, some forked, thin-walled, hyaline; diverticulate 2.4–4.8 x 2.4–4.0 µm, conical to clavate or cylindrical, thin-walled, hyaline. Pleurocystidia absent. Pileipellis cutis; hyphae 2.4–6.4 µm, incrusted,

thin-walled, inamyloid to weakly dextrinoid, hyaline. Pileus trama interwoven; hyphae 4.8–5.6 µm, thin-walled, inamyloid. Stipe tissue monomitic; hyphae 2.4–15.6 µm, parallel, cylindrical, thin to thick-walled up 0.8 µm, weakly incrusted, inamyloid. Stipe vesture common, cheilocystidia-like; main body 16–44 x 4.8–5.8 µm, fusoid to clavate, or irregular shaped, thin-walled, inamyloid, hyaline. Clamp connections present.

Habit, habitat and distribution. Found gregarious on various dicot leaves in botanical garden. Indonesia (Java), USA (Florida), French Guyana, and Argentina (Tucumán).

Specimens examined. Indonesia: Java, West Java, Bogor Botanical Garden, on various dicot leaves, 8 January 2000, D. E. Desjardin 7065, same location, on leaves, 9 January 2000, A.W. Wilson 18.

Notes. This species is closely related to *M. stenophyllus* (Mont.) Singer (Singer 1973). *Marasmiellus stenophyllus* differs in having bigger basidiocarp and sinuous-cylindrical cheilocystidia. Another species which is closely related to *M. stenophyllus* is *M. hirtellus* (Berk. & Br.) Pegler (Singer 1986). It differs from the Indonesian specimens in having sinuous caulocystidia.

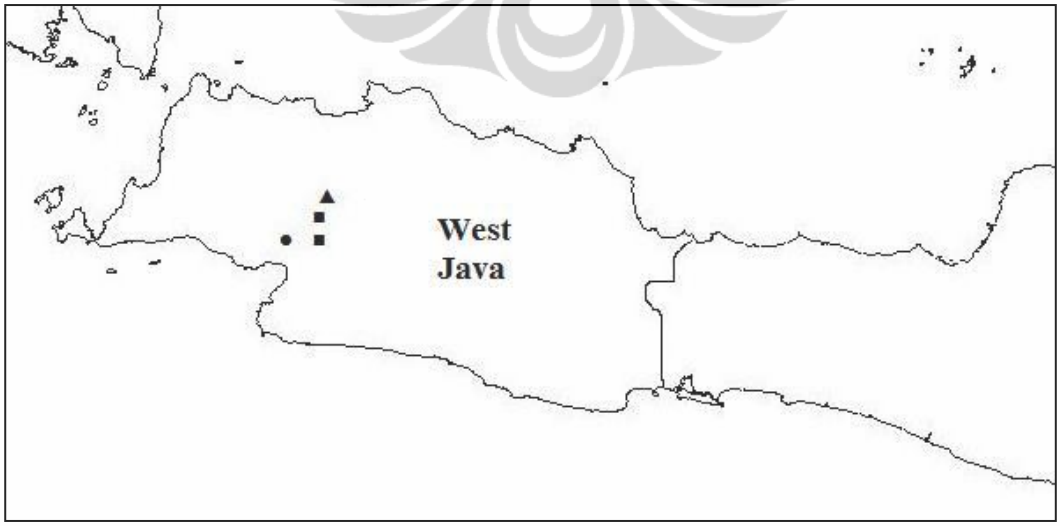

Figure 1.44. Distribution of *M. cikanikiensis* (●), *M. desjardinii* (■) and *M.* cf. *stenophyllus* (▲) in Java.

32. ***Marasmiellus hirtellus*** (Berk. & Br.) Pegler in Kew Bull. Addit. Ser. 6: 130. 1977. Figure 1.45 and 1.47.

Syn. : *Marasmius hirtellus* Berk. & Br. In Journ. Linn. Soc., Bot. 14: 39. 1873.

Type : Central Prov, Kandy District, Peradeniya, on dead herbaceous plants, Nov 1867, Thwaites 102p. p. (K).

Pileus 6–12 mm diam, applanate to plano-convex, glabrous, circular, moist, margin entire to eroded, translucent-striate, sulcate, becoming felted upon drying, brown at center (7E8) to beige at margin (4A2–3), hygrophanous. Context gelatinous-like, grey brown, ½ thick. Lamellae adnate, segmentiform, closed, multiple series of lamellulae, edge entire, 2 mm deep. Stipe 9–22 x 0.8–1 mm, cylindrical, glabrous becoming pruinose upon drying, solid, pliant, found gregarious inserted into leaf, brown at base (7E–F8) lightening to beige/white at top (4A1–2). Odor and taste indistinctive.

Basidiospores 8.0–9.6(10.4) x (2.4)3.2–4.0 µm ($\bar{x}$ = 9.02 ± 0.67 x 3.26 ± 0.32, Q = 2.00–3.33, $\bar{q}$ = 2.79 ± 0.29) n = 25 spores per 1 specimen, cylindrical, smooth, hyaline, inamyloid, thin-walled. Basidia unobserved. Basidioles fusoid to clavate. Cheilocystidia common; main body 12–32 x 8.8–2.4 µm, clavate to broadly clavate, subglobose, globose or irregular in shaped, with diverticulate, thin-walled, hyaline; diverticulate 2.4–8.0 x 0.8–2.4 µm, obtuse, conical to clavate, thin-walled, hyaline. Pleurocystidia absent. Pileipellis cutis; hyphae 5.6–8.8 µm, incrusted, hyaline, dextrinoid at cell walled, other tissue inamyloid. Pileal trama interwoven; hyphae 2.4–8.8 µm, thin-walled, hyaline, inamyloid. Stipe tissue monomitic; hyphae 2.4–10.4 µm, cylindrical, parallel, with a few diverticulate, thin to thick-walled up to 0.8 µm, inamyloid. Stipe vesture common; main body 19.2–48 x 8.0–12 µm, clavate to broadly clavate, thin walled, hyaline, inamyloid. Clamp connections present.

Habit, habitat, and distribution. Gregarious on dicot leaf. Indonesia (Bali), Sri Lanka, and East Africa.

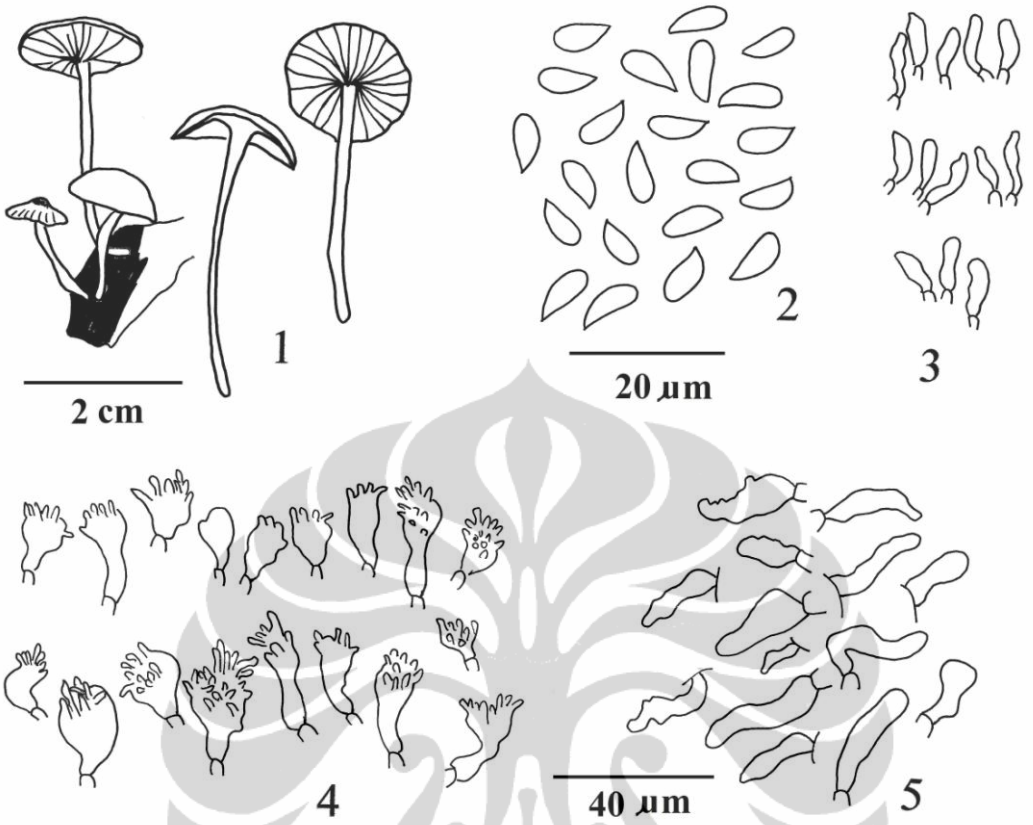

Figure 1.45. *Marasmiellus hirtellus* (Berk. & Br.) Pegler (A.W. Wilson 122); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Stipe vesture; number 3, 4, and 5 use the same scale (scale bar = 40 µm).

Specimen examined. Indonesia: Bali, Lake Tamblingan, on leaf, 20 January 2001, A.W. Wilson 122.

Notes. *Marasmiellus hirtellus* (Berk. & Broome) Pegler is pantropical species which has been reported from Sri Lanka (Pegler 1986) and East Africa (Pegler 1977). Morphologically *M. hirtellus* is very similar to *M. stenophyllus* (Berk. & Broome) Pegler (Pegler 1986). *Marasmiellus hirtellus* has no pale vinaceous-brown at the center, and it presents at *M. stenophyllus* (Berk. & Broome) Pegler.

33. ***Marasmiellus javanicus*** Retnowati sp. nov. Figure 1.46 and 1.47.

Type : Indonesia: Java, West, Bogor Botanical Gardens, on *Salacca zalacca*, 1 December 2010, A. Retnowati 811 (Holotype: BO).

Pileus 8–16 mm diam, convex with flattened disc, strongly hygrophanous; margin incurved; surface dull, dry, glabrous, off-white. Context unobserved. Lamellae adnate, subdistant, with 2–3 series of lamellulae, moderately broad, off-white. Stipe 3–5 x 0.1–0.2 mm, cylindrical, central, insititious, with a small bull at the base, white. Odor and taste indistinctive.

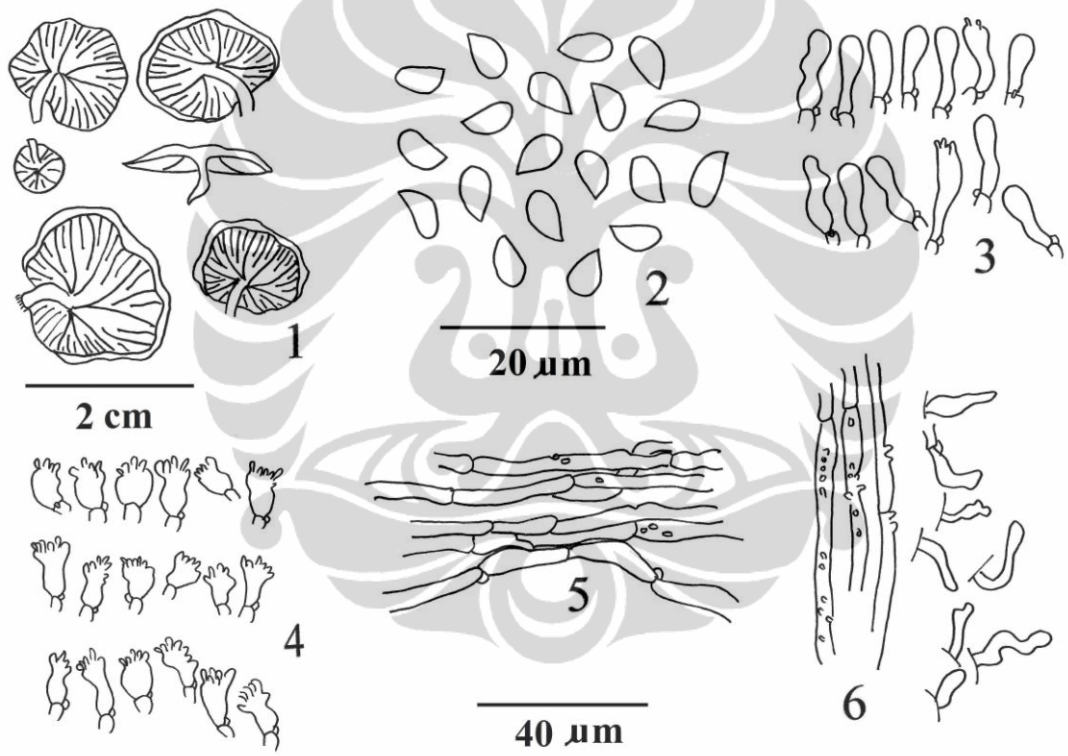

Figure 1.46. *Marasmiellus javanicus* Retnowati (A. Retnowati 811); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis; 6. Hyphae of stipe with diverticulate and stipe vesture; number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

Basidiospores 7.2–8.8(9.6–10.4) x 4.0–5.6 µm ($\bar{x}$ = 8.72 ± 0.8 x 4.99 ± 0.3, Q = 1.3–2.0, $\bar{q}$ = 1.75 ± 0.1) n = 25 spores per 1 specimen, ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 23.2–32 x 8.0–8.8 µm, clavate, 4-spored.

Basidioles clavate. Cheilocystidia common, composed of *Siccus*-type broom cells; main body 11.2–19.2 x 7.2–9.6 µm, clavate to broadly clavate, thin-walled, hyaline; diverticulate 1.6–3.2 x 1.6 µm, conical, obtuse, thin-walled, hyaline. Pleurocystidia absent. Pileipellis cutis with diverticulate; hyphae 4.8–10.4 µm, parallel, not incrusted, thin-walled, hyaline. Pileal trama interwoven; hyphae 2.4–8.8 µm, thin-walled, hyaline. Stipe tissue monomitic; hyphae 1.6–9.6 µm, cylindrical, parallel, hyaline, thin to thick-walled up to 0.8 µm, diverticulate, inamyloid. Stipe vesture uncommon; main body 24–24.8 x 6.4–7.2 µm, fusoid to clavate or irregular in shaped, thin-walled, hyaline. Clamp connections present.

Habit, habitat, and distribution. Solitary to gregarious on monocot wood (*Costus* sp., *Salacca* sp.). Indonesia (Java).

Etymology: *javanicus* refers to the island of Java where the species was collected.

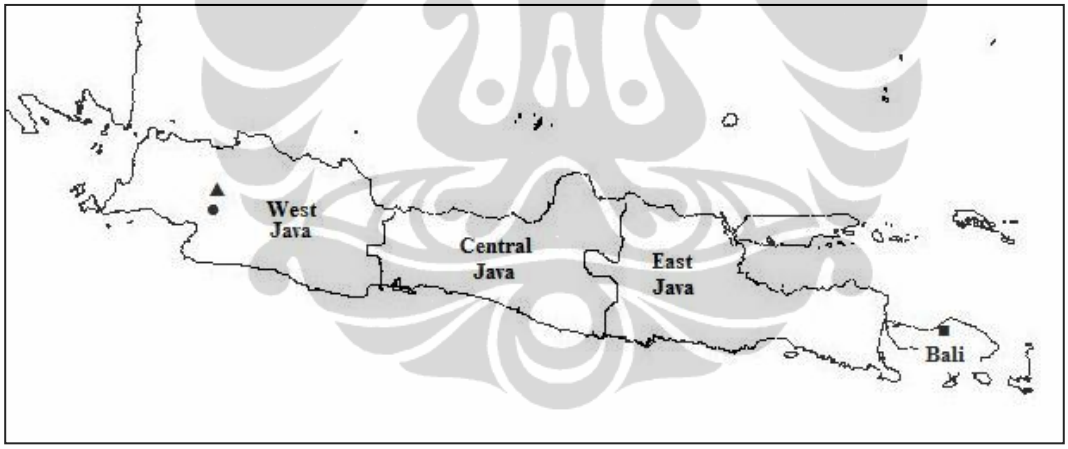

Figure 1.47. Distribution of *M. cibodasensis* (●), *M. hirtellus* (■), and *M. javanicus* (▲) in Java and Bali.

Specimens examined. Indonesia: Java, West, Bogor Botanical Gardens, on *Costus* sp., 1 December 2010, A. Retnowati 808; same location, on *Costus* sp., 1 December 2010, A. Retnowati 809-B; same location, on *Costus* sp., 1 December 2010, A. Retnowati 810; same location, on *Salacca* sp., 1 December 2010, A. Retnowati 811; same location, on *Salacca* sp., 1 December 2010, A. Retnowati 813.

Notes. This species grows on some different monocots plants, such as *Salacca zalacca* (*Arecaceae*) and *Costus dinklagii* (*Costaceae*). There are several species of section *Dealbati* which grow on monocotyledon plant; i.e *M. dealbatus* (Berk. & Curtis) Singer, *M. graminis* Murrill, however, no characters of those species match with characters of this species.

34. ***Marasmiellus*** aff. ***hirtellus*** (Berk. & Br.) Pegler, Kew Bull. Addit. Ser. 6: 130–131. 1977. Figure 1.48 and 1.50.

Syn. : *Marasmius hirtellus* Berk. & Br. in Journ. Linn. Soc., Bot. 14: 39. 1873.

Type : Central Prov, Kandy District, Peradeniya, on dead herbaceous plants, Nov 1867, Thwaites 102 p.p (K).

Pileus 4–10 mm diam, convex becoming plano-convex to plane-wavy depressed, dull, moist to dry, subtranslucent, rugulo-striate; disc light brown (7D4), margin slightly paler, elsewhere pale orangish white (5A2). Context moderately broad, off-white. Lamellae adnate, distant, with 2–3 series of lamellulae, moderately broad (–1.5 mm), intervenose and anastomosing in age, pale brownish green (6C3). Stipe 4–7 x 0.5 mm, insititious, central to eccentric, tough, terete, curved, appressed pubescent, light brown (7D5) overall, apex paler when young. Odor and taste indistinctive.

Basidiospores (7.2)8.0–8.8 x 4.0–4.8 µm ($\bar{x}$ = 8.29±0.56 x 4.32 ± 0.40, Q = 1.67–2.20, q = 1.93 ± 0.11; n = 25 spores per 1 specimen), fusoid, smooth, hyaline, inamyloid, thin-walled. Basidia 16–17.6 x 6.4–8.0, clavate, 4-spored. Basidioles fusoid to clavate. Cheilocystidia common, main body 20–24 x 4.8–8.0, with diverticulate, fusoid to clavate, or irregular in shaped, thin-walled, hyaline. Pleurocystidia absent. Pileipellis cutis; hyphae 3.2–5.6 µm, thin-walled, hyaline, inamyloid to weakly dextrinoid. Pileus trama interwoven; hyphae 3.2–5.6 µm, thin-walled, inamyloid. Stipe tissue monomitic; hyphae 2.4–7.2, cylindrical, parallel, hyaline, thin-walled, weakly dextrinoid. Stipe vesture common; main body 24–32 x 4.8–5.6, fusoid to clavate, thin-walled, hyaline. Clamp connections present.

Habit, habitat, and distribution. Gregarious on ginger leaves. Indonesia (Java).

Specimen examined. Indonesia: Java, Mount Halimun National Park, loop trail from Cikaniki, ca. 1900 m asl, on ginger leaves, 6 January 1999, D.E. Desjardin 6870.

Notes. *Marasmiellus hirtellus* (Berk. & Broome) Pegler is a member of section *Candidi*. The major character of this section *Candidi* is spores longer than 10 µm (Singer 1973), however, *M. hirtellus* showed several different size of basidiospores. Basidiospores size are 5–6.5 x 2.7–3.5 µm (Srilankan specimen), 5.7–7.5 x 3.5–4.5 µm (East African specimen), and (7.2)8.0–8.8 x 4.0–4.8 µm (Indonesian specimen).

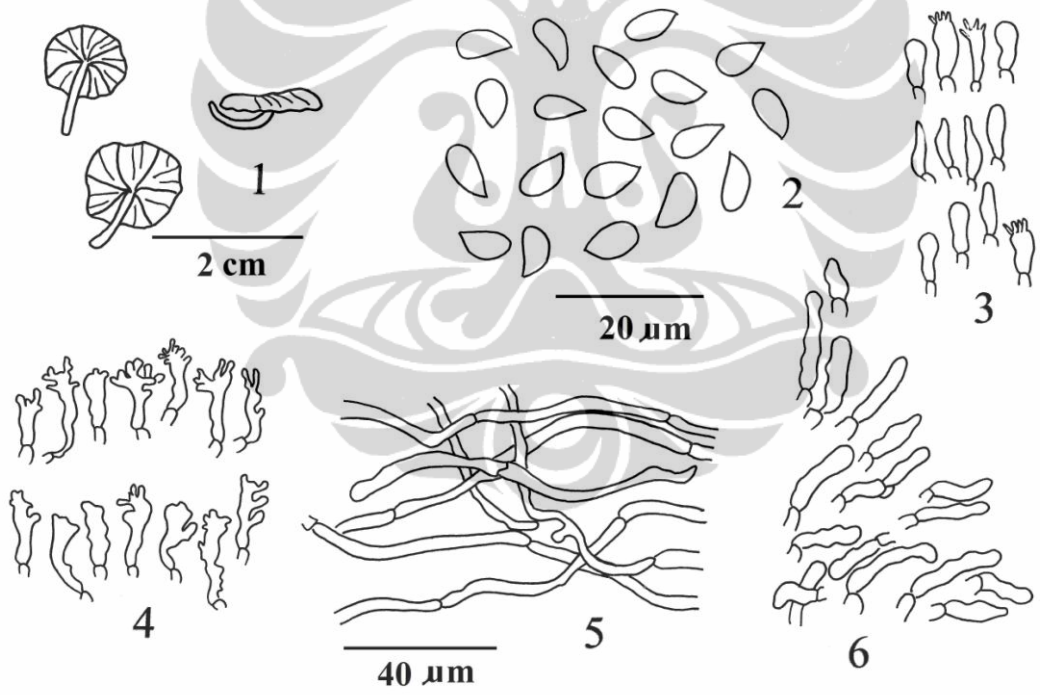

Figure 1.48. *Marasmiellus* aff. *hirtellus* (Berk. & Br.) Pegler (D.E. Desjardin 6870); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis; 6. Stipe vesture; number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

Section *Stenophylloides* Singer, Beih. Nova Hedwig. 44: 73. 1973.

One species of *Marasmiellus* in Java and Bali was a members of section *Stenophylloides*, namely *M. bisporus*. The description was presented below:

35. *Marasmiellus bisporus* Retnowati sp. nov. Figure 1.49 and 1.50.
Type : Indonesia, Java, Cibodas Botanical Garden, on wood, 16 April 2000, A. Retnowati 242 (Holotype: BO).

Pileus 2.5–18 mm diam, convex, non-hygrophanous, translucent-striate, margin straight, thin striation; surface slightly moist, smooth, glabrous; white. Context moderately broad up to 3 mm, concolorous with pileus. Lamellae adnate, subdistant (13–16), with 1 series of lamelluale, narrow up to 1.5 mm, non-marginate; white. Stipe 2–4 x 0.25–1 mm, cylindrical with tappered at the top, eccentric, solid; smooth, glabrous; white; a small disc at the base with white base tomentose. Taste and odor indistinctive.

Basidiospores (4.0)4.8–5.6(6.4) x 2.4–3.2 µm ($\bar{x}$ = 4.99 ± 0.48 x 2.82 ± 0.41, Q = 1.5–2.67, $\bar{q}$ = 1.81 ± 0.30, n = 25 spores per 1 specimen), ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 16–18.4x3.2–4.0 µm, clavate, 2-spored. Basidioles fusoid to clavate. Cheilocystidia common; main body 20–34.4 x 9.2–14.4 µm, clavate to subglobose, globose or irregular in shaped, with diverticulate, thin-walled, hyaline, inamyloid. Pleurocystidia absent. Pileipellis composed of *Rameales*-structure and setae; main body 14.4–32.8 x 9.6–16 µm, clavate to subglobose or irregular in shaped, smooth, thin-walled, hyaline, inamyloid; setae numerous; main body 17.6–64 x 2.4–3.2 µm, cylindrical to slender fusoid, thin to thick-walled up to 1.6 µm, arising among the pilei cells. Pileal trama not clearly seen, due to wrinkled tissue. Stipe tissue monomitic; hyphae 4.0–17.6 µm, cylindrical, parallel, thin-walled, hyaline, thin-walled, hyaline, inamyloid to weakly dextrinoid. Stipe vesture common; main body 16–37.6 x 4.0–6.4 µm, fusoid to clavate, thin-walled, hyaline. Clamp connections present.

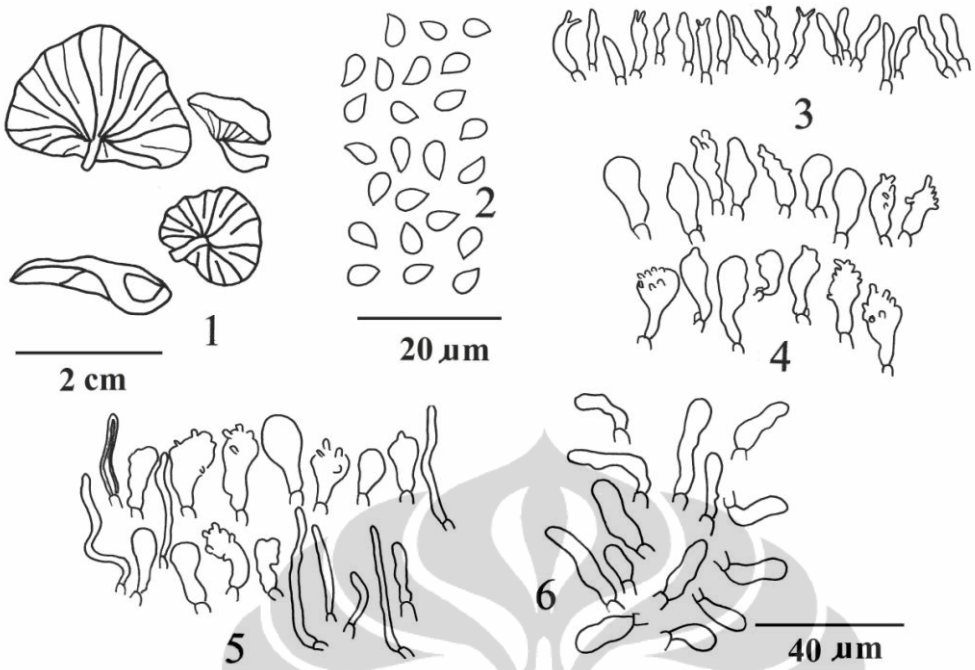

Figure 1.49. *Marasmiellus bisporus* Retnowati (A. Retnowati 242); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis with setae; 6. Stipe vestures; number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

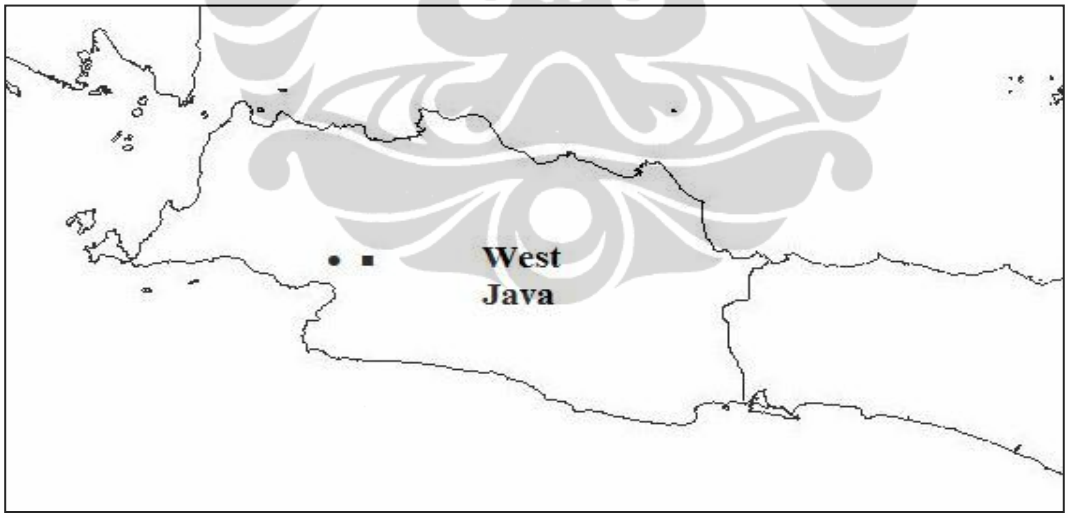

Figure 1.50. Distribution of *M.* aff. *hirtellus* (●) and *M. bisporus* (■) in Java.

Habit, habitat, and distribution. Gregarious on wood. Indonesia (Java).

Specimen examined. Indonesia: Java, Cibodas Botanical Garden, on wood, 16 April 2000, A. Retnowati 242.

Etymology: *bisporus* refers to the number produced basidiospores (two basidiospores).

Notes. The major characters of this species are the presence of setae at the pileus and 2-sterigmata which produce 2 spores. Examining of all members of section *Stenophylloides* (Singer 1973; Pegler 1977, 1983, 1986; Antonín & Noordeloos 1993) which possess setae do not match with javanese specimen. The Javanese specimen has a smallest basidiospores compared to all described species of *Marasmiellus* section *Stenophylloides*.

4. DISCUSSIONS

4.1 *Marasmiellus* species found in Java and Bali

A total of 35 species of *Marasmiellus* belonging to 5 sections (*Dealbati*, *Rameales*, *Marasmiellus*, *Stenophylloides*, and *Candidi*) were recorded from Java and Bali. They were grouped based on Singer's infrageneric classification. The 35 species consisted of 25 species in Java, 7 species in Bali, and 3 species were found both in Java and Bali.

The specimens of *Marasmiellus* were used in this study were collected from 10 locations which consisted of 22 sites in Java and Bali (Table 1.1). It was noted that the number of *Marasmiellus* species in Java is higher than Bali (Table 1.2). This result was caused by ecological differences, particularly on the degree of temperature and humadity, between Java and Bali. Based on Whitten *et al*. (1996) the eastern part of Java and Bali has a drier climate than the western part of Java due to the moonson wind. This monsoon wind blew from Australia in the middle of the year, and has a major influence in central and east-central Java and Bali. Normally, this season starts in March and extend almost to January, giving a dry season of about ten months, which is not favourable for mushrooms growth. Personal experience suggests that the best periodic for collecting *Marasmiellus* in Java and Bali is throughout the rainy season (from October to April), and an intensive collecting during the high of the mushroom season (November to January) are required to collect specimens in good quality.

Seven species of *Marasmiellus* were discovered in this study, i.e *M. inodermatoides, M. longisiccus, M. bolivarianus, M. tamblingensis, M. umbilicatus, M. hirtellus, M. dimidiatus, M. delicius,* and *M. subnigricans* were found in Bali only (Table 1.2). Those seven species possess the *Siccus*-type broom cells. The *Siccus*-type broom cells are the sterile cells which characterize most of marasmioid fungi, and its function is to minimize the evaporation, particularly at the tropical forest which has high temperature. However, such of cheilocystidia were also characterized the *Marasmiellus* species in Java, so that *Siccus*-type broom cells were not a specific character of *Marasmiellus* in Bali. One species in Bali, *Marasmiellus longisiccus*, is characterized by long setulae up to 17.6 µm at the the tip of cheilocystidia. The number of setulae were 2–3, and it becomes an important character to distinguish the species from others. Setulae of *M. longisccus* was the longest setulae among the *Marasmiellus* species in Java and Bali.

Among 22 sampling sites in Java and Bali, Mount Halimun National Park has the highest number of *Marasmiellus* species, followed by Cibodas Botanical Garden, and Eka Karya Botanical Garden. There has been remarkable indication that there was a correlation of altitude and humidity to the species occurrence at sampling sites.

4.2 New species and new combination of *Marasmiellus* in Java and Bali

Among 35 described species of *Marasmiellus* in Java and Bali, 17 species were candidates for new species, and one species of *Marasmiellus* found was a new combination. The rest of them were known species of *Marasmiellus*. The high number of new species in this study (45 % of described species) indicated that Java and Bali is a strategic area for discovering new species of *Marasmiellus*. The finding of 12 new species of other marasmioid fungi, the genus *Marasmius*, in Java and Bali by Desjardin *et al*. (2000) ensure that Java and Bali are the best habitat for marasmioid fungi. These figures were important reason to establish Java as protected area for mushrooms.

The number of new species of *Marasmiellus* in Java and Bali (45% of described species) was not remarkable, because it was comparable to previous

results of studies on several genera in under-explored areas. Horak (1979) studied the genus *Astrosporina* in Indomalaysia and Australasia, and 21 species of 30 described species (70 %) were new to science. Monograph of the genus *Marasmius* in South east Asia, reported that 14 species of 34 described taxa (41 %) were new to science (Desjardin & Horak 1997). Corner (1996) reported 132 new species (78%) from 168 described species of white spore mushrooms in Malesia.

Another finding of this study was *M. nugatorius* as new combination. Corner (1996) published monograph on the genera *Marasmius, Chaetocalathus, Crinipellis, Heiomyces, Resupinatus, Xerula,* and *Xerulina* based on specimens, which were collected in Malay Peninsula and Borneo. Many *Marasmiellus* which he described are hidden under the generic name *Marasmius* due to his broad generic concept. He described 100 *Marasmius,* and some 5-7 species probably belong to *Marasmiellus*. While examining all protoloques of *Marasmius* species in Corner's monographs (1996), one species was considered to represent character of *Marasmiellus* section *Rameales,* and it was *Marasmius nugatorius* Corner (1996). Specimen of *Marasmius nugatorius* was collected from Bukit Timah, Singapore. In this study, *Marasmius nugatorius* was treated as *Marasmiellus nugatorius*. The characters which transferred the species from genus *Marasmius* into genus *Marasmiellus* was *Rameales*-structures of pileipellis with terminal and subterminal cells finely and densely verrucose.

In general, results of this study can be a supported data for making conservation decision at the national level. The nature conservation planning is expected to stop the loosing forest in Java and Bali, and it is the most important activities in conserving the biological diversity.

5. CONCLUSION

Thirty five species of *Marasmiellus* were recorded in Java and Bali, and 17 of them were new to science (*M. globosus, M. zingiberius, M. rifaii, M. dimidiatus, M. reniformis, M. haurbentesis, M. longisiccus, M. clavatus, M. tamblingensis, M. diverticulatus, M. piperus, M. pruinosus, M. cikanikiensis, M. javanicus, M. cibodasensis*, *M. desjardinii*, and *M. bisporus*), and 1 species was a

new combination (*M. nugatorius*). According to Singer's infrageneric classification, they belong to 5 sections; i.e *Rameales*, *Stenophylloides*, *Marasmiellus*, *Candidi*, and *Dealbati*.

6. RECOMMENDATIONS

The actions for future study on the genus *Marasmiellus* must:

1. Do more fieldworks in Java and Bali or other islands in Indonesia. Describing and determining more specimens in Java and Bali will yield more species of *Marasmiellus*.
2. Support the nature conservation.

The result of taxonomic study of the genus *Marasmiellus* in these islands becomes one of important data for making conservation decision at the national level. Lossing biological diversity in Java and Bali is going rapidly due to lossing habitat in Java and Bali. To maintain the biological diversity in Java and Bali is important to establishing Java and Bali as protected areas.

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TOPIC 2

PHYLOGENETIC STUDY OF THE GENUS *MARASMIELLUS* IN JAVA AND BALI BASED ON MORPHOLOGICAL AND MOLECULAR ANALYSIS

Atik Retnowati

Department of Biology, Faculty of Mathematics and Natural Sciences

University of Indonesia

ABSTRACT

The objectives of this study were to analyse the phylogenetic interrelationship of the genus *Marasmiellus* in Java and Bali based on morphological and molecular characters and to clarify the relationship of the genus *Marasmiellus* and *M. juniperinus* to its closely related genera. Sixty eight of fungal specimens which represented of 35 species of *Marasmiellus* in Java and Bali were used for phylogenetic study. These 35 species of *Marasmiellus* were grouped into 5 sections (*Dealbati*, *Rameales*, *Marasmiellus*, *Stenophylloides*, and *Candidi*) based on Singer's infrageneric classification. Characters used for morphological analysis, i.e type of pileipellis, presence or absence of cheilocystidia, basidiospores size, stipe attachment, and pileus color etc., were scored manually and analyzed by using Maximum Parsimony method. Molecular study employed the sequence data of ITS region of rDNA. Analysis of morphological characters and ITS region of rDNA using MP and NJ methods produced phylogenetic trees which revealed that sections within Singer's infrageneric classification were polyphyletic. The result did not support traditional classification, i.e Singer's infrageneric classification. Additional analysis on the relationship the genus *Marasmiellus* and *M. juniperinus* to its closely related genera based on ITS region of rDNA using MP method showed that the genus *Marasmiellus* was polyphyletic.

Keywords: ITS region of rDNA, *Marasmiellus*, morphology, phylogenetic study, Singer's classification.

1. INTRODUCTION

The genus *Marasmiellus* was first described by Murrill (1915), and it has traditionally been divided into 10 sections by Singer (1986), i.e *Defibulati*, *Nigripedes*, *Tetrachroi*, *Stenophylloides*, *Candidi*, *Dealbati*, *Tricholores*, *Rameales*, *Distantifolii*, and *Marasmiellus*. Sections in Singer's classification were based on

combination of macro-micromorphological characters; i.e type of pileipellis (*Rameales*-structure, cutis with or without diverticulate), presence or absence of cheilocystidia, basidiospores sizes, stipe attachment (central, eccentric), and pileus color (pigment or non-pigmented), etc. Most mycologists (Pegler 1977, 1983, and 1986; Antonín & Noordeloos 1993, and Desjardin 1997) who work on the genus *Marasmiellus* have accepted the classification of the genus into 10 sections.

A few phylogenetic studies on *Marasmiellus* have included the 10 sections of Singer's infrageneric classification, and only one study by Wilson and Desjardin (2005) in which they reported sections of the genus *Marasmiellus* as a part of his study on the relationship of marasmioid and gymnopoid fungi. However, in their analysis they included only 4 sections of *Marasmiellus*, i.e section *Marasmiellus*, *Rameales*, *Candidi*, and *Dealbati*, so that the relationship of 10 sections of *Marasmiellus* have not resolved completely.

The genus *Marasmiellus* (Murrill) has been treated as a member of the family *Tricholomataceae* (Singer 1986), and it is one of the genus within marasmioid fungi group. The term of marasmioid fungi refers to the genera which morphologically are similar to the genus *Marasmius*. Based on Desjardin (1987), the group consists of several genera, i.e *Baespora*, *Crinipellis*, *Marasmiellus*, *Marasmius*, *Micromphale*, and *Strobilurus*, and a few other genera might be considered marasmioid fungi, i.e *Xeromphalina*, *Omphalina*, and *Mycena*. According to Wilson and Desjardin (2005), marasmioid fungi are marcescent, basidiomes rather tough and persistent, convex to conical, striate to sulcate pilei, adnate to adnexed lamellae, and typically tough, and filiform stipes. Marcescent was originally defined for the genus *Marasmius* which means revives if moistened after having been dried.

At present, marasmioid fungi are usually compared to gymnopoid fungi group (i.e *Collybia*, *Rhodocollybia*, and *Gymnopus*). Wilson and Desjardin (2005) mentioned in their paper that gymnopoid fungi form relatively putrescent basidiomes, convex and often non-striate pileus, variable attached lamellae and more robust, non-filiform stipe. Taxonomically both groups are notoriously difficult and controversial.

Mata *et al*. (2004) published a paper which indicated a taxonomical problem of marasmioid and gymnopoid group. The position of *M. juniperinus* Murrill, as a type species of *Marasmiellus*, in the phylogenetic tree of *Marasmiellus* to its closely related genera nested into *Gymnopus* clade. Mata *et al.* (2004) used molecular data based on ribosomal large subunit (LSU) DNA and Internal Transcribed Spacer (ITS) sequences in his analysis. In their methods, they used a very limited species of *Marasmiellus*, i.e *M. opacus*, *M. juniperinus*^{T}, and *M. stenophyllus*. Their phylogenetic tree showed that *M. juniperinus* nested among species in the genus *Gymnopus* with a low bootstrap value. This result becomes a taxonomical problem of the genus *Marasmiellus*. Other *Marasmiellus* species nested together with other closely related genera (*Gymnopus*, *Rhodocollybia*, *Lentinula*, and *Omphalotus*) in all major clades. Regarding this result, Mata *et al.* (2004) proposed *M. juniperinus* be *Gymnopus* sinonym. The result of Mata *et al*. (2004) was not supported by Wilson and Desjardin (2005) who provided molecular data based on nLSU rDNA for the phylogenetic analysis. Wilson and Desjardin (2005) included more taxa of *Marasmiellus* than previous paper (Mata *et al.* 2004). The 84 sequences based on nLSU rDNA used in their analysis, and 10 sequences of them were *Marasmiellus* sequences. These 10 sequences represented 7 species of *Marasmiellus* (*M. synodicus*, *M. juniperinus*, *M. rameales*, *M. opacus*, *M. scorodonius*, *M. candidus*, and *M. palmivorus*) were used to construct phylogenetic tree using Bayesian method. The phylogenetic tree showed that *M. juniperinus* was separated from *G. fusipes*, a type species of *Gymnopus* with a low support of bootstrap value. The result of Wilson and Desjardin (2005) indicated *Marasmiellus* was unable to be transferred to *Gymnopus*. To resolve this problem, including more *Marasmiellus* taxa and closely related genera in the analysis, it was expected that the relationship of *Marasmiellus* will be elucidated more clearly.

In this study, morphological characters and more sequences of ITS rDNA of *Marasmiellus* were used in order to analyse the interrelationship of the genus *Marasmiellus* in Java and Bali performed by Maximum Parsimony method and to

clarify the relationship of the genus *Marasmiellus* and *M. juniperinus* to its closely related genera.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Fungal specimens

The 35 species of *Marasmiellus* found in Java and Bali were examined for their micro-macromorphological characters. The specimens were identified according to Singer' infrageneric classification (Singer 1973). Additional species used in this study were *Marasmiellus juniperinus*^{T} (type species of *Marasmiellus*), and *Crinipellis brunneipurpurea* as outgroup. *Marasmiellus juniperinus* was borrowed from the Herbarium of the University of Tennessee, Knoxville, Tennessee, USA (TENN).

2.1.2 Chemical reagents

Chemical reagents which were used in this study were *Cetyl Trimetil Ammonium Bromide* (CTAB) and *Tris-acetate-EDTA* (TAE) buffer. One litter of CTAB consists of CTAB 20 gram, 1 M tris (TE) pH 8 (100 ml), 5 M NaCl (280 ml), 0.25 M Ethylene Diamine Tetra-acetic Acid (EDTA) pH 8 (80 ml), and PVP. Another reagent was *Tris-acetate-EDTA* (TAE) buffer which was used in electrophoresis. One hundred ml of TAE 50x consists of trisbase 24.2 gram, 5.71 ml of *Glacial Acetic Acid*, and 10 ml of EDTA 0.5 M pH 8. For DNA extraction, CTAB and Nucleon Phytopure Genomic Extraction Kit (GE Healthcare) were used. Polymerase Chain Reaction (PCR) used the universal primers pair ITS4 (forward-5' TCCTCCGCTTATTGATATGC 3') and ITS5 (reverse-3' GGAAGTAAACGTAACAAGG 5') (White *et al.* 1990).

2.2 Methods

2.2.1 DNA extraction

Genomic DNA was extracted from dried herbarium specimens (ca. 20 mg) and fresh materials. A small part of pileus, including context and lamellae, was taken. Extraction of genomic DNA was done by CTAB method (Doyle & Doyle 1987) and Nucleon Phytopure Genomic Extraction Kit (GE Healthcare).

2.2.2 DNA amplification

Internal Transcribed Spacer (ITS) regions were amplified by Polymerase Chain Reaction (PCR) using universal primers pair ITS4 (forward-5' TCCTCCGCTTATTGATATGC 3') and ITS5 (reverse-3' GGAAGTAAACGTAACAAGG 5') (White *et al.* 1990). Polymerase Chain Reaction was performed in 25 µl reaction volumes containing 1 µl of a template DNA, 0.5 µl of each primer (ITS4 and ITS5), 0.5 µl DMSO, 12.5 µl Green master mixed (GoTaq), and 15 µl of NFW. The amplification program comprised 35 cycles of 95° C for 30s, 55° C for 15s, and final extension at 72° C for 5 min. Polymerase Chain Reaction (PCR) process was performed on Takara PCR Machine.

2.2.3 Electrophoresis

Electrophoresis was done in order to analyse the DNA from PCR products. The PCR products were visualized in 1% TAE agarose gel (Vivantis). The 2 µ of PCR products were filled into agarose gel and electrophoresis performed for 15-25 minutes at 100 Volt. Agarose gel was placed into ethidium bromide solution for 15 minutes, then set into UV transilluminator to observe the DNA fragments. The DNA fragments were photographed by ATTA printgraph.

2.2.4 Sequencing

PCR products were sent to Macrogen (http://www.dna.macrogen.com) to get sequencing results using the same primers as in PCR, i.e ITS4 and ITS5.

2.2.5 Sequence analysis

Sequences generated as a part of this project will be deposited in the GenBank. Every sequence was subjected to its homology search by BLAST in GenBank (http://www.ncbi.nlm.nih.gov).

Sequence editing was performed by Bioedit and initial sequence alignment by using Clustal X with excluded gaps (Thomson *et al*. 1997). Maximum Parsimony (MP) analysis was conducted using PAUP* 4.0 beta 10 Win (Swofford 1998). All characters were of type 'unord', and have equal weight. Gaps were treated as "missing" characters in MP analysis. Bootstrap value was performed to see the branch robustness in phylogenetic tree (Felsenstein 1985). Two data sets of molecular sequences based on ITS region were analyzed separately. Another method used in this study was the Neighbor-joining method (Saitou & Nei 1987) calculated by Kimura's 2 parameter method.

2.2.6 Character analysis

Based on morphological descriptions of 35 species of *Marasmiellus* in Java and Bali (Table 2.1), 35 morphological characters were chosen to be

Table 2.1. List of specimens used for phylogenetic analysis based on morphological characters.

No.	Species	Section	No.	Species	Section
1.	*M. purpureoalbus*	*Marasmiellus*	19.	*M. delicius*	*Rameales*
2.	*M. globosus*	*Marasmiellus*	20.	*M. diverticulatus*	*Rameales*
3.	*M. inodermatoides*	*Marasmiellus*	21.	*M. piperus*	*Rameales*
4.	*M. ignobilis*	*Marasmiellus*	22.	*M. pruinosus*	*Rameales*
5.	*M.* aff. *concolor*	*Marasmiellus*	23.	*M. umbilicatus*	*Rameales*
6.	*M. epochnous*	*Marasmiellus*	24.	*M. nanus*	*Rameales*
7.	*M. zingiberius*	*Marasmiellus*	25.	*M. subnigricans*	*Candidi*
8.	*M. rifaii*	*Marasmiellus*	26.	*M. setulosipes*	*Candidi*
9.	*M. nugatorius*	*Marasmiellus*	27.	*M. albofuscus*	*Candidi*
10.	*M. dimidiatus*	*Marasmiellus*	28.	*M. cikanikiensis*	*Dealbati*
11.	*M. reniformus*	*Marasmiellus*	29.	*M. desjardinii*	*Dealbati*
12.	*M. haurbentesis*	*Marasmiellus*	30.	*M. cibodasensis*	*Dealbati*
13.	*M. longisiccus*	*Marasmiellus*	31.	*M.* cf. *stenophyllus*	*Dealbati*
14.	*M. bolivarianus*	*Marasmiellus*	32.	*M. hirtellus*	*Dealbati*
15.	*M. pernambucensis*	*Marasmiellus*	33.	*M. javanicus*	*Dealbati*
16.	*M. idroboi*	*Marasmiellus*	34.	*M.* aff. *hirtellus*	*Dealbati*
17.	*M. clavatus*	*Rameales*	35.	*M. bisporus*	*Stenophylloides*
18.	*M. tamblingensis*	*Rameales*			

analyzed by PAUP. Character states were variation of morphological characters of 35 species of *Marasmiellus* in Java and Bali, and they were scored manually (Table 2.2). Morphological characters used by Singer (1973) in his infrageneric classification were applied for describing *Marasmiellus* in Java and Bali.

Table 2.2. List of morphological characters used in the analysis of interrelationship of *Marasmiellus* species in Java and Bali.

No.	Character	Character state and coding
1.	Shape of basidiocarp	pleurotoid=0; pleurotoid to collybioid=1; collybioid=2
2.	Pileus shape from side view	convex=0; plano convex=1; conic=2
3.	Pileus shape from face view	semiorbicular=0; circular=1
4.	Surface	dry=0; moist=1
5.	Disc	depressed=0; flat=1; umbonate=2
6.	Striation	not-striate=0; striate=1; sulcate=2
7.	Margin	entire/straight=0; incurved=1; outcurved=2; wavy=3
8.	Pileus surface	glabrous=0; pruinose=1; suede-like=2
9.	Pileus surface	smooth=0; wrinkled=1
10.	Concentric ridge around disc	absent=0; present=1
11.	Odor	not distinctive=0; distinctive=1
12.	Lamellae attachment	free=0; adnexed=1; adnate=2; decurrent=3
13.	Spasing	distant=0; subdistant=1; close=2
14.	Anastomosing/interveined	absent=0; present=1
15.	The number of lamellulae series	0-2 series=0; 3-5 series=1
16.	Lamellae size	narrow=0; broad=1; moderately broad=2
17.	Stipe attachment on lamellae	absent=0; eccentric=1; lateral=2; central=3
18.	Stipe surface	absent=0; glabrous=1; pruinose=2; granulose=3
19.	Stipe attachment on substrate	non-insititious=0; insititious=1
20.	Bulb at the base of stipe	absent=0; present=1
21.	Base tomentose	absent=0; present=1
22.	Spores shape	cylindrical=0; ellipsoid=1; sub-globose=2
23.	The number of sterigmata	2=0; 4=1
24.	Cheilocystidia	absent=0; *Globulares*-shape=1; irregular shape=2; *Siccus*-type=3
25.	Pleurocystidia	absent=0; *Globulares*-type=1; irregular shape=2
26.	Pileipellis	cutis=0; cutis with diverticulate=1, *Rameales*-structure=2
27.	Hyphae	non-interwoven=0; interwoven=1
28.	Terminal cells	absent=0; present=1
29.	Sklerocystidia/pileocystidia	absent=0; present=1
30.	Diverticulate	absent=0; present=1
31.	Incrustation	absent=0; present=1
32	Diverticulate on stipe	absent=0; present=1
33.	Stipe vesture	absent=0; present=1
34.	Basidioles	absent=0; fusoid present=1
35.	Pileus color	non-pigmented=0; pigmented=1

3. RESULTS

3.1 The length of amplification ITS regions of rDNA by PCR

The fragments of ITS regions were in the range of 600-700bp. Nine sequences of *Marasmiellus* were generated in this study (Table 2.3) and 5 fragments of them were presented in Figure 2.1, and 31 sequences (Table 2.4) were obtained from GenBank.

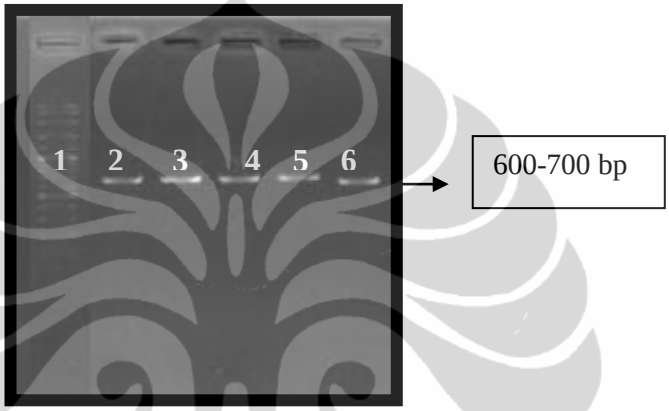

Figure 2.1. Visualization of PCR products (1 = ladder (100bp); 2 = specimen AR 809; 3 = specimen AR 809-B; 4 = specimen AR 810; 5 = specimen AR 811, and 6 = specimen AR 813. The length of fragments were in range of 600-700 bp. AR 809, AR 809-B, and AR 810 found on *Costus dinklagii*; AR 811 and AR 813 found on *Salacca zalacca* (Gaertn.) Voss.

Sequence length of ITS region rDNA of five specimens of *M. javanicus* for this study were ranged from 641bp to 683bp (AR809_B = 641bp; AR811^{T} = 646bp; AR813 = 662bp; AR810 = 670bp; AR809 = 683bp). Three other *Marasmiellus* sequences generated in this study were *M. albofuscus* (AR 817), *M. delicius* (AR 790), and *M. dimidiatus* (AR 748). Their sequence length were 827bp, 776bp, and 723bp.

Table 2.3. List of *Marasmiellus* specimens sequenced (ITS1, 5.8S, ITS2) for this study, itemized by Singer's infrageneric classification.

Species	Section	Collection number	GenBank accession number
Marasmiellus dimidiatus	*Marasmiellus*	AR 789	It will be deposited
Marasmiellus dimidiatus	*Marasmiellus*	AR 748	It will be deposited
Marasmiellus javanicus	*Dealbati*	AR 809	JQ586345
Marasmiellus javanicus	*Dealbati*	AR 809-B	JQ586347
Marasmiellus javanicus	*Dealbati*	AR 810	JQ586344
Marasmiellus javanicus	*Dealbati*	AR 811	JQ586343
Marasmiellus javanicus	*Dealbati*	AR 813	JQ586346
Marasmiellus delicius	*Rameales*	AR 790	It will be deposited
Mrasmiellus albofuscus	*Candidi*	AR 817	It will be deposited

3.2 Phylogenetic relationship of the genus *Marasmiellus*

3.2.1 Based on morphological data

Parsimony analysis of the data set based on 35 morphological characters of 37 species, with *Crinipellis* as outgroup, resulted 100 trees (35 characters, 2 parsimony uninformative characters, 33 parsimony informative characters). Four major clades were recovered (Figure 2.2):

(i). Group 1, composed of 6 species of sect. *Marasmiellus* (*M. zingiberius, M. nugatorius, M. juniperinus, M. rifaii, M. reniformis,* and *M. longisiccus*), 2 species of sect. *Rameales* (*M. umbilicatus* and *M. nanus*), 1 *Marasmiellus* sect. *Candidi* (*M. setulosipes*), and 1 species of sect. *Stenophylloides* (*M. bisporus*).

(ii). Group 2, included 7 species of sect. *Marasmiellus* (*M. bolivarianus, M. globosus, M. dimidiatus, M. inodermatoides, M.* aff. *concolor*, *M. epochnous*, and *M. ignobilis*) and 2 species of sect. *Dealbati* (*M.* cf. *stenophyllus* and *M. hirtellus*).

(iii). Group 3 consisted of *M. pernambucensis* (sect. *Marasmiellus*), *M. albofuscus* (sect. *Candidi*), and *M. cikanikiensis* (sect. *Dealbati*).

(iv) Group 4 comprised 6 species of sect. *Rameales*. *Marasmiellus purpureoalbus, M. idroboi, M. haurbentesis, M. albofuscus, M. cikanikiensis,* and *M. desjardinii*

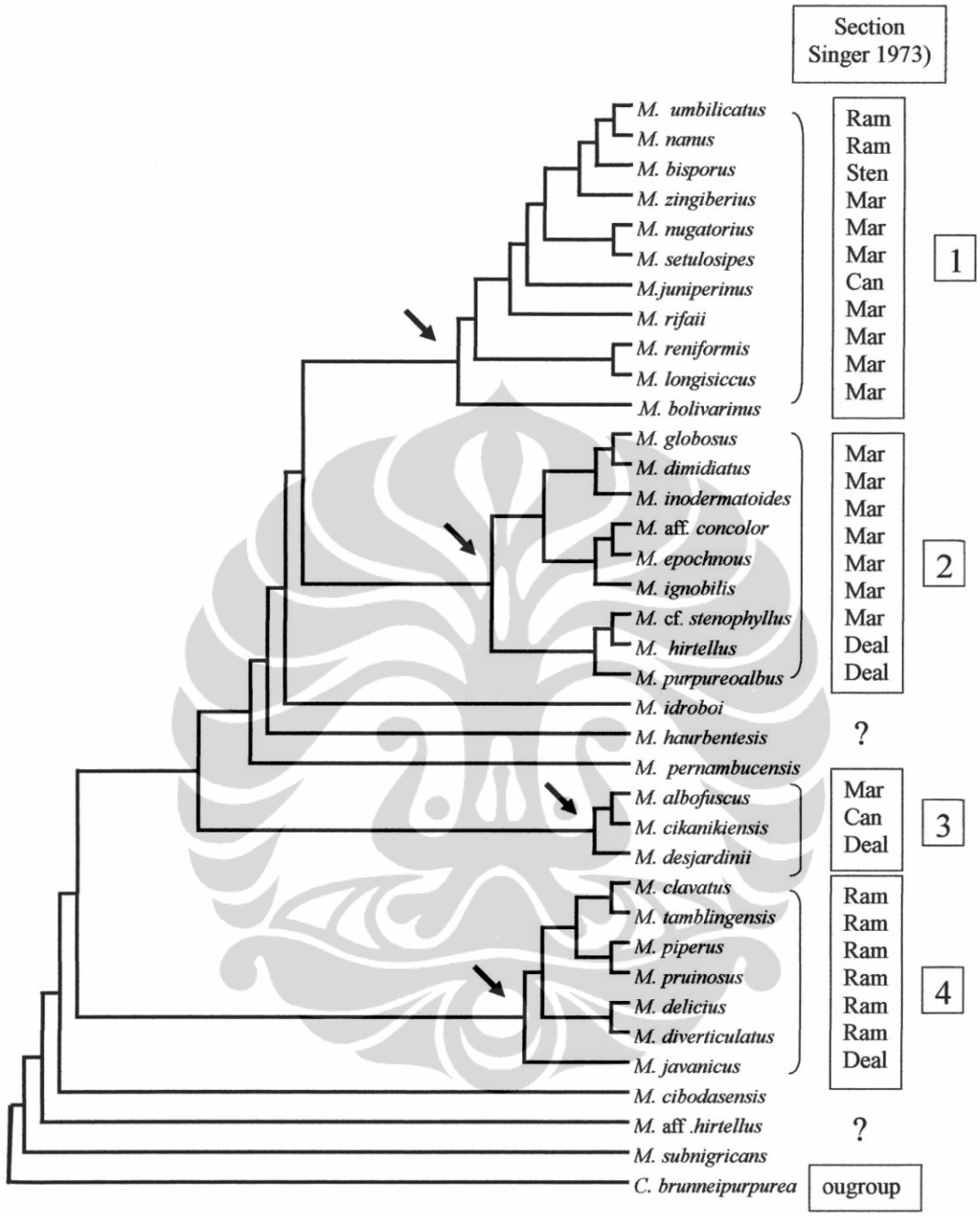

Figure 2.2. Phylogenetic tree of interrelationship of *Marasmiellus* species in Java and Bali based on morphological data. *Crinipellis brunneipurpurea* was as outgroup. The tree was constructed by using MP method (CI = 0.2247; RI = 0.7753).

appeared to be a basal to all clades. Clades which were produced in the analysis were not supported by bootstrap value (less than 50%).

Clades in phylogenetic tree based on morphological characters using MP analysis consisted of several species which belong to different sections. For example, 17 *Marasmiellus* species of section *Marasmiellus* nested into 3 different clades, i.e clade 1, 2, and 3. They nested together with other *Marasmiellus* species which belong to other sections, i.e *Candidi*, *Rameales*, and *Dealbati*. Phylogenetic tree based on morphological data (Figure 2.2) showed that several morphological characters shared by more than one section, lineage 1: (i). *Rameales*-structure of pileipellis shared by sect. *Marasmiellus*, *Rameales*, *Stenophylloides*, and *Candidi*; (ii). Cutis of pileipellis and large spores more than 10 µm shared by sect. *Candidi* and S*tenophylloides*; lineage 2: (i). The absent of pileocystidia is shared by sect. *Marasmiellus* and *Dealbati*. According to Vellingga (2003), the shared characters by multiple sections are caused by the very small number of morphological characters on the generic level, particularly on mushroom, for phylogenetic analysis.

3.2.2 Based on ITS region of rDNA

Eighteen sequences based on ITS region (including ITS1, 5.8S, and ITS2) representing of 13 species of *Marasmiellus* were analyzed in order to see the interrelationship of the genus *Marasmiellus*. Another forty one sequences based on ITS region representing 36 species which belong to 7 genera (*Marasmiellus*, *Gymnopus*, *Marasmius*, *Rhodocollybia*, *Lentinula*, *Collybia*, and *Omphalotus*) was used in the analysis to find out the relationship of the genus to its closely related genera.

Parsimony analysis of the data set based on ITS rDNA of 13 species of *Marasmiellus* and 3 species of *Omphalotus* as outgroup were analyzed. The maximum-parsimony analysis produced 2 parsimonious trees of 721 steps long, 92 constant characters, 38 parsimony-uninformatives, and 309 parsimony-informative. One of a maximum-parsimony trees was presented (CI = 0.728; RI = 0.815) (Figure 2.3). Phylogenetic tree based on ITS rDNA showed that there were 4 major clades were recovered:

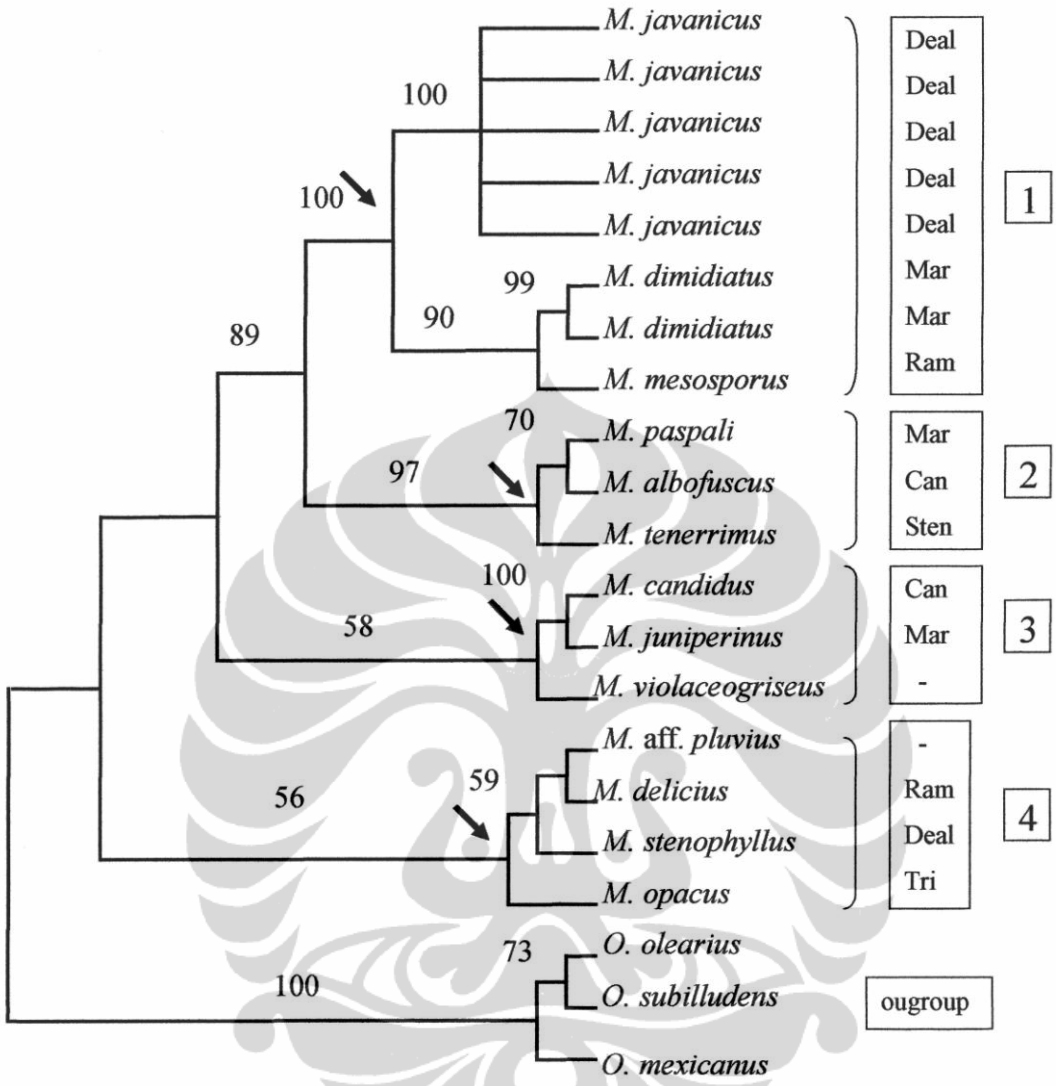

Figure 2.3. Phylogenetic tree of interrelationship of *Marasmiellus* species in Java and Bali based on molecular data of ITS rDNA. *Omphalotus olearius*, *O. subilludens*, and *M. mexicanus* were used as outgroups. The tree was constructed by using MP method (CI = 0.728; RI = 0.815).

(1). Group 1 consisted of three species from different sections: *M. javanicus* (sect. *Dealbati*), *M. dimidiatus* (sect. *Marasmiellus*), and *M. mesosporus* (sect. *Rameales*);

(ii). Group 2 consisted of *M. paspali*, *M. albofuscus*, and *M. tenerrimus*;

(iii). Group 3 contained *M. candidus*, *M. juniperinus*, and *M. violaceogriseus*;

(iv). Group 4 consisted of *M.* aff. *pluvius*, *M. delicius*, *M. stenophyllus*, and *M. opacus*.

Clade 1 and 2 supported well by high bootstrap value (100% and 98), and clade 3 and 4 supported by low bootstrap value of 58 % and 56 %.

Table 2.4. Fungal species and GenBank accession number used in phylogenetic analysis.

Species	GenBank accession number
Marasmiellus candidus	HQ604791
Marasmiellus violaceogriseus	HQ533014
Marasmiellus paspali	EF175515
Marasmiellus stenophyllus	DQ450032
Marasmiellus aff. *pluvius*	DQ450029
Marasmiellus opacus	DQ450005
Marasmiellus juniperinus T	AY256708
Marasmiellus tenerrimus	FJ596840
Marasmiellus mesosporus	AB517375
Marasmius cladophyllus	HQ248211
Marasmius androsaceus	DQ444316
Gymnopus lodgeae	AY256705
Gymnopus dichrous	AY256702
Gymnopus biformis	AY256699
Gymnopus dryophilus	AY256698
Gymnopus confluens	AY256697
Gymnopus cylindricus	AY256696
Gymnopus polyphyllus	AY256695
Gymnopus earleae	AY256694
Gymnopus junquilleus	AY256693
Gymnopus aquosus	AY256691
Gymnopus fusipes T	AY256711
Gymnopus dryophila	FJ467354
Gymnopus luxurians	AY639421
Rhodocollybia f. *butyracea*	AY256689
Rhodocollybia maculate	AY256688
Lentinula raphanica	AY256687
Collybia tuberosa T	DQ830807
Collybia subnuda	GQ452055
Omphalotus mexicanus	AY313274
Omphalotus oleareus	AY313277
Omphalotus subilludens	AY313282

To clarify the relationship of *Marasmiellus* to its related genera, the data set of sequences based on ITS rDNA of 33 species of *Marasmiellus*, *Gymnopus*, *Marasmius*, *Collybia*, *Rhodocollybia*, *Lentinula*, and 3 species of *Omphalotus* as outgroup were analyzed. The analysis of ITS rDNA sequence resulted in 8 most-parsimonious trees based on MP method, with tree length 1441, CI=0.486, and

RI=0.748, a total of 595 base pairs, 366 parsimony-informative characters, 28 uninformative characters, and 77 constant characters.

Phylogenetic tree of *Marasmiellus* and its closely related genera based on ITS rDNA showed that there were 4 major clades in figure 2.4, and labeled 1-4. Clade 1 included *Gymnopus*, *Marasmiellus*, *Collybia*, and *Rhodocollybia*. Clade 2 consists of 8 species which belong to four genera, *Gymnopus*, *Marasmius*, *Collybia*, and *Marasmiellus*. This clade has a bootstrap support of 64.7 %. Clade 3 majority composed of *Marasmiellus* species, and *Collybia tuberosa* nested at this clade. Clade 4 consists of 5 species which belong to *Gymnopus*, *Marasmiellus*, and *Lentinula*. In this lineage, *G. fusipes* (a type species of genus *Gymnopus*) was associated to *M. juniperinus* (a type species of genus *Marasmiellus*) and *G. luxurians*. The position of *M. juniperinus* and *G. fusipes* in the phylogenetic tree of *Marasmiellus* and its closely related genera based on ITS rDNA showed that the clade of *Marasmiellus* had a low bootstrap support (less than 50%).

Another analysis used to understand the position *M. juniperinus* to other *Marasmiellus* species and *G. fusipes* was the Neighbor-joining (NJ) method (Saitou & Nei 1987). Phylogenetic tree based on ITS rDNA by using NJ method showed that there were 5 clades recovered, and the position of *M. juniperinus* clustered at the same clade as *G. fusipes, M. candidus,* and *G. luxurians* (Figure 2.5). Long branches attraction occurred at position of those 4 species at the phylogenetic tree due to more changing of nucleotides at those branches. Phylogenetic tree based on ITS rDNA using NJ method showed that *M. juniperinus* and *G. fusipes* was at the same clade with a low bootstrap value (less than 50%), and it supported the result of the MP method.

This result was the same as previous research (Mata *et al*. 2004). In previous paper, Mata *et al.* (2004) used very limited taxa of *Marasmiellus*, i.e. *M. opacus*, *M. stenophyllus*, and *M. juniperinus*. In the phylogenetic reconstruction based on ITS region, all *Marasmiellus* taxa appeared basal to clades dominated by *Gymnopus* taxa. *Marasmiellus juniperinus* was associated with *G. luxurians* and *G. polygrammus* in all analysis, but with less than 50 % bootstrap support (Figure 2. 6).

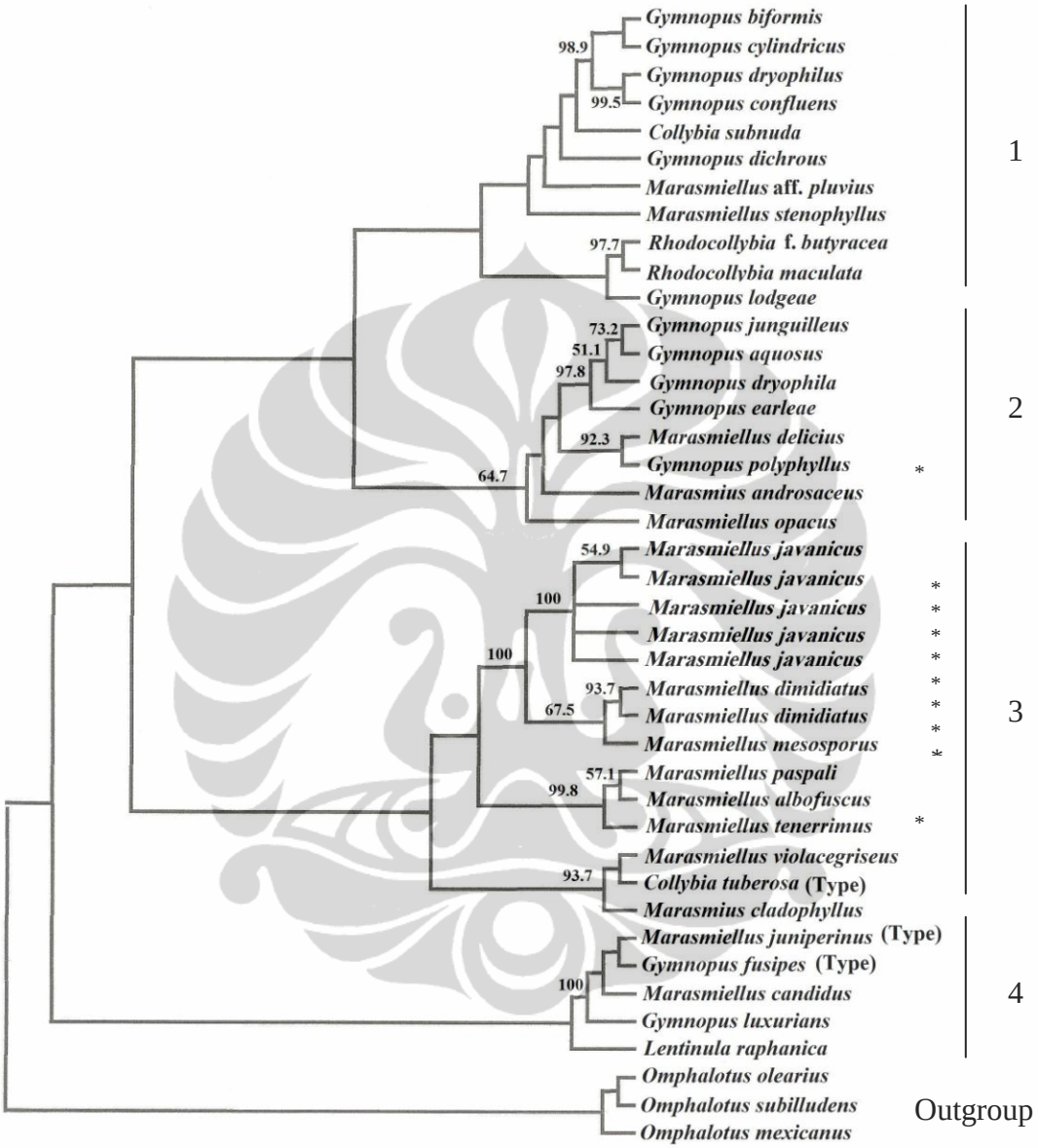

Figure 2.4. Phylogenetic tree of *Marasmiellus* and its related genera based on ITS rDNA. It represents the most parsimonious tree (CI = 0.486; RI = 0.748; data obtained in this study indicated by asterisk (*)). The tree was constructed by using MP method. Bootstrap value which was less than 50% was not mentioned in the figure.

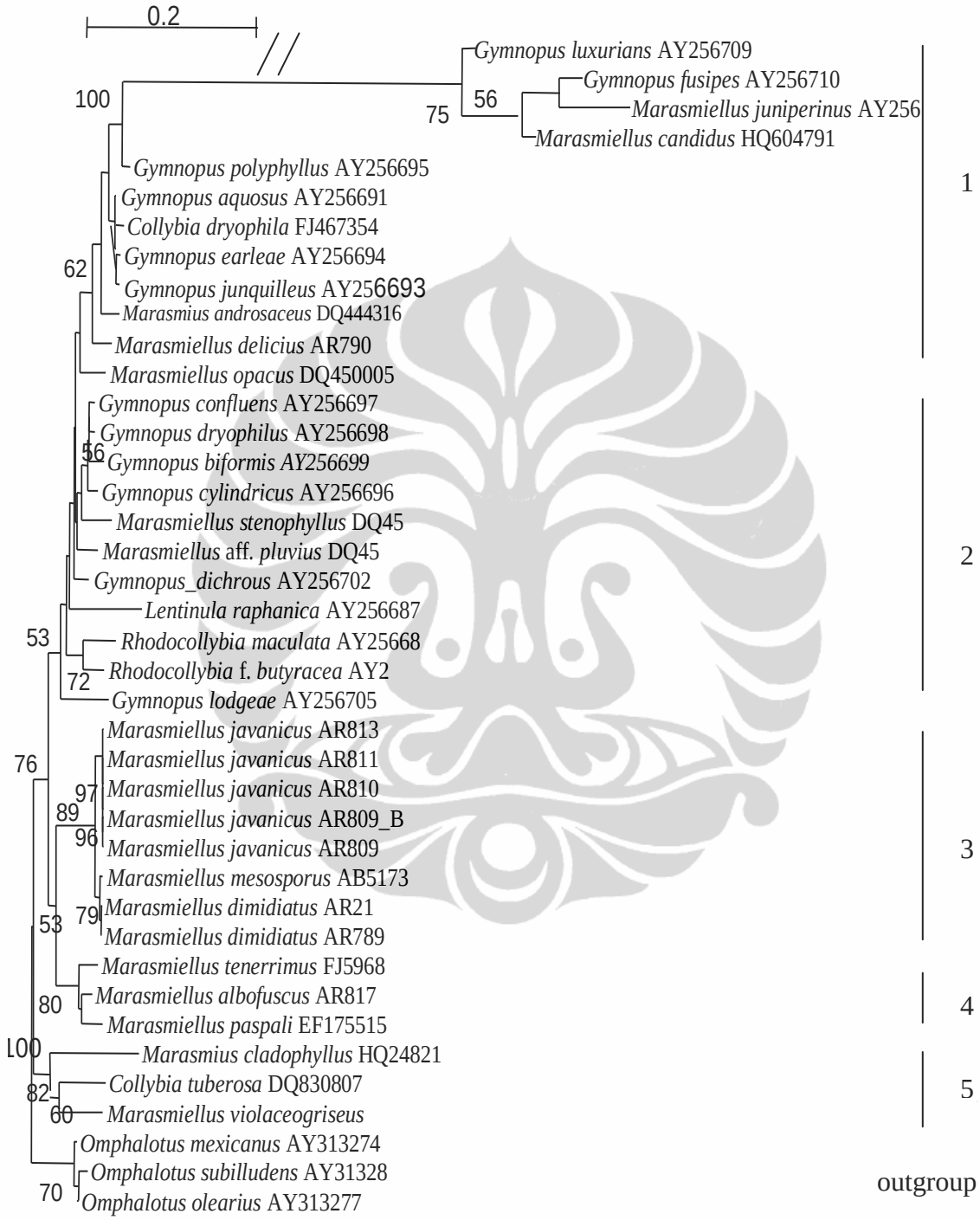

Figure 2.5. Phylogenetic tree of *Marasmiellus* and its related genera based on molecular data of ITS rDNA. The tree was constructed by using NJ method.

4. DISCUSSIONS

4.1 Interrelationship of species of *Marasmiellus* in Java and Bali

Phylogenetic tree based on morphological characters showed that 35 species of *Marasmiellus* in Java and Bali were grouped into 4 clades. Each clade consisted of different sections. The tree indicated that the sections within Singer's infrageneric classification were polyphyletic. The polyphyly of the sections of genus *Marasmiellus* indicated that characters used by Singer in his classification did not show the evolutionary relationship, in other words the characters were not homolog.

To resolve the polyphyly of the sections of the genus *Marasmiellus*, molecular data based on ITS region of rDNA was applied in this study. The ITS rDNA has been widely used in taxonomy and molecular phylogeny. Internal Transcribed Spacer has higher degree of variation than other regions of rDNA (White *et al*. 1990). With high variation of nucleotides, ITS has proven especially useful for elucidating relationships among species level, infrageneric level, and closely related genera. However, in this study, sequences of ITS region of rDNA did not resolve the interrelationship in the genus *Marasmiellus*.

In this study, phylogenetic tree based on ITS region of rDNA showed that sections within Singer's infrageneric classification were polyphyletic. Molecular phylogenetic based on ITS regions resulted in this study were not consistent with traditional classification based on morphological characters, i.e. Singer's classification of *Marasmiellus*.

In contrast, several studies on different genera within *Tricholomataceae* using ITS region rDNA has successfully resolved their phylogenetic problems among species, infrageneric and genera level. Oda *et al.* (1999) used ITS rDNA to re-classify the infrageneric classification of the genus *Amanita* in order to get the monophyly of the genus. Thirty six species of Japanese *Amanita* were studied based on nucleotide sequences of the ITS rDNA. Molecular analysis of this study on the genus *Amanita* supported the traditional classification system which based on morphological data. Re-classification system was applied on one of the sub-genus of *Amanita*. This new classification was more robust than previous system,

and relationship of some closely related taxa and interspecies can be clearly resolved. Another phylogenetic study based on ITS rDNA on other genera within *Tricholomataceae* was investigated by Dávalos *et al*. (2003). Dávalos *et al*. (2003) used ITS rDNA to analyse the infrageneric classification of the genus *Gymnopilus*. Traditionally the genus *Gymnopilus* was classified into 2 primary groups, *Annulati* and *Gymnopilus*, based on the presence or absence of a membranous partial veil. The analyses of ITS rDNA sequence data supported the monophyly of the genus, but the result did not cover the traditional subgroups.

The results of this study suggested that Singer's infrageneric classification should be re-considered in order to establish a modern classification which based on monophyly of the genus *Marasmiellus*. This new modern classification is more robust than previous system, and relationship of some closely related taxa and interspecies can be clearly resolved. To establish a new modern classification of the genus *Marasmiellus*, molecular characters of *Marasmiellus* species have to be available, and they represent the species of 10 sections within the Singer's infrageneric classification. Preferably, specimens examined in Singer's monograph (1973) are borrowed, however, they are old specimens. It is impossible to amplify the old specimens, otherwise they have to be re-collected. Most of those specimens were collected in 1900-1960. For example *M. hondurensis* (Murrill) Singer which was collected from Belice in 1906 by Peck (Singer 1973).

A very limited sequences of ITS regions rDNA which were applied in this study might be the reason of unresolve phylogenetic relationship of sections within Singer's infrageneric classification. Only 18 sequences of *Marasmiellus* was used construct a phylogenetic tree by using MP method. Nine of them were generated from this study, and the remaining sequences were obtained from GenBank. Preferably, the sequences of 35 species of described *Marasmiellus* in Java and Bali were amplified, and were included in the analysis, however, the DNA extraction and sequence DNA from those old specimens were unsuccessful. Many of the specimens used in this study were from old herbarium specimens (more than 10 years), because a few new collected specimens were available. Several kits of DNA extraction have been used, and there was no DNA of specimens successfully

amplified. Moreover, the availability of sequence data of *Marasmiellus* in GenBank is very limited, so that phylogenetic tree based on morphological and sequence ITS rDNA was unable to be compared. As a result, the interrelationship of *Marasmiellus* species was not completely resolved. At present, 33 sequences of M*arasmiellus* are available in GenBank, and 7 sequences of them were identified up to genus level.

4.2 Relationship of the genus *Marasmiellus* to its related genera

Phylogenetic tree of *Marasmiellus* and its closely related genera based on ITS rDNA produced 4 major clades. It is surprising that types species of *Marasmiellus* (*M. juniperinus*) and *Gymnopus* (*G. fusipes*) were nested at the same clade (clade 4). Futhermore, the sequence aligment of ITS rDNA of *Marasmiellus* species, *M. juniperinus*, and other closely related genera presented that sequence of *M. juniperinus* was different from other *Marasmiellus* species, and it was much closely related to *G. fusipes* (Figure 2.6). However, because there was a low bootstrap support in the phylogenetic tree, this position might not be significant. More additional taxa in the analysis will change the results.

It was noted that other *Marasmiellus* species nested together with other closely related genera (*Gymnopus*, *Rhodocollybia*, *Lentinula*, and *Omphalotus*) in all major clades. Phylogenetic tree based on ITS rDNA suggested that *Marasmiellus* was polyphyletic.

The result in this study was the same as previous study by Mata *et al.* (2004). Mata *et al*. (2004) proposed *Marasmiellus* was a synonym of *Gymnopus*, because *M. juniperinus* as a type genus of *Marasmiellus* nested into *Gymnopus* clade (Figure 2.7). This proposal needs further analysis and more taxa included in the analysis.

Another study by Wilson and Desjardin (2005) analyzed the relationship of marasmioid and gymnopoid fungi based on nLSU region of rDNA, and they included several species of *Marasmiellus* in their phylogenetic tree. The result of Wilson and Desjardin (2005) did not support Mata *et al*. (2004). Wilson and Desjardin (2005) provided molecular data based on nLSU rDNA for the phylogenetic analysis, and included more taxa of *Marasmiellus* than previous

paper (Mata *et al.* 2004). Ten sequences based on nLSU rDNA of *Marasmiellus* which represented 7 species of *Marasmiellus* (*M. synodicus*, *M. juniperinus*, *M. rameales*, *M. opacus*, *M. scorodonius*, *M. candidus*, and *M. palmivorus*) were used to construct phylogenetic tree using Bayesian method (Figure 2.8). The phylogenetic tree showed that *M. juniperinus* was separated from *G. fusipes*, a type species of *Gymnopus* with a low support of bootstrap value. The result of Wilson and Desjardin (2005) indicated *Marasmiellus* was unable to be transferred to *Gymnopus*. However, this changing status needs further analysis and more additional taxa.

Following descriptions of *M. juniperinus* and *G. fusipes* illustrated that they are morphologically different. *Marasmiellus juniperinus* is a member of marasmioid group, and *G. fusipes* is a member of gymnopoid group.
Based on protoloque, *Marasmiellus juniperinus* was characterized by pileus digitaliformis, 1.5 cm diam, surface brown, glabrous, especially to the margin, sulcate; lamellae broad, solid, duplicate insertis, intervenose absent, adnate, white at first, becoming white brown to pale brown; stipe generally with short distinctly eccentric stipe, solid, dull, sublateral, glabrous to pruinose, 1–2.5 mm long, 0.1–1 mm wide. *Marasmiellus juniperinus* grew gregariously on wood of *Juniperinus barbadensis* in Cinchona, Jamaica, South America.

Additional micro-description of *M. juniperinus* was examined from representative species of *M. juniperinus* loaned from the Herbarium of the University of Tennessee, Knoxville, Tennessee, USA (TENN). Basidiospores 7.2–8.8 x 4.0–4.8 µm, ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 20 x 6.4 µm, clavate. Basidioles fusoid to clavate. Cheilocystidia absent. Pleurocystidia absent. Pileipellis cutis, hyphae 3.2–4.8 µm, thin to thick-walled up to 0.8 µm, hyaline, incrusted, inamyloid. Pileal trama interwoven, thin-walled, hyaline, inamyloid. Stipe tissue monomitic; hyphae 2.4–12.0 µm, hyaline, thin to thick-walled up to 1.6 µm, hyaline, parallel, weakly incrusted to strongly incrusted, inamyloid. Clamp connection absent. Stipe vesture absent.

Meanwhile, *G. fusipes* was characterized by pileus 4 cm diam, sticky, covered with flesh, convex, glabrous, slightly rimosove, umbonate evanescent,

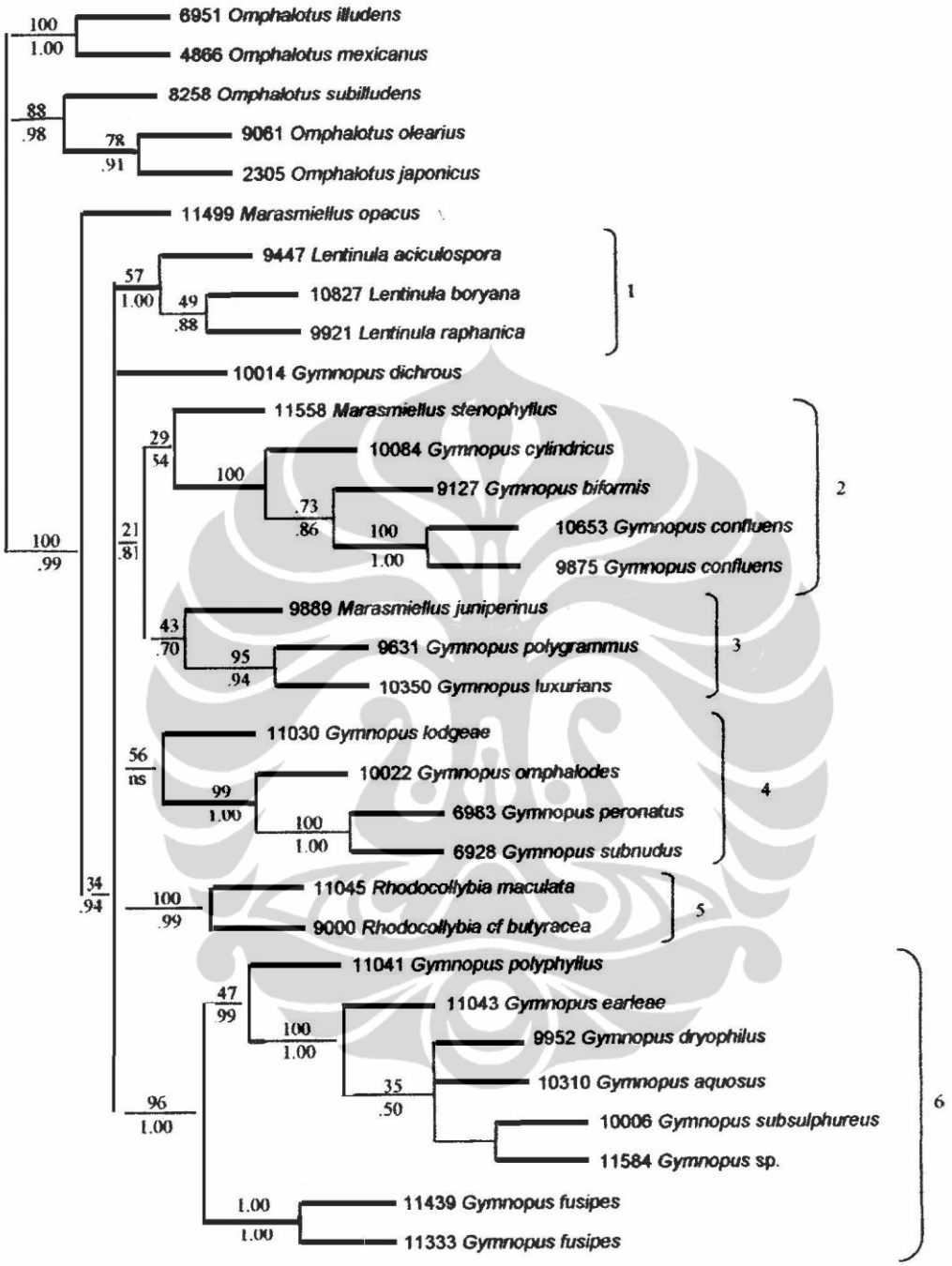

Figure 2.7. Phylogenetic tree of *Marasmiellus* and its related genera based on ITS region (Mata *et al*. 2004).

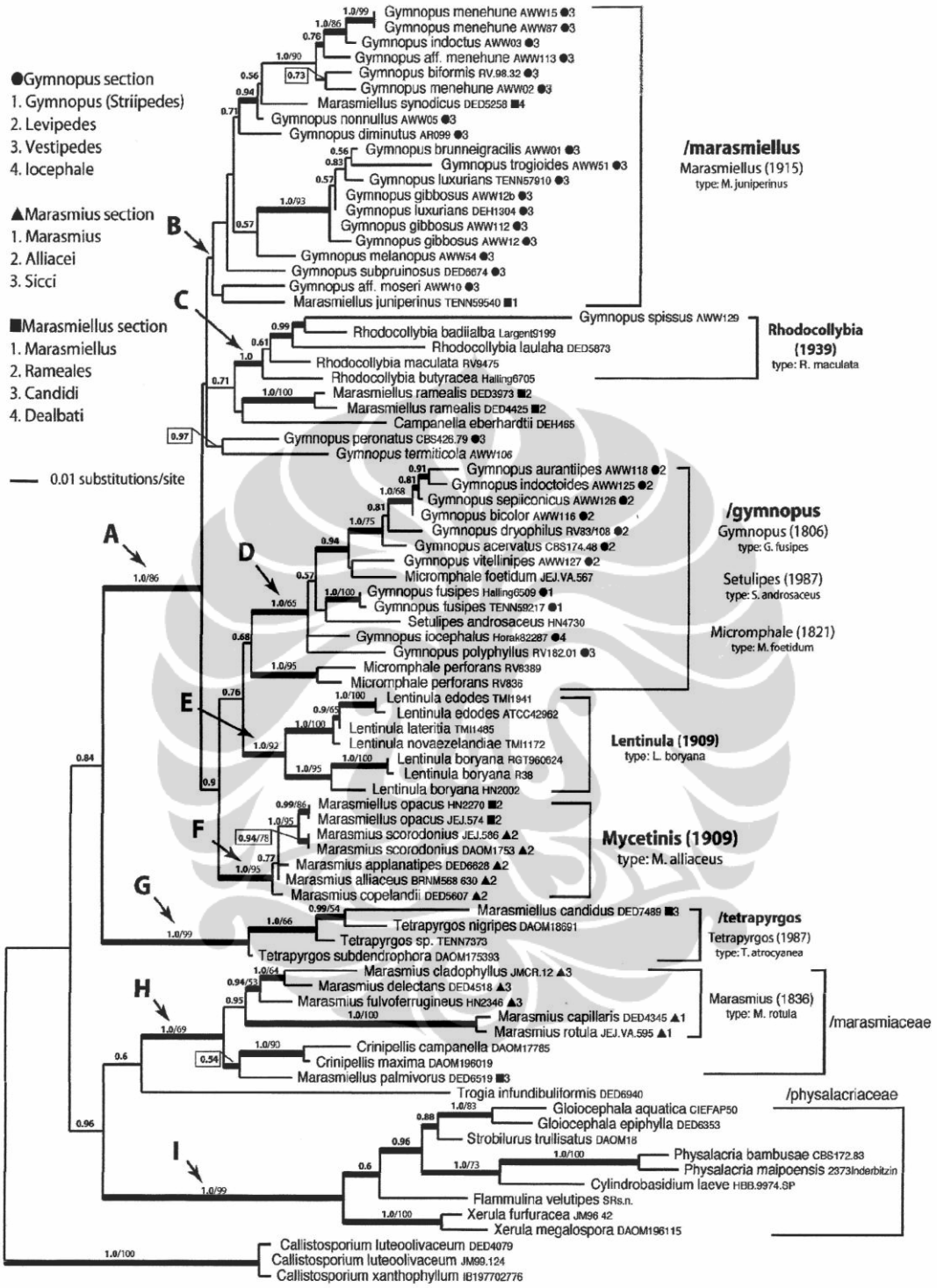

Figure 2.8. Phylogenetic tree based on nLSU rDNA using Bayesian Method of relationship of *Marasmiellus* and its closely related genera (Wilson and Desjardin 2005)

rufescenti-vaccine, expalleus; lamellae submaculatae, adnexed, withdraws, wide, distant; stipe 6–16 cm long, 1–2.5 cm wide, hollow, swollen, sulcate, fusiform, root base. Basidiospores 4–6 x 3–4 µm. It grew on trunk of *Quercus* in Europe (Saccardo 1925).

At present, phylogenetic relationship of *Marasmiellus* to its closely related genera and *M. juniperinus* to gymnopoid group has not been resolved until more species are added to the analysis.

5. CONCLUSIONS

The analysis of interrelationship of the genus *Marasmiellus* in Java and Bali based on morphologhical characters and molecular of ITS rDNA resulted that sections within Singer's infrageneric classification were polyphyletic. Morphological characters and molecular of ITS rDNA did not support the traditional classification, i.e Singer's infrageneric classification of *Marasmiellus*. Therefore, Singer's infrageneric classification should be reconsidered in order to establish a modern classification which based on monophyly of the genus *Marasmiellus*.

The analysis of relationship of the genus *Marasmiellus* to its closely related genera (*Marasmius*, *Gymnopus*, *Collybia*, *Rhodocollybia*, *Lentinula*, and *Omphalotus*) based on ITS rDNA resulted that the genus *Marasmiellus* was polyphyletic. More taxa of *Marasmiellus* are necessary to elucidate the relationship of *Marasmiellus* more clearly.

4. RECOMMENDATIONS

After analysing the phylogenetic relationship of *Marasmiellus* species in Java and Bali, and phylogenetic relationship of *Marasmiellus* to its closely related genera, recommendations for future study on the genus *Marasmiellus* are:

1. Singer's infrageneric classification should be reconsidered in order to establish a modern classification which is based on monophyly of the genus *Marasmiellus*. Morphological and molecular data of ITS region of rDNA supported the result of this study that sections within Singer's infrageneric classification was polyphyletic.
2. The molecular data of ITS rDNA generated in this study were very limited due

to the unsuccessful DNA extraction. To generate more molecular data, it is necessary to extract the fresh basidiomes of *Marasmiellus*.

3. Relationship of *Marasmiellus* and its closely related taxa (*Gymnopus*, *Marasmius*, *Collybia*, *Lentinula*, and *Rhodocollybia*) is still a problem. Since ITS region of rDNA used in this study was unable to determine the relationship of the species treated, multilocus analysis by using the translation elongation factor 1-alpha (tef) and beta-tubulin (*ben*A) protein coding genes as well as intergenic spacer (IGS) regions of the nuclear ribosomal genes are necessary to evaluate the phylogenetic and evolutionary relationship among species in the genus *Marasmiellus* and its closely related taxa.

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TOPIC 3

DESCRIPTION OF A NOVEL SPECIES, *MARASMIELLUS JAVANICUS*, BASED ON MORPHOLOGICAL AND MOLECULAR ANALYSIS

Atik Retnowati

Department of Biology, Faculty of Mathematics and Natural Sciences

University of Indonesia

ABSTRACT

Five specimens of *Marasmiellus* have been collected from Bogor Botanical Garden in December 2010. The specimens found on different substrates, i.e. *Costus dinklagii* K. Schum and *Salacca zalacca* (Gaertn.) Voss. Morphological and molecular analysis were carried out to identify the specimens to species rank. Morphological data were obtained by examining their micro-macro morphological characters. Characters used for molecular analysis were five sequences based on Internal Transcribed Spacer regions of rDNA of *Marasmiellus* specimens were analysed. Morphologically, *M. javanicus* was similar to *M. hondurensis* (Murrill) Singer, however, the ITS rDNA of *M. hondurensis* was unavailable in database. Consequently, the phylogenetic relationship of these species could not be determined. Using the available ITS rDNA sequences of *Marasmiellus* species in GenBank, the phylogenetic tree using Maximum Parsimony method revealed that *M. javanicus* was closely related to *M. mesosporus*. *Marasmiellus javanicus* and *M. mesosporus* have 94% sequence similarity, and they differed in 27 nucleotides (6%) which supported morphological analysis that *M. javanicus* was a distinct species.

Keywords: ITS region of rDNA, *Marasmiellus*, morphology, molecular analysis, new species, sequence similarity.

1. INTRODUCTION

The genus *Marasmiellus* was first described by Murrill (1915), and it is widely distributed throughout tropical and subtropical areas. The genus is a very important fungus for playing a significant ecological role in tropical forests (Singer 1973).

It is known that *Marasmiellus* is a genus with a low variation of morphological character. In fact, the use of morphological characters alone will

be problematic in recognizing species because of the possibility of grouping morphologically similar. Thus, molecular data has to be employed in order to support morphological data, and ribosomal DNA (rDNA) of ITS region was chosen as a molecular marker in this study. According to White *et al*. (1990) Internal Transcribed Spacer has higher degree of variation than other regions of rDNA. With high variation of nucleotides, it has becomes widely used region for identifying of various fungal species within *Tricholomataceae*. Arruda *et al*. (2005) used ITS rDNA sequence data to support morphological characters to propose a new species of *Crinipellis brasiliensis*, and in Ammirati *et al.* (2007), *Cleistocybe*, as a new genus, was described by using morphological characters and sequence data of ITS rDNA.

During regular collecting trips to Bogor Botanical Garden in the course of taxonomic study of a Javanese *Marasmiellus*, one candidate of new species was found growing on *C. dinklagii* K. Schum and *S. zalacca* (Gaertn.) Voss. To elucidate the specimens found in Bogor Botanical Garden, analysis of their morphological characters and molecular data of ITS rDNA was carried out.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Fungal specimens

Fungal specimens used in the study were collected by Atik Retnowati (AR) in Bogor Botanical Garden in December 2010. Three specimens were found on *C. dinklagii* K. Schum (AR 809, AR 809-B, and AR 810), and two specimens were found on *S. zalacca* (Gaertn.) Voss (AR 811 and AR 913). They were examined for their macro-micromorphological characters. Fungal specimens used in the study are kept in Herbarium Bogoriense (BO).

2.1.2 Chemical reagents

Chemical reagents which were used in this study were *Cetyl Trimetil Ammonium Bromide* (CTAB) and *Tris-acetate-EDTA* (TAE) buffer. One litter of CTAB consists of CTAB 20 gram, 1 M tris (TE) pH 8 (100 ml), 5 M NaCl (280 ml), 0.25 M Ethylene Diamine Tetraacetic Acid (EDTA) pH 8 (80 ml), and PVP. Another reagent was *Tris-acetate-EDTA* (TAE) buffer which was used in electrophoresis. One hundred ml of TAE 50x consists of trisbase 24.2 gram, 5.71 ml of *Glacial Acetic Acid*, and 10 ml of EDTA 0.5 M pH 8.

2.2 Methods

2.2.1 Sampling methods

Sampling was conducted by Purposive Random Sampling with particular finding on *Marasmiellus* specimens. Basidiocarps were collected representing as many growth stages as were available in order to examine variability within the species. Each specimen was put in plastic boxes. They were identified tentatively in the field station.

2.2.2 Morphological observations

Many taxonomically important characters were observed only in the field on habit of growth, odor, taste, substrate type, and plant associates. Odor was checked by smelling the fresh basidiome, and taste was checked by taking a small tissue of basidiome and taste it. Notes on macromorphological features were obtained in the field soon after collecting, paying particular attention to macromorphological features: a). size, shape, surface, color, and margin characteristics of different parts of the pileus; b). thickness, texture, color, taste, and odor of the flesh; c). attachment, spacing, color and edge color of lamella; d). size, shape, color, and surface features of stipe; the attachment of the stipe to the substrate; and f). type of substrate. Brief notes were completed on fungus habit and habitat. Color notation was based on Kornerup & Wancher (1983). Micromorphological data were obtained from dried specimens and carried out in the laboratory. All micro characters were studied from dried specimens

rehydrated in distilled water and 3% of KOH solution, with the use of Melzer's reagent or Congo Red dye (Largent 1973). Spore sizes were based on measurements of 25 basidiospores per specimen.

2.2.3 Molecular characterization using ITS rDNA sequence data

2.2.3.1 DNA extraction

Genomic DNA was extracted from dried herbarium specimens (ca. 20 mg) and fresh materials. A small part of pileus, including context and lamellae, was taken. Extraction of genomic DNA was done by CTAB method (Doyle and Doyle 1987) with modifications and Nucleon Phytopure Genomic Extraction Kit (GE Healthcare).

2.2.3.2 DNA amplification

Internal Transcribed Spacer (ITS) regions were amplified by Polymerase Chain Reaction (PCR) using universal primers pair ITS4 (forward-5' TCCTCCGCTTATTGATATGC 3') and ITS5 (reverse-3' GGAAGTAAACGTAACAAGG 5') (White *et al.* 1990). Polymerase Chain Reaction was performed in 25 µl reaction volumes containing 1 µl of a template DNA, 0.5 µl of each primer (ITS4 and ITS5), 0.5 µl DMSO, 12.5 µl Green Master Mixed (GoTaq), and 15 µl of NFW. The amplification program comprised 35 cycles of 95° C for 30s, 55° C for 15s, and final extension at 72° C for 5 min. Polymerase Chain Reaction (PCR) process was performed on Takara PCR Machine. The PCR product was stored 4°C.

2.2.3.3 Electrophoresis

Electrophoresis was done in order to analyse the DNA from PCR products. The PCR products were visualized in 1% TAE agarose gel (Vivantis). The 2 µ of PCR products were filled into agarose gel and electrophoresis performed for 15-25 minutes at 100 Volt. Agarose gel was placed into ehtidium bromide solution for 15 minutes, then placed UV transilluminator to observe the DNA fragments. The DNA fragments were photographed by ATTA printgraph.

2.2.3.4 Sequencing

PCR products were sent to Macrogen (http://www.dna.macrogen.com) to purify and sequence the PCR products. Primers used in the sequencing reactions were the same as in the PCR reactions, i.e ITS4 and ITS5.

2.2.3.5 Sequence analysis

Sequences generated as a part of this project will be deposited in the GenBank. Every sequence was subjected to its homology search by BLAST in GenBank (http://www.ncbi.nlm.nih.gov).

Sequence editing was performed by Bioedit and initial sequence alignment by using Clustal X with excluded gaps (Thomson *et al*. 1997). Maximum Parsimony (MP) analysis was conducted using PAUP* 4.0 beta 10 Win (Swofford 1998). All characters were of type 'unord', and have equal weight. Gaps were treated as "missing" characters in MP analysis. Bootstrap value was performed to see the branch robustness in phylogenetic tree (Felsenstein 1985).

3. RESULTS AND DISCUSSIONS

3.1 The amplification variations of ITS regions rDNA in *Marasmiellus* spp.

The fragments of PCR products were in the range of 600-700bp. Five sequences of *Marasmiellus javanicus* were generated from this study (Table 3.1) and their fragments were presented in Figure 3.1. The remaining sequences were obtained from GenBank (Table 3.2).

Sequence length of ITS region rDNA of five specimens of *M. javanicus* for this study were ranged from 641bp to 683bp (AR809-B = 641bp; AR811^{T} = 646bp; AR813 = 662bp; AR810 = 670bp; AR809 = 683bp). All sequences were aligned, and final alignment for 5 sequences consisted of 634bp. The variations occurred at total of 15 nucleotides (2.4% of total nucleotides; indels=14 nucleotides, substitution=1 nucleotide) (Fgure 3.2). The BLAST search resulted that *M. javanicus* is closely related to *M. mesosporus*, and they have 94% sequence similarity.

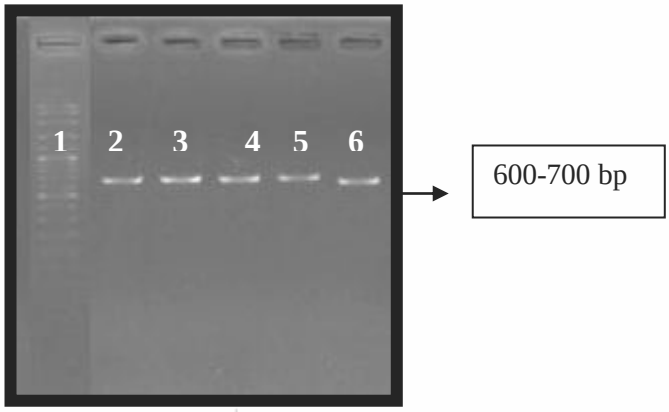
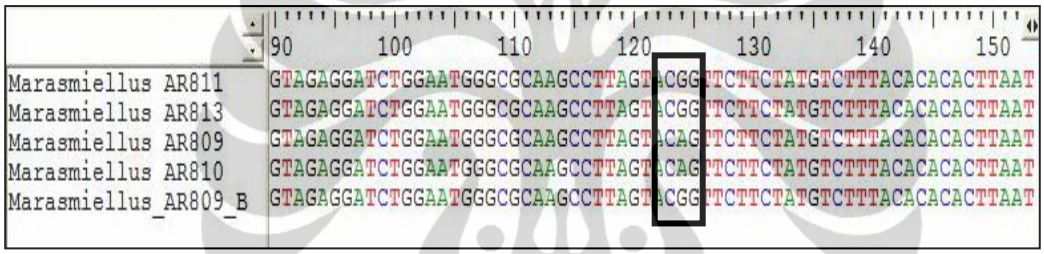


Figure 3.1. Visualization of PCR products (1 = ladder (100bp); 2 = specimen AR 809; 3 = specimen AR 809-B; 4 = specimen AR 810; 5 = specimen AR 811, and 6 = specimen AR 813. The length of fragments were in range of 600-700 bp. AR 809, AR 809-B, and AR 810 found on *Costus dinklagii*; AR 811 and AR 813 found on *S. zalacca* (Gaertn.) Voss.

a)



b)

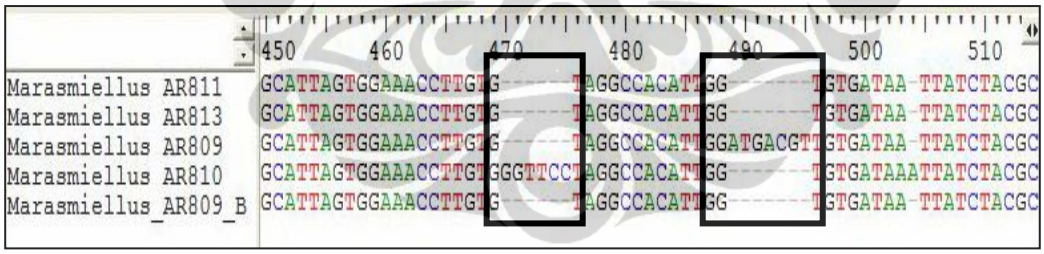


Figure 3.2. Nucleotide variation of ITS rDNA of five specimens (AR 811, AR 813, AR 809, AR 809-B, and AR 810) of *M. javanicus*; a) nucleotide variations from 90bp to 153bp; b) nucleotides variations from 450bp to 514bp. Nucleotides variations presented in boxes.

Table 3.1. List of specimens of *Marasmiellus javanicus* generated in this study and their GenBank accession number.

Species	Section	Coll. number	GenBank accession number
Marasmiellus javanicus	*Dealbati*	AR 809	JQ586345
Marasmiellus javanicus	*Dealbati*	AR 809-B	JQ586347
Marasmiellus javanicus	*Dealbati*	AR 810	JQ586344
Marasmiellus javanicus	*Dealbati*	AR 811	JQ586343
Marasmiellus javanicus	*Dealbati*	AR 813	JQ586346

Table 3.2. *Marasmiellus* species from GenBank and their accession numbers used in phylogenetic analysis.

Species	GenBank accession number
Marasmiellus candidus	HQ604791
Marasmiellus violaceogriseus	HQ533014
Marasmiellus paspali	EF175515
Marasmiellus stenophyllus	DQ450032
Marasmiellus aff. *pluvius*	DQ450029
Marasmiellus opacus	DQ450005
Marasmiellus juniperinus (Type)	AY256708
Marasmiellus tenerrimus	FJ596840
Marasmiellus mesosporus	AB517375
Omphalotus mexicanus	AY313274
Omphalotus oleareus	AY313277
Omphalotus subilludens	AY313282

3.2 Morphological characterization

Marasmiellus javanicus found on monocotyledon (*C. dinklagii* K. Schum and *S. zalacca* (Gaertn.) Voss) in Bogor Botanical Garden (Figure 3.3). There were several species of section *Dealbati* which grew on monocotyledon plants; i.e *M. dealbatus* (Berk. & Curtis) Singer and *M. subgraminis* (Murrill) Singer (Singer 1973), however, morphological characters of *M. dealbatus* and *M. subgraminis* were different from *M. javanicus*.

Marasmiellus javanicus was similar to *M. hondurensis* (Murrill) Singer which was collected from Belice in 1906 by Peck (Singer 1973). The characters similarities of *M. javanicus* and *M. hondurensis* were off-white, same size of width, convex with flattened disc of pileus; subdistant lamellae; tomentose and insititious stipe; cutis with diverticulate of pileipellis, and presence of stipe vesture. *Marasmiellus hondurensis* was different from *M. javanicus* in having longer stipe (6–22 mm diam), fusoid basidiospores, *Rotalis*-type of cheilocystidia, and *M. hondurensis* was found on dicotyledon plant.

3.3 Molecular characterization

Phylogenetic tree construction using MP method. Several characters were missing, and scored as "?". Heuristic searches were conducted under the following conditions: the starting tree was obtained via stepwise addition with the addition as simple, the maxtrees setting was 100, branch-swapping algorithm was

tree-bisection-reconnection (TBR). According to Felsenstein (1985) branch robustness was evaluated by 1000 bootstrap replications.

Result of a maximum-parsimony analysis produced 1 parsimonious tree of 817 steps long, 83 constant characters, 38 parsimony-uninformatives, and 302 parsimony-informative. The single tree of a maximum-parsimony was presented, and it was shown in Figure 3.2 (CI = 0.728; RI = 0.815). Phylogenetic tree using MP analysis can be revealed that *M. javanicus* was closely related to *M. dimidiatus* and *M. mesosporus* Singer (1973). The clade has 100% of bootstrap support.

At present the availability of sequence data of *Marasmiellus* in GenBank is very limited. The 33 sequences of M*arasmiellus* are available in GenBank, and there is no sequence based on ITS rDNA of *M. hondurensis*. Thus, *M. hondurensis* was unable to be compared to *M. javanicus* genetically. To construct phylogenetic tree of *Marasmiellus*, twenty sequences based on ITS rDNA of *Marasmiellus* specimens which represented of 16 *Marasmiellus* species were analysed and phylogenetic tree showed that the most closely related species to *M. javanicus* were *M. mesosporus*. *Marasmiellus javanicus* (Type: A. Retnowati 811) and *M. mesosporus* have 94% sequence similarity. They differ in 27 nucleotides which were 7 nucleotides of insertion and deletion, and 24 nucleotides of substitution (A↔G=11, C↔T=13). The sequence similarity between *M. javanicus* to *M. mesosporus* less than 99% indicated that *M. javanicus* was a distinct species from *Marasmiellus mesosporus* and other known species (Figure 3.3). The morphological characters differences of *M. javanicus*, *M. hondurensis*, and *M. mesosporus* were presented in Table 3.3.

Table 3.3. The morphological differences of *M. javanicus* and its closely related species.

Morphological characters	**Species**		
	M. javanicus	***M. mesosporus***	***M. hondurensis***
Basidiomes	Pleurotoid	Collybioid	Collybioid
Stipe attachment	Eccentric stipe	central stipe	Central to slightly eccentric
Basidiospores	7.2–8.8(9.6–10.4) x 4.0–5.6 µm	(8)10–13(14) x (5)7-8(9) µm	7–8.5 x 3.3–3.5 µm
Cheilocystidia	*Siccus*-type broom cells	Coralloid-like	Rotalis-type
Pileipellis	Cutis with diverticulates	Cutis, with poorly developed *Rameales*-structures	Cutis, repent and smooth hyphae, with diverticulate

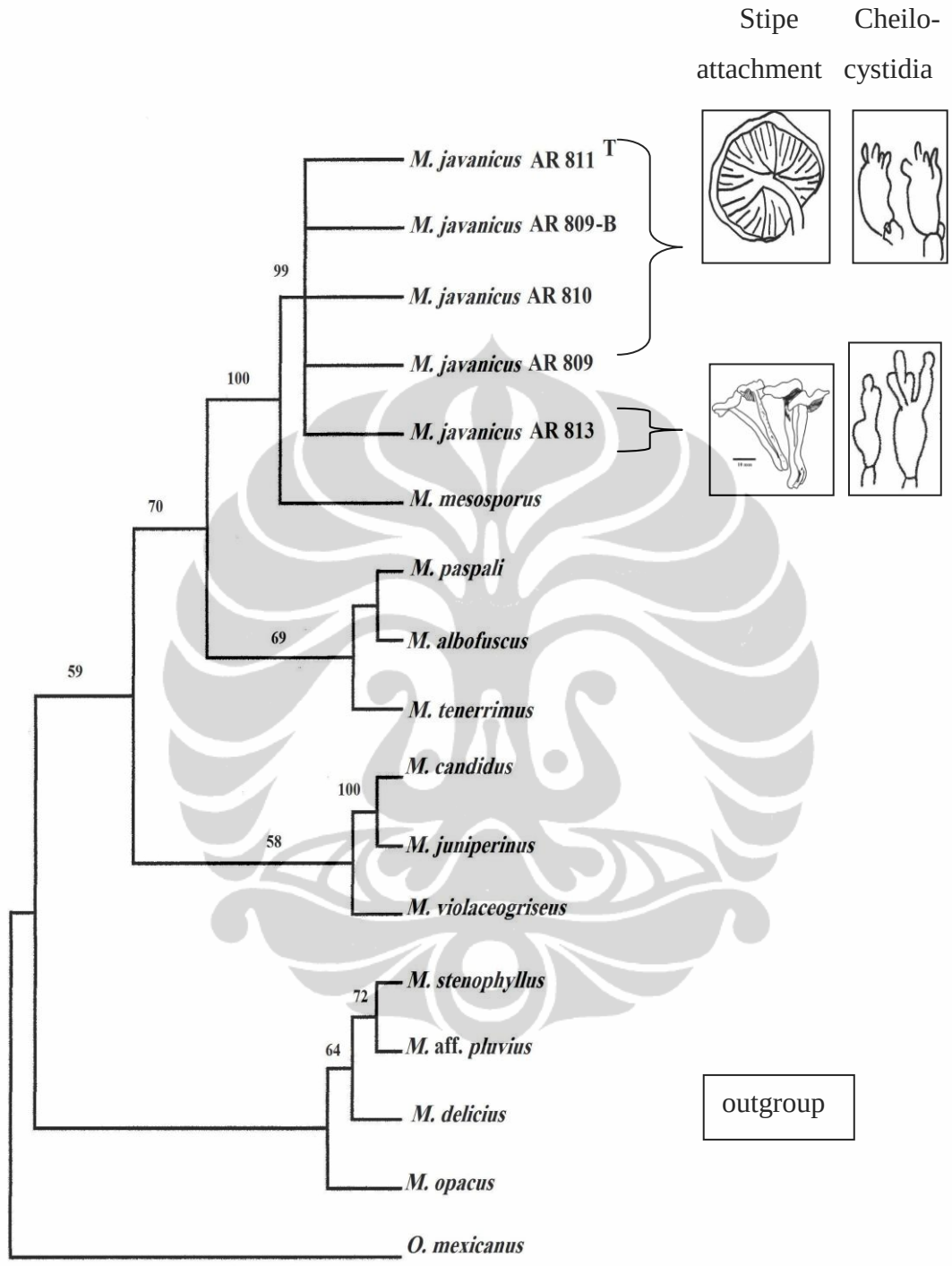


Figure 3.3. Phylogenetic tree of *M. javanicus* and other *Marasmiellus* species based on ITS rDNA (CI = 0.728; RI = 0.815). The tree was constructed by using Maximum Parsimony method.

3.4 Description of *Marasmiellus javanicus* sp. nov.

Marasmiellus javanicus Retnowati, Gandjar, & Sjamsuridzal sp. nov. (Figure 3.4 & 3.5).

Mycobank No.: MB 564070

Pileus 8–16 mm latus, convexus, off-album. Lamellae adnatae, subdistantes. Stipe 3–5 x 0.1–0.2 mm, centrum, insititius, tomentose ad basim, candida ad apicem, ad basim rufulus. Basidiosporae 7.2–8.8(9.6–10.4) x 4.0–5.6 µm, ellipsoideae. Cheilocystidia conspicue diverticula, clavata; diverticula 1.6–3.2 x 1.6 µm, conica. Pleurocystidia nulli. Pileipellis cutem, diverticula, hyphae 4.8–10.4 µm, incrustatae nulli. Caulocystidia 24–24.8 x 6.4–7.2 µm, fusoida vel irregularia. Fibulae presentes. Solitarius vel gregarius ad lignum monocotyledoneum (*C. dinklagii* K. Schum et *S. zalacca* (Gaertn.) Voss. Holotypus: Indonesia, Java, West, Bogor Botanical Garden, 1 December 2010, A. Retnowati 811 (BO). Host: *Salacca zalacca* (Gaertn.) Voss.

Pileus 8–16 mm diam, convex with flattened disc, strongly hygrophanous; margin incurved; surface dull, dry, glabrous, off-white. Context unobserved. Lamellae adnate, subdistant, with 2–3 series of lamellulae, moderately broad, off-white. Stipe 3–5 x 0.1–0.2 mm, cylindrical, central, insititious, with a densely white tomentose, white at the apex, reddish brown at the base. Odor and taste indistinctive.

Basidiospores 7.2–8.8(9.6–10.4) x 4.0–5.6 µm ($\overline{x}$ = 8.72 ± 0.8 x 4.99 ± 0.3, Q = 1.3–2.0, $\overline{q}$ = 1.75 ± 0.1) n = 25 spores per 1 specimen, ellipsoid, smooth, hyaline, inamyloid, thin-walled. Basidia 23.2–32 x 8.0–8.8 µm, clavate, 4-spored. Basidioles clavate. Cheilocystidia common, composed of *Siccus*-type broom cells; main body 11.2–19.2 x 7.2–9.6 µm, clavate to broadly clavate, thin-walled, hyaline; diverticulate 1.6–3.2 x 1.6 µm, conical, obtuse, thin-walled, hyaline. Pleurocystidia absent. Pileipellis cutis with diverticulate; hyphae 4.8–10.4 µm, parallel, not incrusted, thin-walled, hyaline. Pileal trama interwoven; hyphae 2.4–

Figure 3.4. *Marasmiellus javanicus* grows on different substrates: a. On *Costus dinklagii*; b. On *Salacca zalacca* (Gaertn.) Voss (Scale bar = 0.5 mm).

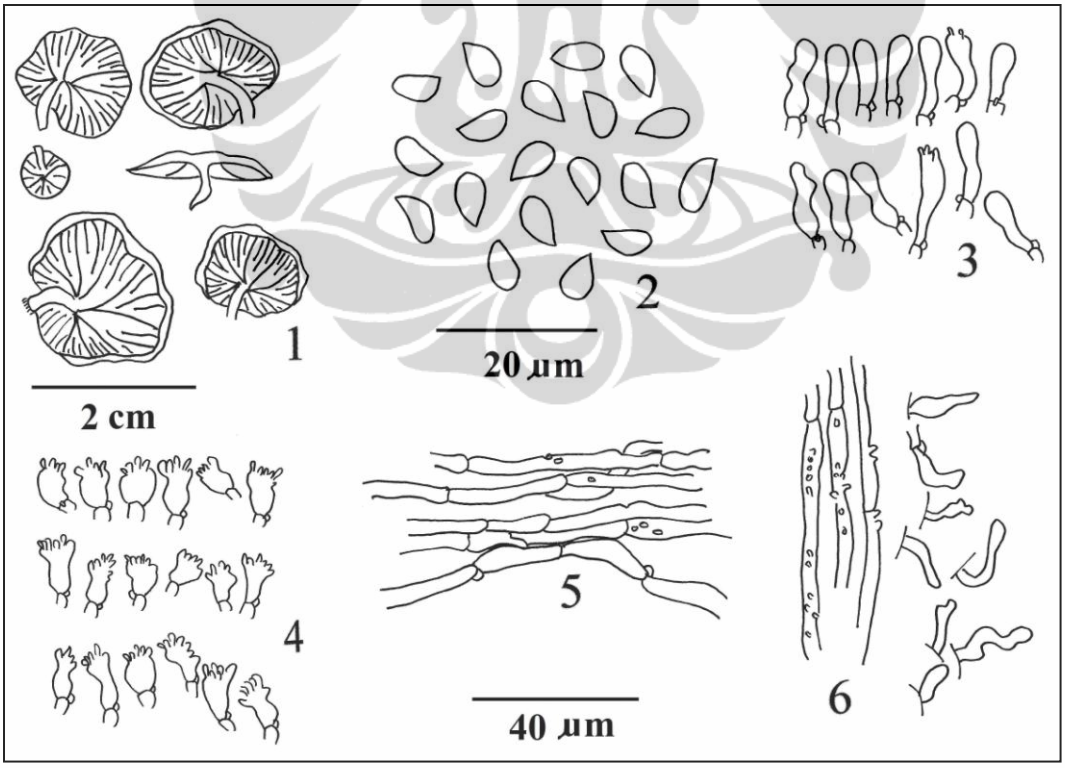


Figure 3.5. *Marasmiellus javanicus* Retnowati (A. Retnowati 811); 1. Basidiomes; 2. Basidiospores; 3. Basidia-Basidioles; 4. Cheilocystidia; 5. Pileipellis; 6. Hyphae of stipe with diverticulate and stipe vesture; number 3, 4, 5, and 6 use the same scale (scale bar = 40 µm).

8.8 µm, thin-walled, hyaline. Stipe tissue monomitic; hyphae 1.6–9.6 µm, cylindrical, parallel, hyaline, thin to thick-walled up to 0.8 µm, diverticulate, inamyloid. Stipe vesture uncommon; main body 24–24.8 x 6.4–7.2 µm, fusoid to clavate or irregular in shaped, thin-walled, hyaline. Clamp connection present.

Distribution: Java.

Habitat: Solitary to gregarious on *C. dinklagii* K. Schum or *S. zalacca* (Gaertn.) Voss.

Etymology: *javanicus* refers to the island of Java where the species was discovered.

Materials examined. Indonesia: Java, West, Bogor Botanical Gardens, on *C. dinklagii*, 1 December 2010, A. Retnowati 809; same location, on *C. dinklagii*, 1 December 2010, A. Retnowati 809-B; same location, on *C. dinklagii*, 1 December 2010, A. Retnowati 810; same location, on *S. zalacca* (Gaertn.) Voss, 1 December 2010, A. Retnowati 811; same location, on *S. zalacca* (Gaertn.) Voss, 1 December 2010, A. Retnowati 813.

Holotype: Indonesia, Java, West, Bogor Botanical Garden, on *S. zalacca* (Gaertn.) Voss, 1 December 2010, A. Retnowati 811.

4. CONCLUSIONS

Marasmiellus javanicus was confirmed in this study as a new species supported by morphological characters and molecular analysis based on ITS rDNA. This new species was different from other known *Marasmiellus* species, i.e *M. hondurensis*, *M. mesosporus*. Based on ITS rDNA using MP method, the sequence similarity of *M. javanicus*, type species, to *M. mesosporus* was 94%. Sequence similarity was less than 99% supported morphological analysis that *M. javanicus* was a distinct species.

5. RECOMMENDATIONS

Discovering the new species provides the information for making conservation decision at the policy maker. As a result, more explorations of *Marasmiellus* at the under-explored areas are highly needed and recommended. By describing and determining more species, the mycologists have enough data to provide recommendation for nature conservation.

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GENERAL DISCUSSIONS

The development of fungal taxonomic study has increased rapidly. Morphological data at first became the major study to distinguish taxa, but at present several approaches have been followed by taxonomists to recognize organisms. However, morphological approach is still predominantly important before doing other approaches. Taxonomists who focus mainly on morpholological data are then interested in learning how other approaches can help them to solve the problem they are facing. In fact, they are excited when one method, such as molecular data, yield the unpredictable result. Then they will try to ensure themselves that other methods can support their research.

The taxonomic problem in the genus *Marasmiellus* is an example among hundred problems in fungal taxonomy in which morphological and molecular data have been used comprehensively. *Marasmiellus* was first described by Murrill (1915), and it has a low variation of morphological characters. As a result, its members are difficult to be identified into species based on morphological characters alone. Internal Transcribed Spacer (ITS) region was used in this study, because it was expected to help in recognizing species.

The ITS rDNA has been widely used to try to resolve phylogenetic relationship of fungi at infrageneric and species level. Oda *et al*. (1999) re-classified the infrageneric classification of the genus *Amanita* based on molecular data of ITS rDNA. They made a molecular phylogenetic study of Japanese *Amanita* species in order to re-arrange the taxonomic treatments within the genus *Amanita*. Thirty six species of Japanese *Amanita* were studied based on nucleotide sequences of the ITS region. Molecular analysis of this study on the genus *Amanita* supported the traditional classification system which based on morphological data. Re-classification system was applied on one of the sub-genus of *Amanita*. This new classification is more robust than previous system, and relationship of some closely related taxa and interspecies can be clearly resolved. Other phylogenetic studies based on ITS rDNA on other genera within *Tricholomataceae* were done by Arruda *et al*. (2005) and Ammirati *et al.* (2007).

Thirty five species of *Marasmiellus* in Java and Bali have been described. Seventeen species of them are new to science, and 1 species as a new combination. All species were treated as a monograph based Singer's infrageneric classification (1973). This classification is widely used by mycologists who work on *Marasmiellus* (Pegler 1977, 1983, & 1986, Desjardin 1997).

It was noted that the 35 species consist of 25 species from Java, 7 species from Bali, and 3 species occur both in Java and Bali. The number of *Marasmiellus* species in Java is higher than in Bali. The different type climate of Java and Bali made this possible, and based on Whitten *et al*. (1996) the eastern part of Java and Bali has a drier climate than the western part of Java due to the moonson wind. This monsoon wind blew from Australia middle of the year, and has a major influence in central and east-central Java and Bali. Normally, this season starts in March and extend almost to January, giving a dry season of about ten months, which is not which is not favourable for mushrooms growth. Corner (1996) published monograph on the genera *Marasmius, Chaetocalathus, Crinipellis, Heiomyces, Resupinatus, Xerula*, and *Xerulina* based mainly on specimen collected in Malay Peninsula and Borneo. Many *Marasmiellus* which he described were hiding under the generic name of *Marasmius* due to his broad generic concept. He described 100 *Marasmius,* and some 5-7 species probably belong to *Marasmiellus*. For example *Marasmius nugatorius* described by Corner (1996) based on material collected from Bukit Timah, Singapore, treated in this study as *Marasmiellus nugatorius*. Examination of species protologue, the characters of this species is considered to represent a species of *Marasmiellus* section *Rameales*. The characters which moved the species from genus *Marasmius* into genus *Marasmiellus* is *Rameales*-structures of pileipellis with terminal and subterminal cells finely and densely verrucose.

The high number of new species in this study (45 % of described species), is accepted, and it is comparable to result of studies on several genera in under-explored areas. Study on the genus *Astrosporina* in Indomalaysia and Australasia produced 21 new species from 30 described species (70 %) (Horak 1979). Monograph of the genus *Marasmius* in Southeast Asia mentioned that 14 species of 34 described taxa (41 %) were a new to science (Desjardin & Horak 1997).

Corner (1996) reported that 132 new species (78%) from 168 described species of white spore mushrooms in Malesia.

Singer's infrageneric classification consists of 10 sections, and the genus *Marasmiellus* in Java and Bali represented 5 sections of them. Sections in Singer's classification were based on combination of macro-micromorphological characters; i.e type of pileipellis (*Rameales*-structure, cutis with or without diverticulate), presence or absence of cheilocystidia, basidiospores sizes, stipe attachment (central, eccentric), and pileus color (pigment or non-pigmented). Analyses the phylogenetic relationship based on morphological and molecular characters were performed by Maximum Parsimony method. Phylogenetic tree based on morphological and molecular characters produced the same topology, and clades consisted of multiple sections. The phylogenetic tree showed that that sections within Singer's infrageneric classification were polyphyletic. Phylogenetic analyses based on morphological and molecular characters of ITS rDNA resulted in this study were not consistent with traditional classification based on morphological characters, i.e. Singer's classification of *Marasmiellus*. Thus, Singer's infrageneric classification should be re-considered in order to establish a modern classification which based on evolutionary relationship.

Marasmiellus is closely related to some genera, *Marasmiellus*, *Gymnopus*, *Collybia*, *Rhodocollybia*, and *Lentinula*. Mata *et al*. (2004) presented how those genera nested into phylogenetic tree based on ITS rDNA. *Marasmiellus juniperinus*, as a type genus of *Marasmiellus*, and some other species of *Marasmiellus* nested into different clades, and it was concluded that the *Marasmiellus* was polyphyletic. In this study, phylogenetic tree of *Marasmiellus* and its related genera was contructed by adding nine sequences of *Marasmiellus* from Java and Bali, 2 sequences of *Marasmius*, and 3 sequences of *Collybia*. The result of the PAUP analysis presented that *M. juniperinus* nested into the same clades as *Gymnopus fusipes* (type genus of *Gymnopus*), thus it confirmed the result of Mata *et al*. (2004).

The result of Mata *et al*. (2004) was not supported by Wilson and Desjardin (2005) who provided molecular data based on nLSU rDNA for the phylogenetis analysis. Wilson and Desjardin (2005) included more taxa of

Marasmiellus than previous paper (Mata *et al.* 2004). The 84 sequences based on nLSU rDNA used in their analysis, and 10 sequences of them were *Marasmiellus* sequences. These 10 sequences represented 7 species of *Marasmiellus* (*M. synodicus, M. juniperinus, M. rameales, M. opacus, M. scorodonius, M. candidus,* and *M. palmivorus*) were used to construct phylogenetic tree using Bayesian method. The phylogenetic tree showed that *M. juniperinus* was separated from *G. fusipes*, a type species of *Gymnopus* with a low support of bootstrap value. The result of Wilson and Desjardin (2005) indicated *Marasmiellus* was unable to be transferred to *Gymnopus*. To resolve this problem, including more *Marasmiellus* taxa and closely related genera in the analysis, it was expected that the relationship of *Marasmiellus* will be elucidated more clearly.

Morphologically, *Marasmiellus juniperinus* and *Gymnopus fusipes* are different, but genetically they are similar. Nucleotides of both species are different from other *Marasmiellus* species and other *Gymnopus* species in this study. In this case, *M. juniperinus* probably have to be transfered to *Gymnopus*, and taxa as belonging to *Marasmiellus* are left to seek a new genus name. However, this changing status needs further analysis and more additional taxa.

At present, to resolve the taxonomical problem of the genus *Marasmiellus*, molecular data is needed.

GENERAL CONCLUSIONS

Morphologically taxonomic study of the genus *Marasmiellus* revealed that there are 35 species in Java and Bali among these 17 of them are new to science (*M. globosus, M. zingiberius, M. rifaii, M. dimidiatus, M. reniformis, M. haurbentesis, M. longisiccus, M. clavatus, M. tamblingensis, M. diverticulatus, M. piperus, M. pruinosus, M. cikanikiensis, M. javanicus, M. cibodasensis, M. desjardinii*, and *M. bisporus*), and 1 species is a new combination (*M. nugatorius*). They are distributed of 5 out of the existing 10 sections in the genus, namely sect. *Marasmiellus*, sect. *Rameales*, sect. *Dealbati*, sect. *Candidi*, and sect. *Stenophylloides*.

The result of analysis of the phylogenetic tree based on morphological and molecular performed by MP method was that sections within Singer's infrageneric classification was polyphyletic.

Relationship of *Marasmiellus* and its closely related taxa (*Gymnopus*, *Marasmius*, *Collybia*, *Lentinula*, and *Rhodocollybia*) was investigated based on ITS rDNA performed by Maximum Parsimony Analysis (MP). The result of the analysis was that *Marasmiellus* was polyphyletic.

To elucidate the specimens of *Marasmiellus*, morphological and molecular data were applied comprehensively. *Marasmiellus javanicus* confirmed as a new species. Morphologically it was different from other *Marasmiellus* species, namely *M. hondurensis*, and genetically *M. javanicus* was different from *M. mesosporus* with sequence similarity of 94%. The sequence similarity was less than 99% indicated that *M. javanicus* was a distinct species from *Marasmiellus mesosporus* and other known species.

RECOMMENDATIONS

After finishing the taxonomic study of the genus *Marasmiellus* in Java and Bali, several recommendations for future study on the genus *Marasmiellus* are as follows:

1. To describe more species of *Marasmiellus*, more fieldworks in Java and Bali or other islands in Indonesia are needed.
2. The discovering of 17 new species of *Marasmiellus* species in Java and Bali in this study indicated that Java and Bali are strategic area for exploring *Marasmiellus*. This result becomes one of important data for making conservation decision at the national level. Lossing biological diversity in Java and Bali is going rapidly due to destructions of habitat in Java and Bali. To maintain the biological diversity in Java and Bali is important to establishing Java and Bali as protected areas.
3. Singer's infrageneric classification should be reconsidered in order to establish a modern classification which based on evolutionary relationship. Morphological and molecular data of ITS region of rDNA supported the result of this study that sections within Singer's infrageneric classification was

polyphyletic. To establish a new modern classification of the genus *Marasmiellus*, more molecular data of *Marasmiellus* species are needed.

4. The molecular data of ITS rDNA generated in this study were very limited due to the unsuccessful of DNA extraction. Therefore, the techniques of genomic DNA extraction should be optimized.

5. Relationship of *Marasmiellus* and its closely related taxa (*Gymnopus*, *Marasmius*, *Collybia*, *Lentinula*, and *Rhodocollybia*) is still a problem. The hyphothesis of previous studies was *Marasmiellus* and its closely related genera are polyphyletic. To support this hyphothesis, multilocus analysis by using the translation elongation factor 1-alpha (tef) and beta-tubulin (*ben*A) protein coding genes as well as intergenic spacer (IGS) regions of the nuclear ribosomal genes are necessary to evaluate the phylogenetic and evolutionary relationship among species in the genus *Marasmiellus* and its closely related taxa.

6. At present, several studies start exploring the economic value of *Marasmiellus*, and some of them have successfully discovered the chemical compound on this genus. *Marasmiellus troyanus* is an example. Nemergut *et al.* (2000) used litter rot of *M. troyanus* as an environment bioremediation agent. *Marasmiellus troyanus* is currently used in several researches to reduce Benzo[a]pyrene (B[a]p) in the environment. This B[a]p is a carcinogenic polyaromatic hydrocarbon resulting from an incomplete combustion production of fossil fuels. Another study found a novel cytokine production inhibitor produced by a basidiomycete which is *Marasmiellus* sp. As a result, the bio-compound in *Marasmiellus* has to be explored.