

Multilocus phylogenetic reconstruction of the Clavariaceae (Agaricales) reveals polyphyly of agaricoid members

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Abstract: The genus *Camarophylloopsis* contains species with lamellate (agaricoid) basidiomes in the family Clavariaceae (Agaricales), a group otherwise dominated by club-like (clavarioid) or branched (coralloid) forms. Previous studies have suggested that species classified in *Camarophylloopsis* occur in two independent lineages. We reconstructed a multilocus phylogeny of the *Clavaria-Camarophylloopsis-Clavicornona* clade in the Clavariaceae using RNA polymerase II second largest subunit (*rpb2*), nuclear ribosomal 28S, and nuclear ribosomal ITS1-5.8S-ITS2 regions data and detected three independent groups of agaricoid fungi, including the genera *Camarophylloopsis*, *Hodophilus*, and *Lamelloclavaria* gen. nov., which distinctly differ in their pileipellis structure. In all, nine major lineages within the *Clavaria-Camarophylloopsis-Clavicornona* clade were recovered: *Clavaria* sensu stricto, *Camarophylloopsis* sensu stricto, *Hodophilus*, the *Clavaria pullei* clade, the *Clavaria fumosa* clade, *Lamelloclavaria* gen. nov., the *Clavaria atrofusca* clade, *Holocoryne* (= *Clavaria* sect. *Holocoryne*), and *Clavicornona*. *Clavaria* is paraphyletic and represented by five clades. Additional gene sampling is necessary to determine and confirm relatedness of these lineages before splitting *Clavaria* into additional genera.

Key words: Agaricomycetes, Basidiomycota, evolution, systematics

INTRODUCTION

The family Clavariaceae Chevall. sensu stricto, comprising seven genera and at least ca. 125 species (Birkebak et al. 2013), contains a diverse assemblage of basidiome morphologies (FIG. 1) (Dentinger and McLaughlin 2006, Matheny et al. 2006, Larsson 2007, Birkebak et al. 2013). The most frequent morphotype

is a single club-shaped (clavarioid) or branched (coralloid) basidiome represented by the genera *Clavaria* L.: Fr. (abbreviated *Cl.*), *Clavulinopsis* Overeem, *Ramariopsis* (Donk) Corner and *Mucronella* Fr. *Mucronella* occupies a well-supported position sister to the remaining groups in the family (Birkebak et al. 2013). *Clavulinopsis* and *Ramariopsis* form a well-supported monophyletic group. All agaricoid members of the family (with a differentiated pileus, stipe, and lamellate hymenophore) are currently classified in the single genus *Camarophylloopsis* Doty. The genus *Hyphodontiella* Å. Strid produces resupinate morphotypes and, according to Birkebak et al. (2013), is poorly supported as the sister group to a clade of *Clavicornona-Clavaria-Camarophylloopsis*. The genus *Clavicornona* produces basidiomes that are inflated upward and have a sterile upper surface but lack lamellar modification of the hymenophore. Although traditionally classified in the Hygrophoraceae Lotsy, the agaricoid genus *Camarophylloopsis* has a phylogenetic affinity with the Clavariaceae (Matheny et al. 2006) and was later shown as nested within the genus *Clavaria* (Birkebak et al. 2013). In the latter work, *Camarophylloopsis* was recovered in two separate clades, but resolution and support were insufficient in this single gene study to reject the monophyly of *Camarophylloopsis*. The two clades recovered corresponded to *Camarophylloopsis* subgenus *Camarophylloopsis* and *Camarophylloopsis* subgenus *Hodophilus* (Singer) Arnolds, which can be separated by the structure of the pileipellis.

The aims of this study are to investigate the systematics of the genus *Camarophylloopsis* further with the following objectives: (i) to produce a supermatrix to test the monophyly of the genus *Camarophylloopsis*; (ii) to assess the taxonomic relationships between agaricoid Clavariaceae members and other genera of the family; and (iii) to propose a modified taxonomic arrangement based on these new evolutionary relationships.

MATERIALS AND METHODS

Morphological examinations.—Macromorphological descriptions were prepared from fresh collections. Color nomenclature standards followed Kornerup and Wanscher (1967). All micro-morphological characters were observed under an Olympus CX-41 light microscope with an oil-immersion lens at a magnification of 1000×. All drawings of microscopic structures, with the exception of basidiospores, were made with a camera lucida using an Olympus U-DA drawing attachment at a projection scale of 2000×. Basidiospores were scanned with an Artray Artcam 300MI camera and measured

Submitted 23 Dec 2015; accepted for publication 20 Apr 2016.

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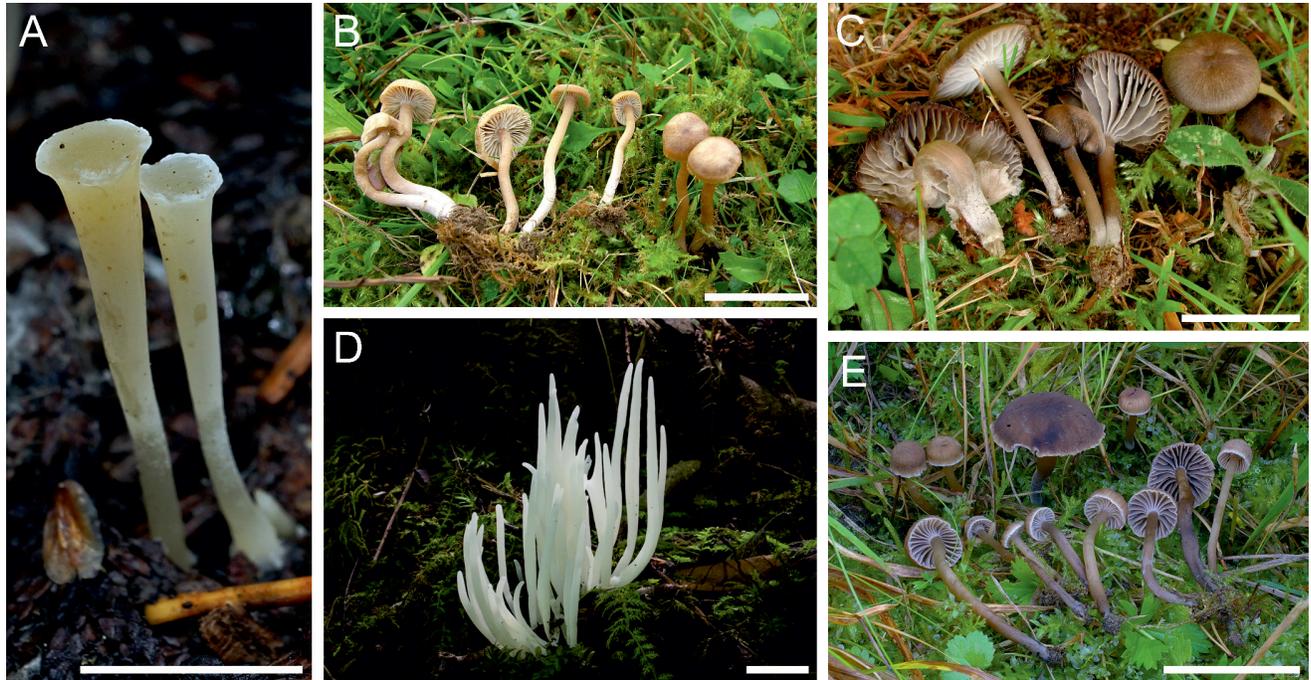


FIG. 1. Diversity of basidiome morphology in the *Clavaria-Camarophyllopsis-Clavicornia* clade of the Clavariaceae. A. *Clavicornia taxophila* (cantharelloid, photograph by Sava Kristic). B. *Camarophyllopsis schulzeri* (agaricoid, photograph by Soňa Jančovičová). C. *Lamelloclavaria petersenii* (agaricoid, photograph by Stefan Jacobsson). D. *Clavaria fragilis* group (clavarioid, photograph by Mike Wood). E. *Hodophilus foetens* group (agaricoid, photograph by Zuzana Egertová). Scale bar = 1 cm.

by QuickPHOTO MICRO 2.1. Enlarged scanned pictures of basidiospores were used for measuring with an accuracy of 0.1 μm and for making line drawings. Microscopic structures were examined on desiccated herbarium specimens in Congo red solution with ammonia after a short treatment in warm aqueous 10% KOH. Q-value is the length/width ratio of the basidiospores. Measurements exclude ornamentation. Statistics for measurements of microscopic characters are based on 30 measurements and given as a mean value plus/minus standard deviation; values in parentheses provide measured minimum or maximum values. Amyloidity and dextrinoidity of basidiospores were tested in Melzer's reagent (Moser 1978).

DNA extraction, PCR, and sequencing.—Protocols of Birkebak et al. (2013) were followed for DNA extraction, PCR, and sequencing. The primer pairs ITS1F-ITS4 (White et al. 1990, Gardes and Bruns 1993) were used to amplify the ITS region. Combinations of LR0R-LR7, LR0R-LR5, or LR0R-LR16 (<http://sites.biology.duke.edu/fungi/mycolab/primers.htm>) were used to amplify and sequence the 28S region. The primer pair b6F and b7.1R (Matheny 2005) were used to amplify and sequence the most variable region of the *rpb2* gene.

Taxon sampling.—171 total taxa were analyzed as a supermatrix, 167 of which are in the *Clavaria-Camarophyllopsis-Clavicornia* clade as recovered by Birkebak et al. (2013) (SUPPLEMENTARY TABLE I). Two species of *Clavulinopsis* and *Ramariopsis* each were used as outgroups based on Birkebak

et al. (2013). The supermatrix consisted of a total of 299 DNA sequences, of which 193 were newly produced by this study (41 *rpb2*, 77 28S, 75 ITS). The majority of the remaining sequences were included from previous works by Birkebak et al. (2013) and Kautmanová et al. (2012). Twenty-one percent of individuals are represented by three loci, 31% by two loci, and 48% by 28S only. Specimens were identified morphologically based on most recent key to identification (Petersen 1988, Knudsen and Vesterholt 2012). Morphotypes showing discrepancies with the phylogenetic analysis (showing polyphyly or with uncertain delimitation) are labelled as species groups or complexes.

Phylogenetic analysis.—Alignments for individual regions were assembled using ClustalX (Larkin et al. 2007) and manually adjusted by eye in MacClade 4.08 (Maddison and Maddison 2005). Individual alignments were concatenated in SeaView 4 (Gouy et al. 2010). gBlocks 0.91 (Castresana 2000, Talavera and Castresana 2007) was used to exclude ambiguously aligned sites. PartitionFinder (Lanfear et al. 2014) was used to identify the best partition scheme and molecular models under the AIC criterion. The sequence alignment files have been deposited in TreeBASE (19107). Maximum likelihood (ML) phylogenetic reconstruction was performed with RAxML 7.4.2 (Stamatakis 2006) implemented in RAxML GUI (Silvestro and Michalak 2012) with 1000 bootstrap replicates. Bayesian inference (BI) was performed in MrBayes 3.2.2 (Ronquist et al. 2011) running 10 000 000 generations and sampling parameter states and trees every 10 000 generations. To ensure that convergence had been reached, the

average standard deviation of split frequencies was monitored to ensure that it fell below 0.01, and trace files of all parameters were examined to ensure proper mixing. A 25% burn-in was used. The approximately unbiased test (AU test, Shimodaira 2002) was performed in CONSEL (Shimodaira and Hasegawa 2001) to evaluate whether agaricoid taxa could constitute a monophyletic group and whether species assigned to the genus *Clavaria* could also form a monophyletic group without being statistically worse than unconstrained topologies. Five constraint trees were made in MacClade.

RESULTS

Phylogenetic reconstruction.—The concatenated alignment included a total of 2860 nucleotide positions (28S 1395 sites, ITS 985 sites, *rpb2* 698 sites). A total of 449 ITS sites were excluded from the alignment before phylogenetic analyses. Five partitions were applied (28S, ITS, and *rpb2* codon positions), each according to a GTR+GAMMA+I model of nucleotide substitution in RAxML and MrBayes.

BI and ML analyses yielded nearly identical phylogenetic reconstructions with only minor incongruences among a few short internodes. The BI tree is shown (FIG. 2). Eight major clades and two individual branches were recovered in both reconstructions, all of which receive strong statistical support with the exception of the *Holocoryne* clade and the isolated position of *Cl. fuscoferruginea*. Five clades are composed of species with clavarioid basidiomes traditionally classified in the genus *Clavaria*, whereas two separate clades and a single branch contain agaricoid species exclusively. The cantharelloid genus *Clavicornia* is sister to the entire *Clavaria* sensu lato-*Camarophylloopsis* sensu lato clade.

One of the agaricoid clades corresponds to the genus *Camarophylloopsis* sensu stricto (*Camarophylloopsis* subgenus *Camarophylloopsis*) typified by *Hygrophorus schulzeri* Bres. The second separate agaricoid clade corresponds to *Hodophilus* (*Camarophylloopsis* subgenus *Hygrotrama* section *Hodophilus*) typified by *Hygrophorus foetens* W. Phillips. The single individual agaricoid branch is represented by one collection from northern Europe (Finland) that could not be identified as any published species or placed in any genus and is described below as new (*Lamelloclavaria petersenii*). AU tests significantly reject the monophyly of *Camarophylloopsis* sensu lato (monophyly of all agaricoid taxa) and the monophyly of *Clavaria* ($p < 0.01$). In addition, constraint topologies of *Hodophilus* + *Lamelloclavaria*, *Camarophylloopsis* sensu stricto + *Lamelloclavaria*, and *Camarophylloopsis* sensu stricto + *Hodophilus*, were rejected ($p < 0.05$). All three agaricoid groups can be distinguished based on pileipellis morphology (FIG. 3).

The resulting clavarioid clades are not all well supported in their placement in both phylogenetic reconstructions. However, *Clavaria* sensu stricto (typified by *Cl. fragilis* Holmsk.: Fr.) is well supported as the sister group to *Camarophylloopsis* sensu stricto. The *Cl. pullei* clade (containing species identified as *Cl. pullei* Donk and *Cl. atroumbrina* Corner) is sister to *Hodophilus* with moderate support. The branch represented by the new genus *Lamelloclavaria* is the sister group to the *Cl. fumosa* clade (with *Cl. fumosa* Pers.: Fr. and *Cl. zollingeri* sensu auctorum) with strong support. A residual group of uncertain affinity (*Cl. atrofusca* clade) contains some darkly pigmented taxa. The *Holocoryne* clade is weakly supported as monophyletic and is composed of two subclades united by the presence of basidia with bifurcated bases; however, neither of these two subgroupings are strongly supported. The *Holocoryne* clade corresponds to the genus *Holocoryne* (Fr.) Bonord. typified by *Cl. falcata* Pers.: Fr. *Holocoryne* has not been accepted at the rank of genus by most authors since its original elevation to generic rank in 1851 (Bonorden 1851) and would require many new combinations. Among the two *Holocoryne* subgroupings, there are several nested clades with strong ML support that correspond morphologically to species groups defined by different colors of single club-shaped basidiomes: white in *Cl. falcata* and its relatives, yellow in the *Cl. flavipes/Cl. argilacea* group, dark gray in *Cl. greletii*, vinaceous-red in two undetermined species (JMB10061001 and ADM1311), and brownish-pink in *Cl. incarnata*. AU tests significantly reject the monophyly of *Clavaria* as currently circumscribed ($p < 0.01$).

TAXONOMY

Lamelloclavaria Birkebak & Adamčík gen. nov.

Mycobank MB810134

Typification: *Lamelloclavaria petersenii* Birkebak & Adamčík.

Diagnosis: A genus in the family Clavariaceae producing agaricoid basidiomes similar to *Camarophylloopsis* and *Hodophilus* but with a rimulose non-hygrophanous pileus and a pileipellis that is a cutis. Basidiospores oblong, inamyloid, not dextrinoid, thin-walled, hyaline. Clamp connections absent in all tissues.

Lamelloclavaria petersenii Adamčík & Birkebak sp. nov.,

FIGS. 1c, 3c, 4

Mycobank MB810135

Typification: FINLAND. ETELÄ-HÄME: Hyytiälä Forestry Field Station, on ground among the grass, near road margin, 61°50'47"N, 24°17'7.5"E, near *Acer pseudoplatanus*, *Betula* sp., *Populus tremula*, 6 Sep 2005, S. Adamčík (**holotype** SAV F-3493).

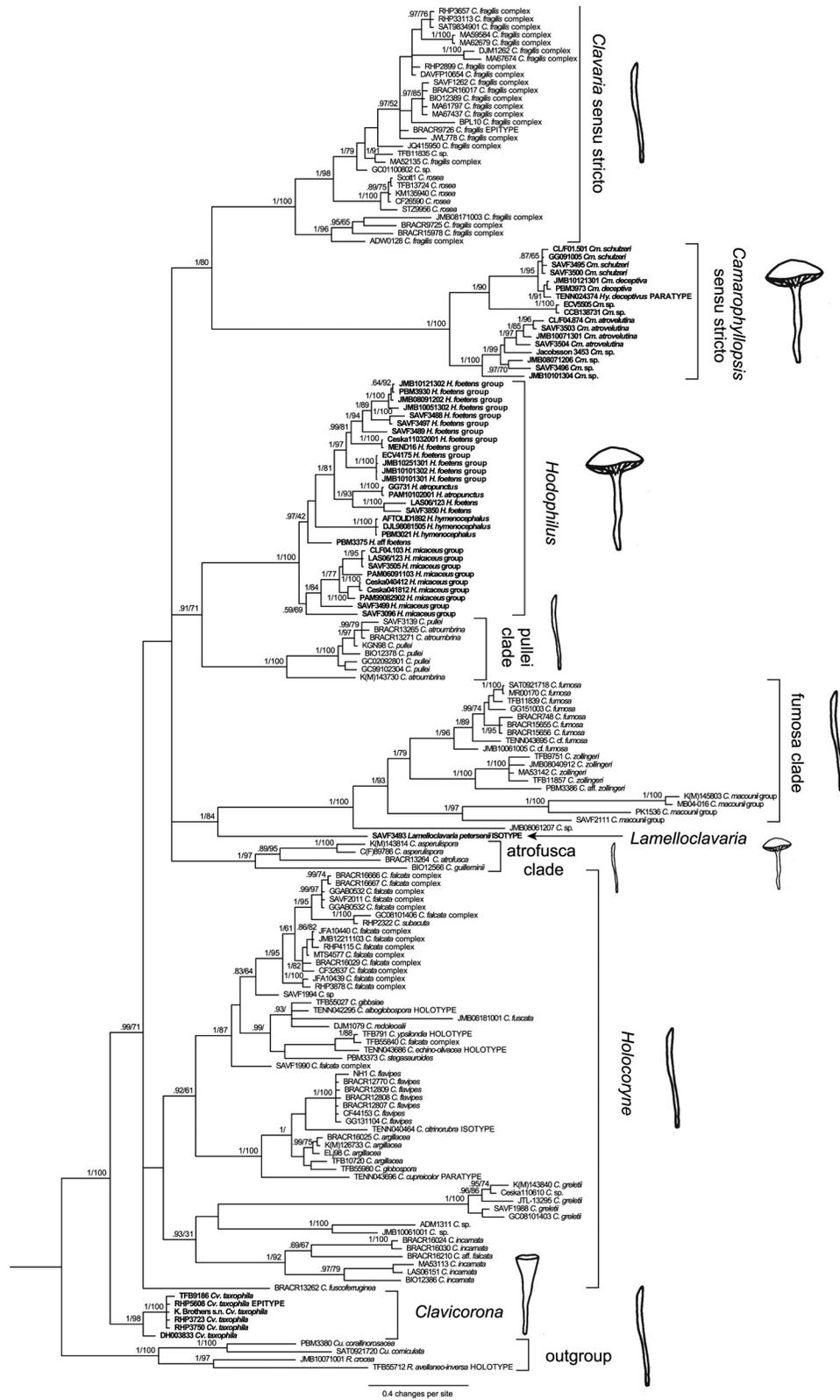


FIG. 2. Bayesian majority rule consensus tree using the ITS, 28S and *rpb2* loci depicting relationships between agaricoid, cantharelloid, and agaricoid forms of the *Clavaria-Camarophyllopsis-Clavicornora* clade. Support values are indicated above the nodes as follows: Bayesian posterior probability/ML bootstrap value. If the topology was not present in the best ML tree, the space after the slash is left blank. Support values are only indicated for nodes with either a posterior probability at or higher than 0.90 or a bootstrap value over 60. Legend: *Cl.* = *Clavaria*, *Cm.* = *Camarophyllopsis*, *Cu.* = *Clavulinopsis*, *Cv.* = *Clavicornora*, *H.* = *Hodophilus*, *Hy.* = *Hygrophorus*, *R.* = *Ramariopsis*.

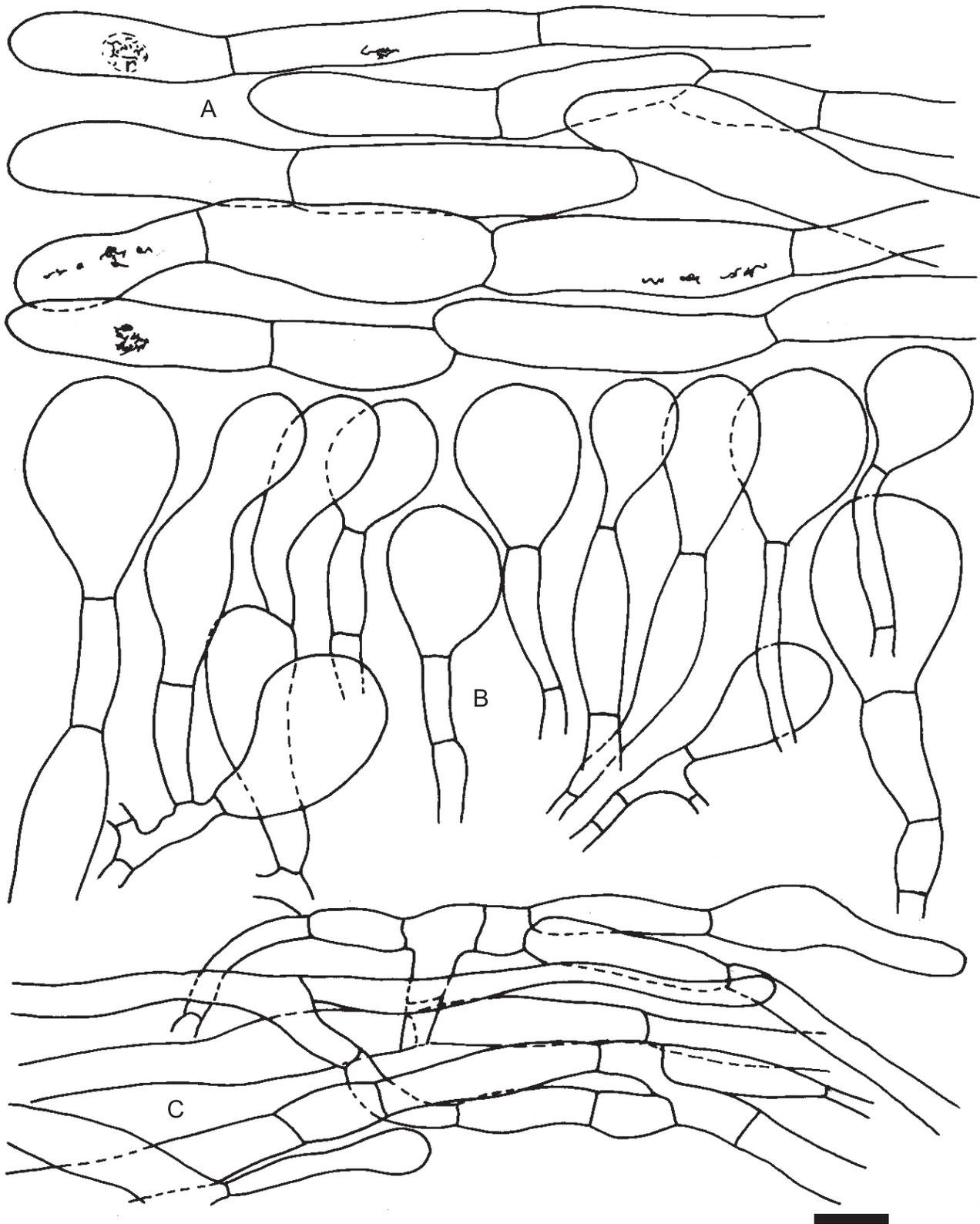


FIG. 3. Comparison of pileipellis structure of different genera of lamellate Clavariaceae. A. Cutis of *Camarophyllopsis* with numerous repent hyphal terminations composed of chains of ellipsoid or cylindrical cells. B. Hymeniderm of *Hodophilus* with obpyriform or sphaeropedunculate hyphal terminations. C. Cutis of *Lamelloclavaria* with scattered hyphal terminations with frequent branching or anastomoses. Scale bar = 10 μ m.

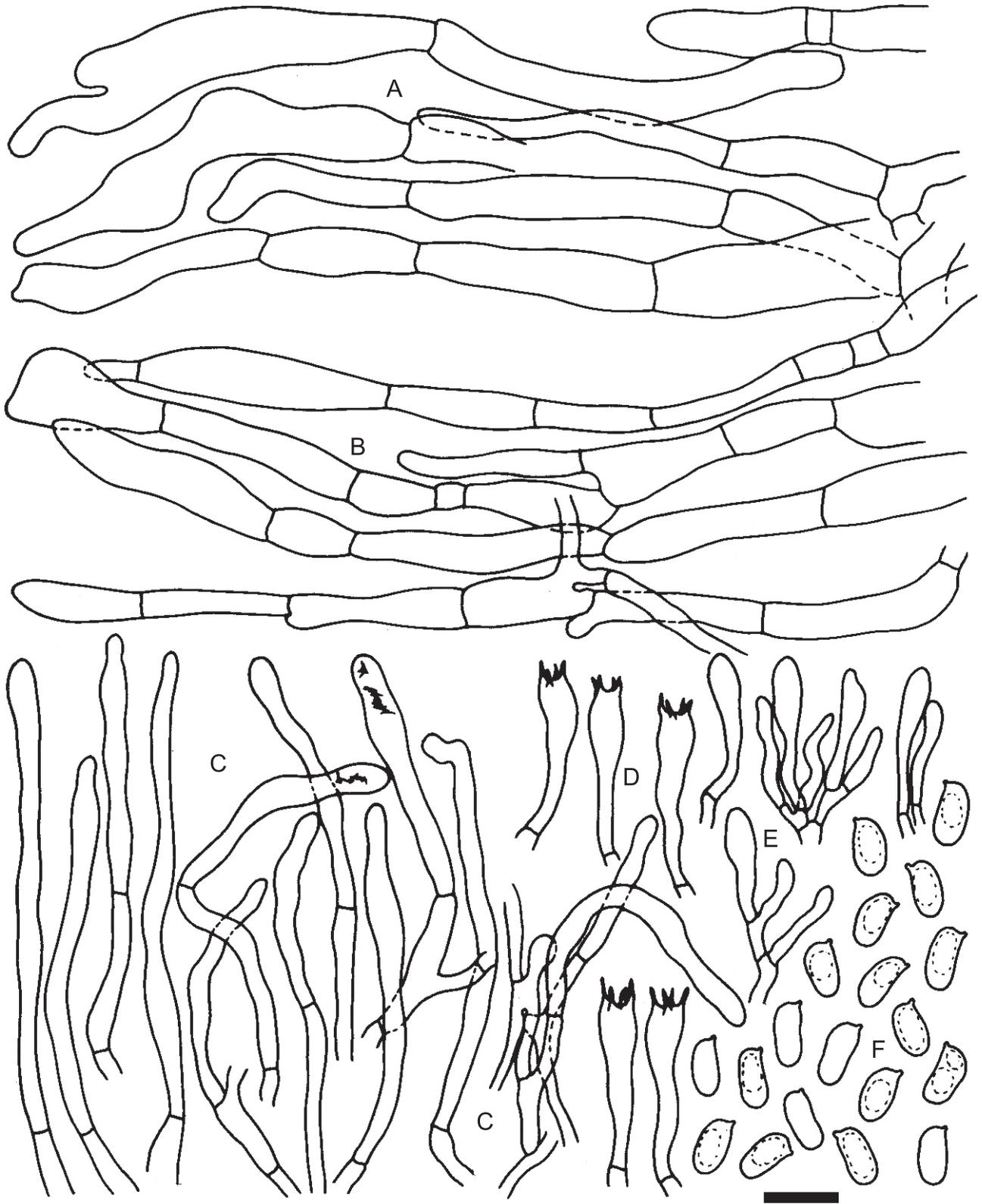


FIG. 4. *Lamelloclavaria petersenii* (holotype). A. Hyphal terminations in pileipellis near the pileus disc. B. Hyphal terminations in pileipellis near the pileus margin. C. Caulocystidia. D. Basidia. E. Basidioles. F. Basidiospores. Scale bar = 10 μm ; 5 μm for basidiospores.

Etymology: Generic name in reference to the phylogenetic relatedness to the genus *Clavaria* but distinct in producing lamellae. Specific epithet in honor of Dr Ronald H. Petersen and his contributions to fungal systematics, taxonomy, and evolution of basidiome morphology, particularly with respect to the Clavariaceae sensu lato.

Pileus 8–15 mm diam, initially convex, later nearly plane, rarely slightly depressed, often with a small papilla on the disc; margin involute when young, becoming straight, nonstriate; surface non-hygrophanous, dry, finely rimulose; color hair brown (5E4), sepia brown (5F4), dark blond (5D3) to nougat brown (5D3), more or less uniformly colored. Stipe 16–20 mm long \times 1–2 mm diam, cylindrical, sometimes eccentric, smooth and shiny, finely granulose near the extreme apex, concolorous with the pileus, base sometimes white tomentose. Lamellae 18–24, lamellulae 1–3, entirely adnate to slightly decurrent, edges entire; birch gray (5C2), dust gray (5D2) or drab (5E3); relatively thin. Context compact and elastic, pale grayish, becoming black, especially near and on the surface when dry; taste mild, lacking a distinctive odor. Spore deposit not observed.

Basidiospores (5.0–)5.2–5.9(–6.2) \times (2.5–)2.7–2.9(–3.0) μm , av. 5.5 \times 2.8 μm , $Q = (1.83\text{--}) 1.88\text{--}2.14$ (–2.35), av. $Q = 2.01$, phaseoliform to oblong, sometimes with a central constriction, hyaline, smooth, inamyloid, not dextrinoid, usually with one large vacuole, thin-walled, hilar appendage 0.4–0.6 μm long. Basidia 4-spored, 22–26.5(–28) \times 5–6 μm , av. 24.5 \times 5.5 μm , hyaline, clavate, attenuated and flexuous toward base. Basidioles cylindrical to narrowly clavate, often flexuous, 2–4.5 μm diam. Hymenium without cystidia, lamellae edge fertile. Subhymenium sharply delimited from parallel hyphae of lamellar trama, pseudoparenchymatic, ca. 10–15 μm deep, trama of the lamellae composed of parallel, ca. 3–10 μm diam, hyphae that are often anastomosed and sparsely branched, often with very short cells (ca. 10–25 μm), but sometimes also longer (50–100 μm long). Pileipellis near margin of the pileus a cutis, composed of relatively thin layer of relatively numerous, repent hyphal terminations with pale brownish intracellular pigment, terminal cells frequently larger, ventricose, fusiform, broadly clavate to lageniform, occasionally narrow cylindrical, occasionally with irregular nodules, (6–)13.5–43(–78) \times (3–)4.5–10(–13.5) μm , av. 28.4 \times 7.5 μm ; basal cells usually shorter and sometimes intermingled with very short (shorter than 10 μm) cells, with or without constrictions at the septa, usually forming chains of 2–4 (or more) unbranched cells; subpellis and trama of the pileus of ca. 5–12 μm diam, parallel, hyaline hyphae, that are very variable in length, usually shorter than 50 μm and intermingled with very short (up to 10 μm) elements, often anastomosed, scarcely branched.

Hyphal terminations near center of the pileus, also a cutis, but of more dispersed and shorter hyphae, some composed of a single cell or lateral branch without a septum arising from horizontally oriented hyphae, with terminal cells more irregular and often nodulose, (13.5–)25.5–51(–66) \times (2–)4–8(–10) μm , av. 38.2 \times 5.9 μm , occasionally with intracellular crystals observed in Congo red. Caulocystidia dispersed or in small clusters, thin-walled, repent or ascending, with terminal cells measuring (14–)25–56(–69) \times 2.5–4.5(–9) μm , av. 40.4 \times 3.5 μm , typically narrow, moniliform and often flexuous, obtuse to slightly constricted near the apex, mostly cylindrical to narrowly clavate, with pale brownish intracellular pigments and occasionally with dispersed crystals visible in Congo red. Trama of stipe of comparatively wider hyphae than caulocystidia, often thick walled, but otherwise similar to those in the pileus trama. Clamp connections absent in all tissues.

Note.—The designation of the genus *Lamelloclavaria* is based on the new species *L. petersenii*, which is based on a single collection from Finland. The combination of the broadly adnate to decurrent lamellae, likely white spore print, finely rimulose gray cap with a small papilla, absence of clamp connections on hyphae and phaseoliform to oblong, small basidiospores makes this species striking in the field and under the microscope. This genus is not treated in recent keys to agaricoid fungi occurring in Nordic countries (Knudsen and Vesterholt 2012), and despite the effort of the authors and other mycologists, it has not been re-collected during the last ten years. It would appear that we are dealing with an extremely rare but conspicuous species. The genus is easily distinguished from other members of the Clavariaceae by its filamentous pileipellis (with repent dispersed hyphal terminations) and basidiospore shape. The combination of the rimose pileus surface, small stature, occurrence on ground and phylogenetic placement differentiate it from lamellate species in the Hygrophoraceae.

Hodophilus R. Heim ex R. Heim, Rev. Mycol. 30:231. 1966.

\equiv *Hodophilus* R. Heim, Champignons d'Europe 2:196. 1957; nom. inval. (Art. 39.1), nom. nudum.

\equiv *Camarophylloopsis* subgenus *Hygrotrama* section *Hodophilus* (R. Heim ex R. Heim) Arnolds, Mycotaxon 25:642. 1986.

Typification: *Hygrophorus foetens* W. Phillips, Grevillea 7:74. 1878.

Species: **Hodophilus foetens** (W. Phillips) Birkebak & Adamčík, comb. nov.

Mycobank MB810136

\equiv *Hygrophorus foetens* W. Phillips, Grevillea 7:74. 1878 (Basionym).

Hodophilus atropunctus (Pers.: Fr.) Birkebak & Adamčík, comb. nov.
MycoBank MB810137

≡ *Agaricus atropunctus* Pers., Syn. Meth. Fung. 2:353. 1801 (Basionym).

Hodophilus hymenocephalus (A.H. Sm. & Hesler) Birkebak & Adamčík, comb. nov.
MycoBank MB810138

≡ *Hygrophorus hymenocephalus* A.H. Sm. & Hesler, Lloydia 5:14. 1942 (Basionym).

Hodophilus micaceus (Berk. & Broome) Birkebak & Adamčík, comb. nov.:
MycoBank MB810139

≡ *Hygrophorus micaceus* Berk. & Broome, Ann. Mag. Nat. Hist. Ser. 5, 3:207. 1879 (Basionym).

Note.—This genus can be distinguished from *Camarophylloopsis* s.str. and *Lamelloclavaria* by the pileipellis, which is composed of typically perpendicular, broadly inflated, globose, obpyriform to sphaeropendunculate terminal elements (FIG. 3b) (a hymeniderm). The four species recombined here are widely accepted and well known. Several more species will likely be transferred from *Camarophylloopsis* to *Hodophilus* based on pileipellis morphology, but are awaiting morphological study and DNA sequencing to confirm their exact placement.

Nomenclatural note: R. Heim (1966) published several invalid combinations that lacked citation of a basionym. These and one additional combination are made above.

Camarophylloopsis Herink, Sborn. Severoces. Musea, Prir. Vedy 1:61. 1958.

Typification: *Hygrophorus schulzeri* Bres., Fungi Tridientini 4/5:57. 1884.

Species: *Camarophylloopsis schulzeri* (Bres.) Herink, Sborn. Severoces. Musea, Prir. Vedy 1:62. 1958.

Camarophylloopsis atrovelutina (Romagn.) Argaud, Doc. Mycol. 31:47. 2002.

Camarophylloopsis deceptiva (A.H. Smith & Hesler) Bon, Doc. Mycol. 26:20. 1996.

Note.—This genus can be distinguished from *Hodophilus* and *Lamelloclavaria* by the pileipellis composed of chains of erect, ascending or repent, subcylindrical to ellipsoid end cells without distinctly inflated terminal elements (FIG. 3a) There are likely more species in the genus than listed above, but these are awaiting detailed morphological study and DNA sequencing.

DISCUSSION

Three independent origins of agaricoid basidiomes have occurred in the Clavariaceae, specifically within the *Clavaria-Camarophylloopsis-Clavicornia* subgroup within

the family. Taxa with simple club-shaped basidiomes (*Clavaria*) are paraphyletic and from which the agaricoid taxa, *Camarophylloopsis* sensu stricto, *Hodophilus*, and *Lamelloclavaria* are derived. This entire group is sister to the cantharelloid lineage *Clavicornia*. Phylogenetic trees constrained to make the three agaricoid lineages monophyletic were statistically rejected in preference to optimal ML and BI topologies. In addition, when clavarioid lineages were forced to be monophyletic the constraint was also significantly worse than optimal topologies that favor a paraphyletic *Clavaria*.

Recently, concerns have been raised over the proliferation of newly named genera of macrofungi and guidelines raised for introducing new genera (Vellinga et al. 2015). One taxonomic approach to the problem here would necessitate a one-genus solution, lumping the three agaricoid genera into *Clavaria*, the oldest generic name available, thus requiring the transfer of *Camarophylloopsis*, *Hodophilus*, and *Lamelloclavaria* to *Clavaria*. Similar one-genus solutions have been proposed for *Trametes* (Justo and Hibbett 2014) and *Russula* and *Cortinarius* (Peintner et al. 2002, Lebel and Tonkin 2007). We do not favor such a disposition now due to uncertainty of the phylogenetic placement of some of the residual *Clavaria* clades. An alternative approach, because *Clavaria* is paraphyletic, would entail splitting the genus into additional smaller genera thereby reducing *Clavaria* sensu stricto, for example, to a small assemblage of species in the *Cl. fragilis* complex along with *Cl. rosea* Dalman ex Fr. We are hesitant to do this at this time because several of the residual clades currently considered as *Clavaria*, again, are lacking robust phylogenetic placement, including the poorly supported and isolated position of *Cl. fuscoferruginea*. Future work is required to consider delimitation of the *Holocoryne*, *Cl. pullei*, *Cl. atrofusca*, and *Cl. fumosa* clades on morphological and ecological grounds and whether such circumscriptions would be useful at a generic level.

ACKNOWLEDGMENTS

The authors would like to thank Pierre-Arthur Moreau, Christine Braaten, Adolf and Oluna Ceska, Zuzana Egertová, Emma Harrower, Stefan Jacobsson, Hailee Korotkin, Sava Kristic, Pierre-Arthur Moreau, Marisol Sánchez-García, Else Vellinga, and Mike Wood for their contribution of specimens, photographs, and/or assistance with fieldwork. We also thank the staff and curators of the herbaria at GB, MICH, NY, OSC, SAV, TENN, UBC, and WTU for providing loans and other assistance. We appreciate contributions made by Soňa Jančovičová in preparation of line drawings. This work was supported by an NSF Doctoral Dissertation Improvement Grant (DEB-1210302) to JMB and PBM and by a grant from the Slovak-American Foundation and the

national grant Vega 02/0075/14 to SA. The authors appreciate constructive comments from two reviewers, associate editor Andrew Methven, and discussion with Jean-Marc Moncalvo on an earlier version of this manuscript.

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