Diversity of Chroogomphus (Gomphidiaceae, Boletales) in Europe, and typification of C. rutilus

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Abstract: In this study, eight species of Chroogomphus are recognized from Europe: C. britannicus, C. aff. filiformis, C. fulmineus, C. cf. helveticus, C. mediterraneus, C. cf. purpurascens, C. rutilus, and C. subfulmineus. Different candidates for the application of the name C. rutilus are evaluated and the best fit to the description is selected; lecto- and epitypes are chosen to fix the name. Chroogomphus fulmineus and C. mediterraneus are also epitypified and a new species, C. subfulmineus, is described. The infrageneric classification is revised and a new subgenus Sicicogomphus and three new sections, Confusi, Filiformes, and Fulminei are introduced. The former sections Chroogomphus and Floccigomphus are elevated to subgeneric level. Comparison of the ITS regions (nuc DNA ITS1-5.8S-ITS2) of all species studied shows that there is a minimum interspecific difference of 1.5%, with the exception of the two species belonging to sect. Fulminei which differ by a minimum of 0.9%. Ecological specimen data indicate that species of Chroogomphus form basidiomes under members of Pinaceae, with a general preference for species of Pinus. Five European species have been recorded under Picea, while Abies and Larix have also been recorded as tree associates, although the detailed nutritional relationships of the genus, involving other suilloid fungi in particular, have yet to be fully clarified.

Key words: DNA barcode ITS molecular systematics new taxa taxonomy

INTRODUCTION

Originally a subgenus of Gomphidius (Singer 1948), Chroogomphus was elevated to generic status by Miller (1964). More recent molecular analyses have shown the genus to be monophyletic (Miller & Aime 2001, Miller 2003, Li et al. 2009). Species of Chroogomphus are characterized by basidiomata with pale orange to ochraceous lamellae when young, an ochraceous pileal trama, a moist to viscid pileipellis, and hyphae at the base of the stipe that have an amyloid reaction in Melzer’s reagent. By contrast, species of the sister genus Gomphidius are characterised by basidiomata with pallid to white lamellae when young, a pallid to white pileal trama, a glutinous pileipellis and veil and non-amyloid to dextrinoid hyphae at the base of the stipe (Miller 1964, Miller & Aime 2001, Li et al. 2009). Both genera have deciduous lamellae and basidiospore deposits that are grey to black. The genus Chroogomphus has been further divided into two sections: Chroogomphus, with a pileipellis of compressed gelatinised hyphae, and Floccigomphus (originally recognised as a subgenus of Gomphidius), with non-gelatinised, tomentose to fibrillose innate hyphae in the pileipellis (Miller 1964). However, these divisions have since been shown not to represent monophyletic lineages (Li et al. 2009). Chroogomphus also includes one species, C. albipes, which is currently unique in the genus due to the scotiod basidiomata. Because of this and other unusual morphological characters, this species was originally described as Secotium albipes (Zeller 1948) and then recombined as Brauniellula albipes (Smith & Singer 1958); however, molecular studies have shown that it belongs to Chroogomphus (Miller 2003, Li et al. 2009). Brauniellula would normally have priority over Chroogomphus, but the latter name was conserved over it by Aime & Miller (2006); it is now accepted as the correct name of the genus.

Six species currently assigned to Chroogomphus have type localities in Europe: C. britannicus A. Z. M. Khan & Hora 1978, C. corallinus O.K. Mill. & Watling 1970, C. fulmineus (R. Heim) Courtec. 1988, C. helveticus (Singer) M.M. Moser 1967, C. mediterraneus (Finschow) Vila et al.
Previous studies have reported that *Chroogomphus* is associated with other suilloid fungi, namely *Rhizopogon* and *Suillus*, but is also able to form ectomycorrhizas with species of *Pinaceae* (Agerer 1990). Similarly, when studying the closely-related genus *Gomphidius*, Olsson et al. (2000) concluded that *G. roseus* was a parasite on *Suillus bovinus*, as opposed to, or possibly as well as, being an ectomycorrhizal partner of conifers. The detailed resource relationships of *Chroogomphus*, and of *Gomphidiaceae* in general, remain unclear and lie beyond the scope of the current study.

In this paper we aim to: (1) provide a clearer picture of the overall species diversity of *Chroogomphus* in Europe; (2) typify *C. rutilus* in order to fix the application of this sanctioned name; and (3) provide an updated infrageneric classification.

**MATERIALS AND METHODS**

**Morphological examination**

The following descriptions of macromorphological characters of the specimens studied were based on notes taken from fresh collections and associated photographs, with the exception of *C. britannicus* whose description is based on the protologue. The colour nomenclature in the description of *C. britannicus* follows Ridgway (1912). A total of 43 specimens were examined, the majority of these were from RBG Kew’s collection (K), the Botanical Museum of the University of Helsinki (H), the herbarium of the Faculty of Pharmacy, Lille (LIP), the Mycological Collection of the Institute of Biodiversity and Ecosystem Research, Sofia (SOMF), and the private fungarium of M. Loizides.

Micromorphological characters were observed using light microscopy. Dried tissue fragments of lamellae, pileipellis, stipe and basal mycelium were mounted in Melzer’s reagent or a 10 % potassium hydroxide (KOH). Melzer’s reagent was used for all measurements and for testing the colour reactions of tissues. For each specimen, measurements of 20 mature spores (obtained from natural spore deposits or naturally discharged spores on the stipe apex) and 10 cystidia were recorded. For the novel species described in this study, a minimum of 30 spores and 20 cystidia were measured from each specimen. Each range of values contains a minimum of 90 % of the measurements made and values shown in brackets indicate the extremes of the recorded ranges. Q is used to indicate the length/breadth ratio of the spores. Mean values are indicated by “av.”. The pileipellis of specimens was observed by taking scalp and cross-sectional samples and mounting them in Melzer’s reagent.

**DNA extraction, PCR amplification, sequencing and data analysis**

DNA was extracted from dried material (lamellae) with the NucleoSpin Plant kit (Macherey-Nagel, Düren, Germany) or the REDExtract-N-Amp™ Plant PCR Kit (Sigma-Aldrich, St Louis, MO), following the manufacturer’s instructions. Primers ITS 1F, ITS 4b and ITS 4 (White et al. 1990, Gardes & Bruns 1993) were used to amplify ITS regions and ITS 1F and ITS 4 were used in direct sequencing. PCR amplification and sequencing followed Liimatainen et al. (2014) and Richard et al. (2015).
Sequences were assembled and edited with Sequencher 4.1 (Gene Codes, Ann Arbor, MI). Our phylogenetic analysis included the newly-generated sequences together with selected published sequences of Chroogomphus specimens downloaded from GenBank and UNITE (Kõljalg et al. 2013). Identical sequences sourced from the same geographical region (country, state or province/territory) were excluded. Several Gomphidius, Rhizopogon and Suillus sequences were chosen as outgroup species following Li et al. (2009), although a slightly different range of species was used. The ITS alignment of 89 sequences was produced with MAFFT v. 7.0 (Katoh & Standley 2013) under default settings. The ITS alignment was manually adjusted in Seaview (Galtier et al. 1996). The alignment obtained is composed of 915 nucleotides (including gaps) and is available at TreeBASE under accession S22668 (http://www.treebase.org/treebase-web/home.html). Sequences were subjected to Maximum Likelihood (ML) analysis as implemented in RAxML version 8 (Stamatakis 2014) with 1000 bootstrap replicates under the GTR+GAMMA model.

Genetic differences within and between species were calculated for paired sequences by dividing the number of indels and/or substitutions found in the ITS1+5.8S+ITS2 regions by the length of the shortest sequence in the pair.

RESULTS

Phylogenetic analysis

Analysis of the ITS regions of the specimens resulted in the phylogenetic tree shown in Fig 1. Eight European species of Chroogomphus were recovered. However, the European status of one of these, here referred to as C. aff. filiformis 1, is currently based on a single ITS sequence downloaded from GenBank which was originally obtained from a Pinus cembra ectomycorrhizal root-tip in Austria. Further sampling is therefore required to support its formal recognition as a distinct species. The remaining seven are based on multiple good quality sequences. The phylogenetic analysis revealed several clades of species with high bootstrap support (BS value mainly > 85), which are proposed as new sections and subgenera herein (see below). The subgenera Chroogomphus, Floccigomphus, and Siccigomphus received BS values of 94, 100, and 100 respectively. Within subgenus Chroogomphus, sect. Chroogomphus has a BS value of 86, sect. Confusi 100, sect. Filiformes 78, and sect. Fulminei 100.

All European species included in this analysis show intraspecific variation of less than 1 % and receive bootstrap support of over 90%, with the exception of those in sect. Fulminei. All species examined can also be identified based on their macro- and micromorphological characters (see below). Interspecific variation is over 1.5 % in all cases except within sect. Fulminei. The two species in this section differ by less than 1 % in some cases, yet inspection of the ITS regions of the two reveals 5 diagnostic nucleotide differences, confirming the presence of two separate but closely-related species.

TAXONOMY

In the following list of taxa, formal names are only applied to specimens based on molecular and morphological matching type materials. Inclusion of “cf.” within a name (C. cf. purpurascens and C. cf. helveticus) indicates that types and type-derived sequences have not been analysed, and the corresponding descriptions only include elements from sequenced materials.

Chroogomphus (Singer) O.K. Mill., Mycologia 56: 529 (1964).

Basionym: Gomphidius subgen. Chroogomphus Singer, Pap. Mich. Acad. Sci. 32: 150 (1948) ["1946"].

Type: Chroogomphus rutilus (Schaeff.Fr.) O.K. Mill. 1964.

Description: Basidiomata small to large, usually expanding fully but secotioid in one species. Pileus subconical to plane, surface smooth or fibrillose, dry to somewhat viscid to viscid; varying in colour from ochraceous-orange to reddish-brown through to purplish, vinaceous or leaden-grey. Lamellae typically decurrent, pale orange to ochraceous-orange when young, though often coloured grey by black spores; in C. mediterraneus rarely purple, becoming greyish orange to wood-brown with age. Trama of the pileus and stipe pale orange to orange-yellow. Veil on stipe ephemeral, fibrous, sometimes forming a thin ring on the upper part of the stipe. Spore deposit blackish. Stipe basal mycelium composed of amyloid hyphae. Basidiospores boletoid, smooth, dark, blackish, weakly to strongly dextrinoid. Cystidium cylindrical to fusiform, thick- or thin-walled.

Ecology and distribution: Found throughout the Northern Hemisphere in coniferous forests, primarily under species of Pinus, but also under other species of Pinaceae.

Currently included subgenera: Chroogomphus, Floccigomphus, and Siccigomphus.

Notes: The genus Chroogomphus can be distinguished from the sister genus Gomphidius by the typically orange-yellow pileal trama, amyloid mycelium at the base of the stipe, and pale orange to ochraceous lamellae when young. Species of Gomphidius have a white to pallid pileal trama, non-amyloid mycelium at the base of the stipe, and white to pallid lamellae when young. The genus Chroogomphus receives high bootstrap support as a monophyletic taxon. The group can be further divided into three subgenera and five sections/clades based on morphological characters which are supported by the molecular data.

Chroogomphus subgen. Chroogomphus

Type: Chroogomphus rutilus (Schaeff.) O.K. Mill. 1964.

Description: Basidiomata small to large, usually expanding fully but secotioid in one species. Pileus subconical to plane, surface smooth or fibrillose, somewhat viscid to viscid, but reported to be dry in the C. britannicus protologue; varying in colour from ochraceous orange to reddish brown through

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Fig. 1. Phylogeny resulting from the RaXML analysis of ITS regions. Bootstrap values greater than 50 % are indicated above branches. The sequences originating from type specimens are in boldface. HT = holotype; ET = epitype.
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Lamellae typically decurrent, pale to ochraceous orange when young, in C. mediterraneus rarely purple, though often coloured grey by spores. Spore deposit blackish. Basidiospores boletoid, smooth, dark, blackish, weakly to strongly dextrinoid. Cystidia cylindrical to subclavate to subfusiform. Lamellar trama hyphae amyloid or non-amyloid. Pileipellis of somewhat narrow hyphae in a layer which is gelatinised to some degree.

Ecology and distribution: Throughout the Northern Hemisphere in coniferous forests, primarily under species of Pinus, but also under other species of Pinaceae.

Currently included sections: Chroogomphus, Confusi, Filiformes, Fulminei, and Vincicolores.

Notes: Species of subgenus Chroogomphus are distinguished by having a pileipellis composed of gelatinised hyphae which are typically narrow (1.5–8.0 μm wide), but may be broader in species of section Confusi (1.5–12.5 μm). The pileipellis hyphae of species in the Asian/North American subgenus Floccigomphus and the circumboreal Siccigomphus are non-gelatinised and usually broader: (5–)7–13(–25) μm (Miller & Aime 2001) and 4–17 μm respectively (Fig. 2).

Chroogomphus sect. Chroogomphus

Type: Chroogomphus rutilus (Schaeff.) O.K. Mill. 1964.

Description: Basidiomata medium to large. Pileus subconical to plane, sometimes umbo nate, often fibrillose, somewhat viscid to viscid; pale reddish pink to reddish brown to vinaceous brown when mature. Lamellae decurrent to adnate. Stipe often quite long (>30 mm). Basal mycelium whitish to salmon to purple-pink. Trama of the pileus and stipe ochraceous to salmon-ochraceous to orange-yellow, often brighter at the base of the stipe. Basidiospores boletoid, smooth, dark, blackish, weakly to moderately dextrinoid, narrow. Cystidia cylindrical to subfusiform, thick-walled in some species. Lamellar trama composed of amyloid hyphae. Pileipellis of narrow, gelatinised hyphae.

Ecology and distribution: Throughout Eurasia, in coniferous and mixed forests forming associations primarily with species of Pinus (both subgenera Pinus and Strobus), but also with other species of Pinaceae.

Currently included species: C. orientirutilus, C. cf. purpurascens, and C. rutilus.

Notes: Species of sect. Chroogomphus all have a lamellar trama composed of amyloid hyphae, a character shared with species of sect. Fulminei, though members of the latter section have an orange-apricot pileus when young, red to pinkish patches on the stipe, especially at the base, and a trama at the base of the stipe coloured either dark grey or olivaceous green. The species of section Confusi are best distinguished from this section by their non-amyloid lamellar trama hyphae (Fig 3).

The current delimitation of section Chroogomphus differs from that of Miller (1964), who originally characterized it as having species with a viscid pileus of somewhat appressed, gelatinised hyphae and included C. jamaicensis, C. ochraceus, and C. vinicolor, as well as C. rutilus. This concept corresponds with subgenus Chroogomphus as described here, which contains the same species and is defined by similar morphological characters.

Chroogomphus rutilus (Schaeff.) O.K. Mill., Mycologia 56: 543 (1964).

(Figs 3B, 4A, 5A, 6A)

Basionym: Agaricus rutilus Schaeff., Fung. Bavar. Palat. 4: 24 (1774); nom. sanct. (Fries 1821).
Fig. 3. Degree of amyloidity of the lamellar trama of European species of Chroogomphus. A. Subgen. Siccigomphus and sect. Confusi in subgen. Chroogomphus are characterised by species with reduced amyloidity in the lamellar trama; non-amyloid lamellar trama of C. mediterraneus (H6029004). B. Other sections of subgenus Chroogomphus have distinctly amyloid lamellar trama; amyloid lamellar trama of C. rutilus (K(M)198589).

Description (a few measurements based on notes accompanying one, non-epitype, collection are also included): Pileus 20–90 mm, conical when young, then low convex to almost plane in age, sometimes umbonate; margin inrolled; surface somewhat viscid, fibrillosome with some appressed reddish brown scales, sometimes shining; pale reddish brown to yellow-brown, often more distinctly yellow close to the margin, to vinaceous brown, often turning a deep reddish brown when dried. Lamellae decurrent to adnate, very crowded to somewhat crowded, colour not recorded when very young, spores soon colouring the lamellae pale to medium grey. Stipe 40–130 × 6–30 mm, cylindrical, often tapering towards the base, upper part pale reddish to pale yellow, sometimes with a pink hue, becoming deeper yellow towards the base, with a few filamentous veil remnants at the stipe apex. Basal mycelium white. Trama of the pileus and stipe not recorded. Taste and odour not distinctive.

Basidiospores boletoid, smooth, dark, blackish, weakly to moderately dextrinoid, (14.0–)16.0–21.5–23.0 × 5.5–7.0(–7.5) μm, av. = 18.0 × 6.2 μm, av. range = 16.7–20.5 × 5.9–6.4 μm, Q = (2.09–)2.43–3.63–4.03), Q av. = 2.94, Q av. range = 2.69–3.47. Basidia bispores or tetrasporic, 38–72 × (9–)10–14 μm, long clavate. Pleuro- and cheilocystidia 101–220 × 11–22 μm, av. = 137.2 × 16.7 μm, av. range = 125.3–158.2 × 13.8–19.0 μm, cylindrical to subfusiform, often thick-walled (walls to 3.0 μm), hyaline in KOH, hyaline to yellow in Melzer’s. Lamellar trama composed of amyloid hyphae. Pileipellis of gelatinised hyphae, 1.5–8.0 μm diam, av. 3.8 μm, mostly non-amyloid with some scattered amyloid elements. Hyphae of the basal mycelium cylindrical, 4.0–12.5 μm diam, with a thick amyloid coating of blue granules when observed in Melzer’s, though hyphae are sometimes smooth; clamp connections observed, but uncommon.

ITS sequence (GenBank MG457852) distinct from other members of sect. Chroogomphus. This species is most closely related to C. orientirutilus (GenBank EU706328, holotype), from which it differs in the ITS regions by 17 substitutions and indel positions, a similarity of 97.4 %.

Synonyms: Agaricus viscidus L., Sp. pl. 2: 1173 (1753); fide Fries (1821). Agaricus rufescens J.F. Gmel., Syst. Nat., 13th edn 2(2): 1406 (1792); nom. illegit. Agaricus gomphus Pers., Icon. Desc. Fung. Min. Cognit. 2: 51 (1800). Agaricus viscidus [f.] atropunctus Pers., Syn. Meth. Fung. 2: 292 (1801). Agaricus viscidus [f.] communis Alb. & Schwein., Consp. Fung.: 158 (1805). Gomphidius viscidus [*] testaceus Fr., Epicr. Syst. Mycol.: 319 (1838). Types: Sowerby, Col. Fig. Engl. Fungi Mushr. 1: tab. 105, 1805 (as Agaricus rutilus; – lectotypus hic designatus, – Estonia: Voru Maakond: antsla vald, in coniferous forest, 27 Aug. 2010, V. Liiv (TU106902 (TU(M), epitypus hic designatus, MBT379498).

Gomphidius testaceus (Fr.) Mussat, in Saccardo, Syll. Fung. 15: 152 (1901). Gomphidius viscidus f. testaceus (Fr.) Kavina, Trav. Mycol. Tchecoslov. 1(2): 6 (1924). Gomphidius rutilus f. testaceus (Fr.) Pilát & Dermek, Hrib. Huby: 163 (1974). Chroogomphus testaceus (Fr.) Přihoda, in Přihoda et al., Kap. Atlas Hub: 237 (1987). Gomphidius littigius Britzelm., Bot. Centralbl. 54: 71 (1893). ? Chroogomphus corallinus O.K. Mill. & Watling, Notes Roy. Bot. Gard. Edinb. 30: 391 (1970).

Types: Schaeffer, Fung. Bavar. Palat. 1: tab. 55, 1762 (lectotypus hic designatus, MBT379513). – Germany: Baden-Württemberg: Schwarzwald, Seedorf (ca 2 km SW), alt. 670–680 m, coniferous forest of Picea abies, on limestone, 27 Aug. 2009, H. Döring & Schwarzwalder Pilzlehrsaul (K(M)198589 – epitypus hic designatus, MBT379497; GenBank MG457852).

Description (a few measurements based on notes accompanying one, non-epitype, collection are also included): Pileus 20–90 mm, conical when young, then low convex to almost plane in age, sometimes umbonate; margin inrolled; surface somewhat viscid, fibrillosome with some appressed reddish brown scales, sometimes shiny; pale reddish brown to yellow-brown, often more distinctly yellow close to the margin, to vinaceous brown, often turning a deep reddish brown when dried. Lamellae decurrent to adnate, very crowded to somewhat crowded, colour not recorded when very young, spores soon colouring the lamellae pale to medium grey. Stipe 40–130 × 6–30 mm, cylindrical, often tapering towards the base, upper part pale reddish to pale yellow, sometimes with a pink hue, becoming deeper yellow towards the base, with a few filamentous veil remnants at the stipe apex. Basal mycelium white. Trama of the pileus and stipe not recorded. Taste and odour not distinctive.

Basidiospores boletoid, smooth, dark, blackish, weakly to moderately dextrinoid, (14.0–)16.0–21.5–23.0 × 5.5–7.0(–7.5) μm, av. = 18.0 × 6.2 μm, av. range = 16.7–20.5 × 5.9–6.4 μm, Q = (2.09–)2.43–3.63–4.03), Q av. = 2.94, Q av. range = 2.69–3.47. Basidia bispores or tetrasporic, 38–72 × (9–)10–14 μm, long clavate. Pleuro- and cheilocystidia 101–220 × 11–22 μm, av. = 137.2 × 16.7 μm, av. range = 125.3–158.2 × 13.8–19.0 μm, cylindrical to subfusiform, often thick-walled (walls to 3.0 μm), hyaline in KOH, hyaline to yellow in Melzer’s. Lamellar trama composed of amyloid hyphae. Pileipellis of gelatinised hyphae, 1.5–8.0 μm diam, av. 3.8 μm, mostly non-amyloid with some scattered amyloid elements. Hyphae of the basal mycelium cylindrical, 4.0–12.5 μm diam, with a thick amyloid coating of blue granules when observed in Melzer’s, though hyphae are sometimes smooth; clamp connections observed, but uncommon.

ITS sequence (GenBank MG457852) distinct from other members of sect. Chroogomphus. This species is most closely related to C. orientirutilus (GenBank EU706328, holotype), from which it differs in the ITS regions by 17 substitutions and indel positions, a similarity of 97.4 %.
The original description of *C. rutilus* is ambiguous. Schaeffer describes a species with a pileus at first subconical and reddish brown, then flatter and striate and finally depressed at the centre with a pale earthy colour. The lamellae become reddish brown and the stipe is concolorous, stout and curved at the attenuated base. The veil is filamentous, there is no annulus, and the species is found in arid forests. In the protologue, Schaeffer (1774) refers to his plate 55 which illustrates a species with a reddish brown fibrillose pileus that is conical when young, becoming low convex to plane with age, with decurrent, brownish-grey lamellae. In deciding upon an epitype to support the lectotypification of *C. rutilus* and stabilise the application of this name, we also considered specimens we have assigned to *C. britannicus* and *C. mediterraneus*, both of which are known to occur in Germany, comparing their original descriptions with Schaeffer’s original concept of *C. rutilus*. Due to the greyish lamellae illustrated in the young basidiomata in the lectotype (a character absent in *C. britannicus* and *C. mediterraneus*), we chose to retain the concept of *C. rutilus* adopted in the recent molecular studies of Miller & Aime (2001), Li et al. (2009), and Martín et al. (2016). Applying the name *C. rutilus* to this species should also ensure that further confusion over names is minimised. The species described in this paper as *C. subfulmineus* was not considered for epitypification, even though it has been named *C. rutilus* in previous studies (Miller & Aime 2001, Li et al. 2009 as *C. *rutilus*), since it is so far unknown from Germany.

Comparison of the available ITS sequence data suggests that the name *C. corallinus* is a synonym of *C. rutilus* as originally proposed by Miller (2003). Although Miller’s proposal is based on the placement of a single sequence derived from *C. corallinus* collected in the UK, he did not specify whether the holotype (collected in 1969) had been sequenced. *C. corallinus* was originally described by Miller & Watling (1970) and the type locality is a conifer plantation near Loughborough, England, from where several collections were made between 1968–1970. One of these collections is most likely (R. Watling, pers. comm.) the source of the sequence in Miller (2003) and so it is possible that the sequenced basidiome was produced by the same mycelium as the holotype. On the other hand, there are some troubling differences between the morphological characters in the original description of *C. corallinus* and those in our current concept of *C. rutilus*. The cystidia of *C. corallinus* are described in Miller & Watling (1970) as “thin-walled, rarely thick-walled”, whereas in *C. rutilus* the reverse is true. The differing texture of the pileipellis is also noteworthy. The pileal surface of the Loughborough collections was described as “matt”, “dry” “woolly”, “tomentose” and “velvety” (Watling 1969, 1970, 2004, Miller & Watling 1970, Watling & Hills 2005) leading to an initial misidentification as *C. helveticus* (Watling 1969). The inference is that the dry aspect of the pileus is due to the non-gelatinised pileipellis hyphae; indeed, this is stated by Watling & Hills (2005). Nevertheless, following the limited sequence-based evidence of synonymy in Miller (2003), Watling & Hills (2005) considered the observed variation
in pileipellis texture and cystidial wall thickness, which had previously been used to distinguish C. corallinus and assign it to section Floccigomphus, as falling within their revised concept of C. rutilus. However, the degree of morphological difference is such that a sequencing and microscopic study of the holotype of C. corallinus is needed to confirm it as a taxonomic synonym of C. rutilus.

Numerous other names in the literature have been synonymised with C. rutilus, and many of their descriptions refer to the Schaeffer plate used to lectotypify that species here. We also regard Chroogomphus testaceus as a synonym. The original description refers to Sowerby’s figure of Agaricus rutilus, plate 105, which is used for lectotypification here. In choosing an epitope for C. testaceus, we selected the specimen which most closely resembles the lectotype. Of all the European species studied, the current concept of C. rutilus seems to provide the best match with that of C. testaceus.

Specimen details of downloaded European sequences: Czech Republic: Ústecký: Roudnice nad Labem, with Pinus subgenus Pinus sp., 30 Aug. 2008, J. Borovicka (HKAS 55294 (KUN), FJ652071). – Estonia: Voru Maakond: Noorootsi vald, Aulepa, with bushes of Juniperus, 08 Sep. 2007, I. Saar (TU101333 (TU(M)), UNITE UDB019693). Viljandi: Lilli, in temperate broadleaf forest, 20 Sep. 2015, [Collector unknown] (TU116830 (TU(M)), UDB025603). – Poland: [Ecology unknown], 13 Oct. 2014, [Collector unknown], ID PAN 592 (GenBank KM085388); [Ecology unknown], 13 Oct. 2014, [Collector unknown], ID PAN 762 (GenBank KM085373). – Russia: Kirov Oblast: Zuevskiy, Kyrov, with Pinus subgenus Pinus sp., 16 Aug. 2006, B. Tolgor HMJAU 4665 (JLAU, GenBank EU791582). – Switzerland: [Ecology unknown], O. K. Miller (OKM24401, GenBank AF205649).

Specimen details of downloaded Asian sequences: China: Yanting: [Ecology unknown], [Collector unknown] (GenBank HM049562). Huairou: [Ecology unknown], [Collector unknown] (GenBank HM049561); [Ecology unknown], [Collector unknown] (GenBank KM488533). Jiilin: Jiilin Agriculture University, with Pinus subgenus Pinus sp., 8 Sep. 2004, J. R. Wang HMJAU 3681 (JLAU, GenBank EU791580). – South Korea: Ulsan-do: Nari-basin, [Ecology unknown], 16 Oct. 2012, [Collector unknown] (KA12 1684, GenBank KR673618); Nasujeon, [Ecology unknown], 23 Sep. 2013, [Collector unknown] (KA13 2103, GenBank KR673676).

Chroogomphus cf. purpurascens (Lj.N. Vassiljeva) M.M. Nazarova, in Wasser, Nizsh. Rast. Grivy Mokh. Sovetsk. Dal’nego Vostoka 1: 378 (1990). (Figs 4B, 5B, 6B)
Fig. 5. Basidiomata of selected Chroogomphus species. A. C. rutilus (TU106902). B. C. cf. purpurascens (K(M)233762). C. C. mediterraneus (K(M)237593). D. Atypical C. mediterraneus (K(M)237779). E. C. fulmineus (LIP 0401320). F. C. cf. helveticus (H7019100). G. C. subfulmineus (LIP 0401318, holotype). H. C. subfulmineus (LIP 0401323, showing colour of the trama). Not to scale; bar applies to F only. Photographs: A, Vello Liiv; B, and C, Geoffrey Kibby; D, Mel Oxford; E, Pierre-Arthur Moreau; F, Kare Liimatainen; and G and H, Michael Loizides.
Notes: *Chroogomphus* cf. *purpurascens* has narrower spores than other members of the genus (width av. 5.2–6.1 μm), and it is also distinguishable, at least when young, due to its pink to purplish pilules. In some instances, young basidiomata also have a stipe with lilac pinkish pruina.

A study of the type material is currently lacking for *C. purpurascens* and specimens sequenced by Li et al. (2009) from “eastern Russia” were of European Russian origin far from the type locality. Several characters in the specimens we have examined match Vassiljeva’s (1950) original description, such as the colour of the pileus and the size of the spores, but until a type study and associated sequence analysis has been carried out the identification cannot be confirmed. Vassiljeva’s collections, if preserved, are expected to be in the Institute of Biology and Soil Science, Far Eastern Branch, Russian Academy of Sciences (Vladivostok, VLA).

Specimens examined: **Brazilia**: Burgas Province: Malko Tarnovo, Strandzha, with *Pinus nigra*, 18 Oct. 2014, B. Assayov (SOMF29761). Biлагоевград Province: West Frontier mts, Ozgrahden Mt, with *Pinus nigra*, 21 Nov. 2014, B. Assayov (SOMF29762, GenBank MG457863).

- **Channel Islands**: Jersey: St Brelade, Rue du Pont Marquet, Jersey Lavender Farm, JE3 8DS, in woods under *Pinus sylvestris*, 28 Oct. 2016, G.G. Kibby (K(M)233762, GenBank MG457854).

- **Finland**: Varsinais-Suomi: Lohja, Vappula, NNW-shore of the pond Jusolanlampi, in grass-herb forest of *Picea abies*, 29 Aug. 1999, U. Nummela-Salo & P. Salo (HMJAU 3489, GenBank MG457855).

Specimen details of downloaded European sequences: **Czech Republic**: Ústecký: Roudnice nad Labem, with *Pinus subgenus Strobus sp.*, 14 Sep. 2008, J. Borovichka (HKAS 55295 (KUN), GenBank FJ652072). – **Germany**: Hesse: Marburg, with *Pinus subgenus Strobus sp.*, [Collector unknown] (HKAS 54925 (KUN), GenBank FJ481128).

- **Russia**: Kirov Oblast: Nikitintsy, with *Pinus subgenus Strobus sp.*, 12 Aug. 2006, B. Tolgor (HMJAU 4633 (JLAU, GenBank EU706332); Falyarchy, with *Pinus subgenus Strobus sp.*, 15 Aug. 2006, B. Tolgor (HMJAU 4634 (JLAU, GenBank EU706333)).

Specimen details of downloaded Asian sequences: **China**: Jiilin: Changchun, Jingyuetan National Forest Park, with *Pinus subgenus Strobus sp.*, 20 Sep. 2004, J. R. Wang (HMJAU 3489 (JLAU, GenBank EU706330); Changchun, Jingyuetan National Forest Park, with *Pinus subgenus Strobus sp.*, 24 Aug. 2004, J. R. Wang (HMJAU 3687 (JLAU, GenBank EU706331)).

*Chroogomphus* sect. **Confusi** Niskanen, Scambler & Limat., sect. nov.

MycoBank MB823592

**Etymology**: Named after the type species of the section.

**Diagnosis**: The mostly non-amyloid hyphae of the lamellar trama distinguish the species of this section from the others of the subgenus *Chroogomphus* that have amyloid lamellar trama hyphae.

**Type**: *Chroogomphus confusus* Y.C. Li & Zhu L. Yang 2009.

**Description**: Basidiomata small to large, one species secotioid. **Pileus** subconical to plane, sometimes umboinate, somewhat viscid to viscid; wood-brown to brownish orange to cream-orange, rarely purple. **Lamellae** extremely decurrent to adnate. *Basal mycelium* whitish to grey to yellowish ochre. Trama of the pileus and stipe orange to orange-yellow. **Basidiospores** boletoid, smooth, dark, blackish, weakly to strongly dextrinoid. **Cystidia** cylindrical to subfusiform, thin-walled. **Lamellar trama** composed of mostly non-amyloid hyphae. **Pileipellis** of somewhat narrow to narrow, gelatinised hyphae.

**Ecology and distribution**: Known from North America and Eurasia, in coniferous and mixed forests, found primarily under species of *Pinus* subgen. *Pinus*, but also under other species of *Pinaceae*.

Currently included species: *C. cf. albipes*, *C. asiaticus*, *C. confusus*, and *C. mediterraneus*. The ITS sequences of *C. asiaticus* (GenBank AF205664 Nepal, holotype, *Pinus roxburghii*, *Alnus nepalensis* forest; GenBank AF205666 Nepal, *Pinus roxburghii* forest) were short and thus not included in our analysis. However, the phylogenetic analysis of Miller & Aime (2001) shows that these specimens belong in this section. The holotype sequence of *C. confusus* (GenBank EF423621) was also omitted from our analysis due to its short length, though this is shown to cluster with the other specimens of *C. confusus* in Li et al. (2009).

Notes: Some features of the above description do not apply to the unusual secotioid species, *C. albipes* (syn. *Brauniellula albipes*). All other members of the section form basidiomata above ground. Species of sect. *Confusi* have reduced amyloidity in the lamellar trama. Although the degree of amyloidity may vary to some extent and some species have weakly scattered amyloid elements, these should generally be scarce enough to avoid confusion with species of other sections of subgenus *Chroogomphus*. *Chroogomphus* cf. *albipes* has been described as having an amyloid trama in previous studies (Miller 2003), but the precise location was not specified. No collections of *C. cf. albipes* were available for study, but in Smith & Singer’s (1958) description there is no indication that the lamellar trama has amyloid elements. This section received high bootstrap support in our phylogenetic analysis.

**Chroogomphus mediterraneus** (Finschow) Vila et al., *Errortari* 3: 68 (2006). (Figs 3A, 5C–D, 6C)

*Basionym*: *Gomphidius mediterraneus* Finschow, Veroff. Uberseemus, Bremen A 5: 43 (1978).

**Types**: **Spain**: *Balearian Islands*: Eivissa, Sant Josep de sa Talaia, Puig d’en Serra, alt. 200 m, under *Pinus halepensis*, 08 Nov 1973, H. Kuhbier [det. G. Finschow] (BREM 2060 – holotype); *ibidem*, alt. 250–300 m, under *Pinus halepensis*, 18 Nov 2012, A. Serra (hb. Siquier, JLS 3539 – *epitypus hic designatus*, MBT37523; GenBank LT219430).

**Description**: Pileus 30–70(–90) mm, hemispherical to convex or more rarely subconical when young, becoming low convex to applanate or weakly umbilicate with age, rarely also weakly umboinate, margin usually inrolled, surface innately fibrillose, subviscid to dry; colour when young ranging from dark
charcoal-grey to olivaceous grey, paling in age to olivaceous brown, vinaceous brown, ochraceous brown or pinkish brown, often with ochraceous orange, pinkish, or cream-orange patches, rarely the whole pileus purple, becoming dark purplish vinaceous to blackish brown when dried. Lamellae moderately to deeply decurrent and distinctly arcuate, distant, when young covered with a fugacious, orange cortinoid veil soon disappearing, ochraceous orange to deep apricot-orange when young and remaining so for a long time, rarely purple, gradually mottled from maturing spores and finally pale brown to olivaceous brown at full maturity; edges more or less smooth and concolorous or slightly paler. Stipe 30–90 × 5–20(–30) mm, cylindrical to fusiform, often flexuous and rooting, apricot-orange to ochraceous buff, frequently with dark remnants of cortinoid veil at the apex, covered in orange or pinkish fibrils below, occasionally with a pinkish flush. Basal mycelium tomentose, distinctly ochraceous yellow or more rarely dull ochraceous cream. Trama of the pileus and stipe uniformly apricot-orange, sometimes vaguely darkening towards the base. Taste and odour weak, somewhat sour; more distinctly acidic in overripe basidiomata.

Basidiospores boletoid; subfusoid to ellipsoid, smooth, thick-walled, dark, blackish, weakly to strongly dextrinoid, (14.0–)15.0–18.5(–20.5) × (5.0–)6.0–7.5(–8.0) μm, av. 16.9 × 6.6 μm, av. range 16.3–18.0 × 5.9–6.8 μm, Q = (1.87–)2.11–2.96(–3.36), Q av. 2.57, Q av. range 2.43–2.70. Basidium bisporic or tetrasporic, 40–75 × 9.5–14 μm, long clavate. Pleuro- and cheilocystidia 91–153 × 11–22 μm, av. 122.3 × 15.3 μm, av. range 108.0–130.5 × 13.8–18. 3 μm, cylindrical to subfusciform or subutriform, sometimes subcapitate, thin-walled (to 1.0 μm), but occasionally also thick-walled (to 2.0 μm), frequently with coarse lateral encrustations; hyaline to brown in KOH, hyaline in Melzer’s. Lamellar trama composed of inamyloid hyphae, yellow to pinkish in Melzer’s. Pileipellis of somewhat gelatinised or gelatinised hyphae, 1.5–12.5 μm diam, av. 5.3 μm, mostly inamyloid, with some scattered amyloid elements. Hyphae of the basal mycelium cylindrical, 4.0–16.0 μm diam, with a thick amyloid coating of blue granules when observed in Melzer’s; clamp connections observed, but uncommon.

ITS sequence (GenBank MG457831) distinct from the other members of section Confusi. This species is most closely related to C. confusus, from which it differs in the ITS regions by 13 substitutions and indel positions, a similarity of 98.0 %.

Ecology and distribution: Forming basidiomes in autumn, winter and spring in coniferous and mixed forests, particularly in rich grass-herb forests in the north of its range, more commonly in thermo- and meso-Mediterranean pine forests in the south, often with mixed sclerophyllous vegetation in the understory. It is found under species of Pinus subgen. Pinus, mainly P. halepensis and P. brutia in the Mediterranean range, but in other parts of Europe also with P. sylvestris, P. halepensis, and P. nigra, with a single record under Picea and another one under Larix. Contrary to the specific epithet, C. mediterraneus is very widely distributed, reaching as far north as Scotland and Finland, although it may be endemic in Europe. Basidiomata have been observed several times in direct contact with basidiomata of Rhizopogon cf. luteolus, R. cf. roseolus, and R. cf. vulgaris.

Notes: Chroogomphus mediterraneus is a species of remarkable plasticity (Siquier et al. 2016), but differs from...
all other European members of the genus, with the notable exception of C. cf. helveticus (subgen. Siccigomphus), in lacking an amyloid reaction in the lamellar trama. Apart from the ecology, it can be distinguished from C. cf. helveticus because the latter has a yellow to orange-apricot, dry pileus when young and broader pileipellis hyphae (to 17 μm wide) that are not embedded in a gelatinised layer. As also noted by Martín et al. (2016), the distinctly ochraceous yellow or ochraceous cream mycelium at the stipe base is usually an excellent diagnostic field character separating this species from C. rutilus, which has a whitish or cream basal mycelium. The deep orange lamellae of C. mediterraneus, which maintain this colour until late in maturity, might be another useful character for discriminating this species from C. rutilus. This needs to be more thoroughly evaluated when further molecularly-confirmed collections become available.

Epitypification of C. mediterraneus with a modern sequenced specimen (JLS 3539, GenBank LT219430) was necessary since three previous attempts at sequencing the holotype of C. mediterraneus in different laboratories were unsuccessful (Martín et al. 2016). The selected specimen is from the type locality (topotype) and its morphological characters match the description of the holotype (Martín et al. 2016). Collections previously reported as “C. rutilus” in Cyprus (e.g. Loizides et al. 2011) all corresponded to C. mediterraneus, greatly extending the species’ biogeographical range towards the eastern Mediterranean.

Additional specimens examined: Bulgaria: Blagoevgrad Province: West Frontier mts, Logodzah village, with Pinus nigra, 22 Sep. 2014, B. Assyov (SOMF29763, GenBank MG457857). – Cyprus: Troodos, under P. nigra subsp. pallasiana, 18 Nov. 2014, M. Loizides ML41111/1, FR2015390 (GenBank MG457867). – Finland: Uusimaa: Porvoo, Bjurböle, NE side of Meteorititie, E from Mäntymäki, in grass-herb forest dominated by Betula pendula, 11 Sep. 1997, U. Nummela-Salo & P. Salo (H6016157, GenBank MG457834). – France: Savoie: Chambéry, M. Durand MDH03 (LIP 0401328, GenBank MG457839). – Germany: Thuringia: Ilmenau, between Oberporzitz and Unterporitz, with Picea, 28 Sep. 2016, R.A. Fortey (K(M)233760, GenBank MG457835); Ilmenau, on the road to Unterporitz, with Pinus sp. (Betula also present), 28 Sep. 2016, P.A. & K. Cavanagh (K(M)233761, GenBank MG457833). – Greece: [Locality unknown], under Pinus sp., 1 Nov. 2014, E. Papadopoulou FR2015401 (GenBank MG457868). – United Kingdom: Wales: Monmouthshire, Hardwick Plantation nr. Highmoor Hill, Laxir woodland, 17 Dec 2017, M. Oxford & W. Thomas (K(M)237779, GenBank MH037154). Scotland: Mid-Perthshire, Black Wood of Rannoch, with Pinus (Betula also present), 24 Aug. 2015, T. Niskanen TN15-015 (K(M)200317, GenBank MG457837); TN15-014 (K(M)200316, GenBank MG457838); South Aberdeenshire, Linn of Dee, with Pinus sylvestris, 27 Aug. 2003, S. Kelly (K(M)175418, GenBank MG457832). – Spain: Castilla-La Mancha: Puente de Vadillos, Hoz de Beteta, near Pinus sylvestris, P. nigra, 1 Nov 2017, G. Kibby (K(M)237593, GenBank MH037155).

Specimen details of downloaded sequences: Spain: Balearen Islands: Eivissa, Sant Josep de sa Talaia, es Cap Falcó, alt. 0–25 m, under Pinus halepensis, 4 Dec. 2009, J. L. Siquier & J. C. Salom (JLS 2917, GenBank LT219429); Formentera, Torrent de Cala Saona, under Pinus halepensis, 7 Dec. 2008, J. L. Siquier & J. C. Salom (JLS 3006, GenBank LT219431); Mallorca, Pollença, Puig de Son Vila, alt. 100–200 m, under Pinus halepensis, 21 Nov. 2009, J. L. Siquier & J. C. Salom (JLS 2887, GenBank LT219432); Menorca, Es Mercadal, Sa Roca, alt. 180–240 m, under Pinus halepensis, 14 Nov. 2011, J. L. Siquier & J. C. Salom (JLS 3384, GenBank LT219433). Teruel: Mora de Rubielos, Puerto de San Rafael, alt. 1400 m, under Pinus nigra and P. sylvestris, 6 Oct. 2009, J. L. Siquier & J. C. Salom (JLS 2775, GenBank LT219434).

Chroogomphus sect. Filiformes Niskanen, Scambler & Limat., sect. nov. MycoBank MB823593

Etymology: Named after the type of the section.

Diagnosis: The combination of a yellow basal mycelium and amyloid lamellar tratal hyphae distinguish this section from others of subgenus Chroogomphus.

Type: Chroogomphus filiformis Y.C. Li & Zhu L. Yang 2009.

Description: Basidiomata small to large. Pileus subconical to plane; greyish orange to orange to ochreous when mature; subviscid to viscid. Lamellae decurrent. Basal mycelium yellowish. Basidiospores boletoid, smooth, dark, blackish, weakly to strongly dextrinoid. Cystidia cylindrical to subclavate to subfusiform. Pileipellis of narrow, gelatinised hyphae.

Ecology and distribution: Known from North America and Eurasia, in coniferous and mixed forests forming basidiomes primarily under species of Pinus (subgenera Pinus and Strobus), but also under other species of Pinaceae.

Currently included species: C. britannicus, C. filiformis, C. aff. filiformis 1, C. aff. filiformis 2, C. cf. ochraceus, and C. aff. ochraceus “Canada”.

Notes: Other sections with amyloid lamellar tratal hyphae do not have a yellow basal mycelium, and although C. mediterraneus (sect. Confus) does have yellow mycelium, it lacks amyloid hyphae in the lamellar trama.

Chroogomphus britannicus A.Z.M. Khan & Hora, Trans. Brit. Mycol. Soc. 70: 155 (1978). (Figs 2B, 6D)

Types: United Kingdom: England: Berkshire (vice-county 22), Mortimer, Benyon’s Inclosure, in plantation of Pinus sylvestris, 22 Nov 1971, A. Z. M. N. A. Khan (K(M)77895 – holotype); ibidem, in plantation of P. sylvestris, 29 May
Description (macroscopic features based on the original description by Khan & Hora 1978): Pileus to 17 mm, convex, margin inrolled, smooth; yellowish orange near ‘Ochraceous-Orange’ to ‘Ochraceous-Buff’, dry or slightly viscid when moist. Lamellae decurrent, thick, ‘Light Vinaceous-Cinnamon’ to ‘Light Pinkish-Cinnamon’ when young, becoming ‘Wood Brown’ with age. Stipe to 60 × 8 mm, tapering below to 6 mm at the base, concolorous with the pileus or paler. Basal mycelium yellowish. Trama of the pileus and stipe ‘Pale Yellow Orange’ to ‘Capucine Buff’. Taste and odour not distinctive.

Basidiospores boletoid, smooth, dark, blackish, weakly to strongly dextrinoid, (17.0–18.0–23.5–(26.5) × (6.0–)6.5–8.0–(9.0) μm, av. 20.3 × 7.1 μm, av. range 18.7–21.1 × 6.7–7.1 μm, Q = (2.31–)2.51–3.17–(3.46), Q av. 2.87, Q av. range 2.76–2.99. Basidia bisporic or tetrasporic, 36–64 × 9.5–12.5 μm, long clavate. Pleuro- and cheilocystidia 105–200 × 12–28 μm, av. 152.0 × 16.5 μm, av. range 130.5–169.2 × 13.8–24.0 μm, cylindrical to subfusiform, rarely capitate, thinned-walled (to 1.0 μm), hyaline to brown in KOH, hyaline to yellow in Melzer’s. Lamellae composed of amyloid hyphae. Pileipellis of gelatinised hyphae, 1.5–7.0 μm diam, av. 3.9 μm, mostly inamylloid with some scattered amyloid elements. Hyphae of the basal mycelium cylindrical, 4.5–14.0 μm diam, with a thick amyloid coating of coarse, blue granules when observed in Melzer’s; clamp connections not observed.

ITS sequence (GenBank MG457847) distinct from other members of sect. Filiformes. This species is most closely related to C. cf. ochraceus (EF619654), from which it differs in the ITS regions by 18 substitutions and indel positions, a similarity of 97.3 %.

Ecology and distribution: Mainly in coniferous forests and acid heath dominated by Pinus sylvestris, though it has once been recorded under Picea. The type locality is a pine plantation indicating that it is able to occur (or at least persist for a few years) in anthropogenic habitats. Basidiomata are produced in the autumn, from late August to late November. This species has one of the most northern distributions of the genus, with two specimens collected from Finland’s northern boreal regions. Although it is recorded in the UK, from where it was originally described, it is currently only known there from the type materials collected in 1971 and 1972.

Notes: *Chroogomphus britannicus* is notable for having longer spores than most other members of the genus, with the exception of *C. fulmineus* and *C. subfulmineus*. However, it can be distinguished from *C. fulmineus* by the slightly broader spores and the considerably coarser amyloid granules on the hyphae of the basal mycelium (Fig. 7A). *Chroogomphus subfulmineus*, on the other hand, does have an overlapping distribution, with collections from Britain and Finland, and the two species have similarly broad spores, but again *C. britannicus* has coarser amyloid granules on the hyphae of the basal mycelium. The absence of reddish to pink patches on the stipe, and lack of olivaceous trama at the stipe base of *C. britannicus* should also enable positive identification. In our phylogenetic analysis, *C. britannicus* clusters close to *C. filiformis*, from which it can be distinguished due to its slightly broader and longer spores. *Chroogomphus filiformis* is currently only known from China.

A morphological examination of the original material of *C. britannicus* was carried out during this study. The characters of both the holotype and paratype were found to conform to those of the more recent collections, but comparison with Khan & Hora’s (1978) original description of *C. britannicus* highlighted a significant difference in the description of the pileipellis. It is originally described as having an “epicutis of non-agglutinated, interwoven, inamyloid hyphae”, yet we found it to have a gelatinous (agglutinated) outer layer of hyphae (Fig. 2B). It may be that the fresh material possessed an overlying dry layer, accounting for the “filamentous, dry pileal surface”, which might have subsequently receded into the gelatinous layer during drying.

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**Fig. 7.** Hyphae of the basal mycelium with a thick amyloid coating of blue granules when observed in Melzer’s reagent: A. *Chroogomphus britannicus* (H6045578) with coarse amyloid granules. B. *C. subfulmineus* (DG56) with finer amyloid granules. Bar = 10 μm. Photographs: Ross Scambler.
Successful sequencing of the paratype of *C. britannicus* confirmed that it does not match any sequences published in previous studies of *Chroogomphus*, and yet it clustered with six other specimens sequenced during this study (not all included in the phylogeny), as well as two ITS2 sequences from GenBank. This is possibly due to a lack of sequenced collections from northern Europe in earlier studies. Only the ITS2 region of the paratype was successfully recovered and for this reason it has been omitted from the phylogeny. It is a species distinct from *C. britannicus* sensu Martín et al. (2016; GenBank AF205639) which we describe below as *C. subfulmineus*.

Additional specimens examined: **Finland**: Etelä-Karjala: Panikkala, Kirkonkylä, Sikoharju, *Pinus sylvestris* dominated heath, 13 Sep. 2003, V. Haikonen (H6059351; GenBank MG457844). Satakunta: Honkajoki, Kivimäki, SE of the Silkainen-Honkajoki road, with *Pinus sylvestris* in pine-dominated heath on sandy soil, 22 Sep. 2006, E. Oehnenoja (H6045578, GenBank MG457847); Silkainen, east of Katesilmankallio, west of Kaakunkiilmet, dry pine-dominated acid heath, old track, 20 Sep. 2006, E. Oehnenoja (H6059327, GenBank MG457845). **Perä-Pohjanmaa**: Kemijärvi, lower south slope of the fjell Pyhätunturi, coniferous forest dominated by *Pinus sylvestris*, *Betula pendula*; GenBank MG457842). **Rovaniemi**: Pisavaara Strict Nature Reserve, acid pine forest of *Pinus sylvestris*, 19 Sep. 2009, J. Kinnunen (H6025417, GenBank MG457846). – **Germany**: Thuringia: east of Ilmenau, under *Picea abies*, 1 Oct. 2016, A. Henrici (K(M)237359, GenBank MG457843).

**Specimen details of downloaded sequences**: **Swedish**: [Ecology unknown], 6 Dec. 2014 [Collector unknown] (GenBank KM493150, only ITS2 region). [Locality unknown], [Ecology unknown], 12 Dec. 2016, [Collector unknown] (GenBank KU062814, only ITS2 region).

**Chroogomphus aff. filiformis**

**Notes**: This species is currently known only from a single ITS sequence from GenBank clustering close to *C. filiformis*. More specimen data are required to study this species properly.

**Specimen details of downloaded sequences**: **Austria**: Haggen, in subalpine forest of *Pinus cembra*, ectomycorrhizal root tip, 13 Dec. 2014, [Collector unknown] (GenBank KM504402).

**Chroogomphus sect. Fulminei**

**Notes**: As with sect. *Chroogomphus*, these species have a lamellar trama composed of amyloid hyphae. However, species of sect. *Chroogomphus* typically have a pileus that is reddish brown or pink to purplish when young, rather than apricot-orange or reddish (as seen in the centre of the pileus of *C. subfulmineus* in Fig 5G). Reddish to pink patches towards the base of the stipe are mostly absent in sect. *Chroogomphus*, and the trama at the base of the stipe is either salmon-ochraceous or orange-yellow in colour, rather than dark grey or olivaceous green. Species of sect. *Fulminei* should not be confused with *C. filiformis* of sect. *Filiformes*, which may also have a pinkish stipe base when *dried*, but differs in the other characters mentioned above.

**Chroogomphus fulmineus** (R. Heim) Courtec., *Docoms Mycol.* 18 (72): 50 (1988). (Figs 5E, 6E)

**Basionym**: *Gomphidius viscidus* var. *fulmineus* R. Heim, *Trab. Mus. Nat. Cienc. Nat.*, ser. *Bot.* 15: 68 (1934).

**Types**: **Spain**: Catalunya: Dos Rius, 30 Oct. 1932, R. Heim [Champ. Catalogne n°28, as “*Gomphidius viscidus var. fulgens*”] (PC0706649 – *lectotypus hic designatus*, MBT379515). – **France**: Corsica: Haute-Corse, Balagne, Forêt de Bonifatu, in woodland over granite, with *Pinus pinaster*, 20 Nov 2013, [Collector unknown] (K(M)190394 – *epitypus hic designatus*, MBT379522; GenBank MG457856).

**Description**: *Pileus* 10–45 mm, subconical to convex when young, becoming low convex with age, margin inrolled, surface slightly fibrillose with age, somewhat viscid to viscid; apricot-orange when young, sometimes with patches of light pink, then dark brown to leaden grey with age. *Lamellae* decurrent, somewhat crowded, colour not recorded when very young, spores soon colouring the lamellae pale to medium grey, then faded brown at maturity. *Stipe* 30–80 × 4–10 mm, cylindrical, often tapering towards the base, ochraceous orange to apricot-orange, then dark brown to leaden grey, with reddish to pinkish patches which increase in frequency towards the base, with a few filamentous, white veil remnants at the stipe apex, covering the lamellae when young. *Basal mycelium* whitish. *Trama* of the pileus and upper part of the stipe pale ochraceous orange, dark grey to black with olivaceous hints at the very base. *Taste and odour* not recorded.

**Basidiomata** boletoid, smooth, dark, blackish, weakly to strongly dextrinoid, (18.0–)19.0–24.0–(25.5) × (5.5–)6.0–
7.0(–8.0) μm, av. 21.2 × 6.5 μm, av. Range 20.6–22.4 × 6.3–6.6 μm, Q (–2.78)9.4–3.67(–3.81), Q av. 3.26, Q av. range 3.11–3.39. Basidia bisporic or tetrasporic, 48–63 × 9.5–12.5 μm, long clavate. Pleuro- and cheilocystidia 79–165 × 13–19 μm, av. 125.0 × 14.3 μm, av. range 110.6–133.4 × 13.8–14.8 μm, cylindrical to subfusiform, thin-walled (to 1.0 μm), some medium to large brown encrustations visible in KOH, hyaline in Melzer’s. Lamellar trama composed of amyloid hyphae. Hyphae of gelatinised hyphae, 3.0–8.0 μm diam, av. 5.4 μm, mostly inamyloid with some scattered amyloid elements. Pileipellis of amyloid hyphae, 4.5–16.0 μm diam, with a thick amyloid coating of blue granules when observed with Melzer’s; clamp connections not observed.

Hyphae of the basal mycelium composed of amyloid hyphae. Lamellar trama medium to large brown encrustations visible in KOH, hyaline and predominantly fusoid cystidia. Basidia are bisporic or tetrasporic, 48–63 × 9.5–12.5 μm, long clavate. Pleuro- and cheilocystidia 79–165 × 13–19 μm, av. 125.0 × 14.3 μm, av. range 110.6–133.4 × 13.8–14.8 μm, cylindrical to subfusiform, thin-walled (to 1.0 μm), some medium to large brown encrustations visible in KOH, hyaline in Melzer’s. Lamellar trama composed of amyloid hyphae. Hyphae of gelatinised hyphae, 3.0–8.0 μm diam, av. 5.4 μm, mostly inamyloid with some scattered amyloid elements. Pileipellis of amyloid hyphae, 4.5–16.0 μm diam, with a thick amyloid coating of blue granules when observed with Melzer’s; clamp connections not observed.

ITS sequence (GenBank MG457856) is distinct from the other members of sect. Chroogomphus. This species is most closely related to C. subfulmineus (MG457866), from which it differs in the ITS regions by five substitutions and indel positions, a similarity of 99.1 %.

Ecology and distribution: Known from coniferous and acidophilous coastal forests, to 700 m elev. in Corsica at supramediterranean levels, found mainly under acidophilous coastal forests, to 700 m elev. in Corsica and the Mediterranean, and as far north as Scotland, UK. Basidiome formation has been observed close to Rhizopogon roseolus, Suillus bellinii, and S. collinitus, albeit without direct basidiomatal contact.

Notes: Chroogomphus fulmineus usually has a smaller pileus than other members of the genus, and the spores are longer and have higher Q values on average (Q av. range 3.11–3.39) than any other Chroogomphus species studied. The sister species, C. subfulmineus, produces larger basidiomata, to 100 mm across, has somewhat wider spores, with lower average Q values (Q av. range 2.12–3.12) and broader cystidia. Across the genus, cystidal size tends to be highly variable, but between these two species at least, the difference in width appears to be consistent. Examination of the trama also reveals differences between the two species. That of C. fulmineus is pale ochraceous orange at the stipe apex, and dark grey to black with greenish tints at the base, whereas the trama of C. subfulmineus is brighter yellow at the stipe apex and then faintly olivaceous at the stipe base. Morphologically, C. fulmineus may also be confused with C. britannicus (sect. Filiformes), however, that species has slightly broader spores, coarser amyloid granules on the hyphae of the basal mycelium and predominantly fusoid cystidia.

The original material of Gomphidius viscidus var. fulmineus (Heim et al. 1934) had never been revisited before. It was recently rediscovered at PC, with other collections from Catalonia cited in the same paper, collected by Heim during a one-month foray in autumn 1932. Only one packet labelled “Gomphidius viscidus var. fulgens”, with one sketch and a single young specimen (“Dos Rius, 30-X [1932], n°28”), here designated as lectotype, could be found as original material. There was also a handwritten description associated with the packet details of another collection, provisionally named “Gomphidius unicolor”, used in the original description of G. viscidus var. fulmineus, with line drawings of fresh specimens coded with the Séguy colour chart reproduced by Heim et al. (1934: pl. 1, fig. 3). By deduction, this last collection probably came from “Environs de Girona, échantillons apportés à l’exposition, 5-XI” as cited in the protologue.

To assess the current application of the name C. fulmineus, Heim’s original description was compared with the known European species of Chroogomphus. The macromorphological characters, in particular the small basidiomata, apricot-orange colour of the pileus, the vinaceous red stipe base and black to greenish trama of the stipe base, are all in accordance with the current species concept. The single basidiome in the lectotype collection is very young, but taking that into consideration, the spore measurements from the type specimen, 18.5–22 × 6–7.5 μm, av. 19.7 × 6.8 μm, fit well with our observations and also other micromorphological characters accord with our species. An attempt was made to sequence the holotype of C. fulmineus, however, due to the specimen’s age this was unsuccessful. We therefore considered it necessary to designate specimen K(M)190394 as a modern epitype.

Considering the disjunction between North American and European species of Chroogomphus, and in expectation of thorough type revisions of North American taxa, the synonymy between C. fulmineus and C. ochraceus, proposed by Singer (1986: 736), and later by Villarreal & Heykoop (1996), is thought to be doubtful and is not retained here.

Additional specimens examined: France: Corse du Sud: Bastelica, in pine forest with Pinus pinaster, 19 Nov. 2014, P.-A. Moreau PAM14111904 (LIP 0401321, K(M)237214, GenBank MG457864). Pas-de-Calais: Le Touquet-Paris-Plage, in acidophilous coastal forest with Pinus pinaster, 11 Nov. 2014, E. Bastien & P.-A. Moreau PAM14111104 (LIP 0401320, K(M) 237215). – Spain: Castilla-La Mancha: Albalate de las Nogueras, near Pinus sylvestris, 3 Nov 2017, [Collector unknown] (K(M)237592). – United Kingdom: Scotland: Morayshire, Aviemore, with Pinus sp., 20 Aug 2017, M. Tortelli (K(M)237988).

Species details of downloaded sequences: Italy: Liguria: Imperia, San Remo, with Pinus pinaster, 30 Oct. 2010, [Collector unknown] (GenBank HM545722). – Spain: Jaén: Arroyo Frio, Sierra de Cazorla, under Pinus halepensis and P. pinaster, 4 Nov. 2013, J.L. Siquer (JLS 3264, GenBank LT219435).

Chroogomphus subfulmineus Niskanen, Loizides, Scambler & Liimat., sp. nov.

Figs 5G–H and 6F–G

MycoBank MB823599

Etymology: Named for its similarity to Chroogomphus fulmineus.

Diagnosis: The sister species, C. fulmineus, produces considerably smaller, viscid basidiomata <45 mm with more vivid orange colours, and a pale ochraceous orange trama at the stipe apex becoming dark grey to black at the stipe base.

Type: Cyprus: Troodos, under Pinus brutia, 18 Nov. 2014, M. Loizides (LIP 0401318 – holotype, GenBank MG457866; K(M)237213, hb. M. Loizides ML411812/1 – isotypes).
Description: Pileus (25–)40–80(–100) mm, hemispherical to subconical when young, expanding to convex or low convex with age, rarely indistinctly umbonate, margin somewhat inrolled; pileal surface innately fibrillose, mostly dry to somewhat viscid in humid conditions, never glutinous, reddish orange to copper-orange when young, then reddish brown, purple-brown or leaden grey in age, sometimes remaining reddish-orange at the centre. Lamellae subdecurrent to decurrent, at first covered with a fugacious, cortinoid, pinkish yellow to straw-coloured veil soon disappearing, somewhat crowded, dingy ochraceous to ochraceous brown when young, subdistant at full maturity (~12 per cm) and coloured olivaceous grey to sepia-brown from the spores; lamellar edges smooth and concolorous. Stipe 55–100 × 5–20 mm, fusiform-rooting and strongly tapering towards the base, covered in reddish, purple-red or orange-red fibrils on an ochraceous yellow to ochraceous buff background, apex often with a pinkish band. Basal mycelium orange to ochraceous orange. Trama of the pileus and stipe straw-yellow to yolk-yellow at the stipe apex, faintly to somewhat olivaceous at the base when sectioned. Taste and odour sourish, somewhat citrus-like.

Basidiospores boletoid, subfusoid to ellipsoid, smooth, thick-walled, dark, blackish, weakly to moderately dextrinoid, sparsely guttulate in water, (16.0–)17.0–24.0(–26.0) × (6.0–)7.0–8.0(–8.5) µm, av. 20.6 × 7.0 µm, av. range 17.5–21.6 × 6.4–7.7 µm, Q = (2.03–)2.18–3.37(–3.71), Q av. 2.81, Q av. range 2.29–3.12. Basidia bisporic or tetrasporic, 30–75 × (8–)9.5–14 µm, long clavate. Pleuro- and cheilocystidia 80–185 × 10–27 µm, av. 140.6 × 17.1 µm, av. range 133.4–155 × 16.2–19.0 µm, subcylindrical, subtriform, or subcapitate, thin-walled (to 1.0 µm), hyaline to brown in KOH, hyaline in Melzer's; encrustations not seen. Lamellar trama composed of amyloid hyphae. Pileipellis composed of gelatinised, sparsely septate hyphae 1.5–7.0 µm diam, av. 4.3 µm, mostly inamyloid, with some scattered amyloid elements. Hyphae of the basal mycelium cylindrical, 3.0–11.0 µm diam, with a thick amyloid coating of blue granules when observed in Melzer's; clamp connections not observed.

ITS sequence (GenBank MG457866) distinct from the other members of section Chroogomphus. This species is most closely related to C. fulmineus (GenBank MG457856), from which it differs in the ITS regions by 5 substitutions and indel positions, a similarity of 99.1 %.

Ecology and distribution: In coniferous forests and plantations, found with species of Pinus subgenus Pinus, mainly P. sylvestris and P. nigra on acidic substrates, and so far not recorded forming basidiomes under other coniferous genera. Producing basidiomata in the autumn, from early August to early November. Known from northern and southern Europe. There is currently a lack of collections from central Europe; however, the presence in Cyprus and the UK suggests it may also occur in intermediate localities.

Notes: Chroogomphus subfulmineus is a large species with typically dull reddish colours, a more or less dry or only slightly viscid pileus, an orangish mycelium, and a deep yolk-yellow trama at the stipe apex becoming somewhat olivaceous at the stipe base.

Microscopically, C. fulmineus also has narrower cystidia on average (av. range 13.8–14.8 µm). The species has longer spores than most other members of the genus, with the exception of C. fulmineus and C. britannicus. However, spores of C. subfulmineus are generally broader than those of C. fulmineus, and in the case of the holotype (LIP 0401318) they were especially broad (Fig 6F–G), a character which appears to be consistent throughout all collections of this species from Cyprus. The spores of C. britannicus are also broad, and the two species may be indistinguishable based on this character alone, but C. britannicus has coarser amyloid granules on the hyphae of its basal mycelium. It also differs in its lack of reddish to pink colouration towards the stipe base, as well as in the colour of its trama. Chroogomphus britannicus is further described as a very small, ochraceous orange to ochraceous buff species not exceeding 20 mm across, with predominantly fusoid cystidia (Khan & Hora 1978), a feature not seen in our collections of C. subfulmineus.

In previous analyses, the names C. rutilus or “C. rutilus” (Miller & Aime 2001, Li et al. 2009) and, more recently, the name C. britannicus (Martin et al. 2016), have been provisionally applied to this species. However, successful sequencing of the 40-year-old type of C. britannicus in this study, has demonstrated this taxon to be phylogenetically, as well as morphologically distinct from C. subfulmineus.

Additional specimens examined: Cyprus: Troodos, under Pinus nigra subsp. pallassiana on serpentinite soil, 6 Nov. 2014, M. Loizides ML41116/1, LIP 0401319; LIP 0401323, GenBank MG457865). United Kingdom: Scotland: Moray, Culbin Forest, plantation of Pinus sylvestris and P. nigra (Betula pubescens also present), 8 Aug. 2003, D. Genney IA09 (UNITE UDB001530; ibidem, 10 Oct. 2003, D. Genney DI56 (ABDF, UNITE UDB001529).

Specimen details of downloaded sequence: Finland: Inarin Lappi: Utsjoki, Kevo, ecology unknown, O. K. Miller OKM17238 (GenBank AF205639).

Vinicolores
Currently included species: C. cf. jamaicensis, and C. cf. vinicolor.

Ecology and distribution: Known from North America, basidiomata under species of Pinus.

Notes: This clade receives high bootstrap support in our analysis, yet we are hesitant to designate it formally as a section since the specimen data available are not based on type materials. Here we leave it unranked pending further study.

Chroogomphus subgen. Floccigomphus (Imai) Niskanen, Scambler, & Limat., comb. nov. MycoBank MB823595
Basionym: Gomphidius sect. Floccogomphus Imai, J. Fac. Agric., Hokkaido Imp. Univ. 43: 285 (1938).
Type: Chroogomphus tomentosus (Murrill) O.K Mill. 1964 (syn. Gomphidius tomentosus Murrill 1912).

Description (based on Miller & Aime 2001): Basidiomata small to medium sized. Pileus conical to convex, umbonate,
Chroogomphus in Europe

Currently included species: C. pseudotomentosus, and C. tomentosus.

Notes: Species of subgen. Floccigomphus can be defined by their somewhat broad, non-gelatinised pileipellis hyphae and amyloid lamellar trama. In addition, the cystidia are thick-walled.

Chroogomphus subgen. Siccigomphus

Currently included species: C. pseuderotomentosus, and C. tomentosus.

Notes: Species of subgen. Floccigomphus can be defined by their somewhat broad, non-gelatinised pileipellis hyphae and amyloid lamellar trama. In addition, the cystidia are thick-walled.

Description: Basidiomata small to medium sized. Pileus subconical to almost plane, appressed fibrillose-scaly, surface dry. Lamellae typically decurrent, pale to ochraceous-orange when young though later coloured grey by spores. Basidiospores boletoid, smooth, moderately to strongly dextrinoid. Cystidia cylindrical to subfusiform, thin-walled. Lamellar trama composed of inamyloid hyphae. Pileipellis of somewhat broad to broad hypheae not embedded in a gelatinous layer.

Ecology and distribution: Found in Eurasia and North America, in coniferous and mixed forests under species of Pinus (both subgenera Pinus and Strobus), and other species of the family Pinaceae, e.g. Picea abies.

Chroogomphus cf. helveticus (Singer) M.M. Moser, in Gams, Kl. Krypt.-Fl., 3rd edn 2b (2): 51 (1967).

Description: Pileus 30–50 mm, convex when young, later low convex, surface dry, felty-scaly-fibrillose, though scales not very evident in wet weather or in old basidiomata; yellow to orange-apricot when young, often with a pinkish or even violaceous tint, turning ochraceous orange when handled. Lamellae somewhat decurrent, medium-spaced, yellow to pale orange when young, later greyish from the spores. Stipe 30–60 × 10–16 mm, cylindrical, often tapered at the base, yellow to pale orange, becoming reddish orange when handled, with a few filamentous veil remnants at the stipe apex. Basal mycelium pale to ochraceous yellow to pinkish. Trama of the pileus and stipe yellow to yellowish orange, brown at the very base of the stipe. Taste and odour not distinctive.

Basidiospores boletoid, smooth, dark, blackish, moderately to strongly dextrinoid, 15.0–19.0(–20.0) × 6.0–7.5 μm, av. 17.2 × 6.7 μm, av. range 16.0–18.0 × 6.4–7.0 μm, Q (2.38–)2.40–2.75(–2.80), Q av. 2.59, Q av. range 2.58–2.60. Basidia bisporic or tetrasporic, 35–55 × 8(–11)–(12.5) μm, long clavate. Pleuro- and cheilocystidia 73–133 × 12–19 μm, av. 108.8 × 14.8 μm, av. range 104.0–113.0 × 14.5–15.0 μm, cylindrical to subfusiform, thin-walled (to 1.5 μm), hyaline to brown in KOH, sometimes with encrustations, hyaline in Melzer’s. Lamellar trama composed of inamyloid hyphae, yellowish to weakly pink in Melzer’s. Pileipellis of non-gelatinised hyphae, 4.0–17.0 μm diam, av. 9.6 μm, mostly inamyloid with some scattered amyloid elements. Hyphae of the basal mycelium cylindrical, 4.5–11.0 μm diam, with a thick amyloid coating of blue granules when observed in Melzer’s; clamp connections not observed.

ITS sequence (GenBank MG457859) distinct from other members of subgen. Siccigomphus. This species is most closely related to C. roseolus (EU706329), from which it differs in the ITS regions by 10 substitutions and indel positions, a similarity of 98.5 %.

Ecology and Distribution: In coniferous and mixed forests, basidiomes formed at least under Picea abies, one record also from a mixed forest under Larix decidua. Producing basidiomata in the autumn, in September. Occuring in the Alps, Carpathians, and high mountains of the Balkans.

Notes: This is currently the only reported European species belonging to subgen. Siccigomphus, and so can be distinguished from the others by the broad, non-gelatinised pileipellis hyphae. Chroogomphus roseolus is morphologically similar, but is only known from China and Pakistan (Li et al. 2009, Razak et al. 2016).

This species was originally described from basidiomes under Pinus cembra, a 5-needled pine, in Switzerland (Singer 1950). However, none of the collections examined in this study
have been recorded under this tree species. Li et al. (2009) state that this species is associated with members of Pinus subgen. Strobus. However, two of the three specimens they studied were first sequenced by Miller & Aime (2001) who did not mention any such ecological relationships. In the literature, a subspecies C. helveticus subsp. tatrensis (Pilát) Kuthan & Singer 1976 is reported with Picea and 2-needled pines and is distinguished from C. helveticus subsp. helveticus that occurs with 5-needled pines (Singer & Kuthan 1976, Breitenbach & Kränzlin 1991). We here refer to our species provisionally as C. cf. helveticus, based on the name that has been applied to it in previous studies (Miller & Aime 2001, Li et al. 2009, Martin et al. 2016), but further study of this taxon is needed since no type material of C. helveticus has been examined yet.

Specimens examined: Austria: Tyrol: Ötztaler Alpen, Sölden, in mixed woodland with Larix decidua, 2 Sep. 2002, S. E. Evans (K(M)105170, GenBank MG457860). – Slovakia: Žilina: Liptovska Kotlina basin, Važec, in meadows and stands along creeks N of the village, in woods of Picea abies, 30 Sep. 2003, T. Niskanen & K. Liimatainen F03-1673 (HT019100, GenBank MG457859).

Specimen details of downloaded sequences: Austria: Klausoden, ecology unknown, O. K. Miller OKM21376 (GenBank AF205642). – Czech Republic: Central Bohemian Region: Neustupov, ecology unknown, [Date and collector unknown] (HKAS 55293 (KUN), GenBank FJ652070). – Germany: Bavaria: under conifers, 26 Sep. 1996, M. Kronfeldner (GenBank GU187514). – Switzerland: Alp Claire, ecology unknown, O. K. Miller OKM24410 (GenBank AF205650).

Key to the European species of Chroogomphus

Each of the seven described European Chroogomphus species can be identified using the key below. Some sections and subgenera are monotypic in Europe, in which case these are included preceding their representative species.

1 Pileus hyphae non-gelatinised ................................................................. 2
   Pileus hyphae gelatinised ........................................................................ 3. subgen. Chroogomphus

2 (1) Lamellar trama amyloid; cystidia thick-walled ...................................................... subgen. Floccigomphus
   Lamellar trama non-amyloid; cystidia thin-walled ........................................ 4. subgen. Siccigomphus, C. cf. helveticus

3 (1) Lamellar trama inamyloid or with very few amyloid elements .......................... sect. Confusi, C. mediterraneus
   Lamellar trama distinctly amyloid ............................................................... 4

4 (3) Basal mycelium yellowish .................................................................................. sect. Filiformes, C. britannicus
   Basal mycelium whitish to salmon to purple-pink to orange ................................. 5

5 (4) Reddish patches towards base of stipe ............................................................. 6. sect. Fulminei
   Yellow-orange to dark brown towards base of stipe ......................................... 7. sect. Chroogomphus

6 (5) Q av. usually > 3.1; cystidia narrow (av. <15 μm wide); trama at base of stipe dark grey to black ............... C. fulmineus
   Q av. usually < 3.1; cystidia broad (av. >15 μm wide); trama at stipe base faint to somewhat olivaceous
   ....................................................................................................................... C. subfulmineus

7 (5) Cystidia thick-walled (to 3 μm) .............................................................................. C. rutilus
   Cystidia thin-walled (to 1 μm) .............................................................................. C. cf. purpurascens

DISCUSSION

European species of Chroogomphus

Eight species are currently recognized from Europe. One of them, C. cf. helveticus, belongs to subgen. Siccigomphus whereas all other species belong to subgen. Chroogomphus. Six of the species received high support in our phylogenetic analysis and had an intraspecific variation of less than 1%; the interspecific variation was over 1.5%. The only exception was between the species pair C. fulmineus and C. subfulmineus, where there was an interspecific variation of 0.9%, and intraspecific variations of 0.8% (C. fulmineus) and 0.4% (C. subfulmineus). This corresponds to the findings from other groups of subclass Agaricomycetinae as well, for example in Cortinarius and the family Lyophyllaceae, a threshold value of 99% was found to be suitable for distinguishing species in the majority of lineages, although some morphologically distinguishable species had an even higher threshold value, likely indicating a recent radiation (Niskanen et al. 2011, Bellanger et al. 2015, Garnica et al. 2016).

Morphology-based identification

Through careful examination of specimens, we have been able to delimit the currently known European species of Chroogomphus on the basis of morphology, using mainly microscopic characters. These delimitations are supported by our molecular data, and to some extent also by our geographical and ecological data. In summary, important distinguishing characters for species identification are: colour of pileus when young, colour of trama, spore size (length, width, and Q value), amyloidity of the lamellar trama, wall thickness of cystidia, gelatinisation of the pileipellis and diameter of the pileipellis hyphae. The bruising colour of basidiomata is also a potentially useful character in identification, but this feature is
not included in our descriptions as macromorphological data are currently lacking for some species. One character which does not appear to be useful is spore dextrinoidity, as this was found to vary considerably within the same species. Another character which might be of limited value in *Chroogomphus* is the presence of clamp connections, as these can be rare or absent and so their presence can be difficult to determine with certainty. However, based on preliminary observations, studying the clamps from the looser mycelial strands from the mycelial mat could give better results; at least these hyphae are not heavily incrusted with amyloid granules that can sometimes obscure clamp connections. Further observations of this microcharacter in good material are needed.

Though not every species can be defined by single characters, combinations of characters along with geographical and ecological data should in most cases allow for positive identification. Infrageneric clades can mostly be distinguished by an assessment of gelatinisation within the pileipellis and/or the amyloidity of the lamellar trama.

When distinguishing between *Chroogomphus* and its sister genus *Gomphidius*, it is important to note that lamellae in young specimens of *Chroogomphus* are not always the pale orange to ochraceous, rarely purple, colour that characterizes the genus, but are often coloured grey by spores long before maturity. This character should therefore be observed in the youngest possible specimens to avoid confusion.

**Ecology and distribution**

Within their infrageneric taxa, species of *Chroogomphus* do not appear to be confined to narrow geographic regions. Subgen. *Siccigomphus* has representatives across the Northern Hemisphere, as do sections *Confusi* and *Filiformes*. Members of *Floccigomphus* are found in North America and Asia, but not Europe; whilst those of sect. *Chroogomphus* are found throughout Eurasia, but not North America. Sect. *Fulminei* and *Viniclores* have narrower distributions and are found in Europe and North America respectively.

Species of *Chroogomphus* can occur in a broader habitat range than has been suggested in previous studies (Miller 2003, Li et al. 2009). Five species, *C. britannicus*, *C. helveticus*, *C. mediterraneus*, *C. purpurascens*, and *C. rutulus*, have now been reported producing basidiomes under *Picea* and other coniferous genera besides *Pinus*. In the light of this evidence, and considering the observations made by Agerer (1990), their occurrence is determined by the presence of the mycorrhizal partner and to unknown extent also by the presence of *Rhizopogon/Suillus* species. The exact biological nature, and degree of specificity of these relationships, is not currently well understood and should be investigated further. Future collections of *Chroogomphus* should include notes on the presence of any *Rhizopogon* or *Suillus* present in the vicinity.

**Infrageneric classification**

The infrageneric clades recovered in our analysis all receive high bootstrap support (BS >75) and generally correspond well to those found in previous studies (Miller 2003, Li et al. 2009, Martin et al. 2016). The subgenera *Floccigomphus* and *Siccigomphus* as defined here are recovered with high support in all three studies. Furthermore, all the sections of subgen. *Chroogomphus* are found in all previous analyses. The study of Miller (2003) also shows subgen. *Chroogomphus* as monophyletic. However, in both Li et al. (2009) and Martin et al. (2016) some sections of the subgenus are not grouped with the main clade, but in those cases the topology of the tree is not well supported.

In our phylogenetic analysis, subgen. *Floccigomphus* formed the basal clade in the genus. This position indicates that subgen. *Floccigomphus*, along with subgen. *Siccigomphus*, which clusters nearby, represent ancestral clades within the genus. This would suggest that species of *Chroogomphus* may have originally lacked a gelatinised layer in the pileipellis, and that this feature emerged during subsequent evolution, along with an overall narrowing of the pileipellis hyphae.

By formally recognising sections and subgenera within *Chroogomphus*, we aim to stabilise the groups that have already been proven to exist by both molecular and morphological data. Establishing infrageneric taxa is important in consolidating the affinities of closely-related species. By defining these groups morphologically, it becomes easier to observe the evolution of characters within the genus. It is also convenient for future studies wherein infrageneric taxa can be referred to by name, reducing confusion. Although some sections currently lack unifying morphological features, we aim to have established a robust infrageneric framework upon which future studies of the genus *Chroogomphus* can be built.

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**REFERENCES**

Agerer R (1990) Studies on ectomycorrhizae XXIV. Ectomycorrhizae of *Chroogomphus helveticus* and *C. rutulus* (*Gomphidiaceae*, *Basidiomycetes*) and their relationship to those of *Suillus* and *Rhizopogon*. *Nova Hedwigia* 50: 1–63.

Aime MC, Miller OK (2006) (1709) Proposal to conserve the name *Chroogomphus* against *Brauniellula* (*Gomphidiaceae*, *Agaricales*, *Basidiomycota*). *Taxon* 55: 228–229.

Antonini D, Antonini M (2002) Macromicieti nuovi, rari o specifici della regione mediterranea. *Fungi non Delineati* 22: 1–72.

Bellanger JM, Moreau P-A, Corriol G, Bidaud A, Chalange R, et al. (2015) Plunging hands into the mushroom jar: a phylogenetic framework for *Lyophyllaceae* (*Agaricales*, *Basidiomycota*). *Genetica* 143: 169–194.

Breitenbach J, Kränzlin F (1991) *Fungi of Switzerland*. Vol. 3. Lucerne: Edition Mykologia.

Fries EM (1821) *Systema Mycologicum*. Vol. 1. Griefswald: E. Fries EM (1821) *Systema Mycologicum*. Vol. 1. Griefswald: E. Fries.
