



## Phylogeny of iguanian lizards inferred from 29 nuclear loci, and a comparison of concatenated and species-tree approaches for an ancient, rapid radiation

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### ABSTRACT

Iguanian lizards form a diverse clade whose members have been the focus of many comparative studies of ecology, behavior, and evolution. Despite the importance of phylogeny to such studies, interrelationships among many iguanian clades remain uncertain. Within the Old World clade Acrodonta, Agamidae is sometimes found to be paraphyletic with respect to Chamaeleonidae, and recent molecular studies have produced conflicting results for many major clades. Within the largely New World clade Pleurodonta, relationships among the 12 currently recognized major subclades (mostly ranked as families) have been largely unresolved or poorly supported in previous studies. To clarify iguanian evolutionary history, we first infer phylogenies using concatenated maximum-likelihood (ML) and Bayesian analyses of DNA sequence data from 29 nuclear protein-coding genes for 47 iguanian and 29 outgroup taxa. We then estimate a relaxed-clock Bayesian chronogram for iguanians using BEAST. All three methods produce identical topologies. Within Acrodonta, we find strong support for monophyly of Agamidae with respect to Chamaeleonidae, and for almost all relationships within agamids. Within Pleurodonta, we find strong Bayesian support for almost all relationships, and strong ML support for some interfamilial relationships and for monophyly of almost all families (excepting Polychrotidae). Our phylogenetic results suggest a non-traditional biogeographic scenario in which pleurodonts originated in the Northern Hemisphere and subsequently spread southward into South America. The pleurodont portion of the tree is characterized by several very short, deep branches, raising the possibility of deep coalescences that may confound concatenated analyses. We therefore also use 27 of these genes to implement a coalescent-based species-tree approach for pleurodonts. Although this analysis strongly supports monophyly of the pleurodont families, interfamilial relationships are generally different from those in the concatenated tree, and support is uniformly poor. However, a species-tree analysis using only the seven most variable loci yields higher support and more congruence with the concatenated tree. This suggests that low support in the 27-gene species-tree analysis may be an artifact of the many loci that are uninformative for very short branches. This may be a general problem for the application of species-tree methods to rapid radiations, even with phylogenomic data sets. Finally, we correct the non-monophyly of Polychrotidae by recognizing the pleurodont genus *Anolis* (*sensu lato*) as a separate family (Dactyloidae), and we correct the non-monophyly of the agamid genus *Physignathus* by resurrection of the genus *Istiurus* for the former *Physignathus lesueurii*.

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### 1. Introduction

The squamate reptile clade Iguania represents a remarkably successful vertebrate radiation (1602+ species, representing ~18% of all squamates; Uetz et al., 2010) whose monophyly has been corroborated in both morphological (e.g., Conrad, 2008; Estes et al., 1988) and molecular (e.g., Townsend et al., 2004) phyloge-

netic studies. Morphological characters strongly support Iguania as the sister taxon of a clade containing all other extant squamates (i.e., Scleroglossa; Conrad, 2008; Estes et al., 1988). However, multiple studies based on nuclear DNA sequence data (e.g., Townsend et al., 2004; Vidal and Hedges, 2005; Wiens et al., 2010) instead root the squamate tree within Scleroglossa and strongly support placement of iguanians in a deeply nested clade that also contains snakes and anguimorphs (i.e., Toxicofera; Vidal and Hedges, 2005).

Iguanians include many of the most common and familiar lizards from throughout the world, such as anoles (*Anolis*), spiny

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lizards (*Sceloporus*), horned lizards (*Phrynosoma*), flying lizards (*Draco*), and chameleons (various genera). Because of the ease with which they can be studied and the great morphological, ecological, and taxonomic diversity within the group, iguanians have been the focus of many comparative studies incorporating phylogeny, encompassing a wide variety of subjects including adaptive radiation (Losos and Miles, 2002), diversification rates and patterns (Harmon et al., 2003), and the evolution of reproductive modes (Schulte and Moreno-Roark, 2010), social signals (Ord and Martins, 2006), ornaments (Ord and Stuart-Fox, 2006; Wiens, 1999), herbivory (Cooper and Vitt, 2002; Espinoza et al., 2004), ecomorphs (Melville et al., 2006), and mitochondrial genomes (Macey et al., 1997, 2000a). However, despite the prominence of phylogeny in these studies, phylogenetic relationships among many iguanian clades remain surprisingly uncertain.

Extant iguanians are divided into two major clades, Acrodonta and Pleurodonta (Frost et al., 2001). All extant acrodonts occur in the Old World, and include Chamaeleonidae and Agamidae (~183 and 412 species, respectively; Uetz et al., 2010). Chameleons are monophyletic based both on morphological (Frost and Etheridge, 1989) and molecular data (Townsend and Larson, 2002; Townsend et al., 2009). However, morphological phylogenetic studies have not supported agamid monophyly (e.g., Conrad, 2008; Frost and Etheridge, 1989). Some molecular phylogenetic studies have found strong support for agamid monophyly (Honda et al., 2000; Melville et al., 2009; Townsend et al., 2004) but others have not (Macey et al., 2000b; Schulte and Cartwright, 2009). Using morphological data, Conrad (2008) found chamaeleonids to be deeply nested within agamids as the sister taxon of the Southeast Asian species *Physignathus cocincinus*. In all other studies cited above, monophyly of agamids *sensu stricto* (i.e., excluding *Uromastix* and *Leiolepis*, which together are sometimes recognized as a family-level clade; e.g., Moody, 1980; Russell, 1988) was recovered. However, in some analyses *Uromastix* and/or *Leiolepis* have been placed outside a clade containing other agamids and chameleons (e.g., Frost and Etheridge, 1989; Macey et al., 1997). Monophyly of Agamidae *sensu stricto* is well supported by nuclear DNA data (Hugall et al., 2008; Melville et al., 2009; Schulte and Cartwright, 2009; Townsend et al., 2004), and monophyly of some more exclusive agamid clades (i.e., Agaminae, Amphibolurinae, Draconinae) is well established (Hugall et al., 2008; Macey et al., 2000b; Townsend et al., 2004). Agaminae and Draconinae appear to be sister taxa (Hugall et al., 2008; Macey et al., 2000b; Melville et al., 2009; Schulte and Cartwright, 2009; Townsend et al., 2004). However, relationships among the Agaminae + Draconinae clade, Amphibolurinae, and Hydrosaurinae (containing the single genus *Hydrosaurus*) are still unclear.

The predominantly New World clade Pleurodonta (~908 species; Uetz et al., 2010) was subdivided by Etheridge and de Queiroz (1988) into eight major clades based on morphology. Subsequently, because of the lack of morphological support for the monophyly of Iguanidae *sensu lato* (=Pleurodonta), Frost and Etheridge (1989) elevated these eight major pleurodont clades to the rank of families. In contrast to Frost and Etheridge (1989), Macey et al. (1997) provided strong mitochondrial DNA (mtDNA) support for monophyly of Pleurodonta (which they recognized as family Iguanidae) and for monophyly of its eight major constituent clades (which they ranked as subfamilies of their Iguanidae). Frost et al. (2001) found that Polychrotidae was not monophyletic based on analyses of mtDNA and morphology, and also noted that monophyly of Tropicuridae was doubtful (Macey et al., 1997; Schulte et al., 1998; Titus and Frost, 1996). Frost et al. (2001) therefore presented a revised taxonomy with 11 clades ranked as families: Corytophanidae, Crotaphytidae, Hoplocercidae, Iguanidae (*sensu stricto*), Leiocephalidae (=Leiocephalinae of the Tropicuridae of Frost and Etheridge (1989)), Leiosauridae (whose members were

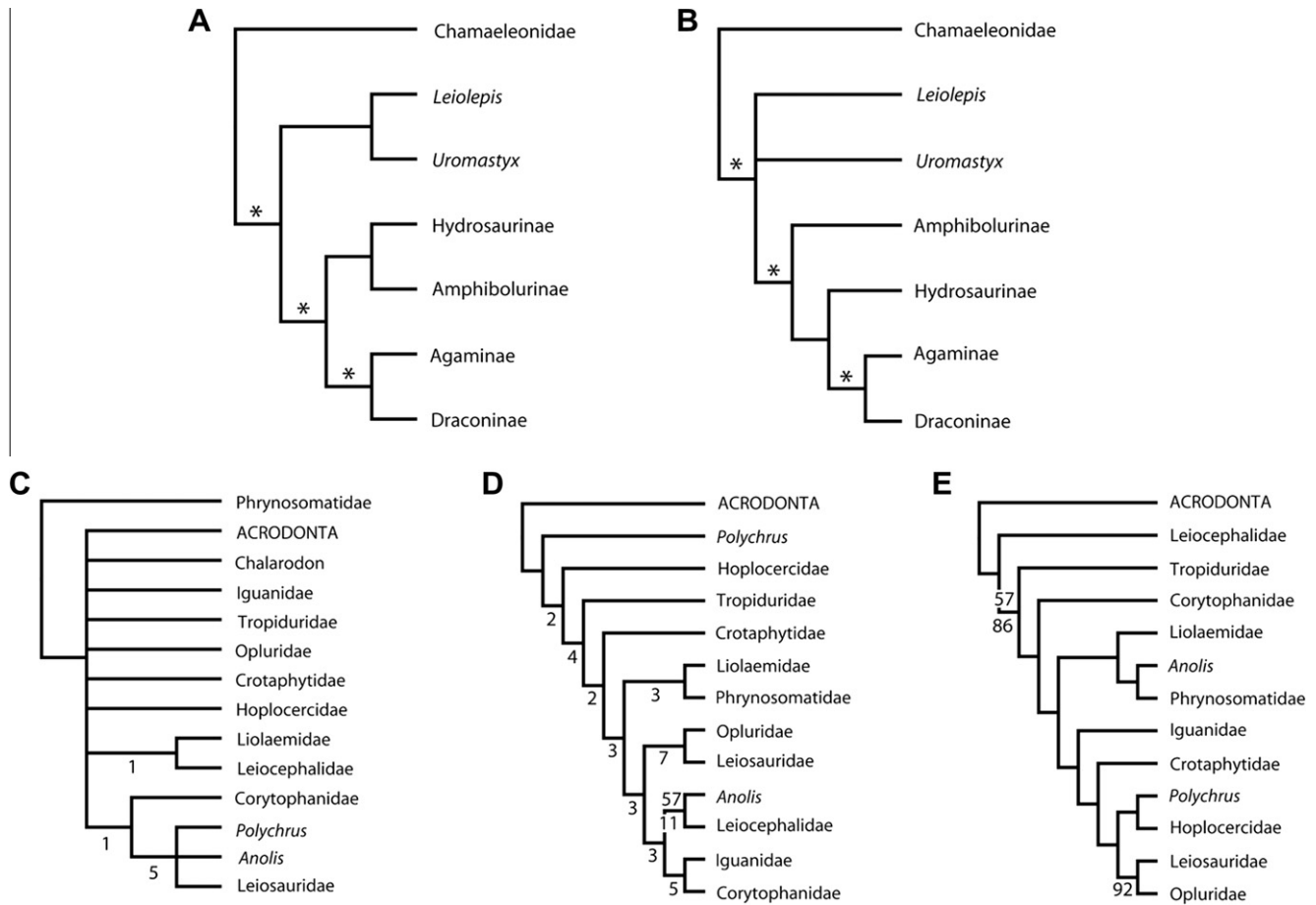
part of Polychrotidae of Frost and Etheridge (1989)), Liolaemidae (=Liolaeminae of the Tropicuridae of Frost and Etheridge (1989)), Opluridae, Phrynosomatidae, Polychrotidae (*sensu stricto*; i.e., containing only *Anolis* and *Polychrus*), and Tropicuridae (=Tropicurinae of the Tropicuridae of Frost and Etheridge (1989)).

Schulte et al. (2003b) also found non-monophyly of Polychrotidae and Tropicuridae (both *sensu* Frost and Etheridge, 1989). However, given a lack of statistical rejection of these clades, Schulte et al. (2003b) chose to provisionally continue their recognition as metataxa of uncertain monophyly (albeit at the subfamilial level, following the convention of Macey et al. (1997)). Using the terminology of Frost et al. (2001) for the constituent taxa, *Anolis*, Leiosauridae, and *Polychrus* are encompassed by Polychrotinae\*, and *Leiocephalus*, Liolaemidae, and Tropicuridae fall under the Tropicurinae\* of Schulte et al. (2003b) (asterisks denote metataxon status). Schulte et al. (2003b), whose mtDNA-based study remains the most taxonomically complete molecular analysis of pleurodont phylogeny, also found no evidence for monophyly of the clade *Anolis* + *Polychrus* (=Polychrotidae of Frost et al. (2001)), and thus identified a total of 12 deeply divergent pleurodont clades connected by very short and mostly poorly supported internodes.

Townsend et al. (2004) conducted phylogenetic analyses using mtDNA (sampling 10 of the 12 major pleurodont clades) and the nuclear protein-coding loci *RAG1* (10 clades) and *CMOS* (5 clades), but found weak support for relationships amongst pleurodont clades. Noonan and Chippindale (2006) sampled 10 major pleurodont clades for four nuclear protein-coding loci (*BDNF*, *CMOS*, *NT3*, and *RAG1*) and found strong Bayesian support for *Sceloporus* (Phrynosomatidae) as the sister taxon of all other pleurodons (but erroneously found Phrynosomatidae to be polyphyletic; see Section 4), and also found a strongly supported Leiosauridae + Opluridae clade. Schulte and Cartwright (2009) sampled all 12 major pleurodont clades using the nuclear genes *RAG1* and *TSHZ1*. These authors found significant Bayesian credibility support (Pagel and Meade, 2004, 2005) from *RAG1* alone for Leiocephalidae as the sister taxon of all other iguanians, and for a sister-taxon relationship between Leiosauridae and Opluridae, but all other relationships were weakly supported. Noonan and Sites (2010) added the nuclear genes *RNF* and *ZFX1b* to the Noonan and Chippindale (2006) dataset; this study failed to recover the Leiosauridae + Opluridae clade, and support for the basal position of *Sceloporus* (Phrynosomatidae) was weak. However, strong support was found for the clades Iguanidae + Opluridae and Crotaphytidae + (Iguanidae + Opluridae). Fig. 1 shows previous hypotheses of iguanian relationships based variously on morphological and DNA sequence data and illustrates the lack of consensus regarding pleurodont phylogeny.

To clarify higher-level iguanian relationships, we obtained DNA sequence data for 29 nuclear protein-coding loci for 47 iguanian and 29 outgroup taxa. Several of these loci are here used for the first time in a phylogenetic study. We performed maximum-likelihood and Bayesian concatenated phylogenetic analyses, and also used a Bayesian relaxed-clock approach (with BEAST; Drummond et al., 2006; Drummond and Rambaut, 2007) to simultaneously estimate topology and divergence times with this dataset.

Pleurodont iguanians represent an ancient group (at least Late Cretaceous in age; Conrad and Norell, 2007) that seems to have split into several deep lineages over a relatively short period of time (e.g., Schulte et al., 2003b; Townsend et al., 2004). This pattern of rapid branching may have created substantial opportunities for incomplete lineage sorting (ILS), which in turn could compromise concatenated phylogenetic analyses (e.g., Edwards et al., 2007; Kubatko and Degnan, 2007). In the last few years, new multilocus phylogenetic methods have emerged with the goal of reconstructing the species tree within which individual gene trees are embedded. These methods explicitly incorporate the



**Fig. 1.** Previous phylogenetic hypotheses of iguanian relationships, all modified from original sources for this figure. (A) Mitochondrial *ND2* acrodont phylogeny from Melville et al. (2009). Asterisks indicate Bayesian posterior probabilities (PP) > 0.99. (B) Nuclear *RAG1* acrodont phylogeny from Melville et al. (2009). Asterisks indicate PP > 0.99. (C) Morphological pleurodont phylogeny from Conrad (2008). Numbers under branches indicate Bremer support. (D) Mitochondrial *ND2* pleurodont phylogeny from Schulte et al. (2003b). Numbers above branches indicate maximum-parsimony bootstrap proportions (BP) > 50%, numbers under branches indicate Bremer support. (E) Nuclear *RAG1/TSHZ1* pleurodont phylogeny from Schulte and Cartwright (2009). Numbers above branches indicate maximum-likelihood BP > 50%, numbers below branches indicate Bayesian credibility values > 0.5.

coalescent process and the expected discordance among genes resulting from ILS (e.g., Edwards et al., 2007; Maddison and Knowles, 2006). Although these methods have been most often applied to recent radiations (e.g., Brumfield et al., 2008; Knowles and Chan, 2008; Leaché, 2010), their use for more ancient rapid radiations has also been promoted (Edwards et al., 2007; Whitfield and Lockhart, 2007). We therefore took advantage of the large number of independent loci in our dataset to apply a coalescent-based species-tree approach to phylogenetic inference within pleurodons, and to compare the results to those from the concatenated analyses. Our results may have important implications for the application of these methods to other ancient, rapid radiations.

## 2. Materials and methods

### 2.1. Taxon and gene region sampling

A total of 75 squamate species were sampled, along with the rhynchocephalian outgroup species *Sphenodon punctatus*. The 75 squamate species included 47 iguanian species (20 acrodonts and 27 pleurodons) and 28 outgroup species chosen to span the phylogenetic diversity within all other major squamate clades (see Electronic Supplementary Materials [ESM] for the complete list of taxa). Within acrodont iguanians, Chamaeleonidae was represented by *Brookesia brygooi* and *Chamaeleo calypttratus*, thus spanning the

deepest genetic divergence within chameleons (Townsend et al., 2009). The monogeneric agamid clades Uromastycinae, Leiolepineae, and Hydrosaurinae were each represented by one species. All other major agamid clades (Agaminae, Amphibolurinae, and Draconinae) were represented by multiple genera with the goal of spanning the earliest divergences within these clades (based on Macey et al. (2000b)). Within pleurodons, sampling included all 12 major clades outlined in Etheridge and de Queiroz (1988), Frost et al. (2001), and Schulte et al. (2003b). Where applicable, multiple genera were sampled that spanned the presumed earliest divergences within these clades, based on these previous studies.

DNA sequence data were obtained from a total of 29 nuclear protein-coding gene regions, with a total alignment length of 23,688 included base pairs (bp). Most of these gene regions were selected and developed following Townsend et al. (2008). Primer sequences for all loci can be downloaded (along with guide alignments) from the Squamate Reptile Assembling the Tree of Life (AToL) website (<http://archive.fieldmuseum.org/deepscale>). Some sequences were used in previous phylogenetic studies of squamates (e.g., Wiens et al., 2008, 2010), but a total of 1482 new sequences were generated for this study. Some species could not be amplified for some genes (despite multiple attempts and design of new primers). Nevertheless, taxon coverage for individual genes varied from 86% to 100% (average 96%; Table 1), and within iguanians was slightly higher, as detailed in Table 1. In some cases, when sequence data for one or more genes was unobtainable for

**Table 1**  
Gene region characteristics and taxon coverage (number of species sampled).

Locus	Taxon coverage		Characters (24,473)		
	All (76)	Iguania (47)	Total	Variable <sup>a</sup>	Pars. inf. <sup>a</sup>
ADNP	72	45	771	226	133
AHR	68	42	442	170	84
AKAP9	74	46	1475	759	434
BACH1	71	47	1299	611	353
BDNF	75	46	670	142	72
BHLBH2	69	47	770	280	166
BMP2	74	45	636	159	96
CAND1	76	47	759	153	71
DNAH3	76	46	722	256	162
ENC1	73	45	888	228	142
FSHR	75	47	753	234	133
FSTL5	74	47	622	166	97
GPR37	65	41	509	147	90
INHBA	70	45	811	255	144
MKL1	71	43	978	351	207
MSH6	71	46	1086	421	272
NGFB	76	47	554	189	112
NKTR	73	45	1246	740	481
NT3	76	47	510	175	104
PNN	75	46	987	416	226
R35	76	47	726	330	196
RAG1	76	47	1091	346	211
SLC30A1	76	47	528	180	104
SLC8A1	75	46	996	271	178
SLC8A3	75	47	1077	304	188
SNCAIP	74	45	498	200	119
TRAF6	76	47	651	246	151
UBN1	66	45	732	351	230
ZEB2	76	47	885	208	104
Averages	73.2 (96%)	45.8 (97%)		Total = 8514	Total = 5060

<sup>a</sup> Values for iguanians only.

an originally targeted species, composite taxa were formed by substituting sequence data from species closely related to the original species (but only one such case involved an iguanian). Simulations suggest that analyses using an appropriate composite-taxon strategy should generally be at least as accurate as those in which certain gene-region/taxon combinations are coded as missing data (Campbell and Lapointe, 2009). See the ESM for museum/collection information of specimens and GenBank accession numbers of sequence data.

## 2.2. Molecular data and main phylogenetic analyses

### 2.2.1. Data collection and manipulation

Standard methods of DNA extraction, amplification, and Sanger sequencing were used. Data collection was divided between the labs of T.W.R., J.W.S., and J.J.W. All sequences for a given gene were generated in the same lab, and the same individual specimen was generally used for a given species in all three labs. Nucleotide sequences were translated to amino acid sequences to check for stop codons and facilitate alignment by eye using MacClade v 4.0 (Maddison and Maddison, 2000). The few positions deemed ambiguously aligned by this process were excluded from all analyses. Gaps were treated as missing data in all analyses. Final alignments used for all analyses in this study are available on TreeBASE at <http://purl.org/phylo/treebase/phylovs/study/TB2:S11448>.

### 2.2.2. ML phylogenetic analyses and tests of alternative hypotheses

Phylogenetic analyses were conducted on the partitioned concatenated dataset under maximum likelihood (ML) using RAXML v7.2.6 (Stamatakis, 2006). These analyses used a separate partition for each codon position in each gene (87 partitions total, see below for justification). The GTR +  $\Gamma$  model was used for all data partitions. RAXML only uses the GTR model, and use of a separate

parameter for invariant sites (I) is not recommended given the large number of rate categories used for  $\Gamma$  to accommodate rate variation (25 instead of the usual four). Support for individual branches was assessed using the nonparametric bootstrap (Felsenstein, 1985) via the rapid bootstrap procedure of Stamatakis et al. (2008) with bootstrapping stopped automatically using a frequency-based criterion (Pattengale et al., 2010). Maximum-likelihood bootstrap proportions (MLBS)  $\geq 70\%$  were considered strong support (Hillis and Bull, 1993; Wilcox et al., 2002).

MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001) was used to conduct Bayesian analyses under a time-free model (i.e., without estimating divergence dates). Posterior probability (PP) support values  $\geq 0.95$  were considered strong support for individual clades (e.g., Alfaro et al., 2003; Erixon et al., 2003; Huelsenbeck and Rannala, 2004). The best-fitting model of sequence evolution was chosen for each data partition for three different partitioning strategies (each gene, each codon position of each gene, and combined first and second codon positions of each gene) using the Akaike Information Criterion (AIC) as implemented in MrModelTest (Nylander, 2004). MrBayes analyses were performed on the concatenated data under three partitioning schemes (one partition per gene [29 partitions], one partition for each codon position in each gene [87 partitions], and 29 genes by two partitions [58 partitions; combined first and second positions, third positions]). The relative fit of different partitioning strategies was evaluated using Bayes factors (Kass and Raftery, 1995) to compare the harmonic means of log-likelihood scores from the posterior distribution. The best-fitting partitioning strategy was used for all reported RAXML and MrBayes analyses of the concatenated data. However, additional analyses were also run using the alternative partitioning schemes to check for sensitivity to this factor.

Two separate MrBayes runs (each consisting of two independent analyses) were conducted for each dataset. Within each run, the average standard deviation of split frequencies (ASDSF) and the potential scale reduction factor (PSRF) statistics from MrBayes were used to evaluate topological and branch-length convergence, respectively. Additional analyses were conducted if individual runs failed to converge or if topology and/or support values differed between runs. To help spot potential problems with overestimation of branch lengths (BL) due to the influence of BL priors (Marshall, 2010), we also examined relative evolutionary rates of the codon partitions to see that they made biological sense (i.e., second codon positions slowest, followed by first positions, and then the more rapidly-evolving third positions), and we compared the Bayesian inferred BL to those of the optimal ML tree to verify that they were similar. All RAXML and MrBayes analyses were performed via the CIPRES 2 portal (Miller et al., 2009) at the San Diego Supercomputer Center.

Bayesian phylogenetic methods have been criticized for potential overestimation of support in certain situations (Cummings et al., 2003; Simmons et al., 2004). One source of potential bias may be the inability of most Bayesian phylogeny programs (including MrBayes and BEAST) to sample trees containing polytomies, leading to inflated support for some nodes (Lewis et al., 2005). To investigate the effect this phenomenon may have had on our results, we also performed Bayesian analyses on the concatenated data set using the program Phycas (Lewis et al., 2009), which can be set to allow unresolved nodes in sampled trees. Although partitioning of data is allowed by Phycas, for our dataset a partitioned analysis was not computationally feasible (estimated to take >6 months). We therefore ran unpartitioned analyses using a GTR + I +  $\Gamma$  model under alternative scenarios that either allowed or did not allow polytomies, and we compared differences in resolution and support between the two scenarios. Three replicate analyses were run for each scenario. All analyses completed 75,000–100,000 cycles, and convergence and mixing were

evaluated by examination of the cumulative split posterior-probability plots and split-sojourn plots produced by Phycas. The impracticality of partitioned Phycas analyses with our dataset makes direct comparisons with our MrBayes and BEAST results problematic. However, the differing treatment of polytomies should account for any substantial differences in topology or support values between the two Phycas runs. If certain nodes lose support (or collapse) when polytomies are allowed, this could suggest potential overestimation of support for these nodes in our partitioned MrBayes and BEAST analyses.

We also statistically tested specific previous phylogenetic hypotheses in an ML framework using the approximately unbiased test (AU; Shimodaira, 2002), implemented in the program CONSEL version 0.1 (Shimodaira and Hasegawa, 2001). Neither Polychrotinae\* nor Tropicodurinae\* (Schulte et al., 2003b) were monophyletic in our main analyses, nor did we recover *Anolis* + *Polychrus* (=Polychrotidae of Frost et al. (2001); see Section 3). We therefore ran three additional ML analyses using RAxML, each constrained to recover one of these clades. We used PAUP\* (Swofford, 2002) to produce unpartitioned site-wise log-likelihoods for each of these alternative phylogenetic hypotheses, and also for the unconstrained ML tree from our main analysis. The constrained trees were simultaneously compared to the ML tree in CONSEL (Shimodaira and Hasegawa, 2001) to determine if any of the alternatives could be rejected at the 0.05 level.

### 2.2.3. BEAST phylogenetic and divergence-time analyses

BEAST v1.4.6 (Drummond and Rambaut, 2007) was used to conduct relaxed-clock Bayesian analyses to simultaneously estimate topology and divergence times. As in the MrBayes analyses, PP support values  $\geq 0.95$  were considered strong support. Evolutionary rates along branches followed an uncorrelated lognormal distribution, and a Yule speciation process was imposed for all analyses. All BEAST analyses were run to achieve an effective sample size (ESS) of at least 200 for all estimated parameters once burnin was removed. Three replicate runs were conducted for each configuration. Initial analyses were run without data, and parameter estimates from these runs were compared to those from the data-included analyses using Tracer (Rambaut and Drummond, 2004) to check the influence of the priors on the results. Tracer was used to examine overall likelihood and individual parameter estimates in the BEAST output for evidence of proper mixing and convergence. Runs were combined using LogCombiner, and maximum credibility trees with divergence time means and 95% highest probability densities (HPDs) were produced using Tree Annotator (both part of the BEAST package). BEAST files can be found in the ESM.

Divergence-time estimates were constrained by placing age calibrations on a total of 18 nodes in the tree corresponding to the oldest known fossils of various groups (11 within Iguania, seven among outgroup taxa; see ESM). Fossil ages were generally equated to the age of the upper (more recent) limit of the stratum from which they came. In some cases, when a specific age for a fossil was given in the paper in which it was originally described, this age was used. For each calibration point, we used BEAUTi (part of the BEAST package) to construct a translated-lognormal (TL) distribution (i.e., lognormally distributed, with an offset from zero roughly equal to the age of the fossil; Drummond et al., 2006; Hedges and Kumar, 2004; Ho, 2007). Assuming that the geologic stratum and the phylogenetic affinities of a fossil are correctly identified, the minimum age of the clade to which a fossil belongs should be well-defined, and thus can be specified with a relatively hard bound (Yang and Rannala, 2006). However, the lizard fossil record is too patchy to reliably place a maximum on the age of a clade using as evidence the absence of that clade in deeper, more ancient strata. A lognormal distribution, with its steep drop-off in

probability at one end, and more gradually sloping tail at the other, is a good fit to this scenario. Specific values of the zero-offset, mean, and standard deviation for each TL distribution were chosen such that the more recent bound of the 95% HPD was placed at a point approximately 1% more recent than the estimated fossil age, and the bulk of the probability was placed near the estimated fossil age. We left (somewhat arbitrarily) long probability tails for the soft maxima of node ages. See the ESM for specific distribution parameters for all calibrations. Squamate monophyly was constrained in all analyses, but no other topological constraints were enforced.

One calibration likely to have a major effect on our results was the putative stem acrodont iguanian *Bharatagama*, from the Middle Jurassic Kota Formation of India (Evans et al., 2002). This fossil is considerably older than other known iguanians, but because it is known only from jaw fragments, its identification as a crown iguanian is best considered tentative. BEAST analyses were therefore conducted both with and without this fossil calibration.

### 2.3. Supplemental analyses focused on pleurodont relationships

Support for some relationships in the pleurodont portion of the tree varied between analysis methods and most deep branches were very short (see Section 3). For these reasons, we performed additional analyses on various data subsets to determine the breadth of support for phylogenetic patterns found in the 29-locus concatenated analyses.

#### 2.3.1. Rooting of pleurodents

Placement of the pleurodont root is an important issue and could be problematic in pleurodents given their potentially rapid basal radiation (e.g., Schulte et al., 2003a,b). To assess the stability of the root within pleurodents, we performed additional ML and Bayesian concatenated analyses with reduced numbers of outgroups (i.e., various combinations of acrodonts, both with and without additional non-iguanian outgroups; see ESM for additional details on methods and trees).

#### 2.3.2. Individual-gene and supplemental concatenated-gene analyses

To evaluate support across the sampled loci for strongly supported pleurodont clades inferred by the concatenated analyses (see Section 3), MrBayes analyses using all taxa were also performed on individual gene regions (partitioned by codon position). While strong support (or even topological congruence) should not necessarily be expected across all individual loci for these clades, even moderate support from many loci would suggest that our concatenated results were not being determined by only a few genes, and thus would bolster our confidence in the reality of these clades.

To evaluate congruence between concatenated and single-gene rooted pleurodont topologies, the posterior distributions from the single-gene analyses were filtered in PAUP\* (Swofford, 2002) using constraint trees. Each constraint tree contained a single resolved node corresponding to one of seven key pleurodont clades from the concatenated analysis (see Section 3). The number of trees passing through the filter was divided by the total number of trees in the posterior to give the PP of the focal pleurodont clade for each individual gene. However, topological incongruence may be exaggerated if only rooted topologies are considered. Incongruence between concatenated and single-gene analyses could also arise simply from differences in the placement of the pleurodont root, even if each analysis yielded identical unrooted pleurodont topologies (e.g., a paraphyletic assemblage at the base of a rooted tree could become a clade if the tree were rooted at a different point; see Fig. 2). To evaluate the role that rooting may have played in the assessment of discordance between single-gene and

concatenated results, the filtering procedure was repeated, but with non-pleurodonts removed from the constraint trees. The pruned trees were then loaded as backbone constraints in PAUP\*. This type of constraint allows a basal paraphyletic assemblage to pass through the filter and be counted as a match, thus allowing a more liberal assessment of congruence between individual gene trees and the unrooted pleurodont tree from the concatenated analyses.

Many of the single-gene analyses provided very weak support for (or did not even recover) certain pleurodont nodes that were strongly supported in the concatenated analyses (see Section 3). As mentioned above, this could indicate that only a few loci are driving support for the strongly supported nodes. Alternatively, there may be widespread support for a given node across loci that is only apparent when the loci are combined in a concatenated analysis (i.e., hidden support; Gatesy and Baker, 2005; Gatesy et al., 1999). To investigate this possibility, we performed seven supplemental concatenated MrBayes analyses. In each of these analyses, loci that gave the highest levels of individual-gene support for one of seven strongly supported focal pleurodont nodes were excluded (the number of loci excluded varied among the seven analyses; see Section 3). If an analysis shows strong support for the focal node after removing these particular loci, this would indicate that character support for this node is relatively widespread across loci.

The loci employed for this study varied substantially in their information content (see Section 3). It is possible that only certain loci have enough informative characters to resolve the deep, short branches at the base of pleurodonts. To evaluate the possibility that a relatively few, highly variable loci were largely driving support for deeper pleurodont nodes, we performed additional concatenated analyses that included only the top seven most variable loci, and compared topology and support values to those of the 29-gene analyses.

### 2.3.3. Pleurodont species-tree analyses

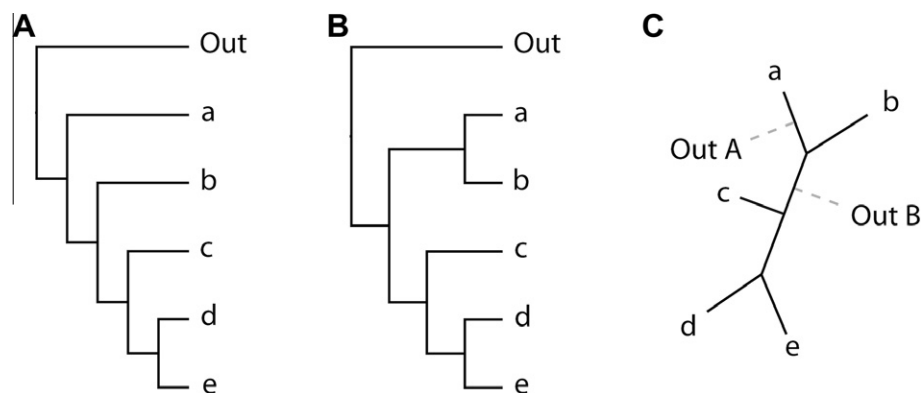
Coalescent-based species-tree analyses were performed using BEST (Bayesian Estimation of Species Trees) v2.3 (Liu, 2008). This method treats gene trees as random variables from a statistical distribution, the shape of which is determined by the length (time) and width (ancestral population size) of the branches of the underlying species tree. Under certain conditions (short, wide branches), incomplete lineage sorting can cause substantial incongruence between gene and species trees (Maddison, 1997). Patterns of gene-tree incongruence (incorporating information on branch lengths and widths) can therefore be fitted to the model (species tree)

most likely to have generated them. BEST (Liu, 2008) is an adaptation of MrBayes (Huelsenbeck and Ronquist, 2001) that estimates separate gene trees while simultaneously estimating the species tree that generated them. Importantly, this method explicitly accounts for uncertainty in the individually estimated gene trees, and allows the separate (but correlated) gene tree estimates to influence each other throughout the analysis (Liu, 2008).

Taxa used for the species tree analyses were limited to the pleurodont iguanian clade plus the single agamid outgroup *Leiopeltis belliana*, chosen arbitrarily from a set of acrodont taxa for which we had data from all genes. We restricted the analysis to pleurodonts because (a) the phylogenetic structure within Pleurodonta observed in our own and previous traditional analyses suggests it may have been more prone to incomplete lineage sorting than the acrodont iguanians (i.e., multiple pleurodont subclades connected by generally very short branches), (b) wider taxon sampling would have introduced considerable amounts of missing data, which are potentially problematic for this method (e.g., Thomson et al., 2008), and (c) the computational burden for a larger dataset would have made the analyses impractical. Two genes (*AHR* and *GPR37*) that were missing for multiple pleurodont taxa were excluded; thus, the final BEST dataset consisted of 27 loci and 28 taxa, with only one missing ingroup sequence (*BMP2* for *Corytophanes cristatus*).

Our initial BEST analyses used the default parameter values ( $\alpha = 3$  and  $\beta = 0.003$ ) of the inverse gamma distribution prior on effective population size ( $\theta$ ), but these analyses failed to converge (average standard deviation of split frequencies  $>0.1$  for several genes) after 80 million generations. Therefore, a broader prior distribution with a higher mean for  $\theta$  was created by setting  $\alpha = 3$  and  $\beta = 0.1$ . Under the broader prior, the analyses appeared to reach stationarity more rapidly (see Section 3). Following the recommendation of BEST's authors (L. Liu, pers. comm.), instead of running multiple chains within each run (as typically done with MrBayes), we ran several separate analyses with different random starting points and one chain per run, and compared our results.

Many of our individual loci appear to be largely uninformative at short, deep branches in the pleurodont tree (see Section 3). Since BEST analyses loci individually to produce gene trees from which the species tree is inferred (albeit with some indirect communication between loci; Liu, 2008), it seems possible that inclusion of large numbers of loci that are uninformative for these branches might adversely affect species-tree estimation. To test this hypothesis, we performed an additional BEST analysis that used only the seven most variable loci, and compared the results to the 27-gene species tree.



**Fig. 2.** Effect of root position when assessing tree congruence. (A) A rooted reference topology to which other topologies are compared. (B) Alternative topology differing from A only in the position of the ingroup root. If a particular Bayesian posterior distribution of trees contained 30% Topology A and 70% Topology B, for example, simply screening this posterior for the presence of clade (a + b) would show low congruence (30%) with the reference tree. However, filtering the posterior using the unrooted reference ingroup topology achieved in PAUP\* by means of a backbone constraint tree with the outgroup removed, (C) would show perfect (100%) congruence with the reference tree. Dashed lines in C show alternative rooting positions along the unrooted network.

### 2.3.4. Historical biogeographic analysis of pleurodents

We used dispersal–extinction–cladogenesis analysis (DEC; Ree and Smith, 2008) in Lagrange v.20090327 (<http://code.google.com/p/lagrange/downloads/list>) to determine the most likely distribution of ancestral pleurodents. This likelihood-based modeling method specifies rates of transition between geographic ranges along phylogenetic branches, and uses this to estimate ancestral-area likelihoods at branching points in the tree. This method requires an ultrametric tree, and we used the pleurodont portion of the BEAST chronogram (without *Bharatagama*) for this purpose. The online “Lagrange configurator” (<http://www.reelab.net/lagrange/configurator/index>) was used to produce the Python script that was run locally.

All 27 pleurodont taxa were included in the analysis. Because our taxon sampling is relatively limited, it would not always make sense to code each included taxon according to its true distribution. Specifically, if a particular included taxon has a distribution that is obviously unrepresentative of its parent clade, it could mislead the analysis to include its distribution here. For example, *Brachylophus* occurs in Fiji, yet few would argue that a South Pacific origin for iguanids is even remotely plausible. Given that we only have three iguanids in our analysis, it seems unreasonable to label one-third of our iguanid taxa as “Oceanic.” We chose to deal with this situation by coding clade distributions as blocks corresponding to the 12 major pleurodont iguanian clades (i.e., within each clade, all members were coded identically according to that clade’s major distributional area [or ancestral area if relevant evidence exists]). Major clade distributions were coded as follows: Corytophanidae = Central America, defined here as the area south of Mexico and north of Colombia (for historical biogeographic reconstruction within this group see Vieira et al. (2005)); Crotaphytidae = North America (defined here as New World north of Central America); Hoplocercidae = South America; Iguanidae = North America and Central America (see Wiens and Hollingsworth (2000) for evidence that South American taxa are nested within a North/Central American and West Indian clade); Leiocephalidae = West Indies; Leiosauridae = South America; Liolaemidae = South America; Opluridae = Madagascar; Phrynosomatidae = North America; *Anolis* = West Indies and South America (see Nicholson et al. (2005) for evidence that the North and Central American taxa originated via dispersal from the West Indies); *Polychrus* = South America (one phylogenetically nested species’ distribution extends into Central America; see Frost et al., 2001); and Tropicuridae = South America.

For this analysis, no ancestral areas were excluded *a priori*, but in the “adjacency matrix” it was stipulated that the following areas were not adjacent: North America and South America, North America and Madagascar, Central America and Madagascar, West Indies and Madagascar. No temporal limitations were placed on dispersal. Two ancestral areas were allowed at a given node.

## 3. Results

### 3.1. Concatenated phylogenetic analyses of the full data

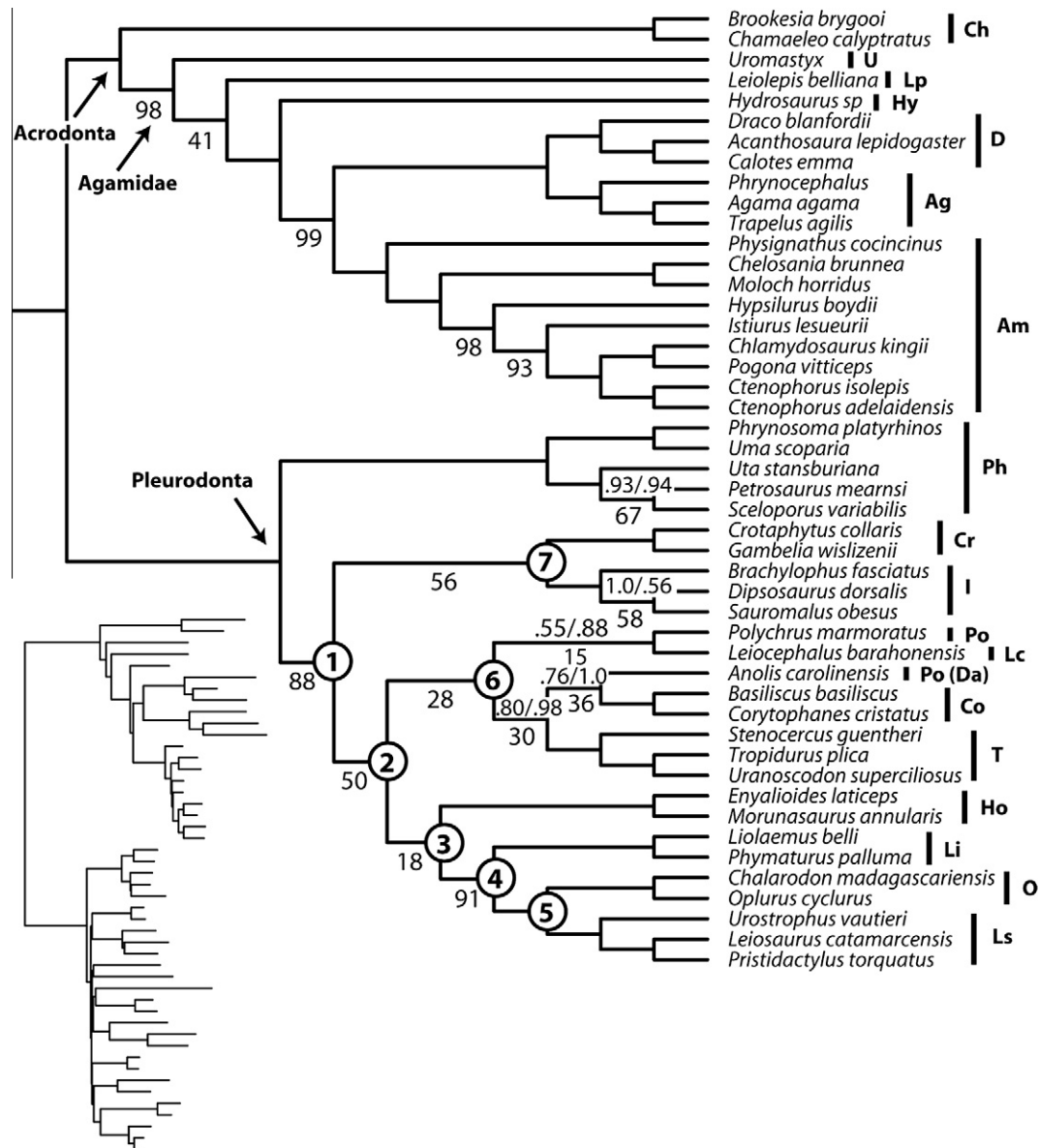
Analysis of Bayes factors strongly favored the 87-partition scheme (each of 29 genes partitioned by codon positions; Bayes factor  $\geq 212$  in all comparisons), and all results reported here for the full concatenated dataset from standard Bayesian (MrBayes) and ML analyses used this partitioning scheme. In the standard Bayesian (MrBayes) analyses, relative rates inferred for the different codon positions were consistent with expectations, and branch lengths were comparable to those in the ML tree (results not shown), suggesting that Bayesian branch lengths were not grossly inaccurate. BEAST analyses ran for 500,000,000 generations,

sampling every 10,000 generations. The first 25% of samples were removed as burnin. Analyses partitioned by codon position (i.e., 87 partitions) did not achieve adequate ESS values for some rate parameters, but analyses that combined first and second positions did; reported results are from this second partitioning scheme (i.e., 58 partitions). Topology, support values, and divergence time estimates were similar between the two partitioning schemes. Visual inspection of parameter estimates from BEAST runs with and without data, respectively, showed markedly different values. This suggests that the data, and not the initial priors alone, are informing our results.

Likelihood and Bayesian concatenated analyses (with both MrBayes and BEAST) all yielded identical overall topology estimates (Fig. 3). Reciprocal monophyly of acrodont and pleurodont iguanians was maximally supported by all analyses (i.e., MLBS = 100%, PP = 1.0). Within acrodonts, both concatenated Bayesian analyses (MrBayes and BEAST) found maximal support for all clades (PP = 1.0), and MLBS values were  $\geq 93\%$  for all but one clade (Fig. 3). Chamaeleonidae was strongly supported as the sister taxon of all other acrodonts, rather than being nested inside agamids (MLBS for agamids = 98%). *Uromastyx* was excluded from a clade containing all remaining agamids (though with only 41% MLBS), and within this more exclusive clade, *Leiolepis* was the sister taxon of a clade (MLBS = 100%) comprising the remaining acrodonts (i.e., Agamidae *sensu stricto*). Monophyly of all major agamid clades (=subfamilies) within this group was maximally supported (but note that Hydrosaurinae, which consists of the single genus *Hydrosaurus*, was represented here by a single species). Hydrosaurinae was strongly supported (MLBS = 99%) as the sister taxon of a clade including Amphibolurinae and the sister clades Agaminae and Draconinae (Fig. 3). Within Amphibolurinae, the Southeast Asian *P. cocincinus* was the sister taxon of a clade containing all other sampled species, including *P. lesueurii*. The Australian genera *Chelosania* and *Moloch* formed a clade that was sister to the remaining species, leaving the primarily New Guinean *Hypsilurus* as the sister taxon of all other sampled amphibolurine species.

Within pleurodents (Fig. 3), all analyses found maximal support for monophyly of 9 of 11 families: Phrynosomatidae, Crotaphytidae, Iguanidae, Corytophanidae, Hoplocercidae, Opluridae, Liolaemidae, Tropicuridae, and Leiosauridae (the monogeneric Leiocephalidae was represented by a single species, *Leiocephalus barahonensis*) (Table 2). However, Polychrotidae was found to be polyphyletic, as *Anolis* and *Polychrus* do not appear to be closely related. All analyses also found strong support for three higher-level groupings. First, Phrynosomatidae is the sister taxon of all other pleurodents (MrBayes/BEAST PP > 0.95; MLBS = 88; Node 1 of Fig. 3). Second, a sister-taxon relationship between Leiosauridae and Opluridae was maximally supported in all analyses (Node 5 of Fig. 3). Third, Liolaemidae is the sister taxon to this leiosaurid–oplurid clade (MrBayes/BEAST PP > 0.95; MLBS = 91; Node 4 of Fig. 3).

Several other higher-level pleurodont clades were strongly supported by the Bayesian analyses, but were weakly supported by MLBS. A clade comprising Crotaphytidae and Iguanidae (MrBayes/BEAST PP > 0.95; MLBS = 56; Node 7 of Fig. 3) is the sister taxon of a clade containing all other non-phrynosomatid pleurodents (MrBayes/BEAST PP > 0.95; MLBS = 50; Node 2 of Fig. 3). This latter clade in turn consists of two primary subclades: within one of these (MrBayes/BEAST PP = 1.0; MLBS = 28; Node 6 of Fig. 3), *Polychrus* (Polychrotidae) and *Leiocephalus* (Leiocephalidae) are sister taxa (MrBayes/BEAST PP = 0.55/0.88; MLBS = 15), as are *Anolis* (Polychrotidae) and Corytophanidae (MrBayes/BEAST PP = 0.76/1.0; MLBS = 36). Thus, our results suggest polyphyly of Polychrotidae. Corytophanidae and *Anolis* together form the sister group of Tropicuridae (MrBayes/BEAST PP = 0.80/0.98; MLBS = 30). Within the other primary subclade (MrBayes/BEAST PP > 0.95; MLBS = 18;



**Fig. 3.** Results of Bayesian (MrBayes, BEAST) and likelihood (RAxML) analyses, with key suprafamilial pleurodont clades marked by circled numbers. Non-iguanian outgroups have been removed from the figure for clarity. Unmarked nodes have PP > 0.95 and MLBS = 100%. For other nodes, MrBayes/BEAST posterior probability (PP) support is shown above the node, with ML bootstrap (MLBS) support shown below. Inset shows ML phylogram. Ch, Chamaeleonidae; U, Uromastixinae; Lp, Leiolepidinae; Hy, Hydrosaurinae; D, Draconinae; Ag, Agaminae; Am, Amphibolurinae; Ph, Phrynosomatidae; Cr, Crotaphytidae; I, Iguanidae; Po, Polychrotidae (note that *Anolis* now belongs to the family Dactyloidae [Da; see Section 4]); Lc, Leiocephalidae; Co, Corytophanidae; T, Tropiduridae; Ho, Hoplocercidae; Li, Liolaemidae; O, Opluridae; Ls, Leiosauridae.

Node 3 of Fig. 3, Hoplocercidae is the sister taxon to the strongly supported clade consisting of liolaemids + (leiosaurids + oplurids).

The unpartitioned Phycas analyses that sampled only fully resolved trees (i.e., similar to our MrBayes analyses, except without data partitioning) resulted in an iguanian topology identical to that in Fig. 3, although Nodes 3 and 7 from Fig. 3 were not significantly supported in the Phycas analyses (possibly due to the absence of partitioning relative to the partitioned MrBayes analyses). Relative to these “no-polytomy” analyses, Phycas analyses that did allow polytomies showed reduced support at some pleurodont nodes (see ESM for both trees). Those nodes showing a >0.03 reduction in PP include those for the clade *Sceloporus* + *Petrosaurus* (0.81–0.57), the clade *Polychrus* + *Leiocephalus* (0.93 to <0.50), and Nodes 3, 6, and 7 of Fig. 3 (0.80 to <0.50, 0.91–0.77, and 0.99–0.80, respectively). These results suggest that support for these nodes might be overestimated in our main (MrBayes and BEAST) concatenated analyses (but note that some reductions in support might also be

due to the absence of partitioning). However, the clade *Anolis* + *Polychrus* was completely absent in trees from the posterior distributions of all Phycas analyses, which suggests that evidence against polychrotid monophyly was not necessarily overestimated (see discussion of polychrotid non-monophyly below).

Monophyly of both Polychrotinae\* and Tropidurinae\* (Schulte et al., 2003b) was rejected by the AU test ( $P = 0.000$  and  $P = 0.038$ , respectively), suggesting that these metataxon names are no longer needed. However, monophyly of Polychrotidae (*Anolis* + *Polychrus*) could not be rejected ( $P = 0.215$ ).

### 3.2. Divergence time estimates

Separate BEAST analyses with the *Bharatagama* calibration included and excluded resulted in a crown-Iguania root age of 165 Mya (million years ago) and 123 Mya, respectively. Other nodes within Iguania were less affected by inclusion of this fossil.

**Table 2**  
Individual-gene support for pleurodont iguanian families.<sup>a</sup>

Locus <sup>b</sup>	Families <sup>c</sup>								
	Phrynosomatidae	Crotaphytidae	Iguanidae	Corytophanidae	Tropiduridae	Hoplocercidae	Liolaemidae	Opluridae	Leiosauridae
NKTR	*/*	*/*	*/*	*/*	*/*	*/*	*/*	*/*	*/*
AKAP9	*/*	*/*	*/*	*/*	*/98	*/*	*/*	*/*	*/*
BACH1	*/*	*/*	*/*	*/*	*/*	*/*	*/*	*/*	*/*
MSH6	*/*	*/*	*/*	*/*	*/89	*/*	*/*	*/*	*/*
UBN1	*/*	*/*	*/*	*/*	–/–	*/*	0.88/76	*/*	*/*
PNN	*/*	*/*	*/99	*/*	*/97	*/*	*/*	*/*	*/*
RAG1	*/*	*/*	*/99	*/*	*/96	*/*	*/*	*/*	*/*
MKL1	*/*	*/*	*/99	*/*	*/97	*/98	*/98	*/*	*/*
R35	*/*	*/*	*/*	*/98	*/95	*/*	*/*	*/*	*/*
SLC30A1	*/97	0.95/80	*/*	*/*	–/–	*/98	0.93/60	*/*	*/98
SLC8A3	*/*	*/*	*/99	*/*	–/–	*/*	*/65	*/*	*/*
BHLBH2	*/76	*/93	*/91	*/84	*/*	*/91	*/80	*/97	*/*
DNAH3	*/99	*/99	*/*	*/*	0.93/65	*/98	0.93/71	*/*	*/*
TRAF6	*/91	*/*	*/*	*/*	0.98/56	*/99	*/87	*/*	*/*
INHBA	*/*	*/*	*/97	*/*	0.45/–	*/99	*/64	*/*	*/96
ENC1	*/*	*/97	*/99	*/*	0.95/66	*/*	0.99/73	*/*	*/99
FSHR	*/72	*/*	*/99	*/88	0.93/68	*/99	0.58/33	*/*	*/*
ADNP	*/*	*/*	*/94	*/*	0.99/63	*/*	*/97	*/*	*/*
SNCAIP	*/86	*/*	*/75	*/*	–/–	*/99	0.9/61	*/99	*/87
NGFB	*/*	*/*	*/90	*/*	*/81	*/*	*/98	*/*	*/99
ZEB2	*/99	0.95/76	*/89	*/*	0.88/53	*/95	0.74/53	*/*	*/96
SLC8A1	*/*	*/*	*/*	*/*	–/–	*/*	*/75	*/*	*/*
NT3	*/*	*/*	*/99	*/99	*/96	*/98	0.73/60	*/*	*/98
FSTL5	0.8/30	*/*	*/77	*/*	0.57/15	*/99	0.73/53	*/*	*/83
BMP2	*/96	*/*	*/80	na	0.57/25	*/99	*/86	*/*	*/88
GPR37	*/93	na	0.99/64	*/96	–/–	na	0.96/65	*/*	na
AHR	*/94	*/96	*/98	*/99	0.53/27	na	0.7/64	*/99	na
BDNF	*/74	*/95	*/92	0.96/85	–/–	*/90	*/83	*/97	–/–
CAND1	0.94/46	*/72	0.99/61	*/96	–/–	0.76/73	*/81	*/*	*/97

<sup>a</sup> Shown as Bayesian posterior probability (PP)/maximum-likelihood bootstrap (MLBS) percentage. Asterisks represent PP = 1.0 or MLBS = 100%. Dashes mean the family was not monophyletic, and “na” means missing data precluded evaluation.

<sup>b</sup> Loci are listed from top to bottom in order of decreasing number of variable characters.

<sup>c</sup> Note that Leiocephalidae, *Anolis*, and *Polychrus* are each represented by single taxa in our dataset and are thus not included.

When *Bharatagama* was included, ages were generally 8–12% older within acrodonts and <1.5% older within pleurodonts (see [ESM](#) for both full chronograms, with all outgroups to *Iguania* included). Our preferred (more conservative) analysis excluded *Bharatagama*. In this tree ([Fig. 4](#)), chamaeleonids diverged from other acrodonts around 93 Mya, and the *Uromastix* and *Leiolepis* lineages diverged around 87 and 82 Mya, respectively. The remaining four major extant lineages of agamids originated from around 70–80 Mya. Crown pleurodonts began their diversification around 73 Mya, and 11 of the 12 major extant lineages appeared over the next ~10 million years. The most recent family-level split, between leiosaurids and oplurids, occurred about 53 Mya.

### 3.3. Supplemental analyses of pleurodont relationships

#### 3.3.1. Rooting of pleurodonts

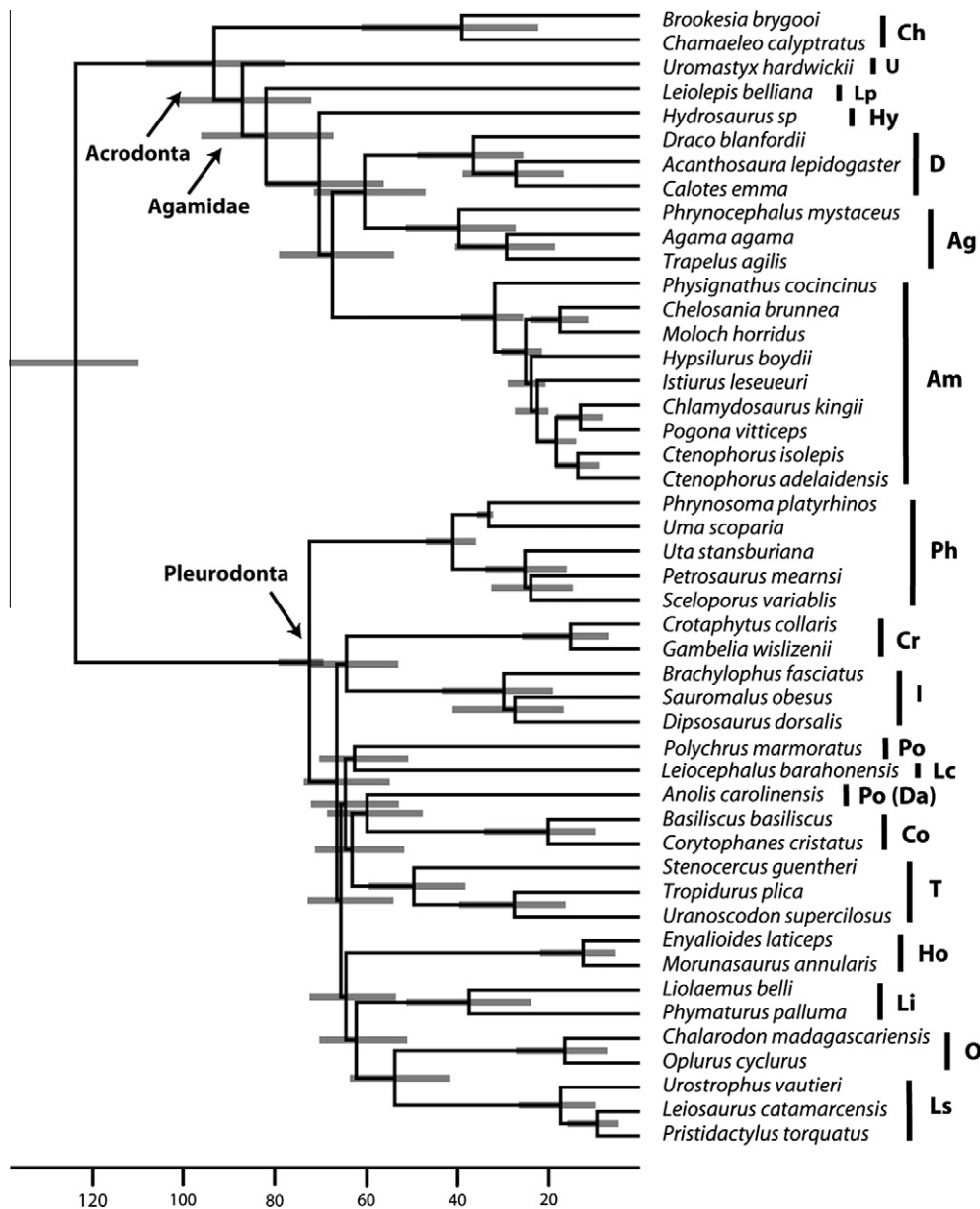
Phylogenetic results for pleurodonts showed some sensitivity to reduced outgroup sampling, but only when all non-iguanian outgroups were excluded. RAxML and MrBayes analyses using various combinations of acrodonts as the only non-pleurodont taxa always resulted in pleurodont root placement along the long branch separating *Leiocephalus* from all other pleurodonts (see [ESM](#)). Interestingly, when these trees were rerooted at phrynosomatids (i.e., giving the same rooting as the 76-taxon concatenated analyses), only one minor topological difference relative to the 76-taxon analyses remained. Specifically, crotaphytids were no longer the sister taxon of iguanids (see Node 7 of [Fig. 3](#)), but were instead the sister taxon of all pleurodonts except iguanids and phrynosomatids (results not shown). Inclusion of additional non-acrodont outgroups (i.e., anguimorphs and/or snakes) along with some or all acrodonts consistently gave the same pleurodont rooting and

topology as the concatenated analyses of all taxa shown in [Fig. 3](#) (see [ESM](#) for specific taxon combinations used). Thus, this specific root placement appears to be dependent on adequate sampling of multiple successive outgroups to pleurodonts. These rooting results were unaffected by the different partitioning schemes (i.e., 87 vs. 58 vs. 29 partitions; results not shown).

#### 3.3.2. Individual gene analyses

Bayesian (MrBayes) analyses of individual genes generally supported the monophyly of most pleurodont families, but yielded poor resolution and support for most interrelationships among these clades (see [ESM](#) for all individual gene trees). Filtering the individual-gene posteriors using backbone constraint trees from which outgroups had been removed resulted in (at most) minimal increases in PP for the seven key nodes from [Fig. 3](#). This result suggests that conflicts between the concatenated and individual-gene topologies were not due to differences in the rooting of pleurodonts (results not shown).

For all but one of the seven focal suprafamilial pleurodont clades (i.e., Node 5 of [Fig. 3](#)), support is generally very low (PP < 0.05) across most of the 29 individual loci ([Table 3](#)). Overall, for these six nodes, the average levels of support (i.e., PP) across loci range from 0.003 to 0.083. Node 5 (Leiosauridae + Opluridae clade) has considerably higher support across loci (mean PP = 0.286) than the other nodes, but even this clade is strongly supported (i.e., PP ≥ 0.95) by only three of the 29 individual loci (*NKTR*, *RAG1*, and *MKL1*). Only one other clade (i.e., Node 4) is strongly supported in the individual-gene analyses, and this is only by a single gene (*RAG1*). At the most extreme, Node 6 receives the lowest support across the loci (mean PP = 0.003), and is recovered with PP > 0.01 for only three genes ([Table 3](#)).



**Fig. 4.** Bayesian chronogram produced by BEAST analyses with 29 genes (based on mean age estimates). Non-iguanian taxa have been removed for clarity. Shaded bars represent 95% highest probability densities. Numbers on scale bar are millions of years before present. Clade name abbreviations as in Fig. 3.

### 3.3.3. Concatenated analyses of data subsets

In these analyses, we included all taxa but excluded loci that gave the highest support for particular nodes in the single-gene analyses, with the goal of detecting hidden support for clades (i.e., support that is only apparent in combined analyses). Any cut-off point below  $PP = 0.95$  is inevitably arbitrary; as an empirical choice, we excluded loci that gave  $PP > 0.07$  in their respective individual-gene analyses (indicated as bold in Table 3).

Two distinct patterns were found (Table 4). First, for Node 5, when only 13 of the 29 loci were analyzed (i.e., those that showed the lowest support for the node in individual analyses, marked by unbolded text in Table 3), the concatenated  $PP$  for this node was 1.0, just as in the full 29-locus concatenated analyses. Similarly, Node 4, which had the second-highest mean  $PP$  across all individual loci (Table 3), was recovered with  $PP = 0.44$  in a concatenated analysis using 24 loci, only two of which individually supported the node with  $PP > 0.02$ . These results for Nodes 4 and 5 indicate the presence of hidden congruence among genes that individually provide very little support.

In contrast, we found very little Bayesian support for the other five nodes after removing only a handful of loci, despite strong support for these nodes in the 29-locus analyses. For example, although Node 1 was strongly supported in all the full 29-locus concatenated analyses and exhibited the third highest mean  $PP$  across loci, there was no support for this node in a 24-gene concatenated analysis (Table 4). Thus, unlike Nodes 4 and 5, there appears to be little or no hidden congruence, and the support for this clade is primarily coming from the five loci excluded from the supplemental analysis. Likewise, for Nodes 3 and 7, respective analyses of 24 and 25 concatenated genes yielded zero occurrences of the focal node in the posterior distribution (Table 4). For Nodes 2 and 6, only a single gene was excluded, leaving 28 genes, and still there was zero support (note that there were actually no loci that supported Node 6 with  $PP > 0.07$ ; *ADNP* was the highest with  $PP = 0.05$ , so we excluded this locus).

Supplemental Bayesian and ML concatenated analyses using only the top seven most variable loci (Fig. 5A) strongly support Nodes 1, 4, and 5 of Fig. 3. These are the only three interfamilial

**Table 3**  
Individual-locus Bayesian support for suprafamilial pleurodont nodes (Fig. 3) strongly supported in the concatenated Bayesian analyses.

Locus <sup>a</sup>	Posterior probabilities (in % form for clarity) for nodes strongly supported in Bayesian (MrBayes) concatenated analyses <sup>b</sup>						
	1	2	3	4	5	6	7
NKTR	2.90	0.08	<b>7.13</b>	<b>14.18</b>	<b>99.84</b>	1.83	<b>9.66</b>
AKAP9	<b>16.31</b>	0.00	0.00	0.00	<b>37.87</b>	0.00	<b>7.21</b>
BACH1	<b>51.71</b>	<b>76.78</b>	<b>7.11</b>	<b>23.72</b>	<b>73.13</b>	0.96	<b>89.63</b>
MSH6	1.79	0.00	0.00	0.00	0.65	0.00	0.66
UBN1	0.29	0.01	0.00	0.02	<b>58.53</b>	0.00	0.04
PNN	<b>23.28</b>	0.88	<b>9.22</b>	<b>78.24</b>	<b>53.05</b>	0.00	3.72
RAG1	4.43	0.00	<b>13.48</b>	<b>99.81</b>	<b>98.51</b>	0.00	0.00
MKL1	5.77	0.00	0.00	0.00	<b>99.19</b>	0.00	0.40
R35	0.18	0.00	0.00	0.00	2.91	0.00	0.00
SLC30A1	0.21	0.00	0.01	0.00	0.33	0.48	0.00
SLC8A3	0.72	0.01	0.38	4.81	<b>18.89</b>	0.02	1.88
BHLBH2	3.41	0.26	0.03	0.45	<b>22.72</b>	0.00	0.53
DNAH3	0.04	0.01	0.00	0.01	0.18	0.00	2.83
TRAF6	0.07	0.01	0.02	<b>11.19</b>	<b>41.03</b>	0.00	3.29
INHBA	1.11	0.00	0.03	0.29	5.34	0.00	0.00
ENC1	6.27	1.88	0.08	0.07	<b>10.38</b>	1.38	6.29
FSHR	0.05	0.00	0.00	0.16	<b>7.23</b>	0.01	1.12
ADNP	0.49	0.02	<b>10.88</b>	1.05	<b>8.93</b>	4.70	<b>13.16</b>
SNCAIP	0.03	0.00	0.00	0.02	1.19	0.00	0.19
NGFB	2.93	0.00	0.00	0.00	0.74	0.00	0.14
ZEB2	0.03	0.01	0.00	0.03	3.11	0.00	0.56
SLC8A1	<b>29.08</b>	0.10	0.00	1.69	<b>28.33</b>	0.00	2.23
NT3	4.06	0.00	0.01	0.03	4.78	0.00	1.27
FSTL5	0.02	0.00	0.00	0.02	3.54	0.00	0.26
BMP2	3.70	0.23	0.03	4.03	<b>65.01</b>	0.00	0.18
GPR37	0.48	0.00	0.00	0.09	0.13	0.00	0.02
AHR	0.09	0.00	0.01	1.94	6.15	0.00	0.01
BDNF	5.83	0.00	0.00	0.00	0.00	0.00	0.00
CAND1	<b>25.38</b>	0.00	0.00	0.01	<b>76.62</b>	0.00	1.43
Overall means (st. dev.)	6.57 (11.81)	2.77 (14.24)	1.67 (3.81)	8.34 (23.15)	28.56 (34.17)	0.32 (0.95)	5.06 (16.58)
Bold means (st. dev.)	29.15 (13.44)	76.78 (n/a)	9.57 (2.7)	45.43 (40.79)	45.04 (34.11)	n/a	29.19 (36.12)
Plain means (st. dev.) <sup>c</sup>	1.83 (2.19)	0.12 (0.38)	0.02 (0.08)	1.04 (2.52)	1.6 (1.67)	0.32 (0.95)	0.86 (1.13)

Note. All MrBayes analyses were run for 16 million generations, 25% of trees removed as burn-in. Bold percentages are those above an arbitrary threshold of 7% used to exclude genes from supplemental concatenated analyses (see text and Table 4).

<sup>a</sup> Loci are listed from top to bottom in order of decreasing number of variable characters.

<sup>b</sup> See numbered nodes from Fig. 3. Bold values indicate those genes excluded from supplementary concatenated analyses.

<sup>c</sup> i.e., Support from loci used in the supplementary concatenated analyses summarized in Table 4.

**Table 4**  
Supplemental MrBayes concatenated analyses showing effect of excluding loci that provide the highest levels of posterior probability (PP) support in individual-gene analyses for key nodes within Pleurodonta.<sup>a</sup>

Key node <sup>a</sup>	No. of included loci <sup>b</sup>	PP
1	24	0.0
2	28	0.0
3	24	0.0
4	24	0.44
5	13	1.0
6	28	0.0
7	25	0.0

<sup>a</sup> Numbered nodes from Fig. 3.

<sup>b</sup> Excluded loci for each analysis are those with PP > 7% (bold in Table 3), except Node 6, for which no locus gave PP > 7%. For this node, ADNP (PP = 4.7%) was excluded.

nodes strongly supported by both MLBS and PP from the 29-locus analyses (Fig. 3).

### 3.3.4. Incongruence between concatenated and individual-gene trees

Bayesian analyses of individual genes (with MrBayes) showed some strongly supported incongruence with the concatenated results for pleurodonta. Nine of the estimated gene trees exhibited strong support (PP ≥ 0.95) for clades that were incongruent with strongly supported clades in the full 29-locus concatenated analyses (Table 5; see also individual-gene trees in Online supplement). However, only one of these strongly supported alternative clades (Crotaphytidae + Opluridae) was supported by more than one gene

(NGFB and SLC30A1). Four of these nine genes (MSH6, NGFB, NKTR, SLC30A1; Table 5) conflicted strongly with the most strongly supported suprafamilial clade from the 29-locus concatenated analysis (Node 5: Leiosauridae + Opluridae).

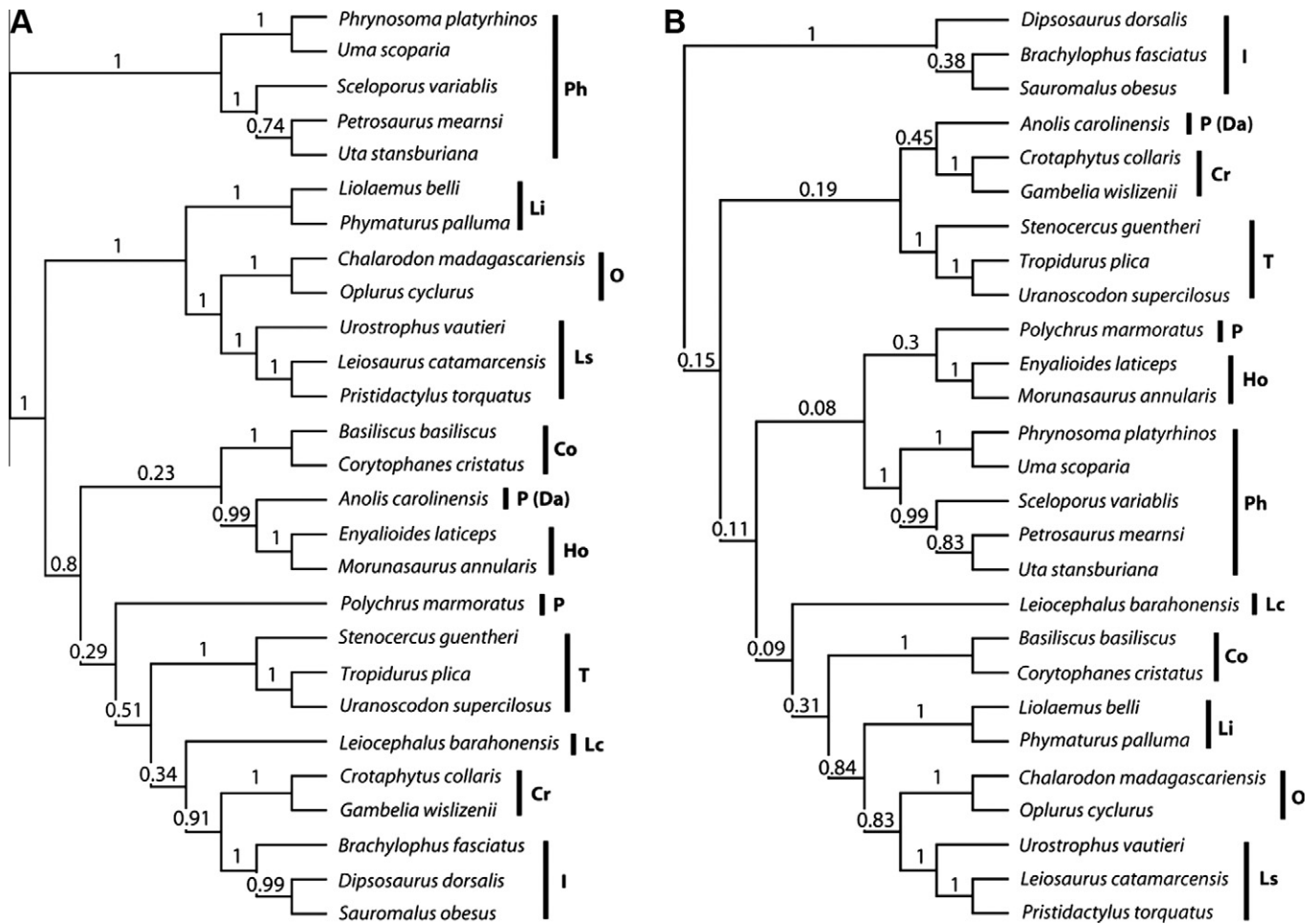
### 3.3.5. Historical biogeography of pleurodonta

The topology agreed on by all concatenated analyses (Fig. 3) recovers two clades that are predominantly North- and Central American in their distributions (phrynosomatids, and crotaphytids + iguanids) sequentially diverging at the base of the pleurodont tree. All the remaining major clades are distributed principally in Central and South America, with the exception of the oplurids in Madagascar. Thus, a simple parsimony argument suggests a North American origin for extant pleurodonta, with later expansion into Central and South America.

Likelihood analysis (using Lagrange) returned a 96% probability that the most recent common ancestor of phrynosomatids + other pleurodonta (i.e., the pleurodont root) was distributed north of South America (North America, Central America, or both), and an 88% probability that the most recent common ancestor of (iguanids + crotaphytids) + other non-phrynosomatid pleurodonta (Node 1, Fig. 3) was distributed north of South America. These results thus confirm the parsimony-based inference of an origin in the Northern Hemisphere with later southward expansion.

### 3.4. Coalescent-based species tree analyses

For the 27-gene coalescent-based species-tree (BEST) analysis of pleurodonta, four replicate analyses (2 runs each) were performed



**Fig. 5.** Bayesian analyses using the top seven most-variable loci. Numbers at nodes represent posterior probabilities. (A) Results from 76-taxon MrBayes analysis (non-pleurodont outgroups have been removed for clarity). (B) Results from 28-taxon BEST analysis (outgroup taxon *Leiolepis belliana* has been removed for clarity). Clade name abbreviations as in Fig. 3.

**Table 5**

Alternative clades (discordant with concatenated trees) supported by Bayesian PP  $\geq 0.95$  in individual-gene analyses.

Alternative clade composition	Supporting loci
1. All except <i>Polychrus</i> , <i>Anolis</i> , Leiocephalidae, Corytophanidae, and Tropiduridae	ADNP
2. Corytophanidae, Hoplocercidae, Leiocephalidae, Leiosauridae, and Opluridae	AKAP9
3. <i>Anolis</i> , Hoplocercidae, Leiocephalidae	DNAH3
4. All except <i>Leiocephalus</i>	FSTL5
5. All except <i>Anolis</i>	MSH6
6. Corytophanidae, Opluridae	MSH6
7. Crotaphytidae, Opluridae	NGFB, SLC30A1
8. Iguanidae, Leiosauridae	NKTR
9. <i>Anolis</i> , Phrynosomatidae	R35
10. Crotaphytidae, Liolaemidae	R35

using 100 million generations each. The average standard deviation of split frequencies (ASDSF) for individual loci was generally between 0.01 and 0.03, with no obvious correlation of values for particular loci among analyses. Consensus topologies were identical for three of these analyses, with very similar PP. The fourth analysis showed some topological differences, but all at very poorly supported nodes, and likelihood values were worse for this analysis. We report here the combined consensus topology from the first three analyses (Fig. 6). These analyses found maximal support for the monophyly of the same major clades (i.e., families) strongly supported in the concatenated ML and Bayesian analyses (Figs. 3 and 6). However, relationships among these clades were for the

most part quite different in the BEST analyses, and support was universally weak. The BEST analysis did recover the leiosaurid + oplurid clade (Node 5 of Fig. 3), which was maximally supported in all concatenated analyses, but the 27-gene BEST PP support for this clade is only 0.57 (Fig. 6). One additional higher-level clade (*Anolis* + corytophanids) was shared between the BEST and concatenated analyses, but this clade received weak support in all but the BEAST concatenated analysis.

Relative to the 27-gene BEST analysis of Fig. 6, the BEST analysis using only the seven most variable loci (Fig. 5B) recovered a more strongly supported topology that more closely resembled the 29-gene concatenated tree. Support for the leiosaurid + oplurid

clade increased (PP = 0.83), and liolaemids were recovered as the sister taxon to this group with PP = 0.84 (Fig. 5B). This latter node is congruent with strongly supported results from the concatenated analyses (Fig. 3), but is absent from the 27-gene BEST species tree (Fig. 6).

## 4. Discussion

### 4.1. Major phylogenetic findings

Our phylogenetic analyses of 29 nuclear loci clarify several aspects of higher-level iguanian relationships. First, within acrodonts, we show that chamaeleonids are the sister taxon to a monophyletic Agamidae *sensu lato*, in agreement with some other molecular analyses (Honda et al., 2000; Melville et al., 2009; Townsend et al., 2004). Further, we provide the first strong support for the placement of the enigmatic agamid clade Hydrosaurinae (consisting of the single Southeast Asian genus *Hydrosaurus*). Macey et al. (2000b), Melville et al. (2009), Schulte and Cartwright (2009), and Okajima and Kumazawa (2010) all placed *Hydrosaurus* as the sister taxon to the agamine–draconine clade with weak support. Our results instead strongly support its position as the sister taxon of all other agamids excluding *Uromastix* and *Leiolepis*. The amphibolurine phylogeny of Hugall et al. (2008) contained a weakly supported clade comprising the Australian *Chelosania* and *Moloch* and the New Guinean *Hypsilurus* as the sister taxon to all remaining Australian taxa. However, our study strongly supports a *Chelosania* + *Moloch* clade as the sister taxon to a clade containing *Hypsilurus* + all remaining Australian taxa. Relationships amongst these remaining Australian taxa agree with previous molecular studies (e.g., Hugall et al., 2008; Schulte et al., 2003a). Finally, we recovered maximal Bayesian support for the genus *Uromastix* (found from Africa to South Asia) as the sister taxon of a clade

containing the Southeast Asian *Leiolepis* and all remaining agamids (see also Macey et al., 2000b; Schulte and Cartwright, 2009). However, we still consider this result somewhat tentative because MLBS support was very low.

Our concatenated and species-tree analyses strongly support each of the pleurodont families that were supported in other recent molecular studies (e.g., Frost et al., 2001 [molecular data only]; Schulte et al., 2003b), but like these studies, we also find Polychrotidae to be polyphyletic. The concatenated analyses solidify support for some previously hypothesized suprafamilial pleurodont clades and also provide strong support for some relatively novel relationships. Interestingly, for several of these clades there is a disparity between Bayesian and ML support levels. We describe these pleurodont results in more detail below.

All concatenated analyses maximally supported the sister relationship between the Malagasy Opluridae and the austral South American Leiosauridae (i.e., Node 5 of Fig. 3). Such a relationship between leiosaurids and oplurids was first weakly suggested by the mtDNA data of Schulte et al. (2003b). Noonan and Chippindale (2006) found strong Bayesian support for this relationship from four nuclear genes (three of which are also included among the 29 used here). Our concatenated analyses also recovered strong support for the austral South American family Liolaemidae as the sister taxon of Opluridae + Leiosauridae (Node 4 of Fig. 3). This relationship was also recovered (with weak support) by Noonan and Chippindale (2006), but surprisingly neither this clade nor the leiosaurid–oplurid clade was recovered in the expanded study by Noonan and Sites (2010), which incorporated these same data plus two additional nuclear loci.

We find strong support for the placement of the predominantly North American clade Phrynosomatidae as the sister taxon of all other pleurodonts (Node 1 of Fig. 3). Previous morphological (Conrad, 2008; Frost and Etheridge, 1989) and mtDNA-based (Macey et al., 1997; Schulte et al., 2003b) phylogenetic studies have

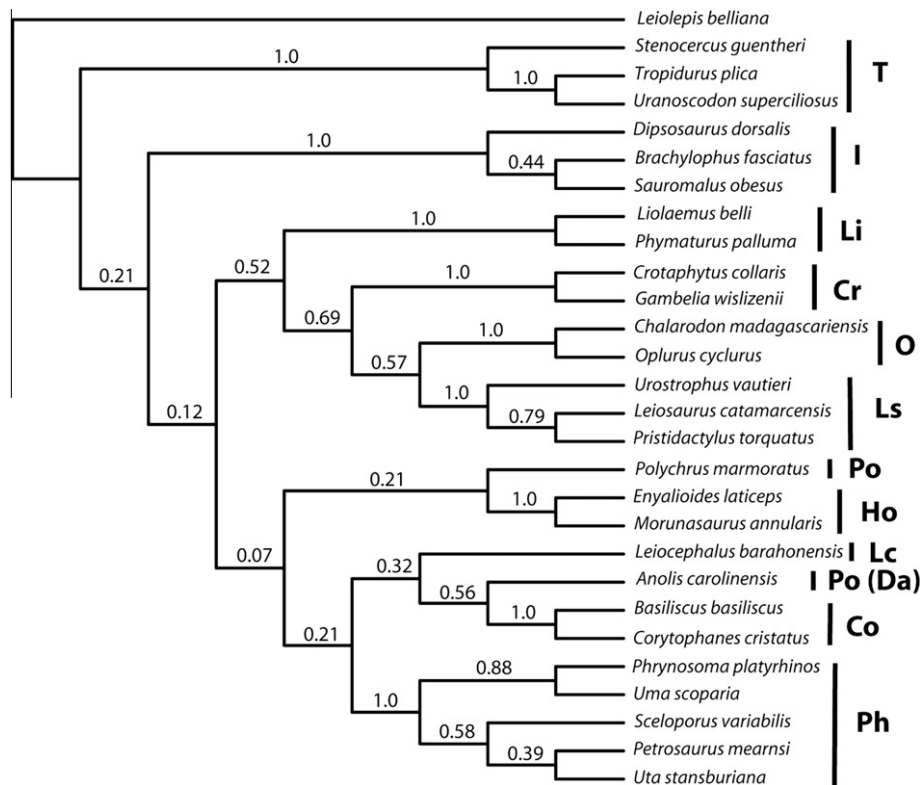


Fig. 6. Cladogram from the 27-locus BEST analysis of pleurodont iguanians. Numbers above branches represent posterior probabilities. Clade name abbreviations as in Fig. 3.

produced conflicting, weakly-supported hypotheses for the phylogenetic placement of phrynosomatids. Interestingly, Conrad's (2008) morphological analysis did recover phrynosomatids as the sister taxon of a clade containing all other pleurodonts, although acrodonts were nested within this clade as well. Noonan and Chippindale (2006) provided strong Bayesian (but weak ML bootstrap) support for *Sceloporus* as the sister taxon of all remaining representative pleurodonts based on nuclear and mtDNA data. A similar but weakly supported result was found in the expanded study of Noonan and Sites (2010). In each of these two studies, however, the monophyly of Phrynosomatidae was strongly rejected, apparently because the putative “*Phrynosoma*” sequences used in both are from a crotaphytid (i.e., the *Phrynosoma* and *Crotaphytus* are identical in Fig. 3 of Noonan and Chippindale (2006)). Phrynosomatids are a diverse clade of pleurodonts whose members (particularly *Sceloporus*) have received much attention from comparative biologists (e.g., Harmon et al., 2003; Leaché and Sites, 2009; Wiens, 1999, 2000). Thus, determining the relative phylogenetic position of this clade is of great interest for phylogeny-based comparative studies.

#### 4.2. Biogeographic implications

Our phylogenetic results suggest an intriguing biogeographic pattern in which the relatively basal pleurodont clades occur predominantly in North America and Central America (Crotaphytidae, Phrynosomatidae, Iguanidae), and more nested taxa are associated with South America and lower Central America. The oldest pleurodont fossils are from northern Asia (Conrad and Norell, 2007) and North America (Conrad et al., 2007), which is consistent with a scenario of Northern Hemisphere origin and later expansion to the south. Our Lagrange analysis also supports this interpretation, and thus corroborates the general hypothesis of Borsuk-Bialynicka and Alifanov (1991) and Gao and Hou (1995) based on multiple ancient pleurodont fossils from Asia and North America. This hypothesis stands in sharp contrast to the traditional view of pleurodont biogeography (e.g., Estes, 1983), which posits a South American origin and later northward dispersal. We note that the oldest North American pleurodont fossil (i.e., *Afairiguana avius*, Early Eocene) was inferred to be most closely related to the South American leiosaurids based on morphological data (Conrad et al., 2007), but a more definitive assessment of the phylogenetic placement of this important fossil should await a combined analysis of the molecular and morphological evidence (e.g., Wiens et al., 2010). Further, the age of this fossil is not inconsistent with a North American origin for crown pleurodonts.

Our placement of liolaemids as the sister taxon of leiosaurids + oplurids is also biogeographically intriguing, as both liolaemids and leiosaurids occur primarily in austral South America. Our estimate for the divergence between the South American leiosaurids and Malagasy oplurids is 53 Mya (95% HPD = 44–62 Mya), which differs considerably from the estimate of 90 Mya (95% confidence interval [CI] = 67–118 Mya) by Noonan and Chippindale (2006). Furthermore, our estimate is more recent than any of the land connections to Madagascar cited by Noonan and Chippindale (2006). Interestingly, Ali and Aitchison (2009) cast doubt on the idea that a complete landbridge ever existed between Antarctica and Madagascar, suggesting that some overseas dispersal was inevitable. However, Antarctica has clearly served as a conduit for interchange between South America and Australia for several vertebrate groups (e.g., Darst and Cannatella, 2004; Harshman et al., 2008; Krenn et al., 2005; Nilsson et al., 2010; Wiens et al., 2006b), including some of similar age to our estimates for leiosaurids and oplurids (e.g., ~50 Mya for pelodyadine hylids; Wiens et al., 2006b). Thus, Antarctica seems likely to have connected ancestral leiosaurids and oplurids as well.

#### 4.3. Comparison of divergence times

Estimated divergence times for iguanian taxa have varied widely in recent molecular phylogenetic studies. Okajima and Kumazawa (2009) estimated the iguanian root at ~200 Mya using whole mitochondrial genomes. These authors further estimated the pleurodont root at ~160 Mya and, because oplurids were the sister taxon of all other pleurodonts in their tree, they proposed Gondwanan vicariance to explain their disjunct distribution. Okajima and Kumazawa (2010) estimated basal acrodont divergences to be considerably more recent (~100 Mya), again using mitogenomic data.

Wiens et al. (2006a) and Hugall et al. (2007) both used a penalized likelihood approach (Sanderson, 2002) to estimate divergence times with data from the nuclear protein-coding gene *RAG1*. Though these studies used different calibration points, they found similar ages for iguanians (146 and 142 Mya, respectively), pleurodonts (75 and 79 Mya), and acrodonts (79 and 80 Mya), all of which are substantially younger than the mitochondrial estimates (Okajima and Kumazawa, 2009, 2010). Relative to the nuclear-based studies, our age estimates using 29 nuclear loci with BEAST are somewhat younger for Iguania (123 Mya if the putative fossil acrodont *Bharatagama* is excluded), roughly the same for Pleurodonta (73 Mya), and somewhat older for Acrodonta (93 Mya).

Noonan and Chippindale (2006) and Noonan and Sites (2010) used dated phylogenies constructed from multiple nuclear loci to investigate biogeographic origins of the Malagasy oplurids and Fijian *Brachylophus*, respectively. They found considerably older iguanian divergences than any of the other nuclear DNA-based studies. In fact, their inferred ages for Iguania and Pleurodonta (~200 Mya and 160 Mya, respectively) were on par with the mitochondrial estimates (Okajima and Kumazawa, 2009), and their estimate for Acrodonta was even older (~150 Mya). However, this may be an artifact of a misidentified sequence. One of the calibration points used in both studies was a “*Crotaphytus–Phrynosoma*” split constrained to be >13 Mya. As noted previously, the “*Phrynosoma*” sequences used in these studies were almost certainly from a crotaphytid. This artifact (i.e., negligible sequence divergence corresponding to a 13 Mya divergence event) presumably pushed divergence estimates to be considerably earlier than they otherwise would have been. This hypothesis is also supported by the supplementary dating analyses of Noonan and Sites (2010), which used only deep vertebrate calibration points and estimated much more recent divergence times.

Finally, Torres-Carvajal and de Queiroz (2009) analyzed mitochondrial data from the *ND2* region to estimate divergence times within hoplocercid iguanians using BEAST (Drummond and Rambaut, 2007). These authors used no fossil calibrations, but instead used an empirical *ND2* rate estimate of 0.65% divergence per lineage per million years (Macey et al., 1998) to set the branch rate prior in BEAST. Interestingly, although the mean estimates for the age of the *Enyalioides–Morunasaurus* split differ between Torres-Carvajal and de Queiroz (2009) and the present study (27.4 and 13.3 Mya, respectively), the 95% HPDs for this node do overlap, despite the very different datasets and calibration methods employed.

#### 4.4. Influence of individual genes and hidden support on the concatenated tree

We found interesting results regarding the ability of the 29 loci and Bayesian and likelihood methods to strongly resolve the ancient, rapid radiation of pleurodonts, which may have important implications for all studies that try to resolve similar groups. In terms of concatenated Bayesian PP, we recovered a remarkably well-supported pleurodont tree. Seven of ten (70%) suprafamilial

nodes were supported by  $PP > 0.95$  (Fig. 3), a vast improvement over all previous studies. However, given the extremely short internodes at the base of the pleurodont tree (Fig. 3 inset), the marked disparity between ML and Bayesian support values at several nodes, and the potential for inflation of Bayesian support values (Cummings et al., 2003; Simmons et al., 2004), some caution is warranted.

Our supplemental analyses allowed us to explore the breadth and nature of this support in more detail. The results of the single-gene and supplemental concatenated Bayesian analyses make intuitive sense for the leiosaurid–oplurid clade (Node 5 of Fig. 3). Most individual genes showed some tendency to support this node (Table 3), and even when only the 13 genes showing the lowest levels of support for this clade were included (each with  $PP < 0.07$ ), the resulting analysis still found  $PP = 1.0$  for this clade. This is the pattern we would expect if many or all genes shared the same history, but some of them individually contained too little information relative to stochastic noise to support the node. This result thus potentially supports the idea that concatenation can overcome misleading homoplasy, thus allowing hidden support for a given node to emerge (Gatesy and Baker, 2005; Gatesy et al., 1999).

However, single-gene Bayesian analyses suggest that support is not widespread across the 29 loci for the five other well-supported higher-level pleurodont nodes. In fact, for all except the leiosaurid–oplurid clade, there was essentially no discernable support for these nodes from most of the individual loci (Table 3), despite their strong Bayesian support in the concatenated analyses. Furthermore, supplemental concatenated analyses in which we removed the few loci that showed some tendency to support these nodes likewise resulted in weak to nonexistent  $PP$  support (Table 4). The lack of support in these supplemental concatenated analyses was somewhat surprising, especially for Nodes 1 and 4 which were supported by MLBS of 88% and 91%, respectively (in addition to having Bayesian  $PP = 1.00$ ). Bootstrap values are fundamentally different from Bayesian posterior probabilities, and are generally more conservative (Alfaro et al., 2003; Wilcox et al., 2002). It was therefore somewhat unexpected that nodes with high support from both  $PP$  and MLBS in the main (29 loci) concatenated analyses would have such scant Bayesian support from the great majority of loci, both taken singly and as concatenated subsets encompassing the majority of the data. The fact that  $PP$  support becomes very low when only a few loci are excluded (Table 4) suggests that this small number of loci may be driving the strong support in the main analyses (although this does not necessarily mean that those nodes are therefore incorrect, see below).

At least part of the explanation may lie in the relative information content of each locus. The deep internodes in question are very short (Fig. 3 inset), and gene regions that have fewer base pairs and/or are relatively slowly-evolving may simply not contain enough variable sites to recover the shortest branches (i.e., not enough time between splitting events for mutations to accumulate without faster rates of change and/or longer sequences). Indeed, leaving Node 5 aside (the more widely supported leiosaurid–oplurid clade), Table 3 shows that if the 29 loci are ranked by their number of variable characters, the top seven genes (< 25% of the loci) account for >70% of the more strongly supported nodes (i.e., those with  $PP$  values shown in bold). Also, supplemental Bayesian concatenated analyses using only these seven loci strongly support Nodes 1, 4, and 5 (Fig. 5A). These are the only three interfamilial nodes strongly supported by both MLBS and  $PP$  from the 29-locus analyses (Fig. 3). Although relatively few loci seem to be providing the support for some of these key nodes, this does not mean that the support is misleading; it may simply reflect the greater suitability of these loci to resolve these difficult branches. Furthermore, all 29 loci almost uniformly provide very strong support for the pleurodont families (Table 2).

#### 4.5. Comparing species-tree and concatenated analyses for an ancient, rapid radiation

We found that concatenation produced a different and more strongly supported set of suprafamilial pleurodont relationships than did the coalescent-based species-tree approach (i.e., BEST), a pattern also seen in several other studies (e.g., Brumfield et al., 2008; Leaché, 2010; Thomson et al., 2008). Unfortunately, it is difficult to know which of these results better reflects the true evolutionary history of the group. For example, Edwards et al. (2007) showed through simulations that concatenation could result in strongly supported, incorrect trees. However, in their study BEST recovered the true topology with strong support, in contrast to our results (i.e., even though the true species tree is unknown, the estimated species tree is weakly supported at all suprafamilial nodes). Leaché (2010) suggested that lower support from explicit species-tree methods might be related to uncertainty in estimation of population-level parameters, whereas a concatenated analysis does not require estimating these parameters. The difficulty of accurately inferring such parameters may be greater with older radiations such as pleurodont iguanians (~70 Mya).

We hypothesize that including many loci that are not informative for very short branches may also impede accurate estimation using species-tree methods, such that a large number of loci may actually become a liability. Given that species-tree analyses are based on estimated gene trees, if a substantial number of loci are unable to resolve certain species relationships individually, a species-tree analysis including these loci might result in a weakly supported (and possibly incorrect) species tree (e.g., see simulation results in Heled and Drummond (2010) and Liu et al. (2008)). In contrast, in concatenated analyses there is the potential for “hidden support” to emerge, and for a few loci to drive the strong resolution of relationships even when most other loci are relatively uninformative.

This potential explanation seems consistent with the varying support for the leiosaurid–oplurid clade across our analyses. Although this clade was strongly supported by multiple individual loci, many others showed no discernable support (Table 3). Yet, when all genes were concatenated, all analyses recovered the clade with very strong support (Fig. 3). In contrast to the concatenated analysis, the 27-gene BEST analysis found low support for the clade (Fig. 6). Intriguingly, BEST analyses using only the top seven most-variable loci recovered the leiosaurid–oplurid clade with stronger support than did the 27-gene analysis ( $PP = 0.83$  vs.  $0.57$ ; Fig. 5B). It also recovered the liolaemid–oplurid–leiosaurid clade with relatively high  $PP$  (0.84). This clade was completely absent in the BEST analysis of all 27 loci, but strongly supported by all methods in the concatenated analysis. Taken together, these results suggest that including many loci that are too short or slow to resolve the shortest branches individually may lead to reduced support (and accuracy) for these branches in species-tree analyses. Thus, the weak support in our estimated species tree for pleurodons (and the conflict with the concatenated results) may reflect problems in the species-tree analysis, rather than erroneous concatenated results.

#### 4.6. Taxonomic implications and resurrection of *Dactyloidae* and *Istiurus*

Our results generally support recent higher-level classifications of iguanians, with the important exception of Polychrotidae. Analyses of morphological data have traditionally found a sister-taxon relationship between *Polychrus* and *Anolis* (e.g., Frost and Etheridge, 1989), but all published analyses of molecular data have contradicted this finding (Frost et al., 2001; Schulte and Cartwright, 2009; Schulte et al., 2003b; this study). Frost et al. (2001) defined

the family Polychrotidae to contain *Polychrus* and *Anolis* based on their morphology-only and combined DNA + morphology analyses, using direct optimization (via POY; Wheeler et al., 1996) in a parsimony framework. In contrast, Schulte et al. (2003b) failed to recover this clade with DNA-only (ML and maximum-parsimony [MP]) and DNA + morphology (MP) analyses, using conventional alignment methods and a much larger molecular data set.

Although Polychrotidae (*sensu* Frost et al., 2001) could not be rejected by the AU test, our results provide other convincing evidence for polyphyly of this clade, and suggest that partitioning of this family is necessary. Although the relationships of *Anolis* and *Polychrus* are not strongly resolved by all methods, the clade (*Polychrus* + *Anolis*) is completely absent from the posterior distributions of trees in all Bayesian analyses of the 29-locus concatenated data (BEAST, MrBayes, and Phycas), and is therefore strongly rejected in a Bayesian framework. This clade is also unsupported by the species-tree analysis. Further, the *Anolis*–*Polychrus* clade appears in only two of the 29 single-gene MrBayes trees (NGFB [PP = 0.5] and NKTR [PP = 0.15]), and only one of the single-gene RAxML trees (NKTR [MLBS = 17%]; see ESM for all trees), always with weak support. We think that unanimous agreement among genes and strong support from all methods is not a realistic expectation for higher-level pleurodont relationships. Given these results and those of previous studies, we prefer to correct the apparent polyphyly of Polychrotidae. We therefore limit the name Polychrotidae to include only the genus *Polychrus*, and resurrect the family name Dactyloidae for the genus *Anolis sensu* Poe (2004), i.e., including the anoline genera *Chamaeleolis*, *Chamaelinorops*, *Ctenonotus*, *Dactyloa*, *Norops*, *Phenacosaurus*, and *Semiurus* of Guyer and Savage (1986), following Article 40.1 of the ICZN. We note that the name Dactyloae (=Dactyloidae; Fitzinger, 1843) is a senior subjective synonym of Anolidae (Cope, 1864), and thus Dactyloidae has priority as the family-level name for this clade (though we acknowledge that Anolidae would be more intuitive). We also acknowledge the possibility that (at least in theory) future analyses might reveal *Anolis* and *Polychrus* to be sister taxa. However, even under this scenario, the new taxonomy that we propose would still be correct and would reflect well-supported, monophyletic groups.

Several molecular studies have also found strong evidence for non-monophyly of the agamid genus *Physignathus* (Hugall et al., 2008; Macey et al., 2000b; Schulte et al., 2003a), and our own analyses of 29 nuclear loci confirm this finding. The southeast Asian species *P. cocincinus* (Cuvier, 1829) is the sister taxon to the entire Australasian agamid radiation, which includes its sole congener, the Australian species *P. lesueurii* (Gray, 1831). The older *P. cocincinus* is the type species of *Physignathus*, and therefore should retain the genus name. Gray (1831) suggested the genus name *Lophura* for all species of *Physignathus*. However, *Lophura* had earlier been used to designate a genus of phasianid birds (pheasants; Fleming, 1822); this usage continues today and thus the name is unavailable. Duméril and Bibron (1837) placed both *P. cocincinus* and *P. lesueurii* in the genus *Istiurus*. This genus name is not currently used and is therefore available. We therefore resurrect the genus *Istiurus* with the sole species *Istiurus lesueurii*.

## 5. Conclusions

Iguanian lizards are one of the most diverse groups of squamates and have been the subject of numerous comparative evolutionary studies. Therefore, a well-supported higher-level phylogeny for this group is an important goal. For acrodonts, our study has provided strong support for almost every major clade. For pleurodonts, we have estimated a well-resolved tree from the 29 concatenated loci that is fully concordant between methods

and includes some relatively novel relationships. However, the support for some of the short interfamilial branches varies between methods, with strong support for nearly all branches using Bayesian methods and variable support from likelihood bootstrapping. Further, many of these inter-family relationships are discordant with a species-tree estimate (from BEST), which found uniformly weak support for alternative relationships. Our analyses of these patterns suggest that the support in the concatenated analysis for these interfamilial pleurodont relationships is determined by hidden support (in some cases) and a small number of faster-evolving loci (in others). Interestingly, our results suggest that including many loci that are too short or slowly-evolving to be informative for very short branches may be problematic for species-tree analyses, and this may have caused the estimates from the species-tree and concatenated analyses to differ in our study. Given these results, we suggest that analyses using species-tree methods should place a premium on including relatively long sequences from more variable loci, even if fewer loci are sampled (e.g., Heled and Drummond, 2010; Liu et al., 2008).

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.07.008.

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