

Towards Resolving the Interordinal Relationships of Placental Mammals

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Here we show that progress towards a reliable phylogeny for placental mammals at the ordinal level continues apace. We draw especially upon insights from the recent “International Symposium on the Origin of Mammalian Orders” held at The Graduate University of Advanced Study, Hayama, Japan (21–25 July 1998), particularly work not incorporated in the remainder of this issue or published elsewhere. Abstracts to talks and posters presented at this meeting can be found at www.utexas.edu/ftp/depts/systbiol/. The talks fell into three main sections, which we will now consider, followed by a summary where we present our current best estimate of the tree for placental mammals.

The Age of Intraordinal Divergences

Perhaps even more than the tree of relationships, the ages molecular divergence times are suggesting have caused greatest consternation to morphologists. Part of this seems to be semantics. For example, when some authors suggest that perissodactyls originated well back in the late Cretaceous, it is not always clear if they mean the stem group Perissodactyla (i.e., all species more closely related to the living perissodactyls than to any other order); the crown group Perissodactyla (the last common ancestor of horses, rhinos, and tapirs, plus all its descendants); or perhaps the synapomorphy (morphologically identified group. The difference is not just the age of the group (in this case perhaps 80 versus 55 million years), but also the “identification of the group.” Modern orders (crown groups) are generally well marked by synapomorphies

expected to be found in fossils (e.g., rodent teeth and bat wings). In contrast, the earliest characteristics of stem groups are much less well known, and depend upon a robust phylogeny to assist identification. Thus, there is little controversy among traditional morphologists that multiple stem groups crossed the KT boundary (e.g., figures in Gregory, 1910; Novacek, 1993).

David Archibald (San Diego State University, California, pers. comm.) argued that since practically all extant placental orders, well defined by synapomorphies, first appear reliably in the period 55 to 65 million years ago (yet none with confidence are older), then they were almost certainly all generated in this period. He supports this view with the claimed high quality of the fossil record through this time, and the near coincident appearance of many new groups. A possible counterargument is that many faunas dispersed widely near the K/T boundary, and their previously restricted ranges remain poorly sampled (e.g., in Africa and India). If molecules are to directly challenge the prevalent morphological viewpoint, they must show that some ordinal crown groups are certainly older than the KT boundary at 65 million years, after modeling all acknowledged sources of calibration error (Waddell and Penny, 1996). The best candidates for finally breaking the KT crown group barrier are rodents, cetartiodactyls, bats, xenarthrans, primates, and the core insectivores, or Eulipotyphla (hedgehogs, shrews, solenodon, and moles).

Related to this issue is an examination of the rate of extinctions, origins, and trends in size, through time. John Alroy (1999), clearly showed that something dramatic happened around the KT boundary. This work helps to cement traditional hypotheses such as

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increasing mammalian body size from this time onwards (e.g., Gregory, 1910). Unfortunately, it does not address the issues of interordinal relationships far older than the KT boundary, phylogenetic inferences of early placental biogeography, or the actual pattern and progress of morphological evolution viewed on an accurate phylogeny, with an accurate time scale; rather, those are promises held for future molecular analyses.

The Resolution of Cetartiodactyla

An important outcome of the conference was at last what seemed to be a consensus of morphologists and molecular workers that the super order Cetartiodactyla was not only real, but its internal subdivision is likely to be radically different from traditional views. The work of Thewissen and Madar (1999) erodes one of the last bastions to this viewpoint, since newly discovered ankle bones of the earliest whales show features that ally them with artiodactyls, to the exclusion of their supposed extinct sister group(s), mesonichids.

The case for the tree [(Tylopoda,(Suidae, ((Cetacea, Hippopotamidae), Ruminantia))) or (camels,(pig,((whale, hippo), ruminants)))] was already good, and is now even stronger as shown by the sequence analysis work of Gatesy et al. (1999). The three unique and well defined insertion retroposons reported in Shimamura et al. (1997) have been readily accepted as conclusive evidence for the clade (whales, hippos, ruminants). This is because the methods of validation used in that paper (e.g., sequencing up and downstream of the insertion) clearly showed the insertions were present in the taxa mentioned, but did not occur (i.e., were not just absent or unidentified) in all the other extant artiodactyl taxa, plus an outgroup. Further retroposon evidence presented by Norihiro Okada at the conference (Nikaido and Okada, unpubl.) expands these data. In particular, three clear and independent retroposons are uniquely present in whales and hippos, while a fourth is clearly present in all cetartiodactyls except camels (the evidence that they were never in camels precludes the need for an outgroup). Taken together these data were perceived

by those present as a very substantial body of phylogenetic evidence in excess of that for any other superordinal group.

We feel it is now only appropriate to name these clades. Whippomorpha = Cetacea + Hippopotamidae, with the name a latinization of the colloquial term coined by Gatesy et al. (1996) to describe the novel "Whippo" hypothesis that whales and hippos are closest relatives. Cetruminantia = Ruminantia + Whippomorpha, with the name a latinized concatenation of Cetacea and ruminant; an obvious name for this group. Lastly, Artiofabula = Suidae + Cetruminantia, the name being derived from "artios" (a Greek word which can mean complete, or perfect of its kind; of numbers, even) and "fabula" (which means "fable"). Thus this clade was the perfect fable (at least until very recently), since gross morphology has long convinced science that camels should be with the ruminants, hippos belong with pigs (and peccaries), and whales should be anywhere but within the well defined "Artiodactyla". All these taxa are defined as crown groups, that is the last common ancestor of all their living members, plus all descendants of this ancestor. It is our hope these names will serve as a further step to convince others not at the meeting that these hypotheses deserve fair recognition.

Placing Bats, Pangolins, and Core Insectivores

The conference also saw new results suggesting that these three orders, which have previously been hard to place, may be finding a home. Indeed, the very concept of core insectivores was shaken by the study of Stanhope et al. (1998), as presented by Mike Stanhope (Queens University, Belfast, UK), with the African endemic insectivores, tenrecs, and golden mole, forming a clade (Afrosoricida) as part of a larger Afrotheria.

Analysis of seven genes with all or nearly all placental orders represented showed considerable congruence in a study presented by Peter Waddell. For simplicity, analyses discussed here use neighbor joining, with observed distances (using aminoacids when possible). One group that appeared repeatedly was bats + ferungulates + pangolin. For example, if 1 = Car-

nivora, 2 = Pholidota, 3 = Perissodactyla, 4 = Artiodactyla, 5 = Cetacea, 6 = Chiroptera, 7 = Lipotyphla, then: clade 1–6 was recovered with 12S + val-tRNA + 16S rRNA genes concatenated (16 orders sampled in total), α -crystallin gave the clade 1–6 (16 orders), ND1 gave the clade 1–5 (12 sampled orders, but no bats), γ -fibrinogen gave clade 1–5 (13 orders, no bats or insectivores), interphotoreceptor retinoid binding protein (IRBP) gave a group 1–7 (18 orders), and von Willebrand factor (vWF) gave 1–3,6 (18 orders). The sequences and alignments used are published rRNA, IRBP, and vWF, from Stanhope et al. (1998) minus tenrec, and for the rRNA minus their new sequences except fruit bat and shrew; ND1, Cao et al. (1998) plus elephant; α -crystallin, de Jong et al. (1993); γ -fibrinogen, Gatesy et al. (1999).

All the genes above are congruent with an expanded ferungulate group, except for IRBP and vWF. Based on other data, particularly complete mtDNA sequences, the group 1,3,4,5 is thought likely to be correct (e.g., Xu et al. 1996, but no 2 or 6 sampled there). In Springer et al. (1999), the feature of taxon 7 coming out among members of group 1–6 is linked to non-stationary base composition at the third position of hedgehog. If vWF and IRBP are concatenated, the group of 1–6 does appear. Such congruence is highly unlikely to be due purely to chance (for any single pair of genes less than 1 in 10^3 by chance, based on counting the incidence of this group over all binary trees), while only two of the datasets have any known interdependence. Further, the bootstrap support regarding this group ranges up to 90% for single genes (e.g., with ND1). Clearly, there is a case of remarkable congruence to be further studied, but apparently best explained at present as historical signal. The clade 1–5 is called Fereuungulata, and clade 1–6, Scrotifera (Waddell et al. 1999, see also cover illustration). These data contradict the Edentata hypothesis (Pholidota + Xenarthra), but do not yet confirm a specific sister taxon relationship of Pholidota + Carnivora (but γ -Fibrinogen, IRBP, and α -crystallin, do show this).

Dorothy Pumo (Hofstra University, New York) presented strong evidence based on

a complete mtDNA molecule that bats are sister to the fereuungulates (see Pumo et al. 1998, although no pangolin was sampled). The support for bats plus fereuungulates forming a clade was near 100%, while the position of bats sister to the fereuungulates (i.e., consistent with the monophyly of Fereuungulata) was supported near the 90% level, but with some uncertainty. Thus, this result and that of the previous paragraph fit together very well, and together they make an even stronger case. Pumo (pers. comm.) went on to explore the relevance of such a finding. She noted there is much limb variation within this group, which appears to be highly derived within the mtDNA tree of placental mammals. For instance, wings, hooves, and at least two different adaptations to an aquatic environment (also digging limbs if we include pangolin) all apparently arose in a relatively short period of evolutionary time, from an ancestral general limb, perhaps with traits common among rodents, primates, and lagomorphs.

Analysis of COII, COB, and mtRNA genes suggested a split core Lipotyphyla (Michiko Onuma, Mitsubishi Institute of Life Sciences, Japan). The hedgehog showed a basal eutherian position (as with complete hedgehog mtDNA analyses), but a musk shrew, shrew mole, and Japanese mole clustered with bats, with this group sister to the 'fereuungulate' taxa Cetartiodactyla, Perissodactyla, and Carnivora (but other possibilities could not be excluded due to finite sequence length; Onuma, pers. comm.). Short pepsinogen sequences including a shrew by Yuichi Narita (Nagoya University) placed it basal amongst the eutherians sampled, but with little statistical support.

Thus, a confident placement of the core Lipotyphla eludes us. The direct association of bats with insectivores is interesting, but was only apparent in one gene (IRBP) in the analyses of PJW mentioned above. Otherwise, with both mole and hedgehog present (12S-16S, vWF) they clustered together and not directly with bats (although an NJ analysis of an enlarged alignment of α and β hemoglobins put hedgehog deepest of placentals, but shrews and moles sister to carnivores; c.f. Stanhope et al., 1998). Our best

interpretation at present is Eulipotyphla is probably monophyletic, and may lie sister to the bat + fereuungulate group. Interestingly, this is about where hedgehog mtDNA came to rest in the analyses of Sullivan and Swofford (1997).

The Emerging Mix of New and Old

Lastly, we outline our current expectations for how the placental tree may resolve. An updated protein-based ML reanalysis of the latest (July 1998) mtDNA sequences (including the unpublished bat and elephant), was presented to the conference by Masami Hasegawa and Yin Cao (Institute of Statistical Mathematics, Tokyo). As in Pumo et al. (1998) the bat groups 100% with the perissodactyls, carnivores, and cetartiodactyla, but support for monophyly of the latter clade, and, therefore, the sister relationship, with bat is near 80%. Also, like Waddell et al. (1999), the grouping of armadillo and elephant was strong. A surprise in these analyses was that the dormouse sequence of Reyes et al. (1998), rather than adding evidence in favor of rodent paraphyly, catalyzes both rodent monophyly, then rabbits plus rodents, to give the traditional Glires (as in some earlier ProtML analyses; Waddell et al., 1999). Glires appears closer to the ferungulates than primates, contradicting earlier findings of rodents branching closer to the root. Liu and Miyamoto (1999) also consider Glires based on distinct data sets, finding strong support with morphology, but nothing clear in their sample of molecules. Thus, the earlier suggestion of "rodents deep" might turn out to be due to poor taxon sampling and/or inappropriate modeling of the substitution process. If so, it may become yet another warning of the possible pitfalls in molecular phylogenetics.

The cover illustration is our present best estimate of placental relationships based on our own extensive analyses of published and unpublished data. Clade names not already mentioned are: Pseudoungulata (false ungulates), Laurasiatheria (from the area of Laurasia or Europe + Asia + North America), and Zooamata (or animal friends, as the group contains cats, dogs, horses, etc.) Each of these taxa is a crown group. The taxa at

the tree's tips are a mix of orders and super orders.

Of other groups on our cover tree, the traditional grouping of Paenungulata and the new Afrotheria and Afrosoricida receive solid support in Stanhope et al., (1998), Springer et al. (1999), and Liu and Miyamoto (1999). The grouping of Xenarthra plus Afrotheria has more equivocal support (Waddell et al., 1999). A suggested true Archonta, or Euarchonta, (of Primates, Dermoptera, Scandentia) holds possibilities (e.g., Pumo et al., 1998; Liu and Miyamoto, 1999). If it is incorrect, a Scandentia + Lagomorpha grouping or Dermoptera + Scandentia seem likely alternatives to the cover tree, (e.g., Bailey, et al., 1992, Liu and Miyamoto, 1999). The interrelationships of these putative groups is most uncertain, but indications are that they may not coalesce until the mid to early Cretaceous, perhaps 90 to 130 MaBP. Thus, new analyses and data are suggesting that the placental tree is resolving in a way that echoes some of the earlier work of Gregory (1910) and Simpson (1945), but in considerably modified form.

In conclusion, the rate at which the higher order placental tree appears to be resolving is surprisingly rapid, and within a couple of years we may be able to infer the whole tree with some degree of confidence. Undoubtedly, there will be mistakes along the way, but these will often concern the misplacement of one or two orders by one or two steps through the tree. Accordingly, our expectation is that the tree shown on the cover may well have more correct clades than any published to date.

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