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ORIGINAL ARTICLE

Gymnopilus maritimus (Basidiomycota, Agaricales), a new species from coastal psammophilous plant communities of northern Sardinia, Italy, and notes on *G. arenophilus*

L. Guzmán-Dávalos · A. Ortega · M. Contu ·
A. Vizzini · A. Rodríguez · A. R. Villalobos-Arámbula ·
A. Santerre

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Abstract The new species *Gymnopilus maritimus* is described from coastal plant communities of *Juncus maritimus*, growing on sandy soil or on decaying plants, from northwestern Sardinia (Italy). The distinguishing features of *G. maritimus* are: (1) an unusual habitat, (2) robust basidiomata, (3) mild taste, and (4) big and strongly warted spores. The new species is compared with the micromorphologically similar species *G. fulgens* sensu auct. Brit. p.p. and the biogeographically and ecologically similar species *G. arenophilus,* as well as with other European species. A photograph of fresh material, drawings of the main micromorphological features, and FESEM and optical microscope microphotographs of basidiospores are added. Furthermore, some notes on micromorphological characters of *G. arenophilus* are presented and its distribution area enlarged with a record from France. A key for the European species of *Gymnopilus* morphologically, ecologically, and/or biogeographically related to *G. maritimus* is presented. The phylogeny inferred from ITS rDNA sequences revealed that *G. maritimus* represents an independent species and that it is not related to *G. arenophilus* or *G. fulgens*. It is the sister group of the clade containing *G. imperialis* and *G. spectabilis*, but with a bootstrap support below 50%. The characters shared by the species in this clade are: (1) robust basidiomata, (2) pileus fibrillose or scaly-fibrillose, and (3) spores longer than 8 µm, dextrinoid and strongly warted. *Gymnopilus imperialis* and *G. spectabilis* differ by the basidiomata with membranous ring in the stem, living on conifers or decaying wood, and having narrower or wider spores, respectively.

Keywords Agaricales · Basidiospores · DNA sequences · Mediterranean ecosystems

Introduction

During fieldwork carried out by M.C. in coastal sand dunes near Olbia, northern Sardinia, Italy, a very distinctive *Gymnopilus* was repeatedly collected growing near the base of *Juncus maritimus* Lam. (Juncaceae). The unusually large basidiospores placed this fungus into *Gymnopilus*, subgenus *Gymnopilus* P. Karst., section *Macrospori* Guzm.-Dáv.

Taxonomical novelties: *Gymnopilus maritimus* Contu, Guzm.-Dáv., A. Ortega & Vizzini

L. Guzmán-Dávalos (✉) · A. Rodríguez
Departamento de Botánica y Zoología,
Universidad de Guadalajara,
Apdo. Postal 1-139, Zapopan, Jal. 45101, Mexico
e-mail: lguzman@cucba.udg.mx

A. Ortega
Departamento de Botánica, Universidad de Granada,
18071 Granada, Spain
e-mail: aortegad@ugr.es

M. Contu
via Traversa via Roma,
12 – 07026,
Olbia, Sardinia, Italy
e-mail: marcocontu@interfree.it

A. Vizzini
Dipartimento di Biologia Vegetale, Università di Torino,
Viale Mattioli 25,
10125 Torino, Italy
e-mail: alfredo.vizzini@unito.it

A. R. Villalobos-Arámbula · A. Santerre
Departamento de Biología Celular y Molecular,
Universidad de Guadalajara,
Zapopan, Jal., Mexico

(Guzmán-Dávalos 1995). Because of the unique characters of those collections, we were not able to identify them with any described species. Based on macroscopic features, habitat, and biogeography, we thought that it could be *G. arenophilus* A. Ortega & Esteve-Rav.; however, this species has different characters (e.g. taste weakly bitterish, shape and ornamentation of basidiospores) (Ortega and Esteve-Raventós 2005). In addition, we compared it to several European taxa, with special emphasis on Mediterranean species and those that have a similar habitat and/or morphological resemblance. We are proposing the Sardinian collections as a new species to science, which is described below. Furthermore, a molecular study supports that *G. maritimus* is different from *G. arenophilus, G. fulgens* (J. Favre & Maire) Singer and other *Gymnopilus* species assumed to be closely related.

Materials and methods

Morphological analyses

Micromorphological observations were made from sections mounted in 3% KOH, or gill fragments mounted in KOH, Melzer's reagent, lugol, cotton blue, and cresyl blue. The terms for the descriptions mainly refer to Vellinga (1998) and in some cases to Largent (1986). Basidiospore shape was determined according to the Q (length–width ratio) (Bas 1969) of at least 20 mature and randomly selected basidiospores. The measurements include the basidiospore ornamentation but not the apiculus and were made in KOH at 1,000x with a calibrated optical micrometer in a Zeiss K-7 optical microscope. Likewise, the basidiospore warts were measured using an image analyzer coupled in a Zeiss Axioscop 40 microscope. The length in basidia measurements includes sterigmata. The microphotographies were made through a Zeiss Axioscop 40 microscope and a FESEM (field electron scanning electron microscope) of a Zeiss model 1539 Geminis type. The herbarium and authors abbreviations follow Holmgren et al. (1990) and Kirk and Ansell (1992), respectively.

Sampling for the DNA study

In this study, 6 specimens of *Gymnopilus* were sequenced, 14 samples were sequenced in a previous work (Guzmán-Dávalos et al. 2003), and 4 additional sequences were obtained from GenBank (Table 1).

DNA extraction, amplification and sequencing

DNA was extracted from small pieces (ca. 4 mg) of the pileus (including pileipellis, context, and lamellae), using the protocol of salt-extraction with 1% PVP (Aljabani and Martínez 1997). Polymerase chain reaction (PCR) was performed to amplify the internal transcribed spacer 1 (ITS1), the 5.8S rRNA gene and the internal transcribed spacer 2 (ITS2) as follows: each 40 µl reaction contained 27.8 µl of MilliQ water, 4 µl of 10 X Taq reaction buffer without $MgCl_2$, 2 µl of 50 mM $MgCl_2$, 1 µl of 10 mM dNTP, 2 µl of 2 µg/µl Bovine Serum Albumine (BSA), 1 µl of each 10 µM primer, 0.2 µl of Taq DNA polymerase (5U/µl), and 1 µl of DNA template. All reagents were from Invitrogen® except BSA which was purchased from Sigma. Negative controls, without DNA template, were always included to detect contamination in the reagents. Primers ITS1G and ITS4G (Guzmán-Dávalos et al. 2003) were used to amplify the region of interest. PCR amplifications were performed in a MJ Research PTC-100 thermocycler as described by Guzmán-Dávalos et al. (2003), except that the annealing temperature was 52°C. PCR products were separated by electrophoresis in 1.2% 1X TBE agarose (Invitrogen®) gel and then stained with ethydium bromide 200 ng/ml (Sigma).

Sequencing reactions were performed with BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) in a 20-µl final volume following the manufacturer's protocols and using the same primers as in the PCR reactions. Sequencing reactions were purified with AutoSeq™ G-50 column (Amersham Biosciences), and finally 18 µl formamide was added. Sequence visualization was done by capillary electrophoresis on an ABI-Prism 310 Genetic Analyzer (Applied Biosystems). Sequences were edited with Chromas 1.45 (McCarthy 1996–1998). Assembly of sequence fragments and alignment of sequences were carried out with MacClade 4.0 PPC (Maddison and Maddison 2000). Alignments were checked by eye and manually corrected when necessary using MacClade 4.0. New sequences have been submitted to GenBank (accession numbers EU518417–EU518422).

Phylogenetic analyses

Twenty-four ITS rDNA sequences from 20 taxa were used in the analyses (Table 1). Maximum-parsimony of equally weighted characters was performed with PAUP* 4.0b10 (Altivec) (Swofford 2000). Gaps were treated as missing characters. A Branch and Bound search was conducted under the following conditions: (1) initial upper bound was computed by stepwise addition; (2) addition sequence was furthest; (3) only minimal trees were kept; (4) branches collapsed if maximum branch length is zero; (5) MulTrees option in effect. Support for the branches was obtained from 1,000 bootstrap replications, with the same conditions as above. The resulting trees were rooted using *Galerina paludosa* (Fr.) Kühner based on Moncalvo et al. (2002) and

Table 1 *Gymnopilus* specimens used for molecular purposes in this study

DNA no.	Species	Collector, number and herbarium	Country	Date of collection	GenBank no.
221	*G. arenophilus* A. Ortega & Esteve-Rav.	L. Alcoba & A. Ortega (GDA-47387)	Spain	Jan. 19, 2001	EU518422
223	*G. arenophilus*	L. Alcoba & A. Ortega (GDA-47384, **Isotype**)	Spain	Nov. 1, 2000	EU518421
	G. decipiens (W.G. Sm.) P.D. Orton				AF325660[a]
253	*G. flavus* (Bres.) Singer	J. Gómez s.n. (GDA-44211)	Spain	Jan. 1, 1996	EU518420
222	*G. fulgens* (J. Favre & Maire) Singer	F. Esteve-Raventós (AH-30751)	Spain	Sept. 6, 2003	EU518417
119	*G. imperialis* (Speg.) Singer	Gómez 18197 (F-1051880)	Costa Rica	July 11, 1982	AY280986[b]
1	*G. lepidotus* Hesler	G. Guzmán 30374 (XAL)	Mexico	Sept. 14, 1991	AY280989[b]
219	*G. maritimus* Contu, Guzm.-Dáv., A. Ortega & Vizzini	M. Contu s.n. (IBUG, **Holotype**)	Italy	Jan. 15, 2006	EU518419
220	*G. maritimus*	M. Contu s.n. (IBUG)	Italy	Jan. 22, 2006	EU518418
153	*G. nevadensis* Guzm.-Dáv. & Guzmán	M.L. Fierros 568 (IBUG)	Mexico	Aug. 3, 1994	AY280995[b]
	G. odini (Fr.) Bon & P. Roux				AF325659[a]
2	*G. penetrans* (Fr. : Fr.) Murrill	L. Guzmán-Dávalos 8196 (IBUG)	Switzerland	Oct. 29, 1999	AY280999[b]
26	*G. penetrans*	L. Guzmán-Dávalos 8223 (IBUG)	France	Nov. 19, 1999	AY281000[b]
150	*G. penetrans*	L. Guzmán-Dávalos 8215 (IBUG)	The Netherlands	Nov. 13, 1999	AY811002[b]
104	*G. picreus* (Pers.) P. Karst.	T. Ahti 52192 (H, IBUG)	Finland	Sept. 16, 1994	AY281003[b]
113	*G. robustus* Guzm.-Dáv.	C.L. Ovrebo 3528 (IBUG, PMA)	Panama	May 25, 1997	AY281004[b]
52	*G. sapineus* (Fr.) Maire	I. Kytövuori 90-2488 (H)	Finland	Oct. 9, 1990	AY281007[b]
100	*G. spectabilis* (Fr.) A.H. Sm.	T. Ahti 49093 (H, IBUG)	Finland	Sept. 27, 1990	AY281010[b]
5	*G. subpurpuratus* Guzm.-Dáv. & Guzmán	L. Guzmán-Dávalos 5303 (IBUG)	Mexico	Aug. 2, 1991	AY281016[b]
	G. turficola M.M. Moser & H. Ladurner				AF325669[a]
151	*G. underwoodii* (Peck) Murrill	L. Guzmán-Dávalos 6248 (IBUG)	Guatemala	Aug. 9, 1992	AY281017[b]
138	*G. validipes* (Peck) Hesler	M.A. Vincent 6403 (ENCB)	USA	Oct. 2, 1993	AY281018[b]
144	*Galerina autumnalis* (Peck) A.H. Sm. & Singer	L. Guzmán-Dávalos 5246 (IBUG)	Mexico	Oct. 15, 1990	AY281020[b]
	G. paludosa (Fr.) Kühner				AJ585448[c]

[a] Moser et al. (2001)

[b] Guzmán-Dávalos et al. (2003)

[c] Gulden et al. (2005)

Rees et al. (2003). In addition, *G. autumnalis* (Peck) A.H. Sm. & Singer was also selected as an outgroup, because of its close relationship to *G. marginata* (Batsch) Kühner, which was found in the sister group of the gymnopilus clade by Rees et al. (2002).

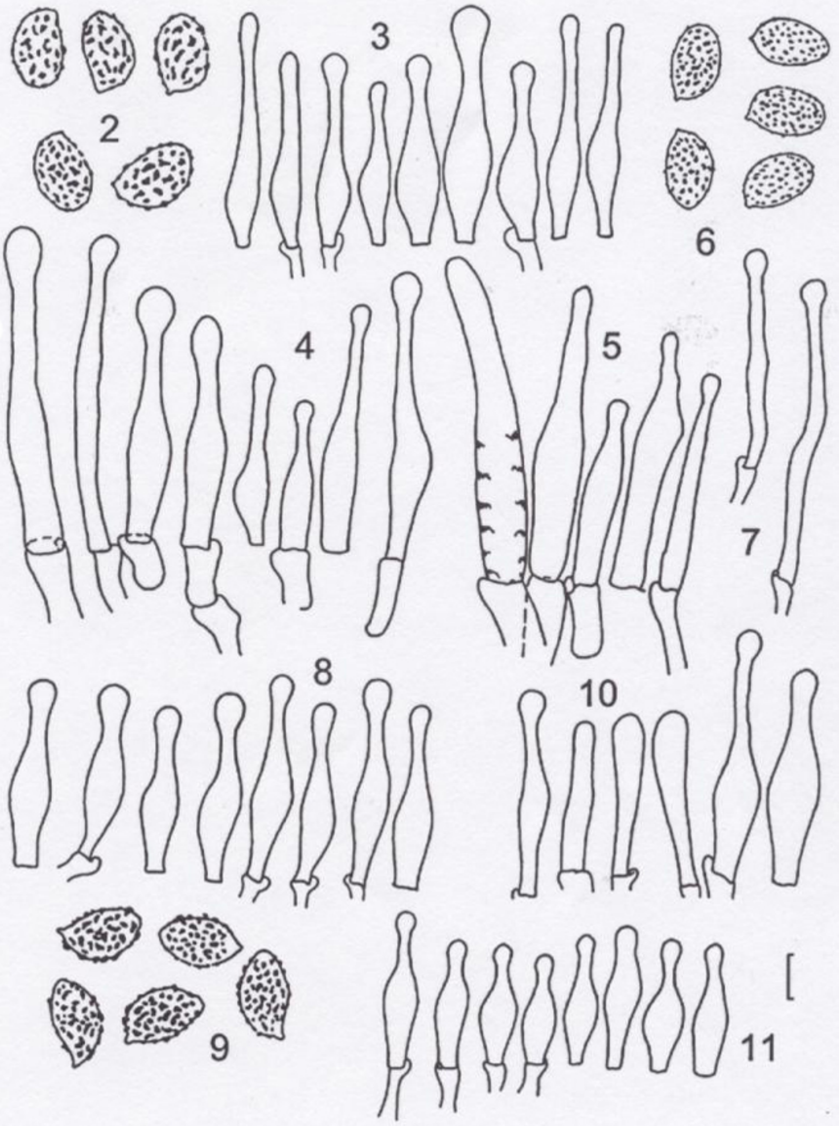
Taxonomy

Gymnopilus maritimus Contu, Guzm.-Dáv., A. Ortega & Vizzini, **sp. nov.** Figs. 1, 2–5, 12–19

MycoBank: MB 511502

Etymology: the name refers to the characteristic habitat, which is coastal sand dunes, on sandy soil or decaying plants of *Juncus maritimus*.

Fig. 1 *Gymnopilus maritimus* (from holotype). Basidiomata. *Bar* 20 mm

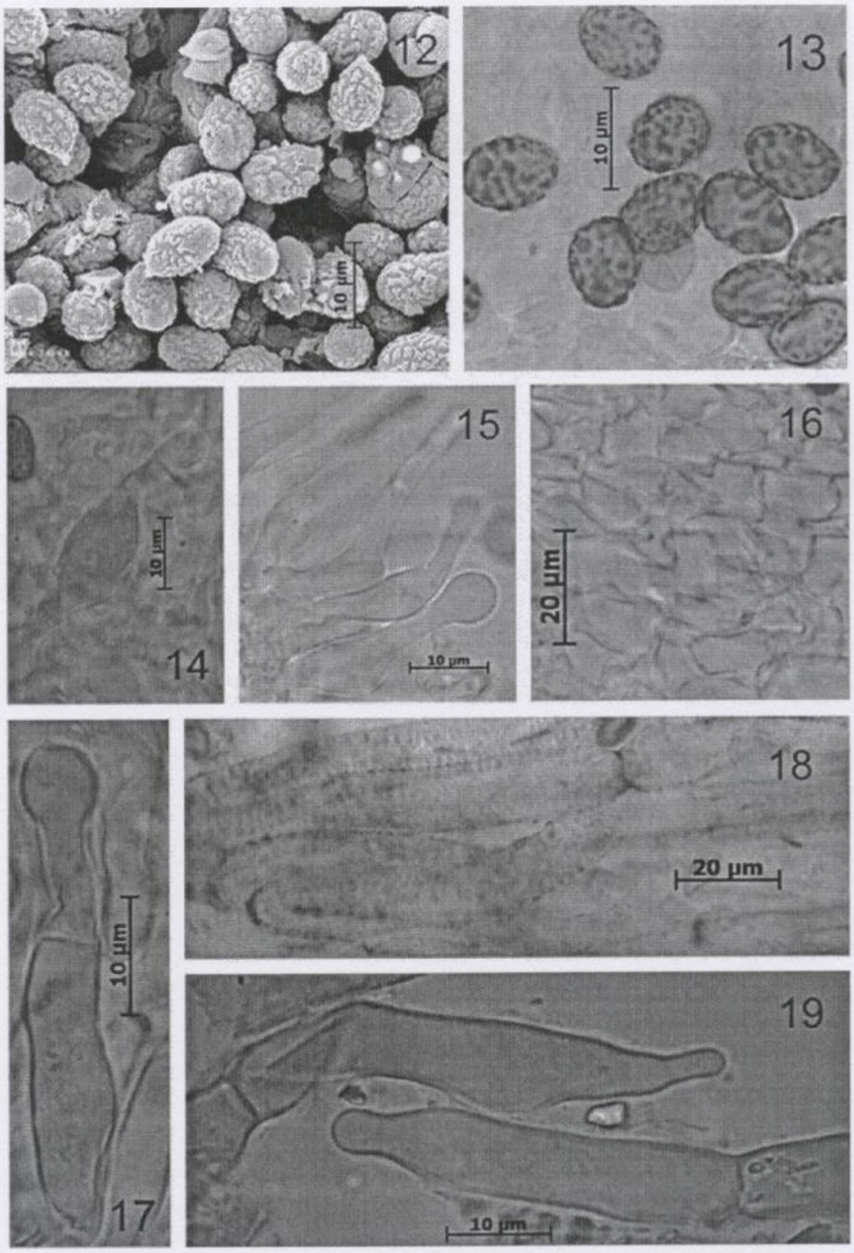


Figs. 2–11 **2–5** *Gymnopilus maritimus* (from holotype). **2** Basidiospores. **3** Cheilocystidia. **4** Caulocystidia. **5** Terminal elements of the pileipellis. **6–8** *Gymnopilus arenophilus* (from isotype, IBUG). **6** Basidiospores. **7** Caulocystidia. **8** Cheilocystidia. **9–11** *Gymnopilus fulgens* (from AH-30751). **9** Basidiospores. **10** Caulocystidia. **11** Cheilocystidia. *Bars* **2, 6, 9**=5 µm, **3–5, 7–8, 10–11**=8 µm

Pileus 15–70 mm, convexus vel plano-convexus, saepe late umbonatus, fibrillosus, fulvus, versus marginem flavus, fibrillis aurantiis praeditus, siccus. *Lamellae* adnatae vel sinuatae, flavae deinde aurantio-ochraceae, in senectute ferrugineae. *Stipes* 35–110×4–8 mm, centralis, cylindricus vel basim versus attenuatus, fibrilloso-striatus, flavidus deinde pileo subconcolor. *Velum* araneosum, fugax. *Caro* flava, basim versus stipitis brunnea. Odor debilis; sapor mitis vel levissime amarus. *Basidiosporae* 8.0–10.5×5.5–7.5 µm, Q = (1.25) 1.3–1.6 (1.7), ferrugineae, ellipsoideae, apice obtuso praeditae, verrucis conspicuis vel mediis praeditae, dextrinoideae. *Basidia* tetraspora, clavata. *Pleurocystidia* nulla. *Cheilocystidia* 30–42×6–10.5 µm, apex 2–7 µm latus, lageniformia, subutriformia, capitulata vel subcapitulata, hyalina vel contentu flavido praedita. *Pileipellis* ex hyphis cylindricis laxe intertextis, 2.5–12 µm latis, manifeste incrustatis. *Caulocystidia* 24–60×3–9 µm, apex 3–7 µm latus, cylindracea vel lageniformia, capitulata. *Fibulae* numerosae.

Holotypus: Sardinia, prov. Sassari, Olbia, Pittulongu, 15 Januarius 2006, M. Contu (IBUG).

Pileus 15–70 mm, convex to plano-convex but never expanding, depressed at centre in old specimens, sometimes low and broadly umbonate; margin undulate; surface fibrillose, dry, dull, golden-yellow towards the margin, rufous to reddish-orange, with orange fibrilles elsewhere, stained dark orange-brown, some basidiomata with minute fibrillose squamules. *Lamellae* thick, adnate to sinuate, ventricose, subdistant, yellow, ochre-orange and finally ferruginous, stained orange-brown or darker; edges paler



Figs. 12–19 *Gymnopilus maritimus* (**12** from isotype, **13–19** from holotype). **12** Basidiospores (FESEM). **13** Basidiospores. **14** Cheilocystidium. **15** Cheilocystidium. **16** Radial pileus trama. **17** Caulocystidium. **18** Pileipellis. **19** Terminal elements of the pileipellis

than gill faces. *Stipe* 35–110×4–8 mm, cespitose, central, cylindrical to tapering downwards, dry, fibrillose-striate, yellowish, completely reddish-brown when bruised; base with or without whitish or cream mycelium. *Veil* arachnoid, evanescent. *Context* variable, up to 15 mm thick, yellow, brown in the lower part of the stipe, unchanging, dark brown when dry. Odor not distinctive; taste mild or somewhat bitterish. *Spore print* rusty-brown. *KOH* blackish-red on dry cap. *Basidiospores* [N=17, n=190] (7.5) 8.0–10.5 (11.5) × 5.5–7.5 (8.0) µm ($\bar{x} = 9.5 \times 6.5\ \mu m$) [including ornamentation], Q = (1.25) 1.3–1.6 (1.7), ellipsoid, few broadly ellipsoid, apex obtuse, in few subattenuate, slightly thickened wall, verrucose; warts large to medium (0.5–2 µm); germ pore and plage absent, suprahilar depression absent or indistinct, orange-yellow or orange-brown in KOH, dextrinoid in Melzer's reagent and lugol, not metachromatic; ornamentation cyanophilic. *Basidia* 24–35×7–9 (10.5) µm, clavate with central constriction, four-spored, clamped, hyaline, with yellowish or yellowish-brown content, sterigmata 1.6–5.0 (7) µm long. *Cheilocystidia* 30–42 (50) × 6.0–10.5 µm, apex 2–7 µm wide, lageniform, subutriform, apex capitate or subcapitate, thin-walled, hyaline or yellowish, with or without granulose content. *Pleurocystidia* not observed. *Hymenophoral trama* subparallel; hyphae 1.5–13.5 µm wide, with thin to moderately thick wall, yellowish, with clamp connections at septa. *Subhymenium* ramose. *Pileus trama* radial, consisting of hyphae with thin to moderately thick walls, yellowish, some with granulose yellowish-brown content. *Pileipellis* a cutis, which has tendency to form a trichoderm in the older specimens or in the squamules; hyphae 2.5–16.0 (20) µm wide, septate, yellowish or brownish, with yellowish-brown or orange-brown encrusted pigment in bands, with clamp connections; some cylindrical or subclaviform terminal free elements present towards the disc, with granulose orange-brown content, or with encrusted orange-brown pigment in bands. *Caulocystidia* in tufts at the stipe apex, 24–60×3–9 µm, cylindrical or narrowly lageniform, with 3–7 µm wide, apex capitate or subcapitate, some with long neck, with thin to moderately thick wall (especially towards apex), clamped, hyaline or yellowish, with or without granulose yellowish-brown content. A yellowish pigment dissolves when lamellae are mounted in KOH.

Habitat Gregarious to cespitose, in coastal sand dunes with pure stands of *Juncus maritimus*. The vegetation belongs to the phytosociological order *Juncetalia maritimi* Br.-Bl. 1931 (Braun-Blanquet 1952). Approximately 10 m from the sea, growing near the base of living plants on sandy soil or on decaying plants. Winter (end of October to January).

Known distribution Only known from the type locality in Sardinia. It has been collected in three different sites of the same locality; the distance among the sites is approximately 20 m from the first to the second, and 10 m from the second to the third.

Materials examined Italy, Sardinia, prov. Sassari, Olbia, Pittulongu, leg. M. Contu 15.1.2006. Holotype (IBUG), Isotype (GDA-52913). Ibidem, 22.1.2006 (GDA-52914, IBUG), 25.1.2006 (Herbarium Contu), 25.10.2006 (GDA-52915), 28.12.2006 (GDA-52916), 30.12.2006 (GDA-52917), 1.1.2007 (GDA-52918).

Microscopical description of *Gymnopilus arenophilus* A. Ortega & Esteve-Rav., Persoonia 18 (4): 506, 2005

Figs. 6–8

Basidiospores [N=4, n=80] 8.5–10.5 (11.0) × 5.5–6.5 (7.0) µm ($\bar{x} = 9.5 \times 6\ \mu m$) [including ornamentation], Q= 1.5–1.7, ellipsoid to oblong, amygdaloid, apex obtuse to attenuate, slightly thickened wall, verrucose; warts small to medium (0.2–1 µm); germ pore, plage and suprahilar depression absent. *Basidia* 26–35×7–9 µm, clavate with central constriction, four-spored, clamped, hyaline, with yellowish or yellowish-brown content, sterigmata 2.5–5 µm long. *Cheilocystidia* 28.0–38.5×6–9 µm, apex 3.0–5.5 µm wide, lageniform, apex capitate or subcapitate. *Pleurocystidia* very rare, 22–28×5–8 µm, shape similar to cheilocystidia. *Hymenophoral trama* subparallel; hyphae 3–14 µm wide, with thin to moderately thick wall, septate, hyaline or yellowish, with clamp connections. *Subhymenium* ramose. *Pileus trama* radial, with some intermixed hyphae close to the pileipellis; hyphae thin to moderately thick-walled, yellowish, some with granulose yellowish content. *Pileipellis* a cutis which has a tendency to be an ixocutis, scarcely gelified; with interwoven hyphae, 3–16 µm wide, yellowish, yellowish-ochre to yellowish-brown, with yellowish-brown or orange-brown encrusted pigment in bands. *Caulocystidia* in tufts at the stipe apex, 27–57×3–9 µm, cylindrical, narrowly lageniform or lageniform, few subutriform, with 3.0–5.6 µm wide, apex capitate or subcapitate, with long neck, thin-walled, clamped, yellowish, with or without granulose yellowish-brown content. A yellowish pigment dissolves when lamellae are mounted in KOH.

Materials examined France, Poqueroles, en troupes sur debris de bois et aiguilles de pins d'Alep (R. Roux private herbarium, Exsi. 2194). Spain, Sevilla, Aznalcázar, pinar de Aznalcázar, leg. L. Alcoba & A. Ortega, 2.12.2000. Holotype (GDA-47384), Isotype (IBUG). Ibidem, 19.1.2001 (GDA-47387).

Key of the morphologically, ecologically and/or biogeographically European species related to *G. maritimus*

- 1. Fruitbodies robust, medium to large-sized, pileus up to 200 mm diam. Stipe with a membranaceous ring. Species lignicolous. *G. spectabilis*
- 1*. Fruitbodies small to medium-sized, pileus up to 90 mm diam. Stipe without membranaceous ring. Species terricolous or lignicolous. 2
- 2. Coastal or continental sand-dunes habitat. 3
- 2* Different habitat. 6
- 3. Fruitbodies small-sized, pileus up to 25 mm diam. Arachnoid veil absent (only recorded in sand-dunes from England). *G. fulgens sensu* Orton *p.p.*
- 3* Fruitbodies small to medium-sized, pileus up to 90 mm diam. Arachnoid veil present (recorded in sand-dunes from diverse European countries). 4
- 4. Basidiospores 7–9.5×3.5–5.5 µm (rare in the Mediterranean region). *G. decipiens*
- 4* Basidiospores 7.5–11.5×5.5–8 µm (typical of the Mediterranean region). 5
- 5. In coastal or continental sand-dunes, under *Pinus* sp. Basidiospores with small to medium-sized warts (0.2–1.0 µm). *G. arenophilus*

- 5* In coastal sand-dunes, with pure stands of *Juncus maritimus*. Basidiospores with large to medium-sized warts (0.5–2.0 μm). *G. maritimus*
- 6. Growing on stumps or sawdust. Only recorded from Corsica. 7
- 6* Growing on soil. Wider geographical distribution. 8
- 7. Arachnoid veil absent. Basidiospores non-dextrinoid. *G. corsicus*
- 7* Arachnoid veil present. Basidiospores dextrinoid. *G. spadiceus*
- 8. Among bryophytes on burnt soils. Basidiospores larger than 6 μm. 9
- 8* Graminicolous, mainly associate with tufts of *Dactylis glomerata*. Basidiospores 4.5–6.0 × 3.5–4.5 μm. *G. flavus*
- 9. Fruitbodies with a sweet taste. Basidiospores 8–11 × 5–8 μm. *G. fulgens sensu str.*
- 9* Fruitbodies with a bitter or farinaceous taste. Basidiospores 8–11 × 4.5–6.0 μm. *G. pseudofulgens*

Phylogenetic analyses

After the introduction of gaps, the alignment of the 24 ITS sequences resulted in 745 nucleotide positions. Both ends of the sequences and ambiguous regions were excluded from the analysis; in total, 586 characters were included and 102 of these were parsimonious informative. Equally weighted parsimony analysis resulted in one tree, shown as a phylogram in Fig. 20, with a tree length of 318 steps (CI excluding uninformative characters = 0.540, RI= 0.704, RC=0.465).

Morphologically, there are three similar species to *G. maritimus*: *G. arenophilus, G. decipiens* and *G. fulgens*, but the molecular analysis places them into different clades.

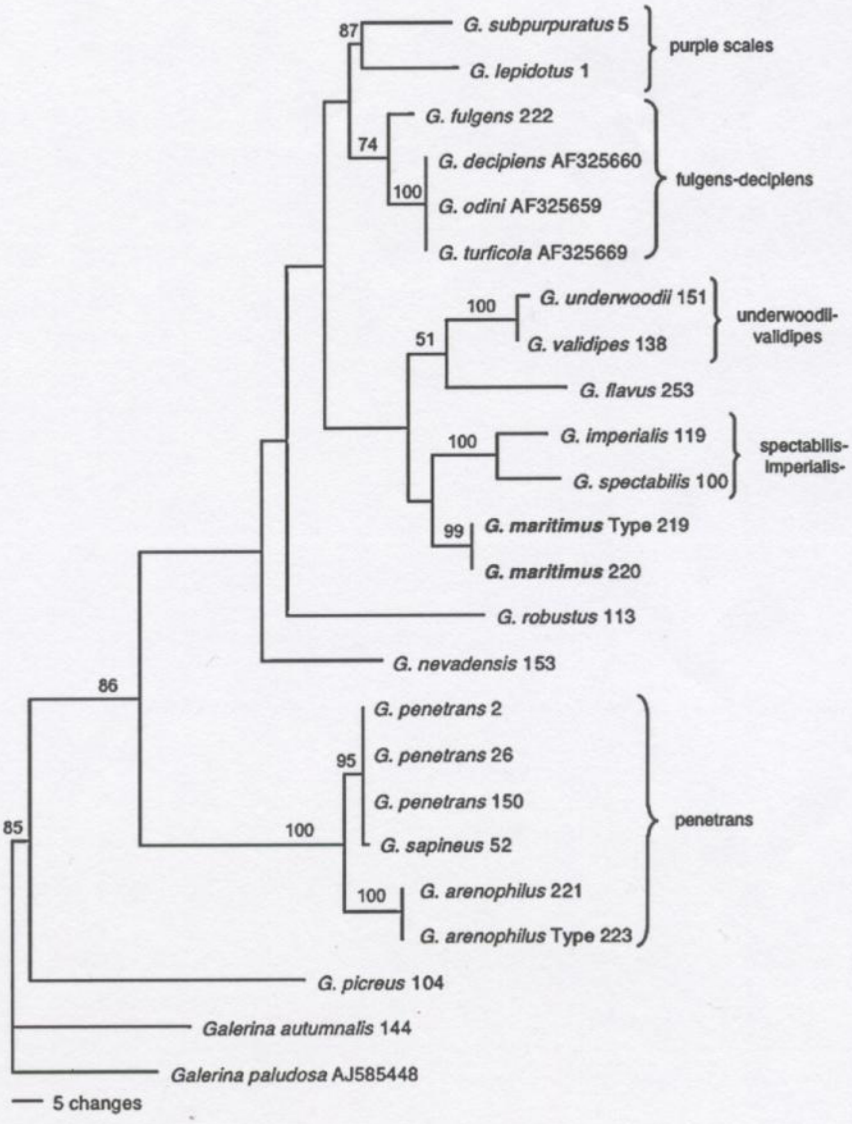


Fig. 20 Phylogram of the most-parsimonious tree with a length of 318 steps (CI excluding uninformative characters = 0.540, RI=0.704, RC=0.465) of 24 ITS rDNA sequences of 18 species of *Gymnopilus* and two species of *Galerina* as the outgroup. Bootstrap values >50% are given above each branch

The most closely related species to *G. maritimus* with the ITS rDNA data resulted *G. imperialis* and *G. spectabilis.*

Discussion

The more distinguished characteristics of *Gymnopilus maritimus* are: (1) the fulvous colour of the fibrillose cap surface, (2) mild taste, (3) large basidiospores, and (4) arenicolous habitat. The habitat of *G. maritimus* is very particular, and we do not know any other taxa growing in similar ecosystems (near the base of *Juncus maritimus*, on sandy soil and/or decaying plants). The species of *Gymnopilus* are saprotrophs or root parasites and have less specialization level than the ectomycorrhizal macrofungi. Therefore, it is possible that *G. maritimus* could grow on different substrates.

According to the literature (see below), there are five species similar to *G. maritimus*: G. *arenophilus, G. decipiens* (W.G. Sm.) P.D. Orton, *G. flavus* (Bres.) Singer, *G. fulgens sensu* Orton p.p., and *G. pseudofulgens* Romagn. From them, we consider *G. arenophilus* and *G. fulgens* to be the most alike (Table 2).

Gymnopilus arenophilus has been collected from Spain (Ortega and Esteve-Raventós 2005) and France (Roux 2006, as *G. penetrans,* see below). It grows in continental areas, always under or near *Pinus* species (e.g. *P. pinea*, *P. halepensis*), on sandy soil, occasionally on burnt-sandy soil, sometimes attached to wood chips, charcoal, burned woody debris, or woody debris buried in the sand. In the protolog, the pleurocystida were indicated as "not seen" (Ortega and Esteve-Raventós 2005); however, they are present but very scarce. Although the distribution area of *G. arenophilus* corresponds with pine woodlands on continental sand dunes (Ortega and Esteve-Raventós 2005), recently it was collected in pine forests on coastal sand dunes from the province of Huelva (Spain) (unpublished data). Besides, the material identified as *G. penetrans* (Fr.) Murrill published by Roux (2006: 725), was checked by us and corresponds to *G. arenophilus*. This record enlarges the distribution area of this species. Furthermore, some records of *G. fulgens* represent *G. arenophilus*. For example, Gómez-Busutil et al. (1996) in their study about the agarics fruiting in continental *Pinus pinaster* forests from Central Spain (Segovia province), recorded *G. fulgens* growing on sandy soil and sometimes on *Pinus* wood debris. We have studied this material and it matches *G. arenophilus*. Also, Bon and Chevassut (1989) seemingly collected *G. fulgens* from Mediterranean ecosystems; its large basidiospores, basidiospore ornamentation, taste and habitat fit well with the concept of *G. arenophilus*.

Although *G. maritimus* and *G. arenophilus* are biogeographically and ecologically similar species, there are significant morphological differences between them (Table 2): (1) pileus surface fibrillose to minutely squamulose versus glabrous to fibrillose, and (2) the shape and ornamentation of spore are different (Q=1.3–1.6 versus 1.5–1.7; warts large or medium versus small to medium).

Orton (1993) recorded *G. fulgens* from sand-dune heaths based on some specimens collected by Watling in Scotland. This particular habitat is also recorded by Ludwig (2001). According to the literature (e.g. Hesler 1969; Orton 1993; Ludwig 2000, 2001; Bon and Roux 2002; Roux 2006), the size, shape and ornamentation of the spores of *G. fulgens* (8.8–10.8×5.2–7.2 µm in AH-30751, Fig. 9) are similar to *G. maritimus* (see Table 2), but other characters are strongly

Table 2 Comparison of important characters of *Gymnopilus maritimus* and morphologically similar species

Characters/species	*G. maritimus*	*G. arenophilus*	*G. fulgens*
Pileus / stipe (diameter in mm)	10–70 / 4–8	10–50 / 4–7	5–30 / 1.5–2.5
Pileus ornamentation	Fibrillose to minutely squamulose	Glabrous to fibrillose	Glabrous to fibrillose
Veil	Arachnoid	Arachnoid	Absent
Basidiospores			
Size (µm)	(7.5) 8.0–10.5 (11.5) × 5.5–7.5 (8.0)	8.5–10.5 (11.0) × 5.5–6.5 (7.0)	8.8–10.8×5.2–6.8 (7.2)
Q (length/width ratio)	(1.25) 1.3–1.6 (1.7)	1.5–1.7	1.45–1.8
Shape	Ellipsoid, few broadly ellipsoid	Ellipsoid to oblong	Ellipsoid to oblong
Apex	Obtuse, few subattenuate	Obtuse or attenuate	Attenuate, some with apical papilla
Warts	Large to medium (0.5–2 µm)	Medium to small (0.2–1 µm)	Large to medium (0.5–2 µm)
Suprahilar depression	Absent	Absent	Commonly present
Plage	Absent	Absent	Poorly developed
Cheilocystidia (µm)	30–42×6–10.5	28–38.5×6–9	20–29×5.6–8
Taste	Mild	Weakly bitterish	Sweet
Habitat	Sandy soil or dead plants in *Juncus* stands	Sandy soil in *Pinus* forest	Peat bogs

divergent: smaller and slender versus bigger and robust basidiomata; veil absent versus arachnoid veil present; spore apex attenuate sometimes with apical papilla (Vellinga 1998) versus spore apex obtuse or few subattenuate, and smaller versus larger cheilocystidia and caulocystidia (Figs. 10, 11). In addition, *G. fulgens* sensu stricto refers to a taxon with very particular ecological requirements, on peaty soil among *Sphagnum* and other mosses (cf. Favre and Maire 1937; Kühner and Romagnesi 1953; Moreno 1980; Holec 2005; Horak 2005), and for this reason it is a different species from *G. maritimus*. In Spain, Moreno (*loc. cit.*) proposed *G. fulgens* var. *luteicystis* Moreno, whose morphological characters, in particular smaller spores (7–8.5×4–5 µm), and habitat are distinctly different to *G. maritimus*.

Gymnopilus flavus is also present in the Mediterranean area. It grows in holm oak woodlands (Ortega et al. 2000), pine–oak or pine forests with sandy soil (Conca-Ferrús et al. 2006), and in grasslands (Moreno and Checa 2002). However, it is easily distinguished from *G. maritimus*, since it is a graminicolous species (Bon and Roux 2002; Holec 2005), mainly associated with tufts of *Dactylis glomerata* (Kühner and Romagnesi 1953; Orton 1993; Ludwig 2000, 2001; Conca-Ferrús et al. 2006), and its spores are smaller (5–6×3.5–4.2 µm in GDAC 44211).

Gymnopilus pseudofulgens was collected in a *Pinus* forests in the Mediterranean area in Italy (Monti et al. 1992), growing among bryophytes and *Calluna vulgaris* (Ericaceae), on burnt soils. But the characters of this collection, according to Monti *et al.* (*loc. cit.*), are different from *G. maritimus*: (1) smaller basidiomata: pileus up to 25 mm diam., stem 1–3 mm wide, and (2) different spores: 9–11×4.8–5.5 µm, with smaller warts. Bon and Roux (2002) characterized *G. pseudofulgens* as a carbonicolous species with large spores showing a marked plage.

Gymnopilus decipiens grows on burnt soil, sandy soil or among mosses (Moser 1983; Høiland 1990; Orton 1993; Ludwig 2001; Horak 2005). For growing on sandy soil, it may be reminiscent of *G. maritimus*; however, according to Moser (1983), Høiland (1990), Orton (1993), Bon and Roux (2002) and Holec (2005), it has different spores (7.0–9.5×3.5–5.5 µm).

Two rare Mediterranean taxa from Corsica and southern France are *G. corsicus* Romagn. and *G. spadiceus* Romagn. (Romagnesi 1976). Both grow on stumps or sawdust in *Pinus* woodlands. The first one is easily distinguished, since it does not present arachnoid veil, and it has non-dextrinoid spores (Romagnesi 1976). On the other hand, the relationship between *G. maritimus* and *G. spadiceus* is closer, because it presents (1) robust basidomata (pileus up to 80 mm diam., stipe up to 70×15 mm), (2) evanescent arachnoid veil, and (3) dextrinoid, with large warts spores. Nevertheless, *G. maritimus* is distinguished by (1) its different habitat: the Romagnesi's fungus is lignicolous, on *Pinus* wood, and (2) dissimilar shape and size of the spores: oblong, Q=1.7–2.0 (according the Romagnesi's drawing), and 7.2–9.0 (10) × (4.5-) 5–7 µm (Romagnesi 1976). Another arenicolous species is the North American *G. arenicola* Hesler. However, it has smaller basidiospores: 6.5–8.5 (9.5)×4.0–5.0 µm (A.H. Smith 47448, MICH, holotype) or 7–8×3.5–4.5 µm according to Hesler (1969).

The ITS rDNA analysis (Fig. 20) also shows that the specimens from Sardinia are different and are described here as the new species *G. maritimus*. An unexpected result, inferred from the phylogenetic analysis, was finding three morphological similar species, i.e. *G. arenophilus*, *G. fulgens* and *G. maritimus*, in three different clades, indicating an ecological convergence in the case of *G. arenophilus* and *G. maritimus*, and a morphological convergence in the case of *G. fulgens* and *G. maritimus*.

Gymnopilus maritimus is the sister group of the *spectabilis–imperialis* clade (Fig. 20) (Guzmán-Dávalos et al. 2003), but with a bootstrap support below 50%. According to Guzmán-Dávalos et al. (2003) this clade includes *G. imperialis* (Speg.) Singer, *G. junonius* (Fr.) P.D. Orton, *G. pampeanus* (Speg.) Singer and *G. spectabilis* (Fr.) A.H. Sm., among others. *Gymnopilus maritimus* and the *spectabilis–imperialis* clade share the following features: (1) robust basidiomata (although *G. maritimus* is the species with the smallest basidiomata of the clade), (2) pileus fibrillose or scaly-fibrillose, and (3) large spores (longer than 8 µm), dextrinoid and with coarse warts. These characters would explain its phylogenetic relationship. On the other hand, the members of this clade are clearly different from *G. maritimus*, because they have a well-developed membranous ring in the stipe, live on dead wood from conifers or deciduous trees, and have narrower (*G. junonius*, *G. pampeanus*, *G. spectabilis*) or wider spores (*G. imperialis*). Within this clade, only *G. junonius* and *G. spectabilis* fruit in Europe (Bon and Roux 2002). *Gymnopilus pampeanus* could be an ecological and geographical variant of *G. spectabilis* or of *G. junonius* (Rees and Strid 2001; Holec 2005). *Gymnopilus junonius* and *G. spectabilis* are considered as synonyms by the majority of the European authors; however, Bon and Roux (2002) and Guzmán-Dávalos (unpublished data) treat them as independent species.

Gymnopilus arenophilus forms a highly supported clade (100%) with *G. penetrans* sensu lato (Fig. 20) in the *nevadensis–penetrans* clade according to Guzmán-Dávalos et al. (2003) (*penetrans* clade here, Fig. 20). As Ortega and Esteve-Raventós (2005) indicated, this fungus is morphologically similar to *G. penetrans*, but differs in having bigger basidiospores 8.5–10.5×5.5–6.5 µm (Fig. 12) versus 7–9×4.5–5.5 µm, absent or very rare versus very common and conspicuous pleurocystidia, and a weakly bitterish versus strongly bitter taste. In Fig. 20, *G. nevadensis* is basal to the clade formed by *G. robustus* Guzm.-Dáv. and the purplish-scales, *fulgens–decipiens* and *spectabilis–imperialis* clades. This result does not agree with Guzmán-Dávalos et al. (2003), who found *G. nevadensis* in the *nevadensis–penetrans* clade with 56% of bootstrap support and a posterior probability above 95%.

Gymnopilus fulgens groups with *G. decipiens, G. odini* (Fr.) Bon & P. Roux and *G. turficola* M.M. Moser & H. Ladurner (*fulgens–decipiens* clade, Fig. 20), with a bootstrap of 74%. All of the members of this cluster have small basidioma and grow in Europe, on soil, burnt substrates, or peat (Moser 1983; Moser et al. 2001; Bon and Roux 2002). Among them, the basidiomata of *G. fulgens* and *G. turficola* lack veil. Moser et al. (1983) found that *G. turficola*, *G. decipiens* and *G. odini* grouped together with *G. penetrans*, with a bootstrap of 100%, and, on the other hand, they found *G. sapineus* (Fr.) Maire in a different clade. However, the *G. penetrans* ITS sequence they used (AF325663) is different from other sequences of this species in GenBank (www.ncbi.nlm.nih.gov/blast/). In a study on the genus *Galerina*, Gulden et al. (2005) included 10 LSU sequences of *Gymnopilus*. From them, one sequence of *G. penetrans* was related to *G. decipiens* and *G. odini*, and other sequence of *G. penetrans* showed a close relationship to *G. sapineus*. Based on Gulden et al. (2005), we assume that there are two species involved. It is generally accepted that *G. penetrans* sensu stricto and *G. sapineus* are closely related (e.g. Orton 1993; Holec 2005) or even synonyms (Høiland 1990), and this is in accordance with Guzmán-Dávalos et al. (2003). Also Peintner et al. (2001), using *Gymnopilus* as outgroup of sequestrate Cortinariaceae, showed *G. penetrans* as related to *G. sapineus*. However, according to Rees et al. (2002), the collections of *G. sapineus* were nested in two different clades, one related with *G. penetrans*, and the other with *G. moabus* Grgur., an Australian fungus with purplish-scaly pileus. It is evident that there is a complex of Gymnopili under the names *G. penetrans* and *G. sapineus*.

The *fulgens–decipiens* clade was not considered by Guzmán-Dávalos et al. (2003). This clade contains only European species and is related (but without statistical support) to species of *Gymnopilus* with purplish-scaly pilei (purple scales clade here, Fig. 20, or *aeruginosus–luteofolius* and *lepidotus–subearlei* clades in Guzmán-Dávalos et al. 2003), which mainly have a tropical distribution (Guzmán-Dávalos et al. 2008). On the other hand, the *Gymnopilus* members that stain greenish or bluish also have purplish-scaly pileus. However, *G. turficola* (*fulgens–decipiens* clade) has olive-brown pileus and bluish to yellow-green lamellae, but without purple scales on the pileus (Moser et al. 2001).

Finally, the other morphologically similar species, *G. flavus*, is not related to *G. maritimus*. It forms a group with a 51% of bootstrap support with the *underwoodii–validipes* clade (Fig. 20) (Guzmán-Dávalos et al. 2003). The affinities among these species are not obvious. *G. underwoodii* (Peck) Murrill and *G. validipes* (Peck) Hesler have a medium to large basidiomata, squamulose pileus and grow in America versus small to medium basidiomata, fibrillose-tomentose pileus and grows in Europe for *G. flavus*. On the other hand, the basidiospores are similar in *G. flavus* and *G. underwoodii*, but bigger in *G. validipes* (Hesler 1969; Guzmán-Dávalos 1996; Holec 2005).

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