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A microCT protocol for the visualisation and identification of domesticated plant remains within pottery sherds

Aleese Barron*, Tim Denham

School of Archaeology and Anthropology, Banks Building, Australian National University, Canberra, ACT 2601, Australia

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ABSTRACT

In tropical, arid and semi-arid environments, archaeobotanical preservation is often relatively poor and, historically, archaeobotanical extraction techniques have been inconsistently applied. As a result, the surface impressions of plants in organic-tempered pottery sherds have been relied upon to explore questions of past human-plant relationships, including domestication. Traditional imaging techniques used to study the morphology of plant impressions have significant limitations including being restricted to imaging visible external surfaces and the difficulty of analysing three-dimensional morphologies in two dimensional images. These limitations can now be overcome through microCT scanning, a major methodological advance, which is relatively non-destructive and enables high resolution and *in situ*, three-dimensional visualisation of internal organic inclusions and impressions. This paper outlines the protocol for image capture, visualisation and qualitative analysis of domesticated rice (*Oryza sativa*) spikelet bases and husks, among other organic and inorganic materials, preserved in pottery.

1. Introduction

The investigation of cereal domesticates in some parts of the world is heavily reliant on the identification of husks or spikelet bases from impressions and inclusions within pottery. For instance, current interpretations of the antiquity and spread of domesticated rice (*Oryza sativa*) in Island Southeast Asia is heavily reliant on a relatively small number of rice husk impressions and inclusions in pottery sherds, for instance from Gua Sireh, c. 3850 ± 260 BP (Bellwood et al., 1992) and Anadarayan, c. 3400 ± 125 BP (Snow et al., 1986), despite the absence of comparable archaeobotanical data for cultivars, contemporaneous palaeoecological shifts suggestive of agricultural practices and material cultural evidence for a transition in human subsistence strategies (Bulbeck, 2008; Donohue and Denham, 2010). Pottery-based inferences regarding the presence and dispersal of domesticates generally occur where macrobotanical preservation is poor and archaeobotanical recovery (macrofossil and microfossil) is not systematically undertaken, which includes regions of the perhumid tropics, as well as arid and semi-arid savannah (Amblard and Pernes, 1989; Fuller et al., 2007a; Manning et al., 2011; Winchell et al., 2017). Despite widespread application, existing methods for the identification of domesticated cereals within pottery sherds are limited.

Here, a microCT protocol is presented as a methodological advance over existing techniques to investigate organic inclusions within pottery sherds at archaeological sites, primarily because it enables high resolution and *in situ*, three-dimensional examination of organic inclusions and impressions. The preliminary results for pottery sherds from multiple early Neolithic sites in Vietnam have indicated the capability of microCT to address this archaeological problem (Barron et al., 2017). In this paper, a detailed protocol for the microCT investigation of domesticated (and other) plant remains in pottery sherds is presented. Current methods are reviewed and limitations highlighted, with specific reference to the dispersal of domesticated rice in Southeast Asia. The new protocol for the microCT investigation of pottery sherds details sample preparation, image capture and processing, and creation of 3D visualisations. The methodological advantages of microCT over current techniques and its future potential for the investigation of archaeobotanical remains within ceramics and similar materials, such as mudbrick, are indicated.

2. Identifying domesticated rice in pottery

Until recently, archaeobotanical field methods were not systematically applied in Island Southeast Asia and few macrobotanical assemblages were recovered for analysis (Paz, 1999, 2002, 2005; Castillo and Fuller, 2010). Consequently, the analysis of surface impressions

* Corresponding author.
E-mail address: Aleese.Barron@anu.edu.au (A. Barron).

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and inclusions of rice in pottery has greatly influenced regional models of the spread of rice cultivation (Yen, 1982; Snow et al., 1986; Bellwood et al., 1992; Thompson, 1992; Doherty et al., 2000; Vanna, 2002). These small hints of rice in archaeological contexts have been extrapolated to build complex models of agricultural dispersal (such as Bellwood, 1996, 1997, 2005; Higham and Lu, 1998; Higham, 2002; Diamond and Bellwood, 2003). Arguably, the quantity and quality of the fragmentary evidence does not justify claims for large-scale cultivation and/or consumption especially in Island Southeast Asia (Paz, 1999; Bulbeck, 2008; Castillo and Fuller, 2010; Donohue and Denham, 2010; Barton, 2012; Barker and Richards, 2013; Denham, 2013). In addition, the majority of instances of early archaeological rice in the region have not been analysed in terms of whether they were harvested from wild or domesticated rice populations. Recent work in mainland Southeast Asia, particularly modern-day Vietnam and Thailand, has sought to redress the lack of direct archaeobotanical evidence by applying extensive and systematic archaeobotanical sampling, including spikelet base analysis (Castillo, 2011; Castillo et al., in press).

2.1. Differentiating wild and domesticated rice

The identification of domesticated rice (*Oryza sativa*) is based on the discrimination of domestic-type spikelet bases from those of the wild progenitor complex (*Oryza rufipogon* and *Oryza nivara*; Fuller et al., 2010), as well as potentially other species of wild rice. Several methods have been used to differentiate wild species from domesticated rice varieties. As for other seed-propagating domesticates, the measurement and comparison of grain size has been widely used to infer domestication status (*e.g.*, Chen and Hedges, 1994; Jiang and Liu, 2006). People preferentially selected plants, either consciously or unconsciously, with larger grain sizes to maximise germination in cultivated soils and to maximise nutritional output. While this method of differentiation is still employed, it is not necessarily diagnostic as there is often significant overlap between grain sizes of different rice species, mature and immature grains, as well as between wild and domesticated types (Fuller et al., 2007b, 2007c, 2010; Liu et al., 2007; Crawford, 2012).

Other methods employed to differentiate domesticated rice focus on changes in the shape and size of diagnostic rice phytoliths, namely the bulliform (Fujiwara, 1976; Lu et al., 2002; Huan et al., 2015) and double-peaked glume cells (Zhao et al., 1998; Gu et al., 2013; Wu et al., 2014) produced by the leaves of rice plants. The anthropic and environmental contributions to changes in the morphologies of these phytoliths are poorly understood and have yet to be definitively linked to the cultural processes of domestication. Therefore the robustness of phytoliths for the determination of domesticated rice requires further study (Harvey and Fuller, 2005; Fuller et al., 2009).

Currently, the most robust methodology to differentiate domesticated from wild rice is the morphological examination of the abscission scar on spikelet bases. The technique was first developed by Thompson (1992) on archaeobotanical remains from Khok Phanom Di, Thailand. Thompson observed the natural dispersal mechanism of rice plants leaves a distinctive scar on the spikelet base, namely, the point where the seed attaches to, and detaches from, the plant. This scar can be differentiated morphologically as either wild or domesticated, with allowance for the identification of intermediate and immature grains (Fuller et al., 2009; Crawford, 2012; Zheng et al., 2016), enabling a determination of domestication status. The proportions of wild and domesticated spikelet bases can then be used as a potential indicator of stage in the domestication process for an archaeobotanical assemblage (Fuller et al., 2007b, 2009, 2010).

Fuller et al. (2009) and Fuller (2011) have refined Thompson's technique for the investigation of early, rice-based agricultural sites in China. Their work highlighted a third type of spikelet base morphology, in addition to wild and domesticated types, consistently identified in archaeobotanical assemblages: an immature type removed from the plant before reaching full maturation (Fuller and Qin, 2008). Immature spikelet bases are present in significant numbers at early agricultural sites in the Yangtze River Valley during all stages of the domestication process (Fuller and Qin, 2008; Fuller et al., 2010; Fuller, 2011; Deng et al., 2015). The occurrence of immature spikelet bases reflects the asynchronous rate of maturation expected within a wild plant population.

Additionally, the transition from wild to domesticated rice, like other crops, is likely to be a continuum during the initial domestication episode (Crawford, 2012). Although spikelet bases are characterised as either ‘wild’ or ‘domesticated’, intermediate forms may be present. Currently, intermediate stages along a plausible domestication continuum have not been clearly identified from imaging of spikelet bases, but may potentially be differentiated through cellular level investigation of the abscission scar (Zheng et al., 2016).

2.2. Rice in pottery

The methods employed to analyse rice and other organic inclusions in tempered pottery have yielded mixed results. The most common technique is to view sherd surfaces under an optical microscope or scanning electron microscope (SEM) and photograph or image, respectively, surface impressions of organic tissues that were combusted during the pottery firing process (Yen, 1982; Snow et al., 1986; Thompson, 1992; Doherty et al., 2000; Vanna, 2002). While organic patterning and structures can be identified in these surface impressions they can only present a partial view of three dimensional objects and do not necessarily capture the morphology of the abscission scar on the spikelet base needed to differentiate between species and determine domestication status. Namely, the distinctive ‘checkerboard’ patterning of rice husk impressions in pottery has often been used to identify the introduction of rice agriculture into Island Southeast Asia, yet it need not be definitive; the key differentiating criterion to identify the presence of the domesticated *Oryza sativa* is the presence of non-shattering spikelet bases.

While surface imaging of rice husk impressions in pottery can indicate the potential presence of rice, they are not usually diagnostic of domesticated rice or the development of cereal agriculture. For example, husk impressions do not enable discrimination between *O. sativa* and a wild progenitor such as *O. rufipogon* (Fuller et al., 2009). Furthermore, spikelet bases are hard to identify, describe and image in surface impressions using optical and electron microscopy due to the partial nature of negative imprints visible on the pottery surface. These characteristics make the probability of finding even a single spikelet base per sherd extremely low. Even if spikelet base impressions are identified on pottery surfaces it is difficult to image them at the correct angle for the necessary morphological assessment of the abscission scar.

Imaging problems can be resolved by taking moulds of the pottery surface in order to view a positive replica of combusted organics. Silicone dental impression material is often used to take moulds of organic impressions in order to identify the plant species present (*e.g.*, Winchell et al., 2017). Moulds are then imaged using an optical or electron microscope. Although enabling three-dimensional imaging of spikelet base impressions, the use of moulds is limited by the occurrence of relevant morphological characteristics, namely, spikelet base impressions exposed on the surface of pottery sherds.

Archaeobotanists have attempted to manually remove preserved rice husks from Southeast Asian pottery sherds for morphological analysis (Thompson, 1992). This process involves breaking sherds to reveal concealed organic tissues within the clay matrices. The fragile organic tissues are then removed manually using tweezers and viewed under a microscope in the hope of finding rice husks and spikelet bases for analysis. When Thompson carried out this process, the rate of successfully extracting and imaging spikelet bases was extremely low (Thompson, 1992: 190–193). In most cases, the organic tissues would be too damaged to be able to confidently determine domestication