Análise filogenética de *Pristimantis* Jiménez de la Espada, 1870, um gênero megadiverso de anfíbios (Anura, Brachycephaloidea, Craugastoridae)

Phylogenetic analysis of *Pristimantis* Jiménez de la Espada, 1870, a megadiverse genus of amphibians (Anura, Brachycephaloidea, Craugastoridae)

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RESUMO

O gênero *Pristimantis* possui 500 espécies de anfíbios anuros com distribuição no noroeste da América do Sul, concentrado principalmente nos Andes. Devido ao grande número de espécies, morfologicamente diversas, o gênero encontra-se atualmente dividido em 11 grupos de espécies, com 312 espécies não alocadas em grupos. O grande número de espécies não alocadas em grupos se deve especialmente pela falta de dados morfológicos para definir e diagnosticar esses grupos, e pelo fato dos grupos anteriormente reconhecidos nas décadas de 70, 80 e 90, serem em sua grande maioria não monofiléticos. O presente trabalho tem como objetivo uma análise filogenética do gênero *Pristimantis*, utilizando caracteres fenotípicos e genotípicos através de uma análise de evidência total utilizando homologia dinâmica e parcimônia. *Pristimantis* não resultou monofilético e algumas de suas espécies foram realocadas no gênero *Tachiramantis*. Os grupos de espécies em *Pristimantis* foram reformulados com diagnoses propostas. No total 25 grupos de espécies são reconhecidos.
The genus *Pristimantis* has 500 species of anurans with distribution in northwest South America, specially in the Andes. Due to the large number of species morphologically diverse, the genus was divided in 11 species groups, with 312 species not allocated in any of them. The great number of species not allocated in species groups is due to the lack of morphological characters to better define and diagnose these groups, and because most of the anteriorly recognized groups in the literature of 70, 80 and 90s decades of the last century were not recovered monophyletic in the most recent phylogenetic analyses. The present study aims to perform a phylogenetic analysis of *Pristimantis*, with phenotypic and genotypic characters, through total evidence with dynamic homology and parsimony. As a result, *Pristimantis* is not monophyletic and some of its species were reallocated in the genus *Tachiramantis*. The species groups within *Pristimantis* were reformulated with proposed diagnosis. In total, 25 species groups are here defined.
1. INTRODUCTION

1.1. Brachycephaloidea and its historical background

Brachycephaloidea is currently composed of three families, Brachycephalidae, Craugastoridae and Eleutherodactylidae, comprising more than 1000 species (Padial et al., 2014). Also known as terraranans, species of this group are direct-developing frogs occurring in the Neotropics (Hedges et al., 2008). Besides the direct development, in which there is no tadpole stage, the other suggested synapomorphies for this group are terrestrial clutch composed by large unpigmented eggs (Lynch, 1971), and presence of the egg tooth in the embryos (Frost et al., 2006). More recently, Taboada et al. (2013) suggested new tentative synapomorphies of the urogenital and vascular systems.

Historically, most of this group was considered Eleutherodactylini (Lynch, 1971) or Eleutherodactylinae (Lutz, 1954), as part of the highly polyphyletic Leptodactylidae. Most species were, for a long time, allocated in Eleutherodactylus, then considered the largest vertebrate genus with more than 700 species (Crawford & Smith, 2005). Izecksohn (1988) suggested the relationship between the Eleutherodactylinae (Leptodactylidae) and Brachycephalus (Brachycephalidae), based on the direct development of both groups, and later Pombal (1999) described the presence of the egg-tooth in Brachycephalus embryos, a trait restricted to these two groups. Darst & Cannatella (2004) molecular phylogeny showed the non-monophyly of Leptodactylidae and found Eleutherodactylinae to be related to Brachycephalus, although no nomenclatural change was made. Frost et al. (2006), with a broad sample of amphibians, also found in their molecular phylogeny the same results. Leptodactylidae was broken into monophyletic families, and Eleutherodactylinae was moved to Brachycephalidae.

Heinicke et al. (2007) greatly increased sampling of Brachycephalidae, from 11 species in Frost et al. (2006) to 276. Three major clades were found: the first one from the Caribbean, the second from Central America, and the third from northwestern South America. A small fourth clade was also found from southeast South America. The clade from Central America retained the name Eleutherodactylus with the subgenera Pelorius, Euhyas, and Syrrhophus. The clade from Central
America corresponded to *Craugastor*, a subgenus elevated to genus by Crawford & Smith (2005), and *Eleutherodactylus binotatus* [= *Haddadus binotatus*]. The clade from northwestern South America corresponded to most species groups recognized in *Eleutherodactylus*. To this clade, the name *Pristimantis* Jiménez de La Espada, 1870 was available and had priority. *Ischnocnema* Reinhardt & Lutken, 1862 was applied to the small clade from southeast South America.

Hedges *et al.* (2008) expanded the sampling of Heinicke *et al.* (2007) to 326 species, and reviewed the taxonomy inside the family. They divided Brachycephalidae in four families, and proposed the unranked name Terrarana for the group. Brachycephalidae was restricted to *Brachycephalus* and *Ischnocnema*, whereas Eleutherodactylidae was applied to *Eleutherodactylus*, *Diasporus*, *Adelophryne*, and *Phyzzellaphryne*. Two new families were erected: *Craugastoridae* to accommodate *Craugastor* and *Haddadus* (genus proposed to *Eleutherodactylus binotatus*), and *Strabomantidae* to accommodate 16 genera, including *Pristimantis*. *Strabomantidae* was divided in two subfamilies, *Holoadeninae* with six genera and *Strabomantinae* with 10 genera.

Heinicke *et al.* (2009) described a new genus and family based on three specimens from Guyanese tepuis sampled in their molecular phylogeny, *Ceuthomantis* and *Ceuthomantidae*, sister group of Terrarana. It was included in Terrarana and considered its basal lineage. Also in this study, it was proposed that Hemiphractidae and Terranana were sister groups, and the name Orthobatrachia was given to this clade.

Padial *et al.* (2009) performed a molecular phylogenetic analysis including for the first time specimens of *Yunganastes*, considered tentatively as subgenus of *Pristimantis* by Hedges *et al.* (2008). Due to its position outside *Pristimantis*, which is more related to the other genera of *Strabomantidae*, *Yunganastes* was elevated to genus.

In an all-inclusive phylogenic analysis of amphibians using the available sequence data from Genbank, Pyron & Wiens (2011) failed to recover a monophyletic *Strabomantidae* and synonymized this family with *Craugastoridae*. *Strabomantinae* was restricted to *Strabomantis*, and *Pristimantinae* was erected to accommodate *Phrynopus*, *Lynchius*, *Oreobates*, and *Pristimantis*.

Canedo & Haddad (2012) recovered *Ceuthomantis* more related to other families of Hyloides sensu Frost *et al.* (2006), than with Brachycephaloidea, and
*Euparkerella* was sampled for the first time, being confirmed in Holoadeninae, Craugastoridae. In that study, analyses based on distinct optimality criteria conflicted with respect to the relationships between families and subfamilies within Craugastoridae; some corroborate Pyron & Wiens (2011) and others Hedges *et al.* (2008).

Padial *et al.* (2014) analyzed the molecular data available for terraranans until February 2012 with distinct optimality criteria (parsimony, maximum likelihood, and posterior probability) and alignment strategies (tree-alignment (= dynamic homology, Wheeler, 1996) and similarity alignment (= static alignment), to point out differences caused by distinct assumptions. In this paper, the authors suggest the resurrection of the name Brachycephaloidea Günther, 1858 as a formal name for Terrarana, and propose the later as a common name for the group. The trees used to review the taxonomy of Brachycephaloidea were the most parsimonious trees constructed with tree-alignment. *Ceuthomantis* is recovered as sister group of *Pristimantis* + *Yunganastes*. The three genera compose the new subfamily Ceuthomantinae (Padial *et al*., 2014b), and Ceuthomantidae was then synonymized with Craugastoridae. The other subfamilies of Craugastoridae are Craugastorinae and Strabomantinae that were also reformulated.

Pyron (2014) expanded the dataset of Pyron & Wiens (2011) with new available sequences from Genbank. As a result, *Ceuthomantis* was recovered as sister-group of Brachycephaloidea.

Different optimality criteria and nucleotide homology have been applied to phylogenetic analyses in Brachycephaloidea (see Padial *et al.*, 2014). Static or similarity alignment, and maximum likelihood or posterior probability optimality criteria were broadly applied (Heinicke *et al*., 2007 and 2009; Hedges *et al*., 2008; Padial *et al*., 2009, Pyron & Wiens, 2011, Fusinatto *et al*., 2013, Canedo & Haddad, 2012, Mendoza *et al*., 2015, Pinto-Sánchez *et al*., 2012, Ortega-Andrade & Venegas, 2012, Rivera-Prieto *et al*., 2014, Rivero-Correa & Daza, 2016, Padial *et al*., 2014). While parsimony with similarity or tree alignment was considerably less performed (Canedo & Haddad, 2012, Padial *et al*., 2014, Rivero-Correa *et al*., 2016). The position of *Ceuthomantis* and the relationships within the genera of Craugastoridae are more unstable relative to the phylogenetic systematics of Brachycephaloidea (see Fig. 8 in Padial *et al*., 2014).
1.2. *Pristimantis* and its historical background

*Pristimantis* is now the largest genus of vertebrates, with 500 species (Frost, 2016). They occur throughout most of South America, from lowlands, in the Pacific versant, Amazon basin, Atlantic Forest, and Cerrado, to almost 4000 m of elevation in the Andes of Colombia, Ecuador, Peru, and Venezuela, where they are more abundant. Eleven species occur in Central America, and three species occur in the Caribbean (Hedges et al., 2008).

*Pristimantis* was described by Jiménez de la Espada in 1841, by monotypy, to *Pristimantis galdi*, from Ecuador. Later, it was synonymized to *Eleutherodactylus* by Stejneger (1904), and finally resurrected by Heinicke et al. (2007), as mentioned above.

Hedges et al. (2008) reviewed the taxonomy of Brachycephaloidea, defined and diagnosed its genera and infrageneric units, including *Pristimantis*. Three subgenera were recognized: *Pristimantis*, *Hypodictyon*, and *Yunganastes*. *Yunganastes* was posteriorly elevated to genus by Padial et al. (2009). The subgenera *Pristimantis* was then divided in 16 species groups with 34 species not allocated in any of them. These groups and diagnoses presented in Hedges et al. (2008) were taken directly from Lynch & Duellman (1997), and most of them were not recovered as monophyletic in their analysis.

Canedo & Haddad (2012) conducted an analysis with emphasis in *Ischnocnema*. These authors recovered the *Ischnocnema ramagii* species group, *I. ramagii*, *I. paulodutrai*, and *I. vinhai*, nested within *Pristimantis*. As a result, they transferred these species to *Pristimantis*, expanding its distribution into the Atlantic Forest of northeastern Brazil.

Pinto-Sanchéz et al. (2012) added sequences of 31 species of *Pristimantis* to 107 species of Brachycephaloidea available in Genbank. Neither subgenera *Pristimantis* and *Hypodictyon* nor 10 of the 16 species groups in *Pristimantis* were recovered as monophyletic. However, no taxonomic changes were made. Other phylogenetic analyses of *Pristimantis* added new sequences to the ones previously available in Genbank (Mendoza et al., 2015, Ortega-Andrade & Venegas, 2012, Rivera-Prieto et al., 2014, Rivero-Correa & Daza, 2016, Rivero-Correa et al., 2016).

The non-monophyly of most previously recognized species groups is evident in all of the mentioned papers above, however the species groups were only reviewed...
in Padial et al. (2014). The authors did not recognize the subgenera Pristimantis and Hypodyction, and made changes in the content of some of their unranked species groups in order to define exclusively monophyletic clades. Eleven species groups were then recognized, and 312 species were not allocated in any of them.

1.3. History of the species groups in “Eleutherodactylus”

As mentioned above, most of the species in Eleutherodactylinae were in one genus, Eleutherodactylus. As an attempt to deal with the increasing number of species in the genus, some authors proposed infrageneric units based on specific morphological characters (e.g. Cochran & Goin, 1960; Schwartz, 1967; Lynch, 1968, 1971, 1975, and 1976; Lynch & Duellman, 1980 and 1997, Savage, 1987).

Cochran & Goin (1960) organized the 33 Colombian species of Eleutherodactylus in four groups. The groups were based mainly on texture of the venter, leg length, total length, and frontoparietal crests. Lynch (1968) proposed a grouping of the southeastern Brazilian species, and Lynch (1975) proposed two groups of the broad-headed Eleutherodactylus from Central and South America, now under Craugastor and Strabomantis (Hedges et al., 2008).

Lynch (1976) proposed species groups for the South American species. Four units were established, based on relative length of finger I and II, and texture of venter. Each unit was divided in several groups. The author clarifies that these divisions do not reflect evolutionary relationships.

Infrageneric Unit IA- binotatus, biporcatus, discoidalis, and fitzingeri groups.
Infrageneric Unit IB- ramagii and sulcatus groups.
Infrageneric Unit 2A- lacteus and parvus groups.
Infrageneric Unit 2B- auriculatus and unistrigatus groups.

Posteriorly, Lynch and Duellman (1980) proposed the use of assemblies as categories within species groups, in special for the “E. unistrigatus” group, which already contained more than 100 species assigned. In this work, the Ecuadorian species from the Amazonian slopes of the Andes were categorized in the already recognized groups, and the unistrigatus group was divided in 16 assemblies. More
groups were subsequently created while new species were being described (e.g. Lynch, 1992). The creation of these groups and assemblies was, at least for some authors, admittedly phenetic (Lynch & Duellman, 1997).

Lynch (1986), based on the condition of the trigeminal nerve relative to the *M. adductor mandibulæ*, suggested as synapomorphy for the *Eleutherodactylus* group of Central America, the E-condition, in which the nerve passes medial to the adductor muscle. This group was later recognized as *Craugastor* Cope, 1862 by Crawford & Smith (2005).

Hedges (1989), based on an alloenzyme analysis mostly of Caribbean species, proposed the recognition of five subgenera in *Eleutherodactylus*: *Eleutherodactylus*, *Pelorus*, *Syrrhophus*, *Euhyas*, and *Swartzius*. These groups were later recognized as genera by Frost et al. (2006).

Lynch & Duellman (1997) recognized all subgenera of Hedges (1989) and *Craugastor*. In *Eleutherodactylus*, they recognized the *abbotti*, *binotatus*, *myersi*, *sulcatus*, *conspicillatus*, and *martinicensis* series. The 63 species of *Eleutherodactylus* from Western Ecuador were allocated in 12 species groups within the subgenera *Eleutherodactylus*. The authors also listed all species of *Eleutherodactylus* and their respective subgenus, series, and species group.

This last review of infrageneric groups in *Eleutherodactylus* by Lynch & Duellman (1997) was the main reference for the 16 groups of *Pristimantis* as per Hedges *et al.* (2008), where most species were previously identified as *Eleutherodactylus*.

As mentioned above, most of these groups were not recovered as monophyletic by Hedges *et al.* (2008) and subsequent analyses. Only in 2014, Padial *et al.* made modifications in the groups’ contents to make some of them monophyletic. Moreover, the two subgenera, *Hypodyction* and *Pristimantis*, were not recognized. Eleven species groups were redefined, ~62% of the genus was not allocated in any of them. These groups are: *P. bellona*, *P. chalceus*, *P. conspicillatus*, *P. danae*, *P. devillei*, *P. galdi*, *P. lacrimosus*, *P. leptolophus*, *P. loustes*, *P. myersi*, and *P. ridens*. *Pristimantis bellona* and *P. loustes* species groups do not have any species sampled in any phylogenetic analysis so far. *Pristimantis leptolophus* species group was recently sampled in a study focused on the group, where it was sampled for the first time (González-Durán *et al.*, 2016, in press). *Pristimantis chalceus* and *P.
galdi did not have their monophyly tested because only one species was sampled for each group in previous analyses.

Besides the changes in content, very little was made about the diagnosis of these groups, which are, in most cases, general and with many overlapping characters. The assignment of species to them is usually problematic, and rely exclusively on the molecular characters and phylogenetic arrangements; the same problems occur to the assignment of species to the genus.

2. OBJECTIVES

• Test the monophyly of Pristimantis and its species groups, in a total evidence analysis, with genotypic and phenotypic characters.
• Based on the topological results, review the taxonomy of Pristimantis in order to propose subgeneric monophyletic units, and, if possible, enhanced morphological diagnoses.
CONCLUSION

In this study we expanded the sampling of Pristimantis in a phylogenetic context, and concluded that the genus is not monophyletic due to the position of the species of Sierra Nevada de Santa Marta, one species from Serrania del Perijá, and one species of the Cordillera Oriental in Colombia, here transferred to Tachiramantis.

Based on our topological results we propose the creation of new species group within the genus, and the reformulation of P. chalceus, P. galdi, P. leptolophus, P. myersi, and P. ridens species groups.

Many species still remain not allocated in species group and must still be evaluated about their phylogenetic position.

REFERENCES


