'Early Rice Agriculture in Bangladesh: Methodological and Archaeobotanical Perspectives'

Md. Mizanur Rahman

Hertford College
School of Archaeology
University of Oxford

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A scenario of harvesting paddy rice from a field by sickle in the early Bengal was depicted on a terracotta plaque (Chandraketugarh c. 300 BCE).

Dedicated

to the

Rice Farmers

who laid the solid foundation of
human society in early Bangladesh.

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Abstract

The origin and domestication of Asian rice remains a complex and contentious topic. Among the three main varieties—*indica*, *aus*, and *japonica*—both *indica* and *aus* are believed to have originated and been domesticated in India and Bangladesh, respectively. However, a paucity of comprehensive archaeobotanical evidence from South Asia has hindered efforts to elucidate these issues. Additionally, establishing methodological parameters for the taxonomic identification of rice varieties is essential for assessing their origin and domestication. This research uses present-day rice landraces to develop models to investigate an archaeobotanical rice assemblage recovered from Wari-Bateshwar (c. 800-200 BCE) in the Lower Brahmaputra Valley, Bangladesh. The study focuses on three key objectives:

1. A charring experiment to determine the optimal heating regime that produces charred rice resembling well-preserved archaeological specimens (Paper 1);
2. An evaluation of two methods—morphometrics and Geometric Morphometrics (GMM)—for identifying rice varieties using modern accessions of *japonica*, *indica*, and *aus*—and application of these refined techniques to archaeological rice remains from Wari-Bateshwar (Paper 2); and
3. An analysis of rice spikelet base morphology from Wari-Bateshwar to understand the domestication status of local rice and to address hypotheses regarding the origin and domestication of Asian rice varieties, including *aus* (Paper 3).

The controlled charring experiment reveals that a low-temperature, short-duration heating regime of 230°C for 2-3 hours in a reduced oxygen environment is ideal for producing modern charred rice that closely resembles well-preserved archaeological specimens (Paper 1).

The morphological study confirms, first, that the morphometric length/width ratio of *japonica* and *aus* rice overlaps significantly, complicating their distinction, whereas *indica* rice shows clear separation. By contrast, a newly derived GMM model achieves an 83% accuracy rate in separating varieties as represented by present-day landraces (Paper 2). When applied to archaeological assemblages from Wari-Bateshwar, the model detects all three Asian rice varieties, *japonica*, *indica*, and *aus* (Paper 2). Furthermore, this study represents a rice chronology in the Bengal frontiers at which *japonica* and *indica* arrived by the 6th century BCE, and the *aus* type became domesticated by the early 4th century BCE.

Finally, the analysis of archaeological rice spikelet bases from Wari-Bateshwar indicates a reduction in shattering and an increase in non-shattering traits through the stratigraphic sequence, indicating a local rice domestication process and hence supporting the multiple domestication hypothesis for Asian rice varieties (Paper 3).

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1. Introduction

1.1. Overview

Rice is one of the world's most important staple crops, being cultivated on all continents except Antarctica and providing nutrition to around half the global population. Rice cultivation remains the single most important source of employment and income for rural people (GRiSP 2013). Asia is regarded as the birthplace of influential rice varieties and accounts for nearly 90% of rice production globally. Besides botanical and evolutionary aspects, rice cultivation emerged as the basis of agricultural societies in East and South Asia (Fuller *et al.* 2016). Despite recent advances in early rice research, the domestication, dispersal and diversity of early Asian rice present many gaps in knowledge. This is due in part to the patchy distribution of archaeobotanical research and also to the need for further methodological development. This study aims to explore rice cultivation dynamics at the archaeological site of Wari-Bateshwar (WB) in the Lower Brahmaputra Valley, Bangladesh, by developing and applying new methods to the analysis of archaeobotanical rice remains.

Cultivated Asian rice belongs taxonomically to *Oryza sativa* L. in the grass family (Poaceae). Asian rice is further subdivided into varieties according to their distinct genetic structure: *japonica*, *indica, aus* and aromatic (Grist, 1965; Chang, 1976; Oka 1988; Garris *et al*. 2005; McNally *et al.* 2009; Schatz *et al.* 2014; Civáň *et al.* 2015; Travis *et al.* 2015; Choi *et al.* 2017). Rice can be distinguished wild and domesticated based on their genetic and morphological traits. Domesticated type rice is non-shattering, and it can germinate spontaneously while wild type is typically shattering and predominantly dormant. Both categories are grown in almost similar habitats while two types of wild rice are predominant in Asia such as perennial *Oryza rufipogon* and annual *Oryza nivara (*Vaughan *et al.* 1989*)*. Between wild and domesticated, researchers have found a distinct type of rice which is characterised as wild-cultivated and termed as intermediate (Oka 1988). Furthermore, Asian rice has been classified predominantly based on botanical inferences wild, domesticated, intermediate, cultivated, weedy and local landraces (Oka 1988; 16-23) while upland, lowland, irrigated, rainfed, deepwater, and tidal rice are grouped based on growing environments (Khush 1984).

1.2. Archaeobotanical Rice Domestication Research in South Asia

The early rice story in South Asia is as yet patchy, and questions surrounding the process of domestication in the region and beyond are hotly debated. Taxonomic identification of archaeological rice at the varieties level is fundamental to these debates. It has been suggested that two Asian rice varieties originated in South Asia, *indica* in the Ganges (Fuller *et al*. 2010; Fuller 2011; Bates *et al.* 2017; Silva *et al.* 2018) and the *aus* variety in central India or Bangladesh and adjoining West Bengal and Assam regions (Civáň *et al.* 2015). It has also been suggested that Chinese *japonica* rice interacted with rice in the subcontinent, giving rise to *indica* and *aus* (Fuller *et al.* 2010; Fuller 2011; Huang & Han 2016). Thus, both of these hypotheses require further archaeobotanical investigation.

The earliest rice exploitation evidence in South Asia has been recorded at Lahuradewa in the Middle Ganges, dated 6409 BCE (8359 cal BP), where charred grains were discovered (Tewari *et al.* 2008, p. 350). There is another date for possible incipient rice exploitation (PRL3031 9570 ± 120 uncal BP, Tewari *et al*. 2008) that raises debate (Fuller *et al.* 2010). Thus, the more broadly accepted date of incipient rice exploitation in the Middle Ganges was the 7th millennium BCE revealed at Lahuradewa (Tewari *et al*. 2003, 2005; 2006; 2008; Saraswat 2004, 2005; Singh 2005a,b; Pokharia 2011).

Tewari *et al.* (2008) claimed that the charred rice grains along with spikelets (n=26) discovered at Lahuradewa, specifically in phase 1A, were domesticated based on three criteria: morphometrics, husk patterns, and the presence of non-shattering rice Spikelet bases. However, this interpretation has been challenged by Fuller *et al*. (2010) who argued that grain morphometrics is not a reliable indicator of rice domestication (Thompson 1996; Harvey 2007) Instead, better evidenced through the shattering or non-shattering morphology of rice is spikelet bases. However, despite their excellent preservation with attached spikelet bases and intact grains, the small number of rice spikelet bases (n=4; Tewari *et al.* 2006, p. 49; 2008; Pokharia, 2011) limits the ability to draw conclusive argument on domestication status. Moreover, Fuller *et al.* (2010) suggested that the morphometrics of the grains from Lahuradewa closely resemble those of wild rice, indicating that the site's rice use may involve pre-domestication rice gathering and cultivation.

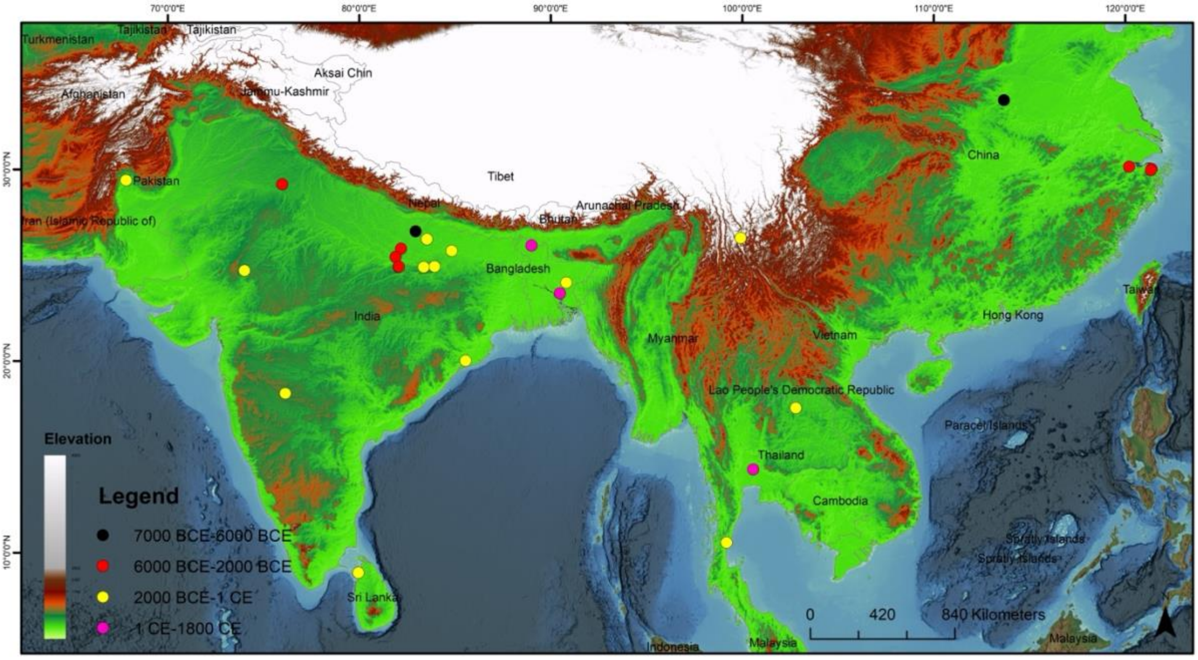


Fig. 1. Map showing some important archaeological sites within the regions from which archaeological rice has been recovered; frontiers location of Bangladesh.

In Phase 1B (c. 2700 BC) at Lahuradewa, rice emerged as a dominant component of the archaeobotanical assemblage. However, the challenge of determining rice's domestication status in this phase remained similar to that of the earlier phase due to low sample numbers, reliance on morphometrics, and the limited numbers of rice spikelet bases available. In the same phase some non-native crops such as wheat, barley, and lentils, as well as non-native animals like sheep and goats, were recorded. Additionally, ceramics reminiscent of Indus Valley styles, such as dish-on-stand shapes, were identified (Tewari *et al.* 2008; Pokharia 2011). The barley found at the site has been dated to approximately 4300 years BP (Tewari *et al.* 2008; Pokharia 2011).

Archaeological rice remains have also been found at several other sites in the Ganges, including the Mesolithic sites of Damdama (Kajale 1990) and Chopani-Mando, as wild-type rice grain impressions in pottery (Sharma and Misra 1980). Neolithic sites such as Jhusi and Hetapatti also provide early rice evidence (Sharma and Misra 1980; Varma *et al.* 1985; Kajale 1990; Pal 2008, 2016). However, the dates for these sites remain subject to debate (Fuller *et al.*, 2010). From the available evidence, it appears that rice cultivation spread throughout India and adjoining regions from the Middle Ganges.

Rice cultivation spread from the Ganges to different regions of the subcontinent including the Indus by the beginning of the 3rd millennium BCE (Bates 2022). Fuller and Madella (2002; 336–337) suggested that rice was not an important crop in Indus agriculture, while Fuller and Qin (2009) inferred that rice cultivation did not play a role in Indus agriculture until the late Harappan period, c. 2200 BCE. Emerging data from recent research (Petrie *et al.* 2016; Bates *et al.*, 2017; Petrie and Bates 2017) suggest that a long rice cultivation from the mature Harappan period in the Indus region. Rice cultivation reached humid coastal regions of Eastern India, such as the sites of Golbai Sasan and Gopalpur, around 1500-1000 BCE (Kingwell-Banham 2018a), while it reached dry South India and Sri Lanka by c. 500 BCE (Kingwell-Banham 2019) and c.200 BCE (Kingwell-Banham 2018b), respectively.

A recent study revealed a significant archaeobotanical rice assemblage from Wari-Bateshwar, Bangladesh – the study sites of this thesis –by c. 400-300 BCE; this assemblage contained rice grains resembling the East Asian *japonica* variety but none resembling *indica* (Rahman *et. al.* 2019, 2020). This finding raises questions about the adoption of *indica* in early Bangladesh, given that Early Historic Bangladesh (c. 500 BCE to 500 CE) was connected with the Middle Ganges basin, where *indica* rice is thought to have originated. Vikrampura (900-1000 CE) and Sotisher Danga (c. 1300-1800 CE) are another two archaeological sites in Bangladesh in which evidence of rice cultivation has been found, indicating widespread rice cultivation in the Bengal frontier zone (Rahman 2018; Rahman *et. al.* 2019).

The origins of *indica* rice are debated. A prominent model is the proto-*indica* hypothesis of its origin and domestication (Fuller 2011). According to this view, local rice cultivation began in the Ganges by the 7th millennium BCE and was not domesticated until the beginning of the 2nd millennium BCE before arriving *japonica* in the Ganges. The locally cultivated rice might have been a semi-domesticated or intermediate-type regarded as proto-indica. According to this theory, *indica* rice could not have been fully domesticated until Chinese *japonica* rice arrived in the Ganges. With introgression between the locally cultivated proto-*indica* rice and domesticated *japonica*, domestication genes were transferred from *japonica* to proto-*indica*, through which this variety became fully domesticated *indica* by around 1500 BCE (Fuller 2011).

An absence of morphometric measurements of archaeobotanical rice from the Ganges basin limits our understanding of the existence and adoption of Chinese *japonica* rice, since morphometric characteristics distinguish the main forms of Asian rice at the variety level, e.g., *indica* and *japonica* rice (Castillo *et al*. 2016). Despite initial evidence of rice spikelet bases (domesticated type mature Harappan phase-17, late Harappan phase-2) at Masudpur by c.

2200 BCE (Bates *et al.* 2017), empirical evidence for *indica* domestication is scarce, and the origins of this variety are yet to be unequivocally demonstrated. This remains one of the most important unresolved issues in the archaeobotany of the Ganges Basin and South Asia (Fuller 2006; Bates 2022).

The morphometrics approach to distinguish *japonica* and *indica* rice has been applied to archaeobotanical rice grains from a restricted number of archaeological sites: Tokwa in the Middle Ganges, Golbai Sasan and Gopalpur in Odisha, Eastern India; Perur and Kodumanal Tamil Nadu in South India (Kingwell-Banham 2015; Kingwell-Banham *et al*. 2018a); Mantai in Sri Lanka (Kingwell-Banham *et al.* 2018b); and Wari-Bateshwar, Vikrampura (Rahman *et al*. 2020) and Sotisher Danga (Rahman 2018) in Bangladesh. The identification of *japonica* at Wari-Bateshwar and the absence of *indica* (Rahman *et. al.* 2020) raises questions about the adoption of *indica* in early Bangladesh, given that Early Historic Bangladesh engaged in trade with the Middle Ganges basin, where rice *indica* is thought to have originated.

Another unresolved issue is the origin of *aus* rice. Research on modern rice genetics has suggested that this distinct variety of rice (Londo *et al.* 2006; Kovach *et al.* 2007; Civáň *et al.* 2015), beyond the standard *japonica* and *indica*, could have emerged around c. 500 BCE (Choi *et al.* 2017), and that this development might have taken place in Bangladesh or neighbouring Central to Eastern India, West Bengal and Assam or, less probably, Myanmar (McNally *et al.* 2009; Travis *et al.* 2015). The first archaeobotanical evidence of charred *aus* rice grain was reported at Sotisher Danga in Bangladesh (Rahman 2018), but this needs reassessment using refined methodological approaches. It has been hypothesised that a kind of rice from Southeast Asia, the *Champa* rice of Vietnam, which contributed to *aus* rice, diffused from Bangladesh around c.700 CE (Barker 2011). Thus, the issues of *aus* rice origin and the mechanisms of its spread are related to a long historical entanglement with distinct regions of South Asia.

Investigation of this question primarily depends upon the taxonomic identification of *aus* rice. It should be noted that, despite recent archaeobotanical research in Bangladesh, the introduction of *indica* and *japonica* and the origins of *aus* rice are not yet clearly understood. This thesis seeks to address this gap in knowledge.

A recent hypothesis suggests that, despite their similar environments and diversity of crop taxa, agricultural prehistories in South Asia and Southeast Asia were largely distinct until a relatively late period (Fuller *et al* 2016). In Bangladesh the earliest existing evidence of Southeast Asian crops – such as rice (*Oryza sativa japonica*) – dates to 3rd century BCE (Rahman *et al.* 2020), while South Asian crops such as mung bean (*Vigna radiata*) and horsegram (*Macrotyloma uniflorum*) entered mainland Southeast Asia around the 1st century BCE, potentially through maritime connections (Rahman *et al.* 2020; Castillo *et al.* 2016).

There is a lack of firm evidence for routes of crop diffusion from China to Bangladesh and northeast India (Silva *et al* 2018). The southern Himalayan-Tibetan route, over the Arunachal, Assam (India) via Bangladesh to northern India and other land routes, or branches of the southern Himalayan-Tibetan route over Thailand and Myanmar to Bangladesh, would be potential pathways for diffusion of crops from China to South Asia, but are poorly studied due to insufficient archaeobotanical evidence from Southwest China and Bangladesh (Silva *et al.* 2015; Stevens *et. al.* 2016). There is evidence of overland rice diffusion from China to South Asia, probably through the Hexi corridor, around the 3rd to 2nd millennia BCE, but the mechanisms are as yet poorly understood and could include human migration (Higham 2014; Castillo 2017; Stevens and Fuller 2017).

1.4. Further Scope for Rice Archaeobotany in Bangladesh

Beyond domestication *per se*, a lack of past crop husbandry reconstruction in Bangladesh and adjacent regions using methods such as [functional] weed ecology and stable isotope approaches limits our understanding of how agriculture shaped societies and demography. It would be useful to combine isotope analysis on rice (archaeological and modern) in South Asia including Bangladesh with functional weed ecology to reconstruct crop husbandry practices, including tillage method, time of sowing, irrigation and drainage, fallowing or rotation, manuring and weeding (cf. Bogaard *et al.* 1999, 2016; Charles *et al.* 1997; Jones 2002).

Combining functional weed ecology and stable carbon and nitrogen isotope analysis of archaeobotanical crop remains has proved a useful technique to explore aspects of early crop husbandry in western Eurasia, including crop water status and manuring (Araus *et al.* 1997; 1999; Bogaard *et al.* 2007; 2013; Mueller-Bieniek *et al* 2019) but has as yet had little application in South Asia (but see Fiorentino *et al.* 2015; Lancelotti *et al.* 2013) and specifically on rice cultivation (Nayak *et al.* 2022). Notably, crop cultivation intensity (labour inputs per unit area) may be pivotal to understanding the emergence of new ways of life, e.g., rural form to urban life, emerging trading communities and so on (Jones *et al.* 1999). Incorporating functional weed ecology and carbon and nitrogen isotope analysis methods proved useful for characterising semi-arid land management in Southwest Asia, including assessment of practices such as irrigation, manuring, tillage and hand-weeding, resulting in the inference that early urban centres in northern Mesopotamia were sustained by extensive low-input cereal farming (Bogaard *et al.* 2016; 2018; Styring *et al.* 2017). More recently, low-input extensive agriculture has been linked with the early medieval European agricultural 'revolution' (Hamerow *et al.* 2020). Comparable understanding is as yet scarce on rice in South

Asia, including Bangladesh. Rice research reported in this thesis is a first step towards establishing the basis for ecological characterisation of early rice growing systems in Bangladesh. Initial evidence of a rice cultivation system has been recognised in Medieval (c.1300-1800 CE) Sotisher Danga, Bangladesh, at which wet rice cultivation system traced dominant throughout the period, along with the presence of significant upland dry rice cultivation system based on analysing weed ecology (Rahman 2018). The vast floodplain, alongside relative uplands and highly diverse seasonal variation with wet and dry conditions in association with changing dynamics of land formation into the delta, could allow an understanding of the diverse mechanism of ecology for early rice cultivation systems in early Bangladesh.

1.4. The Research Context of the Study Site

Bangladesh stands at the juncture of South Asia (or the Indian subcontinent), mainland Southeast Asia and China. At the geographical 'crossroads' of these regions, the dispersal and adoption of the different rice varieties were plausibly subject to diverse influences. Despite significant recent development of archaeobotanical research in India (e.g. Fuller *et al.* 2011; Pokharia *et al.* 2014, 2017; Petrie and Bates 2017), China (e.g. Zhao 2011; Stevens *et al.* 2016; D'Alpoim Guedes 2019) and Southeast Asia (Castillo 2013; Castillo *et al.* 2018), broader regional interrelations including with Bangladesh remain poorly understood, particularly as regards the timing and routes of the dispersal of rice varieties. In addition to questions about crop diffusion into Bangladesh, another set of questions concerns the ecology and consequences of rice cultivation that were discussed in the previous section.

The archaeological site of Wari-Bateshwar (c.500-100 BCE) is studied in this thesis, a well-known Early Historic urban and trading centre of early Bangladesh. The site provides the basis of an archaeobotanical synthesis of Bangladesh covering the Late Prehistoric to early

Historic (Iron Age) period and opens up a comparative perspective between Bangladesh and north-eastern Indian sites. Until now, Wari-Bateshwar has been known solely as a site of the early-historic period, with no stratigraphic sequence traced into prehistory, despite some prehistoric tool types being found as surface finds. A recently excavated trench indicated a c.800-700 BCE depositional layer (Hu *et al.* 2020), from which a significant number of archaeobotanical remains were recovered for this study, with the potential to investigate earlier agricultural practice in the Lower Brahmaputra Valley. Wari-Bateshwar has the potential to inform on early rice use and potential domestication, along with the role of agriculture for later urban developments.

The study site can be characterized by its topographic features, which likely shaped the development of agricultural regimes. Wari-Bateshwar is situated in the Pleistocene uplands along with vast adjoining floodplains (Fig. 2.1). New archaeobotanical evidence from the study site has the potential to shed new light on the nature of early agriculture, especially for rice and its production strategies, and on the emergence and development of urbanization. In both topics, the biogeographical position of Bangladesh is fundamental.

1.5. Aims and Research Questions

The primary research question guiding this project is: what forms of rice were managed at WB in the Lower Brahmaputra Valley during the 1st millennium BCE, and what methodological developments are needed to address this question? More specifically, this research aims to understand the archaeobotany of rice in the Lower Brahmaputra Valley through the examination of modern experimental and archaeological rice. This study will investigate three aspects of rice: 1. the optimal charring conditions for replicating well-preserved rice grains experimentally, as found in archaeological deposits; 2. the potential of morphometrics and geometric morphometrics for distinguishing the Asian rice varieties on the basis of experimentally charred grains of genetically characterise landraces from Bangladesh and 3. the radiocarbon chronology and domestication status of early rice from the study site of WB.

The preservation state impacts the identification of archaeobotanical cereal grains since it determines their shape and size. Heat exposure during processing and cooking as well as accidental charring of stored materials leads grains to char (or carbonise), the precise conditions determining their preservation state (Braadbaart *et al.* 2004; Braadbaart 2008; Stroud 2021). This is further supported by Charles *et al.* (2015) through the classification of experimentally charred glume wheat grains, linking the condition of charred grains with temperature and duration of heating. The preservation can range from well-preserved to highly distorted.

Moreover, preservation conditions of archaeological cereal grains are influenced by factors such as environmental variables as well as taphonomic conditions. Furthermore, the temperature at which grains are charred can affect their preservation state, with specific temperature ranges yielding better-preserved specimens (e.g., López-Dóriga 2015). This highlights the importance of experimental archaeology in understanding the preservation dynamics of grains. The temperature regime at which archaeological rice is well-preserved or heavily distorted remains to be established. Thus, a controlled rice charring experiment was conducted as part of this thesis to ascertain the optimal conditions under which modern charred rice resembles well-preserved archaeological rice. The specific objective of this component of the project is as follows (Paper 1): to ascertain the impact of different temperature regimes on morphological changes through a charring experiment on grains of modern rice landraces from Bangladesh in order to determine the temperature and duration regime for replicating well-preserved archaeological grains.

As already outlined, rice is the staple crop of Bangladesh and is thought to have had this status since the Late Prehistory. There are distinct histories of rice cultivation, diffusion and domestication in mainland China, South Asia and Southeast Asia. Recent genetic research indicates that three Asian rice varieties – *japonica*, *indica*, and *aus* – were domesticated separately and independently and that *aus* rice could have originated in Bangladesh and/or neighbouring Eastern Indian states like Assam and West Bengal (Civáň *et al.* 2015). However, successful archaeobotanical identification of the rice varieties is not only needed because of the multiple/independent hypotheses but also more widely to address the single vs multiple origins debate. Thus, the identification of these rice from an archaeological context is fundamental for exploring the domestication of *aus*, *indica* and *japonica* varieties.

Geometric Morphometrics (GMM) has a growing application in archaeobotany to distinguish grains of closely similar cereal taxa to, for example, variety level based on shape, an approach that has not yet been applied to distinguish Asian rice varieties. Given the importance and likely deep history of three Asian rice varieties in Bangladesh, it is crucial to distinguish them in order to investigate the mechanisms of rice origins and dispersals on a detailed level, building on existing studies of archaeobotanical rice in Bangladesh (Rahman 2018; Rahman *et. al.* 2019: Rahman *et.al.* 2020). Against this background, the relevant research objectives are as follows (Paper 2):

1. to assess the suitability of current morphometric methods for distinguishing *japonica*, *indica* and *aus* rice; and
2. to explore the potential and complementarity of GMM to separate the Asian rice varieties.

Finally, and as outlined above, the domestication of the various Asian rice varieties is debated, and archaeological evidence is needed to complement modern genetic insights. Genetic research suggests at least three different ways that could follow the origin and domestication of Asian rice. The single-origin hypothesis proposed that Asian rice varieties originated and domesticated once from the same wild progenitor of *Oryza rufipogon* in the Lower Yangtze Valley (Vaughan *et al.* 2008; Molina *et al.* 2011). On the contrary, the multiple domestication hypothesis of Asian rice has two contrasted observations of multiple origins but single *de novo* domestication (Choi *et al.* 2017; Huang & Han 2016; Choi & Purugganan 2018) and multiple independent (*de novo*) domestication (Civáň *et al.* 2015; Civáň and Brown 2017; 2018). Multiple independent origins but single domestications occurred in particular regions under specific ecological and human management conditions (there is a large literature on independent origins and multiple domestications; for example, Hunt *et al.* 2018; Harris 1990; Vavilov [1992(1927)]).

However, according to multiple origin and single domestication hypothesis, Asian rice varieties had independent separate origins, but that *de novo* domestication occurred initially in *O. sativa ssp. japonica*. This was followed by introgression, where early *japonica* contributed to the domestication of proto-indica and proto-aus, eventually leading to the development of domesticated *indica* and *aus* varieties. On the other hand, multiple independent domestication hypotheses proposed that the rice varieties originated from distinct geographical regions and received domestication genes from their wild progenitors rather than introgression from *japonica*.

Domestication traits in crops are diverse, of which non-shattering rachis and greater seed size are most identifiable archaeologically. It has been observed that non-shattering traits manifest more rapidly and frequently than seed size changes (Fuller 2007). Therefore, rice Spikelet base morphology is considered the most reliable and archaeologically traceable indicator for assessing the domestication process. Fuller *et al.* (2009) provide identification criteria to distinguish the spikelet bases of wild, domesticated and immature rice. They used this approach to document *japonica* rice domestication processes in the Lower Yangtze Valley from around 4500 BCE. This evidence charts a local evolutionary transformation from wild to domesticated *japonica* rice.

Botanists have also observed a further type of rice that may be relevant archaeobotanically (Sharma and Shastry 1965; Morishima *et al.* 1980; Sano *et al*. 1980; Ng *et al*. 1981; Oka

1988): a wild-cultivated or intermediate type of rice in India and Thailand. This could be a feral domesticated variety of weedy rice that might have served as the immediate progenitor of domesticated rice rather than a pure wild form of rice. Identification criteria for such an intermediate type of rice is needed to assess its archaeological potential, entailing further investigation of modern rice forms to establish its morphology through ethnographic study.

Archaeobotanical evidence from Bangladesh is critical for investigating the nature and context of early Asian rice in Bangladesh. The following objectives are framed (Paper 3):

1. to establish morphological criteria for distinguishing intermediate-type of rice spikelet bases on the basis of modern collections, and to apply these criteria in order to assess their relevance in the WB rice assemblage and sequence; and
2. to assess rice spikelet base types and shattering behaviour at WB, in conjunction with the presence of wild and domesticated type grains, in order to draw out implications for the current debate over single versus multiple rice origins.

1.6. Research Approach

In order to address the research questions and objectives framed above, a multi-stranded methodological approach is needed. A controlled charring experiment will be followed to ascertain the temperature regimes on the morphological characteristics of Asian rice varieties (Paper 1). The experiment will establish the specific temperature and exposure conditions that produce modern rice grains with preservation characteristics similar to those of well-preserved archaeological rice grains.

Castillo *et al.* (2016) successfully utilised morphometrics to identify rice at the variety level, e.g., *indica* and *japonica*. This study should extend this approach to separate the *aus* variety and the previously identified two varieties (Paper 2). Morphometrics, precisely length/width ratio, was used to distinguish between rice varieties.

GMM identifies cereal varieties by analysing shape variations through imaging and outline analysis. This method integrates coordinates, landmarks, and semi-landmarks to provide a comprehensive understanding of morphological differences. Utilising the R statistical software package of Momocs (Bonhomme *et al.* 2014; 2017) offers solutions for this analysis. The morphology of rice spikelet bases determined by observing modern materials will be followed to distinguish wild and domesticated rice to inform on questions surrounding rice domestication (Paper 3).

Two sets of rice samples from the WB17 and WB16 trenches of Wari-Bateshwar will be sent to the Oxford Radiocarbon laboratory to obtain AMS dates. These samples were securely sourced from the trenches' stratigraphic layers, and statistical calibration methods will be used to measure radiocarbon dates for the distinct layers.

References

Araus, J. L., Febrero, A., Buxó, R., Rodríguez-Ariza, M. O., Molina, F., Camalich, M. A. D., Martín, D. & Voltas, J. 1997. Identification of Ancient Irrigation Practices based on the Carbon Isotope Discrimination of Plant Seeds: A case study from the South-East Iberian Peninsula. *Journal of Archaeological Science,* 24**,** 729-740.

Araus, J. L., Febrero, A., Catala, M., Molist, M., Voltas, J. & Romagosa, I. 1999. Crop water availability in early agriculture: evidence from carbon isotope discrimination of seeds from a tenth millennium BP site on the Euphrates. *Global Change Biology,* 5**,** 201-212.

Barker, R. 2011. The Origin and Spread of Early-Ripening Champa Rice: It's Impact on Song Dynasty China. *Rice* Pp:184–186.

Bates *et al.* 2017. Approaching rice domestication in South Asia: New evidence from Indus settlements in northern India. *Journal of Archaeological Science,* 78**,** 193-201.

Bates, J. 2022. The Fits and Starts of Indian Rice Domestication: How the Movement of Rice Across Northwest India Impacted Domestication Pathways and Agricultural Stories. *Frontiers in ecology and evolution,* 10.

Bogaard, A., Palmer, C., Jones, G., & Charles, M. 1999. A FIBS approach to the use of weed ecology for the archaeobotanical recognition of crop rotation regimes. *J Archaeol Sci* 26**,** 211–224.

Bogaard, A., Styring, A., Ater, M., Hmimsa, Y., Green, L., Stroud, E., Whitlam, J., Diffey, C., Nitsch, E., Charles, M., Jones, G., & Hodgson, J. 2018. From Traditional Farming in Morocco to Early Urban Agroecology in Northern Mesopotamia: Combining Present-day Arable Weed Surveys and Crop Isotope Analysis to Reconstruct Past Agrosystems in (Semi) arid Regions, Environmental Archaeology, 23:4, 303-322, DOI: 10.1080/14614103.2016.1261217

Bogaard, A., Hodgson. J., Nitsch, E., Jones., G., Styring., A., Diffey. C., Pouncett. J., Herbig. C., Charles. M., Ertug. F., Tugay. O., Filipovic. D., Fraser, R. 2016. Combining functional weed ecology and crop stable isotope ratios to identify cultivation intensity: a comparison of cereal production regimes in Haute Provence, France and Asturias, Spain. *Veget Hist Archaeobot* 25**,** 57–73

Bogaard, A., Heaton, T. H. E., Poulton, P. & Merbach, I. 2007. The impact of manuring on nitrogen isotope ratios in cereals: archaeological implications for reconstruction of diet and crop management practices. *Journal of Archaeological Science,* 34**,** 335-343.

Bogaard *et al.* 2013. Crop manuring and intensive land management by Europe's first farmers. *Proceedings of the National Academy of Sciences,* 110**,** 12589.

Bonhomme, V., Picq, S., Gaucherel, C. & Claude, J. 2014. Momocs: Outline analysis using R. *Journal of statistical software,* 56**,** 1-24.

Bonhomme, V., Forster, E., Wallace, M., Stillman, E., Charles, M., Jones, G., 2017. Identification of inter- and intra-species variation in cereal grains through geometric morphometric analysis, and its resilience under experimental charring. J. Archaeol. Sci. 86, 60–67. https://doi.org/10.1016/j.jas.2017.09.010

Braadbaart F, van der Horst J, Boon JJ, van Bergen PF (2004) Laboratory simulations of the transformation of emmer wheat as a result of heating: the change of the physical, bulk chemical and molecular composition. *J Therm Anal Calorim* 77:957–973

Braadbaart, F. 2008. Carbonisation and morphological changes in modern dehusked and husked Triticum dicoccum and Triticum aestivum grains. *Veget Hist Archaeobot* 17**,** 155–166

Castillo, C. 2013 The archaeobotany of Khao Sam Kaeo and Phu Khao Thong: the agriculture of late prehistoric Southern Thailand: Unpublished PhD thesis; Institute of Archaeology, University College London.

Castillo, C., Tanaka, K., Sato, Y.-I., Ishikawa, R., Bellina, B., Higham, C., Chang, N., Mohanty, R., Kajale, M. & Fuller, D. 2016. Archaeogenetic study of prehistoric rice remains from Thailand and India: evidence of early japonica in South and Southeast Asia. *Archaeological and Anthropological Sciences,* 8**,** 523-543.

Castillo, C. C., Polkinghorne, M., Vincent, B., Suy, T. B. & Fuller, D. Q. 2018. Life goes on: Archaeobotanical investigations of diet and ritual at Angkor Thom, Cambodia (14th–15th centuries CE). *The Holocene,* 28**,** 930-944.

Chang, T.T. 1976. The origin, evolution, cultivation, dissemination, and diversification of Asian and African rices. *Euphytica,* 25**,** 425-441.

Charles, M., Jones, G. & Hodgson, J. G. 1997. FIBS in Archaeobotany: Functional Interpretation of Weed Floras in Relation to Husbandry Practices. *Journal of Archaeological Science,* 24**,** 1151-1161.

Charles, M., Forster, E., Wallace, M. & Jones, G. 2015. "Nor ever lightning char thy grain" 1 : establishing archaeologically relevant charring conditions and their effect on glume wheat grain morphology. *Science and Technology of Archaeological Research,* 1**,** 1-6.

Choi, J. Y., Platts, A. E., Fuller, D.Q., Hsing, Y-I., Wing. R. A., & Purugganan. M. D. 2017. The Rice Paradox: Multiple Origins but Single Domestication in Asian Rice. *Mol. Biol. Evol.,* 34(4)**,** 969–979.

Choi, J. Y. & Purugganan, M. D. 2018. Multiple origin but single domestication led to Oryza sativa. *G3 (Bethesda),* 8**,** 797-803.

Civáň, P., Craig, H., Cox, C. J., & Brown, T. A. 2015. Three geographically separate domestications of Asian rice. *Nat Plants.* 1: 151-64.

Civáň, P., Brown, T. A. 2017 Origin of rice (Oryza sativa L.) domestication genes. *Genet Resour Crop Evol*, 64**,** 1125–1132.

Civáň, P. Brown, T.A. 2018. Role of genetic introgression during the evolution of cultivated rice (Oryza sativa L.). *BMC Evolutionary Biology,* 18:57. DOI: https://doi.org/10.1186/s12862-018-1180-7

D'alpoim Guedes, J. & Aldenderfer, M. 2019. The Archaeology of the Early Tibetan Plateau: New Research on the Initial Peopling through the Early Bronze Age. *Journal of Archaeological Research,* 28**,** 339–392.

Fiorentino, G., Ferrio, J. P., Bogaard, A., Araus, J. L., Riehl, S. 2015. Stable isotopes in archaeobotanical research. *Veget Hist Archaeobot* 24**,** 215–227.

Fuller, D.Q. 2006. Agricultural Origins and Frontiers in South Asia: A Working Synthesis. *Journal of World Prehistory,* 20**,** 1-86.

Fuller, D. Q. 2007. Contrasting Patterns in Crop Domestication and Domestication Rates: Recent Archaeobotanical Insights from the Old World. *Ann Bot,* 100**,** 903-924

Fuller, D. Q. 2011. Pathways to Asian Civilizations: Tracing the Origins and Spread of Rice and Rice Cultures. *Rice (New York, N.Y.),* 4**,** 78-92.

Fuller, D. Q. & Qin, L. 2009. Water management and labour in the origins dispersal of Asian rice. *World Archaeology* 41**,** 88–111.

Fuller, D. Q., Qin, L., Zheng, Y., Zhao, Z., Chen, X., Hosoya, L. A., Sun G-P. 2009. The Domestication Process and Domestication Rate in Rice: Spikelet bases from the Lower Yangzte. *Science,* 323**,** 1607-1610.

Fuller, D. Q. & Madella, M. 2009. Banana cultivation in South Asia and East Asia: a review of the evidence from archaeology and linguistics. *Ethnobotany Research & Applications* 7**,** 333–51.

Fuller, D., Sato, Y.-I., Castillo, C., Qin, L., Weisskopf, A., Kingwell-Banham, E., Song, J., Ahn, S.-M. & Etten, J. 2010. Consilience of genetics and archaeobotany in the entangled history of rice. *Archaeological and Anthropological Sciences,* 2**,**

Fuller, D.Q., Weisskopf, A. & Castillo, C. 2016. Pathways of rice diversification across Asia. *Archaeology International*. No. 19, pp. 84–96.

Garris *et al.* 2005. Genetic structure and diversity in Oryza sativa L. *Genetics*, 169**,** 1631.

GRiSP (Global Rice Science Partnership). 2013. Rice Almanac, 4th edition. Los Baños (Philippines): International Rice Research Institute.

Grist, D. H. (1965). *Rice*. London: Longman.

Hamerow, H., Mckerracher, M., Forster, E., Bogaard, A., Charles, M., Holmes, M., Thomas, R., Ramsey, C., Stroud, E. & Neil, S. 2020. An integrated bioarchaeological approach to the medieval 'agricultural revolution': a case study from Stafford, England, c.Ad 800–1200. *European Journal of Archaeology*.

Harvey, E. 2007. Early agricultural communities in northern and eastern India: An archaeobotanical investigation. Unpublished PhD dissertation, Institute of Archaeology, University College London.

Harris, D. R. 1990. Vavilov's concept of centres of origin of cultivated plants: its genesis and its influence on the study of agricultural origins. *Biological Journal of the Linnean-Society* 39**,** 7-16.

Higham, C. 2014. *Early mainland Southeast Asia: from first human to Angkor,* Bangkok, River Books.

Hunt, H. V., Oliveira, H. R., Lister, D. L., Clarke, A. C., & Przelomska, N. A.S. 2018. The Geography of Crop Origins and Domestication: Changing Paradigms from Evolutionary Genetics. *In:* Lightfoot, E., Liu, X., & Fuller, D. Q. (ed.), *Far from the hearth, essays in honour of Martin K. Jones.* Cambridge, UK: McDonald Institute for Archaeological Research. 165-176.

Huang, X. & Han, B. 2016. Rice domestication occurred through single origin and multiple introgressions. *Nat Plants,* 2**,** 15207-15207.

Hu, G., Wang, P., Rahman, S. M., Li, D., Alam, M. M., Zhang, J., Jin, Z., Fan, A., Chen, J., Zhang, A., & Yang, W. 2020. Vicissitudes experienced by the oldest urban center in Bangladesh in relation to the migration of the Brahmaputra River. *Journal of Quaternary Science; 35(8) 1089–1099.*

Jones, G., Bogaard, A., Halstead, P., Charles, M. & Smith, H. 1999. Identifying the intensity of crop husbandry practices on the basis of weed floras. *The Annual of the British School at Athens,* 94**,** 167-189.

Jones, G. 2002. Weed ecology as a method for the archaeobotanical recognition of crop husbandry practices. *Acta Palaeobotanica,* 42**,** 185-193.

Kajale, M. D. 1990. "Some Initial Observations on Palaeobotanical Evidence for Mesolithic Plant Economy from Excavations at Damdama, Pratapgarh, Uttar Pradesh," *In:* Chakrabarti, N. C. G. A. S. (ed.) *Adaptation and Other Essays.* Santiniketan Viswa Bharti.

Khush, G.S. 1984. Terminology for rice growing environment. In: IRRI (Eds.), Terminology of rice growing environments. Manila, IRRI.

Kingwell-Banham, E. 2015. Early rice agriculture in South Asia. Early rice agriculture in South Asia. Identifying cultivation systems using archaeobotany. Unpublished PhD thesis. University College London (UCL).

Kingwell-Banham *et al.* 2018a. Archaeobotanical Investigations into Golbai Sasan and Gopalpur, Two Neolithic-Chalcolithic Settlements of Odisha. *Ancient Asia,* 9**,** 1–14.

Kingwell-Banham, E., Bohingamuwa, W., Perera, N., Adikari, G., Crowther, A., Fuller, D. Q., & Boivin, N. 2018b. Spice and rice: pepper, cloves and everyday cereal foods at the ancient port of Mantai, Sri Lanka. *Antiquity* 92 1552–1570

Kingwell-Banham, E. 2019. Dry, rainfed or irrigated? Reevaluating the role and development of rice agriculture in Iron Age-Early Historic South India using archaeobotanical approaches. *Archaeological and anthropological sciences,* 11**,** 648

Lancelotti, C., Caracuta,V., Fiorentino, G., Madella, M., & Ajithprasad, P. 2013. Holocene Monsoon Dynamics and Human Occupation in Gujarat: Stable Isotopes Analyses on Plant Remains. *Journal of Multidisciplinary Studies in Archaeology,* 1**,** 288-300.

Kovach, M. J., Sweeney, M. T., & Mccouch, S. R. 2007. New insights into the history of rice domestication. *Trends Genet,* 23**,** 578-587.

Londo, J. P., Chiang, Y. C., Hung, K. H., Chiang, T. Y. & Schaal, B. A. 2006. Phylogeography of Asian wild rice, Oryza rufipogon, reveals multiple independent domestications of cultivated rice, Oryza sativa. *Proc Natl Acad Sci U S A,* 103**,** 9578-9583.

López-Dóriga, I. L. 2015 An experimental approach to the taphonomic study of charred hazelnut remains in archaeological deposits. *Archaeol Anthropol Sci* 7**,** 39–45

McNally *et al.* 2009. Genome wide SNP variation reveals relationships among landraces and modern varieties of rice. *Proceedings of the National Academy of Sciences,* 106**,** 12273-12278.

Mueller-Bieniek, A., Nowak, M., Styring, A., Lityńska-Zając, M., Moskal-Del hoyo, M., Sojka, A., Paszko, B., Tunia, K. & Bogaard, A. 2019. Spatial and temporal patterns in Neolithic and Bronze Age agriculture in Poland based on the stable carbon and nitrogen isotopic composition of cereal grains. *Journal of Archaeological Science: Reports,* 27.

Molina, J., Sikora, M., Garud, N., Flowers, J. M., Rubinstein, S., Reynolds, A., Huang, P., Jackson, S., Schaal, B. A., Bustamante, C. D., Boyko, A. R. & Purugganan, M. D. 2011. Molecular evidence for a single evolutionary origin of domesticated rice. *Proc Natl Acad Sci U S A,* 108**,** 8351-8356.

Morishima, H., Sano, Y., & Oka, H.I. 1980. Observations on wild and cultivated rices andcompanion weeds in the hilly areas of Nepal, India and Thailand: Report of study-tour in tropical Asia, 1979. Misima: Rep. Natl. Inst. Genetics.

Nayak, A., Basa, K. K., Boivin, N. L., Fuller, D. Q., Mohanty, R. K., Kingwell-Banham, E., Murphy, C., Roberts, P. J., Lee-Thorp, Julia., Bogaard, A. 2022. A stable isotope perspective on archaeological agricultural variability and Neolithic experimentation in India. *Journal of Archaeological Science* 141. https://doi.org/10.1016/j.jas.2022.105591

Ng, N. Q., Chang, T. T., Williams, J. T. & Hawkes, J. G. 1981. Morphological studies of Asian rice and its related wild species and the recognition of a new Australian taxon. *Biological Journal of the Linnean Society,* 16**,** 303-313.

Oka, H. I. 1988. *Origin of cultivated rice,* Tokyo: Japan Scientific Societies Press; Elsevier; Exclusive sales rights for the U.S.A. and Canada, Elsevier Science Pub. Co.

Pal, J. N. 2008. The early farming culture of the middle Ganga plain with special reference to the excavations at Jhusi and Hetapatti. *Pragdhara* 18**,** 263–281.

Pal, J. N. 2016. Mesolithic foragers of the Ganges plain and adjoining hilly. *In:* G. R. Schug, S. R. W., & K. A. R. Kennedy (ed.) *A Companion to South Asia in the Past, Blackwell Companions to Anthropology.* Chichester John Wiley.

Petrie, C. A. & Bates, J. 2017. 'Multi-cropping', Intercropping and Adaptation to Variable Environments in Indus South Asia. *Journal of World Prehistory,* 30**,** 81.

Petrie, C. A., Bates, J., Higham, T. & Singh, R. N. 2016. Feeding ancient cities in South Asia: dating the adoption of rice, millet and tropical pulses in the Indus civilisation. *Antiquity,* 90**,** 1489-1504.

Pokharia, A. K., Sharma, S., Tripathi, D., Mishra, N., Pal, J. N., Vinay, R. & Srivastava, A. 2017. Neolithic-early historic (2500-200 BC) plant use; the archaeobotany of Ganga Plain, India. *Quaternary International,* 443**,** 223-237.

Pokharia, A. K. 2011. Palaeoethnobotany at Lahuradewa: a contribution to the 2nd millennium BC agriculture of the Ganga Plain, India. *Current Science,* 101**,** 1569-1578.

Pokharia, A. K., Kharakwal, J. S. & Srivastava, A. 2014. Archaeobotanical evidence of millets in the Indian subcontinent with some observations on their role in the Indus civilization. *Journal of Archaeological Science,* 42**,** 442-455.

Rahman, M. 2018. Agricultural Extension in Medieval Northern Bangladesh: Archaeobotanical evidence from Setesher Danga: Unpublished MSc dissertation, UCL Institute of Archaeology.

Rahman, M. Murphy, C., Weisskopf, A., Champion, L, Castillo, C, & Fuller, D. Q. 2019. Wari-Bateshwar and Vikrampura: Successful Case Studies in Archaeobotany, Bangladesh. *Man and Environment.,* XLIV (1)**,** pp: 62-72.

Rahman, M. Castillo. C. C., Murphy, C., Rahman, S. M., & Fuller, D. Q. 2020. Agricultural systems in Bangladesh: the first archaeobotanical results from Early Historic Wari-Bateshwar and Early Medieval Vikrampura. *Archaeological and Anthropological Sciences*.

Sano, Y., Morishima, H. & Oka, H.-I. 1980. Intermediate perennial-annual populations of Oryza perennis found in Thailand and their evolutionary significance. *The Botanical Magazine Tokyo,* 93**,** 291-305.

Saraswat, K. S. 2004. Plant economy in ancient Malhar. *Pragdhara,* 4**,** 137-171.

Saraswat, K. S. 2005. Agricultural background of the early farming communities in the Middle Ganga Plain. *Pragdhara,* 15**,** 145-177.

Schatz, M. C., Maron, L. G., Stein, J. C., Hernandez Wences, A., Gurtowski, J., Biggers, E., Lee, H., Kramer, M., Antoniou, E., Ghiban, E., Wright, M. H., Chia, J.-M., Ware, D., McCouch, S. R. & McCombie, W. R. 2014. Whole genome de novo assemblies of three divergent strains of rice, Oryza sativa, document novel gene space of aus and indica. *Genome biology,* 15**,** 506.

Sharma, G. B., & Misra, B. B. 1980. *Excavations at Chopani-Mando (Belan Valley) 1977-1979?: Epipalaeolithic to Protoneolithic.* Allahbad, University of Allahabad.

Sharma, S.D., & Shastry, S.V.S. 1965 Taxonomic studies in the genus Oryza L. III. O. rufipogon Griff. sensu stricto and O. nivara Sharma et Shastry nom. Nov. *Indian J Genet Plant Breed* 25**,** 157–167.

Silva, F., Stevens, C., Weisskopf, A., Castillo, C., Qin, L., Bevan, A. & Fuller, D. 2015. Modelling the Geographical Origin of Rice Cultivation in Asia Using the Rice Archaeological Database. *PLoS One,* 10(9)**,** 1-21.

Silva, F., Weisskopf, A., Castillo, C., Murphy, C., Kingwell-Banham, E., Qin, L. & Fuller, D. Q. 2018. A tale of two rice varieties: Modelling the prehistoric dispersals of japonica and proto-indica rices. *Holocene (Sevenoaks),* 28**,** 1745-1758.

Singh, I. B. 2005a. . Quaternary palaeoenvironments of the Ganga plain and anthropogenic activity. *Man Environ,* 30**,** 1–35.

Singh, I. B. 2005b. Landform development and palaeovegetation in Late Quaternary of the Ganga Plain: Implications for anthropogenic activity. *Pragdhara* 15**,** 5–31.

Stevens *et al.* 2016. Between China and South Asia: A Middle Asian corridor of crop dispersal and agricultural innovation in the Bronze Age. *The Holocene,* 26**,** 1541-1555.

Stroud, E., Charles, M., Bogaard, A., Hamerow, H. 2023. Turning up the heat: Assessing the impact of charring regime on the morphology and stable isotopic values of cereal grains. *Journal of Archaeological Science* 153

Styring, A., Charles, M., Fantone, F. *et al.* 2017. Isotope evidence for agricultural extensification reveals how the world's first cities were fed. *Nature Plants* 3. DOI: 10.1038/nplants.2017.76

Tewari, R., Srivastava, R. K., Singh, K. K., Saraswat, K. S., & Singh, I. B. 2003. Preliminary report of the excavation at Lahuradewa, District Sant KabirNagar, Tewari, R., Srivastava, K. K., Singh, K. K., Vinay, R., Trivedi, R. K., & Singh, G. C. 2005. Recently excavated sites in the Ganga Plain and North Vindhyas: some observations regarding the pre-urban context. *Pragdhara* 15**,** 39–49.

Tewari, R., Srivastava, R. K., Singh, K. K., Saraswat, K. S., Singh, I. B., Chauhan, M. S. 2006 Second preliminary report of the excavations at Lahuradewa district Sant Kabir Nagar, U.P.?: 2002-2003-2004 & 2005-06. *Pragdhara* 16**,** 35–68.

Tewari *et al.* 2008 Early farming at Lahuradewa. *Pragdhara* 18**,** 347–373.

Thompson, G. B. 1996. Ethnographic Models for Interpreting Rice Remains. *In:* Thosarat, C. H. A. R. (ed.) *The Excavation of Khok Phanom Di a Prehistoric Site in Central Thailand; Subsistence and Environment: The Botanical Evidence.* London: The Society of Antiquaries of London.

Travis, A. J., Gareth. J. N., Datta, S., Sarma, R., Dasgupta, T., Savio, F. L., Macaulay, M., Hedley, P. E., McNally, K. L., Sumon, M. H., Islam, M. R., & Price, A. H. 2015. Assessing the genetic diversity of rice originating from Bangladesh, Assam and West Bengal. *Rice,* 8:35.

Varma, R. K., Misra, V. D., Pandey, J. N., & Pal, J. N. 1985 A preliminary report on the excavations at Damdama (1982-1984). *Man Environ,* 9**,** 45–65.

Vaughan, D. A. 1989. *The genus Oryza L.: current status of taxonomy,* Manila, Philippines, International Rice Research Institute.

Vaughan, D. A., Lu, B.-R. & Tomooka, N. 2008b. Was Asian Rice (Oryza sativa) Domesticated More Than Once? *Rice (New York, N.Y.),* 1**,** 16-24.

Vavilov, N.1992(1927). *Origin and geography of cultivated plants*. Cambridge: Cambridge University Press.

Vaughan, D. A., Lu, B.-R. & Tomooka, N. 2008. The evolving story of rice evolution. *Plant science (Limerick),* 174**,** 394-408

Zhao, Z. 2011. New Archaeobotanical Data for the Study of the Origins of Agriculture in China. *Current Anthropology,* 52**,** S2

Paper_01: Assessing the Effect of Experimental Charring on Grain Morphology in Asian Rice Genotypes

Abstract

Rice occurs widely on archaeological sites in charred form, including early agricultural sites in Bangladesh with well-preserved rice grains. Experimental charring of modern rice grains in a controlled environment is needed to establish optimal heating conditions for replicating well-preserved archaeological rice. This replication is needed to assess the effects of heating on rice grain morphology – with implications for taxonomic identification to variety or subspecies level – as well as on rice biomolecular composition. Experimental charring of rice grains of three Asian rice genotypes, *aus*, *japonica*, and *indica*, revealed that a relatively restricted set of low-temperature conditions (230^0C heating for 2-3 hours in a reducing environment) produced rice grains equivalent to well-preserved undistorted archaeological charred rice. Temperatures above 240^0C cause substantial distortion of rice grain morphology and reduce the potential for accurate identification to subspecies level, and for related biomolecular work.

Keywords: Archaeobotany, *Aus*, Charring, Distortion, Genotypes, *Indica*, *Japonica*, Rice

1.1 Introduction

Rice, *Oryza sativa L.*, has been a staple cereal grain in South, Southeast and East Asia since prehistory. Research to establish the origin, domestication and geographical distribution of rice and its chronological framework is a multidisciplinary inquiry including archaeology, genetics and evolutionary biology, and these fields have seen an acceleration in data acquisition along with advances in theory and analytical techniques in recent decades (Fuller

2007; Fuller *et al*. 2009; Civan *et al*. 2015; Castillo *et al*. 2016; Gutakar *et al*. 2020; Ishikawa *et al*. 2020) It is now believed that at least three distinct rice genotypes, *japonica*, *indica* and *aus,* emerged between the mid-5th millennium and the late first millennium BC (Fuller 2011; Choi *et al.* 2017). How rice spread between China, Southeast Asia and South Asia are of primary interest to researchers working in this area, but much remains to be clarified, including the possibility of a route of dispersal through Bangladesh to India (Diamond & Bellwood 2003; Van Driem 2011; Fuller *et al.* 2016; Stevens *et al*. 2016).

Current research suggests that the three rice genotypes identified arose and were domesticated in China (*japonica*), India (*indica*) and Bangladesh (*aus*), respectively. Genetic studies of modern collections of rice variants revealed that *japonica* originated from the wild progenitor *Oryza rufipogon* and was domesticated through a *de novo* domestication process through the ~6th to 4th millennia BC, while *indica* originated from the wild progenitor *Oryza nivara* through introgressive hybridization with domesticated *japonica* in the 2nd millennium BC. In the case of *aus* rice, it has been proposed that the genotype likely originated in Bangladesh through introgressive hybridization with *japonica* and *indica* in the late 1st millennium BC (Gross and Zhao 2014; Civan *et al*. 2015; Choi *et al*. 2017). The *aus* group differs ecologically from *indica* and *japonica* through early maturation and drought tolerance, features of potential importance for the establishment of rice cultivation in Bangladesh (Choi *et al.* 2017).

Archaeobotanically, the distinction between the three genotypes is more difficult to establish, reliant as it is on relatively minor variations in grain and chaff morphology. These issues are further complicated when the plant material is exposed to the types of heating conditions relevant to charred archaeological preservation. Whilst the origin and domestication of *japonica* is well documented archaeobotanically (Fuller *et al.* 2009; Zheng *et al.* 2016), those of the other two major genotypes *indica* and, particularly, *aus,* have been little studied. This may happen for two reasons. First, there has been a lack of routine archaeobotanical sampling programmes accompanying regular excavation in Bangladesh. Secondly, rice chaff, particularly, spikelet bases, has not been systematically collected due to the use of large-aperture mesh sizes in flotation systems in India.

Charring is the most common form of preservation of archaeobotanical rice remains recovered from archaeological sites in early rice-growing regions. Once charred, accidentally or deliberately, the material can be preserved in various contexts, e.g., middens, hearths, pits, floors, and fills. The heating of grain under conditions that allow preservation in the sediment can, potentially, cause major changes in the appearance of the grain, possibly making identification difficult. In archaeological sites of South and Southeast Asia along with China, large numbers of rice grains have been found from the Neolithic to pre-modern period, particularly in Bangladesh, in which ~90% of cereal grains are rice that is very well preserved and relatively undistorted, demonstrating excellent preservation conditions (Deng *et al.* 2015; Pokharia *et al.* 2016; Rahman *et a*l 2019). Thus, recognising the conditions of rice charring that result in well-preserved, undistorted grains is critical for developing a morphologically reliable method of taxonomic identification.

Simple morphometric (L/W ratio based) analysis (e.g., Castillo *et al.* 2016) and geometric morphometric (shape analysis) methods (e.g., Bonhomme *et al.* 2017) have been used to identify cereal grains to the (sub)species level. Underpinning the successful development of these analyses is an understanding of the impact of heating regimes on the plant material (e.g., Charles *et al.* 2015). Despite the archaeological importance of rice, rice charring has been less studied than that of other Old-World crops (e.g., wheat and barley), where the impact of heating conditions, i.e., the rate and duration of the heating event combined with the availability of oxygen, on grain and chaff, has previously been investigated (e.g., Wilson 1984; Boardman and Jones 1990; Braadbaart 2008; Yang *et al.* 2011).

Beyond identification and morphological features, well-preserved charred grains have additional value in providing stable isotope data useful for reconstructing growing conditions and crop husbandry (Fraser *et al.* 2011; Wallace *et al*. 2013; Bogaard *et al.* 2014b), strontium isotope ratios for tracing location (Bogaard *et al.* 2014a, Styring *et al.* 2019) and other biomolecular information such as ancient DNA (aDNA) that can enhance taxonomic identification (Castillo *et al.* 2016) and inform understanding of evolutionary development (Brown 1999; Seco-Morais & Matheson 2008; Bunning, Jones and Brown 2012).

Building on recent studies in experimental charring of rice (Castillo *et al*. 2019, White *et al.* 2019), there is a need to carry out further work to establish the impact of controlled heating regimes on rice grain morphology. Specifically, differences in response to monitored heating conditions on each genotype need to be assessed and compared with well-preserved archaeological rice. Forms of preservation have significant impacts on grain morphology that can be established through experimental charring (as in hulled wheat, Charles *et al*. 2015). The purpose of this paper is to determine the optimal range of conditions under which rice becomes preserved by charring in a form that resembles that encountered archaeologically, and the impact of these conditions on grain morphology. It seeks to chart the range of conditions under which rice can be preserved in a relatively undistorted form suitable for identification to the highest taxonomic level and hence of particular interest for morphological study, dating, aDNA and stable isotope research.

1.2. Background

Extensive experimental work has been conducted on modern plant parts to understand how archaeobotanical material survives on archaeological sites. Charred archaeobotanical plant parts, the most commonly preserved form on archaeological sites, result not only from heating activities but are also affected by long-term taphonomic processes. While it is difficult to replicate all the possible conditions affecting an archaeobotanical assemblage, certain aspects of the process can be investigated.

Two methods have been used in the experiments carried out to assess the impact of different heating conditions on the shape, size and appearance of plant material (Smith and Jones 1990; Braadbaart and Bergen 2005; Castillo *et al.* 2019). First, muffle furnaces have been used in laboratory environments to produce well-controlled heating conditions and to assess cause and effect (e.g., Boardman and Jones 1990; Braadbaart *et al.* 2004a, 2004b; Charles *et al.* 2015). Secondly, open air fires and ovens have been used to mock up real-life processes, albeit with less control over heating conditions (Gustafsson 2000; Guarino-Sciarrillo 2004; Sieveris & Wadley 2008).

Most of the charring experiments conducted so far have been on Old World cereals such as wheat and barley (Robert and Robertson III 1971; Boardman and Jones 1990; Heier, *et al.* 2002; Braadbaart *et al.* 2004b; Braadbaart *et al.* 2005; Braadbaart and Bergen 2005; Fraser *et al.* 2013; Charles *et al.* 2015; Bonhomme *et al.* 2017; Berihuete-Azorín 2019), oat and rye (Gustafsson 2000; Guarino & Sciarrillo 2004), but also mustard (Märkle & Rösch 2008) and pulses (Braadbaart *et al.* 2004a; Guarino-Sciarrillo 2004; Walsh 2017), along with some North American crops, e.g., maize and sunflower (Wright 2003; Braadbaart *et al.* 2007; Dezendorf

2013). In contrast East and South Asian crops are less well studied though not completely overlooked, including rice (Garton 1979; Castillo *et al*. 2019; White *et al*. 2019) and millet (Märkle-Rösch 2008; Motuzaite-Matuzeviciute *et al*. 2012; Fraser *et al*. 2013; Walsh 2017; Castillo *et al*. 2019). Fruits, nuts, and crop weeds have been less studied, with some exceptions including grapes (Smith and Jones 1990; Guarino-Sciarrillo 2004), African fruits and nuts (Sieveris & Wadley 2008) and weeds (Wright 2003).

This experimental work has established a broad set of distortion and preservation biases caused by heating. Extending indices proposed by Hubbard and Al Azm (1990), Charles *et al*. (2015) recorded the effects of experimental heating on glume wheat grains (einkorn and emmer) using indexes for five variables: colour, distortion, blistering, lines, and shininess. Broadly this latter work served to refine models of charring by demonstrating that well-preserved, undistorted archaeobotanical glume wheat grain strongly resembled modern material exposed to a relatively narrow of conditions, at temperatures of 230^0 to 260^0C, time durations of 2-6 hours and with variable levels of oxygen availability (low to high). Complementary work by Nitsch *et al.* (2015) showed that within the optimal range of conditions, the impact on stable carbon and nitrogen isotope measurements was small and predictable. Outside this optimal range, the grain was either insufficiently heated to undergo the chemical transformations enabling it to survive in archaeological deposits or, at higher temperatures, the grain became severely distorted in appearance to the point where identification to species, or even genus level, was no longer reliable (Charles *et al*. 2015). Critically, this study also concluded that grain that was not fully blackened during heating could still survive archaeologically as the grain chemistry had become significantly transformed to limit biological degradation. This suggests that well-preserved archaeological grain material has been exposed to relatively little damage, with correspondingly less alteration of aDNA and stable isotopic values.

Most rice experimental charring to date has been conducted to test the effect of heat on grain size, with an emphasis on grain shrinkage. The results of these experiments have been variable, with some evidence demonstrating that the length, width, and thickness of rice caryopses are changed proportionately by temperatures between 200^{0}C and 300^{0}C (Garton 1979; Castillo *et al*. 2019) while White *et al*. (2019) showed no significant morphological change (L/W/T) in grain heated to 225^{0}C for 45 minutes. An investigation to distinguish rice varieties based on simple morphometric measurements (L/W) was presented by Castillo *et al*. (2016) for *indica* and *japonica*, but a full set of data relating to degree of distortion, colour, and temperature under relevant heating conditions is not yet available. Moreover, Castillo *et al*. (2019) showed that rice survives better in husked than dehusked form. Charring experiments on *japonica* and *indica* rice varieties have also demonstrated different responses towards temperature, with *japonica* less impacted by high temperatures than *indica* (Garton 1979).

The present study is designed to refine understanding of optimal rice preservation conditions, following an approach established in the study of einkorn and emmer grain charring by Charles *et al.* (2015) and complementing previous work on rice charring. The importance of understanding the effects of heating on grain morphology is needed not only to reassess simple morphometric approaches to identifying rice varieties but also to apply geometric morphometric approaches to rice grain (Bonhomme *et al*. 2017), an important development for the reliable identification of cereal grains in archaeobotanical assemblages to species level and beyond.

1.3. Materials and Methods

1.3.1. Samples and sites

Accessions of the three different rice genotypes, *japonica*, *indica* and *aus*, were collected from the germplasm centre (Genetic Seed Division) of the Bangladesh Rice Research Institute (BRRI), which manages rice collections by BRRI scientists from different parts of Bangladesh.

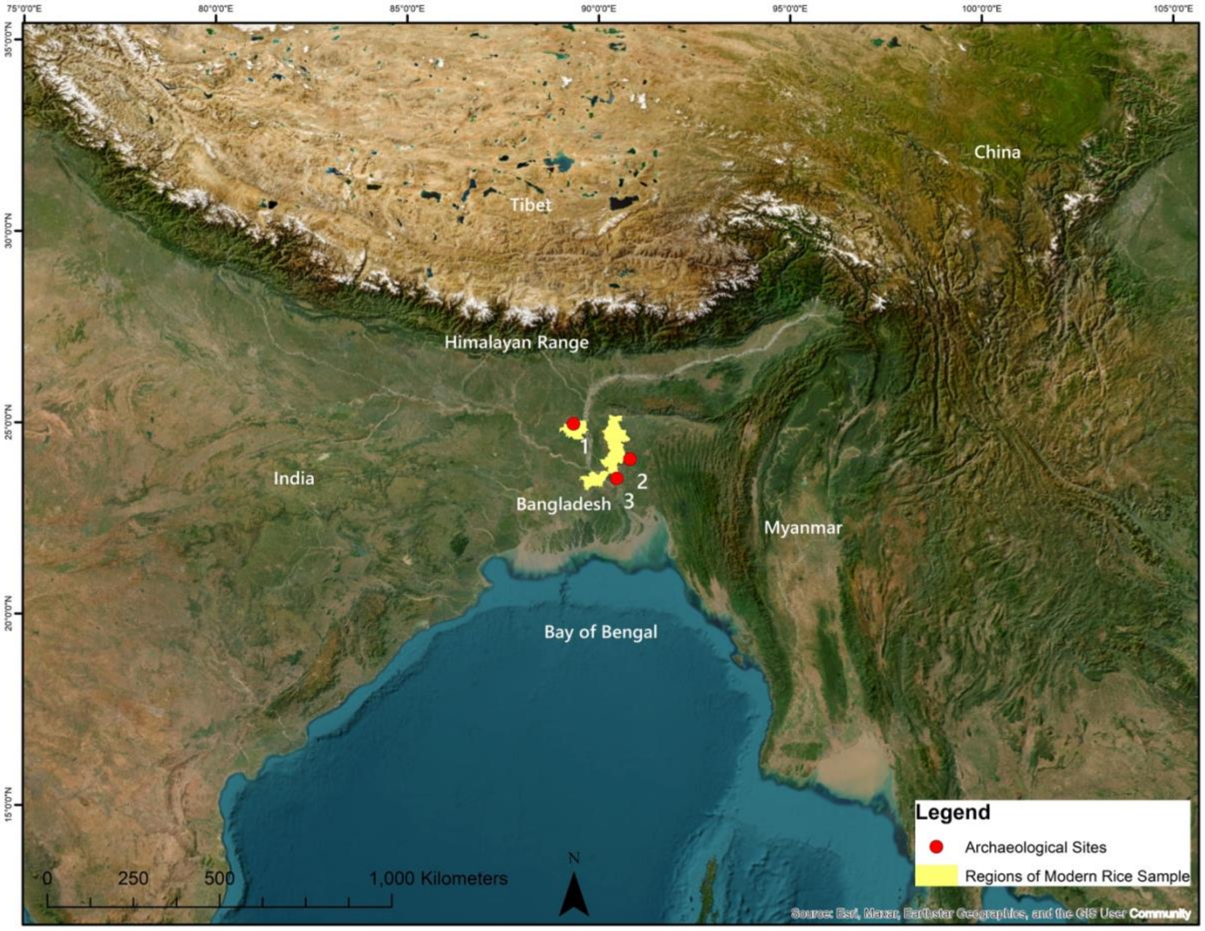


Fig. 1: The map showing the regions and sites from which modern and archaeological rice were sampled and used to compare. 1=Mahasthangarh; 2=Wari-Bateshwar; 3=Nateshwar.

Wang *et al*. (2013) assessed 150 local rice germplasm samples from Bangladesh and categorised them genetically as *japonica*, *indica* or *aus*. Thus, *japonica* and *indica* rice accessions for this study were selected following Wang *et al*. (2013).

For *aus* rice, two landraces—aus-02 and aus-03—were selected by the criteria established by Wang *et al*. (2013), while aus-01 was chosen based on local farmers' perceptions, as it is widely cultivated in the region as an aus rice variety (Details of modern rice genotypes subjected to charring are shown in Table 2).

Table 7: The archaeological sites from which archaeological rice grains have been collected for this study.

No.	Name of site	Code name	Period	Condition
1	Wari-Bateshwar	WB	*c*. 800-200 BC	Mostly well-preserved, undistorted.
2	Mahasthangarh	MH	*c*.300BC-AD1200	
3	Nateshwar	NAT	*c*. 700-1100 AD	

Table 8: List of the modern rice accessions subjected to experimental charring in this study (BRRI = Bangladesh Rice Institute Research; T = transplanted; B = Broadcast; *selected based on ecotype as widely cultivated *aus* rice in Bangladesh; for genotypes see Wang *et al*. (2013).

Genotypes	Code	Local name	Ecotype	BRRI accession	Place of collection
Aus*	A-01	Jugli	Aus	1860	Faridpur
Aus	A-02	Sada boro	T-Aman	1997	Mymensingh
Aus	A-03	Kunail boro	Boro	1714	Faridpur
Indica	I-01	Tapi/Topa	Boro	62	Dhaka
Indica	I-02	Dudh sail	Aus	2178	Bogra
Indica	I-03	Dudh Shar	T-Aman	21	Dhaka
Japonica	J-01	Nenia	B-Aman	1823	Bogra
Japonica	J-02	Dad sail	Boro	4786	Mymensingh
Japonica	J-03	Nepa	B-Aman	61	Dhaka

Well-preserved archaeological rice grains to compare with the modern charred rice genotypes were recovered from three archaeological sites in Bangladesh, Wari-Bateshwar (WB, *c*. 8th century BCE to onwards), Mahasthangarh (MH, c. 3rd century BCE) and Nateshwar (NAT, c. 7th to 10th century CE) (Fig.1; Table 1).

1.3.2. Charring experiment

The current experiment was carried out following the charring methods set out by Charles *et al.* (2015), in which a preheated high-precision muffle furnace/oven was used to heat the selected rice grains, in the Archaeobotany Laboratory of the School of Archaeology, University of Oxford. Five to 15 rice grains of each taxon were used per charring regime, which included both reducing and oxidizing conditions. To better compare the impact of charring between modern and archaeological rice, paddy rice was dehusked manually before putting into the oven. It is because the husk has been destroyed due to charring and it has rarely been found at archaeological sites despite the controversial opinion on charring impact on husked and dehusked cereal grain (For no impact; Braadbaart and Bergen 2005; Charles *et al*. 2015, for impact; Castillo 2019). In both reducing and oxidizing conditions, grains were packed in foil and placed in 250ml Pyrex beakers; for reducing conditions, the beaker was filled with sand while the beaker was kept without sand for oxidized conditions. In each heating session, a thermocouple was placed into one of the beakers. Temperatures ranging between 220^0C-260^0C were used to heat the samples, with up to 2^0C variation continuously monitored using the thermocouple attached to a datalogger. After the predetermined heating duration, the beakers were removed from the oven and cooled to room temperature. A low-power stereomicroscope (x7 to x45 magnification) was used to observe changes on the whole body of charred grain and in cut grain sections. Two grains, one for reduced and one for oxidized conditions, were selected from each charring (temperature and time) regime for observing the grain's internal structure by cutting the grains into apical and embryo halves using a scalpel. Finally, a Leica Z-6 APO microscope with a digital camera (Leica DFC495) was used to capture photographs of whole grains and cut grain sections.

The heated material was assessed according to five variables (summarised scores and attributes shown in Tables 4-6; full detail provided in Annex 2, Table 1): four used by Charles *et al*. (2015) and Hubbard and al Azm (1990) – colour, distortion, blistering, and shininess – plus a fifth, based on the degree of cracking.

The five variables recorded are:

1. Colour: The colour of the outer epidermal layer and the cut grain sections (inner surface) were recorded using Munsell's colour chart as a guide to distinguish shades of brown from black.
2. Distortion: The level of average grain distortion in the sample was scored based on the scale of Hubbard and al-Azm (1990) modified by Charles *et al*. (2015), from 1 (= unchanged) to 5 (=extremely distorted). The final point used on this scale, 5 (=fused and mostly unidentifiable), corresponds to the final point in Charles *et al*. (2015). The least distorted/best preserved archaeological rice would have a score of 1-2, demonstrating that the grain has gone through the minimum morphological change along with negligible shortening and rounding.
3. Blistering: Due to heat, grain caryopses can produce bubbles that have been considered diagnostic of heating conditions.
4. Shininess: Uncharred rice grain is dull in lustre and can become consistently shiny depending on temperature and duration.
5. Cracking: Different types of cracks (longitudinal, elongated, heterogenic, and ranges of cracks; small to large) were recorded on the caryopses.

Table 9: Scoring for the effects of charring on rice grains following Hubbard and Al Azm (1990) and Charles *et al*. (2015), using a 5-point ranging from 1= undistorted to 5= extremely distorted/fused. Shading indicates equivalence to well-preserved undistorted archaeological rice grain.

Score	Colour	Distortion	Internal structure
1	Uncharred	Unchanged	Unchanged
2	Light brown to pale	Slight to perfect (No distortion)	Dense no voids.
3	Medium brown	Slight to moderate	Dense, no voids but possible expansion cracks)
4	High dark brown	Grossly distortion.	Less dense, no voids but possible expansion cracks.
5	Black	Extremely distorted	Less dense, voids.

1.3.3. Scanning Electron Microscopy (SEM)

Both charred modern rice grains and well-preserved archaeological rice grains were selected for SEM examination in order to make detailed observations on the cut grain sections. For this, two rice grains from each charring regime (temperature and duration), one from reduced and one from oxidized conditions, were selected and mounted on stubs. Before mounting, each rice grain was cut perpendicular to the latitudinal axis using a scalpel to visualize the core endosperm. Afterwards, the whole cross-section was examined before taking photos at x50 and x200 magnification, capturing the whole section and the area adjacent to the aleurone layer, respectively.

1.4. Results

Tables 4, 5, and 6 summarise’ the rice charring results in terms of colour, distortion, and internal structure, respectively (Detailed results are presented in Appendix- A) and Figs 2, 4, and 6 show visual evidence of changes due to charring.

1.4.1. Colour

Grain cross-sections at 240^{0}C and above are substantially blackened (Table 4 and Figs 2, 4, and 6). All the grains appear substantially black externally while *indica* turns black at 220^{0}C after 4 hours. At 230^{0}C grain blackening is more variable at short time exposures, with *aus* and *japonica* material not fully blackening at shorter time exposures though *indica* is fully black after 2 hours.

Table 10. Summary of colour for the three Asian rice varieties after heating for 2-6 hours at temperatures ranging from 220^{0}C to 260^{0}C under reducing and oxidizing conditions; 2= light brown to pale, 3= medium dark brown, 4=High dark brown, 5=Black.

Rice type		*Aus*					*Indica*					*Japonica*			
Attributes		Color					Color					Color			
Temp (°C)		2	2	2	2		2	2	2	2		2	2	2	2
Hour		20	30	40	60		20	30	40	60		20	30	40	60
Reducing (Sand)	2	2	4	4	5		4	5	5	5		2	4	5	5
	3	3	4	5	5		4	5	5	5		3	4	5	5
	4	3	4	5	5		5	5	5	5		4	5	5	5
	6	3	5	5	5		5	5	5	5		4	5	5	5
Oxidizing (No sand)	2	2	4	4	5		5	5	5	5		2	4	4	5
	3	3	5	5	5		5	5	5	5		3	5	5	5
	4	4	5	5	5		5	5	5	5		4	5	5	5
	6	4	5	5	5		5	5	5	5		4	5	5	5

All the grains are black after 6 hours. At 220^{0}C the difference in grain charring between *indica* and the other two varieties is also apparent, with *indica* grains turning black and the other two remaining dark brown, even after 6 hours.

1.4.2. Distortion

Table 5 and Figs. 2, 4 and 6 show that grains become distorted even at 220^0C after 2 hours for *aus* and *japonica* and after 6 hours for *indica* with low alteration of grain morphology.

Table 11. Summary of distortion scores for the three Asian rice varieties after heating for 2-6 hours at temperatures ranging from 220^0C to 260^0C under reducing and oxidizing conditions; 1=no distortion (unchanged), 2= Low distortion (perfect grain), 3= Medium distortion (likely perfect grain), 4= Grossly distorted (likely shorter and rounded), 5=Extremely distorted (Fused). Red = rice closely resembles well-preserved archaeological rice grain; orange = grain unlikely to preserve archaeologically; no fill colour = does not resemble well-preserved archaeological rice grain.

Rice type		*Aus*					*Indica*					*Japonica*			
Attributes		Distortion					Distortion					Distortion			
Temp(°C)		220	230	240	260		220	230	240	260		220	230	240	260
Hour															
Reducing (Sand)	2	2	3	4	5		1	2	4	5		1	2	4	4
	3	3	4	4	5		1	4	4	5		2	2	4	4
	4	3	4	4	5		1	4	4	5		2	2	4	4
	6	4	4	4	5		2	4	4	5		2	2	4	4
Oxidizing (No sand)	2	2	4	4	5		3	4	4	5		3	4	4	4
	3	3	4	4	5		4	4	4	5		4	3	4	4
	4	3	4	4	5		4	4	4	5		4	4	4	4
	6	4	4	4	5		4	4	4	5		4	4	4	4

At 240^0C the grains are prone to significant distortion and at 260^0C the contents of the grain have ‘oozed out’ causing them to fuse while *japonica* shows resistance to this fusion.

All types of rice become substantially shorter at 240^0C even after 2 hours of heating while no changes appear to be visible at 220^0C and negligible changes materialize at 230^0C which grain size remains closer to the modern rice grain. Table 5 shows that all rice is more substantially distorted under high oxygen availability than under reduced conditions, even at a low temperature of 220^0C for 2 hours.

1.4.3. Internal Structure and Cellular Changes

Table 6 demonstrates that significant internal changes impacting cell structures typically occur at 230^0C after 3 hours in *aus* and *japonica* and after 4 hours in *indica*. Cut grain sections (Figs. 3, 5, 7) start to show disappearing cell boundaries and clearing of cell contents along with, occasionally, separation of the aleurone layer and pericarp at 240^0C as observed at both lower and higher magnification. A more open network of moderately large empty cells and substantially rounded grain morphology appears at 240^0C in cross-section in all genotypes.

At 260^0C a more open network appears along with relatively large cells without contents. At 220^0C no noticeable changes are observed, with clear cell boundaries evident at low magnification and cells with full contents observed at higher magnification. Modern rice cut sections show clear cell boundaries at lower magnification while cells with filled contents are apparent at higher magnification (Figs. 3, 5, 7).

1.4.4. Overall Summary

While broadly similar patterns are observed in all three varieties, there is evidence that *indica* is more sensitive to heating than the other two. This is manifest in the grain darkening earlier and in more pronounced distortion. Heating conditions matching those of well-preserved archaeological grain occur in the range of 230^0c for 2-3 hours regimes across all genotypes.

Heating at 240^0C causes substantial alteration of grain morphology, while heating to 260^0C results in extremely distorted and often fused grain, though *japonica* is less prone to fusion.

Table 12. Scores for internal grain structure of the three Asian rice varieties after heating for 2-6 hours at temperatures ranging from 220^0C to 260^0C under reducing and oxidizing conditions; 2 = dense, no voids; 3 = dense no voids but possible expansion cracks; 4 = less dense, no voids but possible expansion cracks; 5 = less dense, voids.

Rice type		*Aus*					*Indica*					*Japonica*			
Attributes		Internal structure					Internal structure					Internal structure			
Temp (^{o}C)		2	2	2	2		2	2	2	2		2	2	2	2
Hour		20	30	40	60		20	30	40	60		20	30	40	60
Reducing (Sand)	2	2	2	4	5		2	2	4	5		2	3	4	5
	3	2	3	4	5		2	3	4	5		2	3	4	5
	4	2	3	4	5		2	3	4	5		2	3	4	5
	6	2	4	5	5		2	4	5	5		2	4	5	5
Oxidizing (No sand)	2	2	2	4	5		2	2	4	5		2	3	4	5
	3	2	3	4	5		2	3	4	5		2	3	4	5
	4	2	3	4	5		2	3	4	5		2	3	4	5
	6	3	3	5	5		2	3	4	5		3	4	4	5

Figure 2. Low power microscopy of modern uncharred and charred *aus* rice grains heated from 220^{0}C to 260^{0}C; cross-section x2.00 Mag. (top row); whole body grain x0.57 Mag. (bottom row).

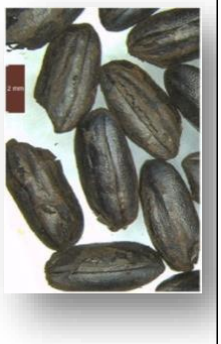
Uncharred	*220^{0}C*	*230^{0}C*	*240^{0}C*	*260^{0}C*
				
				

Figure 3. SEMs photograph of modern unheated and heated *aus* rice cross section range between 220^{0}C to 260^{0}C. The top row shows low magnification, and the bottom shows a high-magnification photo.

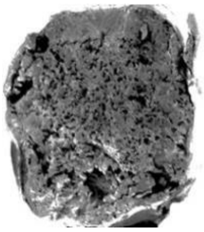
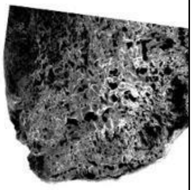
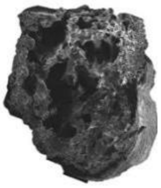
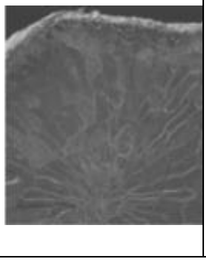
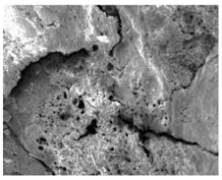
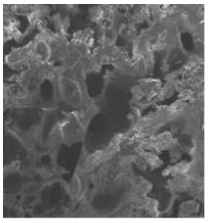
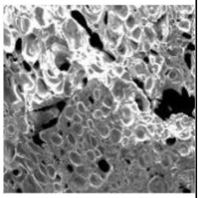
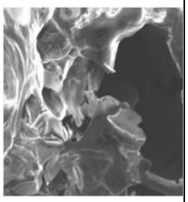
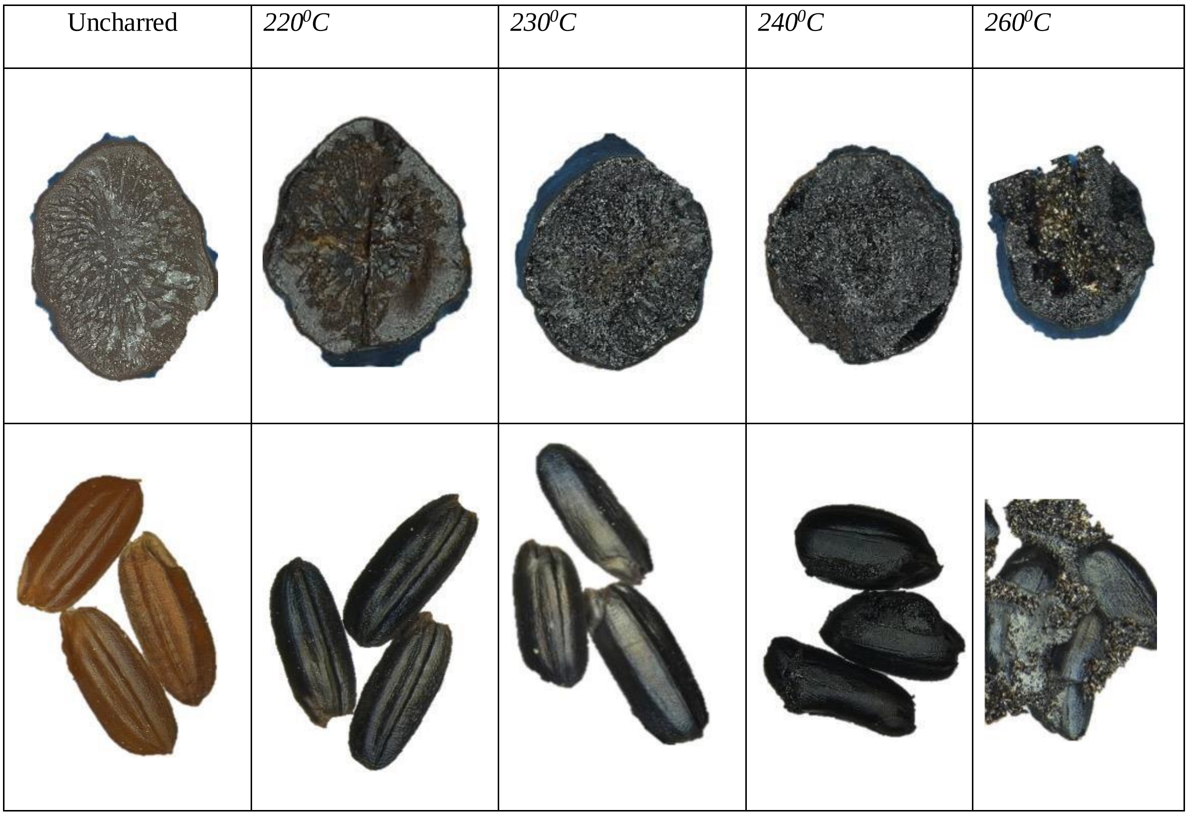
Mag.	Uncharred	*220^{0}C*	*230^{0}C*	*240^{0}C*	*260^{0}C*
X50					
X200					

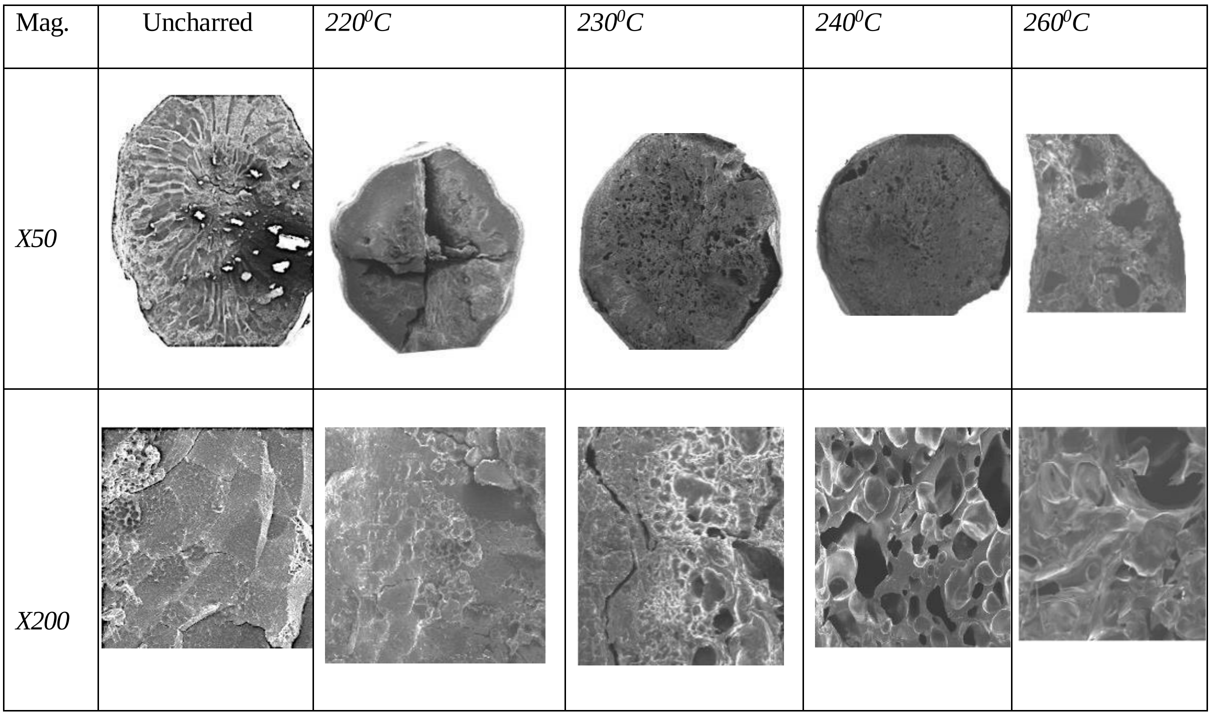
Figure 4. Low power microscopy of modern charred and uncharred *indica* rice grain, heated from 220^0C to 260^0C, cross-section x2.00 Mag. (top row); whole-body grain x0.57 Mag. (bottom row).



1.4.5. Documenting Archaeological Rice

Well-preserved archaeological rice grains mostly exhibit a thoroughly black colour on both outer and inner surfaces, while cut sections of WB rice are sometimes dark brown (Fig. 8). Archaeological rice caryopses exhibit occasional low distortion (S=2-3), simple cracking and small-scale external bulging. Cross-sections show the disappearance of fissures along with 'paste'-like smooth inner texture in which some cells are cleared of contents along with some more open voids. Separation of the aleurone layer and the pericarp is visible in some cut sections (MH & NAT) (Fig.9). Cut surfaces appear dense with occasional cracks and cavities. Well-preserved archaeological rice in the cut section appears obovoid rather than likely rounded equivalent to charred rice grain below 240^0C.

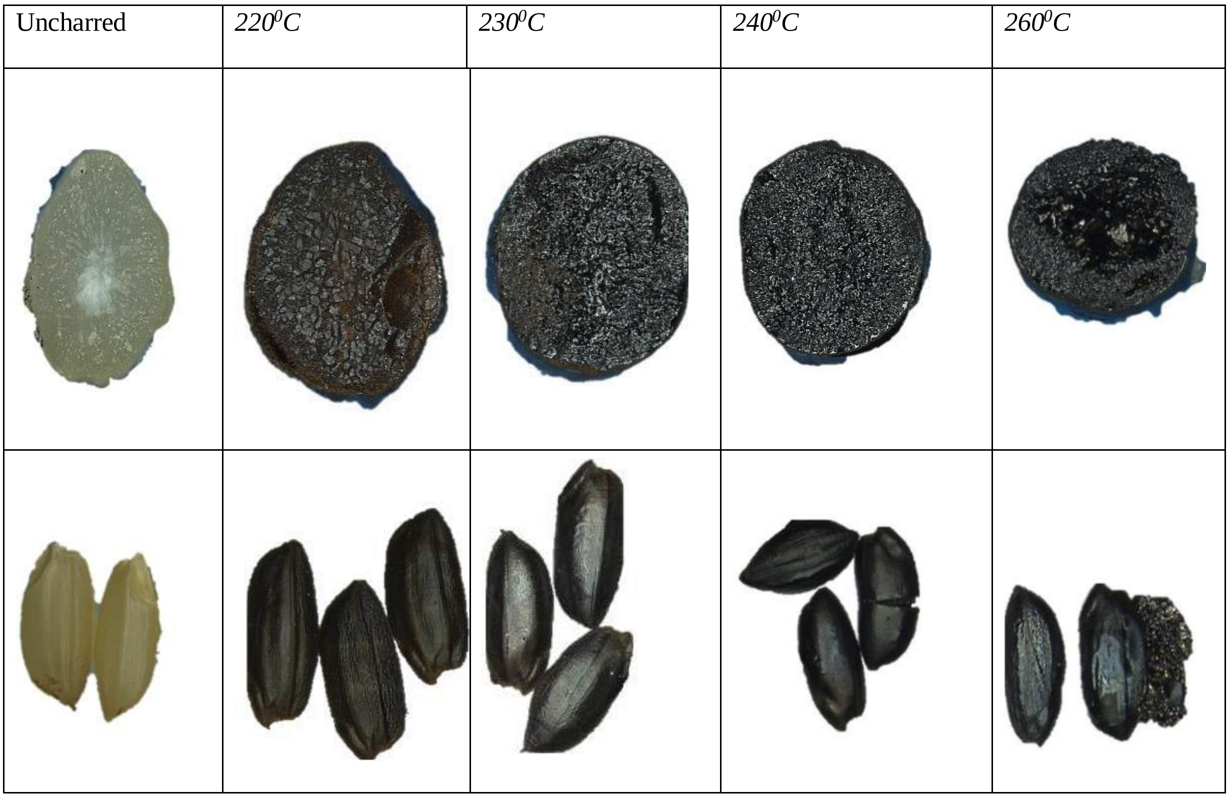
Figure 5. SEMs photograph of modern unheated and heated *indica* rice cross section range between 220^0C to 260^0C. The top row shows low magnification, and the bottom row shows high magnification photo.



1.5. Discussion

This experiment successfully replicated charred, undistorted rice grains as observed in early rice archaeobotanical assemblages from Bangladesh (Fig. 9A & 9 B). Grain colour (black) appears to be the first indicator of grain carbonization. Table 4 indicates that different rice genotypes turn black externally and internally under low temperatures and oxidized conditions. The process of changing grain colour reflects chemical alteration by dextrinization and Maillard reaction (Charles *et al.* 2015). After the onset of Maillard reactions, the conversion of sugars to melanoidins can continue despite a drop in temperatures. As a result, grain can continue to darken progressively over time even without prolonged exposure to heat. In other words, grain may not be blackened fully during the heating regime, but rather may become fully black over time. In that case, the internal structure of the grain is more important than the colour (Styring *et al.* 2013).

Figure 6. Low power microscopy of modern japonica rice grain heated between 220^0C to 260^0C (cross-section x2.00 Mag. in the top row and whole-body grain x0.57 Mag. in the bottom row.



Rice follows a comparatively narrow range of thermal pathways to become charred (Table 4, Figs 2, 4, 6,). This observation matches well with previous work on rice charring by White *et al*. (2019) and Garton (1979), and with work on other large-seeded cereal grains, e.g., wheat and barley (Boardman and Jones 1990; Charles *et al.* 2015), and spelt wheat (Berihuete-Azorìn *et al.* 2019). In addition, reducing conditions broadens the temperature range under which rice grains become carbonized with minimum distortion, resembling well-preserved archaeological material.

Figure 7. SEMs photograph of modern unheated and heated japonica rice cross section range between 220^0C to 260^0C. The top row shows low magnification, and the bottom row shows high magnification photo.

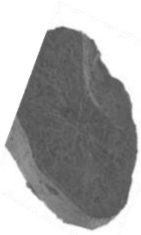
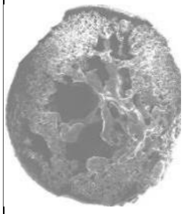
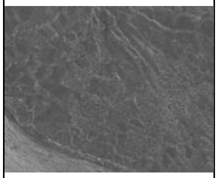
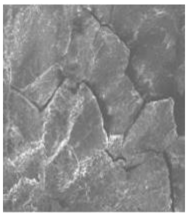
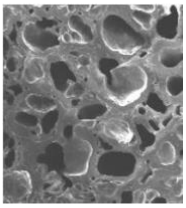
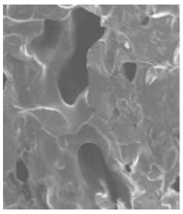
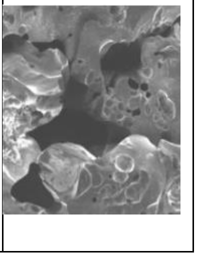
Mag.	Uncharred	2200C	2300C	2400C	2600C
X50					
X200					

Figure 8. Well-preserved archaeological rice grains from three archaeological sites in Bangladesh: WB, MH, *and* NAT. The top row shows a cross-section, and the bottom row shows well-preserved undistorted caryopsis with minor distortion (cracking and bulging).

Mag.	WB	NAT	MH
Cut sectionx2.00 magnification			
Whole grain x0.57 magnification			

The successful replication of charred rice at 230^0C for 2-3 hours under low temperatures (Table 5) contradicts the results of experimental rice charring by Castillo (2019), perhaps due to uncontrolled heating regimes in the latter experiment. The author reported rice charring through heating to high temperatures, e.g., 600-900^{0}C, for at least three hours. Our controlled heating experiments, however, produced completely unrecognisable and highly fused charred rice remains at 260^{0}C for 2 hours for *aus* and *indica*. Moreover, rice charring under very high temperatures would not be expected to yield surviving rice plant parts on archaeological sites.

Additional evidence for the relevance of low-temperature heating comes from the co-occurrence of chaff remains. Chaff and straw are more vulnerable to being reduced to ash than cereal grains (Boardman and Jones 1990; Thompson 1996, Castillo *et al.* 2019). Paleas and lemmas of rice spikelets, along with sterile lemmas, are more vulnerable still than spikelet bases (Thompson 1996; Castillo *et al.* 2019) and hence their presence in an archaeobotanical assemblage indicates low temperature and short duration of heating. This is the case in several early rice assemblages from Bangladesh under study by the present author (i.e., MH-14, WB-17, NAT-14 & 18). The occurrence of undistorted charred rice grains along with significant chaff, spikelet bases and fragments of palea and lemma strongly indicates a low temperature and duration heating regime.

Grain shrinkage (L/W/T ratio) due to carbonization has not, substantially, been observed in this experiment at low temperatures up to 230^{0}C for 2-3 hours under reduced and oxidized conditions through macroscopic examination. These results corroborate the rice charring findings of Garton (1979), White *et al.* (2019) and Charles *et al*. (2015) for einkorn and emmer but again contradict the rice charring results of Castillo *et al*. (2019), perhaps due to uncontrolled temperature regimes in the latter. Gross morphology, e.g., shape and size, of carbonized cereal grains are important for the identification of species or variety levels using simple morphometric or geometric morphometric techniques (Castillo *et al*. 2016; Bonhomme *et al.* 2017). Temperatures for transforming seed morphology may vary among species. Rice morphological modification under comparatively high temperatures, at 240^0C and above, alters *indica* and *aus* rice to resemble *japonica* more closely through substantial shortening, in length and breadth. Such morphological changes have closely corroborated those in emmer, bread wheat and macaroni wheat (Braadbaart and Bergen 2005), which all become identical at 290^0C and above, and the same is true of spelt wheat (Berihuete-Azorìn *et al.* 2019). These results on grain morphological changes imply that well-preserved undistorted archaeological grain should be carefully selected for the application of simple morphometric and GMM techniques. The next step in future work will be to use the results of experimental charring reported here to inform these morphometric approaches as a basis for distinguishing the three Asian rice genotypes.

This experiment potentially revealed differences in responses to heating among the three rice genotypes, with *japonica* showing more resistance to heat than *aus* and *indica*. This observation agrees with work by Garton (1979) and presumably reflects physical and chemical properties. For example, the number of aleurone layers in rice endosperm varies amongst varieties and grain parts (Juliano 1993). Moreover, aleurone layers were found to be thicker in short grain (*japonica* like) than long grain (*indica* and *aus* like) (del Rosario 1968 quoted from Juliano 1993). Five to six aleurone layers have been reported in *japonica* while in indica three have been noted (Matsuo 1955 and Nakako & Kobayashi 1959 quoted from Ahn 1993;82). Those inter-varietal differences in the number of aleurone layers could also be influenced by the degree of grain maturity and the environmental conditions (Hoshikawa 1967; 1968 quoted from Ahn 1993;82). Thus, the number of aleurone layers, grain maturity, environmental and agroecological conditions or other chemical properties could contribute to

9 A			
Experimentally charred modern rice cross section, *aus*, *indica* and *japonica* (left to right) at 230^{0}C, reduced condition			
Cross section of well-preserved archaeological rice grains collected from WB, NAT, and MH (left to right)			
9 B			
Experimentally charred modern rice, *aus*, *indica* and *japonica* (left to right) at 230^{0}C in∫reduced condition			
Well-preserved archaeological rice grains collected from WB, NAT, and MH (left to right)			

Figure 9. **A**. Comparison of the cut section of modern charred Asian rice genotypes (top) with archaeological rice cut section (bottom); Similar pattern in grain morphology (longitudinal), cell content cleared, often aleurone layer or pericarp separated from endosperm, occasional dry crack viewed in both modern charred and archaeological rice; **B-**comparing rice, grain morphology appeared externally, between modern charred Asian genotypes (top) with archaeological rice (bottom); both black and undistorted. Archaeological rice collected from Bangladesh, WB, NAT, and MH sites.

variable thermal reactions between *japonica* and the other two Asian rice genotypes, *indica* and *aus*. Since the aleurone layer can survive well in charred rice, Ahn (1993;82) suggested using this criterion to distinguish *japonica* and *indica* archaeobotanically, though in practice this is difficult to apply routinely.

1.6. Implication of Rice Charring in Future Archaeological Research

The results of experimental rice charring presented in this paper should be useful for further studies in archaeology and the wider interdisciplinary investigation of early rice. The surface appearance and general morphology of charred archaeological rice grains can be used to estimate the conditions under which charring has occurred. Based on the current work, it can be proposed that rice grains charred at temperatures up to c. 230^0C are most appropriate for taxonomic analysis of morphology and for assessment of seed size changes for domestication (Fuller 2007; Fuller, Asouti and Purugganan 2012). Like other types of cereal grain, e.g., glume wheat, rice grain charred at over 240^0C is less suitable for morphological study and identification to the species level and beyond (cf. Charles *et al.* 2015). The establishment of relevant charring temperatures is also useful for assessing the representation of grain, straw, and chaff (cf. Boardman and Jones 1990). Charring conditions are also a key consideration for the successful application of biomolecular analysis. Previous experimental study indicates that the impact of charring on stable isotope values in the temperature range of $2000\text{-}260^0$C is small and predictable (e.g., Heaton *et al.* 2009; Kanstrup *et al.* 2012; Fraser *et al.* 2013; Nitsch *et al.* 2015). Plant aDNA has been detected in grains charred experimentally up to 225^0C for 2-3 hours or more (Threadgold and Brown 2003, Fig.3). Such low-temperature charring of well-preserved cereal grain is associated with the extraction of fragmented aDNA from emmer, spelt and bread wheat and rice in a range of studies (e.g., Allaby, Jones and Brown 1994; Bunning *et al.* 2012; Castillo *et al.* 2016; Schlumbaum and Jacomet 1998).

1.7. Conclusion

In this study, experimental charring of grains of the three Asian rice genotypes successfully replicated well-preserved archaeological rice under a relatively low temperature, 230^{0}C, and particularly under reducing conditions. Heating at 240^{0}C under both reduced and oxidized conditions caused gross distortion to grain morphology, resulting in shorter and more rounded grains. The implication is that rice heated to 240^{0}C or above would be difficult or impossible to identify to the level of genotype/variety. Moreover, charring conditions may complicate morphological analysis (e.g., length/width, or GMM) since *aus* and *indica* become shorter and more rounded, thus approaching the shape of *japonica*. Experimental charring shows that oxidized conditions yield more distorted grains, even at low temperatures and for short durations, compared with grains charred under reducing conditions. The *japonica* variety shows more heat tolerance than the other two genotypes. In future work, detailed (geo)morphometric measurements before and after charring are needed to refine understanding of the impact of heating on taxonomic identification to genotype level. Moreover, well-preserved rice grains corresponding to undistorted experimentally charred specimens externally and in grain cross-section are most suitable for further taxonomic identification and biomolecular analysis.

References

Ahn, S. 1993. Origin and differentiation of domesticated rice in Asia – Review of archaeological and botanical evidence. Unpublished PhD thesis. University of London, University College London (United Kingdom).

Allaby, R. G., Jones, M. K. & Brown, T. A., 1994. DNA in charred wheat grains from the Iron Age hillfort at Danebury, England. *Antiquity,* 68, 126-132.

Berihuete-Azorín, M., Stika, H.-P., Bourliva, A., Papadopoulou, L. & Valamoti, S-M. 2019. "Fresh from the oven": experiments on triticum spelta and a protocol for carbonising specimens for archaeobotanical comparison collections. *Journal of archaeological science, reports*, 26, 101865.

Boardman, S., Jones, G., 1990. Experiments on the effects of charring on cereal plant components. *Journal of Archaeological Science,* 17, 1-11.

Bogaard, A., Henton, E., Evans, J. A., Twiss, K. C., Charles, M., Vaiglova, P. & Russell, N. 2014a. Locating Land Use at Neolithic Çatalhöyük, Turkey: The Implications of 87Sr/86Sr Signatures in Plants and Sheep Tooth Sequences. *Archaeometry,* 56, 860-877.

Bogaard, A., Charles, M., Livarda, A., Ergun, M., Filipovic, D., & Jones, G., 2014b. "The archaeobotany of mid-later occupation levels at Neolithic Çatalhöyük." *In:* Hodder., I. (ed.) *Humans and Landscapes of Catalhyük, Reports from the 2000–2008 Seasons,* Los Angeles: University of California at Los Angeles.

Bonhomme, V., Forster, E., Wallace, M., Stillman, E., Charles, M. & Jones, G. 2017. Identification of inter- and intra-species variation in cereal grains through geometric morphometric analysis, and its resilience under experimental charring. *Journal of Archaeological Science,* 86, 60-67.

Braadbaart, F., Wright, P. J., van der Horst, J. & Boon, J. J., 2007. A laboratory simulation of the carbonization of sunflower achenes and seeds. *Journal of analytical and applied pyrolysis,* 78, 316-327.

Braadbaart, F. & van Bergen, P. F. 2005. Digital imaging analysis of size and shape of wheat and pea upon heating under anoxic conditions as a function of the temperature. *Vegetation history and archaeobotany,* 14, 67-75.

Braadbaart, F., Bakels, C. C., Boon, J. J. & van Bergen, P. F., 2005. Heating experiments under anoxic conditions on varieties of wheat. *Archaeometry,* 47, 103-114.

Braadbaart, F., Boon, J. J., Veld, H., David, P. & van Bergen, P. F., 2004a. Laboratory simulations of the transformation of peas as a result of heat treatment: changes of the physical and chemical properties. *Journal of archaeological science,* 31, 821-833.

Braadbaart, F., van der Horst, J., Boon, J. & van Bergen, P.F., 2004b. Laboratory simulations of the transformation of emmer wheat as a result of heating. *J. Therm. Anal. Calorim.,* 77, 957-973.

Braadbaart, F. 2008. Carbonisation and morphological changes in modern dehusked and husked Triticum dicoccum and Triticum aestivum grains. *Veget Hist Archaeobot* 17, 155–166

Brown, T. A. 1999. How ancient DNA may help in understanding the origin and spread of agriculture. *Philosophical Transactions: Biological Sciences,* Vol. 354, pp. 89-98.

Bunning, S. L., Jones, G. & Brown, T. A. 2012. Next generation sequencing of DNA in 3300-year-old charred cereal grains. *Journal of archaeological science,* 39, 2780-2784.

Castillo, C. C. 2019. Preservation bias: is rice overrepresented in the archaeological record? *Archaeological and Anthropological Sciences,* 11, 6451–6471.

Castillo, C., Tanaka, K., Sato, Y.-I., Ishikawa, R., Bellina, B., Higham, C., Chang, N., Mohanty, R., Kajale, M. & Fuller, D. 2016. Archaeogenetic study of prehistoric rice remains from Thailand and India: evidence of early japonica in South and Southeast Asia. *Archaeological and Anthropological Sciences,* 8, 523-543.

Civáň, P., Craig, H., Cox, C. J., and Brown, T. A., 2015. Three geographically separate domestications of Asian rice. *Nat Plants,* 1: 15164, doi:10.1038/nplants.2015.164.

Charles, M., Forster, E., Wallace, M. & Jones, G. 2015. "Nor ever lightning char thy grain" 1: establishing archaeologically relevant charring conditions and their effect on glume wheat grain morphology. *Science and Technology of Archaeological Research,* 1, 1-6.

Choi, J. Y., Platts, A. E., Fuller, D.Q., Hsing, Y-IE., Wing, R. A., & Purugganan, M. D., 2017. The rice paradox: multiple origins but single domestication in Asian rice. *Mol. Biol. Evol.,* 34(4), 969–979.

Dezendorf, C. 2013. The Effects of Food Processing on the Archaeological Visibility of Maize: An Experimental Study of Carbonization of Lime-treated Maize Kernels. *Ethnobiology letters,* 4, 12-20.

Deng, Z., Qin, L., Gao, Y., Weisskopf, A. R., Zhang, C. & Fuller, D. Q. 2015. From early domesticated rice of the middle Yangtze basin to millet, rice and wheat agriculture: Archaeobotanical macro-remains from Baligang, Nanyang Basin, Central China (6700-500BC). *PloS One,* 10, e0139885-e0139885.

Diamond, J. & Bellwood, P. 2003. Farmers and their languages: The first expansions. *Science,* 300, 597-603.

Fraser, R. A., Bogaard, A., Charles, M., Styring, A. K., Wallace, M., Jones, G., Ditchfield, P. & Heaton, T. H. E., 2013. Assessing natural variation and the effects of charring, burial and pre-treatment on the stable carbon and nitrogen isotope values of archaeobotanical cereals and pulses. *Journal of archaeological science,* 40, 4754-4766.

Fraser, R. A., Bogaard, A., Heaton, T., Charles, M., Jones, G., Christensen, B. T., Halstead, P., Merbach, I., Poulton, P. R., Sparkes, D. & Styring, A. K. 2011. Manuring and stable nitrogen isotope ratios in cereals and pulses: towards a new archaeobotanical approach to the inference of land use and dietary practices. *Journal of Archaeological Science,* 38, 2790-2804.

Fuller, D. Q. 2011. Pathways to Asian civilizations: Tracing the origins and spread of rice and rice cultures. *Rice,* 4, 78-92.

Fuller, D. Q. 2007. Contrasting patterns in crop domestication and domestication rates: recent archaeobotanical insights from the old world. *Ann Bot,* 100, 903-924.

Fuller, D. Q., Qin, L., Zheng. Y., Zhao. Z., Chen. X., Hosoya. L. A., Sun. G-P., 2009. The domestication process and domestication rate in rice: spikelet bases from the lower Yangtze. *Science,* 323, 1607-1610.

Fuller, D. Q., Asouti, E. & Purugganan, M. D. 2012. Erratum to: Cultivation as slow evolutionary entanglement: comparative data on rate and sequence of domestication. *Vegetation history and archaeobotany,* 21, 147-147.

Fuller, D. Q., Weisskopf, A.R., Castillo, C.C. 2016. Pathways of Rice Diversification across Asia. *Archaeology International,* No. 19, pp. 84–96.

Garton, D. 1979. A study of the effects of charring on rice grains. Unpublished B.A. thesis in the Institute of Archaeology, UCL. London.

Gross, B. L. & Zhao, Z. 2014. Archaeological and genetic insights into the origins of domesticated rice. *Proc Natl Acad Sci U S A,* 111, 6190-6197.

Guarino, C. & Sciarrillo, R. 2004. Carbonized seeds in a protohistoric house: results of hearth and house experiments. *Vegetation history and archaeobotany,* 13, 65-70.

Gutaker, R. M., Groen, S. C., Bellis, E. S., Choi, J. Y., Pires, I. S., Bocinsky, R. K., Slayton, E. R., Wilkins, O., Castillo, C. C., Negrão, S., Oliveira, M. M., Fuller, D. Q., Guedes, J. A., Lasky, J. R. & Purugganan, M. D., 2020. Genomic history and ecology of the geographic spread of rice. *Nat Plants,* 6, 492-502.

Gustafsson, S. 2000. Carbonized cereal grains and weed seeds in prehistoric houses—an experimental perspective. *Journal of archaeological science,* 27, 65-70.

Heaton, T. H. E., Jones, G., Halstead, P. & Tsipropoulos, T., 2009. Variations in the 13C/ 12C ratios of modern wheat grain, and implications for interpreting data from Bronze Age Assiros Toumba, Greece. *Journal of archaeological science,* 36, 22242233.

Heier, A., Evans, J. A. & Montgomery, J. 2009. The potential of carbonized grain to preserve biogenic (87) SR/ (86) SR signatures within the burial environment. *Archaeometry,* 51, 277.

Hubbard, R. & Al Azm, A. 1990. Quantifying preservation and distortion in carbonized seeds; and investigating the history of friké production. *Journal of archaeological science,* 17, 103-106.

Ishikawa, R., Castillo, C. C. & Fuller, D. Q. 2020. Genetic evaluation of domestication-related traits in rice: implications for the archaeobotany of rice origins. *Archaeological and anthropological sciences,*12. 10.1007/s12520-020-01112-3

Kanstrup, M., Thomsen, I. K., Mikkelsen, P. H. & Christensen, B. T. 2012. Impact of charring on cereal grain characteristics: linking prehistoric manuring practice to δ15N signatures in archaeobotanical material. *Journal of archaeological science,* 39, 2533-2540. Juliano, B. O. 1993. *Rice in human nutrition,* Rome & Los Banos, Laguna (Philippines), FAO & IRRI.

Märkle, T. & Rösch, M. 2008. Experiments on the effects of carbonization on some cultivated plant seeds. *Vegetation history and archaeobotany,* 17, 257-263.

Motuzaite-Matuzeviciute, G., Hunt, H. V. & Jones, M. K. 2012. Experimental approaches to understanding variation in grain size in Panicum miliaceum (broomcorn millet) and its relevance for interpreting archaeobotanical assemblages. *Vegetation history and archaeobotany,* 21, 69-77.

Nitsch, E. K., Charles, M. & Bogaard, A., 2015. Calculating a statistically robust δ13C and δ15N offset for charred cereal and pulse seeds. *Science and technology of archaeological research,* 1, 1-8.

Pokharia, A. K., Sharma, S., Tripathi, D., Mishra, N., Pal, J. N., Vinay, R. & Srivastava, A. 2016. Neolithic−Early historic (2500–200 BC) plant use: The archaeobotany of Ganga Plain, India. *Quaternary International,* 443, 223-237.

Rahman,M., Murphy.C., Weisskopf, A. R., Champion, L., Castillo, C., AND Fuller, D. Q. 2019. Wari-Bateshwar and Vikrampura: Successful Case Studies in Archaeobotany, Bangladesh. *Man and Environment.*, XLIV (1), pp: 62-72.

Robert, B. S. & Robertson, W. III., 1971. Moisture and Seed Carbonization. *Economic botany,* 25, 381.

Schlumbaum, A., Neuhaus, J.-M. & Jacomet, S., 1998. Coexistence of Tetraploid and Hexaploid Naked Wheat in a Neolithic Lake Dwelling of Central Europe: Evidence from Morphology and Ancient DNA. *Journal of archaeological science*, 25, 1111-1118.

Seco-Morais, J. & Matheson, C.D., 2008. Archaeology and ancient DNA: assessing domestication. *In:* Suárez, A. R. and Vásquez, M. N (ed.) *Archaeology research trends.* New York: Published by Nova Science Publishers, Inc.

Sievers, C. & Wadley, L., 2008. Going underground: experimental carbonization of fruiting structures under hearths. *Journal of archaeological science,* 35, 2909-2917.

Smith, H. & Jones, G. 1990. Experiments on the effects of charring on cultivated grape seeds. *Journal of archaeological science,* 17, 317-327.

Stevens, C. J., Murphy, C., Roberts, R., Lucas, L., Silva, F. & Fuller, D. Q. 2016. Between China and South Asia: A Middle Asian corridor of crop dispersal and agricultural innovation in the Bronze Age. *The Holocene,* 26, 1541-1555.

Styring, A. K., Evans, J. A., Nitsch, E. K., Lee-thorp, J. A. & Bogaard, A. 2019. Revisiting the potential of carbonized grain to preserve biogenic 87Sr/86Sr signatures within the burial environment. *Archaeometry,* 61, 179-193.

Styring, A. K., Manning, H., Fraser, R. A., Wallace, M., Jones, G., Charles, M., Heaton, T. H. E., Bogaard, A. & Evershed, R. P. 2013. The effect of charring and burial on the biochemical composition of cereal grains: investigating the integrity of archaeological plant material. *Journal of Archaeological Science,* 40**,** 4767-4779.

Styring, A. K., Evans, J. A., Nitsch, E. K., Lee-Thorp, J. A., and Bogaard, A. 2019. 'Revisiting the potential of carbonized grain to preserve biogenic 87Sr/86Sr signatures within the burial environment', *Archaeometry,* 61, (1):179-93.
doi: https://doi.org/10.1111/arcm.12398.

Thompson, G. B. 1996. Ethnographic Models for Interpreting Rice Remains. *In:* THOSARAT, C. H. A. R. (ed.) *The Excavation of Khok Phanom Di a Prehistoric Site in Central Thailand; Subsistence and Environment: The Botanical Evidence.* London: The Society of Antiquaries of London.

Threadgold, J. & Brown, T. A., 2003. Degradation of DNA in artificially charred wheat seeds. *Journal of archaeological science,* 30, 1067-1076.

Van driem, G. 2011. Lost in the sands of time somewhere north of the Bay of Bengal. *Himalayan Languages and Linguistics,* 12, 11-38.

Wallace, M., Jones, G., Charles. M., Fraser, R., Halstead. P., T.H.E. Heaton & A. BOGAARD 2013. Stable carbon isotope analysis as a direct means of inferring crop water status and water management practices. *World Archaeology,* 45, 388-409.

Walsh, R. 2017. Experiments on the effects of charring on Setaria italica (foxtail millet). *Vegetation history and archaeobotany,* 26, 447-453.

Wang, M., Zhu, Z., Tan, L., Liu, F., Fu, Y., Sun, C. & Cai, H. 2013. Complexity of indica-japonica varietal differentiation in Bangladesh rice landraces revealed by microsatellite markers. *Breeding Science,*63,227-232.

White, C., Toro, F. & White, J. 2019. Rice carbonization and the archaeobotanical record: experimental results from the Ban Chiang ethnobotanical collection, Thailand. *Archaeological and Anthropological Sciences,* 11, 6501-6513.

Wilson, D. G. 1984. "The Carbonization of Weed Seeds and Their Representation in Macrofossil Assemblages." In Plants and Ancient Man: Studies in Palaeoethnobotany; Proceedings of the 6th Symposium of The International Work Group, Groningen, Netherlands, May 30-June 3, 1983, Viii+344p, edited by W. Van Zeist and W. A. Casparie, 201–206. Rotterdam, Netherlands; Boston, MA, USA: A.A. Balkema [Illus. Maps].

Wright, P. 2003. Preservation or destruction of plant remains by carbonization? *Journal of Archaeological Science,* 30, 577-583.

Yang, Q., Li, X., Liu, W., Zhou, X., Zhao, K., and Sun, N., 2011. "Carbon isotope fractionation during low-temperature carbonization of foxtail and common millets." Organic Chemistry 42:713–719.

Zheng, Y., Crawford, G. W., Jiang, L. & Chen, X. 2016. Rice Domestication Revealed by Reduced Shattering of Archaeological rice from the Lower Yangtze valley. *Sci Rep,* 6, 28136-28136.

Appendix-A

Detailed charring result of 3 Asian rice, *aus*,*indica*, and *japonica*. For colour; 1=unchanged, 2=light brown to pale, 3=Medium brown, 4=High dark brown, 5=Black. For distortion: 1=Unchanged, 2= perfect (No distortion to slight), 3= Slight to moderate, 4= Gross distortion, 5= Extremely distorted. For Blister: 1= Unchanged, 2= Perfect (No blistering to slight), 3= Slight to moderate, 4= Grossly blistered, 5= Extremely blistered. For Crack: 1=Unchanged, 2= Perfect (No crack to slight), 3= Slight to moderate, 4= Grossly cracked, 5= Extremely cracked.For shininess; 1=Unchanged/dull, 2= Low, 3= Medium, 4= High, 5= Extreme; For internal structure; 1=Unchanged/Filled, no voids, 2= Dense, no voids but cracks, 3= Less dense, cracks, 4= Less dense, cavities, 5= Large voids.

Rice geno types	Attributes		Color				Distortion				Blister				Crack				Shininess				Internal structure			
	Temp (ºC)	Hour	220	230	240	260	220	230	240	260	220	230	240	260	220	230	240	260	220	230	240	260	220	230	240	260
*Aus*_ 01	Reduced 02 (Sand)	2	2	4	4	5	2	3	4	5	1	3	4	5	2	2	4	5	2	3	4	5	2	3	3	5
		3	3	4	5	5	3	4	4	5	1	3	4	5	2	3	4	5	3	3	4	5	2	4	4	5
		4	3	4	5	5	3	4	4	5	1	4	3	5	2	4	4	5	3	4	4	5	2	4	4	5
		6	3	5	5	5	4	4	4	5	2	4	4	5	3	4	4	5	3	5	5	5	3	4	5	5
	Oxidized (No sand)	2	2	4	4	5	2	4	4	5	1	3	4	5	2	4	4	5	2	3	3	5	2	3	3	5
		3	3	5	5	5	3	4	4	5	1	3	4	5	2	4	4	5	3	4	5	5	2	4	4	5
		4	4	5	5	5	3	4	4	5	1	4	3	5	3	4	4	5	3	4	5	5	2	4	4	5
		6	4	5	5	5	4	4	4	5	2	4	4	5	4	4	4	5	3	5	5	5	3	4	5	5
Ind_ 01	Reduced 02 (Sand)	2	4	5	5	5	1	2	4	5	1	1	4	5	1	3	4	5	2	4	5	5	2	2	4	5
		3	4	5	5	5	1	4	4	5	1	2	4	5	1	4	4	5	2	4	5	5	3	3	4	5
		4	5	5	5	5	1	4	4	5	1	3	4	5	1	4	4	5	3	4	5	5	3	3	4	5
		6	5	5	5	5	2	4	4	5	1	3	4	5	3	4	4	5	4	4	5	5	3	4	4	5
	Oxidized (No sand)	2	5	5	5	5	3	4	4	5	1	2	2	5	3	4	4	5	5	5	5	5	2	2	4	5
		3	5	5	5	5	4	4	4	5	1	2	3	5	4	4	4	5	5	5	5	5	3	3	4	5
		4	5	5	5	5	4	4	4	5	1	2	3	5	4	4	5	5	5	5	5	5	3	3	4	5
		6	5	5	5	5	4	4	4	5	2	2	3	5	4	4	5	5	5	5	5	5	3	4	4	5
Japo _01	Reduced 02 (Sand)	2	2	4	5	5	1	2	4	4	1	1	1	4	1	1	4	4	2	3	4	5	2	3	4	5
		3	3	4	5	5	2	2	4	4	1	1	2	4	1	2	4	4	2	4	5	5	2	3	4	5
		4	4	5	5	5	2	2	4	4	1	1	3	4	1	2	4	4	3	4	5	5	2	3	4	5
		6	4	5	5	5	2	2	4	4	1	1	4	4	2	1	4	4	4	4	5	5	2	4	5	5
	Oxidized (No sand)	2	2	4	4	5	3	4	4	4	1	2	4	4	3	4	4	4	2	3	4	5	2	3	4	5
		3	3	5	5	5	4	3	4	4	1	2	4	4	4	3	4	4	2	5	4	5	2	3	4	5
		4	4	5	5	5	4	4	4	4	1	2	4	4	4	4	4	4	3	4	4	5	2	3	4	5
		6	4	5	5	5	4	4	4	4	2	3	4	4	5	4	4	4	3	4	5	5	3	4	4	5

2. Paper 2: An Integrated Approach to Archaeological Evidence of Asian Rice Identification: Traditional Morphometrics and Geometric Morphometrics (GMM).

Abstract

Taxonomic identification is fundamental to understanding the origins, domestication and dispersal of Asian rice varieties. Genetic studies have identified three distinct Asian rice varieties—*japonica*, *indica*, and *aus*—each with its own geographical origin. A refined method of rice identification at the variety level is needed to provide archaeological insights into these perspectives. Following a controlled rice charring experiment, this study integrates two methodologies, Morphometrics and Geometric Morphometrics (GMM), to distinguish the rice varieties. While morphometric analysis shows an overlap between *aus* and *japonica*, GMM enables the successful separation of all three rice varieties. Application of these methods to archaeobotanical rice grains from Wari-Bateshwar, combined with new AMS radiocarbon dates, reveals archaeological evidence for the *aus*-type rice alongside *japonica* and *indica* varieties by the 6th century BCE.

2.1 Introduction

Rice (Oryza sativa L.) serves today as a major staple cereal crop, underpinning the nutritional sustenance of over half the global population by contributing 21% of dietary caloric intake and 15% of protein consumption (IRRI *et al.* 2013). Its cultivation spans 144 nations across every continent apart from Antarctica. Most rice cultivation (>90%) occurs within Asia, where a multitude of rice varieties flourish (IRRI *et al.* 2013). The cultural and societal constructs from regions stretching from the Yangtze to the Middle Ganges and the Ganges-Brahmaputra-Meghna (GBM) delta have been profoundly influenced by the cultivation and consumption of rice over millennia (Smith & Mohanty 2018; Fuller 2020). In the GBM delta, there has been little work on fundamental aspects of the evolution and development of rice cultivation, including taxonomic identification of varieties and the reconstruction of agrarian systems.

There are three genetic rice varieties, *Oryza sativa japonica* (henceforth *japonica*), *Oryza sativa indica* (henceforth *indica*), and *aus* cultivar types have been categorised as circum-*aus* by Wang *et al.* (2018). In this paper, we have the term '*aus*' in italic font to refer to the genetic group, while 'aus' in non-italic font refers to seasonal ecotype.

This paper presents new research aimed at assessing approaches to the identification of archaeological rice based on three modern charred rice varieties grown in the GBM delta, *indica*, *japonica*, and *aus*. This study builds on complementary work to establish relevant charring conditions for well-preserved archaeological rice grains of these varieties (Paper 1).

Previously, rice identification was assessed using grain length and width measurements (morphometrics) to distinguish two varieties, *indica* and *japonica* (Castillo *et al*. 2016). The present study compares approaches based on morphometrics with Geometric Morphometrics (GMM) and also considers a third variety, *aus*, alongside *indica* and *japonica*. GMM, recognised for its potential to enhance and refine the taxonomic identification of ancient cereals (Bonhomme *et al*. 2017; Wallace *et al.* 2018; Roushannafas *et al*. 2022; Jeanty *et al.* 2024), potentially offers a useful tool for analysing rice varieties. This study aims to facilitate taxonomic identification of archaeobotanical rice samples from the GBM delta. By assessing morphometrics and GMM approaches to the three genetically distinct rice varieties collected within Bangladesh, this study offers a comparative framework to enhance the resolution of archaeological rice grain identification in the GBM region and beyond.

A further objective of this study is to apply refined taxonomic approaches to the identification of new archaeobotanical rice samples collected from the site of Wari-Bateshwar (WB) in

Bangladesh. This aspect of the study relates to the hypothesis that *aus* rice originated in Bangladesh, a country with a long, albeit undefined, history of cultivating this variety (Civáň *et al.* 2015; Travis *et al.* 2015; Choi *et al.* 2017). These new results will be considered together with previous identifications of *indica* and *japonica* in early Bangladesh (Rahman 2018; Rahman *et al.* 2020) in order to review the introduction and potential co-occurrence of different rice varieties at WB. The temporal frame of WB is also reassessed in this paper through the new AMS radiocarbon dating of rice grains through the whole stratigraphic sequence (Rahman 2007; Rahman *et al.* 2012; Rahman *et al.* 2020; Hu *et al.* 2022).

2.2. Setting the scene - *Geographical and archaeological setting*

2.2.1. Early Archaeological History of Bangladesh

Within the rich archaeological heritage of Bangladesh, its historical periods have received more attention than its prehistory. Coins, inscriptions, diagnostic ceramics, beads, architecture and fortification walls at sites such as Wari-Bateshwar and Mahasthangarh support the hypothesis of early urban settlements from around the 3rd century BCE. Epigraphic evidence of rice cultivation at Mahasthangarh (c. 200 BCE), coupled with initial archaeobotanical findings from Wari-Bateshwar (400-300 cal BCE), Vikrampura (900-1100 cal CE), and Sotisher Danga (c.1300-1800 cal CE), suggest diverse agricultural practices involving rice, pulses, oilseeds, millets, cotton, and fruits in ancient Bangladesh (Rahman 2018; Rahman *et al.* 2019; 2020). Subsequently, a proliferation of human settlements and Buddhist religious sites across the country appeared in the second half of the 1st millennium CE. Wet rice cultivation may have been introduced by the early 14th century CE, the medieval period, along with wheat and barley introduction (Rahman 2018), in which periods more urban centres developed, and settlements expanded. Intensive agricultural practice and expansion alongside population growth are inferred in medieval Bengal. It has been inferred that rice was a predominant staple crop in each period of the early history of Bangladesh, whose origin, evolution, and dispersal have yet to be traced (Rahman 2018; Rahman *et al.* 2019; 2020).

2.2.2 The study site and its surroundings

Wari-Bateshwar (WB), encompassing the adjacent villages of Wari and Bateshwar in central Bangladesh's lower Brahmaputra valley, constitutes a significant archaeological fort settlement. The historical fort consists of an inner and outer fort, surrounded by mud rampart

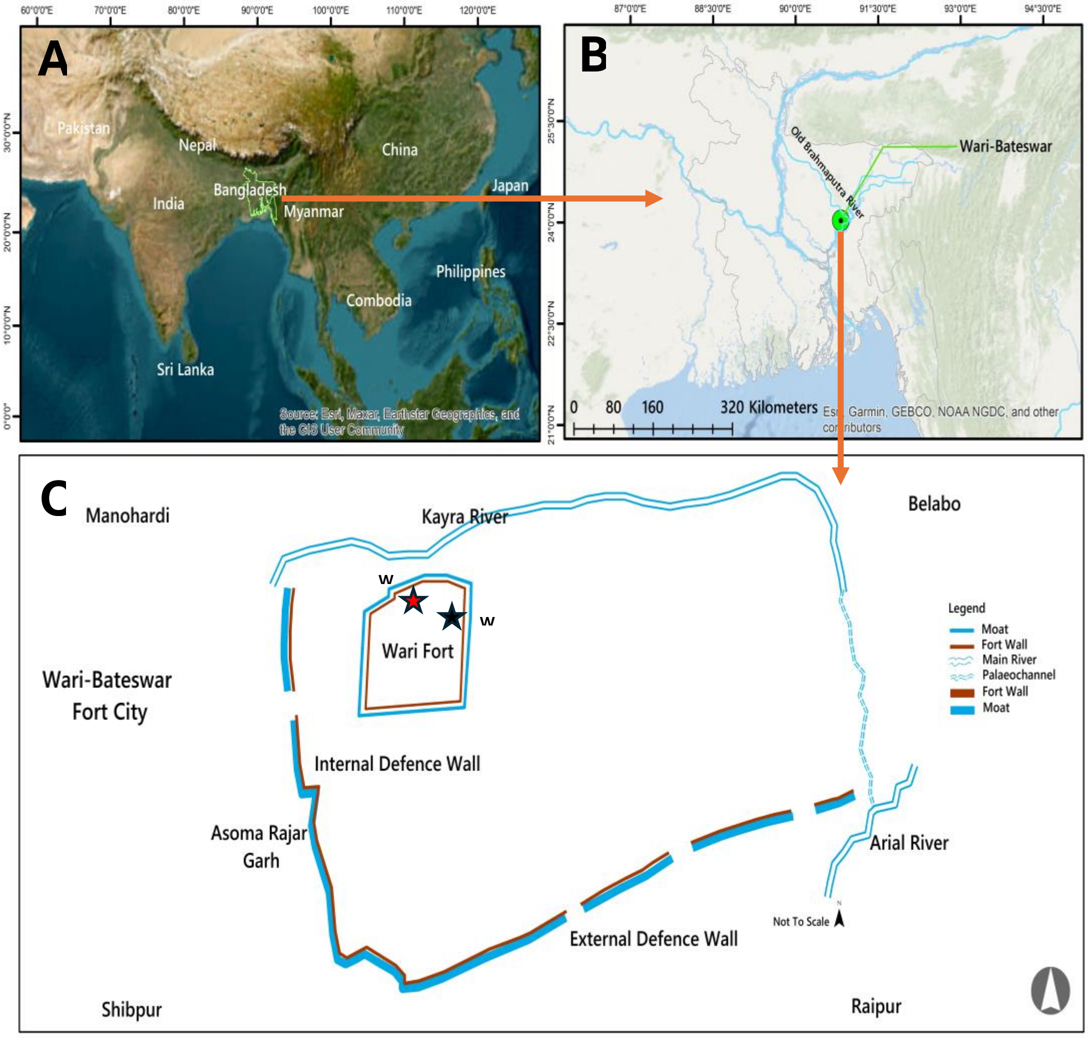


Figure 1: A. Shows the location of Bangladesh within the broad geographical settings of South, Southeast, and China. B. shows Bangladesh and the site location within it. C. Shows the drawing of the site features, outer defense, inner defense, moats, fort areas and the trench location within the Wari fort.

walls alongside moats (Fig. 1). The inner fort, within the village of Wari, measures ~600 m x 600 m and is identified as the core settlement of this area where most artifacts are concentrated (Fig. 1). In contrast, the outer fort, known locally as 'Asom Rajar Garh' (a wall of the king. Asom), remains largely unexplored but features a rampart extending roughly 5 km in length (Fig. 1). The site is perched on an isolated fringe of the Pleistocene Formation of the Madhupur Tract, characterised by higher ground flanked by marshlands. The diverse array of cultural materials uncovered at WB—including hand axes, coins, ceramics, beads, and metal objects—remains to be thoroughly analysed based on distinct stratigraphic sequences and radiocarbon dating (Rahman & Pathan 2018; Alam 2018). Nevertheless, these artifacts highlight Wari-Bateshwar's role as a dynamic trade centre within the regional networks of South and Southeast Asia, underscoring its significant socio-economic, political, and cultural significance (Rahman & Pathan 2012).

2.2.3 Origin and Diversity of Asian Rice

The evolutionary development of domesticated rice varieties, based on modern genetic research, is summarised in Fig. 2. This research suggests that *O. rufipogon*, a wild perennial grass, is the common wild ancestor from which all the currently cultivated rice varieties evolved (Chang 1976; Oka 1988; Cheng *et al.* 2003). It is postulated that *O. rufipogon* is the progenitor of domesticated *O. sativa japonica* (*japonica* rice) (Oka 1988; Morishima, Sano, and Oka 1992; Khush 1997). *O. nivara*, a wild annual variety, arose from perennial *O. rufipogon* (Sharma & Shastry 1965; Chang 1976; Oka 1988). An intermediate type of wild rice has been traced in India and Thailand that might evolve from both rufipogon and nivara type wild progenitors, which may well have been an immediate progenitor of modern domesticated rice (Govindaswami 1957; Sharma & Shastry 1965; Oka & Chang, 1962; Morishima *et al.* 1980; Sano *et al.* 1980; Oka 1988). This hypothesis was formulated based on higher grain yield, polymorphic genetic characteristics, and adaptive evolutionary traits (Sano *et al.*, 1980).

Genetic studies suggest that the origin and domestication of Asian rice varieties were shaped by multiple events occurring in different geographic regions (Yang *et al.* 2012; Civáň *et al.* 2015; Choi & Purugganan 2018). Recent archaeological evidence from the Lower Yangtze Valley of China (Zheng *et al.* 2016) may indicate that the *japonica* variety originated from an intermediate progenitor (could be a variety of rufipogon) rather than directly from a wild progenitor of rufipogon. It has been proposed that domesticated *indica* (*Oryza sativa indica*) originated from an intermediate type of wild rice, and might be cultivated *nivara* or *proto-indica*, which acquired domestication genes from *japonica* through introgression, eventually giving rise to the *indica variety* (Huang *et al.* 2012; 2016; Yang *et al.* 2012; Fuller 2011).

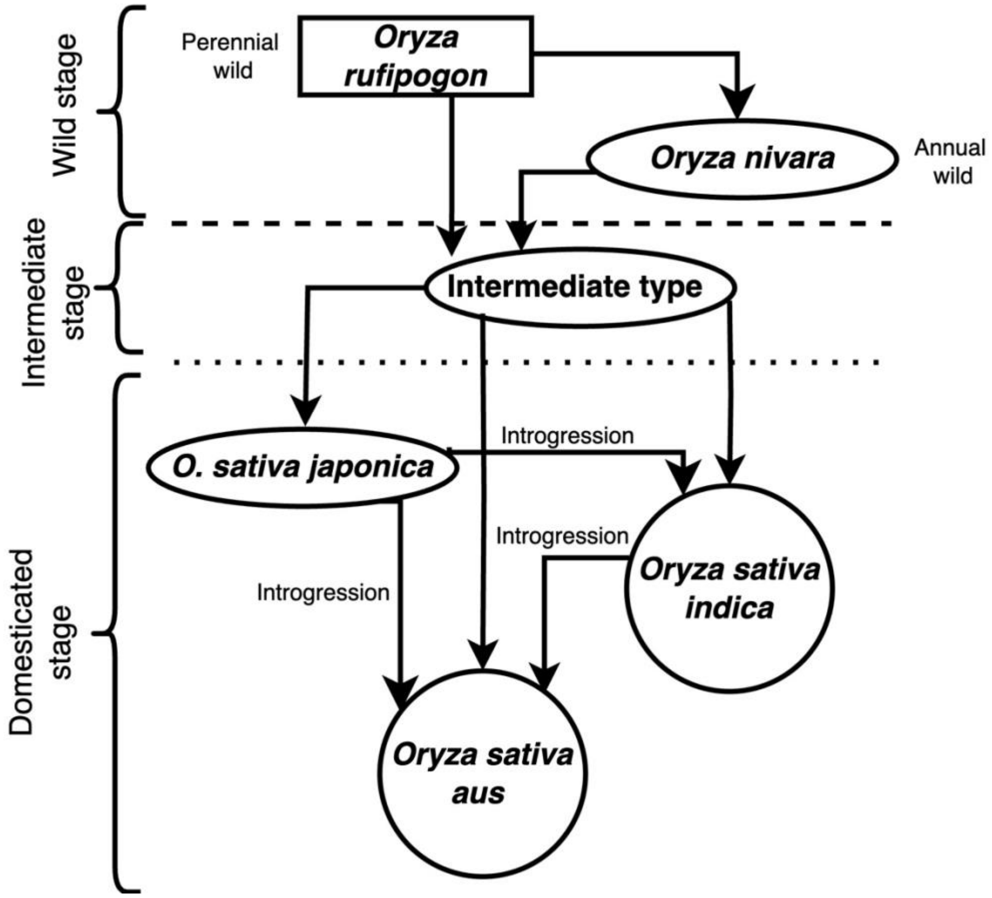


Figure 5: The flow chart illustrates the origin of Asian rice varieties (Excluding aromatic variety).

A similar process might explain the origin of *aus* rice, where an intermediate type of rice (could be a type of *nivara*) received domestication genes from both *japonica* and *indica* through introgression, leading to the domesticated *aus* rice (*Oryza sativa aus*) (Huang *et al.* 2016; Choi *et al.* 2017; Choi & Purugganan 2018). Genetic evidence further indicates that *indica* and *aus* originated from closely related wild progenitors of *O. nivara*, with both varieties maintaining distinct monophyletic lineages (Huang *et al.* 2012).

2.2.4. Identification of Asian Rice and Methodological Approaches in Archaeobotany

Identification of rice in archaeological studies has focussed on two predominant aims: rice identification at the variety level and the rice domestication process. This study focuses on the taxonomic identification of rice grain at the variety level. Work by Fuller *et al.* (2007) and Castillo *et al*. (2016) highlighted the usefulness of morphometric measurements for identifying rice grains to the variety level (Table 1). This approach uses grain measurements (length, width, thickness, length: width ratio) for identifying varieties. Subsequently, Castillo *et al*. refined the morphometrics method to identify archaeological *japonica* and *indica* from South and Southeast Asia. A comprehensive methodological framework that includes the *aus* variety, alongside *japonica* and *indica*, remains to be developed.

Table 1 Morphometric measurements were used to distinguish *japonica* and *indica*.

Name of subspecies	Grain Length: Width ratio	
	Fuller *et al*. 2007	Castillo *et al*. 2016
Indica	>2.5mm	>2.2mm
Japonica	<2.3mm	<2.0mm
Indica & *Japonica* (overlap)		2.0-2.2mm

Geometric Morphometrics (GMM) has advanced the study of shape variation in archaeobotany and combines imaging and analysis of outlines through coordinates, landmarks, and semi-landmarks, using the R statistical package Momocs (Bonhomme *et al*. 2014). Recently, GMM methods for archaeobotanical identification of cereals and fruits have expanded (Table 2) and have proved useful for tracing inter and intra-species morphological variation in wheat (Bonhomme *et al*. 2017), barley (Ros *et al*. 2014; Bonhomme *et al*. 2017; Wallace *et al.* 2018; Jeanty *et al.* 2024), foxtail, and browntop millet (García-Granero *et al*. 2016), and grapes (Orrù *et al*. 2013). This approach has also been instrumental for differentiating between wild and domesticated grains, including charred and uncharred material (Bonhomme *et al*. 2017; Jesus *et al*. 2021; Roushannafas *et al*. 2022). Integrating GMM with traditional morphometrics and ancient DNA (aDNA) analysis has established refined identification criteria for grapes and Southwest Asian pulses (Bouby *et al.* 2020; Tarongi *et al.* 2020; 2024). Moreover, computer-aided image analysis has facilitated the study of local grape cultivation (Orrù *et al*. 2013).

Table 2: Methods used for morphological identification of cereals, pulses, and fruits.

Methods used	Cereal/Fruits	Reference
GMM	Wheat, Barley, Poppy, Foxtail and browntop millet,	Bonhomme *et al*. 2017; Wallace *et al*. 2018; Jesus *et al*. 2021; Roushannafas *et al*. 2022; Jeanty *et al*. 2024
Combining morphometrics (L/W ratio) and GMM	Pulses (Lentil, Grass pea, and Broad bean)	Tarongi *et al*. 2020; 2024.
Combining morphometrics and aDNA	Rice	Castillo *et al*. 2016
Image analysis	Grape	Orrù *et al*. 2013
Combining GMM and aDNA	Grape	Bouby *et al*. 2020

These methodological advances are promising for rice identification at the variety level and could contribute to understanding the evolutionary processes of cereals and fruits in archaeobotany (cf. Wallace *et al.* 2018).

2.3 Materials and Methods

A comparative experimental study of relevant modern rice Varieties is essential for developing a method to identify archaeological rice grains to variety level. Thus, we used two types of material, modern and archaeological, for this study.

2.3.1 Approaches

As defined by Rohlf (1990), morphometrics represents a quantitative methodology aimed at describing, analysing, and elucidating shapes and their variations. It finds significant application within biology, encompassing macroscopic and microscopic organisms and facilitating comparisons of phenotypic structures through morphological measurements. The present study uses two methods for quantifying the morphological diversity in three Asian rice varieties: morphometrics and Geometric Morphometrics (GMM), as outlined by Xu & Bassel (2020).

2.3.1.1. Morphometrics

The morphology of rice grains, characterised by their grain length (GL), grain width (GW), and grain thickness (GT), varies significantly and is determined by the influence of multiple quantitative trait loci (QTLs). While the functional implications of specific genes within these QTLs are understood, others remain unidentified (Azizi *et al.* 2019). For example, the GS5 allele has been shown to increase GW (Li *et al.*, 2011; Xu *et al.* 2015), whereas the GS9 allele reduces it, thus influencing grain shape towards either a bold or slender phenotype (Zhao *et al.* 2018). Furthermore, the dwarf 11 (D11) gene is associated with smaller grain size (Tanabe *et al.* 2005); in contrast, the OsbHLH107 gene elongates grain size (Yang *et al.* 2018). Interestingly, some genes like qGL3 have a regulatory effect on all three physical dimensions—GL, GW, and GT—indicating a complex genetic basis for grain size variation (Hu *et al.* 2012; Qi *et al.*, 2012; Zhang *et al.* 2012).

The genetic diversity underpinning grain size suggests the potential for distinguishing between different genetic varieties of Asian rice. The selection of morphometric variables is critical and varies with the analytical technique, with variables such as grain length-width ratio (GLW) being pivotal for identifying *japonica* and *indica* rice grains in previous work (Fuller *et al.*, 2007; Castillo *et al.*, 2016). Notably, it is argued that not all variables need to be utilised for every species analysis; instead, a selective approach that best suits the research objectives should be adopted (Rohlf, 1990). In this context, we utilise the GLW ratio, among other variables, to assess the differentiation of rice varieties.

2.3.1.2 Geometric Morphometrics (GMM)

In this study, Geometric Morphometrics (GMM) was employed to analyse the shape of rice grains. Given the continuous nature of rice grain shape, an outline analysis method was chosen instead of a landmark-based approach. This analysis utilised Elliptical Fourier Transformation (EFT) (Giardina & Kuhl 1977; Kuhl & Giardina 1982) to convert the grain outlines into a series of coefficients through harmonic coordinates, enabling efficient shape analysis by computation without the need for equally distributed coordinates (Bonhomme *et al.* 2014). The analysis was conducted using the 'Momocs' package in R, built upon Claude's (2008) work and compiled by Bonhomme *et al.* (2014).

2.3.2. Materials

2.3.2.1. Modern reference material

Collecting modern grains of genetic rice varieties was a significant step in developing a reliable method of rice genotype identification. Traditionally, the cultivation of rice in Bangladesh during the Aus, Aman, and Boro seasons led to the classification of local landraces by season rather than by genetic variety. Previous research on rice landraces in Bangladesh identified the genetic varieties *japonica*, *indica*, and *aus* (Wang *et al*. 2013, Supplementary), enabling the collection of genetically identified rice grains for this study. These traditional landraces have been cultivated and developed by local farmers of Bangladesh to adapt to its landscapes over time.

Modern genetic rice materials for this study were collected from the Bangladesh Rice Research Institute's (BRRI) rice germplasm centre. We followed Wang *et al.* (2013) in our selection of samples. In total, nine modern rice accessions were selected for the experiment: three accessions each for *aus indica* and *japonica* (Table 3). Jugli, a local landrace, is widely cultivated in the WB region as *aus* rice; this is the only accession in this study without genetic confirmation of the variety.

Table 3: Information on modern rice accessions used to establish identification criteria for this study.

Varieties	**Code**	**Local name**	**Ecotype**	**BRRI accession No.**	**Place of collection**
*Aus**	A1	Jugli	Aus	1860	Faridpur
Aus	A2	Sada boro	T-Aman	1997	Mymensingh
Aus	A3	Kunail boro	Boro	1714	Faridpur
Indica	I1	Tapi/Topa	Boro	62	Dhaka
Indica	I2	Dudh sail	Aus	2178	Bogra
Indica	I3	Dudh Shar	T-Aman	21	Dhaka
Japonica	J1	Nenia	B-Aman	1823	Bogra
Japonica	J2	Dad sail	Boro	4786	Mymensingh
Japonica	J3	Nepa	B-Aman	61	Dhaka

2.3.2.2. Charring modern rice reference materials

Fifteen grains were selected per accession for experimental charring to incorporate the effect of charring on archaeological rice grains. The modern grain accessions were heated at 230^{0}C for two hours under low O_2 availability in a preheated high-precision oven in the Archaeobotany Laboratory of the School of Archaeology, University of Oxford, with a digital datalogger used to monitor the continuous heating temperature fluctuation. These conditions were previously shown to replicate well-preserved archaeological rice grains (Paper 1).

2.3.2.3. Archaeological rice material

The second dataset used in this study is archaeological rice from the site of WB (Figure 1). The excavation was conducted at WB in two different trenches and seasons, with excavation in 2016 (WB 16) and 2017 (WB 17). These are separate excavations from those previously reported archaeobotanically (Rahman *et al.* 2020). Bucket flotation (Fuller 2007; Pearsall 2015) was used to recover plant materials from the sediment and mesh bags with aperture size of *c*. 300 μ to intercept charred plant parts such as rice spikelet bases. All trenches at WB were small sondages (2 x 2 m or 4 x 4 m), excavated to understand the stratigraphic sequence rather than to uncover large-scale settlement features. Large sediment samples for flotation (*c*. 60 litres) were taken from each stratigraphic layer.

Most rice grains recovered at WB were well-preserved, undistorted and maintained distinct shapes (Paper 1), which are the essential criteria for GMM analysis. The archaeological rice grains selected for this study from the WB17 and WB16 trenches at Wari-Bateshwar met these criteria. This approach provides a fundamental basis for the taxonomic identification of rice varieties. The optical identification of rice was carried out using a Leica MZ8 stereo microscope at the Archaeobotany Laboratory, School of Archaeology, University of Oxford. Archaeological wild rice could be distinguished as long, thin or small grains, and these were excluded from this study, which is focused on domesticated rice varieties for taxonomic identification.

Further assessment of wild rice remains is considered in relation to the domestication process in Paper 3. Previous studies have identified the overlapping morphometrics of wild and domesticated rice (Fuller *et al*. 2007, 2008). Immature rice grains were also excluded from this study, which focused instead on well-developed rice grains. Tables 4-5 present the details of the archaeological material included in this study.

Table 4: Numbers of archaeological rice gains (n = 117) included in this study from trench WB16

Layer	Number of grains
1	17
2	21
3	28
4	38
5	10
6	2

Table 5: Numbers of archaeological rice gains (n = 305) included in this study from trench WB17

Layer	Number of grains
1	9
2	97
3	117
4	82

2.3.3. Photography

Three orthogonal photographs, lateral, dorsal, and polar, were taken for outline shape analysis by GMM, of which two views, lateral for length and width and polar for breadth, were also used for recording morphometric measurements (Fig. 3). Each photograph was captured at the Same magnification (x1.00) with a scale bar (2 mm). The lateral or side view was obtained by placing the grain on its side. with the ventral groove in profile on the Left and the embryo’s bottom right.

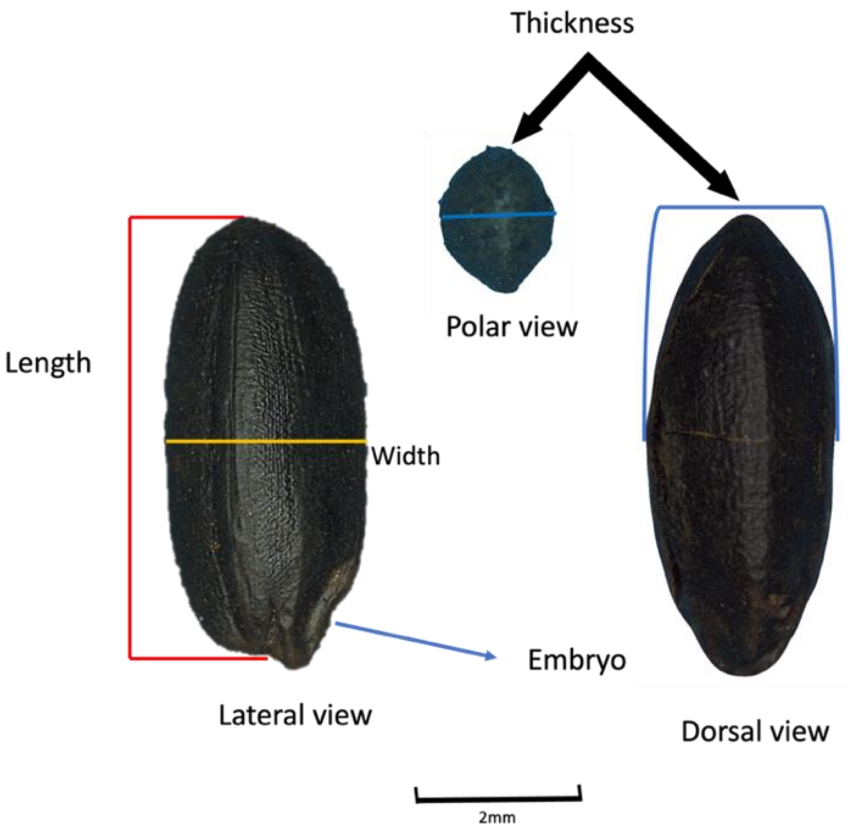


Figure 6: The photograph shows three views, lateral, dorsal and polar, of a rice grain and the way of measuring length, width, and thickness for morphometrics study. The different colours are used to mention different parts.

The dorsal view was obtained by placing the grain with its ventral side down and the dorsal up under the microscope. The polar or cross-section view was obtained by photographing the grain's apex end-on. Each seed was positioned manually, keeping the grain straight. Photographs were taken by Leica Z6 APO Z-stack microscope using a Leica DFC495 camera at the University of Oxford's Archaeobotany Laboratory. A single unique code has been assigned for every grain and preserved so that the grain will be accessible for further investigation as needed. The above procedure was employed for both modern and archaeological rice grains.

2.3.4. Morphometrics measurements

The morphometrics measurement values of each rice grain were obtained using ImageJ v. 1.53a (Schneider *et al*. 2012). Three measurements were recorded: length, width and thickness. The length measurement corresponds to the lateral view’s maximum longitudinal dimension and the width to the maximum perpendicular horizontal dimension (Fig. 3).

Thickness measurement corresponds to the grain’s maximum horizontal dimension in the polar view (Fig. 3). Measurements were taken of maximum dimensions, excluding the embryo part because this is often missing in archaeological specimens. Morphometrics measurements were recorded and log-transformed in a csv. file automatically created by image J.

2.3.5. Image processing

An outline is necessary for GMM analysis, which requires masking raw images. After capturing grain images under the microscope, each image was transferred into Adobe Photoshop 2023, where images were masked by omitting background and cleaning the edge and silhouette of the image to capture the shape. Subsequently, outlines were created of the three views (lateral, dorsal and polar). Before masking, the embryo part (if remaining) was excluded from both modern and archaeological grain images.

2.3.6. AMS radiocarbon dating

The stratigraphic profile of WB 17 comprises 13 layers, with only the bottom four considered in this study; the upper layers were excluded due to a lack of archaeobotanical evidence. Conversely, the stratigraphic profile of WB16 encompasses six layers, and each layer yielded archaeobotanical remains along with artifacts. A comprehensive radiocarbon chronology of the Wari-Bateshwar archaeological site was conducted by submitting a total of 11 samples to the Oxford Radiocarbon Accelerator Unit (ORAU). These samples were sourced from the site excavation trenches, of which six samples were procured from trench WB16 and five from trench WB-17, of which one came from a natural vegetation layer that was finally excluded from this study. The archaeobotanical remains submitted for dating are derived from bulk sediment samples processed by flotation from individual stratigraphic layers. Seeds were selected as short-lived items (Bayliss 2015). The radiocarbon dates from stratigraphic layers were analysed in a Bayesian framework (Buck *et al.* 2006; Bronk Ramsey 2009).

The radiocarbon dates of the stratigraphic layers were subjected to calibration using the IntCal 20 atmospheric calibration curve (Reimer *et al.* 2020) and to Bayesian modelling using OxCal 4.4.4 (Bronk Ramsey 2021). The model was constructed utilising the *Sequence* command.

2.3. 7. Quantitative analysis

2.3.7.1. Morphometric analysis

Morphometric differences among the three rice varieties were explored predominantly using the grain length-width (GLW) ratio to establish a modern framework following Castillo *et al.* (2016). Each grain's length and width were extracted separately for this measurement. Afterwards, the GLW ratio of each grain was calculated by dividing each grain's length by its width. Morphometric measurements of archaeological rice grains were extracted following the same procedure as for modern rice.

2.3.7. 2. Geometric Morphometrics (GMM) Analysis

An outline analysis was conducted on the three rice varieties through Elliptic Fourier Analysis, and the grain views were used to study grain shape geometry. A multivariate statistical approach, Principal Component Analysis (PCA), was initially used to reduce the number of dimensions, summarising the shape variation in fewer variables. Linear Discriminant Analysis (LDA) was used to analyse the coordinates of grains to distinguish shape and variance in order to separate varieties from each other. Leave-one-out cross-validation was conducted. Archaeobotanical rice grains were introduced as unknown cases.

2.4. Results

2.4.1 Radiocarbon Chronology

Ten samples from secure stratigraphic contexts at Wari-Bateshwar yielded reliable radiocarbon dates: four samples from trench WB17 and six from trench WB16 (Tables 6-7). The Bayesian modelling (Ramsey 2009; 2021; Reimer *et al.* 2020) estimates the commencement of the occupation at WB17 between 758-523 cal BCE and at WB16 between 765-535 cal BCE (Table 6 & 7). These dates place the site as the earliest known human occupation in Bangladesh (cf. Alam & Salles 2001; Rahman & Pathan 2018) and contemporary with the Neolithic occupation of Northeast India currently known (Padhan 2023). The Bayesian models (Figs 4 -5) reflect a broad plateau in the calibration curve of the first millennium BCE.

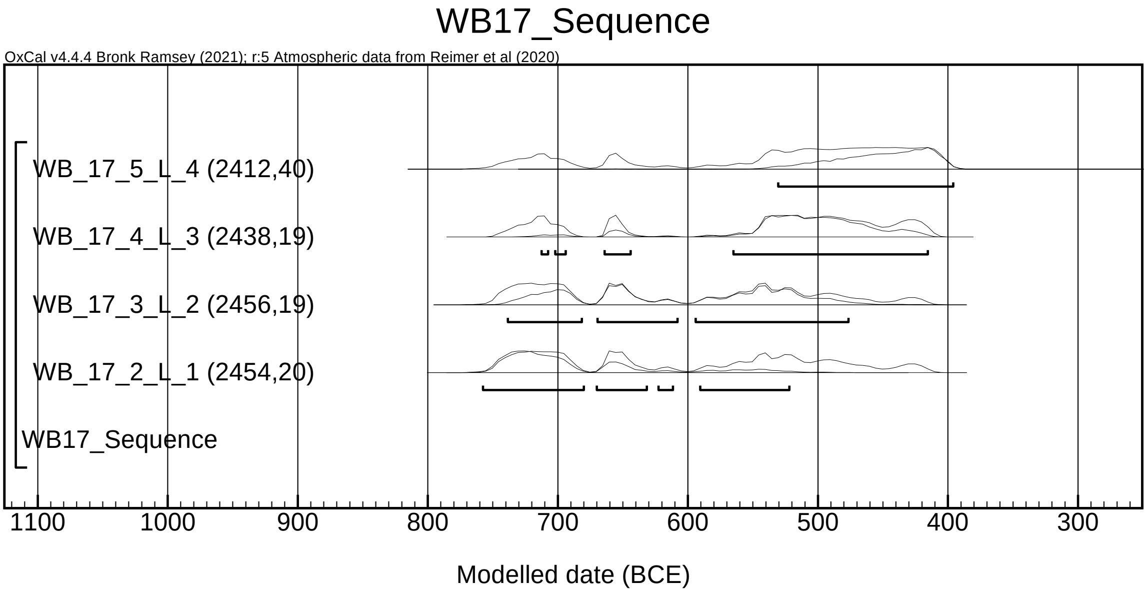


Figure 7: The Bayesian modelled sequence of WB17.

Table 6: AMS radiocarbon dates of Wari-Bateshwar (WB17)

Sample code	Layer number	Lab code	C^{14} age (BP)	Calibrated 2 **σ** (BCE), Modelled		Median (BCE)	Confidence intervals, (CI)%
				From	To		
WB17_2	1	OxA-41706	2454 ± 20	758	523	710	97.5
WB17_3	2	OxA-41707	2456 ± 19	739	477	587	95.8
WB17_4	3	OxA-41708	2438 ± 19	713	416	507	98.3
WB17_5	4	OxA-X-3144-40	2412 ± 40	531	397	447	95.9

Modelled and calibrated (two-sigma) dates for WB17, with a sequence that starts as early as the mid-8th century BCE and ends as late as the beginning of the 4th century BCE (Table 6). Fig. 5 shows the Bayesian modelled and calibrated (two-sigma) dates for WB16, again beginning as early as the mid-8th century BCE and ending as late as the 2nd century BCE (Table 7). In combination, the two trenches document the site occupation maximum of around 550 years, from the mid-8th to the 2nd century cal BCE.

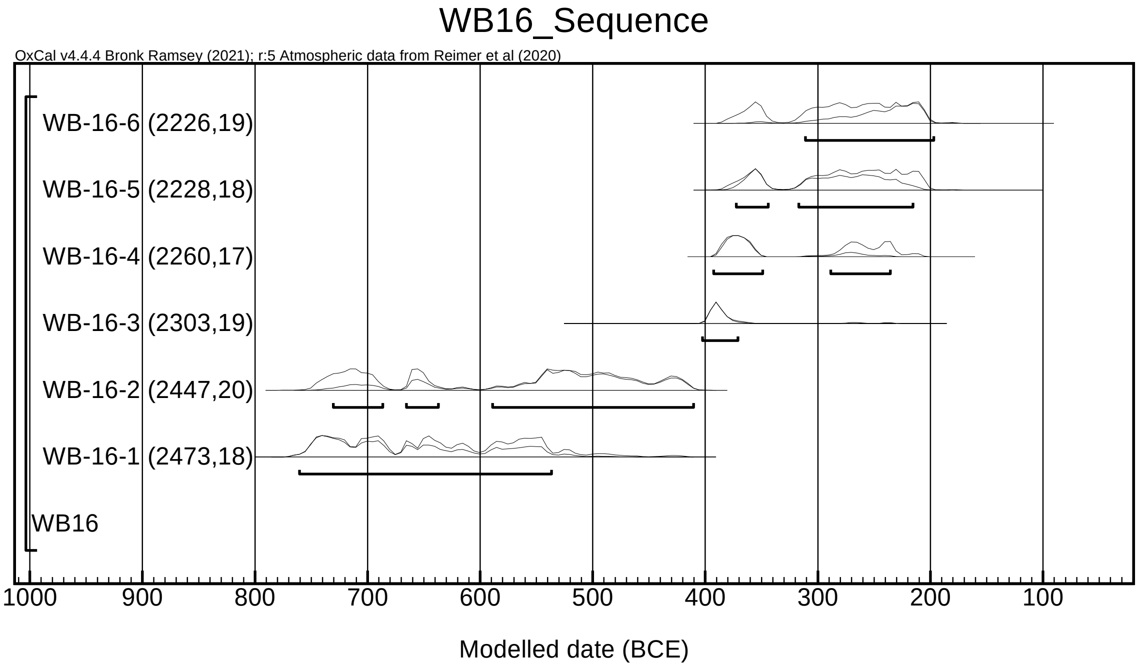


Figure 5: The Bayesian modelled sequence of WB16.

Table 7: AMS radiocarbon dates of Wari-Bateshwar (WB16).

Sample code	Lab code	C14 age (BP)	Calibrated 2 σ (BCE), Modelled.		Median (BCE)	Confidence intervals, (CI)%	Phase
			From	To			
WB16-1	OxA-41616	2473 ± 18	765	535	675	96	1
WB16-2	OxA-41541	2447 ± 20	735	410	515	97	
WB16-3	OxA-41540	2303 ± 19	405	370	390	98	2
WB16-4	OxA-41617	2260 ± 17	395	235	370	98	3
WB16-5	OxA-41618	2228 ± 18	375	215	285	97	
WB16-6	OxA-41542	2226 ± 19	315	195	235	96	

2.4.2. Morphometry of Modern Rice Varieties

Figure 6 and Table 8 provide the results for the GLW ratio of modern charred varieties of *aus*, *indica*, and *japonica* rice. These results show that the *indica* variety is distinct, with GLW values ≥ 2.31 mm. Conversely, *aus* and *japonica* varieties overlap completely, indicating that it is not possible to differentiate between them using morphometric analysis (See details in appendix A_1 for modern morphometrics).

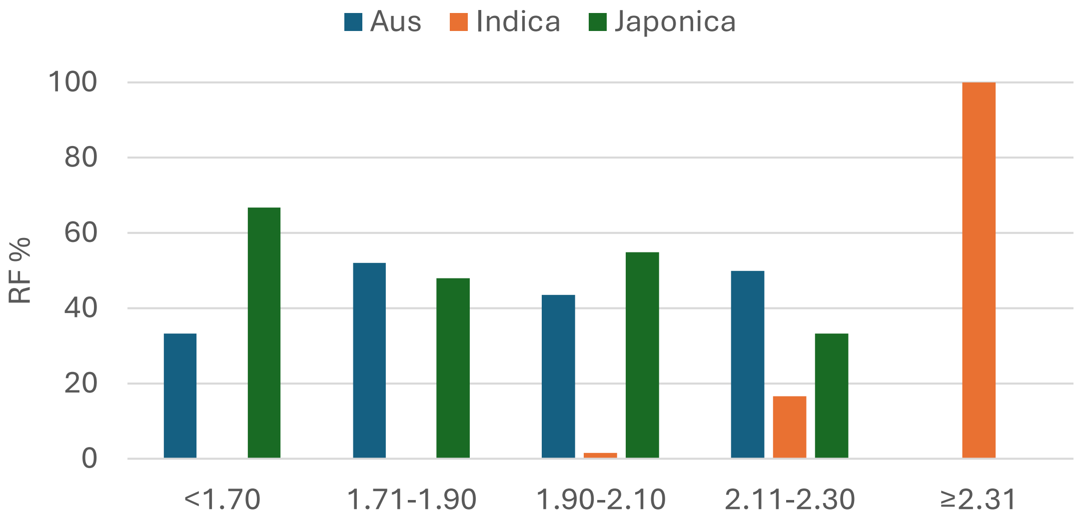


Figure 6: Bar chart showing the GLW ratios for the three modern charred rice varieties (*aus*, *indica* and *japonica*).

Table 8: The table summarises the GLW ratio measurements of the three Asian rice varieties. GLW values below 2.31 are indistinct as regards rice variety.

Genetic types	GLW ratio	Comments
Japonica-Aus mixture	≤1.70-2.29	Entirely overlapping. Not identifiable.
Indica	≥2.31	Identifiable, ≥2.31 probable *indica*.
Aus+Japonica +Indica	2.06-2.30	Not identifiable.
Aus+Indica	2.06-2.30	Not identifiable.

The present study differs from Castillo *et al.* (2016), wherein the *japonica* and *indica* rice varieties were distinguished based on the GLW ratio. The current research suggests that only *indica* rice can be reliably identified using this metric when the *aus* variety is included.

Furthermore, variations in grain size across different geographical regions, as well as the local ecology of rice cultivation, could potentially contribute to morphometric differences.

2.4.3. Identifying Rice Varieties Using GMM

This study compared three modern charred populations of Asian rice, *aus*, *indica*, and *japonica*, to establish a framework using GMM to reclassify the grains. The study has two stages: first, it examines which photographic views work well to distinguish the varieties, in which 15 grains were assigned per variety. In the second stage, a total of 306 grains were used to train the model, and 102 grains were assigned from each variety.

2.4.3.1. Selection of Photographic view and Development of a GMM Model

The assessment of classification precision for three modern charred rice varieties—*aus*, *indica*, and *japonica*—was conducted by examining various morphological perspectives, as delineated in Table 9. The perspectives in question encompassed the dorsal and lateral orientations, a composite of dorsal and lateral, and a synthesis of lateral, dorsal, and polar orientations. For the *aus* variety, an optimal classification accuracy of 80% was attained using the lateral view. Conversely, the amalgamation of multiple views did not enhance the precision of classification, as evidenced by a decline to a mere 24% when all three views were integrated. The *indica* variety was reclassified with an accuracy of 93% when viewed laterally, but this metric diminished to 62% when incorporating the lateral, dorsal, and polar perspectives. Similarly, the *japonica* variety exhibited an accuracy of 87% using the lateral view; however, a significant reduction to 36% was observed when dorsal and lateral views were combined, rising to 56% upon adding the polar view. These outcomes indicate that the lateral view affords the highest and most consistent accuracy in identifying rice varieties. The PCA classification of the rice genotype's view similarly indicates that the lateral view distinguishes the varieties most successfully (Fig. 6).

Table 10 shows the results from LDA on modern charred rice, which indicate successful reclassification, particularly for *indica* at 93% accuracy, while *aus* and *japonica* achieved 78% each. Leave-one-out cross-validation resulted in an 83% accuracy rate overall.

Table 9: LDA results of the photographic view to accuracy test in identifying rice varieties.

Variety	View	Class accuracy (%)
Aus	Dorsal	46
	Lateral	80
	Dorsal+Lateral	46
	Dorsal+Lateral+Polar	24
Indica	Dorsal	86
	Lateral	93
	Dorsal+Lateral	66
	Dorsal+Lateral+Polar	62
Japonica	Dorsal	67
	Lateral	87
	Dorsal+Lateral	36
	Dorsal+Lateral+Polar	56

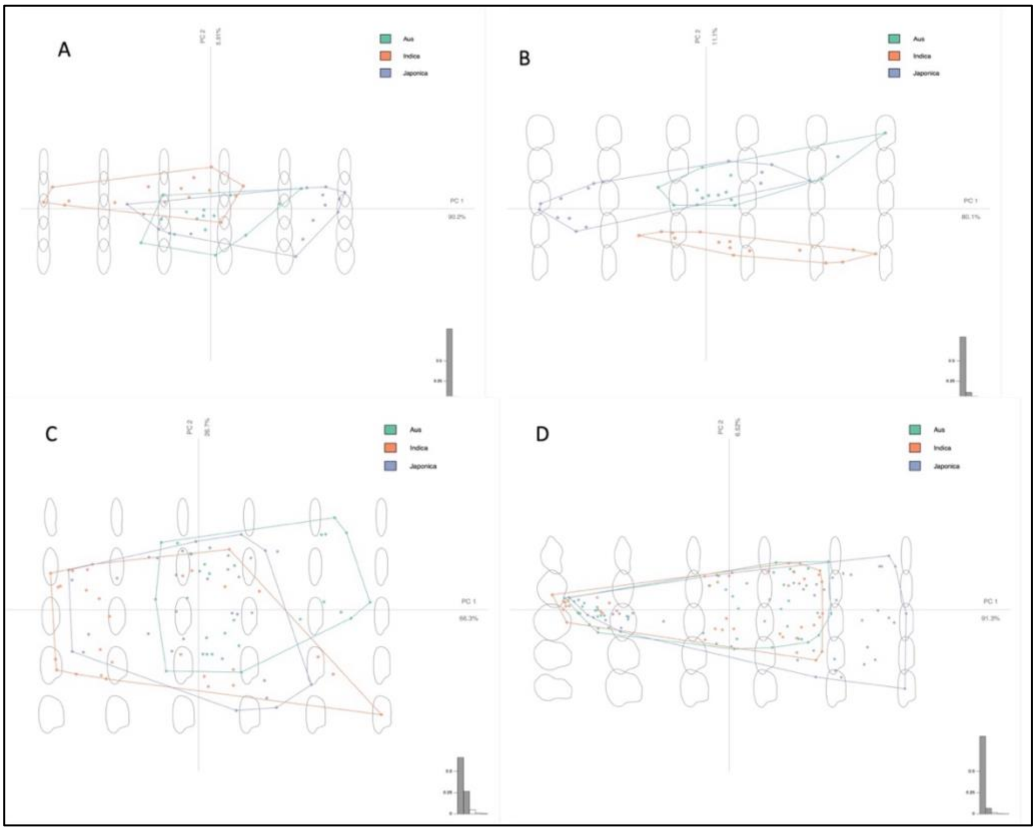


Figure 7: A PCA plot of different views, A. Dorsal B. Lateral, C. Dorsal + lateral, D. Dorsal + Lateral + Polar views, of *aus*, *indica* and *japonica* rice varieties show through lateral view the best distinction between the rice varieties are shown.

Table 10: LDA results of the modern rice reclassification using the GMM model developed.

Actual	Classified as		
	Aus	*Indica*	*Japonica*
Aus	80	0	22
Indica	6	95	1
Japonica	21	1	80
Class accuracy	78.4	93.2	78.4
Leave-one-out cross-validation			83.3% (256/307)

2.4.4. Comparing Archaeobotanical Rice from Wari-Bateshwar of Bangladesh

To classify rice into a variety levels, the morphological analysis of archaeological rice grains was conducted through a comparative assessment against modern charred specimens based on the lateral view. A total of 421 archaeological rice grains, procured from Wari Bateshwar span a maximum chronological range from the mid8th to the beginning of the 2nd century BC E. Figs. 8 & 9 summarise the relative frequency (percentage %) of rice varieties identified through GMM at WB17 and 16, respectively.

Corresponding with chronology, the outcomes are presented for the four layers from WB17 (Fig. 8), dating from c. 800 BCE to 400 BCE. Identifying aus-type rice (n=3) in the earliest layer (Layer 1) marks the first archaeological evidence of this rice variety. Data suggest that japonica-type rice was also present in the early layer (L-1) (n=5), representing its earliest documented occurrence in the Lower Brahmaputra Valley of the GBM delta. A single indica type rice grain was also documented in this layer.

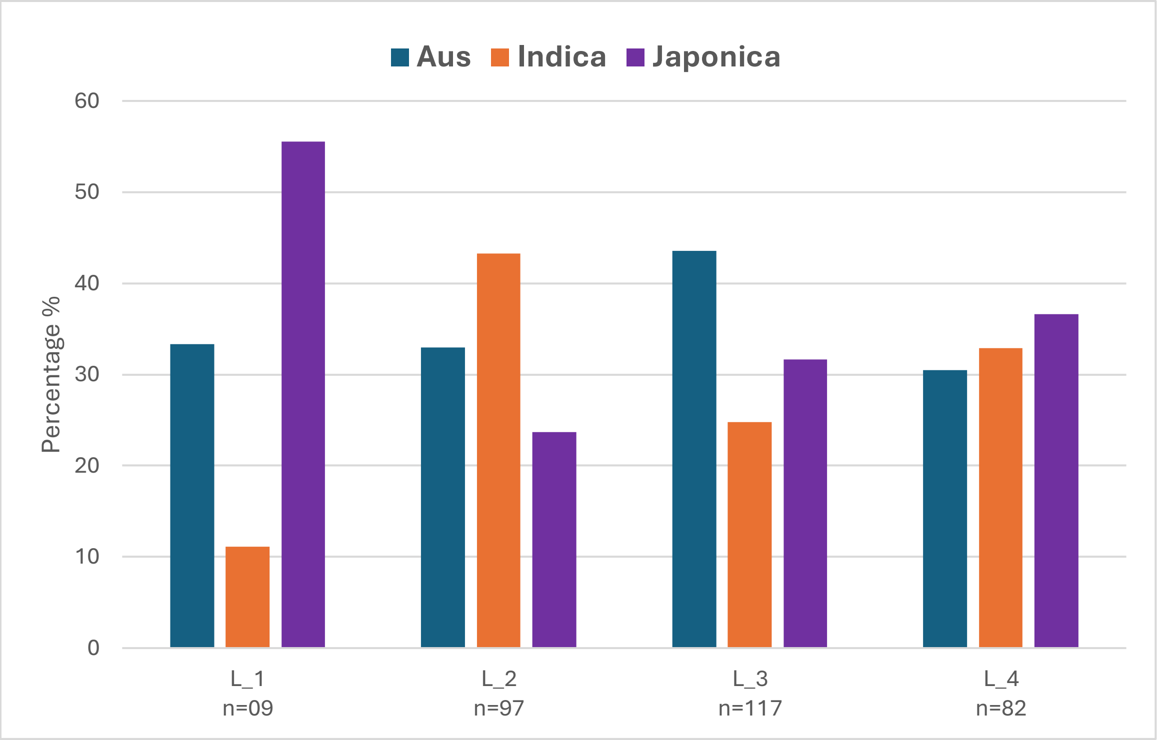


Figure 8: Results of archaeological rice identification by GMM of WB17

In layer 2, the data suggest a dominant presence of indica-type rice at 43% (n=42) alongside japonica and aus-types at 24% (n=23) and 33% (n=32), respectively, marking the unambiguous initial introduction of indica-type rice in the Lower Brahmaputra Valley of the GBM delta. This layer provides robust evidence of diverse rice cultivation. In layer 3, the result shows the predominance of aus type rice at 44% (n=51), along with 32% japonica type (n=37)) and 25% indica type (n=29) rice, reflecting consistency in rice cultivation practices with diverse varieties. Layer 4 shows 37% (n=30) japonica type, 33% (n=27) indica type, and 30% (n=25) aus-type rice presence, suggesting that all three types of rice might have been cultivated with almost similar significance.

Conversely, Fig. 9 for WB16 suggests continuous cultivation of three Asian rice varieties at the site. The data suggest a gradual increase in *indica* rice cultivation through time, from layer 1 to 5 while layer 6 exhibits so small number of grains (n-2) to be extracted meaningful explanation. Layer 1 accounted for *indica* type rice at ~30% (n=5) to layer 5 at 70% (n=7) and decrease of *aus* type rice and vice versa from ~41% (n=7) to 30% (n=3) emphasising increasing dependency on *indica* variety. In the last two layers, 5 & 6, the absence of *japonica* and *aus* respectively might indicate less extensive rice cultivation compared to earlier layers. The overall picture is one of diverse rice cultivation through the sequence of which *indica* exhibits dominant variety.

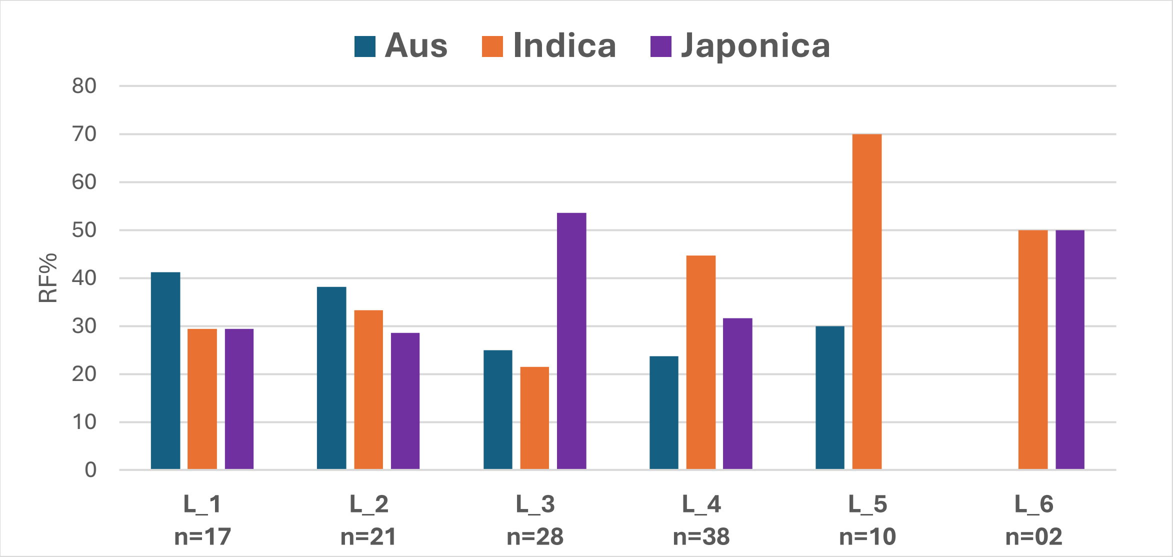


Figure 9: Results of Archaeological rice identification by GMM of WB16

2.5. Discussion

5.1.1. Archaeological Chronology of Wari-Bateshwar and Rice of Early Bangladesh

Radiocarbon dating of two stratigraphic sequences at Wari-Bateshwar documents human occupation from as early as the mid-8th century BCE to as late as the beginning of the 2nd century BCE.

This evidence complements later dates from previous studies at the site (Rahman *et al.* 2012; Rahman & Pathan 2013; 2018; Hu *et al.* 2020). Wari-Bateshwar is currently the oldest site in Bangladesh with evidence for rice cultivation. The radiocarbon dates from WB fit well with relative dating from material culture such as diagnostic ceramics and silver punch-marked coins from the site. The latest layer at WB, just below the surface soil, yielded Northern Black Polished Ware (NBPW) and Black Slipped Ware (BSW) ceramics, suggesting dates no later than the 1st century BCE. The relative dates of the ceramics are in the Middle Ganges ~ 7th to 2nd century BCE (Kanungo 2021), and in West Bengal, the 5th century BCE to the 2nd century BCE (Singh 2017). The same cultural materials, e.g., ceramics and coins, have been unearthed at Mahasthan, in the northern part of Bangladesh, by a French-Bangladesh joint excavation which documented occupation dating back to the 3rd to 2nd centuries BCE (Elaigne 2001). Thus, the date proposed in this study aligns with the relative chronology of cultural materials.

Hu *et al.* (2020) estimated a 4.3 ka BP (2300 BCE) date for layer 1 of WB17. In contrast, our study obtained a radiocarbon date of 758-589 cal BCE for the same layer. This discrepancy highlights a stark difference between the dates. Hu *et al.* based their date on wood, while our study used charred rice grains for radiocarbon dating. The latter method is likely more reliable, as rice grains are short-lived and directly related to human activity, whereas the wood may have been part of the natural vegetation and less indicative of the specific archaeological context.

The GMM results presented here suggest that *japonica* and *indica* were present at WB by the 6th century BCE (layer 1 for both WB17 and WB16). This chronology has wider implications for regional connections. These early occurrences of *japonica* and *indica* could be associated with the first farmer migration into the region (Diamond & Bellwood 2003; Bellwood 2005). *Indica* rice cultivation was noted in Odisha, Eastern India, by the mid-1st millennium BCE

(Kingwell-Benham 2018), while both *japonica* and *indica* rice were cultivated in the Middle Ganges region of North India by c. 1800 BCE (Fuller *et al.* 2010). Finding *japonica* as early as the 6th century BCE from a stratigraphic layer at which no artifacts have been unearthed rather charred archaeobotanical remains might indicate the early rice dispersal through the Southern Routes (H1) suggested by Silva *et al.* (2018).

2.5.2. Comparison of Morphometrics and GMM

Fig. 10 shows the distribution of GLW ratios across both modern (Fig. 10A) and archaeobotanical rice grains (Fig. 10B; See details of morphometrics in Appendix A_1 and A_2 for modern and archaeological identified by GMM respectively). The figure shows a consistent presence of *aus* and *japonica* GLW ratios spanning ≤ 1.70 mm to 1.90 mm in both data sets, alongside the absence of the *indica* variety within the range.

Fig. 10 shows close compatibility between the morphometrics (L/W ratio) of modern charred rice and those of archaeologically identified rice grains at the variety level using GMM. Additionally, GMM provides the probability of each grain identification that might bear a testimony of their distinction. Furthermore, this congruence corroborates the GMM classifications of archaeological rice grains to a variety level. Fig. 11 demonstrates that identified archaeological grains of *japonica*, *indica*, and *aus* varieties show substantial similarity with similar modern accessions in shape. This observation underlines the potential of the GMM approach for accurate rice identifications to variety level.

As Fig. 8 and Table 9 demonstrate, the lateral view of rice grains is key for discrimination among varieties. This is in contrast to wheat and barley identification, which is successfully achieved by combining dorsal and lateral views (Roushannafas *et al.* 2022; Jeanty *et al.* 2024). Tarongi *et al.* (2021) demonstrated that an integrated approach of morphometrics and GMM enhances pulse identification by around 20%-30% better than morphometrics alone, a contrast not observed in rice Varieties classification, potentially due to their subtle variation.

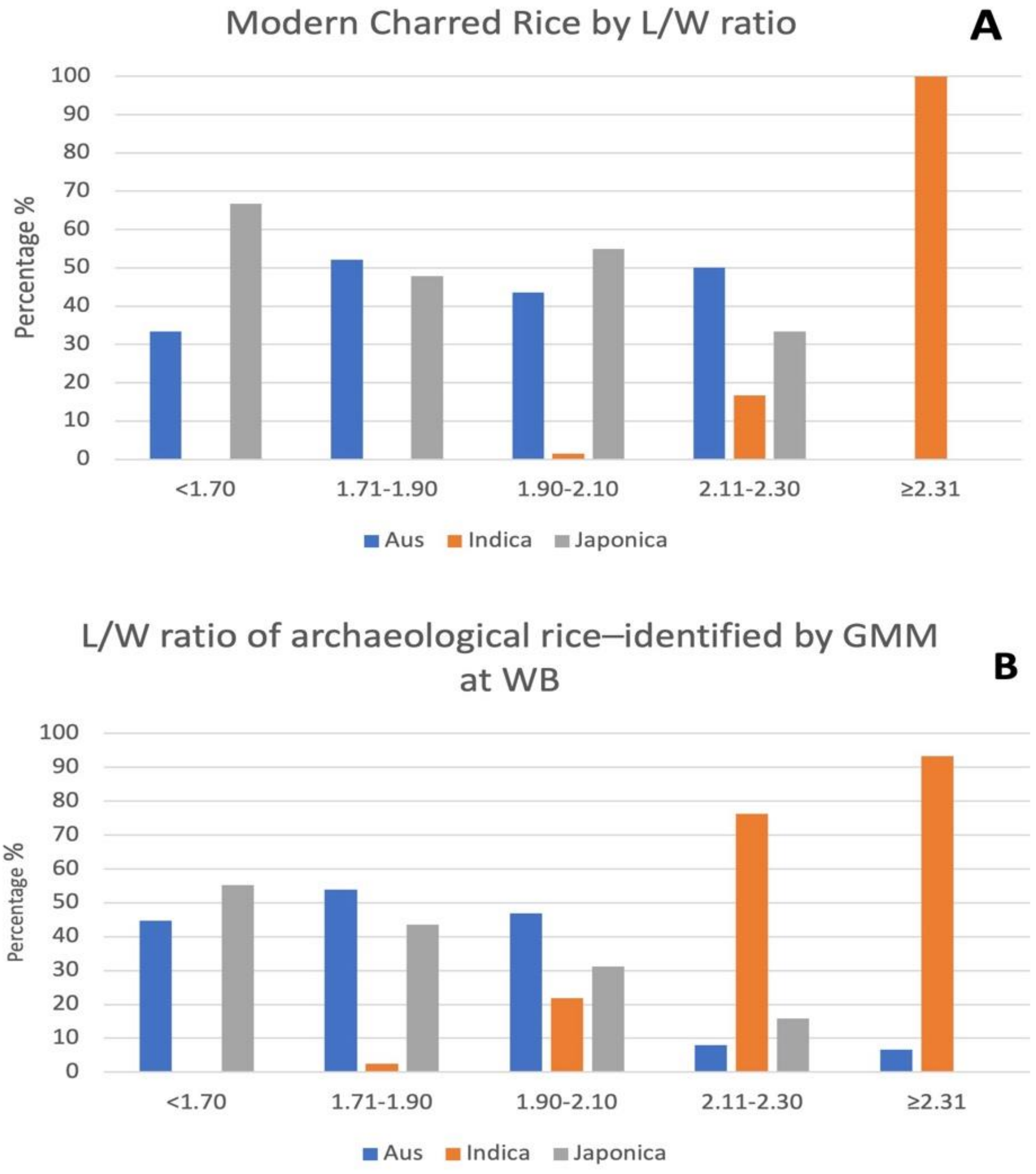


Figure 10: Bar charts comparing A. modern charred rice morphometrics of three Asian rice varieties and B. morphometrics of archaeological rice, classified based on GMM.

Differentiation between wild and domesticated rice through morphometrics has been reported as unsuccessful (Harvey 2007: 223; Fuller et al. 2007) due to overlapping measurements, whereas researchers have successfully distinguished some wild and domesticated cereals using GMM, suggesting the potential of the method for further inquiry in this domain (Bonhomme et al. 2017; Bouby et al. 2021; & Roushannafas et al. 2022). Nonetheless, the study of cereal caryopsis morphology is intricate and influenced by ecological conditions, manuring practices, geographical variances, genetic traits, and functional traits (Gegas et al. 2010; Hodgson *et al. 2020;* Larsson and Bergman *2023).*

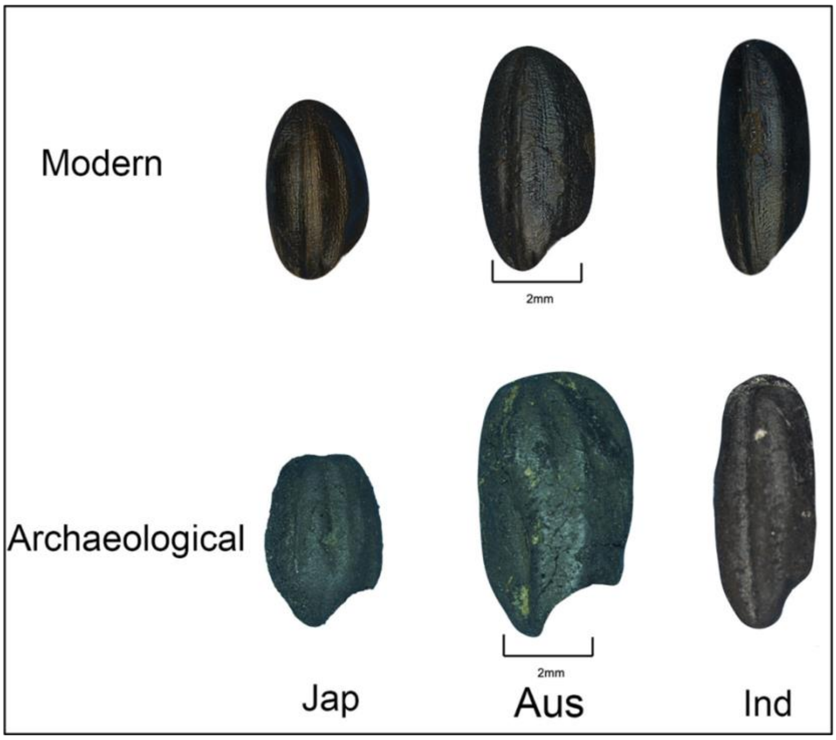


Figure 11: Comparison of modern charred rice (Upper) and identified archaeological rice by GMM (Bottom) illustrating close appearance.

Seed size variation is also a result of evolutionary processes across time and regions (Fuller *et al.* 2017). These diverse factors emphasise the complex interplay of environmental and genetic influences in shaping seed morphology, highlighting the multifaceted nature of this field of study.

2.5.3. *Aus* Rice Cultivation: A New Dimension of Rice Archaeobotany in Asia

The result of archaeological rice identification at WB (Figs 8-9) supports the identification of *aus* rice beyond previously identified *indica* and *japonica* type rice. These findings have significant implications for our understanding of Asian rice varieties. For the first time, we can infer the presence of a third variety in South Asia, with implications for previous investigations of its geographical origin (Civáň *et al.* 2015), including in Bangladesh, where there is a long history of its cultivation (Glaszmann 1987; Wang *et al.* 2013). The identification of *aus* raises the question of its domestication and of the populations that contributed to its evolution (Fig. 2). To confirm the status of wild and domesticated rice at WB, including implications for the emergence of *aus*-type rice, a further inquiry in relation to the chaff morphology is needed (Paper 3), since chaff evidence of shattering/non-shattering offers the most direct evidence of domestication status (Jones 1988; Tanno & Willcox 2006; Fuller *et al.* 2009; Tanno & Willcox 2012; Charles *et al.* 2021).

Archaeological findings suggest that early cultivation of *aus* rice in Bangladesh aligns with expectations given its present-day agronomic characteristics. *Aus* rice is notably drought-tolerant, typically direct-seeded, and cultivated under rainfed upland or dry conditions. Furthermore, this variety exhibits adaptability to floods and heat, matures early, and shows limited sensitivity to photoperiod variations (Garris *et al.* 2005; Bin Rahman & Zhang 2016). Frequent floods (Szabo *et al.* 2016) and droughts (O'Brien *et al.* 2017; O'Connell *et al.* 2018; Perez Arango *et al.* 2021) are standard features for tropical delta regions like Bangladesh which critically shape agroecology and livelihood patterns. These environmental challenges likely influenced early farmers' decision to adopt *aus* rice, recognising its suitability for the local climate and ecological conditions.

Additionally, confirmed identification of *japonica* and *indica* at WB is important for the circumstances of *aus* rice domestication in early Bangladesh (Fig. 2). Multiple studies (Huang & Han 2016; Choi *et al.* 2017; Choi & Purugganan 2018) have suggested that *aus* arose from hybridisation of *japonica* and *indica* with a wild annual rice, Oryza nivara.

5.2.Conclusion

Our research offers an improved methodological framework for rice taxonomic identification by GMM beyond what morphometrics can achieve. The results indicate that differentiation of the three Asian rice varieties is not possible through morphometrics alone. Modern landraces of the three rice varieties from Bangladesh can instead be separated using GMM, based on the lateral view. Application of the new GMM approach to archaeological rice grains from Wari-Bateshwar, Bangladesh, documents the presence of the three Asian rice varieties for the first time, from the 6th century BCE. The results indicate that *aus*-type rice was cultivated in early Bangladesh in the first millennium BCE alongside *japonica* and *indica* varieties.

References

Alam, M. U. 2018. Excavations at Wari-Bateshwar: 2016-17. *In:* Chowdhury, A. M. and Chakrabarti, R. (ed.). *History of Bangladesh: Early Bengal in Regional Perspectives (up to C.1200 CE).* Dhaka: Bangladesh Asiatic Society.

Alam, S., & Salles., J. (ed.) 2001. *France-Bangladesh joint Venture excavations at Mahasthangarh First Interim report.,* Dhaka: Department of Archaeology, Government of Bangladesh.

Azizi, P., Osman, M., Hanafi, M. M., Sahebi, M., Rafii, M. Y., Taheri, S., Harikrishna, J. A., Tarinejad, A. R., Matsharani, S. & Yusuf, M. N. 2019. Molecular insights into the regulation of rice kernel elongation. *Crit Rev Biotechnol,* 39**,** 904-923.

Bayliss, A. 2015. Quality in Bayesian chronological models in archaeology. *World archaeology,* 47**,** 677-700.

Bellwood, P. S. 2005. *First farmers: the origins of agricultural societies,* Malden, Mass., Blackwell.

Bin Rahman, A. N. M. R., & Zhang, J. 2016. Flood and drought tolerance in rice: opposite but may coexist. *Food and Energy Security* 5(2). pp76-88.

Bonhomme, V., Forster, E., Wallace, M., Stillman, E., Charles, M. & Jones, G. 2017. Identification of inter- and intra-species variation in cereal grains through geometric morphometric analysis, and its resilience under experimental charring. *Journal of archaeological science,* 86**,** 60-67.

Bonhomme, V., Picq, S., Gaucherel, C. & Claude, J. 2014. Momocs: Outline analysis using R. *Journal of statistical software,* 56**,** 1-24.

Bouby, L., Wales, N., Jalabadze, M., Rusishvili, N., Bonhomme, V., Ramos-Madrigal, J., Evin, A., Ivorra, S., Lacombe, T., Pagnoux, C., Boaretto, E., Gilbert, M.T.P., Bacilieri, R., Lordkipanidze, D. & Maghradze, D. 2020. Tracking the history of grapevine cultivation in Georgia by combining geometric morphometrics and ancient DNA. *Vegetation history and archaeobotany,* 30**,** 63-76.

Bronk Ramsey, C. 2009. Bayesian analysis of radiocarbon dates. *Radiocarbon***,** 337-360.

Bronk Ramsey, C. 2021. OxCal v4. 4.4. *https://c14. arch. ox. ac. uk/oxcal. html.*

Buck, C. E., Cavanagh, W. G. & Litton, C. D. 1996. *Bayesian approach to interpreting archaeological data,* Chichester, England, Wiley.

Castillo, C., Tanaka, K., Sato, Y.-I., Ishikawa, R., Bellina, B., Higham, C., Chang, N., Mohanty, R., Kajale, M. & Fuller, D. 2016. Archaeogenetic study of prehistoric rice remains from Thailand and India: evidence of early *japonica* in South and Southeast Asia. *Archaeological and Anthropological Sciences,* 8**,** 523-543.

Charles, M., Fuller, D., Roushannafas, T., Bogaard, A. 2021. An assessment of crop plant domestication traits at Çatalh¨oyük. *In:* Hodder, I. (ed.) *Peopling the Landscape of Çatalh¨oyük: Reports from the 2009-2017 Seasons, Çatalh¨oyük Research Project Series* British Institute at Ankara. Pp. 125–136.

Chang, T.T. 1976. The origin, evolution, cultivation, dissemination, and diversification of Asian and African rices. *Euphytica,* 25**,** 425-441.

Cheng, C., Motohashi, R., Tsuchimoto, S., Fukuta, Y., Ohtsubo, H. & Ohtsubo, E. 2003. Polyphyletic origin of cultivated rice: Based on the interspersion pattern of SINEs. *Mol Biol Evol,* 20**,** 67-75.

Choi, J. Y. & Purugganan, M. D. 2018. Multiple origin but single domestication led to Oryza sativa. *G3 (Bethesda),* 8**,** 797-803.

Choi, J.Y., Platts, A.E., Fuller, D.Q., Hsing, Y-I., Wing, R. A., & Purugganan, M.D., 2017. The Rice Paradox: Multiple Origins but Single Domestication in Asian Rice. *Mol. Biol. Evol.,* 34(4), 969–979.

Civáň, P., Craig, H., Cox, C. J., & Brown, T. A. 2015. Three geographically separate domestications of Asian rice. *Nat Plants,* 1: 15164.

Charles, M., Forster, E., Wallace, M. & Jones, G. 2015. "Nor ever lightning char thy grain" 1: establishing archaeologically relevant charring conditions and their effect on glume wheat grain morphology. *Science and Technology of Archaeological Research,* 1**,** 1-6.

Claude, J. 2008. *Morphometrics with R, Use R!,* New York., Springer-Verlag

Diamond, J. & Bellwood, P. 2003. Farmers and their languages: The first expansions. *Science,* 300**,** 597-603.

Elaigne, S. 2001. Northern Black polished ware complex of the oldest stratigraphical sequence at Mahasthan. *In:* Salles, J-F, & Alam. M. S. (ed.) *France-Bangladesh joint venture excavations at Mahasthangarh First Interim Report 1993-1999.* Dhaka: Dept. of Archaeology, Ministry of Culture, Govt. of Bangladesh.

Fuller, D. Q. 2020. Transitions in Productivity: Rice Intensification from Domestication to Urbanisation. *Archaeology International,* 23 (1)**,** pp. 88–103.

Fuller, D. Q. 2011. Pathways to Asian Civilizations: Tracing the Origins and Spread of Rice and Rice Cultures. *Rice (New York, N.Y.),* 4**,** 78-92.

Fuller, D.Q. 2007. "Portable Archaeobotany I: Bucket Flotation"._https://www.youtube.com/watch?v=Nbkfe0B4zBg&t=9s

Fuller, D.Q, Qin. L., Harvey, E. 2008. Evidence for a late onset of agriculture in the Lower Yangzi region and challenges for an archaeobotany of rice. *In:* Sanchez Mazas A, Blench R, Ross MD, Peiros I, Lin, M. (eds). *Past human migrations in East Asia. Matching archaeology, linguistics and genetics.* London: Routledge.

Fuller, D. Q., Harvey, E. & Qin, L. 2007. Presumed domestication? Evidence for wild rice cultivation and domestication in the fifth millennium BC of the Lower Yangtze region. *Antiquity,* 81**,** 316-331.

Fuller, D. Q., Qin. L., Zheng. Y., Zhao. Z., Chen. X., Hosoya. L. A., Sun, G-P. 2009.The Domestication Process and Domestication Rate in Rice: Spikelet bases from the Lower Yangzte. *Science,* 323**,** 1607-1610.

Fuller, D.Q, Sato, Y.-I., Castillo, C., Qin, L., Weisskopf, A., Kingwell-Banham, E., Song, J., Ahn, S-M. & Etten, J. 2010. Consilience of genetics and archaeobotany in the entangled history of rice. *Archaeological and Anthropological Sciences,* 2**,** 115-131.

Fuller, D. Q., Colledge, S., Murphy, C., Stevens, C. J. 2017. Sizing up cereal variation: patterns in grain evolution revealed in chronological and geographical comparisons. *In:* Eraso, J. F., Alustiza, J.A.M., Valbuena, A.A., Díez, M.G. (ed.) *Miscelánea en homenaje a Lydia Zapata Peña (1965-2015).* Bilbao: Spain: Universidad Del País Vasco. Pp-131-149

García-Granero, J.J., Júlia, A.-M., Marco, M. & Carla, L. 2016. Geometric morphometric analysis of Setaria italica (L.) P. Beauv. (Foxtail millet) and Brachiaria ramosa (L.) Stapf, (browntop millet) and its implications for understanding the biogeography of small millets. *Vegetation history and archaeobotany,* 25**,** 303-310.

Garris, A. J., Tai, T. H., Coburn, J., Kresovich, S. & Mccouch, S. 2005. Genetic structure and diversity in Oryza sativa L. (Author Abstract). *Genetics,* 169**,** 1631.

Gegas, V. C., Nazari, A., Griffiths, S., Simmonds, J., Fish, L., Orford, S., Sayers, L., Doonan, J. H. & Snape, J. W. 2010. Genetic Framework for Grain Size and Shape Variation in Wheat. *Plant Cell,* 22**,** 1046-1056.

Giardina, C. R., & Kuhl, F. P. 1977. Accuracy of curve approximation by harmonically related vectors with elliptical loci. *Computer graphics and image processing,* 6**,** 277-285.

Glaszmann, J. C. 1987. Isozymes and classification of Asian rice varieties. *Theor Appl Genet,* 74**,** 21-30.

Govindaswami, S. 1957. Scheme for botanical survey and collection of breeding material from Jeypore Tract of Orissa States, I and II. Cuttack, India.: Mimeogr. Rep. Central Rice Res. Institute.

Harvey, E. 2007. Early agricultural communities in northern and eastern India: An archaeobotanical investigation. Unpublished PhD dissertation, Institute of Archaeology, University college London.

Hodgson, J. G., Marti, G. M., Sera, B., Jones, G., Bogaard, A., Charles, M., Font, X., Ater, M., Taleb, A., Santini, B. A., Hmimsa, Y., Palmer, C., Wilson, P. J., Band, S. R., Styring, A., Diffey, C., Green, L., Nitsch, E., Stroud, E. & Warham, G. 2020. Seed size, number and strategies in annual plants: a comparative functional analysis and synthesis. *Ann Bot,* 126**,** 1109–1128.

Hu, G., Wang, P., Rahman, S. M., Li, D., Alam, M. M., Zhang, J., Jin, Z., Fan, A., Chen, J., Zhang, A., & Yang, W. 2020. Vicissitudes experienced by the oldest urban center in Bangladesh in relation to the migration of the Brahmaputra River. *Journal of quaternary science, 1*–11.

Hu, Z., He, H., Zhang, S., Sun, F., Xin, X., Wang, W., Qian, X., Yang, J. & Luo, X. 2012. A Kelch Motif-Containing Serine/Threonine Protein Phosphatase Determines the Large Grain QTL Trait in Rice. *Journal of Integrative Plant Biology,* 54**,** 979-990.

Huang, X., Kurata, N., Wei, X., Wang, Z.-X., Wang, A., Zhao, Q., Zhao, Y., Liu, K., Lu, H., Li, W., Guo, Y., Lu, Y., Zhou, C., Fan, D., Weng, Q., Zhu, C., Huang, T., Zhang, L., Wang, Y., Feng, L., Furuumi, H., Kubo, T., Miyabayashi, T., Yuan, X., Xu, Q., Dong, G., Zhan, Q., Li, C., Fujiyama, A., Toyoda, A., Lu, T., Feng, Q., Qian, Q., Li, J. & Han, B. 2012. A map of rice genome variation reveals the origin of cultivated rice. *Nature,* 490**,** 497-501.

Huang, X. & Han, B. 2016. Rice domestication occurred through single origin and multiple introgressions. *Nat Plants,* 2**,** 15207-15207.

IRRI, Africa rice & CIAT. 2013. *Rice Almanac,* Manila, Philippines. IRRI.

Jeanty, A., Ros, J., Mureau, C., Dham, C., Lecomte, C., Bonhomme, V., Ivorra, S., Figueiral, I., Bouby, L. & Evin, A. 2024. Identification of archaeological barley grains using geometric morphometrics and experimental charring. *Journal of archaeological science,* 162**,** 105924.

Jesus, A., Bonhomme, V., Evin, A., Ivorra, S., Soteras, R., Salavert, A., Antolín, F. & Bouby, L. 2021. A morphometric approach to track opium domestication. *Sci Rep,* 11**,** 9778-9778.

Jones, G. 1998. Wheat grain identification – why bother? . *Environ. Archaeol,* 2**,** 29–34

Kanungo, A. K., Kulkarni, C., Ingle, V. & Roy, O. 2021. Northern Black Polished Ware in Indian Archaeology: A Study of Spatial and Chronological Distribution. Springer International Publishing.

Kingwell-Banham, E., Harvey, E. K., Mohanty, R. K., & Fuller, D.Q. 2018. Archaeobotanical Investigations into Golbai Sasan and Gopalpur, Two Neolithic-Chalcolithic Settlements of Odisha. *Ancient Asia,* 9**,** 1–14.

Khush, G. S. 1997. Origin, dispersal, cultivation, and variation of rice. *Plant Mol Biol,* 35**,** 25-34.

Kuhl, F. P., & Giardina, C. R. 1982. Elliptic Fourier features of a closed contour. *Computer graphics and image processing,* 18**,** 236-258.

Larsson, M. & Bergman, J. 2023. Experimental approach to evaluate the effect of growing conditions on cereal grain size and its relevance for interpreting archaeological cereal grain assemblages. *Journal of Archaeological Science,* 152.

Li, Y., Fan, C., Xing, Y., Jiang, Y., Luo, L., Sun, L., Shao, Di., Xu, C., Li, X., & Xiao, J., He, Y., Zhang, Q. 2011. Natural variation in GS5 plays an important role in regulating grain size and yield in rice. *Nat Genet,* 43**,** 1266-1269.

Londo, J. P., Chiang, Y. C., Hung, K. H., Chiang, T. Y. & Schaal, B. A. 2006. Phylogeography of Asian wild rice, Oryza rufipogon, reveals multiple independent domestications of cultivated rice, Oryza sativa. *Proc Natl Acad Sci U S A,* 103**,** 9578-9583.

Morishima, H., Sano, Y., & Oka, H. I. 1992. Evolutionary studies in cultivated
rice and its wild relatives. Oxford Surveys Evol. Biol. 8:135–184.
Morishima, H. 1986. Wild progenitors of cultivated rice and their population dynamics. Pp. 3–14 in Rice genetics. International Rice Research Institute, Manila.

Morishima, H., Sano, Y., & Oka, H.I. 1980. Observations on wild and cultivated rices and companion weeds in the hilly areas of Nepal, India, and Thailand: Report of study-tour in tropical Asia, 1979. Misima: Rep. Natl. Inst. Genetics.

O'Brien, M. J., Reynolds, G., Ong, R., & Hector, A. 2017 Resistance of tropical seedlings to drought is mediated by neighbourhood diversity. *Nature Ecology & Evolution,* 1 1643–1648.

O'Connell, C. S., Ruan, L., & Silver, W. L. 2018. Drought drives rapid shifts in tropical rainforest soil biogeochemistry and greenhouse gas emissions. *Nature Communications,* 9**,** 1-9.

Oka, H. I. 1988. *Origin of cultivated rice,* Tokyo: Amsterdam [Netherlands]; New York: New York, N.Y., Japan Scientific Societies Press; Elsevier.

Oka, H. I., Chang, W. T. 1962. Rice varieties intermediate between wild and cultivated forms and the origin of the Japonica type. *Bot. Bull. Acad. Sinica* 3**,** 109-131.

Orrù, M., Grillo, O., Lovicu, G., Venora, G. & Bacchetta, G. 2013. Morphological characterisation of Vitis vinifera L. seeds by image analysis and comparison with archaeological remains. *Vegetation history and archaeobotany,* 22**,** 231-242.

Padhan, T. 2023. New evidence of Neolithic industries from the West Garo Hills, northeastern India. *In:* Tiwari, N., Singh, V. & Mehra, S. B. (ed.), *Quaternary Geoarchaeology of India.* Special Publications, 515, London Geological Society. 125-136.

Pearsall, D. M. 2016. *Paleoethnobotany: a handbook of procedures,* Routledge.

Perez Arango, J. D., Lintner, B. R., Carvalho, L. M. V., & Lyon, B. 2021. Spatial extents of tropical droughts during El Niño in current and future climate in observations, reanalysis, and CMIP5 models. . *Geophysical Research Letters,* 48**,** 1-10.

Qi, P., Lin, Y-S., Song, X-J., Shen, J-B., Huang, W., Shan, J-X., Zhu, M-Z., Jiang, L., Gao, J-P. & Lin, H-X. 2012. The novel quantitative trait locus GL3.1 controls rice grain size and yield by regulating Cyclin-T1;3. *Cell Research,* 22**,** 1666-1680.

Rahman, M. 2018. Agricultural Extension in Medieval Northern Bangladesh: Archaeobotanical Evidence from Setesher Danga. An unpublished MSc dissertation, UCL Institute of Archaeology.

Rahman, M., Murphy, C., Weisskopf. A., Champion. L, Castillo, C., & Fuller, D. Q. 2019. Wari-Bateshwar and Vikrampura: Successful Case Studies in Archaeobotany, Bangladesh. *Man and Environment.,* XLIV(1)**,** pp: 62-72.

Rahman, M., Castillo, C. C., Murphy, C., Rahman, S. M., & Fuller, D. Q. 2020. Agricultural systems in Bangladesh: the first archaeobotanical results from Early Historic Wari-Bateshwar and Early Medieval Vikrampura. *Archaeological and Anthropological Sciences*.

Rahman, S. M. 2007. Pottery *In:* Rahman, S. M. (ed.) *Archaeological Heritage.* Dhaka: Asiatic Society of Bangladesh.

Rahman, S. M., Pathan, H., Rahman, M., Akhter, S., Rayhan, M. 2012. Wari-Bateshwar. *In:* Islam, S. (ed.) *Banglapedia.* 2nd ed. Dhaka: Asiatic Society of Bangladesh.

Rahman, M. & Pathan, S. 2018. Wari-Bateshwar. *In:* Choudhury, A. M. & Chakrabarti., R. (ed.) *History of Bangladesh: Early Bengal in Regional Perspectives (up to C.1200 CE).* Dhaka: Bangladesh Asiatic Society.

Reimer, P. J., Austin, W. E. N., Bard, E., Bayliss, A., Blackwell, P. G., Bronk Ramsey, C., Butzin, M., Cheng, H., Edwards, R. L., Friedrich, M., Grootes, P. M., Guilderson, T. P., Hajdas, I., Heaton, T. J., Hogg, A. G., Hughen, K. A., Kromer, B., Manning, S. W., Muscheler, R., Palmer, J. G., Pearson, C., Van der plicht, J., Reimer, R. W., Richards, D. A., Scott, E. M., Southon, J. R., Turney, C. S. M., Wacker, L., Adolphi, F., Buentgen, U., Capano, M., Fahrni, S. M., Fogtmann-Schulz, A., Friedrich, R., Koehler, P., Kudsk, S., Miyake, F., Olsen, J., Reinig, F., Sakamoto, M., Sookdeo, A. & Talamo, S. 2020. The IntCal20 Northern Hemisphere radiocarbon age calibration curve (0-55 cal kBP). *Radiocarbon,* 62**,** 725-757.

Rohlf, F. J. 1990. Morphometrics. *Annu. Rev. Ecol. Syst.,* 21**,** 299-316.

Ros, J., Evin, A., Bouby, L. & Ruas, M-P. 2014. Geometric morphometric analysis of grain shape and the identification of two-rowed barley (Hordeum vulgare subsp. distichum L.) in southern France. *Journal of archaeological science,* 41**,** 568-575.

Roushannafas, T., Bogaard, A. & Charles, M. 2022. Geometric morphometrics sheds new light on the identification and domestication status of ‘new glume wheat’ at Neolithic Çatalhöyük. *Journal of archaeological science,* 142.

Sano, Y., Morishima, H. & Oka, H-I. 1980. Intermediate perennial-annual populations of Oryza perennis found in Thailand and their evolutionary significance. *The Botanical Magazine Tokyo,* 93**,** 291-305.

Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods,* 9**,** 671-675.

Sharma, S.D, Shastry, S.V.S., 1965 Taxonomic studies in the genus Oryza L. III. O. rufipogon Griff. sensu stricto and O. nivara Sharma et Shastry nom. Nov. *Indian J Genet Plant Breed* 25**,** 157–167.

Silva, F., Weisskopf, A., Castillo, C., Murphy, C., Kingwell-Banham, E., Qin, L. & Fuller, D. Q. 2018. A tale of two rice varieties: Modelling the prehistoric dispersals of japonica and proto-indica rices. *Holocene (Sevenoaks),* 28**,** 1745-1758.

Singh, D. A. S. 2017. The Chronology of Northern Black Polished Ware: Recent Perspectives. *Int. Journal of Social Research Science and Technology,* Nov-Dec; 3(8)**,** 1488-1492.

Smith, M. L. & Mohanty, R. K. 2018. Monsoons, rice production, and urban growth: The microscale management of ‘too much’ water. *Holocene (Sevenoaks),* 28**,** 1325-1333.

Szabo, S., Brondizio, E., Renaud, F. G., Hetrick, S., Nicholls, R. J., Matthews, Z., Tessler, Z., Tejedor, A., Sebesvari, Z., Foufoula-Georgiou, E., Da Costa, S., Dearing, J. A. 2016 Population dynamics, delta vulnerability and environmental change: comparison of the Mekong, Ganges–Brahmaputra and Amazon delta regions. *Sustain Sci,* 11**,** 539–554.

Tanabe, S., Ashikari, M., Fujioka, S., Takatsuto, S., Yoshida, S., Yano, M., Yoshimura, A., Kitano, H., Matsuoka, M., Fujisawa, Y., Kato, H. & Iwasaki, Y. 2005. Novel Cytochrome P450 Is Implicated in Brassinosteroid Biosynthesis via the Characterization of a Rice Dwarf Mutant, dwarf11, with Reduced Seed Length. *Plant Cell,* 17**,** 776-790.

Tanno, K.-I. & Willcox, G. 2006. How Fast Was Wild Wheat Domesticated? *Science,* 311**,** 1886-1886.

Tanno, K.-I. & Willcox, G. 2012. Distinguishing wild and domestic wheat and barley spikelets from early Holocene sites in the Near East. *Vegetation history and archaeobotany,* 21**,** 107-115.

Tarongi, M., Bouby, L., Bonhomme, V. & Alonso, N. 2024. Which pulse is it? Identifying archaeological legumes seeds by means of biometric measurements and geometric morphometrics. *Vegetation history and archaeobotany,* 33**,** 5-14.

Tarongi, M., Bonhomme, V., Evin, A., Ivorra, S., López, D., Alonso, N. & Bouby, L. 2020. A new way of seeing pulses: preliminary results of geometric morphometric analyses of Iron Age seeds from the site of La Font de la Canya (Barcelona, Spain). *Vegetation history and archaeobotany,* 30**,** 77-87.

Travis, A. J., Norton, G. J., Datta, S., Sarma, R., Dasgupta, T,, Savio, F. L., Macaulay, M., Hedley, P.E., McNALLY, K. L., Sumon, M. H., Islam, M. R. & Price, A. H. 2015. Assessing the genetic diversity of rice originating from Bangladesh, Assam and West Bengal. *Rice,* 8:35.

Bonhomme, V., Russell, J., Stillman, E., George, T. S., Ramsay, L., Wishart, J., Timpany, S., Bull, H., Booth, A. & Martin, P. 2019. Searching for the Origins of Bere Barley: a Geometric Morphometric Approach to Cereal Landrace Recognition in Archaeology. *Journal of archaeological method and theory,* 26**,** 1125-1142.

Wang, M., Zhu, Z., Tan, L., Liu, F., Fu, Y., Sun, C., & Cai, H. 2013. Complexity of indica-japonica varietal differentiation in Bangladesh rice landraces revealed by microsatellite markers. *Breeding Science,* 63**,** 227-232.

Wang W, Mauleon R., Hu, Z. *et al.* 2018. Genomic variation in 3,010 diverse accessions of Asian cultivated rice. *Nature* 557**,** 43–49.

Xu, C., Liu, Y., Li, Y., Xu, X., Xu, C., Li, X., Xiao, J. & Zhang, Q. 2015. Differential expression of GS5 regulates grain size in rice. *J Exp Bot,* 66**,** 2611-2623.

Xu, H. & Bassel, G. W. 2020. Linking Genes to Shape in Plants Using Morphometrics. *Annual review of genetics,* 54**,** 417-437.

Yang, C-C., Yoshihiro K. K., Mizuno, H., Wu, J., Matsumoto, T. & Itoh, T. 2012. Independent domestication of Asian rice followed by gene flow from japonica to indica. *Mol Biol Evol,* 29**,** 1471-1479.

Yang, X., Ren, Y., Cai, Y., Niu, M., Feng, Z., Jing, R., Mou, C., Liu, X., Xiao, L., Zhang, X., Wu, F., Guo, X., Jiang, L. & Wan, J. 2018. Overexpression of OsbHLH107, a member of the basic helix-loop-helix transcription factor family, enhances grain size in rice (Oryza sativa L.). *Rice (N Y),* 11**,** 41-12.

Zhang, X., Wang, J., Huang, J., Lan, H., Wang, C., Yin, C., Wu, Y., Tang, H., Qian, Q., Li, J. & Zhang, H. 2012. Rare allele of OsPPKL1 associated with grain length causes extra-large grain and a significant yield increase in rice. *Proc Natl Acad Sci U S A,* 109**,** 21534-21539.

Zhao, D-S., Li, Q-F., Zhang, C-Q., Zhang, C., Yang, Q-Q., Pan, L-X., Ren, X-Y., Lu, J., Gu, M-H. & Liu, Q-Q. 2018. GS9 acts as a transcriptional activator to regulate rice grain shape and appearance quality. *Nat Commun,* 9**,** 1240-14.

Zheng, Y., Crawford, G. W., Jiang, L. & Chen, X. 2016. Rice Domestication Revealed by Reduced Shattering of Archaeological rice from the Lower Yangtze Valley. *Sci Rep,* 6**,** 28136-28136.

Appendix A_1_ Modern Uncharred Rice Morphometrics

Variety=Aus; Local Name=Jugli; BRRI Accession Number=1860

ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio
A101	5.36	2.47	2.17	A113	5.21	2.40	2.18	A129	5.14	2.31	2.22	A141	6.42	2.93	2.19
A102	5.20	2.43	2.14	A114	5.22	2.45	2.13	A130	5.17	2.40	2.15	A142	6.60	3.16	2.09
A103	5.16	2.41	2.14	A115	5.22	2.34	2.23	A131	5.24	2.46	2.13	Mean	5.58	2.62	2.14
A104	4.66	2.36	1.97	A118	5.39	2.52	2.14	A132	5.23	2.28	2.30	Median	5.32	2.45	2.15
A105	5.13	2.43	2.11	A119	5.20	2.38	2.19	A133	6.86	3.47	1.97	STD	0.64	0.35	0.09
A106	5.39	2.51	2.15	A120	5.11	2.41	2.12	A134	6.90	3.34	2.06	Minimum	4.66	2.26	1.93
A107	5.36	2.45	2.19	A121	4.75	2.43	1.96	A135	6.51	2.92	2.23	Maximum	6.90	3.47	2.30
A108	5.22	2.47	2.11	A122	5.18	2.42	2.14	A136	6.87	3.23	2.13				
A109	5.28	2.65	1.99	A123	5.48	2.52	2.18	A137	6.58	3.02	2.18				
A110	5.46	2.37	2.30	A124	5.36	2.43	2.20	A138	6.70	3.30	2.03				
A111	5.45	2.45	2.22	A125	5.22	2.42	2.16	A139	6.65	3.22	2.06				
A112	5.10	2.26	2.26	A126	5.29	2.68	1.97	A140	6.06	3.14	1.93				

Variety=Aus; Local Name=Sada boro; BRRI Accession Number=1997

ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio
A201	5.36	2.47	2.17	A213	5.21	2.40	2.18	A227	5.40	2.35	2.30	A241	6.53	3.39	1.93
A202	5.20	2.43	2.14	A214	5.22	2.45	2.13	A228	5.40	2.48	2.18	A242	6.43	3.20	2.01
A203	5.16	2.41	2.14	A215	5.22	2.34	2.23	A229	5.14	2.31	2.22	Mean	5.62	2.67	2.12
A204	4.66	2.36	1.97	A218	5.39	2.52	2.14	A230	5.17	2.40	2.15	Median	5.32	2.45	2.14
A205	5.13	2.43	2.11	A219	5.20	2.38	2.19	A231	5.24	2.46	2.13	STD	0.71	0.42	0.11
A206	5.39	2.51	2.15	A220	5.11	2.41	2.12	A232	5.23	2.28	2.30	Minimum	4.66	2.26	1.85
A207	5.36	2.45	2.19	A221	4.75	2.43	1.96	A233	6.89	3.56	1.94	Maximum	7.33	3.56	2.30
A208	5.22	2.47	2.11	A222	5.18	2.42	2.14	A234	6.24	3.38	1.85				
A209	5.28	2.65	1.99	A223	5.48	2.52	2.18	A235	6.72	3.54	1.90				
A210	5.46	2.37	2.30	A224	5.36	2.43	2.20	A236	6.92	3.19	2.17				
A211	5.45	2.45	2.22	A225	5.22	2.42	2.16	A237	7.33	3.50	2.09				
A212	5.10	2.26	2.26	A226	5.29	2.68	1.97	A238	7.06	3.31	2.13				

Variety=Aus; Local Name= Kunail boro; BRRI Accession Number=1714

ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio
A301	5.35	2.94	1.82	A313	5.27	2.61	2.02	A327	5.78	2.93	1.98	A341	6.72	3.73	1.80
A302	5.69	2.98	1.91	A314	5.42	2.71	2.00	A328	5.44	2.78	1.96	A342	6.62	3.69	1.79
A303	5.17	2.63	1.97	A315	5.34	2.86	1.87	A329	5.29	2.89	1.83	Mean	5.79	3.04	1.91
A304	5.89	2.87	2.05	A318	5.42	2.95	1.84	A330	5.29	2.67	1.98	Median	5.53	2.92	1.90
A305	5.14	2.66	1.93	A319	5.63	2.97	1.89	A331	5.40	2.73	1.98	STD	0.63	0.39	0.09
A306	5.58	2.97	1.88	A320	5.32	2.66	2.00	A332	5.35	2.84	1.88	Minimum	5.14	2.61	1.66
A307	5.38	2.92	1.84	A321	5.86	2.74	2.14	A335	6.92	3.60	1.92	Maximum	7.25	3.85	2.14
A308	5.55	2.94	1.89	A322	5.24	2.69	1.95	A336	6.39	3.85	1.66				
A309	5.44	2.87	1.90	A323	5.55	2.88	1.93	A337	6.73	3.62	1.86				
A310	5.80	2.92	1.99	A324	5.37	2.95	1.82	A338	7.18	3.73	1.92				
A311	5.45	2.78	1.96	A325	5.57	2.95	1.89	A339	6.40	3.45	1.85				
A312	5.26	2.86	1.84	A326	5.50	2.91	1.89	A340	7.25	3.46	2.09				

Variety=Indica; Local Name= Tapi/Topa; BRRI Accession Number=62

ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio
I101	5.39	2.08	2.59	I113	4.78	2.11	2.26	I125	5.26	2.01	2.62	I137	6.80	3.19	2.13
I102	5.12	2.00	2.56	I114	4.88	2.20	2.22	I126	5.31	2.22	2.40	I138	7.11	3.23	2.20
I103	5.65	2.08	2.71	I115	4.83	1.98	2.44	I127	4.94	2.19	2.25	Mean	5.63	2.34	2.43
I104	4.85	2.15	2.25	I116	5.42	2.10	2.59	I128	4.74	2.11	2.25	Median	5.32	2.15	2.41
I105	4.78	1.95	2.45	I117	5.16	1.99	2.60	I129	4.90	2.12	2.31	STD	0.84	0.42	0.19
I106	5.64	2.08	2.72	I118	5.69	2.07	2.74	I130	4.88	2.16	2.26	Minimum	4.74	1.95	2.13
I107	5.58	2.07	2.69	I119	5.01	2.19	2.28	I131	7.18	3.33	2.16	Maximum	7.18	3.33	2.77
I108	5.21	2.22	2.34	I120	4.79	1.98	2.42	I132	7.17	3.12	2.30				
I109	5.24	2.14	2.44	I121	5.80	2.10	2.77	I133	6.95	2.98	2.34				
I110	5.20	2.03	2.56	I122	5.58	2.06	2.71	I134	7.08	2.58	2.75				
I111	5.34	2.19	2.43	I123	5.38	2.24	2.40	I135	6.80	2.70	2.52				
I112	4.86	2.23	2.18	I124	5.28	2.15	2.45	I136	7.00	3.05	2.30				

Variety=Indica; Local Name= Dudh sail; BRRI Accession Number=2178

ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio
I201	5.52	1.97	2.80	I213	5.40	2.14	2.53	I226	5.65	2.26	2.50	I238	7.08	2.68	2.64
I202	4.91	2.02	2.42	I214	5.18	2.02	2.56	I227	5.25	2.10	2.50	I239	7.20	2.85	2.53
I203	5.42	2.06	2.63	I215	5.08	2.13	2.39	I228	5.11	2.09	2.45	Mean	5.75	2.24	2.56
I204	5.39	2.04	2.64	I217	5.61	2.05	2.74	I229	5.47	2.16	2.53	Median	5.42	2.08	2.53
I205	5.24	2.10	2.49	I218	4.94	2.06	2.40	I230	5.14	2.00	2.57	STD	0.88	0.31	0.13
I206	4.71	2.01	2.34	I219	5.41	2.07	2.61	I231	5.19	2.05	2.53	Minimum	4.71	1.97	2.30
I207	5.23	2.08	2.51	I220	5.47	2.06	2.65	I232	6.69	2.91	2.30	Maximum	7.59	2.96	2.85
I208	5.44	2.06	2.64	I221	5.28	2.14	2.47	I233	6.97	2.69	2.60				
I209	5.35	2.01	2.67	I222	4.81	2.05	2.35	I234	7.59	2.77	2.74				
I210	5.57	2.26	2.47	I223	5.32	2.12	2.51	I235	7.49	2.62	2.85				
I211	5.14	2.06	2.50	I224	5.54	2.07	2.67	I236	7.48	2.96	2.52				
I212	5.00	2.05	2.44	I225	5.42	2.02	2.68	I237	7.54	2.75	2.74				

Variety=Indica; Local Name= Dudh Shar; BRRI Accession Number=21

ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio
I301	5.99	2.11	2.84	I213	6.62	2.24	2.96	I327	5.81	2.20	2.64	I339	9.06	3.28	2.76
I302	6.57	2.26	2.91	I214	6.64	2.25	2.95	I328	5.69	2.11	2.70	I340	9.00	3.15	2.86
I303	6.69	2.19	3.06	I215	6.93	2.10	3.31	I329	6.70	2.13	3.14	Mean	6.97	2.46	2.86
I304	6.85	2.35	2.92	I218	6.16	2.22	2.78	I330	6.63	2.24	2.96	Median	6.65	2.27	2.90
I305	6.42	2.13	3.01	I219	6.65	2.28	2.92	I331	6.66	2.28	2.93	STD	0.99	0.45	0.22
I306	6.35	2.30	2.77	I220	6.71	2.19	3.06	I332	6.92	2.10	3.30	Minimum	5.66	2.08	2.23
I307	6.54	2.19	2.98	I221	6.88	2.35	2.93	I333	7.20	3.12	2.31	Maximum	9.24	3.67	3.31
I308	6.56	2.27	2.89	I222	6.44	2.15	3.00	I334	8.76	3.20	2.74				
I309	6.20	2.27	2.73	I223	6.39	2.35	2.72	I335	8.16	3.67	2.23				
I310	5.83	2.21	2.64	I224	6.78	2.20	3.08	I336	9.24	3.08	3.00				
I311	5.66	2.08	2.72	I225	6.57	2.32	2.83	I337	8.54	3.20	2.67				
I312	6.62	2.10	3.16	I226	6.36	2.31	2.75	I338	8.76	2.98	2.94				

Variety=Japonica; Local Name= Nenia; BRRI Accession Number=1823

ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio
J101	4.03	2.31	1.74	J113	4.34	2.27	1.91	J127	4.16	2.10	1.98	J139	5.93	2.98	1.99
J102	3.73	2.03	1.84	J114	3.95	2.42	1.64	J128	4.96	2.35	2.11	J140	5.42	3.09	1.76
J103	4.14	2.30	1.80	J115	4.15	2.05	2.03	J129	4.41	2.41	1.83	Mean	4.59	2.45	1.88
J104	4.18	2.24	1.87	J118	4.20	2.31	1.82	J130	4.49	2.33	1.93	Median	4.25	2.30	1.87
J105	4.06	2.17	1.87	J119	3.80	2.07	1.83	J131	4.11	2.42	1.70	STD	0.71	0.40	0.10
J106	4.29	2.21	1.94	J120	4.15	2.35	1.77	J132	4.28	2.05	2.08	Minimum	3.73	2.03	1.64
J107	4.08	2.04	2.00	J121	4.20	2.32	1.81	J133	6.15	3.42	1.80	Maximum	6.15	3.42	2.11
J108	4.10	2.22	1.85	J122	4.16	2.20	1.89	J134	5.71	3.08	1.86				
J109	4.29	2.27	1.89	J123	4.38	2.24	1.96	J135	5.78	3.07	1.88				
J110	4.03	2.12	1.90	J124	4.21	2.06	2.04	J136	5.85	3.01	1.94				
J111	4.50	2.27	1.98	J125	4.22	2.25	1.87	J137	5.98	3.09	1.94				
J112	4.16	2.31	1.80	J126	4.38	2.26	1.94	J138	5.56	3.10	1.79				

Variety=*Japoca*; Local Name= Dad sail; BRRI Accession Number=2178

ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio
J201	4.26	1.95	2.18	J213	3.90	2.01	1.94	J227	5.30	2.46	2.15	J243	5.17	2.42	2.14
J202	3.91	1.95	2.00	J214	3.75	1.99	1.89	J228	5.18	2.47	2.10	J244	5.12	2.57	1.99
J203	3.89	1.92	2.03	J215	3.91	1.95	2.00	J229	5.47	2.47	2.22	Mean	4.76	2.30	2.07
J204	4.10	1.89	2.17	J218	5.21	2.45	2.13	J230	5.20	2.62	1.98	Median	5.12	2.45	2.06
J205	4.15	2.01	2.07	J219	5.20	2.55	2.04	J231	4.97	2.57	1.93	STD	0.65	0.29	0.11
J206	3.92	1.93	2.03	J220	5.16	2.44	2.11	J232	5.29	2.56	2.06	Minimum	3.75	1.79	1.86
J207	4.04	1.79	2.26	J221	5.36	2.33	2.30	J237	5.65	2.51	2.25	Maximum	5.65	2.70	2.30
J208	3.87	1.98	1.96	J222	5.41	2.62	2.07	J238	5.11	2.62	1.95				
J209	3.75	2.01	1.86	J223	5.02	2.49	2.02	J239	5.17	2.46	2.10				
J210	3.96	1.88	2.10	J224	5.29	2.42	2.18	J240	5.34	2.40	2.23				
J211	3.90	1.89	2.06	J225	5.12	2.55	2.01	J241	5.52	2.65	2.09				
J212	4.04	1.92	2.11	J226	5.12	2.62	1.96	J242	5.13	2.49	2.06				

Variety=*Japonica*; Local Name= Nepa; BRRI Accession Number=61

ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio	ID	Length	Width	L/W ratio
J301	5.35	2.73	1.96	J313	5.96	2.81	2.12	J325	7.11	3.73	1.91	J337	8.06	3.57	2.26
J302	5.52	2.61	2.11	J314	5.68	2.81	2.02	J326	7.76	3.54	2.19	J338	7.76	3.67	2.11
J303	5.85	2.79	2.10	J315	5.74	2.63	2.18	J327	7.11	3.36	2.12	Mean	6.83	3.26	2.10
J304	5.57	2.72	2.05	J316	6.97	3.56	1.96	J328	7.91	3.60	2.20	Median	7.16	3.54	2.11
J305	5.50	2.50	2.20	J317	7.21	3.43	2.10	J329	7.48	3.65	2.05	STD	0.93	0.44	0.09
J306	5.56	2.72	2.04	J318	7.63	3.67	2.08	J330	7.59	3.37	2.25	Minimum	5.35	2.50	1.91
J307	6.24	2.77	2.26	J319	7.35	3.60	2.04	J331	6.95	3.58	1.94	Maximum	8.09	3.73	2.26
J308	6.00	2.81	2.14	J320	7.68	3.61	2.13	J332	7.30	3.47	2.10				
J309	5.90	2.79	2.12	J321	7.42	3.60	2.06	J333	7.52	3.69	2.04				
J310	5.53	2.55	2.17	J322	8.09	3.58	2.26	J334	7.25	3.54	2.05				
J311	5.96	2.74	2.18	J323	7.67	3.71	2.07	J335	7.85	3.58	2.19				
J312	5.35	2.62	2.04	J324	7.89	3.66	2.15	J336	7.40	3.60	2.06				

Appendix A_2; List of Morphometrics Measurements of Identified Archaeological Rice Varieties by GMM

Grain ID	Site_ Code	Layer	Length	Width	L/W ratio	Grain ID	Site_Code	Layer	Length	Width	L/W ratio
WB17460101	WB17	1	3.78	2.74	1.38	WB17420203	WB17	2	4.38	2.58	1.70
WB17460102	WB17	1	3.66	2.07	1.77	WB17420204	WB17	2	4.52	2.44	1.85
WB17460103	WB17	1	3.82	2.18	1.75	WB17420205	WB17	2	5.14	2.27	2.27
WB17480101	WB17	1	4.04	2.99	1.35	WB17420206	WB17	2	4.84	2.26	2.14
WB17520101	WB17	1	4.57	2.50	1.83	WB17420207	WB17	2	4.42	1.82	2.43
WB17530101	WB17	1	4.38	2.29	1.92	WB17420208	WB17	2	4.08	2.33	1.75
WB17540101	WB17	1	4.95	2.36	2.10	WB17420209	WB17	2	5.24	2.37	2.22
WB17540102	WB17	1	4.99	2.26	2.21	WB17420210	WB17	2	5.60	2.54	2.20
WB17560101	WB17	1	4.74	2.54	1.86	WB17420211	WB17	2	4.85	2.20	2.21
WB17420201	WB17	2	4.83	2.42	1.99	WB17420212	WB17	2	5.55	2.35	2.36
WB17420202	WB17	2	4.86	2.18	2.23	WB17420213	WB17	2	4.5	2.64	1.70
WB17420214	WB17	2	5.32	2.55	2.09	WB17420225	WB17	2	4.77	2.4	1.99
WB17420215	WB17	2	5.22	2.2	2.37	WB17420226	WB17	2	4.86	2.49	1.95
WB17420216	WB17	2	4.92	2.28	2.16	WB17420227	WB17	2	4.96	2.25	2.20
WB17420217	WB17	2	4.02	2.13	1.89	WB17420228	WB17	2	5.87	2.51	2.34
WB17420218	WB17	2	4.7	2.36	1.99	WB17420229	WB17	2	5.79	2.64	2.19
WB17420219	WB17	2	4.71	2.69	1.75	WB17420230	WB17	2	6.04	2.42	2.50
WB17420220	WB17	2	4.64	3.16	1.47	WB17420231	WB17	2	5.46	2.45	2.23
WB17420221	WB17	2	4.45	2.51	1.77	WB17420232	WB17	2	5.36	2.56	2.09
WB17420222	WB17	2	4.8	2.78	1.73	WB17420233	WB17	2	5.08	2.26	2.25
WB17420223	WB17	2	4.64	2.27	2.04	WB17430201	WB17	2	4.38	2.58	1.70
WB17420224	WB17	2	4.91	2.32	2.12	WB17430202	WB17	2	4.32	1.84	2.35
WB17430203	WB17	2	4.82	3.09	1.56	WB17430204	WB17	2	4.38	2.58	1.70
WB17430205	WB17	2	4.83	2.8	1.73	WB17440201	WB17	2	4.96	2.81	1.76
WB17430206	WB17	2	4.43	2.83	1.57	WB17440202	WB17	2	6.49	2.69	2.41
WB17430207	WB17	2	4.7	3.03	1.55	WB17440203	WB17	2	5.68	2.46	2.30
WB17430208	WB17	2	4.8	2.36	2.03	WB17440204	WB17	2	5.51	2.22	2.49

Grain ID	Site_ Code	Layer	Length	Width	L/W ratio	Grain ID	Site_Code	Layer	Length	Width	L/W ratio
WB17430209	WB17	2	5.29	2.6	2.03	WB17440205	WB17	2	5.24	2.23	2.35
WB17430210	WB17	2	5.61	2.85	1.97	WB17440206	WB17	2	5.2	2.56	2.03
WB17430211	WB17	2	5.4	2.67	2.02	WB17440207	WB17	2	6.59	2.46	2.68
WB17430212	WB17	2	5.3	2.55	2.08	WB17440208	WB17	2	6.66	2.46	2.71
WB17430213	WB17	2	4.85	2.26	2.15	WB17440209	WB17	2	5.1	2.31	2.21
WB17430214	WB17	2	5.01	2.31	2.17	WB17440210	WB17	2	5.19	2.42	2.14
WB17430215	WB17	2	5.4	2.51	2.15	WB17440211	WB17	2	5.4	2.56	2.11
WB17430216	WB17	2	6.89	2.62	2.63	WB17440212	WB17	2	5.42	2.48	2.19
WB17430217	WB17	2	4.85	2.26	2.15	WB17440213	WB17	2	5.35	3	1.78
WB17440214	WB17	2	5.43	2.83	1.92	WB17440227	WB17	2	5.05	2.39	2.11
WB17440215	WB17	2	5.3	2.61	2.03	WB17440228	WB17	2	5.86	2.73	2.15
WB17440216	WB17	2	5.2	2.56	2.03	WB17450201	WB17	2	5.70	3.00	1.90
WB17440217	WB17	2	5.35	2.78	1.92	WB17450202	WB17	2	5.75	2.95	1.95
WB17440218	WB17	2	5.05	2.68	1.88	WB17450203	WB17	2	5.41	2.89	1.87
WB17440219	WB17	2	4.57	2.37	1.93	WB17450204	WB17	2	5.80	2.83	2.05
WB17440220	WB17	2	4.49	2.4	1.87	WB17450205	WB17	2	5.67	2.55	2.23
WB17440221	WB17	2	4.09	2.31	1.77	WB17450206	WB17	2	5.38	2.48	2.17
WB17440222	WB17	2	4.67	2.64	1.77	WB17450207	WB17	2	4.90	2.36	2.08
WB17440223	WB17	2	4.96	3.33	1.49	WB17450208	WB17	2	5.80	2.65	2.19
WB17440224	WB17	2	4.85	2.35	2.06	WB17450209	WB17	2	5.53	2.35	2.35
WB17440225	WB17	2	5.2	2.29	2.27	WB17450210	WB17	2	4.60	3.03	1.52
WB17440226	WB17	2	4.91	2.13	2.31	WB17450211	WB17	2	4.46	2.64	1.69
WB17450212	WB17	2	4.35	2.64	1.65	WB17380306	WB17	3	3.64	1.77	2.06
WB17450213	WB17	2	4.36	2.44	1.79	WB17380307	WB17	3	4.59	2.27	2.02
WB17450214	WB17	2	4.70	2.59	1.82	WB17380308	WB17	3	4.38	2.44	1.80
WB17450215	WB17	1	4.88	2.33	2.10	WB17380309	WB17	3	4.9	2.75	1.78
WB17450216	WB17	2	5.30	2.34	2.27	WB17380310	WB17	3	4.86	2.41	2.02
WB17450217	WB17	1	4.81	2.43	1.98	WB17380311	WB17	3	4.33	2.85	1.52
WB17450218	WB17	2	4.76	2.91	1.63	WB17380312	WB17	3	4.75	2.25	2.11
WB17450219	WB17	2	5.79	2.44	2.37	WB17380313	WB17	3	4.44	2.44	1.82
WB17380301	WB17	3	5.35	2.36	2.27	WB17380314	WB17	3	4.51	2.2	2.05
WB17380302	WB17	3	4.49	2.34	1.92	WB17380315	WB17	3	4.51	2.29	1.97

Grain ID	Site_ Code	Layer	Length	Width	L/W ratio	Grain ID	Site_Code	Layer	Length	Width	L/W ratio
WB17380303	WB17	3	4.37	2.29	1.91	WB17390304	WB17	3	4.21	1.98	2.13
WB17380304	WB17	3	3.68	2.07	1.78	WB17390305	WB17	3	4.64	2.4	1.93
WB17380305	WB17	3	4.27	2.20	1.94	WB17390306	WB17	3	4.83	2.68	1.80
WB17380319	WB17	3	4.82	3.02	1.60	WB17390307	WB17	3	4.97	3.05	1.63
WB17380320	WB17	3	4.53	2.53	1.79	WB17390308	WB17	3	5.45	2.94	1.85
WB17380321	WB17	3	5.16	2.27	2.27	WB17390309	WB17	3	4.08	2.2	1.85
WB17380322	WB17	3	5.02	2.07	2.43	WB17390310	WB17	3	4.35	2.31	1.88
WB17380323	WB17	3	5.43	2.41	2.25	WB17390311	WB17	3	4.48	2.49	1.80
WB17380324	WB17	3	5.26	2.18	2.41	WB17390312	WB17	3	4.53	2.57	1.76
WB17380325	WB17	3	5.44	2.36	2.31	WB17390313	WB17	3	4.66	2.22	2.10
WB17380326	WB17	3	5.13	2.26	2.27	WB17390314	WB17	3	5.11	2.27	2.25
WB17380327	WB17	3	5.64	2.55	2.21	WB17390315	WB17	3	4.73	2.2	2.15
WB17380328	WB17	3	5.03	2.56	1.96	WB17390316	WB17	3	5.7	2.78	2.05
WB17380329	WB17	3	4.87	2.56	1.90	WB17400312	WB17	3	4.69	2.69	1.74
WB17390301	WB17	3	4.72	2.24	2.11	WB17400313	WB17	3	5.06	2.27	2.23
WB17390302	WB17	3	4.69	2.48	1.89	WB17400314	WB17	3	4.93	2.53	1.95
WB17390317	WB17	3	5.68	2.29	2.48	WB17400315	WB17	3	4.37	2.41	1.81
WB17390318	WB17	3	5.21	2.49	2.09	WB17400316	WB17	3	4.6	2.25	2.04
WB17400301	WB17	3	5.12	2.13	2.41	WB17400317	WB17	3	4.26	2.42	1.76
WB17400302	WB17	3	5.04	2.75	1.83	WB17400318	WB17	3	4.49	2.26	1.99
WB17400303	WB17	3	4.95	2.58	1.92	WB17400319	WB17	3	4.51	2.57	1.75
WB17400304	WB17	3	4.94	2.34	2.11	WB17400320	WB17	3	4.67	2.68	1.74
WB17400305	WB17	3	4.06	2.77	1.47	WB17400321	WB17	3	4.75	2.31	2.06
WB17400306	WB17	3	4.61	2.14	2.15	WB17400322	WB17	3	5.07	2.93	1.73
WB17400307	WB17	3	3.85	1.88	2.05	WB17400323	WB17	3	4.31	2.98	1.45
WB17400308	WB17	3	4.92	2.6	1.89	WB17400324	WB17	3	4.67	2.56	1.82
WB17400309	WB17	3	4.32	2.71	1.59	WB17400325	WB17	3	4.78	2.6	1.84
WB17400310	WB17	3				WB17400326	WB17	3	4.59	2.36	1.94
WB17400311	WB17	3	5.13	2.73	1.88	WB17400327	WB17	3	4.61	2.26	2.04
WB17380316	WB17	3	4.29	2.62	1.64	WB17400328	WB17	3	5.32	2.61	2.04
WB17380317	WB17	3	4.77	2.29	2.08	WB17400329	WB17	3	4.44	2.37	1.87

Grain ID	Site_ Code	Layer	Length	Width	L/W ratio	Grain ID	Site_Code	Layer	Length	Width	L/W ratio
WB17380318	WB17	3	4.32	2.47	1.75	WB17410322	WB17	3	4.87	2.38	2.05
WB17400330	WB17	3	4.44	1.91	2.32	WB17410323	WB17	3	4.46	2.51	1.78
WB17400331	WB17	3	4.42	2.29	1.93	WB17410324	WB17	3	4.43	2.7	1.64
WB17400332	WB17	3	5.58	2.53	2.21	WB17410325	WB17	3	4.41	2.28	1.93
WB17400333	WB17	3	5.06	2.2	2.30	WB17410326	WB17	3	5.25	2.62	2.00
WB17400334	WB17	3	4.73	2.43	1.95	WB17410327	WB17	3	4.41	2.7	1.63
WB17400335	WB17	3	4.9	2.34	2.09	WB17410328	WB17	3	4.85	2.26	2.15
WB17400336	WB17	3	4.39	2.51	1.75	WB17410329	WB17	3	4.24	2.07	2.05
WB17410301	WB17	3	4.20	1.90	2.21	WB17410330	WB17	3	4.26	2.48	1.72
WB17410302	WB17	3	4.55	2.29	1.98	WB17410331	WB17	3	4.29	2.44	1.76
WB17410303	WB17	3	4.65	2.20	2.11	WB17410332	WB17	3	4.31	2.59	1.66
WB17410304	WB17	3	4.75	2.22	2.14	WB17410333	WB17	3	4.72	2.15	2.20
WB17410305	WB17	3	4.39	2.11	2.08	WB17410334	WB17	3	6.11	2.42	2.52
WB17410306	WB17	3	4.19	2.17	1.94	WB17340401	WB17	4	4.62	2.52	1.83
WB17410307	WB17	3	4.04	2.04	1.98	WB17340402	WB17	4	3.70	1.90	1.95
WB17410308	WB17	3	4.16	2.16	1.93	WB17340403	WB17	4	5.56	2.96	1.88
WB17410309	WB17	3	5.66	2.74	2.07	WB17340404	WB17	4	5.21	2.25	2.31
WB17410310	WB17	3	4.84	2.75	1.76	WB17340405	WB17	4	6.23	2.24	2.78
WB17410311	WB17	3	5.73	2.95	1.94	WB17340406	WB17	4	4.76	2.34	2.03
WB17410312	WB17	3	5.21	2.94	1.77	WB17340407	WB17	4	4.50	2.52	1.79
WB17410313	WB17	3	4.24	2.27	1.87	WB17340408	WB17	4	4.60	2.14	2.15
WB17410314	WB17	3	4.83	2.48	1.95	WB17340409	WB17	4	4.60	2.45	1.88
WB17410315	WB17	3	5.15	2.58	2.00	WB17340410	WB17	4	4.71	1.86	2.54
WB17410316	WB17	3	4.51	2.71	1.66	WB17340411	WB17	4	4.04	2.27	1.78
WB17410317	WB17	3	4.85	2.52	1.92	WB17340412	WB17	4	4.24	2.22	1.91
WB17410318	WB17	3	5.09	2.36	2.16	WB17340413	WB17	4	3.98	2.79	1.43
WB17410319	WB17	3	4.6	2.51	1.83	WB17340414	WB17	4	4.26	2.31	1.84
WB17410320	WB17	3	5.11	2.38	2.15	WB17340415	WB17	4	4.60	2.25	2.04
WB17410321	WB17	3	4.33	2.13	2.03	WB17340416	WB17	4	4.59	2.09	2.19
WB17400330	WB17	3	4.44	1.91	2.32	WB17340417	WB17	4	4.48	2.26	1.98

Grain ID	Site_Code	Layer	Length	Width	L/W ratio	Grain ID	Site_Code	Layer	Length	Width	L/W ratio
WB17340418	WB17	4	4.30	1.84	2.33	WB17350413	WB17	4	4.06	1.83	2.22
WB17340419	WB17	4	4.43	2.25	1.97	WB17350414	WB17	4	4	2.09	1.91
WB17340420	WB17	4	4.53	2.29	1.98	WB17360401	WB17	4	4.84	2.87	1.68
WB17340421	WB17	4	5.34	2.73	1.95	WB17360402	WB17	4	5.40	2.30	2.35
WB17340422	WB17	4	5.60	3.31	1.69	WB17360403	WB17	4	4.45	2.14	2.07
WB17340423	WB17	4	6.21	3.19	1.95	WB17360404	WB17	4	5.03	2.43	2.07
WB17340424	WB17	4	5.16	2.20	2.35	WB17360405	WB17	4	4.45	2.13	2.09
WB17340425	WB17	4	4.02	2.23	1.80	WB17360406	WB17	4	3.82	2.17	1.76
WB17340426	WB17	4	3.93	1.86	2.12	WB17360407	WB17	4	3.66	2.14	1.71
WB17340427	WB17	4	4.07	1.95	2.09	WB17360408	WB17	4	3.74	2.18	1.72
WB17340428	WB17	4	3.77	2.18	1.73	WB17360409	WB17	4	4.94	2.95	1.67
WB17340429	WB17	4	3.90	2.35	1.66	WB17360410	WB17	4	4.29	2.15	2.00
WB17340430	WB17	4	4.67	2.77	1.69	WB17360411	WB17	4	4.57	2.18	2.10
WB17340431	WB17	4	4.32	1.96	2.20	WB17360412	WB17	4	4.42	2.24	1.97
WB17340432	WB17	4	3.86	1.96	1.96	WB17360413	WB17	4	5.06	2.88	1.76
WB17340433	WB17	4	5.01	2.68	1.87	WB17360414	WB17	4	4.76	2.6	1.83
WB17340434	WB17	4	4.24	2.27	1.87	WB17360415	WB17	4	4.33	2.18	1.99
WB17350401	WB17	4	4.81	2.45	1.96	WB17360416	WB17	4	4.46	2.5	1.78
WB17350402	WB17	4	4.49	2.92	1.54	WB17360417	WB17	4	4.33	2.11	2.05
WB17350403	WB17	4	4.54	2.88	1.58	WB17360418	WB17	4	4.63	2.22	2.09
WB17350404	WB17	4	4.26	2.55	1.67	WB17360419	WB17	4	4.64	2.16	2.15
WB17350405	WB17	4	4.84	2.40	2.02	WB17370401	WB17	4	5.41	2.34	2.31
WB17350406	WB17	4	4.13	2.49	1.66	WB17370402	WB17	4	4.96	2.60	1.91
WB17350407	WB17	4	4.47	2.05	2.17	WB17370403	WB17	4	4.81	2.76	1.74
WB17350408	WB17	4	4.33	2.22	1.95	WB17370404	WB17	4	4.22	2.41	1.75
WB17350409	WB17	4	4.75	2.23	2.13	WB17370405	WB17	4	4.04	1.88	2.16
WB17350410	WB17	4	4.41	2.09	2.11	WB17370406	WB17	4	4.07	1.98	2.05
WB17350411	WB17	4	4.16	2.33	1.79	WB17370407	WB17	4	3.43	1.80	1.90
WB17350412	WB17	4	4.15	2.05	2.02	WB17370408	WB17	4	5.89	2.9	2.03

Grain ID	Site_Code	Layer	Length	Width	L/W ratio	Grain ID	Site_Code	Layer	Length	Width	L/W ratio
WB17370409	WB17	4	4.37	2.36	1.85	WB16060211	WB16	2	4.74	2.47	1.92
WB17370410	WB17	4	4.92	2.32	2.12	WB16070201	WB16	2	5.10	2.31	2.21
WB17370411	WB17	4	4.13	2.26	1.83	WB16070202	WB16	2	4.57	2.41	1.89
WB17370412	WB17	4	4.55	2.09	2.18	WB16070203	WB16	2	4.25	2.81	1.51
WB17370413	WB17	4	4.75	2.46	1.93	WB16070204	WB16	2	4.78	2.52	1.89
WB17370414	WB17	4	4.31	2.71	1.59	WB16070205	WB16	2	4.53	2.41	1.88
WB17370415	WB17	4	3.91	2.29	1.71	WB16070206	WB16	2	2.27	1.21	1.88
WB16010101	WB16	1	4.05	2.18	1.86	WB16070207	WB16	2	4.74	2.47	1.92
WB16010102	WB16	1	3.73	1.73	2.16	WB16070208	WB16	2	4.66	2.02	2.31
WB16010103	WB16	1	4.46	2.51	1.78	WB16070209	WB16	2	4.23	2.09	2.03
WB16020101	WB16	1	4.49	2.45	1.83	WB16070210	WB16	2	3.73	2.43	1.53
WB16030101	WB16	1	5.31	2.88	1.84	WB16080201	WB16	2	3.56	1.78	2.00
WB16030102	WB16	1	4.65	2.40	1.94	WB16090201	WB16	2	4.70	2.33	2.01
WB16030104	WB16	1	4.33	2.99	1.45	WB16090202	WB16	2	4.85	2.24	2.17
WB16030105	WB16	1	4.93	1.86	2.66	WB16090203	WB16	2	4.47	1.82	2.46
WB16030106	WB16	1	3.76	1.91	1.97	WB16090204	WB16	2	4.73	2.25	2.10
WB16040101	WB16	1	4.29	2.72	1.58	WB16090205	WB16	2	5.61	2.25	2.49
WB16040102	WB16	1	5.24	2.18	2.41	WB16090206	WB16	2	4.30	2.20	1.96
WB16040103	WB16	1	4.63	2.36	1.96	WB16090207	WB16	2	4.02	2.68	1.50
WB16040104	WB16	1	4.84	2.52	1.92	WB16090208	WB16	2	4.19	2.51	1.67
WB16040105	WB16	1	4.83	2.13	2.27	WB16100201	WB16	2	4.98	2.72	1.83
WB16040106	WB16	2	5.45	2.63	2.07	WB16100202	WB16	2	4.79	2.00	2.39
WB16040107	WB16	2	4.07	2.22	1.84	WB16100203	WB16	2	4.41	2.42	1.82
WB16040108	WB16	2	4.42	2.61	1.69	WB16110301	WB16	3	4.67	2.36	1.98
WB16040109	WB16	2	4.72	2.41	1.96	WB16110302	WB16	3	5.09	2.70	1.88
WB16040110	WB16	2	4.25	2.47	1.72	WB16110303	WB16	3	4.96	2.27	2.18
WB16040111	WB16	2	4.10	2.45	1.67	WB16110304	WB16	3	4.58	2.02	2.27
WB16060202	WB16	2	4.41	2.31	1.91	WB16110305	WB16	3	5.13	2.38	2.15
WB16060203	WB16	2	4.69	2.04	2.30	WB16110306	WB16	3	3.93	2.33	1.69
WB16060205	WB16	2	3.62	1.82	1.99	WB16110307	WB16	3	4.47	2.55	1.76

Grain ID	Site_ Code	Layer	Length	Width	L/W ratio	Grain ID	Site_Code	Layer	Length	Width	L/W ratio
WB16110308	WB16	3	4.40	1.87	2.35	WB16170401	WB16	4	4.65	2.09	2.22
WB16110309	WB16	3	4.20	2.46	1.71	WB16170402	WB16	4	5.19	2.11	2.46
WB16120301	WB16	3	4.52	2.48	1.82	WB16170403	WB16	4	4.89	2.22	2.21
WB16120302	WB16	3	4.86	2.47	1.96	WB16170404	WB16	4	5.96	2.51	2.38
WB16120303	WB16	3	5.37	2.55	2.11	WB16170405	WB16	4	4.75	2.56	1.85
WB16120304	WB16	3	4.35	2.33	1.87	WB16170406	WB16	4	4.29	2.95	1.46
WB16120305	WB16	3	4.29	2.22	1.93	WB16170407	WB16	4	5.14	3.08	1.67
WB16120306	WB16	3	5.34	2.63	2.03	WB16170408	WB16	4	4.70	2.37	1.98
WB16120307	WB16	3	4.42	2.51	1.76	WB16170409	WB16	4	5.33	2.55	2.10
WB16120308	WB16	3	5.43	2.22	2.45	WB16170410	WB16	4	4.99	2.23	2.24
WB16120309	WB16	3	5.04	2.33	2.16	WB16170411	WB16	4	5.01	2.79	1.79
WB16120310	WB16	3	4.57	2.29	1.99	WB16170412	WB16	4	4.53	2.13	2.13
WB16120311	WB16	3	4.23	2.04	2.08	WB16170413	WB16	4	3.98	2.82	1.41
WB16120312	WB16	3	4.80	2.49	1.93	WB16180401	WB16	4	5.08	2.54	2.00
WB16120313	WB16	3	4.00	2.56	1.56	WB16180402	WB16	4	5.26	2.36	2.23
WB16120314	WB16	3	4.56	2.55	1.79	WB16180403	WB16	4	4.82	2.18	2.21
WB16130301	WB16	3	5.34	2.67	2.00	WB16180404	WB16	4	3.87	2.51	1.54
WB16130302	WB16	3	4.33	2.06	2.11	WB16180405	WB16	4	4.81	2.22	2.17
WB16130303	WB16	3	4.76	1.86	2.56	WB16180406	WB16	4	4.75	2.40	1.98
WB16150401	WB16	4	4.18	2.64	1.58	WB16180407	WB16	4	3.99	2.13	1.87
WB16150402	WB16	4	4.63	2.52	1.84	WB16180408	WB16	4	4.33	2.52	1.71
WB16150403	WB16	4	3.93	2.87	1.37	WB16190401	WB16	4	5.34	2.51	2.13
WB16160401	WB16	4	4.84	2.47	1.96	WB16190402	WB16	4	5.24	2.40	2.18
WB16160402	WB16	4	4.93	2.61	1.89	WB16190403	WB16	4	4.95	2.58	1.92
WB16160403	WB16	4	4.80	2.36	2.04	WB16190404	WB16	4	5.47	2.91	1.88
WB16160404	WB16	4	4.13	2.47	1.67	WB16190405	WB16	4	5.11	2.82	1.81
WB16160405	WB16	4	4.96	2.40	2.07	WB16190406	WB16	4	4.44	2.22	2.00
WB16160406	WB16	4	4.65	2.09	2.22	WB16190407	WB16	4	4.44	2.11	2.10
WB16160407	WB16	4	4.43	2.35	1.88	WB16200501	WB16	5	4.46	2.59	1.72
WB16160408	WB16	4	4.20	2.32	1.81	WB16210501	WB16	5	4.34	2.27	1.91

Grain ID	Site_Code	Layer	Length	Width	L/W ratio	Grain ID	Site_Code	Layer	Length	Width	L/W ratio
WB16210502	WB16	5	4.49	2.30	1.95						
WB16210503	WB16	5	4.74	2.36	2.01						
WB16210504	WB16	5	4.88	2.24	2.18						
WB16210505	WB16	5	4.44	2.19	2.03						
WB16210506	WB16	5	5.05	2.11	2.39						
WB16210507	WB16	5	4.51	2.26	2.00						
WB16210508	WB16	5	4.16	2.43	1.71						
WB16210509	WB16	5	4.29	2.38	1.80						
WB16220501	WB16	5	5.06	2.68	1.89						
WB16230501	WB16	5	4.36	2.25	1.94						

Paper 3: New Archaeobotanical Evidence of Rice Domestication Processes at Wari-Bateshwar in the Lower Brahmaputra Valley, Bangladesh

Abstract

This study explores rice domestication status through spikelet base morphology at the 1st millennium BCE site of Wari-Bateshwar in the Lower Brahmaputra Valley, currently the earliest macrobotanical rice assemblage in Bangladesh. The morphology of modern rice populations (wild and domesticated local landraces) from Bangladesh is also examined, and a new ‘intermediate’ category is defined. The archaeobotanical analysis reveals a diachronic increase in the presence of non-shattering spikelet bases through the stratigraphic sequence at Wari-Bateshwar, accompanied by the occurrence of *aus*, *japonica* and *indica*-type rice grains. These findings contribute to the broader discussion of Asian rice domestication.

3.1. Introduction

Rice is one of the world’s key staple cereal crops, sustaining over one-third of the global population. The earliest documented evidence of rice cultivation in Bangladesh dates to the first millennium BCE, as observed at Wari-Bateshwar (Paper 2). Cultivation of *japonica* rice extends back to *c*. 9000 BCE in China (Zhao 1998; Liu *et al*. 2017; Zhang *et al.* 2024), with morphological domestication by 5000-4000 BCE (Fuller *et al*. 2007; Deng *et al*. 2015). Early rice cultivation in India dates back as early as the 7th millennium BCE (Tewari *et al.* 2006), with domesticated *indica* rice by *c*. 1800-1500 BCE (Fuller *et al*. 2010; Fuller 2011; Bates *et al.* 2017; Bates 2022). *Japonica* reached Southeast Asia by c. 2000 BCE (Fuller *et al.* 2010; Castillo 2011, Silva *et al.* 2015), while *indica* arrived by the end of the 1st millennium BCE (Castillo et al. 2016).

The earliest documented timeframe for rice cultivation in early Bangladesh is between 800 and 400 cal BCE in the Lower Brahmaputra Valley (Paper 2). The presence of well-preserved grains of all three Asian rice varieties, *japonica*, *indica* and *aus*, from the beginning of the sequence at Wari-Bateshwar (WB) sheds new light on the development and spread of rice agriculture in early Bangladesh. In particular, the identification of *aus*-type rice provides a basis for further investigation into this variety's origins and the possibility of local domestication processes.

The origin, domestication and dispersal of Asian rice remain highly debated topics in genetics and archaeobotany. Key questions persist about when, how and through which processes rice evolved, how many domestication events occurred, and the geographical regions concerned. Over the past two decades, the discovery of more archaeobotanical and archaeological evidence across broader geographical areas, coupled with advances in methodological approaches in genetics and archaeobotany, has enabled researchers to construct a more complex narrative of the evolutionary processes of rice domestication, its origins and dispersal (for genetics, see Huang *et al*. 2012; Civáň *et al.* 2015; Choi & Purugganan 2018; Gutakar *et al*. 2020; for archaeobotany, see Crawford & Shen 1998; Zhao *et al*. 1998; Fuller *et al*. 2009; 2016; Castillo *et al*. 2016; Zhang *et al*. 2024).

Domestication is widely understood as a protracted co-evolutionary process involving both plant and animal populations through human intervention, marked by archaeologically traceable phenotypic changes between wild and domesticated forms (Diamond 2002; Purugganan 2019; 2022; Bogaard *et al.* 2021). These phenotypic or morphological markers include seed size, shape, loss of seed shattering and disappearance of awns. Human behavioural changes are also associated with domestication, including cultivation and management practices, potentially evidenced through preserved field systems, water management infrastructure and changes in plant-related artifacts (Fuller & Qin 2009). More broadly, domestication has been viewed as a landscape-level process, suggesting that the origins of domesticated plants involved large populations over extensive areas rather than being localised events (Jones *et al.* 2021; Allaby *et al.* 2022).

Rice domestication involved significant changes expressed genetically and phenotypically (Konishi *et al.* 2006; Li *et al*. 2006). Wild and domesticated rice within an archaeological context can be distinguished through the morphology of chaff, specifically the base of the rice spikelet and its features (Thompson 1996, Fuller *et al.* 2009). Comparable techniques are applied to other cereal crops such as wheat and barley (Tanno & Willcox 2006; 2012; Zohary *et al.* 2012). Fuller *et al.* (2009) further refined the understanding of rice spikelet base morphology by integrating ethnographic studies with archaeological evidence from the Lower Yangtze region, identifying distinct non-shattering domestication traits. Zheng *et al.* (2016) added another dimension, suggesting that during the protracted evolutionary process, spikelet base morphology did not transition directly from wild to domesticated forms. Instead, they showed the existence of an 'intermediate' form, perhaps marking a transitional phase in the domestication process. This observation underscores the need for further ethnographic studies to validate and identify such 'intermediate' spikelet base forms in archaeological contexts.

Distinct genotypes or varieties of Asian rice – *aus, japonica,* and *indica* – have been delineated through Geometric Morphometric Methods (GMM), as discussed in Paper 2. At the site of Wari-Bateshwar in Bangladesh, all three varieties have been identified, with *aus, indica* and *japonica* types recovered from the earliest stratigraphic layers onwards. The consistent presence of *aus*-type rice across successive layers at Wari-Bateshwar suggests two potential interpretations: firstly, *aus* rice may have been domesticated locally, and secondly, it might have been introduced to the site from elsewhere. To test these alternative scenarios, analysis of spikelet base morphology within the same stratigraphic sequence is necessary to discern whether the chaff represents a local domestication or dispersal. Moreover, the scenario of local domestication relates to wider discussions of Asian rice origins.

There are two predominant contrasting narratives regarding the domestication of Asian rice. The first, known as the single origin hypothesis, posits that Asian rice was domesticated once in the Lower Yangtze River valley of South China through a de novo domestication process, suggesting a common wild ancestor, *Oryza sativa rufipogon*, for both *japonica* and *indica* (Vaughan *et al.* 2008b; Molina *et al*. 2011).

In contrast, the second narrative, supported by molecular studies, points to significant genetic divergences among cultivated rice varieties throughout Asia. This evidence suggests that at least three major genetic groups emerged from separate geographical domestication events, a perspective defined as the multiple domestication hypothesis (Glaszmann 1987, Cheng *et al.* 2003; Vitte *et al.* 2004; Garris *et al.* 2005; Londo *et al.* 2006; Gross & Zhao 2014; Schatz *et al.* 2014; Travis *et al.* 2015).

A further debate has emerged within the multiple domestication hypothesis regarding whether the domestication process occurred through introgression (multiple domestication by introgression) or via multiple independent de novo events. The introgressive hybridisation model posits that japonica rice developed through a de novo domestication process, while indica and aus were domesticated through genetic adaptations transferred via introgression from japonica, each originating from separate ancestral gene pools (Li *et al*. 1997; Harushima *et al.* 2002; Huang *et al*. 2012; Choi *et al.* 2017; Choi & Purugganan 2018). Complicating this narrative, whole-genome sequencing of wild and cultivated rice has revealed that specific domestication genes—such as *rc*, *prog1*, and *sh4*—are more prevalent in wild accessions than cultivated varieties. This suggests that these alleles existed before domestication and raises the possibility that certain genes in domesticated rice were directly inherited from wild progenitors rather than from previously domesticated rice (Civáň & Brown 2017). Consequently, this finding supports a hypothesis of multiple independent (de novo) domestication events in Asia (Civáň & Brown 2017; 2018).

Additionally, Civáň *et al.* (2015) pointed to Bangladesh as a potential candidate for *aus* rice domestication. Successful separation of the archaeological evidence of three Asian rice varieties, *japonica*, *indica*, and *aus*, using ethnographic and GMM techniques (paper 2) following a controlled rice charring experiment (Paper 1), frames further investigation of rice evolution in South Asia. Thus, archaeobotanical remains, such as rice spikelet bases and identified rice varieties, should be considered impactful, as outlined in Table 1, to unravel these puzzling rice domestication scenarios in Asia, particularly in early Bangladesh.

Fuller (2011) proposed the 'proto-*indica* hypothesis,' which suggested that *indica* rice domestication resulted from the introgression of locally managed wild rice (*O. sativa nivara*) with introduced *japonica* followed by long pre-domestication rice cultivation. This model could parallel the proposed mechanism for the emergence of the *aus* variety, as suggested by evidence for gene flow from *japonica* to other rice genotypes (Huang & Han 2016; Choi & Purugganan 2018).

The *aus* variety, known for its high genetic diversity, is resilient to drought and flooding. It thrives in various conditions, from fully irrigated to rainfed upland dry ecologies and exhibits photoperiod sensitivity and early maturity (Glaszmann 1987; Garris *et al*. 2005; Bin Rahman & Zhang 2016). Early farmers may have selectively cultivated local wild-like feral or weedy types of rice, favouring variants with desirable traits such as ecological adaptability, increased seed size and higher yield (Fuller *et al.* 2010; Fuller 2011). This type of selection led to a local wild type, a regional variant of *O. sativa nivara* (Huang *et al.* 2012; Civáň *et al.* 2015; Choi *et al*. 2017) that could have been morphologically intermediate type. These intermediate forms, observed in South and Southeast Asia, are characterised by improved yield and larger seed sizes compared to typical wild varieties (Sharma and Shastry 1965; Morishima *et al.* 1980; Sano *et al*. 1980; Ng *et al*. 1981; Oka 1988; Vaughan *et al*. 2008a).

Furthermore, these intermediate types could have served as immediate progenitors for domesticated *aus*, potentially receiving domestication genes from introduced *japonica* and *indica*. This proto-*aus* hypothesis is adapted here following the proto-*indica* hypothesis from Fuller (2011). Thus, the domestication pathways for aus rice could be followed by multiple domestication by introgression (Choi *et al.* 2017; Choi and Purugganan 2018) or multiple independent (de novo) domestication models (Civáň *et al.* 2015).

Table 8: Three models of Asian rice domestication together with archaeobotanical evidence expected in relation to the origins of *aus* rice.

Hypothesis/Model	Grain ID	Spikelet base evidence
Single domestication (Vaughan 2008b; Molina et al. 2011)	Grain types other than japonica expected to emerge without associated evidence for loss of shattering	Reduced shattering through time expected only in association with japonica
Multiple domestication (single de novo domestication but multiple local introgressive hybridisation processes) (Huang et al. 2012; Huang & Han 2016, Choi et al. 2017; Choi & Purugganan 2018)	japonica, indica and aus expected to be present	Reduced shattering through time in areas of origin
Multiple de novo domestications (Civáň, et al. 2015, Civáň, and Brown 2017; 2018)	japonica and indica not necessarily present; aus present, potentially on its own	Reduced shattering through time.

The archaeological evidence that elucidates the loss of shattering and identification of specific rice varieties is crucial in clarifying this complexity. Thus far, abundant archaeological rice spikelet base evidence has only been available to inform the domestication process of *japonica* (Fuller *et al.* 2009; Zheng *et al.* 2016). Initial evidence relevant to *indica* has been reported from Masudpur in Northwest India (Bates *et al.* 2017), inferring pre-domestication cultivation or proto-*indica* cultivation. The recovery of unambiguous evidence of archaeological rice spikelet bases documenting a transition from shattering to non-shattering is as yet lacking for *indica* and *aus*. Such archaeological evidence would play a pivotal role in determining which model of rice origins is most plausible, as summarised in Table 1.

This study integrates archaeological evidence of rice spikelet bases and wild-type rice grains with the results of GMM-based identification of associated rice grains from a previous study (Paper 2). The aim is to reassess the model for the origin and domestication of Asian rice in early Bangladesh, particularly the *aus* variety. The study has two specific objectives:

1. to refine morphological criteria for differentiating wild- and domestic-type spikelet bases based on modern collections, and
2. to apply these criteria in the identification of rice spikelet base types at WB in order to draw out implications for the current debate over single versus multiple rice origins.

3.2. Regional and Local Contexts

Situated in the Bengal basin, Bangladesh possesses unique geophysical features. The country is delineated by elevated landforms, encompassing the Himalayas to the north, the Indo-Burma range to the east, and the Chotonagpur plateau to the west. In the central region of the basin, a vast GBM delta is formed by the confluence of the Ganges, Brahmaputra, and Meghna rivers.

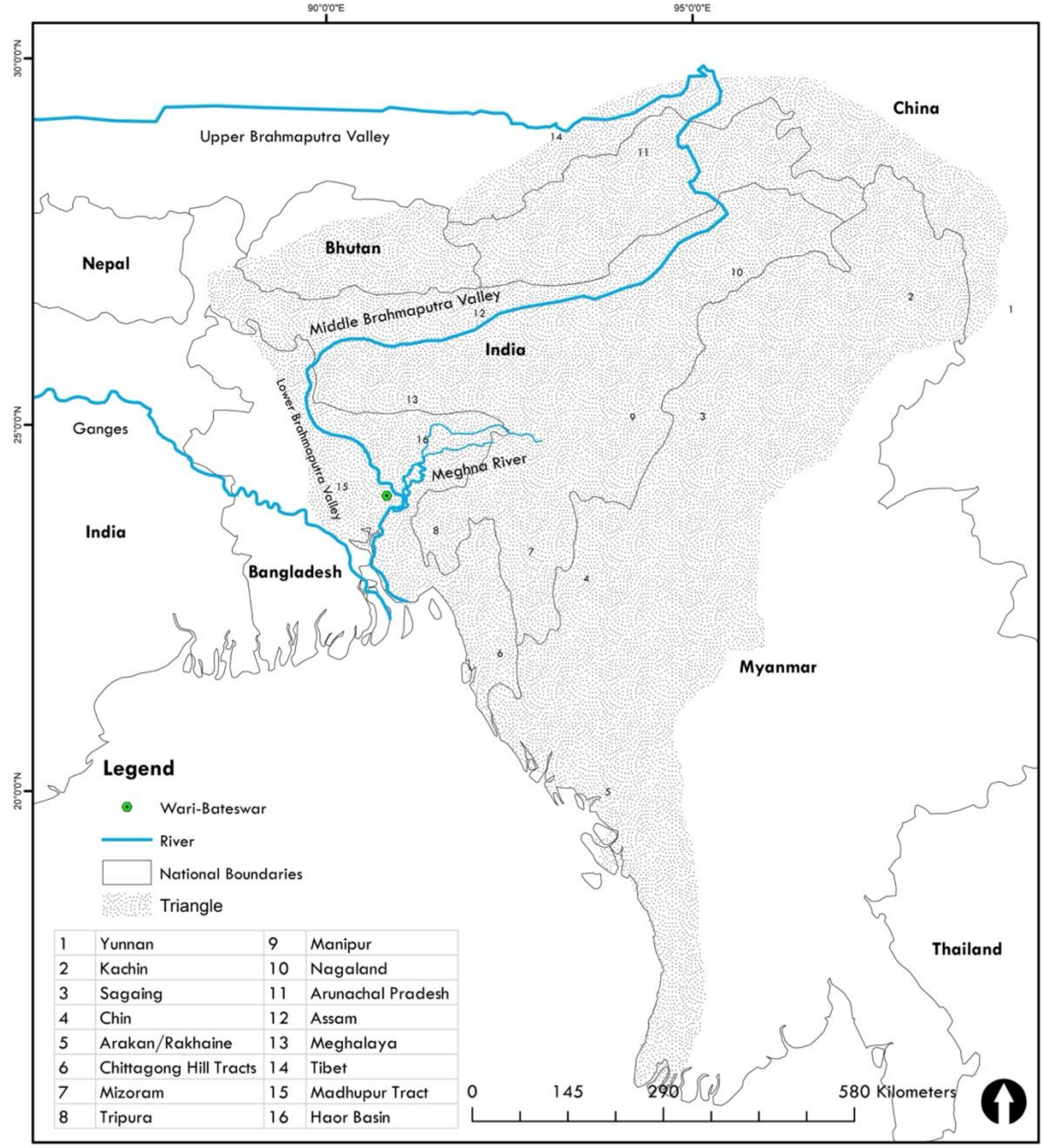


Figure 1: The map shows the location of Wari-Bateshwar within the 'Geographical Triangle' mentioned by Pachuau & Schendel (2022; 1-3), which encompasses diverse hot spots of biodiversity and ecological settings. This map showed the river courses originally drawn by James Renell (1782) and shows the site stands in the Lower Brahmaputra Valley. Additionally, the map also demonstrates the frontiers' location of Bangladesh between South, Southeast Asia and China.

A part of this area also comprises the Eastern Himalayan Triangle (EHT) (Pachuau & Schendel 2022), encompassing lowland floodplains, the Haor Basin wetland, and the Madhupur uplands, which sustain a diverse ecological composition (Islam *et al.* 2013; Saha *et al.* 2021) (See Fig. 1). The triangular region aligns with the Far Eastern Himalayan Landscape (FHL), where three biodiversity hotspots converge – the Himalayas, the Indo-Burma, and the mountain of Southeast China. This area is crucial for harbouring a diverse range of plant and animal species (Sharma 2005; ICIMOD 2018; Sharma & Sharma 2019*)*.

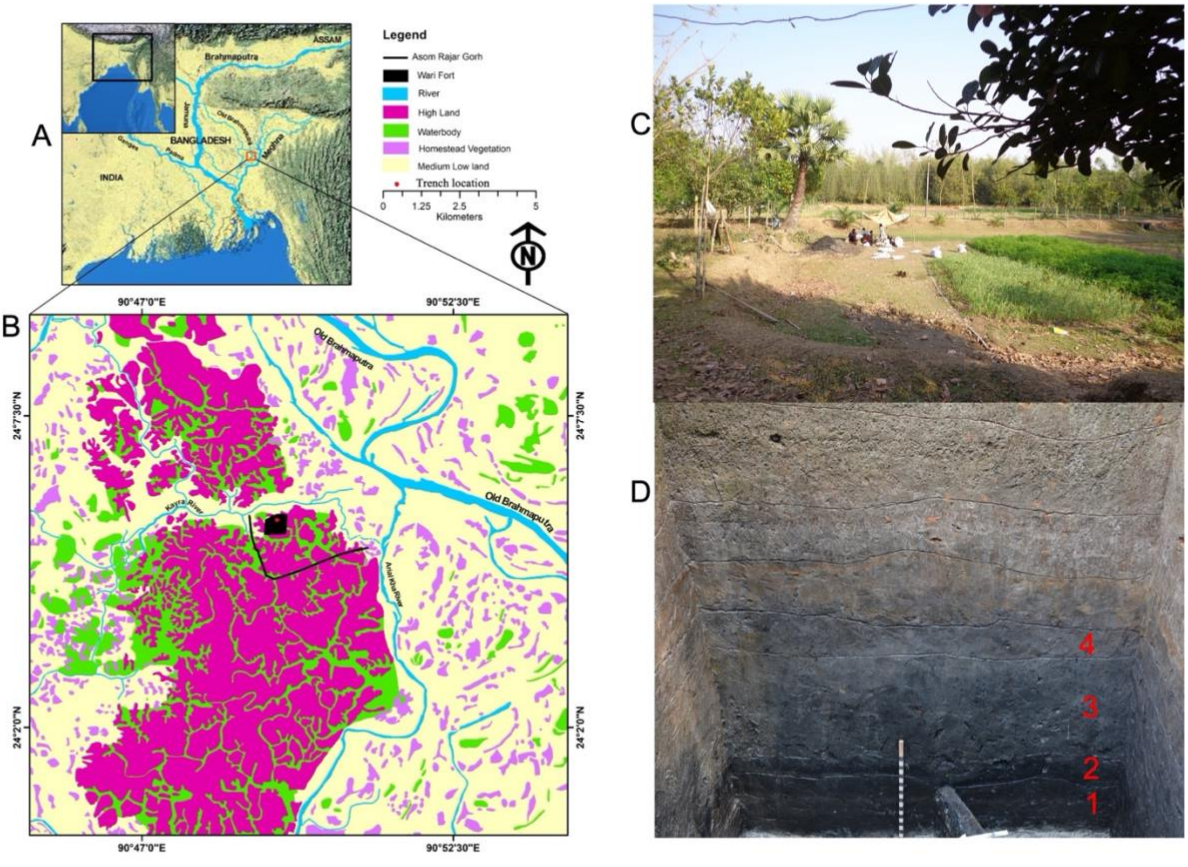


Figure 2: 2A denotes the location of Bangladesh in the frontiers of South and Southeast Asia and the site located in the heart of the Ganges-Brahmaputra-Meghna (GBM) delta. A & B shows the location of the site Wari-Bateshwar and trench WB17. Figure 2B exhibits the Wari Fort along with the outer fort of Asom Rajar Garh, located on and between the diverse landscapes beside the Brahmaputra (now it is called ‘Old Brahmaputra’ due to the shift of the course by the 17th-18th century. Figure 2C shows the general view of the site, and 2D denotes the stratigraphy of the trench from which samples were collected for this study.

The wetlands in this region play a vital role in supporting biodiversity, including freshwater fish, phytoplankton, and other aquatic organisms (Bhuiyan *et al.* 2020; Hasan 2023). Notably, the Haor Basin is renowned for its abundant waterfowl and plant population, highlighting its ecological significance (Parvin & Akteruzzaman 2013).

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The dynamic geomorphological processes of the Brahmaputra River shape the biodiversity of the region. Constant sediment deposition and delta formation result in a dynamic, braided river landscape (Pickering *et al.* 2014; Goodbred *et al.* 2014; Schneider *et al.* 2017). Furthermore, the monsoon season brings heavy rainfall, creating a predominantly humid environment that supports diverse vegetation, including deciduous trees like *gewa* and *sal*. This distinctive environment is particularly prominent in the Madhupur tract (Bookhagen and Burbank 2010; Kumar *et al.* 2022). The Brahmaputra Valley is recognised for its significant biodiversity and distinctive ecological niche. It encompasses a wide array of wild and cultivated species within its floodplain lakes, wetlands, uplands, and forest fragments.

Wari and Bateshwar, two contiguous villages, together form the archaeological site known as Wari-Bateshwar (WB). This site features a mud rampart fortified area spanning 0.5 square kilometres (Fig. 2). Archaeological surveys have identified 52 early settlements within a 25 square kilometres radius surrounding this fortification, the origins of which remain unstudied. Just north of the site, the now-dry Old Brahmaputra riverbed situates this region within the lower Brahmaputra valley.

The site lies on the fringed landscape of the Madhupur tract, a late Pleistocene terrace approximately 7 meters above mean sea level and 2-3 meters above the adjacent floodplain. This terrain features a mixture of broadly dissected lowlands—including perennial and seasonal lakes, bogs, ditches, and canals—medium uplands used for vegetation and current settlements and expansive adjacent river floodplains. The terrace's elevation prevents inundation, allowing habitation, while the floodplain below supports intensified livelihood activities.

Recognised as an early centre of rice cultivation, Wari-Bateshwar saw the cultivation of all three Asian rice varieties by the mid-1st millennium BCE (Paper 2), refining earlier archaeobotanical studies that documented *japonica* rice (Rahman *et al.* 2020). The site has also yielded a diverse array of pulses, millets, and weeds (Rahman *et al.* 2020). Additionally, findings include polished axes, silver punch-marked coins, pottery, glass and stone beads, amulets and pendants, terracotta tile fragments, weights, bronze bangles, a high-tin bronze bowl, a bronze horse, an iron knife and hand axe (Rahman *et al.* 2012), marking it as a significant early historic (Iron Age) 400-300 BCE, site in South Asia and suggesting its role as both a trading centre and river port.

Excavations at WB since 2000 have primarily focused on identifying cultural materials and interpreting them in relation to local and regional cultural development. The initial archaeobotanical study was conducted in trench WB09 (Rahman *et al.* 2020), with subsequent samples collected from the excavation of trenches WB14, WB16, and WB17, the latter of which is the focus of this paper due to considerable findings of rice spikelet bases and charred rice. The trench WB17, known locally as 'Tohar Khet' (Toha's cultivation land), was excavated in 2017 by Mahabub-ul Alam on behalf of the Government Department of Archaeology. Located within the mud fort, approximately 315 m south of the northern wall and 155 m west of the east wall (Fig.2), it reached a depth of 4.1 m, revealing 13 cultural layers. This paper discusses the earliest four layers, which contained thick black sediment; the very first layer (L-1) featured only charred archaeobotanical remains in an aceramic context, while the subsequent layers contained various artifacts and plant materials, suggesting possible use as a waterlogged context with midden disposal. The archaeobotanical remains were found charred rather than uncharred waterlogged preservation.

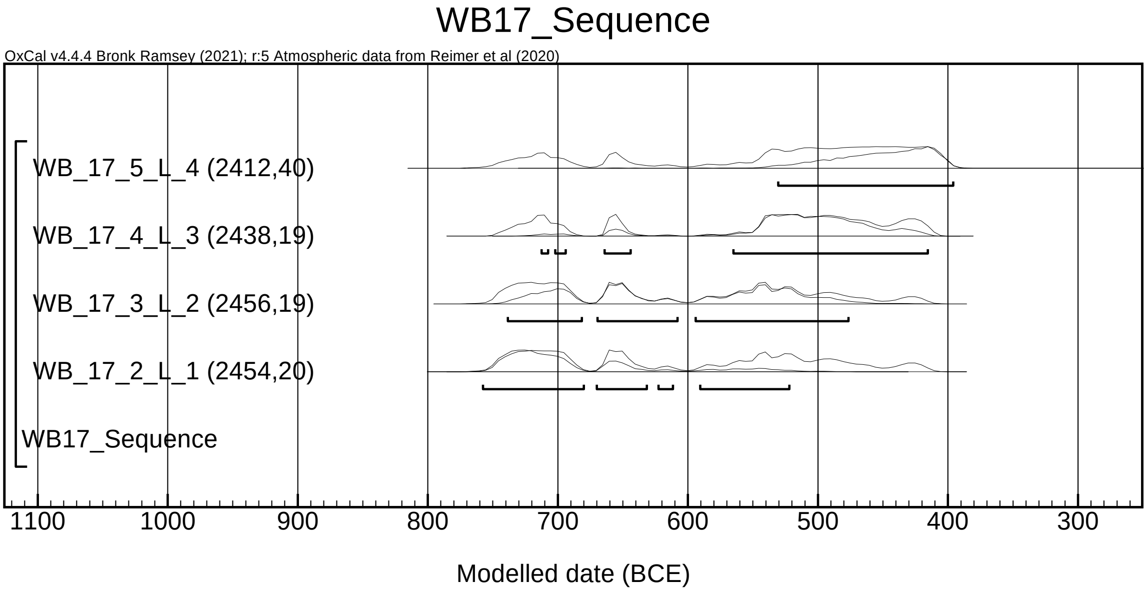

Fig. 3: The modelled radiocarbon sequence of AMS radiocarbon dates from layers 1 to 4 in WB17.

3.3. Materials and Methods

For the classification of archaeological rice spikelet bases, a modern reference collection comprising spikelet bases from 10 populations was utilised (Table 2). The domesticated rice spikelet population was sourced from the Bangladesh Rice Research Institute (BRRI). Three Wild/intermediate-type populations of '*Jhora Dhan*' were collected from a local rice farmer and germplasm conservationist in Tanore, Rajshahi, Bangladesh (Fig. 4; JH_01-03). Noted in literature as wild rice, *Jhora Dhan* is characterised by its shattering nature and propensity to grow beside ponds or in lowland marshy areas (Bin Rahman & Zhang 2013). According to the collector, this population exhibits prostrate tiller morphology, typical of wild rice plants (Ishikawa *et al.* 2020). '*Jhora Dhan*' is translated in Bengali as 'shattering rice', suggesting that it is a form of wild rice. Additional wild population was obtained from the Oxford University Botanical Garden (wild_OxBot) and through archaeobotanist Rubi Wu from the Lower

Yangtze region of China (wild_China) (Fig. 4).

Archaeological rice spikelet bases were collected from the Wari Bateshwar site, specifically from trench WB17; samples from trench WB16 were excluded due to the absence of spikelet base. A bucket flotation is used to recover charred plant parts like rice spikelet bases; we utilise mesh bags with an aperture size of approximately 300μ. Therefore, the floated materials were dried in the field and later sorted, identified and photographed in the laboratory. A total of 24 archaeobotanical samples were selected for study, distributed as follows: 12 samples from layers 1 and 4, each from layers number 2, three, and four. Only samples containing at least 50 spikelet bases were included in the study. Additionally, cultivated and wild types of archaeological rice grains were recovered from WB17 in this study. The selected cultivated type of rice has already been identified by GMM into variety level (Paper 2), while the selected wild type of rice has been assigned here for the first time.

Figure 4: The photographs show modern ethnographic rice, spikelet/paddy of *Jhora Dhan* (A kind of wild & shattering) and wild rice for observation. *Jhora Dhan* type 1-3 (JH 01-03) was collected from Bangladesh, and wild rice was collected from China and Oxford Botanical Garden, respectively.

Table 2: Accessions of modern rice for studying spikelet base morphology.

Category	Number of populations.	another name (If any)	Collection source
Domesticated/ cultivated	5	Aus, Aman, and Boro	BRRI
Wild	2	O. rufipogon	Oxford Botanical Garden and China.
Intermediate (Could be feral domesticated rice introgressed with wild rice).	3	Jhora dhan (Shattering rice)	Personal collection of a local rice collector and preservationist in Bangladesh.

Figure 5. The photographs show different stages of sample collection (A), Collected sediment samples before flotation. B. Processing by bucket flotation. C. Drying of the flots (light fractions) and D. Packed light fractions awaiting microscopic analysis.

Sediment was collected for flotation from each layer of the excavation trench, using a bulk sampling strategy to collect 60L of sediment for each sample where possible. The manual bucket flotation (Pearsall 2015) method was used to recover macro archaeobotanical remains. In this method, a 30L bucket was used to float 20L sediment at a time using mesh bag of a 3 00µ aperture size (Fig. 05).

Distinctions between spikelet bases were made by microscopic observation in the Archaeobotany Laboratory of the University of Oxford. The photographs were taken with two microscopes, e.g., Leica APA-6, DFC-423 camera, x 3.6, in the Archaeobotany laboratory and Scanning Electron Microscope (SEM) in the Research Laboratory of Archaeology and History of Arts (RLAHA) of Oxford University.

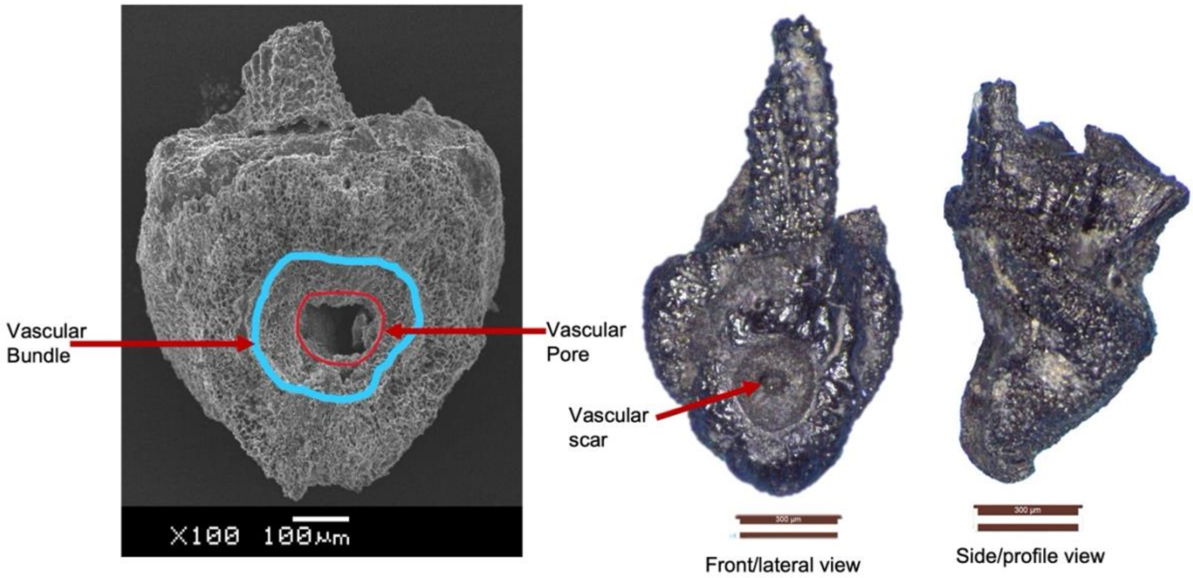

Fig. 6: Photographs of rice spikelet bases marking the various anatomical parts and orientations. We use SEM and general microscopic images to visualise the parts of the spikelet base.

Rice spikelet bases were classified into five categories: domesticated, wild, intermediate, immature and indeterminate/poorly preserved following a comparative examination of ethnographic rice spikelet bases, while Fuller *et al.* (2009) used four categories excluding intermediate.

Ethnographic examination followed to distinguish the morphological structure of wild, intermediate, domesticated, and immature type spikelet bases. This observational examination focused on the morphological characteristics of the vascular bundle, vascular scar, vascular pore, shape of the vascular bundle and profile view of the spikelet base (Fig.6).

Having sorted and identified each category, spikelet bases were calculated according to layer.

3.4 Results

3.4.1Morphological Study of Modern Spikelet Bases

This section presents the results of a morphological study on modern rice spikelet bases to refine the criteria for distinguishing between wild and domesticated rice. Identification markers for spikelet bases are first established and visualised using modern accessions summarised in the methods section. These criteria are then applied to archaeological materials to enhance the accuracy of rice classification in archaeological contexts.

Table 3 presents the details characteristics of the spikelet base morphology identified in archaeological spikelet bases based on ethnographic observation

Types of spikelet base	Morphological characters				
	Vascular pore	Vascular bundle	Front view of vascular bundle	Shape of base	Profile view shape (Side view)
Wild	Smooth, small, unaltered.	Smooth	Circular	Conical	Straight
Intermediate	Comparatively smooth and having protruding pedicel in the pore.	Comparatively smooth	Oval	Flat and wide	Semi straight
Domesticated	Twisted and large hole.	Altered	Dimpled to reniculate, often asymmetrical.	Flat and wide	Curve
Immature		Protruding vascular bundle.			

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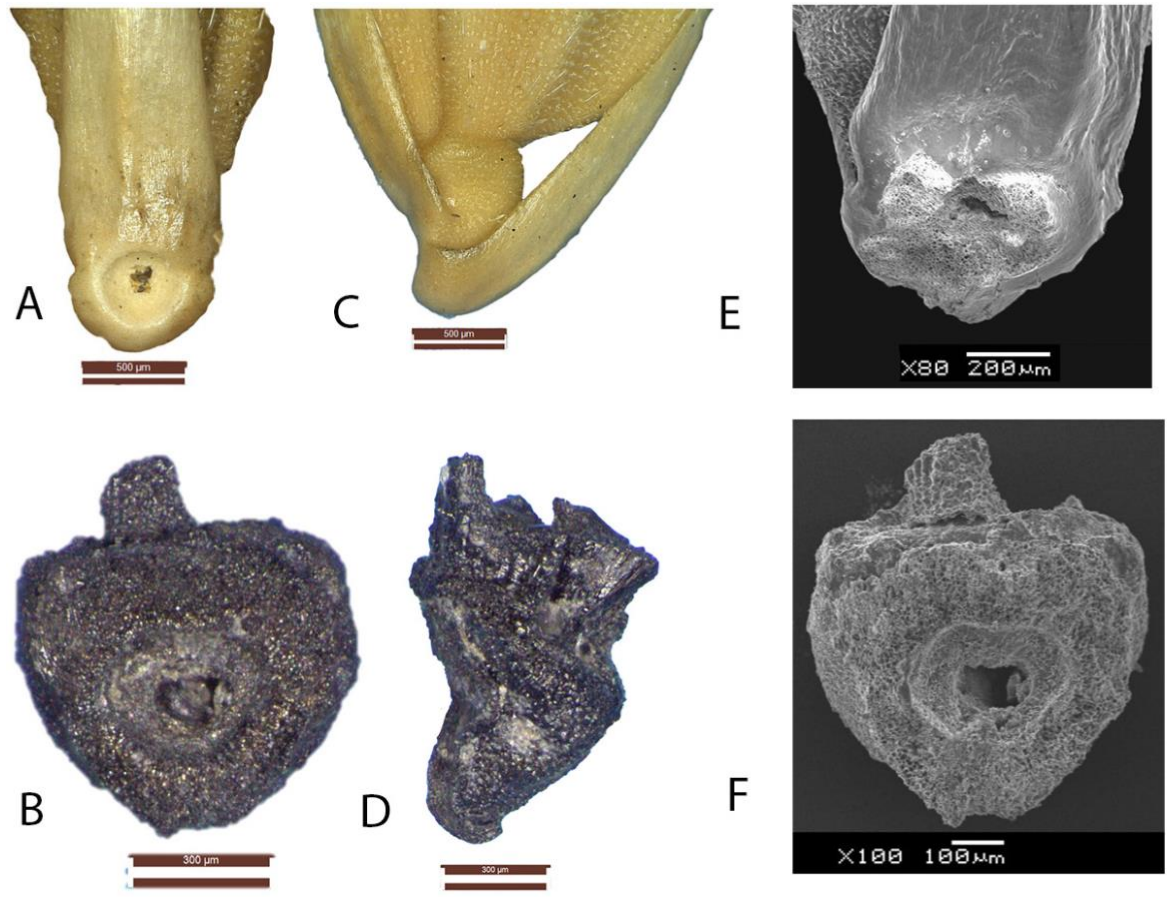
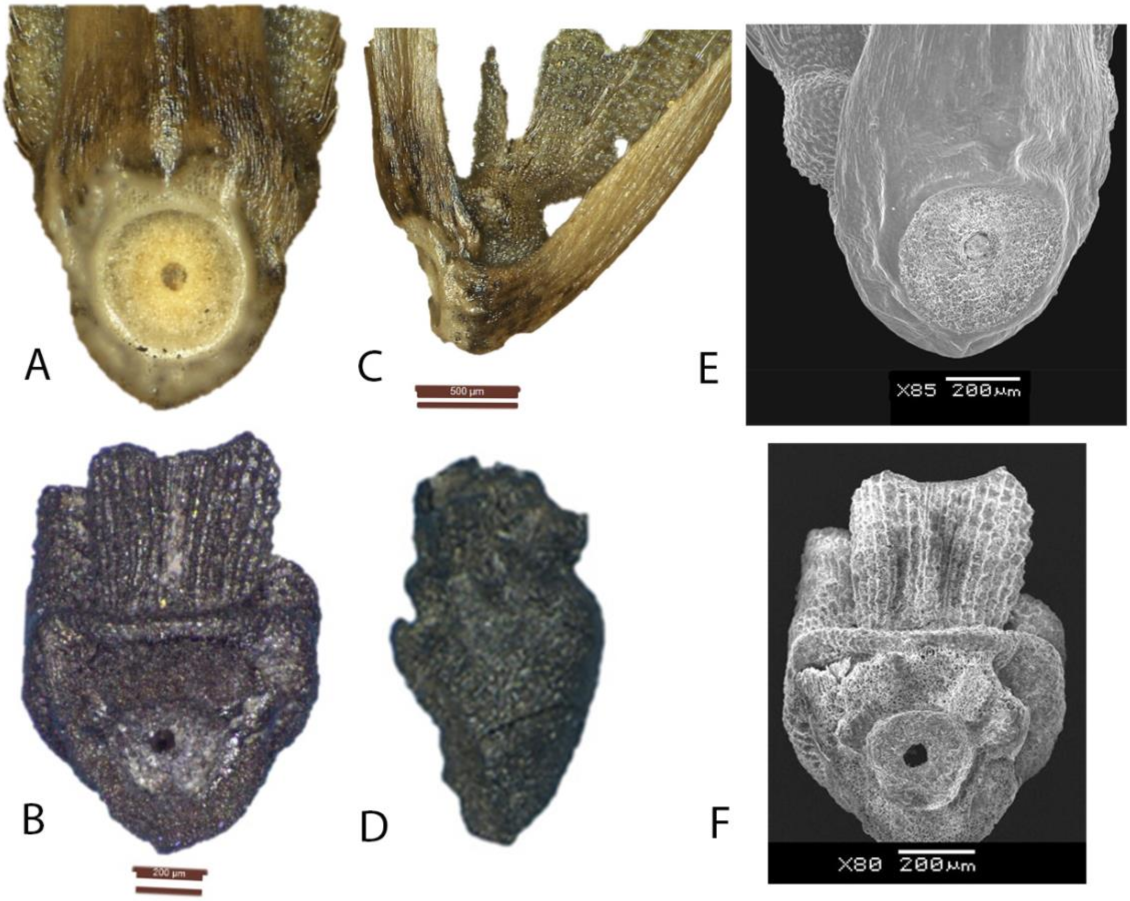
Figure 7: Modern and archaeological domesticated rice spikelet base morphology; the Upper row represents modern specimens, and the lower row shows the archaeological spikelet base. A-B. Front, C-D. Profile, E-F. Lateral-SEM image.



The domesticated type is distinguished by a large irregular vascular pore (see Fig. 7. A, B, E, F) resulting from threshing, often tearing the tissue. The front view of the vascular bundle of this type typically appears dimpled or reniculate and may be somewhat asymmetrical. In profile view, this spikelet base is curved in the middle (see Fig. 7, C, D). Additionally, the base of domesticated spikelet base is broader than that of wild types (Fig.7, B, F). By contrast, wild spikelet bases exhibited a smooth vascular scar and small vascular pore (A, B, E, F) as evidence of natural shattering, while the base has a narrow or subconical shape (Fig. 8, A, B).

Figure 8: Modern and archaeological wild rice spikelet base morphology. The upper and lower rows show modern and archaeological spikelet base photographs, respectively. A-B. Lateral view, C-D. Profile view, E-F. Lateral-SEM image.



Based on ethnographic observation of the rice spikelet base morphology of *Jhora Dhan* (wild/intermediate type), a further type of spikelet base was distinguished. This type (Fig. 9) is characterised by a comparatively smooth vascular bundle, along with subtle signs of pedicel detachment in the vascular scar, often accompanied by a remnant of pedicel within the vascular pore. The front view of the vascular bundle is distinct from the domesticated rice spikelet base, suggesting an intermediate form between wild and domesticated types. This category appears front view oval, and the base is found comparatively wider than the wild type. These features of the spikelet base exhibit subtle differences from wild-type spikelet bases.

Immature type rice spikelet bases were distinguished by protruding vascular bundles from the remnants of the attached rachilla (Fig.10), as noted by Fuller et al. (2009).

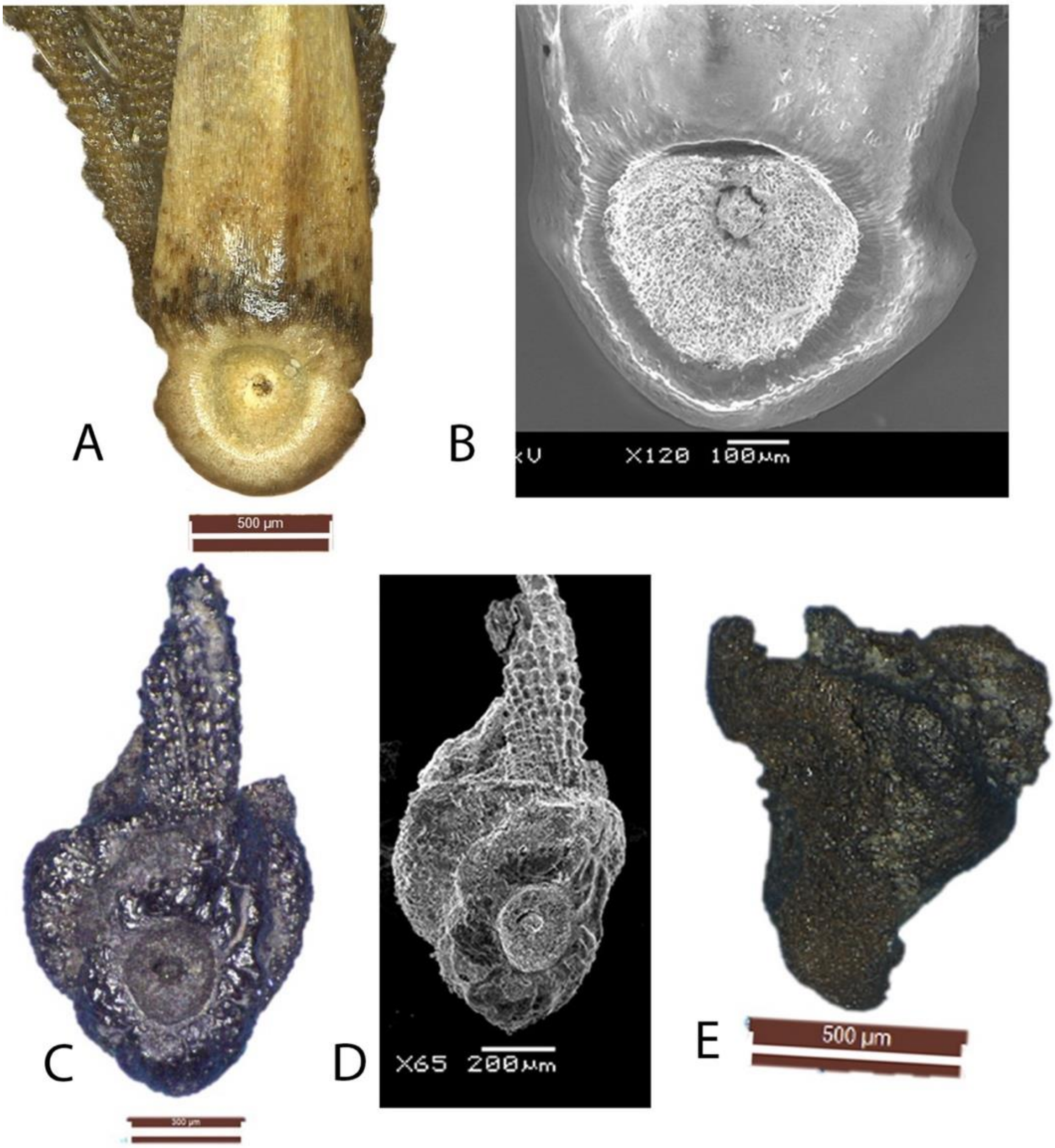


Figure 9: Modern and archaeological intermediate-type rice spikelet base morphology. The upper and lower rows show modern and archaeological spikelet base photographs, respectively. A & C. Lateral B-D: Lateral SEM image, E. Lateral view, slight curve.

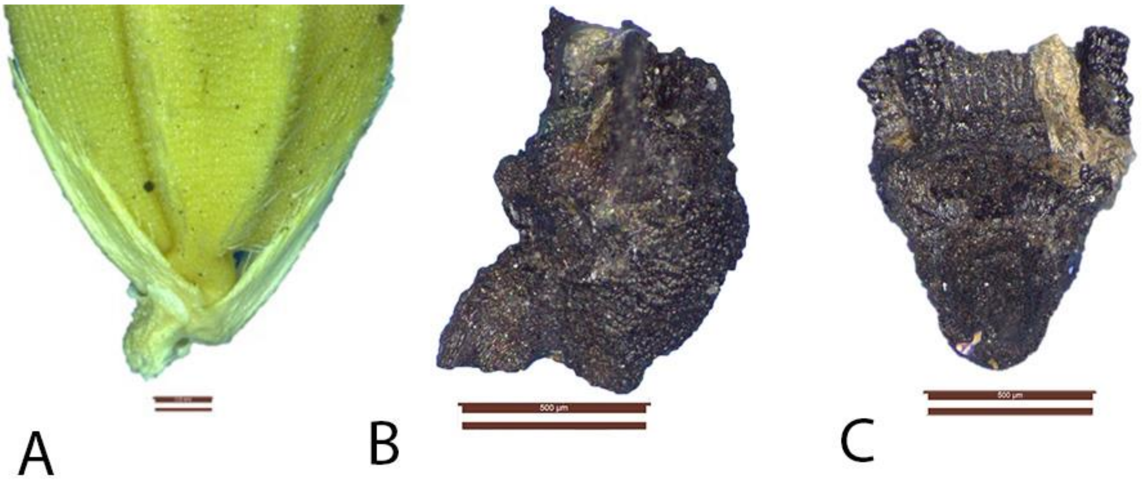


Figure 10: Modern and archaeological immature-type rice spikelet base morphology. A. Profile view (modern), B. Profile view (Archaeological), C. Front view (archaeological).

Overall, the morphological study of ethnographic spikelet base suggests the presence of intermediate type spikelet base along with wild and domesticated rice. Finding such an intermediate type of spikelet base in archaeological and modern populations might corroborate previous studies (Sharma and Shastry 1965; Sano *et al.* 1980; Oka 1988).

3.4.2. Identification of Rice Spikelet Bases

In total, 23644 rice spikelet bases were recovered from Wari-Bateshwar (trench WB17) and classified using the categories defined above on the basis of the modern material (Figs. 7-10, Table 3). Poorly preserved rice spikelet bases lacking clear distinguishing features were classified as indeterminate. All categories were observed in all samples. Fig. 11 and Table 4 show the results of the identification of archaeobotanical spikelet bases. It is apparent that domesticated type, non-shattering rice spikelet bases increase through time in the stratigraphic sequence, from 12% (119/1006) in layer 1 to 56% (1740/3101) in layer 4, while wild type spikelet bases decrease, from 57 % (573/1006) in layer 1 to 9 % (268/3101) in layer 4. Intermediate rice spikelet bases show a tendency to increase through the stratigraphic sequence, from 16% (156/1006) in layer 1 to 32% (1004/3101) in layer 4. Immature and indeterminate rice spikelet bases are a minor presence in all layers.

The period represented is up to a maximum of 350 years according to the Bayesian modelled radiocarbon dates (Fig 3).

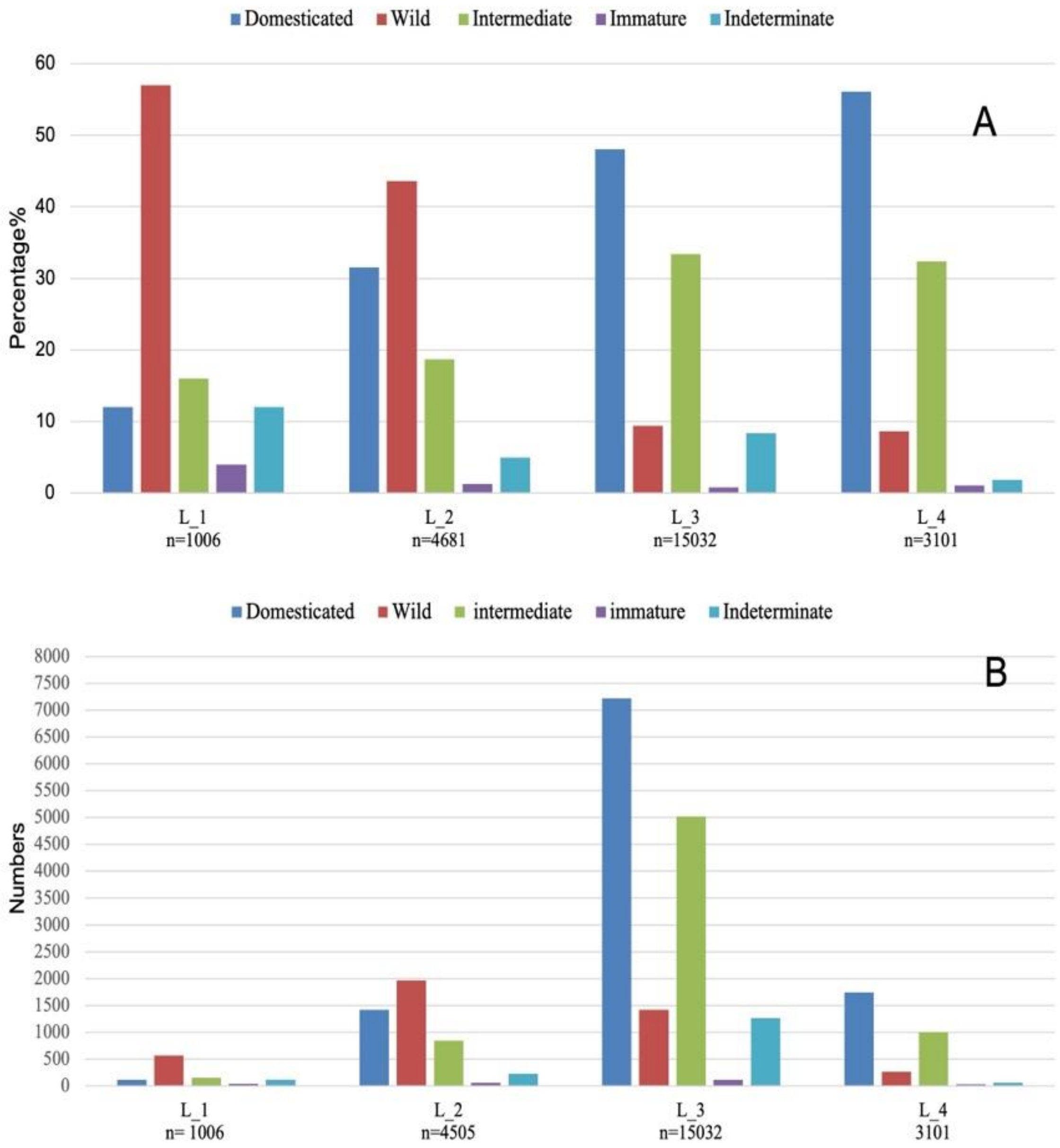


Figure 11: Bar charts A and B showing the proportions of identified rice spikelet base categories by relative frequency (Percentage%) and by numbers, respectively, through the stratigraphic sequence of Trench WB17.

Table 4: Archaeological spikelet base identification results per category and stratigraphic layer by number and relative frequency (%).

Category	Layer 1		Layer 2		Layer 3		Layer 4	
	Number	(%)	Number	(%)	Number	(%)	Number	(%)
Domesticated	119	12	1419	31	7220	48	1740	56
Wild	573	57	1964	44	1416	9	268	9
Inter.	156	16	842	19	5020	33	1004	32
Immature	39	4	56	1	116	1	32	1
Indet.	119	12	224	5	1260	8	57	2
Total	1006	100	4505	100	15032	100	3101	100

3.5. Discussion

The analysis of rice spikelet base morphology (Fig. 11, Table 4) reveals an increasing prevalence of non-shattering types and a decrease in wild types throughout the sequence of WB17, demonstrating a local rice domestication process. Additionally, these results align with the findings from the GMM identified rice varieties (Paper 2) over the same sequence exhibit consistent agreement with the hypothesis that the domestication process of aus rice occurred in the Lower Brahmaputra valley of early Bangladesh between the 8th and 4th centuries BCE. Moreover, these findings reinforce the model of multiple local introgressive hybridisation as summarised in Table 1, in which *japonica* and *indica* varieties were supposed to be present beside the aus variety along with reduced shattering through time in areas of origin (Huang & Han 2016; Choi *et al.* 2017; Choi & Purugganan 2018). Finally, this evidence supports excluding the single origin and domestication hypothesis for Asian rice varieties (Vaughan *et al.* 2008b; Molina *et al.* 2011).

The concept of multiple domestication events is not unique to rice; it has been demonstrated in other crops like barley, with supporting archaeobotanical and genetic evidence found in western Asia and beyond (Tanno & Willcox 2006; Fuller *et al.* 2011; Dai *et al.* 2012; Jones *et* *al.* 2013; Riehl *et al.* 2013). This broader context emphasises the complexity and regional variability of agricultural origins across the ancient world.

The domestication process of Asian rice varieties has been the subject of considerable debate, particularly whether key domestication genes were transferred from previously domesticated *japonica* to *aus* and *indica* through introgression (Huang & Han 2016; Choi & Purugganan 2018) or whether they inherited these genes from their wild progenitor (Civáň and Brown 2017, 2018). However, determining these processes through archaeobotanical evidence of domestication traits is challenging. In the case of WB, the presence of *japonica* and *indica* varieties throughout the WB17 sequence, together with *aus*, supports the idea of introgressive hybridisation. These observations are consistent with the model of multiple origins but with a single de novo domestication (Huang & Han 2016; Choi & Purugganan 2017). Simultaneously, the introgression process complements the proposed *proto-aus* hypothesis, in which '*proto-aus*' or feral weedy rice refers to the early form of *aus* rice cultivated before its full domestication. Based on this hypothesis, early farmers may have selected locally available wild rice varieties with desirable traits for cultivation alongside introduced *japonica* and *indica* rice. This practice would have applied selection pressure and facilitated the gene flow from *japonica* and *indica* to the locally cultivated wild varieties/*proto-aus* through introgression. Such interactions and selection pressures could have played a crucial role in the domestication process of *aus*-type rice in the Lower Brahmaputra Valley of early Bangladesh.

This study has identified an 'intermediate' type of rice spikelet base in modern collections from Bangladesh and archaeological findings from WB (Fig. 9) that could be a feral or weedy rice variety which may correspond to the 'intermediate' type of rice variety identified by rice scientists in South and Southeast Asia (Oka & Chang 1962; Sharma and Shastry 1965;

Morishima *et al*. 1980; Sano *et al*. 1980; Ng *et al*. 1981; Oka 1988; Vaughan *et al*. 2008a). This finding provides an archaeological base to support the hypothesis that the evolutionary routes of cultivated rice were followed through wild/feral-intermediate to domesticated rather than wild/feral to domesticated (Oka & Chang 1962; Oka 1988). More specifically, intermediate type rice was the progenitor of domesticated rice varieties rather than wild varieties. The intermediate types are considered to be wild-cultivated type (Oka & Chang 1962) or proto-type (Fuller 2011), e.g., proto-indica, proto-aus type, or it could be a weedy or feral type such as *Oryza nivara*, *Oryza rufipogon*, *Oryza perennis*, or *Oryza spontanea*. Zheng *et al.* (2016) reported an intermediate type of spikelet base in the Lower Yangtze Valley, which shows similarities to the intermediate type proposed in this paper.

It has been widely recognised that domestication is a protracted process (Purugganan & Fuller 2011; Purugganan 2019), taking place over millennia in the case of cereals like wheat and barley in western Asia (Tanno & Wilcox 2006, 2012) and *japonica*-type rice in China (Zheng *et al.* 2007). Despite having a long tradition of rice collection and cultivation in Northern India from as early as the c. 7th millennium BCE, the *indica* rice domestication process appears to have been completed in the late 3rd millennium to the early 2nd millennium BCE (Fuller 2011; Bates *et al.* 2017), following the introduction of Chinese *japonica* (Silva *et al.* 2018). This evidence further suggests that the domestication process of *indica*-type rice may have been rapid following the arrival of *japonica* in northern India, implying that the presence of previously domesticated variety made faster further domestication events providing domestication genes transfer.

The findings from the present study suggest that a shift from shattering to non-shattering traits at WB occurred over a maximum of 350 years (Fig. 3). This rate of domestication of the *aus* type exhibits comparatively faster than other domestication sequences. The continuous presence of already domesticated rice varieties alongside the *proto* aus type throughout the WB17 sequence (Paper 2) is likely the reason behind the relatively rapid domestication process (e.g., the transformation of spikelet bases) of aus rice.

The evidence of the *aus* rice domestication process found at Wari-Bateshwar in the Lower Brahmaputra Valley indicates that aus rice likely originated locally rather than being introduced elsewhere. Prior genetic studies have suggested that aus rice originated from a broad geographic range extending from Central India to Bangladesh (Civáň et al. 2015). This paper refines this broad geographic scope, providing a more specific context that aligns with the genetic inferences presented by Civáň et al. (2015).

Weiss *et al.* (2006) argued that the presence of a significant number of wild seeds and arable weeds in archaeological sites is indicative of pre-domestication cultivation. Riehl *et al.* (2013) observed that up to 40% of wild types in archaeological sites support pre-domestication cultivation. Applying these arguments to the findings at WB, the modest frequency of *aus*/*proto-aus* rice (RF 30%-44%) throughout the sequence (Fig.8; Paper 2) may indicate a possible pre-domestication cultivation of *proto-aus* at WB. Additionally, the increasing number of wild-type rice grains over the sequence of WB17 (a total of 474 wild type while 305 cultivated type) further supports the possibility of pre-domestication *aus*-type wild rice cultivation at WB. Alternatively, it might have had the option to exist as a part of a natural habitat as a weedy or feral rice variety in crop fields. Notable traits of *proto-aus*, such as resilience to both drought and flood tolerance and early maturity under rainfed conditions, adaptation to long summers and photoperiod neutrality (Garris *et al*. 2005; Bin Rahman & Zhang 2013; 2016; Banaticla-Hilario 2013), along with its high yield traits (Sano *et al.* 1980) likely attracted early farmers of WB to cultivate this variety. Thus, *proto-aus* might have been adapted ecologically and deliberately selected by humans, along with some unintended actions, for their broader sustenance benefits, which may reinforce the *aus* rice domestication process (Jones *et al.* 2021). Further large-scale excavation at WB and studies of weed taxa could further refine our understanding of pre-domestication *aus* rice cultivation at WB.

Fuller (2011) critically reassesses the concept of 'independent origin of rice,' proposing a reconceptualisation highlighting the development of distinct regional rice cultivation patterns. He argues that these patterns should reflect established traditions and management strategies focused on economic sustainability rather than the genetic alterations traditionally associated with domestication. This viewpoint is especially relevant to the *aus* rice domestication process in the Lower Brahmaputra Valley, which illustrates how novel rice cultivation systems in the lower deltaic plains of the Bengal frontier were pivotal in fostering new cultural and political developments. Rice production in the early Wari-Bateshwar was an essential component of food production systems that supported the growing population by feeding and underpinned the development of urban societies, and these agricultural practices played a significant role in the socio-economic transformations of the region.

3.6. Conclusion

This study presents findings based on ethnographic and archaeobotanical evidence of spikelet bases from Bangladesh to explore whether a new category of spikelet base, intermediate type, presents and the domestication status of rice. The study demonstrates a distinct 'intermediate' type of spikelet base presence in the modern collection and so that in the archaeobotanical assemblage, separate from both wild and domesticated forms. This type is characterised by a comparatively smooth vascular bundle, a slightly protruding pedicel at the vascular pore or a subtle sign of pedicel displacement from vascular scar, and an oval vascular profile. Notably, this intermediate form may represent a transitional stage in the evolution of rice, showing phenotypic traits that indicate progression towards domestication supports previous studies (Oka & Chang 1962; Oka 1988). This variety might have been a feral or a weedy type of rice variety that transformed into a domesticated type later.

The transformation of spikelet bases morphology from predominantly shattering to non-shattering is evident at WB17 between 758-398 cal. BCE offers direct attestation of the rice domestication process in the Lower Brahmaputra Valley of Bangladesh. These findings demonstrate the archaeological basis for the multiple origins of domesticated Asian rice (Huang & Han 2016; Choi *et al.* 2017) and support the hypothesis of their separate geographic origin (Civáň *et al.* 2015) and exclude the single-origin hypothesis (Vaughan *et al.* 2008b; Molina *et al.* 2011) (Table 2). Additionally, the presence of two early domesticated rice within the WB17 sequence during the aus rice domestication process suggests that domestication alleles may have been transferred to proto-aus through introgression. This scenario suggests, more specifically, the hypothesis of multiple origins but single (*de novo*) domestication of Asian rice (Choi & Purugganan 2018) instead of the multiple independent (*de novo*) domestication hypothesis (Civáň & Brown 2017; 2018).

References

Allaby, R. G., Stevens, C. J., Kistler, L., & Fuller, D. Q. 2022. Emerging evidence of plant domestication as a landscape-level process. *Trends in Ecology & Evolution,* 37 (3), 268-279.

Banaticla-Hilario, M. C. N., Sosef, M. S. M., Mcnally, K. L., Hamilton, N. R. S. & Van Den Berg, R. G. 2013. Ecogeographic Variation in the Morphology of Two Asian Wild Rice Species, Oryza nivara and Oryza rufipogon. *International journal of plant sciences,* 174**,** 896-909.

Bates, J., Petrie, C. A. & Singh, R. N. 2017. Approaching rice domestication in South Asia: New evidence from Indus settlements in northern India. *Journal of Archaeological Science,* 78**,** 193-201.

Bates, J. 2022. The Fits and Starts of Indian Rice Domestication: How the Movement of Rice Across Northwest India Impacted Domestication Pathways and Agricultural Stories. *Frontiers in ecology and evolution,* 10.

Bhuiyan, M. A. H., Kowser, A., Islam, S., Islam, M. R., Mohid, M., Kakoly, S. A., & Khondker, M. 2020. Phytoplankton in relation to water quality of Tanguar haor ecosystem, Bangladesh: 2. watch tower station. *Dhaka University Journal of Biological Sciences*, 29(1), 9-18. https://doi.org/10.3329/dujbs.v29i1.46526

Bin Rahman, A. N. M. R. & Zhang, J. 2013. Rayada specialty: the forgotten resource of elite features of rice. *Rice (N Y),* 6**,** 41-41.

Bin Rahman, A. N. M. R., & Zhang, J. 2016. Flood and drought tolerance in rice: opposite but may coexist. *Food and Energy Security* 5(2). pp76-88.

Bookhagen, B. and Burbank, D. W. 2010. Toward a complete Himalayan hydrological budget: spatiotemporal distribution of snowmelt and rainfall and their impact on river discharge. *Journal of Geophysical Research: Earth Surface*, 115(F3). https://doi.org/10.1029/2009jf001426

Bogaard, A., Allaby, R., Arbuckle, B. S., Bendrey, R., Crowley, S., Cucchi, T., Denham, T., Frantz, L., Fuller, D., Gilbert, T., Karlsson, E., Manin, A., Marshall, F., Mueller, N., Peters, J., Stépanoff, C., Weide, A., & Larson, G. 2021. Reconsidering domestication from a process archaeology perspective. *World Archaeology,* 53**,** 56-77.

Castillo, C. 2011. Rice in Thailand: The Archaeobotanical Contribution. *Rice (New York, N.Y.),* 4**,** 114-120.

Castillo, C., Tanaka, K., Sato, Y.-I., Ishikawa, R., Bellina, B., Higham, C., Chang, N., Mohanty, R., Kajale, M. & Fuller, D. 2016a. Archaeogenetic study of prehistoric rice remains from Thailand and India: evidence of early japonica in South and Southeast Asia. *Archaeological and Anthropological Sciences,* 8**,** 523-543.

Cheng, C., Motohashi, R., Tsuchimoto, S., Fukuta, Y., Ohtsubo, H. & Ohtsubo, E. 2003. Polyphyletic origin of cultivated rice: Based on the interspersion pattern of SINEs. *Mol Biol Evol,* 20**,** 67-75.

Choi, J. Y., Platts, A. E., Fuller, D.Q., Hsing, Y-I., Wing. R. A., & Purugganan. M. D. 2017. The Rice Paradox: Multiple Origins but Single Domestication in Asian Rice. *Mol. Biol. Evol.,* 34(4)**,** 969–979.

Choi, J. Y. & Purugganan, M. D. 2018. Multiple origin but single domestication led to Oryza sativa. *G3 (Bethesda),* 8**,** 797-803.

Civáň, P., Craig, H., Cox, C. J., & Brown, T. A. 2015. Three geographically separate domestications of Asian rice. *Nat Plants.* 1: 151-64.

Civáň, P., Brown, T. A. 2017 Origin of rice (Oryza sativa L.) domestication genes. *Genet Resour Crop Evol,* 64**,** 1125–1132.

Civáň, P. Brown, T.A. 2018. Role of genetic introgression during the evolution of cultivated rice (Oryza sativa L.). *BMC Evolutionary Biology,* 18:57. DOI: https://doi.org/10.1186/s12862-018-1180-7

Crawford, G. W. & Shen, C. 1998. The origins of rice agriculture: recent progress in East Asia. *Antiquity,* 72 858-66.

Dai, F., Nevo, E., Wu, D., Comadran, J., Zhou, M., Qiu, L., Chen, Z. 2012. Tibet is one of the centres of domestication of cultivated barley. *Proceedings of the National Academy of Sciences of the United States of America.*, 109 pp. 16969-16973.

Diamond, J. 2002. Evolution, consequences and future of plant and animal domestication. *Nature,* 418 700-707.

Deng, Z., Qin, L., Gao, Y., Weisskopf, A. R., Zhang, C. & Fuller, D. Q. 2015. From early domesticated rice of the middle Yangtze basin to millet, rice and wheat agriculture: Archaeobotanical macro-remains from Baligang, Nanyang Basin, Central China (6700-500 BC). *PLoS One,* 10**,** e0139885-e0139885.

Fuller, D. Q., Harvey, E. & Qin, L. 2007. Presumed domestication? Evidence for wild rice cultivation and domestication in the fifth millennium BC of the Lower Yangtze region. *Antiquity,* 81**,** 316-331.

Fuller, D. Q. & Qin, L. 2009. Water management and labour in the origins and dispersal of Asian rice. *World Archaeology,* 41**,** 88-111.

Fuller, D. Q., Qin, L., Zheng, Y., Zhao, Z., Chen, X., Hosoya, L. A., Sun G-P. 2009. The Domestication Process and Domestication Rate in Rice: Spikelet bases from the Lower Yangzte. *Science,* 323**,** 1607-1610.

Fuller, D., Sato, Y.-I., Castillo, C., Qin, L., Weisskopf, A., Kingwell-Banham, E., Song, J., Ahn, S.-M. & Etten, J. 2010. Consilience of genetics and archaeobotany in the entangled history of rice. *Archaeological and Anthropological Sciences,* 2**,** 115-131**.**

Fuller, D. Q. 2011. Pathways to Asian Civilizations: Tracing the Origins and Spread of Rice and Rice Cultures. *Rice (New York, N.Y.),* 4**,** 78-92.

Fuller, D. Q., Willcox, G., & Allaby, R. G. 2011. Cultivation and domestication had multiple origins: arguments against the core area hypothesis for the origins of agriculture in the Near East. *World Archaeology,* 43**,** 628-652.

Fuller, D.Q., Weisskopf, A. & Castillo, C. 2016. Pathways of Rice Diversification across Asia. *Archaeology International*.19, 84–96.

Garris, A. J., Tai, T. H., Coburn, J., Kresovich, S. & Mccouch, S. 2005. Genetic structure and diversity in Oryza sativa L. *Genetics,* 169**,** 1631.

Glaszmann, J. C. 1987. Isozymes and classification of Asian rice varieties. *Theor Appl Genet,* 74**,** 21-30.

Goodbred, S. L., Paolo, P. M., Ullah, M. S., Pate, R. D., Khan, S. R., Kuehl, S. A., & Rahman, W. 2014. Piecing together the Ganges-Brahmaputra-Meghna River delta: use of sediment provenance to reconstruct the history and interaction of multiple fluvial systems during Holocene delta evolution. Geological Society of America Bulletin, 126(11-12), 1495-1510. https://doi.org/10.1130/b30965.1

Gross, B. L. & Zhao, Z. 2014. Archaeological and genetic insights into the origins of domesticated rice. *Proceedings of the National Academy of Sciences,* 111**,** 6190.

Gutakar, R. M., Groen, S. C., Bellis, E. S., Choi, J. Y., Pires, I. S., Bocinsky, R. K., Slayton, E. R., Wilkins, O., Castillo, C. C., Negrão, S., Oliveira, M. M., Fuller, D. Q., Guedes, J. A. D. A., Lasky, J. R. & Purugganan, M. D. 2020. Genomic history and ecology of the geographic spread of rice. *Nat Plants,* 6**,** 492-502.

Harushima, Y., Nakagahra, M., Yano, M., Sasaki, T., Kurata, N. 2002. Diverse variation of reproductive barriers in three intraspecific rice crosses. *Genetics* 160:313–322.

Hasan, M. M. 2023. Commercially important fish biodiversity of hail haor with physicochemical properties and planktonic composition. *Academia Biology*. https://doi.org/10.20935/acadbiol6087

Huang, X. & Han, B. 2016. Rice domestication occurred through single origin and multiple introgressions. *Nat Plants,* 2**,** 15207-15207.

Huang, X., Kurata, N., Wei, X., Wang, Z.-X., Wang, A., Zhao, Q., Zhao, Y., Liu, K., Lu, H., Li, W., Guo, Y., Lu, Y., Zhou, C., Fan, D., Weng, Q., Zhu, C., Huang, T., Zhang, L., Wang, Y., Feng, L., Furuumi, H., Kubo, T., Miyabayashi, T., Yuan, X., Xu, Q., Dong, G., Zhan, Q., Li, C., Fujiyama, A., Toyoda, A., Lu, T., Feng, Q., Qian, Q., Li, J. & Han, B. 2012. A map of rice genome variation reveals the origin of cultivated rice. *Nature,* 490**,** 497-501.

ICIMOD. 2018. "Regional Workshop on Planning Transboundary Technical Collaboration for Landscape Management, 8-9 February 2018, ICIMOD Workshop Report 2018''. Nay Pyi Taw, Myanmar: ICIMOD, Kathmandu.

Ishikawa, R., Castillo, C. C. & Fuller, D. Q. 2020. Genetic evaluation of domestication-related traits in rice: implications for the archaeobotany of rice origins. *Archaeological and anthropological sciences,* 12.

Islam, K. K. and Sato, N. 2013. Protected sal forest and livelihoods of ethnic minorities: experience from Bangladesh. *Journal of Sustainable Forestry,* 32(4), 412-436. https://doi.org/10.1080/10549811.2013.762571

Jones, G., M. P. Charles, M. K. Jones, S. Colledge, F. J. Leigh, D. A. Lister, L. M. J. Smith. 2013. DNA evidence for multiple introductions of barley into Europe following dispersed domestications in Western Asia. *Antiquity,* 87**,** 701-7.

Jones, G., Kluyver, T., Preece, C., Swarbrick, J., Forster, E., Wallace, M., Charles, M., Rees, M., Osborne, C. P. 2021. The origins of agriculture: Intentions and consequences. *Journal of Archaeological Science* 125 https://doi.org/10.1016/j.jas.2020.105290.

Konishi, S., Izawa, T., Lin, S. Y., Ebana, K., Fukuta, Y., Sasaki, T. & Yano, M. 2006. An SNP Caused Loss of Seed Shattering during Rice Domestication. *Science,* 312**,** 1392-1396.

Kumar, A., Mondal, S., & Lal, P. 2022. Analysing frequent extreme flood incidences in Brahmaputra basin, south Asia. *Plos One*, 17(8), e0273384. https://doi.org/10.1371/journal.pone.0273384

Li, Z.K., Pinson, S.R.M., Paterson, A.H., Park, W.D., Stansel, J.W. 1997. Genetics of hybrid sterility and hybrid breakdown in an intersubspecific rice (Oryza sativa L) population. *Genetics* 145:1139–1148

Li, C., Zhou, A. & Sang, T. 2006. Genetic Analysis of Rice Domestication Syndrome with the Wild Annual Species, Oryza nivara. *New Phytol,* 170**,** 185-193.

Liu, T., Liu, Y., Sun, Q., Zong, Y., Finlayson, B., Chen, Z. 2017. Early Holocene groundwater table fluctuations in relation to rice domestication in the middle Yangtze River basin, China. *Quaternary Science Reviews* 79-85.

Londo, J. P., Chiang, Y. C., Hung, K. H., Chiang, T. Y. & Schaal, B. A. 2006. Phylogeography of Asian wild rice, Oryza rufipogon, reveals multiple independent domestications of cultivated rice, Oryza sativa. *Proc Natl Acad Sci U S A,* 103**,** 9578-9583.

McNally, K. L., Childs, K.L., Bohnert, R., Davidson, R.M., Zhao, K., Ulat, V.J., Zeller, G., Clark, R.M., Hoen, D.R., Bureau, T.E. & Stokowski, R. 2009. Genome wide SNP variation reveals relationships among landraces and modern varieties of rice. *Proceedings of the National Academy of Sciences,* 106**,** 12273-12278.

Molina, J., Sikora, M., Garud, N., Flowers, J. M., Rubinstein, S., Reynolds, A., Huang, P., Jackson, S., Schaal, B. A., Bustamante, C. D., Boyko, A. R. & Purugganan, M. D. 2011. Molecular evidence for a single evolutionary origin of domesticated rice. *Proc Natl Acad Sci U S A*, 108**,** 8351-8356.

Morishima, H., Sano, Y., & Oka, H.I. 1980. Observations on wild and cultivated rices and companion weeds in the hilly areas of Nepal, India and Thailand: Report of study-tour in tropical Asia, 1979. Misima: Rep. Natl. Inst. Genetics.

Ng, N. Q., Chang, T. T., Williams, J. T. & Hawkes, J. G. 1981. Morphological studies of Asian rice and its related wild species and the recognition of a new Australian taxon. *Biological Journal of the Linnean Society*, 16**,** 303-313.

Oka, H. & Chang, W. T. 1962. Rice varieties intermediate between wild and cultivated forms and the origin of the Japonica type. *Bot. Bull. Acad. Sinica,* 3**,** 109-131.

Oka, H. I. 1988. *Origin of cultivated rice.* Tokyo: Elsevier Science Pub. Co.

Pachuau, J., & Schendel, W. V. 2022 *Entangled Lives: Human-Animal-Plant Histories of the Eastern Himalayan Triangle*, Cambridge: Cambridge University Press.

Parvin, M. and Akteruzzaman, M. 2013. Factors affecting farm and non-farm income of haor inhabitants of Bangladesh. *Progressive Agriculture*, 23(1-2), 143-150. https://doi.org/10.3329/pa.v23i1-2.16578

Pearsall, D. M. 2015. *Paleoethnobotany: a handbook of procedures,* Walnut Creek, California, Left Coast Press Inc.

Pickering, J., Goodbred, S. L., Reitz, M. D., Hartzog, T. R., Mondal, D. R., & Hossain, M. S. 2014. Late Quaternary sedimentary record and Holocene channel avulsions of the Jamuna and Old Brahmaputra River valleys in the upper Bengal delta plain. Geomorphology, 227, 123-136. https://doi.org/10.1016/j.geomorph.2013.09.021

Purugganan, M. D. & Fuller, D. Q. 2011. Archaeological data reveal slow rates of evolution during plant domestication. *Evolution,* 65**,** 171-183.

Purugganan, M. D. 2019. Evolutionary Insights into the Nature of Plant Domestication. *Current Biology* 29**,** R705–R714.

Purugganan, M. D. 2022. What is domestication? *Trends in Ecology & Evolution,* 37.

Rahman, S. M. & Pathan, M.H. 2013. *Wari-Bateshwar: Shekorer Sandhane.* Dhaka, Prothoma.

Rahman, S. M., Pathan, H., Rahman, M., Akhter, S., Rayhan, M. 2012. Wari-Bateshwar. *In:* Islam, S. (ed.) *Banglapedia.* 2nd ed. Dhaka: Asiatic Society of Bangladesh.

Rahman, M. Murphy, C., Weisskopf, A., Champion, L, Castillo, C, & Fuller, D. Q. 2019. Wari-Bateshwar and Vikrampura: Successful Case Studies in Archaeobotany, Bangladesh. *Man and Environment.*, XLIV(1)**,** pp: 62-72.

Rahman, M. Castillo. C. C., Murphy, C., Rahman, S. M., & Fuller, D. Q. 2020. Agricultural systems in Bangladesh: the first archaeobotanical results from Early Historic Wari-Bateshwar and Early Medieval Vikrampura. *Archaeological and Anthropological Sciences*.

Riehl, S., Zeidi, M., Conard, N. J. 2013. Emergence of Agriculture in the Foothills of the Zagros Mountains of Iran. *Science* 341 65-67.

Saha, S., Hasan, S., Haque, M., & Ahamed, T. 2021. Perception based assessment of ecosystem services of Madhupur sal forest in Bangladesh. European Journal of Agriculture and Food Sciences, 3(1), 39-44. https://doi.org/10.24018/ejfood.2021.3.1.194

Sano, Y., Morishima, H. & Oka, H.-I. 1980. Intermediate perennial-annual populations of Oryza perennis found in Thailand and their evolutionary significance. *The Botanical Magazine Tokyo,* 93**,** 291-305.

Schatz, M. C., Maron, L. G., Stein, J. C., Hernandez, W. A., Gurtowski, J., Biggers, E., Lee, H., Kramer, M., Antoniou, E., Ghiban, E., Wright, M. H., Chia, J.-M., Ware, D., Mccouch, S. R. & Mccombie, W. R. 2014. Whole genome de novo assemblies of three divergent strains of rice, Oryza sativa, document novel gene space of aus and indica. *Genome biology,* 15**,** 506.

Schneider, R., Godiksen, P. N., Villadsen, H., Madsen, H., & Bauer-Gottwein, P. 2017. Application of cryosat-2 altimetry data for river analysis and modelling. Hydrology and Earth System Sciences, 21(2), 751-764. https://doi.org/10.5194/hess-21-751-2017

Sharma, B. 2005. Rotifer communities of floodplain lakes of the Brahmaputra basin of lower assam (N.E. India): biodiversity, distribution and ecology. *Hydrobiologia,* 533(1-3)**,** 209-221.

Sharma, B. K. and Sharma, S. 2019. The biodiverse rotifers (rotifera: eurotatoria) of small wetlands of the Brahmaputra River floodplains of lower and upper assam, northeast India. *Journal of Limnology and Freshwater Fisheries Research,* 5(3), 187-196. https://doi.org/10.17216/limnofish.515981

Sharma, S.D., & Shastry, S.V.S. 1965 Taxonomic studies in the genus Oryza L. III. O. rufipogon Griff. sensu stricto and O. nivara Sharma et Shastry nom. Nov. *Indian J Genet Plant Breed* 25**,** 157–167.

Silva, F., Stevens, C., Weisskopf, A., Castillo, C., Qin, L., Bevan, A. & Fuller, D. 2015. Modelling the Geographical Origin of Rice Cultivation in Asia Using the Rice Archaeological Database. *PLoS One*.

Silva, F., Weisskopf, A., Castillo, C., Murphy, C., Kingwell-Banham, E., Qin, L. & Fuller, D. Q. 2018. A tale of two rice varieties: Modelling the prehistoric dispersals of japonica and proto-indica rices. *Holocene (Sevenoaks),* 28**,** 1745-1758.

Tanno, K.-I. & Willcox, G. 2006. How Fast Was Wild Wheat Domesticated? *Science,* 311**,** 1886-1886.

Tanno, K.-I. & Willcox, G. 2012. Distinguishing wild and domestic wheat and barley spikelets from early Holocene sites in the Near East. *Vegetation history and archaeobotany,* 21**,** 107-115.

Tewari, R., Srivastava, R. K., Singh, K. K., Saraswat, K. S., Singh, I. B., Chauhan, M. S., Pokharia, A. K., Saxena, A., Prasad, V., Sharma, M., 2006. Second preliminary report of the excavations at Lahuradewa district Sant Kabir Nagar, U.P.? 2002-2003-2004 & 2005-06. *Pragdhara* 16**,** 35–68.

Thompson, G. B. 1996. Ethnographic Models for Interpreting Rice Remains. *In:* THOSARAT, C. H. A. R. (ed.) *The Excavation of Khok Phanom Di a Prehistoric Site in Central Thailand; Subsistence and Environment: The Botanical Evidence.* London: The Society of Antiquaries of London.

Travis, A. J., Gareth. J. N., Datta, S., Sarma, R., Dasgupta, T., Savio, F. L., Macaulay, M., Hedley, P. E., McNally, K. L., Sumon, M. H., Islam, M. R., & Price, A. H. 2015. Assessing the genetic diversity of rice originating from Bangladesh, Assam and West Bengal. *Rice,* 8:35.

Vaughan, D. A., Lu, B.-R. & Tomooka, N. 2008a. The evolving story of rice evolution. *Plant science (Limerick),* 174**,** 394-408.

Vaughan, D. A., Lu, B.-R. & Tomooka, N. 2008b. Was Asian Rice (Oryza sativa) Domesticated More Than Once? *Rice (New York, N.Y.),* 1**,** 16-24.

Vitte, C., Ishii, T., Lamy, F., Brar, D. & Panaud, O. 2004. Genomic paleontology provides evidence for two distinct origins of Asian rice (Oryza sativa L.). *Mol Genet Genomics,* 272**,** 504-511.

Tewari, R., Srivastava, R.K., Singh, K.K., Saraswat, K.S., Singh, I.B., Chauhan, M.S., & Pokharia, A. K., Saxena, A., Prasad, V., Sharma, M. 2006. Second preliminary report of the excavations at Lahuradewa District Sant Kabir Nagar, UP, 2002-04 and 2005-06. Uttar Pradesh.

Weiss, E., Kislev, M. E. Hartmann, A. 2006. Autonomous Cultivation Before Domestication. *Science* 312**,** 1608-1610.

Zhang, J., Jiang, L., Yu, L., Huan, X., Zhou, L., Wang, C., Jin, J., Zuo, X., Wu, N., Zhao, Z., Sun, H., Yu, Z., Zhang, G., Zhu, J., Wu, Z., Dong, Y., Fan, B., Shen, C., Lu, H. 2024. Rice's trajectory from wild to domesticated in East Asia. *Science* 384**,** 901–906.

Zhao, Z. 1998. The Middle Yangtze region in China is one place where rice was domesticated: phytolith evidence from the Diaotonghuan cave, Northern Jiangxi. Antiquity 72, 885e897. *Antiquity* 72**,** 885-897.

Zheng, Y., Sun, G., and Chen, X. 2007. Characteristics of the short rachillae o rice from archaeological sites dating to 7000 years ago. *Chin. Sci. Bulletin.* 52, 1654–1660.

Zheng, Y., Crawford, G. W., Jiang, L. & Chen, X. 2016. Rice Domestication Revealed by Reduced Shattering of Archaeological rice from the Lower Yangtze Valley. *Sci Rep,* 6**,** 28136-28136.

Zohary, D., Hopf, M. & Weiss, E. 2012. *Domestication of plants in the old world: the origin and spread of domesticated plants in southwest Asia, Europe, and the Mediterranean basin,* Oxford, Oxford University Press.

6. Discussion and Conclusions

This study aimed to explore the form of rice that was cultivated at Wari-Bateshwar in the Lower Brahmaputra Valley during the mid-1st millennium BCE. The key aims of this research were, first, to establish a basis for recognising well-preserved archaeological rice and reproducing such rice grains through modern rice charring experiments (Paper 1). Secondly, the taxonomic identification of Asian rice at the variety level was investigated and refined by combining Morphometrics and Geometrics Morphometrics (GMM) methods (Paper 2). Thirdly, the status of rice domestication at Wari-Bateshwar in the Lower Brahmaputra valley was assessed on the basis of rice spikelet base morphology (Paper 3).

This concluding chapter is designed to present the key research findings discussed in papers 1-3 in a succinct manner, and corresponding to the research questions set out in the Introduction to the thesis. Synthesised discussion of each aim and conclusion places the key findings of this thesis in context.

4.1 Establishing a Heating Regime for Well-Preserved Archaeological Rice

Most archaeobotanical remains recovered from archaeological sites are charred, and this charred form, along with taphonomic conditions, potentially impacts grain morphology and taxonomic identification. A controlled charring experiment using accessions of present-day rice landraces of the three Asian varieties, and building upon previous studies, has made it possible to identify the heating regime that produces rice resembling well-preserved archaeological rice. The heating experiment was conducted under two conditions: reduced oxygen (with sand) and oxidised (no sand), and four temperature regimes of 220^0C, 230^0C, 240^0C, and 260^0C for 2-, 3-, 4- and 6-hour intervals. The reduced oxygen conditions yielded charred rice grains that closely resemble those found in well-preserved archaeological contexts.

The results indicate that low temperatures and short durations— specifically 230^{0}C for 2-3 hours —produce charred rice that closely resembles well-preserved archaeobotanical rice grains. Conversely, a relatively high temperature of 240^{0}C resulted in significant alterations to the morphology of *aus* and *indica,* causing them to resemble *japonica* rice. Furthermore, exposure to an even higher temperature of 260^{0}C for 2 hours led to extremely distorted rice morphology that makes grain unidentifiable at variety level (Table 5). Notably, *japonica* rice exhibited a relatively higher temperature tolerance than the other two varieties.

The narrow temperature range of the modern rice charring regime closely resembles well-preserved archaeological rice and is consistent with previous rice charring experiments (Garton *et al.* 1979; White *et al.* 2019). Additionally, these findings support several similar experiments conducted on different cereals taxa, including glume wheat and barley (Boardman and Jones 1990; Charles *et al.* 2015), spelt wheat (Berihuete-Azorìn *et al.* 2019) and rye, oat, bread wheat and hulled barley (Stroud *et al.* 2023).

By contrast, the findings of this study contradict the rice charring experiment by Castillo (2019), which suggested that high temperatures and medium duration, 600^{0}C-900^{0}C for at least three hours, work for rice charring. This discrepancy may be attributed to differences in charring methodologies, as Castillo employed an uncontrolled heating regime, whereas this study utilised a controlled charring regime.

4.2. Rice Chronology at Wari-Bateshwar of the Lower Brahmaputra Valley of Early Bangladesh.

A total of 10 AMS radiocarbon dates have been obtained from Wari-Bateshwar, and Bayesian modelling illustrates that the site occupation expanded from at least the 6th century BCE to the early 2nd century BCE. The taxonomic identification of Asian rice, linked to the stratigraphic sequence (Paper 2), shows that *japonica* and *indica* rice were introduced to WB by 758-586 cal BCE (Paper 2). Concurrently, local inhabitants likely managed *aus*-type rice that was not yet fully domesticated, characterised as an intermediate type of wild-cultivated or feral weedy rice, referred to as proto-aus (Paper 3). This proto-aus may have been managed or gathered from feral or weedy species in the fields. Furthermore, proto-aus could have transitioned into fully domesticated *aus* rice, manifesting predominant non-shattering traits by the early 4th century BCE. The radiocarbon dates from WB17 support the inference that the morphological transition from shattering to non-shattering occurred over a maximum period of around 350 years (Fig. 3 & 11; Paper 3).

Dispersal of *japonica* rice from China to South Asia remains to be precisely established. However, Silva *et al.* (2018) proposed three routes for this dispersal, with the Southern Route (H1) suggesting that non-shattering *japonica* rice was introduced to northeast India from southwest China via Myanmar, Assam, and Bangladesh. The findings of this research might indicate this route for *japonica* dispersal to Bangladesh and possibly northeast India by the first half of the 1st millennium BCE (Paper 2). Additionally, this study indicates that *indica* rice was present in the Brahmaputra Valley contemporaneously with *japonica*. The simultaneous presence of *japonica* and *indica* during the mid 1st millennium BCE in the Lower Brahmaputra valley may have facilitated the domestication of the *aus* variety by the early 4th century BCE. This chronology for the domestication process of *aus* aligns with the hypothesis of the earlier domestication of *japonica* and *indica*. To gain a more comprehensive understanding of rice dispersal and its timing, further archaeobotanical sampling across additional sites in the region is essential.

The dates from WB place this site as the earliest known archaeological occupation in Bangladesh (Rahman and Pathan 2012; Rahman *et al.* 2020), making it a contemporary site of neighbouring Neolithic habitation in northeast India (Padhan 2023).

4.3 Identifying Asian Rice: Methodological Contribution

Morphometrics and Geometrics Morphometrics (GMM) are two methodologies utilised to distinguish among the Asian rice genetic varieties: *japonica*, *indica* and *aus*. In this study, 342 modern rice grains and 422 archaeological rice grains (Table 4 & 5; Paper 2) were analysed using both methods. Additionally, nine accessions from the three genetic varieties of modern rice were selected for the study (Table 3; Paper 2).

Following the controlled charring experiment (Paper 1), charred modern rice grains were photographed and measured for grain length (GL), grain width (GW), and grain thickness (GT). The GLW (grain length-to-width) ratio was ultimately calculated to distinguish morphological differences among the varieties (Castillo *et al.* 2016). Castillo refined the rice morphometrics proposed by Fuller *et al.* (2007) and successfully employed the GLW ratio to differentiate between *japonica* and *indica* varieties (Table 1; Paper 2). This study expands our understanding of rice morphometrics to include the *aus* variety, reinforcing the observation that taxonomic identification at the variety level remains complex (Table 8; Paper 2). The taxonomic identification of rice varieties becomes challenging when *aus* rice is included in the analysis. The results reveal that the GLW ratios for *japonica* and *aus* rice significantly overlap (≤1.70-2.29 mm), while the *indica* variety can be distinctly separated with a GLW ratio of ≥2.31mm (Table 8; Paper 2). Despite the apparent morphological differences between *japonica* (short, plump type) and *aus* (long, semi-rounded type), their GLW ratios overlap due to the grain length and grain width proportion. Additionally, factors such as environmental circumstances and cultivation practices —like wet versus dry farming and manuring— can influence grain size, further complicating the identification of rice varieties and the application of GMM methodologies.

The GMM (Geometric Morphometrics) method works quantitatively by analysing the shape geometry of grains, enabled through landmarks or coordinates of outlines. This approach provides a dense and continuous description of the shape perimeter, capturing more detailed shape information than methods that rely solely on sparse landmarks. Higher-order harmonics further allow for the capture of finer details regarding the overall size and shape of the grains. These detailed shape analyses are instrumental in distinguishing the subtle morphological differences among closely related rice varieties, specifically *japonica*, *indica*, and *aus*.

However, dorsal, lateral and polar views are utilised in the analysis of grains (e.g., Bonhomme *et al.* 2017), and the effectiveness of these views for identification may vary depending on their specific application. Linear discriminant analysis (LDA) indicates that the lateral view is particularly effective in distinguishing modern charred rice varieties. By contrast, for glume wheats Roushannafas *et al.* (2022) demonstrated that combining lateral and dorsal views yields the most accurate classifications. The LDA analysis shows an average accuracy of 83% for separating rice varieties, with individual accuracies varying by type: approximately 95% for *indica*, and around 78% each for *aus* and *japonica*.

The accuracy of distinguishing archaeological rice varieties using Geometric Morphometrics (GMM) was evaluated by comparing their morphometric values against traditional morphometric measurements of modern charred rice. This comparison revealed a high degree of comparability between the two sets of accessions, further highlighting the efficacy of GMM in distinguishing rice varieties. The datasets, including outlines of the grains, data frames, and

R scripts, are accessible for further research in an open-access repository on the author's Github account (**https://github.com/zami29ju**).

The findings of this study contrast with those of a previous investigation by Tarongi *et al.* (2021), which combined morphometrics and GMM to distinguish pulse varieties, showing a positive contribution from morphometrics. Such a positive correlation was not observed in identifying rice varieties, potentially due to the subtle morphological differences between the rice varieties.

4.4. *Aus* Rice Cultivation in the Lower Brahmaputra Valley

This study identified *aus* type rice within the archaeobotanical rice assemblage at Wari-Bateshwar in the Lower Brahmaputra Valley. This finding marks the first documentation of archaeobotanical *aus* rice from an archaeological context, supporting genetic inferences that suggest it is a distinct Asian rice variety that emerged later than the *japonica* and *indica* varieties (Garris *et al.* 2005; Londo *et al*. 2006; McNally *et al.* 2009; Choi *et al*. 2017; Huang & Han 2016). Traditionally, *aus* type rice has been cultivated in the eastern regions of South Asia, particularly in Bangladesh, Assam in northeast India, West Bengal, and Odisha in Eastern India (Pearson *et al.* 1999; Garris *et al.* 2005; Wang *et al.* 2013; Travis *et al*. 2015).

Aus rice is genetically distinct, exhibiting a high genetic variation, with 98% of loci identified as polymorphic and an average of 5.1 alleles per locus. Additionally, several chloroplast haplotypes indicate diverse genetic backgrounds (Garris *et al.* 2005). This variety exhibits adaptability to drought and flood conditions, is photoperiod-sensitive and is suited for cultivation as a short-season crop. Modern *aus* landraces are grown in various ecological settings, ranging from rainfed upland to lowland deepwater environments, which reflects their diverse adaptability (Londo *et al.* 2006; Bin Rahman & Zhang 2016;). However, this adaptability often results in lower yields and poorer quality compared to other rice varieties. It can be hypothesised that this rice could have been cultivated or managed by local inhabitants not only in the site region but also in the broad area of the GBM delta. Further archaeobotanical sampling into the other parts of the delta would allow us to understand the extent of *aus* rice c ultivation in the early days.

4.5. Single vs Multiple Rice Domestication; Archaeological Insight from the Lower Brahmaputra Valley

The analysis of archaeological rice spikelet bases from Wari-Bateshwar (WB17) reveals a notable increase in non-shattering types and a corresponding decrease in shattering types (Fig.11; Table 4; Paper 3). This trend is consistent with the archaeobotanical rice identification conducted using Geometric Morphometrics (GMM) across the chronological sequence from the 8th to the 4th century BCE, indicating a local domestication process for *aus* rice. These findings provide initial support for the genetic hypothesis of multiple domestication events in rice (Huang *et al.* 2012; Choi *et al.* 2017; Choi & Purugganan 2018) and exclude the notion of a single domestication occurrence in Asia (Vaughan *et al.* 2008; Molina *et al.* 2011).

Further complicating the discourse, Civáň and Brown (2017) reassessed the whole genome sequences of wild and cultivated rice initially analysed by Huang *et al.* (2012). Their findings revealed the presence of domestication genes in wild rice, reinforcing the conclusion that the domestication genes for cultivated rice varieties are derived from their wild progenitors rather than through introgression. This hypothesis suggests that rice originated in distinct geographic regions, independently inheriting domestication genes from their wild ancestors.

By contrast, Huang & Han (2016) and Choi & Purugganan (2018) re-examined the same accessions of wild and cultivated rice studied by Huang *et al.* (2012). They concluded that domestication genes were transferred from an initially domesticated *japonica* variety to the later domesticated *indica* and *aus* varieties. This led to the proposal of a hypothesis suggesting multiple origins but a single (*de novo*) domestication event (summarised in Table 1 of Paper 3).

This study sheds light on the above debates employing data analysis of archaeological rice grains (Paper 2) and rice spikelet bases (Paper 3) from Wari-Bateshwar. However, understanding the domestication process through archaeobotanical findings is challenging. Finding *japonica* and *indica* rice alongside *aus*/proto-*aus* across the stratigraphic sequence of WB17 (Paper 2) linking with the rice spikelet bases results (Paper 3) implies that during the *aus* rice domestication process, *japonica* and *indica* were consistently present. This presence reinforces the possibility of transferring the domesticated genes to *aus* from *japonica* rather than inheritance from wild progenitors, supporting the hypothesis of multiple origins but single (*de novo*) domestication of Asian rice.

The concept of the "independent origin of rice" has been critically reassessed and reconceptualized by Fuller (2011), who highlights the emergence of distinct regional patterns of rice cultivation. He argues that these patterns should be understood within the framework of established traditions and management practices aimed at economic sustainability, rather than solely focusing on the genetic changes typically associated with domestication. This perspective is particularly relevant to the domestication of *aus* rice in the Lower Brahmaputra Valley, where innovative rice cultivation systems in the deltaic plains of the Bengal frontier significantly contributed to new cultural and political developments.

In early Wari-Bateshwar, rice production emerged as a vital component of the food production systems that not only sustained a growing population but also facilitated the development of urban societies. These agricultural practices played a crucial role in driving the socio-economic transformations of the region.

4.6. Establishing 'Intermediate' Type Rice Spikelet Bases

Morphological analysis of a total of nine accessions of modern wild and domesticated rice spikelet bases from Bangladesh, China and Oxford University's Botanical Garden (Table 2; Paper 3) implies the existence of a type of rice spikelet base that is distinct from typical wild and domesticated types (Fig. 9; Paper 3) (Thompson 1996; Fuller *et al.* 2009). This type is characterised by a relatively smooth vascular bundle, with pedicel material remaining in the vascular pore. These findings align with the archaeological evidence from the Lower Yangtze Valley identified as intermediate type rice spikelet bases (Zheng *et al.* 2016). The intermediate type is distinct from wild and domesticated varieties, where the wild spikelet base is identified through its smooth vascular bundle and small, smooth vascular pore, along with the relatively rounded front appearance of the vascular bundle. We recognise the conical shape for wild type spikelet bases in the increasing number of modern wild accessions as opposed to wide bases for domesticated and intermediate types. Domesticated spikelet bases are characterised by their rough, shattered vascular bundle, large, broken pores, and scars due to tearing. Based on the above criteria in modern rice spikelet bases, archaeobotanical rice spikelet bases are categorised as wild, domesticated, intermediate and immature, while Fuller *et al.* (2009) categorised all but the intermediate type. Finding significant numbers of intermediate type spikelet bases in the archaeobotanical assemblages of WB17 supports the hypothesis that intermediate type rice might have been the immediate progenitor of domesticated rice varieties (Sano *et al.* 1980; Oka 1988). The intermediate type might have been a wild-cultivated variety that could have evolved through introgression between co-occurring wild and cultivated varieties, that subsequently adapted in the local cultivated environment, some being feral or weedy types (Oka 1988; 21).

4.7. Final Remarks

This study has yielded significant findings regarding the history of rice in the frontier zone of Bengal, which sits at the conjunction of South, Southeast and East Asian regions. By establishing a heating regime of 230^{0}°C for 2-3 hours, this research successfully replicated well-preserved archaeological rice grains, facilitating the further development of morphometrics and Geometric Morphometrics (GMM) methods for identifying rice varieties at the varietal level.

The thesis employed GMM techniques to separate modern Asian rice varieties and subsequently applied to the archaeological rice assemblage, allowing for their successful segregation into specific varietal categories. Notably, this research documents the *aus*-type rice for the first time within an archaeological context.

Analysis of the archaeological rice spikelet morphology from Wari-Bateshwar indicated an increasing prevalence of non-shattering types, suggesting that the domestication process of *aus* rice likely occurred in this region. Additionally, modern rice spikelet base studies reveal a distinct intermediate type of spikelet base, supporting the hypothesis that this intermediate type could be the immediate progenitor of domesticated rice varieties.

Moreover, two sets of new radiocarbon dates have played a pivotal role in confirming the expansion of the habitation sequence at Wari-Bateshwar between the 8th century BCE and the early 2nd century BCE. These dates have significantly enhanced our understanding of rice chronology in the Bengal frontier, indicating that *japonica* and *indica* rice were introduced by at least the 6th century BCE, coinciding with an ongoing local *aus* rice domestication process. Ultimately, these findings suggest that the domestication process was completed by the early 4th century BCE.

References

Berihuete-Azorín, M., Stika, H.-P., Bourliva, A., Papadopoulou, L. & Valamoti, S-M. 2019. "Fresh from the oven": experiments on triticum spelta and a protocol for carbonising specimens for archaeobotanical comparison collections. *Journal of archaeological science, reports*, 26, 101865.

Bin Rahman, A. N. M. R., & Zhang, J. 2016. Flood and drought tolerance in rice: opposite but may coexist. *Food and Energy Security* 5(2). pp76-88.

Boardman, S., Jones, G., 1990. Experiments on the effects of charring on cereal plant components. *Journal of Archaeological Science*, 17, 1-11.

Bonhomme, V., Forster, E., Wallace, M., Stillman, E., Charles, M. & Jones, G. 2017. Identification of inter- and intra-species variation in cereal grains through geometric morphometric analysis, and its resilience under experimental charring. *Journal of Archaeological Science,* 86**,** 60-67.

Bronk Ramsey, C. 2009. Bayesian analysis of radiocarbon dates. *Radiocarbon***,** 337-360.

Bronk Ramsey, C. 2021. OxCal v4. 4.4. *https://c14. arch. ox. ac. uk/oxcal. html.*

Castillo, C., Tanaka, K., Sato, Y.-I., Ishikawa, R., Bellina, B., Higham, C., Chang, N., Mohanty, R., Kajale, M. & Fuller, D. 2016. Archaeogenetic study of prehistoric rice remains from Thailand and India: evidence of early *japonica* in South and Southeast Asia. *Archaeological and Anthropological Sciences,* 8**,** 523-543.

Castillo, C. C. 2019. Preservation bias: is rice overrepresented in the archaeological record? *Archaeological and Anthropological Sciences,* 11, 6451–6471.

Charles, M., Forster, E., Wallace, M. & Jones, G. 2015. "Nor ever lightning char thy grain" 1: establishing archaeologically relevant charring conditions and their effect on glume wheat grain morphology. *Science and Technology of Archaeological Research,* 1, 1-6.

Choi, J.Y., Platts, A.E., Fuller, D.Q., Hsing, Y-I., Wing, R. A., & Purugganan, M.D., 2017. The Rice Paradox: Multiple Origins but Single Domestication in Asian Rice. *Mol. Biol. Evol.,* 34(4), 969–979.

Choi, J. Y. & Purugganan, M. D. 2018. Multiple origin but single domestication led to Oryza sativa. *G3 (Bethesda),* 8**,** 797-803.

Civáň, P., Brown, T. A. 2017 Origin of rice (Oryza sativa L.) domestication genes. *Genet Resour Crop Evol,* 64**,** 1125–1132.

Garris, A. J., Tai, T. H., Coburn, J., Kresovich, S. & Mccouch, S. 2005. Genetic structure and diversity in Oryza sativa L. (Author Abstract). *Genetics,* 169**,** 1631.

Garton, D. 1979. A study of the effects of charring on rice grains. Unpublished B.A. thesis in the Institute of Archaeology, UCL. London.

Fuller, D. Q., Harvey, E. & Qin, L. 2007. Presumed domestication? Evidence for wild rice cultivation and domestication in the fifth millennium BC of the Lower Yangtze region. *Antiquity,* 81**,** 316-331.

Fuller, D. Q., Qin. L., Zheng. Y., Zhao. Z., Chen. X., Hosoya. L. A., Sun, G-P. 2009.The Domestication Process and Domestication Rate in Rice: Spikelet bases from the Lower Yangzte. *Science,* 323**,** 1607-1610.

Fuller, D. Q. 2011. Pathways to Asian Civilizations: Tracing the Origins and Spread of Rice and Rice Cultures. *Rice (New York, N.Y.),* 4**,** 78-92.

Huang, X., Kurata, N., Wei, X., Wang, Z.-X., Wang, A., Zhao, Q., Zhao, Y., Liu, K., Lu, H., Li, W., Guo, Y., Lu, Y., Zhou, C., Fan, D., Weng, Q., Zhu, C., Huang, T., Zhang, L., Wang, Y., Feng, L., Furuumi, H., Kubo, T., Miyabayashi, T., Yuan, X., Xu, Q., Dong, G., Zhan, Q., Li, C., Fujiyama, A., Toyoda, A., Lu, T., Feng, Q., Qian, Q., Li, J. & Han, B. 2012. A map of rice genome variation reveals the origin of cultivated rice. *Nature,* 490**,** 497-501.

Huang, X. & Han, B. 2016. Rice domestication occurred through single origin and multiple introgressions. *Nat Plants,* 2**,** 15207-15207.

Londo, J. P., Chiang, Y. C., Hung, K. H., Chiang, T. Y. & Schaal, B. A. 2006. Phylogeography of Asian wild rice, Oryza rufipogon, reveals multiple independent domestications of cultivated rice, Oryza sativa. *Proc Natl Acad Sci U S A,* 103**,** 9578-9583.

McNally, K. L., Childs, K.L., Bohnert, R., Davidson, R.M., Zhao, K., Ulat, V.J., Zeller, G., Clark, R.M., Hoen, D.R., Bureau, T.E. & Stokowski, R 2009. Genome wide SNP variation reveals relationships among landraces and modern varieties of rice. *Proceedings of the National Academy of Sciences,* 106**,** 12273-12278.

Molina, J., Sikora, M., Garud, N., Flowers, J. M., Rubinstein, S., Reynolds, A., Huang, P., Jackson, S., Schaal, B.A., Bustamante, C. D., Boyko, A. R. & Purugganan, M. D. 2011. Molecular evidence for a single evolutionary origin of domesticated rice. *Proc Natl Acad Sci U S A,* 108**,** 8351-8356.

Oka, H. I. 1988. *Origin of cultivated rice.* Tokyo: Elsevier Science Pub. Co.

Padhan, T. 2023. New evidence of Neolithic industries from the West Garo Hills, northeastern India. *In:* Tiwari, N., Singh, V. & Mehra, S. B. (ed.), *Quaternary Geoarchaeology of India.* Special Publications, 515, London Geological Society. 125-136.

Parsons, B. J., Newbury, H. J., Jackson, M. T., & Ford-Lloyd, B. V. 1999. The genetic structure and conservation of aus, aman and boro rices from Bangladesh. *Genetic resources and crop evolution,* 46**,** 587-598.

Reimer, P. J. et al. 2020. The IntCal20 Northern Hemisphere radiocarbon age calibration curve (0-55 cal kBP). *Radiocarbon,* 62**,** 725-757.

Roushannafas, T., Bogaard, A. & Charles, M. 2022. Geometric morphometrics sheds new light on the identification and domestication status of ‘new glume wheat’ at Neolithic Çatalhöyük. *Journal of Archaeological Science,* 142.

Sano, Y., Morishima, H. & Oka, H.-I. 1980. Intermediate perennial-annual populations of Oryza perennis found in Thailand and their evolutionary significance. *The Botanical Magazine Tokyo,* 93**,** 291-305.

Schatz, M. C., Maron, L. G., Stein, J. C., Hernandez, W. A., Gurtowski, J., Biggers, E., Lee, H., Kramer, M., Antoniou, E., Ghiban, E., Wright, M. H., Chia, J-M., Ware, D., Mccouch, S. R. & Mccombie, W. R. 2014. Whole genome de novo assemblies of three divergent strains of rice, Oryza sativa, document novel gene space of aus and indica. *Genome biology,* 15**,** 506.

Silva, F., Weisskopf, A., Castillo, C., Murphy, C., Kingwell-Banham, E., Qin, L. & Fuller, D. Q. 2018. A tale of two rice varieties: Modelling the prehistoric dispersals of japonica and proto-indica rices. *Holocene (Sevenoaks),* 28**,** 1745-1758.

Stroud, E., Charles, M., Bogaard, A., Hamerow, H. 2023. Turning up the heat: Assessing the impact of charring regime on the morphology and stable isotopic values of cereal grains. *Journal of Archaeological Science* 153.

Tarongi, M., Bonhomme, V., Evin, A., Ivorra, S., López, D., Alonso, N. & Bouby, L. 2020. A new way of seeing pulses: preliminary results of geometric morphometric analyses of Iron Age seeds from the site of La Font de la Canya (Barcelona, Spain). *Vegetation history and archaeobotany,* 30**,** 77-87.

Thompson, G. B. 1996. The rice remains. *In:* HIGHAM, C. F. W. (ed.) *The excavation of Khok Phanom Di, a prehistoric site in central Thailand: subsistence and environment: the botanical evidence (the biological remains, part II).* London.: The Society of Antiquaries of London, London.

Travis, A. J., Norton, G. J., Datta, S., Sarma, R., Dasgupta, T,, Savio, F. L., Macaulay, M., Hedley, P.E., McNally, K. L., Sumon, M. H., Islam, M. R. & Price, A. H. 2015. Assessing the genetic diversity of rice originating from Bangladesh, Assam and West Bengal. *Rice,* 8:35.

Vaughan, D.A., Lu, B-R. & Tomooka, N. 2008. Was Asian Rice (Oryza sativa) Domesticated More Than Once? *Rice (New York, N.Y.)*, 1**,** 16-24.

Wang, M., Zhu, Z., Tan, L., Liu, F., Fu, Y., Sun, C., & Cai, H. 2013. Complexity of indica-japonica varietal differentiation in Bangladesh rice landraces revealed by microsatellite markers. *Breeding Science*, 63**,** 227-232.

White, C., Toro, F. & White, J. 2019. Rice carbonization and the archaeobotanical record: experimental results from the Ban Chiang ethnobotanical collection, Thailand. *Archaeological and Anthropological Sciences*, 11, 6501-6513.

Zheng, Y., Crawford, G. W., Jiang, L. & Chen, X. 2016. Rice Domestication Revealed by Reduced Shattering of Archaeological rice from the Lower Yangtze Valley. *Sci Rep*, 6**,** 28136-28136.