



Cyathus lignilantanae sp. nov., a new species of bird's nest fungi (Basidiomycota) from Cape Verde Archipelago

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Abstract

Cyathus lignilantanae sp. nov. is described and illustrated on the basis of morphological and molecular data. Specimens were collected on Santiago Island (Cape Verde), growing on woody debris of *Lantana camara*. Affinities with other species of the genus are discussed.

Resumen

Sobre la base de datos morfológicos y moleculares se describe e ilustra *Cyathus lignilantanae* sp. nov. Los especímenes se recolectaron en la isla de Santiago (Cabo Verde), creciendo sobre restos leñosos de *Lantana camara*. Se discuten las afinidades de esta especie con las del resto del género.

Key words: biodiversity hotspot, Sierra Malagueta Natural Park, gasteromycetes, Agaricales, Nidulariaceae, ITS nrDNA, taxonomy

Introduction

The Cape Verde archipelago is situated in the Atlantic ocean (14°50'–17°20'N, 22°40'–25°30'W), about 750 km off the Senegalese coast (Africa), and is formed by 10 islands (approximately 4033 km²), discovered and colonized by Portuguese explorers in the 15th century. The islands are of volcanic origin and the Pico of Fogo (2829 m) is the largest active volcano in the region. Due to the proximity of the Sahara, the islands are dry, but in those with steep mountains, the humidity is much higher providing a rainforest habitat. Santiago is the largest island with an area of 991 km²; it is dominated by two volcanic mountains: the arch of the Serra da Malagueta (1064 m), in the north, and the Pico d'Antónia (1394 m), at the center of the island.

Geologically Cape Verde archipelago is one of the hotspots of the African plate, a region also considered as a hotspot for diversity of terrestrial (Vasconcelos *et al.* 2012) and marine organisms (Roberts *et al.* 2002). Cape Verde Islands have a number of endemic species of birds (e.g. *Passer iagoensis*, the Iago Sparrow) and reptiles (e.g. *Tarentola gigas*, Cape Verde giant gecko), many of which are endangered by human development (BirdLife International 2015, World Wild Life 2015). At least 92 species of vascular plants are reported as endemic from a total of 659 (Frodin 2001). However, there are few papers on fungi from Cape Verde; some exceptions are Gjaerum (1984), that cites 23 species of rust fungi found in Macaronesia, twelve of them new to Cape Verde; Eckblad & Brochman (1988) that mentions 12 species of gasteroid and secotiod fungi; and Mies (1993) that has a checklist with 370 lichenized and lichenicolous Ascomycota. Even in the global overview of wild edible fungi and their use and importance to people published by Boa (2004), the Cape Verde Islands were one of the few areas that the author mentioned for which there is not information. Recently, new lichenized fungi have been described, three in the genus *Rinodina* (Giralt & van den Boom 2008) and one in the genus *Plectocarpon* (Ert & van den Boom 2012).

During a survey of the corticiaceous fungi from Cape Verde, small branches of *Lantana camara* L. were collected

covered with basidiomes of a Nidulariaceae fungus (Agaricales, Basidiomycota; Matheny *et al.* 2006) from the genus *Cyathus* Haller (1768: 127).

Occurring in several climates and biomes (Brodie 1975, Lloyd 1906), the family Nidulariaceae is composed of five genera of small fungi exhibiting resistant discoid structures called peridioles, where the spores are produced. The genera *Crucibulum* Tulasne & C. Tulasne (1844: 89), *Mycocalia* J.T. Palmer (1961: 58), *Nidula* V.S. White (1902: 271) and *Nidularia* Fries & Nordholm (1817: 2) are recurrent in North and South temperate areas, but rare in tropical countries (Cunningham 1944, Brodie 1975). However, the species of *Cyathus* are very frequent in tropical countries with hot climates (Brodie 1975; Gómez & Pérez-Silva 1988a; Zhao *et al.* 2007; Zhao *et al.* 2008).

Brodie (1975) accepted 43 species into the genus *Cyathus* and distributed them in seven infrageneric groups according to a number of characters, such as whether the peridia are plicate or not. Zhao *et al.* (2007), analyzed ITS and LSU to confirm whether Brodie's morphological groups are well supported; for example, the plications of the peridium appear not to be a phylogenetically informative character. Zhao *et al.* (2007) distributes the species in three clades. The combination of fruit-body coloration, the peridium anatomy and the spore size are the morphological characters that segregate *Cyathus* into the *ollum* group, the *pallidum* group and the *striatum* group.

Since Brodie's paper, or excluded for any reason before this year, 30 new *Cyathus* taxa have been described: *C. discoideus* M. Zang (1980: 33), *C. wutaishanensis* B. Liu, Shangguan & P.G. Yuan (1984: 196), *C. wutaishanensis* var. *yuanguensis* B. Liu & Y.M. Li (1989b: 293), *C. tianshanensis* B. Liu & J.Z. Cao (1987: 316), *C. tianshanensis* var. *tomentosus* B. Liu, J.Z. Cao & Y.M. Li (1989: 109), *C. discostipitatus* B. Liu & Y.M. Li (1989a: 104), *C. hirtulus* B. Liu & Y.M. Li (1989a: 108), *C. taiyuanensis* B. Liu & Y.M. Li (1989a: 105), *C. neimonggolensis* B. Liu & Y.M. Li (1989a: 101), *C. guandishanensis* B. Liu & Y.M. Li (1989b: 290), *C. sichuanensis* B. Liu & Y.M. Li (1989b: 289), *C. yunnanensis* B. Liu & Y.M. Li (1989b: 292), *C. annulatus* var. *wuyishanensis* B. Liu & Y.M. Li (1989a: 110), *C. cornucopioides* T.X. Zhou & W. Ren (1992: 23), *C. novae-zeelandiae* var. *ovisporus* W. Ren & T.X. Zhou (1992: 26), *C. luxiensis* T.X. Zhou, J. Yu & Y.H. Chen (2003: 345), *C. jiayuguanensis* J. Yu, T.X. Zhou & L.Z. Zhao (2004: 243), *C. lijiangensis* T.X. Zhou & R.L. Zhao (2004: 243) and *C. renweii* T.X. Zhou & R.L. Zhao (2004: 245), from China; *C. subglobisporus* R.L. Zhao, Desjardin & K.D. Hyde (2008: 74) from Thailand; *C. morelensis* C.L. Gómez & Pérez-Silva (1988b: 419) from Mexico; *C. stercoreus* f. *halepensis* Mornand (1992: 8) from France; *C. stercoreus* f. *ephedrae* Calonge (1994: 33); *C. olla* f. *brodiensis* T.C. Shinnars & J.P. Tewari (1998: 986) from Canada; *C. amazonicus* Trierweiler-Pereira & Baseia (2009: 74) from Brazilian Amazon forest; *C. calvescens* R. Cruz & Baseia (2014: 174), *C. hortensis* R. Cruz & Baseia (2014: 175), *C. magnomuralis* R. Cruz & Baseia (2014: 177), and *C. parvocinereus* R. Cruz & Baseia (2014: 179), from semi-arid region of Brazil; and *C. thindii* K. Das, Hembrom, A. Paribar & R.L. Zhao (2015: 2). Moreover, besides these new species, the new combination *C. lanatus* (H.J. Brodie 1978a: 32) R.L. Zhao (2007: 394), from USA, the "nomen novum" *C. annictaris* Lloyd (1975: 380) of the previous invalid species *Cyathus fimicola* Lloyd (Stevenson & Cash 1936), and the synonymization of the species *C. gallicus* Mornand (1985: 39), *C. cheliensis* F.L. Tai & Hung (1948: 39), *C. megasporus* W. Ren & T.X. Zhou (1992: 25), *C. gansuensis* B. Yang, J. Yu & T.X. Zhou (2002: 313) and *C. africanus* var. *latisporus* (2003: 346) [now *Crucibulum laeve* f. *gallicum* (Mornand) Mornand (1986: 18), *Cyathus limbatus* Tul. & C. Tul. (1844: 78), *Cyathus poeppigii* Tul. & C. Tul. (1844: 77), *Cyathus pygmaeus* Lloyd (1906: 26), and *Cyathus jiayuguanensis* J. Yu, T.X. Zhou & L.Z. (2002: 314), respectively] has been published.

In Kreisel's (2001) checklist of gasteroid and secotiod fungi, 474 taxa were recorded; among them, 14 *Cyathus* species were registered to West, Central and South Africa, although none from Cape Verde: *C. africanus* H.J. Brodie (1967b: 45), *C. berkeleyanus* (Tul. & C. Tul.) Lloyd (1906: 19), *C. crispus* H.J. Brodie (1974: 1661), *C. limbatus* Tul. & C. Tul. (1844: 78), *C. microsporus* Tul. & C. Tul. (1844: 73), *C. montagnei* Tul. & C. Tul. (1844: 70), *C. nigroalbus* Lloyd (1906: 18), *C. olla* (Batsch) Pers. (1801: 237), *C. pallidus* Berk. & M.A. Curtis (1868: 346), *C. poeppigii*, *C. rudis* Pat. (1924: 532), *C. stercoreus* (Schwein.) De Toni (1888: 40), *C. striatus* (Huds.) Willd. (1787: 399) and *C. triplex* Lloyd (1906: 23). Subsequent works have not pointed to species of *Cyathus* in the Cape Verde Archipelago, neither the common tropical *C. poeppigii* mentioned in many previous papers (Dissing & Lange 1962, Dring 1964, Dring & Rayner 1967, Demoulin & Dring 1975), nor *C. limbatus*, also distributed in Africa (Dissing & Lange 1962, Dring 1964, Demoulin & Dring 1975), nor *C. striatus* (Calonge & Daniëls 1998), which is well-cited around the world.

The aim of this study was to identify and describe *Cyathus* specimens collected in Cape Verde on *Lantana camara* L., using morphological and molecular data. As a result a new species is proposed and described.

Materials and Methods

Taxon sampling and morphological studies:—Two collections of *Cyathus* from Cape Verde were studied. Vouchers are deposited in MA-Fungi herbarium. Field photographs of fresh basidiomata were taken with an Olympus μ Tough camera. Morphological characters were observed macroscopically and microscopically. Colour codes and terms are mostly after Methuen Handbook of Colour (Kornerup & Wanscher 1978). Macromorphological features were analyzed using a stereomicroscope Nikon SMZ1500. Micromorphological features were recorded using a 40 \times objective (magnification of 400 \times) in an optical microscope Nikon eclipse Ni, from the dry samples mounted in 5% KOH. Microphotographs were taken with a dedicated camera Nikon DS-Ri1 and software NIS-Elements AR. Spore measurements are recorded based on thirty basidiospores. Spores are measured in side view. Spore measurements and length/width ratios (Q) are presented as: minimum–maximum and mean (Qm). Spore shapes from the Qm value were in accordance with those proposed by Bas (1969). Herbarium names are after Holmgren *et al.* (1990). The identification follows the key provided by Brodie (1984) for a preliminary species determination, followed by a point-to-point analysis of the morphological features of the Cape Verde specimen compared to the species determined by following the identification key.

In addition, the Holotype of *Cyathus poeppigii* and specimens of the type collection of *C. limbatus* were examined, from the PC Herbarium (National Museum of Natural History—France). All morphological analysis followed the same methodology used in the Cape Verde samples. We could not analyze the Holotype of *Cyathus pedicellatus* due to specific restrictions for the loan.

Molecular analysis:—Genomic DNA was extracted from peridioles of the two collections using DNeasy™ Plant Mini Kit (Qiagen, Valencia, CA), following the manufacturer's instructions, except that lysis buffer incubation was overnight at 55 °C. The primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) were used to amplify the ITS region, both ITS1 and ITS2, including the 5.8S ribosomal RNA cluster and flanking parts of the small subunit (SSU) and large subunit (LSU) nuclear ribosomal gene. Individual reactions were carried out using illustra™ PureTaq™ Ready-To-Go™ PCR Beads (GE Healthcare, Buckinghamshire, UK) as described in Winka *et al.* (1998), following thermal cycling conditions in Martín & Winka (2000). Before sequencing, 20 μ l of the amplification products were cleaned using QIAquick Gel PCR Purification (Qiagen); both strands were sequenced separately at Macrogen (Seoul, Korea), with the primers used in the amplification. Although attempts were made, no sequence was obtained from the samples deposited at the PC Herbarium, probably because the dry specimens were from the first half of the nineteenth century and their DNA was degraded by age.

Sequences were compared with homologous *Cyathus* sequences published in Zhao *et al.* (2007). The alignment was optimized visually. Alignment gaps were indicated as “-” and ambiguous nucleotides were marked as “N”. Two sequences were included as outgroup: *Nidula niveotomentosa* (DQ463358) and *Crucibulum laeve* DQ463357). 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 when reaching 100 trees. Exhaustive searches were conducted without rooting the tree and gaps treated as missing data. To assess homoplasy levels, consistency index (CI) and retention index (RI) were calculated from each exhaustive search. Non-parametric searches (Felsenstein 1985) were used to calculate branch support (MPbs), performing 10000 replicates using the fast-step option. The phylogenetic tree was viewed with FigTree v. 1.3.1 (<http://tree.bio.ed.ac.uk/software/figtree/>) and edited with Adobe Illustrator CS3 v. 11.0.2 (Adobe Systems). A maximum likelihood (ML) analyses was conducted also in PAUP* 4.0b10; as in the MP analysis, 10000 non-parametric bootstrap using the fast-step option was performed to assess branch support (MLbs). Kimura-2-parameter (K2P) pairwise distances were obtained using PAUP* 4.0b10, and a dendrogram was constructed using a neighbor-joining method.

The new consensus sequences have been lodged in the EMLB-EBI database with the accession numbers LN824168 (MA-Fungi 87327) and LN824169 (MA-Fungi 87328).

Results

The two ITS nrDNA sequences obtained in this study were aligned with *Cyathus* sequences included in Zhao *et al.* (2007). A matrix of 792 unambiguously aligned nucleotide positions was produced (467 constant, 151 parsimony-uninformative, and 174 parsimony-informative). The 100 most parsimonious trees gave a length of 533 steps, CI = 0.7786, HI = 0.2214 and RI = 0.8345; one of the 100 MPTs is shown in Fig. 1. The strict consensus tree shows the three main groups indicated in Fig. 1.

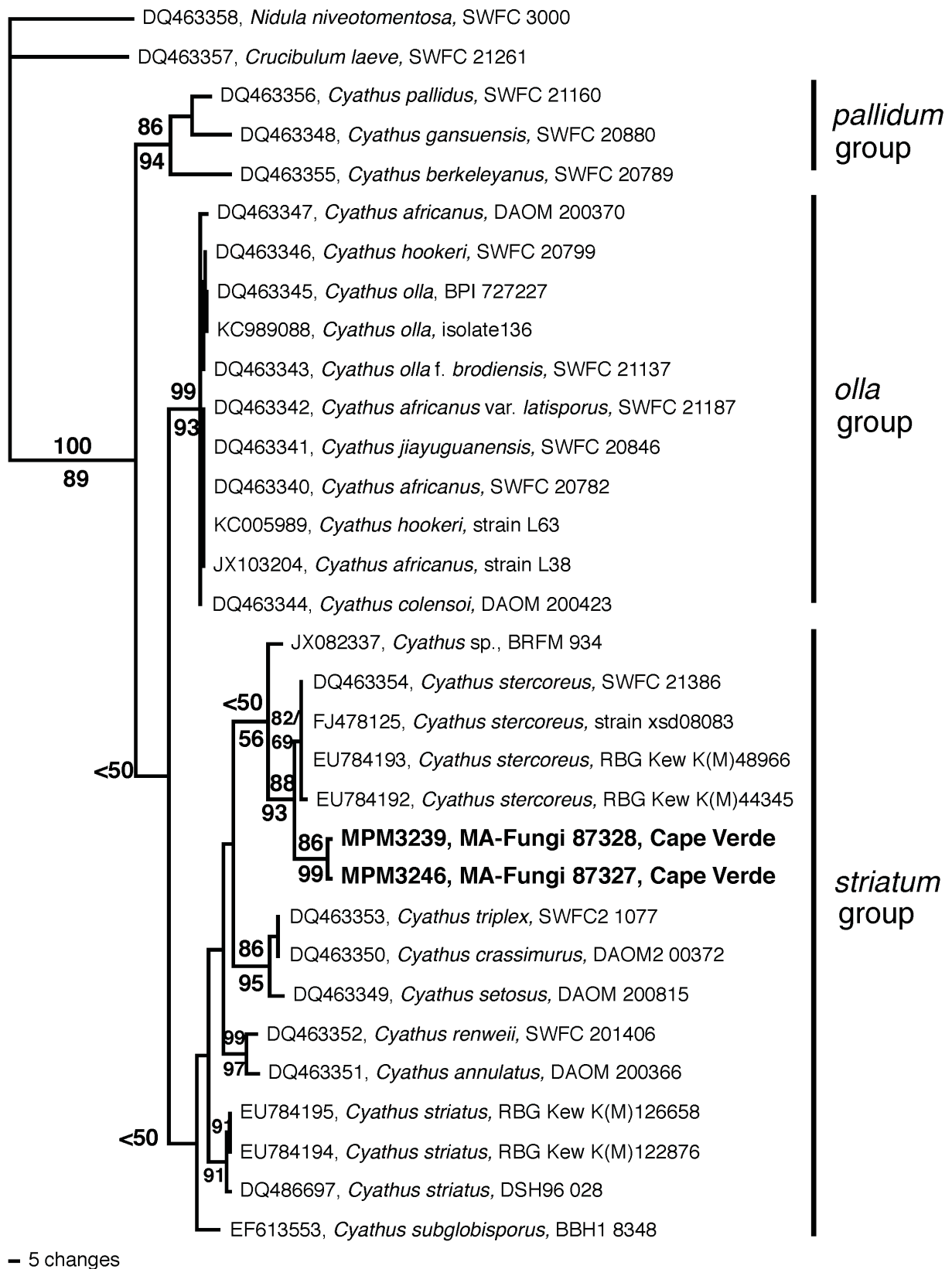


FIGURE 1. One of the 100 MPTs trees for *Cyathus* ITS nrDNA sequences obtained from a heuristic search with PAUP* 4.0b10. Maximum Parsimony bootstrap support ($\geq 50\%$) indicated above branches and Maximum Likelihood bootstrap support ($\geq 50\%$) indicated below branches. Groups according to Zhao *et al.* (2007). Sequences obtained from *Cyathus lignilantanae* Holotype (MA-Fungi 87327) and paratype (MA-Fungi 87328) in bold.

According to the AIC criterion, the substitution model selected by PAUP* 4.0b10 for the maximum likelihood analysis was GTR + I + G. The phylogenetic reconstruction from ML analysis gave three best trees (data not shown), with an overall topology similar to that of the MP strict consensus tree concerning well-supported clades.

The neighbor joining tree obtained from Kimura 2-parameter distances was similar to the MP and ML consensus tree (data not shown), except in the position of *C. triplex* that appeared separated from *C. crassimurus* H.J. Brodie (1971: 1609) and *C. setosus* H.J. Brodie (1967a: 1).

Cyathus species form a monophyletic group with high support (MPbs= 100%; MLbs= 89%), as shown in Fig. 1, are distributed in the three clades mentioned in Zhao *et al.* (2007). The sequences obtained from the two Cape Verde specimens group together (MPbs= 86 %; MLbs= 99 %), in the *striatum* clade, as a sister group of the cluster formed by four sequences of *Cyathus stercoreus*.

Taxonomic Treatment

Cyathus lignilantanae R. Cruz & M.P. Martín, *sp. nov.* (Figs. 2–3)
Mycobank MB811700

Type:—CAPE VERDE, Santiago, St. Domingo, Ruiz Vaz, 15°01'53" N 23°36'33" W, 872 msl, on wooded debris of *Lantana camara*, 12 April 2013, M. Dueñas, M.T. Telleria & M.P. Martín, MPM3246 (holotype, MA-Fungi 87327!)

Diagnosis:—This species has a distinctly plicate peridium, both in external and internal walls, light colored peridioles provided with double layered cortex, spores up to 30 µm in length and its unique ITS sequence.

Peridium infundibuliform (4.6–)6.9–8.2 × (3.9–)5.2–6.9 mm, not expanded at the mouth but tapering at the base, except in small basidiomata of MA-Fungi 87328; conspicuous emplacement 1.7–3.9 mm, reddish brown (6F5 KW or 6F6 KW). Exoperidium hirsute reddish brown (6F5 KW or 6F6 KW), arranged in irregular and flexible tufts, 0.4–0.6 mm, wall conspicuously plicate with 0.3–0.6 mm between the folds. Mouth finely fimbriate, continuum pattern, 0.2–0.4 mm, greyish brown (8F3 KW) to dark brown (7F4 KW). Endoperidium “café-au-lait” or greyish brown (6D3–7F3 KW) to brownish grey (7D2–8E2 KW), conspicuously plicate with 0.2–0.5 mm between the grooves, brightness sometimes contrasting with the exterior, being platinum in the holotype. Peridioles brownish grey (10F2 KW) to greyish brown (10F2–10F3 KW), 2.1–2.3 × 1.8–2.0 mm, circular to elliptical at edges, sometimes irregular to angular; indistinct tunica and double layered cortex. Basidiospores smooth, hyaline, (10.8–)13.0–25.3(–33.1) × (6.9–)9.4–17.0(–19.6) µm, rarely found in microscopic slide, slightly elliptical to elongated (Q = 1.3–2.0), mostly ellipsoid (Qm = 1.5; L = 19.2; W = 12.9), thin wall 1.0–2.1 µm; some spores with apiculus.

Etymology:—The epithet refers to the substrate where this species grows, on woody debris of *Lantana camara*.

Known distribution:—Cape Verde archipelago.

Additional specimens examined:—CAPE VERDE, Santiago, Serra da Malagueta Natural Park, Ruiz Vaz, 15°01'53" N 23°36'33" W, 907 msl, on woody debris of *Lantana camara*, 12 April 2013, M. Dueñas, M.P. Martín & M.T. Telleria, MPM3239 (paratype, MA-Fungi 87328!).

Other specimens examined:—CUBA, on wood debris, 1824 (*Cyathus poeppigii* holotype, PC0167598!; PC017602!). FRENCH GUIANA, 1824, P.A. Poiteau (*Cyathus poeppigii*, PC0167608!). BRAZIL, Rio de Janeiro, 1873 (*Cyathus poeppigii*, PC0167601!). GUADELOUPE, French Antilles, 1873 (*Cyathus poeppigii*, PC0167598!). USA, Organ Mountains, 1873, Gardner (*Cyathus poeppigii*, PC0167606!). MADAGASCAR, Mananara, 02 September 1920, R. Decary (*Cyathus poeppigii*, PC0167607!). BRITISH GUYANA AND SURINAM, 1844, Schomburgk (*Cyathus limbatus* type collection, PC0167583!; PC0167584!; PC0167585!).

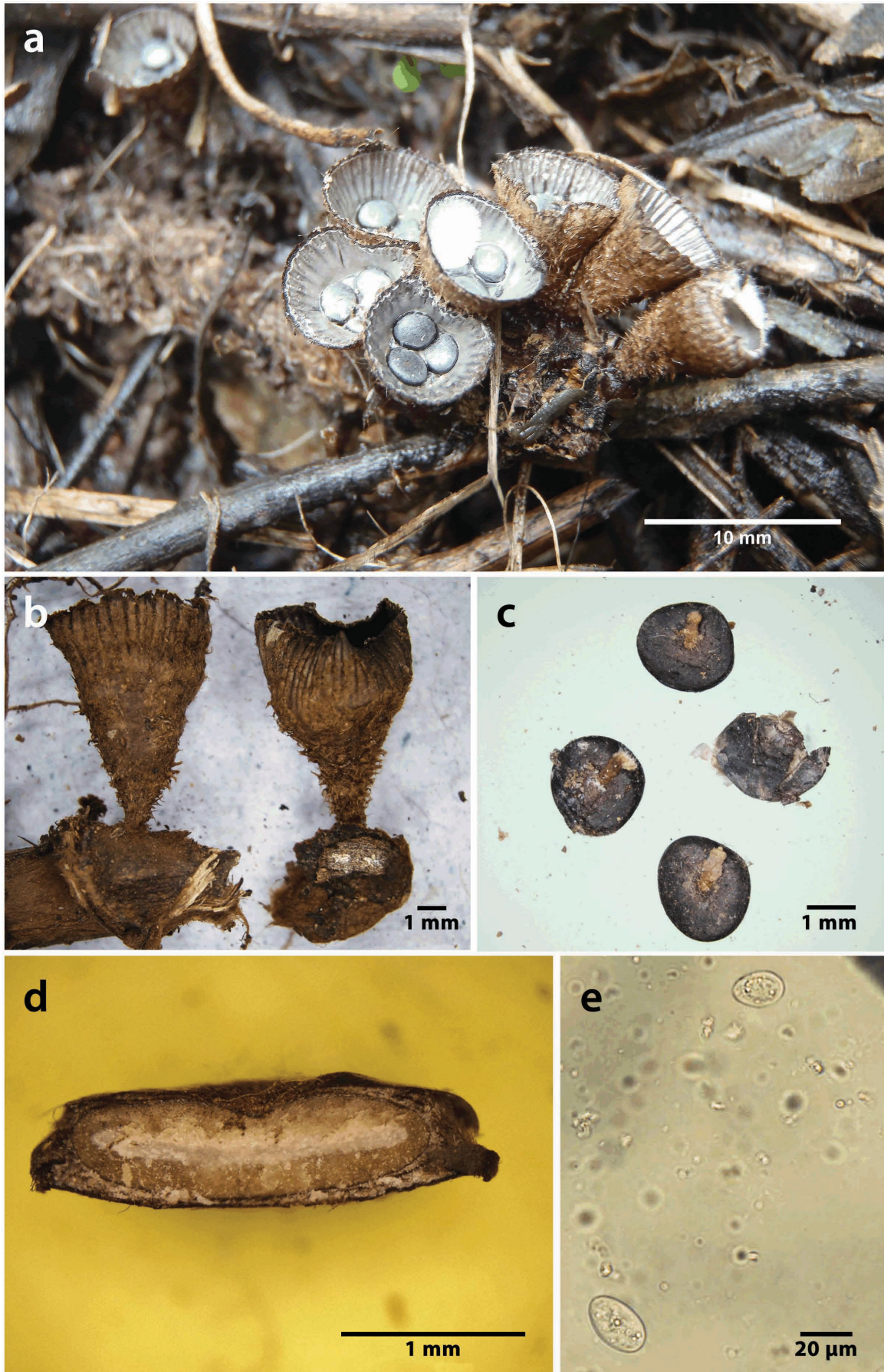


FIGURE 2. *Cyathus lignilantanae* MA-Fungi 87327 (holotype) a. Basidiomata in natural environment. b. Lateral view of basidiomes in stereomicroscope. c. Peridioles. d. Section of peridiole. e. Spores.

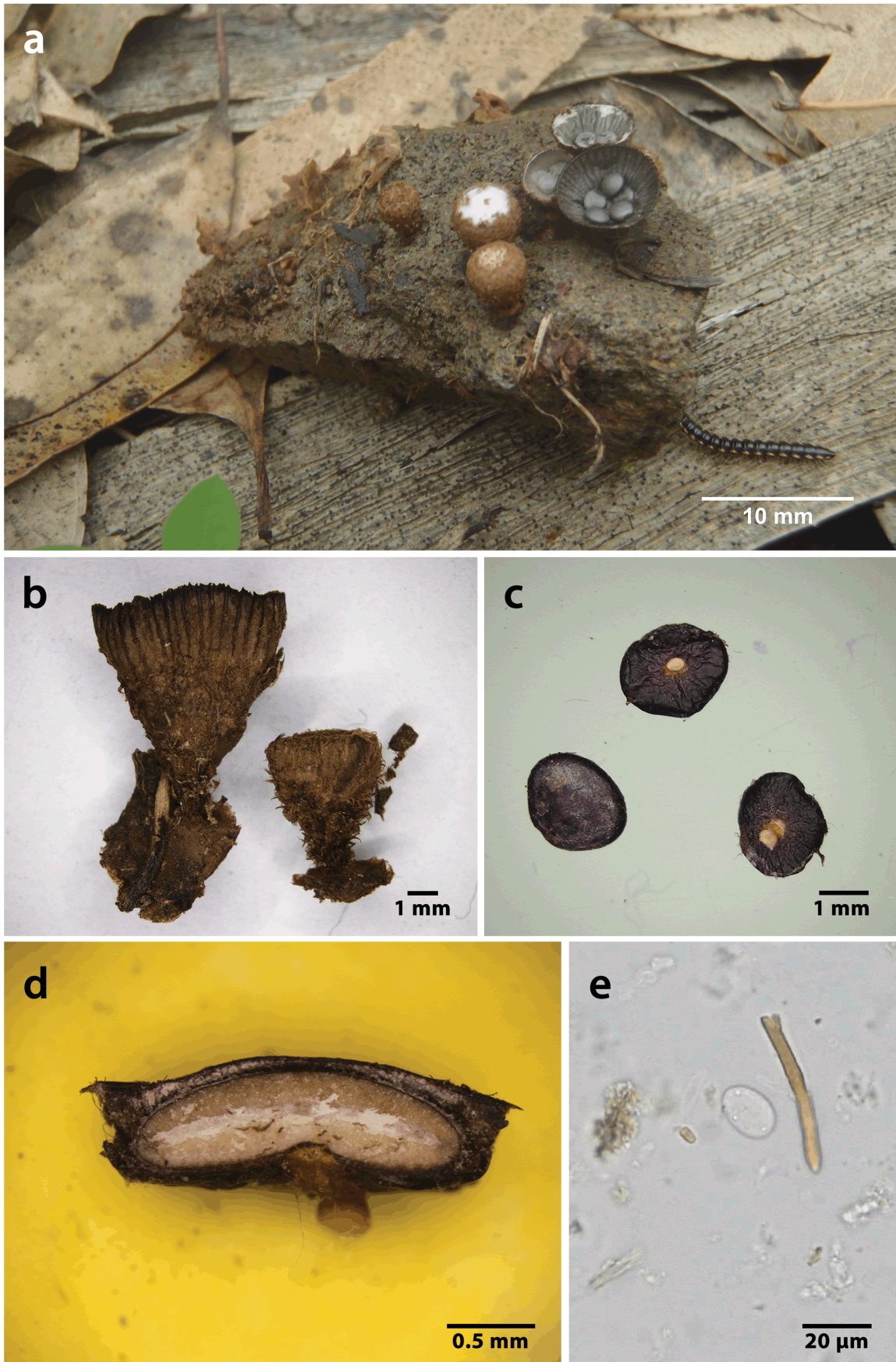


FIGURE 3. *Cyathus lignilantanae* MA-Fungi 87328 (paratype) a. Basidiomata in natural environment. b. Lateral view of basidiomes in stereomicroscope. c. Peridioles. d. Section of peridiole. e. Spore.

TABLE 1. Comparison of morphological diagnostic characters of *Cyathus lignilantanae*, *C. poepigii*, *C. limbatus* and *C. pedicellatus*.

| | <i>C. lignilantanae</i> | <i>C. poepigii</i> | <i>C. limbatus</i> | <i>C. pedicellatus</i> |
|--------------------------|--|--|--|--|
| Herbarium voucher | MA-Fungi 87327 (holotype) | PC0167598 (holotype) | PC0167584 (type collection) | CO-6349 [Brodie (1978) protologue] |
| Basidiome | | | | |
| - sizes | 6.9–8.2 × 5.2–6.9 mm | 6–7.9 × 5–7.1 mm | 8.15 × 7.3 mm | 10–12 × 8–10 mm with distinct pedicel dark brown to black |
| - exoperidium | Hirsute, reddish brown | Hirsute, reddish brown | Woolly to hirsute, brown | Hirsute, very dark brown |
| - tomentum (height) | 0.4–0.6 mm | 0.3–0.6 mm | 0.58–0.78 mm | Not described |
| - exoplication | Conspicuous, 0.3–0.6 mm | Conspicuous, 0.4–0.54 mm | Inconspicuous, 0.65–0.82 mm | Conspicuous, 0.75–1 mm |
| - endoplication | Conspicuous, 0.21–0.48 mm, greyish brown or café-au-lait | Conspicuous, 0.5–0.56 mm, brownish grey or café-au-lait | Inconspicuous, 0.69–0.75 mm, brownish beige | Conspicuous, 0.75–1 mm, color not described |
| Peridioles | | | | |
| - sizes | 2.1–2.3 × 1.8–2 mm | 2–2.2 × 1.8–1.9 mm | 2–2.8 × 1.6–2 mm | 2.5–3 mm |
| - shapes | Circular to elliptical | Circular to elliptical | Angular to elliptical | Circular |
| - colour | Brownish grey to greyish brown | Black | Greyish brown | Not described |
| - cortex | Double cortex | Double cortex | Double cortex | Double cortex ¹ |
| Basidiospores | | | | |
| - sizes | Very rare to found (sometimes one per slide) | Rare to found (but is possible to found 2 or 3 together in many places of the slide) | Many spores per slide | Not described |
| - L and W ratios | (10)13–25.2(33.1) × (6.9) 9.4–17(19.6) µm | 29.47–41.82 × 22.95–33.97 µm | 12–17.2 × 8.4–15.2 µm | 24–27 × 12–14 µm |
| - shapes | L= 19.2 W= 12.9 Slightly elliptical to elongated (Q=1.30–2.00), mostly elliptical (Qm=1.50) | L=37.20 W=26.63 Slightly elliptical to elongated (Q=1.22– 1.81), mostly elliptical (Qm=1.41) | L=14.64 W=11.80 Globose to elliptical (Q= 1.03– 1.60), mostly slightly elliptical (Qm=1.24) | L= 25.5 W=13 ² Ellipsoid, variable in shape, not so much in dimension |
| - wall thickness | 1–2.1 µm | 2.8–5.97 µm | 1.22–3.9 µm | Not described |

¹With doubt, since Brodie (1978) said that the cortex is “probably in reality one layered but lacking in homogeneity”.

²Not included in Brodie (1978), but it has been calculated taking the maximum and minimum size of the spore in this paper.

Discussion

Cyathus lignilantanae has a distinctly plicate peridium, both in external and internal walls, light colored peridioles with double layered cortex, and spores, slightly giant, up to 33 μm in an extreme case (MA-Fungi 87327). According to Brodie's classification (1975) this species could belong to one of the two groups with plicate peridium: group VI (*poeppigii* group; peridia distinctly plicate externally and internally) or group VII (*striatus* group; peridia distinctly plicate internally, sometimes obscurely so externally). However, based on our molecular analyses, *C. lignilantanae* is closely related to *C. stercoreus* that belongs to group V of Brodie's classification (species with peridia not plicate). Moreover, *C. stercoreus* lacks plication, has dark peridioles and globose to subglobose spores (different from slightly elliptical to elongated in *C. lignilantanae*), with the minimum width (17–30 μm) exceeding the maximum value of the new species (9.4–17.0 μm). Also, *C. stercoreus* is found in coprophilous substrate or manure, whereas *C. lignilantanae* occurs on decaying wood. The only morphological similarities between *C. stercoreus* and *C. lignilantanae* are the peridium color, the cortical layer and the spore length, which groups these two species in the *C. striatum* group. According to Zhao *et al.* (2007), in the *C. striatum* group, delimited by the brown, reddish-brown to dark brown peridia, the length of basidiospores are greater than 15 μm .

In Brodie's group VI, according to the size of basidiomata [(4.6)6.9–8.2 \times (3.9)5.2–6.9 mm], size of peridioles (commonly 2 mm in diameter), color of basidiomata (reddish brown) and distance between folds in plication (0.5 mm mean), this species is similar to *C. poeppigii* (Fig. 4a) that also has a double layered cortex [in *C. pedicellatus* protologue of *C. poeppigii* the double layer is not described, but it is drawn on illustrations of Tulasne & Tulasne (1844)]. Although, in *C. lignilantanae* the spores are very rarely found in microscopic slides (sometimes needing to mount more than 10 slides to complete the spore measurements), they are the main features that separate the two species (Table 1). The spores of *C. poeppigii* are slightly elliptical to elongated ($Q = 1.22\text{--}1.81$), mostly elliptical ($Q_m = 1.41$), and very large ($L = 37.20$; $W = 26.63$), 29.47–41.82 $\mu\text{m} \times 22.95\text{--}33.97 \mu\text{m}$, without apiculus; whereas in *C. lignilantanae* the spores are of the same shape ($Q = 1.3\text{--}2.0$; $Q_m = 1.5$), but smaller ($L = 19.2$; $W = 12.9$), 12.2–17.6 \times 8.8–12.0 μm (MA-Fungi 87328) or 13.0–25.3 \times 9.36–17.0 μm (MA-Fungi 87327), with the exception of only one measure of length cited in the description above (33.1 μm), and some of them with terminal apiculus. In Zhao *et al.* (2007) no ITS sequence of *C. poeppigii* was obtained, nor in our study; in our ITS analysis *C. stercoreus* was closer to *C. lignilantanae*, and in the LSU sequence analysis of Zhao *et al.* (2007) also *C. stercoreus* was closer to *C. poeppigii*. However, although barcoding sequences were not available to compare *C. poeppigii* and *C. lignilantanae*, the combination of morphological features included in Table 1 allows differentiation of the two species.

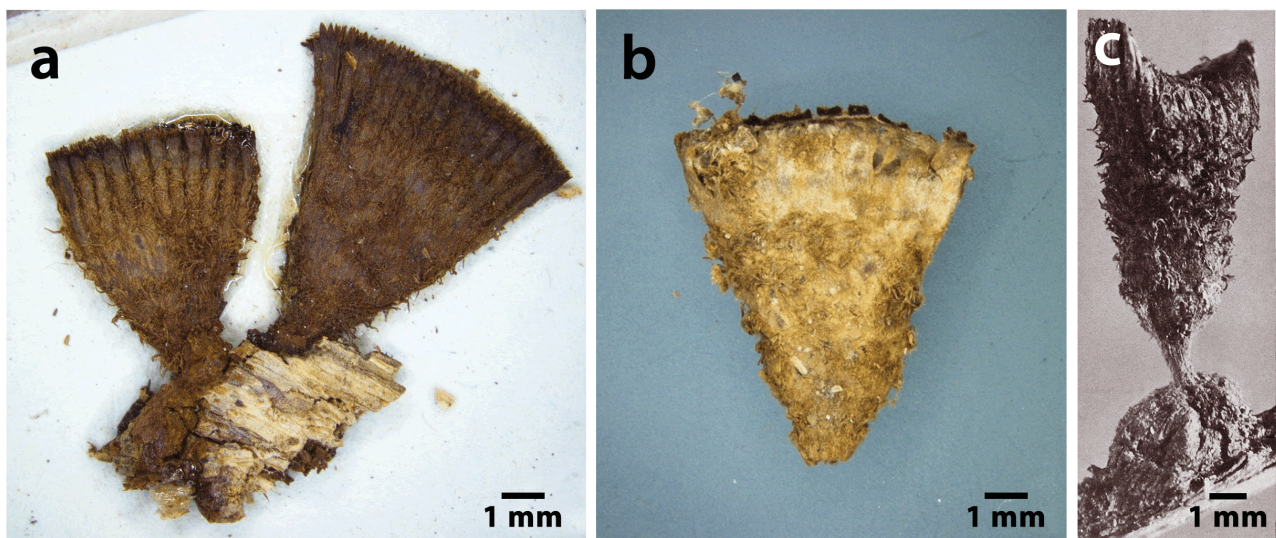


FIGURE 4. Basidiomata of similar species to *Cyathus lignilantanae*. a. *Cyathus poeppigii* (Holotype - PC0167598). b. *Cyathus limbatus*, from the type collection (PC0167584). c. *Cyathus pedicellatus* from Brodie (1978b; Fig. 4; © Canadian Science Publishing or its licensors).

Two other close species with small spores are *C. limbatus* and *C. pedicellatus*. *Cyathus limbatus* (Fig. 4b) presents similar peridium size (8.15 mm in height, 7.3 mm at the mouth in the type collection), tomentum size (0.58–0.78 mm) and the spore wall has a similar thickness (1.22–3.9 μm in *C. limbatus*, 1–2.1 μm in *C. lignilantanae*). The peridioles

are of the same greyish brown color, double layered cortex and size (2–2.8 × 1.6–2 mm in *C. limbatus*). The first striking differences are the peridium characteristics and tomentum: external wall with brown color, and provided with wooly to hirsute tomentum, inconspicuously plicated outside and inside in *C. limbatus*; in contrast to reddish brown external wall color, hirsute and conspicuously plicated peridium of *C. lignilantanae*. The huge amount of spores per slide, their size (12–17.2 × 8.4–15.2 μm in the type collection of *C. limbatus*) and the shape: globose to elliptical (Q= 1.03–1.60), in mean elliptical (Qm= 1.24), are other characteristics that separate these two species.

Concerning *C. pedicellatus* (Fig. 4c) the spores are more similar in size compared with those presented by *C. poeppigii* and *C. limbatus* (24–27 × 12–14 μm in the protologue, 13–25.3 × 9.4–17 in *C. lignilantanae*). The conspicuous plication inside and outside, hirsute peridium and circular peridiole shape (as in *C. lignilantanae*) are other similar features, but the very dark brown peridium, dark brown to black distinct pedicel, peridioles up to 3 mm in diameter and larger peridium (10–12 mm in height and 8–10 mm in the mouth, while *C. lignilantanae* is 6.9–8.2 mm in height and 5.2–6.9 mm wide at the mouth) separate these two species.

On the other hand, the basidiomata with color in different shades of brown, the lack of dark peridioles, and the presence of plicate peridium direct us to *C. striatus* (Huds.) Willd. (1787: 399), in group VII of Brodie's classification. However, differences such as the peridiole with triangular shapes and pale tunica, spores not over 20 μm in length, mouth with setae when young, and single-layered cortex in *Cyathus striatus* is sufficient to exclude this species. Also, our molecular analysis confirms that the two species are very distant in the *striatum* group.

Leaving aside Brodie's groups and *Cyathus* classification keys before 1984, one species published after this year deserves mention: *C. luxiensis*. Based on the plicate peridium and the spores, this species is very similar to *C. lignilantanae*; however, the lack of tomentum at maturity, the peridioles less than 2 μm, the smaller width of basidiospores (8 μm maximum), and the thicker spore wall (2–3 μm; 1–2 μm in *C. lignilantanae*) exclude this species, too.

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