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Amanita species within section Caesareae from the highlands of Laos

Ole Sparre Pedersen

olesped@gmail.com

Retired https://orcid.org/0009-0000-1271-4607

Thomas Læssøe

University of Copenhagen Globe Institute: Kobenhavns Universitet Globe Institute

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Abstract

Specimens of *Amanita* sect. *Caesareae*, collected in Xieng Khouang and Vientiane provinces in Laos from highland forests dominated by members of *Fagaceae* and *Pinaceae*, are studied based on morphological characters and analyses of ITS sequences. Two species, namely *A. fulvocaesarea* and *A. chepangianoides* are reported as new, both found in association with *Fagaceae*, whereas *A. pseudoprinceps and A. subhemibapha*, recently described from China and *A. rubromarginata* from Japan, are confirmed from Laos and found in forests with *Fagaceae* and *Pinaceae*. The phylogenetic analyses to recognize the *Amanita* species showed that the golden-brown to greyish orange *A. fulvocaesarea* and the dominantly white *A. chepangianoides* cluster with *A. aporema, A. chepangiana, A. egregia*, *A. princeps* and *A. pseudoprinceps.* A dichotomous key to identified and likely occurring *Amanita* species in Laos within sect. *Caesareae* is also provided.

Introduction

The genus *Amanita* Pers. (1797: 65) (*Amanitaceae, Agaricales)* is widely occurring in temperate, subtropical, and tropical regions and contains approximately 1,200 binomials of which some 650 are currently accepted species (Yang et al. 2018; Tulloss and Yang 2024). Mycorrhizal tree partners are foremost with *Fagaceae*, *Pinaceae*, *Dipterocarpaceae* and *Betulaceae* (Tulloss and Yang 2024), all with members in Laos (Newman et al. 2007). Currently, three subgenera are accepted within *Amanita* - *Lepidella, Amanitina* and *Amanita* - with subgenus *Amanita* having four sections: *Amanita, Amarrendiae, Caesareae*, and *Vaginatae* (Cui et al. 2018). This study is limited to identified Lao specimens within sect. *Caesareae*. This section, when agaricoid, is characterized by fruitbodies with a striate-sulcate pileus margin, truncate lamellulae, annulate stipes, a non-bulbous stipe base with a saccate volva, inamyloid basidiospores and presence of clamps (Cui et al. 2018). The Amanitaceae.org website lists 101 binomials within sect. *Caesareae* of which 71 have a detailed description (Tulloss and Yang 2024). Sect. *Caesareae* has an almost cosmopolitan distribution with many species found in South and East Asia and in recent years several new species have been published from this region foremost from China, where for example six new species were added by Cui et al. (2018).

Laos lies within the Indo-Burma Biodiversity Hotspot (Myers 1988) and boasts a relative high forest coverage of 56%, spanning from dipterocarp dominated forests at lower altitudes to broadleaved-coniferous and evergreen forests at higher altitudes (MAF 2021). These forest types accommodate numerous ectomycorrhizal fungi, including many *Amanita* species (Læssøe et al. 2018).

Patouillard (1928) was probably the first to publish on Laotian macrofungi among mainly aphyllophoroid species from Vietnam. Another early publication mentioning macrofungi in Laos was the Flora of Laos by Vidal (1958), a French botanist, who included 33 macrofungal species. Much later, this was followed-up by a non-timber forest products handbook (NAFRI 2007), which included 29 common, edible and medicinal fungal species. Recent surveys by mycologists have been carried out at biodiversity conservation areas (Sysouphanthong et al. 2017; Lee et al. 2018; Hong et al. 2019; Lee et al. 2021), at

local community forests (Pedersen and Thammavong 2014) and at local markets (Łuczaj et al. 2021). Macrofungal species described based on material from Laos are rather few, but increased in recent years and includes *Phellinus stratosus* Pat. (Patouillard 1928), *Cyptotrama angustispora* Zhu L. Yang & J. Qin and *C. glabra* Zhu L. Yang & J. Qin (Qin and Yang 2016), *Leucoagaricus houaynhangensis* Sysouphanthong (Sysouphanthong et al. 2018), *Ganoderma nasalaense* Hapuar., Pheng. et K.D. Hyde (Hapuarachchi et al. 2019), *Hebeloma parvisporum* O. S. Pedersen, Læssøe, Beker & Eberh. (Eberhardt et al. 2020), *Cystolepiota pyramidalis* Sysouph. & Thongkl. and *C. rhodella* Sysouph. & Thongkl. (Sysouphanthong et al. 2022), *Nigrocarnea radicata* O. S. Pedersen & Læssøe (van de Peppel et al. 2022), *Leucoagaricus flavus* Sysouph. & Thongkl. and *L. griseosquamosus* Sysouph. & Thongkl. (Sysouphanthong and Thongklang 2022), and *L. laosensis* Sysouph. (Manawasinghe et al. 2022). Læssøe et al. (2018) published the first comprehensive introductory field guide on edible, poisonous and medicinal mushrooms of Northern Laos including 24 *Amanita* species of which three were from sect. *Caesareae*. The authors noted that many specimens need supporting molecular analyses to confirm their identity or make the description of new species possible. This study is the first of its kind from Laos, with the aim to document Lao species of genus *Amanita* sect. *Caesareae*, using morphological characters, molecular phylogenetic evidence and ecological data.

Materials and methods

Basidiomata were collected during the monsoon season from May to September from rich mixed forests, mainly dominated by *Fagaceae* and *Pinaceae* in the highland areas of Xieng Khouang and Vientiane provinces, Laos (Lao People's Democratic Republic). Specimens were deposited in the National Herbarium of Laos (HNL) with duplicates of some collections in C. Index Fungorum (http://www.IndexFungorum.org), the International Index of Plant Names (https://www.ipni.org) and Amanitaceae.org (http://www.Amanitaceae.org) were used as sources for taxonomic names and nomenclature.

Macroscopic characters were described from fresh material with field notes and photographs. Pileal sulcation is annotated as the ratio (R) of the length of the striations from the pileal margin to the centre of the pileus. Stipe length is from the volval base. Colour codes follow Kornerup and Wanscher (1981). Microscopic structures were observed with light microscopy (Brunel SP500 with OMAX 14MP mount microscope camera) from the dried specimens after sectioning and mounting in H_2O and 3% KOH solution and where necessary stained with Congo Red. Basidiospores were tested for amyloidity with Melzer's reagent and were measured in 3% KOH. The abbreviation (n/m/p) means n basidiospores measured from m basidiomata from p collections, using a range notation of the form (a–) b–c (–d), the range b–c contains a minimum of 90% of the measured values, extreme values (a, d) are given in parentheses, m and av. indicate average values, ± standard deviation; Q presents the length/width ratio of spores in side view. Description terminology follows Bas (1969), Tulloss and Rodríguez-Caycedo (2011) and Tulloss and Yang (2024). Sizes of basidiomata are described as small, medium or large with pileus diameter of 30–50 mm, 50–90 mm, and 90–150 mm, respectively (Bas 1969).

DNA extraction and sequencing of the ITS1, 5.8S, ITS2 regions were carried out at Tartu University, Estonia and uploaded into PlutoF cloud database (Abarenkov et al. 2010), following the procedures referred to and described in Voitk et al. (2020). Lao sequences were blasted in Unite website, https://unite.ut.ee/analysis.php (Kõljalg et al. 2020 and Abarenkov et al. 2023) and at NCBI website, http://www.ncbi.nlm.nih.gov/blast and related sequences (supplementary table 1) were retrieved for phylogenetic analyses. Priority was given to similar species, as well as to species representing neighbouring clades within sect. *Caesareae* described in recent publications (Tang et al. 2017; Cui et al. 2018; Kumla et al. 2023). The final ingroup dataset consisted of 38 sequences of which 12 new ITS sequences were from Laos, and *A. pseudosychnopyramis* from sect. *Amanita* was selected as outgroup. Supplied by the NGPhylogeny website http://www.phylogeny.fr/index.cgi (Lemoine et al. 2019) sequences was aligned with MUSCLE (Edgar 2004) and MAFFT v.7.4 (Katoh and Standley 2013) and inspected for reverse sequences in BioEdit (Hall 1999); poorly aligned positions were excluded applying the BMGE (Block Mapping and Gathering with Entropy) software (Criscuolo and Gribaldo, 2010), Maximum Likelihood (ML) phylogenetic analysis were performed by PhyML v.3.3 (Guindon et al. 2010) + Smart Model Selection (SMS) (Lefort et al. 2017) and by Bayesian Interference (BI) analysis by MrBayes v. 3.2.7 (Ronquist et al. 2012). The final trees were edited and visualized using Interactive Tree Of Life (iTOL) v.6 (Letunic and Bork 2021) and Microsoft Office. The BMGE selected 532 and removed 998 characters. MrBayes for BI was performed with 1.000.000 generations and otherwise default settings. The ML tree was examined by HKY85 substitution model with 1,000 replicates, otherwise default settings resulting in the log-likelihood tree value of -3698.83699 for the best scoring tree. The phylogram topology derived from BI and PhyML were similar and only the ML tree is shown in Fig. 1. In comparison, while running ML in MEGA11 (Tamura et al. 2021) a similar topology and bootstrap values were observed. A branch was considered strongly supported if it had ML bootstrap support ≥ 85% and posterior probability (PP) ≥ 0.95. Dataset and final alignments for *A. fulvocaesarea* and *A. chepangianoides* have been deposited in TreeBase (No. xxxxxxx/xxxxxxx and xxxxxxxxxxxx/xxxxxxx, respectively.

Results

Taxonomy

Etymology

Epithet refers to the similarity to *Amanita chepangiana* Tulloss & Bhandary.

Holotype: Laos. Xieng Khouang province, Phoukhout district, Ngodphae village, Na Phouak hamlet, 19°45'43.8"N, 103°15'22.5"E, 1385 m asl., in broadleaved forest with *Quercus* spp. *Castanea* spp. and *Lithocarpus* spp., 23 May 2016, O. Sparre Pedersen and T. Læssøe, OSP20160523–013, HNL501136, as *A.*? *chepangiana* in Læssøe et al. (2018), isotype C-F-169624 (C), GenBank: ITS = xxxxxxxxx

Diagnosis

Amanita chepangianoides is characterized by white basidiomata except the alutaceous-cream centre of the pileus, a relative short pileal margin striation (0.25 R), a snake-skin pattern on the stipe and globose to subglobose spores (8.5–10.5 × 8.5–9.5 µm) and presence of clamps.

Description

Basidiomata (Fig. 2a, f) medium to large. *Pileus* 90–130 mm in diam, initially almost spheric then convex and centrally depressed, slightly viscid, cream (4A2–3) to alutaceous cream (5B4–7B4) at centre and whitish (5A1–2) towards margin; velar remnants absent; margin striate (0.25 R), non-appendiculate; trama white (1A1), unchanging. *Lamellae* free or almost so, plentiful (approximately 100 reaching stripe), white (1A1), edge fimbriate, concolorous when fresh and when dried; *lamellulae* truncate, few of 2–3 lengths. *Stipe* 160–200 × 10–18 mm, white (1A1), cylindrical, slightly tapering upwards, basal bulb absent, covered by white (1A1) squamules, mostly in a snake-skin pattern; context white (1A1), unchanging; *volva* large, ca. 45 mm high and 35 mm wide, flaring, saccate, white (1A1). *Annulus* apical, membranous, white (1A1), upper surface sometimes weakly striate, hanging, fugacious. *Taste* not recorded. *Smell* pleasant, not prominent. *Spore deposit* not observed.

Lamellar trama (Fig. 2b). Mediostratum 20–40 µm wide, composed of fairly abundant ellipsoid to fusiform to cylindrical, thin-walled inflated cells, 25–72 × 17–32 µm; filamentous hyphae abundant, 1–2 µm wide; lateral stratum 37–55 µm wide, composed of ellipsoid to clavate inflated cells (15–40 × 10–15 µm), filamentous hyphae scarce, 1–2 my wide. *Subhymenium* (Fig. 2d) 25–42 µm thick composed of 2–3 layers of subglobose, ovoid, ellipsoid to clavate cells, 12–20 × 9–14 µm. *Lamellar edge* sterile, composed of irregular inflated cells, subglobose to irregular, hyaline, thin-walled, 16–29 × 15–25 µm; filamentous hyphae scattered, 0.5–2 µm wide. *Basidia* (Fig. 2d) 22–35 × 8–14 µm, clavate, mainly 2-spored, few 4-spored; sterigmata 3–6 µm long. *Basidiospores* (Fig. 2e) [40/1/1] (8–) 8.5–10.5 (–11) × (7.5–) 8.5–9.5 (–10.5) µm (av. 9.6 × 8.8 µm), Q = 1.04–1.14 (Qm = 1.07 ± 0.06), globose to subglobose (few broadly ellipsoid), inamyloid, hyaline, thin-walled, smooth; apiculus 1–2 µm my high and 1–1.5 µm wide. *Pileipellis* 80–120 µm thick; upper layer 25–70 µm, gelatinized, composed of radically arranged, filamentous hyphae 2–10 µm wide; lower layer 25–50 µm composed of radically arranged filamentous hyphae, 4–8 µm wide, hyaline to yellowish. *Interior of volva* (Fig. 2c) composed of longitudinally arranged elements; filamentous hyphae very abundant 1–7 µm wide, few branching; inflated cells scattered to locally abundant, subglobose, broadly ellipsoid, ellipsoid to cylindric 25–62 × 15–45 µm, hyaline to yellowish, thin-walled; gelatinized hyphae locally abundant. *Stipe trama* composed of longitudinally arranged, mainly cylindrical terminal cells, thin-walled, 30–240 × 14–65 µm; filamentous hyphae scattered, 3–10 µm wide. *Annulus* composed of interwoven filamentous hyphae and inflated cells; inflated cells rare, subglobose to ellipsoid, 25–55 × 24–35 µm; filamentous hyphae abundant, 2–8 µm wide. *Clamps* presumably present in all parts, but difficult to observe, seen with certainty on stem hyphae.

Habitat

Scattered on rich soil in broadleaved highland forests under *Fagaceae*.

Additional specimen examined

Laos. Xiang Khouang province, Phoukhout district, Ngodphae village, 19°45'43.3"N, 103°15'23.0"E, 1380 m asl., in broadleaved forest with *Quercus* spp., *Castanea* spp. and *Lithocarpus* spp., 23 May 2016, O. Sparre Pedersen and T. Læssøe, OSP20160523-012 (HNL501150).

Discussion

Our ITS phylogenetic analysis (Fig. 1) places *A. chepangianoides* in a larger clade containing *A. chepangiana* Tulloss & Bhandary, *A. fulvocaesarea* O.S. Pedersen & T. Læssøe (described as new in this study), *A. princeps* Corner and Bas, *A. aporema* Boedijn, *A. egregia* D.A. Reid and *A. pseudoprinceps* Y.-Y. Cui, Q. Cai & Zhu L. Yang.

Morphologically, the similar *A. chepangiana* differs by sometimes having yellowish to greyish tinges at centre of the pileus and by subglobose to ellipsoid basidiospores (9.5–12.5 × 8.5–11.5 µm, Q = 1.07–1.28, Qm = 1.17 ± 0.06) (Tulloss and Bhandary 1992; Cui et al. 2018). *Amanita alboumbelliformis* Y.-Y. Cui, Q. Cai & Zhu L. Yang is also similar by sometimes having a cream pileus centre, but it differs by having a longer pilea striation (0.3–0.4 R), larger spores (9–11 × 9–11 µm, Q = 1.0–1.13, Qm = 1.06 ± 0.03), yellowish tinges on the aging annulus and a greyish to brownish volva (Cui et al. 2018). *Amanita egregia* has white basidiomata, but differs by having larger pilei and stipes (up to 200 mm), broadly ellipsoid spores and association with *Eucalyptus* in Australia (Reid 1978).

Amanita fulvocaesarea O. S. Pedersen & T. Læssøe, sp. nov. (Fig. 3)

MycoBank

MB xxxxx.

Holotype: Laos. Xiang Khouang province, Phoukhout district, Ngodphae village, Na Phouang hamlet 19°45'11.9"N, 103°15'29.1"E, 1135 m asl., on soil with *Quercus* spp., *Castanopsis* spp. and *Lithocarpus* spp., 11 May 2015, O. Sparre Pedersen and T. Læssøe, OSP20150511–007, HNL500511, as *A. princeps* in Læssøe et al. (2018), isotype C-F-169623 (C), GenBank Acc. No.: ITS xxxxxxxxx.

Diagnosis: *Amanita fulvocaesarea* is characterized by the initial greyish-orange colours followed by golden brown hues, an umbonate pileus, distinct sulcation (0.3–0.4R), an apical annulus, white non-cracking volva, globose to subglobose basidiospores (9–12 x 9–11.5 µm) and presence of clamps.

Description

Basidiomata medium to large (Fig. 3a, f). *Pileus* up to 140 mm in diameter, first ovoid then convex to applanate with small broad umbo, dry to slightly viscid, centre first greyish-orange (5B4), with age golden brown (5D6) with tinges of greyish-ochraceous (5–8B2) towards margin; veil remnants absent; margin

sulcate (0.3–0.4 R), non-appendiculate; trama white (1A1), unchanging. *Lamellae* free or almost so, whitish (1A1), when dry brownish-orange (6C5), appr. 100 reaching the stipe; *lamellulae* few of 2–3 lengths, truncate; edge fimbriate, sterile, concolorous, when dried whitish-cream (4A1–2). *Stipe* 150–200 ×12–20 mm, white (1A1) smooth to slightly fibrous, tapering towards apex, sometimes almost cylindrical; context white (1A1), hollow; basal bulb absent; *volva* 30–60 mm high and 20–40 mm wide, saccate, thick, white (1A1), not cracking, flaring. *Annulus* white (1A1) on both sides, apical, big, pendent, but rather fugacious; upper surface non-striate. *Taste* not recorded. *Odour* indistinctive. *Spore deposit* not observed.

Lamellar trama (Fig. 3b) bilateral. *Mediostratum* 42–63 µm wide, interwoven, inflated cells thin-walled, hyaline, scattered, fusiform, ellipsoid to clavate 18–180 × 7–27 µm; filamentous hyphae abundant, 1–5 µm wide; lateral stratum 36–64 µm wide with abundant clavate to cylindrical, inflated cells 23–38 × 5–11 µm; filamentous hyphae scarce, 1–2 my wide. S*ubhymenium* (Fig. 3d) 14–34 µm thick, composed of 2–3 layers of subglobose, ellipsoid to irregular cells, 8–21 × 6–15 µm. *Lamellar edge* sterile composed of scattered subglobose, ellipsoid to cylindric, inflated, hyaline, thin-walled cells, 16–36 × 11–18 µm and scattered filamentous, 1–2.5 µm wide. *Basidia* (Fig. 3d) 42–50 × 15–22 µm, clavate, 2- (few) and 4-spored, sterigmata 3–6 µm long. *Basidiospores* (Fig. 3e) [30/1/1] 9–12 (–12.5) × 9–11.5 (–12) µm (av. 11.5 × 11 µm), Q = 1.01–1.08, (Qm 1.04 ± 0.03); globose to subglobose, inamyloid, hyaline, thin-walled, smooth; apiculus 1.5–2 µm high and 1–2 µm wide. *Pileipellis* 135–200 µm thick; upper layer 40–140 µm, gelatinized, composed of radially arranged to interwoven hyphae, 4–11 µm wide, hyaline to yellowish-brown; lower layer 30–75 µm thick, composed of interwoven filamentous hyphae 1.5–3.5 µm wide, hyaline to yellowish. *Volva* dominantly composed of filamentous hyphae; interior surface (Fig. 3c) composed of longitudinally orientated filamentous hyphae, 2–4 µm wide, hyaline, thin-walled; outer surface of similar structure, but with gelatinized filamentous hyphae 2–6 µm wide; inflated cells scattered, subglobose, fusiform to ellipsoid, 15– 90×10–50 µm, hyaline, thin-walled. *Stipe trama* composed of longitudinally arranged, inflated, cylindrical to slightly clavate, hyaline, thin-walled terminal cells, 67–215 × 31–45 µm; filamentous hyphae abundant, 3–5 µm wide. *Annulus* composed of interwoven elements: outer part with inflated cells rare to scattered, subglobose to ellipsoid, 15–34 × 13–23 µm, hyaline, thin-walled; filamentous hyphae very abundant 1–5 µm wide, hyaline, thin-walled; inner part with radially arranged inflated cells, scattered to fairly abundant, hyaline, subglobose to elongate, 20–44 × 8–24 µm; filamentous hyphae abundant, 1–6 µm wide, hyaline, yellow to yellowish-brown, thin-walled. *Clamps* presumably present in all parts, but difficult to observe, seen with certainty on pileipellis hyphae and on stem hyphae.

Habitat and distribution

On rich soil in broadleaved forests with members of *Fagaceae* (Laos) *Fagaceae* and *Pinaceae* (China). Known from Laos (this study) and China (non-described).

Additional specimen examined

Laos. Xiang Khouang province, Phoukhout district, Ngodphae village, 19°45'43.3"N, 103°15'23.0"E, 1381 m asl., in broadleaved forest with *Quercus* spp., *Castanopsis* spp. and *Lithocarpus* spp., 23 May 2016, O. Sparre Pedersen and T. Læssøe, OSP20160523–009 (HNL501130). China. Yunan province, Puer, ca. 1400 m asl., in a forest with *Lithocarpus* and *Pinus*, 6 July 2015, Qing Cai 1349 (KUN-HKAS91972). The analyses of the Chinese taxa are based on Genbank ITS sequences, only.

Discussion

Our phylogenetic analysis (Fig. 1) showed that three *Amanita* specimens: HKAS91972 (ITS = MH508608), HKAS101390 (ITS = MH508603) and HKAS91096 (ITS = MH508607) from China, clustered with *A. fulvocaesarea* with strong ML/PP support. These specimens were briefly mentioned by Cui et al. (2018), but not assigned to any described taxon, due to insufficient morphological observations. For related species in the clade please see the commentary section of *A. chepangianoides*.

Morphologically *A. fulvocaesarea* is similar to *A. pseudoprinceps and A. princeps*. The former differs by a shorter pileal striation (0.2–0.3 R) and subglobose to broadly ellipsoid spores (Q = 1.02–1.23, Qm = 1.12 ± 0.04) (Cui et al. 2018) and in this study (Q = 1.02–1.25, Qm = 1.12 ± 0.08). The latter species differs by a larger (up to 200 mm) brownish to yellow pileus, shorter pileal striation (0.2–0.25 R), a volval outer surface that often cracks and peels into thin brownish patches (Corner and Bas 1962; Yang 2015; Tang et al. 2017) and the association with dipterocarps (Malaysia) (Tang et al. 2017). *Amanita aporema* has some similarities, but it differs by relatively smaller basidiomata and darker coloured pilei (brown to reddish-brown, ca. 60–100 mm in diam.) and longer striations, ca. 0.5 R (Tang et al. 2017; Cui et al. 2018).

Description

Basidiomata (Fig. 4a, d-f) medium sized to large. *Pileus* 60–140 mm in diam, initially hemispheric, then convex to applanate, slightly viscid, olive grey (1E2) to yellowish-brown (5D–E8), darker towards centre and white to yellowish-cream (3–4A3) to white (1A1) at margin; velar remnants absent; margin striate (0.2–0.25 R), non-appendiculate; trama white (1A1), unchanging. *Lamellae* free or almost so, white (1A1) to whitish-cream (4A1–2), plentiful (> 100 reaching stipe), up to 20 mm wide; *lamellulae* truncate, few of 5–6 lengths. *Stipe* 70–150 × 10–20 mm, cylindrical, basal bulb absent, white (1A1) with slightly darker fibrils; context white (1A1), unchanging, hollow; *volva* large, saccate, 30–60 mm high and 20–40 mm wide, white (1A1), sometimes brownish (2B2-4) patches. *Annulus* (Fig. 4f) apical, skirt-like, membranous, 10–20 mm high, white (1A1), partly striate on upper surface. *Taste* not recorded. *Smell* insignificant. S*pore deposit* not observed.

Basidia (Fig. 4b) clavate, 28–54 × 10–17 µm, 4-spored, sterigmata 1.5–7 µm long. *Basidiospores* (Fig. 4c) [120/3/3] (8.5–) 9.5–14.5 (–18.5) × (7.5–) 8–12.5 (–13.5) µm (av. 12 × 10.5 µm), Q = 1.02–1.25 (Qm = 1.12 ± 0.08), subglobose to broadly ellipsoid, very few globose, inamyloid, hyaline, thin-walled,

smooth; apiculus 1.5–2 µm high and 1–1.5 µm wide. *Pileipellis* 95–150 my thick; upper layer composed of radically arranged gelatinized filamentous hyphae, 1–2 µm wide; lower layer composed of radically arranged, hyaline, filamentous hyphae, 4–8 my wide. *Interior of volva* composed of filamentous hyphae 1.5–8 µm wide; inflated cells scattered, ellipsoid, hyaline, hyaline, thin-walled, scattered to locally abundant, 22–55 × 13–25 µm. *Stipe trama* composed of longitudinally arranged, ellipsoid, clavate to cylindric, thin-walled terminal cells, 85–170 × 15–60 µm; filamentous hyphae scattered to abundant, 2–10 µm wide. *Clamps* not observed.

Habitat and distribution

Scattered on soil in mixed forests with members of *Fagaceae*, *Dipterocarpaceae* and *Pinaceae* or without the dipterocarp element, at altitudes 800–1125 m, May to August. Known from southwestern China, Nepal, Laos and Thailand. Common at local markets in Laos.

Specimens examined

Laos. Vientiane province, Thoulakhom district, Vangheua village, 18°20'31.5"N, 102°47'46.6"E 790 m asl., in mixed forest with *Pinus kesiya*, *Dipterocarpus* spp. and *Fagaceae*, 19 May 2012, O. Sparre Pedersen, OSP20120519-001 (HNL500333). Ibid., 18°20'39.7"N, 102°47'36.9"E. 803 m asl., 6 July 2013, O. Sparre Pedersen, OSP20130706-004 (HNL500044). Xieng Khouang province, Pek district, Dong Village, 19°29'39.1"N, 103°16'07.7"E 1123 m asl., in mixed forest including *Pinus kesiya*, *Keteleeria everlyniana* and *Quercus* spp. 22 June 2014, O. Sparre Pedersen, OSP20140622–007 (HNL500154).

Discussion

Amanita pseudoprinceps was recently described from Yunnan, China, from a *Fagaceae* dominated mixed forest at 2300 m altitude (Cui et al. 2018). The Laotian specimens (HNL500044, HNL500155 and HNL500333) match the type in terms of ecology, pileus and stipe colours, striation on pileus, apical placement of annulus, spore size as well as in our phylogenetic analysis (Fig. 1). A minor difference is that Laotian specimens have a hollow stipe in full length, compared to the holotype, which is only hollow in the centre. As discussed above, *A. pseudoprinceps* can be easily confused with *A. princeps*, which differs by a broader pileus (100–200 mm), with light brown to yellowish-brown colours and with brownish cracking patches on the outer surface of the volva and association with dipterocarps (Tang et al. 2017). It also resembles *A. fulvocaesarea* (described as new above), which is smaller, has a longer pileal striation (0.3–0.4 R) and dominantly globose spores. The two other species shown in the clade (Fig. 1), *A. aporema* and *A. egregia* differ from *A. pseudoprinceps* by a brown to reddish-brown pileus and a white basidioma, respectively (Tang et al. 2017; Reid 1978).

Recent work of Łuczaj et al. (2021) surveying markets in Luang Prabang, Laos, as well as an ethnobotanical survey in Yunnan, China by Wang et al. (2022) identified two taxa as *A. princeps* (WA0000072256: ITS = MT 252585 and HKAS122502: ITS = ON394332, respectively). With strong

bootstrap support in our study (Fig. 1) these taxa should be referred to as *A. pseudoprinceps*. See Cui et al. (2018) for further misidentified specimens.

Amanita rubromarginata Har. Takah., Mycoscience 45(6): 372 (2004) (Fig. 5)

Description

Basidiomata (Fig. 5a, b, f) medium sized. *Pileus* 45–90 mm in diam, first almost cylindrical, then convex to applanate, centrally depressed, occasionally with broad umbo, dry or almost so, orange red (8A8) to brownish-red (9C–D8) at centre and orange (5A6–7), yellowish-orange (4AB7) to deep yellow (4A8) at margin; velar remnants absent; margin striate (0.4–0.5 R), non-appendiculate, trama yellowish (4A4–5). *Lamellae* free or almost so, plentiful (ca. 110 reaching stem), yellowish (2A7); edge fimbriate, light orange (5A4) to reddish-orange (7A7); *lamellulae* truncate, few of 3–4 lengths. *Stipe* 70–120 × 7–9 mm, cylindrical to slightly tapering upwards, basal bulb absent, hollow, yellow (2A7–3A7) to light yellow (4A4–5), partially covered by orange (5A6) to orange-red (8A8) squamules in a snake-skin pattern; context light yellow (4A4–5); *volva* thick, large, saccate, 35–40 mm high and 25–30 mm wide, whitish (2A2). *Annulus* subapical, membranous, hanging, dark orange (5A8) to reddish-orange (7A7), upper surface non-striate, edge dentate. *Taste* not recorded. *Smell* indistinct. *Spore deposit* not observed.

Basidia (Fig. 5c) 9–12 x 26–37 µm clavate, 4-spored; sterigmata 2.5–4.5 µm long; basal septa clamped. *Basidiospores* (Fig. 5d) [40/2/2] 6.5–9 (–9.5) × 5.5–7 (–7.5) µm (av. 7.5 × 6.2 µm), Q: 1.13–1.37 (Qm = 1.22 ± 0.7), broadly ellipsoid, few subglobose and few ellipsoid, inamyloid, hyaline, thin-walled, smooth; apiculus small, 0.5 µm high and 1–1.5 µm wide. *Pileipellis* 80–130 µm thick, 2-layered with radically arranged hyphae; upper layer 30–35 µm wide, filamentous hyphae gelatinized, few branching, thin-walled, hyaline, 1–6 µm wide; lower layer 50–100 µm thick, gelatinized; filamentous hyphae 3–8 µm wide, hyaline to yellowish. *Interior of volva* composed of longitudinally arranged elements: filamentous hyphae 2–7,5 µm wide, hyaline to yellowish; inflated cells scattered to abundant ellipsoid to elongate 37–54 × 23–40 µm, hyaline to yellowish, thin-walled. *Stipe trama* composed of longitudinally arranged, mainly cylindrical, terminal cells, 100–150 × 15–30 µm; filamentous hyphae scattered, thin-walled, 3–7 µm wide. *Clamps* not observed with certainty.

Habitat and distribution

Scattered, on soil in mixed highland forests with species of *Pinaceae* and *Fagaceae*. Known from China, Loas (this study) Japan, Malaysia and Thailand.

Specimens examined

Laos. Xieng Khouang province, Pek district, Dong village, 19°29'37.8"N, 103°16'11.2"E1121 m asl., in mixed forest with *Quercus spp.*, *Pinus kesiya*, and *Keteleeria everlyniana*, 14 August 2015, O. Sparre Pedersen and T. Læssøe, OSP20150814-016 (HNL500859); ibid., 19°29'39.8"N, 103°16'05.8"E, 1129 m asl., 16 August 2022, O. Sparre Pedersen and W. Sibounheuang, OSP20220816-005 (HNL502458).

Discussion

Amanita rubromarginata was described from broad-leaved forests in southwestern islands of Japan (Takahashi 2004). It is distinctive by the long striation, i.e. 0.5 R in Cui et al. (2018) (in this study 0.4–0.5 R), the reddish-orange lamellar edge, dark orange to reddish-orange membranous annulus, thick white volva as well as the association with *Fagaceae*. Notably, we have not seen a greyish-yellow discoloration of aged specimens as shown in Terashima et al. (2016). Similarly, this feature was similarly not recorded in the description of this species from China (Cui et al. 2018) and from Thailand (Thongbai et al. 2016; Kumla et al. 2023). *Amanita rubromarginata* somewhat resembles *A. malayensis* L.P. Tang, Zhu L. Yang & S.S. Lee and *A. javanica* (Corner & Bas) T. Oda, C. Tanaka & Tsuda. The former differs by a yellowish to yellowish-orange pileus, concolorous lamellae edge, and the latter differs by less reddish to orange tinges on pileus and annulus. Both of these species are recorded in association with members of *Dipterocarpaceae* (Tang et al. 2017; Cui et al. 2018, Tulloss and Yang 2024). *Amanita subhemibapha* Zhu L. Yang, Y.-Y. Cui & Q. Cai (see below) differs by lacking the dark lamellae edge and yellowish zone in lower pileipellis. In our phylogenetic tree (Fig. 1) the three lao specimens clustered with strong support the *A. rubromarginata* specimens from China and Thailand.

Description

Basidiomata (Fig. 6a, b, e) medium to large sized. *Pileus* up to 100 mm in diam, initially almost cylindrical, then applanate to slightly depressed, not umbonate, dry or almost so, centre brownish-orange (7C6–7) when young then reddish-orange (7A8), orange (5A6, 6A8), at margin orange-yellow (4A8); veil remnants absent; margin striate, 0.2–0.4 R; non-appendiculate; trama white (1A1), unchanging; lower pileipellis with a pale to light yellow (3A3-5) zone, 0.7–0.9 mm wide. *Lamellae* free or almost so, yellowish-white (3A2) to light yellow (4A5), 9–11 mm wide, plentiful (nearly 100 reaching stipe); edge light to pale yellow (3A3–4); *lamellulae* truncate, l–2 shorter of various lengths between full-length lamellae. *Stipe* 80–110 × 8–10 mm, cylindrical, hollow, yellowish-white (3A2), partly to fully covered with reddish-orange (7A8) to dark orange (5A8) fibrils; *volva* saccate, thin, 35–60 mm high and 30–40 mm wide, white (1A1). *Annulus* subapical, membranous, hanging, yellowish-white (3A2) to dark orange (5A8) upper/lower concolorous; upper surface non-striate. *Taste* not recorded. *Smell* indistinct. *Spore deposit* white (1A1).

Basidia (Fig. 6c) 27–55 × 10–16 µm, clavate, 4-spored; sterigmata 2–5 µm long. *Basidiospores* (Fig. 6d) [80/2/2] 7.5–10 (–10.5) × (6–) 6.5–8 (–8.5) µm (av. 8.5 × 7 µm), Q: 1.05–1.39 (Qm 1.23 ± 0.08) broadly ellipsoid to ellipsoid, few subglobose, inamyloid, hyaline, thin-walled, smooth; apiculus 0.5–1 µm high and 1–1.5 µm wide. *Pileipellis* 120–175 µm thick; upper layer 50–100 µm thick, gelatinized, composed of radially to interwoven, thin-walled, hyaline, filamentous hyphae, 2–3 µm wide; lower layer 90–120 µm thick, composed of radially to interwoven filamentous hyphae, 4–15 µm wide, hyaline. *Interior of volval remnants* composed of longitudinally to interwoven arranged elements; filamentous hyphae abundant, 1.5–10 µm wide, hyaline, thin-walled; inflated cells scattered to abundant, broadly ellipsoid, elongate to narrowly cylindrical, hyaline, thin-walled, 38–125 × 30–60 µm. *Stipe trama* composed of longitudinally

arranged, mainly cylindrical, terminal cells, thin-walled, hyaline, 105–200 × 24–46 µm; filamentous hyphae scattered, 1.5–6 µm wide.

Habitat and distribution

Scattered on soil in mixed highland forests at altitudes of ca 1150 m, dominated by members of *Pinaceae* and *Fagaceae*. Know from Southwestern China, Laos (this study) and northern Thailand.

Specimens examined

Laos. Xieng Khouang province, Pek district, Boa village, 19°29'47.3"N, 103°17'39.1"E, 1137 m asl., in mixed forest with *Castanopsis spp., Quercus spp.*, few *Pinus kesiya* and few *Keteleeria everlyniana*, 6 August 2017, O. Sparre Pedersen and W. Sibounheuang, OSP20170806-005 (HNL501890); ibid., Dong village, 19°29'32.7"N, 103°16'06.2"E, 1141 m asl., in mixed forest with *Quercus spp.*, few *Pinus kesiya* and few *Keteleeria everlyniana*, 31 July 2018, O. Sparre Pedersen, OSP20180731–003 (HNL502059), as *A.* cf. *hemibapha* in Læssøe et al. (2018).

Discussion

Amanita subhemibapha was recently described from Yunnan, southwestern China, in association with *Fagaceae* and *Pinaceae* and recognized by the combination of a non-umbonate orange centre of the pileus, striation 0.25–0.35 R, white to cream lamellae, yellowish lamellae edges and broadly ellipsoid to ellipsoid spores (Cui et al. 2018). The Laotian specimens are slightly darker in the yellow and orange colours; the veil remnants on the stipe are more distinct, and the yellow zone in lower pileipellis is also more pronounced. *Amanita subhemibapha* somewhat resembles *A. hemibapha* (Berk. & Broome) Sacc., *A. malayensis, A. kitamagotake* N. Endo & A. Yamada, *A. rubromarginata* and *A. javanica. Amanita hemibapha* differs by having a darker red pileus centre, an orange margin and narrower spores (5.5–6.5 µm) (Vrinda et al 2005; Cui et al. 2018) to more yellowish pileus margin and spore width of 5.5–7 µm (Kumla et al. 2023). *Amanita rubromarginata* and *A. javanica* differ by having longer pileal striation (0.4–0.5 R) and reddish lamellae edges; *A. kitamagotake* differs by having uniformly yellow colours on pileus and stipe surface (Yang 2015; Endo et al. 2017; Cui et al. 2018)d *malayensis* differs by having a whitish-cream stipe and an association with dipterocarps (Tang et al. 2017). Our phylogenetic tree (Fig. 1) indicates that *A. subhemibapha* is closely related to *A. malayensis A. similis* Boedijn and *A. fuscoflava*, Zhu L. Yang, Yang-Yang Cui & Qing Cai. The former differences to *A. subhemibapha* are discussed above and under the discussion heading of *A. rubromarginata; A. similis* differs by a longer pileal striation (0.33–0.6 R) and a greyish brown to olivaceous brown pileal center (Boedijn (1951; Corner and Bas 1962); and *A. fuscoflava* also differs by a longer pilar striation (0.5–0.6 R) and a dark brown pilar center (Cui et al. 2018).

Key to Amanita sect. Caesareae known from and likely to occur in Laos

1. Stipe white to cream with fibrils of same colours 2

Stipe yellowish-white to yellowish, typically with darker fibrils 8

2. Basidiomata entirely white or cream to alutaceous-cream at centre of pileus 3

Basidiomata with yellow-greyish, cream, brown to brownish centre of pileus 5

3. Striation on pileus 0.3–0.4 R; annulus persistent. Pileus yellowish with age; pileus centre cream; stipe white; basidia with 4 sterigmata; spores globose to sub-globose, 9–11 × 9–11 µm, Q = 1.0–1.13, Qm = 1.06 ± 0.03 *A. alboumbelliformis*

Striation on pileus 0.1–0.3 R; annulus fragile 4

4. Pileus centre cream to alutaceous cream, striation 0.25 R. Annulus white; stipe squamules white in snakeskin pattern, basidia mainly with 2 sterigmata; spores globose to subglobose, 8.5–10.5 × 8.5–9.5 µm, Q = 1.04–1.14, Qm = 1.07 ± 0.06 *A. chepangianoides*

Pileus centre white, sometimes with yellowish, yellowish or brownish tint, striation 0.1–0.15R. Annulus with age yellowish; stipe squamules white, bruising yellowish to greyish; basidia with 4 sterigmata; spores sub- globose to broadly ellipsoid, 9.5–12.5 × 8.5–11.5 µm, Q = 1.07–1.28, Qm = 1.17 ± 0.06 *A. chepangiana*

5. Striation on pileus 0.3–0.6 R; spores globose to subglobose 6

Striation on pileus less than 0.3 R; spores subglobose to broadly ellipsoid 7

6. Pileus to 140 mm wide, with broad umbo, striation 0.3–0.4 R, centre first greyish-orange soon golden brown. Stipe white, smooth to slightly fibrous; volval surface not cracking into brownish patches; annulus membranous, basidiospores 9–12 × 8.5–11.5 µm, Qm: 1.04 ± 0.06 *A. fulvocaesarea*

Pileus 60–100 mm wide, slightly depressed, non-umbonate, striation 0.5–0.6 R, centre reddish-brown.

Stipe pale cream, ± darker squamules; anulus fragile; basidiospores 8.5–11 × 8.5–10.5 µm, Qm: 1.06 ± 0.04 *A. aporema*

7. Pileus to 150 mm in diameter, centre yellowish cream, olive-grey to yellowish brown; stipe squamules white to dirty white; volval surface not cracking into brownish patches; basidiospores 9.5–14.5 × 8–12.5 µm, Qm = 1.12 ± 0.08 *A. pseudoprinceps*

Pileus to 200 mm in diameter, centre brown to yellowish-brown; stipe squamules white, volva often cracking and peeling into thin patches; basidiospores 8–11 × 7.5–10 µm, Qm = 1.08 ± 0.05 *A. princeps*

8. Lamellar edge concolorous with lamellae 9

Lamellar edge yellowish or reddish orange 11

9. Pileus uniformly yellowish. Pileus 40–100 mm in diameter, striation 0.3–0.5 R; lamellae yellowish; stipe yellowish to yellow, squamules yellow to orange buff; annulus yellow; basidiospores broadly ellipsoid to ellipsoid, 8.5–13.5 × 6–8.5 µm, Qm = 1.41 ± 0.6 *A. kitamagotake*

Pileus centre and margin of different colours 10

10. Pileus centre red to orange red to yellow, margin yellow. Pileus 60–150 mm in diameter, striation 0.3–0.5R;

lamellae cream to yellowish; stipe yellowish, stipe squamules yellow to orange-yellow in irregular snakeskin pattern; annulus yellow; basidiospores ellipsoid, 7.5–11 × 5.5–7.5 µm, Qm = 1.49 ± 0.13 *A. hemibapha*

Pileus centre yellowish to yellowish orange. Pileus 60–100 mm in diameter, margin pale yellow to maize yellow, striation 0.3–0.4R; stipe yellowish to cream, stipe squamules below annulus yellow; lamellae whitish, edge colour unknown probably concolorous; annulus yellowish white to whitish; basidiospores broadly ellipsoid to ellipsoid, 9–12 × 6–8 µm, Qm 1.39 ± 0.13 *A. malayensis*

11. Lamellar edge reddish orange. Pileus center orange red to brownish-red to red, margin orange to deep yellow, 60–90(130) mm in diameter; striation 0.4–0.5 R; stipe yellowish, stipe squamules orange to orange-red, often in a snakeskin pattern; lamellae yellow, edge light orange to reddish-orange; annulus, dark orange to reddish-orange; basidiospores 6.5–9 × 5.5–7 µm, Qm 1.22 ± 0.07. *A. rubromarginata*

Lamellar edge in yellow colours 12

12. Pileus center brownish-orange to reddish-orange; margin orange yellow, 60–110 mm in diameter; striation 0.2–0.35 R. Lower pileipellis with yellowish zone (0.7–0.9 mm); stipe yellowish white, stipe squamules reddish-orange to dark orange; lamellae yellowish-white to light yellow, annulus yellowish-white to orange; basidiospores 7.5–10 × 6.5–8 µm, Qm: 1.23 ± 0.08 *A. subhemibapba*

Pileus center orange-yellow to ochre yellow, margin paler or buff, 60–90 mm in diameter; striation 0.4–0.5 R. Lamellae whitish, stipe yellowish, stipe squamules orange-buff; annulus orange buff, membranous;

basidiospores 7.5–9 × 5.8–7 µm, Qm: 1.26 ± 0.06 *A. javanica*

Declarations

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Drafts of the manuscript were written by OSP with regular comments and suggestions from TL. Both authors have read and approved the final manuscript. *Datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.* The authors declare no competing interests related to this study.

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Figures

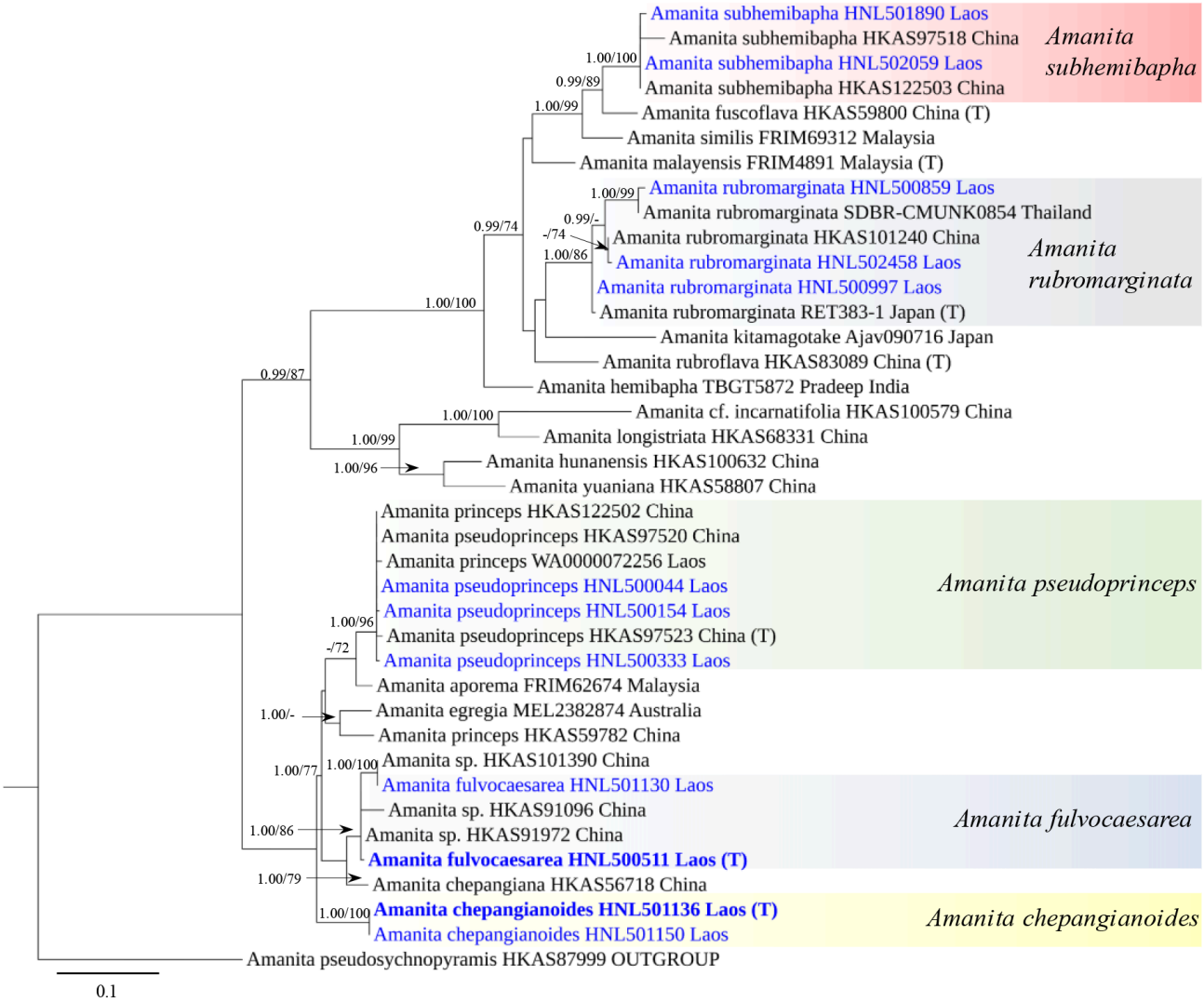


Fig. 1 Phylogenetic tree of ITS derived sequences from Bayesian Interference and Maximum Likelihood analyses of sect. *Caesareae*. Bayesian posterior probabilities (first value) ≥ 0.95 and bootstrap support values for Maximum Likelihood (second value) ≥ 70 are placed near the branches. The ML tree is rooted to *A. pseudosychnopyramis* from sect. *Amanita*. Newly generated Lao sequences used in this study are highlighted in blue. Type specimens are annotated with (T) and proposed new TYPE species are indicated in bold script.

Figure 1

Phylogenetic tree of ITS derived sequences from Bayesian Interference and Maximum Likelihood analyses of sect. *Caesareae*. Bayesian posterior probabilities (first value) ≥ 0.95 and bootstrap support values for Maximum Likelihood (second value) ≥ 70 are placed near the branches. The ML tree is rooted to *A. pseudosychnopyramis* from sect. *Amanita*. Newly generated Lao sequences used in this study are highlighted in blue. Type specimens are annotated with (T) and proposed new TYPE species are indicated in bold script.



Figure 2

Amanita chepangianoides **a**–**e** TYPE, HNL501136: **a** Basidiomata in situ; **b** Lamellar trama: mediostratum and lateral stratum; **c** Inner volva; **d** Hymenium and subhymenium; **e** Basidiospores. **f** HNL501150 (paratype): basidiomata in situ. Scale bars: **a, f** = 30 mm; **b,** = 100 µm; **c** = 30 µm **d, e** = 10 µm



Figure 3

Amanita fulvocaesarea **a-e** TYPE, HNL500511: **a** Basidiomata in situ; **b** Lamellar trama: mediostratum and lateral stratum; **c** Longitudinal section of inner part of volva; **d**Hymenium and subhymenium; **e** Basidiospores. **f** HNL501130 (paratype): basidiomata in situ. Scale bars: **a, f** = 30 mm; **b** = 100 µm, **c** = 30 µm **d, e** = 10 µm



Figure 4

Amanita pseudoprinceps **a**–**c** HNL500333: **a** Basidioma in habitat; **b** Hymenium and subhymenium; **c** Basidiospores. **d** HNL500044: Basidiomata in situ. Scale bars: **a, d-e** = 30 mm; f= 10 mm; **b, c** = 10 µm



Figure 5

Amanita rubromarginata **a**–**d** HNL500859: **a-b** Basidiomata in situ; **c** Basidium; **d** Basidiospores. **e**HNL502458: basidiomata in habitat. Scale bars: **a, b, e** = 30 mm; **c, d**= 10 µm



Figure 6

Amanita subhemibapha **a-d** HNL502059: **a-b** Basidiomata, **c** Hymenium and subhymenium, **d** Basidiospores, **e** HNL501890: basidiomata in situ. Scale bars: **a, e** = 30 mm; **b**= 10 mm **c, d** = 10 µm

Supplementary Files

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- Supplementarytable1AmanitaITSsequencedata.docx