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Agaricus subiculosus, a new species of the genus *Agaricus* sect. *Minores* from Puerto Rico (USA)

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Abstract: *Agaricus subiculosus*, a new species found in Puerto Rico is macro- and microscopically described and illustrated. Phylogenetic analyses were also performed to determine its taxonomical affinities. This species, belonging to *Agaricus* sect. *Minores*, is characterized by its lignicolous habitat growing from a broad white subiculum on degraded bamboo. Notes about its chorology and comparison with allied taxa are also provided.

INTRODUCTION

Puerto Rico, located east of the island of Hispaniola, is part of a group of Caribbean islands called the Greater Antilles. Of the 11 works published on the genus *Agaricus* L. in the Caribbean until the end of the last century (Fries 1851; Berkeley 1852; Berkeley & Curtis 1869; Murrill 1918; Ciferri 1929; Baker & Dale 1951; Heinemann 1961, 1962a, 1962b, 1962c; Pegler 1983), only Murrill (1918) recorded a species collected in Puerto Rico, *Agaricus johnstonii* Murrill, describing this new species from three collections made next to the “Piedras” river. However, Murrill's description does not contain any remarkable character, nor does it include any image to unequivocally interpret this species. For this reason, as Heinemann (1993) pointed out, the different descriptions of this species by different authors (Murrill 1918; Heinemann 1962a; Pegler 1983) do not match well. After Murrill, there is no mention of the genus in Puerto Rico for almost a century until Minter’s (2001) record of *A. endoxanthus* Berk. & Broome on 31 August 1997 in Luquillo locality. However, this specimen collected by D.J. Lodge and deposited in the CMFR herbarium with the number PR4634 (Beatriz-Ortiz, pers. comm.) once sequenced was identified as *A. lodgeae* L.A. Parra, Angelini & B. Ortiz (Parra *et al*. 2018), although later (31 August 2001) *A. endoxanthus* was collected by D.J. Lodge in the same locality (PR6320 deposited in CFMR) as recorded in Parra (2013).

The rest of the taxa that have been cited for Puerto Rico, *A. ciferrianus* L.A. Parra, B. Ortiz & Lodge, *A. lodgeae*, *A. microincrustans* L.A. Parra, B. Ortiz, Lodge & T.J. Baroni and *A. parvisporus* L.A. Parra & B. Ortiz were described in two recent monographs on the genus *Agaricus* in the Caribbean (Parra *et al*. 2018; Ortiz-Santana *et al*. 2021). Thus, before the present work, only six species have been cited for Puerto Rico, which reveals the limited knowledge we have of the diversity of the genus *Agaricus* on this island.

In an effort to contribute to the catalogue of species of the genus *Agaricus* in Puerto Rico we describe *A. subiculosus sp. nov.*, a species with very distinctive morphological and molecular characters that make its identification very simple even in the field.

MATERIALS AND METHODS

Studied collections

The description of this new species is based on the study of two collections collected by one of the authors (K.O.M.) in a copse of ornamental bamboo (*Bambusa vulgaris*) of an urbanization located at 55 m.a.s.l. near the western coast of Puerto Rico from July to September.

Studied basidiomata have been deposited at the University of Alcalá de Henares Herbarium (AH) with duplicates in the private herbarium of L. A. Parra (LAPAM). Herbarium acronyms are from Holmgren & Holmgren (1998).

Morphological characters

Macroscopic descriptions are based on color photographs and field notes from fresh material. Macrochemical reactions were determined from herbarium material. Microscopic descriptions are based on photos and data obtained after examining the basidiomata with a Nikon SE compound microscope. Spore measurements followed the statistical method proposed by Heinemann & Rammeloo (1985). The morphological description of the taxon follows the taxonomic treatments published by Parra (2008, 2013).

DNA extraction, PCR and DNA sequencing

Genomic DNA was isolated from dried specimens following a standard CTAB protocol (Palmer & al., 2008).The following primer pairs were used for PCR amplification and sequencing: ITS1F and ITS4 were used for the internal transcribed spacers 1 and 2 with the 5.8S rDNA (ITS) (Gardes & Bruns 1993; White *et al*. 1990); LR0R (Cubeta *et al*. 1991) and LR5 (Vilgalys & Hester 1990) for the D1–D3 domains of the nuc 28S rDNA (nrLSU) and EF1-983F and EF1-1567R (Rehner & Buckley 2005) for a segment of the translation elongation factor 1-α (*tef1-α*). Sequencing was performed on ABI3730XL Genetic analyzer (Applied Biosystems) at STAB VIDA (Portugal) for the material processed at ALVALAB (Spain).

Sequence alignment and phylogenetic analyses

A combined data set consisting of 99 samples was prepared for phylogenetic analysis. In addition to the six sequences generated from this study, 97 ITS sequences, 67 nrLSU sequences and 63 *tef1-α* sequences were retrieved from GenBank, and the samples' origin 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 generations. 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/).

RESULTS

Phylogenetic analyses

The final alignment consisted of 99 samples and 2042 characters including gaps, representing 95 species from *A.* subg. *Minores*, and *A. campestris* was used as the outgroup. The resulted trees from the ML and Bayesian analysis were very similar, except for few ungrouped samples, such as *A. callacii* (AH42929), *A. campbellensis* (GAL9420), *A. columellatus* (SB-2015), *A. pseudopurpurellus* (ZRL2014063), *A. purpureofibrillosus* (ZRL3080) and *A. wayanadensis* (TBGT18860). The ML tree is presented in Fig. 1, with BS values greater than 50%, and PP values greater than 0.8 are indicated on branches. *Agaricus* subg. *Minores* is monophyletic and comprises three sections: *A.* sect. *Minores*, *A*. sect. *Pantropicales* and *A*. sect. *Leucocarpi*. Our samples are placed in *A*. sect. *Minores* and formed a sister clade to *A. huijsmanii* (LAPAG639) with strong support (99/1).

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 accession number			Geographic origin	Reference
		ITS	LSU	*tef1-α*		
A. aridicola	LAPAG589	KT951331	KX084027	KX198081	Spain	Zhao *et al*. 2016
A. armandomyces T	ZRL2015992	KX684860	KX684882	KX684906	China	He *et al*. 2017
A. arrillagarum	LAPAG810	KF447900	KX083985	KT951592	Spain	Parra 2013
A. badioniveus T	LD2012131	KU975117	–	–	Thailand	Chen *et al*. 2017
A. blatteus T	ZRL2012004	KT951355	KT951457	KT951608	China	Zhao *et al*. 2016
A. bonisquamulosus T	ZRL2010106	KX657047	KX656950	KX684951	China	He *et al*. 2017
A. brunneolus	LAPAG938	KU975082	KX083997	KX198062	Spain	Chen *et al*. 2017
A. brunneolutosus T	MS514	KU975111	KX084006	–	China	Chen *et al*. 2017
A. callacii T	AH42929	KF447899	KX083984	KX198051	Spain	Parra 2013
A. campbellensis T	GAL9420	DQ232644	DQ232657	–	New Zealand	Geml *et al*. 2007
A. campestris T	LAPAG370	JQ903618	KP739803	KR006636	Spain	Kerrigan 2016
A. candidolutescens T	LD2012129	KT951335	KT951525	KT951616	Thailand	Zhao *et al*. 2016
A. catenatus T	ZRL2012104	KX657023	KX656963	KX684957	China	Chen *et al*. 2017
A. cerinipileus T	ZRL2012001	KX657021	KX656957	KX684953	China	Chen *et al*. 2017
A. cf. *kerriganii*	WC912	AY484681	–	–	USA	Geml *et al*. 2004
A. chartaceus T	H6271	JF495048	–	–	Australia	Lebel & Syme 2012
A. coccyginus	ZRL2012576	KT951372	KT951499	KT951596	China	Zhao *et al*. 2016
A. colpetei T	TL2424	JX984565	–	–	Australia	Lebel 2012
A. comtulus	LAPAG303	KU975078	KX083986	KX198052	Spain	Chen *et al*. 2017
A. columellatus	SB-2015	KJ912899	–	–	USA	Bates *et al*. 2016
A. dilatostipes	ZRL2014450	KX656999	KX656941	KX685003	China	He *et al*. 2017
A. dulcidulus	PRM909627	KF447894	–	KX198064	Czech Rep.	Parra 2013
A. edmondoi	LAPAG412	KT951326	KT951481	KT951590	Spain	Zhao *et al*. 2016
A. elongatestipes T	ZRL2013271	KX657002	KX656946	KX684975	China	Chen *et al*. 2017
A. fimbrimarginatus T	LD201250	KU975119	KX084017	KX198076	Thailand	Chen *et al*. 2017
A. flammicolor T	LD201502	KU975114	KX084009	KX198042	Thailand	Chen *et al*. 2017
A. flavoaurantiacus T	MFLU16-0980	KU975107	KX084002	KX198069	China	Chen *et al*. 2017
A. flavopileatus T	MS596	KU975121	KX084022	KX198078	China	Chen *et al*. 2017
A. friesianus	LAPAG592	KT951316	KX083992	KT951594	France	Zhao *et al*. 2016
A. gemlii T	AH44510	KF447891	KX083989	–	Spain	Parra 2013
A. gemloides T	ZRL2014084	KT633271	KX641405	KX684986	China	He & Zhao 2015
A. glabriusculus T	SWAT SH-7	MK751852	–	–	Pakistan	Hussain & Sher 2019
A. globosporus T	ZRL2012656	KX657039	–	KX684968	China	He *et al*. 2017
A. heinemannianus	LAPAG302	KF447906	–	KX198056	Spain	Parra 2013
A. huijsmanii	LAPAG639	KF447889	KT951444	KT951571	Spain	Chen *et al*. 2017
A. iesu-et-marthae	LAPAG41	KF447904	–	–	Spain	Parra 2013
A. indicus T	TBGT16128	OR661746	–	–	India	Arya & Pradeep 2024
A. jacobi	LAPAG52	KF447895	KX083996	KX198061	Spain	Parra 2013
A. jingningensis T	ZRL20151562	KX684877	KX684895	KX684917	China	He *et al*. 2017
A. kerriganii T	AH44509	KF447893	KX083999	KX198066	Spain	Parra 2013
A. laeticulus T	Goossens5272	KX671705	–	–	DR Congo	Chen *et al*. 2017
A. lamelliperditus	MDBF61/96	JX984559	–	–	Australia	Lebel 2012
A. latiumbonatus T	SWAT SH166	MK751861	MK751858	–	Pakistan	Hussain & Sher 2019
A. leucocarpus	LD201226	KU975102	KX083982	KX198049	Thailand	Chen *et al*. 2017
A. leucocarpus T	LD201215	KU975101	KX083981	KX198048	Thailand	Chen *et al*. 2017
A. luteofibrillosus	LD201501	KU975108	KX084003	KX198041	Thailand	Chen *et al*. 2017
A. luteomaculatus	CA331	KF447901	–	KX198053	France	Parra 2013
A. luteopallidus T	LD2012113	KU975124	KX084026	KX198080	Thailand	Chen *et al*. 2017

A. mangaoensis T	ZRL2010056	KX657042	KX656956	KX684946	China	He *et al*. 2017
A. marisae T	LAPAG138	KU975083	KX083998	KX198065	Spain	Zhao *et al*. 2011
A. matrum T	AH44506	KF447896	KX083991	KX198058	Spain	Parra 2013
A. megalosporus	ZRL2012199	KT951367	KT951470	KT951595	Thailand	Zhao *et al*. 2011
A. microviolaceus T	ZRL2012718	KX657033	KX656980	KX684971	China	He *et al*. 2017
A. midnapurensis	CUH AM718	OL467539	–	–	India	Tarafder *et al*. 2022
A. minipurpureus T	ZRL2010058	KX657043	KX656953	KX684947	China	He *et al*. 2017
A. neimengguensis T	ZRL20151845	KX684870	KX684902	KX684924	China	Chen *et al*. 2017
A. pallens	LAPAG441	KF447898	–	KX198067	Spain	Parra 2013
A. palodensis T	TBGT17483	OR661748	–	–	India	Arya & Pradeep 2024
A. parvibicolor T	LD2012116	KP715162	KX084016	KX198075	Thailand	Liu *et al*. 2015
A. parvibrunneus T	ZRL20161053	MG137001	MG196345	MG196351	China	He *et al*. 2018
A. patris T	LD201224	KU975118	KX084012	KX198073	Thailand	Chen *et al*. 2017
A. pseudolutosus	LAPAG454	KT951329	KT951453	KT951602	Spain	Parra 2013
A. pseudopallens T	ZRL20151552	KX684874	KX684891	–	China	Chen *et al*. 2017
A. pseudopurpurellus	ZRL2014063	KX656988	KX641404	KX684985	China	Zhao *et al*. 2016
A. purpurellus	LAPAG944	KU975076	KX083994	KX198060	Czech Rep.	Chen *et al*. 2017
A. purpureofibrillosus T	ZRL3080	JF691542	KX084021	–	Thailand	Zhao *et al*. 2011
A. purpureosquameus T	LE2016047	MF197451	–	–	Thailand	Hyde *et al*. 2017
A. purpureosquamulosus	CUH AM716	OL467541	–	–	India	Tarafder *et al*. 2022
A. robustulus T	CA847	KU975086	KX084034	KX198039	Thailand	Chen *et al*. 2017
A. rufifibrillosus	ZRL20151536	KX684878	KX684893	KX684915	China	He *et al*. 2017
A. rufipileus T	ZRL2014140	KX656991	KX656937	KX684991	China	He *et al*. 2017
A. sodalis T	LD2012159	KP715161	KX084014	KX198074	Thailand	Chen *et al*. 2017
A. sp.	Vellinga2360	AF482831	AF482877	–	USA	Vellinga *et al*. 2003
A. sp.	ZRLLD013	KT951384	KT951516	KT951604	Thailand	Zhao *et al*. 2016
A. sp.	PS036	KU975087	KX084035	KX198036	Thailand	Chen *et al*. 2017
A. sp.	ZRL3056	JF691541	KX084020	–	Thailand	Zhao *et al*. 2011
A. sp.	PYP014	KU975091	–	–	Thailand	Chen *et al*. 2017
A. sp.	TL2307	JF495058	–	–	Australia	Lebel & Syme 2012
A. sp.	ZRL20151437	KX684876	KX684892	KX684914	China	He *et al*. 2017
A. sp.	NTT72	JF514539	–	–	Thailand	Zhao *et al*. 2011
A. sp.	CA935	KU975085	KX084036	KX198034	Thailand	Chen *et al*. 2017
A. sp.	MS386	KU975113	KX084008	KX198044	China	Chen *et al*. 2017
A. sp.	ZRLWXH3064	KX657010	–	–	China	He *et al*. 2017
A. sp.	ZRLWXH3067	KT951387	KT951497	KT951611	China	Zhao *et al*. 2016
A. sp.	GAL3083	EF460374	EF460399	–	USA	Geml *et al*. 2008
A. sp.	ZD1528	KU975104	KX083987	KX198054	China	Chen *et al*. 2017
A. sp.	LD201252	KU975103	–	KX198050	Thailand	Chen *et al*. 2017
A. sp.	MATA774	JF727871	–	–	Mexico	Zhao *et al*. 2011
A. sp.	CA845	KU975084	KX084033	KX198035	Thailand	Chen *et al*. 2017
A. sp.	LAPAM14	KT951312	–	KT951613	Dominican Rep.	Zhao *et al*. 2016
A. sp.	ZRLWXH3161	KT951391	KT951526	KT951615	China	Zhao *et al*. 2016
A. sp.	LAH35900	MK659941	–	–	Pakistan	Unpublished
A. stevensii	FS 06-02-09	KJ877785	–	–	USA	Kerrigan 2016
***A. subiculosus* T**	**AH56332**	**PP313292**	**PP291842**	**PP317134**	Puerto Rico	This study
A. subiculosus	**AH56334**	**PP313293**	**–**	**PP317135**	Puerto Rico	This study
A. viridopurpurascens	Horak68/79	JF514525	–	–	New Zealand	Zhao *et al*. 2011
A. wariatodes	TWM1589	JF495052	JF495030	–	Australia	Lebel & Syme 2012
A. wayanadensis T	TBGT18860	OR661750	–	–	India	Arya & Pradeep 2024
A. yanzhiensis T	ZRL20162082	MG137003	MG196346	–	China	He *et al*. 2018

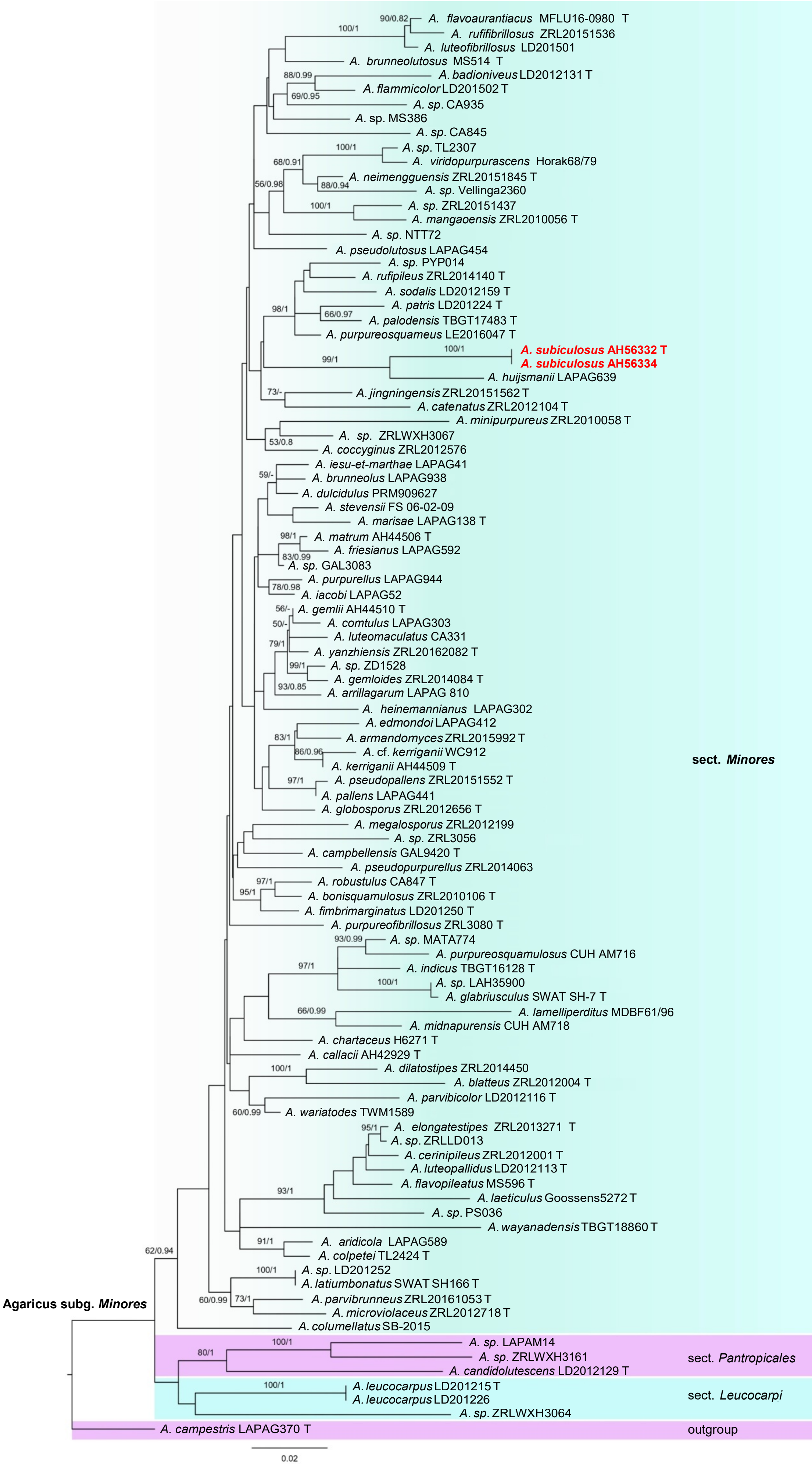
A. flavoaurantiacus MFLU16-0980 T
A. rufifibrillosus ZRL20151536
A. luteofibrillosus LD201501
A. brunneolutosus MS514 T
A. badioniveus LD2012131 T
A. flammicolor LD201502 T
A. sp. CA935
A. sp. MS386
A. sp. CA845
A. sp. TL2307
A. viridopurpurascens Horak68/79
A. neimengguensis ZRL20151845 T
A. sp. Vellinga2360
A. sp. ZRL20151437
A. mangaoensis ZRL2010056 T
A. sp. NTT72
A. pseudolutosus LAPAG454
A. sp. PYP014
A. rufipileus ZRL2014140 T
A. sodalis LD2012159 T
A. patris LD201224 T
A. palodensis TBGT17483 T
A. purpureosquameus LE2016047 T
A. subiculosus AH56332 T
A. subiculosus AH56334
A. huijsmanii LAPAG639
A. jingningensis ZRL20151562 T
A. catenatus ZRL2012104 T
A. minipurpureus ZRL2010058 T
A. sp. ZRLWXH3067
A. coccyginus ZRL2012576
A. iesu-et-marthae LAPAG41
A. brunneolus LAPAG938
A. dulcidulus PRM909627
A. stevensii FS 06-02-09
A. marisae LAPAG138 T
A. matrum AH44506 T
A. friesianus LAPAG592
A. sp. GAL3083
A. purpurellus LAPAG944
A. iacobi LAPAG52
A. gemlii AH44510 T
A. comtulus LAPAG303
A. luteomaculatus CA331
A. yanzhiensis ZRL20162082 T
A. sp. ZD1528
A. gemloides ZRL2014084 T
A. arrillagarum LAPAG 810
A. heinemannianus LAPAG302
A. edmondoi LAPAG412
A. armandomyces ZRL2015992 T
A. cf. kerriganii WC912
A. kerriganii AH44509 T
A. pseudopallens ZRL20151552 T
A. pallens LAPAG441
A. globosporus ZRL2012656 T
A. megalosporus ZRL2012199
A. sp. ZRL3056
A. campbellensis GAL9420 T
A. pseudopurpurellus ZRL2014063
A. robustulus CA847 T
A. bonisquamulosus ZRL2010106 T
A. fimbrimarginatus LD201250 T
A. purpureofibrillosus ZRL3080 T
A. sp. MATA774
A. purpureosquamulosus CUH AM716
A. indicus TBGT16128 T
A. sp. LAH35900
A. glabriusculus SWAT SH-7 T
A. lamelliperditus MDBF61/96
A. midnapurensis CUH AM718
A. chartaceus H6271 T
A. callacii AH42929 T
A. dilatostipes ZRL2014450
A. blatteus ZRL2012004 T
A. parvibicolor LD2012116 T
A. wariatodes TWM1589
A. elongatestipes ZRL2013271 T
A. sp. ZRLLD013
A. cerinipileus ZRL2012001 T
A. luteopallidus LD2012113 T
A. flavopileatus MS596 T
A. laeticulus Goossens5272 T
A. sp. PS036
A. wayanadensis TBGT18860 T
A. aridicola LAPAG589
A. colpetei TL2424 T
A. sp. LD201252
A. latiumbonatus SWAT SH166 T
A. parvibrunneus ZRL20161053 T
A. microviolaceus ZRL2012718 T
A. columellatus SB-2015
A. sp. LAPAM14
A. sp. ZRLWXH3161
A. candidolutescens LD2012129 T
A. leucocarpus LD201215 T
A. leucocarpus LD201226
A. sp. ZRLWXH3064
A. campestris LAPAG370 T
sect. Minores
sect. Pantropicales
sect. Leucocarpi
outgroup
Agaricus subg. Minores
0.02

Fig. 1: Maximum likelihood phylogram of *Agaricus* subg. *Minores* resulting from analysis of ITS, LSU and *tef1-α* 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

TAXONOMY

Agaricus subiculosus Miller, Angelini, L.A. Parra & Linda J. Chen *sp. nov.*
MycoBank: MB 852410

Etymology. Referring to the basidiomata emerging from a *subiculum* covering the substrate

Macroscopic description (Fig. 2)

Pileus 5 – 15 mm diam., at first conical-truncate or convex, then broadly conical, sometimes slightly umbonate, finally almost plane, usually uniformly of a bright and dark purple-pink color, excepting a very narrow white zone on the margin, sometimes or with age becomes darker at the centre and paler towards the margin. Surface radially fibrillose, smooth, dull, strongly flavescent on bruising or with age, covered by scattered white arachnoid remnants of the universal veil. Margin exceeding the lamellae, appendiculate by denticulate white woolly remnants of the partial veil.
Lamellae free, intercalated with lamellulae, not crowded, up to 2.5 mm broad, with finely eroded paler edge under a magnifying glass, at first persistently white, then pinkish-cream, finally blackish-brown.
Stipe 15 – 20 × 1.5 – 2 mm, cylindrical, curved towards the usually bulbous (3 – 4 mm) base, fistulous, provided with an annulus in its upper third, at first entirely white and fibrillose-woolly, then discoloring ochre yellow from the base towards the apex and with scattered fibrils, remaining fibrillose-woolly at the base, sometimes as a peronate sheath, turning yellow when rubbed. Annulus simple, fibrillose-woolly then fibrillose, fragile, evanescent, sometimes completely attached to the margin of the pileus as appendiculate remnants and absent in the stipe.
Subiculum white, covering the surface of the wood (apparently without penetrating it), sometimes exuding amber-coloured drops, crossed by evident mycelial threads from which the primordia emerge.
Context first white becoming yellow to ochre-yellow on cutting. Odour of almonds.

Microscopic description (Fig. 3)

Spores 3.9 – 4.7 × 2.8 – 3.3 µm, on average 4.3 × 3.1 µm, Q = 1.26 – 1.53, on average 1.40, broadly ellipsoidal to ellipsoid, smooth, light brown, mostly uniguttulate, without apical pore.
Basidia 12 – 16 × 6 – 7 µm, tetrasporic, hyaline, clavate or slightly truncated at the apex, sterigmata up to 3 µm long.
Cheilocystidia hyaline, in clusters composed by few elements, only slightly larger than the basidioles, simple, usually claviform (14 –) 17 – 25 × 7 – 9 µm.
Pleurocystidia absent.
Annulus not observable in the dried specimens.
Pileipellis a cutis composed by cylindrical hyphae, 4 – 13 µm diam., the wider the more constricted at septa. The outermost hyphae with a pale reddish-brown diffuse or granular intracellular pigment. Terminal elements abundant with rounded tips.
Subiculum and mycelial threads consisting of hyaline cylindrical hyphae, 1.5 – 4 µm wide, not or slightly constricted at the septa.
Clamp-connections absent in all structures.

Macrochemical reactions

In dried basidiomata the pileus surface is almost black. Therefore, the reagents were applied on the white subiculum and stipe. Schäffer's reaction positive, reddish orange on the subiculum and the stipe. 30% KOH reaction positive, pale yellow, difficult to read on subiculum, yellow-orange on the stipe.

Habit, habitat and distribution: Gregarious in more or less dense groups, lignicolous, growing on dead and partially degraded stems or fallen culms of ornamental bamboo (*Bambusa vulgaris*) in the humid, lowland hills region in very rainy weather. Rare, to date only two collections made from the same place in Puerto Rico.

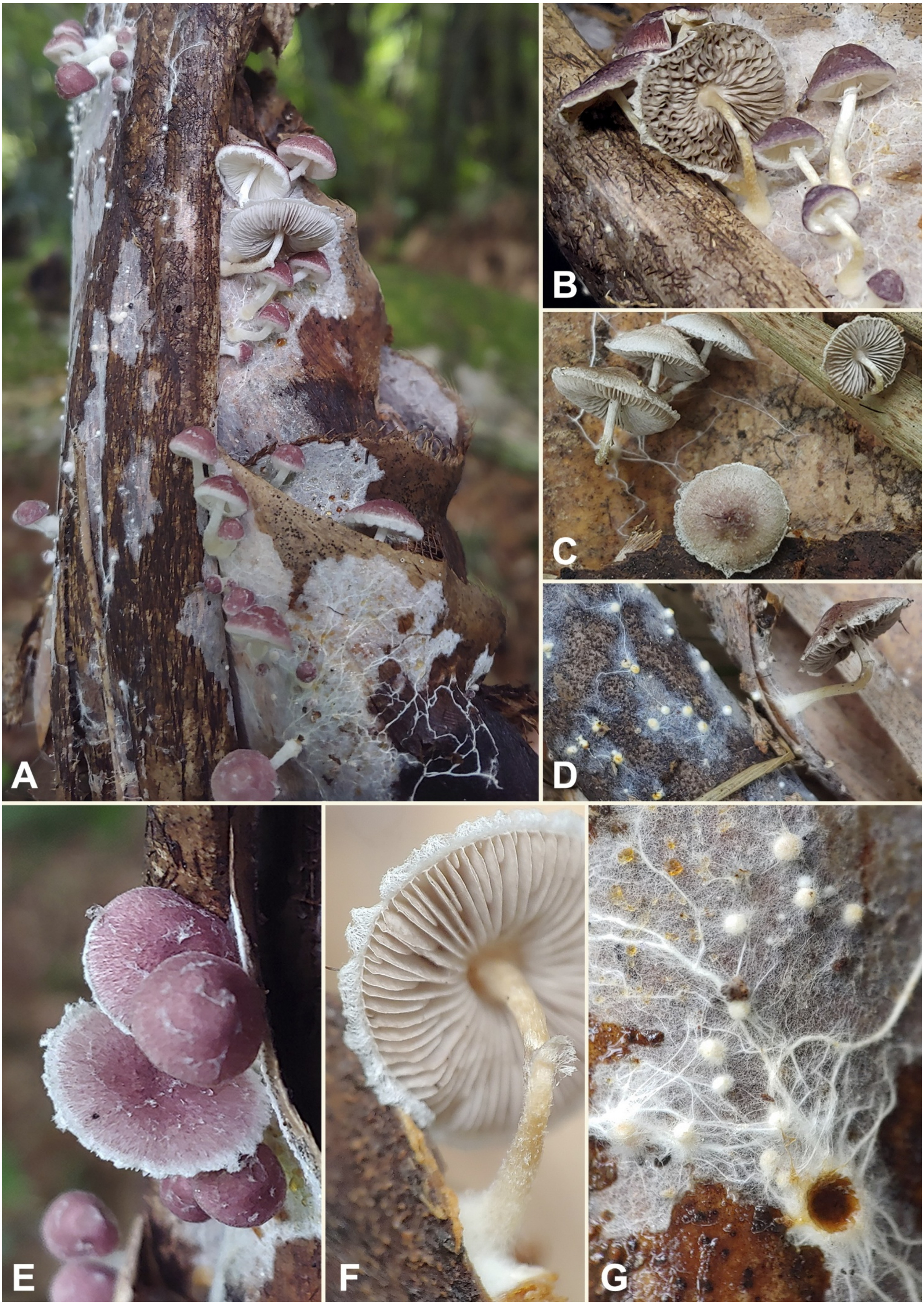

Fig. 2: Macroscopical characters. A-D: General aspect in the field; E: Detail of the pileus surface with velar remnants; F: Detail of the appendiculate margin of the pileus and the annulus; G: Detail of the subiculum. A-B, E: AH56332; C-D, F-G: AH56334. Photographs: Kurt O. Miller

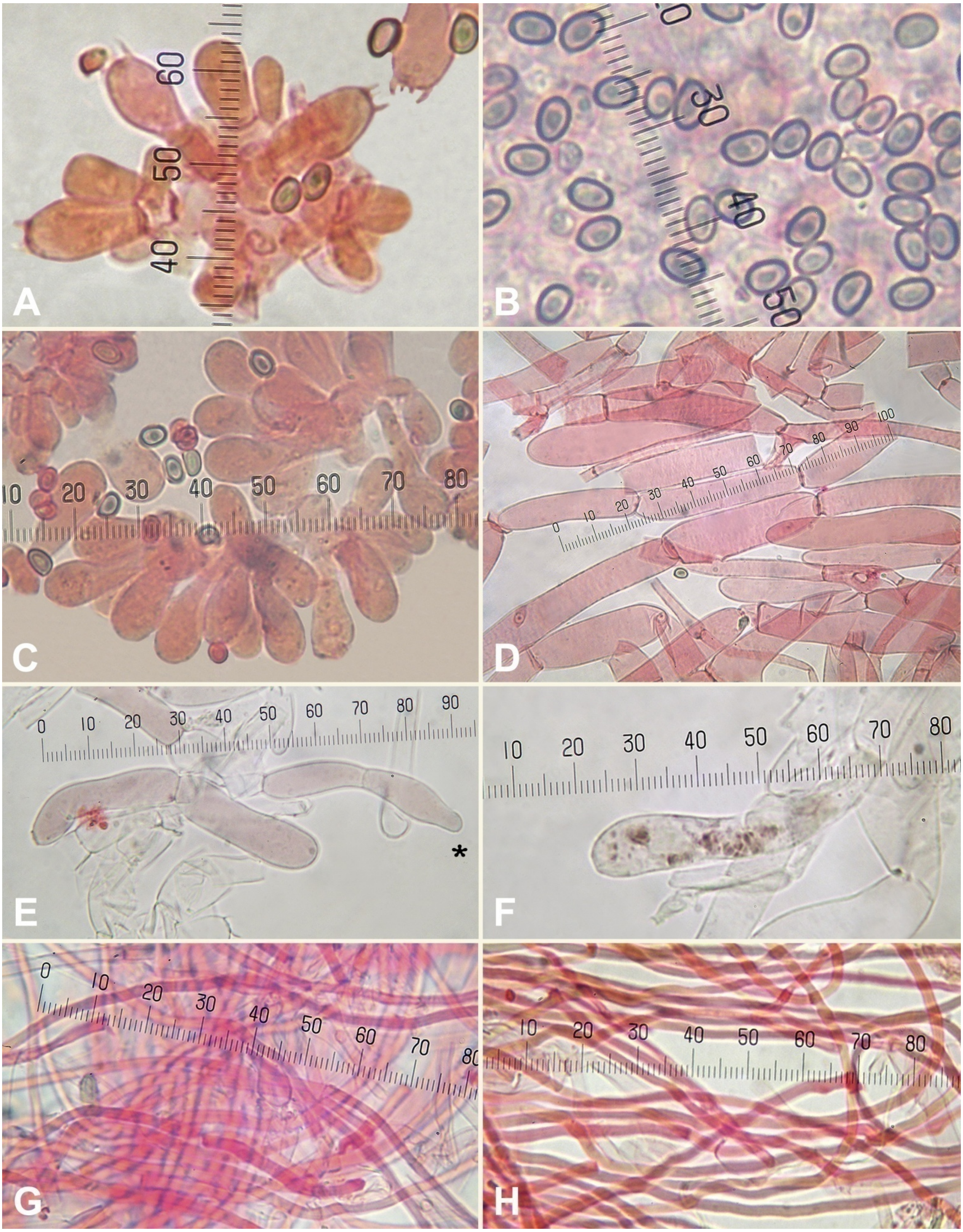

Fig. 3: Microscopical characters. A: Basidia; B: Spores; C: Cheilocystidia; D-F: Pileipellis; E: Some terminal and anteterminal elements with diffuse intracellular pigment. A terminal element is marked with an asterisk; F: Some elements with granular intracellular pigment. G: Subiculum; H: Mycelial threads. A-D and G-H mounted in Congo red, E-F mounted in water. All from AH56332. Photographs: Luis A. Parra.

Typus. USA: Puerto Rico, Mayagüez municipality, Miradero, Urbanización Bellas Lomas, 18°13’28.9’’N; 67°07’58.4’’W, 55 m.a.s.l., on degraded bamboo (*Bambusa vulgaris*) stump in a garden, 21.9.2021, *leg*. *Kurt O. Miller* (Holotype: AH56332).
Additional material examined. USA: Puerto Rico, Miradero, Mayagüez municipality, Urbanización Bellas Lomas, 18°13’28.9’’N; 67°07’58.4’’W, 55 m.a.s.l., on degraded bamboo stump and fallen culms in a garden, 28.8.2022, *leg*. *Kurt O. Miller*; AH56334 (duplicate LAPAM197).

DISCUSSION

Agaricus subiculosus belongs to *A*. sect. *Minores*. Due to the extremely small size, the abundant white cottony velar remnants on the pileus margin, the peculiar growth emerging from a *subiculum* adhering to the wood, and the very small spores, no described species can be confused with this species. Molecularly it is also easy to identify by its species-specific ITS markers tcaga[TC]cg-tt@220-221, and tctctCc-ttt@700 among all the available ITS sequences of *A*. sect. *Minores*. Phylogenetically, *A. subiculosus* is most related to *A. huijsmanii*, however, their ITS and *tef1-α* sequences still differ at more than 20 positions.

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