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THREE NEW SPECIES OF THE GENUS *PSEUDOSPERMA* COLLECTED IN SARDINIA

Abstract

Three new species from Sardinia (Italy), Pseudosperma dominariense, P. coloniense and P. flagellatum, are described in detail, along with colour photographs of their macro and microscopic characters and a comparative study of their closest allies. Additionally, a phylogenetic tree comprising newly discovered species and additional Pseudosperma species from the GenBank (NCBI) and Unite database is also provided.

Riassunto

Tre nuove specie provenienti dalla Sardegna (Italia), Pseudosperma dominariense, P. coloniense e P. flagellatum, sono descritte in dettaglio, corredate da fotografie a colori che ne illustrano le caratteristiche macro e microscopiche e da uno studio comparativo delle specie più affini. Viene inoltre fornito un albero filogenetico comprendente le specie di nuova scoperta e ulteriori specie di Pseudosperma provenienti dai database GenBank (NCBI) e Unite.

Key words Agaricomycetes, Inocybaceae, Phylogeny, Taxonomy.

Introduction

Phylogenetic studies initiated several years ago, which led to the dismantling of the polyphyletic genus *Inocybe* (Fr.) Fr. of classical taxonomy, have demonstrated that also its Sect. *Rimosae* is polyphyletic (MATHENY 2009), its species being found in two different distinct clades: the first clade, called "*Pseudosperma*" (MATHENY 2005; KROPP *et al.* 2013), comprises the species belonging to Sect. *Rimosae* s.s. whereas the second clade, called "*Inosperma*", comprises two subclades in the first of which are placed the species close to *Inocybe maculata* Boud. (clade "*Maculata*") and in the second those of Sect. *Cervicolores* Sing. (clade "*Cervicolores*") of the same genus (LARSSON *et al.* 2009; MATHENY 2009); therefore, ultimately, the clades "*Pseudosperma*" and "*Inosperma*" comprised the species belonging to the sections *Rimosae* and *Cervicolores* of the old taxonomy (KUYPER 1986; STANGL 1989).

In the work of MATHENY *et al.* (2020) the two clades "*Maculata*" and "*Cervicolores*" were included within a new genus called *Inosperma* (Kühner) Matheny & Esteve-Rav., morphologically characterised by ectomycorrhizal species with spores often phaseoliform, basidia hyaline or with necropigment, cheilocystidia sometimes with cyanophilous content, pleurocystidia absent, stipe base sometimes bulbous, context often reddening and odour sometimes characteristic.

Numerous new taxa of genus *Pseudosperma* Matheny & Esteve-Rav. have been described in recent years in Europe in the works of JACOBSSON & LARSSON (2009), CARTERET & REMAUX (2015), CERVINI *et al.* (2020), CERVINI (2022), BANDINI & OERTEL (2020), BANDINI *et al.* (2021, 2022, 2023, 2024), SANNA *et al.* (2024), DONG *et al.* (2025), KLEINE *et al.* (2025).

Pseudosperma Matheny & Esteve-Rav. [= *Inocybe* Sect. *Rimosae* (Fr.) Quél. p.p.] is a genus which comprises overall a total of 133 among species and infraspecific levels worldwide, which can be found in different European ecosystems, according to what is reported on the website Index Fungorum [www.indexfungorum.org/names/names.asp (access carried out on 16 January 2026)]. This genus comprises ectomycorrhizal species with cheilocystidia derived from modified basidia, basidia hyaline and devoid of necropigments, by the absence of pleurocystidia, pileus

fibrillose, often rimose, rarely squamulose, stipe with apex distinctly pruinose, furfuraceous or sometimes floccose, stipe base generally uniform, context not changing colour on rubbing, odour often spermiatic, of green maize or honey, occasionally absent, spores from ellipsoidal to indistinctly phaseoliform (MATHENY *et al.* 2020).

The mycorrhizal biology of the genus *Pseudosperma* is little known beyond those which have been the observations made in the field. However the wide range of habitats in which the species of this genus thrive, such as boreal forests of conifers and broadleaves, coastal dunes and riverbanks (CERVINI *et al.* 2020; BANDINI & OERTEL 2020; SABA *et al.* 2020; BANDINI *et al.* 2021, 2022; MAO *et al.* 2022), suggests that they may establish ectomycorrhizal symbioses with a wide variety of host plants (MATHENY *et al.* 2020). Some species of *Pseudosperma* seem to be specialised, that is engaged in symbiosis with a single host plant or a group of closely related plants (BANDINI & OERTEL 2020; YU *et al.* 2020), but it is presumable that generalist species also exist. Furthermore, as observed by BANDINI & OERTEL (2020), the geographical distribution of most species is little known, leaving ample possibilities to explore the flexibility of this genus.

Over several years the Authors have carried out numerous excursions in different habitats of Sardinia with the aim of studying its fungal species. During these excursions collections of basidiomata were made which the same authors, basing themselves exclusively on macro and micromorphological characters, were not able to classify among the known species of the genus *Pseudosperma*.

In the light of this, it was necessary to carry out in-depth bibliographical research at both European and worldwide level in order to attempt to identify the collected species which are the subject of this study.

In this work *Pseudosperma dominariense*, *P. coloniense* and *P. flagellatum* are proposed as new species on the basis of a detailed morphological examination and molecular phylogenetic analyses, furthermore the main morphological and ecological characters which distinguish them from closely related species will be discussed and we will analyse their evolutionary relationships with the use of a multilocus phylogeny. The internal transcribed spacer (internal transcribed spacer, nrITS) and the large nuclear ribosomal subunit (large nuclear ribosomal subunit, nrLSU) of DNA were sequenced from dried basidiomata.

MATERIALS AND METHODS

Macroscopic and microscopic examination

The study was carried out both on fresh material and on exsiccata of collections of basidiomata found in Sardinia (Italy) and photographed in the field with Canon 450D and Canon 1100D digital cameras.

The preparations were observed with Optika B-383 PLi and Motic BA 400 trinocular microscopes equipped with integrated digital video camera of the same brand for the capture of microscopic images and with Optech B4 binocular microscope without video camera whose microscopic images were acquired with Nikon Coolpix SQ Optec digital camera.

The description of macromorphological characters was carried out on fresh specimens whereas the study of micromorphological characters was carried out on exsiccata using 2% anionic Congo Red and 6% NH_4OH for the preparation of the slides. The exsiccata were previously rehydrated with 5% KOH or with distilled H_2O . The cuticle was observed both in water and in 2% anionic Congo Red.

Spore measurements are the result of a number equal to or greater than 32 measurements obtained from spore deposit, according to what is recommended by PARMASO & PARMASO (1987); spore measurement was carried out on spores belonging to different specimens of the collection.

The data relating to basidiospore dimensions is expressed as (minimum-) mean minus standard deviation – mean – mean plus standard deviation (–maximum) of length × (minimum-) mean minus standard deviation – mean – mean plus standard deviation (–maximum) of width. Qm (= Mean Quotient) is given by the arithmetic mean of the quotients of the single spores; the quotient of a spore (Q) is the ratio length/width. From spore measurements the apiculus was excluded; from basidia measurements the sterigmata were excluded. The data relating to basidia and cystidia dimensions is expressed as (minimum-) mean minus standard deviation – mean plus standard deviation (–maximum); sometimes the value of the mean is also present, which is underlined.

For the description of morphological characters the work of KUYPER (1986) was used, whereas the colour code of RIDGWAY (1912) was used for their definition.

The studied collections were deposited in the Herbaria of the Department of Life and Environmental Sciences, sect. Botany, of the University of Cagliari (CAG) in Italy and that of the University of Alcalá, Alcalá de Henares in Spain (AH). Duplicates of the collections are found in the private herbaria of Alberto Mua (M, Quartu Sant'Elena, Italy) and Massimo Sanna (MS, Cagliari, Italy).

Laboratory procedures and phylogenetic inference

Total DNA was extracted, amplified and sequenced by the companies Alvalab (Oviedo, Spain) and Molecular Solutions LLC (Portland, Oregon, USA) according to their standardised methodologies.

The methodology used by the company Alvalab was that of extraction of total DNA from dried samples according to the standard method using cetyltrimethylammonium bromide (CTAB) (MURRAY & THOMPSON 1980). The nuclear ribosomal internal transcribed spacer (nrITS) of rDNA was amplified and sequenced using the primers ITS-1F, ITS4 and ITS-4B (WHITE *et al.* 1990; GARDES & BRUNS 1993). The nuclear large ribosomal region 28S (nrLSU) of rDNA, instead, was amplified and sequenced using the pair of primers LR0R/LR5 (VILGALYS & HESTER 1990; CUBETA *et al.* 1991). The products obtained from PCR were run on a 1% agarose gel. The chromatograms were checked to detect possible reading errors which were then appropriately corrected manually. Further details on laboratory procedures, including PCR conditions, electrophoresis and purification, are available in ALVARADO E *et al.* (2010, 2021).

Instead, the methodology used by the company Molecular Solutions LLC was that of extracting total DNA from fungal tissue by heating with a Chelex extraction buffer (100 mM Tris with a pH of 8.5, 4% Chelex 100 and 1% Triton X-100) at 99 °C for 20 minutes, then freezing. After thawing, the resulting supernatant was used in PCR. PCR was performed in 25 mL reactions with 1 µL of nrDNA extract, 0.4 mM of each primer, 0.2 mM of the dNTP mixture, 5 µg of bovine serum albumin and 0.5 U of OneTaq Hot Start DNA polymerase (New England Biolabs, Ipswich, MA, USA) in standard OneTaq 1X buffer. PCR conditions were the following: 94 °C for 30 s, followed by 36 cycles of 94 °C for 15 s, 57 °C for 30 s and 68 °C for 60 s, followed by a final extension at 68 °C for 5 min. The primers ITS-1F (GARDEN & BRUNS 1993) and ITS4 (WHITE *et al.* 1990) were used to amplify the ITS1-5.8S-ITS2 region (nrITS). Sequencing was carried out at Eurofins Genomics (Louisville, KY, USA) using the same primers used for PCR. The forward and reverse reads were aligned using the software Geneious 10.2.4 and resolved manually, when necessary.

The nucleotide sequences obtained in this study were initially compared, using the BLASTn algorithm (ALTSCHUL *et al.* 1990) of the website National Center for Biotechnology Information (NCBI), with the sequences present in this database to verify both contamination of the PCR product and to obtain a preliminary taxonomic circumscription at genus level of our samples within the family *Inocybaceae*, then optimising the search of sequences with higher similarity

(MEGABLAST). Subsequently two nucleotide sequence datasets (nrITS and nrLSU) were assembled comparing those available in recent phylogenetic studies on the genus *Pseudosperma* retrieved from the NCBI and *Unite* databases. The final nrITS and nrLSU datasets contained respectively 40 and 13 sequences, including nrITS and nrLSU sequences of *Inocybe oblectabilis* (Britzelm.) Sacc, species used as outgroup. The phylogenetic tree was obtained by creating the Fasta files of the respective datasets containing the nrITS nucleotide sequences and those nrLSU retrieved from the NCBI database, then proceeding to multiple sequence alignment for each marker through the MUSCLE algorithm (EDGAR 2004) of the software MEGA X (KUMAR *et al.* 2018), correcting them manually, following visual inspection and exploratory analysis of the alignment in order to identify taxa with missing data and anomalous divergence patterns, then trimming the ends of the longer sequences in the nrITS alignment which included part of the ribosomal subunits 18S–28S in order to reduce the risks of “noise” in the phylogenetic signal, finally generating a Nexus file as final product of the alignment itself of each analysed dataset. The software MESQUITE version 4.02 (MADDISON *et al.* 2025) was used to perform the concatenation of the datasets of the two markers nrITS and nrLSU then exporting the taxa block produced by the analysis in Phylip and Nexus for Mr. Bayes formats. The concatenated Phylip file was then processed with the program RAXMLGUI 2.0 (EDLER *et al.* 2020) to estimate a multi-locus phylogeny according to the Maximum Likelihood model: the RAXML analysis was conducted using the substitution model GTR+GAMMA for the two delimited partitions (nrITS1+5.8S+nrITS2, nrLSU), 1000 rapid bootstrap pseudoreplications (Rapid Bootstrapping) were implemented to evaluate nodal support, therefore we assumed bootstrap values $\geq 70\%$ as significant for conflicting relationships among the same set of taxa (MASON-GAMER & KELLOGG 1996). Subsequently, we inferred an additional phylogeny in a Bayesian context with the program MrBayes version 3.2.7 (RONQUIST *et al.* 2012) using the concatenated Nexus for Mr Bayes file, which was processed for the calculation of Bayesian Inference. The settings given for the calculation of Bayesian Inference were: 1×10^7 generations, sampling frequency 1000, value of 0.25 for the burn-in setting (therefore the first 25% of the data was discarded as burn-in and the consensus tree based on the 50% majority rule and the corresponding posterior probabilities were calculated from the remaining trees), substitution model GTR+I+ Γ (rate heterogeneity modelled by a gamma distribution with a proportion of invariant sites) and nchain = 4. The nodes of the tree which showed bootstrap support values (BS) equal to or greater than 70% (RAXML analysis) and Bayesian posterior probabilities (PP) equal to or greater than 0.95 (MrBayes analysis) were considered significantly supported. The phylogenetic trees thus obtained were visualised and processed with the program TreeGraph2 v. 2.15.0-887 (STÖVER & MÜLLER 2010).

As outgroup the sequence of the collection of *Inocybe oblectabilis* (Britzelm.) Sacc. JV32005 deposited in GenBank with accession number ON754370 was chosen.

RESULTS

Dataset of molecular sequences

Four nrITS sequences and four nrLSU sequences were generated de novo from the Sardinian collections of the new species presented in this work (**Tab. 1**).

The final nrITS alignment of 40 sequences was 2239 bp long whereas the nrLSU alignment comprised 13 sequences and was 1442 bp long.

The concatenated multi-locus dataset (nrITS1+5.8S+nrITS2, nrLSU) used for the inference of ML and Bayesian phylogenies, composed of 40 sequences, had a total number of 3681 bp, including 638 variable sites of which 299 singleton sites

Table 1. List of the species included in the phylogenetic analysis. The data of the examined collections are provided (species, herbarium number, country, GenBank or UNITE number for the loci nrITS and nrLSU). The sequences in bold were obtained during this study.

Species	Original specimen identification	Voucher/Strain	Country	ITS Accession Number"	LSU Accession Number"
<i>Inocybe oblectabilis</i>	<i>Inocybe oblectabilis</i>	JV32005	Italy	ON754370	ON754370
<i>Pseudosperma bulbosissimum</i>	<i>Inocybe bulbosissima</i>	EL30_06	Sweden	FJ904158	FJ904158
<i>Pseudosperma bulbosissimum</i>	<i>Inocybe bulbosissima</i>	EL6605	Norway	AM882765	AM882765
<i>Pseudosperma calciphilum</i>	<i>Pseudosperma calciphilum</i>	CAG B/5.8.1 holotype	Italy	ON799406	OQ341419
<i>Pseudosperma calciphilum</i>	<i>Pseudosperma calciphilum</i>	CAG 5/3.63	Italy	ON799411	OQ341322
<i>Pseudosperma californicum</i>	<i>Pseudosperma californicum</i>	NY:NY 814859 holotype	USA	OP162981	OP162981
<i>Pseudosperma citrinostipes</i>	<i>Pseudosperma citrinostipes</i>	FHMU3150 holotype	China	MT072898	–
<i>Pseudosperma coloniense</i> sp. nov.	<i>Pseudosperma coloniense</i>	CAG B/5.8.3 holotype	Italy	PX607989	PX607990
<i>Pseudosperma coloniense</i> sp. nov.	<i>Pseudosperma coloniense</i>	GP09M	Italy	PX607986	PX607991
<i>Pseudosperma coloniense</i> sp. nov.	<i>Inocybe fastigiata</i>	AH49047	Spain	PZ019234	PZ017934
<i>Pseudosperma coloniense</i> sp. nov.	<i>Inocybe rimosa</i>	AH00628	Spain	PZ017929	PZ017932
<i>Pseudosperma conviviale</i>	<i>Pseudosperma conviviale</i>	AMB 18243 holotype	Italy	MT095091	MT095115
<i>Pseudosperma conviviale</i>	<i>Inocybe</i> sp.	TUF120558	Estonia	UDB032063 (U)	–
<i>Pseudosperma dominariense</i> sp. nov.	<i>Pseudosperma dominariense</i>	CAG B/5.8.4 holotype	Italy	PX607992	PX607993
<i>Pseudosperma dulcamaroides</i>	<i>Inocybe dulcamaroides</i>	EL112_06	Sweden	FJ904126	FJ904126
<i>Pseudosperma flagellatum</i> sp. nov.	<i>Pseudosperma flagellatum</i>	CAG B/5.8.5 holotype	Italy	PX607994	PX607995
<i>Pseudosperma flavorimosum</i>	<i>Pseudosperma flavorimosum</i>	LAH:35042 holotype	Pakistan	NR169927	–
<i>Pseudosperma gemellum</i>	<i>Pseudosperma</i> sp. JMB- 2022a	XC2002-81 isotype	France	ON129689	–
<i>Pseudosperma gilvum</i>	<i>Pseudosperma</i> sp. NM- 2022c	BJTC FM 1941 holotype	China	OM801910	–
<i>Pseudosperma guttuliferum</i>	<i>Pseudosperma guttuliferum</i>	21581	Italy	JF908233	–
<i>Pseudosperma guttuliferum</i>	<i>Pseudosperma guttuliferum</i>	373	Italy	JF908100	–

Species	Original specimen identification	Voucher/Strain	Country	ITS Accession Number"	LSU Accession Number"
<i>Pseudosperma melleum</i>	<i>Pseudosperma melleum</i>	MCVE:30145 holotype	Italy	NR_171959	MT095114
<i>Pseudosperma melliolens</i>	<i>Inocybe melliolens</i>	G00110921 holotype	France	MN901255	–
<i>Pseudosperma napaeum</i>	<i>Pseudosperma</i> sp. BO- 2020c	SMNS-STU-F-0901463 holotype	Germany	MW010040	–
<i>Pseudosperma obsoletum</i>	<i>Pseudosperma obsoletum</i>	Strain PBM2332	USA	jQ408766	JQ319697
<i>Pseudosperma obsoletum</i>	<i>Inocybe obsoleta</i>	EL1704	Sweden	AM882769	AM882769
<i>Pseudosperma permelliolens</i>	<i>Pseudosperma permelliolens</i>	XC2000-20 isotype	France	ON129680	–
<i>Pseudosperma permelliolens</i>	<i>Pseudosperma permelliolens</i>	XC2006-05	France	ON129682	–
<i>Pseudosperma ponderosum</i>	<i>Pseudosperma ponderosum</i>	MCVE:30144 holotype	Italy	MT095092	MT095116
<i>Pseudosperma rimosum</i>	<i>Inocybe rimosa</i>	PAM03110904	France	FJ904144	FJ904144
<i>Pseudosperma rimosum</i>	<i>Inocybe rimosa</i>	EL7505	Sweden	AM882762	AM882762
<i>Pseudosperma rimosum</i>	<i>Pseudosperma rimosum</i>	"STU:SMNS-STU-F-0901777 ITS variant b epitype"	Germany	OQ324791	OQ324791
<i>Pseudosperma rimosum</i>	<i>Pseudosperma rimosum</i>	"STU:SMNS-STU-F-0901777 ITS variant a Epitype"	Germany	OQ324790	OQ324790
<i>Pseudosperma</i> sp.	<i>Pseudosperma</i> sp.	mushroomobserver.org/482695	USA	ON866523	–
<i>Pseudosperma</i> sp.	<i>Inocybe rimosa</i>	AH30688	Spain	PZ017935	–
<i>Pseudosperma subvolvatum</i>	<i>Inocybe</i> sp.	S448	Estonia	UDB0103230	–
<i>Pseudosperma subvolvatum</i>	<i>Pseudosperma</i> sp.	FQ05 M	Italy	OQ433946	–
<i>Pseudosperma subvolvatum</i>	<i>Pseudosperma</i> sp.	CAG B/5.8.2 holotype	Italy	OR248647	OR248673
<i>Pseudosperma umbrinellum</i>	<i>Inocybe umbrinella</i>	F14488TypeS holotype	"Submitted University of Gothenburg-SWEDEN Collected ITALY"	HM209796	–
<i>Pseudosperma umbrinellum</i>	<i>Pseudosperma umbrinellum</i>	4375	Italy	JF908116	–

Phylogenetic inference

The multi-locus phylogeny produced with RAxML had a Final ML Optimization Likelihood (Final Log-Likelihood) value equal to -10910.434881, whereas the Bayesian analysis produced with MrBayes reached an Average Standard Deviation of Split Frequencies (Average Standard Deviation of Split Frequencies- avgstddev) of 0.005 after 1.245×10^6 generations: the final avgstddev value turned out to be 0.00224, meaning an excellent convergence between the chains. The estimated mean sizes of independent samples (Effective Sample Size - EES) were > 200 for all parameters, with actual values that were generally > 500. Therefore, summarising, the convergence of the MCMC chains was evaluated by means of the Average Standard Deviation of Split Frequencies (<0.01) and the Effective Sample Size, which turned out to be > 200 for all parameters, with values generally > 500 thus demonstrating the reliability of the analysis carried out. Since the phylogenies obtained did not show supported conflicts, the topology inferred according to a Bayesian framework is presented in **Fig. 1**.

The nrITS and nrLSU sequences of the typus of the new species were also compared with some sequences of *Pseudosperma* not yet published whose collections were deposited as *Pseudosperma rimosum* (Bull.) Matheny & Esteve-Rav. and "*Inocybe fastigiata*" (Schaeff.) Quél., kindly shared by Prof. Dr. Fernando Esteve-Raventós, and having herbarium codes AH49047, AH00628 and AH30688; AH is the acronym of the herbarium of the University of Alcalá de Henares (Spain). These collections will be referred to in this study as *Pseudosperma* sp. followed by their herbarium deposit number; their comparison with the three new species presented in the work showed genetic distances in nrITS comprised in the range $0 \div 8.2\%$.

Pseudosperma dominariense forms a well-defined and supported monophyletic clade (BS = 86; PP = 0.96), clearly separated from the collections of the related species *P. subvolvatum* M. Sanna, Mua, Porcu, Marco Casula & A.C. Rinaldi which form a monophyletic clade with supports BS = 67 and PP = 0.95. The stability of the clade supports a consistent phylogenetic isolation, not referable to simple intraspecific variation. The nrITS genetic distance of *P. dominariense* with that of the closest species deposited in the NCBI dataset falls within the range $3.4 \div 3.5\%$, whereas that between the collections of *Pseudosperma* sp. AH00628, AH30688 and AH49047 varies between 8 and 8.2%.

Pseudosperma flagellatum also forms a monophyletic clade pairing with a collection of *Pseudosperma* sp. AH30688 and not nesting within the clade formed by *P. umbrinellum* (Bres.) Matheny & Esteve-Raventós, *P. guttuliferum* (Kühn.) Matheny & Esteve-Raventós and *P. napaeaeum* Bandini & B. Oertel, therefore behaving as an isolated lineage where also the node of the two clades is not strongly but moderately supported (BS = 54; PP = 0.86); the node of the complex "*P. umbrinellum-guttuliferum-napaeaeum*" turns out to be strongly supported (BS = 80; PP = 1) showing it to be formed by a group of species very cohesive among themselves. The clade "*P. flagellatum* – *P. sp.* AH30688", instead, possesses a moderate bootstrap support (BS = 62) but coherent, whereas its posterior probability is high (PP = 0.96) therefore *Pseudosperma* sp. (AH30688) results sister of *P. flagellatum* but whose relationships need further investigations with other future collections. The nrITS genetic distance of *P. flagellatum* with that of the closest species retrieved in the NCBI dataset is comprised in the range 2.7-3.7%, whereas that with *Pseudosperma* sp. AH30688 is 3.1%, with 20 mismatches and 17 gaps, whereas it results to be 3.9% with the collections of *Pseudosperma* sp. AH00628 and AH49047.

Finally, *Pseudosperma coloniense* forms a strongly supported clade (BS = 100; PP = 1) with the collections of *Pseudosperma* sp. AH49047 and AH00628. The monophyletic clade formed by the collections of *P. coloniense* has support BS = 96 and PP = 0.86 which means high topological stability and a probable attempt of recent divergence with respect to the sister taxa. The nrITS genetic distance of *P. coloniense* with the closest species deposited in the NCBI database is comprised in the range $3.9 \div 4.3\%$, whereas that with the collections of *Pseudosperma* sp. AH00628 and AH49047 is 0% this in justification also of the strong support of the clade, whereas that with *Pseudosperma* sp. AH30688 is 4.1%.

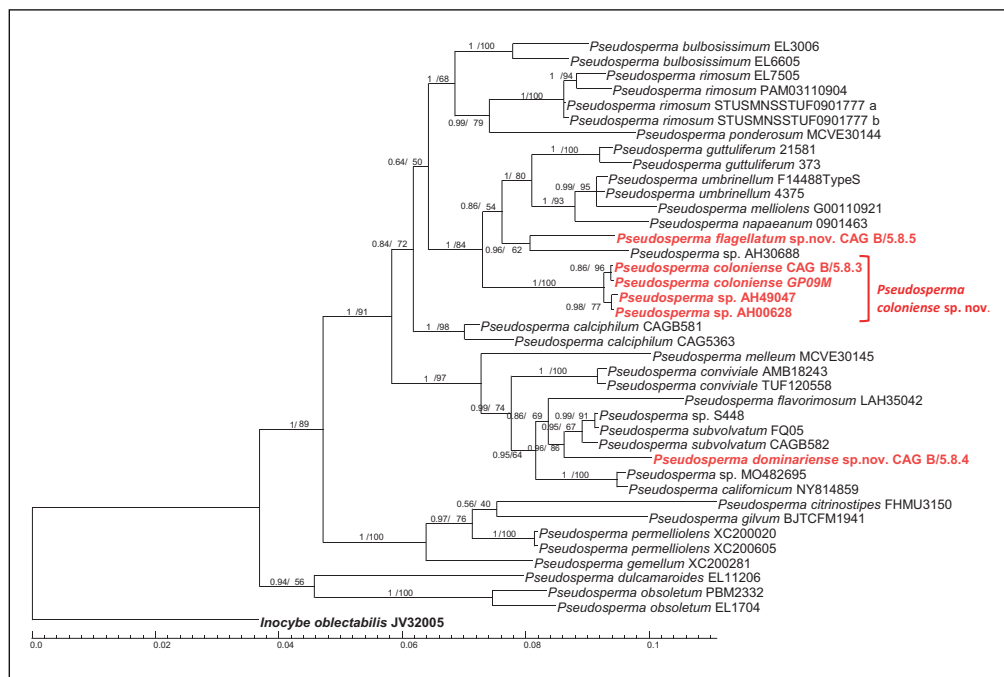


Figure 1. Phylogenetic tree. The phylogram illustrates the evolutionary relationships of the new species of *Pseudosperma*. The topology represented was obtained according to a Bayesian model using MrBayes with nrITS and nrLSU dataset. The values of Posterior Probabilities (PP) and Bootstrap support (BP) are represented on the branches leading to the nodes. *Inocybe oblectabilis* was used as outgroup. The voucher number and the name of the species are indicated in the branch labels. New species are in boldface.

TAXONOMY

Pseudosperma dominariense M. Sanna, A. Mua, M. Melis, M. Casula, A.N.M. Furtado & A.C. Rinaldi sp. nov.

Mycobank number: MB 862723

Original diagnosis

Pseudosperma dominariense is characterized by a pileus with a distinctly acute umbo and an ochre-brown to yellowish-brown cuticle, darker at the disc, with a surface breaking into fibrils and absence of velar remnants. The stipe, whitish at first and later becoming light ochre, is not bulbous and shows pruinosity only at the apex. The spores are smooth, broadly ellipsoidal, occasionally (sub)phaseoliform, measuring (9.3) 10.4–11.4–12 (14) × (5.8) 6.2–7–7.8 (9.2) μm. The cheilocystidia are thin-walled, (sub) cylindrical, claviform, (sub)ventricose or (sub)utriform and they measuring (31) 33–45–53 (58) × (9) 11–15–21 (23) μm. Thin-walled (sub)cylindrical or (sub)claviform caulocystidia are present at the stipe apex. Combined morphological characters and ITS/LSU sequence data genetically distinguish this species from *Pseudosperma subvolvatum*, *P. californicum*, and *P. conviviale*.

Holotypus collected on 23 October 2024 in Italy, Laconi (OR), Loc. "Su Dominariu", in the presence of *Quercus pubescens*, in small groups, leg. Mua A., Sanna M. & Melis M., herbarium CAG B/5.8.4 (holotypus), GenBank: ITS (PX607992), LSU (PX607993)

Etymology the epithet "*dominariense*" refers to the collection location of the holotype "Su Dominariu".

Macroscopic characters

Pileus 2.5-3.5 cm, initially conical, then conico-convex finally plane provided with marked acute umbo, cuticle of ochre-brown colour (Pl. IV, 19, YO-Y, f – Code Ridgway, 1912), brown-yellowish (Pl. IV, 19, YO-Y, d – Code Ridgway, 1912), darker at the disc (Pl. XXIX, 15'', Y-O, k – Code Ridgway, 1912), surface initially compact then broken into fibrils, absence of velar remnants. Margin jagged fissured also deeply, initially involute then plane, also revolute.

Lamellae rather crowded, emarginate or adnate, moderately crowded, ochre then brownish, initially whitish then whitish-greyish (Pl. LI, 23''''', f – Code Ridgway, 1912), then grey-ochre (Pl. XLIV, 21''''', O-YY, b – Code Ridgway, 1912) finally brownish (Pl. XXX, 19'', YO-Y, k – Code Ridgway, 1912) slightly shaded with olive tone, with lamellulae not numerous; edge eroded, white.

Stipe 4-8 × 0.4-0.7 cm, slender, cylindrical, slightly enlarged at the base, not bulbous, surface of whitish colour then light ochre (Pl. XXX, 23'', i, Light Y- Code Ridgway, 1912), sometimes with brown-ochraceous patches (Pl. XV, 17' O-Y, l – Code Ridgway, 1912) in its basal part, pruinose character present in the apical part, slight fibrillose character present along its entire height.

Context fibrous, whitish. Odour slightly spermatic mixed with rancid oil. Taste acidulous.

Microscopic characters

Spores smooth, brown, broadly ellipsoidal, ellipsoidal, also (sub)phaseoliform, obtuse apex, with central guttule, (9.3) 10.4-11.4-12 (14) × (5.8) 6.2-7-7.8 (9.2) μm, Qm = 1.62, n = 105.

Basidia tetrasporic, 31-39 × 11-13 μm, claviform, provided with basal clamp connections.

Cheilocystidia thin-walled, (sub)cylindrical, claviform, (sub)ventricose, (sub)utriform, rounded apex, some provided with 1 septum, (31) 33-45-53 (58) × (9) 11-15-21 (23) μm.



Pseudosperma dominariense. CAG B/5.8.4 (holotypus).

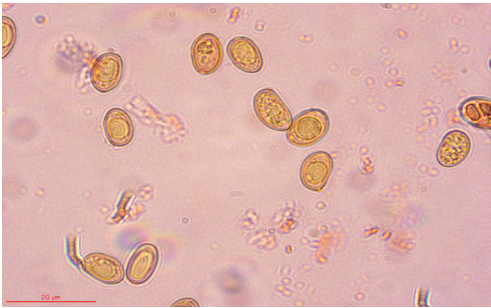
Photo by Massimo Sanna

Caulocystidia thin-walled, (sub)cylindrical, (sub)claviform, rounded apex, 44-54-94 (95) × 10-12-20 (21) µm, only present at the apex, presence of septate piliform elements, 4-6 µm wide, with rounded terminal element.

Suprapellis cutis formed by hyphae 4-10 µm wide provided with encrusting and parietal pigment of brown colour and presence of cytoplasmic pigment of yellow-brownish colour especially in the deeper layers.

Clamp connections present.

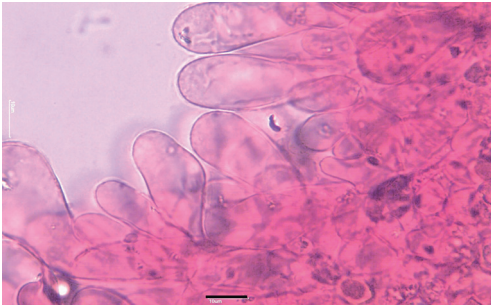
Studied collections 23 Oct 2024, Italy, Laconi (OR), Loc. "Su Dominariu", with presence of *Quercus pubescens* Willd., in small groups, leg. Mua A., Sanna M. & Melis M., herbarium CAG B/5.8.4 (holotypus), GenBank ITS (PX607992), LSU (PX607993); 23 Oct 2024, Laconi (OR), Loc. "Su Dominariu", with presence of *Quercus pubescens*, leg. Mua A., Sanna M. & Melis M., 1940MS.



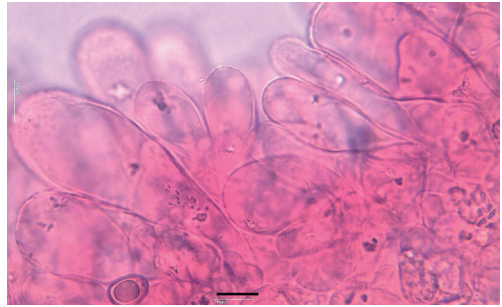
P. dominariense. CAG B/5.8.4 (holotypus). Spores in Congo red (1000 ×). Bar 20 µm. Foto by Mario Melis



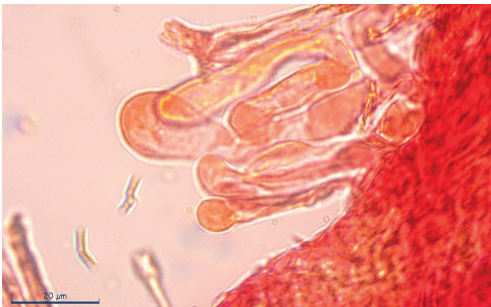
P. dominariense. CAG B/5.8.4 (holotypus). Basidia in Congo red (1000 ×). Bar 10 µm. Photo by Massimo Sanna



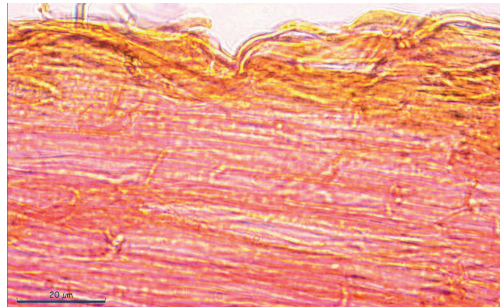
P. dominariense. CAG B/5.8.4 (holotypus). Cheilocystidia in Congo red (1000 ×). Bar 10 µm. Photo by Massimo Sanna



P. dominariense. CAG B/5.8.4 (holotypus). Cheilocystidia in Congo red (1000 ×). Bar 10 µm. Foto by Massimo Sanna



P. dominariense. CAG B/5.8.4 (holotypus). Caulocystidia (1000 ×) in Congo red. Bar 20 µm. Photo by Mario Melis



P. dominariense. CAG B/5.8.4 (holotypus). Pileipellis in Congo red (1000 ×). Bar 20 µm. Photo by Mario Melis

Pseudosperma flagellatum M. Sanna, A. Mua, M. Casula, M. Melis, A.N.M. Furtado & A.C. Rinaldi sp. nov.

Mycobank number: MB 862725

Original diagnosis

Pseudosperma flagellatum is characterized by a pileus that is initially bell-shaped and later plano-convex, with a prominent, well-defined central umbo. The cuticle is brown to yellowish-brown, initially compact and soon becoming strongly rimose, with ephemeral cortiniform velar remnants. The stipe is not bulbous and has a surface covered with whitish longitudinal fibrils. The spores are smooth, broadly ellipsoidal, also (sub)phaseoliform, measuring (7.4) 9.2-10.4-12.5 (13.7) $\times$ (5.4) 5.9-6.9-7.6 (8.4) μm . The cheilocystidia are thin-walled, (sub)claviform, claviform, (sub)cylindrical, rarely subventricose or (sub)utriform, measuring (22) 28-43-44 (47) $\times$ (7) 8.5-12-16 (17) μm . The caulocystidia are (sub)cylindrical or (sub)claviform. Combined morphological characters and ITS/LSU sequence data genetically distinguish this species from *Pseudosperma umbrinellum* and *P. coloniense*.

Holotypus collected on 20 Oct 2022 in Italy, Laconi (OR), Loc. "Su Dominariu", Ippodromo area, in the presence of *Quercus ilex* L., leg. Mua A., Casula M., Sanna M. & Costantini S., herbarium CAG B/5.8.5 (holotypus), GenBank: ITS (PX607994), LSU (PX607995)

Etymology with the cuticle that has the appearance of a surface that has undergone whipping.

Macroscopic characters

Pileus 2.3-5 cm, initially campanulate then conical, then plane with evident well pronounced central umbo. Cuticle of brown colour (Pl. IV, 19 Yo-Y, k – Code Ridgway, 1912), brown-yellowish (Pl. IV, 19 YO-Y, m – Code Ridgway, 1912), initially compact, soon strongly rimose whit diverging fibres showing the whitish context beneath except at the disc where it remains compact. Margin involute then turned downwards, with ephemeral whitish cortiniform velar remnants.

Lamellae rather crowded, adnate or emarginate, with few lamellulae, initially whitish-greyish (Pl. LI, 23''', f – Code Ridgway, 1912), then of light ochre colour (Pl. XXX, 23'', I – Code Ridgway, 1912) finally dark ochre (Pl. XXX, 19'', YO-Y, k – Code Ridgway, 1912), with slight olive shading. Edge slightly crenulate, white.

Stipe 3.5-6.5 $\times$ 0.3-0.8 cm, cylindrical, enlarged in the basal part but not bulbous, surface initially whitish then of ochraceous colour (Pl. XXIX, 17'' O-Y – Code Ridgway, 1912), traversed by longitudinal whitish fibrils and striate, with slight whitish velar remnant at the base of the stipe.

Context fibrous, whitish. Odour spermatic with slight urinary note. Taste acidulous.

Microscopic characters

Spores smooth, brown, broadly ellipsoidal, ellipsoidal, also (sub)phaseoliform, obtuse apex, with central guttule, (7.4) 9.2-10.4-12.5 (13.7) $\times$ (5.4) 5.9-6.9-7.6 (8.4) μm , Qm = 1.59, n = 64

Basidia claviform, tetrasporic, some bisporic, 25.5-36.5 $\times$ 9.3-11.5 μm .

Cheilocystidia (sub)claviform, claviform, (sub)cylindrical, rarely (sub)ventricose or (sub)utriform, thin-walled, rounded apex, basal clamp connections, (22) 28-43-44 (47) $\times$ (7.3) 8.5-12-16 (17) μm , some with 1 septum, some (sub)cylindrical or cylindrical paracheilocystidia present.

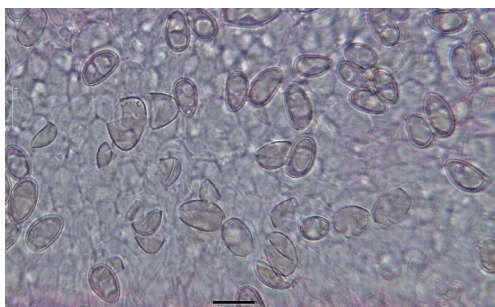
Caulocystidia at the apex of the stipe, thin-walled, (sub)cylindrical, (sub)claviform, (43) 45-61-86 (90) $\times$ (5) 6-8-11 μm , sometimes provided with 1-2 septa, some rare capitate terminal element, pyriform caulocystidioid elements are present.

Suprapellis cutis of hyphae with presence of clamp connections, in some parts the cutis tends to become slightly confused, hyphae 3-8 μm wide, slight encrusting and parietal pigment of brown colour present, cytoplasmic pigment of yellowish colour present especially in the lower layer of the epicutis and in the mediopellis.



Pseudosperma flagellatum. CAG B/5.8.5 (holotypus).

Photo by Massimo Sanna



P. flagellatum. CAG B/5.8.5 (holotypus). Spores in Congo red (1000 ×). Bar 10 μm.

Photo by Massimo Sanna



P. flagellatum. CAG B/5.8.5 (holotypus). Basidia in Congo red (1000 ×). Bar 10 μm.

Photo by Massimo Sanna



P. flagellatum. CAG B/5.8.5 (holotypus). Cheilocystidia in NH_4OH (1000 ×). Bar 10 μm.

Photo by Massimo Sanna

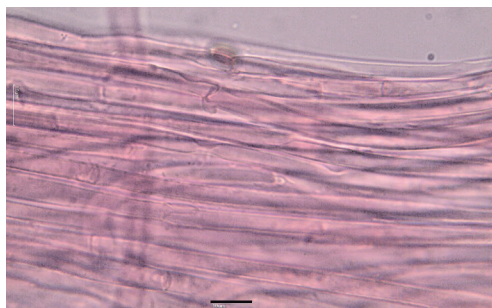


P. flagellatum. CAG B/5.8.5 (holotypus). Cheilocystidia in NH_4OH (1000 ×). Bar 10 μm.

Photo by Massimo Sanna



P. flagellatum. CAG B/5.8.5 (holotypus). Caulocystidia in Congo red (1000 ×). Bar 10 μm.
Photo by Massimo Sanna



P. flagellatum. CAG B/5.8.5 (holotypus). Pileipellis in Congo red (1000 ×). Bar 10 μm.
Photo by Massimo Sanna

Clamp connections present.

Studied collections 20 Oct 2022, Italy, Laconi (OR), Loc. "Su Dominariu", zona Ippodromo, in the presence of *Quercus ilex*, leg. Mua A., Casula M., Sanna M. & Costantini S., herbarium CAG B/5.8.5 (holotypus), GenBank: ITS (PX607994), LSU (PX607995).

Pseudosperma coloniense A. Mua, M. Sanna, M. Melis, M. Casula, A.N.M. Furtado & A.C. Rinaldi sp. nov.

MycoBank number: MB 862726

Original diagnosis

Pseudosperma coloniense is characterized by a pileus that is initially conical, becoming plano-convex, with a broad central umbo. The cuticle is ochraceous brownish to brownish, distinctly rimose at the periphery, and apparently lacking a velipellis. The stipe is cylindrical, whitish, and floccose at the apex. The spores measure (7.7) 8.8–9.6–10.8 (12.5) × (5.6) 5.8–6.3–6.8 (8.7) μm and are ellipsoidal to oblong, smooth. The cheilocystidia measuring (26) 32–52 (53) × (8) 10–15 (18) μm, they are abundant and arranged in tufts and are claviform, fusiform, or cylindrical-claviform, thin-walled. Caulocystidia are frequent at the stipe apex and arranged in tufts. Combined morphological characters and ITS/LSU sequence data genetically distinguish this species from *Pseudosperma umbrinellum*, *P. guttuliferum*, and *P. flagellatum*.

Holotypus collected on 11 Oct 2024 in Italy, Dolianova (CA), Loc. "Sa Colonia", leg. A. Mua, in the presence of *Quercus ilex*, herbarium CAG B/5.8.3 (holotypus), GenBank: ITS (PX607989), LSU (PX607990).

Etymology the epithet "*coloniense*" refers to the collection location of the holotype "Sa Colonia".

Macroscopic characters

Pileus 3–6 cm, initially conical then plane-convex, with broad central umbo. Cuticle dry, ochraceous brownish colour (Pl. XVI, 21'', YO-Y, k – Code Ridgway, 1912) to brownish (Pl. XL, 17''O-Y, I – Code Ridgway) darker and undissociated at the centre, distinctly rimose in the periphery, velipellis apparently absent. Margin initially slightly involute then extended, sometimes revolute, often fissured.

Lamellae colour initially whitish, pale grey in youth (Pl. LI, 23''''', f – Code Ridgway, 1912), then progressively ochre (Pl. XVI, 19', YO-Y, k – Code Ridgway), rather crowded, adnate or emarginate, ventricose at maturity, lamellulae not very numerous. Edge crenulate, white.

Stipe 4–7 × 0.7–1.3 cm, cylindrical, often slightly sinuous, traversed by longitudinal whitish fibrils, floccose above, whitish (Pl. LIII, 10 – Code Ridgway, 1912), with scarce velar remnants in youth.

Context rather thick at the centre of the pileus, whitish. Odour not spermiatic, honeyed-oily, with note of burnt oil. Taste mild.



Pseudosperma coloniense. CAG B/5.8.3 (holotypus).

Photo by Alberto Mua

Microscopic characters

Spores (7.7) 8.8-9.6-10.8 (12.5) × (5.6) 5.8-6.3-6.8 (8.7) μm , $Q = (1.2) 1.4-1.7 (2.1)$, $Q_m = 1.5$, $n = 90$, from ellipsoidal to oblong, smooth, yellow-brownish, with large central guttule.

Basidia (22.5) 25.7-33.3 (36.6) × (8.8) 9.5 - 11 (12) μm , claviform, tetrasporic.

Cheilocystidia (26) 32-52 (53) × (8) 10-15 (18) μm , abundant, gathered in tufts, claviform, fusiform, cylindro-claviform, septate, thin-walled.

Caulocystidia frequent at the apex of the stipe, gathered in tufts, (32) 33-57 (73) × (7) 8-13 μm , cylindro-claviform, cylindrical, cystidioid hairs are present, 24-59 × 2-9 μm , cylindrical, some capitate.

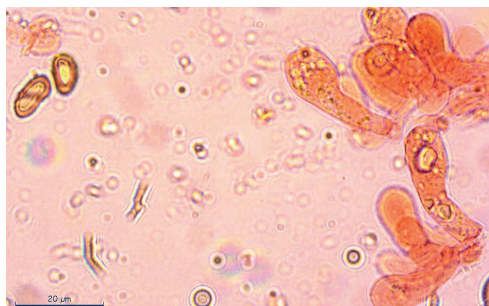
Suprapellis of cutis type, with rare raised terminal elements, hyphae 3.5-6.5 μm wide, finely encrusting parietal and intracellular pigment of yellow-brownish colour, especially in the deeper layers.

Clamp connections observed in all elements.

Studied collections 11 Oct 2024, Italy, Dolianova (CA), Loc. "Sa Colonia", leg. A. Mua, in the presence of *Quercus ilex*, herbarium CAG B/5.8.3 (holotypus), GenBank: ITS (PX607989), LSU (PX607990); 11 Oct 2024, Italy, Dolianova, Loc. "Sa Colonia", leg. A. Mua, in the presence of *Quercus ilex*, GP09M, GenBank: ITS (PX607986), LSU (PX607991); 01 Dec 1975, Spain, Community of Madrid, El Escorial, Alto de la Cruz Verde, with *Pinus pinaster* Aiton, leg. M.T. Saenz, J.J. Salcedo & A. Egaña, AH00628, GenBank: ITS (PZ017929), LSU (PZ017932); 20 Oct 2015, Spain, Catalonia, Sant Feliu de Codina, Monastery of Sant Miquel del Fai, mixed forest of *Quercus ilex* with *Q. pubescens* on slightly basic soil, leg. G. Moreno & E. Marcote, AH 49047, GenBank: ITS (PZ019234), LSU (PZ017934)



P. coloniense. CAG B/5.8.3 (holotypus). Spores in Congo red (1000 ×). Bar 10 μm.
Photo by Mario Melis



P. coloniense. CAG B/5.8.3 (holotypus). Basidia in Congo red (1000 ×). Bar 10 μm.
Photo by Mario Melis



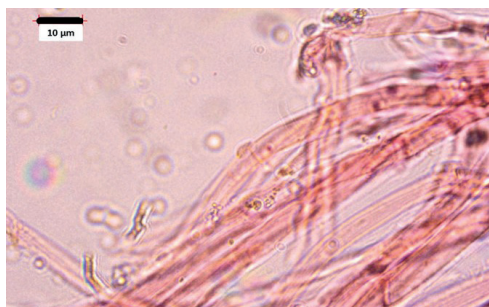
P. coloniense. CAG B/5.8.3 (holotypus). Caulocystidia in Congo red (1000 ×). Bar 10 μm.
Photo by Mario Melis



P. coloniense. CAG B/5.8.3 (holotypus). Cheilocystidia in Congo red (1000 ×). Bar 10 μm.
Photo by Mario Melis



P. coloniense. Collection 11 Oct 2024. Caulocystidia in Congo red (1000 ×). Bar 10 μm.
Photo by Mario Melis



P. coloniense. Collection 11 Oct 2024. Pileipellis in Congo red (1000 ×). Bar 10 μm.
Photo by Mario Melis

Discussion

Pseudosperma dominariense is a small-medium sized species already well characterised macroscopically by the absence of velar remnants on the pileus, fibrillose cuticle, of ochre-brown to brown-yellowish colour, darker at the disc, with marked acute umbo, stipe slightly enlarged at the base but not bulbous, ochre-brownish lamellae with slight olive shading in adult specimens, context with spermatic odour mixed with a note of rancid oil. Microscopically it is characterised by the presence of leiospores from ellipsoidal to (sub)phaseoliform, thin-walled cheilocystidia, with different morphologies, from claviform to (sub)utriform, some septate, caulocystidia present at the apex of the stipe, mostly (sub)clavate, growth under *Quercus pubescens*.

In the phylogenetic tree, elaborated with the results obtained from the Maximum Likelihood (Maximum Likelihood- ML) and Bayesian Posterior Probability (Bayesian Posterior Probability-

BPP) analysis, *P. dominariense* is positioned on a clade together with *P. flavorimosum* Jabeen & Khalid, *P. subvolvatum* Sanna, Mua, Porcu, Casula & A.C. Rinaldi and *P. californicum* (Murrill) Beker & U. Eberh.

The result of the comparative nucleotide ITS analysis of the species carried out on BLAST of the GenBank site (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) provided the result of 96.6% similarity with sequence OR248647 belonging to the holotypus of *P. subvolvatum* (CAG B/5.8.2) (synonymous of *Pseudosperma schweitzingense* Kleine, F. Hampe & M. Scholler).

Pseudosperma flavorimosum Jabeen & Khalid, Pakistani species, is distinguishable from *P. dominariense* by the prominent umbo, lamellae initially yellow-brown then dark brown, stipe also dark-brown, smaller spores, (7.8) 9.5-12.8 (13.31) $\times$ (5.7) 6.3-7.8 (8.9) μm , smaller basidia, (14.8) 26.4-31.2 (36.7) $\times$ (10) 10.5-10.8 (11.8) μm , smaller cheilocystidia, (18.5) 19.2-21.7 (25.3) $\times$ (7.5) 7.7-8.6 (9.1) μm , pileipellis a non-encrusted trichoderma (JABEEN & KHALID, 2020).

Pseudosperma subvolvatum M. Sanna, Mua, Porcu, Marc. Casula & A.C. Rinaldi (synonymous of *Pseudosperma schweitzingense* Kleine, F. Hampe & M. Scholler), recently published, is a medium-sized species, with more compact cuticle, lamellae initially whitish-greyish then brown-olivaceous, longer stipe, with basal velar remnants forming a pseudo-volva, complex odour, different, with herbaceous component, of potato peel mixed with rancid oil and only slight spermatoc note, slightly longer spores, (9.5) 10.4-11.4-12.4 (15) $\times$ (5.5) 6.1-6.7-7.2 (8) μm , smaller cheilocystidia, (25.7) 32.8-38.2-42.7 (62.7) $\times$ (9.2) 11.6-16-19.6 (24.9) μm , slightly gelatinised cutis, growth under *Populus alba* L. and *Q. ilex* (DONG *et al.* 2025).

Pseudosperma californicum (Murrill) Beker & U. Eberh., Californian species found under *Quercus* sp., recently recombined in *Pseudosperma* from *Hebeloma*, possesses spores from cylindrical to ellipsoidal, on average longer, 11-14 $\times$ 6-7.5 μm , sometimes with constrictions and clavate cheilocystidia (EBERHARDT *et al.* 2023).

Pseudosperma umbrinellum (Bres.) Matheny & Esteve-Rav. [= *P. melliolens* (Kühner) Matheny & Esteve-Rav. (2019)] has pilei dark or lurid gray and a cinnamon dark centre, gills from white to argillaceous with white edges, sub-bulbous and white stipe with a brownish base, 30-50 $\times$ 4-6 mm, fibrillose-pruinose with furfuraceous, sub-fiocose apex, veil white cortiniform, odor less, white flesh, spores reniform, 10-14 $\times$ 5.5-6.5 μm , cheilocystidia clavate, sometimes clavate or subfusiform 40-65 $\times$ 10-16 μm , under *Populus nigra* L. (BRESADOLA 1905). In the descriptions made by KÜHNER (1988) and CERVINI *et al.* (2020) as *P. melliolens*, the species would have a slenderer habit, bulbous stipe bases, gills stained with yellow-brown, persistent smell of honey, smaller basidia, 24-38 $\times$ 12-14 μm , slightly smaller spores, (9) 9.9-12.2 (13.5) $\times$ (6) 6.3-7.2 (8) μm , mainly clavate and smaller cheilocystidia, 20-55 $\times$ 8-19 μm , [32-55 $\times$ 8 - 16 μm , on average 39 $\times$ 12 μm , Qm = 3,4 by CERVINI *et al.* (2020)].

Pseudosperma melleum Cervini, Bizio & P. Alvarado, also a Mediterranean species, shows a pileus with ochraceous-orange colours, a much slender stipe up to 10 cm high, a distinctive strong honey-like smell, smaller spores (average 10.6 $\times$ 6 μm , Qm = 1.8), and smaller cheilocystidia (31-42-54 $\times$ 10-13-17 μm . (CERVINI *et al.* 2020).

Pseudosperma permelliolens (Carteret & Reumaux) Matheny & Esteve-Rav. has strong honeyed or virous honeyed smell, spores on average longer and narrower, (11) 11.5-15.5 (16) $\times$ 6-8 μm , Q = 1.8-1.9, cheilocystidia cylindrical or slightly utriform, longer on average, 25-75 (95) $\times$ (6) 10-20 (25) μm , growing under *Fagus sylvatica* L., *Quercus* sp., *Picea abies* (L.) H. Karst., *Larix decidua* Mill., *Betula* sp., *Pinus* sp. (CARTERET & REUMAUX 2015).

Pseudosperma rimosum (Bull.) Matheny & Esteve-Rav. has a bigger size, straw-yellow, tawny or ochre-brown pileus, brown gills with a distinct olive tinge, whitish flesh, straw-coloured on the stipe, spermatoc smell (at least when cut), smaller spores on average, 9-10.8-13 $\times$ 5.4-6.7-7.7 μm , (sub)cylindrical, (sub)phaseoliform or (sub)amygdaliform, longer cheilocystidia, 28-47-70 $\times$ 9-12-18 μm , from (sub)cylindrical to (sub)claviform. (BANDINI *et al.* 2024).

Pseudosperma flagellatum is a small-medium sized species, conical pileus with pronounced umbo, cuticle of brown, brown-yellowish colour, initially compact then rimose except at the disc where it remains compact, on the margin only ephemeral cortiniform remnants, lamellae initially whitish-greyish then dark ochre with slight olive shading, stipe enlarged in the basal part but not bulbous, with slight whitish velar remnant, context with spermatic odour, spores from broadly ellipsoidal to (sub)phaseoliform, thin-walled cheilocystidia, from (sub)cylindrical to claviform, with rounded apex, caulocystidia are present in the apex of the stipe, from (sub)cylindrical to (sub)claviform, some with capitate apex, with pyriform caulocystidioid elements, suprapellis in cutis tending to become confused in some parts and presence of brown encrusting and parietal pigment as well as cytoplasmic pigment of yellow colour.

The result of the comparative nucleotide ITS analysis of the species carried out on BLAST of the GenBank site provided the result of 97.3% similarity with sequence FJ904165 belonging to a collection of *P. umbrinellum* (JV13699) and of 97% with sequence HM209796 of another collection (F14488TypeS) which results to be the holotypus of the same species.

Pseudosperma flagellatum is found on a clade with *Pseudosperma* sp. AH30688, GenBank: ITS (PZ017935)

Basidiomes of *Pseudosperma* sp. AH30688, present context with strong honey odour, slightly larger spores, $10\text{--}14.5 \times 6.5\text{--}8 \mu\text{m}$, smaller cheilocystidia, sub-cylindrical, sub-claviform, sub-utriform, sometimes capitate, also provided with 1-2 septa, $35\text{--}40 \times 11.5\text{--}20 \mu\text{m}$, caulocystidia present in the upper third, cylindrical, sub-cylindrical, also septate, $10 \mu\text{m}$ wide, found in mixed forest of *Pinus halepensis* Mill. and *Quercus ilex*, calcareous soil according to the data provided by Prof. F. Esteve-Raventós (2026).

Pseudosperma guttuliferum (Kühner) Matheny & Esteve-Rav. is an alpine species distinguishable by lamellae of colour +/- olivaceous, inodorous context, slightly larger cheilocystidia, $(27) 34\text{--}46 (55) \times (10) 11\text{--}16 (22) \mu\text{m}$, guttulate in NH_4OH , slightly longer and wider spores, $(10) 11.7\text{--}12.7 (14.5) \times 6.7\text{--}8.7 \mu\text{m}$, growth under *Salix retusa* L. and *S. reticulata* L. (KÜHNER 1988); according to FERRARI (2006) the stipe of this species would be conspicuously bulbous and the cheilocystidia with refractive yellow content in NH_4OH .

Pseudosperma umbrinellum (Bres.) Matheny & Esteve-Rav., often presents an acute umbo, context with distinct spermatic odour, narrower spores, $9\text{--}14 \times 5\text{--}7 \mu\text{m}$, on average $11 \times 6 \mu\text{m}$ and cheilocystidia constricted towards the upper extremity (HEIM 1931).

Pseudosperma napaeum Bandini & B. Oertel, recently published species found in Germany under *Pinus mugo* Turra, *Dryas octopetala* L., *Helianthemum nummularium* (L.) Mill., presents smaller pileus, 1-2.5 cm, lamellae from yellow to yellow-ochre, spores on average wider, $9.1\text{--}14.1 \times 5.9\text{--}9.3 \mu\text{m}$, on average $10.6 \times 7.2 \mu\text{m}$, $Q_m = 1.5$, sub-amygdaliform, slightly shorter basidia, $24\text{--}35 \times 7\text{--}11 \mu\text{m}$, often sub-capitate cheilocystidia of larger dimensions, $23\text{--}60 \times 7\text{--}17 \mu\text{m}$, on average $37 \times 10 \mu\text{m}$, often sub-capitate or papillate caulocystidia (BANDINI & OERTEL 2020).

Pseudosperma ponderosum Cervini, Bizio & P. Alvarado is recognisable by its stocky, massive shape, by its initially copper-brown and not yellowish colouring, as well as by a more abundant cortina, often woolly-floccose in young specimens, by the lamellae and cuticle which frequently tend to become yellow-spotted, complex smell, by the cylindrical-clavate cheilocystidia, relatively narrow and short (CERVINI, BIZIO & ALVARADO 2020).

Pseudosperma conviviale Cervini, Bizio & P. Alvarado originally described from Sardinia does not show a velipellis on the pileus, which is yellowish-ochraceous, the stipe is slender $50\text{--}110 \times 8\text{--}12\text{mm}$, and the smell is honey-like, reminiscent of that of *Inosperma cookie* (Bres.) Matheny & Esteve-Rav., it also shows bigger spores ($11.7 \times 7.2 \mu\text{m}$ on average) and cheilocystidia with dimensions $30\text{--}73 \times 11\text{--}23 \mu\text{m}$ (CERVINI *et al.* 2020).

Pseudosperma rimosum (Bull.) Matheny & Esteve-Rav. has a bigger size, straw-yellow, tawny or ochre-brown pileus, brown gills with a distinct olive tinge, whitish flesh, straw-coloured on

the stipe, slightly longer spores, 9-10.8-13 × 5.4-6.7-7.7 µm, (sub)cylindrical, (sub)phaseoliform or (sub)amygdaliform, longer cheilocystidia, 28-47-70 × 9-12-18 µm, from (sub)cylindrical to (sub)claviform. (BANDINI *et al.* 2024).

Pseudosperma coloniense, third new species presented in this work, is a small-medium sized species, with plane-convex pileus which presents a broad central umbo, cuticle ochraceous brownish to brownish colour, undissociated at the disc and rimose in the peripheral part, apparently lacking a velipellis, without pileic remnants, ochraceous lamellae in adult specimens and in which olive shading is absent, cylindrical stipe, often sinuous, whitish and with scarce velar remnants, context with honeyed-oily odour, cheilocystidia gathered in tufts of claviform, cylindro-claviform or fusiform shape, caulocystidia of cylindrical or cylindro-claviform shape, found under *Quercus ilex*, *Q. pubescens* and *Pinus pinaster*.

The result of the comparative nucleotide ITS analysis of the species carried out on BLAST of the GenBank site provided the result of 96.3% similarity with sequence FJ904165 belonging to a collection of *P. umbrinellum* (JV13699), of 95.7% with sequence HM209796 of another collection (F14488TypeS) which results to be the holotypus of the same species.

Pseudosperma coloniense is found in a well-supported clade (PP = 1; BS = 100) with the collection of *Pseudosperma* sp. AH49047 and that of *Pseudosperma* sp. AH00628.

Different from *P. coloniense*, *P. umbrinellum* often presents an acute umbo, context with distinct spermatoc odour, slightly larger spores, 9-14 × 5-7 µm, on average 11 × 6 µm and cheilocystidia constricted towards the upper extremity (HEIM 1931).

Pseudosperma napaeum Bandini & B. Oertel, species found in Germany under *Pinus mugo*, *Dryas octopetala*, *Helianthemum nummularium*, presents smaller pileus, 1-2.5 cm, lamellae from yellow to yellow-ochre, spores on average longer and wider, 9.1-14.1 × 5.9-9.3 µm, on average 10.6 × 7.2 µm, Qm = 1.5, sub-amygdaliform, slightly narrower basidia, 24-35 × 7-11 µm, often sub-capitate cheilocystidia of slightly larger dimension, 23- 60 × 7-17 µm, on average 37 × 10 µm, often sub-capitate or papillate caulocystidia (BANDINI & OERTEL 2020).

Pseudosperma calciphilum Sanna, Mua, Porcu, Casula, A.C. Rinaldi, Mifsud & Garrido-Ben, recently found in Sardinia, presents a low umbo and evident velar remnants on the pileus, lamellae of beige colour in adult specimens, slightly more robust stipe, with beige-chamois shading and also spotted with rust-brown at the base, context with honeyed-oily odour with herbaceous and spermatoc notes, larger spores, (9) 10.76-12.55-14.34 (17.62) × (5.91) 6.7-7.39-8.09 (9.91) µm with Qm = 1.70, larger basidia, 30.5-44 × 10-15 µm, larger cheilocystidia, 26-71 × 10-23 µm, on average 48.4 × 16 µm, of cylindro-claviform, claviform but also pyriform shape, caulocystidia of larger dimensions and also wider, 45-78-110 × 11-16.9-21 µm, present also in the median third, slightly gelatinised pileipellis (SANNA *et al.* 2024)

Pseudosperma flagellatum, new species presented here, has cuticle of brown, brown-yellowish colour, strongly rimose, ephemeral velar remnants on pileic margin, light ochre lamellae with slight olive shading in mature specimens, stipe initially whitish then of ochraceous colour, larger spores, (7.4) 9.2-10.4-12.5 (13.7) × (5.4) 5.9-6.9-7.6 (8.4) µm, shorter cheilocystidia (22) 28-43-44 (47) × (7) 8.5-12-16 (17) µm, also (sub)ventricose or (sub)utriform, slightly longer caulocystidia, (43) 45-61-86 (90) × (5) 6-8-11 µm, with presence of pyriform caulocystidioid elements.

Pseudosperma guttuliferum (Kühner) Matheny & Esteve-Rav. is an alpine species distinguishable by lamellae of colour +/- olivaceous, inodorous context, slightly wider cheilocystidia, (27) 34-46 (55) × (10) 11-16 (22) µm, guttulate in NH₄OH, larger spores, (10) 11.7-12.7 (14.5) × 6.7-8.7 µm, growth under *Salix retusa* and *S. reticulata* (KÜHNER 1988); according to FERRARI (2006) the stipe of this species would be conspicuously bulbous and the cheilocystidia with refractive yellow content in NH₄OH.

Pseudosperma melleum Cervini, Bizio & P. Alvarado, also a Mediterranean species, shows a pileus with ochraceous-orange colours, a much slender stipe up to 10 cm high, a distinctive

strong honey-like smell, longer spores (average $10.6 \times 6 \mu\text{m}$, $Q_m = 1.8$), and slightly smaller cheilocystidia $31\text{--}42\text{--}54 \times 10\text{--}13\text{--}17 \mu\text{m}$. (CERVINI *et al.* 2020).

Pseudosperma rimosum (Bull.) Matheny & Esteve-Rav. has a bigger size, straw-yellow, tawny or ochre-brown pileus, surface initially smooth, later strongly fibrous, brown gills with a distinct olive tinge, whitish flesh, straw-coloured on the stipe, spermatic smell (at least when cut), larger spores, $9\text{--}10.8\text{--}13 \times 5.4\text{--}6.7\text{--}7.7 \mu\text{m}$, (sub)cylindrical, (sub)phaseoliform or (sub)amygdaliform, longer cheilocystidia, $28\text{--}47\text{--}70 \times 9\text{--}12\text{--}18 \mu\text{m}$, from (sub)cylindrical to (sub)claviform, sometimes septate. (BANDINI *et al.* 2024).

Conclusions

The systematics of the genus *Pseudosperma* continues to be redefined. This work describes three new species, enlarging the number of taxa of the genus and providing information on the distribution and evolutionary history of the genus. The approach that integrates morpho-taxonomic characters with phylogenetic hypotheses supports the distinction of the new taxa from those already known to science.

This study demonstrates how important it is to study the diversity of *Pseudosperma*, in particular in environments which have been under-sampled, such as Mediterranean ecosystems.

In conclusion, the authors consider that the new species here presented, for their macro and micromorphological characters and for the differences between the morphologically and phylogenetically closest species, are to be considered new to science and to be included in the genus *Pseudosperma* Matheny & Esteve-Rav.

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Sitography

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