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Research article

Chlorophyllum tenue sp. nov. (Agaricaceae, Basidiomycota) from the Brazilian Caatinga semiarid region

Erica S. FALCÃO[1,*], Nathalia U. FERRETTI-CISNEROS[2],
Alexandre G.S. SILVA-FILHO[3], Ricardo KOROIVA[4] & Felipe WARTCHOW[5]

[1] Universidade Federal de Pernambuco, Programa de Pós-Graduação em Biologia de Fungos,
Departamento de Micologia, CEP 50740-570, Recife, PE, Brazil.
[2] Universidade Federal da Paraíba, Pós-Graduação em Ecologia e Monitoramento Amabiental,
CEP 58297-000, Rio Tinto, PB, Brazil.
[3] Instituto Federal de Ciência e Tecnologia de São Paulo,
Departamento de Ciências e Matemática (DCM), CEP 01109-010, São Paulo, SP, Brazil.
[4] Universidade Federal da Paraíba, Departamento de Engenharia e Meio Ambiente,
CEP 58059-585, Rio Tinto, PB, Brazil.
[5] Universidade Federal da Paraíba, Departamento de Sistemática e Ecologia,
CEP 58051-970, João Pessoa, PB, Brazil.

[*] Corresponding author: bach11509312@gmail.com
[2] Email: nathaliaferrettic@gmail.com
[3] Email: silvafilhoags@gmail.com
[4] Email: ricardo.koroiva@gmail.com
[5] Email: fwartchow@yahoo.com.br

Abstract. *Chlorophyllum tenue* sp. nov. is described based on morphological and molecular (nrDNA-ITS, nrDNA-LSU) data. The new species belongs to *Chlorophyllum* sect. *Ellipsoidospororum* due to its small sized agaricoid basidiomata with furfuraceous pileal squamules, a loose hymenioderm covering, and basidiospores lacking germ pores. As specific characteristics, the new species has light grayish brown to dark cream pileus with a distinct reddish brown umbo breaking up into appressed light brown to rosy beige irregularly shaped squamules, thin context, cylindrical and subradicant stipe, and stipe surface changing color. Microscopically, the new species has ellipsoid basidiospores smaller than 10 µm long, 4-spored basidia, slender clavate-subcapitate cheilocystidia, and pileus covering formed by elements varying from broadly clavate, clavate to slender clavate sinuous. Additionally, *Chlorophyllum tenue* sp. nov. represents an independent lineage among *Chlorophyllum* sect. *Ellipsoidospororum* and is closely related to *C. africanum*, *C. demangei*, and *C. hortense*.

Keywords. Agaricomycetidae, Neotropics, phylogeny, systematics, taxonomy.

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Introduction

Traditionally, the genus *Chlorophyllum* Massee (Agaricales) was considered monospecific, accommodating only the green-spored species *C. molybdites* (G.Mey.) Massee (Singer 1986). Some white-spored agaricoid taxa with similar habit to *Chlorophyllum* used to be included in *Macrolepiota* Singer or *Leucoagaricus* Locq. ex Singer, and the secotioid ones were considered part of *Endoptychum* Czern. or *Secotium* Kunze (see summary in Vellinga *et al.* 2003, 2011; Vellinga 2004). Ge *et al.* (2018) rearranged the systematics of *Chlorophyllum* based on a multigene phylogeny, delimiting 17 species across six sections, plus another two taxa in a subsequent publication (Loizides *et al.* 2020). Currently, the genus accommodates agaricoid, secotioid and gasteroid species with the hymenidermal pileus covering, mostly smooth stipe (in the agaricoid taxa), and the presence or absence of germ pore in the basidiospores apex, which are white, green or brownish in deposit (Vellinga 2003a, 2003b; Ge & Yang 2006; Ge *et al.* 2018). When present, the germ pore is formed by a depression in the episporium without the hyaline covering as observed in species of *Macrolepiota* (Vellinga 2003b).

Regarding habitat, some members of *Chlorophyllum* are frequently found in anthropic areas of tropical and temperate regions (Noordeloos *et al.* 2001; Ge *et al.* 2018; Dutta *et al.* 2020; Loizides *et al.* 2020; Maubet *et al.* 2022). Few species are reported as toxic, including accidental ingestion of *C. molybdites* by humans (de Meijer *et al.* 2007).

In Brazil, only three species have been recorded: *C. molybdites*, *C. hortense* (Murrill) Vellinga, and *C. rhacodes* (Vittad.) Vellinga (Putzke 1994). However, in the Northeast region of Brazil, only *C. molybdites* has been cited for the states of Bahia (Putzke 1994; Drechsler-Santos *et al.* 2008), Pernambuco (Putzke 1994; Kimbrough *et al.* 1994/1995; Maia & Gibertoni 2002; Drechsler-Santos *et al.* 2008), Ceará (Nascimento & Alves 2014), and Piauí (Alves *et al.* 2019). Here we describe a new member of *Chlorophyllum* from the semi-arid region of the Brazilian Caatinga based on morphological studies and molecular phylogenetic analyses.

Material and methods

Sample collection and morphological study

The specimen was collected during the 2019 rainy season in the 'Reserva Particular do Patrimônio Natural (RPPN) Fazenda Almas' (7°28′45″ S, 36°54′18″ W), municipality São José dos Cordeiros, state of Paraíba, semiarid region of Northeast Brazil. This collection site was already fully described by Ferretti-Cisneros *et al.* (2022). Color codes follow the Online Auction Color Chart (hereafter OAC; Kramer 2004). Macromorphological and micromorphological studies were carried out on fresh collections following the methodology proposed by Singer (1986) for agaricoid fungi analysis. Line drawings were made with the aid of a camera lucida, and photomicrographs were taken using a camera and software (ZEN Microscopy Software™) connected to a Primo Star Zeiss™ microscope (Carl Zeiss Co., Ltd.). The presentation of basidiospore data follows the methodology proposed by Tulloss *et al.* (1992) and slightly modified here. Measurements and statistics are based on 50 basidiospores. The holotype is deposited in JPB (Herbário Lauro Pires Xavier, Departamento de Sistemática e Ecologia, Universidade Federal da Paraíba).

Abbreviations

Q = range of length to width ratio determined from all measured basidiospores
Qm = Q value averaged from all measured basidiospores

Phylogenetic analysis

DNA extraction from the specimen was performed using Qiagen DNeasy Plant Mini Kit. PCR primers for nrDNA-ITS and ITS1/ITS4 (White *et al.* 1990), nrDNA-LSU and LR0R/LR5 (Moncalvo *et al.* 2000). The PCR reactions were proceeded following the method adopted by Barbosa-Silva *et al.* (2022).

Two distinct datasets were constructed: the first including nrDNA-ITS + nrDNA-LSU sequences; and the second with nrDNA-ITS sequences. *Leucoagaricus nympharum* (Kalchbr.) Bon and *L. leucothites* (Vittad.) Wasser were chosen as outgroups in all analyses (according to Vellinga 2003b, 2004; Dutta *et al.* 2020). The datasets were aligned separately using MAFFT ver. 7 (Katoh & Standley 2013) under the E-INS-i criteria. To compute the best-fit model of nucleotide evolution, the nrDNA-ITS region was subdivided into three data partitions: ITS1, 5.8S and ITS2. Maximum Likelihood analyses were performed in RAxML ver. 8.2.X (Stamatakis 2014). The best nucleotide substitution model was selected with BIC (Bayesian Information Criterion) using jModelTest2 ver. 1.6. The evolutionary models selected for the

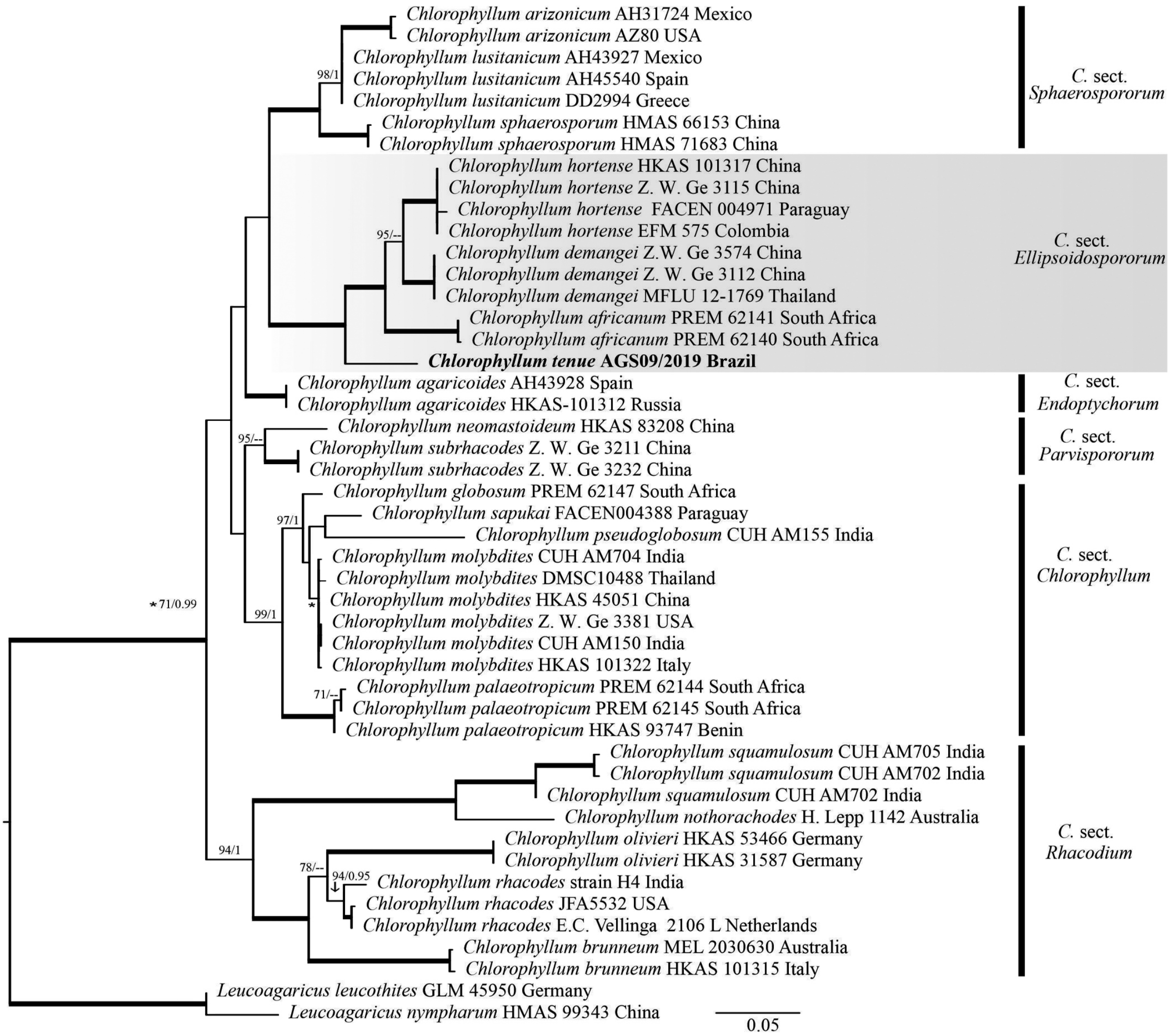

Fig. 1. Phylogenetic tree of species of *Chlorophyllum* based on nrDNA-ITS and nrDNA-LSU. The new species is in bold. Bootstrap values and Bayesian posterior probabilities are indicated if they are equal to or greater than 70% and 0.95, respectively. Thicker lines represent branches with maximum bootstrap values and posterior probabilities (100% BS, 1.0 BPP).

nrDNA-ITS dataset were: ITS1: TIMef+I, 5.8S: TPM2uf, ITS2: HKY+I, whilst the evolutionary models selected for the combined dataset were: ITS1: TrNef+G, 5.8S: K80+I, ITS2: HKY+G and LSU TrN+I. Later, Bayesian inference (BI) were performed using MrBayes ver. 3.1.2 (Ronquist *et al.* 2012), with 2×10^7 MCMC generations, sampling one tree every 1×10^3 generation. RAxML ver. 8.2.X, jModelTest2 ver. 1.6, and MrBayes ver. 3.1.2 software were implemented in the CIPRES Science Gateway ver. 3.1 (Miller *et al.* 2010). The sequences were deposited in NCBI (GenBank) under accession OP852363 for nrDNA-ITS and OP852374 for nr-DNA-LSU (Supp. file 1). The final alignment and the resultant topologies were deposited in TreeBASE, ID: 31387.

Results

Phylogenetic analyses

Two new nrDNA-ITS and nrDNA-LSU sequences were generated in this study (Supp. file 1). The combined data set was composed of 44 terminals with 1705 characters (including introduced gaps), containing 44 and 37 sequences from the nrDNA-ITS region and nrDNA-LSU gene, respectively, representing 21 species of *Chlorophyllum*. Both RAxML analysis (Fig. 1) and Bayesian inference yielded similar tree topologies, including the bootstrap (BS) and posterior probabilities (BPP). The combined phylogeny retrieved the six monophyletic sections from within the genus *Chlorophyllum*: *Chlorophyllum* (99% BS, 1.0 BPP), *Ellipsoidospororum* Z.W.Ge (100% BS, 1.0 BPP), *Endoptychorum* (Czern.) Z.W.Ge (100% BS, 1.0 BPP), *Parvispororum* Z.W.Ge (95% BS, <0.95 BPP.), *Rhacodium* Z.W.Ge (94% BS, 1.0 BPP), and *Sphaerospororum* Z.W.Ge (100% BS, 1.0 BPP), as proposed by Ge *et al.* (2018). The sequences of our new proposed species grouped within the sect. *Ellipsoidospororum* alongside *C. africanum* Z.W.Ge & A.Jacobs (PREM 62141 and PREM 62140, both from South Africa), *C. demangei* (Pat.) Z.W.Ge & Zhu L.Yang (*Z.W. Ge 3112* and MFLU 12-1769 from China and Thailand), and *C. hortense* (*Z.W. Ge 3115*, *Z.W. Ge 3574*, FACEN 004971, HKAS 101317 from China, Paraguay and Colombia), all forming a well-supported clade (100% BS, 1.0 BPP).

Another dataset of nrDNA-ITS included 344 sequences with 950 characters (including introduced gaps). Both RAxML analysis (Supp. file 2) and Bayesian inference yielded similar tree topologies, including the bootstrap and posterior probabilities. The major and well supported clades were collapsed and the sequence numbers were indicated. In this analysis, the newly proposed species *Chlorophyllum tenue* sp. nov. also forms a new lineage in a well-supported clade (81% BS, 0.99 BPP) closely related to *C. africanum*.

Taxonomy

Order Agaricales Underw.
Family Agaricaceae Chevall.
Genus ***Chlorophyllum*** Massee

Chlorophyllum tenue E.Falcão, Ferretti-Cisn., Silva-Filho, Koroiva & Wartchow sp. nov.
MycoBank MB 855028
Figs 2–4

Diagnosis

The species is characterized by the delicate basidiome, light brown to pinkish beige furfuraceous squamules on a light gray to dark cream brown background, cylindrical and slightly radicant stipe, ellipsoid basidiospores 8.7–9.7(–10.7) × 5.6–6.6 µm, 4-spored basidia, slender clavate-subcapitate cheilocystidia, and pileus covering elements varying from broadly clavate, clavate to slender clavate sinuous.

Etymology

From the Latin adjective *tenuis*, *e* = slender, thin, delicate, due to the slender basidiome of the new species.

Fig. 2. *Chlorophyllum tenue* E.Falcão, Ferretti-Cisn., Silva-Filho, Koroiva & Wartchow sp. nov. (holotype, JPB [66971]), general aspect. **A**. Pileus. **B**. Hymenophore. Scale bars = 20 mm.

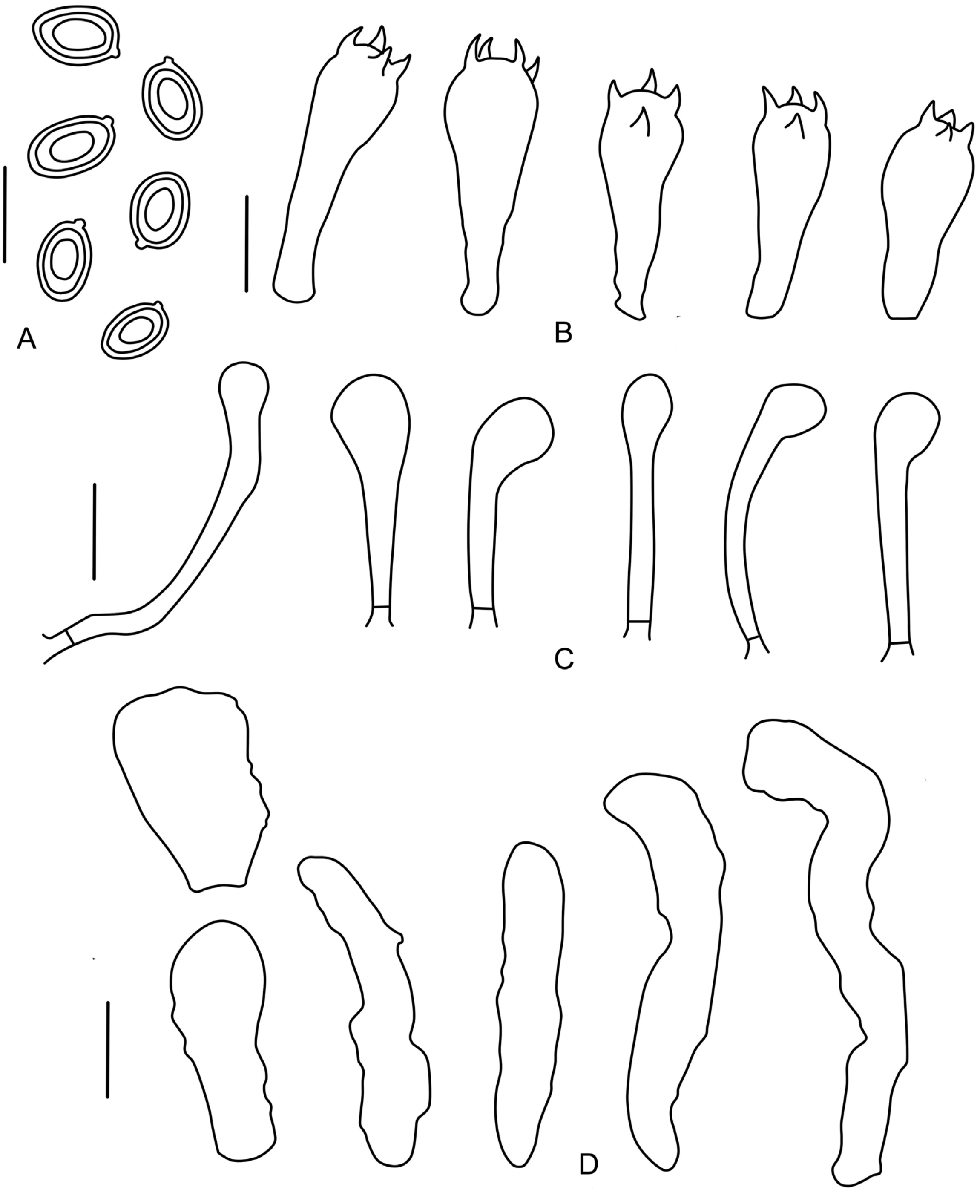

Fig. 3. *Chlorophyllum tenue* E.Falcão, Ferretti-Cisn., Silva-Filho, Koroiva & Wartchow sp. nov. (holotype, JPB [66971]), line drawings of microstructures. **A**. Basidiospores. **B**. Basidia. **C**. Cheilocystidia. **D**. Pileus covering elements. Scale bars = 10 µm.

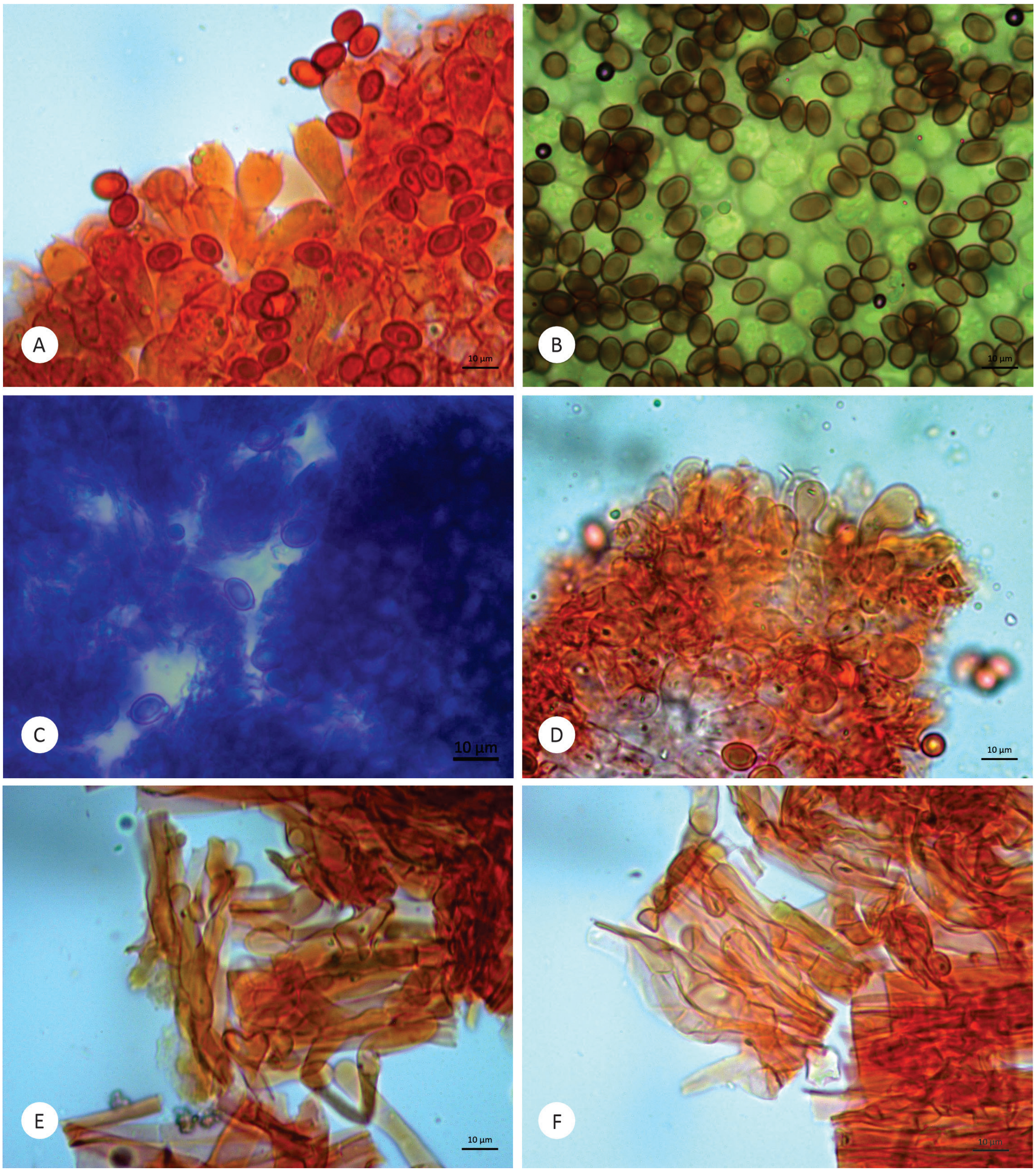

Fig. 4. *Chlorophyllum tenue* E.Falcão, Ferretti-Cisn., Silva-Filho, Koroiva & Wartchow sp. nov. (holotype, JPB [66971]), microstructures. **A**. Basidia. **B**. Basidiospores under Melzer's reagent. **C**. Basidiospores in Cresyl Blue mounts. **D**. Lamella edge with two cheilocystidia. **E**–**F**. Pileus covering elements. Scale bars: A–F = 10 µm.

Type material

Holotype

BRAZIL – **Paraíba** • São José dos Cordeiros; "RPPN Fazenda Almas" [Private Reserve of Natural Patrimony Fazenda Almas]; 7°28′45″ S, 36°54′18″ W; 26 Feb. 2019; *A.G.S. Silva-Filho AGS 09-2019*; solitary on soil among litter in vegetation of Caatinga Biome; JPB [66971].

Description

Basidiome small, slender, lepiotoid. Pileus 42 mm plane-umbonate, surface squamulose, with a distinct reddish brown (OAC 733) umbo breaking up into small appressed scales, light brown (OAC 688) to rosy beige (OAC 689) irregularly shaped, radially oriented, scattered and detersile toward the margin; surface/background light grayish brown to dark cream (OAC 722, 718); margin striated; surface more densely packed toward central disk; context very thin, fragile. Lamellae free, white (OAC 909), subclose, up to 5 mm broad, lamella-edge slightly eroded to smooth, concolorous to side; lamellulae common, of 3 lengths. Stipe 42 × 3 mm, central cylindrical, equal, radicant; whitish to light brown (OAC 909/ 801) to dark ochraceous brown (OAC 796), unchanging when injured; surface squamulose towards the apex, then glabrous and striate bellow the ring; ring with median to slightly superior position, thin with double edge; context white (OAC 909), hollow.

Basidiospores 8.7–9.7(–10.7) × 5.6–6.6 µm, Q = 1.43–1.64, Qm = 1.47, ellipsoid in side view, thin to slightly thick-walled, hyaline, dextrinoid, cyanophilous, metachromatic; germ pore absent. Basidia (19.4–)21.9–24(–27.5) × 8.7–10.7 µm, clavate, hyaline, 4-spored; sterigmata up to 5 µm high. Pleurocystidia absent. Lamella edge sterile, with abundant heterogeneous cheilocystidia. Cheilocystidia 24–27.5 × 5.1–8.2 µm, slender clavate to sometimes broadly capitate, hyaline, thin-walled. Lamella trama regular to subregular, composed by hyphae (3.5–)4.6–5.6 µm in diam., colorless, thin-walled. Pileus covering a hymeniderm with ellipsoid, broadly clavate to sinuously slender clavate terminal elements, pale to brownish pigmented, measuring (11.2–)27.5–34.7(–51) × 6.6–9.7(–14.8) µm, thin-walled. Clamp connections absent from all examined tissues.

Distribution

Known only from the type locality.

Key for identification of the species of Chlorophyllum sect. Ellipsoidospororum (Ge et al. 2018; Dutta et al. 2020; Loizides et al. 2020; this work)

1. Light brown to yellow-brown irregular patches on white to light orange pileus 2
– Furfuraceous squamules arranged on white to light grayish brown or dark cream pileus 3

2. Basidia 2-spored; widely distributed (Africa, Asia and Americas) .... *C. hortense* (Murrill) Vellinga
– Basidia 4-spored; restricted to Southeast Asia................ *C. demangei* (Pat.) Z.W.Ge & Zhu L.Yang

3. Pileus with grey to grey orange furfuraceous squamules on white ground; stipe subcylindrical then slightly enlarged at base, white to light brown, turning brown to red where bruised or handled; annulus simple; cheilocystidia 28–50 × 6–10 µm; pileus covering formed by hymenidermic layer made up of clavate slightly thick-walled elements, with terminal elements mostly narrowly clavate .... *C. africanum* Z.W.Ge & A.Jacobs
– Pileus with reddish brown, light brown to rosy beige squamules on grayish brown to dark cream ground; stipe cylindrical-equal, radicant, not changing color when bruised, annulus upper central, double, thin, white to light brown, cheilocystidia 24–27.5 × 5.1–8.2 µm; pileus covering elements versiform (broadly clavate, clavate to slender clavate sinuous) .... *C. tenue* sp. nov.

Discussion

Chlorophyllum tenue sp. nov. is readily recognized in the field by its agaricoid basidiome, the small pileus covered with light brown to pinkish beige furfuraceous squamules on a light gray to dark cream brown background; the cylindrical equal and slightly radicant stipe with a squamulose surface above and glabrous-striate below the ring; the thin ring with double edge having a median position; and the unchanging color of the stipe surface when injured. Microscopically, the ellipsoid basidiospores (8.7–9.7 × 5.6–6.6 µm) without germ pore, the 4-spored basidia, the clavate-subcapitate cheilocystidia, and the pileus covering as a loose hymeniderm of versiform elements (broadly clavate, clavate to slender clavate sinuous) also characterize the new species (Figs 3–4).

These characteristics are in line with the morphological circumscription of the sect. *Ellipsoidospororum*, principally in the habit and size of the basidiomata, the shape of the pileus squamules, the ellipsoid basidiospores lacking germ pore, and the overall shape of the cheilocystidia and pileus covering elements (Ge *et al.* 2018). Among this section, three other species are known, as follows.

Chlorophyllum africanum, originally described from South Africa, is similar to *C. tenue* sp. nov. in having small basidiomata and ellipsoid basidiospores. However, in *C. africanum* the grey to grey-orange squamules are arranged on a whitish background (Ge *et al.* 2018). Both species also differ in the shape and surface of the stipe: subcylindrical, slightly enlarged at the base and glabrous in *C. africanum* instead cylindrical-equal radicant with squamulose surface above and striate bellow ring in *C. tenue*. Furthermore, *C. africanum* possesses a simple annulus (Ge *et al.* 2018). Microscopically, *C. africanum* differs in having cheilocystidia 28–50 × 6–10 µm, and a pileus covering formed by a hymenidermal layer composed of mostly clavate elements with slightly thick wall, and mostly narrowly clavate terminal ones (Ge *et al.* 2018). In *C. tenue*, although a hymenidermal pileus covering is also present, this structure is formed by versiform elements.

Chlorophyllum demangei from Vietnam and Thailand differs from *C. tenue* sp. nov. in the glabrous and light brown to brown pileus center with concolorous patches around the umbo that disrupt into scattered irregular patches toward margin on a white to pale yellow background; and the cylindrical sometimes slightly wider at the base stipe fibrillose surface with color changing to orange-white during handling. *Chlorophyllum demangei* also has narrowly clavate to slightly narrowly lageniform then cylindrical cheilocystidia, and pileus covering of a trichoderm composed of cylindrical to narrowly clavate elements (Ge *et al.* 2018; Sysouphanthong *et al.* 2021).

Finally, *C. hortense* is found throughout the tropics in diverse habitats (Ge & Yang 2006). Like *C. africanum* and *C. demangei*, it also readily differs from *C. tenue* sp. nov. in having the pileal scales disrupting on a white background. Also, *C. hortense* presents a strong change in color when injured (Dutta *et al.* 2020; Jabeen *et al.* 2021), reaction not observed on *C. tenue*. The Indian collection of *C. hortense* also differs from *C. tenue* in having slightly larger basidiospores (7.9–)9.7–10.5(–11) × (5.4–)6.4–7.0(–7.5) µm, in the presence of 2-spored basidia (contrasting to exclusively 4-spored in *C. tenue*), the subcylindrical to narrowly clavate (or clavate sometimes piriform with a short stem) cheilocystidia, and a trichohymeniderm-like pileus covering with clavate to widely clavate or subfusiform terminal elements (Dutta *et al.* 2020; Jabeen *et al.* 2021).

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Supplementary files

Supp. file 1. Details of the sequences used in this study. The newly generated sequence is in bold. The abbreviation N/A denotes 'not available'. https://doi.org/10.5852/ejt.2025.976.2787.12733

Supp. file 2. Maximum Likelihood Phylogeny of collections of *Chlorophyllum* representatives based on complete nrDNA-ITS with 344 sequences. The new species is in bold. Thicker lines represent branches with maximum bootstrap values and posterior probabilities (100% BS 1.0 BPP). Bootstrap values and Bayesian posterior probabilities are indicated if they are equal to or greater than 70%, and 0.95, respectively. The scale bar represents the expected number of nucleotide changes per site. https://doi.org/10.5852/ejt.2025.976.2787.12735