Chiang Mai J. Sci. 2020; 47(1) : 49-56
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

Lepiota Section *Stenosporae* (Agaricaceae): Two New Records to Lao People's Democratic Republic

Phongeun Sysouphanthong [a,b,c], Naritsada Thongklang [a,b], Chatmongkon Suwannapoom*[d], Wipornpan Nuangmek [d] and Kevin D. Hyde [a,b]

[a] School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand.
[b] Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand.
[c] Ecology Division, Biotechnology and Ecology Institute, Ministry of Science and Technology, P.O.Box: 2279, Vientiane Capital, Lao PDR.
[d] School of Agriculture and Natural Resources, University of Phayao, Phayao Province 56000, Thailand.

*Author for correspondence; e-mail: chatmongkonup@gmail.com

Received: 1 August 2019
Revised: 13 November 2019
Accepted: 16 December 2019

Abstract

Two species of *Lepiota* in section *Stenosporae* (J. Lange) Kühner, *L. aureofulvella* Sysouph., K.D. Hyde, Chukeat. & Vellinga and *L. citrophylla* (Berk. & Broome) Sacc., are firstly recorded from Lao PDR. Full descriptions, colour photographs and line drawings of micromorphology are provided. Furthermore, phylogenetic position of *L. aureofulvella* and *L. citrophylla* based on nrITS sequence data was confirmed and the two species were compared with related species in the section.

Keywords: Basidiomycota, lepiotaceous fungi, phylogeny, taxonomy

1. Introduction

The genus *Lepiota* (Pers.) Gray belongs to the family Agaricaceae comprising 400 white-spored species and has been divided into six sections: *Echinatae* Fay., *Lepiota* (Pers.) Gray, *Lilaceae* M. Bon, *Fuscovinacae* (J. Lange) Kühner, *Ovisporae* (J. Lange) Kühner, and *Stenosporae* (J. Lange) Kühner [1-5]. Members in the section *Stenosporae* are mainly recognized by having cylindrical spores with a spurred base, cutis or a trichodermal structures of pileus covering made up of long erect and slender elements, and clamp connections. According to a review of the genus *Lepiota* and its distribution in East Asia, there are no reports of *Lepiota* from Lao PDR [5, 6]. This study presents two new records of *Lepiota* to Lao PDR, based on evidence from morphological characteristics and nrITS sequence data.

2. Materials and Methods

2.1 Sample Collection and Morphological Study

Specimens were collected from deciduous forests in Xay District, Oudomxay Province during the rainy season (June-October) in 2014. Important information of the collection sites including coordinates of locations, forest types, soil and substrate were recorded. Fresh basidiomata were photographed in the fields. Morphological characteristics followed those by Vellinga [5], and colour annotations of fresh materials were determined based on the colour charts of Kornerup

and Wanscher [7]. The specimens were then dried in an electric food dryer (30–40 °C) for 24 hours and deposited in the National Herbarium of Laos (Herbier National du Laos, HNL). Microscopic characteristics were observed and illustrations were made from dry specimens using an light Olympus CX-41 compound microscope. Water (H_20) and 2.5–10% of KOH were used to observe original colour of microscopic elements; while Melzer's reagent, Cotton blue and Cresyl blue were used for testing spore wall reactions; and Congo red in ammonia for line drawings. Measurements of microcharacters were given in the descriptions, while quotient (Q) of length was also calculated to indicate the basidiospore shape. Technical terms used for description were based on the study by Vellinga [5].

2.2 Molecular Study

DNA was extracted from dried specimens according to the instructions of the Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou, P.R. China). Internal transcribed spacer 1 and 2 (ITS1 & ITS2) sequences were analysed for all collections.. Internal primers ITS1-F and ITS4 were used for PCR [8]. The PCR amplified products were cleaned and sequenced by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. Sequences were edited and contigs assembled using SeqMan program (DNAStar, Madison, WI, USA), and new sequences were deposited in GenBank. All available sequences belonging to *Lepiota* section *Stenosporae* were obtained from the GenBank, and *L. cristata* (Bolton) P. Kumm. was used as an outgroup. A complete data set was aligned using MAFFT version 7.130-win32 [9-10]. The maximum parsimony (MP) tree was constructed by the program PAUP* 4.0 b10 [11], using 1000 heuristic searches, employing TBR branch swapping and random sequence addition, gaps were treated as missing data, and all characters are unordered and equally weighted. Other setting were as follows: multistate taxa interpreted as uncertainty, starting trees were obtained via stepwise addition, one tree was held at each step during stepwise addition, the steepest descent option was not in effect, branches were collapsed (creating polytomies) if minimum branch length was zero, and MulTrees option was in effect. Bootstrap supports were evaluated using 1000 bootstrap replicates with 10 heuristic searches per replicate, random sequence addition and TBR branch swapping. A Maximum Likelihood analysis was executed in the software MEGA version 6 [12-13]. The settings were: Maximum Likelihood as statistical method, 1000 bootstrap replications, using Kimura 2-parameter model, Gamma distributed (G) as rates among sites, and Nearest-Neighbor-Interchange (NNI) as Initial Tree for ML. The final alignments have been submitted to TreeBASE (TreeBase number 23646).

3. Results and Discussion

3.1 Phylogenetic Analysis

The Maximum Parsimony tree is identical to that of Maximum Likelihood. Maximum Parsimony tree is shown in Figure 1, with tree scores for the consistency index (CI) = 0.570, retention index (RI) = 0.753, rescaled consistency index (RC) = 0.429, and homoplasy index (HI) = 0.430, length = 749. Two clades are distinguished. Clade 1 (90% bootstrap support, BS) comprises species with spurred basidiospores and trichodermal pileus covering made up of long cylindrical elements on the top of with short clavate basal elements, and two specimens of *L. citrophylla* from Laos are identical to a specimen from Thailand with 100% BS. Clade 2 (98% BS) comprises species with spurred basidiospores and a cutis pileus covering made up of long cylindrical elements, and a Lao specimen of *L. aureofulvella* is identical to the specimen from Thailand (100% BS).

3.2 Taxonomy

Two species belonging to *Lepiota* sect. *Stenosporae* were described in this study. Each species is provided with a detailed description, colour

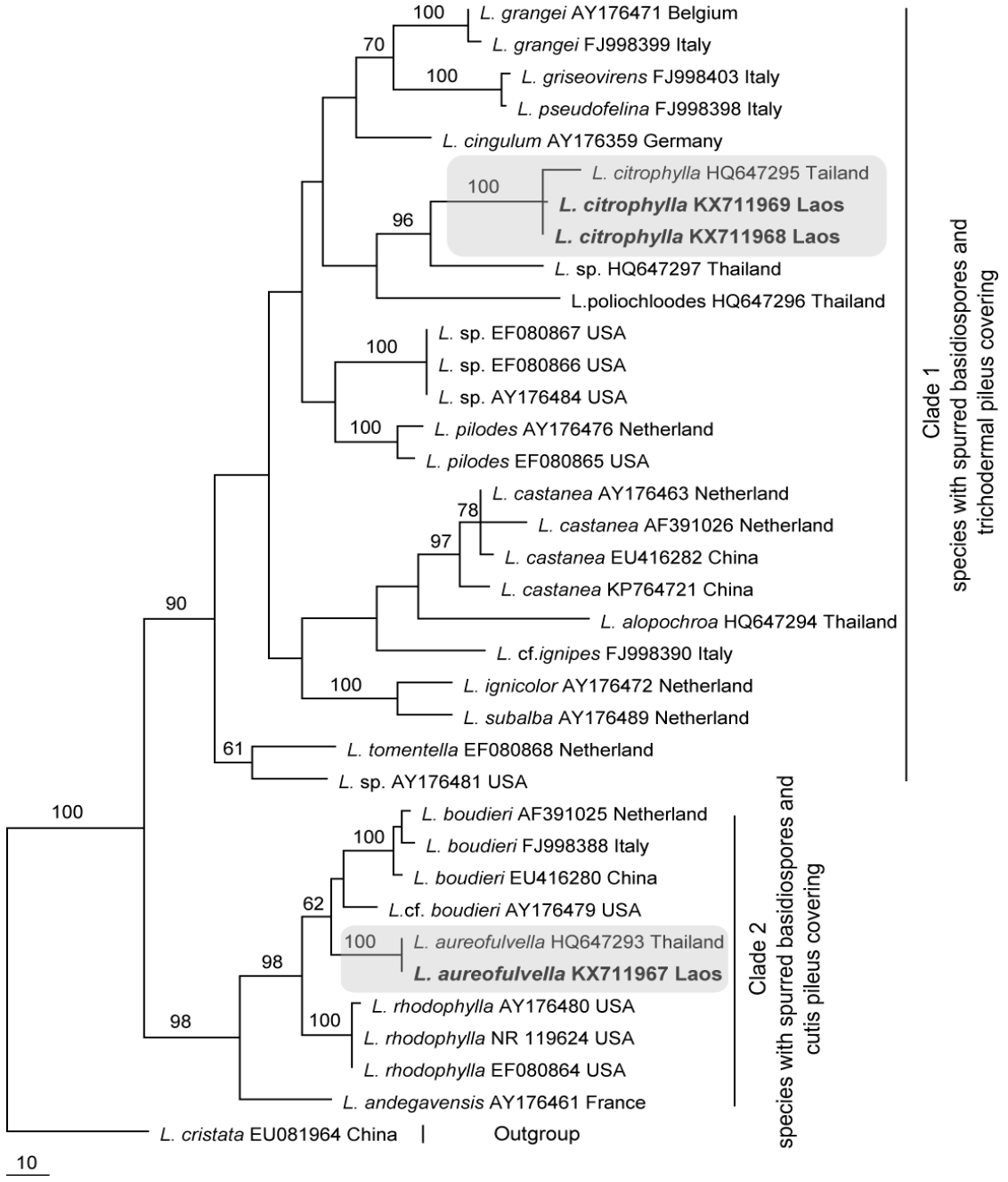

Figure 1. Maximum Parsimony tree of *Lepiota* section *Stenosporae* based on an analysis of nrITS sequences. Bootstrap values over 60% are indicated above the branches. The samples from Laos are marked in bold. GenBank accession numbers and respective countries are given after the name of each collection.

photographs and line drawings of micromorphology. It was found that *L. aureofulvella* has spurred basidiospores and a cutis pileus covering made up of cylindrical elements while *L. citrophylla* has spurred basidiospores and trichodermal pileus covering made up of long cylindrical elements on the top of short clavate elements.

3.3 *Lepiota aureofulvella* Sysouph., K. D. Hyde, Chukeat. & Vellinga, Mycotaxon 117: 53. 2011.
Figures 2, 3

Pileus 18–45 mm, convex to umbonate, expanding to planoconcave, with straight margin; brown to dark brown (6E7–8, 6F7–8) fibrillose around center or umbo, with golden brown to yellowish brown (5D5–8) fibrills to fibrillose squamules towards the margin, background white; margin with yellowish brown (5D5) fibrillose partial veil, exceeding lamellae when mature. Lamellae free, white, ventricose, 4–6 mm wide, somewhat crowded, fragile. Stipe 23–65 × 3–7 mm, cylindrical; covered with white fibrills, with brown to dark brown (6E7–8, 6F7–8) fibrillose at base zone, on white background and slowly turned light brown (6D4) when touched. Annulus in form of an annular zone, made up of white fibrils. Context in pileus white, 3–6 mm wide; in stipe hollow, white. Spore print white.

Basidiospores 5–8.5 × 3.5–5.2 µm, Q = 1.4–2.3, cylindrical to oblong in side view, with truncate to spurred base, triangular or with curved abaxial side, slightly thick-walled, hyaline, dextrinoid, congophilous, cyanophilous, not metachromatic. Basidia 14–20 × 4.5–7.5 µm, clavate, hyaline, 4-spored. Cheilocystidia 15–29 × 4–12 µm, clavate to narrowly clavate, utriform, rarely cylindrical, hyaline, thick-walled. Pleurocystidia absent. Pileus covering a cutis made up of long cylindrical elements to narrowly clavate terminal elements, elements with rounded to slightly tapered tips, 60–145 × 4–15 µm, thick-walled, with brown parietal pigment. Stipe covered with fibrillose squamules similar to pileus covering. Clamp-connections present in all tissues.

Figure 2. Basidiomata of *Lepiota aureofulvella* in the field (HNL502948).

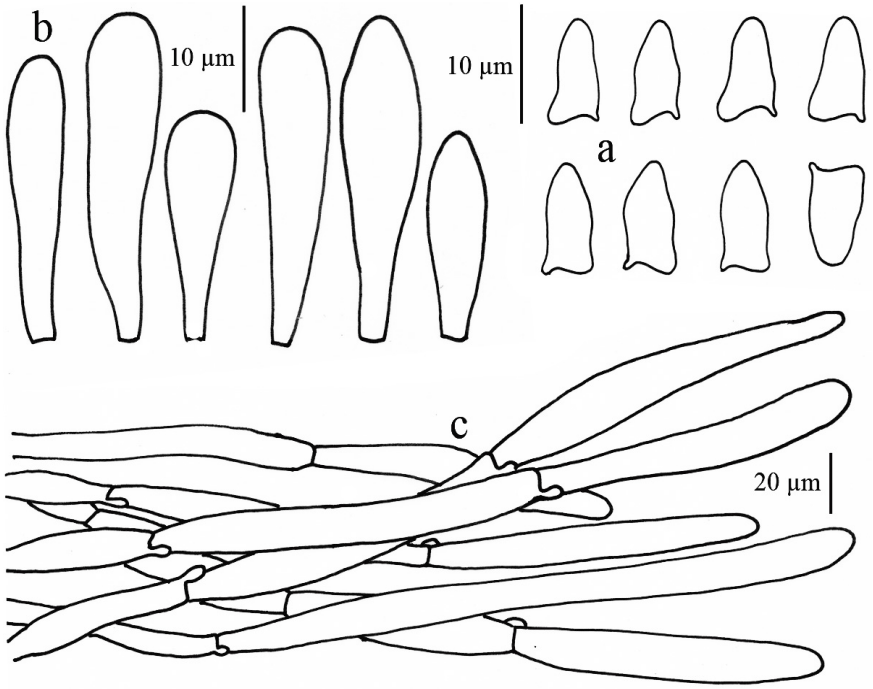

Figure 3. Micromorphological characteristics of *Lepiota aureofulvella* (HNL502948), a. basidospores, b. cheilocystidia, c. a cutis structure of pileus covering.

3.3.1 Habitat and distribution in Laos

Growing in a small groups, saprotrophic, on soil mixed with decayed leaves and wood, found in deciduous forests of Oudomxay Province, northern Laos.

3.3.2 Specimens examined

Laos, Oudomxay Province, Xay District, Houay Houm Village, 7 August 2014, P. Sysouphanthong, HNL502948.

3.3.3 Notes

The species is characterized by golden brown to dark brown pileus covering, white and free lamellae, cylindrical and white stipe, spurred basidiospores, clavate to utriform cheilocystidia, and with pileus/stipe covering in form of a cutis. *Lepiota aureofulvella* was originally described from northern Thailand, and it is the fourth species having spurred basidiospores and a cutis pileus covering of section *Stenosporae* [14]. Specimens described from Laos and Thailand are identical in terms of both morphology and nrITS sequences (Figure. 1).

Three species with spurred basidiospores and a cutis pileus covering are distinguished from *L. aureofulvella*. Firstly, *Lepiota boudieri* Bres. is the most similar in morphology but differs in shorter basidiospores, darker colour of pileus, and its nrITS sequence [5, 14]; the second species, *L. rhodophylla* Vellinga differs in pinkish brown colour of pileus covering and lamellae margin [5]; and the last species, *L. andegavensis* Mornand has dark grey to black colour of pileus covering [15]. The Maximum Parsimony analysis of nrITS sequence showed that the Lao specimen of *L. aureofulvella* is identical to the Thai specimen (Figure. 1).

3.4 *Lepiota citrophylla* (Berk. & Broome) Sacc., Syll. Fung. 5: 57. 1887. **Figures 4, 5**

Figure 4. Basidiomata of *Lepiota citrophylla* in the field (a= HNL502981, b= HNL502963).

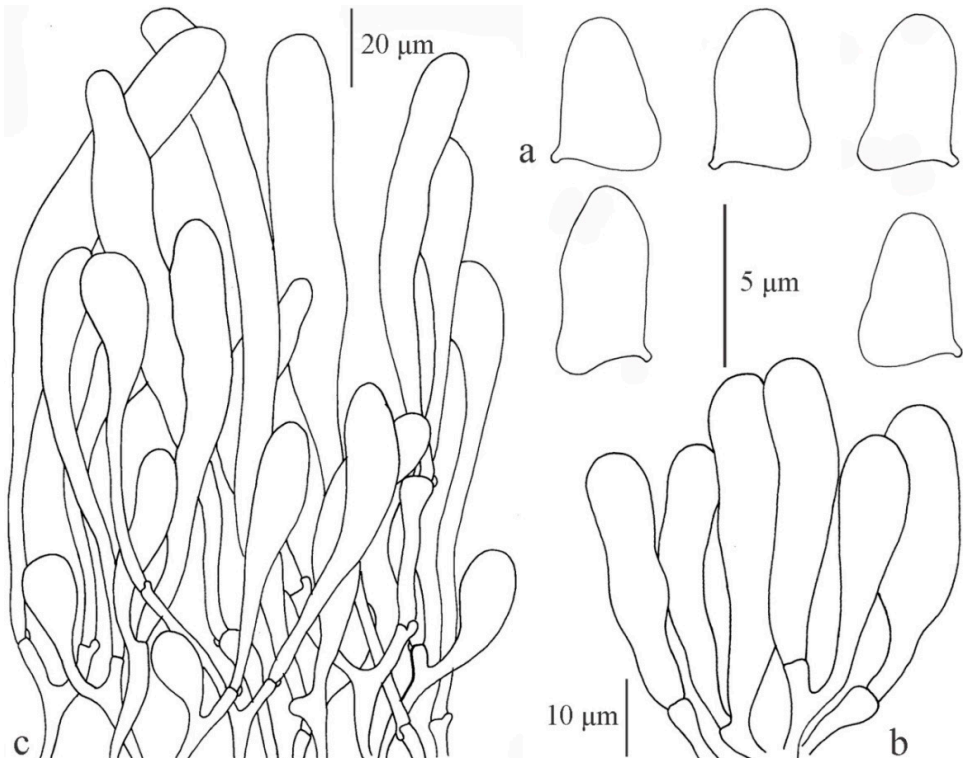

Figure 5. Micromorphological characteristics of *Lepiota citrophylla* (HNL502981), a. basidospores, b. cheilocystidia, c. a trichodermal structure of pileus covering.

Agaricus citrophyllus Berk. & Broome in J. Linn. Soc., Bot. 11: 509. 1871; *Lectotypus* Thwaites 821 (K).

Pileus 18–40 mm, first campanulate, expanding to umbonate, plano concave, with straight to inflexed margin; when young glabrous, golden yellow (5B7) to brown (6D7–8), soon breaking, with glabrous umbo, radially squamulose around umbo toward margin; margin covered with white partial veil. Lamellae free, moderately crowded, ventricose, 3–5 mm wide, pale yellow to light yellow (3A3–4, 4A4–5). Stipe 35–70 × 4–6 mm, cylindrical to subcylindrical; squamulose from annular zone towards the base, squamules concolourous with those on pileus, on light yellow (4D4) background, turned grayish orange (5B4–5) when touched. Annulus in form of an annular zone, made up of concolourous squamules on stipe. Context in pileus pale yellow to light yellow (4D4-5); in stipe hollow, concolourous to surface. Spore print white.

Basidiospores 5.5–8.5 × 3.5–4.5 μm, Q = 1.4–2.2, ellipsoidal to cylindrical, in side view with straight or slightly outgrown spur at base, apex rounded or more acute, hyaline, dextrinoid, congophilous, cyanophilous, not metachromatic. Basidia 15–45 × 5.5–9 μm, mostly clavate, sometimes narrowly clavate, 4-spored, hyaline,thin-walled. Cheilocystidia 25–65 × 4.5–12 μm, narrowly clavate to clavate, rarely oblong with short pedicel. Pleurocystidia absent. Pileus covering a trichoderm, made up of narrowly clavate elements, often wider at apex and narrowed into pedicel, 60–195 × 6.5–20 μm, wall pale brown, with parietal and intracellular brown pigment, with cylindrically hyaline to pale brown hyphae at under layer, 3–7 μm wide, with intracellular pigment. Stipe covering trichodermal, with squamules similar to those in pileus covering. Clamp-connections present in all tissues.

3.4.1 Habitat and distribution in Laos

Occurring in groups, saprotrophic, on soil mixed with decayed leaves and wood; found in deciduous forests of Oudomxay Province, northern Laos.

3.4.2 Material examined

Laos, Oudomxay Prov., Distr., Houay Houm Village, 20 July 2014, P. Sysouphanthong, HNL502981; Kone Kaen Village, 12 Aug 2014, P. Sysouphanthong, HNL502963.

3.4.3 Note

Lao specimen of *Lepiota citrophylla* has mainly pale yellow to light yellow basidiomata, and both pileus and stipe are covered with golden yellow to brown squamules (see Figures 4-5). The morphology of Lao specimens is mostly identical to collections from Thailand and Sri Lanka [16], Trinidad, British West Indies [17], Kenya and Africa [18], India [19], and Thailand [14].

Lepiota citrophylla might be confused in macromorphology with *L. xanthophylla* P.D. Orton because both species have pale yellow to light yellow basidiomata, but *L. xanthophylla* has ovoid basidiospores and belongs to the section *Ovisporae* [20]. The name *Lepiota citrophylla* was used instead of *L. xanthophylla* in Europe [21-22], America [23], and China [24]. The Maximum Parsimony analysis of nrITS sequences showed that the two Lao specimens are identical to the specimen from Thailand. Other yellow species; *Verrucospora verrucispora* (Beeli) E. Horak differs by its angled basidiospores [25-27] (Sysouphanthong, 2013, Horak, 1968, Pegler 1977); *Leucoagaricus houaynhangensis* Sysouph. has yellowish-green basidiomata, basidiospores with a germ pore, clavate to utriform cheilocystidia with short to long appendages, a hymenidermal pileus covering, and lacks clamp-connections in all tissues (Sysouphanthong et al., 2018).

Acknowledgement

A part of this work was supported by the Unit of Excellence 2019 on Biodiversity and Natural Resources Management, University of Phayao (UoE62005), University of Phayao Research Fund (RD62019) and the one faculty one success model, University of Phayao (RM61017, RM62001). In addition, we would like to thank Thailand research fund grants "Study of saprobic

Agaricales in Thailand to find new industrial mushroom products" (Grant No. DBG6180015) and Thailand Science Research and Innovation (TSRI) grant, Macrofungi diversity research from the Lancang-Mekong Watershed and surrounding areas (Grant No. DBG6280009).

REFERENCES

[1] Candusso M. and Lanzoni G., *Fungi Europaei 4. Lepiota s.l.*, Giovanna Biella, Saronno, 1990: 743.

[2] Bon M., *Die Grobpilzflora von Europa 3 Lepiotaceae*, Eching: IHW-Verlag, 1996.

[3] Kirk P.M., Cannon P.F., Minter D.W. and Stalpers J.A., *Ainsworth and Bisby's Dictionary of the Fungi*, 10th Edn., CAB International, 2008.

[4] Singer R., *The Agaricales in Modern Taxonomy. Ed. 4, Koenigstein: Koeltz Scientific Books*, 1986.

[5] Vellinga E.C., *Lepiota* (Pers.: Fr.) S.F. Gray; *in* Noordeloos, M.E., Kuyper, Th.W. and Vellinga, E.C., eds., *Flora Agaricina Neerlandica 5*, Lisse/ Abingdon/Exton (PA)/Tokyo: A.A. Balkema Publishers, 2001: 109-151.

[6] Sysouphanthong P., Hyde K.D., Chukeatirote E. and Vellinga E.C., *Curr. Res. Environ. Appl. Myco.*, 2011; 161-176. Doi10.5943/cream/1/2/3.

[7] Kornerup A. and Wanscher J.H., *Methuen Handbook of Colour,* Eyre Methuen, London, 1978.

[8] Gardes M. and Bruns T.D., *Mol. Ecol.*, 1993; **2**: 113-118. DOI 10.1111/j.1365-294X.1993. tb00005.x.

[9] Katoh K., Misawa K., Kuma K. and Miyata T., *Nucleic Acids Res.*, 2002; **30**: 3059-3066. DOI 10.1093/nar/gkf436.

[10] Katoh K. and Toh H., *Brief. Bioinform.*, 2008; **9**: 286-298. DOI 10.1093/bib/ bbn013.

[11] Swofford D.L., *PAUP*: Phylogenetic Analysis Using Parsimony, Version 4.0b10*, Sinauer Associates, Sunderland, MA, 2004:

[12] Tamura K. and Nei M., *Mol. Biol. Evol.*, 1993; **10**: 512-526.

[13] Tamura K., Stecher G. and Peterson D., Filipski A. and Kumar S., *Mol. Biol. Evol.*, 2013; **30**: 2725-2729. DOI 10.1093/ molbev/mst197

[14] Sysouphanthong P., Hyde K.D., Chukeatirote E., Bahkali A.H. and Vellinga E.C., *Mycotaxon*, 2011; **117**: 53-85.

[15] Mornand J., *Mycology*, 1983; **12(48)**: 41-43.

[16] Pegler D.N., *Kew Bull.*, 1972; **27**: 155-202.

[17] Dennis R.W.G., *Kew Bull.*, 1952; **7**: 459-499.

[18] Pegler D.N., *Kew Bull. Add.,* 1997; **10**: 1-519.

[17] Natarajan K. & Majula B., *Bibl. Mycol.,* 1983; **91**: 563-581.

[20] Chou N.W., *Coll. Res.*, 2010; **23**: 1-7.

[21] Rea C., *British Basidiomycetae. A Handbook to the Larger British Fungi*, Cambridge University Press, 1922.

[22] Kühner R., *Bull. Mens. Soc. Linn. Lyon*, 1934; **3**: 91-93.

[23] Akers B.P. and Sundberg W.J., *Mycotaxon*, 1998; **59**: 429-436.

[24] Liang J.F., Zhuo L.L., Zhong C.L., Chen Y. and Chen Z., *J. Fungal Res.*, 2010; **8**: 63-65.

[25] Sysouphanthong P., Hyde K.D., Chukeatirote E., Bahkali A.H. and Vellinga E.C., *Chiang Mai J. Sci.*, 2013; **40(2)**: 289-293.

[26] Horak E., *Ber. Schw. Bot. Ges.*, 1968; **77**: 362-375.

[27] Pegler D.N., *Kew Bull. Add. Ser.*, 1977; **6**: 1-615.

[28] Sysouphanthong, P., Bouamanivong S., Salichanh T., Xaybouangeun N., Sucharitakul P., Osathanunkul M. and Suwannapoom, C., *Chiang Mai J. Sci.*, 2018; **45(3):** 1287-1295.