<arttitle> Skeletal evidence for Leprosy in India by the Second Millennium B.C.

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<abs> **Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, that affects almost 500,000 people worldwide.[1] The timing of first infection, geographic origin, and pattern of initial transmission of the disease are unknown.[1-3] The earliest accepted textual evidence indicates that leprosy was known in India by 600 B.C. and in Europe by 400 B.C.[6-7] The earliest skeletal evidence was dated 300-200 B.C. in Egypt[8] and Thailand.[9] Here, we report evidence of lepromatous leprosy in skeletal remains from Balathal, a Chalcolithic site (2300-1550 B.C.) in India.[10-11] A middle aged adult male skeleton demonstrates manifestations of *facies leprosa* and rhinomaxillary syndrome, degenerative joint disease, infectious involvement of the**

tibia (periostitis), and injury to the peripheral skeleton. Paleopathological analysis indicates that lepromatous leprosy was present in India by 1800 B.C.. This result supports translations of the *Atharva Veda* that reference leprosy and its treatment in hymns composed before the first millennium B.C..[12] The presence of leprosy in Chalcolithic India also suggests *M. leprae* may have spread out of Asia or Africa during the second or third millennium B.C., at a time when there was substantial interaction between South Asia, West Asia, and Northeastern Africa.[13] Our finding of skeletal evidence in Leprosy in India during the second millennium B.C. should be impetus to search for additional skeletal and molecular evidence of leprosy in human remains from this time period in India and Africa to confirm the origin of the disease.

<p>Leprosy is a debilitating but treatable disease caused by infection with *Mycobacterium leprae*. Although popular conceptions of leprosy are focused primarily on images from Biblical or Medieval times, half a million people worldwide were still suffering from the disease in 2006—primarily in rural areas of Angola, Brazil, Central African Republic, Democratic Republic of Congo, India, Madagascar, Mozambique, Nepal, and United Republic of Tanzania.[1] Leprosy has largely been considered a recent disease that spread with large scale Empires in the Early Historic period after 400 B.C.. [1-3] Recently, a Late Pleistocene model for origin and transmission out of Africa was proposed.[4] We report on the earliest skeletal evidence for the disease in India and interpret this as evidence supporting a Holocene transmission model for the disease.[5] An understanding of the origin and transmission routes of this disease will lead to new insights about the connections between the evolution of infectious diseases and humans.

<p> The history of Leprosy is "interwoven with civilization itself".[14] There is evidence that Leprosy was referenced in an Egyptian papyrus dated to 1550 B.C.,[7] some translations suggest it is mentioned in ancient Indian hymns composed before the first millennium B.C.,[12] and there may be references in the Old and New Testaments of the Bible[3] but this evidence is controversial.[3] The earliest writings that have widely accepted references to the disease are from the South Asian texts *Sushruta Samhita* and Kautilya's *Arthashastra* dated to the 6th century B.C. [2], 4th century accounts of the Greek author Nanzianos[5], a 3rd century Chinese text *Shuihudi Qin Jian*[6], and 1rst century A.D. Roman accounts of Celsus and Pliny the Elder.[6]

<p> The disease became a serious public health problem in Europe during the Middle Ages.[7] Asylums were established by the 7th century in France[18] and skeletal evidence for the disease is well documented for Medieval European samples from England[15-17], Scotland[19], Denmark[20], Italy[21], Czech Republic[18], and Hungary.[22-23] Although historians and other researchers have maintained that Leprosy originated in the Indian subcontinent and spread to Europe after the invasion of Alexander the Great in the fourth century B.C.[3], skeletal evidence for the disease in Asian prehistory is uncommon. Archaeological excavations have yielded skeletal evidence of Leprosy in the 2nd century B.C. in Egypt[8], the 1rst millennium B.C. in Uzbekistan[24], and sites in Thailand occupied from 300 B.C. to 200 A.D..[9] The earliest documented cases in West Asia (Isreal) are from the 9th century A.D..[25]

<p>We report the first skeletal evidence of Leprosy in South Asia from the site of Balathal (24°43' N 73°59' E) located 40 km northeast of Udaipur in the contemporary state of Rajasthan (Fig. 1). There are two phases of occupation represented at Balathal, a town occupied during the Early Historic (200 B.C.-200 A.D.) and a Chalcolithic settlement

(2400-1500 B.C.).[10] The Chalcolithic people of Balathal lived in stone or mud-brick houses, made wheel thrown pottery, copper implements, and practiced dry field agriculture focused on barley (*Hordeum vulgare*) and wheat (*Triticum spp*.). During this phase, also known as the Ahar culture, a large stone enclosure (500 m^2) was built in the center of the settlement. This stone enclosure was filled with stratified layers of vitrified ash from burned cow dung that appeared to have been thrown into this space from the top of the stone wall.[10] Three burials were recovered from this time period—individual 1997-1, 1997-2, and 1999-3. A complete inventory of the state of preservation of the skeletal remains (n = 5) was previously described[11], including two burials recovered from the Early Historic period[11]—individuals 1999-1[i] and 1999-2[ii].[11]

<p>Individual 1997-1 was interred in a tightly flexed posture, resting on its left side[iii]. This individual was uncovered at a depth of 2.66 m in layer 7 of the Northeast Quadrant of trench E3, inside the walled enclosure under 5 stratified layers of burned cow dung. There is one radiocarbon date of cal B.C. 1830 +/- 60 years from layer 7 (trench F4) taken at a depth of 3.17m.[10] The sample is bracket by an earlier date of cal B.C. 2350 +/- 70 years obtained from layer 10 (trench OD, depth 4.0 m) and a later date of cal B.C. 1510 +/- 70 years obtained from layer 4 (Trench B4, depth 1.4 m). These dates indicate that this individual lived *circa* 1800 B.C. (2350-1510 B.C.).

<p> The pelvic morphology indicates that this individual was male, approximately 40 +/-10 years of age based on standard aging techniques of cranial stenosis, dental attrition, and degenerative changes to joint surfaces in the pelvis. He expressed numerous pathological conditions of the skeleton and teeth, including typical manifestations of leprosy, or infection with *Lepromatous leprae.*[2-3]

<p> The skull was relatively complete but the postcranial skeleton is incomplete and more fragmentary. Evidence for bone pathology on the facial skeleton includes erosion/remodeling of the margin of the nasal aperture, atrophy of the anterior nasal spine, bilateral necrosis of the facial aspect of the maxilla, bilateral erosive lesions at the supraorbital region and glabella, and resorption of the alveolar region of the maxilla (Fig. 2a). The palatine process of the maxilla also demonstrates pathological changes including pitting near the midline and in the alveolar region indicating superficial inflammation affected regions that had not already resorbed (Fig. 2b).

<p> Antemortem tooth loss affected the majority of the maxillary teeth, with only the left first molar and fourth premolar remaining *in situ*. There are two large peripical abscesses on either side of the molar. Slight traces of the alveoli remain for the right canine, third premolar, second and third molars and the right second molar is present as an isolated tooth. The molar roots demonstrate a thickening of the apices indicative of hypercementosis. Antemortem tooth loss and alveolar resorption has also affected the mandible (Fig 3). There are 8 teeth *in situ*—right and left central and lateral incisors, canines, right third premolar, and the right third molar. Alveolar resorption and passive eruption in the anterior mandible has exposed an average of 7 mm of root surface in the incisors and canines. Resorption in the left posterior mandible has obliterated the alveoli and only a thin ramus of bone remains (11 mm in height).

<p>In the postcranial skeleton, marginal osteophytes effected most of the joint surfaces present, including the right and left glenoid fossae of the scapulae, left humerus (proximal epiphysis: head and trochanters), right and left ulnae (lunar and radial notches), left radius (distal epiphysis), the vertebral ends of the right and left ribs, left innominate

(around the perimeter of the acetabulum), the right and left femoral heads, and the proximal end of the left tibia (lateral condyle). The fourth through the seventh cervical vertebrae had severe degenerative changes including ventral wedging, osteophytic lipping on the margins of the centra and on the superior and inferior articular surfaces, and spondylolysis, or fusion of the vertebrae (Fig 4a). Similar changes were noted on the lumbar vertebrae (L3-L5). The left pisiform is present and there is a fracture on the articular facet for the triquetral (Fig. 4b). The proximal half of the left and right tibiae are present and the compact bone surface on the right is irregular and evidence for infection (periostitis) is present (Fig. 4c).

<p> The distal end of the right radius, ulna, and left triquetral are present and show no evidence of pathology. Many of the elements in the distal ends of the legs are missing—the distal tibiae, fibulae, and many of the foot bones are missing or damaged postmortem. More specifically, the left medial and intermediate cuneiforms and cuboid are present but damaged postmortem. All five right metatarsals are present though they have also suffered destruction of the articular ends. Seven pedal phalangeal fragments are also present but demonstrate no pathological modification.

<p>The remains of this individual present clear evidence of *facies leprosa*, rhino-maxillary syndrome, degenerative changes to the articular surfaces of the spine and appendicular skeleton, and periostitis on the tibia that represent the classic signs of lepromatous leprosy.[2-3, 20, 26, 28] Evidence for injury to an upper extremity is also commonly associated with a side effect of lepromatous leprosy, skin anaesthesia,[2] Other possible diagnoses, including tuberculoid leprosy and osteomyletis are unlikely. Tuberculoid leprosy, the relatively less infective form of the disease is not associated with disfigurement in the nasal and maxillary region of the facial skeleton.[2] There is no evidence for

involucrae, or sequestering of necrotic bone lesions typical of Osteomyelitis, a bacterial infection of the bones and marrow often the result of injury.[28] Pathological changes indicate a diagnosis of leprosy is likely and thus this individual represents the earliest skeletal evidence for the disease.

<p> Leprosy is found in translations of the *Atharva Veda*, a compilation of hymns concerning disease and its treatment composed in the second millennium B.C..[29] Our biological evidence for the presence of Leprosy in India during the second millennium B.C. broadly supports the accuracy of this translation and indicates that the *Atharva Veda* is the first historical reference to the disease, its pathogenesis and treatment.[12]

> "O Rama, Krishna, and Asikni medicine, thou hast sprung up at night. O Rajani, remove leprosy and whiteness of the body. O medicine, remove the leprosy, remove from him the whiteness of hair and skin, the festering wounds and excruciating pain. May thou regain thy healthy color. O medicine, drive far away the white specks. O medicine, thy quality of absorption in the body removes leprosy, thy quality of sticking removes whiteness of the body. O medicine, highly efficacious art thou, remove from him the painful suppuration of the wound. With my knowledge I have chased away the pallid sign of leprosy, caused by infection on the skin, sprung from the body or from the bones."[12]

<p> More broadly, this evidence can be used to address transmission models for the disease. Although Leprosy is often considered to have a recent origin[2-3, 6-9, 14-28], analysis of rare single nucleotide polymorphisms in contemporary samples of *M. leprae* from

worldwide geographic regions[4] identified two strains of Leprosy segregating in Asia (predominantly Type I) and east Africa (Type II). Because of the low frequency of the Type II strain in Asia, and its high frequency in East Africa, one scenario for Leprosy's origin is that Type II evolved first in East Africa (before 40,000 B.C.) and was later transmitted to Asia (evolving into Type I) and Europe (evolving into Type III), which is also common in West Africa and the Americas.[4]

Alternatively, the Type II strain may have evolved from the Type I strain in Asia much more recently and was then transmitted out of Asia, into Africa and Europe.[4-5] Small sample sizes and potentially biased demographic sampling of *M. leprae* from contemporary populations in the comparative genomics study could explain the absence of the Type II strain in South Asia (n = 4). Sampling issues or fixation of the Type II strain in East Africa (n = 2), combined with contemporary eradication efforts in India may have lead to an underestimate of the putative ancestral Type II strain's historical prevalence in India, and the derived Type I strain's historical prevelance in East Africa.

<p>The Asian origin scenario is more compatible with the natural history of *M. leprae*, which thrives on human contact and may have spread to East Africa during the development of urban life[15-17] and expanding trade networks during the height of the Indus civilization and the "Middle Asian Interaction Sphere." [5,13] The "Middle Asian Interaction Sphere" is a term used to describe political and economic contacts among South and West Asian Bronze Age peoples in the third millennium B.C..[13] There are four core areas to the interaction sphere—Meluhha in the Indus Valley, Turan in Central Asia, Mesopotamia in the Fertile Crescent, and Magan on the Arabian Peninsula. The evidence for inter-regional interaction includes textual sources from Mesopotamia indicating trade relationships with

Meluhha from the Early Dynastic Period (2900-2373 B.C.) to the time of Hammurabi (1792-1750 B.C.). The interpretation of 'Meluhha' as 'Indus' is supported by evidence for trade in raw materials, common artifact styles and motifs among the two regions.[13] Contact among Mesopotamia and the Egyptians began prior to the Early Dynastic period in Egypt (3050-2686 B.C.). South Asia and Northeast Africa were part of a larger regional trade network that stretched across the Arabian Sea. India was not an isolated cul-de-sac for immigration but instead had extensive, wide ranging networks for movements of peoples, goods, and infectious diseases for several millennia B.C. The third millennium interaction sphere is time of incipient urbanization and extensive interaction, which seems a more likely time for transmission of communicable diseases like leprosy than the Late Pleistocene migrations suggested previously.[4-5]

<p>Further research should be done on the geographic origin of the disease using an integrated approach that examines paleopathology and ancient DNA. Ancient DNA from the *Mycobacterium* may be preserved within the sinus cavities of the infected skeleton from Balathal and genomic comparison could provide evidence on whether this strain is closely related to the Type I form identified in Africa and Asia.[4] The first skeletal evidence from Dakhleh Oasis places the disease in Egypt after 400-250 B.C.[30] but there is a reference from Egypt that has been interpreted as evidence of more ancient knowledge of the disease, by 1550 B.C..[7] There may be well-preserved molecular evidence in Egyptian material that has yet to be recognized because individuals were resistant and only expressed the tuberculoid form of the disease, were suffering from a latent infection, or otherwise lacked osseous manifestations. In these individuals, DNA evidence would be required for diagnosis. Until further work is done to identify the origin of the ancestral strain, this

individual from Balathal marks the earliest evidence for lepromatous leprosy, which was present in a North Indian population as early as 1800 B.C., a time during which there was substantial interaction among populations throughout Asia, the Middle East, and Africa.

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<ack>**Authors Contributions** G.R. analyzed and photographed the skeletal material, and wrote the majority of the paper. V.M.T. analyzed the skeletal material and contributed comments to the manuscript. V.N.M. is the principle investigator of the archaeological site and contributed comments to the manuscript. R.K.M. and V.S. Shinde participated in the excavation of the site and contributed comments on the manuscript. K.G. participated in writing the manuscript. M.S. described the genomic data and participated in writing the manuscript.

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<LEGEND>Fig. 1. The site of Balathal. (a) A map of India showing the location of Balathal and a view of the lower town. (b) Photograph of the excavations within the stone enclosure where skeleton 1997-1 was located. This individual was interred in the Chalcolithic deposit (layer 7) of stratified layers of burned cow dung. Associated radiocarbon dates indicate an antiquity of cal B.C. 1800.

<LEGEND>Fig. 2. The cranium of individual 1997-1, a forty year old male. (a) Ventral view demonstrates bilateral erosive lesions at the supraorbital region and glabella, erosion/remodeling of the margin of the nasal aperture, including the anterior nasal spine, bilateral necrosis of the facial aspect of the maxilla, and resorption of the alveolar region of the maxilla with associated antemortem tooth loss. (b) Inferior view of the basicranium demonstrates pathological changes to the palatine process of the maxilla including pitting near the midline and in the alveolar region.

<LEGEND>Fig. 3. Ventral view of the mandible demonstrating root exposure, alveolar resorption, antemortem tooth loss, and a small apical abscess at the left third premolar.

<LEGEND>Fig. 4. Elements demonstrating pathological conditions in the postcranial skeleton. (a) Left lateral view of the cervical vertebrae (C3-C7) demonstrates degenerative changes including ventral wedging, osteophytosis, and spondylolysis. (b) Three views (from the radius, from the triquetral, and the palmar-distal surface) of the left pisiform demonstrating a fracture on the articular surface for the triquetral. (c) Lateral view of the tibia midshaft. Arrow points to periostitis on the compact bone surface.

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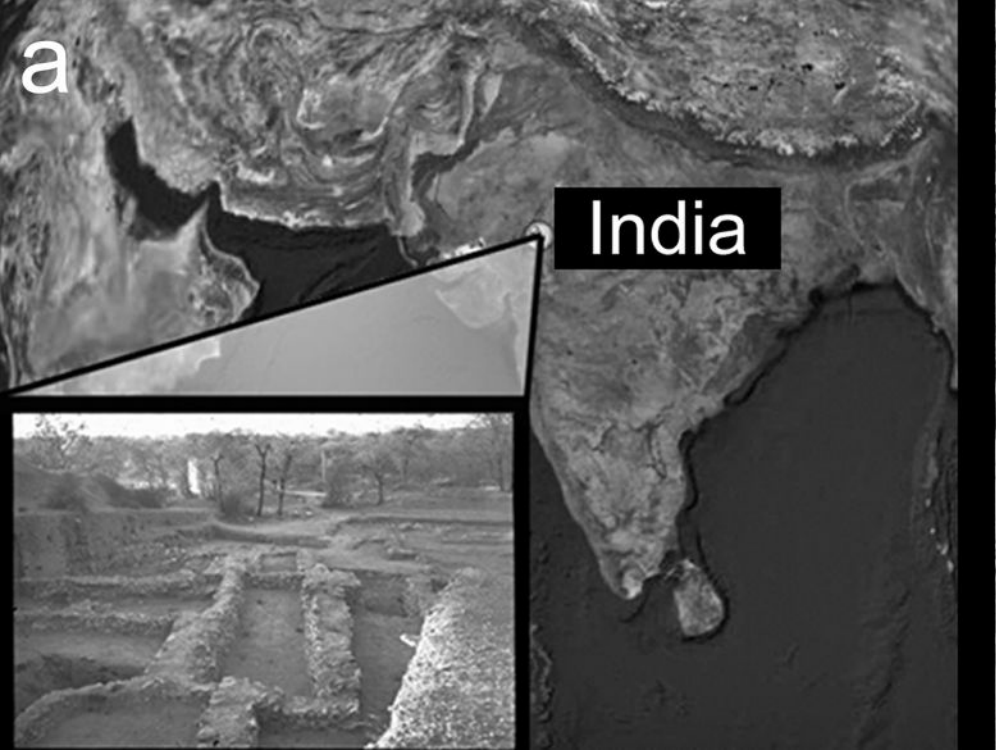
i Burials were numbered using the excavation year followed by the chronological order of the find.

ii Burial 1999-2 is an interesting case in that this individual was buried in a posture resembling *samhadi,* in which the legs are folded, hands rest on the knees, and the index finger and thumb touch to form a circle. It is

customary in Hindu tradition in parts India to bury ascetics in this posture because like children under five, ascetics are considered separate, liminal categories of humans due to their renunciation of ordinary life (Natali C. 2005. *Building Cemeteries, constructing Identities. Funerary practices and nationalist discourse among the Tamil Tigers of Sri Lanka.* Paper presented at the Conference of the British Association of Asian Studies.)

iii Burial 1997-1 was interred underneath 7 stratified layers of cow dung, considered a ritually pure substance in Hindu tradition. It is customary in Hindu tradition in parts of India to bury lepers alive (see Cust, R.N. 1881. Pictures of Indian Life: Sketched with the pen from 1852-1881. London: Trubner and Co.), rather than cremate their bodies, which as diseased, are not an appropriate sacrifice to the Hindu Gods ().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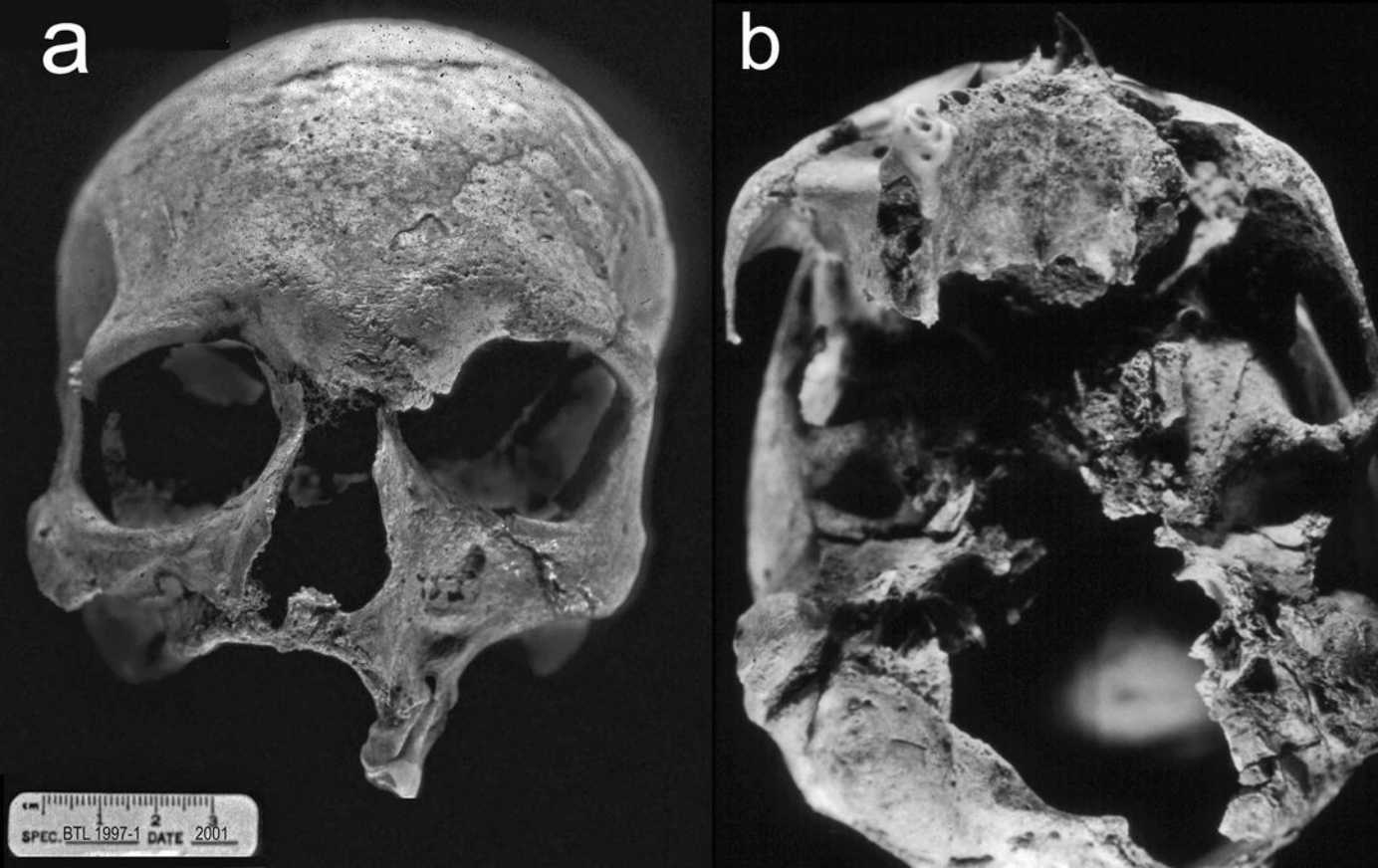
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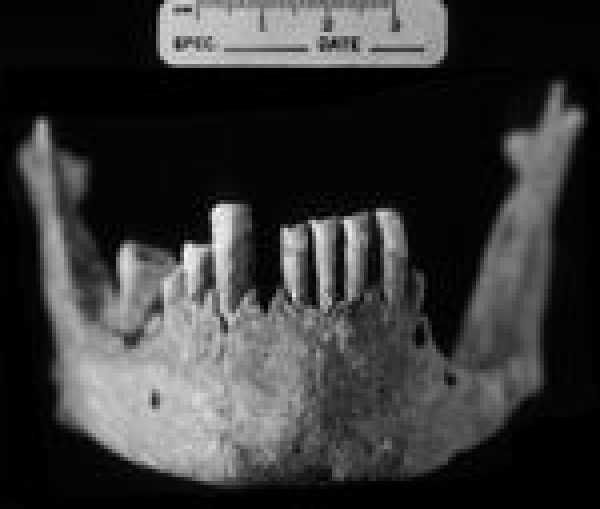
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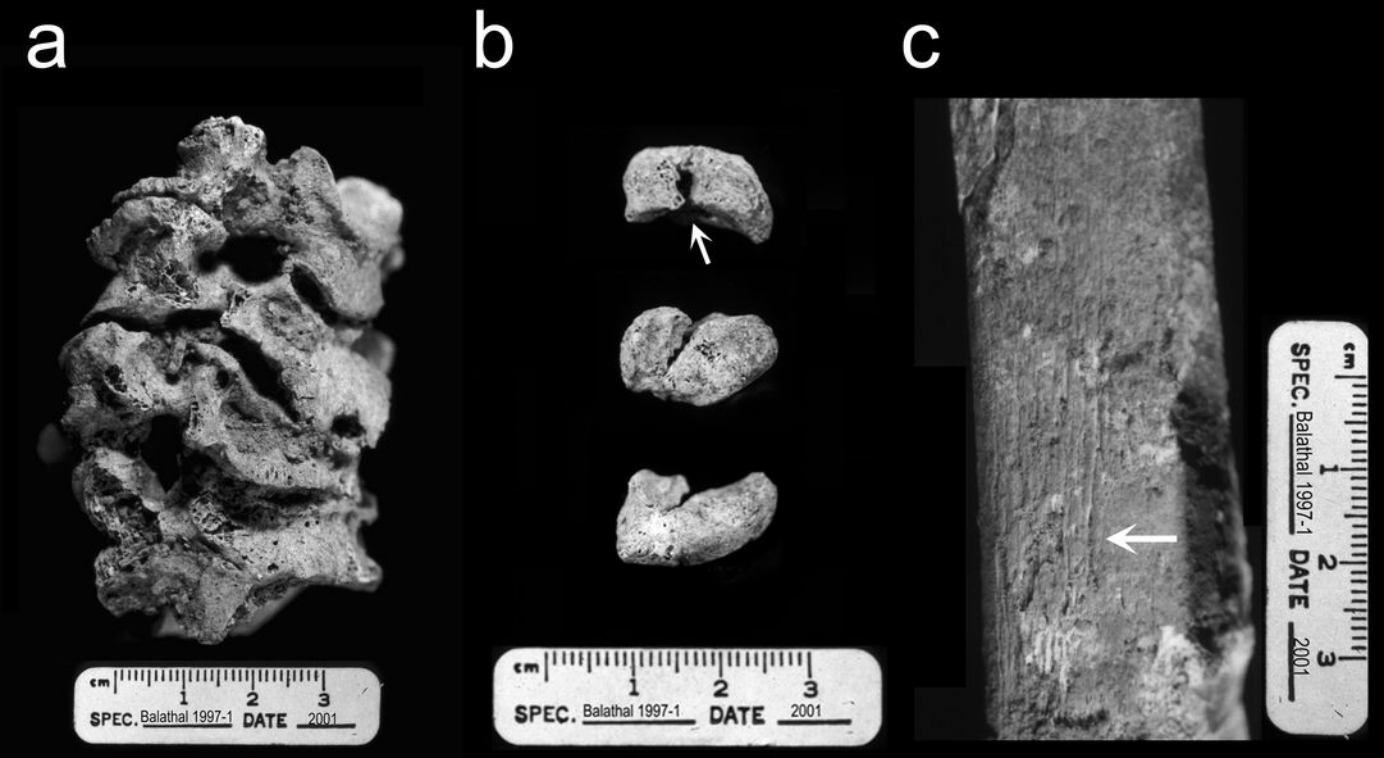
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