

Redescription and epitypification of *Clavaria atrofusca* Velen.

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Clavaria atrofusca was described by Velenovský in 1939 based on a single small collection from the Czech Republic. The species is rather rare and until 2009 it had further only been recorded from Switzerland and Denmark. Since 2009 it has also been collected in Norway, Sweden and in 2014 in the Czech Republic. Because the type locality at Mnichovice has been destroyed and the type collection is fragmentary, we propose an epitype of *Clavaria atrofusca* Velen. based on the recent collection from the Czech Republic, along with the notes on its ecology and distribution.

Key words: *Clavariaceae*, taxonomy, LSU rDNA.

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Velenovského popis *Clavaria atrofusca* z roku 1939 byl založen na jednom malém sběru z České republiky. Tento druh je dosti vzácný a do roku 2009 byl dále znám jen ze Švýcarska a Dánska. Od roku 2009 byl sbírán též v Norsku, Švédsku a roku 2014 v České republice. Protože typová lokalita u Mnichovic byla zničena a typová položka je fragmentární, navrhuje stanovení epitypu *Clavaria atrofusca* Velen., založené na recentním sběru z České republiky, s poznámkami o známé ekologii a rozšíření tohoto druhu.

INTRODUCTION

The genus *Clavaria* was established by Vaillant (1727), accepted by Linné (1753) in his starting point work and sanctioned by Fries (1821). At that time it covered almost all species with club-shaped carpophores, including species currently accepted in other genera and families, e.g. *Ramaria* (*Gomphaceae*) and *Clavulina* (*Clavulinaceae*). The current concept of the genus was proposed by

Corner (1950). The number of currently accepted species distributed worldwide ranges from 28 (Kirk et al. 2008) to 35–36 (Corner 1950, 1970, Petersen 1964, 1965, 1967a, b, c, 1968, 1969, 1971, 1978a, b, 1979).

Clavaria atrofusca Velen. was described by Velenovský (1939) from the Czech Republic. It was collected by the small village of Klokočná near Mnichovice, close to Prague (coordinates approx. 49.958° N, 14.719° E). Until recently only a single record of this species was published from Switzerland by Schild (1971), who gathered a good collection and provided it with a detailed description. Our revision of collections held in European herbaria revealed only one other specimen, collected in 1977 in Denmark (labelled as *C. asperulospora* G.F. Atk.). *Clavaria atrofusca* is a rare, inconspicuous species and all published data are based only on the type collection (Pilát 1958, Parmasto 1965, Corner 1967, 1970) and Schild's paper (Schild 1971). However, the type collection held at PRM is rather small and consists of only a few fragments (Fig. 1) and despite repeated visits the species has never been recollected at the type locality. The habitat has been changed and destroyed by agricultural development and urbanisation.

In 2009 this rare species was collected in Norway, and in 2012 also in Sweden. In 2014 several collections (published here) were recorded in Slovakia. Finally in 2014, i.e. 75 years after its description, *C. atrofusca* was recollected in the Czech Republic by the second author. In this paper, the macro- and microcharacters of fresh basidiomata from this collection and a partial sequence for the 28S subunit of nuclear ribosomal DNA are presented. This collection is proposed as an epitype.

MATERIAL AND METHODS

Morphological observation. Macrocharacters were observed on fresh specimens. Micromorphological characters of the epitype were observed on dried carpophores, using an Olympus BX51 light microscope with an oil-immersion lens at a magnification of 1000×. Spores were scanned with an Olympus C50-50 digital camera and measured using the QuickPhoto micro 3.0 software (Olympus, Japan). Enlarged scanned pictures of spores were used for measurements, with an accuracy of 0.01 µm. The length/width ratio of the spores is expressed as Q. In all specimens, 30 spores were measured to statistically analyse their characteristics (length, width, Q). Values in the descriptions of the species are given as average ± standard deviation; values in parentheses are the 5 and 95 percentiles. All microscopic structures were observed in Melzer's reagent and in a solution of Congo Red in distilled water (0.25 g of Congo Red in 50 ml of distilled water), the objects being kept in the solution for ca. 5 min.

All other specimens were observed using a Nikon Eclipse E200 light microscope with an oil-immersion lens at a magnification of 1000×. Spores were scanned with a Dino Eye Eyepiece camera and measured using the Dino capture 2.0 software. Enlarged scanned pictures of spores were used for measurements, with an accuracy of 0.01 µm. The length/width ratio of the spores is expressed as Q. In all specimens, 20 spores were measured to statistically analyse their characteristics (length, width, Q). Values in the descriptions of species are given as average ± standard deviation; values in parenthesis are the 5 and 95 percentiles. All microscopic structures were observed 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 and 50 ml of distilled water), the objects being kept in the solution for ca. 5 min.

Molecular analyses. Genomic DNA of the epitype specimen was isolated from dry carpophores using a Zymo Research Plant/Seed DNA Miniprep kit (Zymo Research, Orange, USA). Nuclear rDNA containing the partial 28S region (further referred to as LSU) was amplified with forward primer NL1 (5'-GCATATCAATAAGCGGAGGAAAAG) and reverse primer NL4 (5'-GGTCCGTGTTTCAAGACGG) (O'Donnell 1993). PCR products were purified with a Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech Ltd., Taipei, Taiwan). Both strands of PCR fragments were sequenced with NL1/NL4 primers in the Sequencing Laboratory of the Faculty of Science, Charles University in Prague (Czech Republic). The consensus sequence was produced after sequence assembly in the Geneious 6.1.5 program (Biomatters, Auckland, New Zealand).

Phylogenetic analyses. To reveal the phylogenetic position and relationship of *C. atrofusca* to other *Clavaria* species, 34 other LSU sequences obtained in former research and retrieved from GenBank were selected for the analyses including the Norwegian collection of *C. atrofusca* (BRA CR13264, GenBank Accession Nr. JN315785 from the study of Kautmanová et al. 2012). Two *Ramariopsis* species served as outgroup [*R. pulchella* (Boud.) Corner GU299496 and *R. crocea* (Pers.) Corner GU299492]. Sequences were aligned in MUSCLE (Edgar 2004).

Bayesian analysis (BA) was conducted in MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). For BA, a GTR+I+G model of molecular evolution was selected with MrModeltest 2.3 (Nylander 2004). In BA, four Markov chains were run for 2,000,000 generations, sampling every 100th tree with two independent runs per analysis.

Maximum likelihood (ML) analysis was run using the RAxML HPC2 on XSEDE (Stamatakis 2006) tool via LIRMM Science Gateway (Dereeper et al. 2008). A GTR approximation model was used for ML bootstrapping. One hundred bootstrap replicates were performed for branch support assessment, saving the most likely tree and leaving the remaining options as default.

RESULTS

***Clavaria atrofusca* Velen., Novitates Mycologicae: 164 (1939)**

The protologue given by Velenovský (1939) described *C. atrofusca* as: “Solitaria, gracilis, sporoph. simplici, 2–4 cm alto, supra 2–3 mm cr., laevi, atrofusco, firmo, obtuso, basi breviter attenuato, tereti. Sporae hyalinae, laeves, ovato elipt. 5–7.

Ad carbonarium pr. Božkov, ad terram nudam argillaceam infra Klokočná, 9, 1922. – Affinis *Cl. nigritae* Pers. (Bresad. Iconogr. XXIII, 1105), sed multo minor gracilior, non fasciata.”

This description is herein amended based on a morphological study of the collection proposed as an epitype.

Epitype. Czech Republic, Příbram District, Prosenická Lhota, village of Bříšejev, alt. 385–400 m, 49°42'21.865" N, 14°29'07.772" E, 23 Sept. 2014, leg. J. Matouš (PRM 933995).

LSU rDNA sequence deposited in European Nucleotide Archive ID under No. LN868504.

Macrocharacters. Basidiomata solitary or rarely fasciculate (2–3 basidiomata), 15–100 × 1–6.5 mm (av. 54.1 × 2 mm), simple, cylindrical to subclavate, often twisted, rounded or flattened at the top, rarely pointed. Surface smooth, with lines or folds, in dry conditions sometimes finely ridged, colour purplish black (Pantone 1817) when young, when mature fuscous black, matt, paler when dry, brownish black in dried specimens. Sterile basal part short, 5–10 mm, sometimes attenuated at the base, black, slightly glossy, visible, but not distinctly separated from the fertile part, with small white cottony mycelium at the base (Fig. 2). Flesh brittle (breaking when bent for more than 45°), greyish brown, slightly darker than the surface, longitudinally filamentous, silky. Smell and taste insignificant. Spore print white.

Microcharacters. Basidiospores ellipsoid to amygdaloid, rarely almost rounded, attenuated towards the apiculum, with one, rarely two or more greenish oil drops, (5.1)5.4–7.1(7.6) × (3.4)3.5–4.6(4.6) μm (av. 6.3 × 3.9 μm), $Q_{av} = 1.61$ (Fig. 3). Walls thickened, fuscous brownish and roughly warty. Basidia clampless, brownish or hyaline, broadly clavate, 39.1–65.1(66.8) × (7.5)7.6–13.7(15), rarely longer than 60 μm. Hymenium brownish, 52.8–68 μm thick. Subhymenium 22.7–35.8 μm thick, well distinguished from tramal hyphae. Subhymenial cells 3.5–6 μm (av. 4.5 μm) wide, cylindrical, inflated hyphae 7.2–21 μm (av. 12.6 μm). Hyphal cells of trama hyaline, 65.8–303.7 × 5.2–17.2 μm, cylindrical or slightly inflated at septa, thin-walled, with secondary septa.

Habitat (Fig. 4). Sloping meadow below a footpath, at the edge of a small village. Upper part of the meadow dry, probably occasionally mowed, lower part relatively wet, bordering open birch stand.



Fig. 1. Holotype specimen of *Clavaria atrofusca* (Klokočná; PRM147956). Scale bar = 1 cm. Photo I. Kautmanová.



Fig. 2. *Clavaria atrofusca* – epitype (Bříšejev; PRM 933995). Scale bar = 2 cm. Photo J. Matouš.



Fig. 3. Spores and basidia of *Clavaria atrofusca* epitype (PRM 933995). Scale bar = 20 μ m. Photo J. Matouš.



Fig. 4. Epitype locality at Bříšejev near Prosenická Lhota. Photo J. Matouš.

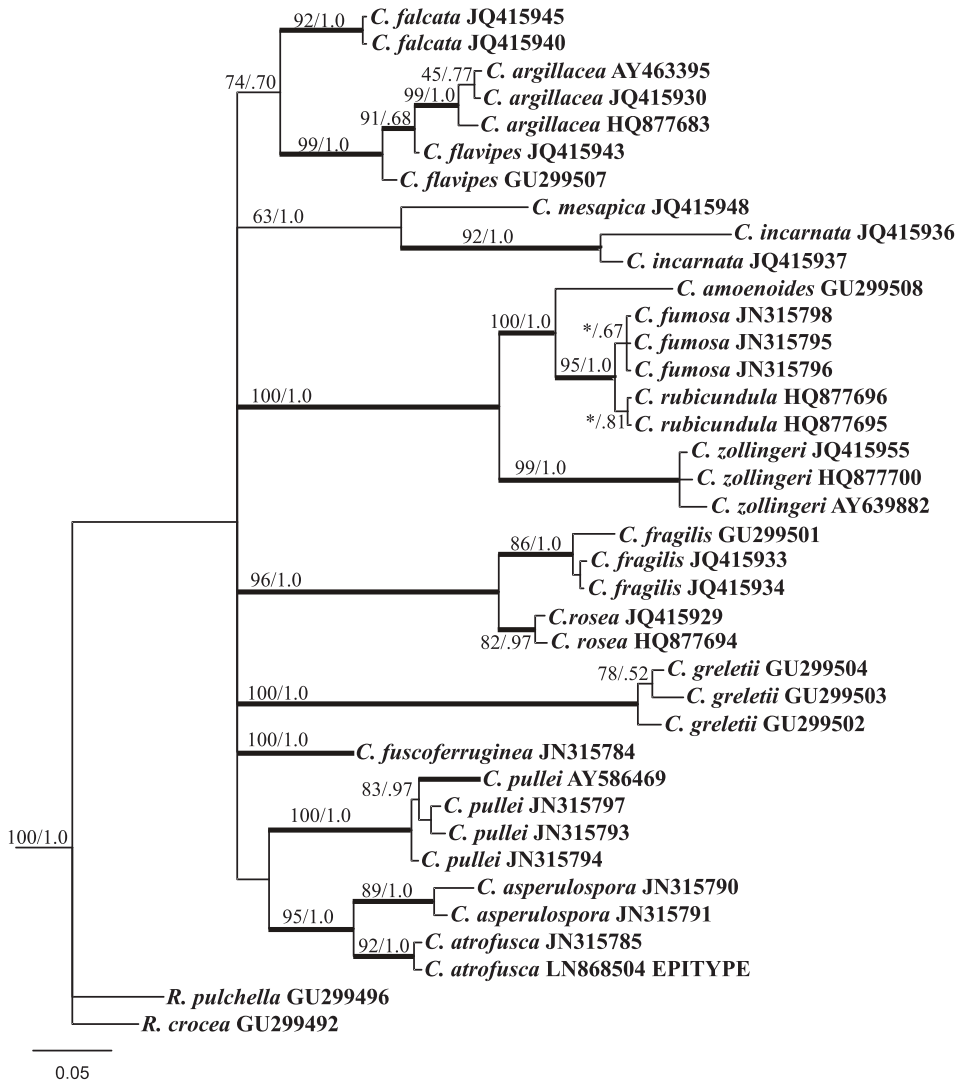


Fig. 5. Majority rule Bayesian phylogram showing phylogenetic relationship of *Clavaria atrofusca* to other related taxa. Numbers above branches represent maximum likelihood bootstrap values (ML) and Bayesian posterior probabilities (BA), respectively.

For information about epitype and Norwegian specimen (sequence JN315785) see Studied specimens. Other sequences used for analysis were retrieved from the GenBank database.

Tab. 1. Dimensions of spores of *Clavaria atrofusca* based on 20 measurements per specimen (30 measurements of epitype specimen). Values are given as average ± standard deviation; values in parentheses are the 5 and 95 percentiles.

Specimen	Length (µm)	Width (µm)	Q av.
PRM 147956 (holotype)	(5.9)6.5– 7.4 –8.3(8.4)	(3.9)4.1– 4.5 –4.9(5.0)	1.64
PRM 933995 (epitype)	(5.1)5.4– 6.3 –7.1(7.6)	(3.4)3.5– 3.9 –4.3(4.7)	1.61
C F-39714 – Denmark	(5.4)5.9– 6.4 –6.9(7.4)	(3.4)3.5– 3.9 –4.2(4.3)	1.67
Schild 140 – Switzerland	(5.2)5.4– 6.1 –6.7(7.1)	(3.0)3.1– 3.5 –4.0(4.5)	1.77
BRA CR13264 – Norway	(6.2)6.3– 7.1 –7.9(8.6)	(3.3)3.5– 4.1 –4.7(4.8)	1.75
BRA CR13272 – Norway	(5.7)5.8– 6.7 –7.6(7.7)	(3.5)3.8– 4.1 –4.5(4.6)	1.64
BRA CR18057 – Sweden	(6.1)6.4– 7.0 –7.6(8.1)	(3.8)3.9– 4.2 –4.5(4.6)	1.68
BRA CR21109 – Slovakia	(6.3)6.4– 7.3 –8.2(8.6)	(3.9)4.0– 4.3 –4.6(4.7)	1.71
BRA CR21601 – Slovakia	(6.4)6.7– 7.3 –7.9(8.1)	(3.9)4.1– 4.4 –4.7(4.8)	1.67
BRA CR21602 – Slovakia	(5.9)5.8– 6.6 –7.3(8.1)	(3.6)3.8– 4.2 –4.7(4.8)	1.56
BRA CR21600 – Slovakia	(6.0)6.2– 6.7 –7.2(7.4)	(3.7)4.0– 4.3 –4.7(4.8)	1.54
BRA CR21603 – Slovakia	(6.1)6.2– 7.0 –7.7(8.5)	(3.6)3.7– 4.1 –4.5(4.8)	1.69

Studied specimens

Czech Republic. Bohemia. Mnichovice-Božkov, village of Klokočná, Sept. 1922, leg. J. Velenovský (PRM 147956, holotype). – Příbram District, Prosenická Lhota, village of Bříšejev, alt. 385–400 m, 49°42'21.865" N, 14°29'07.772" E, 23 Sept. 2014, leg. J. Matouš (PRM 933995, epitype proposed herein).

Switzerland. Near Geneva [Nähe von Genf], in grass under spruce, 26 Sept. 1968, leg. F. Steffen (Schild 140).

Denmark. Bornholm island, Døndalen, on rich black mull in deciduous scrub under *Fraxinus*, 22 Oct. 1977, leg. H. Knudsen as *C. asperulospora* (C F-39714).

Norway. Steinkjer, Skrattasen, in pasture, 64°18'46.93" N, 11°56'29.68" E, alt. 360 m, 5 Sept. 2009, leg. V. Kučera (BRA CR13264, BRA CR13272, PRM 921802, O-F 300032).

Sweden. Dalsland, Bengtfors, Skärbo National Reserve, in pasture, 11 Sept. 2012, leg. I. Kautmanová (BRA CR18057).

Slovakia. Čadca District, village of Korňa, U Žilov settlement, in old abandoned pasture in grass and shrubs, 15 Aug. 2014 (BRA CR21109). – Snina, Sninské rybníky, in mowed lawn, 1 Oct. 2014, leg. V. Kautman (BRA CR21602); *ibid.*, 5 Oct. 2014, leg. V. Kautman (BRA CR21600). – Snina, city park, in lawn, 6 Oct. 2014, leg. J. Pavlík (BRA CR21603, BRA CR21601).

DISCUSSION

The macro- and microcharacters of collection PRM 933995 were identical to those of the holotype of *C. atrofusca* (Fig. 1) and other specimens available (Tab. 1). The sequence of LSU was identical to sequence JN315785 extracted from a collection also identified as *C. atrofusca* in Norway (Kautmanová et al. 2012). Both sequences formed a well-supported clade in the Bayesian analyses, being a sister clade to *C. asperulospora* (Fig. 5), the only other black *Clavaria* species with

finely ornamented spores. *Clavaria asperulospora* was described by Atkinson (1908) from the United States as clustered, wood-brown, 4–7 cm high, 2–3 mm stout, cylindrical, blunt, tapering below, with basidia abruptly clavate $30 \times 10\text{--}12 \mu\text{m}$, 4-spored and spores globose, white, echinulate, pedicellate, 6–7 μm long. *C. asperulospora* is a rare species, known in Europe only from a few collections (Kautmanová et al. 2012). Despite the difference in spore shape and size between *C. asperulospora* and *C. atrofusca*, Petersen (1967b) considered *C. asperulospora* to be the only species of *Clavaria* with rough spores, after which Jülich (1984) and Knudsen (1997) synonymised it with *C. atrofusca*. Corner (1950) accepted only *C. asperulospora* and moved it to another genus as *Ramariopsis asperulospora* (Atk.) Corner, being the only species in the genus with simple fruitbodies. Petersen (1967b) considered this placement incorrect due to the lack of clamps and the different character of the ornamentation. Later, Corner (1967, 1970) accepted *C. asperulospora* in the genus *Clavaria* and also *C. atrofusca*, but only with a brief note about Schild's collection which "will be published and which indicates that the species is more common and maybe overlooked". Only Schild (1971) published a precise and very detailed description of *C. atrofusca* based on a good collection from Switzerland. Also Petersen & Olexia (1969) accepted *C. atrofusca* as a separate species. Finally, Roberts (2007) examined material of *C. asperulospora* from the British Isles and suggested that there are possibly two taxa included under this name, as well as *C. atrofusca*. A detailed delimitation of both species was provided by Kautmanová et al. (2012).

The ecology of *C. atrofusca* is not well known because of the limited number of collections. Although Velenovský (1939) mentioned in the protologue that his collection was found on bare soil at a burnt place, all other records were made in grass in pastures, mowed meadows or city parks often accompanied by various mosses, or among shrubs and trees in old abandoned pastures. Therefore we presume that habitats preferred by the species are semi-natural grasslands and pastures, probably acidic, as none of the known localities is on limestone.

The list of the studied specimens represents probably most of the known records of this rare species. The authors have not been able to find any other published records or herbarium specimens outside Europe. However, the global distribution of *C. atrofusca* is unclear, because the species could be easily overlooked.

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ERRATUM

In the online version of the article, published April 7, 2016, an error occurred in the chapter Studied specimens. The correct locality of the Danish specimen is Bornholm island, Døndalen, and the correct year is 1977. The wrong version was withdrawn and the corrected version published online on April 13, 2016, being the official date of the online publication.

The publisher wishes to thank Henning Knudsen for providing the correct information. We apologise for this inconvenience.