Correspondence

Ancient DNA reveals twenty million years of aquatic life in beavers

Georgios Xenikoudakis[1,*],
Mayeesha Ahmed[2],
Jacob Colt Harris[2], Rachel Wadleigh[2,3],
Johanna L.A. Paijmans[1],
Stefanie Hartmann[1], Axel Barlow[1],
Heather Lerner[2,4,*],
and Michael Hofreiter[1]

With approximately 30 recognised extinct genera, beavers were once a taxon-rich rodent group adapted to both terrestrial and aquatic habitats [1,2]. Today, only two morphologically similar species survive, the Eurasian and the North American beaver [3]. Both are known for their aquatic lifestyle and their woodcutting and engineering behaviour, which allows them to alter the environment and affect sympatric species [3]. Palaeontological studies suggest that aquatic and woodcutting behaviours are derived traits shared only between the extinct group of giant beavers and the extant beaver lineage [1,3]. Here we use 7,686 base pairs of mitochondrial DNA from the extinct giant beaver *Castoroides ohioensis* to investigate when these behaviours originated in beavers. Our phylogenetic analysis retrieves the anticipated sister relationship of giant beavers to the extant beavers and places the time to their common ancestor during the early Miocene, approximately 20 million years ago (mya). Our results are congruent with inferences from the fossil record [1] in suggesting a single evolutionary transition from terrestrial to aquatic life, although they place this event approximately four million years later compared to previous fossil studies [1].

The giant beaver went extinct during the megafaunal extinction at the onset of the Holocene around 12,000 years ago [3] and was one of the largest rodents that has ever lived [4]. We combined DNA hybridisation capture and iterative mapping methods to retrieve around half of a giant-beaver mitochondrial genome (7,686 bp; see the Supplemental Information,

Figure S1). The retrieved sequences indicate advanced DNA fragmentation and nucleotide misincorporations typical for ancient DNA, supporting their authenticity (Figure S2A,C). A pairwise comparison with potential sources of contamination suggested negligible incorporation of exogenous sequences during the giant-beaver mitochondrial assembly (Figure S2B). We then compared the assembled giant-beaver partial mitochondrial genome against a dataset of rodent, lagomorph (hares and rabbits) and primate mitochondrial genomes to determine their phylogenetic affinities. Both maximum likelihood and Bayesian phylogenetic methods returned congruent results (Figures 1 and S2D), strongly supporting a sister-group relationship of the giant beaver to the extant-beaver clade, in agreement with evidence from the fossil record [1], but in contrast with recent palaeoproteomic studies that suggested a closer phylogenetic relationship to *Ictidomys tridecemlineatus* (thirteen-lined ground squirrel) [5]. Using molecular dating, the mean time of divergence between giant and extant beavers is estimated to be 19.7 mya (95% Credibility Interval: 17.4–22.2 mya; Figure 1). Given that giant and extant beavers share an aquatic lifestyle [1], the most parsimonious scenario is that this derived trait evolved in their common ancestor. The estimated age of 19.7 mya thus represents a minimum age for the evolution of aquatic life in beavers.

Extant beavers possess unique adaptations to aquatic life. Examples are the enlarged hind feet, a paddle-like tail used for propulsion in water [1,6], and a 'combing-claw' used to maintain a non-wettable fur while swimming [6]. A primitive form of this specialised claw has already been identified in early Miocene (23–16 mya) fossils of *Steneofiber* [6], an extinct beaver genus distributed across Eurasia [7]. The earliest fossils assigned to *Steneofiber* are considered as progenitors of the common lineage of extant and extinct giant beavers during the Tertiary beaver radiation in Europe [7]. The age of *Steneofiber* fossils overlaps with our molecular estimate for the common ancestor of giant and modern beavers (Figure 1). This implies that the evolution of aquatic traits likely started with *Steneofiber* or its ancestor in Eurasia during the Early Miocene, prior

to the diversification of modern and giant beavers around 20 mya, further supporting a single evolutionary change towards an aquatic lifestyle. This date coincides with the disappearance of terrestrial beavers — known only from North America — from the fossil record, as well as with the appearance of aquatic giant beavers in this region [2]. Around the Oligocene–Miocene boundary ~23 mya ago, the first of a series of glaciations that lasted during the early Miocene (23–16 mya) took place [8]. It is possible that these climatic fluctuations played a role in species turnover in aquatic and terrestrial beavers, but further study is needed to investigate this hypothesis.

Modern beavers perform woodcutting for both food and building material. Studies on isotopes [9] showed that *Castoroides* ate soft aquatic plants, rather than wood. These findings do not directly reject woodcutting for building purposes, although dam and lodge building (engineering) remains an open question due to a lack of fossil evidence. The general similarity of extant beavers reasonably suggests woodcutting behaviour occurred in their common ancestor. In this case, our estimate of their divergence at ~7 mya (95% Credibility Interval: 6.7–8.7 mya; Figure 1) serves as a minimum age for the evolution of woodcutting. The earliest evidence for woodcutting based on cut marks is assigned to the extinct beaver genus *Dipoides* and date to the Pliocene (4–5 mya) [10]. Evidence for extant beavers is even more recent, dating to the Pleistocene [3]. Assuming that *Dipoides* belongs to the giant beaver lineage [1,3], the most parsimonious explanation would be that woodcutting emerged prior to the divergence of the extinct giant and modern beavers during the early Miocene (Figure 1). This would suggest that fossil evidence underestimates the age of this behaviour. Alternatively, if *Dipoides* shares a more recent common ancestor with extant beavers, then woodcutting behaviour would date some time prior to the divergence of modern beavers 7 mya (Figure 1). A molecular phylogenetic study on *Dipoides* subfossils could shed light on these alternative scenarios.

This study provides the first molecular evidence for a single evolutionary change in the behavioural lifestyle of beavers from terrestrial to aquatic
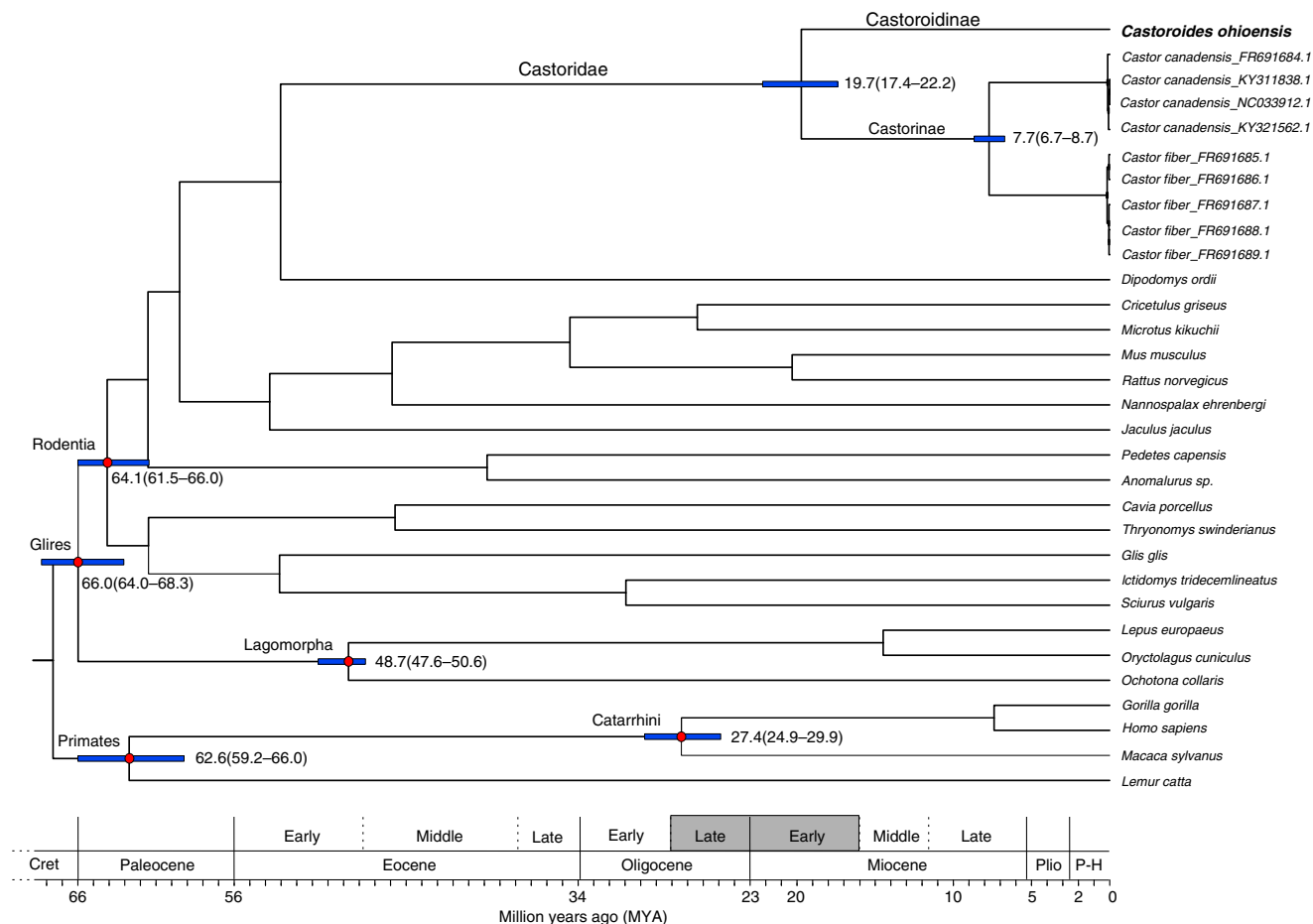
Figure 1. Maximum clade credibility tree.
All nodes returned high posterior probabilities (>0.98). Mean estimates for divergences of Castorinae-Castoroidinae and the European Beaver (*Castor fiber*)–North American beaver (*Castor canadensis*) clade, respectively, are presented in million years ago (mya) next to the corresponding node with node bars depicting the 95% highest posterior density (95% Credibility Interval, shown in parentheses) of the mean estimated age. Nodes marked with a red dot signify constraints based on dated fossils during Bayesian analysis. Scale shown is in million years along with the geological epochs (Cret, Cretaceous; Plio, Pliocene; P-H, Pleistocene-Holocene). The grey bar on the timescale represents the approximate stratigraphic range of *Steneofiber* fossils (adapted from [2]; see also [7] for an overview on beaver taxonomy). The time to the most recent common ancestor of extant and giant beavers is estimated to the early Miocene.

habitats. This change coincided closely with the extinction of terrestrial beavers in North America, suggesting that the two events may share a common, albeit as yet unknown, cause.

SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures, experimental procedures, author contributions and supplemental references and can be found with this article online at https://doi.org/10.1016/j.cub.2019.12.041.

REFERENCES

1. Rybczynski, N.J. (2007). Castorid phylogenetics: Implications for the evolution of swimming and tree-exploitation in beavers. J. Mamm. Evol. *14*, 1–35.
2. Hugueney, M., and Escuillié, F. (1996). Fossil evidence for the origin of behavioral strategies in early Miocene Castoridae, and their role in the evolution of the family. Paleobiology *22*, 507–513.
3. Rybczynski, N.J. (2008). Woodcutting behavior in beavers (Castoridae, Rodentia): estimating ecological performance in a modern and a fossil taxon. Paleobiology, *34*, 389–402.
4. Reynolds, P.S. (2002). How big is a giant? The importance of method in estimating body size of extinct mammals. J. Mamm. Evol. *83*, 321–332.
5. Cleland, T.P., Schroeter, E.R., Feranec, R.S., and Vashishth, D. (2016). Peptide sequences from the first *Castoroides ohioensis* skull and the utility of old museum collections for palaeoproteomics. Proc. R. Soc. B. *283*, 1832.
6. Hugueney, M., and Escuillie, F. (1994). K-strategy and adaptive specialization in *Steneofiber* from Montaigu-le-Blin (dept. Allier, France; Lower Miocene, MN 2a, +23 Ma): first evidence of fossil life-history strategies in castorid rodents. Palaeogeogr. Palaeocl. *113*, 217–225.
7. Stefen, C. (2011). A brief overview on the evolution of european tertiary beavers. Balt. For. *17*, 148–153.
8. Billups, K., Channell, J.E.T., and Zachos, J. (2002). Late Oligocene to early Miocene geochronology and paleoceanography from the subantarctic South Atlantic. Paleoceanography *17*, 1004.
9. Plint, T., Longstaffe, F.J., and Zazula, G. (2019). Giant beaver palaeoecology inferred from stable isotopes. Sci. Rep. *9*, 71–79.
10. Tedford R.H., and Harington C.R. (2003). An Arctic mammal fauna from the Early Pliocene of North America. Nature *425*, 388–390.

¹Institute for Biochemistry and Biology, University of Potsdam, 14476 Potsdam, Germany. ²Joseph Moore Museum, Earlham College, Richmond, IN 47374, USA. ³University of Michigan, Ann Arbor, MI 48109, USA. ⁴Lead Contact.
*E-mail: geoxenik@gmail.com (G.X.), hlerner@gmail.com (H.L.)