

REPLY TO MARTINÓN-TORRES ET AL. AND HIGHAM AND DOUKA: Refusal to acknowledge dating complexities of Fuyan Cave strengthens our case

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Martinón-Torres et al. (1) present a flawed reinterpretation of our work which demands correction. FY-HT-1 and FY-HT-2 were collected from the section walls of the 2011–2013 excavations; details of provenience were provided (2). All of the lingual and much of the occlusal and mesial surfaces of FY-HT-2's enamel is missing (Fig. 1A). Therefore, reconstructions of “deer-like” wear (1) simply bear no resemblance to the preservational reality of the tooth. Also disconcerting is their comparison of FY-HT-2 with images of various deer incisors (Fig. 1A and ref. 1). Confirmation bias aside, a proper comparison would have indicated affinities to recent humans (Fig. 1A), as confirmed by DNA analyses (2). Regarding FY-HT-1, its preservation is visually indistinct from existing samples (see ref. 3). But within-sample variation is clear, and expected, given nonuniform taphonomic processes within the context of a dynamic sedimentary history, as we have demonstrated at Fuyan Cave (2).

Martinón-Torres et al. (1) and Higham and Douka (4) challenge our AMS ¹⁴C dating. We describe our accelerator mass spectrometry (AMS) ¹⁴C procedures (2), which involved similar protocols used previously for Fuyan Cave (3). We are clear about most samples showing poor collagen preservation (2). However, age discrepancies among collagen, CaCO₃, and total organic carbon (TOC) (involving dentin and enamel) results are generally small, indicating low contamination (2). We draw their attention to faunal samples FY3-1 and FY3-5 (2). Importantly, also, the C/N ratio of BA140121 of Liu et al. (3) suggests optimal burial conditions, with a reliable age substantially younger than 80,000 y (i.e., 39,150 ± 270 B.P. ¹⁴C age). The AMS and TOC background of the Peking University laboratory should be far beyond this ¹⁴C age. Contrary to Martinón-Torres et al. (1), dating of BA140121

falls well short of background and actually provides independent confirmation of our results. Importantly, the purpose of our study is to test whether Fuyan Cave contained human remains older than 65,000 y rather than obtaining “true” ages, which it does.

Higham and Douka (4) apply previously published criteria (5) for “reliable” bone ¹⁴C dating to argue that our results do not support the arrival of anatomically modern humans (AMH) <65,000 y ago. These criteria are indicators of degradation (or preservation) of collagen in fossils, but, in principle, collagen degradation should not affect ¹⁴C ages if exogenous carbon is not present. While degraded collagen in fossils may be contaminated, these criteria cannot estimate the amount. While a derivation of 500 y to 10,000 y from true age is generally considered unacceptable for archaeological materials, unreliable ¹⁴C ages simply cannot be assumed to result from a shift in ¹⁴C age from >80,000 y to Holocene age, as Higham and Douka (4) seem to be arguing.

Following Dunbar et al. (6), we undertook testing on a modern bovine bone, a human bone, and a human tooth from a known age historical site prior to ¹⁴C dating of archaeological materials. For ultrafiltration, the gelatinous bone solution was filtered using a Whatman glass microfiber filter (2). We were unable to use molecular ultrafiltration (7) because the samples were too small. Nonetheless, our results demonstrate that our method provided correct ages (Table 1). For the human bone we tested, Higham and Douka (4) would reject our results a priori. Yet the collagen and TOC ¹⁴C ages of the tooth agree well with their known age (Table 1), even though collagen degradation had caused nearly half of the % N and % C to be lost. Furthermore, both the AMS ¹⁴C ages and

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independent DNA tip dates for the same human teeth in our study were in agreement (2). Normally, protein sequences demonstrate marked stability and can exist in older or harsher environments than DNA. The presence of authentic DNA (aDNA) therefore indicates the original collagen (or carbon) should exist. Moreover, pairs of collagen and TOC ¹⁴C ages (e.g., FY 3-5, YJP-1054, and YJP-2936) with reasonable C/N ratios demonstrate these fossils are much younger than 65,000 y BP.

Martinón-Torres et al. (1) also question the authenticity of DNA from FY-HT-2, but our data clearly show otherwise (Fig. 1). Coverage for each position and proportion of reads matching the consensus base for each position are provided herein (Fig. 1B). Average frequencies of the majority base at each position were 99.92%. The low consensus support (~50%) at some positions was due to misalignment around insertions/deletions or C-stretch. The majority of its sequence is evidently from a single individual, as expected for an uncontaminated sample. C/T deamination rates at the 5' end of reads are typical for aDNA. Average deamination patterns over all mapped reads are provided (Fig. 1C). The FY-HT-2 library retained damage in the last nucleotide, which was greater than the suggested threshold (3%) for "double-stranded partial uracil-DNA-glycosylase" protocol (Fig. 1B).

Deciphering site formation processes and history by obtaining absolute dates from flowstones, sediments, charcoal, and mammalian and human fossils, we emphasize that U-Th ages cannot represent the burial age of the paleoanthropological materials at Fuyan Cave. Neither can the optically stimulated luminescence dating of sediments. Only through direct AMS ¹⁴C dating and aDNA analysis of some of the 47 existing human teeth can their true age be understood. We strongly urge Martinón-Torres et al. (1) to do so as a matter of urgency, rather than misrepresenting our findings. We would welcome more research into the arrival time of AMH in southern China, using wide-ranging dating and aDNA techniques. Indeed, this was the impetus for our study (2). To attempt to argue away a large body of geochronological and DNA data, generated using multiple methods and materials, and across five sites, by arguing our standards for prescreening were insufficient, in our opinion, 1) is self-serving and 2) denies the complex depositional, taphonomic, and diagenetic reality of subtropical paleoanthropological caves in southern China. While we agree that our ¹⁴C results should be considered minimum ages, this does not justify using speleothem ²³⁰Th/U dates to represent the age of AMHs, nor can they be used to validate an early settlement of the region, as has been claimed (3, 8, 9). An appearance time for AMH in southern China 50 thousand to 45 thousand years ago, in line with molecular results, is yet to be disproven.

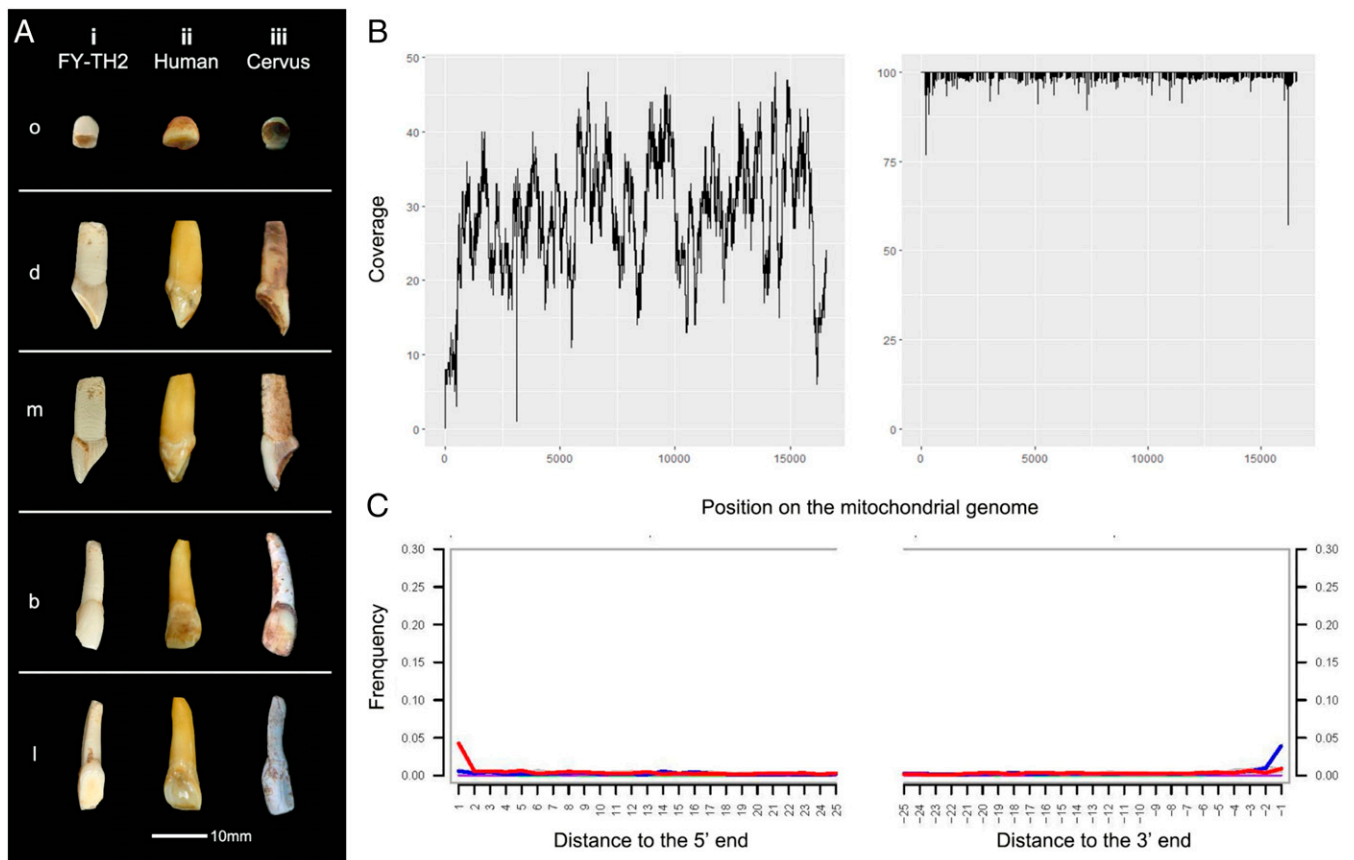


Fig. 1. (A) Fuyan Cave tooth FY-HT-2 (i), a recent human from the Foyemiaowan Han Tomb (ii), and deer of Martinón-Torres et al. (1) (iii). Right column adapted with permission from ref. 1. (B) Number of sequences overlapping each position in the FY-HT-2 mitochondrial genome (coverage, Left) and percentage of sequences carrying an identical base (Right). (C) Frequencies of nucleotide substitutions at the start and end positions of sequences from the FY-HT-2 library.

Table 1. Results of ^{14}C dating, C/N ratio, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ on human teeth from Fuyan Cave, on a modern bovine bone, and on a known-age human bone and tooth used for testing the ^{14}C dating method in ref. 2

Lab code	Sample ID	Type	F ^{14}C	^{14}C age (years B.P.)	Calendric age (years B.P.)	N% by EA	C% by EA	C/N	$\delta^{15}\text{N}$ (air)	$\delta^{13}\text{C}$ (VPDB)
Human teeth from Fuyan Cave										
NTUAMS-5260-C	FY-HT-1	CaCO ₃	0.7976 ± 0.0069	1816 ± 70	1750 ± 90					
NTUAMS-5260-D	FY-HT-1	TOC	0.7388 ± 0.0057	2432 ± 62	2540 ± 130	0.6	2.3	4.5	—	-7.53
NTUAMS-5260b-D	FY-HT-1	TOC	0.7734 ± 0.0058	2382 ± 63	2510 ± 140	0.6	2.3	4.5	—	-7.53
NTUAMS-5259-C	FY-HT-2	CaCO ₃	0.2662 ± 0.0026	10633 ± 79	12580 ± 140					
NTUAMS-5259-D	FY-HT-2	TOC	0.3527 ± 0.0035	8372 ± 80	9380 ± 90	0.04	1.6	46.7	10.89	-16.85
A modern bovine bone from Sichuan Province, China										
NTUAMS-5037-A	bone20181107 (Kang)	Collagen	1.0319 ± 0.0079	-252 ± 62	Modern	14.7	41.7	3.3	5.59	-19.91
NTUAMS-5037-A	bone20181107 (Kang)	Collagen	1.0454 ± 0.0103	-356 ± 80	Modern	14.7	41.7	3.3		
NTUAMS-5038-A	bone20181107 (Chou)	Collagen	1.0482 ± 0.0102	-378 ± 78	Modern	14.8	41.6	3.3	5.60	-19.94
NTUAMS-5038b-A	bone20181107 (Chou)	Collagen	1.0348 ± 0.0080	-275 ± 62	Modern	15.0	42.2	3.3	5.57	-19.75
NTUAMS-5039-A	bone20181107 (TE)	Collagen	1.0487 ± 0.0073	-382 ± 56	Modern	15.5	42.3	3.2	5.60	-19.81
NTUAMS-5039b-A	bone20181107 (TE)	Collagen	1.0485 ± 0.0077	-380 ± 59	Modern	15.3	42.5	3.2	5.61	-19.85
NTUAMS-5039c-A	bone20181107 (TE)	Collagen	1.0563 ± 0.0075	-440 ± 57	Modern	14.9	41.5	3.2	5.59	-19.73
		average	1.0448			15.0	41.9	3.3	5.59	-19.83
		SD	0.0086			0.3	0.4	0.05	0.01	0.09
NTUAMS-5037-D	bone20181107 (Kang)	Bone powder	1.0688 ± 0.0081	-534 ± 61	Modern	3.9	13.0	3.9	6.76	-18.58
A human bone and a human tooth from Yinxu archaeological site (Late Shang Dynasty, about 1146 to ~1046 BC), Anyang, Henan Province, China										
NTUAMS-5397-D	2007AXAT0607m8-a	TOC of tooth	0.7017 ± 0.0064	2846 ± 73	2990 ± 100	1.8	6.2	3.9	10.49	-6.97
NTUAMS-5398-A	2007AXAT0607m8-a	Collagen	0.6996 ± 0.0055	2870 ± 63	3015 ± 100	7.6	20.7	3.2	9.53	-8.13
NTUAMS-5398-D	2007AXAT0607m8-b	TOC of bone	0.7240 ± 0.0065	2594 ± 72	2655 ± 115	0.5	3.8	8.2	10.04	-7.82

Kang, Chou, and TE in Sample ID are the three technicians who undertook the dating work at the NTUAMS laboratory; 2007AXAT0607m8a is a human upper incisor and 2007AXAT0607m8b a human right femur from Yinxu archeological site, historically dated between 1146 BC and 1046 BC (corresponding to calibrated ^{14}C age of 3,096 y B.P. to ~2,995 y B.P.). The ^{14}C age (2σ error) is calculated from the measured $^{14}\text{C}/^{12}\text{C}$ and $^{13}\text{C}/^{12}\text{C}$ of OXII, background and samples. Calendric age (years before 1950 CE) is calibrated from IntCal 13. NTUAMS = National Taiwan University Accelerator Mass Spectrometry; OXII = National Bureau of Standards Oxalic Acid II; EA = elemental analyzer; VPDB = Vienna Pee Dee Belemnite.

- Martinón-Torres *et al.*, On the misidentification and unreliable context of the new “human teeth” from Fuyan Cave (China). *Proc. Natl. Acad. Sci. U.S.A.*, 10.1073/pnas.2102961118 (2021).
- X.-f. Sun *et al.*, Ancient DNA and multimethod dating confirm the late arrival of anatomically modern humans in southern China. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2019158118 (2021).
- W. Liu *et al.*, The earliest unequivocally modern humans in southern China. *Nature* **526**, 696–699 (2015).
- T. F. G. Higham, K. Douka, The reliability of late radiocarbon dates from the Paleolithic of southern China. *Proc. Natl. Acad. Sci. U.S.A.*, 10.1073/pnas.2103798118 (2021).
- F. Brock *et al.*, Reliability of nitrogen content (%N) and carbon: Nitrogen atomic ratios (C:N) as indicators of collagen preservation suitable for radiocarbon dating. *Radiocarbon* **54**, 879–886 (2012).
- E. Dunbar, G. T. Cook, P. Naysmith, B. G. Tripney, S. Xu, AMS ^{14}C dating at the Scottish Universities Environmental Research Centre (SUERC) radiocarbon dating laboratory. *Radiocarbon* **58**, 9–23 (2016).
- T. F. G. Higham, R. M. Jacobi, C. Bronk Ramsey, AMS radiocarbon dating of ancient bone using ultrafiltration. *Radiocarbon* **48**, 179–195 (2006).
- G. Shen *et al.*, Mass spectrometric U-series dating of Huanglong Cave in Hubei Province, Central China: Evidence for early presence of modern humans in eastern Asia. *J. Hum. Evol.* **65**, 162–167 (2013).
- C. J. Bae *et al.*, Modern human teeth from Late Pleistocene Luna Cave (Guangxi, China). *Quat. Int.* **354**, 169–183 (2014).