Ritual complexity in a past community revealed by ancient DNA analysis of pre-colonial terracotta items from Northern Ghana

DOI:

[10.1016/j.jas.2017.01.005](https://doi.org/10.1016/j.jas.2017.01.005)

Document Version

Accepted author manuscript

[Link to publication record in Manchester Research Explorer](#)

Citation for published version (APA):

Robinson, H., Insoll, T., Kankpeyeng, B. W., Brown, K., & Brown, T. (2017). Ritual complexity in a past community revealed by ancient DNA analysis of pre-colonial terracotta items from Northern Ghana. *Journal of Archaeological Science*, 79, 10-18. [2017.01.005]. <https://doi.org/10.1016/j.jas.2017.01.005>

Published in:

Journal of Archaeological Science

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1 **Ritual complexity in a past community revealed by ancient DNA analysis**
2 **of pre-colonial terracotta items from Northern Ghana**

3

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18

19 **Abstract**

20 The pre-colonial 6th–14th century terracotta forms of Koma Land, Northern Ghana, contain
21 cavities which may have been intended to hold liquids. These have been linked to traditional
22 African libation, but the specific nature of their contents is unclear. We used generic
23 polymerase chain reactions that would amplify DNA from a range of plant and fungal
24 species in order to identify remains of libations applied to fourteen terracotta items. We
25 anticipated difficulties in distinguishing genuine ancient DNA sequences from those resulting
26 from contaminating material, and therefore also carried out a series of control experiments
27 to assess the extent to which the samples had become contaminated with exogenous DNA
28 during burial, excavation and downstream analysis. Taking account of the results of the
29 control experiments, as well as the difficulties in assigning matches between ancient DNA
30 sequences and database entries, we provide evidence for the use of three different types of
31 plant – plantain/banana, pine and grasses – in libations associated with the terracotta items.
32 We also identified DNA from *Coniochaeta* yeast within the mouth cavity of one figurine,
33 suggesting that this structure was burnt prior to deposition.

34

35 **Keywords:** Africa, ancient DNA, Koma Land, libations, residue analysis, terracotta

36

37 **1. Introduction**

38 The striking terracottas from Koma Land, Northern Ghana, are a compelling record of
39 a 6th–14th century AD community for which there is no written history or oral tradition
40 (Kröger, 1988). These anthropomorphic, zoomorphic and conical pottery items have drawn
41 considerable attention since anthropological studies and excavation of four mounds in the
42 region in the 1980s (Anquandah, 1987, 1998, 2003; Kröger, 1988), and the excavation of

43 two further mounds in 2007–2011 (Kankpeyeng and Nkumbaan, 2008, 2009; Kröger and
44 Saibu, 2010; Kankpeyeng et al., 2011, 2013; Insoll et al., 2012, 2013).

45 Koma Land, in Northern Ghana, covers an area of approximately 100 km²
46 (Anquandah, 1998; Kankpeyeng et al., 2013) and features hundreds of mounds 4–35 m in
47 diameter, with a prominent mound cluster in the village of Yikpabongo. Some of the mounds
48 contain human remains (Anquandah, 1987), and it has been proposed that others
49 functioned for medicinal rituals or shrine worship (Kankpeyeng et al., 2011), perhaps as
50 places where ritually powerful materials, including the figurines, were deposited (Insoll et al.,
51 2012). The mounds were initially dated by thermoluminescence techniques to the 15th–17th
52 centuries AD, although this chronology was subsequently extended to c.1200–1800 AD
53 (Anquandah, 1998).

54 An early anthropological survey of Yikpabongo and nearby villages equated the
55 locally spoken Mampruli language with the modern Koma language Konni, and concluded
56 that the society which produced the pottery items was continuous with the modern
57 population (Rattray, 1932). However, a subsequent survey recognized Konni as having
58 developed separately (Naden, 1986), and it is now widely accepted that the modern Koma
59 population migrated to this region, most likely arriving after 1880 (Kröger and Saibu, 2010).
60 Local oral traditions recount the discovery of ruins at the site, for which it was named
61 Yipkabongo (‘forest ruins’) (Dagan, 1989). It is suggested that the earlier pottery-producing
62 society may have abandoned their location due to pressures introduced by disease, famine
63 or the slave trade (Insoll et al., 2012, Kankpeyeng et al., 2013). This community is thought to
64 have occupied Yikpabongo between the 6th/7th and 13th/14th centuries AD (Kankpeyeng and
65 Nkumbaan, 2008, 2009; Kankpeyeng et al., 2011, 2013).

66 Many of the terracotta structures recovered from the mounds feature cavities up to 20
67 mm in depth, as visualized by tube current modulation computer tomography (Insoll et al.,

2012, in press). In figurines, these cavities typically originate from the top of the head, ear, nostril or mouth, and in cones the cavities extend downwards from the bowl. It has been suggested that these cavities were reservoirs for libation (Insoll et al., 2012), the liquid ceremonial offerings that can form a component of indigenous African religious practice (Kilson, 1969; Mbiti, 1990; Essien 2014; Essel, 2014).

Pre-colonial West African libation is documented as having been offered with palm wine (Baum 1999; Essel, 2014) and cola nut infusions (Kröger and Saibu, 2010), sorghum or millet beer (Mbiti, 1990; Mulemi, 2004; Kröger and Saibu, 2010), water (Mbiti, 1990; Baum, 1999; Kröger and Saibu, 2010; Essel, 2014), and in some instances, milk (Mbiti, 1990) or animal blood (Baum, 1999). The present Yikpabongo population cultivates pearl millet, sorghum, African rice, yam and cassava (Kröger and Saibu, 2010), all of which can be fermented (Jespersson, 2003; Mulemi, 2004; Mukisa et al., 2012; Lentz, 2013). Additionally, bark/leaf infusions or pastes of 'magic' and medicinal plants (Myren, 2011; van Andel et al., 2012) may have been applied to the items, given the suggested disease and scapegoat themes in some of the figurine designs (Kankpeyeng et al., 2011; Insoll, 2015).

Analysis of ancient DNA (aDNA) from high temperature environments is usually considered inadvisable (Murray et al., 2012; Campana et al., 2013), as heat accelerates DNA hydrolysis and oxidation, reducing the likelihood of DNA sequence recovery (Pääbo and Wilson, 1991; Burger et al., 1999; Krings et al., 1999; Reed et al., 2003; Willerslev and Cooper, 2005; Lorenzen and Willerslev, 2010). Ancient DNA sequences have only infrequently been recovered from warmer latitudes, typically from sites with cool microclimates (Poinar et al., 2003; Gutiérrez-García et al., 2014), such as caves, which were the source of the first ancient African human genome (Gallego Llorente et al., 2015). One notable exception is the recovery of aDNA from 17th century human remains from the tropical Caribbean island of St. Martin (Schroeder et al., 2015). In North Africa, DNA has

93 been shown to degrade to fragments <100 bp in length within 2000 years (Marota et al.,
94 2002; Hekkala et al., 2011; Khairat et al., 2013). However, only a third of Africa shares the
95 'hot desert' climate of the North (Kottek et al., 2006), and aDNA sequences up to 160 bp
96 have successfully been recovered from 2000-year-old desert cattle bones from Mali in West
97 Africa and Eritrea in the East (Edwards et al., 2004; Ascunce et al., 2007). The possible
98 contribution of aDNA studies in Sub-Saharan and central temperate and sub-tropical Africa
99 should therefore not be overlooked, especially in view of the archaeological richness of
100 these regions (e.g. McIntosh, 1994, 2005; Yellen, 1998; Insoll, 2003; Stahl, 2004).

101 Non-destructive, forensic-inspired swab recovery methods can efficiently recover
102 aDNA from pottery, enabling systematic studies of large pottery assemblages without the
103 typical risks associated with sampling delicate artefacts (Foley et al., 2012). Here we report
104 the use of these methods to assess the usage of various terracotta items excavated in
105 Koma Land.

106

107 **2. Materials and methods**

108 *2.1 Terracotta items*

109 Samples were taken from nine terracotta figurines, two cones and three circular
110 disc/horn stoppers from mound YK07 (Kankpeyeng and Nkumbaan, 2008, 2009) and
111 mound YK10-3/YK11 (Kankpeyeng et al., 2011, 2013; Insoll et al., 2012), both in
112 Yikpabongo village, Koma Land (Fig. 1, Fig.2, Table 1). The items collectively span the five
113 Koma Land pottery forms (Kröger, 1988): cones with heads (category I), anthropomorphic
114 figures (II), Janiform figures, (III) animal forms (IV), and other uninterpreted forms (V). These
115 items were excavated over four seasons (Kankpeyeng and Nkumbaan, 2009; Insoll et al.,
116 2012), including two seasons during which excavators wore gloves and implemented
117 packing procedures in anticipation of downstream aDNA analysis (Insoll et al., 2012).

118 Thermal age estimates (Smith et al., 2003) for the excavation series ranged from 18,567–
119 28,929 10°C thermal years, with predicted mean fragment lengths of 13–19 bp, based on
120 the degradation pattern of bone aDNA (Table 2), placing the terracotta items close to the
121 likely threshold for aDNA recovery. Samples were taken with permission of the Ghana
122 National Museum, Accra.

123

124 *2.2 Ancient DNA authentication regime*

125 Sampling was carried out under clean conditions at the Manchester Museum on work
126 surfaces covered with two layers of aluminium foil. Personnel wore protective clothing
127 including forensic suits, face masks, hair nets, goggles and two pairs of sterile gloves at all
128 times, and all utensils and equipment were treated with DNA-Away (Thermo Scientific)
129 before and after use.

130 Ancient DNA analyses were performed in the aDNA laboratories of the University of
131 Manchester in a suite of independent, physically isolated laboratories, each with an
132 ultrafiltered air supply maintaining positive displacement pressure and a managed access
133 system. All surfaces within the laboratories were periodically sterilized by UV irradiation and
134 cleaned with 5% bleach and 70% ethanol, and all utensils and equipment were treated with
135 DNA-Away before and after use. Plasticware such as test tubes were UV irradiated (254
136 nm, 120,000 $\mu\text{J cm}^{-2}$ for 2×5 min, with 180° rotation between the two exposures) before
137 use. Aqueous solutions were similarly irradiated for 15 min. Personnel wore protective
138 clothing as described above at all times. DNA extractions were carried out in a Class II
139 biological safety cabinet in one laboratory within the facility, and PCRs were set up in a
140 laminar flow cabinet in a second, physically-isolated laboratory. All DNA extractions were
141 accompanied by at least two sample blanks (normal extraction but without sample), and
142 every set of eight PCRs was accompanied by at least one PCR blank (set up with water

rather than DNA extract). A positive PCR (i.e. product of correct size and meaningful sequence) was only considered authentic after replication with a second PCR of the same extract.

146

2.3 Sampling and DNA analyses

Samples were taken from various cavities and surfaces from the terracotta items. Following removal of soil with a sterile pick, each cavity was sampled with a sterile rayon swab soaked in Tween 80 + lecithin buffered to pH 7.4 with sodium thioglycollate (Scientific Laboratory Supplies) or, for smaller cavities, with sterile glass wool swabs prepared in the aDNA laboratory, dipped in lysis buffer A (Promega Wizard Magnetic DNA Purification System for Food) at the point of sampling. Tubes of lysis buffer A were also opened on the workbench prior to the sampling of each item to provide the sample blanks.

DNA was extracted from each sample and from associated soil (Promega Wizard Magnetic DNA Purification System for Food), eluted in a final volume of 100 µl, and quantified (Thermo Fisher Scientific Quant-IT DNA Assay Kit and Qubit 2.0 Fluorometer). The significance of possible correlations between the DNA concentrations of extracts and variables including excavation mound, year and sample type (cavity, external surface or soil) was assessed using a linear model, simplified following Crawley (2005), run in R v.3.0.1 (R Development Core Team, 2009).

PCRs were carried out as described by Speirs et al. (2011) using the primers shown in Table 3, using AmpliTaq Gold DNA Polymerase (Thermo Fisher Scientific). To avoid PCR inhibition from residual soil contaminants, extracts were diluted and the equivalent of 0.015–0.150 µl of the undiluted extract used per 30 µl PCR, the amount determined by optimization experiments. Cycling parameters were: 10 min at 95°C; 40–50 cycles of 45 s at 94°C, 1 min at the annealing temperature, 1 min 20 s at 72°C; 7 min at 72°C. PCR products were

168 purified from gels (QIAGEN MinElute PCR Purification Kit) and cloned (Thermo Fisher
169 Scientific CloneJET PCR Cloning Kit) in *Escherichia coli* XL1-Blue (Agilent). Colony PCR
170 was performed in 20 µl reactions comprising 1 × *Taq* buffer (New England Biolabs), 200 nM
171 each primer, 200 mM dNTPs and 0.625 units *Taq* DNA polymerase (New England Biolabs),
172 with cycling at: 95°C for 3 min; 30 cycles of 30 s at 94°C, 30 s at 60°C, 1 min at 72°C; 10
173 min at 72°C. Single-stranded DNA was digested (GE Healthcare Illustra ExoProStar) and
174 the PCR products sequenced (ABi PRISM BigDye Terminator protocol).

175 Amplicons from the original DNA extracts were also sequenced on the GS Junior 454
176 (Roche) platform after multiplex library preparation using the GS FLX Rapid Library MID
177 Adaptors Kit and Titanium emPCR kit, with modifications to optimize short read recovery as
178 described by Penney et al. (2013). All the test reads passed the standard quality controls
179 and control reads were of good quality. As the test read numbers were low, duplicates were
180 not removed prior to downstream processing. Sequences were manipulated in Geneious
181 v.8.0.3 (available from: <http://www.geneious.com/>), and aligned using Seaview v.4.5.3
182 (Galtier et al., 1996; Gouy et al., 2010). Sequences, excluding primer binding sites in the
183 case of PCR products, were compared with the NCBI database using BLASTn (Altschul et
184 al., 1990) using standard parameters (word size 11, DUST filter on, expect value 10,
185 match/mismatch score 2,–3, gap costs existence 5 extension 2). Sequence data and
186 barcodes are curated at the European Nucleotide Archive under project accession number
187 PRJEB11221 (Supplementary Table 2).

188

189 **3. Results**

190 *3.1 DNA concentrations of extracts*

191 The DNA concentrations of the extracts ranged from <0.05–3.32 ng µl⁻¹
192 (Supplementary Table 1). Using a basic linear model, correlations were tested between

193 DNA concentration and excavation year, mound or sample type (cavity, external surface or
194 soil), including a potential interaction between excavation year and mound. The only
195 significant correlation was with sample type ($p = 0.04$), soil samples having a significantly
196 higher DNA concentration than the swab samples taken from cavities or external surfaces.
197 There was no statistical support for a correlation between the DNA concentration of the
198 extracts and excavation year ($P = 0.35$) or mound ($P = 0.63$).
199

200 *3.2 Assessment of modern contamination*

201 Sample blanks (tubes of buffer exposed during sampling of items) and PCR blanks
202 (set up with water instead of extract) occasionally gave single or multiple products of various
203 sizes. Cloning and sequencing indicated that in all but one case these products were primer-
204 dimers or non-specific bacterial amplicons. The single occasion on which an authentic PCR
205 product was obtained from a blank is described below.

206 As well as the sample and water blanks, the extent of exogenous DNA contamination
207 during burial, excavation and/or analysis of the terracotta items was assessed by four
208 different PCRs, carried out in duplicate with each of the DNA extracts. Two of these PCRs
209 were designed to detect DNA from major crops grown in the vicinity of the mounds. The
210 ATPC primer pair (Table 3) targeted the vacuolar ATPase C gene of pearl millet
211 (*Pennisetum glaucum*), a staple regional food crop (Rao et al., 1985; Kröger and Saibu,
212 2010), and the RBSP primer pair (Gyulai et al., 2007) amplified part of the S15 ribosomal
213 protein gene of maize (*Zea mays*), a recently introduced species farmed nearby (Kröger and
214 Saibu, 2010). No extracts gave PCR products of the expected size with either the ATPC or
215 RBSP primer pairs, the only products obtained from these PCRs being shown by
216 sequencing to be either primer-dimers, non-specific bacterial amplicons, or short sequences
217 that could not be identified by BLASTn analysis.

218 Two other PCRs were designed to test for contamination with mammalian and/or
219 human DNA. Four extracts gave products of the expected size with the human-specific
220 mitochondrial HVR1 primers (Table 3; Bouwman et al., 2008). Sequencing of these four
221 HVR1 PCR products, two of which originated from pottery disc stoppers (items YK07-2-85-1
222 and YK07-2-B5-1), one from soil removed from the mouth of the 'large head' (YK07-2-B5.1),
223 and one from the exterior of the 'human head wearing hat' (YK08-A9B9-L7), showed that
224 each contained human DNA. Sequencing placed these amplicons in the HV haplogroup,
225 which is uncommon in present-day Africa, suggesting that they derive from handling during
226 or after excavation. Eight extracts gave products of the expected size with the CYTB primer
227 pair (Table 3), which is designed to amplify part of the mitochondrial cytochrome b gene
228 from mammalian species (Irwin et al., 1991), but sequencing showed these either to be
229 bacterial in origin or unidentifiable.

230 No human DNA was detected from items excavated during seasons 2010–
231 11, indicating that the precautions used to prevent contamination in the field had been
232 effective. One sample blank gave an HVR1 product which matched the sequence of
233 individuals present when samples were taken, but this sequence was not obtained from any
234 of the test extracts.

235

236 *3.3 Identification of possible plant libation offerings*

237 Two PCRs were carried out with primer pairs designed to amplify a variety of plant
238 species that might have been components of libation offerings. The RBCL PCR used
239 primers h1aE and h2aR (Poinar et al., 1998), which amplify part of the chloroplast gene for
240 the large subunit of ribulose-1,5-bisphosphate carboxylase, and the TRNL primer pair were
241 designed by us to amplify part of the chloroplast trnL (tRNA^{Leu}) gene (Table 3). Both primer
242 pairs are generic to multiple land plants, and would be expected to amplify DNA from a

243 range of species. With several extracts, these PCRs resulted in a mixture of products of
244 different lengths. For nine PCRs, two with the RBCL primer pair and seven with the TRNL
245 primers, the products included an amplicon of, or close to, the expected length (Table 4).
246 These amplicons were further examined by purification from the electrophoresis gel followed
247 by cloning and sequencing.

248 The TRNL PCRs from two samples, taken from the mouth cavity of the 'large head
249 form' YK-07-2-B5.1 and the nostril cavity of a 'combined human and animal head' YK11-
250 H13H14-113114 gave products whose sequences closely matched members of the *Musa*
251 and *Ensete* genera, which include bananas and plantains (Supplementary Fig. 1). Ten
252 clones were obtained from the YK07-2-B5.1 PCR, five of which gave matches to the
253 sequence database. Three of these clones gave sequences that were an exact match to a
254 variety of *Musa* species and a single *Ensete* species, *Ensete lasiocarpa*. The two other
255 clones contains single mismatches to the same sequences. A single clone from the YK11-
256 H13H14-113114 PCR gave a sequence with two polymorphisms to the various *Musa*
257 species and *E. lasiocarpa*. Additionally, an RBCL PCR of a sample from the exterior
258 surface adjacent to the mouth cavity of YK07-2-B5.1 gave a weak product of the correct
259 size, which was not replicated in the duplicate PCR. This product could not be cloned, but a
260 partial direct sequence matched the Order Zingiberales, which includes *Musa* and *Ensete*,
261 with the exception of a single indel.

262 The TRNL PCRs of samples taken from the nasal cavity of the 'combined human and
263 animal head' YK11-H13H14-113114, and the exterior of the 'large conical figure' YK07-2-
264 A4-1 yielded sequences whose closest matches were with members of the Order Pinales
265 (Supplementary Fig. 2). Seven clones from YK11-H13H14-113114 and five from YK07-2-
266 A4-1 gave sequences identical to those of various *Pinus* species, and when repeating the

267 query excluding *Pinus* sequences, to *Araucaria araucana* (monkey puzzle). Multiple
268 mismatches were observed to all other database sequences.

269 A sample from the nostril of the 'seated female figure' YK10-D11-3 gave RBCL
270 sequences which were close matches to database entries for various species of the
271 Poaceae (grasses). Four clones exactly matched a sequence conserved in the *Triticum*,
272 *Hordeum*, *Elymus* and *Secale* genera, which comprise widely cultivated cereals, as well as
273 other grass genera (Supplementary Fig. 3A). These sequences had no significant
274 similarities with millet or maize and so are unlikely to be modern environmental
275 contaminants. Three further clones from this PCR product differed by up to three bases,
276 which might indicate DNA damage. An exterior sample, also from the seated female figure,
277 gave a TRNL product that was an exact match to sequences in the *Triticeae* tribe and also
278 to *Lolium perenne* (rye grass). This sequence was replicated across nine clones
279 (Supplementary Fig. 3B).

280 Products of the expected size were also obtained after TRNL PCR of samples from
281 the left mouth cavity of the 'horse and rider' YK07-AB9-L7 and the exterior of the
282 'anencephalic head' YK07-2-A8-1, as well as from a soil sample from a cavity of the 'large
283 head' YK07-2-B5.1. After cloning and sequencing, these products gave no significant
284 matches with entries in the NCBI database (Table 4).

285

286 3.4 Identification of environmental fungi

287 The RDNA primer pair (Table 3) was designed by us to amplify part of the large
288 subunit ribosomal RNA gene and adjacent internal transcribed spacer region from a range
289 of ascomycete fungi. This PCR was intended to test for the presence of yeasts such as
290 *Saccharomyces cerevisiae*, which might indicate that the libations included fermented
291 products. The PCR was successful with three extracts, from the mouth cavity of the 'horse

292 and rider' YK08-AB9-L7, the ear hole of the 'seated female figure' YK10-O11-3, and the
293 exterior of the 'large conical figure' YK07-2-A4-1. As only the product from YK07-2-A4-1C2
294 could be cloned, the products from YK08-AB9-L7 and YK10-O11-3 were sequenced using
295 the GS Junior 454 platform (Supplementary Table 3). The sequences obtained for YK10-
296 O11-3 and YK07-2-A4-1C2 were predominantly primer artefacts, but those from YK08-AB9-
297 L7 fell into two groups, distinguished by a single-nucleotide difference. These sequences
298 were most closely matched with database entries for the yeasts *Coniochaeta africana* and
299 *Coniochaeta gigantospora* (Supplementary Fig. 4).

300

301 **4. Discussion**

302 We used generic PCRs that would amplify DNA from a range of plant and fungal
303 species to identify remains of libations applied to terracotta items from Koma Land, dating
304 from the 9th–14th centuries AD. Thermal age estimates indicated that the items are close to
305 the threshold for aDNA recovery, and we anticipated difficulties in distinguishing genuine
306 aDNA sequences from those resulting from contaminating material. In order to assign an
307 appropriate degree of confidence to our results, we therefore carried out a series of control
308 experiments to assess the extent to which the terracotta items had become contaminated
309 with exogenous DNA during burial, excavation and downstream analysis.

310 PCRs directed at pearl millet and maize, the two major agricultural crops grown in
311 present-day Koma Land, were negative, suggesting minimal contamination with surface
312 vegetation. Sporadic contamination with modern human DNA was detected for items
313 excavated prior to 2010–11, the first season when precautions were taken to prevent
314 handling contamination. For this reason, we made no attempts to assess the use of human
315 products as libations.

316 As well as figurines, we also sampled three circular disc/horn stoppers (YK07-1-85-1,
317 YK07-2-B5-1, YK07-2-C4-L2), which are believed to be repurposed from broken pots. Their
318 actual function is unknown, but they are unlikely to have served the same purposes as the
319 figurines with which they were co-excavated. These stoppers therefore act as negative
320 controls, and are more suitable for this purpose than the outer surfaces of the figurines, as
321 libations might have been poured over the latter as well as applied to cavities. None of these
322 stoppers yielded plant or fungal sequences similar to those that we obtained from some of
323 the figurines. Sample blanks (exposed during sampling of the items) and water blanks
324 (PCRs set up with water rather extract) yielded only sporadic products shown to be primer-
325 dimers or non-specific bacterial amplicons, except for one sample blank that gave a human
326 DNA sequence probably derived from one of the individuals present when samples were
327 taken. With zero evidence for environmental contamination, absence of 'interesting'
328 sequences from the discs, and only sporadic contamination with human DNA, we believe
329 that we are justified in drawing tentative conclusions from those plant and fungal sequences
330 that we detected in samples from the Koma Land figurines.

331 Sequences obtained from generic PCRs must be interpreted with care when
332 comparisons are made by BLASTn analysis. Although we targeted chloroplast sequences
333 that are well-studied in plants, the entries in the NCBI database are far from comprehensive
334 and are biased towards model species and ones with agricultural or economic importance.
335 Many species, especially from less studied ecosystems such as those of West Africa, are
336 under-represented in the database. BLASTn analysis reveals the closest matches to a query
337 sequence, and those matches might display sequence identity, but this does not constitute a
338 definite identification of the query sequence, as the possibility remains that the query
339 sequence derives from a species that is not present in the database. Interpretation of
340 BLASTn results becomes even less specific when the query has non-identity with the

341 closest database match, especially with aDNA where non-identities might be genuine
342 differences between query and closest match, or might be due to miscoding lesions in the
343 query sequence, resulting from damage to the aDNA template.

344 The cavities of two items, the mouth cavity of the 'large head form' YK-07-2-B5.1 and
345 the nostril cavity of a 'combined human and animal head' YK11-H13H14-113114, which
346 were excavated during different years and from different mounds (Table 1), gave TRNL
347 sequences that matched a variety of *Musa/Ensete* (banana and plantain) species. Item YK-
348 07-2-B5.1 also yielded an RBCL sequence which could be assigned to Order Zingiberales.
349 The *Musa* and *Ensete* genera, including bananas and plantains, are non-native to Ghana,
350 and little is known of their West African history (Stover and Simmonds, 1987; Blench, 2009).
351 Plantains have become a dietary staple in West Africa, which might indicate that they were
352 introduced into the region prior to bananas (Tezenas du Montcel et al., 1983; De Langhe,
353 2007). Phytolith studies have placed domesticated banana cultivars in Cameroon by 1000
354 BC (Lejju et al., 2006), suggesting a relatively early introduction of edible Asian cultivars via
355 Central Africa. Plantain and banana cultivars were not described in accounts of the staple
356 crops cultivated at the Koma Land site in 2010, nor do they appear to supplement the
357 current population's regular diet (Kröger and Saibu, 2010). Elsewhere in Sub-Saharan
358 Africa, plantains and bananas are boiled, pounded into fufu paste with cassava, or brewed
359 to produce beer and wine (Tezenas du Montcel et al., 1983; Shale et al., 2012). In the
360 samples that we studied, however, we detected only one fermenting yeast genus,
361 *Coniochaeta*, which primarily ferments rotting wood. We therefore have no evidence to
362 support the use of fermented *Musa/Ensete* products, but cannot discount this possibility.
363 The two figurines from which we obtained these sequences have large, open mouths that
364 lack a rear cavity, in contrast to the cavities or closed mouths found on many other figures,
365 and could therefore have been designed to receive pastes rather than liquids. Solid

366 substances are known to have been associated with figurine cavities produced by cultures
367 such as the Songye (Hersak, 2013), although in these cases the cavities tend to be located
368 in the abdomen and anus, cavity positions which are not represented on the Koma Land
369 figurines.

370 We obtained TRNL sequences matching various *Pinus* species and *Araucaria*
371 *araucana* (monkey puzzle) from the nasal cavity of the 'combined human and animal head'
372 YK11-H13H14-113114, and the exterior of the 'large conical figure' YK07-2-A4-1. These
373 items were recovered from different mounds during different years. Pines are farmed
374 primarily in Southern Ghana, and no stands are present proximal to the study site. Tree bark
375 is commonly boiled in traditional African medicinal rituals to produce infusions perceived to
376 have restorative powers (van Andel et al., 2012). *Pinus halepensis*, *Pinus pinaea*, *Pinus*
377 *brutia* and *Pinus negra* were all established in North Africa prior to the production of the
378 Koma Land figurines (Nicholson and Shaw, 2000), and it is possible that conifer needles,
379 cones or bark were traded from North Africa, as were cowry shells and beads (Anquandah,
380 1998; Insoll et al., 2012). The representation of a horse or camel on item YK08-AB9-L7
381 could suggest an interaction between the Koma Land population and outside groups
382 (Anquandah, 1998; Insoll et al., 2012).

383 We also obtained evidence of grass DNA from the 'seated female figure' YK10-D11-
384 3. The RBCL sequences gave an exact match with a wide range of grass genera, while the
385 TRNL sequence was specific to the Triticeae. Many of the sequence matches belong to
386 widely cultivated cereals, but not to maize and millet, indicating a possible ritual application
387 of grains as pastes or in infusions.

388 The final positive identification that we made was for *Coniochaeta* yeast DNA present
389 in the mouth cavity of the 'horse and rider' YK08-AB9-L7. These sequences were detected
390 only in the second of two samples taken from this cavity, and were not detected in soil

391 removed from the cavity, suggesting that the cavity contained *Coniochaeta* yeasts when the
392 figurine was buried. *Coniochaeta* are early colonizers of post-fire sites, creating a
393 characteristic fungal bloom (Wicklow, 1973; Mahoney and LaFavre, 1981; Innes and
394 Blackford, 2003; Mighall et al., 2007). The figurine YK08-AB9-L7 was excavated from what
395 appears to be scorched earth (Kankpeyeng and Nkumbaan, 2009), although it was unclear
396 whether the figurine was burnt *in situ* or fortuitously positioned within the burnt area. The
397 presence of *Coniochaeta* within the mouth cavity suggests that the item was deliberately
398 burnt. The majority of the Koma Land figurines feature deliberate breaks or missing
399 elements consistent with deconsecration prior to burial (Insoll et al., 2012), and items such
400 as YK07-2-A4-1 also display scorch marks. Burning might therefore have been used as a
401 means of deactivating powerfully perceived spiritual objects, perhaps considered to be
402 invested with some form of personhood (Insoll et al., 2012).

403

404 **5. Conclusion**

405 Despite the Koma Land terracottas having thermal ages placing them at the limits of
406 aDNA analysis, and despite the problems inherent in interpretation of sequences obtained
407 with generic PCRs, we provide evidence for the use of three different types of plant –
408 plantain/banana, pine and grasses – in libations associated with the figurines. We also
409 provide evidence for deliberate burning of one figurine. The use of multiple types of libations
410 using traded exotic commodities, and the practise of selective burning suggests that ritual
411 activities in pre-colonial Koma Land had a complexity that rivals that of current indigenous
412 African religious practices. Further contextual interpretation of our results will contribute to
413 future research on this understudied part of the world pottery record.

414

415 **Acknowledgements**

416 We thank The Ghana Museums and Monuments Board for sampling permissions,
417 Samantha Sportun of the conservation team at The Manchester Museum for assistance with
418 sampling, and Saviour Gidi of the University of Ghana for preparing Figure 1. We thank
419 Romy Müller and The University of Manchester Sequencing Facility for technical advice, and
420 all who continue to work towards the excavation, conservation and exhibition of the Koma
421 Land items. This work was funded by the first author.

422

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619

620 **Figure legends**

621

622 **Fig. 1.** Location of Yikpabongo village in the Builsa district, Northern Ghana.

623

624 **Fig. 2.** Terracotta items from which samples were taken. Scale bars in cm.

625

626 **Supplementary Fig. 1.** Alignment of cloned sequences from the TRNL PCR products of
627 samples YK07-2-B5.1A2 and YK11-H13H14-113114B to their closest BLAST matches,
628 members of the *Musa* genus and *Ensete* (*Musella*) genera. The alignment includes YK07-2-
629 B5.1B2 clone 9, which has some similarity with *Musa/Ensete* but which we do not consider
630 to be a convincing match to these genera.

631

632 **Supplementary Fig. 2.** Alignment of sequences from the TRNL PCR products of samples
633 YK07-2-A4-1C1 and YK11-H13H14-113114B giving BLAST matches to Order Pinales.
634 Matches primarily belong to the *Pinus* genus with the exception of *Araucacia araucana* (not
635 shown). The twelve Pinales clones generated from samples YK07-2-A4-1C1 (five clones)
636 and YK11-H13H14-113114B (seven clones) share a single identical sequence.

637

638 **Supplementary Fig. 3.** Alignment of (A) RBCL sequences from the 'seated female figure'
639 YK10-D11-3, and (B) TRNL sequence from YK10-D11-3 with their closest BLAST matches
640 from the Poaceae family. The same TRNL sequence was obtained from nine separate
641 clones.

642

643 **Supplementary Fig. 4.** Alignment of the most frequently represented 454 sequences from
644 the mouth cavity of the 'horse and rider' YK08-AB9-L7, with their closest BLAST matches in
645 genus *Coniochaeta*.

Table 1

Terracotta items (Insoll et al., 2013) and samples that were taken.

Accession number*	Item description	Sample	Sample description
YK07-2-85-1	circular disc/horn stopper	YK07-2-85-1A YK07-2-85-1B	smooth side patterned side
YK07-2-A4-1	large conical figure	YK07-2-A4-1A YK07-2-A4-1C1 YK07-2-A4-1C2	exterior (black residue) exterior (no residue) exterior (no residue)
YK07-2-A8-1	anencephalic head	YK07-2-A8-1A YK07-2-A8-1B YK07-2-A8-1C YK07-2-A8-1S	interior (mouth) interior (mouth) exterior cavity soil (mouth) 0.32 g
YK07-2-B5-1	circular disc/horn stopper	YK07-2-B5-1A YK07-2-B5-1B	rough side smooth side
YK07-2-B5.1	large head (Mohican head)	YK07-2-B5.1A2 YK07-2-B5.1B2 YK07-2-B5.1S	interior (mouth) exterior (left of mouth) cavity soil (mouth) 0.09 g
YK07-2-C3-1	undecorated cone with skirt	YK07-2-C3-1A YK07-2-C3-1B YK07-2-C3-1A2 YK07-2-C3-1B2 YK07-2-C3-1S	interior exterior interior exterior cavity soil 0.08g

YK07-2-C4-L2	circular disc/horn stopper	YK07-2-C4-L2A YK07-2-C4-L2B	rough side smooth side
YK07-2-O2-1	Janus heads	YK07-2-O2-1A YK07-2-O2-1B YK07-2-O2-1C YK07-2-O2-1SA YK07-2-O2-1SB	exterior interior (large mouth) interior (small mouth) cavity soil (large mouth) 0.29 g cavity soil (small mouth) 0.1 g
YK08-AB9-L7	horse and rider	YK08-AB9-L7A YK08-AB9-L7B YK08-AB9-L7C YK08-AB9-L7S	interior (left mouth cavity) interior (left mouth cavity) exterior cavity soil (mouth) 0.036 g
YK08-A9B9-L7	human head wearing cap	YK08-A9B9-L7A YK08-A9B9-L7B YK08-A9B9-L7S	interior (mouth) exterior cavity soil (mouth) 0.058 g
YK10-D11-3	seated female figure	YK10-D11-3A YK10-D11-3B YK10-D11-3C YK10-D11-3D YK10-D11-3H YK10-D11-3I YK10-D11-3SA	interior (top of head) interior (top of head) exterior (right face) exterior (top of head) interior (left nostril) interior (right nostril) cavity soil 0.095 g
YK10-O11-3	stylised androgynous human head	YK10-O11-3E YK10-O11-3F YK10-O11-3SB	interior (ear hole) exterior (right face) cavity soil (ear) 0.120 g
YK11-H13H14-113114	combined human and animal head	YK11-H13H14-113114A YK11-H13H14-113114B YK11-H13H14-113114C	interior (mouth) interior (right nostril) exterior (right face)

YK11-Q10-L1	clay bicone	YK11-Q10-L1A YK11-Q10-L1B YK11-Q10-L1S	interior (tallest side) exterior (tallest side) cavity soil 0.24 g
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* Mound YK07 was excavated in 2007 (all items with names starting YK07) then again in 2008 (all item names starting with YK08).

Mound YK10-3/YK11 was excavated in 2010 (items starting YK10) and 2011 (items starting YK11). YK07-2-A8-1 is also listed under accession number YK07-2-D5-1 (Insoll et al., 2013).

Table 2

Thermal age estimates for the terracotta items.

Excavation series	Excavation year	Mound	Effective burial temperature (°C)*	Estimated date (BP)	Thermal age estimate (10°C thermal years)*	$\lambda^{\dagger}$
YK07	2007	YK07	28.4–28.7	979–1317 $\pm$ 39	18640–28929	0.056–0.087
YK08	2008				18567–29044	
YK10	2010	YK10-3/YK11	28.4–28.7	780–940 $\pm$ 40	22559–28207	0.067–0.085
YK11	2011				22583–28239	

* Effective burial temperatures and thermal ages were estimated with JRA 1: PrediCtoR (available from <http://thermal-age.eu>) based on excavation depths of 10–20 cm, silt loam soil type, site elevation of 735 m, and water saturation of 15%, storage at 16 $\pm$ 5°C post-excavation, and sample analysis in 2014.

[†] Estimated proportion of nucleotide bases damaged by depurination, based on the degradation pattern of bone aDNA, corresponding to mean fragment lengths of 13–19 bp for items from mound YK07, and 13–16 bp for the items from YK10–3/YK11.

Table 3

Details of PCRs.

PCR	Primer sequences*	Product size (bp)	Target	Reference	Annealing temperature (°C)
ATPC	F: CCGCCCTCGTCTTCTCAT R: ATCGGGCAATGGGTGGCGGA	133	Pearl millet ATPase gene	This study	58
CYTB	F: ATAGACAAAATCCCATTCCA R: TAGTTGTCAGGGTCTCCTAG	125	Mammalian cytochrome b gene	Irwin et al. (1991)	54
HVR1	F: ACAGCAATCAACCCTCAACTATCA R: TGTGCTATGTACGGTAAATGGCTT	131	Human mitochondrial HVR1	Bouwman et al. (2008)	58
RBCL	F: GGCAGCATTCCGAGTAACTCCTC R: CGTCCTTTGTAACGATCAAG	137	Plant RuBisCO gene	Poinar et al. (1998)	58
RBSP	F: AGAAGAAAGAGAAGAAGCACG R: GGACAGCTCGTATTATAACCTGC	143	Maize ribosomal protein gene	Gyulai et al. (2007)	60
RDNA	F: CAGCGGGTACTCCTACCTGA R: GAGAGGTTTCTCTGCGTGCT	136	Ascomycete ribosomal DNA	This study	58
TRNL	F: GGCAATCCTGAGCCAAATC R: GAGTCTCTGCACCTATCCT	80-85	Plant chloroplast trnL gene	This study	58

* F, forward primer; R, reverse primer

Table 4

Results of PCRs with the RBCL and TRNL primer pairs.

PCR	Sample	Sampled region	Highest BLASTn matches*	Number of clones
RBCL	YK07-2-B5.1B2 Large head	exterior	No BLASTn match	2
	YK10-D11-3I Seated female figure	interior	Poaceae $E=2e-35$	7
TRNL	YK07-2-A4-1C1 Large conical figure	exterior	Pinales $E=3e-11$ No BLASTn match	5 2
	YK07-2-A8-1C Anencephalic head	exterior	No BLASTn match	3
	YK07-2-B5.1A2 Large head	interior	<i>Musa/Ensete</i> sp. $E=8e-12$ No BLASTn match	5 5
	YK07-2-B5.1S Large head	cavity soil	No BLASTn match	4
	YK08-AB9-L7B Horse and rider	interior	No BLASTn match	6
	YK10-D11-3D Seated female figure	exterior	Poaceae $E=2e-34$	9

YK11-H13H14-113114B	interior	Pinales $E=8e-11$	7
Combined human and animal		<i>Musa/Ensete</i> sp. $E=4e-29$	1
head		BLASTn match	2

* 'No BLASTn match' denotes that BLASTn analysis only retrieved database entries with low E values and/or high numbers of mismatches. These results include one additional TRNL sequence from YK07-2-B5.1B2 for which the highest BLASTn match was *Musa/Ensete* but with seven differences in a 42 bp sequence (shown in Fig. S1).

Figure 1
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