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Short note

Coprinellus andreorum: a new species from Malta and South America

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Abstract

Coprinellus andreorum sp. nov. is described for the first time from Malta. A full description with illustrations of the macro- and micromorphological characters, as well as its phylogenetic position is provided. This species differs from *Coprinellus aureogranulatus* by the large pleurocystidia, the narrower spores and multidigitate caulocystidia. Some species from sect. *Domestici* are discussed and compared.

Keywords
Agaricales, Psathyrellaceae, *Aureogranulati*, morphology, taxonomy

Introduction

Following a recent clearing of a small area in Buskett (Siggiewi, Malta) several large dead branches of *Ceratonia siliqua* L. were torn down to smaller pieces and dispersed above the soil together with other dead branches from *Quercus ilex* L. The sudden abundance, on the soil, of degraded lignicolous material coupled with abundant rain in the early weeks of October 2020 has resulted in a number of fast growing coprinii appearing over a few days towards the end of October. The list of lignicolous fungi noted in the area were both previously encountered as well as new records for the area and include *Parasola conopilea* (Fr.) Örstadius & E. Larss., *Coprinopsis melanthina* (Fr.) Örstadius & E. Larss., *Coprinellus radians* (Desm.) Vilgalys, Hopple & Jacq. Johnson, *Coprinellus subdisseminatus* (M. Lange) Redhead, Vilgalys & Moncalvo, *Coprinellus xanthothrix* (Romagn.) Vilgalys, Hopple & Jacq. Johnson as well as an unidentified species of *Coprinellus* with yellow-brown coloured velar remnants. The macromorphology of the collected specimens appeared similar to *Coprinellus aureogranulatus* (Uljé & Aptroot) Redhead, Vilgalys & Moncalvo (A. Melzer, pers. comm.) but micromorphology was different (Uljé et al., 1998; Melzer et al., 2016).

The genus *Coprinellus* in the family *Psathyrellaceae* consists of small to large, dark spored, saprotrophic basidiomycete fungi growing on soil, wood and herbivore dung (Nagy et al., 2012; Hussain et al., 2018). The genus has a worldwide distribution with more than 80 species listed in Index fungorum (http://www.indexfungorum.org/). *Coprinellus* has recently been re-evaluated and split into nine different sections based on morphological characters supported by phylogenetic data (Wächter and Melzer, 2020). *Coprinellus aureogranulatus* was originally placed in *Coprinus* subsect. *Setulosi* J.E. Lange by Uljé et al. (1998) based on the presence of pileocystidia, and later in *Coprinellus* sect.

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Micacei (Fr.) D.J. Schaf. by Huang and Bau (2018) but in a recent revision of the *Psathyrellaceae* by Wächter and Melzer (2020) a separate section was created in *Coprinellus* to accommodate *C. aureogranulatus* in sect. *Aureogranulati* Wächt. & A. Melzer. In the present study morphological and phylogenetic studies were conducted to characterize *Coprinellus andreorum* sp. nov.

Materials and Methods

Specimens

Fresh specimens were collected from Buskett (Siggiewi, Malta) on dead branches in a humid environment at 194 m asl in an open area between broadleaf trees. The specimens were dried at 40°C for several hours, over 2 days. A small branch, from which the original specimen was collected, was transferred to a moist chamber (100% relative humidity) at room temperature and a further 3 batches of the same species were collected. The exsiccate were deposited in the Herbarium Senckenbergianum Görlitz (GLM F127896).

Morphological studies

Fresh samples were examined with a light microscope, a few hours after collection. Specimens in the moisture chamber were examined over several hours during growth. Hand cut thin sections were examined microscopically by squash mounts. Pleurocystidia were observed in living specimens, during growth, with a hand lens. Fresh material was examined in water, Congo Red/Phloxine stain (1%) in 5% ammonia solution and toluidine blue (1%).

Dried material was re-examined after wetting with 10% ammonia and treated similarly to fresh material. All measurements were taken on fresh material mounted in water with the aid of Piximètre (downloadable from http://ach.log.free.fr/Piximetre/). Measurements are given on the basis of N number of spores as indicated in each entry using free spores in the mounts. The following abbreviations are used in text L – spore length, W – spore width, B – spore width in side view, Q1 = L/W, Q2 = L/B, Me - mean of L & W.

DNA extraction, amplification and sequencing

DNA extraction, amplification and sequencing of the fungus were performed by Alvalab (Oviedo, Spain) using protocols previously reported (Alvarado et al., 2018; Sammut et al., 2019). The genomic DNA was extracted from dried fruiting bodies and amplification was carried out with the ITS1F-ITS4 primer pair (White et al., 1990). The following molecular phylogenetic markers were used for the phylogenetic analysis: ITS1, 5.8S, ITS2. The nucleotide sequences for the tree inference were taken from NCBI (https://www.ncbi.nlm.nih.gov) (Supplementary Table S1). As outgroup, a set of *Narcissea patouillardii* s.l. sequences were selected.

The initial alignment of the ITS region was performed with MAFFT v7.450 (Katoh and Standley, 2013) using the L-INS-i method and corrected manually. The final maximum likelihood analysis was done with PhyML v3.3.20180621 (Guindon et al., 2010), applying the GTR substitution model and 1000 ML bootstrap inferences were calculated. Of these, 1000 trees were sampled and the best tree was labeled with the ML bootstrap support values over 70%.

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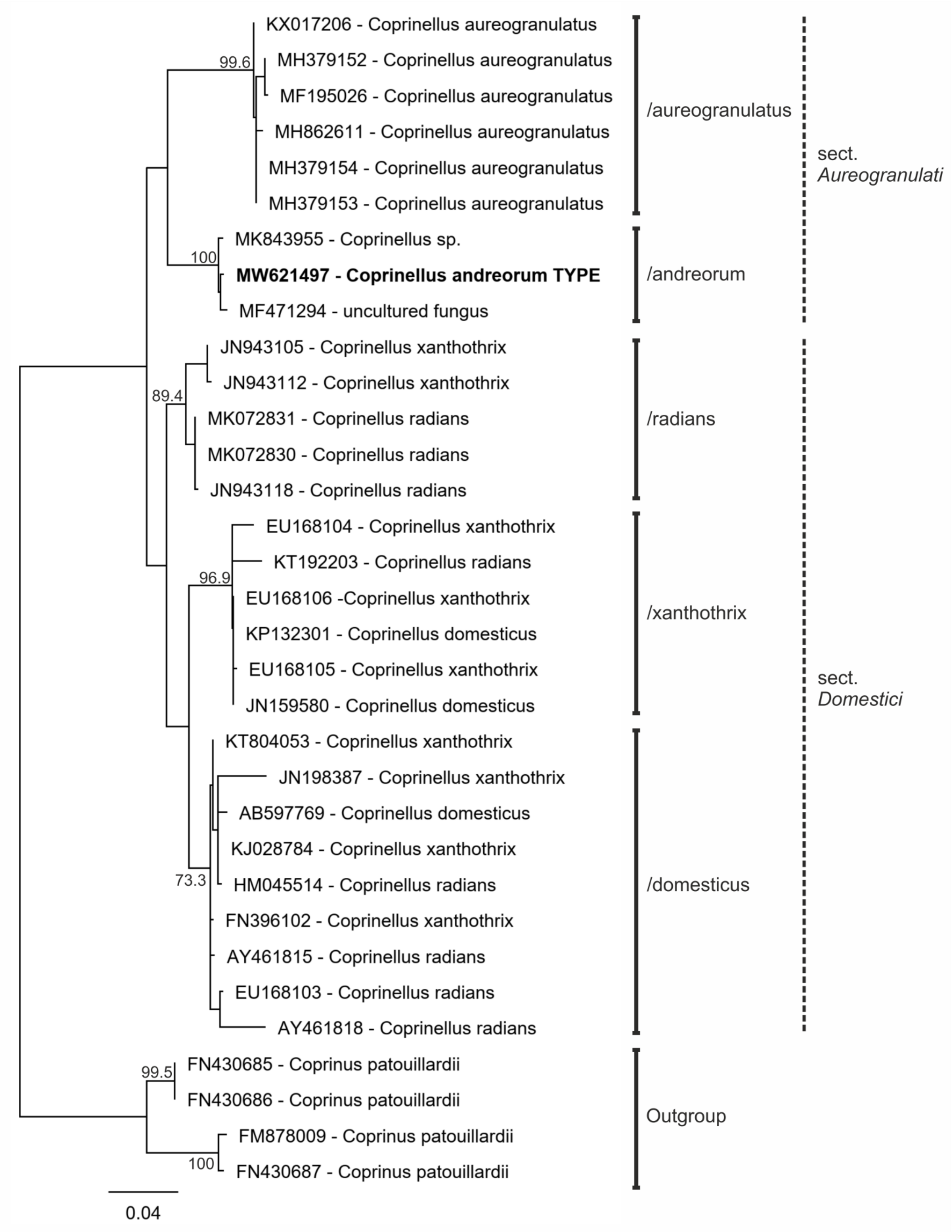

Fig 1. Maximum Likelihood tree based on selected ITS-sequences from specimen of section *Aureogranulati* and *Domestici* applying the GTR-substitution model in PhyML (Guindon et al., 2010). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Only bootstrap support values over 70% are depicted in the tree.

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Taxonomy

Coprinellus andreorum Sammut & Karich *sp. nov.* - Mycobank - MB 838887. Figs. 1, 2, 3; Supplementary Table S2.

Diagnosis: Differs from *C. aureogranulatus*, the closest species, by the voluminous pleurocystidia, narrower spores and multi-furcated caulosystidia.

Typification: MALTA: Siggiewi, Buskett, 35° 51.375N, 14° 23.939E, 194 m asl, on a dead corticated branch of *Ceratonia siliqua* L. on the ground, 29 October 2020, leg. C. Sammut, CS1247 (GLM-F127896); GenBank ITS sequence MW621497, GenBank LSU sequence MW621007, GenBank Tef-1α sequence MW633114.

Etymology: The specific epithet is derived from the name Andreas/André and is dedicated to Andreas Melzer whose collaboration over several years is much appreciated and to André Sammut who accompanied, collected and encouraged the first author on a number of forays.

Pileus 9 – 20 mm diameter when closed, up to 45 mm wide when expanded, campanulate when young to convex to applanate with uprolled margin, initially pale yellow-brown with ochraceous centre changing to pale greyish brown with ochraceous centre as the specimen matures, sulcate striate to almost centre; velar remnants scattered, cream coloured with distinct erect rusty brown tufts. Lamellae L= c. 40 – 60, l= 1 – 3, free, narrow, initially white changing to black, deliquescent. Stipe 30 – 40 x 1 – 3 mm, central, white with visible cystidia; sub-bulbous at base, often with tufts of rust-coloured fibrils 1–3 mm above attachment point, with rust coloured ozonium loosely attached to substrate. Spore print red-brown.

Spores 7.4 – 10.0 × 4.3 – 5.7 x 4.0 – 5.2 µm, ellipsoid in front view, phaseoliform in side view, medium red-brown with rounded base, with central germ pore, 1.6 – 2.0 µm in diameter; apiculus very small; Q1 = 1.59 – 1.92; Q2 = 1.62 – 1.98; N = 30; av. Q1 = 1.75, av Q2 = 1.82; av. L = 7.7 – 8.8 µm, av. W = 4.97 µm, av. B = 4.73 µm. Basidia 17 – 28 x 6.5 – 9 µm, tetrasporic. Pleurocystidia 55 – 110 x 30 – 45 µm, abundant in immature specimens (visible) but rapidly dissolve, ellipsoid to ovoid to subcylindric. Cheilocystidia 40 – 60 x 18 – 26 (base) x 6 – 8 µm (tip), lageniform. Pileipellis composed of a layer of globose cells 20 – 50 µm in diameter and lageniform pileocystidia 65 – 110 x 16 – 31 µm with 4 – 8 µm wide cylindrical necks. Caulocystidia 75 – 180 x 15 – 21 µm with cylindrical neck of 4 – 9 µm at the tips, roughly lageniform but multidigitate (branched) 1–4 digits (2 most common). Veil 15 – 60 x 5 – 20 µm made up of thick walled chains of encrusted brownish coloured subcylindrical or fusoid elements with sub-globose 25 – 50 x 15 – 25 µm terminal elements. Spherical elements 20 – 45 µm in diameter present. Mycelial threads are yellow brown, thick walled (up to 1 µm) filaments, 3 – 5 µm in diameter. Clamps absent.

Habitat: on dead corticated branches of *Ceratonia siliqua* L., scattered, recently cleared open area near *Ceratonia siliqua* L. and *Quercus ilex* L., Buskett, 194 m asl, CS1247, 29/10/20, leg. C. Sammut.

Additional collections examined (cultivated from same branch in moisture chamber on subsequent days): CS1254 (3/11/20), CS1255 (7/11/20) and CS1256 (12/11/20).

Fig. 2. a-m *Coprinellus andreorum* Sammut & Karich sp. nov. a-e – holotype (CS1247); f-g – primordia and ozonium; h, j – isotype (CS1254); i – isotype (CS1255); k-m – isotype (CS1256). Bar f, i - l – 10 mm; g, h, m – 5 mm

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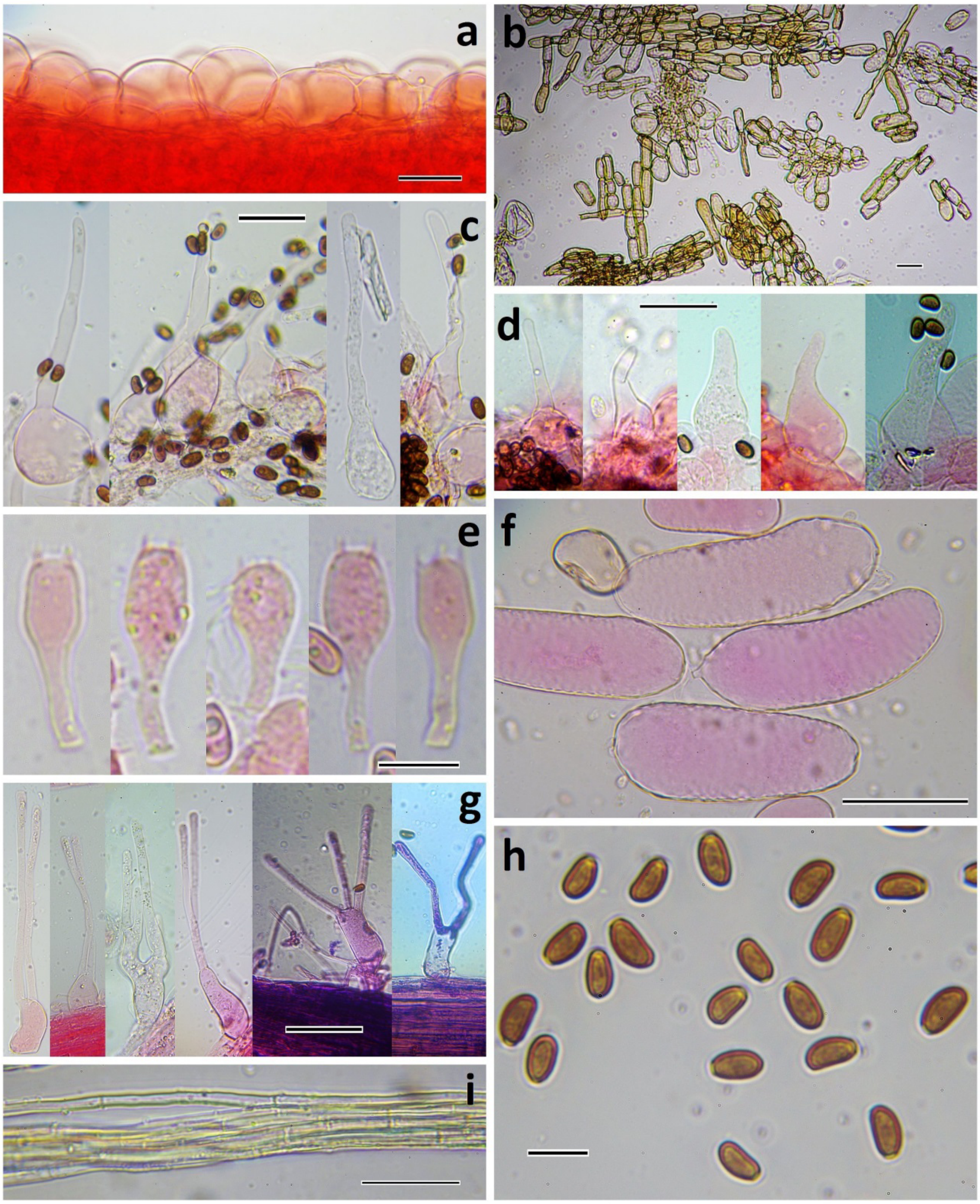

Fig. 3. a-i *Coprinellus andreorum* Sammut & Karich sp. nov. Microscopy: a) pileipellis, b) velar remnants, c) pileocystidia, d) cheilocystidia, e) basidia, f) pleurocystidia, g) caulocystidia, h) spores, i) mycelial hyphae. Scale bar a, b, c, d, i – 25µm; e, h – 10µm; f, g - 50µm. Staining: a, c-f, – Congo Red/Phloxine; b, h, i – unstained; g – 1st set Congo Red/Phloxine, 2nd set – Toluidine blue.

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Results and Discussion

Molecular and morphological studies support the assignment of this fungus to the genus *Coprinellus* sect. *Aureogranulati* Wächt. & A. Melzer as defined in Wächter and Melzer (2020). ITS sequence showed 99.38% and 98.82% similarity with those deposited in GenBank with the accession numbers MK843955 and MF471294, respectively. These accessions form a well-supported clade with the specimen analyzed in this study (Fig. 1). MK843955 corresponds to the voucher BLBS 107 from Brazil (Schünemann, 2019), named as *Coprinellus* sp. MF471294 correspond to the voucher UCUE PC M 22M23 from Ecuador, reported with a best match to AY461815 [voucher SFSU DEH1026 - *Coprinellus radians* (Desm.) Vilgalys, Hopple & Jacq. Johnson] in Maldonado et al. (2020). However, the similarity between these ITS sequences (MF471294 and AY461815) is relatively low (95%) and the identification of the match was not based on a phylogenetic approach (see below).

Coprinellus andreorum and *C. aureogranulatus* are the only known representatives of the section *Aureogranulati* Wächt. & A. Melzer. Their most important characteristics are an ozonium (mycelium mat), a well-developed veil consisting of chains of thick-walled cells as well as the presence of pleuro- and pileocystidia (Wächter and Melzer, 2020). These are rare species, of which there are only a few reports despite their conspicuous habit. *Coprinellus aureogranulatus* was found, in addition to the isotype find in Papua New Guinea (Uljé et al., 1998), so far in Sri Lanka (Dona et al., 2019), India (Priyamvada et al., 2015, 2017; Singh et al., 2017), China (Huang and Bau, 2018), in the Philippines (Lopez et al., 2016), São Tomé (Desjardin and Perry, 2016), and the Seychelles (Melzer et al., 2016). Overall, the main distribution seems to be in areas with high humidity, preferably in tropical or subtropical climates (Fig. 4).

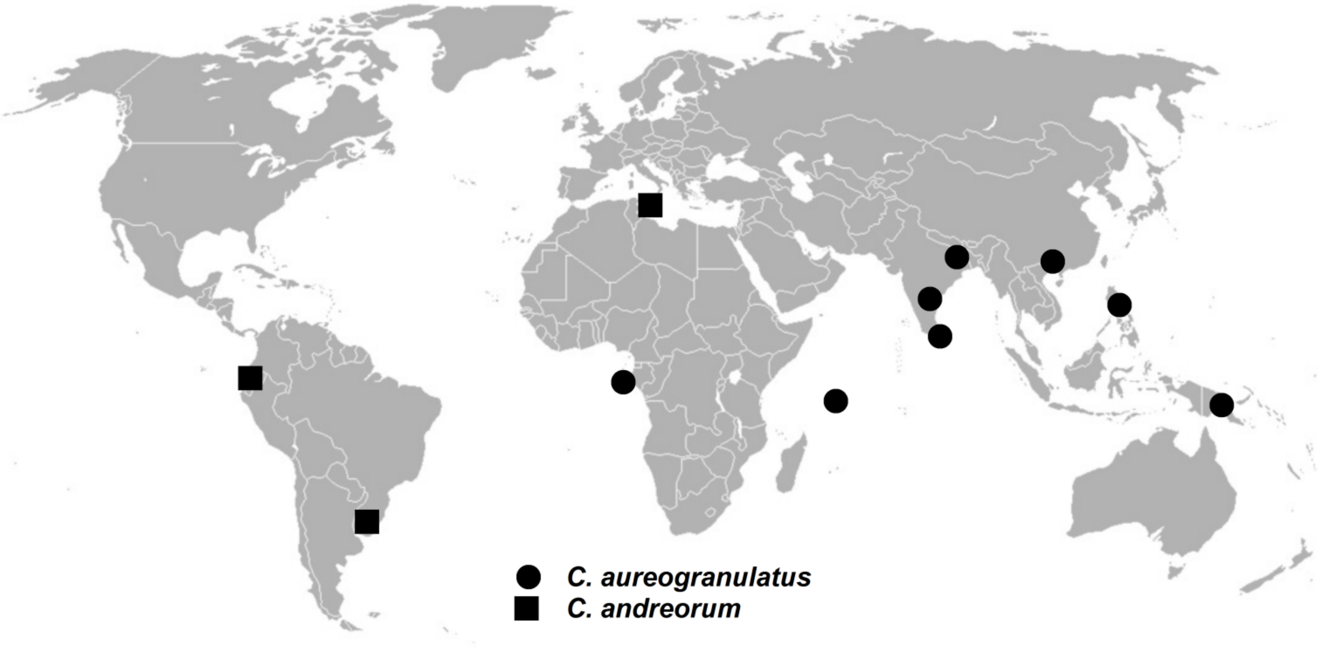

Fig. 4. Distribution of *Coprinellus aureogranulatus* and *C. andreorum*.

The two species in section *Aureogranulati* have many similar macroscopic and microscopic features, so identification might prove difficult without examination of a comprehensive set of characteristics. *Coprinellus andreorum* can be differentiated by the voluminous pleurocystidia, narrower spores and multiply furcated caulocystidia, and possibly by the presence of a more robust velum. Of course, the constancy of these characteristics should be checked in future finds. Neither Schünemann (2019) nor Maldonado et al. (2020) contain descriptions and hence the variability of *C. andreorum* cannot be established at this point, but a reliable determination is currently guaranteed by sequencing of ITS regions. However, there is no doubt about the status of this taxon as an independent species.

The section *Domestici* (Singer) D. J. Schaf. is very closely related. This section contains some species, which sometimes have an ozonium, like *Coprinellus domesticus* (Bolton) Vilgalys, Hopple & Jacq. Johnson, *Coprinellus ellisii* (P. D. Orton) Redhead, Vilgalys & Moncalvo and *Coprinellus radians* (Desm.) Vilgalys, Hopple & Jacq. Johnson. Furthermore, the velum is strongly developed and brownish pigmented. From a macroscopic point of view, confusion with older specimens of *C. andreorum* is quite possible.

Overall, the section *Domestici* is still very incompletely studied; there are major problems with determination and divergent species concepts, especially with *C. radians* (Desm.) Vilgalys, Hopple & Jacq. Johnson and *C. xanthothrix* (Romagn.) Vilgalys, Hopple & Jacq. Johnson. A case in point is highlighted above for AY461815 (Keirle et al., 2004) identified as *Coprinellus radians* but is in reality *Coprinellus domesticus* (compare Wächter and Melzer 2020). With the help of microscopic features, however, the delimitation of all species from the section *Aureogranulati* is unproblematic, because pileocystidia are absent in the section *Domestici*. At the present time confusion with other species is not possible.

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