January 2010

The delimitation of *Flammulina Fennae*

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Mycol Progress (2010) 9:469–484
DOI 10.1007/s11557-009-0654-9

ORIGINAL ARTICLE

The delimitation of *Flammulina fennae*

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Received: 29 October 2009 / Revised: 22 December 2009 / Accepted: 30 December 2009 / Published online: 16 February 2010

Abstract Multivariate morphometric analyses of micromorphological characters measured on 35 specimens of *Flammulina fennae* and related species show that only a combination of spore dimensions and ixohyphidia characters are suitable for delimitation of this species. In order to confirm species identifications based on micromorphology, ribosomal ITS DNA sequences were obtained and compared with those previously deposited in GenBank, and phylogenetic analyses were performed using an ITS dataset of all known *Flammulina* species. All six specimens morphologically determined as *F. fennae* were identified by molecular data. Two of twelve specimens morphologically assigned to *F. velutipes* had *F. elastica* sequences. One ITS sequence of *F. velutipes* appears to be a hybrid: the ITS1 region is homologous to *F. velutipes* and ITS2 is homologous to another *Flammulina* species, *F. rossica*. Variability of morphocharacters observed for *F. fennae* and related species is discussed and compared with the data from previous studies. A key is provided to European taxa of the genus *Flammulina* together with a full description of *F. fennae*.

Keywords *Flammulina velutipes* · *F. ononidis* · *Flammulina* hybrid · Multivariate morphometrics · ITS sequences

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Introduction

When *Flammulina fennae* Bas was described (Bas 1983), two other *Flammulina* species were known in Europe: *F. velutipes* (Curtis) Singer and *F. ononidis* Arnolds. Later, four additional species were recognized: *F. populicola* Redhead et R.H. Petersen, *F. rossica* Redhead et R.H. Petersen, *F. elastica* (Lasch) Redhead et R.H. Petersen, and *F. cephalariae* Pérez-Butrón et Fernández-Vic. Of these, only *F. velutipes* and *F. ononidis* are morphologically similar to *F. fennae*. *Flammulina fennae*, *F. velutipes*, and *F. ononidis* differ from *F. populicola* and *F. rossica* by the absence of sphaeropedunculate or clavate cells in the pileipellis (Adamčík and Ripková 2008; Redhead and Petersen 1999), and from *F. elastica* and *F. cephalariae* in spore dimensions. The spores of *F. elastica* are narrower (the ratio of length and width is 2.5–3), and spores of *F. cephalariae* are longer (the average value of length is 12–16.8 μm) (Pérez-Butrón and Ferdnández-Vicente 2007; Petersen et al. 2009).

Following the taxonomic concept of Bas (1983), more or less accepted until now (Petersen et al. 2009), it seemed easy to distinguish *F. fennae*, *F. velutipes*, and *F. ononidis* by spore size: *F. velutipes* spores were reported as 7−11× (2.5−)3−4 μm, *F. ononidis* as 8.5−12.5×4.5−5.5 μm, and *F. fennae* as 6−8×4−4.5(−5) μm. However, during our study of taxonomy and biogeography of the genus *Flammulina* in

Central Europe (study period 2004–2007), we determined that spore dimensions of several collections corresponded to more than one species and thus misidentification of *F. fennae* as *F. velutipes* was possible. The aim of our paper was therefore: (1) to find the most suitable morphological characters for distinguishing between three similar morphospecies, *F. fennae*, *F. velutipes*, and *F. ononidis*; (2) to delimit *F. fennae* morphologically; (3) to verify the taxonomic value of selected morphological characters with molecular analyses; and (4) to develop a key for European species of the genus *Flammulina*.

Materials and methods

Sampling

Our study is based on 35 *Flammulina* specimens with an average spore length to width ratio (avQ) per collection of up to 2.2. The maximum value for *F. fennae* does not exceed this limit (Bas 1983; Petersen et al. 2009). This is the average value reported for *F. velutipes* and *F. ononidis*. The material examined includes our own specimens deposited in SAV and SLO as well as specimens from herbaria BRA, BRNM, KRAM, M, SAV, and SLO (Table 1). The abbreviations of herbaria are cited in accordance with the Index Herbariorum (Holmgren et al. 1990), and data on specimens are presented in their original form.

Morphological methods

Following recent *Flammulina* concepts (Petersen et al. 2009), typical specimens of *F. fennae*, *F. ononidis*, and *F. velutipes* were selected for examination (Table 1).

Macromorphological characters were observed on fresh material, and micromorphological characters on dried material using an Olympus CX41 microscope and an oil immersion lens. Spores originating from spore prints were measured. Spores were scanned with an Olympus Artcam camera and measured using Quick Micro Photo (version 2.1) software. Enlarged scanned pictures of spores were used for measuring (with an accuracy of 0.1 μm) and for drawing. Fragments of lamellae, stipe, and pileipellis were examined in a solution of Congo Red in ammonia (1 ml of 25% ammonia dissolved in a filtered solution of 1.5 g of Congo Red in 50 ml of distilled water) after a short 5% KOH pre-treatment. Statistics for measurements of micromorphological characters used in the description of *F. fennae* are given as mean value plus/minus standard deviation and are based on 30 measurements per specimen. Values in parentheses give the measured 5 and 95 percentile values. References to colors of macromorphological characters follow Kornerup and Wanscher (1974).

Several hyphal characters from the surface of basidiomata and the hymenium were measured and compared in order to determine their taxonomical importance. Preliminary analyses determined that only spores and ixohyphidia patterns in the pileipellis had distinct differences among all studied species and were suitable for further evaluation as discriminatory characters (data not shown). We observed considerable variation in shape and density of pileocystidia during maturation of basidiomata. Since we were unable to estimate the maturation stage of herbarium specimens included in this study, pileocystidia were omitted from further study. Eight spore and ixohyphidia characters (Table 2, Fig. 1) were measured on all specimens in the study (Table 1). All ixophidial characters were measured on the pileipellis, near the margin of the pileus. Mature basidiomata with expanded pileus and well-developed spores were used. Three specimens (SLO F-1003, SAV F-1445, and M 0065370; see Table 1) were measured twice in order to estimate variability of the characters and were included to multivariate morphometric analyses as test duplicates. Data matrixes of average values of 30 measurements per collection were calculated using the program SAS (SAS Online Doc®, version 9.1). The average value of characters that describe the shape of ixohyphidia (see Table 1, characters IF, IC, IB, and IT) was calculated as the percentage of a given type of ixohyphidia (e.g., 15 positively scored ixohyphidia out of 30 total measurements equals 50%).

In order to create a hypothesis about possible grouping of observed specimens and to estimate the most suitable combination of characters for delimitation of morphotypes, we used multivariate morphometric analyses. Principal component analysis (PCA) (Sneath and Sokal 1973; Krzanowski 1990) is based on average values measured on individual specimens and the correlation matrix among the characters. Each observed specimen is characterized as an object in multivariate space and its position on each dimension is defined by the value of specific measured character. PCA reduces the multidimensionality of the original character space and displays the variation pattern along the first two components extracting most of the variation. Canonical discriminant analysis (CDA) was used to test the hypothesis resulting from PCA (Klecka 1980). In CDA, a discriminant function was derived to maximize variation among the groups and a diagram showing the extent of group separation was produced. The total canonical structure was calculated to reveal correlations of characters with the canonical axis. Characters with a higher value in the total canonical structure have a stronger influence on canonical axes and are therefore more suitable for delimitation of taxa. The classificatory discriminant analysis based on a cross-validation procedure was applied to determine how effectively taxa can be distinguished from each other.

Table 1 Material studied

Specimen	• Locality • Substrate • Collectors	Date	• Herbarium number • Original determination • Morphological determination • Molecular determination	Gene bank number
1	• Slovakia, reservatio Slovenský raj, in reservatio "Hradisko" in loco "Čingov" dicto, ad ripam dextram rivi Hornád, approx. 8 km situ occid. ad oppido distr. Spišská Nová Ves, alt. 520 m • Ad lignum in terre immersum • J. Kuthan, P. Lizoň, L. Hagara	17 Sep 1985	• BRA CR-4429 • *F. fennae* • *F. fennae* • No data	
2	• Slovakia, Bratislava City, the municipal part of Podunajské Biskupice, approx. 0.5 km E of the City Incinerator, alt. approx. 135 m • On wood of fallen rotten trunk of cf. *Acer* sp. • S. Ripková	6 Sep 1999	• SLO F-1002 • *F. fennae* • *F. fennae* • *F. fennae*	FJ914388
3	• Slovakia, Bratislava City, the municipal part of Podunajské Biskupice, the Protected Landscape Area of Dunajské luhy, the locality Topoľové, alt. approx. 130 m, *Salicion albae* • On wood buried in soil under *Ulmus* sp. • S. Ripková	8 Oct 2004	• SLO F-1003 • *F. fennae* • *F. fennae* • *F. fennae*	FJ873393
4	• Slovakia, Bratislava City, the municipal part of Staré Mesto, Nábr. arm. gen. L. Svobodu Street, green area around the block of flats no. 1435, alt. approx. 140 m • On the base of stump of cut deciduous tree cf. *Betula* sp. in association of *Berberis vulgaris* and *Prunus* sp. • S. Ripková	4 Oct 2005	• SLO F-1004 • *F. fennae* • *F. fennae* • *F. fennae*	FJ873394
5	• Germany, Schloßpark Seefeld. • Am Grund un alter Linde, in Moossen • Kupka	Aug 1961	• M 0065379 • *Collybia velutipes* var. "sommerform" • *F. fennae* • No data	
6	• Germany, Bayern: Augsburg, Gögginger Wäldchen 3, bei der Schofweide-Siedlung, MTB 7631 • In einer Fichtenparzelle, auf dem Boden • J. Stangl	17 Oct 1986	• M 0022711 • *F. fennae* • *F. fennae* • No data	
7	• Germany, Nördlich Riedheim, MTB 7527 • Im Reid auf Viehweide unter Bäumen (*Salix* etc.) • M. Enderle	21 Sep1989	• M 0065396 • *F. fennae* • *F. fennae* • No data	
8	• Poland, Krakow city centre, Kopernik Street, at NE edge of the Botanical garden, the urban green area, coord. N 50°03′57.8″, E 19°57′30.1″, alt. approx. 200 m • On stump of cut deciduous tree 0.2–1.5 m above ground, possibly *Acer* sp. • A. Ronikier	23 Nov 2006	• KRAM-F 56109 • *F. fennae* • *F. fennae* • *F. fennae*	FJ873390
9	• Sweden, Skåne, Röddinge idr. pl. • On wood of *Fagus* • T. Foucard	21 Sep 2005	• SAV F-1443 • *F. fennae* • *F. fennae* • *F. fennae*	FJ873391
10	• Russia, St. Petersburg—urban city area close to the Winter Palace • On roots of trunk of a deciduous tree • J. Borovička	21 Sep 2007	• SAV F-1444 • *F. fennae* • *F. fennae* • *F. fennae*	FJ873392
11	• Slovakia, Oščadnica, prope Čadca, alt. 450 m • Ad codicem arbor. frondos • J. Kuthan	5 Nov 1966	• BRA CR-4389 • *F. velutipes* • *F. velutipes* • No data	
12	• Slovakia, Podunajská nížina Lowland, near the village of Nový Tekov, on bank of the Hron River	25 Dec 2004	• SAV F-1445 • *F.* sp.	FJ889519

Table 1 (continued)

Specimen	• Locality • Substrate • Collectors	Date	• Herbarium number • Original determination • Morphological determination • Molecular determination	Gene bank number
	• On fallen trunk of *Populus nigra*		• *F. velutipes*	
	• S. Adamčík		• *F. elastica*	
13	• Slovakia, Podunajská nížina Lowland, the village of Nový Tekov—Marušová, in the area of the old agricultural concern, alt. 200 m	31 Dec 2004	• SAV F-1446 • *F.* sp. • *F. velutipes*	FJ889505
	• On fallen branch of *Juglans regia* (thickness approx. 10 cm) • S. Adamčík		• *F. velutipes*	
14	• Slovakia, Malé Karpaty Mts., the valley of the Sološnický potok Stream, approx. 4 km SE of the church in the village of Sološnica, alt. approx. 300 m	5 Jan 2005	• SAV F-1447 • *F.* sp. • *F. velutipes*	FJ889512
	• On *Alnus* sp. • V. Kučera		• *F. velutipes*	
15	• Slovakia, Malé Karpaty Mts., the valley of the Sološnický potok Stream, approx. 4 km SE of the church in the village of Sološnica, alt. approx. 300 m	10 Jan 2005	• SAV F-1448 • *F.* sp. • *F. velutipes*	FJ889509 FJ889510 FJ889511
	• On stem of *Fagus sylvatica* • V. Kučera		• *F. velutipes*	
16	• Slovakia, Malé Karpaty Mts., the valley of the Sološnický potok Stream, approx. 4.5 km SE of the church in the village of Sološnica, alt. approx. 220 m	10 Jan 2005	• SAV F-1449 • *F.* sp. • *F. velutipes*	FJ889506 FJ889507 FJ889508
	• On dead standing stem of *Carpinus betulus* • V. Kučera		• *F. velutipes*	
17	• Slovakia, Bratislava City, the municipal part of Podunajské Biskupice, the Nature Reserve of Topoľové hony, alt. 132 m	3 Mar 2006	• SAV F-1450 • *F.* sp.	FJ889518
	• On fallen trunk of *Ulmus* sp.		• *F. velutipes*	
	• S. Adamčík		• *F. velutipes*	
18	• Slovakia, the village of Závod, the National Nature Reserve of Abrod, alt. approx. 155 m	3 Mar 2006	• SAV F-1451 • *F.* sp.	FJ889520
	• On submersed roots of *Salix* sp.		• *F. velutipes*	
	• S. Adamčík		• *F. elastica*	
19	• Slovakia, Javorníky Mts., the village of Papradno, the settlement of Dolný Grúnik, alt. approx. 750 m	30 Apr 2006	• SAV F-1452 • *F.* sp.	FJ889513 FJ889514
	• On stump of cf. *Picea abies*		• *F. velutipes*	FJ889515
	• M. Perný, V. Kučera		• *F. velutipes*	FJ889516
20	• Slovakia, Bratislava City, the municipal part of Podunajské Biskupice, the Nature Reserve of Topoľové hony, alt. 132 m	3 Mar 2006	• SAV F-1594 • *F.* sp.	FJ975045
	• On fallen branch of *Fraxinus* sp.		• *F. velutipes*	
	• V. Kučera		• *F. velutipes*	
21	• Germany, Lkr. Garmisch-Partenkirchen: Wettersteingebirge, Bergwald an der Wettersteinalm, alt. 1465 m, MTB 8532/4	18 Sep 1969	• M 0065386 • *F. velutipes*	
	• A. Bresinsky		• *F. velutipes* • No data	
22	• Germany, Bayern, Umgebung von Badwörishofen • An Buche	30 Nov 1970	• M 0065388 • *F. velutipes*	
	• J. Stangl		• *F. velutipes* • No data	
23	• Germany, Bayern, Bad Wörishofen im Kurpark, MTB 8029 • An Buche	28 Sep 1976	• M 0065364 • *F. velutipes*	
	• J. Stangl		• *F. velutipes* • No data	
24	• Germany, Bayern, Murnauer Moor, Weghaus—Köchel • An *Ulmus glabra*	12 Oct 1979	• M 0065362 • *F. velutipes*	

Table 1 (continued)

Specimen	• Locality • Substrate • Collectors	Date	• Herbarium number • Original determination • Morphological determination • Molecular determination	Gene bank number
	• A. Einhellinger		• *F. velutipes* • No data	
25	• Germany, Nationalpark Berchtesgaden, Bärenwald, alt. 1430 m, MTB 8444/1, hochmontaner Fichtenwald • Auf liegenden Fichtenstamm, • H. Schmid-Heckel	10 Sep 1981	• M 0065363 • *F. velutipes* • *F. velutipes* • No data	
26	• Germany, Nationalpark Berchtesgaden, Wachtersteig (Reiteralpe), alt. 1,340 m, MTB 8342/4 • Auf liegenden Stamm v. *Acer pseudoplatanus* • H. Schmid-Heckel	27 Aug1983	• M 0065370 • *F. velutipes* • *F. velutipes* • No data	
27	• Germany, Bayern, Augsburg, Gögginger Wäldchen 1, zwischen Wertach und Kanal, MTB 7631 • Büschelig, auf Stubben • J. Stangl	15 Sep1986	• M 0022710 • *F. fennae* • *F. velutipes* • No data	
28	• Germany, Bayern, Kr. Traunstein, MTB 7942-4, Auwald bei Fridolfing, unter *Cirsium* und *Impatiens* auf überwachsener Kahlschlagfläche im Auwald • Stielbasis aus vergrabener Holzresten (Wurzel) von Auen-Weichholz hervorwachsend • T.R. Lohmeyer	7 Jul 1994	M 0065399 • *F. fennae* • *F. velutipes* • No data	
29	• Czech Republic, Moravskoslezské Beskydy, Frýdek-Místek, u ZOO parku, 49°41′20″N, 18°21′10″E, quadr. 6376a, alt. approx. 300 m • Jednotlivě v trávě • J. Lederer	15 May 1998	• BRNM 652669 • *F. fennae* • *F. velutipes* • No data	
30	• Czech Republic, Ivaň, PP Dolní Mušovský luh, 2.1–3 km JV od kostela v obci, 48°54′39″N, 16°35′51″E, quadr. 7065d, alt. 170 m • Mrtvý stojící *Ulmus* sp. • A. Vágner	5 Oct 2001	• BRNM 666755 • *F. velutipes* • *F. velutipes* • *F. velutipes*	FJ975044
31	• Czech Republic, Jeseníky Mts., along the main road from Šumperk to Ostrava, near the National Nature Reserve of Rašeliniště Skřítek, alt. approx. 850 m • On living standing trunk of *Acer pseudoplatanus* • S. Adamčík	2 Oct 2006	• SAV F-1453 • *F.* sp. • *F. velutipes* • *F. velutipes*	FJ889517
32	• Slovakia, Malé Karpaty Mts., Modra City, left riverside of Žliabok Stream, under the Zámčisko Hill, alt. 370 m • On fallen branch of *Fagus sylvatica* • L. Hagara	24 Nov 2006	• SAV F-1593 • *F. velutipes*. • *F. velutipes* • *F. velutipes*	FJ914389
33	• Slovakia, Cerová vrchovina Mts., 2 km SWW of the village of Chrámec, alt. 250 m, on abandoned pasture, on sandy soil, among herbaceous plants • On roots of *Ononis spinosa* • K. Skokanová	27 Oct 2002	• SAV F-1319 • *F. ononidis* • *F. ononidis* • No data	
34	• Slovakia, Cerová vrchovina Mts., 2 km SWW of the village of Chrámec, alt. 250 m, on abandoned pasture, on sandy soil, among herbaceous plants • On roots of *Ononis spinosa* • S. Adamčík	27 Oct 2004	• SAV F-1318 • *F. ononidis* • *F. ononidis* • No data	
35	• Hungary, Mts. Pilis prope Budakalász • In pascuo • M. Babos, G. Bohus, E. Véssey	1 Nov 1966	• M 0065416 • *F. velutipes* var. *pratensis* • *F. ononidis* • No data	

Table 2 The list of characters measured on *Flammulina* specimens and used for multivariate morphometric analyses

Characters and abbreviations	Measurements
Spores	
SL	Length of spores (μm)
SW	Width of spores (μm)
SQ	Ratio of length and width of spores (Q)
Terminal cells of ixohyphidia near margin of pileus (TCI)	
IF	Proportion of long slender filiform TCI without distinctly inflated part, unbranched or branched
IC	Proportion of coralloid TCI without a distinct central branch and with some inflated part
IB	Proportion of TCI inflated in the basal part, unbranched or branched; if branched, then with one distinct central branch and shorter lateral branches
IT	Proportion of TCI inflated in the terminal part or ± in the middle, unbranched or branched; if branched, then with one distinct central branch and shorter lateral branches
IX	Index of branching of TCI calculated as average of 30 observations and estimated for single ixohyphidia in scale 0–V; the numbers 0–IV correspond to number of branches excluding the main branch, the number V corresponds to ixohyphidia with five and more such branches

In addition, mean values, standard deviations and percentiles were calculated for all characters (exploratory data analysis). Pearson and Spearman rank correlation coefficients were calculated in order to eliminate characters with highly correlated values, that could bias the correlation matrix.

Ecological data (habitat, fruiting period) are based on material examined in this study (Table 1).

Molecular methods

Dikaryon or monokaryon cultures were grown in 30 mls PD broth (24 g/L Difco Potato Dextrose Broth) until the mycelial culture was approx. 2 cm in diameter. The culture was filtered through a fine mesh cloth and blotted to remove excess medium. Approximately 0.25 g was used for DNA extraction. For herbarium samples, a small (3 mm^2) piece of dried pileus was used for DNA extraction. Tissues were ground in 750 μL Carlson lysis buffer with a mortar and pestle then incubated at 74°C for 30 min (Carlson et al. 1991). Particulate material was removed by centrifugation and the supernatant was extracted with 750 μL chloroform: isoamyl alcohol (24:1). The resulting supernatant was removed to a clean microfuge tube and an equal volume of 100% isopropyl alcohol was added to precipitate nucleic acids. The solution was centrifuged immediately. The DNA pellet was washed once with 75% cold ETOH and dissolved in 100 μL TE buffer. The ribosomal ITS region was amplified using the forward primer ITS1F (Bruns and Gardes 1993) and reverse primer ITS4 (White et al. 1990). Cycle parameters were 94°C for 3 min followed by 30 cycles of 94°C for 30 s, 60°C for 60 s and 72°C for 90 s with a final extension at 72°C for 3 min (Jin et al. 1998). PCR products were visualized by gel electrophoresis in 1.5% TBE agarose gels. PCR products were sequenced with primers ITS5 and ITS4 (Bruns et al. 1991). Sanger dideoxy sequencing was performed using primers ITS5 and ITS4 (White et al. 1990). Cloning was accomplished with the Promega pGEM-T cloning vector and JM109 competent cells using the manufacturer's directions followed by Sanger dideoxy sequencing. Sequences of each gene were manually corrected and aligned using the SEQLAB program in the Genetics Computer Group package (GCG 2000). *Flammulina* collections were diagnosed from ITS DNA sequences by alignment with collections previously deposited in GenBank as exemplars by Hughes et al. (1999) and with other collections acquired since then.

Parsimony analysis was carried out using PAUP* 4b (Swofford 2002). Bootstrap support was computed using 1,000 bootstrap replicates. The starting trees were obtained via stepwise addition. One tree was held at each step; max. trees was 1,000. The branch-swapping algorithm was tree-bisection-reconnection. All characters had equal weight and were unordered. The evolutionary model selected by Modeltest (Posada and Crandall 1998) for the ribosomal ITS dataset was GTR + I + Γ. (Rodríguez et al. 1990). Bayesian analysis was performed using MrBayes (Huelsenbeck et al. 2001) using two chains and 500,000 generations with settings appropriate to the GTR + I + Γ model. Chains converged after approximately 10,000 generations.

Results and discussion

Multivariate morphometric analyses of micromorphological characters

The values of Pearson and Spearman coefficients for characters IC (the proportion of coralloid terminal cells of ixohyphidia) and IX (the index of branching) (Table 2,

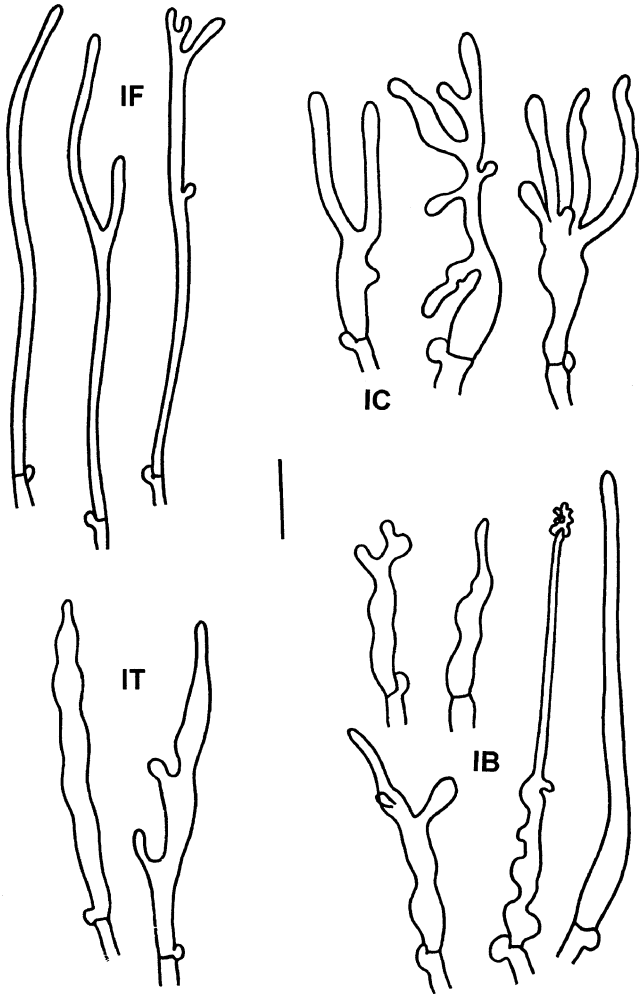

Fig. 1 The types of terminal cells of ixohyphidia (TCI) near margin of pileus of *Flammulina* taxa. *IF* Long slender filiform TCI without distinctly inflated part; *IC* coralloid TCI without a distinct central branch; *IT* TCI inflated in the terminal part or ± in the middle; *IB* TCI inflated in the basal part, with one distinct central branch and shorter lateral branches. For precise descriptions of the abbreviations see Table 2

Fig. 1) were highly correlated and only character IX was used in the data matrix.

The results of a principal component analysis (PCA) graphed in two dimensions (Fig. 2) gave three distinctly separated clusters that corresponded to the traditionally recognized taxa *Flammulina fennae*, *F. ononidis*, and *F. velutipes*. This distribution of characters in the ordination diagram was also supported by results of canonical discriminant analysis (CDA) (Fig. 3). *Flammulina ononidis* was well separated from the two other groups along the second canonical axis and *F. fennae* and *F. velutipes* were separated from each other along the first canonical axis. The highest values (exceeding 0.6) of the total canonical structure expressed within the correlation of characters for the second canonical axis (Can2) were for the characters SL (the length of spores) and SW (the width of spores); the highest values for the first canonical axis (Can1) were for the characters SW, SQ (the ratio of length and width of spores), IT (the proportion of terminal cells of ixohyphidia inflated in the terminal part or ± in the middle), and IX (Table 3). Classificatory discriminant analysis confirmed results of PCA and CDA analyses and resulted in a 100% correct classification for all three taxa. This means that all three groups are well defined by characters used in the morphometric analyses.

The statistical data from our measurements on all three taxa (*F. fennae*, *F. ononidis*, and *F. velutipes*) are given in Figs. 4 and 5. The characters with distinct differences, obtained from multivariate morphometric analyses (Table 3), had non-overlapping rectangles on the graphs. Although the ranges of values of individual measurements were mostly overlapping, the statistical values showed distinct support for taxa delimitation. Accordingly, the average (av.) values of 30 measurements obtained from individual specimens were also only weakly overlapping (dots on the graphs).

Based on our multivariate morphometric analyses of micromorphological characters, characters SL and SW (Figs. 4a, b) are suitable for distinguishing of *F. fennae* and *F. velutipes* from *F. ononidis*; and characters SQ, IC, IT, and IX (Figs. 4c, d, and 5b, d) for distinguishing *F. fennae* from the two other taxa. The characters IF (the proportion of long slender filiform terminal cells of ixohyphidia) and IB (the proportion of terminal cells of ixohyphidia inflated in basal part) are not suitable for distinguishing any of these three taxa (Figs. 5a, c).

Distinguishing F. fennae and F. velutipes from F. ononidis The minimum av. value of SL of 30 measurements on individual specimens of *F. ononidis* was 8.1 µm, the maximum for *F. velutipes* was 8.0 µm, and the maximum for *F. fennae* was 7.2 µm (Fig. 4a). Although collections from *F. velutipes* often reached much higher av. values of SL than 8 µm, only collections with SQ up to 2.2 were used for multivariate morphometric analyses in this study. *F. ononidis* also differed from the two other taxa in having wider spores. The minimum av. value of SW of 30 measurements on individual specimens of *F. ononidis* was 4.2 µm, the maximum for *F. velutipes* was 4 µm (this value was not exceeded even in the set of collections with SQ > 2.2) and the maximum for *F. fennae* reached 4.5 µm only in collection SAV F-1444 from St. Petersburg. All other collections were less than 4.2 µm (Fig. 4b).

Distinguishing F. fennae from F. ononidis and F. velutipes The maximum av. value of SQ of 30 measurements on individual specimen for *F. fennae* ranged up to 1.75 while the minimum for *F. velutipes* was 1.72 (SAV F-1453), but all other collections exceeded the value of 1.75.

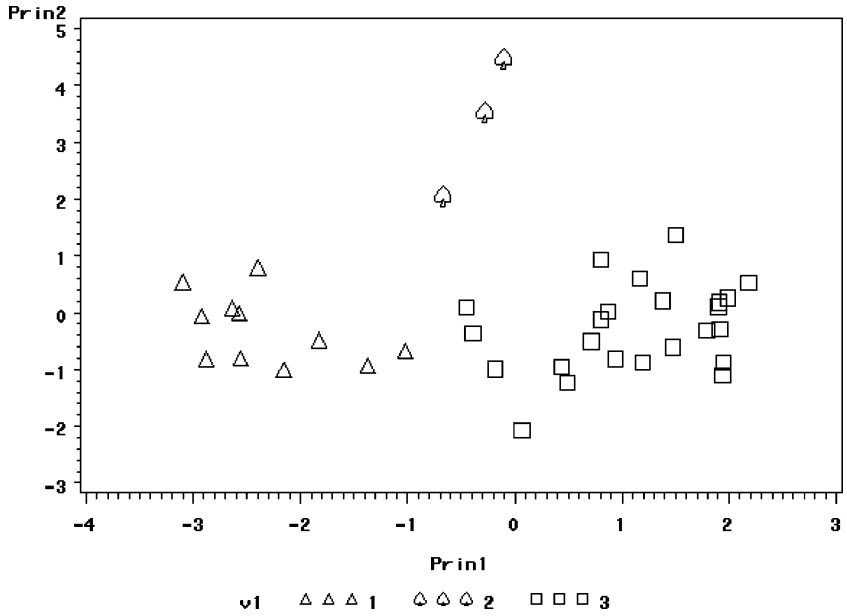

Fig. 2 Ordination diagram of the principal component analysis of average values of characters measured on spores and terminal cells of ixohyphidia near margin of pileus. The first axis account for 41,5% and the second for 22,04% of total variation. *Triangle F. fennae*, *spade F. ononidis*, *square F. velutipes*. The diagram shows results of the multivariate morphometric analyses that reduce multidimensionality of the original character space and displays the variation pattern along the first two components extracting most of the variation. All characters listed in Table 2, with exception of IC, were used for the analysis

Characters IC, IT, and IX were the most suitable for *F. fennae* differentiation, and character IT had the highest statistical support (Table 3, column Can1). The value of IT (the proportion of terminal cells of ixohyphidia inflated in the terminal part or ± in the middle) for *F. fennae* exceeded 0.5 (Fig. 5d), with the exception of two specimens (KRAM F-56109 and BRA CR-4429). The maximum value of IT for *F. velutipes* was 0.37. Terminal cells of ixohyphidia of *F. fennae* were not usually coralloid and were unbranched (defined by characters IC and IX). Most specimens of *F. fennae* had a value of IC less than 0.1 (Fig. 5b) and ixohyphidia were only weakly branched (the value of IX is mostly 0.15) (Fig. 4d). In contrast, coralloid ixohyphidia were frequent in most specimens of *F. velutipes* (value of IC is at least 0.1) and were distinctly branched (value of IX is at least 0.15). There were also two exceptions: one specimen of *F. velutipes* (BRNM 666755) had values of IC = 0 and IX = 0.04 and one specimen of *F. fennae* (BRA CR-4429) had values of IC = 0.25 and IX = 0.25.

Molecular analyses

Results of molecular phylogenetic analyses based on ribosomal ITS sequences are given in Fig. 6. Species, with the exception of *F. ononidis*, formed well-supported

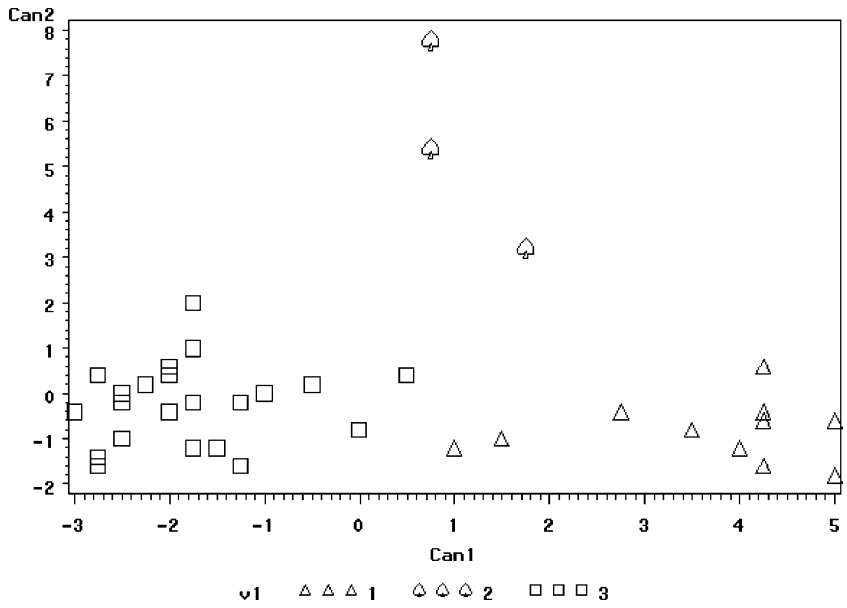

Fig. 3 Ordination diagram of the canonical discriminant analysis of average values of characters measured on spores and terminal cells of ixohyphidia near margin of pileus. The first canonical axis account for 69.04% of the variation among groups. *Triangle F. fennae*, *spade F. ononidis*, *square F. velutipes*. The diagram shows results of analyses, that confirmed delimitation of groups defined by principal component analyses (Fig. 2). All characters listed in Table 2, with exception of IC, were used for the analysis

Table 3 Eigenvector values of the first and the second principal component axis (Prin1, Prin2) calculated in the principal component analysis of average values of 30 measurements on individual *Flammulina* specimens (see Fig. 2). Total canonical structure values of the first and the second canonical axis (Can1, Can2) calculated in the canonical discriminant analyses of average values of 30 measurements on individual *Flammulina* specimens (see Fig. 3)

Character[a]	Prin1	Prin2	Can1	Can2
SL	0.116	0.775	−0.187	**0.943**
SW	−0.399	0.555	**0.663**	**0.654**
SQ	0.525	0.232	**−0.860**	0.282
IF	−0.040	−0.173	−0.234	−0.070
IB	0.089	−0.056	−0.184	−0.05
IT	−0.540	0.102	**0.956**	−0.029
IX	0.498	0.056	**−0.788**	0.035

Significant values shown in bold

[a] For abbreviations of characters see Table 2

clades in both parsimony and Bayesian analyses, but relative relationships between species varied with the analysis. Collections used in this paper fell cleanly within species-specific clades. *Flammulina velutipes* and two collections of *F. elastica* were not morphologically separable in this study. One might expect, therefore, that the two species might be phylogenetically related, and in both Bayesian and Parsimony analyses, *F. elastica* is basal to *F. velutipes*.

European *Flammulina* species can be identified by species-specific motifs in the ITS DNA region (Hughes et al. 1999). Restriction site differences in these variable areas were used to diagnose species without the need for DNA sequencing (Methven et al. 2000). Using the *Flammulina velutipes* ITS1 sequences as a reference, there were variable regions between ITS1 bases 28 and 63 and between ITS1 bases 148 and 149 that were species-specific. *Flammulina velutipes, F. ononidis*, and *F. cephalariae* lacked bases in the latter area while *Flammulina fennae*, *F. rossica*, and *F. populicola* had up to 20 bp of informative sequence (Hughes et al. 1999). The ITS2 region was approximately 313 bp long. Between bases 163 and 237 of ITS2 there was a third variable region that was also species-informative.

As noted previously (Badalyan and Hughes 2004), there are several haplotypes for *F. velutipes* in Europe, and collections were often heterozygous for 1- to 2-bp indels. This was also true of *F. elastica*, requiring cloning in many cases to recover individual haplotypes. *Flammulina fennae* collections, in contrast, exhibited a single haplotype.

ITS sequence data for six specimens of *F. fennae* in this study corresponded to morphological determinations. Petersen et al. (2009) distinguished *F. elastica*, a species phylogenetically related to *F. velutipes*, as differing from *F. velutipes* by the ratio of length and width of spores (Q= 2.5–3). All our collections had a Q value up to 2.2 and, following the concept of Petersen et al. (2009), they were

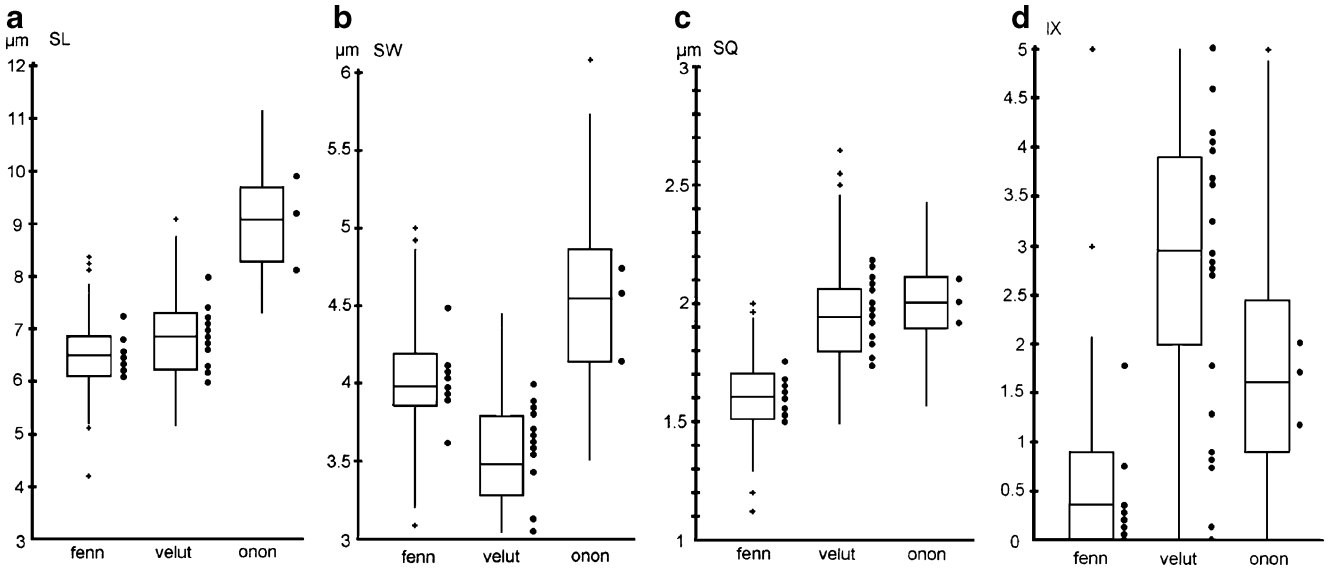

Fig. 4 Comparison the length of spores (*SL*; **a**), the width of spores (*SW*; **b**), the ratio of length and width of spores (*SQ*; **c**) and the index of branching of TCI of terminal cells of ixohyphidia near margin of pileus (*IX*; **d**) measured on specimens *Flammulina fennae* and related taxa. The *box plots* are based on a sample of measurements of all measured specimens in the species. To the *right* of each box plot, average values for each single specimen are labeled with *dots*. *fenn Flammulina fennae* (330 measurements, 10 specimens), *velut F. velutipes* (720 measurements, 22 specimens), *onon F. ononidis* (90 measurements, 3 specimens). The *bottom and top edges* of the boxes are located at the sample 25th and 75th percentiles. The *centre horizontal line* is drawn at the sample average. The *central vertical line* extends from the box as far as the data extend to a distance of at most 1.5 interquartile ranges (an interquartile range is the distance between the 25th and the 75th sample percentiles). Any values more extreme than this are marked with a *cross*

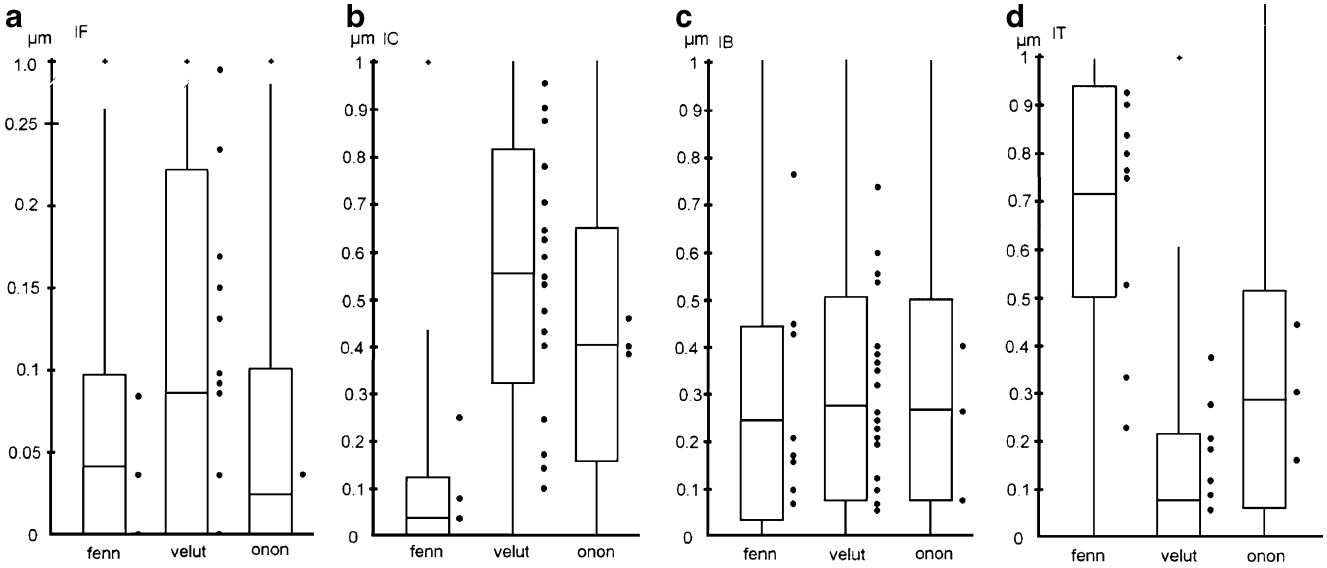

Fig. 5 Comparison the proportion of defined shapes of terminal cells of ixohyphidia near margin of pileus: filiform (*IF*; **a**), coralloid (*IC*; **b**), inflated in the basal part (*IB*; **c**) and inflated on the terminal part or ± in the middle (*IT*; **d**) measured on specimens *Flammulina fennae* and related taxa. The *box plots* are based a sample of on measurements of all measured specimens within the species.To the *right* of each boxplot, average values for each single specimen are labeled with *dots*. *fenn Flammulina fennae* (330 measurements, 10 specimens), *velut F. velutipes* (720 measurements, 22 specimens), *onon F. ononidis* (90 measurements, 3 specimens). The *bottom and top edges* of the boxes are located at the average plus/minus half of standard deviation. The *centre horizontal line* is drawn at the sample average. The *central vertical line* extends from the box as far as the data extend, to a distance of at most 1.5 interquartile ranges (an interquartile is the distance between the average plus half of standard deviation and the average minus half of standard deviation). Any values more extreme than this are marked with a *cross*

morphologically assigned to *F. velutipes*. However, 2 of 12 collections morphologically determined as *F. velutipes* had sequences that corresponded to *F. elastica* as defined by blast sequence homology and by sequence alignments with known exemplars. Discrepancies in morphological and molecular delimitation of *F. velutipes* and *F. elastica* suggest that a critical revision of the current morphological concept of *F. elastica* is needed and, for this reason, we treat both species as the *F. velutipes* complex in the text/key below.

Hughes and Petersen (2001) previously noted that *Flammulina* species were not completely reproductively isolated and that hybrids were possible in vitro. One isolate, SAV F-1450 (culture F-70) from Slovakia, appeared to be a hybrid between *F. velutipes* and another *Flammulina* species. The ITS1 region was *F. velutipes*. The ITS2 variable region appeared to be homologous with *F. rossica*. A similar hybrid was found among *F. velutipes* collections from Argentina where it appeared to be an invasive species. Interestingly, sequences for the Argentine collection and the Slovakian collection SAV F-1450 were the same. There are two possible explanations. Either this collection represents an ancient hybridization event that became widely distributed in Europe and was transported to Argentina by human-mediated activities or the same hybridization event may have happened more than once.

Variability of micromorphological characters

Prior to this study, the delimitation of *F. fennae*, *F. ononidis*, and *F. velutipes* was based primarily on spore characters (Bas 1983; Petersen et al. 2009). However, our multivariate morphometric analyses showed that the length and width of spores was appropriate only for the delimitation of *F. ononidis*. The length and width of spores of *F. fennae* and the *F. velutipes* complex formed a continuous range and, therefore, were not suitable for differentiation of these taxa (Table 4).

Some authors also used pileipellis characters for distinguishing *Flammulina* taxa. Bas (1983) noted that, in borderline cases between *F. fennae* and the short-spored form of *F. velutipes*, the structure of the pileipellis was decisive. He described pileocystidia at the centre of the expanded pileus of *F. fennae* as very crowded and not (or hardly) interspersed with rather simple ixohyphidia; of *F. velutipes* as interspersed with ixohyphidia branching at wide angles. Petersen et al. (2009) emphasized the importance of hyphal tips. The pileipellis of *F. velutipes* was described as a turf of filamentous, often branched hyphal tips with scattered tapering pileocystidia. The pileipellis of *F. fennae* was described as a hymenial layer of almost unbranched, erect hyphal tips often resembling elongate bowling pins with scattered thick-walled pileocystidia.

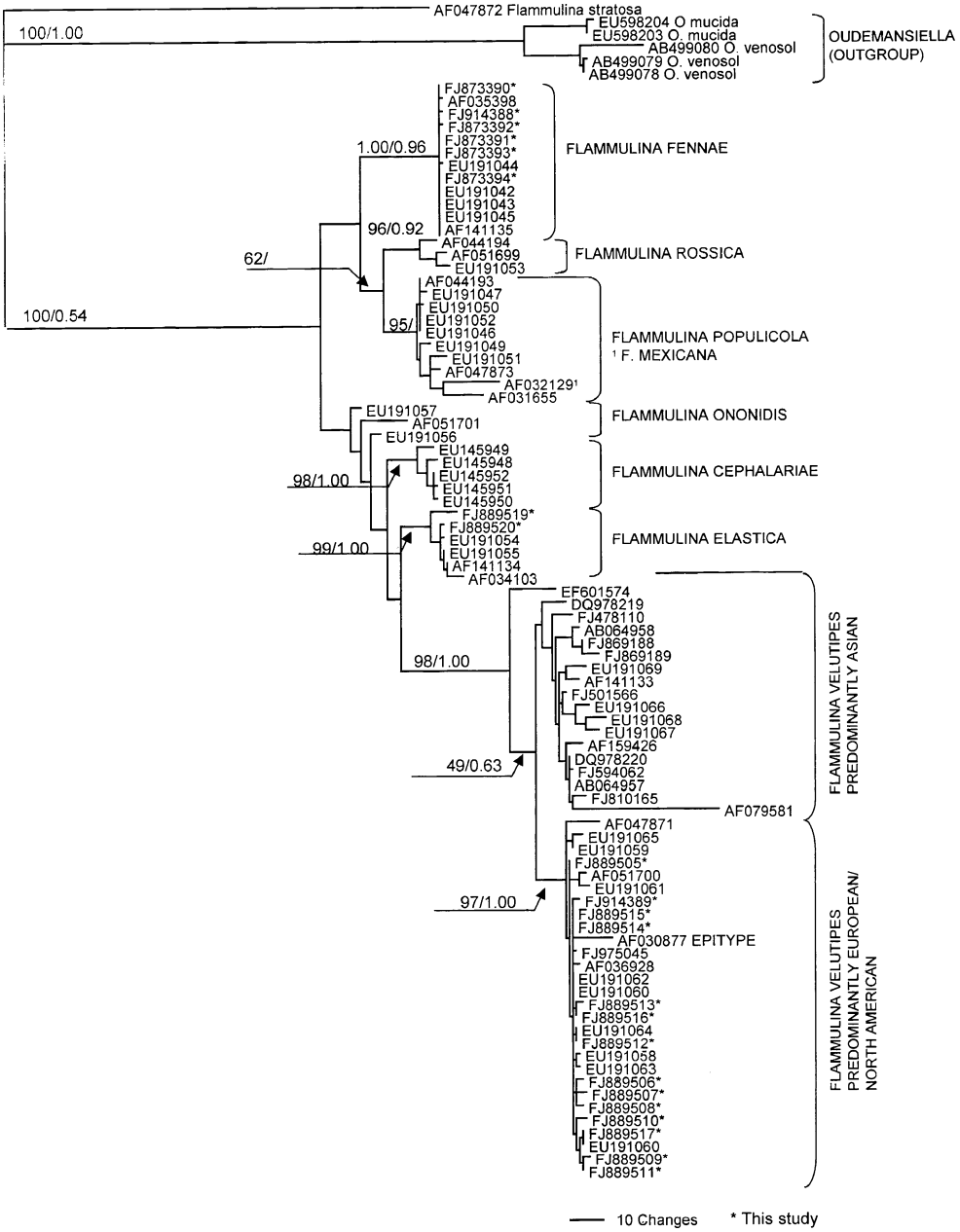

Fig. 6 One of 2,000 most parsimonious trees of length 937 steps. Bootstrap support values ≥50% and Bayesian support values ≥0.50 are given to the *left* of the supported node. The best model of evolution estimated by Model Test was GTR + I + G (General time reversible model with a proportion of invariable sites and a gamma shape distribution). *Collections used in this study

Among all examined specimens with low Q values (the ratio of length and width of spores up to 2.2), the specimens with lanceolate pedunculate and less branched terminal cells of ixohyphidia were morphologically identified as *F. fennae* (Table 1). The group of specimens assigned to *F. velutipes* had considerable variability in shape of ixohyphidia. We recognized three morphological groups: (1) specimens with predominantly coralloid ixohyphidia (sequenced specimens SAV F-1445, SAV F-1446, SAV F-1447, SAV F-1448, SAV F-1449, and SAV F-1450); (2) specimens with various shapes of ixohyphidia (sequenced specimens SAV F-1451, SAV F-1452, SAV F-1453, SAV F-1593, and SAV F-1594); and (3) one specimen (BRNM 666755) with predominantly filiform unbranched ixohyphidia. These three morphotypes did not differ significantly in ITS sequences and, moreover, the first two groups comprised specimens with sequences of both *F. elastica* and *F. fennae* (Table 1).

We have not taken into consideration the density of pileocystidia as reliable for delimitation of *Flammulina* taxa (such as suggested by Bas 1983), because we observed a large variation between juvenile and mature basidiocarps. On the other hand, our morphological studies confirmed the importance of terminal cells of ixohyphidia. In *F. fennae*, the most important morphocharacter seems to be not the number of lateral branches (which is often reduced in some collections of *F. velutipes* complex) but the proportion of terminal cells of

Table 4 The comparison of *Flammulina* spore dimensions

Flammulina species	Source	Length (μm)	Width (μm)	Q
F. velutipes	Arnolds (1977)	6.5–9.5	3–4.5	–
	Klán (1978)	6.7–7.7–10.3	2.6–3.4–4.3	1.86–3.2
	Bas (1995)	6–9.5(–12)	(2.5–)3–4(–5)	(1.85–)2–2.3
	Petersen et al. (2009)	6–9.5	(2.5–)3–4(–5)	2–2.3
	Pérez-Butrón and Fernandéz-Vincente (2007)	6–9.5	3–4	2.5
	Our data (values for *F. velutipes* complex with Q < 2.2)	(5.9–)6.2–7.5(–8.1)	(3–)3.1–3.8(–4.1)	(1.75–)1.81–2.4(–2.13)
F. fennae	Bas (1995)	(5.5–)6–7.5(–8)	(3.5–)4–4.5(–5)	1.4–1.8(–1.95), av. 1.55–1.7
	Petersen et al. (2009)	(5.5–)6–7.5(–8)	(3.5–)4–4.5(–5)	1.5–1.7
	Pérez-Butrón and Fernandéz-Vincente (2007)	6–7.5(–8)	4–4.5(–5)	1.55–1.7
	Our data	(5.6–)5.9–7(–7.4)	(3.5–)3.7–4.3(–4.6)	(1.42–)1.49–1.74(–1.83)
F. ononidis	Arnolds (1977)	(7.5–)8.5–11	(4–)4.5–5.5(–6)	–
	Klán (1978)	9.3–10.5–12.4	4.1–4.6–5.7	1.8–2.75
	Bas (1995)	(7.5–)8.5–12.5(–14)	(4–)4.5–5.5(–6)	(1.6–)1.7–2.45, av. 1.9–2.3
	Petersen et al. (2009)	7.2–13.7	3.7–4.49(–5.4)	–
	Pérez-Butrón and Fernandéz-Vincente (2007)	(7.5–)8.5–13(–14)	(4–)4.5–6	–
	Our data	(7.5–)8.1–10.1(–10.7)	4.1–5(–5.2)	(1.8–)1.85–2.17(–2.33)
F. elastica	Bas (1995) (as *F. velutipes* f. *longispora*)	(7.5–)8–11.5(–12)	(2.5)3–4(–4.5)	2.5–3.05
	Petersen et al. (2009)	(7.5–)8–11.5(–12)	(2.3–)3–4(–4.7)	2.5–3
	Pérez-Butrón and Fernandéz-Vincente (2007)	(7.5–)8–11.5(–12)	(2.3–)3–4(–4.7)	2.5–3

ixohyphidia which are inflated in the upper half (not on basal part) as in most specimens of the *F. velutipes* complex.

In summary, *F. fennae* can be distinguished from *F. ononidis* and the *F. velutipes* complex by a combination of spore and pileipellis characters, i.e., the ratio of length and width of spores, the proportion of a certain type of terminal cells of ixohyphidia and its index of branching.

Macromorphological characters and habitat

Within the genus *Flammulina*, *F. fennae* had a typical combination of macromorphological characters, namely the color of the pileus and stipe shape. The pileus was light colored at the margin (white to pale cream), towards the centre with orange tints (orange-white) and the darkest at centre (grayish orange to dark brown). The presence of darker rusty spots on the pileus was also characteristic. The stipe was cylindrical, fusiform at the base, and often with distinct pseudorhiza (Fig. 7). Based on material examined (Table 1), *F. fennae* fruits in dense clusters on roots or woody fragments buried in soil (seemingly on soil), but also on the base of stumps and fallen rotten trunks. The plant hosts are broad-leaved trees like *Acer*, *Betula*, *Fagus* and *Ulmus*.

In Europe, *F. cephalariae* is another *Flammulina* species with distinct pseudorhiza, by which it is connected to roots of its host *Cephalaria leucantha*. The authors of this species, Pérez-Butrón and Ferdnández-Vicente (2007), considered the macromorphological characters and habitat of *Flammulina* taxa important and used them in the first step in their key for European species of the genus *Flammulina*. Based on this key, *F. elastica*, *F. rossica*, and *F. velutipes* are lignicolous, growing directly from wood in clusters, while *F. cephalariae*, *F. fennae*, *F. ononidis*, and *F. populicola* are terrestrial or growing on roots, gregarious or scattered, sometimes in clusters.

Although the macromorphological characters and habitat of *F. fennae* are considered very distinct, we have also observed some collections of *F. velutipes* complex that look like *F. fennae* (light-colored pilei, growth on roots or woody fragments, i.e., seemingly on soil) and, occasionally some collections of *F. fennae* resembling the *F. velutipes* complex (solitary habit, growth directly on wood, darker-colored pilei). We consider these characters variable within the genus *Flammulina* and therefore not helpful for delimiting species.

Fruiting period

Bas (1983, 1995) considered *Flammulina fennae* to be a summer species, fruiting between April and November. *Flammulina velutipes*, a winter species, fruited throughout the year but was abundant only between September and March, while *Flammulina ononidis* also fruited between September and March.

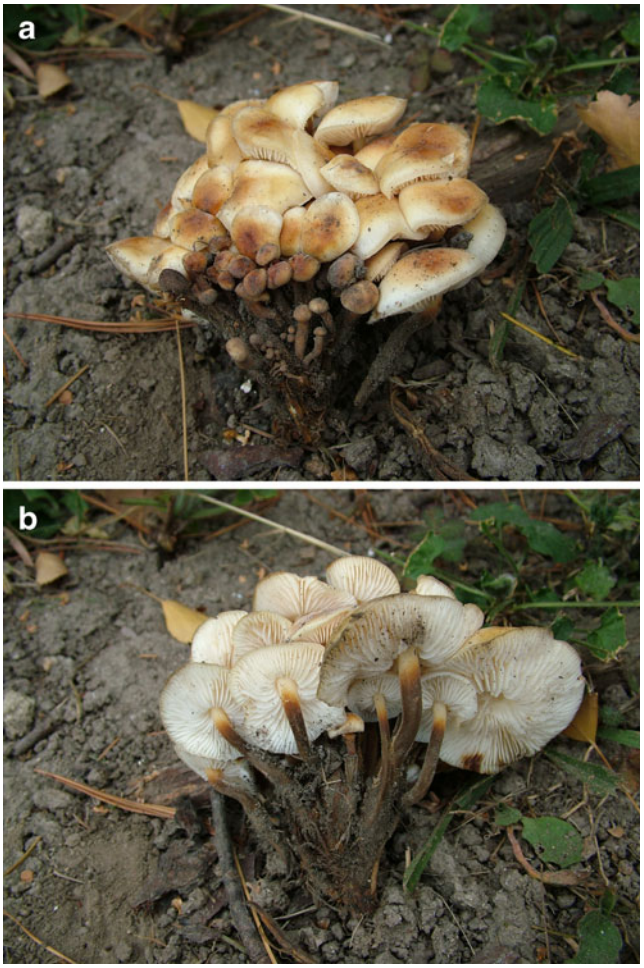

Fig. 7 *Flammulina fennae*: basidiomata (SLO F-1004)

After assessing the phenological data of our *Flammulina* collections (Tables 1 and 5), we determined that of 10 specimens of *F. fennae* collected between August to November, the majority produced basidiocarps in September (5) and October (3). Specimens of the *F. velutipes* complex (22) produced basidiocarps nearly throughout the year with the most in September (4) followed by November, October, January, and March (3 each). We examined only 3 specimens of *F. ononidis* (from October and November) and we are not able to comment its fruiting period.

Flammulina fennae and the *F. velutipes* complex produced basidiomata in autumn and collections from late spring or summer also represented both species (Table 5). Thus, it was difficult to strictly define the fruiting period for individual *Flammulina* species in Europe as some authors have done (see Hagara 1987; Petersen et al. 2009), and phenological data are helpful but not conclusive for correct determination.

Distribution and conservation status

In Europe, *Flammulina fennae* is known from Austria (Krisai-Greilhuber 1999), Belgium (Walleyn and Vandeven 2006), Czech Republic (Antonín 2006; see also Table 1), Denmark (Bas 1995), France (Bas 1995), Germany (Benkert et al. 1996; see also Table 1), Hungary (Bas 1983; see also Table 1), the Netherlands (Bas 1983), Norway (Brandrud et al. 2006), Poland (Komorowska 2000; see Table 1), Russia (Pérez-Butrón and Ferdnández-Vicente 2007; see also Table 1), Slovakia (Hagara 1987; Záhorovská 1997; see also Table 1), Sweden (Gärdenfors 2005; see also Table 1), and Switzerland (Petersen et al. 2009).

F. fennae is classified as threatened in Austria (Krisai-Greilhuber 1999), the Czech Republic (Antonín 2006), Germany (Benkert et al. 1996), Norway (Brandrud et al. 2006), Sweden (Gärdenfors 2005), and Switzerland (Senn-Irlet et al. 2007).

Key to the taxa of *Flammulina* in Europe

1 Spores wider than 4 µm and longer than 8 µm 2
1* Spores narrower than 4 µm and/or shorter than 8 µm 4
2 Ixohyphidia in pileipellis sphaeropedunculate or distinctly inflated, the latter type of ixohyphidia often with lateral nodules or terminal constrictions, on wood of various trees and bushes ***F. rossica***
2* Ixohyphidia in pileipellis not distinctly inflated, on roots of *Ononis spinosa* or *Cephalaria leucantha* 3
3 Spores 8–10 (–12) µm long, on *Ononis spinosa* ***F. ononidis***
3* Spores 12–17 µm long, on *Cephalaria leucantha* ***F. cephalariae***
4 Pileipellis hymeniderm, composed of sphaeropedunculate, mostly unbranched ixohyphidia ***F. populicola***
4* Pileipellis ixotrichoderm, ixohyphidia not sphaeropedunculate 5
5 Ixohyphidia in pileipellis near margin of pileus in major part unbranched and inflated in the terminal part or ± in the middle, lanceolate pedunculate or fusiform pedunculate, average ratio of length and width of spores per collection (avQ) not exceed 1.8 ***F. fennae***
5* Ixohyphidia in pileipellis near margin of pileus in major part distinctly branched, if unbranched than filiform or inflated in basal part, avQ per collection mostly exceeds 1.8 ***F. velutipes* complex**
Note: *F. velutipes* complex also includes *F. elastica*—see above

Table 5 The number of collections of *Flammulina* species in the individual months. Included are only studied specimens with Q (the ratio of length and width of spores) up to 2.2

Species / Month	T[a]	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Flammulina fennae	10	–	–	–	–	–	–	–	1	5	3	1	–
F. velutipes complex	22	3	–	3	1	1	–	1	1	4	3	3	2
F. ononidis	3	–	–	–	–	–	–	–	–	–	2	1	–

[a] *T* Total number of collections

***Flammulina fennae* Bas, Persoonia 12: 52, 1983**

Typus: 'C. Bas 7727, 19 Oct. 1980, Netherlands, prov. Zuid-Holland, Voorschoten, estate "Ter Wadding", (L).'

Description of macromorphological characters (Fig. 7) *Pileus* 15–55 mm, hemispherical, later plano-convex to applanate, at margin inflexed, later straight, often undulate, at center with low broad umbo or flat to slightly depressed; at surface pruinose or slightly velvety, later glabrous, viscid when moist; at margin white to pale cream (4A2), towards the center orange-white (5A2–6A2), at centre grayish orange (5B4), flesh-colored (6B3), rusty (6C4) to dark brown (6F8), often with darker rusty spots; slightly hygrophanous and slightly translucently striate at margin when moist. *Stipe* 45–160×2–8 mm, cylindrical, at base fusiform, often with pseudorhiza, at surface velvety, longitudinally striate, above

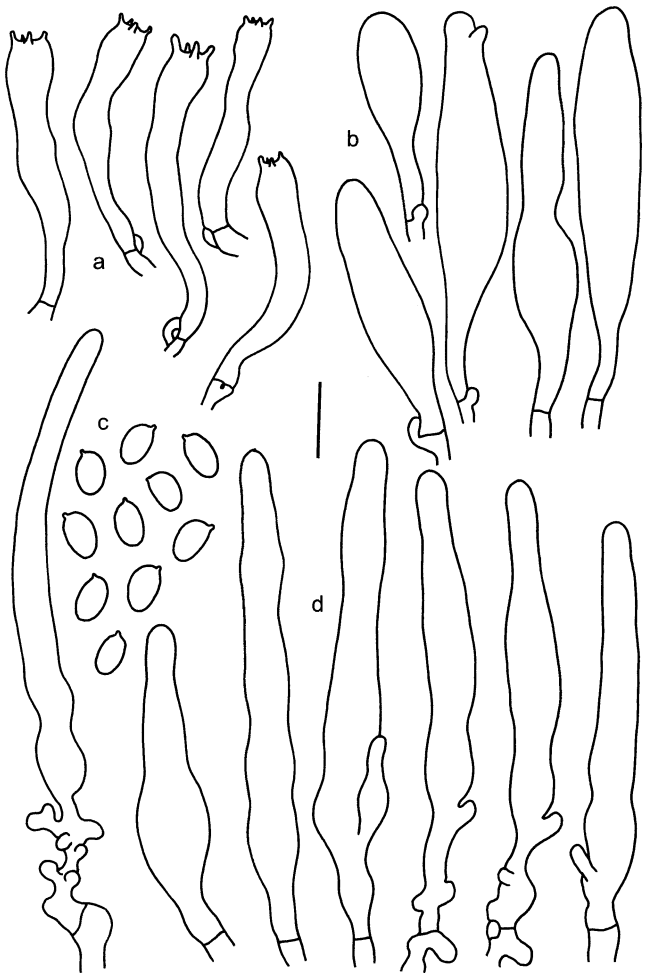

Fig. 8 *Flammulina fennae*: **a** basidia (SAV F-1916, SLO F-1004), **b** pleurocystidia, **c** spores, **d** pileocystidia (BRA CR-4429). *Scale bar* 10 μm

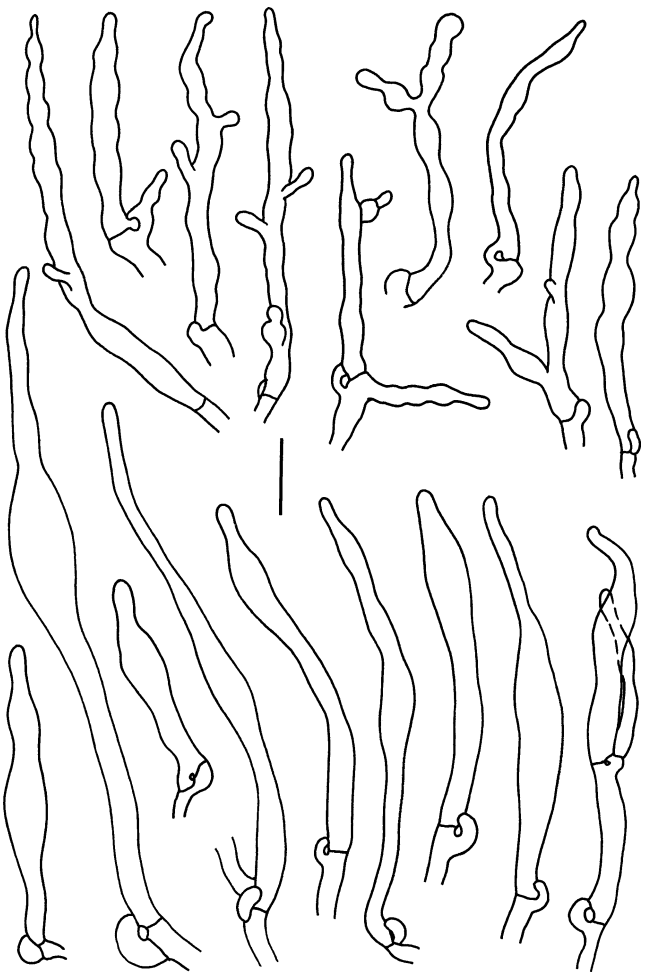

Fig. 9 *Flammulina fennae*: terminal cells of ixohyphidia (BRA CR-4429, SAV F-1915, SLO F-1002). *Scale bar* 10 μm

white to pale cream (4A2), below a narrow orange-brown zone (5C5) passing gradually downwards to dark brown (5F8–6F8; the dark brown part exceeds the half of stipe in mature), solid, later stuffed, tough. *Lamellae* up to 7 mm broad, L=34–52, l=1–3, adnexed or emarginate, white to pale cream (4A2), often rusty spotted when bruised or with age, edge entire and concolorous. *Flesh* elastic, in pileus whitish, in upper part of stipe whitish to yellowish, towards the base ochraceous to brownish, at base brown; smell indistinct to fruity, taste mild to somewhat astringent.

Description of micromorphological characters (Figs. 8, 9) *Spores* (5.6–)5.9–7(–7.4)×(3.5–)3.7–4.3(–4.6) µm, av. 6.5×4 µm, Q=(1.42–)1.49–1.74(–1.83), av. Q=1.62, ellipsoid, smooth, thin walled, inamyloid, hyaline, with short and small hilar appendage. *Basidia* (28–)30–35.5(–38)×5–6 µm, av. 32.9×5.5 µm, narrowly clavate, 4-spored. *Pleurocystidia* approx. 32–57×9–11 µm, mostly fusiform, rarely indistinctly lageniform, obtuse, pedunculate; wall thickened towards the base, thin at apex. *Pileipellis* composed of numerous prominent pileocystidia and ixohyphidia. *Terminal cells of ixohyphidia* (27–)30–62.5(–88)×(3–)3.5–6.5(–9) µm, mostly inflated in the terminal part or ± in the middle, lanceolate pedunculate or fusiform pedunculate, rarely inflated on the base and attenuated, very rarely coralloid; unbranched or with one to three lateral branches or nodules, very rarely with more branches; towards the tips constricted to approx. 2 µm, usually with multiple constrictions (moniliform) along all length. *Pileocystidia* approx. 48–91×7–10 µm, narrowly lageniform, pedunculate, constricted and nodulose on the base, with slightly thickened walls and brownish intracellular pigment. *Surface of stipe* covered by cylindrical hyphae with sparse terminal cells; the terminal cells 26–83×3.5–7 µm, especially towards the base of stipe with thickened wall and brown pigment, towards the gills often moniliform and nodulose, usually slightly narrowed on tips. *Caulocystidia* abundant, mostly longer than 100 µm, of similar shape as pileocystidia. Hyphae in all tissues with *clamp connections.*

Acknowledgements Jan Borovička, Ladislav Hagara, Per Marstad, and Anna Ronikier are acknowledged for providing us with additional material of *Flammulina fennae*. We are grateful to Dagmar Triebel, who corrected information rewritten from the original hand-written text on herbarium sheets of specimens from herbarium M. The Slovak Grant Agency VEGA (grant no. 2/7071/7 and 2/5087/25) supported this study.

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