Mitochondrial genomes and phylogenetic analysis of Central American weakly-electric fishes: *Apteronotus rostratus*, *Brachyhypopomus occidentalis* and *Sternopygus dariensis*

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Abstract

Electric fishes are a diverse group of freshwater organisms with the ability to generate electric organ discharges (EODs) that are used for communication and electrolocation. Over 200 species have originated in South America, but of these, only a few have managed to colonize the Central American Isthmus. Here, we assembled two complete and one nearly complete mitochondrial genomes (mitogenomes) for three Central American Gymnotiformes: *Sternopygus dariensis*, *Brachyhypopomus occidentalis* and *Apteronotus rostratus*. We then explored the three species' phylogenetic position in the context of South American electric fishes. Mitogenomes were organized in the standard fish mitogenome order, and presented sizes of 16,600, 16,540 and 15,940 base pairs (bp) (nearly complete) for *S. dariensis*, *B. occidentalis* and *A. rostratus*, respectively. We uncovered a novel 60 bp intergenic spacer (IGS) located between the *COII* and $tRNA^{Lys}$ genes, which appears to be unique to the Apteronotidae. Furthermore, phylogenetic relationships supported the traditional monophyly of Gymnotiformes, with the three species positioned within their respective family. In addition, the genus *Apteronotus* was placed as the basal taxon of the order. Finally, we found high sequence divergence (13.3%) between our *B. occidentalis* specimen and a sequence previously reported in GenBank, suggesting that the prior mitogenome of *B. occidentalis* represents a different South American species that was misidentified. Indeed, phylogenetic analyses using *Cytochrome b* gene across the genus placed the previously reported individual within *B. bennetti*. Our study provides novel mitogenome resources that will advance our understanding of the diversity and phylogenetic history of Neotropical fishes.

Keywords: Gymnotiformes; Intergenic spacer; Mitogenome; Next generation sequencing; Panama

Introduction

Electric fishes (Teleostei, Gymnotiformes) are a highly diverse group of freshwater organisms that originated in South America (Albert, 2001). One of the defining features of these fishes is their ability to produce electric organ discharges (EODs) that are used for communication and electrolocation (Moller 1995; Bullock et al. 2005). EODs are species-specific electric signals that can be divided into pulse-type and wave-type, depending on the shape and regularity of the discharge. In addition, there is evidence for reproductive character displacement in EOD waveform in this group (Crampton et al., 2011), which has over 200 currently described species.

Electric fishes are widely distributed in lowland freshwater habitats throughout South America (Albert and Crampton, 2005; Hulen et al., 2005). In Central America, however, only 6 species and five genera have been reported thus far, including *Apteronotus*, *Brachyhypopomus*, *Eigenmannia*, *Gymnotus* and *Sternopygus* (Alda et al., 2013; Reis et al., 2003). Despite the high diversity of Neotropical electric fishes, limited genomic resources are currently available for the group, particularly for Central American species. For instance, to date, only nine mitochondrial genomes of Gymnotiformes have been deposited in GenBank (Elbassiouny et al., 2016; Lavoué et al., 2012; Nakatani et al., 2011), but none of these mitogenomes belong to a Central American species. In the case of *B. occidentalis*, it is difficult to determine if the individual from South America previously reported by Lavoué et al. (2012) corresponds to the Central American species, particularly, because *B. occidentalis* presents a wide geographic distribution in South and Central America (Crampton et al., 2016b), and species-level divergence is likely to exist across the species' range (Bermingham and Martin, 1998; Picq et al., 2014). This knowledge gap is important because the dynamic history of the Central American Isthmus has led to a complex evolutionary and phylogeographic history among electric and other primary freshwater fishes (Bermingham

and Martin, 1998; Picq et al., 2014). Thus, generating molecular datasets – including complete mitogenomes – for Central American species of electric fishes is valuable to improve our understanding of diversification in Neotropical environments.

Here, we report for the first time full mitogenome sequences for three Central American weakly-electric fishes: two wave-type species – *Apteronotus rostratus* and *Sternopygus dariensis* – and the pulse-type *Brachyhypopomus occidentalis.* We also compile currently available mitogenomic data to assess the phylogenetic position of the three species within Gymnotiformes. In addition, we estimate genetic distances across complete mitochondrial genomes of three *Brachyhypopomus* individuals available in Genbank. Our study provides novel genomic resources that could facilitate further work on the conservation genetics, phylogenetics, and evolution of Central American Gymnotiformes as well as other freshwater fishes.

2. Materials and methods

2.1. Study site and sampling protocol

We collected three individuals from each of the following species: *A. rostratus, S. dariensis* and *B. occidentalis* in La Hoya stream, which flows into the Chucunaque River in the Darien Province, eastern Panama (N 8.2536, W -77.7189). Fish were detected using wire-electrodes connected to a mini-amplifier (Radio/Shack, Fort Worth, TX), and collected using a hand-net. Fish were euthanized with an overdose of eugenol ($C_{10}H_{12}O_2$) derived from clove oil. Our collecting protocol was authorized by Ministerio de Ambiente (Mi Ambiente; permit number SE/A-100-14) and approved by the Institutional Animal Care and Use Committee (IACUC-16-001) at the Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT AIP).

94 *2.2. Sequencing*

95 Mitochondrial genomes were obtained as the byproduct of Next Generation
96 Sequencing (NGS) of Ultraconserved Elements (UCEs; Faircloth et al., 2012), as part of
97 ongoing studies on the population genomics of the weakly-electric fish *B. occidentalis*. We
98 prepared UCEs libraries following a standard protocol (available from
99 http://ultraconserved.org) using the 500 loci Actinopterygii probe set (Actinopterygians
100 0.5Kv1; Faircloth et al., 2013). Libraries were sequenced using 300 bp paired-end Illumina
101 MiSeq platform (Illumina, San Diego, CA) at the Smithsonian Tropical Research Institute
102 (STRI) Naos Molecular Lab in Panama City, Panama.
103
104 *2.3. Mitogenome assembly and annotation*
105 We followed Aguilar et al. (2016) to generate mitogenomes from UCE sequencing
106 reads. Briefly, we used Illumiprocessor (Faircloth, 2013), which employs Trimmomatic
107 (Bolger et al., 2014) to clean and trim reads. We then assembled all reads using Trinity
108 (Grabherr et al., 2011). Contigs larger than 15,000 bp were subjected to searches of sequence
109 similarity using the BLAST algorithm to compare the query sequences with sequences from
110 GenBank-NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm the presence of the
111 mitochondrial genome. We annotated genes using the MitoFish and MitoAnnotator (Iwasaki
112 et al., 2013) and also inspected alignments manually by comparing them with Genbank
113 reference mitogenomes from *A. albifrons* (Accession no. AB054132), *S. arenatus* (Accession
114 no. KX058571), *B. verdii* (*B. n.sp.* VERD – Accession no. KX058570) and *B. occidentalis*
115 (Accession no. AP011570) in Geneious version 11.1.4 (http://www.geneious.com, Kearse et
116 al., 2012).
117 Nucleotide base composition was also calculated in Geneious version 11.1.4
118 (http://www.geneious.com, Kearse et al., 2012). Strand asymmetry was calculated using the

following formulas: AT-skew=(A–T)/(A+T) and GC-skew=(G–C)/(G+C), which allowed us to measure the nucleotide compositional difference between complete mitogenomes (Perna and Kocher, 1995).

2.4. Phylogenetic analysis

We used MAFFT (Katoh and Standley, 2013) to describe phylogenetic relationships among available mitogenomes of Gymnotiformes. For these analyses, we excluded the control region, and used the characiform *Astyanax paranae* as outgroup (Genbank accession no. KX609386). We tested for the best-fit model based on Akaike Information Criterion (AIC), corrected Akaike Information Criterion (AICc) and Bayesian Information Criterion (BIC), which identified the GTR+I+G model as best-fitting to our data. Bayesian inference (BI) and maximum-likelihood (ML) analysis were performed using the CIPRES Science Gateway v3.3 cluster (Miller et al., 2010). We performed two independent runs of MrBayes v.3.2.6 (Ronquist et al., 2012) using 8,000,000 Markov Chain Monte Carlo iterations (MCMC), with four simultaneous chains, and sampling every 1000 generations. Support for node and parameter estimates were derived from a majority rule consensus of the last 5,000 trees sampled after convergence. In addition, we generated a maximum likelihood phylogeny using RAxML (Stamatakis, 2014) with 1000 bootstrap replicates.

2.5. Genetic divergence in Brachyhypopomus *mitogenomes*

To assess genetic distances among the three *Brachyhypopomus* mitogenomes, we estimated the proportion of nucleotide differences (uncorrected p-distance; Nei and Kumar, 2000) for each protein coding gene separately. Standard errors were calculated using 500 bootstrap replicates in MEGA v7 (Kumar et al., 2016). We also estimated sequence divergence across species of *Brachyhypopomus* using *COI* (e.g., the barcoding gene; Hebert

et al., 2003) data available in Genbank (Bermingham and Martin, 1998; Picq et al., 2014). Finally, to confirm the species identity of available *Brachyhypopomus* mitogenomes, we built a phylogenetic tree using a dataset of 83 *Cyt b* sequences representing 26 species reported in Crampton et al. (2016a).

3. Results and Discussion

3.1. Mitogenome structure

Minimum sequence cover for our three mitogenomes was 26X. The size of the complete mitogenomes was 16,600 bp for *S. dariensis* (Genbank accession no. MH399590) and 16,540 bp for *B. occidentalis* (Genbank accession no. MH399591), while the nearly complete mitochondrial genome of *A. rostratus* had 15,940 bp (Genbank accession no. MH399592). All three mitogenomes contained 2 ribosomal genes (12S and 16S), 22 tRNAs, 13 protein-coding genes (PCGs), as well as a control region (Figure 1). They also contained similar gene counts and organization as in other Gymnotiformes (Elbassiouny et al., 2016; Lavoué et al., 2012; Nakatani et al., 2011).

The nucleotide composition showed a strand bias consistent with the strand asymmetry observed in other fishes (Cheng et al. 2012; Hao et al. 2016). Specifically, the A + T content was 57.8%, 54.1% and 57.1% for *A. rostratus*, *B. occidentalis* and *S. dariensis*, respectively. The average AT-skew was 0.08, ranging from 0.05 in *B. occidentalis* to 0.09 in *S. dariensis*. The average GC-skew was -0.32, ranging from -0.34 in *S. dariensis* to -0.28 in *B. occidentalis*. The three mitogenomes also showed the typical structure of other Gymnotiformes (Elbassiouny et al., 2016). This included the 13 PCGs encompassing ~69% (11440 bp) of the total mitogenome; twelve of which were on the forward strand, while ND6 was on the reverse strand. Overall, we found ~3,791 codons, excluding stop codons, that were predicted for codon usage across the three mitogenomes. The start codon in *S. dariensis* and

169 *B. occidentalis* was a typical ATN codon, but the start codon in the *COI* gene was GTG for
170 all three species, which is consistent with other fish mitogenomes (Satoh et al. 2016; Shi et al.
171 2016). However, *A. albifrons* mitogenome shows alternative start codons in three genes:
172 GTG for *ATPase8* and *ND6*, and ACG for *ND4L*.

173 In fishes, the ACG start codon has been found particularly in the *A. albifrons ND4L*
174 gene (Satoh et al. 2016), but was also recently reported in the *ND1* gene of the perciform
175 *Otolithes ruber* (Guo et al. 2017). The three species shared the TAA stop codon in 3 PCGs
176 (*ND1, ND4L and ND5*), while AGA and AGG stop codons were present in *COI* (except in *B.*
177 *occidentalis*, TAA) and *ND6* (except in *A. rostratus*, AGA), respectively. The remaining
178 PCGs (*ND2, COII, ATPase6, COIII, ND3, ND4* and *Cyt B*) had TAG, TAA or the incomplete
179 stop codons TA/T, which are presumably completed during post- transcriptional
180 polyadenylation (Ojala et al., 1981). This pattern of stop codons is also common in other
181 fishes (Kim et al., 2006; Nakatani et al., 2011). In addition, there were the typical 22 tRNAs
182 predicted by Mitofish and tRNAscan, with a length ranging from 66 bp to 75 bp and
183 including two $tRNA^{Leu}$ and two $tRNA^{Ser}$. The two rRNA genes were located between $tRNA^{Phe}$
184 and $tRNA^{Leu}$ and were separated by $tRNA^{Val}$.

185
186 *3.2. Non-coding regions, intergenic spacers and overlapping*

187 We found small intergenic spacers (IGS) ranging in size from 1–60 bp, and totalling
188 58 bp in *S. dariensis*, 68 bp in *B. occidentalis* and 134 bp in *A. rostratus* (Table. 1). These
189 IGS regions were mostly similar across species and represent a common feature of
190 Gymnotiformes. One of these spacers, with a size of 29 to 31 bp, represents the origin of L-
191 strand replication (OL), and is located between $tRNA^{Asn}$ and $tRNA^{Cys}$. We also found a large
192 IGS of 955 bp that belongs to the Control Region (D-loop) in both *S. dariensis* and *B.*
193 *occidentalis*. This spacer was only partially recovered in *A. rostratus*. We also observed 8
194 gene overlaps with a total of 31 bp; the two longest of which contained 10 bp

(between *ATPase8* and *ATPase6*) and 7 bp (between *ND4L* and *ND4*) (Table 1).

3.3. Novel COII/tRNA-Lys intergenic spacer in Apteronotus

We uncovered a 60 bp IGS between *COII* and $tRNA^{Lys}$ in both *A. rostratus* (from present study) and *A. albifrons* (from GenBank; AB054132). However, this IGS was not found in any of the other available gymnotiform mitogenomes (Figure 2). This spacer showed clear similarity between the two *Apteronotus* mitogenomes that were sequenced independently, which suggests that this IGS represents a unique feature of the genus *Apteronotus*. To our knowledge, this is the first report of a long IGS occurring between the genes *COII* and $tRNA^{Lys}$ in the order Gymnotiformes. In other fishes, the presence of unique IGS has been reported between the genes $tRNA^{Thr}$ and $tRNA^{Pro}$ in Gadiformes (Bakke et al., 1999; Jørgensen et al., 2014), including walleye pollock, *Theragra chalcogramma* (Poulsen et al., 2013), whiting *Merlangius merlangus* and haddock *Melanogrammus aeglefinushiting* (Roques et al., 2006).

Currently, the origin of these IGS and their apparent absence in other Gymnotiformes is not well understood. If we accept a basal phylogenetic position of *Apteronotus* (Figure 3), one possibility is that purifying selection on non-coding regions (Rand, 1993) led to reduction in mitogenome size during the evolutionary history of Gymnotiformes. However, further work is necessary to determine the biological implications of these IGS in Gymnotiformes. Overall, we suggest that comparative studies of this unique mitogenomic feature across species could help elucidate the phylogenetic history of the group. In addition, further work should explore the use of these IGS as genetic markers for the genus Apteronotus or the entire Apteronotidae.

3.4. Phylogenetic and taxonomic implications

Sternopygus dariensis and *S. arenatus*, *A. rostratus* and *A. albifrons* as well as *B. occidentalis*, *B. verdii* (*B.* n.sp. VERD - Genbank KX058570) and *B. occidentalis* (AP011570) showed a monophyletic relationship with respect to each genus (Figure 2), confirming the phylogenetic position of Central American electric fishes within Gymnotiformes as a whole, consistent with Elbassiouny et al. (2016) and Tagliacollo et al. (2016). Our phylogenetic analysis recovered the monophyly of the order Gymnotiformes, and placed *Apteronotus* at the base of the order, in agreement with a recent mitogenomic study (Elbassiouny et al., 2016), but in contrast to the conclusions of Tagliacollo et al. (2016). Whereas the genetic results of the latter study were inconclusive with respect to the position of the apteronotids, their morphology-based tree identified Apteronotidae as a derived group within the Sinusoidea (Sternopygidae and Apteronotidae).

We found over 13% sequence divergence between our complete *B. occidentalis* mitogenome, and the one conspecific mitogenome available in Genbank. At individual genes, our analysis of p-distances revealed values ranging from 12.2% in *COII* to 19.9% in *ND4L*. For protein coding genes, average divergence was 15.6% (Table 2). We believe that our mitogenome represents the correct sequence for *B. occidentalis* given the 99.8% similarity between our sequence and previously sequenced individuals of *B. occidentalis* from Panama (Picq et al., 2014), but only 90.6% similarity with the *B. occidentalis* mitogenome reported by Lavoué et al. (2012; Accession no. AP011570). Indeed, our subsequent phylogenetic analysis of the genus *Brachyhypopomus* using *Cyt b* data from Crampton et al. (2016a) placed that individual within the *B. bennetti* clade from South America rather than with *B. occidentalis* of Central America (Figure S1).

Overall, our study expands our understanding of the evolution and structure of mitochondrial genomes in Central American freshwater fishes. In addition, it generates novel

molecular data that can be used to solve the taxonomic status as well as the phylogenetic history of Neotropical electric fishes.

Disclosure statement

The authors declare that they have no conflict of interest.

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382 Table 1. Characteristics of the mitochondrial genomes of Central American electric fishes.

383 The table shows the mitogenome structure of three species in the following order: *S.*

384 *dariensis, B. occidentalis* and *A. rostratus*.

Amino acid/gene	Start	Stop	Size	Spacer (+) or overlap (-)	Direction	Start Codon	Stop Codon
$tRNA^{Phe}$	36892	68/69/69	68/69/69	0/0/0	F		
12S rRNA	69/70/70	1020/1018/1016	952/949/947	0/0/0	F		
$tRNA^{Val}$	1021/1019/1017	1092/1089/1088	72/71/72	0/0/0	F		
16S rRNA	1093/1090/1089	2766/2750/2760	1674/1661/1672	0/0/0	F		
$tRNA^{Leu}$	2767/2751/2761	2841/2825/2835	75/75/75	1/0/2	F		
ND1 gene	2843/2826/2838	3817/3785/3806	975/960/969	2/4/5	F	ATG/ATT/ATT	TAA/TAA/TAA
$tRNA^{Ile}$	3820/3790/3812	3891/3862/3883	72/73/72	-2/-2/-2	F		
$tRNA^{Gln}$	3890/3861/3882	3960/3931/3952	71/71/71	-1/-1/-1	R		
$tRNA^{Met}$	3960/3931/3952	4029/4000/4020	70/70/70	0/0/0	F		
ND2 gene	4030/4001/4021	5074/5045/5070	1045/1045/1050	0/0/4	F	ATG/ATG/ATA	T-/T-/TAA
$tRNA^{Trp}$	5075/5046/5075	5144/5117/5145	70/72/71	0/1/1	F		
$tRNA^{Ala}$	5145/5119/5147	5213/5186/5215	69/68/69	1/1/1	R		
$tRNA^{Asn}$	5215/5188/5217	5287/5260/5289	73/73/73	31/29/30	R		
$tRNA^{Cys}$	5319/5290/5320	5384/5356/5385	66/67/66	0/1/0	R		
$tRNA^{Tyr}$	5385/5358/5386	5454/5426/5454	70/69/69	1/1/1	R		
COI gene	5456/5428/5456	7021/6987/7015	1566/1560/1560	-5/2/-5	F	GTG/GTG/GTG	AGA/TAA/AGA
$tRNA^{Ser}$	7017/6990/7011	7087/7060/7081	71/71/71	4/4/5	R		
$tRNA^{Asp}$	7092/7065/7087	7160/7133/7155	69/69/69	14/13/11	F		
COII gene	7175/7147/7167	7866/7837/7847	692/691/681	0/0/60	F	ATG/ATG/ATG	TA-/T-/TAA
$tRNA^{Lys}$	7867/7838/7908	7940/7912/7981	74/75/74	1/1/1	F		
ATPase8 gene	7942/7914/7983	8109/8078/8144	168/165/162	-10/-7/-4	F	ATG/ATG/GTG	TAA/TAG/TAG
ATPase6 gene	8100/8072/8141	8782/8754/8823	683/683/683	0/0/0	F	ATG/ATG/ATA	TA-/TA-/TA-
COIII gene	8783/8755/8824	9566/9538/9607	784/784/784	0/1/0	F	ATG/ATG/ATG	T-/T-/T-
$tRNA^{Gly}$	9567/9540/9608	9637/9609/9678	71/70/71	0/0/1	F		
ND3 gene	9638/9610/9680	9987/9959/10030	350/350/351	0/0/6	F	ATG/ATA/ATC	TA-/TA-/TAA
$tRNA^{Arg}$	9988/9960/10037	10057/10029/10106	70/70/70	0/0/0	F		
ND4L gene	10058/10030/10107	10354/10326/10403	297/297/297	-7/-7/-7	F	ATG/ATA/ACG	TAA/TAA/TAA
ND4 gene	10348/10320/10397	11728/11700/11777	1381/1381/1381	0/0/0	F	ATG/ATG/ATG	T-/T-/T-
$tRNA^{His}$	11729/11701/11778	11797/11769/11846	69/69/69	0/0/0	F		
$tRNA^{Ser}$	11798/11770/11847	11865/11836/11913	68/67/67	1/1/0	F		
$tRNA^{Leu}$	11867/11838/11914	11939/11910/11986	73/73/73	0/0/0	F		
ND5 gene	11940/11911/11987	13775/13713/13819	1836/1803/1833	-5/-5/-5	F	ATG/ATG/ATC	TAA/TAA/TAA
ND6 gene	13771/13709/13815	14292/14230/14336	522/522/522	0/0/0	R	ATG/ATG/GTG	AGG/AGG/AGA
$tRNA^{Glu}$	14293/14231/14337	14361/14299/14405	69/69/69	2/5/6	R		

Cyt B gene	14364/14305/14412	15504/15441/15552	1141/1137/1141	0/4/0	F	ATG/ATG/ATG	T-/TAG/T-
$tRNA^{Thr}$	15505/15446/15553	15576/15518/15623	72/73/71	-1/-2/-2	F		
$tRNA^{Pro}$	15576/15517/15622	15645/15585/15691	70/69/70	0/0/0	R		
control region D-loop	15646/15586/15692	1660/16540/15940	955/955/249	0/0/0	F		

385

Table 2. Pairwise genetic distance between specimens of *Brachyhypopomus*. The data represent percentage of uncorrected 'p' genetic distance between *B. occidentalis* vs. *B. occidentalis* (AP011570) and *B. occidentalis* vs. *B. n.sp.* VERD, based on 13 protein-coding genes (PCGs).

	ATPase6	**ATPase8**	**COI**	**COII**	**COIII**	**Cyt B**	**ND1**	**ND2**	**ND3**	**ND4**	**ND4L**	**ND5**	**ND6**
B. occidentalis vs. *B. occidentalis* AP011570	16.7	12.6	14.8	12.2	13.8	14.5	16.5	16.8	17.2	15.7	19.9	16.3	15.6
B. occidentalis vs. *B. n.sp.* VERD	18.4	13.8	14	13.7	13.7	13.3	16	17.5	16.7	15.8	18.2	15.9	18.1

Figure captions

Figure 1. Comparison of the mitochondrial genome of *B. occidentalis* and *A. rostratus* against *S. dariensis*. Rings represent the mitogenome map of. *B. occidentalis* (outer ring), *A. rostratus* (middle), and *S. dariensis* (inner). The protein coding regions are labeled in green, tRNA genes are labeled in pink, rRNA genes are labeled in red and the putative control region is labeled in orange colors. The intensity of color of each of the two outer rings represents the proportion (70 to 100%) of conserved sequence at that region.

Figure 2. Phylogenetic relationships among Gymnotiformes based on MrBayes and RAxML. The phylogeny represents the best-scoring maximum likelihood tree based on complete mitogenomes (excluding the D-Loop). The first number at each node is Bayesian posterior probability and the second number is bootstrap probability of ML analyses. The scale bar indicates relative branch lengths.

Figure 3. Alignment of partial mitogenome sequences of Gymnotiformes species. The 60 bp intergenic spacer, colored in red, is observed in the mitogenome of *A. rostratus* and *A. albifrons*. Individuals in bold represent sequences from the present study. Genes in color are encoded on the light strand, *COII* (grey) and $tRNA^{Lys}$ (blue). Complete and partial stop codons are shown in black. Gaps are represented by dashes.

Fig. 1

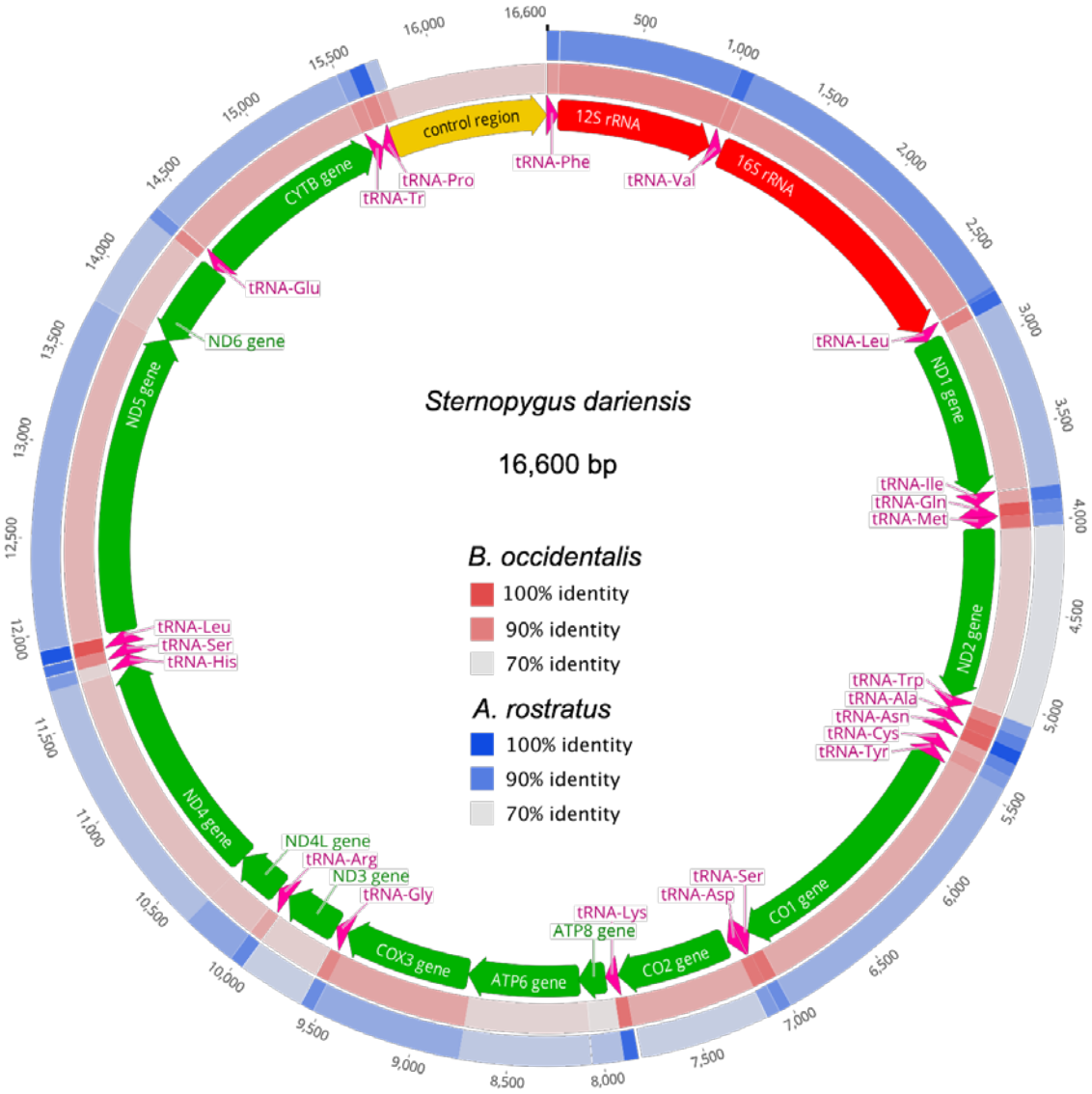


Fig. 2

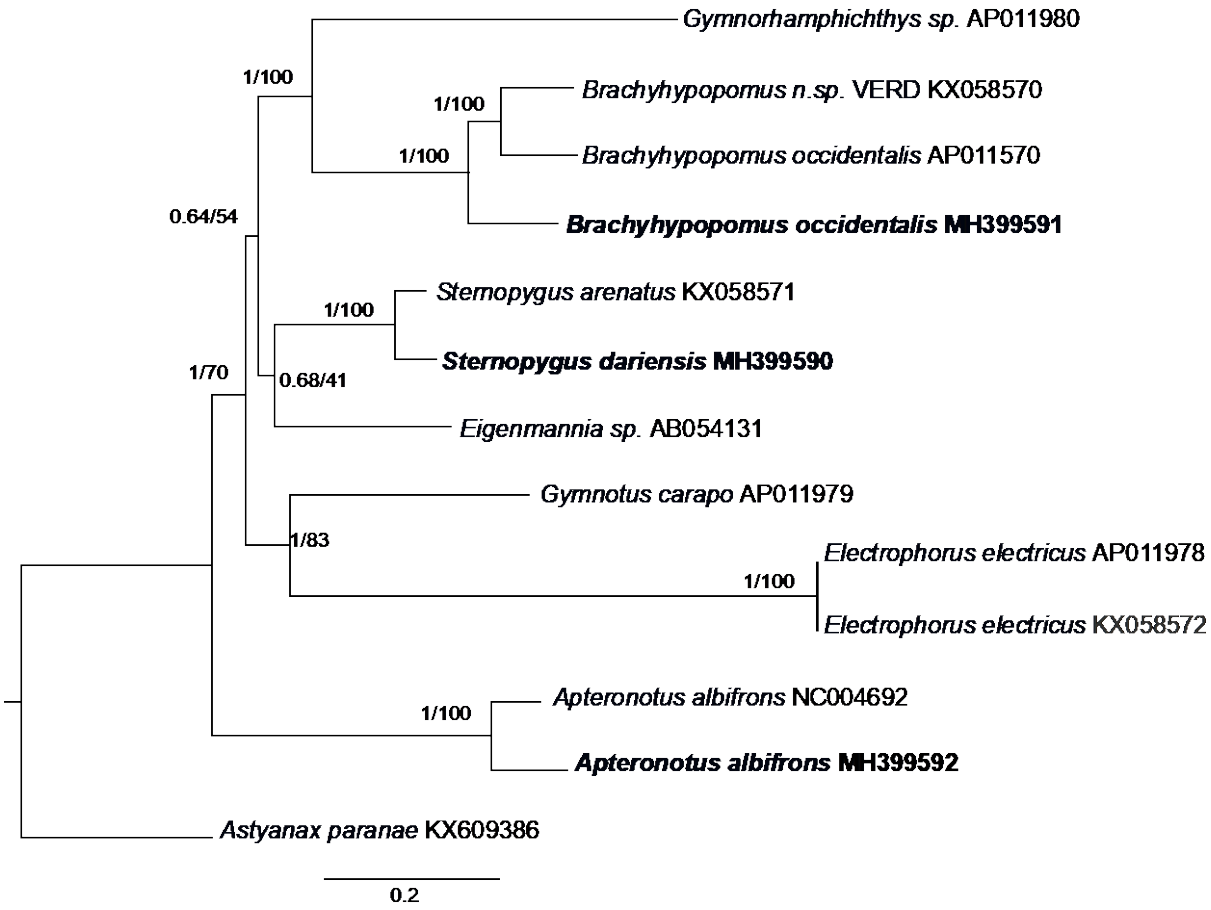
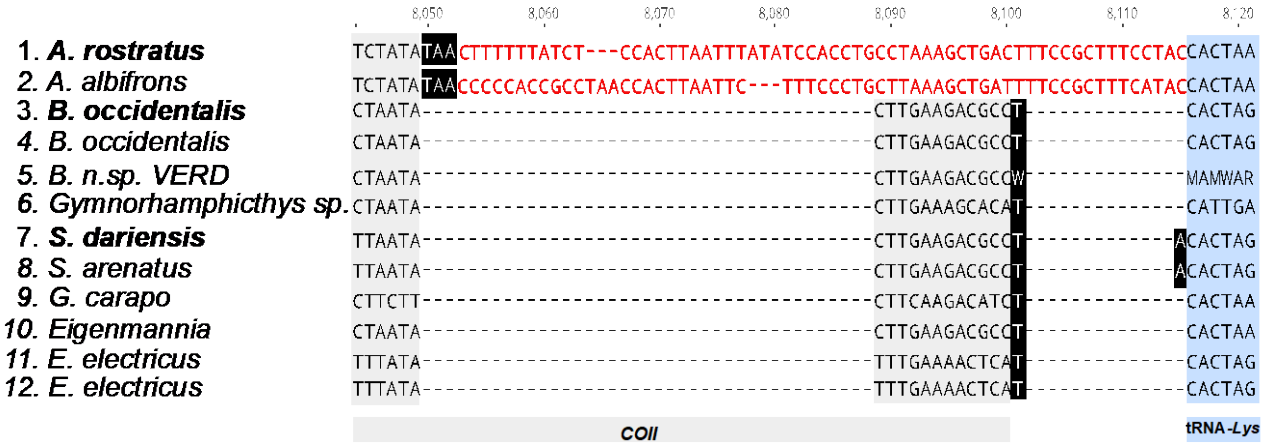


Fig. 3



ATPase6 and *ATPase8*, ATPase subunit 6 and 8 genes; *Cyt B*, cytochrome b gene; *COI-III*, cytochrome oxidase subunits I-III genes; NCR, non-coding region; *ND1–6* and *ND4L*, NADH dehydrogenase subunits 1-6 and 4L genes; ML, maximum likelihood; BI, Bayesian inference; *rRNA*, ribosomal RNA; *16S* and *12S*, large and small subunits of ribosomal RNA genes; *tRNA*, transfer RNA; PCG, protein coding gene; mitogenome, mitochondrial genome; *Ala*, alanine; *Arg*, arginine; *Asn*, asparagine; *Aps*, aspartic acid; *Cys*, cysteine; *Gln*, glutamine; *Glu*, glutamic acid; *Gly*, glycine; *His*, histidine; *Ile*, isoleucine; *Leu*, leucine; *Lys*, lysine; *Met*, methionine; *Phe*, phenylalanine; *Pro*, proline; *Ser*, serine; *Thr*, threonine; *Trp*, tryptophan; *Tyr*, tyrosine; *Val*, valine.

Highlights

1. The mitochondrial genomes of three Central American electric fishes, *Apteronotus rostratus*, *Brachyhypopomus occidentalis* and *Sternopygus dariensis*, are sequenced and characterized.
2. The presence of a novel 60 bp intergenic spacer located between *COII* and $tRNA^{Lys}$ is reported for the first time in Gymnotiformes and may represent a unique feature of the *Apteronotus* mitogenome.
3. Phylogenetic analyses support the position of Central American *A. rostratus*, *B. occidentalis* and *S. dariensis* within monophyletic Gymnotiformes.
4. Genetic divergence and phylogenetic analyses indicate that the mitogenome of *B. occidentalis* (Genbank AP011570) reported previously belongs to *B. bennetti* from South America.

Supplemental material

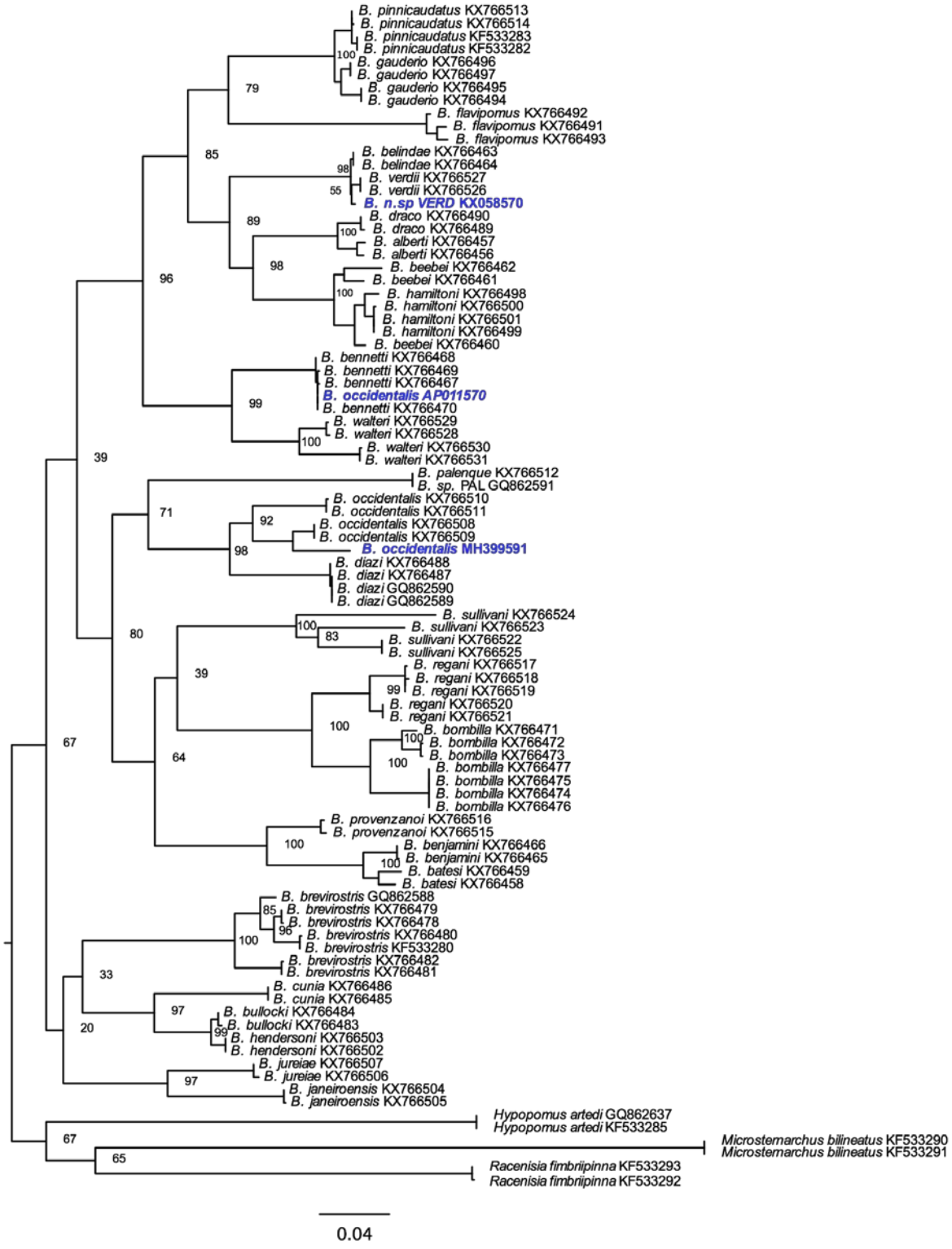


Figure S1. RAxML phylogenetic tree of 26 *Brachyhypopomus* species based on the *Cyt b* gene. Individuals in blue represent mitogenomes reported here (Genbank MH399591) and two additional sequences obtained from GenBank (AP011570, KX058570).