

# Evolution of river dolphins

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The world's river dolphins (*Inia*, *Pontoporia*, *Lipotes* and *Platanista*) are among the least known and most endangered of all cetaceans. The four extant genera inhabit geographically disjunct river systems and exhibit highly modified morphologies, leading many cetologists to regard river dolphins as an unnatural group. Numerous arrangements have been proposed for their phylogenetic relationships to one another and to other odontocete cetaceans. These alternative views strongly affect the biogeographical and evolutionary implications raised by the important, although limited, fossil record of river dolphins. We present a hypothesis of river dolphin relationships based on phylogenetic analysis of three mitochondrial genes for 29 cetacean species, concluding that the four genera represent three separate, ancient branches in odontocete evolution. Our molecular phylogeny corresponds well with the first fossil appearances of the primary lineages of modern odontocetes. Integrating relevant events in Tertiary palaeoceanography, we develop a scenario for river dolphin evolution during the globally high sea levels of the Middle Miocene. We suggest that ancestors of the four extant river dolphin lineages colonized the shallow epicontinental seas that inundated the Amazon, Paraná, Yangtze and Indo-Gangetic river basins, subsequently remaining in these extensive waterways during their transition to freshwater with the Late Neogene trend of sea-level lowering.

**Keywords:** Cetacea; fossil; phylogeny; Odontoceti; Miocene; epicontinental seas

## 1. INTRODUCTION

Four genera of toothed cetaceans comprise the peculiar and poorly known 'river dolphins'. Although several marine delphinids are commonly found in rivers quite far upstream, river dolphins are morphologically and phylogenetically distinct from marine dolphins and most are restricted to freshwater ecosystems. Since the first suggestions of their affinities were advanced in the 19th century (Gray 1863; Flower 1867), the evolutionary relationship of river dolphins to one another and to other odontocetes has remained controversial (Simpson 1945; Kasuya 1973; Zhou 1982; Muizon 1984, 1988a; Fordyce & Barnes 1994; Messenger 1994; Rice 1998). Despite differing in detail, recent morphological systematic studies of modern and fossil taxa (Muizon 1988a,c, 1994; Heyning 1989; Messenger & McGuire 1998) largely corroborated earlier views that each extant lineage is relatively ancient and that river dolphins comprise an unnatural group. Non-monophyly of river dolphins is consistent with their highly disjunct geographical distributions (figure 1): the Amazon river dolphin, *Inia geoffrensis*, and the La Plata river dolphin, *Pontoporia blainvillei*, are found in South America; the Yangtze river dolphin, *Lipotes vexillifer*, and Indian river dolphin, *Platanista gangetica*, inhabit rivers on opposite sides of continental Asia. Placing the four river dolphin lineages within the evolutionary tree of cetaceans can help resolve the confused state of odontocete beta taxonomy (Heyning 1989; Fordyce *et al.* 1985; Fordyce & Barnes 1994; figure 2) and refine our understanding of odontocete evolution.

The difficulties of confronting river dolphin systematics using morphological analyses may relate directly to their

long, independent evolutionary histories. River dolphins are highly modified taxa that have more autapomorphies than shared characters useful for determining their affiliations (Messenger 1994). Furthermore, river dolphin classifications have often assumed monophyly (Simpson 1945; Kasuya 1973; Zhou 1982), although some characters used to unite river dolphins, such as an elongate rostrum and mandibular symphysis, may be primitive for odontocete cetaceans. When existing taxa are few and so distinctly modified that homologous characters are difficult to detect, the fossil record of the group should play an important role in resolving taxonomic relationships (Gauthier *et al.* 1988).

There are various fossil taxa related to extant genera, with the exception of *Lipotes*. Unfortunately, the record is not yet complete enough to determine key character polarities at intermediate stages. The fossil history of river dolphins has a long and confusing treatment in the literature, with many fossils described as members of taxonomic groups no longer recognized; a comprehensive re-examination is needed. A robust hypothesis of the relationships among extant lineages is critical for exploring the biogeographical and evolutionary implications of river dolphin fossils.

Higher-level molecular phylogenetic studies of cetaceans have primarily focused on the relationship between cetaceans and artiodactyls (Graur & Higgins 1994; Montelgard *et al.* 1997) and on the hypothesis of odontocete paraphyly (Milinkovitch *et al.* 1993; Hasegawa *et al.* 1997; Messenger & McGuire 1998). River dolphins were discussed in Arnason & Gullberg's (1996) cytochrome *b* phylogeny of cetaceans, which provided additional evidence for a distinct, though unresolved, position for *Platanista*. Two recent studies have specifically addressed river dolphin phylogeny using DNA sequence analysis. Yang & Zhou (1999) were the first to include all four

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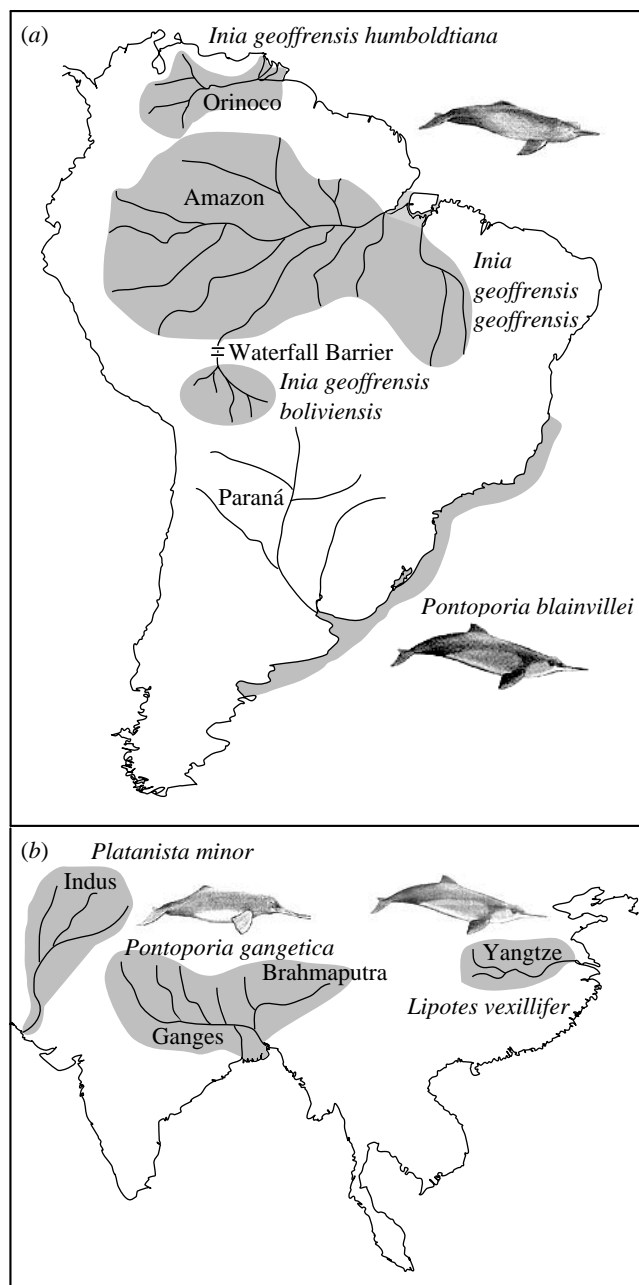


Figure 1. Geographical distribution of extant river dolphins. (a) *Inia geoffrensis humboldtiana* inhabits the Orinoco River system. *I.g. geoffrensis* is found throughout the mainstem Amazon River and its tributaries. *I.g. boliviensis* occurs in the Amazon tributaries of eastern Bolivia, geographically isolated by several hundred kilometres of rapids. *Pontoporia blainvillei* is restricted to coastal South Atlantic waters. (b) *Lipotes vexillifer* is an extremely endangered river dolphin that occurs only in the lower and middle reaches of the Yangtze River. *Platanista minor* inhabits the Indus River system. *P. gangetica* is found in the Ganges–Brahmaputra River system.

river dolphin taxa in a molecular phylogenetic analysis, but their limited data set of only 307 base pairs (bp) of the cytochrome *b* gene is insufficient to address the phylogeny of deeply diverging taxa. In contrast, the molecular phylogeny of Cassens *et al.* (2000) analyses five genes for 19 cetacean species, both nuclear and mitochondrial, yet even this large data set results in low bootstrap values for key nodes in river dolphin phylogeny, particularly under the maximum-likelihood criterion of molecular evolution.

With problematic phylogenies, for which odontocetes certainly qualify, it may be more useful to add taxa rather than to add characters (Hillis 1996; Graybeal 1998). Our approach has been to sample both extensively and broadly from within every primary lineage of odontocete.

Our objective is to reconstruct the evolutionary history of river dolphins. We begin by presenting a hypothesis of the phylogenetic relationships of extant river dolphins based on a multiple mitochondrial gene phylogeny of 29 species of cetaceans. We consider biogeographical and stratigraphical aspects of the fossil record of river dolphins in relation to our phylogenetic hypothesis. Integrating the palaeontological data with known events in Tertiary palaeoceanography, we conclude with a detailed scenario for the evolution of the world's river dolphins in the epicontinental seas of the Middle Miocene.

## 2. MATERIAL AND METHODS

Our data set is comprised of the complete cytochrome *b* (1140 bp), partial 12S (385 bp), and partial 16S (530 bp) mitochondrial genes, for 29 species broadly representative of each primary lineage of odontocete. In addition to sequences available from previous studies of cetacean molecular systematics (Milinkovitch *et al.* 1994; Arnason & Gullberg 1996; LeDuc *et al.* 1999), we sequenced either the ribosomal gene fragments and/or the complete cytochrome *b* for non-overlapping taxa. In all, we generated 44 new sequences (GenBank accession numbers AF334482–AF334525). We analysed sequences of *Inia* of known provenance from Brazil, Peru and Bolivia, as well as *Inia* from GenBank (accession number X92534; Arnason & Gullberg 1996), in order to evaluate the suggestion that the Bolivian form, *Inia geoffrensis boliviensis*, is distinct from *Inia geoffrensis geoffrensis* (da Silva 1994; Pilleri & Gühr 1977). The partial 12S sequence for *Lipotes vexillifer* was not available for this analysis. The mysticete outgroup consists of four species from three families. The taxa in this study, with tissue source, scientific and common names, are listed at the archived web pages of the University of California Museum of Paleontology ([www.ucmp.berkeley.edu/archdata/Hamiltonetal01/river.html](http://www.ucmp.berkeley.edu/archdata/Hamiltonetal01/river.html)), as are the primer sequences, gene sequences, and data set alignments.

Samples were obtained either by biopsy darting, from museum specimens, or from the Genetics Tissue Archive, Southwest Fisheries Science Center, La Jolla, CA, USA. DNA was extracted by standard phenol–chloroform/ethanol precipitation or with the QIAamp DNA extraction kit (Qiagen, Inc., Valencia, CA, USA). After an initial 2 min denaturation at 94 °C, PCR consisted of 35 cycles, 30 s at 94 °C, 45 s at 48–52 °C and 90 s at 72 °C. The products were visualized, cleaned and directly sequenced in both directions on an ABI 377 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were edited with Sequencher v. 3.0 sequence analysis software (GeneCodes Corporation, Ann Arbor, MI, USA) and aligned manually in BioEdit 4.7.8 (Tom Hall). Four sites of ambiguous alignment in the 16S gene were excluded.

All phylogenetic analyses were carried out using PAUP 4.0b3a (Swofford 2000). Tree searches were conducted with optimality criteria of parsimony and maximum likelihood. Twenty replicate searches were made for the maximum-likelihood tree, assuming the HKY85 model of nucleotide evolution (Hasegawa *et al.* 1985) with a transition to transversion (Ti:Tv) ratio of 6.0 and a gamma

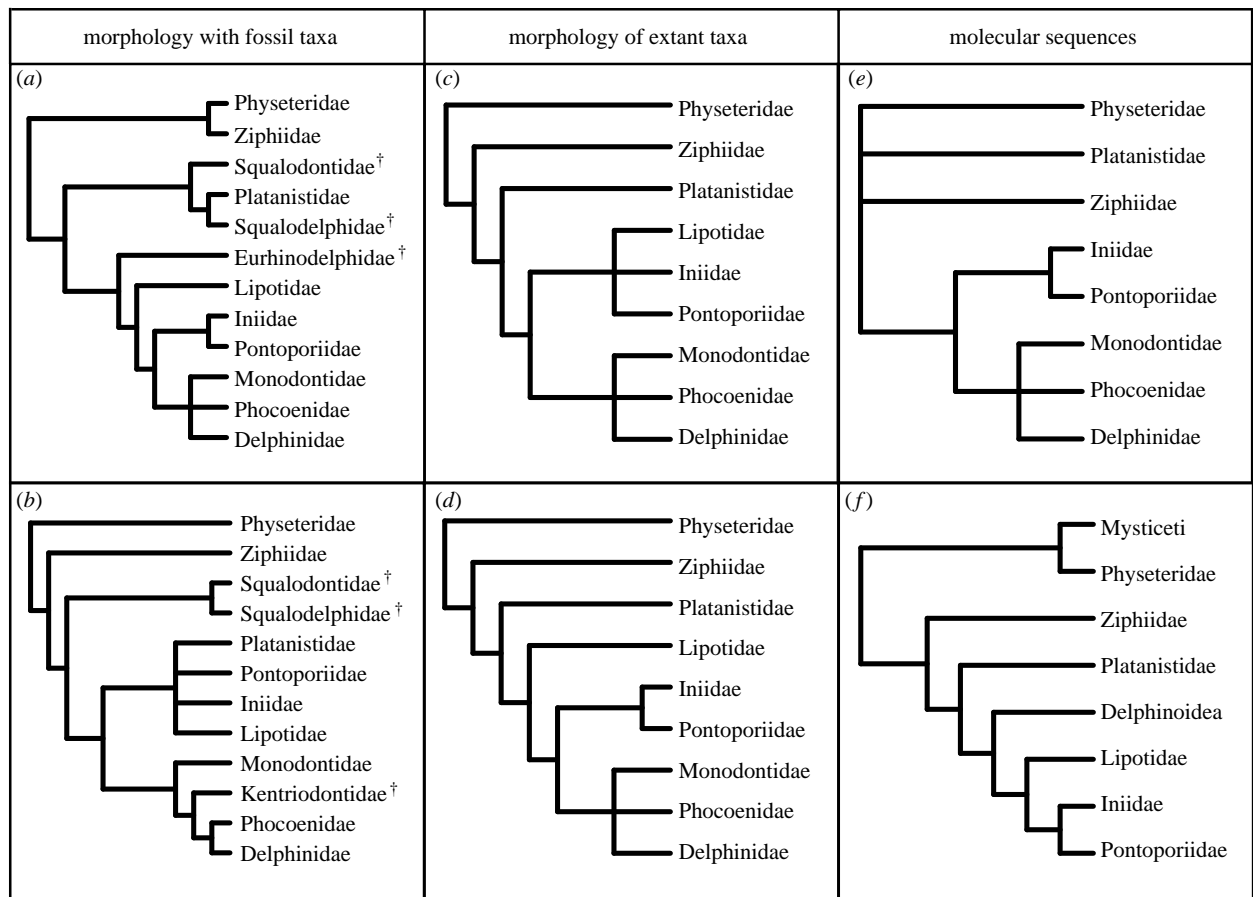


Figure 2. Alternative hypotheses of odontocete phylogeny. Some endings have been emended to standardize taxonomic comparisons. (a) Muizon (1988a, 1991), (b) Barnes (1990); (c) Heyning (1989), (d) Messenger & McGuire (1998); (e) Arnason & Gullberg (1996), (f) Yang & Zhou (1999).

shape parameter of 0.2. The assumed ratio of Ti:Tv and the shape of the distribution of substitution rates were estimated under the criterion of likelihood using trees obtained by both neighbour joining and unweighted parsimony. Parsimony searches (with 1000 replicates) were carried out with a range of differential weighting to assess the impact of these corrections on tree topology. Two bootstrap analyses were performed, one with trees found by neighbour joining (with Jukes–Cantor corrected distances) and one with trees obtained using weighted parsimony (transversions counting six times as much as transitions). Finally, support indices were calculated for each node present in the weighted parsimony analysis (Bremer 1988).

### 3. RESULTS

The maximum-likelihood tree and the consensus of three most parsimonious trees are largely congruent (figure 3). The Physeteridae, represented by *Physeter* and *Kogia*, are basal odontocetes and do not form a clade with Ziphiidae, the beaked whales, contradicting some classifications (Fordyce 1994; Muizon 1991). The long-suspected polyphyly of river dolphins is supported by the mitochondrial sequence data. In both trees, *Platanista gangetica* and *Platanista minor*, representing Platanistidae, are sister to the remaining odontocetes, although bootstrap support for this node is low. The remaining river dolphin taxa (*Lipotes*, *Inia* and *Pontoporia*) are paraphyletically arranged at the base of a well-supported clade that also includes

porpoises, monodontids and modern dolphins, essentially Muizon's concept of the Infraorder Delphinida (Muizon 1988a, 1991). In both analyses, beaked whales compose the sister group to Delphinida (Heyning 1989). The data indicate that non-platanistid river dolphins are the extant representatives of early lineages that diverged from the stem leading to Delphinoidea (porpoises, monodontids and dolphins), supporting their ranking as separate families. Our analysis suggests *Inia* and *Pontoporia* are monophyletic and together form the sister group of Delphinoidea (Muizon 1984), and suggests a distinction between the Bolivian and Amazon forms of *Inia*. The two analyses yield contradicting hypotheses for the relationships within Delphinoidea. The maximum-likelihood tree indicates that porpoises and marine dolphins form a clade, while the weighted parsimony tree groups porpoises with monodontids, a view recently advanced (Waddell *et al.* 2000).

### 4. DISCUSSION

The phylogenetic relationships of river dolphins suggested by our analysis allows for a refined understanding of odontocete systematics and evolution, a long-elusive goal. Just as the extensive adaptations involved in the transition from land mammal to aquatic mammal have obscured cetacean origins, each primary odontocete lineage exhibits a suite of highly derived characters

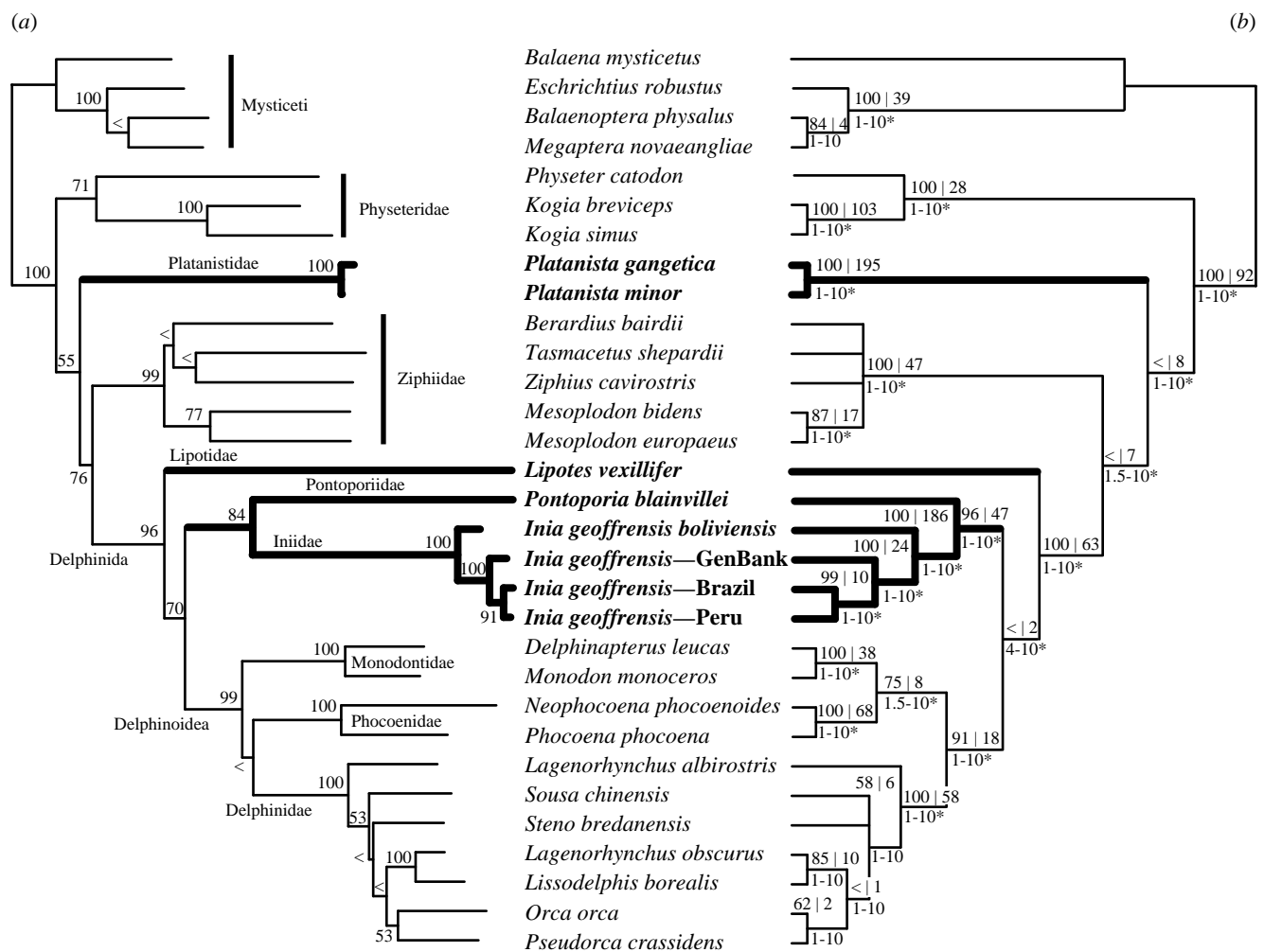


Figure 3. Optimal trees under the criteria of (a) maximum likelihood and (b) parsimony. The maximum-likelihood tree was obtained by carrying out 20 replicate heuristic searches, assuming the HKY85 model of nucleotide evolution with a transition to transversion ratio of 6.0 and a gamma shape parameter of 0.2. Bootstrap values (derived from 1000 replicates of neighbour-joining searches using Jukes–Cantor corrected distances) are shown at the nodes. Values less than 50 are denoted by '<'. The tree to the right is the consensus of three most parsimonious trees of length 5416 found with 1000 replicate heuristic searches. Transversions were weighted six times as heavily as transitions. Above each node are parsimony bootstrap values (1000 replicates) and Bremer support indices, separated by a vertical bar. The range of transition to transversion weighting (from equal to ten times, as well as transversions only, denoted by an asterisk) that yields each clade is reported below each corresponding node. The GenBank accession number for 'Inia-GenBank' is X92534 (Arnason & Gullberg 1996).

without clear evidence of sequential forms. Thus alpha taxonomic assignments are considerably less controversial than higher-level systematics. River dolphins provide an extreme example. Although the generic designations are not disputed, their taxonomic ranks are undecided, and many possible combinations of their interrelationship have been proposed (figure 2). Similarly, the phylogenetic affinities of the remaining odontocete lineages are also unresolved (Heyning 1989; Rice 1998). The placement of the river dolphins among these lineages, as indicated by our molecular analysis, suggests a resolution that is notably concordant with the first appearance of these groups in the fossil record (figure 4).

#### (a) *The fossil record of river dolphins*

The fossil record of pelagic animals is understandably limited. Fossil cetaceans are primarily recovered from rocks that formed in nearshore and continental-shelf depositional environments, and only rarely from deep-sea

settings. During episodes of low sea level, nearshore sediments are eroded, abridging the record. Archaic forms disappear and more advanced groups emerge in successive waves with no clear origins. Many fossil cetaceans are known from single specimens, numerous taxa have been erected on the basis of undiagnostic, isolated or fragmentary bones, and the classification history of extinct cetaceans is long and bewildering. A confident grasp of modern phylogeny will help clarify the relationships of past to present taxa.

Extinct taxa assigned to the Platanistidae are well documented, particularly *Zarhachis* and *Pomatodelphis*, long-beaked Middle to Late Miocene cetaceans recovered primarily from shallow epicontinental sea deposits of the Atlantic coast of North America (Kellogg 1959; Gottfried *et al.* 1994; Morgan 1994; table 1). Possible platanistid relatives are Squalodelphinidae and at least some members of Squalodontidae (Muizon 1994; Fordyce 1994), two well-known, extinct families of archaic, medium-sized

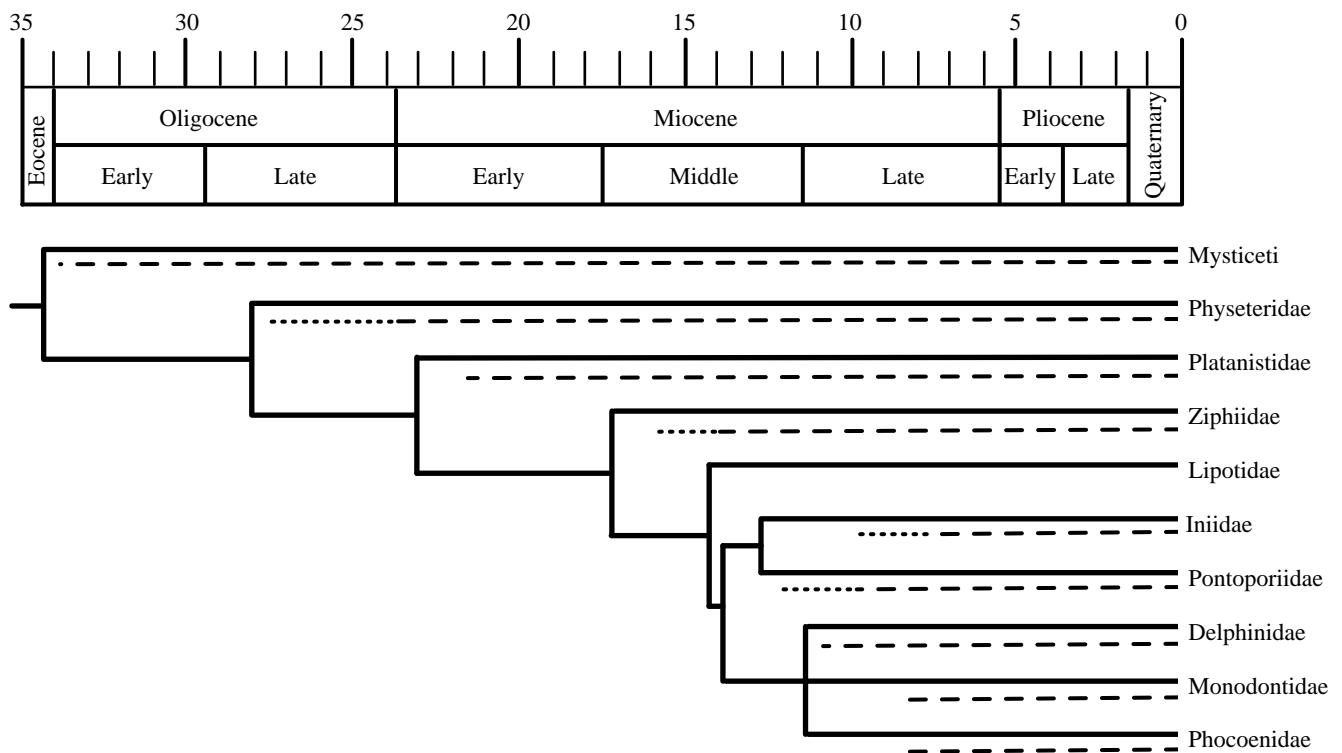


Figure 4. General correspondence between the hypothesized phylogeny and fossil record of Odontoceti. Finer dotting indicates the uncertain dates for some earliest fossil occurrences. Lipotidae is the only clade for which fossils are not yet definitively known.

heterodonts. Other fossil relatives of the Platanistidae include members of the Dalpiaziniidae (Muizon 1994) and Waipatiidae (Fordyce 1994, p. 147). If these lineages are monophyletic, then *Platanista* is the sole extant member of a once-abundant and diverse clade of archaic odontocetes. The side-swimming, blind and highly endangered Indian river dolphin has long been recognized as 'the genus... presenting the greatest total of modifications known in any cetacean' (Miller 1923, p. 41). Both fossil and extant platanistids warrant further investigation for potential insights into cetacean evolution.

The assignment of fossil taxa within non-platanistid river dolphins has been misdirected by inaccurate concepts of the systematic relationship of extant taxa. In most earlier classifications, *Inia* and *Lipotes* were placed together in Iniidae, while *Pontoporia* (*Stenodelphis* in earlier works) was sometimes classified within Delphinidae, the marine dolphins (Miller 1923). For over a century, this concept of Iniidae was a repository for early dolphin-like fossil odontocetes (Kellogg 1944; Rensberger 1969; Wilson 1935). With the description of *Parapontoporia* (Barnes 1984, 1985), an extinct genus considered intermediate between *Lipotes* and *Pontoporia*, subsequent classifications sometimes placed *Lipotes* in the Pontoporiidae (Fordyce & Barnes 1994). Systematic revision and more rigorous diagnosis of fossil taxa leave the majority of generalized small odontocetes outside of Lipotidae, Iniidae and Pontoporiidae. The Lipotidae have essentially no fossil record. A single mandibular fragment from freshwater sediments in southern China, known as *Prolipotes* and tentatively dated as Miocene (Zhou *et al.* 1984), cannot be confirmed as a Lipotid. Both Iniidae and Pontoporiidae are represented by South American fossil relatives

(table 1). With the placement of most previously described 'iniids' in other extinct groups (Muizon 1988b; Cozzuol 1996), the family may be regarded as a freshwater South American endemic. The partial skull, rostral and mandibular fragments known as *Goniodelphis*, from the Early Pliocene Palmetto Fauna of central Florida, are the only fossil remains outside South America that can be considered plausibly as Iniidae (Morgan 1994). However, Muizon (1988b) regarded this material as too incomplete for a confident determination. Significantly, both fossil genera clearly assigned to Iniidae, *Ischyrorhynchus* and *Sauroctes*, are found far south of *Inia*'s present range, occurring only in the fluvial Late Miocene Ituzaingó formation of the Paraná basin, Argentina (with the possible exception of fragmentary mandibular remains reported from Brasil; Rancy *et al.* 1989). The Pontoporiidae have a broader geographical and geological range. Three species of *Parapontoporia* have been described from nearshore shallow water deposits of California and Baja California (Barnes 1985). The members in this Northern Hemisphere genus have been placed in their own subfamily, Parapontoporiinae, based on their asymmetrical cranial vertices. The subfamily Pontoporiinae, identified by symmetrical cranial vertices, is restricted to the Southern Hemisphere. Two fossil genera have been described from the Pisco formation of southern coastal Peru, the Pliocene *Pliopontos*, very similar to *Pontoporia*, and the geologically youngest occurrence of the family, the Middle Miocene *Brachydelphis* (Muizon 1983, 1988c). Another fossil, the Late Miocene *Pontistes*, is found in the Paraná formation, marine sediments of the Paraná basin, Argentina, underlying and adjacent to those with fossil iniids (Cozzuol 1985).

Table 1. *Identification and stratigraphy of fossil river dolphins*

taxon	location	stratigraphy: formation/age	reference
family Platanistidae			
<i>Zarhachis</i>	Maryland	Calvert Formation/Middle Miocene	Kellogg (1924); Gottfried <i>et al.</i> (1994)
<i>Pomatodelphis</i>	Florida	Agricola Fauna, Bone Valley/ Middle Miocene	Kellogg (1959); Morgan (1994)
family Lipotidae			
<i>Proliptotes</i> (?)	Southern China	Miocene (?)	Zhou <i>et al.</i> (1984)
family Pontoporiidae			
<i>Brachydelphis</i>	coastal Peru	Pisco Formation/Middle Miocene	Muizon (1988c)
<i>Pliopontos</i>	coastal Peru	Pisco Formation/Early Pliocene	Muizon (1983), (1984)
<i>Pontistes</i>	Argentina	Paraná Formation/Late Miocene	Cozzuol (1985), (1996)
<i>Parapontoporia</i>	California, Mexico	San Diego/Late Pliocene; Almejas/ Late Miocene	Barnes (1984), (1985)
family Iniidae			
<i>Ischyrorhynchus</i>	Argentina	Ituzaingó Formation/Late Miocene	Cozzuol (1985), (1996)
<i>Saurocetes</i>	Argentina	Ituzaingó Formation/Late Miocene	Cozzuol (1988), (1996)
<i>Goniodelphis</i> (?)	Florida	Palmetto Fauna, Bone Valley/ Late Miocene	Morgan (1994)

### (b) *The evolution of river dolphins*

The Middle Miocene was a time of globally high sea levels, with three significant marine transgressive–regressive cycles recorded worldwide (Haq *et al.* 1987). With the resulting large-scale marine transgressions on to low-lying regions of the continents, shallow epicontinental seas became prominent marine ecosystems. The Indo-Gangetic plain of the Indian subcontinent, the Amazon and Paraná river basins of South America, and the Yangtze river basin of China are vast geomorphic systems whose fluvio-deltaic regions were penetrated deeply by marine waters during high sea-level stands. The shallow estuarine regions created by the mixing of riverine and marine waters probably supported diverse food resources, particularly for aquatic animals able to tolerate osmotic differences between fresh and saltwater systems. We propose that the ancestors of the four extant river dolphin taxa were inhabitants of Miocene epicontinental seas. Draining of the epicontinental seas and reduction of the nearshore marine ecosystem occurred with a Late Miocene trend of sea-level regression, which continued throughout the Pliocene, interrupted by only moderate and relatively brief events of sea-level rise (Hallam 1992). As sea levels fell, these archaic odontocetes survived in river systems, while their marine relatives were superseded by the radiation of Delphinoidea. Cassens *et al.* (2000) also noted the persistence of river dolphins during the radiation of delphinoids. They suggest that extant river dolphin lineages ‘escaped extinction’ by adaptation to their current riverine habitats. All extant organisms have escaped extinction by being adequately adapted to their present circumstances. By integrating phylogenetic, palaeoceanographic and fossil data, we provide an explicit hypothesis for the evolution and modern distribution of river dolphins.

The Indo-Gangetic foreland basin is a broad, flat plain of sediment delivered throughout the Cenozoic by an intricate network of migrating rivers descending from the tectonically dynamic Himalayan mountains (Burbank *et al.* 1996). The increased sea levels of the Middle

Miocene would have inundated large areas of the foreland basin, creating a shallow marine habitat. Fossils have not yet been recovered from these regions, but platanistids are known to have inhabited Miocene epicontinental seas in North America (table 1; Morgan 1994; Gottfried *et al.* 1994). *Platanista* is the only surviving descendant of an archaic odontocete that ventured into the epicontinental seas of the Indo-Gangetic basin, and remained through its transition to an extensive freshwater ecosystem during the Late Neogene trend of sea-level regression. Although the palaeogeography of the two river systems would suggest a history of isolation, the genetic distance we observed in our small sample of *P. gangetica* and *P. minor* is surprisingly low (figure 3).

Several lines of evidence suggest Miocene marine incursions penetrated deeply into continental South America (Hoorn *et al.* 1995; Lovejoy *et al.* 1998). To the north, incursions were along the course of the Amazon river palaeodrainage (Hoorn 1994), and to the south, into the Paraná river basin (Cozzuol 1996). During the highest global stand of Miocene sea levels, the Paraná and Amazon river basins may have been connected, forming an interior seaway that divided the continent, termed the Paranense Sea (Von Ihering 1927). The largely ignored hypothesis of the Paranense Sea is supported by sedimentological data (Räsänen *et al.* 1995) and biogeographical data from foraminifera (Boltovskoy 1991) and molluscs (Nuttall 1990). The existence of the Paranense Sea is consistent with the distribution of both modern and fossil South American river dolphin taxa.

We hypothesize that the dolphins entered the seaway from the north, diversified within its complex fluvial–estuarine–marine system, and colonized its farthest reaches, to the south-west Atlantic Ocean. Lowering of global sea levels drained the inland sea, separating the northern and southern river basins, and isolating the taxa. Iniid ancestors remained in the immense Amazon basin, which was developing its modern transcontinental aspect with the uplift of the Venezuelan Andes and clockwise rotation of its palaeodrainage (Hoorn *et al.* 1995). *Inia*

evolved during the Amazon's transformation to a freshwater system of extraordinary size, diversity and abundance. The Paraná river basin is a fraction of the size of its northern counterpart. The iniid fossil genera *Ischyrorhynchus* and *Sauroctes*, found along the banks of the Rio Paraná, belong to genera that disappeared with the retreat of the continental sea ecosystem. *Pontoporia* followed the marine waters receding from the Paraná basin to colonize the nearshore coastal zone north and south of the La Plata estuary.

Parts of eastern and southern China are low-lying deltaic regions formed of sediments deposited by the area's river systems, such as the Yangtze and the Yujiang. Significant sea-level rise would transform these regions into shallow waterways of mixed fluvial and marine origin. Several fossil locales in nearby Japan confirm the presence of odontocetes in the western Pacific during the Miocene (Ichishima *et al.* 1995), potential colonizers of the Asian epicontinental seas. Our scenario is consistent with the geographical occurrence of the mandibular fragment known as *Prolipotes*, inland of the Yujiang river delta in southern China. If our phylogenetic interpretation is correct, then non-platanistid river dolphins are paraphyletic, and *Lipotes*, like *Platanista*, is the sole surviving taxon of a deeply divergent branch in cetacean evolution.

The ancestry of non-platanistid river dolphins might be found in the progenitors of one of two well-known groups of fossil cetaceans. Eurhinodelphinids were long-beaked, medium-sized odontocetes, sometimes encountered as the dominant vertebrates in Miocene marine fossil formations. In the Tarkarooloo Basin of the Lake Frome region of Southern Australia, eurhinodelphinid fossils from several distinct horizons of the Middle Miocene Namba formation record the adaptation of at least one member of this group to a freshwater environment (Fordyce 1983). Kentriodontids were small to medium-sized odontocetes that are probably basal delphinoids (Barnes 1990). Both groups were widespread, and both have a fossil record extending from the late Oligocene to the Late Miocene. Significantly, some fossil specimens now classified as either kentriodontids or eurhinodelphinids were first described as iniids (Kellogg 1955; Rensberger 1969). Neither eurhinodelphinids nor kentriodontids are likely to have given rise to non-platanistid river dolphins, as each group is diagnosed based on their distinctive morphologies. Nevertheless, a small, long-beaked, polydont Oligocene ancestor of either extinct group is a plausible progenitor of extant Delphinida (*sensu* Muizon). A re-evaluation of both Kentriodontidae and Eurhinodelphinidae in light of our revised understanding of river dolphin phylogeny should provide further insights into the evolution of marine and freshwater odontocetes.

This research was supported by the Remington Kellogg Fund of the Museum of Paleontology, University of California, Berkeley, and the International Fund for Animal Welfare (both to H.H.). A.G.C. is supported by NSF grant EAR-9814845. For generous facilitation of access to tissue samples, we are indebted to B. Curry and K. Robertson at the Southwest Fisheries Science Center, La Jolla, California, to N. Bernal and L. Villalba of the National Museum of Natural History, La Paz, Bolivia, and to J. Mead at the Smithsonian Institution, Washington, DC. R. LeDuc and E. Archer of the Southwest Fisheries Science Center are gratefully acknowledged for cyt *b* PCR primers and

their guidance regarding technical advice. We thank D. Lindberg and J. Lipps for access to the facilities of the Molecular Phylogenetics Laboratory, U.C. Berkeley. The detailed comments of two anonymous reviewers greatly improved the manuscript, which also benefited from the thoughtful comments of J. W. Valentine. This is UCMP publication 1733.

## REFERENCES

- Arnason, U. & Gullberg, A. 1996 Cytochrome *b* nucleotide sequences and the identification of five primary lineages of extant cetaceans. *Mol. Biol. Evol.* **13**, 407–417.
- Barnes, L. G. 1984 Fossil odontocetes from the Almejas formation, Isla Cedros, Mexico. *Paleobios* **42**, 1–46.
- Barnes, L. G. 1985 Fossil pontoporiid dolphins from the Pacific Coast of North America. *Contrib. Sci. Nat. Hist. Mus. LA County* **363**, 1–34.
- Barnes, L. G. 1990 The fossil record and evolutionary relationship of the genus *Tursiops*. In *The bottlenose dolphin* (ed. S. Leatherwood & R. Reeves), pp. 3–26. San Diego, CA: Academic Press.
- Boltovskoy, E. 1991 Ihering's hypothesis in the light of foraminiferal data. *Lethaia* **24**, 191–198.
- Bremer, K. 1988 The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42**, 795–803.
- Burbank, D. W., Beck, R. A. & Mulder, T. 1996 The Himalayan foreland basin. In *The tectonic evolution of Asia* (ed. A. Yin & M. Harrison), pp. 149–188. Cambridge University Press.
- Cassens, I. (and 12 others) 2000 Independent adaptation to riverine habitats allowed survival of ancient lineages. *Proc. Natl Acad. Sci. USA* **97**, 11343–11347.
- Cozzuol, M. A. 1985 The Odontoceti of the 'Mesopotamiense' of the Paraná River ravines. *Invest. Cetacea* **17**, 39–54.
- Cozzuol, M. A. 1988 Una nueva especie de *Saurodelphis* Burmeister, 1891. *Ameghiniana* **25**, 39–45.
- Cozzuol, M. A. 1996 The record of aquatic mammals in southern South America. *Munchner Geowissenschaftliche Abhandlungen* **30**, 321–342.
- da Silva, V. M. F. 1994 Aspects of the biology of the Amazon dolphins genus *Inia* and *Sotalia fluviatilis*. Ph.D. dissertation, St John's College, Cambridge, UK.
- Flower, W. H. 1867 Description of the skeleton of *Inia geoffrensis*. *Trans. Zool. Soc. Lond.* **6**, 87–116.
- Fordyce, R. E. 1983 Rhabdosteid dolphins from the Middle Miocene, Lake Frome Area, South Australia. *Alcheringa* **7**, 27–40.
- Fordyce, R. E. 1994 *Waipatia maerewhenua*, new genus and new species, an archaic late Oligocene dolphin. *Proc. San Diego Soc. Nat. Hist.* **29**, 147–176.
- Fordyce, R. E. & Barnes, L. G. 1994 The evolutionary history of whales and dolphins. *A. Rev. Earth Planet. Sci.* **22**, 419–455.
- Fordyce, R. E., Barnes, L. G. & Miyazaki, N. 1995 General aspects of the evolutionary history of whales and dolphins. *Island Arc* **3**, 373–391 (for 1994).
- Gauthier, J., Kluge, A. G. & Rowe, T. 1988 Amniote phylogeny and the importance of fossils. *Cladistics* **4**, 105–209.
- Gottfried, M. D., Bohaska, D. J. & Whitmore Jr, F. C. 1994 Miocene cetaceans of the Chesapeake group. *Proc. San Diego Soc. Nat. Hist.* **29**, 229–238.
- Graur, D. & Higgins, D. G. 1994 Molecular evidence for the inclusion of cetaceans within the order Artiodactyla. *Mol. Biol. Evol.* **11**, 357–364.
- Gray, J. E. 1863 On the arrangement of the cetaceans. *Proc. Zool. Soc. Lond.* pp. 197–202.
- Graybeal, A. 1998 Is it better to add taxa or characters to a difficult phylogenetic problem? *Syst. Biol.* **47**, 9–17.
- Hallam, A. 1992 *Phanerozoic sea-level changes*. New York: Columbia University Press.
- Haq, B. H., Hardenbol, J. & Vail, P. R. 1987 Chronology of fluctuating sea levels since the Triassic. *Science* **235**, 1156–1162.

- Hasegawa, M., Kishino, H. & Yano, T. 1985 Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* **22**, 160–174.
- Hasegawa, M., Adachi, J. & Milinkovitch, M. C. 1997 Novel phylogeny of whales supported by total molecular evidence. *J. Mol. Evol.* **44**(Suppl. 1), 117–120.
- Heyning, J. E. 1989 Comparative facial anatomy of beaked whales (Ziphiidae) and a systematic revision among the families of extant Odontoceti. *Contrib. Sci. Nat. Hist. Mus. LA County*. **405**, 1–64.
- Hillis, D. M. 1996 Inferring complex phylogenies. *Nature* **383**, 130–131.
- Hoorn, C. 1994 An environmental reconstruction of the palaeo-Amazon River system (Middle–Late Miocene, NW Amazonia). *Palaeogeog. Palaeoclim. Palaeoecol.* **112**, 187–238.
- Hoorn, C., Guerrero, J., Sarmiento, G. & Lorente, M. 1995 Andean tectonics as a cause for changing drainage patterns in Miocene northern South America. *Geology* **23**, 237–240.
- Ichishima, H., Barnes, L. G., Fordyce, R. E., Kimura, M. & Bohaska, D. J. 1995 A review of kentriodontine dolphins: systematics and biogeography. *Island Arc* **3**, 486–492 (for 1994).
- Kasuya, T. 1973 Systematic consideration of Recent toothed whales based on the morphology of the tympano-periotic bone. *Sci. Rep. Whales Res. Inst.* **24**, 87–108.
- Kellogg, A. R. 1924 A fossil porpoise from the Calvert Formation of Maryland. *Proc. U.S. Natl Mus.* **63**, 1–14.
- Kellogg, A. R. 1928 The history of whales—their adaptation to life in the water. *Q. Rev. Biol.* **3**, 29–76, 174–208.
- Kellogg, A. R. 1944 Fossil cetaceans from the Florida Tertiary. *Bull. Mus. Comp. Zool.* **94**, 433–471.
- Kellogg, A. R. 1955 Three Miocene porpoises from the Calvert Cliffs, Maryland. *Proc. U.S. Natl Mus.* **105**, 101–154.
- Kellogg, A. R. 1959 Description of the skull of *Pomatodelphis inaequalis*. *Bull. Mus. Comp. Zool.* **121**, 1–26.
- LeDuc, R. G., Perrin, W. F. & Dizon, A. E. 1999 Phylogenetic relationships among the delphinid cetaceans based on full cytochrome *b* sequences. *Mar. Mamm. Sci.* **15**, 619–648.
- Lovejoy, N. R., Bermingham, E. & Martin, A. P. 1998 Marine incursions into South America. *Nature* **396**, 421–422.
- Messenger, S. 1994 Phylogenetic relationships of platanistoid river dolphins: assessing the significance of fossil taxa. *Proc. San Diego Soc. Nat. Hist.* **29**, 125–133.
- Messenger, S. & McGuire, J. 1998 Morphology, molecules, and the phylogenetics of cetaceans. *Syst. Biol.* **47**, 90–124.
- Milinkovitch, M. C., Orti, G. & Meyer, A. 1993 Revised phylogeny of whales suggested by mitochondrial ribosomal DNA sequences. *Nature* **361**, 346–348.
- Milinkovitch, M. C., Meyer, A. & Powell, J. R. 1994 Phylogeny of all major groups of cetaceans based on DNA sequences from 3 mitochondrial genes. *Mol. Biol. Evol.* **11**, 939–948.
- Miller, G. S. 1923 The telescoping of the cetacean skull. *Smith. Misc. Coll.* **76**, 1–70.
- Montgelard, C., Catzeflis, F. M. & Douzery, E. 1997 Phylogenetic relationships of artiodactyls and cetaceans as deduced from the comparison of cytochrome *b* and 12S rRNA mitochondrial sequences. *Mol. Biol. Evol.* **14**, 550–559.
- Morgan, G. S. 1994 Miocene and Pliocene marine mammal faunas from the Bone Valley formation of central Florida. *Proc. San Diego Soc. Nat. Hist.* **29**, 239–268.
- Muizon, C. de 1983 *Pliopontos littoralis*, un nouveau Platanistidae Cetacea du Pliocène de la côte péruvienne. *C. R. Acad. Sci. Paris*, **296**, 625–628.
- Muizon, C. de 1984 Les vertébrés fossiles de la Formation Pisco (Pérou) II. Les odontocètes du Pliocène Inférieur de Sud-Sacaco. *Edit. Rech. Sur les Civil. Mem.* **50**, 1–188.
- Muizon, C. de 1988a Les relations phylogénétiques des Delphinida. *Ann. Paleon.* **74**, 157–227.
- Muizon, C. de 1988b Le polyphylétisme des Acrodelphidae, odontocètes longirostres du Miocène européen. *Bull. Mus. Natl Hist. Nat. C 4 Ser.* **10**, 31–88.
- Muizon, C. de 1988c Les vertébrés fossiles de la Formation Pisco (Pérou). III. Les odontocètes du Miocène. *Edit. Rech. Sur les Civil. Mem.* **78**, 1–244.
- Muizon, C. de 1991 A new Ziphiidae from the Early Miocene of Washington state and a phylogenetic analysis of the major groups of odontocetes. *Bull. Mus. Natl Hist. Nat. Paris* **3–4**, 279–326.
- Muizon, C. de 1994 Are the squalodontids related to the platanistoids? *Proc. San Diego Soc. Nat. Hist.* **29**, 135–146.
- Nuttall, C. P. 1990 A review of the Tertiary non-marine molluscan faunas of the Pebasian and other inland basins of northwestern South America. *Bull. Br. Mus. Nat. Hist. Geol.* **45**, 165–371.
- Pilleri, G. & Gühr, M. 1977 Observations on the Bolivian (*Inia boliviensis* d'Orbigny, 1834) and the Amazonian bufeo (*Inia geoffrensis* de Blainville, 1817) with description of a new subspecies (*Inia geoffrensis humboldtiana*). *Invest. Cetacea* **8**, 11–76.
- Rancy, A., Boquetin Villanueva, J., Pereira de Souza Filho, J., Santos, J. C. R. & Negri, F. R. 1989 Lista preliminar da fauna do Neógeno da região oriental. *Ameghiniana* **26**, 249.
- Räsänen, M., Linna, A., Santos, J. & Negri, F. 1995 Late Miocene tidal deposits in the Amazonian foreland basin. *Science* **269**, 386–389.
- Rensberger, J. M. 1969 A new iniid cetacean from the Miocene of California. *Univ. Calif. Publ. Geol. Sci.* **82**, 1–43.
- Rice, D. W. 1998 *Marine mammals of the world*. Special Publication no. 4. Lawrence, KS: Marine Mammal Society.
- Simpson, G. G. 1945 The principles of classification and a classification of mammals. *Bull. Am. Mus. Nat. Hist.* **85**, 1–350.
- Swofford, D. L. 2000 *PAUP\*: Phylogenetic analysis using parsimony (\*and other methods)*. Sunderland, MA: Sinauer Associates.
- Von Ihering, H. 1927 *Die Geschichte des Atlantischen Ozeans*. Jena, Germany: Gustav Fischer.
- Waddell, V., Milinkovitch, M. C., Bérubé, M. & Stanhope, M. J. 2000 Molecular phylogenetic examination of the Delphinoidea trichotomy. *Mol. Phylogenet. Evol.* **15**, 314–318.
- Wilson, L. E. 1935 Miocene marine mammals from the Bakersfield Region, California. *Peabody Mus. Nat Hist Bull.* **4**, 1–143.
- Yang, G. & Zhou, K. 1999 A study on the molecular phylogeny of river dolphins. *Acta Theriol. Sinica* **19**, 1–9.
- Zhou, K. 1982 Classification and phylogeny of the Superfamily Platanistoidea, with notes on evidence of the monophyly of the Cetacea. *Sci. Rep. Whales Res. Inst. Tokyo* **34**, 93–108.
- Zhou, K., Zhou, M. & Zhao, Z. 1984 First discovery of a Tertiary platanistoid fossil from Asia. *Sci. Rep. Whales Res. Inst.* **35**, 173–181.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.



*Proc. R. Soc. Lond. B* 268, 549–556 (7 March 2001)

## Evolution of river dolphins

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An error occurred in figure 1*b* (p. 550). The genus for *Platanista gangetica* was incorrectly printed as *Pontoporia*. The corrected figure and its caption appear below.

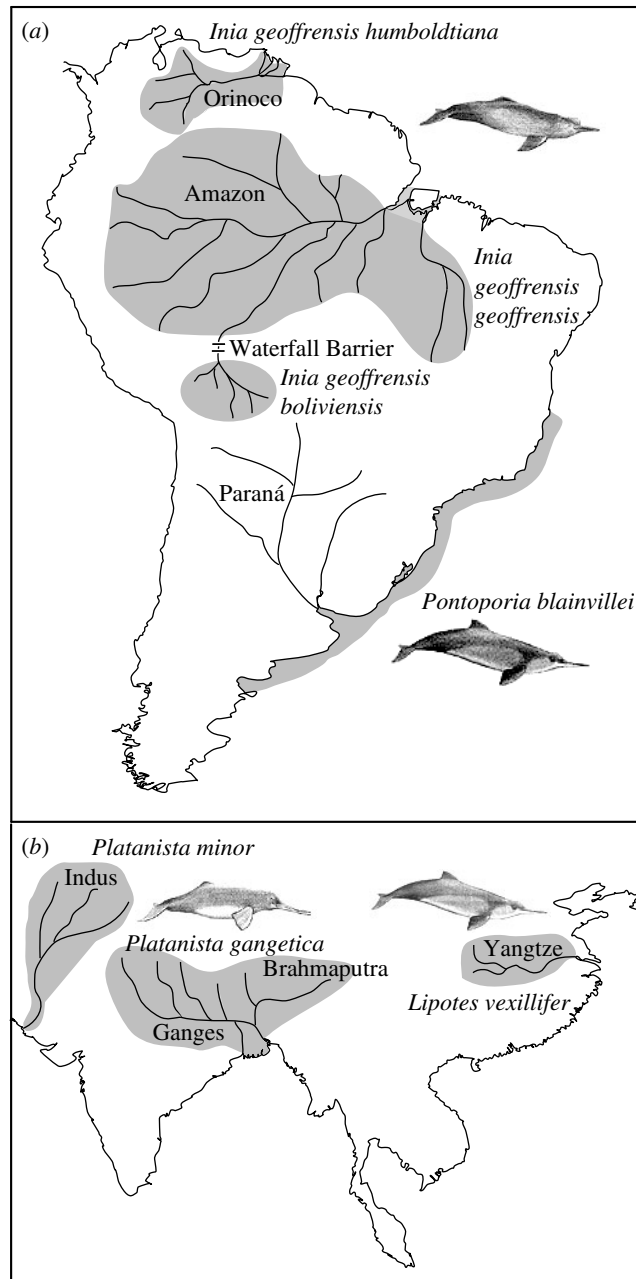


Figure 1. Geographical distribution of extant river dolphins. (a) *Inia geoffrensis humboldtiana* inhabits the Orinoco River system. *Inia geoffrensis geoffrensis* is found throughout the mainstem Amazon River and its tributaries. *Inia geoffrensis boliviensis* occurs in the Amazon tributaries of eastern Bolivia, geographically isolated by several hundred kilometres of rapids. *Pontoporia blainvillei* is restricted to coastal South Atlantic waters. (b) *Lipotes vexillifer* is an extremely endangered river dolphin that occurs only in the lower and middle reaches of the Yangtze River. *Platanista minor* inhabits the Indus River system. *Platanista gangetica* is found in the Ganges–Brahmaputra River system.