

**Notes on Indian species of *Calvatia* and *Langermannia* including *Calvatia natarajanii* sp. nov.**

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**Abstract**

A new species, *Calvatia natarajanii*, is presented as well as brief notes on the distribution of *Calvatia sensu stricto* and *Langermannia* in India. *Calvatia natarajanii* is distinguished by basidiomes having yellowish white to orange-white exoperidium, brownish orange to grayish red, persistent endoperidium, grayish orange to brownish orange cottony gleba, persistent cellular subgleba and globose to subglobose, echinulate basidiospores. It is described, illustrated and compared with the closely related taxa *C. cyathiformis*, *C. fragilis*, *C. rugosa*, *C. aff. rugosa*, *C. pyriformis*, *C. vinosa* and *C. rosacea*. Maximum Parsimony analysis based on ITS sequences confirmed that *C. natarajanii* is closely related to *C. fragilis* and distinct from other morphologically similar species. In addition, a key to the species of *Calvatia* and *Langermannia* recorded in India is presented. Four species recognized under *Calvatia* are excluded from the key since their transfer to *Lycoperdon*.

**Key words:** gasteromycetes, puff balls, Lycoperdaceae, taxonomy, tropical fungi

## Introduction

The genus *Calvatia* Fries (1849: 442) is characterized by small to large, globose to pyriform to turbinate, epigeous basidiomes having pulverulent to cottony gleba made up of eucapillitial threads and distinctly ornamented basidiospores, rudimentary to often well-developed subgleba, and dehiscence of peridium occurring by irregular fragmentation. The genus *Langermannia* Rostkovius (1839: 23) differs from *Calvatia* in having medium to larger basidiomes without subgleba. *Calvatia* is a small genus comprising ca. 40 taxa (Kirk *et al.* 2008) that are distributed worldwide. A few species of *Calvatia*, *C. oblongispora* V.L. Suárez, J.E. Wright & Calonge (2009: 324), *C. guzmanii* C.R. Alves & Cortez (2013: 36), *C. holothurioides* Rebriev (2013: 21) and *C. nodulata* Alfredo & Baseia (2014: 2) have recently been described. The morphotaxonomic demarcation of the genus *Calvatia* is in dispute (Zeller & Smith 1964, Kreisel 1989, 1992, 1994, Calonge & Martin 1990). Also, the phylogenetic analysis for the taxonomic determination of the genus *Calvatia s.s.*, *Langermannia* and other closely related puffball taxa of Lycoperdaceae (Chevallier 1826: 348) is not convincing (Krüger & Gargas 2008, Larsson & Jeppson 2008, Bates *et al.* 2009). In India, the genera *Calvatia* and *Langermannia* are well represented and a total of 11 species is recognized and reported from different regions. Of these, nine species belong to *Calvatia sensu stricto* and the remaining three species belong to *Langermannia*. In the present study, the classification proposed based on the type study of herbarium specimens by Calonge & Martin (1990) and adopted by contemporary researchers (Alves & Cortez 2013, Alfredo *et al.* 2014) is followed to describe the new species.

A taxonomic study on fungi was initiated as a part of the project “Developing a Mushroom Germplasm Bank for Western coast of India with special emphasis on Maharashtra and Goa to commercialize their Nutraceutical and Pharmaceutical potential”. In this paper, we provide brief notes on the distribution of *Calvatia* and *Langermannia* species in India and describe a new species of *Calvatia* collected from the Parsik Hills, Navi Mumbai during the mycological exploration of coastal regions of the Western Ghats of Maharashtra.

## Materials & Methods

*Morphological methods:*—Morphological characters were studied from fresh as well as dried basidiomes. The color names and notations are from Korerup & Wanscher (1978). The microscopic structures were based on young as well as fully mature dried specimens. The peridial layers (obtained by thin hand-made sections), capillitial threads and basidiospores were mounted in water, 10% KOH, 2% phloxine, Lactophenol Cotton Blue and Melzer’s reagent. Seventy randomly selected basidiospores (40 from holotype; 30 from isotype) were measured excluding pedicel and ornamentation. The range of spores with extreme values in parentheses precedes average spore dimensions in parenthesis. The range of the spore quotient (Q) represents the length by the width of the individual basidiospores measured. We followed Krüger *et al.* (2001) to recognize the type of capillitial threads. The holotype is deposited at Ajrekar Mycological Herbarium (AMH), Pune and

the isotype is in the Mycological Herbarium of The Energy & Resources Institute, Western Region (MH-TERI-WRC), Navi Mumbai, Maharashtra.

*Molecular methods:*—Genomic DNA extraction and sequencing were done using the facility available at the National Center for Microbial Resource (NCMR), Pune. The genomic DNA was isolated by the standard phenol/ chloroform extraction method (Sambrook *et al.* 1989), followed by PCR amplification of the ITS regions using universal primers ITS1 [5'-TCC GTA GGT GAA CCT GCG G -3'] and ITS4 [5'-TCC TCC GCT TAT TGA TAT GC -3']. The amplified ITS PCR product was purified by PEG-NaCl precipitation and directly sequenced using an ABI® 3730XL automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA) as per manufacturer's instructions. Sequencing was carried out from both ends so that each position was read at least twice. Assembly was carried out using a Lasergene package followed by NCBI BLAST against sequences from closely related species for identification and constructing the phylogenetic tree (Boratyn *et al.* 2013). The sequence generated was submitted to GenBank.

*Phylogenetic analysis:*—Thirty-five additional sequences of closely related species within *Calvatia sensu lato* and two sequences for the out group were obtained in FASTA format from NCBI for construction of the phylogenetic analysis (Table 1). *Chlorophyllum molybdites* (G. Mey. 1818: 300) Masee (1898: 136) and *Agaricus megacystidiatus* Karun., Guinb. & K.D. Hyde (2014: 775) were selected as an out group (Bates *et al.* 2009). The sequences were manually edited and aligned using Clustal W1.6 module in the MEGA 7.0 software (Kumar *et al.* 2016). Phylogenetic trees were constructed with Maximum Parsimony and Maximum Likelihood (not shown) algorithms with the bootstrap value of 1000 replicates for each tree. The MP tree was obtained using the Subtree-Pruning-Regrafting algorithm with search level five in which the initial trees were obtained by the random addition of sequences with ten replicates. The analysis involved 38 nucleotide sequences. The Maximum Likelihood tree was inferred by Kimura 2-parameter model with Subtree-Pruning-Regrafting-Extensive (SPR level 5) of ML Heuristic Method. Initial tree was Maximum Parsimony with very weak branch swap filters and four search threads. The gaps were treated as missing data that were eliminated.

## Results

A BLASTn search comparing the 700 bp nucleotide sequences of *Calvatia natarajanii* with the closely related sequences of taxa obtained from NCBI database showed 99% similarity with uncultured fungus clone MAT-FN3-2 (JX436280.1) (Query cover 98%) and 98% similarity with uncultured fungus clone MAT-FI1-20 (JX436247.1) (Query cover 93%) and *Calvatia fragilis* (Vittadini 1843: 180) Morgan (1890: 168) (AJ486964) (Query cover 93%). In a phylogenetic tree constructed by Maximum Parsimony method, the sequences of the species fall out into three distinct clades. Clade I comprising of *Calvatia* species with violaceous gleba when mature, Clade II includes *Langermannia* species [*Calvatia pachyderma* (Peck 1882: 54) Morgan (1890: 167) , *C. gigantea*

(Batsch 1786: 237) Lloyd (1904b: 166), *C. fenzlii* Reichardt (1870: 135) and *L. gigantea* (Batsch 1786: 237) Rostk. (1839: 23)] and Clade III consists of *Calvatia* species with non-violaceous gleba when mature. Among the species with violaceous gleba, *C. cyathiformis* (Bosc 1811: 87) Morgan (1890: 168) forms a distinct clade that clusters with *C. fragilis*. *Calvatia natarajanii* clusters with an uncultured fungus clone MAT-FN3-2 (JX436280.1) which in turn clusters with *C. fragilis* from Ghana. In the present study, *Calvatia* cf. *leiospora* Morgan (1895: 39) and *C. chilensis* (AJ486965.1) which have violaceous gleba do not fall into any clade of *C. cyathiformis* or *C. fragilis* in both the phylogenetic tree analysis. It is inferred that a specimen (AH 19509) deposited as *C. cyathiformis* could be recognized as a new species and hence the sequences were generated from the voucher specimen and submitted as *C. chilensis* at NCBI by Morino *et al.* (unpublished study), however no further data is available for *C. chilensis* in Index Fungorum as well as MycoBank. Further study is required to confirm this species.

**TABLE 1.** List of the sequences obtained from GenBank.

S. No.	Species	Voucher/Strain	Geographic origin	GenBank Accession
1	<i>Calvatia cyathiformis</i>	Strain JTT10	Tennessee, Maynardville, USA	MF686508.1
2	<i>C. cyathiformis</i> var. <i>cyathiformis</i>	Voucher AH 25225	Oklahoma, USA	AJ486866.1
3	<i>C. cyathiformis</i>	Isolate MP12	Ontario, Canada	KY706183.1
4	<i>C. fragilis</i>	Strain CFRO	Rhone-Alpes, France	AJ617493.1
5	<i>C. cyathiformis</i> var. <i>cyathiformis</i>	Voucher AH 25232	Rhone, Lyon, France	AJ486864.1
6	<i>C. cyathiformis</i> var. <i>crucibulum</i>	Voucher HAJB 2811	La Habana, Cuba	AJ486869.1
7	<i>C. fragilis</i>	Voucher AH 24114	Corrientes, Argentina	AJ486959.1
8	<i>C. fragilis</i>	Voucher AH 25227	Swat, Kalam, Pakistan	AJ486958.1
9	<i>C. fragilis</i>	Voucher K 56043	Swan River, Australia	AJ486960.1
10	<i>C. fragilis</i>	Voucher AH 18553	Baja California, Mexico	AJ486870.1
11	<i>C. fragilis</i>	Voucher Sydow 941 (M)	South Dakota, USA	AJ486871.1
12	<i>C. fragilis</i>	Voucher Cragin 523 (NY)	Kansas, USA	AJ486957.1
13	<i>C. fragilis</i>	Voucher AH 21915	Madrid, Spain	AJ486961.1
14	<i>C. fragilis</i>	Voucher PAD 3309	Italy	AJ486962.1
15	<i>C. fragilis</i>	Voucher AH 25228	Charaa-Tal, Mangolia	AJ486963.1
16	<i>C. fragilis</i>	Voucher AAH 25226	Kwadoso, Ghana	AJ486964.1
17	Uncultured fungus	Clone MAT-FII-20	Puerto Rico	JX436247.1
18	Uncultured fungus	Clone MAT-FN3-2	Puerto Rico	JX436280.1
19	<i>C. candida</i>	Voucher MJ3514	North Europe	DQ112624.1
20	<i>Calvatia</i> aff. <i>rugosa</i>	Voucher MEL:2382849	NT, Darwin, Australia	KP012724.1
21	<i>C. rubroflava</i>	Strain TFB11269	Urugua Provincial Park, Argentina	KY559335.1
22	<i>Calvatia</i> sp.	Voucher KA08-0253	South Korea	KJ909664.1
23	<i>Calvatia</i> sp.	Voucher KA12-1061	South Korea	KF995352.1
24	<i>C. craniiformis</i>	Voucher TNS Kasuya B734	Japan	KF551248.1
25	<i>C. candida</i>	Strain PB101	Kerala, India	GU939632.1
26	<i>C. holothurioides</i>	Voucher LE 287408	Vietnam	JQ734547.1
27	<i>C. holothurioides</i>	Voucher KA11-0287	South Korea	KJ909662.1
28	<i>C. pachydermica</i>	Voucher AN014692 (ARIZ)	Arizona, USA	EU833653.1
29	<i>C. gigantea</i>	Strain 9-7A	Sichuan, China	HM237179.1
30	<i>C. fenzlii</i>	Strain Jz01	Unknown, could be from China	FJ772413.1
31	<i>Calvatia</i> cf. <i>leiospora</i>	Voucher AN014671 (ARIZ)	Arizona, USA	EU833652.1
32	<i>C. chilensis</i>	Voucher AH 19509	Rancagua, Chile	AJ486965.1
33	<i>Agaricus megacystidiatus</i>	Strain LD2012168	Thailand	KF305947.1
34	<i>Chlorophyllum molybdites</i>	Voucher 4-IX-1994	Arizona, USA	AF482836.1

## Taxonomy

*Calvatia* Fr., *Summa veg. Scand.*, Sectio Post. (Stockholm): 442 (1849).

*Calvatia argentea* (Berk.) Kreisel, *Persoonia* 14(4): 434 (1992).

Distribution:—Tamil Nadu: Chennai (Madras) (Kreisel 1992). The German mycologist Dr. Wight collected this species from Chennai and deposited it at the herbarium of Sir W.J. Hooker. Berkeley described this species as *Bovista argentea* Berkeley (1839: 400). Kreisel (1992) restudied the holotype and made a *comb. nov.* as *Calvatia argentea* (Berkeley 1839: 400) Kreisel (1992: 434). However, this species is poorly described and known only from the type (Kreisel 1992, 1994). Furthermore, molecular data is required for this species to confirm its phylogenetic relationships with other closely related taxa.

*Calvatia candida* (Rostk.) Hollós, *Term. Füz* 25: 112 (1902).

Distribution:—Jammu & Kashmir: Gulmarg (Thind & Thind 1982); KERALA: Ernakulam (Manzur *et al.* unpublished study); Uttar Pradesh: Varanasi (Khare 1976).

*Calvatia craniiformis* (Schwein.) Fr. ex De Toni, in Saccardo, *Syll. Fung.* (Abellini) 7: 106 (1888).

Distribution:—Karnataka: Shankaraghatta, Shanthinagar, (Abrar *et al.* 2008).

*Calvatia cyathiformis* (Bosc) Morgan *J. Cincinnati Soc. Nat. His.* 12(4): 168 (1890).

Distribution:—Chattisgarh (Ahlawat *et al.* 2008); Gujarat: Ahmadabad (Anand Rao 1964); Himachal Pradesh:

Chambaghat, Solan (Sharma *et al.* 1978); Maharashtra: Khandala (Nair & Patil 1977), Amravathi region, Taponeshwar,

Tapovan Goshala (Hedawoo & Mohite 2008); Uttar Pradesh: Varanasi & Raibareli (Khare 1976); West Bengal:

Howrah, Mahisgote, Sankrail block (Chakraborty *et al.* 2012).

This is one of the most easily recognized and widely reported species from India. This species is closely related to *C. fragilis*. However, *C. fragilis* is clearly distinguished from *C. cyathiformis* in the field by having a distinctly plicate fruitbody with reduced to almost nil subgleba. Microscopically, *C. cyathiformis* differs from *C. fragilis* in the strongly echinate basidiospores with broad spines that are up to 2 µm long, whereas, *C. fragilis* has echinate spores with shorter spines up to 0.5 µm long (Cortez *et al.* 2012).

*Calvatia fragilis* (Vittad.) Morgan, *J. Cincinnati Soc. Nat. His.* 12: 168 (1890).

Distribution:—Himachal Pradesh: Rajgarh (Gupta *et al.* 1974); Maharashtra: Satara (Chavan & Barge 1977), Pune [Cunningham 1942 reported as *C. lilacina* (Montagne & Berkeley in Berkeley 1845: 64) Hennings (1904: 205)];

Punjab: Gurdaspur (Ahmad 1941a reported as *C. lilacina*).

*Calvatia fragilis* which is also widely recorded in India closely resembles *C. cyathiformis* (see *C. cyathiformis* for discussion). Some researchers treated this species as *C. cyathiformis* forma *fragilis* (Vittadini 1843: 180) A. H. Smith in Smith and Zeller (1964: 150) (Zeller & Smith 1964, Calonge *et al.* 2005), *C. cyathiformis* subsp. *fragilis* (Vittadini 1843: 180) Dring (1964: 38) (Dring & Rayner 1967) and a synonym (Calonge 1998) of *C. cyathiformis*. However, Kreisel (1992) placed *C. fragilis* as a distinct species and the contemporary researchers (Larsson & Jeppson 2008, Bates *et al.* 2009, Trierweiler-Pereira & Baseia 2009, Cortez *et al.* 2012) also treated it as a separate species. In the present manuscript, *C. fragilis* is retained as a distinct species until conclusive reports are available. *Calvatia lilacina* was synonymized with *C. fragilis* (Kreisel 1992).

*Calvatia longicauda* (Henn.) Lloyd, *Mycol. Writ.* 2(Letter 21): 3 (1908).

Distribution:—Uttranchal: Forest Research Institute Campus & Botanical Survey of India Campus, Dehradun (Bisht *et al.* 2006).

*Calvatia natarajanii* Senthil. & C. Ravindran *sp. nov.* (Figs. 1, 2–3)

MycoBank MB 819531; GenBank MF595877

Diagnosis:—Basidiomes with white to orange-white exoperidium, orange to grayish red, persistent endoperidium, grayish orange to brownish orange cottony gleba, persistent, cellular subgleba, globose to subglobose, echinulate basidiospores ( $3\text{--}7 \times 2.5\text{--}7 \mu\text{m}$ ) and *Calvatia*-type capillitial threads.

Type:—INDIA. Maharashtra State, Thane District, Navi Mumbai, Parsik Hills (19.01°83.95'N, 73.03°30.75'E), early South West monsoon season, 24 June 2016, G. Senthilarasu (holotype AMH 9836!, isotype MH-TERI V1-T1).

Etymology:—The specific epithet '*natarajanii*' refers to the late Prof. K. Natarajan, Center for Advanced Studies in Botany, University of Madras, in honor of his significant contribution to Indian Mycology.

Description:—*Basidiomes* 15–75 mm diam., 10–70 mm high, initially subglobose, becoming pear-shaped to turbinate, attached by the rhizomorph with short white rhizoid at the base, 7–15 mm long. *Exoperidium* smooth to furfuraceous, uniformly white to yellowish white (4A3) when young, becoming pale orange-white (5A2, 5A3, 6A3, 7B4), breaking up into small, polygonal to irregular shaped flaky patches that randomly slough off the apical portion, persisting at base, yellowish white (4A3). *Endoperidium* thin, orange-white (5A2) to pale orange (5A3) to grayish orange (6B3) to brownish orange (7C4–7C5) to grayish red (8C4–8C5), thin, persistent even after drying. *Gleba* 15–30 mm high, initially white to pale yellowish white (2A2), not changing on bruising, becoming yellowish gray (3B2) to beige (4C3), finally becoming light orange (5A4) to grayish orange (5B4) to brownish orange (6C4) on drying, cottony, firm, persistent. *Subgleba* 12–30 mm high, initially undifferentiated from gleba, becoming spongy, cellular, white to yellowish white (4A2) when young, becoming yellowish white (4A2) to pale yellow (4A3) on drying.

*Basidiospores*  $(3)4\text{--}5(7) \times (2.5)4\text{--}5(7)$ ,  $(4.7 \pm 0.56 \times 4.46 \pm 0.61) \mu\text{m}$ ,  $Q=1.0\text{--}1.4$ , globose, rarely subglobose, strongly echinulate, echinulae  $\leq 1.5 \mu\text{m}$  high, yellowish brown in water, cyanophilous, dextrinoid; pedicellate in young basidiomes, pedicel short,  $\leq 3.5 \mu\text{m}$  long, apedicellate in mature basidiomes. *Basidia*  $9\text{--}15 \times 8\text{--}10 \mu\text{m}$ , subglobose to pyriform, thin-walled, tetrasporic, bearing four sterigmata,  $\leq 4 \mu\text{m}$  long, evanescent in mature basidiomes. *Capillitial threads* of the *Calvatia* type; eucapillitial threads  $1\text{--}5.5(6) \mu\text{m}$  diam., hyaline, with yellowish to reddish brown thick wall,  $\leq 1 \mu\text{m}$ , fragile, straight to subundulate, septate, often disarticulated at septum, sparsely dichotomously branched, with abundant small to large perforations; perforations  $1\text{--}16 \times 1\text{--}4 \mu\text{m}$ , circular to ovoid to



elongate-cylindrical. *Paracapillitial threads* absent. *Exoperidial hyphae* comprising of loosely arranged, irregular chains of variable shape, simple pyriform, cylindrical, elongate-clavate, ovoid, dumbbell-shaped to lageniform to irregular,  $23\text{--}120 \times 9\text{--}34 \mu\text{m}$ , rarely branched, hyaline to pale brown, thick-walled,  $\leq 1.5 \mu\text{m}$  diam. *Endoperidial hyphae*  $2\text{--}8 \mu\text{m}$  diam., thick-walled, septate. *Subgleba* hyphae  $1\text{--}5.5 \mu\text{m}$  diam., sometimes inflated to  $11 \mu\text{m}$  diam., often sinuous, branched, aseptate, thick-walled,  $\leq 1 \mu\text{m}$  thick; thin-walled, septate. *Rhizomorphs* with three different hyphae: hyaline hyphae, up to  $11 \mu\text{m}$  diam., septate without lumen, branched; subhyaline to pale brown hyphae,  $1\text{--}7 \mu\text{m}$  diam., aseptate with thin lumen, branched; inner subhyaline to pale brown to golden brown hyphae,  $6\text{--}50 \mu\text{m}$  diam., aseptate, unbranched, thin-walled.



**FIGURE 1.** *Calvatia natarajanii*: **A.** Young basidiome. **B.** Fresh young basidiomes showing gleba and subgleba. **C.** Mature basidiome. **D.**

Colour variations among basidiomes. **E.** Dried basidiome. Photos by: Senthilarasu Gunasekaran.

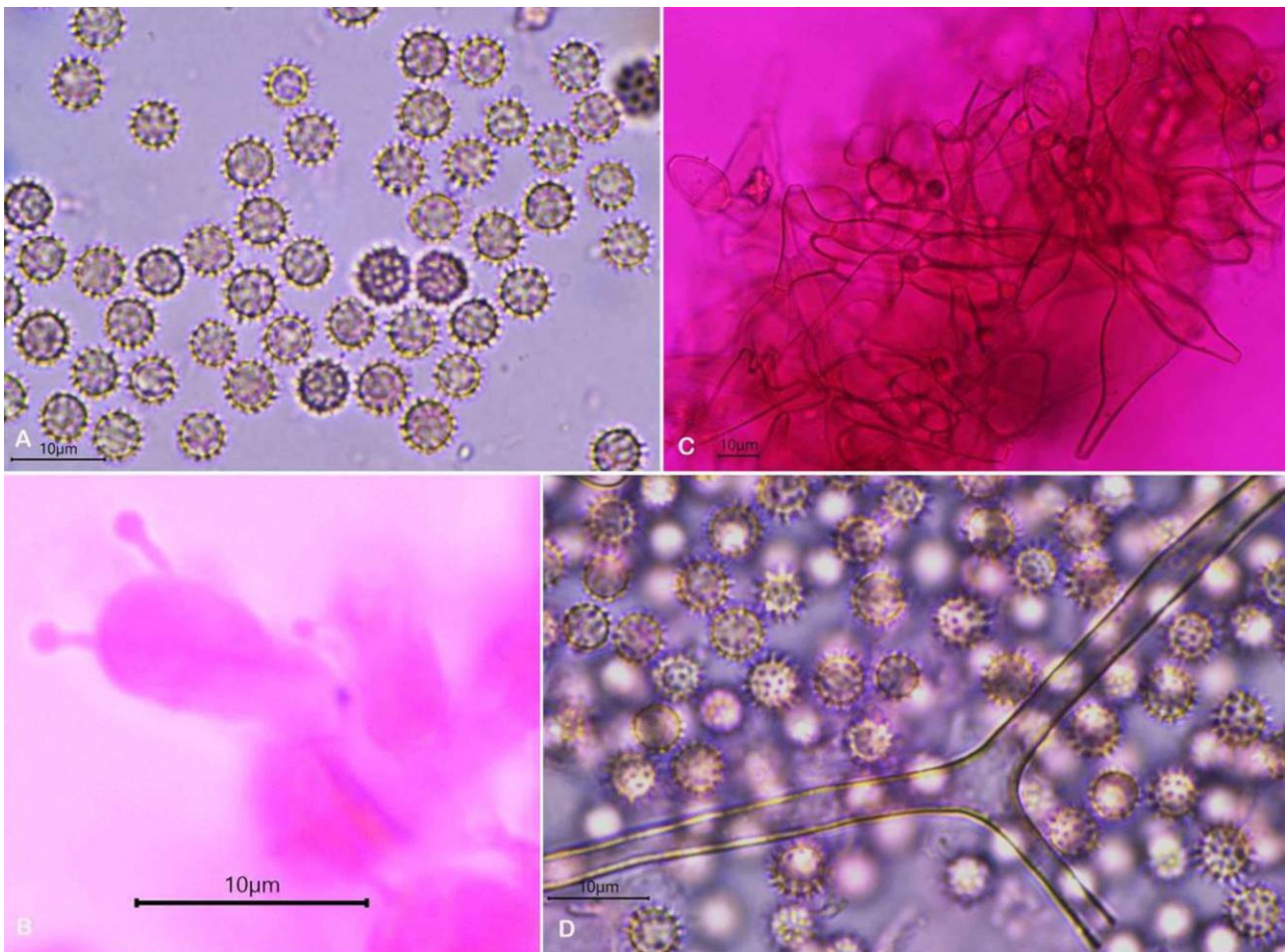
Habitat:—On soil, solitary, scattered to gregarious, under *Samanea saman* (Jacq.) Merr. plantations. Known only from the type locality.

Additional specimens examined:—INDIA. Maharashtra State, Thane District, Navi Mumbai, Parsik Hills ( $19.01^{\circ}83.95^{\circ}\text{N}$ ,  $73.03^{\circ}30.75^{\circ}\text{E}$ ), early South West monsoon season, 13 June 2017 (TERI V13-T1 and in personal collections), *G. Senthilarasu*.

Comments:—Two collections were made from the same locality and more than 20 basidiomes were collected from both the collections. It was observed that the endoperidium was intact in all the collections and the gleba was grayish orange becoming brownish orange on drying.

*Calvatia natarajanii* clusters with the species having violaceous gleba in the phylogenetic tree. However, *Calvatia cyathiformis* and *C. fragilis* differ from *C. natarajanii* macroscopically in having a distinctly violaceous, pulverulent gleba and microscopically in having larger basidiospores (5–7  $\mu\text{m}$ , Kasuya & Retnowati 2006, 5.6–8.0  $\mu\text{m}$ , Bates *et al.* 2009, 6–8.5  $\mu\text{m}$ , Cortez *et al.* 2012 for *C. cyathiformis*, and for *C. fragilis* 6.4–8.0  $\mu\text{m}$ , Bates *et al.* 2009), whereas, *C. natarajanii* has brownish orange gleba and smaller spores (4.7  $\times$  4.4  $\mu\text{m}$ ).

*Calvatia rugosa* (Berkeley & Curtis in Berkeley 1868: 345) Reid (1977: 671) (Cortez *et al.* 2012) closely resembles *C. natarajanii* in having similar shaped and sized basidiomes (23–83 mm diam., 19–66 mm high), similar sized, yellowish brown basidiospores (3–5  $\mu\text{m}$ ) and capillitial threads (2–4.5  $\mu\text{m}$  diam.). However, *C. rugosa* morphologically clearly differs from *C. natarajanii* in having purplish brown to dark brown basidiomes, a granulose to furfuraceous exoperidium with pinkish brown tints at the base, turning yellow on bruising, becoming orange-yellow overall with age and a gleba that exudes distinctly orange-yellow pigment when cut or injured and becomes olive yellow (Cortez *et al.* 2012) to brownish yellow (Bates *et al.* 2009) in age.



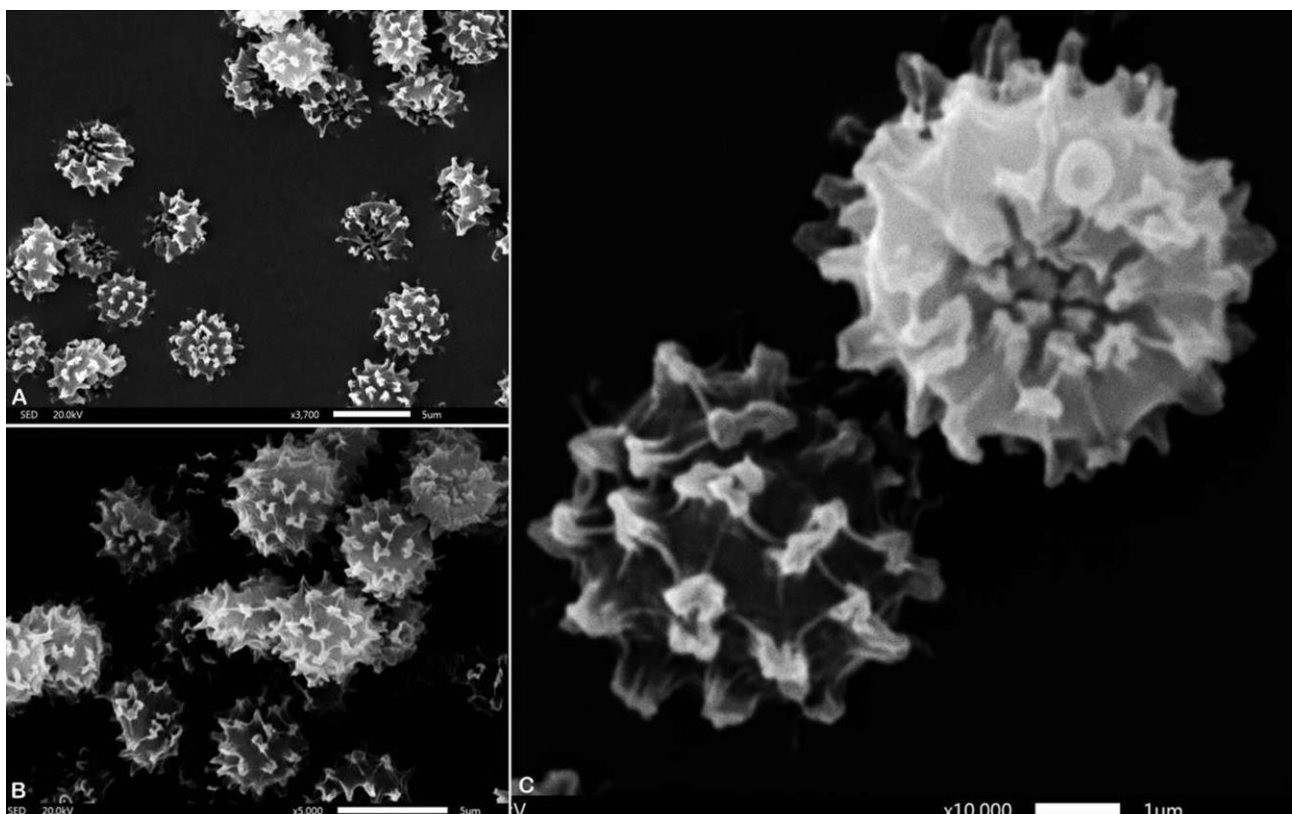
**FIGURE 2.** *Calvatia natarajanii*: **A.** Basidiospores. **B.** Basidia. **C.** Exoperidial hyphae. **D.** Capillitial thread showing branch. Photos by: Senthilarasu Gunasekaran.

*Calvatia rubroflava* (Cragin 1885: 36) Lloyd (1899: 12) is the widely known name in American literature for *C. rugosa*. Based on the description of *Lycoperdon rubroflavum* Cragin (1885: 36), a

basionym of *C. rubroflava* not originally described but reported in Saccardo's *Sylloge Fungorum*, the species is distinguished by the equally rounded basidiomes with an orange-red to orange-brown peridium covered by grayish white spines becoming black, a radicated, wrinkled stipe and an olive orange spore mass with depressed, subglobose basidiospores. In addition, the spores of *L. rubroflavum* are smooth (Cragin 1885, Bates *et al.* 2009).

A species from Sri Lanka, *Lycoperdon rugosum* Berkeley & Curtis in Berkeley (1868: 345), a basionym for the species *C. rugosa*, is characterized by basidiomes having a tomentose peridium, a wrinkled, shortly radicated stipe, a large sterile base confluent with the gleba and yellow, smooth spores Zeller & Smith (1964) synonymized *Lycoperdon rubroflavum* with *Calvatia rubroflava* and characterized the species as having a small amount of homogenous subgleba and subglobose to broadly ovate, weakly ornamented basidiospores. Dring & Reyner (1967) stated that *C. rubroflava* has brightly colored basidiomes becoming yellow on bruising, very little subgleba and typical globose spores. Reid (1977) placed *L. rubroflavum* and *L. rugosum* under *C. rugosa* and described the spores of *C. rugosa* as minutely echinulate. At present, the true identity of *L. rubroflavum*, *L. rugosum* and *C. rubroflava* is not clear.

*Calvatia natarajanii* also resembles *Calvatia* aff. *rugosa* (Bates *et al.* 2009) in having similar sized and shaped basidiomes, similar color of the peridium and similar sized and shaped basidiospores. However, *Calvatia* aff. *rugosa* clearly differs from *C. natarajanii* in having basidiomes that stain yellow when fresh and develop a strong orange coloration in the exoperidium when dry. Furthermore, phylogenetically *Calvatia* aff. *rugosa* nested with *C. rugosa* rather than *C. natarajanii*.



**FIGURE 3.** *Calvatia natarajanii* SEM images of basidiospores: A. 3500 Magnification. B. 5000 Magnification. C. 10000 Magnification.

Photos by: Ravindran Chinnarajan.

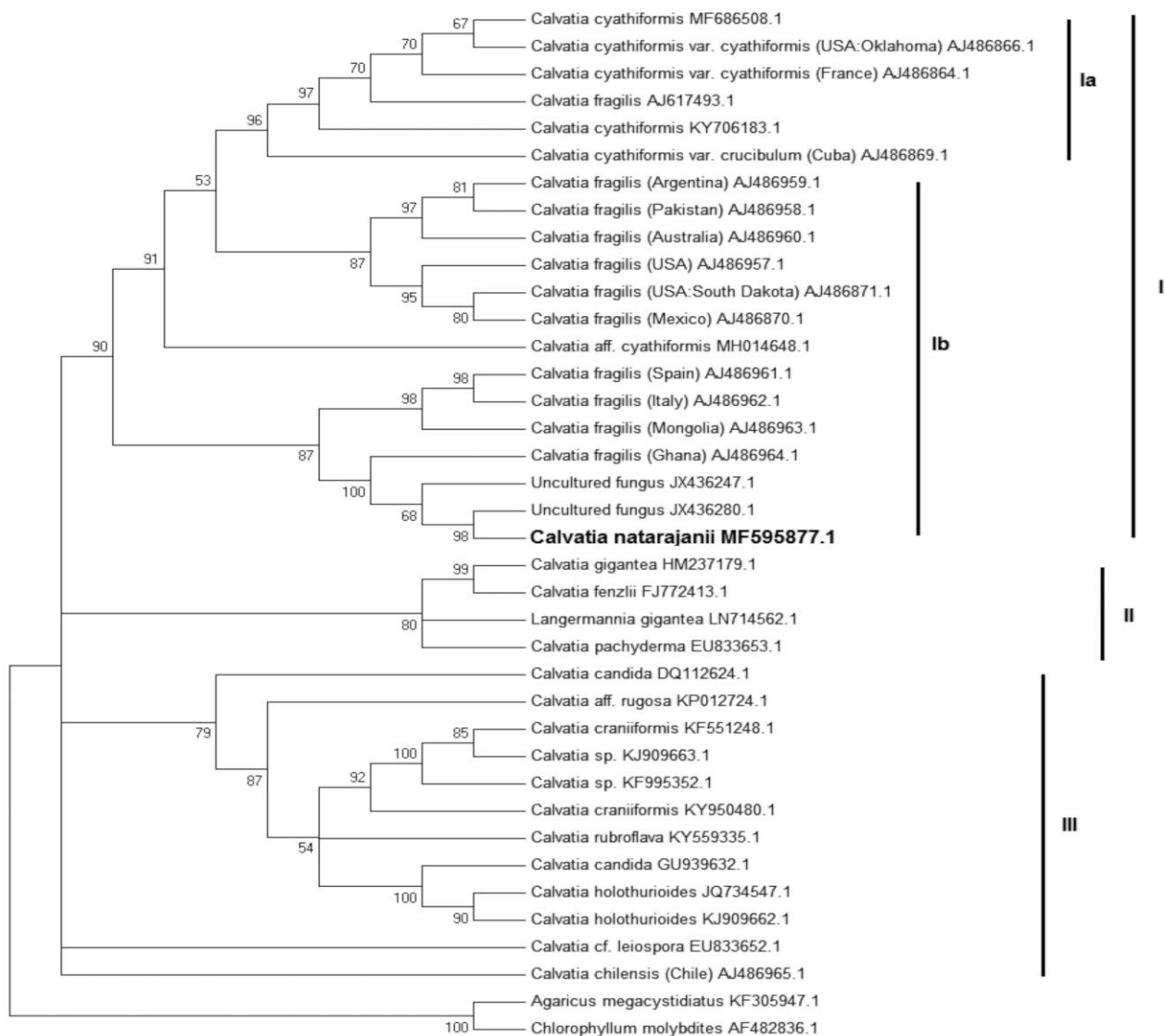
*Calvatia pyriformis* (Léveillé 1846: 164) Kreisel (1992: 433) is macroscopically clearly distinguished from *C. natarajanii* in the larger basidiomes with a chestnut exoperidium and an ochraceous endoperidium becoming rust in color. In addition, the spores of Asiatic specimens of *C. pyriformis* [*C. gardneri* (Berkeley in Berkeley & Broom 1873: 79) Lloyd (1904a: 2)] tend to be broadly ellipsoid (Dring & Rayner 1967).

The other closely related species are *C. vinosa* Kasuya & Retnowati (2006: 286) described from Indonesia and *C. rosacea* Kreisel (1989: 292) described from Macas, Ecuador. *Calvatia vinosa* resembles *C. natarajanii* in the similar sized basidiomes (40–60 × 40–50 mm) with reddish peridium, the ochraceous gleba, the minutely developed subgleba and the somewhat similar sized, basidiospores (4–5.5 µm). However, *C. vinosa* clearly differs from *C. natarajanii* in having a yellowish brown to reddish brown exoperidium with furfuraceous spines, wine red to reddish purple endoperidium, pulverulent gleba and subglobose to ovoid, verrucose basidiospores, whereas, *C. natarajanii* has an orange-white, smooth to furfuraceous exoperidium, a brownish orange to grayish red endoperidium, a cottony gleba and globose to subglobose, echinulate basidiospores. *Calvatia rosacea* differs from *C. natarajanii* in having a pinkish exoperidium, non-pitted capillitial threads and relatively smaller, globose, punctate or finely verruculose basidiospores (3.5–4.1 vs 3.0–6.0 µm).

*Calvatia pyriformis* (Lév) Kreisel, *Persoonia* 14(4): 433  
(1992) *Calvatia gardneri* was synonymized with *C. pyriformis* (Kreisel 1992).

A specimen collected by Thwaites (1006) from Sri Lanka in 1868 was described as *Lycoperdon gautieroides* (Berk. & Br. 1873: 79). Later this species was transferred to *Calvatia gautieroides* (Berk. & Br. 1873: 79) Petch (1919) by Petch (1919, reference not found). Another specimen collected from Sri Lanka was described as *Lycoperdon gardneri* Berkeley in Berkeley and Broom (1873: 79) and was later transferred to *C. gardneri* (Lloyd 1904). However, there is confusion because Dring and Rayner (1967) mentioned that *Lycoperdon gautieroides* is a synonym of *C. gardneri*. *Hippoperdon pyriforme* Léveillé (1846: 164) is a basionym of *Calvatia pyriformis*. Kreisel (1992) made a combination nova *Calvatia pyriformis* based on the specimen *Hippoperdon pyriforme* and synonymized both *Calvatia gardneri* and *Calvatia gautieroides*.

Distribution:—This species has been reported as *C. gardneri* rather than *C. pyriformis* in India. Assam (Lloyd 1904–1919); Maharashtra: Pune (Nair & Patil 1977); Meghalaya: Shillong (Lloyd 1904–1919); West Bengal (Lloyd 1904–1919).



**FIGURE 4.** Maximum Parsimony tree showing phylogenetic position of *Calvatia natarajanii*. Bootstrap values  $\geq 50\%$  are indicated.

*Calvatia rugosa* (Berk. & M.A. Curtis) D.A. Reid, *Kew Bull.* 31: 671 (1977).

Distribution:—Jammu & Kashmir: Kishtwar, Padder region (Kumar & Sharma 2011). Kumar & Sharma (2011) reported this species as *Calvatia rubroflava*. Based on their original description, this species is characterized by medium-sized basidiomes with a cream to white, smooth peridium, a cream colored gleba turning yellow to olive yellow, and smooth to warted, globose basidiospores. However, the illustration clearly depicts that the spores are subglobose to ovoid and warted. Although, it is very difficult to place this species in *C. rugosa* due to inadequate descriptions, at present, it is treated as the same species until further study of the specimen has been carried out for verification.

*Langermannia* Rostk., in Sturm, *Deutschl. Fl.*, 3 Abt. (Pilze Deutschl.) 5: 23 (1838)

*Langermannia bicolor* (Lév.) Demoulin & Dring, *Bull. Jard. Bot. natn. Belg.* 45(3–4): (1975).  
Reported in India as *Calvatia bicolor* (Léveillé 1846: 162) Kreisel (1992: 435)

Distribution:—Andaman & Nicobar Islands: Nicobar islands [Kreisel 1992 reported as *Lasiosphaera fenzlii* Reichardt (1870: 135)]; Maharashtra: Mumbai (Bombay) [Bottomley 1948 reported as *Lanopila wahlbergii* Fries (1849: 151)].

This species was deposited at Kew Herbarium as well as preserved in Kreisel's personal collection.

*Langermannia gigantea* (Batsch) Rostk., in Sturm, *Deutschl. Fl.*, 3Abt. (Pilze Deutschl.) 3: 23 (1839).

Reported as *Calvatia gigantea*.

Distribution:—Jammu & Kashmir: Sonamarg (Cunningham 1942).

Ahmad (1941b) reported a species as *C. gigantea* from Sonamarg, Kashmir. Based on the description as well as comments of the species, the endoperidium has a definite mouth rather than flaking away for disseminating basidiospores. Hence, it could be a species of *Lycoperdon* rather than *C. gigantea*. Further study is required for authentic identification of this species.

*Langermannia pachyderma* (Peck) Kreisel, *Feddes Repert.* 62: 120 (1962).

Reported as *Calvatia pachyderma*.

Distribution:—Himachal Pradesh: Khadralla (Gupta *et al.* 1974).

Excluded species

*Calvatia caelata* (Bull.) Morgan, *J. Cincinnati Soc. Nat. Hist.* 12(4): 169 (1890).

Synonymized with *Lycoperdon utrifforme* Bulliard (1790: 450).

Distribution:—Himachal Pradesh: Babeh Pass & Bashahr state (Ahmad 1941b, Cunningham 1942), Rajgarh (Gupta *et al.* 1974 as *Calvatia elata*).

*Calvatia excipuliformis* (Scop.) Perdeck, *Blumea* 6: 490 (1950).

Synonymized with *Lycoperdon excipuliforme* (Scopoli 1772: 488) Persoon (1801: 143).

Distribution:—Jammu & Kashmir: Gulmarg, Ningal nallah, Pahalgam (Thind & Thind 1982).

*Calvatia lycoperdoides* Kościelny & Wojt., *Roczn. Nauk. rol.* 34: 141 (1935).

Synonymized with *Lycoperdon excipuliforme*.

Distribution:—Jammu & Kashmir: Athkhar region, Bhadarwah (Kumar & Sharma 2011).

*Calvatia saccata* (Vahl) Morgan, *J. Cincinnati Soc. Nat. Hist.* 12(4): 171 (1890).

Synonymized with *Lycoperdon excipuliforme*.

Distribution:—Jammu & Kashmir: Kashmir, Baramulla (Ahmad 1942); Uttar Pradesh: Mussoorie, Arnigadh (Ahmad 1942, Bottomley 1948).

## Key to the Indian species of *Calvatia* and *Langermannia*

1. Subgleba distinctly present, occupying  $\geq 1/3$  of the stipe, or reduced  
2
1. Subgleba absent or reduced  
10
2. Gleba violaceous at maturity  
3
2. Gleba yellowish, grayish, brownish or olivaceous, but not violaceous at maturity  
4
3. Basidiomes (50)60–70(80) mm diam., 75–115 mm high, subglobose, pyriform to turbinate; exoperidium white to pinkish, becoming purplish brown; endoperidium purplish brown; gleba initially white, becoming purplish brown; subgleba  $\geq 20$  mm, white, becoming purplish brown, persistent as a cup like base even after the dispersion of spores; basidiospores mass purplish brown,  $4-7 \times 4-6 \mu\text{m}$ , globose to subglobose, verrucose..... *C. cyathiformis*
3. Basidiomes 50 mm diam., 40 mm high, ovate; exoperidium thin, brownish to wheat brown, becoming purple brown; endoperidium deep purple brown; gleba purple brown; subgleba absent or reduced to  $\leq 20$  mm; basidiospores mass purplish brown,  $3-4.5 \mu\text{m}$ , globose, finely verrucose.....*C. fragilis*
4. Gleba grayish orange to brownish orange to yellowish brown  
5
4. Gleba grayish to olivaceous.  
9
5. Gleba yellowish brown, peridia distinctly staining yellow, basidiomes becoming orange with age  
*C. rugosa*
5. Peridia not staining yellow  
6
6. Gleba grayish orange to brownish orange on drying, cottony, persistent; exoperidium white to orange-white; endoperidium orange to grayish red, persistent, grayish orange to brownish orange; subgleba cellular, pale yellow on drying; basidiospores  $3-7 \mu\text{m}$ , globose to subglobose, echinulate ..... *C. natarajanii*
6. Gleba yellowish brown.  
7
7. Basidiomes 150 mm diam., 100 mm high, initially globose, becoming skull-shaped to strongly wrinkled; peridium initially white, becoming brownish; gleba initially white, becoming yellowish to olive brown; subgleba initially white, becoming yellowish to olive brown; basidiospores  $2.5-3.5 \mu\text{m}$ , globose to subglobose, finely verruculose .... *C. craniiformis*
7. Basidiomes not skull-shaped or distinctly wrinkled.  
8
8. Basidiomes 40–80 mm diam.,  $\leq 70$  mm high, turbinate with long, tapering base; exoperidium dark brown, verrucose; endoperidium orange-yellow; gleba yellowish brown to dark yellowish brown; subgleba cellular, dark yellowish brown; basidiospores  $3.3-4 \times 3.3-3.39 \mu\text{m}$ , globose to subglobose, verruculose.....*C. longicauda*
8. Basidiomes 40–100 mm diam., 20–40 mm high, subglobose, plicate at the base; exoperidium initially white, becoming reddish brown; gleba white; subgleba present; basidiospores  $3.5-5 \mu\text{m}$ , globose to subglobose, warty..... *C. pyriformis*

9. Basidiomes 53–67 mm diam., 25.5 mm high, ovate, depressed; exoperidium silver; endoperidium silver; gleba dark olivaceous brown.....  
.....*C. argentea*
9. Basidiomes 15–35 mm in diam., globose to subglobose; exoperidium yellowish brown to light brown, or grayish white above, pale pinkish below, furfuraceous, fugacious, areolate; endoperidium yellowish brown; gleba white, becoming light to dark brown to grayish brown or olivaceous; subgleba present, reduced, yellowish; basidiospores mass olivaceous brown, 3.5–6.5 µm, globose, verrucose.....  
..... *C. candida*
10. Basidiomes globose; exoperidium smooth; endoperidium persistent in mature basidiomes; gleba olivaceous brown; subgleba absent; basidiospores echinulate .....*L. bicolor*
10. Endoperidium not persistent, breaking apart and sloughing away in mature basidiomes.  
1
11. Basidiomes 50–100 mm diam., globose to subglobose; peridium white becoming grayish yellow to olivaceous brown, smooth; gleba initially white, becoming olive yellow to olive brown; basidiospores 3–4 µm, globose to subglobose, smooth.....  
.....  
..... *L. gigantea*
11. Basidiomes 40–75 mm diam., 35–60 mm high, subglobose to subcylindrical; peridium brown, smooth to granulated; gleba bright olivaceous; subgleba absent; basidiospores mass olivaceous yellow, 4.0–6.5 µm, globose to ovate, smooth.....*L. pachyderma*

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