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Contrasting global-scale evolutionary radiations: phylogeny, diversification, and morphological evolution in the major clades of iguanian lizards

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Parallel evolutionary radiations in adjacent locations have been documented in many systems, but typically at limited geographical scales. Here, we compare patterns of evolutionary radiation at the global scale in iguanian lizards, the dominant clade of lizards. We generated a new time-calibrated phylogeny including 153 iguanian species (based on mitochondrial and nuclear data) and obtained data on morphology and microhabitats. We then compared patterns of species diversification, morphological disparity, and ecomorphological relationships in the predominantly Old World and New World clades (Acrodonta and Pleurodonta, respectively), focusing on the early portions of these radiations. Acrodonts show relatively constant rates of species diversification and disparity over time. In contrast, pleurodonts show an early burst of species diversification and less-than-expected morphological disparity early in their history, and slowing diversification and increasing disparity more recently. Analyses including all species (with MEDUSA) suggest accelerated diversification rates in certain clades within both Acrodonta and Pleurodonta, which strongly influences present-day diversity patterns. We also find substantial differences in ecomorphological relationships between these clades. Our results demonstrate that sister clades in different global regions can undergo very different patterns of evolutionary radiation over similar time frames.

ADDITIONAL KEYWORDS: adaptive radiation – ecomorphology – evolution – reptile.

INTRODUCTION

Adaptive radiations are characterized by rapid speciation accompanied by extensive and correlated divergence in phenotypes and ecology (Schluter, 2000). Some authors have suggested that adaptive radiations collectively explain much of the diversity of life on Earth (e.g. Schluter, 2000). Given this view, adaptive radiation has become a major topic in evolutionary biology in recent years (e.g. Schluter, 2000; Gavrilets & Losos, 2009).

Many classic studies of adaptive radiation have focused on geographically localized radiations. Examples include the sticklebacks of northwestern North America (Schluter, 2000), the cichlids of the rift-lakes in eastern Africa (Meyer, 1993; Kocher, 2004), the finches of the Galapagos Islands (Grant, 1999), and the *Anolis* lizards of the Caribbean (Losos *et al*., 1998; Losos, 2009). A prominent feature of many of these systems is that they are characterized by parallel radiations that generate species with similar phenotypes and ecologies in geographically adjacent but allopatric locations (e.g. stickleback and cichlid ecotypes in adjacent lakes, *Anolis* ecomorphs on adjacent islands). However, few studies have tested whether radiations occurring at the global scale tend to occur in parallel or not. For example, are there similar patterns of species diversification over time? Are

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there similar patterns of morphological evolution? Were similar morphologies generated in each radiation, or are some unique? Are there similar relationships between morphology and ecology in each clade?

Here, we compare two global-scale clades of lizards and evaluate whether their radiations have been similar, in terms of their patterns of species diversification, morphological change, and ecomorphology. Iguanian lizards are arguably the dominant clade of lizards on the planet. With ~1600 currently described species, iguanians comprise one of the most species-rich lizard clades (~28% of all non-snake squamates), and are present and diverse in nearly every major biogeographical region (Uetz, Goll & Hallermann, 2012). Furthermore, although a few other clades have similar diversity (e.g. geckos and skinks with > 1000 species each; Uetz *et al*., 2012), most iguanians are diurnal, heliophilic, and frequently seen, whereas most other lizard clades are nocturnal (e.g. geckos), behaviourally cryptic (e.g. skinks), or else more geographically localized (e.g. cordylids, lacertids, teiids; Pianka & Vitt, 2003; Vitt & Caldwell, 2009). Iguanians include many common and well-known species and clades, such as chameleons, iguanas, anoles (*Anolis*), flying lizards (*Draco*), and spiny lizards (*Sceloporus*). Some iguanian clades have been considered to be adaptive radiations by many criteria (e.g. Pleurodonta; Losos & Miles, 2002; West Indian *Anolis*; Schluter, 2000; Losos, 2009), although testing which iguanian clades meet which criteria is not the focus of our study.

Iguanians are divided into two sister clades (Acrodonta, Pleurodonta) that are largely distinct biogeographically. Acrodonts occur exclusively in the Old World, and include the chamaeleonids of Africa, Madagascar, and adjacent regions, and the agamids, which are diverse in Africa, Asia, and Australia (Vitt & Caldwell, 2009). In contrast, pleurodonts occur almost exclusively in the New World (Vitt & Caldwell, 2009). Pleurodonts are currently divided into 11 families (Corytophanidae, Crotaphytidae, Dactyloidae, Hoplocercidae, Iguanidae, Leiocephalidae, Liolaemidae, Opluridae, Phrynosomatidae, Polychrotidae, Tropiduridae; Townsend *et al*., 2011), although traditional taxonomies recognized a single family (Iguanidae) for all these groups (as do some recent classifications; e.g. Schulte, Valladares & Larson, 2003). Although geographically separated in general, acrodonts and pleurodonts co-occur in Madagascar, where an extensive radiation of acrodonts (Chamaeleonidae) and a small radiation of pleurodonts (Opluridae) are broadly sympatric (Vitt & Caldwell, 2009).

Acrodonts and pleurodonts are generally similar in their overall ecology. For example, both are dominated by relatively small, diurnal, heliophilic, sit-and-wait predators that feed mostly on insects (Pianka & Vitt, 2003; Vitt & Caldwell, 2009). Also, both clades include species that are seemingly specialized for arboreal, terrestrial, and saxicolous microhabitats (Pianka & Vitt, 2003; Vitt & Caldwell, 2009). Thus, acrodonts and pleurodonts may represent parallel radiations, raising the question of whether these radiations have proceeded similarly in each case.

Previous studies have examined many aspects of the evolutionary radiation of iguanian lizards, but have not compared acrodonts and pleurodonts overall. For example, Losos & Miles (2002) used morphological data to test for adaptive radiation across the clades of pleurodont lizards. In a groundbreaking study, Harmon *et al*. (2003) compared patterns of diversification and morphological disparity over time in four clades of iguanian lizards [West Indian *Anolis* (Dactyloidae), the South American liolaemid genus *Liolaemus*, the North and Middle American phrynosomatid genus *Sceloporus*, and Australian agamids]. The relationship between ecology (microhabitat) and morphology has been studied in various clades of iguanian lizards, including agamids (e.g. Collar *et al*., 2010), phrynosomatids (e.g. Bergmann & Irschick, 2010), liolaemids (e.g. Schulte *et al*., 2004), tropidurids (e.g. Vitt *et al*., 1997; Kohlsdorf, Garland & Navas, 2001; Grizante *et al*., 2010), and *Anolis* (e.g. Losos *et al*., 1998; Losos, 2009).

Here, we focus on comparing the evolutionary radiations of pleurodonts and acrodonts. First, we build a new time-calibrated phylogeny for 153 iguanian species. Recent studies have either used few mitochondrial genes to address relationships of many iguanian species and genera (e.g. Macey *et al*., 2000; Schulte *et al*., 2003) or have used many nuclear loci to address relationships among a smaller set of species representing the higher-level clades (Townsend *et al*., 2011). We combine these data sets for the first time here to generate a higher-level phylogeny that includes both many species and many loci. Next we obtain data on morphological variation across 99 species from preserved museum specimens. We also obtain data on microhabitat use from the literature for most of these same species.

Using these phylogenetic and morphological data, we then test three main hypotheses regarding the radiation of pleurodonts and acrodonts. First, we test if patterns of species diversification (speciation – extinction) over time are similar in acrodonts and pleurodonts. Second, we test if patterns of partitioning of morphological disparity over time are similar for the two clades. Third, we test whether morphological variation is related to microhabitat usage in these two groups, if these groups differ in these ecomorphological relationships, and if acrodonts and pleurodonts differ significantly in their morphology

overall. We find that acrodonts and pleurodonts have undergone very different patterns of radiation: acrodonts show relatively constant rates of species accumulation and morphological disparity, whereas pleurodonts show a rapid and early burst of species diversification and temporally varying patterns of morphological disparity. We also show that these groups exhibit somewhat different ecomorphological relationships, despite the overall similarity in their morphologies.

MATERIAL AND METHODS

Phylogeny

To estimate the phylogeny, we combined the nuclear data from Townsend *et al*. (2011) with mitochondrial data from GenBank, mostly from Macey *et al*. (2000) and Schulte *et al*. (2003). The data from Townsend *et al*. (2011) come from 47 iguanian species, each having data for up to 29 nuclear loci (total aligned length of 24 501 bp). We included three species from this same data set from the closely related clade Anguimorpha for use as outgroups (*Heloderma suspectum*, *Shinisaurus crocodilurus*, and *Xenosaurus platyceps*), based on recent higher-level phylogenies from Townsend *et al*. (2004), Wiens *et al*. (2010), and many others. We obtained mitochondrial ND2 data for 156 ingroup and outgroup species, with a total aligned length of 1059 bp. We selected these species to represent as many genera as possible (and to include multiple species for some species-rich genera), and to include species for which nuclear data had been collected and for which morphological data could be obtained. After downloading sequences from GenBank, we removed adjacent tRNAs to facilitate alignment. Sequences were initially aligned using MUSCLE (Edgar, 2004), and alignments were then refined manually to account for amino acid translations and placement of codon positions.

We analysed the combined data matrix for 25 560 bp and 156 taxa in RAxML version 7.2.0 (Stamatakis, 2006). GTR (general time reversible) is the only substitution model implemented in RAxML, but this is also the most general model (all other models are special cases of GTR). We used GTR + Γ following Stamatakis (2006), given that the tree search method we used in RAxML uses 25 rate categories for Γ (instead of the usual four), and thus accounts for potentially invariant sites. We partitioned the data by gene-specific codon positions (90 partitions in total). The phylogenetic analysis used 200 bootstrap replicates combined with a heuristic search for the overall optimal tree every five bootstrap replicates (40 total), using the standard 'f a' option of RAxML. The best fitting tree had a likelihood of –296 781.8.

This analysis contained many species with extensive missing data (i.e. 69% had ND2 data only, and 96% missing data cells each). However, many analyses of real and simulated data suggest that it is possible for phylogenetic analyses to accurately place species with extensive missing data, especially if many characters are sampled overall (see recent review by Wiens & Morrill, 2011). Our results here support this idea. For example, we find that all species are placed in the major clades (families and subfamilies) expected by previous taxonomy and with strong bootstrap support. In addition, most aspects of the phylogeny that are weakly supported in our results (e.g. relationships among many pleurodont families) are also weakly supported in analyses with limited missing data (e.g. Townsend *et al*., 2011).

Divergence dating

We took two approaches to estimating divergence dates. First, we estimated divergence times using the penalized likelihood method in r8s, version 1.7 (Sanderson, 2002, 2003), using the phylogeny and branch lengths estimated from the maximum-likelihood analysis of the 29 nuclear loci and ND2 data. For the second approach, we used the ND2 data alone, and analysed the phylogeny and divergence times simultaneously using the Bayesian uncorrelated lognormal approach (Drummond *et al*., 2006) in BEAST version 1.5.4 (Drummond & Rambaut, 2007). Details of both methods are described in Appendix S1 in the Supporting Information.

For the first analysis, we assumed that missing data should have minimal impact on estimation of branch lengths (e.g. Wiens & Morrill, 2011), and so should not be problematic for penalized likelihood. For the second analysis, we were cautious about the possible impact of missing data on BEAST estimates, and used a gene for which all species had data (i.e. mitochondrial ND2). However, our results using penalized likelihood with 30 loci are generally similar to those using 29 loci with few missing data with BEAST (Townsend *et al*., 2011), whereas estimates based on mtDNA alone with BEAST and limited missing data gave more divergent dates. We therefore generally prefer and use the tree from the penalized likelihood analysis of 30 loci. In theory, we could have performed an analysis of the combined nuclear and mitochondrial data for all 153 taxa using BEAST, but preliminary analyses suggested that this would be problematic (i.e. very slow, given the large numbers of both characters and taxa).

Morphological data

We obtained morphological data for 99 species from 441 preserved museum specimens, representing all

iguanian families (see Appendix S2 for specimen numbers, sample sizes, and data). The 22 variables selected generally follow Losos & Miles (2002), and are as follows: (1) *Snout–Vent Length* (SVL), tip of snout to posterior edge of cloaca; (2) *Tail Length*, posterior edge of cloaca to tip of tail; (3) *Jaw Length*, anterior edge of external ear opening to centre of snout tip; (4) *Jaw Width*, distance between anterior edges of external ear openings; (5) *Head Depth*, top of cranium (between external ear openings) to underside of the mandible; (6) *Eye Length*, maximum length of the external eye opening; (7) *Nostril Height*, maximum height from the base of the nostril to the top of the nostril; (8) *Body Depth*, maximum depth, measured from sternum to dorsum; (9) *Body Width*, maximum width of body; (10) *Shoulder Width*, distance from posterior insertion of left forelimb to the posterior insertion of right forelimb; (11) *Hip Width*, distance from posterior insertion of left hindlimb to posterior insertion of right hindlimb; (12) *Shoulder–Hip Distance*, posterior insertion of forelimb to the posterior insertion of the hindlimb; (13) *Humerus Length*, distance from posterior insertion of humerus on the shoulder girdle to the apex of the elbow; (14) *Antebrachium Length*, apex of the elbow to base of digit 5 (outer edge, level with point between digits 4 and 5); (15) *Manus Length*, lateral insertion of digit 1 to the insertion between digits 3 and 4; (16) *Manus Width*, insertion point between digits 1 and 2 to the insertion point between digits 4 and 5; (17) *Longest (4th) Finger Length*, measured from the insertion point between digits 3 and 4, to the tip of the outstretched claw of digit 4; (18) *Shank Length*, anterior insertion of hindlimb to the apex of the knee; (19) *Crus Length*, apex of the knee to the posterior edge (heel) of the flexed foot; (20) *Foot Length*, posterior edge (heel) of the foot to the insertion point of digits 2 and 3; (21) *Foot Width*, maximum width of foot (including digit 1); and (22) *Longest (4th) Toe Length*, distance from insertion of digits 3 and 4 of pes to the tip of the outstretched claw. Measurements were taken to the nearest 0.01 mm with digital calipers. As much as possible, we selected specimens that appeared to be sexually mature males with unregenerated tails. This selection was done to avoid potentially biasing the results with sexual dimorphism, ontogenetic shape changes, or the reduced length of regenerated tails. These criteria caused us to exclude some specimens, and the sample sizes reported are only a fraction of the total number of specimens considered overall.

Morphological data were not available for some species that were included in the phylogenetic tree. In these cases, we generally obtained morphological data from a congeneric species that was included in the phylogeny. However, if no congener was available, the species could not be included in the comparative morphological analyses. Species for which microhabitat data were unavailable were also removed. The phylogeny used in the ecomorphological analyses ultimately included 90 species representing all iguanian families and subfamilies.

Microhabitat data

To test the relationships between morphology and microhabitat in a phylogenetic context, we assigned each species in the phylogeny (for which morphometric data were available) to a microhabitat category based on data from the literature. Microhabitat data (and corresponding references) are provided in Appendix S2. We were unable to find microhabitat data for a few species and these species were excluded from these analyses. We focused on the microhabitat in which each species is typically active (i.e. not where they rest). We initially focused on three categories of microhabitat use: arboreal (i.e. active in trees, bushes, and other vegetation), saxicolous (active primarily on rocks), and terrestrial (active on the ground). We also performed a separate set of analyses in which we included a semi-arboreal category (for species active in both arboreal and terrestrial microhabitats). Species that are active in both terrestrial and saxicolous microhabitats were treated as saxicolous given that there were few fully saxicolous species and that even partial use of saxicolous microhabitats might require morphological adaptations for using this microhabitat. Semi-arboreal species were treated as arboreal species in analyses where no semi-arboreal category was distinguished. One species (*Sceloporus angustus*) was recorded in all three primary microhabitats and was treated as semi-arboreal (taking into account two of three microhabitats used by this species) or as arboreal (given that all semi-arboreal species were classified as arboreal in the three-category analysis). We examined the influence of ecology on morphology using microhabitat as an independent variable, with three or four (including semi-arboreal) categories.

We used maximum-likelihood reconstruction (e.g. Schluter *et al.*, 1997; Pagel, 1999) to visualize the evolution of microhabitats across the tree. Maximum-likelihood reconstruction was implemented in Mesquite v. 2.74 (Maddison & Maddison, 2010), with the Markov k-state, one-rate model (a single rate for all transitions between all states), and coding each species as belonging to one of three states: 0 (arboreal), 1 (terrestrial), and 2 (saxicolous). Note that this analysis was used for visualization of evolutionary patterns rather than hypothesis testing.

Species diversification (Hypothesis 1)

We compared species diversification between acrodonts and pleurodonts using three approaches, testing the hypothesis that these clades underwent similar patterns of diversification. First, we visually compared plots of lineage accumulation over time for each of the two clades, using the R package *geiger* (Harmon *et al*., 2008, 2009). To avoid biasing the results by incomplete sampling of species, we focused on comparing the early parts of these radiations.

Second, we compared the fit of different models of overall diversification for each clade using the R package LASER (Rabosky, 2006). The models compared included two rate-constant models and three rate-variable models. The two rate-constant models were the pure-birth model (constant, positive, and non-zero speciation rate), and the birth-death model (including a constant non-zero rate of both speciation and extinction). The three rate-variable models were the density-dependent models [logistic (DDL) and exponential (DDX)], and a multi-rate model allowing for two different diversification rates over time (Rabosky, 2006). Under the DDL model, the speciation rate changes (slows) according to a standard logistic growth model depending on the initial speciation rate, the number of lineages at a specific timepoint, and a parameter analogous to the carrying capacity. Under the DDX model, the speciation rate is controlled by the magnitude of change of the initial rate depending on the number of lineages at a specific point in time. In the multi-rate models the speciation rates under pure-birth and birth-death models within a clade are assumed to differ before and after a breakpoint (Rabosky, 2006). For example, the Yule-2-rate model takes into account an initial speciation rate, a final speciation rate, and the moment in time where the rate shifts (breakpoint, optimized during model fitting). We determined the best-fitting model using the Akaike Information Criterion (AIC; Akaike, 1983), based on differences between AIC scores (ΔAIC). A difference of 4 or more was considered sufficient to prefer one model over another (Burnham & Anderson, 2002). We note that both approaches to diversification were necessary, given that two clades could share the same model but still show somewhat different patterns over time.

We performed tests for all iguanians (153 species) and for acrodonts and pleurodonts separately (77 and 76 species, respectively). Given our incomplete sampling of species but nearly complete sampling of major clades, we performed all analyses of diversification and disparity after removing the most recent 20% of the temporal history of the group, and then the most recent 50%. We performed analyses on the optimal tree and then on a set of 360 trees (i.e. from sampling a single tree every 100 000 generations from the posterior distribution of 36 million trees from BEAST; see Appendix S1), for which the mean and 95% confidence intervals were calculated. R code is provided in Appendix S3.

Third, we tested for shifts in diversification rates across iguanians using MEDUSA, a method that includes all species, even if they are not represented in the tree (Alfaro *et al*., 2009). We first estimated the total number of extant species in each major clade (i.e. Chamaelonidae, agamid subfamilies, and pleurodont families; Table S4.5) using Uetz, Goll & Hallermann (2012). Then, we pruned the phylogeny (Fig. 1) to include only these 19 major clades (Fig. S2). The MEDUSA function (in *geiger*) uses stepwise selection of subsequently more complex models (starting with a single birth and death rate for all lineages and adding one breakpoint representing a shift in these rates in each step). The minimum difference in AIC scores for retention of a more complex model was set at 4.

Morphological disparity (Hypothesis 2)

We tested the hypothesis that patterns of morphological disparity over time differ between acrodonts and pleurodonts, using the R package *geiger* (Harmon *et al*., 2008, 2009), and following the general methodology of Harmon *et al*. (2003). Morphological data consisted of mean species values of the 22 characters described above. Data were log-transformed to better meet the assumptions of normality. We compared disparity over time with a null-distribution generated from 1000 simulations of the morphological data on the phylogeny assuming a Brownian motion model. Pairwise disparity values were calculated by finding the average mean-squared Euclidean distance among species pairs. The relative disparity index (RDI) was calculated as the within-subclade disparity relative to among-subclade disparity at a given point in time (where among-subclade disparity is calculated by averaging over all subclades whose lineages were present at that point in time). The RDI was then plotted against time and compared with the expected values given the simulated data. We examined morphological disparity patterns for all sampled iguanians ($N = 90$ species) and for pleurodonts and acrodonts separately (48 and 42 species, respectively). R coding is provided in Appendix S3.

We acknowledge that this analysis is potentially influenced by incomplete sampling of species within clades. However, our primary purpose here is to compare patterns of disparity between acrodonts and pleurodonts, and our level of sampling is similar between these two clades (i.e. all families and subfamilies and most genera have been included from both clades, with similar numbers of species sampled

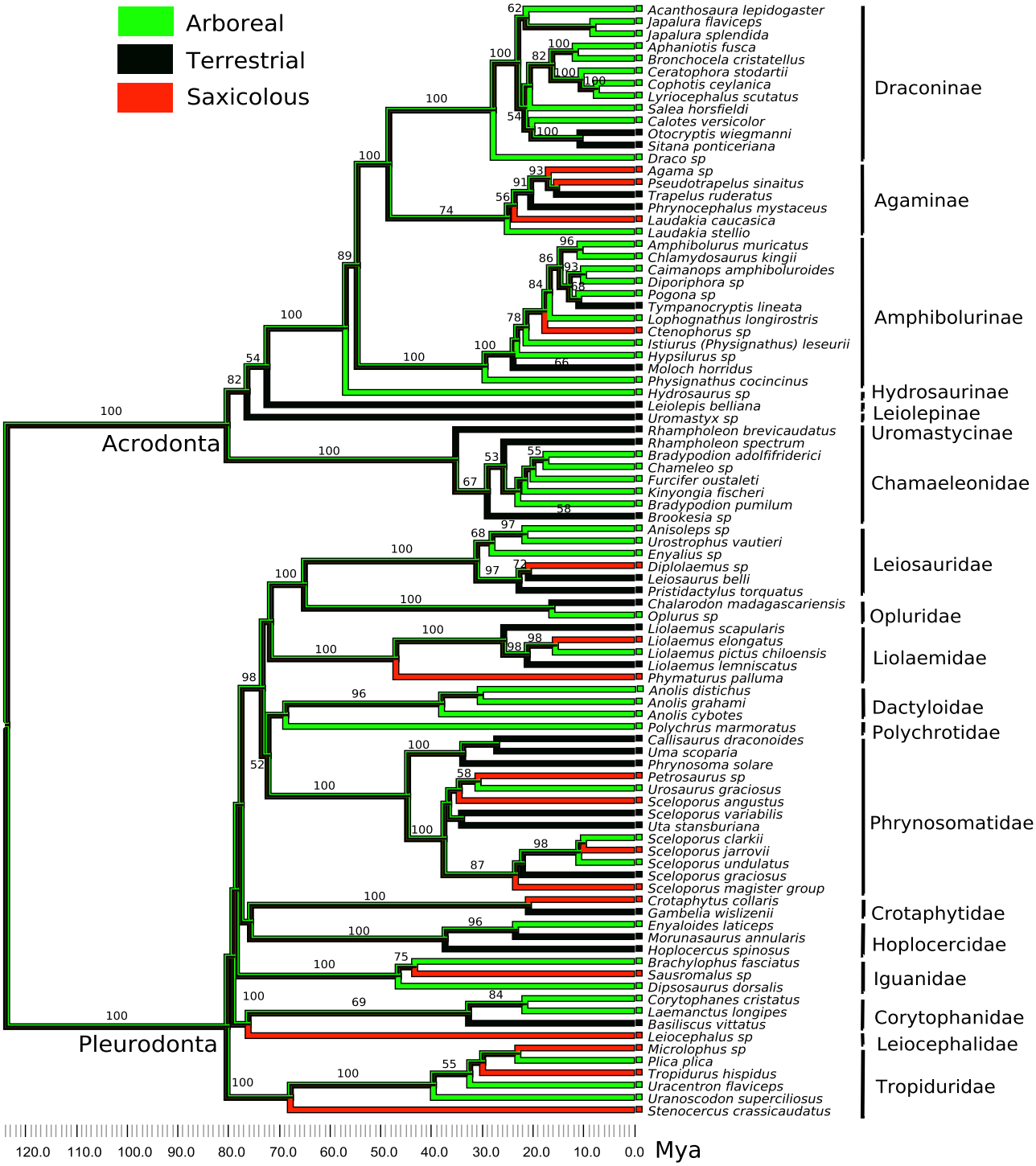

Figure 1. Evolution of microhabitat use in iguanian lizards, shown on a time-calibrated phylogeny based on up to 30 loci per species. Numbers adjacent to branches indicate likelihood bootstrap values > 50%. Branch colours represent microhabitat use: terrestrial (black), arboreal (green), saxicolous (red). Equivocal states are indicated by multicoloured branches. Ancestral states for habitat use were estimated using maximum likelihood under the Markov k-state, one-rate model.

in each). Additionally, the measure of morphological disparity we use is thought to be generally insensitive to sample size (Ciampaglio, Kemp & McShea, 2001).

Ecomorphological relationships (Hypothesis 3)

We used phylogenetic comparative methods to test whether size and shape were associated with microhabitat use in iguanian lizards, and whether these ecomorphological relationships differed in the two clades. We initially removed the effect of size on the morphometric variables using Burnaby's (1966) method, following Adams & Rohlf (2000). Using this approach, the species–morphology matrix containing the log-transformed morphological data was projected on the isometric size vector and returned to the original coordinate system according to the equation

$$X(I - \mu(\mu\mu')^{-1}\mu'),$$

where X is the data matrix, I is the identity matrix, and μ represents the isometric growth vector (Burnaby, 1966; McCoy *et al*., 2006). This method is considered superior to other approaches for removing size from shape data, such as considering size to be the first principal component of a principal components analysis (McCoy *et al*., 2006).

After size-correction, we included the 22 morphological variables in a principal components analysis (PCA), using the *pcaMethods* package in R (Stacklies *et al*., 2007). The PCA was performed on the covariance matrix and 21 axes were retained (note that PC22 has zero-variance in the case of 22 size-corrected variables).

For comparison, we also used phylogenetic PCA (Revell, 2009). Species scores from this analysis still need to be analysed using phylogenetic comparative methods (Revell, 2009). R coding for implementing this and other approaches is provided in Appendix S3.

We used the phylogenetic generalized least squares method (PGLS; Martins & Hansen, 1997) to test the relationship between morphology and microhabitat use. The original non-phylogenetic size-corrected data were converted by multiplication with the variance–covariance matrix derived from the time-calibrated tree using the package *ape* in R (Paradis, Claud & Strimmer, 2004). This can be done under different models of evolution, including Brownian motion (BM) and Ornstein–Uhlenbeck (OU). Prior to conducting these analyses, we tested the fit of the multivariate shape variables to BM and OU models (using *geiger*; Harmon *et al*., 2009), and found that OU models had better fit (results not shown). However, analyses using a BM model gave highly similar results (not shown).

The effects of size were represented by the geometric mean vector obtained after the Burnaby size-correction, and PC1–PC21 represented shape (Table 1). In the case of phylogenetic PCA, PC1 was interpreted as size and PC2–PC22 as shape. Several methods have been used to select the number of PCs for interpretation (e.g. Jollife, 1986; Jackson, 1993). Here, we used three approaches. The main results used PC1–PC11 (together explaining 95% of the total variance). Additionally, we used the broken-stick criterion (which selects PC1 only), and the set of variables that collectively explain 75% of the variance (PC1–PC3). Below, we use PC1–PC11 and size-corrected variables in a traditional PCA.

We first examined the effect of microhabitat use categories on the selected shape PCs simultaneously by performing a multivariate PGLS with a forward selection scheme for the microhabitat categories (i.e. testing for significant differences in shape

Table 1. Comparison of models of diversification for the optimal likelihood tree (Fig. 1)

	Pure-birth	Birth-death	DDL	DDX	Yule-2-rate
20% cut-off					
Iguania	109.4779	111.4779	109.6869	110.4715	**104.6735**
Pleurodonts	99.9379	101.3475	101.9380	101.8657	**94.2954**
Acrodonts	63.4332	62.0967	65.4333	64.6614	61.5063
50% cut-off					
Iguania	79.2924	81.2924	**72.5952**	79.9685	**71.1035**
Pleurodonts	57.5608	59.5608	**20.8408**	41.8693	43.7255
Acrodonts	27.5359	29.5359	27.2777	27.4087	29.1948

The AIC for each model is shown, including rate-constant (pure birth and birth-death), rate-variable (logistic and exponential density-dependent: DDL and DDX, respectively) and the two-rate (Yule-2-rate) models. Model fit was performed for all 153 iguanian species (77 acrodonts and 76 pleurodonts) for the 20 and 50% cut-offs (fitting diversification models on the first 80 or 50% of the time since origin, respectively). Strongly supported models are in bold type (i.e. the AIC differs by ≥ 4 from all other models).

between species in different microhabitat categories). As no R package was readily available for multivariate PGLS, we used a code that allows for multivariate X and Y variables (previously developed by Blankers, Adams & Wiens, 2012; see also Appendix S3). In this function, both predictor and response matrices are corrected for phylogenetic non-independence and the newly derived matrices are implemented in a MANOVA. The degrees of freedom (d.f.) are calculated as $p(N - k)$ and $p\ k$, with p as the number of predictor variables, k as the number of response variables, and N as the sample size, for the denominator and numerator d.f., respectively.

In addition, we examined how individual PCs covary with microhabitat. For this latter analysis, we used the first three PCs as separate response variables in a PGLS with the forward selection scheme for the microhabitat categories. The first three PCs describe variation in body, tail, and limb proportions (see Results).

PGLS was implemented in the R packages *CAIC* (Orme *et al*., 2009) and *nlme* (Pinheiro *et al*., 2011). *CAIC* estimates the strength of phylogenetic signal, and *nlme* estimates the expected variance–covariance matrix under a given evolutionary model (i.e. BM or OU). The best-fitting model was selected using the AIC. The alpha parameter for the OU models was calculated using maximum-likelihood optimization. In general, OU models fit the data better than BM models (Appendix S5, Table S5.4) but both models gave similar results (Appendix S5, Table S5.5).

We tested the robustness of all PGLS results by examining each relationship using 360 alternative trees (from BEAST) and calculating 95% confidence intervals of the statistics (using the model with the lowest AIC on the optimal tree). We also repeated analyses after omitting Chamaeleonidae, given preliminary results showing that this family is highly divergent morphologically from all other iguanians (see Results).

We also performed a series of analyses to test whether acrodonts and pleurodonts generally differ in their morphology. For these analyses we used the clade categories as the independent variable, and tested for significant differences among species in these clades using multivariate shape with PGLS (as described above). Again, we conducted analyses both with and without Chamaeleonidae.

RESULTS

Phylogeny and divergence times

Our phylogenetic results (Fig. 1) are generally similar to those from previous studies of iguanian phylogeny, with some exceptions. Within Acrodonta, relationships among major clades are generally strongly supported and congruent with other recent analyses (e.g. Townsend *et al*., 2011). Chamaeleonids are sister to agamids, and within agamids, we find Uromastycinae weakly supported as sister to all other agamids, and Leiolepinae as sister to all agamids excluding Uromastycinae (as in Townsend *et al*., 2011). Hydrosaurinae is strongly supported as sister to the remaining agamids, and Amphibolurinae is strongly supported as sister to Agaminae and Draconinae (as in Townsend *et al*., 2011).

Within Pleurodonta, monophyly of the currently recognized families is strongly supported, but the relationships among them generally are not (Fig. 1). However, we do find a strongly supported clade that includes oplurids, leiosaurids, liolaemids, phrynosomatids, dactyloids, and polychrotids. This clade is not present in the tree from Townsend *et al*. (2011). Instead, that study shows strong support for phrynosomatids as the sister group to all other pleurodonts. Both studies show strong support for a clade including leiosaurids and oplurids. We note that our analysis contains the same data as Townsend *et al*. (2011) but with the addition of more taxa and more characters (mtDNA). Our unpublished likelihood analyses of 44 nuclear loci also support the clade of oplurids, leiosaurids, liolaemids, phrynosomatids, dactyloids, and polychrotids (J. J. Wiens *et al*., unpubl. data).

Our estimates of divergence dates (Fig. 1) are also generally similar to those of Townsend *et al*. (2011). In that study, the age of the first split within acrodonts is estimated to be ~90 Mya and pleurodonts ~75 Mya. Our analysis of all 30 loci using penalized likelihood show the first split within both clades to be ~80 Mya. In general, most clade ages estimated here (e.g. for families and subfamilies) are within ~10 Myr of those from Townsend *et al*. (2011). Importantly, both studies show a fundamental difference in the timing of splitting events between acrodonts and pleurodonts: in acrodonts, the splits between the major clades (families and subfamilies) occurred over ~30 Myr, whereas in pleurodonts, nearly all major splits occurred over less than 10 Myr (except the more recent leiosaurid–oplurid clade).

Our analyses of divergence dates using BEAST and mtDNA-data only show considerable uncertainty in the phylogeny and some differences in divergence dates (Supporting Fig. S1). For example, even though most major clades (families and subfamilies) remain strongly supported in acrodonts and pleurodonts, many relationships among major clades that were strongly supported by all the data are uncertain in analyses of mtDNA data alone. Furthermore, the divergence dates in acrodonts are somewhat older and those in pleurodonts somewhat younger. These differences and uncertainties in the phylogeny and divergence dates (relative to the penalized likelihood analyses) allow us to evaluate the sensitivity of our

comparative analyses to variation in the estimated topology and divergence dates.

Lineage diversification (Hypothesis 1)

Patterns of diversification differ in acrodonts and pleurodonts, based on plots of lineage accumulation over time for the early part of their evolutionary history (Fig. 2A, B). In acrodonts, there is initially a relatively slow and steady accumulation of lineages over time (with more rapid diversification subsequently), whereas in pleurodonts there is an initial burst of rapid diversification (with more gradual lineage accumulation subsequently).

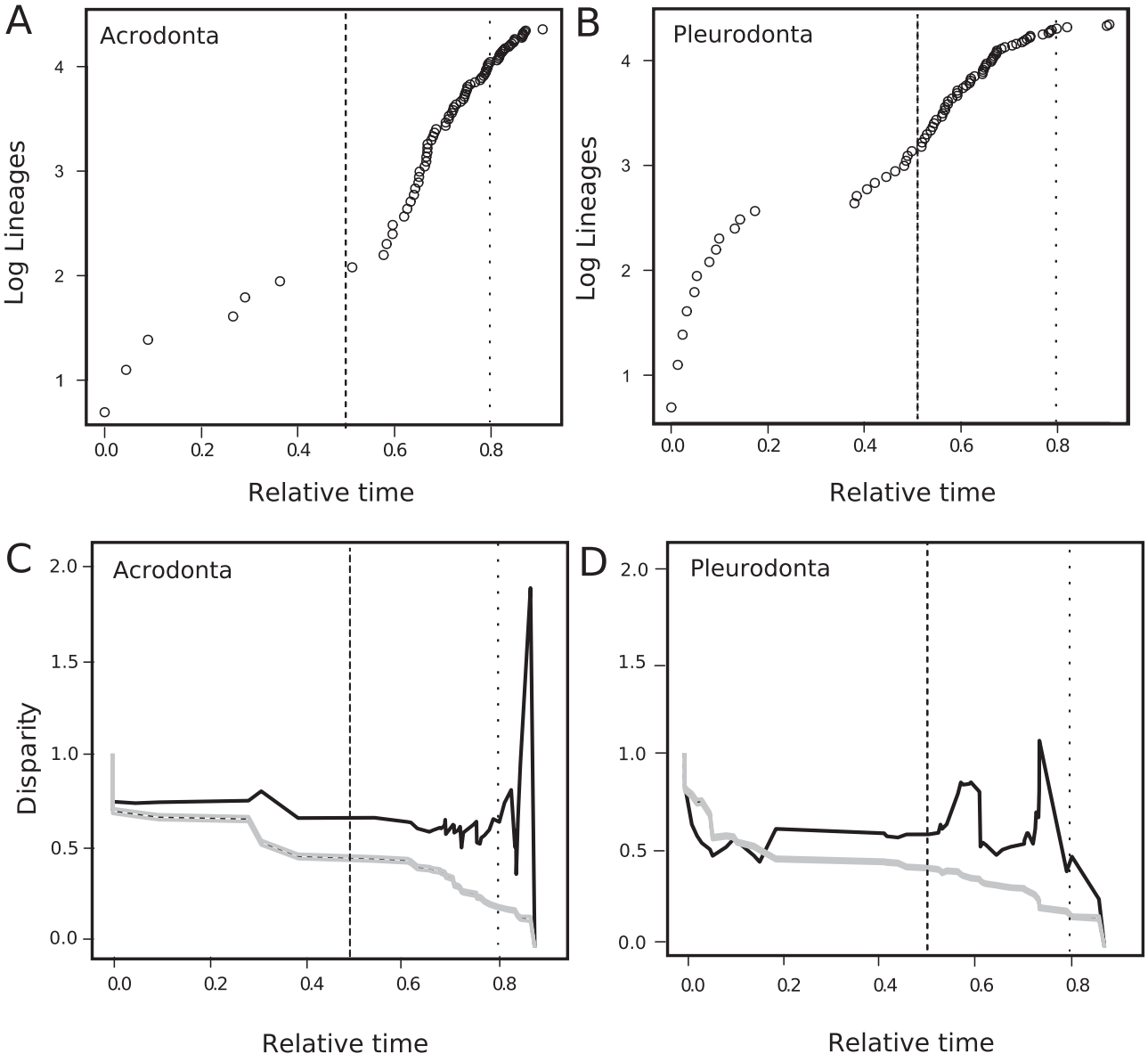

Figure 2. Lineage-through-time (LTT) and disparity-through-time (DTT) plots for pleurodont and acrodont iguanian lizards. LTT plots (A and B) show the logarithm of the cumulative number of lineages through time (relative to the timing of the earliest split within acrodonts and pleurodonts). DTT plots (C and D) show the indices of the actual relative disparity through time (solid line) and of the expected disparity based on 1000 simulations under the Brownian motion model of evolution (broken line), along with lines (solid, grey) indicating the 95% confidence intervals associated with the simulations. The dashed vertical lines indicate the 50% time-slice and the dotted vertical lines indicate the 20% time-slice, which were used to account for incomplete sampling of more recent clades.

Similar patterns are found based on direct comparisons of the fit of different models of diversification. Within Acrodonta (Table 1), a rate-constant model shows similar support to a two-rate or rate-variable model (excluding the most recent 20 or 50% of the clade's history). These results are supported by robustness analyses on the 360 BEAST trees (Appendix S4, Table S4.4). In contrast, within Pleurodonta (Table 1), there is strong support for a rate-variable model with a high initial rate followed by a lower rate (best rate-constant model: AIC = 99.94; Yule 2-rate model: AIC = 94.30) for the earliest 80% and for a logistic growth model (best rate-constant model: AIC = 57.56; DDL model: 20.84) for the earliest 50%. Similar results are found across the 360 trees, although no model was strongly favoured (i.e. all AIC scores differed by less than 4; Appendix S4, Table S4.4).

Using MEDUSA (which incorporates all species, including those not included in the phylogeny), we find two shifts in diversification rates across iguanians, corresponding to increased rates in the pleurodont clade containing Phrynosomatidae, Polychrotidae, Dactyloidae, Liolaemidae, Opluridae, and Leiosauridae, and in the agamid subfamilies Amphibolurinae, Agaminae, and Draconinae (Table S4.6; Fig. S2). These two clades include the majority of pleurodont and acrodont species, respectively (Table S4.6). Importantly, in contrast to the previous two methods that focused on the early history of these groups, this approach suggests that accelerated diversification later in the evolutionary history of both acrodonts and pleurodonts dominates present-day diversity patterns in both clades.

Morphological disparity (Hypothesis 2)

Patterns of accumulation of morphological disparity over time also differ between acrodonts and pleurodonts (Fig. 2C, D). Acrodonts show high and relatively constant levels of disparity over time (higher than expected from simulations), indicating that subclades within Acrodonta contain a substantial proportion of the total variation in the group (Fig. 2C). In contrast, pleurodonts show less disparity than expected early in their history, indicating that there was initially more variation among subclades than within subclades than expected, but disparity within subclades increased subsequently, especially during the second half of the history of the group (Fig. 2D). Overall, disparity is higher in acrodonts (including chameleons: 12.10; excluding chameleons: 10.12) than pleurodonts (6.83).

Ecomorphological relationships (Hypothesis 3)

We first test for overall relationships between morphology and microhabitat across Iguania, and then test whether relationships between ecology and morphology differ between pleurodonts and acrodonts. Across Iguania, species generally cluster based on microhabitat (Fig. 3) and are separated in morphospace (PC1 and PC2) mostly based on tail length (strong negative loading), body width, and limb size (both positive loadings; Table 2). Species with low scores on both PC1 and PC2 generally occupy arboreal microhabitats, while species with high scores on both these PCs are generally terrestrial or saxicolous (but chamaeleonids have high scores on PC1 and low scores on PC2, regardless of microhabitat). Arboreal species tend to have elongated tails, reduced hip and shoulder widths, and narrower bodies. Terrestrial species tend to have shorter tails, wider shoulders and hips, and wider bodies (Fig. 3; Table 2).

Across Iguania, multivariate analysis (using PC1–11, PGLS, and the optimal tree) shows a strong association between shape and microhabitat use (Table 3; $F_{33,237} = 1.9145$, $P = 0.0031$). The results are robust across 360 alternative trees (Appendix S4, Table S4.1). Similarly, we find a significant relationship between microhabitat categories and size ($F_{2,87} = 6.8785$,

Table 2. Eigenvalues, percentage of variance explained, and loadings for the 22 morphological variables on PC1–PC3

Variable	PC1	PC2	PC3
% variance explained	48	20	6
Eigenvalue	0.4838	0.2047	0.0634
Snout–Vent Length	0.066	–0.020	0.064
Tail Length	**–0.457**	**–0.602**	**0.513**
Jaw Length	0.058	–0.042	–0.181
Jaw Width	0.047	0.147	–0.022
Head Depth	**0.206**	**–0.216**	–0.129
Eye Length	0.125	–0.122	**–0.277**
Nostril Height	0.160	0.088	–0.169
Body Depth	**0.220**	–0.155	–0.006
Body Width	–0.031	**0.479**	**0.359**
Shoulder Width	–0.003	**0.331**	0.165
Hip Width	0.006	**0.246**	0.118
Shoulder-Hip Distance	0.103	–0.014	0.185
Humerus Length	0.098	–0.127	**–0.222**
Antebrachium Length	0.127	–0.101	–0.046
Manus Length	0.057	0.064	0.151
Manus Width	**0.287**	–0.096	0.122
4th Finger Length	**–0.358**	0.167	**–0.224**
Shank Length	–0.073	–0.135	–0.189
Crus Length	–0.067	–0.056	**–0.203**
Foot Length	–0.113	–0.048	–0.059
Foot Width	0.140	0.045	**–0.301**
4th Toe Length	**–0.597**	0.166	**0.253**

Bold values indicate the variables with the strongest loadings ($|x| > 0.20$) on that axis.

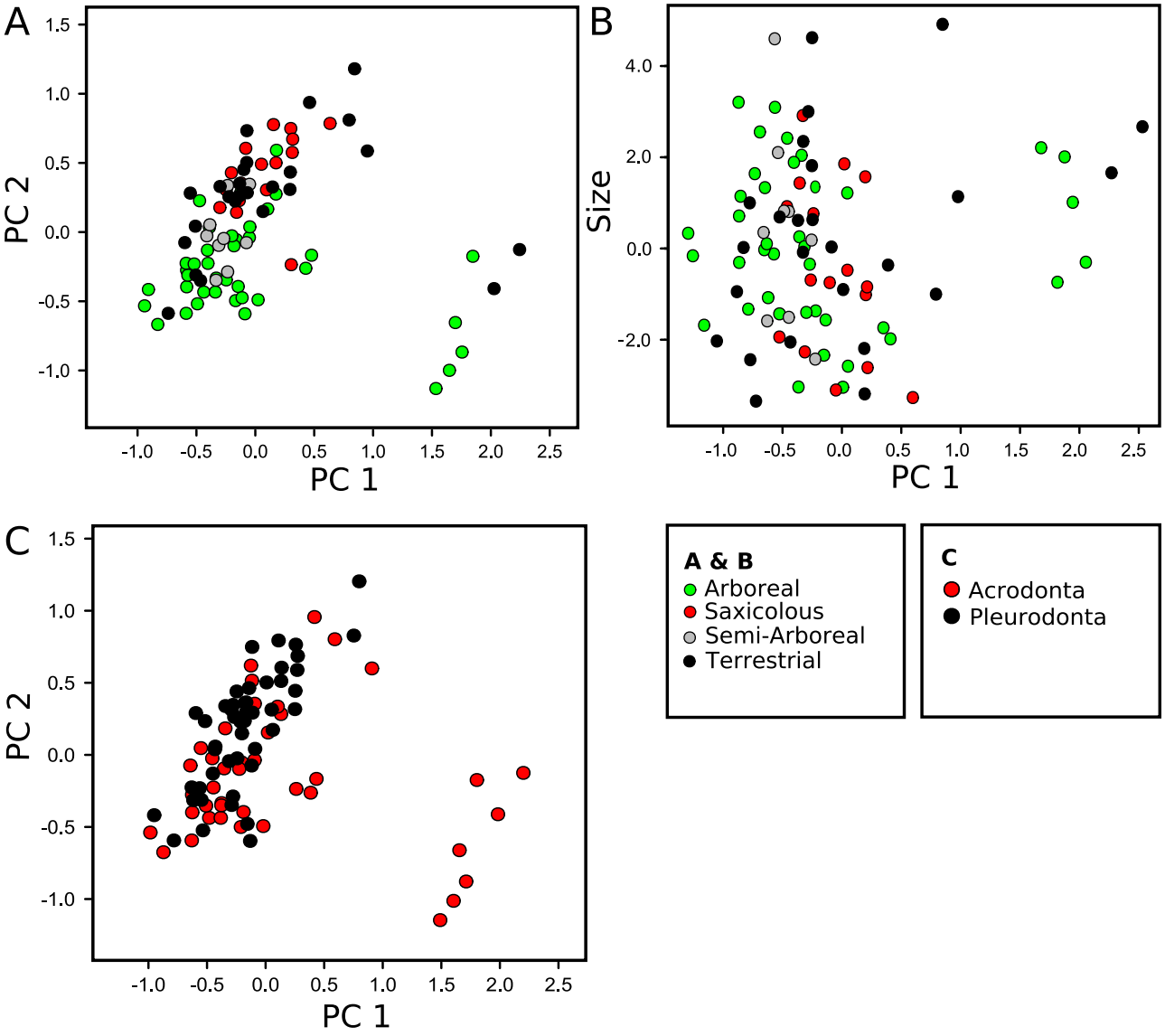

Figure 3. Morphospace distribution of iguanian lizards. (A) Distribution of 90 iguanian lizards (including chamaeleonids in the bottom right-hand corner) along the first two PCs (~68% of variance explained). Colours are based on microhabitat use (arboreal, green; saxicolous, red; semi-arboreal, grey; terrestrial, black). (B) As in (A) but showing morphospace distribution along PC1 and size (geometric mean vector). (C) Same as (A), except coloration is based on clade assignment: Acrodonta (red) versus Pleurodonta (black).

$P = 0.0017$). Examining individual PCs (Table 3) shows that morphological variation is related to microhabitat for both PC1 (explaining ~48% of the total variance and reflecting variation in tail length, head depth, body depth, manus width, digit length, fourth toe length; $F_{2,87} = 4.8451$, $P = 0.0101$) and PC2 (explaining ~20% of total variance, reflecting variation in tail length, body width, shoulder width, hip width, and head depth; $F_{2,87} = 17.6844$, $P = 0.0005$). These results are also supported when using a microhabitat classification scheme that includes semi-arboreal species as a separate category, using both the optimal tree (Appendix S5, Table S5.2) and 360 alternative trees (Appendix S4, Table S4.2).

We also conducted separate analyses on pleurodonts and acrodonts. The loadings of the morphological variables for pleurodonts and acrodonts (including and excluding chamaeleonids) are shown in Table S5.1 (Appendix S5). Within pleurodonts, we find no evidence for an association between microhabitat and multivariate shape (using PGLS and PC1–11; $F_{33,111} = 0.9228$, $P = 0.5919$; Table 3). However, we do

Table 3. Relationship between microhabitat categories and morphology

		Shape*	Size	PC1†	PC2‡	PC3‡
Iguania	*F* (num d.f., den d.f.)	1.9145 (33,237)	6.8785 (2,87)	4.8451 (2,87)	17.6844 (2,87)	1.4942 (2,87)
	P	**0.0031**	**0.0017**	**0.0101**	**0.0005**	0.2301
Pleurodonta	*F* (num d.f., den d.f.)	0.9228 (33,111)	4.5854 (2,48)	10.1684 (2,48)	0.0608 (2,48)	1.5951 (2,48)
	P	0.5919	**0.0154**	**0.0002**	0.9411	0.2141
Acrodonta	*F* (num d.f., den d.f.)	1.7113 (33,93)	3.9701 (2,39)	1.5086 (2,39)	9.6539 (2,39)	1.3987 (2,39)
	P	**0.0236**	**0.0270**	0.2338	**0.0004**	0.259
Acrodonta (excl. chamaeleonids)	*F* (num d.f., den d.f.)	1.4175 (33,66)	3.5782 (2,30)	6.2565 (2,30)	0.4055 (2,30)	5.2015 (2,30)
	P	0.1142	**0.0404**	**0.0054**	0.6702	**0.0115**

Multivariate PGLS was performed on PC1–PC11 which together explain > 95% of the morphological variance. *F*-statistics (degrees of freedom for the numerator and denominator shown in parentheses below as num d.f. and den d.f.), and *P*-values are shown, and *P*-values significant at the 0.05 level are shown in bold type.

*OU models have a higher fit than Brownian motion (BM) models for all multivariate analyses.

†PGLS using BM model (except for Pleurodonta for which an OU model was used).

‡PGLS using OU model (except for Pleurodonta for which a BM model was used).

find a significant association between microhabitat and morphological variation on PC1 ($F_{2,45} = 10.1684$, $P = 0.0002$), but not the other PCs. The relationship between microhabitat categories and size is also significant ($F_{2,48} = 4.5854$, $P = 0.0154$). Similar results are found using different scenarios of microhabitat assignment (Appendix S5, Table S5.2) and using 360 alternative trees (Appendix S4, Table S4.2).

In contrast, within Acrodonta, the multivariate analysis shows a significant association between microhabitat and shape ($F_{33,93} = 1.7113$, $P = 0.0236$; Table 3). The univariate analysis shows a strong relationship between microhabitat and PC2 ($F_{2,39} = 9.6539$, $P = 0.0004$), but not other PCs (Table 3). Again, these results are supported by the robustness analysis using 360 alternative trees (Appendix S4; Table S4.2) and an alternative microhabitat classification scheme (Appendix S5; Table S5.2). However, after excluding Chamaeleonidae (Table 3), multivariate shape shows no association with microhabitat ($F_{33,66} = 1.4175$, $P = 0.1142$), although both PC1 and PC3 show significant associations with microhabitat use (PC1: $F_{2,30} = 6.2565$, $P = 0.0054$; PC2: $P = 0.670$; PC3: $F_{2,30} = 5.2015$, $P = 0.0115$). These results were also supported using the alternative microhabitat assignments (Appendix S5, Table S5.2). A weak relationship between size and microhabitat was found both including ($F_{2,39} = 3.9701$, $P = 0.0270$) and excluding chamaeleonids ($F_{2,30} = 3.5782$, $P = 0.0404$), but this was not supported when a semi-arboreal microhabitat category was included in the analysis (Appendix S5, Table S5.2).

Using phylogenetic PCA instead of Burnaby size correction does not affect the results qualitatively (Appendix 5, Table S5.3). However, the number of PCs included does influence the results. Under the broken stick criterion (PC1 only) both pleurodonts and acrodonts excluding chamaeleonids show significant relationships between morphology and microhabitat use (Table 3; Appendix 5, Table S5.3). Under the 75% criterion (PC1–PC3), neither acrodonts nor pleurodonts show a significant correlation between shape and microhabitat, although *P*-values are only slightly above the 0.05 threshold ($P = 0.0682$ and $P = 0.0895$, respectively; Appendix 5, Table S5.3). Overall, morphology and microhabitat are generally related in iguanian lizards, but the details of this relationship differ between pleurodonts and acrodonts and depend somewhat on the methods used (Appendix 5, Table S5.3).

When we categorize species according to clades instead of microhabitat categories and test for differences in morphology, we find that acrodonts and pleurodonts are somewhat different, but not significantly so ($F_{22,158} = 1.5699$, $P = 0.0615$). This result is sup-

ported by the analysis with 360 alternative trees [mean $F_{22,158}$ (95% CI) = 1.5930 (0.0094), mean P (95% CI) = 0.0580 (0.0025)]. However, support for the association between clade membership and morphology becomes considerably weaker if chamaeleonids are removed ($F_{22,158} = 1.0912$, $P = 0.3631$; results for 360 trees in Table S4.3).

DISCUSSION

In this study, we show that closely related, ecologically similar clades in different global regions can undergo different patterns of evolutionary radiation over similar time frames. We compare the evolutionary radiations of the two major groups of iguanian lizards, which together make up the most species-rich clade of lizards. We find that the New World pleurodont clade and the Old World acrodont clade underwent different patterns of species diversification, morphological divergence, and ecomorphological evolution. Thus, although acrodonts and pleurodonts superficially appear to be parallel radiations, their radiations differ in many ways. We discuss these differences (and their potential causes) in more detail below.

Species diversification and morphological disparity

We find that acrodonts and pleurodonts show different patterns in species diversification (Figs 1, 2) and morphological disparity (Fig. 2) in their early evolutionary histories. What might explain these divergent patterns? Harmon *et al*. (2003) proposed a general hypothesis regarding evolutionary radiations that potentially links these patterns of species diversification and morphological disparity, based on their comparisons of four species-rich subclades within acrodonts and pleurodonts. Harmon *et al*. (2003) suggested that clades that showed early bursts of species diversification would also show greater among-clade than within-clade disparity in their morphology over the same time period (as we find in pleurodonts), mainly because groups with early bursts of radiation have filled or occupied the available niche space, which slows subsequent radiation. Also as predicted, acrodonts do not show an early burst of diversification, and consistently partition more of their morphological variation within clades than among clades (Fig. 2). Thus, our results support the general predictions of Harmon *et al*. (2003).

However, not all studies necessarily support these general patterns. In fact, analyses including dozens of animal clades suggest that early bursts of morphological evolution within clades are relatively rare (Harmon *et al*., 2010), even though such early bursts are predicted for adaptive radiations. Other analyses have shown evidence for slowing diversification over time, including large-scale meta-analyses (e.g. Phillimore & Price, 2008; Morlon, Potts & Plotkin, 2010), but most have not related these patterns of species diversification to morphological evolution. Many of the studies that have combined analyses of morphological evolution and diversification included only a single clade (e.g. Derryberry *et al*., 2011), rather than making comparisons between clades. Furthermore, some studies suggest that diversification and morphological evolution can be uncoupled (e.g. Adams *et al*., 2009). Finally, we note that considering the entire evolutionary history of iguanians (not just the early radiation of each clade) suggests that present-day diversity patterns are dominated by accelerated diversification within more recent clades.

Harmon *et al*. (2003) suggested that clades often show one of two general patterns of radiation in terms of diversification and disparity (as in acrodonts and pleurodonts), but the question remains as to why a given clade will show one pattern or the other. One potential explanation is that clades that show an early burst of diversification and disparity have many subclades occurring in sympatry, and clades with slower initial diversification have subclades that are geographically isolated from each other (Harmon *et al*., 2003). It is not clear to what extent iguanians overall fit this geographical pattern, as both iguanian clades show a mixture of geographically overlapping and non-overlapping subclades (based on range maps from Vitt & Caldwell, 2009, with additional information from Uetz *et al*., 2012). Specifically, within acrodonts, amphibolurines are geographically isolated in Australia, whereas mesic regions of Asia are dominated (in terms of regional species richness) by draconines, and more xeric regions of Asia and Europe are dominated by agamines. Chamaeleonids and agamines are broadly sympatric in Africa. However, other, less-diverse clades are also sympatric with these sub-clades, such as leiolepines and hydrosaurines in Asia and uromastycines in drier regions of Africa and Asia. In the New World, many diverse pleurodont subclades are also geographically isolated from each other (a pattern which is actually inconsistent with the hypothesis of Harmon *et al*., 2003), but there is also substantial overlap between subclades. For example, North and Middle America are dominated by phrynosomatids (although dactyloids are also diverse in Middle America). The Caribbean is dominated by dactyloids and leiocephalids. Northern South America is dominated by tropidurids and dactyloids, and southern South America is dominated by liolaemids and leiosaurids. These distributions suggest a rapid spread and diversification of iguanian clades across the latitudinal span of the New World,

with subsequent radiations in every major biogeographical region. But again, as in acrodonts, less diverse subclades are also broadly sympatric with these dominant subclades in each region (e.g. crotaphytids in North America, corytophanids and iguanids in Middle America, hoplocercids and polychrotids in northern South America). In summary, acrodonts and pleurodonts do not seem to show fundamentally different patterns of clade overlap, and so it is unclear if this geography-based hypothesis explains the divergent patterns of diversification and disparity in these two clades.

Interestingly, there may be similarly contrasting patterns of diversification in other groups of organisms that occur in the same regions. For example, in frogs, hyloids and ranoids are the dominant groups in the New World and Old World, respectively (Vitt & Caldwell, 2009). Analyses of time-calibrated phylogenies suggest that these groups are generally similar in overall age to pleurodonts and acrodonts (~60–100 Myr old), and that they show similar diversification patterns (e.g. Roelants *et al*., 2007; Wiens, 2007). Specifically, the New World hyloids (~60 Myr old) show a pattern of very rapid species diversification (leading to the origins of many family-level clades in a short time), whereas ranoids (~100 Myr old) show longer branches among their major clades (e.g. Roelants *et al*., 2007; Wiens, 2007). Thus, the rapid diversification of New World lineages in these two clades suggests a possible common explanation. However, one difference is that most major hyloid lineages are South American (i.e. either strictly endemic or originated there), whereas pleurodont lineages are more broadly distributed, with many that diversified outside South America (e.g. crotaphytids, corytophanids, iguanids, leiocephalids, phrynosomatids). In summary, our results document striking differences in the patterns of diversification and disparity in the two major clades of iguanian lizards, but the causes of these patterns remain somewhat unclear. Nevertheless, the potential for contrasting patterns of radiation in New World vs. Old World clades should be tested for in other groups, given the similar patterns in frogs and lizards.

Ecomorphological relationships

To our knowledge, relatively few studies have compared ecomorphological relationships between large-scale global radiations. Here, we find notable differences in ecomorphological relationships between acrodonts and pleurodonts (Table 3, Appendix 5). When considering individual PCs (PC1–PC3), acrodonts show a strong relationship between microhabitat and PC2 whereas pleurodonts show a strong relationship between microhabitat and PC1. Examining the contribution of individual morphological variables to each PC (Table 2; Appendix 5, Table S5.1) shows that aspects of morphology related to the stoutness of the body (body width, shoulder width, hip width) are especially related to microhabitat use within acrodonts (PC2), whereas morphology of the extremities (manus width, fourth finger length) appears more important to shape–microhabitat associations in pleurodonts (PC1). However, the ecomorphological relationships within these clades become far more similar when chamaeleonids are removed (i.e. both show no multivariate relationship between morphology and microhabitat, but significant relationships between microhabitat and PC1), or under some PC selection criteria.

The unique morphology of chameleons is a major driver of the distinct patterns of morphological evolution of these clades. Chamaeleonidae is one of the most morphologically unusual groups of tetrapods, both in the variables we measured (Fig. 3), and many others (e.g. horns and crests, turret-like eyes, mitten-like opposable digits, strongly prehensile tail, projectile tongue; Pianka & Vitt, 2003; Vitt & Caldwell, 2009). There is no morphological equivalent to the chamaeleonids within Pleurodonta, even if some lineages have evolved a few similar traits (e.g. Cuban *Anolis* formerly called *Chamaeleolis*; Leal & Losos, 2000). Yet, chamaeleonids are a major clade within Acrodonta that contains nearly one-third of all species. Thus, chamaeleonids strongly illustrate that there have not been fully parallel radiations of iguanian lizards in the New and Old World.

Limitations and caveats

Our study has some important limitations that should be emphasized. The most important is the limited sampling of species at lower taxonomic levels. Even though we included all major clades (and multiple species from many species-rich genera), iguanians contain >1600 species (Uetz *et al*., 2012). Clearly, additional work is needed on patterns of diversification, disparity, and ecomorphology across all iguanian species. However, we reduced the impact of limited taxon sampling on our analyses of diversification and disparity by focusing on the early stages of the acrodont and pleurodont radiations (i.e. excluding the most recent time periods) and by conducting diversification analyses that include all species (i.e. with MEDUSA). Furthermore, limited sampling of species need not necessarily impact estimates of correlation between traits (e.g. if a genus is morphologically specialized for arboreal microhabitats, the correlation between the morphology and microhabitat should be detected regardless of whether the number of species sampled from the genus is one or 100). In

fact, the weak relationship between morphology and microhabitat (e.g. within pleurodonts) might be due in part to combining so many diverse clades.

Another potential limitation of our study is the broad characterization of microhabitats in our ecomorphological analyses. For example, it is clear that there can be important differentiation in morphology within these broad microhabitat categories in iguanians (e.g. different arboreal ecomorphs in *Anolis*; Losos *et al*., 1998; Losos, 2009). Although we think that these broad categorizations were necessary for the large phylogenetic scale used here, more detailed studies within iguanian clades may reveal somewhat different patterns.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Results of Bayesian analysis of topology and divergence times using mitochondrial data (ND2). Numbers adjacent to nodes indicate posterior probabilities of clades, and horizontal purple bars indicate 95% highest posterior density intervals for clade ages. See Appendix S1 for detailed methods.

Figure S2. Pruned phylogeny showing major clades used for analyses of diversification with MEDUSA and results. Node labels correspond to Table S4.6 (node numbers 1–19 at tips not shown) and colours indicate inferred shifts in diversification rates (see Table S4.6).

Appendix S1. Methods for divergence-time estimation.

Appendix S2. Specimen numbers, morphometric and microhabitat data, and associated references.

Appendix S3. Codes for R-based analyses.

Appendix S4. Robustness analyses using 360 alternative trees.

Appendix S5. Additional analyses.