A fifty-locus phylogenetic analysis provides deep insights into the phylogeny of Tricholoma (Tricholomataceae, Agaricales)

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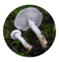

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A fifty-locus phylogenetic analysis provides deep insights into the phylogeny of *Tricholoma* (*Tricholomataceae*, *Agaricales*)

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Key words

ectomycorrhizal fungi
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Abstract As an ectomycorrhizal fungal genus that contains matsutake and other edible mushrooms, *Tricholoma* has great economic and ecological significance. However, the phylogenetic relationships within the genus remain unsettled. To clarify the infrageneric relationships of *Tricholoma*, including the identification of monophyletic subgenera and sections, three phylogenetic analyses were conducted employing single-locus (ITS), five-locus (ITS/*RPB2*/*EF-1α*/*MCM7*/mtSSU) and 50-locus (45 single-copy orthologous genes plus the aforementioned ones) DNA nucleotide sequences. Our data indicated that ITS sequences could serve the species delimitation of *Tricholoma* in most cases and monophyletic groups recognition in some cases, and the five-locus dataset could resolve a section-level phylogeny of this genus, while the 50-locus dataset could clarify the delimitation of subgenera and settle the relationships among sections within this genus. A fifty-locus dataset was firstly employed to construct a robust phylogeny of *Tricholoma*. Based on this, a new infrageneric arrangement for the genus *Tricholoma*, with four subgenera, of which two are in accordance with the previous subgenera *Pardinicutis* and *Sericeicutis*, and eleven sections, is suggested. Subgenus *Pardinicutis*, occupying the basal position, only harbors sect. *Pardinicutis*, while the subg. *Sericeicutis* comprises sects. *Lasciva* and *Sericella* located at the sub-basal position with good support. Subgenus *Terrea* is newly erected here and consists of sect. *Terrea*, sect. *Atrosquamosa* and two as yet unnamed phylogenetic lineages. Besides an unnamed section-level lineage, subg. *Tricholoma* consists of sects. *Genuina*, *Muscaria*, *Rigida*, *Tricholoma*, *Fucata* and *Matsutake*, of which the two latter are newly proposed. The previously defined subg. *Contextocutis* is clustered within subg. *Tricholoma* and is a synonym of the latter. *Tricholoma colossus*, *T. acerbum* and their allies, which used to be allocated in sect. *Megatricholoma* (or genus *Megatricholoma*), are relocated to sect. *Genuina* since they form a strongly supported monophyletic group and share rusty or black spots on lamellae with other species in this section. Taxonomic descriptions of the new infrageneric taxa and a key to subgenera and sections of the genus *Tricholoma* are presented.

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INTRODUCTION

Mycorrhizal fungi play vital roles in forest ecosystems. All species of the genus *Tricholoma* (*Tricholomataceae*, *Agaricales*) are known or supposed to be ectomycorrhizal (ECM), and fungi in this genus mainly form symbiotic associations with trees of the families *Pinaceae*, *Betulaceae*, *Fagaceae*, *Salicaceae*, *Myrtaceae* and *Nothofagaceae* (Bougher 1995, Tedersoo et al. 2010, Bessette et al. 2013, Christensen & Heilmann-Clausen 2013, Sánchez-García & Matheny 2016, Heilmann-Clausen et al. 2017, Reschke et al. 2018). Certain *Tricholoma* species are known to form dual ectomycorrhizal and monotropoid associations, linking trees and monotropoid plants (Bidartondo & Bruns 2001, Leake et al. 2004). The genus harbors several famous delicacy mushrooms known as matsutake, including the 'true' matsutake *T. matsutake* and its allies, such as *T. anatolicum*, *T. bakamatsutake*, *T. fulvocastaneum*, *T. magnivelare*, *T. mesoamericanum* and *T. murrillianum*, many of which have great commercial and cultural significance in East Asia (Zang 1990, Wang et al. 1997, Chapela & Garbelotto 2004, Matsushita et al. 2005, Suzuki 2005, Ota et al. 2012, Heilmann-Clausen et al. 2017), while several other species, such as those in sect. *Pardinicutis*, are known to cause severe gastrointestinal upset (Bessette et al. 2013, Christensen & Heilmann-Clausen 2013, authors' observations), and the edibility of many others remains to be determined (Nieminen & Mustonen 2020).

Tricholoma was erected as a genus by Staude (1857), with *T. flavovirens* as the type species. However, as this species is currently considered a synonym of *T. equestre*, the latter has been regarded as the generic type (Moukha et al. 2013, Heilmann-Clausen et al. 2017). The genus is dominantly distributed in temperate to subtropical ecosystems, and is characterized by fleshy basidiomata, adnexed to emarginate lamellae, a central stipe, white spore prints, smooth basidiospores, simple pileipellis structures and often the absence of well-differentiated cystidia (Kost 1981, Singer 1986, Christensen & Noordeloos 1999, Bessette et al. 2013, Christensen & Heilmann-Clausen 2013, Heilmann-Clausen et al. 2017, Reschke et al. 2018). Traditionally, the basidiospores were treated as inamyloid.

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However, a recent study indicated that the basidiospores of the tested species are weakly amyloid if sufficient time passes (at least 30 min.) or the slide preparation is heated before observation (Vizzini et al. 2020).

A number of infrageneric classifications of *Tricholoma* have been proposed from Friesian times until quite recently. Singer (1986) divided *Tricholoma* in four subgenera, namely *Contextocutis*, *Sericeicutis*, *Pardinicutis* and *Tricholoma*, with nine sections. However, contemporaneous mycologists proposed different treatments that contain seven to eleven sections within this genus (Bon 1984, 1991, Christensen & Noordeloos 1999, Noordeloos & Christensen 1999, Riva 1998, 2003, Christensen & Heilmann-Clausen 2008). For example, sect. *Genuina* in Singer (1986) covers four sections of Noordeloos & Christensen (1999), namely sect. *Imbricata*, sect. *Albobrunnea*, sect. *Caligata* and sect. *Megatricholoma*.

With the advent of molecular phylogenetics, the polyphyly of the *Tricholomataceae* in Singer (1986) was demonstrated by Moncalvo et al. (2002) and Matheny et al. (2006), and recent phylogenetic studies have reduced *Tricholomataceae* s.str. to nine monophyletic genera (Sánchez-García et al. 2014, Vizzini et al. 2016, Sánchez-García et al. 2021). Of these, *Tricholoma* is a monophyletic group since sects. *Leucorigida*, *Iorigida* and *Adusta* in Singer (1986) have been relocated to genera including *Leucocalocybe*, *Macrocybe*, *Melanoleuca* and *Tricholosporum* (Pegler et al. 1998, Noordeloos & Christensen 1999, Yu et al. 2011, Bessette et al. 2013, Christensen & Heilmann-Clausen 2013, Sánchez-García et al. 2014, Angelini et al. 2017, Heilmann-Clausen et al. 2017, Reschke et al. 2018).

The combination of morphological and molecular approaches has become the preferred method to study the systematics of *Tricholoma* since Mankel et al. (1998). Both Heilmann-Clausen et al. (2017) and Reschke et al. (2018) provided comprehensive treatments of this genus including molecular phylogenetic information based on the nuclear ribosomal internal transcribed spacer (ITS) sequences. Heilmann-Clausen et al. (2017) accepted 10 sections that are mainly consistent with previous infrageneric classifications, but with some substantial changes, and Reschke et al. (2018) proposed sect. *Muscaria* to accommodate *T. muscarium* and its allies, and recognized *T. acerbum* and *T. roseoacerbum* as a separated clade, in spite of Heilmann-Clausen et al. (2017) considering these two species as members of sect. *Megatricholoma*.

Furthermore, ITS has also been employed to clarify the taxonomy and phylogeny of several cryptic groups, including the *T. matsutake*, *T. equestre*, *T. sulphureum* and *T. scalpturatum* species complexes (Bergius & Danell 2000, Chapela & Garbelotto 2004, Comandini et al. 2004, Matsushita et al. 2005, Carriconde et al. 2008, Trudell et al. 2017), and two or even multiple gene fragments were used to identify diversity within these groups (Bao et al. 2007, Jargeat et al. 2010, Ota et al. 2012, Moukha et al. 2013). However, only a limited number of species were included in these studies. Although more than 30 species have been described based on phylogenetic analyses in recent years (Christensen & Heilmann-Clausen 2009, Hosen et al. 2016, Heilmann-Clausen et al. 2017, Trudell et al. 2017, Yang et al. 2017, Ovrebo & Hughes 2018, Reschke et al. 2018, Ovrebo et al. 2019, Xu et al. 2020, Trudell & Parker 2021, Ushijimaa et al. 2021, Ayala-Vásquez et al. 2022, Cui et al. 2022, Ding et al. 2022), the infrageneric classification of the genus remains unsettled.

ITS is being used as a universal DNA barcode marker for fungi (Nilsson et al. 2008, Begerow et al. 2010, Schoch et al. 2012). However, to infer the phylogenetic relationships within a large genus like *Tricholoma*, which is likely to have more than 300 species (Christensen & Heilmann-Clausen 2013), ITS sequences alone may be too variable to capture deeper phylogenetic relationships. Although many ITS sequences are accessible for *Tricholoma*, the phylogenetic relationships within this genus are still far from clear. Therefore, the aims of this study are

1. to construct phylogenetic frameworks of *Tricholoma* with single-locus, five-locus and fifty-locus DNA sequence data, and to compare the similarities and differences among them;
2. to evaluate the monophyly of previously proposed subgenera and sections; and
3. to gain deeper understanding of the phylogenetic relationships among subgenera and sections in the genus *Tricholoma*.

MATERIALS AND METHODS

Materials studied

A total of 170 specimens were included in this study, including 167 specimens newly collected from Belarus, Canada, China and Germany and representing all the major clades of *Tricholoma* suggested by previous studies. Three specimens of *Leucopaxillus* and *Pseudotricholoma* were selected as outgroups based on Sánchez-García et al. (2014). Material studied in this study was deposited in the following herbaria: Cryptogamic Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS), Herbarium Marburgense, University of Marburg (MB), Herbarium of Mycology, Jilin Agricultural University (HMJAU) and Alpine Fungarium, Tibet Plateau Institute of Biology (AF). Specimen information and GenBank accession numbers for sequences of five commonly used gene fragments obtained in this study are listed in Table 1, while ITS sequences available in GenBank from previous studies are listed in Appendix 1. Sequences of 45 single-copy orthologous genes obtained in this study were deposited in GenBank (GenBank accession numbers: MW730186–MW730516, MW743318–MW747885, MW774653–MW774779).

Morphological observation and description

Notes of macroscopic descriptions are based on detailed field notes and digital images of fresh basidiomata. Colors of fresh specimens were documented from Kornerup & Wanscher (1981). Microscopic studies were conducted using a Zeiss Axiostar Plus microscope following the standard method described in Yang et al. (2017) and Cui et al. (2018).

DNA extraction, PCR and sequencing

Genomic DNA was extracted from silica-gel dried materials or fungarium materials using the modified CTAB method (Doyle & Doyle 1987). Five gene fragments commonly used in phylogeny, including three protein-coding gene fragments (the second-largest subunit of RNA polymerase (*RPB2*), translation elongation factor 1-*α* (*EF-1α*) and minichromosome maintenance protein 7 (*MCM7*)), and two non-protein coding regions (ITS and mitochondrial small subunit rDNA (mtSSU)) (Matheny et al. 2007, Mouhamadou et al. 2008, Schmitt et al. 2009, Schoch et al. 2012, Sánchez-García et al. 2014), as well as 45 single-copy gene fragments selected from Sato et al. (2017) were amplified to conduct the phylogenetic framework of *Tricholoma*. The primer pairs used in this study, including universal primer pairs and newly designed primer pairs are listed in Appendix 2.

The PCR reactions were conducted on an ABI 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA) or an Eppendorf Master Cycler (Eppendorf, Netheler-Hinz, Hamburg, Germany), and the reactions were conducted using the following profiles: pre-denaturation at 94 °C for 5 min, followed by 35

Table 1 Taxa information and GenBank accession numbers for sequences of five commonly used gene fragments obtained in this study.

Taxon	Voucher	Locality	Typification	GenBank accession				
				ITS	*EF-1α*	*RPB2*	*MCM7*	mtSSU
Tricholoma aff. *album*	HKAS105390	China, Liaoning		MW724441	MW730033	–	–	MW732356
T. aff. *atrosquamosum*	HKAS97910	China, Sichuan		MW724382	MW729977	MW729846	MW730116	MW732309
	HKAS97211	China, Sichuan		MW724388	MW729983	MW729852	MW730122	MW732314
	HKAS106310	China, Yunnan		MW724462	MW730050	–	–	MW732372
	HKAS55164	China, Yunnan		–	–	MW729915	–	MW732386
T. aff. *atroviolaceum*	AF0001640	China, Tibet		MW724455	MW730043	–	MW730169	MW732366
T. albobrunneum	HKAS71269	China, Yunnan		MW724336	MW729930	MW729803	MW730078	MW732266
	HKAS57016	China, Yunnan		MW724391	MW729987	MW729856	MW730126	MW732318
	HKAS68189	China, Yunnan		MW724479	MW730063	MW729914	MW730185	MW732385
T. album	MB-006366	Germany, Hessen		MW724416	MW730009	–	MW730146	–
	MB-006323	Germany, Hessen		MW724421	MW730014	–	MW730151	–
T. argyraceum	HKAS106661	China, Sichuan		MW724415	MW730008	MW729875	MW730145	MW732336
	MB-003509	Germany, Hessen		MW724417	MW730010	MW729876	MW730147	MW732337
	MB-003519	Germany, Hessen		MW724418	MW730011	MW729877	MW730148	MW732338
T. aurantium	HKAS106523	China, Xinjiang		MW724395	MW729990	MW729859	MW730128	MW732320
	HKAS106548	China, Xinjiang		MW724414	MW730007	–	MW730144	MW732335
	HKAS94389	Canada, Ontario		MW724367	MW729962	MW729833	–	–
T. bakamatsutake	HKAS106313	China, Yunnan		MW724402	MW729997	MW729866	MW730135	MW732326
	HKAS107570	China, Yunnan		MW724468	MW730054	MW729906	MW730178	MW732376
	HKAS106301	China, Yunnan		MW724449	–	–	MW730164	–
T. bonii	HKAS78979	China, Yunnan		MW724326	MW729920	–	MW730069	MW732256
	HKAS106563	China, Yunnan		MW724399	MW729994	MW729863	MW730132	MW732323
	HMGAU35946	China, Heilongjiang		MW724393	–	–	–	–
T. boudieri	HKAS74089	China, Yunnan		MW724322	MW729917	MW729791	MW730065	MW732252
	HKAS97163	China, Sichuan		MW724373	MW729968	MW729838	MW730108	MW732300
	HKAS97070	China, Sichuan		MW724437	MW730029	MW729888	MW730158	MW732353
T. cingulatum	HKAS106634	China, Sichuan		MW724406	MW730000	MW729868	MW730137	MW732328
	HKAS106635	China, Sichuan		MW724407	MW730001	MW729869	MW730138	MW732329
	MB-003515	Germany, Hessen		MW724425	MW730017	MW729880	–	MW732342
T. citrinum	HKAS71086	China, Yunnan	Holotype	MW724356*	MW729950	–	MW730097	MW732286
T. equestre	HMGAU22249	Belarus, Gomel		MW724392	MW729988	MW729857	–	MW732319
T. filamentosum	MB-000950	Germany, Baden-Württemberg		MW724422	MW730015	MW729878	MW730152	MW732339
	MB-002942	Germany, Hessen		MW724423	MW730016	MW729879	MW730153	MW732340
T. focale	HKAS106309	China, Yunnan		MW724460	MW730049	MW729902	MW730175	–
T. frondosae type I	HKAS87149	China, Yunnan		MW724346	–	MW729813	–	MW732276
	HKAS98072	China, Sichuan		MW724365	MW729960	MW729831	MW730104	MW732294
T. fulvocastaneum	HKAS107567	China, Yunnan		MW724465	MW730052	MW729904	MW730176	MW732374
	HKAS107568	China, Yunnan		MW724466	MW730053	MW729905	MW730177	MW732375
	HKAS107571	China, Yunnan		MW724469	–	–	–	–
	HKAS107572	China, Tibet	Holotype	MW724472*	MW730057	MW729909	MW730180	MW732379
	HKAS107576	China, Tibet		MW724473*	MW730058	MW729910	MW730181	MW732380
T. highlandense	HKAS74293	China, Yunnan		KY488546*	MW729919	MW729793	MW730067	MW732254
	HKAS76215	China, Sichuan		KY488548*	MW729933	MW729806	MW730080	MW732268
	HKAS70192	China, Yunnan	Holotype	KY488549*	MW729939	MW729811	MW730086	MW732274
	HKAS107590	China, Yunnan		MW724452	–	–	–	–
T. imbricatum	HKAS87886	China, Tibet		MW724327	MW729921	MW729794	MW730070	MW732257
	HKAS112559	China, Yunnan		MW724476	MW730060	–	–	MW732382
T. inocybeoides	HKAS89215	China, Sichuan		MW724375	MW729970	MW729839	MW730109	MW732302
	HKAS106525	China, Xinjiang		MW724396	MW729991	MW729860	MW730129	MW732321
	MB-003516	Germany, Hessen		–	MW730018	MW729881	–	MW732343
T. mastoideum	HKAS97096	China, Sichuan	Holotype	MW724357*	MW729951	MW729823	–	MW732287
	HKAS97105	China, Sichuan		MW724362*	MW729956	MW729827	–	–
T. matsutake	HKAS57470	China, Yunnan		MW724350	MW729945	MW729818	MW730092	MW732280
	HKAS98323	China, Sichuan		MW724385	MW729980	MW729849	MW730119	MW732311
	HKAS106299	China, Yunnan		MW724403	–	–	–	–
	HKAS107569	China, Yunnan		MW724467	–	–	–	–
T. muscarioides	HKAS69737	China, Yunnan		MW724358	MW729952	MW729824	MW730098	MW732288
T. muscarium	HKAS76274	China, Yunnan		MW724339	MW729934	MW729807	MW730081	MW732269
	HKAS106307	China, Yunnan		MW724442	MW730034	–	MW730159	MW732357
T. olivaceoluteolum	HKAS68691	China, Yunnan		MW724353	MW729948	MW729821	MW730095	MW732283
	HKAS89668	China, Yunnan		MW724378	MW729973	MW729842	MW730112	MW732305
T. olivaceotinctum	HKAS50898	China, Sichuan		MW724372	MW729967	MW729837	MW730107	MW732299
	HKAS99374	China, Sichuan		MW724380	MW729975	MW729844	MW730114	MW732307
	HKAS107586	China, Sichuan		MW724405	MW729999	–	–	–
T. olivaceum	HKAS71335	China, Yunnan		MW724338	MW729932	MW729805	MW730079	MW732267
	HKAS68600	China, Yunnan		MW724351	MW729946	MW729819	MW730093	MW732281
T. orienticolossus	HKAS98045	China, Sichuan		MT124444*	MW729958	MW729829	MW730102	MW732292
T. orientifulvum	HKAS105383	China, Liaoning		MW724439	MW730031	MW729890	–	MW732354
	HKAS105381	China, Liaoning		MW724438	MW730030	MW729889	–	–
T. pardinum	MB-006381	Germany, Baden-Württemberg		MW724424	–	–	–	MW732341
T. pessundatum	HKAS97076	China, Sichuan		MW724321	MW729916	MW729790	MW730064	MW732251
	HKAS97827	China, Sichuan		MW724381	MW729976	MW729845	MW730115	MW732308
T. populinum	HKAS106656	China, Sichuan		MW724410	MW730004	MW729872	MW730141	MW732332
	HKAS106657	China, Sichuan		MW724411	MW730005	MW729873	MW730142	MW732333
T. portentosum	HKAS71728	China, Yunnan		MW724343	MW729938	MW729810	MW730085	MW732273

Table 1 (cont.)

Taxon	Voucher	Locality	Typification	GenBank accession				
				ITS	*EF-1α*	*RPB2*	*MCM7*	mtSSU
T. portentosum (cont.)	HKAS97075	China, Sichuan		MW724478	MW730062	MW729913	MW730184	MW732384
	HKAS74375	China, Yunnan		MW724461	–	MW729903	–	–
T. psammopus	HKAS106302	China, Inner Mongolia		MW724436	MW730028	MW729887	–	MW732352
	HKAS106314	China, Gansu		MW724458	MW730046	MW729899	MW730172	MW732369
T. roseoacerbum	HKAS88046	China, Yunnan		MW724332	MW729926	MW729799	MW730075	MW732262
T. saponaceum	HKAS79747	China, Yunnan		MW724335	MW729929	MW729802	MW730077	MW732265
	HKAS97949	China, Sichuan		MW724389	MW729984	MW729853	MW730123	MW732315
	HKAS106668	China, Sichuan		MW724412	–	–	–	–
T. scalpturatum	MB-003485	Germany, Hessen		MW724426	MW730019	MW729882	MW730154	MW732344
	MB-003513	Germany, Hessen		MW724427	MW730020	–	–	MW732345
T. sinoacerbum	HKAS78806	China, Hubei		MW724325	–	–	MW730068	MW732255
	HKAS105349	China, Yunnan		MW724434	MW730026	MW729886	–	MW732350
	HKAS105388	China, Liaoning		MW724440	MW730032	MW729891	–	MW732355
T. sinopardinum	HKAS57199	China, Tibet		KY488550*	MW729944	MW729817	MW730091	MW732279
	HKAS82533	China, Tibet	Holotype	KY488552*	MW729949	MW729822	MW730096	MW732284
	HKAS91129	China, Sichuan		MW724361	MW729955	–	–	–
T. sinoportentosum	HKAS90838	China, Yunnan		MW724359	MW729953	MW729825	MW730099	MW732289
	HKAS90900	China, Sichuan		MW724360	MW729954	MW729826	MW730100	MW732290
T. stans	HKAS87940	China, Yunnan		MW724329	MW729923	MW729796	MW730072	MW732259
	HKAS99382	China, Sichuan		MW724390	MW729985	MW729854	MW730124	MW732316
	HKAS82121	China, Yunnan		MW724344	–	–	–	–
T. stiparophyllum	MB-002925	Germany, Hessen		MW724419	MW730012	–	MW730149	–
	MB-003514	Germany, Hessen		MW724420	MW730013	–	MW730150	–
	HMGAU7821	China, Inner Mongolia		MW724464	–	–	–	–
T. sulphurescens type I	MB-102501	Germany		MF034302*	MW730021	MW729883	–	–
T. sulphureum type I	HKAS55509	China, Yunnan		MW724369	MW729964	MW729835	–	MW732296
	HKAS53473	Germany, Hessen		MW724354	–	–	–	MW732285
T. sulphureum type II	AF0001459	China, Tibet		MW724453	MW730041	–	MW730167	MW732364
	HKAS106305	China, Yunnan		MW724463	MW730051	–	–	MW732373
T. terreum	HKAS69401	China, Yunnan		MW724333	MW729927	MW729800	MW730076	MW732263
	MB-006378	Germany, Hessen		MW724428	MW730022	–	–	MW732346
	HKAS69914	China, Yunnan		MW724459	MW730048	MW729901	MW730174	MW732371
	HKAS52233	China, Yunnan		MW724355	–	–	–	–
T. triste	HKAS68012	China, Yunnan		MW724374	MW729969	–	–	MW732301
	AF0001608	China, Tibet		MW724454	MW730042	–	MW730168	MW732365
T. ustaloides	HKAS74246	China, Yunnan		MW724331	MW729925	MW729798	MW730074	MW732261
T. vaccinum	HKAS87929	China, Yunnan		MW724328	MW729922	MW729795	MW730071	MW732258
	HKAS98037	China, Sichuan		MW724363	MW729957	MW729828	MW730101	MW732291
	HKAS98065	China, Sichuan		MW724364	MW729959	MW729830	MW730103	MW732293
T. virgatum	HKAS97078	China, Yunnan		MW724334	MW729928	MW729801	–	MW732264
	HKAS91176	China, Hubei		MW724368	MW729963	MW729834	–	MW732295
Tricholoma sp. 1	HKAS55189	China, Yunnan		MW724379	MW729974	MW729843	MW730113	MW732306
Tricholoma sp. 2	HKAS106317	China, Gansu		MW724456	MW730044	MW729898	MW730170	MW732367
	HKAS106318	China, Gansu		MW724457	MW730045	–	MW730171	MW732368
Tricholoma sp. 3	HKAS101296	China, Yunnan		MW724446	MW730037	MW729894	MW730162	MW732360
Tricholoma sp. 4	HKAS54922	China, Yunnan		MW724432	MW730024	MW729884	–	MW732348
	HKAS107574	China, Tibet		MW724471	MW730056	MW729908	–	MW732378
Tricholoma sp. 5	HKAS97909	China, Sichuan		MW724383	MW729978	MW729847	MW730117	MW732310
	HKAS99397	China, Sichuan		MW724384	MW729979	MW729848	MW730118	–
Tricholoma sp. 6	HKAS106484	China, Yunnan		MW724349	MW729943	MW729816	MW730090	MW732278
	HKAS49645	China, Yunnan		MW724470	MW730055	MW729907	MW730179	MW732377
Tricholoma sp. 7	HKAS97220	China, Yunnan		–	MW729986	MW729855	MW730125	MW732317
	HKAS106283	China, Yunnan		MW724433	MW730025	MW729885	MW730156	MW732349
	HKAS63047	China, Yunnan		MW724448	MW730038	MW729895	MW730163	MW732361
Tricholoma sp. 8	HKAS74156	China, Yunnan		MW724323	MW729918	MW729792	MW730066	MW732253
	HKAS63020	China, Yunnan		MW724444	MW730035	MW729892	MW730160	MW732359
Tricholoma sp. 9	HKAS99360	China, Sichuan		MW724386	MW729981	MW729850	MW730120	MW732312
	HKAS99361	China, Sichuan		MW724387	MW729982	MW729851	MW730121	MW732313
Tricholoma sp. 10	HKAS70214	China, Yunnan		MW724345	MW729940	MW729812	MW730087	MW732275
	HKAS68655	China, Yunnan		MW724352	MW729947	MW729820	MW730094	MW732282
Tricholoma sp. 11	HKAS106308	China, Sichuan		MW724404	MW729998	MW729867	MW730136	MW732327
	HKAS105344	China, Sichuan		MW724413	MW730006	MW729874	MW730143	MW732334
	HKAS105540	China, Yunnan		MW724447	–	–	–	–
Tricholoma sp. 12	HKAS49388	China, Sichuan		MW724371	MW729966	MW729836	MW730106	MW732298
Tricholoma sp. 13	HKAS81257	China, Yunnan		MW724342	MW729937	MW729809	MW730084	MW732272
Tricholoma sp. 14	HKAS97080	China, Yunnan		MW724337	MW729931	MW729804	–	–
Tricholoma sp. 15	HKAS56154	China, Yunnan		MW724477	MW730061	MW729912	MW730183	MW732383
Tricholoma sp. 16	HKAS106583	China, Yunnan		MW724397	MW729992	MW729861	MW730130	MW732322
	HKAS106610	China, Yunnan		MW724401	MW729996	MW729865	MW730134	MW732325
Tricholoma sp. 17	HKAS92443	China, Yunnan		MW724376	MW729971	MW729840	MW730110	MW732303
	HKAS52260	China, Yunnan		MW724377	MW729972	MW729841	MW730111	MW732304
Tricholoma sp. 18	HKAS73361	China, Yunnan		MW724341	MW729936	MW729808	MW730083	MW732271
	HKAS105356	China, Yunnan		MW724435	MW730027	–	MW730157	MW732351
	HKAS69777	China, Yunnan		MW724451	MW730040	MW729897	MW730166	MW732363
Tricholoma sp. 19	HKAS74195	China, Yunnan		MW724330	MW729924	MW729797	MW730073	MW732260
	HKAS73277	China, Yunnan		MW724340	MW729935	–	MW730082	MW732270
	HKAS108098	China, Yunnan		MW724475	–	–	–	–

Table 1 (cont.)

Taxon	Voucher	Locality	Typification	GenBank accession				
				ITS	*EF-1α*	*RPB2*	*MCM7*	mtSSU
Tricholoma sp. 20	HKAS97051	China, Yunnan		MW724366	MW729961	MW729832	MW730105	–
	HKAS97661	China, Yunnan		MW724398	MW729993	MW729862	MW730131	–
	HKAS101291	China, Yunnan		MW724445	MW730036	MW729893	MW730161	–
Tricholoma sp. 21	HKAS91028	China, Tibet		MW724347	MW729941	MW729814	MW730088	–
Tricholoma sp. 22	HKAS106303	China, Yunnan		MW724450	MW730039	MW729896	MW730165	MW732362
Tricholoma sp. 23	HKAS101281	China, Yunnan		MW724443	–	–	–	MW732358
Tricholoma sp. 24	HKAS74948	China, Yunnan		MW724348	MW729942	MW729815	MW730089	MW732277
	HAKS107581	China, Yunnan		MW724400	MW729995	MW729864	MW730133	MW732324
Tricholoma sp. 25	HKAS106315	China, Gansu		–	MW730047	MW729900	MW730173	MW732370
Tricholoma sp. 26	HKAS106652	China, Sichuan		MW724408	MW730002	MW729870	MW730139	MW732330
	HKAS106653	China, Sichuan		MW724409	MW730003	MW729871	MW730140	MW732331
Tricholoma sp. 27	HKAS107577	China, Sichuan		MW724474	MW730059	MW729911	MW730182	MW732381
Tricholoma sp. 28	HKAS49355	China, Sichuan		MW724370	MW729965	–	–	MW732297
Tricholoma sp. 29	HKAS58046	China, Yunnan		MW724430	MW730023	–	–	–
Tricholoma sp. 30	HKAS79926	China, Tibet		MW724431	–	–	–	–
Leucopaxillus laterarius	HKAS106319	China, Xinjiang		MW724394	MW729989	MW729858	MW730127	–
Leucopaxillus tricolor	MB-000946	Germany, Baden-Württemberg		MW724429	–	–	MW730155	MW732347
Pseudotricholoma metapodium	MB-002938	Germany, Thüringen		–	–	–	–	–

* indicates sequences retrieved from GenBank database.

cycles of denaturation at 94 °C for 50 s, annealing at 50–56 °C for 55 s, elongation at 72 °C for 55 s, and a final elongation at 72 °C for 8 min. The PCR products were purified with a Gel Extraction and PCR Purification Combo Kit (Spin-column) (Bioteke, Beijing, China), and then sequenced on an ABI-3730-XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA) using the same primer pairs as in the PCR amplification. Forward and reverse sequences were assembled and edited with SeqMan (DNA STAR package; DNA Star Inc., Madison, WI, USA).

To generate sequences of the 45 single-copy orthologous gene fragments, a two-step PCR was performed for these representative samples. In the first step, the primers were designed to contain Illumina sequencing primer regions and 6-mer Ns for improved 'chastity' in Illumina sequencing (forward: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNN [specific primer]-3', reverse: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNNNN [specific primer]-3').

The first step was performed in a 25 µL reaction mixture with thermal cycles as follows: pre-denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 40 s, annealing at optimal temperature for 40 s, elongation at 72 °C for 40 s, and a final elongation at 72 °C for 8 min.

The Illumina sequencing adaptors plus the 8 bp identifier indices (Hamady et al. 2008) were added in the subsequent PCR process using a forward and reverse fusion primer (forward, AATGATACGGCGACCACCGAGATCTACAC-index-TCGTCGGCAGCGTC; reverse, CAAGCAGAAGACGGCATACGAGAT-index-GTCTCGTGGGCTCGG). The second step was conducted in a 25 µL reaction mixture and the thermal cycles of the second PCR were as follows: pre-denaturation at 94 °C for 2 min, followed by 8 cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 30 s, elongation at 68 °C for 1 min, and a final elongation at 68 °C for 7 min.

The PCR products were quantified using a Qubit fluorometer (Invitrogen Corporation, California, USA), and the concentrations of the PCR products were then equalized. The adjusted PCR products were pooled, and amplicons of 450–600 bp in length were then excised and extracted using a Zymo DNA Clean & Concentrator-5 and Zymoclean Gel DNA Recovery Kit (Zymo Research Corporation, Irvine City, CA, USA). The amplicon libraries were sequenced with 2 × 300-bp paired-end sequencing on a MiSeq platform using a MiSeq v. 3 Reagent Kit according to the manufacturer's instructions.

After pooling equal volumes of the respective PCR products, the amplicons 450–600 bp in length were excised and extracted using the QIAquick Gel Extraction Kit (Qiagen). The amplicon libraries were sequenced using paired-end sequencing on the MiSeq platform (Illumina, San Diego, CA, USA) using MISEQ v. 3 Reagent Kit according to the manufacturer's instructions.

Phylogenetic analyses

Since GenBank contains a large amount of ITS sequence data for the genus, we employed an ITS dataset to link the new multi-locus datasets to the global understanding of the genus provided by ITS sequences, as well as to infer the phylogenetic relationships between the *Tricholoma* species from different regions. The ITS dataset was aligned with MAFFT v. 7.304b using the E-INS-I strategy and was manually optimized on BioEdit v. 7.0.5. The ambiguously aligned regions were detected and excluded by using Gblocks v. 0.91b with less stringent selection settings.

To further investigate the phylogeny of *Tricholoma*, two multi-locus datasets were compiled. The five-locus dataset included five commonly used gene fragments, namely ITS, *RPB2*, *EF-1α*, *MCM7* and mtSSU; and the fifty-locus dataset included the aforementioned five gene fragments plus 45 single-copy orthologous gene fragments.

For the data obtained from next-generation sequencing, BCL2-FASTQ v. 1.8.4 (Illumina, San Diego, CA, USA) was used to convert the base calls into forward, index1, index2 and reverse FASTQ files. In order to obtain more accurate and reliable results in subsequent bioinformatics analyses, the raw data was pre-processed using an in-house procedure as follows:

1. Sequence reads not having an average quality of 20 over a 30 bps sliding window based on the phred algorithm were truncated, and trimmed reads having less than 75 % of their original length, as well as their paired reads, were removed;
2. Removal of reads contaminated by adapters;
3. Removal of reads with ambiguous bases (N bases), and their paired reads;
4. Removal of reads with low complexity (poly-bases).

Paired-end reads were generated using the Illumina MiSeq platform, and the reads with sequencing adapters, N bases, poly-bases, and low quality bases were filtered out using default

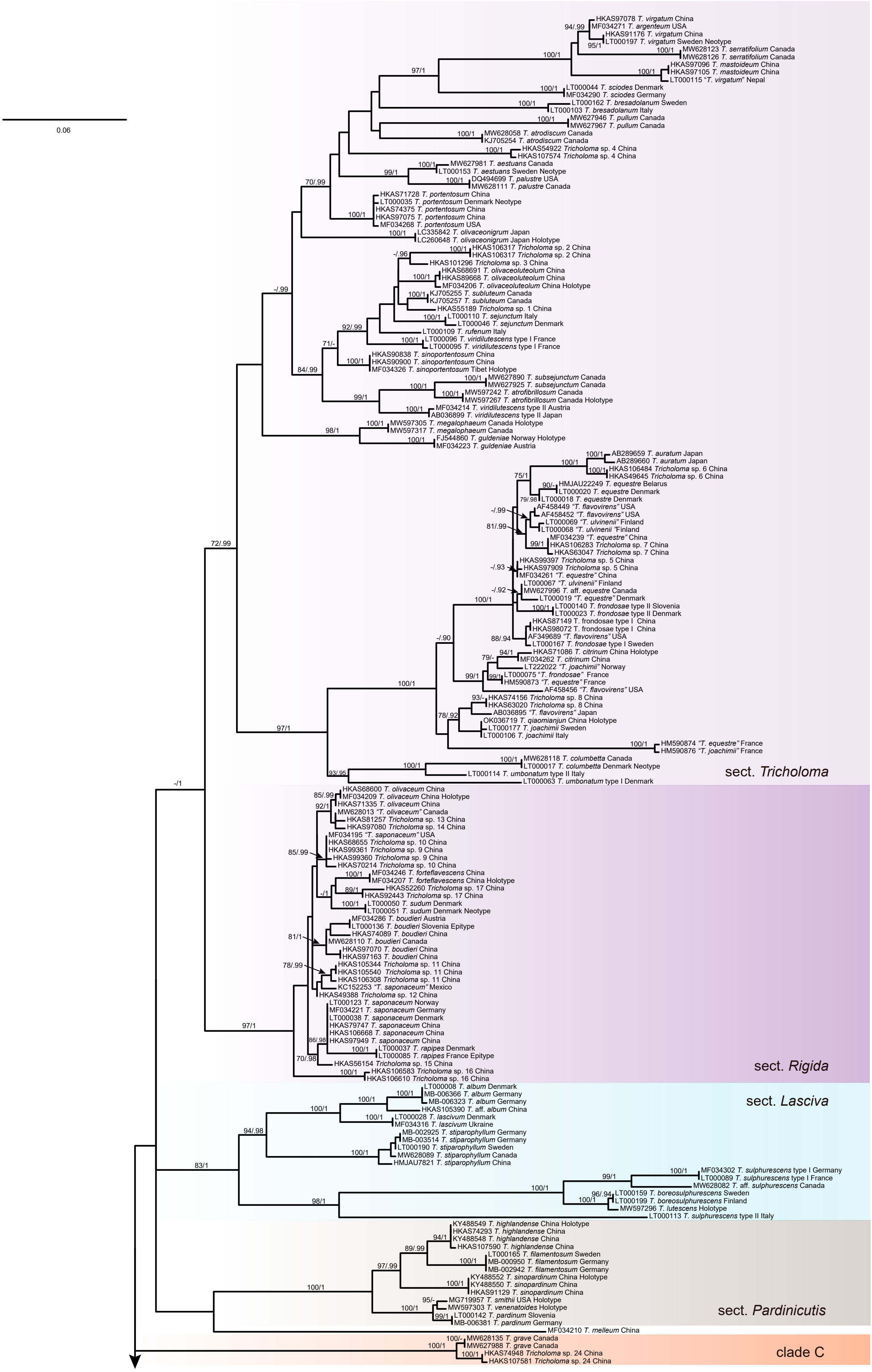

Fig. 1 Maximum likelihood phylogeny of *Tricholoma* species inferred from ITS sequences. Bootstrap (BS) values ≥ 70 and Bayesian posterior probabilities (BPP) ≥ 0.90 are shown above or beneath the individual branches.

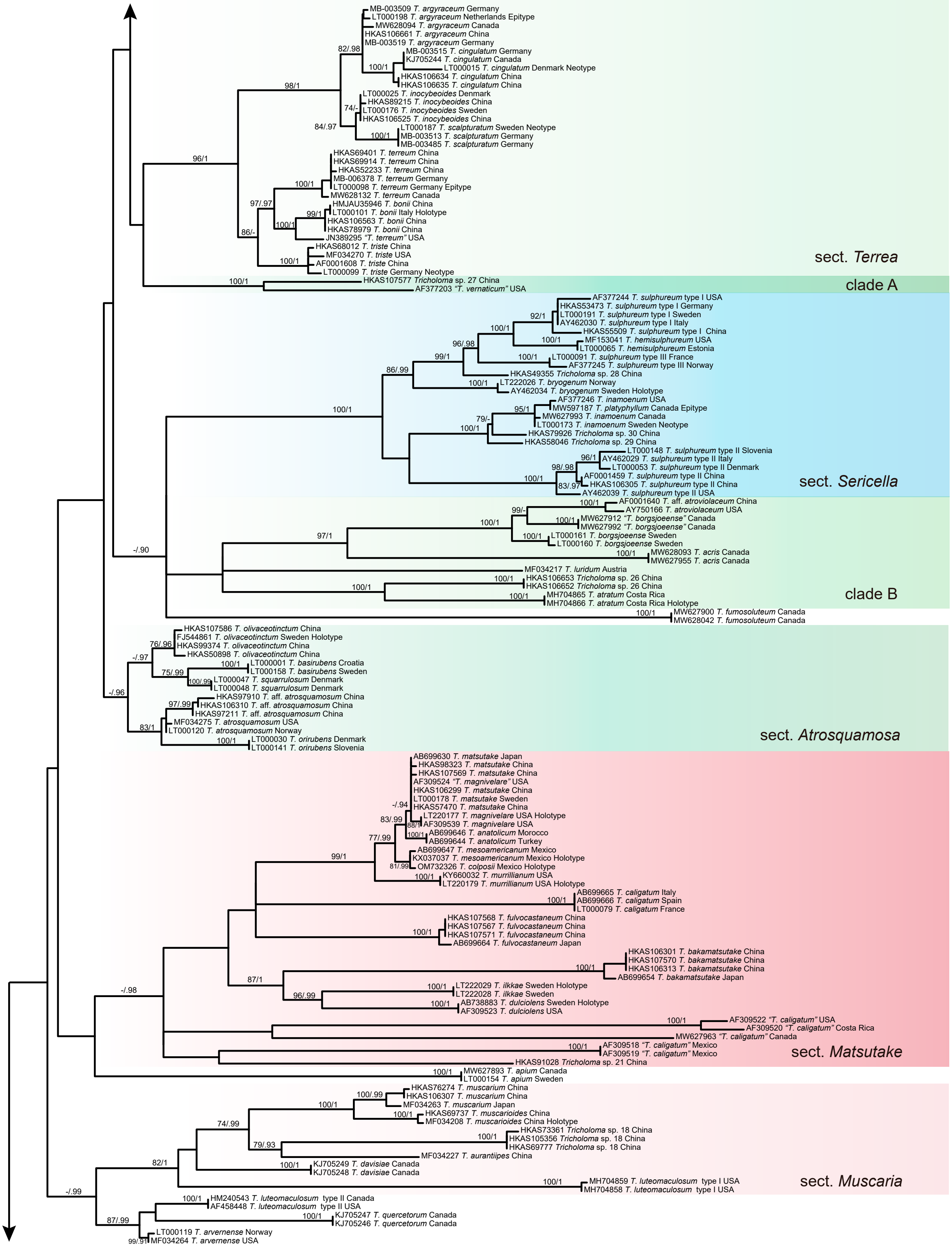

Fig. 1 (cont.)

parameters. If two paired-end reads overlapped with 1) a minimum overlap of 15 bp and 2) a mismatching ratio in the overlapped region ≤ 0.1, the consensus sequence was generated by FLASH v. 1.2.11 (Magoc & Salzberg 2011). Paired-end reads without overlaps were removed. To separate each gene, clean reads were imported in Geneious Prime 2020.0.4, the command 'map to reference' was executed (referring to the same gene in the genome of *T. matsutake* downloaded from JGI, Joint Genomics Institute), and all of the parameters were kept at their default settings, except that 'Sensitivity' was set as 'Medium Sensitivity/Fast'. Reads less than 5 ‰ of the total quantity for each gene were then removed, and the consensus sequences for each gene were generated with 'bases matching' set to 95 %.

The sequences of each gene fragment were aligned with MAFFT v. 7.304b using the E-INS-I strategy and manually optimized on BioEdit v. 7.0.5 (Hall 1999, Katoh et al. 2002). To test for potential conflicts among the 50 gene fragments, Maximum Likelihood analyses and Bayesian Inference were performed on each individual dataset with the same settings as in the con-

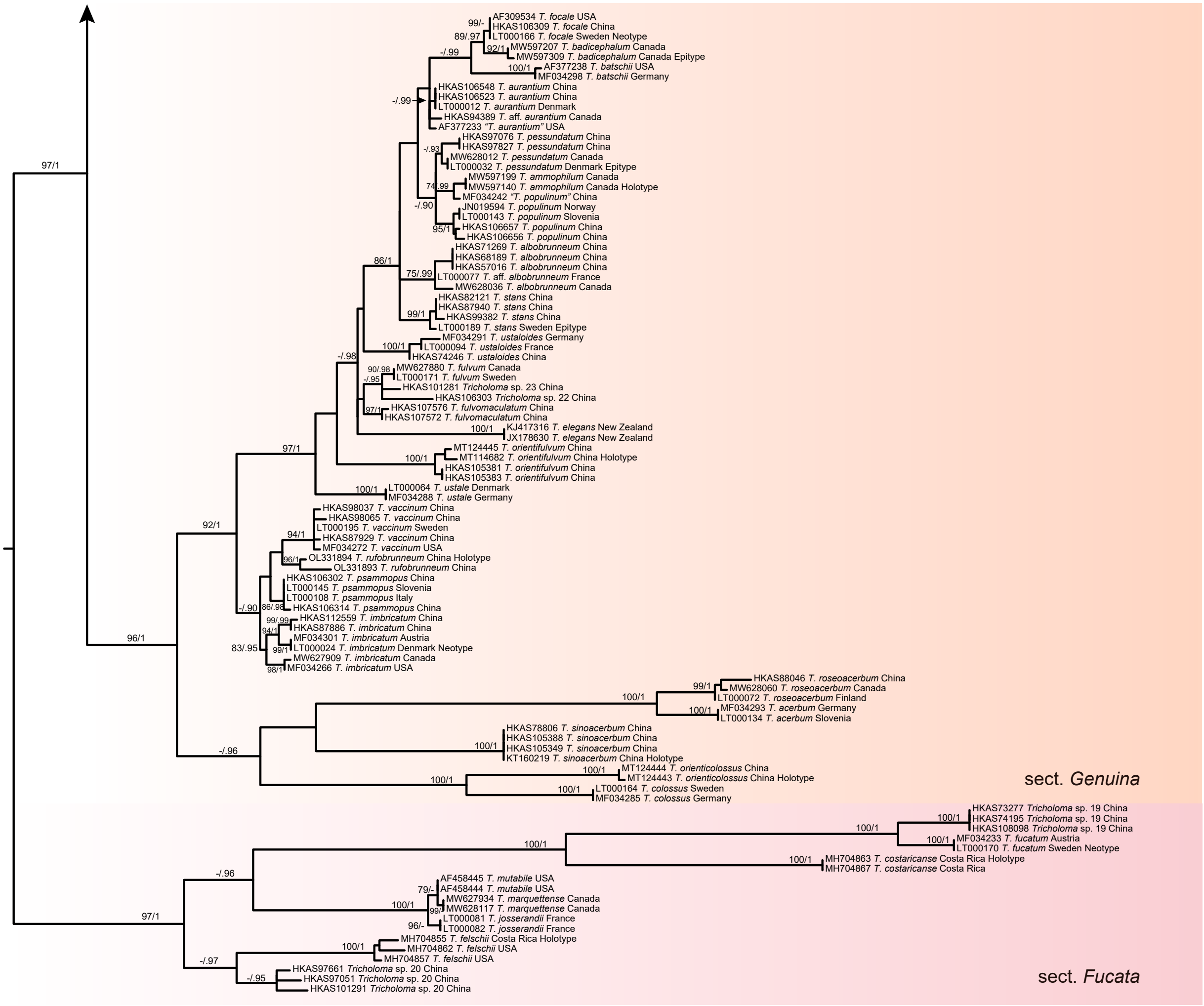

Fig. 1 (cont.)

catenated analysis (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003, Edler et al. 2020). Since no well-supported (BS ≥ 70 %, PP ≥ 0.9) conflict was detected among the topologies of the 50 gene fragments, these datasets were combined with Phyutility v. 2.2 for further phylogenetic analysis. The ambiguously aligned regions in ITS and mtSSU were detected and excluded using Gblocks 0.91b with less stringent selection settings. The intron regions in *RPB2*, *EF-1α* and *MCM7* are retained because they can provide more information than interference. For the 45 single-copy orthologous genes, introns within each gene were excluded from the phylogenetic analyses, as previous studies suggested (Sato & Toju 2019, Li et al. 2020).

Phylogenetic analyses were conducted using Maximum Likelihood (ML) in RAxML GUI 2.0 (Edler et al. 2020) and Bayesian Inference (BI) in MrBayes v. 3.2.2 (Ronquist & Huelsenbeck 2003). The optimal substitution model was determined through MrModeltest v. 2.3 using Akaike Information Criterion (AIC). The best-fit model was GTR + G + I for all three datasets. The final alignments were submitted to TreeBase (No. S27939).

All of the parameters were kept at their default settings for the ML analyses except that the model was set as the best-fit model, and statistical supports were obtained using nonparametric bootstrapping with 1 000 replicates. For the BI analyses, four chains were processed, with the generation set as 30 million using the selected model. The trees were sampled every 100 generations. Other parameters were kept at their default settings. Chain convergence was determined using Tracer v. 1.5 to confirm sufficiently large ESS values (> 200). The sampled trees were subsequently summarized after omitting the first 25 % of trees as burn-in using the 'sump' and 'sumt' commands implemented in MrBayes.

RESULTS

Phylogenetic analyses

A total of 5 714 sequences newly generated from 170 collections were used to conduct three datasets. The ITS dataset comprised 157 ITS sequences generated in this study and 264 ITS sequences retrieved from the GenBank database (other gene fragments of 12 collections were sequenced for the multilocus analyses, although their ITS sequences were provided by GenBank). The aligned length of ITS dataset was 901 bases with 558 variable characters, of which 496 were parsimony informative. 676 sequences from 159 collections make up the five-locus dataset. The aligned length for the five-locus dataset was 3 429 bases with 1 640 variable characters, of which 1 495 were parsimony informative. 131 collections were employed to conduct the fifty-locus dataset. The aligned length of fifty-locus dataset was 13 924 bp with 6 236 variable characters, of which 5 735 were parsimony informative.

The ML and BI analyses produced identical topologies with highly similar support values in each dataset. Therefore, only the ML trees are presented, with Bootstrap (BS) values and Bayesian posterior probabilities (BPP) shown on the branches. The phylogeny trees based on the single-locus, five-locus and fifty-locus datasets are presented in Fig. 1–3, respectively. The schematic dendrograms of dataset II and III are compared in Fig. 4.

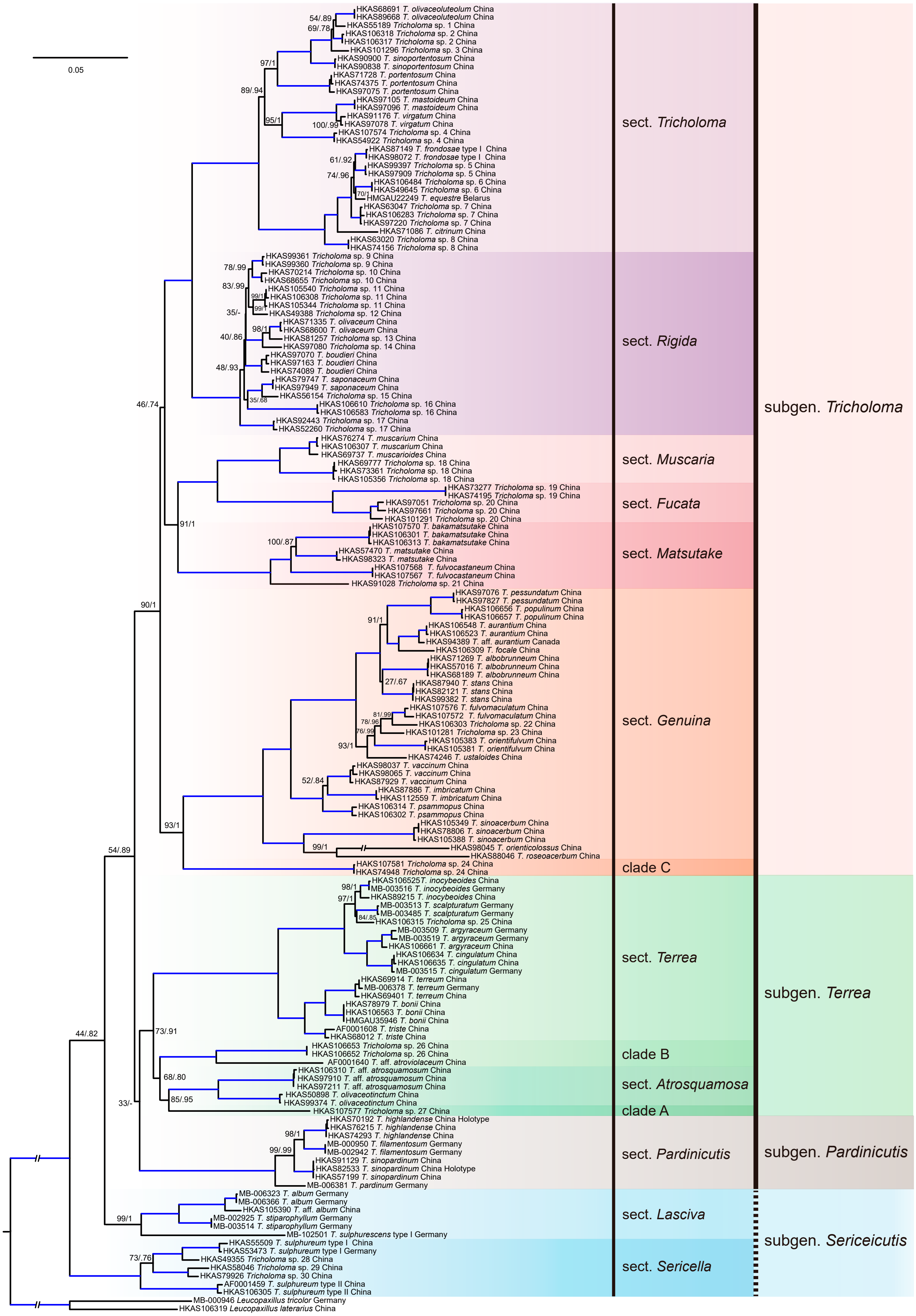

Fig. 2 Maximum likelihood phylogeny of *Tricholoma* using ITS/*RPB2*/*EF-1α*/*MCM7*/mtSSU dataset. Bootstrap (BS) values and Bayesian posterior probabilities (BPP) are shown above or beneath individual branches. Branches with strong statistical support (BS = 100 and BPP = 1) are shown in blue color.

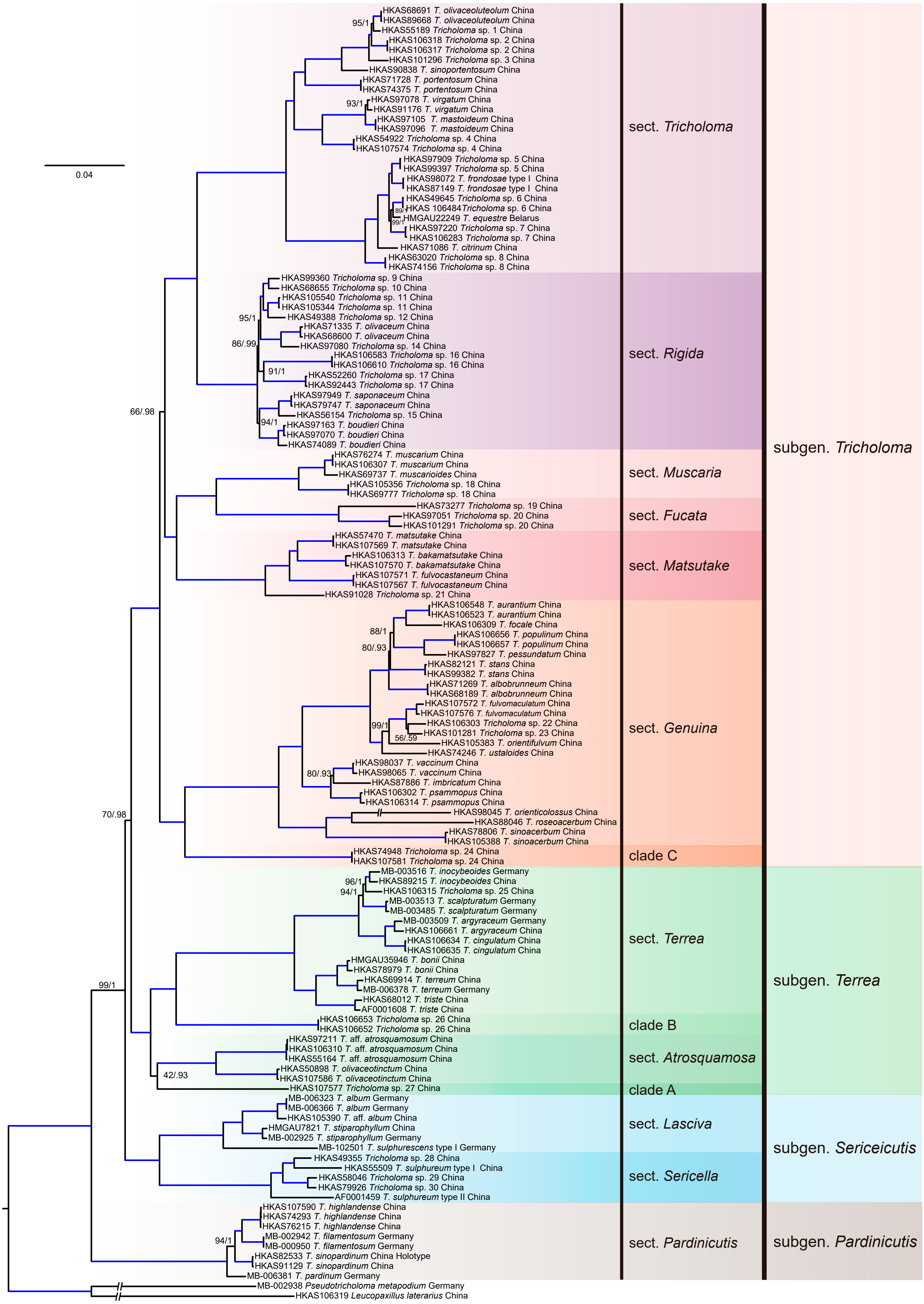

Fig. 3 Maximum likelihood phylogeny of *Tricholoma* using fifty-locus dataset. Bootstrap (BS) values and Bayesian posterior probabilities (BPP) are shown above or beneath individual branches. Branches with strong statistical support (BS = 100 and BPP = 1) are shown in blue color.

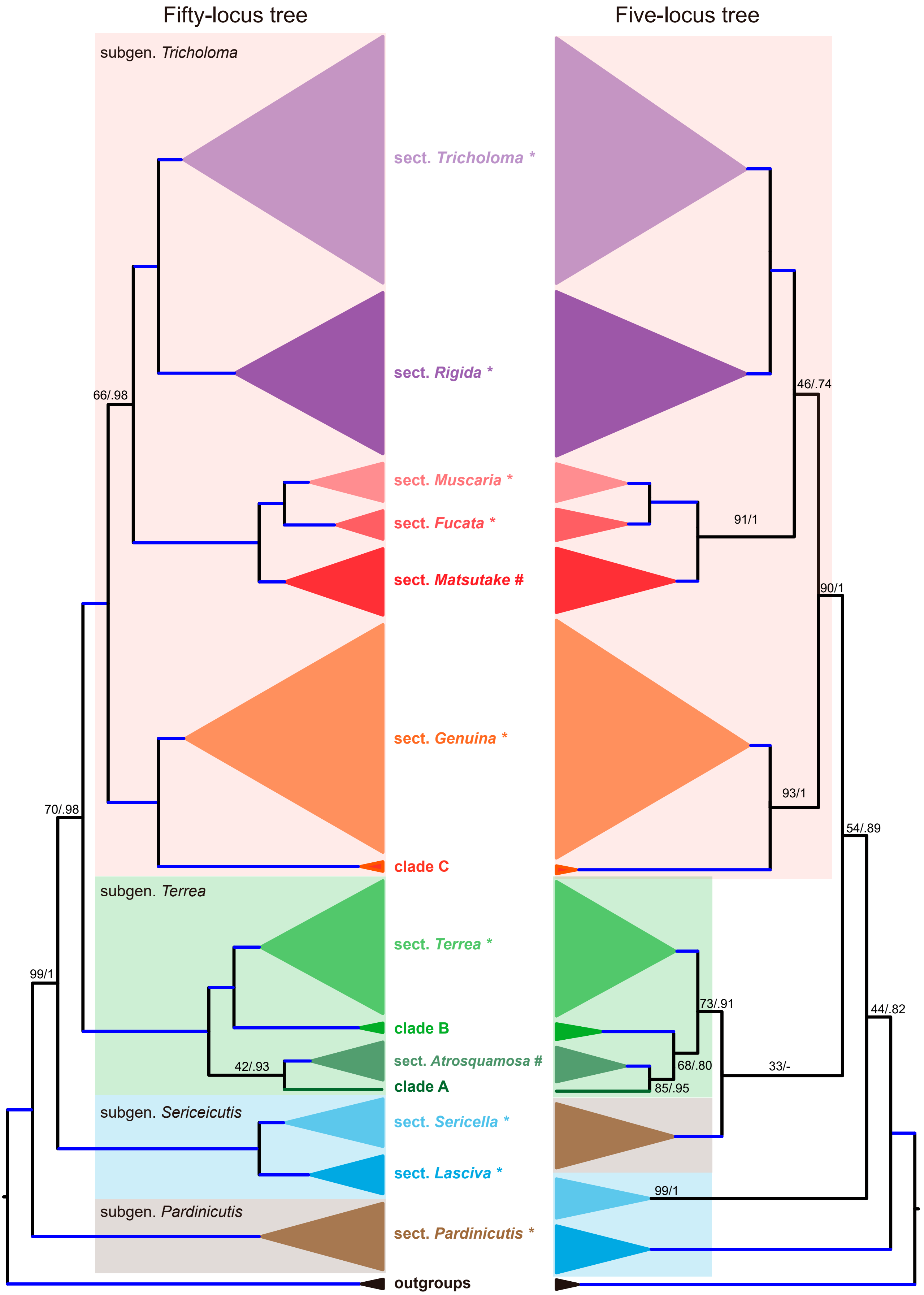

Fig. 4 Schematic dendrograms of the maximum likelihood phylogeny of *Tricholoma* using five-locus and fifty-locus datasets. Bootstrap (BS) values and Bayesian posterior probabilities (BPP) are shown above or beneath individual branches. Branches with strong statistical support (BS = 100 and BPP = 1) are shown in blue color. * indicating that the section was supported in both ML (BS ≥ 70) and BI (BPP ≥ 0.90) analyses based on ITS dataset, # indicating that the section was only supported by BI (BPP ≥ 0.90) analyses based on ITS dataset.

Fig. 5 Fresh basidiomata of the type or representative species of each section in *Tricholoma*. a–b. Representative species of sect. *Tricholoma* (a. *T. frondosae* type I, HKAS98072; b. *T. portentosum*, HKAS97075); c. *T. saponaceum*, type species of sect. *Rigida* (HKAS106668); d. *T. muscarium*, type species of sect. *Muscaria* (HKAS106307); e. a species of sect. *Fucata* (*T.* sp. 19, HKAS108098); f. *T. matsutake*, type species of sect. *Matsutake* (HKAS106299); g. *T. vaccinum*, type species of sect. *Genuina* (HKAS98065); h. *T. terreum*, type species of sect. *Terrea* (HKAS52233); i. *T.* aff. *atrosquamosum*, a species of sect. *Atrosquamosa* (HKAS97211); j. *T.* aff. *album*, a species of sect. *Lasciva* (HKAS105390); k. *T. sulphureum* type II, a species of sect. *Sericella* (HKAS106305); l. *T. sinopardinum*, a species of sect. *Pardinicutis* (HKAS82533). — Scale bars = 2 cm.

ITS phylogeny

A phylogenetic analysis of *Tricholoma* species from different parts of world is presented in Fig. 1. As Fig. 1 and previous studies suggest, ITS sequences could serve the species delimitation of this genus in most cases, while for cryptic groups, such as the *T. equestre* or *T. saponaceum* species complexes, species boundaries are still unclear (Fig. 1). Furthermore, several monophyletic clades were well-supported by ITS phylogenetic analyses (Fig. 1). *Tricholoma* sects. *Pardinicutis* and *Sericella* were strongly supported (BS/BPP = 100/1) monophyletic groups, and seven sections, namely sects. *Fucata*, *Genuina*, *Lasciva*, *Muscaria*, *Rigida*, *Terrea* and *Tricholoma*, were well-supported monophyletic clades (BS ≥ 70, BPP ≥ 0.9). However, sects. *Atrosquamosa* and *Matsutake* were only supported by BI analysis (Fig. 1). An additional three clades (A–C) recognized in other analyses were also delimited, and clade B contained five known species clustering with *T. luridum* but only with weak support.

As Fig. 1 suggests, 48 previously known species are distributed in China. Of these, ten were firstly confirmed with molecular evidence in this study, namely *T. argyraceum*, *T. focale*, *T. inocybeoides*, *T. muscarium*, *T. olivaceotinctum*, *T. ustaloides*, *T. psammopus*, *T. roseoacerbum*, *T. stans* and *T. stiparophyllum*. Besides, 30 new phylogenetic species, and three species closely related to known ones (*T.* aff. *atrosquamosum*, *T.* aff. *album* and *T.* aff. *atroviolaceum*) were uncovered (Fig. 1). The species diversity of *Tricholoma* in China is therefore expanded to 78 species with phylogenetic evidence.

Besides *T. albobrunneum*, *T. cingulatum*, *T. triste*, *T. vaccinum* and *T. viridilutescens*, the occurrence of seven additional species in East Asia, Europe and North America were confirmed, namely *T. boudieri*, *T. focale*, *T. pessundatum*, *T. portentosum*, *T. roseoacerbum*, *T. stiparophyllum* and *T. terreum* (Fig. 1). While several cryptic species groups, such as *T. equestre*, *T. saponaceum* and *T. sulphureum*, may also be widely distributed, the distribution ranges of each species are unclear since the species delimitations are unsettled (Fig. 1).

Five-locus phylogeny

The five-locus dataset was composed of representative species of each major clade selected from the ITS phylogenetic analyses. As Fig. 2 suggests, all eleven previously suggested sections, besides three additional unnamed clades (clades A, B and C), are strongly supported as monophyletic (BS/BPP = 100/1). In addition, several infrageneric phylogenetic relationships were revealed. For instance, sister relationships between sects. *Tricholoma* and *Rigida*, and *Muscaria* and *Fucata* received strong support (BS/BPP = 100/1), and the subgeneric monophyly (subg. *Tricholoma*) comprising sects. *Tricholoma*, *Rigida*, *Muscaria*, *Fucata*, *Matsutake*, *Genuina* and clade C was well-supported (BS/BPP = 90/1). Section *Terrea*, sect. *Atrosquamosa*, clade A and clade B formed a monophyletic group with BS/BPP = 73/0.91, indicating the close relationship of these clades. However, the phylogenetic relationships between sects. *Pardinicutis*, *Sericella* and *Lasciva* were unresolved.

Fifty-locus phylogeny

A fifty-locus dataset was for the first time employed to clarify the phylogenetic relationships of *Tricholoma.* This dataset comprised almost the same taxa and samples as the five-locus dataset. In the phylogenetic tree generated from this dataset (Fig. 3), the subgeneric monophyly comprising sect. *Terrea*, sect. *Atrosquamosa*, clade A and clade B in Fig. 2 was strongly supported (BS/BPP = 100/1). The close relationship between sects. *Sericella* and *Lasciva* was disclosed, and sect. *Pardinicutis* was a separated clade that occupied the basal position of the genus (Fig. 3). Not only were all eleven sections strongly supported as monophyletic, but the monophyly of the four subgenera was also strengthened with strong support (BS/BPP = 100/1). Since the phylogenetic relationships within *Tricholoma* were clearly demonstrated using the fifty-locus dataset, four subgenera can be recognized in this genus.

Phylogenetic relationships of major clades in Tricholoma

Based on the 50-locus phylogenetic tree, three subgenera and nine sections previously proposed were confirmed to be monophyletic and are accepted here, with certain amendments. Furthermore, the new subgenus *Terrea* (for its description see below) is proposed to accommodate the monophyletic group that contains sect. *Terrea*, sect. *Atrosquamosa* and two phylogenetic clades temporarily labeled A and B. The sister clade of sect. *Muscaria* is proposed as sect. *Fucata* (for its description see below), it clustered in subg. *Tricholoma* with other five sections. The clade harboring *T. matsutake* and its allies, which has a close relationship with a clade formed by sects. *Muscaria* and *Fucata*, is proposed as sect. *Matsutake* (for its description see below).

Subgenus *Pardinicutis* harbors only sect. *Pardinicutis* and occupies a basal position in the genus (Fig. 3). Subgenus *Sericeicutis* constitutes sects. *Lasciva* and *Sericella* and is located in a sub-basal position with good support (BS/BPP = 70/0.98).

Species with a dry, gray and radially fibrillose, squamulose to felty pileus surface form the monophyletic subg. *Terrea*, with BS/BPP = 100/1. Within this clade, sect. *Terrea* and clade B form a sister group with strong support (BS/BPP = 100/1), while the close relationship between sect. *Atrosquamosa* and clade A was only supported in the BI analyses (BS/BPP = 42/0.93).

Subgenus *Tricholoma* is the largest subgenus of the genus and consists of six sections, four of which have been previously proposed and two of which are newly erected here (see below). A phylogenetic clade labeled here as C, with unformalized taxonomic status, was uncovered in this subgenus. The subg. *Tricholoma* clusters into three major subclades, each with strong statistical support (BS/BPP = 100/1). The first subclade, formed by sect. *Genuina* and clade C, occupies the basal position within the subgenus. The sub-basal subclade constitutes sect. *Muscaria*, sect. *Fucata* and sect. *Matsutake*, while the last subclade contains a sister group formed by sects. *Tricholoma* and *Rigida*.

TAXONOMIC PART

Based on the phylogenetic analyses, an infrageneric taxonomic treatment of *Tricholoma* is suggested below. Fresh basidiomata of the type or representative species of each section are shown in Fig. 5.

Tricholoma (Fr.) Staude
Type: *T. equestre* (L.) P. Kumm.
I. Subg. ***Pardinicutis*** Singer
Type: *T. pardinum* (Pers.) Quél.
1. Sect. ***Pardinicutis***
Type: *T. pardinum* (Pers.) Quél.
II. Subg. ***Sericeicutis*** Singer
Type: *T. sulphureum* (Bull.) P. Kumm.
2. Sect. ***Lasciva*** Bon
Type: *T. lascivum* (Fr.) Gillet
3. Sect. ***Sericella*** (Fr.) Quél.
Type: *T. sulphureum* (Bull.) P. Kumm.
III. Subg. ***Terrea*** X.X. Ding, X. Xu, G. Kost & Zhu L. Yang
Type: *T. terreum* (Schaeff.) P. Kumm.
4. Sect. ***Atrosquamosa*** Kühner
Type: *T. atrosquamosum* Sacc.
5. Sect. ***Terrea***
Type: *T. terreum* (Schaeff.) P. Kumm.

IV. Subg. ***Tricholoma***
   Type: *T. equestre* (L.) P. Kumm.
   6. Sect. ***Fucata*** X.X. Ding, X. Xu, G. Kost & Zhu L. Yang
      Type: *T. fucatum* (Fr.) P. Kumm.
   7. Sect. ***Genuina*** (Fr.) Sacc.
      Type: *T. vaccinum* (Schaeff.) P. Kumm.
   8. Sect. ***Matsutake*** X.X. Ding**,** X. Xu, G. Kost & Zhu L. Yang
      Type: *T. matsutake* (S. Ito & S. Imai) Singer
   9. Sect. ***Muscaria*** Reschke
      Type: *T. muscarium* Kawam. ex Hongo
   10. Sect. ***Rigida*** (Fr.) Quél.
      Type: *T. saponaceum* (Fr.) P. Kumm.
   11. Sect. ***Tricholoma***
      Type: *T. equestre* (L.) P. Kumm.

Key to the subgenera and sections in Tricholoma

1. Pileus glabrous; lamellae close to distant; odor striking, aromatic or nauseating, recalling tar or gas (subg. *Sericeicutis*) 2
1. Pileus glabrous or felty, radially fibrillose, squamulose to scaly; lamellae crowded to close; odor diverse but not gas-like 3

2. Basidiospores small (length ≤ 8 µm); flesh staining yellow when bruised or after cutting . . . . sect. *Lasciva*
2. Basidiospores large (length > 8 µm); flesh without color change after cutting . . . . sect. *Sericella*

3. Pileus white, gray to gray-black, rarely with a brown or olivaceous tinge 4
3. Pileus color diverse, mostly buff, cinnamon, yellow, olivaceous, orange to red-brown, sometimes white or gray (subg. *Tricholoma*) 6

4. Pileus squamose; basidiospores mostly broadly ellipsoid to ellipsoid; clamp-connections present (subg. *Pardinicutis*) . . . . sect. *Pardinicutis*
4. Pileus mostly squarrulose; basidiospores mostly ellipsoid to elongate; clamp-connections absent or present (subg. *Terrea*) 5

5. Basidiomata small to medium-sized, some staining yellowish when old or bruised, no reddish tinge; odor farinaceous or weak . . . . sect. *Terrea*
5. Basidiomata medium-sized to large, staining reddish when old; odor diverse, often reminiscent of honey, ground pepper or cedar wood . . . . sect. *Atrosquamosa*

6. Flesh often pinkish when bruised or old; clamp-connections present and easily demonstrated . . . . sect. *Rigida*
6. Flesh not changing pinkish when bruised or old; clamp-connections absent, or at least rare 7

7. Pileus whitish, cinnamon, buff, orange to red-brown . . 8
7. Pileus white, gray, yellow to yellow-olivaceous 9

8. Pileus fibrillose to squamose; stipe robust and annulate; odor strong and aromatic; basidiospores predominantly broadly ellipsoid. . . . . sect. *Matsutake*
8. Pileus dry and squamulose, or viscid and smooth; stipe usually without an annulus but sometimes with a ring zone; odor farinaceous; basidiospores predominantly broadly ellipsoid to ellipsoid . . . . sect. *Genuina*

9. Subpellis poorly differentiated . . . . sect. *Tricholoma*
9. Subpellis well-differentiated 10

10. Pileus umbonate to conical, dry, fibrillose to fine-scaly; pileipellis not gelatinized . . . . sect. *Muscaria*
10. Pileus not conical but often with a low umbo, surface viscid, glabrous or radially silky-fibrillose; pileipellis gelatinized . . . . sect. *Fucata*

Tricholoma subg. ***Terrea*** X.X. Ding, X. Xu, G. Kost & Zhu L. Yang, *subg. nov.* — MycoBank MB 838960; Fig. 3, 5h, 6

Etymology. Derived from the name of the type species of the subgenus.

Type species of subgenus. *Tricholoma terreum* (Schaeff.) P. Kumm., Führer Pilzk. (Zerbst): 134. 1871.

Basidioma small, medium-sized to large. Pileus at first convex, plano-convex to applanate when mature, often broadly umbonate at center; surface dry, white, gray to gray-black, covered with felty-tomentose, tomentose to fibrillose squamules, or squarrulose.

Basidiospores predominantly ellipsoid to oblong. Pileipellis a cutis. Clamp connections absent or present. Cheilocystidia present or absent (Fig. 6).

This subgenus comprises two sections, namely sect. *Terrea* and sect. *Atrosquamosa*, as well as two as yet unnamed phylogenetic clades labeled A and B. Twelve known species in sect. *Terrea* and sect. *Atrosquamosa*, namely *T. argyraceum*, *T. atrosquamosum*, *T. basirubens*, *T. bonii*, *T. cingulatum*, *T. inocybeoides*, *T. olivaceotinctum*, *T. orirubens*, *T. scalpturatum*, *T. squarrulosum*, *T. terreum* and *T. triste* are clustered in this subgenus (Fig. 1). In addition, *T. acris*, *T. atratum*, *T. atroviolaceum*, *T.* aff. *atroviolaceum*, *T. borgsjoeense* and two terminal clades labelled as '*T. borgsjoeense*' (MW627912, MW627992) and '*T. vernaticum*' (AF377203), together with two new phylogenetic species, labelled as *Tricholoma* spp. 26 and 27, are also included in this subgenus (Fig. 1). However, *T. vernaticum* was placed in subg. *Contextocutis* by Shanks (1996) due to the presence of clamp connections and the interwoven pileipellis, which are not the features of subg. *Terrea*. Due to the absence of sufficient knowledge about 'AF377203', we prefer not accept this name as a member of this subgenus for the time being. A clade labelled as '*T. borgsjoeense*' from Canada was apart from the *T. borgsjoeense* from Europe, indicating it may be a new phylogenetic species in this subgenus. Besides, *T. luridum* had close relationships with members in clade B, as Fig. 1 suggested. Despite the weak statistical support (BS < 70, BPP < 0.9), this species may also nest in this subgenus. However, further studies are needed to verify this.

Distribution — Species in subg. *Terrea* are found in East Asia, Europe and North America (Fig. 1).

Notes — Members of subg. *Pardinicutis* which have dark gray scales on a whitish pileus may look similar to species of this subgenus. However, they can be easily distinguished by their relatively robust basidiomata and the common presence of clamp connections. *Tricholoma virgatum* and its allies (*T. sciodes* and *T. bresadolanum*) in subg. *Tricholoma* share a gray pileus and may look like species in subg. *Terrea*, but they are typically virgate to fibrillose-squamose, not felty and can be further distinguished by a bitter to acrid taste.

Tricholoma sect. ***Fucata*** X.X. Ding, X. Xu, G. Kost & Zhu L. Yang, *sect. nov.* — MycoBank MB 838961; Fig. 1, 2, 3, 5e

Etymology. Derived from the name of the type species of the section.

Type species of section. *Tricholoma fucatum* (Fr.) P. Kumm., Führer Pilzk. (Zerbst): 130. 1871.

Basidioma small to large. Pileus at first hemispherical to conical with deflexed margin, later convex with a low umbo; surface viscid and glabrous when moist, radially silky fibrillose and shiny when dry, pale gray, dark gray, yellow-brown to brown-olivaceous. Stipe cylindrical to clavate, sometimes tapering downwards, white to pale gray, often punctate or fibrillose. Odor and taste farinaceous.

Basidiospores predominantly broadly ellipsoid. Pileipellis gelatinized, subpellis well-differentiated and composed of short

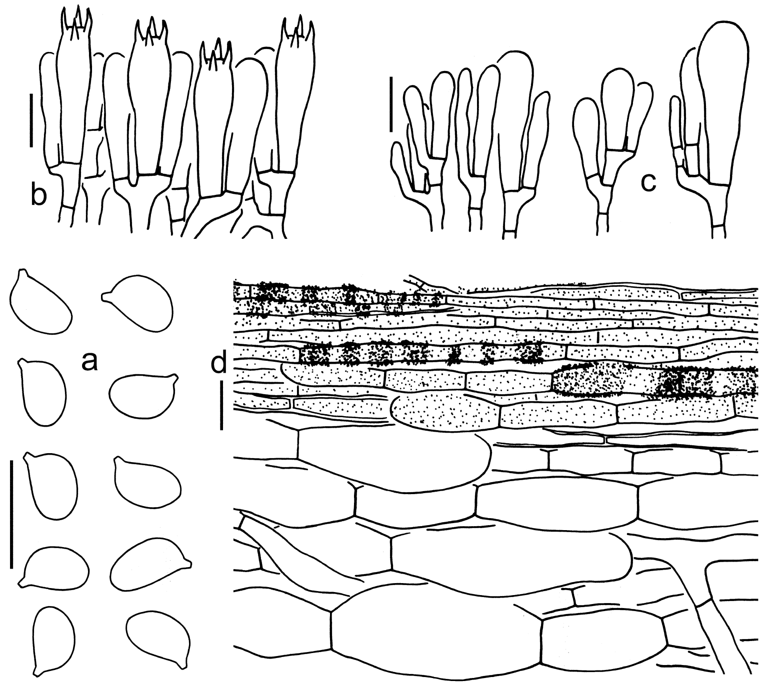

Fig. 6 Microscopical characters of *Tricholoma terreum*, the type species of subg. *Terrea*. a. Basidiospores (HKAS 69401); b. basidia (HKAS 69914); c. cheilocystidia (HKAS 69914); d. pileipellis (HKAS 69401) (b, c. from *L.P. Tang 1453* (HKAS 69914), China, Yunnan Province, Yulong County, in a conifer forest dominated by *Pinus*, 2164 m elev., 4 Aug. 2011; a, d. from *Q. Zhao 680* (HKAS 69401), ibid., 2740 m elev., 11 July 2010. — Scale bars: a–c = 10 μm, d = 20 μm.

inflated elements. Clamp connections absent. Cheilocystidia clavate or cylindrical, sometimes with intracellular pigment. Pleurocystidia absent.

Six known species and two new species are found in this section. Besides the type, the species include *T. costaricense*, *T. felschii*, *T. josserandii*, *T. marquettense*, *T. mutabile*, *Tricholoma* sp. 19 and *Tricholoma* sp. 20 (Fig. 1).

Distribution — Species of sect. *Fucata* are found in East Asia, Europe, and North and Central America (Fig. 1).

Notes — Section *Fucata* is characterized by an umbonate pileus with a viscid, glabrous or radially fibrillose surface, a strong farinaceous odor and taste, a distinct parenchymatoid subpellis, a gelatinized pileipellis and the presence of cheilocystidia. These traits make it relatively easy to recognize within *Tricholoma*. Although sect. *Fucata* has a close relationship with sect. *Muscaria*, the latter can be distinguished by an acute umbo, a distinct yellowish dry pileus and a not gelatinized pileipellis.

Tricholoma sciodes in sect. *Tricholoma* may look similar to species in sect. *Fucata* that share a grayish pileus. However, *T. sciodes* can be easily distinguished by its conical pileus and the bitter to acrid taste. Species of sect. *Terrea* with a gray pileus can be distinguished by their felty to squamulose pilei, small basidiospores with higher Q values and a relatively weak odor. *Tricholoma borgsjoeense* is characterized by an umbonate, tomentose pileus, presence of cheilocystidia and a strong rancid farinaceous odor and taste. However, the subpellis of this species is often not differentiated, and our phylogenetic analyses indicated that this species nested in subg. *Terrea*.

Tricholoma sect. ***Matsutake*** X.X. Ding, X. Xu, G. Kost & Zhu L. Yang, *sect. nov.* — MycoBank MB 838962; Fig. 1, 2, 3, 5f, 7

Etymology. Derived from the name of the type species of the section.

Type species of section. *Tricholoma matsutake* (S. Ito & S. Imai) Singer, Ann. Mycol. 41(1/3): 77. 1943.

Basidioma medium-sized to very large. Pileus at first hemispherical to convex with involute margin, later convex to flattened, with deflexed or straight margin; surface slightly viscid when wet, radially fibrillose, soon breaking up into clay buff, umber, dark gray-brown to dark red-brown, felty scales, margin always paler and woolly with remnants of veil. Stipe cylindrical or tapering downwards, with a large cottony-woolly ring, white, smooth or granulose above the ring, whitish and decorated with bands that share the same color with the pileus under the ring. Odor sweetish and perfumed-fruity; taste mild, aromatic to bitterish.

Basidiospores predominantly broadly ellipsoid. Pileipellis a cutis to a trichoderm. Clamp connections absent. Cheilocystidia absent or scattered. Pleurocystidia absent (Fig. 7).

This section harbors 14 species based on ITS phylogenetic analysis, of which ten are known species, namely *T. anatolicum*, *T. bakamatsutake*, *T. caligatum*, *T. dulciolens*, *T. fulvocastaneum*, *T. ilkkae*, *T. magnivelare*, *T. matsutake*, *T. mesoamericanum* and *T. murrillianum* (Fig. 1). Five sequences named '*T. caligatum*' from Canada, Costa Rica, Mexico and USA split into three distinct clades, and one of the clades may represent *T. glaucescens* (Trudell & Parker 2021). A Chinese collection HKAS91028 labeled as *Tricholoma* sp. 21 is a new phylogenetic species of this section. *Tricholoma colposii* was described recently, however, its ITS sequences from holotype (OM732326) seems extremely close to *T. mesoamericanum* (KX037037, holotype). Further study is needed to clarify its taxonomic status.

Distribution — Species of sect. *Matsutake* are found in East Asia, Europe, North America and Central America. *Tricholoma matsutake* is a well-known species in this section with Holarctic distribution based on ITS phylogenetic analyses (AB699630 from Japan, HKAS 98323 from China, LT000178 from Sweden and AF309524 from USA), while other species, with the exception of *T. anatolicum* and *T. dulciolens*, seem to be restricted to a single continent (Fig. 1).

Notes — Bon (1990) proposed sect. *Caligata* Konrad & Maubl. ex Bon to accommodate species sharing similar morphological traits with *T. matsutake*, and assigned *T. focale* as the type. However, *T. focale* was shown to be nested in sect.

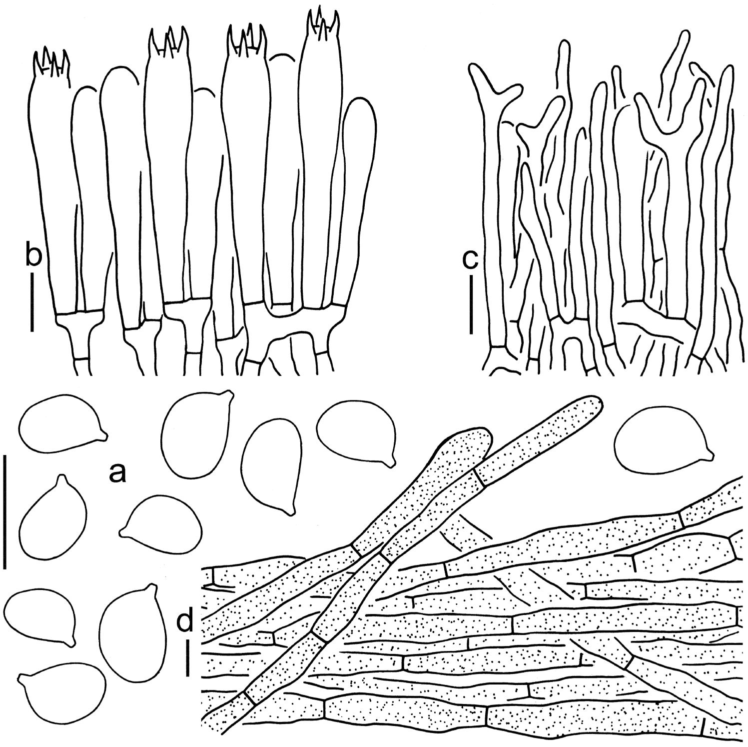

Fig. 7 Microscopical characters of *Tricholoma matsutake* (HKAS 98323), the type species of sect. *Matsutake*. a. Basidiospores; b. basidia; c. cheilocystidia; d. pileipellis. (from *P.M. Wang KD-68* (HKAS 98323), China, Sichuan Province, Kangding County, in a broad-leaved forest dominated by *Fagaceae*, 6 Sept. 2016. — Scale bars: a–c = 10 μm, d = 20 μm.

Genuina as suggested by phylogenetic analyses (Hosen et al. 2016, Heilmann-Clausen et al. 2017, Reschke et al. 2018, Xu et al. 2020, Ding et al. 2022, this study). Therefore, sect. *Matsutake* was proposed to accommodate these species. Members of sect. *Matsutake* can be easily identified by their dry, squamose pilei, distinct woolly rings and the strongly odor. *Tricholoma focale* in sect. *Genuina* with an orange brown pileus and a prominent ring was once considered to have close relationships with members of this section. However, it lacks both the squamose scales and the strong and distinctive odor. Another species with a distinct ring is *T. cingulatum* in sect. *Terrea*, however this species can hardly be confused with members of sect. *Matsutake* because of its slender basidioma, felty grayish cap and farinaceous odor.

DISCUSSION

Comparisons of the single-locus, five-locus and fifty-locus phylogenies

Our three phylogenetic analyses (Fig. 1–3) show that the phylogenetic resolution increases with the number of gene fragments employed.

The ITS dataset is capable to delimit and recognize species in *Tricholoma* as demonstrated by previous studies (Christensen & Heilmann-Clausen 2009, Hosen et al. 2016, Heilmann-Clausen et al. 2017, Trudell et al. 2017, Yang et al. 2017, Ovrebo & Hughes 2018, Reschke et al. 2018, Ovrebo et al. 2019, Xu et al. 2020, Trudell & Parker 2021, Ushijimaa et al. 2021, Ayala-Vásquez et al. 2022, Cui et al. 2022, Ding et al. 2022), and it is even good at recognizing some monophyletic groups (Fig. 1–3). Therefore, ITS phylogenetic analyses can provide insights in species diversity and geographic distribution. For example, five species with Holarctic distribution were detected in Reschke et al. (2018), and seven more were revealed in this study (Fig. 1). In addition, the occurrence of 48 known species of *Tricholoma* in China were confirmed (Fig. 1).

As universal barcoding markers in Fungi, ITS sequences have been widely used in species recognition and delimitation, as well as in ecological studies. In fact, for most species in the genus *Tricholoma*, the only DNA sequences available are ITS sequences. Since type studies are of great significance in taxonomy, and because, in many cases, only ITS sequences can be generated from old type specimens, ITS sequences are valuable and irreplaceable (Horton & Bruns 2001, Nilsson et al. 2008, Schoch et al. 2012, Hibbett et al. 2016).

However, comprehensive phylogenetic results have suggested that the resolution of phylogenetic relationships based solely on ITS sequences is insufficient (Heilmann-Clausen et al. 2017, Reschke et al. 2018; Fig. 1). Although ITS sequences are important in species delimitation of *Tricholoma*, they are not adequate to fully resolve the infrageneric phylogenetic relationships.

It is noticeable that in the five-locus phylogeny, all previous recognized sections were strongly supported as monophyletic groups, indicating that this dataset is useful in the elucidation of section-level phylogenetic relationships among *Tricholoma* species (Fig. 2). And more information about the relationships among some of the sections are also provided by Fig. 2. Meanwhile, in cryptic species groups such as *T. equestre*, *T. saponaceum* and *T. scalpturatum*, multi-locus phylogenetic analyses may be necessary to clarify the species boundaries as previous studies suggested (Jargeat et al. 2010, Moukha et al. 2013, Heilmann-Clausen et al. 2017, Reschke et al. 2018).

However, when the fifty-locus data was employed, the close relationships among the sections were reinforced and a deeper insight into the phylogeny of *Tricholoma* with four strongly supported subgenera was gained (Fig. 3).

Systematic treatments of Tricholoma in the past

To provide a deep insight of the classification history of *Tricholoma*, our taxonomic treatment is listed with four other important classifications from morphological age and two comprehensive studies based on ITS phylogenetic analyses in Table 2.

Table 2 Comparison of six commonly used and our new systematic proposals of *Tricholoma*. Two subgenera and one section in Singer (1986), namely subgen. *Tricholoma*, subgen. *Sericeicutis* and sect. *Tricholoma*, should be divided into two or three different parts based on our analyses separately, and, therefore, these parts were labelled with Roman numerals.

Bon (1984)	Singer (1986)		Riva (1988)	Noordeloos & Christensen (1999)		Heilmann-Clausen et al. (2017)	Reschke et al. (2018)	This study	
Section	Subgenus	Section	Section	Subgenus	Section	Section	Section	Subgenus	Section
Pardinicutis	*Pardinicutis*	*Pardinicutis*	*Pardinicutis*	*Pardinicutis*	*Pardinicutis*	*Pardinicutis*	*Pardinicutis*	*Pardinicutis*	*Pardinicutis*
Inamoena	*Sericeicutis* (part I)	*Sericella*	*Inamoena*	*Sericeicutis*	*Lasciva* *Inamoena*	*Sericella*	*Sericella*	*Sericeicutis*	*Sericella*
						Lasciva	*Lasciva*		*Lasciva*
Atrosquamosa	*Tricholoma* (part I)	*Tricholoma* (part I)	*Atrosquamosa*	*Tricholoma*	*Terrea*	*Terrea*	*Terrea*	*Terrea*	*Terrea*
						Atrosquamosa	*Atrosquamosa*		*Atrosquamosa*
Tricholoma			*Equestria*		*Tricholoma* *Albata*	*Tricholoma*	*Tricholoma*	*Tricholoma*	*Tricholoma*
	Sericeicutis (part II)	*Polyphillina*							
	Tricholoma (part II)	*Tricholoma* (part II)				unsigned	unsigned		*Fucata*
Imbricata		*Genuina*	*Imbricata*		*Imbricata*	*Genuina* (*T. focale*)	*Genuina* "*acerbum* clade" (*T. focale*)		*Genuina* (*T. focale*)
Albobrunnea			*Albobrunnea*		*Albobrunnea*				
					Megatricholoma	*Megatricholoma*	*Megatricholoma*		
					Caligata (*T. focale*)	*Caligata*	*Caligata*		*Matsutake*
not included	not included	not included	not included	not included	not included	not included	*Muscaria*		*Muscaria*
Rigida	*Contextocutis*	*Rigida*	*Rigida*	*Contextocutis*	*Rigida*	*Contextocutis*	*Rigida*		*Rigida*
		Iorigida	not included	/	not included	not included	not included	/	not included
not included		*Leucorigida*	not included	/	not included	not included	not included	/	not included
not included	*Tricholoma* (part III)	*Adusta*	not included	/	not included	not included	not included	/	not included

Section *Pardinicutis* was given a consistent taxonomic status as an independent group in both previous studies and our treatment (Table 2), while for other sections, different delimitations were proposed in different studies. This is especially true for sect. *Genuina*, which has been divided into two or three sections by different authors (Table 2).

Subgenus *Sericeicutis* harboring sects. *Sericella* and *Lasciva* was once accepted as a section-level group based on morphological studies (Bon 1984, Singer 1986, Riva 1988, Noordeloos & Christensen 1999), and two subsections were recognized in it (Bon 1984, Riva 1988). *Sericella* and *Lasciva* were recognized as two separated sections when ITS phylogenetic evidence was provided (Heilmann-Clausen et al. 2017, Reschke et al. 2018). In our fifty-locus phylogenetic analysis, a close relationship between the two sections is clear, and subg. *Sericeicutis* is therefore accepted for the monophyletic group containing sects. *Sericella* and *Lasciva*.

Members of subg. *Terrea* were once treated as one section named *Atrosquamosa* or *Terrea*, this section also contained *T. virgatum* and its allies (Bon 1984, Riva 1988, Christensen & Noordeloos 1999, Noordeloos & Christensen 1999). However, *T. virgatum* and its allies were relocated to sect. *Tricholoma* and two sections, namely *Atrosquamosa* and *Terrea*, were accepted to accommodate species in this group based on ITS inference (Heilmann-Clausen et al. 2017, Reschke et al. 2018). These dispositions were supported in both five-locus and fifty-locus phylogenetic analyses (Fig. 2, 3). Considering the monophyly as inferred by the fifty-locus phylogenetic analysis, the subg. *Terrea* is proposed here to accommodate the two sections (sect. *Atrosquamosa* and sect. *Terrea*) and the additional two unnamed phylogenetic clades (clade A and clade B).

Section *Rigida* was once assigned to subg. *Contextocutis* typified by *T. saponaceum* (Singer 1943, 1986, Noordeloos & Christensen 1999), while in both five- and fifty-locus phylogenetic analyses (Fig. 2, 3), it clustered within subg. *Tricholoma* and had a close relationship with sect. *Tricholoma* with strong support (BS/BPP = 100/1.00). Therefore, the subg. *Contextocutis* is redundant.

Section *Genuina* was redefined in this study since it has been treated very differently throughout the taxonomic history (Table 2). Members of this section were once accepted as two sections, namely sect. *Imbricata* and sect. *Albobrunnea* (Bon 1984, Riva 1988). Genus *Megatricholoma* was proposed to accommodate *T. colossus* (Kost 1984), but was then treated as sect. *Megatricholoma* in *Tricholoma* (Noordeloos & Christensen 1999). ITS phylogenetic analyses in Heilmann-Clausen et al. (2017) supported the inclusion of *T. acerbum* and *T. roseoacerbum* in sect. *Megatricholoma*, while Reschke et al. (2018) recognized these species as an '*acerbum* clade' apart from the last section. In our multi-locus phylogenetic analyses (Fig. 2, 3), both *T. orienticolossus*, a species close to *T. colossus*, and the '*acerbum* clade' were confirmed to cluster with *T. vaccinum* and *T. aurantium* and their allies with strong support (BS/BPP = 100/1.00). Therefore, we prefer to accept sect. *Genuina* as a monophyletic group including *T. vaccinum*, *T. aurantium*, *T. colossus* and *T. acerbum* and their allies, since all these species share rusty, or rarely blackish, spots on the surfaces of the lamellae when bruised or old. In addition, the sect. *Genuina* defined here can also be recognized by ITS dataset with good support (BS/BPP = 96/1.00) as Fig. 1 suggested.

On the contrary, species in sect. *Matsutake* were once considered as members of sect. *Genuina* (Bon 1984, Singer 1986, Riva 1988) or a section named *Caligata* (Noordeloos & Christensen 1999, Heilmann-Clausen et al. 2017, Reschke et al. 2018). However, the type species of sect. *Caligata* was *T. focale* (Bon 1990), which was relocated to sect. *Genuina* (Heilmann-Clausen et al. 2017, Reschke et al. 2018). Although the name *Caligata* is used in Heilmann-Clausen et al. (2017) and Reschke et al. (2018), it is a synonym of *Genuina*. Therefore, we proposed sect. *Matsutake* to accommodate *T. matsutake* and its allies (Table 2). Based on both multi-locus phylogenetic analyses (Fig. 2, 3), sect. *Matsutake* has close relationships with sects. *Muscaria* and *Fucata*, rather than sect. *Genuina*. Although sect. *Matsutake* is a well-studied group within *Tricholoma*, three new phylogenetic species are presented in Fig. 1, indicating the diversity of *Tricholoma* is still largely underestimated in some regions of the world.

The congruence of phylogeny with morphology in Tricholoma sections

Based on our fifty-locus phylogeny, eleven sections are accepted in this work, and they all have good congruence with the morphological characters. As the basal clade of *Tricholoma*, the members of subg. and sect. *Pardinicutis* can be easily distinguished by their relatively robust basidiomata, usually coarsely squamose pilei, common presence of clamp connections and relatively large basidiospores. Therefore, its delimitation has been relatively stable throughout its taxonomic history. Most works only list one or two species in this group (Singer 1986, Noordeloos & Christensen 1999, Heilmann-Clausen et al. 2017, Reschke et al. 2018), while four recently described species expand the species diversity of this group (Yang et al. 2017, Ovrebo & Hughes 2018, Trudell & Parker 2021).

Tricholoma subg. *Sericeicutis* is composed of sects. *Sericella* and *Lasciva*. Species in this subgenus share the sericeous pileus surface, striking odor and presence of clamp connections. Species in sect. *Sericella* are characterized by the white to yellow colors, a strong gas-like odor and large basidiospores, while members of sect. *Lasciva* have a whitish to yellowish gray pileus surface, a strong, complex odor and small basidiospores.

Species with a dry, gray and radially fibrillose, squamulose to felty pileus form the monophyletic subgenus *Terrea*. This subgenus contains two previously recognized sections, namely *Terrea* and *Atrosquamosa* and two new yet unnamed clades labeled A and B. Species in sect. *Terrea* are characterized by small to medium-sized basidiomata and predominantly ellipsoid to oblong basidiospores. Members in *T. terreum* subgroup do not stain yellowish and always have faint smell and taste, while species in *T. scalpturatum* subgroup are characterized by yellowish staining when old, and strongly farinaceous smell and taste. Species in sect. *Atrosquamosa* are characterized by medium-sized to rather large basidiomata becoming red when old, sometimes staining green in the stipe base, diverse odors, often reminiscent of honey, ground pepper or cedar wood, farinaceous to slightly bitter taste, and predominantly broadly ellipsoid to ellipsoid basidiospores. Given that only limited collections have been studied, clades A and B were treated as phylogenetic clades and their taxonomic status remains unformalized.

The remaining six sections all belong to subg. *Tricholoma* in our treatment. The major clade containing *T. vaccinum* was recognized as sect. *Genuina*. This section is characterized by cinnamon, buff, orange to red-brown pilei, and rusty or rarely blackish spots on the lamellae. Based on multi-locus phylogenetic analyses, clade C occupied a basal position in sect. *Genuina* (Fig. 2, 3). As Fig. 1 suggested, a clade labelled as *T. grave* from Canada was clustered in clade C with *Tricholoma* sp. 24 from China. Since only limited collections of *Tricholoma* sp. 24 have been observed, the taxonomic treatment of this clade will require further study.

Our phylogenetic results indicated that sects. *Matsutake*, *Muscaria* and *Fucata* have close relationships within subg. *Tricholoma* (Fig. 2, 3). The members of sect. *Matsutake* are characterized by pale brown to dark red-brown squamose pilei, annulate and robust stipes, large basidiospores with a low Q-value, and often strong and distinctive smells. Species within sect. *Muscaria* are characterized by an umbonate to conical pileus with a dry, fibrillose to fine scaly surface, a not gelatinized pileipellis and a distinct parenchymatoid subpellis. Chemically, the type species of sect. *Muscaria* contains 'tricholomic acid', an unusual amino acid with flycidal properties that are toxic to flies (Takemoto 1967, Hanessian & Vanasse 1987), although the presence of such secondary metabolites in other species in the section is unknown. Four species were accepted in this section, namely *T. aurantiipes*, *T. davisiae*, *T. muscarioides* and *T. muscarium* (Reschke et al. 2018). A clade labeled '*T. luteomaculosum* type I' seems to belong to this section as suggested by Fig. 1. Besides, a clade formed by *T. arvernense*, '*T. luteomaculosum* type II' and *T. quercetorum* have close relationship with this section (Fig. 1), indicating that the delimitation of this section may be extended in the future with further study. The new sect. *Fucata* is characterized by an umbonate pileus with a viscid, glabrous or radially fibrillose surface, a strong farinaceous odor and taste, a distinct parenchymatoid subpellis, a gelatinized pileipellis and the presence of distinct cheilocystidia.

In both Fig. 2 and 3, sects. *Tricholoma* and *Rigida* are sister groups with strong support (BS/BPP = 100/1.00), in spite of their distinctive morphological differences. Section *Rigida* is an easily recognized monophyletic group in both phylogenetic analyses and morphological traits. Members of this section are characterized by smooth and dry pilei and reddening flesh following injury, as well as the presence of abundant clamp connections. In contrast, its sister group, sect. *Tricholoma*, is characterized by a viscid or innately squamulose to silky fibrillose pileus, an unchanging flesh following injury, and the absence or presence of clamp connections.

CONCLUDING REMARKS

In conclusion, ITS sequences can be used to delimit species in the genus *Tricholoma* in most cases and even to recognize several monophyletic groups in some cases, and the five-locus dataset was able to resolve a section-level phylogeny, while the fifty-locus data further supported the observed relationships among sections and the delimitation of subgenera in the genus *Tricholoma*. Based on the current study we have divided genus *Tricholoma* into four subgenera and eleven sections, plus three unnamed section-level clades, which are usually consistent with morphological traits. One new subgenus and two new sections for *Tricholoma* are established here. Subgenus *Contextocutis* should be treated as a synonym of subg. *Tricholoma*.

However, the relationships among subgenera *Tricholoma*, *Terrea* and *Sericeicutis* were only resolved partially, even using the fifty-locus dataset, indicating that more loci sites are needed to completely clarify the phylogenetic relationships within this genus. In addition, more collections of different species from different parts of the world, especially a thorough sampling of the North American species, will contribute a more comprehensive understanding of phylogeny and diversity of *Tricholoma*. Several species did not fall into any of the available sections as Fig. 1 and previous studies suggested (Heilmann-Clausen et al. 2017, Reschke et al. 2018). These species, *T. apium*, *T. arvernense*, *T. fumosoluteum*, *T. luteomaculosum* type II, *T. melleum* and *T. quercetorum*, may need to be studied in the future by multi-locus phylogenetic analyses based on high quality materials.

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Appendix 1 Taxon information and GenBank accession numbers for the sequences retrieved from GenBank.

Taxon	Voucher	Locality	ITS accession	Note	Reference
‘*T. borgsjoeense*’	MQ20-HRL2462-QFB32646	Canada	MW627912		GenBank
	MQ20-YL-CMMF002309	Canada	MW627992		GenBank
‘*T. caligatum*’		Mexico	AF309518		Chapela & Garbelotto 2004
		Mexico	AF309519		Chapela & Garbelotto 2004
		Costa Rica	AF309520		Chapela & Garbelotto 2004
		USA	AF309522		Chapela & Garbelotto 2004
	MQ20-HRL0931-QFB32615	Canada	MW627963		GenBank
‘*T. equestre*’	EqFrPa	France	HM590873		Moukha et al. 2013
	EqFrW	France	HM590874		Moukha et al. 2013
	MC95-187	Denmark	LT000019		Heilmann-Clausen et al. 2017
	MB-301506	China	MF034239		Reschke et al. 2018
	MB-305676	China	MF034261		Reschke et al. 2018
‘*T. flavovirens*’	613	Japan	AB036895		GenBank
	HDT54614	USA	AF349689		Bidartondo & Bruns 2001
	trh545	USA	AF458449		Horton 2002
	trh546	USA	AF458452		Horton 2002
	trh652	USA	AF458456		Horton 2002
‘*T. frondosae*’	MC98-086	France	LT000075		Heilmann-Clausen et al. 2017
‘*T. joachimii*’	JoFr	France	HM590876		Moukha et al. 2013
	O-F167194	Norway	LT222022		Heilmann-Clausen et al. 2017
‘*T. magnivelare*’		USA	AF309524		Chapela & Garbelotto 2004
‘*T. populinum*’	MB-301648	China	MF034242		Reschke et al. 2018
‘*T. saponaceum*’	TB-2010-MEX 15	Mexico	KC152253		GenBank
	DBG:18233	USA	MF034195		Reschke et al. 2018
‘*T. terreum*’	MICH53128	USA	JN389295		GenBank
‘*T. ulvinenii*’	IK931613	Finland	LT000067		Heilmann-Clausen et al. 2017
	JuV13229F	Finland	LT000068		Heilmann-Clausen et al. 2017
	JuV26740F	Finland	LT000069		Heilmann-Clausen et al. 2017
‘*T. vernaticum*’	KMS246	USA	AF377203		Bidartondo & Bruns 2002
‘*T. virgatum*’	MC05-201	Nepal	LT000115		Heilmann-Clausen et al. 2017
T. acerbum	MC00-204	Slovenia	LT000134		Heilmann-Clausen et al. 2017
	MB-002943	Germany	MF034293		Reschke et al. 2018
T. acris	MQ20-HRL1586-QFB32631	Canada	MW627955		GenBank
	MQ20-YL-CMMF003100	Canada	MW628093		GenBank
T. aestuans	MC97-072	Sweden	LT000153	Neotype	Heilmann-Clausen et al. 2017
	MQ20-HL0600-QFB31075	Canada	MW627981		GenBank
T. aff. *albobrunneum*	MQ20-HL1671-QFB32594	Canada	MW628036		GenBank
T. aff. *sulphurescens*	MQ20-YL4160	Canada	MW628082		GenBank
T. albobrunneum	MC99-060	France	LT000077		Heilmann-Clausen et al. 2017
T. album	MC95-159	Denmark	LT000008		Heilmann-Clausen et al. 2017
T. ammophilum	WTU-F-073083	USA	MW597140	Holotype	Trudell & Parker 2021
	WTU-F-073015	USA	MW597199		Trudell & Parker 2021
T. anatolicum	S-3-2	Turkey	AB699644		Ota et al. 2012
	TM-5	Morocco	AB699646		Ota et al. 2012
T. apium	JHC95049	Sweden	LT000154		Heilmann-Clausen et al. 2017
	MQ20-HRL1368-QFB32626	Canada	MW627893		GenBank
T. argenteum	DBG:23372	USA	MF034271		Reschke et al. 2018
T. argyraceum	MEN9491	Netherlands	LT000198	Epitype	Heilmann-Clausen et al. 2017
	MQ19-CMMF002085	Canada	MW628094		GenBank
T. arvernense	MC98020	Norway	LT000119		Heilmann-Clausen et al. 2017
	DBG:18239	USA	MF034264		Reschke et al. 2018
T. atratum	NYBG-REH8158	Costa Rica	MH704865		Ovrebo et al. 2019
	NYBG-REH8263	Costa Rica	MH704866	Holotype	Ovrebo et al. 2019
T. atrodiscum	4660-HRL 1225	Canada	KJ705254		GenBank
	MQ20-HRL3072-QFB32652	Canada	MW628058		GenBank
T. atrofibrillosum	WTU-F-065669	USA	MW597242		Trudell & Parker 2021
	WTU-F-073048	USA	MW597267	Holotype	Trudell & Parker 2021
T. atrosquamosum	O-F64018	Norway	LT000120		Heilmann-Clausen et al. 2017
	DBG:24009	USA	MF034275		Reschke et al. 2018
T. atroviolaceum	C44 EC253	USA	AY750166		Cline et al. 2005
T. aurantiipes	MB-003000	China	MF034227		Reschke et al. 2018
T. aurantium	HDT54945	USA	AF377233		Bidartondo & Bruns 2002
	MC97-227	Denmark	LT000012		Heilmann-Clausen et al. 2017
T. auratum	Tk3	Japan	AB289659		Kikuchi et al. 2007
	Tk6 clone1	Japan	AB289660		Kikuchi et al. 2007
T. badicephalum	UBC-F-16235r	Canada	MW597207		Trudell & Parker 2021
	WTU-F-073095	USA	MW597309	Epitype	Trudell & Parker 2021
T. bakamatsutake	TNS:F-12866	Japan	AB699654		Ota et al. 2012
T. basirubens	MC01-209	Croatia	LT000001		Heilmann-Clausen et al. 2017
	TL5303	Sweden	LT000158		Heilmann-Clausen et al. 2017
T. batschii	KMS436	USA	AF377238		Bidartondo & Bruns 2002
	MB-003027	Germany	MF034298		Reschke et al. 2018
T. bonii	LUG-F8450	Italy	LT000101	Holotype	Heilmann-Clausen et al. 2017
T. boreosulphurescens	SAE9507	Sweden	LT000159		Heilmann-Clausen et al. 2017
	IK971187	Finland	LT000199		Heilmann-Clausen et al. 2017
T. borgsjoeense	JHC95067	Sweden	LT000160		Heilmann-Clausen et al. 2017
	JV95307	Sweden	LT000161		Heilmann-Clausen et al. 2017

Appendix 1 (cont.)

Taxon	Voucher	Locality	ITS accession	Note	Reference
T. boudieri	MC01-600	Slovenia	LT000136	Epitype	Heilmann-Clausen et al. 2017
	MB-002507	Austria	MF034286		Reschke et al. 2018
	MQ20-pat0115	Canada	MW628110		GenBank
T. bresadolanum	MC96-264	Italy	LT000103		Heilmann-Clausen et al. 2017
	CL94-166	Sweden	LT000162		Heilmann-Clausen et al. 2017
T. bryogenum	MC97-101	Sweden	AY462034		Comandini et al. 2004
	O-F52108	Norway	LT222026		Heilmann-Clausen et al. 2017
T. caligatum	TFM-M-L915a	Italy	AB699665		Ota et al. 2012
	SCM:B-4194	Spain	AB699666		Ota et al. 2012
	PH99519	France	LT000079		Heilmann-Clausen et al. 2017
T. cingulatum	4509	Canada	KJ705244		GenBank
	MC96-134	Denmark	LT000015	Neotype	Heilmann-Clausen et al. 2017
T. citrinum	MB-305716	China	MF034262		Reschke et al. 2018
	KUN-HKAS 71086	China	MW724356	Holotype	Cui et al. 2022
T. colossus	MC97-047	Sweden	LT000164		Heilmann-Clausen et al. 2017
	MB-002363	Germany	MF034285		Reschke et al. 2018
T. colposii	MEXU 30413	Mexico	OM732326	Holotype	Ayala-Vásquez et al. 2022
T. columbetta	MC95-181	Denmark	LT000017	Neotype	Heilmann-Clausen et al. 2017
	MQ20-HRL3139-QFB32663	Canada	MW628118		GenBank
T. costaricense	NYBG-REH7997	Costa Rica	MH704863	Holotype	Ovrebo et al. 2019
	NYBG-REH8418	Costa Rica	MH704867		Ovrebo et al. 2019
T. davisiae	2346-QFB-25632	Canada	KJ705248		GenBank
	4689-HRL 1256	Canada	KJ705249		GenBank
T. dulciolens	H:7002022	Sweden	AB738883	Holotype	Murata et al. 2013
		USA	AF309523		Chapela & Garbelotto 2004
T. elegans	OTA:61947	New Zealand	JX178630		Teasdale et al. 2013
	TENN:063711	New Zealand	KJ417316		Sánchez-García et al. 2014
T. equestre	MC94-027	Denmark	LT000018		Heilmann-Clausen et al. 2017
	MC96-155	Denmark	LT000020		Heilmann-Clausen et al. 2017
T. cf. *equestre*	MQ20-pat07101201	Canada	MW627996		GenBank
T. felschii	AGF21	Costa Rica	MH704855	Holotype	Ovrebo et al. 2019
	CSU-CLO4562	USA	MH704857		Ovrebo et al. 2019
	CSU-CLO5177	USA	MH704862		Ovrebo et al. 2019
T. filamentosum	C-F-35924	Sweden	LT000165		Heilmann-Clausen et al. 2017
T. focale		USA	AF309534		Chapela & Garbelotto 2004
	JV97-239	Sweden	LT000166	Neotype	Heilmann-Clausen et al. 2017
T. forteflavescens	HKAS93511	China	MF034207	Holotype	Reschke et al. 2018
	MB-301985	China	MF034246		Reschke et al. 2018
T. frondosae type I	MC95-130	Sweden	LT000167		Heilmann-Clausen et al. 2017
T. frondosae type II	MC96-235	Denmark	LT000023		Heilmann-Clausen et al. 2017
	MC00-225	Slovenia	LT000140		Heilmann-Clausen et al. 2017
T. fucatum	MC97-149	Sweden	LT000170	Neotype	Heilmann-Clausen et al. 2017
	MB-102537	Austria	MF034233		Reschke et al. 2018
T. fulvocastaneum	NTfu-3	Japan	AB699664		Ota et al. 2012
	KUN-HKAS107572	China	MW724472	Holotype	Ding et al. 2022
	KUN-HKAS107576	China	MW724473		Ding et al. 2022
T. fulvum	JHC04-251	Sweden	LT000171		Heilmann-Clausen et al. 2017
	MQ20-YL-CMMF001495	Canada	MW627880		GenBank
T. fumosoluteum	MQ20-YL-CMMF003829	Canada	MW627900		GenBank
	MQ20-JLAB2136-CMMF010550	Canada	MW628042		GenBank
T. grave	MQ20-YL4413	USA	MW627988		GenBank
	MQ20-GUE1476-CMMF014763	Canada	MW628135		GenBank
T. guldeniae	MC95103	Norway	FJ544860	Holotype	Christensen & Heilmann-Clausen 2009
	MB-002988	Austria	MF034223		Reschke et al. 2018
T. hemisulphureum	JV08-364	Estonia	LT000065		Heilmann-Clausen et al. 2017
	FLAS-F-60160	USA	MF153041		GenBank
T. highlandense	HKAS76215	China	KY488548		Yang et al. 2017
	HKAS70192	China	KY488549	Holotype	Yang et al. 2017
T. ilkkae	S-F173364	Sweden	LT222028		Heilmann-Clausen et al. 2017
	S-F513823	Sweden	LT222029	Holotype	Heilmann-Clausen et al. 2017
T. imbricatum	MC94-046	Denmark	LT000024	Neotype	Heilmann-Clausen et al. 2017
	DBG:18375	USA	MF034266		Reschke et al. 2018
	MB-102330	Austria	MF034301		Reschke et al. 2018
	MQ20-YL-CMMF002729	Canada	MW627909		GenBank
T. inamoenum	KMS249	USA	AF377246		Bidartondo & Bruns 2002
	JHC95-042	Sweden	LT000173	Neotype	Heilmann-Clausen et al. 2017
	MQ20-HRL3111-QFB32656	Canada	MW627993		GenBank
T. inocybeoides	MC03-229	Denmark	LT000025		Heilmann-Clausen et al. 2017
	MC97-060	Sweden	LT000176		Heilmann-Clausen et al. 2017
T. joachimii	TRgmb00060	Italy	LT000106		Heilmann-Clausen et al. 2017
	MC98-603	Sweden	LT000177		Heilmann-Clausen et al. 2017
T. josserandii	MC99-053	France	LT000081		Heilmann-Clausen et al. 2017
	MC99-056	France	LT000082		Heilmann-Clausen et al. 2017
T. lascivum	MC00-519	Denmark	LT000028		Heilmann-Clausen et al. 2017
	MB-303096	Ukraine	MF034316		Reschke et al. 2018
T. luridum	MB-002901	Austria	MF034217		Reschke et al. 2018
T. luteomaculosum type I	CSU-CLO4623b	USA	MH704858		Ovrebo et al. 2019
	CSU-CLO4632	USA	MH704859		Ovrebo et al. 2019

Appendix 1 (cont.)

Taxon	Voucher	Locality	ITS accession	Note	Reference
T. luteomaculosum type II	trh1187	USA	AF458448		Horton 2002
	UBC F19693	Canada	HM240543		GenBank
T. lutescens	WTU-F-073078	USA	MW597296	Holotype	Trudell & Parker 2021
T. magnivelare		USA	AF309539		Chapela & Garbelotto 2004
	NYSf2421	USA	LT220177	Holotype	Trudell et al. 2017
T. marquettense	MQ20-HRL1627-QFB32633	Canada	MW627934		GenBank
	MQ20-HRL1003-QFB32618	Canada	MW628117		GenBank
T. mastoideum	KUN-HKAS 97096	China	MW724357	Holotype	Cui et al. 2022
T. matsutake	TNS:F-12850	Japan	AB699630		Ota et al. 2012
	MC03-600	Sweden	LT000178		Heilmann-Clausen et al. 2017
T. megalophaeum	WTU-F-073091	USA	MW597305	Holotype	Trudell & Parker 2021
	WTU-F-073204	USA	MW597317		Trudell & Parker 2021
T. melleum	HKAS93514	China	MF034210	Holotype	Reschke et al. 2018
T. mesoamericanum	MX1	Mexico	AB699647		Ota et al. 2012
	FCME21585	Mexico	KX037037	Holotype	Trudell et al. 2017
T. murrillianum	SAT-16-319-01	USA	KY660032		Trudell et al. 2017
	NY586560	USA	LT220179	Holotype	Trudell et al. 2017
T. muscarioides	HKAS:93512	China	MF034208	Holotype	Reschke et al. 2018
T. muscarium	TNS-F-39016	Japan	MF034263		Reschke et al. 2018
T. mutabile	trh916	USA	AF458444		Horton 2002
	trh1184	USA	AF458445		Horton 2002
T. olivaceoluteolum	HKAS:93510	China	MF034206	Holotype	Reschke et al. 2018
T. olivaceonigrum	TMI 26360	Japan	LC260648	Holotype	Ushijimaa et al. 2021
	TMI 26359	Japan	LC335842		Ushijimaa et al. 2021
T. olivaceotinctum	MC97103	Sweden	FJ544861	Holotype	Christensen & Heilmann-Clausen 2009
T. olivaceum	HKAS93513	China	MF034209	Holotype	Reschke et al. 2018
	MQ20-YL-CMMF003782	Canada	MW628013		GenBank
T. orienticolossus	HAKS99341	China	MT124443	Holotype	Xu et al. 2020
	HAKS98045	China	MT124444		Xu et al. 2020
T. orientifulvum	HAKS107157	China	MT114682	Holotype	Xu et al. 2020
	HAKS107156	China	MT124445		Xu et al. 2020
T. orirubens	JHC93-261	Denmark	LT000030		Heilmann-Clausen et al. 2017
	JHC01-200	Slovenia	LT000141		Heilmann-Clausen et al. 2017
T. palustre	AFTOL-ID 497	USA	DQ494699		Matheny et al. 2006
	MQ20-HRL2173-QFB32641	Canada	MW628111		GenBank
T. pardinum	C-F-96190	Slovenia	LT000142		Heilmann-Clausen et al. 2017
T. pessundatum	JV04-482	Denmark	LT000032	Epitype	Heilmann-Clausen et al. 2017
	MQ20-JLAB931-CMMF009347	Canada	MW628012		GenBank
T. platyphyllum	WTU-F-073003	USA	MW597187	Epitype	Trudell & Parker 2021
T. populinum	O-F63960	Norway	JN019594		Grubisha et al. 2012
	MC00-236	Slovenia	LT000143		Heilmann-Clausen et al. 2017
T. portentosum	MC96-156	Denmark	LT000035	Neotype	Heilmann-Clausen et al. 2017
	DBG:18411	USA	MF034268		Reschke et al. 2018
T. psammopus	MC96-345	Italy	LT000108		Heilmann-Clausen et al. 2017
	MC04-600	Slovenia	LT000145		Heilmann-Clausen et al. 2017
T. pullum	MQ20-HRL2215-QFB32644	Canada	MW627946		GenBank
	MQ20-HRL3138-QFB32662	Canada	MW627967		GenBank
T. qiaomianjun	KUN-HKAS 101303	China	OK036719	Holotype	Cui et al. 2022
T. quercetorum	4447	Canada	KJ705246		GenBank
	4494	Canada	KJ705247		GenBank
T. rapipes	MC03228	Denmark	LT000037		Heilmann-Clausen et al. 2017
	MC98-106	France	LT000085	Epitype	Heilmann-Clausen et al. 2017
T. roseoacerbum	IK881120	Finland	LT000072		Heilmann-Clausen et al. 2017
	MQ20-HRL1010a-QFB32619	Canada	MW628060		GenBank
T. rufenum	MC96-376	Italy	LT000109		Heilmann-Clausen et al. 2017
T. rufobrunneum	KUN-HKAS49069	China	OL331894	Holotype	Ding et al. 2022
	KUN-HKAS90808	China	OL331895		Ding et al. 2022
T. saponaceum	C-F23337	Denmark	LT000038		Heilmann-Clausen et al. 2017
	JHC00-049	Norway	LT000123		Heilmann-Clausen et al. 2017
	MB-002941	Germany	MF034221		Reschke et al. 2018
T. scalpturatum	MC95-165	Sweden	LT000187	Neotype	Heilmann-Clausen et al. 2017
T. sciodes	MC94-007	Denmark	LT000044		Heilmann-Clausen et al. 2017
	MB-002928	Germany	MF034290		Reschke et al. 2018
T. sejunctum	MC95-187	Denmark	LT000046		Heilmann-Clausen et al. 2017
	MC96-314	Italy	LT000110		Heilmann-Clausen et al. 2017
T. serratifolium	MQ20-HRL1047-QFB32621	Canada	MW628123		GenBank
	MQ20-HRL2214-QFB32643	Canada	MW628126		GenBank
T. sinoacerbum	GDGM:44680	China	KT160219	Holotype	Hosen et al. 2016
T. sinopardinum	HKAS57199	China	KY488550		Yang et al. 2017
	HKAS82533	China	KY488552	Holotype	Yang et al. 2017
T. sinoportentosum	HKAS:46084	China	MF034326	Holotype	Reschke et al. 2018
T. smithii	DBG:CLO4513	USA	MG719957	Holotype	Ovrebo & Hughes 2018
T. squarrulosum	JHC93-224	Denmark	LT000047		Heilmann-Clausen et al. 2017
	JHC93-262	Denmark	LT000048		Heilmann-Clausen et al. 2017
T. stans	MC95-145	Sweden	LT000189	Epitype	Heilmann-Clausen et al. 2017
T. stiparophyllum	MC95-117	Sweden	LT000190		Heilmann-Clausen et al. 2017
	MQ20-GUE1522-CMMF014811	Canada	MW628089		GenBank
T. subluteum	2139-QFB-25830	Canada	KJ705255		GenBank
	1065	Canada	KJ705257		GenBank

Appendix 1 (cont.)

Taxon	Voucher	Locality	ITS accession	Note	Reference
T. subsejunctum	MQ20-GUE2528-CMMF014821	Canada	MW627890		GenBank
	MQ20-HL1204-QFB32576	Canada	MW627925		GenBank
T. sudum	JV96-306	Denmark	LT000050		Heilmann-Clausen et al. 2017
	MC98-601	Denmark	LT000051	Neotype	Heilmann-Clausen et al. 2017
T. sulphurescens type I	MC99-063	France	LT000089		Heilmann-Clausen et al. 2017
	MB-102501	Germany	MF034302		Reschke et al. 2018
T. sulphurescens type II	TRgmb00062	Italy	LT000113		Heilmann-Clausen et al. 2017
T. sulphureum type I	HDT32084	USA	AF377244		Bidartondo & Bruns 2002
	C19 AQUI	Italy	AY462030		Comandini et al. 2004
	JHC08-049	Sweden	LT000191		Heilmann-Clausen et al. 2017
T. sulphureum type II	P62 AQUI	Italy	AY462029		Comandini et al. 2004
	DED4539	USA	AY462039		Comandini et al. 2004
	JHC07-236	Denmark	LT000053		Heilmann-Clausen et al. 2017
	MC01-204	Slovenia	LT000148		Heilmann-Clausen et al. 2017
T. sulphureum type III	HO70098	Norway	AF377245		Bidartondo & Bruns 2002
	TF06045	France	LT000091		Heilmann-Clausen et al. 2017
T. terreum	MEN95192	Germany	LT000098	Epitype	Heilmann-Clausen et al. 2017
	MQ20-MCNC3447-CMMF007442	Canada	MW628132		GenBank
T. triste	E3754	Germany	LT000099	Neotype	Heilmann-Clausen et al. 2017
	DBG:22631	USA	MF034270		Reschke et al. 2018
T. umbonatum type I	MC00A01	Denmark	LT000063		Heilmann-Clausen et al. 2017
T. umbonatum type II	TRgmb00651	Italy	LT000114		Heilmann-Clausen et al. 2017
T. ustale	JHC92-299	Denmark	LT000064		Heilmann-Clausen et al. 2017
	MB-002924	Germany	MF034288		Reschke et al. 2018
T. ustaloides	MC99-067	France	LT000094		Heilmann-Clausen et al. 2017
	MB-002929	Germany	MF034291		Reschke et al. 2018
T. vaccinum	MC95-109	Sweden	LT000195		Heilmann-Clausen et al. 2017
	DBG:23466	USA	MF034272		Reschke et al. 2018
T. venenatoides	WTU-F-073089	USA	MW597303	Holotype	Trudell & Parker 2021
T. virgatum	MC97-164	Sweden	LT000197	Neotype	Heilmann-Clausen et al. 2017
T. viridilutescens type I	MC98-061	France	LT000095		Heilmann-Clausen et al. 2017
	MC98-080	France	LT000096		Heilmann-Clausen et al. 2017
T. viridilutescens type II	NA12	Japan	AB036899		GenBank
	MB-002842	Austria	MF034214		Reschke et al. 2018

Appendix 2 The primer pairs used in this study.

Locus	Primer name	Nucleotide sequence (5’–3’)	Reference
ITS	ITS1-F	CTTGGTCATTTAGAGGAAGTAA	Gardes & Bruns 1993
	ITS4	TCCTCCGCTTATTGATATGC	White et al. 1990
EF1-α	*EF1*-983F	GCYCCYGGHCAYCGTGAYTTYAT	Rehner & Buckley 2005
	EF1-1567R	ACHGTRCCRATACCACCRATCTT	Rehner & Buckley 2005
	tef1F	TACAARTGYGGTGGTATYGACA	Morehouse et al. 2003
	tef1R	ACNGACTTGACYTCAGTRGT	Morehouse et al. 2003
RPB2	b*RPB2*-6F	TGGGGYATGGTNTGYCCYGC	Matheny 2005
	b*RPB2*-7.1R	CCCATRGCYTGYTTMCCCATDGC	Matheny 2005
	RPB2-T1F	TGGCTTGCATATCTGTCGGTTCT	This study
	RPB2-T1R	ATATTGGCCATCGTGTCC	This study
MCM7	b*MCM7*-709F	ACNCGNGTRTCVGARGTMAARCC	Schmitt et al. 2009
	b*MCM7*-1348R	GAYTTSGCNACMCCNGGRTCRCCCAT	Schmitt et al. 2009
	MCM7-T1F	TGTTCGTGGCATTGTTACTCGTGT	This study
	MCM7-T1R	CCATCGCCGGTGACTTTC	This study
mtSSU	MS1	CAGCAGTCAAGAATATTAGTCAATG	White et al. 1990
	MS2	GCGGATTATCGAATTAAATAAC	White et al. 1990
ADE12	*ADE12* Forward	AGCATCGGNACMACVAAGAA	Sato et al. 2017
	ADE12 Reverse	CCRAARTCRATRTCVAGCAT	Sato et al. 2017
ARC40	*ARC40* Forward	TGATCACNTCNATYGAYTGGGC	Sato et al. 2017
	ARC40 Reverse	GTCGAYCKGATNGGYTTCTT	Sato et al. 2017
ATP2	*ATP2* Forward	GTYCGYACYATTGCYATGGA	Sato et al. 2017
	ATP2 Reverse	ACGTTGTTGATGAGYTCCTGRAT	Sato et al. 2017
ATP3	*ATP3* Forward	AACATYGAGAAAATYACVAAG	Sato et al. 2017
	ATP3 Reverse	ATACCRCCGCAVAGRCCYTTGTC	Sato et al. 2017
BRX1	*BRX1* Forward	TGGGCTGCRAARACRCCNAAYGG	Sato et al. 2017
	BRX1 Reverse	TGGAARTTNCGGAACCADATYTT	Sato et al. 2017
CAF40	*CAF40* Forward	GCCATYTTCATYGTNCARAARAT	Sato et al. 2017
	CAF40 Reverse	CGYTTBGTGACCATRTCNCCCT	Sato et al. 2017
CBF5	*CBF5* Forward	CTCCTCAAAAAYTAYGAYAA	Sato et al. 2017
	CBF5 Reverse	ACCWGCACCCTGYTGNGAYTT	Sato et al. 2017
CCT3	*CCT3* Forward	AARGCCATGCTSAARATGAT	Sato et al. 2017
	CCT3 Reverse	GARATGATSACGACNGGRTGRAT	Sato et al. 2017
CCT4	*CCT4* Forward	ATGGARGCNTAYTGYTTCCARGC	Sato et al. 2017
	CCT4 Reverse	GCTTGYACRTARTCRTCRATYTT	Sato et al. 2017
CCT5	*CCT5* Forward	GARAAGTTYGMGGAYATGATCAA	Sato et al. 2017
	CCT5 Reverse	TCYTCRATDACVAGCATCTTRTC	Sato et al. 2017
CCT7	*CCT7* Forward	ATYCAYTCCGARAARCCHTTYTT	Sato et al. 2017
	CCT7 Reverse	CGRTAGATGATYTCCCAYTC	Sato et al. 2017
CDC47	*CDC47* Forward	TGCCTNATGGGTGAYCCHGGTGT	Sato et al. 2017
	CDC47 Reverse	ATYGAGATYGTYTGYTGYTCCAT	Sato et al. 2017
CRM1	*CRM1* Forward	ATGGTCAARCCNGARGAGGT	Sato et al. 2017
	CRM1 Reverse	CGCTTYTCVGTYTCYTCRTCT	Sato et al. 2017
DED81	*DED81* Forward	GGCAGATGACNGAYATCATYGG	Sato et al. 2017
	DED81 Reverse	CGCTGRTCRGTRWACCARTARTA	Sato et al. 2017
FRS2	*FRS2* Forward	TTCMGMAAYGAGACNATGGAYGC	Sato et al. 2017
	FRS2 Reverse	TCMARCATYTCDGGNCGGAACAT	Sato et al. 2017
GDI1	*GDI1* Forward	AAGAAGGTSCTYCACATGGA	Sato et al. 2017
	GDI1 Reverse	GCYTCCATYTCBGTRCTBGG	Sato et al. 2017
GSH1	*GSH1* Forward	AAYCCWCATGCNCGHTTYCCGT	Sato et al. 2017
	GSH1 Reverse	CARCADCCCATBCCRAARCCCAT	Sato et al. 2017
GUS1	*GUS1* Forward	ATGGAYTGGGGYAAYGCNATYGT	Sato et al. 2017
	GUS1 Reverse	ARTATCCYTTCCTCTCRAAYTG	Sato et al. 2017
HEM15	*HEM15* Forward	CAGTAYCCBCARTAYAGYTGYAG	Sato et al. 2017
	HEM15 Reverse	TCYCCRCGGTTBACVACYGACAT	Sato et al. 2017
HOM3	*HOM3* Forward	GAGGTGCAYGTBAGYATGGC	Sato et al. 2017
	HOM3 Reverse	ATRACRCANGADATRTTGATCTC	Sato et al. 2017
HSH49	*HSH49* Forward	AGGCDTCKTCKGAYAARAARCA	Sato et al. 2017
	HSH49 Reverse	TCDCCYTTGCCRTCYTTYTTRAA	Sato et al. 2017
ILS1	*ILS1* Forward	GAYGGMAAGAARATGAGCAARAG	Sato et al. 2017
	ILS1 Reverse	AGRATCCAKCGRTCCATVACRTT	Sato et al. 2017
ILV2	*ILV2* Forward	GGNCARCAYCAGATGTGGGC	Sato et al. 2017
	ILV2 Reverse	ACCATNCCYTGGAABTCGTTGTT	Sato et al. 2017
KOG1	*KOG1* Forward	TGTGYATYGCGCARATVTGGG	Sato et al. 2017
	KOG1 Reverse	TCYTCCCARTAGAKCCANGCRCA	Sato et al. 2017
MCM2	*MCM2* Forward	AARCGCATCRTCAARTCYATHGC	Sato et al. 2017
	MCM2 Reverse	TCGTTCATYTTGTCRAAYTCRTC	Sato et al. 2017
MET6	*MET6* Forward	GTCGAYGAGCCNGCYATYCGTGA	Sato et al. 2017
	MET6 Reverse	TAGACACCVGGVCCRATCTGGTT	Sato et al. 2017
MVD1	*MVD1* Forward	TCVYRCAACAACTTCCCYAC	Sato et al. 2017
	MVD1 Reverse	GTCTCGACGGTGCGYTGCATVCC	Sato et al. 2017
NIP1	*NIP1* Forward	CCVTTYCAYATGCAYATCAACAC	Sato et al. 2017
	NIP1 Reverse	ACTTGGCBARCATYTCYTTVAC	Sato et al. 2017
PDB1	*PDB1* Forward	GARTTCATGACNTTBAACTTYGC	Sato et al. 2017
	PDB1 Reverse	AGGAAGACRACRGGRTTNGGRTC	Sato et al. 2017
POL30	*POL30* Forward	CARGCNATGGAYAACTCYCAYGT	Sato et al. 2017
	POL30 Reverse	TCRATRTCCATNAGYTTCAT	Sato et al. 2017
PRE2	*PRE2* Forward	AAGAARGTCATYGARATYAA	Sato et al. 2017
	PRE2 Reverse	GTYTTGTCCCARCCRCARATCAT	Sato et al. 2017

Appendix 2 (cont.)

Locus	Primer name	Nucleotide sequence (5’–3’)	Reference
PRE8	*PRE8* Forward	GCAGCARGCMACDCARTCHGGGT	Sato et al. 2017
	PRE8 Reverse	AGSGCKGTGTGRATNGCGTCYTC	Sato et al. 2017
PUP1	*PUP1* Forward	GCVGACAAGAACTGYGARAAGG	Sato et al. 2017
	PUP1 Reverse	CCGTGWGGRTGGATNGTRAA	Sato et al. 2017
QNS1	*QNS1* Forward	GCNTGYTGGCTBTGGGAYTA	Sato et al. 2017
	QNS1 Reverse	CCCATRTARCANGTRTGGAA	Sato et al. 2017
RIO2	*RIO2* Forward	TCNGCNTCRTGGATGTAYATGTC	Sato et al. 2017
	RIO2 Reverse	ATGAGRATRTTGAAYTCRTT	Sato et al. 2017
RPN11	*RPN11* Forward	GTMGGVTGGTAYCAYTCNCATCC	Sato et al. 2017
	RPN11 Reverse	AGYTCYGTCTTGCGRTARTT	Sato et al. 2017
SAC6	*SAC6* Forward	GAGCTBGAVGAYTGGGTHGAGGT	Sato et al. 2017
	SAC6 Reverse	KRCABTCGTCRAAGAKYTGCAT	Sato et al. 2017
SMC1	*SMC1* Forward	ATCAARTAYCATGCCATGCC	Sato et al. 2017
	SMC1 Reverse	GAGCTRTTVACWTCYTGRTC	Sato et al. 2017
SMC3	*SMC3* Forward	TTCAACTCVAARGTNGAYGARGG	Sato et al. 2017
	SMC3 Reverse	CGGAAVGTMGTYGTRATGAAYTG	Sato et al. 2017
TCP1	*TCP1* Forward	TTYGTCGARGCTGGYGCNATGGC	Sato et al. 2017
	TCP1 Reverse	ACCARTGTCGTNGCRAAGTTYTC	Sato et al. 2017
TRP2	*TRP2* Forward	TACATGTTYTAYYTBGAYTGYGG	Sato et al. 2017
	TRP2 Reverse	GTYARRTGRATGACRTGRCTGAA	Sato et al. 2017
UBA1	*UBA1* Forward	GARTTYGAGAAGGAYGAYGA	Sato et al. 2017
	UBA1 Reverse	GGYTCNGAGAARCCRAAGAA	Sato et al. 2017
UBA3	*UBA3* Forward	GARCAYTGYATHGARTGGGC	Sato et al. 2017
	UBA3 Reverse	GCRATCTTGAANGCYTCRTTRCA	Sato et al. 2017
VMA2	*VMA2* Forward	CARAAGATYCCYATYTTCTC	Sato et al. 2017
	VMA2 Reverse	AGYTGGTANGCRTAGTAYTC	Sato et al. 2017
YSH1	*YSH1* Forward	GACTACTCBCGNGARGARGAYCG	Sato et al. 2017
	YSH1 Reverse	TTCATGGTRTGDATRTANGTYTG	Sato et al. 2017