Summary of Laurasiatheria (Mammalia) Phylogeny

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Abstract: Laurasiatheria is one of the richest and most diverse superorders of placental mammals. Because this group had a rapid evolutionary radiation, the phylogenetic relationships among the six orders of Laurasiatheria remain a subject of heated debate and several issues related to its phylogeny remain open. Reconstructing the true phylogenetic relationships of Laurasiatheria is a significant case study in evolutionary biology due to the diversity of this suborder and such research will have significant implications for biodiversity conservation. We review the higher-level (inter-ordinal) phylogenies of Laurasiatheria based on previous cytogenetic, morphological and molecular data, and discuss the controversies of its phylogenetic relationship. This review aims to outline future researches on Laurasiatheria phylogeny and adaptive evolution.

Keywords: Laurasiatheria; Phylogeny; Mitochondrial DNA; Nuclear genes; Phylogenomics

Placental mammals, which diverged from marsupials about 160 million years ago (Mya) (Luo et al, 2011, Dos Reis et al, 2012), consist of four super-orders, i.e., Afrotheria, Xenarthra, Euarchontoglires, and Laurasiatheria.(Asher & Helgen, 2010; Carter, 2001; Eizirik et al, 2001; Hallström et al, 2011; Kriëgs et al, 2006; Kulemzina et al, 2010; Lin et al, 2002; Murphy et al, 2001a,b; Murphy et al, 2007; Nikolaev et al, 2007; Nishihara et al, 2006; Prasad et al, 2008; Springer et al, 2004; Wildman et al, 2006; Zhou & Yang, 2010; Zhou et al, 2011a). Among them, Laurasiatheria is one of the richest and most-diverse, with an origin and a primary distribution on the supercontinent of Laurasia (including North America, Europe and Asia) during their early history (Springer et al, 2011; Waddell et al, 1999b). Although the root of placental mammals is unclear, Laurasiatheria has been consistently recognized as a sub-clade of Boreoeutheria (=Laurasiatheria+Euarchontoglires) (Dos Reis et al, 2012; Gibson et al, 2005; Hallström & Janke, 2008; Song et al, 2012; Madsen et al, 2001; McCormack et al, 2012; Murphy et al, 2001a, b; Nishihara et al, 2006; Springer & Murphy, 2007; Springer et al, 2011; Wildman et al, 2006). Many Laurasiatheria species have attracted great interest in relation to the conservation of wild animals and are also crucial animal models for studies on adaptive evolution.

As a mammalian group bearing important evolutionary significance and conservation value, Laurasiatheria has long been a focus of researches.

Current classifications of Laurasiatheria recognize six orders, namely Eulipotyphla (hedgehogs, shrews, and moles), Perissodactyla (rhinoceroses, horses, and tapirs), Carnivora (carnivores), Cetartiodactyla (artiodactyls and cetaceans), Chiroptera (bats), and Pholidota (pangolins) (Hallström et al, 2011; Lin et al, 2002; Waddell et al, 1999a; Wildman et al, 2006; Wilson & Reeder, 2005; Zhou et al, 2011a). However, the phylogeny and evolution of Laurasiatheria remain subjects of heated debate and are not yet well-established. The origins of Laurasiatheria have been dated to the Cretaceous, ranging from 100 to 78.5–93 mya according to several molecular estimates (Cao et al, 2000; Hallström & Janke 2008, 2010; Hallström et al, 2011; Hasegawa et al, 2003; Ji et al, 2002; Kitazoe et al, 2007; Meredith et al, 2011; Murphy et al, 2004; Springer & Murphy, 2007; Zhou et al, 2011b). However, most morphological studies place the origins of this super-order in the Paleocene (Asher et
The diversification of mammalian species within Laurasiatherian orders appear to have radiated within 1–4 mya in the Eocene (Hallström & Janke 2008, 2010; Zhou et al, 2011b). So, attempts to clarify relationships among the six Laurasiatheria orders from a variety of studies have encountered serious challenges due to the rapid evolutionary radiations and recent speciation events. Although there has been a general consensus regarding the earliest divergence of the order Eulipotyphla (Dos Reis et al, 2012; Hallström et al, 2011; Lin et al, 2002; Murphy et al, 2001a, b; Nery et al, 2012; Nishihara et al, 2006; Romiguier et al, 2010; Song et al, 2012; Wildman et al, 2006; Zhou et al, 2011a, b), conflicting phylogenetic hypotheses exist for the other orders that evolved subsequently (Figure 1). In this article, we review the higher-level (inter-ordinal) phylogenies of Laurasiatheria based on previous cytogenetic, morphological and molecular data, and discuss the controversies of its phylogenetic relationship. This review aims to outline future research of Laurasiatheria phylogeny and adaptive evolution.

Laurasiatheria phylogeny inferred from cytogenetic and morphological evidence

Early karyotype studies of Laurasiatheria have suggested there are significant differences in chromosome number, morphology and banding pattern among the six orders (Ao et al, 2007; Frönicke et al, 1997; Kulemzina et al, 2009; Nie et al, 2011; Sotero-Caio et al, 2011; Trifonov et al, 2008; Yang & Graphodatsky, 2004; Yang et al, 2006; Ye et al, 2006). Kulemzina et al (2010) investigated the phylogenetic relationships among Laurasiatheria orders by comparing their karyotypes with human karyotype, and proposed the Laurasiatheria phylogeny as (Eulipotyphla, (Cetartiodactyla, (Perissodactyla, Chiroptera), (Carnivora, Pholidota)))) (Figure 1A, Table 1). Their study therefore supports the bas al placement of Eulipotyphla, and the close relationship between Carnivora and Pholidota, as well as that between Perissodactyla and Chiroptera. However, the trichotomy among Cetartiodactyla, Carnivora + Pholidota, and Perissodactyla + Chiroptera is unresolved.

Compared with limited karyotype studies, numerous morphological studies have been conducted on Laurasiatheria phylogeny reaching a variety of conclusions. In early morphological studies, Laurasiatheria monophyly was not even supported (Asher et al, 2003; Novacek, 1992, 2001; Simpson, 1945; Wible et al, 2007). For example, Perissodactyla was originally classified into clade Ungulata (Shoshani & McKenna, 1998), whereas Chiroptera was often associated with
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<th>Study evidence</th>
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Dermoptera (flying lemur) (Asher et al, 2003; Novacek, 1992; Shoshani & McKenna, 1998; Simmons & Geisler, 1998). Gunnell & Simmons (2005) proposed that the grouping of Chiroptera with Dermoptera was spurious due to the homoplastic morphological characters. Based on analyses of 13 placenta features, Wildman et al (2006) first supported that Laurasiatheria formed a monophyletic clade, which was confirmed by later molecular studies, and favored the Laurasiatheria phylogeny as (Eulipotyphla, (Chiroptera, (Cetartiodactyla, (Perissodactyla, (Carnivora, Pholidota)))).

Similar to the karyotype studies, the earliest divergence of Eulipotyphla and the close association between Carnivora and Pholidota were supported. Regarding the phylogenetic placements of the other three orders, however, morphological studies demonstrated more resolutions and debates than the karyotype studies.

Laurasiatheria phylogeny inferred from molecular evidence

Phylogenetic estimate from mitochondrial genes

Due to relatively small effective population size and lack of recombination as well as easy obtainment, mitochondrial DNA (mtDNA) has often been chosen as a preferred molecular marker in early molecular phylogenetics (Bargelloni et al, 2000; Brito & Edwards, 2009; Phillips & Penny, 2003; Sánchez-Gracia & Castresana, 2012; Springer et al, 2001; Sturmbauer & Meyer, 1993; Yu et al, 2007).

Pumol et al (1998) analyzed 12 complete mt genome sequences of five Laurasiatheria orders, placing Chiroptera as the sister group of Cetartiodactyla and Perissodactyla and Carnivora, while positioning Eulipotyphla as the most basal order of placent mammals, thus rejecting Laurasiatheria monophyly. Cao et al (1998) investigated the mt genome sequences of three orders, placing Cetartiodactyla as the sister group of Perissodactyla and Carnivora. By summarizing the results proposed in the “International Symposium on the Origin of Mammalian Orders”, Waddell et al (1999b) suggested the Laurasiatheria phylogeny as (Eulipotyphla, (Chiroptera, (Cetartiodactyla, (Perissodactyla, (Carnivora, Pholidota)))).

These relationships are consistent with those from the morphological study of Wildman et al (2006). Moreover, these relationships are also supported by later analysis of 13 important rare genomic changes (RGCs) (Springer et al, 2004). By adding the mtDNA genome sequences of other orders and by using different tree-building schemes, later studies obtained the same phylogenetic relationships as those from Waddell et al (1999b) and Wildman et al (2006) (Arnason et al, 2002; Arnason & Janke, 2002; Cao et al, 2000; Gibson et al, 2005; Kern & Kondrashov, 2004; Kjer & Honeycutt, 2007; Lin et al, 2002; Reyes et al, 2004) (Figure 1B, Table 1) with the exception of Eulipotyphla positioned as the most basal order of placent mammals, and Cetartiodactyla as the sister group of Perissodactyla and Carnivora (Waddell et al, 1999a).

Conversely, Nikaido et al (2001) and Mouchaty et al (2000) proposed that Eulipotyphla and Chiroptera were clustered together, which were closely related to Perissodactyla, Carnivora and Cetartiodactyla (Figure 1C, Table 1). Jow et al (2002) utilized all mt tRNAs and rRNAs to reconstruct the Laurasiatheria phylogeny among orders except for Pholidota. The resulting tree topology was (Eulipotyphla, (Chiroptera, (Carnivora, (Cetartiodactyla, Perissodactyla)))). (Figure 1D, Table 1). Subsequently, by adding the mt tRNAs and rRNAs of Pholidota, Hudelet et al (2003) claimed the Laurasiatheria phylogeny was (Eulipotyphla, (Chiroptera, (Pholidota, (Carnivora, (Cetartiodactyla, Perissodactyla)))). (Figure 1E, Table 1). Notably, their study rejected the general view of the clustering of Carnivora and Pholidota (Arnason et al, 2002; Arnason & Janke, 2002). Therefore, except for the early divergences of Eulipotyphla and Chiroptera, the phylogenetic relationships among the other four orders are inconsistent with all the previous studies.

Phylogenetic estimate from nuclear genes

Although analyses of mtDNA sequences have provided insights into the phylogeny and evolution of Laurasiatheria, the fact that all genes comprising mt genome are inherited as a single, haploid linkage unit is a well-known limitation on phylogenetic reconstruction because the resulting mt gene trees are unlikely to reflect one independent estimate of the species tree (Giannasi et al, 2001; Johnson & Clayton, 2000; Moore, 1995; Page, 2000; Yu et al, 2004; Yu & Zhang, 2006a). Therefore, when much longer nuclear DNA sequences become widely available, nuclear genes received more and more attention in molecular phylogenetic studies, and demonstrated superiority in resolving deep phylogenies (Springer et al, 1999, 2001). Since 2001, a series of Laurasiatheria studies based on analyses of nuclear genes made important contributions to the resolution of relationships among Laurasiatheria orders, entering into an unprecedented progress.

Madsen et al (2001) first use three nuclear genes, combined with mt tRNA and rRNAs (~5 kb in total), to investigate Laurasiatheria relationships and proposed a tree topology of (((Carnivora, Perissodactyla), (Cetartiodactyla, (Pholidota, (Chiroptera, Eulipotyphla))))) (Figure 1F, Table 1). When another nuclear gene (BRCA1) was added and the taxon of representatives was increased to test the stability of the resulting tree, the tree topology changed to (Eulipotyphla, ((Carnivora, Pholidota),
(Chiroptera, (Cetartiodactyla, Perissodactyla))) (Figure 1G, Table 1), indicating that Laurasiatheria phylogeny varied with the genes and species used in the analyses.

In the same year, by screening 15 nuclear genes (~10 kb) and three mt genes, Murphy et al (2001a) supported a Laurasiatheria phylogeny of (Eulipotyphla, (Chiroptera, ((Cetartiodactyla, Perissodactyla), (Carnivora, Pholidota)))) (Figure 1H, Table 1). Interestingly, their study clustered Cetartiodactyla with Perissodactyla, though this relationship received low statistical support. The subsequent study of Waddell et al (2001) using 12 nuclear genes (~10 kb) and mt genome sequences retrieved the same Laurasiatheria phylogeny and similar nodal supports (Figure 1H, Table 1). These results were also supported by the analysis of 20 nuclear genes and 175 morphological characters by Springer et al (2002). The subsequent study of Waddell et al (2001) using 12 nuclear genes (~10 kb) and mt genome sequences retrieved the same Laurasiatheria phylogeny and similar nodal supports (Figure 1H, Table 1). These results were also supported by the analysis of 20 nuclear genes and 175 morphological characters by Springer et al (2007) (Figure 1H, Table 1).

Remarkably, when the dataset is increased to 19 nuclear genes and three mt genes (~16 kb), Murphy et al (2001b) showed that the Laurasiatheria phylogeny was (Eulipotyphla, (Chiroptera, (Cetartiodactyla, (Perissodactyla, (Carnivora, Pholidota)))) (Figure 1b and Table 1). Differences from other nuclear trees thus mainly lie in the phylogenetic positions of Cetartiodactyla and Perissodactyla. By adding a new nuclear marker (apolipoprotein B gene) to Murphy et al (2001b)'s dataset, Amrine-Madsen et al (2003) achieved the same phylogenetic results (Figure 1B, Table 1). These results were congruent with those from the morphological and mt genome analyses (Arnason et al, 2002; Arnason & Janke, 2002; Springer et al, 2004; Wildman et al, 2006).

Madsen et al (2002) attempted to evaluate the utility of Alpha 2B adrenergic receptor gene in the Laurasiatheria phylogeny, which showed the tree as (Perissodactyla, ((Eulipotyphla, Cetartiodactyla), (Chiroptera, Carnivora, Pholidota))) (Figure 11, Table 1). Surprisingly, Perissodactyla was placed as the basal and Eulipotyphla was the sister-group of Cetartiodactyla. Waddell & Shelley (2003) developed seven nuclear gene fragments and determined the Laurasiatheria phylogeny to be (Eulipotyphla, (Cetartiodactyla, (Perissodactyla, Chiroptera), (Carnivora, Pholidota))) (Figure 1A, Table1).

Asher et al (2003) examined 18 nuclear genes, in combination with three mt genes and 196 morphological characters, and presented the Laurasiatheria phylogeny as (Eulipotyphla, ((Cetartiodactyla, Perissodactyla), (Chiroptera, (Carnivora, Pholidota)))) (Figure 1J, Table 1). Notably, Chiroptera was not placed at the second diverging lineage in Laurasiatheria, as suggested in most previous studies (Madsen et al, 2001; Murphy et al, 2001b), but displayed a close relationship with Carnivora + Pholidota. Using 8 housekeeping genes from four Laurasiatheria orders, Kullberg et al (2006) retrieved a phylogeny of (Chiroptera, (Cetartiodactyla, (Perissodactyla, Carnivora))) (Figure 1J, Table 1). Despite weak support, this result was consistent with most mt phylogenies (Cao et al, 2000; Lin et al, 2002).

Different from those protein-coding nuclear genes commonly used in previous studies, Matthee et al (2007) first utilize three nuclear introns (~6 kb) from 114 Laurasiatheria species to rebuild the relationships among the six orders. Their study supported the phylogeny of (Eulipotyphla, (Perissodactyla, (Cetartiodactyla, (Chiroptera, (Carnivora, Pholidota)))) (Figure 1K, Table 1). However, these relationships were poorly supported. Asher (2007) reanalyzed 19 nuclear and three mt sequences data from Murphy et al (2001b) as well as 196 morphological characters from Asher et al (2003) (Table 1). By incorporating indels of sequence data, the study suggested that the phylogenetic relationships among the Laurasiatheria orders changed with the combinations of characters and the analytic methods.

Based on the above studies, Laurasiatheria phylogeny at the ordinal level remains an outstanding problem in mammalian systematics due to the contradicting conclusions reached under different datasets, especially in the case of the phylogenetic positions of Perissodactyla, Cetartiodactyla and Chiroptera.

**Phylogenomics of Laurasiatheria**

With the increasing availability of genomic data of more species, phylogenetic analysis, which use genomic data to infer evolutionary relationships, is entering a new era (Delsuc et al, 2005; Rokas & Chatzimanolis, 2008; Yu & Zhang, 2006a, b). By performing phylogenomic studies, a reliable phylogenetic tree can be reconstructed using many more characters than those in previous studies, including gene families, large insertion and deletion gene fragments, and gene rearrangement, etc. (Meslin et al, 2011; Wu et al, 2012; Yu et al, 2011). These characters might be useful for distinguishing nodes resulting from rapid radiation episodes such as the Laurasiatheria speciation events.

Bashir et al (2005) attempted to reconstruct the Laurasiatheria phylogeny of four orders (Eulipotyphla, Cetartiodactyla, Perissodactyla, and Carnivora) based on more than 1000 orthologous repetitive elements obtained from publically available genome sequence data. In their study, Perissodactyla and Carnivora were grouped together, whereas the placements of the other two orders remained unclear.

Using retroposed elements as new markers to reconstruct the Laurasiatheria phylogeny with the exception of Perissodactyla, Kriegs et al (2006) determined the tree as (Eulipotyphla, (Chiroptera, (Cetartiodactyla, (Pholidota, Carnivora)))) (Figure 1L, Table 1). On the other hand, Nishihara et al (2006) performed a comprehensive comparison of orthologous
non-coding sequence analyses favored the tree as (1N, Table 1), whereas the combination of coding and (Chiroptera, (Perissodactyla, Carnivora)))) (Figure 1M, Table 1). Their most interesting finding was that Carnivora, Perissodactyla, and Chiroptera were grouped together, excluding Cetartiodactyla and Eulipotyphla, which has not been recovered by previous studies.

Based on 218 protein-coding genes (~200 kb) obtained from the analysis of 18 placental mammalian genomes, Nikolaev et al (2007) suggested that Eulipotyphla diverged first, followed by Chiroptera, and, Cetartiodactyla and Carnivora were sister-group (Figure 1L, Table 1). Subsequently, by searching for informative coding indels within whole-genome sequence data and amplifying them in Laurasiatheria species, Murphy et al (2007) yielded a new tree topology as (Eulipotyphla, (Cetartiodactyla, ((Chiroptera, Perissodactyla), (Carnivora, Pholidota)))) (Figure 1A, Table 1). The close relatedness of Chiroptera and Perissodactyla has been only recovered in karyotype research (Kulemzina et al, 2010).

Prasad et al (2008) reconstructed the phylogeny of Laurasiatheria except for Pholidota based on 1.9 Mb gene regions. The protein-coding sequence analyses supported the tree as (Eulipotyphla, (Carnivora, (Chiroptera, (Perissodactyla, Cetartiodactyla))))) (Figure 1N, Table 1), whereas the combination of coding and non-coding sequence analyses favored the tree as (Eulipotyphla, (Chiroptera, (Perissodactyla, (Carnivora, Cetartiodactyla)))) (Figure 1O, Table 1), showing a lack of consistency in Laurasiatheria phylogeny under different kinds of characters used. The lack of consistency with such large amounts of data can be also evidenced from Hou et al (2009), in which 2705 orthologous genes (~40 Mb) from Cetartiodactyla, Perissodactyla, and Carnivora were used, and different tree topologies were produced under different tree-building methods. When Chiroptera was added into the analysis, they found close relatedness of Perissodactyla with Carnivora, and that of Cetartiodactyla with Chiroptera (Figure 1P, Table 1).

Hallström & Janke (2008) screened 3 012 genes (~280 kb) from four orders with available genome sequences (Eulipotyphla, Chiroptera, Carnivora, and Cetartiodactyla). Their results only recovered the basal placement of Eulipotyphla, and failed to resolve the relationships of the other three orders. The rapid radiation of Laurasiatheria within a narrow time scale was proposed to explain the irresolution using such large amounts of data. By utilizing the third codon position GC content (GC3) of 1 138 protein-coding orthologous genes (~300 kb), Romiguier et al (2010) revealed the Laurasiatheria phylogeny to be (Eulipotyphla, (Cetartiodactyla, (Chiroptera, (Perissodactyla, Carnivora)))) (Figure 1M, Table 1).

Hallström et al (2011) analysed 4 775 protein-coding genes (~6 Mb) screened from the available genome sequences of five Laurasiatheria orders and determined the tree as (Eulipotyphla, (Chiroptera, (Cetartiodactyla, (Perissodactyla, Carnivora)))) (Figure 1B, Table 1). Their results were more consistent with morphological and mt genome analyses as well as nuclear analyses of Murphy et al (2001b). However, Pholidota was not included in the analyses. When the retroposon insertions from these genome sequences were analysed, their study supported the earliest divergence of Eulipotyphla, but failed to resolve the relationships among the other four orders. Additionally, McCormack et al (2011) analysed 683 loci of ultraconserved elements, supporting the tree topology (Eulipotyphla, (Cetartiodactyla, (Chiroptera, (Perissodactyla, Carnivora))))) which was consistent with Nishihara et al (2006) (Figure 1M, Table 1).

By screening protein-coding genes within genome sequence data and amplifying them in Laurasiatheria species, Zhou et al (2011a) analyzed 8 markers (~5 kb) and reconstructed the Laurasiatheria phylogeny as (Eulipotyphla, (Cetartiodactyla, (Chiroptera, (Perissodactyla, Carnivora))))) (Figure 1M, Table 1), consistent with the results from Nishihara et al (2006) and McCormack et al (2011). Subsequently, Zhou et al (2011b) investigated Laurasiatheria phylogeny based on 97 orthologous genes (~46 kb) from six orders. Regardless of datasets and analytic methods used, they obtained an identical tree topology of (Eulipotyphla, (Chiroptera, (Carnivora, Pholidota), (Cetartiodactyla, Perissodactyla)))) (Figure 1H, Table 1). This result was consistent with those from Murphy et al (2001a) and Waddell et al (2001).

Recently, Nery et al (2012) choose 3 733 orthologous genes (~50 Mb) obtained from available genome sequences of five orders, namely Eulipotyphla, Chiroptera, Carnivora, Cetartiodactyla, and Perissodactyla. They obtained a Laurasiatheria phylogeny of (Eulipotyphla, ((Chiroptera, Cetartiodactyla), (Perissodactyla, Carnivora))) with high supports (Figure 1P, Table 1).

As the most comprehensive study so far, Dos Reis et al (2012) studied 11 available whole genomes of five Laurasiatheria orders (except Pholidota). Based on 857 nuclear genes and 153 mt genomes (~2 Gb), they reconstructed the tree topology as (Eulipotyphla, (Cetartiodactyla, (Chiroptera, (Perissodactyla, (Carnivora, Pholidota)))) (Figure 1M, Table 1), which was consistent with Nishihara et al (2006) and McCormack et al (2011).

Most recently, Song et al (2012) analyzed 447 orthologous genes (~7 Mb) and used multispecies coalescent model analysis to investigate the phylogenetic relationships among the five orders of Laurasiatheria.

Perspectives

The phylogeny of Laurasiatheria, which is characterized by rapid species radiations and short internal tree branches, has long been one of the most controversial and challenging problems in mammalian systematics. So far, only the earliest divergence of Eulipotyphla and the close relationship between Carnivora and Pholidota has been generally accepted. The main controversies are concentrated on the phylogenetic positions of the other three orders, i.e., Chiroptera, Cetartiodactyla and Perissodactyla.

References


Phylogenetics, the data flood in, but the challenges remain.


