ARPHA Preprints

PENSOFT

PREPRINT

Author-formatted, not peer-reviewed document posted on 26/07/2024

DOI: https://doi.org/10.3897/arphapreprints.e133080

A new species of *Agaricus*; *Agaricus totalaiiensis* in section *Minores* from Pakistan

Muhammad Ishaq, Muhammad Binyamin khan, Fahim Hussain Shah, Muhammad Fiaz, Abdul Nasir Khalid

ARPHA Preprints *Author-formatted, not peer-reviewed document posted on 26/07/2024* DOI: https://doi.org/10.3897/arphapreprints.e133080

A new species of *Agaricus*; *Agaricus totalaiiensis* in section Minores from Pakistan

Muhammad Ishaq[1]*, Muhammad Binyamin Khan [2, 5, 6], Fahim Hussain Shah [3]*, Muhammad Fiaz[1] & Abdul Nasir Khalid[4]

1 *Department of Botany, Hazara University, Mansehra, Pakistan* **2** *State Key Laboratory of Green Pesticide, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, Guiyang 550025, China* **3** *Department of Plant Sciences, Quaid-i-Azam University Islamabad, 45320, Pakistan* **4** *Institute of Botany, University of the Punjab, Quaid-e-Azam Campus, 54590, Lahore, Pakistan* **5** *Engineering Research Center of Southwest Bio-Pharmaceutical Resources, Ministry of Education, Guizhou University, Guiyang 550025, China* **6** *School of Pharmacy, Guizhou University, Guiyang 550025, China*

Corresponding author: Muhammad Ishaq & Fahim Hussain Shah (ishaqm783@gmail.com; fahimhussain112233@gmail.com)

Abstract

Agaricus is a large genus of mushrooms with having great number of edible species. Subgenus *Minores* sect. Minores contain the richest species diversity. A new species *Agaricus totalaiiensis* described based on morpho-anatomy and molecular data analyses. It is characterized by small to medium-sized basidiomata that are initially parabolic to convex, becoming planate to concave at maturity, dull red or purplish-red to reddish brown at the center with a pale margin, become too red at the center become reddish brown to light radish brown margin at maturity, and large ellipsoid basidiospores. Molecular phylogenetic analyses based on ITS sequences confirmed its identity as a new taxon nested within the subgenus *Minores* sect. Minores.

Keywords

Agaricaceae, Buner district, Diversity, Mushroom, Phylogenetic

Introduction

Agaricus L. (Agaricales), the type genus of Agaricaceae, contains a large number of species with diverse habitats (Zhao et al. 2011; Karunarathna et al. 2016). Several species in this genus has high commercial value, such as *A. bisporus* (J.E. Lange) Imbach and *A. subrufescens* Peck. Regional monographs for *Agaricus* have been published in recent years, such as monographs from Europe (Parra 2008, 2013) and Northern America Kerrigan (2016). These monographs have given some detailed introductions among *Agaricus* and also well documented *Agaricus* species from those areas (Parra 2008, 2013; Kerrigan 2016). Estimates of the number of species of *Agaricus* range from about 200 (Kirk et al. 2008) to 386 Zhao et al. (2011) to over 500 (Parra 2013; Chen et al. 2017; He et al. 2018).

In the last ten years the availability of molecular data has greatly assisted mycologists in the study of the systematic of *Agaricus* (He et al. 2018). Recent comprehensive infra generic studies have recognized six subgenera and 21 sections of this genus (Zhao et al. 2016, Chen et al. 2017). *Agaricus* section *Minores* Fr. was raised to subgenus rank based on phylogenetic information and divergence time Zhao et al. (2016). Section *Minores* is the most species-rich section in *Agaricus*, and 27 Asian species were introduced from the Greater Mekong sub-region and southern China Chen et al. (2017). Now this section contains about 80 named species and is expected to harbor at least 200 species worldwide (Chen et al. 2017; He et al. 2017; 2018). Common morphological characters of *A*garicus sect- *Minores* are as follows: basidiomes slender, small or medium sized; annulus simple, thin; odor of almonds or anise; basidiomes turning yellow on bruising; KOH and Schäffer's reactions positive. However, in general, the morphological characteristics used for species distinction are scarce, especially between closely related species. In such cases, molecular data becomes essential for an unequivocal identification (He et al. 2017; 2018).

Up to date, only 26 species of *Agaricus* were reported from Pakistan (Ahmad et al. 1997; Sultana et al. 1997, Thongklang et al. 2014; Chen et al. 2016; Bashir et al. 2018; Hussain & Sher 2019). In this study we add one new species in sect. *Minores*, based on morpho-anatomical characteristics and phylogenetic analyses.

Materials and methods

Morphologic study

Basidiomes were collected in rainy seasons from Buner District, Pakistan. Photographs and other morphological characteristics of fresh basidiomata, including odor, basidiome size, color using Munsell notation, Munsell (1975). Specimens were dried with heater and kept in Ziploc (brand, deposited in LAH (Herbarium, Department of Botany, University of the Punjab, Lahore, Pakistan). For microscopic studies, tissues of pileus, gills and stipe were mounted in 5% KOH and stained with 1% aqueous Congo red (w/v). Anatomical features examined include basidiospores, basidia, cystidia, pileipellis and stipitopellis. They were observed under light microscope (LABOMED, Labo America, Inc. USA). The abbreviation [n/b/p] indicates 'n' basidiospores measured from 'b' basidiomata from 'p' collections. For basidiospores, L is the average length of the measured basidiospores, Similarly W is the average with of the measured basidiospores, Q is the range of L/W ratio of all the measured basidiospores, Qe is the average L/W ratio of the all measured basidiospores. Shape of the basidiospores is quoted according to Bas (1969).

Macrochemical reactions include the KOH reaction and Schäffer's reaction following Chen et al. (2015).

DNA extraction, amplification and sequencing

A modified CTAB method was used for DNA extraction, following (Bruns 1995). Amplification of the nuclear ribosomal internal transcribed spacer (ITS) locus was done with the fungal-specific ITS1F primer (5′-CTTGGTCATTTAGAGGAAGTAA-3′) (Gardes & Bruns 1993) and the eukaryotic ITS4 primer (5′-TCCTCCGCTTATTGATATGC-3′) (White et al. 1990). Amplified products were sent to Macrogen Inc., Korea, for purification and sequencing.

Sequence alignment and phylogenetic analyses

Consensus sequences were generated from the sequences obtained by both primers (forward and reverse) in BioEdit software v. 7.2.5 (Hall 1999). Sequences of *Agaricus* sect. *Minores*, at NCBI (http://www.ncbi.nlm.nih.gov/) and from published literature He et al. (2018) were added to the datasets. Two members of *A*garicus sect. *Xanthodermatei*, *A. microvolvatulus* Heinem (1971: 6) and *A. xanthodermus* Genev (1876: 32) were chosen as out group taxa. All the GenBank accession numbers are indicated in the ML tree (Fig. 1), and specimens used for the

molecular phylogenetic analyses are listed in (Table 1). Downloaded sequences were then aligned using online MUSCLE v.3.8 (Edgar, 2004) and edited manually with BioEdit v7.0.9 (Hall 1999). Maximum likelihood analyses were performed for individual gene regions via CIPRES Science Gateway Miller et al. (2010), employing RAxML-HPC v.8. Rapid bootstrapping was performed with one thousand bootstraps.

For the bootstrapping phase, the GTRCAT model was selected. The phylogeny from ML analysis was displayed with Fig Tree 1.4.2 (Rambaut 2014) and exported to Adobe Illustrator.

Tabel 1. Taxa and sequences used in the phylogenetic analyses with the new taxon in bold; “T” refers to type.

Species	Collection	ITS
Agaricus armandomyces	ZRL2015991	KX684863
A. candidolutescens T	LD2012129	KT951335
A. edmondoi	LAPAG80	KF447902
A. friesianus	F156208	KF447907
A. friesianus	ZRL20151813	KX684858
A. gemlii	*AH44510*	KF447891
A. gemloides T	ZRL2014084	KT633271
A. glabriusculus T	SWAT SH-7	MK751852
A. glabriusculus	LAH SH-Ag4	MK751854
A.iesu-et-marthae	LAPAG41	KF447904
A. Jacobi	AH44505	NR158300
A. jingningensis T	ZRL20151562	KX684877
A. jingningensis	*ZRL2013405*	KX657009
A. kerriganii T	AH44509	KF447893
A. latiumbonatus T	SWAT SH166	MK751861
A. latiumbonatus	LAH SH-Ag-3	MK751860
A. leucocarpus T	LD201215	KU975101
A. luteomaculatus	LAPAG331	KF447901
A. marisae	LAPAG138	KU975083
A.microvolvatulus	Grinling70109	JF514524
A. matrum T	LAPAG817	KF447896
A. pallens	LAPAG926	KT951315
A. pallens	LAPAG 580	KF447897
***A.totalaiiensis* T**	**LAH97**	MK659941
A.totalaiiensis	**LAH97**	MK659942
A. robustulus	CA1213	KU975089
A. xanthodermus	CA15INRA	AY899271
*A.*sp.	LAPAM14	KT951312
*A.*sp.	ZRLWXH3161	KT951391
A. sp.	MATA774	JF727871
A. sp.	ADK2905	JF514520

ARPHA Preprints *Author-formatted, not peer-reviewed document posted on 26/07/2024* DOI: https://doi.org/10.3897/arphapreprints.e133080

Results

Phylogenetic analysis:

The two sequences of our putative new taxon clustered in sect. *Minores* (Fig.1) as a sister group with *Agaricus glabriusculus* (MK751852, MK751854) from Pakistan differed by 98% and with an unnamed *Agaricus* spp (JF727871) from Mexico, from which they differed by 95% and *Agaricus jingningensis* (KX684877, KX656996) from China, which differs by 94%. Furthermore, it showed 94% identity to *A. edmondoi* (KF44902) and 95% with *A. jacobi* (NR158300, KF447895).

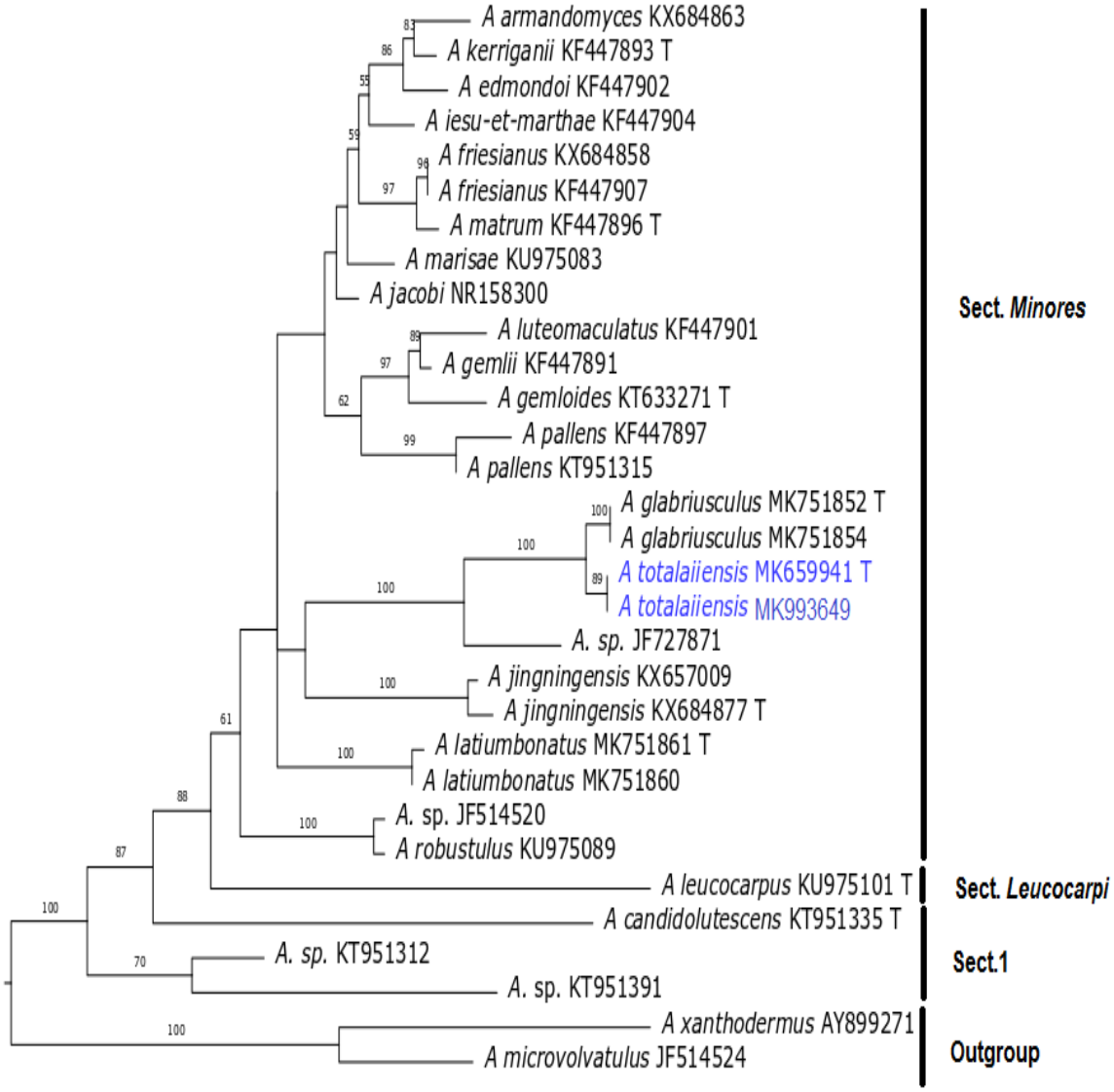

Figure 1. Maximum likelihood (ML) phylogram of *Agaricus* subg. *Minores* based on ITS drawn from dataset of 29 ITS sequences belonging to 27 species of *A.* sect. *Minores* and 2 out group species of *A.* sect. *Xanthodermatei*. The two sequences of the new species are highlighted in blue.

Taxonomy

Agaricus totalaiiensis M. Ishaq, M. Fiaz & A.N. Khalid, *sp. nov*. (Figs.2 & 3)

Mycobank no: MB 831249

Etymology: The specific epithet "*totalaiiensis"* refers to the collection locality.

Diagnosis: This species is distinguished from others in section *Minores* by its pileus color of dull reddish to reddish brown at the center with white to pale margin, almond odor, and large basidiospores.

Figure 2. A-D Basidiomata of A. *totalaiiensis***.** (Scale bars 1cm)

Description: Pileus 18-38mm diam, initially parabolic to convex, becoming plane to concave at maturity, thin-fleshed, surface dull reddish (10R 6/3) to reddish brown (10R 6/8) at the center to nearly white (2.5R 9/2) toward the margin in young stages, becoming purplish-red (10R 3/4) to red (10R 3/6) or reddish brown (10R 6/1) at the center and light reddish brown (10R 7/1) toward margin at maturity, surface smooth, dull, margin smooth, slightly uplifted at maturity. Lamellae free, unequal, crowed with intercalated lamellulae, purplish brown (10R 6/8) to dark purpile brown (10R 5/4), with entire margin. Stipe 15–40 × 4–5 mm, equal but with slightly bulbous base, dull orange (5YR 7/3) to pinkish orange (5YR 7/8), whitish (2.5YR 9/2), hollow, surface finely hairy, Annulus superior, flaccid-membranous, white (2.5R 9/2), margin obtuse. Odor of almond. Basidiospores [n/b/p] [80/4/2] (5–) 5.2–5.8 (–6.2) × (3.1–) 3.3–4.2 (–4.6) µm, L × W =

5.5 × 3.8 µm, Q = (1.1–) 1.4–1.6 (–1.8), Qe = 1.5, broadly ellipsoid to ellipsoid, pale brown in 5%KOH, apiculus prominent, smooth, thick-walled, gutullated. Basidia 21.5–44.3 × 13.4–18 µm, bi-tetrasporic, clavate, pale brown in 5%KOH, thin-walled. Cheilocystidia septate at the base, terminal elements pyriform, 17–34 × 5.6–14.4 µm, thin-walled, with oily contents. Pleurocystidia absent. Pileipellisa hyphae 5.1–8.4 µm wide, branched, septate, thin walled, light brown in KOH. Stipitpellis hyphae 7.8–9.5 µm wide, septate, thin-walled. Clamp connections absent.

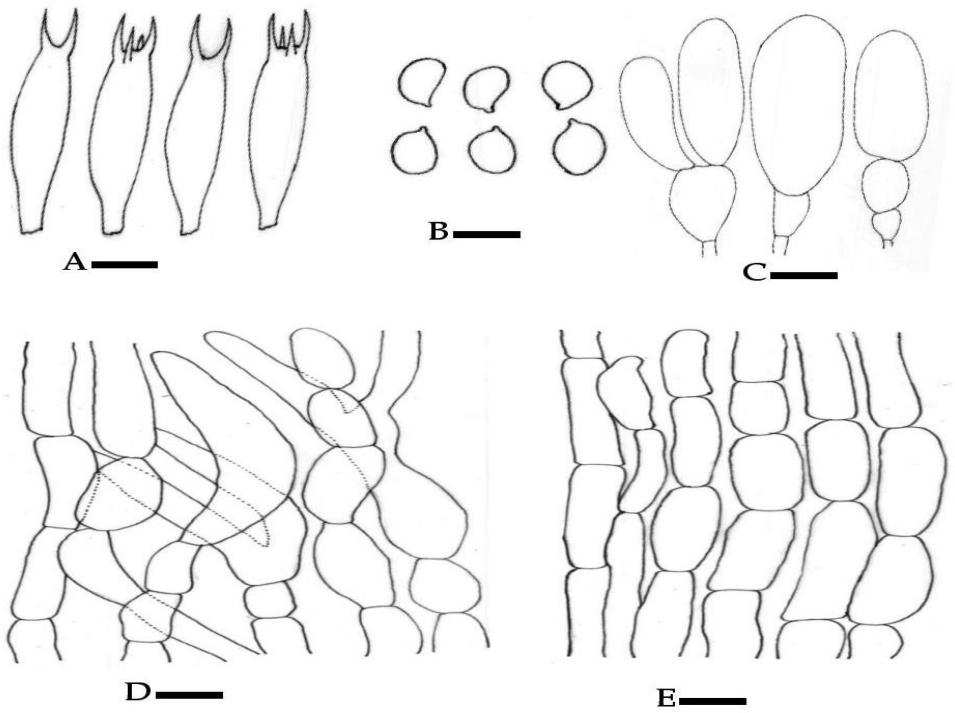

Figure 3. Anatomical features of *Agaricus totalaiiensis*. A. Basidia B. Basidiospors C. Cheilocystidia D. Pileipellis E. Stipitipellis Scale bars: A = 2.4µm, B = 4.5µm, C = 9.1µm, D = 5.6µm ,E = 9.5µm.

Macrochemical reactions: KOH reaction positive, yellow. Schäffer's reaction positive, reddish orange on cap of dry specimen.

Habitat and habitat: Gregarious, saprotrophic on dung in maize field.

Holotype: Pakistan, Khyber Pakhtunkhwa province, Malakand division, Buner district,

Totalai, 416 m asl, on dung in maize field, 16. 08. 2017, Muhammad Ishaq (IB286)

Additional Material examined:—Pakistan, Khyber Pakhtunkhwa province, Hazara division, Battagram district, 590m asl, on dung in maize field, 28.08. 2018, Muhammad Binyamin Khan (BU56)

Discussion

The epithet Minores was initially used in 1874 to lodge small-sized mushrooms (Fries 1874). *Agaricus* section *Minores* currently includes 83described species worldwide. About half of these species are reported from different climatic conditions in Asia, ranging from tropical to sub-tropical and temperate to plateaus. In this section and subgenus, morphological characteristics can overlap among species, so molecular phylogeny analysis is necessary for the identification at the species level He et al. (2017).

Agaricus totalaiiensis is characterized by thin-fleshed pileus of reddish brown disc with white color margins, one third at young stage become reddish brown at maturity, close lamellae have dark purple brown color and white superior flaccid-membranous annulus. Anatomically, it has broadly ellipsoid to ellipsoid spores and septate cheilocystidia. Phylogenetic analysis based on ITS data set, our new species clustered with *A. glabriusculus* S. Hussain & H. Sher, unnamed *Agaricus* spp and *A. jingningensis* M.Q. He &R.L. Zhao. show in (Fig. 1).

However, *Agaricus totalaiiensis*is significantly different from both the two said species based on macro-and micro-morphological characters other than molecular analysis. The large size and plane to concave shape pileus of A. *totalaiiensis*is different from *A. glabriusculus* have small pileus of plano-convex to applanate with slightly umbonate disc shape. Furthermore, *A. totalaiiensis* pileus color is purplish-red to reddish brown while *A. glabriusculus* pileus color of light to moderate pink denser at the center has creamy white background. It also has light yellowish pink lamellae which is dark purpile brown in *A.totalaiiensis*. *A. glabriusculus* has also large basidiospores (5.5–) 6–6.5 (–7.5) × (3–) 4–4.5 (–5.5) µm of ellipsoid to cylindrical shape (Hussain 2019). Another closely related species *A. jingningensis* resembles morphologically with *A.totalaiiensis*in its pileus shape and color but differs by its larger pileus (32-78mm) and stipe 60 × 5 – 7 mm, it sellipsoid.

Basidiospores and small basidia, 12.8–18.4 × 5.5–7.6 µm, He et al. (2017). *Agaricus Jacobi* L.A.

Parra, A. Caball. & Callac, differs by its large (30–75 mm) reddish-pink to reddish-purple pileus and smaller basidiospores, 5.2 × 4.0 µm. The pileus color of *A. edmondoi* L.A. Parra, Cappelli & Callacis similar to our species, but differ by smaller basidiospore (4.8 × 3.3 µm) and larger (20–60 mm) pileus than in our species. *A.kerriganii*L.A. Parra, B. Rodr., A. Caball., M. Martín-Calvo & Callac, shows similarities in and pileus color but differ by smaller basidiospores size (5.0 × 3.4, µm) and large (30–60 mm) pileus (Parra 2013). Based on phylogenetic analyses and morpho-anatomical characteristics, *Agaricus totalaiiensis* introduced here as a new species, that stands out from others in sect-Minores.

Acknowledgements

The authors are thankful to Prof. Dr. Donald H. Pfister, (Farlow Herbarium, Harvard University, MA, USA), Dr. Greg Thorn, (Department of Biology, Biological & Geological Sciences Building, Western University, London, Ontario, Canada N6A 5B7) and Yoshitaka Ono, Ibaraki University, Japan for their valuable comments and suggestions to improve the manuscript.

References

Ahmad S, Iqbal SH, Khalid AN (1997) Fungi of Pakistan. Sultan Ahmad Mycological Society of Pakistan, Lahore. https://fungiofpakistan.com/history

Bas C (1969) Morphology and subdivision of Amanita and a monograph of its section Lepidella. Persoonia 5: 285–579. https://repository.naturalis.nl/pub/531781

Bashir H, Hussain, S, Khalid, AN, Niazi ARK, Parra LA, Calla P (2018) First report of *Agaricus* sect. Brunneopicti from Pakistan with descriptions of two new species. Phytotaxa 357 3:167–178. https://phytotaxa.mapress.com/pt/article/view/phytotaxa.357.3.1

Bruns TD (1995) Thoughts on the processes that maintain localspecies diversity of ecto-mycorrhizal fungi. Plant and Soil 170: 63–73. https://link.springer.com/article/10.1007/BF02183055

Chen J, Zhao, RL, Karunarathna SC, Callac P, Raspé O, Bahkali AH, Hyde KD (2012) Agaricus megalosporus: a new species in section Minores. Cryptogamie, Mycologie 33:145–156. https://www.researchgate.net/publication/235665916

Chen J, Zhao R, Parra LA, Guelly AK, De Kesel A, Rapior S, Hyde KD, Chukeatirote E, Callac P (2015) *Agaricus* section *Brunneopicti*: a phylogenetic reconstruction with descriptions of four new taxa. Phytotaxa 192: 145–168. https://www.biotaxa.org/Phytotaxa/article/view/phytotaxa.357.3.1

Chen J, Parra LA, De Kesel A, Khalid AN, Qasim T, Ashraf A, Bahkali AH, Hyde KD, Zhao R, Callac P (2016) Inter– and intra–specific diversity in *Agaricus endoxanthus* and allied species reveals a newtaxon *A. punjabensis*. Phytotaxa 252:1–16. https://www.biotaxa.org/Phytotaxa/article/view/phytotaxa.252.1.1

Chen J, Callac P, Parra, LA, Karunarathna SC, He MQ, Moinard M, De Kesel, A, Raspé O, Wisitrassamee wong K, Hyde KD, Zhao RL (2017) Study in Agaricus subgenus Minores and allied clades reveals a new American subgenus and contrasting phylogenetic patterns in Europe and Greater Mekong Subregion. *Persoonia:* Molecular Phylogeny and Evolution of Fungi 38, p. 170. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5645183/

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797. https://pubmed.ncbi.nlm.nih.gov/15034147/

Fries GE (1874) Hymenomyc. Eur. Ed. Berling Uppsala.

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-294X.1993.tb00005.x

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98. https://www.scirp.org/reference/ReferencesPapers?ReferenceID=1383440

He MQ, Chen J, Zhou JL, Ratchadawan C, Hyde KD, Zhao RL (2017) Tropic origins a dispersal model for saprotrophic mushrooms in Agaricus section Minores with descriptions of sixteen new species. Scientific reports 7: p.5122. https://www.nature.com/articles/s41598-017-05203-5

He MQ, Hyde KD, Wei SL, Xi YL. Cheewangkoon R, Zhao RL (2018) Three new species of Agaricus section Minores from China. Mycosphere9: 189–201. https://www.mycosphere.org/pdf/MYCOSPHERE_9_2_3.pdf

Hussain S, Sher H (2019) Study in Agaricus section Minores in Pakistan with the description of two new species. Mycological Progress 18: 795–804. https://ouci.dntb.gov.ua/en/works/7BgmOD34/

Karunarathna SC, Chen J, Mortimer PE, Xu JC, Zhao RL, Callac P, Hyde KD (2016) A review of genus *Agaricus* in tropical and humid subtropical regions of Asia. Mycosphere**7**: 417–https://www.mycosphere.org/pdf/Mycosphere_7_4_3.pdf439.

Kerrigan RW (2016) *Agaricus* of North America Memoirs of The New York Botanical Garden Volume 114 ISBN: 978-0-89327-536-5 https://www.amazon.com/Agaricus-America-Memoirs-Botanical-Publisher/dp/0893275360

Kirk P, Cannon P, Minter D, Stalpers J (2008) Dictionary of the Fungi 10th Edition. Walling ford UK. https://www.cabidigitallibrary.org/doi/book/10.1079/9780851998268.0000

Miller MA, Pfeiffer W, Schwartz T, (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway computing environments workshop GCE. New Orleans Louisiana 1:8. https://ieeexplore.ieee.org/document/5676129

Munsell AH (1975) Soil color charts. Munsell™. Baltimore. https://www.scirp.org/reference/referencespapers?referenceid=1517520

Parra LA (2013) Fungi europaei Volume 1A Agaricus L. AllopsalliotaNauta& Bas Parte II Alassio: CandussoEdizioni. https://www.nhbs.com/en/fungi-europaei-volume-1-agaricus-l-english-spanish-book

Rambaut A 2014 FigTree 1.4. 2 software. Institute of Evolutionary Biology Univ. Edinburgh. http://tree.bio.ed.ac.uk/software/figtree/

Thongklang N, Nawaz R, Khalid AN, Chen J, Hyde KD, Zhao RL, Parra LA, Hanif M, Moinard M, Callac P (2014) Morphological and molecular characterization of three *Agaricus* species from tropical Asia Pakistan Thailand reveals a new group in section Xanthodermatei. Mycologia 106:1220–1232. https://www.tandfonline.com/doi/pdf/10.3852/14-076

White TJ, Bruns T, Lee SJWT, Taylor JL (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18: 315–322. https://www.scirp.org/reference/ReferencesPapers?ReferenceID=1356194

Zhao RL, Zhou JL, Chen J, Margaritescu S, Sánchez-Ramírez S, Hyde KD, Callac P, Parra LA, Li GJ, Moncalvo JM (2016) Towards standardizing taxonomic ranks using divergence times-a case study for reconstruction of the *Agaricus* taxonomic system. Fungal Diversity 78: 239–292. https://www.cifor-icraf.org/knowledge/publication/__40168/

Zhao R, Karunarathna S, Raspé O, Parra LA, Guinberteau J, Moinard M, De Kesel A, Barroso G, Courtecuisse R, Hyde KD, Guelly AK (2011) Major clades in tropical. *Agaricus.* Fungal Divers51: 279–296. https://www.academia.edu/12911528/Major_clades_in_tropical_*Agaricus*