



Direct radiocarbon dating and DNA analysis of the Darra-i-Kur (Afghanistan) human temporal bone



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ABSTRACT

The temporal bone discovered in the 1960s from the Darra-i-Kur cave in Afghanistan is often cited as one of the very few Pleistocene human fossils from Central Asia. Here we report the first direct radiocarbon date for the specimen and the genetic analyses of DNA extracted and sequenced from two areas of the bone. The new radiocarbon determination places the find to ~4500 cal BP (~2500 BCE) contradicting an assumed Palaeolithic age of ~30,000 years, as originally suggested. The DNA retrieved from the specimen originates from a male individual who carried mitochondrial DNA of the modern human type. The petrous part yielded more endogenous ancient DNA molecules than the squamous part of the same bone. Molecular dating of the Darra-i-Kur mitochondrial DNA sequence corroborates the radiocarbon date and suggests that the specimen is younger than previously thought. Taken together, the results consolidate the fact that the human bone is not associated with the Pleistocene-age deposits of Darra-i-Kur; instead it is intrusive, possibly re-deposited from upper levels dating to much later periods (Neolithic). Despite its Holocene age, the Darra-i-Kur specimen is, so far, the first and only ancient human from Afghanistan whose DNA has been sequenced.

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1. Introduction

The site of Darra-i-Kur (Cave of the Valley; other spellings include Darra-e Kür; Bābā Darwish is another name of the site) in Afghanistan first came to prominence in the late 1950s with fieldwork and extensive site surveys undertaken by a team of researchers from the American Museum of Natural History (NYC) and the National Museum of Afghanistan (Kabul). Louis Dupree and Abdul Rauf Wardak (Dupree, 1972a) surveyed limestone foothills in northern Afghanistan and recorded more than 100 caves. In the Badakhshan province, near the village of Chinār-i Gunjus Khān (36°44'N, 69°59'E) (Fig. 1) they identified a cave called Darra-i-Kur as potentially promising, after finding Mousterian-like flakes

eroding on the talus slopes leading to the site. Subsequently, in 1966, the site was excavated and three trenches were dug. The first, Trench 1, with prominent Neolithic finds, was 2.5 m wide and reached bedrock ~1 m down at one end, while at the other end there was a large roof fall. A second trench was then excavated in order to avoid the roof fall debris. This was 4 m in length, 2.5 m in width and reached 2.5 m depth before the bedrock was encountered. A third trench was the largest (2.5 m wide and 24 m in length) and extended out of the cave down the talus slope and into deposits supposedly derived from the action of a nearby stream. According to Dupree (1972b) periodic flooding may have been responsible for mixing some of the hearth-related charcoal material found at the site with overlying silts.

The lithic industry of the site comprised implements and debitage made of locally available, poor-quality flint or chert. More than 800 implements, predominantly made using Levallois techniques, were recovered (Dupree and Davis, 1972). The lithics included crude Levallois points (Costa, 2012) and flakes, handaxes,

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Figure 1. Map of Afghanistan showing the location of Darra-i-Kur in the northeastern part of the country (Badakhshan province).

scrapers and flake-blades, and were attributed to the Middle Palaeolithic (Mousterian). Neolithic and Iron Age implements were also found in the sequence toward the upper sections, as well as Late Islamic and modern 20th century material.

The excavators identified enough charcoal for radiocarbon dating, found in association with the Middle Palaeolithic flake implements and cores. Radiocarbon dating was undertaken at the GeoChron Laboratories (Cambridge, Massachusetts, USA) in the early 1970s. However, the laboratory was compelled to amalgamate the charcoal and soil material in order to get enough carbon for dating using a conventional system. The radiocarbon date, produced on total carbon, GX-1122: $30,300 \pm 1900/-1200$ BP (Dupree, 1972b), must be looked at very critically; it is almost certainly a minimum age as the radiocarbon laboratory noted at the time (Krueger pers. comm. in Dupree, 1972b) and echoed by others since (Stringer and Burleigh, 1981).

2. The human fossil

Angel (1972) described a fragmentary human right temporal bone discovered at the Mousterian level of the site (Cut LC 11:200) (Fig. 2). The bone comprised the tympanic and petrosal bones, with the mastoid process and paramastoid crest broken at the base. Angel (1972) compared measurements of the temporal and, specifically, the tympanic bone, which was quite well-preserved, against anatomically modern human and Neanderthal equivalents and suggested that the temporal sat closer to moderns than Neanderthals. In particular, he emphasized that due to the flatness of the tympanic bone and most other features, with the exception of the very large internal auditory meatus and slightly lateral location of the stylomastoid foramen, the temporal bone was modern in appearance. Assuming an age that overlapped with that of Neanderthals, Angel cautiously suggested that the Darra-i-Kur temporal

could be transitional to modern humans rather than a Neanderthal. Bricker (1976) urged caution about inferring too much from the remains due to the fragmentary nature of the find. Arensburg et al. (1981) included the Darra-i-Kur fossil in their study of 140 middle ear bones belonging to three different populations of modern humans and concluded that there was a general homogeneity observed in their results, regardless of antiquity. More recently, in their study of the ossicular chain of La Ferrassie 3, Quam et al. (2013) found that the Darra-i-Kur specimen fell within, but towards the upper end, of the recent *Homo sapiens* range of variation.

The Darra-i-Kur temporal bone is mentioned in various publications because it stands out as one of the very few human remains recovered from this period in Central Asia. It is regularly referred to by a range of authors and in a variety of studies revolving around human paleoanthropology and paleoenvironments of Asia (e.g., Lindly and Clark, 1990; Trinkaus, 2005; Jaubert et al., 2006; Sonakia and de Lumley, 2006; Glantz et al., 2008a, 2008b; Malassé and Gaillard, 2011; Reyes-Centeno, 2016).

Given doubts regarding the precise age and skeletal attribution of the specimen, we decided to test it for the first time by direct dating and by DNA analysis. The bone [Catalog number: NMNH 387961] is housed in the Smithsonian National Museum of Natural History in Washington (DC). We obtained permission for destructive sampling in 2014.

3. Methods and materials

3.1. Sampling procedure

Prior to sampling, the temporal bone was CT scanned to document the morphology for future research and to plan the most appropriate locations from which to take samples. The CT images were acquired using the Skyscan 1173 (Bruker, Belgium) at the

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