

Supplementary Information for the article

Charting Taxa in *Amanita* Section *Amidella* (*Basidiomycota* : *Amanitaceae*)

Submitted to Mycological Progress

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Online Resource 1 – *Amidella* sequence database accessions (UDB refers to the UNITE database, otherwise except for one iNaturalist record, refer to GenBank; last search on December 2, 2024)

Subsection	Stirp	Epithet	ITS	LSU	RPB2	TEF1	BTUB	etc.
Volvatae	Volvata	<i>clarisquamosa</i>		AF024448				
		<i>aff. curtipes</i> 1	KM052544	KU139473				
		<i>pseudovalens</i>	KJ950803 KJ950804	KJ950782 KJ950783				
			MW431348 MW431350	MW431338				
			MW431352 MW431354	MW431339				
				MW431340				
				MW431341				
				MW431342				
				MW431343				
				MW431344				
		<i>curtipes</i>	AY486235 AY486236	EF653960 EF653961				
			EF653963 KJ950805	KJ950784 MW494958				
			MW494955 MW538529	MW494959				
			PP680674					
		<i>sp. QU06</i>	OQ946582					
		<i>sp. whartonensis</i>	ON129192	MK277570 OQ754989				
		<i>volvata</i>	MN722424 MT241886	AF024485 OQ311333				
			MZ667952 MZ668151					
	OM809189 OP104057							
	OP104077/OP681759							
	OP541683 OP681749							
	OQ311333							
Peckiana	<i>brunneomaculata</i>	MH508278 MH508279	MH486410 MH486411	MH485892	MH508698	MH485428		
		MH508280	MH486412 MH486413	MH485893	MH508699	MH485429		
				MH485894	MH508700			
				MH485895	MH508701			

Subsection	Stirp	Epithet	ITS	LSU	RPB2	TEF1	BTUB	etc.
(Volvatae)	(Peckiana)	<i>lanigera</i>	MH508416 MH508417 MH508418 MH508419 MH508420 MH508421 ON794269 PP768065	MH486617 MH486618 MH486619 MH486620 MH486621 MH486622	MH486070 MH486071 MH486072 MH486073 MH486074 MH486075	MH508876 MH508877 MH508878 MH508879 MH508880	MH485590 MH485591	
		<i>peckiana</i>	MH281877 MK966422 OK316971 ON080993 OQ286517 OR026673 OR297618 PP436658	HQ539720 MH620300 OQ286517 OR026673				
		Fulvisquamea	sp. 'Sarawak1'	KY090936 KY090973				
		<i>fulvisquamea</i>	HE814144 LC364238 OQ780689 OQ780690 OQ780691 OW732041 UDB01048294	OQ780671 OQ780672 OQ780673	OQ740051 OQ740052 OQ740053	OQ740069 OQ740070 OQ740071	OQ740087	
Sagittariae		aff. clarisquamosa2	MK388161					
		aff. clarisquamosa1	FJ375331					
		sp. OMS-013	LC667043					
		sp. OMS-017	LC667047					
		sp. montelongense	PP680673					
		aff. vaginata	OR978098					
		sp. sagittaria	MT073012	MT073023				
		sp. 17 GMB-2014	KP012973	KP012973				
Lepiotidae	Lepiotoides	<i>rufobrunnescens</i>	KT865208 KT865209 ON971238 ON971239	KT865210 KT865211 OP363814 OP363815				
		<i>parvirufobrunnescens</i>		MW365377				
		sp. JPN01/sp. OMS-015	LC667045 MN794888/KT779088 MZ420670	KT779075 MZ420670				
		<i>lepiotoides</i> f. <i>subcylindrospora</i>	MN497357 MW431346 MW431347	MN497358 MW431336 MW431337				
		sp. QU05	OQ946543	OQ946543				

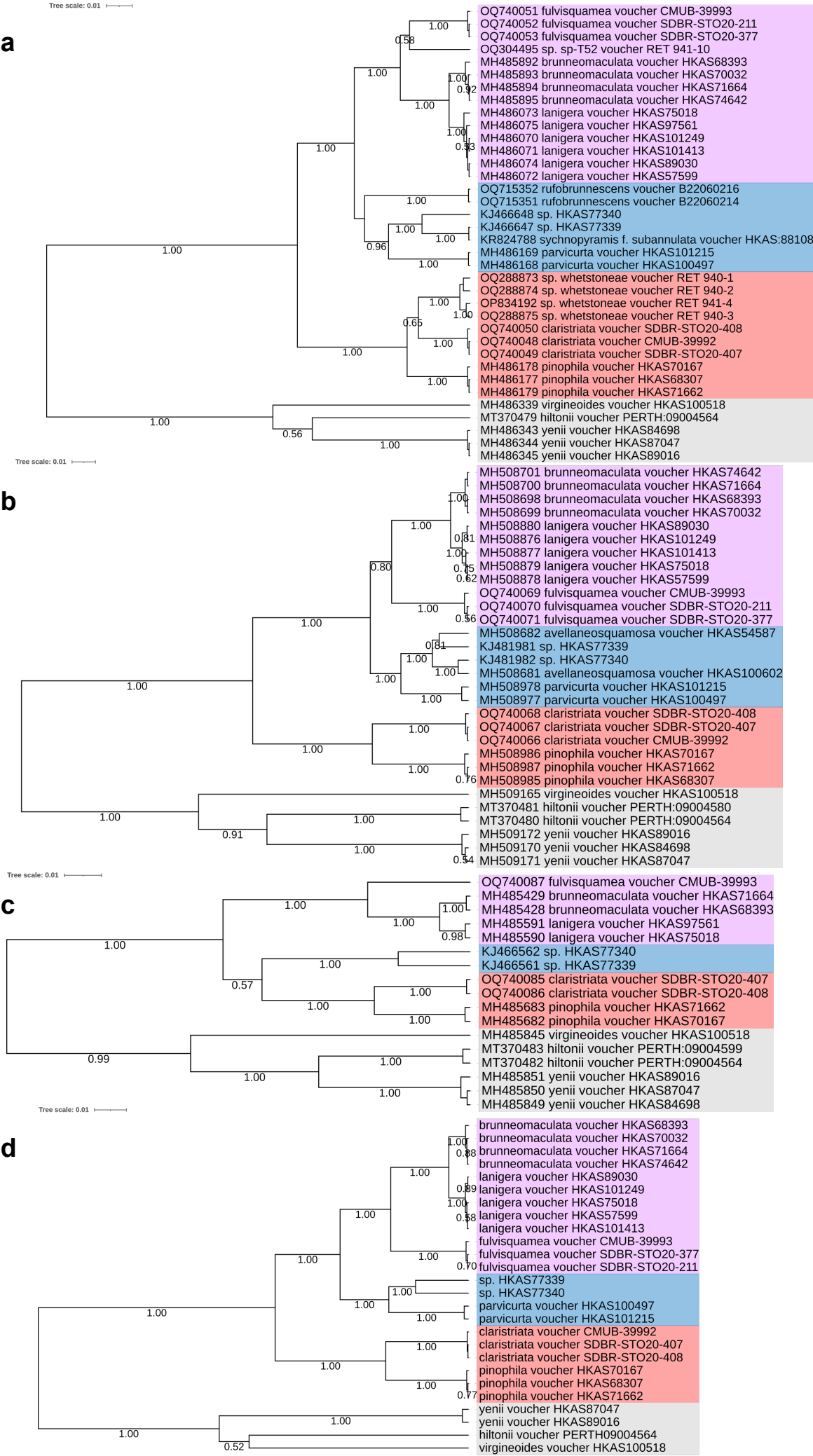
Subsection	Stirp	Epithet	ITS	LSU	RPB2	TEF1	BTUB	etc.
(Lepiotidae)	(Lepiotoides)	sp. IN25	OM809223					
		sp. IN33	MZ668207					
		sp. insolens	ON129236 iNAT:178300376	OQ755010				
		sp. texidella		MT073025				
		sp. canadensis01/sp. IN28	MZ668090 OM972289	OR039770				
			OR039770					
		sp. 19 GMB-2014	KP013009					
		Avellaneosquamosa	aff. avellaneosquamosa2	MH508258	MH486379	MH485873	MH508681	
			<i>parvicurta</i>	MH508489 MH508490	MH486744 MH486745	MH486168		
						MH486169		
		<i>cf. avellaneosquamosa1</i>	MH508259	AF024441 MH486380		MH508682		
		aff. avellaneosquamosa3/ OMS-012	KJ466418 LC667042	KJ466483	KJ466648	KJ481982	KJ466562	
		aff. avellaneosquamosa4/ aff. volvata3	AB509467 JF723273 KJ466417 KM052554	KJ466482	KJ466647 KR824788	KJ481981	KJ466561	
		aff. volvata4	KM052540					
		aff. volvata5	KF245921 KM052547	KF245905				
		aff. avellaneosquamosa5	MH508260					
		sp. IN31	MZ262330 MZ668196 MZ668215 OR978102 PP436548					
		sp. S17/sp. IN41	MK580700 MT241889 MT345230 MW464411 MZ668076 MZ668129 MZ668205 OM809210 OM972560 OP104059/OP681731 OP643234 OP681770 OP749011 OQ389389 OR800094 PP573989	HQ539665 OQ754986 OQ755005				JF707921 HQ539773 HQ539876 HQ539980
		aff. rosea		OQ628446				
		sp. 12 GMB-2014	KP012768					
		Subviscosa	aff. subviscosa	MZ027793				

Subsection	Stirp	Epithet	ITS	LSU	RPB2	TEF1	BTUB	etc.
(Lepiotidae)	(Subviscosa)	<i>subviscosa</i> s.l. (Benin)	HG995511 MW829797 MZ027794 MZ027795 MZ027796 MZ027797 MZ027798 MZ027799 MZ027800 MZ027801 UDB0800069	HG995511 PQ528016 PQ528078 PQ528079				
		<i>subviscosa</i> s.l. (Zambia)	FR731572					
		<i>subviscosa</i> s.l. (Zimbabwe)	UDB0800069 UDB0800100					
		aff. <i>goossensiae</i> KM 12		MT446289				
		sp. TANZ01		MZ358873				
		sp. soil subtropical China 32	OW732032					
		sp. soil subtropical China 33	OW732033					
		sp. clone d28 3Ama	JQ347157					
		sp. 'Sarawak2'		KY090972				
		sp. Thai04	MZ420676	MZ420676				
		sp. TUF116503	UDB025267					
		sp. clone Ama3	AB854647					
		sp. 'Jalisco'	MH160011					
	Pseudomutabilis	sp. 'Lupane'	UDB0800134					
		<i>pseudorufobrunnescens</i>		MK116903 MK120005				
		aff. <i>volvata</i> 2	AB509773					
		sp. 'California'	OR767846 PP407486					
		aff. <i>mutabilis</i>	MH212007	MH620228 MH620308 MK277550				
		aff. <i>volvata</i> 1		AF024487				

Subsection	Stirp	Epithet	ITS	LSU	RPB2	TEF1	BTUB	etc.
Ponderosae	Ponderosa	<i>ponderosa</i>	AY486233 AY486234 EF653962 KJ950785 KJ950786 KJ950787 KJ950788 KJ950789 KJ950790 KJ950791 KJ950792 KJ950793 KJ950794 KJ950795 KJ950796 KJ950797 KJ950798 KJ950799 KJ950800 KJ950801 KJ950802 MW431345 OQ120624 UDB0787041	EF653957 EF653958 EF653959 KJ950779 KJ950780 KJ950781 MW431335				
		sp. whetstoneae	KC841903 KC841904 KC841905 KC841906 KC841907 KC855221 KP284288 KU186814 KU186815 KU186816 KU186817 KU186818 KU186819 KX018800 KX061517 KX061518 KX061519 MK580730 MK580806 MZ267772 MZ411567 MZ668198 MZ668202 MZ668211 ON209212 OP001377 OP035793 OP101157 OP101161 OP749303 OR824528 PP156349 PP516769 PP516840	AF042608 KP284289 KU186811 KU186812 KU186813 KX018806 KX018807 KX018808 KX061530 KX061531 KX061532 KX061533 MK277595 MZ411549 OP001370 OP001371 OP001372 OP001373 OP001374 OP001375 OP035793 OQ754985 OQ755004 OQ755014				
	Claristriata	aff. avellaneosquamosa6	AY436447					
		aff. volvata6/sp. OMS-010	AB015681 KF245922 KF245923 LC667040 OR852537	KF245906 KF245907				

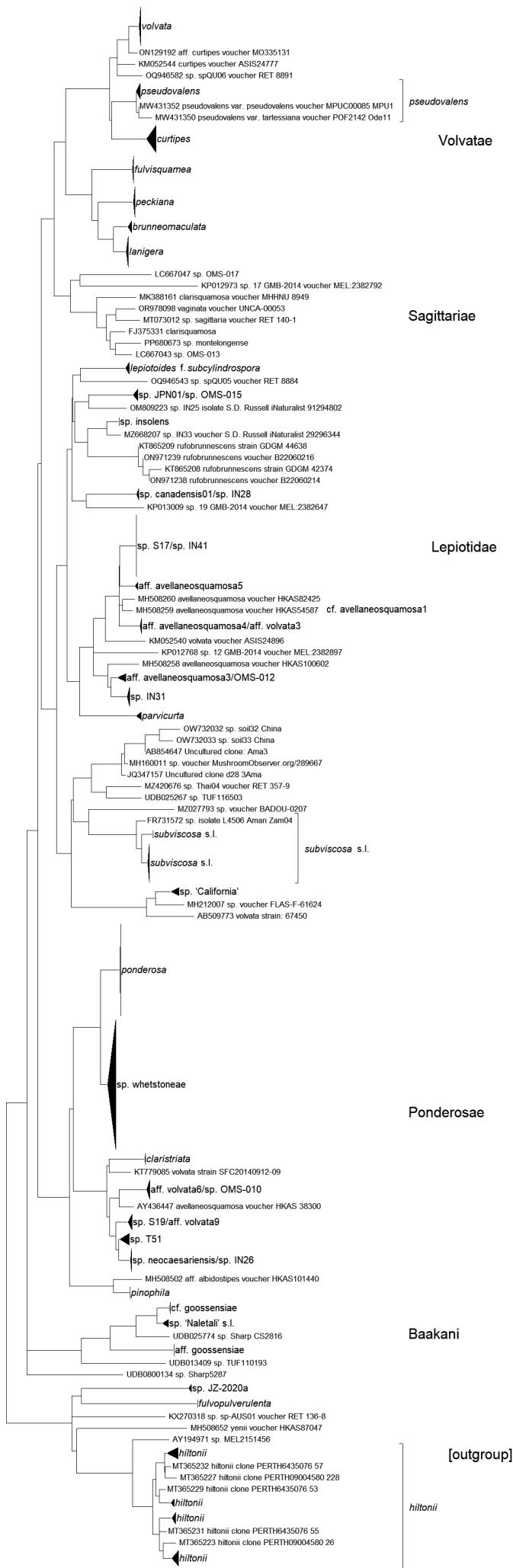
Subsection	Stirp	Epithet	ITS	LSU	RPB2	TEF1	BTUB	etc.
(Ponderosae)	(Claristriata)	aff. volvata7	KT779085	KT779072				
		aff. volvata8		KU139472				
		<i>claristriata</i>	OQ780686 OQ780687 OQ780688	OQ780668 OQ780669 OQ780670	OQ740048 OQ740049	OQ740066 OQ740067	OQ740085 OQ740086	
					OQ740050	OQ740068		
		sp. neocaesariensis/ sp. IN26	MT073016 MZ242065 MZ668089 MZ668091 OM972273 OR297617	MT073024				
		sp. S19/aff. volvata9	FJ596795 FJ596796 MF161251 MZ363616	AF097387 AF097388 MK277571 MZ363616 OQ754990 OQ755007 OR344379				
		sp. T51	MK580770 MZ265216 OP541661	MZ265216				
	Pinophila	aff. albidostipes	MH508502					
		<i>pinophila</i>	MH508503 MH508504 MH508505	MH486758 MH486759 MH486760				
Baakani		aff. goossensiae	MZ345339 MZ345340 MZ345341					
		cf. goossensiae	MZ345342 MZ345343 MZ345344					
		sp. 'Madagascar'	UDB013409					
		sp. Arora-01-560		HQ539698				HQ539802 HQ539909 HQ540012
		sp. Sharp2816	UDB025774					
		sp. 'Naletali' s.l.	UDB0800029 UDB01047904					

Online Resource 2

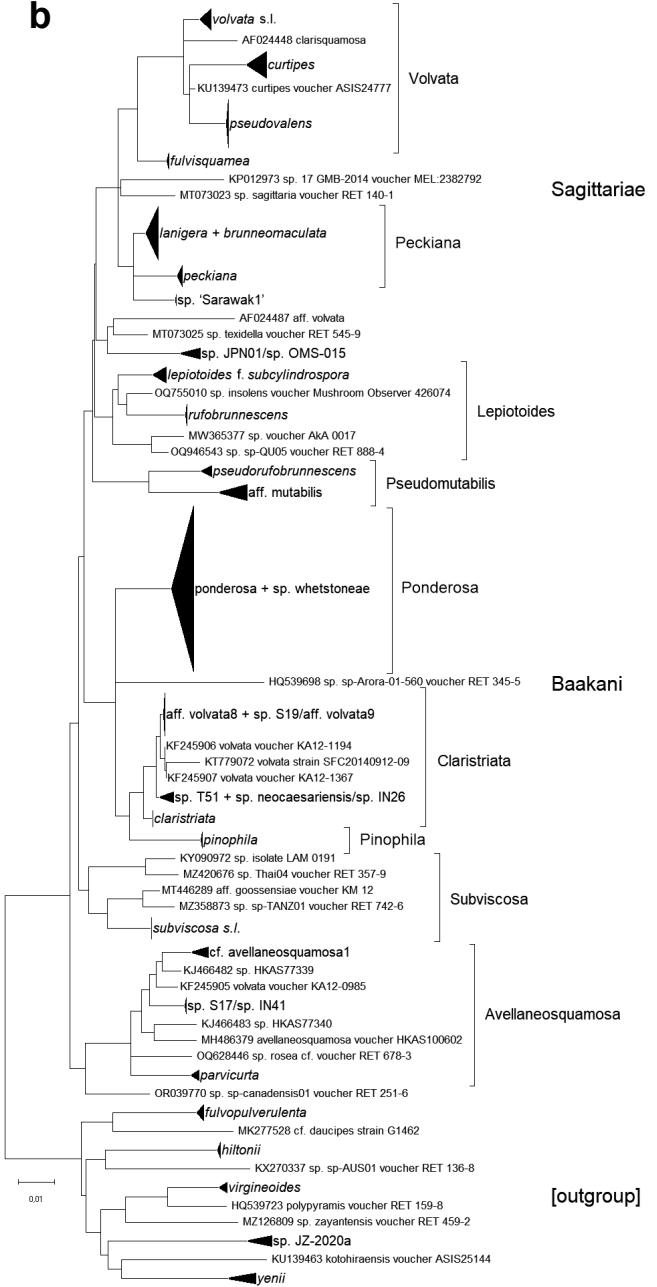


Online Resource 3

a



b



Online Resource 4

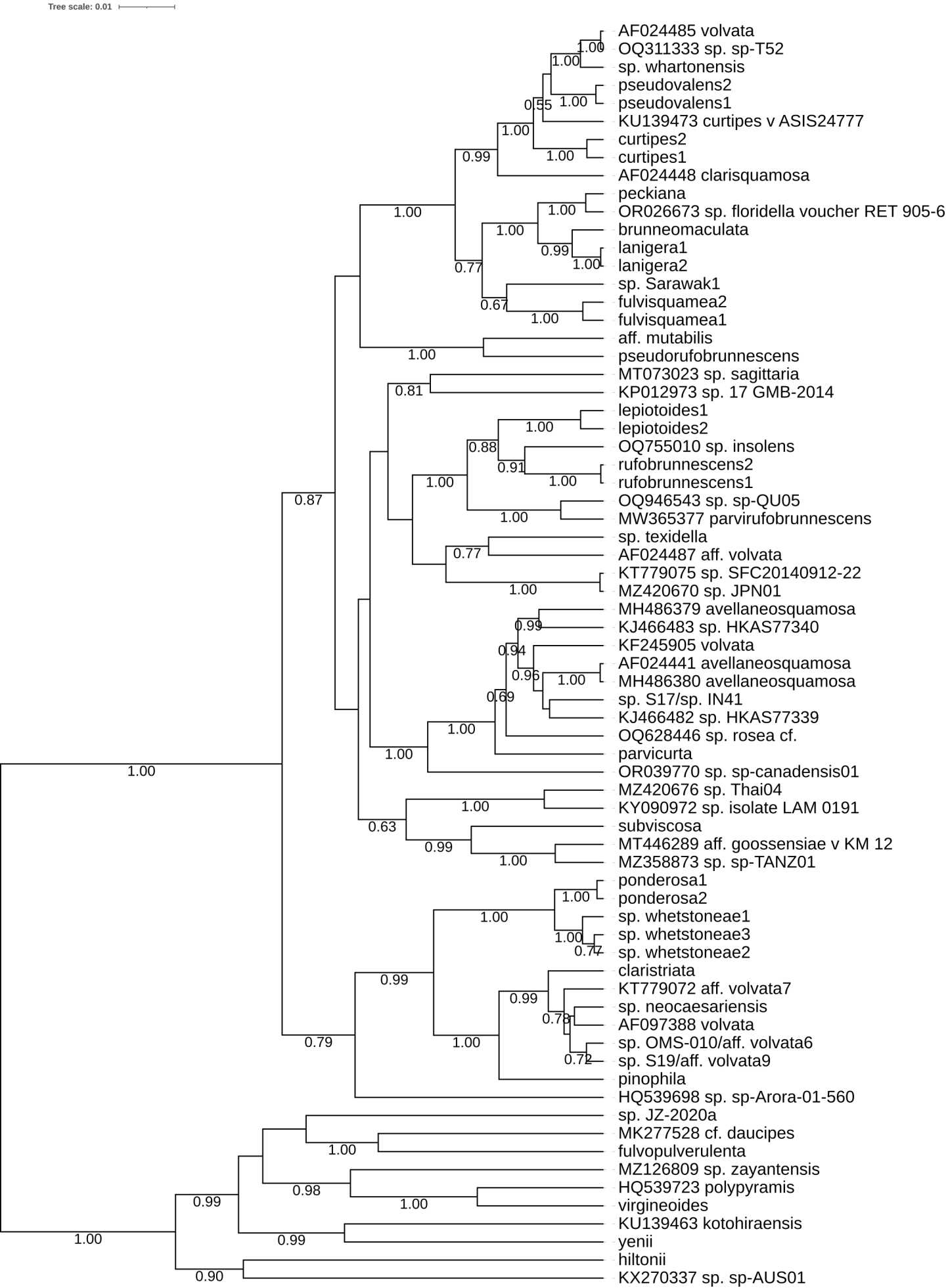
a

Tree scale: 0.01



Online Resource 4

b



Online Resource 5 – Biogeography of the *Amidella* sequences, regions according to Loidi & Vynokurov (2024).

Continent	Biogeographical regions (Kingdom)	Countries	Species clade	Subsection
Africa ^a	Guinean-Congolese (Paleotropical)	Cameroon	aff. <i>goossensiae</i>	Baakani
			cf. <i>goossensiae</i>	Baakani
			aff. <i>goossensiae</i> KM 12	Lepiotidae
	Malagasy (Paleotropical)	Madagascar	sp. 'Madagascar'	Baakani
	Sudano-Zambezian (Paleotropical)	Benin	<i>subviscosa</i> s.l.	Lepiotidae
			aff. <i>subviscosa</i>	Lepiotidae
		Tanzania	sp. TANZ01	Lepiotidae
		Zambia	<i>subviscosa</i> s.l.	Lepiotidae
		Zimbabwe	sp. Arora-01-560	Baakani
			sp. 'Lupane'	Lepiotidae
			<i>subviscosa</i> s.l.	Lepiotidae
			sp. <i>naletali</i> s.l.	Baakani
			sp. 'Sharp2816'	Baakani
Asia	Chinese-Japanese (Holarctic)	South Korea	aff. <i>curtipes</i> 1	Volvatae
			aff. <i>avellaneosquamosa</i> 4/aff. <i>volvata</i> 3	Lepiotidae
			aff. <i>volvata</i> 4	Lepiotidae
			sp. JPN01/sp. OMS-015	Lepiotidae
			aff. <i>volvata</i> 5	Lepiotidae
			aff. <i>volvata</i> 8	Ponderosae
			aff. <i>volvata</i> 7	Ponderosae
			sp. OMS-010/aff. <i>volvata</i> 6	Ponderosae
			sp. OMS-013	Sagittariae
			sp. OMS-017	Sagittariae
		Japan/South Korea Japan	aff. <i>volvata</i> 2	Lepiotidae
			aff. <i>avellaneosquamosa</i> 3/OMS-012	Lepiotidae
		Japan/China		

Continent	Biogeographical regions (Kingdom)	Countries	Species clade	Subsection
Asia	Chinese-Japanese (Holarctic)	China	<i>brunneomaculata</i>	Volvatae
			<i>clarisquamosa</i>	Volvatae
			<i>lanigera</i>	Volvatae
			aff. <i>clarisquamosa</i> 1	Sagittariae
			aff. <i>clarisquamosa</i> 2	Sagittariae
			aff. <i>avellaneosquamosa</i> 2	Lepiotidae
			sp. clone d28 3Ama	Lepiotidae
			aff. <i>avellaneosquamosa</i> 5	Lepiotidae
			<i>parvicurta</i>	Lepiotidae
			aff. <i>volvata</i> 1	Lepiotidae
			cf. <i>avellaneosquamosa</i> 1	Lepiotidae
			aff. <i>avellaneosquamosa</i> 6	Ponderosae
			<i>pinophila</i>	Ponderosae
			aff. <i>albidostipes</i>	Ponderosae
		India	<i>parvirufobrunnescens</i>	Lepiotidae
			<i>pseudorufobrunnescens</i>	Lepiotidae
	Indo-Chinese (Paleotropical)	China	sp. soil subtropical China 32	Lepiotidae
			sp. soil subtropical China 33	Lepiotidae
			<i>rufobrunnescens</i>	Lepiotidae
		Thailand	sp. TUF116503	Lepiotidae
			sp. clone Ama3	Lepiotidae
			sp. Thai04	Lepiotidae
			<i>claristriata</i>	Ponderosae
		Thailand (China, Japan) Malaysia – Sarawak	<i>fulvisquamea</i>	Volvatae
			sp. ‘Sarawak1’	Volvatae
Europe	Eurosiberian (Holarctic)	Portugal	sp. montelongense	Sagittariae
		Spain/France	<i>lepiotoides</i> f. <i>subcylindrospora</i>	Lepiotidae
	Eurosiberian/Mediterranean (Holarctic)	Portugal/France	<i>pseudovalens</i> ^a	Volvatae

Continent	Biogeographical regions (Kingdom)	Countries	Species clade	Subsection	
(Europe)	Eurosiberian/Mediterranean (Holarctic)	Portugal/Spain/France/Italy (UK)	<i>ponderosa</i> ^a	Ponderosae	
	Mediterranean (Holarctic)	Portugal/Spain	<i>curtipes</i> ^a	Volvatae	
North America	Antillean-Mesoamerican (Neotropical)	Mexico	sp. ‘Jalisco’	Lepiotidae	
	Western North American (Holarctic)	USA	sp. ‘California’	Lepiotidae	
	North American Atlantic (Holarctic)	USA	<i>volvata</i>	Volvatae	
			sp. whartonensis	Volvatae	
			<i>peckiana</i>	Volvatae	
			aff. vaginata	Sagittariae	
			sp. sagittaria	Sagittariae	
			aff. mutabilis	Lepiotidae	
			sp. IN25	Lepiotidae	
			sp. canadensis01/sp. IN28	Lepiotidae	
			sp. IN31	Lepiotidae	
			sp. IN33	Lepiotidae	
			sp. S17/sp. IN41	Lepiotidae	
			sp. insolens	Lepiotidae	
			sp. texidella	Lepiotidae	
			sp. neocaesariensis/sp. IN26	Ponderosae	
			sp. S19/aff. volvata9	Ponderosae	
			sp. T51	Ponderosae	
			sp. whetstoneae	Ponderosae	
			Canada	sp. QU05	Lepiotidae
				sp. QU06	Volvatae
Oceania	Tasmanian-SE Australian (Australian)	Australia	aff. rosea	Lepiotidae	
	North Australian (Australian)		sp. 17 GMB-2014	Sagittariae	
			sp. 12 GMB-2014	Lepiotidae	
			sp. 19 GMB-2014	Lepiotidae	

^aThree species listed in Europe are known as fruitbodies in West Mediterranean Africa (Neville & Poumarat, 2004), Mediterranean region (Holarctic).

Online Resource 6 – List of described species tentatively in Section *Amidella*, currently (December 2024) without assigned DNA sequences.

Continent	Countries	Name	Comment
Africa	Democratic Republic of Congo	<i>Amanita fulvosquamulosa</i> Beeli	
		<i>Amanita subviscosa</i> Beeli	Sequences under this name from countries wide apart
	Madagascar	<i>Amanita irreperata</i> nom. prov. E.-J. Gilbert	Possibly the specimen classified as sp. ‘Madagascar’
	Republic of the Congo	<i>Amanita floccosolivida</i> Beeli	possibly not <i>Amidella</i>
		<i>Amanita olivacea</i> Beeli	
	Zambia	<i>Amanita subviscosa</i> sensu Pegler & Shah-Sm.	Basidiospores not fitting the description for the species (Tulloss & Yang 2024). One of the <i>subviscosa</i> s.l. is from Zambia
	“Congo”, “Central Africa”	<i>Amanita goossensiae</i> Beeli	Sequences under that name in clades Lepiotidae and Baakani
Asia	Singapore	<i>Amanita duplex</i> Corner & Bas	
North America	Dominican Republic	<i>Amanita occidentalis</i> O. K. Mill. & D. J. Lodge	
	United States (Eastern)	<i>Amanita dolichopus</i> nom. prov. Tulloss	
	United States (Eastern), Canada	<i>Amanita pseudovolvata</i> nom. prov. Tulloss	
	United States (Western), Mexico	<i>Amanita fallax</i> nom. prov. Tulloss & G. Wright	Could be either sp. ‘California’ or (as suggested in Tulloss & Yang 2024) sp. ‘Jalisco’
Oceania	Australia (NSW)	<i>Amanita pallidogrisea</i> A. E. Wood	possibly not <i>Amidella</i>
	Australia (Tasmania)	<i>Amanita grisea</i> Masee & Rodway	
	Australia (Victoria)	<i>Amanita curta</i> (Cooke & Masee) E.-J. Gilbert	

Online Resource 7

Volvatae



volvata
OQ311333



pseudovalens var. *tartessiana*
MW431341



peckiana
OR026673

Sagittariae



sp. montelongense
PP680673

Lepiotidae



aff. *rosea*
OQ628446



lepiotoides f. *subcylindrospora*
MW431337 MW431347

Ponderosae



sp. whetstoneae
KX061533 KX061519



sp. TANZ01
MZ358873



sp. S17/sp. IN41
OQ755005



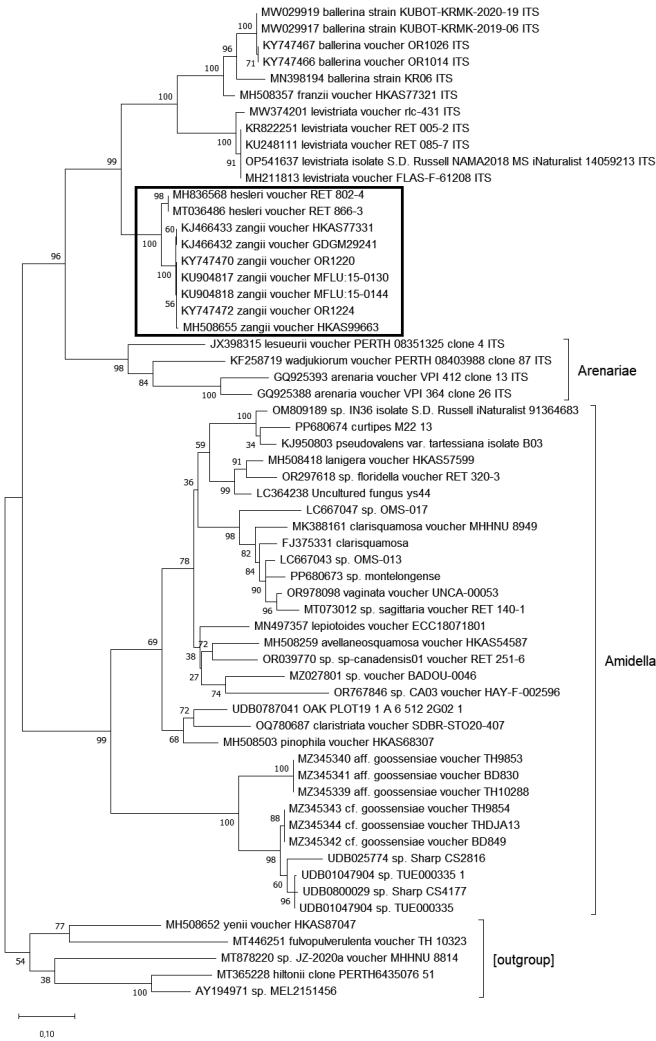
sp. S19/aff. volvata9
OQ754990

Photo credits

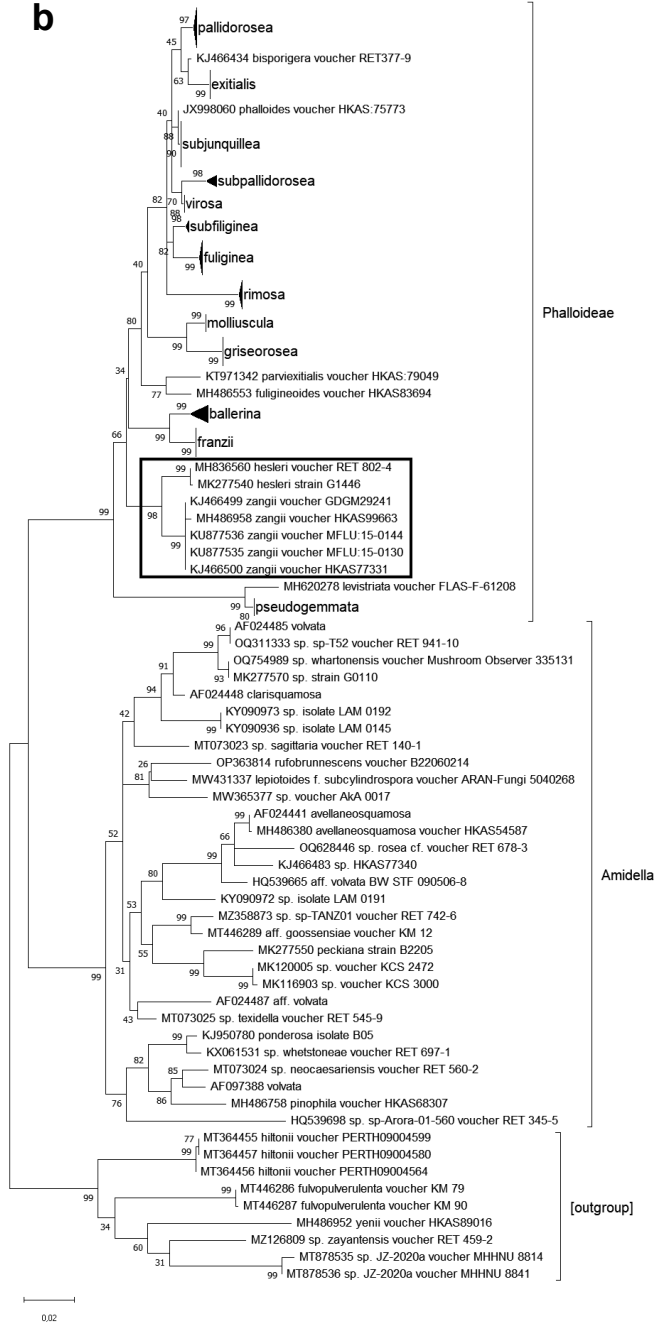
Note: the photos collected from Mushroom Observer, marked MO in the following list, are licensed through the Creative Commons BY-SA 3.0 <https://creativecommons.org/licenses/by-sa/3.0/>

volvata: MO 457058 Amanita sp. 'T52' (image 1341369) Photo by Ron Pastorino (2021) <https://mushroomobserver.org/obs/457058>
pseudovalens var. *tartessiana*: Amanita pseudovalens var. tartessiana. Photo by Ana C. Silva (2015), used with permission.
peckiana: MO 136750 Amanita floridella (image 338679) Photo by Ron Pastorino (2013) <https://mushroomobserver.org/obs/136750>
sp. montelongense: Amanita sp. PA-2024a. Photo by Helder Rodrigues (2022), used with permission.
aff. *rosea*: MO 197733 Amanita rosea (image 503402). Photo by Lucy Albertella (2015) <https://mushroomobserver.org/obs/197733>
lepiotoides f. *subcylindrospora*: Amanita lepiotoides f. subcylindrospora. Photo by Pedro Arrillaga (2006), used with permission.
sp. TANZ01: MO 240597 Amanita sp. 'TANZ01' (images 622776/9) Photos by Johannes Harnisch (2016) <https://mushroomobserver.org/obs/240597>
sp. S17/sp. IN41: MO 415318 Amanita sp. 'S17' (image 1199497) Photo by I. G. Safonov (2020) <https://mushroomobserver.org/obs/415318>
sp. whetstoneae: MO 175265 Amanita whetstoneae (image 448836) Photo by David Wasilewski (2014) <https://mushroomobserver.org/obs/175265>
sp. S19/aff. volvata9: MO 370560 Amanita sp. 'sp-S19' (image 1048387) Photo by I. G. Safonov (2019). <https://mushroomobserver.org/obs/370560>

a



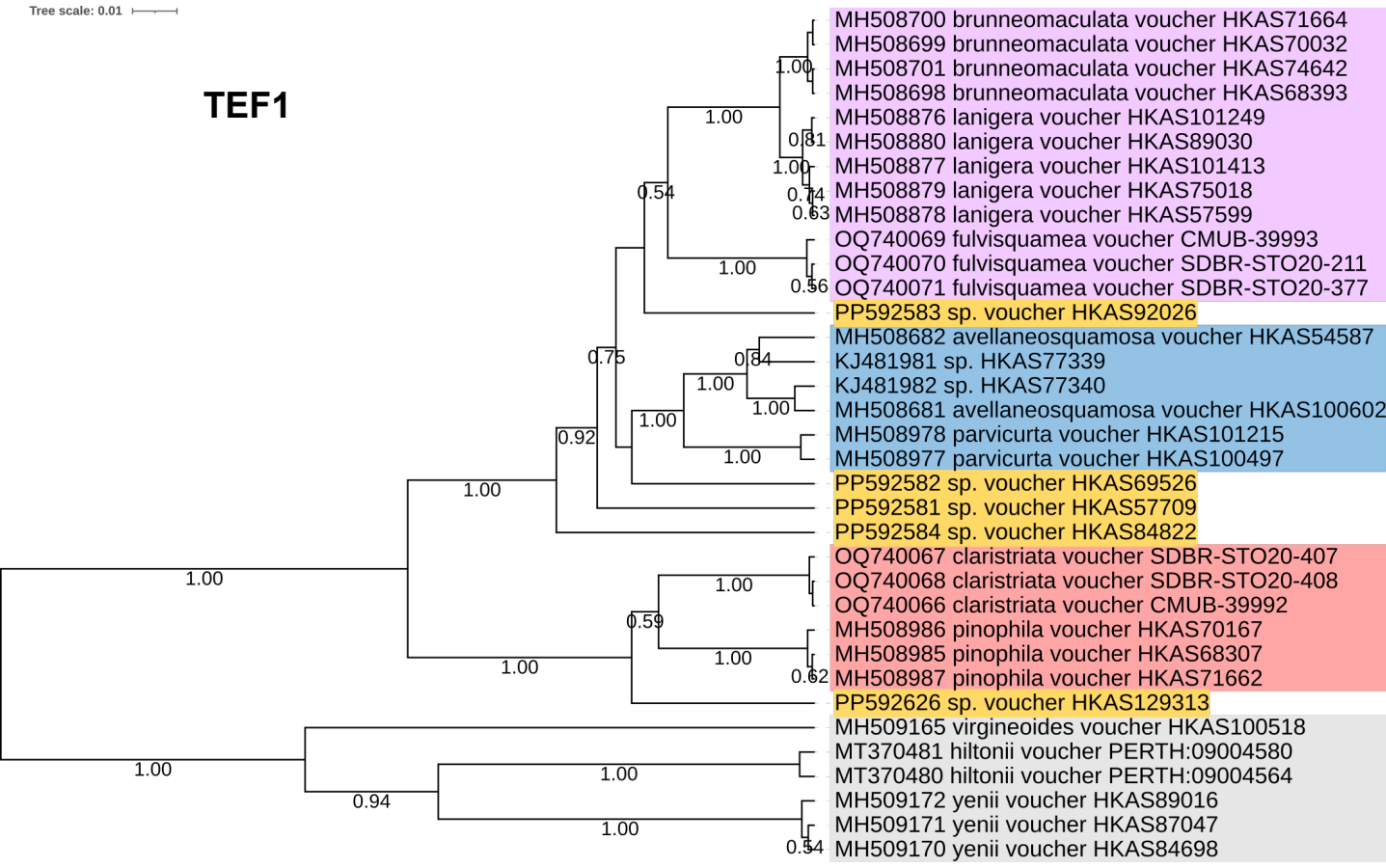
b



Online Resource 9

Tree scale: 0.01

TEF1



Online Resource 10

Targeted PCR amplification from old specimens

Introduction

This procedure is expected to facilitate the retrieval of DNA sequences from old specimens where the degree of DNA degradation is such that genomic DNA fragments of enough length for successful amplification form a residual fraction.

It encompasses two rounds of amplification, the second one consisting of reamplification from samples of the previous round, using taxonomy-selective primers. The latter primers can be used already from the outset.

The choice of primers must be such that the resulting amplicons are relatively short (<400 bp).

A PCR block that can run at different temperatures simultaneously (temperature gradient blocks, for example) will facilitate optimising the primer annealing temperatures.

This procedure enabled the sequencing of ITS regions from specimens over 50 years old (Arraiano-Castilho et al., 2022).

Protocol

1. Prepare an enzyme mix (without primers). For the whole experiment, the Mg^{2+} concentration in the final PCR reaction is set to 2.5 mM, adjusting the volume of $MgCl_2$ to be added depending on the composition of the enzyme buffer. The water volume is such that the enzyme mix fulfils 60% of the final volume. A standard Taq polymerase can be used.
2. Prepare the primer mixes, such that they form 30% of the total volume in each PCR reaction and the final concentration of each primer is 0.5 μM each.
3. First round: set up a 10 μL PCR reaction containing 1 μL of genomic DNA extract, 3 μL of primer mix, and 6 μL of the enzyme mix. Cover each reaction with 10 μL mineral oil.
 - a. Amplify over 40 cycles, allowing 1 minute for the elongation step in each cycle and a final elongation step of 5 minutes.
4. Second round: set up the PCR reactions with 3 μL water, 1 μL PCR solution from the corresponding first-round reaction, 12 μL primer mix, and 24 μL enzyme mix.
 - a. Amplify over 30 cycles, allowing 15–30 seconds for the elongation step in each cycle and a final elongation step of 5 minutes.
5. Analyse the PCR reactions, using a 3 μL sample from each one, by agarose gel electrophoresis (at 1.25% in TBE 0.5X).

Example (possible experiment)

Materials and hypotheses

Two DNA extracts from dried vouchers belonging in *Amanita* section *Amidella*:

- Voucher #1 could be from clade Volvatae or clade Baakani (there are primers for the ITS2 region in both clades);
- Voucher #2 could be from clade Lepiotoides or clade Avellaneosquamosa (there are primers for the LSU gene only for the latter).

Strategies

For #1, amplify with universal primers in the first round, and then with taxon-specific primers in the second round (10 + 2x40 µL). Expected results: positive only for one of the tests (Baakani vs. Volvatae). A positive control such as the one devised for #2 can be added in the second round (10 + 3x40 µL).

For #2, amplify with an Avellaneosquamosa-specific primer in the whole experiment (test), using section-wide primers as positive controls (2x(10 + 40 µL)). Expected results: positive for the control, but positive for the test only if belonging to clade Avellaneosquamosa.

Setup

Enzyme mix (120 µL):

Enzyme buffer 10x (containing 15 mM Mg²⁺)20 µL

MgCl₂, 25 mM.....8 µL

dNTP mix, 4 x 10 mM4 µL

Taq polymerase, 5 U/µL1 µL

Water.....87 µL

Note: if a positive control is added for voucher #1, this mix will be scaled up to 150 µL.

Primers:

#1:

First round (single reaction)

forward ITS3 5'GCATCGATGAAGAACGCAGC, reverse ITS4 5'TCCTCCGCTTATTGATATGC

Mix: 0.5 µL each primer at 10 µM + 2 µL water.

Second round (two reactions, one per test, possibly a third one for the positive control)

Baakani

Forward 5'CAATYTTAAATGCTTTYRG, reverse ITS4 5'TCCTCCGCTTATTGATATGC

Volvatae

Forward 5'AATGAATTAGCAGGGAWATATG, reverse ITS4 5'TCCTCCGCTTATTGATATGC

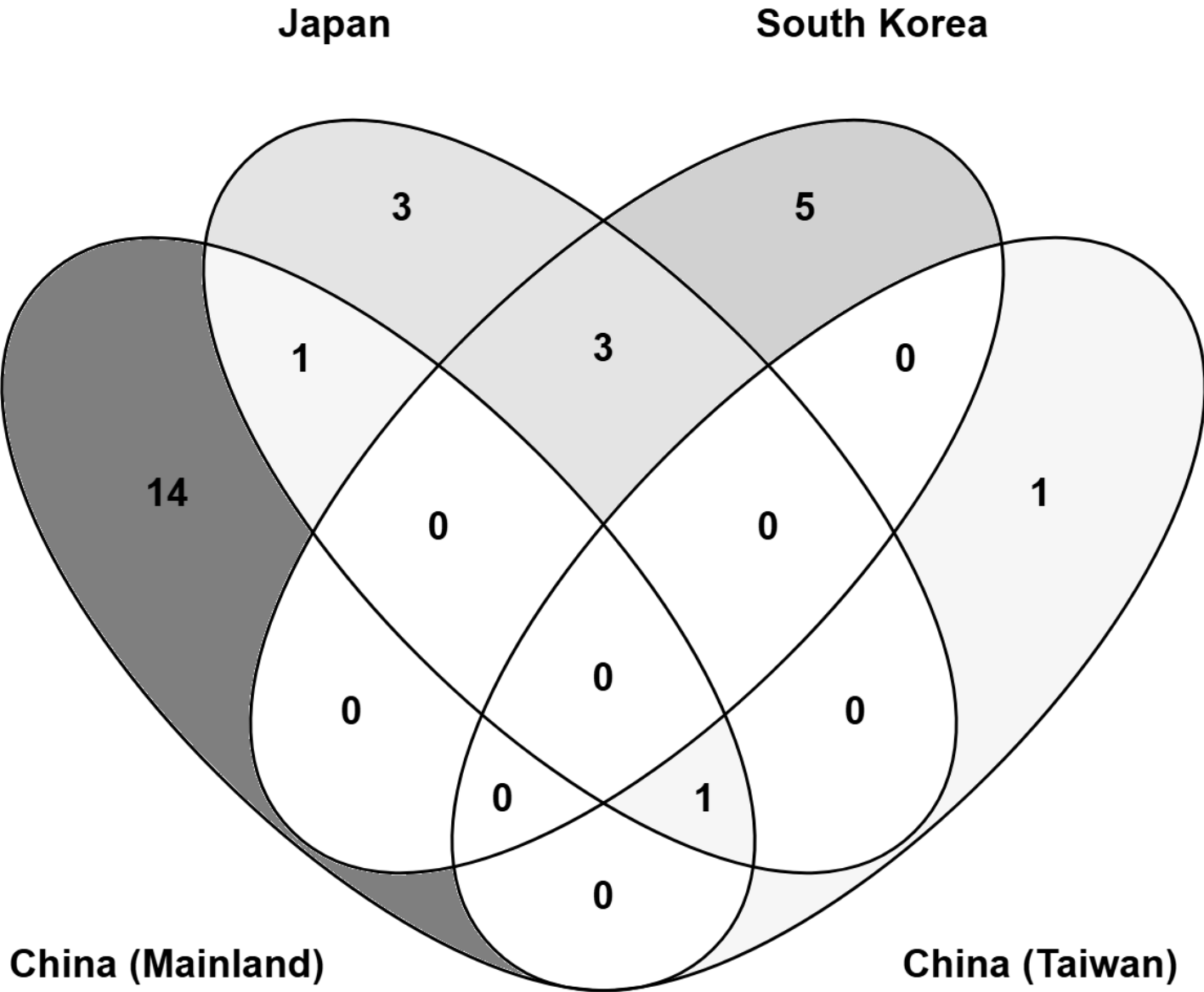
Each mix: 2 µL each primer at 10 µM + 8 µL water.

#2:

Test forward LR0R 5'ACCCGCTGAACTTAAGC, reverse 5'CTTYGAGAGCACACCACAAC

Control forward LR3R 5'GTCTTGAAACACGGACC, reverse 5'CAACATRCATGCTCTAC

Each mix (both rounds): 2.5 µL each primer at 10 µM + 10 µL water.



Supplementary figures legends

Online Resource 2. Phylogenetic trees obtained with BEAST, with posterior probabilities above 50% shown to the left of each node. The sequence labels have the GenBank accession numbers followed by unique identifiers, and the proposed clades are highlighted with the same background colours as in Figure 1. The scale bars are for substitutions per site. a) *RPB2* dataset, tree log likelihood $-2,930.1$. b) *TEF1* dataset, tree log likelihood $-2,513.4$. c) *BTUB* dataset, tree log likelihood $-1,224.3$. d) *LSU:RPB2:TEF1* concatenate dataset, tree log likelihood $-7,681.8$.

Online Resource 3. Phylogenetic trees obtained with MEGA (Kumar et al. 2024) using the Minimum Evolution Method with pairwise uncorrected p-distances, branches collapsed by sequence difference up to a common threshold and labelled according to the epithets listed in Table 4 and Online Resource 1. a) ITS1:ITS2 concatenate dataset, distance threshold 1.25%. b) nLSU dataset, distance threshold 0.8%.

Online Resource 4. Phylogenetic trees for the nLSU produced by clustering algorithms. a) ABGD (Puillandre et al. 2012) tree output, ingroup (*Amidella*) only, for the second init partition with MinSlope = 1.08, each sequence assigned to a group, with background colouring to highlight clusters of two or more sequences, leaving singletons uncoloured. Groups 9 and 21 are paraphyletic in this phylogeny. b) Phylogenetic tree obtained with BEAST, with posterior probabilities above 50% shown to the left of each node, from the aligned centroid sequences derived by USEARCH (Edgar 2010) from the nLSU dataset, using the cluster_fast command (UCLUST algorithm as described in https://drive5.com/usearch/manual/uclust_algo.html) and id option set at 0.992 (degree of similarity), tree log likelihood $-6,309.7$.

Online Resource 7. Representative collections classified in section *Amidella*, identified according to Table 4, with the corresponding GenBank accession numbers, and arranged according to their subclade affinities. All photos untouched, except for cropping as required.

Online Resource 8. Evolutionary analyses using MEGA (Kumar et al. 2024) designed to clarify the phylogenetic positions of the Hesleri clade (boxed). The scale bars are for substitutions per site. a) ITS1:ITS2 concatenate dataset phylogeny comprising members of sections *Phalloideae*, *Arenariae*, *Amidella* and (outgroup) *Roanokenses*, using the Maximum Likelihood method and General Time Reversible model of nucleotide substitutions with Gamma distribution modelling of evolutionary rate differences among sites and invariant sites, log likelihood $-6,075.34$, showing node bootstrap supports above 50% next to the branches. b) nLSU dataset phylogeny comprising members of sections *Phalloideae*, *Amidella* and (outgroup) *Roanokenses*, same method as in a), log likelihood $-6,023.87$.

Online Resource 9. Phylogenetic tree based on the *TEF1* dataset in Online Resource 2b, with five additional sequences (orange background), tree log likelihood $-3,096.5$.

Online Resource 11. Venn diagram built with Venny version 2.1.0 (Oliveros 2007-2015) showing the distribution of 28 *Amidella* species from East Asia represented in this study.

References (Online Resources)

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