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When do species-tree and concatenated estimates disagree? An empirical analysis with higher-level scincid lizard phylogeny

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ABSTRACT

Simulation studies suggest that coalescent-based species-tree methods are generally more accurate than concatenated analyses. However, these species-tree methods remain impractical for many large datasets. Thus, a critical but unresolved issue is when and why concatenated and coalescent species-tree estimates will differ. We predict such differences for branches in concatenated trees that are short, weakly supported, and have conflicting gene trees. We test these predictions in Scincidae, the largest lizard family, with data from 10 nuclear genes for 17 ingroup taxa and 44 genes for 12 taxa. We support our initial predictions, and suggest that simply considering uncertainty in concatenated trees may sometimes encompass the differences between these methods. We also found that relaxed-clock concatenated trees can be surprisingly similar to the species-tree estimate. Remarkably, the coalescent species-tree estimates had slightly lower support values when based on many more genes (44 vs. 10) and a small (~30%) reduction in taxon sampling. Thus, taxon sampling may be more important than gene sampling when applying species-tree methods to deep phylogenetic questions. Finally, our coalescent species-tree estimates tentatively support division of Scincidae into three monophyletic subfamilies, a result otherwise found only in concatenated analyses with extensive species sampling.

1. Introduction

A major conundrum has arisen in the phylogenetic analysis of multi-locus sequence data. In recent years, there has been growing appreciation for the idea that the evolutionary histories of species (species trees) and the genes that they carry (gene trees) can often differ (e.g. Edwards, 2009; Maddison, 1997). To address this problem of discordance between gene and species trees, a variety of methods for explicit species-tree reconstruction have been developed (e.g. Edwards et al., 2007; Heled and Drummond, 2010; Kubatko et al., 2009; Larget et al., 2010; Liu, 2008). Simulation studies show that these methods, particularly those employing Bayesian coalescent models (i.e. BEST, *BEAST), are more accurate than other approaches for inferring species trees, especially traditional concatenated analysis (e.g. Bayzid and Warnow, 2012; Edwards et al., 2007; Heled and Drummond, 2010; Hovmöller et al., 2013; Leaché and Rannala, 2011). The use of *BEAST (Heled and Drummond, 2010), which coestimates gene and species trees using the multispecies coalescent, has become particularly widespread (e.g. according to Google Scholar April 12th, 2014: 580 citations for the paper proposing *BEAST as compared to <200 each for BEST [Liu, 2008], STEM [Kubatko et al., 2009] and BUCKy [Larget et al., 2010]). However, it is widely known (if not explicitly documented) that *BEAST and similar methods can become computationally intractable with large numbers of genes or species. Unfortunately, such large matrices may be the most useful for accurately resolving phylogenies (e.g. Rannala et al., 1998) and for macroevolutionary analyses (e.g. Davis et al., 2013). Other species-tree methods, while computationally more efficient (e.g. Kubatko et al., 2009; Larget et al., 2010), may require complete datasets (all species with data for all genes), making them impractical in many cases. Furthermore, it is unclear whether coalescent species-tree methods (e.g., *BEAST) are preferable to concatenation for deep phylogenetic questions, especially given limited sampling of species and individuals. These and other issues make choosing between concatenated and species-tree analyses an increasingly common problem for empirical studies (McVay and Carstens, 2013).

The current impracticality of coalescent species-tree methods for many phylogenetic studies leaves systematists with several important and unresolved questions: how similar are phylogenetic estimates between concatenated and coalescent species-tree

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approaches? In other words, do concatenated analyses offer a reasonable proxy? Can we make accurate predictions about when estimates from concatenated and species-tree analyses will differ? Specifically, can we examine a tree from concatenated analysis and predict which branches are most likely to be resolved differently using coalescent species-tree methods? Here, we make specific predictions regarding these questions and test them empirically using data from scincid lizards.

Species-tree methods generally rest on the assumptions that gene trees and species trees disagree, that gene trees disagree with one another, and that species-tree methods deal with this discordance better than concatenation (e.g. Edwards, 2009; Edwards et al., 2007; Maddison and Knowles, 2006). Further, methods such as BEST and *BEAST assume that this genealogical discordance is due to incomplete lineage sorting (e.g. Edwards et al., 2007; Heled and Drummond, 2010). Theory suggests incomplete lineage sorting will be more prevalent on shorter branches in the true species tree (all other things being equal; e.g. Degnan and Rosenberg, 2009; Maddison, 1997; Pamilo and Nei, 1988). Empirical multilocus analyses (e.g. Wiens et al., 2008, 2010a, 2012) show that shorter branches in concatenated trees tend to have weaker support (e.g. bootstrap proportions) and greater disagreement with their underlying gene trees (e.g. a smaller proportion of genes will support the short branch in the concatenated tree, and the other genes will support trees that conflict with this branch). Therefore, we predict that estimates from concatenated analysis and species-tree methods will tend to disagree most often on those branches of the concatenated tree that have: (1) shorter estimated branch lengths (assuming that short branches in the concatenated tree reflect short branches in the underlying species tree, and short branches in concatenated trees do seem to reflect short branches in comparable gene trees; Wiens et al., 2008), (2) weaker support (i.e. low bootstraps and/or Bayesian posterior probabilities), and (3) greater disagreement with the underlying gene trees. Although these assumptions may seem simple and uncontroversial, they do have important implications. For example, if discordance with species-tree estimates occurs primarily on weakly supported branches of concatenated trees, then comparative studies that use concatenated trees can incorporate the likely impact of using species-tree methods simply by incorporating uncertainty in the concatenated estimate (e.g. repeating analyses on trees with alternate resolutions of the poorly supported branches).

Another important, unresolved issue is whether coalescent-based species-tree methods are effective for resolving deep phylogenetic splits, especially with limited sampling of individuals and species. So far, *BEAST is most frequently applied to relatively closely related species, and with >1 individuals sampled per species (but see for example Perez et al., 2012; Townsend et al., 2011; Wiens et al., 2012; Williams et al., 2013). Both simulations and empirical analyses have shown that reduced sampling of individuals per species can decrease the accuracy of *BEAST (Camargo et al., 2012; Heled and Drummond, 2010; Hovmöller et al., 2013). Specifically, the lack of multiple individuals per species prevents *BEAST from accurately estimating population sizes for extant taxa (i.e. terminal branches), which may negatively impact estimates of divergence times and possibly topology (Heled and Drummond, 2010). Previous work suggests that sampling additional individuals has a greater effect on accuracy at shallow phylogenetic levels and sampling additional genes is more beneficial for deeper relationships (Heled and Drummond, 2010; Maddison and Knowles, 2006). However, it remains unclear whether limited sampling of individuals may make *BEAST estimates problematic, and whether it is even worth applying to typical higher-level empirical studies in which one tries to estimate deep divergences in species-rich groups with limited sampling of both individuals and species. The impact of limited species sampling (and the relative importance of sampling genes versus species) on species-tree analyses of higher-level relationships is especially poorly known.

Here we compare multispecies coalescent species-tree (i.e. *BEAST) and concatenated estimates using empirical datasets for higher-level scincid lizard phylogeny. Skinks are the most species-rich family of lizards, containing >1560 species (Uetz et al., 2013), and are relatively ancient (crown group ~80–110 million years old; Mulcahy et al., 2012). Skink phylogeny and higher-level classification remain uncertain, despite recent studies based on concatenated analyses of multiple nuclear and mitochondrial genes (e.g. Brandley et al., 2012; Pyron et al., 2013; Wiens et al., 2012). For example, Hedges and Conn (2012) divided Scincidae into seven families (without estimating a large-scale phylogeny), but Pyron et al. (2013) found some of these families to be non-monophyletic. Traditional classifications recognized four subfamilies in Scincidae (Acontinae, Feylininae, Lygosominae, Scincinae; Greer, 1970 and subsequent authors). Brandley et al. (2012) analyzed 56 scincid species and found Scincinae to be paraphyletic with respect to Lygosominae (with Acontinae as sister to the other subfamilies), but did not include Feylininae. In a broad analysis of squamate phylogeny, Wiens et al. (2012) analyzed 44 nuclear loci and included 12 skink species and found Scincinae to be paraphyletic with respect to Feylininae and Lygosominae, with Acontinae as sister to all other scincids. In another large-scale analysis of squamate phylogeny, Pyron et al. (2013) included 683 scincid species for multiple nuclear and mitochondrial genes (but with relatively incomplete gene sampling) and supported monophyly of Acontinae (as sister to all other scincids), Lygosominae, and Scincinae (including Feylininae). We follow this latter classification here. However, as of yet, no studies have used species-tree methods to address higher-level relationships in Scincidae.

In this study, we analyze higher-level scincid phylogeny and test our predictions about concordance of species-tree and concatenated estimates using a dataset of 17 ingroup species and 10 nuclear protein-coding genes (combining published and new data). We also compare concatenated and species-tree estimates from a previously published dataset (Wiens et al., 2012) containing 44 nuclear protein-coding genes for 12 scincid species.

2. Materials and methods

2.1. Sequence data and alignment

We assembled data from 10 nuclear protein-coding loci from 17 species representing all four traditionally recognized subfamilies of Scincidae (Acontinae, Feylininae, Lygosominae, Scincinae), including multiple representatives of the subfamilies Lygosominae and Scincinae, which together include most species and genera of skinks (Uetz et al., 2013). These 17 species spanned many levels of divergence, including multiple species in the species-rich genus *Sphenomorphus*. As outgroups, we included six species representing the families Cordylidae (*Cordylus*, *Platysaurus*), and Gerrhosauridae (*Cordylosaurus*, *Zonosaurus*), and Xantusiidae (*Lepidophyma*, *Xantusia*). Molecular and combined-data analyses of higher-level squamate phylogeny have repeatedly shown that these three families form a strongly supported clade that is the sister group of Scincidae (e.g. Mulcahy et al., 2012; Pyron et al., 2013; Wiens et al., 2010b, 2012). Voucher specimens used are listed in online Appendix A.

For our primary dataset (17 ingroup taxa, 10 genes) we first utilized published sequence data from all 12 scincid taxa included by Wiens et al. (2012) for a subset of 10 relatively well-sampled genes. The 10 genes are (with aligned lengths): AHR = 453 base pairs, BDNF = 669, DNAH3 = 646, ECEL = 480, FSTL5 = 621, GPR = 509, NGFB = 579, PTGER = 492, RAG1 = 996, ZFP36L1 = 606

(total concatenated length = 6051). These 10 gene segments were carefully selected to be: (1) single copy, to avoid paralogy, (2) within a single exon, to facilitate alignment, (3) short enough to be amplified and sequenced with single pairs of reactions (~450–1000 bp), and (4) evolving at an appropriate rate for squamate phylogenetics (see Townsend et al., 2008). All 10 genes have been used successfully in higher-level squamate phylogenetics (e.g. Mulcahy et al., 2012; Townsend et al., 2011;Wiens et al., 2008, 2010b, 2012). For these 10 genes, we then obtained sequences from five additional scincid species (*Egernia whitii*, *Mabuya unimarginatus*, *Prasinohaema virens*, *Sphenomorphus nigriventris*, *Tropidophorus grayi*), all from the subfamily Lygosominae, which contains most species and genera of scincids (Uetz et al., 2013). We also obtained a few additional sequences from the initial set of 12 species. We used standard methods of DNA extraction, amplification, and Sanger sequencing. Primers are given in Townsend et al. (2008), Mulcahy et al. (2012), and Wiens et al. (2012). Given the protein-coding sequences used, alignment was straightforward and done by eye, after translating nucleotide sequences to amino acid sequences with MacClade version 4.0 (Maddison and Maddison, 2000). GenBank numbers are provided in online Appendix B, and the data matrix will be deposited in Dryad upon acceptance.

For each gene, we conducted preliminary phylogenetic analyses using parsimony (PAUP* 4.0b10; Swofford, 2002) to look for any possible contamination or sequencing errors (i.e. different species with identical sequences). Any such erroneous sequences were deleted. However, we did not exclude sequences merely because they conflicted with trees from other genes, to avoid biasing our analyses with regards to congruence between genes.

For the entire dataset, we were not able to obtain sequences for all species for all genes (missing data = 14.6% across the data matrix), despite repeated attempts using multiple primer combinations. However, both empirical and simulation studies suggest that even large amounts of missing data do not necessarily impede accurate estimation with model-based methods for concatenated data (review in Wiens and Morrill, 2011), and recent simulation studies suggest that *BEAST may also be resilient to missing data (Bayzid and Warnow, 2012; Hovmöller et al., 2013).

2.2. Model and partition selection

We used PartitionFinder v.1.1.1 (Lanfear et al., 2012) to select both data partitioning schemes and models of sequence evolution for all concatenated and single-gene datasets. Models and partitions selected are described in Appendix C. PartitionFinder uses maximum likelihood and the information theoretic criterion to select partitioning schemes and models. We changed the models that PartitionFinder compared based on the software used for subsequent phylogenetic analyses (e.g. only GTR+Γ for RAxML analyses). We did not evaluate models that include both a parameter for among-site rate heterogeneity and a parameter for invariant sites (i.e. GTR+I+Γ model), because correlation between these two parameters may make it difficult to estimate both accurately (e.g. Sullivan et al., 1999; Yang, 2006). We used the sample-size corrected Akaike Information Criterion (AICc; Sugiura, 1978) to select models and employed the greedy heuristic algorithm in all cases.

2.3. Likelihood analysis

Maximum likelihood analyses of individual genes and the concatenated data were conducted using RAxML v7.4.2. (Stamatakis, 2006). For each analysis, we conducted 1000 rapid bootstrap replicates combined with 200 replicate searches for the optimal tree, using the "-f a" option. We used the partitions identified above.

2.4. Bayesian concatenated and gene tree analyses

We conducted Bayesian analyses of the concatenated data and separate genes using a standard (non-clock) approach (MrBayes v3.2.1; Ronquist et al., 2012) and using a relaxed molecular clock approach (BEAST v1.7.5; Drummond et al., 2012).

For MrBayes analyses, we applied the preferred models of molecular evolution (estimated as described above) for each partition. We also unlinked priors across all partitions and set the rate prior to "variable", allowing site-specific rates of evolution to vary across partitions (Marshal et al., 2006). We used the default temperatures for chain heating, and the default of four Metropolis-coupled Markov chain Monte Carlo (MCMCMC) chains for the two replicate runs for each analysis. For analyses of each gene, we ran 2×10^6 generations sampled every 1,000. For the concatenated analysis, we used 1×10^7 generations sampled every 1,000. We assessed convergence using diagnostics from the *sump* command. Specifically, we ensured adequate effective sample sizes (ESS) of each parameter (>200), that chains were mixing appropriately, and that the average standard deviation of split frequencies between independent runs reached <0.05. A conservative burnin fraction of 50% was used to obtain the consensus phylogram and posterior probabilities for each bipartition, using the *sumt* command.

We used BEAST v1.7.5 (Drummond and Rambaut, 2007) to perform relaxed clock Bayesian analyses of the concatenated data and each gene. Input files contained partitions for each codon position of each gene. We linked site models in accordance with partitions identified by PartitionFinder, unlinked clock models across all partitions, and linked topology across all partitions. We tested the null hypothesis of a strict molecular clock for each gene (data not partitioned) using likelihood-ratio tests in PAUP* v4.0b10 (Swofford, 2002), and found that all genes rejected the strict molecular clock hypothesis. Thus, we used uncorrelated lognormal relaxed molecular clock models for all partitions, with mean rates estimated from a gamma distribution relative to a partition with an arbitrary fixed rate of 1. For a tree prior, we used a Yule (pure-birth) model with random starting trees. We used 1×10^8 generations (sampled every 10,000) for each individual gene tree, with the number of generations increased to 2.5×10^8 for the concatenated analysis.

We assessed convergence of BEAST analyses using the programs Tracer v1.5 (Drummond and Rambaut, 2007) and Are We There Yet? (AWTY; Nylander et al., 2008). In Tracer, we examined plots of likelihood and other parameters for stationarity and for effective sample sizes >200. In some instances, ESS for the prior and posterior were lower than 200, and this appeared alongside low ESS for particular transition or transversion types in a given partition, suggesting possible model overparameterization. However, likelihood plots appeared stable and with high ESS in all cases. We repeated these analyses to ensure that topologies were consistent between independent MCMC chains. In AWTY, we used the "compare" function to compare the posterior probabilities of bifurcations across duplicate MCMC analyses, and the "cumulative" function to examine stability of topological support throughout the MCMC chains. To ensure consistency of these convergence diagnostics with those used for MrBayes, we also used Tracer and AWTY as described above to diagnose convergence using the two replicate runs of each MrBayes analysis.

We used TreeAnnotator v1.7.5 (Drummond et al., 2012) to summarize the posterior distribution of trees from individual MCMC analyses into maximum clade credibility trees using a conservative burn-in fraction of 50%. Importantly, we did not use BEAST to estimate divergence times per se; thus, estimated branch lengths are in units of substitutions per site and are not ultrametric.

2.5. Species tree estimation

We used *BEAST (Heled and Drummond, 2010) as implemented in BEAST v1.7.5 to infer species trees using the multispecies coalescent. Tree models were linked across partitions within each gene. As in BEAST analyses, substitution models were linked within partitions identified by PartitionFinder, and clock models were unlinked across all codon positions of each gene. We used lognormal relaxed molecular clocks with relative rates estimated and a Yule process species tree prior. Convergence assessment and maximum clade credibility tree construction followed the methods described above for BEAST. The interpretation of branch lengths in *BEAST is not straightforward, and so these values are treated as unitless.

2.6. Analyses of 44 genes

We inferred concatenated and species-tree estimates using a dataset of 44 genes (Wiens et al., 2012), using BEAST and *BEAST. We excluded all taxa except the 12 scincid species and three outgroup species (*Cordylus*, *Cordylosaurus*, *Zonosaurus*). Methods followed those described above, except that each gene was treated as its own partition, with substitution models, clock models, and tree models linked within each gene. We chose not to evaluate separate substitution models for each codon position of each gene, given that preliminary analyses showed that these extra parameters made the species-tree analysis computationally intractable. Substitution models for each gene were selected using jModelTest2 (Darriba et al., 2012). We ran the analysis for 1×10^9 generations sampled every 10,000.

2.7. Analyses of congruence and support

We tested whether those branches in the concatenated tree that differ from the species-tree estimate are short, weakly supported, and in conflict with the underlying gene trees. Therefore, we compared the concatenated trees from the likelihood and Bayesian analyses to the estimated species tree from *BEAST. For each concatenated tree, we classified all 16 nodes within the ingroup as being concordant or discordant with the estimated species tree from *BEAST. We then compared values of these two classes of nodes (for length, support, and gene congruence) using one-sided, nonparametric Mann–Whitney *U* tests in R v2.15.1 (R Core Team, 2012). We used the package exactRankTests (Hothorn and Hornik, 2002), which implements the shift algorithm (Streitberg and Röhmel, 1986) to calculate exact *P*-values, even in the case of tied data. We used a nonparametric approach because Kolmogorov-Smirnov tests (in R) indicated that many of the relevant sampling distributions were non-normal (results not shown). The raw data for all analyses (here and below) are presented in Appendix D.

For branch lengths, we used estimated lengths from the concatenated analysis. A reasonable concern is whether these adequately reflect the true branch lengths in the underlying species tree (although our analyses do not assume this). Previous analyses show that shorter branch lengths in concatenated trees are related to shorter branches in the corresponding gene trees for comparable clades (Wiens et al., 2008), strongly suggesting that these short branches generally reflect limited time between splitting events, rather than artifacts of concatenation. Furthermore, previous analyses show that shorter branches in concatenated trees are associated with weaker support and greater incongruence among genes (e.g. Wiens et al., 2008, 2010a, 2012). We also tested these latter two hypotheses on the 10 gene, 17-taxa skink dataset (see below). For our measure of branch support in the concatenated trees, we used bootstrap values for likelihood analyses and posterior probabilities (Pp) for Bayesian analyses.

To assess whether nodes in the concatenated trees are concordant or discordant with the underlying estimated gene trees we used several measures (following Wiens et al., 2008), including the proportions of concordant genes, strongly concordant genes, and strongly discordant genes. For a given clade in the concatenated tree, we counted a gene as concordant if all the species included in the gene tree from that clade form a monophyletic group. However, not every species had data for every gene. In these cases, we counted a gene as supporting a given clade if the basal species of the clade were present in the separate gene tree. Using the example from Wiens et al. (2008), say that clade (A (B + C)) was present in the concatenated-data tree. If a given gene included taxa A and C, but lacked data for B, and the clade A + C was supported, then this gene was considered as supporting (A (B + C)). If taxon A lacked data for that gene instead, we would consider this to be ambiguous. We then calculated the proportion of concordant genes for each node (the number of concordant genes divided by the number of genes that have relevant sampling for that node, given that not every species had data for every gene, and that some genes were therefore ambiguous for a given node).

We also tallied whether genes that were concordant (or discordant) with a given clade in the concatenated tree showed strong or weak support for that clade. This distinction is important because weakly supported discordance might be the result of stochastic error in estimating the gene tree, whereas strongly supported discordance may indicate distinct genealogical histories (e.g. due to incomplete lineage sorting, introgression, or paralogy). For likelihood analyses, we used a threshold of $\geqslant$70% bootstrap support for classifying a relationship as strongly supported. We acknowledge that a criterion of $\geqslant$95% would be more conservative (and potentially more appropriate) for considering a clade to be strongly supported in a standard phylogenetic analysis, but this criterion was used only for assessing congruence, and is based on the well-established observation that bootstrap values are often biased but conservative (Felsenstein, 2004). For Bayesian analyses, we used the standard threshold Pp $\geqslant$ 0.95 as indicating strong support, as posterior probabilities are less conservative compared to bootstrap values (e.g. Rannala and Yang, 1996; Douady et al., 2003). For each node in the concatenated tree, we evaluated the proportion of genes that strongly supported that clade (among the genes relevant for that clade) and the proportion of genes that strongly supported an alternative relationship.

In the case of discordant relationships, selection of the appropriate support value is not always straightforward (especially when the gene tree was locally very different from the concatenated and/or *BEAST tree). Following Wiens et al. (2008), when a discordant relationship was found, we used the support value from the deepest node in the gene tree that rejected the clade, given the taxon sampling for that gene. For example, if the concatenated or *BEAST tree supported the relationships (A (B (C + D))), and a gene supported the relationships (D (B (A + C))), we considered that gene's support for the clade uniting A + B + C rather than the clade A + C. If two nodes at equal depth formed incongruent clades, we used the larger support value. Overall, we found that in these more ambiguous cases, all the discordant clades involved were usually weakly supported, making the selection of a particular support value less critical (as found by Wiens et al., 2008).

We also quantified strongly supported discordance among genes for a given clade. We used the index of gene conflict from Wiens et al. (2008). This index has its highest values ($\sim$1) when the number of genes strongly supporting a clade is equal to the number strongly rejecting it, and when most genes belong to these two types. In contrast, the index will take low values ($\sim$0) when the concordant and discordant clades are weakly supported or if

the genes all strongly support the relevant clade in the concatenated or *BEAST species tree.

We primarily tested whether branch lengths, support, and concordance in the concatenated tree predicted congruence with the estimated species tree from *BEAST (since in many cases a concatenated tree will be available but a species-tree estimate will not be). However, we also tested whether these same properties of the species tree from *BEAST predicted incongruence with the concatenated trees.

Finally, we assessed correlations between branch lengths, support, and indices of gene congruence and conflict using one-sided Spearman's nonparametric rank correlations with R. Specifically, we predicted that shorter branches in the concatenated tree would have weaker support and greater incongruence with their underlying gene trees. We then tested similar predictions on the estimated species tree.

Overall, we performed only standard statistical analyses rather than phylogenetically corrected analyses (e.g. using independent contrasts; Felsenstein, 1985). In general, we do not expect characteristics of branches (such as their length, support values, and congruence among genes) to show phylogenetic autocorrelation. Furthermore, most phylogenetic comparative methods utilize characteristics of terminal species, and are not designed for internal nodes. Indeed, most phylogenetic comparative methods rely heavily on branch length information, but branch length is one of our key variables.

3. Results

3.1. Comparison of topologies

Maximum likelihood and standard Bayesian analyses of the concatenated 10 gene dataset yielded identical topologies (Fig. 1). These trees show strong support for placing Acontinae as sister group to all other scincids, as in previous studies. These trees also support monophyly of Lygosominae, but show Lygosominae as nested inside a paraphyletic Scincinae. The two branches underlying non-monophyly of Scincinae are weakly supported in the likelihood analysis (bootstrap values <50%) and the Bayesian analysis (Pp = 0.87 and 0.77).

The topology estimated in the Bayesian relaxed clock analysis of the concatenated data (Fig. 2) differs notably from the other concatenated analyses (Fig. 1). Most importantly, this analysis shows strong support (Pp = 0.99) for monophyly of Scincinae (including *Feylinia*), which is paraphyletic in the other concatenated analyses. Within Scincinae, there is strong support (Pp = 1.00) for placing *Plestiodon* with the *Eumeces* + *Scincus* clade, whereas the other concatenated analyses suggest a very different placement for *Plestiodon*, and place *Brachymeles* with the *Eumeces* + *Scincus* clade. Intriguingly, there are differences between the two Bayesian concatenated analyses that are strongly supported by each (Pp > 0.95), specifically regarding the placement of scincine genera. However, all three concatenated analyses agree regarding the placement of Acontinae, and the relationships among the sampled lygosomine species.

The topology from the Bayesian species-tree analysis (*BEAST; Fig. 3) is almost identical to that from the Bayesian concatenated relaxed-clock analysis (BEAST). These topologies differ only in their placement of *Brachymeles*, and the conflicting nodes are weakly supported in both analyses. Thus, as in the relaxed-clock concatenated Bayesian analysis, the *BEAST analysis shows strong support for monophyly of Scincinae, in contrast to the non-clock concatenated analyses.

The 44-gene dataset contains a similar set of taxa, but lacks five lygosomine species present in the 10 gene, 17-species datasets. The previously published concatenated analyses of this dataset (RAxML and MrBayes; Fig. 4ab) also show Lygosominae nested inside a paraphyletic Scincinae (Wiens et al., 2012), although the relationships among scincine genera differ between the 10 gene and 44 gene analyses. The BEAST analysis of the 44 gene dataset (Fig. 4c) supports a monophyletic Scincinae with the exception of *Brachymeles*, which is strongly placed as sister to Lygosominae. The *BEAST analysis (Fig. 4d) again supports a monophyletic Scincinae, but this time with weak support. Again, the standard non-clock likelihood and Bayesian analyses give topology estimates that are identical to each other, but differ from the relaxed clock and species-tree estimates to the same extent (2 of 10 nodes). In this case, the BEAST and *BEAST trees also differ by 2 of 10 nodes. There are conflicting nodes between the relaxed clock and standard Bayesian estimates that are strongly supported by each.

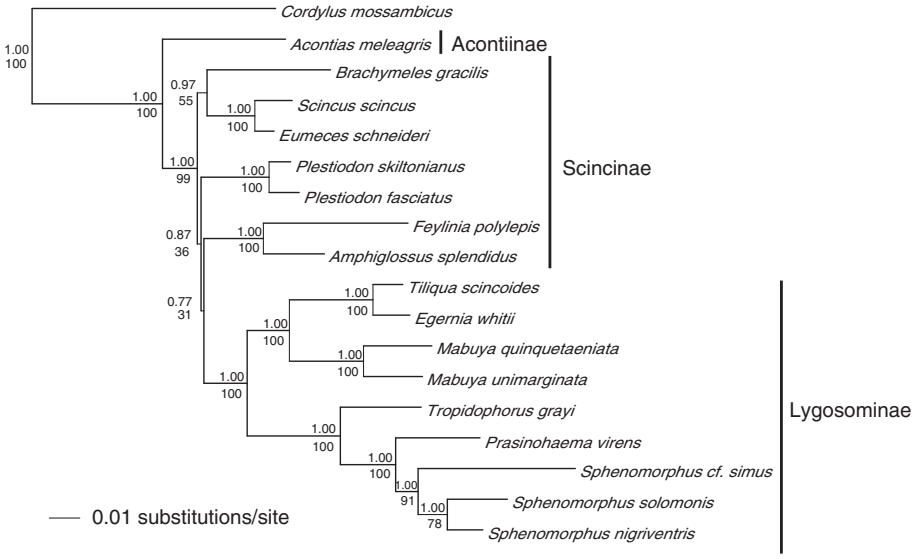

Fig. 1. Estimated tree from standard concatenated analyses of 10 genes for higher-level scincid lizard relationships. Both non-clock maximum likelihood (RAxML) and Bayesian analysis (MrBayes) estimate the same topology. Branch lengths shown are from maximum likelihood. Support values below each branch are from likelihood bootstrap values, values above each branch are Bayesian posterior probabilities. For illustrative purposes, only one outgroup taxon (*Cordylus*) is shown, but all six were included in the analyses.

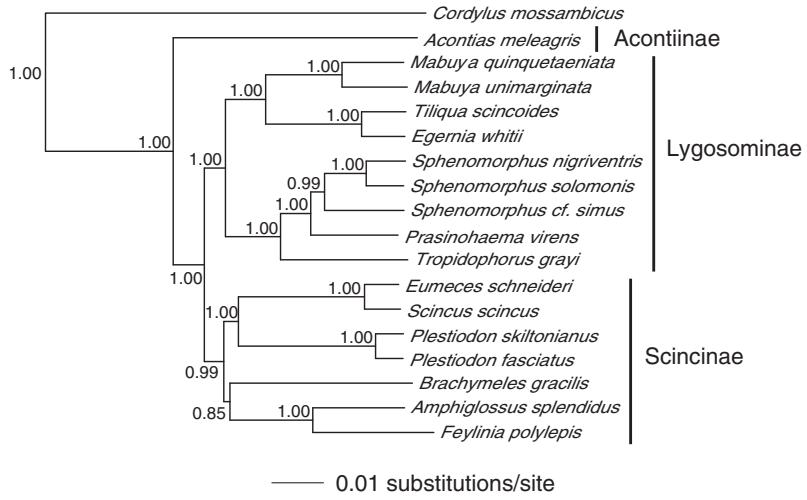

Fig. 2. Estimated tree from concatenated analyses of 10 genes for higher-level scincid lizard relationships based on a relaxed clock Bayesian analysis (BEAST). Values at branches indicate posterior probabilities of clades. For illustrative purposes, only one outgroup taxon (*Cordylus*) is shown, but all six were included in the analyses.

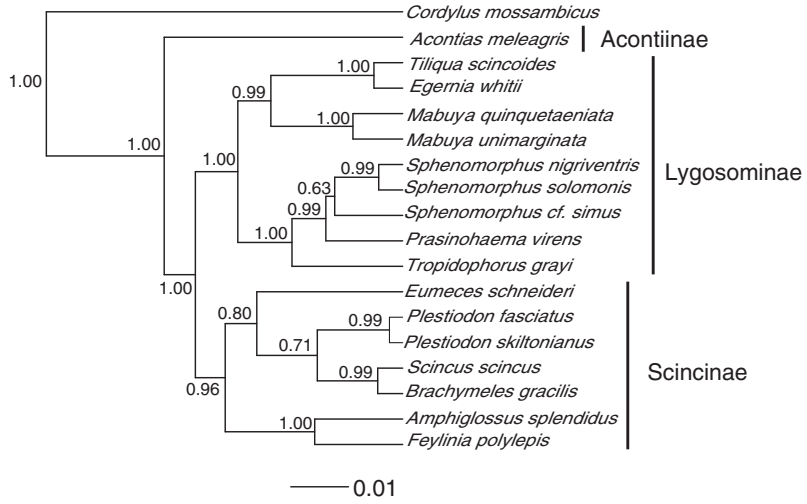

Fig. 3. Estimated tree from Bayesian coalescent species-tree analysis (*BEAST). Values at branches indicate posterior probabilities of clades. For illustrative purposes, only one outgroup taxon (*Cordylus*) is shown, but all six were included in the analyses.

Relationships among scincine genera differ somewhat between the two species-tree analyses (Fig. 3 vs. Fig. 4d), but these differences are only weakly supported in each. Surprisingly, the analysis of 44 genes and 12 skink species has slightly lower mean support values for *BEAST relative to that using 10 genes and 17 species (mean Pp = 0.924 with 44 genes, mean Pp = 0.943 with 10 genes). Although this decrease is not significant ($P = 0.368$ for Mann–Whitney test), the expectation is that increasing the number of genes from 10 to 44 should significantly increase support.

3.2. Analyses of congruence

Our overall goal was to determine what properties of branches from concatenated trees predict whether they will be concordant or discordant with estimates from species-tree methods. We predicted that branches of the concatenated tree that are most likely to differ from the coalescent species-tree estimate are those that are short, weakly supported, and contested by the underlying gene trees. These predictions are generally supported (Table 1). For the concatenated trees from non-clock likelihood and Bayesian analyses, those branches that disagree with the estimated species tree are significantly shorter, more weakly supported, and have a lower proportion of genes that are concordant and strongly concordant with them. However, the proportion of genes that are strongly discordant with the concatenated tree is significantly higher for the likelihood results but not for the non-clock Bayesian results, and the measure of strong conflict among genes is not significant for either method.

The branches in the species tree from *BEAST that conflict with the non-clock concatenated trees (RAxML and MrBayes) tend to be shorter (although the trend is only marginally significant; $P = 0.057$), with significantly weaker support ($P = 0.013$). Separate non-clock analyses of genes show that nodes that are concordant between the concatenated and *BEAST trees do have higher proportions of genes that are concordant and strongly concordant with these branches ($P = 0.002$–0.013; using both Bayesian and likelihood-estimated gene trees), while the proportion of strongly discordant genes is significant for non-clock Bayesian estimated trees ($P = 0.045$) but not likelihood estimated trees ($P = 0.786$).

Branch lengths and support values were significantly, positively correlated among branches within each of the three concatenated

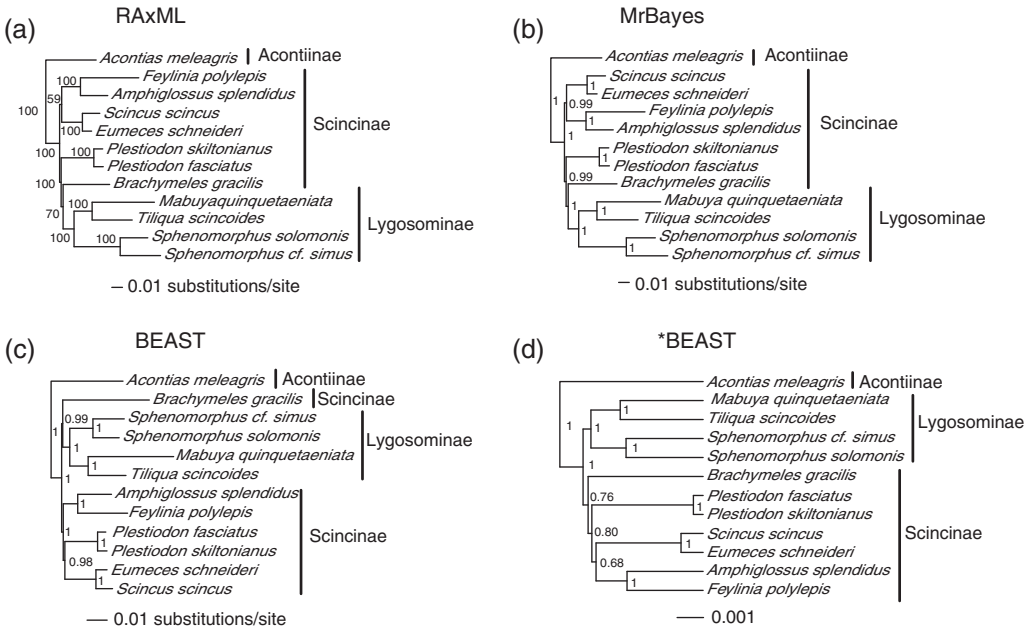

Fig. 4. Estimates of scincid phylogeny based on a dataset of 44 nuclear protein-coding genes, including estimates from (a) concatenated non-clock maximum likelihood analysis (RAxML), (b) concatenated non-clock Bayesian analysis (MrBayes), (c) concatenated relaxed clock Bayesian analysis (BEAST) and (d) Bayesian coalescent species-tree analysis (*BEAST). Trees in (a) and (b) are from the larger squamate-wide analyses in Wiens et al. (2012). The three outgroup taxa included are not shown.

Table 1
Results from one-sided Mann–Whitney *U*-tests comparing the characteristics of branches in the non-clock concatenated trees that are disconcordant or concordant with the estimated species tree from *BEAST. For each variable, medians for the groups of discordant and concordant nodes are given. Asterisks indicate statistically significant results using one-sided tests and a threshold of $P < 0.05$. Proportions of concordant/discordant genes refer to the gene trees estimated using MrBayes, RAxML, or BEAST depending on the method used for the concatenated tree. The conflict index is from Wiens et al. (2008). Note that the common ancestor of Scincidae was included as a node.

Variable	MrBayes medians	MrBayes *P*-value	RAxML medians	RAxML *P*-value
Branch length	Discordant ($n = 3$): 0.0017 Concordant ($n = 13$): 0.0151	0.002*	Discordant ($n = 3$): 0.0011 Concordant ($n = 13$): 0.0166	0.002*
Support (concatenated)	Discordant ($n = 3$): 0.8730 Concordant ($n = 13$): 1.0000	0.002*	Discordant ($n = 3$): 36 Concordant ($n = 13$): 100	0.002*
Support (*BEAST)	Discordant ($n = 3$): 0.9593 Concordant ($n = 13$): 1.0000	0.011*	Discordant ($n = 3$): 0.9593 Concordant ($n = 13$): 1.000	0.011*
Proportion concordant genes	Discordant ($n = 3$): 0.0000 Concordant ($n = 13$): 0.8354	0.002*	Discordant ($n = 3$): 0.0000 Concordant ($n = 13$): 0.8333	0.002*
Proportion strongly concordant genes	Discordant ($n = 3$): 0.0000 Concordant ($n = 13$): 0.5714	0.002*	Discordant ($n = 3$): 0.0000 Concordant ($n = 13$): 0.7143	0.002*
Proportion strongly discordant genes	Discordant ($n = 3$): 0.1000 Concordant ($n = 13$): 0.0000	0.250	Discordant ($n = 3$): 0.1111 Concordant ($n = 13$): 0.0000	0.011*
Conflict index	Discordant ($n = 3$): 0.0000 Concordant ($n = 13$): 0.0000	0.589	Discordant ($n = 3$): 0.0000 Concordant ($n = 13$): 0.0000	0.688

Variable	BEAST Medians	BEAST *P*-value
Branch length	Discordant ($n = 1$): 0.0011 Concordant ($n = 15$): 0.0077	0.0625
Support (Concatenated)	Discordant ($n = 1$): 0.8460 Concordant ($n = 15$): 1.0000	0.0625
Support (*BEAST)	Discordant ($n = 1$): 0.8044 Concordant ($n = 15$): 0.9527	0.1875
Proportion concordant genes	Discordant ($n = 1$): 0.0000 Concordant ($n = 15$): 0.8333	0.0625
Proportion strongly concordant genes	Discordant ($n = 1$): 0.0000 Concordant ($n = 15$): 0.6000	0.188
Proportion strongly discordant genes	Discordant ($n = 1$): 0.0000 Concordant ($n = 15$): 0.0000	1.000
Conflict index	Discordant ($n = 1$): 0.0000 Concordant ($n = 15$): 0.0000	0.625

tree (MrBayes: rho = 0.68, $P = 0.002$; RAxML: rho = 0.87, $P < 0.0001$; BEAST: rho = 0.64, $P = 0.004$), but surprisingly, not the species-tree estimated from *BEAST (rho = 0.30, $P = 0.131$).

Overall, levels of support in the estimated Bayesian species tree are related to concordance and discordance among the separately estimated gene trees (Table 2), except for the proportion of strongly discordant genes estimated using relaxed clock methods (BEAST). The proportions of concordant and strongly concordant genes are significantly related to branch lengths in the species tree and the concatenated trees (Table 2). However, the proportion of

Table 2
Results of one-sided non-parametric Spearman's rank correlations for gene concordance versus support and branch lengths, in the format: method used to estimate gene trees vs. method used to estimate reference tree (for measures of support and branch length). Asterisks indicate statistically significant results using a threshold of $P < 0.05$. Note that the common ancestor of Scincidae was included as a node.

Variable 1	Variable 2	MrBayes vs. *BEAST	RAxML vs. *BEAST	BEAST vs. *BEAST
Proportion of concordant genes	Support	rho = 0.56 $P = 0.0119^*$	rho = 0.47 $P = 0.0315^*$	rho = 0.51 $P = 0.0211^*$
Proportion strongly concordant genes	Support	rho = 0.51 $P = 0.0215^*$	rho = 0.44 $P = 0.04237^*$	rho = 0.51 $P = 0.0223^*$
Proportion strongly discordant genes	Support	rho = −0.79 $P = 0.0001^*$	rho = −0.58 $P = 0.0094^*$	rho = −0.23 $P = 0.1996$
Proportion of concordant genes	Length	rho = 0.67 $P = 0.0024^*$	rho = 0.60 $P = 0.0066^*$	rho = 0.68 $P = 0.0020^*$
Proportion strongly concordant genes	Length	rho = 0.73 $P = 0.0006^*$	rho = 0.60 $P = 0.0066^*$	rho = 0.60 $P = 0.0067^*$
Proportion strongly discordant genes	Length	rho = −0.22 $P = 0.2048$	rho = −0.034 $P = 0.5487$	rho = −0.51 $P = 0.0217^*$

Variable 1	Variable 2	MrBayes vs. MrBayes Concatenated	RAxML vs. RAxML Concatenated	BEAST vs. BEAST Concatenated
Proportion of concordant genes	Support	rho = 0.68 $P = 0.0017^*$	rho = 0.52 $P = 0.0205^*$	rho = 0.51 $P = 0.0207^*$
Proportion strongly concordant genes	Support	rho = 0.68 $P = 0.0018^*$	rho = 0.59 $P = 0.0084^*$	rho = 0.60 $P = 0.0070^*$
Proportion strongly discordant genes	Support	rho = −0.20 $P = 0.2328$	rho = −0.46 $P = 0.0348^*$	rho = 0.13 $P = 0.6868$
Proportion of concordant genes	Length	rho = 0.72 $P = 0.0008^*$	rho = 0.68 $P = 0.0019^*$	rho = 0.77 $P = 0.0003^*$
Proportion strongly concordant genes	Length	rho = 0.81 $P = 7.197e{-5}^*$	rho = 0.74 $P = 0.0005^*$	rho = 0.82 $P = 5.062e{-5}^*$
Proportion strongly discordant genes	Length	rho = −0.29 $P = 0.135$	rho = −0.62 $P = 0.0049^*$	rho = 0.18 $P = 0.7535$

strongly discordant genes is only related to branch lengths in the RAxML concatenated tree (Table 2).

4. Discussion

4.1. Differences between concatenated and coalescent species trees

Species-tree methods may be more accurate than concatenated approaches for estimating phylogenies from multi-locus sequence data under a variety of conditions (e.g. Heled and Drummond, 2010; Leaché and Rannala, 2011), but concatenated approaches remain a widely used approach for many large-scale multi-locus studies (e.g. Blanga-Kanfi et al., 2009; Crawford et al., 2012; Wiens et al., 2012). Here, we address a critical but neglected question related to this issue: are there properties of concatenated trees that will predict their concordance with species-tree estimates? Our results from scincid lizards demonstrate that the answer is yes. We find that branches of the standard non-clock Bayesian and likelihood concatenated trees that are short, weakly supported, and have few genes that are concordant with them are those that tend to have a different resolution in the estimated species tree from *BEAST. Although our results are based on only one group of organisms, these results are broadly congruent with theoretical predictions and related results from previous studies. For example, theory suggests that discordance among genes will tend to be greater on shorter branches due to incomplete lineage sorting (e.g. Maddison, 1997), and previous empirical studies show that short branches will tend to be weakly supported in concatenated analyses and will have greater disagreement with their underlying gene trees (e.g. Wiens et al., 2008, 2010a, 2012). Furthermore, without discordance between gene trees (and gene and species trees), estimated trees from species tree and concatenated analyses are expected to generally be identical (e.g. Edwards, 2009).

4.2. Practical implications

Our results have a particularly important practical application. We find that weakly supported relationships in concatenated trees may be those most likely to have a different resolution when species-tree methods are applied. Thus, incorporating uncertainty in the concatenated analysis may also help make phylogenetic results robust to the future application of species-tree methods. For example, the simple practice of performing comparative analyses on multiple trees sampled from the posterior distribution (from a Bayesian analysis) or from bootstrapping (from a likelihood analysis) should help ensure that the overall results are not compromised by differences between trees from concatenated and species-tree analyses. Nevertheless, it is important to point out that we do find cases where concatenated analysis strongly suggests a relationship that is strongly contradicted by species-tree analysis (i.e. MrBayes versus *BEAST for monophyly of Scincinae), but there are also strongly supported conflicts between different methods for estimating concatenated trees (MrBayes versus BEAST).

4.3. Similarity of relaxed clock concatenated and coalescent species trees

An unexpected result of our study was that in the 10-gene analyses, the relaxed clock concatenated analysis (BEAST) provided an estimate that was almost identical to that from the species-tree method (*BEAST), differing by only one node (which was weakly supported in both trees). The explanation for this pattern is not entirely clear. For example, the individual gene trees estimated by BEAST do not show greater congruence with the *BEAST species-tree estimate than do gene trees estimated by standard Bayesian analysis (Table 2). This suggests that other factors are at work besides the concordance of genes alone. One possibility is that the simple use of a relaxed molecular clock model by both BEAST and *BEAST may explain their similar topological estimates. However, analyses using an alternative relaxed clock method on the 10-gene data set did not support this idea (see Appendix E).

Furthermore, the pattern of greater congruence between relaxed-clock concatenated and species-tree estimates was not repeated in the 44 gene case (Fig. 4). Instead, the standard concatenated, relaxed-clock concatenated, and coalescent species-tree

estimates were all equally dissimilar (Fig. 4), based on the number of shared nodes. Nevertheless, we again found that relaxed clock and standard concatenated analyses gave quite different results, and these differences were actually strongly supported by each method for the Bayesian analyses.

These observations should also be considered in future simulation studies. Specifically, it is important to ensure that differences between concatenated and species-tree estimates are actually due to the use of these two classes of approaches, and not to use of a relaxed clock approach when estimating species trees vs. a non-clock approach when estimating concatenated trees. We note that previous analyses have suggested that relaxed-clock methods may provide more accurate estimates of phylogeny than standard concatenated analyses (using empirical datasets; Drummond et al., 2006) but some results from simulations suggest that standard concatenated analyses are more accurate instead (Wertheim et al., 2010).

4.4. Species-tree methods, deep phylogenies, and sampling of genes and taxa

Our results also offer some insights on the application of coalescent-based species-tree methods to relatively deep divergences and with limited sampling of species and individuals. Species-tree methods (especially BEST and *BEAST) are typically applied to closely related species (e.g. Camargo et al., 2012). Further, *BEAST is explicitly recommended for cases in which >1 individual is sampled per species (Heled and Drummond, 2010). Here, we explored a case where some divergences were very deep (~100 myr), species sampling was limited (relative to the >1560 species in the family), and only a single individual was sampled per species. Even though the true species phylogeny is not known in this case, we nevertheless found the results to be encouraging. Specifically, the species tree results were largely concordant with traditional taxonomy and with a recent (concatenated) analysis based on extensive species sampling, especially with regards to basal placement of Acontinae, monophyly of Lygosominae, and monophyly of Scincinae (including Feylininae). The first two results have also appeared in other studies based on concatenated analyses (e.g. Brandley et al., 2012; Wiens et al., 2012). However, the latter result (Scincinae monophyly) is concordant with traditional taxonomy but among recent molecular analyses has so far appeared only in one with extensive species sampling (683 scincid species; Pyron et al., 2013). Intriguingly, we found that the results from relaxed clock concatenated and species-tree analyses of 10 genes were more concordant with results from extensive species sampling than concatenated analyses based on 44 genes (e.g. concatenated analysis with 44 genes and 12 ingroup taxa does not support monophyly of Scincinae).

In addition, we found that many clades remained weakly supported in the estimated species trees after increasing the sampling of genes from 10 to 44 (but with reduced taxon sampling), and that mean Pp was actually slightly lower with 44 genes and reduced taxon sampling (12 species) than with 10 genes and more extensive taxon sampling (17 species). These results raise the interesting possibility that sampling more species may be more important for the accuracy of coalescent species-tree analyses than sampling more loci, at least for studies of higher-level phylogeny (possibly because of increased errors in estimating the underlying gene trees with poor taxon sampling, leading to errors in the estimated species tree). The relative importance of sampling taxa versus genes has been a major debate in systematics (e.g. Graybeal, 1998; Heath et al., 2008; Poe and Swofford, 1999; Rannala et al., 1998; Rosenberg and Kumar, 2001; Zwickl and Hillis, 2002), but not in reference to species-tree methods. Clearly, the impact of limited taxon sampling on species-tree analyses of higher-level phylogeny should be an urgent area for future research.

4.5. Skink phylogeny

Finally, our results are relevant to the higher-level phylogeny and classification of the largest family of lizards, which have been uncertain and controversial in the recent literature (e.g. Hedges and Conn, 2012; Pyron et al., 2013). Our relaxed-clock concatenated and species-tree results are concordant with a higher-level phylogeny and classification of skinks suggested by fewer loci but more extensive species sampling, (Pyron et al., 2013), as noted above. We argue that the subdivided classification of the clearly monophyletic Scincidae into multiple families (Hedges and Conn, 2012) is unnecessary and disrupts traditional classification (e.g. changing the long-standing definition of Scincidae without any phylogenetic justification). Our results also confirm some aspects of previous concatenated analyses (e.g. Brandley et al., 2012), including placement of Acontinae as sister to other scincids, monophyly of Lygosominae, and placement of Feylininae within Scincinae. However, further resolution of generic and species-level relationships within the major clades of scincids will clearly require more extensive taxon sampling.

5. Conclusions

Using data from scincid lizards, we found that differences between concatenated and coalescent-based species trees are predictable and associated with branches in the concatenated tree that are short, weakly supported, and have fewer concordant gene trees. Importantly, our results suggest that in cases where coalescent-based species trees cannot be estimated (e.g. too many taxa), incorporating uncertainty in the concatenated tree may also incorporate differences between the concatenated tree and estimated species tree. We also found that relaxed-clock concatenated trees provided a better approximation of the coalescent-based species tree in our 10-gene, 17-taxon analyses than standard concatenated analyses. In addition, we found that coalescent-based species trees have lower mean support values when estimated using a dataset with a more than four-fold increase in the number of genes, but a ~30% reduction in taxon sampling. This suggests that taxon sampling could be more important than sampling large numbers of genes when applying coalescent-based species-tree methods to deep phylogenetic questions. Finally, our results from species-tree analyses support division of Scincidae into three monophyletic subfamilies, a result otherwise found only in concatenated analyses with extensive species sampling.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2014.10.004.

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