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Research article

Pseudosperma brunneopilosum, a new species of Inocybaceae from West Africa

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A new species of *Pseudosperma* is described based on collections recently made in Benin in West Africa. *Pseudosperma brunneopilosum* sp. nov. occurs in forests dominated by ectomycorrhizal Fabaceae *Isoberlinia* and Phyllanthaceae *Uapaca* trees. Phylogenetic analysis of combined ITS-LSU-RPB2-TEF1 gene regions shows that *P. brunneopilosum* is unrelated and distinct from several recently described species of *Pseudosperma* also from West Africa. Complete descriptions and illustrations, including photographs and line drawings are presented.

Keywords: Benin, ectomycorrhizal fungi, molecular systematics, phylogeny, taxonomy

Introduction

Biotic inventories, combined with systematic studies, have resulted in the description of many new species of fungi in Inocybaceae Jülich from West Africa in recent years (Aïgnon et al. 2021a, b, 2022, 2023). The Inocybaceae contains seven genera, all of which occur in Africa, except for one, *Nothocybe* Matheny & K.P.D. Latha, which is known only from southern India. This suggests that Africa contains important phylogenetic diversity distributed throughout much of the family. In Africa, about 80 species of Inocybaceae have so far been recorded or described (Heim 1931, Maire and Werner 1937, Malençon and Bertault 1975, Buyck and Eyssartier 1999, Matheny and Watling 2004, Matheny et al. 2017, Aïgnon et al. 2021a, Kaygusuz et al. 2024). The genus *Pseudosperma* Matheny & Esteve-Rav. is characterized by species producing basidiomata often with a rimose pileus, conspicuously fimbriate edges of the lamellae; a fibrillose, furfuraceous or flocculose stipe apex, never smooth throughout; and often elliptic or phaseoliform basidiospores and non-necropigmented basidia (Matheny et al. 2020). *Pseudosperma* currently contains 125 species found worldwide (https://index-fungorum.org/Names/Names.asp). With the exploration of poorly explored areas of

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the globe, the genus is expected to be found in further regions (Bandini and Oertel 2020, Cervini et al. 2020, Jabeen and Khalid 2020, Saba et al. 2020, Yu et al. 2020, Zhao et al. 2022, Sanna et al. 2024). This study presents *P. brunneopilosum* sp. nov. based on collections made in *Isoberlinia* Craib & Stapf ex Holland and *Uapaca* Baill. woodlands in West Africa. A morphological assessment and multigene phylogenetic analyses of ITS, LSU, RPB2 and TEF1 sequence data support the autonomy and distinctiveness of the new species.

Material and methods

Study area and specimen sampling

Fresh basidiomata were sampled during field work in Okpara (9°15′16.99″N, 2°43′23.02″E) woodlands dominated by ectomycorrhizal trees *Isoberlinia doka* Craib & Stapf, *I. tomentosa* (Harms) Craib & Stapf, and *Uapaca togoensis* Pax. The area is situated at tropical latitudes but characterized by a long dry season (October–April) followed by a wet period (May–September). Annual precipitation varies between 1200 and 1400 mm. Specimens were photographed in situ using a digital camera. Colors were recorded according to the Online Auction Color (2004). Basidiomata were dried using an electric dryer at 40°C for 24 h. The holotype is deposited at the mycological herbarium at Parakou University, Benin (UNIPAR).

Morphological analysis

Micromorphological data were obtained from dried samples. Microscopic observations were made in 5% KOH and in 1% Congo red with a microscope. Characteristics of the basidiospores, basidia, cystidia, stipitipellis and pileipellis were investigated and drawn at 1000× magnification. We measured length (L) and width (W) of the basidiospores and calculated the ratio Q = L/W. The spore size range was determined by 40 measurements of basidiospores from each sample. Measurements of basidiospores and basidia excluded the apiculus and sterigmata, respectively. Use of the terms cheilocystidia and caulocystidia follow Kuyper (1986).

DNA extraction, PCR and sequencing

Genomic DNA was extracted from dried specimens following a NuClean Plant Genomic DNA kit (ComWin Biotech). The internal transcribed spacer 1, 5.8S rRNA gene, and the internal transcribed spacer 2 (ITS) and portions of the nuclear large subunit ribosomal RNA gene (LSU), DNA-directed RNA polymerase II subunit (RPB2), and transcription elongation factor 1-alpha (TEF-1) were amplified using primers pairs ITS1F/ITS4 for ITS (Gardes and Bruns 1993), LR0R/LR7 for LSU (Vilgalys and Hester 1990, Cubeta et al. 1991, Rehner and Samuels 1995), bRPB2-6F/bRPB2-7.1R for RPB2 (Matheny 2005), and EF1-983F/EF1-1576R for TEF1 (Rehner and Buckley 2005). All PCR products were purified and sequenced at the Beijing Genomics Institute using the same primers as those used for PCR.

Sequence alignment and phylogenetic analysis

The new sequences in this study were submitted to GenBank (Table 1), and compared with other *Pseudosperma* sequences retrieved from GenBank (Benson et al. 2010). The four gene regions (ITS, LSU, RPB2 and TEF1) were aligned separately using the L-INS-i algorithm as implemented in MAFFT ver. 7.511 (Katoh et al. 2019), and a final concatenated dataset of ITS, LSU, RPB2 and TEF1 was generated using SequenceMatrix ver. 1.7.8 (Vaidya et al. 2011) and edited in AliView ver. 1.26 (Larsson 2014). We tested for the best partitioning scheme and best model for each partition using Modelfinder (Kalyaanamoorthy et al. 2017). Maximum likelihood (ML) analysis was performed using IQ-TREE ver. 2.2.0 (Trifinopoulos et al. 2016). Ultrafast bootstrapping (UFBoot) was performed using 1000 replicates (Hoang et al. 2018). *Mallocybe myriadophylla* (Vauras & E.Larss.) Matheny & Esteve-Rav., *M. subdecurrens* (Ellis & Everh.) Matheny & Esteve-Rav., *M. terrigena* (Fr.) Matheny, Vizzini & Esteve-Rav., and *M. tomentosula* Matheny & Esteve-Rav. were used for rooting purposes based on Matheny et al. (2020).

Results

Phylogenetic analyses

Nineteen (19) new sequences were submitted to GenBank (Table 1). In the concatenated alignment, the ITS locus contained 56 taxa and 660 sites; the LSU locus contained 62 taxa with 1472 sites; the RPB2 locus 31 taxa with 775 sites; and the TEF1 locus was featured for 10 taxa including 1077 sites.

ML analysis nested multigene sequences from six West African specimens into a fully supported species-level clade within the genus *Pseudosperma* (Fig. 1). This lineage is characterized as unique considering a combination of morphological characteristics, ecological association, geographic distribution, and isolated placement within *Pseudosperma*. Phylogenetically (Fig. 1), the Australian species *P. pluviorum* from subtropical rainforest in Queensland is sister to the rest of the genus as shown previously in Matheny and Bougher (2017). *Pseudosperma brunneopilosum* presents a unique position, poorly supported close to the Australian species *P. gracilissimum* and *P. friabile* from USA, among others. The species occupies a rather long branch similar to several other tropical species such as *P. lepidotellum*, *P. neglectum* and *P. pluviorum*, for example.

Taxonomic treatment

Pseudosperma brunneopilosum Aïgnon & Fan sp. nov., Fig. 2a–d, 3–4

MycoBank: MB 835524.

Diagnosis

A species distinguished from other tropical African *Pseudosperma* by the combination of the small basidiomata, brownish hairy and rimose pileus, lighter colored stipe, and isolated phylogenetic position.

Table 1. Taxon sampling information and DNA sequences used for phylogenetic analysis. Bold font indicates the new species.

Species	Voucher	Country	ITS	LSU	RPB2	TEF1	References
Pseudosperma aestivum (Kropp, Matheny & L.J. Hutchison) Matheny & Esteve-Rav.	BK18089706	USA	EU600847	EU600847	EU600846	–	Matheny et al. 2009
P. araneosum (Matheny & Bougher) Matheny & Esteve-Rav.	TENN 066983	Australia	NR_152346	NG_057226	KJ729935	–	unpublished
P. arenarium Y.G.Fan, Fei Xu, Hai J. Li & Vauras	NXYC20201005_01	China	OM304278	OM304287	OM421667	–	Yan et al. 2022
P. arenicola (R.Heim) Matheny & Esteve-Rav.	NL-0392	Hungary	–	MK278219	–	–	Varga et al. 2019
P. beninense Kaygusuz, Bandini, Rühl, Sarawi, Yorou & M. Piepenbr.	AR-22–037	Benin	PP060393	PP060408			Kaygusuz et al. 2024
P. breviterincarnatum (D.E.Stuntz ex Kropp, Matheny & L.J. Hutchison) Matheny & Esteve-Rav.	PBM1914	USA	JQ408750	JQ319677	JQ846465	–	Kropp et al. 2013
***P. brunneopilosum* Aïgnon & Fan**	**HLA0731**	**Benin**	**OR939938**	**OR936777**	–	–	
***P. brunneopilosum* Aïgnon & Fan**	**HLA0780**	**Benin**	**OR939939**	**OR936776**	–	**OR965993**	
***P. brunneopilosum* Aïgnon & Fan**	**HLA0911**	**Benin**	**OR939940**	**OR936780**	–	**OR965988**	**this study**
***P. brunneopilosum* Aïgnon & Fan**	**HLA0910**	**Benin**	**OR939941**	**OR936775**	–	**OR965987**	
***P. brunneopilosum* Aïgnon & Fan**	**HLA0735**	**Benin**	**OQ448196**	**OR936778**	**OR965984**	**OR965997**	
***P. brunneopilosum* Aïgnon & Fan**	**HLA0763**	**Benin**	**OQ448199**	**OR936779**	**OR965981**	**OR965992**	
P. brunneosquamulosum (K.P.D.Latha & Manim.) Matheny & Esteve-Rav.	CAL 1308	India	NR_153172	NG_057276	KX073589	–	Tibpromma et al. 2017
P. bulbosissimum (Kühner) Matheny & Esteve-Rav.	DBG:19916	USA	MH024849	MH024885	MH249788	MK426213	Matheny et al. 2020
P. calciphilum M. Sanna, Mua, Porcu, M. Casula, A.C. Rinaldi, Mifsud & Garrido-Ben	CAG B/5.8.1	Italy	ON799406	OQ341419	OQ348110	–	Sanna et al. 2024
P. cercocarpi (Kropp, Matheny & L.J. Hutchison) Matheny & Esteve-Rav.	BK20069806	USA	MK421964	EU600890	EU600889	MK426214	Matheny et al. 2020
P. cf. *neoumbrinellum*	PBM3766	Australia	KP636852	KP171054	KM555146	–	unpublished
P. citrinostipes Y.G. Fan & W.J.Yu	FYG2909/FHMU3150	China	MT072898	MT071203	MT086749	–	Yu et al. 2020
P. cremeo-ochraceum Kaygusuz, Bandini, Rühl, Sarawi, Yorou & M. Piepenbr.	AR-22–088	Benin	PP060394	PP060409	–	–	Kaygusuz et al. 2024
P. dulcamaroides (Kühner) Matheny & Esteve-Rav.	EL29_08	USA	FJ904127	FJ904127		–	Larsson et al. 2009
P. fissuratum (Matheny & Bougher) Matheny & Esteve-Rav.	PBM2206	Australia	JQ408770	AY732213	JQ421069	–	unpublished
P. flavellum (P.Karst.) Matheny & Esteve-Rav.	BJ920829	Sweden	AM882774	AM882774	–	–	Ryberg et al. 2008
P. flavorimosum Jabeen & Khalid	MSM 0053	Pakistan	MG742419	MG742420	–	–	Jabeen and Khalid 2020
P. friabile (Matheny & Kudzma) Haelew.	PBM2983	USA	–	JN975015	JQ421078	–	Matheny and Kudzma 2019
P. fulvidiscum Y.G. Fan, L.N.Zhao & W.J. Yu	FYG6311	China	OM135592	OM349998	OM747849	–	unpublished
P. gilvum L. Fan & N. Mao	BJTC FM1941	China	OM801910	OM801917	–	–	Mao et al. 2022
P. gracilissimum (Matheny & Bougher) Matheny & Esteve-Rav.	PERTH:08373116	Australia	KP171122	KJ801178	KJ729946		unpublished
P. huginii Bandini & U. Eberh.	SMNS-STU-F-0901564	Austria	NR_173974	MW647628	–	–	Bandini et al. 2021
P. hygrophorus (Kühner) Matheny & Esteve-Rav.	EL97_06	Sweden	FJ904137	FJ904137	–	–	Larsson et al. 2009
P. illudens (Matheny, Bougher & G.M. Gates) Matheny & Esteve-Rav.	TENN 065726	Australia	NR_153126	JQ319699	JQ421068	–	unpublished
P. indicum K.P.D. Latha, P. Haridev & Manim.	DKP-SERB109	India	OR243200	OR243199	OR25389	–	Latha et al. 2023
P. laricis L.Fan & N. Mao	BJTC FM887	China	OM801905	OM801912	–	–	Mao et al. 2022

(Continued)

Table 1. Continued.

Species	Voucher	Country	ITS	LSU	RPB2	TEF1	References
P. lepidotellum (Matheny & Aime) Matheny & Esteve-Rav.	TENN066442	Guyana	JN642233	NG_042597	MH577508	–	Matheny et al. 2012
P. luteobrunneum (K.P.D.Latha & Manim.) Matheny & Esteve-Rav.	CAL 1260	India	NR_153171	NG_057275	KX073588	–	Tibpromma et al. 2017
P. mediterraneum (Kuyper) Bandini, B. Oertel & U. Eberh.	JV14920F	Italy	JQ408748	JQ319676	–	–	unpublished
P. melliolens (Kühner) Matheny & Esteve-Rav.	EL224_06	France	FJ904149	FJ904149	–	–	Larsson et al. 2009
P. mimicum (Massee) Matheny & Esteve-Rav.	PRM 951344	Czech Republic	–	OR602233	–	–	unpublished
P. neoumbrinellum (T. Bau & Y.G.Fan) Matheny & Esteve-Rav.	HMJAU 25742	China	NR_160608	MH047252	–	–	uUnpublished
P. neglectum (E. Horak, Matheny & Desjardin) Haelew.	DED8063	Thailand	EU600829	EU600829	–	–	
P. niveivelatum (D.E. Stuntz ex Kropp, Matheny & L.J. Hutchison) Matheny & Esteve-Rav.	PBM2337	USA	–	JQ313566	AY333776	–	Kropp et al. 2013
P. notodryinum (Singer, I.J.A.Aguiar & Ivory) Matheny & Esteve-Rav.	CO4463	USA	MH578028	MK421970	MH577509	MK426216	unpublished
P. obsoletum (Quadr.) Valade	PBM2332	USA	JQ408766	JQ319697	JQ846490	–	unpublished
P. occidentale (Kropp, Matheny & L.J. Hutchison) Matheny & Esteve-Rav	PBM525	USA	–	AY038321	AY333775	–	Kropp et al. 2013
P. pakistanense (Z. Ullah, S.Jabeen, H. Ahmad & A.N.Khalid) Matheny & Esteve-Rav.	vmt1	Pakistan	MG958608	MG958608	–	–	unpublished
P. perlatum (Cooke) Matheny & Esteve-Rav.	JV10247	Finland	JQ408767	JQ319698	–	–	unpublished
P. pinophilum Saba & Khalid	MSM 0046	Pakistan	MG742414	MG742418			
P. pluviorum (Matheny & Bougher) Matheny & Esteve-Rav.	BRI:AQ794010, PERTH:08556466	Australia	–	NG_057259	KM406221	MK426217	Horak et al. 2015
P. pseudoniveivelatum L.Fan & N.Mao	BJTC FM1660	China	OM801909	OM801915	–	–	Mao et al. 2022
P. quercinum Naseer & Jabeen	ASPC_93	Pakistan	MZ314058	MZ314078	–	–	Naseer et al. 2023
P. renisporum (E. Horak) Matheny & Esteve-Rav.	PDD:105457	New Zealand	MN047354	MN047411	–	–	unpublished
P. rimosum (Bull.) Matheny & Esteve-Rav.	PAM06112703	Corsica	FJ904143	FJ904143	–	–	Larsson et al. 2009
P. rubrobrunneum (K.P.D. Latha & Manim) Y.G.Fan	CAL 1307	India	NR_153173	KX073587	KX073590	–	unpublished
P. singulare Y.G. Fan, L.N.Zhao & W.J. Yu	FYG6363	China	OM135606	OM149381	OM780123	–	Zhao et al. 2022
P. solare Bandini B. Oertel & U. Eberh.	SMNS-STU-F-0901563	Germany	NR_173973	MW647627	–	–	Bandini et al. 2021
P. sororium (Kauffman) Matheny & Esteve-Rav.	PBM3042	USA	JQ408780	JQ319704	JQ421074	–	unpublished
Pseudosperma sp.	MCA704	Japan	JQ408765	JN975007	–	–	Ryberg and Matheny 2012
Pseudosperma sp.	PBM3751	Australia	KP636851	KP171053	KM555145	–	Pradeep et al. 2016
Pseudosperma sp.	TR194-02 (M)	Papua New Guinea	JQ408793	JN975032	JQ421080	–	Ryberg and Matheny 2012
Pseudosperma sp.	MCA562	Japan	JQ408785	JN975016	JQ421077	–	Ryberg and Matheny 2012
Pseudosperma sp.	PBM1889	USA	JQ408787	JQ319707	JQ846493	–	unpublished
Pseudosperma sp.	DV04132011	USA	KP636835	KP171040	–	–	unpublished
P. spurium (Jacobsson & E. Larss.) Matheny & Esteve-Rav.	BK180809723	USA	JQ408794	EU600868	EU600867	MK426219	Matheny et al. 2020
P. squamatum (J.E. Lange) Matheny & Esteve-Rav.	SJ08003/NL-0806	Sweden/ Hungary	FJ904136	MK278247	–	–	Larsson et al. 2009

(Continued)

Table 1. Continued.

Species	Voucher	Country	ITS	LSU	RPB2	TEF1	References
P. squarrosofulvum Kaygusuz, Bandini, Rühl, Sarawi, Yorou & M. Piepenbr.	AR-22–024	Benin	PP060395	**PP060410**	–	–	Kaygusuz et al. 2024
P. stramineum Kaygusuz, Bandini, Rühl, Sarawi, Yorou & M. Piepenbr.	AR-22–008	Benin	PP060396	**PP060411**	–	–	Kaygusuz et al. 2024
P. triacicularе Saba & Khalid	MSM 0039	Pakistan	MG742423	MG742424	–	–	Saba et al. 2020
P. ushae Bandini & G. Bandini	PAM00100606	France	FJ904130	FJ904130	–	–	Larsson et al. 2009, Bandini et al. 2022
P. yunnanense (T.Bau & Y.G.Fan) Matheny & Esteve-Rav.	HMJAU25840	China	MH047250	MG844975	–	–	unpublished
Mallocybe myriadophylla (Vauras & E.Larss.) Matheny & Esteve-Rav.	JV19652F	Finland	Q221106	AY700196	AY803751	DQ435791	Matheny et al. 2020
M. subdecurrens (Ellis & Everh.) Matheny & Esteve-Rav.	REH10168	USA	MH024850	MH024886	MH577503	MK426209	Matheny et al. 2020
M. terrigena (Fr.) Matheny, Vizzini & Esteve-Rav.	JV16431 MR00339 EL117/04	Finland/ Sweden	AM882864	AY380401	AY333309		Matheny et al. 2020
M. tomentosula Matheny & Esteve-Rav.	PBM4138	USA	PBM4138	MK421969	MH577506	MK426210	Matheny et al. 2020

Type: Benin, Borgou Province, region of Tchaourou, Okpara forest, 9°14′40.66″N, 2°43′34.44″E, on soil, in woodland dominated by *Isoberlina doka* and *Isoberlinia tomentosa*, 11 July 2020, leg. L.H Aïgnon (holotype: HLA0735). GenBank accessions: ITS (OQ448196), LSU (OR936778), RPB2 (OR965984), TEF1 (OR965997).

Etymology

brunneopilosum, in reference to brownish densely fibrillose pileus.

Description

Basidiomata small (Fig. 2a–d). Pileus 3–10 mm wide, whitish orange to pale brown (oac902); hemispherical to convex, at times conical, occasionally with a small obtuse umbo; surface dry, densely fibrillose, rimose, bronze and sometimes slightly white towards the margin. Lamellae adnexed, moderately close to subdistant with several tiers of lamellulae, pale brown (oac902) to brownish yellow then yellowish brown, subventricose; edges white-fimbriate, smooth. Stipe 18.0–20.0 × 0.3–0.6 mm wide at the apex and with a slightly swollen base; white to dull yellow or with dull ochraceous yellow ground color; surface with white fibrils all over when young, these wearing away to reveal the pigmented ground color. Odor and taste not remarkable. Basidiospores (8.0) 8.3 –10.3 –12.3 (13.0) × (4.0) 4.6 –5.8 –6.9(7.0) µm, Q = (1.6) 1.7 –1.8 –2 (2.1) µm [n = 40, from three collections], reniform to somewhat amygdaliform or oblong, often with bluntly pointed apices, smooth; wall slightly thickened, brownish yellow to yellowish brown; apiculus not distinctive. Basidia 22–40 × 9–13 µm, 4-spored (occasionally 2-spored), clavate; sterigmata up to 4 µm long, hyaline. Pleurocystidia absent. Cheilocystidia 30–50 × 12–20 µm, thin-walled, clavate to broadly so, mixed with some fusiform and utriform cells. Pileipellis a dense layer of moderately compact hyphae, these yellowish brown and 5–15 µm wide (Fig.3n, 4e). Lamellae trama almost regular; hyphae hyaline, sometimes slightly swollen. Stipitipellis composed of tangled hyphae towards the apex; hyphae 4–15 µm wide, thick-walled but sometimes slightly thin-walled. Caulocystidia 15–35 × 9–15 µm, slender (sub)cylindrical to subclavate, thin-walled, at the stipe apex. Clamp connections present.

Distribution and ecology

Gregarious on soil near *Isoberlinia doka* and/or *Isoberlinia tomentosa* and *Uapaca togoensis*. Benin, West Africa.

Additional specimens examined

Benin, Borgou Province, Region of Tchaourou, Okpara forest.: 9°14′40.66″N, 2°43′34.44″E, on soil, in woodland dominated by *Isoberlinia doka* and/or *I. tomentosa*, 11 July 2020, leg. H. L. Aïgnon, specimen voucher (HLA0731), GenBank accession. ITS (**OR939938**), and LSU (**OR936777**). Ibid, specimen voucher (HLA0763), 10 July 2022, GenBank accession, ITS (**OQ448199**), LSU (**OR936779**), RPB2 (**OR965981**), and TEF1 (**OR965992**). Ibid, specimen voucher (HLA0780), 24 July 2021, GenBank accession, ITS

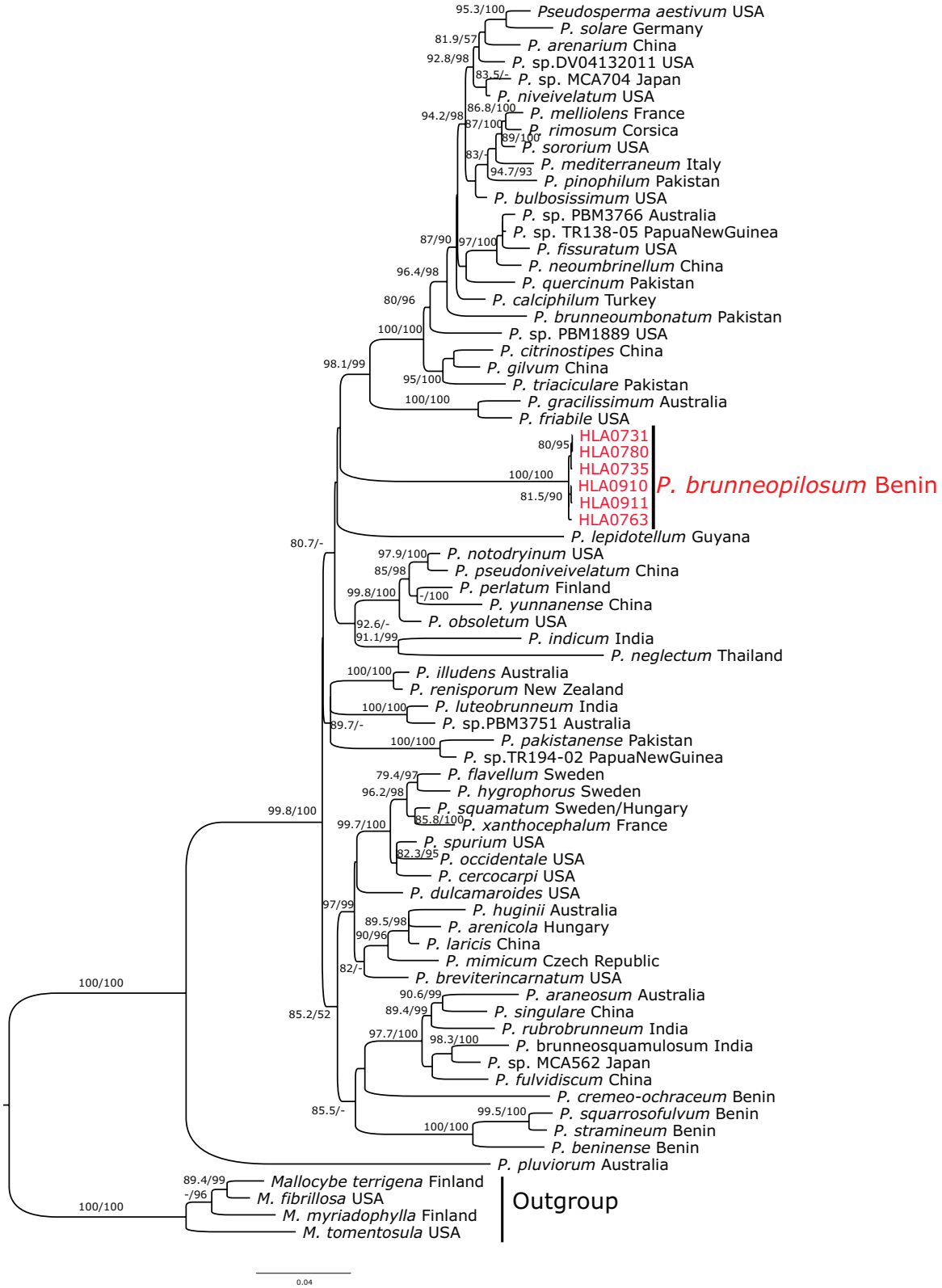


Figure 1. ML tree of ITS, LSU and RPB2 and TEF1 sequences showing the placement of *Pseudosperma brunneopilosum*. Values above or below branches indicate bootstrap proportions SH-aLRT support ≥ 80% / ultrafast bootstrap support ≥ 95% are shown and the geographic origin of each tip is given after the species name.

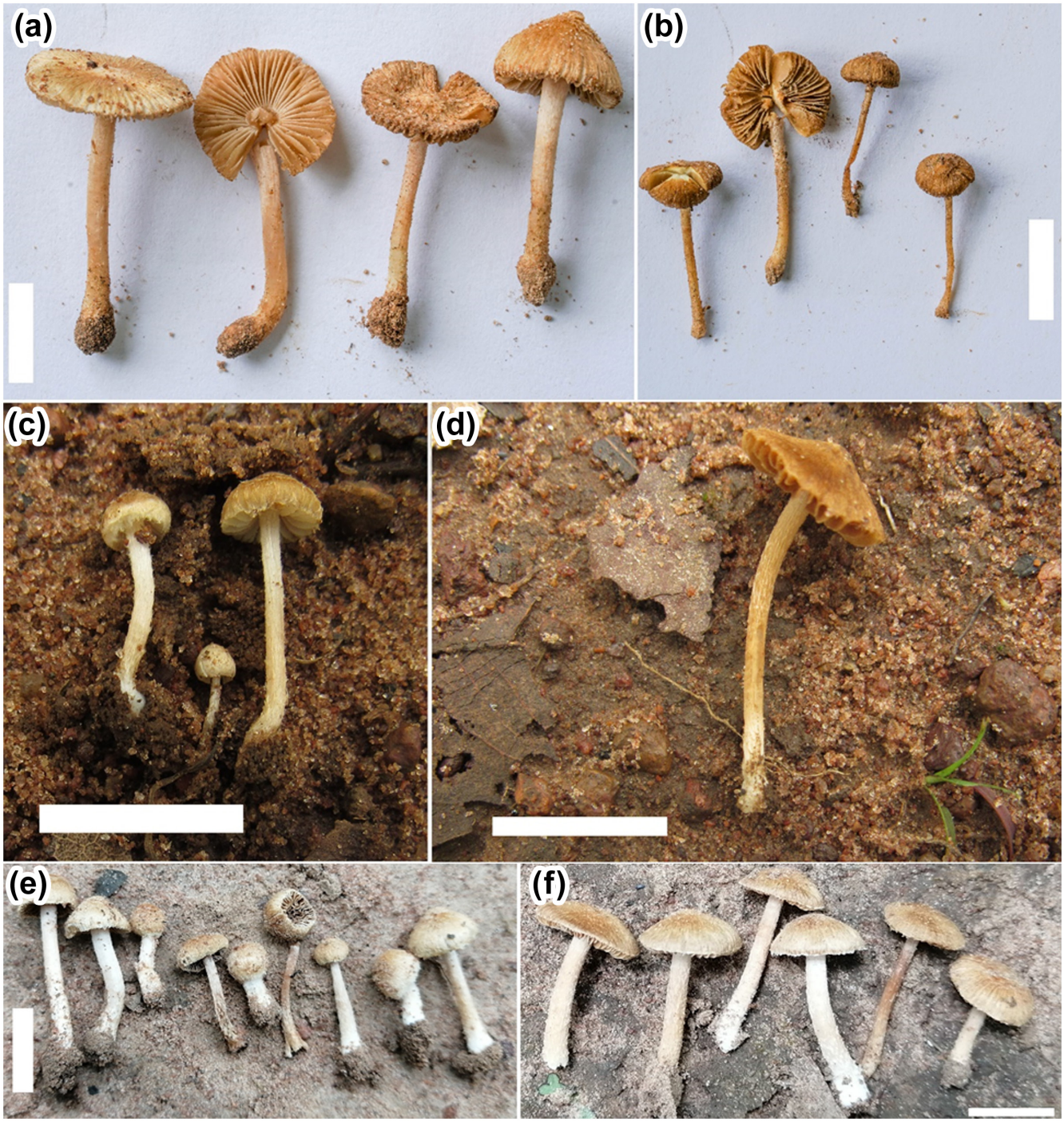


Figure 2. (a–f) = Basidiomes of *Pseudosperma brunneopilosum* (a = HLA0735 holotype, b = HLA0731 c = HLA0910 and d = HLA0911, e = HLA0763, f = HLA0780). Bar = 1 cm. Photos by: H. L. Aïgnon.

(**OR939939**), LSU (**OR936776**), and TEF1 (**OR965993**). Ibid, specimen voucher (HLA0910), 18 Aug. 2022, GenBank accession, ITS (**OR939941**), LSU (**OR936775**), and TEF1 (**OR965987**). Ibid, specimen voucher (HLA0911), 18 Aug. 2022, GenBank accession, ITS (**OR939940**), LSU (**OR936780**), and TEF1 (**OR965988**).

Discussion

This study describes *P. brunneopilosum* sp. nov. from Benin in West Africa as new based on morphological, anatomical, and phylogenetic analyses of sequence data from four gene regions (ITS-LSU-RPB2-TEF1). Analyses without TEF1, which was

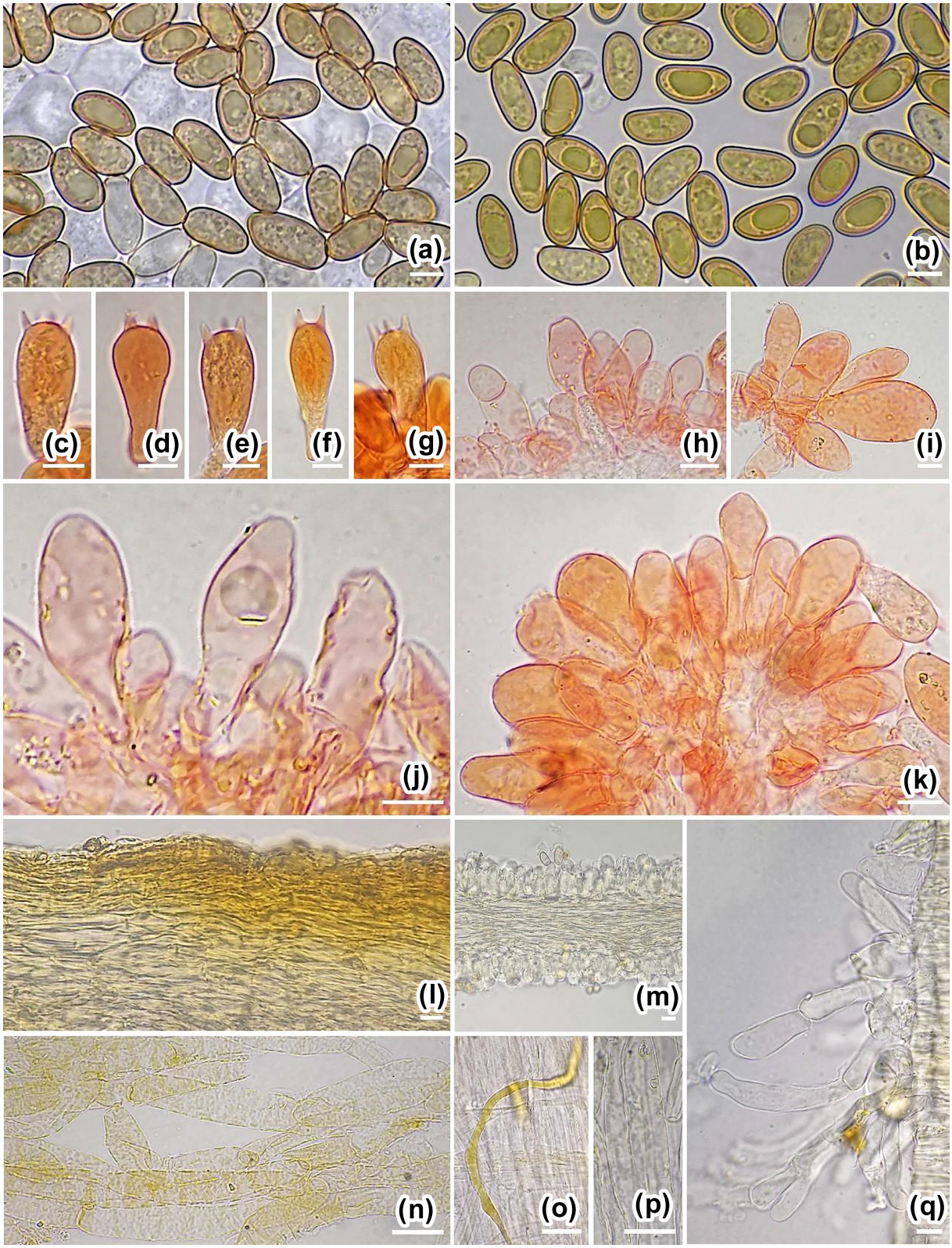


Figure 3. Microscopic features of *Pseudosperma brunneopilosum* (HLA0735, holotype). (a–b) Basidiospores, (c–g) basidia, (h–k) cheilocystidia in clusters, (l) pileipellis, (m) cross section of lamellae, (n) pileipellis hyphae, (o) oleiferous hyphae, (p) stipe trama hyphae, (q) caulocystida. Scale bars: a–q = 10 µm.

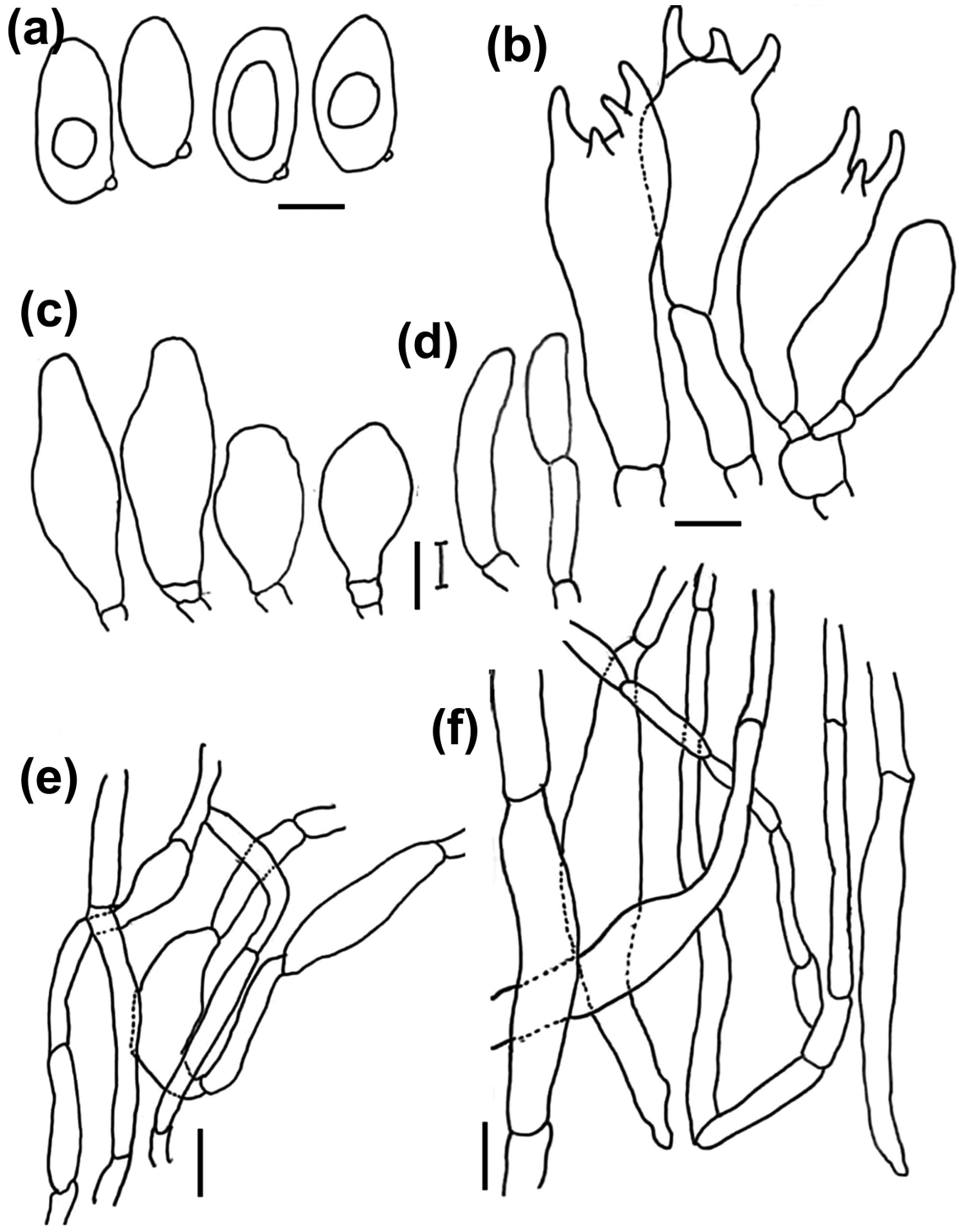


Figure 4. Micromorphology of *Pseudosperma brunneopilosum*, (HLA0735). (a) Basidiospores, (b) basidia, (c) cheilocystidia, (d) caulocystidia, (e) pileipellis, (f) stipitipellis. Scale bars: 5 µm (a–b), 10 µm (c–f).

sparsely represented in our dataset, showed revealed similar results (data not shown). However, phylogenetic results from the combined ITS-LSU-RPB2-TEF1 dataset showed a higher number of resolved nodes than results obtained with ITS-LSU-RPB2 alone (Fig. 5). Alternative analyses (data not shown) with redacted regions of ITS also showed a poorly supported placement of *P. brunneopilosum* in contrast to the result shown in Fig. 1. Nonetheless, no instance of strongly supported gene conflict was observed, and in all analyses *P. brunneopilosum* is distantly isolated from other *Pseudosperma*.

The discovery and description of this species contributes to a better understanding of the diversity of Inocybaceae in

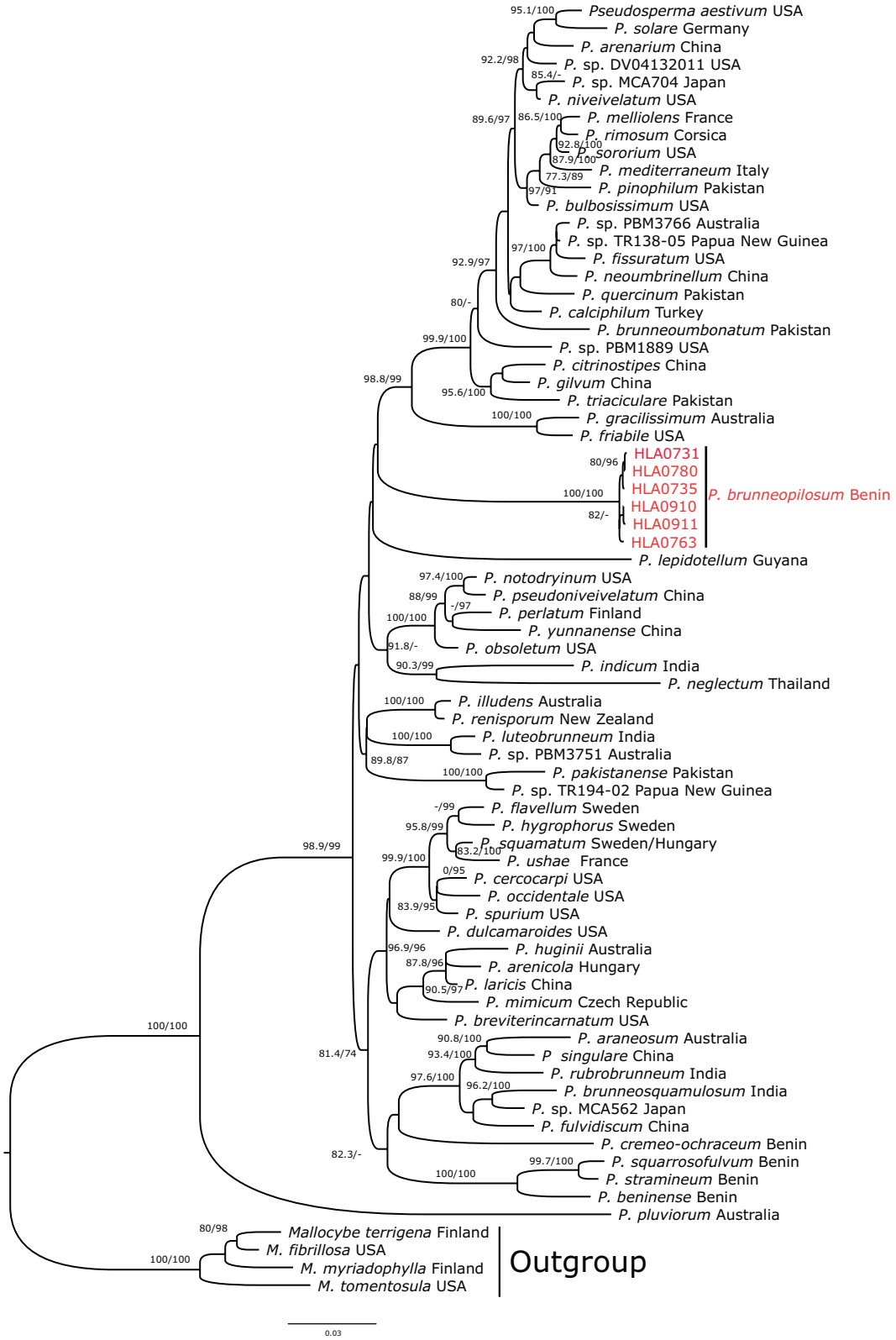


Figure 5. ML tree of ITS, LSU, and RPB2 sequences showing the placement of *P. brunneopilosum*.

West Africa and the evolution of *Pseudosperma*. *Pseudosperma brunneopilosum* is distributed in vegetation dominated by ectomycorrhizal trees *Isoberlinia doka*, *I. tomentosa* and/or *Uapaca togoensis*, like most Inocybaceae species described in Benin (Aïgnon et al. 2021b, c, 2022, 2023). The species is distantly related to and distinct from four other recently described species from Benin by Kaygusuz et al. (2024). Indeed, all five West African species are not monophyletic. This contribution adds to our understanding of the diversity of Inocybaceae in tropical Africa as well as the diversity and global distribution of *Pseudosperma*.

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Author contributions

Hyppolite L. Aïgnon: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Funding acquisition (equal); Investigation (lead); Methodology (equal); Project administration (equal); Writing – original draft (lead); Writing – review and editing (equal). **Yu-Guang Fan**: Conceptualization (equal); Data curation (equal); Funding acquisition (equal); Writing – review and editing (equal). **P. Brandon Matheny**: Supervision (lead); Validation (lead); Visualization (lead); Writing – review and editing (equal).

Data availability statement

This article has no additional data.

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