

Earliest human funerary rites in insular Wallacea 15,500 to 14,700 years ago

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Abstract

The insular region of Wallacea has become a focal point for studying early human evolution in island environments. Here we focus on how socioeconomic adaptations, under changing climatic conditions, influenced the belief systems and burial practices of past foragers at Ratu Mali 2, an elevated coastal cave site on the small, impoverished island of Kisar dated to 15.5–3.7 ka. This multidisciplinary study reveals the impressive flexibility of our species in the most marginal of environments by demonstrating extreme marine dietary adaptations as well as engagement with an extensive trade and exchange network across open seas. A male and a female, interred in a single grave at Ratu Mali 2 by 14.7 ka are the oldest known human burials in Wallacea with established funerary rites. These findings enable exploration of how human societies and belief systems adapted to rising sea levels in Wallacea after the Last Glacial Maximum.

Introduction

The arrival of anatomically modern humans (AMH) into the insular region of Wallacea ca. 46 – 40 ka (1–4) was contemporaneous with some of the earliest maritime adaptations of our species (5–10). These adaptations coincided with substantial climate-mediated sea level fluctuations that influenced island size and ecologies, as well as human migration and evolution (5, 11–16). Wallacea lies between the continental shelves of Sunda and Sahul, to which it has never been connected, and contains the islands of eastern Indonesia (Sulawesi, Nusa Tenggara Timur, and Moluccas) and the independent country of Timor-Leste on Timor (12–13). These unique biogeographical conditions likely contributed to the emergence of biological, cultural, and economic variability seen in contemporary societies in the region today (17–20).

Here, we argue that AMH adaptation to marine environments in Wallacea over several millennia (1–3, 5–10) led to the emergence of social complexity after the Last Glacial Maximum (LGM) ca. 25 – 18 ka (21), when rising sea levels, and generally warmer and wetter conditions (22–24) coincided with expanding populations (25), and the development of large-scale maritime socioeconomic exchange networks and technology (26–30). Furthermore, we argue that over time, these marine adaptations profoundly influenced the beliefs of AMH as expressed in material culture and mortuary practices. While ritualized belief systems are notoriously difficult to identify in the archaeological record, evidence exists of symbolic ornamentation and treatment of the dead that indicates social hierarchies and religious ideologies (31–32). We explore this hypothesis by investigating socioeconomic adaptations on the small island of Kisar through recent research conducted at the Ratu Mali 2 (RM2) cave site (Fig. 1–2). Kisar is critical to this debate because it is one of the smallest islands in the world to be occupied during the Pleistocene ca. 15.5 ka and epitomises human adaptation to a small precarious island environment (11, 33–34).

At RM2, cultural materials were recovered that span the Terminal Pleistocene to mid-Holocene period and include the Neolithic transition. We investigate how socioeconomic adaptations to this small island

influenced early mortuary practices in this insular maritime region. We studied the mortuary practices of two burials interred in a single grave feature at RM2, dated to the earliest settlement of Kisar (representing the earliest evidence of funerary rites in Wallacea), supported by multidisciplinary archaeological science techniques (Bayesian modelling of radiocarbon dates, zooarchaeology, artefact analyses, obsidian geochemical characterization, stable isotopes, and enamel peptide analyses of human skeletal remains).

Ratu Mali 2 site and chronology

Kisar is a small island at 81.15 km² (Fig. 1). Due to its steep offshore bathymetry it would not have changed much in size since human settlement ~ 15.5 ka (33). Kisar is part of the active Banda arc-continent collision zone and has a metamorphic core ringed by both emerged and submerged limestone terraces (35). The island is visible from Timor-Leste 25 km to the southwest and has a narrow reef platform overlooked by uplifted coralline limestone cliffs with few coastal access points. Kisar is drier and relatively more impoverished in terrestrial fauna (small rat, bat, snake, lizard, and bird species) compared to larger islands in the region (33, 36). The current vegetation is dry savannah with woodland patches and the island has few seasonal freshwater sources and low rainfall, which falls mostly during the monsoon season from November to May (33). Kisar is thus more prone to the climatic shifts affecting water and faunal resources that occurred in Wallacea since the LGM (22–24).

RM2 is located 500 m inland from the east coast of Kisar (S 8°04'20.572 E 127°12'42.697) in the upper limestone coastal terrace elevated at 38m asl, and 650 m to the south of Jawalan harbor, which provides access for watercraft and reef foraging (Fig. 1–3). RM2 is a modest-sized cave 40x20 m, with a gently sloping floor, high ceiling (ca. 10 m), and large opening overlooking the surrounding coastal terrace below (Fig. 2–3). SQ A (1 m²) was excavated in nine 5 cm spits (A1-9) near the cave entrance to a depth of 45–55 cm until bedrock was encountered. A further 100 x 50 cm extension (SQ B) at the eastern side of SQ A was excavated to expose a basal grave feature. A further six spits (B1-6) were excavated down to the top of the grave feature. Five layers were recorded in the field based on colour and sediment consistency and the depositional age-depth model based on eight radiocarbon dates suggests three major cultural chronostratigraphic phases from which cultural and bioarchaeological samples were recovered (Fig. 4–14, SI Tables 1–16).

Phase 1 represents the initial use of the cave to inter the dead where two poorly preserved individuals were recorded *in situ* in a single grave. The grave appears to have been dug into the top of Layer 5, a natural layer containing very small amounts of cultural material directly overlying cave bedrock. Direct dating of abalone (*Haliotis varia*) and chiton (*Acanthopleura* spp.) shell adjacent to Burial 2 in the grave fill just above bedrock returned dates of 15,179 – 14,725 cal. BP (S-ANU-58732) and 15,504 – 15,043 cal. BP (Wk-54632) respectively, making this grave contemporaneous with the earliest phase of Kisar settlement at Here Sorot Entapa (HSE) (33), 5.8km southeast of RM2 on the south coast. Abundant marine shell and obsidian artefacts were recovered from the grave fill. An inverted charcoal radiocarbon date (S-ANU-60433) of 1,045–917 cal. BP was obtained in A9 of Layer 5 where intrusive burnt tree roots

were recorded. The model indicates that this inverted date has a 100% probability of being an outlier and is thus justifiably removed from our main model (Fig. 6 and SI Table 16).

Phase 2 is a preceramic habitation characterized by sparse chert artefacts, animal bone and mollusc remains in Layer 4 and 3, with the base of layer 4 dated to 9,424-9,139 cal. BP (S-ANU-60432) overlying the grave feature of Phase 1. An abalone shell (*Haliotis varia*) (Wk-54631) dates its base to 8,642-8,362 cal. BP directly above Burial 2. Two charcoal radiocarbon dates of 7,268-7,165 cal. BP (Wk-54633) and 4,419-4,291 cal. BP (Wk-54634) were obtained from the middle and top of Phase 2 (Layers 4 – 3).

The top of Phase 3 contains more concentrated lithics, animal bone and shell. A single charcoal radiocarbon date 3,972-3,775 cal. BP (S-ANU-60431) in Layer 2 (Spit 2) places the Phase 3 deposits well within the early Neolithic period of ISEA (37). The Phase 3 deposits are clearly distinct from the mid-Holocene deposits of Layer 3 and underlying Layer 4, as Layers – 1 and 2 contain concentrated white/grey ash lenses and charcoal. These upper layers also contained pottery fragments, found mostly in Layer 1 (Spit 1). The site was abandoned soon after but may have been used more recently for goat herding activities.

Dietary adaptations on a small island

Coastal prehistoric sites in Wallacea demonstrate significant marine dietary adaptations that included inshore and offshore fishing, littoral zone shellfish foraging, and in some cases marine turtle exploitation (2–3, 6, 9–1033, 38). Predictably, on Kisar, zooarchaeological data indicate that prehistoric foragers at HSE and RM2 adapted to their small island environment by focusing their subsistence predominantly on the near shore marine resources of the fringing rocky reef. The presence of small amounts of marine finfish (Actinopterygii) in the RM2 deposits (Fig. 7, SI Tables 2–3) reflects a mixture of angling, netting and spearfishing on the reef platform (39). This is consistent with our observations during fieldwork where men were observed spearfishing on the rocky reef at high tide, and women and children were observed angling on the reef from the cliff-edge with small metal fishhooks, similar in size to the small J-shaped fishhooks recovered from HSE since the terminal Pleistocene (33). However, finfish remains at RM2 were not present in anywhere near the same abundance as at HSE, a fishing camp closer to shore (33).

Much like at HSE, intensified foraging of rocky reef shellfish is demonstrated at RM2 by the presence of large abundances of rocky reef molluscs (Fig. 8, SI Tables 4–6), and crab (Decapoda) carapace fragments (SI Table 7). Most crab remains are concentrated in upper levels and there is a general decline over time, indicating their presence in the deposit could be due to post-depositional disturbances. Most shell was concentrated in the Phase 1 grave fill, was sparse during Phase 2 before increasing in deposition intensity by Phase 3. Indeed, closer inspection of the high diversity of marine shellfish (Number of Taxa = 89) at RM2 indicates a generalist reef gleaning strategy as well as a healthy and vibrant littoral reef zone. This is not surprising considering Kisar falls within the coral triangle which is one of the most rich and diverse marine environments on the planet (40). Evenness values indicate

generalist foraging behavior that was focused on a few taxa most of which were from the rocky shore families Neritidae and Chitonidae making up 83% of the MNI recovered. Neritids and chiton, which can be harvested *en-masse*, were important resources in early sites in Wallacea (1, 10). They have predictable behaviours and are found concentrated on the base of rocks in shallow water and in rock pools in the intertidal zone (41). Foraging was thus likely conducted along the narrow intertidal reef with minimal planning required (42), by women and children as observed during fieldwork, and documented ethnographically on other islands in the wider Asia-Pacific region (43–47). While gleaning intensity increased over time during the mid-Holocene, there was no notable change in diversity, evenness, and equitability, suggesting significant increases in settlement intensity by the mid-Holocene but no significant changes in foraging behavior.

Stable carbon isotope studies of human tooth enamel bioapatite indicate that, by the terminal Pleistocene on Alor and Timor, some communities had additionally developed adaptations to terrestrial tropical resources that likely included a greater consumption of endemic plants (48). At Kisar, a greater marine adaptation relative to other sites in the region by the Terminal Pleistocene is supported by the stable isotope data from RM2 (Fig. 9, SI Table 8). The human teeth sampled had values compatible with a heavy reliance on marine resources, but could also reflect wild C₄ resources (48). There is no isotopic evidence for wild C₄ resources on the island of Timor across the same period (48), and Kisar may have once been covered by primary forests and woodlands dominated by C₃ plants (36). Similar $\delta^{13}\text{C}$ values have been observed for humans at Asitau Kuru ~ 40,000 years ago and the sites of Matja Kuru 1 and 2 ~ 11–4,000 years ago and interpreted as heavy marine reliance (48).

Prehistoric terrestrial resource exploitation on Kisar is difficult to assess. Most of the vertebrate remains deposited at RM2, unlike HSE, were tetrapod microfauna (small rodents, lizards, frogs, snakes, birds, and bats) (Fig. 7, SI Tables 2–3). Based on other studies conducted in Wallacea, they likely represent remains deposited by avian predators (49–50). Megafauna and giant rats, found on other islands in Wallacea (1–4, 50–55), were not present on the small island of Kisar at the time of human settlement. The lithic assemblages at HSE and RM2 with few formal tools, show similarities with other lithic assemblages in Wallacea (1, 56) such as on Sulawesi where residue analyses indicate flakes were used to exploit plants (57), and this may have also been the case on Kisar. At RM2, lithic artefacts (n = 126, Fig. 10, SI Tables 9–10), varying between 2.4 mm and 43.7 mm in percussion length, included mostly chert and obsidian flakes, with smaller amounts of quartz, quartzite, a fine-grained silicified limestone and chalcedony. These show core preparation and some instances of anvil rested reduction. Small numbers of dog and goat bones occur in the upper late Holocene deposits at RM2 (Fig. 7, SI Tables 2–3) and HSE (33), associated with the arrival of Neolithic and Metal-Age cultures in the Asia-Pacific (37, 58–60). These are indicative of the socioeconomic transformations that were occurring across the region during this period (37, 61).

The establishment of maritime networks and technology in Wallacea

Historical records, oral histories, linguistic studies and rock art of the late Holocene speak to the degree of maritime movement of peoples, culture and socio-political interactions on Kisar (62–71) suggesting integration within a socio-cultural milieu. Going back further in time, exotic obsidian has been discovered widely dispersed in archaeological sites across Nusa Tenggara Timur (NTT), from Timor to Alor, indicating water transport across Wallacea ca. 15 – 4 ka based on geochemical results (26–29). On Kisar, both HSE (29, 33) and RM2 share this same unsourced exotic obsidian known as Group 1 (Fig. 11), which could originate from one of the nearby volcanic islands in the Banda Arc such as Wetar (33). Most of the RM2 Group 1 obsidian flakes (3.7–21.2 mm in percussion length), were associated with the grave feature and the lower spits of SQ A and SQ B (SI Tables 9–10). Their temporal distribution is consistent with the dating of this maritime exchange network elsewhere (Fig. 10, SI Tables 9–10).

The exotic obsidian overlaps in time with evidence of *Tridacna* shell adze manufacturing, identified at RM2 from 9-3.7 ka (Fig. 12, SI Table 11). Similar adzes are found from the early Holocene in other sites in Wallacea and elsewhere in the Asia-Pacific, and have been linked to improvements in boat construction and an increase in inter-island voyaging (30). The RM2 data provide further support for connectivity in NTT Post-LGM (29, 33) through to the mid-Holocene. However, strontium isotope data from the buried individuals at RM2 (SI Table 8) were not able to provide further insights into mobility of early settlers on Kisar. These data seem to have been influenced by marine food consumption and/or by the sea spray effect (72), and are thus consistent with the two individuals living in an environment exposed to marine conditions.

Prehistoric burials in Island Southeast Asia

AMH remains are present in the earliest archaeological sites in ISEA dating back to 73 ka in Sumatra (73). The earliest burial in ISEA is a flexed primary burial with evidence for amputation dated to 31.5–30 ka at Liang Tebo cave on Borneo (74). However, the majority of identified burials appear in cave sites across the Asia-Pacific from the end of the LGM and appear clustered at the onset of the early Holocene and Neolithic periods (75–79). The RM2 burials dated to 15,504 – 14,725 cal. BP are amongst the oldest burials so far discovered east of Wallace's line with only Liang Lembudu in the Aru Islands, then part of Sahul, older at 18,800 (\pm 2300) (80). Further east in the Pacific the earliest human remains are dated to 12,300 – 11,300 cal. BP at the Pamwak site on Manus in the Bismarck Archipelago (81). To the west in the Sunda region, the Gua Braholo 6 burial on Java dated to 15,371 – 12,698 cal. BP (13,290 \pm 400) overlaps the age of the RM2 burials (82). The two primary flexed individuals interred in one grave at RM2 (Fig. 13), are the oldest known burials in Wallacea, except perhaps those on Roti where disarticulated human remains, probably associated with secondary burials, were recovered from levels dated as early as 23 ka (83). Burials closest in age in Wallacea were found on Alor from Tron Bon Lei in Square B (TBL-1) dated to 11.7–11.3 ka (32, 78, 84), and the Tron Bon Lei Square C burial dated to 17–7 ka (78). Unfortunately, direct dating of the RM2 human petrous bones failed, and these burials had to be dated using shell samples adjacent to Burial 2 within the burial fill where large amounts of shell was deposited. Direct dating of human bones is commonly unsuccessful, due to a lack of well-preserved bone collagen

in the tropics, and just under half of burials in ISEA are indirectly dated by associated shell and charcoal (79).

Maritime belief systems in Prehistoric Island Southeast Asia

Data indicate that ideologies in Wallacea were far more complex than previously thought (31). Shell disc-bead ornaments made out of *Nautilus* sp. From RM2, of the single hole variety (Fig. 12, SI Table 11), are similar to those found on Timor (52; 85–86), Alor (2), and at HSE on Kisar (33) since the early Holocene. These likely represented symbols of cultural identity and social status within a wider community across this region and may have been shared via the same obsidian maritime exchange network that moved the Group 1 obsidian. At RM2, these disc-beads were recovered in the mid-Holocene deposits.

RM2 holds symbolic relevance to the people of Kisar today. The Oraita villagers expressed deference towards a rock cairn/platform constructed adjacent to the excavation at RM2 (Fig. 2–3). This is not unusual since caves have often been documented by researchers to as having been for socio-ritualized ceremonies in Timor (87–88), while caves in ISEA have been used for funerary practices since at least the early Holocene (75–76). Reconstructions of mortuary practices, combined with direct isotopic analyses of human skeletal material, have become an important way to understand past human diet and belief systems in the Asia-Pacific (32, 48, 81, 91). However, mortuary practices in ISEA are not well known for the Pleistocene.

The two RM2 individuals were interred in a single shallow grave, 90 cm in diameter, in a primary burial position and oriented along an east-west axis facing south towards Timor-Leste (Fig. 13). Enamel peptide analysis revealed Burial 1 was male and Burial 2 was female (Fig. 14, SI Table 15). Less than 50% complete, the skeleton of Burial 1 had a highly fragmentary skull but most teeth were intact, as were the ribs and hands, while the lower body was less than 10% complete (SI Table 12). Burial 2's skull was more fragmentary than Burial 1 with only 20% of teeth present, but had 60% of hands, and 30% of legs intact (SI Table 12). Large limestone rocks were placed near the head of each burial (Fig. 13), in similar fashion to the Liang Tebo cave burial on Borneo (74) and the TLB-1 Tron Bon Lei burial on Alor (84). Both RM2 burials were found in an identical flexed position on their right sides, with hands carefully tucked under the head (Burial 1) or chin (Burial 2) (Fig. 13). The significance of the orientation of the burials facing south towards Timor-Leste is unknown. The burials are associated with most of the obsidian flakes recovered from RM2 within the grave fill as well as abundant marine shell – both of these are interpreted as grave good inclusions (SI Table 6). These data reveal socio-ritualized funerary rites, including ceremonial feasting, for a male and female potentially bonded together in life. These are the earliest funerary rites yet recorded in Wallacea and the wider Asia-Pacific and are three millennia older than the rotating circular fishhooks interred with the Tron Bon Lei TLB-1 burial dated to ca. 12 ka (32).

Discussion and Conclusions

These data correlate with findings from other sites in Wallacea that indicate substantial changes in society and demography in the region post-LGM when sea levels, temperatures, and precipitation were rising dramatically (22–24). At this time, populations appear to have increased significantly in the region judging by the substantial increase in settlement intensity at a number of sites in Wallacea (1, 14, 25). Maritime obsidian exchange networks and shell adze technology dramatically expanded with shell disc-bead culture throughout Wallacea and on Kisar after 15.5 ka at HSE (33) and RM2. This may represent the earliest extensive maritime network in human history, with socio-political inter-group connections expanding in a non-linear exponential fashion throughout the Wallacean Archipelago as predicted by Metcalfe's Law (92). Such a network could be beneficial, particularly to the inhabitants of a marginal environment such as Kisar, as this may have reduced the risks of settlement during periods of precipitation decline and reef instability (33).

Eastern Indonesia, post-LGM, saw significant fluctuations between cooler/drier and warmer/wetter conditions as the strength of the Australian-Indonesian Summer Monsoon changed (24). Unexpectedly, occupation intensity was greater during the cooler drier Bølling-Allerød period 14.6–12.9 ka (22) at HSE, and declined after 12.8 ka cal. BP culminating in hiatuses at both HSE and RM2 in the early Holocene (11, 33). It appears that reef platform stability and its impact on marine resource availability may have influenced the desirability of settlement on Kisar more so than precipitation (11). This may explain resettlement of the island, seen at both HSE and RM2, by the mid-Holocene when higher stable sea levels likely resulted in stable productive coral reefs (11). Prior to this, in the terminal Pleistocene and early Holocene, occupation on Kisar was supported by inter-island reciprocity evidenced by the obsidian exchange network. This would have ensured the ability of the Kisar population to relocate to neighbouring network islands during periods of climate instability or reef bleaching on Kisar (33). The disappearance of this obsidian exchange network ca. 4000 BP in Wallacea and on Kisar coincides with the dispersal of Neolithic peoples and food production indicating it was superseded by a new socio-political strategy. Similarities in trade and exchange networks and differences in subsistence behaviours on Kisar compared with other sites on Alor and Timor, indicate that different populations within this regional maritime network had to adapt to differences in marine and terrestrial environments. This could explain variations in subsistence strategies along the diversification/specialization spectrum. In contrast to previous isotope studies on larger islands that demonstrated diversification over time by the end of the Pleistocene (48), an extreme marine diet was critical to human sustainability on impoverished Kisar.

The proliferation of cave burials in the Asia-Pacific post-LGM (77–79, 81–82) perhaps indicates an increase in the use of caves for socio-ritualized purposes (75) as increased network effects coincided with the arrival of diverse populations (93) and led to heightened cultural interaction across Wallacea. The earliest burials in ISEA were typically secondary burials, including Liang Lemdubu on Sahul, as flexed burials became common by the Holocene onset (77–79, 84, 90). RM2 appears to contain the earliest flexed burials in Wallacea, followed by the Tron Bon Lei individual TLB-1 from nearby Alor, dating to ca. 12,000 cal. BP, which was also interred in a primary flexed position, lying on its left side (32, 84). West of Wallace's line, the Liang Tebo flexed burial on Borneo was significantly earlier (74). On Java, Gua Braholo 6, contemporaneous with RM2, was also flexed and interred on the right side on an east-west axis (82).

The similarity of burial treatment between Java, Borneo and the Wallacean islands of Alor and Kisar may indicate that these islands were connected by an expanding cultural network with shared belief systems. Funerary rites at RM2 (shellfish feasting and exotic obsidian) and Tron Bon Lei (fish-hooks, and cobbles coated with red ochre) three millennia later (32, 84) are rare examples of symbolic treatment of the dead, shaped by variability in the cultural and physical environments across Wallacea post-LGM.

Persistent settlement in some of the most marginal ecological conditions globally (1, 94), and now on Kisar, has been argued to demonstrate the unique adaptive flexibility of our species when compared to archaic hominins (94). What drove this novel 'plasticity' remains debated, but the technological change, social networks, and cultural expressions of communication were all likely essential in enabling populations of our species to develop resilience in challenging habitats while maintaining connectivity between populations on a larger scale. Burial traditions, subsistence strategies, and evidence for social connectivity at RM2 provide further evidence of this, and highlight the diverse contexts of human socio-cultural experimentation during the late Pleistocene.

Methods

Excavation and Radiocarbon dating

A 1 m² pit (SQA) was opened near to the entrance of the cave where the slope flattened. The pit location was chosen for its central location near the opening with optimal light and where prehistoric habitation activity was most likely to be focused. SQ A was hand excavated with trowel and hand shovels in 5 cm intervals (spits) within stratigraphic layers down to bedrock (Spits A1-A9). When a burial was encountered in the east baulk this was extended at the eastern side of SQ A with another 100 x 50 cm pit (SQ B) for the purposes of accessing the complete burial feature for excavation. SQ B was also excavated in 5 cm spits down to the top of the burial (spits B1–B6). When the top of the burial was exposed in both SQ A and B, the excavation was further extended into the southeast baulk using spades to expose it in the burial extension area. Once the discrete burial feature was exposed fully in plan, the burial was excavated separately in two 5 cm spits removing sediment and exposing the human skeletal remains *in situ* using brushes, fine wooden skewers and small trowels to reveal the burial position and associated artefacts.

All excavation units, features, and *in situ* charcoal, shell and artefacts were recorded in 3D using a Leica TS09 total station. Charcoal was also collected from the section walls. These sections were hand drawn on graph paper and digitized later. Charcoal was also recovered *in situ* during excavation as well as during sieving and sorting. All material from SQ A (spits 1–9), SQ B (spits 1–6) and from the burial feature was first dry sieved through a 1.5 mm mesh and then bagged by spit and feature for transport to the beach where it was further wet sieved through another 1.5 mm mesh. The material from above the burial extension was discarded without sieving due to time constraints. All retrieved material was then dried and sorted into the following categories: bone, shell, lithics, charcoal, seeds, pottery, ochre, shell artefacts.

In situ marine shell and charcoal were dated at the Australian National University Radiocarbon Dating Centre (95) and Waikato Radiocarbon dating laboratory. All dates were calibrated in OxCal 4.4, using IntCal20 (96) and Marine20 (97), to 95.4% confidence interval (SI Table 1). These radiocarbon dates were put into an age depth depositional model within OxCal 4.4 (98). The age depth model assumes a Poisson (or random) accumulation of sediment (99), calculated from the available age data by averaging the model over many values of k (100). The model interpolation rate was set to a single date per spit, and the unit of depth used was in centimeters (cm). For charcoal dates, we applied the *Charcoal Plus* t-type Outlier Model with a prior outlier probability of 10%, which is specifically designed to account for the inbuilt age of charcoal, while also allowing for some stratigraphic movement in an archaeological context (101–102).

Mortuary practices

Mortuary practice reconstructions used standard data collection methods in the field (103–105) and centered on the anatomical composition of human skeletal material and their spatial distribution in relation to grave goods to reconstruct the initial internment conditions (body position, manipulation) through visual aspects of the burials in plan (photographs and drawings). Once the skeletal material was exposed and carefully cleaned of loose sediment, the burials were photographed *in situ* with datum points plotted in 3D at critical points on the skull, long bones, hands and feet. Both burials were in exceptionally fragile condition, the bones were soft and flaking so excavation and lifting had to be completed with care. Once recorded elements were individually lifted by hand and placed into bags with bubble wrap and stored in plastic containers for transport for study at the archaeology department laboratory in Universitas Gadjah Mada (UGM), Jogjakarta. Biological characteristics consisting of age, sex, stature, biological affinity, and pathological history are difficult to assess due to the poor condition of these individuals, which are also covered in concreted carbonate. They were assessed using standard bioarchaeological methods (103) and will be reported in a future publication.

Faunal analyses

The vertebrate and invertebrate remains were further sorted in the UGM lab by broad taxonomic group for further designation into the lowest taxonomic level possible whether that be order, family, genus or species. Identification and quantification methods followed (1). In most cases identification could only be made to class, order or family level due to limited reference materials available at UGM. Vertebrates were quantified by Number of Identified Specimens Present (NISP) and weight (g). In the case of invertebrates, previously identified archaeological reference specimens were available at the department of archaeology at UGM for comparison with the RM2 material. Molluscs were quantified by Minimum Number of Individuals (MNI) using a non-recurring element for each taxon.

Diversity and evenness values were calculated for mollusc MNI by taxa using PAST4 software (106) to determine changes in foraging strategies, whereby diversity or richness (NTAXA) calculates number of

taxa exploited and evenness indices including Simpsons, Shannon-Wiener H, Equitability e, and Equitability J, calculate relative abundance of taxa being exploited to detect changes in foraging strategies (50, 107). The higher the evenness value the less diverse the assemblage and the more generalist the foraging strategy, conversely the lower the evenness score the greater the focus of foraging on fewer taxa suggesting a more specialized strategy.

Stable carbon ($\delta^{13}\text{C}$), oxygen ($\delta^{18}\text{O}$) isotope analysis

Two human teeth, one from each burial, were selected for stable carbon and oxygen isotope analysis. Stable carbon and oxygen isotope analyses of human and faunal tooth enamel has proven an effective method for reconstructing Pleistocene-Holocene human subsistence in Wallacea. In tropical regions, $\delta^{13}\text{C}$ analysis can be used to distinguish reliance on C_3 resources, which dominate woodland and forests settings and include crops such as rice, versus C_4 resources, which are commonly tropical grasses including crops such as millet (108–111). Plants and animals feeding on plants, in closed canopy forest also have lower $\delta^{13}\text{C}$ than other C_3 plants growing in more open areas thanks to the 'canopy effect' (112). Higher $\delta^{13}\text{C}$ in marine producers than all C_3 terrestrial plants (113–114) facilitates distinction of marine versus terrestrial C_3 consumers (115). While consumption of marine and C_4 resources can lead to overlapping $\delta^{13}\text{C}$, the provision of a detailed faunal baseline and contextual information can tease these factors apart (48). $\delta^{18}\text{O}$ analysis of tooth enamel provides additional mammalian information about imbibed water and consumed food. $\delta^{18}\text{O}$ analysis has also been argued to distinguish terrestrial from marine consumers (116), though this is not currently evident for Wallacean contexts (48).

Air-abrasion was used to clean the selected teeth and remove adhering external material. A diamond-tipped drill was used to obtain 8 milligrams of enamel powder from the full length of the buccal surface of the tooth, representing the entire period of enamel formation. Samples were washed in 1.5% sodium hypochlorite for 60 minutes, followed by three rinses in purified H_2O and centrifuging, before 0.1M acetic acid was added for 10 minutes, followed by another three rinses in purified H_2O . Samples were subsequently lyophilized for 24 hours. 100% phosphoric acid was added to the samples with the gases evolved being measured for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ using a Thermo Gas Bench 2 connected to a Thermo Delta V Advantage Mass Spectrometer at the Max Planck Institute for the Science of Human History, Jena, Germany. $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values were compared against International Standards (IAEA-603 ($\delta^{13}\text{C} = 2.5$; $\delta^{18}\text{O} = -2.4$); IAEA-CO-8 ($\delta^{13}\text{C} = -5.8$; $\delta^{18}\text{O} = -22.7$); USGS44 ($\delta^{13}\text{C} = -42.2$) and in-house standard (MERCK ($\delta^{13}\text{C} = -41.3$; $\delta^{18}\text{O} = -14.4$)). Replicate analysis of MERCK standards suggests that machine measurement error is *c.* $\pm 0.1\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.2\text{‰}$ for $\delta^{18}\text{O}$. Overall measurement precision was based on repeat extracts from a bovid tooth enamel standard ($n = 20$, $\pm 0.2\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.3\text{‰}$).

Strontium ($^{87}\text{Sr}/^{86}\text{Sr}$) isotope analysis

Strontium isotope analysis has been used to assess the mobility and migration patterns of prehistoric humans around the world (117–120), but little work has focused on applying this method in Island Southeast Asia. The method is based on the premise that rocks display variable $^{87}\text{Sr}/^{86}\text{Sr}$ depending on their type, age, and original rubidium (Rb) content. Mineral weathering and erosion of the underlying bedrock, airborne dust (loess), sea-spray, atmospheric deposition, groundwater, and stream water are major contributors to the strontium in soil (121–123). As a result of these processes the biologically available $^{87}\text{Sr}/^{86}\text{Sr}$ of the soil may vary from the $^{87}\text{Sr}/^{86}\text{Sr}$ of the underlying bedrock (124–125). Human tooth enamel is resistant to diagenetic alteration in the burial environment. Strontium purified from enamel should be representative of the biogenic strontium available during the time of tooth mineralization (117, 126–127). The $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of tooth enamel reflects the bioavailable strontium isotope signature of the food and drink a person consumed during the time of tooth mineralization (121).

For radiogenic strontium isotope analysis, two 10–20 mg enamel samples from the same teeth used for the above analyses were prepared and analysed at the class-10, ultra-clean laboratory at the Centre for Trace Element Analysis, Department of Geology, University of Otago, Dunedin, New Zealand. Prior to transferring to the clean lab, the enamel was cleaned through abrasion with a sonicated Dremel® reinforced diamond cutting wheel to remove possible surface contaminants. Any additional adhering materials, such as organic matter or dentin, were also removed. Samples were then transferred to the clean lab and weighed in clean Perfluoroalkoxy alkanes (PFA) vials (Savillex, Eden Prairie, Minnesota, USA) and digested in 2 ml of 3M HNO_3 overnight at 110°C. Samples were then evaporated between 4–8 hours at 110°C. After evaporation, dried samples were dissolved in 2 ml of 3M HNO_3 and subsampled to determine strontium concentration. Strontium was purified manually using established processes of column chemistry (117, 128). Only a single elution was necessary and the sample was evaporated, reconstituted in 2% HNO_3 , subsampled for strontium concentration analysis, and then $^{87}\text{Sr}/^{86}\text{Sr}$ was measured using a Nu Plasma-HR MC-ICP-MS instrument (Nu Instruments Ltd., UK). Data were normalized using repeated measurement of two in-house lab references, NIST SRM 987 and HPS, that bracketed every six samples to monitor the accuracy and reproducibility of the measurements. The in-house, long-term reference value for NIST SRM 987 is 0.71025 ± 0.00002 (2 SD, $n > 200$) and for HPS it is 0.70762 ± 0.00003 (2 SD, $n = 189$). Any instrumental mass fractionation present was corrected using repeated measurement of $^{86}\text{Sr}/^{88}\text{Sr}$, expected to be 0.1194. Procedural blanks were run with each batch of 48 samples and all yielded negligible Sr levels of < 250 pg.

Peptide analysis

Enamel peptide preparation followed established methods (129). First the enamel was washed in 3% H_2O_2 for 30 seconds and quenched in MilliQ H_2O for 30 seconds. The enamel chip was then conditioned in 60 μL of 5% (vol/vol) HCl for 2 minutes. The HCl conditioning solution was removed and discarded. Another 60 μL of 5% (vol/vol) HCl was added to the enamel chip and incubated for 2 minutes for peptide extraction. The HCl extraction solution containing the peptides was then recovered and centrifuged for 5

min at 20,000 rpm to remove any particulates. The extracted peptides were then purified by solid phase extraction on a ZipTip with 0.6 μL C_{18} resin (Millipore) essentially following the manufacturer's instructions. In brief, after rinsing of the C_{18} resin in pure acetonitrile (ACN), the ZipTip was equilibrated in 0.1% formic acid (FA) in water before loading the sample of extracted peptides. The bound peptides were then washed on-tip with 0.1% formic acid (FA) in water and eluted in 60% ACN, 0.1% formic acid (FA) in water. The eluate was dried using a centrifugal vacuum concentrator and stored as dried samples at -80°C .

For peptide analysis by untargeted liquid chromatography-coupled tandem mass spectrometry (LC-MS/MS) samples were reconstituted in 20 μL of 5% ACN, 0.1% FA in water of which 5 μL were loaded into an Ultimate 3000 nano-flow uHPLC system inline coupled to a LTQ-Orbitrap mass spectrometer (Thermo Scientific). Peptides were separated on a 75 μm ID silica emitter tip column that was in-house packed with Aeris 2.6 μm PEPTIDE XB-C18 100 \AA bead material (Phenomenex) on a length of 20 cm. The LC gradient between mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in 90% aqueous ACN) was developed from 5% B to 25% B over 13 min followed by an increase to 40% B over 3 min. and 90% B over 2 min. at a flow rate of 300 nl/min. The LTQ-Orbitrap mass spectrometer was operated in an untargeted data acquisition mode. The precursor ion scan was performed in a m/z window of 400 to 2000 at a resolution of 60,000. The precursor ion scan was followed by 9 data-dependent collision-induced dissociation (CID) fragment ion spectrum acquisitions, of which the first three scans were set up as semi-targeted acquisitions of pre-specified precursor masses if present in the precursor ion scan at a threshold intensity of at least 5000 counts. The following 6 CID acquisitions were set up as data-dependent scans of the 6 most intense ions. The peptide targets included in the precursor ion list were three peptidofoms of the AMELY encoded amelogenin isoform (SMIRPPY at m/z 432.23, $\text{SM}_{\text{OX}}\text{IRPPY}$ at m/z 440.22 and $\text{SM}_{\text{OX}}\text{IRPPYS}$ at m/z 483.74), one peptide specific for the AMELX encoded isoform (SIRPPYPSY at m/z 540.28) and one peptide common to both isoforms (MPLPPHPGHPGYINF at m/z 837.42).

Raw data were analysed by both software-assisted sequence database searches and manual spectrum interpretation. The Proteome Discoverer software (version 2.5, Thermo Scientific) was used for sequence assignment using the Sequest HT program to search the human reference and UniProt amino acid sequence databases. Enzyme cleavage was set to non-specific and methionine oxidation was included as a variable modification. Precursor and fragment ion intensities (area under the curve) were extracted manually using the Qual Browser program of the Xcalibur software package (version 2.0.7, Thermo Scientific).

Based on the results of the untargeted data acquisition approach, a fully targeted high resolution multiple reaction monitoring assay was set up for further confirmation of the presence of the AMELY isoform using a 5600 + Triple Time-Of-Flight mass spectrometer coupled to nano-flow liquid chromatography (AB Sciex). Sample loading and LC conditions were the same as for the untargeted approach. The following precursor ions of selected peptide sequences were targeted. AMELY-specific: SMIRPPY, $\text{SM}_{\text{OX}}\text{IRPPY}$, and

SM_{OX}IRPPYS; AMELX-specific: SIRPPYSY, YEVLTPLK; present in both AMELY and AMELX: LPPHPGHPGYINF. Data were analysed with the Skyline software (version 21.2.0, <https://skyline.ms/project/home/begin.view?>).

Artefacts

Stone artefacts were identified by raw material with flakes, flake fragments, cores and flaked pieces counted using the total number of stone flaked artifacts [TNA] and the minimum number of flakes [MNF] (130). Heat damage was present on some stone artefacts and identified by the presence of crazing, potlids and crenulated surfaces. Pottery was counted by NISP and weight and assessed for form and decoration. Shell artefacts were counted, weighed, photographed and recorded by taxa and evidence of use-wear and/or manufacture.

Obsidian Geochemistry

Obsidian artefacts were analysed semi-quantitatively by portable X-Ray Fluorescence analysis (pXRF), a Bruker Tracer III-SD was employed. Manufacturer recommended settings were used of 40 keV and 42 mA using a 0.1524 mm Cu, 0.0254 mm Ti and 0.3048 mm Al filter in the X-Ray path and a 60 s live-time count at 145 FWHM. The raw counts of the pXRF were calibrated using 40 international standards provided by MURR (131). Each artefact was analysed at two spots. Element concentrations of manganese (Mn), iron (Fe), zinc (Zn), gallium (Ga), thorium (Th), rubidium (Rb), strontium (Sr), yttrium (Y), zirconium (Zr), and niobium (Nb) were calculated. Elemental concentrations were compared to six known obsidian sources in ISEA, four obsidian source regions in the Western Pacific and two identified sources in the Lesser Sunda Islands which are only known by artefact occurrences at sites (Group 1 and 2) (26–29). Discriminant Analysis (LDA) in the PAST4 software was used for comparison (106).

Geochemical analysis of 31 obsidian flakes was conducted. Discriminant function analysis (LDA) and bi-plots of the elemental composition of each artefact against the reference samples were used and the statistical analysis unambiguously sourced all samples to the Group 1 source (Fig. 11). The initial LDA of Mn, Rb, Sr, Y, Zr and Nb was successful insofar that 98% of all sources could be excluded as a possible origin. Only the sources of Uliang Bundoc on Luzon, Philippines, and Group 1 obsidian, assumed to be located in the Lesser Sunda Islands, match the geochemistry of the samples. Unfortunately, these two sources plot closely together and an unambiguous sourcing by LDA alone was not possible. However, additional analysis by bi-plots of selected elemental compositions revealed that the Uliang Bundoc source is not a good match for the RM2 samples. Sr and Nb were selected to investigate the differences between Uliang Bundoc and Group 1 (Fig. 11). Uliang Bundoc has significantly lower values in both Sr and Nb, and can be excluded as possible origin of the artefacts.

Declarations

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Author Contributions

S.C.H directed the research. S.C.H, Mahirta, G.A.Z, Y.R.F and the Oirata community undertook fieldwork. S.C.H and Y.R.F conducted vertebrate analyses. D.M.S., Y.S. and M.L. conducted invertebrate analyses. C.R. conducted pXRF geochemical analyses and interpretations. P.R. conducted isotopic analyses and interpretations. T.M. conducted lithic analyses. S.K. conducted Bayesian age modelling. G.A.Z, S.C.H, R.K., and P.Y. conducted human skeletal element and mortuary practices analyses. R.K. and S.C.H sampled human remains for isotope and aDNA and radiocarbon dating analyses. S.C.H. prepared the manuscript draft and all authors contributed to the final draft.

Data availability

All raw data is presented in the supplementary information of this paper.

Competing interests

The authors declare no conflict of interests

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Figures

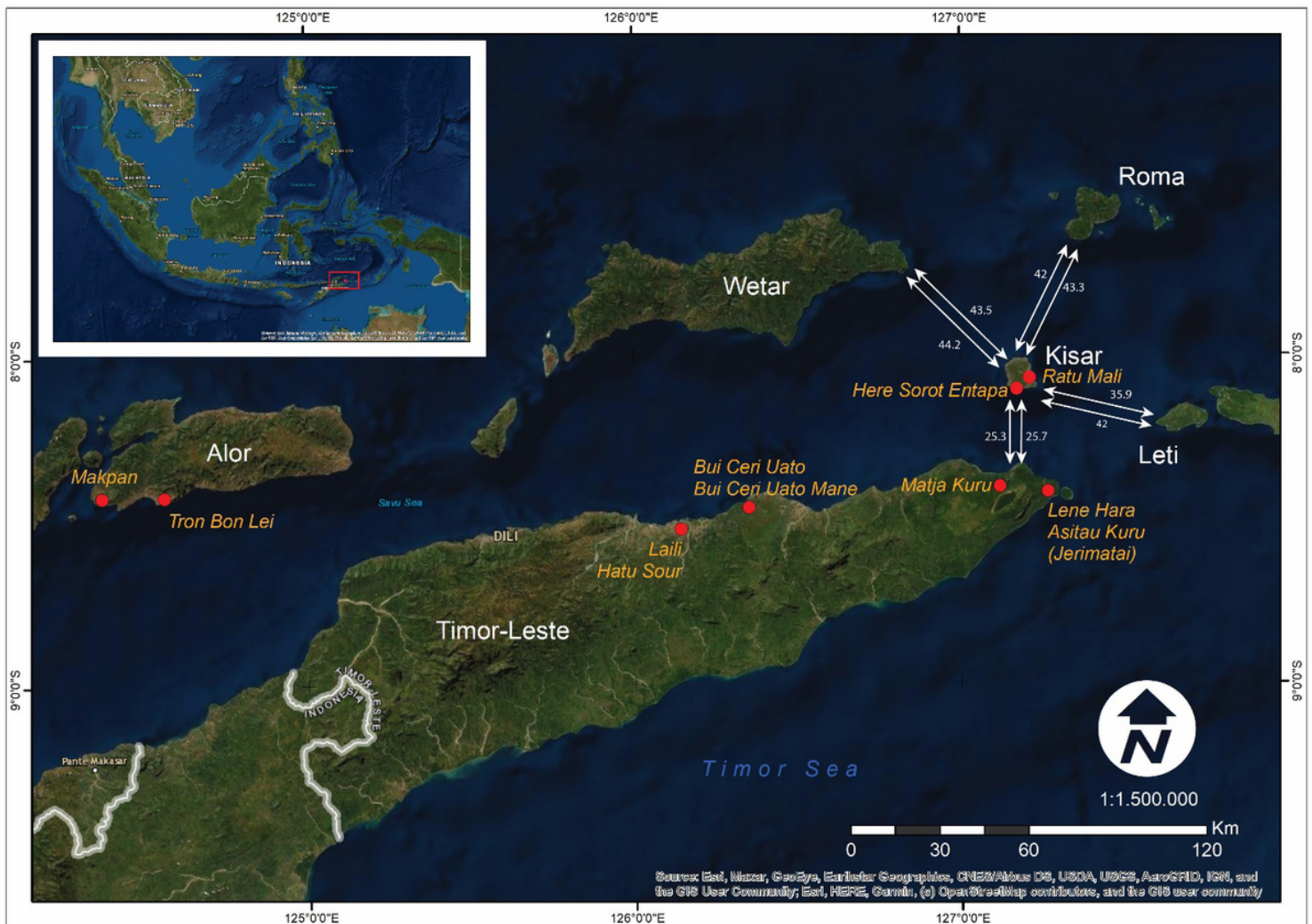


Figure 1

Location of Ratu Mali 2 (RM2), and Here Sorot Entapa (HSE) Kisar Island, Nusa Tenggara Timur, eastern Indonesia



Figure 2

Ratu Mali 2; A) View of Ratu Mali 2 from the coastal flat facing west; B) Ratu Mali 2 and excavation area facing west; C) Ratu Mali 2 cave opening facing east towards the coast; D) Excavation units SQ A, SQ B ext, and unit C ext; E) Ratu Mali 2 facing east towards the cave opening

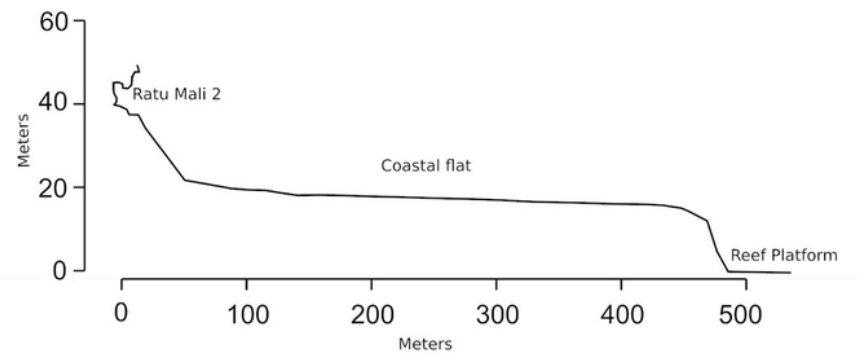
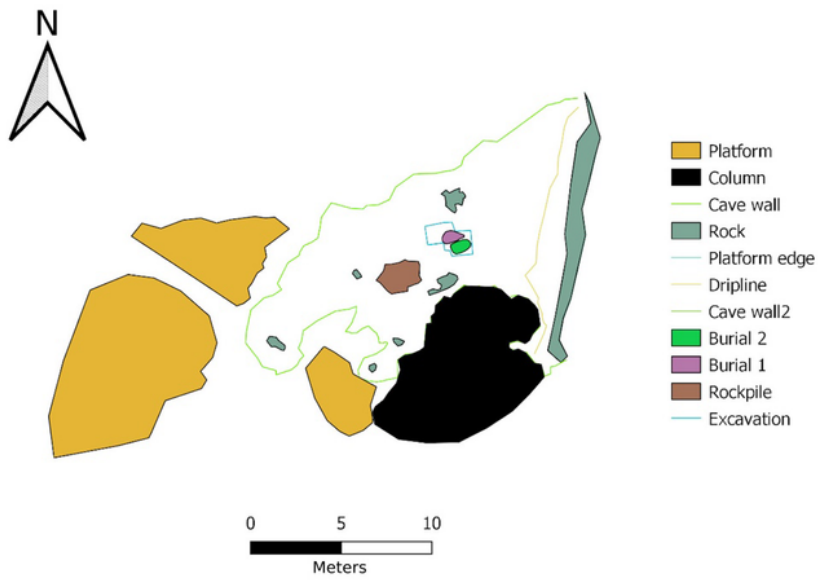


Figure 3

Ratu Mali 2, excavation area, and site plan (above) and transect from cave to sea level (below).

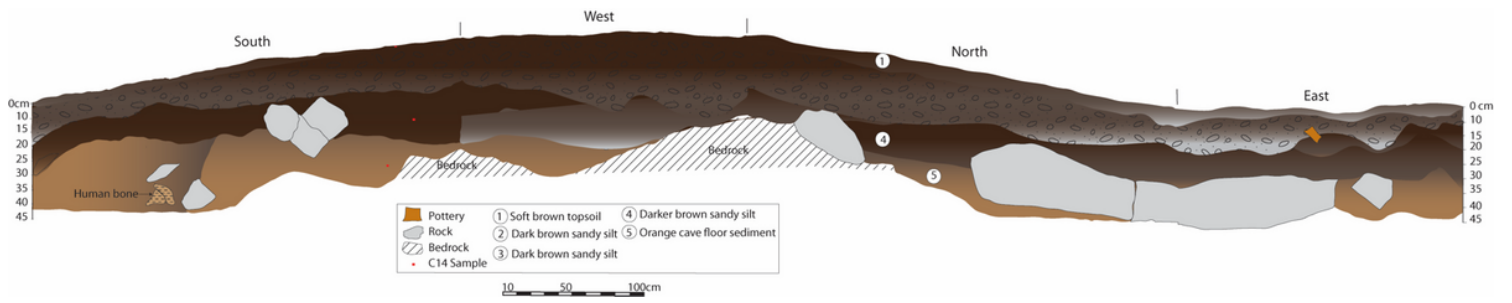


Figure 4

SQ A and SQ B extension Section drawing

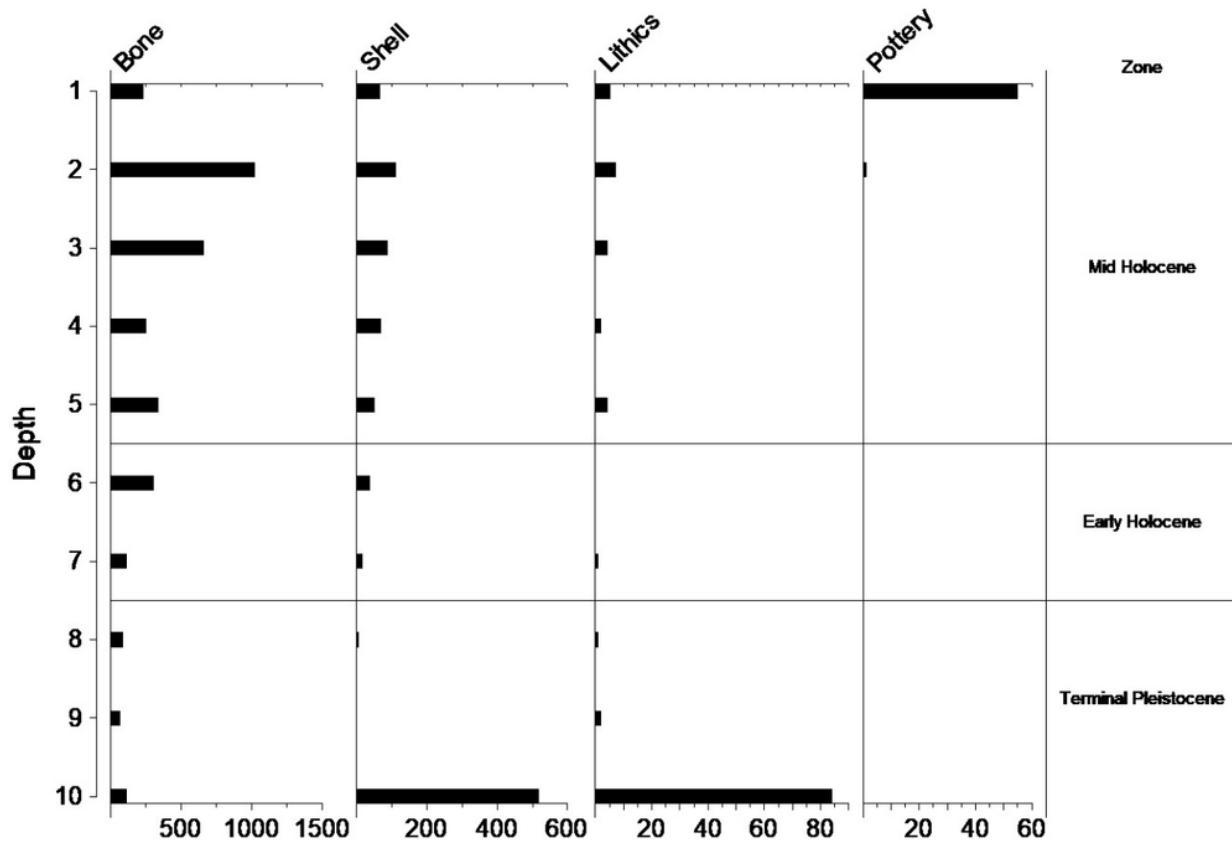


Figure 5

Ratu Mali 2 materials by spit and burial. Depth 1-9=A1-A9, 10=burial feature. NISP for bone, lithics, pottery, MNI for shell in x axis.

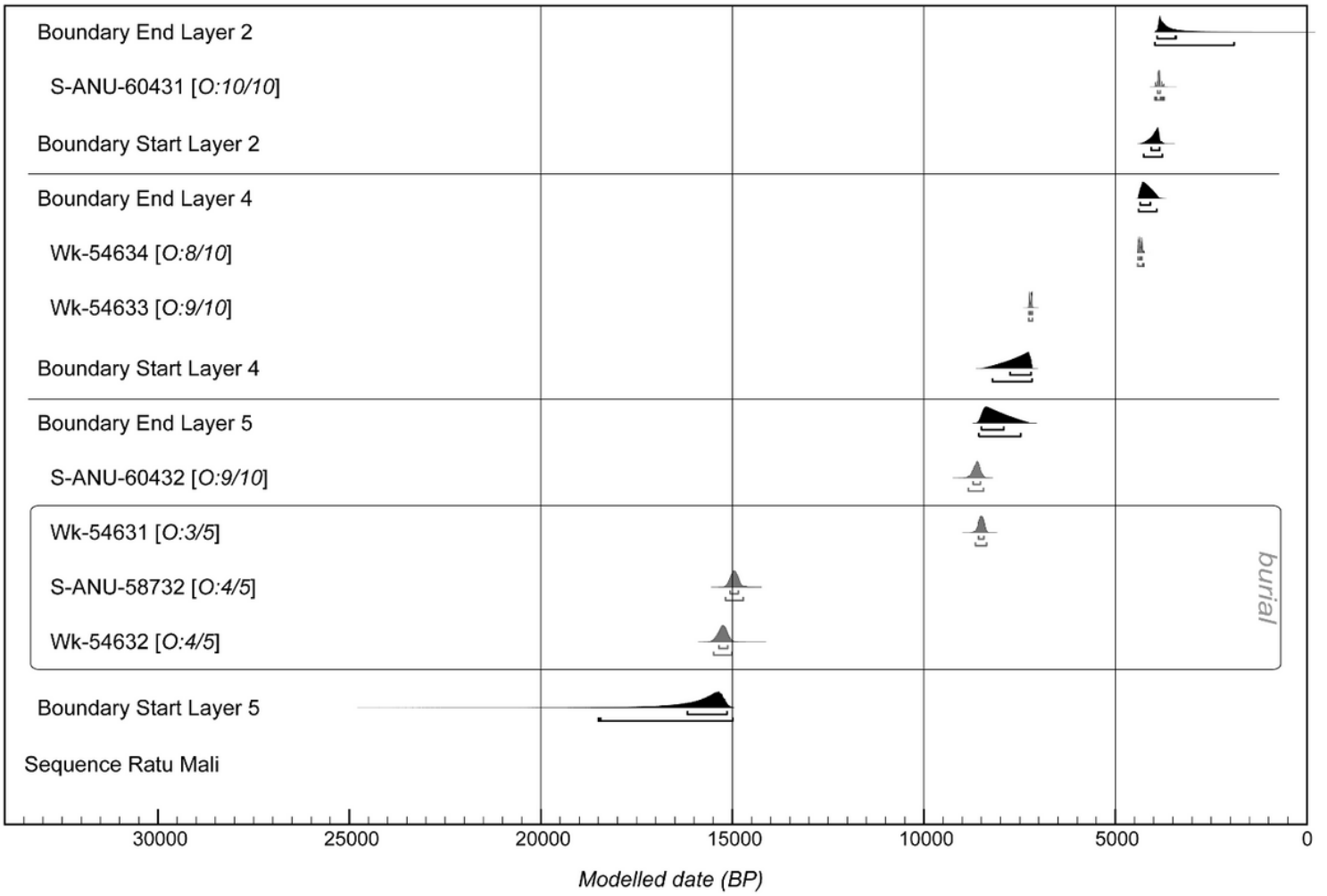


Figure 6

Bayesian age-depth model for Ratu Mali 2

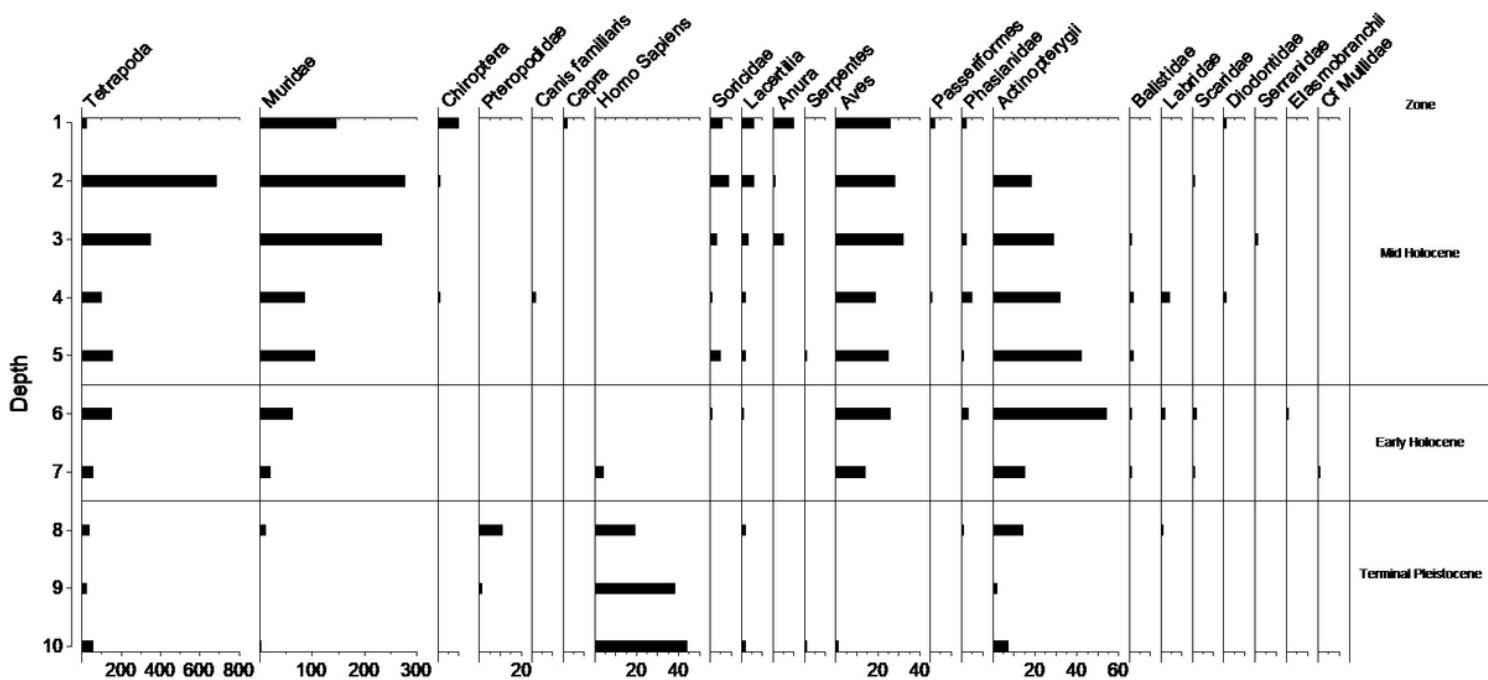


Figure 7

Ratu Mali 2 vertebrates by spit (A1-9) and burial (10)

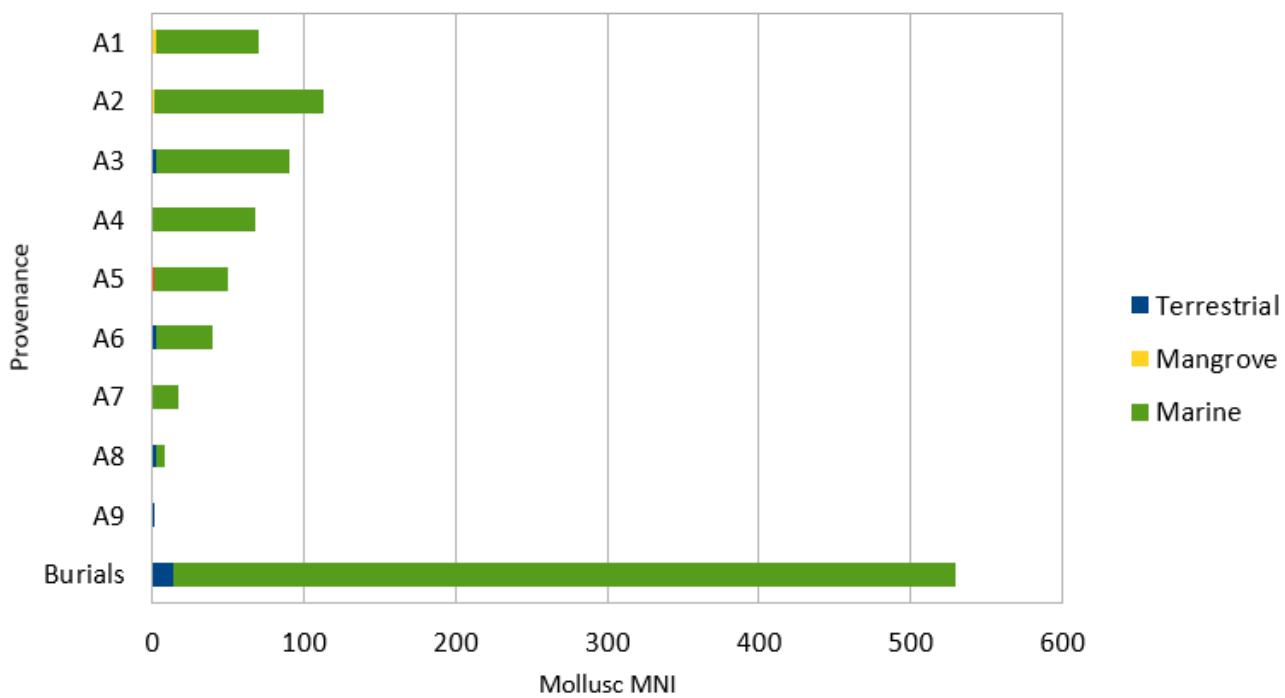


Figure 8

Shell taxa by spit and habitat for Square A

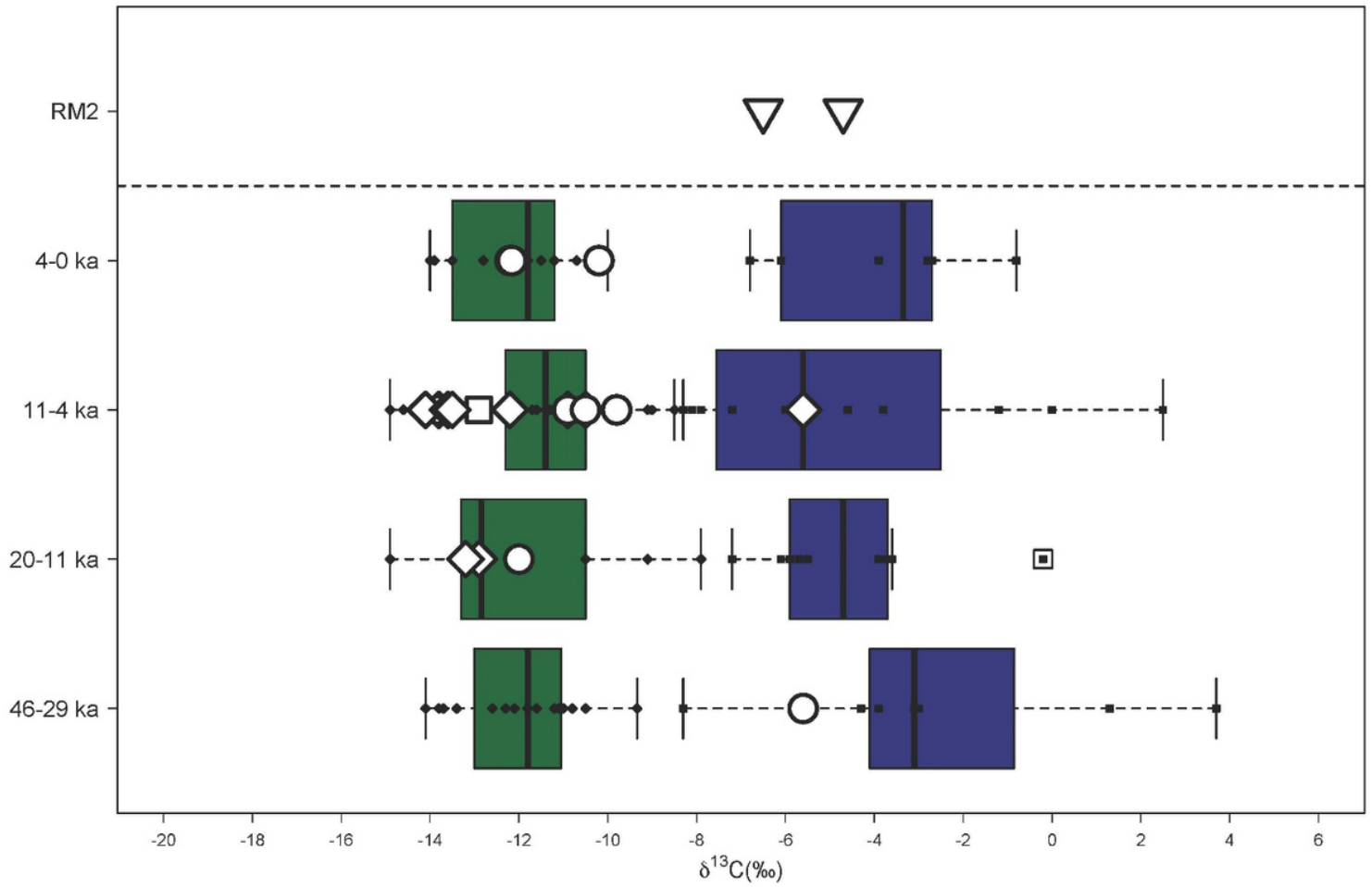


Figure 9

Carbon isotope data from human teeth samples associated with the two Pleistocene individuals buried at Ratu Mali 2 (RM2) in relation to other human teeth from sites in Wallacea after Roberts *et al.* 2020.

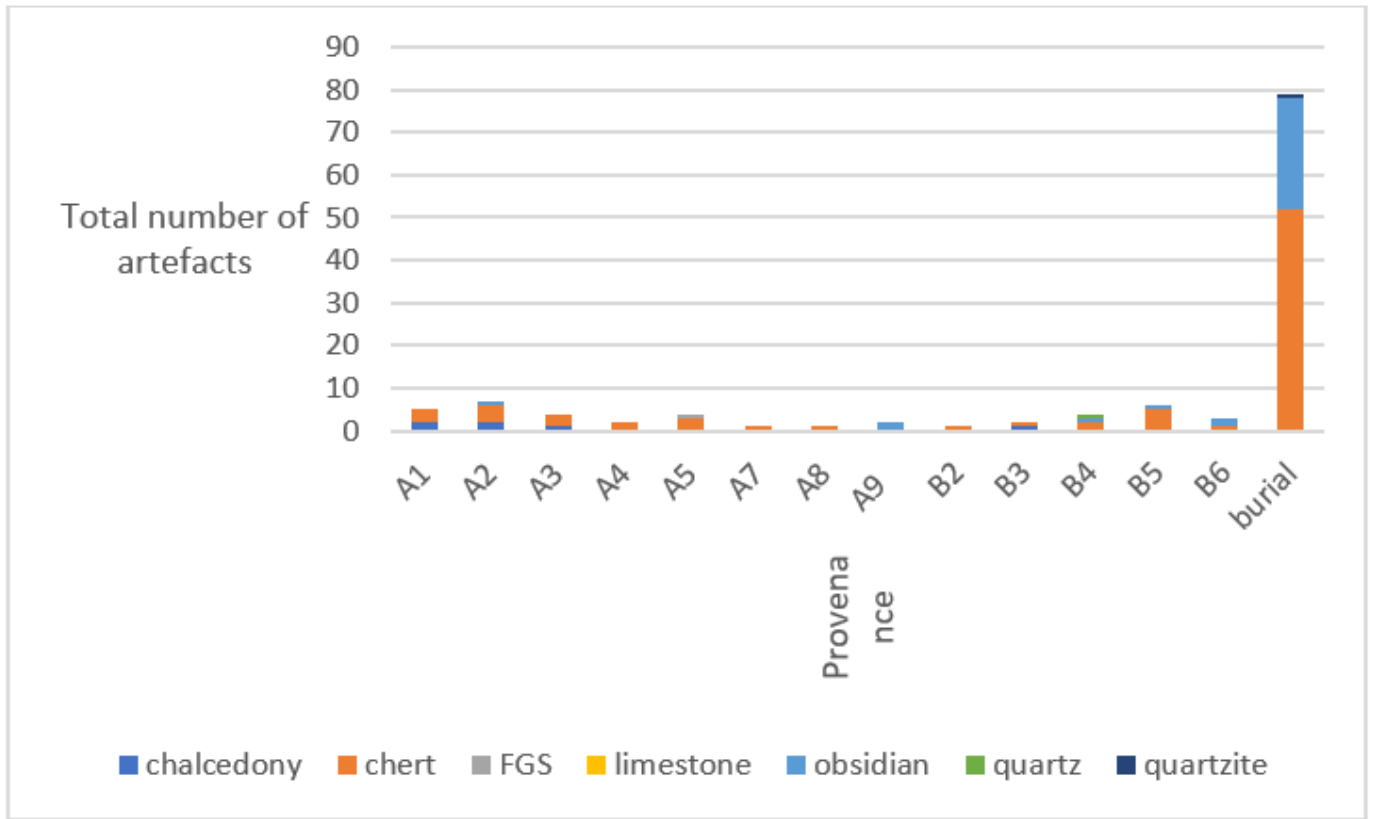


Figure 10

Total number of lithic artefacts by raw material and spit for square A (A1-A9)

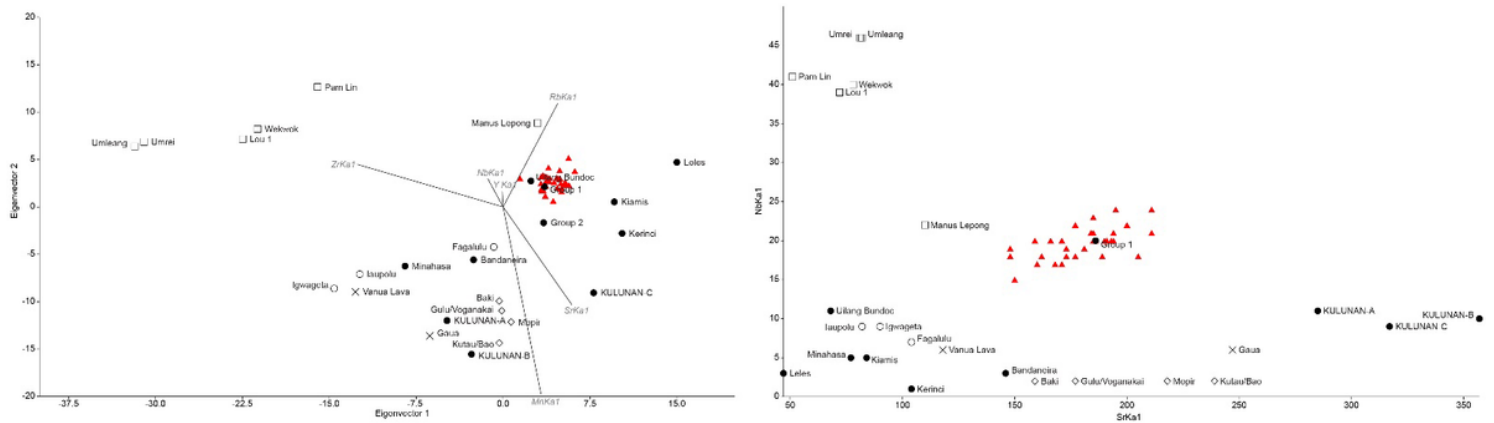


Figure 11

LDA and Bplot for obsidian sources at Ratu Mali 2 compared to other sites in Island Southeast Asia.



Figure 12

Shell artefacts from Ratu Mali 2, arrows indicate percussion impact scars and zones of modification; A) Ground *Tridacna* flake, A5 Layer 4 mid-Holocene, 5 cm scale bar; B) *Tridacna* flake, A2 Layer 2 Neolithic, 5 cm scale bar; C) Modified cowrie (*Cypraeidae*) shell associated with removing the dorsum Pleistocene burial feature, 1 cm scale bar; D) *Nautilus* shell disc-bead, single hole variety, A2 Layer 2 Neolithic, 1 cm scale bar.

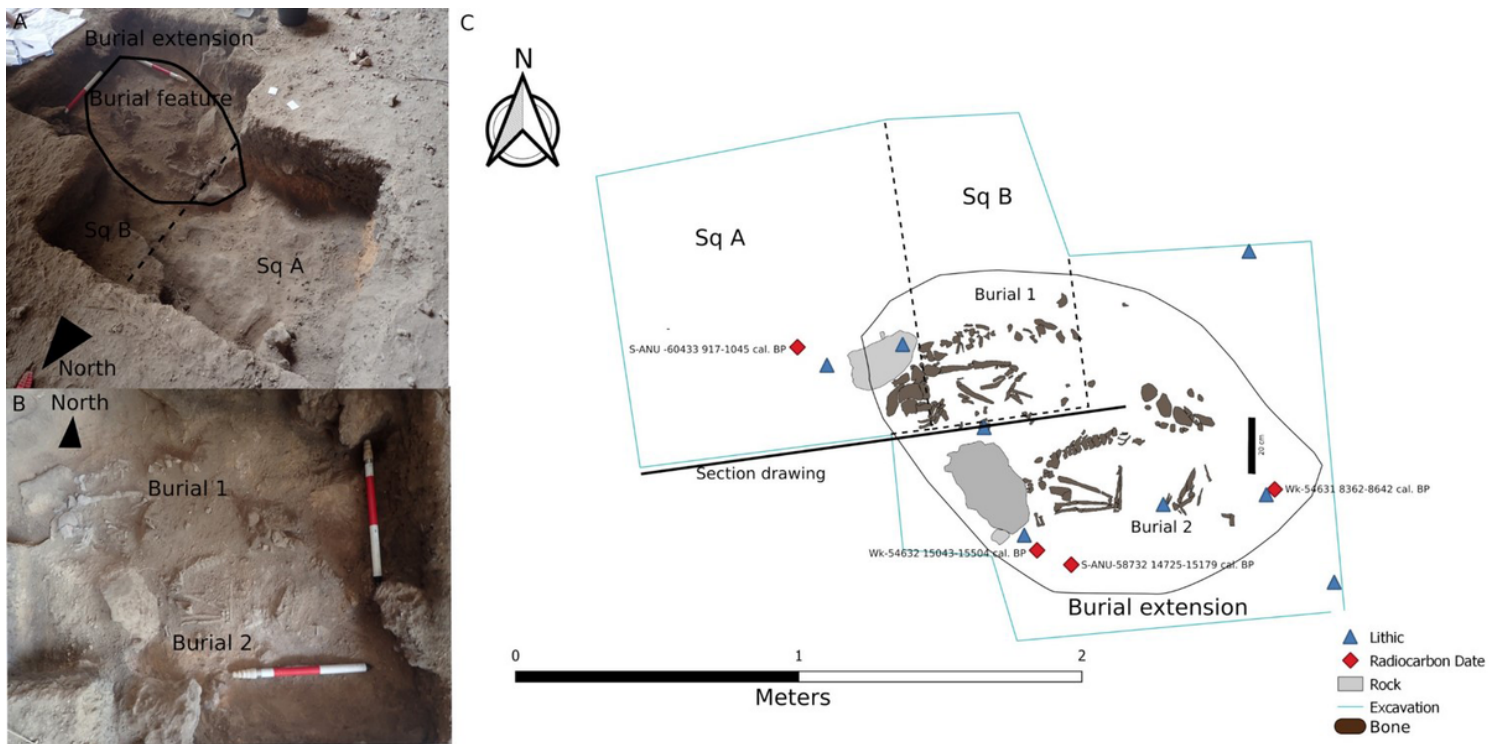


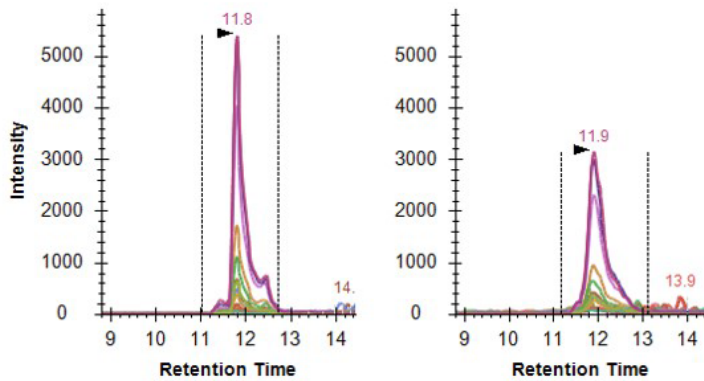
Figure 13

A; Excavation unit including SQ A, SQ B, burial extension and burial feature: B; Burials 1 & 2 photograph: C; Digital excavation plan including grave feature at Ratu Mali 2 with 3D recorded radiocarbon and lithic samples associated with burial 1 & 2.

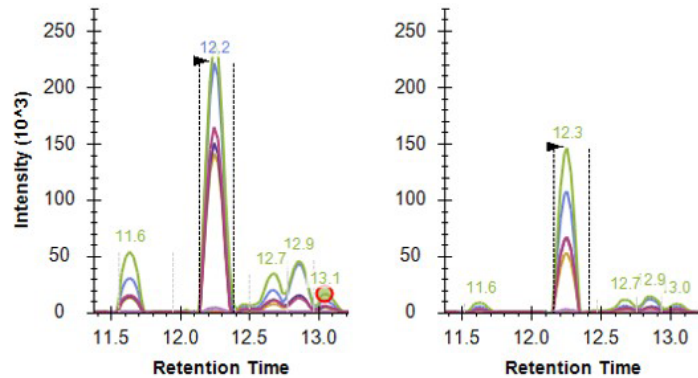
Sample 986

Sample 987

(A) Peptide common to both isoforms: LPPHPGHPGYINF



(B) Peptide specific for AMELX isoform: YEVLTPLK



(C) Peptide specific for AMELY isoform: SIM_{OX}RPPY

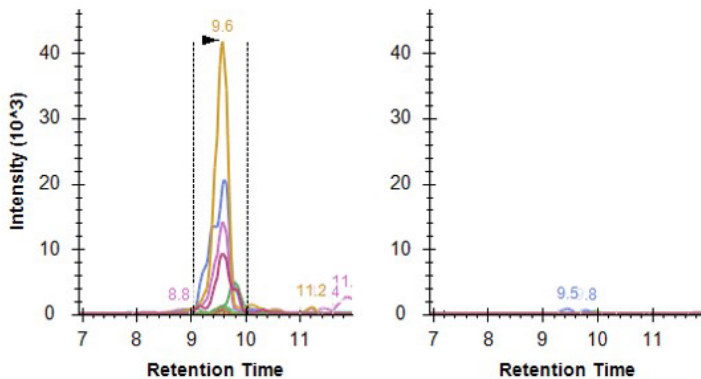


Figure 14

Fragment ion intensities measured by high resolution multiple reaction monitoring. For both samples (986 and 987) a set of peptide precursor ions was selected that are either present in (A) both the female and male isoform AMELX and AMELY, respectively, or are specific for (B) the female isoform or (C) the male isoform. Ion signals for the male isoform AMELY were detected in high intensities in sample 986 (Burial 1) but not in sample 987 (Burial 2) identifying burial 1 as male and Burial 2 as female.

Supplementary Files

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