Letter to the Editor

Monkey Business in Pompeii—Unique Find of a Juvenile Barbary Macaque Skeleton in Pompeii Identified Using Osteology and Ancient DNA Techniques

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The remains of a juvenile monkey were discovered among a collection of repeatedly sorted adult and juvenile human skeletal material stored in Terme del Sarno, Pompeii. A more detailed provenance of these bones is not known, but their placement together with human remains indicates that the monkey was originally found near human remains. Bones of domestic animals were placed separate from those of humans in the same store. The radiocarbon date for this sample (OxA number 7448) of between 50 B.C. and A.D. 140 indicates that the find is authentic. Wild monkeys were not indigenous to this region at this time. The documentation of this unique specimen is the only record of the presence of monkeys in Pompeii, an association which also implies specific trade links.

The Roman towns of Pompeii and Herculaneum and surrounding areas of the Sarno Valley were buried after the sudden volcanic eruption of Mount Vesuvius in A.D. 79. The Pompeii site has a unique environmental situation. The bodies of its inhabitants were engulfed in ash probably no hotter than 200°C (Kent et al. 1981). Furthermore, the bodies were well above the groundwater table such that the ash hardened before the flesh had decayed, leaving a cast of the body and also limiting the microbial decay to purely anaerobic organisms (Sigurdsson et al. 1985). The recovered skeletal remains are well preserved and ideally suited to osteological and ancient DNA (anDNA) examinations.

The juvenile monkey elements discovered are shown in figure 1. The skeleton is obviously that of an immature individual. An approximate reconstruction of body size can be based on the sizes of long bones, the measurements of which are as follows: The right radius is 90 mm in diaphyseal length (DL), with a midshaft circumference (MSC) of 16 mm. For the left ulna, the DL is 99 mm and the MSC is 18 mm. For the right femur, the DL is 108 mm and the MSC is 28 mm. For the left tibia, the DL is 102 and the MSC is 25 mm. The length of the lower limb consists of the lengths of the femur and the tibia the height of the talus and the calcaneus, and allowing for the thickness of the missing epiphyses, it was concluded that the lower limb was about 240 mm long. Since the length of the lower limb roughly approximates the crown-rump length in quadrupedal monkeys, the body weight can be reconstructed from the relationship between body length and body weight for all catarrhines (Henneberg, Hugg, and Townsend 1989). In this relationship, weight equals double the exponent of length times 0.02 (W = 2exp[0.02L]). The body weight of the monkey is estimated as approximately 3 kg. It is noted that due to the inherent inaccuracies of such calculations, this value is an estimate.

Since the skull and dentition are missing, it is very difficult to make an exact taxonomic attribution of the specimen. Taxonomic attribution of macaques on the grounds of skeletal remains is generally difficult, a problem which is further compounded by the absence of much of the skull and dentition. The general morphology of the postcranial skeleton is that of a cercopithecine. The current range of the Barbary macaque is the temperate cedar and oak forests of Morocco and Algeria, although over the last few hundred years, macaques have been imported into Gibraltar (Kavanagh 1983). Hence, it seems most likely that the specimen in question represents a juvenile macaque imported to Pompeii from this region. However, it cannot be excluded that the skeleton belonged to a young baboon (*Papio*) or a vervet monkey (*Cercopithecus*).

Samples were taken for DNA testing from the vertebra, the scapula, a long bone, and a tooth. DNA was extracted by the phenol/chloroform method, followed by a silica column clean-up (Bailey et al. 1996). To authenticate the results, the following eight samples were examined (element and amount of bone powder sampled): vertebra (a, 150 mg), scapula (a, 190 mg), long bone (a, 50 mg), tooth (20 mg), vertebra (b, 50 mg), long bone (b, 50 mg), scapula (b, 50 mg), and long bone (c, 50 mg). The mitochondrial 12S rRNA primers were designed to exclude the amplification of the Hominoidea (including humans) but to include the possibility of amplifying macaques, baboons, and vervets based on previously published sequence information (van der Kuyl et al. 1995*a*, 1995*b*). Primer pair Mon1/Mon2^B was designed to amplify a 220-bp fragment, and Mon1/Mon3^B was designed to amplify a 110-bp fragment. Standard notation is used to describe the nucleotide bases of primers: R = A/G (purine), Y = C/T (pyrimidine), D = A/G/T (not C), H = A/C/T (not G), and V = A/C/G (not T). Mon1 (rev) = TCAATTAAGCACTCTATTCTTAA, Mon2^B (for) = b-TATATACCGCCATCTTCAGC, and Mon3^B (for) = b-GDAARARATGGGCTACATTTTCT. Primers were designed from the published sequences of the mitochondrial NADH dehydrogenase subunit (ND)

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FIG. 1.—The juvenile monkey skeletal remains recovered from the Terme del Sarno, Pompeii. (Scale 1:3). The cranium comprises one triangular 15 × 10-mm fragment of very thin bone of the vault, further unidentifiable, and a nearly complete left temporal bone with a portion of left parietal approximately 10 mm wide attached at the entirely unobliterated squamous suture. The temporal bone is 43 mm long from the asterion to the zygomaticotemporal suture at the end of the zygomatic process, while the highest point on the squama is located about 15 mm above the porion. The axial skeleton is represented by eight thoracic and five lumbar vertebrae, one segment of the sacrum, a complete upper left second rib, one right rib, and three rib fragments. All vertebrae have their arches fused with the bodies but no epiphyseal plates attached. For the appendicular skeleton, the upper limb is represented by the nearly complete right scapula and a fragment of the left scapula including the glenoid fossa and most of the spine, the complete right radius and the complete left ulna, one complete metacarpal and two fragments of other metacarpals. Long bones epiphyses are not present, and diaphyseal ends have characteristic surfaces for growth plates. The hip bone is represented by fragments of the right and left ilium with acetabular parts and most of the auricular surfaces and practically complete left and right ischia. The rest of the lower limb comprises the complete right femur, the left tibia, the proximal fragment of the right tibia, a fragment of the fibular shaft, the left calcaneus and talus and the nearly complete right calcaneus. A navicular and cuboid bones are also present, together with a fragment of a metatarsal. All bones have characteristic surfaces for growth plates.

5 gene (Hayasaka, Fujii, and Horai 1996) to amplify two overlapping fragments and exclude the amplification of human mtDNA. The first region of the ND5 gene (93 bp) was amplified using the primer pair Mon4/Mon5^{B}. Mon4 (for) = TTGGTGCAACTCCAAATAAAAGTA and Mon5^{B} = b-RAGRGHRGCVARAATTGGRA. The second region of the ND5 gene (114 bp) was amplified using the primer pair Mon6/Mon7^{B}. Mon6 (for) = AACYCTYAYCTCCCTAACTCT and Mon7^{B} (rev) = b-AGGCTRRTRATRAARGCATATATT. Where possible, primers were designed to take advantage of mismatches between the Hominoidea and other primates. The primer pairs did not amplify a 30-ng sample of modern human DNA. PCR conditions were 40 cycles of 55°C for 1 min, 72°C for 1 min, 94°C for 45 s, with the exception of Mon1/Mon2B for which the annealing temperature was 56°C. Second-round PCR was not required for any of these samples.

PCR products were cloned using the blunt-ended pCR-Script SK+ cloning kit (Stratagene). An artificially generated template (125 bp) was generated using extended primers which contained ''monkey tails'' identical to primers Mon1 and Mon3^{B} but internally specific to *Bos taurus* (cattle) mtDNA D-loop as a positive control and for quantification. Extended primers are as follows; Moncow1 = TCAATTAAGCACTCTATTCTTAAACGCGGCATGGTAATTAAGC and Moncow3^{B} = b-GYAARARATGGGCTACATTTTCTTTATGTCAAATTCATTCTTGATAG.

Sequencing was carried out manually (Bailey et al. 1996) and by the Florida DNA Sequencing Core Laboratory using ABI Prism Dye Terminator cycle-sequencing protocols developed by Applied Biosystems (Perkin-Elmer). The fluorescently labeled extension products were analyzed on an Applied Biosystems sequencer (Perkin-Elmer). Automated products were sequenced in both directions. The sequences were aligned using CLUSTAL W in the GeneJockey II computer program (Taylor 1993). The phylogenetic relationships of sequence haplotypes were displayed using reduced median networks (Bandelt et al. 1995) which were constructed using the network-building computer program (Röhl and Minh 1997).

12S rRNA mtDNA was recovered from five of the eight extracted Pompeii samples: scapula (a), long bone (a), tooth, scapula (b), and long bone (c). The sequenc-

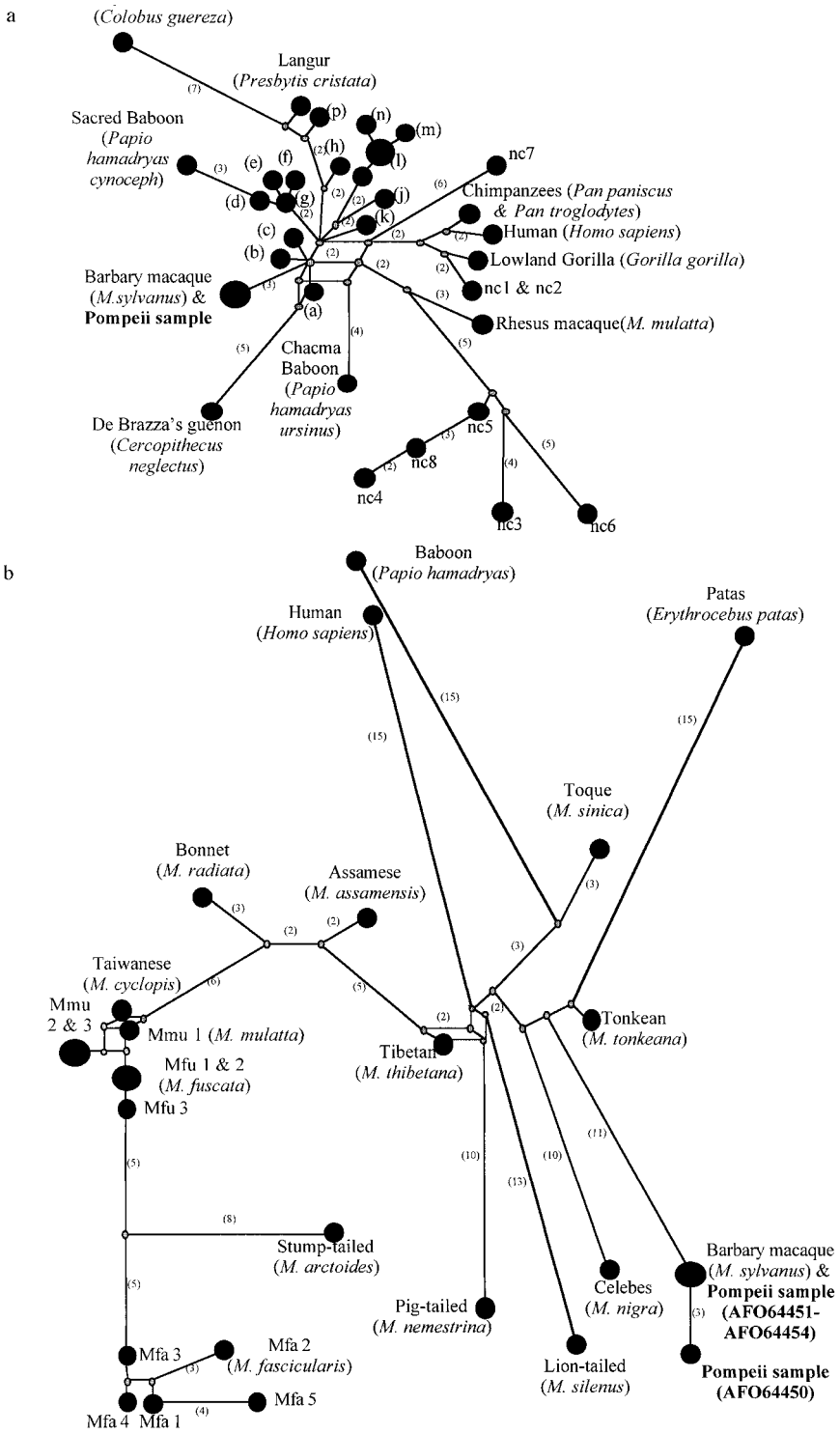

FIG. 2.—*a*, A reduced median network (Bandelt et al. 1995) comparing published African monkey and human nuclear insert sequences of the mitochondrial 12S rRNA region (van der Kuyl et al 1995*a*, 1995*b*) with those recovered from the ancient Pompeii monkey sample. Branch lengths are marked by bracketed numbers, except for those of one mutation, which are unlabeled. a = black mangabey (*Cercocebus aterrimus*), b = diana monkey (*Cercopithecus diana*), c = talapoin monkey (*Miopithecus talapoin*), d = moustached monkey (*Cercopithecus cephus*), e = mandrill (*Mandrillus sphinx*), f = tana mangabey (*Cercocebus galeritus*), g = redtail monkey (*Cercopithecus ascanius*), h = patas monkey (*Cercopithecus patas*), i = green monkey (*Cercopithecus aetiop sabaeus*), j = vervet (*Cercopithecus aetiop pygerythrus*), k = blue monkey (*Cercopithecus mitis*) and spot-nosed guenon (*Cercopithecus nictitans*), l = vervet (*C. aetiop pygerythrus*) and tantalus monkey (*Cercopithecus aetiop tantalus*), m = grivet (*Cercopithecus aetiop aetiop*), n = vervet (*C. aetiop pygerythrus*), p = mona monkey (*Cercopithecus mona*); nc1–nc8 indicate the eight human nuclear inserts. The network clearly shows the relationship of the Pompeii sample to the Barbary macaque and that the Pompeii sample is not closely related to any human or human nuclear insert sequences. The sequences retrieved from the Pompeii monkey do not cluster with any human or human nuclear inserts found by van der Kuyl et al. (1995*b*). *b*, A reduced median network (Bandelt et al. 1995) comparing published macaque sequences of the ND5 gene (Hayasaka, Fujii, and Horai 1996) with those recovered from

es obtained were aligned to the reported sequences of African monkeys (van der Kuyl et al. 1995*a*) and human nuclear inserts (van der Kuyl et al. 1995*b*), displayed in the network (fig. 2*a*). All of the recovered sequences were identical to the Barbary macaque (*Macaca sylvanus*) (van der Kuyl et al. 1995*a*) and have been submitted to GenBank under one entry (AF064449). The next closest related sequences were those of the black mangabey (*Cercocebus aterrimus*), the diana monkey (*Cercopithecus diana*), and the talapoin monkey (*Miopithecus talapoin*), all separated by one transversional and three transitional base changes. No PCR amplification was recorded for the 210-bp product. This was thought to be due to the fragmented nature of the ancient DNA, which is consistent with other reports (Handt et al. 1994).

Four samples yielded PCR products for the first 93-bp segment of the ND5 mtDNA: vertebra (a), scapula (a), tooth, and long bone (b). It was possible to recover DNA for the second 114-bp segment in two samples: tooth and long bone (b). Figure 2*b* shows a network of the ND5 mtDNA sequences retrieved from the Pompeii monkey and data previously published by Hayasaka, Fujii, and Horai (1996). The sequences retrieved were identical (GenBank accession numbers AF064451–AF064454) or most closely related (GenBank accession number AF064450) to the Barbary macaque (Hayasaka, Fujii, and Horai 1996). Identified changes are probably caused by DNA damage (Bailey 1998). The network (fig. 2*b*) clearly demonstrates that the Pompeii monkey belongs to the species of Barbary macaque (*M. sylvanus*).

A PCR product from the scapula (b) sample for the 12S rRNA 110-bp primer pair was cloned, and 30 clones were sequenced. Twenty-two had the same sequence, which was identical to the 12S rRNA *M. sylvanus* sequence (van der Kuyl et al. 1995*a*). One clone had a variant at L01394 numbering as Anderson et al. (1981) (C-A transversion), six clones at L01381 (G-A transition), and one clone at L01381 (G-A) and L01391 (A-G). Cloning error for modern human DNA in this laboratory is 0.1%. The observed number of cloning errors here was 0.9%, an increase which probably reflects damage of ancient DNA (Handt et al. 1994).

Scapula (a) and the tooth were used in the quantification experiment. The results of quantification varied between experiments and, as was the case in other studies (Handt et al. 1994; Krings et al. 1997), it was concluded that the starting material contained less than 1,000 templates (Bailey 1998).

We are confident that the sequences retrieved from the monkey sample are genuine; all probable forms of contamination have been eliminated: (1) all extraction blanks and PCR blanks were clean; (2) no modern macaque DNA has ever been amplified in our lab; (3) the result was compared with, and shown not to be, a human nuclear insert; and (4) only small fragments were amplified, consistent with the DNA being of ancient origin.

The unique find of the monkey remains at Pompeii was analyzed using both osteological and ancient DNA methods. Osteological examination indicated that the juvenile remains were either from a macaque (*Macaca*) or, conceivably, a baboon (*Papio*) or a vervet monkey (*Cercopithecus*). DNA testing using specifically designed primers which excluded the amplification of the Hominoidea but included the Colobine and Cercopithecine monkeys conclusively proved that the remains are those of a Barbary macaque (*M. sylvanus*). The radiocarbon date of the remains is between 50 B.C. and A.D. 140 at the 95% confidence level (OxA number 7448). It is assumed that the skeleton does not postdate the destruction of Pompeii and may well be contemporaneous with the eruption of Vesuvius in AD 79.

Pompeii, like many other cities of the Roman Empire, had extensive trade links (Casson 1984). Many animals are known to have been used by the inhabitants of Pompeii in a wide range of circumstances. This information has been gleaned from the osteological analysis of excavated remains, from contemporary accounts, and from pictorial evidence such as frescos and paintings. However, none of these sources has previously recognized the presence of the Barbary macaque or any other monkey species at Pompeii. A 48-mm-tall bronze figurine of a crudely carved baboon sitting on its haunches has been found in Pompeii, but it is most probably an import from the Nile Valley related to oriental cults popular among Romans of this era and does not depict local use of live monkeys (Cicirelli 1995). Remains of a monkey represent unique archaeological evidence of the trade links with the north coast of Africa. Presumably, the animal—not an indigenous species of this region—had been imported to be kept as a curiosity or pet by an inhabitant of Pompeii.

This result shows that it is possible to extract uncontaminated nonhuman DNA from an ancient sample of a close relative of our own species, even if it has been handled. Human contamination of ancient DNA is a particular problem when working within our own order (Krings et al. 1997; van der Kuyl et al. 1995*b*). It was possible to avoid it by heeding warnings of contamination (Hedges and Schweitzer 1995; Krings et al. 1997; van der Kuyl et al. 1995*b*) and using extant sequence information available in GenBank for primer de-

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the ancient Pompeii monkey sample. The sequence of the DNA recovered from the Pompeii sample was aligned with 22 sequences of the macaque genus and sequences from a baboon, a patas, and a human (Anderson et al. 1981; Hayasaka, Fujii, and Horai 1996). When more than one sample is used from a species, the full name is given initially, and subsequent references to it are abbreviated: Mmu = rhesus monkey (*Macaca mulatta*), Mfu = Japanese monkey (*Macaca fuscata*), and Mfa = crab-eating monkey (*Macaca fascicularis*). Both networks are drawn to a scale at which the bracketed numbers indicate the branch lengths, which are proportional to the numbers of base substitutions separating sequences. The size of the filled circle indicates the number of individuals. The network demonstrates that the ancient sequences belong to the Barbary macaque species.

sign. Our results suggest that molecular phylogenetic studies can use museum specimens of monkeys as long as an appropriate experimental design is used.

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