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Indels ascertain the phylogenetic position of *Coleodactylus elizae* Gonçalves, Torquato, Skuk & Sena, 2012 (Gekkota: Sphaerodactylidae)

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The Neotropical gecko genus *Coleodactylus* Parker 1926 was, until recently, composed of five species: *C. amazonicus* (Andersson 1918), *C. brachystoma* (Amaral 1935), *C. meridionalis* (Boulenger 1888), *C. natalensis* Freire 1999, and *C. septentrionalis* Vanzolini 1980 (Geurgas *et al.* 2008). However, several phylogenetic analyses recovered a polyphyletic *Coleodactylus* (Geurgas *et al.* 2008; Gamble *et al.* 2011a) leading Gamble *et al.* (2011b) to recognize a new genus, *Chatogekko*, for *C. amazonicus*. *Coleodactylus* and *Chatogekko* differ in both morphological and molecular characters. *Coleodactylus* has smooth dorsal scales and five scales forming the unguis sheath, while *Chatogekko* has keeled dorsal scales and four scales forming the unguis sheath (Gamble *et al.* 2011b). Furthermore, all *Coleodactylus* species have two deletions in the protein coding recombination-activating gene 1 (RAG1), one of six base pairs (bp) and another of 18 bp (Gamble *et al.* 2008a; Geurgas *et al.* 2008), while *Chatogekko* has a unique three bp deletion in the RBMX gene and a three bp deletion in the protein tyrosine phosphatase nonreceptor type 12 gene (PTPN12) (Gamble *et al.* 2011b). In addition, *Chatogekko* is differentiated from all other geckos by a unique set of 10 craniofacial features (Gamble *et al.* 2011b).

Coleodactylus elizae Gonçalves, Torquato, Skuk, Sena & Araújo 2012 was recently described from the Maceió municipality in the northeastern state of Alagoas, Brazil. Although the species was allocated to *Coleodactylus*, Gonçalves *et al.* (2012) pointed out the difficulty in determining its generic position using morphological data alone, because *C. elizae* possesses similarities to both *Coleodactylus* and *Chatogekko*. *Coleodactylus elizae* has smooth dorsal scales like the remaining species of *Coleodactylus* but only four scales forming the unguis sheath as found in *Chatogekko*. Here we use a molecular phylogenetic approach to determine the generic status of *C. elizae*.

We extracted genomic DNA from one *C. elizae* sample and two *C. meridionalis* samples using a standard Phenol/Chloroform protocol (Sambrook *et al.* 1989). The *C. elizae* sample (MUFAL 11417) was collected in Murici municipality, 17.22 km away from the type locality at Maceió municipality, state of Alagoas, Brazil. The *C. meridionalis* samples (MUFAL 9663 and 10838) were collected in Murici and Traipu municipalities, state of Alagoas, Brazil and added to our analysis due to sympatry with *C. elizae* in Murici. Two fragments of nuclear genes with diagnostic deletions (PTPN12 and RAG1) were amplified following Gamble *et al.* (2011b) and Geurgas *et al.* (2008) protocols, respectively. Sequencing was performed at Laboratório Central da Universidade Federal de Pernambuco (LabCen/UFPE) using BigDye Terminator v3.1 Cycle Sequencing kit on ABI Prism 3500 Genetic Analyzer Sequencer, and sequences were deposited in the GenBank database (Accession Numbers KT355476–KT355481). Sequences were edited in BioEdit v. 7.0.9 (Hall 1999) and aligned with 43 additional gecko sequences from Genbank using ClustalW (Larkin *et al.* 2007) with default parameters. Sequence alignment included exemplars from all recognized species of *Coleodactylus* and *Chatogekko*, and at least one representative from the closely related genera *Gonatodes*, *Lepidoblepharis*, *Pseudogonatodes*, and *Sphaerodactylus*. Outgroups included four additional sphaerodactylid species, one gekkonid and one eublepharid species. The aligned dataset was 1,828 bp (791 bp of PTPN12 and 1,037 bp of RAG1).

We conducted Bayesian analyses of the concatenated dataset, partitioned by gene, and of each gene separately using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). All analyses included two independent runs, using four parallel Markov Chain Monte Carlo (MCMC) chains per run with 5,000,000 generations, sampled every 1,000 generations. Twenty-five per cent of the total trees were discarded as burn-in, and the remaining trees were used to build a 50% majority rule consensus tree. Convergence was determined by comparing parameters from different runs with Tracer v1.6 (Rambaut *et al.* 2014) and ensuring the average standard deviation of the split frequencies was < 0.01 (Ronquist & Huelsenbeck 2003) and effective sample sizes for all parameters was higher than 200 (Drummond & Rambaut 2007). The Akaike information criterion (AIC), as implemented in jModeltest 2.1.5 (Posada 2008), was used to identify the best-fit models of sequence evolution for each gene, TPM1uf+G for PTPN12 and TPM1+G for RAG1. We tested the hypothesis that *C. elizae* was part of the *Coleodactylus* clade and not *Chatogekko* in a maximum likelihood (ML) phylogenetic framework using the Shimodaira–Hasegawa (SH) test (Shimodaira & Hasegawa 1999) and the Approximately Unbiased (AU) test (Shimodaira 2002). We compared the optimal maximum likelihood tree to a constrained tree that enforced a monophyletic *Chatogekko* + *C. elizae*. ML phylogenies were constructed using RAxML 8.1.24 (Stamatakis 2014) implemented on the CIPRES Science Gateway (Miller *et al.* 2010). Data were partitioned by gene and nodal support estimated using rapid bootstrapping, which was stopped automatically after 204 replicates. The SH and AU tests were implemented in PAUP* v4.0a145 (Swofford 2003) with likelihood scores estimated using the GTR+G+I model and *p*-values generated using 10,000 RELL bootstrap replicates.

Two previously described deletions of six and 18 bp in the RAG1 gene that are diagnostic for *Coleodactylus* (Geurgas *et al.* 2008) were found in *C. elizae*. Furthermore, the *Chatogekko*-specific deletion in PTPN12 (Gamble *et al.* 2011b) was not observed in *C. elizae*. Indels in protein-coding sequences are rare genomic events considered relatively free of homoplasy and provide strong evidence to diagnose clades and clarify phylogenetic relationships (Townsend *et al.* 2004; Geurgas *et al.* 2008; Gamble *et al.* 2008b; Gamble *et al.* 2011b). Bayesian analyses of both the concatenated and individual genes recovered *C. elizae* in a well-supported clade with other *Coleodactylus* species (Fig. 1). Relationships among other sampled taxa were largely concordant with other recently published phylogenies at well-supported nodes (Gamble *et al.* 2011b; Gamble *et al.* 2012b; Gamble *et al.* 2015). We recovered a monophyletic Sphaerodactylini (probability posterior, PP = 1.00) consisting of three major clades: a clade composed exclusively of *Chatogekko*, a clade composed of *Coleodactylus*, *Pseudogonatodes*, *Sphaerodactylus*, and a third clade containing *Lepidoblepharis* and *Gonatodes*. Relationships among these three clades were poorly resolved and received low nodal support, a result consistent with other phylogenies (Gamble *et al.* 2008a; Gamble *et al.* 2011a; Gamble *et al.* 2011b; Gamble *et al.* 2012b; Gamble *et al.* 2015). Within *Coleodactylus*, *C. septentrionalis* was recovered as the sister taxon to all remaining *Coleodactylus* species (PP = 1.00), with *C. brachystoma*, the sister taxon of a clade composed by *C. meridionalis*, *C. elizae* and *C. natalensis*. *Coleodactylus meridionalis* was rendered paraphyletic by *C. natalensis* and *C. elizae*. The paraphyly of *C. meridionalis* has already been commented on (Geurgas *et al.* 2008), and it is apparent that *C. meridionalis* represent a species complex. Thus, a taxonomic revision and a phylogeographic study of *C. meridionalis* species complex is needed to delimit species boundaries in this group. *Coleodactylus meridionalis* is not unusual in this regard and several molecular genetic studies have revealed cryptic species within Gekkota (e.g. Gamble *et al.* 2012a; Agarwal & Karanth 2015; Makhubo *et al.* 2015).

The maximum likelihood tree was broadly similar to the Bayesian phylogeny at well-supported nodes. Both the SH and AU tests indicated the maximum likelihood tree, with *C. elizae* as part of *Coleodactylus* ($-\ln L = 11419.06462$), provided a better fit to the data than the alternative hypothesis that enforced *Chatogekko* + *C. elizae* monophyly ($-\ln L = 11587.59034$; difference $-\ln L = 168.52573$; SH test, $P = 0.000$; AU test, $P = 0.000$). Thus, both topology tests provide strong support for the taxonomic placement of *C. elizae* into *Coleodactylus* allowing us to reject the hypothetical placement of *C. elizae* as part of *Chatogekko*.

The generic allocation of *C. elizae* to *Coleodactylus* has been uncertain since it was described (Gonçalves *et al.* 2012). Possessing smooth dorsal scales like *Coleodactylus* but an unguis sheath composed of four scales like *Chatogekko* suggested affinities with either genus. We resolved this uncertainty by generating a molecular phylogenetic hypothesis that included *C. elizae* thus corroborating its current generic placement. Furthermore, deletions in RAG1 provide unambiguous evidence of the affinity of *C. elizae* with other *Coleodactylus* species and further highlight the important role of indels in taxonomy and systematics.

The presence of four unguis sheath scales in *C. elizae* is unique among *Coleodactylus* species. Our phylogenetic results show that the presence four unguis sheath scales in both *Chatogekko* and *C. elizae* is due to homoplasy not homology. However, it remains unclear whether this represents a loss of one scale or a fusion of two. Therefore, further work is needed to clarify whether the four scales in the unguis sheath in *C. elizae* and *Chatogekko* follow the same ontogenetic pathway.

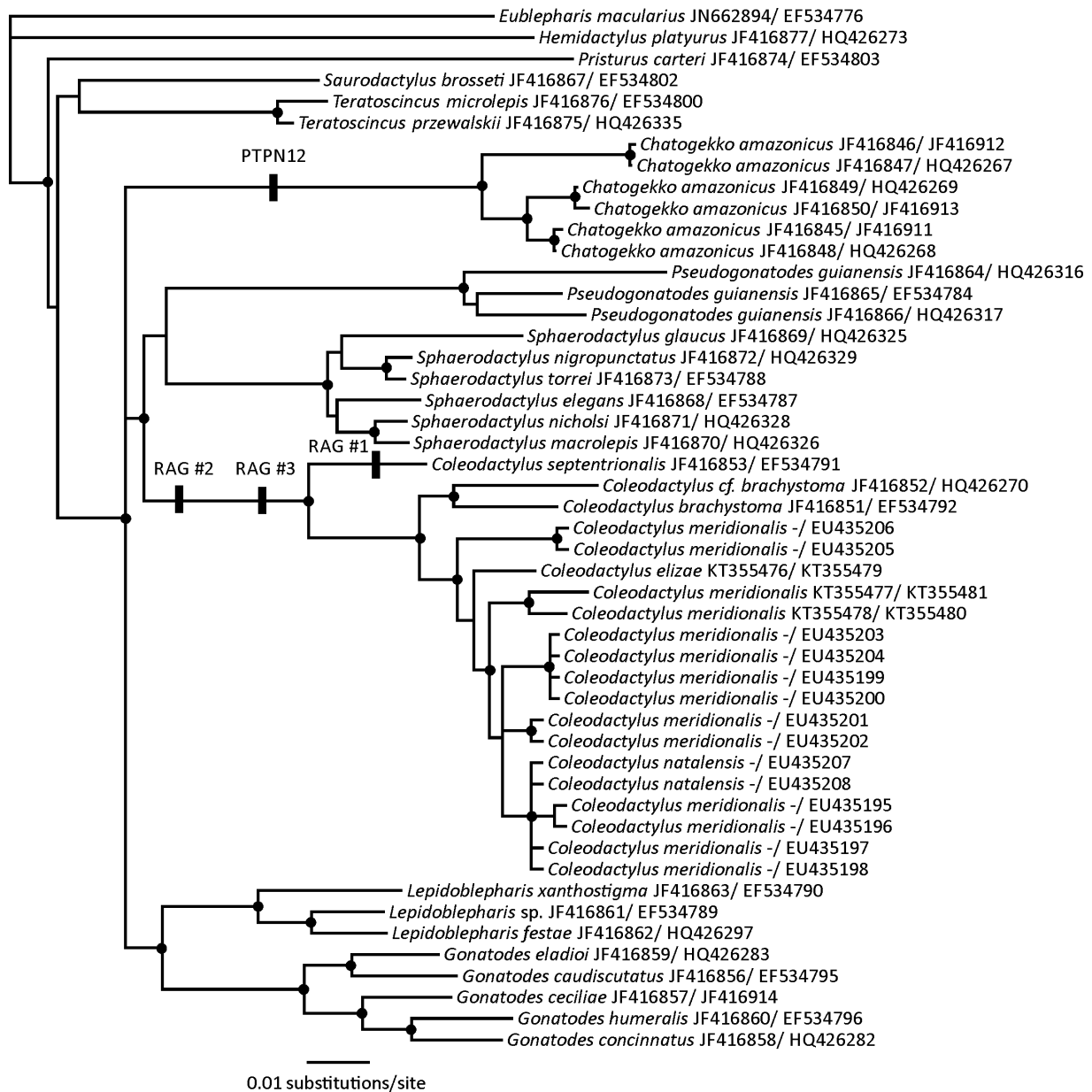


FIGURE 1. Bayesian tree topology (consensus by majority rule 50%) of spheerodactyl geckos obtained from 1828 base pairs of concatenated nuclear genes (RAG1 and PTPN12). Closed circles indicate nodes with posterior probabilities ≥ 0.95 . Indels from protein-coding regions are indicated along with the gene name, PTPN12 in *Chatogekko* clade and RAG1 in *Coleodactylus* clade. RAG1 possessed multiple unique indels and each is numbered sequentially in 5'–3' direction following Gamble *et al.* 2011b. Taxon names are shown on the right followed by GenBank accession number of PTPN12 and RAG1 genes respectively.

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