Evidence for mid-Holocene rice domestication in the Americas

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Abstract

The development of agriculture is one of humankind's most pivotal achievements and questions about plant domestication and the origins of agriculture have engaged scholars for well over a century [1-5], with implications for understanding its legacy on global subsistence strategies, plant distribution, population health[6], and the global methane budget [7]. Rice is one of the most important crops to be domesticated globally, with both Asia (*Oryza sativa* L.) and Africa (*Oryza glaberrima* Steud.) discussed as primary centres of domestication[8]. However, until now the pre-Columbian domestication of rice in the Americas has not been documented. Here we document the domestication of *Oryza* sp. wild rice by the mid-Holocene residents of the Monte Castelo shell mound starting at *ca.* 4000 cal yr BP, evidenced by the selection of increasingly larger seed size. Our data provide evidence for the domestication of wild rice in a region of the Amazon that was also the likely cradle of domestication of other major crops such as cassava (*Manihot esculenta*), peanut (*Arachis hypogaea*) and chilli pepper (*Capsicum* sp.)[4]. The results underlines the role of wetlands as prime habitats for plant domestication worldwide[9-11].

More than half of the world's population depend on rice for more than 20% of their daily calories [12]. Modern global consumption is dominated by varieties of the domesticated Asian (*Oryza sativa* L.) and African (*O. glaberrima* Steud.) species [13], which were domesticated in the early Holocene in the Yangtze River, China[8], and ca. 2000 cal yr BP in West Africa [14]. Long before the introduction of these species to the New World by Europeans in the 18th century[15], wild rice was already a seasonal staple of indigenous subsistence. Growing in seasonally flooded areas that compose up to 10% (1.4M km^2) of lowland South America (Fig. S1), wild rice is a particularly important resource during the rainy season when flooding causes other resources to be dispersed and scarce[16]. Early 16th-19th century historical and ethnographic accounts report extensively on the consumption of wild rice species by indigenous groups in lowland South America (Supplementary Note 1). However, despite the occasional reference to its potential role in pre-Columbian diets[17,18], the domestication of rice has not yet been investigated in this region. *Oryza* sp. phytoliths recovered from mid-Holocene levels of a shell mound in southwestern Amazonia (Fig. 1) dating to ca. 4000 cal yr BP show the progressive selection of larger wild rice seeds by its pre-Columbian residents, whom were already engaged in the cultivation of maize (*Zea mays*) and squash (*Cucurbita* sp.) [4].

Dating back to ca. 10,000 cal yr BP, a diversity of coastal [19] and freshwater [20] shell mounds represent some of the oldest forms of human occupations across lowland South America, some of which are associated with the earliest ceramics on the continent[21]. Our study site, the Monte Castelo residential shell mound is located in the Upper Madeira basin of SW Amazonia, Rondônia state, Brazil. The region is characterised by a seasonally flooded tropical wetland exhibiting gallery forest along the larger streams, which are dotted with anthropogenic shell mounds [20]. Monte Castelo is a 6.3 m high platform-shaped freshwater shell-mound, exhibiting a 160 m long elliptical base (Fig.1) and dating from 9400 cal yr BP[22,23]. The first excavation of Monte Castelo by Miller [24] in 1984, revealed a seven-meter-deep stratigraphy bracketing a long-term occupation from 9130 to 667 cal yr BP (Supplementary Table 1). Miller defined three major and one transitional occupation phases including: Cupim phase (700-685 cm; 9130-7701 cal yr BP), Sinimbu phase (670-275cm; 7701-4822 cal yr BP), Sinimbu-Bacabal transitional stratum (275-220 cm; 4862-4388 cal yr BP) and Bacabal phase (220-30 cm; 4388-

689 cal yr BP)[24]. Renewed excavations at Monte Castelo in 2014 and 2016 by the Laboratory of Tropical Archaeology of the University of São Paulo reached a depth of 640 cm, uncovering ten archaeological strata across the Sinimbu to Bacabal phases dating from 5310 cal. yr BP. to 689 cal yr BP (Fig. 3k).

Four endemic species of the *Oryza* genus occur in lowland Latin America with a tropical-subtropical distribution from 23^0 N in Cuba to 34^0 S in the Paraná River delta, including the diploid (2n = 24, AgpAgp) *O. glumaepatula*, and three tetraploids (2n = 48, CCDD) *O. alta*, *O. grandiglumis* and *O. latifolia* [25] (Fig. S1). The Oryzeae tribe produce four distinct
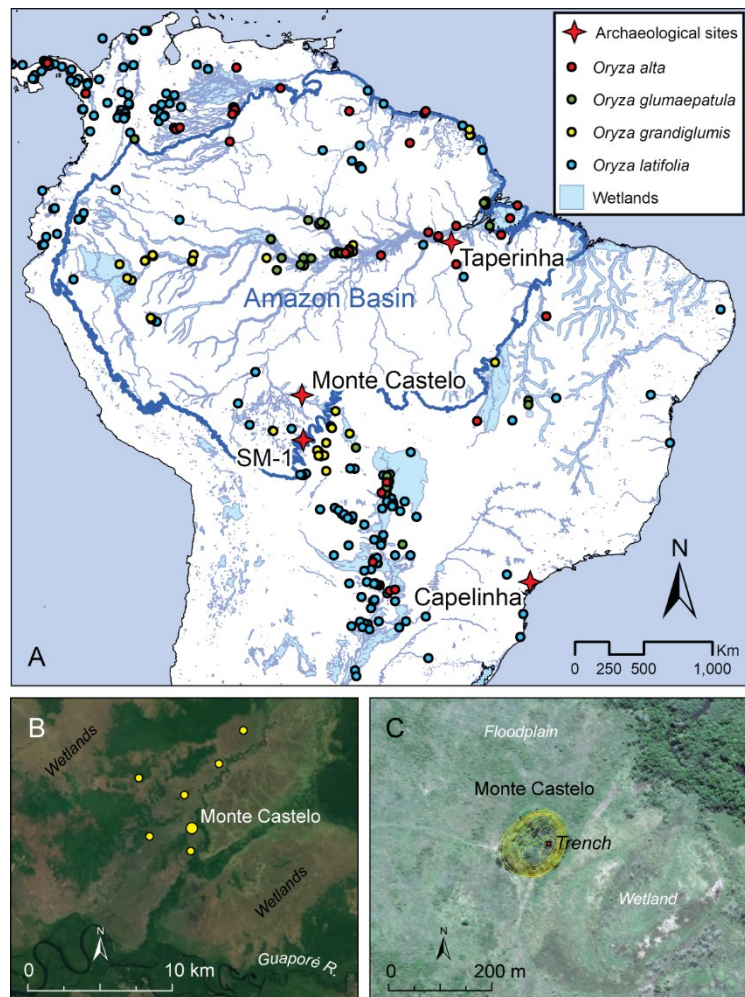

Figure 1. A. Map showing the location of the Monte Castelo and other important early Holocene shell mound sites in South America. B. The Monte Castelo locality, topographical map, and location of the 2014 trench excavation.

phytoliths associated with different parts of the plant: 1) cuneiform keystone bulliform cell phytoliths exhibiting fish-scale decorations on the fan edges are produced in the leaves (Fig. 2d); 2) 'scooped'-shaped bilobates are produced in the leaves and stems (Fig. 2e); 3) double-peaked glume cells (Fig. 2a-b, f-i); and 4) deeply serrated phytoliths, are derived from the epidermis of the seed glume (husk), and are further diagnostic to the *Oryza* genus (Fig. 2c)[26-28]. Pearsall [26] and Zhao et al.[27] have demonstrated a clear correlation between phytolith size and seed size in Asian rice. Zhao et al. [27] devised a discriminant function to differentiate assemblages of wild from domesticated *Oryza* rice using five different size measurements of the double-peaked glume cells including: 1) Top Width (TW): the distance between the two peaks of the projecting hairs; 2) Maximum Width (MW): the width at the point where the glume projection attaches to the base; 3-4) Height of each hair (H1, H2): length from the tip

to the base of the hair, H2 is defined as the smaller measurement; and 5) the Curve Depth (CD): distance from the tip of H1 to the lowest point of the curve (Fig. 3m, Supplementary Note 2).
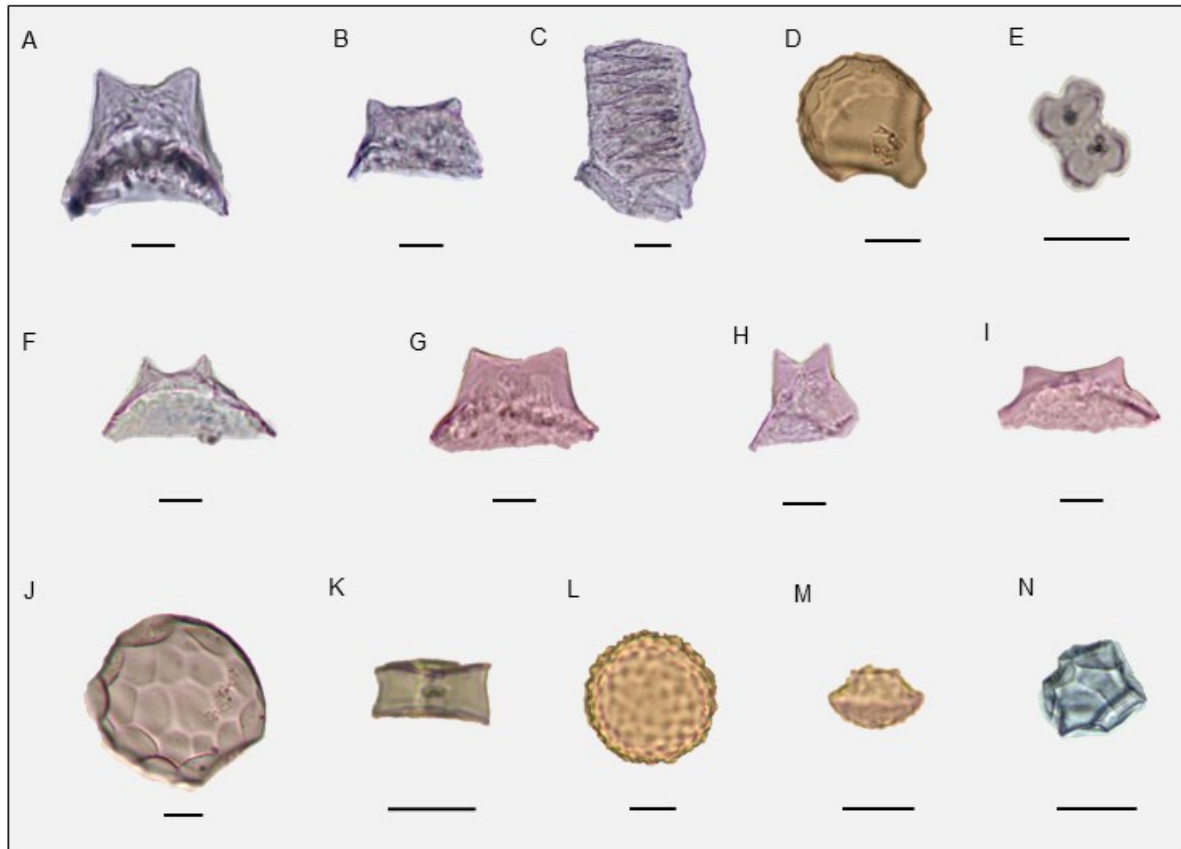

Figure 2. Microphotographs of phytolith morphotypes recovered at the Monte Castelo shell mound and modern reference wild rice species analysed. **A-E**. *Oryza* sp. phytolith morphotypes recovered in the Monte Castelo shell mound: **A.** double-peaked glume (Layer A); **B.** double-peaked glume (Layer J); **C.** deeply serrated body (Layer C); **D.** cuneiform keystone bulliform (Layer D 130-140cm); **E.** scooped bilobate (Layer E). **F-I.** Double-peaked glume phytoliths from modern wild-rice species native to the study area: **F.** *O.alta* (PRI-1); **G.** *O.latifolia* (Arg-5); **H.** *O.grandiglumis* (SO-23); **I.** *O.glumaepatula* (SO-17). **J-N.** Crops and other native edible plants recovered in the Monte Castelo shell mound: **J.** scalloped sphere from the rind of squash (*Cucurbita* sp.)(Layer F); **K.** wavy-top rondel from the cob of maize (*Zea mays*)(Layer C); **L.** large globular echinate from Arecaceae (Layer J); **M.** conical to hat-shaped phytolith from Arecaceae (Layer H); **N.** spherical facetate from Annonaceae (Layer C); Scale bar= 20 μm.

To investigate the potential domestication of wild rice by the Monte Castelo residents we analysed both archaeological samples and modern wild rice reference material. A total of 16 archaeological sediment samples, from across all ten levels uncovered during the 2014 Monte Castelo excavations (Fig. 3), and 19 modern specimens from the four wild species of rice occurring in South America, were analysed for phytoliths following standard procedures[29] (Supplementary Methods, Supplementary Table 2). All archaeological sediment

samples analysed yielded phytoliths of wild rice. Our analysis shows a clear increase in the proportion of rice morphotypes in the total phytolith assemblage from 6.4% on average in the Sinimbu phase occupation (Layers J-H) to 14.4% in the more recent Bacacal phase, suggesting that rice played a larger role in diet over time (Fig. 3f). There is also an increase in the proportion of *Oryza* seed phytoliths from the lower to the upper levels of the mound reflected in the husk:leaf+stem ratio. For example, during the Sinumbú phase (Layers J-I; 280-460cm) *Oryza* sp. seed phytoliths represent on average 3.4% of the total assemblage while Oryzeae leaf and stem phytoliths constitute on average 3%, a 1/1 ratio. During the
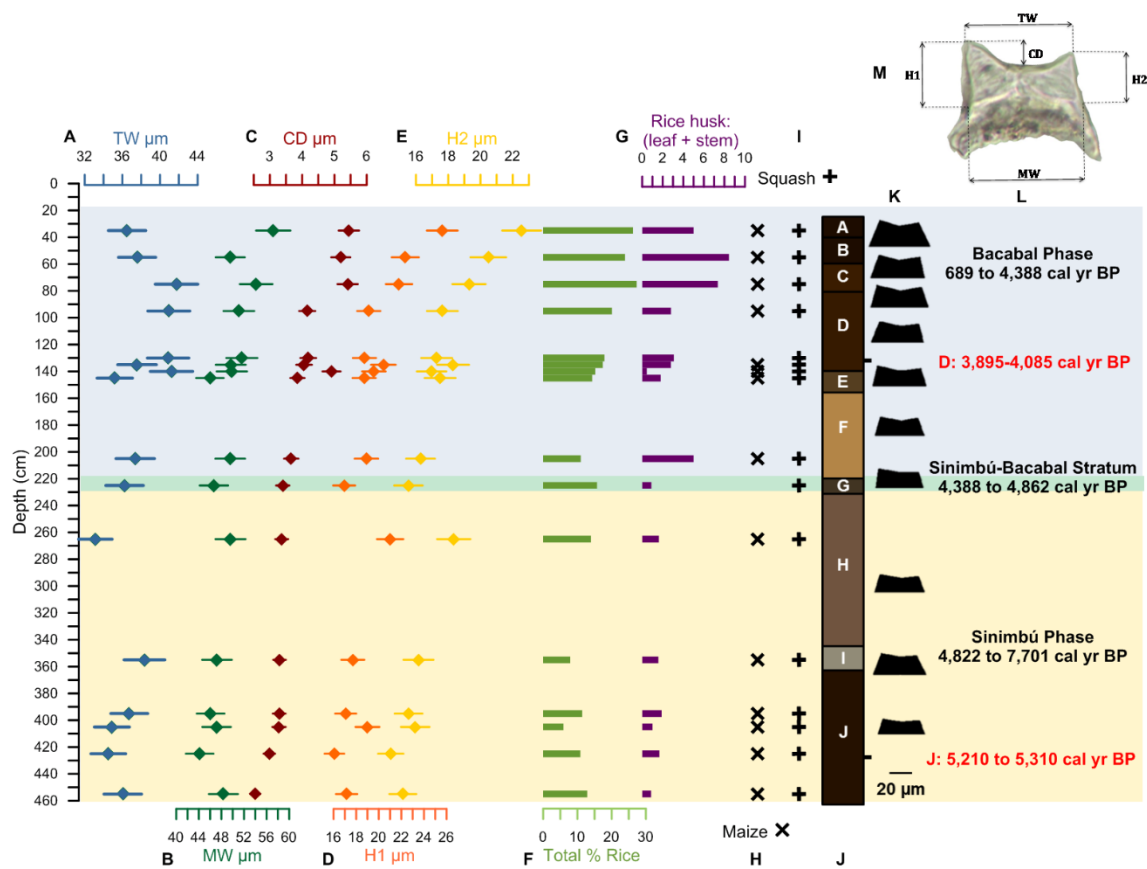

Figure 3. Sketch stratigraphic diagram of the 2014 Monte Castelo shell mound excavation layers showing: **A-E.** Mean and two standard deviations of the metric attributes of *Oryza* sp. double-peaked glume phytoliths: **A.** TW, Top Width, **B.** MW Maximum Width, **C.** CD, Curvature Depth, **D.** H1, Height 1 and **E.** H2, Height 2; **F.** Percentage of rice phytoliths to total phytolith assemblage*;* **G.** *Oryza* husk:leaf+stem ratio; **H.** Presence of *Cucurbita* scalloped spheres; **I.** Presence of *Zea mays* wavy top rondels; **J.** Monte Castelo stratigraphy; **K.** Sketch drawings of double-peaked glume phytoliths using the average of the five metric attributes for each archaeological layer; **L.** Monte Castelo cultural chronology; **M.** Metric attributes of Oryza double-peaked glume phytoliths.

Bacabal occupation (Layers F-A; 30-210 cm) *Oryza* seed phytoliths constitute on average 12% of the total assemblage while leaf phytoliths constitute on average 3.5%, a ratio of 3.4/1,

over three times the relative proportion of seed husks as occur in the Sinimbu occupation (Fig. 3g). The collection and flailing of wild rice in canoes in the Americas should leave leaf and stem bulliform and bilobate phytoliths in the place of harvest while double-peaked and deeply serrated glume phytoliths should be more abundant at residential sites where the grain is brought for consumption. Therefore, the increase in the ratio of husk:leaf+stem Oryzeae phytolith morphotypes suggests that the Monte Castelo residents became more efficient harvesters over time, bringing more grain and fewer leaves to the site (Supplementary Note 2).

The analysis of the average size of the attributes measured on the *Oryza* glume phytoliths (Fig.3 and Fig. S3) shows a gradual increase in Height (H1, H2) and Width (TW, MW) through time. Mean H1 values increase ca. 8μm (17μm to 25μm) and H2 increases ca. 7μm (15μm to 22μm)
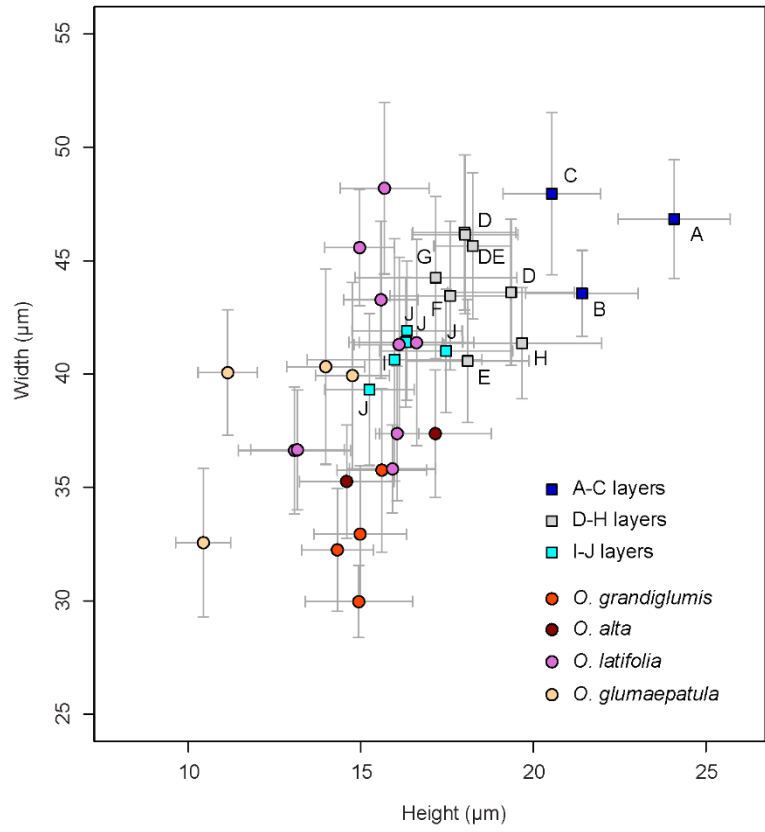

Figure 4. Mean height (H1+H2/2) and width (MW+TW/2) of all *Oryza* phytolith specimens showing that archaeological specimens are larger compared to botanical specimens, and an increase in phytolith size through time.

from Layers J to A. MW increases 9μm (48-57μm) through the stratigraphy. Mean CD values are larger in the upper occupation layers (A-H) compared to its initial dimensions in Layers I-J (Fig. 3). We used Principal Component Analysis (PCA) of modern reference wild species to

determine the variables that best explained phytolith shape differences among specimens, which are the two highly correlated height and width measurements (Supplementary Note 3, Figs. S4-7). Following Zhao et al.[27], therefore, we created a simple model of phytolith size to characterise the changes in phytolith morphology through time. One-way ANOVA with a Bonferroni corrected p-value shows phytoliths in the upper archaeological layers (A - D) are significantly larger than those in Layer J and wild reference specimens (Supplementary Table 3). Fig. 4 illustrates mean height and width of all *Oryza* phytolith specimens, showing an increase in phytolith size through time. The data show a significant shift towards bigger phytoliths compared to wild specimens began in Layers D-E (Fig. 3k) around 4000 cal yr BP. Phytolith size in lower archaeological layers were not significantly different from some botanical specimens (*O. latifolia, O. alta*) (Supplementary Table 2). Phytolith data also show that subsistence strategies of the Monte Castelo residents were based on a mixture of wild and domesticated resources including cultivars such as maize and squash as well as other plants of economic importance including soursop (*Annona* sp.) and palm fruits (Fig. 2 j-m, Fig. S1).

Our results indicate a significant increase in the size of double-peaked glume phytoliths across the Monte Castelo occupation starting around 4000 cal yr BP. Wild rice constituted an important seasonal resource for the Monte Castelo residents, who began to husband wild rice stands at lake or river edges. The phytolith data show that wild rice was modified by human intervention to produce larger grains, exceeding the range of variation found in the lower levels of the Monte Castelo shell mound and the modern populations of wild rice. The arrival of Europeans to the American continent in AD 1492 with the consequent population decimation and impact on cultural practices may have caused the domesticated traits to gradually disappear, a phenomena that has also occurred for other indigenously domesticated species in the Americas[30].

The presence of phytoliths from known cultigens, such as the wavy-top rondels of maize and scalloped spheres from squash, in the strata analysed shows that both crops were commonly grown in the region from at least 5300 B.P. onwards (Fig. S1). This in turn, indicates that the Monte Castelo shell mound residents began to systematically select larger rice seeds when they were already engaged in the cultivation of maize and squash. While in other regions of the Americas, wild grasses such as Setaria decrease in importance or are replaced by maize [31], the opposite trend is apparent in the Monte Castelo record. Wild rice was domesticated and

increased in importance a considerable time after Monte Castelo residents had become engaged in farming practices.

Our study highlights the importance of wetlands for the adoption and intensification of agriculture[9-11]. The results contribute to a broader understanding of how wetlands and the seasonal tropical forests of the Amazon may have been critical for early human settlement and the origins of food production in the Americas. This domestication process took place in a region that was likely the cradle of domestication for cassava, peanuts and chilli peppers pointing to the importance of this region of South America[4].

Our research has implications for sustainable Amazonian futures. Modern intensive breeding for high yield and pest resistance has narrowed the genetic diversity of cultivated rice leaving crops more susceptible to disease and less adaptable to the effects of climate change. Understanding the process of rice manipulation by ancient Native Americans and the role of South American native varieties could help provide more resistant high-yielding varieties, and provide further knowledge for plant breeders interested in the introgression of genes from wild *Oryza* species into modern rice varieties[25].

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Supplementary Information

Evidence for mid-Holocene rice domestication in the Americas

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Supplementary Methods

Phytoliths were identified and counted under a Zeiss Axioscope 40 light microscope at 500X magnification. Phytoliths from slides were scanned at least three times at 200 to count. Phytolith identifications were made using published material for the Neotropics and the Oryzoideae family [1-7] and by direct comparison with the phytolith reference collection of the Archaeobotany and Palaeoecology Laboratory in the Department of Archaeology of the University of Exeter. When possible, we followed the criteria of the ICPN group for naming phytolith types [8]. Identification of Poaceae phytoliths was based on a morphological classification first proposed by Twiss et al. [9], and later modified or refined by various researchers by taking into account criteria based on three-dimensional morphology and other micromorphological features [4,5,10-12]. In addition, each slide was scanned until the first 20 double-peaked glume cells were encountered. Phytolith diagrams were made using C2 software [13]. Following Zhao et al.[6], the five metric attributes (Fig. 3m) were measured from 20 *Oryza* double-peaked glume phytoliths from each of the archaeological and modern samples totalling 700 phytoliths.

Supplementary Note 1. Historical and ethnographic account of rice harvesting and consumption in lowland South America.

In lowland South America, 16th-19th century historical and ethnographic accounts [14-20] report extensively on the consumption of wild rice species by indigenous groups. Similar to the traditional North American canoe-and-flail harvesting method, native South American people were reported to harvest wild rice by beating the grains of mature inflorescences into their canoes with wooden poles [16,19,21,22]. Native Americans living in the Great Lakes region of North America were known to roast the grain, pour it into deerskin-lined pits, and trample it to remove the husks [23]. South American accounts hint towards the importance and culinary practices involving wild rice. For example, De Azara[15] mentions the consumption of an unknown type of rice in southern Paraguay that "… feed a nation of approximately seventy warriors". Cardim [14] mentions that wild rice was mixed with maize to make bread, and Acosta [24] describes its consumption in the form of a fermented brew, similar to wine. Today, the native Guató communities in the Pantanal still consume the wild native species *Oryza glumaepatula* and *O. latifolia* by sun drying the seeds, peeling them, and boiling them [25,26].

Supplementary Note 2. Taxonomy and phytolith production of the Oryzideae.

Taxonomy. Oryzoideae (syn. Ehrhartoideae) is a subfamily of the true grass family Poaceae that includes around 120 species in 20 genera. The Oryzeae tribe within the Oryzoideae subfamily consists of twelve genera and is distributed in tropical and temperate regions worldwide. Five of these twelve genera occur in South America: *Leersia*, *Luziola, Rhynchoryza*, *Zizaniopsis*, and *Oryza* [27,28]. The *Oryza* genus comprises 22 known wild species. Four of them are endemic to Latin America with a tropical-subtropical distribution from Cuba 23^0 N to the Paraná River delta 34^0 S, including the diploid (2n = 24, $A^{gp}A^{gp}$) *O. glumaepatula*, and three tetraploids (2n = 48, CCDD) *O. alta*, *O. grandiglumis* and *O. latifolia*

[29](Fig. S1). Oryza spp. rice are all aquatic emergent macrophytes that grow along rivers, lakes and wetland margins. They include annual, biannual, and perennial species with seedling recruitment occurring during the dry season and a short reproductive phase generally starting directly after the flood peak [30]. *Oryza* spp. have a nutty flavour, and firm consistency. Preliminary studies on *O. glumaepatula* show that it has high levels of total protein, albumin, and glutelin fractions, which compares favourably with *O. sativa* commercial cultivars [31].

Phytolith production. The Oryzoideae subfamily produce four distinct phytoliths associated with different parts of the plant. The Oryzeae tribe produce: 1) cuneiform keystone bulliform cell phytoliths exhibiting fish-scale decorations on the fan edges are produced in the leaves (Fig. 2d) and 2) 'scooped'-shaped bilobates in the leaves and stems (Fig. 2e). The *Oryza* genus produce: 3) double-peaked glume cells (Fig. 2a-b, f-i); and 4) deeply serrated phytoliths both derived from the epidermis of the Oryza seed glume (husk) (Fig. 2c)[5,6,32]. Domestication causes a gradual increase in plant size from wild to domesticate as a result of selective exploitation [33,34]. As the plant become larger, so do the phytoliths. The increase in phytolith size has been documented in *Zea mays* [7], *Cucurbita* [35] and *Musa* bananas [36], where larger fruits and seeds often yield considerably larger phytoliths. Pearsall [5] and Zhao et al.[37] have demonstrated a clear correlation between phytolith size and seed size in Asian rice. Zhao et al. [6] devised a discriminant function to differentiate assemblages of wild from domesticated Oryza rice using five different size and shape measurements of the double-peaked glume cells. These metric attributes are: 1) Top Width (TW): the distance between the two peaks of the projecting hairs; 2) Maximum Width (MW): the width at the point where the glume projection attaches to the base; 3-4) Height of each hair (H1, H2): length from the tip to the base of the hair, H2 is defined as the smaller measurement; and 5) the Curve Depth (CD): distance from the tip of H1 to the lowest point of the curve (Fig. 3m).

The presence of diagnostic *Oryza* phytoliths produced in the different parts of the plant has allowed the detection of crop processing stages[38] and different agricultural techniques [39] in Asia. For example, the distinctive bulliform and bilobate phytoliths from Oryzeae leaves and stalks are representative of the early stages of harvesting and processing, while the *Oryza* husk double-peaked glumes represent later stages of processing, such as pounding, winnowing and storage. The collection and flailing of wild rice in canoes in the Americas should leave leaf and stem bulliform and bilobate phytoliths in the place of harvest while double-peaked and deeply serrated glume phytoliths should be more abundant at residential sites where the grain is bought for consumption.

Supplementary Note 3. Multivariate statistics

Principal Component Analysis (PCA) of the five size measurements of *Oryza* phytoliths was performed on the modern wild reference (botanical) dataset to determine the key variables that define phytolith morphology (Fig. S4). Height (H1, H2) and Width (TW, MW) measurements are each shown to be highly correlated and comprise most of PCA axis 1 (60%). PCA of archaeological material (Fig. S5) and all specimens (archaeological and botanical) (Fig. S6), confirm the findings that Height and Width measurements are key defining variables. All PCA analyses were performed in R package Community Ecology package 'vegan' version (2.2-1)[40].

Supplementary Table 1. Radiocarbon dates from the Monte Castelo shell mound, Rondonia State, Brazil.

Layer	Depth (cm)	Phase	Radiocarbon date B.P.	Cal.yr. B.P.	Material dated	I.D.	Reference
A	10-15		810±70	805-625 (85%)	Charcoal	B103185	Miller (2009)[41]
A	20-30		2,475±105	2,756-2,306 (94.5%)	Shell	SI6843	Miller (2009)
A-D	10-110		2,270±105	2,493-1,992 (92.5%)	Shell	SI6844	Miller (2009)
D	110-120	Bacabal	3,160±70	3,482-3,140 (94%)	Charcoal	B66309	Miller (2009)
D	120-130		3,700±30	4,085-3,895 (95%)	Charcoal	B408413	Neves et al. (2016)[42]
D	130-140		4,810±90	5,662-5,305 (95%)	Charcoal	B66310	Miller (2009)
F	160-170		3,945±110	4,628-3,984 (94%)	Shell	SI6845	Miller (2009)
F	210-220		3,920±85	4,526-4,078 (93,5%)	Shell	SI6847	Miller (2009)
G	230-240		4,455±100	5,318-4,827 (95.4%)	Shell	SI6852	Miller (2009)
H	230-240		4,395±70	5,285-4,822 (92.7%)	Charcoal	SI6848	Miller (2009)
J	430-440	Sinimbú	4,570 ± 30	5,310-5,210 (95%)	Charcoal	B408414	Neves et al. (2016)
**	600-610		6,316±105	7,424-6,938 (95.4%)	Charcoal	SI6850	Miller (2009)
**	600-610		5,065±85	5,923-5,599 (95.4%)	Charcoal	SI6849	Miller (2009)
**	610-620		5,605±95	6,570-6,182 (93.3%)	Charcoal	SI6853	Miller (2009)
**	610-620		5,165±80	6,021-5,655 (92.5%)	Shell	SI6854	Miller (2009)
**	620-630	Cupim	7,010±80	7,959-7,661 (95.4%)	Charcoal	B118274	Miller (2009)
**	650-670		5,970±80	6,970-6,540 (95.4%)	Charcoal	B118275	Miller (2009)
**	685-700		8,350±70	9,478-9,090 (95.4%)	Charcoal	B103187	Miller (2009)

Supplementary Table 2. Provenience of wild rice modern reference material.

Species	Population identification	Hydrographic basin
O.grandiglumis[1]	PA-1	Solimões
O.grandiglumis	SO-23	Solimões
O.grandiglumis	PU-1	Purus
O.grandiglumis	JA-1	Japurá
O.alta	RI-1	Ribeira
O.alta	RI-2	Ribeira
O.glumaepatula	SO-17	Solimões
O.glumaepatula	JA-4	Japurá
O.glumaepatula	RB-9 - Dauau Lake	Negro
O.glumaepatula	Pu-1	Purus
O.latifolia	Arg-11	Paraguai/Paraná
O.latifolia	Arg-5	Paraguai/Paraná
O.latifolia	Arg-7	Paraguai/Paraná
O.latifolia	Arg-8	Paraguai/Paraná
O.latifolia	E-00814355 / 1926*4	
O.latifolia	E-00814354 / 1926*3	
O.latifolia	E-00814353 / 1926*2	
O.latifolia	E-00258728 / 1926*1	
O.latifolia	E-00258733 / 1926*10	
O.sativa	E-00258732 / 1926*9	
O.sativa	E-00258729 / 1926*6	
O.sativa	E-00258730 / 1926*7	

[1] Except for the last eight samples, wild rice seed samples were obtained from a collection of the Genetics Dept., at Escola Superior de Agricultura "Luiz de Queiroz", University of São Paulo.

Supplementary Table 3. Results of pairwise comparison of overall phytolith size (mean Height * mean Width) of archaeological and wild reference specimens using a Bonferroni corrected p-value. Highly significant differences among samples ($p<0.001$) are shaded in dark grey. Significantly different specimens ($p < 0.05 – 0.01$) are shown in light grey. Phytoliths in the A layer are largest compared to all specimens, and phytoliths in the DE layers show a shift towards larger specimens.

	A	B	C	D	DE	E	F	G	H	I	J	OALT	OGG	OGP
B	0.537													
C	1.000	1.000												
D	0.000	1.000	0.622											
DE	0.002	1.000	1.000	1.000										
E	0.000	0.495	0.026	1.000	1.000									
F	0.000	1.000	0.141	1.000	1.000	1.000								
G	0.000	1.000	0.238	1.000	1.000	1.000	1.000							
H	0.002	1.000	1.000	1.000	1.000	1.000	1.000	1.000						
I	0.000	0.056	0.002	1.000	1.000	1.000	1.000	1.000	1.000					
J	0.000	0.000	0.000	0.004	0.522	1.000	1.000	1.000	0.719	1.000				
OALT	0.000	0.000	0.000	0.000	0.006	1.000	0.348	0.193	0.009	1.000	1.000			
OGG	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.059	0.000	1.000		
OGP	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.041	0.000	1.000	1.000	
OLAT	0.000	0.000	0.000	0.000	0.011	1.000	0.982	0.526	0.019	1.000	1.000	1.000	0.001	0.000

Fig. S1. Distribution of *Oryza* species and wetlands in South America. Species occurrences from the Global Biodiversity Information Facility[43]. Wetland areas from the Global Lakes and Wetlands Database, World Wildlife Fund (https://www.worldwildlife.org).
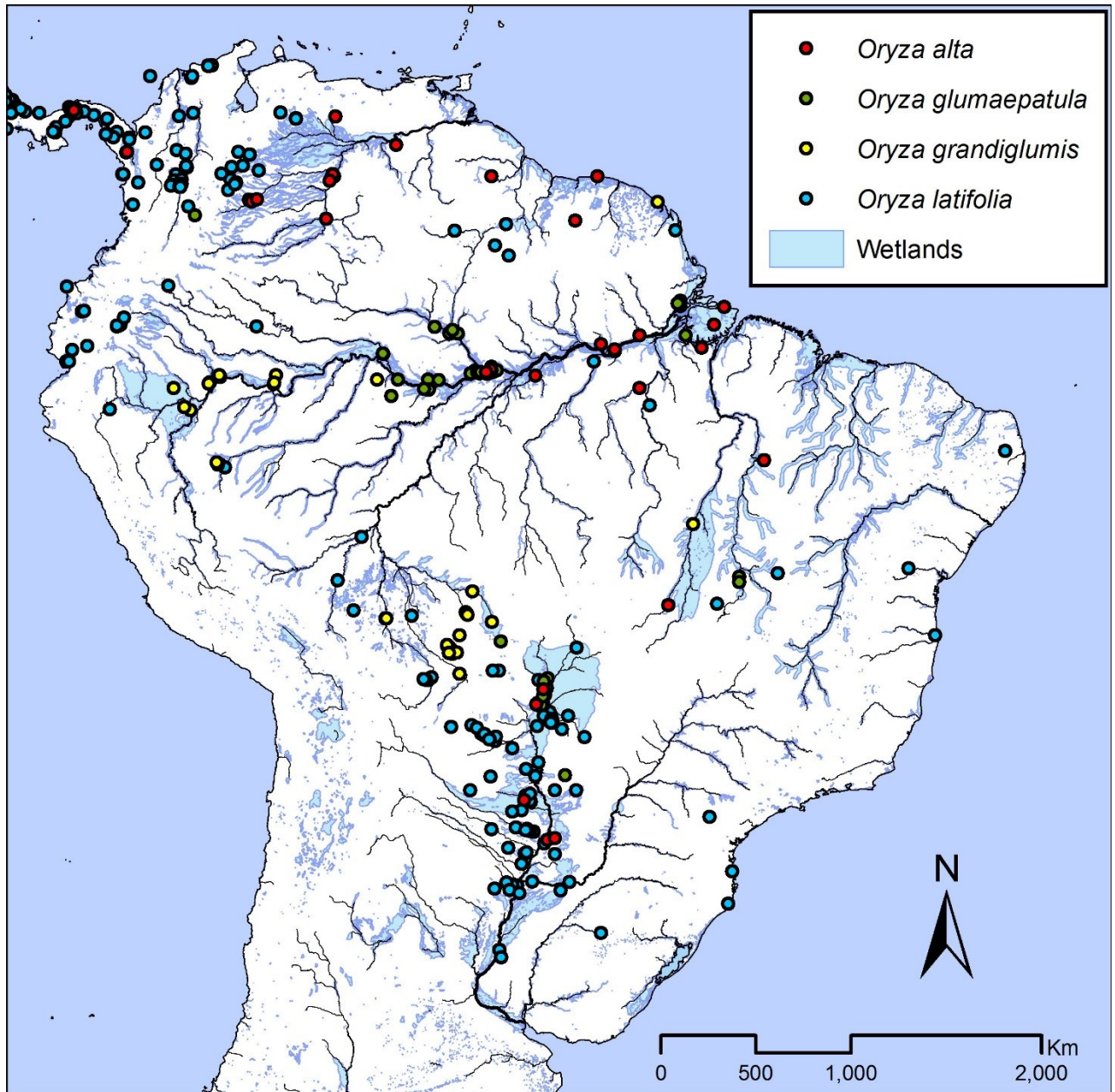
Fig S2. Relative frequencies of phytoliths recovered in the A and C fractions of samples from levels A to J of the Monte Castelo shell mound. Horizontal bars represent relative percentages; circles correspond to the presence of plant taxa lower than 1% in abundance. *Non-diagnostic Poaceae* phytoliths include non-diagnostic bulliforms, rondels and crosses; *Non-diagnostic Arboreal* morphotypes include globular granulates, sclereids and various types of faceted tracheary elements.
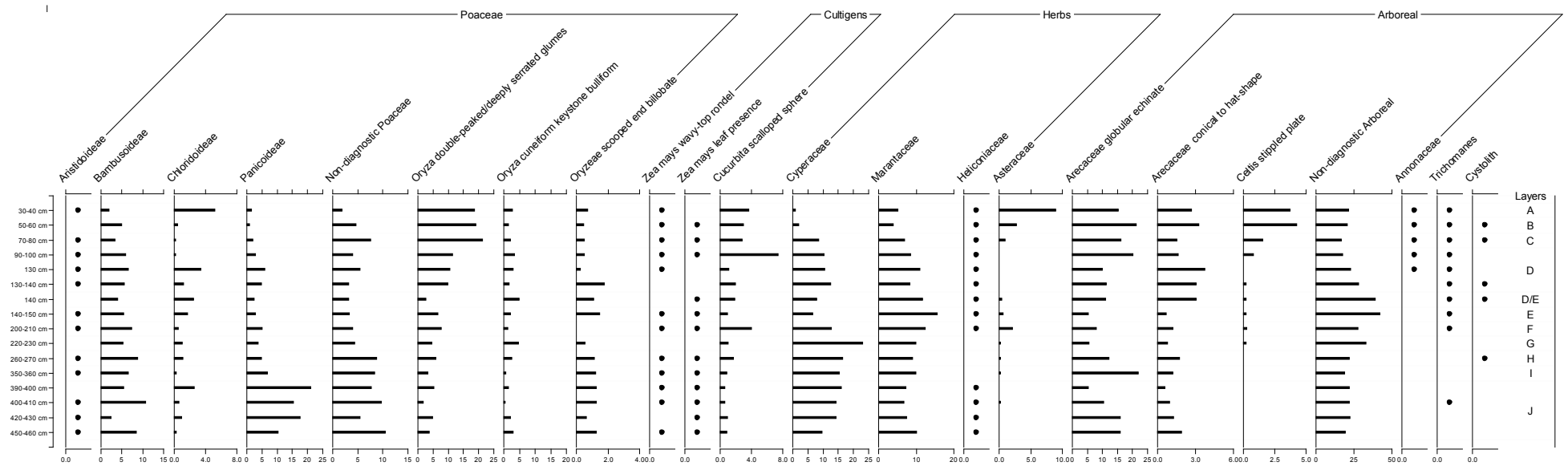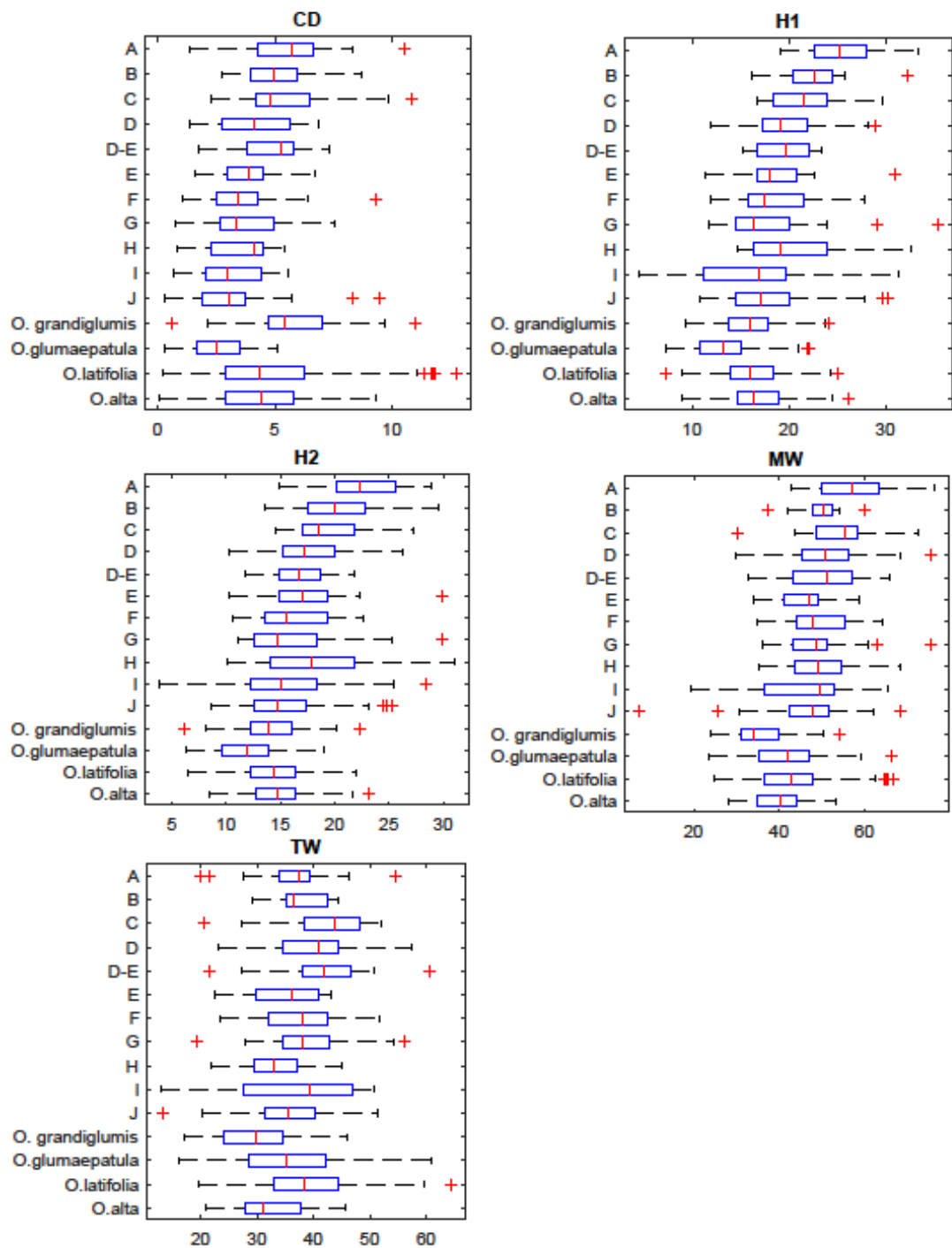
Figure S3. Boxplots of *Oryza* double-peaked glume metric attributes from modern reference species and the Monte Castelo stratigraphy.
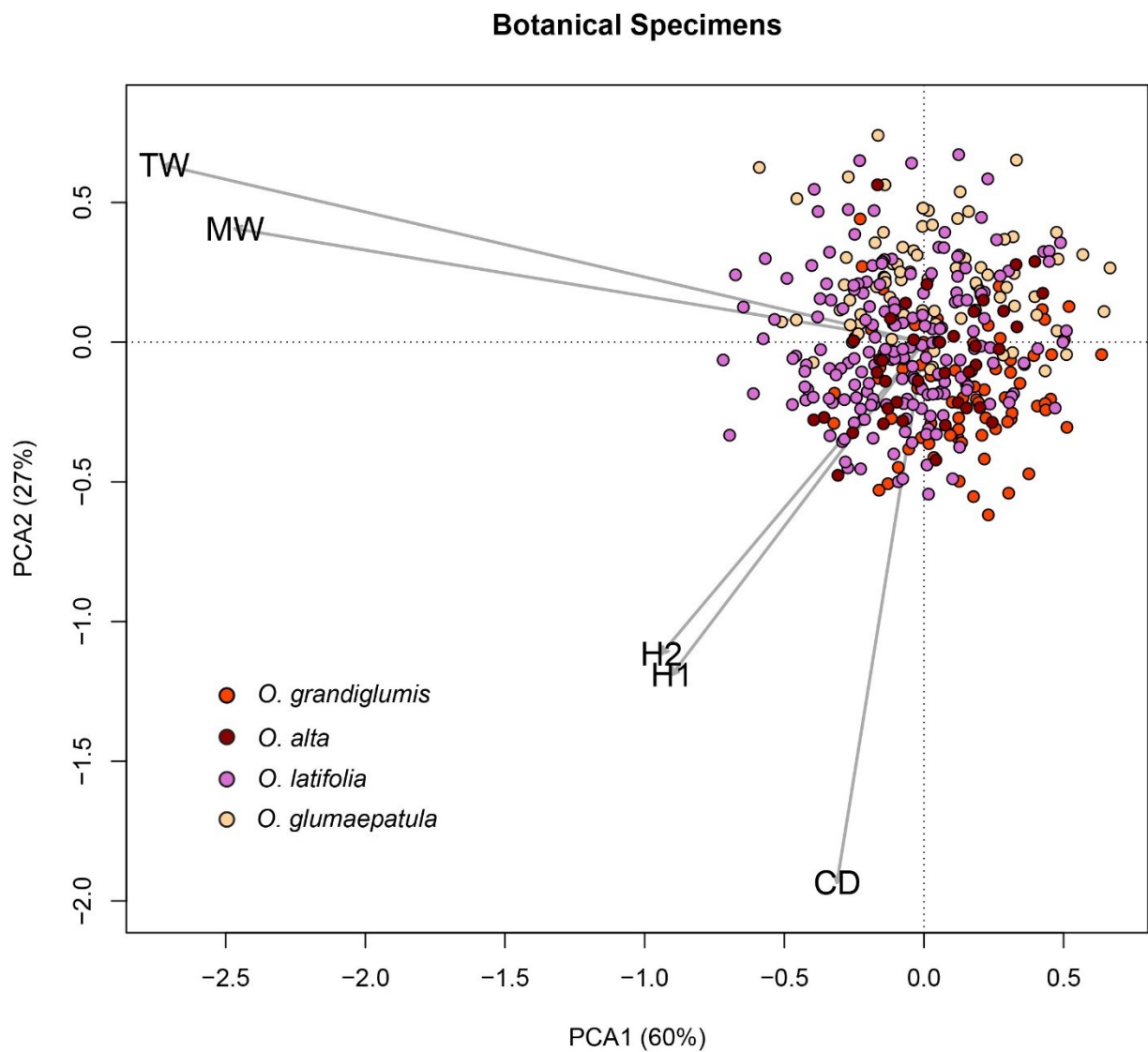
Fig. S4. PCA of all botanical specimens showing that the two Width and Height measurements are highly correlated and correspond to PCA1, explaining 60% of the variance in the dataset. Curvature Depth is correlated to PCA 2, which comprises 27% of the variance.
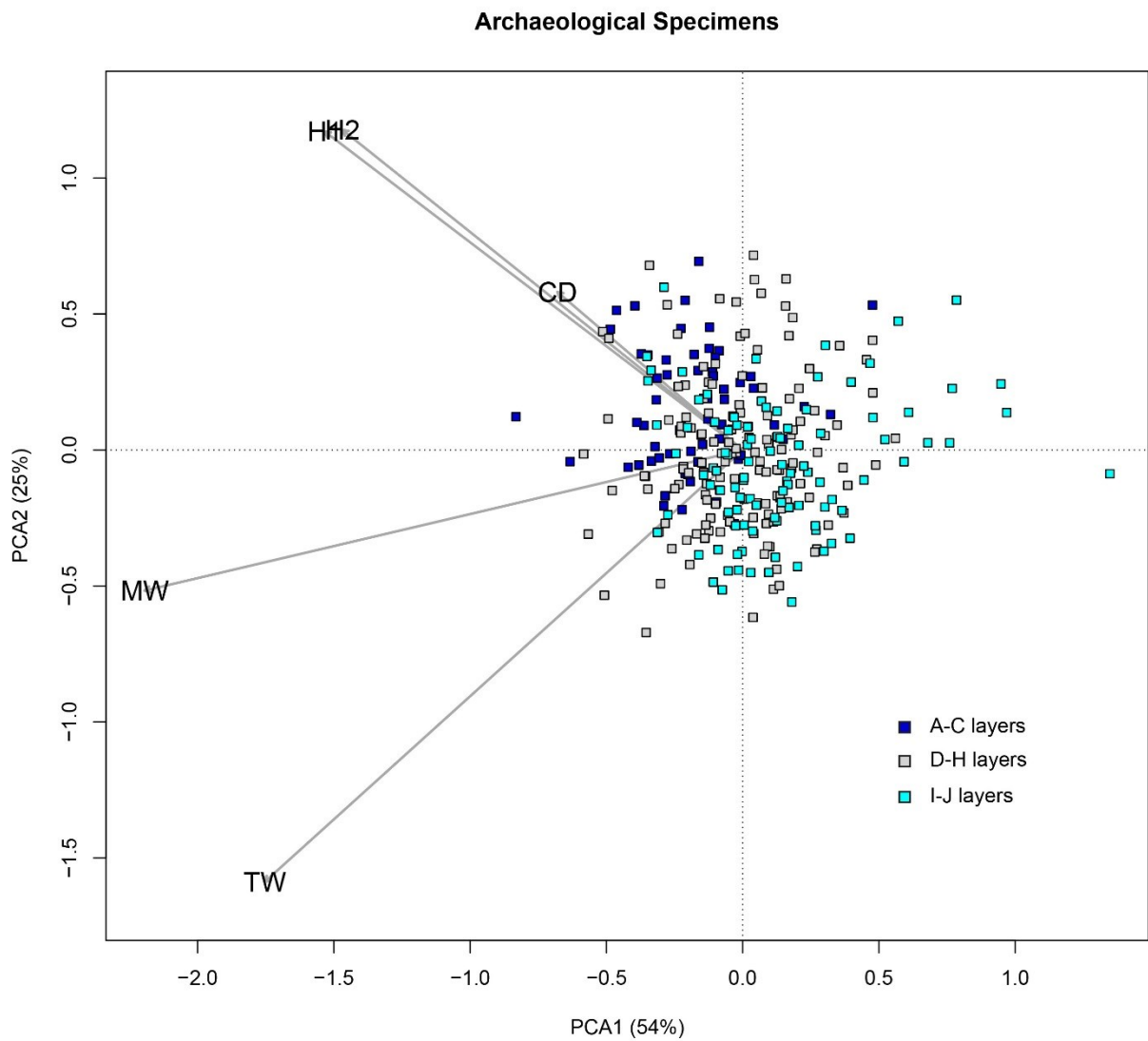
Fig. S5. PCA of archaeological specimens only. Height and width measurements explain most variance in the data and Curvature Depth has a minor contribution to Axis 1 (54%); the species score, however, is low compared to height and width. Axis 2 is correlated to Top Width and Curvature Depth and explains 25% of the variance.
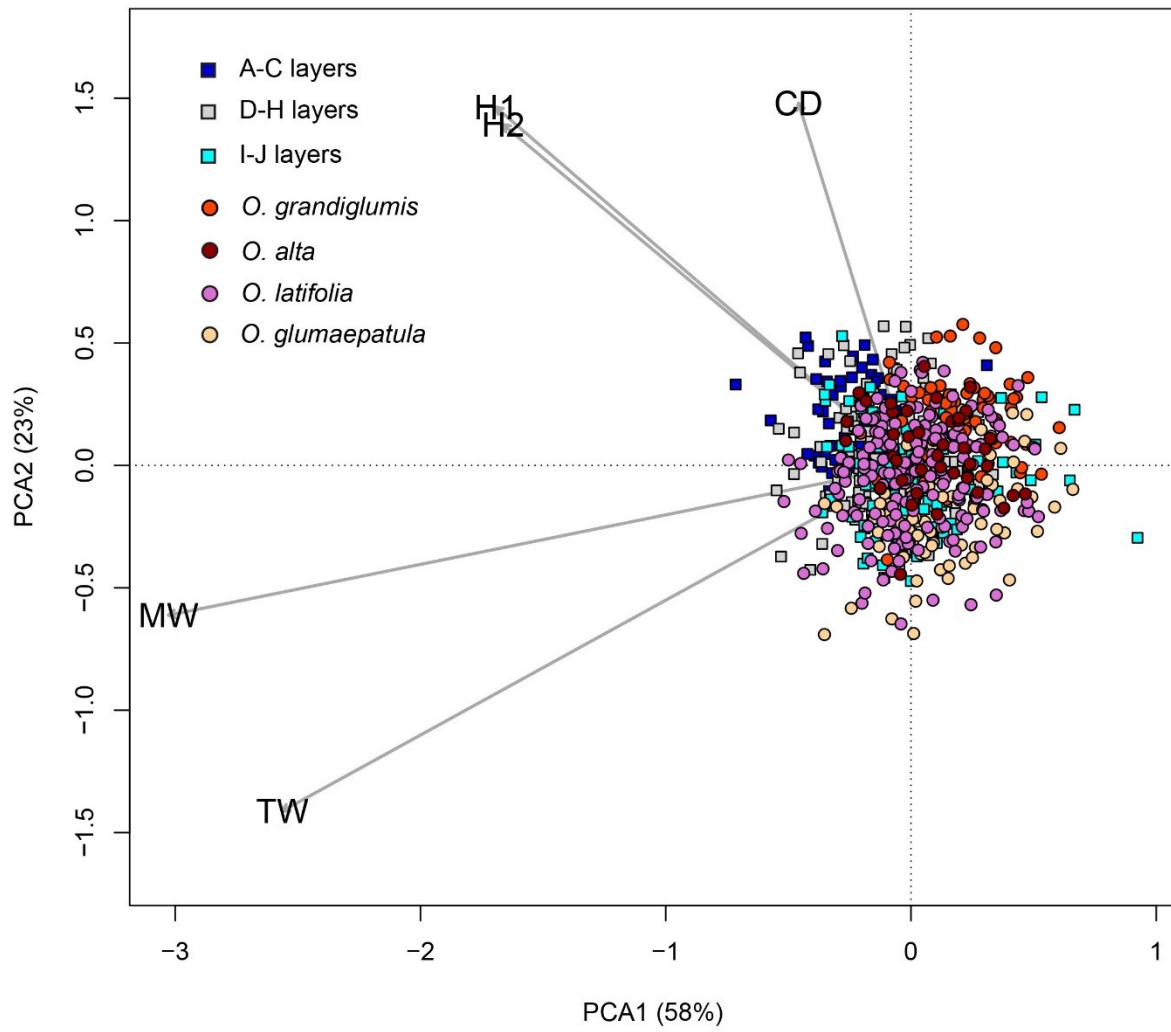
Fig. S6. PCA for all specimens (botanical and total) confirming that Height and Width explain most variance in the dataset. PCA1 explains 58% of the variance. PCA axis 2 is correlated to Curvature Depth and Top Width.
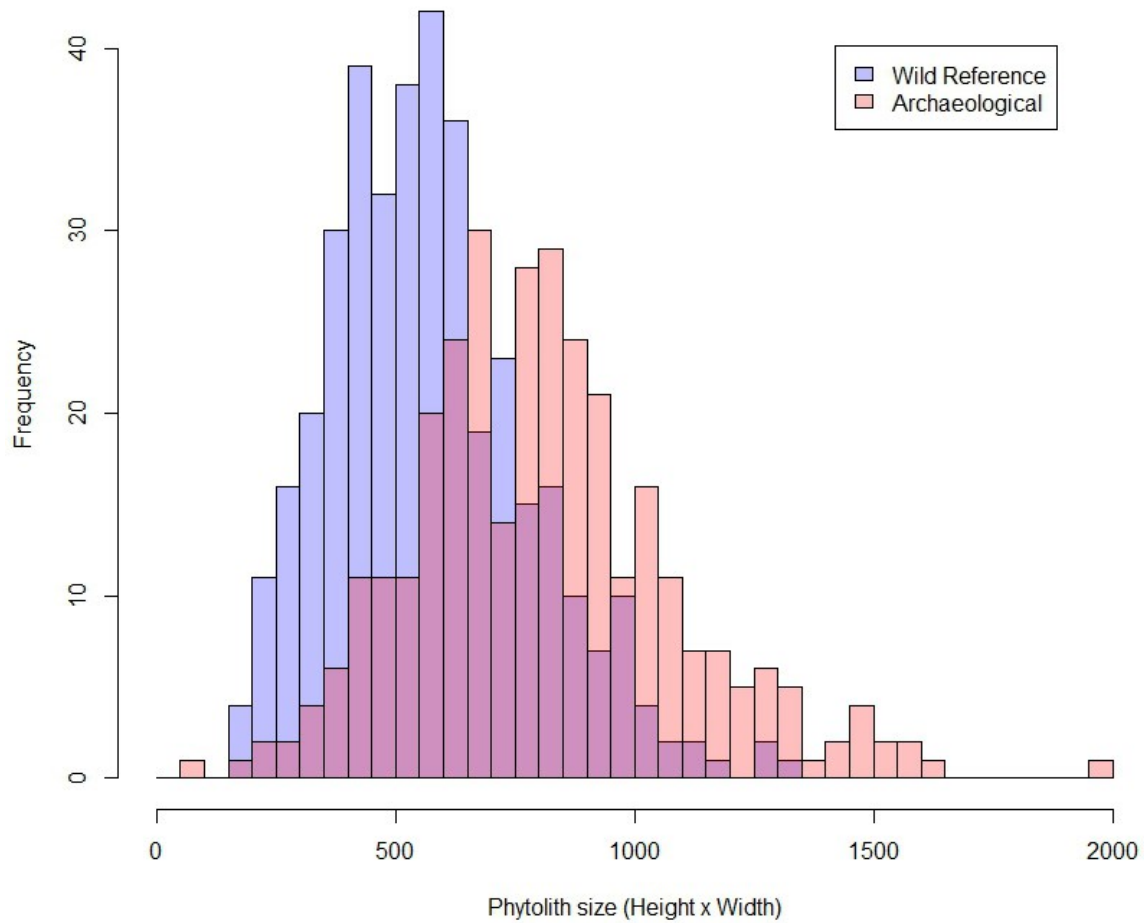
Fig. S7. Distribution of phytolith size among archaeological and wild reference samples, as determined by simple model (MW*TW/2)*(H1*H2/2). A Wilcoxon non-parametric demonstrates the means of the two groups are significantly different (p<0.0001).

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