



Analysing *Aproteles bulmerae* mega bat remains from the Kiowa Rockshelter, Papua New Guinea

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CONTEXT

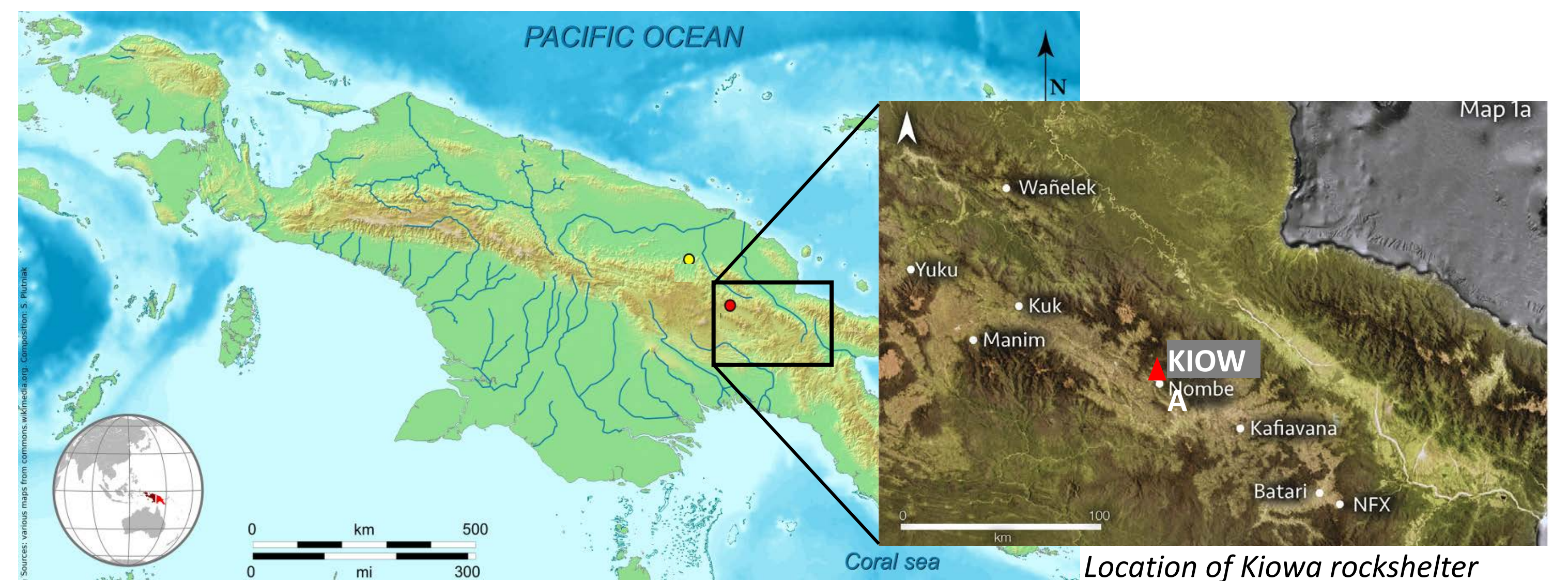
The Kiowa rockshelter (Simbu province, PNG) was first excavated in 1960 by a team led by Susan Bulmer [1].

Located at ~ 1530 masl this rockshelter was used from the late Pleistocene to late-Holocene (12 k to 3 kya).

Lithic assemblages and faunal remains excavated at Kiowa suggest that the site was first used as a hunting camp, with bats being the species most frequently captured.

This led to the large fruit bat (*Aproteles bulmerae*) becoming locally extinct around 10 kya, although found to be extant elsewhere in New Guinea.

We propose a methodological approach to access data from *A. bulmerae* remains.



Location of Kiowa rockshelter (red triangle) (modified from [2])

METHODS + RESULTS

Four mandible fragments from *A. bulmerae* were sent from the Univ. of Papua New Guinea to the CNRS (Univ. of Toulouse, France) for analysis.



Mandible of *A. bulmerae* dated to between 10000 to 12000 years BP used for this study analyses.

1) 3D SCANNING

Samples were scanned using an X-ray microtomographic instrument (Phoenix Nanotom 180) at the CIRIMAT (Univ. of Toulouse).

This non-destructive technique records digital 3-D morphological data, to be used for further analysis.



Phoenix Nanotom 180 scanner used at the CIRIMAT

2) FTIR COLLAGEN ASSESSMENT

Samples were screened to assess the amount of collagen using Fourier transform infrared spectroscopy (FTIR).

This method uses less than 1 mg of sample and induces minimal damage to archaeological samples.

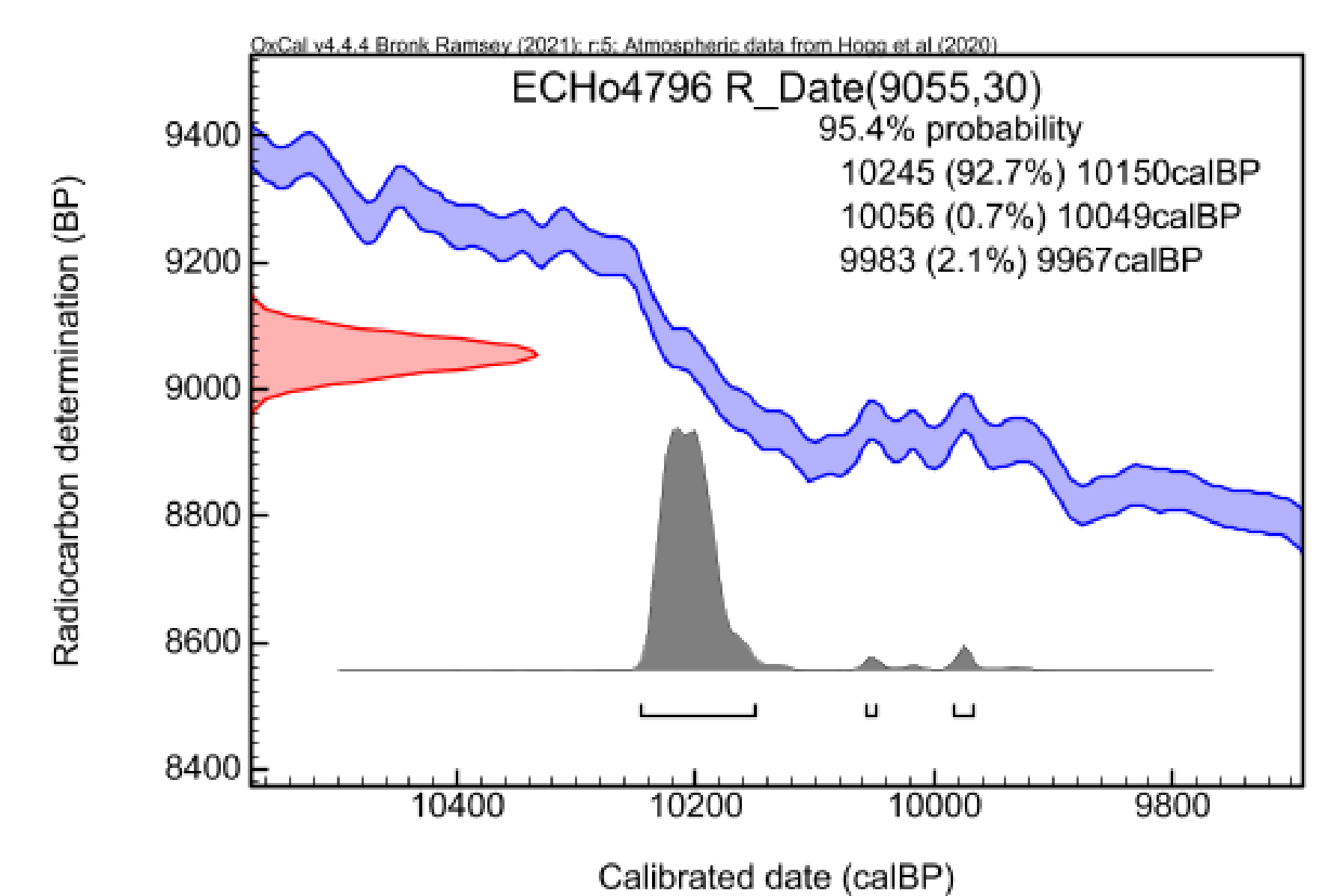
Samples with enough collagen (>3%) were selected for direct 14C dating.

Sample N°	% nitrogen	% collagen	< 0.7 %N wt
K-EE-12-1	0.44	2.42	not datable
K-EE-12-44	0.39	2.16	not datable
K-EE-12-47	0.52	2.85	not datable
K-EE-12-56	0.71	3.91	datable

3) RADIOCARBON DATING

Direct 14C dating was only performed on bone from K-EE-12-56 (sufficient amount of collagen).

The sample was dated to 9062 +/- 34 BP (10245-10150 cal BP) using OxCal v4.4.4 [3] and the SHCal20 Southern Hemisphere curve [4].



4) DNA ANALYSIS

DNA was extracted from the root of a tooth for each sample at the ancient DNA facility of PACEA (Univ. of Bordeaux) following Dabney et al. [5] and used to build Illumina libraries [6] which were shotgun sequenced on NextSeq 500.

Sequenced were aligned against the Egyptian rousette (*Rousettus aegyptiacus*) genome HLrouAeg4.

Analyses retrieved <1% of endogenous DNA for 3 samples. K-EE-12-56 sample did not contain enough DNA to build a library and be sequenced.

Sample N°	N° reads obtained	N° of reads aligned to bat genome	N° of reads aligned after filtering	% endogenous DNA	% endog. DNA after Quality control	Deamination rate (3')	Deamination rate (5')	% GC
K-EE-12-1	690176	31080	172	8.526	0.158	0.0	0.0	80.8
K-EE-12-44	591272	48117	153	15.472	0.217	0.0394	0.25	87
K-EE-12-47	715338	30084	208	8.017	0.170	0.0	0.0	81.6

DISCUSSION

The methodological approach applied to these bone remains minimises the destruction of the sample and the loss of information.

- ✓ 3D scanning **preserves all the morphological information** for further analysis and in case of damage.
- ✓ Sample screening using FTIR analysis identifies only **1 sample with enough collagen** (>3%) for direct C14 dating [7] avoiding partial destruction of the 3 other samples.
- ✓ C14 dating of *A. bulmerae* K-EE-12-56 to around **10200 years old** confirms the presence of this bat species in the lower level (12A) of human occupation at Kiowa [1, 8].
- ✓ We observe an inverse correlation between collagen content and endogenous DNA yields. Collagen survival does not predict DNA preservation, when collagen <5% [9].
- ✓ **Endogenous DNA is low** in Kiowa bat remains suggesting poor preservation of this site, as expected in tropical environments. However, endogenous DNA retrieved is sufficient for further phylogenetic analysis based on the mitochondrial genome.

CONCLUSION

Despite tropical environmental factors favouring the degradation of organic matter, the step-by-step approach follows here: (1) identifies the samples with higher potential for further analysis (14C dating, DNA analysis), and (2) retrieves endogenous DNA for species identification despite poorly-preserved faunal remains.

Reference: [1] Bulmer S 1960. Report on Archeological Field Work in the New Guinea Highlands (10/1959 to 05/1960). Dpt of Anthropology, Univ. of Auckland. Unpublished report. [2] Summerhayes GR et al. 2017. The archaeology of forest exploitation and change in the tropics during the Pleistocene: the case of northern Sahul (Pleistocene New Guinea). *Quat. Int.* 448, 14e30. [3] Bronk Ramsey C 2009. Bayesian analysis of radiocarbon dates. *Radiocarbon*, 51: 337–360. [4] Hogg A et al. 2020. SHCal20 Southern Hemisphere calibration, 0–55,000 years cal BP. *Radiocarbon* 62, 759–778. [5] Dabney J et al. 2013. Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc Natl Acad Sci* 110, 15758. [6] Meyer M et al. 2010. Illumina Sequencing Library Preparation for Highly Multiple. *Cold Spring Harb Protoc.* 2010(6):pdb.prot5448 [7] Lebon M et al 2016. Rapid quantification of bone collagen content by ATR-FTIR spectroscopy. *Radiocarbon* 58, 131–145 [8] Gaffney D et al 2021. Small game hunting in montane rainforests: Specialised capture and broad spectrum foraging in the Late Pleistocene to Holocene New Guinea Highlands. *Quat. Science Reviews* 253, 106742 [9] Kontopoulou I. et al 2020. Screening archaeological bone for palaeogenetic and palaeoprotoeomic studies. *PLoS One.* 2020 Jun 25;15(6):e0235146

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