Bull. Natl. Mus. Nat. Sci., Ser. B, 45(2), pp. 71–76, May 22, 2019

First Record of *Macrolepiota velosa* Vellinga & Zhu L. Yang (Agaricaceae) from Myanmar

Kentaro Hosaka[1]*, Kyung-Ok Nam[1], Wah Wah Linn[2], Mu Mu Aung[2]

[1] Department of Botany, National Museum of Nature and Science,
4–1–1 Amakubo, Tsukuba, Ibaraki 305–0005, Japan
[2] Forest Research Institute, Forest Department, Yezin, Nay Pyi Taw, Myanmar
* E-mail: khosaka@kahaku.go.jp

(Received 12 March 2019; accepted 27 March 2019)

Abstract The mushroom specimen with large-sized fruit body with a distinct volva at base of stipe collected in Myanmar was demonstrated to be *Macrolepiota velosa*, originally described from Yunnan, China. This is the first record of the species outside of China and Thailand, and is the first record from Myanmar. This study extended the known distribution of *M. velosa* to wider areas, indicating the species may be distributed across tropical to subtropical regions of Southeast Asia.

Key words: Agaricales, Agaricomycota, Basidiomycota, biogeography, distribution, fungi, inventory, mushrooms, Southeast Asia, volva.

Introduction

The genus *Macrolepiota* Singer is typically characterized by having medium- to large-sized basidiomata with a scaly pileus, free gills, and thick annulus, but lacking a volva at base of stipe (Largent and Baroni, 1988). However, several species are known to possess a distinct volva and a new genus *Volvolepiota* Singer has been proposed to accommodate them (Singer, 1959). Recent molecular phylogenetic studies indicated that *Volvolepiota* is deeply nested within the rest of *Macrolepiota* (Ge *et al.*, 2012; Vellinga *et al.*, 2003; Vizzini *et al.*, 2011), or it consists of a sister clade to the rest of *Macrolepiota* (Ge *et al.*, 2010). Because of their close affinities, *Volvolepiota* is currently treated as a synonym of *Macrolepiota*.

To clarify fauna and flora (including fungi) of Myanmar and the surrounding areas, a joint research team of National Museum of Nature and Science, Japan, and Forest Research Institute, Myanmar has been conducting biological inventories in Myanmar since 2016. During one of our fieldwork activities in 2016, we have collected a peculiar basidioma with a distinct volva at base of stipe. Further investigations all indicated that our material is *Macrolepiota velosa* originally described from Yunnan, China (Vellinga and Yang, 2003). This is the first record of the species from Myanmar and the second record outside of China.

Colored photographs of macroscopic and microscopic characters of *M. velosa* from Myanmar are provided. In addition, DNA sequence data from the internal transcribed spacer (ITS) region and the large subunit (LSU) of nuclear ribosomal DNA were compared with the materials from China and Thailand.

Materials and Methods

Collecting Sites, Collecting Scheme, and Curation of Specimens

One basidiome of a volvate *Macrolepiota* was collected by the first author from National Kandawgyi Garden, Pyin Oo Lwin, Mandalay Division, Myanmar (N21°59′33.2″, E96°28′16.3″;

elevation ca. 1,100 m) on August 28, 2016. Specimen was photographed and macroscopic observation was conducted. Specimen was cut into half and dried with low heat and good air circulation, using a food dehydrator for 24 hours. In addition to dried materials, small fragments of clean, internal tissue from freshly collected material were cut using a clean, sterile razor blade. Contamination of visible soil particles and other materials was strictly avoided. The tissue fragments were soaked in DMSO buffer (Seutin *et al.*, 1991) with an addition of 100 mM Tris-HCl (pH 8.0) and 0.1 M sodium sulfite (Na_2SO_3) under 4℃, following the procedures of Hosaka (2009) and Hosaka and Castellano (2008).

Specimens collected during the fieldwork were deposited at Forest Research Institute, Myanmar, and the duplicate at the fungal herbarium of the National Museum of Nature and Science, Tsukuba, Japan (TNS). All tissue samples were stored in freezers (−80℃) at the Center for Molecular Biodiversity Research, National Museum of Nature and Science.

Light Microscopy

For light microscopic observations, a small portion from dried basidioma was mounted in 3% (w/v) KOH or Meltzer's reagent on glass slides. Those samples were examined with Olympus BX53 microscope under Nomarski interference contrast. Thirty randomly selected basidiospores were measured under a light microscope at 1000× magnification.

DNA Preparation, PCR, and Sequencing

DNA was extracted from the tissue fragments stored in DMSO buffer. Tissues were ground under liquid nitrogen using a mortar and pestle. DNA extractions used a modified CTAB extraction followed by glass milk purification methods as summarized by Hosaka (2009) and Hosaka and Castellano (2008).

DNA sequence data were obtained from the ITS and LSU regions of the nuclear ribosomal DNA. For amplifying the ITS, the primer combination of ITS5 and ITS4 (White *et al.*, 1990) was used. For amplifying the LSU, the primer combination of LR0R and LR5 (Vilgalys and Hester, 1990) was used. PCR reactions were carried out using 20 μL reaction volumes each containing: 1 μL genomic DNA, 1 μL dNTPs (4 mM), 1 μL of each primer (8 μM), 0.5 units of Taq polymerase (TaKaRa, Tokyo, Japan), 2μL MgCl2 (25 mM), 2 μL Bovine Serum Albumin (BSA). PCR products were electrophoresed in 1% agarose gels stained with ethidium bromide and visualized under UV light. When amplification bands were confirmed, PCR products were then purified using the ExoSap-IT (Millipore, Molsheim, France) and directly sequenced using the Big Dye Terminator Cycle Sequencing Kit on ABI3500 (Applied Biosystems Inc., Foster City, CA, USA), following the manufacturer's instructions.

Phylogenetic Analyses

The obtained raw sequences were edited using ATGC version 7.1.0 (GENETYX Corporation, Tokyo, Japan). Edited sequences were analyzed using the GenBank BLAST search (blastn). Default settings of blastn option were used. Because our sequences (both ITS and LSU) showed a significant hit (more than 99% similarities) with *Macrolepiota velosa*, we have retrieved all available sequences (ITS and LSU) of *M. velosa* for further analyses.

DNA sequences of the ITS and LSU (GenBank accession nos. MK634569 and MK634568, respectively) were aligned manually using the data editor of BioEdit ver. 7.0.1 (Hall, 1999). The dataset was then analyzed by maximum parsimony (MP) analysis. MP analyses were conducted under the equally weighted parsimony criterion using PAUP* version 4.0b10 (Swofford, 2002), with heuristic search option (with TBR and Multrees on). Support for the individual nodes was tested with bootstrap (BS) analysis under the equally-weighted parsimony criterion. BS analysis was based on 100 BS replicates using the heuristic search option (TBR and Multrees options off), with ten random addition sequences.

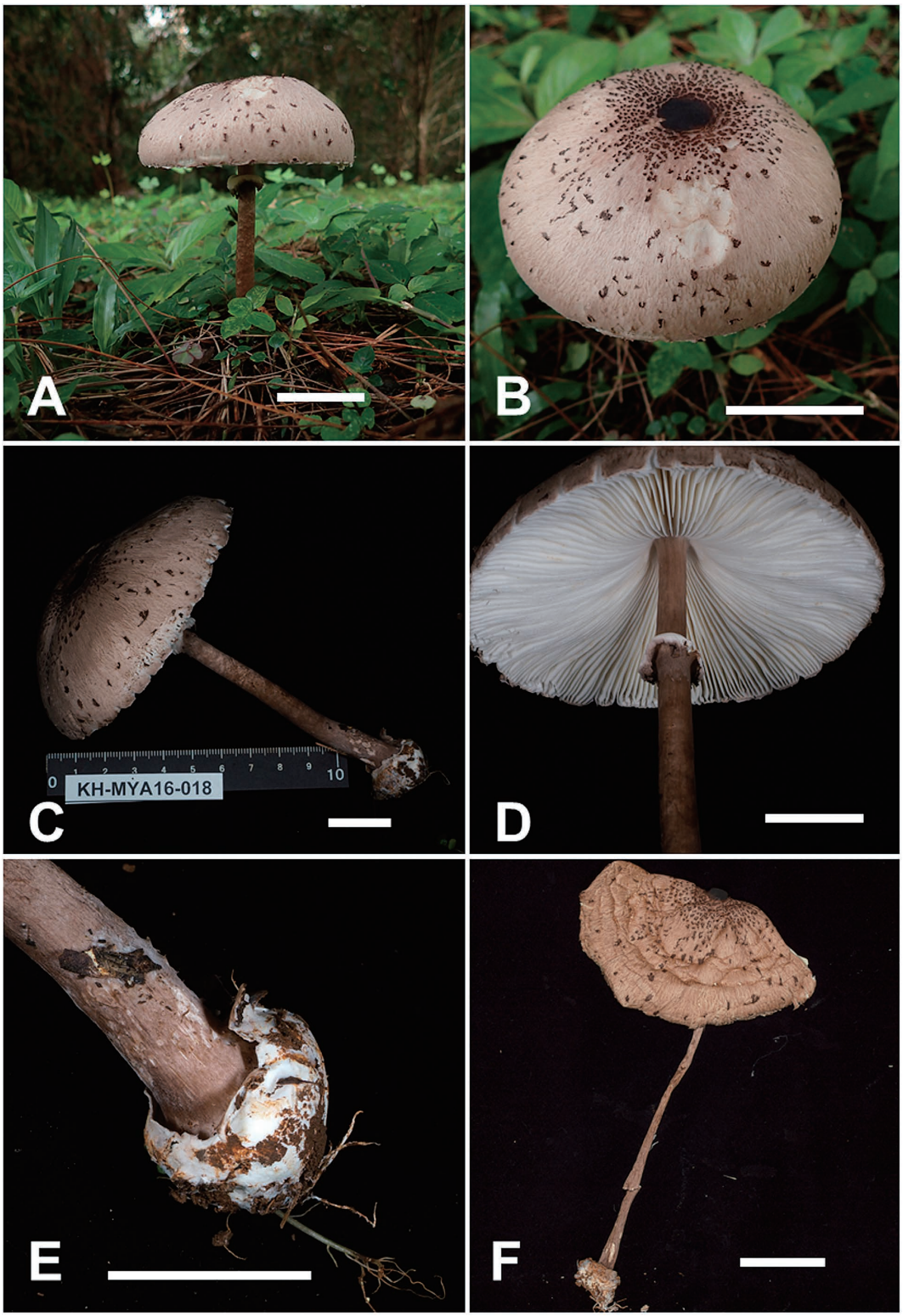

Fig. 1. *Macrolepiota velosa* (KH-MYA16-018). A. Basidioma *in situ*. B. Pileus showing fibrillose surface. C. Basidioma with distinct volva at base of stipe. D. Lamellae and annulus. E. Volva. F. Dried basidioma. Scale bars = 2 cm.

Results and Discussion

Macrolepiota velosa Vellinga & Zhu L. Yang, Mycotaxon 85: 184. 2003.

Basidioma (Fig. 1) large-sized, 14 cm high. **Pileus** 10 cm in diam., plano-convex, with a wide indistinct umbo (Fig. 1B), fibrillose, covered with dense, purplish brown squamules at center, much scarcer near margin (Fig. 1A, B, F). **Lamellae** (Fig. 1D) free, moderately crowded, white. **Stipe** (Fig. 1A, C, D) purplish brown, slightly paler above annulus, cylindrical, 13 × 0.7–0.9 cm, tapering at apex, slightly enlarged at base. **Annulus** (Fig. 1D) ascending, white above, purplish brown below, with margin irregularly waved. **Volva** (Fig. 1C, E) white, limbate, membranous, with white rhizomorphs at base (Fig. 1E). **Taste and odor** indistinct.

Basidiospores (Fig. 2A, B) amygdaloid-ellipsoid in side view, ellipsoid in front view, 8–11 × 5.5–7 µm (average = 9.2 × 6.2 µm), Q (length/width ratio) 1.28–1.68 (average Q = 1.49), smooth, thick-walled, dextrinoid, with api-

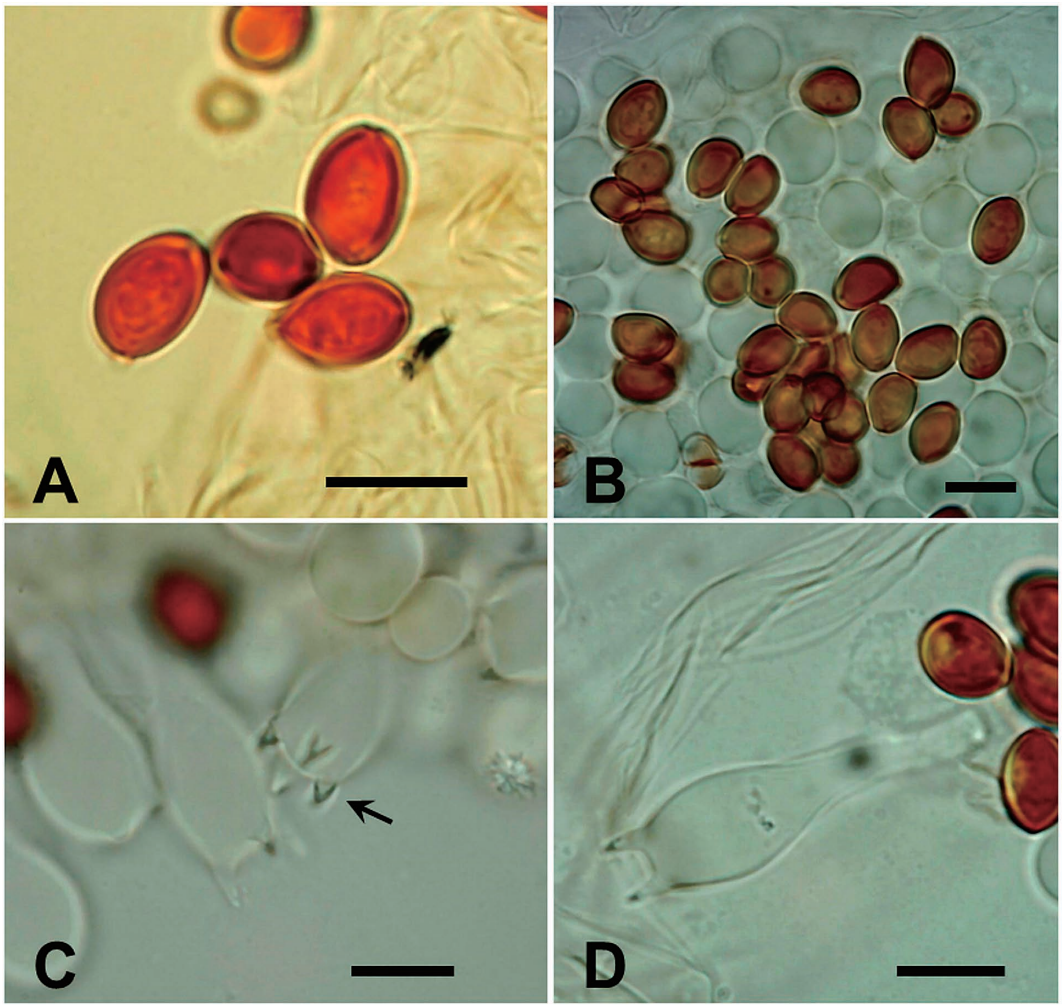

Fig. 2. Light microscopy of *Macrolepiota velosa* (KH-MYA16-018). A. Basidiospores in Meltzer's reagent. B. Basidiospores (reddish brown) with globose lamellar cells (hyaline). C. Basidia showing 4 sterigmata (arrow). D. 2-spored basidia. Scale bars = 10 µm.

cal central germ pore. **Basidia** (Fig. 2C, D) clavate, without clamp connections at base, mostly 4-spored (Fig. 2C) but sometimes 2-spored (Fig. 2D), 27–35 (not including sterigma) × 9–11 μm, sterigma 2–4 μm long. **Cheilocystidia** cylindrical, without clamp connections at base, thin-walled, 52–63 × 6–8.5 μm. **Squamules on pileus** composed of slightly thick-walled brown hyphae with cylindrical to swollen apex, up to 95 × 24 μm.

Habitat and known distribution in Myanmar: Terrestrial, solitary on the ground in predominantly evergreen, broad-leaved forests including Dipterocarpaceae, mixed with bamboos and *Pinus* spp. So far only found in one locality (National Kandawgyi Garden) in Myanmar.

Materials examined: Myanmar: Mandalay Division, Pyin Oo Lwin, National Kandawgyi Garden, alt. 1,100 m, 28 Aug. 2016, K. Hosaka (KH-MYA16-018, deposited as TNS and Forest Research Institute, Myanmar).

Comments: *Macrolepicola velosa* was first described from Yunnan Province, China (Vellinga and Yang, 2003). It was then recorded from Hainan Province, China (Ge *et al.*, 2010). The available information also indicates that the species is also distributed in Thailand (Chiang Mai), retrieved as a GenBank accession nos. KJ524569 and also mentioned by Ge *et al.* (2010). However, we could not find any other published records (e.g., morphological observation) of the specimen from Thailand.

Our morphological observation was consistent with previous descriptions of *M. velosa* (Vellinga and Yang, 2003; Ge *et al.*, 2010). However, the material from Myanmar possesses slightly longer basidia (27–35 × 9–11 μm) than Chinese materials (25–30 × 9.5–11.5 μm). Furthermore, we have infrequently found 2-spored basidia (Fig. 2D), which were not observed by previous studies (Vellinga and Yang, 2003; Ge *et al.*, 2010). These differences are likely intraspecific variations, but further studies are warranted.

The BLAST search indicated that the sequences (ITS and LSU) of Myanmar material shares highest similarities with *M. velosa* from China and Thailand. The top 5 hits of ITS BLAST search were all *M. velosa* (99.03% or higher similarities), and the next hit was "*Macrolepiota* sp. 3" (GenBank accession no. KY927722) from Brazil at 93.83% similarity. The top hit of LSU BLAST search (99.89%) was also *M. velosa* (GenBank accession no. JN940273) from Hainan, China. There was only one CT transition observed between Chinese and Myanmar samples across the whole range of LSU region.

Phylogenetic analyses of the ITS region resulted in 6 equally parsimonious trees (Fig. 3). The alignment consisted of a total of 680 characters, but 675 characters were parsimony uninformative and therefore excluded from the analyses. The ITS phylogeny (Fig. 3) was midpoint rooted, and arguably indicated that the materials from

Fig. 3. One of 6 most parsimonious trees of *Macrolepiota velosa* based on the ITS sequences. The tree was rooted at midpoint. Taxon names are shown as GenBank accession numbers followed by the countries (and more detailed locality, if available) of origin. The number of branch indicates parsimony bootstrap value. The asterisk indicates the node collapsed in the strict consensus.

Hainan was more diverged from the rest of samples from Myanmar, Thailand and Yunnan, China. All materials were, however, separated only by a few substitutions. We therefore conclude that they all represent a single species, *M. velosa*.

Our study revealed a new distribution of *M. velosa* outside of China and Thailand. The species may be distributed in wider areas in tropical to subtropical regions of Southeast Asia. Whether intraspecific variations observed from morphological and molecular analyses actually represent local populations or distinct species warrant further investigation.

Acknowledgments

The authors thank Kazuo Nishibori, Megumi Johnston, and Jun Yamazaki for assisting our curatorial work. We also thank the park rangers and officers in Mandalay Division, Myanmar, for assisting our fieldwork. This study was financed in part by a research project of the National Museum of Nature and Science entitled as "Biological inventory with special attention to Myanmar: investigations of the origin of southern elements of Japanese flora and fauna" and JSPS KAKENHI Grant Number 18KK0210.

References

Ge, Z. W., Chen, Z. H. and Yang, Z. L. 2012. *Macrolepiota subcitrophylla* sp. nov., a new species with yellowish lamellae from southwest China. Mycoscience 53: 284–289.

Ge, Z. W., Yang, Z. L. and Vellinga, E. C. 2010. The genus *Macrolepiota* (Agaricaceae, Basidiomycota) in China. Fungal Diversity 45: 81–98.

Hall, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hosaka, K. 2009. Phylogeography of the genus *Pisolithus* revisited with some additional taxa from New Caledonia and Japan. Bulletin of the National Museum of Nature and Science, Series B (Botany) 35: 151–167.

Hosaka, K. and Castellano, M. A. 2008. Molecular phylogenetics of Geastrales with special emphasis on the position of *Sclerogaster*. Bulletin of the National Museum of Nature and Science, Series B 34: 161–173.

Largent, D. L. and Baroni, T. J. 1988. How to Identify Mushrooms to Genus VI: Modern Genera. Mad River Press, Eureka, California.

Seutin, G., White, B. N. and Boag, P. T. 1991. Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69: 82–90.

Singer, R. 1959. Dos generos de hongos nuevos para Argentina. Boletín de la Sociedad Argentina de Botánica 8: 9–13.

Swofford, D. L. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4.0. Sunderland, Massachusetts: Sinauer Associates.

Vellinga, E. C. and Yang, Z. L. 2003. *Volvolepiota* and *Macrolepiota* — *Macrolepiota velosa*, a new species from China. Mycotaxon 75: 183–186.

Vellinga, E. C., de Kok, R. P. J. and Bruns, T. D. 2003. Phylogeny and taxonomy of *Macrolepiota* (Agaricaceae). Mycologia 95: 442–456.

Vilgalys, R. and Hester, M. 1990. Rapid genetic identification and mapping of enzymatically amplified DNA from several *Cryptococcus species*. Journal of Bacteriology 172: 4238–4246.

Vizzini, A., Contu, M., Chignone, S. and Vellinga, E. 2011. A new volvate *Macrolepiota* (Agaricomycetes, Agaricales) from Italy, with observations on the *M. procera* complex. Mycotaxon 117: 149–164.

White, T. J., Bruns, T., Lee, S. and Taylor, J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. (eds.), PCR protocols, pp. 315–322, Academic Press, New York.