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

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**Key words**
basidiospores
European fungi
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. asperulíspora* and *C. atrofusca* are two distinct species recognized by the shape of their spores, and the name *C. neo-nigra* is a possible synonym of *C. asperulíspora*; 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 atrobádia* is a dubious species, the name being most likely a synonym of *C. fuscoferruginea*; 4) two species with close morphological and phylogenetic affinity, *C. atróbrímna* 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. asperulíspora*, *C. atróbrímna* 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* by Corner (1950): *Clavaria, Clavulinopsis* Overeem and *Ramaropsis* (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* Holmst., 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, *Ramaropsis 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. groletii* 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 B, BIO, C, CUP, GB, K, L, MA, MIC, NCU, PC, PRM, SAM, TENV 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 (Korokin, Czech Republic). The studied material also contained types or original material of C. atrufusca Velen., C. asperulispora G.F. Atk., C. atrobadia Corner, C. neonigrita R.H. Petersen, C. atromunbrina Corner, C. funosa, 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 & Oxelia (1969) and Corner (1950, 1970) were used.

Macromorphological characters were observed on fresh specimens. Chemical reactions to 10 % FeCl₃ 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.

### 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 atrufusca  | BRA CR13272 – Norway             | 5.8 – 6.7        | 3.8 – 4.1        | 1.64| JN315785    |
|                     | BRA CR13264 – Norway             | 6.3 – 7.1        | 3.5 – 4.1        | 1.75| JN315785    |
|                     | PRM 147956 – Czech Rep. (holotypus) | 8.0 – 8.6   | 4.4 – 4.7        | 1.81|             |
|                     | C(F) 39714 – Denmark             | 5.9 – 6.4        | 3.5 – 3.9        | 1.67|             |
|                     | Schmid 140 – Switzerland         | 5.4 – 6.1        | 3.1 – 3.5        | 1.77|             |
| Clavaria asperulispora | CUP(A) 13162 – USA (holotypus)   | 5.0 – 5.2        | 4.6 – 5.0        | 1.04| JN315791    |
|                     | C(F) 89796 – Sweden              | 4.4 – 4.9        | 3.7 – 4.3        | 1.16| JN315790    |
|                     | K(M) 143814 – Great Britain      | 3.6 – 4.0        | 3.2 – 3.6        | 1.13| JN315790    |
| Clavaria neonigrita | TENV 037787 – USA (holotypus)    | 4.8 – 5.5        | 4.3 – 4.8        | 1.15|             |
| Clavaria pullei     | BRA CR13101– Slovakia            | 4.7 – 5.1        | 2.7 – 3.1        | 1.66|             |
|                     | GC 99102304 – France (neotypus)  | 4.8 – 5.3        | 2.8 – 3.2        | 1.64| JN315793    |
|                     | BIO10231 – Spain                 | 5.3 – 5.9        | 3.3 – 3.7        | 1.61|             |
|                     | BIO12378 – Spain                 | 5.0 – 5.4        | 3.1 – 3.3        | 1.62| JN315794    |
| Clavaria atromunbrina | BRA CR13265 – Norway             | 4.8 – 5.5        | 2.7 – 3.0        | 1.88| JN315786    |
|                     | BRA CR13271 – Norway             | 5.1 – 5.7        | 2.7 – 3.1        | 1.86| JN315787    |
|                     | SAV F3139 – Czech Republic       | 5.1 – 5.5        | 2.8 – 3.0        | 1.86|             |
|                     | NCU 2794 – USA (syntypus)        | 5.2 – 5.7        | 2.8 – 3.1        | 1.86|             |
|                     | TENN 032685 – USA                | 5.2 – 5.6        | 2.9 – 3.1        | 1.77|             |
|                     | TENN 031091 – USA                | 5.0 – 5.5        | 2.8 – 3.2        | 1.74| JN315788    |
|                     | TENN 030946 – USA                | 5.1 – 5.5        | 2.9 – 3.2        | 1.74| JN315796    |
|                     | K(M) 143730 – Great Britain      | 5.4 – 5.9        | 3.0 – 3.3        | 1.82| JN315792    |
| Clavaria fumosa     | BRA CR748 – Slovakia             | 6.0 – 6.6        | 3.4 – 3.8        | 1.73| JN315798    |
|                     | BRA CR115655 – Slovakia          | 6.0 – 6.5        | 3.2 – 3.4        | 1.89| JN315796    |
|                     | BRA CR115656 – Slovakia (epitypus) | 6.1 – 6.5      | 3.3 – 3.4        | 1.78| JN315795    |
|                     | BRA CR16039 – Czech Republic     | 5.9 – 6.6        | 3.3 – 3.5        | 1.86|             |
|                     | BRA CR13262 – Norway             | 5.1 – 6.1        | 3.2 – 3.5        | 1.77|             |
|                     | GC00292401 – France              | 5.8 – 6.4        | 3.1 – 3.4        | 1.88|             |
| Clavaria greletii   | SAV F1988 – Slovakia             | 7.2 – 8.0        | 5.8 – 7.3        | 1.20| GU299504    |
|                     | PC 0094981 – France (holotypus)  | 6.6 – 7.6        | 5.9 – 6.7        | 1.13|             |
|                     | GC08101403 – France              | 8.2 – 9.1        | 7.2 – 8.0        | 1.14| GU299502    |
|                     | BRA CR13694 – Slovakia           | 6.3 – 7.3        | 6.5 – 6.7        | 1.13|             |
|                     | K(M) 143840 – Great Britain      | 6.5 – 7.6        | 6.5 – 6.7        | 1.17| GU299503    |
| Clavaria fuscoferruginea complex | BRA CR13262 – Norway       | 6.6 – 7.2        | 3.3 – 3.6        | 1.99| JN315784    |
|                     | MIC 10089 – USA (holotypus)      | 5.6 – 6.0        | 3.1 – 3.4        | 1.80|             |
|                     | NCU 596584 – USA                | 5.4 – 6.0        | 2.6 – 2.9        | 2.12|             |
**Species** | **Country of origin** | **Voucher ID** | **GenBank ID**  
--- | --- | --- | ---  
**Clavaria amoenoïdes** | United Kingdom | K(M) 145803 | JQ415946  
**Clavaria argillosa** | Slovakia | BRA CR16025 | JQ415930  
**Clavaria falcatula** | United Kingdom | BRA CR12873 | JQ415931  
**Clavaria fragilis** | Spain | BRA CR15978 | JQ415932  
**Clavaria guilleminii** | Spain | BRA CR16017 | JQ415933  
**Clavaria incarnata** | Spain | BRA CR16030 | JQ415934  
**Clavaria nigripes** | Denmark | TL 13295 | JN416778  
**Clavaria guillerminii** | Spain | BIO12566 | JQ415939  
**Clavaria rosea** | United Kingdom | K(M) 135940 | JQ415928  
**Clavaria straminea** | Slovakia | BRA CR12809 | JX069827  
**Clavaria zollingeri** | Norway | MA-Fungi 53113 | JQ415948  
**Clavaria pullei** | France | GC 02092801 | J315797  
**Clavaria rosea** | United Kingdom | K(M) 135940 | JQ415928  
**Clavaria pullei** | Denmark | CF 02092801 | JQ415929  
**Clavaria straminea** | Slovakia | BRA CR12809 | JX069827  
**Clavaria zollingeri** | Norway | MA-Fungi 53142 | JQ415955  
**Clavaria sp.** | Spain | BIO12762 | JQ415960  
**Clavariopsis luteolus** | Spain | BRA CR16696 | JQ415959  
**Clavariopsis sp.** | Spain | MA-Fungi 67771 | JQ415957  
**Ramariopsis canaliculata** | Spain | BRA CR16019 | JX12902  
**Ramariopsis sp.** | Spain | BRA CR16696 | JX12902

**Table 2**  
List of collections and LSU nrDNA sequences used in this study for phylogenetic analysis.
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, sampled 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 primers LR3R, LR5 and LR16 retrieved in this study. Sequences successfully amplified with the primers LR3R, LR5 and LR16 retrieved in this study. Altogether, 60 sequences of the LSU nrDNA region were re-

100/1.0
63/88
50/75
99/1.0
62/98
91/1
90/93
7*/
62/98
91/1
90/93
99/1.0
63/88
50/75
100/1.0

Clavaria fumosa
Clavaria fumosa JN315798
Clavaria fumosa JN315795
Clavaria fumosa HQ877695
Clavaria fumosa
Clavaria fumosa JN315795

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.
Clampless *Clavaria* species with dark basidiomata were group-
ed in three different clades: ‘fumosa’, ‘pullei’ and ‘asperulispora’. 
The fumosa clade grouped morphologically diverse species 
without clamps (Fig. 2); three sequences of species with yel-
low, simple basidiomata and oblong spores (corresponding to 
*C. amoenoideas* Corner, K.S. Thind & Anand) formed a well-
supported clade (96 % bs, 1.0 pp); three sequences of a spe-
cies 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 spe-
cies within this clade, *C. fumosa* and *Clavaria* cf. *rubicundula* 
Leathers, were not clearly separated, suggesting their possible 
conspecifity.

Species with elastic, unbranched basidiomata, smooth spores 
and clampless basidia (*C. atrobrmina*, *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).

**Morphological studies**

Spore values for all specimens including detailed morphologi-
cal 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. atrobrmina* were always 
smaller, and the average values of spore length for the other 
species were always higher than 6 μm (Table 1). The vari-
ability 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. atrobrmina* (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.](image)

**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.

![Fig. 6 Comparison of Q value (ratio of length and width) of spores measured 
on studied species.](image)

Based on the length/width ratio of spores (Q value), the *Clava-
ria* 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 el-
lipsoid, 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. atrobr-
mina* 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.

**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 FeCl₃ 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. 9 Basidiomata of Clavaria species with dark coloured basidiomata. a. C. asperulispora C(F) 98786; b. C. atrofuscus 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 $\text{FeCl}_3$, negative. Spores $(5.7–)6.0–8.0(–8.8) \times (3.2–)3.6–4.6(–5.0) \mu m$ (average $6.9 \times 4.1 \mu m$), $Q = 1.64–1.81$ (average $Q = 1.73$), ellipsoid to oblong, black to brownish black, often with thickened walls, ornamented with c. 0.2–0.3 $\mu m$ high warts (Fig. 7b, 8a). Basidia clavate, $38.9–49.5(–50.2) \times 9.0–10.6(–11.1) \mu m$, clampless, mostly tetrasporic. Some basidia and large, obtusely clavate basidioles thick-walled, narrower hypal terminations in hymenium also rarely thick-walled. 

**Hymenium** thickening, 60–80(–95) $\mu m$ deep, subhyphum pseudoparenchymatic, 20–30 $\mu m$ deep, hyphae 2.5–6 $\mu m$ wide, sharply delimited from trama. Trama composed of thin-walled, cylindrical, parallel hyphae, with cells from 50 to more than 100 $\mu m$ long and 5–20 $\mu m$ broad, sometimes constricted at the septa, secondarily septate, some narrower hyphae with intracellulig insect. Surface of the sterile base similar to hymenium, but with shorter hypal 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 atrobrunina** Corner, Ann. Bot. Mem. 1: 691. 1950. — Fig. 7, 9

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

**Basidioïd* 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 $\text{FeCl}_3$, negative. Spores $(5.0–)5.2–6.1(–6.3) \times (2.8–)2.9–3.4(–3.5) \mu m$, (average $5.6 \times 3.2 \mu m$), $Q = 1.74–1.88$ (Qav = 1.81), oblong, often phaseoliform or amygdaliform in side view, hyaline, thick-walled, smooth (Fig. 7c). Basidia clavate, $20.5–42.1(–49.8) \times 5.4–6.5(–8.1) \mu m$, clampless, tetrasporic, some with dark incrustations. Hymenium thickening, 50–80 $\mu m$ deep, subhyphum pseudoparenchymatic, composed of intricate, densely interwoven hyphae, with incrusted pigments, with cells measuring 20–30(–60) $\times 2–6 \mu m$, not sharply delimited from trama. Trama composed of parallel or slightly interwoven, thin-walled, hyaline hyphae, with cells 70–100 $\times 3.5–13 \mu m$, some septa with intracellular pigments, and with few incrustations. Surface of the sterile base cuts, some of the hypal terminations with ochreous intracellulig hyphae. 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; Studied material. Norway: Buskerud, Nedre Elker, Rygsetta, 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 groove 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 $\text{FeCl}_3$, negative. Spores $(6.3–)6.6–8.1(–8.2) \times (3.2–)3.3–3.8(–3.9) \mu m$, (average $7.2 \times 3.6 \mu m$), Qav = 1.75–2.25 (Qav = 1.99), oblong, narrowly dacyroid or amygdaliform in side view, hyaline, smooth, thin-walled (Fig. 7e). Basidia clavate, 50.1–72.0 $\times 4.5–7.2 \mu m$, mostly with ochreous intracellular pigments, clampless, mostly tetrasporic. Hymenium thickening, 65–90 $\mu m$ deep, subhyphum pseudoparenchymatic, 75–85 $\mu m$ deep, composed of 2.5–5.5 $\mu m$ thick hyphae, sharply delimited from trama. Trama composed of hyaline or brown-pigmented, smooth, thick-walled, secondarily septate, parallel hyphae with incrusted cells measuring 20–110 $\times (3–)7–18 \mu m$. Surface of the sterile base smooth, covered by repent hyphae of 3–4 $\mu m$ 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

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

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

Possible synonyms

Description of collection BRA CR13262 — **Basidiomata** in dense clusters, unbranched, fusiform or cylindrical, often twisted and/or laterally compressed, often with a longitudinal groove 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 $\text{FeCl}_3$, negative. Spores $(6.3–)6.6–8.1(–8.2) \times (3.2–)3.3–3.8(–3.9) \mu m$, (average $7.2 \times 3.6 \mu m$), Qav = 1.75–2.25 (Qav = 1.99), oblong, narrowly dacyroid or amygdaliform in side view, hyaline, smooth, thin-walled (Fig. 7e). Basidia clavate, 50.1–72.0 $\times 4.5–7.2 \mu m$, mostly with ochreous intracellular pigments, clampless, mostly tetrasporic. Hymenium thickening, 65–90 $\mu m$ deep, subhyphum pseudoparenchymatic, 75–85 $\mu m$ deep, composed of 2.5–5.5 $\mu m$ thick hyphae, sharply delimited from trama. Trama composed of hyaline or brown-pigmented, smooth, thick-walled, secondarily septate, parallel hyphae with incrusted cells measuring 20–110 $\times (3–)7–18 \mu m$. Surface of the sterile base smooth, covered by repent hyphae of 3–4 $\mu m$ 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. FRAÎNCHE, Savigné (Vienne), sandy grasslands, at the edge of oak forest, Abbé Grelet, 10 Nov. 1913 (PC 0094881).

**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 shining 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 $\text{FeCl}_3$, negative. Spores $(6.4–)7.0–9.0(–9.5) \times (5.6–)6.1–7.8(–8.7) \mu m$, (average $7.9 \times 7.0 \mu m$), Qav = 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. Corioli, 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 FeCl₃ negative. *Spores* (5.0–)5.1–6.0(–6.8) × (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). *Basidium* clavate, 20.2–35.8(–40.0) × 5.1–6.0(–6.2) µm, clampsless, 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*
2. Basidia without clamps .................................. 2
3. Spores thick-walled, distinctly warted ................. 3
4. Spores thin-walled, smooth ............................ 4
5. Spores ellipsoid to oblong (Q = 1.64–1.81) .............................. *C. atrofuscus*
6. Spores globose to subglobose (Q = 1.04–1.16) .............. *C. asperulispora*
7. Basidiomata smaller, up to 5 cm, elastic, solitary or in small clusters of 2–4 .................. 5
8. Basidiomata often large, 5–10 cm, fragile, in dense clusters .................................................. 6
9. Spores with Q = 1.61–1.66 ................................. *C. pullei*
10. Spores with Q = 1.74–1.88 ................................... *C. atrobrinia*
11. Basidiomata pale, beige, brownish or grey-brown, rarely with violet tinge .......................... *C. fuscosa*
12. 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 warty 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* = *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 farnaceous 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 avellaneoaeigrecens* 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. ardosiae R.H. Petersen with broadly ellipsoid spores 8.3–11.2 × 6.8–8.3 µm; pale blue-grey *C. plumbeoargillaec* 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 roughened 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. *rubricundula* 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 other two species with smooth spores and unbranched (but solitary and elastic) basidiomata: *C. pullei* and *C. atrobrinia*. 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 originating from the USA and New Zealand are mixed with our sequences of *C. fumosa*. This finding suggests that they are the 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* Kaufman.

*Clavaria fuscoferruginea* has darker-brown basidiomata without greyish or buff tints and with a distinct, urpique 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 atrobatia* Corner is a species with dubious conception. Corner (1950) published *C. atrobatia* 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. atrobatia* 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. atrombrina* than to *C. fumosa* or *C. fuscofer-

ruginosa*, 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 described it as a species with small whitish basidiomata, reddish stipe, clampsless basidium 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 hypal 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 basidiom apex should be omitted from modern keys to the identification of *Clavaria* species.

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. *Asperulispora* clade (Fig. 4) also contains one sequence of *C. guilemmini* Bourdot & Galzin that was collected and identified by Oliariaga (2009), who described it as a species with small whitish basidiomata, reddish stipe, clampsless basidium and rather small (5.4 × 2.7 μm), smooth, thin-walled spores.

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. atrombrina*, 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 basidium and ovoid spores 4.75–6.75 x 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 basidium 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 = ± 1.8 and C. atrobrina ± 1.8. All sequences of C. atrobrina and C. pullei form one well-supported clade, but within the clade, only two North American collections of C. atrobrina 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. atrobrina 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 cinereocarotata (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 (pulliei clade), although the proximity is not well supported. Clavaria atrobratica 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. asperulisperi 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. atrobrina 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. atrobrina 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. atrobrina. 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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REFERENCES

Atkinson GF. 1908. Notes on some new species of fungi from the United States. Annales Mycologici 6: 54–62.
Bresadola G. 1891. Fungi Tridentini novi, vel nondum delineati, descripti, et iconibus illustrati 1, 1: 62.
Burt EA. 1922. The North American species of Clavaria with illustration of the type specimens. Annals of the Missouri Botanical Garden 9: 1–78.
Coker WC. 1923. The club and coral mushrooms (Clavarias) of the United States and Canada. Dower Publications, Canada.
Cooke WC. 1947. Further notes on Clavarias with several new species. Journal of the Elisha Mitchell Scientific Society 63: 43–67.
Cooke MC. 1879. Mycographia seu icones fungorum: figures of fungi from all parts of the world, vol. 1, 1: 205.
Corner EJH. 1950. A monograph of Clavaria and allied genera. Oxford University Press, United Kingdom.
Corner EJH. 1967. Notes on Clavaria. Transactions of the British Mycological Society 50: 33–44.
Corner EJH. 1970. Supplement to a monograph of Clavaria and allied genera. Nova Hedwigia Beihfte 33: 1–299.
Donk MA. 1933. Revision der Niederländischen Heterobasidiomycetae und Homobasidiomycetae—Aphyllophoraceae. Mededelingen van het Botanisch Museum en Herbarium van de Rijksuniversiteit te Utrecht 9: 74–100.
Fries EM. 1821. Systema mycologicum. Lund, Sweden.
Gårdenfors U (ed). 2005. The 2005 Red Lists of Swedish species. ArtDatabanken, SLU, Sweden.
