Molecular Phylogenetics and Evolution 61 (2011) 784–800

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Phylogenetic relationships of the *Dactyloa* clade of *Anolis* lizards based on nuclear and mitochondrial DNA sequence data

María del Rosario Castañeda [a,b,*], Kevin de Queiroz [b]

[a] Department of Biological Sciences, The George Washington University, 2023 G Street NW, Washington, DC 20052, USA
[b] Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, MRC 162, Washington, DC 20560, USA

ARTICLE INFO

Article history:
Received 27 January 2011
Revised 14 June 2011
Accepted 5 July 2011
Available online 21 July 2011

Keywords:
Anolis
Dactyloa
Phylogenetics
South America
Taxonomy

ABSTRACT

The *Dactyloa* clade, one of two major subgroups of mainland *Anolis* lizards, is distributed from Costa Rica to Peru, including the Amazon region and the southern Lesser Antilles. We estimated the phylogenetic relationships within *Dactyloa* based on mitochondrial (ND2, five transfer-RNAs, COI) and nuclear (RAG1) gene regions using likelihood and Bayesian methods under different partition strategies. In addition, we tested the monophyly of five previously recognized groups within *Dactyloa*. The data strongly support the monophyly of *Dactyloa* and five major clades: eastern, *latifrons*, *Phenacosaurus*, *roquet* and western, each of which exhibits a coherent geographic range. Relationships among the five major clades are less clear: support for basal nodes within *Dactyloa* is weak and some contradictory relationships are supported by different datasets and/or phylogenetic methods. Of the previously recognized subgroups within *Dactyloa*, only the *roquet* series consistently passed the topology tests applied. The monophyly of the *aequatorialis*, *latifrons* (as traditionally circumscribed) and *punctatus* series was strongly rejected, and the monophyly of *Phenacosaurus* (as traditionally circumscribed) yielded mixed results. The results of the phylogenetic analyses suggest the need for a revised taxonomy and have implications for the biogeography and tempo of the *Dactyloa* radiation.

1. Introduction

Anolis (Squamata: Iguanidae) is one of the most diverse groups of vertebrates traditionally ranked as genera, with 377 currently recognized species (Uetz and Etzold, 1996). Its members are distributed from southeastern North America to middle South America, including the West Indies (Etheridge, 1959; Peters and Donoso-Barros, 1970; Schwartz and Henderson, 1991). These lizards are characterized by the presence of adhesive toe pads and brightly colored dewlaps (Etheridge, 1959), and are typically of small size, arboreal habits and insectivorous diet, though there is significant interspecific variation in these traits (Schwartz and Henderson, 1991).

Early systematics studies (Etheridge, 1959; Williams, 1976a,b) divided *Anolis* into two sections (designated alpha and beta) based on the morphology of the caudal vertebrae. Each section was further subdivided into series and species groups based on several other osteological characters (e.g., post-xiphisternal rib formula, presence versus absence of a splenial, shape of the interclavicle). Subsequent phylogenetic studies have used a variety of data including morphology, allozymes, karyotypes, albumin immunology, and DNA sequences (e.g., Brandley and de Queiroz, 2004; Burnell and Hedges, 1990; Creer et al., 2001; Glor et al., 2003; Gorman and Atkins, 1967; Gorman and Kim, 1976; Gorman et al., 1968, 1980, 1983; Jackman et al., 2002; Poe, 1998; Schneider et al., 2001; Shochat and Dessauer, 1981). In these analyses, monophyly of the beta section and of several series and species groups has been supported, though others clearly are not monophyletic, and the phylogenetic relationships within and among some groups remain controversial (e.g., Creer et al., 2001; Giannasi et al., 2000; Glor et al., 2003; Jackman et al., 1999, 2002; Nicholson, 2002; Poe, 2004; Schneider et al., 2001).

Of the major groups within *Anolis*, the most poorly known regarding phylogenetic relationships is the clade designated as M1 (Mainland1) by Pinto et al. (2008) and recognized as the *latifrons* series by Etheridge (1959) and the genus *Dactyloa* by Guyer and Savage (1986; in the last two cases with the exclusion of *Phenacosaurus*; see below). However, the recognition of *Dactyloa* and other groups of anoles as genera (Guyer and Savage, 1986; Savage and Guyer, 1989) is controversial (Cannatella and de Queiroz, 1989; Williams, 1989). Following recent authors (e.g., Brandley and de Queiroz, 2004; de Queiroz and Reeder, 2008; Nicholson, 2002), who have applied the names of some of Guyer and Savage's genera to clades within *Anolis* regardless of rank and not necessarily identical in composition, we use the name *Dactyloa* for the clade

* Corresponding author. Address: P.O. Box 37012, MRC 162, Division of Amphibians and Reptiles, Smithsonian Institution, Washington, DC 20013-7012, USA. Fax: +1 202 633 0182.
E-mail address: castanedar@si.edu (María del Rosario Castañeda).

1055-7903/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2011.07.004

originating with the most recent common ancestor of the species included in the genus *Dactyloa* by Savage and Guyer (1989), which also includes the anoles formerly assigned to the genus *Phenacosaurus* according to the results of recent phylogenetic analyses (e.g. Jackman et al., 1999; Nicholson et al., 2005; Poe, 1998, 2004).

Only a handful of phylogenetic studies have included *Dactyloa* species (Jackman et al., 1999; Glor et al., 2001; Poe, 2004; Nicholson et al., 2005), and most of these have included relatively few, particularly mainland, species of *Dactyloa*. Moreover, most of what is known about the systematics of this group is based on morphological characters, which have been used to recognize six subgroups ranked as species groups by Williams (1976a) and as series by Savage and Guyer (1989): *aequatorialis*, *laevis*, *latifrons*, *punctatus*, *roquet*, and *tigrinus* (Williams, 1976b). The monophyly of these subgroups has never been tested, and there is no published hypothesis describing the relationships among them.

In this study, we present new molecular data for 40 of the 82 currently recognized species of *Dactyloa*, two potentially new *Dactyloa* species and 12 outgroup species (non-*Dactyloa Anolis* and non-*Anolis* Polychrotinae) to resolve the phylogenetic relationships within *Dactyloa*. In addition, we test hypotheses of monophyly of *Dactyloa* including *Phenacosaurus* species, *Dactyloa* excluding *Phenacosaurus* species and five of seven previously recognized species groups/series within *Dactyloa*.

2. Materials and methods

2.1. Taxa and character sampling

We collected new DNA sequence data from 40 species of *Dactyloa* (62 specimens), including representatives of the previously described *aequatorialis*, *latifrons*, *punctatus*, and *roquet* series, as well as *Phenacosaurus*, and two specimens suspected (based on morphological data and geographic distribution) to be new species. Only one representative of the *tigrinus* series was included, but we did not have any representatives of the *laevis* series. In addition, we included 12 species as outgroups: three non-*Anolis* species of Polychrotinae (*Polychrus marmoratus*, *Pristidactylus scapulatus*, *Urostrophus gallardoi*), and nine species representing nine series of non-*Dactyloa Anolis* (*Anolis bimaculatus*, *Anolis cupreus*, *Anolis cuvieri*, *Anolis equestris*, *Anolis lucius*, *Anolis marcanoi*, *Anolis occultus*, *Anolis sagrei*, *Anolis smaragdinus*). Sequenced fragments include two mitochondrial regions: a fragment including the entire NADH dehydrogenase subunit II (ND2), five transfer-RNA ($tRNA^{Trp}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$, $tRNA^{Tyr}$), and the origin for light-strand replication (O_L) (Macey et al., 1997; ~1500 b), and a fragment of the cytochrome oxidase subunit I (COI, ~650 b), as well as one nuclear region: the recombination activating gene (RAG-1, ~2800 b). This selection of genes contains both highly conserved areas that are informative for deeper divergences and rapidly evolving regions that are informative for more recent divergences (Groth and Barrowclough, 1999; Jackman et al., 1999; Miyata et al., 1982). Previously collected sequences for the ND2 region were obtained from GenBank for all three non-*Anolis* Polychrotinae, seven non-*Dactyloa Anolis* and ten *Dactyloa* species. A complete list of samples, with voucher/catalogue and GenBank numbers and collection localities is given in Appendix A.

2.2. Laboratory protocols

Genomic DNA was extracted from liver or muscle tissue using DNeasy Tissue Extraction Kits (QIAGEN Inc.). Polymerase chain reaction (PCR) was used for amplification of the particular genomic regions and performed in a DNA Engine (PTC-200) Peltier Thermal Cycler (MJ Research) and a DNA Engine Dyad® Peltier Thermal Cycler (Bio-Rad Laboratories). Two alternative PCR cycling protocols were used, depending on the combination of primers and their respective optimal annealing temperatures. When both primers had similar optimal annealing temperatures (less than 2° difference), the protocol used was: pre-denaturation at 94 °C for 120 s, followed by 30–35 cycles of denaturation at 95 °C for 30 s, annealing at the average optimal temperature between the two primers for 30 s, and primer extension at 72 °C for 60–120 s. When primers used had a large difference in optimal annealing temperatures (more than 2° difference), the protocol used was: pre-denaturation at 94 °C for 120 s, followed by 5 cycles of denaturation at 95 °C for 30 s, annealing at the higher optimal temperature between the two primers for 30 s, and primer extension at 72 °C for 60–120 s. Then, two sets of 5 cycles each were run with identical denaturation and primer extension conditions as before, but with decreased annealing temperatures (each set decreased by 2–3°). A final set with the annealing temperature set at the lowest optimal annealing temperature between the two primers was run for 20 cycles. Primer extension time was adjusted according to the length of the fragment being amplified (~1 min per 1000 b). PCR products were purified using ExoSAP-IT (USB Corporation) or magnetic beads (AMPure, Agencourt Bioscience Corporation). Cycle sequencing reactions were performed using BigDye Terminator chemistry (Applied Biosystems) directly on purified PCR products. The sequencing protocol used was denaturation at 96 °C for 10 s, annealing at 50 °C for 10 s, and primer extension 60 °C for 240 s for 35 cycles. Sequenced products were purified using Sephadex G-50 columns (SIGMA), and run on an automated sequencer (ABI Prism 3100 and 3730xl Genetic Analyzer, Applied Biosystems). The complete list of primers used in amplification and sequencing reactions is given in Appendix B. Assembly of sequences was performed with SeqMan II (DNASTAR, Inc.).

2.3. Alignment procedures and data matrices

Protein-coding regions were aligned using Clustal X (Thompson et al., 1997), under default gap costs, and subsequently translated into amino acids using MacClade v4.07 (Maddison and Maddison, 2001) to verify the correct translation frame. Genes coding for tRNAs were aligned manually to incorporate secondary structure information, following Kumazawa and Nishida's (1993) structural model for mitochondrial transfer RNAs. Sequences were strictly aligned following this model (i.e., no gaps were introduced in areas with conserved lengths: AA, AC, TΨC and D-stems, junctions between AA- and D-stems, D- and AC-stems, and AC- and TΨC-stems, and the anticodon loop). From the set of tRNA regions that according to this model can exhibit length variation (and potentially result in ambiguous alignments), those that showed length variation were excluded from the analyses.

Three different data matrices were analyzed, one including the nuclear gene region (RAG1), one including both mitochondrial regions (ND2–COI), and one combining all three gene regions (ND2–COI–RAG1).

2.4. Phylogenetic analyses and data partitions

Phylogenetic relationships were estimated using likelihood and Bayesian inference methods. Likelihood analyses were performed with GARLI-PART (Zwickl, 2006) v0.97 and GARLI (Zwickl, 2006) v1.0 using multiple partitioning strategies for each matrix (Table 1). Each analysis was run with 20 replicates using random starting trees (other settings were left as defaults). The models of evolution for the different partitions were selected based on the Akaike Information Criterion (AIC) as implemented in Modeltest (Posada and Crandall, 1998) v3.7. The trees inferred using GARLI v1.0 with an unpartitioned strategy were compared with those inferred using