MYCOLOGIA

Mycologia

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/umyc20

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To cite this article: Marta Cortina-Escribano, Pyry Veteli, Michael John Wingfield, Brenda Diana Wingfield, Martin Petrus Albertus Coetzee, Henri Vanhanen & Riikka Linnakoski (2024) Phylogenetic analysis and morphological characteristics of laccate *Ganoderma* specimens in Finland, Mycologia, 116:6, 1046-1062, DOI: 10.1080/00275514.2024.2381424

To link to this article: https://doi.org/10.1080/00275514.2024.2381424

MYCOLOGIA
2024, VOL. 116, NO. 6, 1046–1062
https://doi.org/10.1080/00275514.2024.2381424

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Phylogenetic analysis and morphological characteristics of laccate *Ganoderma* specimens in Finland

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ABSTRACT

The *Ganoderma lucidum* complex includes fungi with similar morphologies but which are thought to represent different species. The lack of available type material and associated absence of multiple locus sequence data has complicated identification of these fungi. The aim of this study was to clarify the identity of the laccate *Ganoderma* species occurring in Finland by inferring a phylogeny using DNA sequences from available boreal-temperate material. DNA from Finnish isolates together with an older *G. lucidum* isolate originating from the United Kingdom was sequenced, and the morphological features of the Finnish specimens were examined. The phylogenetic analysis of the internal transcribed spacer region (ITS), the elongation factor 1-α (*tef1*), RNA polymerase II subunit (*rpb2*), and partial β-tubulin (*β-tub*) genes revealed that the *G. lucidum* isolate from the United Kingdom did not fall within a well-supported clade with other *G. lucidum* sequences or related species. The Finnish isolates were closely related to the *G. tsugae* lineage in *tef1*, *rpb2*, and *β-tub* phylogenies. However, *G. tsugae* appears morphologically distinct from the Finnish material. The results suggest that *G. tsugae*, or a species phylogenetically closely related to it, may occur in Finland. But further investigation into the relationship between *G. tsugae* and *G. lucidum* from Europe will be needed to clarify the identity of the laccate *Ganoderma* species in Finland.

ARTICLE HISTORY
Received 9 November 2023
Accepted 5 July 2024

KEYWORDS
Basidiomycota; biodiversity; Ganodermataceae; fungal diversity; Polyporales

INTRODUCTION

Ganoderma P. Karst includes a diversity of wood-decaying polyporoid fungal species. Several of these species are agents of tree diseases and thus of interest to tree health specialists (e.g., Coetzee et al. 2011, 2015; Paterson 2007). There are seven *Ganoderma* species known to occur in Europe, namely, *G. adspersum* (Schulzer) Donk, *G. applanatum* (Pers.) Pat., *G. carnosum* Pat., *G. lucidum* (Curtis) P. Karst., *G. pfeifferi* Bres., *G. resinaceum* Boud, and *G. valesiacum* Boud. Of these, *G. adspersum, G. applanatum, G. pfeifferi*, and *G. resinaceum* cause wood decay in living trees (e.g., Schwarze 2001; Terho et al. 2007). The other species are found mostly on dead trees and rarely on roots of living trees.

Two *Ganoderma* species, *G. lucidum* (subgenus *Ganoderma*) and *G. applanatum* (subgenus *Elfvingia*), have previously been recorded in Finland (Niemelä 1982; Niemelä and Kotiranta 1986). Both these taxa are known to include species having similar morphology but that are genetically distinct. They are therefore usually referred to as representing species complexes. In general, laccate specimens are referred to as *G. lucidum* and non-laccate specimens as *G. applanatum*.

In 1881, P. A. Karsten described the genus *Ganoderma* P. Karst, with *G. lucidum* as the only species (Karsten 1881). Karsten (1889) collected several specimens growing on oak (*Quercus robur*), alder (*Alnus* sp.), and spruce (*Picea abies*) wood stumps located in Ruissalo, Merimasku, and Vaasa (southwest and western Finland). The taxonomic history of *G. lucidum* dates back to 1781 when Curtis described *Boletus lucidus* Curtis (now the basionym of *G. lucidum*) using a specimen collected from London, UK, in 1780. The species has been reported worldwide based on identifications relying on morphological characteristics, although many of these collections represent other species. The holotype specimen has been lost and only an illustration of the basidiocarp is available (Curtis 1781),

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/00275514.2024.2381424.

Published online 12 Sep 2024

complicating efforts to stabilize the correct identity of *G. lucidum*. Attempts to locate a neotype specimen from the original type locality (Hyde Park, Peckham, London) have failed (Steyaert 1972). Consequently, this species lacks a type specimen suitable for DNA sequence–based studies that would be required to resolve this question.

Application of DNA sequence–based techniques in fungal taxonomy has improved the differentiation of species that share phenotypic characteristics and that therefore cannot be delineated based on morphology alone. Earlier studies have made use of single-locus sequences to differentiate species in *Ganoderma* and other fungi (Kwon et al. 2016; Moncalvo et al. 1995; Wang et al. 2009). However, several authors suggest that the analysis of a single or low number of genes may not resolve taxonomic questions, as a single (or few) gene tree do not necessarily reflect the species phylogeny (e.g., Rokas et al. 2003; Sun et al. 2022). Therefore, to discriminate between *Ganoderma* species, multilocus phylogenetic analyses have been performed by various authors to resolve problems that have arisen when only a single locus is used (Cabarroi-Hernández et al. 2019; Hapuarachchi et al. 2015; Loyd, Barnes et al. 2018a; Sun et al. 2022; Wang et al. 2012; Zhou et al. 2015). Also, a genealogical concordance phylogenetic species recognition approach that uses the concordance of different gene trees has been employed to resolve species in *Ganoderma* (e.g., Tchotet Tchoumi et al. 2019).

DNA sequences included in previous studies on *Ganoderma* were mainly from genes or regions located on the nuclear genome, with few studies including sequences from the mitochondrial genome. Sequences from the nuclear genome were generated from the internal transcribed spacer region (ITS) region (Cao et al. 2012; Hennicke et al. 2016; Jargalmaa et al. 2017; Loyd, Barnes et al. 2018a; Loyd, Richter et al. 2018b; Moncalvo et al. 1995; Park, Kwon, Son, Yoon, Han, Nam et al. 2012; Park, Kwon, Son, Yoon, Han et al. 2012; Wang et al. 2009; Zhou et al. 2015; Zhang et al. 2017), as well as partial sequences from genes coding the elongation factor 1-α (*tef1*), RNA polymerase II subunit (*rpb2*) (Cao et al. 2012; Jargalmaa et al. 2017; Loyd, Barnes et al. 2018a; Loyd, Richter et al. 2018b; Sun et al. 2022; Zhou et al. 2015), and partial β-tubulin (*β-tub*) (Hennicke et al. 2016; Park, Kwon, Son, Yoon, Han et al. 2012). Other than a small number of ITS sequences, there is no robust DNA sequence data set for Finnish *Ganoderma* specimens.

The aim of this study was to clarify the identity of the laccate *Ganoderma* species occurring in Finland. Phylogenetic and morphological studies on selected boreal-temperate collections from Satakunta and Uusimaa provinces in Finland were performed. We generated sequences from the ITS region and partial *tef1, rpb2*, and *β-tub* genes. These sequences were then compared with available sequence data of *G. lucidum* specimens from Europe and related species from East Asia and North America using phylogenetic methods. The morphological characteristics of selected Finnish specimens, including specimens from Karsten's collection (1889), were investigated and compared with those of other regions and other described species.

MATERIALS AND METHODS

Fungal isolates.—Laccate polypore specimens with a macromorphology similar to *G. lucidum* growing on *Picea abies* and *Betula pubescens* wood stumps were collected from Satakunta and Uusimaa provinces in Finland (TABLE 1). Dikaryotic strains were isolated from 25 specimens by cutting a 0.5 × 0.5 mm piece of tissue from the surface of the freshly collected wild basidiocarps context and transferring this to 2% malt extract agar [MEA: 20 g/L malt extract (VWR International LLC, USA) and 20 g/L agar bacteriological] medium and incubating the cultures at room temperature. Pure cultures were obtained by repeated transfers from the emerging colony margins, and these were stored on 5% MEA medium at 5 C. The dikaryotic state was evident from the presence of clamped septa in the cultures. The fungal isolates are preserved in the Culture Collection of Natural Resources Institute Finland (LUKE), Helsinki, Finland. Isolate CBS 170.30 labeled as *G. lucidum* collected in London and deposited by Kenneth St. G. Cartwright was obtained from the culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands, and included in this study. The isolate was transferred to 2% MEA medium and kept at room temperature.

Herbarium specimens.—Two specimens were collected for morphological characterization, one growing on *P. abies* and another on *B. pubescens*. The specimens were dried at 35 C and frozen for 2 weeks at −20 C to prevent invertebrate damage. The dried basidiocarps were deposited in the Botanical Museum of the University of Helsinki after examination. Herbarium specimens of several species of *Ganoderma* were obtained from the University of Turku Herbarium (Turku, Finland) and the Finnish Museum of National History Botanical Herbarium (Helsinki, Finland) (TABLE 2) for morphological studies. The *Ganoderma* specimens from conifers originated from Finland, Estonia, Russia (Central

Table 1. Strain names, origin and GenBank accession numbers of the sequences used in the phylogenetic analyses.

Species	Isolate	Origin	ITS	*tef1*	*β-tub*	*rpb2*	Reference
G. adspersum	SFC20141001-16	Korea	KY364251	KY393284	–	KY393270	Jargalmaa et al. (2017)
G. adspersum	LGAM 401 = ACAM DD2486	Greece	MG706206	MG837829	–	MG837781	Fryssouli et al. (NP)
G. annulare	KCTC 16803	Brazil	JQ520160	–	JQ675613	–	Park, Kwon, Son, Yoon, Han, Nam et al. (2012)
G. applanatum	IUM 3985	Netherlands	JQ520162	–	JQ675615	–	Park, Kwon, Son, Yoon, Han et al. (2012)
G. applanatum	SFC20150930-02	Korea	KY364258	KY393288	–	KY393274	Jargalmaa et al. (2017)
G. australe	CMW47785	South Africa	MH571686	MH567276	MH567310	–	Tchotet-Tchoumi et al. (2019)
G. australe	HUEFS:DHCR411	Australia	MF436675	MF436677	–	–	Costa-Rezende et al. (2017)
G. boninense	WD 2085	Japan	KJ143906	KJ143925	–	KJ143965	Zhou et al. (2015)
G. curtisii	CBS 100132	USA	JQ781849	KJ143927	JQ675617	KJ143967	Cao et al. (2012); Park, Kwon, Son, Yoon, Han, Nam et al. (2012); Zhou et al. (2015)
G. flexipes	Wei 5491	China	JQ781850	–	–	KJ143968	Cao et al. 2012; Zhou et al. (2015)
G. flexipes	Wei 5494	China	JN383978	–	–	–	Cao and Yuan (2013)
G. gibbosum	GZ14070501	China	MK345432	–	–	MK371436	Hapuarachchi et al. (2019)
G. gibbosum	SFC20150918-08	Korea	KY364271	KY393291	–	KY393278	Jargalmaa et al. (2017)
G. lingzhi	Wu 1006-38	China	JQ781858	JX029976	–	JX029980	Cao et al. (2012)
G. lingzhi	Dai 12374	China	JQ781867	–	–	–	Cao et al. (2012)
G. lingzhi	Cui 9166	China	KJ143907	JX029974	–	JX029978	Zhou et al. (2015)
G. lingzhi	SFC20150624-06	Korea	–	KY393279	–	KY393267	Jargalmaa et al. (2017)
G. lingzhi	MFLU 19-2209	Thailand	–	MN423165	–	MN423132	Luangharn et al. (NP)
G. lucidum	GI-4/CMI-UNIBO Glu5039	Armenia	JN588572	–	–	–	Iotti et al. (NP)
G. lucidum	IUM 4303	Bangladesh	JQ520182	–	JQ675635	–	Park, Kwon, Son, Yoon, Han, Nam et al. (2012)
G. lucidum	FBE 10458	Bulgaria	MG706224	–	–	MG837797	Fryssouli et al. (NP)
G. lucidum	ATCC 46755	Canada	JQ520185	–	JQ675638	–	Park, Kwon, Son, Yoon, Han, Nam et al. (2012)
G. lucidum	HKAS:48969	China	KC222322	–	–	–	Yang and Feng (2013)
G. lucidum	IUM 4242	China	JQ520186	–	JQ675639	–	Park, Kwon, Son, Yoon, Han, Nam et al. (2012)
G. lucidum	Cui 9207	China	KJ143910	KJ143928	–	KJ143970	Zhou et al. (2015)
G. lucidum	Cui 14405	China	MG279182	MG367574		MG367520	Xing et al. (2018)
G. lucidum	DB	China	KX589245	–	–	–	Zhang et al. (2017)
G. lucidum	G.260125-1	China	–	–	PRJNA71455	–	Chen et al. (2012)
G. lucidum	MT 26/10	Czech Republic	KJ143912	KJ143930	–	–	Zhou et al. (2015)
G. lucidum	Dai 11593	Finland	JQ781852	–	–	–	Cao et al. (2012)
G. lucidum	MUS1	Finland	ON598647	MW685379	MW685347	OM810210	This study
G. lucidum	MUS2	Finland	ON598648	MW685380	MW685348	OM810211	This study
G. lucidum	MUS3	Finland	ON598649	MW685381	MW685349	OM810212	This study
G. lucidum	MUS4	Finland	ON598650	MW685382	MW685350	OM810213	This study
G. lucidum	MUS5	Finland	ON598651	MW685383	MW685351	OM810214	This study
G. lucidum	MUS6	Finland	MT334583	MW685384	–	OM810215	This study
G. lucidum	MUS7	Finland	ON598652	–	MW685352	OM810216	This study
G. lucidum	MUS8	Finland	ON598653	MW685385	MW685353	OM810217	This study
G. lucidum	MUS9	Finland	MT334585	MW685386	MW685354	OM810218	This study
G. lucidum	MUS10	Finland	ON598654	–	MW685355	OM810219	This study
G. lucidum	MUS12	Finland	MT334586	MW685387	MW685356	OM810220	This study
G. lucidum	MUS13	Finland	ON598655	MW685388	MW685357	OM810221	This study
G. lucidum	MUS14	Finland	ON598656	MW685389	MW685358	–	This study
G. lucidum	MUS16	Finland	ON598657	MW685390	MW685359	OM810222	This study
G. lucidum	MUS17	Finland	ON598658	–	–	–	This study
G. lucidum	MUS18	Finland	ON598659	MW685391	MW685360	OM810223	This study
G. lucidum	MUS19	Finland	MT334587	MW685392	–	–	This study
G. lucidum	MUS20	Finland	ON598660	MW685393	MW685361	OM810224	This study
G. lucidum	MUS21	Finland	ON598661	MW685394	MW685362	OM810225	This study
G. lucidum	MUS23	Finland	ON598662	MW685395	MW685363	OM810226	This study
G. lucidum	MUS67	Finland	–	MW685396	MW685364	OM810227	This study
G. lucidum	MUS68	Finland	–	MW685397	MW685365	OM810228	This study
G. lucidum	MUS75	Finland	MT334584	–	MW685366	OM810229	This study
G. lucidum	MUS126	Finland	–	MW685398	–	–	This study
G. lucidum	MUS192	Finland	MT334582	–	–	–	Cortina-Escribano et al. (2020)
G. lucidum	M9720	France	KU310900	–	KU310902	–	Hennicke et al. (2016)
G. lucidum	Rivoire 4195	France	KJ143909	–	–	KJ143969	Zhou et al. (2015)
G. lucidum	MUCL 31549	France	MG706230	MG837845	–	MG837804	Fryssouli et al. (NP)
G. lucidum	LGAM 484 = ACAM 2013-0022	Greece	MG706227	MG837842	–	MG837801	Fryssouli et al. (NP)
G. lucidum	BCRC36123/ ATCC 32471	India	EU021459	–	–	–	Wang et al. (2009)
G. lucidum	HA2012-001	Iran	KX765192	–	–	–	Aghajani et al. (NP)
G. lucidum	G1T099	Italy	AM269773	–	–	–	Guglielmo et al. (2007)
G. lucidum	GlCN04	Italy	AM906058	–	–	–	Guglielmo et al. (2008)
G. lucidum	WD565	Japan	AB462322	–	–	AB368126	Sotome et al. (2008)
G. lucidum	WD2038	Japan	EU021456	–	–	–	Wang et al. (2009)

(*Continued*)

Table 1. (Continued).

Species	Isolate	Origin	ITS	*tef1*	*β-tub*	*rpb2*	Reference
G. lucidum	RDA-Yeongji-1/ ASI 7004	Korea	JQ520167	–	JQ675620	–	Park, Kwon, Son, Yoon, Han, Nam et al. (2012)
G. lucidum	IUM 0047	Korea	JQ520174	–	JQ675627	–	Park, Kwon, Son, Yoon, Han et al. (2012)
G. lucidum	KACC 42232	Korea	KT717954	–	–	–	Kwon et al. (2016)
G. lucidum	RYV 33217	Norway	Z37096/ Z37073	–	–	–	Moncalvo et al. (1995)
G. lucidum	FCL191	Poland	JQ627589	–	–	–	Siwulski et al. (NP)
G. lucidum	FCL188	Poland	JN008869	–	–	–	Siwulski et al. (NP)
G. lucidum	ZBS1	Russia	MF419230	–	–	–	Kokaeva et al. (NP)
G. lucidum	GL	Slovakia	MK415269	–	–	–	Gasparcova et al. (NP)
G. lucidum	GL81	Slovenia	KC311369	–	–	–	Tang and Zhang (NP)
G. lucidum	GLS-1	Spain	KT805317	–	–	–	Ozcariz Fermoselle (NP)
G. lucidum	Dai 2272	Sweden	JQ781851	–	–	–	Cao et al. (2012)
G. lucidum	BCRC37033	Taiwan	EU021462	–	–	–	Wang et al. (2009)
G. lucidum	ATCC 64251	Taiwan	JQ520187	–	JQ675640	–	Park, Kwon, Son, Yoon, Han, Nam et al. (2012)
G. lucidum	KCTC 16802	Thailand	JQ520188	–	JQ675641	–	Park, Kwon, Son, Yoon, Han et al. (2012)
G. lucidum	K 175217	UK	KJ143911	KJ143929	–	KJ143971	Zhou et al. (2015)
G. lucidum	HMAS86597	UK	AY884176	–	–	JF915436	Wang et al. (2012)
G. lucidum	CBS 176.30	UK	ON600478	OM810241	OM810242	OM810243	This study
G. lucidum	SP26	USA	AM269772	–	–	–	Guglielmo et al. (2007)
G. lucidum	UMNCA6	USA	MG654067	MG754724	–	–	Loyd, Barnes et al. (2018a)
G. lucidum	UMNUT7	USA	–	MG754726	–	–	Loyd, Richter et al. (2018)
G. lucidum	UMNUT1	USA	–	MG754725	–	–	Loyd, Barnes et al. (2018)
G. lucidum	UMNUT2	USA	MG654068	–	–	–	Loyd, Richter et al. (2018)
G. lucidum	GLVN02	Vietnam	MN636776	–	–	–	Bui and Nguyen (NP)
G. mirabile	CBS 218.36	Philippines	–	–	JQ675645	–	Park, Kwon, Son, Yoon, Han, Nam et al. (2012)
G. multipileum	CWN 04670	China	KJ143913	KJ143931	–	KJ143972	Zhou et al. (2015)
G. oregonense	ASI 7067	USA	JQ520197	–	JQ675650	–	Park et al. (2012)
G. oregonense	CBS 265.88	USA	JQ781875	KJ143933	–	KJ143974	Cao et al. (2012); Zhou et al. (2015)
G. oregonense	UMNAK1	USA	–	MG754740	–	–	Loyd, Barnes et al. (2018)
G. resinaceum	IUM 3651	Czech Republic	JQ520204	–	JQ675657	–	Park, Kwon, Son, Yoon, Han, Nam et al. (2012)
G. resinaceum	LGAM 344 = ACAM DD2380	Greece	MG706245	MG837853	–	MG837816	Fryssouli et al. (NP)
G. resinaceum	CBS 194.76/ BCRC36147	Netherlands	KJ143916	KJ143934	–	–	Zhou et al. (2015)
G. resinaceum	CBS 152.27	UK	JQ520200	–	JQ675653	–	Park, Kwon, Son, Yoon, Han, Nam et al. (2012)
G. resinaceum	HMAS86599	UK	AY884177			JF915435	Wang et al. (2012)
G. sichuanense	HMAS42798	China	JQ781877	–	–	–	Cao et al. (2012)
G. sichuanense	HMAS252081	China	KC662402				Yao et al. (2013)
G. tsugae	CBS 223.48	Canada	Z37054/ Z37079	–	–	–	Moncalvo et al. (1995)
G. tsugae	KCTC6457/ATCC 64795	Canada	JQ520215	–	JQ675668	–	Park, Kwon, Son, Yoon, Han, Nam et al. (2012)
G. tsugae	Dai 3937	China	JQ781853	–	–	–	Cao et al. (2012)
G. tsugae	Dai 15529	China	MG279197	MG367590	–	MG367536	Xing et al. 2018
G. tsugae	JV 0307/4 J	China	–	MG367562	–	MG367505	Xing et al. (2018)
G. tsugae	Cui 14112	China	MG279196	MG367587	–	MG367534	Xing et al. (2018)
G. tsugae	S90	China	–	PRJNA445345	PRJNA445345	–	NA
G. tsugae	KL20	India	FJ655478	–	–	–	Arulpandi and Kalaichelvan (NP)
G. tsugae	AFTOL-ID 771	USA?	DQ206985	–		DQ408116	Matheny et al. (2007)
G. tsugae	ASI 7064/KU-4018	USA	JQ520216	–	JQ675669	–	Park, Kwon, Son, Yoon, Han, Nam et al. (2012)
G. tsugae	KCTC6290/ATCC 64794	USA	–	–	JQ675673	–	Park, Kwon, Son, Yoon, Han, Nam et al. (2012)
G. tsugae	Dai 12760	USA	KJ143920	KJ143940	–	KJ143978	Zhou et al. (2015)
G. tsugae	UMNAZ9	USA	MG654321	MG754763	–	–	Loyd, Barnes et al. (2018)
G. tsugae	UMNMI30	USA	–	MH025362	–	MG754871	Loyd, Richter et al. (2018)
G. tsugae	UMNNC4	USA	MG654329	MG754765	–	MG754872	Loyd, Barnes et al. (2018)
G. tsugae	UMNMN7	USA	MG654327	–	–	–	Loyd, Richter et al. (2018)
G. tsugae	UMNWI22	USA	MG654344	–	–	–	Loyd, Barnes et al. (2018)
T. colossus	CGMCC5.763	Philippines	JQ081068	–	–	JQ081070	Wang et al. (2012)
T. colossus	TC-02	Vietnam	KJ143923	KJ143943	–	–	Zhou et al. (2015)
T. suaveolens	CBS 446.61	Austria	–	–	FJ410378	–	Lesage-Meessen et al. (2011)

Note. NP = Not published.

and Eastern Siberia), Slovakia, China, Canada, and USA, and those from deciduous trees were from Finland, Latvia, Sweden, Romania, and China.

DNA extraction, PCR, and sequencing.—Fungal isolates were grown on 50-mm Petri dishes containing modified orange serum 2% [MOS: 15 g/L orange

Table 2. Herbarium specimens examined.

Species	Specimen	Collector/collection	Date	Country	Region	Host	Host genus
G. carnosum	H7050121	G. Silaghi	16 September 1955	Romania	Transilvania	Deciduous	*Quercus*
	H7055041	P. Vampola exsiccati 139	30 September 1994	Slovakia	Badin	Conifer	*Abies*
G. lucidum	H6049741	P.A. Karsten	1858	Finland	Turku	Deciduous	*Quercus*
	TFU.186924	P. Kunttu 5950	19 November 2009	Finland	Kaarina	Conifer	*Picea*
	H6135371	P. Veteli 410	15 August 2017	Finland	Porvoo	Conifer	*Picea*
	TFU.186580	P. Kunttu 5752	7 October 2009	Finland	Kaarina	Conifer	*Picea*
	TFU.110754	M. Kyröläinen 239	30 August 1993	Finland	Vaasa	Conifer	*Picea*
	TFU.205608	P. Kunttu 7691	10 August 2011	Finland	Kotka	Deciduous	*Prunus*
	H6135369	E. Kalska	2017	Finland	Ylöjärvi	Conifer	*Larix*
	H6135370	P. Veteli 764	1 August 2018	Finland	Helsinki	Deciduous	*Betula*
	TFU.34356	U. Kalamees & K. Kalamees	30 April 1958	Estonia	Viimsi-Krillimäe	Conifer	*Picea*
	TFU.34357	J. Smarods	14 April 1936	Latvia	Vidzene	Deciduous	*Betula*
	H7049337	H. Kotiranta 28986	23 August 2007	Russia	Sakhalin	Conifer	*Abies*
	H7044873	H. Kotiranta 21344	16 August 2006	Russia	Perm region	Conifer	*Picea*
	H7029500	D. Hildebrandt K-05-09 & I. Stepanchikova	5 August 2009	Russia	Kamtschatca	Conifer	*Larix*
	H7050168	Y.C. Dai 2062	13 September 1995	China	NA	Conifer	*Pinus*
	H7050209	Z. Lu 37. J.-D. Zhao & X.-Q. Zhang	1979	China	Heilongjiang	Unknown	Unknown
	TFU.72720	H. Karlsted	9 May 1972	Sweden	NA	Deciduous	*Alnus*
G. oregonense	H7009574	O. Miettinen 19004.1	21 October 2014	USA	Washington	Conifer	*Tsuga*
	H7050171	T. Ahti 51047 & F.M. Rhoades	29 March 1992	USA	Washington	Conifer	*Tsuga*
G.tsugae	H7050170	J. H. Ginns 8800	2 September 1986	Canada	Ontario	Conifer	*Tsuga*
	H7008236	O. Miettinen 16813	14 April 2022	USA	Massachusetts	Conifer	*Tsuga*

serum agar (Berner Oy, Maharashtra, India), 20 g/L Bacto malt extract (Berner), 8 g/L Berner dextrose (Berner), 9 g/L Bacto agar (Berner)] medium or 2% MEA medium and a layer of sterilized cellophane. After 2–3 weeks of growth, DNA was extracted from the isolates using PrepMan Ultra Sample following the manufacturer's protocol (Applied Biosystems, Fosters City, California). DNA concentration was determined using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Madison, Wisconsin). The DNA was diluted to a working concentration of 50 ng/μL with SABAX water (Adcock Ingram, Midrand, South Africa). Four gene regions were amplified for the isolates. These included the ITS and regions of the *tef1, rpb2*, and *β-tub* genes. The primers used to amplify the ITS region were ITS1-F (5′-CTT GGT CAT TTA GAG GAA GTA A-3′) forward primer (Gardes and Bruns 1993) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) reverse primer (White et al. 1990). The primers used to amplify the gene for the *tef1* region were EF595F (5′-CGT GAC TTC ATC AAG AAC ATG-3′) forward primer and EF1160R (5′-CCG ATC TTG TAG ACG TCC TG-3′) reverse primer (Kauserud and Schumacher 2001). The β-tubulin gene region was amplified using β-tubF (5′-CCG GTG CAG GCA TGG GTA CC-3′) forward primer and β-tubR (5′-TGA AGA CGG GGG AAG GGA AC-3′) reverse primer (Park, Kwon, Son, Yoon, Han et al. 2012). The primers used to amplify the *rpb2* gene region were G-RPB2-F1 (5′-CAT CGA GTT CTT GGA GGA GTG G-3′) forward primer and G-RPB2-R1 (5′-CGG AAT GAT GCT GGC ACA GAC A-3′) reverse primer (Cao et al. 2012).

Polymerase chain reaction (PCR) mixtures included 2.5 μL of 10× KAPA Taq BUffer A (Bioline, London, United Kingdom), 0.5 μL of 25 mM $MgCl_2$ (Bioline), 0.5 μL of 10 mM dNTP Mix (Bioline), 1 μL of 10 μM forward primer, 1 μL of 10 μM reverse primer, 0.1 μL of 5 U/μL KAPA Taq DNA polymerase (Bioline), and 0.5 μL of template DNA (1 μL for *tef1* and *rbp2* gene regions). The volume of the mixture was adjusted to 25 μL with SABAX water. PCRs used the following thermal cycling protocol: initial denaturation (95 C for 3 min, 1 cycle), denaturation, annealing, extension (95 C for 30 , 52 C for 30 , 72 C for 1 min, respectively; 35 cycles), final extension (72 C, 10 min, 1 cycle), and hold at 4 C. After PCR amplification, amplicons were stained with GelRed nucleic acid gel stain (Biotium, Hayward, California) and separated using electrophoresis in a 1% agarose gel with 10× Tris-borate-EDTA (TBE) buffer. PCR products were observed under ultraviolet (UV) light and their sizes compared with a GeneRuler 100 bp Plus DNA ladder (Thermo Scientific, Vilnius, Lithuania) using Image Lab software (Bio-Rad, Hercules, California).

PCR products were purified with 8 μL of ExoSAP reagent [5 μL of Exo (exonuclease I; Thermo Fisher Scientific, Vilnus, Lithuania), 100 μL of SAP 1 U/μL (shrimp alkaline phosphatase; Roche, Mannheim, Germany), and 895 μL H_2O] to the remaining 20 μL of post-PCR product. The mixture was incubated at 37 C for 15 min to degrade remaining primers and nucleotides. A second incubation period at 80 C for 15 min was applied to inactivate ExoSAP reagent. The sequencing reactions of the purified PCR products were performed in a 12-μL reaction mixture [0.5 μL of BigDye Terminator v3.1 Ready Reaction Mix (Perkin-Elmer Applied Biosystems, Warrington, UK), 2.1 μL sequencing buffer, 1 μL of 10 mM primer, and 2 μL purified

PCR product]. The thermal cycling conditions followed the protocol of the manufacturer. The PCR products were precipitated with ethanol and 3 M pH 4.6 sodium acetate and dried in a laminar flow overnight. DNA sequencing was performed with an ABI Prism 3100 DNA analyzer (Applied Biosystems, Foster City, California) at the DNA Sequence Facility of the University of Pretoria.

The ITS and *rpb2* gene regions for some of the isolates and all the gene regions of strain CBS 176.30 were sequenced in the Viikki laboratory at the Natural Resources Institute Finland (Helsinki). The protocol used was similar to that described above, but Phusion Green High-Fidelity DNA Polymerase (Thermo Fisher Scientific, Vilnus, Lithuania) was used rather than KAPA Taq in the PCR master mix. The PCR cycling parameters and PCR cleanup protocol were the same as those mentioned above, and the PCR products were sequenced at Macrogen (Amsterdam, The Netherlands).

DNA sequence and phylogenetic analyses.—The forward and reverse sequences were assembled using Geneious 10.2.6 (Biomatters, Auckland, New Zealand). The consensus sequences were queried against the GenBank nucleotide database (https://www.ncbi.nlm.nih.gov/) using BLASTn search for preliminary identification of the isolates. Consensus sequences were deposited in GenBank (TABLE 1). Data sets were generated for each of the four loci (ITS, *tef1, β-tub*, and *rpb2*) separately. The data set included sequences from this study together with reference sequences from previous studies obtained from GenBank (Cao et al. 2012; Loyd, Barnes et al. 2018; Loyd, Richter et al. 2018; Park, Kwon, Son, Yoon, Han, Nam et al. 2012; Park, Kwon, Son, Yoon, Han et al. 2012; Wang et al. 2012; Zhou et al. 2015). Where available, the data sets included sequences from type strains and the closely related species *G. curtisii* (Berk.) Murrill, *G. lingzhi, G. oregonense* Murrill, and *G. tsugae* Murrill as well as strains from different geographic locations to show the heterogeneity among *Ganoderma* species (TABLE 1). Available sequences of *G. lucidum* from Europe, North America, and East Asia were included in all data sets. Available sequences representing *G. tsugae* from isolates originating in Canada, USA, China, and India (only ITS region) were also included in all data sets. Where available, representative sequences for *G. adspersum, G. annulare* (Fr.) Gilb., *G. applanatum, Ganoderma australe* (Fr.) Pat., *G. boninense* Pat., *G. flexipes* Pat., *G. gibbosum* (Blume & T. Nees) Pat., *G. mirabile* (Lloyd) C.J. Humphrey, *G. multipileum* Ding Hou, *G. resinaceum*, and *G. sichuanense* J.D. Zhao & X.Q. Zhang were included in all data sets.

Tropical *Ganoderma* species were not included in the final data sets after running preliminary phylogenetic analyses (data not shown), as they are very distinct from the European *Ganoderma* spp. The names of species shown in TABLE 1 correspond with those given in the GenBank records. *Tomophagus colossus* (Fr.) Murrill and *Trametes suaveolens* (L.) Fr. were selected as outgroup taxa (Tchotet Tchoumi et al. 2018). Individual data sets were compiled and edited using Molecular Evolutionary Genetic Analysis (MEGA) 10.0.5 (Kumar et al. 2018). The data sets were aligned using the online version of MAFFT 7 (Katoh and Standley 2013). The alignment strategies consisted of FFT-NS-i for the ITS data set and G-INS-i (default parameters) for the other data sets.

The phylogenetic analyses were performed using maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI) methods for each data set. ML analysis was conducted with the online version of PhyML 3.0 (http://www.atgc-montpellier.fr/phyml/; Guindon et al. 2010), using Smart Model Selection (SMS; Lefort et al. 2017) and Akaike information criterion (AIC) for substitute model selection. Approximate likelihood-ratio test (aLRT; Anisimova and Gascuel 2006) was selected for branch support estimation.

MP trees were inferred using PAUP 4.0a169 (Swofford 2003) with default parameters for parsimony analysis. A heuristic search with tree bisection reconnection (TBR) branch swapping was used to search tree space. The starting trees were obtained by random stepwise additions of sequences. Branches having a maximum length of zero were collapsed, and the gaps were considered as missing data. The maximum most parsimonious trees to be retained were set to 100.

BI analyses were carried out with MrBayes 3.2.7a (Ronquist et al. 2003) using a Markov chain Monte Carlo (MCMC) simulation. The GTR+G+I substitution model was preselected using the AIC in MrModeltest 3.0 (Nylander 2004). Four runs of MCMC chains for 5 million generations were done using a sample frequency of every 100th generation. The first 25% of the trees sampled were discarded as burn-in, and the posterior probabilities were calculated for the remaining trees. Effective sampling (ESS) and convergence of trees and posterior probabilities were assessed in Tracer 1.7.2 (Rambaut et al. 2018). The phylogenetic trees generated using the different methods were visualized in MEGA and post-edited using Inkscape 1.1.1 (https://inkscape.org/).

Morphological characterization.—The microscopic structures of the selected specimens (TABLE 2) were studied with a Leica DMLB 100T light microscope (Mannheim, Germany). The spore measurements were made using phase-contrast optics at ×1000 magnification in oil immersion (Leica Immersion Oil). Melzer's reagent was used to mount the spores before they were measured. The spore measurements included the myxosporium, the external layer of the spore wall structure. Fifty spores per specimen were measured, and 80% confidence was used for reporting the variation limits. Variation in spore size is reported as in Miettinen et al. (2006): number of spores measured (n), mean length (L), mean width (W), average length divided by average width (Q), and length and width ratio of individual spores (Q'). The whole range variation of L, W, and Q' is reported in parentheses, and the 80% confidence range is given without parentheses.

The terminology used to describe the spore wall structure, spore shape, and spore ornamentation followed Clémençon (2012), Niemela (2016), and Torres-Torres and Guzmán-Dávalos (2012), respectively. The shape of the dermis and the arrangements of the pileipellis dermal elements were described following the terminology of Torres-Torres and Guzmán-Dávalos (2012) and Clémençon et al. (2012). The general descriptive terms for the macroscopic features followed Niemela (1982), and Furtado (1981).

RESULTS

DNA sequences and phylogenetic analyses.—The ITS, *tef1, β-tub*, and *rpb2* gene regions were successfully amplified for most of the isolates. The amplicon size was approximately 650 bp long for the ITS region, 550 bp long for *tef1*, 410 bp long for *β-tub*, and 575 bp long for *rpb2* genes. The ITS, *tef1*, and *β-tub* gene regions were identical for all the Finnish isolates, and nearly identical for the *rpb2* gene region. ITS sequences had high sequence similarity (99–100%) to sequences of *G. lucidum* and *G. tsugae* on GenBank using BLASTn searches. The ITS sequence from the UK isolate (CBS 170.30) showed high sequence similarity to *G. carnosum* and *G. oregonense*. All Finnish strains sequenced in this study grouped together in the phylogenetic trees (FIGS. 1–4). The topology was similar for all the phylogenetic trees generated by ML, MP, and BI. Therefore, only the ML tree for each data set is presented, together with aLRT, bootstrap, and posterior probability values.

The ITS sequence data set included 82 taxa with two outgroup taxa (TABLE 3). The Finnish and UK sequences generated in this study formed a strongly supported clade (ML-aLRT and MP bootstrap values ≥95% and BI-PP ≥0.95) together with 26 isolates of *G. lucidum* from Europe, North America, and East Asia, 13 sequences for *G. tsugae* from East Asia and North America, and one sequence of *G. oregonense* (FIG. 1). Within this large clade, sequences of *G. tsugae* originating from India, USA, and Canada grouped in a subclade with strong statistical support from ML (aLRT = 98%) and BI (PP = 0.95) analyses. Two isolates, also labeled as *G. tsugae*, one from northern Arizona, USA (Loyd, Barnes et al. 2018a), and one from Konkuk University, also of USA origin (Park, Kwon, Son, Yoon, Han et al. 2012), and three isolates from China (Xing et al. 2018) grouped outside of this subclade. The phylogeny based on ITS sequence data was the only one that separated *G. tsugae* originating from USA from the Chinese *G. tsugae*. The remainder of the subclades in the phylogenetic tree were only supported in the ML analysis. Closely related species therefore could not be separated with confidence based on ITS sequence data.

The *tef1* sequence data set included 36 taxa and one outgroup taxon (TABLE 3). The phylogenetic tree generated from *tef1* sequence data (FIG. 2) grouped the UK isolate (CBS 176.30) within a major clade strongly supported by the three phylogenetic methods (aLRT branch support = 100%, MP bootstrap = 100%, and PP = 1) and included sequences of *G. tsugae, G. lucidum*, and *G. oregonense*. Within this clade, the species were separated into respective monophyletic clades with strong statistical support. Sequences for the Finnish isolates grouped with those of *G. tsugae* within a strongly supported clade. This clade included *G. tsugae* isolates from Connecticut, northern Arizona, North Carolina, and Michigan, USA, and from Jilin, China. Sequences of *G. lucidum* from UK, USA, France, Greece, China, and Czech Republic grouped in a strongly supported monophyletic clade. The third clade included *G. oregonense* isolates originating from USA. In contrast to the ITS phylogeny, the *tef1* phylogeny successfully separated closely related species. However, the UK isolate (CBS 176.30) was not placed in any of these clades and its identity remains unclear.

The *rpb2* data set consisted of 34 taxa and one outgroup taxon (TABLE 3). Like the *tef1* phylogeny, the *rpb2* analyses placed the Finnish and the UK *G. lucidum* isolates within a larger clade that included sequences from *G. lucidum, G. tsugae*, and *G. oregonense* (FIG. 3). The phylogenetic analyses also separated the sequences of *G. lucidum* in a subclade that was strongly supported by the three methods (aLRT branch support = 98%, MP bootstrap = 88%, and PP = 0.96). The Finnish isolates formed a clade with isolates representing *G. tsugae* from

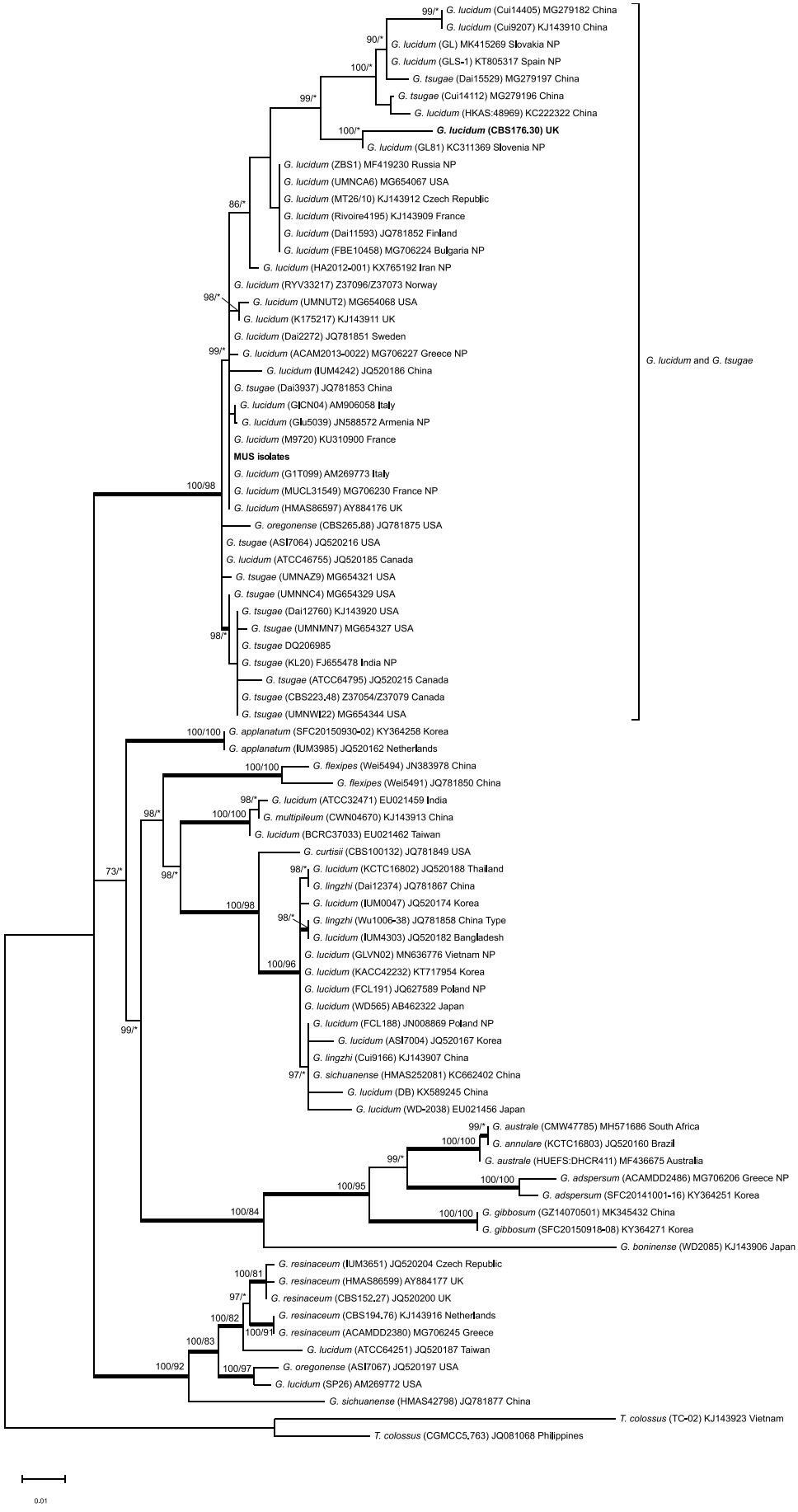

Figure 1. Maximum likelihood analyses based on the ITS sequence data. Branch support (aLRT) for ML and bootstrap values for MP higher than 70% from 1000 replicates are indicated in the nodes. Values below 70% are indicated with an asterisk. BI posterior probabilities higher than 0.95 are indicated by thick branch lines. Isolates sequenced in this study are indicated in bold.

China and USA, but it had statistical support only from the aLRT analysis (89%). Similar to the *tef1* phylogeny, the sequence generated in this study for the *G. lucidum* UK isolate did not group within the *G. lucidum* clade.

The *β-tub* data set included 24 taxa and one outgroup taxon (TABLE 3). The *β-tub* phylogeny formed a strongly supported major clade (aLRT branch support = 100%, MP bootstrap = 97%, and PP = 1) including isolates of *G. lucidum* from Korea, Thailand, China, and Bangladesh, a *G. curtisii* isolate from USA, and a *G. tsugae* isolate also from USA (FIG. 4). Isolate CBS 176.30 from the UK grouped within a well-supported major clade that included sequences from strains of *G. tsugae* and a *G. lucidum* isolate from Canada (aLRT branch support = 100%, MP bootstrap = 95%, and PP = 0.97). Within this major clade, the Finnish isolates formed a strongly supported subclade (aLRT = 100%, MP bootstrap = 96%, and PP = 1) with isolates representing *G. tsugae* from Canada and China. Only one *β-tub* sequence from European *G. lucidum* material was available in GenBank (strain M9720 from France), and it did not group within the East Asian *G. lucidum* clade nor the *G. tsugae* clade.

Morphological characterization.—The Finnish isolates were identical based on DNA sequence comparisons, and the basidiocarps collected displayed only expected phenotypic variation (i.e., color range and shape). The basidiocarps of the Finnish specimens are clearly stipitate, large, and flabelliform. The context of the Finnish specimens presents creamy white color on the stipe and subdermal part, and light brown to beige in the layer next to the concolourous tubes. The specimen H6135370 (southern Finland) is macroscopically representative of *G. lucidum* as understood in Finland (FIG. 6); however, it is also a good example of the problem associated with morphological variation in *Ganoderma* spp. Specimen H6135370 has consistently more narrow spores than is usual for Finnish *G. lucidum*, with average width of only 6.0 microns, and average Q-value of 1.7. This is likely due to the specimen being in the beginning of prime sporulation phase at the time of the collection. The other end of the spectrum is Karsten's oldest collection, H6049741, with spore size with an average of 10.3 × 6.9 Microns µm, Q 1.5 (TABLE 4). The width of the spore in H6049741 is, however, still within the range of typical Finnish laccate *Ganoderma* (6–7 microns).

The Finnish isolates grouped together with *G. tsugae* in the phylogenetic trees, but comparisons of spore and basidiocarp morphology showed that they are slightly distinct from each other. *G. tsugae* does not differ much in spore dimensions from the Finnish specimens (TABLE 4; FIG. 5) but often appears to have slightly longer echinulate. Moreover, *G. tsugae* may have on average slightly longer hymenodermis terminal elements. This is, however, highly dependent on basidiocarp stage. Macroscopically, *G. tsugae* has often a deeper color and shinier appearance than the Finnish herbarium specimens and all the specimens collected in this study. *G. carnosum* fruiting bodies have a darker color and wider spores (>7 microns width) compared with laccate *Ganoderma* originating in Finland.

DISCUSSION

The *G. lucidum* complex is a taxonomically complicated group that invokes considerable debate regarding species boundaries. This arises from a lack of type material, an absence of cultures and, thus, reliable sequence data, as well as the fact that the complex includes several species with overlapping morphological characteristics. In this study, we focused on the phylogenetic analysis and morphological descriptions of north European boreal-temperate material resembling *G. lucidum*. The studied gene regions were nearly identical for all the Finnish isolates and grouped together in the phylogenetic trees. Surprisingly, the Finnish material, widely assumed to correspond with the European *G. lucidum*, grouped with *G. tsugae* in the phylogenetic analyses. However, the morphology of the Finnish material was slightly different from that of *G. tsugae* from North America and northeast Asia.

There are two nomenclatural outcomes for the Finnish laccate *Ganoderma* species. If the laccate *Ganoderma* material from Finland is indeed conspecific with North American *G. tsugae, G. valesiacum* should be considered the valid name, with *G. tsugae* as a later synonym, as was previously suggested (Adaskaveg and Gilbertson 1986; Stalpers 1978). This nomenclatural proposal requires further study of authentic *G. valesiacum* specimens. Alternatively, the Finnish laccate specimen represents *G. lucidum* sensu stricto. Given the lack of type material from the original type locality (UK), an epitype should be selected for this species. The basionym of *G. lucidum* is a sanctioned name by Fries (1821), *P. lucidus*. Thus, morphological examination of material noted by Fries and obtaining DNA sequence data for the material could aid in providing an epitype of *G. lucidum*. If the *P. lucidus* material collected by Fries exists, sequence data from that specimen would resolve the relationship with the Finnish laccate *Ganoderma* species.

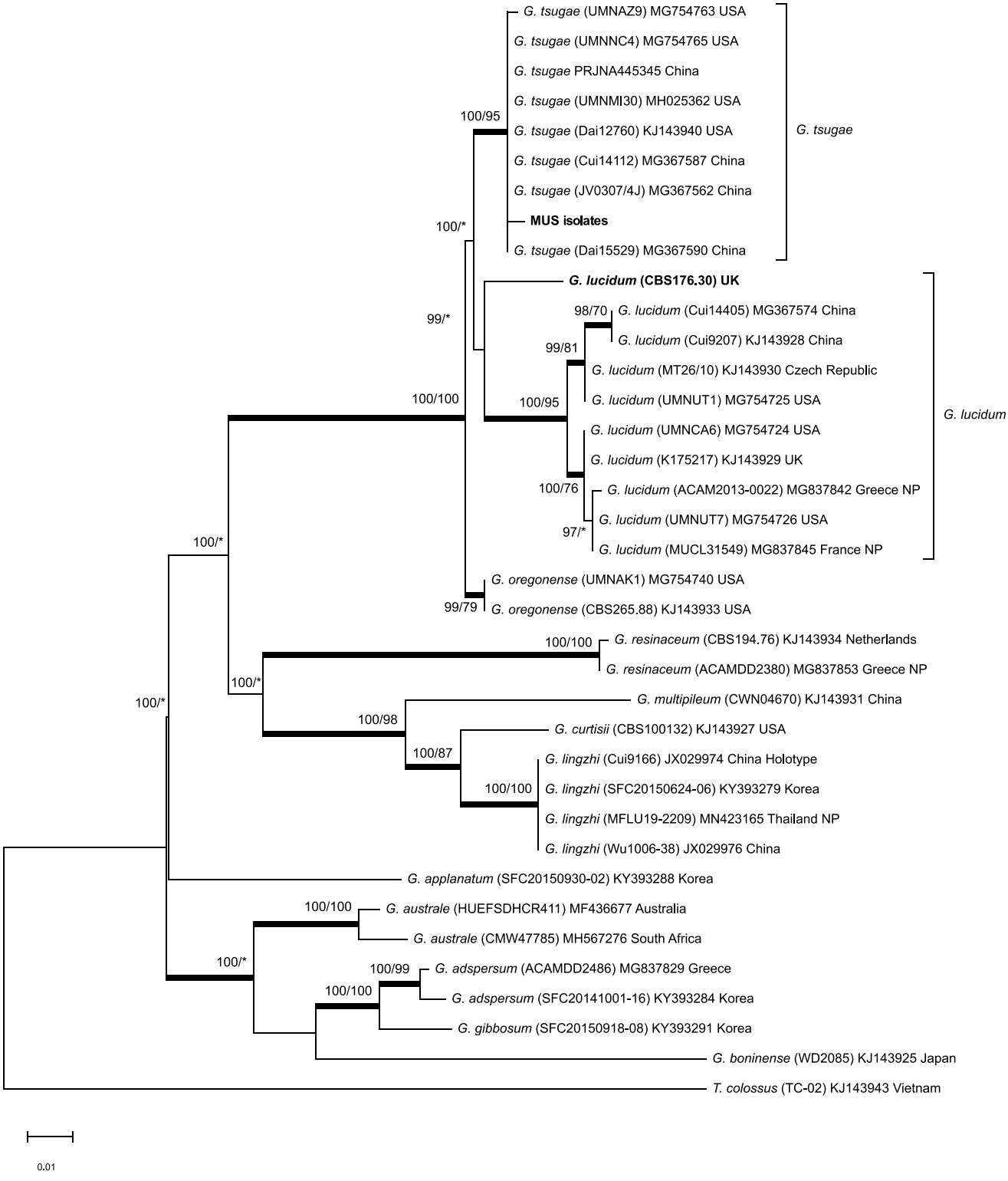

Figure 2. Maximum likelihood phylogenetic tree derived from the *tef1* sequence data including the isolates used for DNA sequencing in this study (in bold). Branch support (aLRT) and bootstrap values ≥70% from 1000 replicates are indicated in the nodes for ML and MP, respectively. Values below 70% are indicated with an asterisk. BI posterior probabilities ≥0.95 are shown in thick lines.

Ganoderma tsugae was first described by Murrill (1902) as a species occurring predominantly on *Tsuga canadensis* (Pinaceae). This tree species is confined to North America; hence, it has been assumed that *G. tsugae* is native to North America (Loyd, Barnes et al. 2018a). *Ganoderma tsugae* has been predominantly reported on conifers such as *Abies* spp. and *Larix* spp., and it is assumed being strictly associated with these

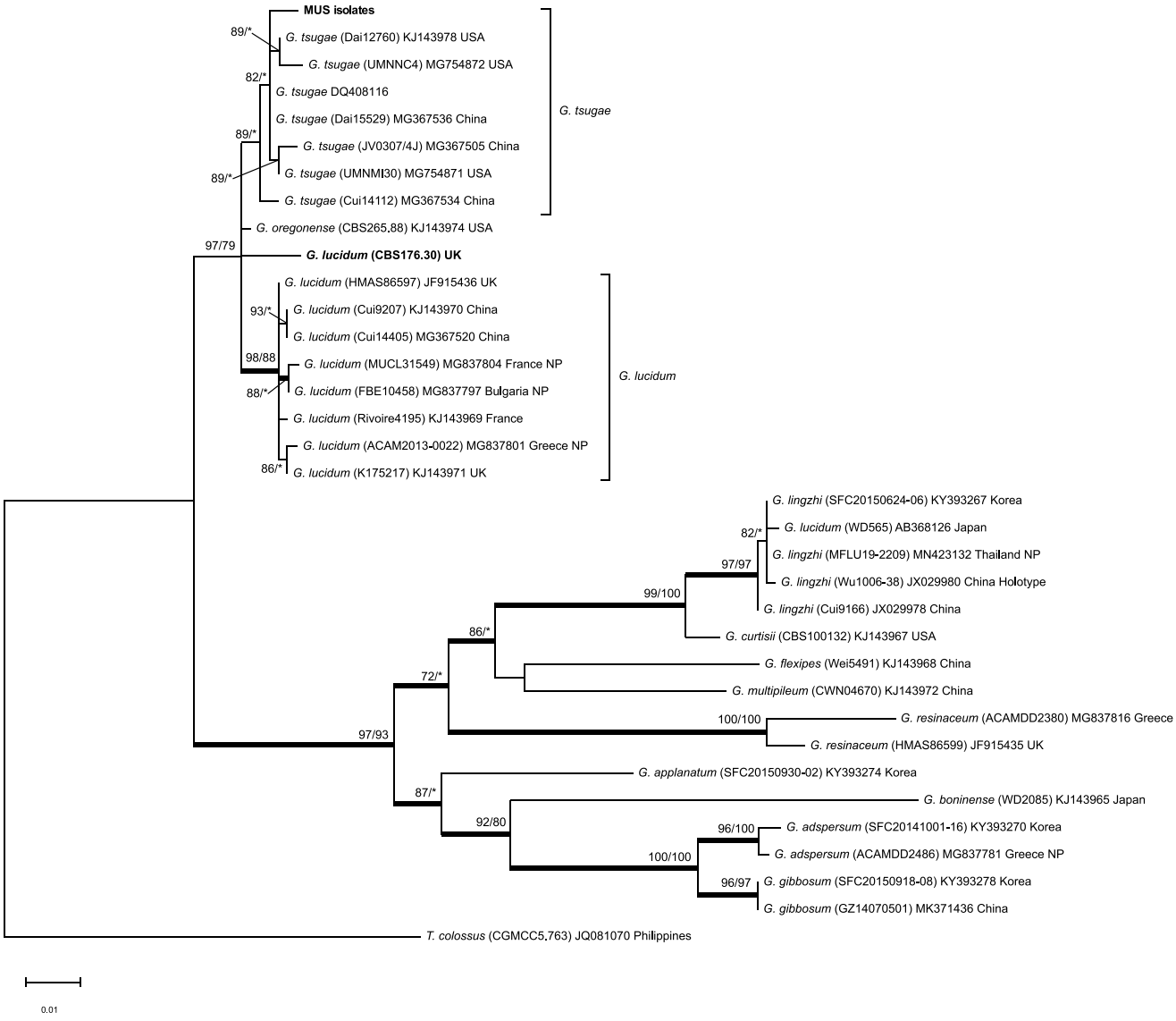

Figure 3. Maximum likelihood phylogenetic tree constructed from the *rpb2* data set. Isolates sequenced in this study are in bold. Branch support (aLRT) and bootstrap values higher than 70% are indicated in the nodes (ML/MP, respectively). Values below 70% are indicated with an asterisk. BI posterior probabilities higher than 0.95 are indicated by thick lines in the branches.

hosts (Loyd, Barnes et al. 2018a; Xing et al. 2018). Adaskaveg and Gilbertson (1988) reported *G. tsugae* occurring also on *Betula* spp. in North America. In his description of *G. tsugae*, Murrill (1902) noted that the specimens found by Karsten (1881) growing on *Picea excelsa* could have been *G. tsugae* rather than "*Ganoderma pseudoboletus*." However, Karsten (1881) reported *G. lucidum* ("*G. pseudoboletus*") occurring on both conifer and deciduous trees. Our results support the observation of Karsten (1881), in which he reported a single species (*G. lucidum* in Fungi Fenniae Exsiccati) growing on both hardwoods and conifers in Finland. As *G. lucidum* commonly grows on conifer substrates in Finland, *G. carnosum*, a European laccate species growing preferably in conifer hosts, has been actively looked for (Niemela and Kotiranta 1986). However, to date, no basidiocarps having spore sizes of *G. carnosum* have been recorded from Finland.

The basidiocarp morphology of the Finnish specimens considered in this study was slightly different from that of *G. tsugae*. Basidiocarps of the *G. tsugae* specimens examined often presented a deeper color and shinier appearance than the Finnish *G. lucidum* material. Overholts (1953) noted similar morphological differences when comparing specimens thought to represent *P. tsugae and P. lucidus* in North America and concluded that they are indeed separate species. The spore characteristics showed that the North

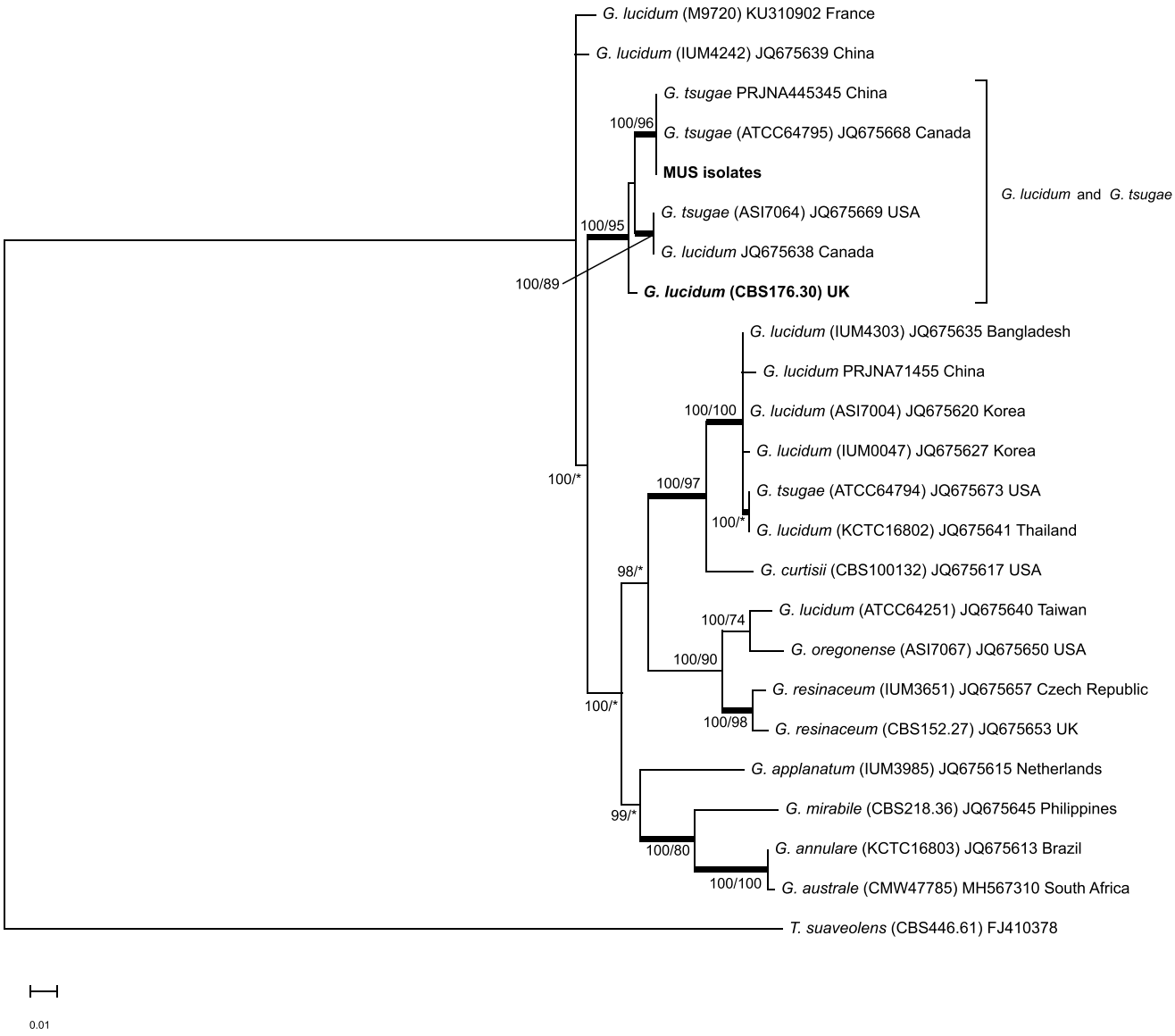

Figure 4. Maximum likelihood phylogenetic tree generated from the *β-tub* data set. Isolates used for DNA sequencing in this study are in bold. ML branch support (aLRT) followed by MP bootstrap values above 70% are indicated in the nodes. Values below 70% are indicated with an asterisk. BI posterior probabilities above 0.95 are indicated in the branches (bold lines).

American *G. tsugae* spores have similar dimensions to those of the Finnish specimens in this study, but with coarser ornamentation. *Ganoderma oregonense*, a species closely related to *G. tsugae*, has generally larger spores (Adaskaveg and Gilbertson 1988; Atkinson 1908; Murrill 1908) compared with the Finnish material examined in this study. However, the spore dimensions may differ at different sporulation phases of the same specimen. Moreover, the natural variation within the populations and the influence of the environmental conditions could also affect morphological characteristics of the basidiocarps (Adaskaveg and Gilbertson 1986). Such variation is well known, and Nuss (1982), for example, reported differences in size and appearance of early and later spores from several *Ganoderma* species, including *G. lucidum*.

Previous phylogenetic studies have separated the North American *G. tsugae* from European *G. lucidum* (Loyd, Barnes et al. 2018a; Sun et al. 2022; Zhou et al. 2015). The ITS data generated in the present study did not delimit strains of European *G. lucidum*, East Asian *G. lucidum*, North American *G. tsugae*, and Chinese *G. tsugae* in distinct clades. The *tef1, rpb2*, and *β-tub* phylogenies separated the European *G. lucidum* and the Finnish strains in different clades, the latter grouping together with *G. tsugae*. This is consistent with a recent taxonomic revision of Ganodermataceae by Sun et al. (2022) in which it was shown that sequence data for the

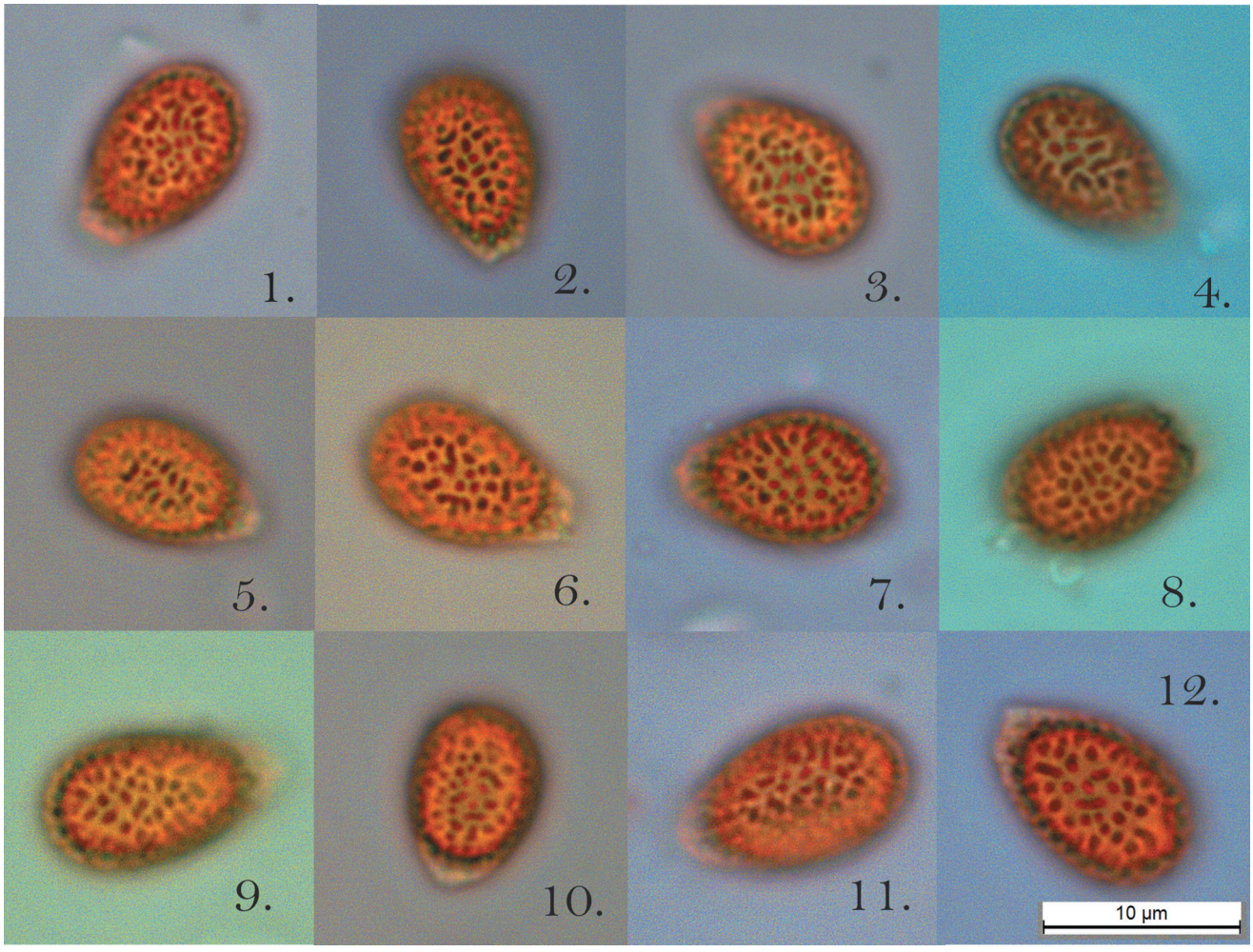

Figure 5. Spore morphology variation of specimens representing *Ganoderma lucidum* (1) H7704873, (2) H6049741, (3) H7029500, (4) H7044337, (5) H7050168, (6) H7050209, (7) H6135371/MUS11, (8) TFU.186580, and (9) kalske2017; *Ganoderma tsugae* (10) H7050170; *Ganoderma oregonense* (11) H7050171; and *Ganoderma carnosum* (12) H7055041.

Table 3. Statistics resulting from the phylogenetic analyses.

			Maximum parsimony								Maximum likelihood	
Data set	No. of taxa	No. of bp	PCC	PIC	No. of trees	Tree length	CI	RI	RC	HI	Substitution model	NST
ITS	84	616	423	137	100	383	0.68	0.898	0.61	0.321	HKY85+G+I	4
tef1	37	583	414	121	100	338	0.68	0.853	0.58	0.32	GTR+G	4
rpb2	35	590	428	122	100	315	0.65	0.832	0.54	0.352	GTR+I	1
β-tub	25	413	396	59	4	188	0.73	0.822	0.6	0.271	GTR+G	4

Note. PIC = number of parsimony informative characters; CI = consistency index; RI = retention index; RC = rescaled consistency index; HI = homoplasy index; NST = number of substitution rate categories.

ITS region alone do not differentiate *Ganoderma* species sufficiently. Previous studies have demonstrated that for species in the *G. lucidum* complex, *tef1* and *rpb2* loci are more informative than the ITS region (Loyd, Barnes et al. 2018a; Loyd, Richter et al. 2018b; Sun et al. 2022).

There are some constraints when constructing phylogenetic trees from *tef1*, *rpb2*, and *β-tub* gene regions for taxonomic purposes. Most important is the lack of sequence data from most or all loci for some crucial species belonging to the *G. lucidum* complex (i.e., *G. carnosum*, *G. mongolicum* Pilát, and *G. valesiacum*). This results in phylogenies based on only the ITS or one additional locus. Rokas et al. (2003) showed that a single locus or low number of loci does not provide support in resolving phylogenetic questions due to incongruent tree topologies that can be generated from different DNA regions. Thus, we suggest employing the genealogical concordance phylogenetic species recognition

Figure 6. Basidiocarp morphology of specimen H6135370 collected from *Betula* sp. stump in the region of Helsinki.

Table 4. Basidiospore characteristics of *Ganoderma* specimens.

Species	Specimen	Country	n Microns (µm)	L variation Microns (µm)	L avg. Microns (µm)	W variation Microns (µm)	W avg.	Q' variation	Q avg.	Host genus
G. carnosum	H7050121	Romania	37	(9.7)10.4–11.7(12.3)	11.1	(6.6)7–7.8(8)	7.4	(1.3)1.4–1.6	1.5	*Quercus*
	H7055041	Slovakia	42	(8.9)10.6–12.6(13.2)	11.6	(6.5)7.1–7.9(8.3)	7.5	(1.3)1.4–1.7(1.8)	1.5	*Abies*
G. lucidum	H6049741	Finland	50	(8.8)9.6–10.9(11.3)	10.3	(5.9)6.5–7.3(7.5)	6.9	(1.4)1.42–1.6(1.7)	1.5	*Quercus*
	TFU.186924	Finland	50	(10)10.1–11.8(12.7)	11	(5.8)6.4–7.5(9)	7	(1.4)1.44–1.7(1.9)	1.6	*Picea*
	H6135371	Finland	50	(9.4)9.8–11.2(11.5)	10.6	(6.3)6.5–7.2(7.6)	6.9	(1.4)1.5–1.6(1.7)	1.5	*Picea*
	TFU.186580	Finland	50	(10)10.4–11.8(12.4)	11.2	(6.3)6.6–7.5(7.8)	7.1	(1.4)1.5–1.6(1.8)	1.6	*Picea*
	TFU.110754	Finland	50	(8.8)10–11.7(12.8)	10.9	(5.8)6.4–7.2(7.5)	6.8	(1.4)1.5–1.7(1.8)	1.6	*Picea*
	TFU.205608	Finland	50	(8.4)9.2–10.5(10.8)	9.8	(5.6)6.2–7.1(7.6)	6.7	(1.3)1.4–1.6(1.7)	1.5	*Prunus*
	H6135369	Finland	50	(8.7)9.3–11.6(12.9)	10.6	(6.1)6.4–7.2(7.5)	6.8	(1.2)1.4–1.7(1.9)	1.6	*Larix*
	H6135370	Finland	30	(8.8)9.0–11.0(11)	10.0	(5.3)5.8–6.1(6.3)	6.0	(1.4)1.5–1.8(1.9)	1.7	*Betula*
	TFU.34356	Estonia	50	(9.6)10.1–11.6(12.4)	10.9	(6)6.5–7.3(8)	7.0	(1.3)1.5–1.7(1.8)	1.6	*Picea*
	TFU.34357	Latvia	30	(8.9)9.3–10.9(11)	10.1	(5.9)6.3–7.2(7.4)	6.8	(1.3)1.4–1.6	1.5	*Betula*
	H7049337	Russia	40	(8.7)9.3–11(11.4)	10.1	(5.4)5.6–6.9(7.2)	6.2	(1.4)1.5–1.7(1.9)	1.6	*Abies*
	H7044873	Russia	50	(8.6)9.1–11.1(12.3)	10.0	(5.5)6–7.3(8.2)	6.6	(1.3)1.4–1.6(1.8)	1.5	*Picea*
	H7029500	Russia	50	(8.9)9.5–11.3(12)	10.4	(6)6.2–7.1(7.6)	6.7	(1.4)1.45–1.7	1.6	*Larix*
	H7050168	China	50	(8.7)9.2–10.5(10.8)	9.8	(5.7)6–6.7(6.9)	6.4	(1.4)1.5–1.7(1.9)	1.6	*Pinus*
	H7050209	China	50	(8.6)9.9–11.5(12.3)	10.7	(5.3)5.9–7(7.2)	6.5	(1.4)1.6–1.8(2)	1.7	Unknown
	TFU.72720	Sweden	50	(9.1)9.9–11.2(13.2)	10.5	(6.1)6.6–7.6(7.7)	7	(1.3)1.4–1.6(1.7)	1.5	*Alnus*
G. oregonense	H7009574	USA	34	(11)12–13(14)	12.4	(7)7.3–8(8.4)	7.8	(1.4)1.5–1. 8(1.8)	1.6	*Tsuga*
	H7050171	USA	50	(10.4)11.1–13.1(13.6)	12.3	(6.6)6.7–7.9(8.3)	7.3	(1.4)1.6–1.8(1.9)	1.7	*Tsuga*
G. tsugae	H7050170	Canada	50	(9.3)9.9–11.1(11.7)	10.5	(6.4)6.5–7.2(7.4)	6.9	(1.4)1.44–1.6(1.7)	1.5	*Tsuga*
	H7008236	USA	34	(9.5)10–12(12)	10.9	(6)6.5–7.4(7.5)	7	(1.3)1.4–1.7(1.8)	1.6	*Tsuga*

Note. Spores measured (n), mean length (L), mean width (W), average length divided by average width (Q), and length and width ratio of individual spores (Q'). The whole range variation of L, W, and Q' is reported in parentheses, and the 80% confidence range is given without parentheses.

approach to further study the relationships between the Finnish *G. lucidum* and related species.

The concept of *G. lucidum* sensu stricto is ambiguous, leading to a difficult delimitation between closely related species. Host-tree specificity, morphological characteristics, interfertility between isolates, and phylogenetic and phylogenomic analyses are some aspects that need to be considered for species recognition. The

present phylogenetic and morphological analyses could not confirm the identity of the laccate *Ganoderma* species of Finland, other than the fact that it is a single taxon that resides in the *G. lucidum* complex. Regarding the nomenclature related to *G. lucidum* sensu stricto, the most convenient approach would be to select an epitype following the recommendations of the International Code of Nomenclature for algae, fungi, and plants. We thus recommend that interfertility tests be performed between *G. tsugae* originating from North America and China, the selected *G. lucidum* sensu stricto epitype, and *G. lucidum* originating from Finland, Siberia, and the UK. Morphological and multilocus phylogenetic studies of less scrutinized *Ganoderma* species (i.e., *G. carnosum, G. mongolicum* Pilát, and *G. valesiacum*) as well as those originating from undersampled boreal regions, such as Siberia, are also needed to resolve the identity of the laccate *Ganoderma* species occurring in Finland.

ACKNOWLEDGMENTS

We thank the University of Turku Herbarium (Turku, Finland) and the Finnish Museum of National History Botanical Herbarium for allowing us to examine their fungal collections. We are also grateful to Prof. Tuan Duong and Dr. Suvi Sutela for their help with our PCRs and to the citizens and field workers who collected the specimens used in this study, and to Luke's and FABI's laboratories and laboratory staff.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the author(s).

FUNDING

This work was supported by Niemi Foundation under grant 20180020 and by the Wihuri Foundation under grant 200042/3b. This work was also supported by Luke Leads under MushValue project 41007-00098000 and InnoFungi project 41007-00158400; by Business Finland Research to Business funding under Natural Antivirals project 42431/31/2020; by Academy of Finland under Antivirals from Forest Biomasses: Structure, Function, and Applicability project 342250; by the Regional Council of North Karelia EAFRD program under MushroomHarvester project EURA2014/8860/09020101/2019/PKARJALA; and by the Regional Council of Lapland EAFRD program under FeedFUNK project EURA2014/10739/09020101/2020/LL. The strains used in this work were collected in MushValue project; Sievi project 68873 funded by EU Rural Development Programme for Mainland Finland 2007–2013; and LUMO-INKA project 2845/31/2015 funded by Tekes.

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