

## ***Clitocybula familia* (Fungi, Agaricales) – taxonomy, distribution, ecology and first records in the Czech Republic and Slovakia**

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The first records of *Clitocybula familia* from the Czech and Slovak Republics were studied and compared with the type specimen of *C. familia* var. *compressa* and several North-American collections of var. *familia* using macro- and microscopic characters as well as molecular data (LSU rDNA). All the collections were identical. Therefore, there is no need to recognise var. *familia* and var. *compressa*, as they represent the same taxon. Detailed macro- and microscopic descriptions are given. In Europe, the species grows on strongly decayed fallen trunks of *Abies alba* and *Picea abies*. All four Central-European localities represent valuable old-growth forests. Therefore, this species may be a bioindicator of such environments.

**Key words:** var. *familia*, var. *compressa*, DNA studies, Europe, North America, old-growth forests, bioindication.

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První nálezy druhu *Clitocybula familia* z České republiky a Slovenska byly srovnány s typovou položkou *C. familia* var. *compressa* a několika severoamerickými sběry typové var. *familia*. Všechny jsou shodné jak co se týká makroskopických, tak i mikroskopických znaků a struktury DNA (LSU). Autoři proto považují var. *familia* a var. *compressa* za identické. Je sestaven podrobný makroskopický a mikroskopický popis druhu. *Clitocybula familia* roste ve střední Evropě na silně zetlelých padlých kmenech jedle a smrku. Všechny čtyři lokality představují přirozené lesy; je tedy diskutováno možné zařazení *C. familia* mezi bioindikační druhy těchto porostů.

## INTRODUCTION

The genus *Clitocybula* represents a small genus with c. 10 taxa in Europe and North America (Barrasa et al. 2006). Two species, *C. lacerata* (Lasch) Singer and *C. abundans* (Peck) Singer occur scattered on decaying wood of conifers in old-growth forests in Central Europe. During field excursions to such habitats, a third European wood-inhabiting taxon, *Clitocybula familia* (Peck) Singer, has been collected in the Czech Republic and Slovakia, representing the first records for both countries.

## MATERIALS AND METHODS

The macroscopic description is based on fresh basidiocarps, microscopic features are described from dried material mounted in H<sub>2</sub>O, 5 % KOH solution, Melzer's reagent and Congo Red. For basidiospores, the factors E (quotient of length and width in any one spore) and Q (mean of E-values) are used. In any collection, 20 spores and 5–20 basidia were measured. For micromorphological terminology, see Bas et al. (1988). Authors of fungal names are cited according to the International Plant Names Index Authors website (<http://www.ipni.org/ipni/authorsearchpage.do>), colour abbreviations follow Kornerup and Wanscher (1983).

The degree of naturalness of forest stands is described using the terms virgin forest, natural forest and near-natural forest (falling under so-called old-growth forests), man-influenced forest and man-made forest (for detailed explanation, see Holec 2008).

Voucher specimens are deposited in the following herbaria: BRNM (Moravian Museum, Dept. of Botany, Brno), BRNU (Masaryk University, Faculty of Sciences, Department of Botany and Zoology, Brno), CB (Museum of South Bohemia, České Budějovice), and PRM (National Museum, Mycological Department, Prague). Six North-American specimens of *Clitocybula familia* from the NY herbarium (New York Botanical Garden, Bronx, USA) have also been studied.

To get insight into the taxonomic position of the fungus under study, nuclear DNA was extracted from selected samples (Tab. 1). A small piece of a dried basidiocarp was extracted using the NucleoSpin<sup>®</sup> Plant II extraction kit (Macherey-Nagel) according to the manufacturer's instructions. The rDNA regions encoding a partial sequence of the 28S rDNA gene, the complete sequence of the 5.8S rDNA gene and the cognate ITS1 and ITS2 regions were amplified by the polymerase chain reaction (PCR) using primer pairs and the PCR regime as described in Borovička et al. (2011). In the case of the old American herbarium collections, semi-nested PCR was used with the primer pairs LROR-NL4 and NL1-NL4 (LROR primer sequence: 5-ACCCGCTGAACTTAAGC-3). The obtained amplicons were purified with isopropanol and both strands were sequenced at

Macrogen Inc., Korea. The DNA sequences were edited using the biological sequence alignment editor BioEdit (Hall 1999) and aligned using the ClustalW Multiple Alignment tool.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura 1980). The tree with the highest log likelihood (−1983.5901) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically as follows. If the number of common sites was < 100 or less than one fourth of the total number of sites, the maximum parsimony method was used; otherwise the BIONJ method with MCL distance matrix was used. A discrete Gamma distribution was used to model evolutionary rate differences between sites [5 categories (+G, parameter = 0.3871)]. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 18 nucleotide sequences. There were a total of 589 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2007). *Baeospora myosura* (GenBank accession number AY207146) was used as outgroup.

**Tab. 1.** *Clitocybula familia* samples selected for molecular study and GenBank accession numbers of their sequences.

Sample ID	Origin	Specimen	LSU	ITS1-5.8S-ITS2
X 02	Czech Rep.	PRM 921866	JF730320	JF730327
X 13	Slovakia	BRNM 736053	JF730323	JF730328
X 14	Slovak Rep.	BRNM 736052	JF730321	–
X 15	Czech Rep.	BRNU 618420	JF730322	–
X 21	USA	NY, Bigelow 16061	JF730325	–
X 22	USA	NY, Bigelow 16757	JF730326	–

## RESULTS

### *Clitocybula familia* (Peck) Singer

- ≡ *Agaricus familia* Peck, Ann. Rep. N. Y. State Mus. Natur. Hist. 23: 79, 1873.
- ≡ *Collybia familia* (Peck) Sacc., Syll. Fung. 5: 241, 1887. ≡ *Gymnopus familia* (Peck) Murrill, North Amer. Fl. 9(5): 365, 1916. ≡ *Baeospora familia* (Peck) Singer, Rev. Mycol. 3: 193, 1938. ≡ *Clitocybula familia* (Peck) Singer, Sydowia 8(1–6): 110, 1954.
- = *Collybia familia* var. *compressa* Romagn., Collect. Bot. 7(2), 58: 1090, 1968.
- ≡ *Clitocybula familia* var. *compressa* (Romagn.) H. E. Bigelow, Mycologia 65(5): 1102, 1973.

Description of collected basidiocarps. Basidiocarps in large dense clusters (often of several dozen basidiocarps). Pileus 15–40 mm broad, convex or obtusely conical when young, then broadly conical, and plano-convex to finally applanate with undulate margin, centre plane to slightly depressed usually with low broad umbo, margin inflexed, soon straight, hygrophanous, slightly translucently striate only at margin when young, finely radially rugulose, slightly radially innately fibrillose or smooth, slightly slippery, sometimes slightly pruinose at centre, beige-brown (5B–C3, 6D–E4) with olivaceous grey tinge when fresh, paler up to pale brownish grey (5B2–C3) or pale beige-grey to whitish (especially at margin) when dried up. Lamellae moderately close, L = 32–40, l = 2–4, emarginate with tooth, distinctly sinuate when old, up to 7 mm high, intervenose especially when old, white when young, pale cream when old, with concolorous, ± finely pubescent edge. Stipe 30–90(150) × 2–4 mm, cylindrical, hollow, often laterally compressed (especially in lower part), slightly broadened at apex, entirely finely pubescent, especially at apex, whitish when young, then brownish grey (6C2, sometimes to beige-grey – 6B2); basal tomentum white to dirty whitish. Context concolorous with the surface of basidiocarps, without distinct smell (very young basidiocarps?) or unpleasant (cucumber-mealy or like rotten cabbage), taste mild to slightly bitterish.

Basidiospores 3.5–5.25(5.5) × 3.5–5.0 µm, average 4.39 × 3.98 µm, E = 1.00–1.43, Q = 1.09–1.21, globose, subglobose to broadly ellipsoid, thin-walled, amyloid, smooth. Basidia 18–28 × 5.0–7.5 µm, 4-spored, narrowly clavate. Basidioles up to 28 × 3.0–7.0 µm, narrowly clavate to cylindrical. Lamellae edge fertile. Marginal cells sometimes scattered, sometimes ± frequent, (16)21–34(40) × 3.0–7.0 µm, cylindrical, narrowly clavate, regular, irregular, some with irregular, sometimes branched narrow rostrum, thin-walled. Trama hyphae cylindrical to fusoid, thin- to slightly thick-walled, smooth, non-dextrinoid, clamped, up to 25(35) µm wide. Pileipellis a cutis of radially arranged, ± cylindrical, 3–10 µm wide hyphae, with adpressed to suberect, cylindrical or fusoid, less frequently subcylindrical, obtuse, slightly thick-walled, smooth or minutely incrustated, subhyaline or pale greyish, up to 55 µm wide terminal cells. Stipitipellis a cutis of cylindrical, smooth, non-dextrinoid, ± slightly thick-walled, up to 6.0 µm wide cells. Caulocystidia numerous, (22)32–55 × 4.0–12(16) µm, cylindrical, fusoid, clavate, regular, (sub)rostrate or slightly irregular, thin-walled.

Ecology. In the Czech Republic and Slovakia, *C. familia* was mostly collected on decaying fallen stems of *Abies alba*, rarely of *Picea abies*. Most American collections lack detailed information on substrates; in one case (Québec, Cain s.n.), a decayed *Acer* is mentioned as the substrate.

**Studied specimens**

Czech Republic. Novohradské hory Mts., Žofínský prales Nature Reserve, alt. c. 750–800 m, decaying coniferous stem, mixed natural forest, 30 Sept. 2008 leg. V. Antonín and V. Kabát, V.A. 08.251 (BRNM 736054). – Ibid., leg. J. Borovička (PRM 921866). – Ibid., alt. 780 m, 48°40'03" N, 14°42'36" E, montane mixed forest (*Fagus sylvatica*, *Picea abies*, *Abies alba*), strongly decayed lying stem of *Abies alba*, 5 Oct. 2009 leg. J. Novotný, det. M. Beran (CB 16204). – Vsetínské vrchy Mts., SE slopes of Cáb hill, Kutáný Nature Reserve, alt. c. 650–750 m, mixed natural/near-natural forest (*Fagus sylvatica*, *Abies alba*), on lying trunk of *Abies alba*, 11 Oct. 2008 leg. D. Dvořák 645/08 (BRNU 618420).

Canada. Ontario, Long Point, L. Temagami, 7 Sept. 1936 leg. R.F. Cain, det. A.H. Smith (NY). – Québec, St. Adolphe, 2 Sept. 1959 leg. R.F. Cain, det. A.H. Smith (NY).

France. Nay, B.-Pyrénées, 2–4 Nov. 1965 leg. J. Beller (PC, holotype of *Clitocybula familia* var. *compressa*).

Slovak Republic. Kremnické vrchy Mts., Badín, Badínsky prales Nature Reserve, alt. c. 750 m, on decaying fallen stem of *Abies alba*, mixed natural forest (*Abies alba*, *Fagus sylvatica*), 28 Sept. 2009 leg. V. Antonín and J. Lederer, V.A. 09.296 (BRNM 736053). – Ibid., N 48°41.321', E 19°03.293', leg. J. Holec, JH 142/2009 (PRM 899163). – Veporské vrchy Mts., Dobroč, Dobročský prales Nature Reserve, alt. c. 800–900 m, on lying stem of *Abies alba*, mixed natural forest (*Fagus sylvatica*, *Abies alba*, *Picea abies*), 29 Sept. 2009 leg. V. Antonín, V.A. 09.310 (BRNM 736052). – Ibid., N 48°40.827', E 19°40.504', alt. 920 m, on decaying fallen trunk of *Picea abies* (diam. 50 cm) covered with mosses, 28 Sept. 2009 leg. J. Holec, JH 154/2009 (PRM 899174).

U.S.A. Idaho, Idaho Co., Papoose Creek, 7 Devil Mts., 3 Sept. 1954 leg. et det. H.E. Bigelow 1940 (NY). – Maine, Franklin Co., Flagstaff Lake Road, Carrabassett, 24 Sept. 1971 leg. et det. H.E. Bigelow 16757 (NY). – Michigan, Luce Co., Tahquamenon, 12 Sept. 1969 leg. W. Habermehl, det. H.E. Bigelow 16061 (NY).

**Anatomic, morphological and molecular characters**

Detailed studies of macroscopic structures showed that all collections studied were fully identical (Tab. 2).

**Tab. 2.** Variability of basidiospore size and basidium length of studied collections of *Clitocybula familia*.

Specimen	Size of basidiospores (µm)	E	Q	Size of basidia (µm)
PRM 921866	(4.0)4.5–5.25 × 3.75–4.25(5.0)	1.00–1.25	1.13	22–28 × 5.5–7.0
PRM 899163	4.0–4.75 × 3.5–4.0	1.10–1.33	1.17	23–25 × 5.0–6.0
PRM 899174	4.0–4.75 × 3.5–4.0(4.75)	1.00–1.25	1.12	22–25 × 5.0–6.0
BRNM 736052	(4.0)4.5–5.0 × 3.5–4.5(4.75)	1.00–1.31	1.12	18–29 × 5.0–6.0
BRNM 736053	(3.5)4.0–5.0 × (3.0)3.5–4.5	1.00–1.43	1.21	up to 25 × 5.0–7.0
BRNU 618420	(3.75)4.0–4.75 × 3.5–4.5	1.00–1.22	1.10	23–26 × 6.5–7.0
CB 16204	(3.0)3.5–4.0 × (2.5)3.0–3.5(4) µm	1.00–1.33	1.20	(21)23–26(28) × (4.0)4.5–5.5
PC, Beller (holotype of var. <i>compressa</i> )	(3.5)4.0–5.0 × 3.5–5.0	1.00–1.25	1.09	19–25 × 5.0–7.0
NY, Smith s.n.	3.5–4.5 × 3.0–3.75(4.0)	1.06–1.25	1.14	20–26 × 5.0–7.0
NY, Cain s.n.	3.75–5.0(5.25) × 3.5–4.25(5.0)	1.00–1.25	1.12	20–22 × 6.0–7.5
NY, Bigelow 1940	4.0–5.0(5.5) × (3.5)3.75–5.0	1.00–1.25	1.10	21–27 × 6.0–7.0
NY, Bigelow 16757	3.75–5.0 × 3.5–4.5	1.00–1.25	1.09	22–24 × 6.0–6.5
NY, Bigelow 16061	4.0–5.0 × 3.5–4.5(5.0)	1.00–1.14	1.08	18–26 × 6.0–7.0

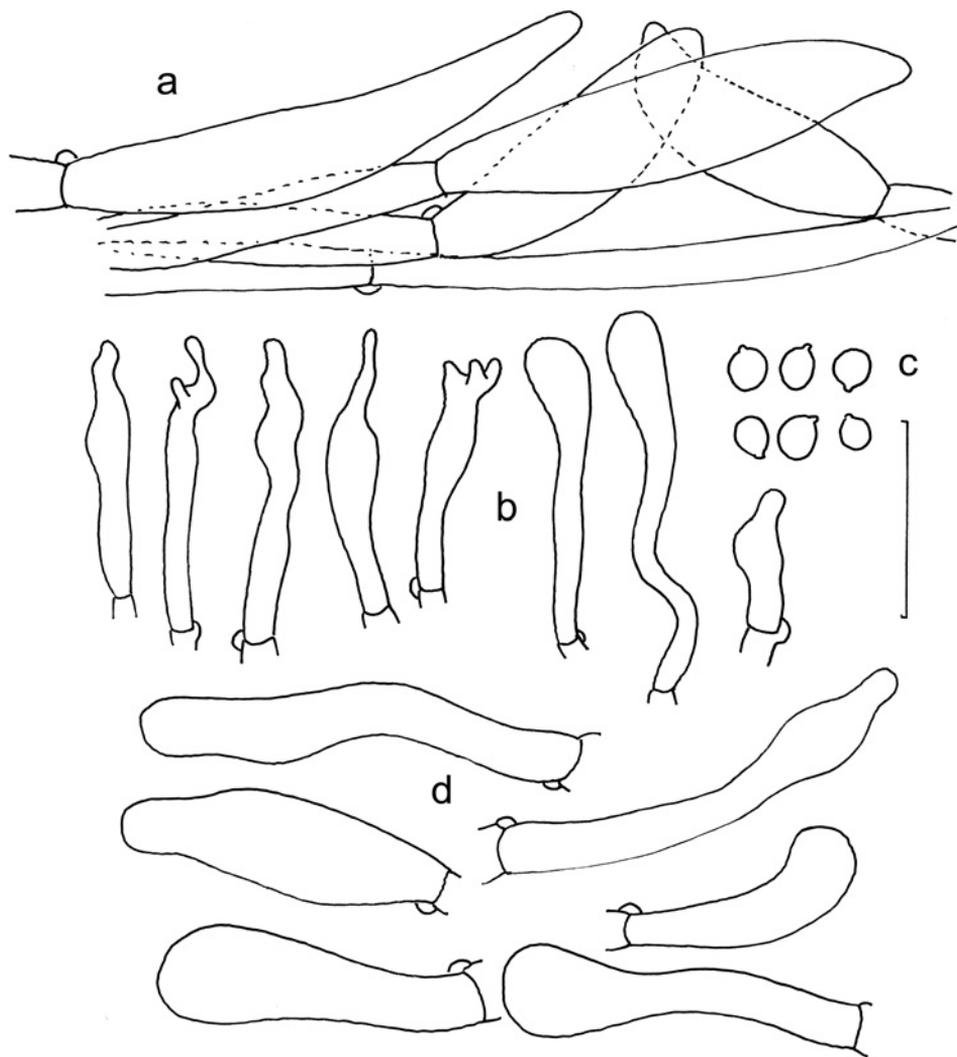
Since our attempts to obtain ITS sequences of most *Clitocybula* samples under study repeatedly failed, we used LSU rDNA data to investigate phylogenetic relationships. The sequences of all *Clitocybula* species under study formed a distinct and well-supported clade. The well-known species *Megacollybia platyphylla* (Pers.) Kotl. et Pouzar also belongs to this clade, and therefore its correct name should be *Clitocybula platyphylla* (Pers.) E. Ludw. Collections of *Clitocybula familia* from Europe and USA formed a distinct and well-supported clade; the American collections differed only in two nucleotide positions (Fig. 2). Only the American collection of *C. familia* (New York, Hamilton Co., Raquette Lake, Long Point, 21 Sept. 1985, Halling 4598) from the NY herbarium was different. It differs both anatomically by slightly larger basidiospores and larger basidia, and also molecularly according to its LSU (data not shown). A detailed study of this specimen and other European *Clitocybula* species will be published separately in a currently prepared paper.

#### DISCUSSION

*Clitocybula familia* is a distinct species differing from other European *Clitocybula* species by growing in larger and denser clusters, having a more or less mycenoid, usually not centrally depressed (or only sometimes when old), smooth or sometimes very slightly innately fibrillose pileus (never distinctly radially fibrillose), and by smaller basidiospores.

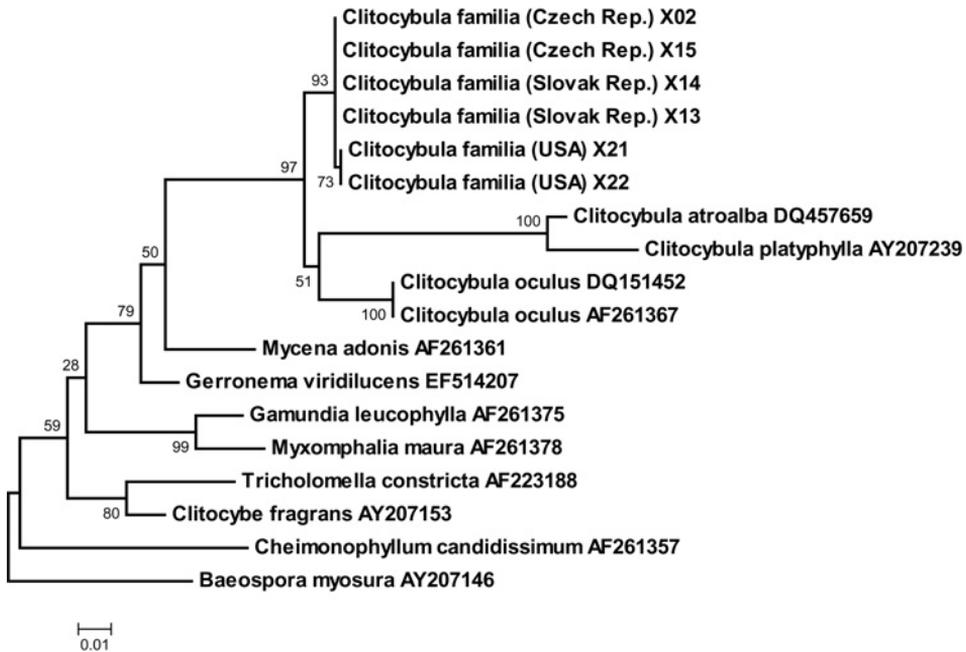
Our collections of *Clitocybula familia* from the Czech Republic and Slovakia agree well with the description by Bigelow (1973) and Lennox (1979), who also revised the type specimen. Bigelow (1973) mentioned slightly smaller basidiospores (3.0–4.0(5.0)  $\mu\text{m}$ ), and neither of them mentioned the presence of marginal cells. According to our observations, sterile marginal cells with transient forms to cheilocystidia are always present on the lamella edges. They can be scattered to rather frequent and are of very variable shape – from indistinct, deformed basidioloid cells to distinct clavate cystidia. Lennox (1979) mentioned the same size of basidiospores (3.5–5.5  $\times$  3.5–4.5  $\mu\text{m}$ ). Basidia are published to be 15–30  $\times$  4.5–5.0  $\mu\text{m}$  (Bigelow 1973) and 15–22  $\times$  4.5–6.0  $\mu\text{m}$  (Lennox 1979) large. Ludwig (2001) described the smell of this fungus as indistinct, sometimes grass-like. Romagnesi (1968) and Ludwig (2001) also mentioned the absence of cheilocystidia (but stated the presence of scattered sterile cells on the lamella edge).

Romagnesi (1968) described *Collybia familia* var. *compressa* from France, which should differ from var. *familia* by several macroscopic characters (pileus colour, less slender basidiocarps, stipe often compressed and flocculose at least at apex). Its formal transfer to *Clitocybula* was made by Bigelow (1973, as *Clitocybula familia* var. *compressa*). However, he did not discuss the difference



**Fig. 1.** *Clitocybula familia*. **a.** pileipellis, **b.** marginal cells, **c.** basidiospores, **d.** caulocystidia. Scale bar = 50  $\mu\text{m}$  for a, 20  $\mu\text{m}$  for b, c, d.

between var. *familia* and var. *compressa*. We compared European and North-American collections according to morphological and anatomical characters and DNA sequences. The collections from both continents were identical.



**Fig. 2.** Phylogenetic placement of *Clitocybula familia* and related *Clitocybula* species inferred from LSU rDNA data. The best tree resulting from the Maximum Likelihood analysis in MEGA5 is presented. Numbers beside the internal nodes are Maximum Likelihood bootstraps. Sequences indicated by X-numbers were obtained during this study, the others were downloaded from the GenBank database (accession numbers are indicated). Tree was rooted using *Baeospora myosura* as outgroup.

In Europe, *Clitocybula familia* was published by Romagnesi (1968, as *Collybia familia* var. *compressa*) from France for the first time. The same find was mentioned in a key by Bon (1978). It has also been found in Sweden (Ludwig 2001, Vesterholt 2008), Austria and Switzerland (Gröger 2006). Thus, our finds represent records from the third and fourth Central–European countries. This species is not included in the key by Horak (2005); however, it is mentioned by Ludwig (2000, 2001) and Moser (1983).

In all four Czech and Slovak localities, *Clitocybula familia* grows on strongly decayed fallen trunks of conifers (*Abies alba* and *Picea abies*). On the other hand, some North American finds are from hardwood. Concerning the habitats of our localities, it grows in natural and near-natural forests (protected as nature reserves). This fact is in concordance with Romagnesi (1968). Four localities are too small number for a final conclusion, but it seems that *Clitocybula familia* is a species of old-growth forests. Consequently, it could be considered an indicator of such forests, at least in Central Europe.



**Fig. 3.** *Clitocybula familia*, typical rich cluster of basidiocarps. Slovakia, Dobročský prales Nature Reserve (PRM 899174). Photo J. Holec.



**Fig. 4.** *Clitocybula familia*, cluster of mature basidiocarps, Czech Republic, Žofínský prales Nature Reserve (PRM 921866). Photo J. Borovička.



**Fig. 5.** *Clitocybula familia*, detail of young basidiocarps. Slovakia, Badínský prales Nature Reserve (PRM 899163). Photo J. Holec.



**Fig. 6.** *Clitocybula familia*, detail of mature basidiocarps. Slovakia, Dobročský prales Nature Reserve (PRM 899174). Photo J. Holec.

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