



***Cyathus tenuicorticalis* (Agaricales, Basidiomycota), a new species from La Selva Biological Station, Costa Rica**

Góis JS¹, Cruz RHSF², Ovrebo CL³ and Baseia IG⁴

¹ Curso de Graduação em Ciências Biológicas, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Av. Senador Salgado Filho, 3000, Campus Universitário, Natal, 59072-970, RN, Brazil.

² Programa de Pós-Graduação em Sistemática e Evolução, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Av. Senador Salgado Filho, 3000, Campus Universitário, Natal, 59072-970, RN, Brazil.

³ Department of Biology, University of Central Oklahoma, 100 North University Drive, Edmond, OK 73034, United States.

⁴ Departamento de Botânica e Zoologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Av. Senador Salgado Filho, 3000, Campus Universitário, Natal, 59072-970, RN, Brazil.

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Abstract

Cyathus tenuicorticalis was found growing on decaying wood from La Selva Biological Station, Costa Rica. Specimens were analyzed using the methodology proposed for the group and specific literature for the genus. The fundamental diagnostic features for this new species are the very thin double-layered cortex in peridioles, exoperidium strongly hirsute, and a platinum bright endoperidium. Description, discussion and photographs of this new species are provided.

Key words – Biodiversity – Bird’s nest fungi – Gasteriod fungi – Nidulariaceae – Taxonomy

Introduction

The genus *Cyathus* Haller consists of organisms with angiocarpic basidiomata in the form of cone, vessel or inverted ball, distinguished by the presence of small internal structures in the fruitbody, which resemble small eggs inside a tiny bird’s nest, providing the etymology of the common name “bird’s nest fungi” (Brodie 1975). According to Brodie (1975, 1984), Lloyd (1906), most of the species are cosmopolitan, with a few endemic ones.

Few studies have been published on the fungi of Costa Rica, with the earliest records being made by Polakowsky (1877), Bommer & Rosseau (1896), Stanley (1927), Weston (1933), Garner (1956). For Costa Rica, there are 17 known species of the genus *Cyathus*: *C. africanus* Brodie, *C. berkeleyanus* (Tul. & C. Tul.) Lloyd, *C. canna* Lloyd, *C. earlei* Lloyd, *C. helenae* Brodie, *C. intermedius* (Mont.) Tul. & C. Tul., *C. julietae* Brodie, *C. limbatus* Tul. & C. Tul., *C. microsporus* Tul. & C. Tul., *C. montagnei* Tul. & C. Tul., *C. novae-zeelandiae* Tul. & C. Tul., *C. olla* (Batsch) ex. Pers., *C. pallidus* Berk. & C. Curtis, *C. poeppigii* Tul. & C. Tul., *C. setosus* Brodie, *C. stercoreus* (Schwein.) De Toni, and *C. striatus* (Huds.) Willd. (Lloyd 1906, Brodie 1975, Calonge et al. 2005). Among the ecological reserves in the country, La Selva Biological Station is one of the most productive in terms of tropical forest research in the world, with several studies on vertebrates, invertebrates and plants (Michener et al. 2009). According to the official list of species for the station, no fungi have been described for La Selva Biological Station (ThreePaths 2018), however,

some works were published with specific groups of these organisms, demonstrating a huge diversity not yet widely known for the Station (Ovrebio & Baroni 1988, Welden & Ovrebo 1989, Miller Jr et al. 1991, Ovrebo 1996, Maas Geesteranus & Ovrebo 1997, Guzmán & Ovrebo 2000, Ovrebo & Baroni 2007, Mata & Ovrebo 2009). Nevertheless, no *Cyathus* species was published for this region until now.

There is still a need for further studies on the genus *Cyathus*, and its wide distribution around the world should be better reported. Due to the lack of specialists, species of the genus are often misidentified, and this directly affects the knowledge of the group's distribution and biodiversity. Thus, the present work aims to increase knowledge about the genus through the description of this new species.

Materials & Methods

Fieldwork and morphology

The specimens were collected in 1986, at La Selva Biological Station and Reserve (Fig. 2a, b). The reserve is located in Costa Rica, 10°25'19"N 84°00'54"W. (Fig. 2a), at the confluence of the Sarapiquí and Puerto Viejo rivers, in the province of Heredia. It covers an area of 1,600ha of tropical rainforest, with an annual rainfall of 4m³ and relatively nutrient-rich soil (McDade 1994).

The sample analyzed followed the proposed methodology for Nidulariaceae fungi described by Lloyd (1906), Brodie (1975, 1984), as well as the morphological characteristics proposed by Cruz (2017). The measurements of the basidiomata (height × width), peridiole size (length × width), emplacement diameter and texture, cortex type, presence or absence of tunic, size of the tomentum in the exoperidium and the upper border (mouth), and the striae pattern in the exo- and endoperidium were analyzed. To determine the color of the structures, the color chart and color dictionary in the Methuen Handbook of Colour (Kornerup & Wanscher 1978) was used, through the code composed by the sequence “page + column + line” in the color chart. For the entire macroscopic analysis we used a Nikon SMZ1500 stereomicroscope (Nikon Corporation, Tokyo, Japan).

Microscopically, the size and shape of the basidiospores were analyzed. Microscopic observation was performed using the optical microscope Nikon Eclipse Ni-U (Nikon Corporation, Tokyo, Japan). Both stereomicroscope and optical microscope had a Nikon DS-Ri1 camera attached and the software NIS-Elements AR v.4.51.00 for obtaining and analyzing the images. For the preparation of slides, the peridioles were immersed in water for 48 hours (adapted from Lloyd 1906), and cut free-hand with the aid of a steel sheet. The peridioles were macerated to a fine powder, and a mounting medium composed of 5% potassium hydroxide (KOH) was added for observation under optical microscopy. The measurements of length and width of 30 basidiospores were obtained, as well as the average length (L), average width (W), the length-width ratio of each spore (Q), and the mean of the ratio (Qm) (Zhao et al. 2008). The spore shape was defined using the Qm value definition proposed by Bas (1969). The identification was carried out using identification keys proposed by specific works (Brodie 1984, Gómez & Pérez-Silva 1988) and identification keys prepared provisionally based on the original descriptions of materials published soon after 1988, and species that were not covered by the identification keys until 1988 (Stevenson 1975, Stevenson & Cash 1936, Brodie 1978, Zang 1980, Liu et al. 1984, Liu & Cao 1987, Gómez & Pérez-Silva 1988, Liu & Li 1989, Ren & Zhou 1992, Mornand 1992, Calonge 1994, Shinnars & Tewari 1998, Chen et al. 2003, Zhou et al. 2004, Zhao et al. 2008, Trierveiler-Pereira & Baseia 2009, Cruz & Baseia 2014, Das et al. 2015, Martín et al. 2015, Crous et al. 2016, Silva et al. 2016, Hyde et al. 2016, Crous et al. 2017a, b).

Results

Taxonomy

Cyathus tenuicorticalis J. S. Góis, R. Cruz, Baseia & Ovrebo, sp. nov.

Figs 1–2c, d

Etymology – In reference to the thin cortical layer of the peridiole.

MycoBank: MB825448

Diagnosis – Peridium infundibuliforme, 7.9 – 10.23 mm altum, 3.07 – 6.82 mm diametrum; exoperidium hirsutus, tomentum plus quam 1 mm longus, brunneus; endoperidium brunneo cinereo, plicatae, platinum; peridiola 2.04 – 2.9 mm × 1.69 – 2.74 mm, badius, circulari vel leniter ellipticis, superficiem lenis vel leniter rugosus, apparent tunica; duplex cortex, tenuissimus; basidiospora leniter ellipticis, 13.7 – 18.01 × 9.64 – 12.75 µm, 1.02 – 1.76 µm murum.

Description – Peridium infundibuliform, 7.9 – 10.23 mm in height, 3.07 – 6.82 mm in width at the upper part, not expanded at the mouth or tapering abruptly at the base. Emplacement 1.62 – 3 mm, conspicuous, tomentose, light brown (7D6). Exoperidium hirsute, dark brown (6F8), with 1.06 – 1.33 mm tomentum, arranged in regular and flexible tufts. External wall inconspicuously plicated, 0.39 – 0.70 mm between the folds. Mouth slightly fimbriated in a continuous pattern, 0.23 – 0.31 mm in height, dark brown (7E8). Endoperidium brownish grey (6F6), conspicuously plicated, 0.49 – 0.72 mm between the folds, bright platinum, contrasting with the exterior. Stipe 1.13 – 1.66 mm, rusty brown (6E8). Epiphragm not observed. Peridioles 2.04 – 2.9 × 1.69 – 2.74 mm in diameter, chestnut brown (6F7), 9 to 13 per basidiomata, circular to slightly elliptical in shape at borders, smooth to slightly rugulose. Tunic present, light brown. Double-layered cortex, very thin, exocortex brown, mesocortex greyish white with compact hyphae, and endocortex dark colored. Basidiospores smooth, hyaline, slightly elliptical to elliptical, apicule absent, 13.7 – 18.01 × 9.64 – 12.75 µm (L= 15.40 µm; W= 10.84 µm; Qm= 1.42 (1.30 – 1.55), with spore wall of 1.02 – 1.76 µm.

Habit – Gregarious, on decaying wood.

Material examined – Costa Rica, Province of Heredia, La Selva Biological Station, on decomposed wood, 27 May 1991, leg. Clark L. Ovrebo, CO3196 (Holotype USJ 109098, Isotype UFRN-Fungos 2962); 7 July 1986, leg. Clark L. Ovrebo, CO2131 (Paratype USJ 109097).

Remarks – *Cyathus tenuicorticalis* is characterized by the strongly hirsute exoperidium with long tomentum (greater than 1 mm) (Fig. 1a), bright platinum in the endoperidium (Fig. 1b), the very thin cortical layer and circular peridioles (Fig. 1c–d), The spores present a very common size for the genus, with a predominantly ellipsoid shape (Fig. 1e). In addition, this species also presents larger peridioles that can be seen easily in the field (Fig. 2c, d).

According to Brodie's (1975, 1984) classification, *Cyathus tenuicorticalis* could be grouped in the group IV (*gracilis*), based on the peridium inconspicuously plicated, tomentum aggregated in tufts, tunic thin and elliptical spores, or in the group VII (*striatus*), based on the exoperidium sometimes inconspicuously plicated, hirsute, tunic present and spores mostly elliptical. However none of these groups presents the complete morphological features, but scattered punctual characteristics, to include *C. tenuicorticalis* correctly in them, a recurring problem in the Brodie's classification due the non-inclusion of species published after 1984.

Morphologically, this species resembles *C. bulleri* due to hirsute exoperidium, the shape and size of peridioles, and the bright platinum in the endoperidium, but it differs in the larger dimensions of the peridium (5.59 – 8 mm in height, 6 – 8 mm in width), greater spacing between external wall plication (0.4 – 0.8 mm), single layered cortex, and larger spores (6.85 – 11.58 × 4.98 – 6.93 µm) (Brodie 1967, Cruz 2017). *C. tenuicorticalis* can also be compared to *C. ellipsoideus* due to the hirsute exoperidium, inconspicuous plication in the external wall, presence of tunic, and elliptical spores, but the latter differs in the smaller basidiomata (6 – 7 mm in height, 5 – 6 mm in width), shorter tomentum (0.3 – 0.6 mm), shorter mouth tomentum (0.1 – 0.15 mm), smaller peridioles, exclusively circularshape (1.53 – 1.83 mm × 1.25 – 1.39 mm), and single layered cortex (Brodie 1974). According to the morphological review done by Cruz (2017), the size of the spores present in the original description (15 – 16 µm × 9 – 10 µm) is not faithful to the type material analyzed, which presented values of 19.44 – 23.75 × 10.59 – 13.95 µm, but it still differs from that species.



Fig. 1 – *Cyathus tenuicorticalis* sp. nov. (Holotype USJ 109098): a Exoperidium hirsute, inconspicuously plicated. b Endoperidium showing plications and bright platinum. c Upper view of peridioles with circular shape, showing the smooth surface. d Peridiole double-layered thin cortex. e Basidiospores elliptical-shaped.

Another similar species is *C. gracilis*, showing similarities in the basidiomata size, hirsute exoperidium, tomentum height at the mouth and double layered cortex (Brodie 1973). However, this species differs from *C. tenuicorticalis* by the external smooth wall, smaller tomentum (0.25 – 0.6 mm), endoperidium smooth to inconspicuously plicated, not bright, and smaller peridioles (1.7 – 1.94 × 1.65 – 1.85 mm in diameter). *C. tenuicorticalis* is also similar to *C. intermedius* due to the presence of hirsute exoperidium, inconspicuously plicated external wall, presence of tunic and tomentum arranged in tufts, but it is differentiated by the smaller dimensions in the basidiomata (4.74 – 5.82 mm in height, 4.97 – 6.04 mm in width), smaller tomentum (0.32 – 0.43 mm), endoperidium inconspicuously plicated and single-layered cortex (Tulasne & Tulasne 1844, Lloyd 1906).

C. tenuicorticalis can be compared with *C. striatus* and *C. berkeleyanus* in group VII (*striatus*) in the classification proposed by Brodie (1975) by the presence of tunic, external wall inconspicuously plicated and the basidiomata size but differs from *C. striatus* by larger emplacement (3.46 – 4.25 mm), smaller peridioles (1.85 – 2.28 × 1.51 – 1.95 mm) and peridium expanded at the mouth. Due the same characteristics, *C. berkeleyanus* can be compared to the proposed species but

differs for the single-layered cortex, smaller basidiospores ($7 - 13 \mu\text{m} \times 5 - 8 \mu\text{m}$) and blacker peridioles.

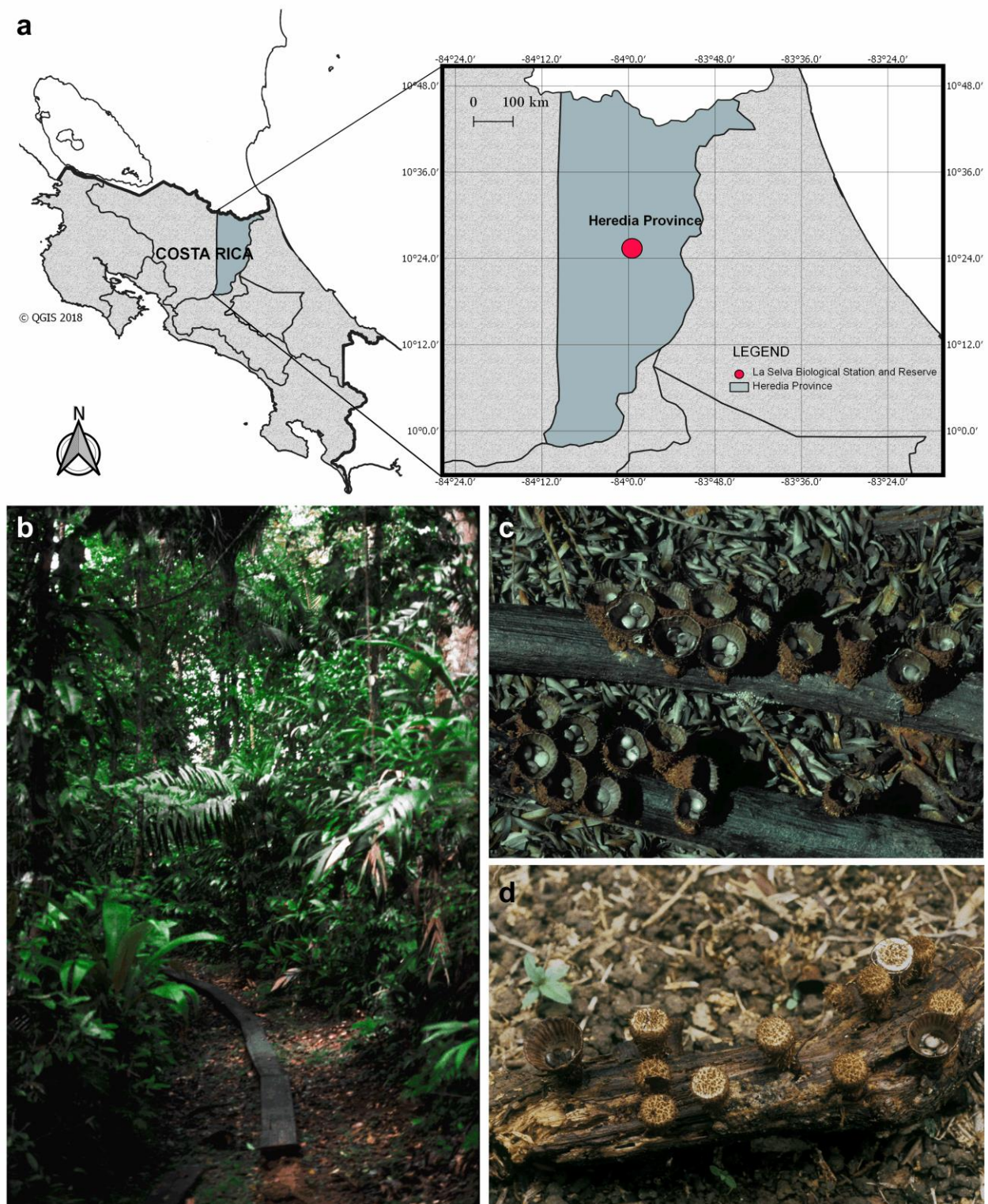


Fig. 2 – a Map showing the collection site, La Selva Biological Station and Reserve – Costa Rica. b Field trail. c Basidiomata *in situ* (Holotype USJ 109098). d Basidiomata *in situ* (Paratype USJ 109097).

There are currently 81 *Cyathus* species known to the world. Many of the new species of the genus are reported using superficial features or characteristics common to other species of *Cyathus*,

which makes the use of more concrete and limiting data indispensable to describe a new species, such as details of the cortical layer and surface of the peridioles, as well as DNA extraction to increase the support of the analysis. Some of the recent descriptions already use data such as these (Crous et al. 2016, 2017a, b). However, it was unfortunately not possible to extract DNA from the specimens studied here, and this may be related to the way the exsiccate was dehydrated and preserved. Even so, the macroscopic and microscopic characteristics, when compared with other *Cyathus* species, allow us to propose *Cyathus tenuicorticalis* as a distinct and reliable species for science.

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