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

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## CONTEXT

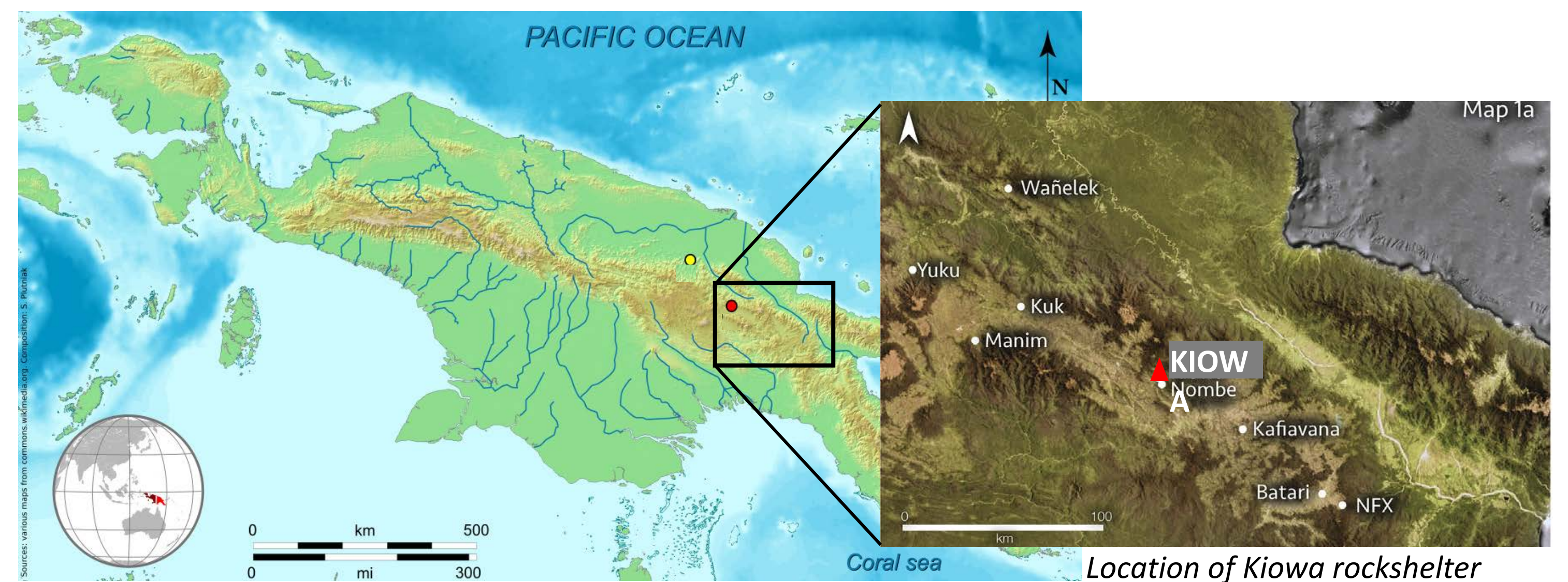
The Kiowa rockshelter (Simbu province, PNG) was first excavated in 1960 by a team led by Susan Bulmer [1].

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.



Mandibles 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.

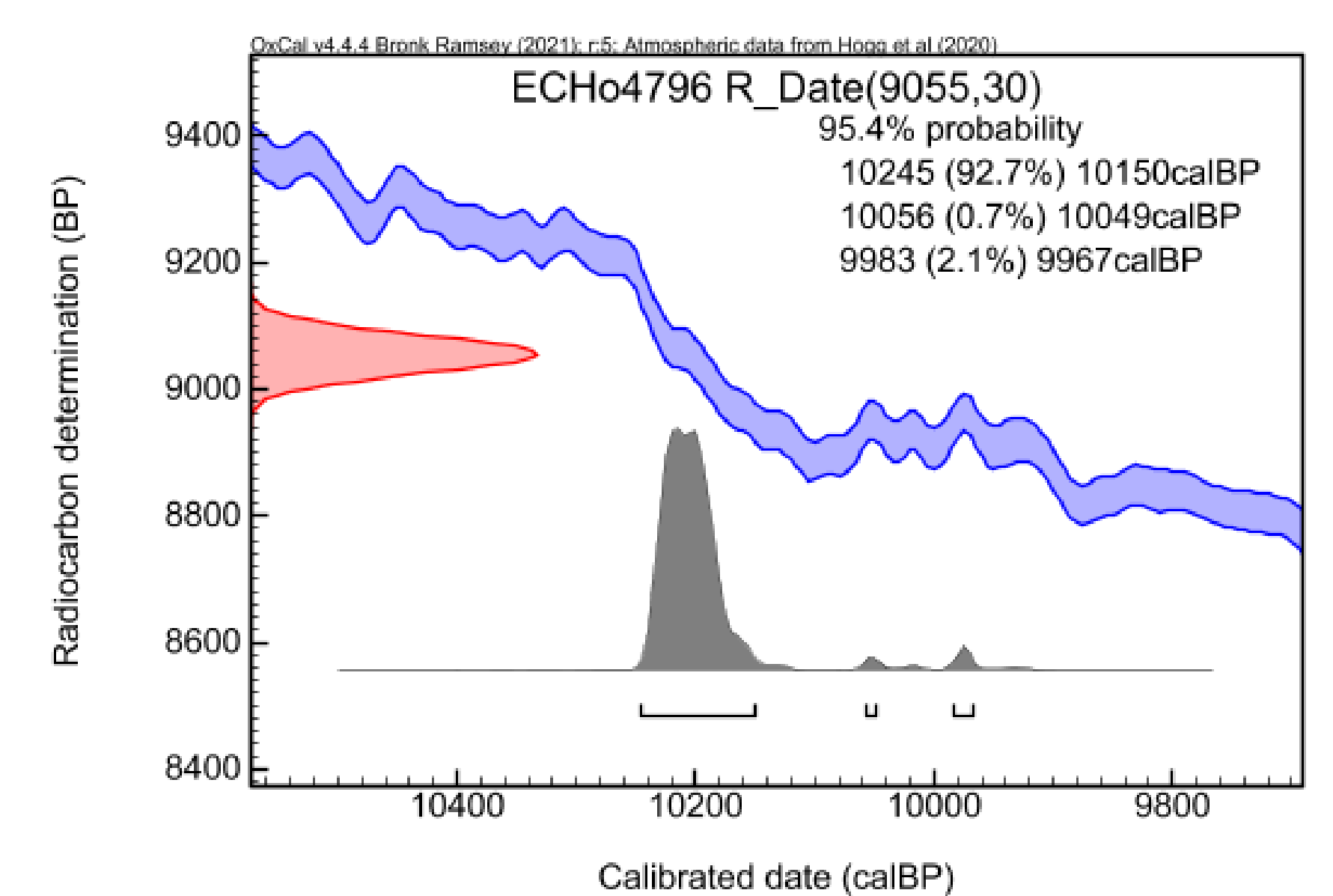
Samples with enough collagen (>3%) were selected for direct 14C dating.

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 palaeoproteomic studies. *PLoS One.* 2020 Jun 25;15(6):e0235146

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