

New species and distinctive geographical divergences of the genus *Sparassis* (Basidiomycota): evidence from morphological and molecular data

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Abstract Species of the genus *Sparassis* in East Asia were investigated using morphology and DNA sequences data. Phylogenetic analyses inferred from sequences of the internal transcribed spacer (ITS), the nuclear gene coding for the ribosomal large subunit (nLSU) and partial gene coding RNA polymerase subunit II (*rpb2*) strongly supported lineages corresponding to morphological features. Three taxa, *S. subalpina*, *S. cystidiosa* f. *flabelliformis* and *S. latifolia* were recognized from East Asia, and the former two taxa are new to science. The occurrence of *S. latifolia* in Japan and in the Russian Far East was confirmed. Geographical divergences of *Sparassis* in the Holarctic were observed. Most species have relatively narrow distribution ranges, and taxa with intercontinental distributions were not detected. Divergence of species in the Northern Hemisphere in different clades appears to have taken place at different times: the *S. latifolia-crispa-radicata* species complex appears to have undergone a recent radiation, while the *S. subalpina-brevipes-spathularia* species complex represents a relatively ancient speciation.

Keywords Cauliflower mushrooms · Morphology · Polyporales · Systematics · Taxonomy

Introduction

The Cauliflower Mushrooms (*Sparassis* Fr.) include species that produce flabellae with an amphigenous hymenium and a central mass giving rise to the flabellae (Desjardin et al. 2004; Wang et al. 2004). Members of *Sparassis* are distributed mainly in temperate forests, and are well known in some regions of the world (Blanco-Dios et al. 2006; Burdsall and Miller 1988; Dai et al. 2006; Desjardin et al. 2004; Kreisel 1983). About 17 names have been assigned to the genus (<http://www.indexfungorum.org>). However, due to morphological plasticity and paucity, delimitation of species in the genus has been difficult. Recent molecular phylogenetic analyses together with morphological data recognized seven species within the genus, two of which are known only from eastern and southeastern Asia (Dai et al. 2006; Desjardin et al. 2004; Wang et al. 2004).

During studies of macrofungi in East Asia (e.g. Du et al. 2012; Feng et al. 2012; Li et al. 2011; Liang et al. 2011; Yang 2011; Yang et al. 2012; Zeng et al. 2012), rich collections of *Sparassis* were made. Taxonomy and biogeographic distribution of East Asia *Sparassis* species were revised based on morphological examination and molecular phylogenetic analyses.

Materials and methods

Specimens and morphological studies Collections were obtained and photographed in the field during 1997–2011 in China, the Russian Far East and Japan, and deposited in the herbarium of cryptogams of Kunming Institute of Botany, Chinese Academy of Sciences (HKAS), and the

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mycological herbarium of Jilin Agriculture University (HMJAU). Macro-morphological characters were observed from fresh specimens. For microscopical examination, dried specimens were cut and squashed in 5–10 % aqueous KOH. Spore sizes are given in the form of Li et al. (2011).

DNA extraction, amplification and sequencing Total DNA was extracted from silica-gel-dried or herbarium materials using the CTAB procedure of Doyle and Doyle (1987). ITS and nLSU gene fragments were amplified with primers ITS5 and ITS4 (White et al. 1990), and LROR and LR7 (Vilgalys and Hester 1990), respectively. Part of the gene encoding the second largest RNA polymerase subunit II (*rpb2*) was amplified with primer pairs RPB2-6F/RPB2-7R (Wang et al. 2004). PCR products were purified using the Bioteke's Purification Kit (Bioteke Corporation, Beijing, China), and were sequenced with an ABI 3730 DNA analyzer and an ABI BigDye 3.1 terminator cycle sequencing kit (Sangon Co., Ltd, Shanghai, China). Sequences generated in this study were submitted to GenBank; accession numbers and specimen data are provided in Table 1.

Sequence alignments and phylogenetic analyses All sequences were aligned with the SATé program (Liu et al. 2009; Wang et al. 2011). SATé alignments were performed with six runs of 50 iterations under default settings of MAFFT for alignment estimation and RAxML for phylogeny estimation using a four-core PC. The best-scored alignment was viewed with BioEdit 5.0.9 (Hall 1999) and manually adjusted when necessary. Final concatenated alignment of three genes (ITS, nLSU and *rpb2*) was deposited in TreeBASE with accession no. SN 11775. The phylogenetic relationships among the taxa were estimated in PAUP* version 4.01 (Swofford 2002) under the Maximum Parsimony (MP) criterion. MP analysis was conducted using a heuristic search strategy with the following settings: the heuristic search option using the tree bisection-reconstruction algorithm. All sites were treated as unordered and equally-weighted, with gaps treated as missing data. Robustness of the internal branches of the resulting trees was tested with bootstrap analyses using 1,000 replications with the step-wise addition option set as simple. *Grifola frondosa* (Dicks.) Gray and *Oligoporus rennyi* (Berk. & Broome) Donk were chosen as outgroups following Wang et al. (2004). Tree scores, including tree length, consistency index [CI], retention index [RI], rescaled consistency index [RC], were also calculated.

Results

The combined ITS + nLSU + *rpb2* dataset consists of 33 taxa and 2,645 aligned nucleotide sites, of which 587 sites (23–62, 85–101, 119–129, 150–283, 306–309, 347–362,

451–749, 755–801, 824–827, 1213–1222, 1336–1337, 2396–2398) were excluded due to alignment ambiguities. In the analyzed 2,058 nucleotides, 1,427 were constant, 631 were variable of which 164 were parsimony uninformative and 467 parsimony informative. Maximum parsimony analysis resulted in four equally best trees of 955 steps, with CI=0.824, RI=0.946, and RC=0.779 (Fig. 1). Tree topologies were, in most parts, similar to that of the most-parsimonious multi-gene tree in Wang et al. (2004). Three major clades (clades I, II, and III) were inferred.

In clade I, fourteen isolates of *S. latifolia* Y.C. Dai & Zheng Wang from China, the Russian Far East and Japan clustered together with a 98 % statistic support, which shared the most recent common ancestor with *S. crispata* (Wulfen) Fr. from Europe and *S. radicata* Weir from North America. Within the cluster of *S. latifolia*, samples were grouped into two subclades (SW China subclade, and Northeast Asia subclade). Each subclade was statistically supported but bootstrap value for the later subclade was relatively low (62 %).

In clade II, four isolates of *S. subalpina* formed a monophyletic group (bootstrap=100 %) with European *S. brevipes* Krombh. and *S. miniensis* Blanco-Dios & Zheng Wang, and *S. spathulata* (Schwein.) Fr. as its sister groups (bootstrap=100 %).

In clade III, three isolates of *S. cystidiosa* f. *flabelliformis* from western Yunnan, which clustered together with 53 % bootstrap support, formed a strongly supported clade with *S. cystidiosa* f. *cystidiosa* Desjardin et Zheng Wang (bootstrap=100 %).

Taxonomy

Sparassis cystidiosa f. *flabelliformis* Q. Zhao, Zhu L. Yang & Y.C. Dai, f. nov.

Mycobank: MB 566348

Figure 2

Etymology Named for its flabelliform flabellae.

Basidioma annual, solitary, up to 25 cm high and 30 cm diam, grayish brown to yellowish brown, darkening with age, becoming brownish to light brown when dry; hyphae in the trama of the flabellum with abundant clamp connections; basidiospores (6.5) 7–9 (9.5) × (5.5) 6–7 (7.5) μm, broadly ellipsoid to subglobose, hyaline; basidia 55–70 × 7–8.5, narrowly elongate-subclavate, basal septa usually with clamp connections; cystidia absent.

Holotype Q. Zhao 1225 (HKAS 59855), 2,400 m, 19 VII. 2011, Gaoligong Mountains, Tengchong County, Yunnan Province, China.

Table 1 Specimens used in molecular phylogenetic studies and their GenBank accession numbers

Name code	Collection & herbarium no.	Locality	ITS	nLSU	<i>rpb2</i>
<i>Sparassis crispa</i> FRA	ILKKA94-1587	FRANCE	AY218427	AY218389	AY218534
<i>S. crispa</i> GER	DORISLABER	GERMANY	AY218442	AY218404	AY218544
<i>S. latifolia</i>	HKAS 55937	CHINA/YN/Lijiang	JN387097	JN387108	JN387119
<i>S. latifolia</i>	HKAS 55938	CHINA/YN/Lijiang	JN387098	JN387109	JN387120
<i>S. latifolia</i>	HKAS 55435	CHINA/YN/Shangri-La	JN387099	JN387110	JN387121
<i>S. latifolia</i>	HKAS 59358	CHINA/YN/Yongping	JN387100	JN387111	JN387122
<i>S. latifolia</i>	HKAS 59355	CHINA/YN/Jianchuan	JN387101	JN387112	JN387123
<i>S. latifolia</i>	HKAS 59356	CHINA/YN/Jianchuan	JN387102	JN387113	JN387124
<i>S. latifolia</i>	HKAS 59357	CHINA/YN/Jianchuan	JN387103	JN387114	JN387125
<i>S. latifolia</i>	HKAS 59854	JAPAN/Hokkaido	JQ743071	JQ743081	JQ743091
<i>S. latifolia</i>	HMJAU 2007	CHINA/JL/Jiaohe	JQ743072	JQ743082	JQ743092
<i>S. latifolia</i>	HMJAU 2955	RUSSIA/Big Khekhstir	JQ743073	JQ743083	JQ743093
<i>S. latifolia</i>	HMJAU 5301	CHINA/JL/Antu	JQ743074	JQ743084	JQ743094
<i>S. latifolia</i> (isotype)	HKAS 74972	CHINA/JL/Antu	JQ743075	JQ743085	JQ743095
<i>S. latifolia</i>	HKAS 74973	CHINA/JL/Changchun	JQ743076	JQ743086	JQ743096
<i>S. latifolia</i>	HKAS 74974	CHINA/JL/Changchun	JQ743077	JQ743087	JQ743097
<i>S. radicata</i> AME	TENN 50232	USA/TN	AY218449	AY218410	AY218546
<i>S. radicata</i> CAN	UBC-F 12464	CANADA	AY218443	AY218405	DQ270672
<i>S. brevipes</i> GER	ILKKA-96-1044	GERMANY	AY218441	AY218403	AY218543
<i>S. spathulata</i> AME7	ZW-Clarku 001	USA/MA	AY218428	AY218391	AY218535
<i>S. spathulata</i> AME8	ZW-Clarku 002	USA/NH	AY218429	AY218392	AY218536
<i>S. spathulata</i> AME11	ZW-Clarku 004	USA/MA	AY218432	AY218395	AY218538
<i>S. miniensis</i>	Lou-Fungi 18390	SPAIN	DQ270675	DQ270676	DQ270674
<i>S. cystidiosa</i> f. <i>cystidiosa</i>	DED 7410	THAILAND	AY256891	AY256890	AY256892
<i>S. subalpina</i> (holotype)	HKAS 57488	CHINA/YN/Lijiang	JN387093	JN387104	JN387115
<i>S. subalpina</i>	HKAS 57511	CHINA/YN/Lijiang	JN387094	JN387105	JN387116
<i>S. subalpina</i>	HKAS 55936	CHINA/YN/Lijiang	JN387095	JN387106	JN387117
<i>S. subalpina</i>	HKAS 55269	CHINA/YN/Jianchuan	JN387096	JN387107	JN387118
<i>S. cystidiosa</i> f. <i>flabelliformis</i> (holotype)	HKAS 59855	CHINA/YN/Tengchong	JQ743078	JQ743088	JQ743098
<i>S. cystidiosa</i> f. <i>flabelliformis</i>	HKAS 59856	CHINA/YN/Tengchong	JQ743079	JQ743089	JQ743099
<i>S. cystidiosa</i> f. <i>flabelliformis</i>	HKAS 59857	CHINA/YN/Tengchong	JQ743080	JQ743090	JQ743100
<i>Grifola frondosa</i>	ZW-Clarku 005	USA/MA	AY218415	AY218413	AY218521
<i>Oligoporus rennyi</i>	KEW 57	Locality unknown	AY218416	AY287876	AY218499

Basidioma (Fig. 2a) annual, solitary, up to 25 cm high, 30 cm diam, composed of a rosette of loosely arranged flabella arising from a poorly developed central core. *Flabellae* mostly extend from a common central mass, contorted, surface glabrous, grayish brown to yellowish brown, darkening with age, becoming brownish to light brown when dry, top margin entire or dissected, wavy, azonate, up to 10 cm broad, 2 mm thickness. *Context* tough, concolorous with surface. *Odor* cheese-like. *Taste* slightly bitter.

Basidiospores (Fig. 2b) [120/3/3] (6.5) 7–9 (9.5) × (5.5) 6–7 (7.5) μm ($Q=(1.08)$ 1.10–1.38 (1.45), $Q_m=1.26\pm 0.09$), broadly ellipsoid to subglobose, hyaline, thin-walled, smooth, non-amyloid, non-dextrinoid. *Basidia* (Fig. 2c) 55–70 × 7–8.5 μm, narrowly elongate-subclavate, 4-spored,

hyaline, usually with a basal clamp connection; basidioles narrowly elongate-subclavate. *Subhymenium* composed of tightly packed, thin- to slightly thick-walled (up to 1 μm thick), cylindrical or vesiculose to irregularly inflated hyphal elements. *Trama* of flabellae a monomitic hyphal system composed of loosely interwoven, hyaline, inamyloid, non-gelatinous, thin- to slightly thick-walled (up to 1.5 μm thick), commonly clamped cylindrical hyphae (Fig. 2d). *Gloeoplerous hyphae* present, refractive, thin-walled, flexuous, irregularly cylindrical to sinuous or strangulate, 2–11 μm diam. *Cystidia* absent.

Habitat and known distribution in China Terrestrial and saprophytic, solitary to scattered on the base of rotten oak

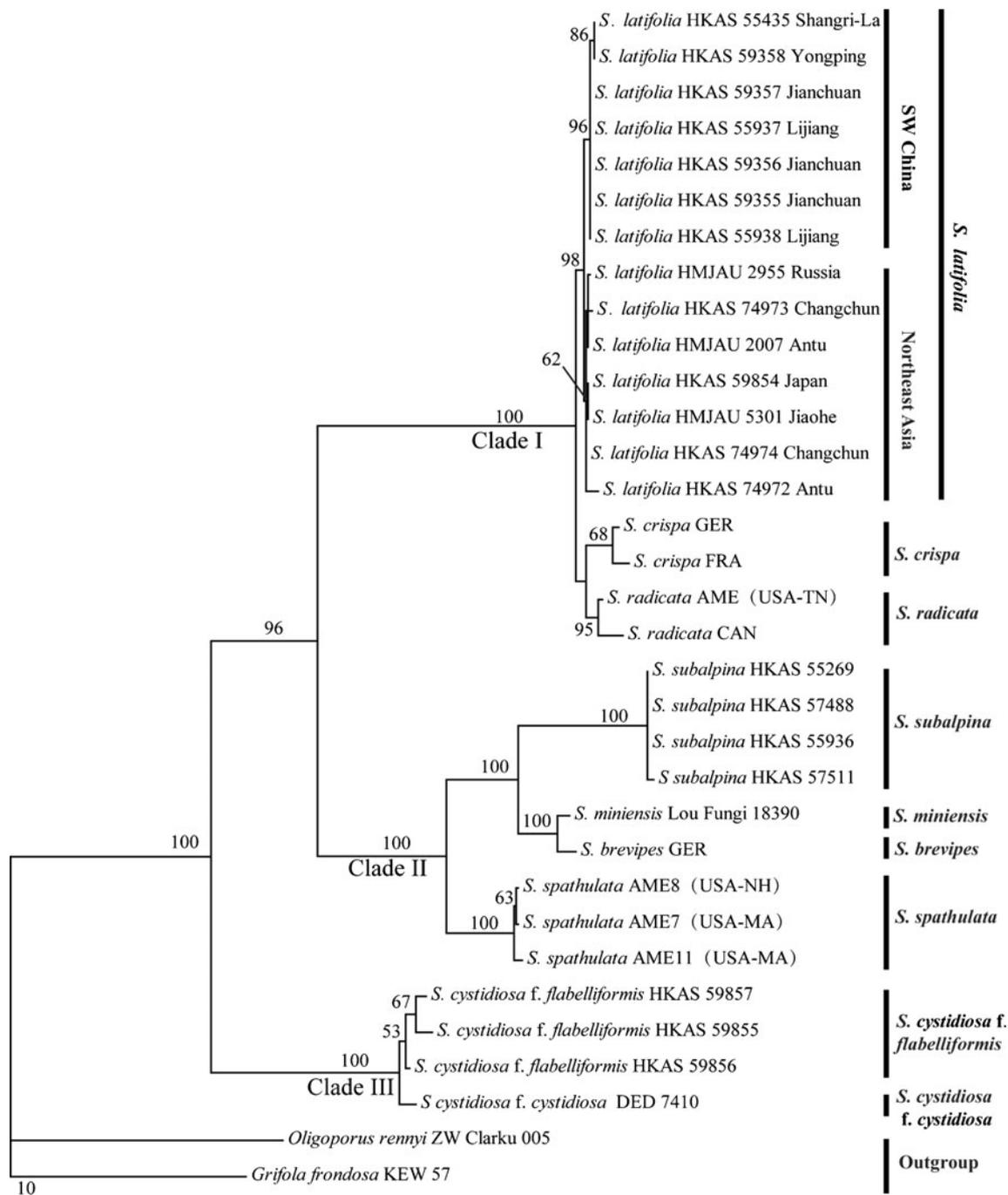


Fig. 1 Phylogenetic relationships of *Sparassis* species inferred from a combined dataset of ITS, nLSU, and *rpb2* sequences. One of the four equally parsimonious trees is shown, with branch lengths proportional

to the number of mutations inferred with parsimony. Bootstrap values greater than 50 % are indicated along nodes

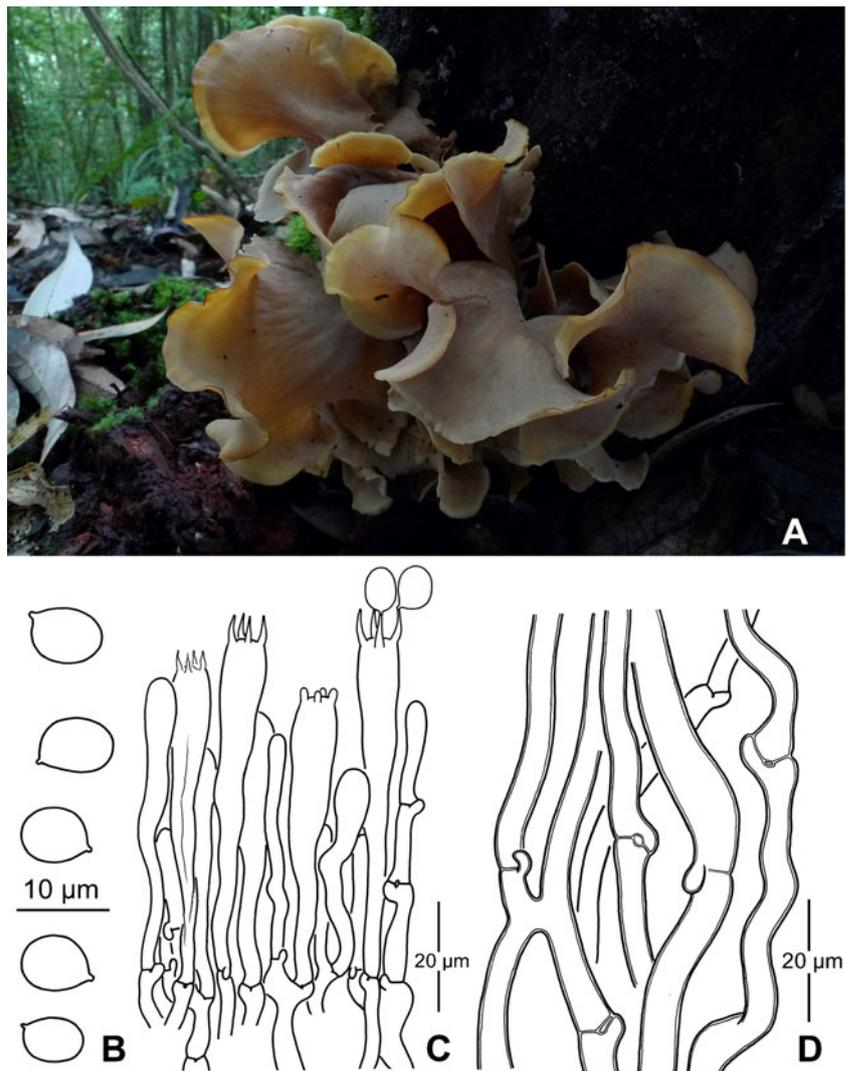
trees (*Quercus* sp.) in evergreen forest. So far only found in southwestern China (Yunnan).

County, Houqiao, alt. 2,000 m, 20. VII. 2011, B. Feng 1070 (HKAS 59857).

Specimens examined CHINA, Yunnan Province: Tengchong County, Gaoligong Mountains, alt. 2,400 m, 19. VII. 2011, Q. Zhao 1225 (HKAS 59855, holotype); the same location, 19. VII. 2011, Q. Zhao 1228 (HKAS 59856); Tengchong

Notes The distinctive characters of *S. cystidiosa* f. *flabelliformis* are the very broad flabellae with non-dissected and only slightly wavy margins, the greyish brown hymenium and relatively large basidiospores. Such traits are very

Fig. 2 *Sparassis cystidiosa* f. *flabelliformis* (holotype). **a** Basidioma. **b** Basidiospores. **c** Basidia at different stage of development. **d** Hyphae in the trama of a flabellum



similar to those of *S. cystidiosa* f. *cystidiosa*. However, *S. cystidiosa* f. *cystidiosa* differs from *S. cystidiosa* f. *flabelliformis* by the common presence of cystidia in the hymenium and its distribution in tropical southeastern Asia (Desjardin et al. 2004). The sister relationship of *S. cystidiosa* f. *flabelliformis* with *S. cystidiosa* f. *cystidiosa* was well supported by the molecular phylogenetic analysis with 100 % bootstrap value (Fig. 1).

Sparassis subalpina Q. Zhao, Zhu L. Yang & Y.C. Dai, spec. nov.

Mycobank: MB 569412

Figure 3

Etymology Named for its occurrence in subalpine regions in southwestern China.

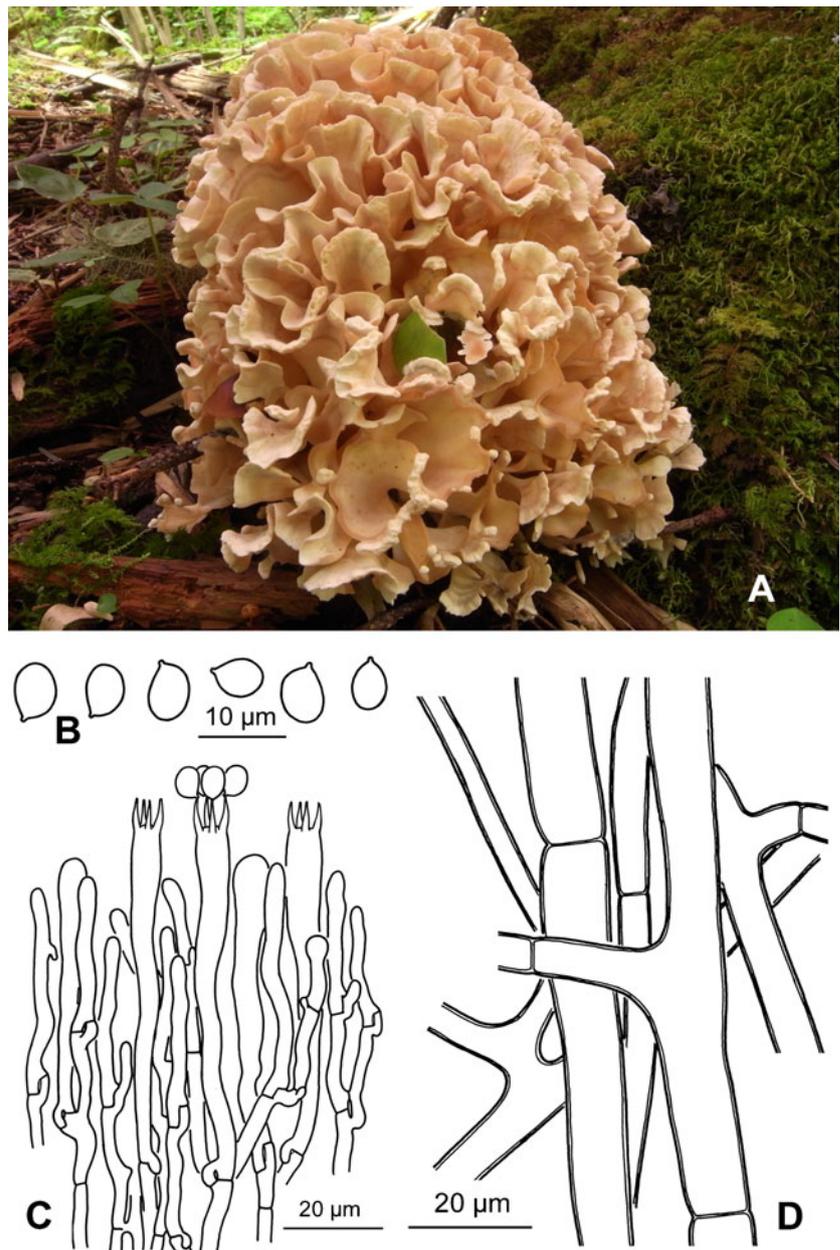
Basidioma annual, solitary, stipitate, up to 16 cm high and 15 cm diam, grayish when fresh, becoming brownish and leathery with age, dirty cream to light brown when dry;

hyphae in the trama of the flabellum without clamp connections; basidiospores (5) 5.5–6.5 (7) × (3.5) 4–5 (5.5) µm, broadly ellipsoid to subglobose, hyaline; basidia 73–85 × 5.5–7.5 µm, narrowly elongate-subclavate, basal septa usually with clamp connections.

Holotype B. Feng 759 (HKAS 57488), 3,050 m, 1. VIII. 2009, Laojun Mountains, Yulong County, Yunnan Province, China.

Basidioma (Fig. 3a) annual, solitary, stipitate, up to 16 cm high and 15 cm diam, composed of a group of flabella arising from a poorly developed central whitish stipe. *Flabellae* mostly extend from a common central mass, contorted, surface finely pruinose to glabrous, grayish and soft when fresh, becoming brownish and leathery with age, dirty cream to light brown when dry, 1–1.5 mm thick; top margin entire or slightly undulate, sometimes tooth-like or slightly lacinate, up to 2 mm in thickness. *Context* tough, concolorous with surface. *Stipe* up to 7 cm long, 3.0 cm

Fig. 3 *Sparassis subalpina* (holotype). **a** Basidioma. **b** Basidiospores. **c** Basidia at different stage of development. **d** Hyphae in the trama of a flabellum



thick at base, thinning out. *Odor* pleasant. *Taste* not recorded.

Basidiospores (Fig. 3b) [120/4/4] (5) 5.5–6.5 (7) × (3.5) 4–5 (5.5) µm ($Q=(1.10)$ 1.2–1.5 (1.57), $Q_m=1.35\pm 0.12$), broadly ellipsoid to subglobose, hyaline, thin-walled, smooth, non-amyloid, non-dextrinoid. *Basidia* (Fig. 3c) 73–85 × 5.5–7.5 µm, narrowly elongate-subclavate, 4-spored, hyaline, usually with a basal clamp connection; basidioles narrowly elongate-subclavate. *Subhymenium* composed of hyaline, thin-walled, tortuous, densely interwoven hyphal segments with common presence of clamp connections. *Trama* of flabellum a monomitic hyphal system composed of simply septate, colorless and hyaline, thin

to thick-walled, frequently branched, interwoven hyphae 4–10 µm diam; clamp connections absent (Fig. 3d). *Gloeoplerous hyphae* present, refractive, thin-walled, flexuous, frequently branched, 4–10 µm diam.

Habitat and distribution Solitary, near the roots of living *Picea likiangensis* (Franch.) Pritz, *Rhododendron* sp. and *Quercus* sp. or near subalpine bamboos in northwestern Yunnan Province. So far this fungus has only been collected in subalpine Yunnan, southwestern China.

Specimens examined CHINA, Yunnan Province, Yulong County, Laojun Mountains, alt. 3,050 m, 11. VII. 2009, B.

Feng 759 (HKAS 57488, holotype); the same location, 11. VII. 2007, Q. Zhao 708 (HKAS 55936); the same location, 12. VII. 2009, B. Feng 782 (HKAS 57511); Jianchuan County, Jianyang, alt. 3000 m, 29. VIII. 2008, S.H. Li 154 (HKAS 55967).

Notes *Sparassis subalpina* is similar to *S. brevipes*, *S. miniensis* and *S. spathulata* by sharing the massed base of the basidioma, scattered and broad flabellae. However, the flabellae of *S. subalpina* are very broad, zonate and with only slightly contorted, thickened margins, while those of *S. brevipes*, originally described from Central Europe and mostly associated with *Abies*, *Fagus* and *Quercus*, are split into pieces that fuse with other flabellae several times below the top margin. In addition, clamp connections are common and abundant in the subhymenium but absent in the remaining parts of the basidioma of *S. subalpina*, while they are absent in all parts of *S. brevipes* (Wang et al. 2004). *Sparassis miniensis*, described based on collections made in forests of *Pinus* spp. from Spain, basidioma is composed of an orange to rose-purplish base, a scattered group of fragile, azonate flabellae with a strongly lacinate margin and sporadic of clamp connections in the subhymenium (Blanco-Dios et al. 2006). *Sparassis spathulata*, originally described from eastern United States and mostly associated with *Pinus* spp. and *Quercus* spp., produces significantly larger basidiomata, slightly longer basidiospores and no clamp connections (Burdall and Miller 1988; Desjardin et al. 2004; Wang et al. 2004).

Sparassis latifolia Y.C. Dai & Zheng Wang, Mycologia 98: 586 (2006).

Figure 4

Basidioma (Fig. 4a) annual, solitary, stipitate, up to 30 cm high and 35 cm diam, composed of numerous loosely arranged flabellae. *Flabellae* mostly extending from a common central mass, broad, dissected and slightly contorted, white to cream when fresh, becoming deep cream to shallow brown with age, pale ochraceous and light brown when dry, azonate, up to 1 cm broad, 1 mm thick, margin wavy to tooth-like. *Stipe* up to 15 cm long, 2 cm thick at base. Flabellar context tough, concolorous with surface. *Odor* pleasant. *Taste* indistinctly acid.

Basidiospores (Fig. 4b) [340/17/17] (4) 4.5–5.5 (6) × (3) 3.5–4 (4.5) μm ($Q=(1.1)$ 1.25–1.43 (1.5), $Q_m=1.306 \pm 0.07$), broadly ellipsoid to subglobose, hyaline, thin-walled, smooth. *Basidia* (Fig. 4c) 55–68 × 5–7 μm, narrowly elongate-subclavate, 4-spored, hyaline, usually with a basal clamp connection; basidioles narrowly elongate-subclavate. *Subhymenium* composed of delicate, hyaline, thin-walled, tortuous, densely interwoven hyphal segments with common presence of clamp connections. *Trama* of flabellum a

monomitic hyphal system composed of simply septate, colorless and hyaline, thin to thick-walled, frequently branched, interwoven hyphae 5–15 μm diam; clamp connections common and abundant (Fig. 4d). *Gloeoplerous hyphae* present, refractive, thin-walled, flexuous, frequently branched, 7–12 μm diam.

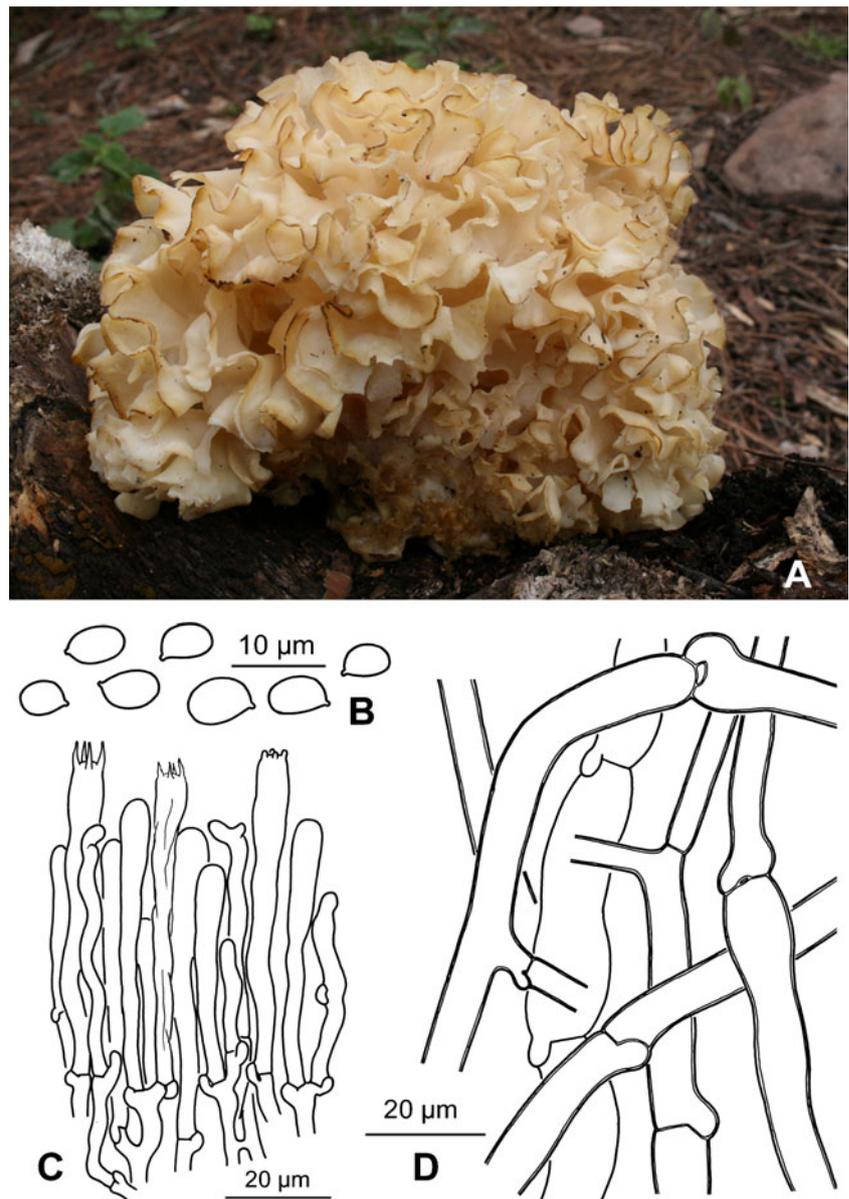
Habitat and known distribution Solitary on the base of conifers and Fagales. Distributed in eastern Russia, Japan and China.

Specimens examined CHINA, Yunnan Province, Lijiang County, Xinzhu, alt. 2,900 m, 19. VIII. 1984, Y. Xian 16 (HKAS 14208 as “*Sparassia crispa*” in Zang et al. 1996); Shangri-La County, Biguo, alt. 3,200 m, 7. VII. 1984, D.G. Ji 76 (HKAS 16741 as “*S. crispa*” in Ying and Zang 1994); Yulong County, Laojun Mountains, alt. 2,600 m, 19. VII. 2007, Q. Zhao 794 (HKAS 55938); the same location, 24. VII. 2007, Q. Zhao 7326 (HKAS 55937); Shangri-La County, Tianchi, alt. 3,200 m, 12. VII. 2008, B. Feng 324 (HKAS 55435); Yongping County, Dapindi, alt. 1,700 m, 20. VII. 2009, S.H. Li 656 (HKAS 59358); Jianchuan County, Jianyang, alt. 2300 m, 12. VII. 2009, S.H. Li 353, 354 and 355 (HKAS 59355, 59356 and 59357 respectively). Sichuan Province, Daocheng County, Julong, 11. VIII. 1984, M.S. Yuan (HKAS 15728 as “*S. crispa*” in Yuan and Sun 2007). Jilin Province, Antu County, Changbaishan, 14. VIII. 1997, Dai 2441 (HKAS 74972, isotype); Antu County, Edaobaihe, 20. VIII. 2007, Y. Wang 5301 (HMJAU 5301); Changchun, Jingyuetan National Forest Park, 14. VII. 2007, DAI 12549 (HKAS 74973); the same location, 24. VIII. 2007, DAI 10269 (HKAS 74974); Jiaohe County, Jiaohe, 20. VIII. 2006, Y.G. Fan 2007 (HMJAU 2007). JAPAN, Hokkaido, alt. 100 m, 12. XI. 2011, Z.L. Yang 5531 (HKAS 59854). RUSSIA, Big khekhstir, 16. VIII. 2004, B. Tolgor 2955 (HMJAU 2955).

Notes Studies on collections from Russian Far East, Japan, northeastern and southwestern China yielded additional information on *S. latifolia*. This taxon is a common species widely distributed under trees of conifers and Fagales in the subtropic alpine and temperate regions in eastern Asia. Macroscopically, *S. latifolia* differs from other species of *Sparassis* by its large, broad, dissected and slightly contorted flabellae. Microscopically, it is characterized by the small spores and the common presence of clamp connections in all tissues of the basidioma.

Sparassis latifolia and *S. subalpina* are similar to each other in that they both produce broad flabellae and in their ecological preference. However, *S. latifolia* has azonate flabellae arising from a relatively more robust stipe, and the common presence of clamp connections not only in the

Fig. 4 *Sparassis latifolia* (HKAS55938). **a** Basidioma. **b** Basidiospores. **c** Basidia and basidioles at different stage of development. **d** Hyphae in the trama of a flabellum



subhymenium but also in other parts of the basidioma, a character shared with *S. crispa* and *S. radicata* (Dai et al. 2006).

Key to the taxa of *Sparassis* known from East Asia and Southeast Asia

- 1 Hyphae in the trama of the flabellum without clamp connections *S. subalpina*
- 1* Hyphae in the trama of the flabellum with abundant clamp connections
- 2 Flabellae broad (up to 30 cm in diam.), azonate, stipitate; hymenophore white to cream; basidiospores $4.5\text{--}5.5 \times 3.5\text{--}4 \mu\text{m}$ *S. latifolia*
- 2* Flabellae broad (10–15 cm in diam.), rosette; hymenophore dark greyish brown; basidiospores $7\text{--}9 \times 6\text{--}7 \mu\text{m}$
- 3 Cystidia common *S. cystidiosa* f. *cystidiosa*
- 3* Cystidia absent *S. cystidiosa* f. *flabelliformis*

Discussion

Biogeographic divergence in *Sparassis*

Supported with molecular phylogenies, 10 morphological taxa, *S. brevipes*, *S. crispa*, *S. cystidiosa* f. *cystidiosa*, *S. cystidiosa* f. *flabelliformis*, *S. latifolia*, *S. miniensis*, *S. radicata*, *S. spathulata*, *S. subalpina*, and *S. sp.* (AUS31), have been recognized within *Sparassis*. It is interesting to note that species of *Sparassis* show distinct geographical distribution patterns, although they are primarily saprophytic (Dai et al. 2006; Desjardin et al. 2004; Wang et al. 2004),

living mainly on decayed wood and debris. The three taxa described in this study have only been found in East Asia so far, while the other six previously described species and one undescribed species also have a restricted distribution, i.e., *S. crispa*, *S. brevipes* and *S. miniensis* in Europe, and *S. crispa* and *S. spathulata* in eastern North America (One Japanese collection, CUP JA1385, regarded as *S. spathulata*, might be introduced from North America as suggested by Wang et al. 2004), *S. radicata* in western North America, while *S. cystidiosa* f. *cystidiosa* is currently known from Thailand only, and undescribed species “*S. sp.* (AUS31)” only known from Tasmania, Australia (Wang et al. 2004). These distribution patterns indicate that *Sparassis* is adapted to temperate environments with a limited number of species from tropical areas. Except for unsolved relationships between European and eastern North American *S. crispa*, no taxa with intercontinental distributions were found to date.

The species that occur in the temperate North Hemisphere are clustered into two well-supported major clades (clades I and II) in the phylogeny (Fig. 1), and closely related species or species pairs were found in Eurasia, East Asia-North America, or Europe-North America in each clade, indicating that broad historical exchange of *Sparassis* could exist in the Holarctic region. This is in accordance with observations on many ectomycorrhizal fungi, such as *Boletus* (Dentinger et al. 2010; Feng et al. 2012), *Chroogomphus* (Li et al. 2009) and *Amanita* (Geml et al. 2006, 2008; Zhang et al. 2004), and many other fungi (Halling 2001; Mueller et al. 2001; Petersen and Hughes 2007; Redhead 1989). This suggests allopatric speciation events have contributed a lot to the current distribution patterns of plants (Donoghue and Smith 2004) and some fungal groups (e.g. Garnica et al. 2011; Hosaka et al. 2008; Vincenot et al. 2012). Furthermore, divergence of species in the temperate North Hemisphere in clades I and II appears to have occurred at different times based on the phylogenetic analysis (Fig. 1). Further studies that include more taxa from Europe and eastern North America are critical for understanding the biogeography of *Sparassis*. The multilocus phylogeny suggest that species in Clade I (the species complex of *S. latifolia-crispa-radicata*) may have undergone a recent radiation as the three species showed limited sequence variation, while the species in Clade II (the *S. subalpina-brevipes-spathularia* species complex) appear to represent a relatively ancient speciation event as the species in different geographical localities (East Asia, Europe, and North America) showed relatively high sequence variation.

Taxonomy of *S. latifolia* in East Asia

Sparassis latifolia was originally described from northeastern China (Dai et al. 2006). It was originally misidentified as *S. crispa* in eastern Asia (Imazeki et al. 1988; Mao 1998,

2000; Ying and Zang 1994; Yuan and Sun 2007; Zang et al. 1996). Dai et al. (2006) discovered that *S. latifolia* and *S. crispa* can be distinguished from each other by their macro- and micro-morphological characters, i.e., the shape of flabellae and the size of spores, as well as their host plant preferences. Our observations show that the long stipe of *S. latifolia* can also be used to separate *S. latifolia* from *S. crispa* in the field. Dai et al. (2006) suggested that “*S. crispa*” collected and reported from far eastern Russia and Japan could be *S. latifolia*. Studies on our collections made from the Russian Far East and northern Japan confirmed the occurrence of *S. latifolia* in these two regions. Furthermore, analyses based on ITS sequences indicate that samples named “*S. crispa*” (GU138863 and GU138864 in GenBank) from South Korea are also closely related to *S. latifolia* from northeast China (data not shown). These morphological and molecular data strongly indicate that *S. latifolia* is an easily distinguishable species with a wide distribution throughout eastern Asia.

With our extensive collections from the current known distribution of *S. latifolia*, interesting intraspecific divergences of this species can be detected. As shown in Fig. 1, isolates of *S. latifolia* were divided into two subclades, the SW China subclade and the Northeast Asia subclade, with relatively high bootstrap support. Although no obvious morphological characters could be found to distinguish them, our molecular data suggests that they may represent two distinctive phylogenetic species separated by geography. Future population genetic studies should provide insights into the evolution of *S. latifolia*.

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