

Integrative assessment of *Panaeolus* and *Psilocybe* species (Agaricales, Basidiomycota) from Uruguay: multigene phylogenetic analyses, psychoactive alkaloid content, and novel taxonomic insights

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Abstract: Coprophilous species of *Panaeolus* and *Psilocybe* are ecologically significant components of dung-associated fungal communities worldwide and include the principal natural producers of the psychoactive alkaloids psilocybin (PSB) and psilocin (PS). Despite their relevance, the diversity of these genera in Uruguay has remained poorly documented. In this study, we conducted an integrative assessment of Uruguayan specimens using multigene phylogenetic analyses, detailed morphological characterization, and quantitative alkaloid profiling. Seven species were confirmed. Five species of *Panaeolus* were identified: *Pa. antillarum*, *Pa. cyanescens*, *Pa. foenisecii*, *Pa. retirugis*, and *Pa. charrua* sp. nov. All species, except *Pa. antillarum*, represent new records for Uruguay, and *Pa. charrua* is described here as a species new to science. Two species of *Psilocybe* were documented: *Ps. cubensis*, previously recorded for Uruguay, and *Ps. stuntzii*, representing a new record for Uruguay and an extension of its known distribution range. Quantitative NMR analyses revealed detectable PSB and/or PS exclusively in *Pa. cyanescens*, *Ps. cubensis*, and *Ps. stuntzii*, with *Ps. cubensis* exhibiting the highest alkaloid concentrations. This study substantially expands the known diversity of coprophilous agarics in Uruguay, provides the first integrative taxonomic framework for these genera in the country, and contributes novel chemical data relevant to both systematics and regulatory contexts.

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INTRODUCTION

Coprophilous fungi play a key role in ecosystems by contributing to the degradation of cellulose and other polymers that are partially or completely digested by herbivores (Richardson 2001). They exhibit specific adaptations to this habitat, including tolerance to dehydration, resistance to UV radiation, and the ability of basidiospores to survive passage through the digestive tract of herbivores (Garnica *et al.* 2007). The introduction of domestic livestock into the Americas in the 15th century (Primo 1992), followed by its dispersal across South America and successive re-introductions of breeds from Asia, North America, and Europe, greatly increased the availability of dung. These conditions likely facilitated the establishment of coprophilous fungi through both the introduction of non-native species—co-introduced with

livestock—and the expansion or substrate shift of native taxa. As a result, the geographic origins of many dung-associated fungi remain difficult to determine, with some lineages suggesting recent introductions, as hypothesized for *Bolbitiaceae* species in the Americas (e.g., Morera *et al.* 2024) and *P. cubensis* in Australia (McTaggart *et al.* 2023); whereas others may have been present in America prior to livestock arrival and later expanded with domesticated animals, as proposed for *P. cubensis* (Bradshaw *et al.* 2026). Consequently, dung-rich environments may host a mixture of native and introduced lineages, including taxa previously undescribed and new to science.

The most conspicuous basidiomata-forming taxa of coprophilous fungi belong to the order Agaricales (*Basidiomycota*) and are distributed across several genera, including *Bolbitis*, *Conocybe*, *Coprinellus*, *Deconica*, *Agrocybe*,

Panaeolus, *Pholiotina*, *Psathyrella*, *Psilocybe*, and *Stropharia* (Dix & Webster 1995, Ramírez-Cruz et al. 2013, Strauss et al. 2022). These fungi can exhibit either an agaricoid habit, in which the pileus expands to expose the lamellae at maturity, or a secotioid habit, in which the lamellae remain enclosed at maturity. Several species within these genera are known to produce psychoactive alkaloids, mainly psilocybin (PSB) and psilocin (PS). The PSB biosynthetic pathways appear to have been evolutionarily conserved as a result of complex evolutionary processes which implies natural selection, convergent evolution and horizontal gene transfer (Meyer & Slot 2023, Schäfer et al. 2025). When PSB is dephosphorylated, it produces PS, a serotonin (5-HT_{2A}) receptor partial agonist that targets the animal central nervous system and is responsible for the psychoactive effects observed in humans (Halberstadt & Geyer 2011, Plazas & Faraone 2023). Species of *Panaeolus* and *Psilocybe* comprise most of the known dung-associated psychoactive fungi, with total alkaloid contents (PSB + PS) ranging from 1.5 to 3 % of dry mass (Guzmán et al. 1998, Allen 2010, Laussmann & Meier-Giebing 2010, Gotvaldová et al. 2022, Batista et al. 2025).

Both *Panaeolus* and *Psilocybe* species have been historically used by diverse cultures for medicinal, religious, and spiritual purposes (Wasson 1957, Ott 1993). More recently, PSB has gained attention in biomedicine due to promising results in treating depression and other psychiatric conditions (Carhart-Harris et al. 2021, Haikazian et al. 2023). Ongoing U.S. FDA-approved phase II clinical trials are testing PSB-assisted therapy for major depressive disorder (Raison 2019, Højlund et al. 2025). In this context, the effects of administration of whole mushrooms in clinical trials have not been fully elucidated; however, recent preclinical studies suggest that they may differ from those of pure PSB, likely due to the contribution of other metabolites acting synergistically (Zhuk et al. 2015, Dörner et al. 2022). Yet, outside the clinical framework, the popular use of these fungi is largely supported by domestic cultivation and wild harvesting (Batista et al. 2025). In such cases, identification is often based on the characteristic bluing reaction of the pileus or stipe when damaged (Lenz et al. 2020).

The taxonomic history of *Panaeolus* and *Psilocybe* dates back to the first half of the 19th century, when both were treated as infrageneric taxa within *Agaricus* (Fries 1821, 1836–1838, 1849). They were subsequently elevated to genus rank by Kummer (1871) and Quélet (1872), respectively. Today, *Panaeolus* comprises approximately 56 species and *Psilocybe* around 150 species, ranging from agaricoid to secotioid forms, which grow on dung, decaying wood, or soil across temperate, tropical, and desert regions worldwide (Ramírez-Cruz et al. 2013, Strauss et al. 2022, Voto & Angelini 2024, Consiglio & Marchetti 2025, He et al. 2026). Both genera have been previously studied in the region, with several species recorded in neighboring countries. In Argentina and Brazil, for example, 17 species of *Panaeolus* and 29 of *Psilocybe* have been reported (Guzmán 2005, Niveiro & Albertó 2012, Silva 2013, Van Court et al. 2022, Strauss et al. 2023, Batista et al. 2025). In Uruguay, knowledge of *Panaeolus* and *Psilocybe* remains limited. To date, the following species have been recorded: *Panaeolus antillarum* (Gerhardt 1996, Sequeira 2017), *Pa. acuminatus* (Rosa-Mato 1939, Felippone 1928), *Pa. papilionaceus* (Herter 1907,

Felippone 1928, Rosa-Mato 1939), *Pa. semiovatus* (as *Pa. phalaenarum*, Felippone 1928), *Psilocybe caeruleoannulata* Singer ex Guzmán (Guzmán 1978), *Ps. uruguayensis* Singer ex Guzmán (Guzmán 1978), *Ps. cubensis* (Sequeira 2013), and *Ps. bullacea* (Felippone 1928). Documenting the diversity of these genera in Uruguay is important both, from a taxonomic perspective and due to their relevance as the main natural producers of PSB, particularly in the context of emerging regulations concerning fungi and their derivatives.

The aim of this study was to analyze the diversity of coprophilous fungi of the genera *Panaeolus* and *Psilocybe* in Uruguay using phylogenetic and morphological approaches, to provide detailed morphological descriptions and to determine the content of the main psychoactive alkaloids (PSB and PS) in the species.

MATERIALS AND METHODS

Sampling

During sampling campaigns conducted in Uruguay between 2021 and 2024 to document coprophilous agaric fungi, surveys were carried out in cattle- and horse-grazed fields across the southern and eastern regions of the country (departments of Montevideo, Colonia, Maldonado, Lavalleja, Rocha, Tacuarembó and Cerro Largo). Basidiomata collected in the field were placed in aluminum-foil envelopes for transport to the laboratory. Tissue samples were aseptically taken for DNA analysis, and basidiomata were subsequently air-dried at 20 °C. Part of the sample was separated for chemical analysis, kept at ≈ 20 °C degrees in darkness, and a representative sample was deposited in the MVHC Herbarium (“Montevideo, Humanidades y Ciencias”). In two cases, anonymous spore-print donations identified as *Ps. cubensis* were received (MVHC 5762 and MVHC 5764); these were cultivated to obtain basidiomata, following the procedure described in Rodríguez et al. (2025).

Morphological analysis

Macro- and micromorphological studies of the basidiomata were conducted following the terminology and methodology of previous authors (Singer 1960, Guzman & Petrarca 1972, Young 1989, Gerhardt 1996, Wright & Albertó 2002, Malyshova et al. 2019, Voto & Angelini 2024, Batista et al. 2025). For each species, we elaborated descriptions covering both macroscopic and microscopic characters. Macroscopic assessments emphasized texture, color following Kornerup & Wanscher (1978), and the dimensions of the pileus, margin, context, lamellae, and stipe. Microscopic structures were examined with a light microscope; small sections of basidiomata were rehydrated in KOH (5 %) and stained with Congo Red (1 %), as needed. Images were captured using an AmScope digital microscope camera. Sections of pileus, lamellae, and stipe were analyzed, and measurements were taken for n = 30 elements of each structure, including: basidiospores, basidia, and cystidia (length × width). Basidiospore dimensions are reported as (a–)b–c(–d), where b–c represents 90 % of the measured values and the extremes (a, d) are given in brackets. The basidiospore length-to-width ratio (Q) and its mean value (Q_m) were also calculated.

Molecular protocols

Genomic DNA extraction was performed from basidiomata tissue fragments following a standard CTAB protocol (Doyle & Doyle 1987) and stored at -20°C . The ITS and LSU regions were amplified with primers ITS4/ITS5 or ITS1F/ITS4B (White *et al.* 1990) and LR0R/LR5 or LR0R/LR7 (Vilgalys & Hester 1990), respectively. For *Psilocybe* specimens, the *rpb1* region was also amplified with primers Rpb1-af/Rpb1-cr (Matheny *et al.* 2002). PCR reactions contained 2 μL of genomic DNA ($\sim 60\text{ ng}$), 16 μL of ultrapure water, 0.25 μL of Taq polymerase (1 U), 0.5 μL of each primer (10 mM), 0.7 μL of 50 mM MgCl_2 , 2.5 μL of dNTPs (2.5 mM), and 2.5 μL of 10 $\times$ buffer, in a final volume of 25 μL . Amplifications were performed in a MultiGene Optimax thermocycler (Labnet International Inc.) under the following conditions: initial denaturation at 94°C for 3 min; 35 cycles of 94°C for 60 s, 50°C for 45 s, and 72°C for 60 s; followed by a final extension at 72°C for 5 min. PCR products were verified by electrophoresis on 1% agarose gels in TBE buffer, stained with EZ-Vision One (Amresco[®]), and visualized under UV light. The purified PCR products were sent to Macrogen (Seoul, Korea) for sequencing. The received sequences were inspected and edited with Bioedit v. 7.0.5.3 (Hall *et al.* 1999) and finally deposited in GenBank (Tables S1, S2).

Phylogenetic analysis

To explore the phylogenetic relationships of *Panaeolus* and *Psilocybe* species, two data sets were constructed, one for each genus, combining the new DNA sequences generated in the present work with sequences retrieved from GenBank (NCBI). Scientific names and GenBank Accession Numbers of sequences are listed in Tables S1 and S2. The *Panaeolus* dataset was composed of ITS and LSU sequences, and *Staktophyllus* was selected as an outgroup (Voto & Angelini 2024, Consiglio & Marchetti 2025). The *Psilocybe* dataset was composed of ITS, LSU, *rpb1* and *tef1* sequences, and *Deconica* was selected as an outgroup (Ramirez-Cruz *et al.* 2013). For each dataset, each region (ITS, LSU, *rpb1* and *tef1*) was individually aligned using the MAFFT online service (Kato *et al.* 2019) using the G-INS-I alignment method. All obtained alignments were manually inspected and adjusted with Bioedit.

The best-fit partitioning strategy and the best-fit model of nucleotide evolution for each dataset was estimated using ModelFinder (Kalyaanamoorthy *et al.* 2017) implemented in the IQ-Tree software (Nguyen *et al.* 2015) with 4 data blocks for *Panaeolus* dataset (ITS1; 5.8S; ITS2; and LSU) and 12 data blocks for *Psilocybe* dataset (ITS1; 5.8S; ITS2; and LSU; *rpb1* 1st, 2nd, and 3rd codon positions; *rpb1* introns; *tef1* 1st, 2nd, and 3rd codon positions; and *tef1* introns), restricting the models to those implemented using MrBayes v. 3.2. (Ronquist *et al.* 2012).

Bayesian inference and maximum likelihood phylogenetic analyses were performed independently to each concatenated dataset using the partition scheme and evolutionary models defined in ModelFinder following Robledo *et al.* (2020, 2021). BI was performed in MrBayes v. 3.2 (Ronquist *et al.* 2012) using the CIPRES science gateway (Miller *et al.* 2010; <http://www.phylo.org/>). Two

independent runs were performed, each one starting from random trees with four simultaneous independent chains, for 10 M generations, sampling parameters every 1000 generations. The burn-in tree discard value was set at 0.25, and remaining trees were used to reconstruct a 50 % majority rule consensus tree and calculate Bayesian posterior probabilities (BPP) of the clades. ML searches were performed in IQ-TREE, with 100 ML initial searches, starting from one randomized stepwise addition parsimony tree. Branch support (BS) was calculated using UFBoot (ultrafast bootstrap approximation implemented in IQ-TREE with 1000 replications) (Hoang *et al.* 2018). A node was considered strongly supported with BPP ≥ 0.95 and/or BS $\geq 95\%$ (Hyde *et al.* 2013, Minh *et al.* 2020). Alignments and phylogenetic trees are available at Zenodo (DOI: 10.5281/zenodo.18852023).

Chemical analysis

For quantitative analyses of PSB and PS, extracts were prepared following Rodríguez *et al.* (2025). Briefly, 0.3 g of a pooled mixture of fruiting bodies (stipe + pileus) were ground, then divided in 3 Falcon tubes and subjected to four consecutive extractions with 1.7 mL methanol under sonication for 30 min ($25\text{--}30^{\circ}\text{C}$). Solvents were combined and removed by vacuum evaporation to obtain dry extracts, which were redissolved in 0.6 mL DMSO- d_6 for quantitative Nuclear Magnetic Resonance (qNMR) analyses on a Bruker Avance Neo 400 spectrometer. qNMR analyses were carried out using the PULse Length based CONcentration (PULCON) method, which allows the determination of the absolute content of target compounds. ^1H -NMR was used for the quantification of PS and ^{31}P -NMR for PSB. Data were expressed as the percentage of PSB or PS by dry mass. Samples were analyzed in triplicate, and results are presented as mean $\pm$ relative standard deviation (RSD). However, in cases where only a single measurement was possible, the corresponding value is reported without an error estimation (Table 1). Finally, we systematically reviewed the literature to document whether each species represented in the phylogenetic analysis had previously been reported as producing PSB and/or PS. For this purpose, we assigned presence or absence to each species in the phylogeny (Figs 1, 2) based on the previously published data (Guzmán & Petrarca 1972, Høiland 1978, Young 1989, Guzman *et al.* 1998, Senn-Irlet *et al.* 1999, Allen 2010, Guzman *et al.* 2012, Borovicka *et al.* 2012, Hyde 2014, Borovicka *et al.* 2015, Wang & Tzean 2015, Li *et al.* 2020, Nkadameng *et al.* 2020, Voto & Angelini 2021, Gotvaldová *et al.* 2022, Strauss *et al.* 2023, Allen *et al.* 2024, Canan *et al.* 2024, Ostuni *et al.* 2024, and the references within).

RESULTS

Phylogenetic analysis

The concatenated *Panaeolus* dataset consisted of 285 sequences (including the outgroup) and 1695 aligned positions including gaps. Within the ITS region (772 bp), 170 sites were parsimony-informative, 61 were singleton, and 541 were constant, while in the LSU region (923 bp), 47 sites were parsimony-informative, 46 were singleton, and 830 were con-

Table 1. Psilocybin (PSB) and psilocin (PS) content expressed as percentage of dry mass in samples of *Panaeolus* and *Psilocybe* species collected in Uruguay.

Species	Voucher or sample	%PSB	%PS
<i>Panaeolus antillarum</i>	MVHC 5787	<LOD	<LOD
<i>Panaeolus charrua</i>	MVHC 5825	<LOD	<LOD
<i>Panaeolus charrua</i>	MVHC 5833	<LOD	<LOD
<i>Panaeolus cyanescens</i>	MVHC 5827	<LOD	<LOD
<i>Panaeolus cyanescens</i>	MVHC 5839	<LOQ	0.06 ± 0.01
<i>Panaeolus cyanescens</i>	MVHC 5842a	0.09	0.2
<i>Panaeolus cyanescens</i>	MVHC 5879	0.28 ± 0.019	0.31 ± 0.035
<i>Panaeolus cyanescens</i>	MVHC 5880	0.04 ± 0.009	0.11 ± 0.009
<i>Panaeolus cyanescens</i>	PC_sample 2 ^a	0.03	0.28
<i>Panaeolus cyanescens</i>	PC_sample 3 ^a	<LOD	0.42
<i>Panaeolus cyanescens</i>	PC_sample 4 ^a	<LOD	0.31
<i>Panaeolus foenisecii</i>	MVHC 5862	<LOD	<LOD
<i>Panaeolus retirugis</i>	MVHC 5797	<LOD	<LOD
<i>Panaeolus retirugis</i>	MVHC 5863	<LOD	<LOD
<i>Psilocybe cubensis</i>	MVHC 5762	<LOD	0.32 ± 0.007
<i>Psilocybe cubensis</i>	MVHC 5849 Sample 1 ^a	0.71	0.209
<i>Psilocybe cubensis</i>	MVHC 5849 Sample 2	<LOD	0.066 ± 0.006
<i>Psilocybe cubensis</i>	MVHC 5849 Sample 3	0.44 ± 0.019	<LOQ
<i>Psilocybe cubensis</i>	MVHC 5850	0.41 ± 0.011	0.22 ± 0.016
<i>Psilocybe cubensis</i>	MVHC 5851	<LOD	0.26 ± 0.006
<i>Psilocybe cubensis</i>	MVHC 5853	0.052 ± 0.009	0.077 ± 0.012
<i>Psilocybe stuntzii</i>	MVHC 5854	<LOD	<LOQ
<i>Psilocybe stuntzii</i>	MVHC 5866	<LOD	<LOD
<i>Psilocybe stuntzii</i>	PU_sample 1	<LOD	<LOQ
<i>Psilocybe stuntzii</i>	PU_sample 2	<LOD	<LOQ

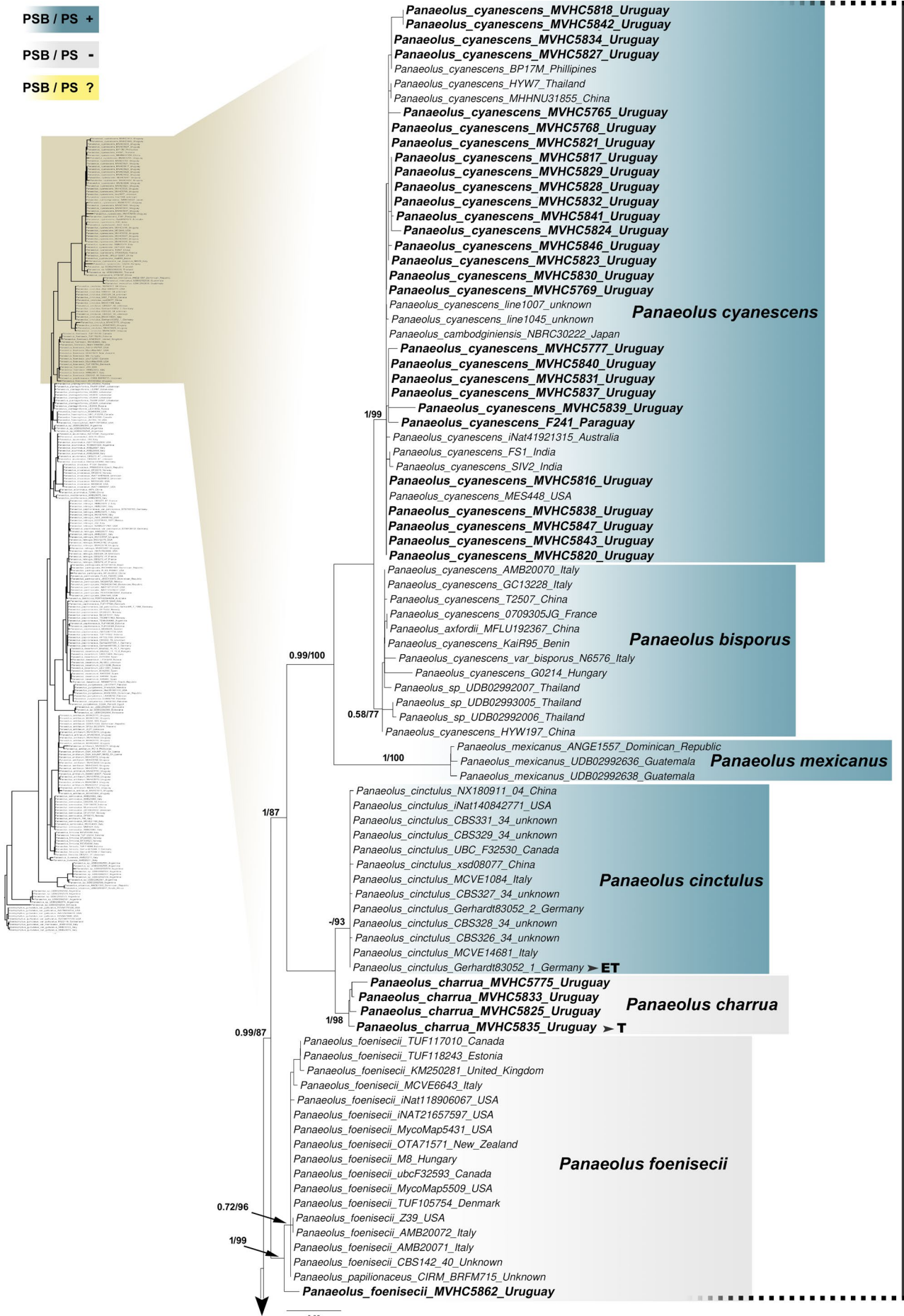
PC and PU samples correspond to pools of basidiomata without associated voucher. For triplicate samples, results are presented as mean ± relative standard deviation (RSD). a: Only a single measurement (n = 1) was possible due to limited sample availability; therefore, the corresponding value is reported without an error estimate. LOD (Limit of detection): 0.017% PS and 0.013% PSB; LOQ (Limit of quantification): 0.034% PS and 0.036% PSB (Rodríguez et al. 2025).

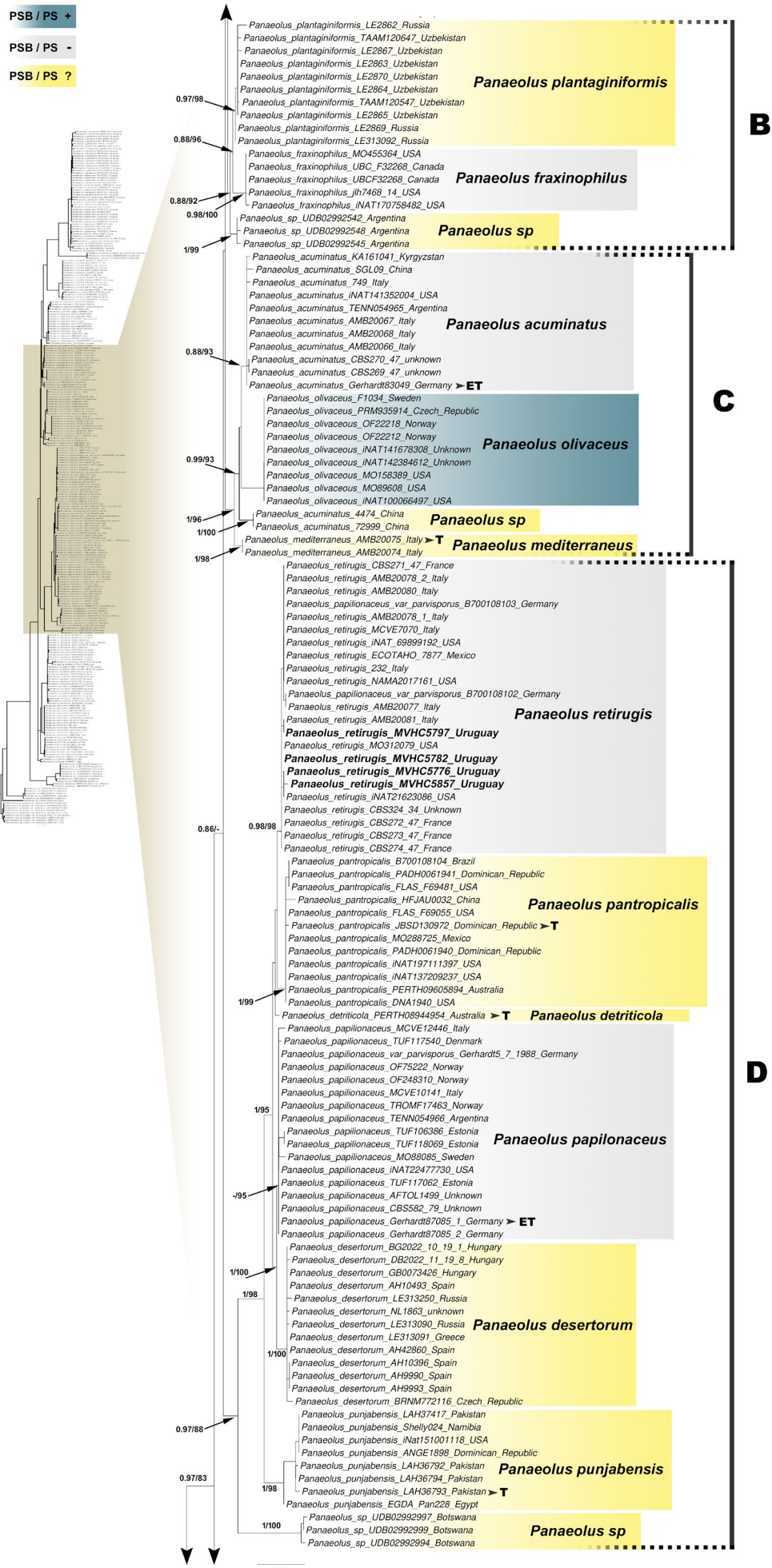
stant. The partitions and evolutionary models selected were: ITS1 + ITS2 (HKY+F+G4), 5.8S + LSU (K2P+I+G4). Maximum Likelihood and Bayesian Inference analyses resulted in highly similar topologies. The ML tree is shown in Figure 1 and has been divided into three panels to facilitate visualization of the major clades and terminal lineages. Nine major clades (A–I in Fig. 1) were consistently recovered, most of them with high support in both analyses (BPP ≥ 0.95 and/or BS ≥ 95 %), indicating stable relationships among the principal lineages of *Panaeolus*. A total of 57 sequences generated in this study were clustered within five species: *Panaeolus antillarum* (BPP: 1, BS: 100), *Pa. cyanescens* (BPP: 1, BS: 99), *Pa. foenisecii* (BPP: 1, BS: 99), *Pa. retirugis* (BPP: 0.98, BS: 98) and an isolated clade *Pa. charrua* sp. nov. (BPP: 1, BS: 98). The *Psilocybe* combined dataset comprised 228 terminals and 3560 total aligned positions including gaps (including the out-group). ITS region contained 720 bp with 241 parsimony-informative sites, 37 singleton, and 442 constant; LSU region 875 bp with 60 parsimony-informative, 50 singleton, and 765 constant; *rpb1* region 1412 bp with 601 parsimony-informative, 216 singleton, and 595 constant; and *tef1* region 553 bp with 183 parsimony-informative, 25 singleton, and 344 constant. The partitions and evolutionary models selected were: ITS1 + ITS2 + *rpb1* 2ndpos (HKY+F+I+G4), 5.8S + *tef1* 1stpos + *tef1* 3rdpos (SYM+I), LSU (K2P+I+G4), *rpb1* 1stpos (F81+F+G4), *rpb1* 3rdpos (HKY+F+G4), *rpb1* introns (HKY+F+G4), *tef1* 2ndpos (GTR+F+I) and *tef1* introns (HKY+F+I). The phylogenetic

trees obtained from ML and BI analyses using the combined data sets showed overall the same topology. The ML tree is shown in Fig. 2 and has been divided into two panels to facilitate visualization of the major clades and terminal lineages. The global tree is divided into two large strongly supported clades (1–2), each with two well-defined clades (A–D) (Fig. 2). Sequences corresponding to 16 Uruguayan samples were clustered in two clades: *Psilocybe cubensis* (BPP:0.77, BS:83) and *Ps. stuntzii* BPP:0.97, BS:83).

Chemical analysis

Quantification of psilocybin (PSB) and psilocin (PS) by qNMR was performed on 25 extracts derived from pooled specimens representing each of the seven species recorded in Uruguay (Table 1): *Pa. antillarum*, *Pa. cyanescens*, *Pa. foenisecii*, *Pa. charrua*, *Pa. retirugis*, *Ps. cubensis*, and *Ps. stuntzii*. Psilocibin was quantified by ¹H-NMR using the signal at 6.27 ppm (H-5 of the indole ring), whereas PSB was quantified by ³¹P-NMR targeting the phosphate resonance at -2.875 ppm. The values obtained for each species are summarized in Table 1. Only three species showed significant PS or PSB contents: *Ps. stuntzii* showed detectable levels of PS but below the quantification limits, *Pa. cyanescens* displayed moderate alkaloid concentrations (PSB up to 0.28 %; PS up to 0.42 %) and *Ps. cubensis* yielded the highest overall contents (PSB up to 0.71 %; PS up to 0.32 %).





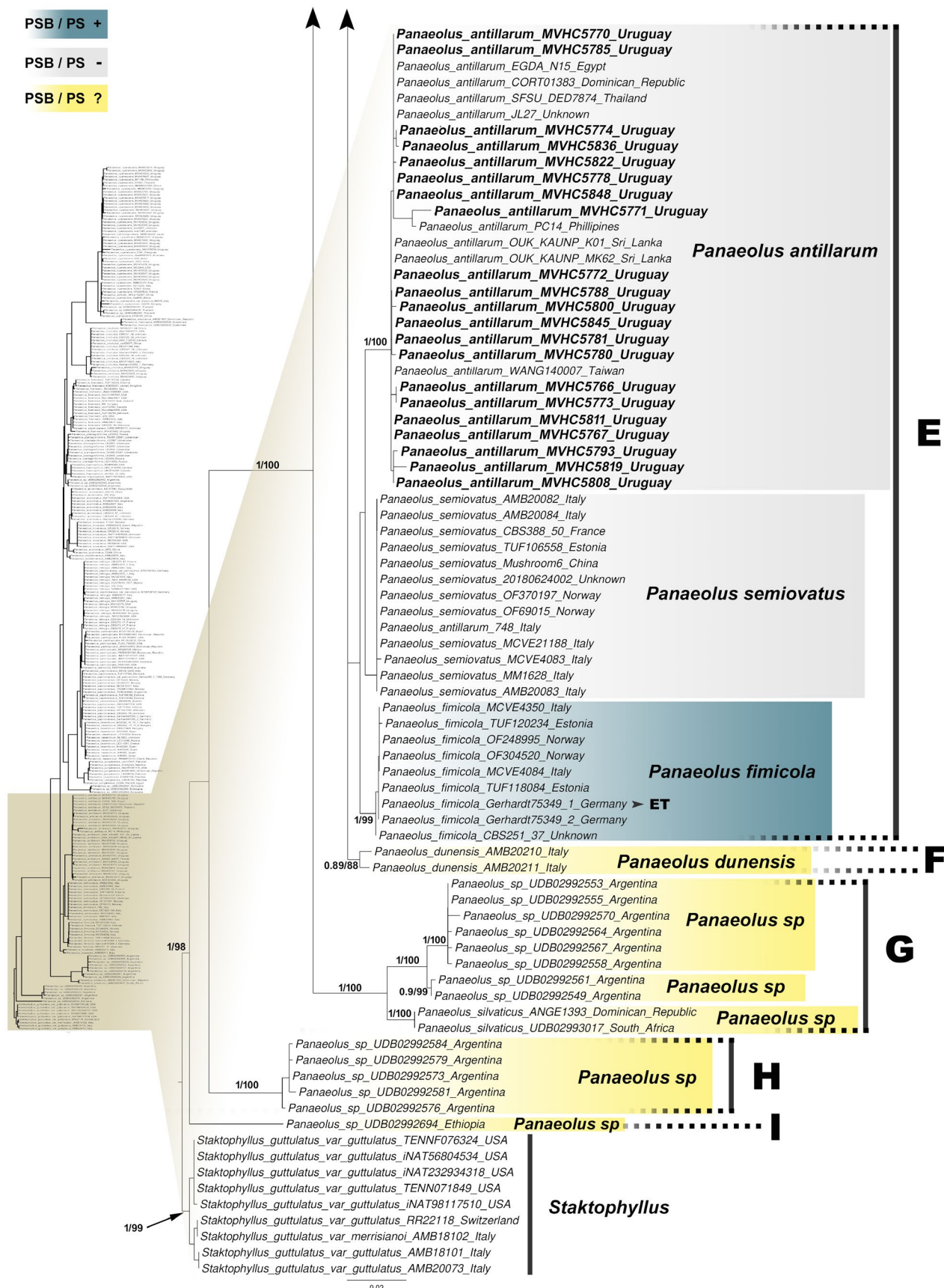


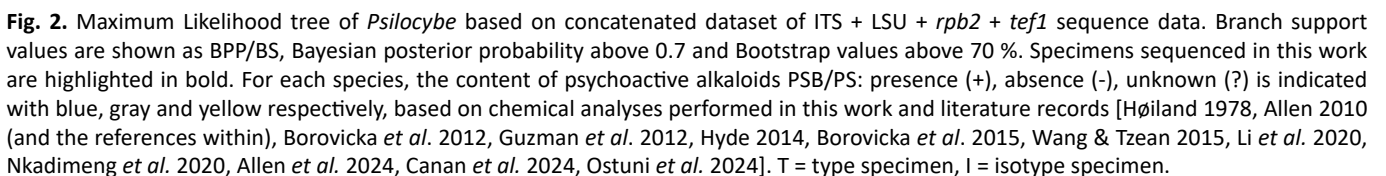
Fig. 1. Maximum Likelihood tree of *Panaeolus* based on concatenated dataset of ITS + LSU sequence data. Branch support values are shown as BPP/BS, ≥ 0.7 and ≥ 70 respectively. Specimens sequenced in this work are highlighted in bold. For each species, the content of psychoactive alkaloids PSB/PS: presence (+), absence (-), unknown (?) is indicated with blue, gray and yellow respectively, based on chemical analyses performed in this work and literature records (Guzmán & Petrarca 1972, Young 1989, Guzman et al. 1998, Senn-Irllet et al. 1999, Allen 2010, Voto & Angelini 2021, Gotvaldová et al. 2022, Strauss et al. 2023 and the references within). T= type specimen, ET= epitype specimen.

PSB / PS +

PSB / PS -

PSB / PS ?





Taxonomy

Based on morphological and phylogenetic analyses, five species of *Panaeolus* were identified, one of them new to science, along with two species of *Psilocybe*. Detailed morphological descriptions based on the studied specimens are presented below. For each species, a taxonomic discussion and the results of alkaloid content analysis are included.

Panaeolus antillarum (Fr.) Dennis, *Kew Bull.* **15**(1): 124. 1961. Fig. 3A–M.

Description: *Pileus* 0.8–10 × 0.7–5 cm, conical to convex or broadly parabolic, occasionally hemispherical to conico-campanulate; surface shiny, dry, non-hygrophanous, and glabrous, becoming wrinkled, lacunose, or irregularly rimose at maturity. Color varying with gray valleys (5B1; Gray) and yellowish-brown ridges (5E5; Yellowish Brown) or creamy-whitish tones (5E3; Pale Orange). When dry, the pileus appears homogeneous, rough, beige (5E7; Yellowish Brown), and shiny, sometimes exhibiting a grayish sheen (5D1; Gray). *Margin* straight to slightly incurved, entire, finely undulating to eroded when dry, concolorous with the pileus surface. *Lamellae* adnate to adnexed, close to crowded; edge entire, smooth, whitish to dark when dry; faces waxy, dark grayish (5F1; Gray) to black, mottled. *Stipe* 2–15 × 0.2–1.2 cm, central, cylindrical. Surface slightly pruinose to longitudinally striate (from apex to base), fibrous, hard, and brittle. Color whitish to yellowish-brown, consistent with the pileus surface. *Context* 0.1–0.2 cm thick, fleshy, whitish. *Veil* absent. *Spore print* blackish. *Basidiospores* (10.3–)14.5–20(–21.8) × (7.3–)8.9–12.9(–13.9) × (–5.8)6.7–10.9(–12.8) μm , $Q = 1.49$ –1.69, $Q_m = 1.69$, limoniform to subhexagonal in frontal view and ellipsoid in lateral view, dark brown to blackish, smooth, thick walls 1.2–1.5 μm thick and a truncated germ pore 1.1–2.5 μm . *Basidia* 16.2–32.5 × 6.4–17.5 μm , irregularly cushion-shaped to pyriform, hyaline, tetrasporic. *Pleurocystidia* 20–56.5 × 10.1–25.8 μm , chrysocystidia, sulphidia type, hyaline, thin-walled, clavate to occasionally cylindrical, some mucronate or papillate, with refractive and irregular greenish-grayish content in KOH. *Cheilocystidia* 13.8–41.9 × 4.3–20 μm , sparse, grouped, polymorphic, cylindrical, short-clavate, fusiform, with broad and obtuse apex or lageniform, hyaline, and thin-walled. *Hymenophoral trama* composed of smooth, hyaline, thin-walled hyphae, cylindrical (up to 4.3 μm) or more commonly inflated (5.6–11.7 μm). *Pileipellis* formed by an epithelium of isodiametric to pyriform cells, smooth, thin-walled, 12.2–44.6 μm diam. *Pileal trama* composed of interwoven, thin-walled hyphae, 3.3–5.1 μm diam. *Stipitipellis* a cutis of interwoven hyaline, thin-walled hyphae, 4.9–7.9 μm diam. *Caulocystidia* 19–69.6 × 3.5–14.7 μm , leptocystidia, cylindrical, hyaline, thin-walled, with an obtuse apex.

Specimens examined: **Uruguay**, Montevideo, Manga, S34°48'26", W56°08'17, 37 m.a.s.l., peri-urban field, on cow dung, 1 Apr. 2021, G. Morera (MVHC 5748, MVHC 5766 and MVHC 5767); Colonia, S33°58'23.4", W57°16'56.7", 20 m.a.s.l., open meadow with cattle, on horse dung, 8 Feb. 2022, G. Morera et al. (MVHC 5770–74, MVHC 5778–80 MVHC 5781, MVHC 5783–96, MVHC 5798–5815); Maldonado, Sierra

de las Ánimas, S34°36', W55°17', 209 m.a.s.l., on cow dung, 3 Mar. 2022, G. Morera & S. Lupo (MVHC 5819, MVHC 5822, MVHC 5836 and MVHC 5845); Tacuarembó, S31°42'40"S, W55°58'44", 137 m.a.s.l., on cow dung, May 2022, S. Lupo (MVHC 5848).

Habit, habitat and distribution: Cosmopolitan in tropical to subtropical regions (Voto and Angelini 2024, Strauss et al. 2023). It was firstly registered in Uruguay by Gerhardt (1996) and recently recorded by Sequeira (2017). Found growing solitary or in groups up to 4 specimens, in horse and cow dung.

Notes: *Panaeolus antillarum* is a coprophilous cosmopolitan species originally described from the Antilles, Dominican Republic (Fries 1828, Dennis 1961), where it was found growing on mule dung. However, it has also been observed on cow, horse, and even elephant dung, among other substrates (De & Perry 2017). It is characterized by basidiomata of medium size, with the pileus convex or broadly parabolic, reaching up to 10 cm diam., with a pale grayish or whitish coloration and lacking annulus. Microscopically, the species is distinguishable by its large basidiospores (up to 21.8 μm in length, subhexagonal in frontal view) and pleurocystidia that are mucronate or papillate, containing refractive and irregular greenish gray content in KOH. These characteristics align with the observations of Guzman & Petrarca (1972), Gerhardt (1996), Kaur et al. (2014) and Voto & Angelini (2024). Phylogenetically, *Pa. antillarum* is closely related to *Pa. semiovatus* and *Pa. fimicola* (Fig. 1), two pale-colored species that also possess sulphidia-type chrysocystidia (Hesler 2013, Consiglio & Marchetti 2025). *Panaeolus antillarum* can be distinguished from *Pa. semiovatus* by the absence of an annulus or any remnants of a marginal veil on the pileus (Voto & Angelini 2024), and from *Pa. fimicola*, which produces markedly smaller basidiospores reaching about 12 μm (Consiglio & Marchetti 2025). *Panaeolus antillarum* was initially classified as a hallucinogenic species (Guzmán 1959); however, recent studies have provided evidence for the absence of psychoactive indole alkaloids such as psilocybin, psilocin, or baeocystin in this species (Young 1989, Allen 2010, Strauss et al. 2023, this work). This misconception could have been due to confusion with other potentially hallucinogenic species, such as *Pa. fimicola* or *Pa. cyanescens*.

Panaeolus charrua Morera, Lupo, Martínez Kopp & Robledo, **sp. nov.** Fig 4A–J. MB 862528.

Etymology: Named after the Charrúa people, Indigenous inhabitants of Uruguay and neighboring regions of southern South America.

Typus: **Uruguay**, Maldonado, Sierra de las Ánimas, S34°36', W55°17' elev. 209 m.a.s.l., on cow dung, 3 Mar. 2022, G. Morera & S. Lupo (**holotype** MVHC 5835).

Description: *Pileus* 0.5–2.5 × 1–2 cm (generally twice as high as wide), mostly secotoid, occasionally conical-umbonate, parabolic, or campanulate; surface opaque, dry, non-hygrophanous, glabrous, smooth to wrinkled, yellowish or light brown (5D6; Yellowish Brown), with concentric

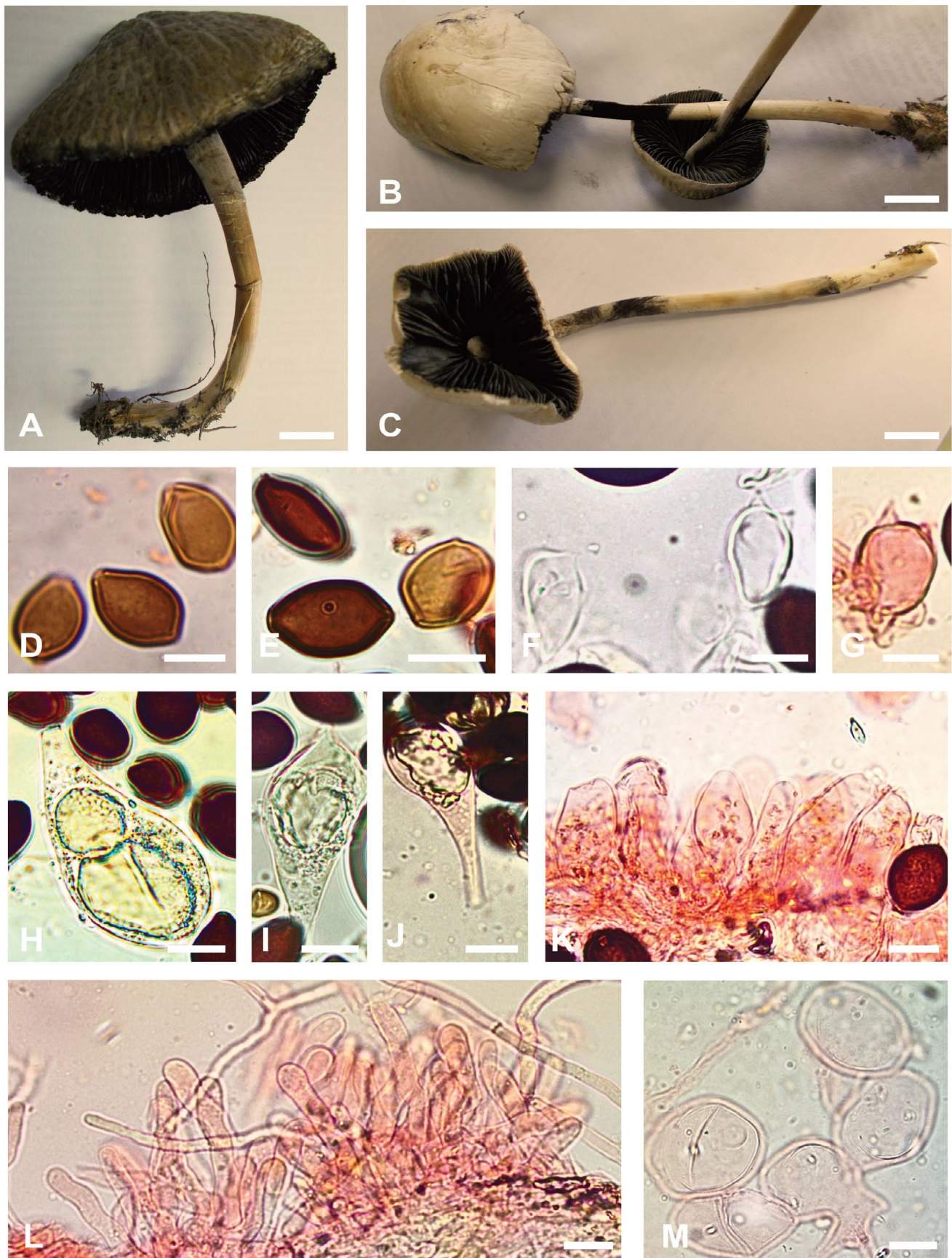


Fig. 3. *Panaeolus antillarum*. Basidiomata. **A.** MVHC 5770. **B, C.** MVHC 5774. Basidiospores. **D, E.** MVHC 5770. Basidia. **F.** MVHC 5770. **G.** MVHC 5780. Pleurocystidia. **H, I.** MVHC 5774. **J.** MVHC 5791. Cheilocystidia. **K.** MVHC 5783. Caulocystidia. **L.** MVHC 5795. Pileocystidia. **M.** MVHC 5780. Scale bars: A–C = 1 cm, D–M = 10 µm.



Fig. 4. *Panaeolus charrua*. Basidiomata. **A, B.** MVHC 5826. Basidiospores. **C.** MVHC 5835. Basidia. **D.** MVHC 5775. **E, F.** MVHC 5826. Cheilocystidia. **G.** MVHC 5835. Caulocystidia. **H.** MVHC 5826. Pileocystidia. **I, J.** MVHC 5775. Scale bars: A, B = 1 cm, C–J = 10 μ m.

striations and tears when fresh. *Margin* irregular, straight, sometimes incurved or nearly attached to the stipe, entire, then eroded, initially concolorous, later darkened (5F4; Olive Brown). *Lamellae* adnate or adnexed, close to crowded, waxy, whitish when fresh (7A2; Pinkish White), dark grayish (5F1; Gray) when mature, not mottled. Edge thick, entire, light brown when fresh, turning grayish. Stipe 6–10 $\times$ 0.1–0.4 cm, cylindrical, striated (longitudinally helicoidal), glabrous, hollow, fibrous, and fistulose. Inner surface dark, outer surface concolorous with the pileus, with darker

longitudinal bruises. *Context* 0.1–0.2 cm, fibrous to corky, whitish to light brown (4A2; Yellowish White). *Veil* absent. *Spore print* dark gray to graphite colored. *Basidiospores* (10–)11.6–15.6(–16) $\times$ (6.9–)7.2–10.1(–10.5) $\times$ (3.8–)5.3–8(–8.6) μ m, $Q = 1.5$ –1.87, $Q_m = 1.85$, ovoid to rhomboid in frontal view, and ellipsoidal in lateral view, with a slightly flattened side. Color light to dark brown, with walls up to 1.3 μ m thick, smooth, with a conspicuous truncate germ pore, up to 1.8 μ m. *Basidia* 12.4–38.9 $\times$ 6–15.1 μ m, clavate to sub-spherical, with 1–4 sterigmata, normal to thickened, sometimes very

long (up to 53 μm), other times branching from a central axis (up to 24 μm). *Pseudoparaphyses* often observed, generally utriform to ovoid, $16.4 \times 10 \mu\text{m}$ in average. *Pleurocystidia* not seen. *Cheilocystidia* $16.4\text{--}40.8 \times 4.8\text{--}14.2 \mu\text{m}$, hyaline, thin-walled, lageniform, sometimes ventricose, rarely cylindrical. *Hymenophoral trama* composed of smooth, irregularly arranged hyphae, filamentous or more commonly inflated up to 12 μm . *Pileipellis* composed of an epithelium of spherical pedunculated cells, $18.4\text{--}51.9 \times 10.6\text{--}33.5 \mu\text{m}$. *Pileocystidia* hyaline, thin-walled, clavate, $17.4\text{--}52.5 \times 4.7\text{--}20.2 \mu\text{m}$. *Stipitipellis* a cutis of longitudinally arranged, yellowish brown hyphae, up to 8 μm wide. *Caulocystidia* $17.1\text{--}45.1 \times 2.4\text{--}12.6 \mu\text{m}$, polymorphic, cylindrical, clavate, sometimes ventricose, with a conspicuous wall in the apice.

Other collections examined: **Uruguay**, Colonia, S33°58'23.4", W57°16'56.7", 20 m.a.s.l., open meadow with cattle, on horse dung, 8 Feb. 2022, G. Morera et al. (MVHC 5775); Maldonado, Sierra de las Ánimas, S34°36'S, W55°17', 209 m.a.s.l., on cow dung, 3 Mar. 2022, G. Morera & S. Lupo (MVHC 5825, MVHC 5826 and MVHC 5833).

Habit, habitat and distribution: So far, it is only known from Uruguay, where it grows gregariously on cow dung, forming groups of up to 20 specimens.

Notes: *Panaeolus charrua* represents a new species characterized by a mostly enclosed pileus that is approximately twice as tall as it is wide, and by whitish to dark grayish, non-mottled lamellae. It presents a long (6–10 cm), longitudinally helicoid-striate stipe. Microscopically, it forms basidia with irregular sterigmata in shape (sometimes branched), length (up to 53 μm), and number (1–4). The general morphology of this new species, the combination of gasteroid and agaricoid basidiomata with *Panaeolus* micromorphology, resembles partially that of species traditionally known as *Galeropsis* (Velenovský 1930) and *Panaeolopsis* (Singer 1969). Historically the species classified in *Galeropsis* were later transferred from one to other genera (e.g., *Galera*, *Bolbitius*, *Conocybe*, *Agrocybe*, *Gastrocybe*, *Secotium*, *Weraroa*, *Cyttarophyllum*, *Psammomyces*), illustrating the taxonomic complexity and circumscription difficulties associated with this group. Since the 2000s, DNA-based studies have revealed the polyphyletic nature of *Galeropsis* (Tóth et al. 2013, Malysheva et al. 2019). On the other hand, *Panaeolopsis*, considered the secotioid counterpart of *Panaeolus*, is now considered an artificial genus and was recently synonymized with *Panaeolus* (Angelini & Voto 2023). *Panaeolus desertorum* and *Pa. plantaginiformis* are two species previously considered in *Galeropsis* (Malysheva et al. 2019). *Panaeolus charrua* differs from *Pa. desertorum* in its obtuse (not acute) pileus apex, smaller basidiospores ($10\text{--}16 \times 6.9\text{--}10.5 \times 3.8\text{--}8 \mu\text{m}$ vs $12.5\text{--}21.7 \times 6.3\text{--}17.7 \mu\text{m}$), and longer sterigmata (53 μm vs 20 μm). Moreover, *Pa. desertorum* lacks cheilocystidia and has a Eurasian distribution (Malysheva et al. 2019). The general macromorphology of the type of *Pa. charrua* is reminiscent of *Pa. plantaginiformis*, however, the color of the lamellae in *Pa. plantaginiformis* is ochraceous brown in contrast with the dark grayish of *Pa. charrua*. Microscopically, both species are very similar (including basidiospore and basidia size, as well as cheilocystidia morphology), but differ by the

absence of caulocystidia in *Pa. plantaginiformis*. Although *Pa. plantaginiformis* has been reported to have a worldwide distribution and has been reported from Argentina (Malysheva et al. 2019), it is phylogenetically unrelated to *Pa. charrua* and so far only known from Russia and Uzbekistan (Fig. 1). For their part, there are 4 ex-*Panaeolopsis* species close to *Pa. charrua*: *Pa. brasiliensis*, *Pa. nirimbii*, *Pa. obtusus*, and *Pa. sanmartinianus*. *Panaeolus brasiliensis* features a stipe with a bulbous base and subglobose basidiospores, unlike what is observed in *Pa. charrua* (Singer 1976). *Panaeolus sanmartinianus*, described from Argentina, differs from *Pa. charrua* by its ring-like basal opening of the mature pileus, smoky-black fusiform basidiospores, and the presence of pileocystidia similar to cheilocystidia (Singer 1969). The macromorphology of *Pa. nirimbii* also resembles that of *Pa. charrua*, however, *P. nirimbii* was described from Australia, growing on soil, and lacks descriptions of aberrant basidia, pseudoparaphyses (Young 1989), or a longitudinally helicoid-striate stipe (Voto & Angelini 2024). *Panaeolus obtusus* is a similar species with smaller basidia (up to 20 μm) that was described from Italy in soil with herbaceous plants (Contu 1998), unlike our specimens, which always grow from dung and show larger basidia (up to 38.9 μm). Moreover, the description by Contu (1998) does not provide details of the pileipellis nor comments on the presence of paraphyses (Contu 1998, Angelini & Voto 2023). Considering its unique morphological, ecological, geographical, and phylogenetic traits, we propose *Pa. charrua* as a new species. Regarding the alkaloid content, no detectable amounts of PSB and PS were observed (Table 1).

***Panaeolus cyanescens* Sacc., Syll. Fung. (Abellini) 5: 1123. 1887. Fig. 5A–L.**

Description: *Pileus* 0.5–4.5 $\times$ 0.5–1.8 cm, conical, campanulate-conical, or broadly parabolic, later becoming convex and eventually appearing flat; when dry, may exhibit an umbo; surface variable in luster, glabrous, dry to slightly subviscid, non-hygrophanous, and tending to become cerulescent upon handling, rimose at maturity. Color initially very pale brown to solid creamy white (3A2; yellowish white), sometimes with fine radial ornamentation, later becoming woody brown (5D5; light brown) with irregular deep cracks (except at the center). *Margin* straight or incurved, initially entire, later irregular and eroded, becoming dark and deeply rimose like the surface, concolorous with the surface or lighter, and cerulescent. *Lamellae* adnate to adnexed, close to crowded, pruinose, gray (5E6; gray) to ashy mottled, becoming blackish at maturity, with edges concolorous to the face or whitish (dark at maturity), entire to slightly serrated in exceptional cases. *Stipe* 2–8 cm $\times$ 0.2–0.5 cm, cylindrical, sometimes with basal mycelium and rarely subbulbous. Texture fibrous-cartilaginous, not fragile; surface longitudinally striated in the upper third, pruinose or fibrillose below, with more pronounced longitudinal striations in dry specimens. Internally yellowish, cerulescent, hollow; surface initially white to light brown, concolorous with the pileus, later yellowish brown and cerulescent. *Context* 0.2 cm thick, fleshy, creamy white (3A2; yellowish white), cerulescent. *Veil* absent. *Spore print* dark violet to blackish. *Basidiospores* (8.7–)11.5–16.1(–17.8) $\times$ (6.4–)7.8–11.7(–12.7) $\times$ (4.2–)5.6–9.8(–12.7) μm , $Q = 1.33\text{--}1.87$, $Q_m = 1.59$, lemon-shaped to



Fig. 5. *Panaeolus cyanescens*. Basidiomata. **A.** MVHC 5765. **B.** MVHC 5818. Basidiospores. **C, D.** MVHC 5769. Basidia. **E.** MVHC 5828. Pleurocystidia. **F.** MVHC 5777. **G.** MVHC 5820. Cheilocystidia. **H, I.** MVHC 5841. **J.** MVHC 5824. Caulocystidia. **K.** MVHC 5838. Pileocystidia. **L.** MVHC 5829. Scale bars: A, B = 1 cm, C–L = 10 μ m.

hexagonal in frontal view and ellipsoidal in lateral view, light brown to commonly coffee-colored, smooth, with a wall thickness of 1.2–1.3 μ m, and a conspicuous truncate germ pore up to 2.5 μ m. *Basidia* 12.3–40.2 $\times$ 4.9–15.8 μ m, cushion-shaped or clavate, tetrasporic. *Pleurocystidia* 45.3–83.5 $\times$ 12.8–22.5 μ m, abundant, chrysocystidia, metuloid, and occasionally subfusoid, with thick walls or apical granulations. *Cheilocystidia* 12.7–56.7 $\times$ 3.4–21.8 μ m, hyaline, thin-walled, polymorphic, appearing lageniform, cylindrical, or pyriform,

with a subcapitate or slightly inflated apex. *Hymenophoral trama* composed of smooth hyphae, regularly arranged, up to 11 μ m wide. *Pileipellis* formed by an epithelium of subglobose, hyaline, thin-walled cells, 17.2–42.5 $\times$ 13.6–31.8 μ m. *Pileocystidia* hyaline, thin-walled, cylindrical, 22.8–96 $\times$ 6.4–36.2 μ m. *Stipitipellis* a cutis composed of longitudinally arranged, yellowish-brown hyphae, up to 14 μ m wide. *Caulocystidia* 18.7–50.5 $\times$ 3.6–17.3 μ m, polymorphic, cylindrical, pedunculated spheroid, subclavate, or lageniform.

Specimens examined: **Uruguay**, Cerro Largo, Paso del Centurión, S32°8'28.9458", W53°43'51.276", 75 m.a.s.l., forest ecosystem, from cow dung, 1 Apr. 2021, *B. Corallo* (MVHC 5768 and MVHC 5769); Colonia, S33°58'23.4", W57°16'56.7", 20 m.a.s.l., open meadow with cattle, on horse dung, 8 Feb. 2022, *G. Morera et al.* (MVHC 5777); Maldonado, Sierra de las Ánimas, S34°36', W55°17', 209 m.a.s.l., on cow dung, 23 Mar. 2021, *G. Morera & S. Lupo* (MVHC 5765); 3 Mar. 2022, *G. Morera & S. Lupo* (MVHC 5816–18, MVHC 5820–5821, MVHC 5823–24, MVHC 5827–5832, MVHC 5834, MVHC 5837–44, MVHC 5846 and MVHC 5847); Maldonado, Sierra de las Ánimas, S34°42'12", W55°14'07, 75 m.a.s.l., on cow dung, 20 Mar. 2024, *S. Lupo* (MVHC 5879 and MVHC 5880).

Habit, habitat and distribution: Cosmopolitan distribution, in all continents except for Antarctica (Gerhardt 1996, Silva-Filho *et al.* 2019). This is the first record of this species for Uruguay. It was found in grasslands within fertilized fields, growing either around or directly on cow dung. It occurs solitarily or gregariously, forming groups of up to 13 specimens.

Notes: In the original description of *Agaricus cyanescens* (Berkeley & Broome 1871), the species was characterized by a dirty whitish to yellowish hemispherical cap, a stipe with a rooting base, and a pronounced cerulescent reaction occurring in various parts of the basidiome. For several decades, the species was segregated into the genus *Copelandia*, primarily due to its intense cerulescent staining and the presence of golden metuloid cystidia with acute apices (Young *et al.* 1989). However, recent molecular phylogenetic analyses have consistently recovered this taxon within *Panaeolus* (Hu *et al.* 2020, Asif *et al.* 2023, Voto & Angelini 2024, Consiglio & Marchetti 2025), thereby supporting its current generic placement. *Panaeolus cyanescens* exhibits a broad, cosmopolitan distribution, with confirmed occurrences across Europe, Africa, Oceania, Asia, and the Americas (Strauss *et al.* 2023). In South America, it has been recorded from Argentina (Batista *et al.* 2025) and Brazil (Singer 1986, Young 1989, Silva-Filho *et al.* 2018). The present study provides the first confirmed record of the species from Uruguay. Morphologically, the species is distinguished by a conical to campanulate pileus that may become broadly convex with age, typically pallid brown to yellowish white, sometimes with blue staining and deep erosions. Lamellae are dark gray to blackish and mottled. The stipe may display basal mycelium forming a pseudorhiza and consistently stains blue when bruised. Basidiospores are light brown to coffee-colored, lemon-shaped to hexagonal in frontal view, and ellipsoid in lateral view. Pleurocystidia are abundant and include both chrysocystidia and metuloid types. Macroscopic and microscopic features correspond closely with previous descriptions and illustrations (Saccardo 1887, Singer 1960, Guzmán & Petrarca 1972, Young 1989, Gerhardt 1996, Consiglio & Marchetti 2025). Nonetheless, certain differences have been noted: Singer (1960) observed slightly longer basidiospores and greater pileocystidial variation were described by Young (1989) documented metuloid caulocystidia; and basidiomata in Consiglio & Marchetti (2025) were notably more pigmented. Basidiospore dimensions reported in the literature show some degree of variability: 13.0–15.0 × 10–12.0 µm

(Bresadola 1912); 11.0–16 × 9.0–12.0 µm (Bresadola 1931); 11.0–13.0(–14.0) × 7.0–9.0 µm (Smith 1948); 13.0–14.0(–19.0) × 8.8–11.0 µm (Singer 1960); 12.0–14.0 × 8.5–11.0 µm (Oláh 1969); and 12.2–14.2 × 7.7–10.6 × 6.0–8.6 µm (Consiglio & Marchetti 2025). These differences may reflect phenotypic plasticity or underlying genetic variation within the species. Two species present in the Neotropics: *Panaeolus cambodginiensis* and *Pa. tropicalis*, can be distinguished by their smaller basidiospores, i.e.: 10–12.5 × 6.5–9 µm and 9–12 × 7–10 µm, respectively (Kaur *et al.* 2014, Batista *et al.* 2025). Additionally, *Pa. cyanescens* differs from *Pa. bisporus* in possessing four-spored basidia (Gerhardt 1996). With respect to psychoactive properties, *Pa. cyanescens* is unequivocally hallucinogenic and ranks among the species with some of the highest documented tryptamine concentrations, reaching up to 2.5 % of the dry fungal biomass (Guzmán 1959, Laussmann & Meier-Giebing 2010, Gotvaldová *et al.* 2022, Cohen *et al.* 2025).

Panaeolus foenisecii (Pers.) J. Schröt., *Botaniste* 17(1–4): 187. 1926. Fig. 6A–G.

Description: Pileus 1–1.5 × 1.5–2.5 cm, conical to conical-campanulate; surface opaque, glabrous, smooth, occasionally with radial striations, dry to moist, not entirely hygrophanous. uniform coffee color (5D6; Light Brown) with a slightly darker center (5E6; Yellowish Brown), fading towards the margin. Margin straight to slightly incurved, entire, concolorous with the surface. Lamellae adnate, close, dark brown (5F8; Yellowish Brown), mottled. Edge entire, smooth, and light brown. Stipe 3–5.5 × 0.2 cm, central, cylindrical, with a smooth to pruinose surface, sometimes with longitudinal striations; fibrous and fragile in consistency, concolorous with the pileus surface, slightly lighter inside. Context up to 0.1 cm thick, fibrous, light brown (3A2; Yellowish White). Veil absent. Spore print dark brownish black. Basidiospores (12.2–)13–17.1(–19.5) × (6.4–)6.7–9.2(–9.3) × (5.7–)6.3–9.3(–9.4) µm, $Q = 1.93–2.13$, $Q_m = 2$, ellipsoid, flattened in lateral view, greenish-brown, walls up to 1.3 µm thick, with a rough ornamentation and a noticeable germ pore 1.5–2 µm. Basidia 21.3–29.9 × 10–11.6 µm, cushion-shaped, ventricose, hyaline, tetrasporic. Pleurocystidia absent. Cheilocystidia hyaline, subcylindrical to subventricose, 12.4–56.6 × 4.3–10.8 µm. Hymenophoral trama composed of smooth, hyaline, thin-walled hyphae, cylindrical, up to 5.5 µm diam. Pileipellis composed of a matrix of inflated, interwoven hyphae in brownish and greenish tones (21.1–39.6 × 17.6–21.4 µm). Stipitipellis composed of a cutis of parallel, yellowish-brown hyphae, up to 8 µm diam. Caulocystidia hyaline, variable in shape, lageniform ventricose with thick walls (21.4–37.9 × 5.9–13.5 µm), commonly cylindrical with an obtuse apex (25.1–41.2 × 2.3–9.6 µm), and rarely irregular, 21.8 × 10.1 µm.

Specimen examined: **Uruguay**, Lavalleja, surroundings of the “Salto del Penitente”, S34°22'20", W55°03'10", 240 m.a.s.l., on horse dung, 23 May 2022, *G. Morera & S. Lupo* (MVHC 5862).

Habit, habitat and distribution: Cosmopolitan, commonly found in temperate grasslands across all continents except Asia and Antarctica (Gerhardt 1996). This is the first record

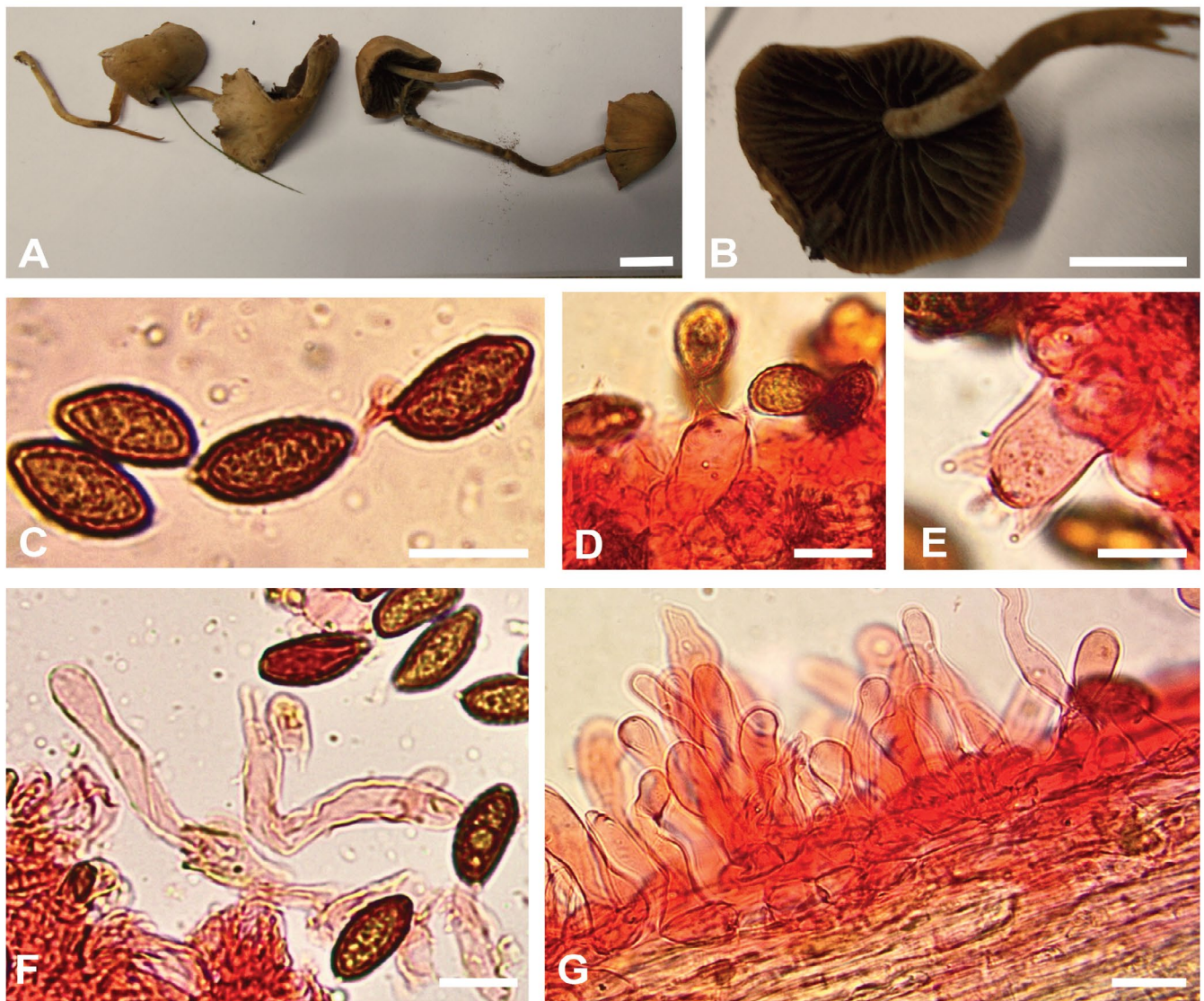


Fig. 6. *Panaeolus foenisecii* MVHC 5862. **A, B.** Basidiomata. **C.** Basidiospores. **D, E.** Basidia. **F.** Cheilocystidia. **G.** Caulocystidia. Scale bars: A, B = 1 cm, C–G = 10 µm.

for Uruguay of this species, found growing gregariously on horse dung, with groups of up to four specimens.

Notes: *Panaeolus foenisecii* was originally described from Germany under the name *Agaricus foenisecii* (Persoon 1801), and was historically classified in the genus *Panaeolina*, established by Maire (1933), due to its verrucose basidiospores (Singer 1986, Young 1989, Guzmán & Petrarca 1972). According to Gerhardt (1996), its distribution includes Europe, Africa, the Americas, and Australia, a pattern further corroborated by phylogenetic analysis of Consiglio & Marchetti (2025). The species typically grows on cow dung and fertilized soils, often found in parks, gardens, or grasslands (Persoon 1801, Consiglio & Marchetti 2025). It is morphologically distinguished by its conical to conical-campanulate pileus, brownish surface, brown spore print, and ornamented basidiospores that appear flattened on one side in lateral view ($12.2\text{--}19.5 \times 5.7\text{--}9.4 \mu\text{m}$). The cystidia are hyaline and variable in shape, though consistently non-pigmented. The specimen MVHC 5862, collected in Uruguay on horse dung, was recovered

in a distinct clade (BPP: 1, BS: 99) from those of the North Hemisphere analyzed by Consiglio & Marchetti (2025) (Fig. 1). Descriptions, figures and illustrations from European (Consiglio & Marchetti 2025; Young 1989), Mexican (Guzmán & Petrarca 1972), and Chinese specimens (He et al. 2026), including measurements of microscopic structures, broadly overlap with our observations. The Uruguayan specimen deviates from the traditional species description in having less polymorphic cheilocystidia (Consiglio & Marchetti 2025) and lacking the pleurocystidia reported by Guzmán and Petrarca (1972). *Panaeolus foenisecii*, like *Pa. olivaceus*, has verrucose basidiospores; however, the two species can be distinguished by their phylogenetic delimitation (Fig. 1), and by their distribution, which is more restricted in the case of *Pa. olivaceus* (Gerhardt 1996). A recently described species from China, *Pa. subfoenisecii*, appears to be closely related with *Pa. foenisecii*, however, this species is characterized by smooth and smaller basidiospores measuring $11.1\text{--}12.8 \times 6.5\text{--}8.3 \mu\text{m}$ (He et al. 2026). Further collections are required to assess whether these differences lie within the intraspecific variation of the species or represent a distinct

taxon. Regarding its potential psychoactivity, *Pa. foenisecii* is considered a non-hallucinogenic species. Although latent psilocybin content has been reported (Ola'h 1969), this claim has been discussed, and many positive findings may have resulted from misidentifications or confusion with other species such as *Pa. subbalteatus* and/or *Pa. castaneifolius* (Stijve 1995). The studied specimen MVHC 5862 did not show significant contents of PSB or PS (Table 1), and based on a thorough review of the literature, *Pa. foenisecii* should be considered non-psychoactive (Allen 2010, Gotvaldová et al. 2022).

***Panaeolus retirugis* (Fr.) Gillet, *Hyménomycètes*: 621. 1878. Fig. 7A–L.**

Description: *Pileus* 1.5–2 × 1.5 cm, conical-campanulate, umbonate; surface shiny or opaque, dry, not hygrophanous, glabrous, with radial or lacunar rugose ornamentation. Color light brown (5C7; Yellowish brown) fading to creamy whitish (4A3; Pale yellow) towards the margin. *Margin* incurved, serrated to eroded, appendiculate, concolorous with the surface. *Lamellae* adnexed, close, with blackish faces (4F5 to 4F3; Olive brown), waxy, and edges concolorous with the face or slightly whitish. *Stipe* 6–9 × 0.2–0.3 cm, central, cylindrical to subbulbous, with pruinose surface at the apex, then fibrillose. Texture fleshy to fibrous, whitish internally, and light brown externally, concolorous with the pileus, later dark with pinkish tones (8F8; Dark brown). *Context* 0.1 cm, fleshy, whitish. *Veil* absent. *Spore print* black. *Basidiospores* (10.2–)12.9–16.6(–17.1) × (7.5–)8.5–11.2(–12) × (4.4–)5.8–9.3(–10.1) µm, $Q = 1.5–1.86$, $Q_m = 1.6$, lemon-shaped to subhexagonal in frontal view, and ellipsoid in lateral view, with a slightly flattened side, light to dark brown, smooth, walls 0.8–1.2 µm thick and a prominent, truncate germ pore up to 2.5 µm diam. *Basidia* 15.4–41.5 × 6.1–14.6 µm, shortly clavate to clavate, to utriform with a central constriction, granulated, tetrasporic. *Pleurocystidia* not observed. *Cheilocystidia* 13.5–33.6 × 2.6–11.8 µm, cylindrical and tortuous to lageniform and ventricose, occasionally subcapitate at the apex, hyaline. *Hymenophoral trama* composed of smooth, inflated hyphae, up to 6 µm wide. *Pileipellis* composed of a palisade of pedunculated spheroid to ampulliform or isodiametric hyphae (hymenodermis), measuring 18.7–47.6 × 8–30.9 µm. *Pileocystidia* 30.4–66.8 × 10.7–47 µm, clavate or irregular, hyaline. *Pileus trama* consisting of interwoven, inflated hyphae up to 20 µm wide and filamentous hyphae up to 4 µm diam. *Stipitipellis* composed of a cutis of parallel, yellowish brown to greenish hyphae up to 5 µm wide. *Caulocystidia* 20.5–59.8 × 2.1–10.7 µm, hyaline, cylindrical, and very abundant.

Specimens examined: **Uruguay**, Lavalleja, surroundings of the “Salto del Penitente”, S34°22'20", W55°03'10", 240 m.a.s.l., on horse dung, 23 May 2022, G. Morera & S. Lupo (MVHC 5857 and MVHC 5863); Colonia, S33°58'23.4", W57°16'56.7", 20 m.a.s.l., open meadow with cattle, on horse dung, 8 Feb. 2022, G. Morera et al. (MVHC 5776, MVHC 5782 and MVHC 5797).

Habit, habitat and distribution: Widely distributed in temperate regions of Europe and America (Guzmán & Petrarca 1972, Voto & Angelini 2024). This constitutes the

first record for Uruguay. Found in fertilized meadow fields or on cow dung, occurring solitarily or in small groups of up to two specimens.

Notes: *Panaeolus retirugis* was originally described from Europe (Fries 1836–1838, Gillet 1878). It is currently understood to have a subcosmopolitan distribution across the Americas and Europe. The species has a complex taxonomic history, having been historically confused with *Pa. papilionaceus* and related species such as *Pa. sphinctrinus*, and *Pa. acuminatus* (Guzmán & Petrarca 1972, Voto & Angelini 2024). Recently, two paratypes specimens of *Pa. papilionaceus* var. *parvisporus* collected in the type locality in Germany, microscopically similar to *Pa. retirugis*, were included in the phylogenetic analyses conforming a clade –including specimens identified as *Pa. retirugis*, which was named *Pa. parvisporus* (Voto & Angelini 2024). Parallely, it was shown that the holotype of *Pa. papilionaceus* var. *parvisporus*, grouped phylogenetically with *Pa. papilionaceus* sensu stricto while *Pa. retirugis* was recovered as an independent lineage (Consiglio & Marchetti 2025), in the same way that it is recovered in our analyses (Fig. 1, Clade D). Morphologically, *Pa. retirugis* is characterized by conical-campanulate, umbonate basidiomata with a light brown surface and distinct radial or lacunar rugose ornamentation. The pileus margin often bears remnants of the partial veil. Basidiospores measure on average 14.8 × 9.9 × 7.6 µm, are lemon-shaped to subhexagonal in frontal view, ellipsoid in lateral view, with slightly flattened walls. Cheilo- and caulocystidia present, the latter reaching up to 60 µm in length, and lacks pleurocystidia. This description agrees closely with those recently provided for the species (Voto & Angelini 2024, Consiglio & Marchetti 2025). *Panaeolus retirugis* differs from *Pa. pantropicalis* by the latter's deeper pileus fissures, smaller basidiospores (averaging up to 13 µm), and smaller cheilocystidia (up to 40 µm). *Panaeolus papilionaceus*, on the other hand, produces larger basidiospores [13.0–18.5(–19.0) × 8.5–13.0 × 7.0–10.0 µm] and belongs to a distinct clade (Fig. 1). *Panaeolus desertorum* develops secotoid basidiomata, while *Pa. punjabensis* differs by exhibiting bluing when handled, lacking an umbonate pileus and veil remnants, and producing fusiform basidiospores (Malysheva et al. 2019, Asif et al. 2023). According to our results, *Pa. retirugis* lacks PSB and PS (Table 1). It has been reported that *Pa. retirugis* may produce anesthetic effects on nervous tissue, suggesting the presence of other bioactive compounds (Guzmán & Petrarca 1972); however, these findings must be interpreted with caution due to the lack of molecular and detailed morphological data, and the historical confusion with closely related species, as discussed above.

***Psilocybe cubensis* (Earle) Singer, *Sydowia* 2(1–6): 37. 1948. Fig. 8A–L.**

Description: *Pileus* 50–120 × 10–25 mm, conical, conico-campanulate, or campanulate, becoming convex to plane at maturity, occasionally with an umbo or papilla; surface smooth, opaque to slightly shiny, glabrous, dry to subviscid, non-hygrophanous, yellowish brown (4A5, Light Yellow) fading towards the margin, becoming darker at maturity (6D7, Light Brown), occasionally with whitish scales when

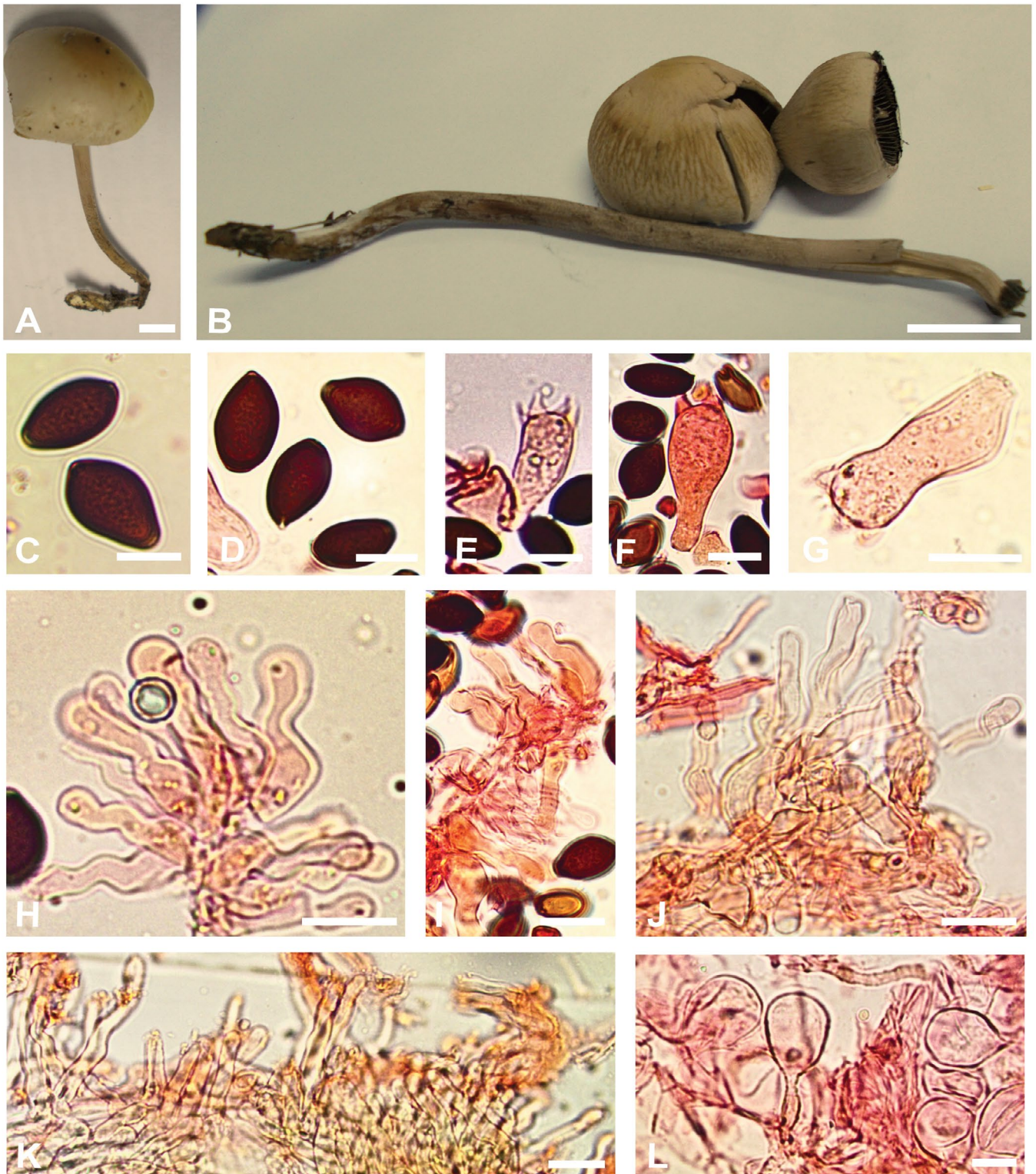


Fig. 7. *Panaeolus retirugis*. Basidiomata. **A.** MVHC 5776. **B.** MVHC 5857. Basidiospores. **C.** MVHC 5776. **D.** MVHC 5797. Basidia. **E, F.** MVHC 5776. **G.** MVHC 5797. Cheilocystidia. **H, I.** MVHC 5776. **J.** MVHC 5797. Caulocystidia **K.** MVHC 5776. Pileocystidia. **L.** MVHC 5776. Scale bars: A, B = 1 cm, C–L = 10 μ m.



Fig. 8. *Psilocybe cubensis*. Basidiomata. **A, B.** MVHC 5853. Basidiospores. **C.** MVHC 5763. **D, E.** MVHC 5853. Basidia. **F.** MVHC 5762. **G.** MVHC 5851. Pleurocystidi. **H.** MVHC 5849. **I.** MVHC 5851. **J.** MVHC 5850. Cheilocystidia. **K.** MVHC 5762. **L.** MVHC 5850. Scale bars: A, B = 1 cm, C–L = 10 μ m.

mature. *Margin* entire, concolorous with the surface or whitish. *Lamellae* light to dark brown (4A2, Yellowish White), turning dark purplish black at maturity (10F5, Purplish Brown), occasionally mottled; adnate to adnexed, close, with an entire whitish edge. *Stipe* 50–120 × 5–25 mm, cylindrical, thickened towards the base, with a bulbous base; white, shiny, turning bluish when bruised; longitudinally striate, fibrous. *Context* up to 20 mm thick, white, fleshy to corky, bluish upon bruising. *Veil* membranous, persistent to absent; may turn bluish but typically appears colored due to spore deposition once the pileus expands. *Spore print* dark purplish. *Basidiospores* (10–)10.4–14.5(–15.4) × (5.8–)6.3–9.2(–9.5) × (5.4–)5.6–8.9(–9.2) μm , $Q = 1.57\text{--}1.77$, $Q_m = 1.67$. Subhexagonal to hexagonal in frontal view, ellipsoid to oblong in lateral view, yellowish-brown, with thick walls up to 1.5 μm , and a conspicuous germ pore up to 2 μm wide. *Basidia* 12.7–37 × 4.8–12.7 μm , cylindrical to clavate, occasionally subutriform, sometimes with a median constriction, hyaline, occasionally granular, and tetrasterigmatic. *Pleurocystidia* 10.1–42.7 × 4.3–14.1 μm , clavate to fusiform, sometimes more ventricose, occasionally subglobose 7.7–22.5 × 5.7–14 μm , hyaline, with apical incrustation, thin-walled. *Cheilocystidia* 13.4–36 × 4.3–10.5 μm , lageniform with short to long necks, sometimes with a subcapitate apex, thin-walled, hyaline. *Hymenophoral trama* regular, composed of hyaline hyphae up to 7 μm wide. *Subhymenium* composed of inflated hyaline hyphae up to 9 μm wide. *Pileus trama* composed of filamentous hyphae up to 8 μm wide. *Pileipellis* an ixocutis of filamentous hyaline hyphae up to 7 μm wide. *Stipitipellis* composed of parallel hyaline hyphae up to 7.5 μm wide.

Specimens examined: **Uruguay**, Lavalaja, surroundings of the “Salto del Penitente”, S34°22'20", W55°03'10", 240 m.a.s.l., on horse dung, 23 May 2022, G. Morera & S. Lupo (MVHC 5856); Rocha, Bosque de Ombúes, S34°22'8.4", W53°52'48.7", 10 m.a.s.l., 3 Apr. 2018, G. Morera & S. Lupo (MVHC 5763), 5 May 2022, G. Morera & S. Lupo (MVHC 5849-53); Montevideo, from anonymous users, Apr. 2021, (MVHC 5762 and MVHC 5764).

Habit, habitat and distribution: With a pantropical distribution (Guzmán 1983, Guzmán 1995), this species was previously recorded in Uruguay (Sequeira 2013). Characteristically, it grows on cow dung, occurring either solitarily or gregariously in groups of up to 4–5 specimens.

Notes: In 1906, Earle described the species *Stropharia cubensis*, which was later transferred to the genus *Psilocybe* by Singer (1948). This species has a broad distribution, typically growing on bovine and horse dung, or, more rarely, in rich pasture soils. *Psilocybe cubensis* is one of the most widely known and cultivated PSB-containing mushrooms worldwide, due to its psychoactive effects (Hawksworth & Wiltshire 2011, Gotvaldová et al. 2022). Uruguayan specimens exhibit robust basidiomata (up to 12 cm in pileus diam.), with a conical to plane pileus, occasionally presenting an umbo. The pileus surface is glabrous, yellowish brown, while the lamellae become dark purplish black at maturity. The stipe is white, sometimes slightly discolored, and bruises bluish when handled. Microscopically, the species is characterized by basidiospores measuring 10–15.4 × 5.8–9.5

× 5.4–9.2 μm , subhexagonal to hexagonal in frontal view, ellipsoid to oblong in lateral view, and yellowish-brown in color. Pleurocystidia are variable, while cheilocystidia are lageniform with short to long necks (with pileocystidia and caulocystidia absent). These features are consistent with previous descriptions by Singer & Smith (1958), Wright & Albertó (2002), Batista et al. (2025), and the phylogenetic placement established by Ramírez-Cruz et al. (2013). Singer & Smith (1958) and Batista et al. (2025) reported longer basidiospores, reaching up to 17 μm in length. Notably the sequence of the specimen Guzmán 35102, corresponding to *Ps. subcubensis* in Ramírez-Cruz et al. (2013), clusters within our *Ps. cubensis* clade (BPP: 0.77, BS: 83; Fig. 2). Several studies have suggested that the only distinguishing feature between *Ps. cubensis* and *Ps. subcubensis* is the size of the basidiospores. However, in addition to phylogenetic evidence, we have observed a significant overlap in spore size of our specimens with the measurements documented for *Ps. subcubensis* by Guzmán (1995), Keller et al. (1999), and Guzmán et al. (2014), suggesting that *Ps. subcubensis* could be considered a synonym of *Ps. cubensis* in future taxonomic revisions. *Psilocybe natalensis* and *Ps. maluti* are phylogenetically related species, but the former differs by its smaller spore size (8.8–10.1 × 5.5–6.0 μm), as documented by Windsor et al. (2025), while the latter exhibits a secotoid habit (Van Der Merwe et al. 2024). Additionally, *Psilocybe chuxiongensis* is extremely similar in macromorphology to *Ps. cubensis* but differs by lacking an annulus and being reported exclusively from China (Ma et al. 2014). The samples analyzed in Uruguay showed a wide range of variation in tryptamine content, reaching a maximum of 0.91% (PSB + PS) of dry weight in MVHC 5849 (Table 1).

Psilocybe stuntzii Guzmán & J. Ott, *Mycologia* 68(6): 1261. 1977. [1976]. Fig. 9A–J.

Description: *Pileus* 10–25 × 3–15 mm, conical, almost hemispherical to plano-convex, with a well-defined umbo, papilla-like; surface smooth, shiny, translucent-striate, glabrous, nearly viscid when moist, hygrophanous, yellowish brown (5D6; Light Brown) to dark or olive-brown (4F8; Olive Brown), homogeneous or with a darker center and lighter sectors. *Margin* entire to eroded, striate, concolorous with the surface, olive-brown, hygrophanous, cerulescent. *Lamellae* non-homogeneous brown (6D6; Dark Brown) with violet mottling, adnexed, close, edge irregular and concolorous with the face. *Stipe* 10–50 × 1–4 mm, cylindrical, straight to sinuous, tapering towards the base, hollow, yellowish-brown to dark brown (5E6; Yellowish Brown); semi-translucent when moist, base conrescent, with fibrillose surface and longitudinal striations, fibrous, bearing cerulescent mycelial projections. *Veil* membranous, persistent, cerulescent. *Context* 1–2 mm, gummy, whitish to brownish, concolorous with the stipe, cerulescent. *Spore print* dark violet. *Basidiospores* (7.9–)9–12.3(–13.4) × (4.1–)5.2–7.6(–8.3) × (4.4–)5–7.4(–7.8) μm , $Q = 1.52\text{--}1.85$, $Q_m = 1.69$, ellipsoid, subangular to subrhomboid in frontal view, ellipsoid to oblong in lateral view, yellowish-brown, with thick walls up to 1.2 μm and a conspicuous germ pore up to 1.7 μm . *Basidia* 9.4–32.1 × 4.5–11.8 μm , cylindrical to clavate, sometimes with a median constriction, hyaline,

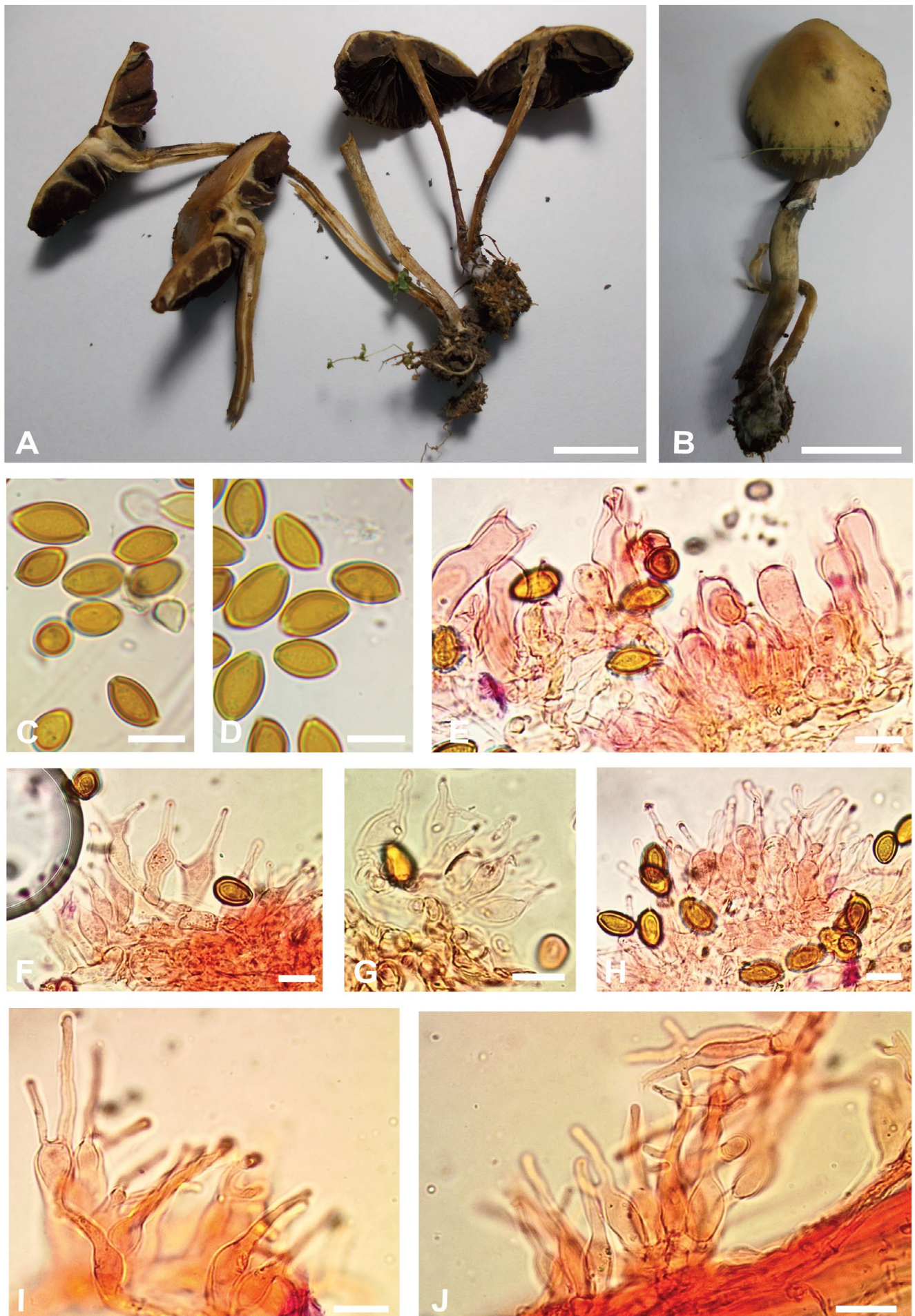


Fig. 9. *Psilocybe stuntzii*. Basidiomata. **A.** MVHC 5854. **B.** MVHC 5855. Basidiospores. **C, D.** MVHC 5854. Basidia. **E.** MVHC 5858. Cheilocystidia. **F.** MVHC 5858. **G.** MVHC 5874. **H.** MVHC 5858. Caulocystidia. **I, J.** MVHC 5870. Scale bars: A, B = 1 cm, C-J = 10 μ m.

occasionally granular, bi- to tetrasporic. *Pleurocystidia* absent. *Cheilocystidia* 12.3–33.8 × 2.9–10.2 µm, lageniform with a long neck, occasionally with a short neck, sometimes cylindrical or ventricose; apex with refractive content, occasionally bifurcated; hyaline. *Hymenophoral trama* regular, composed of hyaline hyphae up to 7 µm wide. *Subhymenium* with inflated hyaline hyphae up to 9 µm wide. *Pileal trama* composed of filamentous hyphae up to 5 µm wide. *Pileipellis* of hyaline hyphae up to 7 µm diam. *Stipitipellis* formed by parallel hyphae with incrustations, up to 5 µm wide. *Caulocystidia* 10.5–33.9 × 3.5–13.2 µm, lageniform with a long neck, sometimes bifurcated, hyaline. Clamp connections present.

Specimens examined: Uruguay, Lavalleja, surroundings of the “Salto del Penitente”, S34°22'20", W55°03'10", 240 m.a.s.l., 23 May 2022, growing in manured soil, in fields with cattle, G. Morera & S. Lupo (MVHC 5854-55, MVHC 5858-61, MVHC 5864-66, MVHC 5869-5878).

Habit, habitat and distribution: *Psilocybe stuntzii* was previously only recorded from North America (Guzmán & Ott 1976) and is now reported for Uruguay, suggesting it may be an amphitropical species. It is associated with horse and cow dung and is often found growing in groups directly from the soil, with as many as eight specimens observed together.

Notes: *Psilocybe stuntzii* was described from Washington State (USA) as a common gramincolous species in the region, used recreationally due to its psychoactive properties (Guzmán & Ott 1976). It is characterized by a papillate, lustrous pileus that may exhibit light ochraceous tones, violaceous lamellae, and a bluish annulus. Microscopically, it has basidiospores measuring 8.2–12.6 × 6–7.7 × 5.5–6.6 µm, lentiform in frontal view and ellipsoid in side view, no pleurocystidia, and long-necked cheilocystidia up to 27.5 µm long. These characters match closely with those observed in Uruguayan specimens; however, another blue-ringed species with highly overlapping micromorphological features, *Ps. caeruleoannulata* (≡ *Ps. uruguayensis*), has previously been recorded from Uruguay and southern Brazil (Guzmán 1978, Silva 2013). It is possible that specimens described as *Ps. caeruleoannulata* from Uruguay actually belong to *Ps. stuntzii*; nevertheless, this hypothesis requires confirmation through including sequences of the holotype kept at BAFC herbarium in phylogenetic analyses, unfortunately we were unable to access the type materials. *Psilocybe semilanceata* is a related species, but differs in lacking a distinct bluish annulus and in having a wavy stipe (Cacialli et al. 1996). Regarding the alkaloid content, *Ps. stuntzii* has been reported as hallucinogenic, with psilocybin and psilocin concentrations below 1 % (Guzmán & Ott 1976, Windsor et al. 2025), and even lower levels have been recorded in specimens from Brazil and Uruguay (Stijve & Demeijer 1993, this work).

DISCUSSION

Recent advances in molecular studies of species diversity within the genera *Panaeolus* and *Psilocybe* have improved our understanding of their evolutionary relationships and have contributed to refining species circumscription and re-

solving species complexes (Ramírez-Cruz et al. 2013, Voto & Angelini 2024, Consiglio & Marchetti 2025, He et al. 2026). In this study, we sequenced and analyzed 57 *Panaeolus* and 16 *Psilocybe* Uruguayan specimens, placing them within phylogenies comprising 285 and 228 terminals, respectively, in order to determine their phylogenetic placement and relationships with previously documented taxa. To construct these phylogenies, the most widely represented loci were used for each genus: ITS and LSU for *Panaeolus*, and ITS, LSU, *rpb1*, and *tef1* for *Psilocybe*.

In *Panaeolus*, a total of 29 clades were recovered (Fig. 1), representing distinct lineages. Of these, 20 correspond to previously described species included in earlier phylogenies (Voto & Angelini 2024, Consiglio & Marchetti 2025), one represents a new species (described here), and eight clades correspond to species that have not yet been identified. Five of these clades, representing undescribed species, occupy basal positions in the phylogeny. Although several terminal clades were recovered in a pattern comparable to earlier studies, the overall topology is not fully congruent and remains difficult to compare with the most recent large-scale phylogenetic treatments (Voto & Angelini 2024, Consiglio & Marchetti 2025). Also, the subgenera proposed by He et al. (2026) are likewise not recovered, with the exception of subgenus *Panaeolus*, corresponding to our clade D (Fig. 1). These differences may be attributable, at least in part, to the broader taxon sampling incorporated in the present analyses, which nearly doubles the number of terminal taxa included in previous studies.

In the case of *Psilocybe*, the topology recovered terminal clades previously shown in multigen phylogenetic analyses (Ramírez-Cruz et al. 2013, Canan et al. 2024, Ostuni et al. 2024). In the phylogenetic tree of Fig. 2, we show four clades (A–D), within clade A, the three groups ‘mexicanae’, ‘cordiosporae’, and ‘zapotecorum’, each of which includes several representatives of *Psilocybe* sections sensu Guzmán (1983, 1995), represent more comprehensively sampled versions of what has been shown in previous works (Ramírez-Cruz et al. 2013). Within clade B two strongly supported groups can be recognized here named as ‘semilanceatae’ and ‘atrobrunneae’ according to *Psilocybe* sections of Guzmán (1983, 1995) and Singer (1986) respectively. Clade C includes two groups, here named as ‘cyanescens’ and ‘serbica’, corresponding with *Psilocybe* stirps sensu Singer (1986) and Noordeloos (2011) respectively. Finally, within clade D, two other strongly supported groups are recognized, one is congruent with previous works, i.e. ‘cubensae’ (Ramírez-Cruz et al. 2013) and the other, also recovered previously (Canan et al. 2024, Ostuni et al. 2024) is here named as ‘subaeruginascens’ (due *Ps. subaeruginascens* was the first described species in the group).

Combined morphological and phylogenetic analyses of Uruguayan specimens allowed the identification and description of five *Panaeolus* species, of which only *Pa. antillarum* had been previously recorded for Uruguay (Gerhardt 1996, Sequeira 2017). In turn, *Pa. retirugis* was likely previously misidentified as *Pa. papilionaceus* and *Pa. acuminatus* (Herter 1907, Felippone 1928, Rosa-Mato 1939), likely due to its affinities with this species. The remaining taxa — *Pa. cyanescens*, *Pa. charrua* sp. nov. and *Panaeolus foenisecii* — represent new records for the country, and

one also represents a new species to science. For *Psilocybe*, two species were found and described: *Psilocybe cubensis*, which had been previously recorded for Uruguay (Sequeira 2013), and *Ps. stuntzii*, which represents a novel record for the country. This taxon may previously have been confused with *Psilocybe caeruleoannulata* Singer ex Guzmán and/or *Ps. uruguayensis* Singer ex Guzmán (Guzmán 1978).

The contents of PS and PSB exhibited wide variation among species and samples, which may be attributed to differences in sample origin and to the intrinsic instability of tryptamines. PSB can be hydrolyzed to PS under acidic or humid conditions via endogenous phosphatases present in fungal tissues (Gartz 1994). In addition, it has been demonstrated that ground mushrooms stored under different conditions show significant loss of tryptamine content after one year, with storage in darkness at 20 °C being comparatively the most stable condition (Gotvaldová *et al.* 2021). The time elapsed between mushroom collection and chemical analysis is another factor likely contributing to PSB/PS variability (Bradshaw *et al.* 2022, Miller *et al.* 2024). For example, *Ps. stuntzii* showed only low levels of PS (up to 0.021 %), which are markedly lower than those reported in other studies (Windsor *et al.* 2025). However, these samples were analyzed after more than one year of storage, which likely contributed to reduced tryptamine contents. In *Ps. cubensis*, pooled specimens MVHC 5762, MVHC 5849 sample 2, and MVHC 5851 contained no detectable PSB and had been stored for over one year. In contrast, MVHC 5849 samples 1 and 3 and MVHC 5850, all stored for less than one year, contained PSB levels ranging from 0.41 to 0.71 %. An exception was MVHC 5853, which, despite being analyzed within one year, showed comparatively low contents of both PSB and PS. For *Pa. cyanescens*, all samples were analyzed within the collection year, with the exception of MVHC 5827, which exhibited the lowest tryptamine levels and was quantified two years after collection. In the remaining specimens, PSB content ranged from 0 to 0.28 %, while PS varied from 0.06 to 0.42 %. PSB and PS contents in *Psilocybe cubensis* are consistent with previously documented ranges, whereas *Pa. cyanescens* showed lower concentrations than those reported in previous studies (Gotvaldová *et al.* 2022).

No alkaloids were detected (<LOD) in *Pa. foenisecii*, *Pa. antillarum*, and *Pa. retrugis*, consistent with previous reports describing these taxa as non-psychoactive (Guzmán & Petrarca 1972, Allen 2010). In *Pa. charrua*, no PSB or PS was detected, however, given the lack of previous records, the time elapsed between collection and measurement, and the presence of closely related psychoactive species, we cannot exclude the possibility that this species may produce significant alkaloid contents under different conditions.

Regarding PSB and PS occurrence, these alkaloids were detected in 7 of the 29 phylogenetic species of *Panaeolus* recognized in Figure 1. In contrast, within *Psilocybe*, among the 42 recovered phylogenetic species (Fig. 2), only one (*Ps. atrobrunnea*) has been confirmed to lack PSB/PS production. It has been suggested that PSB/PS occurrence in *Panaeolus* is sporadic across the phylogeny, potentially resulting from an ancestral acquisition followed by multiple losses, or alternatively from independent acquisition events (Hu *et al.* 2020, Meyer & Slot 2023). However, as shown in Fig. 1, there is a notable clustering of producer species within Clade A. In

contrast, the widespread distribution of PSB/PS production in *Psilocybe* supports the hypothesis of a single ancestral acquisition (Meyer & Slot 2023).

CONCLUSIONS

In the present study, we applied an integrative approach combining phylogenetic, morphological, and chemical analyses to investigate Uruguayan specimens of *Panaeolus* and *Psilocybe*. Seven species were documented. Within *Panaeolus*, five species were recognized, including *Pa. antillarum*, the only taxon previously recorded in Uruguay; three new country records (*Pa. cyanescens*, *Pa. foenisecii*, and *Pa. retrugis*); and one species new to science (*Pa. charrua*). Two *Psilocybe* species were identified: *Psilocybe cubensis*, previously reported from Uruguay, and *Ps. stuntzii*, representing a new national record and an extension of its known distribution. The discovery of several previously unrecorded taxa highlights the still underexplored fungal diversity of Uruguay and underscores the need for continued systematic studies. Among the studied taxa, only *Pa. cyanescens* and the two *Psilocybe* species tested positive for psychoactive alkaloids (PSB/PS). In the context of emerging regulations concerning fungi and their derivatives, these results provide an evidence-based framework for prioritizing species with confirmed alkaloid production in future scientific and regulatory efforts.

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Supplementary material

Table S1. Sequences of *Panaeolus* species used in the phylogenetic analyses. New sequences generated in this study are highlighted in bold. An emdash (—) indicates missing data.

Table S2. Sequences of *Psilocybe* species used in the phylogenetic analyses. New sequences generated in this study are highlighted in bold. An emdash (—) indicates missing data.