

# Earliest evidence for surgical amputation

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## **Abstract**

The prevailing view regarding the evolution of medicine is that the emergence of agricultural societies around 10,000 years ago (the 'Neolithic Revolution') gave rise to a host of health problems that were previously unknown among non-sedentary foraging populations, stimulating the first major innovations in prehistoric medico-socio-cultural practices1,2. Such changes included the development of more advanced surgical procedures, with the oldest known indication of an 'operation' formerly held to consist of a farmer with a surgically amputated arm from a Neolithic site in France, dating to around 7,000 years ago3. This accepted case of amputation would have required comprehensive knowledge of human anatomy and considerable technical skill and has thus been viewed as the earliest evidence in world history for a complex medical act3. Here, however, we report the discovery of skeletal remains from Borneo of a young individual who had their lower left leg surgically amputated, probably as a child, at least 31,000 years ago. The individual survived the surgical procedure, living for at least another six to nine years before intentional burial within Liang Tebo cave—located in East Kalimantan, in a limestone karst area that hosts some of the world's earliest dated rock art4. This unexpectedly early evidence for successful limb amputation implies that modern human foraging groups had developed sophisticated medical knowledge and skills long before the Neolithic farming transition.

### Main

The Sangkulirang-Mangkalihat Peninsula (SMP) of East Kalimantan (Indonesian Borneo) is host to an extensive limestone karst landscape (~4,200 km²) which during the late Pleistocene was located close to the extreme easternmost edge of the Eurasian continental landmass, Sunda (Fig. 1a). This rugged karst terrain harbours numerous caves and rockshelters that abound with archaeological evidence for prehistoric human occupation, including figurative rock art dating to at least 40 ka⁴. However, a significant gap in Pleistocene archaeological records, particularly human skeletal remains⁵-10, exists in the region. Liang Tebo—a large three-chambered limestone cave (~160 m³) with preserved rock art in the uppermost chamber—is situated approximately 2.5 km from, and 165 m above, the Marang River (Fig. 1b; Extended Data Fig. 1). In 2020, following geophysical survey, a 2 x 2 m trench was excavated in the central floor area of the largest chamber. This area was excavated to a depth of 1.5 m without reaching bedrock, revealing nine major stratigraphic units (SU) and a burial feature comprising a fully articulated single adult inhumation (designated TB1), first exposed at 0.87 m depth in squares C and D (Extended Data Fig. 2).

#### **Burial feature**

The Liang Tebo burial feature exhibited a strongly defined stratigraphic boundary and distinctive infilling sediment (grave fill), demonstrating the grave cuts into and modifies SU8. The bottom of the ovate-shaped grave cut terminated in SU8 and did not continue into underlying SU9 (Extended Data Fig. 2). A portion of the western margins of the burial cut was clearly visible when partially cross-sectioned by the western excavation wall (Extended Data Fig. 2). Limestone rocks were positioned above the head and

each arm of the individual, immediately atop grave infill (Extended Data Fig. 3). These apparent burial markers, coupled with strong feature boundaries, being unique to all other associated horizontal strata (Extended Data Fig. 2 and 3), confirm the burial is a 'manufactured' stratum and a deliberate human grave<sup>11-13</sup>. TB1 was interred lying on their back in an almost north to south alignment (310°N), with left and right legs flexed—the right with the knee at the chest, and left knee flexed below the pelvis (underneath femur), with the left hand inferior and the right superior, to the pelvic girdle (Fig. 2a). Minimal movement of fragile bone elements suggests rapid sedimentation and decomposition within a confined space<sup>12,13</sup>. Cultural materials recovered from the burial include flaked chert artefacts and a 22 x 17 mm nodule of red ochre (natural earth pigment), the latter recovered near the mandible (Fig. 2b).

The TB1 burial feature and skeleton was removed in 32 episodic stages (R1 to R32), each accompanied by laser scanning and photography (Extended Data Fig. 3). TB1 is well preserved (Supplementary Information): the reassembled skeleton reveals 75% bone presence, with all teeth present and intact (Fig. 2c; Extended Data Fig. 5), and is therefore considered relatively complete. The individual is classified as an anatomically modern human (*Homo sapiens*) based on a range of morphological considerations (Supplementary Information). The combination of epiphyseal fusion, pubic symphysis, and auricular surface stages, as well as dental formation techniques indicate that TB1 was a young adult approximately 19–20 years of age at time of death (Supplementary Information). The cranium and pelvis display intermediate sex traits and hence gender is indeterminate (Supplementary Information). In comparison to the stature of other prehistoric individuals with morphological and morphometric affinity to pre-Last Glacial Maximum (LGM) skeletons from Asia, the TB1 individual is typical in stature for males and more than 1 Standard Deviation (SD) taller than the mean for most females (Extended Data Table 1).

#### **Dating**

Immediately above the grave cut, within SU7, a charcoal sample returned an Accelerator Mass Spectrometry (AMS) radiocarbon (<sup>14</sup>C) age of 31,133 to 30,437 calibrated radiocarbon years before present (cal BP) at 95.4% probability (D-AMS38332), providing a stratigraphic *terminus ante quem* for the inhumation of TB1 (Extended Data Table 2). In addition, a charcoal sample within the burial feature, collected amongst the pelvic girdle, returned an age estimate of 31,110 to 30,437 cal BP (D-AMS38337). Charcoal recovered from SU9, the stratum underlying the burial feature, provides a stratigraphic *terminus post quem* with an age estimate of 31,519 to 31,054 cal BP (D-AMS38338). Thus, associated radiocarbon dating of charcoal indicates an age estimate for the TB1 burial feature between 31,519 to 30,437 cal BP, with a mean of 30,978 cal BP. Radiocarbon dating from overlying SUs confirms subsequent human occupation at the site transitioning the LGM and Holocene towards the surface (Extended Data Table 2), with each sample's depth measurement displaying strong and significant correlation with mean calibrated age (r = 0.990, r<sup>2</sup> = 0.981, F = 253.942, p = 0.001). This positive age-depth relationship supports an argument for minimal deposit reworking.

In addition to radiocarbon dating of charcoal, a combined Uranium-series and Electron Spin Resonance (ESR) dating technique was undertaken on a sample of TB1's left mandibular molar ( $M_3$ ) and returned an

age estimate of  $25.4 \pm 4.3$  ka, and is thus within error (2-sigma) of the  $^{14}$ C burial context age. Both Uranium-series analysis in isolation and radiocarbon dating of the skeletal remains were unsuccessful owing to the lack of a sufficient amount of uranium and collagen in the sample, respectively. In sum, we infer a secure Late Pleistocene age of between 31.5 and 30 ka for TB1, making this the oldest intentional burial of a modern human currently known from Island Southeast Asia.

### Evidence for surgical amputation

Careful excavation of the burial feature containing TB1 revealed the complete absence of the lower left leg (Fig. 3; Extended Data Fig. 3 and 4). Recovered left tibia and fibula shaft fragments, flexed underneath the left femur, presented unusual distal bony growth (Fig. 3; Extended Data Fig. 4). The opposite leg was articulated, with all right foot bones (n = 26) recovered within the grave (Fig. 3a). Remodelled bone covers kerf surfaces identified on the left distal tibia and fibula shaft fragments, demonstrating healing (Fig. 3bf; Extended Data Fig. 4; Supplementary Information). This evidence indicates that TB1's lower left leg was removed through deliberate surgical amputation of the distal tibia and fibula. The trauma pattern observable is not consistent with clinical descriptions of non-surgical amputation, except in cases of modern trauma where a large metal blade or mechanical processes have been involved 14-17. Non-surgical amputations, commonly a result of accidents, do not cause clean oblique sectioning and are not clinically recorded to sever the lower limb of both tibia and fibula, as is the case for TB1. Blunt force trauma from an accident or animal attack typically cause comminuted and crushing fractures 18, features that are absent from the clearly simple and oblique amoutation margin of TB1. Remodelling has enclosed the inferior margin of the fibula (Fig. 3e,f), indicating TB1 died approximately six to nine years after the initial trauma—confirming this was not a fatal pathology. There is no evidence for infection of the left limb, the most common complication of an open wound without antibiotic treatment. The lack of infection further rules out the probability of animal attack, such as a crocodile bite, which present with very high probability of complications from infection due to microbes entering the wound from the animal's teeth<sup>19</sup>. Partial consolidation of bone between the left tibia and fibula and complete closure of the distal end of the left fibula (Fig. 3b,e,f; Extended Data Fig. 4) are consistent with late-stage amputation changes 14. The small size of the left tibia and fibula compared with the right suggests a childhood injury, as the bones did not continue growing (Fig. 3a). The severe bone thinning of the left tibia and fibula is also suggestive of the heavily restricted use of the left leg resulting in musculoskeletal disuse atrophy<sup>20</sup> (Extended Data Fig. 4). Some thinning of the cortical margins of the right tibia suggests that TB1 was rarely ambulatory owing to the incapacitating nature of the injury to the lower left leg (Extended Data Fig. 4).

## Discussion

The surgical amputation of TB1's lower left leg some 31,000 years ago has important implications for our understanding of the evolution of human medico-socio-cultural practices. Evidence for surgery in the time before written records is scarce. Until now the earliest primary evidence of advanced medical knowledge, including amputation, was restricted to Holocene cases 1,21,22—earlier reports of deliberate amputation of

limbs among Neanderthals are now considered inconclusive, albeit they remain examples of medical care intervention<sup>23</sup>. Furthermore, it has long been a commonly held view among western scholars that healthcare systems and medical procedures of historically known foraging societies are, and were, rudimentary. It is recognised that traditional healing practices typically involve extensive knowledge of plant-based medicinal remedies<sup>24</sup>. Surgical intervention and treatment of patients, however, are held to have been poorly developed among small-scale foraging communities, being generally limited to procedures such as suturing lacerations, cranial trephination, and various body modification practices like tooth avulsion, scarification, and genital 'mutilation' (e.g., circumcision). The prevailing assumption has been that more complex surgeries lay beyond the capabilities of foraging societies past and present. The surgical removal of body parts, specifically, is thought to have been confined mostly to phalangeal (finger segment) amputation for symbolic purposes (e.g., as a mourning rite)<sup>24</sup>. Concerning the history of amputation surgery per se, historical accounts vary from ancient Roman sources to advances in surgical procedures developed during the past few centuries<sup>1</sup>. Review of the latter<sup>1,25</sup> provides details of modern clinical procedures of amputation, exemplifying the level of anatomical understanding, hygiene, surgical skill, and required apparatus for success (the latter being synonymous with patient survival). In western societies, successful surgical amputation only became a medical norm within the past 100 years<sup>1</sup>. Before modern clinical developments, including antiseptic, it was widely thought that most patients undergoing amputation surgery would have died, either at the time of amputation from blood loss and shock or from subsequent infection—scenarios leaving no skeletal markers of advanced healing.

With regards to TB1, we infer that the Late Pleistocene 'surgeon(s)' who amputated this individual's lower left leg must have possessed detailed knowledge of limb anatomy and muscular and vascular systems to prevent fatal blood loss and infection. They must also have understood the necessity to remove the limb for survival<sup>26</sup>. Furthermore, during surgery, the surrounding tissue including veins, vessels, and nerves, were exposed and 'negotiated' in such a way that allowed this individual to not only survive but continue living with altered mobility. Intensive post-operative nursing and care would have been vital, such as temperature regulation, regular feeding, bathing, and movement to prevent bed sores while the individual was immobile<sup>26</sup>. The wound would have been regularly cleaned, dressed, and disinfected, likely using locally available medicinal botanical resources to prevent infection<sup>27,28</sup>. While it is not possible to determine whether infection occurred post-surgery, this individual did not suffer from infection severe enough to leave permanent skeletal markers and cause death. It is supposed that life without a lower limb (combined with other traumas; Extended Data Fig. 6; Supplementary Information) in a karstic landscape presented a series of challenges—several of which can be assumed to have been overcome by a high degree of community care<sup>29,30</sup>.

In sum, the discovery of this exceptionally old evidence for deliberate amputation demonstrates the surprisingly advanced level of medical expertise developed by early modern human foragers in the Late Pleistocene on the eastern margins of Sunda. We infer that the comprehensive knowledge of human anatomy, physiology, and surgical procedures evident within TB1's community is likely to have been developed through trial and error over a long period of time and transmitted inter-generationally through

oral traditions of learning. Notably, we do not think it is the case that this 'operation' was a rare and isolated event in the Pleistocene history of this region, or that this particular foraging society had achieved an unusually high degree of proficiency in this area. Risk of death from trauma and disease has always been with us, and complex medical acts, such as lower limb amputation, are likely to have been more commonplace in the pre-agricultural past of our species than is broadly assumed at present. Our understanding of this aspect of *H. sapiens* prehistory, however, may be affected by poor preservation of pathological bone, as well as by preconceptions about the 'primitive' nature of earlier medico-socio-cultural practices, especially among non-sedentary foraging populations.

# **Main References**

- 1. Roberts, C.A. Palaeopathology and archaeology: The current state of play, in: *The Archaeology of Medicine*. *BAR International Series* **1046**, 1-20 (2002).
- 2. Richards, M.P. A brief review of the archaeological evidence for Palaeolithic and Neolithic subsistence. *Euro. J. Clin. Nutrition* **56**(12), 1270-1278 (2002).
- 3. Buquet-Marcon, C. et al. The oldest amputation on a Neolithic human skeleton in France. *Nat Prec* (2007).
- 4. Aubert, M. et al. Palaeolithic cave art in Borneo. Nat 564, 254-257 (2018).
- 5. Brumm, A. et al. Skeletal remains of a Pleistocene modern human *(Homo sapiens)* from Sulawesi. *PLos ONE* (9), e0257273 (2021).
- 6. O'Connell, J. et al. When did Homo sapiens first reach Southeast Asia and Sahul? *Proc. Nat. Acad. Sci.* **115** (34), 8482-8490 (2018).
- 7. Oxenham, M. & Buckley, H. *The Routledge Handbook of Bioarchaeology in Southeast Asia and the Pacific Islands*. Routledge, London. (2016).
- 8. Samper-Carro, S.C. et al. Burial practices in the early mid-Holocene of the Wallacean Islands: A subadult burial from Gua Makpan, Alor Island, Indonesia, *Quat. Int.* **603**, 125-138 (2021).
- 9. Curnoe, D. et al. Deep Skull from Niah Cave and the Pleistocene peopling of Southeast Asia. *Front. Ecol. Evo.* **4**, 75 (2016).
- 10. Westaway, K.E. et al. An early modern human presence in Sumatra 73,000-63,000 years ago. *Nat* **548**, 322-325 (2017).
- 11. Pettitt, P. The Palaeolithic Origins of Human Burial. Routledge, London. (2011).
- 12. Gargett, R.H. Middle Palaeolithic burial is not a dead issue: The view from Qafzeh, Saint-Césaire, Kebara, Amud, and Dederiyeh. *J. Hum. Evol.* **37**, 27-90 (1999).

- 13. Martinón-Torres, M. et al. Earliest known human burial in Africa. Nat 593, 95-100 (2021).
- 14. Barber, C.G. Immediate and eventual features of healing in amputated bones. *Ann. Surg.* **90**, 985 (1929).
- 15. Donnally III, C. et al. Orthopedic injuries associated with jet-skis (personal watercrafts): A review of 127 inpatients. *Orthop. & Traumatol Surg. & Res.* **104**, 267-271 (2018).
- 16. Pennoyer, G.P. Traumatic amputation of the thigh, complicated by both tetanus and gas gangrene with recovery. *J. Am. Med. Assoc.* **95**, 342-343 (1930).
- 17. Sherk, V.D. et al. BMD and bone geometry in transtibial and transfemoral amputees. *J Bone Min. Res.* **23**, 1449-1457 (2008).
- 18. Aydin, K. Cokluk, C. A fracture of unilateral pars interarticularis of the axis: a case report. *Turkish Neurosurgery* **17**, 155-157. (2007).
- 19. Wamisho, B.L. et al. Ward round-crocodile bites in Malawi: Microbiology and surgical management. *Malawi Med. J.* **21**(1), 29-31 (2009).
- 20. Sievänen, H. Immobilization and bone structure in humans. *Arch. Biochem. Biophys.* **503**, 146-152 (2010).
- 21. Juengst, S.L., Chavez, S.J. Three trepanned skulls from the Copacabana Peninsula in the Titicaca Basin, Bolivia (800 BC-AD 1000). *Int. J. Paleopathology* **9**, 20-27 (2015).
- 22. Zhou, Y. et al. Early evidence of trepanation along the Yellow River Basin in Neolithic China. *Archeol. Anth. Sci.* **12**, 176 (2020).
- 23. Spikins, P. et. al. Living to fight another day: The ecological and evolutionary significance of Neanderthal healthcare. *Quat. Sci. Rev.* **217**, 98-118 (2019).
- 24. Ackerknecht, E.H. The role of medical history in medical education. *Bull. Hist. Med.* **21** (2), 135-45 (1947).
- 25. Burns, K.R. Forensic Anthropology Training Manual. Routledge, London. (2015).
- 26. Wen, A.P.Y. et al. Successful ankle replantation in two cases with different presentations. *Archives Plast. Surg.* **47**, 182 (2020).
- 27. Az-Zahra, F.R. et al. Traditional knowledge of the Dayak Tribe (Borneo) in the use of medicinal plants. *Biodiversitas* **22**, 4633-4647 (2021).
- 28. Caniago, I., Stephen, F.S. Medicinal plant ecology, knowledge and conservation in Kalimantan, Indonesia. *Econ Bot* **52**, 229-250 (1998).

29. Oxenham, M.F. et al. Paralysis and severe disability requiring intensive care in Neolithic Asia. *Anthro. Sci.* **117**, 107-112 (2009).

30. Tilley, L., Oxenham, M.F. Survival against the odds: Modeling the social implications of care provision to seriously disabled individuals. *Int. J. Paleopathology* **1**, 35-42 (2011).

# **Methods**

### **Ground Penetrating Radar**

Geophysical survey using Ground Penetrating Radar (GPR) and Electrical Resistivity Tomography (ERT) was conducted in the chambers of Liang Tebo (Extended Data Fig. 1). GPR data collection was undertaken using a Malå X3M with a 500 Mhz antenna utilising a time window of 62 ns with 1024 samples, a trace interval of 2 cm and four stacks. Data were processed using ReflexW software with a suite of filters, including Move Start time, Dewow, Energy Decay, Bandpass Butterworth, Background Remove and Time Cut. ERT data collection was undertaken using a ZZ Flash Res-64 using an electrode spacing of 0.5 m, collected in Wenner and Dipole-Dipole arrays with *k* values of 20 and a Dipole-Dipole *l* value of 5. Acquisition was undertaken with 120V, an on-time of 1.2 and an off-time of 0.2 seconds. Data was output using ZZ RData Check software, then inverted in Res2D using the robust scheme, and displayed with a colour scale constructed using the Jenks Breaks feature with ArcGIS.

#### Excavation

Sedimentary features within the deposit, such as hearths and all other sediment changes, were excavated separately following stratigraphic boundaries. Homogenous sediments, when encountered, were excavated in arbitrary excavation units (XU), measuring between 1 cm and 5 cm in thickness. Materials and sedimentary features were recorded with 3D plotting and laser scanning, using a Leica MS60 Robotic Total Station. All artefacts larger than ~19 mm in maximum dimension were plotted in 3D, and all stratigraphic features were laser-scanned. All sediments were sieved using 1.5 mm screens, while feature sediments (including those surrounding the burial) were sieved using a soft nylon 0.5 mm screen. Whether recovered in situ or from sieved residues, all artefacts can be precisely associated with both a stratigraphic unit (SU) and an excavation unit (XU). Cultural materials recovered throughout include stone artefacts, ochre, shell, faunal remains, and macrobotanical remains, with a total lack of ceramic and metal finds. Human remains and all other delicate artefacts were excavated using handheld softwood tools to prevent damage, with other sediments removed via fine leaf trowel. First encountered at 0.87 m depth in the western squares, the TB1 burial feature had a strongly defined stratigraphic boundary with distinctive infill sediment: revealing the grave cuts into SU8. The latter unit was marked by a very different colour and texture—a weakly cemented white (10YR 8/1) calcitic silt (Extended Data Fig. 2)—making grave cut boundaries particularly distinctive (Extended Data Fig. 3). The thin western margins of the burial cut partially cross-sectioned by the western excavation wall, served to define these stratigraphic relationships in profile (Extended Data Fig. 2). Feature boundaries of the burial were unique to

surrounding and overlying strata, constituting a 'manufactured' stratum<sup>31</sup> modifying SU8. These observations rule out placement of the body into natural crevices or deposition via natural processes<sup>32, 33</sup>, and instead support an interpretation of a deliberately excavated grave cut into SU8. Placement of large stratigraphically analogous limestone rocks as burial markers (Extended Data Fig. 3) further distinguished the upper surface of the grave and supports the case of deliberate burial. A red ochre (earth pigment) nodule adjacent TB1's mandible on the left clavicle (Fig. 2b) is likely to be a mortuary good placed near the mouth. Anatomical integrity and articulation of unstable joints, the first to decompose, support a primary and relatively undisturbed burial (Fig. 2a).

#### **Dating**

Throughout the nine SUs (Extended Data Fig. 2), a total of 10 in situ radiocarbon dating samples (charcoal plotted in 3D during excavation) were dated by (AMS<sup>14</sup>C) at the Direct AMS laboratory, in Seattle U.S.A (Extended Data Table 2). Dates are calibrated using OxCal v. 4.4, with the Northern Hemisphere Atmospheric curve [IntCal20]<sup>34</sup>.

Coupled uranium-series and Electron Spin Resonance (ESR) dating was undertaken at Southern Cross University at the GARG facility on a left mandibular molar (M<sub>3</sub>). The tooth was first cut in half using a rotating diamond saw with a blade of 300 microns, before being polished to 5 micron smoothness. The sample was then analysed for uranium-series isotopes and concentration in both dentine and enamel using a laser ablation NWR ESI 213 laser coupled with a MC-ICPMS Neptune XT from Thermo Fisher to calculate the internal dose rate. An enamel fragment was then measured on a Freiberg MS5000 ESR X-band spectrometer and irradiated with the Freiberg X-ray irradiation chamber. ESR intensities were extracted from the merged spectra obtained on the angular variation measurements<sup>35</sup> (e.g., Extended Data Fig. 7), after correcting for baseline, subtraction of isotropic signals, and assessment of NOCORS contribution using the published protocol<sup>36,37</sup> (e.g., Extended Data Fig. 7). Dose response curve were obtained using the MCDOSE 2.0 software<sup>38</sup> (Extended Data Fig. 7). All age calculations were carried out with the DATA program<sup>39</sup>.

### Osteology

Bone preservation is assessed both in terms of completeness (how much of the skeleton is there) and taphonomy (post-depositional processes that have affected the bones). Skeletal and dental completeness, post-depositional processes including colour change, root damage, animal scavenging marks, sun and water exposure, post-mortem breakage and surface erosion were each assessed<sup>40,41</sup>.

The TB1 individual was morphologically an adult, therefore adult age-at death estimation techniques were applied. Pubic symphysis and auricular surface degeneration stage methods were compared to standards<sup>42, 43</sup>. Different fusion timings of the various epiphyses allow for a narrow age estimate in late teenage years to early adulthood. Epiphyses (growth plates) that do not fuse until early adulthood such

as the medial end of the clavicle were assessed following Schaefer et al.<sup>44</sup>. Dental eruption, wear, and formation methods supplemented these age estimation protocols <sup>45-49</sup>.

Regression equations are applied for estimating stature from the maximum length of long bones. The right femur and tibia were considered the most valuable bone for stature estimation due to its relationship in contributing to stature and preservation. Australo-Melanesian populations rather than East or Southeast Asian populations are likely to provide better estimates for pre-Neolithic individuals from Southeast Asia. 'American Black' stature estimate standards are used<sup>50-51</sup> due to similar proportions of the contribution of maximum tibia lengths, with 10 mm adjustments to the maximum tibial lengths<sup>51</sup>. Estimates for comparative pre-Neolithic hunter-gatherers in Southeast Asia have traditionally been estimated from modern Asian populations in the United States, even if they predate migration of groups with morphological affinity to modern East Asians to the region. Therefore, these stature estimates are provided for comparison to other pre-Neolithic modern humans.

A full skeletal assessment of abnormal bone changes was completed. Lesions (any pathological bone loss, growth, or deformity) were recorded following revised standard protocols<sup>52-54</sup>. Bone lesion location, aspects affected, percentage of bone affected by lesion, and bone type affected (cortical, trabecular and/or medullary canal) were recorded to assess spatial distribution of lesions. The level of healing, margin definition, presence of necrotic bone (sequestrum), presence of shape changes to the bone, focality (focal, multifocal, or diffuse), laterality, symmetricity and lesion size were recorded to reconstruct progression and pattern of disease for differential diagnosis. Lesions were compared against clinical and palaeopathological literature to determine possible candidates for disease origin (aetiology of the disease). Trauma analysis (e.g., fractures) followed protocols<sup>55</sup> to describe the mechanism of injury, force, type and time of trauma, and degree and complications to healing.

#### Methods references

- 31. Martinón-Torres, M. et al. Earliest known human burial in Africa. Nature 593, 95-100 (2021).
- 32. Pettitt, P. The Palaeolithic Origins of Human Burial. Routledge, London. (2011).
- 33. Gargett, R.H. Middle Palaeolithic burial is not a dead issue: The view from Qafzeh, Saint-Césaire, Kebara, Amud, and Dederiyeh. *J. Hum. Evol.* **37**, 27-90 (1999).
- 34. Reimer, P.J. et al. The IntCal20 Northern Hemisphere Radiocarbon Age Calibration Curve (0–55 cal kBP). *Radiocarbon* **62**(4), 725-757 (2020).
- 35. Joannes-Boyau, R. & Grün, R. A comprehensive model for  $CO_2$  radicals in fossil tooth enamel: Implications for ESR dating. *Quat. Geochron.* **6**, 82-97 (2011).
- 36. Grün R., Aubert M., Joannes-Boyau R., & Moncel M.H. High resolution analysis of uranium and thorium concentrations as well as U-series isotope distributions in a Neanderthal tooth from Payre using

- laser ablation ICP-MS. Geochimica Cosmochimica Acta. 72, 5278-5290 (2008).
- 37. Joannes-Boyau, R. Detailed protocol for an accurate non-destructive direct dating of tooth enamel fragment using Electron Spin Resonance. *Geochronometria* **40**, 322-333 (2013).
- 38. Joannes-Boyau, R., Duval, M., & Bodin, T. MCDoseE 2.0. A new Markov Chain Monte Carlo program for ESR dose response curve fitting and dose evaluation. *Quat. Geochron.* **44**, 13-22 (2018).
- 39. Grun, R., The DATA program for the calculation of ESR age estimates on tooth enamel. *Quat. Geochron.* **4**, 231-232 (2009).
- 40. Buikstra, J.E. & Ubelaker, D.H. Standards for Data Collection from Human Skeletal
- Remains: Proceedings of a Seminar at the Field Museum of Natural History, Arkansas. Archaeological Survey Research Series **44**, (1994).
- 41. McKinley, J. Compiling a Skeletal Inventory: Disarticulated and Co-Mingled Remains, in: Brickley, M. & McKinley, J. (Eds.), *Guidelines to the Standards for Recording Human Remains*. BABAO/ Institute of Field Archaeologists, Reading (2004).
- 42. Brooks, S., Suchey, J.M. Skeletal Age Determination Based on the Os Pubis: A Comparison of the Acsádi-Nemeskéri and Suchey-Brooks Methods. *J. Hum. Evol.* **5**, 227-238 (1990).
- 43. Lovejoy, C.O. et. al. Chronological metamorphosis of the auricular surface of the Ilium: A new method for the determination of adult skeletal age at death. *Am. J. Phys. Anthropol.* **68,** 15-28 (1985).
- 44. Schaefer, M., Black, S.M., Scheuer, L. *Juvenile Osteology: A Laboratory and Field Manual*. Elsevier, Academic Press, London (2009).
- 45. Ubelaker, D.H. *Human Skeletal Remains: Excavation, Analysis, Interpretation*. Taraxacum Press, Washington (1989).
- 46. Moorrees, C.F., Fanning, E.A., Hunt, E.E. Age variation of formation stages for ten permanent teeth. *J. Dent. Res.* **42,** 1490-1502 (1963).
- 47. Scott, E.C. Dental wear scoring technique. Am. J. Phys. Anthropol. 51, 213-217 (1979).
- 48. Reid, D.J. Dean, M.C. Brief communication: The timing of linear hypoplasias on human anterior teeth. *Am. J. of Phys. Anthropol.* **113**, 135-139 (2000).
- 49. Reid, D.J. Dean, M.C. Variation in modern human enamel formation times. *J. Hum. Evol.* **50**, 329-346 (2006).
- 50. Trotter, M. Gleser, G.C. Estimation of stature from long bones of American whites and negroes. *Am. J. Phys. Anthropol*, **10**, 463-514 (1952).

- 51. Jantz, L.M., Jantz, R.L. Secular change in long bone length and proportion in the United States, 1800–1970. *Am. J. of Phys. Anthropol.* **110**, 57-67 (1999).
- 52. Littleton, J., Kinaston, R. Ancestry, age, sex, and stature: Identification in a diverse space, in: *Forensic Approaches to Death, Disaster and Abuse*, 155-176 (2008).
- 53. Buckley, H.R. *Health and Disease in the Prehistoric Pacific Islands*. British Archaeological Reports International Series **2792**, Oxford (2016).
- 54. Ortner, D.J. *Identification of Pathological Conditions in Human Skeletal Remains*. Academic Press, San Diego (2003).
- 55. Lovell, N.C. Trauma analysis in paleopathology. Am. J. of Phys. Anthropol. 104, 139-170 (1997).

# **Declarations**

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#### **Author contributions:**

T.M., I.D.H., A.A.D.P.: excavation of site and burial, analysis of remains, conception and writing of manuscript. M.A. and A.B. conceived the study and contributed to manuscript. Site access, project coordination, and field logistics was facilitated by P.S., M.R., A.A.O., F.T.A., I.M.G., M.A.R.E, B.I., S.A. M.V. conducted Osteological analyses, and I.M. Geophysical survey. R.J.B. conducted the U-Series and ESR dating analyses.

### Competing interest declaration:

Authors declare no conflict of interest.

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# **Figures**

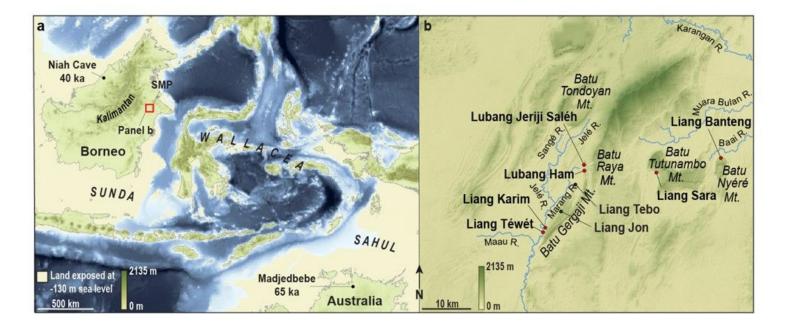


Figure 1

**Location of Liang Tebo. a,** Sunda, the continental shelf region encompassing the present-day island of Borneo during periods of lowered sea-levels, is situated to the west of Wallacea, and northwest of the Pleistocene low sea-level landmass of Sahul (Australia-New Guinea). The Sangkulirang-Mangkalihat Peninsula is adjacent to the easternmost edge of Sunda. **b,** Liang Tebo and surrounding archaeological sites, including those with dated Late Pleistocene rock art (shown in red). Map source, Shuttle Radar Topography Mission 1 Arc-Second Global by NASA/NGS/USGS; GEBCO\_2014 Grid, version 20150318 (http://gebco.net). Base maps generated using ArcGIS by M. Kottermair and A. Jalandoni.

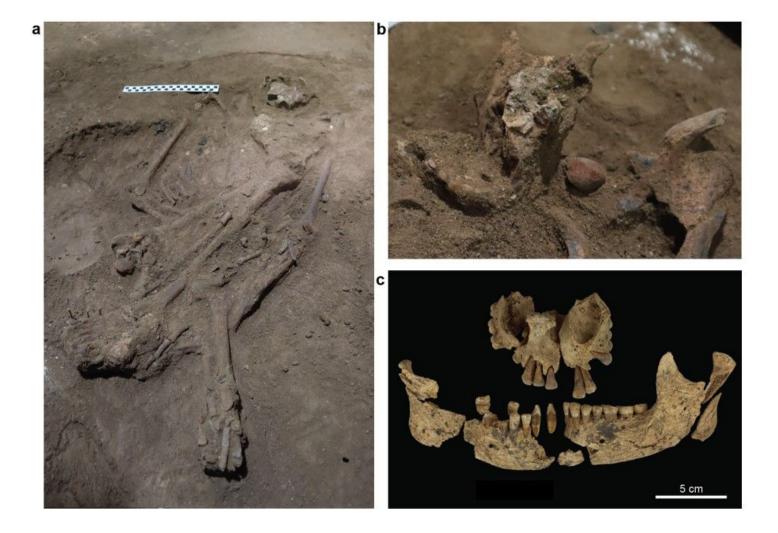


Figure 2

**Liang Tebo burial feature. a,** Single adult inhumation (TB1) showing burial position, with right knee brought to chest and complete right foot, and left knee flexed below the pelvis with tibia and fibula underneath the femur. **b,** In situ nodule of red ochre (earth pigment) adjacent to the mandible. **c,** Maxilla and mandible.



Figure 3

**Surgically amputated site of left tibia and fibula. a,** TB1 left and right legs with pelvic girdle demonstrating the complete absence of the lower left leg. **b,** Left tibia and fibula showing kerf surface, atrophy, and necrosis. **c,** Left tibia and fibula radiograph. **d-f,** Remodelled bone covering kerf surfaces demonstrating post-amputation healing. **d-f,** Images taken using Olympus DSX1000 Digital Microscope.

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