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KARIN TAK CAVE, LESSER CAUCASUS, AS A POTENTIAL SITE FOR GENETIC RECONSTRUCTION OF PALAEOECOLOGY

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Fossils and sediments preserved in caves are an excellent source of information for investigating past biodiversity, palaeoecology, and human activity. Until recently, studies have relied on morphology-based palaeontological records, however recent advances in molecular analytical methods offer excellent potential for extracting a greater array of information from the available data. Karin Tak is one such cave that is characterized by a high rate of ancient biomolecules preservation, which creates wide possibilities for reconstructing ecology from Pleistocene to our days. Thus, Karin Tak cave can be considered as a regionally significant site where the unique preservation conditions provide deep molecular insights into palaeoecology and palaeodemography.

Lesser Caucasus – Karin Tak cave – ancient DNA – Palaeoecology

Քարանձավներում պահպանվող բրածոները և նստվածքաշերտերը հանդիսանում են հնագույն կենսաբազմազանության, էկոլոգիայի և մարդկային գործունեության ուսումնասիրության համար հարուստ աղբյուր: Մինչև վերջերս հետազոտությունները կատարվում էին հնէաբանական նյութի մորֆոլոգիական տվյալների հիման վրա, սակայն մոլեկուլային վերլուծական մեթոդների զարգացումները հնարավորություն են ընձեռում պեղածո նյութից ստանալ առավելագույն տեղեկություններ: Քարին Տակ քարանձավը այդպիսի հնավայրերից մեկն է, որը բնութագրվում է հնագույն կենսամոլեկուլների պահպանման բարձր մակարդակով, ինչը լայն հնարավորություն է ստեղծում վերականգնել էկոլոգիական պատկերը պլեյստոցենից առ այսօր: Այսպիսով, Քարին Տակ քարանձավը կարող է դիտարկվել որպես տարածաշրջանային արժեքավոր հնավայր, որտեղ պահպանման եզակի պայմանները հնարավորություն են տալիս բացահայտել հնագույն էկոլոգիական և ժողովրդագրական առանձնահատկությունները:

Փոքր Կովկաս – Քարին Տակ քարանձավ – հնագույն ԴՆԹ – հնագույն էկոլոգիա

Органические останки и отложения, находящиеся в пещерах, являются богатым источником информации для изучения древнего биоразнообразия, палеоэкологии и деятельности человека. До недавнего времени исследования в этой области основывались на морфологических характеристиках палеонтологических находок, однако достигнутые успехи в молекулярно-аналитических методах позволяют извлечь больший объем информации из этих данных. Карин Так – одна из таких пещер, которая характеризуется высоким уровнем сохранности древних биомолекул, что создает широкие возможности для реконструкции экологии региона начиная с плейстоцена до наших дней. Поэтому данный памятник можно рассматривать как регионально важный объект, уникальные условия сохранности органического материала в котором позволяют детально изучить палеоэкологию и палеодемографию.

Малый Кавказ – Пещера Карин Так – древняя ДНК – палеоэкология

Vertebrate fossil assemblages are an important source of information in understanding prehistoric life and evolutionary processes. In particular, morphological analyses of this kind of records can reveal species that existed in the past, help identify the evolutionary relationships of extinct and extant taxa, assist palaeoenvironmental reconstruction, and provide deep insights into the evolutionary and ecological impacts of environmental changes [3]. However, the accuracy of fossil identifications mainly relies on the easily observable morphological characteristics, making the classification of fragmented or taxonomically-mixed bone records challenging, if not impossible [8]. This can result in lumping distinct species together or, in case of incomplete fossil material, over-splitting species. However, various molecular strategies have been developed for those options when morphology-based identification proved problematic.

Over the past two decades, ancient DNA (aDNA) research has proved to be a useful complement to the morphological study of fossils; this approach is rapidly growing in popularity, accessibility, and applicability. Since its advent in the mid-1980s [6, 9] the field of aDNA has brought powerful tools for studying past. In particular, aDNA provides access to genomic data covering hundreds of thousands of years, allowing answering evolutionary, ecological, social, and environmental questions, especially regarding the ways the humans have interacted with other species and modified past ecosystems and environments [2]. Ancient DNA research has made a massive progress in its rather short history, extending greatly with the advent of next-generation sequencing (NGS) technologies. Novel sequencing approaches have made it possible to regularly acquire data from dozens of variable positions in the genome from increasingly diverse sources drawn from archaeological, paleontological, and archival materials [5]. Further, developments in aDNA sequencing have now enabled the mapping of entire nuclear genomes of fossils. The information acquired even from a single genome can provide direct insights into the demographic history of past generations. Such data are often lacking in an area of study reliant on incomplete fossil assemblages and chronologies [10]. Overall, studies using aDNA have addressed a wide range of questions regarding taxonomy, phylogeny, palaeodiet, palaeoclimate, population dynamics and interspecies relationships [4].

A necessary premise for aDNA research is the sufficient biomolecular preservation. Cave systems represent an ideal environment for palaeontological investigation since they often contain relatively complete stratigraphic deposits coupled with stable environmental proxies, as minimal temperature and humidity fluctuations that proved to be favourable for DNA preservation [5].

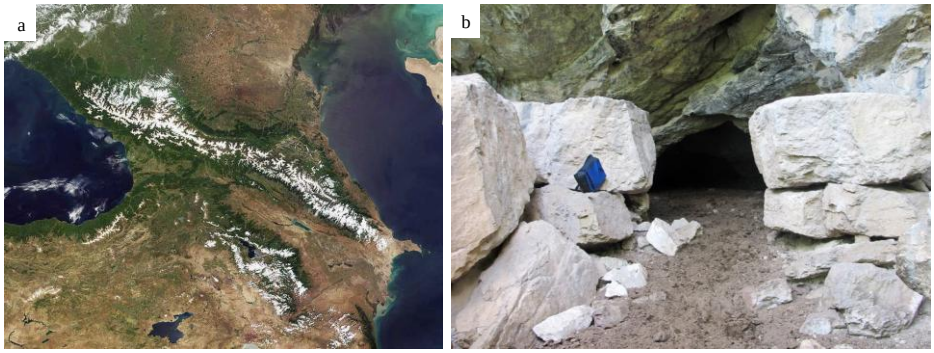

Fig. 1. a) Geographical position of Karin Tak cave within the Lesser Caucasus. b) The entrance of the site. The cave opening is approximately 50 meters below the ridgeline and has an entrance facing west. Limestone blocks have fallen from the thickly bedded limestone formations containing the cave and its caverns.

Karin Tak cave (fig. 1) in Artsakh Republic is one of such undisturbed sites that contain Late Pleistocene to Holocene sediment infill together with hominid remains, obsidian stone tools and contemporaneous flora and fauna. The scientific importance of the cave derives from the presence of over 42,000 years old fossiliferous sediments that contain vertebrate fossils with fair to moderate chemical preservation allowing aDNA research. Excellent biomolecule conservation in this site is confirmed by the high-quality ancient DNA (aDNA) yielded from human tooth (ca. 6,900 years ago) [7] and preliminary screening of samples indicating up to 70% of endogenous DNA content.

As is most commonly the case, the archaeological excavations in Karin Tak mainly yielded highly fragmented and morphologically indistinct bones, which are futile in compiling faunal assemblages morphologically (fig. 2). To complement traditional morphology-based taxonomy, for the first time in the region, a bulk-bone metabarcoding method was applied. This approach employs metabarcoding to characterize DNA extracted from a powder of morphologically unidentifiable bone fragments to provide records of faunal diversity [8]. The method involves (1) simultaneous extraction of aDNA from multiple unidentifiable bone fragments; (2) amplification of short, 'diagnostic' regions of mitochondrial genes by polymerase chain reaction; and (3) sequencing (via NGS) of these amplicons to identify the species present by comparison with a genetic database of known species [3, 4, 8].

Fig. 2. Bone fragments >125 μm recovered from 4 liters of sediment from Karin Tak. Stratigraphic age > 42,000 years.

Genetic screening of vertebrate fossils has revealed a high diversity in animal taxa, inhabiting the region between ca. >42,000 and 25,683-24,803 years ago. Overall, the results indicated continuity in a faunal composition of the region throughout the Late Pleistocene, with the presence of only a few extinct taxa. This allows suggesting that the onset of the Last Glacial Maximum (LGM) did not cause major turnover in fauna in the region, and during the abovementioned timespan, the Karin Tak cave was located at the boundary between arid subtropical and humid climate regions, a pattern preserved till the modern days [1]. Further exploration of the cave will include a larger number of animal fossils and botanical remains in order to thoroughly test the hypothesis of refugium for the Lesser Caucasus.

Based on these results we consider Karin Tak cave to be an archaeological site of great regional importance, where the remarkable preservation conditions allow detailed molecular reconstruction of the palaeoecology. The outcomes achieved so far highlight the potential of the ongoing exploration that will have a significant contribution to our understanding of early human occupation, demographic history and its relationships with the dwelling environment of the region since the Pleistocene.

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