Supplementary Text

Radiocarbon Dating of Man Bac Human Remains

Radiocarbon dates for charcoal throughout the stratigraphic layer above the sterile layer within which the burials were found date to 4016–3524 cal B.P. (95% CI), and relative dating of archaeological material provides supportive evidence for a secure window of time for the burials in question in this paper (Oxenham et al. 2011). Archaeological material indicates a contemporaneous relationship with the burials and the stratigraphic layer wherein the charcoal was obtained for radiocarbon analysis.

Human rib and tooth samples from both individuals (MB05 M20 and MB07 H2 M29) were sent to the University of Waikato Radiocarbon Dating Laboratory. Insufficient collagen yields meant radiocarbon dating could not be attempted.

Radiocarbon dating of four human rib samples from other burials at Man Bac was undertaken at the Oxford Radiocarbon Accelerator Unit (ORAU) at the University of Oxford. Prior to AMS dating we screened bones for their %nitrogen content to assess collagen preservation. The majority were below our ideal threshold of ~0.76% Brock et al. (2010a). However, values still indicated preserved collagen is likely to be present in some samples (Table S1).

We extracted collagen from bones that passed or nearly passed the %nitrogen screening (those in bold and in italics in Table S1) using methods previously outlined in Brock et al. (2010b) using the following chemical pretreatment protocol (coded AF):

- Coarsely ground bone powder was loaded into a glass test tube (weights of bone are provided in Table S1).
- A sequence of 0.5 M HCl, 0.1 M NaOH, and 0.5 M HCl was used to treat the bone, interspersed with rinsing with ultra-pure (MilliQ™) water between each reagent.
- Crude collagen was gelatinized in pH3 solution at 75°C for 20 hours.
- The gelatin solution was filtered using a polyethylene Eezi-filter™ whose pore size ranges between 45–90 μm, precleaned by thorough rinsing and ultrasonication and the insoluble residues discarded.
- The filtered gelatin was then pipetted into a precleaned ultra-filter (Sartorius Vivaspin™ 15 30kD MWCO) and centrifuged at 2,500–3,000 rpm until 0.5–1 ml of the >30 kD gelatin fraction remains (typically 20–40 min.) (for the human bone this was not applied due to the low sample size of the recovered collagen).

Table S1. Samples analyzed for % nitrogen. Samples in bold were later successfully dated. Those in italics were attempted but failed to yield sufficient collagen for dating. Those in neither bold nor italics were not attempted.

Individual	Sample	wt %N
MB05 M34	*MB05 M34 13*	*0.434*
MB07 H2 M5	MB07 H2 M5 8	0.264
MB05 M31	**MB05 M31 6**	**0.629**
MB05 M20	MB05 M20 19	0.281
MB07 H2 M30	*MB07 H2 M30 5*	*0.402*
MB05 M28	MB05 M28 20	0.059
MB07 H2 M10	MB07 H2 M10 9	0.206
MB05 M15	**MB05 M15 18**	**0.689**
MB05 M29	*MB05 M29 1*	*0.730*
MB07 H1 M9	*MB07 H1 M9 21*	*0.448*
MB07 H2 M32	MB07 H2 M32 4	0.259
MB07 H2 M12	**MB07 H2 M12 10**	**0.955**
MB07 H2 M2	MB07 H2 M2 7	0.170
MB07 H2 M19	MB07 H2 M19 11	0.345
MB07 H1 M4	MB07 H1 M4 16	0.097
MB07 H1 M11	*MB07 H1 M11 14*	*0.595*
MB05 M11	MB05 M11 17	0.291
MB07 H2 M1	MB07 H2 M1 2	0.167
MB07 H1 M10	*MB07 H1 M10 15*	*0.443*
MB07 H2 M24	**MB07 H2 M24 12**	**0.728**

Table S2. Radiocarbon dates of human bone from Man Bac. P number denotes the ORAU code and OxA denotes the reference number for the radiocarbon date. ^{14}C age B.P. denotes the conventional radiocarbon age, expressed in years B.P. with B.P. being before A.D. 1950. AF is the ORAU pretreatment code for ultrafiltered collagen as outlined in Brock et al. (2010a). Stable isotope ratios are expressed in ‰ relative to VPDB and AIR with a mass spectrometric precision of ±0.2‰ for C and ±0.3‰ for N. Yield represents the weight of ultrafiltered collagen in milligrams. %Yld is the percent yield of extracted collagen as a function of the starting weight of the bone analyzed ("Used" also in mg). %C is the carbon present in the combusted gelatin. CN is the atomic ratio of carbon to nitrogen and is acceptable if it ranges between 2.9–3.5 Brock et al. (2010a).

OxA	P Number	Sample	^{14}C age B.P.	± value	PCode	Used	Yield	%Yld	%C	δ^{13}C (‰)	δ^{15}N (‰)	CN
X-2636-24	38655	MB05 M15 18	3539	29	AF	1050	4.53	0.4	37.6	–16.9	11.1	3.3
32378	38653	MB05 M31 6	3505	28	AF	1050	5.95	0.6	39.7	–17.6	11.3	3.4
32379	38658	MB07 H2 M12 10	3453	28	AF	950	5.67	0.6	39.6	–17.3	11.1	3.3
32380	38661	MB07 H2 M24 12	3406	35	AF	1010	5.67	0.6	39.9	–17.6	11.8	3.4

- This gelatin was freeze-dried ready for combustion in a CHN analyzer.

The ultrafiltration step was originally described by Brown et al. (1988) and the ORAU has used the Sartorius and Vivaspin filters since 2000. The precleaning steps are undertaken after the protocols outlined in Brock et al. (2010a).

Combusted gelatin samples were analyzed using a PDZ-Europa Robo-Prep biological sample converter (combustion elemental analyzer) coupled to a PDZ-Europa 20/20 mass spectrometer operating in continuous flow mode using an He carrier gas. This enables $\delta^{15}N$ and $\delta^{13}C$, nitrogen and carbon content and calculation of C:N atomic ratios. VPDB was the standard for $\delta^{13}C$ values. Graphite was produced by reacting the sample CO_2 over an iron catalyst in an excess H_2 atmosphere at 560°C. AMS radiocarbon measurement was carried out using the ORAU 2.5MV HVEE accelerator. Radiocarbon ages were calculated with reference to Stuiver and Polach (1977) in years B.P.

Radiocarbon dates of bone are reported in Table S2 along with their context information. Bones were uniformly poorly preserved in terms of collagen, with all successfully dated samples > than 1% wt. collagen (the effective threshold in the ORAU). Six samples produced no collagen and therefore could not be dated (Table S3). All other analytical parameters measured, including the carbon to nitrogen atomic ratio, were acceptable.

We used OxCal 4.3 (Ramsey 2001) and the INTCAL13 calibration curve (Reimer et al. 2013) to calibrate the radiocarbon data.

Table S3. Samples from Man Bac that failed to yield a radiocarbon determination. P number denotes the ORAU code.

P Number	Sample	Lab Comment
38652	MB05 M34 13	Failed due to no yield
38654	MB07 H2 M30 5	Failed due to very low yield
38656	MB05 M29 1	Failed due to no yield
38657	MB07 H1 M9 21	Failed due to no yield
38659	MB07 H1 M11 14	Failed due to very low yield
38660	MB07 H1 M10 15	Failed due to no yield

Calibration

Calibration of the AMS determinations was undertaken using OxCal 4.3 (Ramsey 2001). We observe the possibility of a slight reservoir effect based on (1) the presence of marine and freshwater fish and other mammals in the archeozoological assemblage, and (2) the stable isotopes of carbon and nitrogen that we

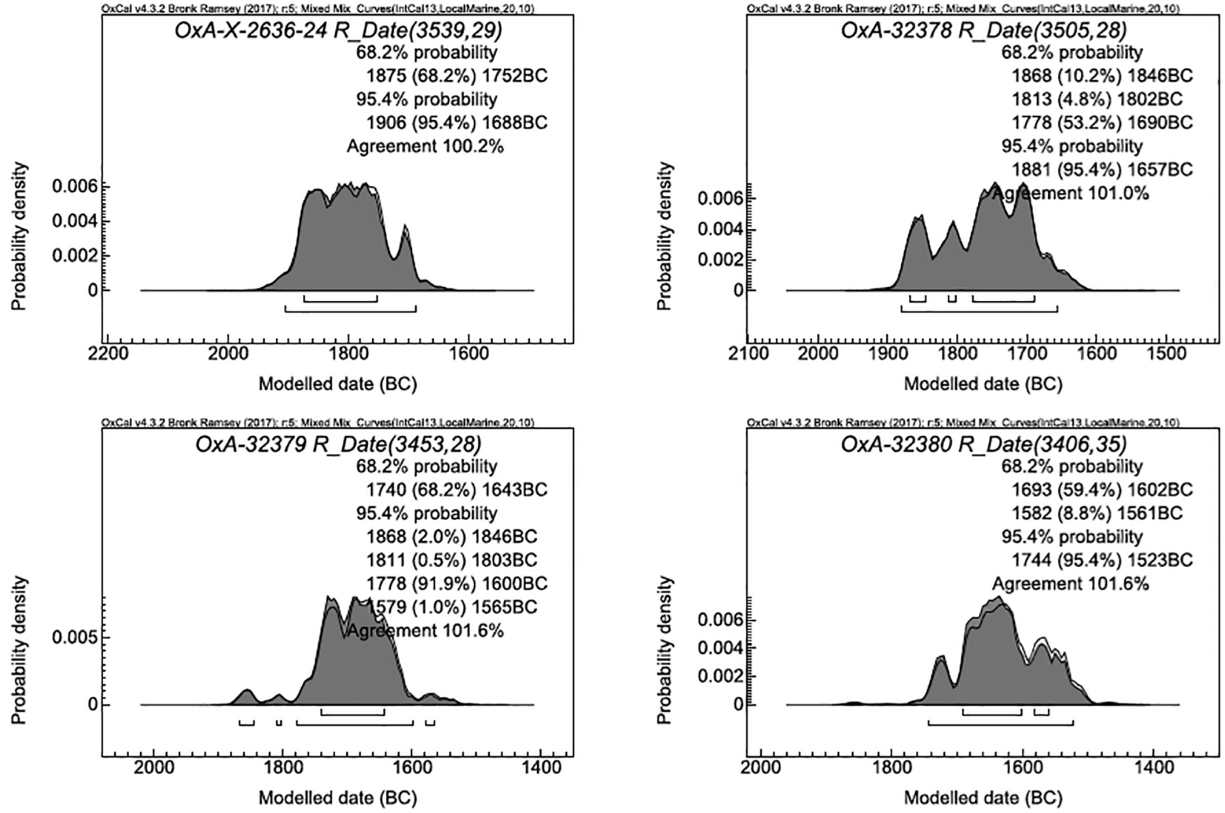

Figure S1. Calibrated results from the four AMS dates obtained from Man Bac. See text for details.

Table S4. Calibrated age ranges for samples in Table S2, corrected using the data outlined in the text.

	Years B.C. 68% CI Range		Years B.C. 95% CI Range	
Name	From	To	From	To
R_Date OxA-X-2636-24	–1875	–1752	–1906	–1688
R_Date OxA-32378	–1868	–1690	–1881	–1657
R_Date OxA-32379	–1740	–1643	–1868	–1565
R_Date OxA-32380	–1693	–1561	–1744	–1523

measured as part of the radiocarbon dating procedure (see Table S2).

There is a dearth of stable isotope evidence from humans from this area in the prehistoric period which makes it difficult to obtain reliable estimates of reservoir uptake in humans of the period. Similarly, in terms of modern collected material of known age for calculation of the reservoir age, there is little reported by Reimer and Reimer (2001) for the Vietnam region. On Hon Tre Island, Bolton et al. (2016) report a measurement on a 1950 *Porites* sample, citing a reservoir age of 261 ± 22 B.P., with a ΔR value of −9 ± 20 years.

Nam et al. (2011) have published fish carbon and nitrogen isotope results from modern catches to consider trophic level effects. This is important data in terms of understanding the baseline for fish, shellfish and marine mammal isotopic values. Interestingly, the values are very similar to those we obtained from the humans from Man Bac, raising the possibility that the people there might have been consuming quite significant quantities of fish or that the fish have values that are not dissimilar to values for foods of terrestrial origin. The lack of a wider trophic level study of the food web and a baseline for marine and freshwater resources raises challenges to reliably correcting for the reservoir effect. We have therefore estimated a rather conservative 20% marine protein uptake for Man Bac with an uncertainty of 10% and used the ΔR value of −9 ± 20 years with a mixed marine curve approach in OxCal. We caution that the true calendar ages of the Man Bac samples may need to be revisited as more data become available that enables us to estimate more precisely the degree of marine food in the diet. For the time being, this is our best estimate of the calendar age. The results of the analysis are shown in Figure S1 and also in Table S4.

References

Bolton, Annette, Nathalie F. Goodkin, Ellen R. M. Druffel, Sheila Griffin, and Sujata A. Murty. 2016. Upwelling of pacific intermediate water in the South China Sea revealed by coral radiocarbon record. *Radiocarbon* 58(1):37–53.

Brickley, Megan, and Rachel Ives. 2010. *The Bioarchaeology of Metabolic Bone Disease*. Academic Press, Oxford, UK.

Brock, Fiona, Thomas Higham, Peter Ditchfield, and Christopher Bronk Ramsey. 2010a. Current pretreatment methods for AMS radiocarbon dating at the Oxford Radiocarbon Accelerator Unit (ORAU). *Radiocarbon* 52(1):103–112.

Brock, Fiona, Thomas Higham, and Christopher Bronk Ramsey. 2010b. Pre-screening techniques for identification of samples suitable for radiocarbon dating of poorly preserved bones. *Journal of Archaeological Science* 37(4):855–865.

Brown, Thomas A., D. Earle Nelson, John S. Vogel, and John R. Southon. 1988. Improved collagen extraction by modified longin method. *Radiocarbon* 30(2):171–177.

Frangos, Constantinos C., Giagkos M. Lavranos, and Christos C. Frangos. 2011. Higoumenakis' sign in the diagnosis of congenital syphilis in anthropological specimens. *Medical Hypotheses* 77(1):128–131.

Hackett, Cecil John. 1951. *Bone Lesions of Yaws in Uganda*. Blackwell Scientific Publications, Oxford, UK.

Hackett, Cecil John. 1975. An introduction to diagnostic criteria of syphilis, treponarid and yaws (Treponematoses) in dry bones, and some implications. *Virchows Archive A* 368(3):229–241.

Hackett, Cecil John. 1976. *Diagnostic Criteria of Syphilis, Yaws and Treponarid (Treponematoses) and of Some Other Diseases in Dry Bones*. Springer, Berlin.

Harper, Kristin N., Molly K. Zuckerman, Megan L. Harper, John D. Kingston, and George J. Armelagos. 2011. The origin and antiquity of syphilis revisited: An appraisal of old world pre-Columbian evidence for treponemal infection. *American Journal of Physical Anthropology* 146(S53):99–133. DOI: 10.1002/ajpa.21613.

Lewis, Mary E. 2017. *Paleopathology of Children: Identification of Pathological Conditions in the Human Skeletal Remains of Non-Adults*. Academic Press, London.

Nam, Nguyen Thanh, Todd William Miller, Nguyen Xuan Huan, Vu Trung Tang, and Koji Omori. 2011. Integrating community structure and stable isotope analysis to assess a heavily exploited coastal marine ecosystem off central Vietnam. *Fisheries Research* 110(2):268–276.

Ortner, Donald J. 2003. *Identification of Pathological Conditions in Human Skeletal Remains*. 2nd ed. Academic Press, San Diego, USA.

Oxenham, Marc F., Hirofumi Matsumura, and Nguyen Kim Dung. 2011. *Man Bac: The Excavation of a Neolithic Site in Northern Vietnam. The Biology, Terra Australis 33*. ANU ePress, Canberra, Australia.

Ramsey, Christopher Bronk. 2001. Development of the radiocarbon calibration program. *Radiocarbon* 43(2A):355–363.

Rasool, M. N., and S. Govender. 1989. The skeletal manifestations of congenital syphilis. A review of 197 Cases. *The Journal of Bone and Joint Surgery. British Volume* 71(5):752–755.

Reimer, Paula J., and Ron W. Reimer. 2001. A marine reservoir correction database and on-line interface. *Radiocarbon* 43(2A):461–463.

Reimer, Paula J., Edouard Bard, Alex Bayliss, J. Warren Beck, Paul G. Blackwell, Christopher Bronk Ramsey, Caitlin E. Buck, Hai Cheng, R. Lawrence Edwards and Michael Friedrich. 2013. IntCal13 and Marine13 radiocarbon age calibration curves 0–50,000 years cal BP. *Radiocarbon* 55(4):1869–1887.

Snoddy, Anne Marie E., Hallie R. Buckley, Gail E. Elliott, Vivien G. Standen, Bernardo T. Arriaza, and Siân E. Halcrow. 2018. Macroscopic features of scurvy in human skeletal remains: A literature synthesis and diagnostic guide. *American Journal of Physical Anthropology* 167(4):876–895. DOI: 10.1002/ajpa.23699.

Stuiver, Minze, and Henry A. Polach. 1977. Discussion reporting of ^{14}C data. *Radiocarbon* 19(3):355–363.