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Forked Tongues Revisited: Molecular Apomorphies Support Morphological Hypotheses of Squamate Evolution

Caleb D. McMahan1,2, Layla R. Freeborn3, Ward C. Wheeler4, and Brian I. Crother5

Incongruence between morphological and molecular-based phylogenetic hypotheses has been reported across a wide range of taxa. Specifically, morphological and molecular hypotheses of squamate phylogeny have been consistently incongruent and have been notoriously difficult to reconcile. With the ever-rising popular view of the superiority of molecular data over morphological data, studies have been biased toward seeking explanations for homoplasy in the morphological data. We propose considering approaches that do not make a priori assumptions about the superiority of one type of data over another. We present two case studies using the proto-oncogene c-mos, as well as a large multilocus dataset, to examine apomorphy distributions across hypotheses. This approach reveals novel insights into data incongruence currently plaguing squamate phylogeny, with direct implications for other scenarios of phylogenetic incongruence.

Incongruence between molecular and morphological phylogenetic hypotheses has been reported across a wide range of taxa, including woodcreepers (Irestedt et al., 2004), cetaceans and artiodactylans (OLeary et al., 2003; OLeary and Gatesy, 2008), placental mammals (Lee and Camens, 2009), haptosaurians (Burreson and Reece, 2006), pickerelweed (Graham et al., 1998), hexapods (Bitsch et al., 2004), fishes (Betancur-R., 2009), and squamates (Vidal and Hedges, 2005; Losos et al., 2012). Numerous aspects of analyses (e.g., taxon sampling, outgroup selection, number and types of characters/genes included, chosen method of analysis, species vs. gene trees) may help explain the discordance between phylogenies derived from these different data types; however, the issue still receives considerable attention in the literature, and deservedly so, as it is largely unresolved (Hermsen and Hendricks, 2008; Lee and Camens, 2009; Losos et al., 2012). Nevertheless, many evolutionary biologists are quick to prefer molecular hypotheses to morphological, which begs the conclusion that the molecular data and inferred phylogeny are somehow superior to the morphological (e.g., Crother and Presch, 1992; Hedges and Sibley, 1994; Hedges and Maxson, 1996; Scotland et al., 2003; Wiens, 2004). For example, it is common practice in the study of trait evolution to map characters over molecular trees (termed the molecular scaffold approach; Hermsen and Hendricks, 2008). However, this method makes the implicit and unwarranted assumption that the molecular hypothesis is the best-inferred hypothesis (Mooi and Gill, 2010).

Squamata (lizards, snakes, amphisbaenians) comprises a group of approximately 8,000 species with a diversity of ecological, behavioral, and physiological adaptations. Since Camp’s initial, pre-cladistic work (Camp, 1923), numerous morphology-based analyses, often including numerous fossil taxa, have obtained results strikingly similar to the landmark analysis (Wu et al., 1996; Evans and Barbadillo, 1998; Lee, 1998; Caldwell, 1999; Lee and Caldwell, 2000; Evans et al., 2005; Conrad, 2008). However, the results of molecular studies (Townsend et al., 2004; Vidal and Hedges, 2005; Albert et al., 2009; Eckstut et al., 2009; Wiens et al., 2010, 2012; Pyron et al., 2013) sharply contradict morphology-based hypotheses. Losos et al. (2012) published a thought-provoking overview of the stark incongruence between morphological and molecular phylogenetic hypotheses of squamates and brought to light the need for investigation into the factors leading to this incongruence. In short, all morphological studies of squamate phylogeny (regardless of taxon sampling and characters) have recovered a sister relationship between the Iguania and Scleroglossa; however, no molecular study has ever recovered this relationship. Furthermore, there is little to no consensus across molecular studies as to the earliest divergence within squamates. By way of assessing the incongruence, Conrad (2008) fit his morphological data onto two molecular phylogenies (Townsend et al., 2004; Vidal and Hedges, 2005) of squamates and found hypotheses less parsimonious by 175 and 171 steps, respectively. Gauthier et al. (2012) were also unsuccessful in finding morphological characters from their dataset to support molecular hypotheses of squamate evolution, noting a necessary 51–77 reversals (“cases in which iguanians must re-evolve the unmodified ancestral lepidosaurian conditions . . .”) for molecular hypotheses of the position of Iguania to be accurate (Gauthier et al., 2012). Additionally, Gauthier et al. (2012) found not a single synapomorphy (ambiguous nor unambiguous) that supported gene tree hypotheses for a nested Iguania within either Autarchoglossa or Scleroglossa, much less any part of Anguimorphia.

Here, we aim to address the incongruence between molecular and morphology-based hypotheses of squamate interrelationships. We investigate character support for the two major clades (i.e., earliest divergence within Squamata) by employing the opposite of the molecular scaffold approach (thus mapping molecular data onto...
a morphological topology. We first address whether there are molecular apomorphies for morphological hypotheses of squamate phylogeny, then compare results to support on molecular trees. By investigating morphological/molecular incongruence in squamate relationships, we present a method for examining similar scenarios of phylogenetic incongruence in other taxa.

MATERIALS AND METHODS

To employ our method, we chose two large molecular datasets. The molecular study of Eckstut et al. (2009) uses only the highly conserved proto-oncogene \textit{c-mos}, but we chose to use the dataset and phylogeny from this study given that it is one of the most complete in terms of taxon sampling (>600 spp.) for a single gene. Second, we used the molecular study of Pyron et al. (2013), as this multi-locus dataset consists of 12 genes for 4161 species of squamates. The morphological phylogeny of Conrad (2008) was utilized because this study has the greatest depth of taxon sampling of both extant and extinct lineages. Given that the \textit{c-mos} dataset is only one gene, taxa were compared between Conrad (2008) and Eckstut et al. (2009) to determine genera present in both analyses (\( n = 35 \)). \textit{C-mos} sequences for individuals (one per species for as many available species per genus) were taken from the original, aligned data file from Eckstut et al. (2009), and these sequences were compiled. Given the breadth of taxon and locus sampling, the entire Pyron et al. (2013) dataset was used.

Two tree topologies were constructed for each dataset using Mesquite (Maddison and Maddison, 2011)—one with the morphological Iguania + Scleroglossa relationship, followed by: Teiidae/Gymnophthalmidae + remaining Squamata (following \textit{c-mos} tree) and Dibamidae + remaining Squamata (following Pyron et al., [2013] tree). Topologies are shown in Figures 1 and 2. Using PAUP* version 4.0a14 (Swofford, 2002), each molecular dataset was mapped onto both the molecular and morphological topologies via the Describe Trees > List of apomorphies option (ACCTRAN optimization). The numbers of unambiguous and ambiguous character state changes were recorded for each clade, as well as standard descriptive statistics for each topology (Consistency Index [CI], Homoplasy Index [HI], Retention Index [RI]).

RESULTS

Descriptive statistics (CI, HI, and RI) are relatively similar for both datasets, regardless of topology (Figs. 1, 2). For the \textit{c-mos} dataset for squamates, results show that a number of molecular apomorphies support an Iguania clade and sister Scleroglossa clade (Fig. 1A), the most internal sister relationship consistently recovered in all morphological squamate phylogenies to date. There are 14 unambiguous (thus unique and unreversed) and 51 ambiguous character state changes diagnosing the Iguania, and 24 unambiguous and 26 ambiguous changes diagnosing its sister Scleroglossa. In stark contrast, however, there is far less support for the Teiidae/Gymnophthalmidae and sister clade consisting of remaining Squamata.

For the multi-locus dataset of Pyron et al. (2013), a similar pattern was observed as for analysis of the \textit{c-mos} dataset. Sixteen unambiguous and 954 ambiguous character state changes diagnose the Iguania, and 19 unambiguous and 977 ambiguous character state changes diagnose a sister Scleroglossa (Fig. 2A). Once again, there is less support for the Dibamidae and sister clade consisting of remaining Squamata.
squamates, as recovered in Pyron et al. (2013). Only three unambiguous and 729 ambiguous character state changes support this position of Dibamidae, with three unambiguous and 511 ambiguous apomorphies supporting a sister clade consisting of other squamates (Fig. 2B).

**DISCUSSION**

**Distant outgroups.**—Our results show that numerous apomorphies from both the c-mos sequence dataset and multi-locus dataset unambiguously support a monophyletic lineage of Iguania, as well as its sister clade, the Scleroglossa. Despite different topologies inferred from phylogenetic analysis of the c-mos dataset and multi-locus dataset of Pyron et al. (2013), in both cases these molecular data seem to better fit the morphological topology (Conrad, 2008; and others), at least for the most internal relationships within squamates. We contend that molecular-based squamate phylogenies may be misled by the genetic divergence between the only reasonable outgroup (tuatara; Rhynchocephalia: Sphenodon spp.) and the ingroup. For the molecular datasets used in the present study, tuatara constitutes the longest branch of both respective trees. Furthermore, although Rhynchocephalia is still considered the closest extant sister lineage to Squamata within the Lepidosauromorpha, recent work has shown that the numerous differences between squamates and rhynchocephalians are no longer interpreted as plesiomorphic; but instead are considered derived (Conrad, 2008). The latter interpretation suggests a long evolutionary separation between rhynchocephalians and squamates, with the potential for several extinct lepidosauromorphian lineages; indeed, a number of fossil taxa exist that are phylogenetically intermediate between tuataras and squamates (e.g., Eolacertilia; however, detailed position of these lineages between tuataras and squamates remains uncertain; Conrad, 2008). These critical taxa would be impossible to be accounted for in a molecular dataset and obviously contribute to the divergence between tuataras and squamates.

Wheeler (1990), regarding the inference of phylogeny with DNA sequence data, clearly showed that distant outgroups are essentially random data connections to the ingroup. The immediate consequence of such a connection would be a random placement of the root, and consequences of this are discussed at length in Hillis and Wiens (2000). Given that the distance from tuataras to Squamata is greater than the average distance across all Squamata, we can consider this outgroup distant and consider the root determined by it to be suspect. If *Sphenodon* is a functionally random outgroup, it could root the tree at the longest branch of the ingroup (Wheeler, 1990). To us, this is further convincing evidence that *Sphenodon* is a functionally random outgroup for these two molecular datasets and may explain the unusual relationships in this and other DNA-based trees. One important caveat to consider is that like most squamate phylogenetic studies, we have only a single outgroup taxon. In this case, the use of additional outgroup taxa (e.g., Wiens et al., 2012) only introduces even more distantly related lineages and exacerbates this issue.

**Homoplasy.**—A second possible explanation for the incongruence between molecular and morphological-based topologies concerns homoplasy. The claim has often been made that morphology is inferior to molecular data for phylogeny reconstruction because morphology is rife with homoplasy (Hedges and Sibley, 1994; Hedges and Maxson, 1996; Scotland et al., 2003; Wiens et al., 2010). However, concluding convergence of morphological traits based on the mapping of these traits onto a molecular tree (i.e., the molecular scaffold approach) only indicates convergence...
based on that topology the data were fitted to—only discovering incongruence, which is not evidence of superiority of one dataset over another for phylogeny reconstruction. Based on our reverse scaffold approach, it appears there is so much homoplasy in the c-mos and multi-locus datasets that those characters easily distribute on the morphological tree as unambiguous and ambiguous synapomorphies for the earliest divergence within Squamata, with more characters than on the trees inferred from each dataset themselves. Additionally, numerous authors have argued that these patterns of homoplasy are what one would expect for misplaced taxa in a phylogeny (Gauthier et al., 1988, 2012; Donoghue et al., 1989).

Of course additional explanations for the incongruence (see Introduction above) are equally plausible and should also be investigated. Clearly the overwhelming evidence gathered from phylogenetic incongruence among hypotheses of squamate evolution, indicates that the amount of data (i.e., number of loci) is not the problem, nor is the taxon sampling (e.g., Gauthier et al., 2012; Pyron et al., 2013). Regardless of how these variables are adjusted, corroboration is never achieved between the two sources of data. Reeder et al. (2015) utilize a total evidence approach to study the phylogeny of Squamata and claimed to have solved the incongruence problem. However, our results show the problem is not resolved. It is interesting to us that they interpret the “unexpected placements for fossil taxa” as a better hypothesis than wondering if there may be a problem.

Most prior work has asked the question “why do morphological data not recover molecular relationships?” We ask the reverse “why do molecular data not recover the morphological relationships?” We argue that consideration of both these questions is an important and crucial step in attempting to understand incongruence between these two groups of hypotheses. The morphological scaffold approach identifies 50 c-mos characters and 996 characters across 12 loci supporting the Scleroglossa, thus excluding the Iguania from the Scleroglossa. While there are clearly synapomorphies supporting some of the internal molecular clades (thus similar descriptive statistics for both topologies within each dataset), the stark incongruence in the position of Iguania still begs explanation. We propose the hypothesis that the molecular data are incongruent with morphological hypotheses of squamate evolutionary relationships not because the molecular data are correct or that the morphology is rife with homoplasy, but because there may be an inherent analytical problem with the molecular data. What this problem is, we are uncertain, but we can point to the distant outgroup as a starting place.

These results should be of immediate interest to systematists, as well as others who utilize phylogenies, as we suspect that if this morphological scaffold method were employed on other scenarios of molecular-morphological phylogenetic incongruence, similar results may be obtained. Future work could be aimed at including vigorous statistical tests of both the molecular and morphological hypotheses of squamate evolution, indicating that the amount of data (i.e., number of loci) is not the problem, nor is the taxon sampling (e.g., Gauthier et al., 2012; Pyron et al., 2013). Regardless of how these variables are adjusted, corroboration is never achieved between the two sources of data. Reeder et al. (2015) utilize a total evidence approach to study the phylogeny of Squamata and claimed to have solved the incongruence problem. However, our results show the problem is not resolved. It is interesting to us that they interpret the “unexpected placements for fossil taxa” as a better hypothesis than wondering if there may be a problem.

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LITERATURE CITED


