Supplementary Material

Density separation of petrous bone powders for optimised ancient DNA yields

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Supplementary Note 1
Archaeological site descriptions

Prague 5 – Jinonice district, Holman's Garden Centre site
Prague, Central Bohemia, Czech Republic
This flat La Tène culture cemetery was discovered in the area of the former Holman's Garden Centre in Prague. The site was uncovered by archaeological rescue excavations associated with the construction of the Prague metro in the 1980s (1984-1986; City of Prague Museum). In addition to the La Tène graves, this polycultural site also yielded graves from the Neolithic, the Eneolithic and Early Bronze Age (Únětice culture).

A total of about 55 La Tène graves containing the skeletal remains of 65 individuals were discovered. The preservation of the skeletal remains was generally poor, although the site was not excavated completely. Thirty-seven individuals were included in the analyses.
Only the anthropological analyses of the excavated skeletal remains have been completely published thus far (Velemínský and Dobisíková 1998). The complex archaeological and scientific evaluation will be summarised in a monograph that is currently in preparation (Sankot 2022). The site has been dated to the 4th-3rd centuries BC on the basis of archaeological artefacts, more precisely to the LT B1b to LT C1b phases. Usually, inhumation burials are in a supine position, most often with the head towards the NNE, N or NE. A genetic profile has been carried out on some individuals from the burial site, including grave 18 (Patterson et al. 2022).

- *Grave 18*. Skeleton: inhumation burial, supine, head towards the north–east. Sex: anthropology – M, DNA – M. Age: adult, 30-50. Grave goods: iron fibula. Archaeological dating: La Tène culture. Radiocarbon dating: not available. NM Prague, Inv. No.: P7A 16113. (Sankot 2022; Velemínský and Dobisíková 1998; Patterson et al. 2022, Master_ID I16269).
- *Grave 27*. Skeleton: inhumation burial, supine, left hand on the pelvis, head towards the north–east. Sex: anthropology – ? (M?), DNA – M. Age: adult, 30–50. Grave goods: iron arm arm-ring, bronze bracelet. Archaeological dating: La Tène culture. Radiocarbon dating: not available. NM Prague, Inv. No.: P7A 16119. (Sankot 2022; Velemínský and Dobisíková 1998).
- *Grave 35*. Skeleton: inhumation burial in a slightly crouched position on the supine, left hand points, going to the pelvis, head towards the north–west. Sex: anthropology – ?, DNA – M. Age: adolescent, 12–18. Grave goods: iron bracelet fragments. Archaeological dating: La Tène culture. Radiocarbon dating: not available. NM Prague, Inv. No.: P7A 16122. (Sankot 2022; Velemínský and Dobisíková 1998).

Prague 5 – Malá Ohrada
Stodůlky area, Central Bohemia, Czech Republic
Rescue excavation due to the construction of the "Lužiny" prefab housing estate (J. Kovářík) in 1979–1980. On an area of ca 1.5 ha more than 500 settlement features (Funnel Beaker culture, Late and Final Bronze Age, Hallstatt period, Roman Iron Age) and burials (Jordanów culture – one grave, Petriščáková et al. 2016; Corded Ware culture – four graves, Buchvaldek and Kovářík 1993; Bell Beaker culture – one grave, Olalde et al. 2018; Únětice culture; Early Middle Ages) were excavated. One Corded Ware grave (Nr. 10) and 18 burials from 17 Únětice culture graves were analysed for aDNA, including grave 25 (Patterson et al 2022). The Únětice culture finds have not yet been published in detail and are only mentioned elsewhere (Smejtek 2005, 451); a monograph is being prepared (K. Petriščáková).

- *Grave 25*. Skeleton: unknown position (documentation missing). Sex: anthropology - ? (M?), DNA - M. Age: adult, 30-50. Grave goods: without finds. Archaeological dating: Únětice culture. Radiocarbon dating: not available. (Petriščáková 2011, 68-69, fig. 16; Patterson et al. 2022, Master_ID 16110). NM Prague, Inv. No.: P7A 38770. (Petriščáková 2011, 68-69, fig. 16; Patterson et al. 2022, Master_ID I16110).

Necropolis of Castel Sozzio
Municipality of Civitella D'Agliano, Viterbo, Italy

The Necropolis of Castel Sozzio is located in the Middle Tiber Valley, approximately 80 km in a straight line north of Rome, on the slopes of the hills looking at the river on its western side. This region was very important, at least since the Roman period, when it was exploited for agricultural purposes. In the Late Roman and Early Medieval periods, the situation changed and this area became a strategic border in the struggles between the Byzantines and the Goths, and, then, the Lombards.

The excavation of the necropolis was resumed in 2020 under the direction of Emanuela Borgia (Dipartimento di Scienze dell'Antichità, Sapienza Università di Roma) with an official Excavation Concession by the Soprintendenza Archeologia, Belle Arti e Paesaggio per l'Area Metropolitana di Roma, la Provincia di Viterbo e l'Etruria Meridionale, now Soprintendenza Archeologia, Belle Arti e Paesaggio per la Provincia di Viterbo e l'Etruria Meridionale (Borgia 2021).

The necropolis was connected to a rural settlement that grew up upon a Roman *villa* or *vicus*. This settlement was probably located to the east of the funerary area, but its remains are still to be investigated. At the present time, a total of 56 tombs were investigated. The burials are carved in lines in the local travertine bedrock or in the clay deposits, and pertain to different typologies: chest tombs made with, and covered by stone slabs, *cappuccina* graves, and pit graves. The tombs are mainly oriented west-east, with the head of the deceased looking east; only a few of them are oriented north-south and in this case, the deceased usually looks south. We can recognize at least four overlapping phases of tombs, pertaining to different periods of use of the funerary area. A large part of the graves was reused various times (as multiple burials reveal), often respecting the previous burials, whose bones could be moved to a sort of "ossuary-pit" near the tomb or reorganised within the tomb itself. The reuse of tombs was a highly complex procedure that forced the (maybe different) communities using the cemetery into negotiating and formalising, or even ritualizing, the way in which bodies were acted on.

All this information proves the protracted use of the necropolis in terms of time, even if the chronology of the single tombs and of their different phases of use is difficult to establish, due the almost total lack of grave goods. A preliminary interpretation based on stratigraphic data, on the few elements of furniture, and on the large reuse of *spolia* from a Roman villa in the tombs suggests a date between the late 5th-early 6th and the 7th century AD.
The tombs from which the analysed samples were taken are chiefly chest graves (tomb 7, tomb 15, tomb 16, tomb 19), whereas tomb 27 and tomb 32 are pit graves. They contained both single and multiple burials.

- *Tomb 7*, oriented west-east, contained two superimposed depositions, the uppermost of which (individual n. 1, US 175) is analysed in this work.
- *Tomb 15* is oriented west-east, and appears to be as one of the most recent in the necropolis due to stratigraphic reasons. It revealed a very complex burial situation, as it contained one primary deposition (US 210) and 8 skulls well-orderly arranged around it (US 207); one of the latter is analysed in this work (skull 5).
- *Tomb 16* is oriented north-south, and contained a single individual (US 208). It is as recent as tomb 15.

- *Tomb 13/19A* is oriented west-east, and encompassed two burials, one of which in primary deposition that is analysed here (US 162).
- *Tomb 27 and Tomb 32* are pit graves (the former deposition is numbered US 193). The latter is the most recent among those analysed here, because it was carved over an earlier chest tomb. It encompassed one single individual (US 321).

Supplementary Note 2
Supplementary References

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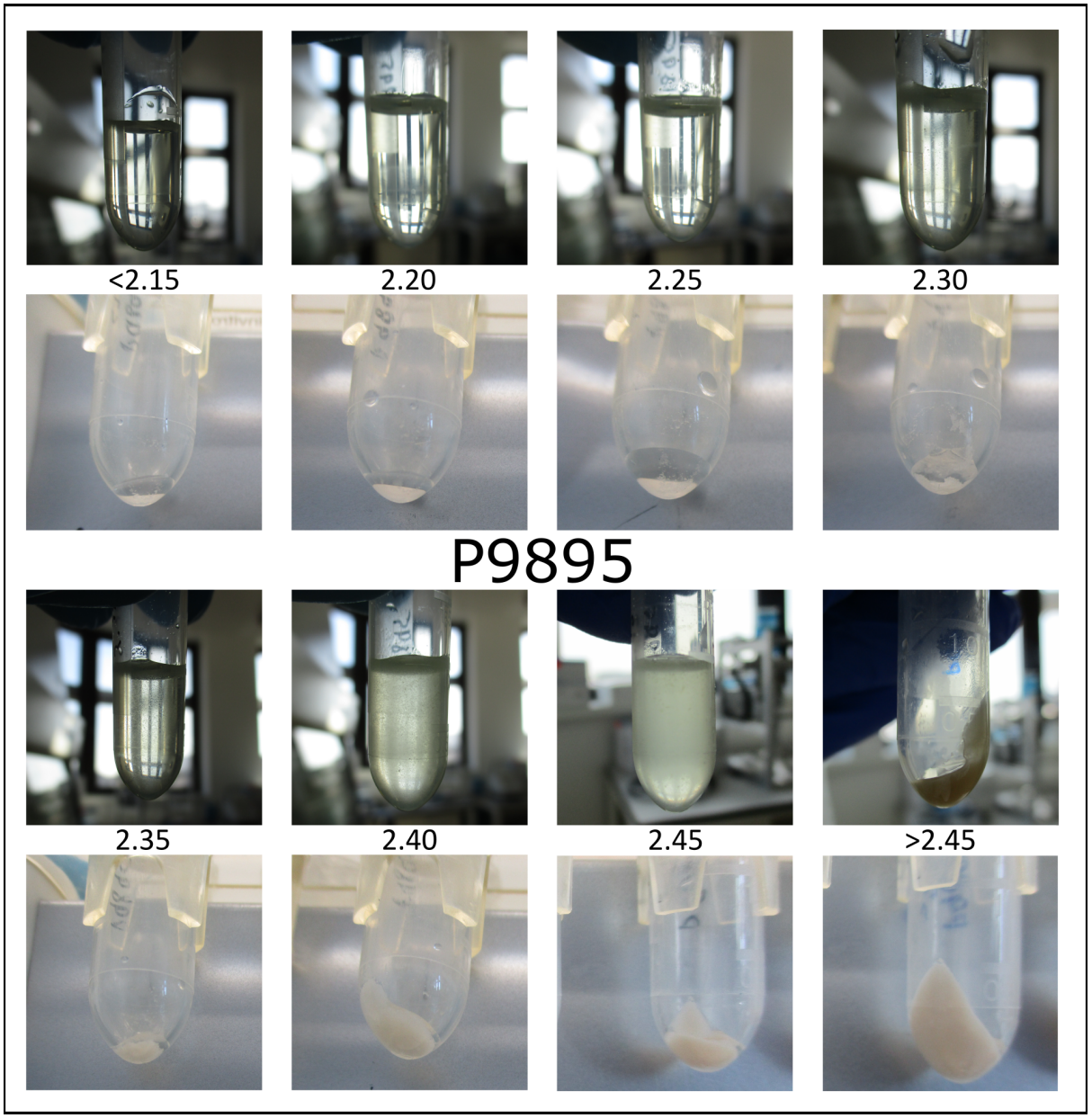
Supplementary Figure S1

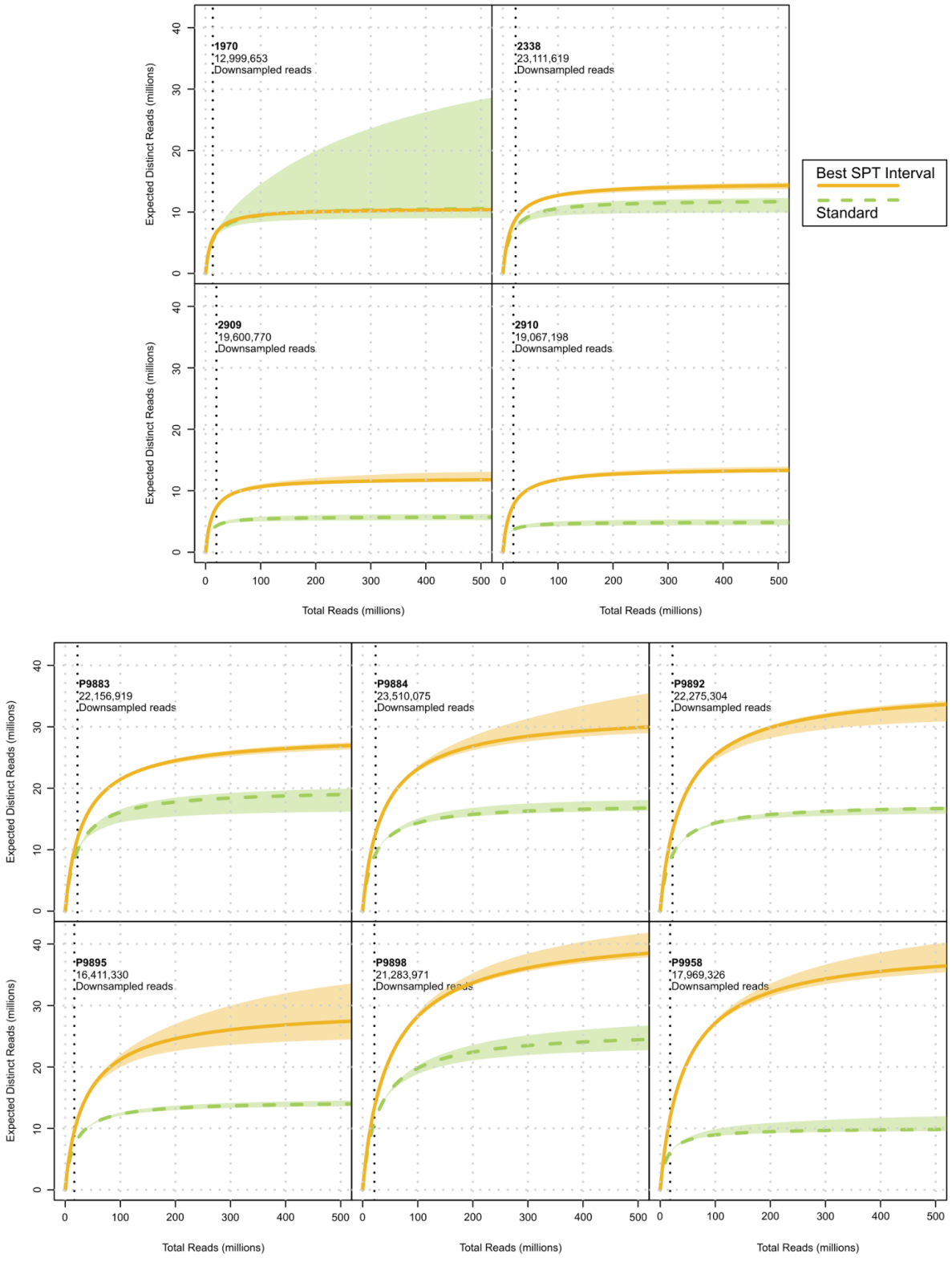


Supplementary Figure S1. Bone powder of P9895 suspended in SPT solution (top rows) and after re-pelleting and TE washes (bottom rows), for each separated interval, demonstrating increasing suspension turbidity and pellet sizes.

Supplementary Figure S2

Supplementary Figure S2: Bone powder powder of all samples after re-pelleting and TE washes, for each separated interval, demonstrating increasing pellet sizes.

Supplementary Figure S3



Supplementary Figure S3. Library complexity curves, based on the yield of expected distinct reads for a theoretically larger sequencing effort, using the *lc_extrap* function of the software *preseq*. Vertical dotted lines represent the number of reads each sample's best SPT interval and standard extraction were randomly downsampled to. Shaded areas represent 95% confidence intervals. Results shown here for up to 500 million total reads, and in **Figure 5** for up to 100 million.