

# Hippopotamus and whale phylogeny

Arising from: J. G. M. Thewissen, L. N. Cooper, M. T. Clementz, S. Bajpai & B. N. Tiwari *Nature* 450, 1190–1194 (2007)

Thewissen *et al.*<sup>1</sup> describe new fossils from India that apparently support a phylogeny that places Cetacea (that is, whales, dolphins, porpoises) as the sister group to the extinct family Raoellidae, and Hippopotamidae as more closely related to pigs and peccaries (that is, Suina) than to cetaceans. However, our reanalysis of a modified version of the data set they used<sup>2</sup> differs in retaining molecular characters and demonstrates that Hippopotamidae is the closest extant family to Cetacea and that raoellids are the closest extinct group, consistent with previous phylogenetic studies<sup>2–3</sup>. This topology supports the view that the aquatic adaptations in hippopotamids and cetaceans are inherited from their common ancestor<sup>4</sup>.

To conduct our analyses, we started with the same published matrix that Thewissen *et al.* modified<sup>2</sup>, but included all taxa (but see Appendix Fig. 2 where *Andrewsarchus* is removed from our results), retained the molecular partition that was derived from an earlier study<sup>5</sup>, added the three anthracotheres included by Thewissen *et al.*<sup>1</sup>, replaced 'Raoellidae' with their character codings for *Indohyus* and *Khirtharia*, included most of the changes and additions to pakicetids that Thewissen *et al.*<sup>1</sup> suggested (see Appendix), and then conducted cladistic analyses of the entire matrix, with all characters weighted equally<sup>6</sup> (see Appendix), as well as with homoplastic characters down-weighted<sup>7</sup>. We consider it particularly important to include molecular data because they are widely recognized as being instrumental in reconstructing mammalian phylogeny<sup>8</sup>.

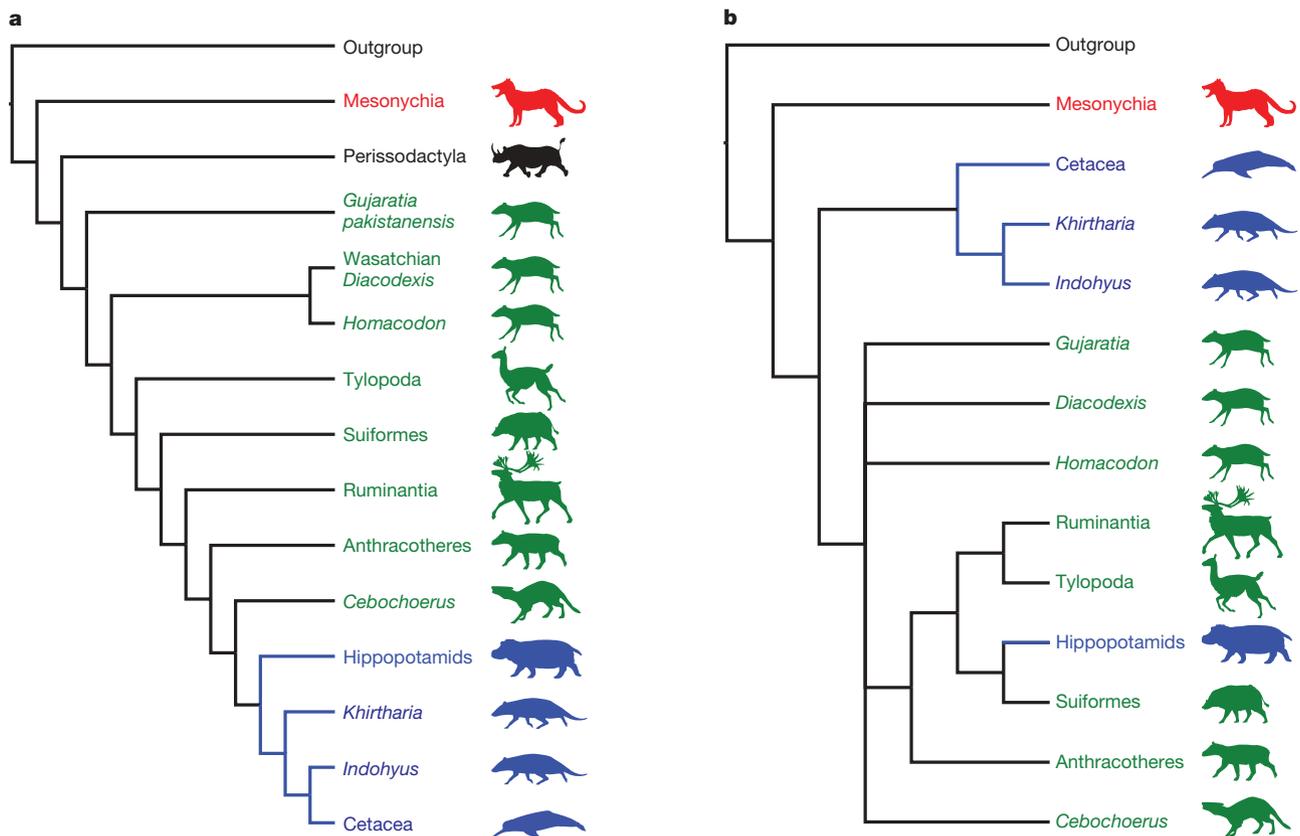
In the equal weights and differential weights analyses, Hippopotamidae is the closest extant taxon to Cetacea, and in those that include

fossils, Raoellidae or the raoellid *Indohyus* is more closely related to Cetacea than is Hippopotamidae (Fig. 1). Hippopotamidae is the exclusive sister group to Cetacea plus Raoellidae in the analysis that down-weights homoplastic characters, although in the equally weighted analysis, another topology was equally parsimonious. In that topology, Hippopotamidae moved one node out, being the sister group to an *Andrewsarchus*, Raoellidae and Cetacea clade. In neither analysis is Hippopotamidae closer to the pigs and peccaries than to Cetacea, the result obtained by Thewissen *et al.*<sup>1</sup>. In all our analyses, pachyostosis (thickening) of limb bones and bottom walking, which occur in hippopotamids<sup>9,10</sup>, are interpreted to have evolved before the pachyostosis of the auditory bulla, as seen in raoellids and cetaceans<sup>1</sup>. Pachyostosis of the bulla, referred to as the involucrum, is thought to be an adaptation for underwater hearing<sup>11</sup>. Thus, by looking at the limited pachyostosis in hippopotamids, we can speculate that pachyostosis in the ancestors of whales initially evolved to counteract buoyancy, but was later co-opted for underwater hearing as it spread to the cranium. This and other hypotheses like it are tenable using our trees, but not those of Thewissen *et al.*<sup>1</sup>, and underscore the importance of including different classes of data in phylogenetic analyses.

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**Figure 1 | Effect of molecular data on the phylogeny of cetaceans and terrestrial relatives.** **a**, Simplified version of the single most parsimonious tree from the present study when homoplastic characters are down-weighted. **b**, Simplified version of the strict consensus shown in Fig. 2 of Thewissen *et al.*<sup>1</sup>. The carnivorous to omnivorous mesonychians are in red, terrestrial

cetartiodactyls (even-hoofed ungulates) are in green, and aquatic to semi-aquatic cetartiodactyls are in blue. Branches are coloured (blue) to show when aquatic adaptations, such as bottom walking, evolved. In **a** these features may have evolved earlier, depending on the behaviour of anthracotheres and cebochoerids. Details of the tree structure in **a** are available in the Appendix.

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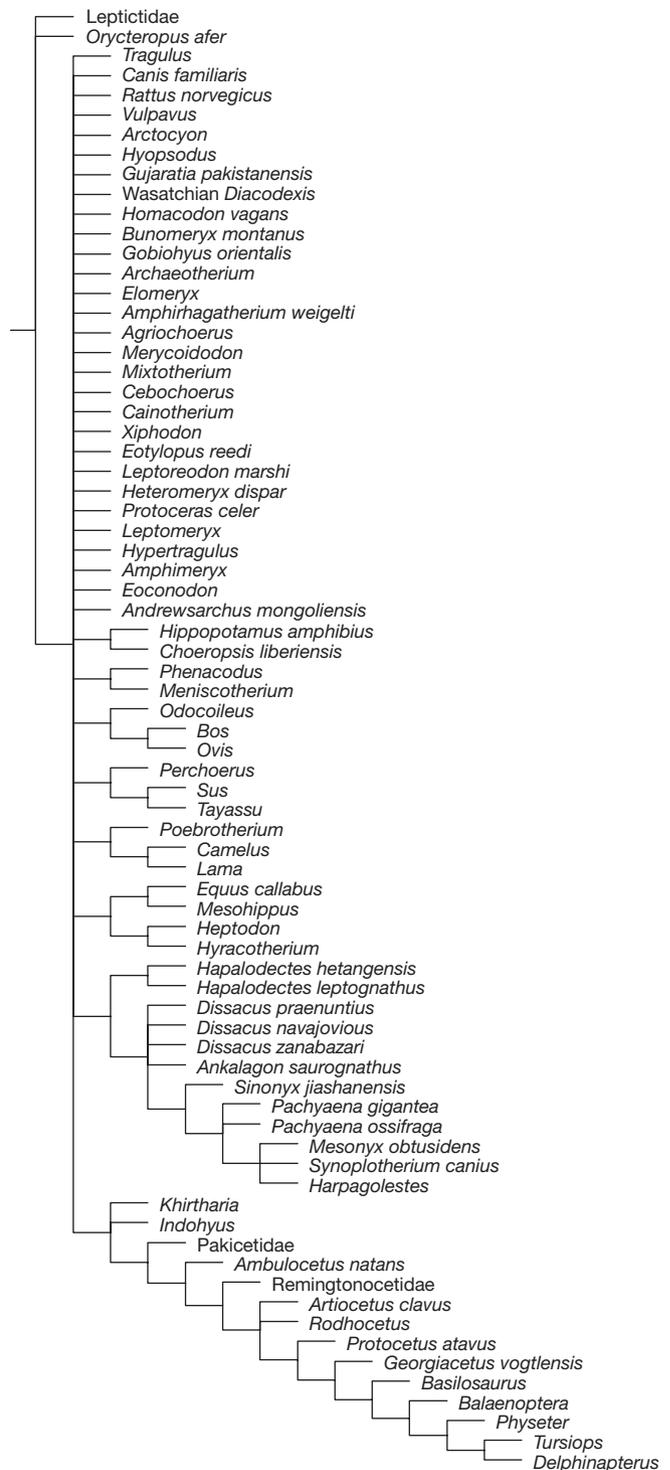
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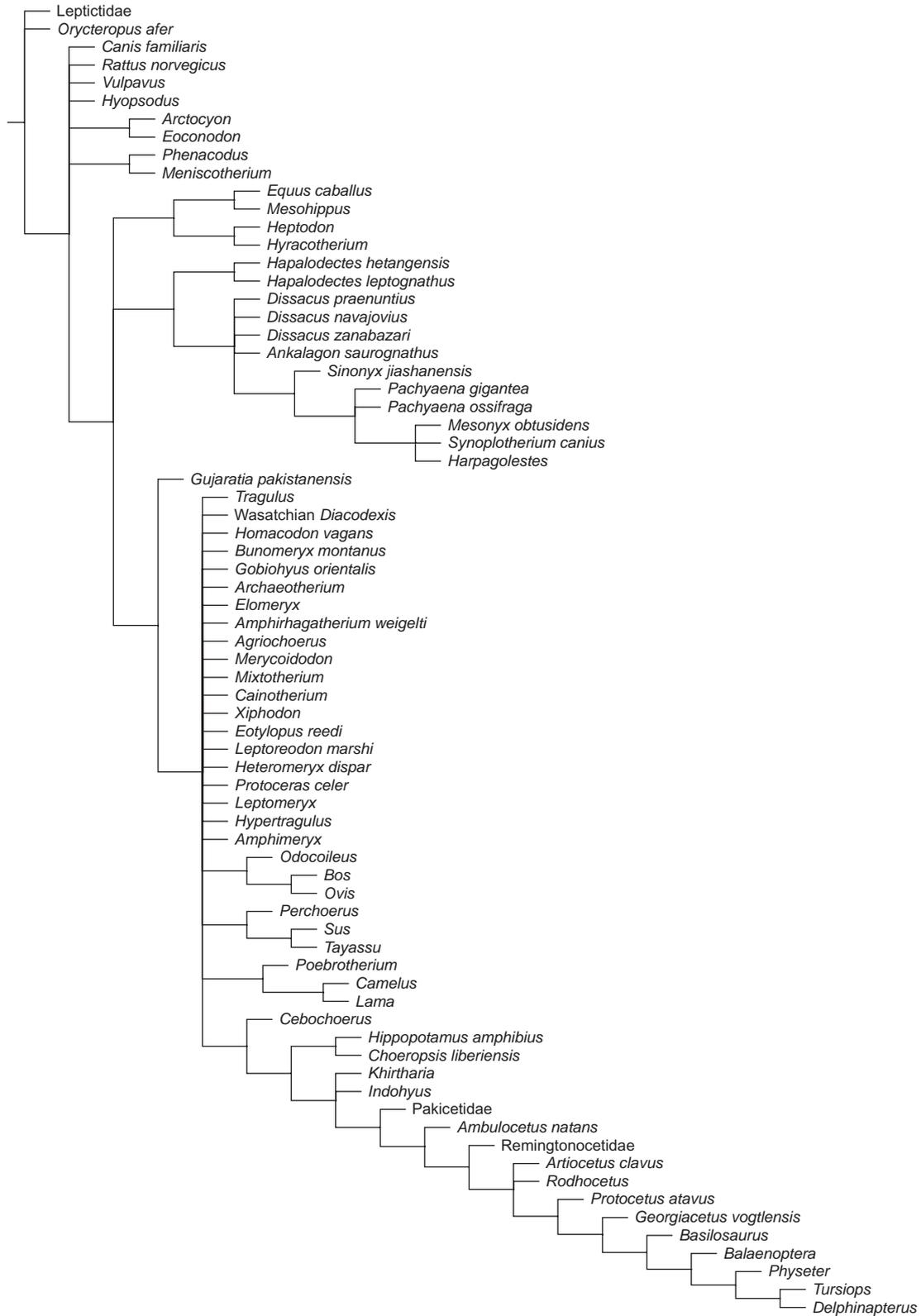
## APPENDIX

Three character codings for pakicetids from Thewissen *et al.*<sup>1</sup> were not followed here: character 38 was coded as state 1, character 142 also as state 1, and character 105 as state 2. These character codings are based on J.H.G.'s examination of pakicetid specimens currently housed at the Department of Anatomy, Northeastern Ohio Universities College of Medicine. The entire matrix used in the reanalysis and the tree files are available at <http://www.cetacea-evolution.org/main/>, which is supported by National Science Foundation grant DEB-0640361.

Phylogenetic analyses were conducted with the computer application TNT<sup>6</sup>. Two separate analyses were conducted: one with all characters given equal weight (Appendix Figs 1 and 2) and another that uses implied weighting (Appendix Fig. 3), which dynamically down-weights characters that show greater homoplasy during the analysis. The degree of down-weighting is set by the constant  $k$ , and the default value of 3 was used. Both analyses used the default settings for a 'New Technology Search', which uses sectorial searches and tree fusing. Exceptions to the defaults are that the heuristic searches were terminated when the minimum length was found 1,000 times and the maximum number of trees saved was set to 10,000.



**Appendix Figure 1 | Strict consensus of equal-weights parsimony analysis.** A total of 1,584 most parsimonious trees of 33,490 steps was found. The tree files can be found at <http://www.cetacea-evolution.org/main/> and the strict consensus of these trees is shown.



**Appendix Figure 2 | Reduced consensus of equal-weights parsimony analysis.** Much of the lack of resolution in the strict consensus (Fig. 1) can be attributed to differing positions for the extinct taxon *Andrewsarchus*. If it

is pruned from all 1,584 most parsimonious trees, then the reduced consensus is as shown.



**Appendix Figure 3 | Differential weights parsimony analysis.** The analysis using implied weights with  $k = 3$  found only one optimal tree, which is

shown. This tree is the basis for the simplified tree in the main text and has a fit of 2329.20679.

# Thewissen et al. reply

Replying to: J. H. Geisler & J. M. Theodor *Nature* 458, doi:10.1038/nature07776 (2009)

The analysis of Geisler and Theodor<sup>1</sup> confirms our main phylogenetic result<sup>2</sup>, that raellids are, or include, the sister group to cetaceans. Their study expands on our findings by inferring that hippopotamids are the sister group to the combined raellid–cetacean clade, whereas our paper had explicitly stated that our data could not address the position of the extant artiodactyl families.

Geisler and Theodor<sup>1</sup> place confidence in their results with regard to hippopotamids by stating that their analysis is consistent with “previous phylogenetic studies”. However, one of the two articles that they cite<sup>3</sup> was published after the publication of our paper, and they do not cite a recent paper<sup>4</sup> that disagrees with their (and our own) results.

Geisler and Theodor<sup>1</sup> execute two analyses, one with equally weighted characters and one with down-weighted characters (Appendix Fig. 3 of their paper). They state that in both analyses Hippopotamidae is the closest extant taxon to Cetacea, but their Appendix is inconsistent with this statement. In the Appendix, a large polychotomy lies at the base of the raellid–cetacean clade in the equally weighted analysis. This polychotomy dissolves when *Andrewsarchus* is excluded from the study, which results in a strict consensus tree where hippopotamids are indeed the sister group to the raellid–cetacean clade. Instead of discussing this consensus tree, Geisler and Theodor<sup>1</sup> mention that the equally weighted analysis yielded two most parsimonious trees: one showing hippopotamids as the sister group to the raellid–cetacean clade and the other showing (apparently undeleted) *Andrewsarchus* as more closely related to the raellid–cetacean clade than to hippopotamids. Notably, it took the deletion of *Andrewsarchus* before hippopotamids could be identified as the raellid–cetacean sister group in the unweighted analysis of the Appendix of Geisler and Theodor<sup>1</sup>; the text seems to identify *Andrewsarchus* as a possible close relative of hippopotamids and this is consistent with another recently published cladogram<sup>4</sup>. *Andrewsarchus* is poorly known, and this may result in its unstable phylogenetic position, although it may still be part of the hippopotamid–raellid–cetacean clade.

Geisler and Theodor<sup>1</sup> agree with us that the phylogenetic link between cetaceans and raellids is closer than that between cetaceans and hippopotamids, and we consider the former link to be more robust than the latter. We also believe that improving fossil collections of poorly known taxa is important in advancing understanding of cetacean relationships. Hippopotamids are certainly closely related to cetaceans, yet they appear about 35 million years after the first cetaceans, and their origins are in Africa, not the birthplace of cetaceans.

Geisler and Theodor<sup>1</sup> further elaborate on our results by discussing the origin of osteosclerosis of limbs and bulla, calling this process pachyostosis (for definitions, see refs 5, 6). Osteosclerosis, but not pachyostosis, of the limbs occurs in hippopotamids, raellids and paki-cetids, whereas pachyosteosclerosis of the bulla occurs in raellids and pakicetids, but not in hippopotamids. Experimental studies with lipoprotein-receptor-related proteins in transgenic mice suggest that pachyostosis and osteosclerosis are underlain by different genetic mechanisms<sup>7</sup>. The mechanisms that lead to such hyperostoses deserve further study, and such a study is necessary to test the speculation of Geisler and Theodor<sup>1</sup> that hyperostoses of the limbs and bulla are related. Such a study should be rooted in a sound understanding of the developmental controls of bone formation, a field that is growing rapidly<sup>8</sup> and that promises to elucidate cetacean evolution considerably.

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