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The status of bioluminescent fungal species in Singapore

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Abstract. Three species of bioluminencent fungi have been identified in Singapore: *Filoboletus manipularis*, *Mycena chlorophos* and *Neonothopanus nambi*. *Filoboletus manipularis* is a rediscovery while *Mycena chlorophos* and *Neonothopanus nambi* are new records for the country. All three species can be found in secondary forests in Singapore.

Key words. Mycenaceae, Omphalotaceae, macrofungi diversity

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INTRODUCTION

Bioluminescence is a visually fascinating trait in a fungus. Current knowledge holds that bioluminescent fungi are mushroom-forming basidiomycetes belonging to the mycenoid lineages (mostly Mycenaceae), the *Omphalotus* lineage (Omphalotaceae) and the *Armillaria* lineage (Physalacriaceae) (Desjardin et al., 2008). Fungal bioluminescence is a chemical reaction that occurs in fungi leading to constant light emission with maximum intensity in the range 520–530 nm (Desjardin et al., 2008) and has been shown to be of a single luminescent mechanism (Oliveira et al., 2012). It has been hypothesised to be a by-product or secondary metabolite of a biological process, with a possible role in lignin degradation (Lingle, 1989; Bermudes et al., 1992; Deheyn & Latz, 2007; Desjardin et al., 2007, 2008). Although it has been hypothesised that fungi with glowing mycelium may potentially attract animals that might translocate it to more favourable environs (Deheyn & Latz, 2007), the significance of complementary animal dispersal for basidiomes, that are capable of producing hundreds of easily wind-dispersed spores per night, remains ambiguous. Furthermore, the loss of luciferase clusters from the genome in many *Mycena* species seems to indicate a reduced ecological significance of bioluminescence for these fungi (Ke et al., 2020). The functions or benefits of bioluminescence in fungi therefore remain obscure.

The biodiversity of fungi in Singapore has not been very much studied since the time of E. J. H. Corner, former assistant director of the Singapore Botanic Gardens. Corner collected specimens and made many observations during his time with the Gardens from 1923–1943. However, most of his works were only published long after he left Singapore. Most of the material he collected also left with him in order for him to continue his studies on them, but at least 100 of his voucher specimens are deposited at the Herbarium of the Singapore Botanic Gardens (SING). A list of his fungal works can be found in his obituary (Watling, 2001). More recently, Turner et al. (1994) reported a literature checklist on fungi in Singapore and three bioluminescent fungi species were recorded by Rodda (2012). However, the species in these publications were not vouchered nor formally validated. This paper confirms the presence of three bioluminescent fungi in Singapore: *Filoboletus manipularis* (Berk.) Singer, *Mycena chlorophos* (Berk. & M.A.Curtis) Sacc. and *Neonothopanus nambi* (Speg.) R.H.Petersen & Krisai.

MATERIAL AND METHODS

Night surveys were conducted from 2020–2022 in specific areas in parks and nature reserves around Singapore that had day-sightings of potential bioluminescent fruiting bodies. Surveys were conducted on moonless nights, after dusk (c. 1900–2200 hours). Visual sweeps were conducted while slowly walking along boardwalks and existing mapped trails. Once a bioluminescent fruiting body was located, collection involved removing the entire basidiome. Photo-documentation was conducted in-situ when possible, with bioluminescence shots being taken on the 'Pro' mode with a timed exposure on a Samsung S20FE handphone camera.

Basidiomes were processed following Halling (1996) and Buyck et al. (2010). Basidiomes were air-dried in a food dehydrator at no more than 40ºC. After drying, they were placed in zip-lock bags (to avoid rehydration) along with accompanying pictures and the collection label. Various parts of the fungi were first hydrated with potassium hydroxide (KOH, 4%) and stained with a staining reagent, Congo red, a saturated solution in ammonium hydroxide that renders the microscopic features more visible. The morphological features of the specimens were then examined using a Differential Interference Contrast BX53 compound microscope (Olympus Corporation) and imaged with a DP22 mounted camera (Olympus Corporation). Specimens were identified using Largent (1986), Largent et al. (1980), Largent & Baroni (1988) and Chew et al. (2015) and then further confirmed by referring to original descriptions of the species.

Molecular methods were further used to confirm the identity of *Neonothopanus nambi* in order to distinguish it from morphologically similar species. DNA was extracted from a clean tissue sample with EZ-DNA Genomic DNA Isolation Kit (Omega Bio-Tek). DNA amplification of the ITS and LSU genes were conducted following protocols in Chew et al. (2015). PCR products were sent to Apical Scientific Sdn. Bhd. to be sequenced. Identification was confirmed by a BLAST against GenBank database records.

Records of the Herbarium of the Singapore Botanic Gardens (SING) were examined and a literature review was conducted to find previous records of the species in Singapore. Specimens collected from this study are lodged at SING.

BIOLUMINESCENT FUNGI OF SINGAPORE

Filoboletus manipularis (Berk.) Singer **(Mycenaceae)**

(Fig. 1)

Filoboletus manipularis (Berk.) Singer, Lloydia 8: 215 (1945). — Basionym: *Favolus manipularis* Berk., Hooker's J. Bot. Kew Gard. Misc. 6: 229 (1854). — *Mycena manipularis* (Berk.) Sacc., Syll. fung. (Abellini) 5:272. (1887). — *Poromycena manipularis* (Berk.) R. Heim. Revue Mycol., Paris 10 (1-4): 35 (1945); Corner, Nova Hedwigia Beih. 109: 261 (1994). — *Mycena manipularis* (Berk.) Métrod. Prodrome à une flore mycologique de Madagascar 3: 87 (1949). — *Mycena manipularis* var. *micropora* (Kawamura) Corner. Trans. Brit. Mycol. Soc. 37: 267 (1954). — TYPE: Sri Lanka, coll. Thwaites G. H. K., 1851, s.n.

Description. Fruiting bodies can range from pure white, beige, greyish, pinkish, yellowish to brown; pileus to 35 mm diameter, conical when young, convex to plane, with slight or broad to acute umbo when mature; hymenophore with squarish, honeycomb-like pores.; spores 5.9–7.9 × 4.2–5.1 µm, Q = 1.23–1.56, ellipsoid to broadly ellipsoid, smooth hyaline with a single large guttule; basidia thin-walled, subclavate 6.8–15.9 × 6.1–6.9 µm, sterigmata-4, 1.5–4.9 µm; cheilocystidia 39.0–76.0 × 8.3–10.4 µm; pleurocystidia absent; pileocystidia 23.8–54.4 × 3.8–6.7 µm; caulocystidia 31.8–58.8 × 3.5–12.6 µm; pileus hyphae inflated 4.8–37.8 µm wide; clamps present.

Occurrence in Singapore. Seen in nature reserves and parks throughout Singapore, typically on fallen wood in secondary forests. Never in urban neighbourhood parks.

Specimens examined. Lower Peirce Reservoir Park, coll. Lee S., 26 March 2021, SL1733 (SING0336449); Mandai, Track 15, coll. Lee S., 13 May 2021, SL1780 (SING0325638); Nee Soon Freshwater Swamp Forest, coll. Lee S. et al., 3 November 2020, SL1614 (SING030080); Pulau Ubin, coll. Lee S., 5 May 2021, SL1763 (SING0325599); Singapore Botanic Gardens, rainforest, coll. Lee S. et al., 8 March 2017, SL1149 (SING0227403).

Distribution. Australia, Brazil, China, Christmas Island, India, Japan, Lao People's Democratic Republic, Malaysia, New Guinea, New Caledonia, Philippines, Taiwan**,** Singapore, Sri Lanka. (GBIF.org, 2021a, Singer, 1945),

Habitat. Gregarious to caespitose on dead or decomposing dicot wood.

Provisional conservation assessment for Singapore. Common.

Remarks. There was a previous mention of *Filoboletus manipularis* being found in Singapore and that someone had shown Corner its glowing properties (Corner, 1954). However, the first collection in the SING was from 2009 and this is the first confirmation of its presence in Singapore based on examination of voucher specimens. The record of this species would therefore be considered a rediscovery since nothing has been published on it since 1954. Vydryakova et al. (2014) has shown that the patterns of luminosity in this species are very variable with different clumps and that it seems that certain strains glow while others do not. In their study, basidiomes were either luminescent at the pileus or stipe, or the entire fruiting body or none at all. In Singapore, observations of this species have also revealed a range of characters—totally non-luminescent clumps, faintly luminescent overall and some with more luminosity in the hymenophore than in other parts. Despite the wide morphological variation, clumps with a combination of such characteristics have been found to be of the same species (Vydryakova et al., 2014; Chew et al., 2015).

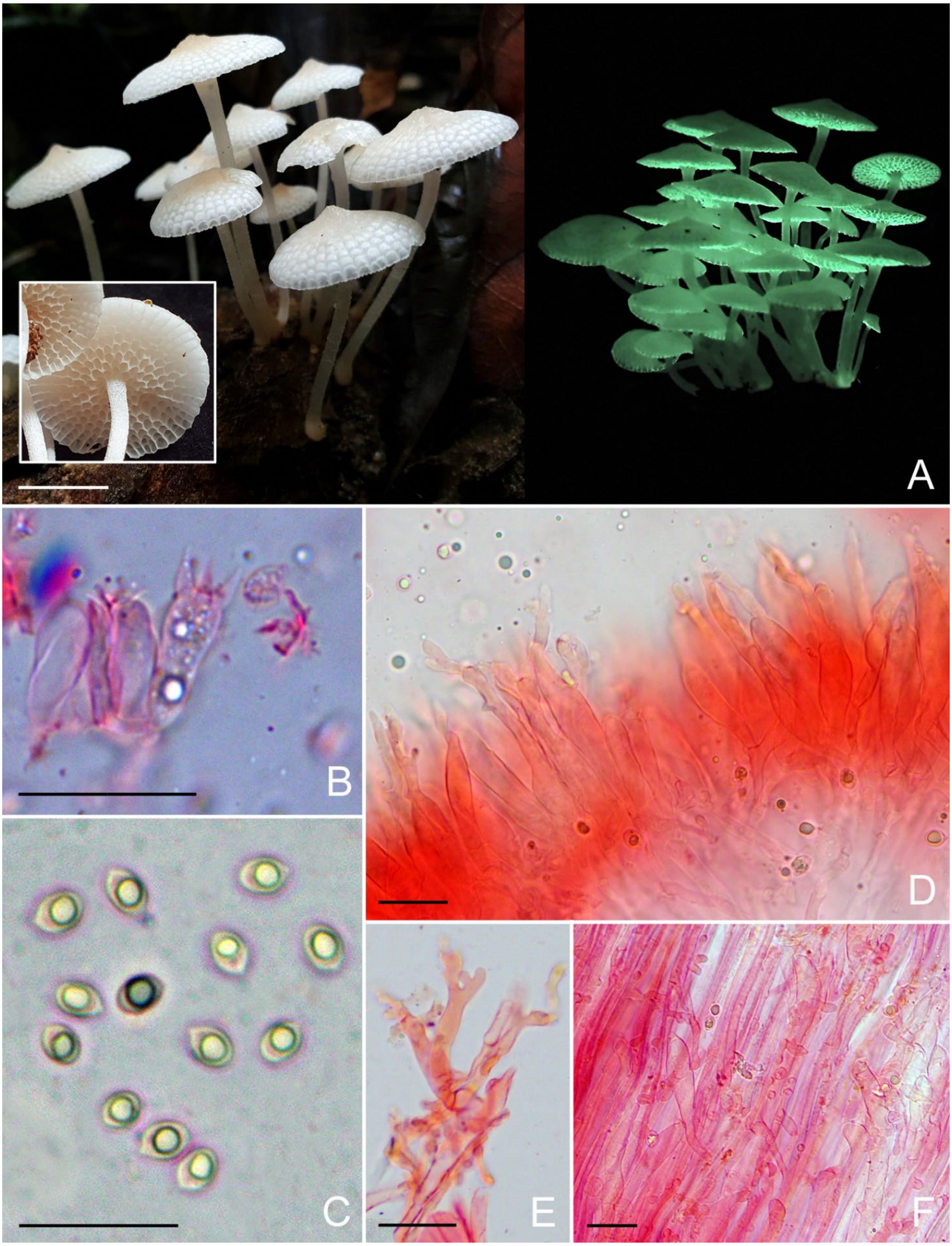

Fig. 1. *Filoboletus manipularis* (Berk.) Singer. A, (left) in situ; (right) fruiting body in the dark; inset shows poroid hymenophore. B, basidia. C, spores. D, cheilocystidia. E, pileocystidia. F, caulocystidia. Scale bars represent 20 µm, except in (A), where it represents 1 cm. (Photographs by: Serena Lee).

Mycena chlorophos (Berk. & M. A. Curtis) Sacc. **(Mycenaceae)**
(Fig. 2)

Mycena chlorophos (Berk. & M. A. Curtis) Sacc., Syll. Fung. (Abellini) 5: 301 (1887). — Basionym: *Agaricus chlorophos* Berk. & M.A. Curtis Proc. Amer. Acad. Arts & Sci. 4: 113 (1860). — *Agaricus cyanophos* Berk. & M.A. Curtis, Proc. Amer. Acad. Arts & Sci. 4: 113 (1860). — *Mycena cyanophos* f. *carolinensis* Kobayasi, Bot. Mag., Tokyo 53: 161 (1939). — TYPE: Japan, coll. Wright, C., no date, s.n.

Description. Pileus white, plicate, broadly convex and covered with a viscid membrane (separable layer) that is glutinous when fresh; largest to 1.55 cm; stipe sub-translucent, cylindric,1.39 × 0.11 cm, not sticky, with a basal disk; basidium c. 19.08–20.5 × 8.03 µm, sterigmata-4, c. 5.4 µm; spores hyaline, c. 8.7 × 6.11 µm, Q = 1.3–1.4, ellipsoid to broadly ellipsoid; not clearly amyloid; cheilocystidia 56.46–68 × 13.42 µm, ventricose and acuminate, some just clavate and others mucronate; pileipellis an ixocutis with the pileus terminal cells embedded in a gelatinous matrix, cells pedunculate 38.95–45 × 17.64–29 µm; pleurocystidia 17.76–22. 92 × 3.85–5.09 µm; clamps present in all tissues.

Occurrence in Singapore. Seen in nature reserves and parks where it is often moist, typically in secondary forests on fallen monocot wood. Never in urban neighbourhood parks.

Specimens examined. Lower Peirce Reservoir Park, coll. Lee S. et al., 7 Jan 2021, SL1650 (SING0303180); Mandai, Track 15, coll. Lee S. et al, 13 May 2021, SL1778 (SING0325605), coll. Yap, G., 18 Dec 2020, SL1642 (SING0303170).

Distribution. Australia, Brazil, Costa Rica, Indonesia, Japan, Korea, Malaysia, Micronesia (Federated States of), Singapore, Vietnam (GBIF.org, 2021b).

Habitat. Mostly solitary, sometimes gregarious, on decomposing monocot sheaths and stems or on other decomposing wood.

Provisional conservation assessment for Singapore. Common.

Remarks. *Mycena chlorophos* was mentioned as common in Malaya (Corner, 1954) with no specific mention of it being in Singapore. For this species, herbarium records are of those collected from 2020 only, when specimens were collected for this study during the night surveys. It may have previously been collected as a *Mycena* sp. when no one yet knew of its luminescent feature. As there are far too many unknown *Mycena* spp. in Singapore, without observing this feature, species-level identification is next to impossible at the moment. It is the brightest of the three species presented here and has very intense luminescence in the pileus and lamellae but with a weakly luminescent stipe, while the centre of the pileus is very dark and give the impression that it is umbilicate. This taxon is here presented as a new record for Singapore.

Neonothopanus nambi **(Speg.)** R.H. Petersen & Krisai **(Omphalotaceae)**
(Fig. 3)

Neonothopanus nambi (Speg.) R.H. Petersen & Krisai Persoonia 17(2): 210 (1999). — Basionym: *Agaricus nambi* Speg., Anal. Soc. Cient. Argent. 16: 247 (1883). — TYPE: Paraguay, coll. Balansa B, December 1879, 3374.

Description. *Neonothopanus nambi* has been found to be small c. 2 cm and rather cantharellus-like to over 10 cm in diameter, irregular to flabelliform in shape, white to dirty yellowish-white and glabrous; pileus margin inrolled when young, plane to uplifted when mature; lamella decurrent, white to yellowish; stipe almost sessile, eccentric to lateral, solid; spores hyaline, white en-mass, 5–(5.9)–7 × 2.4–(3)–3.6 µm, inamyloid, Q = 1.68–2.36 µm, elongate to cylindric; basidium 21.4–24.2 × 4.4–6.1 µm, sterigmata-4, 2.2–4.1 µm; cheilocystidia 29.6–35.0 × 5.2–5.5 µm; pleurocystidia ventricose 25.8–32.5 × 4.2–5.2 µm; lamella hyphae 1.1–2.7 µm wide; clamps present.

Occurrence in Singapore. Seen in nature reserves and parks, in drier areas. Never in urban neighbourhood parks.

Specimens examined. Bukit Timah Nature Reserve, coll. Lee S. et al., 30 Jun 2020, SL1532 (SING0295955); MacRitchie Reservoir Park, coll. Lee S. et al., 13 Aug 2020, SL1527 (SING0295968); Pulau Ubin, coll. Lee S., 5 May 2021, SL1761 (SING 0323717); Singapore Botanic Gardens, rainforest, Lee S., 4 Jun 2021, SL1791 (SING0325639).

Distribution. Australia, French Guiana, Hong Kong, Japan, Madagascar, Malaysia, Paraguay, Singapore (GBIF.org, 2021c, Petersen & Krisai-Greilhuber, 1999).

Habitat. On living roots of dicot trees and decomposing wood.

Provisional conservation assessment for Singapore. Common.

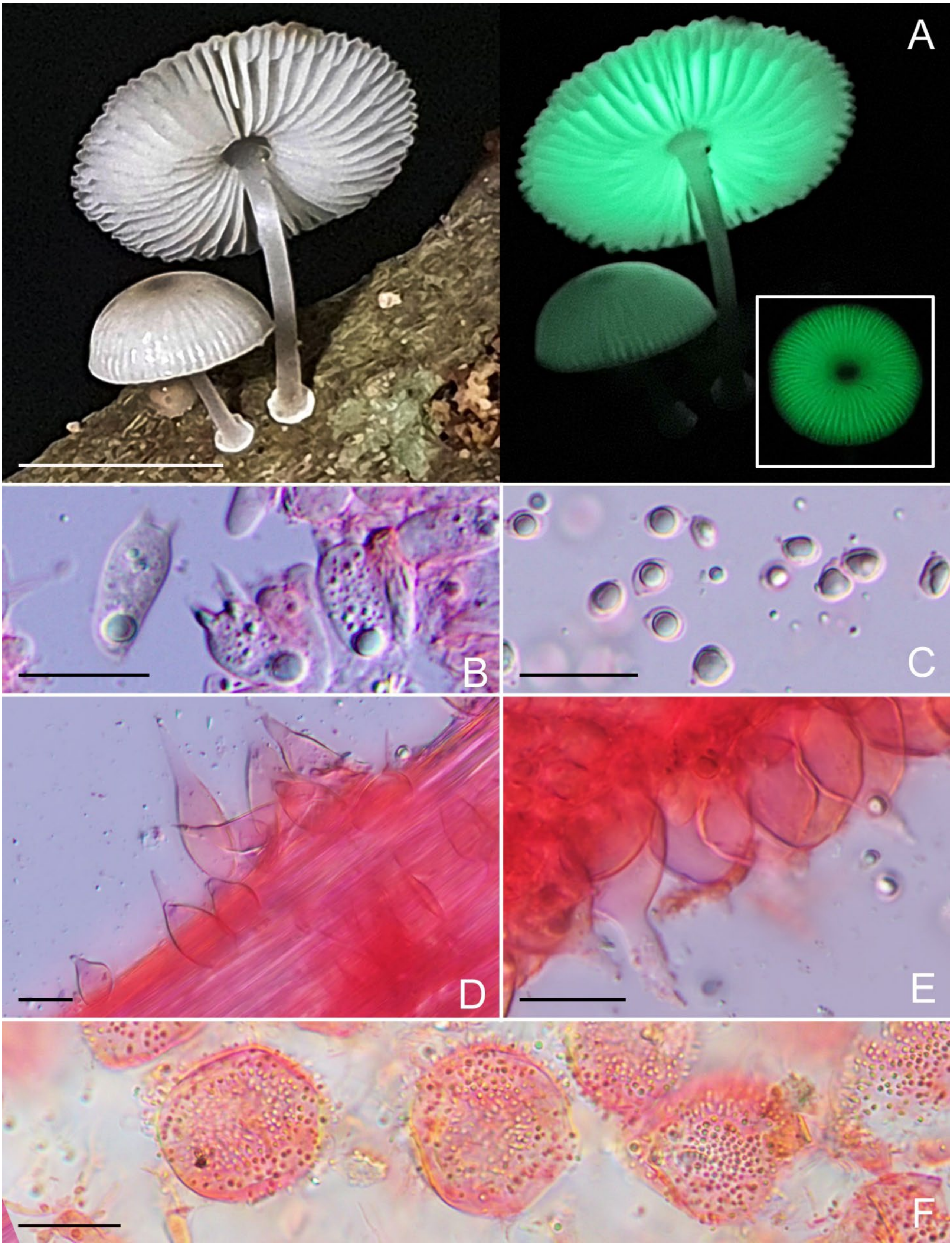

Fig. 2. *Mycena chlorophos* (Berk. & M.A.Curtis) Sacc. A, (left) in situ with light; (right) fruiting body in the dark; inset shows top view of the pileus. B, basidia. C, spores. D, caulocystidia. E, cheilocystidia. F, surface view of pileipellis terminal cells embedded in gelatinous matrix of the pileus. Scale bars represent 20 µm, except in (A), where it represents 1 cm. (Photographs by: Serena Lee).

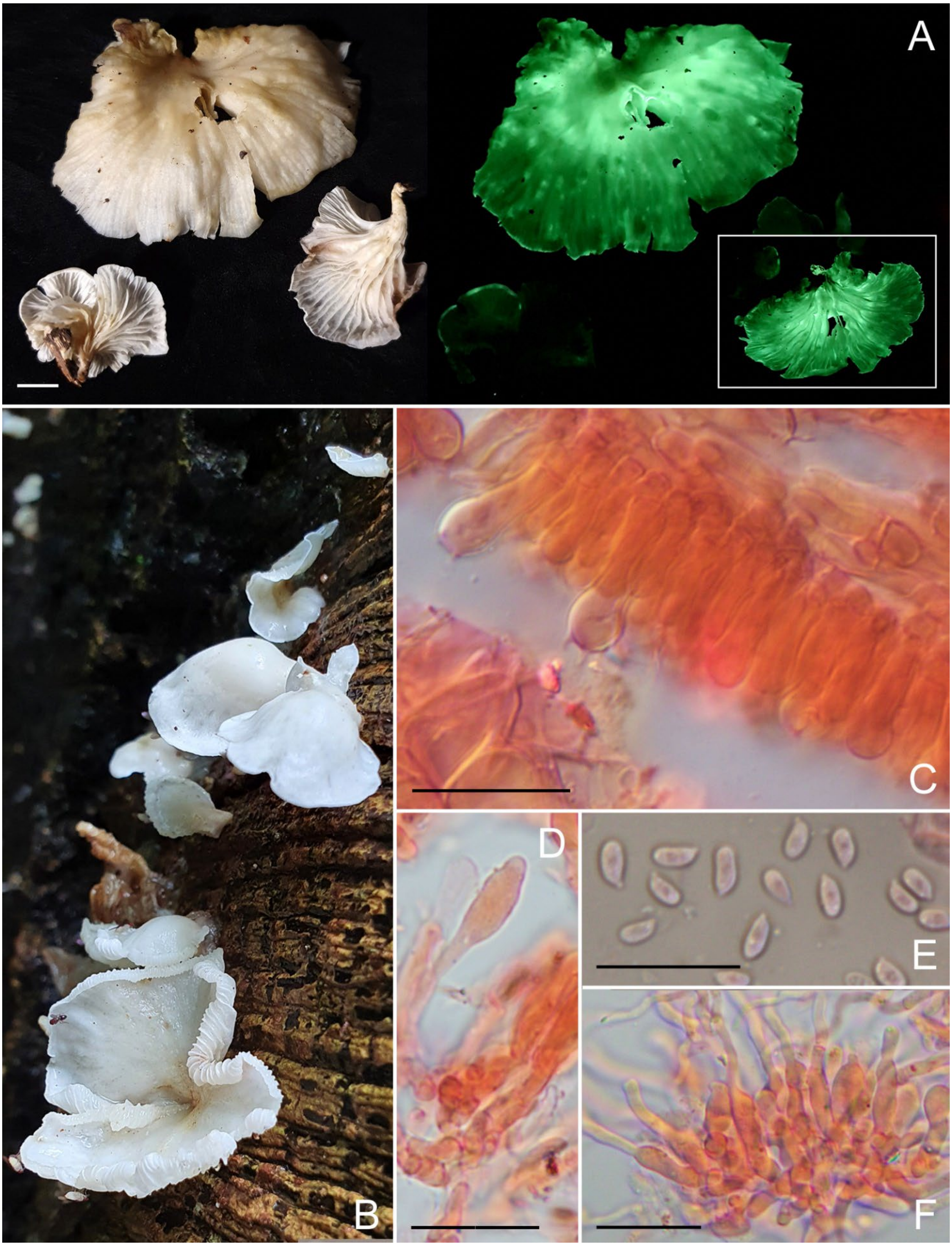

Fig. 3. *Neonothopanus nambi* (Speg.) R.H. Petersen & Krisai. A, (left) under florescent light; (right) fruiting body in the dark; inset shows underside of the basidiome in the dark. B, fruiting body in situ growing on the bark of a live tree. C, basidia. D, pleurocystidia. E, spores. F, cheilocystidia. Scale bars represent 20 μm, except in (A), where it represents 1 cm. (Photographs by: Serena Lee).

Remarks. *Neonothopanus nambi* is a rather cosmopolitan species (Petersen & Krisai-Greilhuber, 1999) and is a new record for Singapore. The SING database reveals that the earliest and only collections of this species were from surveys of this study. Since then, it has been spotted in many other parts of Singapore by the Mushroom Spotters (Singapore) a public group on Facebook (Mushroom Spotters (Singapore), n.d.). It seems unlikely that this fungus would have been missed by E. J. H. Corner because of his extensive collecting efforts in Singapore when he was the Assistant Director of the Botanic Gardens from 1929–1941 (van Steenis-Kruseman & van Steenis, 1950). One possibility is that *Neonothopanus nambi* had in fact been collected from Singapore previously but if the collections had occurred in the daytime (when luminescence cannot be observed), the species may have been mistaken as similar-looking, but non-luminescent *Pleurotus eugrammus* var. *eugrammus* (Mont.) Dennis or *Pleurotus eugrammus* var. *radiciola* Corner (Corner, 1981). As the micro-morphological characters of *Neonothopanus nambi* were too nondescript and its form too similar to these other species, molecular sequencing was undertaken to confirm its identity. It is luminescent in all parts of the basidiome and appears to be the dimmest of the three species recorded in this study.

CONCLUSIONS

This study confirms the presence of three bioluminescent fungal species in Singapore: *Filoboletus manipularis* and *Mycena chlorophos* from the mycenoid lineage, and *Neonothopanus nambi* from the *Omphalotus* lineage. Even though *Filoboletus manipularis* seems ubiquitous now, a significant amount of time passed from its first reported sighting in Corner (1954) before its first vouchered collection in 2009. This study therefore confirms its rediscovery. *Mycena chlorophos* is a new record for Singapore. *Neonothopanus nambi* as assessed with molecular techniques and by matching to its type description, is also a new record for Singapore.

While molecular techniques are not always necessary nor practical and cannot be relied upon as the sole method to identify fungal species accurately, in this study, DNA sequencing was integral to the confirmation of *Neonothopanus nambi*, a species that was originally described from Paraguay ((Petersen & Krisai-Greilhuber, 1999). Herbarium records through time are important as physical proof but photo-documentation of the species is equally important. Fruiting bodies change a lot after they are dried and many characters are lost after drying. This loss is doubly felt when no proper descriptive notes accompany the specimen voucher.

With the proper documentation of species with photographs, voucher specimens, and in one case, DNA sequences, this study formalises the presence of three bioluminescent fungi in Singapore in order to include them into the national list of macro-fungi diversity.

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