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A NEW SPECIES OF *ROMAGNESIELLA* (BASIDIOMYCOTA)
AND NOTES ON SOME SARDINIAN COLLECTIONS OF *R. CLAVUS*

Abstract

The new species Romagnesiella sanctae-christinae is proposed on morphological basis on the ground of a collection from central Sardinia. The new form Romagnesiella clavus f. mediterranea is described for mediterranean records of R. clavus from northern Sardinia. Descriptions of the material collected, taxonomical notes and macroslides of fresh or dry basidiomata and sketches of the main micromorphological features are added.

Riassunto

Viene proposta, come nuova specie, su base morfologica, Romagnesiella sanctae-christinae, rinvenuta nella Sardegna centrale. Ritrovamenti di Romagnesiella clavus effettuati nella Sardegna centrale vengono descritti come una nuova forma di questa specie. Le due entità vengono descritte, tassonomicamente discusse e vengono aggiunte illustrazioni a colori di materiale fresco o secco e schizzi dei principali caratteri micromorfologici.

Key words: Basidiomycota, Agaricales, Romagnesiella, *R. clavus* f. *mediterranea*, *R. sanctae-christinae*, taxonomy, key.

Introduction

The genus *Romagnesiella* has been recently introduced by MATHENY & AL. (2014) to accommodate *Galerina clavus* Romagn. (1942), an unusual species originally described from France and collected in several regions of central Europe. Although SINGER & SMITH (1964) place this species in *Galerina*, section *Pseudotubaria* Singer & A.H. Smith, genetic studies have proved that this placement is artificial and that Romagnesi's agaric belongs to a genus on its own right.

In this paper a new form of *Romagnesiella clavus* is proposed for the mediterranean collection made by M.C. and other AA (see below), which proved to be different by the type form both from morphological and molecular point of view.

A new species, viz. *Romagnesiella sanctae-christinae*, is also described based on a collection made by M.C. in central Sardinia.

Material and methods

Morphological analysis

Basidiomata were studied from herbarium material (personal herbarium of M. Contu) consulting descriptive notes and colour slides or digital pictures when available. Color designations in the format "(5E7)" refer to plate, column, and row of KORNERUP & WANSCHER (1967). Microscopic observations were made in 5 % KOH and Melzer's reagent (0.5 g I, 1.5 g IK, 20 g chloral hydrate, 20 cl H₂O). Spore dimensions are estimated from a natural deposit on the stipe surface and from pictures taken on a digital camera Moticam1000 connected to a microscope Nacet Andromede 0181. Measurements on screen and estimations are processed under the software Mycomètre 2.02 (FANNECHÈRE, 2009). 1st and 9th deciles (D1,9) and average value (italic) are given according to FANNECHÈRE (2005, 2009). Nomenclature follows MATHENY & AL.

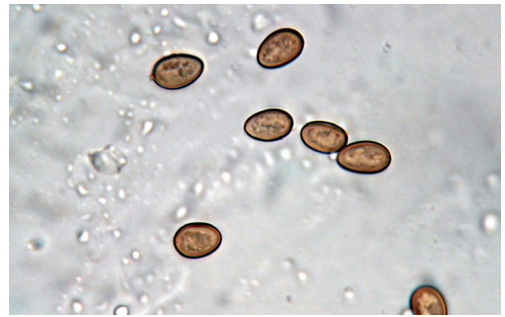


Romagnesiella sanctae-christinae

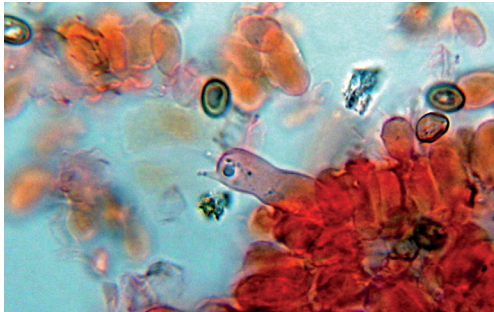
Photo by Marco Contu



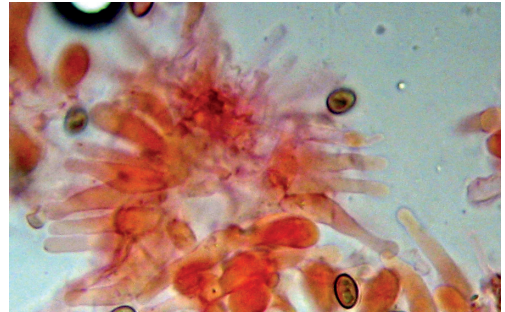
R. sanctae-christinae. Spores. Photo by Mariano Curti



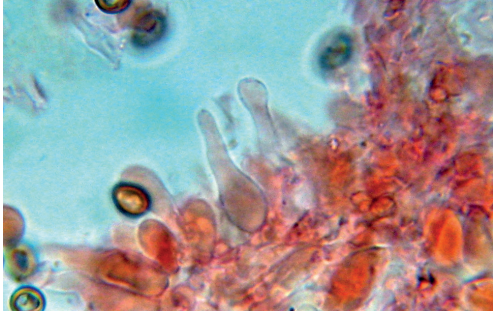
R. sanctae-christinae. Spores. Photo by Mariano Curti



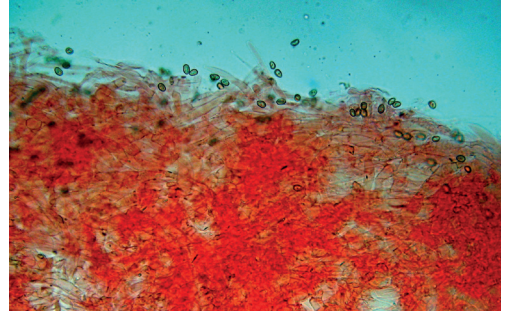
R. sanctae-christinae. Basidium. Photo by Mariano Curti



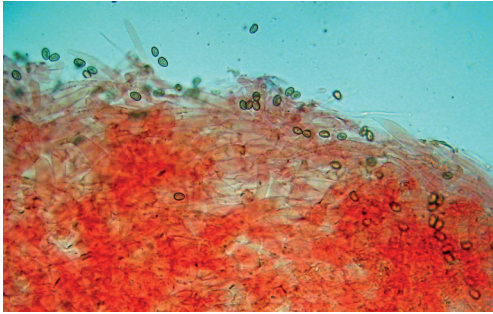
R. sanctae-christinae. Cheilocystidia. Photo by Mariano Curti



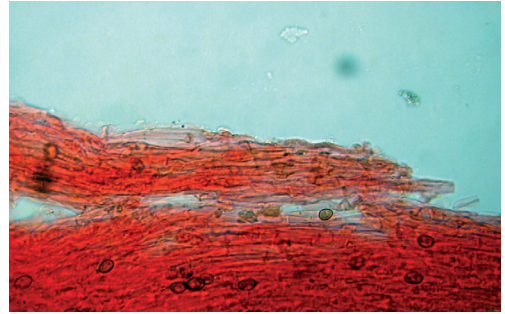
R. sanctae-christinae. Cheilocystidia. Photo by Mariano Curti



R. sanctae-christinae. Pileipellis. Photo by Mariano Curti



R. sanctae-christinae. Pileipellis. Photo by Mariano Curti



R. sanctae-christinae. Stiptipellis. Photo by Mariano Curti

TAXONOMY

Romagnesiella sanctae-christinae Contu & P.-A. Moreau, spec. nov., Index fungorum registration number: IF550995.

Pileus 2-10 mm, very thin, convex then expanding to plano-convex, not umbonate, glabrous, dry, smooth, fulvous-brown, fading buff, entirely striate when fresh, without veil remnants.

Lamellae distant, thick, triangular, uncinately adnate, brown to chestnut-brown, with a concolorous crenulate edge.

Stipe 5-20 × 3-5 mm, often flexuose, cylindrical, thickened downwards, concolorous with the pileus or paler, surface nearly smooth, subsericeous; mycelium white.

Context very thin, ochraceous, unchanging; smell and taste not distinctive.

Spore print unknown as for the colour.

Spores (6.0)7.2-7.7-8.5 × (4.5)4.8-5.2-5.5(5.8) μm, Q = 1.35-1.49-1.60, ovo-ellipsoidal, slightly flattened and ovoid in front view, dark brown when fresh, pale rusty ochre on exsiccatum and in KOH, wall thickened up to 0.5 μm, sometimes with a minute germinative pore at apex.

Basidia 19-24 × 7-8.5 μm, 4-spored, shortly cylindro-clavate strongly strangulate at mid length, necrobasidia abundant and collapsed, reddish brown in KOH. Subhymenium narrow, 6-8 μm thick, shortly ramose.

Hymenophoral trama regular, with slender punctuate hyphae 2-5 μm wide and large ellipsoidal articles 12-25 μm wide intermixed, smooth to slightly incrustate.

Lamella edge (possibly eroded due to age) fertile, with **basidia** intermixed with clusters of hardly differentiated fasciculate; **cheilocystidia**, 14-32 × 3.5-5 μm, slightly refringent, narrowly

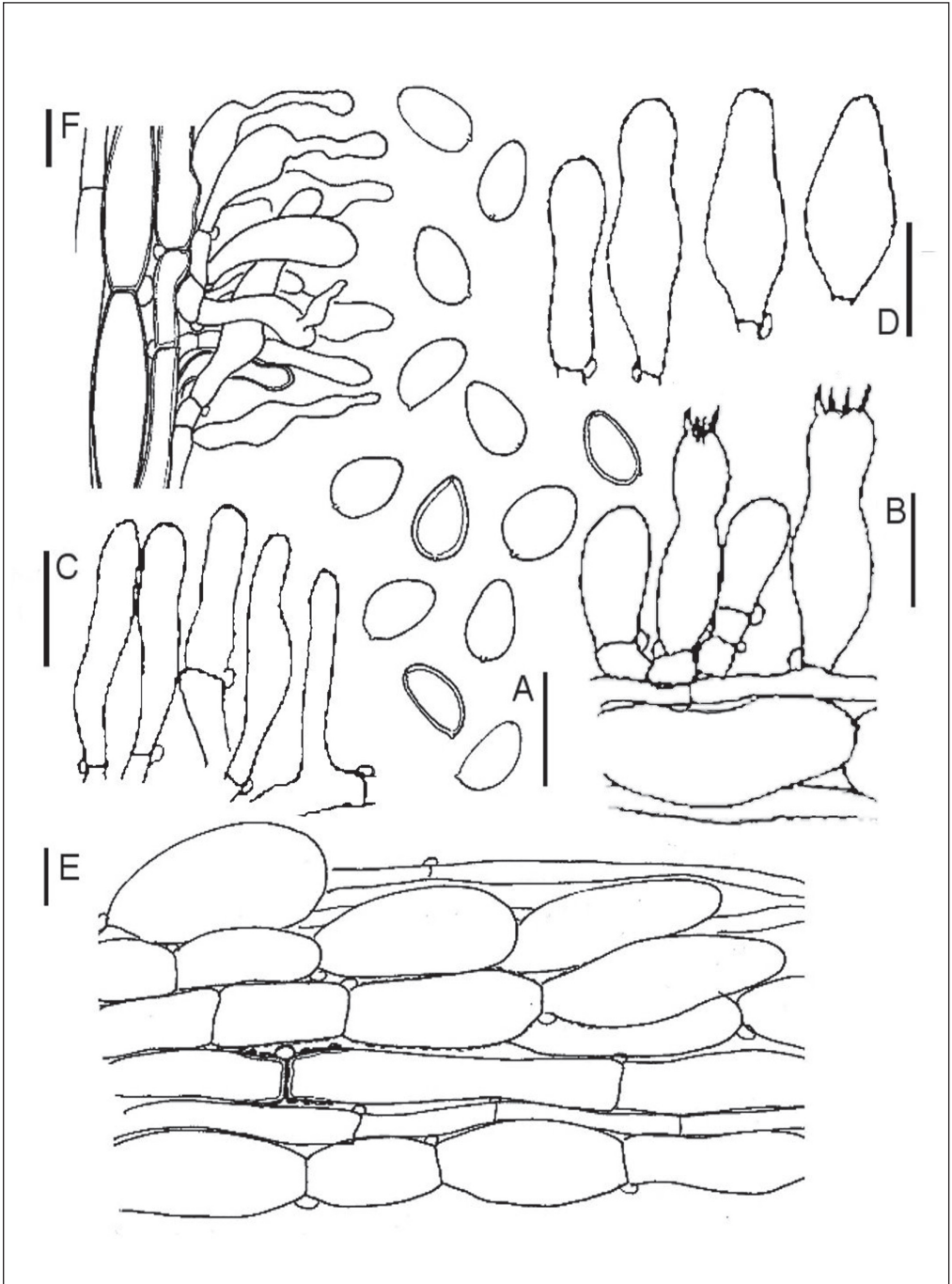


Fig. 1. *Romagnesiella sanctae-christinae* sp. nov. **A:** spores; **B:** basidia and subhymenium; **C:** cheilocystidia; **D:** pleurocystidia; **E:** pileipellis, radial cut; **F:** stipitipellis, radial cut. Scale bar = 10 μ m.

Drawn by Pierre-Arthur Moreau

cylindrical to slightly clavate, sometimes septate; **pleurocystidia** 20-24 × 7.5-9 µm, only slightly protruding, cylindrical to utriform, sometimes slightly clavate, not unfrequent but hardly differentiated from basidioles.

Pileipellis made up of short, cylindrical to fusiform +/- erected articles, 32-55 × 6-14 µm, slightly thick-walled, not incrustated or only in depth, reddish ochre in KOH, especially thickened at septa towards context; suprapellis represented by sparse filamentous hyphae 2-4.5 µm wide, thin-walled, smooth.

Pileus context made up of cylindrical hyphae 2.5-7 µm wide, with thickened wall reddish in KOH, smooth.

Stipitipellis at apex with clusters of fasciculate caulocystidia, 20-38 × 4-6 µm, clavate to cylindrical or appendiculate with tortuous or attenuate neck up to 25 × 3 µm, some with yellow refringent content; superficial hyphae 2-5 µm wide, slender, smooth, with yellow slightly thickened wall.

Clamp connections present at all septa.

Ecology: in troops on moist soil in a grassy, open area, in a basic land. Only known from the type locality in Sardinia (Italy).

Material studied: ITALY; Sardinia, prov. Nuoro, Abbasanta, archaeological site of Santa Cristina, in troops, on moist soil in a grassy, open area, in a basic land, 25 Nov 1997, leg. M. Contu (CAG, isotypus in TENN).

This species has been observed only once, but it takes a very interesting position by its intermediate characters between *Pachylepyrium* and *Romagnesiella*: basidiospores minutely pored, more thick-walled than other species in *Romagnesiella* and more egg-shaped in front view like *Crassisporium funariophilum* (M.M. Moser) Matheny, P.-A. Moreau & Vizzini and allied species, and short basidia. The presence of abundant necrobasidia and small hardly differentiated pleurocystidia, exclude an alternative classification in other genera such as *Psilocybe* (*Deconica*) or *Galerina*.

In the field this probably very rare species could be confused with several members of *Deconica*, but it easily differentiated by the differently tinged basidiospores, dark brown in fresh material but much paler in herbarium material or in alkali and not turning red in KOH. Spore morphology somewhat reminds that of *R. clavus*, different by smaller, more elongate basidiospores, larger cystidia, incrusting pigment on hyphae of pileal surface, and a cutis-like pileal structure.

R. clavus f. *mediterranea* is sharply different in the granulose-fibrillose covering layer of the pileus, the larger basidiospores not so dark in the fresh basidiomata, obviously larger cystidia and pileipellis arrangement.

Despite intensive research in the site where the thus far unique collection of this species was made, no additional material could be obtained.

Romagnesiella clavus* f. *mediterranea Contu & P.-A. Moreau f. nov., Mycobank registration number: MB 519561.

Synonyms: *Galera hypnorum* s. Malençon & Bertault (1970: 554) and unpublished notes of G. Malençon, MPU; *Galerina clavus* s. Hausknecht (1993: 47), s. Moreau (2009: 199).

Mycobank registration number: MB 519561.

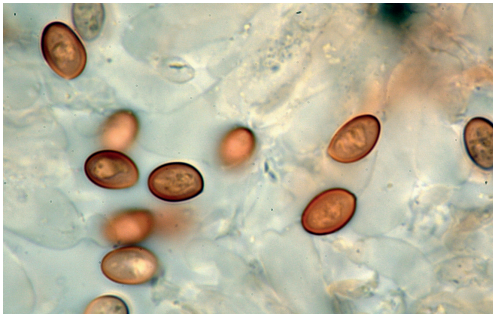
Bibliography – HAUSKNECHT (1993: 47-48; description, picture); MALENÇON in MOREAU (2009: 199-200, description, illustration).

This new form differs by type in having somewhat larger basidiospores [(7.0) 7.6–8.25–9.0 (10) × (4.0) 4.9–5.19–5.5 (5.7) µm, Q = 1.48–1.59–1.70], slightly larger cystidia, and a filamentous pileipellis with a more or less continuous suprapellis of slender cylindrical hyphae, with sparse slightly erected terminal elements. Typus: Italia, Sardinia: Golfo di Marinella, 10.I.2007, leg. M. Contu s.n. (holotypus: LIP; isotypus: herb. M. Contu).

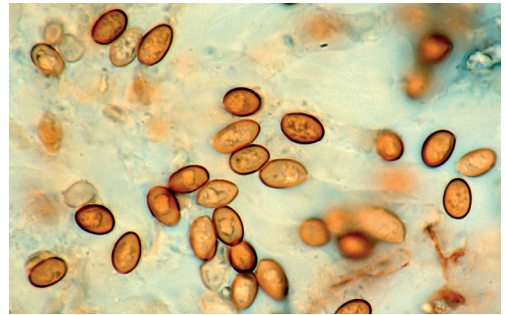


Romagnesiella clavus f. *mediterranea*

Photo by Luigi Perrone



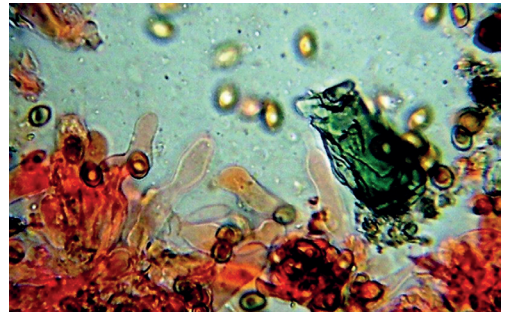
R. clavus f. *mediterranea*. Spores. Photo by Mariano Curti



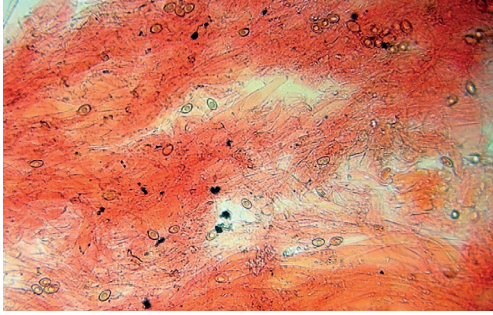
R. clavus f. *mediterranea*. Spores. Photo by Mariano Curti



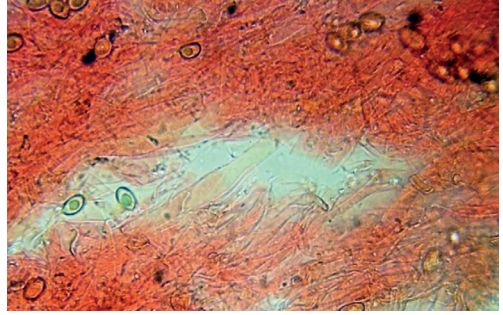
R. clavus f. *mediterranea*. Spores. Photo by Mariano Curti



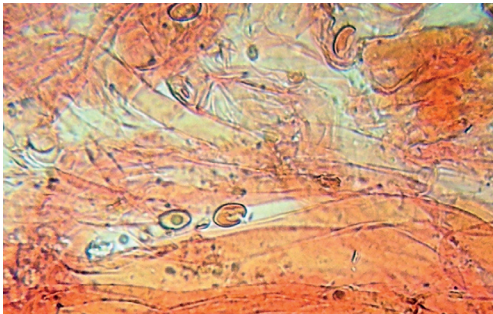
R. clavus f. *mediterranea*. Cheilocystidia. Photo by Mariano Curti



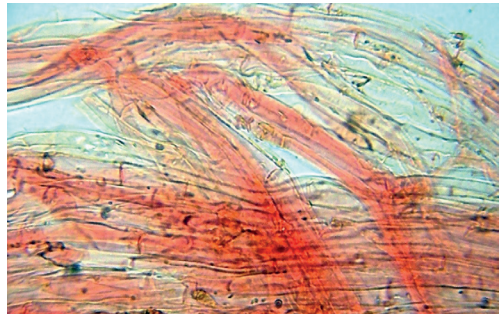
R. clavus f. *mediterranea*. Pileipellis. Photo by Mariano Curti



R. clavus f. *mediterranea*. Pileipellis. Photo by Mariano Curti



R. clavus f. *mediterranea*. Pileipellis. Photo by Mariano Curti



R. clavus f. *mediterranea*. Pileipellis. Photo by Mariano Curti

Pileus 1-15 mm diam., thin, at first conico-convex then hemispherical-convex then convex-flattened to expanding, not umbonate, dry, very hygrophanous, rusty-fawn but soon russet to russet-buff, with a silky covering of white fibrils, striate at margin when very fresh, without veil remnants.

Lamellae distant, thick, triangular, adnate, fulvous or rusty-fawn, with a paler crenulate edge.

Stipe 5-15 × 0.5-1.5 mm, often flexuose, cylindric, slightly thickened downwards, concolorous with the pileus, surface with white fibrils and striate, often white-pruinose; mycelium white.

Context very thin, fulvous to rusty, unchanging; smell not distinctive or slightly mealy; taste mild.

Spore print rusty brown.

Spores (7.0)7.6-8.25-9.0(10) × (4.0)4.9-5.19-5.5(5.7) μm, Q = 1.48-1.59-1.70 (on hymenium up to 9.5-12 × 5.5-6 μm from 2-spored basidia), narrowly ovoid-ellipsoidal, bright ochre yellow in KOH, smooth, slightly thick-walled (up to 0.2 μm thick), multiguttulate in KOH, slightly dextrinoid, without germ pore.

Basidia 25-38 × 6.5-9 μm, 4-spored (rarely 2-spored with long abnormal sterigmata), clavate often strangulate beneath the apex, hyaline, often thick-walled when old, necrobasidia frequent with bright yellow content originating from deep subhymenium.

Subhymenium 8-12 μm thick, ramose with short cylindrical to trapezoidal elements.

Hymenophoral trama (mediostratum) regular, made of long slender hyphae 3.5-8 μm wide, intermixed with numerous large, vesicular to subglobose elements up to 35 μm wide, with irregularly incrusting, punctuate to granular pigment.

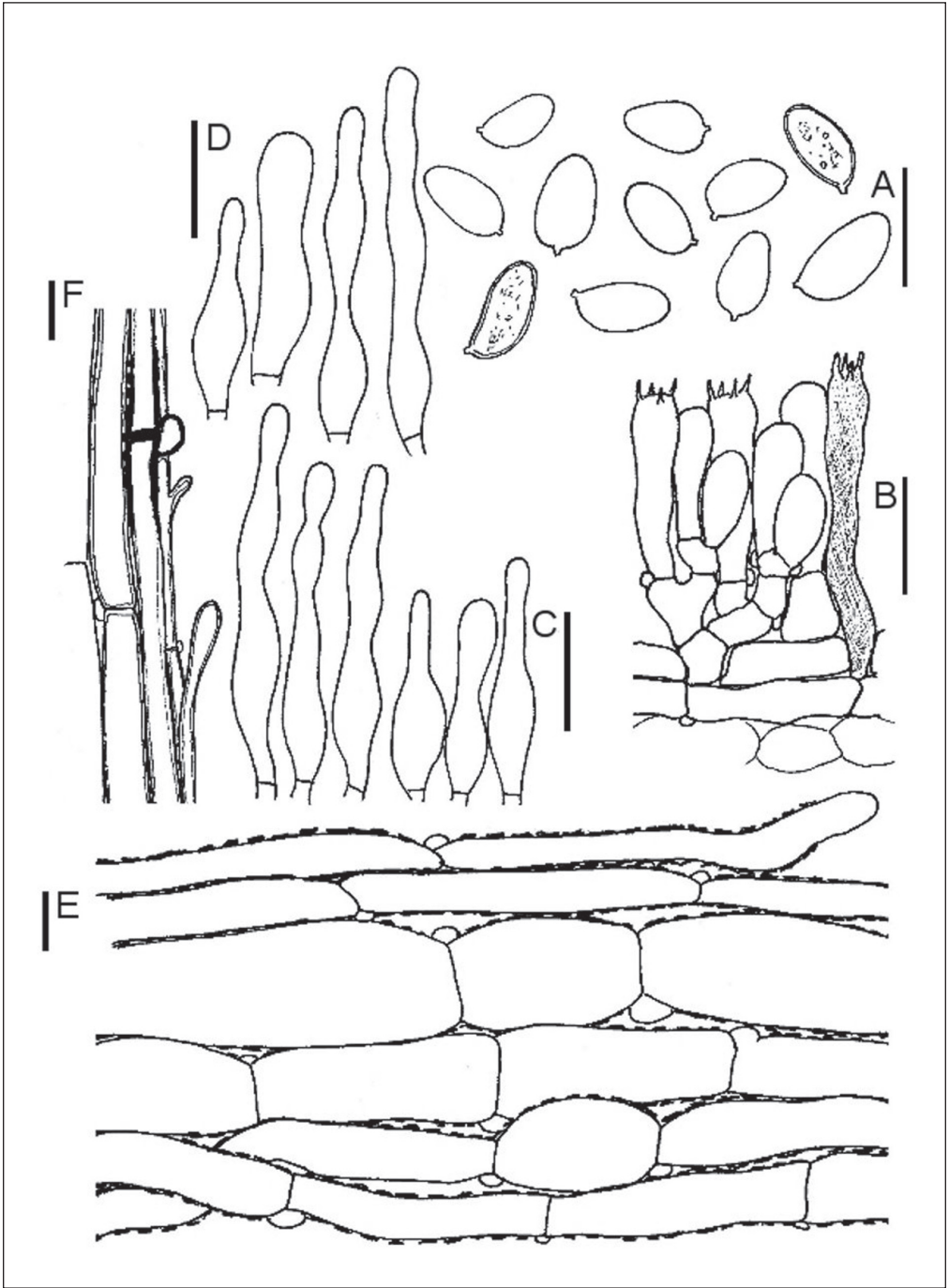


Fig. 2. *Romagnesiella clavus* f. *mediterranea* f. nov. A: spores; B: basidia and subhymenium; C: cheilocystidia; D: pleurocystidia; E: pileipellis, radial cut; F: stipitipellis, radial cut. Scale bar = 10 μ m. Drawn by Pierre-Arthur Moreau

Lamella edge sterile, with **cheilocystidia** $32\text{--}58 \times 6\text{--}8.5(12) \mu\text{m}$, polymorphic, mostly narrowly lageniform often with elongate +/- flexuous neck and +/- enlarged base, also shorter, lageniform to cylindrical +/- strangulate at mid-length, thin-walled and hyaline, often covered by colourless granulations of $0.5\text{--}1 \mu\text{m}$ diam; **pleurocystidia** scarce, especially towards edge, $22\text{--}60 \times 4\text{--}10.5 \mu\text{m}$, fusiform to lageniform, apex swollen or acute, hyaline, mostly thin-walled but sometimes also slightly thick-walled, not encrusted.

Pileipellis composed of a filamentous +/- continuous suprapellis of slender cylindrical hyphae, $4\text{--}8.5 \mu\text{m}$ wide, with sparse slightly erected terminal articles, strongly incrustated, reddish ochre in KOH, only apex of terminal articles smooth and paler; subpellis made of cylindrical to +/- inflated articles $15\text{--}25 \mu\text{m}$ wide, short and subglobose to rather long, coarsely incrustated, reddish ochre in KOH. Pileus context made of cylindrical slender hyphae $3.5\text{--}8 \mu\text{m}$ wide intermixed with cylindrical +/- inflated articles $7\text{--}35 \mu\text{m}$ wide, all coarsely incrustated.

Stipitipellis without cystidia, made of slender hyphae $1.5\text{--}3.5 \mu\text{m}$ wide, with few appressed terminal cells, wall slightly thickened, especially bright yellow and incrustated at septa. Stipe context of parallel hyphae $3\text{--}15 \mu\text{m}$ wide, slender and wider intermixed, smooth; no gleoplerous hyphae seen.

Clamp connections present, frequent.

Ecology in troops on moist sandy soil, in grassy open areas, often near the coast. Rare, only known from peninsular Italy (Toscana), Sardinia, and North Africa (Morocco).

Material studied: ITALY, Sardinia, prov. Olbia-Tempio Pausania, Golfo di Marinella, on sandy soil in a coastal grassland, in troops, leg. M. Contu, 10 Jan. 2007 (holotype: LIP; isotype: herb. pers. M. Contu), 14 Jan 2007, 15 Dec 2007 (TENN063957), 27 Dec 2007, 9 Oct. 2008; id. Aglientu, loc. Riu Li Saldi, on sandy soil, in a coastal pine-wood, leg. M. Contu, 23 Dec. 1995 (Z+ZT).

North African and Sardinian collections of *Galerina clavus*, as described by HAUSKNECHT & ZUCCHERELLI (1993) or MALENÇON (in MOREAU 2009: 199) diverge sensitively from continental collections by somewhat larger basidiospores [$(7.0)7.6\text{--}8.25\text{--}9.0(10) \times (4.0)4.9\text{--}5.19\text{--}5.5(5.7) \mu\text{m}$, $Q = 1.48\text{--}1.59\text{--}1.70$], slightly larger cystidia, and a filamentous pileipellis with a more or less continuous suprapellis of slender cylindrical hyphae, with sparse slightly erected terminal elements. It is envisaged that Mediterranean collections may represent a distinct although very close taxon. Such a collection from Sardinia was sequenced (C15122007, leg. M. Contu), for which only ITS1 could be obtained ITS1 reveals nine site differences with *R. clavus* PAM6090110, four of which are polymorphic in C15122007, therefore we are describing the collections from Sardinia as a new form of *R. clavus*.

This new form can be easily distinguished from *R. clavus* by the rusty to rusty-fawn tinges of the pileus, the larger basidiospores, the slightly larger cystidia and by the arrangement of the pileipellis, which is made up of a superficial layer of short articles that are lobate, digitate, puzzle-like, fusiform or pyriform, while in *R. clavus* f. *mediterranea* the pileipellis is made up of a filamentous or more or less continuous suprapellis of slender cylindrical hyphae, with sparse slightly erected terminal articles and absence of inflated cells. We could only obtain the ITS1 of *R. clavus* f. *mediterranea*, however, a blastn comparison in GenBank with *R. clavus* (coll. PAM6090110, as *Tubaria minima*) reveals nine site differences, four of which are polymorphic in *R. clavus* f. *mediterranea*.

"*Galerina clavus*" described by HAUSKNECHT (1993) is identical to *R. clavus* f. *mediterranea* in micromorphological features. The revision of collections and field notes by G. Malençon kept at herb. MPU (MOREAU, 2009: 199) revealed that Malençon's *Galerina hypnorum*, collected in Morocco and published without description in MALENÇON & BERTAULT (1970: 554), belonged to the "*Galerina*" *clavus* complex and is also referable to *R. clavus* f. *mediterranea*. Malençon's manuscript notes and drawings are reproduced in MOREAU (2009).

Key to European species of *Romagnesiella*:

1. Spores egg-shaped in front view, with minute germ pore. Spores $7.2-8.5 \times 4.8-5.5 \mu\text{m}$. Basidia $< 25 \mu\text{m}$ long. In basic grasslands..... *R. sanctae-christinae* sp. nov.
- 1*. Spores elliptical to cylindrical in front view, without germ pore. Basidia $> 25 \mu\text{m}$ long.... 2
2. Pileipellis a cutis of coarsely incrustated hyphae with few erected articles. Spores $7.6-9.0 \times 4.9-5.5 \mu\text{m}$. Mediterranean grasslands, mainly on sandy soils..... *R. clavus* f. *mediterranea* f. nov.
- 2*. Pileipellis a cutis with transition to a trichoderm with numerous polymorphic terminal articles with smooth wall. Spores $6.2-10.3 \times 3.9-5.5 \mu\text{m}$. Mineral-rich substrates: road sides, dunes, gardens, etc..... *R. clavus* f. *clavus*

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