



Research papers

Historical use of grasses in South Indian palm-leaf manuscript production reconstructed from phytolith analysis[☆]

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ABSTRACT

We collected thirty fallen off fragments of palm-leaf manuscripts (PLM), from four manuscript collections. Aiming to identify grasses used in the 18-19 centuries for PLM preparation, we analysed these samples for grass silica short cell phytoliths (GSSCP). Due to the complex, diverse phytolith sources and limited literature, we first examined reference grass material from herbaria collected in South India and SE Asia with comparable climates. Material from thirty grass species across six subfamilies was studied. In total, 36 GSSCP phytolith morphotypes were described; subfamily-specific assemblages were identified. All individual morphotypes were analysed for positive and negative co-occurrence patterns, which were then used in the interpretation of the results of phytolith analysis of the PLM samples. We revealed 28 GSSCPs in PLM samples. Preservation of the phytolith material varied from sufficient to low. Many cells demonstrated abnormal morphology, and some GSSCPs were forced into the surface of the palm leaves, mechanically broken, half-brushed off and eroded, providing evidence of active mechanical forces applied to the surface of PLMs during their production. In PLM samples provenancing from Kerala, *Arundo donax*, *Phragmites* sp., Bambuseae, and Chloridoideae (most likely *Desmostachya bipinnata*) were revealed. In PLM samples from Tamil Nadu, *Phragmites* sp., domestic rice (possibly with admixture of Arundinoideae and/or Chloridoideae), and Panicoideae material were evidenced. Evidence of *Aristida* sp. is most likely attributed to historical contamination. The main difference in the PLM samples reflects the amount of preserved material historically accumulated on the folio surfaces as well as the original provenance of the PLMs.

1. Introduction

Grasses (Poaceae) are among the most widely studied plant families in terms of phytolith research, due to the abundance and distinctiveness of their phytoliths (Strömberg et al., 2018; ICPT 2.0, 2019; Rashid et al., 2019; Wang et al., 2022; Cordova, 2023). The high silica content of Poaceae leads to the formation of robust and easily identifiable grasses-specific phytoliths, which can be preserved in archaeological, historical, and sedimentary contexts for millennia (Strömberg et al., 2018; Rashid

et al., 2019; Wang et al., 2022). These phytoliths have been extensively characterized at the subfamily, tribe and not rarely at the genus levels, offering a high degree of resolution for plant identification. In particular, as Cordova discussed in their introduction (2023), grass silica short cell phytoliths (GSSCP) have distinct morphologies enough to relate it to the source plants subfamilies and the environment conditions such plants are adapted to (Fredlund and Tieszen, 1994; Fredlund and Tieszen, 1997; Alexandre et al., 1997; Krishnan et al., 2000; Lu and Liu, 2003; Strömberg, 2004; Piperno, 2006a; Biswas et al., 2016; Naskar and Bera, 2018a; Naskar et al., 1918). As a result, grass phytoliths provide an

Abbreviations: Cent, century; DCA, detrended correspondence analysis; EFEO, École Française D'extrême-Orient in Puducherry, India; GSSCP, grass silica short cell phytoliths; h, Hour(s); H₂O₂, hydrogen peroxide; HCl, hydrochloric acid; IFP, French Institute of Puducherry, India; P, probability; PCA, principal component analysis; PLM, palm-leaf manuscript; S, South; SE, Southeast; SEM, Scanning Electron Microscopy; SUB Hamburg, State and University Library (Carl von Ossietzky Staats- und Universitätsbibliothek Hamburg), University of Hamburg, Germany; TPS, total phytolith sum; VRC / VMB, Thrissur, Kerala Manuscript Preservation Centre and the Vedic Research Centre (VRC), Vadakke Madham Brahmaswam (VMB) in Thrissur, India.

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Nomenclature

The phytolith morphology and terminology is based on the International Code for Phytolith Nomenclature (ICPN, Neumann et al., 2019). Plant taxonomy is followed the Angiosperm Phylogeny Group (APG 2016).

invaluable source of data for reconstructing past environments, climates, agricultural practices, and cultural uses of plant materials (e.g. Twiss et al., 1969; Terrell and Wergin, 1981; Piperno, 2006b).

Grasses are particularly important in the study of human-environment interactions and the area-specific historical plant usage, as they have played significant roles in both subsistence practices and material culture across diverse cultures worldwide. Due to their widespread presence, abundance in almost all terrestrial ecosystems and availability, grasses are used excessively. Phytoliths from grasses can reveal detailed information about domesticated and wild species utilized by humans (e.g. Yost and Blinnikov, 2011; Zhao et al., 1998; Gu et al., 2013, 2016a; Huan et al., 2015; Tao et al., 2019; Wang et al., 2022), enabling insights into the development of agricultural systems, and cultural uses of plant materials (e.g. Radomski and Neumann, 2011; Piperno, 2006b, 2009). One example of the latter is the grass application for brushing, polishing, smoothing, fire fuel and ritual materials during the complex, multi-step palm-leaf manuscripts (PLM) production, which entails practices that are specific to individual manufacturers, historical times and geographic areas. PLMs have been central to South Indian literary and religious traditions, yet the specifics of their production - particularly the materials and techniques employed - remain largely unknown, leaving a gap in our understanding of the botanical resources utilized. As the practice of PLMs production has almost completely ceased nowadays and that it has never been properly documented, the retrospective reconstruction based on the palaeoecological methods remains the only reliable way to reveal its features and peculiarities. Historical accounts and publications (mainly gray and non-academic, though) provide relatively large and diverse but inconsistent, rather subjective and controversial data that are difficult to verify (Poliakova et al., 2026). Among the available information, data on the plants and plant products historically used in the preparation of PLMs is remarkably scant, including the preparation step that consists of brushing and polishing the leaves prior to the text inscription (Sah, 2002; Meher, 2009; Alahakoon, 2012).

To the best of our knowledge, these practices as well as the PLMs themselves, were never an object of the botanical, palaeoecological or palaeoethnobotanical studies. Phytolith analysis offers a viable method for investigating such historical practices (Poliakova et al., 2025, 2026; Poliakova and Ciotti 2026). To start contributing this novel, broad and scientifically perspective research, this study aims to reveal historical use of grasses in the preparation of South Indian PLMs through phytolith analysis. During the preparation process, close contact of palm leaves with the non-palm plant material leaves material traces and micro-fragments of “exotic” (i.e. non-palm) material on the surface of PLMs. By examining phytoliths from the surfaces of PLMs and comparing them with a reference collection derived from plants mentioned in religious and ritual literature, we seek to identify the grass subfamilies and, possibly, the specific taxa employed in the PLM production. This approach will not only shed light on the materials used but also contributes to our general understanding of the cultural, historical, ecological and ethnobotanical contexts of PLM production.

2. Material and methods

2.1. Reference grass material

Thirty samples from historical herbarium collections in South India and SE Asia, representing six grass subfamilies, were studied as the reference material: Arundinoideae (2 species), Bambusoideae (3), Chloridoideae (4), Pooideae (1, *Hordeum vulgare* L.), Oryzoae (3), and Panicoideae (17). Table 1 presents the list of species chosen for this study. The selection was based on a preliminary review of the literature that mentions any use of grasses related to PLM production and traditional conservation. As this literature is scarce and controversial (Poliakova et al., 2026), and in the absence of reliable sources describing the historical use of plants (including grasses) for PLM production, we also used Hindu texts to gather data on grasses important for religious and cultural practices. Furthermore, we considered that in some regions of India PLM production has had a ritualistic value (Taylor, 2012; De Simini, 2016). We assumed that religious and other traditional spiritual practices are the most conservative and, therefore, should be the most similar to historical ones. Taking into account that multiple environmental factors may influence phytolith production in grasses (Rosen and Weiner, 1994; Madella et al., 2009; Jie et al., 2010; Jenkins et al., 2011; Liu et al., 2013; Dey et al., 2015; Li et al., 2016; Bourel and Novello, 2020; Biswas et al., 2021, 2024), we used reference materials from areas with the same or comparable climate and geography as the regions where our research samples were collected (see Table 2 and Fig. 1). A complete list of the Poaceae samples analysed for phytoliths is provided in the Supplementary Material S1.

When it comes to the identification of phytolith morphologies from the research material, namely, phytoliths described from the surface of the PLM micro-fragments (see Table 2), we combined traditional approaches based on visual comparison with regional reference collections (e.g. Twiss et al., 1969; Piperno, 1988; Mulholland and Rapp, 1992; Gallego and Distel, 2004; Neumann et al., 2019) and quantitative approaches, especially for unclear and doubtful cases, using 2D outlines (Liu et al., 2016; Cai and Ge, 2017) and 3D outlines (Gallaher et al., 2019). The identification of phytolith subtypes within each category of GSSCP is based on a range of descriptive shape characteristics of both two-dimensional and three-dimensional structures, as detailed in Boyd et al. (2024).

In each case, a registered phytolith was aimed to be assigned to a grass subfamily as in Pearsall (1978), Piperno (1984), Alexandre et al. (1997), Zhijun (1998), Harvey and Fuller (2005), Piperno et al. (2009), Ball et al. (2016). When preservation allowed, genera were recognized (e.g. as in Brown, 1984; Twiss, 1992; Mulholland and Rapp, 1992; Piperno and Pearsall, 1998; Zhao et al., 1998; Piperno and Sues, 2005; Piperno, 2006a; Yost and Blinnikov, 2011; Gu et al., 2013, 2016a; Rudall et al., 2014; Huan et al., 2015; Tao et al., 2019; Wang et al., 2022; Cordova, 2023). If the phytolith morphology suggests a certain identification but does not look exactly like reference plant material, phytolith morphotypes are marked with “cf.”

2.2. Palm-leaf manuscript samples

Microsamples (i.e. fallen off fragments of 2–5 mm²) of the thirty palm-leaf manuscripts were taken from the collections of the French Institute of Puducherry, India (IFP, 5 *Borassus flabellifer* manuscripts), École Française d'Extrême-Orient in Puducherry, India (EFEO, 5 *Borassus flabellifer* manuscripts), Kerala Manuscript Preservation Centre and the Vedic Research Centre (VRC), Vadakke Madham Brahmaswam (VMB), in Thrissur, Kerala, India (10 *Borassus flabellifer* manuscripts), and from the State and University Library (Carl von Ossietzky Staats-

Table 1

List of Poaceae analysed for phytoliths and mentioned in the literature on the PLM production and preservation, traditional use of grasses, the Hindu texts and/or grasses important for religious practices

NN	Plant name	Poaceae subfamily	Literature on PLM production and/or traditional preservation	Religious texts and practices, ethnobotanical literature
1	<i>Arundo donax</i> L.	Arundinoideae	NA	(!) Boddupalli, 2024
2	<i>Bambusa arundinacea</i> (Retz.) Willd.	Bambusoideae	NA	(!) Boddupalli, 2024
3	^a Bambusoidea (unspecified)	Bambusoideae	Harinarayana, 1995; Subramaniam, 1995; Sah, 2002; Meher, 2009; Perumal, 2021; Wilson and Rice, 2019	Amirthalingam, 2013
4	<i>Chrysopogon zizanioides</i> (L.) Nash	Panicoideae	Sah, 2006	NA
5	<i>Coix lacrymajobi</i> L.	Panicoideae	NA	Lata et al., 2022; (!) Boddupalli, 2024
6	<i>Cymbopogon citratus</i> (DC.) Stapf	Panicoideae	Agrawal, 1984; Ghosh, 2012; Harinarayana, 1995; Joshi, 1995; Subramaniam, 1995; Sah, 2002; Nishanthi and Wijayasundara, 2022; Padmakumar and Sreekumar, 2003; Sahoo and Mohanty, 2004; Sahoo and Mohanty, 2007; Kumar et al., 2009; Perumal, 2013; Wilson and Rice, 2019	NA
7	<i>Cymbopogon nardus</i> (L.) Rendle	Panicoideae	Agrawal, 1984; Geethecharya, 1995; Joshi, 1995; Prajapati, 1995; Sheth, 1995; Subramaniam, 1995; Vijayan, 1995; Sah, 2002; Padmakumar and Sreekumar, 2003; Sahoo and Mohanty, 2004; Sahoo and Mohanty, 2007; Sah, 2006; Kumar et al., 2009; Ghosh, 2012; Perumal, 2013; Mahilrajana et al., 2014; Sharma, 2015; Aye, 2018; Aye et al., 2020	NA
8	<i>Cynodon dactylon</i> (L.) Pers.	Chloridoideae	NA	Majupuria, 2009; Nanditha, 2014; Namboothiri, 2019;

Table 1 (continued)

NN	Plant name	Poaceae subfamily	Literature on PLM production and/or traditional preservation	Religious texts and practices, ethnobotanical literature
9	<i>Dendrocalamus strictus</i> Nees.	Bambusoideae	NA	Mahdihassan, 1987; Kavitha et al., 2019; Nithya and Thangaraju, 2022; Lata et al., 2022; (!) Boddupalli, 2024
10	<i>Desmostachya bipinnata</i> (L.) Stapf	Chloridoideae	NA	Kavitha et al., 2019; Prakash, 2024; Nanditha, 2014; Roshen, 2014; Mahdihassan, 1987; (!) Boddupalli, 2024
11	<i>Echinochloa crus-galli</i> (L.) P. Beauv.	Panicoideae	NA	(!) Boddupalli, 2024 (mentioned as <i>Echinochloa frumentacea</i>)
12	<i>Eleusine indica</i> (L.) Gaertn.	Chloridoideae	NA	(!) Boddupalli, 2024
13	<i>Eragrostis eragrostis</i> (L.) Wolf	Chloridoideae	NA	(?) Prakash, 2024
14	<i>Imperata cylindrica</i> (L.) P. Beauv.	Panicoideae	NA	Mahdihassan, 1987
15	<i>Hordeum vulgare</i> L.	Pooideae	NA	Lata et al., 2022; (!) Boddupalli, 2024
16	^a <i>Oryza sativa</i> L.	Oryzeae	Alahakoon, 2012; Chakravarti, 1897; Gunawardana, 1997; Sah, 2002; Padmakumar and Sreekumar, 2003; Panda, 2006; Meher, 2009; Wilson and Rice, 2019	Kavitha et al., 2019; Lata et al., 2022; (!) Boddupalli, 2024
17	^a <i>Oryza rufipogon</i> Griff.	Oryzeae	NA	(!) Boddupalli, 2024 (misspelled as <i>Oryza rufipogan</i>)
18	<i>Panicum dichotomiflorum</i> Michx.	Panicoideae	Joshi, 1995; Sahoo and Mohanty, 2004, 2007	
19	<i>Panicum miliaceum</i> L.	Panicoideae	NA	(?) Nithya and Thangaraju, 2022; (!) Boddupalli, 2024
20	^a <i>Phragmites australis</i> (Cav.) Trin. ex Steud.	Arundinoideae	Mahaparata, 1995; Sheth, 1995; Van Dyke, 2009; Wilson and Rice, 2019	NA
21	<i>Polytoca gigantea</i> (J.Koenig) Mabb.	Panicoideae	NA	(!) Boddupalli, 2024 (mentioned as <i>Chionachne gigantea</i>)
22	<i>Setaria italica</i> (L.) P.Beauv.	Panicoideae	NA	(!) Boddupalli, 2024
23	^a <i>Saccharum officinarum</i> L.	Panicoideae	NA	Balkrishna et al., 2019; Lata et al., 2022; (!) Boddupalli, 2024
24	^a <i>Saccharum spontaneum</i> L.	Panicoideae	NA	(!) Boddupalli, 2024

(continued on next page)

Table 1 (continued)

NN	Plant name	Poaceae subfamily	Literature on PLM production and/or traditional preservation	Religious texts and practices, ethnobotanical literature
25	<i>Triplidium bengalense</i> (Retz.) H.Scholz	Panicoideae	NA	(!) Boddupalli, 2024 (mentioned as <i>Saccharum benghalense</i>)
26	<i>Thysanolaena latifolia</i> (Roxb. ex Hornem.) Honda	Panicoideae	NA	Lata et al., 2022
27	<i>Triticum</i> sp./ <i>Triticum aestivum</i> L.	Panicoideae	starch of wheat, wheat flour paste as ink binders, 'maida paste' (Meher, 2009), wheat flour paste as ink binder (Prajapati, 1995)	(?) Nithya and Thangaraju, 2022 (!) Boddupalli, 2024
28	<i>Vetiveria zizanioides</i> (L.) Nash	Panicoideae	Sah, 2006	(!) Boddupalli, 2024
29	^b <i>Zea mays</i> L.	Panicoideae	Meher, 2009	NA
30	^{a,c} <i>Zizania</i> sp.	Oryzaeae	NA	NA

^a Evidenced by phytoliths on the surface of the PLMs in the previous study (Poliakova et al., 2025).

^b Only starch as ink binder is mentioned in literature.

^c Region-specific evidences: found only on the surface of the PLMs from Burma/Myanmar, (?) stays when it was not clear which exact plant is meant in the cited source, (!) stays when the exact scientific name (or its synonym) is directly mention in the cited source, NA stays if no reliable reference found.

und Universitätsbibliothek), University of Hamburg, Germany (SUB Hamburg, 5 *Borassus flabellifer* manuscripts and 5 *Corypha umbraculifera* manuscripts). Palm species used for manuscript preparation were identified microscopically. Manuscript sampling was performed as described in Poliakova et al. (2025). The origin of the PLMs was established on paleogeographical grounds. Each cultural subregion of India, in our case Kerala and Tamil Nadu more specifically, has historically adopted one or more characterizing scripts that makes establishing the broad geographical provenance of each manuscript rather certain. Furthermore, all manuscripts included in this study contain further information about the place (e.g. village name) and/or time of production, which is provided by the scribe in rather short statements called “colophons”. Time wise, all manuscripts date back to the 18/19 century. Due to environmental and storage conditions, it is extremely rare that older PLMs may have survived till now (Ciotti and Franceschini, 2016).

2.3. Phytolith analysis

Reference grasses material as well as research PLM samples were dried at 50–60 °C using a hot-air oven and then ashed at 550 °C for 3 h in a muffle furnace, followed by a 12-h cooling period. Phytolith extraction was performed as described in D’Agostini et al. (2022) and Poliakova et al. (2025) using 10% hydrochloric acid (HCl) for 30 min in *quantum satis* and 10–15 ml of 10% hydrogen peroxide (H₂O₂) for 3–5 h. A tablet of *Lycopodium clavatum* (number of spores 20,848 ± 1546, batch numbers 1031 and 113, serie 78, Stockmarr, 1971) was added to each sample at the first step of chemical treatment in order to estimate the phytolith concentration (amount of phytoliths per milliliter of studied material). No vortexing was applied, possibly to reduce additional disturbance and mechanically induced degradation of the material. To avoid potential cross-contamination, reference grass material and manuscript samples were processed separately on different days. In both cases, new and sterile laboratory glassware was used. At the final step, samples were sieved through a 150 µm mesh. Greater tissue fragments

were analyzed separately under the microscope or stereoscope. Due to the low initial grass phytolith concentration in the PLM material, each residue was sieved over a nylon 10-µm filter to remove smaller unidentifiable fractions and concentrate research material, if needed, this step was repeated several times. Ashing and all wet laboratory preparations were partly performed at the Department of Palynology and Climate Dynamics, University of Göttingen, and partly at the Institute of Plant Sciences and Microbiology (IPM), University of Hamburg, Germany.

Final residues were kept in the fridge (5 °C) in distilled water, slides for light microscopy were prepared with sterile liquid glycerin, since this allows rotation of the micro-objects and ensures better investigation of phytolith morphology. Permanent slides of the research material were prepared with Canadian balsam. Microslides were examined under a light microscope at magnifications of x400, x600, and x1000. To separate phytoliths with ambiguous appearance and random mineral particles, polarized light microscopy was applied. A minimum of 100 grass phytoliths were counted per sample. We did not study silica sand in this research. Photo-documentation was performed using Leica and Zeiss Axiostar Plus microscopes at the Department of Palynology and Climate Dynamics (University of Göttingen) and at the Thünen Institute of Wood Research (Hamburg-Bergedorf).

2.4. Phytoliths morphology and identification

In this paper, we focus on the grass silica short-cell phytoliths (GSSCP) as the most typical and easily recognized morphotypes found exclusively within Poaceae and which allow unequivocal diagnostics of the grass material presence. These include BILOBATE (Fig. 2: A–F), POLYLOBATE (see Fig. 2: G–I), CRENATE (Fig. 2: J–L), CROSS (Fig. 3: A–F), SADDLE (including ‘long saddles’ sensu Kondo et al., 1994 and ‘SADDLE-like’ as well as ‘PLATEAUED SADDLE’ phytoliths of *Phragmites* sp. as described by Piperno and Pearsall, 1998; Fig. 3: G–L), RONDEL (including equidimensional saddle (see Fig. 4: A–J), and TRAPEZOID (Fig. 4: K–L) as it was formally established by Neumann et al. (2019) and by Dai et al. (2023). GSSCPs were sub-categorized in detail following Naskar and Bera (2018b, see also literature within) and Boyd et al. (2024).

In addition, attention was paid to the BULLIFORM phytolith morphotypes (Fig. 4: M) as suggested by Chen et al. (2020). Non-diagnostic phytolith morphotypes of Poaceae (mainly ELONGATE PSILATE, ELONGATE DENTATE, ELONGATE DENDRITIC, cf. ELONGATE BULBOUS, ELONGATE SINUATE, ACUTE BULBOSUS, BLOCKY, cf. PSILATE PAPILLAR and ANNULAR/HELICAL TRACHEARY) known for all Poaceae regardless their taxonomy and geographical origin (e.g. Strömberg, 2004; Cordova, 2013; Biswas et al., 2016; Naskar et al., 2018; Neumann et al., 2019) as well as their identifiable fragments, were counted separately as ‘OTHERS’ and were not included in the statistical and verification analyses.

Microphotographs and microslides are kept at the Department of Palynology and Climate Dynamics, University of Göttingen, and are available on request from the first author. Plant taxonomy of the phytolith source material followed the guidance of the Angiosperm Phylogeny Group (AGP, 2016). Scanning Electron Microscopy (SEM) was applied in order to perform proper identification of the phytoliths registered in the reference grass material and in PLM samples. A small part of grass tissue, herbarium specimen, or manuscript fragment was cut with an extra-sharp razor blade and placed on the SEM specimen stubs with conductive and adhesive carbon tabs. In the case of liquid ash samples, a drop of material was placed with a clean pipette on the specimen stubs. After drying and sticking the dried powder on the stub, the samples were coated with gold (SEM coating system SC 510, BIO-Rad). For imaging, signals from the back-scattered (BSD) and secondary electron detector (SED) of the field emission electron microscope were combined. Samples were examined in high vacuum using the field emission scanning electron microscope Hitachi S520 at the Johann Heinrich von Thünen Institute for Wood Research, Hamburg-Bergedorf.

Table 2

List of PLM samples analysed for Poaceae phytoliths from the manuscript surfaces

Sample name	Manuscript code	Palm leaves used as writing support	Manuscript collection	Provisional age and origin of the manuscripts	Sampling date
RE1	RE 04137	<i>Borassus flabellifer</i>	IFP, Puducherry	19 cent. / Karisathan viladage or Karisastha Temple in Tamil Nadu	28.03.2025
RE2	RE 10718	<i>Borassus flabellifer</i>	IFP, Puducherry	19cent. / Anniyur village, Tamil Nadu	04.04.2025
RE3	RE 10726	<i>Borassus flabellifer</i>	IFP, Puducherry	19 cent. / Kilaiyur, Tamil Nadu	04.04.2025
RE4	RE 10822	<i>Borassus flabellifer</i>	IFP, Puducherry	19 cent. / Vedaranyam, Tamil Nadu	04.04.2025
RE5	RE 10829	<i>Borassus flabellifer</i>	IFP, Puducherry	19 cent. / Thiruvavur, Tamil Nadu	03.04.2025
EO1	EO 0102	<i>Borassus flabellifer</i>	EFEO, Puducherry	19 cent. / Alwarthirunagari in Valutivalanatu region, Tamil Nadu	02.07.2024
EO2	EO 0137	<i>Borassus flabellifer</i>	EFEO, Puducherry	19 cent. / Alwarthirunagari in Valutivalanatu region, Tamil Nadu	27.06.2024
EO3	EO 0143	<i>Borassus flabellifer</i>	EFEO, Puducherry	19 cent. / Alwarthirunagari in Valutivalanatu region, Tamil Nadu	28.06.2024
EO-4	EO 0442	<i>Borassus flabellifer</i>	EFEO, Puducherry	19 cent. / Alwarthirunagari in Valutivalanatu region, Tamil Nadu	02.07.2024
EO-5	EO 0541	<i>Borassus flabellifer</i>	EFEO, Puducherry	19 cent. / Alwarthirunagari in Valutivalanatu region, Tamil Nadu	30.06.2024
TR1	EAP 1304 B 0005	<i>Borassus flabellifer</i>	VRC / VMB, Thrissur	18-19 cent. / Thrissur, Kerala	04.07.2024
TR2	EAP 1304 B 0720	<i>Borassus flabellifer</i>	VRC / VMB, Thrissur	18-19 cent. / Thrissur, Kerala	04.07.2024
TR3	EAP 1304 B 0088	<i>Borassus flabellifer</i>	VRC / VMB, Thrissur	18-19 cent. / Thrissur, Kerala	04.07.2024
TR4	EAP 1301 B 0013	<i>Borassus flabellifer</i>	VRC / VMB, Thrissur	18-19 cent. / Thrissur, Kerala	04.07.2024
TR5	EAP 1304 B 0735	<i>Borassus flabellifer</i>	VRC / VMB, Thrissur	18-19 cent. / Thrissur, Kerala	04.07.2024
AL1	DKA 005 TVP 00012	<i>Borassus flabellifer</i>	VRC / VMB, Thrissur	18-19 cent. / Aluva, Kerala	04.07.2024
AL2	DKA 005 TVP 00010	<i>Borassus flabellifer</i>	VRC / VMB, Thrissur	18-19 cent. / Aluva, Kerala	03.07.2024
AL3	DKA 005 TVP 00001	<i>Borassus flabellifer</i>	VRC / VMB, Thrissur	18-19 cent. / Aluva, Kerala	03.07.2024
AL4	DKA 005 TVP 00008	<i>Borassus flabellifer</i>	VRC / VMB, Thrissur	18-19 cent. / Aluva, Kerala	03.07.2024
AL5	DKA 005 TVP 00003	<i>Borassus flabellifer</i>	VRC / VMB, Thrissur	18-19 cent. / Aluva, Kerala	03.07.2024
BM2	Cod. Palmb. II 209 (35.3209)	<i>Borassus flabellifer</i>	SUB Hamburg	1885 / Tamil Nadu, India	08.04.2023
BM3	Cod. Palmb. I 33 (35.3033)	<i>Borassus flabellifer</i>	SUB Hamburg	1802 / Tamil Nadu, India	19.04.2023
BM4	Cod. Palmb. I 24 (35.3024)	<i>Borassus flabellifer</i>	SUB Hamburg	19 cent. / Tamil Nadu, India	26.04.2023
BM5	Cod. Palmb. I 170 (35.3170)	<i>Borassus flabellifer</i>	SUB Hamburg	19 cent. / Tamil Nadu, India	26.04.2023
BM6	Cod. Palmb. I 51 (35.3051)	<i>Borassus flabellifer</i>	SUB Hamburg	1840 / Tamil Nadu, India	26.04.2023
CM13	Cod. Palmb. 35.3194	<i>Corypha umbraculifera</i>	SUB Hamburg	18–19 cent. / Tamil Nadu, India	13.09.2023
CM14	Cod. Palmb. 35.3192	<i>Corypha umbraculifera</i>	SUB Hamburg	18–19 cent. / Tamil Nadu, India	13.09.2023
CM15	Cod. Palmb. 35.3249	<i>Corypha umbraculifera</i>	SUB Hamburg	18–19 cent. / Tamil Nadu, India	15.09.2023
CM16	Cod. Palmb. 35.3041	<i>Corypha umbraculifera</i>	SUB Hamburg	18–19 cent. / Tamil Nadu, India	15-18.09.2023
CM17	Cod. Palmb. 35.3044	<i>Corypha umbraculifera</i>	SUB Hamburg	18–19 cent. / Tamil Nadu, India	20.09.2023

Notes: IFP – French Institute of Puducherry (India), EFEO – École Française D'extrême-Orient in Puducherry (India), VRC / VMB, Thrissur - Kerala Manuscript Preservation Centre and the Vedic Research Centre (VRC), Vadakke Madham Brahmaswam (VMB) in Thrissur, India, SUB Hamburg - State and University Library (Carl von Ossietzky Staats- und Universitätsbibliothek Hamburg), University of Hamburg, Germany. Cent. stays for century.

2.5. Phytolith diagrams

Phytolith diagrams were prepared with TILIA/TILIA.GRAPH software (Grimm, 2004). For the research material, groups of samples with statistically similar phytolith assemblages were represented as local zones in the diagrams and were established based on a number-constrained cluster analysis by sum of squares implemented with CONISS for TILIA (Grimm, 1987). For prior studies of the reference grass material, the same clustering methods were used to check if the phytolith composition from the reference plants' tissues reflects the taxonomical origin of these plants. In both cases - the reference and the research PLM material - the stability of the classification and the sharpness of the clusters (probability, P , $0 < P \leq 1$) were tested using

bootstrap resampling performed in MULTIV (Pillar and Orlóci, 1996; Pillar, 1999). Phytolith diagrams compare the phytolith assemblages from the analyzed samples and present relative abundances (percentages) of individual phytolith types, which were calculated based on the total phytolith sum (TPS), excluding malformed, eroded, or largely broken phytoliths (i.e. indeterminate ones). The sums of the phytoliths from different grass subfamilies are presented, as well as the phytolith concentrations. Phytolith diagrams for the reference and research material are given in Figs. 5 and 8, respectively. The full phytolith diagram for the reference grass material is provided in the Supplementary Material S2. Raw phytolith counts for both the reference grass material and the research PLM material are provided in Supplementary Materials S2 and S3, respectively.

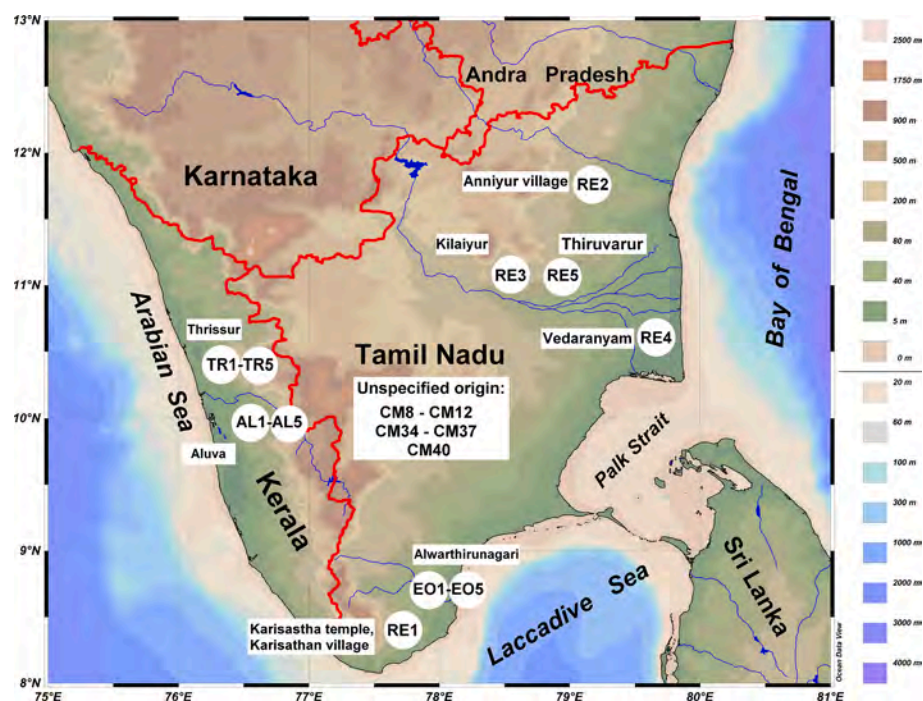


Fig. 1. Research material origin. Mapping is based on the metadata obtained from the manuscript collections and associated with the codes of the samples PLMs. Map is created using QGIS (2009) and the Ocean Data View software and a base ground map (Schlitzer, 2015), available from: <http://odv.awi.de>.

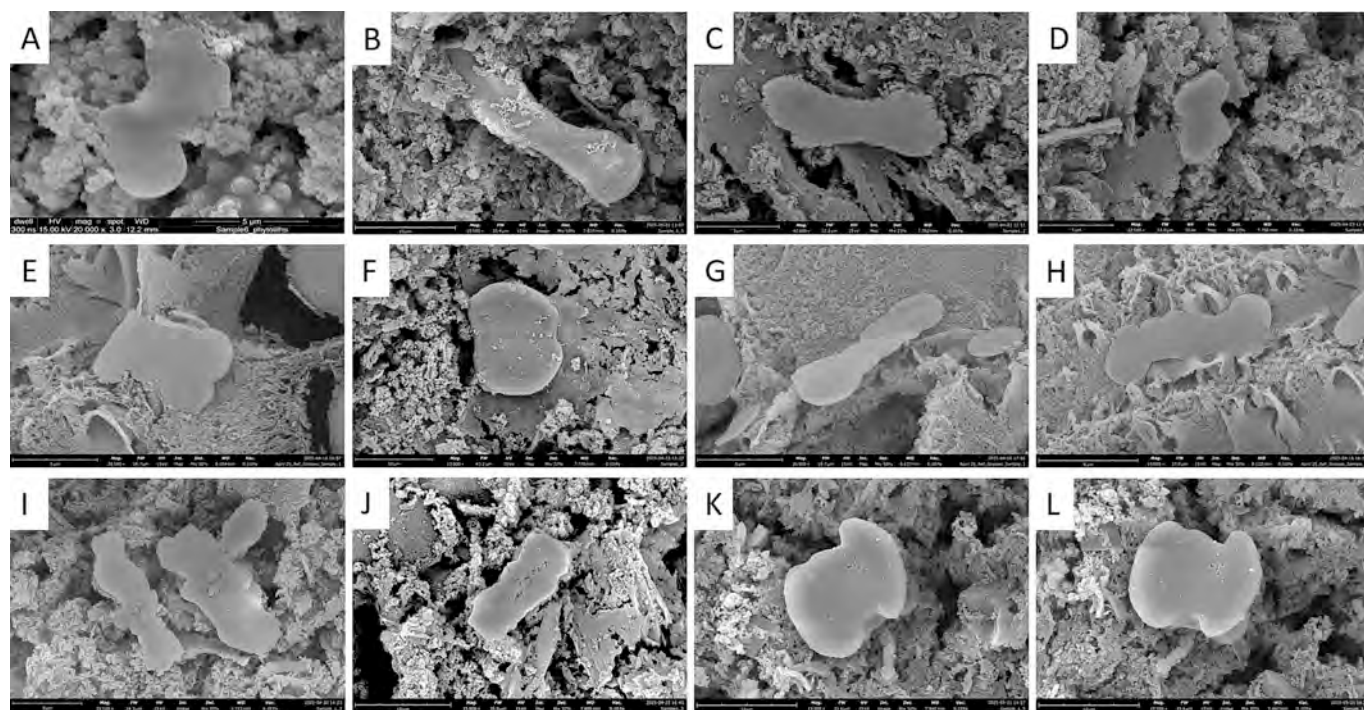


Fig. 2. Grass silica short-cell phytoliths (GSSCP) described from reference material and PLM samples. BILOBATE morphotypes (A–F), POLYLOBATE morphotypes (G–I), CRENATE morphotypes (J–L).

2.6. Phytolith co-occurrence matrix and probabilistic co-occurrence

To estimate the relationships among phytolith morphotypes, that is, to assess positive (if certain phytolith morphotypes regularly occur together in the same grass species material) or negative co-occurrence (if the presence of certain phytolith morphotypes is not or almost never evidenced for the same grass species material) and to further reveal the

use of grasses for PLM preparation, we calculated a co-occurrence comparison matrix based on the presence-absence of the phytolith morphotypes and performed probabilistic co-occurrence analysis as described in Boyd et al. (2024). The comparison (presence-absence) matrix is based on 630 pairings of morphotypes, using a significance threshold of 0.01. Only the most common 15 morphotypes (out of a total 36) from our 30 reference grass species were used for the final matrix,

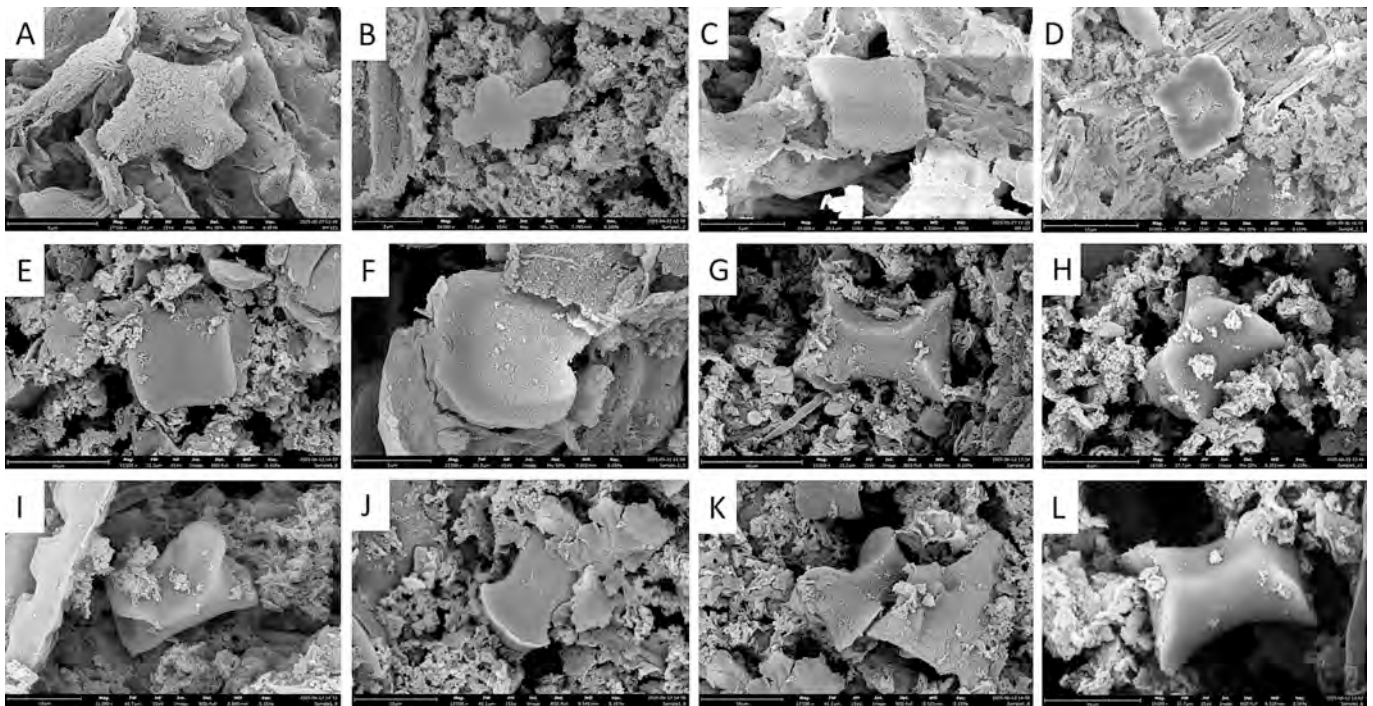


Fig. 3. Grass silica short-cell phytoliths (GSSCP) described from reference material and PLM samples. CROSS morphotypes (A–F), SADDLE morphotypes (G–L).

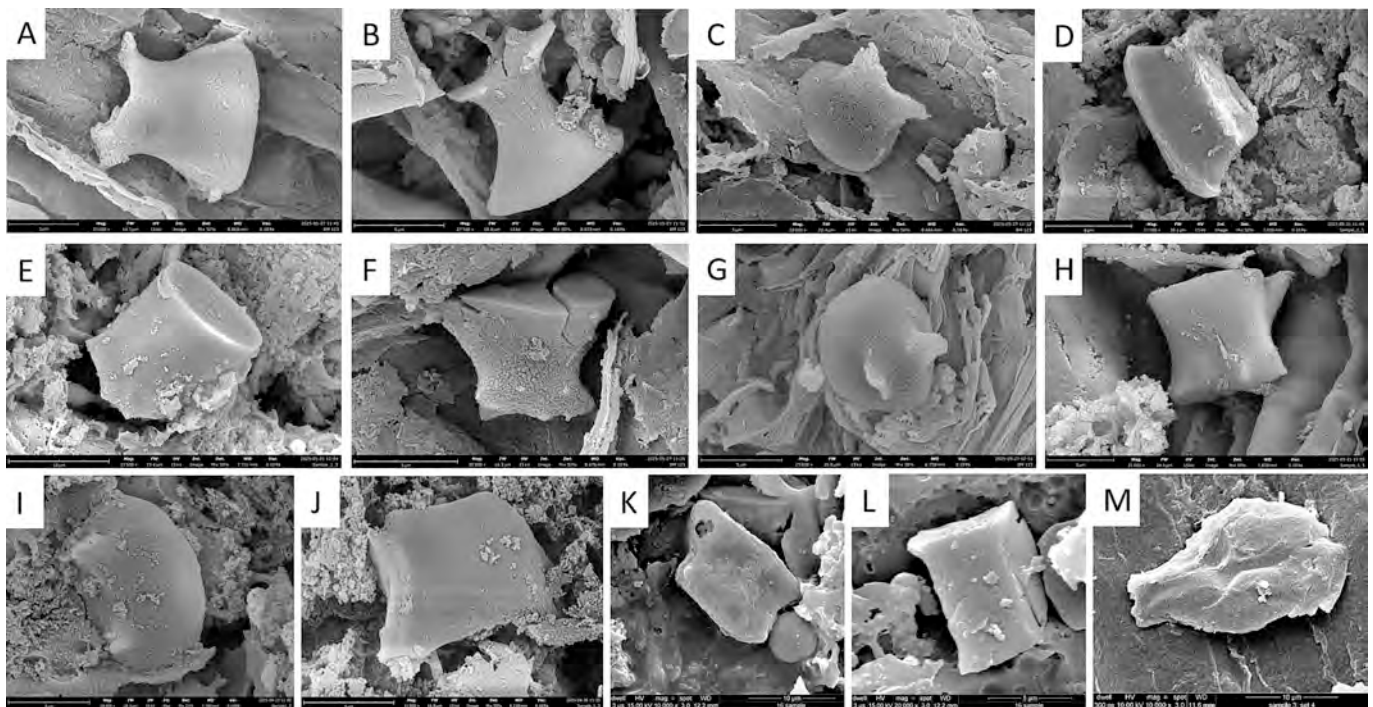


Fig. 4. Grass silica short-cell phytoliths (GSSCP) described from reference material and PLM samples. RONDEL morphotypes (A–J), TRAPEZOID morphotypes (K–L), BULLIFORM FLABELLATE morphotype (M).

rare types were excluded as not significant for the analysis.

2.7. Multivariate data analysis

Multivariate data analysis was performed in CANOCO for Windows 5.10 (Ter Braak and Smlauer, 2018) on the reference grass material and subsequently on the research PLM material. In the first case, the relationship between (1) phytolith type morphology and (2) phytolith type

diversity (i.e. the number of phytolith types registered within the tissues of certain reference grass species) was studied. The results obtained in this ordination were used in interpretations of the phytolith analysis of the PLM research material. In the latter case, we attempted to reveal whether the phytolith associations - both in terms of morphological diversity and the number of phytolith types registered within the specific manuscript sample - allow for the identification of potential grass sources.

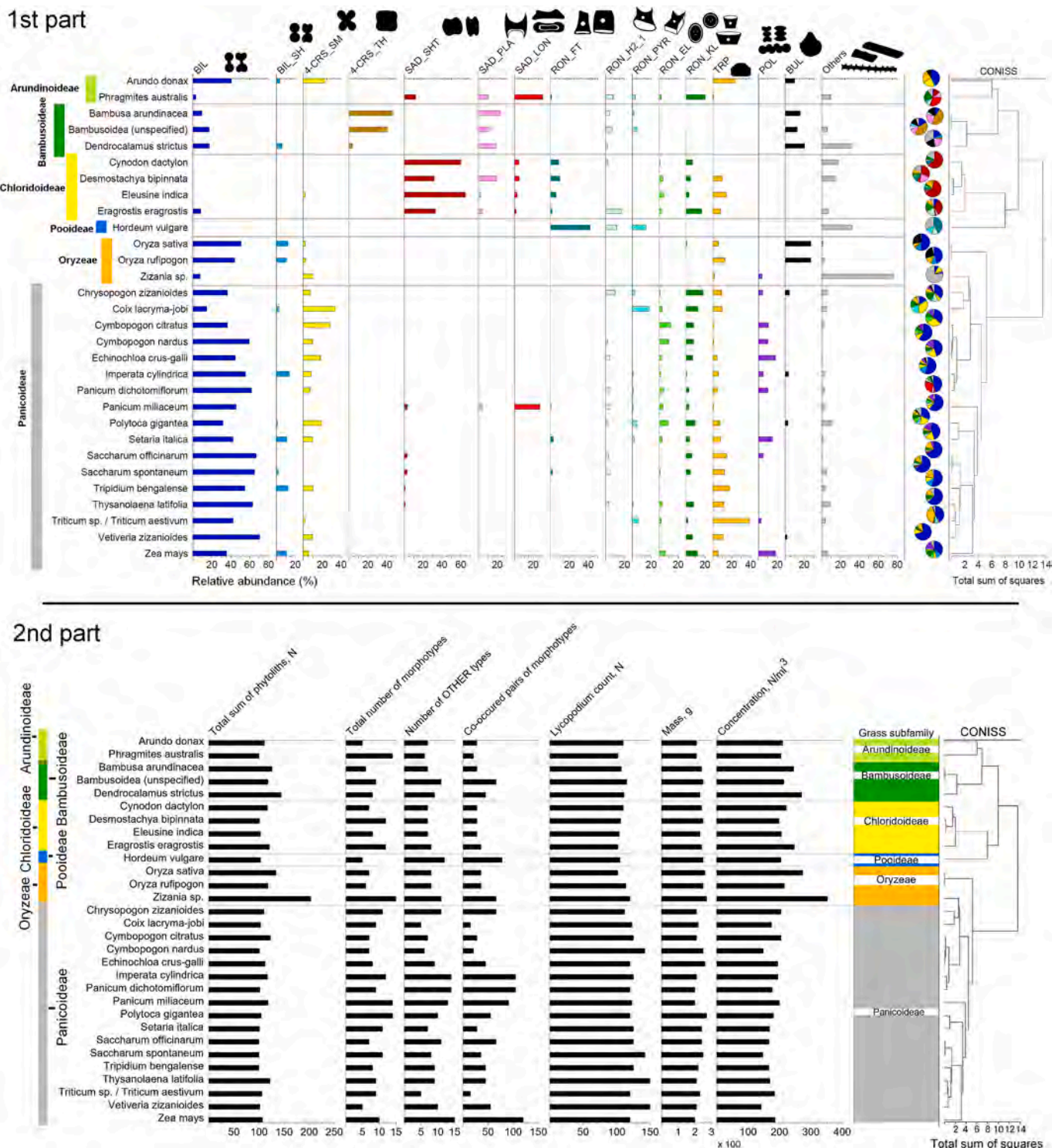


Fig. 5. Diagram of phytolith analysis performed on the reference grass material including 15 most common phytolith morphotypes, pie-charts of the species-specific cumulative spectra of the most diagnostically important morphotypes, and the results of cluster analysis (CONISS). Only phytolith morphotypes accounted for more than three grass species and/or demonstrating abundance of >5% TPS included. Non-diagnostic morphotypes are summarized as 'OTHERS' (OTH). Concentration is calculated per ml of sample volume.

For each ordination, a detrended correspondence analysis (DCA, Hill and Gauch, 1980) was first conducted to determine the length of the composition turnover gradient, which resulted in a value of 1.0 for the reference grass samples and 1.3 for the PLM samples. This indicated that a linear approach was appropriate, and a principal component analysis (PCA) was conducted. The ratio of λ_1 plus λ_2 over the total variance,

equivalent to R^2 (Jongman et al., 1987), was used to express the total variance explained. In each ordination, all phytolith data were used without any transformation or other standardization. To reduce the effect of rare phytolith types, only those present in at least two samples and with a percentage value greater than 2% in at least one sample were included in the final analysis.

Ordination stability was checked using the MULTIV software (Pillar and Orłóci, 1996; Pillar, 1999) as described above. To evaluate the significance of the PCA axes, the null hypothesis was tested using a nonparametric Monte Carlo permutation test (Manly, 1992). Under the null hypothesis, for both the reference and the research PLM material, no relationships are expected between the variation in phytolith percentages and sample type.

3. Results

3.1. Reference grass material

3.1.1. Phytolith morphotype diversity

Altogether, 36 principal phytolith morphotypes were described for the studied 30 grass taxa (Fig. 5). The most common GSSCP morphotype was BILOBATES (BIL), registered in 26 reference grass species across five subfamilies. BILOBATES were dominant in the morphological spectra of *Vetiveria zizanioides* (L.) Nash (71% of total phytolith sum, TPS), *Thysanolaena latifolia* (Roxb. ex Hornem.) Honda (63%), and *Panicum dichotomiflorum* Michx. (62%, Fig. 5), all belonging to the Panicoideae subfamily. Other common and widespread morphotypes included TRAPEZIFORM phytoliths (TRP), found in 25 species, and predominant in *Triticum sp./Triticum aestivum* (L.) (39% of TPS, Panicoideae), *Arundo donax* (L.) (23%, Arundinoideae), and *Eleusine indica* (L.) Gaertn. (15%, Chloridoideae). KEELED RONDELS (RON_KL) were described from 22 species, most abundantly in *Chrysopogon zizanioides* (L.) Nash (18% of TPS, Panicoideae) and *Eragrostis eragrostis* (L.) Wolf (17%, Chloridoideae). ELLIPSOID RONDEL (RON_EL) appeared to be a common morphology within 19 grass species, prevailing in the reference samples of Panicoideae, specifically *Cymbopogon citratus* (DC.) Stapf (12% of TPS), *Cymbopogon nardus* (L.) Rendle (10%), and *Polytoca gigantea* (J. Koenig) Mabb. (10%). 4-LOBED SYMMETRIC CROSSES (4-CRS_SM) were registered in 18 species, mainly in *Coix lacryma-jobi* (L.) (34%) and *Cymbopogon citratus*

(DC.) Stapf (28%), both from Panicoideae. TWO-HORNED RONDEL, type 1 (RON_H2_1, Fig. 5), was observed in 16 reference grass species, predominantly in *Eragrostis eragrostis* (L.) Wolf (17%, Chloridoideae) and *Hordeum vulgare* (L.) (12%, Pooideae).

The summary diagram (Fig. 5) demonstrates the 15 most common phytolith morphotypes, including those already described, one can clearly observe how the taxonomical position of reference grasses is reflected in the CONISS clustering. Cumulative spectra of diagnostically important morphotypes are given alongside in the form of pie charts. Other (OTH) morphotypes comprised non-diagnostic Poaceae phytoliths, such as ELONGATE DENDRITIC, ELONGATE ENTIRE, ELONGATE SINUATE, and ELONGATE DENTATE types with various transitional forms, as well as flat rectangular epidermal silicified cells, papillate (nipple-shaped) morphotypes, minor amounts of graminaceous stomata, ACUTE BULBOSUS, and BLOCKY phytoliths (Metcalf, 1960; ICPN 2.0, 2019). A full representation of GSSCP production across all 90 reference samples can be seen in the Supplementary Material S1.

3.1.2. Multivariate data analysis

The PCA-based ordination of the grass reference samples demonstrated a measure of goodness of fit equal to 0.87. The first two dimensions of the linear ordination (Fig. 6) account for 46% and 21% of the total variance of data. The ordination results show that grasses are grouped in clouds according to their taxonomic positions within related subfamilies, as also demonstrated by clustering (Fig. 3, CONISS). The two exceptions in PCA (Fig. 4A) are *Phragmites australis* (Cav.) Trin. ex Steud. (Arundinoideae), which is grouped together with Chloridoideae grasses, and *Zizania* sp. (Oryzeae), grouped together with Panicoideae.

The first case is easily explained by the high predominance of SADDLES, which are the morphotypes characterized by both *Phragmites* (52% of various SADDLES, all accounted for together with the long or PLATEAUED SADDLE being the species diagnostic morphotype (Cordova, 2023). The case of *Zizania* sp. is more likely a statistical artifact, not only because of

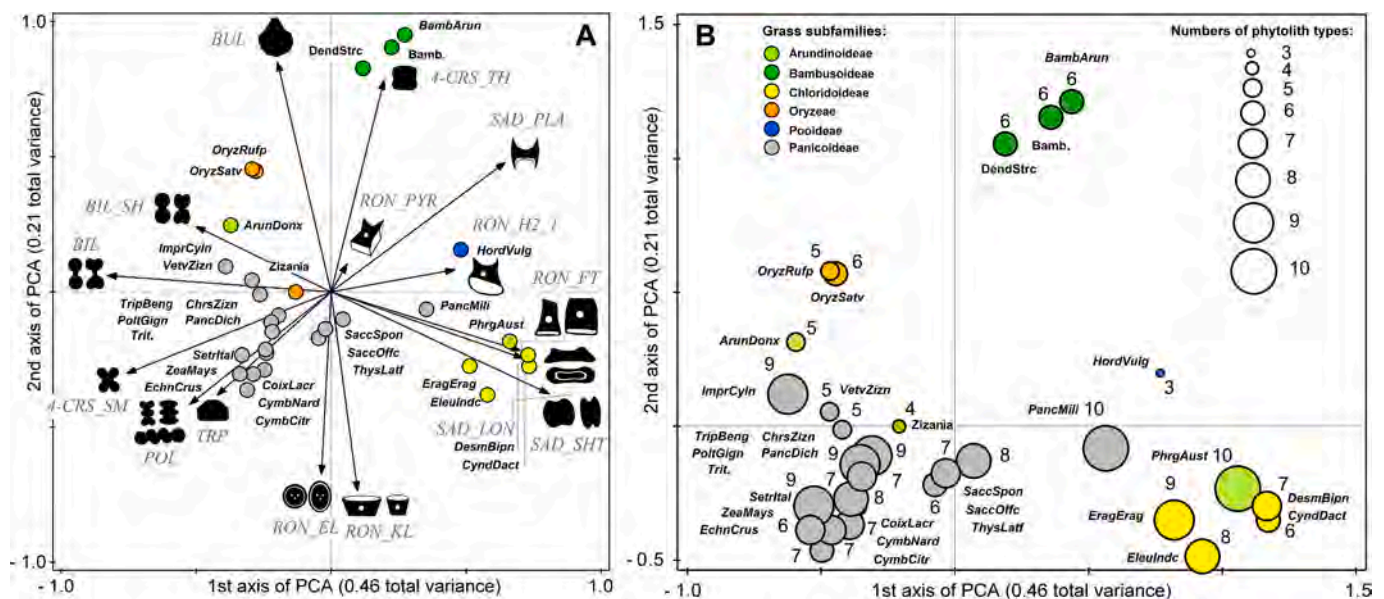


Fig. 6. Results of principle component analysis (PCA) illustrating (A) statistical relations of the 15 most common and abundant phytolith types (shown with arrows and black-shadow contours) and the reference grass species (shown with coloured circles, colours are subfamily-specific, address to the graphical legend) and (B) phytolith morphotype diversity within the reference grass species clustered within the ordination field. Size of circles indicated mean number of phytolith types registered within the given species. For abbreviations of the phytolith types see Table 3. Reference grass species are abbreviated as following: ArunDonx. - *Arundo donax*, BambArun. - *Bambusa arundinacea*, Bamb. - *Bambusoidea* (unspecified), ChrsZizn - *Chrysopogon zizanioides*, CoixLacr - *Coix lacryma-jobi*, CymbCit - *Cymbopogon citratus*, CymbNard - *Cymbopogon nardus*, CyndDact - *Cynodon dactylon*, DendStrc - *Dendrocalamus strictus*, DesmBipn - *Desmostachya bipinnata*, EchnCrus - *Echinochloa crus-galli*, EleulIndc - *Eleusine indica*, EragErag - *Eragrostis eragrostis*, ImprCyn - *Imperata cylindrica*, HordVulg - *Hordeum vulgare*, OryzSanv - *Oryza sativa*, OryzRufp - *Oryza rufipogon*, PancDich - *Panicum dichotomiflorum*, PancMili - *Panicum miliaceum*, PhrgAust - *Phragmites australis*, PoltGign - *Polytoca gigantea*, Setrital - *Setaria italica*, SaccOffc - *Saccharum officinarum*, SaccSpon - *Saccharum spontaneum*, TripBeng - *Tripsacum bengalense*, ThysLatf - *Thysanolaena latifolia*, Trit. - *Triticum sp.* / *Triticum aestivum*, VetvZizn - *Vetiveria zizanioides*, ZeyMays - *Zea mays*, Zizania - *Zizania* sp.

the mass effect caused by Panicoideae species - those heavily prevailing in our reference list (17 species out of 30) - which drive the patterns of analysis, but also because of the presence of typical BILOBATES (7% of TPS) and 4-LOBED SYMMETRIC CROSSES (10%) characteristic for Oryzeae. Types unique to *Zizania* sp. are very rare or absent in other grasses (i.e. 4-LOBED ASYMMETRIC CROSSES, 4-LOBED CROSSES WITH CLEFT BASE LOBES, 5-LOBED, AND 6-LOBED CROSSES), as well as specific rondels (e.g. wavy-top RONDELS of type 1 and type 2, the latter being a TRAPEZIFORM RONDEL with an asymmetric wavy top), which have been previously described only in *Zizania* and *Zea* species. However, none of these types were included in the PCA analysis in order to obtain a clearer picture by reducing the effect of rare morphotypes grouped together with 'OTHERS'.

As for subspecies-specific associations, the 4-LOBED SYMMETRIC CROSS with thick lobes (4-CRS_TH) showed a positive correlation with Bambusoideae ($r^2 = 0.84$ at $p < 0.05$, Fig. 6A), specifically with *Bambusa arundinacea* and unspecified bamboos, whereas *Dendrocalamus strictus* Nees demonstrated a slightly weaker correlation with 4-CRS_TH ($r^2 = 0.68$) due to the presence of BULLIFORM (BUL) phytoliths, which in this ordination were also associated with Oryzeae ($r^2 = 0.53$). Most of Panicoideae are well-correlated with various BILOBATES ($r^2 = 0.61$), 4-LOBED SYMMETRIC CROSS (4-CRS_SM, $r^2 = 0.50$), POLYLOBATE (POL, $r^2 = 0.58$), and TRAPEZIFORM (TRP, $r^2 = 0.66$) morphotypes. The presence of saddle and rondel morphotypes in the morphospectrum of *Panicum miliaceum* L. moved it towards the Chloridoideae cloud, thus, its correlation with their typical phytoliths remained weak ($r^2 = 0.21$ – 0.32). Diverse SADDLES and flat RONDEL (lower phytoliths, RON_FT) demonstrated a positive correlation with Chloridoideae ($r^2 = 0.55$ – 0.72), specifically – saddles are better correlated with *Eleusine indica* and *Eragrostis eragrostis* (L.) Wolf, and rondels are associated with *Cynodon dactylon* (L.) Pers. and *Desmostachya bipinnata* (L.) Stapf. Other rondels, including the flat RONDEL (RON_FT), two-horned rondel type 1 (RON_H2_1), and pyramidal RONDEL (RON_PYR), show positive correlations with *Hordeum vulgare* L. As Fig. 6(B) demonstrates, the highest diversity of morphotypes within our reference list characterizes *Panicum miliaceum* and *Phragmites australis*, each with ten different morphotypes described. For *Eragrostis eragrostis*, *Chrysopogon zizanioides*, *Imperata cylindrica* (L.) P. Beauv., *Polytoca gigantea*, and *Setaria italica* (L.) P. Beauv., we identified nine phytolith types. The least morphotype diversity was observed in *Hordeum vulgare*, it was limited to two or three of the most common types of rondels (listed above), with a less commonly observed oval RONDEL (RON_OV) that was not included among the most common 15 diagnostic types (see the full phytolith morphological spectra in the Supplementary Material S1).

3.2. Positive and negative relationships of the phytolith morphotypes in reference material

The comparison matrix is based on 120 morphotype pairings (Table 4). Analysis of morphotype relationships in the grass reference material demonstrated the strongest positive co-occurrence between BIL and TRP (registered together in 23 samples, Fig. 7A), RON_KL and TRP (in 21 samples), BIL and RON_KL (19 samples), TRP and 4-CRS_SM, RON_EL (both pairs registered in 18 samples), BIL and 4-CRS_SM (17 samples), BIL and RON_EL (16 samples), and BIL and RON_H2_1, 4-CRS_SM, RON_KL (all three pairs registered in 14 samples). The strongest negative co-occurrence (morphotypes never registered together in the 30 species examined, $p = 0.01$) was observed for 4-CRS_SM with 4-CRS_TH, for 4-CRS_TH with SAD_SHT, for RON_FT with RON_H2_1, RON_PYR, RON_KL, TRP, POL, and BUL, for SAD_SHT with BUL, for SAD_PLA with POL, and for SAD_LON with POL and BUL (Fig. 7B).

3.3. Palm-leaf manuscript material

3.3.1. Phytolith morphotype diversity

We analyzed in total 30 PLM fragments from four manuscript collections, 28 different GSSCPs were registered (Fig. 8). Interestingly,

Table 3

All phytolith morphotypes registered in our reference grass material and their evidenced source plants according to the previous phytolith studies.

Acronyms, schemes	Phytolith morphotypes	Evidenced source plants	References
		Panicoideae, Arundinoideae, Bambusoideae	Piperno, 2006a; Biswas et al., 2016, Neumann et al., 2019
		Oryzeae, Danthonioideae, Pharoideae	Gu et al., 2013; Ma et al., 2016
		Bambusoideae, Oryzeae, or Panicoideae grasses	Kealhofer and Piperno, 1998; Dai et al., 2023
		BILOBATE TALL narrow	Kealhofer and Piperno, 1998; Dai et al., 2023
		Bambusoideae, Oryzeae, or Panicoideae grasses	Novello et al., 2012, Cordova, 2013
		WIDE LOBE BILOBATES in Panicoideae	Neumann et al., 2017
		TALL BILOBATES widely produced across subfamilies	Neumann et al., 2017; Novello et al., 2012; Cordova, 2013
BIL	BILOBATE	Arundinoideae, Bambusoideae, Oryzeae, Panicoideae	This study
BIL_SH	BILOBATE WITH SHORT OR REDUCED SHANK	Bambusoideae, Panicoideae	This study
BIL_LS	BILOBATE WITH LONG SHANK	Bambusoideae, Panicoideae	This study
BIL_KS	BILOBATE WITH KEELED SHANK	Bambusoideae, Chloridoideae, Panicoideae	This study
4-CRS_SM	4-LOBED SYMMETRIC CROSS		
4-CRS_TH	4-LOBED SYMMETRIC CROSS WITH THICK LOBES	CROSSES are observed in Panicoideae, Arundinoideae, Bambusoideae, Danthonioideae, Chloridoideae	Biswas et al., 2016, Neumann et al., 2017, Neumann et al., 2019
4-CRS_ASM	4-LOBED ASYMMETRIC CROSS		
4-CRS_CLF	4-LOBED CROSS WITH CLEFT BASE LOBES	CROSSES with ampliate cleft lobes: <i>Zizania</i> sp.	Yost and Blinnikov, 2011
5-CRS	5-LOBED CROSS	4-LOBED CROSSES: Panicoideae	Boyd et al., 2024
6-CRS	6-LOBED CROSS		
SAD_SHT	SHORT SADDLE	Arundinoideae, Chloridoideae, Panicoideae	This study
SAD_COL	COLLAPSED SADDLE	Bambusoideae	Strömberg, 2004, Biswas et al., 2016, Neumann et al., 2017, Naskar and Bera, 2018b
SAD_PLA	PLATEAU SADDLE	<i>Dendrocalamus</i> sp.	Wang et al., 2022
SAD_TAL	TALL SADDLE	Arundinoideae, Bambusoideae	Piperno and Pearsall, 1998, Biswas et al., 2016, Neumann et al., 2017, Neumann et al., 2019
		<i>Phragmites australis</i>	Cordova, 2023
		Bambusoideae	Kondo et al., 1994, Strömberg, 2004, Biswas

(continued on next page)

Table 3 (continued)

Acronyms, schemes	Phytolith morphotypes	Evidenced source plants	References
		Bambusoideae, Oryzeae, or Panicoideae grasses	et al., 2016, Naskar and Bera, 2018a
SAD_LON 	LONG SADDLE	Bambusoideae	Kealhofer and Piperno, 1998; Dai et al., 2023
SAD 	SADDLE UNSPECIFIED	Chloridoideae, Bambusoideae Bambusoideae, Oryzeae, or Panicoideae grasses Chloridoideae SADDLES, double Chloridoideae SADDLES	Gu et al., 2016b; Li et al., 2014; Tao et al., 2019 Twiss et al., 1969b; Biswas et al., 2016, Neumann et al., 2019; Cordova, 2023 Kealhofer and Piperno, 1998; Dai et al., 2023 Cordova, 2023
RON_FT 	FLAT RONDEL (TOWER)	Arundinoideae, Chloridoideae, <i>Hordeum vulgare</i> , Panicoideae	This study
RON_H2_1 	TWO-HORNED RONDEL, TYPE 1	Arundinoideae, Bambusoideae, Chloridoideae, <i>Hordeum vulgare</i> , Panicoideae	This study
RON_H2_2 	TWO-HORNED RONDEL, TYPE 2	Arundinoideae, Chloridoideae, Panicoideae	This study
RON_H3 	THREE-HORNED RONDEL	Arundinoideae, Chloridoideae, Panicoideae	This study
RON_H4 	FOUR-HORNED RONDEL	Arundinoideae	This study
RON_PYR 	PYRAMIDAL RONDEL	Arundinoideae, Bambusoideae, <i>Hordeum vulgare</i> , Panicoideae	This study
RON_EL 	ELLIPSOID RONDEL	Arundinoideae, Chloridoideae, Panicoideae	This study
RON_KL 	KEELED RONDEL	Arundinoideae, Chloridoideae, Panicoideae	This study
RON_OV 	OVAL RONDEL	Chloridoideae, <i>Hordeum vulgare</i> , Panicoideae	This study
RON_HRN 	SPOOL/HORNED RONDEL	Arundinoideae, Bambusoideae, Chloridoideae, Panicoideae	This study
RON_WT_1 	WAVY-TOP RONDEL, TYPE 1	Oryzeae	This study
RON_WT_2 	WAVY-TOP RONDEL, TYPE 2 OR TRAPEZIFORM RONDEL with asymmetric wavy top	Oryzeae	This study
RON_REN_1S 	RENIFORM RONDELS with large central sinus (can be seen both in	Oryzeae	This study

Table 3 (continued)

Acronyms, schemes	Phytolith morphotypes	Evidenced source plants	References
RON_REN_2S 	side- and in top-view) RONDEL with two opposing sinuses (seen in top-view)	Oryzeae	This study
RON_CS 	RENIFORM RONDELS with central sinus (seen in side-view)	Oryzeae	This study
RON_REN 	Other RENIFORM RONDELS	Oryzeae	This study
RON_CON	RONDELS with concave base, top and sides	<i>Zizania</i> sp.	Yost and Blinnikov, 2011; "WIDE SADDLES" by Brown, 1984; "SADDLE-TOPPED SHORT TRAPEZOID" by Ollendorf et al., 1988; and "PLATEAUED SADDLES" by Piperno, 2006b; also named as "BIDOLABRIFORM-PATELLIFORM" by Bombin, 1984. Twiss et al., 1969a; Biswas et al., 2016, Neumann et al., 2019
RON	RONDEL unspecified	All Poaceae subfamilies	
TOW 	COMPLEX TOWER	All Poaceae subfamilies	Biswas et al., 2016, 2021
TRP 	TRAPEZIFORM	Arundinoideae, Chloridoideae, Oryzeae, Panicoideae	This study
POL 	POLYLOBATE	Panicoideae, Bambusoideae, Chloridoideae, Arundinoideae	Twiss et al., 1969b; Biswas et al., 2016, Neumann et al., 2017
POL_CLF 	POLYLOBATE with cleft base lobes	Oryzeae	This study
BUL 	BULLIFORM	Oryzeae <i>Dendrocalamus</i> sp.	Neumann et al., 2019; Gu et al., 2013; Ma et al., 2016 Wang et al., 2022

samples characterized by the highest phytolith type diversity for this study, namely BM4 (with a total of 17 types), BM6 (15 types), and BM2, CM14, and CM16 (14 morphotypes in each of these three samples), as well as those where unique and rare phytolith types are registered (BM4, BM5, CM15, CM16), come from the SUB Hamburg, where the manuscripts have been stored since the time when they were obtained, mostly in the 19th century. Furthermore, the manuscripts were never brushed, cleaned, or treated in any other way (just seldom flipped through, as for example when they were digitized). Therefore, they can be expected to retain most of the originally accumulated historical material on their surface. Clustering (CONISS, Fig. 8) shows that the first two clusters derived for PLM samples - and thus representing the largest statistically significant difference - are the clusters of samples coming from Indian (IFP, EFEO, VRC/VMB) and German (SUB Hamburg) collections, except for sample AL5. This sample is artificially grouped with BM and CM samples because of the comparable amounts of BILOBATES with short or reduced shank (BIL_SH) and KEELED RONDEL (RON_KL) phytoliths, neither of which alone, is diagnostic for any grass subfamily or other taxonomic group. The clear statistical separation of the samples from SUB Hamburg supports that the main difference in our PLM material lies not in the region of original provenance, but in the amount of preserved phytolith material on the surface, which is obviously greater on the surface of the uncleaned and untreated PLMs from SUB Hamburg.

Table 4
Heat map of probabilistic co-occurrence between the most common 15 GSSCP morphotypes, using a presence-absence matrix of species and morphotypes across 30 reference grass species.

Compared pares of morphotypes:																
		BIL	BIL_SH	4-CRS_SM	4-CRS_TH	SAD_SHT	SAD_PLA	SAD_LON	RON_FT	RON_H2_1	RON_PYR	RON_EL	RON_KL	TRP	POL	BUL
	BIL	26/29	11	17	1	7	6	3	5	14	12	16	19	23	11	10
	BIL_SH	15	11/15	9	1	1	3	1	1	3	4	6	7	10	3	6
	4-CRS_SM	6	7	18/7	0	2	1	1	2	4	7	12	14	18	10	7
	4-CRS_TH	2	3	3	3/3	0	1	1	0	0	0	2	0	0	0	0
	SAD_SHT	2	4	12	26	10/27	5	6	7	7	3	8	9	9	1	0
	SAD_PLA	17	0	1	7	16	8/17	5	5	6	4	5	5	5	0	3
	SAD_LON	10	12	1	3	7	13	6/14	6	4	2	6	6	5	0	0
	RON_FT	11	15	25	2	4	11	24	9/25	6	3	8	8	1	0	0
	RON_H2_1	15	10	12	17	3	6	13	20	16/20	9	10	12	10	5	5
	RON_PYR	10	14	6	10	16	2	2	10	17	13/17	10	10	10	5	5
	RON_EL	1	1	1	1	1	1	0	0	1	1	19/1	19	18	9	3
	RON_KL	1	16	17	25	12	17	27	2	4	12	26	22/27	21	10	5
	TRP	24	1	15	19	22	14	16	24	3	7	15	26	25/27	11	7
	POL	7	6	0	3	7	6	4	4	6	2	3	5	7	11/7	2
	BUL	5	16	15	1	9	14	14	6	9	15	1	5	11	17	10/17

Key to heat matrix:



In diagonal cells as the first number before the backslash stays a number of diagnosed phytoliths registered in the reference grass material, as the second number stays a number of diagnosed phytoliths registered in the PLM material. Above the diagonal stays the number of the reference grass material samples in which compared morphotypes co-occurred; below the diagonal stays the number of the PLM samples in which compared morphotypes co-occurred

In diagonal cells as the first number before the backslash stays a number of diagnosed phytoliths registered in the reference grass material, as the second number stays a number of diagnosed phytoliths registered in the PLM material. Above the diagonal stays the number of the reference grass material samples in which compared morphotypes co-occurred, below the diagonal stays the number of the PLM samples in which compared morphotypes co-occurred

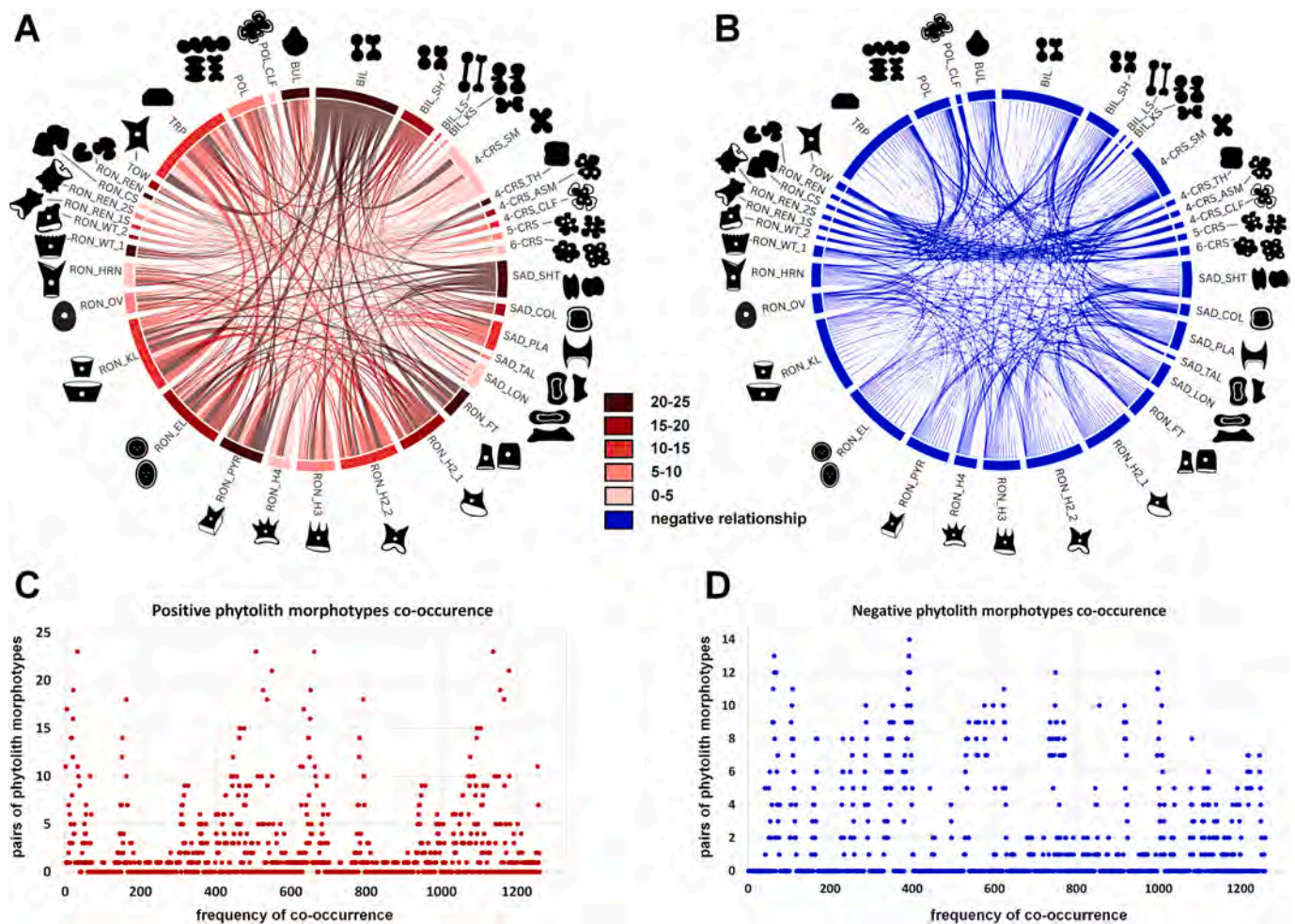


Fig. 7. Pairwise comparison matrix based on the total 1260 pair-cases of all 36 phytolith morphotypes registered across 30 reference grass species showing (A) – positive co-occurrence (when paired morphotypes were registered together at least in one case), (B) – negative co-occurrence (when paired morphotypes do not registered together in the analyzed material), (C) – frequency of the phytolith morphotype negative co-occurrence per amount of compared pairs, (D) – frequency of the phytolith morphotype positive co-occurrence per amount of compared pairs.

The average number of phytolith morphotypes per sample is 10.3 ± 2.9 , with a maximum of 17 morphotypes registered in sample BM4 (collection of SUB Hamburg) and a minimum of 5 types described in samples RE4 (archive of IFP, India) and AL5 (VRC/VMB collection, Thrissur, India). Other samples characterized by relatively high phytolith type diversity include TR3 (VRC/VMB collection, Thrissur, India) and BM6 (collection of SUB Hamburg), both having 15 phytolith types, as well as BM2, CM14, and CM15, all three from SUB Hamburg, each with 14 phytolith morphotypes. The concentration of phytoliths in PLM samples is generally low, averaging $13,481 \pm 2,113$ phytoliths per milliliter, with a maximum of 10,282 phytoliths per milliliter registered in sample TR4 (VRC/VMB collection, Thrissur, India).

The most common morphologies found in PLM samples are listed in Table 5 and include those most frequently observed in the grass reference material, except for the OVAL RONDELS (RON_OV), which are described from 10 samples with an average percentage of 1.60 ± 1.5 , and the 4-lobed symmetric CROSSES (4-CRS_SM), which are seen in only 7 samples with an average relative abundance of 4.14 ± 1.6 . Other RENIFORM RONDELS (RON_REN) are observed in 10 samples at minimal percentages (1.10 ± 0.3). Other morphologies are registered even more rarely in the PLM samples (see Fig. 5 and Supplementary Material S4). Phytolith types registered only once and in a single sample include BIL_KS (sample BM-4), RON_EL (BM-4), RON_HRN (CM-15), and RON_CS (BM-5). In addition, five SAD_COL cells were registered exclusively in CM-16. In the PLM material analyzed here, unlike in the

reference grass material, certain phytolith morphologies were not found at all, namely 4-CRS_CLF, 5-CRS, 6-CRS, RON_H4, RON_WT (both types), RON_WT_2, and RON_REN_2S (for morphotype abbreviations, please consult Table 3). The diversity of 'OTHERS' morphotypes does not differ from that of the reference grass material either in terms of morphotypes or statistically ($r = 0.42 \pm 0.7$, $P \leq 0.5$, $p < 0.01$) however, broken blocky and amorphous phytoliths predominate in the PLM samples.

3.3.2. Multivariate data analysis

The PCA-based ordination of PLM samples shows a measure of goodness of fit equal to 0.60. The first two dimensions of the linear ordination (Fig. 9A) account for 17% and 29% of the total variance of the data. The results demonstrate that samples tend to group in clouds according to the provisional origin of their source manuscripts, but there are clear exceptions. The strongest statistical similarity was observed for EO1-EO5 samples (ca. 0.82, $p < 0.01$) and TR1-TR5 (0.64, $p < 0.01$). Samples CM14-CM17 demonstrate some weak similarity (0.44, $p < 0.01$), but they are clearly different from CM13 due to the high proportion of BILOBATES (58% vs. 17–20% in other samples) and the relatively low percentage of trapezoids (8% vs. 10–14% in others). In contrast, samples RE1-RE5 do not group together based on phytolith assemblages' similarity. Only RE2 and RE5 appeared to be somewhat similar (0.37, $p < 0.01$). Samples from BM2 to BM6 are also significantly different (0.09, $p < 0.01$). These results are comparable to the CONISS-based clustering

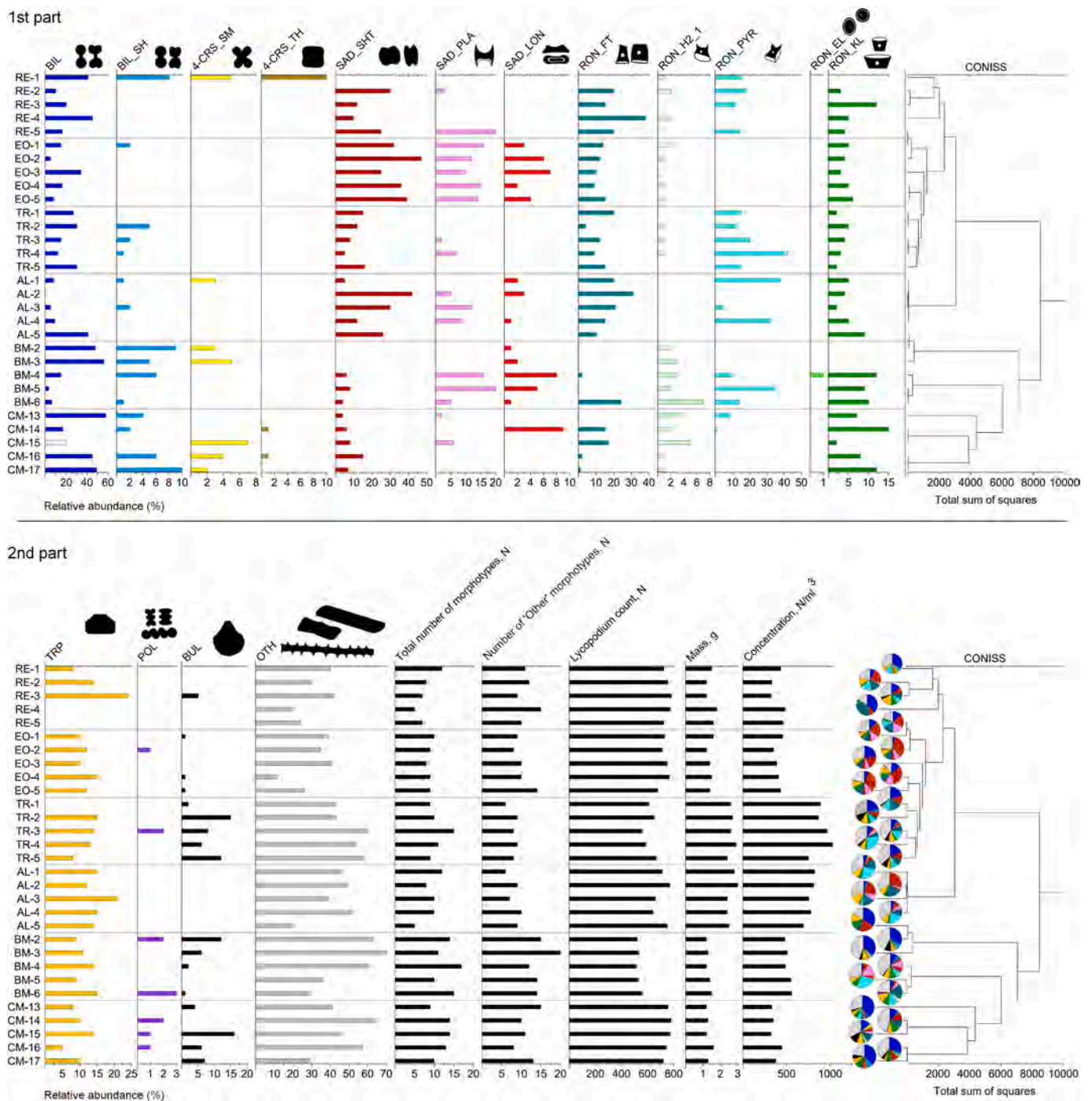


Fig. 8. Diagram of phytolith analysis performed on the PLM material including 15 most common phytolith morphotypes, pie-charts of the phytolith morphotype spectra, and the results of cluster analysis (CONISS). Only phytolith morphotypes accounted for more than three grass species and/or demonstrating abundance of $> 5\%$ TPS included. Non-diagnostic morphotypes are summarized as 'OTHERS' (OTH). Concentration is calculated per ml of sample volume. Total sum of phytoliths (TPS) for each sample is 100.

performed independently of the ordination (see Figs. 8 and 9). Only three sample groups are clearly separated by clustering: samples from SUB Hamburg versus those from Indian collections (except AL5, which has comparable amounts of BIL_SH and RON_KL as the BM and CM samples and is therefore integrated into the lower stratified cluster). The third well-separated group consists of EO1-EO5 (Fig. 8). In terms of probability analysis, only these three groups are supported with $P \geq 55$ ($p < 0.01$); otherwise, clustering shows that the separation is unreliable ($P < 40$ with $p < 0.01$).

As for PLM samples and specific phytolith morphotypes, a clear separation can be seen between samples correlated with BILOBATES and CROSSES (1st group, Fig. 9B) and those correlated with SADDLES and RONDELS (2nd group). These groups are negatively correlated with each other. The first group includes TR2, BM2, BM3, and CM13-CM17 (ca. 0.57, $p < 0.01$). In contrast, EO1-EO5 (ca. 0.64, $p < 0.01$) and AL2, AL3, AL5, RE4 (ca. 0.51, $p < 0.01$) are positively correlated with the second group of morphotypes (Fig. 9B). Along with them, samples RE2, RE5, BM5, and BM6 (0.30, $p < 0.01$) are weakly positively correlated. The only RONDEL

Table 5

Main phytolith types registered in the PLM samples and parameters of the phytolith analysis performed on PLM samples

Phytolith type	N samples	Max %	Min %	Average %	STD
BIL	29	58	3	24.28	16.6
SAD_SHT	27	47	4	17.96	12.9
RON_KL	27	15	2	6.04	3.6
TRP	27	24	5	12.48	3.9
RON_FT	25	38	1	14.84	8.5
RON_H2_1	20	7	1	2.15	1.6
SAD_PLA	17	20	2	10.24	5.8
RON_PYR	17	42	1	17.94	11.2
BUL	17	16	1	6.18	4.8
BIL_SH	15	10	1	4.27	2.9
SAD_LON	14	9	1	3.86	2.6
RON_OV	10	6	1	1.60	1.5
RON_REN	10	2	1	1.10	0.3

N samples – number of samples where this phytolith type is registered, Max % – maximal percentages of this phytolith type registered in PLM samples, Min % – minimal percentages of this phytolith type registered in PLM samples, Average % – mean percentages of this phytolith type registered in PLM samples, STD – standard deviation of related phytolith type percentages.

type sharing an ordination relationship with the first group is RON_H2_2. TRP phytoliths are very common in PLM material, present in almost all samples (except RE4, RE5, and TR1), so their presence cannot be attributed to any specific sample or group of samples. RON_PYR is positively correlated with TR1-TR5 (0.52, $p < 0.01$) as well as with RE1-RE3 and RE5 (0.48, $p < 0.01$). Sample BM4 is positioned in the middle of the ordination chart and cannot be characterized by any specific morphotype (Fig. 9B).

4. Interpretation and discussion

4.1. Grass phytolith spectra in the reference material

Based on observations and calculations of phytoliths extracted from 30 key grass species likely being used in PLM production and religious rituals, we described clear differences in the morphotype spectra of the reference species. The morphology of grass phytoliths is usually consistent within a subfamily (Chen et al., 2020), and this was

statistically supported in this study.

4.1.1. Arundinoideae

The subfamily Arundinoideae in this study is represented by two grasses (namely, *Arundo donax* L. and *Phragmites* sp.) with significant differences in their phytolith morpho-spectra. For *Arundo donax*, Chauhan et al. (2011) described patterns comparable to ours, including the highest frequency of BILOBATE phytoliths (according to Shakoor et al., 2016, these are NARROW BILOBATES, and we also observed that particular morphology), along with BULLIFORM CONCAVE (in the leaves) and knobbed spine phytoliths (in the stems). We agree with previous conclusions that these characteristics, together with a relatively high presence (23% of TOS, Fig. 2) of TRAPEZIFORM phytoliths and the total absence of RONDELS, can be used for the identification of *Arundo donax* based on its phytolith fingerprint. Marker morphotypes of *Arundo donax* also include long trapezoids and triangular shapes (we assume these are TRAPEZIFORM phytoliths with distinctly shortened upper bases of the TRAPEZOID). Shakoor et al. (2016), in their freshly collected material from Badani (district Pathankot, Northern India), from both leaves and synflorescences, also described POLYLOBATE phytoliths, although in our material these morphologies were registered rarely. SADDLE and CROSS morphotypes can be present (Metcalf, 1960; Chauhan et al., 2011), although Shakoor et al. (2016) considered the reports of SADDLE morphotypes from *Arundo donax* as controversial. They indicated that, although they "did not recover SADDLES from the leaf dry ash of *Arundo donax* L.," these morphologies "were visualized in situ in the leaf epidermis." We did not register SADDLES in our *Arundo* samples.

As for *Phragmites* sp., TALL SADDLES are the phytolith marker of the genus (Boyd et al., 2024). These morphologies are well known to be exclusively present in species of *Phragmites* sp., being particularly abundant in leaves (Piperno and Pearsall, 1998 - described as 'PLATEAUED SADDLES', Novello et al., 2012; Cordova, 2013; Boyd et al., 2024). Although comparable phytoliths have also been reported from Chloridoideae in minor amounts (Novello et al., 2012; Ghosh et al., 2011; Lu and Liu, 2003), their morphologies are not exact. The dominance of TALL SADDLES should be attributed to *Phragmites*, especially if accompanied by high frequencies of TALL TOWER RONDELS (also described from culms and inflorescences of *Phragmites* species by Boyd et al., 2024) and large roundish FLABELLATE BULLIFORM phytoliths with pointed tops (Lu et al.,

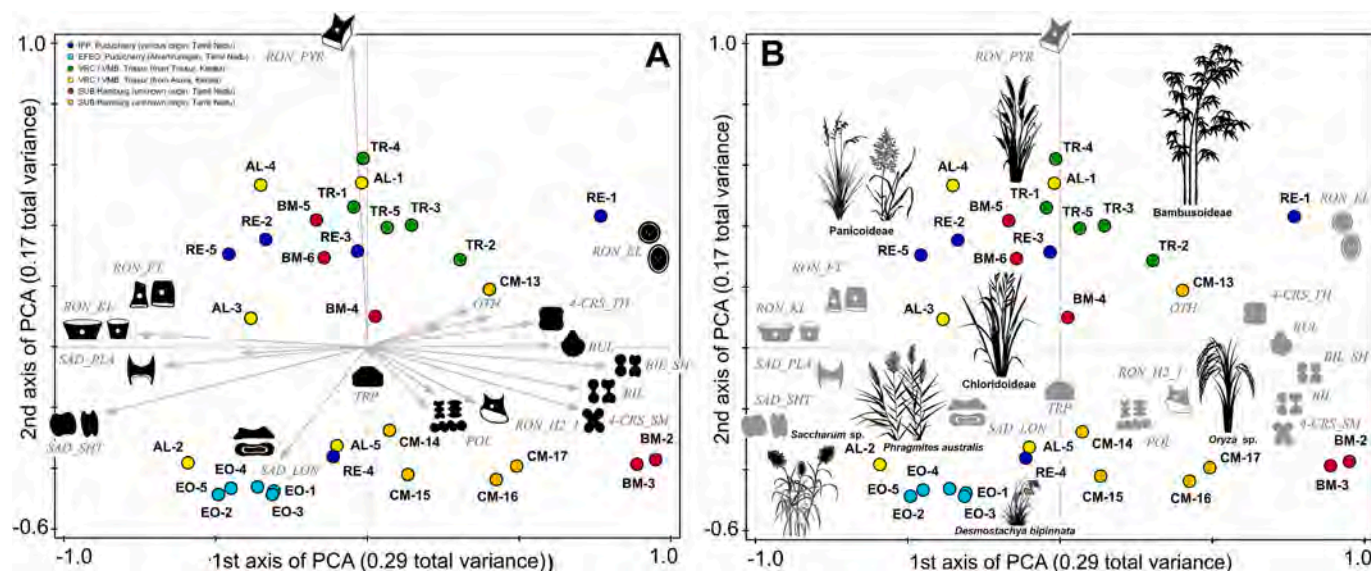


Fig. 9. Results of principle component analysis (PCA) illustrating (A) statistical relations of the 15 most common and abundant phytolith types (shown with arrows and black-shadow contours) and the reference grass species (shown with coloured circles, colours are subfamily-specific, address to the graphical legend) and (B) phytolith morphotype diversity within the reference grass species clustered within the ordination field. Size of circles indicated mean number of phytolith types registered within the given species. For abbreviations of the phytolith types see Table 3.

2006; Chen et al., 2020). These authors suggested using these specific BULLIFORM phytoliths as a marker of the *Phragmites* genus "when found alongside TALL SADDLES," although we observed great variability in the FLABELLATE BULLIFORM phytoliths, often within the same specimen, and therefore recommend applying this suggestion with caution and always considering other indicators. Shakoor et al. (2016), in India, also pointed out other RONDEL phytoliths (especially common in the synflorescences) and CROSSES described in the material of *Phragmites karka* (Retz.) Trin. ex Steud. We did not observe these phytoliths in our material from *Phragmites australis* (Cav.) Trin. ex Steud. In contrast, other forms of SADDLES (short and long varieties) and a rather high number of KEELED RONDELS (average 20% of TOS) were recorded. These latter morphotypes may be used as support if the primary marker (i.e. TALL SADDLES) is clearly present but never alone.

4.1.2. Bambusoideae

Although SADDLE PLATEAUED (SAD_PLA) phytoliths alone cannot be used as a definitive marker of Bambusoideae grasses, since they are also known from Arundinoideae (Piperno and Pearsall, 1998; Biswas et al., 2016, 2024; Neumann et al., 2017; Neumann et al., 2019) and CROSSES (including 4-CRS_TH) are produced in Panicoideae, Arundinoideae, Dathonioideae, and Chloridoideae (Biswas et al., 2016; Neumann et al., 2017; Neumann et al., 2019), a combination of BILOBATES (BIL), 4-LOBBED SYMMETRIC CROSS WITH THICK LOBES (4-CRS_TH), SADDLE PLATEAUED (SAD_PLA), with BULLIFORM (BUL) phytoliths, can be used as a marker for Bambusoideae. We did not register long saddles phytoliths (Fig. 2) in our reference material; however, other authors report these morphotypes as evidence for large tropical bamboos (Gu et al., 2016b; Li et al., 2014; Tao et al., 2019). It is doubtful that, in the current state of the art, we can clearly distinguish genera within this very large subfamily: India is considered a bamboo diversity hotspot (The National Bamboo Mission NBM, 2008; Bamboo Phylogeny Group (BPG), 2012; Biswas et al., 2024), especially in its moist deciduous and semi-evergreen forests in the northeast and the tropical moist deciduous forests in East and Southeast India (Sharma and Nirmala, 2015). Three tribes - namely Bambuseae (tropical woody bamboo), Olyreae (mostly tropical herbaceous bamboo), and Arundinarieae (bamboo of elevated temperate regions) (Zhang et al., 2020) - consist of about 29 genera and 148 species of Bambusoideae (Motomura et al., 2006). All attempts to study phytoliths in bamboos support the great variety of their morphologies and morphometrics (Piperno, 1988; Piperno and Pearsall, 1998; Lu et al., 1995, 2006; Madella et al., 2005; Bremond et al., 2008; Dickau et al., 2013; Naskar and Bera, 2018a; Tao et al., 2019; Biswas et al., 2016, 2024). Additionally, Bambusoideae grasses have often been recommended to be distinguished based on combinations and body parameters of SADDLE tall and SADDLE collapsed morphologies, as these morphotypes are considered exclusive to this subfamily (Madella et al., 2005; Dickau et al., 2013). However, Neumann et al. (2019) specifically warned against this, citing the similarity of these types in certain projections and in solid media, which do not allow object rotation under the light microscope. Taking this into account - and noting that the calculation of co-occurrence for these morphotypes did not reveal any reliable negative patterns (moreover, the only positive co-occurrence for SAD_TAL is probable with 4-CRS_TH, see Fig. 7A) - in this study, we refrain from making any assumptions solely based on patterns of SAD_TAL versus SAD_COL morphologies, regardless of their relative abundances in the samples.

The only clearly distinguishable genus appears to be *Dendrocalamus*, characterized by the bamboo-like appearance of BULLIFORM FLABELLATE phytoliths (Wang et al., 2022). These are found abundantly (ca. 20% of TPS in our samples, Fig. 2) and are typically present in combination with SADDLE TALL, SADDLE COLLAPSED, BILOBATE (including those with short or reduced shank), SADDLE PLATEAUED, and TALL TOWER RONDELS (Biswas et al., 2016, 2024). Gu et al. (2016b) suggested distinguishing *Dendrocalamopsis* sp. from *Bambusa* sp. and *Dendrocalamus* sp. based on the morphology of BULLIFORM FLABELLATE cells and their high frequencies. However, high production of these phytoliths can also indicate elevated

transpiration rates or water stress conditions (Bremond et al., 2005), which demand high transpiration or occur under low moisture conditions (Bremond et al., 2005; Messenger et al., 2010; D'Agostini et al., 2023). This makes sense in the wet tropics, with their high and relatively constant humidity, but can be misleading in regions with seasonality or fluctuations of floods and droughts, such as South India.

4.1.3. Chloridoideae

This subfamily is characterized by a high presence of subfamily-specific SADDLES (Mulholland, 1989; Lu and Liu, 2003; Piperno, 2006a; Chauhan et al., 2011; Novello et al., 2012; Neumann et al., 2017; Cordova and Scott, 2010; Cordova, 2013; Boyd et al., 2024; Karmakar et al., 2024) and, in general, dominance of the SHORT SADDLE ("squat saddles" in Boyd et al., 2024) - ranging from 31% of TPS in *Desmostachya bipinnata* to 65% in *Eleusine indica*. Karmakar et al. (2024) showed that in the northern parts of the Western Ghats (India), lobate phytolith types (i.e. BILOBATE, CROSS, and POLYLOBATE) were observed along with SADDLES and some RONDELS. These morphotypes were also previously observed in *Eleusine* and *Eragrostis* from Northern India (Ghosh et al., 2011; Naskar and Bera, 2018b, 2022). These types are also reported for modern Chloridoideae grasses collected in the Sunderbans delta (Northeast India, Naskar and Bera, 2018a). In contrast, Jattisha and Sabu (2015) reported no presence of RONDELS in chloridoids from Punjab (North India). In our case, in material from South India, these lobate phytoliths are either absent altogether (except for BILOBATE in *Eragrostis eragrostis* (L.) Wolf) or registered in very small amounts (not more than ca. 2% of TPS of 4-LOBBED SYMMETRIC CROSSES in *Eleusine indica*, and even lower or absent in other species). Furthermore, almost all lobate phytoliths in our material appeared to be negatively correlated or not correlated with SADDLES (Table 2 and Fig. 4).

To add more, Boyd et al. (2024) indicate that Eleusineinae shows a reduced abundance of TOWER RONDELS and a higher abundance of wide saddles, this pattern is also seen in our data but unsupported statistically (the correlation for both morphotypes with *Eleusine indica* is 0.67% at $p = 0.1$, with a probability of 0.52, which makes our calculations in this case unreliable). The same lack of statistical support was observed when we attempted to discriminate *Cynodon dactylon* and *Desmostachya bipinnata*. The absence of PLATEAU SADDLES and TRAPEZIFORM phytoliths in *Cynodon dactylon* is a clear phenomenon, but it cannot be used as a marker. First, because of the principle that "absence of evidence is not evidence of absence", second, because of the variability in the morphological spectra of chloridoids as described above. Furthermore, these statistical cases highlight the warning against using multivariate statistics as the sole tool for describing relationships between dependent variables with an unclear dependency structure - such as samples and the relative abundances of proxies of interest (e.g. phytoliths) - without controlling for the quality of the data in the ordination process.

4.1.4. Pooideae

Only one Pooideae species was relevant to our study, which is clearly not representative of the entire subfamily. *Hordeum vulgare* L. is characterized by a strong dominance of various RONDELS in the GSSCP spectrum (in our material - up to 100% of TPS overall), with a clear predominance of FLAT RONDELS (tower, ca. 42%) and OVAL RONDELS (31%), which are known palaeoecological markers of the species (e.g. Kaplan et al., 1992; Rosen, 1992; Tubb et al., 1993; Ball et al., 1993, 1999, 2001, 2009; Naskar and Bera, 2018b; Naskar et al., 2018). *Hordeum* usually produces a large number of phytoliths (Ball et al., 1999; Rosen, 1992; Tubb et al., 1993), especially in the inflorescence (Naskar and Bera, 2018a). The presence of long DENDRITIC and/or papillae phytoliths, especially if articulated aggregations are noticeable, along with a high abundance and concentration of RONDELS and the virtual absence of TRAPEZIFORM phytoliths, should allow for quite reliable identification of *H. vulgare*.

4.1.5. Oryzoideae

The Oryzoideae subfamily is represented in our study by two notably different genera, namely *Oryza* and *Zizania*. A large amount of BILOBATES with scooped lobes was present in all reference samples (up to 63% in *Oryza sativa*, Fig. 2), which is a distinct marker of the subfamily (Pearsall, 1978; Lu and Liu, 2003 - described as "the phytolith with truncated margins at both ends of its lobes"; Yost and Blinnikov, 2011; Huan et al., 2015; Hilbert et al., 2017; Neumann et al., 2017; Chen et al., 2020; Boyd et al., 2024). In our material, the amount of BILOBATES with short or reduced shank in rice samples was overall quite high (up to 10–11% of TPS), which was also statistically described by Bourel and Novello (2020) in comparison to BILOBATES of Panicoideae, Aristidoideae, and Chloridoideae, indicating that Oryzoideae (and also Panicoideae) "have a higher probability to produce BILOBATES with a base length less than 25 μm ." Calculations by Lu and Liu (2003) on the BILOBATES of Oryzoideae (including *Oryza*, *Leersia*, and *Zizaniopsis*) demonstrated as much as approximately 70% production of BILOBATES with shank lengths shorter than one-third of the lobes. However, the authors note that comparable morphologies are produced in "significant abundance" by *Panicum amarum*. This suggests that BILOBATES with short shanks can support the presence of Oryzoideae grasses in palaeoecological material, but they should not be used as sole indicators. Furthermore, attributing the shortened shank of BILOBATES in rice to regional climate and environmental conditions - as previously suggested - is likely incorrect, since this is a taxonomic, not an ecological, feature. The presence of very typical and distinctive symmetric and asymmetric 4- to 6-LOBED CROSSES, as well as diverse RONDELS (e.g. PYRAMIDAL, ELLIPSOID, KEELED, and especially TWO-HORNED RONDELS, see diagram in Supplementary S1), detailed by Yost and Blinnikov (2011), indicates the presence of *Zizania*. The combination of these phytolith types is characteristic of this Southeast Asian and American genus.

4.1.6. Panicoideae

In this study, Panicoideae are represented by 17 species, which produce a wide variety of phytoliths (6 or more types, Fig. 4B). Among our reference species, only *Phragmites australis* produces a comparable number of phytolith types (approximately 10, Figs. 2 and 4B). As in previous studies (e.g. Pearsall, 1978; Lu and Liu, 2003), we observed diverse and abundant BILOBATES: up to 60–67% in *Panicum dichotomiflorum*, *Saccharum* species, and *Thysanolaena latifolia*, and about 70% of TPS in *Vetiveria zizanioides*. We did not identify any specific phytolith markers exclusive to this subfamily.

KEELED RONDELS are indeed frequent in Panicoideae (Piperno and Pearsall, 1998; Fahmy, 2008; Cordova, 2013) and were observed in all reference species except *Tripidium bengalense*. They are also abundant, accounting for about 12% of TPS in *Coix lacryma-jobi*, *Zea mays*, and up to 20% in *Chrysopogon zizanioides*. However, Boyd et al. (2024) indicate that similar morphologies are found in *Enneapogon* sp. (Chloridoideae), so these cannot be treated as unique markers for Panicoideae. ELLIPSOID RONDELS are correlated with *Saccharum* species ($r = 0.57$ for *Saccharum spontaneum* and $r = 0.63$ for *Saccharum officinarum*, $p < 0.01$), as well as with *Thysanolaena latifolia* ($r = 0.65$, $p < 0.01$). Nonetheless, we observed similar RONDELS in Chloridoideae species, even in comparable amounts (e.g. in *Eleusine indica*, see Figs. 2 and 4A).

In the case studies reported from Africa (Novello et al., 2012; Cordova, 2013) and from northern Australia (if they occur together with short BILOBATES and several specific CROSSES, Boyd et al., 2024), wide BILOBATE morphologies are associated with Panicoideae. However, although we observed WIDE BILOBATES, Bourel and Novello (2020) demonstrated statistically that Oryzoideae also have a high probability of producing comparable BILOBATES. It remains unclear whether WIDE BILOBATES can be used as indicators of Panicoideae presence in paleoecological material from South India. POLYLOBATE morphologies show a strong positive correlation with some Panicoideae grasses, such as *Coix lacryma-jobi*, both *Cymbopogon* species, *Echinochloa crus-galli*, *Setaria italica*, and *Zea mays* (r^2 values range from 0.55 to 0.70, $p < 0.01$). This

aligns well with previous studies (e.g. Cordova and Scott, 2010; Cordova, 2013; Neumann et al., 2017), but differs from the findings of Fahmy (2008) and Boyd et al. (2024), who report various POLYLOBATES from Chloridoideae and Micrairoideae species.

Based on our analyses, we may suggest separating only several Panicoideae grass groups and genera based on their phytolith spectra. For *Saccharum* species, characteristic morphologies include short, wide, and elongated roundish BILOBATES in large abundances (Negrão and Driemeier, 2022), along with TRAPEZIFORM morphotypes in at least one-third of the BILOBATES' abundance, as well as SHORT SADDLES, KEELED RONDELS, and TWO-HORNED RONDELS. POLYLOBATE morphologies may (or may not) be present. Species separation is not feasible, especially since not only interspecies but also intergeneric hybrids have been reported for *Saccharum* sp. (e.g. Watson et al., 1992). Moreover, the phytolith morphology spectra of sugar canes can be confounded with those of Nepalese broom grass (*Thysanolaena latifolia*), which is a very common tall grass in India and is widely used for broom making - exported to Thailand and northern provinces of Laos (Sounthone and Viloune, 1998). The culms are also reported to be used in paper-making and small-scale construction, apparently for roofing (Niveditha and P Niveditha and Balarama, 2016). To distinguish the *Saccharum* sp./*Thysanolaena latifolia* group from *Setaria italica*, the presence of 4-LOBED SYMMETRIC CROSSES and POLYLOBATE phytoliths should be observed. The last two types are always present in *Setaria*.

Although BULLIFORM phytoliths are seldom observed in Pooideae (Chen et al., 2020), separation of *Vetiveria zizanioides* could be feasible if small BULLIFORM asymmetrical phytoliths with weakly cuneiform endings, wider tops, and weakly curved bottoms are observed along with BILOBATES (which are strongly dominant, as mentioned above), TRAPEZIFORM phytoliths, 4-LOBED SYMMETRIC CROSSES and KEELED RONDELS as the only RONDEL type in the material. If the described marker BULLIFORM phytoliths are absent (which can be the case, as they are not always produced, not abundant, and/or not well preserved), distinguishing *Vetiveria* remains doubtful. *Chrysopogon* species produce TALL FOUR-LOBE CROSSES (Boyd et al., 2024), which are "always more frequent than POLYLOBATES," with BILOBATES being more frequent than RONDELS. More than half of the BILOBATES are with short shanks, whereas BILOBATES with convex-concave ends are absent (Krishnan et al., 2000). This phytolith-based description of *Chrysopogon* sp. collected in Tamil Nadu (South India) clearly defines its morphological spectrum. TRAPEZIFORM types and TWO-HORNED RONDELS in *Chrysopogon zizanioides* are present but never dominate. *Panicum (miliaceum)* is characterized by the most diverse spectrum of SADDLES and RONDELS, with the highest percentages of LONG SADDLES (~21% of TPS). This may cause confusion with *Phragmites australis*, although reed-specific TALL SADDLES are always absent in *Panicum*. This makes the genus distinguishable from its most common confuser (the reed) and from other Panicoideae, which rarely produce the same amounts and diversity of SADDLES.

Large ($\geq 21 \mu\text{m}$) WAVY-TOP RONDELS are diagnostic phytoliths of *Zea mays* (Iriarte, 2003; Piperno, 2006a; Yost and Blinnikov, 2011). Hsu et al. (2000) refer to the same size BULLIFORM cells in maize leaves, but we did not observe any in our reference material. Other morphologies are redundant.

4.2. Grass phytoliths on the surface of PLMs: preservation and peculiarities

Grass husks (i.e. inflorescence bracts including glumes, lemmas, and paleas) and straw (dried stems and leaves) used for smoothing, cleaning, and polishing the surface of palm leaves during PLM preparation - to make them flat and suitable for engraving or writing (Poliakova et al., 2025) or when the ink has been applied and the excess needs to be removed - should be considered the main reasons for the diverse and abundant numbers of GSSCPs on the surface of PLMs we studied.

Both the use of grass material and the application of mechanical force are evidenced in regular microscopic observations: GSSCPs are

seen forced into the surface of the palm leaf (Figs. 2B, 3E, 3F, 3H, 3J, 3K, 4B, 4F, 4I), or in between the filaments of other non-palm plant material (Figs. 4B, 4F, 4G, 4H), mechanically broken (Figs. 4B, 4F), eroded (Figs. 3A, 4K), and as thin-brushed, flattened, smoothed phytoliths (Figs. 2A, 2E, 2G, 2H, 2I, 3B). There is hardly any other factor or process than brushing that could result in similar effects on phytoliths.

In general, the preservation quality of phytoliths in PLM samples can be described as ranging from sufficient to low. Many cells exhibit abnormal morphology not typically seen in phytoliths from reference grasses or sediments commonly used in botanical, archaeological, and paleoecological investigations. These abnormalities include unusually sharp features, brushed-off surfaces, cuts, cracks, and patina covers – these features are regularly observed in our PLM samples regardless of the manuscript's origin or collection. Such features can be misleading in terms of correct morphological identification. Conversely, these peculiarities also serve as evidence of active mechanical force applied to the surface of the palm leaves during their preparation for writing and/or at the end of the writing process to remove the excess ink. Palm leaves are naturally tough, especially the mature *Borassus* leaves. The grass phytolith material from the surface of all studied PLM samples demonstrates a similar level of degradation, except for phytoliths found in dust that has settled as a very thin, last material layer. This dust merely represents environmental contamination from storage conditions (which can be easily checked by comparing dust swabs from archive shelves and PLM dust contamination). However, as we demonstrated earlier (Poliakova et al., 2025), this contamination does not significantly contribute to the overall data and cannot compromise the results of phytolith analysis. Moreover, random phytolith fragments from dust contamination (if there are any) always demonstrate a stronger degree of erosion, which not only indicates their reworked nature, but also clearly separates them from the grass morphologies settled on the PLM folios' surface or driven into it during manuscript manufacturing.

4.3. Potential sources of grass material from the surface of PLMs

4.3.1. Possible use of Arundinoideae grasses for PLM preparation

Based on the presence of genus-typical NARROW BILOBATE phytoliths (Chauhan et al., 2011; Shakoor et al., 2016), we suggest the presence of *Arundo donax* grass material in samples TR1, TR4, and AL1. However, in TR1, TRAPEZIFORM phytoliths are absent, and RONDELS as well as SADDLES (including SAD_SHT and cells exclusively characteristic of other grass groups) are frequent and abundant in all three samples. PLATEAUED SADDLES of *Phragmites* sp. (Novello et al., 2012; Cordova, 2013; Boyd et al., 2024) are observed in samples EO1 - EO5, as well as in RE5, BM4, and BM5. However, the positions of the last three samples in the PCA plot (Fig. 9) are centered or shifted upwards due to the relatively high presence of RON_PYR. The complex nature of their phytolith assemblages precludes interpretation of their predominant material type. Most likely, several grasses were used in the preparation and curating of these PLMs. In addition, not only *Phragmites* but also Bambusoideae may develop SAD_PLA with a confusing appearance - especially under light microscopy (Biswas et al., 2016; Neumann et al., 2017; Cordova, 2023). Both reeds and bamboo styluses were used for engraving the letters on palm leaves, they could also have been used as sources of ash used for inks, but the last hypothesis needs more material evidence.

Arundo donax and *Phragmites* sp. are cosmopolitan tall reeds that frequently grow at water edges (Chen et al., 2020). These genera also have a widespread distribution - both globally and regionally - in temperate and tropical areas (Shakoor et al., 2016). Thus, it is difficult to determine whether the plant sources used for PLM production were local or regional. The use of these grasses in PLM preparation may suggest that the production site was located near a freshwater source, such as a river, lake, or pond bank.

4.3.2. Use of bamboos

OBLONG CONCAVE SADDLES (i.e. very TALL SADDLES) from leaf epidermis are diagnostic for Bambusoideae (Piperno and Pearsall, 1998; Yost et al., 2018) and are clearly observed in samples TR2, TR3, and TR5. The presence of these, in combination with BILOBATES, 4-CRS_TH, SAD_PLA, and BUL, makes it almost certain that bamboos (presumably leaf material) were used in the preparation of the source manuscripts. Regarding RE1, its grouping with all other samples described here should have been due to the maximal abundance of 4-CRS_TH (10%), but the presence of this morphotype alone - without the typical co-occurrence of PLATEAU SADDLES and BULLIFORM cells - does not allow an unequivocal interpretation. The presence of TALL SADDLES (SAD_TAL) in CM14 is controversial and could be attributed either to the use of Bambusoideae or Panicoideae grasses (Kealhofer and Piperno, 1998; Dai et al., 2023) in the preparation of CM14 or to the use of rice (Kondo et al., 1994; Strömberg, 2004; Biswas et al., 2016; Naskar and Bera, 2018b). The presence of LONG SADDLES in the same sample (at a low percentage, i.e. 9%) and our calculations of GSSCP co-occurrence in the reference grass material collected from South India and Southeast Asia (Fig. 7) make the use of Bambusoideae, especially *Dendrocalamus* sp. or other woody bamboos, more probable (Gu et al., 2016; Li et al., 2014; Tao et al., 2019). However, this does not entirely exclude the presence of Oryzoideae material.

4.3.3. Use of Desmostachya bipinnata and other Chloridoideae grasses

Relatively large amounts of SADDLES and FLAT RONDELS (TOWER phytoliths, RON_FT) indicate that Chloridoideae grasses were very likely used for the preparation and/or historical handling specifically of the EO1-EO5 manuscripts (EFEO collection, Puducherry, India). These manuscripts are from Alwarthirunagari or nearby areas (Table 1) and, probably, were produced by a limited number of individuals belonging to the same priestly family or small priestly community practicing a specific form of devotion to Vishnu as the supreme deity, called Sri Vaishnavism. Samples AL2, AL3, AL5, as well as TR1-TR5, have a complicated nature in terms of phytolith assemblages, although they are also characterized by relatively high abundances of SADDLES typical for Chloridoideae grasses (including subfamily-specific types) and distinct RONDEL morphologies (Boyd et al., 2024; Karmakar et al., 2024). This supports the assumption that C4 Chloridoideae grasses were involved in the preparation of these manuscripts, even if not predominantly, likely in combination with other grasses.

There is "no ritualistic activity without the usage of plant products" (Boddupalli, 2024) in Hindu rituals. Several Chloridoideae grasses hold sacred value, among which *Desmostachya bipinnata*, also known as "kusha", "darbha", or "pavitram", which has been a key part of many Hindu religious rituals since ancient times. For example, rings made from this grass, known as "darbha pavitram", are worn by priests and individuals during prayer rituals and when handling meaningful temple items. This grass is also used, even until now, in the decoration of temples (personal observations in Tamil Nadu). The production of a PLM may (but not necessarily must) be a ritual act, hence kusha grass may have been involved. However, manuscripts are generally not (at least not in the context of South Indian Hinduism) used in rituals. The texts of some of them may have been read aloud to an audience and that would be the only ritual in which they could have been used, although we cannot prove which manuscripts were used for such an activity. On the other hand, most texts of the sampled manuscripts have religious content, and they used to be actively handled by people performing rituals, or kept in the houses of priests and their families, who were expected to perform daily rituals. Besides, brushes with kusha (called *kūrccam* in Tamil, for instance) could have been used for oiling the manuscript at a final step of preparation. Thus, in the absence of credible literature, ethnobotanical surveys and experiments, it is difficult to evaluate, if the contact with brush(es) was enough to leave any amount of phytoliths on the PLM surface.

Other members of Chloridoideae that were possibly used include *Cynodon dactylon*, commonly referred to as "durva" and "doob" and associated with the god Ganesha, as well as *Imperata cylindrica* (locally also known as "darbha grass"). Our observations in Tamil Nadu (2024–2025) and Kerala (2024) show that both grasses are still used in temples as decorations and offerings to gods. Note also that Ganesha was often invoked as tutelary deity before copying a text in an empty manuscript. Hence, one cannot exclude the use of ritual grasses at certain stage(s) of PLM preparation for the EO1–EO5, AL2, AL3 (less for AL5, TR1–TR5) manuscripts, or their handling and/or worship with these grasses, which could explain the presence of their traces on the surface of these particular PLMs. Although, to accumulate such amount of Chloridoideae grass material as evidenced in EO1–EO5, close and/or long-term contact of palm leaves with these grasses would be needed.

4.3.4. Use of domesticated rice

The use of grass material is not only revealed by material analysis, but is also repeatedly described in the literature. The use of ink, which is carbon black (soot) mixed with an unspecified ink-binder and rubbed into the text incised on the palm leaves, then wiped off with water and cleaned with rice bran or hot sand, is described by Sah (2002). Indeed, we have clear evidence for the use of rice in South India. Rice produces three distinctive phytolith types: BILOBATE phytoliths in leaves and stems, double-peaked phytoliths in the husk, and BULLIFORM phytoliths in the leaves (Lu et al., 1997; Huan et al., 2015). In addition, the use of double-peaked rice phytoliths is suggested by Gu et al. (2013) and Zhao et al. (1998) to distinguish domesticated rice from wild rice based on multivariate linear discriminant function analysis and three-dimensional measurements. As we observed only very few double-peaked phytoliths in the PLM samples, there is not enough evidence to apply the proposed method. However, based on the simultaneous presence of BILOBATE phytoliths with scooped lobes (Pearsall, 1978; Lu and Liu, 2003) as well as BILOBATES with short or reduced shank (Bourel and Novello, 2020) in CM14, CM15, CM16, CM17, supported by the presence of highly decorated (with 8–12 decorations, Huan et al., 2015) BULLIFORM phytoliths in the same samples (Figs. 8 and 9), speaks for the use of domesticated rice. Chen et al. (2020) also indicated the presence of "cuneiform to nearly circular" grass BULLIFORM phytoliths in Oryzoideae, Bambusoideae, Arundinoideae and Chloridoideae. However, the total absence of NARROW BILOBATES and knobbed spine phytoliths in this research material (Shakoor et al., 2016) excludes the use of Arundinoideae for CM13–CM17 manuscripts, in our opinion. The use of Chloridoideae is also very unlikely due to the absence of subfamily-specific SADDLES (e.g. Novello et al., 2012; Neumann et al., 2017; Karmakar et al., 2024), SHORT SADDLES (Boyd et al., 2024), and the abundant presence of diverse RONDELS, which contradicts regional observations (Jattisha and Sabu, 2015). The indication of rice uses for *Corypha umbraculifera* manuscripts from SUB Hamburg was already reported in our previous study on phytoliths from PLMs (Poliakova et al., 2025). The use of any other Oryzoideae grasses for PLM preparation in South India is not evidenced in this research.

4.3.5. Use of Panicoideae grasses

As for Panicoideae, interpretation of their historical use in PLM production is the most difficult. On one hand, diverse Panicoideae grasses are known to be important in plant-related Hindu practices (see Table 1) and even directly in PLM making, such as *Chrysopogon zizanioides* (Sah, 2006), *Cymbopogon citratus* and *C. nardus* (e.g. Agrawal, 1984; Sah, 2002; Nishanthi and Wijayasundara, 2022; Kumar et al., 2009; Perumal, 2013; Wilson and Rice, 2019) - these two grasses are used most frequently, especially for their oil, *Panicum dichotomiflorum* (Joshi, 1995; Sahoo and Mohanty, 2004, 2007), *Vetiveria zizanioides* (Sah, 2006; Boddupalli, 2024). On the other hand, Panicoideae are mainly diagnosed at the subfamily level. Clear separation of genera is doubtful to impossible, especially when several grasses may have been applied simultaneously or one after another. To avoid misinterpretation,

and considering that in PLMs phytolith material comes from different, unconnected, and largely unknown sources, we apply a conservative approach. Diverse BILOBATES in combination with TALL FOUR-LOBE CROSSES in RE1 could be an indicator of *Chrysopogon* species (Boyd et al., 2024), though KEELED RONDELS frequent in Panicoideae (Piperno and Pearsall, 1998; Fahmy, 2008; Cordova, 2013) are absent in these samples. However, the presence of only two POLYLOBATE phytoliths, one TWO-HORNED RONDEL, and as little as 8% of trapeziform morphotypes does not support this interpretation. POLYLOBATES are generally scarce in our research material, only in BM2 and BM6 are three cells observed. In BM6, all typical panicoid phytoliths are present, and TRAPEZIFORM cells are also frequent enough to conclude on the presence of Panicoideae material. In the case of BM2, with rare SADDLES and RONDELS, which are not frequent enough, a positive conclusion is doubtful.

Samples AL5 and, to some extent, RE4 are special cases, both are characterized by short, wide, and ELONGATED ROUNDISH BILOBATES in quite large abundance (41% and 45%, respectively). In the presence of SHORT SADDLES and KEELED RONDELS, this could indicate possible application of *Saccharum* species (Negrão and Driemeier, 2022), which are C4 grasses, as well as the already discussed Chloridoideae evidenced in samples EO1–EO5 and AL2, AL3. The group of samples clustered in opposition to them (i.e. TR2, BM2, BM3, CM13–CM17, and RE2, RE5, BM5, BM6) cannot be grouped together according to the prevailing grass material revealed in them, but all these are C3 plants. Hence, the clear sample ordination depicted in Fig. 9 should be attributed to the nature of grasses in terms of their carbon fixation pathway, not only their taxonomy. The use of *Triticum* sp. and *Zea mays* for PLM preparation is uncertain. Prajapati (1995) and Meher (2009) indicate the use of wheat flour paste as an ink binder. Use of maize starch for the same purpose is also mentioned (Meher, 2009). As for the phytoliths, our research material does not provide enough evidence for either use. Single cells described from the PLM surface are not enough to make any credible assumptions, even in the case of very characteristic ones, i.e. LARGE CROSSES ($\geq 21 \mu\text{m}$) together with WAVY-TOP RONDELS (Iriarte, 2003; Piperno, 2006a; Witteveen et al., 2024). These are observed in samples of several manuscripts from Tamil Nadu, but their random and scarce nature should be attributed to contamination rather than to the purposeful use of the source material.

4.3.6. Evidence for presence of other grass material on the surface of PLMs

Although it is impossible to make a clear conclusion about the presence of *Hordeum vulgare* in any of our samples, the phytolith spectrum of TR1 - with abundant TOW and RON_FT phytoliths, the absolute absence of TRP, and the dominance of long dendritic cells in the category of 'OTHERS' - suggests it could have been used for this manuscript. Lata et al. (2022) and Boddupalli (2024) indicate the importance of barley for various rituals, but so far, we do not have evidence of its use in any practices involving PLMs. Thus, it must be admitted that our knowledge on the subject is incomplete. LONG and NARROW BILOBATE morphotypes, which Boyd et al. (2024) described as 'BILOBATES with narrow, convex or flat lobes and very long shanks', are exclusively characteristic of *Aristida* species, which were not included in our reference material. The cultural and ethnobotanical significance of *Aristida* spp., aside from use as sweeping brooms, handcraft weaving, and thatching, is not extensive. We observed these BILOBATES in RE2–RE5 (in most cases broken, with completely or partly brushed-off lobes), TR1, TR2 (burned and fire-deformed phytoliths), and in EO3. As *Aristida* brooms are commonly used in temples and yards up to the present day, their use alongside the processing of palm leaves for manuscript making is possible, and thus *Aristida* spp. phytoliths might represent historical contamination. Similarly, OVAL RONDELS with "tent-like" or "keeled" ridges in top view, as described by Fredlund and Tieszen (1994) and Yost and Blinnikov (2011) as specific morphology of reed canary grass (*Phalaris* cf. *arundinacea* L.), may also be present. This decorative tall grass has also been used historically as a raw material for paper pulp production and for fuel (Andersson and Lindvall, 1997).

5. Importance of historically accumulated material on the surface of manuscript folios

Clear statistical separation of samples in the CONISS-based cluster analysis (Fig. 8) shows that the main difference in phytolith assemblages occurred not due to the original provenance of the manuscripts (although that was the second most important factor), as initially expected, but rather due to the amounts of preservation of material accumulated on individual folios over time. PLM samples from SUB Hamburg, which have never been brushed or cleaned since collection, are clustered separately from those in the three Indian archives, where manuscripts undergo, or used to undergo in the past, regular curation, including brushing and sometimes re-oiling with lemongrass or citronella oil. While such curation is meant to ensure care and preservation, it not only removes dust but may also unintentionally remove or disrupt the ultra-thin layers of valuable material that have accumulated due to preparation, preservation, transportation, reading, and handling. This effect is especially pronounced on the first and last folios and the folio margins that experience the most contact. These layers, built up over centuries, provide a unique opportunity for future scholars to study the material history of manuscripts. In palaeoecology, with both traditional methods (such as phytolith analysis presented here) and modern molecular approaches, these layers are a significant source of data, as PLM production practices have almost disappeared today and were never properly documented. Statistically verified multi-proxy palaeoecological reconstruction is now the only way to investigate them. Minimally invasive sampling - based on collecting and analyzing manuscript fragments that have fallen off - can help answer questions about manuscript provenance, the history of PLM preparation, trading, looting, handling, and conservation. However, each additional cleaning removes more material, reducing the possibility of recovering centuries-old information. We strongly suggest reconsidering current brushing and cleaning practices, especially recurrent ones. Moreover, swabbing for pest identification, microbiome or fungi analysis, DNA studies, proteomics, and metabolomics should be performed only minimally, with a lot of care, from the margins of selected folios, and must never be allowed along the entire folio.

6. Summary and conclusions

Fallen off palm-leaf manuscript samples from South India, namely from Kerala (15 samples of *Borassus flabellifer* manuscripts) and from Tamil Nadu (10 samples of *Borassus flabellifer* manuscripts and 5 samples of *Corypha umbraculifera* manuscripts), were analysed for GSSCPs to reveal the pattern of grasses applied for PLM preparation. This study represents the first attempt in paleoecology to mathematically describe positive and negative co-occurrence patterns of phytolith morphologies using reference research material (grass herbarium samples) and to apply these patterns for the quantitative interpretation of novel research material (i.e. PLM samples) containing grass phytoliths from unknown sources, with the aim of identifying those sources. The results provide unique insights into historically lost manuscript-making practices and can potentially serve future palaeoecological reconstruction. Based on the data obtained and analysed in this study, the following conclusions can be drawn:

- Phytolith analysis of PLM samples allows for the study of regional and subregional peculiarities in PLM production in South India and reveals these differences at the level of grass subfamilies (in some cases at the level of genera, i.e. *Saccharum* sp. and *Oryza* sp.) with reliable statistical significance.
- Grass phytolith diversity in the PLM samples from South India comprises at least 28 different GSSCPs, with an average number of 10.3 ± 2.9 morphotypes per sample. The amount of GSSCPs in PLM

samples is generally low, research material requires several rounds of filtration in order to reach a concentration suitable for preparation of the microslides.

- Preservation of material varies from sufficient to low, with many phytoliths being mechanically broken, half-brushed off, eroded, and covered with patina. Many GSSCPs are forced into the surface of the palm leaves. Phytoliths with abnormal, unnatural morphology are regularly observed. This, on one hand, can hinder correct identification of the phytoliths, and requires the use of liquid instead of solid mounting media to ensure rotation of the cells in the slides, good optics, and strong skills in microscopy. On the other hand, these features provide additional evidence of active mechanical use of grass material when brushing, smoothing, and polishing palm leaves prior text writing or after inking.
- Both CONISS-based clustering and PCA-based ordination, applied independently, reveal that in most cases samples of manuscripts with closer geographical proximity demonstrate more similarity. This is the case with EO1-EO5 samples (all originally from Alwarthirunagari in Valutivalanatu region, Tamil Nadu, the probability of similar origin is ca 0.82 at $p < 0.01$) and with TR1-TR5 (all provisionally from Thrissur, Kerala, similarity is ca 0.64 at $p < 0.01$).
- In ordination, two significantly different groups of PLM samples were identified. For the first group (EO1-EO5, AL2, and AL3) Chloridoideae grass material was evidenced. The second group includes samples TR2, BM2, BM3, CM13-CM17, and RE2, RE5, BM5, BM6, where the use of rice, bamboos, and panicoids was evidenced. The difference between groups reflects the nature of grasses in terms of their carbon fixation pathway (C4 in the first group vs. C3 in the second group), not only their taxonomy.
- In PLM samples from Kerala, *Arundo donax* (TR1, AL1), *Phragmites* sp. (EO1-EO5), Bambuseae (TR2, TR3, TR5), Chloridoideae (EO1-EO5, AL2, AL3 - most likely *Desmostachya bipinnata*, possibly with some role of *Cynodon dactylon* and/or *Imperata cylindrica*, as main grass source, AL5, TR1-TR5 as admixture) were identified.
- In PLM samples from Tamil Nadu, *Phragmites* sp. (RE5, BM4, BM5), domestic rice (CM14-M17, with possible admixture of Arundinoideae and/or Chloridoideae material), and Panicoideae (RE1, BM2) were identified. For RE1, either woody bamboo or rice, or a mixture of both, was used. For CM14, woody bamboo *Dendrocalamus* sp. was most likely applied, but the presence of panicoid grass material is also possible.
- The main difference in the PLM samples lies not only in the manuscript's original provenance, but also in the amount of preserved material historically accumulated on the folio surfaces. Manuscript samples from SUB Hamburg, which have never been cleaned since collection, were separated from PLMs stored in Indian manuscript archives, where brushing was or is a recurrent practice (as in Kerala archives, EFEO and IFP Puducherry).
- To conserve unique material accumulated on PLM folios and make it available for future studies, practices of brushing and cleaning manuscripts should be reconsidered. Sampling involving swabbing has to be applied with control and restrictions.

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Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.revpalbo.2026.105610>.

Data availability

The datasets analyzed for this study can be found in the: Zenodo depository.

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