MYCOSCIENCE 57 (2016) 384–392

Available online at www.sciencedirect.com

MYCOSCIENCE

ISSN 1340-3540 (print), 1618-2545 (online)

journal homepage: www.elsevier.com/locate/myc

Short communication

Barcoding sequences clearly separate *Chroogomphus mediterraneus* (Gomphidiaceae, Boletales) from *C. rutilus*, and allied species

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ARTICLE INFO

Article history:
Received 7 April 2016
Received in revised form
14 June 2016
Accepted 18 June 2016
Available online 29 July 2016

Keywords:
Basidiomycota
Gomphidius mediterraneus
ITS nrDNA
Morphological features
Systematics

ABSTRACT

Chroogomphus mediterraneus, a species described from the Balearic Islands, is confirmed as belonging to the genus *Chroogomphus*, instead of the genus *Gomphidius*, as it appears in taxonomic databases. *Chroogomphus mediterraneus* shows macroscopic features similar to *Chroogomphus fulmineus* and *C. rutilus*, species with which it has been confused in the literature. However, analyses based on comparison of barcoding sequences (internal transcribed spacers of nuclear ribosomal DNA), and a review of the morphological features, support *C. mediterraneus* as a separate species close to *C. albipes*, a gasteroid fungus, and *C. confusus*, a species described from Yunnan, China. *Chroogomphus mediterraneus* is also reported for the first time in the Iberian Peninsula.

Gomphidius mediterraneus Finschow was described from Ibiza (Balearic Islands, Spain), growing under *Pinus halepensis* (Finschow 1978, 1984); there are no records of this species from other parts of the World. As indicated in Li et al. (2009) and mentioned by other authors (Miller 1964, 2003; Miller and Aime 2001), the species of the genus *Gomphidius* are characterized by pallid to whitish lamellae in young basidiomata, a white to pallid pileal trama, and a glutinous pileipellis, as well as a mycelium at the base of the stipe with dextrinoid to non-amyloid hyphae; these characters are not present in *G. mediterraneus*. Vila et al. (2006), based on the protologue and the original iconography from Finschow (1978), made a combination to *Chroogomphus mediterraneus* (Finschow) Vila, Pérez-de-Greg. & G. Mir. However, since they

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http://dx.doi.org/10.1016/j.myc.2016.06.004

published the combination in a local journal, at present, both in Index Fungorum and Mycobank, this species is included in the genus *Gomphidius*. Vila et al. (2006) were correct in making the combination of *C. mediterraneus*, since the species of the genus *Chroogomphus* are mainly characterized by basidiome with pale orange to ochraceous lamellae when young, with an ochraceous pileal trama, a moist to viscid pileipellis, as well as a mycelium at the stipe base formed by amyloid hyphae (Li et al. 2009). Vila et al. (2006) also mentioned that the relationship between *C. mediterraneus* and *C. rutilus* (Schaeff.) O.K. Mill. should be reviewed, since both species share many morphological characters.

During the study of Agaricales of the Balearic Islands (Constantino and Siquier 1996, 2006, 2011; Siquier et al. 2009; Siquier and Salom 2013), we came across many specimens in different stages of development, which could be identified as *Chroogomphus mediterraneus*. This was a good opportunity to review morphological characters and to compare this species with *C. rutilus*, as well as with other similar species, such as *Chroogomphus fulmineus* (R. Heim) Courtec. Moreover, barcoding sequences (internal transcribed spacer region of ribosomal DNA sequences, ITS nrDNA; Schoch et al. 2012), could be obtained to clearly delimitate *C. mediterraneus* from those the barcoding sequences already available in public DNA databases, mainly discussed in Miller and Aime (2001), Miller (2003), and Li et al. (2009).

Thus, the aim of this paper was to delimitate *C. mediterraneus* using a barcoding approach with an accurate revision of the microscopic characters that could be used to clearly identify this species, even from herbarium specimens. Morphological characters were also compared with similar species, especially *C. fulmineus* and *C. rutilus*, as well as with other allied species. Moreover, a revision of earlier records of Gomphidiaceae from the Balearic Islands has been performed. In this paper, we report the first record of *C. mediterraneus* for the Iberian Peninsula.

Fifteen collections of Gomphidiaceae from the Balearic Islands and one from Teruel (Spain) were studied. Vouchers are deposited in JLS (Jose Leonardo Siquier) and JCS (Joan Carles Salom) personal herbaria. Field photographs of fresh basidiomata were taken with Olympus C-7070 (Olympus Imaging Corporation, Tokyo), Nikon D300S and Nikon D7000 (Nikon Corporation, Tokyo) cameras. Morphological characters were observed macroscopically and microscopically. Macromorphological features were analyzed using a stereomicroscope Olympus SZ61 (Olympus Corporation, Tokyo). Micromorphological features were recorded at the magnification of 40× and 100× using an Olympus BX40 and BX51 (Olympus Corporation, Tokyo) from the dry samples mounted in 5% KOH, water, ammoniacal congo-red 1%, and Melzer's reagent. Microphotographs were taken with a dedicated camera Fujifilm Fineprix JX (Fujifilm Corporation, Tokyo), Panasonic DMC-FS50 (Panasonic Corporation, Osaka), Olympus C-7070 and Amscope MU 900 (AmScope, Irvine, California). Spore measurements are based on thirty basidiospores, which were measured in side-view. Spore measurements and length/width ratios (Q) are presented as minimum–maximum and mean (Qm) values. Spore shapes from the Qm values were in accordance with those proposed by Bas (1969). The holotype of *G. mediterraneus*, one collection of *C. fulmineus* and two collections of *C. rutilus* were also studied. Herbaria names follow Holmgren et al. (1990).

Total DNA was extracted from herbarium material using a modified CTAB procedure of Doyle and Doyle (1987) or a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The internal transcribed spacer (ITS) regions, including the 5.8S nrDNA were amplified by the polymerase chain reaction (PCR) with the primer pair ITS1-F/ITS4 (White et al. 1990; Gardes and Bruns 1993). PCR reactions contained 2 μL DNA solution (adjusted to approximately 100 ng), 5 μL PCR reaction buffer, 5 μL dNTP mix (0.2 mM), 2 μL each of the primers ITS1-F and ITS4 (5 μM), and 1.5 U TaqDNA polymerase. The final volume was adjusted to 50 μL with distilled sterile H_2O. The amplification conditions were set as follows: denaturation at 95 °C for 4 min, 34 cycles of 30 s at 94 °C, 2 min at 52 °C, 1 min at 72 °C, and a final extension of 5 min at 72 °C. The PCR products were purified using the BioTeke Purification Kit (BioTeke Corporation, Beijing, China), and sequenced with an ABI 3730 DNA analyzer (Life Technologies, Carlsbad, CA) and an ABI BigDye3.1 terminator cycle sequencing kit (Sangon Co., Ltd, Shanghai, China); the same primers described above for PCR were used for the sequencing reactions. Sequences of specimens JLS 3539 and JLS 3624 were retained at the ALVALAB (Oviedo, Spain). Consensus sequences where assembled using Sequencher (Gene Codes Corporation, Ann Arbor, MI). Prior to the alignment, sequences were compared with homologous sequences in EMBL/GenBank/DDBJ (Cochrane et al. 2011) and UNITE (Kõjalg et al. 2005) using the BLASTn algorithm (Altschul et al. 1997) to check for contamination. A preliminary identification of the new sequences obtained was done through UNITE database (http://unite.ut.ee) species hypotheses (SHs) search (Kõljalg et al. 2013). The PlutoF multiple sequence alignments obtained in UNITE were merged and manually adjusted using Se-Al v.2.0a11 (Rambaut 2002). Six sequences under *Rhizopogon* sp. were chosen as the outgroup, since their sequence values in the BLASTn search were next highest after *Chroogomphus* and *Gomphidius* sequences. Alignment gaps were indicated as "-" and ambiguous nucleotides were marked as "N".

Maximum parsimony (MP) analyses were performed using the program PAUP* 4.0b10 for Macintosh (Swofford 2003); as indicated in Telleria et al. (2013), with a default setting to stop the analyses at 100 trees. Exhaustive searches were conducted, without rooting the tree, and gaps treated as missing data. Tree scores, including tree length, consistency index (CI), and retention index (RI) were calculated from each exhaustive search. Non-parametric searches (Felsenstein 1985) were used to calculate branch support (bootstrap, bs), performing 10000 replicates using the fast-step option. The phylogenetic tree was edited with Adobe Illustrator CS3 v. 11.0.2 (Adobe Systems, Spain). Genetic distances were also calculated in PAUP*v. 4.0b10 using Kimura-2-parameter model (Kimura 1980), and a dendrogram was constructed using neighbor-joining (NJ).

No ITS sequences were obtained from the holotype of *C. mediterraneus* by any of the laboratories testing (Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, China; Real Jardín Botánico-CSIC, Spain; and ALVALAB, Spain). However, good sequence was obtained from specimen JLS 3539 collected from the same locality as the holotype; and this

specimens share the same features as the revised holotype. Using the barcoding sequence from specimen JLS 3539, we can confirm that this taxon belongs to the genus *Chroogomphus* and not *Gomphidius*; thus, the generic position in the database Index Fungorum and Mycobank should be corrected. The Blast search of this sequence showed the highest value match with *C. asiaticus* O.K. Miller & Aime, a species described from Nepal; however, the sequences from *C. asiaticus* have only the ITS1 region, and this species was excluded from the barcoding analyses. As indicated in Table 1, these two species differ in the pileus size and color, as well as in the identity of the putative hosts.

The nine new ITS nrDNA sequences obtained in this study were aligned with 95 sequences included in UNITE databases. A matrix of 798 unambiguously aligned nucleotide positions was produced (455 constant, 69 parsimony-uninformative, and 274 parsimony-informative). The 100 most parsimonious trees gave a length of 826 steps, CI = 0.5545, HI = 0.4894 and RI = 0.8897; one of the 100 MPTs is shown in Fig. 1. The strict consensus tree showed the main groups indicated in Fig. 1; as did the NJ tree based on K2P distances (data not shown). Using *Rhizopogon* sequences as outgroup, the Gomphidiaceae sequences grouped together with high support (bs = 100%). The basal species was *Gomphidius borealis* O.K. Miller (species hypothesis SH5-00563), and the rest of the sequences were distributed in at least 16 SH clades (including a clade formed by six specimens from the Balearic Islands that do not fit into any SH clade). Among these clades, seven *Gomphidius* species (*G. glutinosus* (Schaeff.) Fr., SH-011317; *G. smithii* Singer, SH-011320; *G. subroseus* Kauffman, SH5 011316; *G. oregonesis* Peck, SH-011319; *G. nigricans* Peck, SH-011318; *G. roseus* (Fr.) Fr., SH5 007907; *G. maculatus* Fr., SH5-000562) grouped into the same clade formed by *C. vinicolor* (Peck) O.K. Mill. and *C. jamaicensis* (Murrill) O.K. Mill., both SH-000558.

The 17 main clades (including the basal *G. borealis* SH-000563), in general had high support (bs > 75%), except the clade of *C. vinicolor* SH-000558 (bs = 71%), *C. glutinosus* SH-011317 (bs = 73%), *C. ochraceus* (Kauffman) O.K. Mill. (bs = 61%) and *C. albipes* (Zeller) O.K. Mill., SH-000564 (bs = 50%). In many cases, the SH number corresponded to a described species, such as the already mentioned *G. borealis* SH 000563; but in other cases the SH number included more than one species, although in well-supported separated groups (e.g., SH5-000556 or SH-000557). Also, the SH5-000556 grouped 15 sequences identified as *C. rutilus* (including the two new sequences from the MA-Fungi specimens), five sequences under *C. orientirutilus* Yan C. Li & Zhu L. Yang and six under *C. purpurascens* (Lj. N. Vassiljeva) M.M. Nazarova.

As mentioned before, the specimens from the Balearic islands, as well as the collection from Teruel, morphologically identified as *C. mediterraneus*, formed a highly supported clade (bs = 93%), which is a sister group of *C. albipes* SH5-000564 (early name *Brauniellula albipes* (Zeller) A.H. Sm. & Singer). In Fig. 1, these two species are close to *C. confusus* Yan C. Li & Zhu L. Yang. *Chroogomphus mediterraneus* and *C. confusus* share a number of features as indicated in Table 1, but they differ in the pileus color, as well as in the putative host. Also, as shown in Fig. 1, both *C. fulmineus* and *C. rutilus* clades are quite far away from the *C. mediterraneus* clade. Overall *C. mediterraneus* is morphologically very similar to *C. fulmineus* and *C. rutilus*, but in the second morphological revision *C. mediterraneus* can be distinguished from *C. rutilus* based on a microscopic feature mentioned in Li et al. (2009), i.e., namely the wall thickness of the cystidia (Table 1). In *C. rutilus*, the cystidium walls are ~2.5 μm thick, whereas in *C. fulmineus* and *C. mediterraneus* the cystidia have thin walls (~1 μm thick), as in *C. confusus*, the sister species of the clade formed by *C. mediterraneus* and *C. albipes*. As indicated in Table 1, other features allow separation of *C. fulmineus* from *C. mediterraneus*, such as the basidiome and mycelium colors; also, as with *C. rutilus*, the spores and cystidia of *C. fulmineus* are usually bigger that in *C. mediterraneus*.

Molecular techniques are commonly used to overcome taxonomic problems posed by the limitation of morphological characters or in cases where morphological characters are in conflict, ambiguous or missing (Hillis 1987; Bruns et al. 1991; Hibbett 1992). In this study, DNA sequences from ITS, including the 5.8S nrDNA subunit, were analyzed separately and in combination with a re-evaluation of some morphological criteria to define the species *Chroogomphus mediterraneus*. In our analyses, both *C. fulmineus* and *C. rutilus* clades are well separated from the *C. mediterraneus* clade. Interestingly *C. mediterraneus* is the sister species of *C. albipes* (≡ *Brauniellula albipes*), a gasteroid *Chroogomphus* that has lost the capacity for forcible basidiospore discharge as mentioned in Miller and Aime (2001), but without any statistic support. The other close species in our analyses is *C. confusus*, a species described from China; however, the color of the basidiome and mycelium (Table 1), and the different putative hosts offers a method to discriminate *C. mediterraneus* from *C. confusus*.

In the Blast search of the GenBank nucleotide database of the sequences generated from *C. mediterraneus* the closest hits were sequences of *C. asiaticus* (Miller and Aime 2001; Miller et al. 2002), a small species with reddish brown to brownish orange pileus (13–15 mm broad), described from Nepal. However, as indicated under methodology above we do not include these sequences in our barcoding analyses, since they were not complete (ITS2 region was missing). More specimens of *C. asiaticus* should be analyzed to obtain the ITS2 sequences, since the complete ITS sequence is crucial to delimitate this species from *C. mediterraneus* using a barcoding approach. For example, in Telleria et al. (2010), the ITS1 region alone did not allow separation of the corticioid fungi *Hyphodermella corrugata* (Fr.) J. Erikss. & Ryvarden from *H. rosae* (Bres.) Nakasone. However, the ITS 2 region clearly separated both taxa as belonging to two different species, since in this region 11 bases were different, and the molecular analyses grouped the specimens analyzed in two well-supported clades.

Our revision of the morphological characters of *C. mediterraneus, C. confusus*, *C. fulmineus* and *C. rutilus*, confirm that the basidiome color, as well as other morphological features that have been described traditionally to separate Gomphidiaceae species, such as the pileipellis (Table 1), could help to delimit *C. mediterraneus* from similar species: in *C. mediterraneus* the basidiome is subviscid with internal subgelatinized fibrils, whereas in *C. asiaticus*, *C. confusus* and *C. fulmineus* it is subviscid with internal, non-gelatinized fibrils, and in *C. rutilus* they are viscid with internal gelatinized fibrils; this character allows one to separate *Chroogomphus* species from China (Li et al. 2009). Also, although it has not been

Table 1 – Comparison of different characters of *Chroogomphus mediterraneus* and close species.

	C. mediterraneus Protologue (Finschow 1978) and our observations from the holotype	*C. mediterraneus* All collections examined in this study, excluding the holotype	*C. rutilus* Collections examined MA-Fungi 66940 and MA-Fungi 68920	*C. fulmineus* Collection examined JLS 3624	*C. confusus* Protologue (Li et al. 2009)	*C. asiaticus* Protologue (Miller and Aime 2001)
Pileus color	Brown violet with silver effect	Grayish, ochraceous-orange, cream-orange, vinaceous to dingy vinaceous brown	Grayish, ochraceous-orange, vinaceous to dingy vinaceous brown	Brown reddish, orange reddish, ruby red with purple or yellowish tones	Brownish orange to orange or orange yellow some times with reddish tinge	Orange to brownish orange
Pileus size	3–5 cm	2.5–7(–8) cm	2–8(–10) cm	3.5–5 cm	1.3–4 cm	1.3–1.5 cm
Pileipellis	Subviscid with innated subgelatinized fibrils	Subviscid with innated subgelatinized fibrils	Viscid with innated gelatinized fibrils	Subviscid with innated no gelatinized fibrils	Viscid with innated no gelatinized fibrils	Viscid with innated no gelatinized fibrils
Stipe size	2.5–5 × 0.7–1.1 cm (Finschow's watercolor)	3–9 × 1.1–1.9 cm	5–8(–12) × 0.5–1.5 cm	5–9 × 0.5–1.5 cm	2.5–8 × 0.5–1 cm	3.2 × 0.4 cm
Mycelium color	Our observations: Yellow to yellowish ochre	Cream to yellowish ochre	Cream to yellowish ochre	Vinaceous to violet	Gray to whitish	Grayish yellow
Basidiospores	Protologue: 14–15(–19) × 6–7(–8) μm Our observations: 15.5–19(–20.2) × 6.5–8	(15–)16–19 × (5.5–)6–8 μm	16.5–19(–21) × (5.5–)6–8 μm	16–22(–25) × 6–8 μm	15–20(–21) × 5.5–7(–7.5) μm	12–20 × 5–8.5 μm
Wall cystidia[a]	Thin walled, ≤1.2 μm	Thin walled, ≤1.2 μm	Thick walled, up to 2.70 μm	Thin walled, ≤1.2 μm	Thin walled, ≤1.2 μm	Thin walled, ≤1.2 μm
Habitat	*Pinus halepensis*	*Pinus halepensis, P. sylvestris, P. nigra*	*Pinus sylvestris, P. pinea, P. pinaster, P. halepensis*	*Pinus halepensis, P. pinaster, P. pinea*	*Pinus yunnanensis, P. densata P. densiflora, P. koraiensis, Abies* spp., *Picea* spp.	*Pinus roxburgii, Alnus nepalensis*

[a] Character observed in young specimens.

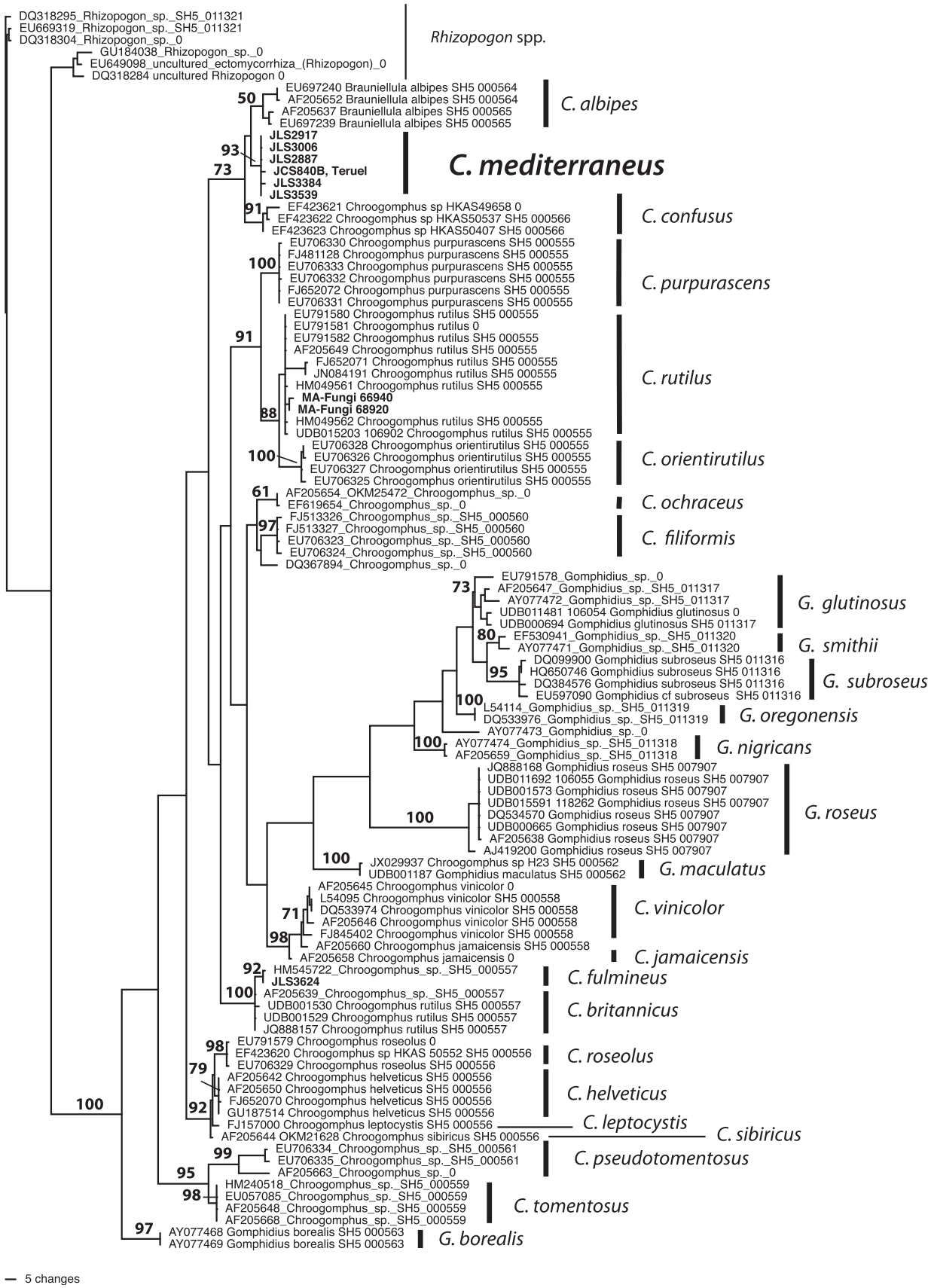

Fig. 1 – One of the 100 most parsimonious trees inferred from a heuristic search of ITS nrDNA sequences of Gomphidiaceae, using *Rhizopogon* sequences as outgroup. Each sequence with the accession number from GenBank or UNITE, the identification name used by the authors of the sequences, and the SH number obtained in the UNITE search (May 2015). New

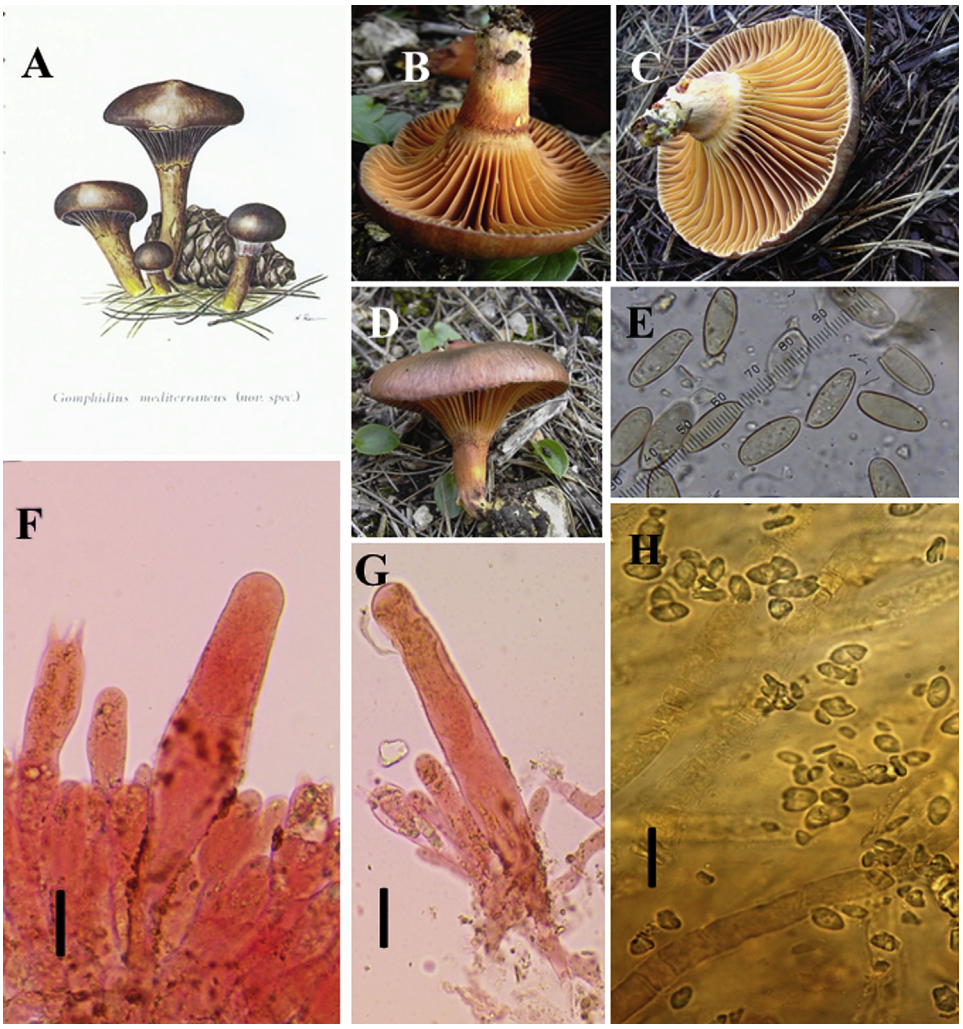

Fig. 2 – *Gomphidius mediterraneus*. A: Original painting BREMEN 2060. B–D: JLS 3539. E: Basidiospores BREMEN 2060. F, G: Basidia and cystidia JLS 3539. H: Crystals in pileipellis JCS 840B. *Bars*: D–I 20 μm.

considered by other authors, the mycelium color seems to be a good taxonomic feature to discriminate *C. mediterraneus* (cream-color to yellow ochraceous) from *C. fulmineus* (vinaceous to violet).

Moreover, in our study, with the specimen from Teruel, the presence of *C. mediterraneus* in the Iberian Peninsula has been confirmed. Probably, more collections preserved in herbaria from the Iberian Peninsula, could belong to *C. mediterraneus* instead of *C. rutilus*. The presence of cystidia with thin walls is a good morphological feature that helps to separate *C. mediterraneus* from *C. rutilus*, without obtaining the ITS sequences; however, the wall size is not always easy to check in adult basidiomes. Li et al. (2009) showed that many specimens from China cited as *Chroogomphus tomentosus* (Murrill) O. K. Mill., a species described from North America, were in fact *C. pseudotomentosus* O.K. Mill. & Aime (Li 2007). Also, according to Li et al. (2009) the presence of *C. rutilus* in China or in East Asia is doubtful since the identifications have not been corroborated with molecular analyses. Similarly, Sulzbacher et al. (2016) showed that many sequences located in GenBank under *Rhizopogon luteolus* Fr. & Nordholm, the type species of the genus and one of the most frequent *Rhizopogon* species in Europe, corresponds to *Rhizopogon verii* Pacioni described from Tunizia.

The revision of the previous records of Gomphidaceae from the Balearic Islands revealed that many specimens of *C. mediterraneus* were cited as belonging to *G. viscidus* (Rolland 1904; Malençon and Bertault 1972; Aguasca et al. 1992), and *C. rutilus* (Schilling 1987; Llistosella and Aguasca 1990; Aguiló 1994; Kajan et al. 1995; Constantino and Siquier 1996, 2006, 2011; Escandell and Escandell 1999; Siquier et al. 2009). These taxa should be eliminated from the fungal checklist of

sequences indicated in bold. Clades named according to the Index Fungorum and Mycobank, except for specimens from Balearic Islands in which *Chroogomphus mediterraneus* is indicated instead of the name that appears in the fungal names databases. Percentage of bootstrap values >50% included above branches.

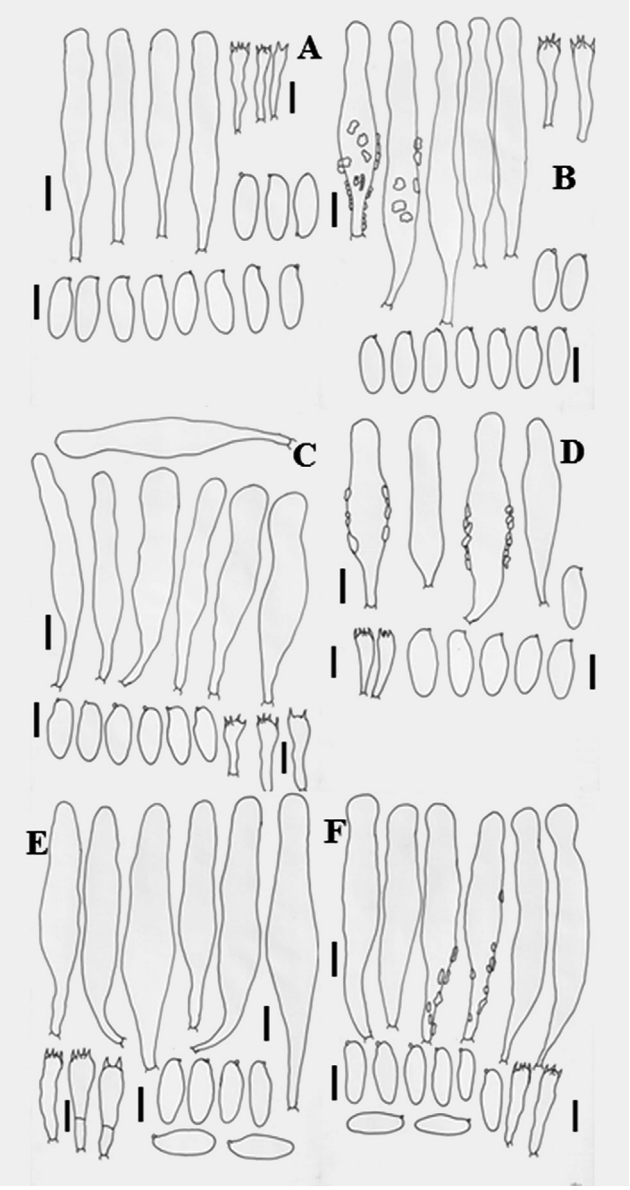

Fig. 3 – *Chroogomphus mediterraneus*, microscopical characters (cystidia, basidia and spores). A: JLS 2887. B: JLS 2917. C: 3006. D: JLS 3384. E: JLS 3539. F: JLS 3847. *Bars*: cystidia and basidia 20 μm; spores 10 μm.

the Balearic Islands where only two Gomphidiaceae species should be maintained: *C. mediterraneus* and *G. tyrrhenicus* D. Antonini & M. Antonini.

Taxonomic treatment

Chroogomphus mediterraneus (Finschow) Vila, Pérez-De-Greg. & G. Mir, Errotari 3: 68, 2006.
Basionym: *Gomphidius mediterraneus* Finschow, Veröffentlichungen aus dem Überseemuseum Bremen 5: 43, 1978. MB 314675.

An updated and complete description of microscopic features of *C. mediterraneus* is provided based on the specimens examined in this study, including the holotype (Figs. 2, 3). *Pileipellis* ixo-trichoderma made up of parallels to interwoven subgelatinized hyphae up to 9 μm in diam, some encrusted with brown-yellowish pigments. *Pileal trama* hyaline to orange-brown in Melzer's reagent, with some amyloid cells (in the web version). *Pleuro-* and *cheilocystidia* (90–) 100–175 × (13–)16–20(–22.5) μm, numerous, cylindrical, elongate-clavate to clavate, with lateral constrictions, thin walls (up to 1 μm), with yellow-brown to brown incrusted material. *Basidia* 47–62.5 × 10–12.5(–14) μm, clavate (in JCS 840B and JLS 3539 frequently septate at the base), (2–)4-spored. *Basidiospores* (15.5–)16–19(–20) × (5.5–)6–7.5(–8) μm, Q = (2.16–)2.46–2.75, Q_m = 2.57, smooth, subfusiform to fusiform, subelliptic in face-view with short apiculus and thin walls, yellow ochraceous in water; mature spores in Melzer's reagent dextrinoid. Clamp-connections not seen.

Holotype: Spain, Balearic Islands, Eivissa, Puig d'en Serra, close to "es Cubells", Sant Josep de sa Talaia, alt. 200 m, 8 Nov 1973, leg. H. Kuhbier, det. G. Finschow, Herbarium BREM 2060 (Herbarium Übersee Museum, Bremen).

Specimens examined: SPAIN, Balearic Islands, Eivissa: Sant Antoni de Portmany, Puig de Can Maimó, alt. 230 m, 5 Dec 2008, JLS 2588; Sant Josep de sa Talaia, es Cap Falcó, alt. 0–25 m, 4 Dec 2009, leg. J.L. Siquier and J.C. Salom, JLS 2917 (duplicate JCS 795B) (ITS nrDNA sequence LT219429); Sant Josep de sa Talaia, es Coscollar, alt. 200–250 m, 17 Nov 1994, leg. J.L. Siquier and J.C. Salom, JLS I-168B; Sant Joan de Llabritja, cala Llenya, alt. 10–50 m, 5 Dec 2009, JLS 2919; Santa Eulària des Riu, Talaia de Sant Llorenç, alt. 175–225 m, 5 Dec 2009, JLS 2944; Sant Josep de sa Talaia, Serra de sa Cova Santa, alt. 75–125 m, 6 Dec 2009, JLS 2952; Sant Josep de sa Talaia, torrent de ses Alfabis, alt. 0–50 m, 6 Dec 2009, JLS 2962; Sant Antoni de Portmany, ses Planes d'en Francolí, alt. 175–200 m, 8 Dec 2009, JLS 3038; Sant Josep de sa Talaia, Puig d'en Serra, alt. 250–300 m, 18 Nov 2012, leg. A. Serra, JLS 3539 (ITS nrDNA sequence LT219430); ibidem, 9 Dec 2014, leg. J.L. Siquier and J.C. Salom, JLS 3847 (duplicate JCS1319B). Formentera: Torrent de Cala Saona, 7 Dec 2008, leg. J.L. Siquier and J.C. Salom, JLS 2666; idem, JLS 2679; idem, JLS 3006 (ITS nrDNA sequence LT219431). Mallorca: Banyalbufar, camí des Correu, alt. 300–450 m, 15 Nov 2005, JLS 2054; Pollença, Puig de Son Vila, alt. 100–200 m, 21 Nov 2009, leg. J.L. Siquier and J.C. Salom, JLS 2887 (ITS nrDNA sequence LT219432). Menorca, Es Mercadal, Sa Roca, alt. 180–240 m, 14 Nov 2011, leg. J.L. Siquier and J.C. Salom, JLS 3384 (ITS nrDNA sequence LT219433). SPAIN, Teruel. Mora de Rubielos, Puerto de San Rafael, alt. 1400 m, 6 Oct 2009, leg. J.L. Siquier and J.C. Salom, JCS840B (duplicate JLS 2775) (ITS nrDNA sequence LT219434).

Habitat: in the Balearic Islands, *C. mediterraneus* is found in *Pinus halepensis* forests with *Pistacia lentiscus*, *Juniperus phoenicea*, *Juniperus oxycedrus*, *Myrtus communis*, *Ampelodesmos mauritanica*, *Erica multiflora*, *Arbutus unedo* and *Cistus* spp. In the Iberian Peninsula (Teruel), under other pine species, such as *Pinus nigra* and *P. sylvestris*.

Other specimens examined: *Chroogomphus fulmineus*—SPAIN, Jaén. Sierra de Cazorla, Arroyo Frío, *Pinus halepensis*, *Pinus pinaster*, *Populus alba* and *Quercus rotundifolia*, 4 Nov 2013, leg. J.L. Siquier, JLS 3624 (ITS nrDNA sequence LT219435). *Chroogomphus rutilus*—SPAIN, Navarra. Cilveti, alt. 750 m, *P. sylvestris*, MA-Fungi 66940 (ITS nrDNA

sequence LT219436). SPAIN, TERUEL. Rubielos de Mora, alt. 1112 m, coniferous and cupressaceae, MA-Fungi 68920 (ITS nrDNA sequence LT219437).

Acknowledgments

We are thankful to Dr. Yan-Chun Li (Kunming Institute of Botany, China) for kindly providing the majority of the sequences included in this study; to Prof. Roy Watling (UK) for constructive comments and suggestions; to all colleagues of Funga Cultural Balear (F.C.B.); to L. A. Parra (Burgos, Spain), involved in the long discussions about the identity of *C. mediterraneus*; to H. Kuhbier (Bremen, Germany) for sending the duplicate of the type collection; to M. Dueñas (curator MA-Fungi herbarium, RJB-CSIC) for the loan of *C. rutilus* specimens; to B. Grauwinkel (Berne, Germany) for sending the original photographic material of holotype; to T. Serra "Miquelet" (Santa Gertrudis–Eivissa, Spain) for his photographic material of *C. mediterraneus*; to D. Merino (Jaén, Spain) for his information about *C. fulmineus*, and to J. Planas (Mallorca, Spain) for help with photography. Thanks to Dr. Marian Glenn (Seton Hall University, New Jersey, USA) for her kind English revision.

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