CZECH MYCOL. 60(2): 221–230, 2008

Flammulina ononidis – a new species for Slovakia

SOŇA RIPKOVÁ[1], SLAVOMÍR ADAMČÍK[2] and VIKTOR KUČERA[2]

[1] Comenius University in Bratislava, Faculty of Natural Sciences, Department of Botany, Révová 39, SK-811 02 Bratislava, Slovakia
ripkova@fns.uniba.sk
[2] Institute of Botany SAS, Dúbravská cesta 14, SK-845 23 Bratislava, Slovakia
slavomir.adamcik@savba.sk, viktor.kucera@savba.sk

Ripková S., Adamčík S. and Kučera V. (2008): *Flammulina ononidis* – a new species for Slovakia. – Czech Mycol. 60(2): 221–230.

Although described in 1977 from Germany, *Flammulina ononidis* Arnolds was found in Slovakia in 2002 for the first time. Macro– and micromorphological characters of the Slovak collections are described and illustrated. The delimitation of *F. ononidis* is discussed and the knowledge of its distribution, ecology, biology and threat is summarised.

Key words: fungi, Basidiomycota, *Xerulaceae*, distribution, ecology, biology.

Ripková S., Adamčík S. a Kučera V. (2008): *Flammulina ononidis* – nový druh pre Slovensko. – Czech Mycol. 60(2): 221–230.

Hoci bol druh *Flammulina ononidis* Arnolds opísaný z Nemecka v roku 1977, na Slovensku sme ho prvýkrát našli až v roku 2002. V práci uvádzame opis a vyobrazenie makro– a mikromorfologických znakov našich slovenských zberov. Hodnotíme ohraničenie *F. ononidis* a sumarizujeme poznatky o jeho rozšírení, ekológii, biológii a ohrozenosti.

INTRODUCTION

There are many fungal names with epithets referring to a specific host-fungus interaction and *Flammulina ononidis* is one of them. As a new species, *Flammulina ononidis* Arnolds was described in 1977 based on material from Germany and growing on *Ononis spinosa* (Arnolds 1977). Although *O. spinosa* is widespread in Slovakia, with the exception of the Vihorlat Mts., Východné Beskydy Mts. and Bukovské vrchy Mts. (Chrtková and Jasičová 1988), we confirmed the occurrence of *F. ononidis* in the country only in the year 2002.

The aim of the paper is to describe the first collections of *F. ononidis* for Slovakia, to compare the characters with published data and to summarise the current knowledge of its distribution, ecology, biology and threat.

MATERIAL AND METHODS

More than 300 *Flammulina* specimens deposited in herbaria in Austria (W), Czech Republic (BRNM), Germany (M) and Slovakia (BRA, SAV and SLO) were examined during our study of the taxonomy and biogeography of the genus *Flammulina* in Central Europe in the years 2004–2007. However, besides our two specimens from Slovakia (SAV F-1318, SAV F-1319), only one specimen from Hungary (M 0065416) was identified as *F. ononidis*. The macromorphological characters were observed in fresh material (SAV collections), the micromorphological characters in dried material (M and SAV collections) under an oil immersion lens with an Olympus CX41 light microscope. Fragments of lamellae, stipe and pileipellis were examined in 5 % KOH and a solution of Congo Red in ammonia (1 ml of 25 % ammonia dissolved in a filtrated solution of 1.5 g of Congo Red and 50 ml of distilled water). The measurements of microscopical characters are given as mean value plus/minus standard deviation and are based on 30 measurements per specimen. Values in parentheses are measured minimum or maximum values. Q is the ratio of length and width of the spores. The abbreviations of herbaria are cited in accordance with the Index Herbariorum (Holmgren et al. 1990). Data on specimens are presented in their original form. References to colours follow Kornerup and Wanscher (1974).

RESULTS AND DISCUSSION

Flammulina ononidis Arnolds, Westfälische Pilzbriefe 11(3–4): 33, 1977

Holotype. "Oeynhausen prope Detmold, 6 Okt. 1976, E. Arnolds 3628 in Rijksherbarium, Leiden (L)."

Description. Pileus 15–30 mm, hemispherical when young, later planoconvex to applanate and sometimes with umbo when mature; surface rough, shiny, viscid when wet, slightly hygrophanous and slightly translucently striate on margin when older; margin brownish yellow to golden brown (5C7–5D7), towards centre light orange (5B5), at centre darker caramel to cinnamon brown (6C6–6D6), light brown (6D7–6D8) to rusty brown (6E8). Stipe 40–95 × 2.5–4 mm, cylindrical, base rooting and sometimes fusiform; surface velvety, longitudinally striate; in upper part almost white or yellowish white to pale yellow (3A2–3A3), in lower part bright lemon yellow when young (3B8), later light brown (5D8) to chocolate brown (6E4–6F4) at base when mature; hollow. Lamellae 3–5.5 mm wide, L = 24–43, l = 3(–7), adnexed to adnate-emarginate, edge entire, ivory white (4B3) with orange shade in frontal view. Flesh elastic; in pileus pale yellowish (paler than 4A3), in upper part of stipe ivory white (4B3), in base of stipe wax yellow (3B5); smell indistinct; taste mild to somewhat pleasantly sweetish (Figs. 1, 2).

Fig. 1. *Flammulina ononidis*: basidiocarps (SAV F-1319). Photo by M. Vašutová.

Fig. 2. *Flammulina ononidis*: basidiocarp (SAV F-1319). Photo by M. Vašutová.

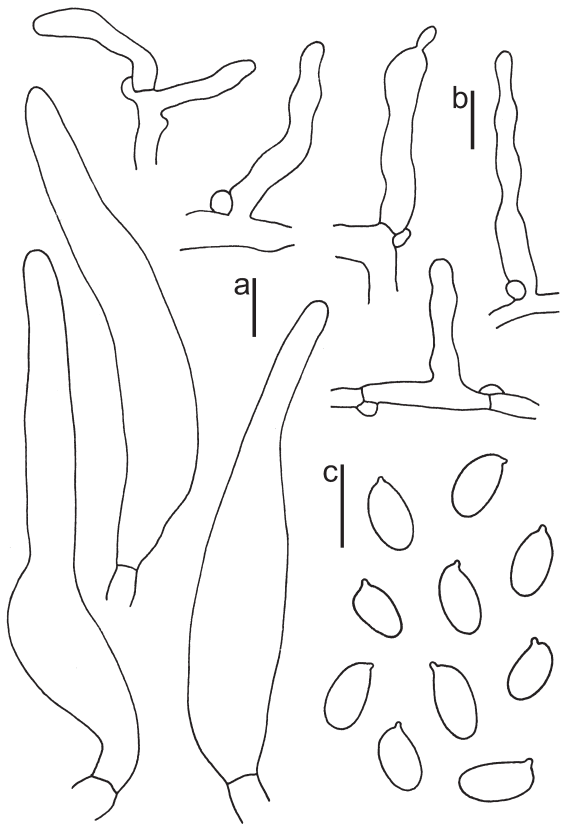

Fig. 3. *Flammulina ononidis*: a – caulocystidia, b – hyphal terminations on stipe surface, c – spores (SAV F-1319). Scale bars = 10 µm.

Basidia 4-spored, narrowly clavate, (25.5–)28–33(–35.5) × 6.5–7 µm, av. 30.7 × 6.8 µm. Spores (7.3–)8.1–10.1(–11.2) × (3.5–)4.1–5(–6) µm, av. 9.1 × 4.5 µm, Q = (1.62–)1.84–2.19(–2.43), av. Q = 2.01, broadly ellipsoid to indistinctly amygdaliform, smooth, hyaline (Fig. 3). Pleurocystidia (33.5–)36–54(–78) × (8–)10.5–16(–19.5) µm, av. 45 × 13.3 µm, lageniform, fusiform-pedunculate, ellipsoid-pedunculate, broadly clavate, thick-walled (c. 0.5 µm); more frequent towards the edge of lamellae (Fig. 4). Cheilocystidia similar to pleurocystidia, abundant. Pileipellis composed of ixohyphidia and pileocystidia. Terminal cells of ixohyphidia c. 27–65 × 3.5–7.5 µm, mostly of three types: 1) coralloid, 2) branched with one central stem and lateral branches (usually not with more than 5 branches), 3) unbranched; all of them usually with constricted or attenuated tips (1–2 µm wide at the tips), thin-walled (Fig. 5). Pileocystidia c. 68–105 × 7.5–12 µm, narrowly lageniform, often pedunculate-constricted and sinuose at base, thick-walled (0.5–1 µm) (Fig. 6). Surface of stipe made up of scarce

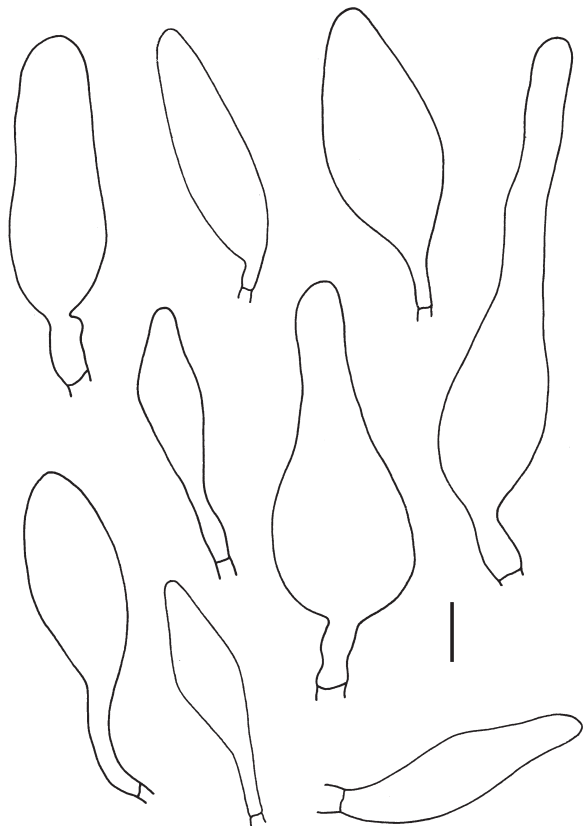

Fig. 4. *Flammulina ononidis*: pleurocystidia (SAV F-1318). Scale bar = 10 µm.

caulocystidia and cylindrical thin-walled hyphae (Fig. 3). Caulocystidia similar to pileocystidia but wider (c. 12–15 µm). Hyphae thin-walled, moniliform and slightly constricted towards the tips (sometimes also appendiculate), with short terminal cells (c. 30–50 × 4.5–7 µm); in upper part of stipe thin-walled and hyaline, in basal part with brownish pigment and thick-walled (Fig. 3).

Published figures and/or descriptions. Anonymus 2 (on-line), Arnolds (1977), Bas (1995), Cetto (1994), Hagara et al. (2005), Kotlaba (1995), Kříž (on-line), Petersen et al. (on-line).

Notes on biology and delimitation. There is also another *Flammulina* taxon with its epithet referring to a specific host, namely *Flammulina velutipes* var. *lupinicola* Redhead et R.H. Petersen. In comparison with the typical variety, it can be recognised by larger spores (Tab. 1) and habitat on *Lupinus arboreus* Sims in coastal dunes in California (Redhead and Petersen 1999). The similar spore size (Tab. 1) and the growth on a leguminous host (*Fabaceae*) in

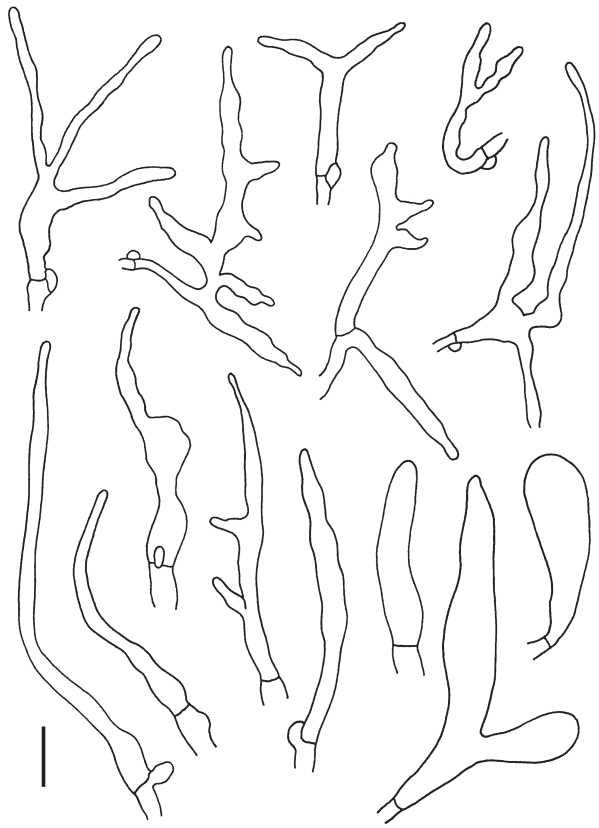

Fig. 5. *Flammulina ononidis*: terminal cells of ixohyphidia (SAV F-1319). Scale bar = 10 µm.

both taxa, *F. velutipes* var. *lupinicola* and *F. ononidis*, involved discussions about their relationship (Redhead and Petersen 1999). Pairing experiments (Petersen et al. 1999) revealed that *F. ononidis* is compatible with all infraspecific taxa within *F. velutipes*, namely with *F. velutipes* var. *lupinicola*, *F. velutipes* var. *velutipes* (Curtis) Singer and *F. velutipes* var. *lactea* (Quél.) Bas. Such sexual compatibility suggests a close genetic relationship between these taxa. However, based on ribosomal ITS sequences (Hughes et al. 1999), *F. velutipes* together with its varieties formed an individual clade, and *F. ononidis* was clustered with *F. elastica* (Lasch) Redhead et R.H. Petersen. Using two restriction enzymes (*Hae* III and *Bst* F51), isolates of *F. ononidis* showed a unique restriction fragment pattern (3–1) within the genus *Flammulina* (the pattern is characterised by an additional *Hae* III site near the end of the ITS2 region, creating fragments of c. 55 bp, 100 bp, 220 bp and 440 bp; and no restriction site for Bst F51) (Methven et al. 2000). For both mating and molecular analyses, only limited material of *F. ononidis* was available (single culture from CBS) and Petersen et al. (on-line) therefore hopes to delineate the

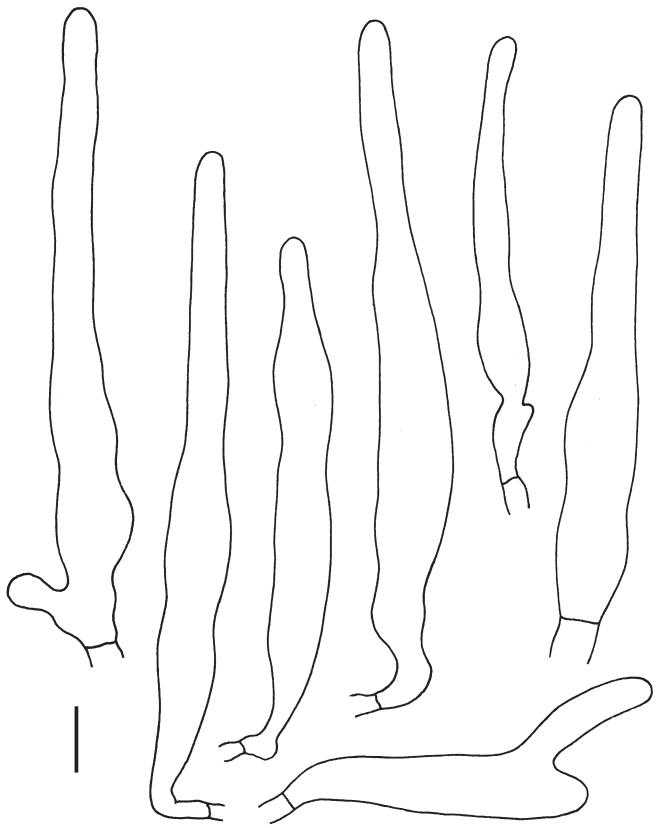

Fig. 6. *Flammulina ononidis*: pileocystidia (SAV F-1319). Scale bar = 10 µm.

position of *F. ononidis* better in a phylogenetic reconstruction using more collections as well as cultures from them.

The latest described new *Flammulina* species, *F. cephalariae* Pérez-Butrón et Fernández-Vic., also has its epithet referring to the host *Cephalaria leucantha* (L.) Roem. et Schult. Its distribution seems to be limited to Losa Valley in the province of Burgos (Northern Spain) (Pérez-Butrón and Fernández-Vicente 2007). In comparison with *F. ononidis*, *F. cephalariae* has distinctly larger spores (Tab. 1).

Considering that the yellow-brownish slimy pileus and the rooting velvety stipe are typical characters of most *Flammulina* taxa, the habitat (namely association with *Ononis spinosa*) seems to be the only useful character for identification of *F. ononidis* in the field. Despite this fact, we recommend to check the identification also microscopically (especially if the host is unknown). However, *F. ononidis* is also microscopically similar to other *Flammulina* taxa. It has a pileipellis structure similar to that of *F. velutipes* and *F. elastica*, and spore measurements are necessary to distinguish these taxa. *F. ononidis* has spores on aver-

age wider than 4 µm and with a length-width ratio of up to 2.2. *F. velutipes* and *F. elastica* have spores on average mostly not wider than 3.5 µm (Tab. 1) and/or with a length-width ratio of at least 2.2 (according to our unpublished observations). The delimitation of *F. ononidis* based on spore dimensions is well discussed by Klán (1978) and Bas (1983). Our measurements of spores of *F. ononidis* fit well to those published by Arnolds (1977) and Bas (1983) (Tab. 1).

For detailed nomenclatural comments, see Arnolds (1977).

Tab. 1. Comparison of spore size of some *Flammulina* taxa.

Source of measurements of some *Flammulina* taxa		spore length	spore width	Q	av. Q
F. ononidis Arnolds (1977)		(7.5) 8.5–13 (14)	(4) 4.5–6	–	–
F. ononidis Bas (1983)		(7.5) 8.5–12.5 (14)	(4) 4.5–5.5 (6)	(1.6) 1.7–2.45	1.9–2.3
F. ononidis our observations	SAV-F 1318	(7.9) 8.5–10 (11)	(4.1) 4.3–4.9 (5.1)	(1.78) 1.85–2.17 (2.37)	2.01
	SAV-F 1319	(7.3) 7.5–8.7 (10.4)	(3.5) 3.9–4.5 (5.1)	(1.62) 1.77–2.07 (2.26)	1.92
	M 0065416	(8.6) 9.4–10.6 (11.2)	(4.1) 4.3–5.2 (6)	(1.76) 1.94–2.28 (2.43)	2.11
F. velutipes var. lupinicola Redhead and Petersen (1999)		7–14.8	3.7–6.6av. 4.5–5.4	–	–
F. cephalariae Pérez-Butrón and Fernández-Vicente (2007)		(9.2) 12–16.8 (17)	(5) 5.6–7.6 (8)	2	–
F. velutipes (Petersen et al. on-line)		6–9.5	3–4	–	2–2.3
F. elastica (Petersen et al. on-line)		8–11.5	3–4	–	2.5–3

Ecology. *Flammulina ononidis* is a saprophyte inhabiting roots and stem bases of *Ononis spinosa* from August to March. It usually fructifies solitarily, less often in small to large groups. The species seems to prefer grasslands on calcareous soils in stands of the plant communities *Bromion erecti* Koch 1926 and *Cirsio-Brachypodion pinnati* Hadač et Klika 1944; it has also been mentioned from calcareous dunes and river dikes (Antonín 2006, Bas 1995, Hagara et al. 2005, Klán 1978, Kotlaba 1995).

We have found *F. ononidis* on an abandoned pasture on sandy soil among herbaceous plants. Its basidiocarps were produced on roots of *Ononis spinosa* in October (see Material studied).

We consider the knowledge of the ecology of *F. ononidis* insufficient, which corresponds with its rarity and little number of collections. We would therefore not be surprised if the spectrum of habitats as well as host plants were more wider, as indicated by Urbonas et al. (1986) who mentioned their collection of *F. ononidis* from another leguminous host, namely from *Trifolium pratense* L.

Distribution. *Flammulina ononidis* is hitherto known from Austria (Krisai-Greilhuber 1999), Croatia (Tkalčec et al. 2005), the Czech Republic (Antonín 2006), Belgium (Walleyn and Vandeven 2006), Denmark (Petersen and Vesterholt 2003), France

(Anonymus 1, 2 on-line), Germany (Arnolds 1977, Krieglsteiner 1978), Hungary (Babos 1968, Cetto 1994, the collections reported here), Estonia (Urbonas et al. 1986), Italy (Petersen et al. on-line), Russia (Petersen et al. on-line), Slovakia (our collections reported here) and Turkey (Pekşen and Karaca 2003).

Threat. *Flammulina ononidis* is classified as endangered in Austria (Krisai-Greilhuber 1999), Croatia (Tkalčec et al. 2005), the Czech Republic (Holec and Beran 2006) and Germany (Benkert et al. 1996). It is argued that the species is threatened by agricultural measures, e.g. fertilisation and habitat transformation (Antonín 2006). Steps in preserving *F. ononidis* should, therefore, involve the preservation of suitable habitats and continuation of the traditional management activities, e.g. cultivation without pesticides and herbicides, hand-mowing and extensive grazing (Biber 1986, Kotlaba 1995).

Material studied

Slovakia, Cerová vrchovina Mts., 2 km SWW of the village of Chrámec (near the town of Rimavská Sobota), alt. 250 m, on abandoned pasture, on sandy soil, among herbaceous plants, on roots of *Ononis spinosa*, 27 Oct 2002, leg. K. Skokanová (SAV F-1319). Ibid., 27 Oct 2004, leg. S. Adamčík (SAV F-1318).

Hungary, Pilis Mts., prope Budakalász, in pascuo, 1 Nov 1966, leg. M. Babos, G. Bohus, E. Véssey (M 0065416 as *Flammulina velutipes* var. *pratensis*).

ACKNOWLEDGEMENTS

Anna Ronikier (Institute of Botany PAS, Krakow, Poland) is acknowledged for reading and commenting on the manuscript. Thanks also go to Natália Yehorová (Institute of Botany SAS, Bratislava, Slovakia) for her advisory note on the habitat of our collections. The Slovak Grant Agency VEGA (grants no. 2/7071/7 and 2/5087/25) supported this study.

REFERENCES

ANONYMUS 1 (on-line): La modernisation des zones naturelles d'intérêt ecologique, faunistique et floristique en région Nord Pas-de-Calais. – http://www.nord-pas-de-calais.ecologie.gouv.fr/IMG/pdf/informations_generales-2.pdf. [accessed 30 Apr 2008].

ANONYMUS 2 (on-line): *Flammulina ononidis* Arnolds. – http://www.pharmanatur.com/Mycologie/Flammulina%20ononidis.htm. [accessed 30 Apr 2008].

ANTONÍN V. (2006): *Flammulina ononidis* Arnolds. – In: Holec J. and Beran M. (eds.), Červený seznam hub (makromycetů) České republiky, Příroda, Praha, 24: 116.

ARNOLDS E. (1977): Einige Pilze eines Halbtrockenrasen bei Detmold (Westfalen). – Westfäl. Pilzbriefe 11: 29–39.

BABOS M. (1968): Adatok Magyarország ritka kalaposgombáinak és pöfetegféléinek ismeretéhez III. A budakalászi legelögombái. – Fragm. Bot. 6: 23–40.

BAS C. (1983): *Flammulina* in western Europe. – Persoonia 12: 51–66.

BAS C. (1995): Genus *Flammulina* P. Karst. – In: Bas C., Kuyper Th. W., Noordeloos M. E. and Vellinga E. C. (eds.), Flora agaricina neerlandica, Vol. 3, p. 170–173, Rotterdam.

BENKERT D., DÖRFELT H., HARDTKE H. J., HIRSCH G., KREISEL H., KRIEGLSTEINER G. J., LÜDERITZ M., RUNGE A., SCHMIDT H., SCHMITT J. A., WINTERHOFF W., WÖLDECKE K. and ZEHFUSS H. D. (1996): Rote Liste der Großpilze Deutschlands. – In: Ludwig G. and Schnittler M. (eds.), Rote Listen gefährdeter Pflanzen Deutschlands, Schriftenreihe für Vegetationskunde 28, p. 377–426, Bonn.

BIBER J. (1986): Další krok v ochraně vzácných a ohrožených druhů hub. – Mykol. Listy no. 23: 12–13.

CETTO B. (1994): I funghi dal vero, Vol. 6. – 722 p. Trento.

CHRTKOVÁ A. and JASIČOVÁ M. (1988): *Ononis spinosa* L. – In: Bertová L. (ed.), Flóra Slovenska IV/4, p. 246–247, Bratislava.

HAGARA L., ANTONÍN V. and BAIER J. (2005): Veľký atlas húb. – 432 p. Praha.

HOLEC J. and BERAN M., eds. (2006): Červený seznam hub (makromycetů) České republiky. – Příroda, Praha, 24: 1–282.

HOLMGREN P. K., HOLMGREN N. H. and BARNETT L. C., eds. (1990): Index Herbariorum 1: The herbaria of the world. – 693 p. Bronx, New York.

HUGHES K. W., MCGHEE L. L., METHVEN A. S., JOHNSON J. E. and PETERSEN R. H. (1999): Patterns of geographic speciation in the genus *Flammulina* based on sequences of the ribosomal ITS-5.8S-ITS2 area. – Mycologia 91(6): 978–986.

KLÁN J. (1978): *Flammulina ononidis* Arnolds, ein besonderer Steppen-Samtfussrübling in der Tschechoslowakei. – Česká Mykol. 32: 205–214.

KORNERUP A. and WANSCHER J. H. (1978): Methuen handbook of colour, 5th ed. – 248 p. London.

KOTLABA F., ed. (1995): Červená kniha ohrozených a vzácnych druhov rastlín a živočíchov SR a ČR. Vol. 4. Sinice a riasy, huby, lišajníky, machorasty. – 224 p. Bratislava.

KRIEGLSTEINER G. J. (1974): Der Hauheckel-Rübling, *Flammulina ononidis* Arnolds 1977 auch in Süddeutschland. – Südw. Pilzrundsch. 14: 1–7.

KRISAI-GREILHUBER I. (1999): Rote Liste gefährdeter Großpilze Österreichs. – In: Niklfeld H. (ed.), Rote Listen gefährdeter Pflanzen Österreichs, Bundesministerium für Umwelt, Jugend und Familie, p. 229–266, Graz.

KŘÍŽ M. (on-line): Plamienka ihlicová (*Flammulina ononidis* Arnolds). – http://www.nahuby.sk/obrazok_detail.php?obrazok_id=30800&poradie=1. [accessed 30 Apr 2008].

METHVEN A., HUGHES K. W. and PETERSEN R. H. (2000): *Flammulina* RFLP patterns identify species and show biogeographical patterns within species. – Mycologia 92(6): 1064–1070.

PÉREZ-BUTRÓN J. L. and FERNÁNDEZ-VICENTE J. (2007): Una nueva especie de *Flammulina* P. Karsten, *F. cephalariae* (*Agaricales*) encontrada en España. – Revista Catalana de Micologia 29: 81–91.

PEKŞEN A. and KARACA G. (2003): Macrofungi of Samsun Province. – Turkish J. Bot. 27: 173–184.

PETERSEN R. H., HUGHES K. W. and REDHEAD S. A. (on-line): The genus *Flammulina*, a Tennessee tutorial. – http://www.bio.utk.edu/mycology/Flammulina/default.html [accessed 28 Jul 2008].

PETERSEN R. H., HUGHES K. W., REDHEAD S. A., PSURTSEVA N. and METHVEN A. S. (1999): Mating system in the *Xerulaceae* (*Agaricales*, Basidiomycotina): *Flammulina*. – Mycoscience 40: 411–426.

PETERSEN J. H. and VESTERHOLT J. (2003): De danske svampenavne – en kommenteret navneliste. – 76 p. Ebeltoft. – http://www.mycokey.com/MycokeyDK/DKkeysPDFs/DeDanskeSvampenavne.pdf [accessed 30 Apr 2008].

REDHEAD S. A. and PETERSEN R. H. (1999): New species, varieties and combinations in the genus *Flammulina*. – Mycotaxon 71: 285–294.

TKALČEC Z., MEŠIĆ A. and MATOČEC N. (2005): Crveni popis gljiva HR. – http://www.dzzp.hr/publikacije/Crveni%20popis%20gljive.pdf [accessed 30 Apr 2008].

URBONAS V., KALAMEES K. and LUKIN V. (1986): Conspectus florum agaricalium fungorum (*Agaricales* s. l.) Lithuaniae, Latviae et Estoniae (Materies 1778–1984 annorum). – 138 p. Vilnius.

WALLEYN R. and VANDEVEN E., eds. (2006): Standaardlijst van Basidiomycota en Myxomycota van Vlaanderen en het Brussels Gewest. – 143 p. Brussel. www.inbo.be/docupload/3062.pdf [accessed 30 Apr 2008].