

## Full Length Research Paper

# Morphology and molecular taxonomy of *Hymenagaricus mlimaniensis* species nov: A new Basidiomycota mushroom from Mlimani main campus, Tanzania

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*Hymenagaricus mlimaniensis* Mwanga & Tibuhwa sp. nov. is described from Dar es Salaam Mlimani Main Campus in the semi protected natural tropical forest left in the Dar es Salaam city. The species superficially looks like *Agaricus* and its difference to the closest taxa in *Hymenagaricus* genus is both morphologically and genetically presented. The species is distinctively characterized from the closest *H. pallidodiscus* Reid & Eicker and *H. alphitichrous* (Berk. & Broome) Heinem by having the distinctive pink-reddish colour of the disc, whiter diminutive fibril on the pink-reddish background, lack of developed cortinate veil, possession of smooth margin and microscopically, the presence of clamp connections which are lacking in the two closest taxa. This study thus, describe *H. mlimaniensis* sp. nov. as a new species in *Hymenagaricus* genus based on both macro-micromorphology and molecular markers.

**Key words:** *Hymenagaricus*, taxonomy, Mlimani, Tanzania, *Agaricus*, mushroom.

## INTRODUCTION

The genus *Hymenagaricus* was described in 1981 by Heinemann as a new genus in Agaricaceae in Bulletin du Jardin Botanique national de Belgique / Bulletin van de National Plantentuin van België, Vol. 51, pp. 465-466. It is among the small Agaricaceae genus with so far 16 described species (Table 1). It is known to have a wide paraetropical distribution, mainly known from countries including Taiwan, Sri Lanka, Ghana, Kenya, South Africa, Thailand and Tanzania although the diversity of its species remains poorly known. Before the description of this new genus, its members were formally

lumped together in the genus *Agaricus* L. that superficially looks similar to *Hymenagaricus* especially the possession of dark brown gills in mature specimens (Heinemann, 1981).

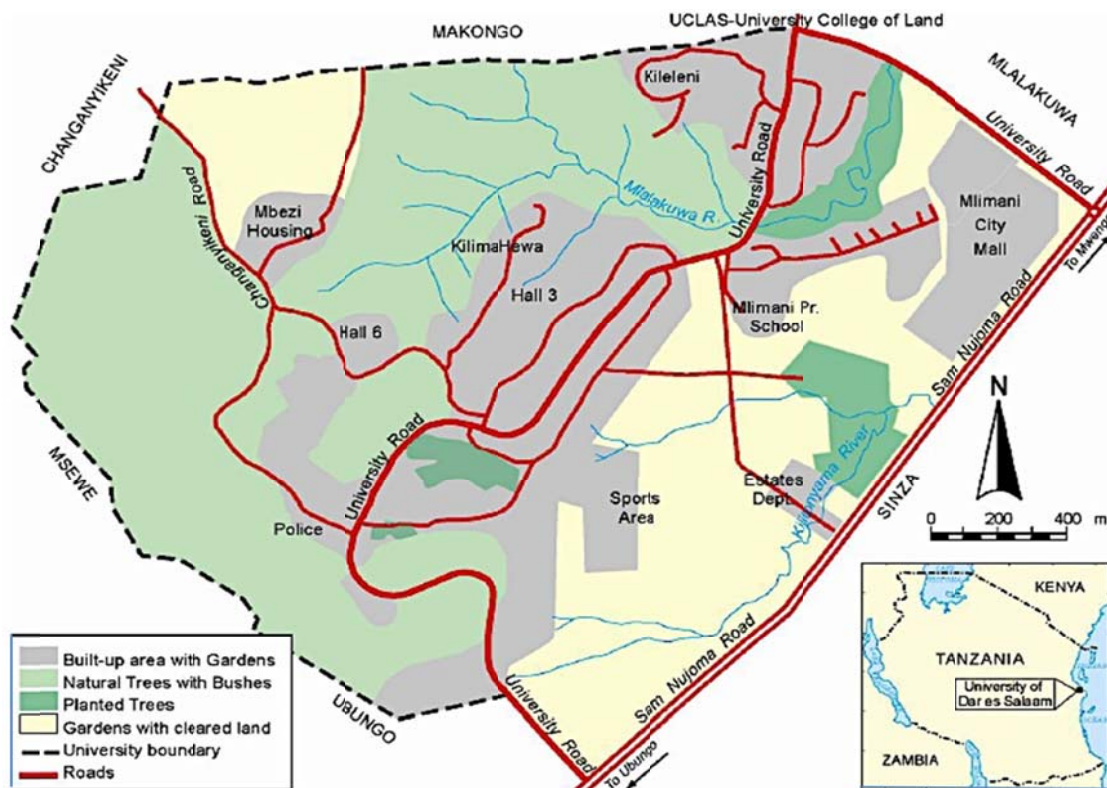
In Tanzania, the diversity of mushroom in Agaricaceae in particular the close genus *Agaricus* is limited to the conventional work done by Härkönen et al. (1995, 2003), Tibuhwa et al. (2012) and Tibuhwa (2011). For example, Tibuhwa (2011) carried out an inventory of the composition of macrofungi community belonging to Basidiomycetes within UDSM Mlimani Main Campus,

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**Table 1.** Described species in the genus *Hymenagaricus* H.

S/N	Described <i>Hymenagaricus</i> species and their synonymy	Iconography*
1	<i>Hymenagaricus alphitochrous</i> (Berk. & Broome) Heinem Synonymy: <i>Agaricus alphitochrous</i> Berk. & Broome <i>Hymenagaricus alphitochrous</i> (Berk. & Broome) Heinem var. <i>alphitochrous</i> <i>Hymenagaricus alphitochrous</i> var. <i>pegleri</i> Heinem. <i>Lepiota alphitochroa</i> (Berk. & Broome) Sacc. <i>Psalliota alphitochroa</i> (Berk. & Broome) Petch	Bull. Jard. Bot. natn. Belg. 51(3/4): 466 (1981) J. Linn. Soc., Bot. 11(no. 56): 511 (1871) Bull. Jard. Bot. natn. Belg. 51(3/4): 466 (1981) Bull. Jard. Bot. natn. Belg. 55(3-4): 494 (1985) Syll. fung. (Abellini) 5: 64 (1887) Ann. R. Bot. Gdns Peradeniya 4(2): 54 (1907)
2	<i>Hymenagaricus ardosiaecolor</i> (Heinem.) Heinem. Synonymy: <i>Agaricus ardosiaecolor</i> Heinem. <i>Hymenagaricus ardosiaecolor</i> (Heinem.) Heinem. var. <i>ardosiaecolor</i> <i>Hymenagaricus ardosiaecolor</i> var. <i>rufidulus</i> Heinem.	Bull. Jard. Bot. natn. Belg. 55(3-4): 493 (1985) Bull. Jard. Bot. Brux. 26(1): 118 (1956) Bull. Jard. Bot. natn. Belg. 55(3-4): 493 (1985) Bull. Jard. Bot. natn. Belg. 55(3-4): 493 (1985)
3	<i>Hymenagaricus caespitosus</i> D.A. Reid & Eicker	S. Afr. J. Bot. 61(6): 293 (1995)
4	<i>Hymenagaricus calicutensis</i> Heinem. & Little Flower	Bull. Jard. Bot. natn. Belg. 54(1-2): 163 (1984)
5	<i>Hymenagaricus canoruber</i> (Berk. & Broome) Heinem. & Little Flower Synonymy: <i>Agaricus canoruber</i> Berk. & Broome <i>Psilocybe canorubra</i> (Berk. & Broome) Sacc.	Bull. Jard. Bot. natn. Belg. 54(1-2): 153 (1984) J. Linn. Soc., Bot. 11(no. 56): 554 (1871) Syll. fung. (Abellini) 5: 1052 (1887)
6	<i>Hymenagaricus cylindrocystis</i> Heinem. & Little Flower	Bull. Jard. Bot. natn. Belg. 54(1-2): 156 (1984)
7	<i>Hymenagaricus epipastus</i> (Berk. & Broome) Heinem. & Little Flower Synonymy: <i>Agaricus epipastus</i> Berk. & Broome <i>Fungus epipastus</i> (Berk. & Broome) Kuntze <i>Stropharia epipasta</i> (Berk. & Broome) Sacc.	Bull. Jard. Bot. natn. Belg. 54(1-2): 166 (1984) J. Linn. Soc., Bot. 11(no. 56): 553 (1871) Revis. gen. pl. (Leipzig) 3(2): 479 (1898) Syll. fung. (Abellini) 5: 1018 (1887)
8	<i>Hymenagaricus fuscobrunneus</i> D.A. Reid & Eicker	S. Afr. J. Bot. 64(6): 356 (1998)
9	<i>Hymenagaricus hymenopileus</i> (Heinem.) Heinem. Synonymy: <i>Agaricus hymenopileus</i> Heinem. <i>Hymenagaricus kivuensis</i> Heinem. <i>Hymenagaricus laticystis</i> Heinem.	Bull. Jard. Bot. natn. Belg. 51(3/4): 466 (1981) Bull. Jard. Bot. natn. Belg. 50(1-2): 41 (1980) Bull. Jard. Bot. natn. Belg. 54(1-2): 290 (1984) Bull. Jard. Bot. natn. Belg. 55(3-4): 493 (1985)
10	<i>Hymenagaricus nigrovinosus</i> (Pegler) Heinem. Synonymy: <i>Agaricus nigrovinosus</i> Pegler	Bull. Jard. Bot. natn. Belg. 51(3/4): 466 (1981) Kew Bull., Addit. Ser. 6: 327 (1977)
11	<i>Hymenagaricus ochraceoluteus</i> D.A. Reid & Eicker	S. Afr. J. Bot. 64(6): 357 (1998)
12	<i>Hymenagaricus olivaceus</i> Heinem.	Bull. Jard. Bot. natn. Belg. 55(3-4): 494 (1985)
13	<i>Hymenagaricus pallidodiscus</i> D.A. Reid & Eicker	Mycotaxon 73: 169 (1999)
14	<i>Hymenagaricus rufomarginatus</i> D.A. Reid & Eicker	S. Afr. J. Bot. 64(6): 357 (1998)
15	<i>Hymenagaricus subaeruginosus</i> (Berk. & Broome) Heinem. & Little Flower Synonymy: <i>Agaricus subaeruginosus</i> Berk. & Broome <i>Fungus subaeruginosus</i> (Berk. & Broome) Kuntze <i>Stropharia subaeruginosa</i> (Berk. & Broome) Sacc.	Bull. Jard. Bot. natn. Belg. 54(1-2): 160 (1984) J. Linn. Soc., Bot. 11(no. 56): 554 (1871) Revis. gen. pl. (Leipzig) 3(2): 480 (1898) Syll. fung. (Abellini) 5: 1013 (1887)
16	<i>Hymenagaricus taiwanensis</i> Zhu L. Yang, Z.W. Ge & C.M. Chen, in Ge, Chen & Yang	Mycol. 29(3): 261 (2008)

\*Index Fungorum <http://www.indexfungorum.org/names/Names.asp>; in August 2014.



**Figure 1.** University of Dar es Salaam Mlimani main Campus showing study sites (After Tibuhwa 2011).

which is the study site, based on morphological characterisation. In her study, she established 18 families, 29 genera and more than 21 species of basidiomycetes but the *Hymenagaricus* genera were not reported. The *Hymenagaricus* is only known in the country from a single collection by Pegler (1977) who reported on one species *Hymenagaricus alphitochrous* (Berk. & Broome) Heinem. This study describes a new species in the genus *Hymenagaricus*, based on macro-micromorphological and molecular markers (ITS), the genus being reported for the second time in the country record.

## MATERIALS AND METHODS

### Study site

The intensive study on the Agaricus like mushroom was carried out at the UDSM Mlimani main Campus (Figure 1) for 3 years (2011-2014). The Campus is situated on the western side of the city of Dar es Salaam 6°48' South, 39°27' East (-6.8000, 39.2833), on the observation hill, 13 km from the city centre. In Dar es Salaam city in Tanzania, the only big unintentionally conserved natural trees forming huge thicket bushes, is observed at the Mlimani main Campus of the University of Dar es Salaam. The area occupies 1625 acres out of which 20% of the total area is occupied by buildings and roads while the remaining 80% constitutes a uniquely complex ecosystem which supports a wide range of organisms including Macro fungi as noted by Tibuhwa (2011). A

remarkable feature of the studied area is its enormous orography, geological, floristic diversity as well as different land use units (natural trees, planted trees and gardens) that give rise to its macrofungi diversity described in Tibuhwa (2011).

### Species description

Macroscopic characters are all based on fresh material observed in the field and laboratory. Microscopic characterisations were done using dried material mainly in Congo red mixed with 10% of ammonia. Each feature (basidia, basidiospore, hyphae system) were selected randomly and measured twenty times and the statistical average was taken to represent the measurement of the observed feature. Measurement was done direct on the MOTIC digital microscope using in built microscopic scale. They were given as (min) min-SD - AV -max-SD (max) Q; whereby min = lowest value recorded for the measured feature, max = highest value, AV = arithmetic average and SD = standard deviation. Q stands for "quotient length/width" and is given as MINQ - AvQ- MAXQ in which AvQ stands for the mean quotient for the measured spores. Colour codes refer to Kornerup and Wanscher (1962).

### Molecular study

#### DNA extraction and PCR amplification

Genomic DNA was isolated from fruit body using protocol that was developed by the Department of Molecular Biology and Biotechnology of University of Dar es Salaam with some few modifications. The DNA was extracted from fruit bodies dried in

**Table 2.** Taxa included in the phylogenetic analysis.

Species	Voucher	Collection no.	ITS-Accession #
<i>Agaricus subsaharianus</i>	ZNM1	Zuhura 7.2013	KM360157
<i>Agaricus subsaharianus</i>	ADK 4733	Hama, O. et al.	ADK4732
<i>Agaricus arvensis</i>	ZNM 15	Zuhura 15.2013	KM1360158
<i>Agaricus arvensis</i>	ADK 2564	Schaeff	JF514518
<i>Agaricus porphyrocephalus</i>	CA 856	Møller	JF797202
<i>Agaricus porphyrocephalus</i>	Z NM21	Zuhura 21.2013	KM360156
<i>Hymenagaricus species nov.</i>	ZNM A	Zuhura A.2013	MB809981
<i>Hymenagaricus species</i>	CA801	Heinemann	JF727859.1
<i>Agaricus fiscofibrillosus</i>	CA800	Møller	JF727862
<i>Agaricus fuscifibrillosus</i>	ZNM7	Zuhura 7.2013	KM1360155
<i>Agaricus campestris</i>	ZNM 19	Zuhura 19. 2014	KM1360161
<i>Agaricus campestris</i>	CA 819	Møller	JF727860
<i>Agaricus species</i>	ZNM 18	Zuhura	MB810237
<i>Agaricus species</i>	ZNM 18 (2)	Zuhura	MB810237
<i>Hymenagaricus ordosiicolor</i>	ZNM 4	Zuhura 2014	KM1360160
<i>Hymenagaricus ordosiicolor</i>	LAPAF9	Heinemann	JF727840

silica gel using the CTAB method. Amplification of 5.8S rRNA gene for assessing ITS length variation was done using primer ITS 1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) as described by White et al. (1990). PCR amplification products were electrophoretically separated on 1.5% agarose gel prepared in 1X TAE then stained in ethidium bromide (5 mg/ml). The gel was then run for at 90 V for 45 min followed by patterns visualization on UV transilluminator, and photographing using digital camera. The genomic DNA was amplified using ITS 4 (TCCTCCGCTTATTGATATGC) and ITS 5 (GGAAGTAAAAGTCGTAACAAGG) primers. The generated sequences of the species were compared to those available in the GenBank database at: (<http://www.ncbi.nlm.nih.gov/BLAST/>) using the BLASTN search as detailed in Tibuhwa et al. (2012).

### Phylogenetic analyses

The generated sequences in this study which were later used in phylogenetic analysis were submitted to gene Bank and their accession number, together with those obtained from the Genbank are summarised in Table 2. The sequences were aligned using ClustalW Olgorith (Higgins et al., 1990) of Mega Align 4.03 followed by manual editing. One sequence of non *Agaricus*-like (*Aspergillus niger*) was included in sequence analysis to serve as an out-group. A maximum likelihood (ML) tree was constructed using Kimura 2-parameter model. Bootstrap analyses with 1000 replicates were used to evaluate the stability of clade (Kimura, 1980).

## RESULTS AND DISCUSSION

### *Hymenagaricus mlimaniensis* Mwanga and Tibuhwa sp. nov.

Etymology: from Mlimani University Main *Campus*, of the University of Dar es Salaam, Tanzania.

**Pileus:** Medium sized 3-5 cm diameter, plano convex,

with central disc which is distinctive pink - reddish leaving the rest covered with white diminutive fibril on the pink-reddish background.

**Margin:** smooth.

**Colour:** unicolorous cream 1A1-2, except the disc at the broad umbo 8CD5-6.

**Stipe:** 2-6.5 x3 - 5b cm, irregularly cylindric, centrally positioned, with superior ring in young stage which disappears in age.

**Lamellae:** free, crowd spaced, pale pink color when young then turn chocolate brown in age. **Context:** white, turning reddish pink on exposure. Smell mushroomy.

**Basidiospores** ellipsoid, (4.0)5.2-5.6(6.9) x (4.3) 3.6-3.7(3.5)  $\mu\text{m}$ , Q = 1.25 (1.3) 1.4.

**Basidia:** clavate, four spored, (16)10.5-14.6(9.5) x (4.2) 4.5-4.8(5.3)  $\mu\text{m}$ .

**Cystidia:** Not observed.

**Hyphae:** thick walled, 3.1-7  $\mu\text{m}$  broad, septate with clamp connection.

**Lamellae-edge:** Fertile, composed of tetra basidiospores (Figure 2b and d).

### Studied material

Tanzania, Dar es Salaam city Mlimani main *Campus*, alt. 780 m, S 06°77.20' E 39°21.31', near Darajani, 15 March 2013, ZNMA (Holotypus, DSM), Dar es Salaam Mlimani main *Campus*, alt. 816 m, S04°54.52' E29°36.06', near academic bridge 29 April 2012, DT2012-25 (DSM).

*H. mlimaniensis* species nov. can be recognized in the field by its small sized basidiome coupled with distinctive diminutive fibrils covering the whole fruit body including the stipe, that are easily removed on touch, or falling down naturally such that they are seen



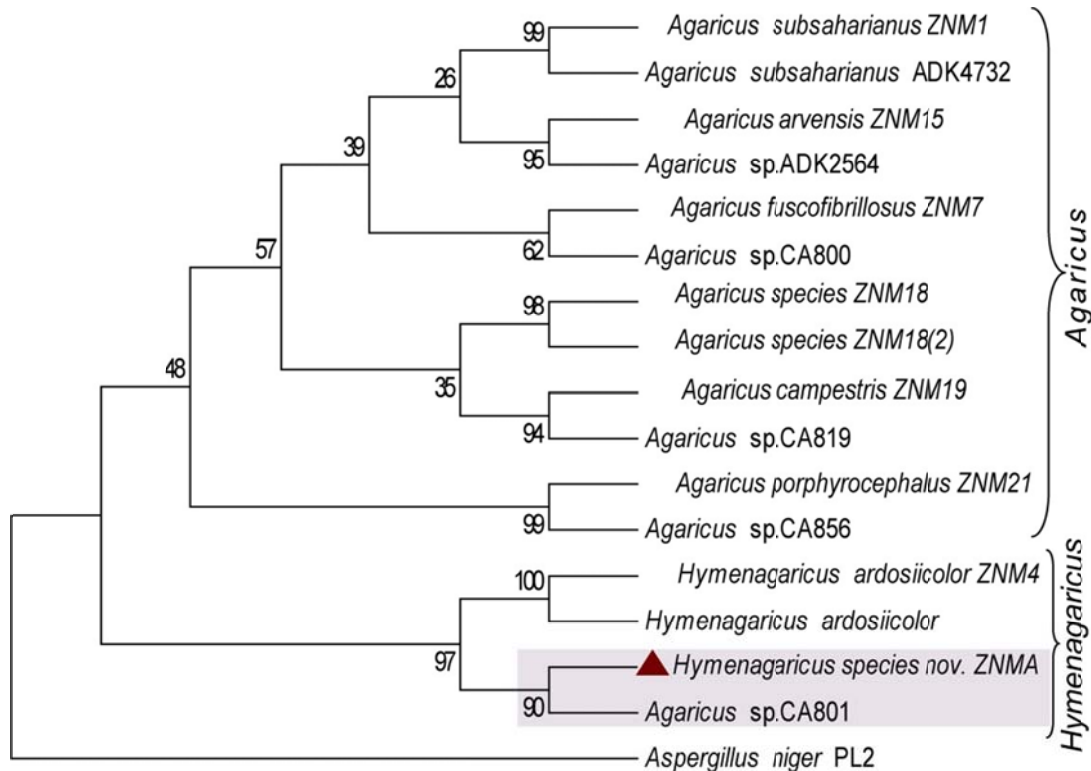
**Figure 2** *Hymenagaricus mlimaniensis* (holotypes) (a - c) fruit bodies in the natural habitat, (d) microscopic features (i) basidiospores (ii) Basidia (iii & iv) hymenium and section through pileipellis with clamp connections) (Photo taken March 15, 2013, microscopic drawing from holotype, scale bar =10  $\mu$ m).

on the grasses beneath and surroundings like white flakes (Figure 2b). Furthermore, the distinctive pink - reddish disc at the broad umbo (Figure 2a-c) contrasting the cream white-cream diminutive fibrils on the reddish pink background demarcate the species. The fibrils are so conspicuous and look more or less like those observed in some *Amanita* species.

The South African species *Hymenagaricus pallidodiscus* described by Reid & Eicker (1999) was also reported as having a distinctive disc on the pileus centre which is pale grey-buffy, elsewhere with white minute granular scales on the white background. However, *H. mlimaniensis* differs markedly in the distinctive pink-reddish colour of the disc and whiter diminutive fibril on the pink-reddish background. The strongly developed cortinate veil in the South African taxa also is missing in *H. mlimaniensis* but short superior annulus which disappear with age. Another close taxa is *Hymenagaricus alphitochrous* also known

from Tanzania (Pegler, 1977). *H. mlimaniensis* is delineated from *H. alphitochrous* by having smooth margin unlike the striate margin in the latter. Microscopically, the presence of clamp connections in *H. mlimaniensis* demarcates it from the two close species (*H. pallidodiscus* and *H. alphitochrous*) with similar microscopic characters but both lacking clamp connections observed in *H. mlimaniensis*.

In phylogenetic analysis the species seems to belong to *Hymenagaricus* H. and it forms a distinct clade with *Hymenagaricus ardosicolor* (Figure 3). The analysis carried out in this study using ITS data set, from the maximum likely hood, two major clades of genus *Agaricus*, and non *Agaricus* (*Hymenagaricus ardosicolor*, *Hymenagaricus mlimaniensis* species nov. (ZNM4) and *Agaricus* specie (CA.801) were retrieved. Identification of *H. ardosicolor* and species ZNM4 has a well-supported bootstrap value 100, thus the studied species ZNM4 is the *Hymenagaricus ardosicolor*



**Figure 3** Phylogenetic tree by Neighbour-joining showing *Hymenagaricus mlimaniensis* species nov. in *Hymenagaricus* distinct from *Agaricus* clade. (Bootstrap value 1000).

while the proposed new species *H. mlimaniensis* species nov. form a separate clade with an identified species *Agaricus* (CA 801) with low support value of 90 which show that they are not the same. Nevertheless, this monophyletic clade of the unknown *Agaricus* species (CA 801) with the proposed new species suggests that this unknown *Agaricus* could belong to *Hymenagaricus* genus and not *Agaricus*.

This study thus recommends for further study on this taxa from the GenBank (*Agaricus* CA 801) to ascertain its identity. Likewise, in the ITS data set analysis, this proposed species forms a sister clade with *H. ardosicolor* and their basal root form a main clade with a support value of 97 which puts them together in the same genus leaving out the rest of the *Agaricus* species clade with less support value of 48 since the species in the genus are so diverse and represent an independent line of evolution (Vellinga, 2004). The *Hymenagaricus* species are similar with members in the *Agaricus* clade by sharing some morphological feature such as dark brown gills in mature specimen, presence of rings and gills with free attachment (Heinemann, 1981; Ge et al., 2008).

## Conclusion

*H. mlimaniensis* Mwanga & Tibuhwa sp. nov. is described

from Dar es Salaam Mlimani Main Campus in the semi protected natural tropical forest left in the Dar es Salaam city. The species contributes to increase of another species within the genus *Hymenagaricus* raising for number of species and the species divulge to the academic society in general.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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