Correspondence

Molecular phylogenetic relationships among Pristimantis summit populations in the eastern tepui chain: insights from P. aureoventris (Anura: Craugastoridae)

Daniel Jablonski¹, Daniel Grula¹, César L. Barrio-Amorós² & Philippe J. R. Kok³

¹) Department of Zoology, Comenius University in Bratislava, Mlynská dolina, Ilkovičova 6, 842 15 Bratislava, Slovakia
²) Doc Frog Expeditions, Apartado Postal 220-8000, San José, Pérez Zeledón, San Isidro del General, 11901, Costa Rica
³) Amphibian Evolution Lab, Biology Department, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium

Corresponding authors: Daniel Jablonski, Philippe Kok, e-mails: daniel.jablonski@balcanica.cz; philippe.kok@vub.be

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No less than 500 nominal species are currently recognized in the frog genus Pristimantis, the most speciose vertebrate genus in the world (Frost 2016). Only twenty of these species are distributed in Pantepui (area sensu Kok 2013b, see also Kok & Barrio-Amorós 2013, Rojas-Runjaic et al. 2013), nine of them being restricted to tepui summits and tepui upper slopes (Kok 2013a, Rojas-Runjaic et al. 2013). According to McDiarmid & Donnelly (2005) and Kok (2013a, b), several undescribed Pristimantis species could still be expected in this poorly explored region. Pristimantis has a complex taxonomy and biogeographical history (Padial et al. 2014), and since most tepui summits and slopes have been inadequately sampled (Kok 2013b) any additional information about tepui Pristimantis populations is crucial. Furthermore, any data about the distribution of tepui species are important in terms of conservation because tepui summit organisms might seriously be threatened by global warming (e.g., Rödder et al. 2010, Kok et al. 2016a).

Pristimantis aureoventris Kok, Means & Bossuyt, 2011 is currently only known from the type locality (the summit of Wei-Assipu-tepui at ca. 2,210 m elevation along the border between Guyana and Brazil), and from the upper slopes of the northern tip of Mount Roraima in Guyana at ca. 2,305 m elevation (Kok et al. 2011). No other records are known from neighboring tepuis or intervening upland/highland areas, but a putative new Pristimantis species from the summit of Mount Roraima has been reported to possibly be conspecific with P. aureoventris (Kok et al. 2011, see below). The geographically closest tepui summit species to P. aureoventris is P. yuruaniensis, which occurs on the summit of Yuruaní-tepui, about 19 km (airline) NW of Wei-Assipu-tepui. Both taxa inhabit similar environments, and although phenotypically similar, these two species diverge in a number of morphological characters (e.g., size, skin texture), colour pattern (including sexual dichromatism), and advertisement call (Kok et al. 2011). In addition, P. aureoventris exhibits a high degree of pattern polymorphism, while P. yuruaniensis is barely variable. Molecular phylogenetic analyses indicated that populations of these two species are reciprocally monophyletic (Kok et al. 2012). Mägdefrau & Mägdefrau (1994) suggested the presence of P. yuruaniensis on the summit of Kukenán-tepui (located 10 km SE of Yuruaní-tepui, airline), mostly based on overall similarities of specimens and similar calls to the human ear (but apparently none of the Kukenán specimens was collected, nor the call of that population recorded). Rödder & Jungfer (2008) mentioned the population from the summit of Kukenán-tepui as P. cf. yuruaniensis pending additional data and analyses, thus not ruling out that the specimen from Kukenán-tepui illustrated in Rödder & Jungfer (2008: 64) could belong to an undescribed species. Tissue samples from two Pristimantis individuals from the summit of Kukenán-tepui were recently available and we conducted molecular analyses to elucidate their affinities to either P. aureoventris or P. yuruaniensis.

Kukenán-tepui lies in the Estado Bolívar in Venezuela and reaches a maximum elevation of 2,650 m above sea level (a.s.l.). Its summit area is estimated to be 20.63 km² and is covered by “pioneer vegetation on sandstone summits” according to McDiarmid & Donnelly (2005). To date, only two amphibian species have been recorded from the summit of Kukenán-tepui: Oreophrynella nigra and Pristimantis cf. yuruaniensis, with no addition since
the first herpetological exploration on its summit in 1977

Table 1. List of taxa used in this study, with localities and GenBank accession numbers.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Country</th>
<th>Locality</th>
<th>GenBank voucher</th>
<th>GenBank accession number</th>
<th>Reference</th>
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<td>3513</td>
<td>KY495891</td>
<td>This study</td>
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<td>Kok et al. 2012</td>
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Total genomic DNA was extracted from the two samples using the Qiagen DNeasy Blood and Tissue Kit following manufacturer’s protocols. DNA was amplified for a fragment of the widely used 16S ribosomal RNA standard phylogenetic marker (16S) with primers 16Sar-L and 16Sbr-H (Palumbi et al. 1991). PCR products were sent to Macrogene Inc. (Amsterdam, the Netherlands) for purification and sequencing. The two novel sequences were deposited in GenBank under accession numbers KY495891 and KY495892. The new sequences were combined with (1) available 16S GenBank sequences for P. aureoventris and P. yuruaniensis; and (2) selected 16S sequences of closely related Pantepui Pristimantis species (Table 1). For intraspecific relationships within P. aureoventris we only used the distinct haplotypes published in Kok et al. (2012), not the all-individual dataset. The 16S fragments (563 bp) were aligned using Clustal W algorithm (Thompson et al. 1994) as is implemented in BioEdit (Hall 1999). Alignments were checked by eye and low quality ends trimmed. Ambiguously aligned region/gaps were ignored for the subsequent analysis. We used a network approach (Posada & Crandall 2001) to infer inter-individual relationships. First, we generated a phylogenetic network of closely related species of Pantepui Pristimantis (see above) using the Neighbor-Net algorithm (Bryant & Moulton 2004) implemented in the software SplitsTree 4.10 (Huson & Bryant 2006). To assess the support for the observed structure, bootstrap analysis was performed with 1000 replicates. Nodes were considered strongly supported if they received bootstrap values > 70%. This type of analysis is a powerful tool for inferring and visualizing conflicting and consistent evidence
Figure 1. Individuals of *Pristimantis aureoventris* from Kukenán-tepui and their habitat. (A) Dorsolateral view of an unsexed individual; (B) Ventral view of the same individual; (C) Dorsolateral view of a male; (D) Ventral view of the same individual; (E) Locality of capture (arrow in F) on the summit of Kukenán-tepui; and (F) Macrohabitat on Kukenán-tepui. All photos by DG except 1F (P. Fend).
in the dataset (Huson & Bryant 2006). Second, we constructed a haplotype network for *P. aureoventris* and *P. yuruaniensis* using the 95% limit of parsimony as implemented in TCS 1.21 (Clement et al. 2000).

Both methods supported the identification of the two individuals from Kukenán-tepui as *Pristimantis aureoventris*, which is the first evidence of the presence of *P. aureoventris* in Venezuela. As indicated in the network, the sequences obtained from the new specimens clearly cluster with sequences of *P. aureoventris* (JQ74251–54, 58), all of them being very close to *P. yuruaniensis* (JQ742160), the most closely related species according to previous phylogenetic analyses (Kok et al. 2012). Both new sequences from Kukenán-tepui are distinct haplotypes separated from haplotypes from Mount Roraima by one and/or two mutation steps, and by three and four mutation steps from haplotypes from Wei-Assipu-tepui.

The newly discovered population of *Pristimantis aureoventris* is located (airline distances) 11 km from the single known locality of *P. aureoventris* on Mount Roraima (northern upper slopes), 13 km from the type locality of *P. aureoventris* (Wei-Assipu-tepui), and 14 km from Yuruani-tepui (type locality of *P. yuruaniensis*). Coloration and pattern of the Kukenán individuals (Figs 1A–D) are consistent with the colour pattern variation observed in *P. aureoventris* as illustrated in Kok et al. (2011).

The mechanisms underlying the species/populations structure on a single tepui and among isolated tepui summits are still not fully understood. Kok (2013b) and Kok et al. (2016b) hypothesized an intricate pattern of multiple nonexclusive processes to explain species diversification in the area, and suggested that different lineages may have responded differently to the same historical events. It seems indeed difficult to find a generalized evolution pattern applicable to all tepui summit amphibian species/populations. For example, although the current distribution of *Oreophrynella quelchii* (Wei-Assipu-tepui and Mount Roraima) and *O. nigra* (Kukenán-tepui and Yuruani-tepui) would suggest closer affinities within each of these two pairs of tepuis, our results indicate that this is not relevant to *Pristimantis*. Our observations also suggest that *Pristimantis* sp. "J" from the summit of Mount Roraima in Venezuela (see McDiarmid & Donnelly 2005) could be *P. aureoventris*, but tissue samples and specimens from that population are necessary to corroborate this assumption.

**Figure 2.** Mitochondrial 16S rRNA neighbor-net networking of selected *Pristimantis* species from Pantepui using SplitsTree. Scale bar represents 1% sequence divergence; numbers correspond to bootstrap value.
Figure 3. (A) Location of the eastern tepui chain (enlarged red rectangle) in northern South America; (B) Map of the eastern tepui chain (modified from Kok et al. 2011) showing the distribution of known haplogroups of *Pristimantis aureoventris* on Kukenán-tepui, Mount Roraima and Wei-Assipu-tepui, and of *P. yuruaniensis* on Yuruani-tepui; and (C) Haplotype network inferred for 16S rRNA dataset in TCS under the 95% parsimony threshold of *P. aureoventris* and the closely related *P. yuruaniensis*. Colours are concordant with those used in Fig. 3B.

Acknowledgments

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References


