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When did anoles diverge? An analysis of multiple dating strategies

Cristian Román-Palacios[a,b,*], Jose Tavera[a], María del Rosario Castañeda[a,c]

[a] Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Universidad del Valle, A. A. 25360 Cali, Valle del Cauca, Colombia
[b] Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721-0088, USA
[c] Departamento de Ciencias Biológicas, Facultad de Ciencias Naturales, Universidad Icesi, Calle 18 No. 122-135, Cali, Valle del Cauca, Colombia

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ABSTRACT

Whereas most of the studies that discuss the evolutionary divergence of *Anolis* lizards have dated the clade's crown group in between 31 and 64 Ma, a single study has recovered a significantly older age for the same node (87 Ma). These differences also entail notable consequences on the preferred biogeographical hypothesis for the whole clade. Here we analyze a total of seven dating strategies by combining three calibration sources in independent BEAST runs to infer the most probable divergence timing for anole lizards (a mitochondrial rate for ND2 gene, the *Anolis dominicanus* fossil, and a group of fossils assigned to the Priscagamines, Iguanines, and *Idontosaurus* clades). Based on the estimated timing, we also addressed whether chronograms differ the most in deeper or shallower nodes by exploring the trend in the standard deviation of mean ages between chronograms across time. Next, we focus on the pattern for a single shallow node by hypothesizing the biogeography of the island-endemic Malpelo anole (*Anolis agassizi*), and evaluating the temporal congruence between the species' divergence and the island geology. The estimated set of ages suggests that anoles most likely diverged 72 Ma (71–73 Ma), with the crown group established around 58 Ma (51–65 Ma). Dispersal is therefore supported as the major driver in the biogeography of the group (and in Caribbean lineages in particular). Our analyses also indicated that (1) rate-based analyses pulled dates toward younger ages, (2) the differences in node ages between chronograms decrease towards the tips regardless of the position of the constrained node, and that (3) the estimated age for deep nodes (e.g. *Anolis* stem) is highly influenced when deep nodes are also constrained. The latter two results imply that the estimated age for shallower nodes is largely unaffected by the used temporal constraint. The congruence of all chronograms for the Malpelo anole also supports this finding. *Anolis agassizi* was found to have diverged before the emergence of Malpelo island in each analysis (anole: 19–31 Ma vs. Malpelo island: 16–17 Ma). Finally, we recommend when performing absolute dating analyses to first test for sequence saturation in the analyzed dataset (especially when calibrations are based on molecular rates). Our study also points out the importance of using multiple node constraints, especially when placed deeply in the tree, for fossil–based divergence dating analyses.

1. Introduction

With more than 400 species, *Anolis* lizards are among the most diverse vertebrate groups (Uetz and Hošek,2016). Despite anoles playing a central role in our understanding of several evolutionary processes such as diversification dynamics (e.g. Kolbe et al., 2011), trait evolution (e.g. Mahler et al., 2013), ecological opportunity (e.g. Algar et al., 2016), and biogeography (e.g. Campbell-Staton et al., 2012), the clades' divergence timing is still under debate (Townsend et al., 2011; Nicholson et al., 2012; Castañeda et al., 2014; Prates et al., 2015; Poe et al., 2017). Here we primarily discuss *Anolis* divergence timing by comparing the estimated ages of chronograms calibrated based on a set of well-supported divergence evidences (see below; Table 1).

To date, multiple studies have estimated the stem age of *Anolis* lizards. However, there is considerable variation among studies based on different methods and data sources (e.g. fossils and molecular clocks). Initially, Townsend et al. (2011) used a concatenated dataset of 29 protein-coding genes to estimate the stem age of *Anolis* in 65.5 Ma (50–70 Ma; anole species = 1). Later, Daza et al. (2012), using an updated morphological dataset from Conrad (2008), estimated the same node in ~ 50 Ma (anole species = 1). Next, Blankers et al. (2012) used the dataset presented by Townsend et al. (2011) in addition of a protein-coding mitochondrial gene (ND2), to estimate the *Anolis* stem age in about 70 Ma (~61–82 Ma; anole species = 3). Mulcahy et al. (2012), provided two different ages for the *Anolis* stem clade based on 25 protein-coding loci: 25–75 Ma as estimated by BEAST dating,

* Corresponding author at: Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721-0088, USA.
E-mail address: cromanpa94@email.arizona.edu (C. Román-Palacios).

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Table 1
Description of calibration sources used in dating analyses with details on the specified parameters for each. For both fossil calibrations, mean and standard deviation corresponded to 1. Probability density on prior distribution (95%) based on BEAST.

Code	Calibration source	Priors on age	Probability density	Description	References
F1	*Anolis* fossils	Lognormal Offset = 17	17.1–21.3	This node comprises all three described *A. dominicanus* amber fossils. The phylogenetic position has been widely discussed, and recently assigned to the *chlorocyanus* series (minimum age)	De Queiroz et al. (1998); Sherratt et al. (2015); Poe et al. (2017)
F2	Pleurodonta (Iguania) fossils	Lognormal Offset = 70	71.0–74.3	Includes the fossils assigned to Priscagamines, Iguanines, and *Isodontosaurus*. Their position was based on phylogenetic analyses. These fossils constraints the minimum age of the stem group of Pleurodonta clade (Iguania)	Conrad (2008); Mulcahy et al. (2012); Poe et al. (2017)
Rate	Mitochondrial evolutionary rate	0.65%/lineage/Ma	–	Several time-calibrated phylogenies in *Anolis* are based on the evolutionary rate for certain mitochondrial genes. We used the rate estimated from *Laudakia* (Agamidae)	Macey et al. (1998, 1999)

and ~ 80 Ma using a penalized likelihood method (anole species = 1). Nicholson et al. (2012), based on two protein-coding and six non-protein genes, found one of the oldest estimates for the stem-clade *Anolis* (ca. 95 Ma; anole species = 189). Prates et al. (2015), using seven protein-coding genes and one RNA region, estimated the *Anolis* stem age in about 82 Ma (71–99 Ma; anole species = 33). More recently, Poe et al. (2017), using the most comprehensive species-level phylogeny for anoles based on a combined dataset of morphological and three protein-coding genes, dated the stem age of the group in ca. 71 Ma (69–72 Ma; anole species = 379). In contrast, fewer papers have provided age estimates for the *Anolis* crown group. Blankers et al. (2012) estimated this node in about 38 Ma (~31–47 Ma), Nicholson et al. (2012) in ~87 Ma (no range provided by the authors), Prates et al. (2015) in 49 Ma (38–63 Ma), and Poe et al. (2017) estimated the same node in about 51 Ma (46.3–64.4 Ma).

The estimated age of the *Anolis* crown group has a significant impact on interpreting the biogeography of the clade (especially Caribbean lineages; Crother and Guyer, 1996; Hedges, 1996; Hedges et al., 1994). Two major hypotheses have been proposed to explain the current distribution of Caribbean *Anolis*. Vicariance–driven biogeography is supported when older ages are inferred for the crown group (e.g. Guyer and Savage, 1986). In contrast, younger ages imply dispersal as a major biogeographical driver in anoles (e.g. Hedges et al., 1992). Even though most analyses have supported a predominantly dispersal–driven biogeography in Caribbean anoles (e.g. Hedges et al., 1992; Calsbeek and Smith, 2003; Poe et al., 2017), Nicholson et al. (2012) found support for a vicariance–driven scenario for the same lineages, which was initially proposed by Guyer and Savage (1986).

Previous studies have suggested that the age estimates presented in Nicholson et al. (2012) are flawed. We highlight two lines of evidence that have challenged Nicholson's et al. (2012) results. First, several authors have agreed on the low likelihood of hypotheses based on vicariance to explain the historical patterns of Antillean anoles (e.g. crown–age estimations suggest that anoles' lineages are younger than the island system; see discussion in Poe et al., 2017 and references therein). Secondly, the position of one of the fossils used by Nicholson et al. (i.e. *Anolis electrum*) is still unsupported within the anole phylogeny (Castañeda et al., 2014; Poe et al., 2017), and its placement seems to be pulling node ages towards older dates.

Here, we primary aim to address when anoles diverged. We follow an approach that can be easily extended to other taxonomic groups with debatable or problematic divergence date estimates. First, we used three different calibration sources to estimate divergence times across the tree: the ND2 mitochondrial rate, the age of the *Anolis dominicanus* fossil, and multiple fossils to calibrate the Pleurodonta crown group. Each calibration source was analyzed independently and in all possible combinations. Then, we compared the estimated likelihood values of the seven chronograms using Bayesian two-sample t-tests and Bayes factors to answer: Are chronograms based on multiple evidence (i.e., combinations of calibration sources) better explanations of the anole evolutionary timing than divergence hypotheses based on a single calibration sources?

Second, we discuss how the use of different time–estimation strategies might lead to different date estimates across the phylogeny. To date, most discussions about the timing of *Anolis* diversification have focused on its basal divergence (e.g. stem or crown groups). However, it is widely known that node ages are highly variable when different constraints are applied to molecular clocks (e.g. molecular rates vs node constraints; Mello and Schrago, 2014; van Tuinen, 2015). We addressed whether chronograms differed most in deeper or shallower nodes by exploring the change over time in the standard deviation of node ages between chronograms. Furthermore, we analyzed the effect of the position of fossil constraints (i.e. deep or shallow in the tree) regarding the variation in mean age estimates across the phylogeny to test whether lower between-chronograms variation in dates is associated to deep or shallow nodes. This topic has been rarely discussed in the literature (Mello and Schrago, 2014; Duchêne et al., 2014).

Third, we focused on the pattern between chronograms for a single shallow node. Specifically, we compared the estimated divergence of *Anolis agassizi* to the emergence of the island to which this species is endemic to (Malpelo island). This oceanic island is located 380 km off the Pacific coast of Colombia and probably emerged 16–17 Ma (Hoernle et al., 2002). We suggest that both the degree of isolation and the known age of this oceanic island provide an ideal scenario to evaluate the congruence between biogeography and evolutionary timing for this island-endemic anole.

2. Methods

2.1. Phylogenetics

Our analyses were based on 73 taxa comprising 68 *Anolis* species from the major taxonomic series (see Williams, 1976a, 1976b). We also included five outgroup species representing the Pleurodontid genera *Enyalioides*, *Polychrus*, *Pristidactylus*, *Oplurus*, and *Urostrophus*. Taxonomic sampling for both ingroup (see Jackman et al., 1999, Castañeda and de Queiroz, 2011) and outgroup follow previous studies (Conrad, 2008; Townsend et al., 2011), and aims to maximize the coverage across major anole series without sampling every single species in the anole phylogeny.

We sampled both fast– and slow–evolving genes to estimate the phylogenetic relationships and divergence dates among anoles. Our molecular sampling comprises the mitochondrial NADH dehydrogenase subunit II (ND2, c. 1500 bp), the five-adjacent transfer-RNA ($tRNA^{Trp}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$, $tRNA^{Tyr}$), the origin of light-strand replication (O_L), and a fragment of the cytochrome oxidase subunit I (COI, c. 650 bp), and the nuclear recombination-activating gene (RAG-1, c. 2800 bp). A list of species and accession numbers is provided in Table A.1.

Protein coding genes (ND2, COI, and RAG-1) were aligned in