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# *AGARICUS MANZOLLII* AND *AGARICUS PERMIANUS* TWO NEW SPECIES IN *AGARICUS* SECT. *MINORES*

## Abstract

*The paper presents exhaustive descriptions of Agaricus manzollii and A. permianus, two new species belonging to A. sect. Minores, illustrated with colour photographs of their macro- and microscopic characters as well as of the respective growing stations in the Italian Alps. The study includes also a morphological and phylogenetic comparison of the new taxa with their closest allies. The Italian version of this paper is available on the AMER website www.ameronlus.it/ojs/index.php/rmr.*

## Riassunto

*Il lavoro presenta descrizioni esaurienti di Agaricus manzollii e A. permianus, due nuove specie appartenenti ad A. sez. Minores, illustrate da fotografie a colori dei caratteri macro- e microscopici come anche delle rispettive stazioni di crescita nelle Alpi italiane. Lo studio include anche una comparazione morfologico-filogenetica con i taxa viciniori. La versione italiana di questo lavoro è disponibile sul sito AMER www.ameronlus.it/ojs/index.php/rmr.*

**Key words** *Agaricus*, section *Minores*, *Agaricus manzollii, Agaricus permianus,* Alps, Dolomites, Italy, Norway.

## Introduction

In our previous contribution to the knowledge of the mycological flora of the genus *Agaricus* L. of the Italian Alps (Parra *et al*. 2018), it was noted that the study of taxa living at a height above 2,000 m a.s.l. in the Dolomites is very difficult due to the extreme climatic conditions of the alpine zone. Likewise, the study of taxa occurring in the Norwegian lower alpine area is also demanding, because of its harsh environmental conditions. Accordingly, the fruiting of alpine *Agaricus* species is usually very poor, occurs in a short span late in the summer and only in favourable years.

*Agaricus manzollii* and *A. permianus*, the two species belonging to *A.* sect. *Minores* (Fr.) Henn. here proposed as new, were collected only twice (2018 and 2019) and once (2009) respectively, despite the repeated attempts that have been made to gather additional material ever since.

For this reason, much as we would have preferred to underpin the publication of new species with a higher number of collections, which would also have permitted us to gain a better insight into their variability, it was decided to publish them all the same because of their rarity and because their geographical distribution is confined to relict areas. Incidentally, this might be the very reason why there is such a dearth of records for both species. Finally, we think that gaining a better knowledge of the species inhabiting very restricted alpine zones could prove helpful for the protection and conservation of the areas where they live.

## Material and methods

### *Macro- and microscopic description*

The description of the macro- and the microscopic characters of the specimens is based on fresh material of both species and follows the same format as in Parra (2008). Microscopic observations were carried out on dry material, previously rehydrated with 5% ammonia and observed in Congo red or in water with a Nikon SE microscope. The spore measures were taken following Heinemann & Rammeloo (1985) with the spore and Q ratio average values underlined.

The holotypes of *A. manzollii* and *A. permianus* were deposited in the herbarium of Museo di Storia Naturale di Venezia (MCVE). In the private herbaria of L.A. Parra (LAPAG) are also conserved the isotypes of both *A. manzollii* and *A. permianus*, while in the herbaria of R.J. Ferrari (FRJ) and E. Bizio (eb) are preserved, respectively, an additional isotype of *A. manzollii* and another of *A. permianus.*

References and acronyms of institutional herbaria follow Holmgren & Holmgren (1998).

### *Molecular analysis*

Total DNA was extracted following a standard CTAB protocol. PCR amplification of the internal transcribed spacer regions (ITS 1+2) of the ribosomal DNA was performed with primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al*. 1990). Sequencing was performed by STAB VIDA LDA. (Caparica, Portugal), with the primers used for PCR. A combined dataset including 103 samples were prepared for phylogenetic analysis (**Table 1**). In addition to the three sequences produced from this study, 100 ITS sequences, 69 nrLSU sequences and 65 *tef1-α* sequences were retrieved from GenBank, and were used in the previous studies (Parra *et al*. 2024; Wang & Bau 2024), and the origin of the samples and their GenBank accession numbers are given in **Table 1**. Sequences were aligned, for each region independently using MAFFT (Katoh & Standley 2013), then manually adjusted in BioEdit v. 7.0.4 (Hall 2007). The maximum likelihood (ML) analysis was performed in RAxMLHPC2 v. 8.2.12 (Stamatakis 2014) as implemented on the Cipres portal (Miller *et al*. 2010), under a GTRGAMMA model with one thousand rapid bootstrap (BS) replicates. The combined dataset was partitioned into ITS, LSU, *tef1-α* regions. The best substitution model for each partition was inferred with the program MrModeltest 2.2 (Nylander 2004): GTR+I+G for both ITS and LSU, SYM+I+G for *tef1-α*. Bayesian inference (BI) analysis was performed in MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). Two runs of six Markov chains were run for one million generations and sampled every 100th generation. Burn-in was determined by checking the likelihood trace plots in Tracer v. 1.6 (Rambaut *et al*. 2014) and subsequently discarded. The outputs were displayed in FigTree v. 1.4.0. (http://tree.bio.ed.ac.uk/software/figtree/) (**Fig. 1**).

**Table 1**. Specimens and sequences used for the phylogenetic analyses. New species and new sequences are in bold. "T" refers to type specimens.

| **Taxon** | **Specimen** | **GenBank code** | | | **Geographic origin** |
|---|---|---|---|---|---|
| | | **ITS** | **LSU** | ***tef1-α*** | |
| *A. aridicola* | LAPAG589 | KT951331 | KX084027 | KX198081 | Spain |
| *A. armandomyces* T | ZRL2015992 | KX684860 | KX684882 | KX684906 | China |
| *A. arrillagarum* | LAPAG810 | KF447900 | KX083985 | KT951592 | Spain |
| *A. badioniveus* T | LD2012131 | KU975117 | - | - | Thailand |
| *A. blatteus* T | ZRL2012004 | KT951355 | KT951457 | KT951608 | China |
| *A. bonisquamulosus* T | ZRL2010106 | KX657047 | KX656950 | KX684951 | China |
| *A. brunneolus* | LAPAG938 | KU975082 | KX083997 | KX198062 | Spain |
| *A. brunneolutosus* T | MS514 | KU975111 | KX084006 | - | China |
| *A. callacii* T | AH42929 | KF447899 | KX083984 | KX198051 | Spain |
| *A. campbellensis* T | GAL9420 | DQ232644 | DQ232657 | - | New Zealand |

(to be continued)

| **Taxon** | **Specimen** | **GenBank code** | | | **Geographic origin** |
|---|---|---|---|---|---|
| | | **ITS** | **LSU** | ***tef1-α*** | |
| *A. campestris* T | LAPAG370 | JQ903618 | KP739803 | KR006636 | Spain |
| *A. candidolutescens* T | LD2012129 | KT951335 | KT951525 | KT951616 | Thailand |
| *A. catenatus* T | ZRL2012104 | KX657023 | KX656963 | KX684957 | China |
| *A. cerinipileus* T | ZRL2012001 | KX657021 | KX656957 | KX684953 | China |
| *A.* cf. *kerriganii* | WC912 | AY484681 | - | - | USA |
| *A. chartaceus* T | H6271 | JF495048 | - | - | Australia |
| *A. coccyginus* | ZRL2012576 | KT951372 | KT951499 | KT951596 | China |
| *A. colpetei* T | TL2424 | JX984565 | - | - | Australia |
| *A. columellatus* | SB-2015 | KJ912899 | - | - | USA |
| *A. comtulus* | LAPAG303 | KU975078 | KX083986 | KX198052 | Spain |
| *A. dilatostipes* | ZRL2014450 | KX656999 | KX656941 | KX685003 | China |
| *A. dulcidulus* | PRM909627 | KF447894 | - | KX198064 | Czech Republic |
| *A. edmondoi* | LAPAG412 | KT951326 | KT951481 | KT951590 | Spain |
| *A. elongatestipes* T | ZRL2013271 | KX657002 | KX656946 | KX684975 | China |
| *A. fimbrimarginatus* T | LD201250 | KU975119 | KX084017 | KX198076 | Thailand |
| *A. flammicolor* T | LD201502 | KU975114 | KX084009 | KX198042 | Thailand |
| *A. flavoaurantiacus* T | MFLU16-0980 | KU975107 | KX084002 | KX198069 | China |
| *A. flavopileatus* T | MS596 | KU975121 | KX084022 | KX198078 | China |
| *A. friesianus* | LAPAG592 | KT951316 | KX083992 | KT951594 | France |
| *A. gemlii* T | AH44510 | KF447891 | KX083989 | - | Spain |
| *A. gemloides* T | ZRL2014084 | KT633271 | KX641405 | KX684986 | China |
| *A. glabriusculus* T | SWAT SH-7 | MK751852 | - | - | Pakistan |
| *A. globosporus* T | ZRL2012656 | KX657039 | - | KX684968 | China |
| *A. heinemannianus* | LAPAG302 | KF447906 | - | KX198056 | Spain |
| *A. huijsmanii* | LAPAG639 | KF447889 | KT951444 | KT951571 | Spain |
| *A. iesu-et-marthae* | LAPAG41 | KF447904 | - | - | Spain |
| *A. indicus* T | TBGT16128 | OR661746 | - | - | India |
| *A. jacobi* | LAPAG52 | KF447895 | KX083996 | KX198061 | Spain |
| *A. jingningensis* T | ZRL20151562 | KX684877 | KX684895 | KX684917 | China |
| *A. kerriganii* T | AH44509 | KF447893 | KX083999 | KX198066 | Spain |
| *A. laeticulus* T | Goossens5272 | KX671705 | - | - | DR Congo |

(to be continued)

| Taxon | Specimen | GenBank code | | | Geographic origin |
|---|---|---|---|---|---|
| | | ITS | LSU | *tef1-α* | |
| *A. lamelliperditus* | MDBF61/96 | JX984559 | - | - | Australia |
| *A. latiumbonatus* T | SWAT SH166 | MK751861 | MK751858 | - | Pakistan |
| *A. leucocarpus* | LD201226 | KU975102 | KX083982 | KX198049 | Thailand |
| *A. leucocarpus* T | LD201215 | KU975101 | KX083981 | KX198048 | Thailand |
| *A. lodgeae* | LAPAM14 | KT951312 | - | KT951613 | Dominican Republic |
| *A. luteofibrillosus* | LD201501 | KU975108 | KX084003 | KX198041 | Thailand |
| *A. luteomaculatus* | CA331 | KF447901 | - | KX198053 | France |
| *A. luteopallidus* T | LD2012113 | KU975124 | KX084026 | KX198080 | Thailand |
| *A. mangaoensis* T | ZRL2010056 | KX657042 | KX656956 | KX684946 | China |
| *A. marisae* T | LAPAG138 | KU975083 | KX083998 | KX198065 | Spain |
| *A. matrum* T | AH44506 | KF447896 | KX083991 | KX198058 | Spain |
| ***A. manzollii* T** | **MCVE 31982** | PP864485 | PP909787 | PP911358 | Italy |
| ***A. manzollii*** | **JH-A1-18** | PP864486 | - | - | Norway |
| *A. manzollii* | GAL3083 | EF460374 | EF460399 | - | USA |
| *A. manzollii* | GAL8696 | EF460368 | - | - | USA |
| *A. megalosporus* | ZRL2012199 | KT951367 | KT951470 | KT951595 | Thailand |
| *A. microviolaceus* T | ZRL2012718 | KX657033 | KX656980 | KX684971 | China |
| *A. midnapurensis* | CUH AM718 | OL467539 | - | - | India |
| *A. minipurpureus* T | ZRL2010058 | KX657043 | KX656953 | KX684947 | China |
| *A. neimengguensis* T | ZRL20151845 | KX684870 | KX684902 | KX684924 | China |
| *A. pallens* | LAPAG441 | KF447898 | - | KX198067 | Spain |
| *A. palodensis* T | TBGT17483 | OR661748 | - | - | India |
| *A. parvibicolor* T | LD2012116 | KP715162 | KX084016 | KX198075 | Thailand |
| *A. parvibrunneus* T | ZRL20161053 | MG137001 | MG196345 | MG196351 | China |
| *A. patris* T | LD201224 | KU975118 | KX084012 | KX198073 | Thailand |
| ***A. permianus* T** | **MCVE 31981** | PP864487 | - | - | Italy |
| *A. pseudolutosus* | LAPAG454 | KT951329 | KT951453 | KT951602 | Spain |
| *A. pseudopallens* T | ZRL20151552 | KX684874 | KX684891 | - | China |
| *A. pseudopurpurellus* | ZRL2014063 | KX656988 | KX641404 | KX684985 | China |
| *A. purpurellus* | LAPAG944 | KU975076 | KX083994 | KX198060 | Czech Republic |
| *A. purpureofibrillosus* T | ZRL3080 | JF691542 | KX084021 | - | Thailand |

(to be continued)

| Taxon | Specimen | GenBank code | | | Geographic origin |
|---|---|---|---|---|---|
| | | ITS | LSU | *tef1-α* | |
| *A. purpureosquameus* T | LE2016047 | MF197451 | - | - | Thailand |
| *A. purpureosquamulosus* | CUH AM716 | OL467541 | - | - | India |
| *A. robustulus* T | CA847 | KU975086 | KX084034 | KX198039 | Thailand |
| *A. rufifibrillosus* | ZRL20151536 | KX684878 | KX684893 | KX684915 | China |
| *A. rufipileus* T | ZRL2014140 | KX656991 | KX656937 | KX684991 | China |
| *A. sodalis* T | LD2012159 | KP715161 | KX084014 | KX198074 | Thailand |
| *A.* sp. | Vellinga2360 | AF482831 | AF482877 | - | USA |
| *A.* sp. | ZRLLD013 | KT951384 | KT951516 | KT951604 | Thailand |
| *A.* sp. | PS036 | KU975087 | KX084035 | KX198036 | Thailand |
| *A.* sp. | ZRL3056 | JF691541 | KX084020 | - | Thailand |
| *A.* sp. | PYP014 | KU975091 | - | - | Thailand |
| *A.* sp. | TL2307 | JF495058 | - | - | Australia |
| *A.* sp. | ZRL20151437 | KX684876 | KX684892 | KX684914 | China |
| *A.* sp. | NTT72 | JF514539 | - | - | Thailand |
| *A.* sp. | CA935 | KU975085 | KX084036 | KX198034 | Thailand |
| *A.* sp. | MS386 | KU975113 | KX084008 | KX198044 | China |
| *A.* sp. | ZRLWXH3064 | KX657010 | - | - | China |
| *A.* sp. | ZRLWXH3067 | KT951387 | KT951497 | KT951611 | China |
| *A.* sp. | ZD1528 | KU975104 | KX083987 | KX198054 | China |
| *A.* sp. | LD201252 | KU975103 | - | KX198050 | Thailand |
| *A.* sp. | MATA774 | JF727871 | - | - | Mexico |
| *A.* sp. | CA845 | KU975084 | KX084033 | KX198035 | Thailand |
| *A.* sp. | ZRLWXH3161 | KT951391 | KT951526 | KT951615 | China |
| *A.* sp. | LAH35900 | MK659941 | - | - | Pakistan |
| *A. stevensii* | FS 06-02-09 | KJ877785 | - | - | USA |
| *A. subiculosus* T | AH56332 | PP313292 | PP291842 | PP317134 | Puerto Rico |
| *A. viridopurpurascens* | Horak68/79 | JF514525 | - | - | New Zealand |
| *A. velutinosus* T | HMJAU 67768 | OR690296 | OR690376 | OR711546 | China |
| *A. wariatodes* | TWM1589 | JF495052 | JF495030 | - | Australia |
| *A. wayanadensis* T | TBGT18860 | OR661750 | - | - | India |
| *A. yanzhiensis* T | ZRL20162082 | MG137003 | MG196346 | - | China |

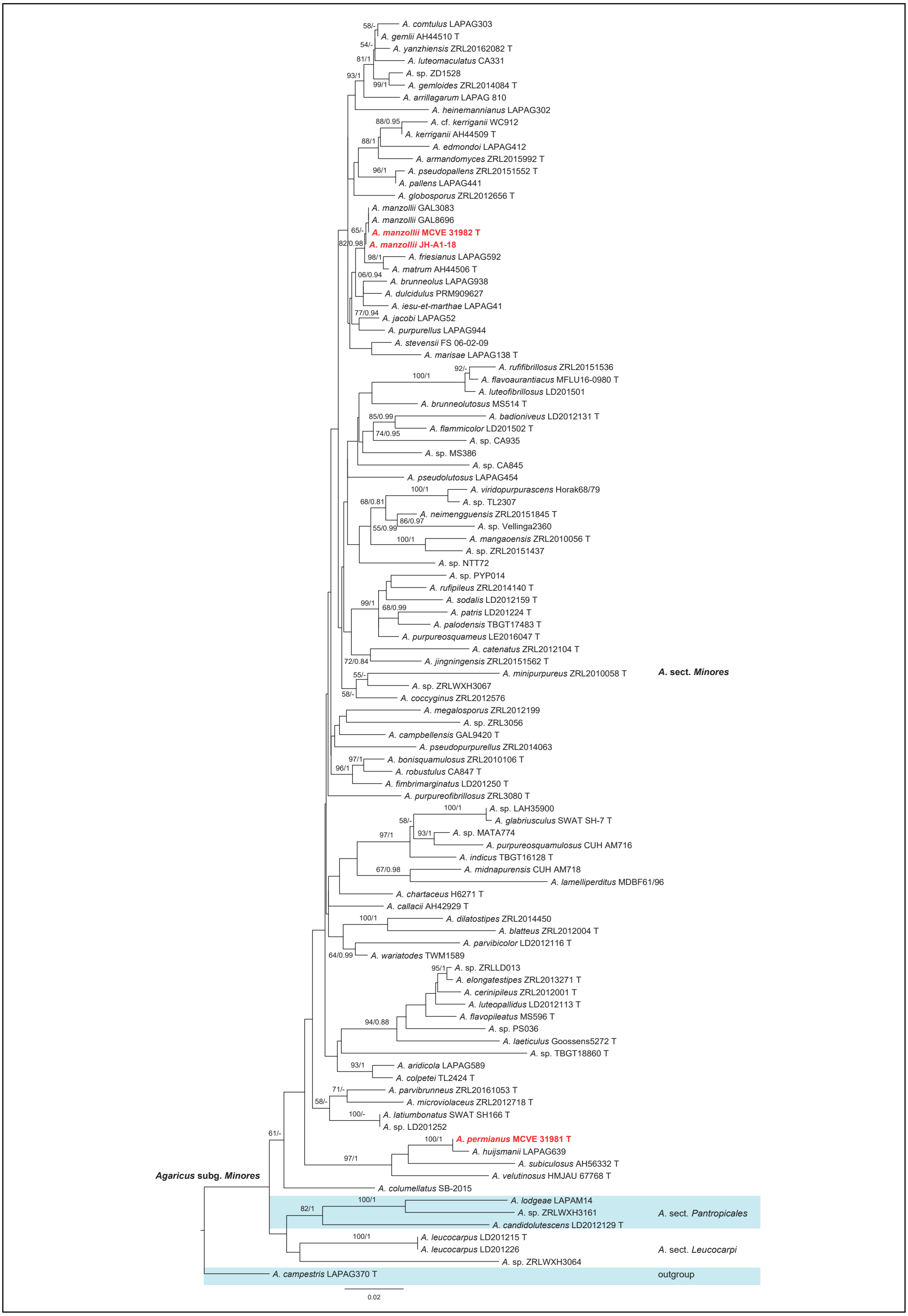

**Fig. 1**. Maximum likelihood phylogram of *Agaricus* subg. *Minores* resulting from analysis of ITS, LSU and tef1-$\alpha$ sequence data. The best scoring RAxML ITS tree is rooted with *A. campestris*. The bootstrap support values greater than 50% and Bayesian posterior probabilities greater than 0.8 are indicated. New species are in red. T = Type.

***Agaricus manzollii*** L.A. Parra, R.J. Ferrari, Weholt, A. Fellin, Cappelli & Linda J. Chen sp. nov. (**Fig. 2**).
MycoBank: MB 854245

**Holotype** ITALY: Trentino-Alto Adige, San Vigilio di Marebbe (BZ), Gran Fanes (Fanes-Senes-Braies Nature Park), at about 2,150 m a.s.l., 46°35'38.643"N, 12°1'18.135"E, 30/08/2019, *leg*. R.J. Ferrari, MCVE 31982.

**Etymology** in honour of the memorable president of the Mycological association AMB group of Brunico (Bolzano-Italy), Lucio Manzolli.

**Diagnosis**

*Agaricus manzollii belongs to A. sect. Minores and is phylogenetically close to, but distinct from, A. hujismanii and A. matrum. Microscopically, the new species differs in the spores that are larger than in the latter two species and in the lack of cheilocystidia, which instead are abundant in both of them.*

**Macroscopic description**

**Pileus** 1.5-3 cm, at first truncate-conical, finally applanate with depressed center and incurved margin. Surface dry, dull, subtly radially fibrillose, when young, whitish with a cream to pale greyish discal area, which is unbroken or appressed-scaly, becoming yellow when rubbed and, with time, becoming sordid greyish-ochre in the centre and leaden grey on the margin, on the margin, margin hardly exceeding the lamellae by 0.5 mm.

**Lamellae** free, crowded, with intercalated lamellulae, first whitish, finally dark brown-red, almost black with a paler edge.

**Stipe** 2-3 × 0.5-0.7 cm, clavate to slightly bulbous at the base (1 cm), with an annulus in its upper half, smooth, white to pale ochraceous above and below the annulus, discolouring yellow when rubbed or with age at the base.

**Annulus** superous, simple, very narrow, thin, fragile, submembranous, white, becoming yellow with time or when rubbed.

**Context** white, unchanging or slightly yellowing in the stipe base. Odour of anise.

**Microscopic description**

**Spores** 4.9-$\underline{5.5}$-6 × 3.6-$\underline{4}$-4.3 µm, Q = 1.28-$\underline{1.39}$-1.56, broadly ellipsoid to ellipsoid, dark brown, smooth, thick-walled, lacking an apical pore and usually uniguttulate.

**Basidia** 15-22 × 6-8 µm, usually 4-spored, some 1-, 2- or 3-spored, clavate or slightly truncate at the apex, hyaline or with diffuse dark brown pigment, with sterigmata up to 5 µm long.

**Cheilocystidia** absent.

**Pleurocystidia** absent.

**Lower surface of the annulus** composed of cylindrical hyphae, 3-12 µm wide, the wider hyphae more constricted at the septa, more contorted and branched in comparison with the pileipellis hyphae, without inflated elements, hyaline. Terminal elements not found.

**Pileipellis** a cutis of cylindrical hyphae 2-11 µm wide, not or slightly constricted at septa, hyaline, without internal or parietal pigments. Terminal elements with rounded apex.

**Clamp connections** absent in all the structures that have been studied.

**Macrochemical reactions** Schäffer and KOH reactions were not tested on fresh material. On dried material, Schaeffer's reaction was positive red on the pileus and stipe base. KOH reaction hardly perceivable due to the dark ochraceous yellow colour of the dried material.

**Habit, habitat and distribution** gregarious in small groups (two or three basidiomes), in the second half of the summer, in alpine areas with *Dryas octopetala* L. mats. Known to date only

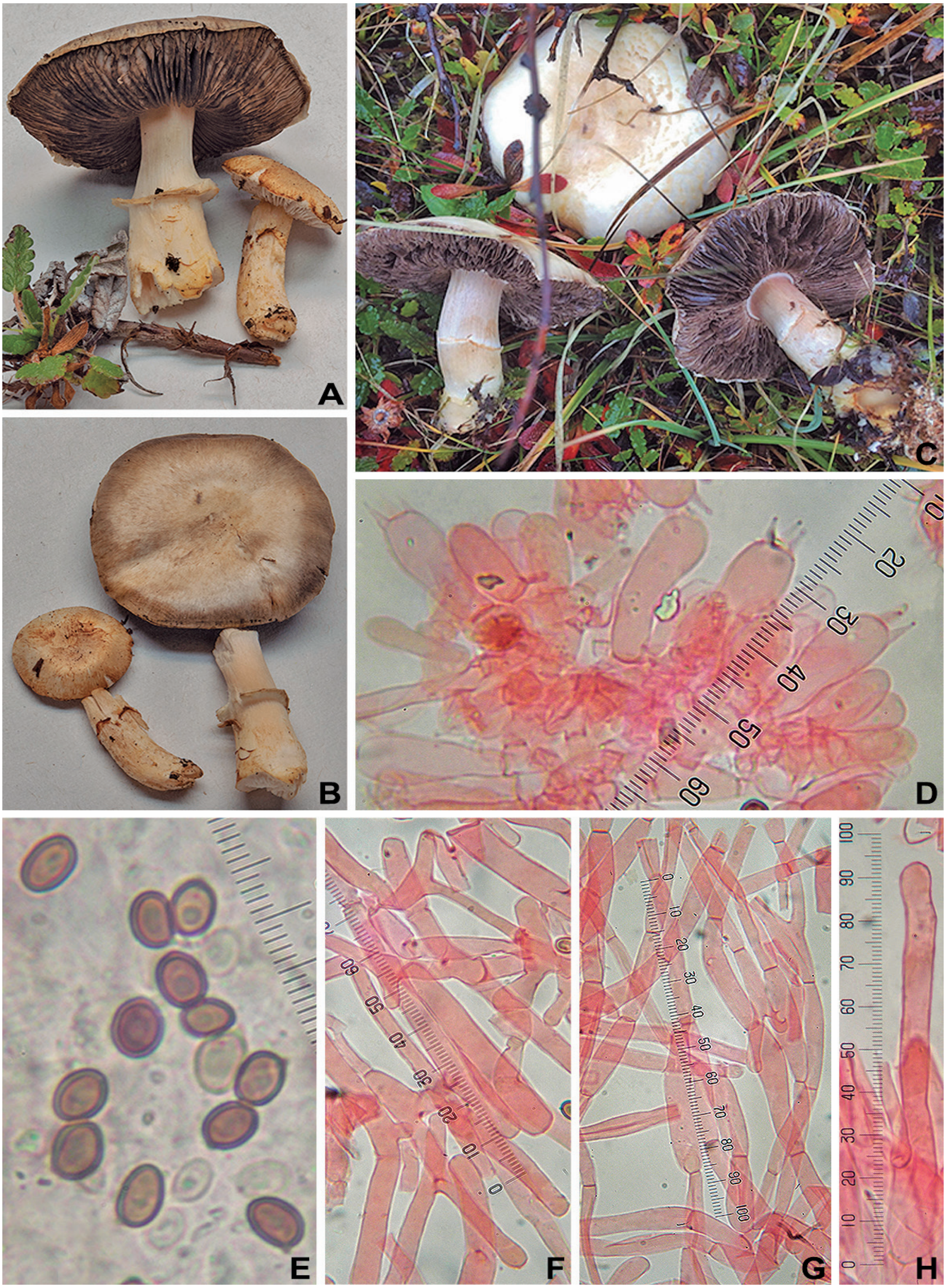

**Fig. 2**. *Agaricus manzollii*. **A-B**. *Ex situ*; **C**. In habitat; **D**. Basidia; **E**. Spores; **F**. Annulus hyphae; **G**. Pileipellis hyphae; **H**. Terminal element of the pileipellis. **A-B**: MCVE 31982. **C**: JH-A1-18. **E-H**: LAPAG1417.

Photos **A-B**: Renato J. Ferrari. **C**: J. Høgås. **D-H**: Luis A. Parra from Italian and Norwegian alpine areas (**Fig. 3**), and Alaskan tundra in the USA (Geml *et al*. 2008). The collections available until now suggest that this species prefers very cold habitats.

**Material examined:** ITALY, Trentino-Alto Adige, loc. San Vigilio di Marebbe (BZ), Gran Fanes (Fanes-Senes-Braies Nature Park), at about 2,150 m a.s.l., 46°35′38.643″N, 12°1′18.135″E, 30/08/2019, *leg*. R.J. Ferrari, MCVE 31982 (holotype); FRJ072-2019 and LAPAG1396 (isotypes). NORWAY: reg. Helgeland, county Norland, loc. Rana, at about 650 m a.s.l., 66°07′37.4″N, 14°23′10.7″E, 16/09/2018, *leg.* J. Høgås, JH-A1-18 (duplicate LAPAG1417).

## TAXONOMY

### Comments on the phylogenetic analysis

Geml *et al*. (2008) included two Alaskan collections, identified as GAL3083 and GAL8696 in his work on artic species of *Agaricus*, collected in Artic tundra. The ITS sequences of these two collections were deposited in GenBank under the codes EF460374 and EF460368 respectively and they have an ITS sequence 100% identical to that of the holotype of *A. manzollii*. The Norwegian collection shows 5 heteromorphic positions in which one allele from the other collections is present. Therefore, despite the enormous distance between the collecting sites, genetically the species does not show great variability in the ITS region.

Comparing the ITS sequence of *A. manzollii* with all available ITS squences of *A*. sect. *Minores*, it lacks any species-specific ITS markers. According to our phylogenetic analysis, *A. manzollii* is resolved as a sister clade to *A. friesianus* L.A. Parra, Olariaga & Callac and *A. matrum* L.A. Parra, A. Caball. S. Serrano, E. Fern. & Callac with strong support (82/0.98). However, *A. manzollii* differs from *A. friesianus* at 6 positions in the ITS region and 18 positions in the *tef1-α* region, and from *A. matrum* at 8 positions in both ITS and *tef1-α* regions.

**Fig. 3**. Gran Fanes. Collecting site of *Agaricus manzollii*. Photo by Renato J. Ferrari

### Morfological comments

*Agaricus manzollii* is characterized by a whitish pileus with a pale greyish centre, lacking any pink or purple tones. The annulus is simple, very narrow and fragile and a context, white, unchanging or slightly yellowing at the stipe base with an anise-like odour. Microscopically, the spores are 4.9-$\underline{5.4}$-6 × 3.6-$\underline{3.9}$-4.3 µm and the cheilocystidia are completely lacking. Its distribution appears to be confined to areas with extremely cold conditions, such as tundra or alpine areas. The Alaskan collections were published without any morphological description or illustration (Geml *et al*. 2008) and their morphological characters are unknown.

In the mycological literature, there is hardly any mention of alpine collections of *Agaricus* sect. *Minores*, in which the mention is accompanied at least with a short description. Kühner (1974) recorded two collections of "*Agaricus rubellus* (Gillet) Sacc." [*nom. illeg. non Agaricus rubellus* Scop., *qui est Entoloma rubellum* (Scop.) Gillet] in Swedish Lapland at 700 m a.s.l., and two collections of *Agaricus purpurellus* in the "Parc National de la Vanoise" of the French Alps at 2,150 m a.s.l., in both cases with presence of *Dryas* as in our collections of *A. manzollii*. However, in all these collections the basidiomes had pilei with purple or vinaceous tinges and abundant, distinctive cheilocystidia. Lucchini (1997) also mentions two collections of "*Agaricus semotus* Fr." in the Swiss Alps at 1,950 m and 2,260 m a.s.l., in alpine meadows, but this species has always been interpreted as having a pileus with a pinkish to purple center and distinctive cheilocystidia, which fits with the description and photograph provided of one of them.

Phylogenetically the taxa most closely related are *A. friesianus* L.A. Parra, Olariaga & Callac and *A. matrum* L.A. Parra, A. Caball. S. Serrano, E. Fern. & Callac, but both species have smaller spores and abundant cheilocystidia. In addition, *A. friesianus* has a pileus with pinkish or purplish tones on the disc, while in *A. matrum* the pileus surface is entirely white.

## *Agaricus permianus* L.A. Parra, Bizio, Cappelli & Linda J. Chen sp. nov. (**Fig. 4**).

MycoBank: MB 854246

**Holotype** ITALY, Trentino-Alto Adige, Moena (TR), loc. Forcella Grana, at approximately 2,300 m a.s.l., 46°21′23′′N; 11°45′37′′E, 18/08/2009, *leg*. E. Bizio, A. Aiardi, A. Castellan & C. Zoldan, MCVE 31981.

**Etymology** from the Latin *permianus*, with reference to the Permian period, because the Permian quartziferous porphyries constitute the substrate of the ground where the new species was found.

### Diagnosis

*Agaricus permianus belongs to A. sect. Minores and is phylogenetically close to, but distinct from, A. huijsmanii, the latter differs also in the white pileus lacking any pinkish or violet tinges and in its growth in Fagus forests. Further, microscopically, both its spores and cheilocystidia are smaller than those of the new species.*

### Macroscopic description

**Pileus** 3-6 cm, at first hemispherical, then convex, finally more or less applanate with incurved margin. Surface dry, dull, at first radially fibrillose, then often cracking into large polygonal scales in a more or less broad central area. When young, with appressed, radially arranged vinaceous-purple fibrils on a whitish background, denser in the centre, with time the areolate centre and the radially arranged fibrils become sordid violaceous-brown on an ochraceous background, becoming yellow on rubbing. Margin exceeding the lamellae by 2 mm, appendiculate with small annulus remnants.

**Lamellae** free, crowded, with intercalated lamellulae, first whitish, then greyish-pink, finally dark brown almost black, with paler edge.

**Stipe** 4-8 × 0.8-1 cm, clavate to slightly bulbous at the base (1.5 cm), with an annulus in its upper half, above the annulus white and smooth, below the annulus white, smooth or slightly fibrillose, staining yellow when rubbed or with age at the base, with abundant white rhizomorphs.

**Annulus** superous, simple, very narrow, thin, fragile, submembranous, white, becoming yellow with time or when rubbed.

**Context** white, unchanging or slightly yellowing in the stipe base. Odour of anise, clearly of almonds when the pileus margin is bruised.

### Microscopic description

**Spores** 4.8-$\underline{5.4}$-6.1 × 3.7-$\underline{3.9}$-4.2 µm, Q = 1.25-$\underline{1.38}$-1.49, broadly ellipsoid to ellipsoid, dark brown, smooth, thick-walled, lacking an apical pore, usually uniguttulate.

**Basidia** 15-20 × 5-9 µm, usually 4-spored, clavate or slightly truncate at the apex, hyaline, with sterigmata up to 3 µm long.

**Cheilocystidia** very abundant, hyaline, usually simple, globose, sphaeropedunculate, pyriform or broadly clavate, 6-52 × 5-45 µm.

**Pleurocystidia** absent.

**Lower surface of the annulus** composed of cylindrical hyphae, 2-6 µm wide, not or slightly constricted at the septa, without inflated elements, hyaline. Terminal elements not observed.

**Pileipellis** a cutis of cylindrical hyphae 2-14 µm wide, the broader the more constricted at septa. Terminal elements scarce, with round apex.

**Clamp connections** absent in all the structures that have been studied.

**Macrochemical reactions** Schäffer and KOH reactions were not tested on fresh material. On dried material Schaeffer's reaction was positive red at the stipe base, unreadable on the pileus surface. KOH reaction is hardly perceivable due to the dark ochraceous-yellow colour of the dried material.

**Habit, habitat and distribution** gregarious in small groups (only three basidomes found) in the second half of the summer, in an alpine mat of dwarf willows, *Salix retusa* L., on a substrate of extrusive rocks (Permian quartziferous porphyries). Known to date only from the Italian Alps (**Fig. 5**).

**Material examined:** ITALY, Trentino-Alto Adige, Moena (TR), loc. Forcella Grana, at approximately 2,300 m a.s.l., 46°21'23''N; 11°45'37''E, 18/08/2009, *leg*. E. Bizio, A. Aiardi, A. Castellan & C. Zoldan, MCVE 31981 (holotype); eb09081808 and LAPAG1395 (isotypes).

## TAXONOMY

### Comments on the phylogenetic analysis

Comparing ITS sequence of *A. permianus* with all the available ITS sequences of *A.* sect. *Minores*, it lacks any species-specific ITS markers. According to our phylogenetic analysis, *A. permianus* is closely related to *A. huijsmanii* with full support (100/1). However, without considering the heteromorphisms, their ITS sequences still differ at five positions.

### Morfological comments

*Agaricus permianus* is characterized by a pileus with vinaceous-purplish fibrils, a simple, very narrow annulus, which is fragile and evanescent and a white unchanging context, at times slightly yellowing only at the stipe base. The spores measure 4.8-$\underline{5.4}$-6.1 × 3.7-$\underline{3.9}$-4.2 µm and the cheilocystidia are well developed and abundant, measuring 6-52 × 5-45 µm.

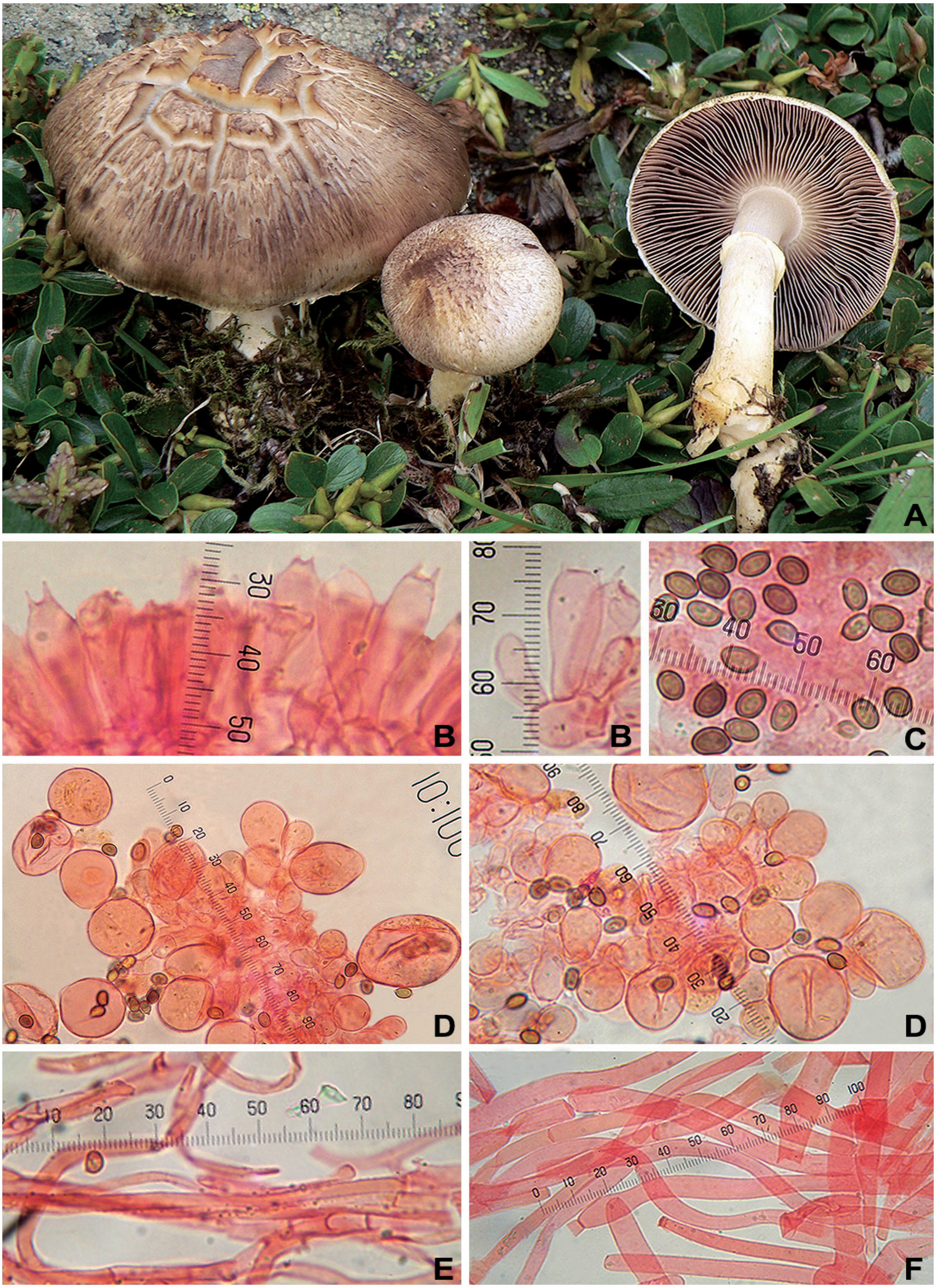

**Fig. 4**. *Agaricus permianus*. **A**. In habitat; **B**. Basidia; **C**. Spores; **D**. Cheilocystidia; **E**. Annulus hyphae; **F**. Pileipellis hyphae; **A**: MCVE 31981. **B-F**: LAPAG1395. Photos: **A**: Enrico Bizio. **B-F**: Luis A. Parra.

**Fig. 5**. Forcella Grana. Luogo di raccolta di *Agaricus permianus*. Foto di Enrico Bizio

The alpine collections of Kühner (1974) and Lucchini (1997) belonging to *A.* sect. *Minores* mentioned in the taxonomic comments of *A. manzollii*, differ in having cheilocystidia either much narrower or absent.

Phylogenetically the taxa most closely related are *A. velutinosus* T. Bau & S.E. Wang, *A. subiculosus* K.O. Mill., Angelini, L.A. Parra & Linda J. Chen and, especially, *A. huijsmanii* Courtec. *Agaricus velutinosus* differs in having much smaller basidiomes, a velutinous pileus and stipe surfaces and much narrower cheilocystidia (19-32 × 7-13 µm). *Agaricus subiculosus* is a tropical lignicolous species with a minute pileus (0.5-1.5 cm), growing from a subiculum attached to the substrate, has much smaller spores (3.9-4.7 × 2.8-3.3 µm) and basidiole-like cheilocystidia. *Agaricus huijsmanii* has a different habitat (*Fagus* sp.), a white pileus without any pink or purple tinges and both spores (on average 5 × 3.4 µm) and cheilocystidia (9-25 × 9-12 µm) are smaller.

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