Flammulina species from China inferred by morphological and molecular data

Ge, Z.W.[1,4], Yang, Z.L.[1,*] , Zhang, P.[2], Matheny, P.B.[3], and Hibbett, D.S.[3]

[1]Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, China
[2]College of Life Science, Hunan Normal University, Changsha 410081, Hunan Province, China
[3]Department of Biology, Clark University, 950 Main Street, Worcester, Massachusetts 01610, USA
[4]Graduate School of the Chinese Academy of Sciences, Beijing 100039, China

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Morphological, phylogenetic and biogeographic studies were carried out on Chinese collections of *Flammulina.* It is revealed that at least four species [*F. rossica, Flammulina* sp. (HKAS 51191)*, F. velutipes* and *F. yunnanensis*] occur in China. *Flammulina yunnanensis* is described as new based on morphological and molecular data. *F. rossica*, a new record to China, is confirmed to have a Holarctic distribution. *Flammulina* sp. has a hymeniform suprapellis but is phylogenetically close to *F. velutipes.* Analyses of the ITS/5.8S rDNA sequences of *Flammulina* species suggest that collections of *F. velutipes* from China are more closely related to a Canadian population rather than to those of Europe and the USA.

Key words: distribution, enoki-take, internal transcribed spacer, new taxon, species diversity

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[*]Corresponding author: Yang Z.L.; e-mail: fungi@mail.kib.ac.cn

Introduction

There has been considerable interest in studying the macrofungi of China (Nuytinck *et al*., 2006; Dai *et al*., 2007; Wang et al., 2007; Cui *et al*., 2008; Yang and Ge, 2008; Zheng and Liu, 2008; Zhuang and Yang, 2008). In this study we report on collections of *Flammulina* from southwestern, central and northeastern China. *Flammulina* (*Physalacriaceae, Agaricomycetidae*) (Binder *et al*., 2006; Matheny *et al*., 2007; Petersen, 2007, 2008) is a major genus containing edible mushrooms with a cosmopolitan distribution. Because of its nutritional and medical properties, *F. velutipes* (Curtis) Singer is one of the six most actively cultivated mushroom species in the world with a production over 300,000 tons per year by the end of 20th century (Psurtseva, 2005), and has received much attention (Lamour, 1989; Yokoyama, 1991; Psurtseva and Mnoukhina, 1998; Petersen *et al*., 1999; Methven *et al*., 2000; Hughes and Petersen, 2001). Traditionally, this genus was regarded as monotypic with only the type species *F. velutipes* although the variation was noted (Buchanan, 1993). However, several new species were recognized based on more detailed studies of inclusive worldwide collections in the last three decades, and 12 species (17 taxa, including varieties and forms) are currently accepted: *F. callistosporoides* (Singer) Singer, *F. elastica* (Lasch) Redhead & R.H. Petersen, *F. fennae* Bas, *F. ferrugineo-lutea* (Beeli) Singer, *F. mediterranea* (Pacioni & Lalli) Bas & Robich, *F. mexicana* Redhead, Estrada & R.H. Petersen, *F. ononidis* Arnolds, *F. populicola* Redhead & R.H. Petersen, *F. rossica* Redhead & R.H. Petersen, *F. similis* E. Horak, *F. stratosa* Redhead, R.H. Petersen & Methven, and *F. velutipes* (Curtis) Singer (Arnolds, 1977; Horak, 1980; Bas, 1983; Bas and Bobich, 1988; Redhead and Petersen, 1999; Redhead *et al*., 1999; Redhead *et al*., 2000; Singer 1964, 1969). A phylogenetic study of *Flammulina* showed that terminal taxa for the ITS tree are concordant with both morphology and mating studies, supporting the division of *Flammulina* into different morphological species (Hughes *et al*., 1999).

However, materials used in the study were mainly from Europe, North America and to a lesser extent from eastern Asia, and only one collection was from China.

In contrast to the situation in Europe and America, *Flammulina* has not been critically studied in China, and few reports can be found. Except for the collection GDGM 4637, which was regarded as *F. fennae* by Bi *et al.* (1994), all other Chinese *Flammulina* collections were lumped as *F. velutipes* or *Collybia velutipes* (in Chinese "Donggu", or "Jinzhengu") (Lohwag, 1937; Tai, 1979; Wang and Zang, 1983; Bi *et al.*, 1994; Ying and Zang, 1994; Teng, 1996). The objectives of the present study are: (i) to revise the taxonomy of putative Chinese collections of *Flammulina*; (ii) to characterize Chinese species of *Flammulina* using morphology and molecular data; (iii) to clarify phylogenetic relationships of Chinese *Flammulina* species with their counterparts from other continents; and (iv) to provide details on the distribution and biogeography of *F. rossica*.

Materials and methods

Specimens and morphological descriptions

Macro-morphological descriptions are based on the field notes and color slides of basidiomes. Color codes of the form "5A3" that indicate the plate, row, and color block are from Kornerup and Wanscher (1981). Herbarium codes used follow Holmgren *et al.* (1990) with one exception: Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS), which is not listed in the index or relevant publications. Micro-morphological data were obtained from the dried specimens after sectioning and mounting in 5% KOH solution for study under a light microscope. In the descriptions of basidiospores, the abbreviation [*n*/*m*/*p*] shall mean *n* basidiospores measured from *m* fruit bodies of *p* collections; *Q* is used to mean "length/width ratio" of a spore in side view; ***Q*** means average *Q* of all basidiospores ± sample standard deviation; *x* means range of basidiospore length × width.

DNA extraction, PCR and sequencing

Genomic DNA was extracted from 15 Chinese materials and a German collection of *Flammulina* (Table 1). Sampling localities of all the Chinese collections were showed in Fig. 3. DNA was isolated with a SDS miniprep following the protocol of Wang *et al.* (2002), or using a modified CTAB procedure of Doyle and Doyle (1987), or using the E.Z.N.A. Fungal DNA Kit (Omega Bio-tek, Doraville, Georgia, USA). ITS/5.8S rDNA were amplified using primers ITS1F and ITS4 (White *et al.,* 1990, Gardes and Bruns, 1993). PCR products were purified using a QIAquick PCR purification kit (Qiagen Science, Maryland, USA). Sequencing was performed using a Big-dye terminator cycle sequencing kit (Applied Biosystems, Foster City, California, USA) following the manufacturer's protocol. Sequencing primers for the ITS regions were ITS1F and ITS4. Sequencing reactions were purified using Pellet Paint (Novagen, Madison, Wisconsin, USA) and were run on an Applied Biosystems 377 XL automated DNA sequencer. Sequence chromatograms were compiled with Sequencher 4.1 software (GeneCodes Corporation, Ann Arbor, Michigan, USA). Sequences generated in this study were deposited in Genbank with accession numbers EF601574, DQ486704 and EF599844-EF595857.

Phylogenetic analyses

16 ITS sequences were assembled manually in MacClade 4.0 (Maddison and Maddison, 2000) with the data set of Hughes *et al.* (1999), which was downloaded from TreeBASE (accession no. SN235) (http://www.treebase.org/treebase/). Gaps were treated as "fifth base". The alignment is available at TreeBASE (accession no. SN3411).

The ITS data set included 34 samples of *Flammulina*. *Flammulina stratosa* was designated as the outgroup because a more inclusive prior phylogenetic analysis (including genera *Xerula* and *Oudemansiella*) suggests that it is basal to all other *Flammulina* species (results not shown). Phylogenetic relationships were estimated in PAUP* (Swofford, 2004) under the Maximum Parsimony (MP) criterion. MP analysis in PAUP* used a heuristic search strategy with the following settings: Multistate taxa interpreted as uncertainty; starting tree(s) obtained via stepwise addition; addition sequences with random option of 1000 replicates; held 10 trees at each step during

Table 1. Specimens of *Flammulina* used in molecular studies and GenBank accession numbers.

Species	Collections	Location and month of colletion	Substrate	GenBank accession #
F. elastica	TENN 56057	Austria: Vienna, 27-XI-1994	On *Salix alba*	AF034103
F. elastica	TENN 54689	Netherlands: Leiden, 21-I-1997	On *Salix*	AF141134
F. elastica	HKAS 52018	Germany: Marburg, XI-2004		EF595849
F. fennae	Th.Kuyper 2220	Netherlands: Utreght, Breukelen, 16-XI-1982		AF141135
F. fennae	TENN 54172	Switzerland: Canton Graubunden, 16-X-1995	On *Alnus incana*	AF035398
F. mexicana	TENN 52894	Mexico: Est. Tlaxcala, 11-VII-1993	On *Senecio cinerarioides*	AF032129
F. ononidis	TENN 54743	Germany: location and date unknown		AF051701
F. populicola	TENN 53636	Sweden: Uplland, vic. Uppsala, 7-XI-1994	On *Populus*	AF047873
F. populicola	TENN 54171	United States: Alaska, Anchorage, 17-IX-1995		AF044193
F. populicola	Halling 6536	United States: California, Sierra Co.	On *Alnus* or *Populus*	AF031655
F. rossica	I. Bullakh	Russia: Terr. Primorsk, IX-1994		AF051699
F. rossica	TENN 54169	United States: Alaska, 14-IX-1995	On *Salix*	AF044194
F. rossica	HKAS 46076	China: Tibet, Changdu, 7-VIII-2004	On *Salix*	EF595845
F. rossica	HMJAU 20588	China: Jilin, Zuojia, 16-IX-2000		EF595847
F. rossica	HKAS 43699	China: Tibet, Leiwuqi, 8-VIII-2004	On *Salix*	EF595846
F. rossica	HKAS 45970	China: Tibet, Changdu, 27-VII-2004	On *Salix*	EF595850
F. rossica	HKAS 32154	China: Sichuan, Xiangcheng, 16-VII-1998	On rotten wood of *Salix*	EF595856
F. rossica	HKAS 32155	China: Sichuan, Daocheng, 4-VII-1998	On *Picea*	EF595855
F. rossica	HKAS 7930	China: Jilin, Baihe, 15-VIII-2004	In *Betula* forest	EF595852
F. sp.	HKAS 51191	China: Tibet, Mozhugongka, 2-IX-2006	On the base of a dead trunk	EF601574
F. stratosa	TENN 56240	New Zealand: South Island, 17-V-1994		AF047872
F. yunnanensis	HKAS 32774	China: Yunnan, Lushui, 29-IX-1998	In forest with *Schima* trees	DQ486704
F. yunnanensis	HKAS 41344	China: Yunnan, Longlin, 27-VIII-2002	On a dead fagaceous plant	EF595857
F. velutipes	TENN 56008	Canada: British Columbia.		AF141133
F. velutipes	TENN 54748	Netherlands: Prov. Zeeland, 23-XI-1981		AF036928
F. velutipes	K 28262	United Kingdom: Surrey, Ham, 3-I-1995		AF030877
F. velutipes	TENN55402	United States: California, 24-XI-1996	On *Lupinus arboreus*	AF047871
F. velutipes	TENN 56028	United States: Michigan, 19-XI-1995		AF051700
F. velutipes	HKAS 49485	China: Yunnan, Kunming, 9-VII-2004	Cultivated	EF595844
F. velutipes	HKAS 51962	China: Hubei, Wuhan, 20-XI-2006	On *Broussonetia papyrifera*	EF595848
F. velutipes	HKAS 47767	China: Hunan, Changsha, 24-XII-2004	On *Broussonetia papyrifera*	EF595853
F. velutipes	HKAS 47768	China: Hunan, Changsha, 24-XII-2004	On *Broussonetia papyrifera*	EF595854
F. velutipes	HKAS 51988	China: Jilin, Changbai Mt., 27-X-2006	On *Betula platyphylla*	EF595851
F. velutipes	FH DH97-080	China: Sichuan, Gongga Mt., 17-VIII-1997	On dead hard wood	AF159426

stepwise addition; tree-bisection-reconnection (TBR) branch-swapping; "MulTrees" option not in effect. 500 MP bootstrap replicates were done also using heuristic search with the same search parameters as above. The best-fit model to the ITS data set was estimated using Modeltest 3.7 (Posada and Crandall, 1998, 2001). Bayesian analysis was also performed on this data set in MrBayes 3.1.2 (Huelsenbeck *et al.,* 2001, Huelsenbeck *et al.,* 2002; Ronquist and Huelsenbeck, 2003). A preliminary run of 200,000 generations using four Metropolis-Coupled Monte Carlo Markov chains was done to estimate how many generations were required for likelihood scores to reach stationarity. This result then dictated our burn-in value for a second run of 2,000,000 generations also using four chains. A total of 19,000 trees, among 20,000 sampled, was used to calculate posterior probabilities.

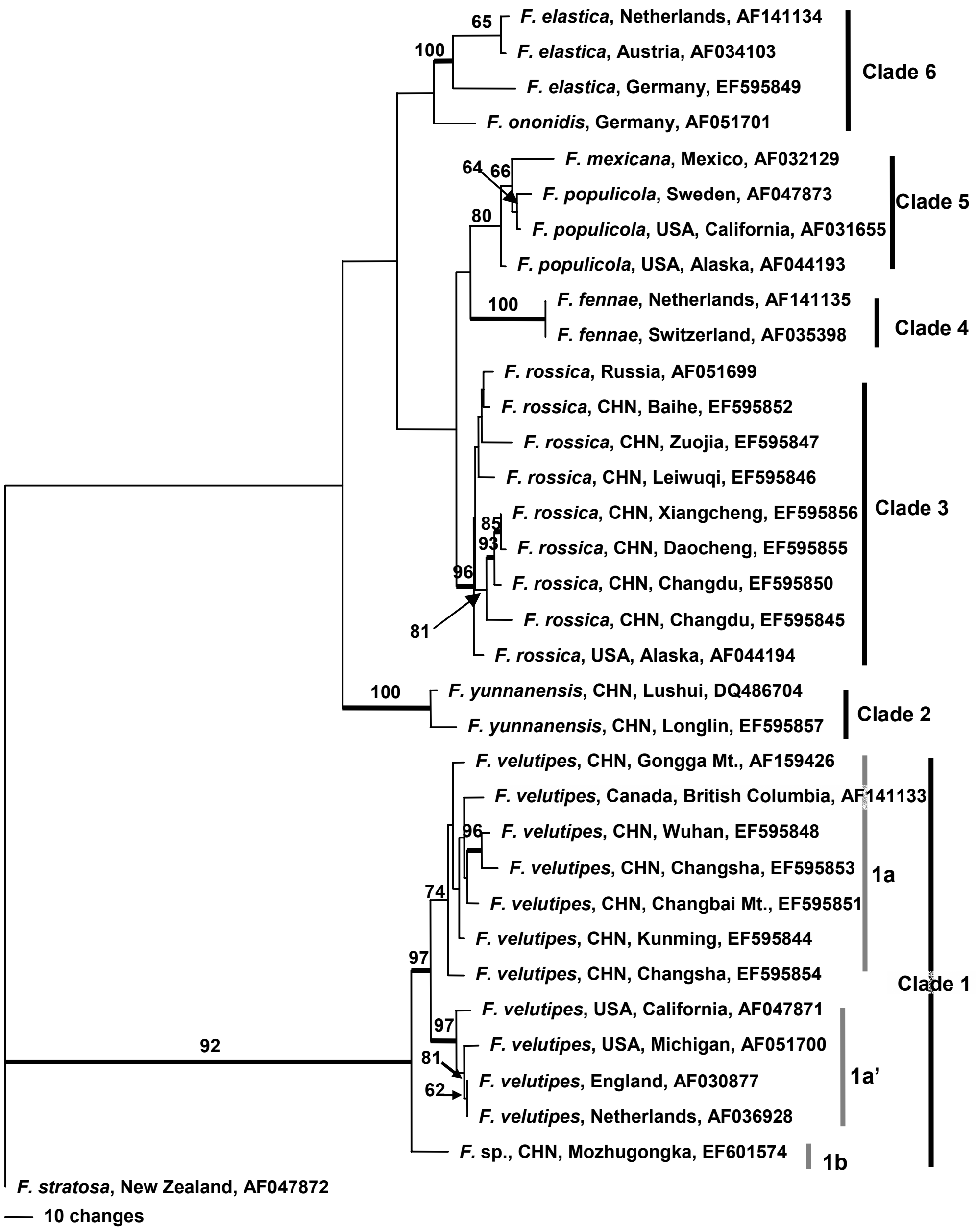

Fig.1. One of the 11 most parsimonious trees based on ITS/5.8S rDNA sequences. Bootstrap support values greater than 50% are indicated above the branch. Thick branches indicate Bayesian posterior probabilities value is greater than 0.90 for that clade.

Results

Taxonomy

Examinations of the Chinese collections resulted in four species based on morphology: *F. velutipes*, *F. rossica,* and two undescribed species, one of which is described below. Two collections, namely GDGM 4637and 4553, regarded as *F. fennae* and *F. velutipes* separately by Bi *et al.* (1994) are not members of *Flammulina* but of *Gymnopus* based on the form and the size of the basidiospores and the pileipellis of repent hyphae. The collection Handel-Mazzetti 12966 (WU), regarded as *Collybia velutipes* by Lohwag (1937), was restudied and treated as an undescribed taxon of *Flammulina* by Horak (1987). However, this accession might be a species of *Xeromphalina* if the basidiospores are amyloid, as mentioned by Horak (1987). Our reexamination of the collection showed that the basidiospores are non-amyloid, elongate to subcylindric, 5.5-7.5

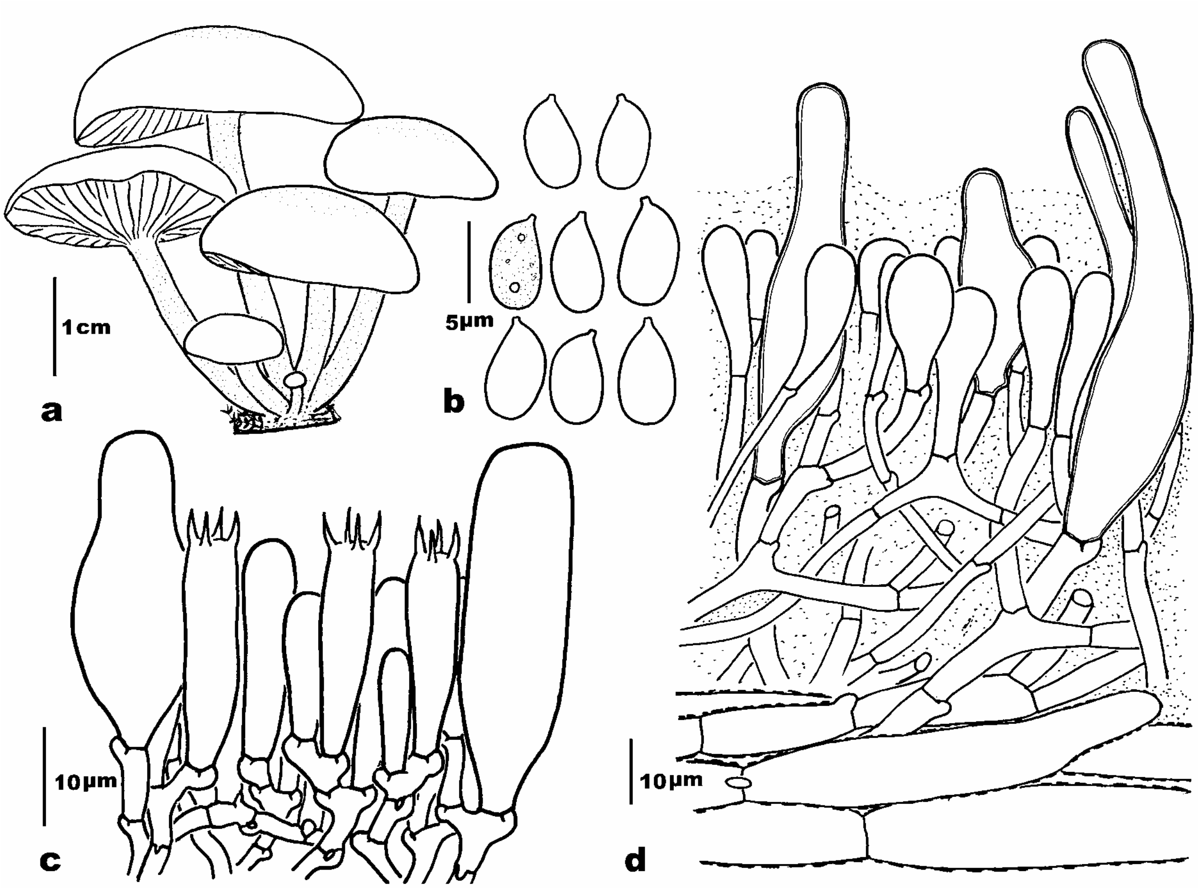

Fig 2. *Flammulina yunnanensis*. (HKAS41344-**holotypus**). **a.** Basidiomata. **b.** Basidiospores. **c.** subhymenium, and hymenium with pleurocystidia and basidia. **d.** Radial section of pileipellis.

× 2.3-3.2 µm. The pileipellis consists of an ixohyphidia (4.1-5.5 µm in width) with interspersed clavate elements and fusoid-lanceolate pileicystidia (90-120 × 8-12 µm). It is a member of *Flammulina* without any doubt. However, the width of the spores and the structure of the pileipellis don't fit the concepts of any known species. Due to the scanty of the material, the taxonomic issue can only be elucidated when more collections become available.

The characters of the Chinese *F. velutipes* agree well with the relative descriptions (eg. Bas, 1983; Redhead and Petersen, 1999), except that the ixohyphidia composing the suprapellis of all the Chinese *F. velutipes* collections are not highly differentiated (not so extensively branched as those collections from Europe) and usually branch at shorter angles. *Flammulina rossica* has not been reported from China before. Except that the basidiomata sometimes are not pale-colored and the protrudings of the clavate elements composing the suprapellis are sometimes longer and narrower, all other characters of the Chinese collections agree well with the relative descriptions of *F. rossica* (Redhead and Petersen, 1999).

With regard to the undescribed species, one, *Flammulina* sp., is sister to two clades of *F. velutipes* (Fig. 1). But it is characterized by a hymeniform suprapellis that is different from suprapellis of *F. velutipes* which is composed of ixohyphidia. However, the basidiomata of the single collection were immature when dried, and thus, this species can only be formally described when more collections become available. The other, *F. yunnanensis*, is described herein:

Flammulina yunnanensis Z.W. Ge & Zhu L. Yang, **sp. nov.**

MycoBank MB 512371

Etymology: the epithet refers to the locality where the type of this species was collected.

Pileus 1.5-3.5 cm latus, convexus vel applanato-convexus, luteus vel ranunculinus, centro pallide aurantiaco vel armentiaco, subviscidus vel viscidus. *Lamellae* sinuatae vel adnexae, cremeae vel albidae. *Stipes* 3-6 × 0.3-0.7 cm, subcylindricus, subeaqualis vel sursum attenuatus. *Caro* alba, non-discolorans. *Basidia* 24-32 × 9.5-12.5 µm, angusticlavata, 4-sporigera. *Basidiosporae* (5)5.4-6.5(7) × 3-4 µm, ellipsoideae, interdum obovoideae vel lacrymoideae, hyalinae, non-dextrinoideae. *Pleurocystidia* et cheilocystidia lageniformia vel ventricosa, hyalina vel luteo-brunnea, 29-45 (56) × 10-16 µm. *Suprapellis* hymeniformis. *Fibulae* praesentes.

Holotypus: Z.L. Yang 3275 (HKAS 41344), 27 August 2002, Xiaoheishan Nature Reserve, Longlin County, Yunnan, China.

Basidiomata (Fig. 2a) in clusters on wood. *Pileus* 1.5-3.5 cm in diam., convex to broadly convex; surface smooth, yellow (3A6-7), butter yellow (4A5), to buttercup yellow (4A7), center light orange (5A5), melon yellow (5A6), grayish orange (5B5), to apricot yellow (5B6), subviscid to viscid when wet, margin translucently striate. *Lamellae* sinuate to adnexed, crowded to subdistant, cream to yellowish white (2A3), edge even; lamellulae 3-4 tiers, not forked. *Stipe* central, 3-6 × 0.3-0.7 cm, subcylindrical, subequal to slightly attenuate upward, apical part cream to yellowish, lower part concolourous with pileus or darker, surface pruinose to pubescent, not viscid. *Context* white, unchanging; taste mild.

Basidiospores (Fig. 2b) [60/4/2] (5)5.5-6.5(7) × 3-4 µm (x = 5.94 ± 0.42 × 3.35 ± 0.35 µm), Q = 1.5-2(2.33) ($\boldsymbol{Q}$ = 1.78 ± 0.16), ellipsoid, sometimes obvoid or lacrymoid, smooth, hyaline, thin-walled, without germ pore, non-amyloid, with an apiculus 1-1.5 µm long. *Basidia* (Fig. 2c) 24-32 × 9.5-12.5 µm, narrowly clavate, hyaline, thin-walled, 4-spored; sterigmata up to 3 µm long. *Pleurocystidia* (Fig. 2c) lageniform to ventricose, abundant to scattered, projecting up to 20 µm beyond the surface of the hymenium, 29-45(56) × 10-16 µm, hyaline to yellowish, slightly thick-walled. *Cheilocystidia* similar to pleurocystidia both in size and form. *Lamellar trama* regular to somewhat interwoven, composed of colorless filamentous hyphae 6-15 µm in width. *Suprapellis* (Fig. 2d) 60-80 µm thick, moderately gelatinized, with a hymeniform layer composed of hyaline to brownish, slightly thick-walled, narrow clavate, clavate to sphaeropedundiculate elements (14-25 × 5-10 µm) terminated on brownish filamentous hyphae of 2.5-6 µm in width, interspersed with typical reddish brown, lageniform to ventricose pileocystidia (50-75 × 12-15 µm); ixohyphidia absent; *subpellis* composed of yellow-brown to brownish filamen-tous hyphae 4-12 (16) µm in width, often with intra-cellular, yellow-brown pigments. *Clamp connections* abundant in every part of basidioma.

Habitat and known distribution: gregarious on dead trunk of a fagaceous (*Quercus* or *Lithocarpus*) plant or on dead wood in a forest with *Schima* sp. (*Theaceae*); fruiting between August and September in southern subtropical Yunnan, southwestern China at 2000-2100 m elev.

Additional material examined: China, Yunnan Province, Lushui County, 29 Sept. 1998, M. Zang 12949 (HKAS 32774), alt. 2000 m.

Molecular phylogeny

Aligned sequences of ITS were 844 sites long. Among these, 589 characters are constant, 122 variable characters are parsimony-uninformative, and 133 characters parsimony-informative. The genetic divergence among all the sequences ranged from 0% (between two collections of *F. velutipes* from Netherlands and England, as well as *F. fennae* from Switzerland and Netherlands) to 14.38% (between *F. rossica* HKAS 32154 and *F. stratosa*). The mean sequence genetic divergence is 3.56%. The mean genetic divergence between *F. yunnanensis* and other known species is 4.75% (HKAS 41344) or 4.73% (HKAS 32774), while the mean genetic divergence between *F.* sp. and all other known species is 3.39%, and the mean divergence between *F.* sp. and *F. yunnanensis* is 4.9%.

Parsimony analysis of the ITS data set resulted in 11 equally parsimonious trees of 473 steps (CI =0.717, RI = 0.844, RC = 0.605), one of the most parsimonious tree was shown in Fig. 1. Six major clades were recovered as supported by bootstrapping: (1) the *F. velutipes*-*Flammulina* sp. lineage (Clade 1, bootstrap 92%), (2) the *F. yunnanensis* lineage (Clade 2, bootstrap 100%), (3) the *F. rossica* lineage (Clade 3, bootstrap 96%), (4) the *F. fennae* lineage (Clade 4, bootstrap 100%), (5) the *F. populicola*-*F. mexicana* lineage (Clade 5, bootstrap 80%), and (6) the *F. elastica*-*F. ononidis* lineage (Clade 6). The *F. yunnanensis* lineage, consisting of two collections of *Flammulina* from southern subtropical Yunnan, southwestern China, formed a unique clade of its own with strong bootstrap support. In the well supported *F. velutipes* clade, all of the collections from China clustered with a

Canadian accession with moderate bootstrap support (74%, Clade 1a), and are the sister group to the European-North American *F. velutipes* (Clade 1a′). All of the Chinese collections of *F. rossica* clustered with their counterparts from eastern Russia and Alaska. These formed a well supported *F. rossica* clade (96%), with the Alaska collection at the base. Collections from southwestern China form a clade with moderate bootstrap (81%), except one from Leiwuqi uniting with two collections from northeastern China and one collection from far-eastern Russia. *Flammulina mexicana* is nested within the *F. populicola* group (Clade 5), thus made *F. populicola* paraphyletic, this result is same with that in Hughes *et al.* (1999) in which they discussed in detail.

For the Bayesian analysis, TrN+I+G including a proportion of invariable sites with gamma-distributed substitution rates at the remaining sites was selected by Akaike information criterion (AIC) as the best-fit model to the ITS data set. The Bayesian analysis recovered the six similar major clades (*F. velutipes-Flammulina* sp., *F. yunnanensis*, *F. rossica, F. fennae*, *F. populicola-F. mexicana,* and *F. elastica*) as in MP analysis but with different topological rearrangements within each clade. Clades with Bayesian posterior probabilities value greater than 0.9 are indicated by thick branches in Fig. 1.

Discussion

Several morphological traits are useful to distinguish species of *Flammulina*. These include basidiospore size, suprapellis structure and substrate and habitat, as showed by our own and other studies.

Flammulina yunnanensis is morphologically distinct from all other known species of the genus because of the following combination of features: (i) small ellipsoid to obvoid or lacymoid basidiospores; (ii) the hymeniform suprapellis composed of brownish, slightly thick-walled clavate terminal elements without ixohyphidia; and (iii) the preference to fagaceous plants and other broad-leaved trees in warm areas as its substrate. Both MP and Bayesian analyses of the ITS data set constantly showed *F. yunnanensis* forms a clade of its own with strong bootstrap supports suggesting that it is a unique species.

Flammulina yunnanensis is similar to *F. populicola* Redhead & R.H. Petersen, in bearing a hymeniform suprapellis and small basidiospores. However, *F. populicola*, originally described from western North America and then found in Sweden, has larger ellipsoid basidiospores (6-8.7 × 4-4.8 μm). Furthermore, *F. yunnanensis* occurs on rotten trunks of fagaceous plants and other broad-leaved trees in warm regions in southwestern China, whereas *F. populicola* most frequently fruits on the ground at the base of *Populus* trees in temperate regions (Redhead and Petersen, 1999). *Flammulina yunnanensis* is also similar to *F. rossica* Redhead & R.H. Petersen on account of the hymeniform suprapellis. However, the latter, originally described from far-eastern Russia, usually bears paler basidiomata, considerably larger basidiospores (7.4-11 × 3.8-4.5 μm), and clavate terminal cells often with protrusions at the apex in the suprapellis. In addition, *F. rossica* usually occurs on *Salix*, *Populus* or *Picea* in temperate or alpine regions (Redhead and Petersen, 1999; data of the authors in the present paper).

Among the five collections of *F. rossica* from southwestern China, four of them formed a well supported subclade (bootstrap value 81%) within the clade of the species (Fig. 1), suggesting that populations in the core region of southwestern China are much more closely related with each other than with other populations within China (Fig. 3). The collection from Leiwuqi did not cluster with the other four, which may imply that the Leiwuqi population might have limited gene flow with the other four, and is probably located outside of the core region of southwestern China both geographically and ecologically. In fact, the habitat in Leiwuqi was relatively drier than in the other four places. *Flammulina rossica* was previously regarded as a trans-Beringian distributed species (Hughes *et al.*, 1999). More recently, this species has also been found in Western Europe, Japan, interior Russia, northern Thailand (Badalyan and Hughes, 2004; Nishizawa *et al.*, 2003, as *F. velutipes*; Petersen and Hughes, 2007). Thus, *F. rossica* should be regarded as a Holarctic element, and can not only be distributed in areas with low elevations (HKAS 7930, alt. 840 m) in

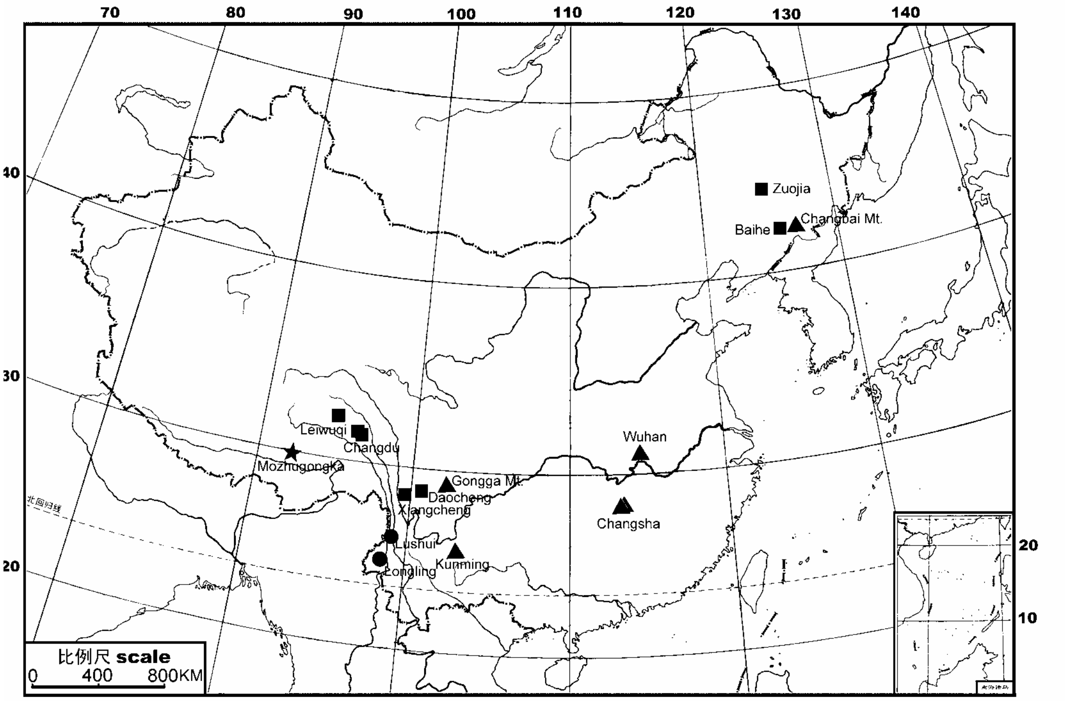

Fig 3. Sampling localities of *Flammulina yunnanensis* (circles), *F.* sp. (stars), *F. rossica* (squares) and *F. velutipes* (triangles) in China.

temperate northeastern China but also in alpine or subalpine areas with much high elevations (HKAS 43699, alt. 4380 m) in subtropical southwestern China.

Flammulina velutipes occurs mostly on substrates of *Salix* and *Populus*, *Fraxinus*, *Sambucus*, and other deciduous trees. Three Chinese collections (HKAS 47767, 47768, and 51962) were found fruiting on dead trunk of *Broussonetia papyrifera* (*Moraceae*). *Flammulina velutipes* is commonly found in winter months of the year in Europe and North America, thus earning the name "winter mushroom". However, it can also be collected in early autumn in China (Table 1). *Flammulina velutipes* has a worldwide distribution pattern probably due to human mediation, and fruits either saprotrophically on dead wood or as a parasite of living trees (Kreisel, 1961; Vellinga, 1996, Hughes, 1999; Petersen and Hughes, 2007). In regard to the biogeographic relationships among populations of *F. velutipes*, our study suggests that strains of eastern Asia are more closely related to the one from the western coast of Canada than those of Europe and the US.

Flammulina sp. (Clade 1b) clustered with *F. velutipes* (Clade 1a and Clade 1a′) with strong statistic support (Fig. 1). Only according to the molecular analysis may it be regarded as a lineage of the latter species. However, its hymeniform suprapellis without ixohyphidia significantly differs from that of the latter taxon. Because of the single collection of this species consisting of only basidiomata without basidiospores, it will be described when collections with mature basidiomata become available.

Concluding Remarks

This study demonstrates that four species of *Flammulina* are distributed in China. The eastern Himalayas and Hengduan Mountains region in southwestern China, one of the world's 34 hotspots of biodiversity, may be another diversity center of this group (Fig. 3) besides Europe in which five *Flammulina* species are distributed (Hughes *et al.*, 1999). *Flammulina velutipes* and *F. rossica* are by far the two most common species in China. The former is widely cultivated in East and Southeast Asia as a delicacy. A strain of *F. rossica* from western North America was found to be compatible with a strain of *F. velutipes* of European origin, which indicates interspecific hybridization is possible in *Flammulina* under laboratory conditions (Hughes and Petersen, 2001). Our findings that at least four *Flammulina* species occur in China will provide information on genetic variability that can be exploited in improving strains of *Flammulina* for commercial use both genetically and productively.

This study provides information on the species diversity, phylogeny and biogeography of the genus *Flammulina* in China. To understand the evolution and biogeography of the genus *Flammulina* as a whole, molecular analysis of larger DNA domains, further sampling and mating test (Tan *et al*., 2007) may be helpful.

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