

1 Discovery of the closest free-living relative of the domesticated “magic mushroom” *Psilocybe cubensis* in  
2 Africa

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30 **Keywords:** psychedelic mushroom, psilocybin, diversity, Zimbabwe, Southern Africa

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34 **Abstract:**

35 The “magic mushroom” *Psilocybe cubensis* is cultivated worldwide for recreational and medicinal uses.  
36 Described initially from Cuba in 1904, there has been substantial debate about its origin and  
37 diversification. The prevailing view, first proposed by the *Psilocybe* expert Gastón Guzmán in 1983, is  
38 that *P. cubensis* was inadvertently introduced to the Americas when cattle were introduced to the  
39 continents from Africa and Europe (~1500 CE), but that its progenitor was endemic to Africa. This  
40 hypothesis has never been tested. Here, we report the discovery of the closest wild relative of *P.*  
41 *cubensis* from sub-Saharan Africa, *P. ochraceocentrata* nom. prov. Using DNA sequences from type  
42 specimens of all known and accessible African species of *Psilocybe*, multi-locus phylogenetic and  
43 molecular clock analysis strongly support recognizing the African samples as a new species that last  
44 shared a common ancestor with *P. cubensis* ~1.5 million years ago (~710k - 2.55M years ago 95% HPD).  
45 Even at the latest estimated time of divergence, this long predates cattle domestication and the origin of  
46 modern humans. Both species are associated with herbivore dung, suggesting this habit likely  
47 predisposed *P. cubensis* to its present specialization on domesticated cattle dung. Ecological niche  
48 modeling using bioclimatic variables for global records of these species indicates historical presence  
49 across Africa, Asia, and the Americas over the last 3 million years. This discovery sheds light on the wild  
50 origins of domesticated *P. cubensis* and provides new genetic resources for research on psychedelic  
51 mushrooms.

52 **INTRODUCTION:**

53 *Psilocybe cubensis* (Earle) Singer is the most widely known, collected, and cultivated "magic  
54 mushroom" in the world (A. J. Bradshaw et al., 2022). *P. cubensis* was first described as *Stropharia*  
55 *cubensis* Earle from a cattle-grazed field in Cuba in 1904 and today is globally distributed where it is  
56 common in association with domesticated cattle across subtropical and tropical regions of America,  
57 Asia, and Australia (T. Froese et al., 2016; Guzman, 2005; McTaggart et al., 2023; Thomas et al., 2002). *P.*  
58 *cubensis* is one of the core species of psychoactive mushrooms used traditionally and  
59 contemporaneously for cultural and spiritual ceremonies across Mexico, and has been domesticated  
60 with many strains developed by an active social subculture due to its ease of cultivation (Castro Jauregui  
61 et al., 2022; Guzmán, 2008; Van Court et al., 2022). *P. cubensis* is also the target of ongoing biochemical  
62 and biomedical studies for drug discovery and whole organism therapies for a wide range of psychiatric  
63 illnesses (Blei et al., 2020; Brownstien et al., 2024; Fricke et al., 2017; Lerer et al., 2024; Matsushima et  
64 al., 2009; Shahar et al., 2024; Zhuk et al., 2015). Yet, despite the cultural, scientific and medical  
65 importance of *P. cubensis*, we know little of its specific ecology or evolutionary origins.

66 The prevailing hypothesis, first proposed by Guzmán (1983), is that *P. cubensis* originated in  
67 Africa and was transported to the Americas by Spanish colonizers during the 15th and 16th centuries.  
68 This hypothesis was largely predicated on the speculation that other species closely related to it remain  
69 to be discovered in Africa, a prediction rooted in the observation that the African continent is historically  
70 undersampled for *Psilocybe* (Piepenbring et al., 2020; Tsakem et al., 2024). The closest relatives of *P.*  
71 *cubensis* were recently shown to be from both Asia and Africa, but sampling from these and other  
72 regions remains vastly incomplete and definitive statements about origins and biogeography have  
73 remained untenable (A. J. Bradshaw et al., 2024). Interestingly, despite substantial popular knowledge  
74 and conspicuousness of *P. cubensis* in cattle-grazed pastures, it has not been officially confirmed from  
75 subtropical or tropical Africa, although casual reports of a *P. cubensis* lookalikes have appeared in peer-  
76 reviewed literature (Froese et al. 2016) and online databases such as iNaturalist and MycoPortal. *P.*  
77 *cubensis* does occur in Asia, recorded for Thailand (Ma et al. 2014) and India based on an ITS sequence  
78 matching *P. cubensis* in GenBank (accession OK165610.1), and multiple records of it occur across South  
79 and Southeast Asia in iNaturalist and MycoPortal.

80 One obstacle to determining the existence of named species in a given place at a given time is  
 81 the availability of type specimens as references. Molecular identification is particularly sensitive to the  
 82 lack of type reference sequences because sequences in public databases can carry names that have not  
 83 been validated through comparison with them. In the absence of type reference sequences, this can  
 84 result in a broad misapplication of names resulting in a collective misunderstanding of species'  
 85 identities, ecologies, and distributions. The recent study by Bradshaw et al. (2024) explicitly targeted  
 86 type specimens for whole genome sequencing in an effort to remedy this long-standing problem in  
 87 *Psilocybe*. While many type specimens were included in the study, it was not exhaustive, and critical  
 88 types such as *S. cubensis* and species known only from Africa were not included.

89 Recent fieldwork across sub-Saharan Africa between 2013 and 2022 resulted in multiple  
 90 specimens of an unknown *Psilocybe* sp. that is superficially similar to *P. cubensis* in habit, habitat, and  
 91 general appearance. Upon further comparison of microscopic and molecular characters with the type of  
 92 *S. cubensis* and other *P. cubensis* specimens from around the world, the recognition of the African  
 93 specimens as a distinct species is warranted, provisionally named *Psilocybe ochraceocentrata*. A multi-  
 94 locus molecular phylogenetic analysis was used to confirm its close relationship to *P. cubensis*, and  
 95 divergence dating, ecological niche modeling, and species distribution modeling were used to predict  
 96 when, in time, these two lineages diverged and where they were likely to be found over the last 3  
 97 million years.

98  
 99 **MATERIALS AND METHODS:**

100 Collections and Sampling

101 Six collections of mushrooms resembling *P. cubensis* were collected across Zimbabwe and South Africa,  
 102 occurring on or near decomposing herbivore dung. All collections were made on public or private land  
 103 with the owner's permission. Sporocarp collections were air-dried over low heat and preserved from  
 104 insect damage using naphthalene. All other voucher details and deposition are reported in Table 1.

105  
 106 **Table 1: Sample and voucher information**

Sample name	Voucher designation	Determination	Locality	Type status	Collection year	Type authorship	Holding Institution	Herbarium Code	C strain number	Accession JFIS	Accession JF14	Accession RFB5	Accession JF587	NRA	BioSample
<i>Psilocybe</i> sp. Africa_Zimbabwe_CS-5008	<i>Psilocybe</i> sp.	<i>Psilocybe ochraceocentrata</i> sp. nov.	Zimbabwe (Manicaland South Province)	Holotype	2013	This publication	Natural History Museum of Zimbabwe	BUL	BUL0013	PQ015824	PQ017397	PQ017404	PQ017412	SRR30663520	SAMN243585585
<i>Psilocybe</i> Africa_Zimbabwe_CS-5703	<i>Psilocybe</i> sp.	<i>Psilocybe ochraceocentrata</i> sp. nov.	Zimbabwe (Matabeleland South Province)	N/A	2018	This publication	Pierre Fragarum of C. Slings	N/A	CS-5703	PQ015825	PQ017398	PQ017405	PQ017413	SRR30663510	SAMN243585590
<i>Psilocybe</i> sp. Africa_Zimbabwe_CS-4809	<i>Psilocybe</i> sp.	<i>Psilocybe ochraceocentrata</i> sp. nov.	Zimbabwe (Manicaland Central Province)	N/A	2013	This publication	Pierre Fragarum of C. Slings	N/A	CS-4809	PQ015826	PQ017399	PQ017406	PQ017414	SRR30663518	SAMN243585593
ED	<i>Psilocybe ochraceocentrata</i> sp. nov.	<i>Psilocybe ochraceocentrata</i> sp. nov.	South Africa (Kwa-Zulu Natal province)	N/A	2022	This publication	Stellenbosch University	N/A	<i>Psilocybe ochraceocentrata</i> -T1	P9999211	N/A	N/A	N/A	N/A	N/A
ET	<i>Psilocybe ochraceocentrata</i> sp. nov.	<i>Psilocybe ochraceocentrata</i> sp. nov.	South Africa (Kwa-Zulu Natal province)	N/A	2022	This publication	Stellenbosch University	N/A	<i>Psilocybe ochraceocentrata</i> -T1	P9999212	N/A	N/A	N/A	N/A	N/A
Eladina	<i>Psilocybe ochraceocentrata</i> sp. nov.	<i>Psilocybe ochraceocentrata</i> sp. nov.	South Africa (Kwa-Zulu Natal province)	N/A	2022	This publication	Stellenbosch University	N/A	<i>Psilocybe ochraceocentrata</i> -Eladina	P9999213	N/A	N/A	N/A	N/A	N/A
<i>Stropharia cubensis</i> NY-761563 Holotype	<i>Stropharia cubensis</i>	<i>Psilocybe cubensis</i> (Earle) Singer	Cuba	Holotype	1906	Sydéniak 2(1-6): 87 (1948)	New York Botanical Garden	NY	NY-761563	PQ015827	Not recorded	PQ017407	PQ017415	SRR30663517	SAMN243585591
<i>Psilocybe jaliscana</i> IBUC-1710 Isotype	<i>Psilocybe jaliscana</i>	<i>Psilocybe cubensis</i> (Earle) Singer	Mezcuic, Jalisco	Isotype	2000	Decom. Mycol. 2(100: 116): 46 (2000)	Universidad de Guadalajara	IBUC	IBUC-43710	PQ015828	PQ017400	PQ017408	PQ017416	SRR30663516	SAMN243585592
<i>Psilocybe similis</i> Lx Pii nr. resq. HOLOTYPE	<i>Psilocybe similis</i>	<i>Psilocybe similis</i> Gartz, D.A. Reid, M.L. Sosa & Pickett	KwaZulu-Natal	Holotype	1994	Integrations 6: 29 (1994)	University of Leuven	LZ	6266	PQ015829	PQ017401	PQ017409	PQ017417	SRR30663518	SAMN243585594
<i>Psilocybe wynnandensis</i> XAL-18000-1-321a Holotype	<i>Psilocybe wynnandensis</i>	<i>Psilocybe wynnandensis</i> K.A. Thomas, Masarik & Grunwaldt	India, Kerala	Holotype	2002	Myxotaxa 83: 198 (2002)	Instituto de Ecología, A.C.	XAL	Not Recorded	PQ015830	PQ017402	PQ017410	PQ017418	SRR30663514	SAMN243585595
<i>Psilocybe baralensis</i> XAL-T119a Isotype	<i>Psilocybe baralensis</i>	<i>Psilocybe baralensis</i> K.A. Thomas, Schmidt & Grunwaldt	India, Kerala	Isotype	2002	Myxotaxa 83: 196 (2002)	Instituto de Ecología, A.C.	XAL	Not Recorded	PQ015831	PQ017403	PQ017411	PQ017419	SRR30663513	SAMN243585596
<i>Stropharia spumosa</i> Holotype_KM 169812	<i>Stropharia spumosa</i>	<i>Psilocybe spumosa</i> (Pegler) Grunwaldt	Kenya	Holotype	1977	Biblioth. Mycol. 459: 459 (1977)	Royal Botanic Gardens, Kew	KM	KM 169812	PQ015832	PQ015231	PQ015232	PQ015233	SRR30663519	SAMN243585597

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108

109 Genomic sequencing, assembly, and barcode extraction from Type specimens.

110 Type specimens of the Cubensae complex (Table 1) were extracted and sequenced in the same manner  
111 as (A. J. Bradshaw et al., 2024). Hymenophore fragments (5 to 15 mg) from dried fungarium samples  
112 were homogenized by placing them in 2.0 mL screw-cap tubes containing a single 3.0-mm and 8 × 1.5-  
113 mm stainless steel beads and shaking them in a BeadBug™ microtube homogenizer (Sigma-Aldrich,  
114 #Z763713) for 120 s at a speed setting of 3,500 rpm. DNA extraction of mechanically homogenized  
115 samples was performed using a Phenol-chloroform DNA extraction protocol. Lysis was performed with  
116 Monarch® Genomic DNA Purification Kit (NEB, #T3010S) following the manufacturer’s protocol for  
117 Tissue Lysis with an overnight incubation at 56 °C , using a volume of 500 ul lysis buffer, 10ul of  
118 Proteinase K and increasing the amount of wash buffer to 550 µL during each of the wash steps.  
119 Overnight incubation was then followed by a 2-hour incubation with 4ul of RNase A, after which total  
120 lysate was placed in homemade Phase Lock gel tubes made using Dow Corning™ High Vacuum Grease  
121 (ASIN:B001UHMNWO) along with an equal volume of OmniPur® Phenol:Chloroform:Isoamyl Alcohol  
122 (25:24:1, TE-saturated, pH 8.0) solution (MilliporeSigma, Calbiochem #D05686) and then mixed by  
123 gentle inversion for 15 min using a fixed speed tube rotator. After mixing, tubes were centrifuged at  
124 maximum speed (14,000×g) for 10 min; then, the aqueous (top) layer was transferred to a new phase-  
125 lock gel tube and the process repeated. DNA precipitation of the aqueous phase was performed by  
126 adding 5 M NaCl to a final concentration of 0.3 M and two volumes of room temperature absolute  
127 ethanol, inverting the tubes 20× for thorough mixing followed by an overnight incubation at –20 °C. The  
128 next day, DNA was pelleted by centrifugation at 14,000×g for 5 min. The DNA pellet was washed twice  
129 with freshly prepared, ice-cold 70% ethanol, air-dried for 15 min at room temperature, and then  
130 resuspended in 150 µL of Elution Buffer from the Monarch® Genomic DNA kit.

131  
132 DNAs were then cleaned using the Zymo Research™ DNA Clean & Concentrator-5 (#D4003) kit, with the  
133 5:1 binding buffer protocol to account for the short fragments common of older herbarium samples.  
134 Cleaned gDNAs were submitted to the High Throughput Genomics Core at the University of Utah, where  
135 sequencing libraries were prepared using the Nextera™ DNA Flex Library Prep (Illumina®, #20018704)  
136 and sequenced on a full lane of Illumina® NovaSeq 6000 PE 2 × 150 bp using an S4 flow cell. SRA  
137 accession and biosample numbers are provided in Table 1. Raw reads were trimmed using fastP v0.23.4  
138 (Chen et al., 2018), assembled using MetaSPades v3.15.5 (Nurk et al., 2017; Pribelski et al., 2020), and  
139 barcodes were extracted using Pathracer v3.16.0.dev (Shlemov & Korobeynikov, 2019) with custom  
140 hidden Markov models (A. Bradshaw et al., 2023). Genome assembly stats and resulting BUSCO (Simão  
141 et al., 2015) scores are reported in Supplementary Table 2.

#### 142 143 DNA barcoding and sequencing of additional Specimens

144 The Internal transcribed spacer (ITS) DNA barcodes, commonly used for species delineation (Schoch et  
145 al., 2012), of specimens “T1,” “T2,” and “Harding” were amplified using the primers set ITS1 and ITS4  
146 (White et al., 1990). All ITS sequences were sequenced using Sanger sequencing and processed in the  
147 same manner as Van Der Merwe et al. (2024). gDNA from dried basidiomata was extracted using the  
148 Zymo Research™ Quick-DNA Fungal/BacterialMiniprep kits (#D6005). Approximately 10 mg of fungal  
149 tissue was used for extraction, which was carried out according to the manufacturer’s instructions.  
150 Successful DNA extraction was visualized on a 1% agarose gel with ethidium bromide. Amplified  
151 barcodes were sequenced at the Central Analytical Facility, Stellenbosch University, using an ABI 3730xl  
152 DNA analyzer (Thermo Fisher Scientific™, Waltham, Massachusetts) with a 50 cm capillary array and  
153 POP-7. All ITS sequences were deposited into NCBI GenBank (Sayers et al., 2019) with accession  
154 numbers reported in Table 1.

#### 155 156 Phylogenetic Inference

157 Four independent loci were selected for phylogenetic analysis: the nuclear ribosomal internal  
158 transcribed spacers (ITS), translation elongation factor 1-alpha (Ef1a), the largest subunit of RNA  
159 polymerase II (RPB1), and the second largest subunit of RNA polymerase II (RPB2). In addition to newly  
160 generated sequences, selected sequences from NCBI GenBank (Sayers et al., 2019) and the UNITE  
161 database `sh_general_release_dynamic_04.04.2024` (Abarenkov et al., 2010), including sequences from  
162 recently named species from Africa and elsewhere (Canan et al., 2024; Ostunii et al., 2024; Van Der  
163 Merwe et al., 2024) and sequences from type specimens (A. Bradshaw et al., 2023; A. J. Bradshaw et al.,  
164 2024); Supplementary data, Table 1), were used for phylogenetic analysis. ITS sequences were trimmed  
165 at the 5' and 3' end conserved motifs 5'- CATT- and -GACCT-3' for downstream analysis (Dentinger et  
166 al., 2010). Only the coding sequence of the protein-coding genes were used. ITS barcodes were  
167 partitioned into ITS1, 5.8S, and ITS2, using ITSx v1.1.3 (Bengtsson-Palme et al., 2013), aligned separately  
168 using MAFFT v7.490 with the flags `--maxiterate 1000 --localpair` for slow but accurate analysis (Kato,  
169 2002). Concatenation of multiple sequence alignments of the three ITS partitions was achieved using  
170 SEGUL 0.22.1 (Handika & Esselstyn, 2024). Phylogenetic analyses were performed using IQTREE2 2.3.6  
171 with the flags `-m MFP+MERGE -bnni -bb 1000`, to enable automatic model finder (Kalyaanamoorthy et  
172 al., 2017) and 1000 ultrafast bootstrap replicates (Minh et al., 2013). Evolutionary models were  
173 automatically determined for ITS1, 5.8S, and ITS2 partitions separately, and for each codon position for  
174 EF1a, RPB1, and RPB2. Edges were linked across all partitions. Phylogenetic trees were rendered in  
175 FigTree (<http://tree.bio.ed.ac.uk/software/figtree/>).

176

#### 177 Molecular dating

178 A reduced dataset was constructed using sequences of the three protein-coding loci for all specimens  
179 represented by two or more loci. A concatenated matrix was constructed using Segul (Handika &  
180 Esselstyn, 2022), and each codon position was given its own partition. A root calibration normally  
181 distributed around a mean of 67.6 Ma (SD +- 6) following Bradshaw et al. (2024), and a calibrated Yule  
182 speciation model were used to estimate divergence times. Bayesian inference was conducted using  
183 BEAST2 v 2.7.7 (Bouckaert et al., 2019) with Markov Chain Monte Carlo (MCMC) sampling to estimate  
184 posterior distributions of phylogenetic parameters. The best fitting models of evolution determined with  
185 automatic model finder in IQTREE (Nguyen et al., 2015) were used for each partition. A chain length of  $1 \times 10^8$   
186 steps was used, sampling every 5000 steps to ensure a thorough parameter space exploration.  
187 Convergence and effective sample sizes (ESS) of parameter estimates were assessed using Tracer v1.7.2  
188 (Rambaut et al., 2018), with ESS values above 200 considered indicative of sufficient mixing and  
189 convergence. The resulting posterior tree distribution was downsampled to every 2000 trees using  
190 LogCombiner due to computational limitations and then summarized using TreeAnnotator v1.10  
191 (Drummond & Rambaut, 2007) to produce a maximum clade credibility tree, with node heights  
192 representing median posterior estimates. The annotated tree was visualized using FigTree as above,  
193 with posterior probabilities indicated on nodes to reflect the robustness of inferred relationships and  
194 95% highest posterior density (HPD) of estimated divergence times indicated with bars.

195

#### 196 Species Distribution Modeling

197 To predict the ranges of *P. cubensis* and *P. ochraceocentrata*, we conducted species distribution  
198 modeling (SDM) using GPS coordinates of known collections and the contemporary 19 bioclimatic  
199 variables at 2.5M resolution (Fick & Hijmans, 2017) as environmental predictors. In addition, we sought  
200 to predict their historical ranges by conducting SDM of *P. cubensis* using four 19 bioclimatic datasets  
201 modeled to have occurred during the Current age, Anthropocene (1979 – 2013), last interglacial (LIG)  
202 ~130KYA, Pleistocene, MIS19 (~787 KYA) and the Pliocene ~3.3Mya (Brown et al., 2018; Dolan et al.,  
203 2015; Hill, 2015; Karger et al., 2017) at 2.5M resolution accessed from paleoclim.com (Brown et al.,  
204 2018). All collection GPS coordinates used for SDM were pulled from MycoPortal (Miller & Bates, 2017)  
205 entries, including data from Mushroom Observer (<https://mushroomobserver.org/>) and iNaturalist  
206 (<https://www.inaturalist.org>). All data points were filtered to remove non-wild collections, including the  
207 removal of all entries with specific mentions of samples being cultivated, confiscated by police, or those  
208 labeled as known cultivated strains of *P. cubensis*. The locations of all collections and observations were  
209 plotted using GPS coordinates over a global map with R packages ggmap v. 4.0.0 (Kahle & Wickham,  
210 2013) and sf v1.0-16 (Pebesma, 2018), and then visualized using ggplot2 v3.5.1 (Wickham, 2016).

211 To account for lumping of *P. ochraceocentrata* in prior records of *P. cubensis*, SDM for all  
212 environmental datasets was conducted both with and without African collections of *P. cubensis*, using  
213 the SDM R package (v1.2-46). We tested six of the most common SDM models ("bioclim",  
214 "domain.dismo", "glm", "gam", "rf", and "svm"), using 1000 random points as "absence" points to  
215 validate the model (Supplement Data). The best-performing model, according to AUC, COR Deviance,  
216 TSS, MCC, and F1 score, was chosen for each environmental dataset.

217

#### 218 Microscopy and Morphological analysis

219 Microscopic analysis on specimens CS-3006, CS-5783, and CS-3309 was performed using a Leitz Wetzlar  
220 Orthoplan microscope with a Leitz Wetzlar drawing tube. All spore measurements were taken from  
221 thirty spores whose side-view was clearly visible. Spore prints were studied using Melzer's Reagent, and  
222 sections of dried lamellae were studied in Congo Red. Color descriptions were derived from (Rayner,  
223 1970), and codes are recorded parenthetically for each specimen. Microscopic analysis of additional  
224 specimens T1, T3, and Harding was performed using Methods outlined in Van Der Merwe et al. (2024).  
225 The length and width were measured, and the length/width quotient (Q) was calculated and reported in  
226 Supplementary Table 1 for all samples.

227

## 228 **RESULTS**

### 229 Molecular systematics

230 Independent phylogenetic analysis of ITS, EF1a, and RPB2 all recovered African specimens originally  
231 identified as *P. cubensis*, *P. cf. cubensis*, and *P. cf. natalensis* as a monophyletic group sister to a clade  
232 containing sequences from the holotype of *Stropharia cubensis* Earle (= *Psilocybe cubensis*) (Fig. 1) with  
233 strong bootstrap support (ITS=97%, EF1a=99%, RPB2=100%). These same African sequences were not  
234 reciprocally monophyletic with respect to sequences of *P. cubensis* using RPB1. Sequences of the types  
235 of the other known species of *Psilocybe* endemic to Africa were consistently recovered in other clades.  
236 Sequences from the holotype of *Stropharia aquamarina* Pegler were placed sister to *Psilocybe*  
237 *wayanadensis* K.A. Thomas, Manim. & Guzmán and other species from Asia and Australia (BS; ITS=100,  
238 EF1a=92, RPB1=88, RPB2=97, Concat=100). Sequences from the holotype of *Psilocybe natalensis* Gartz,  
239 D.A. Reid, M.T. Sm. & Eicker did not match any publicly available sequences and was placed sister to  
240 *Psilocybe chuxiongensis* T. Ma & K.D. Hyde (typified from Yunnan, China) and *Psilocybe maluti* B. Van der  
241 Merwe, Rockefeller & K. Jacobs (typified from South Africa) ( BS; ITS=99, Concat=99). Sequences of the  
242 holotype of *Psilocybe jaliscana* Guzmán and the sequences of *Psilocybe subcubensis* Guzmán were  
243 nested within a clade of sequences from *P. cubensis* across all loci. Sequences from the holotype of  
244 *Psilocybe keralensis* K.A. Thomas, Manim. & Guzmán was recovered in Clade I, and its ITS sequence was  
245 identical to the ITS from the type specimen of *Psilocybe ingeli* B. Van der Merwe, Rockefeller & K.  
246 Jacobs.

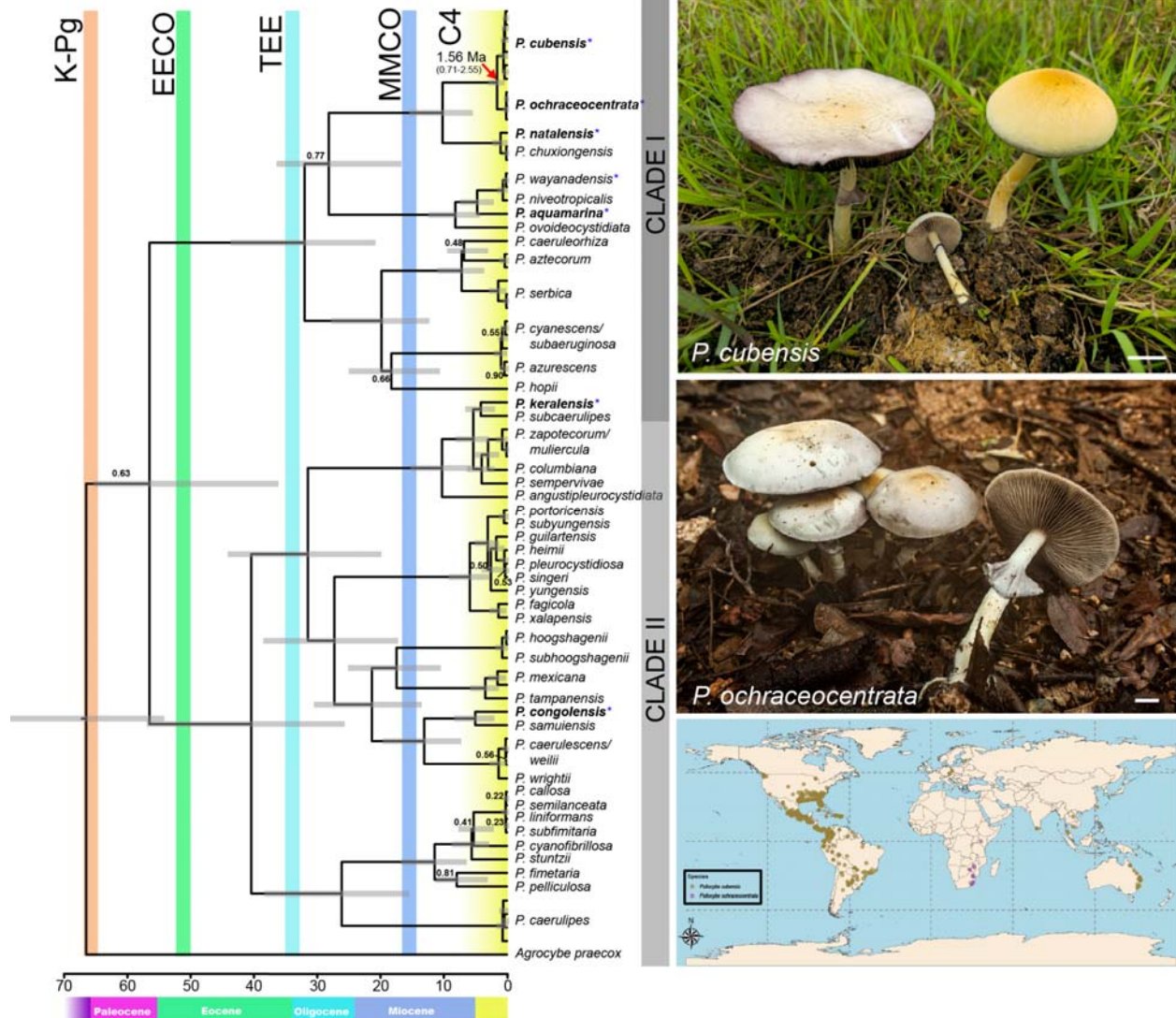
247

#### 248 **Molecular dating and geological timeline**

249 Due to the close relationship between *P. ochraceocentrata* and *P. cubensis*, we performed molecular  
250 dating using Bayesian inference with BEAST of a concatenated dataset of EF1a, RPB1, and RPB2 (Figure  
251 1). Phylogenetic reconstruction generated a tree with highly supported nodes (posterior probability  
252 >95%). Only a minority of nodes received less than a 0.95 posterior probability and most of these were  
253 near the tips among closely related species and species complexes. Molecular dating places the MRCA  
254 of *P. ochraceocentrata* and *P. cubensis* at ~1.56 million years ago (MYA)(0.71-2.55 95% HPD). This  
255 estimated divergence date corresponds to the Pleistocene epoch (2.5 MYA - 11.7 KYA) following the  
256 mass emergence of grass biomes in warm climates with the evolution of the C<sub>4</sub> photosynthetic pathway  
257 (8 to 3 MYA) (Edwards et al., 2010).

258

259



260  
 261 **Figure 1.** Phylogeny, photo documentation, and geographical distribution of *Psilocybe ochraceocentrata*.  
 262 LEFT: Maximum clade credibility chronogram of *Psilocybe* spp. resulting from Bayesian divergence  
 263 analysis of three loci (EF1a, RPB1, RPB2). All nodes received >95% posterior probability except for  
 264 internal nodes with posterior probabilities indicated. Shaded bars at nodes represent 95%HPD. Shaded  
 265 bars have been removed from nodes where descendants represent multiple individuals of a species for  
 266 improved readability. The mean divergence time for the common ancestor of *P. cubensis* and *P.*  
 267 *ochraceocentrata* is indicated by a red arrow. Scale bar at the bottom represents the estimated time  
 268 using a root calibration normally distributed around a mean of 67.6 Ma (SD +/- 6) following Bradshaw et  
 269 al. (2024). Geologic epoch time scale is approximate. Colored vertical bars represent noteworthy global  
 270 events that illustrate correlation of major divergences within *Psilocybe*: the K-Pg event ~65 MYA, the  
 271 Eocene Epoch Climate Optimum (EECO), the Terminal Eocene Event (TEE), the Mid-Miocene Climate  
 272 Optimum (MMCO), and the emergence of C4 grass biomes (C4). Right: in situ photos of *Psilocybe*  
 273 *cubensis* (BD1406, top), *P. ochraceocentrata* (CS3006, middle), and records of *P. cubensis* (gold squares)  
 274 from Mycoportal, iNaturalist, and Mushroom Observer with African records reinterpreted as *P.*  
 275 *ochraceocentrata* (Purple squares).

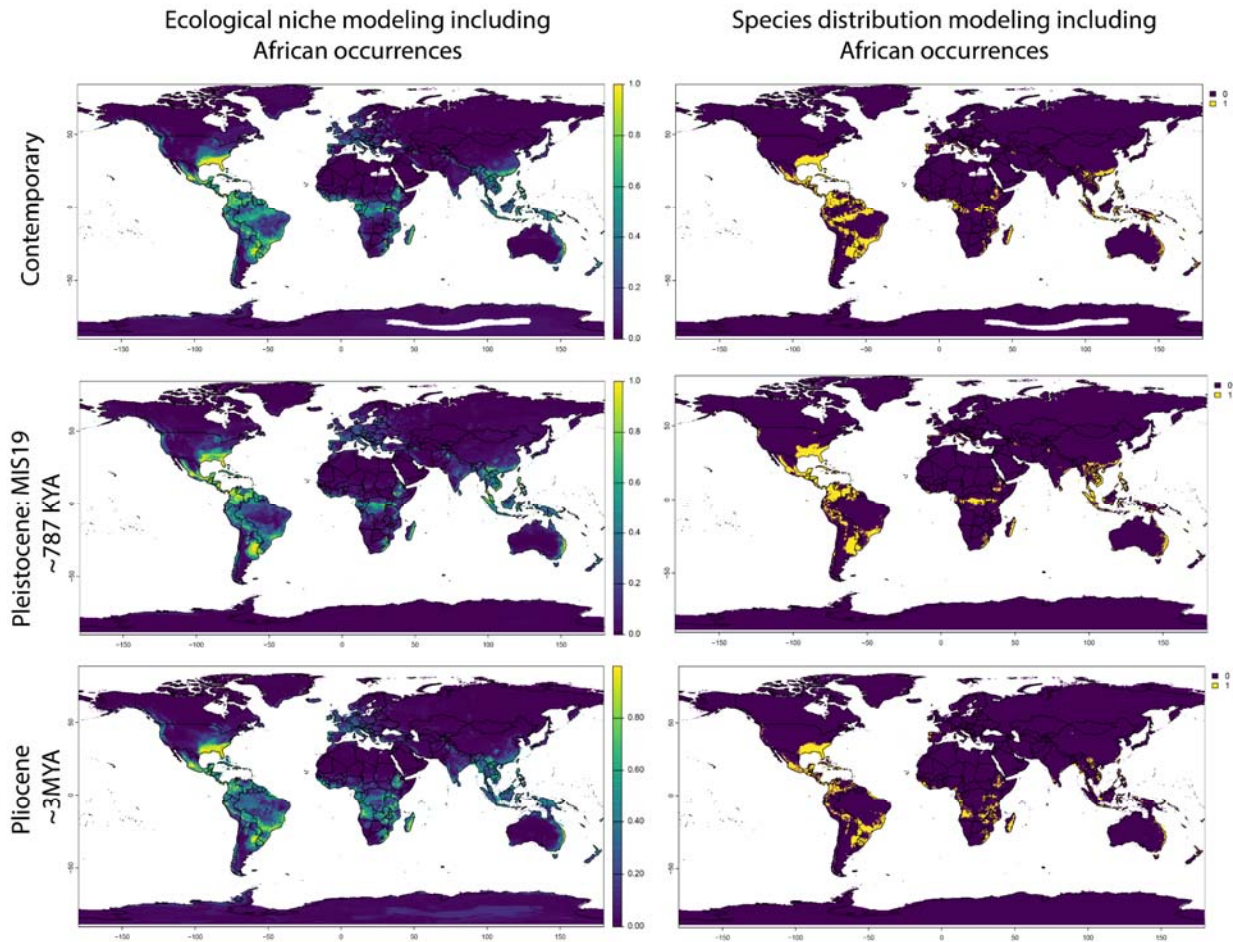
276  
 277

**Ecological niche modeling and species distribution of *P. cubensis***



278 Due to the close relatedness of *P. ochraceocentrata* and *P. cubensis*, we chose to investigate the  
279 theoretical species distribution of *P. cubensis* through time using ecological niche modeling (ENM) and  
280 species distribution modeling (SDM). To do so, we used publicly available data from MycoPortal, which  
281 includes data from iNaturalist and Mushroom Observer. Occurrence data was filtered for only those  
282 with georeferences and removed any entries that suggested occurrences were acquired from non-  
283 natural sources, such as cultivation. After filtering, we found 1001 occurrences, 12 of which were from  
284 the African continent (Supplementary data). Due to the inability to authenticate specimens from Africa  
285 as *P. cubensis*, and sparse literature reports of its distribution across Africa and India (Guzmán, 2014;  
286 Thomas & Manimohan, 2003), we performed ENM and SNM in two ways. The first analysis included all  
287 geopoints (minus confirmed *P. ochraceocentrata* specimens), assuming that *P. cubensis* specimens from  
288 Africa were correctly identified (Figure 2). The second analysis removed African specimens, assuming  
289 that these specimens are *P. ochraceocentrata* (Supplementary Figure 5). ENM and SDM were done using  
290 multiple geological datasets, ranging from the Pliocene (~3MYA) to the Modern day (Figure 2,  
291 Supplementary data).

292 Both data sets exhibited high similarity with ENM and SDM across time, predicting similar  
293 species distributions (Figure 2, Supplementary Figure 5). The most extensive predicted ranges occurred  
294 across the Southern United States, Central America, and South America (ENM >0.8, SDM =1), with a  
295 presence across Southern Africa, Southeast Asia, and Australasia, albeit as a more restricted range  
296 (ENM <0.5, SNM=1) (Figure 2). Across time, ENM and SDM indicated reduced distribution across Africa,  
297 while the Americas, South East Asia, Australasia, and Europe exhibited an increased presence and  
298 distribution up to the Last Interglacial (LIG) ~130 thousand years ago (KYA). Post LIG into contemporary  
299 times, ENM and SDM predict distributions more in line with the Pleistocene, MIS19 (~787 KYA) and the  
300 Pliocene (~3MYA), but with a slightly increased predicted presence in Africa and distribution across  
301 Southeast Asia and Australasia remaining similar to the LIG (Figure 2).



302  
303 **Figure 2.** Ecological niche modeling (ENM) and Species distribution modeling (SDM) of *Psilocybe cubensis*  
304 and *P. ochraceocentrata* through time. All occurrence data for *P. cubensis* from public sources (likely  
305 including *P. ochraceocentrata* misidentified as *P. cubensis* in Africa) across three time scales since the  
306 mid-Pleistocene. **Top Row:** Contemporary (Modern day), **Middle Row:** Pleistocene, MIS19 (~787 KYA) ,  
307 **Bottom Row:** Pliocene (~3MYA). **Left:** ENM with distribution likelihood indicated as a heat gradient from  
308 purple (0%) to yellow (100%). **Right:** SDM with predicted species presence as present (1, yellow) or  
309 absent (0, purple).

310

### 311 **Taxonomy**

312 ***Psilocybe ochraceocentrata*** C. Sharp, A. Bradshaw, B. Dentinger & B. van der Merwe **nov. sp.**

313 Index Fungorum Registration #: IF 902894

314 Holotype Deposition: Natural History Museum of Zimbabwe, BUL8013

315 GenBank Accession: PQ315824 (ITS), PQ317397( EF1a), PQ317404 (RPB1), PQ317412(RPB2)

316 SRA Accession:

317

318 **Etymology:**

319 Pileus with a yellow-ochre center.

320

321 **Diagnosis:**

322 Typus: Zimbabwe, Matabeleland South Province, The Farmhouse, Kezi Road, Matobo Hills. QDS 2028A4.

323 In thick leaf litter in mixed deciduous woodland on granitic sand. 14 Jan 2013. Collectors C. Sharp & R.

324 Aldridge. Holotype: CS3006 (Field ID, Private Fungarium of C. Sharp), Holotype split: Natural History

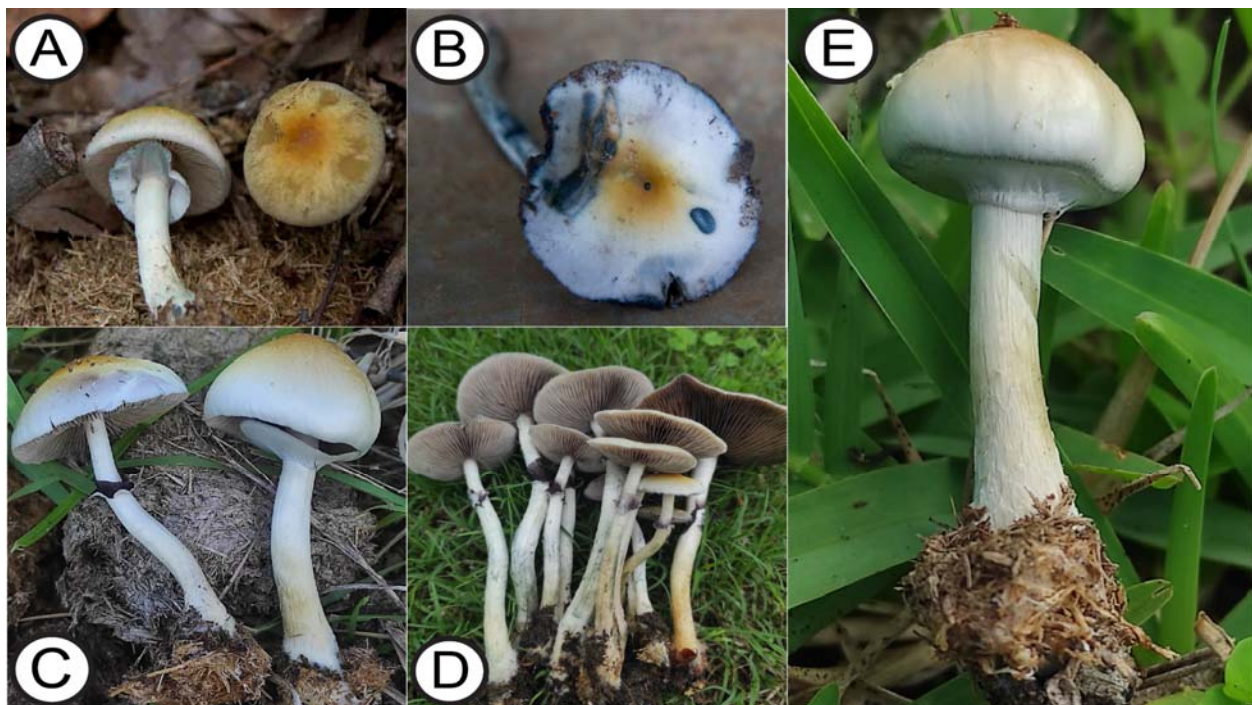
325 Museum of Zimbabwe, BUL 8013.

326

327 **General field description:**

328 **Fruiting body** medium-sized, up to 105 mm tall and growing in tight clusters; a pale fungus that bruises  
329 blue-green. **Pileus** 65-75 mm diam.; first cream-colored, then vinaceous-grey (115) with center ochreous  
330 (44) to fulvous (43); pale yellow towards margin; convex then planate, often undulating; surface finely  
331 and radially silky with a dull sheen. **Flesh** bright cream-coloured, firm to pithy. **Margin** first down-curved;  
332 edge smooth often with radial cracking. **Lamellae** adnate; first pale then greyish-sepia (106) to brown-  
333 vinaceous (84) to very dark sepia and almost black near margin; face of gill speckled as spores ripen; to  
334 12 mm deep, thin, papery and fragile; edge thin, smooth or finely scalloped, pale; sparsely to  
335 moderately crowded with lamellulae, 6-8/cm. **Stipe** central; up to 95 mm long x 9-10 mm; cream-  
336 coloured, bluing where handled; cylindrical, often twisted; apex minutely tufted (x10), streaked  
337 longitudinally, dull sheen, base often with white silky hairs. **Flesh** fibrous in walls and center hollow. **Ring**  
338 median to higher; white first then with dark spores; membranous, striate, very fragile and soon clinging  
339 to stipe and disintegrating. **Mycelium** is white to cream-coloured, forming a thick, compact mat amongst  
340 leaf litter. **Bruising** instantly to blue-green (94) where handled or damaged. **Odour** of typical mushroom.  
341 **Spore-print colour** dark vinaceous-grey (116) to purplish-grey (128).

342



343

344 **Figure 3:**

345 (A) *Psilocybe ochraceocentrata*. Holotype collection (CS-3006). Photo credit: C.Sharp,(B) Older  
346 fruiting body of *Psilocybe ochraceocentrata*; color change possibly due to water logging,  
347 (C)*Psilocybe ochraceocentrata* found on dung; (D) Cluster of *Psilocybe ochraceocentrata* (South  
348 Africa). Photo credit: Talan Moul, (E) Young sporocarp of *Psilocybe ochraceocentrata* (Harding).  
349 Photo credit: Talan Moul.

350

351 **Habit and habitat:**

352 Miombo woodland, mixed deciduous woodland, all on granitic sand; CS3309 was found growing on old,  
353 decomposed herbivore dung of unknown origin.

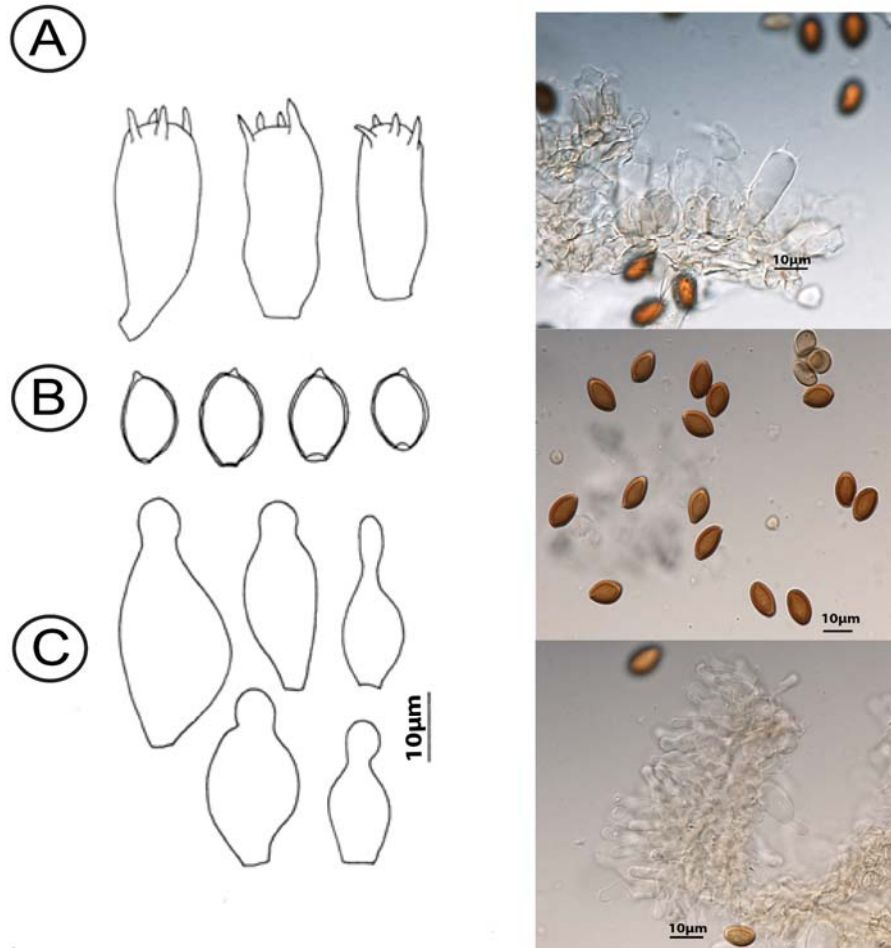
354

355 **Macroscopic Description:**

356 **Fruiting body** in a tight cluster, to 105 mm tall. **Pileus** to 65 mm diameter; first cream-colored to pale  
357 vinaceous-grey (115) with ochreous (44) or fulvous (43) center; first convex then planate; surface finely  
358 streaked radially with dull sheen. **Flesh** cream-coloured, firm. **Margin** straw-yellow (40), down-curved,  
359 smooth; some radial cracking. **Lamellae** greyish-sepia (106) to brown vinaceous (84), face speckled with  
360 ripening spores; adnate; thin, papery, fragile; edge finely scalloped and pale; with lamellulae. **Stipe** to 95  
361 mm long x 9 (apex) – 10 (mid) – 10 (base) mm; cylindrical, twisted or not; apex minutely tufted (x10  
362 lens), longitudinally streaked to silky-hairy at the base. **Flesh** hollow with fibrous walls. **Ring** just above  
363 median, white, striate, membranous to clinging, and very fragile. **Bruising** immediately to glaucous sky-  
364 blue (93) to glaucous blue-green (94). **Odour** typical mushroom. **Spore-print** dark vinaceous-grey (116)  
365 to purplish-grey (128). **Chemical reactions:** not recorded  
366

367 **Microscopic description:**

368 **Figure 4:**



369

370 Microscopic illustrations and microscopic feature morphology of *Psilocybe ochraceocentrata*: (A) Basidia  
371 from Holotype specimen CS-3006; (B): Basidiospores from Holotype specimen CS-3006; (C)  
372 Cheilocystidia from specimen CS-5783. Microscopic images are derived from specimen *P.*  
373 *ochraceocentrata* "Harding."

374 **Spores derived from Holotype CS-3006:** Ranges are given parenthetically with mean values underlined;  
375 ellipsoid, a few lenticulars; thick-walled, smooth-walled with germ-pore; (11)11.8(12.5) x (6.5)7.6(8.0)  
376 µm; Q = (1.44)1.55(1.71). **Basidia** elongate or clavate and variable in size, 22-38 x 8-12.5 µm; four  
377 sterigmata, 4-5 µm long with rounded or acute apex.

378 **Notes from additionally examined samples:**

379 [1] **Spores** can be highly variable in size; note CS3309 has much smaller spores with a few in the extreme  
380 range (Supplementary Table 1)

381 [2] **Spores** shape ellipsoid, some collections having more lens-shaped spores than others; thick-walled,  
382 smooth-walled with apical germ-pore. **Basidia** elongate or clavate; 22-30 x 8-11  $\mu\text{m}$ ; four sterigmata, 4-  
383 5 $\mu\text{m}$  long with rounded or acute apex. **Cystidia** no pleurocystidia were observed; despite numerous  
384 sections, no cheilocystidia were observed in the Type collection but very clear in CS3309 and CS5783;  
385 capitate in shape, thin-walled, 21-26 x 9-13 $\mu\text{m}$  across the widest part. **Hyphae** clamp connections  
386 observed in the Type.

387 [3] CS-2680. Mashonaland Central Province, Mukuvisi Woodlands, Harare. QDS 1731C3. 18 Jan 2012.  
388 Collector Daniel Nyamajiwa. The spore range is a good match even if a bit smaller, e.g.  
389 (10)10.8(11.5)[13] x [6.5](7)7.4(8)  $\mu\text{m}$  but shape slightly different, Q = (1.33)1.45(1.57) (Supplementary  
390 Table 1).

391 [4] Even though the ochre-yellow center is diagnostic, one noticeable morphological difference is that  
392 the green-blue pigments turn black and remain dark while the specimen is fresh. This may be due to the  
393 older fruiting bodies or because they contain more water in a very wet season. All signs of green or black  
394 coloration are absent in the dried samples (Figure 2, B).

#### 395 Synonomizations

396

397 ***Psilocybe cubensis*** (Earle) Singer, *Sydowia* 2(1-6): 37 (1948)

398

399 Basionym:

400 *Stropharia cubensis* Earle, *Inf. an. Estac. Cent. agr. Cuba* 1: 240 (1906)

401

402 Synonyms:

403 =*Naematoloma caerulescens* Pat., *Bull. Soc. mycol. Fr.* 23(2): 78 (1907)

404     ☐ *Hypholoma caerulescens* (Pat.) Sacc. & Trotter, *Syll. fung. (Abellini)* 21: 212 (1912)

405     ☐ *Psilocybe cubensis* var. *caerulescens* (Pat.) Singer & A.H. Sm., *Mycologia* 50(2): 269 (1958)

406 =*Stropharia cyanescens* Murrill, *Mycologia* 33(3): 279 (1941)

407     ☐ *cubensis* var. *cyanescens* (Murrill) Singer & A.H. Sm., *Mycologia* 50(2): 269 (1958)

408 =*Psilocybe jaliscana* Guzmán, *Docums Mycol.* 29(no. 116): 46 (2000)

409

#### 410 **DISCUSSION:**

411 *Psilocybe* has become a world-renowned genus of mushrooms, primarily due to their  
412 psychoactivity. Currently, *Psilocybe* mushrooms have been targeted as a source of natural products for  
413 use in a global mental health crisis (Carhart-Harris et al., 2017; Daniel & Haberman, 2017; Gandy et al.,  
414 2020; Johnson & Griffiths, 2017). However, due to the issue of legality in their collection,  
415 characterization of their diversity and studies of their biology have been severely stifled. Despite the  
416 ~160 species of true *Psilocybe* described around the world, most were described in the Americas  
417 (Borovička et al., 2011; Guzmán, 2014; Guzman et al., 2013; Johnston & Buchanan, 1995; Ma et al.,  
418 2014; Picker & Rickards, 1970). In contrast, only seven species of *Psilocybe* (including *P.*  
419 *ochraceocentrata*) have been typified from Africa, ranging from the cedar forests of Northern Africa  
420 (*Psilocybe mairei*) to the grassland of South Africa (*Psilocybe maluti*) (Guzmán, 2014; Van Der Merwe et  
421 al., 2024); many more still undocumented species are predicted.

422 The subject of psychoactive mushrooms, in particular *Psilocybe*, and their proposed medical  
423 benefits is rapidly gaining interest globally, and no less so in Zimbabwe. Recreational use in Zimbabwe is  
424 generally isolated and often dependent on the availability of imported products, likely the frequently  
425 cultivated *P. cubensis* (Musshoff et al., 2000). However, historical indigenous knowledge of these fungi is  
426 lacking outside of Mesoamerica, making traditional-use claims challenging to validate. However, there  
427 have been suggestions that access to the information has been restricted due to a sense of protected  
428 information among traditional healers (T. Froese et al., 2016; Guzmán, 2014; Van Der Merwe et al.,  
429 2024). This issue is likely a consequence of the historical lack of mycological studies across Africa, which  
430 remains one of the most understudied geographic locals for fungal diversity (Antonelli et al., 2024; Crous  
431 et al., 2006). Consequently, the people of Zimbabwe's use of *Psilocybe* for ceremonial or medicinal  
432 purposes is unknown.

433 *Psilocybe* has been previously grouped into four major sections, including the “Cordisporae”,  
434 “Mexicanae,” “Zapotecorum,” and “Cubensae” sections. Of these sections, the Cubensae has had little  
435 documentation of novel species. The Cubensae section includes species found across Central America,  
436 Southeast Asia, India, and Africa, including the most iconic species *P. cubensis* (A. J. Bradshaw et al.,  
437 2024; Guzmán, 1983, 1995; Ramírez-Cruz et al., 2013). However, the abundance of diversity within the  
438 section remains uncertain, primarily due to a limited representation of type specimens. Type specimens  
439 serve as the authoritative description of a species, and their representation is essential to characterize  
440 new species definitively. In particular, some specimens of the Cubensae complex, such as *Psilocybe*  
441 *jaliscana* (typified from Mexico) and *Psilocybe aquamarina* (typified from Kenya), were thought to be  
442 synonymous with *P. cubensis* and *P. subcubensis*, respectively (Guzman 2014, and personal  
443 Communication, Virginia Ramírez-Cruz and Alonso Cortés-Pérez). While the case for *Psilocybe jaliscana*  
444 being synonymous with *P. cubensis* is true, the same is not true for *P. aquamarina*, which is reported  
445 here as genuinely novel.

446 Further, a new issue arises when publicly deposited data with type specimens is validated. The  
447 commercially sold “Natal Super Strength (NSS)” (OK491080.1) strain of *P. natalensis* (typified from  
448 KwaZulu-Natal) does not match the type specimen of *P. natalensis*. Instead, four of the five publicly  
449 deposited sequences cluster with *P. ochraceocentrata*, indicating misidentification. This could lead to  
450 future regulatory issues and confusion over the species identity of commercially sold *Psilocybe* strains.  
451 Misidentifications at these levels illustrate the difficulty of identifying many species of *Psilocybe*, which  
452 often exhibit plastic morphology, necessitating type specimen validation for taxonomic accuracy and  
453 stability. This phenomenon is not unique to the Cubensae complex, as it has also been shown to occur  
454 across the genus multiple times in commonly collected species (Awan et al., 2018; A. J. Bradshaw et al.,  
455 2022, 2024). Without the ability to accurately describe what species are being consumed medically or  
456 being commercialized, we jeopardize future work in understanding the species-specific secondary  
457 metabolism of *Psilocybe*. With this work, we explicitly targeted type specimens, allowing for direct and  
458 accurate comparison of future specimens of *Psilocybe* from around the world. Notably, we provide the  
459 molecular data from the holotype of *Stropharia cubensis* Earle, confirming the identity of the majority of  
460 commercially available strains of “magic mushrooms” as *P. cubensis* for the first time.

461 *Psilocybe cubensis* is a globally distributed species whose origin is debated. Our ecological  
462 modeling suggests that *P. cubensis* could have been found historically but discontinuously across the  
463 Americas, Southeast Asia, Southern and Central Africa, and Australasia between 0.71 and 2.55MYA  
464 (Figure 2, Supplementary data). While there is an extensive predicted range, it has been shown that *P.*  
465 *cubensis* from Australia has relatively low genetic diversity, suggesting recent arrival with domesticated  
466 cattle to the continent (McTaggart et al., 2023). There are also no authenticated specimens of *P.*  
467 *cubensis* from Africa and only two sequences of it from India (iNat144581118, OK165610.1). While it is  
468 possible that *P. cubensis* may occur naturally in Africa, *P. ochraceocentrata* has a strikingly  
469 morphological and genetic similarity and shares its almost exclusively ruminant coprophilic habit, which  
470 is likely to lead to misidentification. Our divergence analysis suggests that their MRCA likely originated  
471 alongside the large herbivores, possibly during the expansion of the C4 grasslands in East Africa 1.8-1.2  
472 MYA (Cerling, 1992; Cerling et al., 1988). Coincidentally, this is also the period when *Homo erectus*  
473 became the dominant hominin in East Africa and the first to spread from Africa through Eurasia via the  
474 Levantine corridor alongside large herbivores, including bovids (Antón & Swisher, 2004; Belmaker,  
475 2010; Dennell & Roebroeks, 2005; Zhu et al., 2018). These major migration events present a possible  
476 avenue for dispersal of the MRCA of *P. cubensis* and *P. ochraceocentrata* from Africa and their  
477 subsequent divergence in Asia and Africa after aridification lead to loss of habitat in the intervening  
478 region.

479 *P. cubensis* was typified from Cuba in 1904 and is regularly found across the Americas, where it  
480 is associated with herbivore dung. However, cattle did not reach the Americas until 1493 (Sluyter, 2023)  
481 during the European colonization of Mesoamerica. While the origins and modern domestication of  
482 cattle are still debated, there is no question that their evolutionary origins reside in the Old World as  
483 opposed to the New World. Domestication of large grazing cattle (*Bos taurus*) and Asian zebu (*Bos*  
484 *indicus*) occurred between ~8-10KYA. The MRCA of both of these domesticated animals, aurochs (*Bos*  
485 *primigenius*), occupied a range from northern Africa to both coasts of Eurasia (Hanotte et al., 2002; Pitt  
486 et al., 2019; Zeuner, 1963), overlapping with estimated historic species distribution of *P. cubensis*.

487 Precolonial presence of *P. cubensis* in the Americas is not known, but its estimated divergence  
488 from *P. ochraceocentrata* and ecological niche modeling suggest it may have existed there before the  
489 arrival of Europeans. Bison (*Bison* spp.) are bovids that dominated grasslands in the Americas  
490 throughout the Pleistocene and may have facilitated the precolonial arrival and spread of *P. cubensis*.  
491 Bison are thought to have arrived from Asia to the Americas in multiple waves, with the first occurring  
492 ~195–135KYA during the LIG and then the second ~45–21KYA (D. Froese et al., 2017). After their arrival,  
493 bison became one of the dominant herbivores in the Americas and were widely distributed across North  
494 America and Central America. Bison are known ecological engineers, expanding and maintaining  
495 grassland ecosystems wherever they roam (Gates et al., 2010; Ripple et al., 2015), making them  
496 important vectors for coprophilic fungi, potentially including *P. cubensis*. The predicted ranges that we  
497 see in the ENM and SDM results overlap with reported range of bison across the Americas, including  
498 their co-occurrence across the coastlines of Alaska and the Pacific Northwest, and the peninsula of  
499 Florida during the LIG (Supplementary data). The occurrence of *P. cubensis* in the Antilles archipelago is  
500 most likely a recent development since no large herbivore existed that could have supported it except  
501 for giant ground sloths (*Megalocnus* spp.), although this seems unlikely given these were probably  
502 browsers with grasses making up a limited portion of their diets (Dantas et al., 2023). While we cannot  
503 confirm the Americas are an origin of *P. cubensis*, we also cannot rule it out with the presently available  
504 data. However, the inferred divergence of *P. cubensis* and *P. ochraceocentrata* millions of years ago  
505 rather than hundreds of years makes the hypothesis that *P. cubensis* was brought to the Americas from  
506 its ancestral home in Africa, where it then would have to have gone extinct, less parsimonious.



507 The recent characterization of novel species of *Psilocybe* from Africa (Van Der Merwe et al.,  
508 2024) indicates that more diversity remains to be described there. The research landscape of *Psilocybe*  
509 has been heavily stigmatized due to the near-global government restrictions on possession of the  
510 psychoactive compounds psilocybin and psilocin. As a consequence of this long-standing impediment,  
511 research on *Psilocybe* has a rich history of cooperation between citizens and professional scientists. The  
512 study presented here was made possible by the collections and observations of numerous citizen  
513 scientists, analysis with modern phylogenetic analysis and ecological modeling, and validation against  
514 type specimens maintained in museum collections. Together, these resources provided a robust,  
515 collaborative framework that enabled the discovery of *P. ochraceocentrata* and further refined our  
516 understanding of the possible geographic origins of one of the world's most infamous mushrooms, *P.*  
517 *cubensis*. More investment and less regulation on the collecting of fungi, including species and  
518 specimens predicted but not proven to contain controlled substances, is the only way to accelerate  
519 scientific discovery to gain a more complete understanding of biodiversity and its importance to human  
520 well-being before it is lost.

521

#### 522 **ACKNOWLEDGEMENTS:**

523 The Authors would like to acknowledge Virginia Ramírez-Cruz and Laura Guzmán-Dávalos for assistance  
524 in identifying difficult-to-find specimens. Sariah VanderVeur and Toma Ipsen for assistance in sample  
525 preparation and help with technical lab duties. We would also like to thank the holding institutions who  
526 provided material, much of which is rare and irreplaceable: Instituto de Ecología (INECOL, XAL),  
527 University of Leipzig (LZ), Universidad de Guadalajara (IBUG), and Royal Botanic Gardens KEW (K). We  
528 would also like to thank Talan Moulton for documenting and collecting the South African specimens of  
529 *Psilocybe ochraceocentrata* used in this study. Dr. David Minter is thanked for his thoughts and  
530 information on correct Latin designation.

531

#### 532 **Data availability:**

533 All extracted DNA barcodes have been deposited on NCBI GenBank with corresponding accession  
534 numbers reported in Table 1. All raw genomic sequencing data has been deposited in the Short Read  
535 Archive (SRA) under Bioproject number PRJNA1159811; Biosample accession numbers are reported in  
536 Table 1. All Type specimens derived molecular data has also been provided for RefSeq designation and  
537 curation. Any code or specific script requests should be sent to the corresponding author.  
538 Supplementary data, including microscopic features, genome assembly statistics, sequence alignments,  
539 raw phylogenetic trees, and ecological and species distribution modeling outputs, can be downloaded  
540 from the DRYAD data repository at <https://doi.org/10.5061/dryad.5x69p8df2>.

541

#### 542 **Conflict of interest:**

543 The authors declare that there is no conflict of interest.

544

#### 545 **List of Supplementary Figures:**

546 SF1 ML tree of ITS

547 SF2 ML tree of EF1a

548 SF3 ML tree of RPB1

549 SF4 ML tree of RPB2

550 SF5 ENM and SDM without African occurrence data

551

#### 552 **List of Supplementary Tables:**

553 ST1 Microscopic characteristics and measurements

554 ST2 Genome assembly and BUSCO statistics

555

556 **Supplementary data:**

557 All occurrence data pulled from MycoPortal, including filtered sets used for ENM and SDM

558

559 **References:**

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