Coalescent-based phylogenetic inference from genes with unequivocal historical signal suggests a polytomy at the root of the placental mammal tree of life

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38 Abstract

39 Finding the correct place of the root of the Placentalia tree of life is an unsolved problem in
 40 mammalian systematics. Three major competing hypotheses were proposed, alternating the position
 41 of the supraordinal taxa Afrotheria, Xenarthra, or Atlantogenata as sister lineages to the remaining
 42 placentals. While all three phylogenetic associations were inferred by studies conducted so far,
 43 recent assessments applying heterogeneous models and coalescent-based phylogenetic methods
 44 found robust support for the Atlantogenata hypothesis. Current developments in theoretical
 45 phylogenetics, such as the demonstration that concatenating genes may bias the likelihood function,
 46 and that summary coalescent-based phylogenetic methods are sensitive to errors in gene tree
 47 estimation, calls for a reevaluation of the early placental split problem. We investigated the
 48 phylogenetic relationships between placental superorders by restricting the analysis to subsets of
 49 genes with unequivocal phylogenetic signal. In contrast to previous works, we show that the
 50 hypothesis of a polytomy at the placental root could not be rejected under the multispecies
 51 coalescent model. This result endorses conclusions from analyses of retroposon insertion patterns.
 52 We provide an analytical framework to assess empirical polytomies employing summary coalescent
 53 methods and topological tests, helping the investigation of poorly resolved nodes in the tree of life.

54

55 Introduction

56 Rooting the placental tree of life is a major challenge in mammalian systematics (Teeling
57 and Hedges, 2013). Three hypotheses are generally considered regarding the phylogenetic
58 association of the major mammalian lineages, positioning Afrotheria (e.g. elephants and hyraxes;
59 (Asher, 2007; McCormack et al., 2012; Murphy et al., 2001; Nikolaev et al., 2007; Nishihara et al.,
60 2007; Romiguier et al., 2013), Xenarthra (e.g., armadillos and sloths; (Kriegs et al., 2006; O’Leary
61 et al., 2013; Waddell et al., 2001), or Atlantogenata, i.e., Afrotheria + Xenarthra (Hallström et al.,
62 2007; Hallström and Janke, 2008; Kjer and Honeycutt, 2007; Meredith et al., 2011; Morgan et al.,
63 2013; Murphy et al., 2007; Prasad et al., 2008; Song et al., 2012; Tarver et al., 2016; Wildman et al.,
64 2007), as the sister group to the remaining placentals (**Figure 1**). So far, studies employing different
65 data sources and methods, including genomic data, were unable to unambiguously resolve the early
66 placental split, motivating the proposition that the first divergences of the placental tree were not
67 strictly bifurcating (Churakov et al., 2009; Hallström and Janke, 2010; Nishihara et al., 2009).
68 Alternatively, other works suggested that this phylogenetic incongruence may be attributed to the
69 effects of incomplete lineage sorting (ILS) (Song et al., 2012), long branch attraction (LBA)
70 (Romiguier et al., 2013), and model misspecification (Morgan et al., 2013; Tarver et al., 2016).
71 Notwithstanding, assessments of this topological conflict adopting heterogeneous substitution
72 models (Morgan et al., 2013; Tarver et al., 2016) and summary coalescent methods (Song et al.,
73 2012; Tarver et al., 2016) found robust support for the Atlantogenata hypothesis, which was also
74 recovered when previously published datasets were re-examined with best-fitting models (Tarver et
75 al., 2016).

76 Despite these results, controversy remains, as methods employed in previous analyses are
77 not free of biases from different sources. For instance, it has been shown that, when sequences are
78 concatenated for carrying out supermatrix analysis, only a small fraction of sites may be responsible
79 for choosing between alternative topologies (Shen et al., 2017). Moreover, it has been demonstrated

that the likelihood function used in phylogenetics is inappropriate when genes are concatenated, resulting in positively misleading estimates under high levels of gene trees/species tree incongruence (Degnan and Rosenberg, 2006; Kubatko and Degnan, 2007; Mendes and Hahn, 2018; Roch and Steel, 2015), which might be the case of the early placental split (Song et al., 2012; Tarver et al., 2016). On the other hand, methods that avoid concatenation by accounting for the long-term effects of ILS were criticized in the context of placental phylogeny (Springer and Gatesy, 2016). The criticism lies on the fact that summary coalescent-based phylogenetic methods treat gene trees as error-free observations (Song et al., 2012). As maximum likelihood trees are point estimates, treating them as observations imply overconfidence, which may bias phylogeny estimation by approaches derived from the multispecies coalescent (MSC) model (Xi et al., 2015). This issue has been largely ignored by analyses of the placental tree relying on coalescent-based methods (McCormack et al., 2012; Song et al., 2012; Tarver et al., 2016). It is thus conceivable that previous studies on placental phylogeny were impacted by genes with ambiguous signal for resolving the early divergences.

Following this reasoning, in this work, we were prompted to investigate, under the MSC model, which phylogenetic hypothesis regarding the early placental split was significantly favored when only subsets of phylogenetically informative genes were employed. We examined the mammalian phylogeny from two subsets of genes trees that either presented unequivocal phylogenetic resolution for the early placental split or consisted of topologies that did not overfit data.

100

101 **Material and Methods**

102 Our analysis was carried out using 14,526 orthologous coding sequences retrieved from the
 103 OrthoMam v9 data base (Douzery et al., 2014), which were filtered to consist solely of genes whose
 104 maximum likelihood (ML) tree contained an outgroup and at least one representative of each
 105 placental superorder, namely, Xenarthra, Afrotheria, Laurasiatheria and Euarchontoglires.
 106 Furthermore, to reduce the phylogenetic problem to a 4-terminals tree, which makes the topological
 107 space computationally accessible, only ML trees wherein superorders were recovered as
 108 monophyletic were kept. We thus focused on the phylogenetic resolution of the early placental
 109 divergence, allowing us to evaluate all the three possible topological hypotheses (**Figure 1**). After
 110 this filtering step, our dataset was composed of 5,862 alignments, hereafter referred to as the *full*
 111 dataset.

112 Gene tree estimation and choice of substitution models were performed with the IQ-TREE
 113 software (Kalyaanamoorthy et al., 2017; Nguyen et al., 2014). The statistical support for the three
 114 topological configurations at the root was assessed by the approximately-unbiased (AU) test
 115 (Shimoidara, 2002), also implemented in IQ-TREE. Genes with unequivocal phylogenetic signal,
 116 i.e., those with exclusive support for a single topology, were grouped, constituting the *AU* dataset.
 117 Finally, we also built a dataset encompassing genes that, under the corrected Akaike information
 118 criterion (AICc) (Hurvich et al., 1998), rejected the hypothesis of a polytomy between the
 119 mammalian superorders, suggesting a strictly bifurcating tree as the best fit model. Species tree
 120 inference was conducted independently for each dataset (*full*, *AU*, and *AICc*) employing the
 121 coalescent-based algorithm implemented in the Astral-III software (Zhang et al., 2017), which also
 122 performs hypothesis testing of polytomies according to the MSC model expectation (Sayyari and
 123 Mirarab, 2018). A significance level of 5% was used throughout this study. Astral was also used to
 124 estimate branch lengths in coalescent units, which is relevant for interpreting gene tree distribution
 125 in light of the MSC, in particular to investigate whether the branching pattern that defines the deep

126 splits between the major lineages of placentals lies in the anomaly zone (Degnan and Rosenberg,
127 2006).

128

129 Results

130 The distribution of ML gene trees supporting each of the three possible phylogenetic
131 hypotheses of the early placental split was approximately equivalent in the *full* dataset. Out of 5,862
132 gene trees, 1,910 (32.6%) supported the Atlantogenata hypothesis, whereas 2,045 (34.9%)
133 supported Afrotheria, and 1,907 (32.5%) favored the Xenarthra tree. Therefore, the majority of gene
134 trees, as well as MSC-topology from Astral analysis, supported the Afrotheria tree. The coalescent-
135 based polytomy test also rejected the null hypothesis of a polytomic topology for this distribution of
136 gene trees ($p = 0.04$). However, when each gene from the *full* dataset was subjected to the AU test,
137 we found that only 101 genes (0.02%) presented unequivocal phylogenetic signal for a single
138 topology concerning the early placental split (the *AU* dataset). Each of the three possible topological
139 associations were represented with the following frequencies among these gene trees: 37.6%
140 supporting Atlantogenata, 22.8% Afrotheria and 39.6% Xenarthra. When Astral analysis was run
141 using the *AU* dataset, the Xenarthra topology was inferred as the species tree, although the null
142 hypothesis of a polytomy at the root of placental mammals was not rejected ($p = 0.08$).

143 According to the AICc test, 2,079 gene trees, nearly 35% of the *full* data set, overfit data.
144 The distribution of topologies inferred from the remaining 3,782 genes that favored a strictly
145 bifurcating tree as the best fit model (the *AICc* dataset) presented a similar frequency of each early
146 placental phylogenetic hypothesis, namely, Atlantogenata: 1,259 (33.3%), Afrotheria: 1,308
147 (34.6%), and Xenarthra: 1,216 (32.2%). Astral inferred the Afrotheria as the placental species tree
148 using the *AICc* dataset, but the hypothesis of a polytomic association among superorders was also
149 not rejected ($p = 0.29$). Branch lengths establishing the pattern of the early placental split in the *full*,
150 *AU*, and *AICc* datasets measured 0.0235, 0.0924, and 0.0167 coalescent units respectively (**Table**
151 **1**).

152

153

154 Discussion

155 Our results indicated that when genes with significant phylogenetic signal were used,
 156 Afrotheria or Xenarthra were the likely sister lineages of the remaining placentals. However, in both
 157 datasets, the hypothesis of a polytomy at the root of the placental tree was not rejected. When it
 158 comes to phylogenomics, all three phylogenetic hypotheses depicted in **Figure 1** were obtained by
 159 combining different methods and datasets. For instance, support for the Afrotheria tree was found
 160 when datasets less prone to LBA artifacts were employed (Romiguier et al., 2013), and when
 161 ultraconserved elements were analyzed (McCormack et al., 2012). With a single exception (Kriegs
 162 et al., 2006), the Xenarthra tree has been rarely recovered by molecular data, although it stands as
 163 the preferred hypothesis of morphologists (O’Leary et al., 2013). In our analyses, we found no
 164 support for the Atlantogenata tree. This hypothesis was recently endorsed by Tarver *et al.* 2016,
 165 who implemented MSC methods and argued that correcting for heterogeneous substitution
 166 processes supports Atlantogenata as sister lineage to the remaining placentals. On the other hand,
 167 most previous works have relied on concatenated supermatrices (Hallström et al., 2007; Hallström
 168 and Janke, 2008; McCormack et al., 2012; Morgan et al., 2013; Nikolaev et al., 2007; Nishihara et
 169 al., 2007; Romiguier et al., 2013; Wildman et al., 2007), which may be affected by the few-
 170 sites/large-effect issue (Shen et al., 2017). In face of these conflicting results, our study indicates
 171 that this lack of resolution for the early placental split might be due to the failure of the distribution
 172 of topologies from genes with significant phylogenetic signal to reject a polytomic association
 173 among mammalian superorders.

174 In this sense, it is meaningful to investigate whether the branching pattern of the early
 175 placental split lies in the anomaly zone, the region of parameter space where the most common gene
 176 tree is incongruent with the species tree (Degnan and Rosenberg, 2006). Even though it has been
 177 argued that the anomaly zone is unlikely to be a problem when studying empirical datasets (Huang
 178 and Knowles, 2009), the branch lengths supporting resolutions for the early placental split in all

179 species trees inferred here were shorter than ~ 0.156 coalescent units (**Table 1**). Therefore, the
 180 theoretical possibility of obtaining anomalous gene trees distribution exists when investigating
 181 supraordinal relationship of placental mammals. Under this scenario, phylogenetic inference from
 182 concatenated sequences will be biased (Degnan and Rosenberg, 2006; Kubatko and Degnan, 2007;
 183 Mendes and Hahn, 2018; Roch and Steel, 2015).

184 By restricting our analysis to subsets of genes with significant phylogenetic signal, our study
 185 also avoided setbacks of summary coalescent methods (e.g., Astral) when gene tree uncertainty is
 186 not accounted for. It is worth noting that, although Tarver *et al.* (2016) implied that few genes have
 187 unequivocal signal for resolving the placental root by likelihood comparisons, which we
 188 corroborated via proper topological tests, the authors did not restrict their MSC phylogeny inference
 189 to this subset of unequivocal genes. Instead, they controlled for errors in gene trees by filtering
 190 topologies with non-parametric bootstrap thresholds (Felsenstein, 1985). The bootstrap, however, is
 191 not a formal statistical test between alternative topologies, and such procedure may have resulted in
 192 topological overconfidence.

193 The hypothesis of a polytomy at the root of placentals has been previously advocated by
 194 studies relying on retroposon insertions as phylogenetic markers (Churakov *et al.*, 2009; Nishihara
 195 *et al.*, 2009). Alternatively, using supermatrices, Hallström and Janke (2010) argued that the
 196 phylogenetic relationship among placental superorders is best represented by a phylogenetic
 197 network. While the proposition of a placental root polytomy is not entirely novel, this is the first
 198 coalescent-based analysis supporting this view, contradicting previous phylogenomic studies that
 199 were possibly affected by the very issues that motivated our reexamination of this problem
 200 (Hallström *et al.*, 2007; Hallström and Janke, 2008; McCormack *et al.*, 2012; Morgan *et al.*, 2013;
 201 Nikolaev *et al.*, 2007; Nishihara *et al.*, 2007; Romiguier *et al.*, 2013; Song *et al.*, 2012; Tarver *et al.*,
 202 2016; Wildman *et al.*, 2007). Our results were not able to devise whether the placental root is the
 203 case of a soft (dataset limited) or hard (real) polytomy (Whitfield and Lockhart, 2007), but we

showed how the prior examination of the historical signal of genes, associated with a control for systematic biases, impacts the resolution of a hard phylogenetic problem.

As conservative as our approach was, it illustrated how the MSC model may be used to test for polytomies in empirical datasets (Edwards, 2009). Future application of this framework is promising in unveiling rapid radiations in the deeper nodes of the tree of life (Whitfield and Lockhart, 2007). As has been argued by Shen et al. (Shen et al., 2017), branches that fail to reject a polytomy should be considered unresolved, implying a more accurate view of the signal available in data. We here provided evidence that this is the case of the early split between superorders of Placentalia.

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339

340 **Table 1.** Summary of results from analyses employing three different filters on the alignments from
341 OrthoMam.

Dataset	Number of genes	Gene tree distribution (Atlg : Afro : Xnrt)	Astral species tree	Null hypothesis of polytomy	Branch length in coalescent units
Full	5,862	32.6 : 34.9 : 32.5	Afrotheria	Rejected ($p = 0.04$)	0.0235
AU	101	37.6 : 22.8 : 39.6	Xenarthra	Not rejected ($p = 0.08$)	0.0924
AICc	3,782	33.3 : 34.6 : 32.2	Afrotheria	Not rejected ($p = 0.3$)	0.0167

342

343 **Figure legend.**

344 **Figure 1.** The three phylogenetic hypotheses for the position of the root of placental mammals (a).
 345 Distribution of gene tree topologies in the *full*, *AICc*, and *AU* datasets, with their respective species
 346 tree resolution inferred with Astral-III. The *p*-value of the polytomy test (above) and lengths in
 347 coalescent units (bellow) were given for branches responsible for resolving the polytomy at the root
 348 (b).