Small-bodied, insectivorous Nyctitheriidae are known in the Palaeogene fossil record almost exclusively from teeth and fragmentary jaws and have been referred to Eulipotyphla (shrews, moles and hedgehogs) based on dental similarities. By contrast, isolated postcrania attributed to the group suggest arboreality and a relationship to Euarchonta (primates, treeshrews and colugos). Cretaceous–Palaeocene adapisoriculid insectivores have also been proposed as early euarchontans based on postcranial similarities. We describe the first known dentally associated nyctitheriid auditory regions and postcrania, and use them to test the proposed relationship to Euarchonta with cladistic analyses of 415 dental, cranial and postcranial characteristics scored for 92 fossil and extant mammalian taxa. Although nyctitheriid postcrania share similarities with euarchontans likely related to arboreality, results of cladistic analyses suggest that nyctitheriids are closely related to Eulipotyphla. Adapisoriculidae is found to be outside of crown Placentalia. These results suggest that similarities in postcranial morphology among nyctitheriids, adapisoriculids and euarchontans represent separate instances of convergence or primitive retention of climbing capabilities.

1. Background

Euarchonta is a eutherian clade supported by numerous molecular analyses that includes the modern orders Dermoptera, Scandentia and Primates [1,2]. Euarchontan mammals probably evolved from an arboreal ancestor [3,4] and many proposed euarchontan synapomorphies are also associated with climbing capabilities [1]. The oldest known (approx. 64.85 Ma [2]) euarchontan, earliest Palaeocene Purgatorius, has been supported as a stem primate (e.g. [5,6]), and older and more primitive euarchontans outside the crown-radiation have been lacking in the fossil record [1].

Possibly filling this gap, adapisoriculids from the Cretaceous of India (Deccanolestes) and Early Palaeogene of Europe and Africa (Afrodon and Bustylus) and the Eocene European nyctitheriid Cryptotopos have been hypothesized to be early euarchontans based on postcranial morphology [7–11]. Considering dental morphology alone, Deccanolestes was considered similar to palaearcticids [12], and Adapisoriculidae and Nyctitheriidae were classified as ‘Lipotyphla’...
A cladistic analysis of fossil and modern mammals found *Decanolestes* and Paleocene adapiforms to be closely related to each other, but not to Euarchonta [14]. Here, we test the hypothesis of nyctitheriids as euarchontans using new data from partial skeletons of North American Paleocene nyctitheriids in a revised cladistic analysis, and evaluate the utility of using an isolated morphological data partition.

2. Material and methods

Fossils of Paleocene nyctitheriids *Plagioctenodon rosei* and *Plagioctenodon thewisseni* used in this study include dentally associated crania and postcrania (electronic supplementary material, S1–S2). These fossils were recovered from Late Paleocene freshwater limestones in the Willwood Formation, WY, USA [15].

Cladistic analyses using maximum parsimony in TNT [16] were performed on a character matrix (415 characters, 92 taxa, electronic supplementary material, see S1) modified from previously published work (e.g. [14,17]) that includes dental, cranial and postcranial data. Twelve taxa were added to the matrix, including three nyctitheriids (electronic supplementary material, S1I). Unconstrained and constrained analyses were performed (see the electronic supplementary material, S3 for detailed methodology and results). Constraints were based on recent, well-supported molecular clades [18] and were used to test whether the position of nyctitheriids and adapiforms would be affected when the tree topology was more congruent with molecular results, owing to the discrepancies sometimes found between morphological and molecular studies (e.g. [19]).

To test competing evolutionary hypotheses of convergence or primitive retention of arboreal characters at certain nodes, the maximum likelihood of character states was estimated using a strict consensus of results from an analysis using molecular constraints for extant taxa (electronic supplementary material, S4).

3. Nyctitheriid postcranial morphology

(a) Humerus

The humerus of *Plagioctenodon* (figure 1a,b) has a spherical head and a small greater tuberosity that does not extend proximally above the head. The lesser tuberosity protrudes medially, with a broad muscle attachment site. The distal humerus (figure 1a,c) is wide, with a large medial epicondyle, a shallowly oblique trochlea separated from the capitulum by a groove, and a nearly spherical capitulum with a small greater tuberosity that does not extend proximally above the head. The lesser tuberosity protrudes medially, with a broad muscle attachment site. The distal tuberosity protrudes medially, with a broad muscle attachment site.

The humeral morphology (electronic supplementary material, figure S15–1) indicates that nyctitheriids had considerably mobile shoulder and elbow joints [20]. Two characteristics, a medially protruding lesser tuberosity on which powerful medial rotators of the arm insert and a spherical capitulum that allows considerable rotation of the radius in pronation and supination of the forearm, are proposed synapomorphies for Euarchonta [1]. The presence of these euarchontan-like features in nyctitheriid postcrania has caused past confusion in identifying isolated bones, but dentally associated nyctitheriid humeri are used here to reallocate humeri previously attributed to micromomyid pleurodontally associated nyctitheriid humeri are used here to reallocate humeri previously attributed to micromomyid pleurodonty.

(b) Tarsals

The astragalus (figure 1d–f) and calcaneum (figure 1g–i) of *Plagioctenodon rosei* are similar in morphology to those of isolated tarsals reported as *Cryptotopos*? [8], supporting the attribution of those fossils to Nyctitheriidae. The astragalus has a shallow trochlear groove, with a higher lateral than medial trochlear crest. A broad, convex sustentacular facet is on the plantar surface of the neck and is confluent both medially and laterally with the rounded navicular facet, creating a circular fossa between the two facets. The calcaneal facet is evenly convex and oblique to the long axis of the calcaneum. Distomedial to it is a rectangular sustentacular with a round, slightly concave sustentacular facet. A small distal sustentacular facet is distolateral to the sustentacular facet, but not connected to it. The peroneal process is positioned proximal to the cuboid facet and is deeply grooved on its lateral surface. The cuboid facet is shallowly concave and oblique to the long axis.

The tarsal bones (electronic supplementary material, figure S15–3) indicate arboreal capabilities [3], including three traits proposed as euarchontan synapomorphies [1,8,9]: (i) conformity of the astragalar sustentacular and navicular facets, (ii) a calcaneal distal sustentacular facet, and (iii) a concave calcaneal cuboid facet. These characteristics aid in inversion of the foot via a greater degree of movement between the astragalus and calcaneum (via the confluent astragalar sustentacular and navicular facets and calcaneal distal sustentacular facet) and between the calcaneum and cuboid (via the concave calcaneal cuboid facet).

4. Nyctitheriid auditory morphology

The auditory region of *Plagioctenodon* (figure 1j–k) includes a petrosal with a laterally positioned epitympanic petrosal wing and ventral promontorial grooves associated with the medial entrance of the internal carotid artery to the inner ear and the stapedial and promontorial branches of that artery. Both taxa have an open canal for the facial nerve and show no evidence of a fully ossified bulla. *Plagioctenodon rosei* has a fusiform ectotympanic ring with a malleolar sulcus. All of these characteristics are shared by most eulipotyphlans, although some may be primitive within Eutheria [6].

5. Results

A strict consensus of the results from the unconstrained analysis (electronic supplementary material, figure S13–1) recovers a poorly resolved clade that includes euarchontans, xenarthrans, Glires, and some afrotherians to the exclusion of a clade that includes eulipotyphlans, other afrotherians and nyctitheriids. The strict consensus of the analysis constraining only extant taxa recovers Placentalia as a polytomy consisting of Afrotheria + Xenartha + fossil Glires + Euarchonta + Eulipotyphla that is sister to some fossil members of Laurasiatheria (figure 2a; electronic supplementary material, figure S13–2). Nyctitheriidae is in a clade with the extant eulipotyphlans supported by 23 synapomorphies, most of which are cranial (electronic supplementary material, SI3).
A strict consensus tree (figure 2b; electronic supplementary material, figure SI3–3) of the results of the analysis constraining extant taxa and three fossil taxa closely approximates molecular relationships within crown Placentalia [18]. Nyctitheriidae is supported in a clade with Eulipotyphla, but like the other two analyses, Bremer supports for that relationship are very low. All three analyses find Adapisoriculidae outside of Placentalia.

A test of ancestral character states (electronic supplementary material, SI4) suggests that common arboreal traits
among euarchontans, nyctitheriids and adapisoriculids; competing hypotheses: (i) a close phylogenetic relationship and euarchontans could be interpreted as support for three postcranial similarities between nyctitheriids, adapisoriculids has supported euarchontan relationships [7,9,10,11]. The insectivorous mammals [12,13], whereas postcranial evidence vide support for eulipotyphlan (e.g. [22]) and euarchontan [8]. Dental and postcranial morphology have been argued to provide support for a common crown placental ancestor.

between Adapisoriculidae and Euarchonta are likely convergent. Results for nyctitheriids and euarchontans are mixed, with half of the characters (mostly tarsal) probably convergent and the other half (all humeral) retained from a common placental ancestor.

6. Discussion

Dental and postcranial morphology have been argued to provide support for eulipotyphlan (e.g. [22]) and euarchontan [8] affinities of Nyctitheriidae, respectively. The dental morphology of some nyctitheriids is also like that of bats (e.g. [23]). Similarly, the dental morphology of adapisoriculids has been used to suggest relationships with various insectivorous mammals [12,13], whereas postcranial evidence has supported euarchontan relationships [7,9,10,11]. The postcranial similarities between nyctitheriids, adapisoriculids and euarchontans could be interpreted as support for three competing hypotheses: (i) a close phylogenetic relationship among euarchontans, nyctitheriids and adapisoriculids; (ii) separate instances of convergence on arboreal positional behaviours; or (iii) a primitive state of arboreality in Early Placentalia that was later lost in most modern placental groups other than Euarchonta.

Our cladistic results do not support nyctitheriids or adapisoriculids as euarchontans. Postcranial morphology is highly modified by adaptations for climbing in euarchontans and other mammals [3], and while some of these postcranial characteristics are clearly synapomorphies for Euarchonta, their convergent acquisition in other clades could lead to erroneous phylogenetic hypotheses if postcranial data are considered in isolation. Our results suggest that the presence of arboreal traits in adapisoriculids is mostly due to convergence, whereas in nyctitheriids, most humeral similarities to euarchontans are due to retention of climbing adaptations from a common crown placental ancestor [2] and all tarsal similarities are convergent. Our study highlights the importance of using all morphological evidence in phylogenetic analyses whenever data from more than one partition (e.g. cranial, dental and postcranial) are available.

Figure 2. Phylogenetic relationships of Nyctitheriidae within Eutheria. Strict consensus of (a) 2839 most parsimonious trees (MPT) resulting from cladistic analysis forcing (using only extant species) monophyly of Afrotheria, Xenarthra, Boreoeutheria, Euarchontoglires and Eulipotyphla and (b) 237 MPTs resulting from cladistic analysis forcing (using fossil and extant species) monophyly of Afrotheria, Xenarthra, Boreoeutheria, Euarchontoglires, Laurasiatheria and Eulipotyphla. Bold text indicates taxa previously hypothesized as euarchontans. Fos. CCA, fossil representatives of Carnivoramorpha, Condylarthra and Artiodactyla; Xen., Xenarthra; Fos. Gli., fossil representatives of Gliridae. *Collapsed nodes.
Data accessibility. The datasets supporting this article can be found in the electronic supplementary material.

Acknowledgements. We thank P. D. Gingerich, G. F. Gunnell, R. C. Hulbert, Jr for assistance with specimens; J. Van Houten for assistance in micro-CT scanning; J. R. Bourque, D. M. Boyer, A. R. Wood for helpful discussions; two anonymous reviewers for helpful comments; and the Willi Hennig Society for supporting TNT. This is University of Florida contribution to Paleobiology 680.

Author contributions. C.L.M. and J.I.B. conceived the study; C.L.M., S.G.B.C. and J.I.B. collected data. C.L.M. ran the cladistic analysis. All authors (C.L.M., S.G.B.C., J.I.B., M.T.S. and E.J.S.) contributed to analysis of data and writing of the manuscript.

Funding statement. C.L.M. was funded by the Miss Lucy Dickinson Fellowship and the University of Florida Geological Sciences Graduate Award.

Conflict of interests. We have no competing interests.

References


