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

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

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**Abstract**

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

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

## 1. Introduction

The striking terracottas from Koma Land, Northern Ghana, are a compelling record of a 6th–14th century AD community for which there is no written history or oral tradition (Kröger, 1988). These anthropomorphic, zoomorphic and conical pottery items have drawn considerable attention since anthropological studies and excavation of four mounds in the 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 $km^2$
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 $15^{th}$–$17^{th}$
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 $6^{th}$/$7^{th}$ and $13^{th}$/$14^{th}$ 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.,

68 2012, in press). In figurines, these cavities typically originate from the top of the head, ear,
69 nostril or mouth, and in cones the cavities extend downwards from the bowl. It has been
70 suggested that these cavities were reservoirs for libation (Insoll et al., 2012), the liquid
71 ceremonial offerings that can form a component of indigenous African religious practice
72 (Kilson, 1969; Mbiti, 1990; Essien 2014; Essel, 2014).
73 Pre-colonial West African libation is documented as having been offered with palm
74 wine (Baum 1999; Essel, 2014) and cola nut infusions (Kröger and Saibu, 2010), sorghum
75 or millet beer (Mbiti, 1990; Mulemi, 2004; Kröger and Saibu, 2010), water (Mbiti, 1990;
76 Baum, 1999; Kröger and Saibu, 2010; Essel, 2014), and in some instances, milk (Mbiti,
77 1990) or animal blood (Baum, 1999). The present Yikpabongo population cultivates pearl
78 millet, sorghum, African rice, yam and cassava (Kröger and Saibu, 2010), all of which can
79 be fermented (Jespersson, 2003; Mulemi, 2004; Mukisa et al., 2012; Lentz, 2013).
80 Additionally, bark/leaf infusions or pastes of 'magic' and medicinal plants (Myren, 2011; van
81 Andel et al., 2012) may have been applied to the items, given the suggested disease and
82 scapegoat themes in some of the figurine designs (Kankpeyeng et al., 2011; Insoll, 2015).
83 Analysis of ancient DNA (aDNA) from high temperature environments is usually
84 considered inadvisable (Murray et al., 2012; Campana et al., 2013), as heat accelerates
85 DNA hydrolysis and oxidation, reducing the likelihood of DNA sequence recovery (Pääbo
86 and Wilson, 1991; Burger et al., 1999; Krings et al., 1999; Reed et al., 2003; Willerslev and
87 Cooper, 2005; Lorenzen and Willerslev, 2010). Ancient DNA sequences have only
88 infrequently been recovered from warmer latitudes, typically from sites with cool
89 microclimates (Poinar et al., 2003; Gutiérrez-García et al., 2014), such as caves, which were
90 the source of the first ancient African human genome (Gallego Llorente et al., 2015). One
91 notable exception is the recovery of aDNA from 17$^{th}$ century human remains from the
92 tropical Caribbean island of St. Martin (Schroeder et al., 2015). In North Africa, DNA has

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

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

## 2. Materials and methods

### *2.1 Terracotta items*

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

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

*2.2 Ancient DNA authentication regime*

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

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

143 rather than DNA extract). A positive PCR (i.e. product of correct size and meaningful
144 sequence) was only considered authentic after replication with a second PCR of the same
145 extract.
146
147 *2.3 Sampling and DNA analyses*
148 Samples were taken from various cavities and surfaces from the terracotta items.
149 Following removal of soil with a sterile pick, each cavity was sampled with a sterile rayon
150 swab soaked in Tween 80 + lecithin buffered to pH 7.4 with sodium thioglycollate (Scientific
151 Laboratory Supplies) or, for smaller cavities, with sterile glass wool swabs prepared in the
152 aDNA laboratory, dipped in lysis buffer A (Promega Wizard Magnetic DNA Purification
153 System for Food) at the point of sampling. Tubes of lysis buffer A were also opened on the
154 workbench prior to the sampling of each item to provide the sample blanks.
155 DNA was extracted from each sample and from associated soil (Promega Wizard
156 Magnetic DNA Purification System for Food), eluted in a final volume of 100 µl, and
157 quantified (Thermo Fisher Scientific Quant-IT DNA Assay Kit and Qubit 2.0 Fluorometer).
158 The significance of possible correlations between the DNA concentrations of extracts and
159 variables including excavation mound, year and sample type (cavity, external surface or soil)
160 was assessed using a linear model, simplified following Crawley (2005), run in R v.3.0.1 (R
161 Development Core Team, 2009).
162 PCRs were carried out as described by Speirs et al. (2011) using the primers shown
163 in Table 3, using AmpliTaq Gold DNA Polymerase (Thermo Fisher Scientific). To avoid PCR
164 inhibition from residual soil contaminants, extracts were diluted and the equivalent of 0.015–
165 0.150 µl of the undiluted extract used per 30 µl PCR, the amount determined by optimization
166 experiments. Cycling parameters were: 10 min at 95°C; 40–50 cycles of 45 s at 94°C, 1 min
167 at the annealing temperature, 1 min 20 s at 72°C; 7 min at 72°C. PCR products were

purified from gels (QIAGEN MinElute PCR Purification Kit) and cloned (Thermo Fisher Scientific CloneJET PCR Cloning Kit) in *Escherichia coli* XL1-Blue (Agilent). Colony PCR was performed in 20 µl reactions comprising 1 × *Taq* buffer (New England Biolabs), 200 nM each primer, 200 mM dNTPs and 0.625 units Taq DNA polymerase (New England Biolabs), 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 min at 72°C. Single-stranded DNA was digested (GE Healthcare Illustra ExoProStar) and the PCR products sequenced (ABi PRISM BigDye Terminator protocol).

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

## 3. Results

### *3.1 DNA concentrations of extracts*

The DNA concentrations of the extracts ranged from <0.05–3.32 ng $\mu l^{-1}$ (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

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

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

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

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

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

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

*3.4 Identification of environmental fungi*

The RDNA primer pair (Table 3) was designed by us to amplify part of the large subunit ribosomal RNA gene and adjacent internal transcribed spacer region from a range of ascomycete fungi. This PCR was intended to test for the presence of yeasts such as *Saccharomyces cerevisiae*, which might indicate that the libations included fermented 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 9$^{th}$–14$^{th}$ 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.

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

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

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

## 5. Conclusion

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

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

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

| | | | |
|---|---|---|---|
| YK11-Q10-L1 | clay bicone | YK11-Q10-L1A | interior (tallest side) |
| | | YK11-Q10-L1B | exterior (tallest side) |
| | | YK11-Q10-L1S | cavity soil 0.24 g |

* 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)* | λ† |
|---|---|---|---|---|---|---|
| YK07 | 2007 | YK07 | 28.4–28.7 | 979–1317 ± 39 | 18640–28929 | 0.056–0.087 |
| YK08 | 2008 | | | | 18567–29044 | |
| YK10 | 2010 | YK10-3/YK11 | 28.4–28.7 | 780–940 ± 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 ± 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<br>R: ATCGGGCAATGGGTGGCGGA | 133 | Pearl millet ATPase gene | This study | 58 |
| CYTB | F: ATAGACAAAATCCCATTCCA<br>R: TAGTTGTCAGGGTCTCCTAG | 125 | Mammalian cytochrome b gene | Irwin et al. (1991) | 54 |
| HVR1 | F: ACAGCAATCAACCCTCAACTATCA<br>R: TGTGCTATGTACGGTAAATGGCTT | 131 | Human mitochondrial HVR1 | Bouwman et al. (2008) | 58 |
| RBCL | F: GGCAGCATTCCGAGTAACTCCTC<br>R: CGTCCTTTGTAACGATCAAG | 137 | Plant RuBisCO gene | Poinar et al. (1998) | 58 |
| RBSP | F: AGAAGAAAGAGAAGAAGCACG<br>R: GGACAGCTCGTATTATAACCTGC | 143 | Maize ribosomal protein gene | Gyulai et al. (2007) | 60 |
| RDNA | F: CAGCGGGTACTCCTACCTGA<br>R: GAGAGGTTTCTCTGCGTGCT | 136 | Ascomycete ribosomal DNA | This study | 58 |
| TRNL | F: GGCAATCCTGAGCCAAATC<br>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<br>Large head | exterior | No BLASTn match | 2 |
| | YK10-D11-3I<br>Seated female figure | interior | Poaceae *E*=2e-35 | 7 |
| TRNL | YK07-2-A4-1C1<br>Large conical figure | exterior | Pinales *E*=3e-11<br>No BLASTn match | 5<br>2 |
| | YK07-2-A8-1C<br>Anencephalic head | exterior | No BLASTn match | 3 |
| | YK07-2-B5.1A2<br>Large head | interior | *Musa/Ensete* sp. *E*=8e-12<br>No BLASTn match | 5<br>5 |
| | YK07-2-B5.1S<br>Large head | cavity soil | No BLASTn match | 4 |
| | YK08-AB9-L7B<br>Horse and rider | interior | No BLASTn match | 6 |
| | YK10-D11-3D<br>Seated female figure | exterior | Poaceae *E*=2e-34 | 9 |

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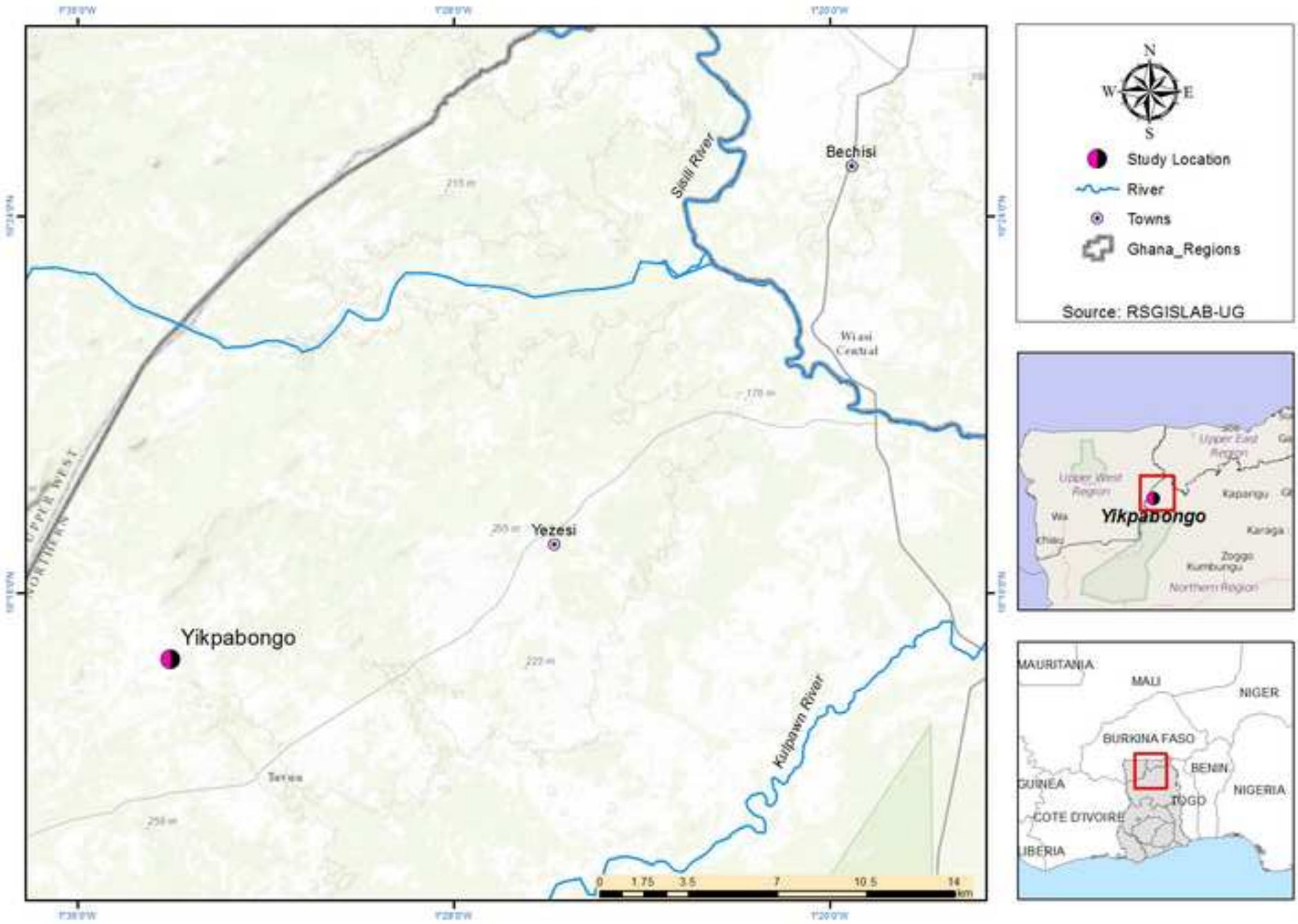

Figure 1

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