Early Tertiary mammals from North Africa reinforce the molecular Afrotheria clade

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The phylogenetic pattern and timing of the radiation of mammals, especially the geographical origins of major crown clades, are areas of controversy among molecular biologists, morphologists and palaeontologists. Molecular phylogeneticists have identified an Afrotheria clade, which includes several taxa as different as tenrecs (Tenrecidae), golden moles (Chrysochloridae), elephant-shrews (Macrossedilidae), aardvarks (Tubulidentata) and paenungulates (elephants, sea cows and hyracoids). Molecular data also suggest a Cretaceous African origin for Afrotheria within Placentalia followed by a long period of endemic evolution on the Afro-Arabian continent after the mid-Cretaceous Gondwanan breakup (approx. 105–25 Myr ago). However, there was no morphological support for such a natural grouping so far. Here, we report new dental and postcranial evidence of Eocene stem hyrax and macroscelidid from North Africa that, for the first time, provides a congruent phylogenetic view with the molecular Afrotheria clade. These new fossils imply, however, substantial changes regarding the historical biogeography of afrotheres. Their long period of isolation in Africa, as assumed by molecular inferences, is now to be reconsidered inasmuch as Eocene paenungulates and elephant-shrews are here found to be related to some Early Tertiary Euramerican ‘hyposodontid condylarths’ (archaic hoofed mammals). As a result, stem members of afrotherian clades are not strictly African but also include some Early Paleogene Holartic mammals.

Keywords: molecules versus fossils; Afrotheria; Africa; Eocene; hyrax; elephant shrew

1. INTRODUCTION

Recent molecular analyses have proposed well-resolved higher level phylogenies for living eutherian mammals in recognizing four superordinal clades: Laurasiatheria (shrews, moles, hedgehogs, bats, cetaceans, even- and odd-toed ungulates, carnivorans and pangolins), Euarchontoglires (rodents, rabbits, primates, flying lemurs and tree shrews), Xenarthra (anteaters, sloths and armadillos) and Afrotheria, which includes tenrecs (Tenrecidae), golden moles (Chrysochloridae), elephant-shrews (Macroscelididae), aardvarks (Tubulidentata) and elephants, sea cows and hyraxes (Paenungulata; Murphy et al. 2001a,b; Springer et al. 2003, 2004). For Afrotheria, there is a broad molecular consensus for its monophyly deriving from various nuclear and mitochondrial genes as well as from rare genomic changes that constitute probable synapomorphies (Madsen et al. 2001). In contrast, morphological data have until now failed to provide strong support for Afrotheria (Shoshani & McKenna 1998; Archibald 2003; Robinson & Sciffert 2004; Zack et al. 2005). In the absence of a comprehensive fossil record for stem members of crown Afrotheria, most high-level phylogenetic analyses have been based primarily on the morphology of extant afrotheres (Asher et al. 2003). However, modern afrotheres seem inadequate for reconstructing morphological phylogenies because their long period of endemism in Africa favoured the emergence of morphological convergences with laurasiatherian mammals due to similar selection pressures. Stem members of Afrotheria are thus highly critical for resolution of this phylogenetic issue because they exhibit morphological patterns that have not been overwritten by subsequent evolution and further specializations.

Intensive palaeontological work in Early to Middle Eocene North African localities in Algeria (Gour Lazib) and Tunisia (Chambi) have led to the recovery of several new fossils of mammals. Based on biostratigraphic studies,
the Gour Lazib formation has been assigned either a late Ypresian or Early Lutetian age (Tabuce et al. 2004) and the Chambi locality an Ypresian age (Hartenberger et al. 2001). The newly discovered Algerian fossils document rodents, primates (particularly assigned to Algeripithecus, the oldest alleged anthropoid), creodonts, chiropterans, insectivores and hyraxes. For the last clade, we have recovered several fragmentary upper and lower jaws as well as tarsal bones of Microhyrax lavocati (Sudre 1979; Tabuce et al. 2001a), which considerably improves our knowledge of this basal hyrax. From Chambi, we collected additional remains (teeth and tarsal bones) of Chambius kasserinensis (Hartenberger 1986), the oldest known and most primitive elephant-shrew, whose postcranial anatomy was so far unknown. These fossils extend back the date of the oldest macroscelidean records with associated dental and postcranial remains by ca 30–40 Myr.

2. RESULTS

(a) Description and comparisons

Chambius is a small mammal with an estimated body weight of 13 g according to the equation of Legendre (1989). Its upper molars appear to be more primitive than those of more recent Nementchatherium (Tabuce et al. 2001b) and Herodotius (Simons et al. 1991) in having a robust metaconule, a more prominent parastyle and a comparatively more reduced hypocone (figure 1). On the basis of their tooth characters, basal elephant-shrews have been compared with ‘hyopsodontid condylarths’, notably with the North American Haplomylus and the European louisson Microhyus, and these studies suggested ‘condylarth’ affinities for basal elephant-shrews. This hypothesis was recently strengthened on tarsal characters shared between Haplomylus (Zack et al. 2005), Microhyus (Tabuce et al. 2006) and extant elephant-shrews. Chambius tarsal bones reveal specializations for rapid running and/or jumping terrestrial locomotion. The pulley-shaped and the semicircular crests of the astragalar trochlea are indicative of lateral stability and dynamic movements (Lewis 1989); the tibio-astragalar motion was certainly limited to flexion–extension. The calcaneus of Chambius is long both proximally and distally to subtarsal joints, a morphology also characteristic of cursorial and/or saltatorial mammals (Tabuce et al. 2006). The calcaneus bears an ectal facet oriented at nearly 90° to the long axis of the bone. This character, observed in both living elephant-shrews and Haplomylus, constitutes a derived condition within eutherians (Zack et al. 2005). Microhyus differs in having a
proximodistally elongated crestentic ectal facet. Microhyus differs also from Chambius, Haplomylus and modern elephant-shrews in the lack of a fibular facet. Chambius and Microhyus share a large peroneal process. This plesiomorphic condition (Szalay & Decker 1974; Hooker 2001) sets apart Chambius from both modern macroscelids and Haplomylus. The astragalus of Chambius, as in living elephant-shrews, typically has a sustentacular facet shifted to the medioplantar side of the bone. This character occurs only in the living elephant-shrews Petrodromus, Elephantulus, Rhynchocyon (unknown in Macroscelides), as well as in Scandentia (Meng et al. 2003). Chambius presents a cuboid facet on the astragalus as in living elephant-shrews. In addition, as in modern macroscelids, the Chambius astragalus displays a large concave depression, called a cotylar fossa, for articulation with the medial malleolus of the tibia. This character also occurs in some ‘condylarths’, notably in the louisinine (Tabuce et al. 2006) and apheliscine (Zack et al. 2005) ‘hyopsodontids’. Following these comparisons, it appears that Chambius tarsal bones represent a morphological intermediate between Haplomylus and living elephant-shrews, thus reinforcing the relationships between some archaic Euramerican ‘ungulates’ and members of Afrotheria.

Hyraxes have been classified among the ‘ungulates’ by morphologists since the end of the nineteenth century but recently assigned to Afrotheria by molecular biologists. They represented, during the Paleogene, the most diverse group of terrestrial ‘ungulates’ in Africa (Rasmussen 1989; Schwartz et al. 1995). Microhyrax, from the early-middle Eocene of Gour Lazib (Algeria), is one of the smallest known hyraxes with a body mass estimated
around 3 kg (Schwartz et al. 1995). *Microhyrax* displays a mosaic of primitive and derived dental characters (Tabuce et al. 2001a; figure 2). On one hand, lower premolars are simple relative to other Paleogene hyraxes: the metaconid is small on premolar (P)_{2-4}, and the talonid is reduced, giving only a strong hypoconid and a salient cristid obliqua. On the other hand, lower molars are reminiscent to those of *Megalohyrax*, *Titanohyrax*, *Antilohyrax*, *Sagatherium*, *Thyrohyrax* and *Selenohyrax* from the Eocene/Oligocene of the Fayum (Egypt) in showing derived structures such as the interrupted protocristid and hypocristid (Rasmussen 1989). *Microhyrax* is, however, clearly distinct from these taxa in its simple premolars and the morphological pattern of upper molars. Molar (M)_{1-3} display evident primitive characters such as small parastyle and mesostyle, weakly W-shaped ectoloph and paracoronal swelling (Tabuce et al. 2001a). *Microhyrax* is also typical for the increasing reduction of the metacone from M1 to M3.

Functionally, the tarsal bones of *Microhyrax* show no cursorial adaptations given the short tuber calcanei and moderately grooved astragalar trochlea, which indicates cursorial adaptations given the short tuber calcanei and the talonid is reduced. But, as the living *Dendrohyrax* is characterized by its mobile midtarsal joint (Fischer 1986). Basal perissodactyls differ from *Microhyrax* in having a saddle-shaped navicular facet. The peculiar combination of all tarsal characters strongly supports a relationship between *Microhyrax* and tethytheres (proboscideans and sirenians). The head of the astragalus in *Microhyrax* is characterized by a navicular facet with no trace of cuboid facet. This serial arrangement, called taxocody, differs from the diplarthral or alternating arrangement observed in perissodactyls. Taxocody characterizes Proboscidea, Sirenia, Embrithopoda (putative tethytheres), more derived hyraxes (Rasmussen et al. 1990) and several ‘condylarth’ species of *Microhyrax* is, however, clearly distinct from these taxa in its simple premolars and the morphological pattern of upper molars. Molar (M)_{1-3} display evident primitive characters such as small parastyle and mesostyle, weakly W-shaped ectoloph and paracoronal swelling (Tabuce et al. 2001a). *Microhyrax* is also typical for the increasing reduction of the metacone from M1 to M3.

(b) Phylogenetic analysis
On the basis of a recent phylogenetic analysis, including the tarsal anatomy of modern hyraxes and elephant-shrews, the monophyly of Afrotheria was not supported (Zack et al. 2005). Zack et al. (2005) scored the operational taxonomic units (OTU) Macroscelidea as a chimera based on the tarsal characters of the living *Rhinocyon* with the dental features of the Eocene *Chambius*. The OTU *Hyracoidae* was also established on a composite morphology based on several Eocene, Oligocene and modern hyraxes. We modified the analysis of Zack et al. (2005) in removing these taxonomic chimeras and in scoring the tarsal and dental morphology of *Microhyrax* (hryax) and *Chambius* (macroscelid) from new paleontological evidence of both taxa. Other macroscelidids are represented by *Rhinocyon* (Pliocene to Holocene) and *Myohyrax* (Miocene; Butler 1995). In addition to hyraxes and macroscelidids, we supplemented the analysis by sampling the afrotherian orders known since the Eocene: proboscideans (N. koholense) and sirenians (Protosiren). The phylogenetic relationships within the paenungulate clade (see Gheerbrant et al. (2005a) for an overview of paenungulate classification and history) were assessed in coding Anthracobune (‘Anthracobunidae’ from the early-middle Eocene of Indo-Pakistan), Arsinotherium (Embrithopoda from the late Eocene? early Oligocene of Africa) and Phenacolepbus (a putative archaic embrithopod from the late Palaeocene of China and Mongolia). Other representatives of modern ‘ungulates’ are Diazodexis (Cetartiodactyla) and Hydrochoerus (Perissodactyla). As for archaic ‘ungulates’, the matrix includes at least one representative of each of the ‘condylarth’ families: two arctocyonids (*Molodus* and *Crichius*), a mesonychid (*Disaccus*), a peripithyid (*Mithrandir*), a mioclaenid (*Caenodon*), a phanocodontid (*Ectocion*), five ‘hypodontodents’ (*Hypodus*, *Apheliscus*, *Haplolemus*, *Paschatherium* and *Microhyrax*) and Protagulatus (‘condylarth’ incertae sedis, specified as outgroup). In order to minimize homoplasy, the taxonomic dataset exemplifies only groups that existed in the time period corresponding to the early paleontological record of ‘ungulates’ and afrotheres. Despite their incomplete fossil record, early ‘ungulates’ and afrotheres present a major phylogenetic advantage as they are expected to provide morphological patterns that have not been overwritten by subsequent evolution (our primary assumption). Except for *Numidotherium* and *Protopistes*, for which cranial remains are documented, most selected afrotheres, especially *Chambius* and *Microhyrax*, are only known by dental and tarsal remains. In this context, we have limited...
the analysis to dental and tarsal features as a means of minimizing the effect of missing data.

The data matrix contains 52 characters and 23 taxa (electronic supplementary material). We analysed the matrix using heuristic search option in PAUP v. 4.0b10 software (Swofford 2003) with the tree-bisection-reconnection branch-swapping algorithm, a random addition sequence and 1000 replications. An initial analysis of the dataset generated 105 equally most parsimonious trees of 225 steps each. The strict consensus tree is unresolved (figure 3a). A successive approximations reweighting by the method of Farris (1969) was performed, reweighting characters according to their rescaled consistency index, as a means of reducing the misleading effect of homoplasmous characters. This analysis yielded three equally most parsimonious trees. The strict consensus presented in figure 3b shows the monophyly of an Afrotheria clade. In that topology, paenungulates, lousinines, aspheliscines and macroscelidids appear to be pectinately arranged. Among the paraphyletic paenungulates, Arsinotherium occupies a basal position. Since its original description, the systematics and phylogenetic position of Arsinotherium is disputed: Andrews (1906) related this genus to hyraxes, while, more recently, Court (1992a) and Gheerbrant et al. (2005a) considered Arsinotherium as the sister group of proboscideans or tethytheres, respectively. Arsinotherium exhibits an autapomorphic dental morphology. As such, it differs substantially from basal tethytheres in showing an unusual case of hypsodonty and in having a very peculiar bilophodonty of its cheek teeth. Court (1992b) hypothesized that this morphological dental pattern could derive from a hyper-specialized dilambdodonty. It seems to us that the homology of selected dental characters scored in Arsinotherium with respect to the other ingroup taxa is not attested, thereby we excluded that genus from the phylogenetic analysis.

In that new phylogenetic framework, heuristic searches generated four equally most parsimonious trees of 214 steps each. The strict consensus presented in figure 4a is much more resolved than the previous analysis. The paenungulate, tethythere and macroscelid clades appear monophyletic, while lousinines and aspheliscines are polyphyletic. These results (figure 4a) are particularly interesting since they are consistent with current molecular phylogenies on high-level relationships within Afrotheria (figure 4b). For the first time, morpho-paleontological analyses recover underdemonstrated relationships between some stem afrotheres: paenungulates are allied with macroscelids and far from the clade including Perisodactyla (here Hyracoferatherium) and Cetartiodactyla (here Diacodexis; figure 4c). The paenungulate clade is composed of hyraxes plus tethytheres: proboscideans, ‘anthracobunids’, Phenacolophus and sireniens. In addition, European lousinines are related to paenungulates, and the North American apherline Haploplomylus appears as the sister group of macroscelids (figure 4a).

3. PALEOBIOGEOGRAPHIC IMPLICATIONS

In view of our results, and considering the strong molecular evidence for the monophyly of Afrotheria, the early biogeography history of this super-order must be amended since stem afrotheres are not only restricted to Africa but also include some Euramerican ‘hypso-dontids’ and Asian tethytheres. The occurrence in Asia of afrotheres during the Early Paleogene was already suspected in as much as ‘anthracobunids’ from the Indian subcontinent and Phenacolophus from central Asia were described as Tethytheria (see Gheerbrant et al. (2005a) for a summary). Therefore, stem afrotherians appeared both in Africa and in Holarctic area, which substantially changes the paleobiogeographic scenario derived from molecular inferences. Molecular phylogenies argue for an unusual case of endemism in Africa of Afrotheria from the Cretaceous to the Miocene (approx. 105–20 Myr ago; Madsen et al. 2001; Springer et al. 2003), while our results suggest the existence of dispersal events between Africa, Euramerica and Asia. According to the fossil record (figure 5), such events might have occurred around the Palaeocene/Eocene (P/E) boundary during which numerous intercontinental mammalian dispersals occurred (Bowen et al. 2002; Tabuce &
Figure 4. (a) Strict consensus of 4 equally parsimonious trees recovered from a parsimony analysis of 52 morphological characters, all characters are unordered. Tree length = 214 steps, consistency index = 0.35, retention index = 0.55. (b) molecular phylogeny of afrotheres resulting from Springer et al. (2004). (c) morphological phylogenetic position of selected afrotheres resulting from Shoshani & McKenna (1998); the majority of morphological studies consider Afrotheria to be polyphyletic: Macroscelidea are allied with Glires (Lagomorpha and Rodentia) among Anagalida; and Paenungulata (hyraxes, sirenians and proboscideans) are included in the super-order Ungulata. Green colour indicates, in (a) and (b), members of living afrotherian orders; in (c), blue colour indicates Anagalida, and red colour indicates Ungulata.

Figure 5. (Caption opposite.)
Figure 5. (Opposite.) Phylogeny, fossil record and molecular inferences for Afrotheria. Solid lines show phylogenetic relationships of afrotheres resulting from a parsimony analysis of 52 morphological characters (figure 4). Geological ranges of the selected early Paleogene afrotheres are indicated by grey bold lines. Known stratigraphic distribution of modern afrotherian orders are indicated by black bold lines. Dashed lines represent the molecular phylogeny and divergence times for afrotheres estimated by Springer et al. (2003).

Marivaux 2005; Gingerich 2006; Gheerbrant & Rage 2006). A question then arises about the direction of these afrotherian migrations, which depend on the geographical location considered wherein afrotherians were likely to have originated. Two scenarios are conceivable. The first one suggests Palaeocene Laurasian origins for Afrotheria, as expected by both our phylogenetic and current biostatigraphic datasets. Nevertheless, this scenario fails if we consider a Cretaceous origin for afrotheres as molecular studies suggest (figure 5). If these molecular estimates are valid, the second scenario implies a Cretaceous African origin for Afrotheria, followed by dispersal to Euramerica (apheliscines and louisinines) and Asia (‘anthracobunids’ and Phenacolophus) before the P/E boundary. However, the Cretaceous and Palaeocene fossil record of African mammals is too poorly documented to test this scenario and Late Cretaceous fossil mammals are urgently needed. Therefore, only a more complete African fossil record will allow a relevant morphological versus molecular debate regarding the origin, evolution and subsequent migrations of afrotherians.

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