

# Stropharia misionensis and Stropharia viridis, two new species from native forests of Northern Argentina (Agaricomycetes, Agaricales)

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## Research Article

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# Abstract

Northern Argentina hosts tropical and subtropical forests with a rich but still underexplored diversity of Agaricales fungi. In this study, we describe two new species of *Stropharia* found growing on decaying wood in native forests: *Stropharia viridis*, from the *Cedrela* forest within Baritú National Park (Salta, Andean Yungas), and *Stropharia misionensis*, from the San Antonio Strict Natural Reserve (Misiones, Alto Paraná Atlantic Forests). Phylogenetic analysis based on the nuclear internal transcribed spacer (nrITS) region and the large subunit (nrLSU) of the nuclear ribosomal DNA placed both species in well-supported clades within *Stropharia*, thereby confirming their placement as distinct taxa within the genus. *Stropharia viridis* is characterized by a dull green to grayish yellow, viscid pileus, sinuate lamellae, and thick-walled inamyloid basidiospores; *S. misionensis* has a white pileus densely covered with brownish-red squamules, a membranous superior annulus, and subangular spores < 10 µm long. Both taxa present smooth and thick-walled, inamyloid basidiospores, and clamp connections in all tissues. Complete taxonomic descriptions, field photographs, drawings, photographs captured with the scanning electron microscope (SEM) of the basidiospores, and comparisons of similar and closely related species are provided. Additionally, UV fluorescence microscopy images reveal the presence of fluorescent cystidia in *S. misionensis*. Finally, since both species are restricted to decaying wood within native forests currently threatened by habitat loss, it is proposed that they should also be considered at risk.

## Introduction

In the provinces of Misiones and Salta, located in northern Argentina, there are various natural areas that preserve a high biodiversity of fauna, flora, and fungi. These regions have been the focus of numerous mycological studies over the years, which have reported a significant diversity of fungal taxa (Spegazzini 1912, 1919; Singer 1950a, b; Singer and Digilio 1951; Singer 1953, 1955, 1959, 1960; Singer and Morello 1960; Singer 1965a, b, 1969, 1970, 1973a, b, 1975, 1976; Wright and Wright 2005; Lechner et al. 2006; Grassi et al. 2016). In the last decades, several studies have highlighted the high macrofungal diversity present in northern Argentina, particularly in the Yungas and the Selva Paranaense. Niveiro and Albertó (2012) compiled the macrofungal taxa of the Argentine Yungas and estimated that only 23–46% of the Agaricales had been recorded, suggesting that a substantial number of species remain undocumented. In Misiones, similar conclusions were reached by Lechner et al. (2006), Grassi et al. (2016) and Lining (2021), who emphasized the presence of a rich, still-undescribed macrofungal community. Wright and Wright (2005) documented a mycobiota comprising 374 species of Basidiomycota in the Iguazú National Park, including a wide range of agarics, polypores, and gasteroid fungi. More recently, Nouhra et al. (2018) described structured communities of Agaricomycetes along altitudinal gradients in the montane cloud forests of the Yungas, with a predominance of saprotrophic and ectomycorrhizal taxa.

The genus *Stropharia* (Fr.) Quél. is the type genus of the family *Strophariaceae* Singer & A.H. Sm., comprising approximately 120 species (CABI Bioscience and Landcare Research 2020), and typified by *Stropharia aeruginosa* (Curtis) Quél. Although the genus has been regarded as a synonym of *Psilocybe* (Fr.) P. Kumm. (Noordeloos 1999; Kirk et al. 2008), molecular studies have supported its recognition as

an independent genus (Moncalvo et al. 2000, 2002; Matheny et al. 2006). The genus is characterized by saprotrophic mushrooms with medium to large basidiomata, viscid to dry pileus, hygrophanous or not, and glabrous to squamous surface; a central stipe with annulus, viscid or dry; white to deep brownish lamellae, sometimes with violaceous tinges, and a brown to violaceous-black spore print. Spores are smooth and thick-walled, typically inamyloid; cystidia are present as cheilocystidia and pleurocystidia, sometimes with chrysocystidia; pileipellis in cutis or ixocutis, occasionally with erect cells; and acanthocytes—large spiked structures—that usually are present in the mycelium, rhizomorphs, and stipe base mycelium (Singer 1986; Watling and Gregory 1987; Noordeloos 2011; Ryman 2012). The presence of acanthocytes has been considered a diagnostic feature for the genus and has been emphasized in several species descriptions from South America (Cortez and Silveira 2007, 2008; Seger et al. 2016). Foundational works by Singer and Smith (1946) and Smith (1979) helped define the morphological boundaries of *Stropharia*, particularly within *Strophariaceae*, and recent studies continue to refine its taxonomy and species diversity in tropical and subtropical regions.

*Stropharia* is a genus with a broad global distribution and is represented by species recorded from all continents (Seger et al. 2016). Several species have been described from temperate and tropical regions, particularly in the Americas and Asia, contributing to the growing understanding of the genus's morphological and ecological diversity (Desjardin and Hemmes 2001; Bandala et al. 2005; Cortez and Silveira 2007, 2008; Bau and Meng 2008; da Silva et al. 2009; Senthilarasu and Singh 2013; Tian and Bau 2014; Seger et al. 2016; Vizzini et al. 2017; Khan et al. 2019; Tian et al. 2021; Zhang et al. 2024; Feng et al. 2025). In South America, taxonomic contributions from Brazil and Argentina have expanded the known diversity of the genus, often emphasizing diagnostic features, such as acanthocytes and chrysocystidia. Although some species, such as *Stropharia rugosoannulata* F.H. Møller, have been reported as edible (Dai et al. 2010), and others, such as *Stropharia indigocephala* Pegler & Young, have shown medicinal potential (Dai and Yang 2008). The genus remains primarily studied for its taxonomic complexity and ecological roles in wood decay and nutrient cycling. *S. rugosoannulata* has demonstrated nematophagous activity through acanthocyte-mediated trapping mechanisms, with effective immobilization and degradation of plant-parasitic nematodes, such as *Meloidogyne hapla* Chitwood and *Panagrellus redivivus* Linneo (Luo et al. 2006; Zouhar et al. 2013). Its capacity to degrade organic pollutants via extracellular enzymes like laccases and manganese peroxidases has positioned it as a promising agent in bioremediation (Hu et al. 2021). Furthermore, its integration into crop rotation systems has shown to enhance soil fertility, increase microbial diversity, and promote nutrient recycling, particularly in models such as rice–*S. rugosoannulata* and sunflower–*S. rugosoannulata* rotations (Tang et al. 2022; Dou et al. 2025; Lu et al. 2025).

The Argentinean Yungas, situated in the northwest, are considered an extension of the Amazonian Domain (Cabrera 1994). This region is characterized by a marked altitudinal gradient and a complex climate, with seasonal fog, persistent cloud cover, and a wide range of environmental conditions including high temperatures, drought, elevated humidity, frosts, and occasional snowfall during winter. Rainfall is concentrated between November and February, while the rest of the year is relatively dry. The Yungas host a high diversity of tree species, with more than 230 recorded taxa (Grau and Brown 2000;

Brown et al. 2005). In the northeast, the Selva Paranaense, located in Misiones province, forms part of the Atlantic Forest and is recognized for its exceptional biodiversity (Bitetti et al. 2003; Izquierdo et al. 2011). The climate in this area is humid and subtropical, with abundant rainfall mainly between November and February, and temperatures ranging from 16°C to 22°C, with summer peaks reaching 40°C and occasional frosts in winter. The forest composition includes a wide variety of native and exotic tree species, with dominant genera, such as *Araucaria* Juss., *Aspidosperma* Mart. & Zucc., *Campomanesia* Ruiz & Pav., *Cedrela* P. Browne, *Mimosa* L., *Ocotea* Aubl., *Nectandra* Rol. ex Rottb., and *Parapiptadenia* Brenan. Despite their ecological importance, both the Argentine Yungas and the Selva Paranaense, face increasing threats due to unsustainable logging, extensive livestock farming, and subsistence hunting, among other pressures, which contribute to the degradation and loss of biodiversity in these ecosystems.

An excursion to the north of Argentina, in the provinces of Salta and Misiones, provided the opportunity to document several collections of *Stropharia*, within Baritú National Park and San Antonio Reserve. Sampling efforts in Salta, in northwestern Argentina, were focused on upper temperate montane ecosystems (1500–2500 m a.s.l.). This area is dominated by trees of the genus *Cedrela* P. Browne, from which collections of the newly proposed species *Stropharia viridis* A.P. Martínez, J.M. Suárez, J. Aliaga & B.E. Lechner were obtained. The genus *Cedrela* is native to Argentina (Zuloaga and Morrone, 1999) and is distributed mainly in South America, with five species recorded in the country, three of which are exclusive to the northwest (*C. balansae* C. DC., *C. lilloi* C. DC., *C. saltensis* Zapater & del Castillo) and two from the northeast (*C. fissilis* Vell., *C. odorata* L.) (Zapater et al. 2004). Two new species of agaric fungi were described by Martínez et al. (2024) from the *Cedrela* forest in Baritú National Park, highlighting the fungal diversity associated with these native ecosystems. Sampling efforts in Misiones focused on the San Antonio Strict Nature Reserve, located in northeastern Argentina within the Atlantic Forest. This area is composed of trees belonging to the genera *Araucaria*, *Aspidosperma*, *Cedrela* and *Nectandra*, where collections of the newly proposed species *Stropharia misionensis* A.P. Martínez, J.M. Suárez, J. Aliaga & B.E. Lechner were found. The native forest in this reserve plays a crucial role in maintaining ecological balance, supporting biodiversity, and providing habitat for numerous plant species (Fundación Vida Silvestre Argentina, 2025).

This study contributes to the taxonomic knowledge of *Stropharia* in South America by describing two new species from native forests in northwestern and northeastern Argentina. Both taxa, *Stropharia viridis* and *Stropharia misionensis*, are known only from their type localities, which are ecologically distinctive and under conservation pressure. Species delimitation was based on an integrative approach combining morphological and molecular data (ITS and LSU), supporting their recognition as distinct taxa. These findings emphasize the importance of documenting fungal diversity in threatened ecosystems and provide a foundation for future conservation and research initiatives.

## Material and methods

### Specimens studied

Specimens were collected in 2023 within Baritú National Park (Salta Province) and San Antonio Strict Nature Reserve (Misiones Province), both located in northern Argentina. Collections were obtained in forests dominated by trees of the genus *Cedrela*, locally referred to as the “Cedral” site, and in native Atlantic Forest ecosystems characterized by the presence of *Araucaria* species. All specimens were carefully documented and preserved following standard methodologies for Agaricales (Largent 1986). Voucher specimens were deposited in the Mycological Herbarium of the Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (BAFC).

## Morphological studies

Macroscopic descriptions were based on fresh basidiomata, with colors determined according to Kornerup and Wanscher (1987). Microscopic observations followed standard protocols for Agaricales (Lechner 2021). Longitudinal radial sections of the pileus were made to examine the pileipellis; transverse sections of the lamellae were used to describe basidia, cystidia, and spores; and longitudinal sections of the stipe were prepared to characterize caulocystidia. Various mounting media and stains were employed to enhance visibility of structures and reactions: 3% KOH, 1% aqueous phloxine, and Melzer’s reagent. Microscopic analyses were conducted using a Leica DM750 bright field microscope, and images were captured with a Leica iCC50 W integrated camera. All structures were measured, and the following abbreviations were used: Q (length/width ratio of spores), Q<sub>e</sub> (mean Q value), N (number of spores measured), and Me (mean spore size). Illustrative figures were hand-drawn to complement the photographic documentation of the two new *Stropharia* species. For basidiospore imaging, a FE SEM SUPRA40 scanning electron microscope (Carl Zeiss AG) was used. Fluorescence imaging was performed using a Zeiss LSM980 confocal microscope with Airyscan-2 and a Leica DM750 microscope equipped with UV illumination.

## DNA extraction, PCR conditions, and sequencing

Genomic DNA was extracted from fresh basidiomata using the NaOH-based protocol described by Steiner et al. (1995). PCR amplification was performed using the primer pairs ITS4–ITS5 (White et al. 1990) for the internal transcribed spacer (ITS) region and LR0R–LR5 (Vilgalys and Hester 1990) for the partial large subunit (LSU) region. The amplification protocol for ITS consisted of an initial denaturation at 95°C for 2 min, followed by 40 cycles of 95°C for 30 s, 55°C for 45 s, and 72°C for 1 min, with a final extension at 72°C for 15 min. For LSU, the program included an initial denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 15 min. PCR products were visualized on 1% agarose gels and sent to Macrogen Inc. (South Korea) for sequencing. Raw sequences were manually edited using BioEdit v7.2.5 (Hall 1999, 2004) and compared against existing data using BLAST searches in GenBank. Newly generated sequences were deposited in the GenBank database.

# Phylogenetic analyses

Phylogenetic analyses were conducted to assess the placement of the two new *Stropharia* species within the genus. Two datasets were constructed: one based on the internal transcribed spacer region including the 5.8S gene (ITS1–5.8S–ITS2) and the other based on the large subunit (LSU, 28S). A third concatenated dataset combining ITS and LSU sequences was also assembled. Reference sequences were selected based on previous studies (Vizzini et al. 2017; Khan et al. 2019; Tian et al. 2021; Zhang et al. 2024; Feng et al. 2025) and BLAST best hits from GenBank. Species of the genus *Psilocybe*, which are phylogenetically distinct from *Stropharia*, were used as outgroup taxa (Feng et al. 2025). All related information is presented in Table 1.

Sequence alignments were performed separately for each locus using MAFFT v7 (<https://mafft.cbrc.jp/alignment/server/>) and manually refined in BioEdit v7.2.5 (Hall 1999, 2004). Maximum Likelihood (ML) analyses were conducted using RAxML-NG under the GTR + G model, with node support estimated via 1000 bootstrap replicates. Bayesian Inference (BI) analyses were performed in MrBayes v3.2.6 (Ronquist et al. 2012), with two parallel runs of 15,000,000 generations, discarding the first 25% of sampled trees as burn-in. Nodes with bootstrap values  $\geq 60\%$  and Posterior probability (PP) values  $\geq 0.75$  were retained in the final consensus tree. Resulting phylogenetic trees were visualized using FigTree v1.4.0 (Rambaut 2009).

Table 1. Species name and GenBank accession numbers of the sequences included in this study for the *Stropharia* phylogenetic analyses.

## Taxonomy

Taxonomic treatment followed the guidelines of the International Code of Nomenclature for algae, fungi, and plants (ICNafp; Turland et al. 2018). Names and synonyms were verified using Index Fungorum and the Authors of Fungal Names database (CABI Bioscience and Landcare Research 2020). Herbarium acronyms were cited according to Thiers (2012). Reference descriptions of related taxa were consulted from the following sources: Morgan (1908), Redhead (1984), Bandala and Montoya (1993), Desjardin and Hemmes (2001), Cortez and Silveira (2008), Cortez (2008), da Silva et al. (2009), Senthilarasu and Singh (2013), Tian and Bau (2014), Seger et al. (2016), Vizzini et al. (2017), Khan et al. (2019), Lee et al. (2020), Tian et al. (2021), Hausknecht and Greilhuber (2024), Zhang et al. (2024), Kotkova et al. (2024), Feng et al. (2025). The new taxa were registered in the MycoBank database under accession numbers MB#861022 and MB#861114.

## Results

### Molecular phylogeny

The Maximum Likelihood (ML) and Bayesian Inference (BI) analyses of the combined ITS-LSU dataset for *Stropharia* and related taxa (Fig. 1) revealed that the collections corresponding to *Stropharia viridis* and *S. misionensis* form strongly supported monophyletic clades within their respective species groups. Eight sequences, including four ITS and four LSU, were newly generated for the phylogenetic analyses. The final alignment comprised 47 sequences with a total length of 1581 base pairs, of which 1151 sites were conserved and 430 were variable. The resulting topology is congruent with previous phylogenetic frameworks proposed by Vizzini et al. (2017), Khan et al. (2019), Tian et al. (2021), Zhang et al. (2024), and Feng et al. (2025).

## Taxonomy

1 ***Stropharia viridis*** A.P. Martínez, J.M. Suárez, J. Aliaga & B.E. Lechner, sp. nov. (Fig. 2).

MycoBank: MB#861114

*GenBank*: rDNA ITS: PX124085, PX124086; LSU: PX124144, PX124145.

## Etymology

The specific epithet *viridis* is derived from the Latin word “green”, referring to the characteristic dull green coloration of the pileus observed in fresh basidiomes. This color trait is one of the most distinctive macroscopic features of the species.

*Diagnosis*: *Stropharia viridis* is characterized by a viscid pileus of dull green (26E8) to grayish yellow (28A8–2C5), with remnants of the veil especially near the margin. The lamellae are sinuate, light brown in color. The stipe is central, longitudinally striate, with white fibrils. The cheilocystidia are cylindrical to clavate-capitate, with thickened and ornamented walls.

## Holotype

ARGENTINA, Salta, Parque Nacional Baritú, Bosque El Cedral, 1700 m., 03/03/2023, 22° 37' 11.8" S, 64° 45' 12.3" W, leg. Agustín P. Martínez, Juan M. Suárez & Joaquín Aliaga (BAFC53517).

## Description

Basidiomata (Fig. 2) medium-sized. Pileus 10–45 mm in diameter, convex to campanulate, dull green (26E8) when young, umbonate and vivid green to grayish yellow (28A8–2C5) at maturity; hygrophanous, with a few white (1A1) scales near the margin. Margin irregularly wavy, slightly inrolled, appendiculate; surface smooth, viscid; context fleshy. Lamellae sinuate to adnate, 4–20 mm wide, light brown (6D5), with a whitish and slightly irregular edge; with lamellulae intercalated. Stipe central, 5–8 mm wide, terete, solid, fibrillose, longitudinally striate, equal, white to grayish yellow (2B3), covered with white fibrils (1A1) below the pileus attachment zone; annulus absent.

Basidiospores (Fig. 3a; Fig. 5. a–b)  $6.4\text{--}8.6 \times 4.2\text{--}4.9 \mu\text{m}$ ,  $Me = 7.5 \times 4.4 \mu\text{m}$ ,  $Qe = 1.7$ ,  $N = 30$ ; ellipsoid, smooth, with a small truncate germ pore, slightly thick-walled ( $0.7 \mu\text{m}$ ), yellowish orange (4B7). Basidia (Fig. 3b)  $21.3\text{--}31 \times 4.7\text{--}6.9 \mu\text{m}$ , clavate, tetra- and mostly bisporic, hyaline. Cheilocystidia (Fig. 3c)  $52.2\text{--}66.4 \times 9.1\text{--}11.9 \mu\text{m}$ , cylindro-clavate, capitate, sometimes with constrictions and slightly thickened, rugose walls containing grayish yellow (4B6) pigment; emitting pale green (26A4) fluorescence under UV excitation (Fig. 4). Pleurochrysocystidia (Fig. 3d)  $38.9\text{--}53.5 \times 10.1\text{--}13 \mu\text{m}$ , lageniform, mucronate, abundant on the lamellar sides, some completely filled with subhyaline to grayish yellow (4B6) contents and irregular in shape. Pileipellis (Fig. 3e) a cutis with a gelatinized layer  $45\text{--}65 \mu\text{m}$  thick, composed of ornamented (tiger-striped) hyphae,  $1.9\text{--}4.6 \mu\text{m}$  wide, with encrusted grayish yellow (4B6) pigment. Stipitipellis a cutis with caulocystidia (Fig. 3f)  $8.7\text{--}15.6 \mu\text{m}$  wide,  $24.3\text{--}51.9 \mu\text{m}$  long, claviform, pyriform, or capitate, with rugose walls bearing grayish yellow (4B6) pigment. Clamp connections present in all tissues.

### Habit and habitat

Gregarious or in clusters. This species has been collected only in the Baritú National Park, Salta, within a forest mainly composed of long-lived *Cedrela* spp. (Meliaceae Juss.) specimens.

### Commentary

The collections of *Stropharia viridis* form a highly supported monophyletic group (PP = 1.00; BS = 100) within the clade comprising *S. aeruginosa*, *S. albonitens* (Fr.) Quél., *S. caerulea* Kreisel, *S. daliensis* Y.L. Feng, Y. Fang and X. Guo, *S. inuncta* (Fr.) Quél., and *S. microaeruginosa* J.Z. Xu. This placement confirms its close relationship with taxa characterized by greenish to bluish pileus tones, ellipsoid spores with moderately thick walls, pleurochrysocystidia, and a gelatinized pileipellis.

Macroscopically, *S. viridis* is easily distinguished by its viscid, greenish pileus (dull green to grayish yellow) with veil remnants near the margin, light brown sinuate lamellae, and a longitudinally striate stipe covered with white fibrils. The presence of ornamented, thick-walled cheilocystidia with capitate apices is a key diagnostic feature, absent in most related species.

Among the phylogenetically closest taxa, *S. aeruginosa* and *S. caerulea* differ by their bluish pileus tones, persistent annulus, and habitat on soil rather than decayed wood (Vagner and Silveira 2008). *Stropharia albonitens* and *S. inuncta* share the viscid pileus and gelatinized pileipellis but lack ornamented cheilocystidia and exhibit larger basidiospores (Breitenbach and Kränzlin 1995). *Stropharia microaeruginosa* and *S. daliensis* differ by their smaller basidiomes, bluish pileus, and terrestrial habit (Zhang et al. 2024; Feng et al. 2025). A superficially similar species, *S. pseudocyanea* (Desm.) Morgan, can be separated by its bluish-green stipe, larger spores, and cheilocystidia without thickened or ornamented walls (Redhead 1984; Bandala and Montoya 1993).

These combined morphological and molecular characters support the recognition of *S. viridis* as a distinct species associated with decayed wood under *Cedrela* spp. in the Atlantic Forest of northeastern

Argentina.

*Material examined: Stropharia viridis.* ARGENTINA, Salta, Parque Nacional Baritú, Bosque El Cedral, 1700 m asl., 03/03/2023, 22° 37' 11.8" S, 64° 45' 12.3" W, leg. Agustín P. Martínez, Juan M. Suárez & Joaquin Aliaga (BAFC53517). Parque Nacional Baritú, Bosque El Cedral, 1700 m asl., 03/03/2023, 22° 37' 15.9" S, 64° 45' 11.5" W, leg. Agustín P. Martínez, Juan M. Suárez & Joaquin Aliaga (BAFC53518).

2 ***Stropharia misionensis*** A.P. Martínez, J.M. Suárez, J. Aliaga & B.E. Lechner, sp. nov. (Fig. 6).

## MycoBank

MB#861022

*GenBank:* ITS: PX124083, PX124084; LSU: PX124146, PX124147.

## Etymology

The specific epithet refers to Misiones Province (northeastern Argentina), referring to the locality where the type material was collected.

*Diagnosis: Stropharia misionensis* is characterized by its brownish red squamulose pileus over a white background, stipe with concolorous scales, membranous superior annulus, lamellae with eroded edge, and subangular spores less than 10 µm in length.

## Holotype

ARGENTINA, Misiones, Reserva Natural Estricta San Antonio, 500 m asl., 14/06/2023, 26° 1' 20.4" S, 53° 47' 58.40" W, leg. Agustín P. Martínez, Juan M. Suárez & Joaquin Aliaga (BAFC53520).

## Description

Basidiomata (Fig. 6) medium-sized. Pileus 20–50 mm in diameter, convex to plane or concave; surface white (1A1), covered with appressed–imbricate brownish red (11C7) squamules, more concentrated toward the center; dry; margin irregularly wavy and appendiculate, straight to slightly uplifted; context fleshy, up to 8 mm thick, white (1A1). Lamellae sinuate, 15–35 mm long, grey (6C1) to brownish grey (6C2), edge irregular and eroded, moderately broad, close. Stipe central, 50–80 × 5–8 mm, cylindrical, solid, fibrillose, equal or slightly tapering toward the base, white (1A1), covered with concolorous scales, becoming pale yellow (1A3) when handled; annulus membranous, superior, rugulose.

Basidiospores (Fig. 7a, 5b) (5.6)5.9–6.9(7.4) × (3)3.5–4.1(4.5) µm, *Me* 6.1 × 3.9 µm, *Qe* = 1.7, *N* = 30; ellipsoid, slightly angular, ovate, subhexagonal to subrhomboid in side view, smooth, with a truncate germ pore, wall slightly thickened (0.5–0.7 µm), greyish yellow (4C7) in KOH. Basidia (Fig. 7b) 24.8–34.3 µm, clavate, predominantly tetrasporic, occasionally bisporic, hyaline; sterigmata 6 µm long. Lamellar edge sterile, entirely covered with cheilocystidia (Fig. 7c) 29.9–49 × 10–19.3 µm, pyriform, hyaline.

Pleurochrysocystidia (Fig. 7d) 29.5–41.3 × 8.6–12.3 µm, abundant on lamellar faces, lageniform, apex mucronate, sometimes bifurcate, contents subhyaline. Hymenophoral trama pseudoparenchymatous, composed of thin-walled, hyaline hyphae. Pileipellis (Fig. 7e) a regular cutis, with elements of the squamules erect and inflated, 8.5–20 µm wide, greyish yellow (4C5) in KOH. Stipitipellis composed of hyaline, thin-walled, clamped hyphae, 2–17 µm diam.; caulocystidia not observed. All tissues clamped.

### Habit and habitat

solitary to gregarious, growing on decayed wood, in mixed forest vegetation. Known only from the type locality in San Antonio, Misiones Province, Argentina.

### Commentary

The collections of *S. misionensis* form a highly supported monophyletic group, with high posterior probability (PP) and bootstrap support (BS) values, within the clade comprising *S. rugosoannulata*, *S. lignicola* E.J. Tian, *S. hardii* G.F. Atk., *S. subrugosoannulata* J.Z. Xu and *S. acanthostipitata* Angelini & Vizzini, with *S. acanthostipitata* as the sister clade. The macroscopic features of *S. misionensis*—a white pileus covered with brownish-red squamules, a membranous superior annulus, and brownish-grey lamellae with irregular edges—clearly distinguish it from other species of this clade.

The phylogenetically closest species recovered in this study possess acanthocysts. These structures are typically present in the rhizomorphs of the basidiome, occasionally on the stipe, and in the case of *S. acanthocystis* Cortez & R.M. Silveira, even in the hymenium. The new taxon *S. misionensis* was found without basal rhizomorphs and without acanthocysts in any part of the basidiome.

*Stropharia acanthostipitata* has a yellowish to brown pileus, emarginate lamellae, and lacks a membranous annulus, and microscopically, it differs from *S. misionensis* by its irregularly shaped chrysocystidia with yellowish contents, smaller cheilocystidia, pileipellis with a gelatinized layer, stipitipellis with diverticulate cauloleptocystidia and caulochrysocystidia, and the presence of acanthocysts (Vizzini et al. 2017). In addition, other species of the genus exhibit distinctive features: *S. acanthocystis* is distinguished by hymenial acanthocytes mixed with the basidia and cystidia, *S. apiahyna* by a persistent membranous annulus and absence of cheilocystidia, and *S. araucariae* by a dark brown pileus and cheilocystidia as chrysocystidia (Vagner and Silveira 2008). Another closely related and macroscopically similar species, *S. subrugosoannulata*, differs by having adnate to adnexed lamellae with reddish tones (titian red to terra-cotta, 7D6–7D7), a non-persistent annulus, growth on soil under bamboo, smaller basidia and basidiospores (13.8–16.8 µm), lageniform cheilocystidia, and acanthocysts in the basal mycelium of the stipe (Zhang et al. 2024). *Stropharia lignicola* is easily distinguished by its yellowish pileus tones, viscid surface, adnate lamellae, clavate pleurocystidia with yellowish content, clavate cheilocystidia, and the presence of acanthocysts both in the hymenium and basal mycelium (Tian et al. 2021). *Stropharia rugosoannulata*, differs from *S. misionensis* by its larger and smooth pileus, darker and violaceous lamellae, and terrestrial habitat (Breitenbach and Kränzlin 1995). Lastly, *Stropharia jilinensis* T. Bau & E.J. Tian also exhibits a scaly pileus, but with grayish-violet

(17D4–18D4) to yellowish-brown (5D5) tones, a viscid surface, lamellae with crenate or dentate edges, and a persistent membranous annulus. Microscopically, it differs by having yellowish, tetrasporic basidia and versiform cheilocystidia—clavate to subfusoid, flexuous–filamentous, sometimes branched, and arranged in chains of clavate to subglobose cells (Tian and Bau 2014).

These combined morphological and molecular features support the recognition of *S. misionensis* as a distinct and novel species endemic to the Atlantic Forest of northeastern Argentina.

Material examined: *Stropharia misionensis*. ARGENTINA, Misiones, Reserva Natural Estricta San Antonio, 500m asl., 14/06/2023, 26° 1' 20.4" S, 53° 47' 58.40" W, leg. Agustín P. Martínez, Juan M. Suárez & Joaquin Aliaga (BAFC53519). Reserva Natural Estricta San Antonio, 500m asl., 14/06/2023, 26° 1' 24.4" S, 53° 47' 42.4" W, leg. Agustín P. Martínez, Juan M. Suárez & Joaquin Aliaga (BAFC53520).

## Discussion

The Agaricales represent one of the most species-rich orders within the Basidiomycota, comprising over 40,000 described species and exhibiting remarkable morphological and ecological diversity (Kalichman et al. 2020; Dong et al. 2025). Fungi kingdom plays essential roles in ecosystems as decomposers of organic matter, mutualists in mycorrhizal associations, and contributors to nutrient cycling and soil formation (Matheny et al. 2006; Ruiz-Dueñas et al. 2021). Their conservation is increasingly recognized as critical, not only for maintaining biodiversity but also for preserving ecosystem functionality and their potential applications in medicine, biotechnology, and agriculture (Heilmann-Clausen et al. 2015; Haelewaters et al. 2022).

In this study, we describe two new species of *Stropharia* (*S. misionensis* and *S. viridis*) from native forests of northeastern and northwestern Argentina. These findings underscore the richness of fungal diversity in underexplored ecosystems such as the Atlantic Forest and the montane Yungas, both considered biodiversity hotspots (Wright and Wright 2005). The integration of morphological, ecological, and molecular data provides robust support for the recognition of both taxa as distinct species within the genus *Stropharia*, which continues to expand with recent discoveries in South America and Asia (Vizzini 2017; Zhang et al. 2024; Hausknecht and Greilhuber 2024).

Phylogenetic analyses based on ITS-LSU sequences positioned *S. viridis* within a clade comprising *S. inuncta*, *S. caerulea*, *S. aeruginosa*, and *S. microaeruginosa*, while *S. misionensis* was placed within the group that includes *S. rugosoannulata*, *S. subrugosoannulata*, *S. lignicola*, *S. acanthostipitata*, and *S. hardii*. In both cases, the new species formed highly supported monophyletic groups, reinforcing their taxonomic significance and independent evolution (Vizzini et al. 2017; Khan et al. 2019; Tian et al. 2021; Zhang et al. 2024; Feng et al. 2025).

From a conservation perspective, these species are of particular concern. *Stropharia misionensis* is known only from a single locality within the Reserva Natural Estricta San Antonio, a protected remnant of Atlantic Forest, while *S. viridis* was found exclusively in the Parque Nacional Baritú, in association with

long-lived *Cedrela* trees. Their apparent endemism, restricted distribution, and habitat specificity suggest that both species may be vulnerable to environmental changes and habitat degradation (Amaranthus 1998; Sridhar 2013). These findings align with broader patterns observed in macrofungi, where many species remain undescribed and are often confined to threatened ecosystems (Heilmann-Clausen et al. 2015).

The documentation and formal description of these fungi contribute to the understanding of fungal diversity in South America and provide a scientific basis for their potential inclusion in conservation assessments such as the IUCN Red List of Threatened Species (IUCN 2025). The application of IUCN criteria to fungi has gained momentum in recent years, with over 500 fungal species now assessed globally, and given their restricted distribution and habitat specificity, *S. misionensis* and *S. viridis* may qualify under criteria such as B1 (limited extent of occurrence) and D2 (very restricted area of occupancy), which are designed to identify species at risk due to geographic limitation and potential threats (Heilmann-Clausen et al. 2015; Haelewaters et al. 2022). These initiatives are essential to ensure that fungi are adequately represented in biodiversity policies and protected under environmental legislation.

Our findings reinforce the importance of continued fieldwork, taxonomic research, and molecular studies in native forests, which remain reservoirs of undescribed fungal biodiversity. The discovery of *S. misionensis* and *S. viridis* in protected areas highlights the role of conservation areas not only in preserving known biodiversity but also in enabling the discovery of new taxa. Moreover, these results emphasize the need to integrate fungi into broader conservation frameworks, recognizing their ecological roles and the urgency of protecting their habitats (Sridhar 2013; Heilmann-Clausen et al. 2015). Strengthening fungal representation in biodiversity policies and environmental legislation is a necessary step toward a more inclusive and effective conservation strategy.

## Declarations

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**Author contribution** All authors contributed to the studies described in the paper and read and approved the final manuscript.

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**Availability of data and materials** DNA sequence data generated or used in the current study are deposited at GenBank as set out in Table 1 of the present paper.

**Ethics approval and consent to participate:** All authors confirm that no research involving humans or animals was involved in the current study, that there are no issues relating to animal welfare relating to the current study and that they have approval to participate in the current study.

**Consent for publication:** All authors have given explicit consent to the submitted paper and to the inclusion of their data in it.

**Competing interests:** The authors declare no competing interests.

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## Table 1

Table 1 is available in the Supplementary Files section.

## Figures

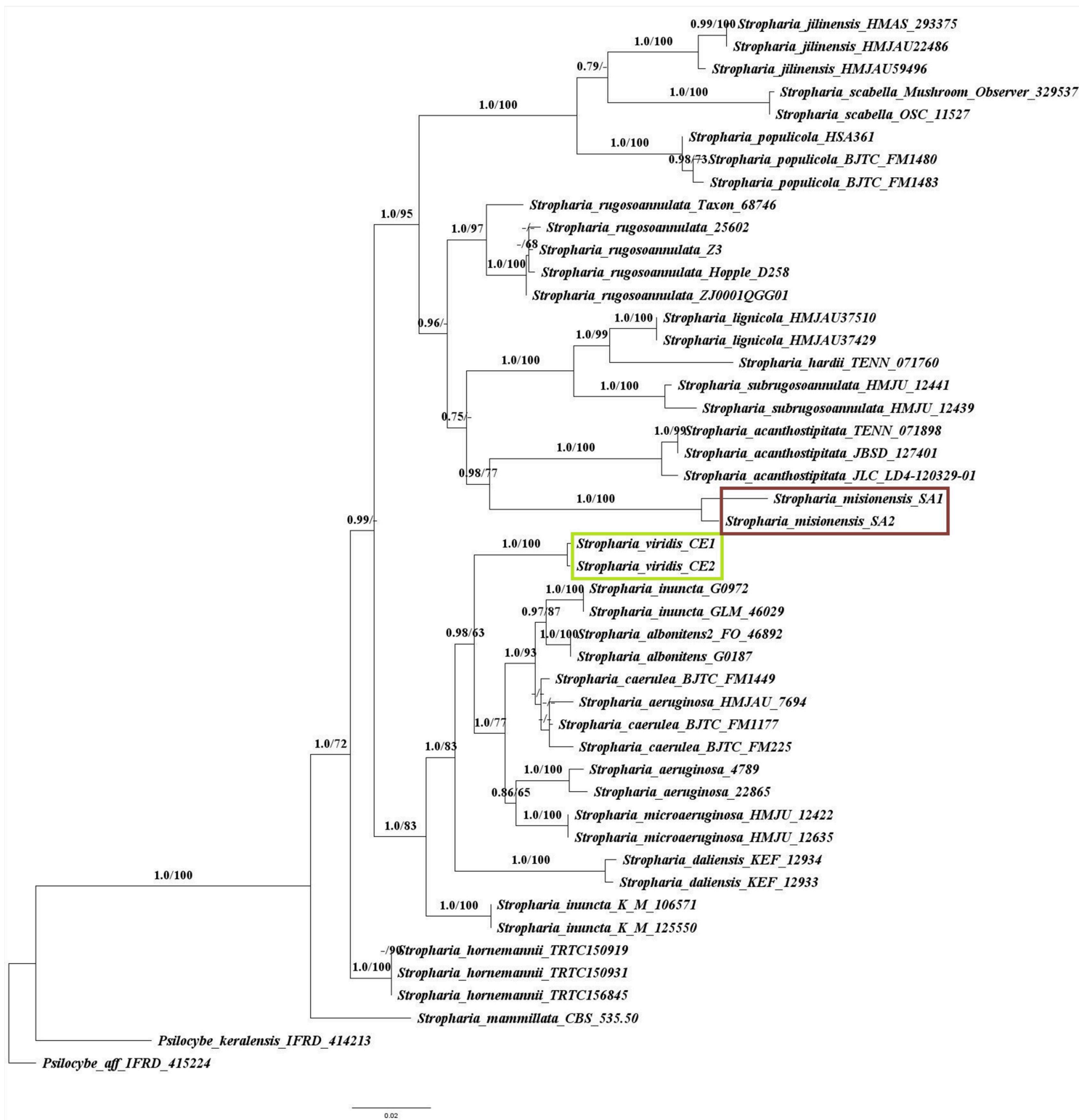


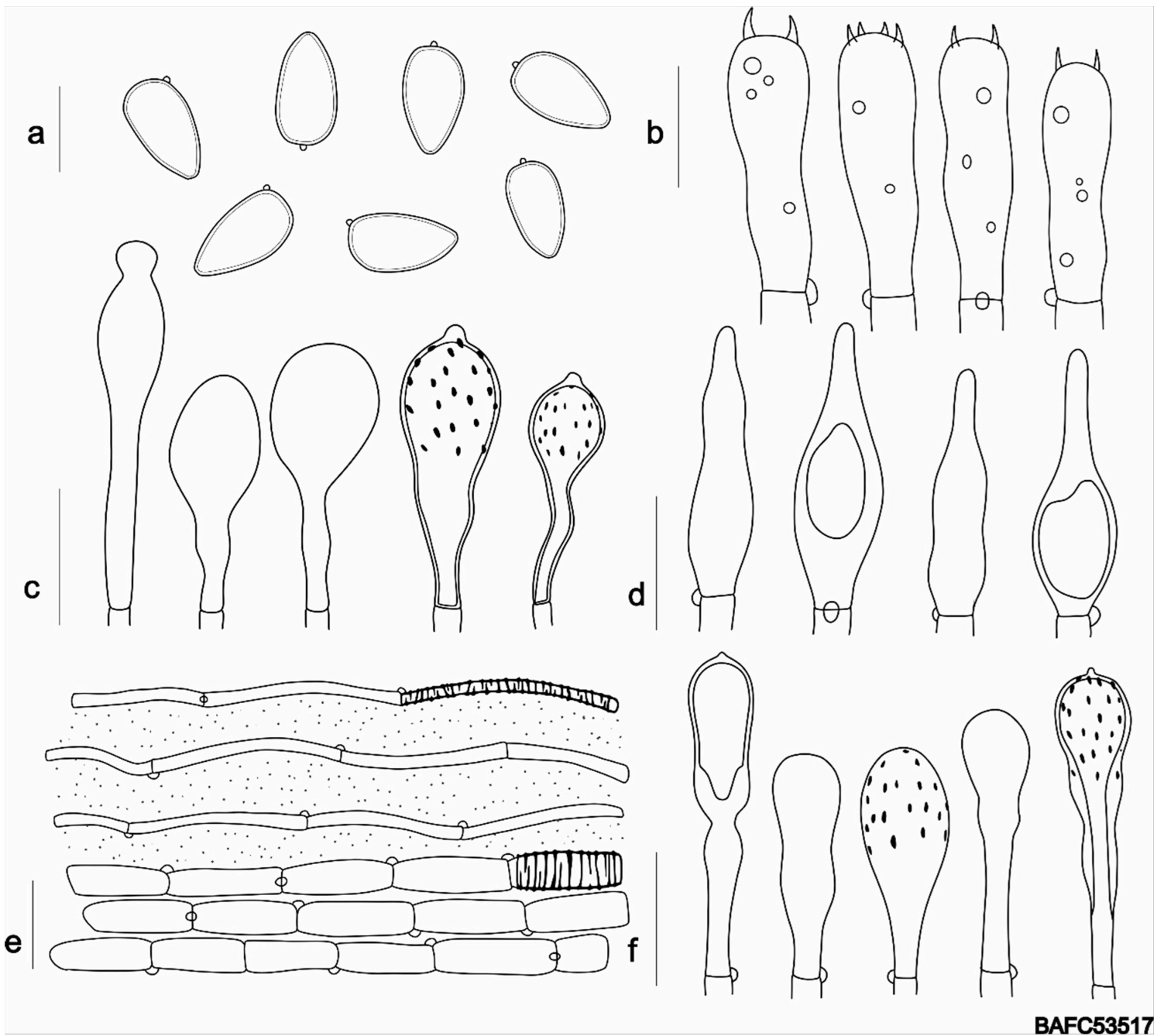
Figure 1

Cladogram derived from Maximum Likelihood (ML) and Bayesian Inference (BI) analyses of the combined ITS-LSU dataset, showing the phylogenetic placement of *Stropharia viridis* and *Stropharia misionensis*.



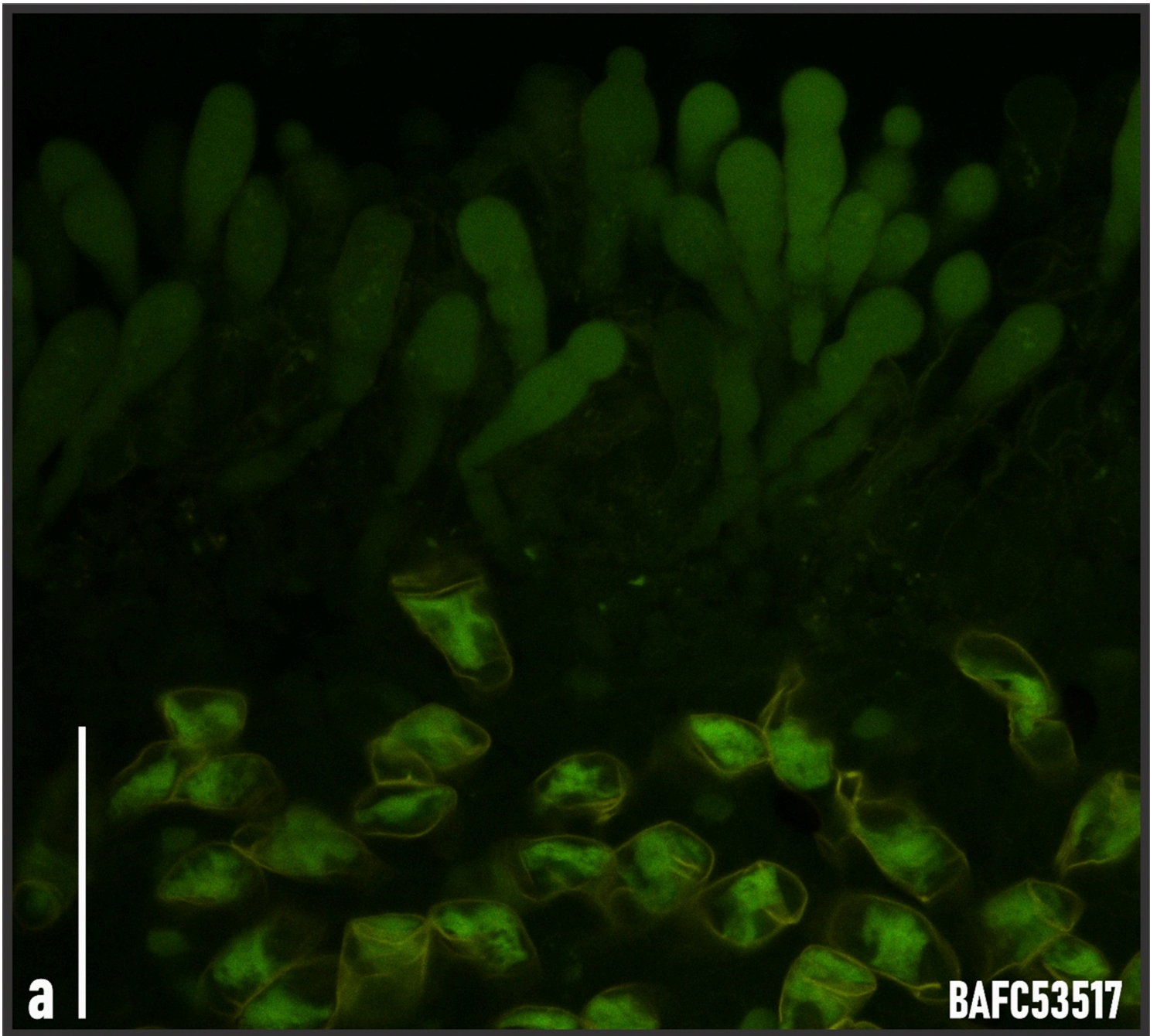
Figure 2

Basidiomata of *Stropharia viridis* under normal light (top) and UV light at 365nm (bottom) BAFC53517 (Holotype). Scale bars = 15 mm. Images by Agustín P. Martínez.



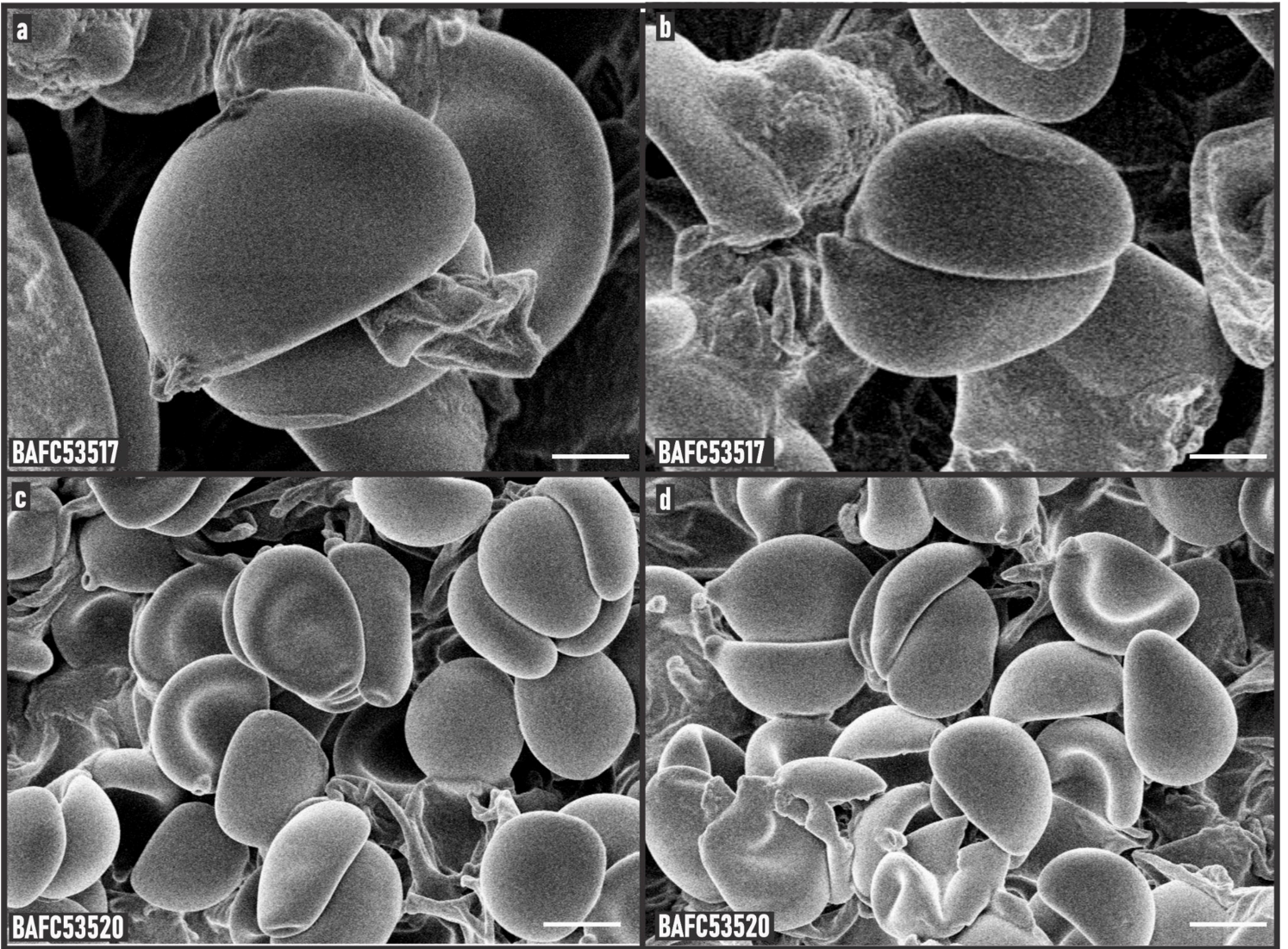
**Figure 3**

Microscopic characters of *Stropharia viridis* BAF53517 (Holotype). a, Basidiospores. b, Basidia. c, Cheilocystidia. d, Pleurochrysocystidia. e, Cells of pileipellis. f, Caulocystidia. Scale bars, a = 7  $\mu\text{m}$ , b = 10  $\mu\text{m}$ , c = 20  $\mu\text{m}$ , d = 20  $\mu\text{m}$ , e = 6  $\mu\text{m}$ , f = 20  $\mu\text{m}$ . Draws by Juan M. Suárez.



**Figure 4**

Fluorescence microscopy image captured with a 63x objective under spectral excitation at 405 nm. a, Cheilocystidia (top) and Pleurochrysocystidia (bottom). Scale bars, a = 50  $\mu$ m.



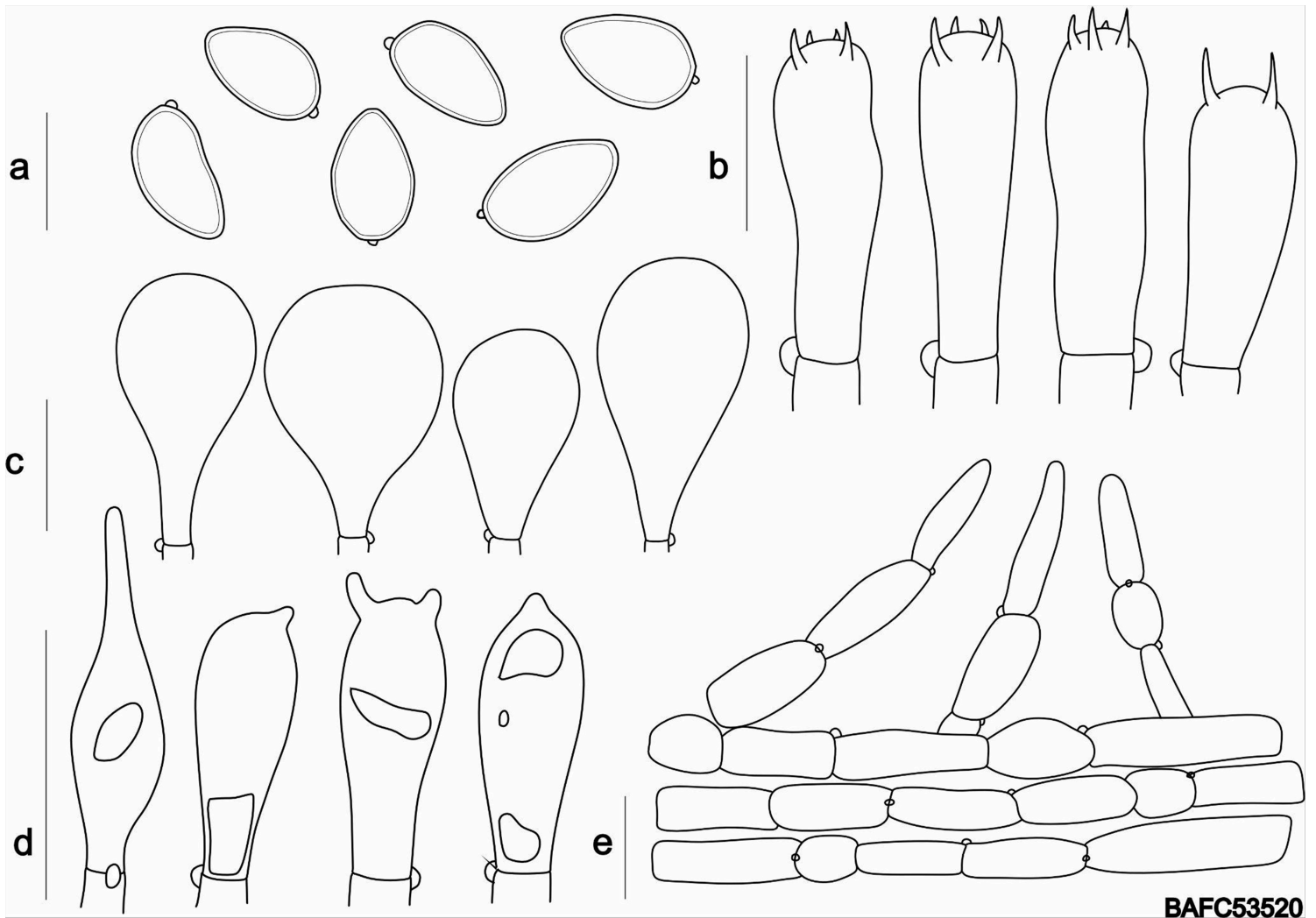
**Figure 5**

a–b, SEM photos of *Stropharia viridis* BAFC53517 (Holotype). c–d SEM photos of *Stropharia misionensis* BAFC53520 (Holotype). Scale bars = 3  $\mu$ m.



Figure 6

Basidiomata of *Stropharia misionensis*, BAFC53520 (Holotype). Scale bars = 20 mm. Images by Agustín P. Martínez.



**Figure 7**

Microscopic characters of *Stropharia misionensis*, BAFC53520(Holotype). a, Basidiospores. b, Basidia. c, Cheilocystidia. d, Pleurochrysocystidia. e, Cells of pileipellis. Scale bars, a = 10  $\mu\text{m}$ , b = 15  $\mu\text{m}$ , c = 20  $\mu\text{m}$ , d = 30  $\mu\text{m}$ , e = 20  $\mu\text{m}$ . Draws by Juan M. Suárez.

## Supplementary Files

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