COMMUNICATION

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Vadivelu Kumaresan, Chakravarthy Sariha, Thokur Sreepathy Murali & Gunasekaran Senthilarasu

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Occurrence of gilled fungi in Puducherry, India

Vadivelu Kumaresan 1,2, Chakravarthy Sariha 2, Thokur Sreepathy Murali 3 & Gunasekaran Senthilarasu 4

1,2 Department of Botany, Kanchi Mamunivar Government Institute for Postgraduate Studies and Research, Puducherry 605008, India. 3 Department of Biotechnology, Manipal School of Life Sciences, Manipal Academy of Higher Education, Manipal, Karnataka 576104, India. 4 Weikfield Foods Private Ltd., Jamdar Hills Road, Bakori, Pune, Maharashtra 412207, India.

Abstract: Thirty-three species of gilled fungi belonging to 23 genera and 14 families were recorded from Puducherry, southern India. Agaricaceae were represented by eight species, followed by Psathyrellaceae (5), Lyophyllaceae & Marasmiaceae (3 each), Hymenogastraceae, Pleurotaceae, Pluteaceae, & Polyporaceae (2 each), and Biannulariaceae, Bobbitiaceae, Omphalotaceae, Schizophyllaceae, Strophariaceae, & Tricholomataceae (1 each). Fourteen species of agarics are new reports from Puducherry. Chlorophyllum rhacodes, Lactocollybia epia, Leucoagaricus meleagris, and Schizophyllum commune were widely distributed. Phylogenetic relationships of the abundant species C. rhacodes, L. epia, and L. meleagris were inferred by maximum likelihood method.

Keywords: Agarics, Agaricaceae, mushrooms, phylogeny, southern India, taxonomy.
INTRODUCTION

Gilled fungi belonging to *Agaricales* Underw. constitute ~10% of fungal species described so far (Kirk et al. 2008). It has been assumed that India hosts one-third of the global fungal taxa (Manoharachary et al. 2005) and hence there is an urgent need to document fungi in the unexplored parts of this country covering all possible habitats and seasonal variations. This will help in maintaining the germplasm of these important fungi, as well as to screen these macrofungi for their unique and versatile metabolic potential.

Gilled fungi in Puducherry have not been extensively studied. Studies on the diversity of macrofungi in adjacent areas are by Mani & Kumaresan (2009a,b). Thirty species of white-spored agarics have been reported from Puducherry (Kumaresan et al. 2011), although their identity was not confirmed by phylogenetic inferences. With the rapid deterioration of natural habitats due to human activity, it has become imperative to record these fungi before they become extinct. The study becomes even more interesting considering the fact that these basidiomata are ephemeral, especially the gilled fungi. Moreover, scientists have taken recourse to molecular techniques for identification of these poorly-studied organisms. Many Indian species are called after their North American or European lookalikes (Cannon & Kirk 2007). Sequencing the internal transcribed spacer region for as many fungi as possible from different regions will help immensely in creating or adding to the existing sequence database, to resolve the identities of species complexes and uncover new taxa.

MATERIALS AND METHODS

Basidiomata were sampled during the rainy season of 2007–2009 and 2016–2019 from different places in Puducherry, located 160 km south of Chennai on the southeastern coast of India. The area has a tropical climate and receives a mean annual rainfall of around 126 cm during the north-east monsoon in the months of October–December. During collection, photographs of fresh specimens were taken and morphological characters of fresh basidiomata such as colour (Kornerup & Wanscher 1978), size, and gill attachment were recorded in the field (Senthilarasu & Kumaresan 2018). Dried basidiomata were sealed in zip lock polythene covers after labeling for further microscopic studies. Samples are maintained in the mushroom herbarium collection in the Department of Botany, Kanchi Mamunivar Government Institute for Postgraduate Studies and Research, Puducherry, India.

**Microscopic examination**

Thin hand-made sections of the pileus and gills were taken and revived in 5–10 % KOH and stained with phloxine (1 %). Microscopic features were recorded following Largent (1977). Approximately, 30 basidiospores sections were measured, excluding the apiculus. The spore quotient (Q) was obtained by dividing the mean length by the mean width in profile view.

**DNA extraction and PCR amplification**

Few nuclear ribosomal internal transcribed spacer sequences are available for a majority of the species observed here, thus we isolated whole genomic DNA and amplified the ITS sequence to compare it with available sequences in the NCBI database.

The pure fungal culture of *Leucoagaricus meleagris* was inoculated onto potato dextrose agar and grown for 10 days at 26 °C, and the mycelia were processed for genomic DNA isolation (Paranetharan et al. 2018). Dried basidiomata of *Chlorophyllum rhacodes* and *Lactocollybia epia* were processed for genomic DNA isolation following the method of Gardes & Bruns (1993). Using the fungal specific primers ITS1F (CTTGGTGATTAGAGGAAGTAA) and ITS4B (CAGGAGACTTTGACGCGTCCAG) (Gardes & Bruns 1993), a PCR reaction was performed to amplify the internal transcribed spacer (ITS) region. The PCR mix consisted of PCR buffer, forward and reverse primers (10 μM each), dNTPs (4 mM), Taq Polymerase (1 U), DMSO (1 %), MgCl₂ (25 mM) and genomic DNA (10–25 ng).

The PCR amplification was performed as follows: 95 ºC for 10 min, 30 cycles of 95 ºC for 30 s, 55 ºC for 30 s and 72 ºC for 60 s; and 72 ºC for 10 min. The PCR products were purified and sequenced using ABI 3130 genetic analyzer using primers ITS1F and ITS4B.

**Phylogenetic analyses**

Sequences were compared using NCBI Blast. Sequences with significant matches were selected and aligned using ClustalW (Thompson et al. 1994), checked visually and edited as required, and evolutionary trees were inferred using the maximum likelihood approach (Kimura 1980) using MEGA v6.06 (Tamura et al. 2013). Bootstrap analysis (1,000 replicates) was performed to calculate the branch support (Felsenstein 1985).
RESULTS

A total of 33 species of gilled fungi from 23 genera in 14 families were recorded from Puducherry. Of these, eight species from four genera belonged to Agaricaceae, constituting the dominant family among the 14 agaric families. Psathyrellaceae was represented by five species from three genera, Lyophyllaceae by three species from one genus and Marasmiaceae by three species from three genera, and Hymenogastraceae, Pleurotaceae, Pluteaceae, and Polyporaceae by two species each (Table 1, Images 1–3).

TAXONOMY

**Agaricus endoxanthus** Berk. & Broome, J. Linn. Soc., Bot. 11(no. 56): 548 (1871).

Pileus 40–90 mm diam., convex to plano-convex with broad umbo, dark brown (6E8) to henna brown (7E8) at disc, fading towards margin, pileus easily peeling off, surface dry, appressed fibrilllose, margin decurved, entire. Lamellae free, crowded, reddish-brown (8D6), edge smooth. Stipe 45–100 5–13 mm, central, terete, broadened towards base, white, greyish-brown (5D3) near base, fleshy fibrous, hollow, surface smooth. Annulus superior, membranous, large. Basidiospores 4.5–6.0 × 3–4.5 µm, Q= 1.46, ovoid to ellipsoid, brown, thick-walled.

On ground, in groups. (PY096).

**Agaricus trisulphuratus** Berk., Ann. Mag. nat. Hist., Ser. 5 15: 386 (1885).

Pileus 20–30 mm diam., globoso-canaplanate to convex, surface with cadmium orange (5A8) to salmon orange (6C4) with thick pulverulent veil, later fading away, margin appendiculate. Lamellae free, dark henna brown (7E8), crowded. Stipe 25–45 × 2–4 mm, terete, equal, surface below the annulus concolorous with the pileus and covered by pulverulent veil. Annulus superior, membranous, large. Basidiospores 4.5–6.0 × 3–4.5 µm, Q= 1.46, ovoid to ellipsoid, brown, thick-walled.

On ground, solitary. (PY109).

**Agrocybe manihotis** Pegler, Kew Bull. 21(3): 508 (1968).

Pileus 30 mm diam., convex, greyish–orange (5B3), smooth, margin decurved, entire. Lamellae adnexed, brownish grey (5C2), crowded. Stipe 45 × 5 mm, central, concolorous with the pileus, cartilagenous, smooth. Spore-print brown. Basidiospores 10.5–12 × 6.5–7.5 µm, Q= 1.61, ellipsoid, thick-walled with truncated germ pore, brown. Pleurocystidia pyriform, 32–45 × 16–20

### Table 1. Gilled fungal species recorded from Puducherry, India.

| Family         | Genus                  | Species                                |
|----------------|------------------------|----------------------------------------|
| Agaricaceae    | Agaricus               | Agaricus endoxanthus Berk. & Broome    |
|                |                        | Agaricus trisulphuratus Berk.          |
| Chlorophyllum  |                        | Chlorophyllum molybdites (G. Mey.) Massae* |
| Leucoagaricus  |                        | Leucoagaricus meleagris (Gray) Singer  |
| Leucoagaricus  |                        | Leucoagaricus serenus (Fr.) Bon & Boffard* |
| Leucocrinus    |                        | Leucoagaricus cepistipes (Sowerby) Pat.* |
| Leucocrinus    |                        | Leucocrinus cepistipes (Sowerby) Pat.* |
| Biannulareaae  | Macrocybe              | Macrocybe loboyensis (R. Heim) Pegler & Lodge |
| Bolbitiaceae   | Panaeolus              | Panaeolus cyanescens Sacc.*            |
| Hymenogastraceae| Gymnopilus             | Gymnopilus subtropicus Hesler           |
| Nauvoria       |                        | Nauvoria conicopipilata (Henn.) Sacc.* |
| Lycophyllaceae | Termitomyces           | Termitomyces clypeatus R. Heim          |
| Marasmiaceae   | Crinellis              | Crinellis megalospora (Beeli) R. Heim*  |
| Lactocylobia   |                        | Lactocylobia epig (Berk. & Broome) Pegler** |
| Tetrapyrus     |                        | Tetrapyrus nigripes (Fr.) E. Horak*     |
| Omphalotaceae  | Marasmiellus           | Marasmiellus confluentes (Pers.) J.S. Oliveira |
| Pleurotaceae   | Hohenbuehelia         | Hohenbuehelia atrocoerulea (Fr.) Singer* |
| Pleurotus      |                        | Pleurotus ostreatus (Jacq.) P. Kumm.*  |
| Pluteaceae     | Volvariella            | Volvariella hypophysis (Fr.) Shaffer*   |
| Polyporaceae   | Lentinus               | Lentinus cladopus Lév.*                 |
| Lentinus       |                        | Lentinus squarrosum Mont.*              |
| Psathyrellaceae| Coprinopsis            | Coprinopsis lagopus (Fr.) Redhead, VIlgaly & Moncalvo |
| Parasola       |                        | Parasola pilqatilis (Curtis) Redhead, VIlgaly & Hopple |
| Pithyrella     |                        | Pithyrella candolleiana (Fr.) Maire     |
| Parasolal      |                        | Pithyrella glaucescens Dennis           |
| Schizophyllaceae| Schizophyllum          | Schizophyllum commune Fr.*              |
| Stephariaceae  | Agrocybe               | Agrocybe manihotis Pegler               |
| Tricholomataceae| Lepista               | Lepista hyalodes (Berk. & Broome) Pegler** |

*The species have already been recorded with brief descriptions in Kumaresan et al. (2011). The remaining species are recorded for first time from Puducherry. *Incertae sedis.
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Image 1. A—Agaricus endoxanthus | B—Agaricus trisulphuratus | C—Gymnopilus subtropicus | D&E—Leucocoprinus meleagris | F—Marasmiellus confluens | G—Macrocybe lobayensis. © Vadivelu Kumaresan.
μm. Cheilocystidia broadly clavate to cylindric, 24–30 × 8–10 μm.

On ground along the grass, solitary. (PY1746).

**Coprinopsis lagopus** (Fr.) Redhead, Vilgalys & Moncalvo, in Redhead, Vilgalys, Moncalvo, Johnson & Hopple, Taxon 50(1): 229 (2001).

Pileus 30–45 mm diam., plano-convex to plane, initially yellowish-brown (5D8) at the disc, becoming brown (6E8), brownish-orange (5C5, 5C4) towards margin, surface dry, margin plane, crenate, plicate-striate. Lamellae adnate, subdistant, width 3 mm, teak brown (6F5), edge smooth. Stipe 30–55 × 2–4 mm, central, terete, with slightly bulbous base (10 mm diam.), white, surface with striations and superficial pruinose scales, cartilagenous, hollow, small collar like ring at the base. Rhizomorphs present. Spore-print black. Basidiospores 9.5–12 × 5.5–7 μm, Q = 1.78, ellipsoid to elongate-ellipsoid, truncated by apical germ-pore, black, smooth.

Scattered, on ground. (PY098).

**Gymnopilus subtropicus** Hesler, Mycol. Mem. 3: 41 (1969).

Pileus 20–60 mm diam., convex to plane, apricot yellow (5B6) fading towards the margin to butter yellow (4A5), squamulose at the disc reddish-brown (9E8), greyish ruby (12D7) in young, surface dry, margin decurved, entire. Lamellae adnate with decurrent tooth, close, greyish-orange (5B4), gill edge smooth, lamellulae of 5 lengths, width 5 mm. Stipe 30–50 × 3–8 mm, terete, hollow, butter yellow (4A5), base hygrophanous to reddish-brown (9F8), fleshy fibrous, striate due to appressed scales. Spore-print brownish-orange. Basidiospores 5.5–8 × 4–5 μm, Q = 1.51, ellipsoid, brown, verruculose.

On palm trunk, in groups. (PY119).

**Leucoagaricus meleagridis** [Gray] Singer, Lilloa 22: 422 (1951) [1949].

Pileus 25–35 mm diam., convex to expanded convex, broadly parabolic when young, dark brown (8F8) at the disc, white towards the margin, surface pruinose, margin decurved, entire. Lamellae free, white, crowded. Stipe 60–110 × 5–8 mm, central, terete, expanding towards the base, fleshy fibrous, smooth, solid. Anulus superior. Spore-print white. Basidiospores 6–8 × 5–6 μm, Q = 1.53, broadly ellipsoidal to ellipsoid, slightly truncated with germ-pore, hyaline, dextrinoid with a thickened wall, guttulate. Pleurocystidia absent. Cheilocystidia 25–45 × 10–15 μm ellipsoidal to short cylindric with pronounced mucronate apex.

On decaying wood, in groups and scattered. (PY19111).

**Macrocybe lobayensis** (R. Heim) Pegler & Lodge, in Pegler, Lodge & Nakasono, Mycologia 90(3): 498 (1998).

Pileus 50–120 mm diam., convex, white, plane, dry, margin decurved, entire. Lamellae adnate, whitish to cream, crowded. Stipe 40–100 × 15–35 mm, central, white, fleshy fibrous, smooth, solid. Spore-print white. Basidiospores 4–6 × 3–4.5 μm, Q = 1.32, broadly ellipsoid to ellipsoid, thin-walled, hyaline.

On ground, on soil root interface, solitary. (PY19126).

**Marasmiellus confluens** (Pers.) J.S. Oliveira, in Oliveira, Vargas-Isla, Cabral, Rodrigues & Ishikawa, Mycol. Progr. 18(5): 734 (2019).

Pileus 15–25 mm diam., convex to plane, dry, reddish brown (9E8) at the disc, brown (6D8) towards the margin, margin decurved, striate. Lamellae adnexed, white to yellowish-white (1A2), crowded. Stipe 25–60 × 2–3 mm, central to slightly eccentric, concolorous with the pileus, terete to compressed. Spore-print white. Basidiospores 5–6.5 × 2–3 μm, Q = 2.34, elongate to cylindric, nearly fusoid, hyaline, inamyloid. Pleurocystidia absent. Cheilocystidia 32–40 × 3.5–5.5 μm, cylindric to subfusoid, flexuous, often somewhat lobed and diverticulate.

On leaf litter in groups, scattered. (PY1931).

**Panaeolus cyanescens** Sacc., Syll. fung. (Abellini) 5: 1123 (1887).

Pileus 20–35 mm diam., convex to conico-convex, disc brownish-grey (5C3), yellowish-white (4A2) to yellowish-grey (4B2), towards margin, surface dry, smooth, becoming bluish-green on bruising, margin decurved, entire. Lamellae adnate to adnexed, close, yellowish-brown (5D8) to rawumber (5F8). Stipe 50–60 × 2–3 mm, terete, equal, yellowish white (4A2) to yellowish-grey (4B2), cartilagenous, hollow, surface superficially pruinose, bluish-green on bruising. Basidiospores 11.5–14 × 7–8.5 μm, Q = 1.65, lenticular, limoniform in faceview, elongate-ellipsoid in side view, blackish-brown, smooth apically truncated by a germ-pore.

On soil and decaying litter, in groups. (PY092).

**Parasola plicatilis** [Curtis] Redhead, Vilgalys & Hopple, in Redhead, Vilgalys, Moncalvo, Johnson & Hopple, Taxon 50(1): 235 (2001).

Pileus 20–25 mm diam., membranous, convex to plane, greyish-yellow (4B5) at the disc, grooves orange white (6A2), olive brown (4D8) elsewhere, surface dry,
plicate striate, margin plane, crenate. Lamellae free, brownish grey (4D2), subdistant. Stipe 85–100 × 1–2 mm, central, terete, white, cartilagenous, smooth, inserted. Basidiospores 11.5–14.5 × 8.5–10.5 µm, Q= 1.47, lenticular, ellipsoid in side view, with abaxially inclined germ-pore, black, smooth.

Solitary, on ground. (PY065).

*Psathyrella candolleana* (Fr.) Maire, in Maire & Werner, Mém. Soc. Sci. Nat. Maroc. 45: 112 (1937).

Pileus 20–35 mm diam., convex to broadly campanulate, brown (6E8) to brownish-orange (5C4), margin appendiculate. Lamellae adnexed, dark brown (9F7), crowded. Stipe 40–70 × 3–4 mm, central, white, terete, smooth, hollow. Spore-print dark brown. Basidiospores 6–7.5 × 3.5–4.5 µm, Q= 1.69, ellipsoid to ellipsoid, with an apical germ-pore, black, smooth.

On ground, in groups and scattered. (PY108).

*Psathyrella glaucescens* Dennis, Kew Bull. 15(1): 128 (1961).

Pileus 10–40 mm diam., conico-convex to convex, pale orange (5A3) to brownish-orange (6C4), margin white to light grey (1C3), surface dry, smooth, margin appendiculate. Lamellae adnate, brownish-orange (7C4) to greyish red (8C4). Stipe 30–70 × 2–4 mm, white, silky fibrillose, cartilaginous, hollow. Basidiospores 6.5–8 × 4–5 µm, Q = 1.63, ellipsoid, purplish-brown, apically truncated by a germ-pore.

On ground, in groups. (PY003).
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Termitomyces clypeatus R. Heim, Bull. Jard. bot. État Brux. 21: 207 (1951).

Pileus 40–70 mm diam., convex to expanded convex with a spinaform perforament, broadly parabolic when young, surface dark brown (7F8) at the disc, fading towards the margin, smooth, margin decurved, entire. Lamellae adnexed to free, pinkish white (8A2), crowded. Stipe 50–60 × 8–10 mm, central, terete, expanding towards the base, fleshy fibrous, smooth, solid. Pseudorrhiza present. Spore-print pink. Basidiospores 5–7 × 3–4 µm, Q= 1.62, ellipsoid to elongate ellipsoid, hyaline, guttulate. Pleurocystidia pyriform. Chielocystidia subglobose.

On soil, solitary to scattered. (PY1878).

Phylogenetic analysis

The sequences obtained from Chlorophyllum rhacodes, Leucoagaricus meleagris and Lactocollybia epia have been deposited in GenBank with the accession numbers MT229200, MT229202, KU320581, respectively. We constructed maximum likelihood trees to compare our sequences to understand their phylogenetic relationship with related sequences from the database (Figures 1–3). The phylogenetic tree generated using ITS dataset for C. rhacodes and related species included 28 nucleotide sequences. The tree with the highest likelihood (-2549.8398) is depicted (Figure 1). For constructing the tree, all positions with less than 95 % site coverage were eliminated and the final dataset included 537 positions. The ITS sequence of C. rhacodes from this study (MT229200) was placed in the same subclade containing sequence from Gorakhpur, India (MH820354) with 100 % support. The maximum likelihood tree generated for ITS sequence of L. meleagris and its other related species included 17 nucleotide sequences. The tree with the highest likelihood (-1609.0537) is depicted (Figure 2). The final dataset included 604 positions after removing all positions with less than 95 % site coverage. Our isolate (MT229202) clustered in the same subclade with other L. meleagris isolate (GQ249888) from Rajasthan, India with 100 % bootstrap support. For L. epia and its related isolates, the maximum likelihood tree generated included 20 nucleotide sequences and the tree with the highest likelihood (-3410.7721) is shown (Figure 3). The final dataset included 412 positions after removing all positions with less than 95 % site coverage. Our isolate (KU320581) clustered together with L. epia (MN523272), an isolate obtained from China, and showed 100 % bootstrap support.

DISCUSSION

Puducherry does not have any major forest, but there are patches of tropical dry evergreen forest and small areas of sacred groves and mangroves (Ponnuchamy et al. 2013). Therefore, not much litter deposition occurs to create conditions favourable for litter fungi. Studies on the occurrence of agarics in Puducherry resulted in recording more gilled fungi from soil as substrate including A. endoxanthus, A. trisulphuratus, C. molybdites, C. rhacodes, L. serenus, P. cyaneoscens, three species of Termitomyces, V. hypophyts, C. lagopus, P. picatilis, three species of Psathyrella, A. manihtis, L. hyalodes, and M. lobayensis. Most of the dark-spored species recorded in the present study were reported by Natarajan & Raman (1983) in tropical dry evergreen forest areas. This shows that forest type plays an important role in determining agaric species composition (Küffer & Senn-Illet 2005). The 10 dark-spored species along with four white-spored ones recorded in the present study are reported for the first time from Puducherry (Table 1). Among the three species of Psathyrella sampled in the present study, P. candolleana is known to be widely distributed (Manjula 1983; Natarajan et al. 2005; Farook et al. 2013; Amandeep et al. 2015a). Interestingly, a total of 53 species of Psathyrella have been recorded from India (Amandeep et al. 2015a); however, P. glaucescens and P. obtusata recorded in the present study have so far not been reported from southern India. Similarly, the genus Termitomyces, one of the mushrooms of tribal importance (Varghese et al. 2010), was represented by three species, of which T. microcarpus has been reported widely (Karun & Sridhar 2013).

Vellinga (2002) based on similarities in morphology and molecular studies transferred a few species previously placed in Macrolepiota Singer or Lepiota (Pers.) Gray, into Chlorophyllum. Most of the Chlorophyllum species occur in arid habitats in subtropical to tropical regions (Ge et al. 2018). In India, C. rhacodes is known to be widely distributed and recorded as Macrolepiota rhacodes earlier (Manjula 1983; Amandeep et al. 2015b). We found C. rhacodes to occur in a number of places in Puducherry and the identity of the species was confirmed through ITS sequence analysis by constructing maximum likelihood based phylogenetic tree (Figure 1). Interestingly, phylogenetic analysis of ITS sequences from two species which occurred widely in Puducherry showed that L. meleagris (Syn: Leucocoprinus meleagris) (Figure 2) clustered with L. meleagris reported from Rajasthan, India while L. epia (Figure 3) formed a tight cluster with L. epia reported earlier from China.
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