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First report of *Mycena clavata* (Fungi, *Agaricales*) in the Czech Republic including notes on its taxonomy, phylogenetic position and ecology

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The rare gilled fungus *Mycena clavata* is reported from the Czech Republic for the first time. It was found on large fallen trunks of *Picea abies* in Boubínský prales virgin forest, the best preserved montane old-growth forest in the country. The basidiomata occurred in the lower half of the trunks, either on their upper or lateral sides, on bark covered by mosses, on bare bark, or directly on wood covered by mosses, in wet times of the year. Descriptions and photographs of macro- and microcharacters are provided and data on the distribution and ecology of *M. clavata* are summarised. The species was sequenced for the first time. ITS-LSU rDNA sequences confirmed the species' distinct position and showed that *M. clavata* belongs to a moderately supported clade consisting of various *Mycena* and *Hemimycena* species, some of them recently transferred to the vaguely delimited genus *Phloeomana*.

Key words: Europe, Boubínský prales virgin forest, Bohemian Forest, ITS-LSU rDNA sequences, *Phloeomana*.

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Holec J., Kolařík M. (2017): *Mycena clavata* (Fungi, *Agaricales*) – první nález v České republice a poznámky k její taxonomii, fylogenetickému postavení a ekologii. – Czech Mycol. 69(1): 1–14.

Vzácná lupenatá houba *Mycena clavata* je poprvé publikována z České republiky. Byla nalezena na několika mohutných padlých kmenech smrku v Boubínském pralese, našem nejlépe zachovaném přirozeném lese. Plodnice se vyskytovaly ve spodních částech kmenů, buď na jejich horní nebo boční straně, na mechaté kůře, holé kůře, nebo přímo na mechem porostlém dřevě, ve vlhkých obdobích roku. Článek obsahuje popis makro- a mikroznaků, fotografie plodnic a jsou v něm shrnuty údaje o evropském rozšíření a ekologii *M. clavata*. Druh byl také vůbec poprvé sekvenován. Sekvence ITS-LSU rDNA potvrdily hodnotu *M. clavata* jakožto samostatného druhu a ukázaly, že patří do středně dobře podpořeného kladu obsahujícího různé druhy rodů *Mycena* a *Hemimycena*, z nichž některé – včetně *M. clavata* – byly nedávno přeřazeny do vágně vymezeného nového rodu *Phloeomana*.

INTRODUCTION

Mycena clavata (Peck) Redhead (Redhead 1986) is a rare gilled fungus described from North America as *Omphalia clavata* Peck (Peck 1898). It is characterised by small basidiomata with long decurrent lamellae, non-amyloid, broadly amygdaliform to subglobose spores, fusiform to lageniform cheilocystidia, pileipellis hyphae with some elongated outgrowths, and encrusting pigmentation in lower layers of the pileipellis and pileitrama (Ronikier & Aronsen 2007, Aronsen 2016, Aronsen & Læssøe 2016). Its taxonomy and synonymy were perfectly elaborated by Ronikier & Aronsen (2007). The authors showed that *Mycena clavata* is known from montane and boreal regions of North America (Canada, USA) and Europe (Denmark, France, Switzerland, Norway, Poland). In addition, reliable data on its occurrence are known from Sweden (Emmett et al. 2012), Finland (Bonsdorff et al. 2015), Spain (Pérez-de-Gregorio 2015), Belgium (Aronsen & Læssøe 2016), and Italy (Robich 2016). No sequences of *M. clavata* are available in GenBank.

Recently, *M. clavata* has been transferred to the genus *Phloeomana* Redhead (Redhead 2013). The genus currently comprises 6 species formerly belonging to *Mycena*, mostly to sect. *Hiemales*: *P. alba* (Bres.) Redhead, *P. clavata* (Peck) Redhead, *P. hiemalis* (Osbeck) Redhead, *P. minutula* (Sacc.) Redhead, *P. neospeirea* (Singer) Gminder, and *P. speirea* (Fr.) Redhead (Redhead 2013, 2016a, 2016b, Gminder 2016). Unfortunately, Redhead's and Gminder's contributions contain merely formal combinations, but no discussion on delimitation of *Phloeomana* nor the systematic position of *M. clavata*.

In 2015, the first author repeatedly found *M. clavata* on fallen trunks of *Picea abies* in Boubínský prales virgin forest, Czech Republic. The species is new to both this locality (compare Holec et al. 2015) and the country.

The aims of our paper are:

1. to characterise the first Czech records of *M. clavata* morphologically and molecularly and compare them with the similar taxa *M. speirea* (Fr.) Gillet and *M. hiemalis* (Osbeck) Quél., occurring at the same locality,
2. to show the phylogenetic and systematic position of *M. clavata* using DNA sequences,
3. to summarise and discuss the current knowledge of its ecology and distribution.

Material and methods

Field work. Field data were obtained during a project named "Monitoring of natural forests in the Czech Republic" coordinated by the Silva Tarouca Research Institute for Landscape and Ornamental Gardening (RILOG) and focused on the fungal diversity of the thickest fallen trunks in selected old-growth forests. Thanks to the existence of stem position maps containing habitat/dendrometric data on all standing and fallen trees, it was possible to identify trunks inhabited by *Mycena clavata* (RILOG ID numbers) exactly. The decay stage of the trunks was estimated as follows:

1: wood hard, almost impossible to penetrate with a knife point,
2: wood slightly softened, a knife point penetrates just a few millimetres deep,
3: wood soft, a knife penetrates several centimetres deep, the wood can be impressed with fingers and rather large wood parts can be removed with a knife.

Morphology. Macrocharacters of fresh fruitbodies are described according to fresh material documented with field notes and photographs. Microscopic mounts were made in Congo Red, 5% KOH solution, Melzer's reagent, and studied under an Olympus BX-43 light microscope equipped with Nomarski differential interference contrast. In each collection studied, 20 randomly selected mature spores were measured in 5% KOH. Spore sizes are presented in the form of the main range (5–95 percentile values), complemented with minimal and maximal values in parentheses. For descriptive terminology, see Bas et al. (1988).

Voucher specimens are kept in the PRM herbarium (National Museum, Mycological Department, Prague, Czech Republic).

DNA study. DNA from dried specimens (see Records and studied specimens) was isolated as described by Holec & Kolařík (2013). ITS-LSU rDNA was amplified using primers ITS1F and LR6, and the same primers, together with ITS4 and NL1, were used for sequencing (see Holec & Kolařík 2013 for details). BlastN similarity search identified *M. clavata* as related to *M. hiemalis* and *Hemimycena gracilis* (Quél.) Singer. Thus, species related to these two taxa according to Osmundson et al. (2013) and Matheny et al. (2006) were used for comparison (only sequences of the relevant length and alignable to our data were used). Other sequences were selected based on a BlastN similarity search and subsequent phylogenetic analyses. ITS region sequences were aligned using MAFFT 6 using the Q-INS-i algorithm (Katoh on-line, Katoh & Toh 2008). There were a total of 44 sequences (seven obtained in this study) and 862 positions in the dataset, of which 468 were variable and 88 singletons. Alignment was curated using Gblocks ver. 0.91b (Talavera & Castresana 2007) resulting in 642 positions of which 378 were variable and 60 singletons. Maximum likelihood (ML) and Maximum parsimony (MP) analyses were conducted with 1,000 bootstrap replicates and default

parameters in MEGA 7.0 (Tamura et al. 2011) (Fig. 4). Bayesian searches (MB) were conducted with MrBayes 3.0 (Ronquist & Huelsenbeck 2003) and 1.5 million replicates estimated together with burn-in value in Tracer v. 1.4 (Rambaud & Drummond 2007). The best substitution model (HKY+G) used in ML and MB analyses was determined in MEGA 7.0. *Flammulina velutipes* (Curtis) Singer was chosen as an outgroup, being a taxon standing outside of the lineage of *Hemimycena gracilis* in Matheny at al. (2006).

Sequences obtained in this study have been entered into the EMBL database and their accession numbers are cited below together with their voucher numbers. Other sequences shown in the phylogenetic tree (Fig. 4) were obtained by Matheny et al. (2006) and Osmundson et al. (2013), except for those of *Henningsomyces candidus* (Pers.) Kuntze (obtained by Bodensteiner et al. 2004) and two sequences of *Mycena olida* Bres. (Kim et al. 2015, Telfer et al. 2015). The sequence of *Mycena adonis* (Bull.) Gray comes from an unpublished study by Berube et al.

Abbreviations. alt. – altitude, BB – provisional tree identification number in the Boubínský prales virgin forest, L – number of lamellae reaching the stipe, l – number of lamellulae between a pair of neighbouring lamellae, not. – „notavit", i.e. recorded and identified in the field without collecting a voucher, Q – length/width quotient of each spore measured, Q_{min} – minimum value of Q per collection, Q_{max} – maximum value of Q per collection, Q_{av} – average Q of all spores measured per collection, RILOG ID – trunk identification number in the trunk parameters database stored by the Silva Tarouca Research Institute for Landscape and Ornamental Gardening (RILOG).

Records and studied specimens with sequence accession numbers

For more data on segments in the Boubínský prales National Nature Reserve, see Holec et al. (2015: 163). Sequence accession numbers are in bold.

Mycena clavata

Czech Republic. Bohemia. Šumava Mts., near the village of Zátoň, Boubínský prales National Nature Reserve, fenced core area: segment BP1d, montane virgin forest (*Picea abies*, *Fagus sylvatica*, *Abies alba*), alt. 955 m, *Picea abies*: fallen trunk, RILOG ID 103513 = BB04, decay stage 1, lower half, on bark, 2 Jun 2015 leg. & det. J. Holec, JH 30/2015 (PRM 935279). – Ibid., segment BP1d, alt. 960 m, *Picea abies*: fallen trunk, RILOG ID 103272 = BB05, decay stage 2, lower half, on mossy bark, 2 Jun 2015 leg. & det. J. Holec, JH 34/2015 (PRM 935283; sequence no. **LT671449**). – Ibid., segment BP1e, alt. 1035 m, *Picea abies*: fallen trunk, RILOG ID 104175 = BB14, decay stage 1, lower half, on mossy bark, 3 Jun 2015 not. J. Holec. – Ibid., segment BP1d, alt. 1000 m, *Picea abies*: fallen trunk, RILOG ID 115583 = BB19, decay stage 1, lower half, on mossy bark, 3 Jun 2015 not. J. Holec. – Ibid., segment BP1d, alt. 980 m, *Picea abies*: fallen trunk, RILOG ID 105479 = BB18, decay stage 2, lower half, on mossy bark, 3 Jun 2015 leg. & det. J. Holec, JH 95/2015 (PRM 935333; sequence no. **LT671448**). – Ibid., segment BP1b, alt. 940 m, *Picea abies*: fallen trunk, RILOG ID 102950 = BB02, decay stage 2, lower half, on bark, 26 Sep 2015 not. J. Holec. – Ibid., segment BP1d, alt. 980 m, *Picea abies*: fallen trunk, RILOG ID 105479 = BB18, decay stage 2, lower half, on bark, 28 Sep 2015 leg. &

det. J. Holec, JH 375/2015 (PRM 935560; sequence no. **LT671447**). – Ibid., segment BP1d, alt. 960 m, *Picea abies*: fallen trunk, RILOG ID 103384 = BB06, decay stage 3, lower half, on mossy wood, 5 Nov 2015 not. J. Holec.

Mycena speirea

Czech Republic. Bohemia. České Švýcarsko (Bohemian Switzerland) National Park, c. 3.4 km NNE of Hotel Lípa, village of Vysoká Lípa, Zlé díry sandstone gorge, near-natural mixed forest (*Fagus*, *Picea*, *Acer pseudoplatanus*), alt. 340 m, *Fagus sylvatica*: decayed fallen trunk covered with mosses, 21 Sep 2010 leg. & det. J. Holec, JH 85/2010 (PRM 922296; sequence no. **LT671445**). – České Švýcarsko National Park, ca. 2.8 km SEE of the church in the village of Růžová, Růžovský vrch hill ("Růžák"), near-natural *Fagus* forest with admixed *Betula* and *Picea*, alt. 400 m, *Fagus sylvatica*: fallen trunk covered with mosses, 7 Nov 2012 leg. L. Edrová & J. Holec, det. J. Holec, JH 200/2012 (PRM 860810; sequence no. **LT671446**). – Šumava Mts., near the village of Zátoň, Boubínský prales National Nature Reserve, fenced core area: segment BP1d, montane virgin forest (*Picea abies*, *Fagus sylvatica*, *Abies alba*), alt. 990 m, *Picea abies*: fallen trunk, RILOG ID 106809 = BB20, decay stage 2: starting decay, trunk exterior slightly softened, 28 Sep 2015 leg. & det. J. Holec, JH 360/2015 (PRM 935546; sequence no. **LT671444**).

Mycena hiemalis

Czech Republic. Bohemia. České Středohoří Protected Landscape Area, Korozluky, Jánský vrch National Nature Monument, on bark chip of a broadleaved tree lying in detritus, 3 Sep 2012 leg. & det. M. Kříž (PRM 923718). – Šumava Mts., near the village of Zátoň, Boubínský prales National Nature Reserve, segment BP2a, natural montane forest (*Fagus sylvatica*, *Picea abies*, *Abies alba*), alt. 1050 m, *Fagus sylvatica*: on mossy bark of decaying fallen trunk, 4 Sep 2013 leg. & det. J. Holec, JH 185/2013 (PRM 922923; sequence no. **LT671450**).

RESULTS

Mycena clavata (Peck) Redhead, Mycologia 78: 523, 1986 Figs. 1–3

≡ *Omphalia clavata* Peck, Ann. Rep. New York State Mus. 51: 285, 1898
≡ *Phloeomana clavata* (Peck) Redhead, Index Fungorum no. 291: 1, 2016
= *Mycena phaeophylla* Kühner, Le genre *Mycena*: 590, 1938
= *Mycena thujina* A.H. Sm., North American species of *Mycena*: 361, 1947

Macrocharacters. Basidiomata growing solitarily or in small groups. Pileus 4–12 mm broad, thin, membranous, hemisphaerical to paraboloid, later convex with slightly flattened upper part, at first slightly, then distinctly radially grooved in outer part, margin crenate and slightly overlapping, colour brown to grey-brown at centre, pale brown-ochre to whitish-ochre with olive tinge towards the margin, surface dry, matt, finely silvery pruinose („givré"). Lamellae sparse, L = 14–17, l = 1–3, subdecurrent when young, later decurrent to deeply decurrent, beige greyish whitish, paler towards the edge, edge even. Stipe 15–25 × 1–1.5 mm, cylindrical, often curved, same colour as pileus, paler towards apex, finely silvery pruinose, distinctly whitish hairy to strigose in basal part.

Microcharacters. Basidiospores variable in size and shape, smooth, hyaline, non-amyloid, non-dextrinoid, with small but distinct hilar appendix, 7.0–10.0 × (5.0)5.5–7.5(8.0) µm, mostly subglobose to broadly ellipsoid, but also broadly ovoid to broadly amygdaliform with obtuse apex (see Bas et al. 1988: p. 59, fig. 18), rarely with slightly conical apex, some spores flat at adaxial side in side view or rarely with a slight suprahilar depression, mature ones with large refractive droplet, immature ones (with granular content) more prolonged, almost ellipsoid. Basidia 22–30 × 6–8 µm, narrowly clavate, 2-spored, rarely 1-spored, clampless, sterigmata 4–6 µm long. Cheilocystidia forming a sterile band, 35–50 × 6–10 µm, cylindrical fusiform to narrowly lageniform, flexuose, hyaline. Pleurocystidia absent. Pileus cuticle a cutis of hyaline hyphae densely covered with small unbranched outgrowths measuring 2–4 × 1–2 µm and scarce longer outgrowths 10–30 × 2–4 µm („pileocystidium-like hairs"), mostly unbranched, flexuose, rarely branched at apex, hyphae of lower cuticle layers distinctly brown encrusted. Stipe cuticle consisting of densely arranged parallel hyphae covered with a net or tufts of hyphae 4–6 µm broad, flexuose, partly moniliform, some of them perpendicularly projecting („caulocystidium-like hairs"), smooth or with sparse short outgrowths, in places covered with encrusting pigment. Clamp connections not observed. No parts of fruitbody amyloid or dextrinoid.

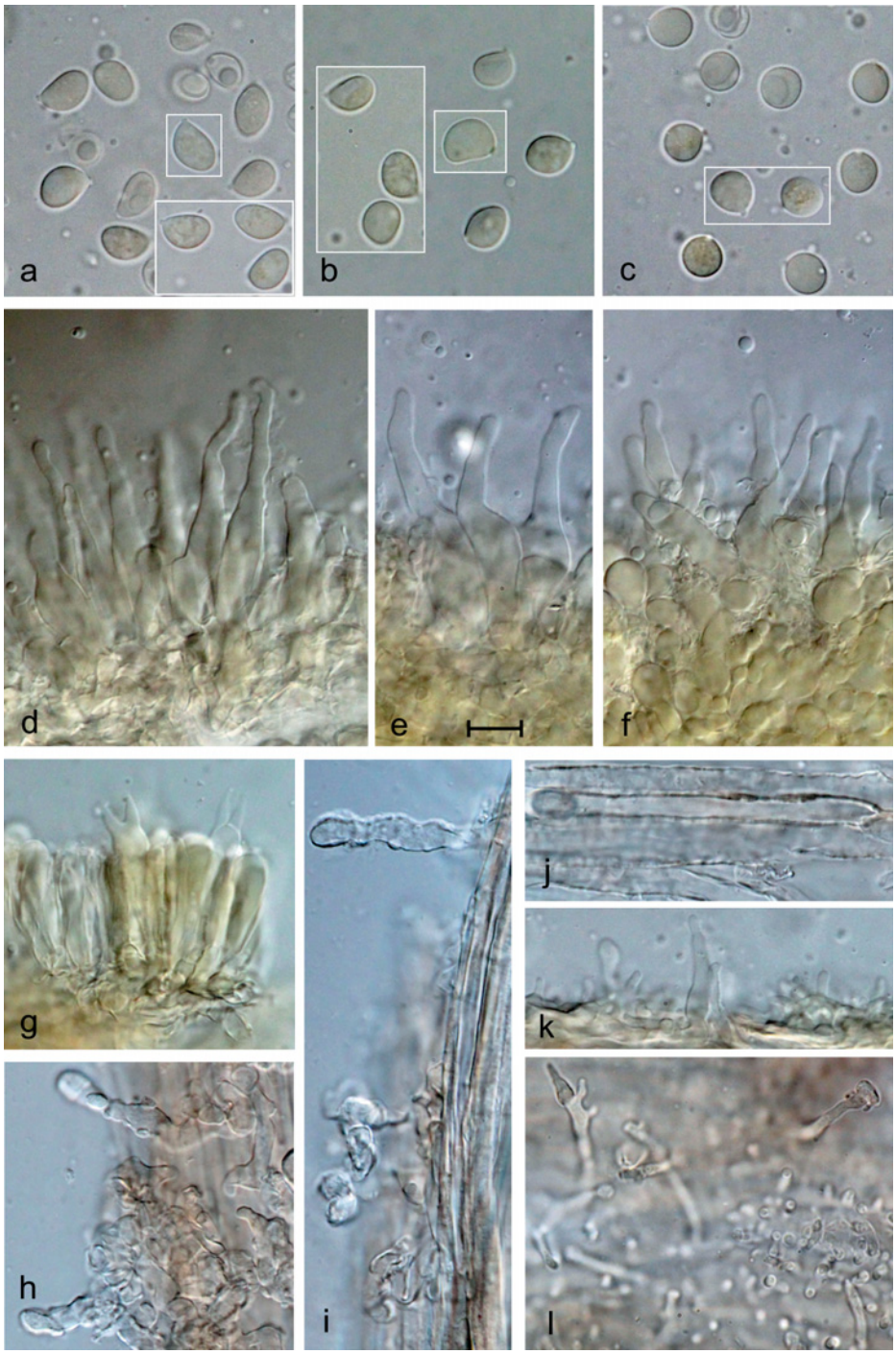
Tab. 1. Comparison of spore size values.

Voucher	Spore length (µm)	Spore width (µm)	Q_{min}	Q_{max}	Q_{av}
PRM 935279	7.0–8.0	(5.0)5.5–6.0	1.17	1.60	1.34
PRM 935283	8.0–10.0	6.0–6.5(7.0)	1.31	1.58	1.44
PRM 935333	(7.5)8.0–9.0(10.0)	6.0–7.5(8.0)	1.13	1.58	1.28
PRM 935560	(7.0)7.5–8.0(9.0)	(6.0)6.5–7.0	1.07	1.29	1.16

DNA sequences. *Mycena clavata* ITS rDNA sequences (638 bp) showed five variable positions. The most similar sequences deposited in NCBI GenBank and identified using BlastN belonged to *Mycena hiemalis* (92%, JF908445, Osmundson et al. 2013) for the ITS region and *Mycena amabilissima* Peck (97%, DQ490644, Matheny et al. 2006) for the LSU region. Phylogenetic analysis placed our *M. clavata* specimens in a single, fully supported clade (Fig. 4). The species belongs to a moderately supported clade (1.00/69/100) which consists of various *Mycena* and *Hemimycena* species. Among them, *Mycena speirea* forms a well-supported clade with *M. maurella* Robich and *M. oregonensis* A.H. Sm. Another well-supported lineage consists of *M. olida* Bres., *M. alba* (Bres.) Kühner (incl. *M. capillaripes* Peck) and *M. hiemalis*. Phylogenic analysis did not separate *M. hiemalis* from *M. alba*. Two other species, *Hemimycena gracilis* and *Mycena leptophylla* (Peck) Sacc., formed separate lineages inside the discussed clade.

Fig. 1. *Mycena clavata*, Boubínský prales virgin forest, Czech Republic (PRM 935333; for details, see Records and studied specimens). Photo J. Holec.

Fig. 2. *Mycena clavata*, Boubínský prales virgin forest, Czech Republic (PRM 935283; for details, see Records and studied specimens). Photo J. Holec.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The closest relatives of this entire group belong to members of the Hydropoid clade sensu Matheny et al. (2006), but it is not clear which of the identified lineages (e.g. lineage of *Clitocybula* with *Megacollybia*, lineage of *Henningsomyces*, lineage of *Mycenella*, and lineage of *Mycena dura* Maas Geest. & Hauskn.) are the most related due to the low statistical support.

E c o l o g y. In Boubínský prales virgin forest (Czech Republic, Šumava Mts. = Bohemian Forest), *M. clavata* was repeatedly found on large fallen trunks (diameter measured at breast height 100–130 cm, length 34–52 m; data taken from RILOG database) of *Picea abies*, mostly in initial stages of decay (1–2, only once in stage 3), at altitudes of 955–1035 m above sea level. In all cases the basidiomata occurred in the lower half of the trunks, either on their upper or lateral sides, four times on bark covered by mosses, three times on bare bark, once directly on wood covered by mosses. They were observed in early June, late September and early November, i.e. in wet parts of the year. The trunks where *M. clavata* was found were also inspected in August 2015 after a period of hot and dry weather but no basidiomata were observed. All records are from the strictly protected core area of Boubínský prales National Nature Reserve, which is covered by old-growth montane *Picea-Fagus-Abies* forest never cut nor managed by foresters, i.e. representing a true virgin forest with an unusually high amount of living and dead wood in all age classes and stages of decay.

Discussion

Taxonomy, synonymy, similar species

The macrocharacters of our records well agree with detailed descriptions of *Mycena clavata* (Ronikier & Aronsen 2007, Aronsen 2016, Aronsen & Læssøe 2016). Our basidiomata have rather short stipes (15–25 mm), whereas the cited authors mention stipe lengths of up to 80 mm. Concerning microcharacters, in all collections studied by us only 2-spored and rarely 1-spored basidia are present. The spores are highly variable in size and shape but fall exactly within the range given for spores from 2-spored basidia by Emmett et al. (2012: 7–10 × 5–8 µm). The spores from 4-spored basidia should be smaller (Emmett et al. 2012: 6–9 ×

◀ **Fig. 3.** *Mycena clavata*, microcharacters. **a–c** – basidiospores (spores bordered by a thin white line are from other places of the same microscopic mount); **d–f** – cheilocystidia; **g** – basidia and basidiolae; **h, i** – stipe cuticle with caulocystidium-like hairs; **j** – brown encrusted hyphae from lower layer of pileus cuticle; **k, l** – pileus cuticle hyphae covered by a combination of small and longer outgrowths. Scale bar for all figures = 10 µm. Vouchers: PRM 935283 (a, l), PRM 935333 (b, d, g, h, i, j, k), PRM 935560 (c, e, f). Photo J. Holec.

5–7 µm). We observed two types of spores: fully mature ones, typical by their more rounded shape (subglobose to broadly ellipsoid), slightly larger size, and presence of 1(–3) refractive droplets; and (probably immature) ones of a more prolonged shape, with a thinner wall and granular content lacking the droplets. This fact is partly responsible for the rather high spore size variation within one collection, however, there are also differences in size and shape between the collections (not overlapping size and different Q_{av}, see Tab. 1). The average Q value of our collections ranges from 1.16 to 1.44, which is in accordance with values given by Emmett et al. (2012: 1.2–1.5). The average Q of all spores measured by us is 1.31, which perfectly agrees with the Q_{av} of 1.3 given by Aronsen (2016) as a diagnostic character of *M. clavata* compared to *M. speirea*. We did not observe cheilocystidia with branched necks and clavate cheilocystidia with excrescences as reported by Ronikier & Aronsen (2007) and Aronsen (2016). Elborne & Læssøe (1982) noticed that spores of *M. clavata* appear to be covered by small holes, i.e. not smooth. This fact was confirmed by SEM photographs by Ronikier & Aronsen (2007), showing numerous small depressions on the spore surface. We were able to see spots of different thickness and colour (especially when focusing on spore outlines, but also on the spore surface under Nomarski differential interference contrast; Fig. 3c) looking like lunar craters.

In Boubínský prales virgin forest, we frequently observed and studied *Mycena speirea* (Fr.) Gillet, a species most similar to *M. clavata*. We can confirm the key distinguishing characters of *M. clavata* stressed by Ronikier & Aronsen (2007) and Aronsen (2016): subglobose to broadly ellipsoid spores with a Q_{av} of ca. 1.3 (*M. speirea*: ellipsoid to amygdaliform, Q_{av} about 1.9); cylindrical fusiform to lageniform cheilocystidia (*M. speirea*: subcylindrical), pileipellis hyphae densely covered with short outgrowths and sparse long hairs (*M. speirea*: outgrowths not so dense and shorter), and encrusting pigment in lower layers of the pileipellis (*M. speirea*: intracellular). We can add another distinguishing character: the obtusely conical apex of some spores (which makes such spores very striking), which is typical of all collections seen by us. In our opinion, *M. clavata* is distinguishable from *M. speirea* already in the field by its broader, more grooved pileus and less crowded and much more deeply decurrent lamellae. The strigose stipe base is also characteristic.

In our opinion, *M. clavata* sensu Robich (2016) is not very characteristic – the depicted basidiomata are too tiny, long and narrow, the spore shape is atypical (globose to subglobose only, absence of a broadly conical apex) and the cheilocystidium neck is unusually moniliform.

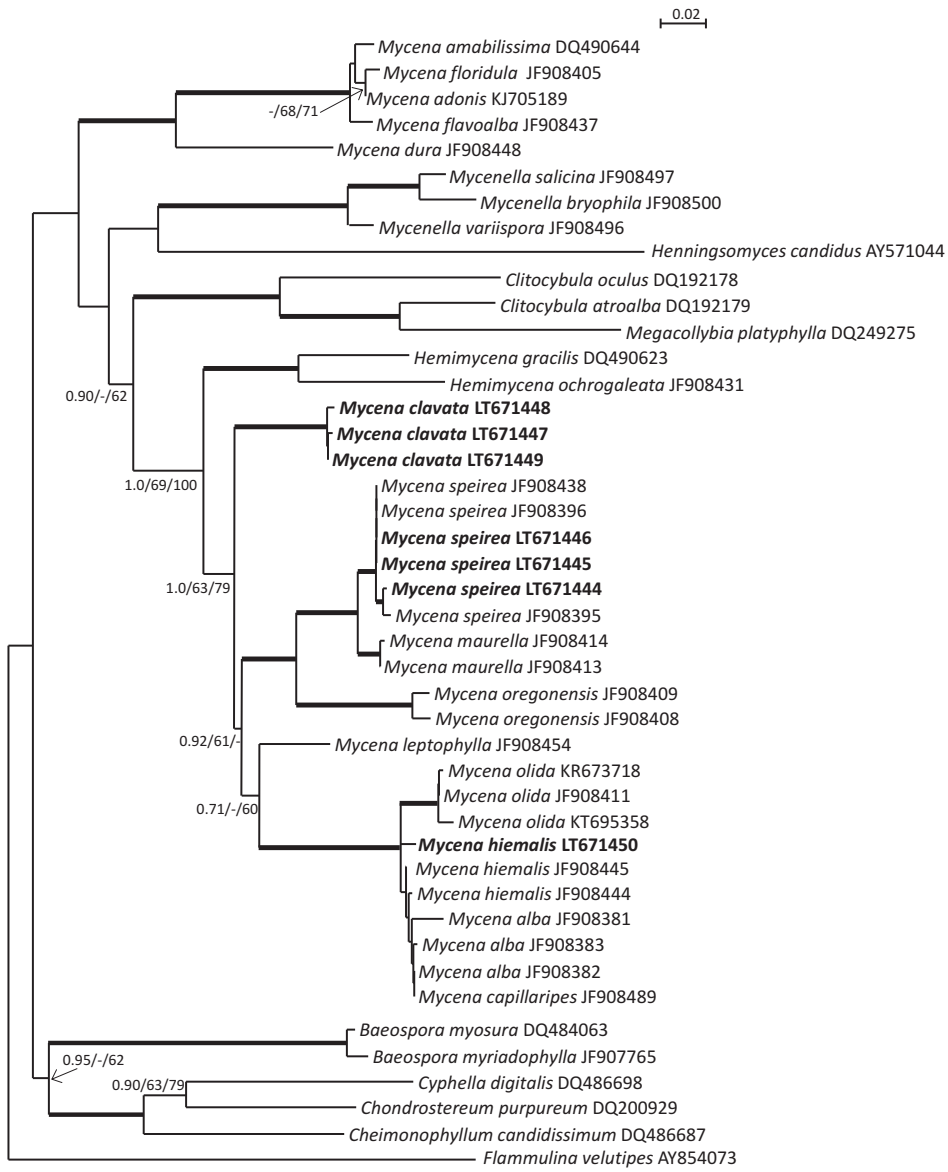

Fig. 4. Evolutionary relationships of studied *Mycena* and related taxa collections, reconstructed using Bayesian inference. Bootstrap values (≥ 60) from Maximum parsimony and Maximum likelihood analyses are showed after Bayesian posterior probabilities (≥ 0.7). Sequences of collections printed in bold were obtained during this study. Thick branches are fully supported by all methods used (1.00/100/100). *Flammulina velutipes* was used as an outgroup.

Phylogeny

Our molecular study confirmed the species' distinct position of *Mycena clavata*. It also showed the phylogenetic position of *M. clavata* and some other (not all) species of *Phloeomana* (Fig. 4), a genus created by using phenotypic data only (Redhead 2013). We showed that these species (including *P. speirea*, type species of *Phloeomana*) belong to a moderately supported clade consisting of various *Mycena* and *Hemimycena* species. This suggests that the current concept of *Phloeomana* (Redhead 2013, 2016a, 2016b, Gminder 2016) is premature and should be revised after inclusion of all *Phloeomana* species and a larger set of *Mycena* and *Hemimycena* representatives into a broadly based and multi-gene phylogenic study. Such a study is highly desirable as the genus *Mycena* is clearly polyphyletic (Moncalvo et al. 2002, Matheny et al. 2006). Therefore, for the time being we prefer to use the name *Mycena clavata*.

Matheny et al. (2006) did not use ITS sequences in their analyses, thus their data are not directly comparable to ours. The ITS region is obviously too variable to obtain a fine resolution of the *M. clavata* phylogeny with respect to more distant taxa.

Ecology and distribution

Mycena clavata was described from dead prostrate trunks of *Thuja plicata* (Peck 1898) in the USA. Ronikier & Aronsen (2007) characterised *M. clavata* as a species „growing solitary or in clusters on bark of coniferous wood or on coniferous litter, once found on deciduous wood (Elborne & Læssøe 1982) … known from North America and Europe … in Europe in mountainous habitats as well as in northern part of the continent". They enumerate *Pinus*, *Picea*, *Abies*, and *Juniperus* wood and litter (twigs, needles, mossy pieces of bark, mossy wood, decayed mossy trunk, mossy base of conifer trunk) as substrates and *Pinus* plantage, fern/*Urtica* stand under *Pinus*, litter under *Juniperus*, mixed forest, old forest, Carpathian *Pinus mugo* stands, Alpine bogs and alder forests as habitats (altitude up to 2000 m in the Alps, Switzerland). In Spain, *M. clavata* was found on twigs of *Abies alba* on southern slopes of the Pyrenees, alt. 1250 m (Pérez-de-Gregorio 2015). The Italian record is from *Cupressus* litter (fallen needles and twigs) covered by mosses (Robich 2016). Ronikier & Aronsen (2007) conclude that *M. clavata* is a mountain-boreal species. This is in contradiction with records reported from Belgium (Aronsen & Læssøe 2016), the Netherlands (NDFF & NMV on-line), and Germany (DGfM on-line) which originate from lowlands and, simultaneously, outside the boreal zone. However, identification of the internet records should be revised.

Our records are from the best-preserved montane *Picea-Fagus-Abies* virgin forest in the Czech Republic hosting unique mycobiota (Holec et al. 2015). No

other records are known from this country in spite of intensive study of Czech virgin, natural, and near-natural forests carried out in the past decade. This could indicate that *M. clavata* strictly prefers the virgin forest environment (long forest continuity, high amount of dead wood, humid microclimate etc.). The same ecological preferences were observed in Finland (Bonsdorff et al. 2015: „spruce dominated old-growth forests, on the bark of fallen, large trunks of *Picea abies*"). However, the habitats listed in the previous paragraph show that *M. clavata* occurs at man-influenced and man-made sites, too. It is more probable that the species prefers sites having high air humidity, i.e. in oceanic parts of Europe (see the distribution below, almost exclusively represented by countries neighbouring with sea), in montane habitats and in old-growth forests, where humidity is maintained under a dense cover of trees and thanks to the water reserves in dead wood covered by mosses (a typical substrate of *M. clavata*).

Mycena clavata is currently well-documented from Canada, USA, Spain, France, Belgium, Switzerland, Denmark, Norway, Sweden, Finland, Poland (see Introduction for references), and newly from the Czech Republic (this paper). In all these countries it is a rare species known from just a few sites.

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