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A NEW SPECIES OF GENUS *ROMAGNESIELLA* (*AGARICALES*) FROM THE MEDITERRANEAN REGION

Abstract

In this work Romagnesiella clavus f. mediterranea Contu is elevated to the rank of autonomous species with the name Romagnesiella contui sp. nov in honor of the Italian mycologist Marco Contu. This is based on the examination of a paratypus of the aforementioned form existing in the TENN herbarium. Furthermore, the macroscopic, microscopic and molecular examination of the taxon is carried out, comparing it with neighboring species. New collections coming from Spanish and French territories are studied. A French collection from the Alsace region is proposed as a new holotypus collection.

Key words *Basidiomycota, Crassisporiaceae*, new taxa, phylogeny, taxonomy.

Introduction

The genus *Romagnesiella* Contu, Matheny, P.-A. Moreau, Vizzini & A. de Haan, was proposed by Matheny *et al*. (2014), to honor the famous French mycologist Henri Romagnesi (1912-1997). It was first classified in the family *Strophariaceae* Singer & A.H. Sm., but later moved to *Crassisporiaceae* Vizzini, Consiglio & M. Marchetti, (validated in *Index Fungorum* 386: 1, 2019). According to Matheny *et al*. (2014), *Romagnesiella* is characterized by: "*Basidiomata naucorioid, lamellae distant, adnate to subdecurrent, pileus dry, not hygrophanus, stipe smooth, without a partial veil. Basidiospores smooth, more or less ovate, not subangular, yellow in water mounts, reddish ochre in KOH, not dextrinoid, germ pore absent, necrobasidia numerous; cheilocystidia present, edges of lamellae smooth and (sub)sterile, pleurocystidia present but dispersed and infrequent, pileipellis filamentous, hymenophoral trama regular, clamp connections frequent. On unburnt soil or sand among mosses and grasses*".

The type species of *Romagnesiella* is *R. clavus* (Romagn.) Contu, Matheny, P.-A. Moreau, Vizzini & A. de Haan (Matheny *et al*. 2014). It was originally described in genus *Galerina* Earle (Romagnesi 1942), and later moved to *Naucoria* (Fr.) P. Kumm., subgenus *Tubaria* W.G. Smith (Kühner & Romagnesi 1953). Currently, two species more are known in *Romagnesiella*: *R. sanctae-christinae* Contu & P.-A. Moreau (Contu & Moreau 2014) and *R. campestris* Musumeci (Musumeci 2021), as well as *R. clavus* f. *mediterranea* Contu & P.-A. Moreau (Contu & Moreau 2014), whose status is here revisited with new DNA data.

Materials and methods

Macroscopical studies were conducted on fresh specimens. In situ images were taken with a Canon EOS 600D camera or else or NIKON 7600 Coolpix series device. Images of microscopical structures were taken with a binocular Leica DME microscope with acromatic objectives or a NIKON Eclipse 2000 microscope. Preparations for microscopy were mounted in the usual reagents (distilled water, Melzer reagent, KOH 5%, Congo Red). Spore measures exclude the apiculus. French specimens are preserved in LUG herbarium (Museum of Natural History of Lugano, Switzerland), while Spanish material is preserved at AH (Herbarium of the University of Alcalá, Alcalá de Henares, Spain).

Total DNA was extracted from dry specimens employing a modified protocol based on Murray & Thompson (1980). PCR reactions (Mullis & Faloona 1987) included 35 cycles with an annealing temperature of 54 ºC. The primers ITS1F and ITS4 (White *et al*. 1990, Gardes & Bruns 1993) were employed to amplify the ITS rDNA region, LR0R and LR5 (Vilgalys & Hester 1990, Cubeta *et al*. 1991) were used for the 28S rDNA region, and bRPB2-6F2 (reverse of bRPB2-6R2), and bRPB2-7R2 for the RNA polymerase II second largest subunit (rpb2) gene (Matheny *et al*. 2007). PCR products were checked in 1% agarose gels, and amplicons were sequenced with one or both PCR primers. Sequences were corrected to remove reading errors in chromatograms. BLASTn (Altschul *et al*. 1990) was used to select the most closely related ITS sequences from the International Nucleotide Sequence Database Collaboration public database (INSDC, Arita *et al*. 2021) and Unite (Nilsson *et al*. 2018). The sequences employed are listed in **Table 1**. Sequences first were aligned in MEGA 5.0 (Tamura *et al*. 2011) with its Clustal W application and then realigned manually as needed to establish positional homology. The resulting alignment was loaded in MrBayes 3.2.6 (Ronquist *et al*. 2012), where a Bayesian analysis was performed (one partition, two simultaneous runs, four chains, temperature set to 0.2, sampling every 100th generation) until the average split frequencies between the simultaneous runs fell below 0.01 after 0.23 M generations. Finally, a full search for the best-scoring maximum likelihood tree was performed in RAxML 8.2.12 (Stamatakis 2014) using the standard search algorithm (same partitions, GTRGAMMAI model, 2000 bootstrap replications). The significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

TAXONOMY

Romagnesiella contui G. Moreno, Musumeci & Perrone, sp. nov. (**Figures 1-5**)

= *R. clavus* f. *mediterranea* Contu & P.A. Moreau, *Rivista Micologica Romana, Boll. AMER* 93 (3): 7 (2014)

Mycobank: 857538

Etymology: dedicated to the Italian mycologist Marco Contu, for his many research works on the taxonomy of *Agaricales* s. lato.

Classification: *Crassisporiaceae, Agaricineae, Agaricales*

Studied material: FRANCE: Haut-Rhin, Alsace, Petit Landau, open sunny area, among grass, calcareous-argillaceous soil, 13 October 2020, *leg.* Enzo Musumeci, (holotype LUG 20715, isotype EM 5978-20 in E. Musumeci pers. herb.), Genbank (ITS = PV056473, LSU = PV110129, RPB2 = PV114888). SPAIN: Madrid, Colmenarejo, Prado Ibarra, open sunny area, among grass, acidic sandy soil, 885 m, *leg.* F. Prieto-García & J.C. Zamora, 7 January 2012 (AH 46569). Ibid. *leg.* F. Prieto-García, 28 November 2012 (AH 51161), Genbank (ITS = PV056476, LSU = PV110130, RPB2 = PV114889). Ibid. 16 December 2012 (AH 51169), Genbank (ITS = PV056474, RPB2 = PV114890). Ibid. 20 January 2012 (AH 51168). Ibid. 24 January 2012 (AH 51167). Ibid. 31 January 2012 (AH 51166). Ibid. 16 December 2013 (AH 51170). Ibid. 18 December 2014 (AH 51163). Ibid. *leg.* F. Prieto-García & A. González, 29 November 2014 (AH 51164), Genbank (ITS = PV056475, LSU = PV110128). Ibid. *leg.* F. Prieto-García, 18 November 2015 (AH 51162). Ibid. 19 November 2015 (AH 51161). Ibid. 5 December 2018 (AH 49358).

Macroscopical description

Pileus 4-9 mm in diam., first subglobose to hemispherical-convex, finally extended-flattened, sometimes with a central umbo or a subtle depression in mature specimens. Surface weakly hygrophanous, slightly fibrillose when young, sometimes coated with a whitish pruine, cuticle felting to smooth, minutely rugose-subsquamose, with a striated margin when mature. Color fawn brown to reddish brown or brick brown, recalling that of *Tubaria*.

Table 1. ITS *Romagnesiella* sequences.

Species	Herbarium	ITS
Crassisporium funariophilum	Mushroom Observer 366861	MT703796
Romagnesiella campestris	**EM6411-20 - FRANCE: Niffer - HOLOTYPE**	**MZ092917**
Romagnesiella campestris	**ALV27140 EM6141-20 - FRANCE: Niffer - ISOTYPE**	**PV056478**
Romagnesiella clavus	20Rom01 - GERMANY: Brandenburg	OM501088
Romagnesiella clavus	G3560 - ESTONIA	UDB0338719
Romagnesiella clavus	G3568 - ESTONIA	UDB0390126
Romagnesiella clavus	G3602 - LATVIA	UDB0303830
Romagnesiella clavus	G4271 - ESTONIA	UDB0494596
Romagnesiella clavus	G4789 - ESTONIA	UDB0554928
Romagnesiella clavus	LIP: PAM06090110 - FRANCE - EPITYPE	NR_171207
Romagnesiella clavus	O-F-21838 - NORWAY - Oslo	UDB036680
Romagnesiella clavus	TUF132100 - ESTONIA	UDB0780314
Romagnesiella* cf. *clavus	**ALV28070 EM1571-19**	**PV056477**
Romagnesiella clavus (uncultured)	Tienshan - CHINA	OW847407
Romagnesiella contui (as *R. clavus* f. *mediterranea*)	Contu15122007 - ITALY: Sardinia - PARATYPE	HQ832447
Romagnesiella contui (uncultured, from Sorghum)	OTU736 - USA: California	MK018997
Romagnesiella sp.	iNAT:197113528 - USA: California	PQ160944
Romagnesiella sp.	iNAT:200284839 - USA: California	PQ144480
Romagnesiella sp. (uncultured, from soil Zea mays field)	09S10C53 - GERMANY: Lower Saxony	HG937047
Romagnesilla contui	**ALV19885 AH51164**	**PV056475**
Romagnesilla contui	**ALV28069 EM5978-20, LUG 20715 - HOLOTYPE**	**PV056473**
Romagnesilla contui	**ALV28982 AH51161**	**PV056476**
Romagnesilla contui	**ALV28984 AH51169**	**PV056474**

Lamellae spaced, with scarce thin lamellulae, slightly decurrent, sometimes bifurcated, ferruginose-brown to flesh-brown, lamellulae lighter in color.

Stipe 8-17 × 1-2 mm, cylindrical, concolor with the pileus, surface coated with whitish fibrils sometimes forming a ring at the top.

Flesh thin, smell and taste fungal, not defined.

Microscopical description

Spores (10-)9.7-5.9 × 6.4-4.2 µm, av. 7.8 × 5.3 µm, Q = (1.9-)1.8-1.2, Q_{av} = 1.5 (n = 30), smooth, not amyloid, not dextrinoid, ochraceous-brown in KOH 3%, ellipsoid to ovoid in front view,

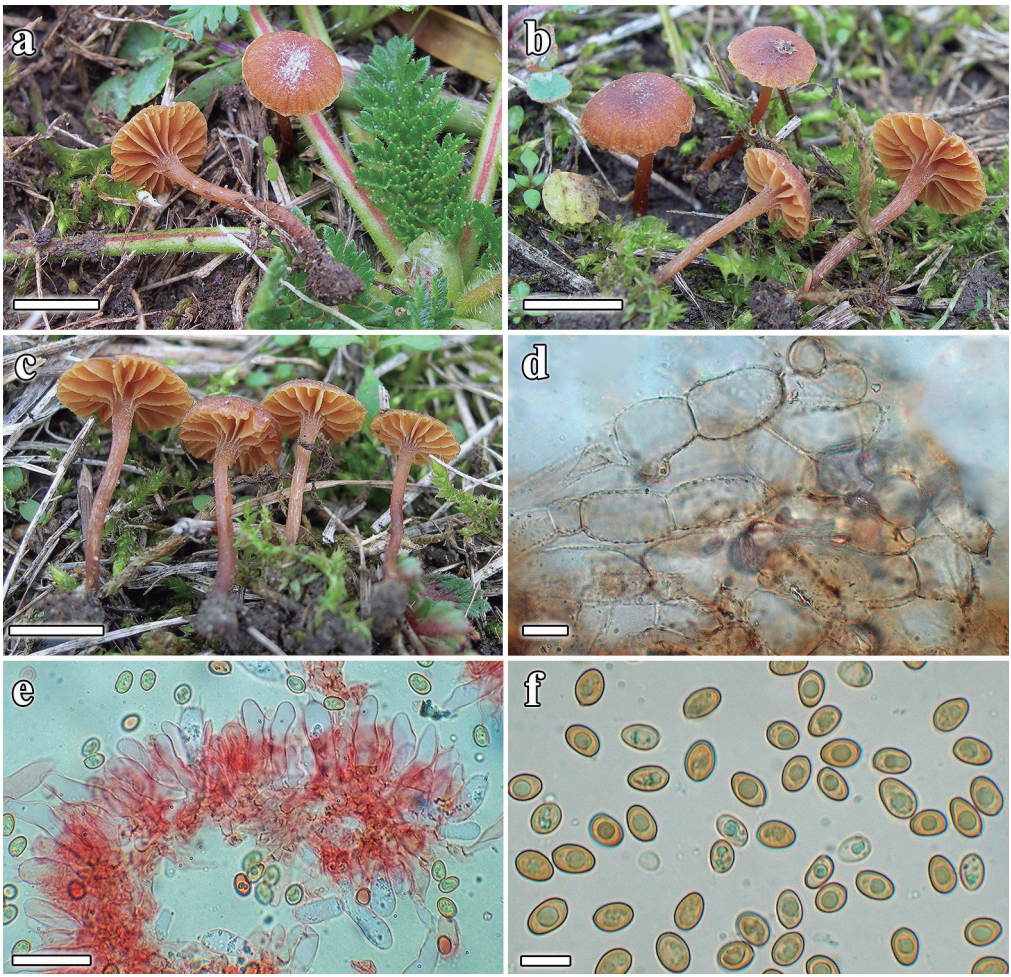

Figure 1. *Romagnesiella contui* Moreno, Musumeci & Perrone, holotype LUG 20715: **a-b**. Habitat; **c**. Detail of basidiomata; **d**. Epicutis; **e**. Cheilocystidia; **f**. Spores. Scale bars: **a-c** = 5 mm, **d** = 5 µm, **e** = 25 µm, **f** = 10 µm. Photos by Enzo Musumeci

ellipsoid in side view, with coarse walls (× 1 µm), generally lacking a central depression. Germ pore absent, apiculus not conspicuous.

Basidia 27-38 × 7–9 µm, tetrasporic, rarely bisporic, subclaviform, frequently with brownish-ochre to reddish brown content.

Hymenophoral trama formed by thick cylindrical hyphae (× 6-12 µm, sometimes reaching 15-26 µm), pigmented and encrusted, not metachromatic.

Cheilocystidia 25-45 × 7-10 µm variable in shape, sublageniform to cylindrical, subclaviform or subutriform, apex sometimes inflated, shrinked or with a sinuose-submoniliform neck.

Pleurocystidia scarce or rare, similar to cheilocystidia.

Epicutis not jellified, hyphae superficial (× 3-6 µm), parallel, sometimes entangled-intertwined, strongly pigmented (brownish-ochre) and incrusted. Incrusting pigment arranged on surface as short grooves or conspicuously incrusting with large adhering plates.

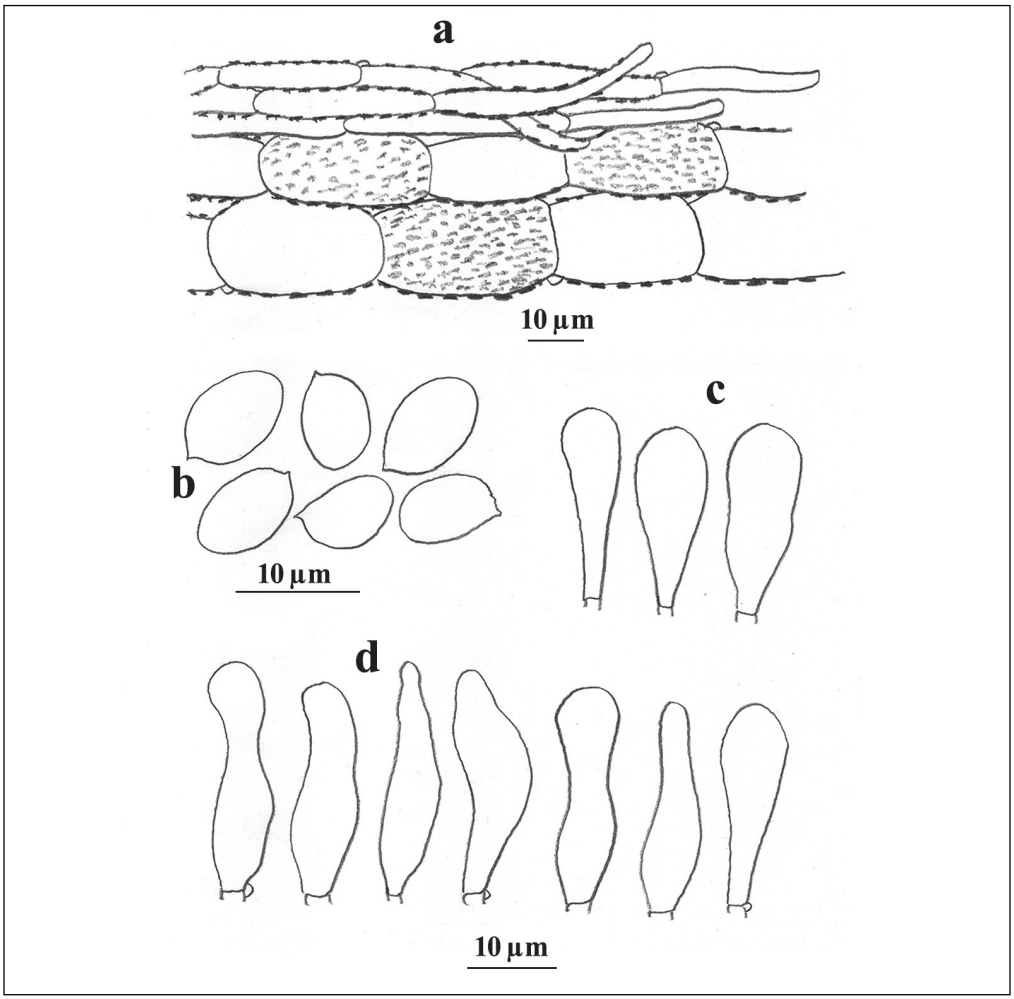

Figure 2. *Romagnesiella contui* Moreno, Musumeci & Perrone, holotype LUG 20715: **a**. Epicutis; **b**. Spores; **c**. Caulocystidia; **d**. Cheilocystidia and pleurocystidia. Drawings by Enzo Musumeci

Subcutis formed by subcylindrical hyphae (× 4-10 µm) mixed with a subcellular structure with variable elements (15-58 × 15-45 µm), pigmented and strongly incrusting.

Caulocutis formed by superficial hyphae (× 3-7 µm), slightly pigmented and incrusting, with larger hyphae (× 4-11 µm) at the central trama, also pigmented and incrusting.

Caulocystidia 18-52 × 5-10 µm, scarce, claviform to pyriform, rarely differently shaped.

Clamp connections present.

Discussion

Romagnesiella contui is characterized by its small size 4-9 mm in diam., a fibrillose pileus in young basidiomes, striate when mature, spores slightly wider and more ovoid than *R. clavus*, and an epicutis formed by entangled to parallel cylindrical hyphae, lacking claviform to pyriform erect tips.

Figure 3. *Romagnesiella contui* Moreno, Musumeci & Perrone, paratypes: **a**. Habitat (AH 51168); **b**. Pileus surface with fibrils (AH 51168); **c**. Detail of lamellae and stipe with a fibrillose ring (AH 51168); **d**. Stipe with fibrillose ring (AH 51168); **e**. Basidiomata in situ and detail of lamellae and fibrillose stipe (AH 46569); **f**. Basidiomata and detail of the lamellae (AH 51165). Scale bars: **b-d** = 1 mm, **c-f** = 5 mm. Photos by Francisco Prieto-García

Figure 4. *Romagnesiella contui* Moreno, Musumeci & Perrone, paratypes: **a**. Details of basidiomata (AH 51164); **b**. Striated pileus and detail of lamellae (AH 51162). Scale bars: **a-b** = 5 mm. Photos by Francisco Prieto-García

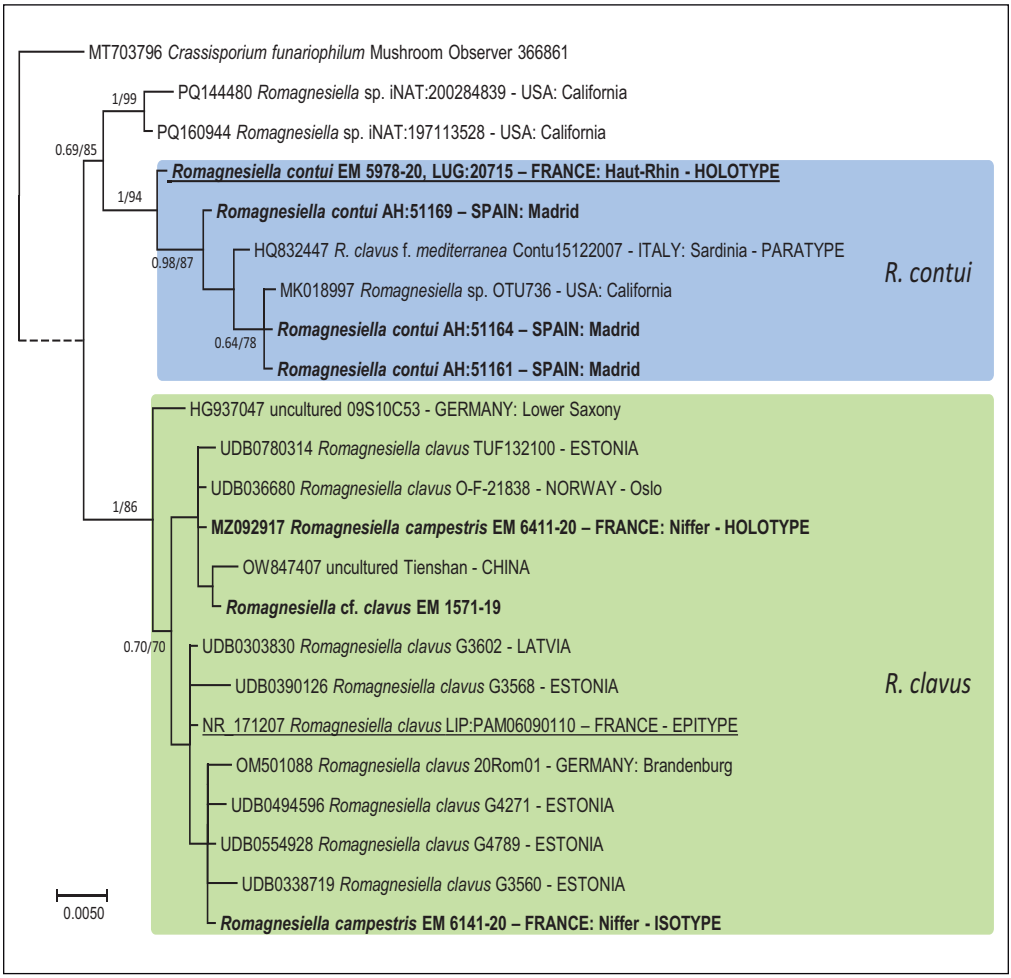

Figure 5. A 50% majority rule ITS rDNA consensus phylogram of the genus *Romagnesiella* (*Crassisporiaceae*, with *Crassisporium funariophilum* as outgroup) obtained using MrBayes from 1725 sampled trees. Nodes were annotated if they were supported by ≥ 0.95 Bayesian posterior probability (left) or ≥ 70% maximum likelihood bootstrap proportions (right). Sequences newly generated in this study are in bold.

Romagnesiella clavus is morphologically and phylogenetically close, but it does not have a striate pileus when mature, its spores are slightly narrower (5.6-)6.2-6.7-7.3(-8.5) × (3.6) 3.9-4.2-4.4 (5.0) µm, and it has an epicutis with abundant claviform to pyriform erect tips. For this reason, *R. contui* was originally described as a Mediterranean form of *R. clavus* (Contu & Moreau 2014). Both taxa can be confused with species of genus *Galerina* or *Tubaria*, leading to several erroneous interpretations, as described in Contu & Moreau (2014) and Musumeci (2021, 2024).

Romagnesiella sanctae-christinae, described by Contu & Moreau (2014), differs from *R. contui* because of the small germ pore of its spores. The only known collection is that of the holotype found in Sardegna (Italy), it is supposed to be deposited at CAG herbarium (Università degli Studi di Cagliari), with additional material stored at Marco Contu's personal herbarium, and an isotype in TENN herbarium (University of Tennessee Herbarium). However, curators at CAG herbarium were not able to find it there, and the material at Contu's herbarium seems

equally lost. Dr. B. Matheny, curator at TENN, reported that all attempts to sequence the isotype failed in PCR, and very few material is left, suggesting that additional collections are necessary.

He also stated that a paratype of *R. clavus* f. *mediterranea* (TENN-F-063957) was already sequenced by their team, and uploaded to genbank database as "*Pachylepyrium* sp." Contu15122007. The holotype collection of *R. clavus* f. *mediterranea* deposited at LIP and the paratype supposedly present in the personal herbarium of Marco Contu were not located either, rendering the paratype at TENN herbarium the only known type collection of this taxon. The species named *R. contui* in the present work is therefore supposed to be the same as *R. clavus* f. *mediterranea*, at least based on the data available from the paratype stored at TENN. The conservation of type material at properly funded specialized institutions is capital to solve taxonomical issues. Ideally, type collections should include multiple specimens to allow different studies, as done here with *R. contui*.

Finally, the basidiomes of *R. campestris* Musumeci are small sized (from a few mm to 10 mm or more), resembling those of *R. contui*. It differs from this species because of it naucorioid habit, spores 6.5-10.5 × 4.2-5 µm, more elongated on average, but never so much ovoid, the absence of pleurocystidia, and a strong farinaceous-rancid smell recalling that of *Macrocystidia cucumis* (Pers.) Joss. (Musumeci 2021). Genetically, the ITS rDNA sequences obtained from the holotype of *R. campestris* (EM6411-20) and the isotype (EM6414-20) are not statistically distinct from homologous sequences of *R. clavus* (**Figure 5**). Whether the morphologically deviant *R. campestris* is actually independent from *R. clavus* should be further explored with additional samples and multiple genetic markers. In order to have a correct comparative analysis, the epitypus collection of *R.clavus* should also be further examined with other molecular markers as a reference point for future collections.

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