A large carnivorous mammal from the Late Cretaceous and the North American origin of marsupials

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Marsupial mammal relatives (stem metatherians) from the Mesozoic Era (252–66 million years ago) are mostly known from isolated teeth and fragmentary jaws. Here we report on the first near-complete skull remains of a North American Late Cretaceous metatherian, the stagodontid *Didelphodon vorax*. Our phylogenetic analysis indicates that marsupials or their closest relatives evolved in North America, as part of a Late Cretaceous diversification of metatherians, and later dispersed to South America. In addition to being the largest known Mesozoic therian mammal (node-based clade of eutherians and metatherians), *Didelphodon vorax* has a high estimated bite force and other craniomandibular and dental features that suggest it is the earliest known therian to invade a durophagous predator–scavenger niche. Our results broaden the scope of the ecomorphological diversification of Mesozoic mammals to include therian lineages that, in this case, were linked to the origin and evolution of marsupials.

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marsupial mammals are today overshadowed by placentals in diversity and geographic extent. However, before the Cretaceous–Palaeogene mass extinction 66 million years ago (Myr ago), metatherians (the stem-based clade of living marsupials and their closest relatives) ranged widely across northern landmasses; they also surpassed eutherians (the stem-based clade of living placentals and their closest relatives) in both taxonomic and morphological richness. Although studies of comparative development and physiology have shed light on constraints that might have influenced this clade changeover, critical gaps in our understanding of early metatherian evolution can only be addressed with more anatomically complete fossils of these taxa. Unfortunately, skulls of early metatherians are scarce and distributed unevenly across time, space and phylogeny. Of the 69 Cretaceous species, relatively complete skulls have been described for only four Asiatic taxa, all phylogenetically distant from the origin of marsupials. The 60 species from North America (NA) are known almost exclusively from teeth and fragmentary jaws. Consequently, previous phylogenetic analyses of early metatherians have either relied on mostly dental data, or excluded most NA Late Cretaceous stem metatherians. Their results have implied that the clade of NA Late Cretaceous stem metatherians diverged early (>100 Myr ago) from the lineage leading to marsupials and the latter predominantly evolved in South America (SA) (but see ref. 12 for an opposing non-cladistic viewpoint). Until now, it has been unclear whether this scenario reflects (i) a meaningful evolutionary pattern, in which NA Late Cretaceous metatherians had only minor influence on the origin of marsupials or (ii) an artefact of incomplete fossil data and/or inadequate analysis.

The four specimens described herein, a near-complete skull (NDGS 431), a snout (UWBM 94084) and two maxillae (UWBM 94500, SCNHM VMa 20) from the Hell Creek Formation (upper Maastrichtian, 69–66 Myr ago) of Montana and North Dakota, are assigned to the stagodontid metatherian Didelphodon vorax on the basis of cheek-tooth features. This taxon has long captivated palaeontologists as the largest Cretaceous metatherian with shearing molars and bulbous premolars, leading to the hypothesis of its specialized role in Late Cretaceous food webs, perhaps as a predator or scavenger capable of crushing hard objects (for example, bone, shell). Postcranial elements attributed to D. vorax have spurred further speculation that this taxon was semiaquatic (but see ref. 14).

We incorporate new anatomical data from these four specimens of D. vorax into a phylogenetic analysis that show that marsupials or their closest relatives evolved in NA, as part of a Late Cretaceous diversification of metatherians, and soon thereafter dispersed to SA. We also show that D. vorax is the largest known Mesozoic therian mammal and has a high estimated bite force and other craniomandibular and dental traits that suggest it is the earliest known therian to evolve diphagous predator–scavenger behaviour. Taken together, these results indicate that the ecomorphological diversification of Mesozoic mammals extended to therian lineages, including those near the origin of marsupials.

Results

Description. In dorsal view (Figs 1d,h and 2c), the skull is broad and triangular with a short rostrum (~1/3 skull length), robust, wide zygomata, narrow postorbital breadth, small braincase, small sagittal crest and well-developed lambdoidal crest. Lacking a prominent postorbital process, the orbital region is confluent with the temporal region. In lateral view (Figs 1b,g and 2e), the skull has a low, flat profile, even after post-mortem deformation is accounted for. The skull length of the younger adult individual (NDGS 431) is 94.7 mm and its maximum width is 59.3 mm; the mature adult (UWBM 94084) scales to 122.1 and 77.4 mm, respectively (see also Supplementary Figs 1–10, Supplementary Tables 1–3 and Supplementary Note 1 for additional figures, measurement data and extended description of the specimens).

Phylogenetic relationships. As a result of the specimens reported herein, the skull of D. vorax is the best-known among all Cretaceous metatherians. To understand the impact of these new data, we performed a parsimony analysis of dental and craniomandibular characters (Supplementary Note 2) scored for 48 metatherians and closely related taxa. For the first time, all relevant Cretaceous and Palaeogene metatherian taxa known by relatively complete skulls were included in the same data matrix (Supplementary Data 1). We obtained 18 equally parsimonious trees with tree length = 601, consistency index = 0.364 and retention index = 0.667. Our resulting strict consensus tree (Fig. 3 and Supplementary Note 3) agrees in most aspects with previous analyses, including support for a monophyletic Stagodontidae, in which the early Campanian–early Maastrichtian Eodelphis and the Albion–Cenomanian Paradiens are successive sister taxa to Didelphodon. We fail to recover a monophyletic Australodelphia (dasyurids + Dromiciops), in contrast with the weight of previous morphological and molecular phylogenetic studies, but this might be an artefact of sparse sampling of these clades in our analysis. Placement of Herpetotherium and Mimoperadectes–Peradectes in the crown clade is novel but differs only slightly from previous topologies. A more striking result is that the main dichotomy within Marsupialiformes places NA Cretaceous stem taxa (including Didelphodon) with marsupials to the exclusion of SA Palaeogene stem taxa (for example, borhyaenids, Mayulestes and Pucadelphys) and the ‘Gurlin Tsav skull’. Bremer support for this topology is low, but alternatives are several steps longer and, in some cases, significantly different (Supplementary Note 4). Synapomorphies for this clade of Asiatatherium, NA stem marsupialiforms and marsupials are (i) double-rank prevallum/postvalidum shearing; a lower molar with (ii) talonid subequal to wider than trigonid and (iii) entoconid subequal to larger than hypoconid and/or hypoconulid; and presence of (iv) a larcral tubercle and (v) postpalatine torus (Supplementary Data 2). Homoplasy is prevalent within Marsupialiformes (low consistency index) and dental features in particular seem prone to homoplasy; for example, the ‘Gurlin Tsav skull’ + SA stem marsupialiforms and the Stagodontidae convergently evolved features, such as a well-developed upper-molar postmetacrista, presumably as adaptations for carnivory. However, the relatively high retention index implies that homoplasies often serve as synapomorphies in our analysis and contribute to the moderately resolved consensus tree.

Palaeobiology. Body mass estimates from total-skull length (TSL) and upper-molar-row length show that D. vorax ranged from 2.4 kg (2.1–2.7 kg) for the young adult individual (NDGS 431) to 5.2 kg (4.2–6.2 kg) for the older adult individual (UWBM 94084, Supplementary Table 4). Using the body mass estimate of the older adult individual and a formula derived from modern comparative data, we estimated the maximum prey size of D. vorax to have been 5.0 kg (95% confidence intervals = 2.2–11.4 kg; Supplementary Table 5).

The estimated maximal bite force at the canines (CBS), which is based on measurements of the skull model of the younger adult individual (NDGS 431), is 218 N (Supplementary Fig. 11 and Supplementary Tables 6 and 7). To account for the body size
putative hard-object feeder27. Crushing capabilities (RPS of extant carnivorans and dasyuromorph marsupials with 3.27 and 4.99 (Supplementary Table 8); it overlaps or exceeds the 201 exceeds that of all other measured taxa. Than that of most larger-bodied extant and fossil taxa, its BFQ of 28, corroborating the BFQ results.

The relative premolar size (RPS) of D. vorax ranges between 3.27 and 4.99 (Supplementary Table 8); it overlaps or exceeds the RPS of extant carnivores and dasyuromorph marsupials with crushing capabilities (RPS = 3.00–3.90)25,26, and the RPS of the early Palaeocene archaic ungulate Periptychus carinidens (body mass = 10.1 kg; p4 width = 9.6 mm; RPS = 4.44), which is also a putative hard-object feeder27.

The canine bending strength estimates of D. vorax are proportionally greater than those for extant canids and more similar to those for extant felids and hyaenids (Supplementary Fig. 12 and Supplementary Tables 9 and 10). Bending strength estimates about the mediolateral axis (Sy) of the canine suggest that D. vorax generated proportionally greater bite forces at the canines compared with canids28, corroborating the BFQ results. The high estimates about the anteroposterior axis (Sx) suggest that the canines of D. vorax likely could have withstood substantial oblique or mediolateral stresses induced from either contact with bone during a deep bite, struggling prey during a killing bite or bone crushing performed by adjacent teeth28. The mandibular bending strength estimates of D. vorax are greater than those of the Virginia opossum Didelphis virginiana but less than those of the Tasmanian devil Sarcophilus harrisii (Supplementary Fig. 13 and Supplementary Table 11). Loads in the dorsoventral plane (Zx/L, Supplementary Fig. 13a) generally correspond to bite forces, which tend to scale with body size29; thus, these results are also consistent with results from our bite force analysis. Dorsoventral buttressing in D. vorax specifically increases below the ultimate premolar and molars, likely to accommodate larger stresses induced by crushing hard objects with its premolars. The capacity to withstand large labiobucular loads, as seen in the mandible of D. vorax (Zy/L, Supplementary Fig. 13b), often reflects the need to resist lateral movements of a prey animal struggling under the bite of a predator or from torsional stresses induced by hard-object feeding (for example, bones and shells)29. The relative force profile (Supplementary Fig. 13c) shows that while the mandible of D. vorax was adapted to withstand large bite forces posteriorly, it also maintained strong buttressing against labiobucular forces to deal with the torsional stresses noted above (Zx/Zy < 2.00).

The results of the dental microwear analysis (Supplementary Fig. 14 and Supplementary Tables 12–15) show that all specimens of D. vorax cluster together and that the range of variation is similar to that observed within many extant taxa (for example, Bassariscus astutus). D. vorax clusters with extant specimens with high numbers of puncture pits and hypercoarse scratches, low numbers of pits and average numbers of scratches (that is, principal coordinate analysis (PCoA) 1 scores near average and negative PCoA 2 scores). Although the presence of numerous puncture pits on the enamel surface of D. vorax is consistent with a hard-food diet, similar numbers of puncture pits are also found in animals that do not have a hard-food diet (for example,
Elephantulus rufescens

signature of omnivores: Procyon cancrivorus, overlaps with the microwear signatures of two plant-dominated families, Glasbiidae, Pediomyidae, Stagodontidae, Alphadontidae and marsupials (including Mimoperadectes–Peradectes + Herpetotherium), diverged in NA by the Albir–Cenomanian (100 Myr ago) to late Santonian (85 Myr ago). Accompanying this taxonomic radiation, and consistent with other recent findings, were increases in marsupialiform body size (up to 5.2 kg) and dietary diversity, from insectivory to omnivory, frugivory and carnivory, consuming a range of soft to hard food items. This ecomorphological expansion was broadly coincident with and perhaps related to the onset of adaptive radiations in multituberculates and flowering plants. Most of this NA marsupialiform diversity, however, was lost gradually from the late Campanian to Maastrichtian (79–66 Myr ago) and then abruptly during the Cretaceous–Palaeogene mass extinction (66 Myr ago). Meanwhile, the focus of marsupialiform evolution shifted to SA. Palaeocene SA stem marsupialiforms represent their own monophyletic radiation, rather than successive outgroups to crown marsupials, with implied ghost lineages that stretch back to at least the early Campanian (84–81 Myr ago) in Asia and presumably NA, considering its intermediate position between Asia and SA. Likewise, our results push the origin of crown marsupials back to the early Palaeocene (65 Myr ago), based on the age of the oldest known fossils of Peradectidae, and possibly to the late Campanian (79–74 Myr ago), depending on the herpetotherid affinities of certain fossils. The latter age would be at least 20 Myr ago older than the earliest definitive marsupial fossils not in the Peradectidae or Herpetotheriidae (Itaboraian of Brazil, ca. 54–52 Myr ago), but would be congruent with recent molecular estimates. The NA roots for multiple, successive outgroups also constrain marsupials or a recent ancestor to a NA origin and dispersal to SA during the Late Cretaceous or earliest Palaeocene—an inference corroborated by other biotic connections (multituberculates and hadrosaurines) and geological evidence of a land connection between NA and SA.

Novel morphology from the specimens reported herein broadens the ecological diversity of NA Cretaceous metatherians. The new specimens support the hypothesis that D. vorax was a powerful predator–scavenger. New body mass estimates show that, among Mesozoic mammals, D. vorax was larger than all other therians and rivaled by only a few non-therians. The body mass estimate of 5.2 kg for UWBM 94084 (Supplementary Table 4) is comparable in size to the Virginia opossum D. virginiana (1.9–6.0 kg) and American badger Taxidea taxus (4.0–12.0 kg). Owing in part to energetic constraints, small- to medium-sized extant predators (<15 kg) mostly feed on small vertebrates and invertebrates that are a small fraction of their own body mass. Accordingly, an adult D. vorax would have preyed on mainly small Late Cretaceous molluscs, fish, amphibians, lizards, turtles and mammals; however, its maximum prey size is estimated to have been 5.0 kg (95% confidence intervals of 2.2–11.4 kg; Supplementary Table 5), which would have included some small and juvenile dinosaurs.

Analysis of skull biomechanics points to an even wider predatory range for D. vorax than would be expected from its body size alone. Its relatively short, broad and robust skull conferred mechanical advantages via shorter jaw outlevers, more space in the temporal region for jaw adductor musculature and increased resistance to torsional loadings. Its estimated maximal bite force at the canines (CBF = 218 N) is greater than that of most small- to medium-sized extant mammals (<15 kg) and only slightly less than that of the river otter L. canadensis (233 N) and European badger Meles meles (244 N; Supplementary Tables 6 and 7). The canine BFO of D. vorax (BFO = 201) indicates that its ‘pound-for-pound’ relative bite force exceeded that of all other measured extant and fossil mammals, including the marsupial lion Thylacoleo carnifex† (193), Tasmanian devil S. harrisii (166), dire wolf Canis dirus† (157), African lion Panthera leo (116) and...
spotted hyaena *Crocuta crocuta* (114; Supplementary Table 7). BFQs greater than 100 typically only occur in bone-cracking specialists and taxa that occasionally hunt or scavenge on prey larger than themselves, such as the honey badger *Mellivora capensis*, striped hyaena *Hyaena hyaena* and spotted hyaena *C. crocuta*23,24.

Other craniodental features strengthen our inference that *D. vorax* was durophagous and possibly osteophagous. Its broad, convex premolars with the protocone as a stress concentrator closely approximate an 'ideal' crushing form37. They require less force than concave shapes to induce crack formation in prey items and are more resistant to tooth fracture than tall- or narrow-cusped shapes37. Even the flat premolars of later wear stages, while not as effective at crushing, incur lower strain values37. Moreover, the RPS25 of *D. vorax* (RPS of P3 = 3.27–4.99; Supplementary Table 8) matches or exceeds those of extant carnivores and dasyuromorph marsupials with crushing capabilities (RPS = 3.00–3.90)25. The rounded cross-sectional shape of the canines of *D. vorax* resembles those of extant hyaenids and extinct bone-crushing dogs (borophagines) and dire wolves28 (Supplementary Fig. 12 and Supplementary Tables 9 and 10). This canine shape resists bending in all directions, for
example, from deep bites contacting bone or from stresses incurred from adjacent premolars crushing hard objects. D. vorax also has a robust lower jaw that exhibits strong dorsoventral buttressing below the ultimate premolar and molars, likely to accommodate large stresses induced by crushing hard objects with the premolars. Anteriorly, the cross-sectional shape of the lower jaw is more rounded and likely reflects strengthening of the symphysis for crushing with the anterior teeth and/or for handling torsional stresses of struggling prey (Supplementary Fig. 13 and Supplementary Table 11). Antemortem canine tooth breakage, such as in UWBM 94084 and NDGS 431, is also significantly more common in durophagous mammals than in carnivores, insectivores or omnivores.

However, the skull of D. vorax differs from those of highly specialized bone crackers. While its reconstructed cranial profile is relatively tall at the snout, as in the molluscivorous sea otter *Enhydra lutris*, it lacks the characteristic vaulted forehead of *Dolichorhina* and *Hesperotomia* (Fig. 1). The evolutionary constraint that shaped the cranial morphology of the metatherian *D. vorax* differed from those of either bone-cracking specialists (Supplementary Fig. 14 and Supplementary Tables 12–15). These results perhaps also reflect that as *D. vorax* had relatively little time (10 Myr) to evolutionarily diverge from its late Campanian sister genus (*Eodelphis*) with smaller, trenchant premolars and a gracile lower jaw, it lacked the characteristic vaulted forehead of *Dolichorhina* and *Hesperotomia* (Fig. 1). The evolutionary constraints that shaped the cranial morphology of the metatherian *D. vorax* differed from those of either bone-cracking specialists; 3) akin to non-bone-cracking canids (for example, *coyotes*); D. vorax did not regularly deploy its high bite force because its skull was not well adapted for very high stresses; instead, D. vorax had a broad dietary range that included hard-object food items as well as other vertebrate and invertebrate material and vegetation. Dental enamel microwear of *D. vorax* favours this last explanation: analysed specimens show lower frequencies of pits than in other osteophages and are spread across the microwear space defined by extant mammals, from durophagous carnivores and omnivores, like the river otter *L. canadensis* and crab-eating raccoon *P. cancrivorus*, to small vertebrate and insect predators, like the ring-tailed cat *B. astutus*, and taxa that mainly feed on plants, like the kinkajou *Potos flavus* and muskrat *O. zibethicus* (Supplementary Fig. 14 and Supplementary Tables 12–15). These results perhaps also reflect that as the skull shape of *D. vorax* varied with ontogeny, as in extant marsupials, so too did skull function and diet. Together, our data indicate that *D. vorax* was a powerful predator–scavenger in Late Cretaceous ecosystems with considerable faculties for cracking bone, invertebrate shell and/or turtle shell, but lacked some modifications of larger, more advanced osteophages, and *D. vorax* likely supplemented its diet of small vertebrates, carrion and/or molluscs with insects and plant matter.

Our study adds to a growing body of exceptional fossil discoveries and innovative quantitative analyses that has revealed an unexpectedly rich ecomorphological diversity of Mesozoic mammals. This recasting of mammals from the Age of Dinosaurs, however, has previously focused on ‘dead-end’ non-therian lineages. Until recently, there has been little evidence that therians, which comprise 99.9% of all living mammals, underwent significant ecomorphological radiation during their more than 100 Myr of Mesozoic evolution. *D. vorax* highlights a previously under-appreciated radiation of Late Cretaceous NA stem marsupialiforms and its direct link to the origin and evolution of living marsupials.

Methods

**Fossil specimens and their geologic age and context.** The four specimens of *D. vorax* in this study are curated at three repositories: the North Dakota Geological Survey State Fossil Collection at the North Dakota Heritage Center State Museum in Bismarck, North Dakota, USA (NDGS); Sierra College Natural History Museum, Rocklin, California, USA (SCNHM); and University of Washington Burke Museum of Natural History and Culture, Seattle, Washington, USA (UWBM). The most complete specimens, NDGS 431 and UWBM 94084, from the primary sites for description of the skull morphology (Supplementary Note 1) and reconstruction (Fig. 2) of *D. vorax* with supplemental anatomical details incorporated from UWBM 94500 and SCNHM VMma 20.

NDGS 431 (Supplementary Figs 1 and 2) is a partial cranium with parts of the zygoma, maxilla, palate and P3–M4. The braincase, basioccipital and petrosals are also preserved, although some aspects are crushed (Supplementary Figs 7–9). The semicircular canals of the inner ear are complete on the right side only, as revealed by computed tomography (CT) imaging (Supplementary Fig. 10). These results perhaps also reflect that as *D. vorax* had relatively little time (10 Myr) to evolutionarily diverge from its late Campanian sister genus (*Eodelphis*) with smaller, trenchant premolars and a gracile lower jaw, as in extant *D. vorax* varied with ontogeny, as in extant marsupials, so too did skull function and diet. Together, our data indicate that *D. vorax* was a powerful predator–scavenger in Late Cretaceous ecosystems with considerable faculties for cracking bone, invertebrate shell and/or turtle shell, but lacked some modifications of larger, more advanced osteophages, and *D. vorax* likely supplemented its diet of small vertebrates, carrion and/or molluscs with insects and plant matter.

**Micro-CT scanning and processing.** The main part of NDGS 431 was scanned at Pennsylvania State University. Resolution of each CT slice was 1.024 × 1.024 pixels, the interpixel spacing was 0.0672 mm and interslice spacing was 0.0673 mm. The associated parts of NDGS 431 were scanned at the University of Washington (UW) Santana Lab. Resolution of each CT slice was 1.024 × 1.304 pixels, and pixel size was 0.0867 mm. UWBM 94084 was scanned at the SAAM laboratory at the UW School of Medicine. Resolution of each CT slice was 668 × 1.968 pixels, and pixel size was 0.0344 mm. UWBM 94500 and SCNHM VMma 20 were scanned at the Duke University MICRO-CT Facility at 0.0205 mm resolution. CT imagery of *Didelphodon* was compared with that of the extant didelphids *D. virginiana* and *D. marsupialis minimus* that were described at the University of Texas at Austin CT Facility (www.digimorph.org/specimens).

A composite model of the cranium was assembled in Geomagic Studio 2014.1.0 (3D Systems, Rock Hill, South Carolina, USA). Digital isolation of the petrosals, segmentation of the bony labyrinth and measurements of segmented digital endosteal casts were performed in Avizo 7.0 (VIS Imaging) for the previously used methods. Orthogonal planes through the cranium were defined following methods modified from Rodgers. Points along the medial sutures in the posterior half of the cranium were used to define the vertical sagittal plane (XZ). A modified Ried’s line from the centre of the ventral rim of the infraorbital foramen to the outer margin of the external auditory meatus was used to define the horizontal (frontal) plane (XY). Normally, the inferior rim of the orbit is used, but neither orbit is preserved on the specimen. The ventral rim of the infraorbital
foramen is preserved on the left side of the cranial only, but the dorsal rims of both external auditory meati are present. The axial plane (V2) intersects the centres of the dorsal rims of the external auditory meati and is perpendicular to the sagittal and horizontal planes.

**Phylogenetic analysis.** We used a data matrix of 164 characters (67 dental, 86 cranial and 11 mandibular) that were scored for 48 taxa (13 outgroup taxa, 10 deltatheriodans and 25 marsupialiforms). The data matrix was mainly derived from Rougier et al.2,9,51,52 but with modifications and additions from other previous studies.9,11,51,52 For the taxa for which we could not find data, we updated the scores of Didelphodon for 41 characters in the matrix; 34 were previously unknown (°) and seven were known but revised with new information. Didelphodon is now the best-known Cretaceous marsupialiform in the data set (88% of characters scored versus 72% in Bi et al.°). To this data matrix, we also added two NA Cenozoic marsupialiforms (Herpetotherium and Mimoperadectes informed by Peradectes) that had previously been included in other data matrices11,20 but not in the matrices of Rougier et al.2,9,51,52 or its derivatives.° Scores for Herpetotherium and Mimoperadectes—Peradectes were largely based on previous studies11,20 and our own observations. A few scores for Pseudaludis, Madyuctus, Suctotherium and some South American taxa were modified from Rougier et al.11,20 on the basis of Horovitz et al.11 and our own observations. We used Appendix B of Horovitz and Sánchez-Villagra53 for character correspondence across studies. The character list and scores for Didelphodon are in Supplementary Note 2. We submitted our data matrix (Supplementary Data 1) to a new technology search in TNT (version 1.1)54, using the sectorial, ratchet, drift and tree-fusing characters as additive.

**Testing alternative tree topologies.** To test whether the topology of our strict consensus tree is robust, we compared it with two alternative topologies. Previous analyses have generally placed NA Cretaceous stem marsupialiforms as an early diverging marsupialiform group or as a grade distant from the crown group, and SA stem marsupialiforms have been placed as sister taxon to Marsupialia2,9,11,20 or to Herpetotheriidae + Marsupialia11,20. To compare these alternatives with our strict consensus tree, we conducted Templeton tests in TNT (Supplementary Note 4).

**Constraining the timing of the origin of Marsupialia.** Marsupialia is a crown clade formed by the most recent common ancestor of extant metatherians, plus all of its descendants.5 According to our phylogenetic analysis, Mimoperadectes—Peradectes and Herpetotherium are included along with extant marsupials (Dromiciops, Diprotodon and Marmosa) in Marsupialia. The origin of crown group marsupials is thereby constrained by the age of the oldest fossils attributed to Marsupialia: the oldest known species of Peradectes (for example, P. minor) are from the early Palaeocene (middle Eocene of Wyoming, USA, specimens not scored), whereas the oldest known species of Horiportherium (for example, H. comstocki) are from the earliest Eocene (late Paleocene of Wyoming, USA, specimen not scored), and 55 Myr ago, according to the age of the oldest member of the monophyletic Herpetotheriidae5,7,20 we can further extend its lineage back to at least the oldest member of this clade. Williamson and Lofgren58 placed Golderdelphys stocki, which is known by four isolated molars, in the Herpetotheriidae. These specimens are from the middle Tiffanian-age Golder Formation (middle Paleocene of Wyoming, USA). We added two NA Cenozoic marsupialiforms (Herpetotherium and Mimoperadectes) informed by Peradectes that had previously been included in other data matrices11,20 but not in the matrices of Rougier et al.2,9,51,52 or its derivatives.° Scores for Herpetotherium and Mimoperadectes—Peradectes were largely based on previous studies11,20 and our own observations. A few scores for Pseudaludis, Madyuctus, Suctotherium and some South American taxa were modified from Rougier et al.11,20 on the basis of Horovitz et al.11 and our own observations. We used Appendix B of Horovitz and Sánchez-Villagra53 for character correspondence across studies. The character list and scores for Didelphodon are in Supplementary Note 2. We submitted our data matrix (Supplementary Data 1) to a new technology search in TNT (version 1.1)54, using the sectorial, ratchet, drift and tree-fusing characters as additive. Previous studies have generally placed NA Cretaceous stem marsupialiforms as an early diverging marsupialiform group or as a grade distant from the crown group, and SA stem marsupialiforms have been placed as sister taxon to Marsupialia2,9,11,20 or to Herpetotheriidae + Marsupialia11,20. To compare these alternatives with our strict consensus tree, we conducted Templeton tests in TNT (Supplementary Note 4).

**Body mass estimates.** Numerous predictive body mass equations have been developed from regression analyses of craniodental measurements of extant mammals22,29. To estimate body mass in D. vorax, we chose to use formulae developed from an all-dasyuromorphian data set22. We deemed this data set as appropriate for the Dasyuromorphia because it includes a fairly taxonomic group of marsupials and they adequately approximate the craniodental morphology of Didelphodon. This equation also provided reasonable body mass estimates for a sample of D. virginiana with tag mass data (data not presented here). We estimated body masses of four specimens of D. vorax: (1) the relatively complete skull of a young adult (UMORL 413); the most complete dentary of an individual (UMW 94084); the near-complete dentary of young adult (UWBM 212193); and the more fragmentary dentary of a young adult (UCMP 159909). For NDGS 431, we used the formulae that use TSL and upper-molar-occclusal-row length (UMORL). For UWM 94084, we estimated TSL based on the scaling force calculations are based on the contributions of the temporalis muscle and their largest known prey items. To correct for logarithmic transformation bias52, Meers23 calculated a Ratio estimator (RE) correction factor according to the method of Snowdon56. Because that RE correction factor was not reported, we reconstructed it from the regression formula and the appendix of comparative data in Meers23. We applied Meers’ formula and the reconstructed correction factor to three different body mass estimates from D. vorax (Supplementary Table 4). The estimates and 95% confidence intervals are presented in Supplementary Table 5.

**Bite force analysis.** To estimate bite force in D. vorax and other taxa, we replicated Thomason’s method using two-dimensional images of digital surface models instead of photographs of dry skulls9,49. In this analysis, we used the composite model of the CT scanned D. vorax cranial material. Stereolithography files of skulls (Dromiciops fasciatus, D vitaminana and D. vorax) were modified from the University of California (UCAL M-2517) and the more fragmentary dentary of young adult (UMORL). Therefore, we chose to use formulae that include the following: the estimated physiological cross-sectional area of the muscle groups involved in jaw adduction; the length of the lever arms of each muscle force vector about the temporal—mandibular joint (TMJ), and the length of the outlever; or distance from the TMJ axis to the bite point. Our bite force calculation required the bite force from Functional anatomy of mammals (35° Myr ago). Body mass estimates from the scaled TSL. We estimated body masses of four specimens of D. vorax: (1) the relatively complete skull of a young adult (UMORL 413); the most complete dentary of an individual (UMW 94084); the near-complete dentary of young adult (UWBM 212193); and the more fragmentary dentary of a young adult (UCMP 159909). For NDGS 431, we used the formulae that use TSL and upper-molar-occclusal-row length (UMORL). For UWM 94084, we estimated TSL based on the scaling force calculations are based on the contributions of the temporalis muscle and their largest known prey items. To correct for logarithmic transformation bias52, Meers23 calculated a Ratio estimator (RE) correction factor according to the method of Snowdon56. Because that RE correction factor was not reported, we reconstructed it from the regression formula and the appendix of comparative data in Meers23. We applied Meers’ formula and the reconstructed correction factor to four different body mass estimates from D. vorax (Supplementary Table 4). The estimates and 95% confidence intervals are presented in Supplementary Table 5.

**Estimate of maximum prey size of D. vorax.** Predator body size constrains the maximum body size of prey that it can kill. For energetic and biomechanical reasons, small- to medium-sized predators (<15–20kg) most commonly feed on small vertebrates and invertebrates that are only a small fraction of their own body mass22,49,64. Meers64 developed a regression formula to predict maximum prey size from a predator’s body mass, using a large data set of extant predators and their largest known prey items. To correct for logarithmic transformation bias52, Meers23 calculated a Ratio estimator (RE) correction factor according to the method of Snowdon56. Because that RE correction factor was not reported, we reconstructed it from the regression formula and the appendix of comparative data in Meers23. We applied Meers’ formula and the reconstructed correction factor to four different body mass estimates from D. vorax (Supplementary Table 4). The estimates and 95% confidence intervals are presented in Supplementary Table 5.
Our measurements and bite force estimates are presented in Supplementary Table 6. We could only estimate bite forces for D. vorax using the smaller, more complete skull (NDGS 431); however, we would predict larger bite force estimates for the larger UWBM 94084. In light of the nearly identical canine bite force estimates for S. harrisii by us and by Wroe et al.24 using different specimens, we have confidence that our results for the other taxa are consistent with those presented in Table 1 of Wroe et al.24 Bite force scales with body mass23,24, such that comparison of bite force estimates across a wide range of body sizes is problematic. To adjust for this body size allometry and to enable comparisons of relative bite forces, Wroe et al.24 estimated BFQ, which utilizes the residuals of the regression of log body mass and log estimated bite force. Supplementary Table 7 contains the body mass and canine bite force data from Table 1 of Wroe et al.24 and this study. From that data set, we performed a regression of log body mass versus log CBs. The regression formula was then used to calculate new BFQ values for all taxa. Some BFQs differ slightly from those in Table 1 of Table 1 of Wroe et al.24 due to the data set-specific regression formulae. For D. vorax, we used the maximum body mass estimate for NDGS 431 (Supplementary Table 4) as a conservative estimate of BFQ (lower body mass estimates produce higher BFQs).

Relative premolar size. Van Valkenburgh27 and Hartstone-Rose28 discriminated among dietary categories in carnivorans and marsupials using a number of cranial and dental indices. In particular, they found that RPS was greatest among durophagous taxa compared with all other dietary categories. RPS was calculated for D. vorax as described in Appendix 2 of Van Valkenburgh28: the maximum width of the ultimate premolar (in mm) was divided by the cusp root of body mass (in kg).

Canine bending strength analysis. Van Valkenburgh and Ruft29 investigated the biomechanical link between mandibular shape and feeding behaviour in extant carnivores by modelling the mandibular body as a solid beam and, in turn, estimating its resistance to bending forces in the anteroposterior and mediolateral directions. We applied those methods to specimens of D. vorax, D. virginiana, S. harrisii and T. taxus (Supplementary Table 9). All measurements were taken with digital calipers on actual specimens as shown in Fig. 1 of Van Valkenburgh and Ruft29 and according to the methods in their text. We then added our data to their data set and made bivariate plots to examine the canine shape of D. vorax in the broader context of extant mammalian predators (Supplementary Fig. 12 and Supplementary Table 10).

Mandibular bending strength analysis. Therrien29 investigated the biomechanical link between mandibular shape and feeding behaviour in large carnivores by modelling the canine tooth as a solid beam, and, in turn, estimating its resistance to bending forces in the anteroposterior and mediolateral planes. Given the same material properties of the bone, a dorsally deep mandibular body is better able to withstand forces in the dorsoventral plane, whereas a mediolaterally wide mandibular body is better able to resist bending forces in the mediolateral plane. We applied their methods to specimens of D. vorax, D. virginiana and S. harrisii (Supplementary Table 11). All measurements were taken with digital calipers on actual specimens as shown in Fig. 2 of Therrien29 and according to the methods in that text. Because metatherians differ from extant carnivorans in dental formula, we took measurements at different interdental gaps: canine p2–p3; p3–m1; m1–m2; m2–m3; and post m4. Cross-sectional dimensions and, in turn, bending strengths can vary along the mandibular body in a way that correlates with feeding behaviours in extant carnivorans29. For comparison with taxa in Figs 3–7 of Therrien29, we plotted our data as mandibular force profiles in Supplementary Fig. 13.

Low-magnification dental microwear analysis. Dental microwear is used as a proxy to infer feeding behaviour of individual animals. It is quantified by counting pits and scratches on the enamel of the occlusal surface of the teeth and has been widely used to study the diet of a variety of mammals69,70. These features reflect the material properties of the food and exogenous items eaten1. Specifically, a high density of scratches suggests the ingestion of abrasive foods (including plant materials and grit), whereas abundant pits indicate a diet composed of hard foods from both plant (for example, seeds and nuts) and animal sources (for example, bones and shells)72,73. By quantifying the microwear of species with known diets, a comparative data set can be built and then used to estimate the diet of taxa for which food intake is unknown, including extinct species. Although multiple studies have quantified the microwear of numerous extant mammals exhibiting different feeding strategies, analyses of inter-observer error demonstrate that each investigation of the diet of fossil taxa should develop its own comparative data set74.

We therefore quantified microwear in 16 modern mammals. We focused our study on small mammals with body masses comparable to those of Mesozoic taxa in general, and Didelphodon in particular, including numerous marsupials. Their diets range from carnivory to herbivory, including omnivores, insectivores and animals capable of durophagy (Supplementary Table 12). Diets of extant mammals were taken from the literature. On the basis of the dominant food items consumed by animal, we categorized each in one of five dietary categories: carnivory; animal-dominated omnivory; omnivory; plant-dominated omnivory; and herbivory. All specimens sampled were adult individuals from museum collections collected in the wild (the diet of a captive animal is not representative of the wild behaviour of that animal). Because the microwear features counted by different observers cannot be compared, only one researcher (J.J.C.) counted pits and scratches on the tooth surface of the specimens sampled. We used low-magnification stereomicroscopy to quantify microwear in our sample. Specimens were observed under a Leica MZ 9.5 microscope with a × 2.0 objective. We excluded from our data set fossil specimens with taphonomic damage75. We assessed intra-observer error by counting a subset of 20 specimens, including a carnivore (L. canadensis), two animal-dominated omnivores (N. narica and T. glis) and a plant-dominated omnivore (P. tryodactylus), twice and comparing the two independent counts (Supplementary Table 13). The intra-observer consistency in microwear counts was calculated using Spearman’s rank-order correlation coefficient, which is not as sensitive to outliers and non-normal distributions as other coefficients. Because most of the pits are small pits and most of the scratches are cross scratches, we also ran correlation analyses for these two categories of microwear features. The number of microwear features counted for each specimen was normalized by the total number of microwear features counted for the specimen to account for differences in the number of microwear features across specimens. The percentage of each category of microwear features for each specimen was used in a multivariate analysis of the microwear signature (that is, combined percentages of the different categories of microwear features). Because of the non-normal and nonlinear nature of our data, which include many zeros, we used PCoA to construct a multivariate microwear space to visualize similarities between the microwear signature of D. vorax and that of extant mammals (Supplementary Fig. 14). The analysis of microwear features was run in R 3.1.3 (rel. 78) using RStudio 0.98.1103 (ref. 79) and the package vegan 2.3–4 (ref. 80).

Data availability. The data sets generated or analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

G.P.W. wrote the manuscript with input from all authors; all authors contributed to the morphological and functional analyses; G.P.W. and E.G.E. performed the phylogenetic analyses; J.W.H. and G.P.W. led field work and stratigraphic analysis.

Additional information

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