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Title:

Evidence for mid-Holocene rice domestication in the Americas

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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, with implications for understanding its legacy on global subsistence strategies, plant distribution, population health, and the global methane budget . 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. 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 increasingly larger rice husk phytoliths. 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.). The results underlines the role of wetlands as prime habitats for plant domestication worldwide.

More than half of the world's population depend on rice for more than 20% of their daily calories [1]. Modern global consumption is dominated by varieties of the domesticated Asian (*Oryza sativa* L.) and African (*O. glaberrima* Steud.) species [2], which were domesticated in the early Holocene in the Yangtze River, China[3], and ca. 2000 cal yr BP in West Africa [4]. In North America, *Zizania* wild rice was so important to the subsistence economy of several Upper Great Lakes Native American tribes that some early-twenty century ethnologists designated this region as a distinct 'wild rice culture area'[5]. Wild rice was already a seasonal staple of indigenous subsistence in South America long before the introduction of Old World species in the 18th century[6]. Growing in seasonally flooded areas that compose up to 10% (1.4M km^2) of lowland South America (Fig. 1), wild rice is a particularly important resource during the rainy season when flooding causes other resources to be dispersed and scarce[7]. Early 16th-19th century historical and ethnographic accounts report extensively on the consumption of wild rice species by indigenous groups in this region. 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[8-11]. South American accounts hint towards the importance and culinary practices involving wild rice. For example, De Azara[12] mentions the consumption of an unknown type of rice in southern Paraguay that "… feed a nation of

88 approximately seventy warriors". Cardim[13] mentions that wild rice was mixed with maize to

89 make bread, and Acosta[14] describes its consumption in the form of a fermented brew, similar

90 to wine. Locally known as "arroz-de-pato" (duck rice) or "arroz-do-brejo" (swamp rice)

91 today, wild rice is stilly consumed as a valuable source of carbohydrates when other food

92 resources are scarce by riverine communities across the Amazon. It is still gathered and

93 consumed in various modern localities close to the study site along the Guapore River such as

94 Costa Marquez and Santo Antônio, where the communities used to manage wild rice stands

95 until the first half of the twentieth century. This kind of landscape management can still be

96 observed in other parts of the Amazon, such as in wild rice fields of the municipality of

97 Manaquiri, in the lower Solimoes river basin[15] (Supplementary Figure 1b). In the Pantanal,

98 the native Guató communities consume the wild native species *Oryza glumaepatula* and *O.*

99 *latifolia* by sun drying the seeds, peeling them, and boiling them [16]. However, despite the

100 occasional reference to its potential role in pre-Columbian diets [17,18], the domestication of rice

101 has not yet been investigated in this region. Increasingly larger *Oryza* sp. husk phytoliths

102 recovered from mid-Holocene levels of a shell mound in southwestern Amazonia (Fig. 1)

103 dating to ca. 4000 cal yr BP show the progressive selection of larger wild rice seeds by its

104 pre-Columbian residents, whom were already engaged in the cultivation of maize (*Zea mays*)

105 and squash (*Cucurbita* sp.) [19].

106

107 **Taxonomy and domestication phytoliths of the *Oryza*.**

108 Oryzoideae (syn. Ehrhartoideae) is a subfamily of the true grass family Poaceae

109 that includes around 120 species in 20 genera. The Oryzeae tribe within the Oryzoideae

110 subfamily consists of twelve genera and is distributed in tropical and temperate regions

111 worldwide. Five of these twelve genera occur in South America: *Leersia*, *Luziola*,

112 *Rhynchoryza*, *Zizaniopsis*, and *Oryza* [20,21]. The *Oryza* genus comprises 22 known wild

113 species. Four of them are endemic to Latin America with a tropical-subtropical distribution

114 from Cuba 23^0 N to the Paraná River delta 34^0 S, including the diploid (2n = 24, AgpAgp) *O.*

115 *glumaepatula*, and three tetraploids (2n = 48, CCDD) *O. alta*, *O. grandiglumis* and *O.*

116 *latifolia*[22] (Fig.1). *Oryza* spp. rice are all aquatic emergent macrophytes that grow along

117 rivers, lakes and wetland margins. *Oryza alta*, *O. grandiglumis*, and *O. latifolia* are perennial

118 species, while *O. glumaepatula* can be annual, biannual or perennial depending on the

119 geographical location [23,24]. *Oryza* spp. have a nutty flavour, and firm consistency. Preliminary

120 studies on *O. glumaepatula* show that it has high levels of total protein, albumin, and glutelin

121 fractions, which compares favourably with *O. sativa* commercial cultivars[25]. Wild rice can

122 also be stored and can be rather productive. Although, not directly comparable to *Oryza*, the

123 traditional canoe-and-flail harvesting of *Zizania* wild rice in North America yield about 125

124 kg/ha[26], while modern domesticated shattering resistant cultivars, yields have been reported

125 as high as 1,680 kg/ha in Minnesota and twice that amount in California[27].

126   The Oryzoideae subfamily produce four distinct phytoliths associated with different

127 parts of the plant. The Oryzeae tribe produce: 1) cuneiform keystone bulliform cell phytoliths

128 exhibiting fish-scale decorations on the fan edges are produced in the leaves (Fig. 2d) and 2)

129 'scooped'-shaped bilobates in the leaves and stems (Fig. 2e). The *Oryza* genus produce: 3)

130 double-peaked glume cells (Fig. 2a-b, f-i); and 4) deeply serrated phytoliths both derived

131 from the epidermis of the *Oryza* seed glume (husk) (Fig. 2c)[28-30]. The presence of diagnostic

132 *Oryza* phytoliths produced in the different parts of the plant has allowed the detection of crop

133 processing stages[31] and different agricultural techniques[32] in Asia. For example, the

134 distinctive bulliform and bilobate phytoliths from Oryzeae leaves and stalks are

135 representative of the early stages of harvesting and processing, while the *Oryza* husk double-

136 peaked glumes represent later stages of processing, such as pounding, winnowing and

137 storage.

138   Domestication is a process that causes genetic changes in populations such that the

139 average phenotype diverges from the range found in wild populations[18]. Domestication

140 causes a gradual increase in plant size from wild to domesticate as a result of selective

141 exploitation[33]. As the plant become larger, so do the phytoliths. The increase in phytolith size

142 has been documented in *Zea mays*[34], *Cucurbita*[35] and *Musa bananas*[36], where larger fruits

143 and seeds often yield considerably larger phytoliths. Pearsall[29] and Zhao et al.[30] have

144 demonstrated a clear correlation between increasing phytolith size and domestication in

145 Asian rice based on the analysis of 27 accessions of domestic rice, originated from China,

146 and 79 specimens from the nine wild rice species considered ancestral to rice distributed

147 geographically in South and Southeast Asia. These authors[30] devised a discriminant function

148 to differentiate assemblages of wild from domesticated *Oryza* rice using five different size

149 measurements of the double-peaked glume cells including: 1) Top Width (TW): the distance

150 between the two peaks of the projecting hairs; 2) Maximum Width (MW): the width at the

151 point where the glume projection attaches to the base; 3-4) Height of each hair (H1, H2):

152 length from the tip to the base of the hair, H2 is defined as the smaller measurement; and 5)

153 the Curve Depth (CD): distance from the tip of H1 to the lowest point of the curve (Fig. 3m).

154 Further comparative research by Lu et al.[37] including hundreds of grass species from China

155 and Gu et al[28] including wild and domesticated rice species from East Asia have confirmed

156 their results.

157

158 **Archaeological background: the Monte Castelo shell mound.** Dating back to ca. 10,000

159 cal yr BP, a diversity of coastal and freshwater [38] shell mounds represent some of the oldest

160 forms of human occupations across lowland South America, some of which are associated

161 with the earliest ceramics on the continent[39]. Our study site, the Monte Castelo residential

162 shell mound is located in the Upper Madeira basin of SW Amazonia, Rondônia state, Brazil.

163 The region is characterised by a seasonally flooded tropical wetland exhibiting gallery forest

164 along the larger streams, which are dotted with anthropogenic shell mounds [38]. Monte Castelo

165 is a 6.3 m high platform-shaped freshwater shell-mound, exhibiting a 160 m long elliptical

166 base (Fig. 1c) and dating from 9400 cal yr BP[40,41]. The first excavation of Monte Castelo by

167 Miller [42] in 1984, revealed a seven-meter-deep stratigraphy bracketing a long-term

168 occupation from 9130 to 667 cal yr BP (Supplementary Table 1). Miller defined three major

169 and one transitional occupation phases based on stratigraphy, artefact content and sixteen

170 radiocarbon dates including: Cupim phase (700-685 cm; 9130-7701 cal yr BP), Sinimbu

171 phase (670-275cm; 7701-4822 cal yr BP), Sinimbu-Bacabal transitional stratum (275-220

172 cm; 4862-4388 cal yr BP) and Bacabal phase (220-30 cm; 4388-689 cal yr BP)[42]. Renewed

173 excavations at Monte Castelo in 2014 and 2016 by the Laboratory of Tropical Archaeology

174 of the University of São Paulo expanded the previous excavation by E. Miller reaching a

175 depth of 640 cm. They uncovered ten archaeological strata across the Sinimbu to Bacabal

176 phases dating from 5310 cal. yr BP. to 689 cal yr BP (Fig. 3k; Supplementary Figure 3;

177 Supplementary Note 1)[40]. The stratigraphy shows a sequence of construction events

178 evidenced by unburnt entire *Pomacea* shell layers, occupation floors marked by lenses of

179 crushed shells, primary burials and human-created dark soils. Sample collection for

180 microfossil analysis was carried out in undisturbed sectors of each of the layers and targeted

181 samples were collected from particular features such as burials (Supplementary Note 1;

182 Supplementary Figure 3; Supplementary Table 3).

183

184 **Results and Discussion**

185 To investigate the use and potential domestication of wild rice by the Monte Castelo

186 residents we analysed both archaeological samples and modern wild rice reference material.

187 A total of 16 archaeological sediment samples, from across all ten levels uncovered during

188 the 2014 Monte Castelo excavations (Fig. 3; Supplementary Table 3), and 19 modern

189   specimens from the four wild species of rice occurring in South America (Supplementary

190   Table 2), were analysed for phytoliths following standard procedures[34] (Methods,

191   Supplementary Table 2 and 3). Each slide was scanned until the first 20 double-peaked glume

192   cells were encountered. Following Zhao et al.[30], the five metric attributes (Fig. 3m) were

193   measured from 20 *Oryza* double-peaked glume phytoliths from each of the archaeological

194   (16) and modern samples (19) totalling 700 phytoliths.

195   Phytolith preservation was excellent in all context analysed. All archaeological

196   sediment samples analysed yielded phytoliths of wild rice. Our analysis shows a clear

197   increase in the proportion of rice morphotypes in the total phytolith assemblage from 6.4% on

198   average in the Sinimbu phase occupation (Layers J-H) to 14.4% in the more recent Bacacal

199   phase, suggesting that rice may have played a larger role in diet over time (Fig. 3f).

200   At Monte Castelo, there is also an increase in the proportion of *Oryza* seed phytoliths

201   from the lower to the upper levels of the mound reflected in the husk:leaf+stem ratio. For

202   example, during the Sinumbú phase (Layers J-I; 280-460cm) *Oryza* sp. seed phytoliths

203   represent on average 3.4% of the total assemblage while Oryzeae leaf and stem phytoliths

204   constitute on average 3%, a 1/1 ratio. During the Bacabal occupation (Layers F-A; 30-210

205   cm) *Oryza* seed phytoliths constitute on average 12% of the total assemblage while leaf

206   phytoliths constitute on average 3.5%, a ratio of 3.4/1, over three times the relative proportion

207   of seed husks as occur in the Sinimbu occupation (Fig. 3g). The collection and flailing of

208   wild rice in canoes in the Americas should leave leaf and stem bulliform and bilobate

209   phytoliths in the place of harvest while double-peaked and deeply serrated glume phytoliths

210   should be more abundant at residential sites where the grain is brought for consumption.

211   Therefore, the increase in the ratio of husk:leaf+stem Oryzeae phytolith morphotypes

212   suggests that the Monte Castelo residents became more efficient harvesters over time,

213   bringing more grain and fewer leaves to the site.

214   The analysis of the average size of the attributes measured on the *Oryza* glume

215   phytoliths (Fig. 3 and Supplementary Figure 5) shows a gradual increase in Height (H1, H2)

216   and Width (TW, MW) through time. Mean H1 values increase ca. 8μm (17μm to 25μm) and

217   H2 increases ca. 7μm (15μm to 22μm) from Layers J to A. MW increases 9μm (48-57μm)

218   through the stratigraphy. Mean CD values are larger in the upper occupation layers (A-H)

219   compared to its initial dimensions in Layers I-J (Fig. 3). We used Principal Component

220   Analysis (PCA) of modern reference wild species to determine the variables that best

221   explained phytolith shape differences among specimens, which are the two highly correlated

222   height and width measurements (Supplementary Note 2, Supplementary Figures 6-9).

223   Following Zhao et al.[30], therefore, we created a simple model of phytolith size to characterise
224   the changes in phytolith morphology through time. Results of a one-way ANOVA show that
225   mean phytolith size varies significantly among layers and pairwise comparison (with
226   Bonferroni corrected p-value) shows phytoliths in the upper archaeological layers (A - D) are
227   significantly larger than those in Layer J and wild reference specimens (Supplementary Table
228   4). Fig. 4 illustrates mean height and width of all *Oryza* phytolith specimens, showing an
229   increase in phytolith size through time. The data show a significant shift towards bigger
230   phytoliths compared to wild specimens began in Layers D-E (Fig. 3k) around 4000 cal yr BP.
231   Phytolith size in lower archaeological layers were not significantly different from some
232   botanical specimens (*O. latifolia, O. alta*) (Supplementary Table 4). The gradual increase in
233   *Oryza* husk phytolith dimensions since the basal layers of the Monte Castelo shell mound
234   suggest that the Monte Castelo residents may have been manipulation *Oryza* by at least 5000
235   cal yr BP. Phytolith data also show that subsistence strategies of the Monte Castelo residents
236   were based on a mixture of wild and domesticated resources including cultivars such as
237   maize and squash as well as other plants of economic importance including palm fruits and
238   possibly soursop (*Annona* sp.) (Fig. 2 j-m).

239   Our results indicate a significant increase in the size of double-peaked glume
240   phytoliths across the Monte Castelo occupation starting around 4000 cal yr BP. Wild rice
241   constituted an important seasonal resource for the Monte Castelo residents, who began to
242   husband wild rice stands at lake or river edges. The phytolith data show that wild rice was
243   modified by human intervention to produce larger grains, exceeding the range of variation
244   found in the lower levels of the Monte Castelo shell mound and the modern populations of
245   wild rice. The possibility that the increase in dimensions of husk phytoliths may be a result of
246   selection for large seeds during collection from wild plant stands is countered by fact that no
247   husk phytoliths with larger dimensions than the domesticated ones have been found on the
248   modern wild rice specimens.

249   *Oryza alta*, *O. grandiglumis*, and *O. latifolia* are perennial species, while *O.*
250   *glumaepatula* can be annual, biannual or perennial depending on the geographical location
251   [23,24]. Although we cannot distinguish specific *Oryza* species using phytoliths, it is likely that
252   the Monte Castelo residents were targeting the annual varieties of *O. glumaepatula* due to
253   their generally larger-scale seed production compared to perennials, as seen with other cereal
254   grains[43]. The specific husbandry practices that led to this process of domestication are
255   unknown; however, native North Americans increased natural *Zizania* wild rice stands by
256   mixing wild rice seeds into clay, rolling it into a ball and dropping the clay ball into the

257 water[27]. It is not unlikely that the Monte Castelo residents may have seeded the Guapore

258 basin wetland margins with a similar practice. With this technique, larger seeds might have

259 been indirectly selected because they would germinate better from the clay balls, eventually

260 leading to domestication. In addition, like traditional societies in India today, they may have

261 practised burning of enriched rice patches during the dry season to remove competing

262 vegetation after rice grains were embedded safely in the soil. To what extent the selection of

263 non-shattering types contributed to the fact that the Monte Castelo residents became more

264 efficient harvesters, as shown by the increase in husk:leaf+stem ratio, is something we cannot

265 directly detect with phytolith analysis, since phytoliths cannot document the

266 presence/absence of this key domestication syndrome trait.

267   It is interesting to note that the apparent major role of rice in the diet of the Monte

268 Castelo residents, as well as the beginning of its domestication, coincides with a rapid

269 increase in precipitation in the Amazon. As summarised by Iriarte et al.[44], the palaeoclimate

270 records from southern Amazonia and adjacent regions influenced by the South American

271 Low Level Jet show a consistent long-term trend of increasing precipitation starting during

272 the mid-Holocene (~6k cal. yr BP), showing a rapid rise up to 4k cal. yr BP, and then

273 continued to increase slightly towards the present. This higher precipitation would likely have

274 expanded the spatial extent of wetlands across the basin and possibly made the flooding

275 season longer. Since wild rice is a particularly important resource during the rainy season in

276 wetlands and floodplains when flooding causes other resources to be disperse and scarce, the

277 increase precipitation would have likely made wild rice a critical seasonal resource, which

278 may have, in turn, led populations to focus on its manipulation, which ultimately led to its

279 domestication. Further work is needed on this hypothesis.

280   The presence of phytoliths from known cultigens, such as the wavy-top rondels of

281 maize and scalloped spheres from squash, in the strata analysed shows that both crops were

282 commonly grown in the region from at least 5300 B.P. onwards (Fig. 3, Supplementary

283 Figure 4). This in turn, indicates that the Monte Castelo shell mound residents began to

284 systematically select larger rice seeds when they were already engaged in the cultivation of

285 maize and squash. While in other regions of the Americas, wild grasses such as *Setaria*[45] or

286 marsh-eleder[46] decrease in importance or are replaced by maize, the opposite trend is

287 apparent in the Monte Castelo record. Wild rice was domesticated and increased in

288 importance a considerable time after Monte Castelo residents had become engaged in farming

289 practices.

290   The arrival of Europeans to the American continent in AD 1492, with the consequent
291   population decimation and impact on cultural practices, caused the domesticated traits to
292   gradually disappear. The loss of domesticated varieties is a phenomena that has also occurred
293   for other indigenously domesticated species in both South[18] and North America[46]. A case in
294   point similar to *Oryza* is the 'extinct cultigen' marsh-elder (*Iva annua*), a member of the
295   Asteraceae family greatly appreciated for its achene oil content, which was originally
296   domesticated in southeastern North America and then abandoned with the introduction of
297   maize[46]. As in our case study, the achenes of marsh elder from the earlier archaeological
298   sequences are not much larger than the modern ones, but the achenes from the more recent
299   archaeological contexts are much larger than any existing races of *Iva annua* today. In the
300   case of rice, some varieties are in the process of de-domestication today; modern studies of
301   Californian weedy rice show how reversions to non-domestic or wild-traits (such as seed
302   shattering, presence of awns) can occur following abandonment[47]. In our case study, it is
303   likely that the wind-pollinated wild rice progressively hybridised with the domesticated one,
304   with the consequent return to the wild characteristics seen today.

305   Our study highlights the importance of wetlands for the adoption and intensification
306   of agriculture [48,49]. The results contribute to a broader understanding of how wetlands and the
307   seasonal tropical forests of the Amazon may have been critical for early human settlement
308   and the origins of food production in the Americas. This domestication process took place in
309   a region that was likely the cradle of domestication for cassava, peanuts and chilli peppers
310   pointing to the importance of this region of South America[19].

311   Our research has implications for sustainable Amazonian futures. Modern intensive
312   breeding for high yield and pest resistance has narrowed the genetic diversity of cultivated
313   rice leaving crops more susceptible to disease and less adaptable to the effects of climate
314   change. Understanding the process of rice manipulation by ancient Native Americans and the
315   role of South American native varieties could help provide more resistant high-yielding
316   varieties, and provide further knowledge for plant breeders interested in the introgression of
317   genes from wild *Oryza* species into modern rice varieties[22].

318

319   **Methods**
320   *Phytolith analysis*. Phytoliths were identified and counted under a Zeiss Axioscope 40 light
321   microscope at 500X magnification. Phytolith identifications were made using published
322   material for the Neotropics and the Oryzoideae family[29,30,34] and by direct comparison with
323   the phytolith reference collection of the Archaeobotany and Palaeoecology Laboratory in the

324   Department of Archaeology of the University of Exeter. A minimum of 200 phytoliths were

325   counted per slide. Following Zhao et al.[30], the five metric attributes (Fig. 3m) were measured

326   from 20 *Oryza* double-peaked glume phytoliths from each of the archaeological (16) and

327   modern samples (19) totalling 700 phytoliths.

328
329
330
331   **Figure 1**. **a**. Distribution of *Oryza* species, wetlands in South America, and important early
332   Holocene shell mound sites in South America . Species occurrences from the Global
333   Biodiversity Information Facility[50]. Wetland areas from the Global Lakes and Wetlands
334   Database, World Wildlife Fund (https://www.worldwildlife.org). **b.** Map showing the
335   location of the Monte Castelo. **c.** The Monte Castelo locality, topographical map, and
336   location of the 2014 trench excavation.

337

338   **Figure 2**. Microphotographs of phytolith morphotypes recovered at the Monte Castelo shell
339   mound and modern reference wild rice species analysed. **a-e**. *Oryza* sp. phytolith
340   morphotypes recovered in the Monte Castelo shell mound: **a.** double-peaked glume (Layer
341   A); **b.** double-peaked glume (Layer J); **c.** deeply serrated body (Layer C); **d.** cuneiform
342   keystone bulliform (Layer D 130-140cm); **e.** scooped bilobate (Layer E). **f-i.** Double-peaked
343   glume phytoliths from modern wild-rice species native to the study area: **f.** *O.alta* (PRI-1); **G.**
344   *O.latifolia* (Arg-5); **h.** *O.grandiglumis* (SO-23); **i.** *O.glumaepatula* (SO-17). **J-N.** Crops and
345   other native edible plants recovered in the Monte Castelo shell mound: **J.** scalloped sphere
346   from the rind of squash (*Cucurbita* sp.)(Layer F); **k.** wavy-top rondel from the cob of maize
347   (*Zea mays*)(Layer C); **l.** large globular echinate from Arecaceae (Layer J); **m.** conical to hat-
348   shaped phytolith from Arecaceae (Layer H); **n.** spherical facetate from Annonaceae (Layer
349   C); Scale bar= 20 µm.

350

351   **Figure 3.** Sketch stratigraphic diagram of the 2014 Monte Castelo shell mound excavation
352   layers showing: **a-e.** Mean and 95% confidence intervals of the metric attributes of *Oryza* sp.
353   double-peaked glume phytoliths (N=700): **a.** TW, Top Width, **b.** MW Maximum Width, **c.**
354   CD, Curvature Depth, **d.** H1, Height 1 and **e.** H2, Height 2; **f.** Percentage of rice phytoliths to
355   total phytolith assemblage; **g.** *Oryza* husk:leaf+stem ratio; **h.** Presence of *Cucurbita*
356   scalloped spheres; *i.* Presence of *Zea mays* wavy top rondels; **j.** Monte Castelo stratigraphy;
357   **k.** Sketch drawings of double-peaked glume phytoliths using the average of the five metric
358   attributes for each archaeological layer; **l.** Monte Castelo cultural chronology; **m.** Metric
359   attributes of *Oryza* double-peaked glume phytoliths. Box and whisker plots for all metrics are
360   shown in Supplementary Figure 5.

361
362   **Figure 4.** Mean height (H1+H2/2) and width (MW+TW/2) of all Oryza phytolith specimens
363   (N=700), shown with 95% confidence intervals, demonstrating that archaeological specimens
364   are larger compared to botanical specimens, and an increase in phytolith size through time.

365
366
367   **Data availability.** The dataset analysed is available from corresponding author upon request.
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369
370   **References**

371

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488 **Author contributions**

489 LH, JI and EGN designed research; EGN, FP, MS and CAZ performed archaeological
490 excavations at Monte Castelo; LH undertook phytolith analysis; BSW carried out statistical
491 analyses; EV provided *Oryza* reference collection samples for analysis; JI and LH led the
492 writing of the paper with inputs from all other authors.

493

494 **Additional information**

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502 **Competing interests**

503 The authors declare no competing financial interests.

A

B

C

Wetlands

Wetlands

Monte Castelo

Wetlands

Guaporé R.

0   10 km

Floodplain

Monte Castelo

Trench

Wetland

0   200 m

Archaeological sites

Oryza alta

Oryza glumaepatula

Oryza grandiglumis

Oryza latifolia

Wetlands

Taperinha

Amazon Basin

Monte Castelo

SM-1

Capelinha

N

0   250 500   1,000   Km

A TW μm: 32 36 40 44

C CD μm: 3 4 5 6

E H2 μm: 16 18 20 22

G Rice husk: (leaf + stem): 0 2 4 6 8 10

I Squash +

Depth (cm): 0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 420, 440, 460

B MW μm: 40 44 48 52 56 60

D H1 μm: 16 18 20 22 24 26

F Total % Rice: 0 10 20 30

H Maize ✕

Bacabal Phase
689 to 4,388 cal yr BP

D: 3,895-4,085 cal yr BP

Sinimbú-Bacabal Stratum
4,388 to 4,862 cal yr BP

Sinimbú Phase
4,822 to 7,701 cal yr BP

J: 5,210 to 5,310 cal yr BP

20 μm

M TW, CD, H1, H2, MW, L, K, J