

## Additional Species of *Inocybe* (Agaricales, Inocybaceae) from Indonesia

### Catatan baru jenis *Inocybe* (Agaricales, Inocybaceae) dari Indonesia

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#### Abstract

Three species of *Inocybe* are newly reported from three different locations in Indonesia (Bogor Botanical Garden, Haurbentes (West Java), and Mt. Seraya (Bali)). Complete descriptions of *Inocybe assimilata* Britzelm., *Inocybe hydrocybiformis* (Corner & E. Horak) Garrido, and *Inocybe* cf. *viraktha* K. P. D. Latha & Manim. are provided. *Inocybe assimilata* is a member of *Inosperma* clade, meanwhile *I. hydrocybiformis* and *I. cf. varaktha* are members of *Inocybe* clade. Art line and photos of basidiomata, microscopic features, SEM images of basidiospores and cystidia are presented.

Key words – Basidiomycota – new records – taxonomy

#### Abstrak

Tiga jenis *Inocybe* dilaporkan sebagai rekaman baru dari tiga lokasi berbeda di Indonesia (Kebun Raya Bogor, Haurbentes (Jawa Barat), dan Gunung Seraya (Bali)). Deskripsi lengkap *Inocybe assimilata* Britzelm., *Inocybe hydrocybiformis* (Corner & E. Horak) Garrido, dan *Inocybe* cf. *viraktha* K.P.D. Latha & Manim. disertakan dalam tulisan. *Inocybe assimilata* merupakan anggota klade *Inosperma*, sedangkan *I. hydrocybiformis* dan *I. cf. varaktha* merupakan anggota klade *Inocybe*. Art line atau line drawing dan foto dari tubuh buah, karakter mikroskopis, gambar basidiospora dan sistidia dengan SEM disajikan dalam tulisan ini.

Kata kunci – Basidiomycota – rekaman baru – taksonomi

#### Introduction

Seven species of Indonesian *Inocybe* have been reported (Horak 1979; 1980; Retnowati *et al.* 2019). Those were *I. petchii* Boedijn, *I. Angustifolia* (Corner & E. Horak) Garrido, *I. titi-buensis* Kobayasi, *I. fuscospinulosa* Corner & Horak, *I. olorinata* Horak, *I. subgeophylla* Hennings, and *I. stellata* E. Horak, Matheny & Desjardin. The species reported here will contribute to the distribution information of the *Inocybe* species in Indonesia.

The genus has very distinctive macro-characters; *i.e.* brownish basidiomata, fibrillose or squamulose pileus and stipe, and brownish lamellae. However, species determination is

impossible without observing the microscopic characters, particularly shape and ornamentation of basidiospores, pleurocystidia and cheilocystidia (Pegler & Young 1972). The variation of spores' shape and ornamentation is considered taxonomic characters to identify the genus at the species level. Several species of the genus have smooth, nodulose, or spinose basidiospores. The limitations to examine those ornamentations by using the light microscope can be supported by the Scanning Electron Microscope (SEM). Three species here are the first reported for the Indonesian mycota and are representative of *Inosperma* and *Inocybe* clade with smooth and nodulose spore ornamentations (Latha & Manimohan 2017).

## Materials and Methods

To help the identification process, notes on macroscopic characters were made in the field, and colors of fresh basidiomes relate to the color codes of Kornerup & Wanscher (1967). Description of the microscopic characters was made from dried materials rehydrated in KOH (5% aqueous solution), Congo Red (5%), or Melzer's reagent. The microscopic study was done under a light microscope. Ornamentations of basidiospores and cystidia were observed by thermoscientific Quattro S Scanning Electron Microscope (SEM) at Integrated Laboratory of Bioproduct (i-lab), BRIN. The examined specimens are kept in the Herbarium Bogoriense (BO), Indonesia.

## Results

### Ecological aspect of *Inocybe*

*Inocybe* is one of the ectomycorrhizal genera associated with specific plants in tropical lowland or submontane forests. Based on the several published data, there are recognized several potential ectomycorrhizal plant associations; *i.e.* Dipterocarpaceae (Pradeep *et al.* 2016), Pinaceae, Fagaceae, and Salicaceae, Fabaceae (Matheny *et al.* 2012), Myrtaceae, and Tiliaceae (Horak 1980). Of the species documented here, *I. assimilata* has been recorded in association with *Araucaria cunninghamii* at Bogor Botanical Garden. Meanwhile, *I. hydrocybiformis* is found in the artificial Dipterocarpaceae forest of Haurbentes, mostly *Shorea* spp. The *I. cf. viraktha* was collected at the Bukit Panulisan, Bali, and associated with an undetermined dicot plants.

### Taxonomy Descriptions

1. *Inocybe assimilata* Britzelm. Fig. 1–3.

Type: Singapore, Havelock Road, 21.XI.1913, Burkill 250 (K, holotype).

Basidiomata medium to large-sized. Pileus 2.5–6 cm in diam, convex to plano-convex with an umbo; surface dry, plicate–striate towards the margin, margin slightly incurved when very young, becoming decurved to somewhat straight with age, or rather wavy; golden brown (5D7) to brown (5E7). Lamellae adnate, crowded with 2 series of lamellulae, broad, golden brown (5D7) to brown (5E7). Stipe 4.5–8 × 0.5–1.2 cm, central, terete, equal or slightly tapering at above, solid, surface fibrillose towards the base, base enlarged; golden brown (5D7) to brown (5E7), or paler, whitish at the base with a very thin basal tomentose. Odor and taste not distinctive. Context brown, thick.

Basidiospores 7.5–9 × 5.5–6.4 μm ( $x_m = 8.33 \pm 0.42 \times 5.85 \pm 0.32$ ,  $Q = 1.26–1.958$ ,  $Q_m = 1.43 \pm 0.09$ ,  $n = 25$  spores per 1 specimen), ovoid, thick-walled, smooth, brown. Basidia 19.4–27.5 × 5.9–10 μm, clavate, thin-walled, hyaline, 4-spored. Basidioles 20.7–26.2 × 8.3–9.7 μm, clavate, thin-walled. Cheilocystidia common; 22.3–36.6 × 14.5–16.5 μm, clavate to broadly clavate, thin-walled, hyaline to yellowish white. Pleurocystidia absent. Pileipellis a cutis; made up of cylindrical hyphae; 3.2–7.5 μm diam, thin to slightly thin-walled, incrusting, hyaline to yellowish white. Stipe tissue monomitic; hyphae 5.3–10.4 μm diam, parallel, cylindrical, thin to thick-walled up to 2.4 μm, hyaline. Caulocystidia common at the apex, absent at the base; 22.1–27.3 × 8.1–9.4 μm, clavate, thin-walled, hyaline. Clamp connection present.

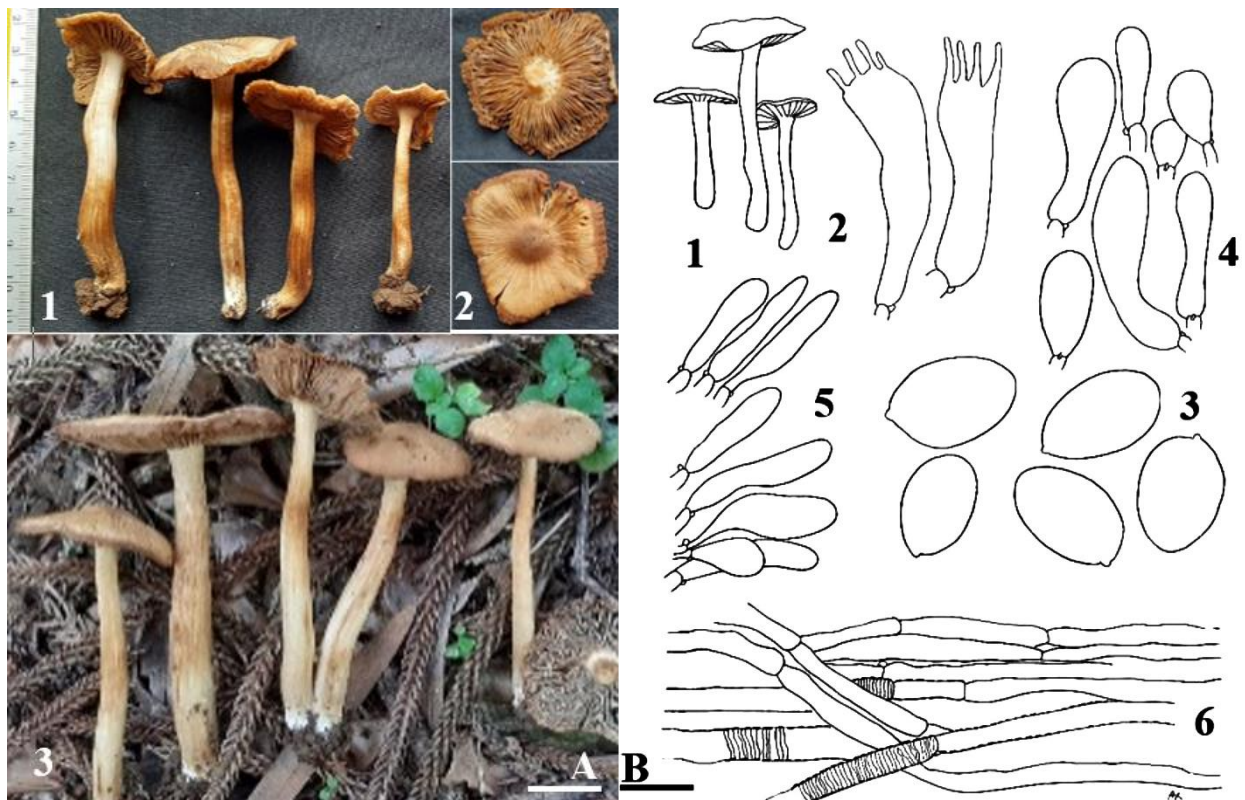
Distribution: Indonesia (Java), Malaysia, Singapore, Papua New Guinea, Solomon Island.

Habit and habitat: Gregariously on soil at Bogor Botanical Garden under the *Araucaria cunninghamii*.

Material examined: Indonesia, Java, West Java, Bogor, Botanical Garden, 12 August 2021, Halu 9 (A. Retnowati 1586, BO!), collected by the team of Halucinogenic mushroom project, identified by A. Retnowati.

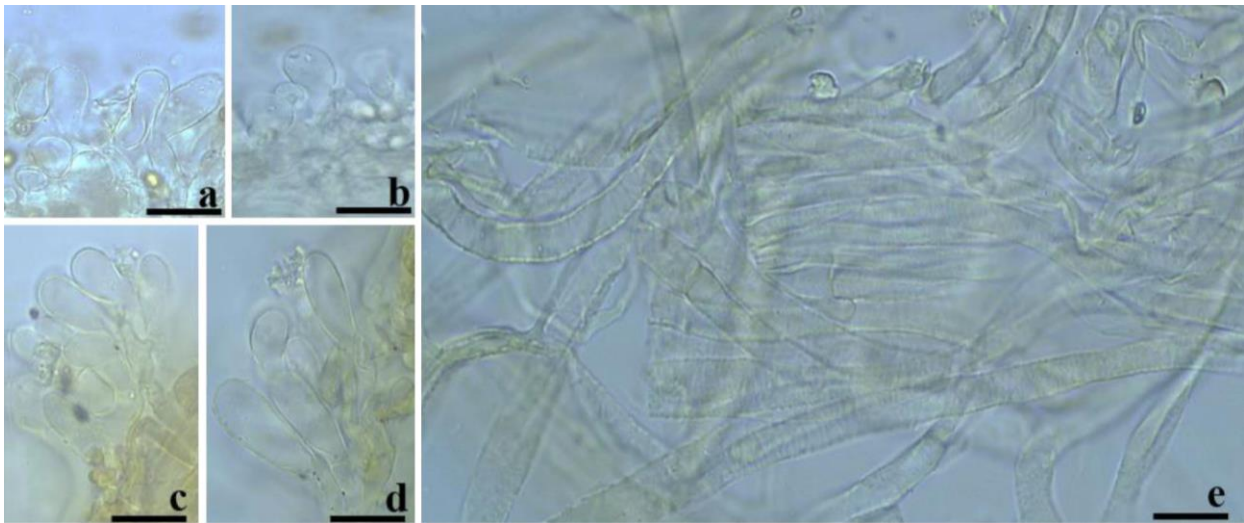
Notes. The *Inocybe assimilata* Britzelm. is the current name of *I. umbrina* that was described by Horak (1980). The species is characterized by medium to large-sized basidiomata; 2.5–6 cm in diam., convex to plano-convex with an umbo, and golden brown to brown; crowded and adnate lamellae, small ( $7.5\text{--}9 \times 5.5\text{--}6.4 \mu\text{m}$ ) and ovoid basidiospores; cheilo and caulocystidia present, pleurocystidia absent; cutis and incrustated pileipellis.

A very close species that shares several morphological characters is *I. althoffiae* Horak (1980). However, it differs in having the smaller size of basidiomata, larger basidiospores ( $8.5\text{--}10 \times 3.5\text{--}4 \mu\text{m}$  vs  $7.5\text{--}9 \times 5.5\text{--}6.4 \mu\text{m}$ ), and different shapes of basidiospores (cylindric to subfusoid vs. ovoid) (Table 1). Those two species grew on different substrates; *I. assimilata* on *Araucaria cunninghamii*. Meanwhile, *I. althoffiae* was found on *Althoffia* sp. (Tiliaceae) (Horak 1980).

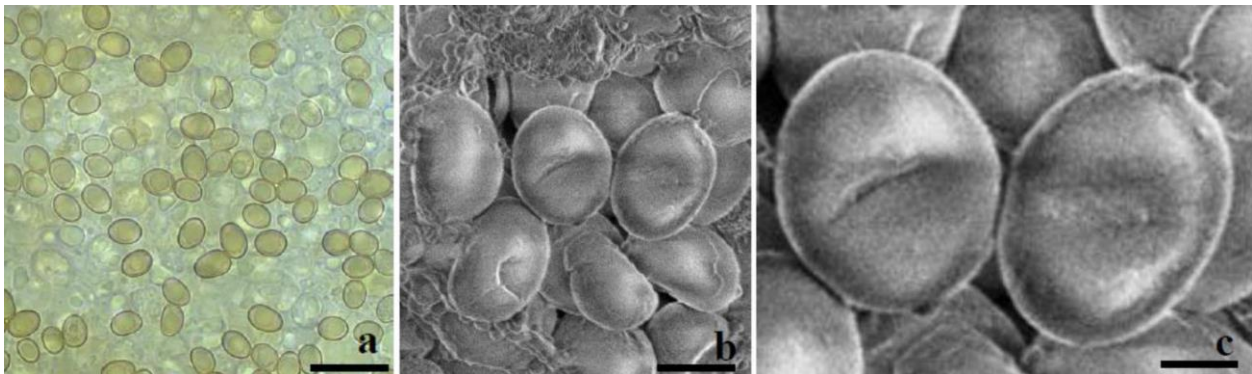


**Fig. 1.** *Inocybe assimilata* (from A. Retnowati 1586): A. 1–2. Basidiomata; 2. Pileus; and 3 Basidiomata at the habitat; B. Line art: 1. Basidiomata; 2. Basidia; 3. Basidiospores; 4. Cheilocystidia; 5. Caulocystidia; and 6. Pileipellis. Scale bar: A: 1–2 = 3 cm, and 3 = 2.3 cm; B: 1 = 2.5 cm; 2 = 11  $\mu\text{m}$ ; 3 = 4.5  $\mu\text{m}$ ; 4 = 15.7  $\mu\text{m}$ ; 5 = 16  $\mu\text{m}$ ; and 6 = 15  $\mu\text{m}$ . Drawings by A. Retnowati.





**Fig. 2.** Microscopic characters of *I. assimilata* (from A. Retnowati 1586): a–b. Cheilocystidia; c–d. Caulocystidia; and e. Incrustated pileipellis. Scale bar: a–b = 30  $\mu\text{m}$ ; c–d = 18  $\mu\text{m}$ ; and e = 10  $\mu\text{m}$ .



**Fig. 3.** Basidiospores of *I. assimilata* (from A. Retnowati 1586): a. Spores under the light microscope; b–c. Spores under SEM. Scale bar: a = 22  $\mu\text{m}$ ; b = 6.4  $\mu\text{m}$ ; and c = 3  $\mu\text{m}$ .

2. *Inocybe hydrocybiformis* (Corner & E. Horak) Garrido. Fig. 4–5.

Type: Singapore Botanical Garden, Gardens Jungle, 5 Sep. 1940, leg. E.J.H. Corner (E, holotype; ZT78–47, isotype).

Basidiomata medium. Pileus 1.5–2.2 cm diam., at first convex or expanded with small umbo, becoming expanded with acute umbonate; margin plicate–striate towards the margin, straight in young age, then decurved; surface dry to wet, slightly hygrophanous, covered with a very little scale, scurvy, appressed–fibrillose at some part, crenate; brown overall (7E6). Lamellae adnexed, close with 2 series of lamellulae, medium to broad, concolorous with pileus (7E6). Stipe 3–4  $\times$  0.2–0.35 cm, central, cylindrical, equal or slightly tapering at base, surface fibrillose towards the base, apex slightly enlarged, concolorous with pileus. Odor and taste not distinctive. Context brown, thin to slightly thick.

Basidiospores (9.58–9.10)8.86–7.01  $\times$  7.68–5.48  $\mu\text{m}$  ( $x_m = 8.18 \pm 0.63 \times 6.69 \pm 0.50 \mu\text{m}$ ,  $Q = 0.95–1.46$ ,  $Q_m = 1.23 \pm 0.13$ ,  $n = 25$  spores per 1 specimen), conspicuously echinulate up to 2  $\mu\text{m}$ , conical to cylindrical, thick-walled, brown. Basidia uncommon, 34  $\times$  10.6  $\mu\text{m}$ , clavate, 4-spored. Basidioles uncommon, 22.2  $\times$  9.4  $\mu\text{m}$ . Cheilocystidia 21.11–38.52  $\times$  5.86–12.31  $\mu\text{m}$ ,

cylindrical to clavate, thin-walled, hyaline to yellowish brown; paracystidia 10.75–17.24 × 7.23–8.64 μm, clavate or vesiculose, thin-walled, hyaline. Pleurocystidia absent to rare. Pileipellis cutis, 4.64–7.53 μm, thin, hyaline to yellowish brown. Caulocystidia absent. Stipe tissue monomitic; hyphae 4.56–8.26 μm diam, parallel, cylindrical, thin to thick-walled up to 2.4 μm, hyaline to yellowish brown. Clamp connection present.

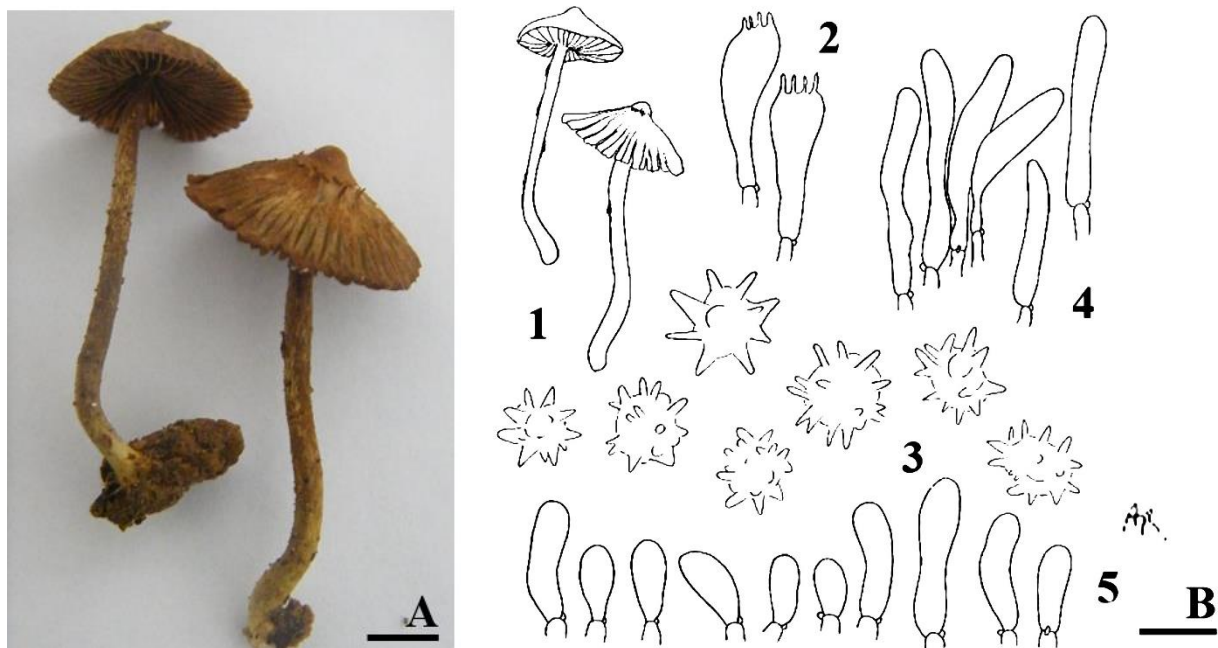
Distribution: Indonesia (Java), Thailand, Malaysia, Singapore, and India.

Habit and habitat: Gregariously on soil at artificial Dipterocarpaceae forest of Haurbentes, mostly *Shorea* spp.

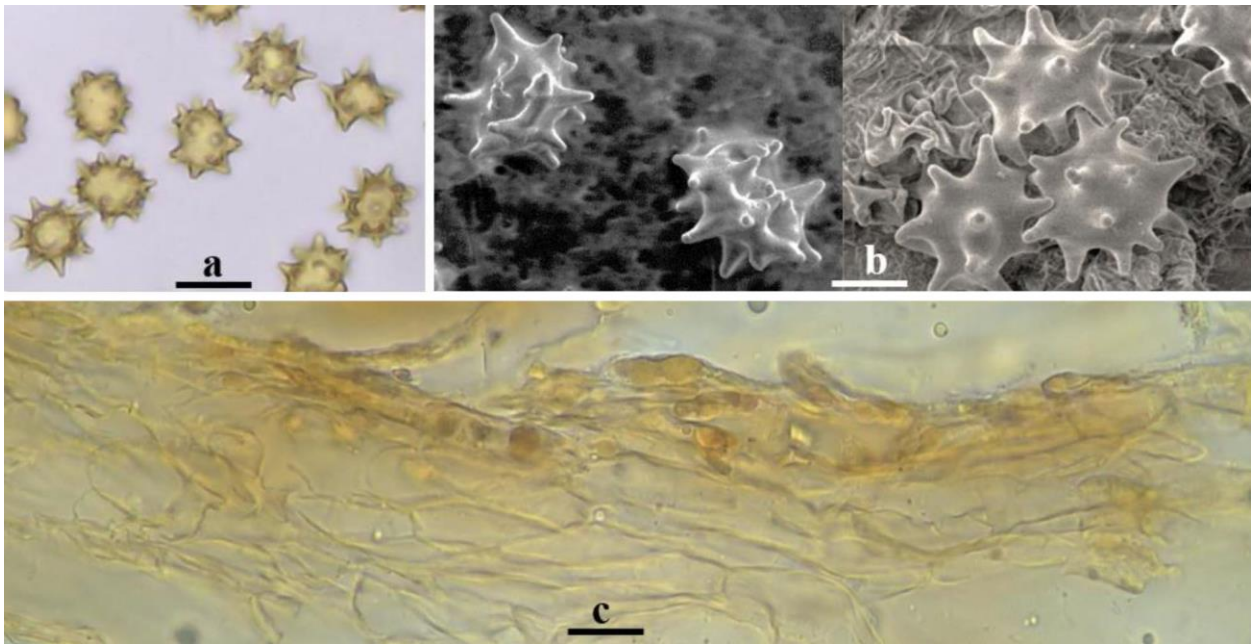
Material examined: Indonesia, Java, West Java, Jasinga, Haurbentes Forest, 4 June 2009, A. Retnowati 654 (BO!), collected and identified by A. Retnowati.

Notes. *Inocybe hydrocybiformis* is distinguished by having medium basidiomata; convex or expanded with small umbo, and expanded with acute umbonate in age, brown overall pileus; adnexed lamellae, 2 series of lamellulae; fibrillose stipe; echinulate basidiospores, cylindrical to clavate cheilocystidia, pleuro-caulocystidia absent, cutis pileipellis. The Indonesian material matches all characters of the Singapore material, differing only in the absence of caulocystidia.

The species has been reported from Singapore (Horak 1979), Thailand, Singapore, and Malaysia (Horak et al. 2015), and from Kerala (India) (Latha & Manimohan 2017). This Indonesian *I. hydrocybiformis* confirms another distribution record of species in South East Asia.



**Fig. 4.** *Inocybe hydrocybiformis* (from A. Retnowati 654): A. Basidiomata; B. Line art: 1. Basidiomata; 2. Basidia; 3. Basidiospores; 4. Cheilocystidia; and E. Paracystidia. Scale bar: A= 0.7 cm; B: 1 = 1.3 cm; 2 = 17 μm; 3 = 7.5 μm; 4 = 12 μm; and 5 = 7.7 μm. Drawings by A. Retnowati.



**Fig. 5.** Microscopic characters of *I. hydrocybiformis* (from A. Retnowati 654): a. Basidiospores seen under light microscope; b. Basidiospores under SEM; and c. Cutis pileipellis. Scale bar: a = 11  $\mu\text{m}$ ; b = 4  $\mu\text{m}$ ; and c = 7.5  $\mu\text{m}$ .

3. *Inocybe* cf. *viraktha* K. P. D. Latha & Manim. Fig. 6-7.

Type: India, Kerala State: Wayanad District, Kuruva islets on river Kabani, 26 September 2013, K. P. Deepna Latha, DKP230 (CAL 1357, holotype).

Basidiomata small to medium. Pileus 1.4–2.2 cm in diam., at first convex, then expanded flat, center depressed or with shallow umbo in age; margin non-striate, slightly incurved in young age to straight or slightly decurved, crenate; surface slightly wet, non-hygrophanous, appressed fibrillose; light brown (5D4). Lamellae adnexed, close, with 2 series of lamellulae, medium, thick, light brown (5D4). Stipe 1.5–2  $\times$  0.2–0.4 cm, central, equal, cylindrical, solid, fibrillose; light brown (5D4) with paler at the base. Odour and taste not distinctive. Context whitish brown, thick up to 0.2 cm.

Basidiospores 6.24–7.72  $\times$  4.15–4.89 (5.38)  $\mu\text{m}$  ( $x_m = 6.33 \pm 0.49 \times 4.63 \pm 0.40$ ;  $Q = 1.11$ – $1.60$ ;  $Q_m = 1.37 \pm 0.13$ ,  $n = 20$  spores per 1 specimen), nodulose, cylindrical, thick-walled, brown. Basidia 17.52–25.11  $\times$  5.53–8.21  $\mu\text{m}$ , clavate, 4-spored. Basidioles 23.23  $\times$  7.61  $\mu\text{m}$ , clavate. Cheilocystidia 22.48–45.33  $\times$  12.14–16.52  $\mu\text{m}$ , metuloid, broadly fusoid, thin to thick-walled up to 5.21  $\mu\text{m}$ , crystal present, hyaline to yellowish brown; paracystidia 12.25–14.24  $\times$  7.23–8.64  $\mu\text{m}$ , clavate or vesiculose, thin-walled, hyaline. Pleurocystidia similar to cheilocystidia. Pileipellis cutis; hyphae 6.3–10 in diam., thin-walled, not incrustated, hyaline to yellowish white. Stipe tissue monomitic; hyphae 5.0–8.7  $\mu\text{m}$  diam, cylindrical, parallel, thin to thick-walled, hyaline to yellowish brown. Caulocystidia common, present along the stipe; similar to cheilocystidia, 42–77  $\times$  13–18.7  $\mu\text{m}$ , metuloid, clavate, broadly clavate, thick walled 3.9–6.20  $\mu\text{m}$ , yellowish brown. Clamp connection present.

Distribution: Indonesia (Bali), India (Kerala).

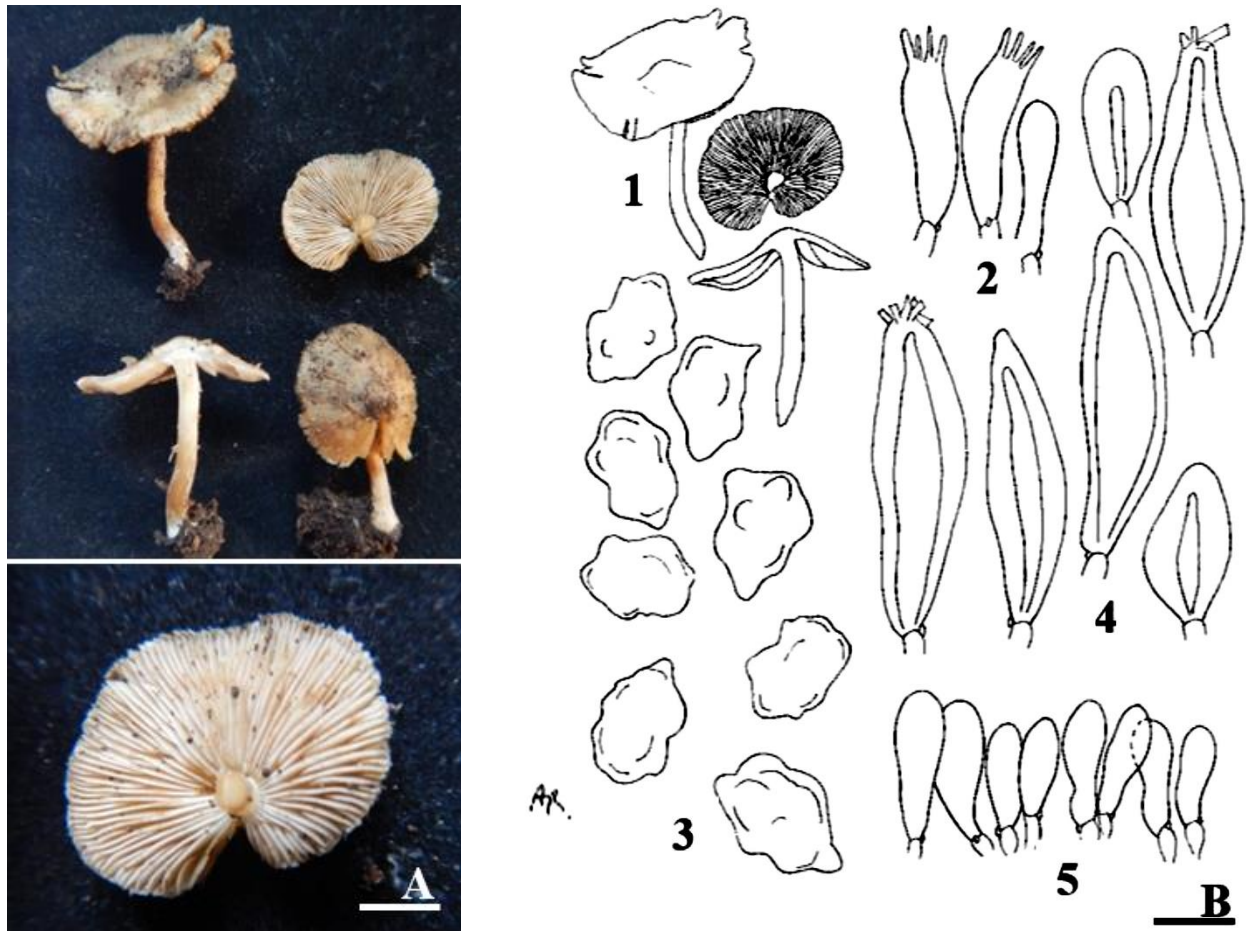
Habit and habitat: Gregariously on soil.

Material examined: Indonesia, Bali, Bangli District, East and West of Bukit Penulisan, 3 May 2015, A. Retnowati 1129 (BO!), collected and identified by A. Retnowati.

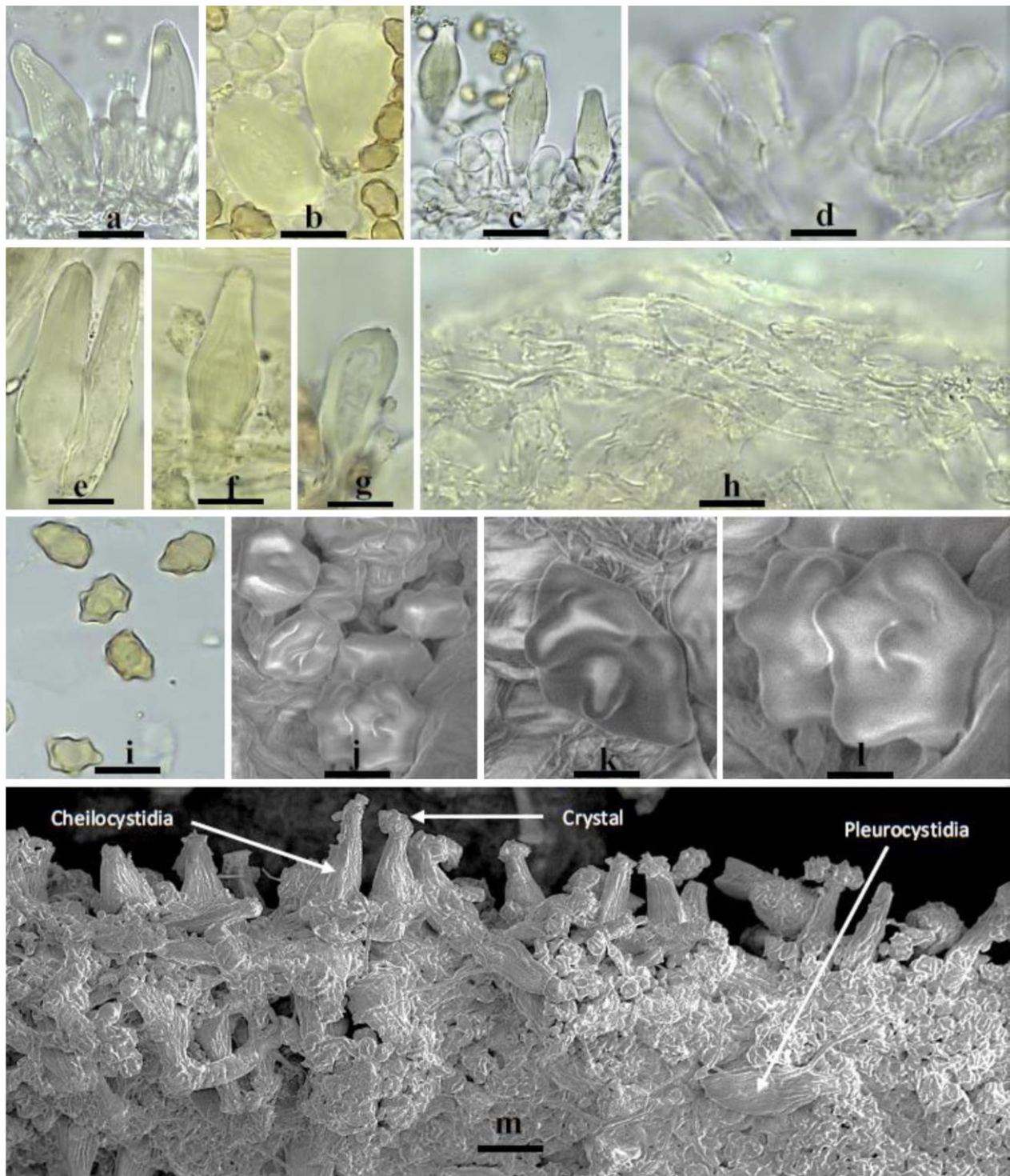


Notes. The species is characterized by having the small to medium basidiomata, convex to expanded flat with umbo, light brown pileus, non-striate margin; closed lamellae, adnexed; nodulose and thick-walled basidiospores; metuloid hymeniall cystidia with crystal present, paracystidia present, cutis and incrusted pileipellis. Our material matches the morphology for *I. viraktha* and is tentatively identified as 'aff' until molecular data are available. Table 1 shows the morphological differences *I. cf. viraktha* and *I. viraktha*. The only significantly different is the size of basidiospores ( $6.24\text{--}7.72 \times 4.15\text{--}4.89$  (5.38)  $\mu\text{m}$  vs.  $7.5\text{--}10 \times 5\text{--}8$   $\mu\text{m}$ ).

The species that share the similarity of morphological characters with *I. cf. viraktha* are *I. fragilissima* E. Horak, Matheny & Desjardin and *I. neoumbrina* Kobayasi. The Indonesian material and Indian material of *I. cf. viraktha* are slightly different in having smaller size of basidiospores ( $6.24\text{--}7.72 \times 4.15\text{--}4.89$  (5.38)  $\mu\text{m}$  vs.  $7.5\text{--}10 \times 5\text{--}8$   $\mu\text{m}$ ), the number of lamellulae (2 series vs. 1 series).



**Fig. 6.** A. Basidiomata of *I. cf. viraktha* (from A. Retnowati 1129); and B. Line art: 1. Basidiomata; 2. Basidia-basidioles; 3. Basidiospores; 4. Metuloid cystidia (cheilocystidia, pleurocystidia and caulocystidia); and 5. Paracystidia. Scale bar: A: 1 = 1 cm; and 2 = 0.5 cm; B: 1 = 1  $\mu\text{m}$ ; 2 = 10  $\mu\text{m}$ ; 3 = 3.5  $\mu\text{m}$ ; 4 = 10.5  $\mu\text{m}$ ; and 5 = 5.7  $\mu\text{m}$ . Drawings by A. Retnowati.



**Fig. 7.** Microscopic characters of *I. cf. viraktha* (from A. Retnowati 1129): a–c. Cheilocystidia-Pleurocystidia; d. Paracystidia; e–g. Variation of caulocystidia; h. Cutis pileipellis; i. Basidiospores seen under the light microscope; j–l. Basidiospores seen under SEM; and m. Metuloid cystidia (Cheilocystidia-Pleurocystidia) at lamellae surface. Scale bar: a = 11  $\mu\text{m}$ ; b = 14.5  $\mu\text{m}$ ; c = 16  $\mu\text{m}$ ; d = 4  $\mu\text{m}$ ; e–g = 11  $\mu\text{m}$ ; h = 5  $\mu\text{m}$ ; i = 3.4  $\mu\text{m}$ ; j = 4.4  $\mu\text{m}$ ; k = 2  $\mu\text{m}$ ; l = 1.8  $\mu\text{m}$ ; and m = 18  $\mu\text{m}$ .



**Table 1.** Morphological differences *Inocybe cf. viraktha* and *I. viraktha*.

Characters	<i>I. cf. viraktha</i> (Indonesia)	<i>I. viraktha</i> (India)
<b>Pileus</b>		
Size	1.4–2.2 cm in diam.	0.8–2.9 cm in diam.
Shape	at first convex, then expanded flat, center depressed or with shallow umbo in age	convex or plano-convex with a small umbo
Color	light brown	reddish brown at the centre, brownish orange on the fibrils, orange grey elsewhere
<b>Lamellae</b>		
Attachment	adnexed	adnate
Lamellulae	2 series	1 series
<b>Stipe</b>		
Size	1.5–2 × 0.2–0.4 cm	0.9–4.5 × 0.2–0.4 cm
<b>Microscopic characters</b>		
Basidiospores	6.24–7.72 × 4.15–4.89 (5.38) μm nodulose	7.5–10 × 5–8 μm nodulose to somewhat angular
Cheilocystidia	22.48–45.33 × 12.14–16.52 μm metuloid with crystal	36–62 × 17–24 μm metuloidal with crystals
Pleurocystidia	similar to cheilocystidia	similar to cheilocystidia
Paracystidia	12.25–14.24 × 7.23–8.64 μm	15–36 × 7–19 μm
Caulocystidia	similar to cheilocystidia	similar to cheilocystidia
Pileipellis	cutis	cutis
Clamp connection	present	present

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