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RESEARCH ARTICLE

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European species of *Clavaria* (*Agaricales*, *Agaricomycetes*) with dark basidiomata – a morphological and molecular study

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Key words

basidiospores
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LSU nrDNA
phylogeny

Abstract *Clavaria* species with dark basidiomata occurring in Europe were analysed using morphological and molecular methods. Morphological analyses revealed four groups containing seven *Clavaria* species with dark basidiomata. Phylogenetic analysis of the LSU nrDNA region confirmed the separate positions of all seven *Clavaria* species within the genus. All sequences were grouped in four well-supported clades, mostly corresponding to defined morphological species. The results of the molecular study are inconsistent with the infrageneric classification of *Clavaria* based on the presence or absence of clamps on the bases of basidia and two widely accepted subgenera. *Clavaria* and *Holocoryne* appear to be polyphyletic. A new approach in species delimitation is presented: 1) *C. asperulispora* and *C. atrofusca* are two distinct species recognized by the shape of their spores, and the name *C. neo-nigrita* is a possible synonym of *C. asperulispora*; 2) species with clustered fragile basidiomata, *C. fumosa* and *Clavaria* cf. *fuscoferruginea*, which are almost identical in shape and size of spores differing only in the darker basidiomata of the latter, are phylogenetically unrelated; 3) *Clavaria atrobadia* is a dubious species, the name being most likely a synonym of *C. fuscoferruginea*; 4) two species with close morphological and phylogenetic affinity, *C. atroumbrina* and *C. pullei*, are distinguished based on the more oblong and narrower spores of the former. Comparison of European and North American material suggests the transatlantic nature of the distribution of *C. asperulispora*, *C. atroumbrina* and *C. fumosa*.

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INTRODUCTION

The study focuses on European species of the genus *Clavaria* L. (Basidiomycota, Agaricomycetes, *Agaricales*, *Clavariaceae*) with dark (grey, brown or black) basidiomata. The colour of the basidiomata is a basic identifying characteristic of *Clavaria* species in the field or from brief diagnoses, and this distinction is reflected in the species identification keys (e.g., Corner 1950, Knudsen 1997, Roberts 2007). All recently accepted *Clavaria* species with dark basidiomata are consistently accepted as members of this single genus since the delimitation of the three genera of *Clavariaceae* Chevall. by Corner (1950): *Clavaria*, *Clavulinopsis* Overeem and *Ramariopsis* (Donk) Corner.

Knowledge of *Clavaria* species with dark basidiomata compared with other *Clavariaceae* groups is complicated by the following facts: 1) most species are reported as extremely rare (e.g. Gärdenfors 2005, Holec & Beran 2006); and 2) morphologically similar collections from distant areas are often recognized as different species. Certain species of *Clavariaceae* are considered almost cosmopolitan. For example, *Clavaria fragilis* Holmsk., described from Europe, is also reported from Australia, North America, Brazil, China, Costa Rica, Indonesia, Japan, the Solomon Islands and South Africa (Burt 1922, Corner 1950, 1967). The other European species, *Ramariopsis pulchella* (Boud.) Corner and *Clavulinopsis laeticolor* (Berk. & M.A. Curtis) R.H. Petersen, are reported from the USA, South America and New Zealand, *C. laeticolor* is also known from Malaysia (Corner 1970, Petersen 1971, 1978, 1988). In contrast, most *Clavaria* species with dark basidiomata have been reported only from one continent.

Clavaria species with dark basidiomata have been placed in both subgenera of *Clavaria*: subgenus *Clavaria* and subgenus *Holocoryne* Corner. The latter is distinguished by the presence of loop-like clamps at the bases of its basidia (Corner 1950). Subgenus *Clavaria* contains 13 species with dark basidiomata worldwide, while only two species with dark basidiomata are classified within the second subgenus (Corner 1950, 1970, Petersen 1978). Kautmanová et al. (2012) published a phylogenetic study on *Clavariaceae* based on analyses of LSU nrDNA region. Their study suggests that subg. *Holocoryne* is polyphyletic and that one species with dark basidiomata, *C. greletii* Boud., is unrelated to other species with clamp-bearing basidia included in the study. The position of *C. fumosa* Pers. – another species with dark basidiomata – was confirmed within subg. *Clavaria*, but that study did not include representatives of other *Clavaria* species with dark basidiomata to elucidate their classification and relationships.

The rareness of *Clavaria* species with dark basidiomata and the lack of studies comparing material from different continents resulted in various interpretations of names and inconsistent numbers of accepted species in the literature. In Europe, Corner (1950, 1970) accepted seven species, Knudsen (1997) five species (Nordic countries) and Roberts (2007) four species (excluding *C. fumosa* from 'dark clavarias' as a species with paler basidiomata).

The aim of this study is to provide a taxonomic revision of European *Clavaria* species with dark basidiomata and a critical evaluation of their classification. Our analysis is based on morphological and molecular characters examined on recently collected material, as well as types and other relevant herbarium material that was available.

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MATERIAL AND METHODS

Morphological study

Studied specimens were field-collected by the authors or loaned from herbaria BIO, C, CUP, GB, K, L, MA, MICH, NCU, PC, PRM, SAV, TENN and UPS (abbreviations according to Theirs (continuously updated)) and from the private herbaria of E. Schild (Zürich, Switzerland), G. Corriol (Bagnères de Bigorre, France), O. Jindrich (Horovice, Czech Republic) and S. Valda (Kokorín, Czech Republic). The studied material also contained types or original material of *C. atrofusca* Velen., *C. asperulispora* G.F. Atk., *C. atrobadia* Corner, *C. neonigrita* R.H. Petersen, *C. atroumbrina* Corner, *C. fumosa*, *C. greletii* and *C. fuscoferruginea* Leathers. Loans were selected to cover specimens included in important published descriptions and authentic material determined by R.H. Petersen (NCU, TENN), P. Roberts (K) and H. Knudsen (C). A total of 93 specimens of *Clavaria* with dark basidiomata from nine European countries, the USA and Ecuador were examined. Delimitations of species as defined by Petersen & Olexia (1969) and Corner (1950, 1970) were used.

Macromorphological characters were observed on fresh specimens. Chemical reactions to 10 % $FeCl_3$ were tested on the hymenium surface of fresh basidiomata. Micromorphological characteristics were observed on fresh and dried basidiomata using an Olympus CX-41 microscope with an oil-immersion lens at a magnification of 1500×. All microscopic structures were observed in a solution of Congo Red in ammonia. Spores were scanned with the Olympus Artcam camera and measured using Quick Micro Photo (v. 2.1) software. Enlarged scanned pictures of spores were used for measurements, with an accuracy of 0.1 µm. The length/width ratio of the spores was given by Q. Measurements excluded spore ornamentation. Thirty-five selected specimens were measured to statistically analyse spore characteristics (length, width, Q; 30 spores per specimen, at least 3 specimens per species). Average values and ranges are listed for all studied specimens in Table 1 and were based on 30 measurements per specimen. Box plot charts were prepared with the statistical software R (www.r-project.org) based on all measurements for individual species and were edited and supplemented by additional average values for each specimen by CorelDRAW X5 software. Values in the descriptions of species are given as average ± standard deviation; values in parenthesis are for the 5th and 95th percentiles.

Specimens examined

Clavaria asperulispora – Sweden, Uppsala, Predikstolen, 30 Aug. 1988, C(F) 89786. – United Kingdom, West Lancashire, Eaves Wood, 16 Oct. 2006, K(M) 143814.

Clavaria atrobadia – India, Arunachal Pradesh, West Kameng County, Bombdila, 24 Aug. 1981, TENN 044236. – USA, New York, Ithaca, as *C. nigrita* Bres. NCU 596584.

Clavaria atrofusca – Czech Republic, Mnichovice, Božkov, Klokočná, Sept. 1922, PRM 147956, holotypus. – Denmark, Sjaelland, under Fraxinus, 22 Oct. 1997, C(F) 39714. – Norway, Skrattåsen, Steinkjer, 5 Sept. 2009, BRA CR13272; ibid., BRA CR13264. – Sweden, Dalsland, Bengtsford, Skärbo National reserve, 11 Sept. 2012, BRA CR18057. – Switzerland, Nähe von Genf, 26 Sept. 1968, Schild 140.

Clavaria atroumbrina – Czech Republic, Orlické hory Mts, Uhřínov village, Antonínovo údolí, 19 Oct. 2008, SAV F3139. – Norway, Akershus, Nesodden, Røer, 9 Sept. 2009, BRA CR13265; Buskerud, Nedre eiker, Ryghsettra, 11 Sept. 2009, BRA CR13271. – Sweden, Uppsala, 30 Sept. 1927, UPS-F-120973; Viker par., 12 Sept. 1965, UPS-F-120972; Gotland, Alskog par.,

Table 1 List of specimens included in morphological and molecular studies. Values of spore characters are 5 percentile/average/95 percentile of 30 measurements. Average values are in **bold**.

Species	Herbarium number – Country	Length of spores	Width of spores	Qav	GenBank No.
Clavaria atrofusca	BRA CR13272 – Norway	5.8–**6.7**–7.6	3.8–**4.1**–4.5	**1.64**	
	BRA CR13264 – Norway	6.3–**7.1**–7.9	3.5–**4.1**–4.7	**1.75**	JN315785
	PRM 147956 – Czech Rep.(holotypus)	8.0–**8.6**–9.5	4.4–**4.7**–5.1	**1.81**	
	C(F) 39714 – Denmark	5.9–**6.4**–7.2	3.5–**3.9**–4.2	**1.67**	
	Schild 140 – Switzerland	5.4–**6.1**–6.7	3.1–**3.5**–4.0	**1.77**	
Clavaria asperulospora	CUP(A) 13182 – USA (holotypus)	5.0–**5.2**–5.5	4.6–**5.0**–5.4	**1.04**	
	C(F) 89786 – Sweden	4.4–**4.9**–5.5	3.7–**4.3**–4.7	**1.16**	JN315791
	K(M) 143814 – Great Britain	3.6–**4.0**–4.6	3.2–**3.6**–4.0	**1.13**	JN315790
Clavaria neonigrita	TENN 037787 – USA (holotypus)	4.8–**5.5**–5.9	4.3–**4.8**–5.3	**1.15**	
Clavaria pullei	BRA CR13101– Slovakia	4.7–**5.1**–5.6	2.7–**3.1**–3.4	**1.66**	
	GC 99102304 – France (neotypus)	4.8–**5.3**–5.7	2.8–**3.2**–3.6	**1.64**	JN315793
	BIO10231 – Spain	5.3–**5.9**–6.6	3.3–**3.7**–4.2	**1.61**	
	BIO12378 – Spain	5.0–**5.4**–5.9	3.1–**3.3**–3.7	**1.62**	JN315794
Clavaria atroumbrina	BRA CR13265 – Norway	4.8–**5.5**–6.1	2.7–**3.0**–3.1	**1.88**	JN315786
	BRA CR13271 – Norway	5.1–**5.7**–6.5	2.7–**3.1**–3.5	**1.86**	JN315787
	SAV F3139 – Czech Republic	5.1–**5.5**–5.9	2.8–**3.0**–3.3	**1.86**	
	NCU 2794 – USA (syntypus)	5.2–**5.7**–6.2	2.8–**3.1**–3.5	**1.86**	
	TENN 032685 – USA	5.2–**5.6**–6.2	2.9–**3.1**–3.3	**1.77**	
	TENN 031091 – USA	5.0–**5.5**–6.2	2.8–**3.2**–3.5	**1.74**	JN315788
	TENN 030948 – USA	5.1–**5.5**–6.1	2.9–**3.2**–3.5	**1.74**	JN315789
	K(M) 143730 – Great Britain	5.4–**5.9**–6.4	3.0–**3.3**–3.5	**1.82**	JN315792
Clavaria fumosa	BRA CR748 – Slovakia	6.0–**6.6**–7.4	3.4–**3.8**–4.5	**1.73**	JN315798
	BRA CR115655 – Slovakia	6.0–**6.5**–7.5	3.2–**3.4**–3.7	**1.89**	JN315796
	BRA CR115656 – Slovakia (epitypus)	6.1–**6.5**–7.0	3.3–**3.4**–4.0	**1.78**	JN315795
	BRA CR16039 – Czech Republic	5.9–**6.6**–7.5	3.3–**3.5**–3.9	**1.86**	
	BRA CR13277 – Norway	5.5–**6.1**–6.7	3.2–**3.5**–3.8	**1.77**	
	GC02092401 – France	5.8–**6.4**–7.1	3.1–**3.4**–3.7	**1.88**	
Clavaria greletii	SAV F1988 – Slovakia	7.2–**8.0**–9.1	5.8–**7.3**–7.5	**1.20**	GU299504
	PC 0094981 – France (holotypus)	6.6–**7.6**–8.8	5.9–**6.7**–7.6	**1.13**	
	GC08101403 – France	8.2–**9.1**–9.9	7.2–**8.0**–9.0	**1.14**	GU299502
	BRA CR13694 – Slovakia	6.3–**7.3**–8.6	5.6–**6.5**–7.6	**1.13**	
	K(M) 143840 – Great Britain	6.3–**7.6**–9.0	5.5–**6.5**–7.7	**1.17**	GU299503
Clavaria fuscoferruginea complex	BRA CR13262 – Norway	6.6–**7.2**–8.1	3.3–**3.6**–3.8	**1.99**	JN315784
	MICH 10089 – USA (holotypus)	5.6–**6.0**–6.6	3.1–**3.4**–3.5	**1.80**	
	NCU 596584 – USA (authentic material of *C. atrobadia*)	5.4–**6.0**–7.0	2.6–**2.9**–3.3	**2.12**	

27 Sept. 1991, UPS-F-012880; Dalarna, Ludvika, Sorvik, 27 Aug. 1993, UPS-F-141056; Bälinge par., Lövstalöt, 2 Sept. 1998, UPS-F-012854; Närke, Snavlunda, 20 Aug. 1998, GB-007 5100. – United Kingdom, Wales, Carmarthenshire, Llanarthne, Gelli Aur Country Park, 24 Oct. 2006, K(M) 143730. – USA, North Carolina, Chapel Hill, (as *C. nigrita* Coker), 28 July 1917, NCU 2794; North Carolina, Great Smokey Mountains National Park, 23 July 1963, TENN 030948; ibid., 9 Aug. 1963, TENN 031091; ibid., 8 Dec. 1966, TENN 032073.

Clavaria fumosa – Czech Republic, Liberec, Machnín village, 10 Sept. 2010, BRA CR16039; Liberec, Rašovka, 16 Sept. 2010, BRA CR16683; ibid., 16 Aug. 2011, BRA CR 16738. – Denmark, Roskilde, 18 Sept. 2000, C(F) 71569; Grejs Bakke 4 Sept. 2001, C(F) 42757; Egtved, Tågelundgård Plantage, 14 Sept. 2001, C(F) 42613. – France, Vallon de Crastes, 24 Sept. 2002, GC 02092401. – Norway, Mokk, 3 Sept. 2009, BRA CR13277; Steinkjer, Skrattåsen, 5 Sept. 2009, BRA CR13276; Oppdal, Slettsvol, 6 Sept. 2009, BRA CR13270; Akershus, Nesodden Røer, 9 Sept. 2009, BRA CR13275. – Slovakia, Čadca, Raková, 12 Oct. 1974, BRA CR8969; Važec, 11 July 1987, BRA CR8970; Liptovský Hrádok, Borová Sihoť, Kameničné hill, 14 July 1988, BRA CR8810; Malužiná village, Michalovo valley, 15 July 1999, BRA CR938; ibid., 25 Aug. 2002, BRA CR840; ibid., 8 Sept. 2006, BRA CR8909; ibid., 9 Sept. 2006, BRA CR15655; ibid., 24 Aug. 2008, BRA CR12847; ibid., 28 Aug. 2008, BRA CR12837; ibid., 30 Aug. 2010, BRA CR748; ibid., 1 Sept. 2010, BRA CR16077; ibid., 18 Sept. 2010, BRA CR15926; ibid., 19 Sept. 2010, BRA CR15034; Vršatské Podhradie, 17 Sept. 2002, SAV F1989; Zuberec, Mačie diery Nature Reserve, 16 July 2007, BRA CR15656; Kováčová village, 19 Sept. 2009, BRA CR15971; Lomná village, 19 Sept. 2009, BRA CR16088; Pusté Pole village, 3 Sept. 2010, BRA CR15914; Rudno nad Hronom village, Drastvica hill, 9 Sept. 2010, BRA CR16172; Korňa village, Žilovci settlement, 19 Sept. 2010, BRA CR16083; Muránska Zdychava village, 14 Oct. 2010, BRA CR16612. – Spain, Berastegi, Artaleku, 17 Aug. 2002, BIO9598; ibid., 12 Oct. 2007, BIO12566. Bilbao, Larrinagatxu, Izurtza, 16 Oct. 2007, BIO12384. – Sweden, Dalsland, Mellerud, Ryrhalvön, 14 Sept. 2012, BRA CR18077; ibid., BRA CR17963; Dalsland, Bengtsfors, Skärbo National reserve, 11 Sept. 2012, BRA CR17989.

Clavaria fuscoferruginea – USA, Michigan, Emmet Co., Pellston hills, 11 Aug. 1951, MICH 10089, holotypus.

Clavaria cf. *fuscoferruginea* – Norway, Buskerud, Nedre Eiker, Ryghsettra, 11 Sept. 2009, BRA CR13262.

Clavaria greletii – Denmark, Sjælland, Røsnæs, 7 Oct. 2007, TL-13295 (Thomas Laessoe private herbarium). – Ecuador, Cotopaxi province, Union del Toachi village, Otongachi, 28 Mar. 2011, BRA CR16384. – France, Savigné (Vienne), Pelouses sablonneuses, 10 Nov. 1913, PC 0094981, holotypus; Aude, Roquefeille, Tourbière du Pinet, 14 Oct. 2008, GC 08101403. – Norway, Vestfold, Tjome, Moutmarka, 8 Sept. 2009, BRA CR13285. – Slovakia, Hostovice village, Hostovické lúky, 9 Oct. 2005, SAV F1988; Piešťany, Banka village, 27 Oct. 2007, BRA CR12345; ibid., 11 Sept. 2010, BRA CR 16723; Žilina, Višňové village, 21 Sept. 2008, BRA CR13111; Muráň village, Predná Hora, 10 Oct. 2008, BRA CR13677; ibid., 10 Oct. 2008, BRA CR13694; ibid., 4 Oct. 2011, BRA CR16702; Zvolen town, 8 Oct. 2009, BRA CR16023; Nová Bošáca village, Blažejová Nature Reserve, 17 Sept. 2010, BRA CR16078; Kováčová village, 3 Oct. 2010, BRA CR16173. – Sweden, Dalarna, Stora Tuna, Tuna-Hästberg, 29 Sept. 1998, 98108AJ (Anders Janols private herbarium); ibid., 5 Sept. 2009, 09058AJ. – United Kingdom, Leicestershire, Ashby de la Zouch, New Lount Nature Reserve, 7 Oct. 2006, K(M) 143840.

Clavaria neonigrita – USA, Michigan, 19 Sept. 1964, TENN 037787.

Clavaria nigricans – Chile, Region Metropolitana County, Santiago, Quinta Normal, V.1918, TENN 040208.

Clavaria nigripes – USA, Tennessee, County Blount, town Townsend, 2.VI.1974, TENN 039634.

Clavaria pullei – Czech Republic, Orlické hory Mts, Uhřínov village, 19 Oct. 2008, SAV F3139; Sychrov, castle park, 14 Oct. 2012, BRA CR18123. – France, Loiret, Estouy, 'les Vaux', 23 Oct. 1999, BRA CR17657; Hautes Pyrenées, Boguéra de Bigone, Ravin de la Tapère, 28 Sept. 2002, GC02092801 (Giles Corriol private herbarium). – Norway, Akershus, Nesodden, Røer, alt. 110 m, in grass in the forest road, leg. V. Kučera, 9 Sept. 2009, BRA CR13265; Buskerud, Nedre Eiker lake, Rhygsettra, 11 Sept. 2009, BRA CR13271. – Slovakia, Sološnica village, 26 Aug. 2008, BRA CR13101. – Spain, Córdoba, Arroyo Pedroches, 4 Dec. 2003, BIO10231; Larrinagatxu, Izurtza, 5 Dec. 2003, BIO12378.

Clavaria rufobrunnea – USA, California, Humboldt Co., Orick, 4 Dec. 1937, MICH 10098, holotypus.

DNA extraction, amplification and sequencing

DNA was extracted from dried and fresh basidiomata. The LSU nrDNA sequences were obtained from 80 isolates of *Clavariaceae*, 20 of them (GU299491–GU299510) were published in Kautmanová et al. (2012), additional 60 specimens, 19 of them with dark basidiomata, are listed in Table 1 and 2. DNA was extracted and purified with a DNeasy Plant Mini Kit (Qiagen, Crawley, West Sussex, UK) or PowerSoil™ DNA Isolation Kit (Mo-Bio, Carlsbad, CA, USA) following the manufacturer's protocol.

Primers for LSU (LR0R, LR5, LR16, LR3R and LR7; Moncalvo et al. 2000) were used for PCR and sequencing. Reaction mixes and PCR cycling regimes were done according to Kautmanová et al. (2012).

All PCR and cycle sequencing reactions were run on a DNAEngine Peltier Thermal Cycler (BIO-RAD USA), PTC-200 Peltier Thermal Cycler (MJ Research), Gene Amp PCR System 9700 (Applied Biosystems) or Mastercycler® ep thermocycler (Eppendorf), the last according to Tomšovský et al. (2010). After amplification, samples were purified and sequences were obtained according to Kautmanová et al. (2012).

Sequences were edited using Sequencer 4.7. software (Gene Codes, Ann Arbor, Michigan, USA) by overlapping the unidirectional reads.

All DNA sequences were submitted to BLAST and used to query the nucleotide collection using default settings. Sequences obtained in this study were analysed together with others retrieved from the GenBank database. Multiple alignment was performed by MEGA v. 4 (Tamura et al. 2007).

Table 2 List of collections and LSU nrDNA sequences used in this study for phylogenetic analysis.

Species	Country of origin	Voucher ID	GenBank ID
Clavaria amoenoides	United Kingdom	K(M) 145803	JQ415946
	Finland	BRA CR16717	JQ415947
Clavaria argillacea	Slovakia	BRA CR16025	JQ415930
	United Kingdom	K(M) 126733	JQ415931
Clavaria falcata	Slovakia	BRA CR16666	JQ415961
	Slovakia	BRA CR16667	JQ415962
	United Kingdom	AB 0532	JQ415935
	Slovakia	BRA CR16029	JQ415940
	France	GC 08101406	JQ415941
	Denmark	C(F) 32637	JQ415945
Clavaria fragilis	Spain	BRA CR15978	JQ415932
	Spain	BRA CR16017	JQ415933
	Spain	BIO12389	JQ415934
	Spain	MA-Fungi 61797	JQ415949
	Spain	MA-Fungi 52135	JQ415950
	Spain	MA-Fungi 59584	JQ415951
	Spain	MA-Fungi 62679	JQ415952
	Spain	MA-Fungi 67437	JQ415953
	Spain	MA-Fungi 67674	JQ415954
Clavaria greletii	Denmark	TL 13295	JN416778
Clavaria guilleminii	Spain	BIO12566	JQ415939
Clavaria incarnata	Slovakia	BRA CR16210	JQ415960
	Slovakia	BRA CR16024	JQ415936
	Slovakia	BRA CR16030	JQ415937
	Spain	BIO12386	JQ415938
	Spain	MA-Fungi 53113	JQ415948
Clavaria pullei	France	GC 02092801	JN315797
Clavaria rosea	United Kingdom	K(M) 135940	JQ415928
	Denmark	C(F) 26590	JQ415929
Clavaria straminea	Slovakia	BRA CR12809	JQ415942
	Slovakia	BRA CR12808	JQ415943
	Slovakia	BRA CR12807	JQ415944
	United Kingdom	C(F) 44153	JQ415958
Clavaria zollingeri	Norway	MA-Fungi 53142	JQ415955
Clavaria sp.	Spain	BIO12762	JQ415960
	France	GC 01100802	JQ415956
	Slovakia	SAV F1994	JQ415963
	Slovakia	BRA CR16032	JX069825
	Slovakia	SAV F2000	JX069826
	Spain	BRA CR16695	JX069827
	Spain	BIO 10433	JX069828
Clavulinopsis luteoalba	Denmark	BRA CR16669	JQ415959
Clavulinopsis sp.	Spain	MA-Fungi 67771	JQ415957
	Estonia	SAV F2087	JX112903
Ramariopsis corniculata	Spain	BRA CR16019	JX112904
Ramariopsis. sp.	Slovakia	BRA CR16696	JX112902

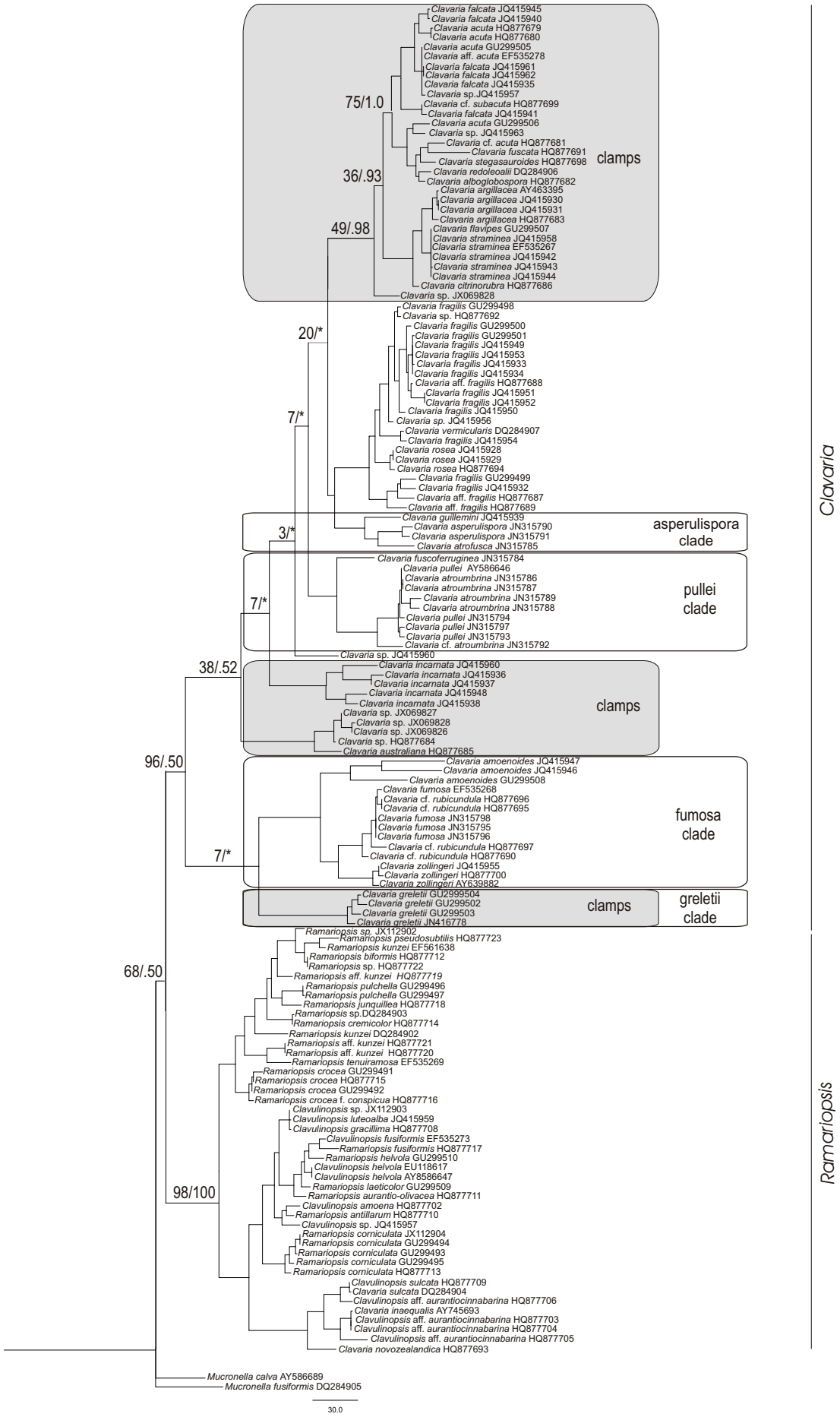

Fig. 1 Maximum parsimony phylogram inferred from LSU nrDNA sequences of selected *Clavariaceae* species (tree length = 2200, CI = 0.3191, RI = 0.7969 and RC = 0.2543). Numbers above branches represent maximum parsimony bootstrap values (bs) and Bayesian posterior probabilities (pp), respectively. Percentage of bootstrap values < 50 % and Bayesian posterior probabilities values < 0.5 are marked by an asterisk (*). Inside of shaded boxes are sequences of species with clamps on bases of basidia.

Phylogenetic analysis

Sequences retrieved in this study and used for phylogenetic analyses are listed in Table 1 and 2. Maximum parsimony (MP) was conducted in PAUP* 4.0b.10 (Swofford 2003) and Bayesian analysis in MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). For Bayesian analysis, a GTR+I+G model of molecular evolution was selected with MrModeltest 2.3 (Nylander 2004). Nonparametric bootstrapping with 1 000 heuristic bootstrap replicates holding a single tree at each step, Max trees set to 1 000, and TBR branch swapping with all characters treated as unordered and gaps treated as missing data were used to assess branch support in the MP analysis. In Bayesian analyses, four Markov chains were run for 2 000 000 generations, sampling every 100th tree with two independent runs per analysis.

RESULTS

Molecular results

Altogether, 60 sequences of the LSU nrDNA region were retrieved in this study. Sequences successfully amplified with the combination of primers LR0R/LR7 were 1306–1752 bp long. When this combination failed, primers LR3R, LR5 and LR16 were used, and their products were 730–933 bp in length. An additional 83 sequences of *Clavaria*, *Clavulinopsis* and *Ramariopsis* and two *Mucronella* Fr. sequences serving as the outgroup were retrieved from GenBank. These sequences were 571–1966 bp long. A final set of 143 sequences was aligned by ClustalW and adjusted manually. This alignment is available at TreeBASE as submission 13184. The unalignable positions 612–654 were removed, and all sequences were truncated at the ends to equal lengths of 838 bp. Out of the total 838 characters, 427 characters were constant, 75 variable characters were parsimony-uninformative, and 336 were parsimony-informative. The 100 most parsimonious trees (MPTs) were obtained (tree length = 2200, CI = 0.3191, RI = 0.7969 and RC = 0.2543) in the parsimony analysis under a heuristic search. Almost identical tree topologies were generated after parsimony and Bayesian analyses. One of the most parsimonious trees is shown in Fig. 1.

The generic circumscription of the genus *Clavaria* follows Corner (1950), except *C. novozealandica* R.H. Petersen, treated by Petersen (1988) in *Clavaria* subg. *Clavulinopsis*. All *Clavaria* species included in our phylogenetic study formed a well-supported, monophyletic group (Fig. 1). The concept of two subgenera of *Clavaria* (subg. *Clavaria* and subg. *Holocoryne*) defined by the presence or absence of clamps was not supported due to the polyphyly of the respective groups though bootstrap and posterior probability values were low. Six well-supported monophyletic groups, each characterized by the presence or absence of clamps, were recognized.

Sequences of *C. greletii* with clamps on the bases of basidia (Fig. 2), and the only dark species of subg. *Holocoryne* included in our study, formed a well-supported '*greletii*' clade (100 % bs, 1.0 pp).

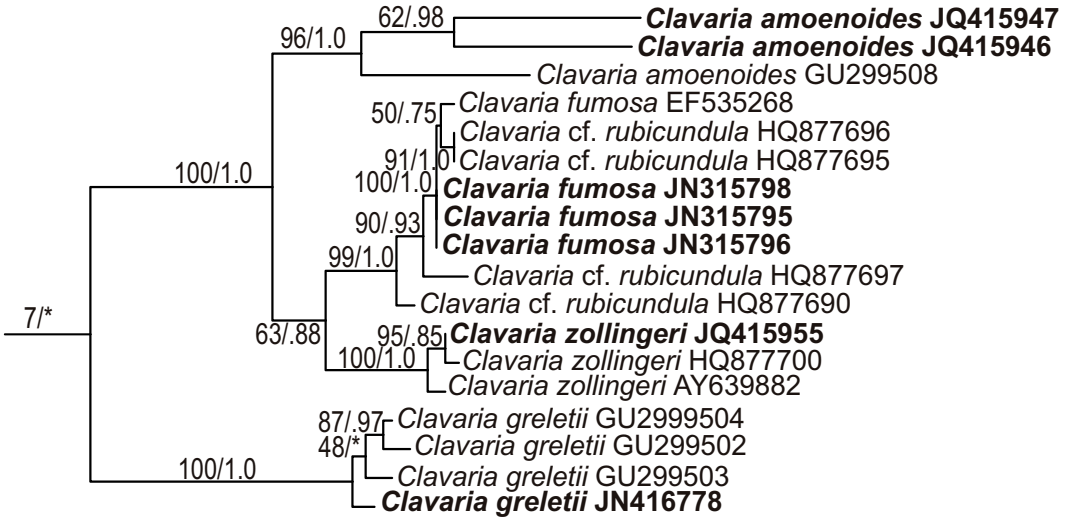

Fig. 2 Phylogenetic relationship within the '*fumosa*' clade and '*greletii*' clade. Numbers above branches represent maximum parsimony bootstrap values (bs) and Bayesian posterior probabilities (pp), respectively. Percentage of bootstrap values < 50 % and Bayesian posterior probabilities values < 0.5 are marked by an asterisk (*). Sequences obtained during this study are in **bold**. For complete maximum parsimony phylogram with details see Fig. 1.

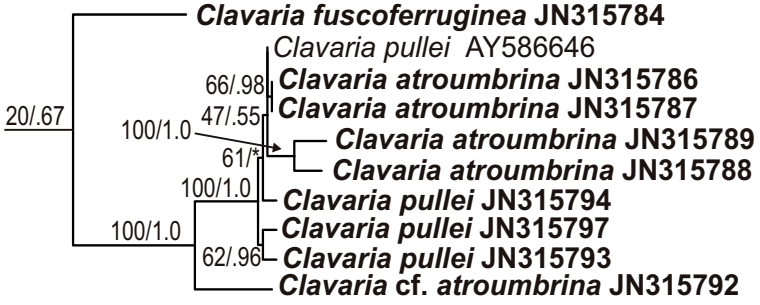

Fig. 3 Phylogenetic relationship within the '*pullei*' clade. Numbers above branches represent maximum parsimony bootstrap values (bs) and Bayesian posterior probabilities (pp), respectively. Percentage of bootstrap values < 50 % and Bayesian posterior probabilities values < 0.5 are marked by an asterisk (*). Sequences obtained during this study are in **bold**. For complete maximum parsimony phylogram with details see Fig. 1.

Clampless *Clavaria* species with dark basidiomata were grouped in three different clades: '*fumosa*', '*pullei*' and '*asperulispora*'. The *fumosa* clade grouped morphologically diverse species without clamps (Fig. 2): three sequences of species with yellow, simple basidiomata and oblong spores (corresponding to *C. amoenoides* Corner, K.S. Thind & Anand) formed a well-supported clade (96 % bs, 1.0 pp); three sequences of a species with branched, violet basidiomata and ovoid to subglobose spores (*C. zollingeri* Lév.) clustered in a highly supported clade (100 % bs, 1.0 pp); and 8 sequences of species with very brittle, pale grey-brown, clustered, simple basidiomata grouped in a highly supported clade (99 % bs, 1.0 pp). However, two species within this clade, *C. fumosa* and *Clavaria* cf. *rubicundula* Leathers, were not clearly separated, suggesting their possible conspecificity.

Species with elastic, unbranched basidiomata, smooth spores and clampless basidia (*C. atroumbrina*, *C. pullei* Donk and *Clavaria* cf. *fuscoferruginea*) formed the *pullei* clade (Fig. 3). Within this clade, the position of the single sequence of *Clavaria* cf. *fuscoferruginea* was differentiated but the delimitation of the other two species was not well supported.

Two species without clamps on the bases of their basidia and with ornamented spores (*C. asperulispora* and *C. atrofusca*) fell within the well-supported *asperulispora* clade. Though there were only four sequences in this clade, the close relationship of *C. asperulispora* sequences was well supported (100 % bs, 1.0 pp) (Fig. 4).

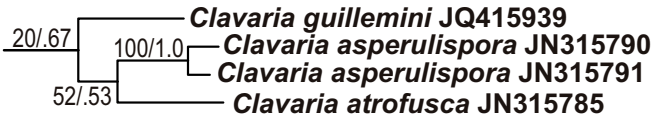

Fig. 4 Phylogenetic relationship within the '*asperulispora*' clade. Numbers above branches represent maximum parsimony bootstrap values (bs) and Bayesian posterior probabilities (pp), respectively. Percentage of bootstrap values < 50 % and Bayesian posterior probabilities values < 0.5 are marked by an asterisk (*). Sequences obtained during this study are in **bold**. For complete maximum parsimony phylogram with details see Fig. 1.

Morphological studies

Spore values for all specimens including detailed morphological analyses are listed in Table 1 and variability of spore length and Q value within each species are presented in Fig. 5 and 6. The spore length for the majority of species overlapped but the average values measured for individual collections did not (Fig. 5). The average values of spore length measured for individual collections of *C. asperulispora* (including the type of *C. neonigrita*), *C. pullei* and *C. atroumbrina* were always smaller, and the average values of spore length for the other species were always higher than 6 µm (Table 1). The variability of spore length seems to be lower in some species than in others. Species with low variability of spore length were *C. fumosa*, *C. pullei* and *C. atroumbrina* (coefficient of variation of all measurements not exceeding 8 %). *Clavaria atrofusca* and *C. greletii* had rather variable spore length (coefficient of variation 13–14 %). Great differences were observed between the spore length for the type (mean length = 8.6 µm) and other specimens (mean length = 7.1 µm) of *C. atrofusca.*

The species did not differ remarkably in spore width, except *C. greletii*, which had spores of a special shape, always wider, on average, than 6 µm. Spores of species with ornamentation were, on average, wider (3.5–5 µm) than those of species with smooth spores (3–3.8 µm) (Table 1).

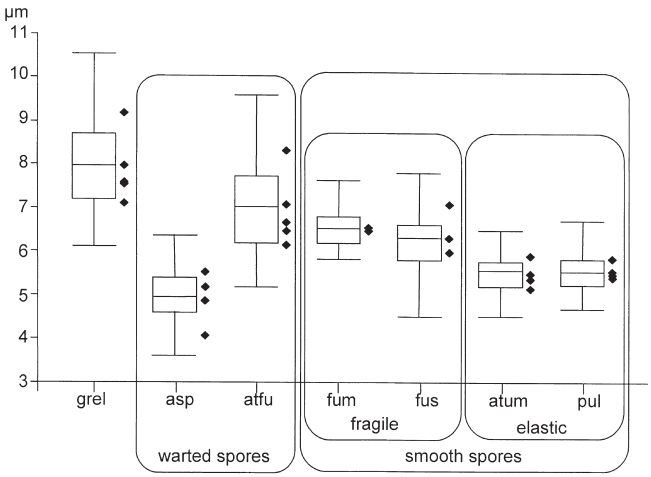

Fig. 5 Comparison of length of spores measured on studied species. The box plot statistics refer to R stat program (www.r-project.org) and are based on all measurements used in the study. Horizontal line crossing the rectangle is labelled at mean value. The dots right of the box are average values of 30 measurements obtained from individual collections listed in Table 2. Morphological groups of species without clamps are outlined by boxes, transparent boxes delimit species with warted spore ornamentation or smooth spores, respectively, shaded boxed delimit species with fragile or elastic basidiomata. – grel = *C. greletii*, asp = *C. asperulispora*, atfu = *C. atrofusca*, fum = *C. fumosa*, fus = *C. fuscoferruginea* complex, atum = *C. atroumbrina*, pul = *C. pullei*.

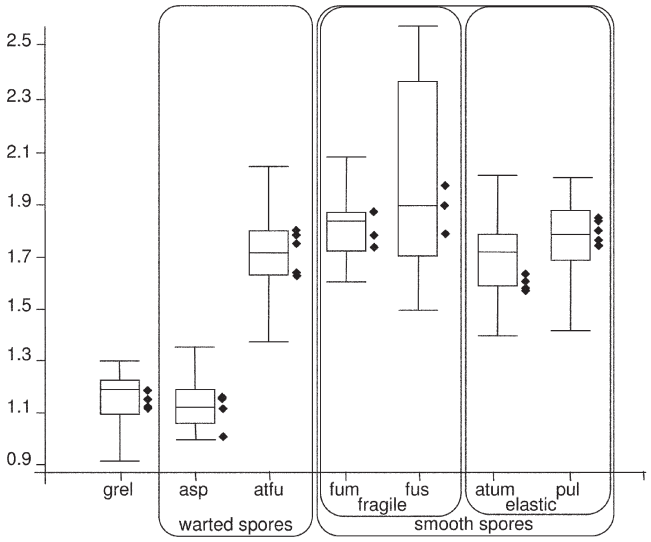

Fig. 6 Comparison of Q value (ratio of length and width) of spores measured on studied species. The box plot statistics refer to R stat program (www.r-project.org) and are based on all measurements used in the study. Horizontal line crossing the rectangle is labelled at mean value. The dots right of the box are average values of 30 measurements obtained from individual collections listed in Table 2. Morphological groups of species without clamps are outlined by boxes, transparent boxes delimit species with warted spore ornamentation or smooth spores, respectively, shaded boxed delimit species with fragile or elastic basidiomata. – grel = *C. greletii*, asp = *C. asperulispora*, atfu = *C. atrofusca*, fum = *C. fumosa*, fus = *C. fuscoferruginea* complex, atum = *C. atroumbrina*, pul = *C. pullei*.

Based on the length/width ratio of spores (Q value), the *Clavaria* species with dark basidiomata formed two morphological groups (Fig. 6). Spores of *C. atrofusca* (including the type of *C. neonigrita*) and *C. greletii* were subglobose to broadly ellipsoid, with Q values not exceeding 1.2 on average. The Q value of the remaining species, with ellipsoid to oblong spores, were on average 1.6–2. The difference between *C. atroumbrina* and *C. pullei* deserves to be explained in detail. These

species differed in Q values: species with Qav less than 1.7 were interpreted as *C. pullei*, while the Qav of 1.74–1.88 was interpreted as *C. atroumbrina*. High infraspecific variability of Q value was observed, especially in the *C. fuscoferruginea* complex (this species complex includes the type, Bresadola's specimen of *C. atrobadia* NCU 596584 and the recent Norwegian collection BRA CR13262), with a coefficient of variation of 12 %. However, since we included only three specimens of this complex in the study, the estimation of species variability will need observation of additional material.

Morphological analysis does not show any difference in spore characteristics between two species with warted spores: *C. asperulispora* (4.0–5.2 × 3.6–5.0 µm, Qav = 1.04–1.16) and *C. neonigrita* type (5.5 × 4.8 µm, Qav = 1.15). Due to lack of DNA sequences of *C. neonigrita*, we did not find characters separating *C. neonigrita* from *C. asperulispora.* Within the *C. fuscoferruginea* complex (species with clustered and fragile basidiomata), the average spore length of the type of *C. fuscoferruginea* was the same as observed in the authentic material of *C. atrobadia*, but they differed in spore width and Q value (Table 1). The recent Norwegian collection BRA CR13262 (with field characteristics identical to *C. fuscoferruginea*) had larger (longer and wider) spores than both type specimens, and it had an intermediate Q value. Spore measurements of both species were included in our statistical analysis (Fig. 5, 6), together with *C. fumosa* (a species with clustered, fragile, but paler basidiomata) with spores similar to those of the *C. fuscoferruginea* complex.

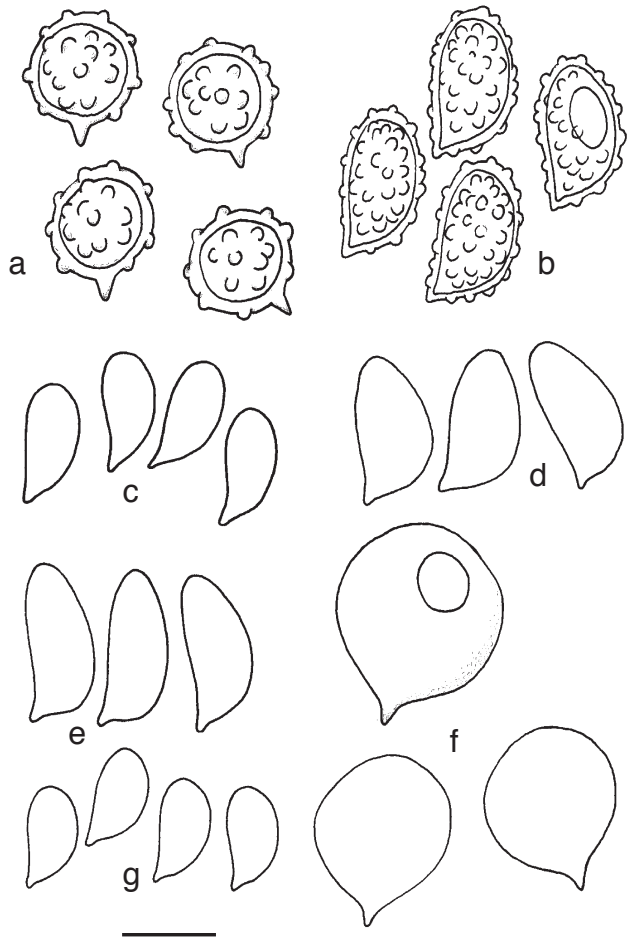

Fig. 7 Basidiospores of *Clavaria* species with dark coloured basidiomata. a. *C. asperulispora*; b. *C. atrofusca*; c. *C. atroumbrina*; d. *C. fumosa*; e. *Clavaria* cf. *fuscoferruginea*; f. *C. greletii*; g. *C. pullei.* — Scale bar = 5 µm.

TAXONOMY

Clavaria asperulispora G.F. Atk. (as '*asperulospora'*), Ann. Mycol. 6: 55. 1908. — Fig. 7, 8, 9

≡ *Ramariopsis asperulispora* (G.F. Atk.) Corner (as '*asperulospora'*), Ann. Bot. Mem. 1: 638. 1950.

Holotypus. USA, New York State, Ithaca Co., Ground Fall Creek Woods, Whetzel, N.H. Long, 3 Aug. 1902 (CUP 13182).

Basidiomata solitary or in small clusters of 2–3, unbranched, cylindrical or irregularly clavate, obtuse and often flattened near the apex, 30–80 mm tall, smooth, dark brown or black, sometimes with an olivaceous tint, tapering towards the base. Fertile portion 2–8 mm broad, sterile base indistinctly delimited, 1–2 mm broad (Fig. 9a). *Flesh* elastic, odour indistinct, taste mild. Reaction to $FeCl_3$ negative. *Spores* (3.7–)4.2–5.3(–5.5) × (3.4–)3.7–4.9(–5.1) µm (average 4.7 × 4.3 µm), Q = 1.04–1.16 (Qav = 1.11), globose to broadly ellipsoid, hyaline, slightly thick-walled, with numerous scales and warts (Fig. 7a, 8b). *Basidia* abruptly clavate, 41.8–68.2(–71.0) × 8.1–11.2(–12.1) µm, clampless, mostly tetrasporic. *Hymenium* thickening, 50–70 µm deep, *subhymenium* pseudoparenchymatic, c. 30 µm deep, composed of hyphae of 3–5 µm wide, interwoven, short cells, sharply delimited from trama. *Trama* composed of parallel hyphae, with cells 70 to more than 100 µm long and 10–15 µm wide, secondarily septate. *Surface of the sterile base* similar to hymenium but without basidia and basidioles. *Pigments* dark grey or blackish, intracellular, present in some hyphae in hymenium and subhymenium. *Clamp connections* absent in all tissues.

Clavaria atrofusca Velen., Novit. Mycol.: 164. 1939. — Fig. 7, 8, 9

Holotypus. Czech Republic, Mnichovice, Božkov, Klokočná, J. Velenovský, Sept. 1922 (PRM 147956).

Basidiomata solitary or in clusters of 2–3, unbranched, cylindrical or tapering towards the apex, obtuse when young, subacute in maturity, 20–50 mm tall, smooth, matt, fuscous black, shortly stipitate, sometimes attenuated at the base. Fertile portion 2–3 mm broad, sterile base indistinctly delimited, 1–2 mm broad (Fig. 9b). *Flesh* elastic, smell indistinct, taste mild.

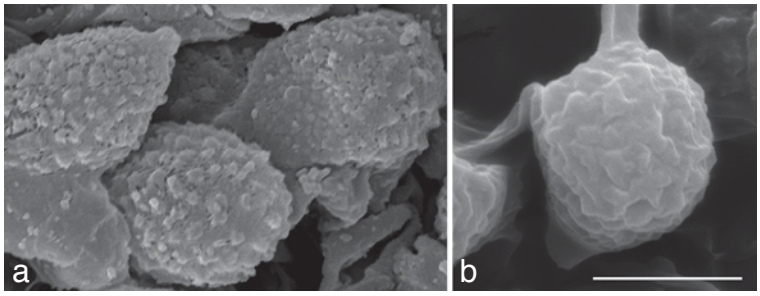

Fig. 8 SEM image of different character of basidiospore ornamentation. a. *Clavaria atrofusca* BRA CR13264; b. *Clavaria asperulispora* C(F) 98786. — Scale bar = 3 µm.

Fig. 9 Basidiomata of *Clavaria* species with dark coloured basidiomata. a. *C. asperulispora* C(F) 98786; b. *C. atrofusca* BRA CR13264; c. *C. atroumbrina* BRA CR13271; d. *C. pullei* (neotype) BRA CR17567; e. *Clavaria* cf. *fuscoferruginea* BRA CR13262; f. *C. fumosa* (epitype) BRA CR15656; g. *C. greletii* BRA CR16173. — Scale bar = 1 cm.

Reaction to $FeCl_3$ negative. *Spores* (5.7–)6.0–8.0(–8.8) × (3.2–)3.6–4.6(–5.0) µm (average 6.9 × 4.1 µm), Q = 1.64–1.81 µm (Qav = 1.73), ellipsoid to oblong, black to brownish black, often with thickened walls, ornamented with c. 0.2–0.3 µm high warts (Fig. 7b, 8a). *Basidia* clavate, 38.9–49.5(–50.2) × 9.0–10.6(–11.1) µm, clampless, mostly tetrasporic. Some basidia and large, obtusely clavate basidioles thick-walled, narrower hyphal terminations in hymenium also rarely thick-walled. *Hymenium* thickening, 60–80(–95) µm deep, *subhymenium* pseudoparenchymatic, 20–30 µm deep, hyphae 2.5–6 µm wide, sharply delimited from trama. *Trama* composed of thin-walled, cylindrical, parallel hyphae, with cells from 50 to more than 100 µm long and 5–20 µm broad, sometimes constricted at the septa, secondarily septate, some narrower hyphae with intracellular pigment. *Surface of the sterile base* similar to hymenium, but with shorter hyphal terminations that are often thick-walled and with rare, mostly sclerified basidia. *Pigments* dark, intracellular in hymenium and subhymenium present. *Clamp connections* absent in all tissues.

Clavaria atroumbrina Corner, Ann. Bot. Mem. 1: 691. 1950. — Fig. 7, 9

= *Clavaria nigrita* auct. non Pers.: Coker, Clavar. U.S.: 43. 1923.

Syntypus. USA, North Carolina, Orange Co., Chapel Hill, in moss and thin grass, under elms and crepe myrtle in lawn, W.C. Coker, 28 July 1917 (NCU 2794, as *Clavaria nigrita*).

Basidiomata solitary or in small clusters of 2–3, unbranched, cylindrical, sometimes slightly clavate, subacute or obtuse, 20–40 mm tall, dark brown, rufous, yellowish or blackish brown, tips blackening with age. Fertile part 1–3 mm broad, sterile base indistinctly delimited, up to 1–2 mm broad, paler, yellowish brown, base white, finely cottony (Fig. 9c). *Flesh* elastic, without smell, taste mild. Reaction to $FeCl_3$ negative. *Spores* (5.0–)5.2–6.1(–6.3) × (2.8–)2.9–3.4(–3.5) µm, (average 5.6 × 3.2 µm), Q = 1.74–1.88 (Qav = 1.81), oblong, often phaseoliform or amygdaliform in side view, hyaline, thin-walled, smooth (Fig. 7c). *Basidia* clavate, 20.5–42.1(–49.8) × 5.4–6.5(–8.1) µm, clampless, tetrasporic, some with dark incrustations. *Hymenium* thickening, 50–80 µm deep, *subhymenium* pseudoparenchymatic, composed of intricate, densely interwoven hyphae, some with incrusted pigments, with cells measuring 20–30(–60) × 2–6 µm, not sharply delimited from trama. *Trama* composed of parallel or slightly interwoven, thin-walled, hyaline hyphae, with cells 70–100 × 3.5–13 µm, some septa with intracellular pigments, and with few incrustations. *Surface of the sterile base* cutis, some of the hyphal terminations with ochraceous intracellular pigments. *Pigments* dark, intracellular, present especially in basidia, also in hymenium and subhymenium. *Clamp connections* absent in all tissues.

Clavaria fumosa Pers.: Fr., Ann. Bot. (Usteri) 15: 31. 1795; Syst. Mycol. 1: 483. 1821. — Fig. 7, 9

Typus (designated here): L0115746, herb. C.H. Persoon.

Ind. loc. The Netherlands, Hab passim autumno ad terram subhumidam ut plurimum.

Epitypus (designated here): Slovakia, Západné Tatry Mts, Zuberec village, Mačie diery Nature Reserve, alt. 800 m, in dry mowed meadow, on limestone, leg. I. Kautmanová, 16 June 2007 (BRA CR15656).

Note. Type specimen, though in good state, lacks any data about date and site of the collection. Epitype is supplemented also by DNA sequence JN315795.

Basidiomata in dense groups, unbranched, fusiform, cylindrical, often twisted, obtuse when young, in maturity subacute to acute, becoming hollow with age, 40–100 mm tall, pale beige, pale greyish brown, sometimes with a violet tint, almost white at the base, tips darkening to brown when drying, surface smooth. Fertile portion 2–5 mm broad, sterile base indistinctly delimited, 2–3 mm broad (Fig. 9f). *Flesh* whitish, extremely fragile, without smell, taste mild. Reaction to $FeCl_3$ negative. *Spores* (6.0–)6.1–7.0(–7.4) × (3.2–)3.4–3.9(–4.1) µm (average 6.5 × 3.5 µm), Q = 1.73–1.89 (Qav = 1.8), oblong, dacryoid or amygdaliform in side view, hyaline, thin-walled, smooth (Fig. 7d). *Basidia* clavate, 47–78 × 8.5–9.8(–10.5) µm, clampless, mostly tetrasporic. *Hymenium* thickening, 60–85 µm deep, *subhymenium* pseudoparenchymatic, c. 30–50 µm deep, composed of hyphae of 2–3 µm wide, loosely interwoven, sharply delimited from trama. *Trama* composed of parallel hyphae, with distinctly inflated cells 20–50(–70) × 5–20(–30) µm that are constricted at septa and often secondarily septate. *Surface of the sterile base* covered by repent hyphae with dispersed, cylindrical or clavate terminal cells measuring c. 15.5–28 × 3–5 µm. *Pigments* and incrustations not observed. *Clamp connections* absent in all parts.

Clavaria* cf. *fuscoferruginea Leathers — Fig. 7, 9

Possible synonyms

= *Clavaria fuscoferruginea* Leathers, Mycologia 48: 281. 1956.

= *Clavaria atrobadia* Corner, Ann. Bot. Mem. 1: 691. 1950.

= *Clavaria nigrita* auct. non Pers.: Bres., Iconogr. Mycol. (Milan): 1105. 1881.

Studied material. Norway, Buskerud, Nedre Eiker, Ryghsettra, in mowed meadow, V. Kautman, 11 Sept. 2009 (BRA CR13262).

Description of collection BRA CR13262 – *Basidiomata* in dense clusters, unbranched, fusiform or cylindrical, often twisted and/or laterally compressed, often with a longitudinal grove on each side, obtuse to subacute, becoming hollow with age, 30–70 mm tall, smooth or longitudinally rugose, fertile part dark brown to cinnamon brown, drying to blackish brown, contrasting with the yellowish coloured base. Fertile portion 2–7 mm broad, sterile base sharply delimited, 2–3 mm broad (Fig. 9e). *Flesh* pale brown or yellowish brown, fragile, smell indistinct, taste mild. Reaction to $FeCl_3$ negative. *Spores* (6.3–)6.6–8.1(–8.2) × (3.2–)3.3–3.8(–3.9) µm (average 7.2 × 3.6 µm), Q = 1.75–2.25 (Qav = 1.99), oblong, narrowly dacryoid or amygdaliform in side view, hyaline, smooth, thin-walled (Fig. 7e). *Basidia* clavate, 50.1–72.0 × 4.5–7.2 µm, mostly with ochraceous intracellular pigments, clampless, mostly tetrasporic. *Hymenium* thickening, 65–80(–90) µm deep, *subhymenium* pseudoparenchymatic, 75–85 µm deep, composed of 2.5–5.5 µm thick hyphae, sharply delimited from trama. *Trama* composed of hyaline or brown-pigmented, smooth, thin-walled, secondarily septate, parallel hyphae with inflated cells measuring 20–110 × (3–)7–18 µm. *Surface of the sterile base* smooth, covered by repent hyphae of 3–4 µm wide. *Pigments* dark, intracellular, present in hymenium only, incrustations absent. *Clamp connections* absent in all parts.

Clavaria greletii Boud., Bull. Soc. Mycol. France 33: 13. 1917. — Fig. 7, 9

Holotypus. France, Savigné (Vienne), sandy grasslands, at the edge of oak forest, Abbé Grelet, 10 Nov. 1913 (PC 0094981).

Basidiomata solitary, unbranched, cylindrical or irregularly clavate, sometimes flattened, obtuse, tapering towards the base, 30–100 mm tall, smooth or finely granulose, black when young, later greyish black to ash-grey, smooth, black and shinning towards the base. Fertile portion 2–5 mm broad, sterile base distinctly delimited, 1–2 mm broad (Fig. 9g). *Flesh* fragile, smell indistinct, taste mild. Reaction to $FeCl_3$ negative. *Spores* (6.4–)7.0–9.0(–9.5) × (5.6–)6.1–7.8(–8.7) µm (average 7.9 × 7.0 µm), Q = 1.13–1.20 (Qav = 1.15), subglobose to ellipsoid, hyaline, smooth, rarely with scattered large warts (asterosporic

form), thin-walled (Fig. 7f). *Basidia* clavate, 44.5–58.2(–60.1) × 7.8–8.9 µm, with loop-like clamps at the bases, tetrasporic. *Hymenium* thickening, 65–80 µm deep, *subhymenium* pseudoparenchymatic, c. 30–40 µm deep, composed of short-celled, interwoven hyphae of 2–5 µm wide, sharply delimited from trama. *Trama* composed of thin-walled, parallel hyphae, with cells often more than 100 µm long and either 3–15 µm broad and cylindrical or 15–20 µm broad and slightly inflated, secondarily septate. *Surface of the sterile base* cutis. *Pigments* ochraceous, intracellular, present in some hyphae in trama and subhymenium, some basidia also pigmented. *Clamp connections* absent in all parts except of basal septa of basidia and basidioles.

Clavaria pullei Donk, Meded. Bot. Mus. Herb. Rijks Univ. Utrecht 9: 86. 1933. — Fig. 7, 9

Typus. Type specimen L(M)2861 cited in Donk (1933) is lost.

Neotypus (designated here). France, Loiret, Estouy, 'les Vaux', alt. 45 m, on calciphilous meadow outgrown by *Juniperus communis*, G. Corriol, 23 Oct. 1999 (BRA CR17657).

Basidiomata solitary or in small clusters of 2–3, unbranched, cylindrical, sometimes slightly clavate, subacute or obtuse, 20–40 mm tall, dark brown, red brown, or yellowish brown, tips blackening with age, paler yellowish brown towards the base, base white and finely cottony. Fertile part 1–3 mm broad, sterile base indistinctly delimited, up to 1–2 mm broad (Fig. 9d). *Flesh* pale brown to yellowish brown, elastic, smell indistinct, taste mild. Reaction to $FeCl_3$ negative. *Spores* (5.0–)5.1–6.0(–6.5) × (2.8–)2.9–3.4(–3.5) µm, (average 5.4 × 3.3 µm), Q = 1.61–1.66 (Qav = 1.63), oblong, often phaseoliform or amygdaliform in side view, hyaline, thin-walled, smooth (Fig. 7g). *Basidia* clavate, 20.2–35.8(–40.0) × 5.1–6.0(–6.2) µm, clampless, bi- or tetrasporic. *Hymenium* thickening, 25–35(–60) µm deep, *subhymenium* pseudoparenchymatic, 20–40 µm deep, composed of intricate and often anastomosed hyphae of 1.5–4.5(–6) µm wide, not sharply delimited from trama. *Trama* composed of parallel or slightly interwoven hyphae, with thin-walled cells that are mostly longer than 100 µm and 3–14 µm broad, secondary septa rare. *Surface of the sterile base* cutis, some of the terminal hyphae with ochraceous pigment. Brown necropigments present in collapsed basidia and also occasionally in narrow (c. 3 µm thick) hyphae in the trama. *Clamp connections* absent in all parts.

KEY TO IDENTIFICATION OF DARK-COLOURED EUROPEAN CLAVARIA SPECIES

1. Basidia with loop-like clamps at the base . . . . . *C. greletii*
1. Basidia without clamps 2
2. Spores thick-walled, distinctly warted 3
2. Spores thin-walled, smooth 4
3. Spores ellipsoid to oblong (Q = 1.64–1.81) . . . *C. atrofusca*
3. Spores globose to subglobose (Q = 1.04–1.16) . . . . . *C. asperulispora*
4. Basidiomata smaller, up to 5 cm, elastic, solitary or in small clusters of 2–4 5
4. Basidiomata often large, 5–10 cm, fragile, in dense clusters 6
5. Spores with Q = 1.61–1.66 . . . . . *C. pullei*
5. Spores with Q = 1.74–1.88 . . . . . *C. atroumbrina*
6. Basidiomata pale, beige, brownish or grey-brown, rarely with violet tinge . . . . . *C. fumosa*
6. Basidiomata dark, tobacco or reddish brown, when drying darker blackish brown . . . . . *C. fuscoferruginea* complex

DISCUSSION

Our molecular study did not confirm the infrageneric classification introduced by Corner (1950) and widely accepted in the literature (Corner 1970, Petersen 1978). This happened mainly because species without clamps at the base of the basidia, and classified in single subgenus *Clavaria*, formed three phylogenetically distant groups. Additionally, *C. greletii* was unrelated to other species with clamps at the base of basidia in the subg. *Holocoryne*. All *Clavaria* species with dark basidiomata formed four distinct morphological groups: 1) species with clamps on the bases of basidia; 2) species without clamps and with clustered, large and fragile basidiomata; 3) species without clamps and with dark, solitary basidiomata and warted spores; and 4) the same as the third except with smooth spores. Each morphological group is discussed separately below.

1. *Species with clamps at the base of the basidia*

The single known European species with dark basidiomata and loop-like clamps at the bases of the basidia is *C. greletii*. This species has been treated as a member of subg. *Holocoryne*, but it is isolated and distant from the type of the subgenus (*C. falcata* Pers. = *C. acuta* Sowerby) in our phylogenetic tree. Relatively large, subglobose spores are typical for this species. Ornamentation observed on part of the spores in certain collection is probably caused by environmental conditions, as has been suggested by Knudsen (1996) for *C. falcata* and *C. incarnata* Weinm. In the field, the species is characterized by a clearly delimited sterile basal part, greyish tints of the basidiomata and a farinaceous appearance of the surface, especially during dry conditions, but this aspect is not always distinct.

Several species with dark basidiomata and basidia with clamps has been described worldwide, however, most of them are extremely rare, often known only from type specimens. *Clavaria avelaneonigrescens* S. Imai, described from Japan (Imai 1930), differs from *C. greletii* by its smaller spores. Corner (1950) and Parmasto (1965) considered these two species related. Petersen (1988) described from New Zealand and Australia cacao-brown *C. cupreicolor* R.H. Petersen and four grey species – purplish grey *C. ardosiaca* R.H. Petersen with broadly ellipsoid spores 8.3–11.2 × 6.8–8.3 µm; pale blue-grey *C. plumbeoargillacea* R.H. Petersen with globose to subglobose spores 8.3–10.4 × 7.9–9.7 µm; and mouse grey *C. muscula* R.H. Petersen and *C. musculospinosa* R.H. Petersen with broadly ellipsoid spores, which are smaller (6.7–8.1 × 5.2–6.3 µm) and smooth in the former and larger (9.4–11.2 × 7.9–9.0 µm) and conspicuously roughned in the latter. Further molecular studies are necessary to resolve the relationships within this group.

2. *Species without clamps at the base of the basidia with clustered, large and fragile basidiomata*

Morphological delimitation of this group does not correspond to the molecular results because sequences morphologically determined as *C. fumosa* and *Clavaria* cf. *rubicundula* retrieved from GenBank (Table 2) are clustered in one clade separate from the sequence of *Clavaria* cf. *fuscoferruginea*. The latter sequence is in the same clade as two other species with smooth spores and unbranched (but solitary and elastic) basidiomata: *C. pullei* and *C. atroumbrina*. The phylogenetic distance between *Clavaria* cf. *fuscoferruginea* and *C. fumosa* is especially interesting because both species are indistinguishable using only spore characteristics.

Clavaria fumosa is the best-known and most likely the most common species with dark basidiomata. This species was described from Europe (Persoon 1796) and is considered to be the only species of the genus *Clavaria* with dark basidiomata that has a cosmopolitan distribution; it is known to be found in

Bolivia (Corner 1970), the USA (Coker 1923) and Java (van Overeem 1923). Sequences of *Clavaria* cf. *rubicundula* retrieved from GenBank and originating from the USA and New Zealand are mixed with our sequences of *C. fumosa*. This finding suggests that they are most likely conspecific and may confirm the transatlantic nature of their distribution. Voucher specimens of *Clavaria* cf. *rubicundula* sequences were characterized by broader spores with a Qav = 1.55 (New Zealand collection HQ877697), lack of vinaceous or red colour similar to a typical *C. fumosa* (USA collection HQ877690) or by narrower spores with Qav = 2.01 or 2.06 (USA collections HQ877695 and HQ877696) (J. Birkebak, pers. comm.). Judging from the original diagnosis of *C. rubicundula* (Leathers 1956), there is no other distinctive characteristic than the iodine smell that can be used for recognizing the two species. The decision about the possible conspecificity of these two species requires additional morphological revision of the corresponding herbarium material and analyses of other molecular markers. *Clavaria fumosa* is the only species of this morphological group reported from Europe so far. In addition to *C. rubicundula*, three other similar species were described from North America: *C. fuscoferruginea*, *C. rufobrunnea* Coker and *C. fumosoides* Kauffman.

Clavaria fuscoferruginea has darker-brown basidiomata without greyish or buff tints and with a distinct, unripe smell of peanuts (Leathers 1956). The only sequence determined as *Clavaria* cf. *fuscoferruginea* included in our study was from a Norwegian collection (BRA CR13262). The colour of the basidiomata was distinctly darker than is known in *C. fumosa*, and molecular analyses confirmed that this specimen is unrelated to *C. fumosa*. Except for the lack of smell of the dried specimen, other characteristics suit the original description of *C. fuscoferruginea* by Leathers (1956). However, Leathers himself admitted that the smell was not always recognizable. Comparison of the spore size of the type with the Norwegian specimen revealed differences in spore length (reflected also in the Q value): the spores of the Norwegian specimens are longer and wider (Table 1). As no recent North American material or respective sequences in GenBank were available, the proximity of these transatlantic populations should be examined in future studies.

Clavaria atrobadia Corner is a species with dubious conception. Corner (1950) published *C. atrobadia* as 'nomen novum' for '*C. nigrita* Bres.', a species originally described from Europe by Persoon (1797). The concept of *C. nigrita* Pers. is also dubious because according to Cooke (1879), part of the authentic material determined by Fries (1821) is *Geoglossum* Pers. (Fries sanctioned the name). Corner (1950) mentioned that he did not examine this species personally, and his brief description of *C. atrobadia* is almost a literal translation of the Latin description of *C. nigrita* by Bresadola (1881): the basidiomata are clustered, fragile, reddish brown, turning to blackish brown when drying and with a faint smell, thus resembling *C. fuscoferruginea*, especially in colour. The spores are described (Bresadola's observations adopted by Corner) as smaller and correspond better to *C. atroumbrina* than to *C. fumosa* or *C. fuscoferruginea*, but considering the quality of light microscopy at that time, this information may be misleading and urgently needs revision. We included in our study a specimen determined as *C. nigrita* by G. Bresadola (NCU 596584), who sent it to W.C. Coker (R.H. Petersen, pers. comm.). Spores of this specimen are identical in length to the type of *C. fuscoferruginea*, but they are narrower and have a higher Q value (Table 1). *Clavaria atrobadia* had not been reported after its re-description by Corner (1950), but further taxonomic studies may confirm that this is identical to *C. fuscoferruginea*.

The third North American species, *C. rufobrunnea*, described from California but published invalidly without a Latin diagnosis (Coker 1947), differs only by the smaller size of basidiomata and darker colour. Corner (1950) suggested that this is another possible synonym of *C. fumosa.* The other North American species, *C. fumosoides*, has cystidia in its hymenium (Coker 1947) and most likely belongs to the genus *Alloclavaria* Dentinger & D.J. McLaughlin.

3. *Species without clamps on basidia, with solitary basidiomata and warted spores*

Among species with simple basidiomata and warted spores, most authors accept only a single species in Europe: *C. atrofusca*, described from Czechoslovakia − currently the Czech Republic (Velenovský 1939). Some European authors (Jülich 1984, Nitare 1988, Knudsen 1997) applied the name *C. asperulispora* − described from the USA (Atkinson 1908) for this species, offering *C. atrofusca* in synonymy. Petersen & Olexia (1969) and Corner (1970) treated *C. atrofusca* and *C. asperulispora* as two distinct species. Our morphological study revealed a difference in the Q value of spores, confirmed by molecular support that clearly distinguished two morphologically and phylogenetically related species. The *asperulispora* clade (Fig. 4) also contains one sequence of *C. guilleminii* Bourdot & Galzin that was collected and identified by Olariaga (2009), who described it as a species with small whitish basidiomata, reddish stipe, clampless basidia and rather small (5.4 × 2.7 µm), smooth, thin-walled spores.

Another species with warted spores originates from the USA: *C. neonigrita*, with cystidia on the sterile base of basidiomata (Petersen & Olexia 1969). No recent collections of this species were found. Spore measurements of the type suggest its affinity to *C. asperulispora*, in agreement with the original diagnosis (Petersen & Olexia 1969). Thick-walled cystidia on the sterile base may correspond to thick-walled basidia descending as thick-walled hyphal terminations towards the base of basidiomata, and such features have been observed in restricted numbers in several collections of both *Clavaria* species with dark basidiomata and warted spores.

Petersen & Olexia (1969) used the apex of basidiomata as the determining characteristic in the key to the species of *Clavaria* with spiny spores (we treat spore ornamentation as warted in this paper). They interpreted the apex of *C. atrofusca* as rounded to turbinate, similar to *C. neonigrita*, and this characteristic was used for its delimitation from *C. asperulispora* (the apex being acute). The original diagnosis of Atkinson (1908), descriptions of European authors (Nitare 1988, Roberts 2007), and our recent observations described the basidiomata of *C. asperulispora* as having blunt and often flattened apices. On the other hand, blunt apices have also been observed on *C. atrofusca*. Schild (1971) reported basidiomata of *C. atrofusca* with blunt-rounded apices that are rarely acute, and we also confirmed the presence of subacute apices in some of our recent collections. Considering the variability of basidiomata and the confusion in the literature, the shape of the basidiome apex should be omitted from modern keys to the identification of *Clavaria* species.

4. *Species without clamps on basidia, with solitary basidiomata and smooth spores*

This group also includes two species described from two continents and treated variously in the literature. *Clavaria pullei*, described from Europe (Donk 1933), and *C. atroumbrina*, described from the USA (originally under the name *C. nigrita* by Coker 1923), are synonymized by some authors (Jülich 1984, Knudsen 1997) though separated by others (Corner 1970, Roberts 2007). Roberts (2007) noted the differences in the spores of the two species, but he was uncertain about their delimitation due to a lack of comparative type studies. *Clavaria pullei* has never been re-described. According to the

original diagnosis (Donk 1933), it is defined as bearing bisporic basidia and ovoid spores '4.75–6.75 × 4.25 µm'. As the type specimen cited in protologue was not found in L and is probably lost, and no other collection of *C. pullei* has been found there, we propose a neotype, based on the specimen from France, which has been sequenced.

Although we did not confirm the presence of bisporic basidia in our recent collections, we applied the concept of wider spores and classified collections with Qav = 1.7 as *C. pullei*. Our spore measurements of the two species were very similar (Table 1). We tested the hypothesis that two species could be delimited by Q value, *C. pullei* with Qav = ± 1.6 and *C. atroumbrina* ± 1.8. All sequences of *C. atroumbrina* and *C. pullei* form one well-supported clade, but within the clade, only two North American collections of *C. atroumbrina* have high support. Eight of nine sequences included in our study support our hypothesis of the two-species concept; the exception is the sequence from Great Britain JN315792 (K(M)143730), which is distantly placed from all eight of those sequences.

All three of our collections determined as *C. pullei* originated from south-western Europe (France and Spain) and form one well-supported clade. In contrast, the sister clade of *C. atroumbrina* is composed of collections from North Carolina (USA) and Norway, and it confirms the transatlantic nature of its distribution. The British specimen might represent a third species, but more sequences and samples for morphological data and also more field observations are necessary to answer this and other questions. In this study, we adopted the concept of two species because it seems to be the most probable explanation of the morphological and genetic variability of the group.

Notes on the remaining Clavaria species with dark basidiomata

Of the remaining species, *C. nigricans* Lloyd, described from Chile (Lloyd 1917), has small, black basidiomata and smooth, oval spores. Corner (1950) considered this to be a dubious species, perhaps allied with *Clavulina cinereoatra* (Rick) D.A. Reid & Hedger, which was described from Brazil (Rick 1906) and has very large subglobose spores and a white stem that later becomes straw-coloured.

CONCLUSIONS

This study includes seven *Clavaria* species with dark basidiomata classified into four morphological groups. Our phylogenetic analysis of the LSU region does not support the infrageneric classification of these species in two subgenera, based on the presence of loop-like clamps on the bases of basidia. Representatives of both subgenera fall within polyphyletic groups. The morphological groups recognized in this study correspond to four well-supported clades suggested by our phylogenetic analysis (with the exception of *Clavaria* cf. *fuscoferruginea*):

a) Species with clamps at the bases of basidia are represented by a single European species (*C. greletii*) which is also characterized by subglobose, relatively large, smooth spores.
b) Species without clamps at the bases of basidia and with clustered fragile basidiomata, represented by *C. fumosa* and *Clavaria* cf. *fuscoferruginea* in Europe, are members of two unrelated groups. Sequences of *C. fumosa* are clustered in a clade that includes species with basidiomata of variable form and colour. Together with sequences of extra-European collections of *Clavaria* cf. *rubicundula*, they form one clade, and judging from the description of *C. rubicundula*, this species is most likely synonymous with *C. fumosa*. *Clavaria* cf. *fuscoferruginea* is not related to *C. fumosa*, but it is related to other species with dark, simple, elastic basidiomata, clampless basidia and smooth spores (*pullei* clade), although the proximity is not well supported. *Clavaria atrobadia* is a species with dubious conception that is most likely the synonym of *C. fuscoferruginea*.
c) Our phylogenetic analyses show strong support for the recognition of two species with warted spores, simple basidiomata and clampless basidia. This is in agreement with spore characteristics: *C. asperulispora* has subglobose spores (Qav up to 1.2), and *C. atrofusca* has distinctly narrower, ellipsoid spores (Qav up to 1.6). The apices of their basidiomata have been variously interpreted in the literature; this characteristic appears to be quite variable and is useful only for orientation in the field (*C. atrofusca* often has acute tips), being useless for dried specimens.
d) Both phylogenetic and morphological analyses revealed the close affinity of *C. atroumbrina* and *C. pullei*, species with simple and elastic basidiomata, clampless basidia and smooth spores. We delimited these species based on the wider spores (with Qav up to 1.7) of the latter. Sequences of *C. pullei* form a monophyletic group, but sequences of *C. atroumbrina* are paraphyletic because of a single sequence that is sister to all others in the *pullei* clade. Our study does not contain suitable data to explain the position of the isolated sequence determined as *C. atroumbrina*. We treat both species separately in this paper, but further studies based on more samples and more sequenced genes are required as the positions of sequences in this clade are not well supported.

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