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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* 1, *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 *Siccigomphus* 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 rDNA 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

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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, an ephemeral fibrous veil, 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 decurrent 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 secotioid 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.*

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2006, and *C. rutilus* (Schaeff.) O.K. Mill. 1964. However, the application of these names remains open to interpretation as no type material has been sequenced to date. In addition, there are differing opinions regarding synonymy, as well as uncertainties regarding the delimitation of taxa and identification of specimens due to overlapping morphological characters. There is, therefore, disagreement over the total number of species of *Chroogomphus* thought to occur in Europe and in individual European countries. *C. rutilus* was considered to be the only European species by Miller (1964) and, more recently, Knudsen & Taylor (2012) regarded this as the only species occurring in northern Europe. Similarly, it is the only currently accepted species on the British and Irish checklist (Legon & Henrici 2005). By contrast, from the latest molecular study by Martín *et al.* (2016) it can be inferred that at least six species occur in Europe.

Members of the genus *Chroogomphus* occur throughout the Northern Hemisphere, with only one species, *C. papillatus*, reported from the Southern Hemisphere (Raitelhuber 1974). It is notable that currently there is no molecular evidence of any of the species having a distribution encompassing both North America and Eurasia. Moreover, there do not appear to be many species with an intercontinental distribution across both Europe and Asia (Miller & Aime 2001, Li *et al.* 2009, Martín *et al.* 2016); *C. rutilus* occurs in both Europe and Asia; and *C. purpurascens*, originally described from the former Soviet Union, is now known also to occur in Europe (Li *et al.* 2009).

The most useful morphological characters for distinguishing similar species of *Chroogomphus* include: thickness of the cystidial wall, width of hyphae in the pileipellis and spore size. The gelatinization of hyphae in the pileipellis can also be a useful character, as can the colour of the mycelium at the base of the stipe (Miller & Aime 2001, Li *et al.* 2009, Martín *et al.* 2016).

Species of *Chroogomphus* are found in coniferous forests dominated by *Pinaceae*. Miller described the genus as forming basidiomes under a variety of conifers including *Larix*, *Picea*, *Pinus*, *Pseudotsuga*, and *Tsuga* (Miller 1964), and some North American species such as *C. tomentosus* and the East Asian *C. pseudotomentosus* are recorded from under several tree genera (Miller & Aime 2001, Li *et al.* 2009). However, recent studies have shown that *Chroogomphus* basidiomes are primarily found under species of *Pinus*, especially in Europe. Also, some species are found only forming basidiomes under members of *Pinus* subgen. *Pinus*, whilst others form these only with *Pinus* subgen. *Strobos* (Li *et al.* 2009). In Europe, subgenus *Strobos* contains the native five-needled species *Pinus cembra* and *P. peuce*. All other native European *Pinus* species are two-needled and belong to subgenus *Pinus*. Unlike *Chroogomphus* species, those belonging to the sister genus *Gomphidius* are not found with members of *Pinoideae* but only with the other *Pinaceae* subfamilies *Piceoideae*, *Lariceoideae*, and *Abietoideae* (Miller 2003, Li *et al.* 2009), although some exceptions to this have been recorded: *G. nigricans* Peck 1897 with *Pinus strobus* (Miller 2003), *G. roseus* (Fr.) Fr. 1838 with *Pinus* spp. (Knudsen & Taylor 2012), and *G. tyrrhenicus* D. Antonini & M. Antonini 2004 with *Arbutus unedo* and *Quercus ilex* (Antonini & Antonini 2002, Vila *et al.* 2006).

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 mouting 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 REExtract-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 (Kato & 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 GTRGAMMA 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. *Cystidia* 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

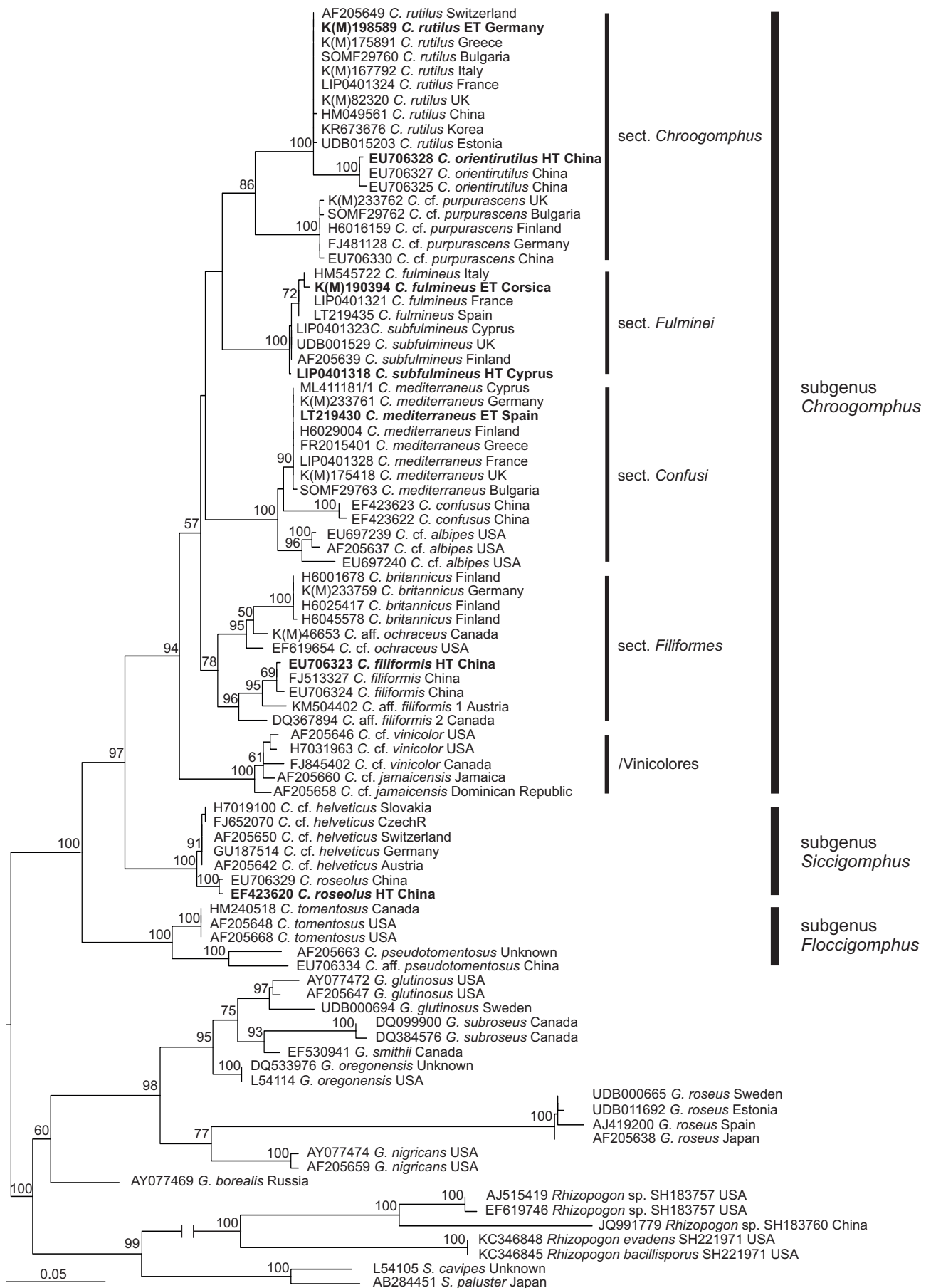


Fig. 1. Phylogeny resulting from the RaXML analysis of ITS regions. Bootstrap values greater than 50 % are indicated above branches. The sequences originating from type specimens are in boldface. HT = holotype; ET = epitype.

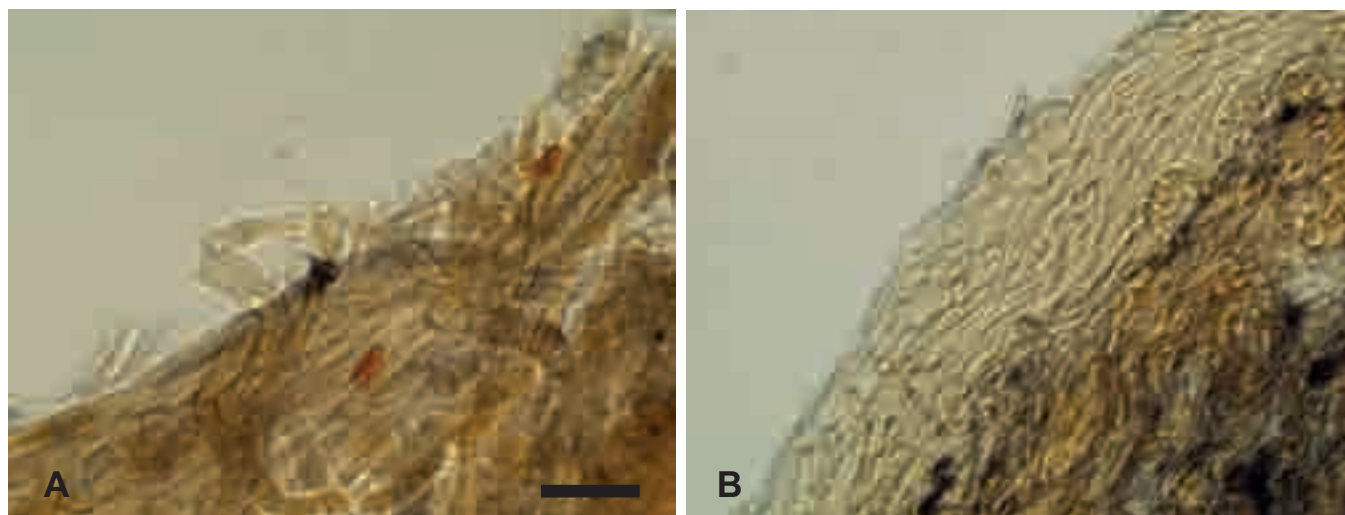


Fig. 2. The two different types of pileipellis found in European species of *Chroogomphus*. **A.** Subgenera *Siccigomphus* and *Floccigomphus* are characterized by species with broad pileipellis hyphae that lack a gelatinous layer; non-gelatinised pileipellis hyphae of *C. cf. helveticus* (H7019100). **B.** Species of subgen. *Chroogomphus* have narrower pileipellis hyphae embedded in a gelatinous layer; these gelatinised pileipellis hyphae are found in all other European species, in this example, *C. britannicus* (K(M)77895, holotype). Bar = 50 μ m. Photographs: Ross Scambler.

to purplish, vinaceous or leaden grey. *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 *Vinicolores*.

Notes: Species of subgenus *Chroogomphus* are distinguished by having a pileipellis composed of gelatinised hyphae which are typically narrow (1.5–8.0 μ m wide), but may be broader in species of section *Confusi* (1.5–12.5 μ m). 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) μ m (Miller & Aime 2001) and 4–17 μ m respectively (Fig. 2).

Chroogomphus sect. Chroogomphus

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

Description: *Basidiomata* medium to large. *Pileus* subconical to plane, sometimes umbonate, 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 *Strobis*), 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 tramal 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).

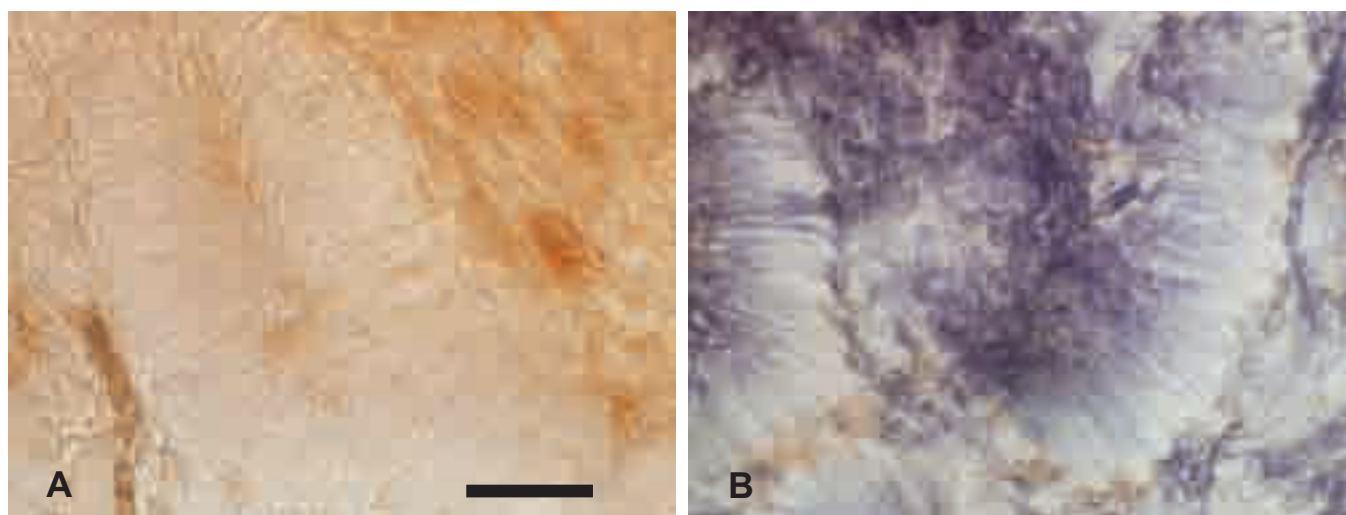


Fig. 3. Degree of amyloidity of the lamellar trama of European species of *Chroogomphus*. **A.** Subgen. *Siccigomphus* and sect. *Confusi* in subgen. *Chroogomphus* are characterised by species with reduced amyloidity in the lamellar trama; non-amyloid lamellar trama of *C. mediterraneus* (H6029004). **B.** Other sections of subgenus *Chroogomphus* have distinctly amyloid lamellar trama; amyloid lamellar trama of *C. rutilus* (K(M)198589). Bar = 50 μ m. Photographs: Ross Scambler.

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 [β .] *atropunctus* Pers., *Syn. Meth. Fung.* 2: 292 (1801).

Agaricus viscidus [α .] *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**, MBT379514). – **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. Tchechoslov.* 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 litigiosus 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 & Schwarzwälder Pilzlehorschau (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, fibrillose 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 \times 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) \times 5.5–7.0(–7.5) μ m, av. = 18.0 \times 6.2 μ m, av. range = 16.7–20.5 \times 5.9–6.4 μ m, Q = (2.09–)2.43–3.63–4.03, Q av. = 2.94, Q av. range = 2.69–3.47. *Basidia* bispore or tetrasporic, 38–72 \times (9–)10–14 μ m, long clavate. *Pleuro-* and *cheilocystidia* 101–220 \times 11–22 μ m, av. = 137.2 \times 16.7 μ m, av. range = 125.3–158.2 \times 13.8–19.0 μ m, cylindrical to subfusiform, often thick-walled (walls to 3.0 μ m), hyaline in KOH, hyaline to yellow in Melzer's. *Lamellar trama* composed of amyloid hyphae. *Pileipellis* of gelatinised hyphae, 1.5–8.0 μ m diam, av. 3.8 μ m, mostly non-amyloid with some scattered amyloid elements. *Hyphae of the basal mycelium* cylindrical, 4.0–12.5 μ m 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 %.



Fig. 4. The two different types of pleuro- and cheilocystidia found in European species of *Chroogomphus*. **A.** Thick-walled cystidia, only found in *C. rutilus* (K(M)198589). **B.** Thin-walled cystidia, found in all other European species; *C. cf. purpurascens* (K(M)233762). Photographs: Ross Scambler.

Ecology and distribution: In coniferous and mixed forests, but also in more urban environments such as lawns in parks and cemeteries. Basidiomes primarily found under species of *Pinus* subgenus *Pinus*, though it has also been found under *Picea* and *Abies*. Producing basidiomata in the autumn, from mid-August to mid-October. Known as a common species throughout Europe as far north as Estonia, but to date not from Fennoscandia, it also occurs in parts of Asia as far east as China and Korea.

Notes: *Chroogomphus rutilus* usually has quite large basidiomata, and is the only European member of the genus to have thick-walled cystidia (Fig 4A). This is the micromorphological character that sets it apart from other species most clearly. The few photographs of the species we have seen suggest that the lamellae could be truly grey from the beginning. If this is confirmed in future observations, it would set *C. rutilus* apart from all the other European members of the genus. Indeed, the presence of pale orange to ochraceous lamellae in young basidiomata is currently regarded as a characteristic of the genus.

The name *C. rutilus* was first used by Schaeffer (1774) in Germany, and has since been applied broadly throughout Europe. The epitypified concept of *C. rutilus* is in accordance with that recognised by Miller & Aime (2001), Li *et al.* (2009), and Martín *et al.* (2016), however it should be noted that the phylogenies published by these authors also include other

clades of downloaded sequences labelled as *C. rutilus* or "*C. rutilus*" which represent the newly recognized *C. subfulmineus* (see below).

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 misdetermination 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 epitype 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*.

Additional specimens examined: **Bulgaria:** Samokov. Govedarts, Rila Mountain, near *Picea abies*, *Pinus sylvestris* also present in the forest, 14 Sep. 2014, B. Assyov (SOMF29760, GenBank MG457861). – **Channel Islands:** Jersey: St Brelade, Red Houses, Parc du Pont Marquet, parkland under *Pinus radiata*, 18 Oct 2015, N.W. Legon (K(M)235485, GenBank MH037156). – **France:** Nord: Le Touquet-Paris-Plage, under *Pinus* sp., 30 Sep. 2014, A. Thorel PAM14093001 (LIP 0401324, GenBank MG457862). Savoie: Bessans, with *Pinus sylvestris*, 6 Sep. 2002, P.-A. Moreau PAM02090618 (LIP 0401326); Jarrier, with *Pinus sylvestris*, 18 Sep. 2014, M. Durand MDH01 (LIP 0401325). – **Greece:** Trikala: South Pindos Mountains, Amarantos, with *Abies borisii-regis*, 18 Oct. 1999, A. E. Hills (K(M)175891, GenBank MG457853); Voras, alt. 1800 m, with *Pinus* sp., Dec. 2014, A. Triantaphyllos PAM14120001 (LIP 0401327). – **Italy:** Trento: Peregine Valsugana, Susa, with *Pinus* sp., 8 Sep. 2002, A. E. Hills (K(M)167792, GenBank MG457851). – **Romania:** with *Pinus sylvestris*, 2014, M. M. Pop (FR2015502). – **United Kingdom:** England: Kent, Tunbridge Wells cemetery, near *Pinus* sp., 17 Oct. 2005, E. W. Brown (K(M)135802, GenBank MG457848); Somerset, Crewkerne Grammar School grounds, near *Pinus* sp., 28 Oct. 1975, J. Keylock (K(M)108451, GenBank MG457850). N. Ireland: Fermanagh, Inisherik, Crom Estate, with *Pinus sylvestris*, 15 Oct. 2000, E. E. Emmett (K(M)82320, GenBank MG457849).

Specimen details of downloaded European sequences: **Czech Republic:** Ústecský: Roudnice nad Labem, with *Pinus* subgenus *Pinus* sp., 30 Aug. 2008, J. Borovicka (HKAS 55294 (KUN), FJ652071). – **Estonia:** Voru Maakond: Noarootsi 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: Zuyevsky, Kirov, 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:** Yanqing: [Ecology unknown], [Collector unknown] (GenBank HM049562). Huairou, [Ecology unknown], [Collector unknown] (GenBank HM049561); [Ecology unknown], [Collector unknown]

(GenBank KM488533). Jilin: Jilin Agriculture University, with *Pinus* subgenus *Pinus* sp., 8 Sep. 2004, J. R. Wang HMJAU 3681 (JLAU, GenBank EU791580). – **South Korea:** Ulleung-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. Griby Mokh. Sovetsk. Dal'nego Vostoka 1: 378 (1990). (Figs 4B, 5B, 6B)

Description (some macroscopic features based on Li et al., 2009): *Pileus* 20–50 mm, conical when young, low convex in age, with inrolled margin, surface viscid, sometimes slightly fibrillose; pink to purplish when young, becoming pale brown to brownish red to greyish red to dark purple with age. *Lamellae* adnate to decurrent, somewhat crowded to distant, salmon-ochraceous then greyish to pale brown with age. *Stipe* 35–55 × 7–10 mm, cylindrical, sometimes tapering towards the base, pale yellow to pale brown, becoming darker towards the base, sometimes with a lilac pinkish pruina in young specimens, with some filamentous veil remnants at the stipe apex. *Basal mycelium* salmon to purple pink. *Trama* of the pileus and stipe salmon-ochraceous, sometimes greyish green at the very base. *Taste and odour* not distinctive.

Basidiospores boletoid, smooth, dark, blackish, moderately dextrinoid, (14.0–)15.0–18.0(–19.0) × 5–6.5 μm, av. = 16.6 × 5.7 μm, av. range = 16.0–17.2 × 5.2–6.1 μm, Q = (2.57–)2.63–3.52(–3.72), Q av. = 2.95, Q av. range = 2.83–3.06. *Basidia* tetrasporic, rarely bisporic, 37–60 × 9–12.5 μm, long clavate. *Pleuro- and cheilocystidia* 89–196 × 11–17 μm, av. = 123.5 × 14.2 μm, av. range 114.5–132.5 × 13.5–15.0 μm, cylindrical to subfusiform, thin-walled (to 1.0 μm), hyaline to deep brown in KOH, hyaline in Melzer's. *Lamellar trama* composed of amyloid hyphae. *Pileipellis* of gelatinised hyphae, 3.0–8.0 μm diam, av. 5.5 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; clamp connections observed, but uncommon.

ITS sequence (GenBank MG457855) distinct from other members of section *Chroogomphus*. This species is most closely related to *C. rutilus*, from which it differs in the ITS regions by 34 substitutions and indel positions, a similarity of 94.7 %.

Ecology and distribution: In coniferous and mixed forests. Li et al. (2009) reported that *C. cf. purpurascens* occurs with members of *Pinus* subgen *Strobus*. Our collection data indicate that this species has a broader range of associates including *P. sylvestris* and *P. nigra*. One collection has also been reported as made from a *Picea abies* dominated forest, however the presence of *Pinus* in that forest cannot be ruled out. Producing basidiomata in the autumn, from mid-August to mid-November. This species is found across most of Europe, as far north as Finland, west to the Channel Isles, and east as far as Russia (Kirov). It is also known from parts of Asia, including far eastern Russia, from where it was first described, and China.



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 pileus. 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: **Bulgaria:** Burgas Province: Malko Tarnovo, Strandzha, with *Pinus nigra*, 18 Oct. 2014, B. Assyov (SOMF29761). Blagoevgrad Province: West Frontier mts, Ograzhden Mt, with *Pinus nigra*, 21 Nov. 2014, B. Assyov (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 (H6016159, GenBank MG457855).

Specimen details of downloaded European sequences: **Czech Republic:** Ústecký: Roudnice nad Labem, with *Pinus* subgenus *Strobos* sp., 14 Sep. 2008, J. Borovicka (HKAS 55295 (KUN), GenBank FJ652072). – **Germany:** Hesse: Marburg, with *Pinus* subgenus *Strobos* sp., [Collector unknown] (HKAS 54925 (KUN), GenBank FJ481128). – **Russia:** Kirov Oblast: Nikitintsy, with *Pinus* subgenus *Strobos* sp., 12 Aug. 2006, B. Tolgor HMJAU 4633 (JLAU, GenBank EU706332); Falyonsky, with *Pinus* subgenus *Strobos* sp., 15 Aug. 2006, B. Tolgor HMJAU 4634 (JLAU, GenBank EU706333).

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

Chroogomphus sect. **Confusi** Niskanen, Scambler & Liimat., **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 umbonate, 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.*, *Errotari* 3: 68 (2006).

(Figs 3A, 5C–D, 6C)

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

Types: **Spain:** *Balearic 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*, MBT379523; 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 umbonate, margin usually inrolled, surface innately fibrillose, subviscid to dry; colour when young ranging from dark

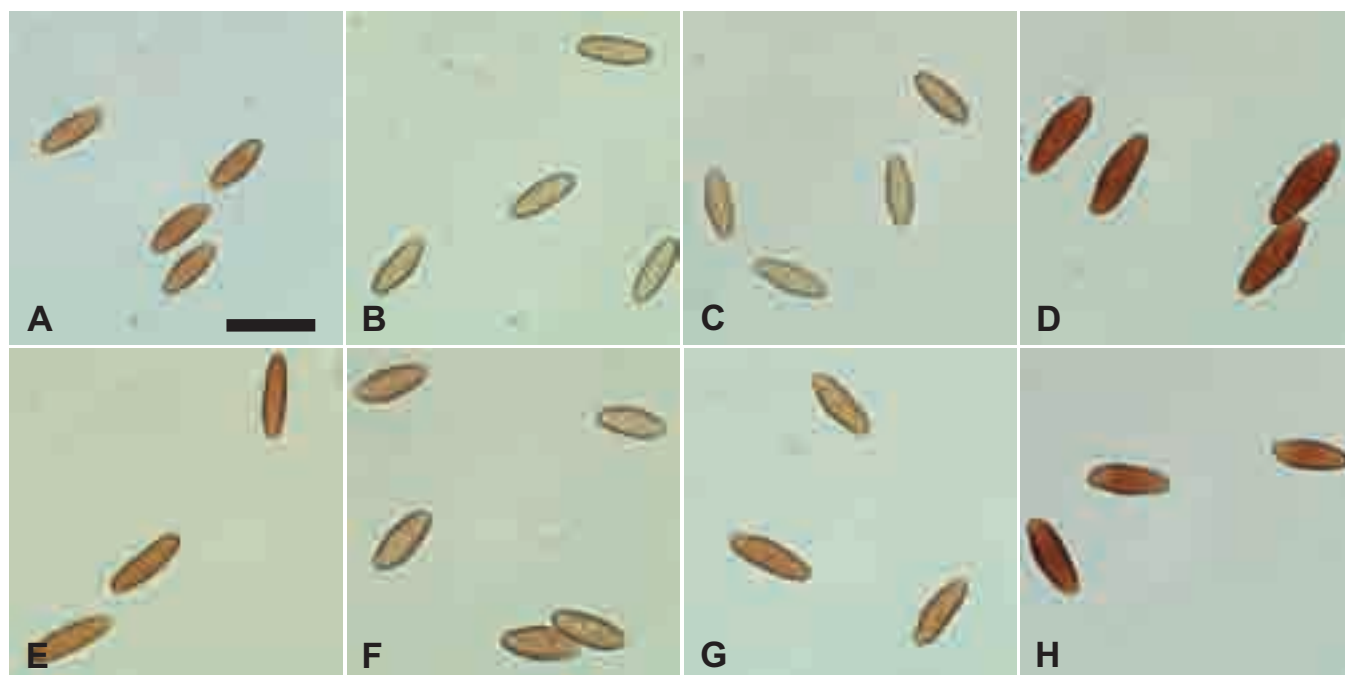


Fig. 6. Basidiospores of *Chroogomphus* species: **A.** *C. rutilus* (K(M)198589). **B.** *C. cf. purpurascens* (K(M)233762). **C.** *C. mediterraneus* (K(M)233761). **D.** *C. britannicus* (H6045578). **E.** *C. fulmineus* (LIP 0401320). **F.** *C. subfulmineus* (LIP 0401318, holotype, with unusually broad spores). **G.** *C. subfulmineus* (UDB001529). **H.** *C. cf. helveticus* (H7019100). The degree of spore dextrinoidity does not appear to be a consistent character within species. Bar = 20 μ m. Photographs: Ross Scambler.

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 \times 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) \times (5.0–)6.0–7.5(–8.0) μ m, av. 16.9 \times 6.6 μ m, av. range 16.3–18.0 \times 5.9–6.8 μ m, Q = (1.87–)2.11–2.96(–3.36), Q av. 2.57, Q av. range 2.43–2.70. *Basidia* bisporic or tetrasporic, 40–75 \times 9.5–14 μ m, long clavate. *Pleuro- and cheilocystidia* 91–153 \times 11–22 μ m, av. 122.3 \times 15.3 μ m, av. range 108.0–130.5 \times 13.8–18.3 μ m, cylindrical to subfusiform or subutriform, sometimes subcapitate, thin-walled (to 1.0 μ m), but occasionally also thick-walled (to 2.0 μ m), 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 μ m diam, av. 5.3 μ m, mostly inamyloid, with some scattered amyloid elements. *Hyphae of the basal mycelium* cylindrical, 4.0–16.0 μ m 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, Logodazh village, with *Pinus nigra*, 22 Sep. 2014, *B. Assyov* (SOMF29763, GenBank MG457857). – **Cyprus:** Troodos, under *P. nigra* subsp. *pallasiana*, 18 Nov. 2014, *M. Loizides ML411181/1, FR2015390* (GenBank MG457867). – **Finland:** *Uusimaa:* Porvoo, Bjurböle, NE side of Meteorittitie, E from Mäntymäki, in grass-herb forest dominated by *Betula pendula*, 11 Sep. 1997, *U. Nummela-Salo & P. Salo* (H6016157, GenBank MG457834). *Varsinais-Suomi:* Lohja, Virkkala, E part of Pähkinäniemi, very rich, dry grass-herb forest with calcareous bottom, 29 Aug. 1999, *U. Nummela-Salo & P. Salo* (H6016160, GenBank MG457836). *Etelä-Karjala:* Lappeenranta, Ihalainen, Mattila, S from the highway, NE of the Russian military cemetery, on dry heath forest dominated by *Pinus sylvestris*, with rich calcareous bottom, 5 Sep. 2003, *U. Nummela-Salo & P. Salo* (H6002491, GenBank MG457839). *Etelä-Häme:* Padasjoki, Kasiniemi, Viitaniemi, in herb-rich mesic forest, 5 Sep. 2011, *V. Haikonen* (H6029004, GenBank MG457831). – **France:** *Savoie:* Chambéry, les Charmettes, with *Pinus* sp., 11 Nov. 2014, *M. Durand MDH03* (LIP 0401328, GenBank MG457839). – **Germany:** *Thuringia:* Ilmenau, between Oberporlitz and Unterporlitz, with *Picea*, 28 Sep. 2016, *R.A. Fortey* (K(M)233760, GenBank MG457835); Ilmenau, on the road to Unterporlitz, 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, *Larix* 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:** *Balearic 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 & Liimat., **sect. nov.**
Mycobank MB823593

Etymology: Named after the type of the section.

Diagnosis: The combination of a yellow basal mycelium and amyloid lamellar tramal 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 ochraceous 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 *Strobos*), 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 tramal hyphae do not have a yellow basal mycelium, and although *C. mediterraneus* (sect. *Confusi*) 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

1972, A. Z. M. N. A. Khan (K(M)206849 – paratype, GenBank MG457841).

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, thin-walled (to 1.0 μm), hyaline to brown in KOH, hyaline to yellow in Melzer's. **Lamellar trama** composed of amyloid hyphae. **Pileipellis** of gelatinised hyphae, 1.5–7.0 μm diam, av. 3.9 μm, mostly inamyloid 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.

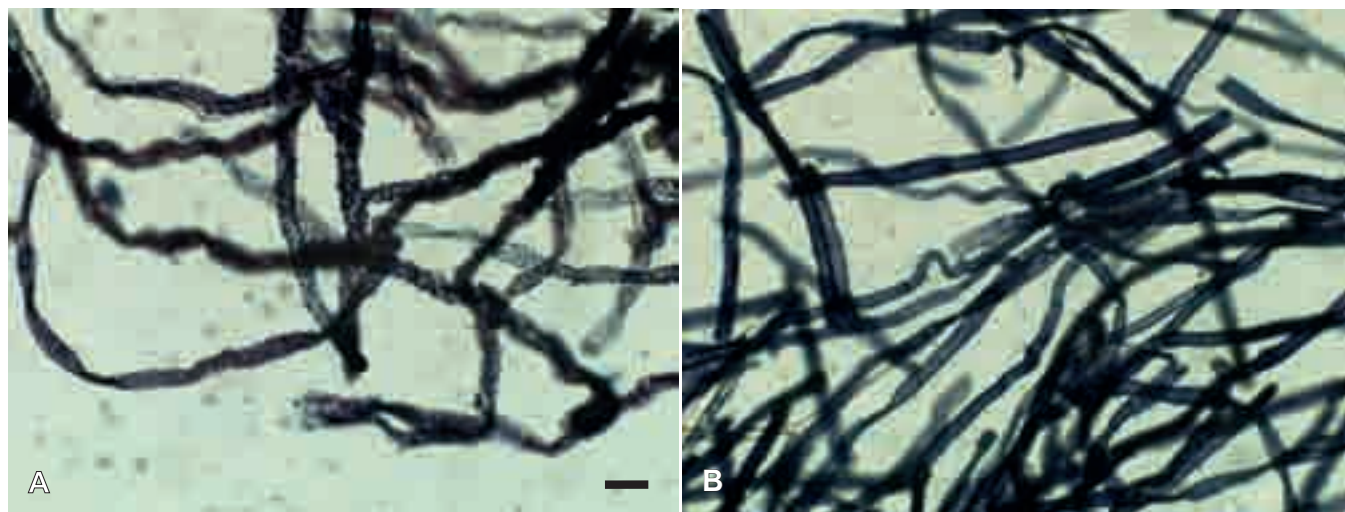


Fig. 7. Hyphae of the basal mycelium with a thick amyloid coating of blue granules when observed in Melzer's reagent: **A.** *Chroogomphus britannicus* (H6045578) with coarse amyloid granules. **B.** *C. subfulmineus* (DG56) with finer amyloid granules. Bar = 10 μm. Photographs: Ross Scambler.

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:* Parikkala, Kirkonkylä, Sikoharju, *Pinus sylvestris* dominated heath, 13 Sep. 2003, V. Haikonen (H6059351, GenBank MG457844). *Satakunta:* Honkajoki, Kivimäki, SE of the Siikainen-Honkajoki road, with *Pinus sylvestris* in pine-dominated heath on sandy soil, 22 Sep. 2006, E. Ohenoja (H6045578, GenBank MG457847); Siikainen, east of Katselmankallio, west of Kaakkurilammet, dry pine-dominated acid heath, old track, 20 Sep. 2006, E. Ohenoja (H6059327, GenBank MG457845). *Perä-Pohjanmaa:* Kemijärvi, lower south slope of the fjell Pyhätunturi, coniferous forest dominated by *Pinus sylvestris*, *Betula* and *Picea abies*, 27 Aug. 2008, E. Ohenoja (H6001678, 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*, 1 Oct. 2016, A. Henrici (K(M)233759, GenBank MG457843).

Specimen details of downloaded sequences: **Sweden:** [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* 1

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* Niskanen, Scambler & Liimat., sect. nov.**
MycoBank MB823594

Etymology: Named after the type of the section.

Diagnosis: The combination of whitish to orange basal mycelium, reddish patches on the stipe, dark or olivaceous trama at the base of the stipe, and amyloid lamellar trama hyphae distinguish this section from others of subgenus *Chroogomphus*.

Type: *Chroogomphus fulmineus* (R. Heim) Courtec. 1988.

Description: *Basidiomata* medium to large. *Pileus* low convex, surface smooth, fibrillose with age, dry to viscid; ochraceous orange, reddish orange to apricot-orange when young, dark

brown, purple-red to leaden grey when mature. *Lamellae* subdecurrent to decurrent. *Stipe* with patches of pinkish to vinaceous red, especially towards the base. *Trama* of the base of the stipe dark or olivaceous. *Basal mycelium* whitish to orange.

Ecology and distribution: Found in northern and southern Europe, in coniferous and acidophilous coastal or mountainous forests under species of *Pinus* subgen. *Pinus*. To date, species of this clade have not been found with tree species other than those belonging to subgen. *Pinus*.

Currently included species: *C. fulmineus*, and *C. subfulmineus*.

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.,**
Docums Mycol. **18** (72): 50 (1988).
(Figs 5E, 6E)

Basionym: *Gomphidius viscidus* var. *fulmineus* R. Heim,
Trab. Mus. Nac. 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.

Basidiospores 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 \times 6.5 μm , av. Range 20.6–22.4 \times 6.3–6.6 μm , Q (–2.78)2.94–3.67(–3.81), Q av. 3.26, Q av. range 3.11–3.39. *Basidia* bisporic or tetrasporic, 48–63 \times 9.5–12.5 μm , long clavate. *Pleuro- and cheilocystidia* 79–165 \times 13–19 μm , av. 125.0 \times 14.3 μm , av. range 110.6–133.4 \times 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. *Pileipellis* of gelatinised hyphae, 3.0–8.0 μm diam, av. 5.4 μm , mostly inamyloid with some scattered amyloid elements. *Hyphae of the basal mycelium* cylindrical, 4.5–16.0 μm diam, with a thick amyloid coating of blue granules when observed in 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 *Pinus pinaster*, though it has also been recorded forming basidiomes under *P. halepensis* and *P. pinea*. Producing basidiomata in autumn, from late October to November. This species occurs throughout 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, cystidial 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 revised 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 \times 6–7.5 μm , av. 19.7 \times 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 Villareal & 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).

Specimen 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 Cazorta, under *Pinus halepensis* and *P. pinaster*, 4 Nov. 2013, J.L. Siquier (JLS 3264, GenBank LT219435).

Chroogomphus subfulmineus Niskanen, Loizides, Scambler & Liimat., **sp. nov.**
MycoBank MB823599
(Figs 5G–H and 6F–G)

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 ML411181/2 – 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, subutriform, 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* (Martín *et al.* 2016), have been provisionally applied to this species. However, successful sequencing of the 40-year-old paratype 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. *pallasiana* on serpentine soil, 6 Nov. 2014, *M. Loizides* ML41116/1, LIP 0401319; *ibidem*, ML4193/1 (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* DG56 (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, & Liimat., **comb. nov.**

Mycobank MB823595

Basionym: *Gomphidius* sect. *Floccigomphus* 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,

fibrillose, dry; pale to dark orange. *Lamellae* decurrent, light orange when young, vinaceous with age. *Basidiospores* broadly elliptic, dextrinoid. *Cystidia* fusiform, thick-walled. *Lamellar* trama composed of strongly amyloid hyphae. *Pileipellis* of somewhat broad to broad, non-viscid hyphae.

Ecology and distribution: Known from North America and Asia, associated with species of *Pinus* and other species of the family *Pinaceae*.

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. They form a basal clade within the genus alongside subgen. *Siccigomphus*, the species of which also have broad, non-gelatinised pileipellis hyphae but lack amyloid elements in the lamellar trama and have thin-walled cystidia.

Chroogomphus subgen. Siccigomphus Niskanen, Scambler, & Liimat., **subgen. nov.**
MycoBank MB823597

Etymology: Referring to the dry pileus of its species.

Diagnosis: The combination of broad, non-gelatinised pileipellis hyphae, inamyloid lamellar trama and narrow spores with a low Q value (Q av. <2.60) distinguish this subgenus from others of genus *Chroogomphus*.

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

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 hyphae 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 *Strobis*), and other species of the family *Pinaceae*, e.g. *Picea abies*.

Currently included species: *C. cf. helveticus*, *C. cf. leptocystis*, *C. roseolus*, and *C. cf. sibiricus*. The ITS sequences of *C. cf. leptocystis* (GenBank FJ157000, OKM2981, USA, ID) and *C. cf. sibiricus* (GenBank AH009856, OKM21628, Korea) were short and thus not included in our analysis. However, the phylogenetic analyses of Miller & Aime (2001) and Li *et al.* (2009) show that those specimens belong in this subgenus.

Notes: Members of subgen. *Siccigomphus* can be defined by their broad, non-gelatinised pileipellis hyphae, inamyloid lamellar trama and narrow spores with a low Q value (Q av. <2.60). Species of subgen. *Floccigomphus* have similar pileipellis characters to those of this subgenus, but the two

groups can be easily distinguished by the amyloidity of the lamellar trama: species of *Floccigomphus* have an amyloid lamellar trama. In addition, the cystidia are thick-walled.

Chroogomphus cf. helveticus (Singer) M.M. Moser, *in Gams, Kl. Krypt.-Fl.*, 3rd edn **2b** (2): 51 (1967). (Figs 2A, 5F and 6H)

Basionym: *Gomphidius helvetica* Singer, *Schweiz. Z. Pilzk.* **28**: 198 (1950).

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, Martín *et al.* 2016), but further study of this taxon is needed since no type material of *C. helveticus* has been examined yet.

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. <i>Chroogomphus</i> |
| 2 (1) | Lamellar trama amyloid; cystidia thick-walled | subgen. <i>Floccigomphus</i> |
| | Lamellar trama non-amyloid; cystidia thin-walled | subgen. <i>Siccigomphus</i> , C. cf. helveticus |
| 3 (1) | Lamellar trama inamyloid or with very few amyloid elements | sect. <i>Confusi</i> , C. mediterraneus |
| | Lamellar trama distinctly amyloid | 4 |
| 4 (3) | Basal mycelium yellowish | sect. <i>Filiformes</i> , C. britannicus |
| | Basal mycelium whitish to salmon to purple-pink to orange | 5 |
| 5 (4) | Reddish patches towards base of stipe | 6. sect. <i>Fulminei</i> |
| | Yellow-orange to dark brown towards base of stipe | 7. sect. <i>Chroogomphus</i> |
| 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 0.6% (*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

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 (H7019100, 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).

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 incrustated 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 *Vinicolores* 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. rutilus*, 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, Martín *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 Martín *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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