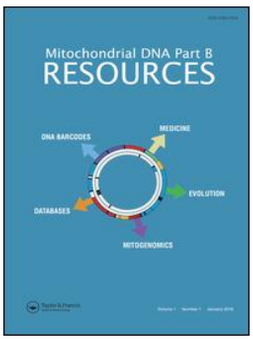

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MITOGENOME ANNOUNCEMENT

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The mitochondrial genomes of five frog species of the Neotropical genus *Ischnocnema* (Anura: Brachycephaloidea: Brachycephalidae)

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ABSTRACT

We report the mitogenomes for five species of the *Ischnocnema guentheri* series, being the first described for this genus of brachycephalid frogs. We assembled mitogenomes from anchored hybrid enrichment data and recovered the 13 protein-coding genes, 22 tRNA genes, and two rRNA genes for all species. The general structure agrees with most previously sequenced neobatrachians, with two exceptions: the origin of replication of L-strand (O_L) was found between tRNA-A and tRNA-N, and the position of tRNA-L and tRNA-T, which are dispersed in the control region. We provide a phylogenetic tree with outgroups, which is consistent with previous phylogenetic hypotheses.

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The Neotropical genus *Ischnocnema* (Reinhardt and Lütken 1862) comprises 37 species (Frost 2018) of leaf-litter dwelling frogs divided into five species series and three species unassigned to any series (Taucce at al. 2018). Within this genus, the *I. guentheri* series comprises 10 species distributed all over the southern and central portions of the Atlantic Forest, throughout seven Brazilian states and the Argentinean province of Misiones (Frost 2018). The series has a challenging taxonomy, with notable intra and inter-specific morphological variation (Heyer 1984), and some of its members may actually represent complexes of morphologically cryptic species (Gehara et al. 2013). Herein, we provide complete or nearly complete metagenome sequences for half (five) of the currently recognized species of the *I. guentheri* series, assembled from anchored hybrid enrichment data (Lemmon et al. 2012): *I. erythromera* (Heyer 1984), *I. guentheri* (Steindachner 1864), *I. nasuta* (Lutz 1925), *I. oea* (Heyer 1984) (one specimen each), and *I. henselii* (Peters 1870) (five specimens). Voucher specimens and tissue samples are housed in the CFBH or LGE collections (acronyms follow Sabaj 2016).

We extracted total DNA from ethanol-preserved muscle or liver tissues using the DNeasy Qiagen® kit following manufacturer's protocols. DNA was eluted to a volume of 100 µl and quantified using a Qubit fluorometer dsDNA BR Assay Kit (Thermo Fisher Scientific Inc., Waltham, MA). Extractions were sent to the Center for Anchored Phylogenomics (Tallahassee, FL) to be sequenced with a method for anchored hybrid enrichment analysis (Lemmon et al. 2012). Samples were pooled after indexing and hybrid enrichments were performed with probes designed for anchored loci from amphibians (Barrow et al. 2018; Heinicke et al. 2018). Mitochondrial sequences were recovered as bycatch during the process of hybrid enrichment. Sequencing was carried out on an Illumina HiSeq2500 sequencer. For mitochondrial genomes assemblies, each lane of raw sequence reads was first concatenated per sample and quality-trimmed using Trimmomatic (Bolger et al. 2014). Then, we used MITObim v1.9 (Hahn et al. 2013) using as reference the mitogenome of *Eleutherodactylus atkinsi* (GenBank number: JX564864) to assemble the mitogenome of *Ischnocnema oea*. Next, we checked the quality and coverage of this new mitogenome and used it to assemble the remaining specimens of *Ischnocnema*. Assemblies were checked for quality by mapping the mitochondrial reads recovered by MITObim to the final fasta file with Geneious R11 (Kearse et al. 2012). We also used Geneious R11 to test for mitogenome circularity and completeness using the 'De novo assemble' tool. Regions with low coverage (less than eight reads) were manually edited to unknown nucleotides ('N'). The preliminary annotation of final mitochondrial genomes was carried out by MITOS 2 (Bernt, Donath, Jühling, et al. 2013), available online at http://mitos2.bioinf.uni-leipzig.de/index.py, and verified by alignment with *Eleutherodactylus atkinsi*. The protein-coding regions were checked to confirm no indels or stop codons were present. The new mitogenomes have been deposited in GenBank under accession numbers MH492729–MH492737.

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Supplemental data for this article can be accessed here.

We recovered the typical 13 protein-coding genes, two rRNA genes and all 22 tRNA genes for all specimens, except *I. guentheri*. The mitogenome sequences of *I. nasuta*, one specimen of *I. henselii* and *I. guentheri* are complete and circular, but we could not annotate the tRNA-T in *I. guentheri*, probably because it is in a low coverage region. Other specimens are incomplete only in the control region (Supplementary Table S1; available at https://figshare.com/s/1537a00d5bf67288030e). Generally, the gene order observed agrees with most previously sequenced neobatrachian frogs (Zhang et al. 2013), with two main exceptions: the position of the putative origin of replication of the L-strand (O_L), which we found between tRNA-A and tRNA-N (cluster WA(O_L)NCY) and not between tRNA-N and tRNA-C (cluster WAN(O_L)CY); and the organization of the LTPF cluster in the 5′ of the 12S rRNA gene, which differs in the *Ischnocnema* by the tRNA-L and tRNA-T being located within the control region (see Supplementary Figure S1). We also found a new arrangement for the cluster LTPF in *I. erythromera*, in which the tRNA arrangement is PLTF. These tRNAs clusters are known to be a hotspot of gene rearrangement in amphibians (San Mauro et al. 2006; Bernt, Braband, et al. 2013; Zhang et al. 2013) and the rearrangements in these regions are normally interpreted as a result of replication errors near the replication origins O-L and O-H (Kurabayashi and Sumida 2013).

For phylogenetic inference, *Ischnocnema* sequences were aligned to publish complete or near complete genomes of four outgroups (Table 1) using the software MAFFT v.7 (Katoh and Standley 2013). To avoid ambiguous alignments, we used only protein-coding and rRNA genes in the analyses. Search for the best partition scheme and best fitting nuclear substitution models was performed with PartitionFinder 2.1.1 (Lanfear et al. 2017). Phylogenetic analyses were performed under Bayesian inference (BI), maximum likelihood (ML), and maximum parsimony (MP) optimality criteria with the software MrBayes 3.2.6 (Ronquist et al. 2012), RAxML 8.2.11 (Stamatakis 2014), and TNT 1.5 (Goloboff and Catalano 2016), respectively. The best partition scheme with respective best-fitting substitution models and details on each phylogenetic analysis are given in Supplemental Online Material.

The three phylogenetic analyses are congruent and show all *Ischnocnema* species as a fully supported clade which is placed with high support as sister group to *Eleutherodactylus atkinsi* (Figure 1). The two species of *Pristimantis* (Jiménez de la Espada 1870) also appear as a fully supported clade. *Ischnocnema erythromera* is the sister species of all other *Ischnocnema* in our tree and *I. guentheri* and *I. henselii*, as well as *I. nasuta* and *I. oea*, appear, respectively, as sister species. The only clade in our phylogeny that did not receive strong support was *I. nasuta* + *I. oea,* but only in the ML analysis (56% of bootstrap replicates). These results are congruent with the previous phylogenetic hypothesis encompassing all these *Ischnocnema* species based on a selection of mitochondrial and nuclear genes (Canedo and Haddad 2012 Taucce et al. 2018). The mitogenomes assembled here provide important

Table 1. GenBank accession numbers, collection numbers, local of collection, and geospatial coordinates of specimens of the *Ischnocnema, Gastrotheca, Pristimantis,* and *Eleutherodactylus* species used in this study.

Species	GB accession number	Voucher	Local of collection	Coordinates (decimal degrees)
I. erythromera		MH492735CFBH 40985	Teresópolis, RJ, Brazil	−22.45386, −42.99235
I. guentheri		MH492737CFBH 27440	Rio de Janeiro, RJ, Brazil	−22.96192, −43.28912
I. henselii		MH492729CFBH41854	Bertioga, SP, Brazil	−23.73123, −46.17280
I. henselii		MH492732CFBH 35837	Miracatu, SP, Brazil	−24.28223, −47.46796
I. henselii		MH492733CFBH 27554	São Bonifácio, SC, Brazil	−27.87721, −48.94057
I. henselii		MH492731CFBH 39280	São Francisco do Sul, SC, Brazil	−26.22797, −48.68011
I. henselii		MH492730LGE 10099	San Pedro, Misiones, Argentina	−26.90000, −53.86667
I. nasuta		MH492734CFBH 40981	Nova Friburgo, RJ, Brazil	−22.28923, −42.67095
I. oea		MH492736CFBH 40987	Santa Teresa, ES, Brazil	−19.90706, −40.54034
G. pseustes	JX564866	TNHC 62492	Tixán, Chimborazo, Ecuador	–
P. thymelensis	JX564889	TNHC-GDC 14370	–	–
P. fenestratus	KT221610	–	Carolina, MA, Brazil	–
E. atkinsi	JX564864	MVZ 241209	Isla de la Juventud, Cuba	21.65495, −82.75922

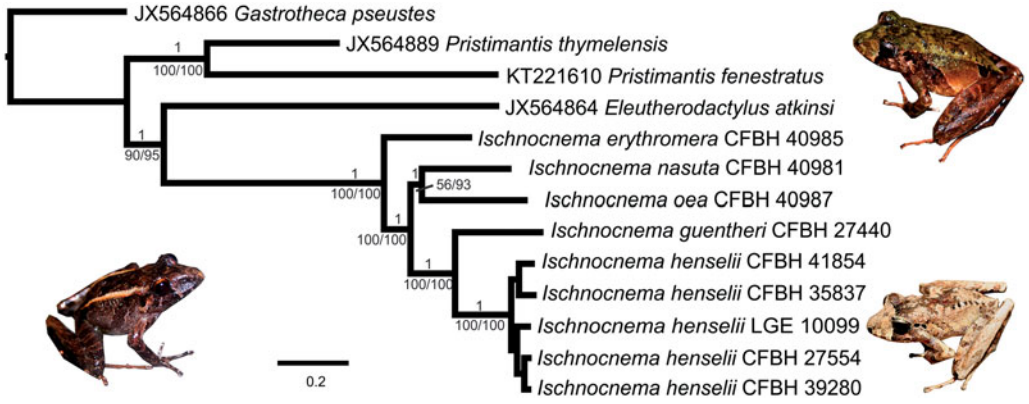

Figure 1. The 50% majority rule consensus tree from Bayesian inference analysis of mitogenomic sequences of Ischnocnema and four outgroups. Numbers above branches are posterior probabilities and numbers below branches are maximum likelihood bootstrap replicates (left) and maximum parsimony jackknife replicates (right). No support below species level is shown. Pictures show *Ischnocnema nasuta* (left), *I. erythromera* (above, right), and *I. henselii* (below, right).

information regarding the relationships within the *I. guentheri* species series and their genomic evolution.

Disclosure statement

No potential conflict of interest was reported by the authors.

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