Flammulina species from China inferred by morphological and molecular data

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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

Article Information
Received 12 June 2007
Accepted 3 March 2008
Published online 30 September 2008
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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 Mnozhina, 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. ferrugineolutea (Beeli) Singer, F. mediterranea (Pacioni & Lalli) Bas & Robich, F. mexicana Redhead, Estrada & R.H. Petersen, F. ononisidis Arnolds, F. populicola Redhead & R.H. Petersen, F. rossica Redhead & R.H. Petersen, F. similis E. Horak, F. stratos 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 morphological 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 \(\times\) 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 stratosata* 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Collections</th>
<th>Location and month of collection</th>
<th>Substrate</th>
<th>GenBank accession #</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. elastica</em></td>
<td>TENN 56057</td>
<td>Austria: Vienna, 27-XI-1994</td>
<td>On <em>Salix alba</em></td>
<td>AF034103</td>
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<tr>
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<td>AF141134</td>
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<td><em>F. elastica</em></td>
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<td>Germany: Marburg, XI-2004</td>
<td></td>
<td>EF595849</td>
</tr>
<tr>
<td><em>F. fennae</em></td>
<td>TENN 54172</td>
<td>Switzerland: Canton Graubunden, 16-X-1995</td>
<td>On <em>Alnus incana</em></td>
<td>AF035398</td>
</tr>
<tr>
<td><em>F. mexicana</em></td>
<td>TENN 52894</td>
<td>Mexico: Est. Tlaclaca, 11-VII-1993</td>
<td>On <em>Senecio cinerarioide</em></td>
<td>AF032129</td>
</tr>
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<td><em>F. ononidis</em></td>
<td>TENN 54743</td>
<td>Germany: location and date unknown</td>
<td></td>
<td>AF051701</td>
</tr>
<tr>
<td><em>F. populicola</em></td>
<td>TENN 54171</td>
<td>United States: Alaska, Anchorage, 17-IX-1995</td>
<td></td>
<td>AF044193</td>
</tr>
<tr>
<td><em>F. populicola</em></td>
<td>Halling 6536</td>
<td>United States: California, Sierra Co.</td>
<td>On <em>Alnus or Populus</em></td>
<td>AF031655</td>
</tr>
<tr>
<td><em>F. rossica</em></td>
<td>I. Bullakh</td>
<td>Russia: Terr. Primorsk, IX-1994</td>
<td></td>
<td>AF051699</td>
</tr>
<tr>
<td><em>F. rossica</em></td>
<td>TENN 54169</td>
<td>United States: Alaska, 14-IX-1995</td>
<td>On <em>Salix</em></td>
<td>AF044194</td>
</tr>
<tr>
<td><em>F. rosica</em></td>
<td>HKAS 46076</td>
<td>China: Tibet, Changdu, 7-VII-2004</td>
<td>On <em>Salix</em></td>
<td>EF595845</td>
</tr>
<tr>
<td><em>F. rosica</em></td>
<td>HKAS 20588</td>
<td>China: Jilin, Zuoja, 16-IX-2000</td>
<td>On <em>Salix</em></td>
<td>EF595847</td>
</tr>
<tr>
<td><em>F. rosica</em></td>
<td>HKAS 43699</td>
<td>China: Tibet, Leiuqu, 8-VIII-2004</td>
<td>On <em>Salix</em></td>
<td>EF595846</td>
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<tr>
<td><em>F. rosica</em></td>
<td>HKAS 45970</td>
<td>China: Tibet, Changdu, 27-VII-2004</td>
<td>On <em>Salix</em></td>
<td>EF595850</td>
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<tr>
<td><em>F. rosica</em></td>
<td>HKAS 32154</td>
<td>China: Sichuan, Xiangcheng, 16-VII-1998</td>
<td>On rotten wood of <em>Salix</em></td>
<td>EF595856</td>
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<tr>
<td><em>F. rosica</em></td>
<td>HKAS 32155</td>
<td>China: Sichuan, Daocheng, 4-VII-1998</td>
<td>On <em>Picea</em></td>
<td>EF595855</td>
</tr>
<tr>
<td><em>F. rosica</em></td>
<td>HKAS 7930</td>
<td>China: Jilin, Baihe, 15-VIII-2004</td>
<td>In <em>Betula</em> forest</td>
<td>EF595852</td>
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<tr>
<td><em>F. sp.</em></td>
<td>HKAS 51191</td>
<td>China: Tibet, Mozhuangongka, 2-IX-2006</td>
<td>On the base of a dead trunk</td>
<td>EF601574</td>
</tr>
<tr>
<td><em>F. stratosa</em></td>
<td>TENN 56240</td>
<td>New Zealand: South Island, 17-V-1994</td>
<td>In forest with <em>Schima</em> trees</td>
<td>AF047872</td>
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<tr>
<td><em>F. yunnanensis</em></td>
<td>HKAS 32774</td>
<td>China: Yunnan, Lushui, 29-IX-1998</td>
<td>On a dead fagaceous plant</td>
<td>EF595857</td>
</tr>
<tr>
<td><em>F. yunnanensis</em></td>
<td>HKAS 41344</td>
<td>China: Yunnan, Longlin, 27-VIII-2002</td>
<td></td>
<td>DQ486704</td>
</tr>
<tr>
<td><em>F. velutipes</em></td>
<td>TENN 56008</td>
<td>Canada: British Columbia.</td>
<td>On a dead fagaceous plant</td>
<td>EF595857</td>
</tr>
<tr>
<td><em>F. velutipes</em></td>
<td>K 2826</td>
<td>United Kingdom: Surrey, Ham, 3-I-1995</td>
<td></td>
<td>AF036928</td>
</tr>
<tr>
<td><em>F. velutipes</em></td>
<td>TENN55402</td>
<td>United States: California, 24-XI-1996</td>
<td>On <em>Lupinus arboreus</em></td>
<td>AF047871</td>
</tr>
<tr>
<td><em>F. velutipes</em></td>
<td>TENN 56028</td>
<td>United States: Michigan, 19-IX-1995</td>
<td>On <em>Broussonetia papyrifera</em></td>
<td>AF051700</td>
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<tr>
<td><em>F. velutipes</em></td>
<td>HKAS 49485</td>
<td>China: Yunnan, Kunming, 9-VII-2004</td>
<td>Cultivated</td>
<td>EF595844</td>
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<td><em>F. velutipes</em></td>
<td>HKAS 47767</td>
<td>China: Hunan, Changsha, 24-XII-2004</td>
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<td>EF595853</td>
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<td><em>F. velutipes</em></td>
<td>HKAS 47768</td>
<td>China: Hunan, Changsha, 24-XII-2004</td>
<td>On <em>Broussonetia papyrifera</em></td>
<td>EF595854</td>
</tr>
<tr>
<td><em>F. velutipes</em></td>
<td>HKAS 51988</td>
<td>China: Jilin, Changbai Mt., 27-X-2006</td>
<td>On <em>Betula platyphylla</em></td>
<td>EF595851</td>
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<td><em>F. velutipes</em></td>
<td>FH DH97-080</td>
<td>China: Sichuan, Gongga Mt., 17-VIII-1997</td>
<td>On dead hard wood</td>
<td>AF159426</td>
</tr>
</tbody>
</table>

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 4637 and 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
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× 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 pileicytidia (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 protrusions 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, subequalis vel sursum

**Holotypus:** Z.L. Yang 3275 (HKAS 41344), 27 August 2002, Xiaoleishan 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 (5B7), to apricot yellow (5B6), subviscid to viscid when wet, margin transversely striate. *Lamellae* sinuate to adnexed, crowded to subdistant, to subdistant, to subequal to slightly attenuate upward, apical part cream to yellowish white (2A3), edge even; lamellulae adnexed, crowded to subdistant, cream to translucently striate.

**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) (Q = 1.78 ± 0.16), ellipsoid, sometimes obvoid or larcymoid, 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 subviscid, 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. *Cheilocystidia* and *Pleurocystidia* in 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.

**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 between *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
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.

Acknowledgements

We would like to thank the curators of HMJAU, GDGM and WU for loaning the specimens of Flammulina. Mr. Bo Wang and Mr. Hanyi Zhang are acknowledged for providing us a few interesting Flammulina collections. Members of the Hibbett Laboratory at Clark University assisted us with technical issues. Professor S.M. Badalyan (Yerevan State University, Armenia) is thanked for sharing valuable literature. Comments on the manuscript by Dr. Ronald H. Petersen (University of Tennessee) are greatly appreciated. This work was supported by the Knowledge Innovation Program of the Chinese Academy of Sciences (No. KSCX2-YW-G-025), by the National Science Fund for Distinguished Young Scholars (No. 30525002) of the National Natural Science Foundation of China (NSFC) and by the National Basic Research Program of China (No. 2009CB522300). The field work was supported by the Knowledge Innovation Program of the Chinese Academy of Sciences (No. KSCX2-YW-G-025) and the National Natural Science Foundation of China (2009CB522300). The field work was supported by the Knowledge Innovation Program of the Chinese Academy of Sciences (No. KSCX2-YW-G-025) and the National Basic Research Program of China (No. 2009CB522300).

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