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ORIGINAL ARTICLE

Taxonomic reintroduction of the holarctic saprotrophic fungus *Crepidotus cinnamomeus*

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Abstract
Crepidotus is a genus of common saprotrophic fungi well known especially in the Northern Hemisphere, but distribution patterns of individual species are not sufficiently understood. We redefined a taxonomic circumscription of *Crepidotus cinnamomeus* based on morphological and molecular congruencies between the type material and recent collections. The species is well delimited from other similar and currently accepted species of the genus. *Crepidotus cinnamomeus* was found to have a broad holarctic distribution with occurrences in North America, Europe and Asia where it grows on twigs and branches of deciduous trees and shrubs in preferably cold humid habitats. Here we present the first multilocus phylogeny of the genus, including portions of the *RPB2* gene. Our study highlights the importance of sufficient sampling from broader areas supported by sequence data, which is essential for estimation of species delimitation, distribution and correct name assignment for *Crepidotus* species.

Keywords *Crepidotus variabilis* · Intercontinental distribution · Multilocus phylogeny · Saprotrophs · Type study

Introduction

Crepidotus (Fr.) Staude (*Crepidotaceae*, *Agaricales*) is a well-defined fungal genus with typically pleurotoid basidiomata, lamellate hymenophore and brown spore print. Members of this genus are saprotrophs, growing mainly on dead wood, less frequently on other plant remnants or soil, exceptionally on thalli of bryophytes or on fruiting bodies of other fungi (Pilát 1948; Consiglio and Setti 2008; Hausknecht and Krisai-Greilhuber 2010). Early molecular studies suggested that the traditional morphology-based genus concept corresponds to a monophyletic origin of this genus (Aime et al. 2002; Jančovičová et al. 2022). The infra-generic relationships are, however, not fully resolved mainly due to incomplete sampling. The genus has a cosmopolitan distribution with multiple underexplored areas (Singer 1986, https://www.inaturalist.org/taxa/118288-Crepidotus).

Currently, new morphological and molecular *Crepidotus* profiles providing reliable species identifications are scarce. This prevents estimation of species distribution limits. Recent large-scale phylogenetic studies demonstrated that a part of some saprotrophic phylogenetic lineages include species with broad holarctic or cosmopolitan distributions (Ševčíková et al. 2022; Schünemann et al. 2024). In *Crepidotus*, some species are probably endemic to a single continent, e.g. the North American species *C. brunnescens* Hesler & A. H. Sm. (Jančovičová et al. 2017). However, other species originally described from North America have also been reported elsewhere, e.g. *C. alabamensis* Murrill from India (Kumar et al. 2022) and *C. cinnabarinus* Peck from Europe

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(Senn-Irlet 2012). These studies relied on analyses of the LSU or ITS nrDNA regions and morphology.

Our study is focused on *C. cinnamomeus* Hesler & A. H. Sm. described from Idaho in western North America and published in the first comprehensive North American monograph of the genus *Crepidotus* by Hesler and Smith (1965). There are 125 accepted taxa in this monograph, including 78 species new to science. However, many of these species are known only from this book and their names are not used. As far as we know, *C. cinnamomeus* has not been reported or accepted by any other literature in North America or outside the continent after its first publication. The sequence obtained from isotype of this species (TENN-F-026166) is more than 99.2% similar to publicly available sequences from Sweden (Jančovičová et al. 2020) and China (Ge and Bau 2020). The aim of this study is to define taxonomic circumscription of *C. cinnamomeus* and assess its geographical distribution based on publicly available sequence data.

Materials and methods

Sampling

Sampling included the isotype of *Crepidotus cinnamomeus* (TENN-F-026166) and additional samples collected by the authors with DNA sequence similarity > 90% to the isotype ITS sequence. The authors' collections originated from Montana (USA), Sweden and Spain. Specimens collected by authors were deposited in the SLO herbarium (Comenius University Bratislava).

DNA extraction, PCR and sequencing

Total genomic DNA was extracted from dried material using the EZNA Fungal DNA Mini Kit (Omega Bio-Tek Inc., Norcross, GA, USA) following the manufacturer´s instruction. Three nuclear loci were amplified and sequenced: (i) ITS1-5.8S-ITS2 rDNA (ITS); (ii) D1–D2 domains of 28S rDNA (LSU) and (iii) the region between domains 6 and 7 of the nuclear gene encoding the second largest subunit of RNA polymerase II (*RPB2*). The ITS region was amplified with primer pairs ITS5-ITS4 (White et al. 1990), and for the LSU primers LR0R and LR5 were used (Moncalvo et al. 2000) with PCR conditions according to Jančovičová et al. (2022). Primers bRPB2-6F and bRPB2-7.1R (Matheny 2005) were used to amplify the *RPB2* region. PCR conditions were as follows: 1 min at 95 °C, 1.5 min at 55 °C, an increase of 1 °C per 5 s to 72 °C and 2 min at 72 °C, repeated 35 times and finalized for 10 min at 72 °C. Amplification of DNA was performed in a PCR reaction mix consisting of approximately 2 ng/µl of template DNA, forward and reverse primers (10 pmol/µl), 5 × HOT FIREPol® Blend Master Mix (Solis BioDyne, Tartu, Estonia) and molecular grade water added up to 20 µl. The target fragments were purified using ExoSap-IT (Thermo Fisher Scientific, Wilmington, Delaware, USA). Sequencing was performed at the SEQme sequencing company (Dobříš, Czech Republic).

Phylogenetic analyses

A multilocus phylogenetic analysis was supported by samples of species with phylogenetic affinity or morphological similarity to *Crepidotus cinamomeus* (Jančovičová et al. 2020): *C. variabilis* (Pers.) P. Kumm., *C. variabilis* var. *trichocystis* Hesler & A. H. Sm., *C. neotrichocystis* Consiglio & Setti, *C. kubickae* Pilát and *C. cesatii* (Rabenh.) Sacc. *Crepidotus applanatus* (Pers.) P. Kumm. and *C. malachius* (Berk. & M.A. Curtis) Sacc. were selected as representatives of unrelated members of the genus. Two samples of *Simocybe* P. Karst. were selected as an outgroup (Matheny et al. 2020). Samples used in the phylogenetic analyses are listed in Table 1.

Raw sequence files were edited in Geneious version R10 (Kearse et al. 2012). Intra-individual polymorphic sites having more than one signal were marked with NC-IUPAC ambiguity codes. Datasets for each marker were aligned separately by MAFFT version 7 using the strategy E-INS-i (Katoh and Standley 2013), manually edited in Geneious version R10 (Kearse et al. 2012) and concatenated using SeaView v.4.5.1 (Gouy et al. 2010). The final alignment was analysed with Maximum Likelihood (ML) and Bayesian inference (BI). For ML, the aligned dataset was loaded as a fasta file at the Cipres Science Gateway (Miller et al. 2010) and analysed using RAxML-HPC2 on XSEDE 8.2.12 (Stamatakis 2014). The alignment was partitioned under default settings with a GTR + GAMMA model with 1000 bootstrap iterations. For BI, the aligned fasta files were converted to the nexus format using Mesquite 3.61 (Maddison and Maddison 2023) and further analysed using MrBayes 3.2.6. (Ronquist et al. 2012) on XSEDE at the Cipres Science Gateway (Miller et al. 2010). Bayesian runs were computed independently twice with four MCMC chains for 10 million generations until the standard deviation of split frequencies fell below the 0.01 threshold. The convergence of runs was visually assessed using the trace function in Tracer 1.6 (Rambaut et al. 2014). The results were further edited with TreeGraph 2 (Stöver and Müller 2010) and graphically improved in CorelDRAW X5 (Ottawa, Canada).

The UNITE database (https://unite.ut.ee/) was searched using massBLASTer tool for all sequence variants assigned to *C. cinnamomeus* by multilocus analysis. Sequences in species hypothesis (SH) and individual sequences with similarity > 95% were included in additional analysis of the ITS region. The final ITS alignment and ML analysis were processed the same way as described for the multilocus dataset. The results of both multilocus and ITS analyses were further

Table 1 The list of ITS, LSU and *RPB2* sequences used in the phylogenetic analyses. Sequences generated in this study are in bold

Taxon name	Sample number	Country of origin	ITS accesion number and source	LSU accesion number and source	*RPB2* accesion number and source
Crepidotus applanatus	SLO 1492	Slovakia	MF621029 Jančovičová et al. 2017	MF621023 Jančovičová et al. 2017	**PP928836**
Crepidotus applanatus	SLO 1821	Slovakia	MF621028 Jančovičová et al. 2017	MF621022 Jančovičová et al. 2017	**PP928837**
Crepidotus cesatii	SLO 2613	Germany	**PP874212**	**PP874221**	**PP928826**
Crepidotus cesatii	SLO 2607	Slovakia	**PP874214**	**PP874223**	**PP928828**
Crepidotus cesatii	SLO 2616	Slovakia	**PP874213**	**PP874222**	**PP928827**
Crepidotus cesatii	TUF105761	Denmark	UDB034217 https://unite.ut.ee/cite.php	–	–
Crepidotus cesatii	TUF120093	Estonia	UDB023726 https://unite.ut.ee/cite.php	–	–
Crepidotus cesatii	TUF137290	Italy	UDB07673142 https://unite.ut.ee/cite.php	–	–
Crepidotus cf. *cesatii*	Montri-256	Switzerland	MK028394 Hofstetter et al. 2019	–	–
Crepidotus cf. *cesatii*	Montri-5	Switzerland	MK028393 Hofstetter et al. 2019	–	–
Crepidotus cinnamomeus **ISOTYPE**	TENN-F-026166	USA, Idaho	**PP874208**	–	–
Crepidotus cinnamomeus	SLO 2811	USA, Montana	**PP874215**	**PP874224**	**PP928829**
Crepidotus cinnamomeus	SLO 2778	Spain	**PP874217**	**PP874226**	**PP928831**
Crepidotus cinnamomeus	SLO 2780	Spain	**PP874216**	**PP874225**	**PP928830**
Crepidotus cinnamomeus	SLO 2407	Sweden	MT055897 Jančovičová et al. 2020 as *Crepidotus* sp.	**PP874228**	**PP928833**
Crepidotus kubickae	SLO 708	Slovakia	**PP874211**	**PP874220**	**PP928825**
Crepidotus kubickae	SLO 703	Slovakia	**PP874209**	**PP874218**	**PP928823**
Crepidotus kubickae	SLO 716	Slovakia	**PP874210**	**PP874219**	**PP928824**
Crepidotus malachius	SLO 479	Slovakia	MF621033 Jančovičová et al. 2017	MF621027 Jančovičová et al. 2017	**PP928834**
Crepidotus malachius	SLO 1497	Slovakia	MF621032 Jančovičová et al. 2017	MF621026 Jančovičová et al. 2017	**PP928835**
Crepidotus neotrichocystis **HOLOTYPE**	MCVE 22213	Italy	MT055895 Jančovičová et al. 2020	–	–
Crepidotus neotrichocystis	CS1150	Malta	OL672745 Sammut 2021	–	–
Crepidotus reticulatus	HMJAU 37086	China	MF461345 Ge & Bau 2020	–	–
Crepidotus sp.	iNaturalist observations/90967590	USA, Arizona	OM343180 https://www.inaturalist.org/.	–	–
Crepidotus sp.	4248_417	Lithuania	MT236579 Marčiulynas et al. 2020	–	–
Crepidotus subsphaerosporus	TUF111908	Italy	UDB07672099 https://unite.ut.ee/cite.php	–	–
Crepidotus variabilis	SLO 2018	Slovakia	MT055890 Jančovičová et al. 2020	OM832583 Jančovičová et al. 2022	**PP928838**
Crepidotus variabilis	SLO 2021	Slovakia	MT055889 Jančovičová et al. 2020	OM832585 Jančovičová et al. 2022	**PP928839**
Crepidotus variabilis	SLO 2423	Slovakia	MT055887 Jančovičová et al. 2020	OM832584 Jančovičová et al. 2022	**PP928840**

Table 1 (continued)

Taxon name	Sample number	Country of origin	ITS accesion number and source	LSU accesion number and source	*RPB2* accesion number and source
Crepidotus variabilis var. *trichocystis* **PARATYPE**	MICH 34852	USA, Idaho	MT055896 Jančovičová et al. 2020	**PP874227**	**PP928832**
Simocybe sp.	PBM3031	USA, Tennessee	GQ893023 Matheny et al. 2020	GQ892979 Matheny et al. 2020	HQ832444 Matheny et al. 2020
Simocybe phlebophora	PBM3089	New Zealand	MK421963 Matheny et al. 2020	MK421967 Matheny et al. 2020	MK415449 Matheny et al. 2020

edited with TreeGraph 2 (Stöver and Müller 2010) and graphically improved in CorelDRAW X5 (Ottawa, Canada).

Morphological analyses

Macromorphological characters of recent collections of *Crepidotus cinnamomeus* were observed from fresh material. Colour codes followed Kornerup and Wanscher (1978). Dried specimens were used to examine micromorphological characters. Microscopic structures were prepared in ammoniacal Congo red after a short pre-treatment in 3% aqueous solution of KOH. The structures were measured directly under an Olympus BX41 light microscope using an oil-immersion lens at a magnification of 1000×. Drawings of microscopic structures were made with a camera lucida using an Olympus U-DA drawing attachment at a projection scale of 2000×. Statistical calculations were based on 30 (spores, basidia, basidioles, cheilocystidia and terminal cells of pileipellis) and 20 (other microscopic structures) measurements per specimen. Q = ratio of length and width of spores. The range of microscopic characters was given as the minimum, maximum (in the parenthesis), average ± standard deviation, and average values. Morphological groups and frequency of cheilocystidia were adopted from Jančovičová et al. (2020); spore ornamentation was described according to Senn-Irlet (1995); other morphological terminology followed Vellinga (1988).

Results

Phylogenetic analyses

Phylogenetic analysis of combined ITS, LSU and *RPB2* included 22 samples (Fig. 1). Three major groups were supported within *Crepidotus*: *C. applanatus* and *C. malachius* formed distinct species-level lineages, whereas all other species were grouped into an inclusive clade (ML = 96, BI = 1.00). Within this larger inclusive clade, all non-singleton species clades received strong ML and BI support, but there was no support among internodes. Within the *C. cinnamomeus* lineage, there was no support for sample grouping based on geography.

A UNITE search resulted in retrieval of five SHs with at least one sequence more similar than 95% to *C. cinnamomeus* (SH1185163.09FU 99.85%, SH1185183.09FU 96.54%, SH1185159.09FU 96.45%, SH1185148.09FU 95.25%, SH1185185.09FU 95.21%) and one sequence not included in any SH (UDB07673142 96.05%). In the ITS tree (Supplementary file 1), only one new sample OM343180 was placed in the *C. cinnamomeus* lineage. This sample was published on iNaturalist (https://www.inaturalist.org/observations/90967590) and originated from Arizona, USA. Seven other retrieved sequences were placed in *C. cesatii*, and two formed a clade that included a molecular annotation of the *C. neotrichocystis* holotype.

Taxonomy

Crepidotus cinnamomeus Hesler & A. H. Sm., North American species of *Crepidotus*: 109. 1965. Figs. 2, 3

Original diagnosis: *Pileus 8–20 mm latus, sessilis, dimidiatus vel inaequalis, obscuro-albus deinde cinnamomeus, fibrillosus. Lamellae confertae densae, angustae demum medio-latae, pallidae deinde cinnamomeae vel rubido-brunneae. Sporae 5–6.2 × 3.3–4(4.2) µm, ellipsoideae, punctatae. Basidia 22–28 × 4–6 µm, di- et tetraspora. Pleurocystidia desunt; cheilocystidia 32–42 × 5–8 µm. Cuticula sine magno discrimine, hyphas erectas sine colore gerens. Fibulatae adsunt. Specimen typicum in Herb. Univ. Mich.; lectum prope Priest Lake, Idaho, Oct. 1, 1956, A. H. Smith 53816.* Holotype: MICH-F-5525. Isotype: TENN-F-026166.

Description of the isotype of *Crepidotus cinnamomeus* (TENN-F-026166, *Smith 53816*, Idaho, isotype).

Basidiomata ca. 10–12 mm wide; pileus surface not scaly, buff to warm buff in colour when dried; lamellae appear subdistant or only moderately close, moderately deep, pale brown to light yellowish brown.

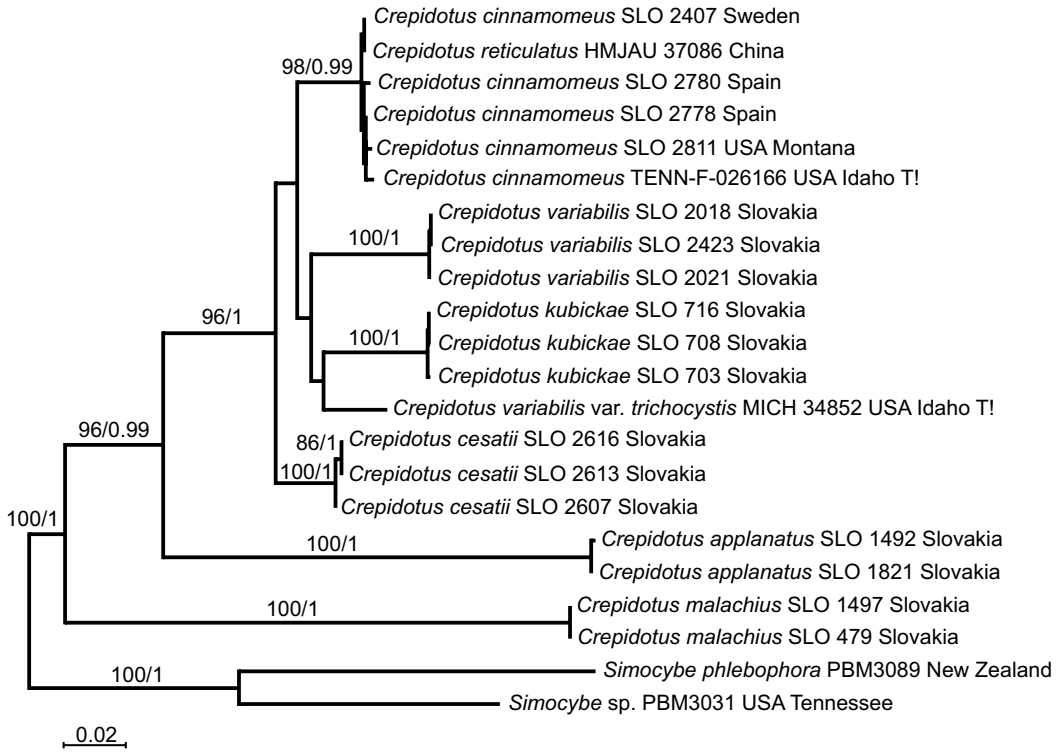


Fig. 1 Phylogram generated by ML analysis based on combined sequence data of ITS, LSU and *RPB2*. ML bootstrap support values greater than 50% and Bayesian posterior probabilities greater or equal to 0.90 are indicated above or below the nodes. Sequences originated from type collections are indicated with "T!"

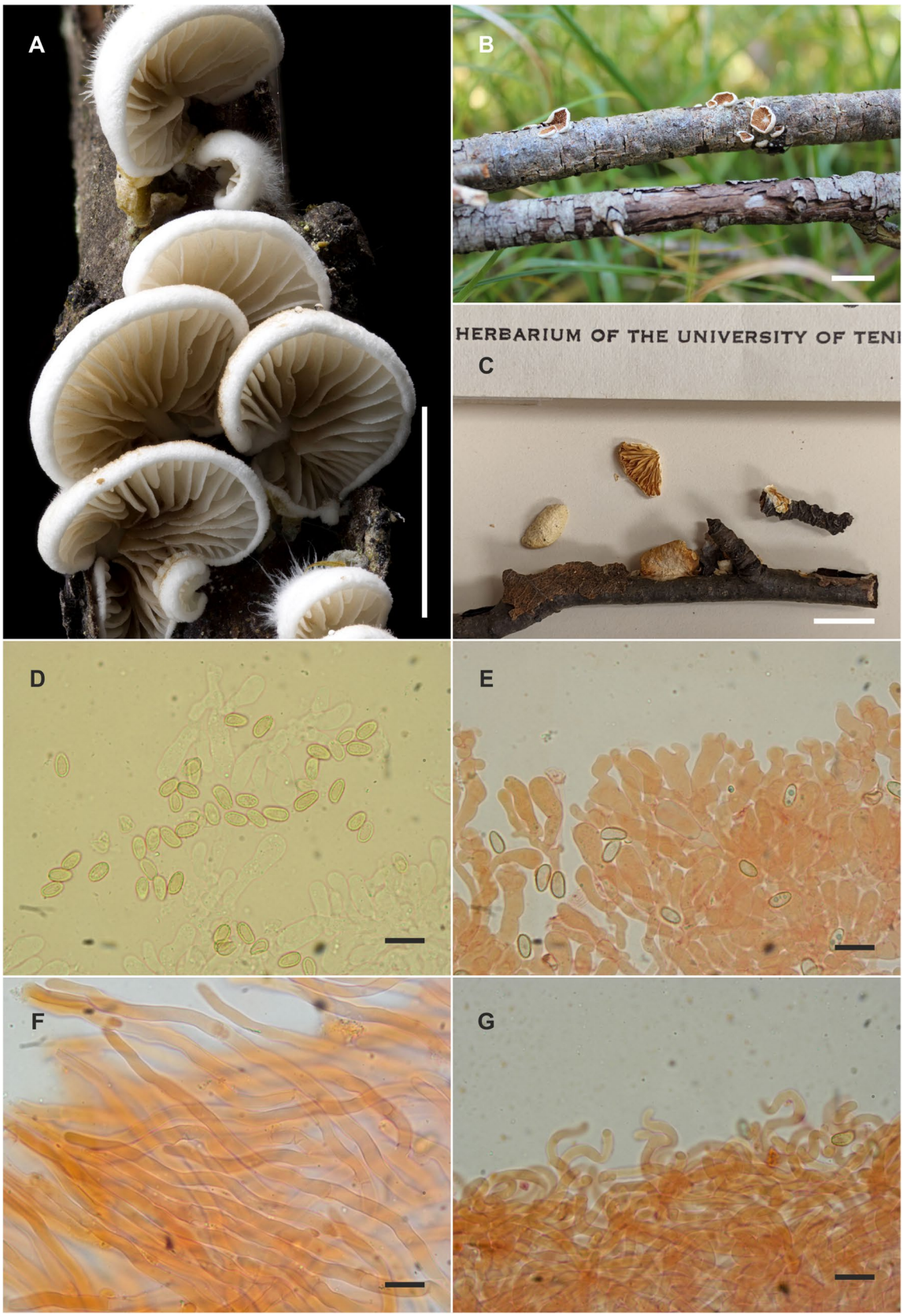
Basidiospores (4.9)5.6–6.1–7(7.3) × (3)3.2–3.4–3.7(3.8) µm, Q = (1.44)1.58–1.79–2.06(2.09) (*n* = 33/1), ellipsoid, oblong or amygdaliform with rounded or bluntly pointed apices, surface punctate, wall slightly thickened, pale yellowish brown to pale yellowish, hilar appendix very small and not conspicuous. Basidia 23–29 × 5–7 µm, mostly 4-sterigmate, slenderly clavate, not pigmented. Pleurocystidia absent. Cheilocystidia (19)21.7–26–30.3(34) × (4)4.4–6–7.4(8.5) µm, variably or irregularly shaped: mostly rostrate, sometimes clavate, lobate, forked and antler-like, at times flexuous, thin-walled, not pigmented. Lamellar tramal hyphae cylindrical, smooth, thin-walled, lacking pigment, cells 3–12 µm wide. Clamp connections present in all parts.

Description of recent collections of *Crepidotus cinnamomeus*

(SLO 2407, SLO 2778, SLO 2780, SLO 2811).

Basidiomata pileate, sessile, laterally or dorsally attached to the substrate, or with a rudimental lateral stipe, gregarious or clustered in groups. Pileus 3–17 mm in diameter; when young and mature rounded flabelliform or reniform (as seen from above); when young hemispherical, rarely campanulate, with age convex, plano-convex to applanate (as seen from aside); not hygrophanous; margin long involute, then inflexed, entire or lobed, not translucently striate; surface of young basidiomata white to orange-white (5A2), when mature golden blond (5C4) to brown (5D6-oak brown or 5E6-mustard brown); sericeous to velutinous, in one case (SLO 1096) when mature brownish orange (6C4-red-haired) to light brown (6D4-camel) flocculose; at the point of attachment white mycelial tomentum. Stipe (if present) cylindrical, curved, 1 × 0.5 mm, whitish, pubescent. Lamellae l = (1)3–7, L = 14–24(32), 0.5–1.5 mm wide, ventricose, adnexed, when young white to orange-white (5A2), when mature greyish orange (5B4), light brown (6D6-cinnamon) to brown (6E5, 6E6-leather brown, 6E7); lamellae edges entire or irregularly serrulate, when young concolorous, when mature paler (whitish) than the lamellae sides. Context up to 0.5 µm thick, yellowish white (3A2), smell and taste indistinct. Spore print light brown (6D6-cinnamon).

Basidiospores (5.2)5.8–6.5–7.2(9) × (3)3.1–3.4–3.7(4) µm, Q = (1.71)1.81–1.93–2.26(2.29), oblong, yellowish to yellowish brown, verruculose (punctate under light microscope), hilar appendix not conspicuous. Basidia 4-spored, rarely 2-spored, (16)18.8–20.7–22.7(25) × (5)5.7–6.1–6.5(7.5) µm, clavate, hyaline, thin-walled. Basidioles (10.5)14–17.4–20.7(27) × (3.5)4.5–5.2–6(7) µm, clavate, hyaline, thin-walled. Pleurocystidia absent. Cheilocystidia (13)19.1–24–28.9(40) × (3)4.6–5.9–7.2(10.5) µm, often forked and antler-like, sometimes rostrate, diverticulate and lobate,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◂**Fig. 2** *Crepidotus cinnamomeus*. **A** Fresh basidiomata in detail (Montana, SLO 2811). **B** Field aspect of basidiomata (Spain, SLO 2778). **C** Dried basidiomata of isotype (Idaho, TENN-F-026166). **D** Basidiospores. **E** Cheilocystidia. **F** Terminal cells near pileus centre. **G** Terminal cells near pileus margin (D–G: Montana, SLO 2811, D stained in KOH, E–G stained in Congo red). Scale bars A–C = 1 cm, D–G = 10 µm. Photos: A: T. B. Wheeler; C: P. B. Matheny; B, D, E, F, G: S. Jančovičová

rarely clavate, at apex obtuse, hyaline, thin-walled. Lamellar trama of 3–11 µm wide, almost parallel, flexuous, irregularly inflated, intricate, occasionally anastomosed or branched, hyaline, thin-walled or up to 0.5 µm thick-walled hyphae. Pileipellis ca. 200 µm deep, composed of ascending to erect, usually unbranched and two-celled, loosely arranged, hyaline, thin-walled hyphae forming a transition from cutis to trichoderm. Terminal cells near pileus centre (38)52.7–83 .5–114.3(164) × (3)3.1–3.6–4.1(5) µm, cylindrical, straight or slightly flexuous, occasionally nodulose, at some places fasciculated, apically tapering and ca. 0.5–1 µm narrower than near the septum; near pileus margin (21)29.7–48.5–67 .3(78) × (2.5)2.6–3.3–3.9(5) µm, flexuous, angulate, twisted, often branched, with lateral nodes or coralloid. Clamp connectiones present in all parts.

Note: We did not observe any distinct differences among spore and cheilocystidia dimensions. Our study revealed high variability of cheilocystidia shape among collections. These included mostly rostrate, sometimes clavate, lobate, forked and antler-like in TENN-F-026166; mostly rostrate, often forked and antler-like, and never diverticulate in the collection from Sweden (SLO 2407); mostly forked and antler-like, and only sometimes rostrate and diverticulate in the Spanish collections (SLO 2778, SLO 2780); often forked, antler-like and diverticulate and only sometimes rostrate in the collection from Montana (SLO 2811). The pileipellis of all examined collections showed the same variation pattern (Figs. 2 and 3; Supplementary file 2).

Specimens examined: United States, Idaho, Priest Lake, on *Betula* branches, 1st of October 1956, leg. A. H. Smith *Smith 53816* (TENN-F-026166), isotype. United States, Montana, Lake County, Jocko River Canyon, 6606 Jocko Canyon Road, 47°10′52.6″N, 113°37′2.8″W, 1080 m asl., mixed riparian forest with *Populus*, *Thuja*, *Betula* and *Picea*, on wood/bark of fallen decaying branch of *Betula?* or *Populus?*, ca. 5–15 mm in diam., 1st of July 2020, leg. T. B. Wheeler *TBW 8159* (SLO 2811). Spain, Pyrénées Mts., Huesca Province, (Javierre) municipality, Valle de Pineta, near Cinca river, 42°38′08″N, 00°10′54″E**,** 1170 m asl., mixed riparian forest with *Pinus sylvestris*, *Prunus spinosa*, *Salix*, on wood/bark of fallen twig of *Rubus*, 2–4 mm in diam., 5th of October 2022, leg. S. Jančovičová (SLO 2780); ibidem, on bark of fallen decaying branch of *Salix* cf. *purpurea*, ca. 15 mm in diam., 5th of October 2022, leg. S. Jančovičová (SLO 2778). Sweden, Västernorrland County, Âse village, ca. 1 km W of the village or ca. 2 km SW of the Âsetjärnen (lake), 62°31′03″N, 16°02′04″E, 160 m asl., moist brook forest with *Alnus*, *Betula*, *Picea*, *Salix*, on wood and bark of fallen decaying branch of deciduous tree (*Betula?)*, ca. 5–10 mm in diam., 27th of August 2018, leg. S. Jančovičová (SLO 2407).

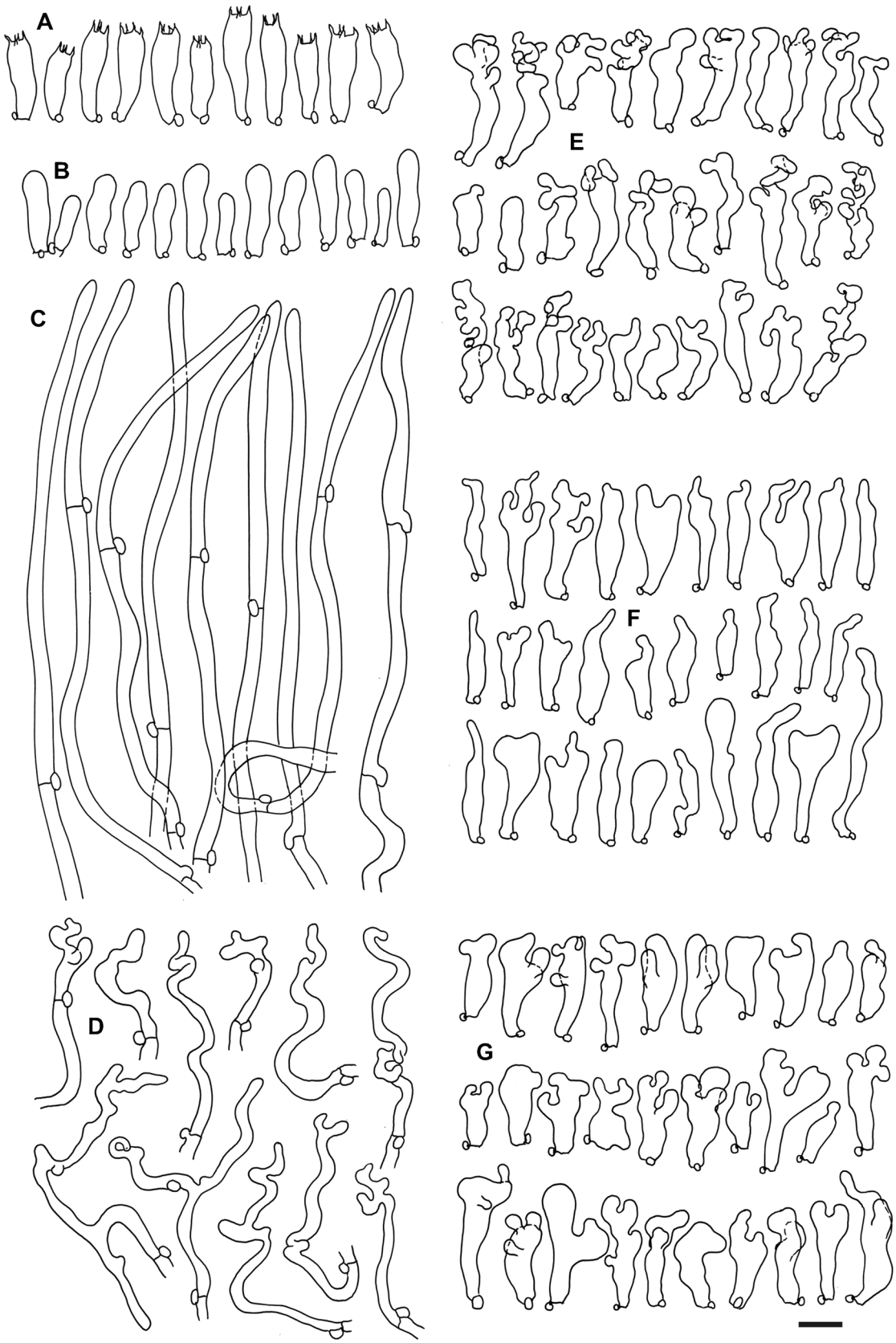
Discussion

This study provides the first report and molecular and morphological delimitation of *Crepidotus cinnamomeus*, a species originally described by Hesler and Smith (1965) and placed in *Crepidotus* subgen. *Dochmiopus* (Pat.) Pilát, sect. *Dochmiopus* Consiglio & Setti. Within this section (in the sense of Hesler and Smith 1965), species were distinguished mainly by the colour of the pilei: white in *C. subsphaerosporus* (J. E. Lange) Kühner & Romagn., *C. variabilis* var. *variabilis* and *C. variabilis* var. *trichocystis*; pale cinnamon pileus in *C. cinnamomeus*; lemon yellow in *C. subcroceitinctus* Hesler & A. H. Sm.; and orange-buff to warm-buff in *C. croceitinctus* Peck.

According to our observations, *C. cinnamomeaus* does not have cinnamon tints on pileus, which can also be seen on the isotype (Fig. 2). On the other hand, yellow and orange tints on pilei of two other species are probably good diagnostic characters such as in case of e.g. *C. luteolus* Sacc. and *C. crocophyllus* (Berk.) Sacc. respectivelly (Senn-Irlet 1995).

Morphologically similar species with white pilei include *C. variabilis* var. *variabilis*, *C. variabilis* var. *trichocystis*, and recently described *C. neotrichocystis* (Consiglio and Setti 2008). Although Consiglio and Setti (2008) presented *C. variabilis* var. *trichocystis* as a misapplied name of *C. neotrichocystis*, our phylogenetic study confirmed that var. *trichocystis* deserves a species rank (Fig. 1).

Crepidotus variabilis var. *variabilis* differs from *C. cinnamomeus* by consistently larger cheilocystidia (usually in average longer than 30 µm and wider than 7 µm), which is demonstrated and statistically well documented in the study by Jančovičová et al. (2020) that also included one of our collection of *C. cinnamomeus* (SLO 2407). The cheilocystidia shape of these two species, *C. variabilis* and *C. cinnamomeus*, show high variability among collections, but both species have some cystidia types that were rare or absent in the other species (see Fig. 3 and Fig. 7 in Jančovičová et al. 2020). This study confirmed that *C. cinnamomeus* can be distinguished from *C. variabilis* var. *trichocystis* and *C. neotrichocystis* by more elongated


A
B
C
D
E
F
G

◂**Fig. 3** *Crepidotus cinnamomeus*. **A** Basidia. **B** Basidioles. **C** Terminal cells near pileus centre. **D** Terminal cells near pileus margin. **E** Cheilocystidia (A–E: Montana, SLO 2811). **F** Cheilocystidia (Sweden, SLO 2407). **G** Cheilocystidia (Spain, SLO 2778). Scale bar = 10 µm. Drawings: S. Jančovičová

spores (average Q > 1.8), which was also suggested in our previous study (Jančovičová et al. 2020, Fig. 3).

Other species mentioned as similar to *C. cinnamomeus* by Hesler and Smith (1965) include *C. subsphaerosporus*, but the species name is invalid and its concept is unclear, probably referring to *C. kubickae* (Jančovičová and Pennycook 2012). Both *C. kubickae* and a similar species *C. cesatii* are clearly different from *C. cinnamomeus* by spores wider than 4.2 µm (Ripková 2009). Delimitation of *C. cinnamomeus* and all similar species is presented in the identification key (Supplementary file 3).

One of sequences retrieved from GenBank was MF461345 from China. This sequence number with name "*Crepidotus* sp." appeared in the publication by Ge and Bau (2020), in their table of specimens used in the phylogenetic analysis and in the tree. The associated voucher number HMJAU37086, however, refers to the holotype of *C. reticulatus* T. Bau & Y.P. Ge: "Holotype: CHINA. Guangdong Province: Zhaoqing City, Dinghu Mountain, 17 Jun 2015, Tian, Liu & Zhang HMJAU37086 (HMJAU). Gene sequences ex-holotype: MF461346 (ITS)." As can be seen, authors cited different ITS sequence number MF461346, which is not a sequence similar to *C. cinnamomeus*. *Crepidotus reticulatus* is a species described as having a coral red pileus and spores ellipsoid to ovoid, with ridges or partial reticulation. Because of such differences from *C. cinnamomeus*, we think that the sequence number MF461345, which is placed in our phylogenies in *C. cinnamomeus* clade (Fig. 1), is incorrectly paired with the type specimen of *C. reticulatus* and actually represent a record of the former species from China. *Crepidotus cinnamomeus* was not included in the monographic work by Tolgor et al. (2022).

Boreal and temperate regions are estimated to have lowest endemicity of fungal species, which is in agreement with our discovery of the wide distribution for *C. cinnamomeus* (Tedersoo et al. 2022). Previous studies reported broad intercontintenal species distributions in *Crepidotus* based on morphology or phylogenetic analyses. Phylogenetic studies reporting *Crepidotus* species from different continents were based only on ribosomal DNA regions (ITS and LSU), and either they lacked sequences from continents from where reported species were described (Kumar et al. 2020; Na et al. 2022), or they resulted in an inconclusive outcome (Kasuya et al. 2014). Glacial dynamics have driven widely distributed species of temperate, boreal and arctic environments into isolated refugia with subsequent contact, which resulted in complex genetic structure of geographically distant populations with specific ITS variability. This ITS variability is sometimes non-concordant with other loci in the fungal genome. Multi-locus phylogenetic analysis that include protein-coding regions may help to elucidate differences within and between species in ITS region (Kauserud 2023). The phylogenetic study of *Inocybaceae* (sister family to *Crepidotaceae*) confirmed existence of relatively high number of species distributed in Europe and North America (Matheny et al. 2020). This, together with our study, suggests that wide species distributions across the holarctic region of the Northern Hemisphere may be a common distribution pattern also in *Crepidotus*.

The redescription of *C. cinnamomeus* is only the first step towards to understand species delimitation, richness and nomenclature of North American species. As the result of technological tools available at the time when the monograph of the genus was published (Hesler and Smith 1965), species diagnoses were brief, which led to incorrect estimation of species richness. For example, in case of studies on North American *Hebeloma* species described by Hesler and Smith during the 1970s and 1980s, recent studies revealed a high species overestimation (Eberhardt et al. 2023). The holarctic distribution of species described in this study suggests that the nomenclatural assignment in *Crepidotus* will be even more difficult task because names from other continents must be also considered.

From available material used in this study it seems that *Crepidotus cinnamomeus* prefers humid habitats such as riparian *Salix* shrub or riparian *Alnus* forest. While twigs and branches of *Betula*, *Salix* and *Rubus* were identified as probable substrates, woody remnants of other plants were present at collecting sites, including *Populus* and *Alnus* are potential substrates. The substrate varied from 2 mm thick twigs to 15 mm thick branches. The elevation ranged from 160 m in Sweden to 1170 m in Spanish Pyrenees. We think that the absence of the species in warmer temperate and Mediterranean areas suggest that its occurrence is rather limited by a preference of a cooler climate.

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Author contribution Study conception and design were prepared by Soňa Jančovičová and Slavomír Adamčík. Morphological analysis were performed by Soňa Jančovičová and P. Brandon Matheny; molecular analysis by Katarína Adamčíková, Mary G. Graddy, P. Brandon Matheny, Chance R. Noffsinger and Tim B. Wheeler; phylogenetic analysis by Slavomír Adamčík and Miroslav Caboň. The first draft of the manuscript was written by Soňa Jančovičová and Slavomír Adamčík and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The DNA sequences produced in this study are available on NCBI GenBank (https://www.ncbi.nlm.nih.gov).

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

References

Aime MC, Baroni TJ, Miller OK (2002) *Crepidotus thermophilus* comb. nov., a reassessment of *Melanomphalia thermophila*, a rarely collected tropical agaric. Mycologia 94:1059–1065. https://doi.org/10.1080/15572536.2003.11833161

Consiglio G, Setti L (2008) Il genere *Crepidotus* in Europa. A.M.B, Fondazione Centro Studi Micologici, Vincenza

Eberhardt U, Schütz N, Bartlett P, Beker HJ (2023) Many were named, but few are current: The *Hebeloma* of Hesler, Smith, and coauthors. Mycologia 115:813–870. https://doi.org/10.1080/00275514.2023.2230538

Ge YP, Bau T (2020) Descriptions of six new species of *Crepidotus* from China. Mycosystema 39:238–255. https://manu40.magtech.com.cn/Jwxb/CN/https://doi.org/10.13346/j.mycosystema.190345

Gouy M, Guindon S, Gascuel O (2010) SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol Biol Evol 27:221–224. https://doi.org/10.1093/molbev/msp259

Hausknecht A, Krisai-Greilhuber I (2010) Die Gattung *Crepidotus* in Österreich. Österr Z Pilzk 19:53–91

Hesler LR, Smith AH (1965) North American species of *Crepidotus*. Hafner Publishing Company, New York, London

Hofstetter V, Buyck B, Eyssartier G, Schnee S, Ginfro K (2019) The unbearable lightness of sequenced-based identification. Fungal Divers 96:243–284. https://doi.org/10.1007/s13225-019-00428-3

Jančovičová S, Pennycook SR (2012) Lectotypification of *Crepidotus variabilis* var. *subsphaerosporus*. Mycotaxon 120:423–426

Jančovičová S, Adamčik S, Looney BP, Caboň M, Čaplovičová M, Kopáni M, PennyCook SR, Adamčiková K (2017) Delimitation of European *Crepidotus stenocystis* as different from the North American species *C. brunnescens* (*Crepidotaceae*, *Agaricales*). Phytotaxa 328:127–139. https://doi.org/10.11646/phytotaxa.328.2.3

Jančovičová S, Adamčíková K, Caboň M, Adamčík S (2020) How variable is *Crepidotus variabilis*? Phytotaxa 449:243–264. https://doi.org/10.11646/phytotaxa.449.3.4

Jančovičová S, Adamčíková K, Caboň M, Adamčík S (2022) Phylogeny of *Crepidotus applanatus* look-alikes reveals a convergent morphology evolution and a new species *C. pini*. J Fungi 8:489. https://doi.org/10.3390/jof8050489

Kasuya T, Uno K, Hosaka K (2014) Reexamination of *Crepidotus crocophyllus* (*Basidiomycota*, *Fungi*) in Japan, with reference to its phylogenetic placement. Univ Bull Chiba Inst Sci 7:159–166

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software, version 7: improvements in performance and usability. Mol Phylogenet Evol 30:772–780. https://doi.org/10.1093/molbev/mst010

Kauserud H (2023) ITS alchemy: On the use of ITS as a DNA marker in fungal ecology. Fungal Ecol 65:101274. https://doi.org/10.1016/j.funeco.2023.101274

Kearse M, Moir R, Wilson A et al (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649. https://doi.org/10.1093/2Fbioinformatics/2Fbts199

Kornerup A, Wanscher JH (1978) Methuen Handbook of Colour, 3rd edn. Methuen, London

Kumar M, Aime MC, Vrinda KB, Pradeep CK (2020) Two new species and new record of *Crepidotus* (*Agaricomycetes*) from India. Aust Syst Bot 33:380–391. https://doi.org/10.1071/SB19033

Kumar AM, Pradeep CK, Aime MC (2022) New species and new records of *Crepidotus* (*Crepidotaceae*) from India. Mycol Prog 21:311–326. https://doi.org/10.1007/s11557-021-01751-3

Maddison WP, Maddison DR (2023) Mesquite: a modular system for evolutionary analysis, version 3.81. http://mesquiteproject.org. Accessed 5 May 2024

Marčiulynas A, Marčiulynienė D, Lynikienė J, Gedminas A, Vaičiukynė M, Menkis A (2020) Fungi and Oomycetes in the irrigation water of forest nurseries. Forests 11:459. https://doi.org/10.3390/f11040459

Matheny PB (2005) Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*; *Agaricales*). Mol Phylogenet Evol 35:1–20. https://doi.org/10.1016/j.ympev.2004.11.014

Matheny PB, Hobbs AM, Esteve-Raventós F (2020) Genera of *Inocybaceae*: New skin for the old ceremony. Mycologia 112:83–120. https://doi.org/10.1080/00275514.2019.1668906

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the gateway computing environments workshop (GCE). New Orleans, LA, USA, pp 1–8

Moncalvo J-M, Lutzoni FM, Rehner SA, Johnson J, Vilgalys R (2000) Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Syst Biol 49:278–305. https://doi.org/10.1093/sysbio/49.2.278

Na Q, Liua Z, Zengb H, Chenga X, Ge Y (2022) *Crepidotus yuanchui* sp. nov. and *C. caspari* found in subalpine areas of China. Mycoscience 63:1–11. https://doi.org/10.47371/mycosci.2021.10.004

Pilát A (1948) Monographie des espèces européennes du genre *Crepidotus* Fr. Atlas Des Champignons De L'europe 6:1–84

Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) Tracer version 1.6. http://beast.bio.ed.ac.uk/tracer. Accessed 5 May 2024

Ripková S (2009) *Crepidotus kubickae* – a forgotten name. Mycotaxon 110:271–281

Ronquist F, Teslenko M, van der Mark P et al (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542. https://doi.org/10.1093/sysbio/sys029

Sammut C (2021) Further additions to the mycobiota of Malta. Ecol Mediterr 47:85–135

Schünemann BLB, Reginato M, da Silveira RMB (2024) Palaearctic origin and repeated dispersal over the world shaped the biogeographic history of the saprophytic genus *Coprinopsis* (*Psathyrellaceae*, *Basidiomycota*). J Biogeogr 51:982–991. https://doi.org/10.1111/jbi.14805

Senn-Irlet B (1995) The Genus *Crepidotus* (Fr.) Staude in Europe. Persoonia 16:1–80

Senn-Irlet B (2012) *Crepidotus* (Fr.) P. Kumm. In: Knudsen H, Vesterholt J (eds) Funga Nordica Nordsvamp, Copenhagen, pp 977–980

Ševčíková H, Malysheva E, Ferisin G et al (2022) Holarctic species in the *Pluteus romellii* clade. Five new species described and old names reassessed. J Fungi 8:773

Singer R (1986) The Agaricales in Modern Taxonomy, 4th edn. J. Cramer, Germany

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stöver BC, Müller KF (2010) TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. BMC Bioinforma 11:7. https://doi.org/10.1186/1471-2105-11-7

Tedersoo L, Mikryukov V, Zizka A et al (2022) Global patterns in endemicity and vulnerability of soil fungi. Glob Change Biol 28:6696–6710. https://doi.org/10.1111/gcb.16398

Tolgor B, Fan YG, Yang SS, Ge YP (2022) Flora fungorum sinicorum, vol. 53 (*Inocybaceae*). Science Press, Beiiing [in Chinese]

Vellinga EC (1988) Glossary. In: Bas C, Kuyper TW, Noordeloos ME, Vellinga EC (eds) Flora Agaricina Neerlandica, vol. 1. A.A. Balkema, Rotterdam, Brookfield, pp 54–64

White TJ, Bruns S, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protocols: a guide to methods and applications. Academic Press, New York, pp 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1