Plant and Fungal Systematics 67(2): 75–85, 2022
DOI: https://doi.org/10.35535/pfsyst-2022-0009

ISSN 2544-7459 (print)
ISSN 2657-5000 (online)

Introducing a new member of the genus *Chlorophyllum*: *Chlorophyllum sapukai* sp. nov., and new records from Paraguay

Yanine Maubet[1*], Michelle G. Campi[1], Enzo Cristaldo[1] & Gerardo L. Robledo[2,3,4*]

Article info

Received: 23 Aug. 2022
Revision received: 20 Dec. 2022
Accepted: 22 Dec. 2022
Published: 31 Dec. 2022

Associate Editor

Bryn T. M. Dentinger

Abstract. Discovered in Paraguay, *Chlorophyllum sapukai* is a new species based on morphological characters and multigene molecular phylogenetic analyses (ITS, LSU, RPB2 and TEF1 markers). Macroscopically, the species is characterized by large agaricoid basidiomata, with a hemispherical to broad convex pileus, a yellowish to cream buff surface of the pileus covered centrally with plate-like squamules. Microscopically, the new species is characterized by abundant cheilocystidia (35–50 × 14–20 µm), truncate basidiospores with a defined germ pore (9–10 × 6.5–7.5 µm), a pileus covering arranged as a trichodermial palisade at the squamules. *Chlorophyllum hortense* is recorded for the first time for Paraguay and the distribution of *C. molybdites* is expanded. Detailed morphological descriptions, field photographs of the species and the phylogenetic position of the *Chlorophyllum* species occurring in Paraguay are presented. A key to neotropical *Chlorophyllum* species is also provided.

Key words: *Agaricaceae*, multigene phylogenetic analyses, neotropics, new taxa, taxonomy

Introduction

Chlorophyllum Massee (*Agaricaceae*, *Agaricales*, *Basidiomycota*) is a monophyletic genus typified by *Chlorophyllum molybdites* (G. Mey.) Massee. The genus comprises a morphologically diverse group that includes species with agaricoid, secotioid, and gasteroid forms; the presence of a smooth stipe, a hymenidermal pileus covering (in agaricoid species) and green-brown or white, hyaline basidiospores with a germ pore usually present (Vellinga 2002, 2003; Vellinga & De Kok, 2002; Loizides et al. 2020). The number of documented species of *Chlorophyllum* increased during the last decade from 16 (Kirk et al. 2011) to 28 currently accepted species due to the use of molecular tools (Crous et al. 2015; Ge et al. 2018; Dutta et al. 2020). Based on multigene phylogenetic analyses, six infrageneric sections have been proposed: sect. *Chlorophyllum* Massee, sect. *Endoptychorum* (Czernajew) Z. W. Ge, sect. *Ellipsoidospororum* Z. W. Ge, sect. *Parvispororum* Z. W. Ge, sect. *Sphaerospororum* Z. W. Ge, and sect. *Rhacodium* Z. W. Ge. (Ge et al. 2018). It is noteworthy that only two specimens from South America have been included in any phylogenetic analysis despite the widespread presence of the genus in the region (Johnson 1999; Ge et al. 2018).

Chlorophyllum is widely distributed throughout the world, often growing in urban and ruderal habitats with a preference for tropical and subtropical regions (Vellinga 2004; Kirk et al. 2011). In South America, there have been previous records of the genus based exclusively on morphological data. For example, *C. molybdites* (G. Mey.) Massee, *C. hortense* (Murrill) Vellinga and *C. rhacodes* (Vittad.) Vellinga have been identified in Brazil and Argentina (Alves et al. 2019; Dominguez et al. 2021), *C. rhacodes* has been cited in Chile (Salazar-Vidal et al. 2017), and lastly, only *C. molybdites* has been reported in Paraguay (Campi et al. 2018). Additionally, four species described from the Caribbean and South America have been considered synonyms of *C. molybdites: Annularia camporum* Speg., *Chlorophyllum esculentum* Masse, *Agaricus glaziovii* Berk., and *Agaricus guadelupensis* Pat.

To address this gap in published scholarship regarding *Chlorophyllum* species in the Global South, we collected and analyzed *Chlorophyllum* specimens in Paraguay through combined morphological and phylogenetic

[1] Universidad Nacional de Asunción, Facultad de Ciencias Exactas y Naturales, Lab. Análisis de Recursos Vegetales, área Micología, San Lorenzo, Paraguay
(Maubet, ORCID: 0000-0002-0322-6238; Campi, ORCID: 0000-0001-8809-0159; Cristaldo, ORCID: 0000-0002-2221-3395)
[2] CONICET. Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina
(Robledo, ORCID: 0000-0002-3840-1104)
[3] Universidad Nacional de Córdoba. Facultad de Ciencias Agropecuarias, BioTecA3 – Centro de Biotecnología Aplicada al Agro y Alimentos, Córdoba, Argentina
[4] Fundación Fungicosmos, Córdoba, Argentina
[*] Corresponding authors e-mails: ymaubet@gmail.com, gerardo.robledo@agro.unc.edu.ar

multigene analyses. Furthermore, we discussed the taxonomic status of the distinct species recorded in Paraguay, including descriptions and illustrations of macro- and micro-morphological characteristics. Additionally, we introduced a key for identifying *Chlorophyllum* species in South America.

Materials and methods

Morphological study

Specimens were collected in the eastern region of Paraguay in the departments Canindeyú, Central, and Guairá. Samples were prepared and analyzed in the Plant Resources Laboratory, Mycology Lab, in the *Facultad de Ciencias Exactas y Naturales* (FACEN) of the *Universidad Nacional de Asunción*. The macroscopic descriptions were based on fresh material, according to the guidelines proposed by Lodge et al. (2004). For microscopic analysis, free-hand sections of basidiomata were mounted in 3–5% (w/v) aqueous potassium hydroxide (KOH) and 1% (w/v) aqueous phloxine or Congo red. Cresyl blue and Melzer's reagent were used to test the metachromatic and dextrinoid reactions of the basidiospores, respectively. The description of the pileal surface (pileipellis) is according to Largent et al. (1977), who classifies two types of pileipellis based on hyphal arrangement of the elements as follows: a) cutis = more or less parallel to the surface; called a parallelocutis when the elements are parallel to the surface b) derm = perpendicular to the surface (anticlinal); types of derm include: b.1) trichodermial palisade composed of filiform elements unequal in length, arranged anticlinally which reach more or less the same level and b.2) hymeniform layer composed of elements similar to those observed in the hymenium, but lacking cell resembling basidia. Description of sterile elements on the hymenium and cystidia shape follows Largent et al. (1977). The notation [n/m/p] was used to indicate the numbers of basidiospores measured, where n indicates the number of basidiospores measured from m basidiomata of p collections, and the measurement contains a minimum of 90% of the values. Additionally, Q is defined as the length/width ratio of a basidiospore, and Q_{av} represents the average Q of all basidiospores studied. Micrographs of basidiomata were taken with a BOECO BM-800 optical microscope coupled with a B-CAM14 camera.

DNA isolation, PCR amplification, and cycle sequencing

DNA extraction and amplification were performed on the dried basidiomata following a standard CTAB protocol (Góes-Neto et al. 2005). Sequencing was conducted by Macrogen, Inc. The internal transcribed spacer (ITS) regions, including ITS1, 5.8S and ITS2, were amplified with the primer pair ITS1F/ITS4b (Gardes & Bruns 1993; White et al. 1990). The 5′ end of the 28S large subunit (LSU) of the nuclear ribosomal RNA was amplified with primers LR0R (Cubeta et al. 1991) and LR7 (Vilgalys & Hester 1990). Thermocycler conditions for ITS markers were: initial denaturalization at 95°C for 5 min, followed by 15 cycles of denaturalization at 94°C for 30 s, annealing at 55°C for 45 s, extension at 72°C for 1 min; followed by 25 cycles of denaturalization at 94°C for 30 s, annealing at 52°C for 45 s, extension at 72°C for 1 min, and a final extension at 72°C for 10 min; for LSU initial denaturalization at 94°C for 85 s, followed by 37 cycles of denaturalization at 94°C for 1 min, annealing at 47°C for 45 s, extension at 72°C for 2 min; final extension at 72°C for 7 min. Thermocycler conditions for RPB2 were set following methods per Kuo & Ortiz-Santana (2020) utilizing primers bRPB2-6F and bRPB2-7.1R (Matheny 2005). Thirteen total sequences (6 nrITS; 6 nrLSU; 1 RPB2) were generated for the purpose of this study and were deposited in GenBank.

Sequence alignment and phylogenetic analyses

The newly generated DNA sequences were combined with sequences retrieved from GenBank to construct three data sets: the first composed of 152 ITS sequences, the second and third dataset were composed of sequences from 71 *Chlorophyllum* specimens with 3 (ITS, LSU and RPB2) and 4 markers (ITS, LSU, RPB2 and TEF1), respectively. Scientific names of species and GenBank accession numbers of the sequences included in the multigene analyses are listed in Table S1. Sampling was based on the most recent and extensive works regarding the genus (Ge et al. 2018; Dutta et al. 2020; Loizides et al. 2020; Sysouphanthong et al. 2021) (Table S1). *Leucoagaricus barssii* and *Leucoagaricus nympharum* were selected as outgroup (Vellinga et al. 2003). The sequences were aligned with MAFFT 7 (Katoh et al. 2019) using the G-INS-i alignment method. Alignments were manually inspected and adjusted using MEGA 6 (Tamura et al. 2013). Utilizing IQ-Tree software (Nguyen et al. 2015), ModelFinder (Kalyaanamoorthy et al. 2017) was employed for both datasets to estimate the best-fit partitioning strategy and the best-fit model of nucleotide evolution by data blocks. The partitions and evolutionary models selected for the first data set were ITS1, ITS2 (HKY+F+G4) and 5.8S (K2P). For the multigene datasets, the partitions and evolutionary models selected were the following: ITS1, ITS2 (HKY+F+G4), 5.8S, RPB2 1stpos (K2P+I), LSU, RPB2 3stpos (GTR+F+I+G4) and RPB2 2stpos (GTR+F+G4) for the 3 marker analysis; and ITS1, ITS2 (HKY+F+G4), 5.8S, TEF1 1stpos and TEF1 3rdpos (K2P+I), LSU, RPB2 1stpos and RPB2 2ndpos (K2P+I+G4), RPB2 3rdpos and TEF1 intron (K2P+I) and TEF1 2ndpos (HKY+F+G4) for the 4 marker analysis. Bayesian inference (BI) was performed following Robledo et al. (2020) in the CIPRES science gateway (Miller et al. 2010; http://www.phylo.org/). Maximum likelihood (ML) searches were conducted with IQ-TREE. The analyses initially involved 100 ML searches, each starting from one randomized stepwise addition parsimony tree. Branch supports were calculated using the ultrafast bootstrap approximation (UFBoot) in IQ-TREE with 1,000 replications (Hoang et al. 2018). A node was considered strongly supported with a Bayesian posterior probability (BPP) ≥ 0.95 or bootstrap support (BS) ≥95% (Hyde et al. 2013; Minh et al. 2020).

Results

Phylogenetic analyses

The ITS dataset contained 152 terminals and 778 characters, of which 249 were parsimony informative and 455 were variable. The analyses of Maximum likelihood (ML) and Bayesian inference (BI) resulted in similar topologies, and the ML tree is presented in Figure S1.

The multigene dataset of 3 markers (ITS, LSU and RPB2) included 71 terminals and 2303 characters, of which 124 were parsimony informative, 638 were variable, and 51 were constant. Both analysis, ML and BI, resulted in similar topologies, and the ML tree is presented in Figure S2. The multigene dataset of 4 markers (ITS, LSU, RPB2 and TEF1) included 71 terminals and 2863 characters, of which 167 were parsimony informative, 855 were variable, and 81 were constant. Both analysis, ML and BI, resulted in similar topologies, and the ML tree is presented in Figure 1.

The recovered topologies are consistent with previous works (Ge et al. 2018; Loizides et al. 2020; Dutta et al. 2020) and the six main clades previously recognized as sections, i.e. sect. *Chlorophyllum,* sect. *Ellipsoidospororum*, sect. *Endoptychorum,* sect. *Rhacodium*, sect. *Parvispororum* and sect. *Sphaerospororum* were recovered with maximum support.

The studied specimens represented three different taxa. Four specimens were confirmed as *Chlorophyllum molybdites*; one specimen as *Chlorophyllum hortense* grouping with samples from Bolivia, Colombia, USA, and China within sect. *Ellipsoidospororum*. Finally, one specimen was confirmed as an independent lineage clustering with *C. pseudoglobosum* within sect. *Chlorophyllum*, both related to *C. molybdites*. Morphological analysis of this specimen showed characters that differ from the other *Chlorophyllum* known species. Based on these morphological and phylogenetic evidence, we propose a new species described below.

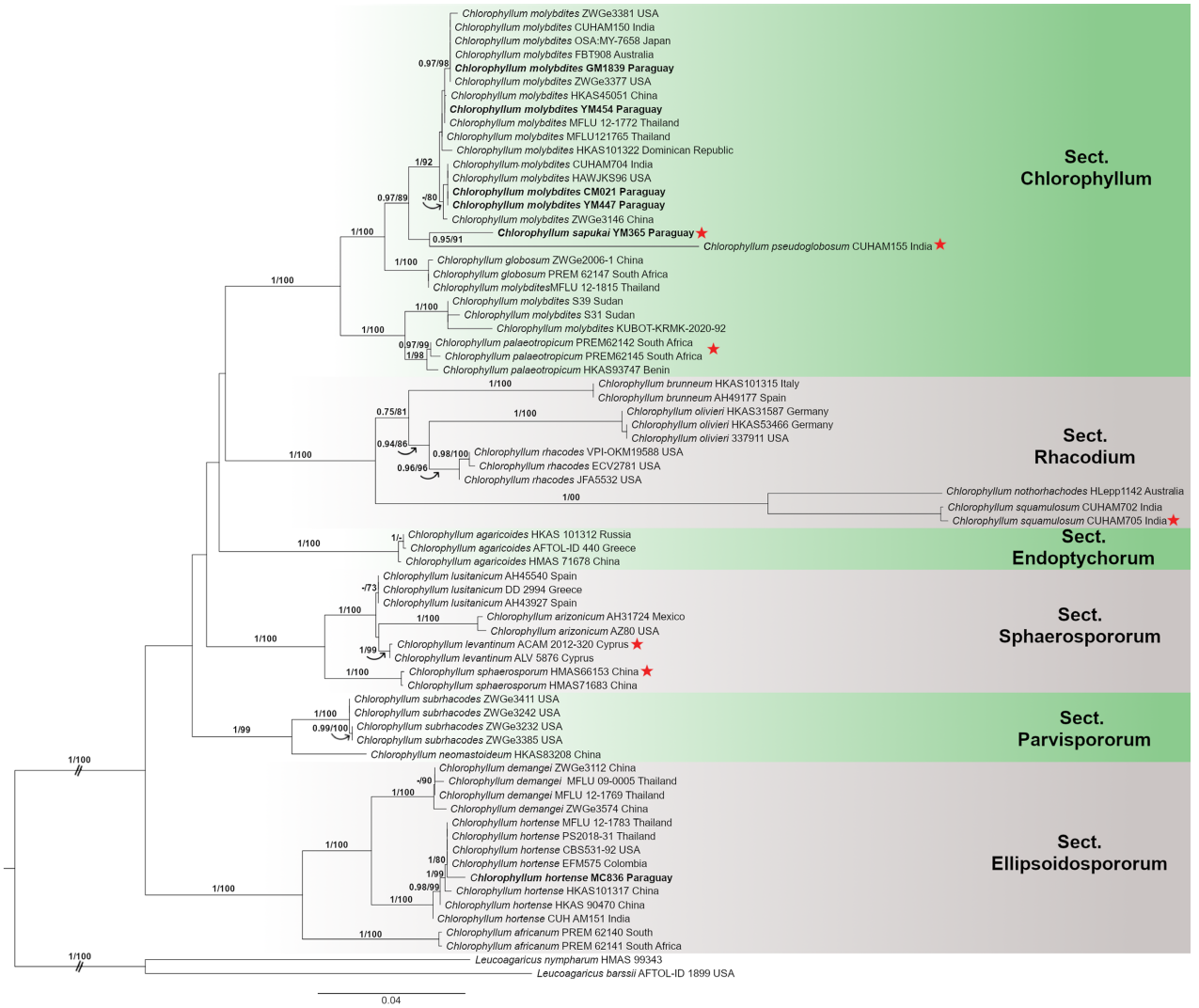

Figure 1. Maximum likelihood tree based on concatenated datasets of ITS + LSU + RPB2 + TEF1 sequence data. Newly generated sequences for the present study are in bold. Bayesian posterior probability (BPP) above 0.7 and Bootstrap (BS) values above 70% are shown above the branches. Red star = type specimen.

Taxonomy

Chlorophyllum sapukai Maubet, Campi & Robledo, sp. nov. (Fig. 2A–G)

MycoBank MB 844577

Type: Paraguay: Canindeyú Department, Mbaracayú Forest Nature Reserve, 24°08′53.1″S, 55°25′21.8″W, 29 January 2021; Y. Maubet 365 (FACEN 004388, holotype).

Etymology: the epithet "sapukai" is a word in the Guaraní language meaning "scream," and it refers to the scream of joy that the researchers gave when they found the specimen.

Description. Basidiomata agaricoid, large. Pileus 70–190 mm diam., hemispherical to convex at first, expanding to convex to broadly convex with age, whitish to cream buff, covered with two types of scales: (i) tiny, fibrillose, tufted, yellowish to cream buff squamules

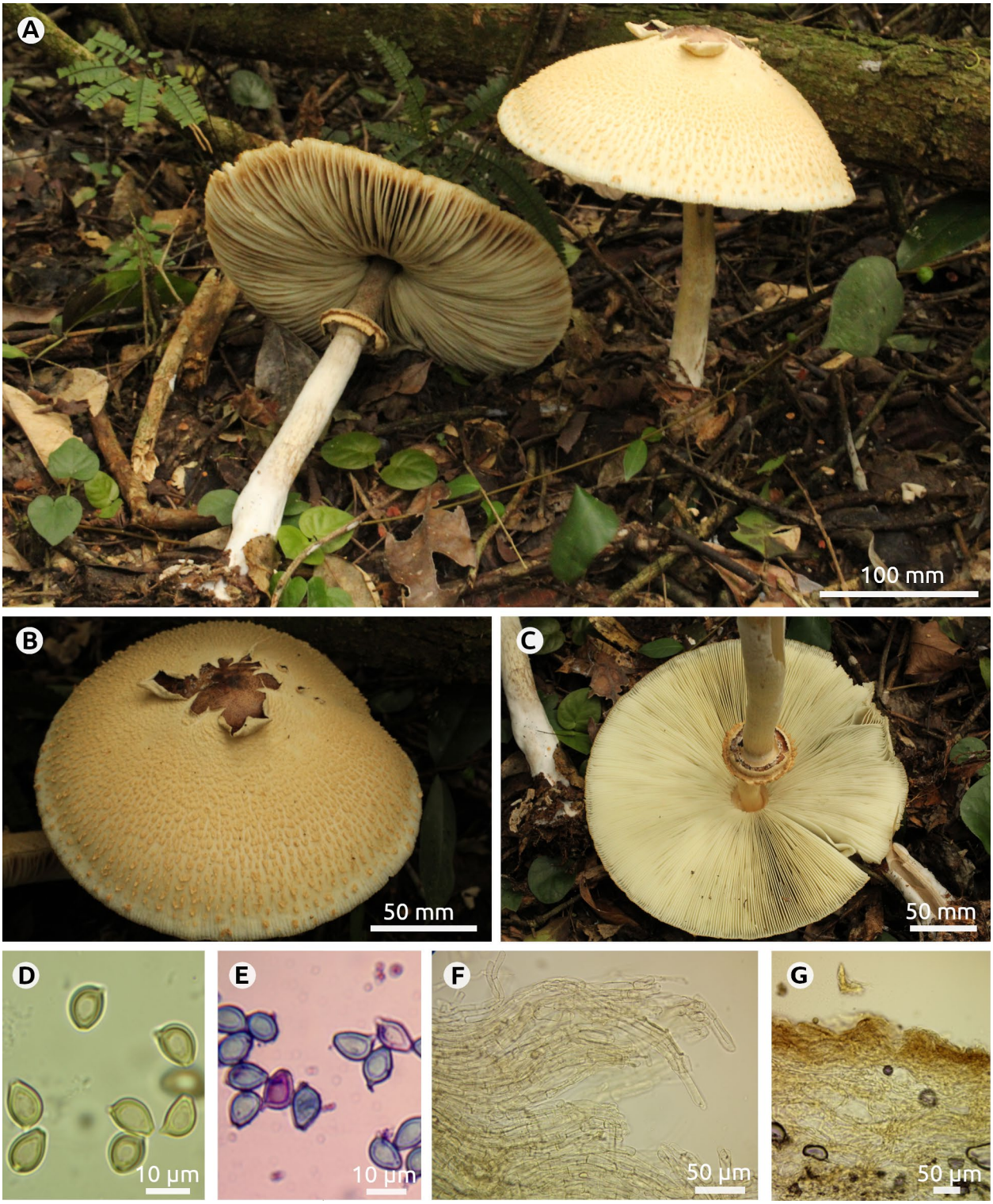

Figure 2. Morphological features of *Chlorophyllum sapukai* (FACEN 004388, holotype). A – fresh basidiomata; B – detail of the pileus showing scales; C – detail of the ring; D – basidiospores in KOH; E – basidiospores in cresyl blue; F – pileus covering at the squamules; G – pileus covering at the plate-like scales.

becoming scaly to minutely squarrose scaly toward the margin, evenly covering the surface; in the top-center covered with (ii) plate-like scales that are brownish, ocher to dark brown; surface short-striate; white margin slightly exceeding the lamellae (Fig. 2). Lamellae free; with 1–2 series of lamellulae, white to off-white when young, turning whitish to greenish-white when mature and greenish to grayish-brown when dry; crowded; 6–11 mm broad; edge whitish. Stipe 150–270 × 15–38 mm, subcylindrical, central, hollow, straight, or curved, with an annulus at the upper part, brown to chestnut towards the apex, not changing when bruised; surface fibrillose, shiny, pearlescent, covered with whitish fibrils below the annulus; base slightly enlarged covered with white, cottony mycelium. Context 4–6 mm thick in pileus, white, discoloring to cream buff when dry. Annulus double; upper portion concolorous to the stipe surface; lower surface white to yellowish-cream, with a distinct reddish-brown to brown margin; upper edge lacerated. Spore print greyish-green to yellowish-green. Odor foul, like bleach.

Basidiospores [60/2/1] 9–10 × 6.5–7.5 µm (mean 9.8 × 8.0 µm), Q = 1.34–1.37, Q_{av} = 1.2, amygdaliform to ovoid in side and frontal view with truncated apex, smooth and greenish to chestnut in KOH when mature, variably congophilic, weakly dextrinoid reaction observed in some immature basidiospores from young basidiome, thick-walled with central germ pore, metachromatic. Basidia 26–34 × 9.5–11.5 µm, clavate, hyaline, 4-spored. Cheilocystidia 35–50 × 14–20 µm, abundant in clusters, clavate to broadly clavate, hyaline to sometimes with brownish to fuscous brown vacuolar pigment. Pleurocystidia absent. Lamellar trama made up of slightly interwoven subcylindrical hyaline hyphae 3.5–9(13) µm diam. Pileus covering a parallelocutis composed of hyphae 3.5–10 µm diam., sometimes with vacuolar yellowish pigment, turning inflated 7.5–13(–17) µm diam. towards the pileus trama; the hyphae become erect at the squamules forming more or less a trichodermial palisade (Fig. 2F), terminal elements cylindrical, 23–50(–107) × 7.5–12.5(–17.5) µm diam. Plate-like scales detaching from the pileus, composed of hyphae disposed in a cutis, with vacuolar brown pigment (Fig. 2G). Pileus trama hyphae 5.5–12(–14.5) µm diam.; septate, hyaline, thin-walled. Caulocystidia absent. Stipitipellis hyphae (4.2–)5–10(–16) µm diam.; parallel, septate, hyaline, rarely branched, non-incrusted, thin-walled; numerous non-septate hyphae emerging from the stipitipellis up to 7 µm diam.; refringent with rounded apex. Stipititrama hyphae similar to stipitipellis hyphae 8–11.5(–16) µm; hyaline, parallel, thin-walled, septate with scarce clamp connections. Annulus hyphae from the external portion 3–7 µm diam.; tightly arranged, often branched; hyaline rarely with brown refringent internal content, thin-walled; internal portion composed of similar hyphae, slightly thicker 3.5–8 µm diam. Clamp connections not observed.

Distribution. So far, only reported in soil of humid tropical forest in the Atlantic Forest of Alto Paraná ecoregion, Paraguay.

Remarks. Macroscopically, the species is characterized by large agaricoid basidiomata, a hemispherical to broad convex pileus with a yellowish to cream buff surface that is covered centrally with plate-like scales and yellowish spore print. Microscopically, it is distinguished by a pilear covering in a parallelocutis that becomes a trichodermial palisade at the squamules with yellowish vacuolar pigments and non-differentiated cylindrical terminal elements. *Chlorophyllum sapukai* is similar to *C. pseudoglobosum*, *C. palaeotropicum* and *C. molybdites. Chlorophyllum sapukai* can be distinguished from these three species by its larger basidiomata with strong yellowish coloration of its pileus surface plus other features (Table 1). *Chlorophyllum pseudoglobosum* also differs by presenting basidiospores with a strong dextrinoid reaction, slightly smaller cheilocystidia, and a white spore print, along with its distribution in India (Crous et al. 2015). When compared to *C. palaeotropicum, C. sapukai* does not exhibit the same subglobose basidiospores that lack a germ pore and truncated apex, as well as differing by a trichodermal pileus covering with differentiated terminal elements (Ge et al. 2018). Additionally, *Chlorophyllum sapukai* resembles *C. globosum*, but *C. globosum* is characterized by a strong dextrinoid reaction of the basidiospores, a pileus covering as a hymenidermal layer and being known from Africa and Asia (Ge et al. 2018). Finally, *C. molybdites* represents a morphologically variable species, but differs in the coloration of the pileus which is never yellow, disposition of the pileus cover elements at the squamules as a hymenidermal layer with differentiated terminal elements (Alves et al. 2019; Sysouphanthong et al. 2021). Another species with similar macroscopically appearance is *Clarkeinda trhacodes* (Berk.) Singer, but it differs by having a smaller pileus covered with brownish squamules and presenting a volva. In addition, microscopy reveals smaller basidiospores that have only been recorded for South-eastern Asia (Hosen & Ge 2012; Sysouphanthong et al. 2021).

After comparing these results with descriptions of neotropical *Chlorophyllum* from South America, the most relevant morphological difference is the coloration of the pileus and squamules and the pileus covering at the squamules of *C. sapukai* disposed as a trichodermial palisade with non-differentiated, cylindrical terminal elements (Table 1).

Chlorophyllum hortense (Murrill) Vellinga

(Fig. 3A–G)

MycoBank MB 374396

Description. Basidiomata agaricoid, small to medium-sized. Pileus 20–65 mm wide, conical when young and convex to plano-convex when mature; surface smooth, glabrous, yellowish-white with creamy-yellowish squamules concentrating on its central honey-sepia umbo 20 mm wide and 10 mm tall; margin striate. Lamellae free, crowded, white, turning yellowish-green when dry, up to 5 mm broad. Stipe 30–45 × 3–8 mm, up to 10 mm wide at the base, central, cylindrical, hollow, fibrous, yellowish-white, turning crimson red when damaged. Annulus

Table 1. Comparative table between *Chlorophyllum* species, based on specimens studied and literature.

Species Reference Origin	Basidiomata		Pileipellis	Basidiospores		Cheilocystidia (shape and size)	Clamp connection
	Pileus (size and shape)	Scales		Shape and size	IKI/cresyl blue		
Chlorophyllum molybdites Massee 1898 Brazil	Ø 15–20 cm	ND	ND	7–8 × 5 µm	ND	ND	ND
Chlorophyllum molybdites Alves et al. 2019 Brazil	Ø 5–11 cm Y: OV, SuH Mat: Pl-C	Brownish, uplifted or flat, concentrated near the center	Palisade; differentiated terminal elements	EL; ELO 9.4–11 × 6–8 µm	+ / ND	B-Cl; SPH 18–38 × 14–20 µm	ND
Chlorophyllum molybdites Ge & Yang 2006 China	Ø 8–17 cm Y: H, C Mat: Pl-C	Covered with brown squamules	Palisade; differentiated terminal elements	B-AM 9–10 × 6.5–8 µm	+ / ND	B-Cl; SPH (numerous) 21–40 × (9) 13–21 µm	ND
Chlorophyllum molybdites Sundberg 1971 USA	Ø 4.5–15.5 cm Y: OV, OB Mat: Pl-C	Central disc diffracted to areolate scaly, elsewhere scales appressed, fibrillose toward the margin	ND	OV; S-EL 10–12 × 7.7–9 µm	+ / –	N-CL; B-CL; SPH; SSC (abundant to sometimes scattered) 21.9–47.4 × 10.3–25.5 µm	Always present in pileus trama
Chlorophyllum molybdites Dutta et al. 2020 India	Ø 4.8–9.3 cm Y: C / B-C Mat: Pl-C / AP	Scales, entire at the disc, elsewhere disrupting into small patches	Palisade; differentiated terminal elements	EL 10–12 × 7.5–7.9 µm	+ / ND	PY; SPH 22–26.5 × 15–18 µm	Not found
Chlorophyllum molybdites This study Paraguay	Ø 7–11.5 cm Y: SuH Mat: C / B-C	Scales, entire at the disc, elsewhere disrupting into small patches	Hymenidermal layer; differentiated terminal elements	B-AM 9.5–12 × 7.5–8.5 µm	+ / ±	B-CL; OV 25–47(–50) × 13.5–22 µm	Variably, present in some specimens
Chlorophyllum sapukai This study Paraguay	Ø 7–19 cm Y: H / C Mat: B-C	Fibrillose, tufted, yellowish adpressed squamules; dark brownish to orange, plate-like squamule at center	Trichodermial palisade at the squamules, not differentiated terminal elements	AM; OV 9–10 × 6.5–7.5 µm	± / +	CL; B-CL (abundant in clusters) 35–50 × 14–20 µm	Not found
Chlorophyllum pseudoglobosum Crous et al. 2015 India	Ø 4.5–6.2 cm Y: C (distinct umbo at the disc)	Scales in concentrically arranged patches, continuous on disc, elsewhere disrupted	Hymeniderm, differentiated terminal elements	SuG; EL 10–10.7 × 8–8.5 µm	+ / +	B-CL 32–38× 17–18 µm	In stipitipellis and stipe trama
Chlorophyllum globosum Ge et al. 2018 China	Ø 5–20 cm, Y: OV / SuG / P Mat: C / B-C	Squamules forming a felted or fibrillose background, intact at disc, elsewhere diffracting	Hymeniderm, differentiated terminal elements	B-AM; OV in frontal view 11.5–12 × 8.5–9 µm.	+ / +	CL (occasional long stalk) 42–65 × 18–29 µm	Not observed
Chlorophyllum palaeotropicum Ge et al. 2018 South Africa	Ø 5–10 cm Y: H / C Mat: C / B-C	Fibrillose, tufted, reddish-white to brownish-orange squamules; brownish to orange grey, plate-like squamules at the centre	Trichoderm, differentiated terminal elements	EL, OBL in side and frontal view 8.5–11 × 7–9 µm	+ / +	CL; B-CL; N-CL 20–55 × 10–15 µm	Not common, in young basidia and tissue of annulus

IKI+ = dextrinoid reaction, ND = not described, Y = young, Mat = mature, OV = ovoid, SuH = subhemispheric, Pl-C = plano-convex, H = hemispherical, C = convex, OB = obtuse, B-C = broadly convex, AP = applanate, SuG = subglobose, P = parabolic, EL = ellipsoid, ELO = elongate, B-AM = Broadly amygdaliform, S-EL = shortly ellipsoid, OBL = oblong, B-CL = broadly clavate, CL = clavate, N-CL = narrowly clavate, SPH = sphaeropedunculate, SSC = subsaccate, Py = Pyriform

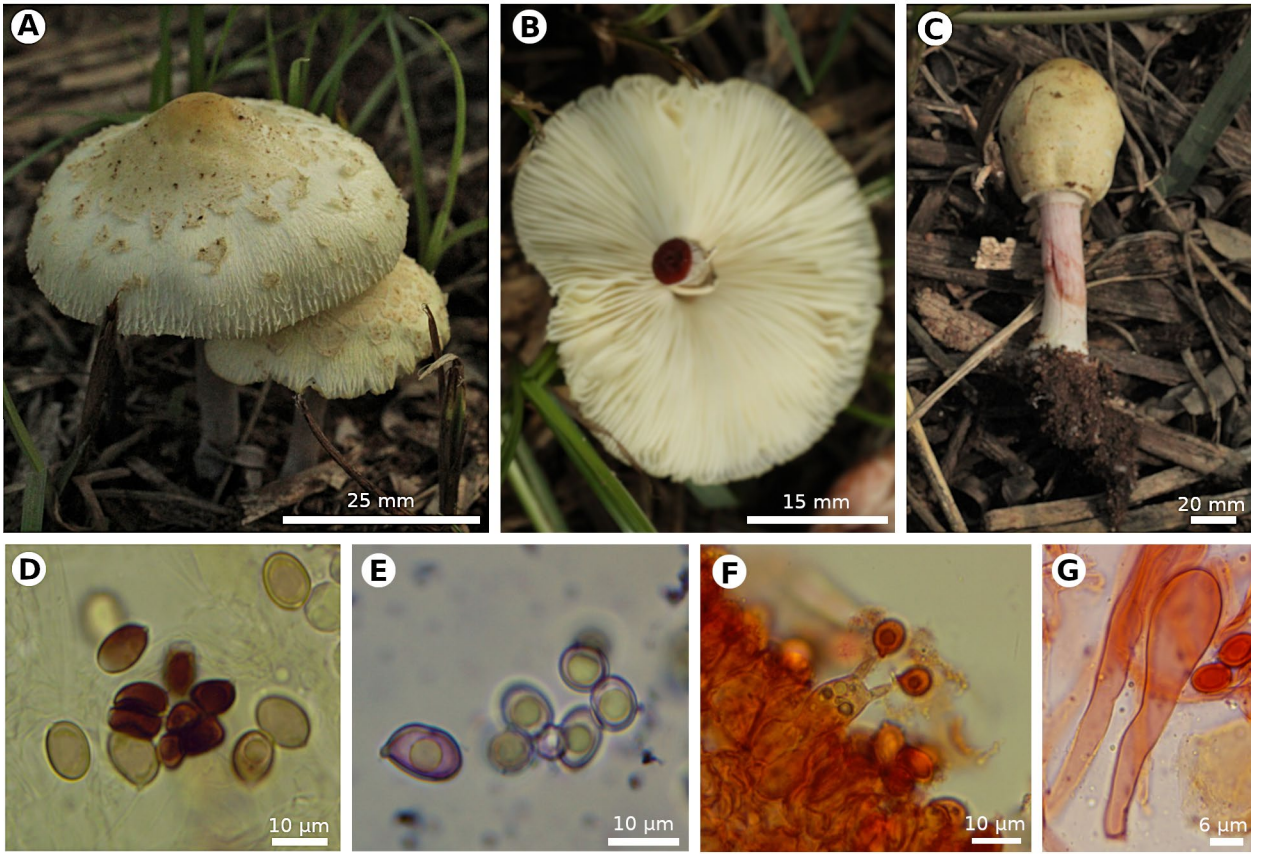

Figure 3. Morphological features of *Chlorophyllum hortense* (FACEN 004971). A – fresh basidiomata; B – cut of the stipe showing strong coloration change; C – bruised stipe also showing coloration change; D – basidiospores in Melzer; E – basidiospores in cresyl blue; F – two-spored basidium; G – differentiated terminal element of the squamules on pileus.

double, ascending; color similar to stipe. Basidiospores [90/3/1] 8.2–13.7 × 5.0–8.9 µm (mean 9.8 × 6.8 µm), Q = 1.40–1.46, Q_{av} = 1.4, broadly ellipsoid to slightly ovoid; yellowish-green in KOH; congophilic and metachromatic, mature basidiospores strongly dextrinoid, smooth, thick-walled, without germ pore; with sublateral hilar appendix. Basidia 25–34 × 8–11 µm; clavate, thin-walled, hyaline, 2–sterigmate; sterigmata up to 6.7 µm long. Pleurocystidia absent. Cheilocystidia 34–60 × 8–11 µm, clavate, cylindrical to narrowly cylindrical, slightly flexuose, thin-walled. Lamellar trama made up of subregular, thin-walled, hyaline hyphae 3–14 µm wide. Pileus covering radially arranged as a cutis of thin-walled hyphae 2.3–7.2 µm wide, hyaline in KOH. Terminal elements differentiated in squamules on pilear surface, 31–112 × 8–17 µm, narrowly clavate to broadly clavate; cylindrical, hyaline, thin-walled. Stipitipellis a cutis of parallel hyphae longitudinally arranged, 2.4–5.7 µm wide; yellowish in KOH. Annulus made up of hyaline to yellowish interwoven hyphae 2.1–6.3 µm wide. Clamp connections rarely observed in stipiticutis; absent in all other tissues analyzed.

Specimen examined. PARAGUAY. Central Department, Ita city, Ñanduá Country House, 25°33′49.9″S, 57°19′52.52″W, gregarious, on grassy soil, modified by the presence of farm animals, 09 April 2022, M. Campi 836 (FACEN 004971).

Distribution. Widespread in tropical and subtropical regions. In South America, recorded in Bolivia (Ge et al. 2018); Brazil (Sobestiansky 2005; Nascimento & Alves 2014), Colombia (Johnson 1999 [as *Leucoagaricus hortensis*]; Luna-Fontalvo et al. 2021), and in Argentina (Domínguez et al. 2021).

Remarks. *Chlorophyllum hortense* was first described from Alabama, USA as *Lepiota hortensis*, and it was moved through other genera given its morphological characteristics until phylogenetic analysis confirmed its placement within *Chlorophyllum* (Vellinga 2002; Vizzini et al. 2014). *C. hortense* is characterized by medium-sized, whitish basidiomata with whitish to yellowish scales on the pileus and a yellowish umbo. It also displays a strong reddening reaction when the stipe is bruised, a double annulus, basidiospores without a germ pore, 2-spored basidia and cylindrical cheilocystidia (Vellinga 2003). The morphological characteristics of the studied specimen coincide with those previously mentioned.

Section *Ellipsoidospororum* comprises three species: *Chlorophyllum africanum* Z. W. Ge & A. Jacobs, *Chlorophyllum demangei* (Pat.) Z. W. Ge & Zhu L. Yang, and *C. hortense* (Ge et al. 2018). *Chlorophyllum africanum* is similar to *C. hortense* in the size of the basidiomata, ellipsoid basidiospores, and subcylindrical cheilocystidia, but differs in the presence of 4-spored basidia and the absence of a change in coloration when bruised and that it is known only from South Africa (Ge et al. 2018). *Chlorophyllum demangei* is also very similar to *C. hortense*, differing by the 4-spored basidia and so far, only known

from Southeast Asia (Ge et al. 2018; Sysouphanthong et al. 2021).

This is the first report of the species *Chlorophyllum hortense* for Paraguay.

Chlorophyllum molybdites (G. Mey.) Massee
(Fig. 4A–E)

MycoBank MB 604726

Description. Basidiomata agaricoid, medium-sized. Pileus 70–115 mm diam., convex when young, becoming broadly convex to plano-convex when mature, sometimes displaying a small umbo at the center with white to cream surface, covered radially with appressed, concolorous tiny scales, covered at the center with plate-like scales in some specimens scattering into small pieces towards the margin, in age areolate-scaly; center scales greyish-brown, brownish-orange to light brown, or greyish-red; margin entire. Context white to cream. Lamellae free, crowded with 1–2 series of lamellulae, white to cream when young turning greenish at maturity; even edges, sometimes slightly wavy; 4–5 mm broad. Stipe 50–130 × 5–7 mm, central, cylindrical, hollow, chestnut to brownish-cream turning brownish upon bruising or when dried; bulbous base (9–13 mm diam.) covered with abundant cottony white mycelium. Annulus double; membranous, movable; white to cream on upper side and lower side to concolorous, brownish plate-like scales. Spore print pale green. Odor earthy, mushroom-like.

Basidiospores [300/10/4] (8.5–)9.5–12(–14.5) × (6.7–)7.5–8.5(–9) µm (mean 10.4 × 7.6 µm), Q = 1.3–1.4, Q_{av} = 1.37, broadly amygdaliform, pale green, thick-walled, smooth with a truncated germ pore, dextrinoid, some immature basidiospores staining bluish-violet in cresyl blue. Basidia 31.5–38(–42) × 11.5–13.5 µm, clavate, thin-walled 4-spored, hyaline or with yellowish content; guttules variably present; sterigmata 2–4 µm long. Subhymenium coralloid. Pleurocystidia absent. Cheilocystidia 25–47(–50) × 13.5–22 µm, broadly clavate to ovoid, rarely spheropedunculate, yellowish or with golden-brown pigmentation, thin-walled; scattered to abundant. Pileus covering a hymenidermal layer of densely packed thin-walled hyphae at the squamules (Fig. 4E), terminal elements clavate to subfusiform, 20–32(–47) × 11–17 µm. Lamella trama subparallel to irregular hyphae (4)6.5–13 µm diam.; hyaline, thin-walled. Pileus trama made up of interwoven, hyaline, thin-walled hyphae 4–5 µm diam. Stipitipellis a cutis made up of parallel to subparallel, hyaline to pale yellow, thin-walled, hyphae 4–10(–15) µm diam., sometimes refringent. Stipe trama comprising parallel, hyaline, thin-walled hyphae. Annulus made up of hyaline, rarely branched, thin-walled hyphae 3–6.5(10) µm diam. Caulocystidia absent. Clamp connections rarely found in some specimens, completely absent in others.

Specimens examined. PARAGUAY. Guairá Department, Colonia Independencia city, Tilinski Hotel, 25°42′41.2″S, 56°12′20.4″W, terrestrial, found on soil, grass or on roadside, 20 November 2021, Y. Maubet 447, FACEN 004890; Central

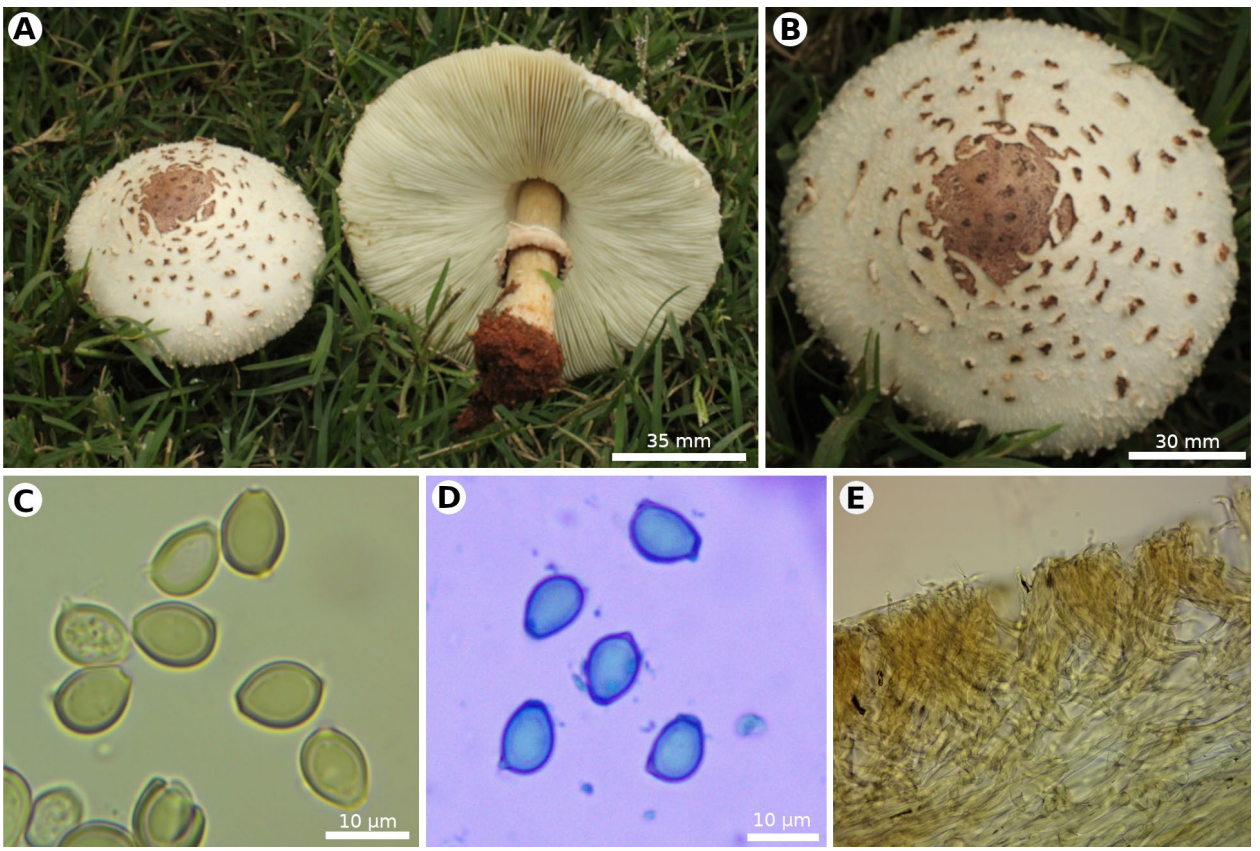

Figure 4. Morphological features of *Chlorophyllum molybdites*. A – fresh basidiomata (FACEN 004302); B – detail of pileus (FACEN 004302); C – basidiospores in KOH (FACEN 004891); D – basidiospores in cresyl blue (FACEN 004302); E – pileus covering at the squamules (FACEN 004889).

Department: San Lorenzo city, University campus (UNA), 25°20′08.6″S, 57°31′03.2″, 30 January 2018, C. Mancuello 021, FACEN 004302; Central Department: San Lorenzo city, University campus (UNA), 25°20′03.1″S, 57°31′05.4″W, 11 October 2021, Y. Maubet 454, FACEN 004891; Central Department: Ypané city, 25°27′3.31″S, 57°30′59.78″W, 30 December 2019, G. Marín 1839, FACEN 004889.

Distribution. In Paraguay, widespread in the South, recorded from the Guaira and Central departments, in the ecoregions of Atlantic Forest and Humid Chaco. Distributed worldwide including Africa, Asia, Central America, North America, Oceania and South America (Reid & Eicker 1991; Natarajan & Kaviyarasan 1991; Vellinga 2001; Ge & Yang 2006; Alves et al. 2019). In South America, recorded in Argentina (Raithelhuber 1974, 1988, 1991, 2004; Singer 1950, 1969; Singer & Digilio, 1952; Spegazzini 1926a, b; Wright & Albertó 2002; Romano et al. 2013), Brazil (Massee 1898; De Meijer et al. 2007; Alves et al. 2019), Colombia (Franco-Molano et al. 2010), and Venezuela (Dennis 1970).

Remarks. The morphological characteristics that have been used to define the species include (i) agaricoid basidiomata with medium to large sized pileus, (ii) the free, crowded lamellae that turn greenish white to pale green when dry, (iii) thick-walled, pale green, ellipsoid basidiospores with a prominent truncated apex without a hyaline cover, and (iv) pyriform to spheropedunculate cheilocystidia (Ge & Yang 2006; De Meijer et al. 2007; Dutta et al. 2020).

In South America, *Chlorophyllum molybdites* was originally documented in Brazil (Massee 1898). The closest material to the type locality is from the state of Piauí in northern Brazil (Alves et al. 2019). Our specimens are consistent with the description of the specimens identified in Northern Brazil and with specimens identified in USA (Sundberg 1971). The only observed difference was that the Paraguayan specimens displayed larger basidia and cheilocystidia than those from Brazil (Alves et al. 2019).

Key to Neotropical *Chlorophyllum* species

1 Spore print green to yellowish **2**
Spore print whitish **3**

2(1) Squamules with a hymeniderm structure with differentiated terminal elements; basidiospores dextrinoid . . . . . ***C. molybdites***
Squamules with erected hyphae as a trichodermial palisade with non-differentiated terminal elements; basidiospores weakly dextrinoid when immature . . . . . ***C. sapukai***

3(1) Basidiospores with a germ pore. . . . . ***C. rhacodes***
Basidiospores without a germ pore . . . . . ***C. hortense***

Discussion

From the results of the multigene analyses, the Paraguayan specimens *Chlorophyllum molybdites* and the newly identified *C. sapukai*, cluster within sect. *Chlorophyllum*, whilst *Chlorophyllum hortense* clustered within sect. *Ellipsoidosporum* (Figs 1, S1–S2). Morphologically, members of section *Chlorophyllum* possess agaricoid basidiomata with central, plate-like scales on the pileus, olive to greenish-white, ellipsoid to amygdaliform, thick-walled, truncate basidiospores (except *C. palaeotropicum*, which possesses subglobose basidiospores without a germ pore), and broadly clavate to sphaeropedunculate cheilocystidia (Ge et al. 2018). The morphological characteristics of *C. sapukai* match the general description of section *Chlorophyllum*.

Chlorophyllum molybdites appears to be a polymorphic species and presents variations in macro- and micromorphology between specimens throughout its distribution. For instance, pileus size can range from medium 5−11 cm in diam. (Alves et al. 2019; Dutta et al. 2020) to large 15–20 cm in diam. (Massee 1898; Sundberg 1971; Ge & Yang 2006). Basidiospore sizes have been reported as 9–12 × 6.5–9 µm (Sundberg 1971; Ge & Yang 2006; Alves et al. 2019; Dutta et al. 2020), but other specimens are described as having smaller basidiospores (7–8 × 5 µm) (Massee 1898). Additionally, while the presence of clamp connections in the pileus trama is described for some specimens (Sundberg 1971), clamp connections were rarely found or absent in most other specimens (Vellinga 2003; Alves et al. 2019; Dutta et al. 2020). In this study, specimens presented varying characteristics. Microscopically, the samples FACEN 004890, FACEN 004302, and FACEN 004891 presented many cheilocystidia, whilst in sample FACEN 004889 were scarce.

Despite these morphological differences, our multigene analysis revealed *Chlorophyllum molybdites* as a single, strongly supported lineage, including specimens from India, China, USA, Australia, the Dominican Republic, and now Paraguay. However, within the multigene analysis, a clade of sequences identified in GenBank as *C. molybdites* from Sudan and India clustered as a sister group of the species *C. palaeotropicum* and could comprise a new taxon, which suggests that the characteristics used to morphologically identify *C. molybdites* may be unreliable to differentiate it from other species within sect. *Chlorophyllum*.

Additionally, four species described from Neotropical South America and Caribbean have been considered synonyms of *C. molybdites*: *Agaricus glaziovii* Berk. from Brazil, *Agaricus guadelupensis* Pat. from Guadaloupe island, *Annularia camporum* Speg. from Argentina and *Chlorophyllum esculentum* Masse from Guyana. The original descriptions of these synonyms, despite being short and concise, mention characters that do not coincide with *C. sapukai*. The color of the pileus surface is whitish in the four mentioned species, and the size of the basidiospores is slightly different, smaller in *C. esculentum* (7–8 × 5 µm) and larger in the other species: 11–12 × 8 µm in *Annularia camporum* (Spegazzini 1898), 11 × 8 µm in *Agaricus guadelupensis* (Patouillard 1899) and 16 µm in *Agaricus glaziovii* (Berkeley 1880).

This is the first systematic approach towards describing the diversity of *Chlorophyllum* in Paraguay. The identification of a new species, *C. sapukai*, shows that the diversity of *Chlorophyllum* in the Neotropics still needs further research, particularly including a large geographic

sampling were *Chlorophyllum molybdites* specimens identified based on morphology have been reported.

Acknowledgements

This work was carried out within the framework of the project PINV18-699 Development of molecular tools for the taxonomic identification of fungi from 3 Ecoregions of Paraguay, funded by the Consejo Nacional de Ciencias y Tecnología (CONACYT). The authors thank Professor Griselda Marín, Claus Brehm and Amambay Ruiz Díaz for the field assistance. Anonymous reviewers are generously acknowledged for discussions and comments that improved the manuscript. Additionally, Sarah Torhan, MSc. is gratefully recognized for her revision of the manuscript in English and for her valuable contributions to the writing.

We thank the Fundación Moisés Bertoni and the Facultad de Ciencias Exactas y Naturales for facilitating both the field and laboratory work

Supplementary electronic materials

Figure S1. Maximum likelihood tree based on dataset of 152 ITS sequence data. Newly generated sequences for the present study are in bold. Genbank accession numbers precede the name of each terminal. Bayesian posterior probability (BPP) above 0.7 and Bootstrap (BS) values above 70% are shown above the branches. Download file

Figure S2. Maximum likelihood tree based on concatenated datasets of ITS + LSU + RPB2 sequence data. Newly generated sequences for the present study are in bold. Bayesian posterior probability (BPP) above 0.7 and Bootstrap (BS) values above 70% are shown above the branches. Red star = type specimen. Download file

Table S1. Taxon sampling: voucher specimens/cultures and GenBank accession numbers. New sequences generated in this study are in bold. (T) = type specimen. Download file

References

Alves, M. H., da Cruz, M. O. & do Nascimento, C. C. 2019. First record of *Chlorophyllum molybdites* (G. Mey.) Massee (*Basidiomycota*, *Agaricaceae*) from Piauí state, Brazil. *Check List* 15(4): 695–699. https://doi.org/10.15560/15.4.695

Berkeley, M. A. 1880. Fungi Brasiliensis provincia Rio de Janeiro a clar Dr. Glaziou lecti (p. 31–34). In: Warming E. (ed.), *Symbolae and floram brasiliae centralis cognocedam. Videnskabelige meddelelser fra den Naturhistoriske forening i Kjöbenhavn*, 31.

Campi, M., de Madrignac, B. & Flecha, A. 2018. *Hongos del Campus Universitario U.N.A.* Facultad de Ciencias Exactas y Naturales UNA, Paraguay.

Cubeta, M. A., Echandi, E., Abernethy, T. & Vilgalys, R. 1991. Characterization of anastomosis groups of binucleate *Rhizoctonia* species using restriction analysis of an amplified ribosomal RNA gene. *Molecular Plant and Pathology* 81: 1395–1400. https://doi.org/10.1094/Phyto-81-1395

Crous, P. W., Wingfield, M. J., Guarro, J., Hernández-Restrepo, M., Sutton, D. A., Acharya, K., et al. 2015. Fungal Planet description sheets: 320–370. *Persoonia* 34: 167–266. https://doi.org/10.3767/003158515X688433

Dennis, R. W. G. 1970. Fungus flora of Venezuela and adjacent countries. *Kew Bulletin Additional Series* 3: 1–531.

Dominguez, L. S., Crespo, E. M., Daniele, G. M., Gómez-Montoya, N., Moyano, A. J., Nouhra, E., Robledo, G. & Urcelay, C. 2021. *Hongos del Centro de Argentina, Guía ilustrada de las especies más comunes, venenosas y comestibles*. Ecoval, Argentina.

Dutta, A. K., Bera, S., Paloi, S., Rakshit, S., Tarafder, E., Sherpa, A. R. & Acharya, K. 2020. Lepiotaceous fungi of West Bengal, India: the genus *Chlorophyllum*. *Phytotaxa* 451(2): 113–131. https://doi.org/10.11646/phytotaxa.451.2.2

Franco-Molano, A. E., Corrales, A. & Vasco-Palacios, A. M. 2010. Macrohongos de Colombia II. Listado de especies de los órdenes *Agaricales*, *Boletales*, *Cantharellales* y *Russulales* (Agaricomycetes, *Basidiomycota*). *Atual Biología* 32 (92): 89–114.

Gardes, M. & Bruns, T. D. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Ge, Z. W. & Yang, Z. L. 2006. The genus *Chlorophyllum* (*Basidiomycetes*) in China. *Mycotaxon* 96: 181–191.

Ge, Z. W., Jacobs, A., Vellinga, E. C., Sysouphanthong, P., van der Walt, R., Lavorato, C., An, Y. F. & Yang, Z. L. 2018. A multi-gene phylogeny of *Chlorophyllum* (*Agaricaceae*, *Basidiomycota*), new species, new combination and infrageneric classification. *MycoKeys* 32: 65–90. https://doi.org/10.3897/mycokeys.32.23831

Góes-Neto, A., Loguercio-Leite, C. & Guerrero, R. T. 2005. DNA extraction from frozen fieldcollected and dehydrated herbarium fungal basidiomata: performance of SDS and CTAB-based methods. *Biotemas* 18(2): 19–32.

Hoang, D. T., Chernomor, O., von Haeseler, A., Minh, B. Q. & Vinh, L. S. 2018. UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* 35: 518–522. https://doi.org/10.1093/molbev/msx281

Hosen, I. & Ge, Z. W. 2012. *Clarkeinda trachodes* (*Agaricales*, *Basidiomycetes*), first record from Bangladesh. *Mycotaxon* 118(1): 331–336. https://doi.org/10.5248/118.331

Hyde, K. D., Udayanga, D., Manamgoda, D. S., Tedersoo, L., Larsson, E., Abarenkov, K., Bertrand, Y. J. K, Oxelman, B., Hartmann, M., Kauserud, H., Ryberg, M., Kristiansson, E. & Nilsson, R. H. 2013. Incorporating molecular data in fungal systematics: a guide for aspiring researchers. *Current Research in Environmental and Applied Mycology* 3: 1–32.

Johnson, J. 1999. Phylogenetic relationships within *Lepiota* sensu lato based on morphological and molecular data. *Mycologia* 91(3): 443–458. https://doi.org/10.2307/3761345

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K., Von Haeseler, A. & Jermiin, L. S. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 14(6), 587–589. https://doi.org/10.1038/nmeth.4285

Katoh, K., Rozewicki, J. & Yamada, K. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20(4): 1160–1166. https://doi.org/10.1093/bib/bbx108

Kirk, P. M, Cannon P. F., Minter D. W. & Stalpers, J. A. 2011. *Dictionary of Fungi*. CABI, USA.

Kuo, M. & Ortiz-Santana, B. 2020. Revision of leccinoid fungi, with emphasis on North American taxa, based on molecular and morphological data. *Mycologia* 112(1): 197–211. https://doi.org/10.1080/00275514.2019.1685351

Largent, D. L., Johnson, D. & Watling, R. 1977. *How to Identify Mushrooms to Genus III: Microscopic Features*. Mad River press Inc., Eureka, C.A., USA.

Lodge, D. J., Ammirati, J. F., O'Dell, T. E., Mueller, G. M., Huhndorf, S. M., Wang, C. J., Stokland, J. N., Schmit, J. P., Ryvarden, L., Leacock, P. R, Mata, M., Umaña, L., Wu, Q. & Czederpiltz, D. L. 2004. Terrestrial and lignicolous macrofungi. In: Mueller, G. M., Bills, G. F. & Foster, M. S., *Biodiversity of Fungi, Inventory and Monitoring Methods*, pp. 127–158. USGS Publications Warehouse, USA. https://doi.org/10.1016/B978-012509551-8/50011-8

Loizides, M., Alvarado, P., Polemis, E., Dimou, D. M., Zervakis, G. I., Thines, M., Tellef, S., Konstantinou, G. & Gube, M. 2020. Multiple evolutionary origins of sequestrate species in the agaricoid genus *Chlorophyllum*. *Mycologia* 112(2): 400–422. https://doi.org/10.1080/00275514.2020.1712179

Luna-Fontalvo, J. A., Barrios, A., Abaunza, C., Ramírez-Roncallo, K. & Negritto, M. A. 2021. New records of *Agaricaceae* (*Agaricales*, *Basidiomycota*) from Colombia. *Check List* 17(5): 1383–1393. https://doi.org/10.15560/17.5.1383

Massee, G. E. 1898. Fungi exotici, I. *Bulletin of Miscellaneous Informations of the Royal Botanical Gardens Kew* 6: 113–136.

Matheny, P. B. 2005. Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*; *Agaricales*). *Molecular Phylogenetics & Evolution* 35: 1–20. https://doi.org/10.1016/j.ympev.2004.11.014

Meijer, A. A. R. de, Amazonas, M. A. L. de A., Rubio, G. B. G. & Curial, R. M. 2007. Incidences of poisonings due to *Chlorophyllum molybdites* in the state of Paraná, Brazil. *Brazilian Archives of Biology and Technology* 50(3): 479–488. https://doi.org/10.1590/S1516-89132007000300014

Miller, M. A., Pfeiffer, W. & Schwartz, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop* (GCE), pp. 1–8. New Orleans, LA. https://doi.org/10.1109/GCE.2010.5676129

Minh, B. Q., Hahn, M. W. & Lanfear, R. 2020. New methods to calculate concordance factors for phylogenomic datasets. *Molecular Biology and Evolution* 37: 2727–2733. https://doi.org/10.1093/molbev/msaa106

Nascimento, C. C. & Alves, M. H. 2014. New records of *Agaricaceae* (*Basidiomycota*, *Agaricales*) from Araripe National Forest, Ceará State, Brazil. *Mycosphere* 5(2): 319–332. https://doi.org/10.5943/mycosphere/5/2/6

Natarajan, K. & Kaviyarasan, V. 1991. *Chlorophyllum molybdites* poisoning in India – A case study. *Mycologist* 5(2): 70.

Nguyen, L. T., Schmidt, H. A., Von Haeseler, A. & Minh, B. Q. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Patouillard, N. T. 1899. Champignons de la Guadeloupe. *Bulletin de la Société Mycologique de France* 15: 191–209.

Raithelhuber, J. 1974. *Hongos argentinos I*. Compañía Impresora Argentina, Argentina.

Raithelhuber, J. 1988. *Flora mycologica argentina: hongos II*. Ed. Stuttgart. Mycosur, Argentina.

Raithelhuber, J. 1991. *Flora mycologica argentina: hongos III*. Ed. Stuttgart. Mycosur, Argentina.

Raithelhuber, J. 2004. *Nueva flora micológica Argentina*. Stuttgart. Mycosur, Argentina.

Reid, D. A. & Eicker, A. 1991. A comprehensive account of *Chlorophyllum molybdites*. *Botanical Bulletin Academia Sinica* 32: 317–334.

Robledo, G. L., Palacio, M., Urcelay, C., Vasco-Palacios, A. M., Crespo, E., Popoff, O., Põldmaa, K., Ryvarden, L. & Costa-Rezende, D. H. 2020. Mystery unveiled: *Diacanthodes* Singera lineage within the core polyporoid clade. *Systematics and Biodiversity* 19: 725–741. https://doi.org/10.1080/14772000.2020.1776784

Romano, G. M., Iannone, L., Novas, M. V., Carmarán, C., Romero, A. I., Lopez, S. E. & Lechner, B. E. 2013. Hongos tóxicos en la ciudad de Buenos Aires y alrededores. *Medicina* 73: 406–410.

Salazar-Vidal, V. E., Dibán, M. J. & Ponce, S. 2017. Revisión del género *Macrolepiota* Singer (*Agaricales*, *Basidiomycota*) en Chile. *Boletín Micológico* 32(1): 13–18.

Singer, R. 1950. Type studies on Basidiomycetes IV. *Lilloa* 23: 147–246.

Singer, R. 1969. Mycoflora australis. Beih. *Nova Hedwigia* 29: 1–405.

Singer, R. & Digilio A. P. L. 1952. Pródromo de a flora agaricina Argentina. *Lilloa* 25: 6–461.

Spegazzini, C. 1898. Fungi Argentini novi vel critici. *Anales del Museo Nacional de Historia Natural Buenos Aires* 6: 81–288.

Spegazzini, C. 1926a. Contribución al conocimiento de la flora micológica de las Sierras de Córdoba. *Boletín de la Academia Nacional de Ciencias* 29: 113–190.

Spegazzini, C. 1926b. Observaciones y adiciones a la micología argentina. *Boletín de la Academia Nacional de Ciencias* 28(3/4): 267–351.

Sobestiansky, G. 2005. Contribution to a Macromycete Survey of the States of Rio Grande so Sul and Santa Catarina in Brazil. *Brazilian Archives of Biology and Technology* 48: 437–457.

Sundberg, W. J. 1971. The genus *Chlorophyllum* (*Lepiotaceae*) in California. *Madroño* 21(1): 15–20.

Sysouphanthong, P., Thongklang, N., Liu, J. K. & Vellinga, E. C. 2021. Description of lepiotaceous fungal species of the genera *Chlorophyllum*, *Clarkeinda*, *Macrolepiota*, *Pseudolepiota*, and *Xanthagaricus*, from Laos and Thailand. *Diversity* 13(12): 666.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30(12): 2725–2729. https://doi.org/10.1093/molbev/mst197

Vellinga, E. C. 2001. *Chlorophyllum* Mass. In: Noordeloos, M. E., Kuyper, T. H. W. & Vellinga, E. C. (eds), *Flora Agaricina Neerlandica* 5, pp. 74–75. A. A. Balkema Publishers, Netherlands.

Vellinga, E. C. 2002. New combinations in *Chlorophyllum*. *Mycotaxon* 83: 415–417.

Vellinga, E. C. 2003. *Chlorophyllum* and *Macrolepiota* (*Agaricaceae*) in Australia. *Australian Systematic Botany* 16: 361–370. https://doi.org/10.1071/SB02013

Vellinga, E. C. 2004. Ecology and distribution of lepiotaceous fungi (*Agaricaceae*) – a review. *Nova Hedwigia* 78: 273–299.

Vellinga, E. C & de Kok, R. P. J. 2002. Proposal to conserve the name *Chlorophyllum* Massee against *Endoptychum* Czern. (*Agaricaceae*). *Taxon* 51: 563–564.

Vilgalys, R. & Hester, M. 1990. Rapid genetic identification and mapping of enzymatically amplified DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Vizzini, A., Perrone, L., Gelardi, M., Contu, M., Li, T., Zhang, M. & Xia, W. 2014. A new collection of *Chlorophyllum hortense* (*Agaricaceae*, *Agaricales*) from south-eastern China: molecular confirmation and morphological notes. *Rivista Micologica Romana* 91(1): 3–19.

White, T. J., Bruns, T., Lee, S. & Taylor, J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D. H., Shinsky, J. J. & White, T. J. (eds), *PCR protocols: a guide to methods and applications*, pp. 315–322. Academic Press, San Diego.

Wright, J. E. & Albertó, E. O. 2002. *Guía de hongos de la Región Pampeana I. Hongos con laminillas*. Buenos Aires, L.O.L.A.