

Points of View

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Morphology, Paleontology, and Placental Mammal Phylogeny

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Placental mammals constitute the most intensively studied large clade of organisms. Deciphering their relationships has important implications not only to understand evolutionary processes (Bininda-Emonds et al., 2007) but also to inform many aspects of biomedical research (Springer and Murphy, 2007). Springer et al. (2007) have recently discussed limitations of morphological analysis of placental phylogeny compared with reconstructions based on molecular data. They presented a pessimistic view of the potential of morphological characters for use in high-level placental mammal phylogeny reconstruction, as the morphological data set they scrutinized (from Asher et al., 2003) does not recover the four major clades of mammals (Afrotheria, Xenarthra, Laurasiatheria, and Euarchontoglires) that these authors regard as overwhelmingly supported.

In their contribution to the recurring debate on the “adequacy of morphology” (from their title), Springer et al. (2007) have provided empirical data to support their claims of the inadequacy of a morphological data set for resolving high-level mammalian phylogeny. Here, we would like to address several issues relevant to the arguments of Springer et al., including (1) their use of congruence of individual partitions to a “true” tree; (2) their generalization across morphological data sets based on their evaluation of one matrix; (3) their emulation of extinction as a heuristic tool; and (4) the role of paleontology and neontology in eutherian systematics. We end our commentary by discussing how “morphology” (comprising both paleontological and neontological characters) has not yet been fully exploited as a source of data for phylogenetic research.

CONGRUENCE AND THE “TRUE” MAMMALIAN TREE

The molecular evidence for the four clades of mammals discussed by Springer et al. (2007) is very strong by any measure and is growing at a rapid pace (Nikolaev et al., 2007; Wildman et al., 2007; Hallström et al., 2007). We are very close to having a solid phylogeny of the major groups of extant placental mammals in which to

examine the evolution of character complexes. Even so, there is not total agreement among molecular biologists, and debate remains on the identity of the placental root (e.g., Kullberg et al., 2006; Kriegs et al., 2006) as well as the possibility of a carnivoran-primate clade (Cannarozzi et al., 2007; Huttley et al., 2007), contrary to Springer et al.’s preferred four-clade topology. Other discrepancies among recent publications include the placement of Afrotheria basal and an anteater-sloth clade (Springer et al., 2007: fig. 1), as opposed to Atlantogenata basal with an anteater-armadillo clade (Murphy et al., 2007: fig. 6). To use congruence as a means by which to question the reliability of an entire class of data (which Springer et al. do for hard-tissue morphology), confidence in the “correct” topology must be high indeed. Springer et al. (2007) are obviously confident in their tree and deserve a lot of the credit for assembling data that support it and being among the first to articulate its structure (cf. Murphy et al., 2001). In terms of the four-clade division of Placentalia, we suspect that they are largely correct; and we note that most genomic studies from 2007 that sample across placental orders support their interpretation (e.g., Wildman et al., 2007). Nevertheless, the fact that debate remains comprises one of the reasons why we consider their use of a single topology to question an entire class of data (e.g., “morphology”) as premature.

To qualify as the kind of “positively misleading” signal that some phylogeneticists recommend excluding a priori (e.g., Lecointre and Deleporte, 2005), a “bad” data partition should converge on a resolved, supported, and incorrect topology. Whether or not this is the case for “morphology” as a whole would require analysis of a substantially larger morphological data set than that published by Asher et al. (2003). As summarized below, even for this much more limited skeletodental matrix, no single, well-supported alternative to the four-clade model is present. At worst, the morphological data set of Asher et al. (2003) can be characterized as providing an ambiguous signal regarding high-level placental relations, one which can be (and was) resolved upon combination with other data sets, such as the DNA

concatenation of Murphy et al. (2001). For this reason, Asher et al. (2003) did not base their phylogenetic conclusions on morphology alone, but rather on the topology produced by the combined DNA-morphological signal.

Data-centric methods to improve congruence of individual partitions among each other exist (e.g., Wheeler, 1995), and indeed the application of one such method was a central focus of Asher et al. (2003). By differentially weighting classes of base mutation, indel and morphological characters, "sensitivity analysis" seeks to maximize congruence of individual partitions with one another, as measured by indices derived from treelength or topology (Asher et al., 2003: fig. 1). Hence, the central concern of Springer et al. (2007), that the morphology partition of Asher et al. (2003) is not sufficiently congruent with topologies based on genetic data, was actually highly relevant to the methodology used in Asher et al. (2003), who explicitly identified weighting schemes based on indices of congruence to maximize the agreement between the morphological characters with the 22 other partitions in their combined analysis. To the extent that it is desirable to maximize congruence across data partitions (and some argue it is not, e.g., Grant and Kluge, 2003), we would certainly agree that the sensitivity analysis as applied in that study could be expanded upon considerably; for example, by not tying weights of morphology to indel characters. Nevertheless, even with increased weight given to morphology and indels, Asher et al. (2003) not only supported afrotherian monophyly, but also favored a topology consistent with the four clades, albeit with a different location for the placental root (within rodents). Further refinement of that data set has been published (Asher et al., 2005; Asher, 2007), and indeed it is only with the inclusion of the morphological data set that parsimony, applied to a modified 22-gene data set (based on Roca et al. [2004] and including indel characters), reconstructs Placentalia with a root in Afrotheria. Using only DNA, murid rodents occupy the basal-most placental branch (Asher, 2007: fig. 4).

Springer et al. (2007) employ individual genes in their analysis that do not support the four-clade division of Placentalia. For example, analyses of vWF, A2AB, or GHR in isolation (Springer et al., 1997: fig. 1b, c; Douady et al., 2004) do not resolve the placental root, nor are the four clades consistently supported using such individual loci. Other individual genes do not fully reconstruct well-established orders (e.g., ND6 and Lagomorpha; epsilon-globin and Chiroptera; see Waddell and Shelley, 2003). Springer et al. (2007) themselves figure a tree that "fails" to reconstruct the tarsier in its well-corroborated position close to anthropoid primates, as supported by some nuclear DNA concatenations (Waddell and Shelley, 2003; Poux et al., 2005), morphology (Ni et al., 2003), and rare genomic changes (Schmitz et al., 2001, 2005).

We would not argue that GHR, vWF, or other individual data sets, including morphology, should be eschewed because of apparent incongruence with a popular, even well-corroborated, signal. In cases where a phylogeny is already known, there is obviously no need to conduct a phylogenetic analysis. We may soon reach the time

where the mammalian tree is fully established and when new data are used primarily for questions beyond the high level interrelations among extant clades. However, the ambiguity surrounding the affinities of many extinct mammals, as Springer et al. (2007) note, is tremendous, and is clearly worth pursuing with potentially relevant data.

GENERALIZATION ACROSS MORPHOLOGICAL DATA SETS

Springer et al. (2007) concluded that morphological data are unreliable for resolving affinities of placental mammals: "there is fundamental incongruence between molecular and morphological data at the level of placental interordinal relationships. ... [M]orphological studies of eutherian interordinal relationships have failed to separate homology and homoplasy and have consistently been misled by the latter" (p. 682).

Among other reasons, this is objectionable because they base their conclusions on a single morphological data set (Asher et al., 2003), which is not the only one available with relevance for placental mammals (e.g., Horovitz 2004; Luo and Wible, 2005), including one recently published (Wible et al., 2007). Analyzed in isolation, the skeletodentally focused morphology matrix of Asher et al. (2003: fig. 2) does not resolve most basal nodes around which the four clades are built. However, constraining the morphological data set to support either monophyletic Euarchontoglires or Xenarthra yields trees that are only slightly longer than the shortest tree. Based on Winning Sites and Templeton tests, and using Asher et al.'s (2003) morphological data set, these topologies cannot be rejected at a significance level of 0.05. Although a monophyletic Afrotheria, Laurasiatheria, or the four clades simultaneously do conflict with the morphological data from Asher et al. (2003), alternatives such as Lipotyphla are not strongly supported. Dermoptera + Chiroptera receives the highest branch support (4) for a clade that conflicts with the four-clade hypothesis; yet in a previous publication, Springer et al. applied another morphological data set (that of Simmons and Geisler, 1998) to investigate high-level phylogenetic questions in an analysis that samples members of both clades (Springer et al., 2001).

The image that Springer et al. (2007) portray is one of misleading morphological data that support such clades as Volantia, Lipotyphla, and Anagalida. However, the validity of these groups has been hotly debated among morphologists for many years (cf. chapters in MacPhee, 1993). Morphology has generally allowed a wide range of interpretation on phylogenetic questions, ranging from an afrotherian-like clade outlined by LeGros Clark and Sonntag (1926), exclusion of golden moles from Lipotyphla (Broom, 1916), Proboscidea + Sirenia + Hyracoidea (Novacek, 1986), Dermoptera + Primates (Beard, 1993), a paraphyletic Chiroptera (Pettigrew, 1986), and Hyracoidea + Perissodactyla (Fischer, 1989). This diversity of interpretation conveys the tremendous variety of morphological data and the fact that individual researchers generally focus on particular subsets thereof (basicranial

morphology, neurology, cranial soft tissues, placentation, etc.). It also highlights the fact that performance of morphology from Asher et al. (2003) has no necessary bearing on other skeletodental data sets, much less those focusing on reproduction, neurology, or development (see discussion below). Indeed, such morphological data have yet to be incorporated into any large-scale phylogenetic analysis, including most shared anatomical characteristics of Afrotheria that have recently come to light (Werdelin and Nilssone, 1999; Bedford et al., 2004; Mess and Carter, 2006; Sánchez-Villagra et al., 2007; Seiffert, 2007; Asher and Lehmann, 2008). A valid critique of previous, cladistic applications of "morphology" to placental phylogeny is that of Robinson and Seiffert (2004: 48); i.e., that they remain limited to a relatively small component of mammalian phenotypic diversity. Researchers have been attempting to improve the Web accessibility of morphological databases (Thacker, 2003; Asher, 2007), which will hopefully lead to better integration across anatomical data sets in the long term. In fact, expanding the scope of morphological phylogenetics in this way is a major component of a recently funded NSF Tree of Life proposal (<http://mammaltree.informatics.sunysb.edu>), a collaborative project that involves most authors in the Springer et al. (2007) publication.

DATA RAREFACTION AND EMULATING EXTINCTION

As quoted above, Springer et al. (2007) concluded that morphological data have "misled" systematists interested in placental mammal interordinal phylogeny. This is based in part on a data rarefaction technique that they refer to as "pseudoextinction." For placental mammals, this procedure was previously applied by Asher and Hofreiter (2006), who artificially treated living terminals as fossils in order to gauge the congruence yielded by 22 morphological characters known for an incomplete fossil tenrecid, compared with the topology produced by a combined morphology-DNA data set. In this case, data available from one of the fossils yielded a largely congruent phylogenetic signal with the combined data set (showing varying degrees of nonresolution) for most living genera of tenrecids. Based on this, Asher and Hofreiter inferred some confidence in the placement of the fossil. However, they noted that for another part of the tenrecid tree, morphology supports an incongruent signal, with semiaquatic Malagasy and mainland tenrecs in a clade together, as opposed to the sequence and combined data sets, which strongly support the position of the Malagasy semiaquatic tenrec with terrestrial species. Asher and Hofreiter concluded (2006: 190):

If the morphological data used here are misleading regarding a semiaquatic tenrec clade, why do we then combine them with our GHR data? ... [M]ost individual datasets are not in their entirety either "true" or "false"; but are themselves mosaics of variable character-data that may provide resolution at different levels in any given tree (Gatesy et al., 2003). Combined data sets enable recognition of phylogenetic signals that would remain obscure with the analysis of subdivisions thereof (Gatesy et al., 1999; Gatesy and Baker, 2005).

Rather than disregarding the morphological data set due to its incongruent signal on the placement of semiaquatic tenrecs, Asher and Hofreiter (2006) combined it with another data set (nuclear GHR sequences), which yielded a single, well-resolved topology that is congruent with other, independent studies of relevant phylogenetic data for the well-supported nodes they have in common (Olson and Goodman, 2003; Poux et al., 2005). Because of their inclusion of morphological data, Asher and Hofreiter (2006) were able to present the first cladistic hypotheses on the affinities of fossil tenrecs.

Artificial extinction can be used to roughly gauge the performance of a subset of data in placing extinct taxa in a phylogeny but is insufficient to make broad generalizations about the quality of data for mammal phylogeny reconstruction. It is already widely recognized that morphology is reliable in recognizing orders, at least as evidenced by congruence with molecular data (Archibald, 2003). So at what point does homoplasy start to affect our ability to correctly recover relationships among mammals? In addressing this question, it should be remembered that there is no objective difference between orders, superorders, or other Linnean ranks, which have been assigned to various mammalian clades quite fluidly (cf. McKenna and Bell, 1997). For those working on Recent taxa only, the crown group of an order and the entire order are equivalent, but this is not the case for paleontologists. Convincing morphological data have been put forth to support the recognition of fossil taxa as stem groups for the crown of many mammalian orders; including Cetartiodactyla (O'Leary, 2001; Geisler and Uhen, 2003, 2005), Carnivora (Wesley-Hunt and Flynn, 2005), Rodentia and Lagomorpha (Asher et al., 2005), Primates (Bloch and Boyer, 2002), Hyracoidea (Gheerbrant et al., 2007), and Sirenia (Domning, 2001). As noted above, some superordinal groups were first recognized at least in part (Archonta, Cetartiodactyla) or entirely (Glires, Paenungulata) on morphological evidence. Clearly, then, morphology provides useful information for some mammalian superorders as well. Hence, when an extinct taxon such as *Apheliscus* is reconstructed as a close relative of elephant shrews (Zack et al., 2005), this hypothesis deserves to be evaluated on its own merits, for example, by examining its nodal support and testing its robustness to the addition of characters and taxa with further phylogenetic scrutiny.

Although in one part of their paper they acknowledge the "primacy" (p. 673) of morphological data to infer phylogeny of fossil taxa, this seems to be quite conditional. In fact, Springer et al. (2007: 682) express "difficulty" in accepting "the assumption that morphological data, and methods for analyzing these data, give reliable phylogenetic solutions for the placement of key fossil taxa" (p. 674). They also clearly imply an answer of "no" to their question "can we trust morphological cladistic analyses that place extinct aspheliscines [*sic*] within or at the base of Afrotheria?" Springer et al. (2007) take exception to Asher et al. (2003), Zack et al. (2005), and Tabuce et al. (2007) for highlighting the possibility that there are older crown afrotherians in northern continents than

southern continents (see below). The means by which this hypothesis can be rejected, if it is in fact false, is not to categorically dismiss the data (morphology) and analyses upon which it has been presented. Rather, it is to find a Paleocene, or even Cretaceous, fossil mammal from a southern continent (either through fieldwork or examination of existing fossil collections) and demonstrate its status as an afrotherian with a phylogenetic analysis, for which morphological data remain the near-exclusive means by which we recognize such animals.

THE ROLE OF PALEONTOLOGY IN EUTHERIAN SYSTEMATICS

The majority of works on morphology-based cladistic analysis are conducted by palaeontologists who concentrate on skeletal characters, and this has led to the wrong perception that the study of morphology in placental systematics is equivalent to the study of fossils. This is not the case. The amount and quality of data that can be gathered from living placentals is much richer, including those on organ systems rarely or never preserved in fossils. Discussions on the role of paleontology in systematics abound, and the same fundamental points raised by Patterson (1981) and A. B. Smith (1998) are still relevant (see also Peterson et al., 2007).

Comprehensive morphological analyses focusing on high-level eutherian phylogeny and including many fossils are rare. One of the best examples of our ignorance on the high-level phylogeny of placental mammals concerns the large radiation of South American "ungulates." These animals include many forms known from complete skeletons (Flynn and Wyss, 1998; MacFadden, 2006) that until 2004 had not yet been included in a comprehensive analysis of eutherian phylogeny, as the many students of these groups have been concerned primarily with issues of stratigraphic correlation and paleoecology (e.g., Flynn et al., 2003). Only Horovitz (2004) presented a cladistic analysis (which includes more taxa and characters than the morphology matrix of Asher et al. (2003) with postcranial data sampling four South American ungulates and several North American and European condylarths. Her results were highly incongruent with the topology advocated by Springer et al. (2007).

Does the "failure" of her morphological matrix to recover the "accepted" topology (Springer et al., 2007) serve as an indictment of morphological data in general to resolve the phylogeny of extinct, South American ungulates? Of course not; nor does the failure of the morphology matrix of Asher et al. (2003) to reconstruct the four superordinal clades of Springer et al. have great relevance to her study. Horovitz (2004) represents an important start to the process of analyzing data available for these extinct clades, for which data on cranial morphology (e.g., Flynn et al., 2003) and enamel microstructure (Rensberger and Pfretschner, 1992), among other bodies of data, have yet to be included. Had there been more congruence with the four-clade topology of modern placental clades, we would regard this no less positively than other cases of congruence (cf. Wheeler, 1995). However, as stated above, the lack thereof is a reason to en-

rich the morphological sample to try and resolve the discrepancy.

CONCLUSION: HAVE WE EXHAUSTED MORPHOLOGY-BASED STUDIES OF PHYLOGENY IN PLACENTALS?

The long history of comparative anatomy has led some to believe that we have exhausted the potential of morphological characters in phylogenetic analysis (Scotland et al., 2003), especially as it concerns placental mammals. Yet information on the skeleton is incomplete. We do not even know the identity of many foramina and bones in many species (Starck, 1995), and only recently have there been synthetic efforts to overcome this (Wible, 2003; Wible and Gaudin, 2004; Giannini et al., 2006). Even the identity of certain cranial bones remains obscure for certain groups, such as the jugal of lipotyphlans. Only recently have developmental data been presented that demonstrate the presence of the jugal bone in *Talpa europaea* (Goswami and Prochel, 2007). This late discovery regarding such a common European mammal illustrates the fact that much in the way of character data remains to be investigated or rescued from the old literature.

Many character complexes have not been integrated into larger phylogenetic analyses, although some studies clearly indicate their potential in providing autapomorphies for higher-level clades. This includes, for example, placentation (Vogel, 2005; Mess and Carter, 2006), the ethmoidal region (Ruf, 2004), and the integument (Ade, 1998; Hill, 2005). The phylogenetic signal in organs such as the brain has been studied by few authors (Johnson and Kirsch, 1993). Most of these complexes are not included in broadly sampled morphological analyses (Asher et al., 2003; Horovitz, 2004), which due to their paleontological orientation concentrate on skeletal anatomy. The studies of endocasts by Macrini and colleagues (e.g., Macrini et al., 2007) represent an area of paleontology that is delivering a new source of characters. In the absence of advance knowledge on how extinct clades fit into the Tree of Life, optimism as to the potential value of such new characters is no less well-founded than Springer et al.'s generalization that morphological data are as a rule unreliable for high-level mammalian phylogeny. Certainly it is better for morphologists to collect more data and explore new character complexes than to assume without justification that they will be phylogenetically misleading.

Lee (2006) has demonstrated that recent morphological study of squamate reptiles has yielded a steadily improved understanding of their phylogeny. We agree with his observations that not only does novel morphological scrutiny of relatively well-studied groups, such as squamates and mammals, continue to yield important scientific results, but also that morphologically "well-studied" groups are the exception: "for most of the tree of life, morphology has either not been described at all, or has not been rigorously analyzed phylogenetically—let alone exhausted" (Lee, 2006; see also Britz and Johnson, 2002).

We also agree with Springer et al. (2007: 682) that "new methods for coding and analyzing morphological

characters should be explored." Potential avenues in this direction include morphometric approaches to character definition (MacLeod and Forey, 2002), methods to incorporate continuous data (Goloboff et al., 2006), and the consideration of modularity and possible non-independence among characters (O'Keefe and Wagner 2001; Polly, 2005; Goswami, 2006) in character weighting and definition. A particularly novel area in this context involves what we call "quantitative comparative ontogeny," which relates to the study of heterochrony in two of its manifestations: allometric and sequential (Smith, 2001). Giannini and colleagues (e.g., Giannini et al., 2004) have presented in a series of papers data on the allometric relations among parts of the marsupial skull. Similar data for placentals could be then plotted on phylogenies to examine the evolution of growth trajectories. Concerning ontogenetic sequences, the development of the event-pairing method and related applications (cf. Jeffery et al., 2005) may provide a way to incorporate developmental data on relative timing into phylogenetic analysis (Harrison and Larsson, 2006).

The call by Springer et al. (2007) for character analyses that consider developmental correlation among parts is in principle well founded, given the problems associated with a numerical approach led by instrumentalization (Rieppel, 2007). A pervasive issue is that no matter how integrative the atomization of morphological diversity may be, character definition itself will still involve subjectivity (Cartmill, 1994). Nevertheless, in whatever fashion morphological data are coded, they remain among the best (and often only) means by which we can incorporate the real data afforded to us via the fossil record into reconstructing the mammalian component of the Tree of Life.

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The Taming of the Skew: Estimating Proper Confidence Intervals for Divergence Dates

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Estimating divergence dates from molecular phylogenies permits researchers to test evolutionary hypotheses that cannot be explored using the fossil record alone. For example, estimating the age of nodes on a tree aids in testing the possibility of dispersal versus vicariance, assessing the origin and survival of groups across major geological time periods, and inferring times of divergence and rates of molecular evolution (Barker et al., 2004; Simmons, 2005; Teeling et al., 2005; Bossuyt et al., 2006), hypotheses that could not be credibly examined without statistically sound methods of date inference using DNA sequences and phylogenetic trees. The formal and strict definition of rate constancy and a molecular clock as proposed by Zuckerkandl and Pauling (1962) may not be valid in many situations and across a broad spectrum of taxa and genes (Wu and Li, 1985; Britten, 1986; Sanderson, 2002; Thorne and Kishino, 2006). Therefore, methods that can infer divergence dates utilizing relaxed-clock algorithms have grown in popularity. Two leading methods of statistical inference are commonly applied that do not require clock-like behavior from the data; relaxed clocks using Bayesian inference (BI) or penalized likelihood (PL; Sanderson, 2002, 2003; Thorne et al., 1998; Thorne and Kishino, 2002). Though BI methods have some measures of error estimation built into

the date inference, PL methods lack a simple protocol for assessing uncertainty in the estimation of divergence dates.

For any method of statistical inference, it is necessary to have some measure of error in order to assess confidence. This is particularly important for divergence date estimation, where calculated uncertainty may be very large, and thus include the null hypothesis. There are three primary methods of calculating confidence intervals for a likelihood function: (i) the likelihood profile, which summarizes the likelihood shape in terms of the curvature of the likelihood surface at the maximum likelihood estimate (MLE) using Fisher Information; (ii) the bootstrap, which produces a frequency distribution of samples with the shape of the likelihood function; and (iii) the Bayesian credible interval, which combines maximum likelihood estimation with prior information on parameters (Clark, 2007). These methods may all be used to produce error estimates for divergence dates using PL (first two methods) or BI (last method).

The first of the two widely used methods of divergence dating is Bayesian inference with a relaxed clock, implemented in the programs MultiDivTime (Thorne and Kishino, 2002) and BEAST (Drummond and Rambaut, 2003). Bayesian methods without an expectation of constant substitution rates use a stochastic model for inferring rates of change along a branch while applying calibration reference priors that are external to the

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