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Research article

Clitopilus cretoalbus sp. nov. (Entolomataceae, Agaricales), a new species from Pakistan

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Abstract. A new species, *Clitopilus cretoalbus* A.Izhar, Zaman, M.Asif, H.Bashir, Niazi & Khalid sp. nov., is described herein based on several collections from Punjab, Pakistan. It is characterized by a clitocyboid to somewhat omphaloid stature combined with a white pileus, decurrent lamellae, an almost central to slightly eccentric whitish relatively long stipe, the occurrence of cheilocystidia, and basidiospores with 6 to 9 ridges in polar view. Molecular phylogenetic analyses of nrITS and nrLSU performed using the maximum likelihood method supported the novelty of this Pakistani species and its placement within the genus *Clitopilus* section *Scyphoides*. A comparison with other morpho-anatomically close species confirmed that the newly described species is distinct from others.

Keywords. Basidiomycota, morphology, phylogeny, taxonomy, subtropical.

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Introduction

The Entolomataceae Kotl. & Pouzar (Basidiomycota R.T.Moore) is a highly diversified family of Agaricales Underw. and comprises over 1500 described species globally (Co-David *et al.* 2009; Baroni *et al.* 2011; Kluting *et al.* 2014; Jian *et al.* 2020a, 2020b). *Clitopilus* (Fr. ex Rabenh.) P.Kumm. is a small genus within this family which was proposed by Kummer (1871) with *C. prunulus* (Scop.) P.Kumm. as the type species. This genus includes saprotrophic or mycoparasitic species and is widely distributed, particularly in northern temperate areas. A few reports are from the southern hemisphere, with only 30 of

the previously described species recorded to date (Singer 1986; Baroni & Watling 1999; Moncalvo *et al.* 2002; Kirk *et al.* 2008; Hartley *et al.* 2009; Crous *et al.* 2012; Raj & Manimohan 2018).

Clitopilus is characterized by basidiomata that are clitocyboid, omphaloid, or pleurotoid, mostly whitish or occasionally greyish or brownish, with pink or pinkish-brown spore prints, subdecurrent to decurrent lamellae, ellipsoid basidiospores with evenly cyanophilic walls that possess 5 to 12 longitudinal ridges which appear angular in a polar view, and with hyphae lacking clamp connections (Singer 1946, 1986; Noordeloos 1988, 2008; Kluting *et al.* 2014; Jian *et al.* 2020a, 2020b).

The systematic position of *Clitopilus* was confirmed by Matheny *et al.* (2006). Afterwards, the genus *Clitopilus* included *Rhodocybe* Maire (Co-David *et al.* 2009). However, molecular phylogenetic analyses have provided powerful tools for identification at genus level leading to the separation of *Clitopilus* and the allied genera (*Rhodocybe*, *Clitocella* Kluting, T.J.Baroni & Bergemann and *Clitopilopsis* Maire) (Baroni & Matheny 2011; Kluting *et al.* 2014). The new section level classification, viz. sect. *Clitopilus* (Fr. ex Rabenh.) P.Kumm., sect. *Crispi* S.P.Jian & Zhu L.Yang and sect. *Scyphoides* Singer. of *Clitopilus* was given by Jian *et al.* (2020a, 2020b).

Previously, four species in *Clitopilus* have been reported from Pakistan based on morpho-anatomical features, *C. apalus* (Berk & Broome) Petch, *C. peri* (Berk. & Broome) Petch, *C. pinsitus* (Fr.) Joss. and *C. scyphoides* (Fr.) Singer. Recently, two species *Clitopilus chichawatniensis* N.Fatima, Usman & Khalid and *Clitopilus shanglayensis* S.U.Rehman, M.Fiaz, Khalid, B.M.Khan, A.Hazrat, M.Yahya & M.A.Khan have been reported (Table 1), based on DNA sequence data (Ahmad *et al.* 1997; Sultana *et al.* 2011; Fatima *et al.* 2022; Rehman *et al.* 2022). In this study, four collections of *Clitopilus* were gathered from four different locations (viz. Bahawalpur, Sheikhupura, Swat, and Lahore of Pakistan), representing a new species. It is described in this paper with an illustrated morphological description and molecular evidence. This is the third species of the genus *Clitopilus* from Pakistan that is described based on morphological and molecular evidence.

Material and methods

Sampling sites

The type specimen was collected from district Sheikhupura, over loamy textured soils located at 31°42′40″ N, 73°59′16″ E . In summer, the district has extremely high temperatures followed by a heavy monsoon, average annual rainfall is 635 mm (Mukhtar *et al.* 2021; Shaheen *et al.* 2019). Common plant species of the district include *Albizia lebbeck* (L.) Benth., *Ficus benghalensis* L., *Capparis decidua* (Forssk.) Edgew., *Dalbergia sissoo* Roxb. ex DC. and *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb. (Kazi 1961). Other sampling locations included Jhok Reserve Forest in Lahore, the Lal Suhanra National Park adjacent to the Cholistan desert in Punjab Province, and Tehsil Kabal in district Swat, Khyber Pakhtunkhwa Province, Pakistan (Sheikh 1993; Ilyas *et al.* 2015; Khan *et al.* 2015; Ali *et al.* 2018; Bashir *et al.* 2020).

Morpho-anatomical protocols

The samples were photographed, dried, and packed in zip-loc bags. The morphological characters were noted and, for observation of anatomical features, a light microscope (LABOMED, Labo America, Inc. USA) was used. Chemicals such as 5% KOH (potassium hydroxide), Congo red, and Melzer's reagent were used in the anatomical analysis. The notation [n/l/p] indicates that measurements were made on n basidiospores in l basidiomata from p collections. The following abbreviations are used for all spores under analyses: avl = average length, avw = average width, Q = length/width and avQ = average Q value (Bas 1969; Yu *et al.* 2020). Specimens were deposited in Lahore Herbarium (LAH), Institute of Botany, University of the Punjab, Lahore.

Table 1. Comparison of important characters of *Clitopilus cretoalbus* A.Izhar, Zaman, M.Asif, H.Bashir, Niazi & Khalid sp. nov. and related species.

Taxa	Pileus	Basidia	Basidiospores	Cheilocystidia	Caulocystidia	Origin	References
C. albominutellus	3–8 mm, pale cream, concave to funnel-shaped	12–5 × 5–8 µm	4.5–7.5 × 2.5–3.5 µm, ellipsoid to ovoid	absent	absent	Germany	Ludwig 2001
C. apalus	20–30 mm, white, umbilicate to infundibuliform	up to 25 µm, clavate	6–8.5 × 4.5–5.5 µm, broadly oval	present, size and shape not specified	absent	Sri Lanka	Petch 1917; Ahmad *et al.* 1997
C. bigelowii	6–8 mm, gray, convex to umbilicate to infundibuliform	16–23 × 6.4–7.2 µm, clavate	5.6–7.6 × 3.2–4.5 µm, ellipsoid	absent	26–40 × 3–5 µm, cylindric to subcapitate	USA	Baroni 1995
C. chichawatniensis	50–65 mm, surface white to light pale, pleurotoid	25–32 × 7.2–11 µm, clavate to broadly clavate	6.17–8.4 × 4.75–6.6 µm, ovoid to ellipsoid	absent	absent	Pakistan	Fatima *et al.* 2022
C. cretoalbus sp. nov.	25–30 mm, white to chalk white, concave to infundibuliform or umbilicate	13–25 × 6.3–8 µm, clavate	6.4–8.5 × 3.6–5.5 µm, oblong to broadly ellipsoid or amygdaliform	17–30 × 5.5–8.8 µm, polymorphic, subcylindrical, lageniform, some filiform	absent	Pakistan	this study
C. peri	8–22 mm, white, convex to infundibuliform	16–18 × 5–7 µm,	5–8 × 3–5 µm, ellipsoid	absent	absent	Sri Lanka	Petch 1917; Pegler 1977a, 1977b; Ahmad *et al.* 1997; Baroni & Watling 1999
C. pinsitus	Up to 60 mm, white to pinkish, convex to flattened	19.6–30 × 6–8.8 µm, clavate	6.7–8.7 × 4.1–5.2 µm	absent	absent	France	Josserand 1937; Ahmad *et al.* 1997
C. scyphoides	5–25 mm, white, convex to umbilicate to infundibuliform	17–28 × 6.5–8.5 µm, clavate	7.5–11.5 × 4–5.5 µm, ellipsoid to oblong	absent	absent	Europe	Singer 1946; Ahmad *et al.* 1997; Sultana *et al.* 2011
C. shanglayensis	50–60 mm, white, light yellowish to yellowish red, umbonate	19–22 × 5–6 µm, clavate	7–8 × 4–5 µm, ellipsoid to amygdaliform	absent	absent	Pakistan	Rehman *et al.* 2022

Table 2 (continued on two next pages). Taxa used in phylogenetic analyses. Newly generated sequences are shown in **bold**. * = holotype.

Taxon	Voucher	GenBank accession		Locality	References (where sequence was published for the first time)
		nrITS	nrLSU		
C. abprunulus	KUN-HKAS 107041	MT345049	–	China	Jian *et al.* 2020b
C. abprunulus	KUN-HKAS 107042	MT345047	–	China	Jian *et al.* 2020b
C. abprunulus	KUN-HKAS 107040	MT345048	–	China	Jian *et al.* 2020b
C. albidus	CAL 1319	MF926596	MF926595	India	Raj & Manimohan 2018
C. amygdaliform	KUN-HKAS 87950	MN061290	MN065680	China	Jian *et al.* 2020a
C. amygdaliform	KUN-HKAS 81125	MN061291	MN065681	China	Jian *et al.* 2020a
C. amygdaliform	KUN-HKAS 60406	MN061292	–	China	Jian *et al.* 2020a
C. apalus	M536	–	AF261287	USA	Moncalvo *et al.* 2002
C. austroprunulus	MEN2009062	KC139085	–	Netherlands	Crous *et al.* 2012
C. austroprunulus	MEN2009001	KC139084	–	Netherlands	Crous *et al.* 2012
C. baronii	AMB 18363	MN855369	–	Italy	Consiglio & Setti 2019
C. brunneiceps	KUN-HKAS 80211	MN061293	–	China	Jian *et al.* 2020a
C. brunneiceps	KUN-HKAS 73123	MN061294	–	China	Jian *et al.* 2020a
C. brunneiceps	HMJAU 23509	MN061296	–	China	Jian *et al.* 2020a
C. brunneiceps	KUN-HKAS 104510	MN061295	–	China	Jian *et al.* 2020a
C. cf. *prunulus*	KUN-HKAS 75845	–	MN065693	China	Jian *et al.* 2020a
C. cf. *scyphoides*	UWO:SI11	KY706197	–	Canada	Hay *et al.* 2018 (unpubl.)
C. cf. *scyphoides*	KUN-HKAS 104511	MN061329	MN065720	China	Jian *et al.* 2020a
C. cf. *scyphoides*	T-782	KC176289	–	USA	Thorn *et al.* 1996
C. chalybescens	MFLUCC130808	KP938184	–	Thailand	Jatuwong *et al.* 2017
C. chalybescens	SDBR-CMUUP0039	–	MK764940	Thailand	Kumla *et al.* 2019
C. chichawatniensis	LAH37431	ON980767	ON980764	Pakistan	Fatima *et al.* 2022
C. chichawatniensis	LAH37432	ON980766	ON980763	Pakistan	Fatima *et al.* 2022
C. chichawatniensis	LAH37433	ON980765	–	Pakistan	Fatima *et al.* 2022
C. chrischonensis	TO HG1994	HM623128	–	Switzerland	Vizzini *et al.* 2011
C. crispus	KUN-HKAS 90506	MN061312	MN065702	China	Jian *et al.* 2020a
C. crispus	KUN-HKAS 102670	MN061313	–	China	Jian *et al.* 2020a
C. crispus	KUN-HKAS 84667	–	MN065705	China	Jian *et al.* 2020a
C. crispus	KUN-HKAS 87018	–	MN065706	China	Jian *et al.* 2020a
C. crispus	KUN-HKAS 104507	–	MN065707	China	Jian *et al.* 2020a
C. crispus	KUN-HKAS 102670	–	MN065704	China	Jian *et al.* 2020a
C. crispus	KUN-HKAS 90508	-	MN065703	China	Jian *et al.* 2020a
C. cystidiatus	TO AV131	HM623130	–	Switzerland	Vizzini *et al.* 2011
C. cystidiatus	E. Arnolds 03-27	KC885964	–	Netherlands	Morgado *et al.* 2016
C. cystidiatus	ME Noordeloos 200350	–	GQ289147	Netherlands	Co-David *et al.* 2009
C. doimaesalongensis	MFLUCC130806	KP938183	–	Thailand	Jatuwong *et al.* 2017
C. fasciculatus	297071	MG551863	–	USA	Rockefeller 2018 (unpubl.)
C. fusiformis	SAAS1038	KY385634	–	China	Wang *et al.* 2017
C. fusiformis	KUN-HKAS 104517	–	MN065689	China	Jian *et al.* 2020a
C. fusiformis	KUN-HKAS 104516	–	MN065688	China	Jian *et al.* 2020a
C. fusiformis	KUN-HKAS 104515	–	MN065690	China	Jian *et al.* 2020a
C. fusiformis	KUN-HKAS 104514	–	MN065687	China	Jian *et al.* 2020a
C. fusiformis	KUN-HKAS 104513	–	MN065686	China	Jian *et al.* 2020a
C. fusiformis	SAAS1892	KU751777	–	China	Wang *et al.* 2017
C. giovanellae	S.F.14368	EF413030	EF413027	Spain	Moreno *et al.* 2007
C. giovanellae	AH 19780	–	EF413026	Spain	Moreno *et al.* 2007
C. hobsonii	CBS 399.79	FJ770402	–	UK	Hartley *et al.* 2009
C. hobsonii	K(M) 122842	MN855372	–	UK	Consiglio & Setti 2019
C. hobsonii	CBS 269.36	FJ770395	–	UK	Hartley *et al.* 2009

Table 2 (continued). Taxa used in phylogenetic analyses. Newly generated sequences are shown in **bold**.

Taxon	Voucher	GenBank accession		Locality	References (where sequence was published for the first time)
		nrITS	nrLSU		
C. hobsonii	CBS 455.86	FJ770385	–	UK	Hartley *et al.* 2009
C. hobsonii	CBS 270.36	FJ770398	–	UK	Hartley *et al.* 2009
C. hobsonii	QYL-10	–	OK655769	China	Peng *et al.* 2021
C. hobsonii	NL-19	–	OK655770	China	Peng *et al.* 2021
C. kamaka	PDD 96106	NR_137867	–	New Zealand	Cooper 2014
C. kamaka	KA12-0364	KR673433	–	Korea	Kim *et al.* 2015
C. lampangensis	SDBR-CMUNK0047	MK764934	MK773856	Thailand	Kumla *et al.* 2019
C. lampangensis	SDBR-CMUJK0147	MK764933	MK764935	Thailand	Kumla *et al.* 2019
C. orientalis	CAL1613	MG345134	MG321558	India	Raj & Manimohan 2018
***C. cretoalbus* sp. nov.**	**Skp 106**	**ON229117**	–	**Pakistan**	**this study**
***C. cretoalbus* sp. nov.**	**AG 130**	**ON241754**	–	**Pakistan**	**this study**
***C. cretoalbus* sp. nov.**	**GHSS 02**	**ON326583**	–	**Pakistan**	**this study**
***C. cretoalbus* sp. nov.**	**Skp 107**	**ON229118**	–	**Pakistan**	**this study**
***C. cretoalbus* sp. nov. ***	**SKP 102**	**ON117610**	**ON229505**	**Pakistan**	**this study**
***C. cretoalbus* sp. nov.**	**MN 16**	**OM935685**	**OM934826**	**Pakistan**	**this study**
C. passeckerianus	P121	MG282460	–	South Korea	Yan *et al.* 2017 (unpubl.)
C. passeckerianus	P73	KY962489	–	South Korea	Yan *et al.* 2017 (unpubl.)
C. passeckerianus	P78	KY962494	–	South Korea	Yan *et al.* 2017 (unpubl.)
C. passeckerianus	CBS 330.85	FJ770388	–	UK	Hartley *et al.* 2009
C. passeckerianus	CBS 299.35	FJ770386	–	UK	Hartley *et al.* 2009
C. passeckerianus	DSMZ 1602	FJ770409	–	UK	Hartley *et al.* 2009
C. passeckerianus	DSMZ 901	FJ770406	–	UK	Hartley *et al.* 2009
C. pinsitus	CBS 401.79	FJ770387	–	UK	Hartley *et al.* 2009
C. pinsitus	CBS 623.70	FJ770403	–	UK	Hartley *et al.* 2009
C. pinsitus	G. Immerzeel 1990-11	–	GQ289148	Netherlands	Co-David *et al.* 2009
C. prunulus	HMJAU 4521	MN061302	MN065692	China	Jian *et al.* 2020a
C. prunulus	G.v. Zanen F96065	KC885965	–	Netherlands	Morgado *et al.* 2016
C. prunulus	KUN-HKAS 96158	MN061301	MN065691	China	Jian *et al.* 2020a
C. prunulus	CBS 129.42	FJ770389	–	England	Hartley *et al.* 2009
C. prunulus	CBS 227.93	FJ770408	–	England	Hartley *et al.* 2009
C. prunulus	ME Noordeloos 2003-09-14	KR261096	GQ289149	Netherlands	Morgado *et al.* 2016
C. prunulus	XSD-33	EU273512	–	China	Jiang *et al.* 2007 (unpubl.)
C. prunulus	CCBAS 775	FJ770407	–	UK	Hartley *et al.* 2009
C. ravus	KUN-HKAS 107043	MT345050	–	China	Jian *et al.* 2020b
C. reticulosporus	WU 27150	–	HM164412	Netherlands	Co-David *et al.* 2009
C. rugosiceps	KUN-HKAS 107044	MT345046	–	Macedonia	Jian *et al.* 2020b
C. rugosiceps	KUN-HKAS 59455	–	MN065696	China	Jian *et al.* 2020a
C. rugosiceps	KUN-HKAS 57003	–	MN065694	China	Jian *et al.* 2020a
C. rugosiceps	KUN-HKAS 73232	MN061305	MN065695	China	Jian *et al.* 2020a
C. scyphoides	CBS 127.47	MH856181	MH867707	France	Vu *et al.* 2019 (unpubl.)
C. scyphoides	CBS 400.79	FJ770401	–	UK	Hartley *et al.* 2009
C. scyphoides	CBS 171.48	FJ770390	–	UK	Hartley *et al.* 2009
C. scyphoides	T-777	KC176282	–	USA	Thorn *et al.* 1996
C. shanglayensis	LAH35495	MK348559	MK344431	Pakistan	Rehman *et al.* 2022
C. sinoapalus	KUN-HKAS 102807	MN061319	MN065710	China	Jian *et al.* 2020

Table 2 (continued). Taxa used in phylogenetic analyses. Newly generated sequences are shown in **bold**.

Taxon	Voucher	GenBank accession		Locality	References (where sequence was published for the first time)
		nrITS	nrLSU		
C. sinoapalus	KUN-HKAS 82230	–	MN065712	China	Jian *et al.* 2020a
C. sinoapalus	KUN-HKAS 77037	–	MN065713	China	Jian *et al.* 2020a
C. sinoapalus	KUN-HKAS 102737	–	MN065709	China	Jian *et al.* 2020a
C. sinoapalus	KUN-HKAS 101191	–	MN065711	China	Jian *et al.* 2020a
Clitopilus sp.	WU27150	KC885966	–	Netherlands	Morgado *et al.* 2016
Clitopilus sp.	BAB-4967	KR155057	–	India	Patel *et al.* 2015 (unpubl.)
Clitopilus sp.	BAB-4959	KR155049	–	India	Patel *et al.* 2015 (unpubl.)
Clitopilus sp.	BAB-4914	KR155011	–	India	Patel *et al.* 2015 (unpubl.)
Clitopilus sp.	BAB-5056	KR155101	–	India	Patel *et al.* 2015 (unpubl.)
Clitopilus sp.	BAB-5214	KT186178	–	India	Patel *et al.* 2015 (unpubl.)
Clitopilus sp.	BAB-5178	KR349632	–	India	Prajapati *et al.* 2015 (unpubl.)
Clitopilus sp.	BAB-5215	KT188594	–	India	Prajapati *et al.* 2015 (unpubl.)
Clitopilus sp.	BAB-5216	KT188595	–	India	Prajapati *et al.* 2015 (unpubl.)
Clitopilus sp.	BAB-4962	KR155052	–	–	Patel *et al.* 2015 (unpubl.)
Clitopilus sp.	BAB-4761	KR154990	–	India	Patel *et al.* 2015 (unpubl.)
Clitopilus sp.	KUN-HKAS 68389	–	MN065700	China	Jian *et al.* 2020a
Clitopilus sp.	TB8067	–	GU384612	USA	Baroni *et al.* 2011
Clitopilus sp.	110114MFBPZH0762	MW488359	–	China	Zhou 2021 (unpubl.)
Clitopilus sp.	Z-20	–	KC176284	USA	Thorn *et al.* 1996
Clitopilus sp.	Z-20	–	KC176283	USA	Thorn *et al.* 1996
C. subscyphoides	CAL 1326	MF927542	MF946580	India	Raj & Manimohan 2018
C. umbilicatus	KUN-HKAS 80370	MN061325	MN065717	China	Jian *et al.* 2020a
C. umbilicatus	KUN-HKAS 104509	MN061327	MN065719	China	Jian *et al.* 2020a
C. umbilicatus	KUN-HKAS 80289	–	MN065715	China	Jian *et al.* 2020a
C. umbilicatus	KUN-HKAS 80945	–	MN065718	China	Jian *et al.* 2020a
C. umbilicatus	KUN-HKAS 80310	MN061324	MN065716	China	Jian *et al.* 2020a
C. velutinus	CORT014618	MN784991	–	Dominican Republic	Baroni *et al.* 2020
C. yunnanensis	KUN-HKAS 82076	MN061306	–	China	Jian *et al.* 2020a
C. yunnanensis	KUN-HKAS 59712	MN061307	–	China	Jian *et al.* 2020a
C. yunnanensis	HMJAU 24677	MN061309	MN065699	China	Jian *et al.* 2020a
C. yunnanensis	KUN-HKAS 104518	MN061308	–	China	Jian *et al.* 2020a
Outgroups					
Clitocella fallax	CBS605.79	AF357018	–	Switzerland	Hofstetter *et al.* 2002
Clitocella orientalis	KUN-HKAS 75664	MN061332	–	China	Jian *et al.* 2020a
Lepista irina	PBM 2291	–	DQ234538	USA	Matheny *et al.* 2007
Lepista ovispora	E. Arnolds 05-183	–	GQ289207	Netherlands	Co-David *et al.* 2009
Tricholoma palustre	AFTOL-ID 497	–	AY700197	USA	Matheny & Hibbett 2004
Tricholoma vaccinum	H. v.d. Burg 2004-11-03	–	GQ289219	Netherlands	Co-David *et al.* 2009

Molecular protocols

By following the protocol of Bruns (1995), the DNA was extracted using the CTAB method. For amplification of the Internal Transcribed Spacer (ITS) region of the nuclear ribosomal DNA, primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') (Gardes & Bruns 1993) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used, and for amplification of the Large Subunit (LSU) rDNA, primers LROR (5'-ACCCGCTGAACTTAAGC-3') (Cubeta *et al.* 1991) and LR5 (5'-TCCTGAGGGAAACTTCG-3') (Vilgalys & Hester 1990; Ge *et al.* 2014) were used. For the sequencing, amplified products were sent to TsingKe China. Consensus sequences of samples were

created by combining the forward and reverse sequences using BioEdit software ver. 7.0.4.1 (Hall 1999). Sequences were BLAST searched at NCBI (https://www.ncbi.nlm.nih.gov/) and closely related sequences from the genus *Clitopilus* were downloaded from GenBank. For nrITS-based phylogeny, *Clitocella fallax* (Quél.) Kluting, T.J.Baroni & Bergemann and *Clitocella orientalis* S.P.Jian & Zhu L.Yang were selected as outgroup taxa, while in the nrLSU-based phylogeny, *Lepista ovispora* (J.E.Lange) Gulden, *Lepista irina* (Fr.) H.E.Bigelow, *Tricholoma vaccinum* (Schaeff.) P.Kumm., and *Tricholoma palustre* A.H.Sm. were used as outgroup taxa (Morgado *et al.* 2016; Jian *et al.* 2020b). Sequences were aligned using MUSCLE by EMBL-EBI, Wellcome Genome Campus, Hinxton, Cambridgeshire, UK (http://www.ebi.ac.uk/Tools/msa/muscle/). The final nrITS dataset contained 98 sequences and nrLSU dataset consisted of 60 sequences including outgroup taxa (Table 2). Maximum likelihood (ML) trees were constructed using CIPRES Science Gateway online employing RAxML ver. 8 (Miller *et al.* 2010).

Results

Phylogenetic analysis of nrITS dataset

The fragment size of the target region was 664 bp. The nrITS alignment contained a total of 921 characters, of which 607 were conserved, 280 were variable, 196 parsimony informative and 79 were singletons. The nrITS sequences of new species show 96% similarity with an unpublished and unidentified species of *Clitopilus* from China (MW488359), followed by *C. kamaka* J.A.Cooper (NR_137867) with 96% identity. In the nrITS tree (Fig. 1), *C. giovanellae* (Bres.) Singer (EF413030) reported from Spain is also closely related to the new species separated by strong bootstrap support (BS = 75%). The newly generated sequences of the Pakistani species clustered with other species from section *Scyphodies* (Morgado *et al.* 2016; Wang *et al.* 2017; Raj & Manimohan 2018; Kumla *et al.* 2019; Baroni *et al.* 2020a, 2020b; Fatima *et al.* 2022; Rehman *et al.* 2022).

Phylogenetic analysis of nrLSU dataset

The nrLSU sequences of *C. cretoalbus* sp. nov. were 911 and 912 bp long. The nrLSU alignment contained 938 characters, of which 771 were conserved, 153 were variable, 104 parsimony informative and 55 were singletons. In the nrLSU-based phylogram (Fig. 2), the new species formed a distinct clade sister to *C. giovanellae* (EF413026, EF413027).

Taxonomy

Phylum Basidiomycota R.T.Moore
Class Agaricomycetes Doweld
Order Agaricales Underw.
Family Entolomataceae Kotl. & Pouzar
Genus *Clitopilus* (Fr. ex Rabenh.) P.Kumm.

Clitopilus cretoalbus A.Izhar, Zaman, M.Asif, H.Bashir, Niazi & Khalid sp. nov
Mycobank: MB843564
Figs 3–4

Diagnosis

Clitopilus cretoalbus sp. nov. is close to *C. scyphoides* but the latter differs by its hygrophanous pileus with smaller diameter (3–6 mm), short stipe (4–9 mm), small colourless basidiospores (5–7 × 3.5–4.5 µm), absence of cystidia and pale yellow pileipellis.

Etymology

The specific epithet '*cretoalbus*' refers to chalk white colour of basidiocarps.

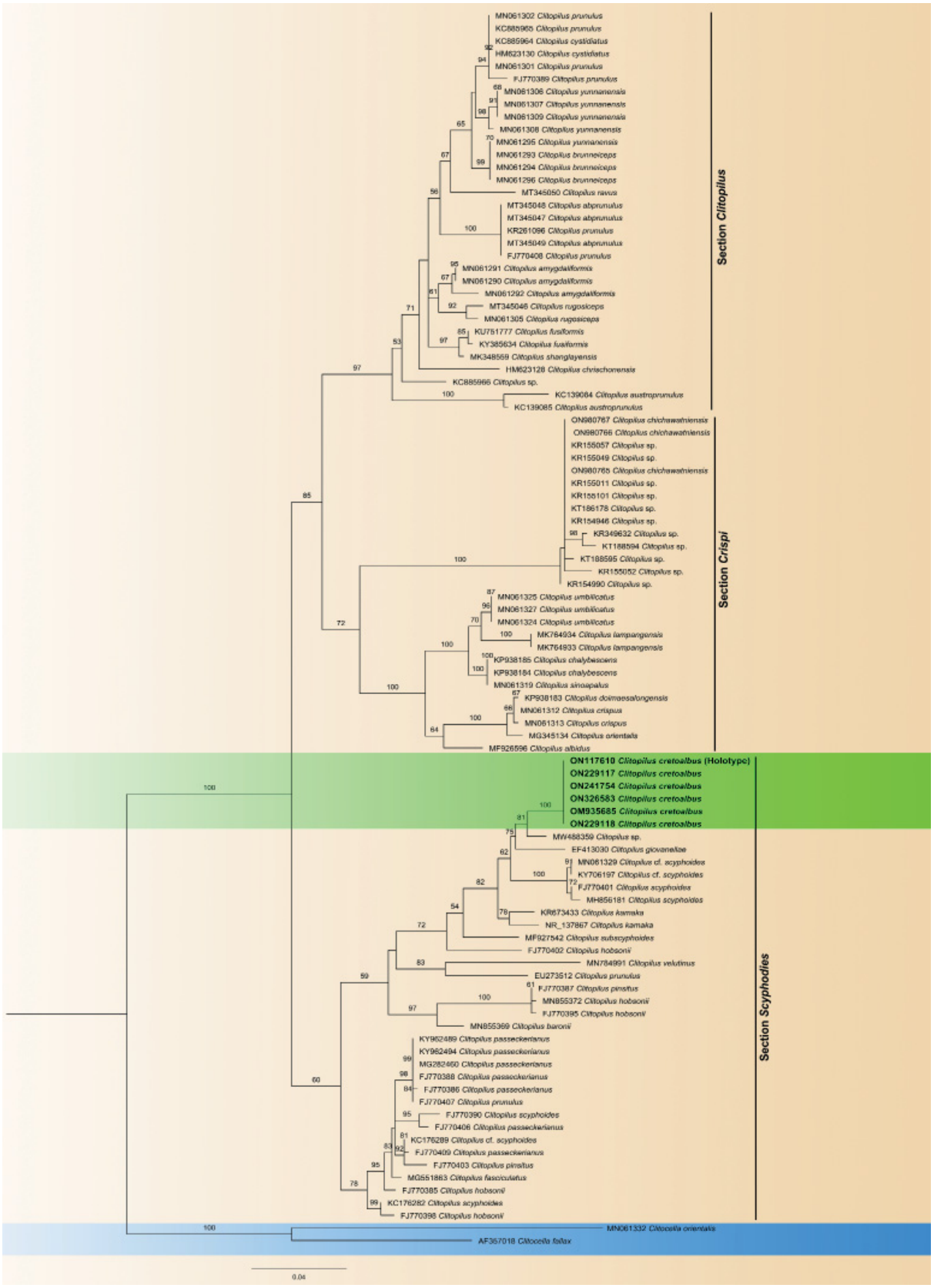

Fig. 1. Phylogenetic tree of *Clitopilus cretoalbus* A.Izhar, Zaman, M.Asif, H.Bashir, Niazi & Khalid sp. nov. based on nrITS sequences. Sequences generated from Pakistani collections are in **bold**.

Type material

Holotype

PAKISTAN – **Punjab Province** • Sheikhupura; 31°42′40″ N, 73°59′16″ E; 236 m a.s.l.; 3 Aug. 2017; *A. Izhar Skp102*; GenBank nos: ON117610 (nrITS), ON229505 (nrLSU); LAH[35709].

Additional material examined

PAKISTAN – **Punjab Province** • Sheikhupura; 31°42′40″ N, 73°59′16″ E; 236 m a.s.l.; 20 Jul. 2018; *A. Izhar Skp106*; GenBank no.: ON229117 (nrITS); LAH[37112] • same data as for preceding; 12 Aug. 2021; *A. Izhar Skp107*; GenBank no.: ON229118 (nrITS); LAH[37113] • Lahore, Jhok Reserve Forest; 31°25′36.01″ N, 74°7′11.13″ E; 217 m a.s.l.; 23 Aug. 2020; *A.N. Khalid MN16*; GenBank nos: OM935685 (nrITS), OM934826 (nrLSU); LAH[37017] • Bahawalpur, Lal Suhanra National Park, under *Bombax ceiba* L.; 31°26′28″ N, 74°8′7″ E; 125–140 m a.s.l.; 5 Sep. 2020; *M. Asif AG130*; GenBank no.:

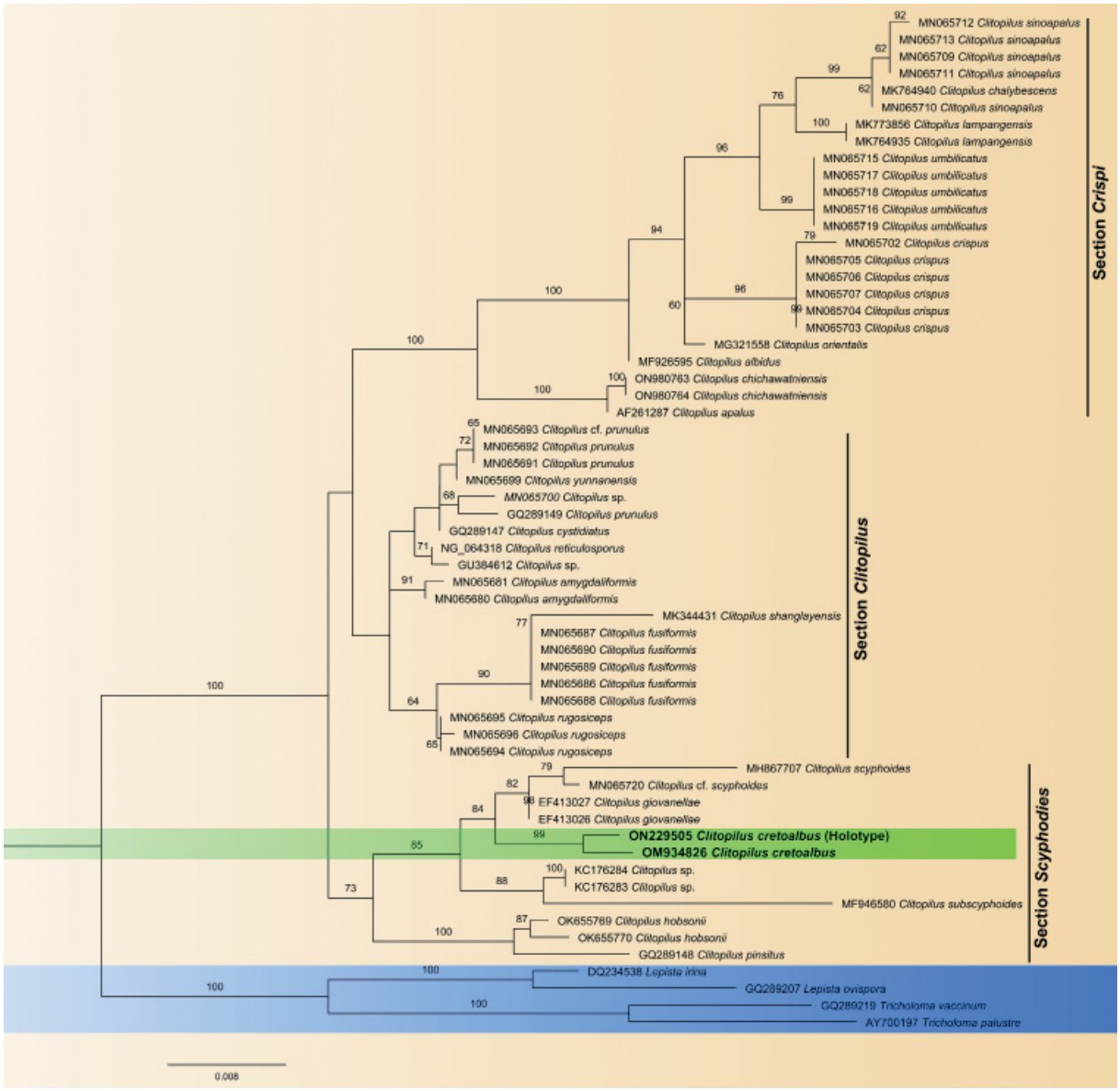

Fig. 2. Phylogenetic tree of *Clitopilus cretoalbus* A.Izhar, Zaman, M.Asif, H.Bashir, Niazi & Khalid sp. nov. based on nrLSU sequences. Sequences generated from Pakistani collections are in **bold**.

ON241754 (nrITS); LAH[37111] • Khushab, Noorpur Thal, Peelowains; 32°20′ N, 72°20′ E; 185 m a.s.l.; 8 Aug. 2021; *Z. Khan & J. Khan PW18;* GenBank no.: OM935686 (nrITS); LAH[37016]. – **Khyber Pakhtunkhwa Province** • Swat, Tehsil Kabal, Village Deolai; 34°34′ N, 72°08′ E; 1000 m a.s.l.; 25 Jul. 2021; *H. Bashir GHSS02*; GenBank no.: ON326583(nrITS); LAH[37126].

Fig. 3. A–F. Morphology of *Clitopilus cretoalbus* A.Izhar, Zaman, M.Asif, H.Bashir, Niazi & Khalid sp. nov. **A–B**. Basidiomata of *Skp102* (holotype, LAH35709). **C–D**. Basidiomata of *Skp106* (LAH37112) in natural habitat. **E–F**. Basidiomata of *MN16* (LAH37017). Photographs by Aiman Izhar, Muhammad Asif & Zaman Khan. Scale bars = 10 mm.

Description

Basidiomata clitocyboid, infundibuliform, or omphaloid, usually very small in size. Pileus 25–30 mm, concave, infundibuliform or umbilicate, context very fragile, white (2.5YR9/2), later chalk white, pale yellow at the disc (5Y 8/3), small brown patches develop when touched or dried, umbo never found, surface smooth to finely fibrillose under the lens, margins inrolled, becoming relatively straight towards maturity, cracks or furcations develop at pyramidal portions. Lamellae adnate to deeply decurrent, very thin, membranous, somewhat distant to close, sometimes bifurcated, white (2.5YR9/2) concolorous to

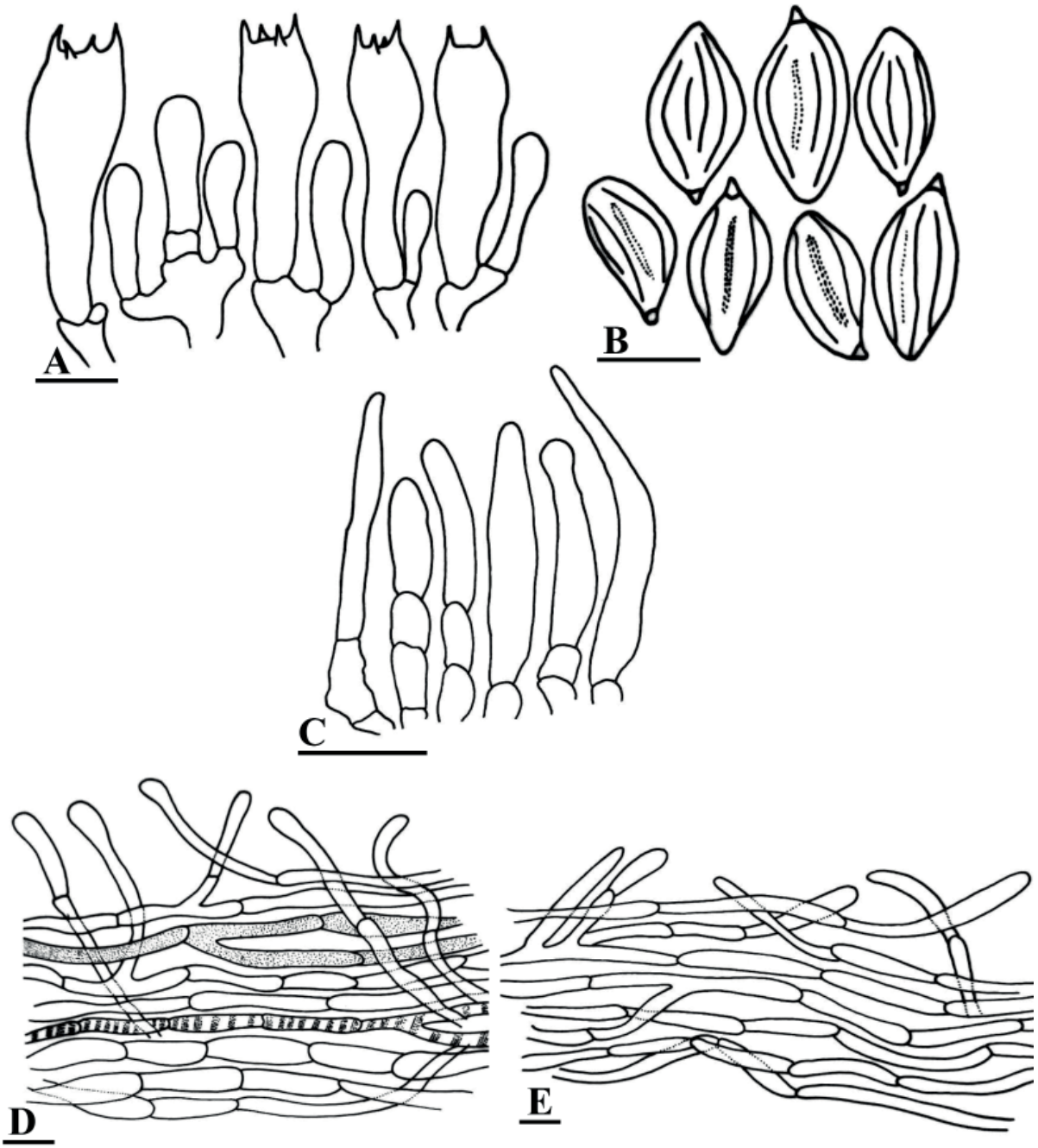

Fig. 4. A–F. Microscopic characters of *Clitopilus cretoalbus* A.Izhar, Zaman, M.Asif, H.Bashir, Niazi & Khalid sp. nov. (*Skp102*). **A**. Basidia. **B**. Basidiospores. **C**. Cheilocystidia. **D**. Pileipellis. **E**. Stipitipellis. Drawings by Aiman Izhar. Scale bars = 10 μm.

pileus, up to 1.8 mm in height, edges entire, lamellulae abundant, present in 1–3 tiers. Stipe 10–25 × 1–4 mm, almost central, slightly eccentric in a few specimens, sub-cylindrical, concolorous to pileus, surface smooth or minutely pubescent, white mycelium at the base. Odor farinaceous.

Basidiospores [100/5/5] (4.5–)6.4–7.5(–8.5) × (3.6–)3.9–4.6(–5.5) µm, avl × avw = 6.7 × 4.4 µm, Q = 1.2–1.54, avQ = 1.5, oblong, broadly ellipsoid or amygdaliform, hyaline to pale in 5% KOH, thin-walled, smooth-walled, frequent lipidic guttules, apiculus prominent, mostly isolated, few in tetrads. Basidia 13–25 × 6.3–8 µm, avl × avw = 18.7 × 7.2 µm, clavate, hyaline in 5% KOH, 4-spored, rarely 2-spored, sterigmata up to 3–3.4 µm in length, no clamp connections at base. Cheilocystidia 17–30 × 5.5–8.8 µm, avl × avw = 23 × 7 µm, polymorphic, subcylindrical, lageniform, some filiform, septate, few with apical or lateral outgrowths, resembling end cells of pileipellis, hyaline to pale in 5% KOH, often present in clusters. Pleurocystidia not found. Sub-hymenium irregular in texture, composed of 2–3 layers of ellipsoid hyphal elements, 3–13 × 4–8 µm. Pileipellis consists of a cutis of hyaline to pale cylindrical thin-walled hyphae 4–8 µm wide, avw = 6.2 µm, few pigmented brownish, hyphae parallel, often interwoven, many isolated and clustered ascendingly, terminal elements 5–8 µm wide. Pileal trama consists of subregular, hyaline, thin-walled, relatively inflated hyphae 8–14 µm wide, few oleiferous hyphae present. Stipitipellis a cutis composed of hyaline, loosely arranged or interwoven hyphae 4–5.5 µm wide, thin-walled, many clustered ascendingly.

Habitat

Scattered or in small groups on nutrient-rich loamy soil and gregarious in grassy plots.

Known distribution

Currently only known from Pakistan.

Discussion

The genus *Clitopilus* is here enriched by the addition of a new species, *C. cretoalbus* sp. nov. *Clitopilus cretoalbus* is distinguished from other species in having small-sized, pleurotoid to omphaloid basidiomata, convex to concave pileus, being less than 30 mm in diameter, and having basidiospores with many transverse ridges or folds (Singer 1946, 1978, 1986; Pegler & Young 1975; Jian *et al.* 2020a).

Clitopilus cretoalbus sp. nov. is morphologically similar to some members of the section *Scyphoides* such as *C. scyphoides* (Fr.) Singer (the type species of the section), *C. giovanellae*, *C. velutinus* T.J.Baroni & Angelini, *C. subscyphoides* W.Q.Deng, T.H.Li & Y.H.Shen and *C. prunulus* (Scop.) P.Kumm. (Kummer 1871; Singer 1942; Moreno 2007; Deng *et al.* 2013; Baroni *et al.* 2020; Jian *et al.* 2020a).

Based on DNA sequence data, the closest European taxon is *C. giovanellae* known from Italy and Spain. It differs from our new species by a smaller (5–15 mm), pale grey to grey-ochraceous, slightly longer stipe (20 mm), smooth, rather smaller (5–8 × 3–4 µm) basidiospores and the absence of any type of cystidia (Moreno *et al.* 2007). The nrITS sequences of *C. giovanellae* and *C. cretoalbus* sp. nov. differed by 19 nucleotide positions and the nrLSU sequences by 18 nucleotides positions. The basidiomata of *C. cretoalbus* are similar to those of another European species, *C. scyphoides*, which can be distinguished by producing a whitish grey, pallid, pink occasionally reddish-brown pileus surface, longer basidiospores (7.5–11.5 µm), a stipe that is eccentric lateral or absent, and more longitudinally ridged basidiospores (5–10 significant ridges) (Singer 1946; Jian *et al.* 2020a). Based on molecular data, *Clitopilus kamaka*, originally described from New Zealand by Cooper (2014), showed an identity of 96% with *C. cretoalbus* but significantly differs from our new species by producing a very small but lateral stipe in mature specimens, smooth basidiospores when basidiomata are fresh, lacking any type of cystidia, pileipellis with no encrusted pigments, and with basidiomata growing on rock surfaces. Based on DNA sequence

data, *C. subscyphoides*, described from China and India, is closer to *C. cretoalbus* but is separated by its hygrophanous pileus with smaller diameter (3–6 mm), short stipe (4–9 mm), colourless small basidiospores (5–7 × 3.5–4.5 μm), absence of cystidia and pale yellow pileipellis (Deng *et al.* 2013; Raj & Manimohan 2018).

The phylogenetic tree inferred from the nrITS dataset showed that *C. hobsonii* (Berk.) P.D.Orton, a wide-ranging species of both temperate and tropical regions, is closely related to our new species but the small diameter pileus (2–12 mm), adnexed lamellae, eccentric stipe in mature specimens, slightly longer basidiospores (6–9.6 μm), lack of cystidia as well as the absence of any encrusted material in pileipellis and growth on a diverse variety of habitats (plant materials, soil, rock, even on termite mounds) make it a significantly different species (Singer 1978; Baroni & Watling 1999, 2000).

The recently described tropical species *C. velutinus* from the Dominican Republic differs from *C. cretoalbus* sp. nov. by its longer (20–40 mm) much broader (4–7 mm), eccentric stipes, larger basidiospores (7–9 × 4.5–6 μm) and hyaline pileipellis (Baroni *et al.* 2020). *Clitopilus prunulus*, occurring in Europe, North America, Russia, and the Dominican Republic, is distinct from our new species by variable colours of the cap, ranging from pale grey to orange grey, large size (pileus up to 70 mm broad), predominantly larger basidiospores (8.8–16 × 4.5–6.4 μm), and absence of any type of cystidia (Scopoli 1772; Kummer 1871; Singer 1946; Noordeloos 1988; Yang 2007; Baroni *et al.* 2020; Jian *et al.* 2020a). Morphologically, *C. cretoalbus* can be confused with *C. bigelowii* T.J.Baroni but the latter species differs by its smaller (diameter less than 10 mm) grey-coloured cap, greyish lamellae with pinkish tones, small-sized basidiospores (5.6–7.6 × 3.2–4.5 μm), lack of hymenial cystidia, and reddish-brown pileipellis, whereas its stipitipellis is yellowish-brown pigmented having significant caulocystidia (Singer 1946; Josserand 1955; Contu 1992; Baroni 1995).

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