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Phylogeny

Ancient mitochondrial DNA reveals convergent evolution of giant short-faced bears (Tremarctinae) in North and South America

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The Tremarctinae are a subfamily of bears endemic to the New World, including two of the largest terrestrial mammalian carnivores that have ever lived: the giant, short-faced bears *Arctodus simus* from North America and *Arctotherium angustidens* from South America (greater than or equal to 1000 kg). *Arctotherium angustidens* became extinct during the Early Pleistocene, whereas *Arctodus simus* went extinct at the very end of the Pleistocene. The only living tremarctine is the spectacled bear (*Tremarctos ornatus*), a largely herbivorous bear that is today only found in South America. The relationships among the spectacled bears (*Tremarctos*), South American short-faced bears (*Arctotherium*) and North American short-faced bears (*Arctodus*) remain uncertain. In this study, we sequenced a mitochondrial genome from an *Arctotherium* femur preserved in a Chilean cave. Our molecular phylogenetic analyses revealed that the South American short-faced bears were more closely related to the extant South American spectacled bear than to the North American short-faced bears. This result suggests striking convergent evolution of giant forms in the two groups of short-faced bears (*Arctodus* and *Arctotherium*), potentially as an adaptation to dominate competition for megafaunal carcasses.

1. Introduction

The spectacled bear (*Tremarctos ornatus*) is the only living member of Tremarctinae, a previously diverse group of bears endemic to the Americas. The now-extinct Pleistocene diversity of Tremarctinae comprised the Florida spectacled bear (*Tremarctos floridanus*), South American short-faced bears (*Arctotherium*—five species; [1]) and North American short-faced bears (*Arctodus*—two species; [2]). These species ranged in size from the relatively small *Arctotherium wingei* (approx. 150 kg; [3]) to the giant short-faced bears *Arctodus simus* and *Arctotherium angustidens*, which may have attained body masses exceeding 1000 kg [4,5]. In addition, tremarctine bears displayed a diversity of foraging strategies, ranging from carnivorous/omnivorous (e.g. *Arctodus simus*, *Arctotherium angustidens*) to largely herbivorous (e.g. *Arctotherium wingei*, *T. ornatus*) [6–9]. The evolution and biogeography of this diverse group of bears is enigmatic, and currently lacks a robust phylogenetic framework.

Putative representatives of both *Arctodus* and *Tremarctos* are known from the Late Pliocene fossil record of North America [2,10,11], and genetic data suggest these two genera diverged during the Late Miocene or Early Pliocene [12]. In contrast, the earliest representative of *Arctotherium* appears in the Early Pleistocene fossil record of South America [1,4,10]. Concordantly, it is generally accepted that *Arctotherium* arose as a distinct clade following dispersal of North American ancestors to South America after the Panamanian Isthmus formed approximately 3 million years ago (Ma) [4,13–15], an event that precipitated a period of extensive faunal interchange between North and South America known as the great American biotic interchange (GABI) [16–18]. Conversely, *Tremarctos* does not appear in the South American fossil record until the Holocene, suggesting that the extant spectacled bear descends from an independent, later dispersal event.

Previous palaeontological studies have considered the short-faced bears (*Arctotherium* and *Arctodus*) as forming a monophyletic clade [19–21]. The proportionally larger and wider molars of both *Arctodus* and *Arctotherium* set them apart from other bears [19–21], and the cranium and mandible morphology of *Arctodus simus* and *Arctotherium angustidens* are strikingly similar [6]. However, the similarities between *Arctodus* and *Arctotherium* could alternatively result from convergent evolution driven by adaptation to the same ecological niche and/or feeding behaviour, as dental and mandible characters are frequently correlated with diet [8]. Similarly, the huge size of *Arctodus simus* and *Arctotherium angustidens* may have been convergent, and linked to their ecology by allowing predation on large herbivores and competition for carcasses. Consequently, resolving the phylogenetic relationships among these taxa would have important implications for our understanding of body size and foraging strategy evolution in tremarctines.

We sequenced a near-complete mitochondrial genome from a specimen of *Arctotherium* and compared these new data with previously published sequences from the extant spectacled bear *T. ornatus*, the North American giant short-faced bear *Arctodus simus*, and a number of additional outgroups.

2. Methods

Analyses were performed on an *Arctotherium* femur collected from Cueva del Puma, Chile (complete right femur, no. 32104, Centro de Estudios del Hombre Austral, Instituto de la Patagonia, Universidad de Magallanes). DNA extraction and genomic library preparation were performed in the ancient DNA laboratory at the Australian Centre for Ancient DNA, University of Adelaide. The *Arctotherium* genomic library was enriched for mitochondrial DNA, and sequenced on an Illumina MiSeq; the resulting reads were assembled into a mitochondrial genome. We aligned the *Arctotherium* mitochondrial genome sequence to 13 previously published carnivoran sequences, used PartitionFinder v. 1.1.1 [22] to determine optimal partitioning and substitution models, and performed phylogenetic analyses with RAxML v. 8.2.0 [23] and BEAST v. 1.8.0 [24]. See the electronic supplementary material for detailed methods.

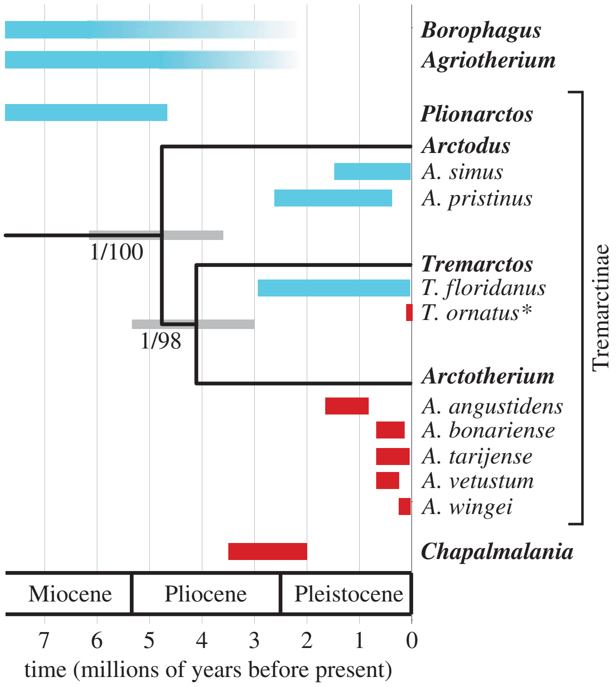

Figure 1. Relationships among tremarctine genera resulting from phylogenetic analysis of our mitochondrial genome dataset. Nodes reflect mean age estimates, whereas grey bars reflect 95% highest posterior densities (HPDs). Branch support values (BEAST posterior probability/RAxML bootstrap %) are given for each clade. The approximate temporal range of taxa of interest (see main text) is plotted based on the fossil record, and coloured according to distribution (North America, blue; South America, red). The extant taxon is marked with an asterisk.

3. Results

Our analyses recovered relationships among outgroup taxa that were consistent with previous genetic studies ([12,25], but see [26]). Within Tremarctinae, we recovered strong support for a clade comprising *Tremarctos ornatus* and *Arctotherium* to the exclusion of *Arctodus simus* (Bayesian posterior probability = 1.0, maximum-likelihood bootstrap percentage = 98%; figure 1). Our mean estimate for the time of divergence between *Tremarctos* and *Arctotherium* was 4.1 Ma (95% highest posterior density, HPD = 3.0–5.3 Ma), and 4.8 Ma for the most recent common ancestor of *Tremarctos*, *Arctotherium* and *Arctodus* (95% HPD = 3.6–6.2 Ma). In general, node age estimates from our molecular dating analyses were slightly younger than those of a previous study of ursid mitochondrial DNA [12], although our 95% highest posterior densities (HPDs) overlapped with theirs substantially for equivalent nodes. These differences likely arise from our less restrictive calibration on the root of the tree, which allowed for the possibility that the Eocene *Paristis* is not a true member of the bear lineage. Conversely, a study of nuclear DNA obtained dates for the radiation of Ursinae that were much younger than ours [26], likely as a result of our conservative constraint on the crown-age of Ursidae, which permitted this node to substantially predate its first unequivocal fossil representative (electronic supplementary material).

4. Discussion

Our results suggest that the North and South American short-faced bears (*Arctodus* and *Arctotherium*, respectively) do not form a monophyletic clade (figure 1), contrary to the suggestions of previous palaeontological studies [19]. In addition, our molecular dating analyses indicate that *Arctotherium*, *Arctodus* and *Tremarctos* all diverged from one another during the Late Miocene or Pliocene. This inferred timeframe suggests that the Miocene/Pliocene genus *Plionarctos* is ancestral to the Quaternary tremarctine genera. Our observations are consistent with the idea that giant representatives of *Arctodus* and *Arctotherium* evolved independently in both North and South America during the Pleistocene [4], as all known *Plionarctos* specimens are relatively small-bodied (as was the earliest occurring species of *Arctodus*, *Arctodus pristinus*). The largest tremarctine bears (*Arctodus simus* and *Arctotherium angustidens*) appear to have been among the most inclined towards carnivory, although plant matter would likely still have made up a substantial proportion of their diets [6–8]. Consequently, the convergent morphological evolution of giant short-faced bears may have occurred as an adaptation for securing and scavenging large carcasses, as none of the tremarctine bears was specialized for active predation [27,28].

Many carnivoran species inhabited North America during the Pliocene and Pleistocene, including wolves (*Canis lupus*/*Canis dirus*), lions (*Panthera leo*) and the sabre-toothed cats (e.g. *Smilodon*, *Xenosmilus*, *Homotherium*). During the Pliocene, carcasses resulting from carnivore kills were probably scavenged by the 'bone-crushing dogs' (*Borophagus* spp.; [29]) or the bear *Agriotherium* [27], because the only known North American hyaenid (*Chasmaporthetes ossifragus*) does not appear to have been as well equipped for bone-crushing as extant *Hyena* and *Crocuta* species [30]. However, both *Agriotherium* and *Borophagus* appear to have become extinct by the end of the Pliocene, which may have vacated an ecological niche that *Arctodus* subsequently exploited. This explanation is compatible with the observation that the giant *Arctodus simus* is only known from the Pleistocene, whereas the temporal range of the smaller-bodied *Arctodus pristinus* overlaps with *Borophagus* and *Agriotherium*. It is also possible that changes in the large herbivore community during the Pleistocene—for example, the immigration of bison into North America—may have played a central role in the evolution of *Arctodus simus*.

The oldest known *Arctotherium* specimens are giant-sized [3,8,10], suggesting either that their size evolution occurred very rapidly or that fossils from the early stages of *Arctotherium* evolution have not yet been recorded. Our molecular dating estimates are compatible with both possibilities. A substantial proportion of the 95% HPD for our estimated time of divergence between *Arctotherium* and *Tremarctos* is distributed in the Early Pliocene, suggesting that *Arctotherium* may have existed in North and/or Central America for several million years without being detected in the fossil record (but see [31]) or perhaps even island-hopped to mainland South America prior to formation of the Isthmus of Panama approximately 3 Ma. However, our date estimates also allow for a Late Pliocene origin of *Arctotherium*, approximately coincident with the establishment of direct land connection between North and South America. This latter hypothesis is more consistent with the fossil record, as the earliest unequivocal records of *Arctotherium* are from the Early Pleistocene of South America (Ensenadan age).

The first recorded *Arctotherium* specimens in South America occur alongside the earliest known South American records of several other carnivorans: the sabre-toothed cats *Smilodon* and *Homotherium*, the puma (*Puma concolor*), the jaguar (*Panthera onca*), some large 25–35 kg canids, and several smaller less than 15 kg mustelids, canids, felids and mephitids [14,15, 32,33]. This Ensenadan carnivore guild was dramatically more diverse than the communities recorded in immediately preceding layers, which included only a handful of carnivorous mammal species and none larger than approximately 30 kg [13,14,33]. Thus, species migrating southwards into South America during the GABI would initially have encountered an abundance of large mammalian herbivores and a paucity of carnivores [14,17,33]. Further, there would have been no South American mammals specialized for scavenging, because the large (more than 30 kg) procyonid *Chapalmalania*, which appears to have filled this niche [34], became extinct in the Late Pliocene/Early Pleistocene. Thus, a lack of competition for carcass scavenging during this period of faunal turnover may have driven the evolution of the giant *Arctotherium angustidens* [4].

Data accessibility. Data from this study are available on GenBank (KU886001) and the Dryad Data Repository (http://dx.doi.org/10.5061/dryad.v7f30).

Authors' contributions. A.C., J.J.A., F.P., A.P., L.S. and F.M. conceived of the study. S.C.B., K.J.M. and P.B. performed the laboratory work. S.C.B., K.J.M., L.S., B.W.S. and F.P. significantly contributed to the design of the analyses. K.J.M wrote the manuscript. All authors helped with interpretation of results and critically revised the manuscript. All authors approve the final version of this manuscript and agree to be held accountable for all aspects of the work performed.

Competing interests. The authors declare no competing interests.

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