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Holocene grinding stones at Madjedbebe reveal the processing of starchy plant taxa and animal tissue

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ABSTRACT

The functional study of ground stone artefacts and the analysis of charred plant remains together demonstrate that plant foods played a significant role in the diets of Aboriginal Australians through all occupation phases at the Pleistocene-aged archaeological site of Madjedbebe. Here we report studies of three sandstone grinding stones from the Holocene levels of the site, one associated with a radiocarbon age of 690 cal. BP, and the others with an age of 8320 cal. BP. The functional analyses involved technological studies combined with brightfield microscopy, starch grain analysis, biochemical testing and gas chromatography-mass spectrometry (GC–MS). All three tools had usewear consistent with plant processing, with two having abrasive smoothing and polish characteristics typical of seed-grinding. Significant quantities of starch were recovered from each artefact and demonstrate the early Holocene processing of waterilly (*Nymphaea violacea*) and possibly kapok bush root (*Cochlospermum fraseri*), cheeky yam (*Amorphopallus galbra*) and long yam (*Dioscorea transversa*). In addition to starchy plant foods, one of the tools was used for processing animal tissue, as indicated by biochemical testing and GC–MS analysis, inferring a multi-functional use.

1. Introduction

Globally, grinding stones are typically used as food processing tools and have been documented archaeologically from as early as 780,000 years ago (Goren-Inbar et al., 2002). In Australia it has been argued that grinding/pounding stones played a key role in exploiting hard-to-digest starchy plant foods. In the arid and semi-arid zones of the continent, grass seeds, other hard-cased seeds, tubers and corms along with pulverized animals such as lizards and small mammals, formed important dietary components (e.g., Balme et al., 2001; Cane, 1987; 1989; 1980; 1986; Gorecki et al., 1997; Gould, 1977; Latz, 1995; O'Connell et al., 1983; Smith, 1985). Functional studies that incorporate technological, usewear and residue analyses have demonstrated the use of grinding/ pounding stones to process grains and other starchy foods from at least 25–32,000 years ago in inland central and southeast Australia (Fullagar and Field, 1997; Fullagar et al., 2008; 2015) and possibly even earlier in northern Australia (Clarkson et al., 2015; 2017b; Hayes, 2015). Other functional studies have shown that grinding stones from more recent

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contexts were used to process animal tissues (e.g., Balme et al., 2001; Fullagar et al., 2015; 2017; Hayes, 2015; Owen et al., 2019; Smith et al., 2015; Stephenson, 2015), pigments (e.g., Balme et al., 2001; Hayes, 2015; Huntley et al., 2020), toxic nuts and fruits (e.g., Cosgrove et al., 2007; Field et al., 2009; Smith, 1982) and various plant components including leaves, seeds, sporocarps, roots, fruits, non-toxic nuts and kernels (e.g., Balme et al., 2001; Fullagar and Wallis, 2012; Fullagar et al., 2017; Hayes et al., 2018a; Owen et al., 2019; Pardoe et al., 2019; Smith et al., 2015; Veth et al., 1997; Wallis et al., 2020). The aim of this paper is to report the results of a functional analysis of three Holoceneaged sandstone grinding implements from the Madjedbebe rock shelter in northern Australia (Clarkson et al., 2017a; 2017b), associated with radiocarbon ages of 690 cal. BP (L49, UPGS 2) and 8320 cal. BP (GS3).

2. Background

Functional studies of artefacts require multiple lines of evidence for confident interpretations of tasks and use. These include tool design and morphology, wear traces, experimental studies, residues (including microscopically visible structures and invisible, adsorbed molecules), archaeological context, and relevant historical and ethnographic data (Fullagar, 2014; Rowan and Ebeling, 2008). In Australia, grinding stones recovered from archaeological contexts occur mostly as small fragments, making it difficult to extrapolate their original shape or size. Consequently, the best indicators of artefact function are specific usewear traces and associated residues in combination with tool-use experiments (Fullagar et al., 2015). Experimental studies of grinding stones have indicated that usewear can be diagnostic of certain tasks and types of processed material over a variety of stone materials (e.g., sandstone, basalt, schist) (e.g., Adams, 2014; Dubreuil, 2004; Fullagar et al., 2012; 2017; Hamon, 2008; Hayes, 2015; Hayes et al., 2018b). The presence of use-related residues can potentially indicate tool function with identification of the worked material(s). Mechanically damaged residues are more common on grinding stones compared with other tools owing to the mechanical forces associated with grinding and pounding that cause their structural bonds to be broken, altering their microscopic diagnostic features and making them difficult to identify visually. The use of staining agents has made it possible to identify damaged, degraded or otherwise physically altered residues by binding with specific compounds to cause a distinguishable colorimetric change. A variety of staining agents are available that target specific compounds/groups of compounds and are routinely used in archaeological investigations (e.g., Fullagar et al., 2015; Hayes, 2015; Owen et al., 2019; Smith et al., 2015; Stephenson, 2015).

Secure identification of use-related residues relies on relevant modern comparative reference libraries (e.g., starch, phytoliths, hair, feathers, etc. for the region of interest). Molecular data can be obtained using spectrographic and spectrometric techniques. The identification of plant taxa from starch grains was originally explored by Reichert (1913), who noted that the various shapes and sizes of starch grains can often be (broadly) correlated with the source plant taxa. Starch grains are insoluble carbohydrates formed in the cells of the plant and often have characteristic shapes that vary between species. They consist of an initial growth point—called the hilum—which is surrounded by layers of amylase and amylopectin (forms of carbohydrate) giving the starch its characteristic granular shape (Evert, 2006). Because starch grains are composed entirely of carbohydrate, it is not possible to identify species based on their chemical composition, so taxonomic identification is made based on their morphology.

Over the past two decades there has been an increasing number of reports attributing starch grains to specific plant taxa based on features such as grain shape, maximum length through hilum, and the presence or absence of some morphological features such as fissures, surface 'folds' or lamellae (e.g., Liu et al., 2010; 2014; 2004; Louderback et al., 2017; Musaubach et al., 2013; Piperno et al., 2000). Other researchers have reported descriptions of starch from various families/genera (e.g.

Hall et al., 1989; Mercader et al., 2018) and have noted the difficulties in attributing specific identifying features to any Genus or Family. An important issue is the qualitative nature of these reported claims. Currently, there is no consensus on protocols for such studies and, in some cases, the outcome has been facilitated by the fact that there is an apparently limited number of possible plant taxa available/exploited. Of these, some starches appear to have diagnostically distinct grain shapes/ sizes. Nonetheless, the process of starch grain identification relies heavily on expert input, and the claims for identification to plant taxa are often, in our view, largely speculative.

One of the common measures for starch grain descriptions is maximum length through the hilum for >100 grains, presented as the size range for that species. Field et al. (2009) examined a range of starch grain attributes and demonstrated that this attribute was the best separator for distinguishing between species or excluding noncontributing species (see also Fullagar et al., 2008). Nonetheless, the variability of shape and size within one species has often been argued to exceed that variability between species, not only in length but also in grain shape (Mercader et al., 2018). To improve the positive outcomes for analysis of unknown ancient starch assemblages, a technique was developed for both starch grain features and the subsequent identification of robust classifiers (Coster and Field, 2015; 2018; Field et al., 2016). Rather than using automatic shape recognition, which can alter the perceived shape, in this technique, each starch grain is manually traced and the hilum position located via a digitizing tablet using micrographs of these grains. A range of geometric attributes from the traced starch grain shape can then be extracted and the radial Fourier decomposition of the outline about the hilum position determined. These characteristics can then be compared statistically across large populations of grains derived from the reference species for more reliable identifications.

Non-visible residues may occur on stone tools as films or fine layers of material that can only be detected via chemical analysis using presumptive biochemical tests or spectrographic (e.g., FTIR, Raman microscopy) and spectrometric techniques (e.g., gas chromatography mass spectrometry [GC–MS]). Biochemical tests are used to screen for groups of compounds that indicate the presence of fatty acids, lipids, carbohydrates, proteins and other compound groups. Because these tests are unable to identify individual compounds and only groups of compounds, they are less sensitive than other methods of residue characterization such as GC–MS (see below) and only suitable to be used as an initial screening test. Although the use of biochemical tests in archaeological investigations has a very limited application (but see Fullagar et al., 2015; Hayes, 2015; Matheson and Veall, 2014), it has proven to be useful for screening for certain compounds, requiring only a small amount of residue in solution.

GC-MS is a highly-specific spectrometric method of residue characterization that can identify organic compounds within a residue mixture. Because organic materials at archaeological sites have a biological origin, they occur as highly complex mixtures that become more complex as a result of human activity—for example, pounding animal tissue creates a mixture of bone, collagen and blood, as does the mixing of many biological materials during food preparation and the grinding of different plant foods (Evershed, 2008). This complexity is further increased in archaeological residues that have also experienced compositional alterations due to decay and the mixing of multiple organic components in surrounding sediments during burial. GC-MS provides a means for identifying the molecular components of complex biological mixtures by separating them and characterizing them in detail, making it possible to distinguish the origins of multiple constituents (Evershed, 2008). The method involves creating a "chemical fingerprint" of the residue components within a mixture, then the various components of the residue mixture can be matched with reference data to identify their biological origin. Although most often applied in archaeological investigations to study lipids on ceramics and pot sherds, GC-MS analysis of use-related residues extracted from flaked



Fig. 1. Madjedbebe rock shelter and surrounds. (A) Map of the Northern Territory showing the location of Madjedbebe rock shelter and Kakadu National Park boundary. (B) Arnhem Land Plateau from above. (C) Excavations at Madjedbebe rock shelter, 2012. (Photos: C. Clarkson).



Fig. 2. Illustration and 3D scans of grinding implements examined in this study. From top to bottom: L49, an upper sandstone grinding stone with numerous flake scars; UPGS 2, a small sandstone grinding flake, probably removed from L49; and GS3, a sandstone grinding implement with one ground edge and abrasive smoothing on both surfaces. (Illustrations: Angeliki Theodoropoulou).

Details of the analysed grinding stones from Madjedbebe.

			-	-			
GS no.	Spit/ square	Phase	Age (cal. BP)	Mass (g)	$L \times W \times D$ (mm)	GS Type and Stone Material	Description
L49	C2/5	7	~690	539	154 × 113 × 18	Upper grinding stone fragment; sandstone	 One ground surface with numerous flake removals along the edges Convex cross section Plotted <i>in situ</i>
UPGS 2	C2/5	7	~690	3	$31\times17\times3$	Flake, ground on dorsal surface, cf. L49; sandstone	- One ground surface - Convex cross section - Recovered from sieve residues
GS3	E1/21	5	~8320	408	$\frac{123\times87\times}{28}$	Complete grinding stone; sandstone	 One ground edge (grinding wear on two opposing surfaces), cf. bulga knife Flat cross section Plotted in situ

stone tools (e.g., Luong et al., 2017; 2018; 2019) and grinding stones (e. g., Buonasera, 2007; Hayes, 2015; Mazzia and Flegenheimer, 2015) has also shown some promise.

3. Madjedbebe rock shelter

The grinding stones analysed in this study were recovered from the Holocene levels of Madjedbebe rock shelter-a stratified sandstone rockshelter site located in the Jabiluka Mineral Lease adjacent to the surrounding Kakadu National Park in the Northern Territory. Madjedbebe is located at the head of a low-gradient sand-apron that developed at the base of the Arnhem Land Plateau, approximately 20 m above sea level (Kamminga and Allen, 1973; Roberts et al., 1990; Clarkson et al., 2015; 2017a; 2017b) (Fig. 1). Excavations were carried out in collaboration with the Mirarr Traditional Owners and are described in detail elsewhere (Clarkson et al., 2015; 2017a; 2017b; 2018; Florin et al., 2020). The excavated cultural material include stone artefacts, pigments, shellfish, human burials, faunal remains and charred plant remains. These finds occur in three dense bands ("pulses") with intermediate deposits having fewer artefacts. Seven depositional phases have been identified, six of which are clear occupational phases that have yielded stone artefacts including grinding stones.

The three grinding stones reported here were recovered from Phase 7 (~690 cal. BP) and Phase 5 (~8320 cal. BP) during excavations undertaken in 2012 (Fig. 2; Fig. SII). Grinding stones L49 and GS3 were recovered *in situ* from C2/5 and E1/21 (dated to ~690 years cal. BP and ~8320 years cal. BP; Clarkson et al., 2017a; 2017b), respectively; their exact locations were plotted precisely in 3D using a total station. The remaining specimen, UPGS 2, also recovered from C2/5, was recovered from the sieve during on-site sorting (Table 1). Following excavation, each grinding stone was placed in an individual plastic zip-lock bag and protected with bubble wrap and stored in clean plastic storage tubs at the University of Wollongong. The grinding stones were sampled and analyzed shortly after excavation using the appropriate protective equipment (i.e., powder free gloves) to avoid contamination, and handling was kept to a minimum.

4. Methods

Functional analysis of the three sandstone grinding stones/fragments from Madjedbebe included six components:

- Morphological analysis of grinding stone tool technology/ manufacture;
- 2. Usewear analysis using various optical light microscopes;
- 3. Starch grain analysis;
- 4. Analysis of other extracted residues using transmitted light microscopes and biochemical stains;
- 5. Biochemical residue analysis using a variety of presumptive chemical tests for groups of compounds; and

Table 2

Summar	v of	residue	extractions	removed	from	each	artefact

Tool ID	Pipette extractions (distilled water)	Pipette extractions (EWA solvent)	Ultrasonic Bath (Ancient Starch)	Sediment sample
L49	$1 \times \text{ground}$ surface $1 \times \text{unground}$ surface	$2 \times \text{ground}$ surface	Partial immersion 2 Min	Collected (brushed from artefact)
UPGS 2	$1 \times unground$ surface	$1 \times \text{ground}$ surface	Comp. submersion 2 Min	Not collected
GS3	2 × ground surface 1 × unground surface	$2 \times ground$ surface	Partial immersion 2 Min	Collected (brushed from artefact)

 GC–MS analysis to characterize specific molecules in extracted residue mixtures.

Experimental reference libraries that document usewear and residue traces on Australian sandstone/quartzite grinding/pounding implements have been published elsewhere and assist with our final functional interpretations (see Hayes, 2015; Hayes et al., 2018b).

4.1. Usewear analysis

Usewear on all three grinding tools was examined and documented under low magnification (up to x45) using an Olympus SZ61 stereozoom microscope with an external fibre optic, 150 Watt halogen light source (Olympus LG-PS2) and a Leica MZ16A stereo-zoom microscope with an automated Z-stacking function. Multifocal images were obtained using a DFC320 Leica camera and stacked to create a focused image using Leica LAS V4.4 software. Both microscopes were effective at highlighting the occurrence of broad striations (mostly furrows) across the grinding surfaces. The degree of surface levelling and grain rounding was best observed at lower magnifications, where multiple grains could be viewed in context.

Artefact surfaces were also examined under high magnification using an Olympus metallographic microscope (model BX51) with vertical incident light (brightfield and darkfield) with objective lenses of x50, x100, x200, and x500 and polarizing filters. Detailed examination of use-polish, micro-fractures, micro-striations (including sleeks) and residues located at lower elevations of the surface was undertaken. Micrographs of these features were captured with an Olympus Infinity 2 camera.

4.2. Optical residue analysis

Residue samples were extracted from the used and unused surfaces of each grinding stone using distilled water and a tri-solvent mixture of

List of staining agents applied to extracted residues from each grinding stone. *After* Fullagar et al., 2015 (Table 2).

Staining agent	Chemical formula	Stained material	Colour change
Congo Red	C ₃₂ H ₂₂ N ₆ O ₆ S ₂ Na ₂	Gelatinized starch Damaged starch Cellulose Plant cell walls	Red
Iodine potassium iodide	IKI	Starch Cellulose	Purple
Methylene blue	C16H18N3SCI	Cellulose	Blue
Safranin	C ₂₀ H ₁₉ CIN ₄	Lignin Plant cell walls Plant cell nuclei	Red
Orange G	$C_{16}H_{10}N_2Na_2O_7S_2$	Collagen Keratin	Orange

acetonitrile, ethanol and distilled water with an adjustable pipette and a disposable nylon pipette tip (Table 2). Residue samples were prepared by mounting 5–15 μ L of sample on a clean glass slide (cleaned with ethanol or acetone) with a clean glass cover slip. Slides were scanned with an Olympus BX-51 metallographic microscope and images were captured with an Olympus DP72 Microscope Camera.

A selection of plant-specific stains (Congo Red, Iodine Potassium Iodide, Methylene Blue, Safranin) and one animal-specific stain (Orange G) were applied to slide-mounted samples to confirm the presence of any highly degraded, fragmented or amorphous plant or animal tissues that

Table 4

Curated starchy plant species (21) included in this study.

were unable to be recognized microscopically (Table 3). Approximately $5 \ \mu L$ of staining solution was applied to the edge of the coverslip using a pipette and left for at least 10 min to ensure adequate development time. The stain solution was then rinsed out with distilled water using a pipette. Slides were then examined using the transmitted light microscope to assess colour changes.

4.3. Starch analysis

After pipette extractions had been taken from all three grinding stones, further samples were collected by partial (GS3, L49) or complete (UPGS 2) immersion in an ultrasonic bath (Table 2). The resultant sample plus water was transferred to a 50 ml Falcon tube and centrifuged for 3 min at 3000 RPM. Starch (and phytoliths) were isolated using heavy liquid separation (Sodium polytungstate, Specific Gravity 2.35) by centrifugation for 15 min at 1000 RPM. Following rinsing, samples were slide-mounted in distilled water and examined using a Zeiss Axioskop II brightfield transmitted light microscope under Differential Interference Contrast (DIC). Images were captured using a Zeiss HRc camera, and archived using Zeiss Axiovision software. Starch grain images were subsequently traced with a WACOM Intuos Pen Tablet (CTH-480) using a graphical user interface (GUI) developed in MATLAB by Adelle Coster (MATLAB Release 2014b, The MathWorks, Inc., Natick, MA, USA). The position of the hilum was marked and other morphological features such as fissures, lamellae and facets were noted.

To identify plant taxa from starch grains, a reference collection of well-curated starches of known taxonomic origin from relevant

Genera/Species	Family	Language name (Kundjeyhmi)/ common name	Plant parts	# of grains	Pounded/ ground	References
Amorphophallus galbra (MJB60)	Araceae	An-didjkanku/cheeky yam	Tuber	163	Y	Fox and Garde:119, 2018; Smith, 1991
Buchanania obovata (UOM2779)	Anacardiaceae	An-bikkurrudj/green plum	Fruit	126	Y	Fox and Garde:46, 2018; Atchison et al., 2005
Boerhavia diffusa *	Nyctaginaceae	Tar vine	Root	120	Y	Crawford:47, 1982; O'Connell et al., 1983
Brachychiton Paradoxus (MJB63)	Malvaceae	Kurrajong	Seed	151	Y	Low, 1991. Note restricted to east Arnhem Land; Franklin and Bate, 2013
Brachychiton sp. [^]	Malvaceae	Kurrajong	Seed	166	Y	Fox and Garde, 2018
Carpentaria acuminata (MJB)	Arecaceae	Carpentaria palm	Apical pith	114	Y#	Smith, 1991:15
Causonis cf. trifolia*	Vitaceae	An-kodbe/ three leaved vine	Root	145	Ν	Fox and Garde:151, 2018; Crawford:56, 1982
Cochlospermum fraseri (MJB31)	Bixaceae	An-djedj/kapok bush	Root	133	Y^	Fox and Garde:52, 2018; Crawford:63, 1982
Curculigo ensifolia*	Hypoxidaceae	An-mulbirrk/Anburda/grass vam	Corm	121	Y	Fox and Garde:128, 2018; Crawford:42, 1982
Dioscorea transversa (UOM3095)	Dioscoreaceae	Karrbarda/Long yam	Tuber	113	Ν	Fox and Garde, 2018:158
Ipomoea cf. abrupta; cf. dunlopii	Convulvulaceae	An-burre/An-karrbilk/bush potato	Root	241	Ν	Fox and Garde, 2018:160
I. aff. abrupta (MDB5854)*	Convulvulaceae	An-karrbilk/bush potato	Root	126	Y	Fox and Garde, 2018:161
I. graminea [@]	Convulvulaceae	Bush potato	Root	132	Ν	Fox and Garde:163, 2018; Crawford:69, 1982
Livistona benthamii	Arecaceae	Babinjbabinj/cabbage palm	Apical pith	100	Y	Fox and Garde, 2018:174
L. humilis (UQM3092)	Arecaceae	An-marrabbi/An-kurllurrudj/ common sand palm	Pith	179	Y	Fox and Garde, 2018:176
Marsilea drummondii*	Marsileacae	Nardoo	Sporocarp	200	Y	AVH; not sure it was used here
Nelumbo nucifera (MJB62)	Nelumbonaceae	Wurrmarninj/lotus lily	Seed	182	Y	Fox and Garde, 2018:134
Nymphaea violacea (MDB5961)*	Nymphaeaceae	Wayuk/Karwar/waterlily	Corm	174	Y	Identified by Matt Barrett Fox and Garde, 2018:140
Nymphaea sp. (UQM2770)	Nymphaeaceae	Wayuk/Karwar/Madjakkalang/ Yaldanj waterlily	Seed	250	Y	Fox and Garde, 2018:136-141
Nymphoides indica (UQM3078)	Menyanthaceae	Water snowflake	Seed	127	?	Smith, 1991:44
Sorghum plumosum	Poaceae	Plume Sorghum	Seed	111	Y	Arndt, 1961; Turner:49–50, 1895
Typhonium sp. (MJB61)	Araceae	An-djanek	Corm	140	Y	Fox and Garde, 2018:144–145
Vigna vexillata var. augustifolia (UQM3228)	Fabaceae	An-kornak	Root	148	Y	Thomson, 1939

drought food; # babies; *Kimberley collection at UNSW; ^Kimberley UOW; @Central Australia.

Biochemical tests applied to residues extracted from the three Holocene-aged Madjedbebe grinding stones.

Biochemical test	Compound groups detected	Origin of compound groups
Bradford Assay	Proteins	Plant and/or animal
Copper triethanolamine diphenyl- carbazide (Falholt)	Fatty Acids	Plant and/or animal
Iodine potassium iodide	Starch	Plant
Hemastix®	Haemoglobin (blood)	Animal
Diphenylamine	Carbohydrates	Plant and/or animal
Phenol-Sulphuric Acid (PSA)	Carbohydrates	Plant and/or animal

geographic region was constructed. Comparative starch reference material was compiled from field collections made in Arnhem Land and the Kimberley region of Western Australia (WA) and analysed in the UNSW laboratory. In particular the study has benefited from access to the 1972 Ian Crawford collection (from the Kimberley), accessioned at the WA Museum and curated by Dr Moya Smith, the East Kimberley collection curated by Dr Jenny Atchison accessioned at the University of Wollongong (UOW), and Kimberley field collections by Matt Barrett, India Dilkes-Hall, Richard Cosgrove and Judith Field accessioned at the University of New South Wales (UNSW). The Arnhem Land reference collections were compiled by S.A.F., M.N., Dj.Dj. and E.H. The final reference list included 21 samples from 18 genera (Table 4).

Reference specimens were prepared by grinding various components of each plant taxa in a glass mortar and pestle, smearing the resulting material on a dry slide, and slide mounting with 50% glycerol/water solution before sealing with a clear coverslip and nail varnish. Examination was then undertaken using a brightfield microscope. In order to generate predictor variable values for the starch assemblages from the different taxa, a minimum of 100 grains per single specimen were photographed and traced. Statistical classifiers were constructed from the metrics of the starch grain outlines and hilum placement to separate one species from another across the reference set. Using this process, over 20,000 classifiers of different configurations were constructed and tested; however, it was determined that none provided sufficient clarity to confidently separate the different reference species to a level where they could be attributed to a source (plant species). Thus, an alternate approach to identifying unknown starch grains was developed, used in concert with the classifier outputs. Population comparisons of the normalized and aligned grain shapes (scaled so that the average radius about the hilum was equal to one), in combination with an assessment of the physical size and other abstracted metrics were used to reduce the candidate species. The new method allowed the determination of the correspondence of grains from unknown starch assemblages to the populations of grains from the reference species. The comparative approach was coupled with manual expert inspection to assess the plant species of origin.

4.4. Biochemical testing

Biochemical tests including the Bradford Assay, Copper triethanolamine diphenyl-carbazide (cf. "Falholt" test of Fullagar et al., 2015), Iodine potassium iodide, Hemastix® and the Diphenylamine and the Phenol-Sulphuric Acid test were undertaken on residue mixtures extracted from artefact surfaces. These tests were used to screen for groups of compounds that indicate protein, fatty acids, starch, haemoglobin and carbohydrates, respectively (Table 5). Each test was performed on a small portion of sample ($<5 \mu$ L) and observed for a subsequent positive reaction, indicated by a specific colour detected with an EpochTM Multi-Volume Spectrophotometer System. Standard measurements for the detection of these compound groups were made

Table 6

Summary of usewear	documented or	n archaeological	tools.
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Macro/Low magnification					
L49 UPGS 2 GS3	.49 High grain levelling; high grain rounding; macro striations JPGS 2 High grain levelling; high grain rounding; macro striations GS3 Ground edge: High grain levelling; moderate rounding; macro striations Surface 1: Moderate grain levelling; moderate grain rounding				
High magni	High magnification				
L49 UPGS 2 GS3	Reticular polish with high smoothing; multi-directional striations Reticular polish; multi-directional striations Ground edge: Low polish development Surface 1: Undulating, domed polish; multi-directional striations				

Table 7

Summary of residues documented/detected on archaeological tools. 'Not considered use-related *Identified with stains.

Optical residue Starch Starch Starch	
analysis (pipette Damaged starch* Damaged starch* Damaged	
extractions) Phytoliths Cellulose* starch*	
Cellulose Plant tissue/lignin* Cellulose	
Plant tissue/ Red pigment Plant tissue/	
lignin* Collagen fibre*^ lignin*	
Red + yellow Red pigment [*] Collagen [*]	
pigment^ Red pigment^	
$Starch \ analysis \qquad n=204 \ grains \qquad n=133 \qquad \qquad n=228$	
Possible origin: Possible origin: Origin:	
Cochlospermum Cochlospermum Nymphaea	
fraseri/ fraseri violacea corm	
Amorphophallus	
galbra;	
Dioscorea	
transversa	
Biochemical tests Starch Starch Starch	
Carbohydrates	
Fatty acids Carbohydrate	s
Fatty acids	
GC–MS Unspecified plant Unspecified Unspecified	
plant	
Animal fat	
Degraded blo	od
Handling	
contaminatio	1

on blood protein, corn starch, cooking oil, and a combination of sucrose and glucose, respectively (see Supplementary Information). The readings obtained from the measured standards were considered the minimum values for detection. To assess the possibility of environmental contamination, associated sediment samples were also tested.

4.5. Gas chromatography mass spectrometry

Desiccated residue samples from the water and tri-mixture solvent extractions were prepared for GC–MS by adding 500 μ L of acetonitrile to sample tubes, and allowed to sit for 24 h. The acetonitrile was then transferred to a separate glass vial using a pipette ensuring that no particulate material was present. Before sealing, all oxygen was removed from the glass vial by purging the vial with nitrogen gas and sealing it with an aluminium cap. GC-MS analysis was performed using a Varian model 450 gas chromatograph coupled with a Varian model 300-MS quadrupole mass spectrometer fitted with FactorFourTM capillary column (VF5ms, 30 m \times 0.25 mm ID, DF = 0.25 μm), following the methods described by Crowther et al. (2015:380). The chemical compounds recovered from each residue mixture were identified following the characterization of their ion spectra and the ionization peaks (e.g., the molecular ion, M+ peak, $M\,+\,1$ peak and the various ionization peaks M-15 peaks), using Varian MS Workstation Version 6 and the NIST98 Mass Spectral Database (National Institute of Standards and



Fig. 3. Surfaces of the three grinding stones, documented under the stereo-zoom microscope. (A) Grinding wear on L49, note the highly smoothed and levelled grains on the higher regions of the surface and the more angular grains on the lower portions of the surface that appear unmodified. (B) Grinding wear on UPGS 2; (C) Grinding wear on GS3. (Photos: E. Hayes).

Technology). Compounds were then cross-referenced with published data to enhance taxonomic identification.

5. Results

Our functional analysis has indicated that all three artefacts functioned as grinding stones, with usewear and starch grains providing insight into the specific worked materials. Tables 6 and 7 provide summary data for the usewear and residues documented on each of the three implements using various approaches of analysis. More detailed data are presented in the Supplementary Material. Locations of usewear images and residue samples for each stone are shown in Figs. SII–3.

5.1. Grinding stone morphology/tool descriptions

All three artefacts were made from locally sourced sandstone characterized by a strongly cemented matrix, with rounded, well sorted finegrained quartz (150–200 μ m) making up 96.9% of the sedimentary matrix. The remaining matrix is composed of crystalline illitic clays including kaolin (0.2%) and illite (2.9%) (Hayes et al., 2018b). The sandstone is relatively hard (7 on Mohs hardness scale) compared with other Australian sandstones, which are typically comprised of more illitic clays making them softer and less well-cemented.

All three artefacts had macroscopically visible grinding wear on one or two surfaces and varied in size, ranging from 3 to 539 g (Table 1). L49



Fig. 4. Usewear documented under high magnification on the ground surfaces of L49 (top row), UPGS 2 (middle row) and GS3 (bottom row). (A-B) Reticular usepolish with highly smoothed surface morphology cf. silica gloss from plant processing (C) Polish on L49 with multi-directional striations from stone-on-stone contact; (D-F) Polish on UPGS 2. (G-H) Polish on GS3. (Photos: E. Hayes).



Fig. 5. Residues documented in water extractions sampled from the ground surfaces of L49 (top row), UPGS 2 (middle row) and GS3 (bottom row). (A) Intact plant cells stained with Congo Red; (B) amorphous plant tissue stained with Safranin; (C) Damaged starch grain stained with Congo Red. (D) Starch grains contained within a plant cell, stained with Congo Red. (E) Plant sieve cells stained with Congo Red; (F) Cellulose fibre stained with Methylene Blue. (G) Starch grain stained with IKI; (H) Plant sieve cell (photographed in cross-polarised light); (I) Amorphous collagen tissue stained with Orange G. (Photos: E. Hayes).

is a fragment that has been split transversely with grinding wear present on the upper convex surface (Fig. 2). The convex shape of the ground surface indicates that it was used as an upper grinding stone (cf. "hand stone", "active stone"). It has several negative flake scars on the ground surface along the edge boundary; these may indicate deliberate shaping of the edge or possibly an effort to rejuvenate the ground surface. UPGS 2 is a small flake with grinding wear on the dorsal surface that is convex in section, indicating that it derived from an upper grinding stone-—possibly L49. Both artefacts were found in the same excavation context (i.e., Spit/Square C2/5, Phase 7, see Table 1), but could not be directly refitted. GS3 was recovered from E1/21 (Phase 5) and has wear on the margins of both surfaces to form one ground edge with an edge angle of ~45°. Both surfaces are flat in section and have minimal abrasive smoothing away from the edge.

5.2. Usewear analysis

A summary of the usewear features documented under various magnifications on each artefact is presented in Table 6. All three had macroscopic (visible to the naked eye) usewear indicative of stone-onstone grinding formed during tool manufacture (GS3) or from incidental contact with another stone during use (L49 and UPGS 2). The presence of levelled grains and both micro- and macro-striations provides unequivocal evidence that the sandstone was modified by grinding (Fig. 3).

L49 has one convex surface with highly smoothed, interconnecting zones of levelled and well-rounded grains on the uppermost regions. In the lower regions of the ground surface grains appear to be minimally modified, being angular under low magnification (Fig. 3A). Striations

are only just visible at low magnification, occurring as fine, parallel alignments on the uppermost smoothed regions. Under high magnification, polish appears well developed on the smoothest zones of the ground surface. The polish is bright (highly reflective) and has a reticular texture whereby the highest zones of the quartz grains are well polished compared to the lowermost regions, indicating the grinding of a harder material (Fig. 4A-C). Some of the more extensively polished regions have highly reflective, smoothed zones that resemble silica polish, possibly caused by processing siliceous plants or seeds with a siliceous outer seed husk (Fig. 4A-B). Micro-striations of varying widths occur on grains with the most developed and extensive polish (Fig. 4C).

UPGS 2 has slightly different usewear than that documented on L49—under low magnification, the grains on UPGS 2 are more rounded and there are fewer interconnected levelled zones (Fig. 3B). Striations are common and are mostly oriented in the same direction. At high magnification, a well-developed, reticular use-polish occurs across the entire ground surface, sometimes with fine, parallel alignments (Fig. 4D–F). Unlike that documented on L49, where polish appeared highly smooth in some locations, the polish on UPGS 2 had a rougher texture and is consistent with the processing of a small, hard material, such as seeds. Polish is absent on the exposed grains of the unground surface but micro-fracturing is common and probably occurred when the flake was detached from the larger grinding stone. The lack of developed polish on the unground surface indicates that the polish documented across the ground surface is use-related and not taphonomic.

GS3 has macroscopically visible striations along both sides of the ground edge that most likely represent wear associated with its manufacture (i.e., stone-on-stone grinding to deliberately shape the edge) rather than use. Under low magnification, grains comprising the ground



Fig. 6. Starch grains documented in sonicated water extractions sampled from the ground surfaces of L49 (top row), UPGS 2 (middle row) and GS3 (bottom row). (A) Cluster of starch grains from L49, photographed with polarised light. (B) Tuber-like grain from L49, photographed with DIC. (C) Compound starch grains documented in extractions from L49. (D) Intact cluster of starch grains from UPGS 2, photographed with polarised light. (E-F) Examples of compound starch grains from UPGS 2, photographed with DIC. Note the grain on lower right of image (F) with tuber-like morphology. The black material is carbonised particles. (G-H) Examples of starch grains from GS3, photographed with DIC. (Photos: J. Field).

edge are moderately rounded and levelled, with more marked rounding on one of the surfaces (Surface 1-Fig. SI1). Some small chipping/ flaking is also present along the ground edge but may be the result of post-depositional/excavation damage. Under high magnification, polish appears similar on both surfaces but is only weakly developed with a patchy/discontinuous distribution and is not diagnostic of worked material. Away from the manufacture-ground edge, another patch of distinctive wear was documented on Surface 1 (Fig. SI1), consisting of moderately rounded and levelled grains (Fig. 3C). Under high magnification, polish in this zone was markedly different to that documented on the ground edge, appearing brighter with an undulating, domed morphology that is consistent with polishes documented on sandstone plant- processing tools (Fig. 4G-I). The polish extends into the lower recesses of the grains to indicate a softer plant material. Fine striations were also documented in some of the polished zones but typically only occurred as shallow scratches with no common orientation (Fig. 4I).

5.3. Optical residue analysis

Visible residues were documented on all three artefacts, both *in situ* (directly on the stone surface) under the microscope, and in solvent extractions prepared on glass slides (Table 7). All artefacts had a fine coating of silty sediment that was brushed from the surface prior to examination/residue sampling. Clusters of red and yellow pigment grains were documented on all three grinding stones, both on the ground and unground surfaces. These residues are not considered to be use-related, as they occur in low abundance with no consistent distribution. Instead, we attribute these residues to incidental contact with

haematite during burial, and potentially from handling during excavation. For UPGS 2, which was recovered from the sieve, pigment residues may have been deposited as a result of the artefact coming into contact with haematite pieces during the sieving process.

Plant residues were documented on all three artefacts, both during in situ observations and in residue extractions (Table 7). Plant tissue documented on L49 included cellulose fibres, lignin and both intact and gelatinized starch grains, identified with staining agents Safranin and Congo Red (Fig. 5A-C). Amorphous organic material later identified as plant tissue was documented in residue extractions sampled from UPGS 2, and included cellulose fibres, starch grains and various other plant cells (e.g., sieve cells) (Fig. 5D–F), identified with plant-specific staining agents Methylene Blue and Congo Red. In addition, a single collagen fibre was also documented on UPGS 2, identified with a protein-specific stain Orange G, but its isolated occurrence suggested it was not userelated and has instead been attributed to modern handling contamination. Residues in extracted solutions sampled from GS3 included cellulose fibres, lignin, woody tissue, plant sheaths, starch grains and collagen fibres, identified with staining agents Safranin, IKI and Orange G, respectively (Fig. 5G-I). As multiple collagen fibres were documented on the abraded edge of GS3, we consider them to be related to use.

5.4. Ancient starch analysis

All three grinding stones yielded significant numbers of starch grains: L49, n = 204; UP GS2, n = 133; and GS3 n = 228. The measurement data generated from the 21 reference plant species have indicated potential matches with the unknown starch assemblages



Fig. 7. Stacked and aligned grain shapes, centred about the hilum, for the three Madjedbebe grinding stones analysed in this study. Radii are in μ m and grains are centred at their hilum, GS3 (n=228); L49 (n=204); UPGS 2 (n=133). Note that all plots are on the same scale.



recovered from the archaeological tools. Micrographs of representative grains from each reference plant species are shown in Fig. SI2. Stacked and aligned grain shapes for the three assemblages are presented in Fig. 7 and indicate both the tightly constrained assemblage in GS3 and the variability in L49. Hierarchical clustering of the normalised grain shapes allowed the identification of subgroups of uniform within-subgroup similarity for the reference species and the archaeological samples. The variation is further illustrated in Table SI2 that clearly shows the subgroups of grain shapes in each sample. Box-plots of maximum length through the hilum of subgroups of unknown and the reference set grains suggest the exclusion of a number of the taxa based on this measurement (Fig. SI3).

The GS3 starch assemblage had one major subgroup that was confidently attributed to the waterlily (*Nymphaea violacea* MDB5961) corm (Figs. 7–9)—an aquatic plant usually found in still freshwater environments and identified as a starch staple (see Russell-Smith et al., 1997). The plant part most commonly known to be used are the seeds, which are usually ground into a paste or flour and cooked (Fox and Garde, 2018:140). Seed starch grains are very small and generally <10 μ m in maximum dimension. There have also been observations of the roots/corms of these plants being pounded or ground prior to

consumption (e.g., Clarke, 2014).

Two major subgroups were identified in the L49 starch assemblage and represented two particularly distinctive shapes: small faceted and compound grains (n = 102 grains, 50% of the assemblage); and larger, eccentric hilum, ovoid grains (n = 90 grains, ~44%) (Fig. 6). The former grains most closely aligned with *Cochlospermum fraseri* and *Amorphophallus galbra*, and the latter with *Dioscorea transversa*. Grinding stone UPGS 2, possibly a part of L49, had only one clear subgroup of grains and these most closely aligned to *C. fraseri* (Figs. 6–7; Fig. SI2).

5.5. Biochemical tests

Presumptive biochemical tests detected three compound groups in residue extractions taken from each tool, including carbohydrates (both sugars and starch) and fatty acids (Table 7). Starch was the most commonly detected compound group with positive IKI test readings for all three grinding stones (Table SI9). Given the abundance of starch identified in sonicated residue extractions, positive IKI readings were expected. Other carbohydrate groups were detected on UPGS 2 and GS3 with the PSA and Diphenylamine tests, respectively, in addition to fatty acids, detected with the Falholt test. Proteins were not detected with the



Fig. 9. Plants and associated starch grains known to be consumed by Mirarr people that potentially match the starch grains found on the archaeological tools. (A) *Nymphaea violacea* (Wayuk/Karwarr/waterlily) and starch from the edible corm. (Photos: *left*: ©M.Fagg 1987, supplied by the Australian Plant Image Index; *middle*: S. A. Florin; *right*: J. Field); (B) *Dioscorea transversa* (Karrbarda/Kayawal/long yam) and starch from the edible tuber. (Photos: *left, middle*: S.A. Florin; *right*: J. Field); (C) *Cochlospermum fraseri* (An-djedj/kapok bush) and starch from the edible root. (Photos: *left, middle*: S.A. Florin; *right*: J. Field); (D) *Amorphophallus galbra* (An-didj-kanku/cheeky yam) and starch from the edible tuber. (Photos: *left, middle*: @R.L. Barrett; *right*: J. Field).

Bradford Assay test in any of the extracted residue solutions but were detected in sediment samples from L49 and GS3. The Hemastix® test was negative for all artefacts, indicating an absence of blood or iron-rich minerals (e.g., haematite, manganese). The precise amounts of each of the detected compound group are given in the Supplementary Information (Table SI9), with positive compound groups listed for each artefact below.

5.6. Gas chromatography mass spectrometry

Organic compounds were detected via GC–MS in residue solutions extracted with the tri-mixture solvent (EWA) from L49 and GS3, including plant and animal-derived compounds to indicate plant processing (L49) and multifunctional use (GS3) (Table 8). Among other compounds, the plant derived compound 2-ethylhexanoic acid was detected in residue extractions from both L49 and GS3, indicating that

GS no.	Compound detected	Origin
L49	2-hydroxypropanoic acid	Lactic acid
	oxanilic acid	Unknown
	2-ethylhexanoic acid	Plant
UPGS 2	None detected	-
GS 3	N- <i>tert</i> -butylacetamide	Unknown
	2-ethylhexanoic acid	Plant
	2,4-diphenyl-4-methyl-2(E)-pentene	Unknown
	2-phenyl-2-oxophenyl-propane	Unknown
	hexadecanoic acid	Plant, animal, beeswax, handling contamination
	cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta., 4.alpha.)-	Unknown
	2,4-bis(dimethylbenzyl)-6-t-butylphenol	Plant
	N-tert-butylacetamide	Unknown
	4-(1,1,3,3-tetramethylbutyl) phenol	Unknown
	2-phenyl-2-oxophenyl-propane	Unknown
	dodecandioic acid	Plant
	(2,8,12,18-tetraethyl-3,7,13,17-tetramethyl-21H, 23H-porphinato(2-)-N21,N22,N23,N24)-, (SP-4–1)-	Degraded porphyrin (blood cell)
	2,4-diphenyl-4-methyl-2(E)-pentene	Unknown
	cis-10-heptadecenoic acid	Animal fat
	hexadecanoic acid butyl ester	Plant

Compounds detected with GC–MS on L49 (ground surface), UPGS 2 (ground surface) and GS3 (ground edge of Surface 1). For references, see Supplementary Information Table SI10. Note that some compounds may originate from more than one source.

both tools were used to process an unknown plant material. Animalderived compounds including *cis*-10-heptadecenoic acid (found in animal fats) (Igwe and Okwu, 2013) and (2,8,12,18-tetraethyl-3,7,13,17tetramethyl-21H, 23H-porphinato(2-)-N21,N22,N23,N24)-, (SP-4-1)- (a degraded blood-protein) were also detected in extractions sampled from GS3, and indicate that this stone was used for processing both plant and animal material. Interestingly, no compound groups were detected in residue extractions sampled from UPGS 2.

6. Discussion

Based on multiple lines of evidence, we argue that the primary function of the three grinding stones from Madjedbebe was plant processing, specifically, the grinding/pounding of starchy plants. The evidence includes three data sets that indicate plant processing: (1) usewear including a bright, reticular polish that has also been documented on experimental sandstone grinding tools used for processing seeds and other plants; (2) residues, including microscopically visible plant tissues and abundant starch grains; and (3) chemical confirmation of plant-derived compounds and compound groups detected with biochemical tests and GC–MS.

We suggest that UPGS 2, a flake with grinding wear on its dorsal surface, and L49, a larger grinding stone fragment with negative flake removals (Fig. 2), were once parts of a complete tool that was used for multiple plant processing tasks. Both artefacts were found in the same depositional context and have at least one assemblage of starch that possibly derives from the same plant taxa (*Cochlospermum fraseri*). The second distinctive starch grain assemblage documented on L49 (and closely aligned to *Dioscorea transversa*) was absent in extractions sampled from UPGS 2, indicating that L49 was used to process a second species of starchy plant after a flake (UPGS 2) had been removed.

Interestingly, organic residues were not detected via GC–MS in extractions sampled from UPGS 2, despite this method of analysis being more sensitive than the biochemical tests that had indicated the presence of starch, carbohydrates and fatty acids (Table 7). We attribute the absence of residues detected by GC–MS to the small sample size of the extracted residue solution. Only a single sample from the ground surface was extracted from this tool using the EWA solvent. This sample was used for both biochemical and GC–MS analyses, with GC–MS measurements made after biochemical tests on the remaining extracted material after adding additional solvent to the near-empty solution. Future work should consider more extensive sampling with high volumes of extracted material to explore this limitation in more detail.

The usewear on UPGS 2 and L49 is consistent with seed grinding,

despite the absence of starch grains originating from grass and other silica-rich seed plants. Grasslands comprised of *Oryza* sp. occur within the catchment of Madjedbebe and make up an important seed grass in this area. Although we currently have no reference material for *Oryza* sp., the starch grains on UPGS 2 and L49 are not characteristic of grass seeds. We attribute the bright developed polish identified in the usewear study to an earlier stage in its use-life, when it was used to process relatively hard, small seeds of a silica-rich plant. Hence, we suggest that both tools were used to process multiple plant species, including soft starchy plants and hard seeds, at different stages in the use-life of the artefact.

The third grinding stone, GS3, has a deliberately ground edge that was used for a variety of tasks. These include the processing (by chopping and pounding/grinding) of starchy plants, and most significantly, waterlily (*Nymphaea violacea*), that was a staple food at the time of European contact. GS3 also preserves evidence for the processing of animal tissue, recognized by the presence of collagen fibres, animals fats and a degraded blood, the latter two of which were detected chemically with GC–MS. The shape of the ground edge would make this a suitable scraping and chopping tool, useful for a variety of tasks.

There are a considerable number of starchy plants utilized by Aboriginal people in northern Australia, and this alone presents a unique challenge in our identification of plant taxa. Our reference plant material included only 21 species from potentially hundreds of edible starchy plants in the region. It also became obvious in the initial investigative stages that it was difficult to construct a classifier that could discriminate between species across the broad spectrum of the plants being analysed. One of the problems with identifying unknown starch to plant taxa is that morphological variability within a species can exceed that observed between species and the large overlap in shape types that also occur (see also Mercader et al., 2018). Very few plant starches are diagnostic (with few exceptions such as nardoo/Marsilea drummondii), while some starch producing species are very difficult to separate (e.g., seed starch from Nymphoides sp., Nymphaea sp. and Portulaca oleracea) (Fig. SI2). Since the beginning of the study, over 30,000 starch grains have been traced from single and duplicate reference samples. The starch grain database is continually evolving and already there is a robust dataset with which we have been able to continually test and improve the method for classification of grains from their geometric properties (Coster and Field, 2018). We are continuing to pursue improvements to the method with each study undertaken (Field et al., 2020; Gaffney et al., 2020; Lape et al., 2018; Owen et al., 2019; Shaw et al., 2019)-in-built in the approach is the notion that a "one size fits all" classification system simply does not work for all microfossils; and this is particularly so in the

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case of these samples.

The absence of identifiable grass starch grains on grinding stones with distinct seed-grinding usewear from Madjedbebe is curious. We are confident in our interpretations of both the usewear and residue traces and note that sometimes these may indicate different tasks through the life of the artefact. It is possible that the lack of starch grains originating from grass seeds could be taphonomic, or that different analytical techniques record different phases of the use-life of an implement (e.g., the last use of discarded tools).

Functional analysis of stone artefacts provides a useful means for recognizing tool function, past human activities and subsistence practices. This functional study of Holocene grinding stones from Madjedbebe has demonstrated the value of multi-disciplinary studies in attributing tool function and identifying specific tasks. Each of the methods described and presented in this paper would have been less powerful had they been carried out individually, but together build on multiple lines of evidence that provide an important window on the use life of this durable material culture.

7. Conclusion

Three Holocene-aged sandstone grinding implements from Madjedbebe were used to process multiple varieties of starchy plants, other plants and animal tissue. Ancient starch grain analysis has confidently identified *Nymphaea violacea* (waterlily), a contact period staple of the Mirarr people, as the species of plant processed by GS 3 in the early Holocene, with likely *Cochlospermum fraseri* (kapok bush), *Amorphophallus galbra* (cheeky yam) and *Dioscorea transversa* (long yam) on the other two. GC–MS data also indicated the presence of animal residues on GS3, to indicate multi-functional tool use. The diversity of functions identified on these tools indicates that people were processing a wide variety of plant and animal foods at Madjedbebe during the Holocene, with contact period staple plant foods in use since at least the early Holocene

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The language, images and information contained in this publication includes reference to Indigenous knowledge including traditional knowledge, traditional cultural expression and references to biological resources (plants and animals) of the Mirarr people. The source Indigenous knowledge is considered "Confidential Information"; traditional law and custom applies to it and the Mirarr people assert ownership over it. Any Mirarr-related language, images and information are published with the consent of Gundjeihmi Aboriginal Corporation as the representative of the Mirarr people for the purposes of education and specifically for use only in the context of this published work. Please contact Gundjeihmi Aboriginal Corporation to request permission to refer to any Indigenous knowledge in this publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jasrep.2020.102754.

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