

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

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## 21 **Abstract**

22 Electric fishes are a diverse group of freshwater organisms with the ability to generate  
23 electric organ discharges (EODs) that are used for communication and electrolocation. Over  
24 200 species have originated in South America, but of these, only a few have managed to  
25 colonize the Central American Isthmus. Here, we assembled two complete and one nearly  
26 complete mitochondrial genomes (mitogenomes) for three Central American Gymnotiformes:  
27 *Sternopygus dariensis*, *Brachyhypopomus occidentalis* and *Apteronotus rostratus*. We then  
28 explored the three species' phylogenetic position in the context of South American electric  
29 fishes. Mitogenomes were organized in the standard fish mitogenome order, and presented  
30 sizes of 16,600, 16,540 and 15,940 base pairs (bp) (nearly complete) for *S. dariensis*, *B.*  
31 *occidentalis* and *A. rostratus*, respectively. We uncovered a novel 60 bp intergenic spacer  
32 (IGS) located between the *COII* and tRNA<sup>Lys</sup> genes, which appears to be unique to the  
33 Apteronotidae. Furthermore, phylogenetic relationships supported the traditional monophyly  
34 of Gymnotiformes, with the three species positioned within their respective family. In  
35 addition, the genus *Apteronotus* was placed as the basal taxon of the order. Finally, we found  
36 high sequence divergence (13.3%) between our *B. occidentalis* specimen and a sequence  
37 previously reported in GenBank, suggesting that the prior mitogenome of *B. occidentalis*  
38 represents a different South American species that was misidentified. Indeed, phylogenetic  
39 analyses using *Cytochrome b* gene across the genus placed the previously reported individual  
40 within *B. bennetti*. Our study provides novel mitogenome resources that will advance our  
41 understanding of the diversity and phylogenetic history of Neotropical fishes.

42

43 **Keywords:** Gymnotiformes; Intergenic spacer; Mitogenome; Next generation sequencing;

44 Panama

## 45 **Introduction**

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

54 Electric fishes are widely distributed in lowland freshwater habitats throughout South  
55 America (Albert and Crampton, 2005; Hulen et al., 2005). In Central America, however, only  
56 6 species and five genera have been reported thus far, including *Apteronotus*,  
57 *Brachyhypopomus*, *Eigenmannia*, *Gymnotus* and *Sternopygus* (Alda et al., 2013; Reis et al.,  
58 2003). Despite the high diversity of Neotropical electric fishes, limited genomic resources are  
59 currently available for the group, particularly for Central American species. For instance, to  
60 date, only nine mitochondrial genomes of Gymnotiformes have been deposited in GenBank  
61 (Elbassiouny et al., 2016; Lavoué et al., 2012; Nakatani et al., 2011), but none of these  
62 mitogenomes belong to a Central American species. In the case of *B. occidentalis*, it is  
63 difficult to determine if the individual from South America previously reported by Lavoué et  
64 al. (2012) corresponds to the Central American species, particularly, because *B. occidentalis*  
65 presents a wide geographic distribution in South and Central America (Crampton et al.,  
66 2016b), and species-level divergence is likely to exist across the species' range (Bermingham  
67 and Martin, 1998; Picq et al., 2014). This knowledge gap is important because the dynamic  
68 history of the Central American Isthmus has led to a complex evolutionary and  
69 phylogeographic history among electric and other primary freshwater fishes (Bermingham

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

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

## 82 **2. Materials and methods**

### 83 *2.1. Study site and sampling protocol*

84 We collected three individuals from each of the following species: *A. rostratus*, *S.*  
85 *dariensis* and *B. occidentalis* in La Hoya stream, which flows into the Chucunaque River in  
86 the Darien Province, eastern Panama (N 8.2536, W -77.7189). Fish were detected using wire-  
87 electrodes connected to a mini-amplifier (Radio/Shack, Fort Worth, TX), and collected using  
88 a hand-net. Fish were euthanized with an overdose of eugenol (C<sub>10</sub>H<sub>12</sub>O<sub>2</sub>) derived from clove  
89 oil. Our collecting protocol was authorized by Ministerio de Ambiente (Mi Ambiente; permit  
90 number SE/A-100-14) and approved by the Institutional Animal Care and Use Committee  
91 (IACUC-16-001) at the Instituto de Investigaciones Científicas y Servicios de Alta  
92 Tecnología (INDICASAT AIP).

93

## 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

119 following formulas:  $AT\text{-skew}=(A-T)/(A+T)$  and  $GC\text{-skew}=(G-C)/(G+C)$ , which allowed us  
120 to measure the nucleotide compositional difference between complete mitogenomes (Perna  
121 and Kocher, 1995).

122

#### 123 2.4. Phylogenetic analysis

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

137

#### 138 2.5. Genetic divergence in *Brachyhypopomus* mitogenomes

139 To assess genetic distances among the three *Brachyhypopomus* mitogenomes, we  
140 estimated the proportion of nucleotide differences (uncorrected p-distance; Nei and Kumar,  
141 2000) for each protein coding gene separately. Standard errors were calculated using 500  
142 bootstrap replicates in MEGA v7 (Kumar et al., 2016). We also estimated sequence  
143 divergence across species of *Brachyhypopomus* using *COI* (e.g., the barcoding gene; Hebert

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

148

### 149 **3. Results and Discussion**

#### 150 *3.1. Mitogenome structure*

151 Minimum sequence cover for our three mitogenomes was 26X. The size of the  
152 complete mitogenomes was 16,600 bp for *S. dariensis* (Genbank accession no. MH399590)  
153 and 16,540 bp for *B. occidentalis* (Genbank accession no. MH399591), while the nearly  
154 complete mitochondrial genome of *A. rostratus* had 15,940 bp (Genbank accession no.  
155 MH399592). All three mitogenomes contained 2 ribosomal genes (12S and 16S), 22 tRNAs,  
156 13 protein-coding genes (PCGs), as well as a control region (Figure 1). They also contained  
157 similar gene counts and organization as in other Gymnotiformes (Elbassiouny et al., 2016;  
158 Lavoué et al., 2012; Nakatani et al., 2011).

159 The nucleotide composition showed a strand bias consistent with the strand  
160 asymmetry observed in other fishes ([Cheng et al. 2012](#); [Hao et al. 2016](#)). Specifically, the A +  
161 T content was 57.8%, 54.1% and 57.1% for *A. rostratus*, *B. occidentalis* and *S. dariensis*,  
162 respectively. The average AT-skew was 0.08, ranging from 0.05 in *B. occidentalis* to 0.09 in  
163 *S. dariensis*. The average GC-skew was -0.32, ranging from -0.34 in *S. dariensis* to -0.28 in  
164 *B. occidentalis*. The three mitogenomes also showed the typical structure of other  
165 Gymnotiformes (Elbassiouny et al., 2016). This included the 13 PCGs encompassing ~69%  
166 (11440 bp) of the total mitogenome; twelve of which were on the forward strand, while ND6  
167 was on the reverse strand. Overall, we found ~3,791 codons, excluding stop codons, that were  
168 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 *NDI* gene of the perciform  
175 *Otolithes ruber* ([Guo et al. 2017](#)). The three species shared the TAA stop codon in 3 PCGs  
176 (*NDI*, *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<sup>Leu</sup>* and two *tRNA<sup>Ser</sup>*. The two rRNA genes were located between *tRNA<sup>Phe</sup>*  
184 and *tRNA<sup>Leu</sup>* and were separated by *tRNA<sup>Val</sup>*.

### 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<sup>Asn</sup>* and *tRNA<sup>Cys</sup>*. 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



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

196

### 197 3.3. Novel *COII*/tRNA-Lys intergenic spacer in *Apteronotus*

198 We uncovered a 60 bp IGS between *COII* and tRNA<sup>Lys</sup> in both *A. rostratus* (from  
199 present study) and *A. albifrons* (from GenBank; AB054132). However, this IGS was not  
200 found in any of the other available gymnotiform mitogenomes (Figure 2). This spacer showed  
201 clear similarity between the two *Apteronotus* mitogenomes that were sequenced  
202 independently, which suggests that this IGS represents a unique feature of the genus  
203 *Apteronotus*. To our knowledge, this is the first report of a long IGS occurring between the  
204 genes *COII* and tRNA<sup>Lys</sup> in the order Gymnotiformes. In other fishes, the presence of unique  
205 IGS has been reported between the genes tRNA<sup>Thr</sup> and tRNA<sup>Pro</sup> in Gadiformes (Bakke et al.,  
206 1999; Jørgensen et al., 2014), including walleye pollock, *Theragra chalcogramma* (Poulsen  
207 et al., 2013), whiting *Merlangius merlangus* and haddock *Melanogrammus aeglefinus* (Roques et al., 2006).

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

218

### 219 3.4. Phylogenetic and taxonomic implications

220 *Sternopygus dariensis* and *S. arenatus*, *A. rostratus* and *A. albifrons* as well as *B.*  
221 *occidentalis*, *B. verdii* (*B.* n.sp. VERD - Genbank KX058570) and *B. occidentalis*  
222 (AP011570) showed a monophyletic relationship with respect to each genus (Figure 2),  
223 confirming the phylogenetic position of Central American electric fishes within  
224 Gymnotiformes as a whole, consistent with Elbassiouny et al. (2016) and Tagliacollo et al.  
225 (2016). Our phylogenetic analysis recovered the monophyly of the order Gymnotiformes, and  
226 placed *Apteronotus* at the base of the order, in agreement with a recent mitogenomic study  
227 (Elbassiouny et al., 2016), but in contrast to the conclusions of Tagliacollo et al. (2016).  
228 Whereas the genetic results of the latter study were inconclusive with respect to the position  
229 of the apteronotids, their morphology-based tree identified Apterontidae as a derived group  
230 within the Sinusoidea (Sternopygidae and Apterontidae).

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

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

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

## 246 **Disclosure statement**

247 The authors declare that they have no conflict of interest.

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## 255 **References**

- 256 Aguilar, C., De León, L.F., Loaiza, J.R., McMillan, W.O., Miller, M.J., 2016. Extreme  
257 sequence divergence between mitochondrial genomes of two subspecies of White-  
258 breasted Wood-wren (*Henicorhina leucosticta*, Cabanis, 1847) from western and central  
259 Panamá. *Mitochondrial DNA* 27, 956–957.
- 260 Albert, J.S., 2001. Species diversity and phylogenetic systematics of American knifefishes  
261 (Gymnotiformes, Teleostei). Museum of Zoology, University of Michigan, Ann Arbor,  
262 MI.
- 263 Albert, J.S., Crampton, W.G.R., 2005. Diversity and phylogeny of neotropical electric fishes  
264 (Gymnotiformes), in: Bullock, T.H., Hopkins, C.D., Popper, A.N., Fay, R.R. (Eds.),  
265 *Electroreception*, Springer Handbook of Auditory Research. Springer New York, pp.  
266 360–409.
- 267 Alda, F., Picq, S., De León, L.F., González, R., Walz, H., Bermingham, E., Krahe, R., 2013.  
268 First record of *Gymnotus henni* (Albert, Crampton and Maldonado, 2003) in Panama:

- 269 phylogenetic position and electric signal characterization. Check List 9, 655–659.
- 270 Bakke, I., Shields, G.F., Johansen, S., 1999. Sequence characterization of a unique intergenic  
271 spacer in Gadiformes mitochondrial DNA. Mar. Biotechnol. 1, 411–415.
- 272 Bermingham, E., Martin, A.P., 1998. Comparative mtDNA phylogeography of neotropical  
273 freshwater fishes: testing shared history to infer the evolutionary landscape of lower  
274 Central America. Mol. Ecol. 7, 499–517.
- 275 Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina  
276 sequence data. Bioinformatics 30, 2114–2120.
- 277 Bullock, T.H., Fay, R.R., Hopkins, C.D., Popper, A.N., 2005. Electroreception. Springer  
278 Handbook of Auditory Research. Springer. New York.
- 279 Cheng, J., Ma, G.Q., Song, N., Gao, T.X., 2012. Complete mitochondrial genome sequence  
280 of bighead croaker *Collichthys niveatus* (Perciformes, Sciaenidae): a mitogenomic  
281 perspective on the phylogenetic relationships of Pseudosciaenidae. Gene 491, 210–223.
- 282 Crampton, W.G.R., de Santana, C.D., Waddell, J.C., Lovejoy, N.R., 2016a. Phylogenetic  
283 systematics, biogeography, and ecology of the electric fish genus *Brachyhypopomus*  
284 (Ostariophysi: Gymnotiformes). PLoS One 11, e0161680.
- 285 Crampton, W.G.R., de Santana, C.D. de, Waddell, J.C., Lovejoy, N.R., 2016b. A taxonomic  
286 revision of the Neotropical electric fish genus *Brachyhypopomus* (Ostariophysi:  
287 Gymnotiformes: Hypopomidae), with descriptions of 15 new species. Neotrop. Ichthyol.  
288 14. <https://doi.org/10.1590/1982-0224-20150146>
- 289 Crampton, W.G.R., Lovejoy, N.R., Waddell, J.C., 2011. Reproductive character displacement  
290 and signal ontogeny in a sympatric assemblage of electric fish. Evolution 65, 1650–  
291 1666. <https://doi.org/10.1111/j.1558-5646.2011.01245.x>
- 292 Elbassiouny, A.A., Schott, R.K., Waddell, J.C., Kolmann, M.A., Lehmborg, E.S., Nynatten,  
293 A.V., Crampton, W.G.R., Chang, B.S.W., Lovejoy, N.R., 2016. Mitochondrial genomes  
294 of the South American electric knifefishes (Order Gymnotiformes). Mitochondrial DNA  
295 Part B 1, 401–403.
- 296 Faircloth, B.C., 2013. Illumiprocessor: a trimmomatic wrapper for parallel adapter and  
297 quality trimming. doi: 10.6079.
- 298 Faircloth, B.C., McCormack, J.E., Crawford, N.G., Harvey, M.G., Brumfield, R.T., Glenn,  
299 T.C., 2012. Ultraconserved elements anchor thousands of genetic markers spanning  
300 multiple evolutionary timescales. Syst. Biol. 61, 717–726.
- 301 Faircloth, B.C., Sorenson, L., Santini, F., Alfaro, M.E., 2013. A phylogenomic perspective on  
302 the radiation of ray-finned fishes based upon targeted sequencing of Ultraconserved

- 303 Elements (UCEs). PLoS One 8, e65923.
- 304 Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis,  
305 X., Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke,  
306 A., Rhind, N., di Palma, F., Birren, B.W., Nusbaum, C., Lindblad-Toh, K., Friedman,  
307 N., Regev, A., 2011. Full-length transcriptome assembly from RNA-Seq data without a  
308 reference genome. Nat. Biotechnol. 29, 644–652.
- 309 Guo, C.-C., Liu, M., Lin, J.-J., Dai, F.-Q., 2017. Complete mitochondrial genome and the  
310 phylogenetic position of the tigertooth croaker *Otolithes ruber* (Perciformes:  
311 Sciaenidae). Mitochondrial DNA Part B 2, 132–133.
- 312 Hao, G., Wu, Q., Zhong, H., Zhou, Y., 2016. Complete mitochondrial genome of  
313 *Pterophyllum scalare* (Perciformes, Cichlidae). Mitochondrial DNA A 27, 4215–4216.
- 314 Hebert, P.D.N., Cywinska, A., Ball, S.L., deWaard, J.R., 2003. Biological identifications  
315 through DNA barcodes. Proc. Biol. Sci. 270, 313–321.
- 316 Hulen, K.G., Crampton, W.G.R., Albert, J.S., 2005. Phylogenetic systematics and historical  
317 biogeography of the Neotropical electric fish *Sternopygus* (Teleostei: Gymnotiformes).  
318 System. Biodivers. 3, 407–432.
- 319 Iwasaki, W., Fukunaga, T., Isagozawa, R., Yamada, K., Maeda, Y., Satoh, T.P., Sado, T.,  
320 Mabuchi, K., Takeshima, H., Miya, M., Nishida, M., 2013. MitoFish and  
321 MitoAnnotator: a mitochondrial genome database of fish with an accurate and automatic  
322 annotation pipeline. Mol. Biol. Evol. 30, 2531–2540.
- 323 Jørgensen, T.E., Bakke, I., Ursvik, A., Andreassen, M., Moum, T., Johansen, S.D., 2014. An  
324 evolutionary preserved intergenic spacer in gadiform mitogenomes generates a long  
325 noncoding RNA. BMC Evol. Biol. 14, 182.
- 326 Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7:  
327 improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.
- 328 Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S.,  
329 Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond,  
330 A., 2012. Geneious Basic: an integrated and extendable desktop software platform for  
331 the organization and analysis of sequence data. Bioinformatics 28, 1647–1649.
- 332 Kim, B.-C., Kang, T., Kim, M., Kim, C.-B., 2006. The complete mitogenome of *Rhodeus*  
333 *uyekii* (Cypriniformes, Cyprinidae). DNA Seq. 17, 181–186.
- 334 Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis  
335 version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.
- 336 Lavoué, S., Miya, M., Arnegard, M.E., Sullivan, J.P., Hopkins, C.D., Nishida, M., 2012.

- 337           Comparable ages for the independent origins of electrogenesis in African and South  
338           American weakly electric fishes. PLoS One 7, e36287.
- 339 Miller, M.A., Pfeiffer, W., Schwartz, T., n.d. Creating the CIPRES Science Gateway for  
340           inference of large phylogenetic trees, in: 2010 Gateway Computing Environments  
341           Workshop (GCE), IEEE, pp. 1–8.
- 342 Moller, P., 1995. Electric fishes: history and behavior. Chapman & Hall. London.
- 343 Nakatani, M., Miya, M., Mabuchi, K., Saitoh, K., Nishida, M., 2011. Evolutionary history of  
344           Otophysi (Teleostei), a major clade of the modern freshwater fishes: Pangaeon origin  
345           and Mesozoic radiation. BMC Evol. Biol. 11, 177.
- 346 Nei, M., Kumar, S., 2000. Molecular Evolution and Phylogenetics. Oxford University Press,  
347           New York.
- 348 Ojala, D., Montoya, J., Attardi, G., 1981. tRNA punctuation model of RNA processing in  
349           human mitochondria. Nature 290, 470–474.
- 350 Perna, N.T., Kocher, T.D., 1995. Patterns of nucleotide composition at fourfold degenerate  
351           sites of animal mitochondrial genomes. J. Mol. Evol. 41, 353–358.
- 352 Picq, S., Alda, F., Krahe, R., Bermingham, E., 2014. Miocene and Pliocene colonization of  
353           the Central American Isthmus by the weakly electric fish *Brachyhyppopomus occidentalis*  
354           (Hypopomidae, Gymnotiformes). J. Biogeogr. 41, 1520–1532.
- 355 Poulsen, J.Y., Byrkjedal, I., Willassen, E., Rees, D., Takeshima, H., Satoh, T.P., Shinohara,  
356           G., Nishida, M., Miya, M., 2013. Mitogenomic sequences and evidence from unique  
357           gene rearrangements corroborate evolutionary relationships of myctophiformes  
358           (Neoteleostei). BMC Evol. Biol. 13, 111.
- 359 Rand, D.M., 1993. Endotherms, ectotherms, and mitochondrial genome-size variation. J.  
360           Mol. Evol. 37, 281–295.
- 361 Reis, R.E., Kullander, S.O., Ferraris, C.J., Junior, C., 2003. Checklist of the freshwater fishes  
362           of Central and South America. EDIPUCRS Porto Alegre Brasil Brasil.
- 363 Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B.,  
364           Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian  
365           phylogenetic inference and model choice across a large model space. Syst. Biol. 61,  
366           539–542.
- 367 Roques, S., Fox, C.J., Villasana, M.I., Rico, C., 2006. The complete mitochondrial genome of  
368           the whiting, *Merlangius merlangus* and the haddock, *Melanogrammus aeglefinus*: a  
369           detailed genomic comparison among closely related species of the Gadidae family. Gene  
370           383, 12–23.

- 371 Satoh, T.P., Miya, M., Mabuchi, K., Nishida, M., 2016. Structure and variation of the  
372 mitochondrial genome of fishes. *BMC Genomics* 17, 719.
- 373 Shi, X., Tian, P., Lin, R., Huang, D., Wang, J., 2016a. Characterization of the complete  
374 mitochondrial genome sequence of the globose head whiptail *Cetonurus globiceps*  
375 (Gadiformes: Macrouridae) and its phylogenetic analysis. *PLoS One* 11, e0153666.
- 376 Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of  
377 large phylogenies. *Bioinformatics* 30, 1312–1313.
- 378 Tagliacollo, V.A., Bernt, M.J., Craig, J.M., Oliveira, C., Albert, J.S., 2016. Model-based total  
379 evidence phylogeny of Neotropical electric knifefishes (Teleostei, Gymnotiformes).  
380 *Mol. Phylogenet. Evol.* 95, 20–33.
- 381

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 <sup>Phe</sup>	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 <sup>Val</sup>	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 <sup>Leu</sup>	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 <sup>Ile</sup>	3820/3790/3812	3891/3862/3883	72/73/72	-2/-2/-2	F		
tRNA <sup>Gln</sup>	3890/3861/3882	3960/3931/3952	71/71/71	-1/-1/-1	R		
tRNA <sup>Met</sup>	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 <sup>Tyr</sup>	5075/5046/5075	5144/5117/5145	70/72/71	0/1/1	F		
tRNA <sup>Ala</sup>	5145/5119/5147	5213/5186/5215	69/68/69	1/1/1	R		
tRNA <sup>Asn</sup>	5215/5188/5217	5287/5260/5289	73/73/73	31/29/30	R		
tRNA <sup>Cys</sup>	5319/5290/5320	5384/5356/5385	66/67/66	0/1/0	R		
tRNA <sup>Tyr</sup>	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 <sup>Ser</sup>	7017/6990/7011	7087/7060/7081	71/71/71	4/4/5	R		
tRNA <sup>Asp</sup>	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 <sup>Lys</sup>	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 <sup>Gly</sup>	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 <sup>Arg</sup>	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 <sup>His</sup>	11729/11701/11778	11797/11769/11846	69/69/69	0/0/0	F		
tRNA <sup>Ser</sup>	11798/11770/11847	11865/11836/11913	68/67/67	1/1/0	F		
tRNA <sup>Leu</sup>	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 <sup>Glu</sup>	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 <sup>Thr</sup>	15505/15446/15553	15576/15518/15623	72/73/71	-1/-2/-2	F		
tRNA <sup>Pro</sup>	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		

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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
<i>B. occidentalis</i> vs. <i>B. occidentalis</i> 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
<i>B. occidentalis</i> vs. <i>B. n.sp.</i> 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<sup>Lys</sup>* (blue). Complete and partial stop codons are shown in black. Gaps are represented by dashes.

Fig. 1

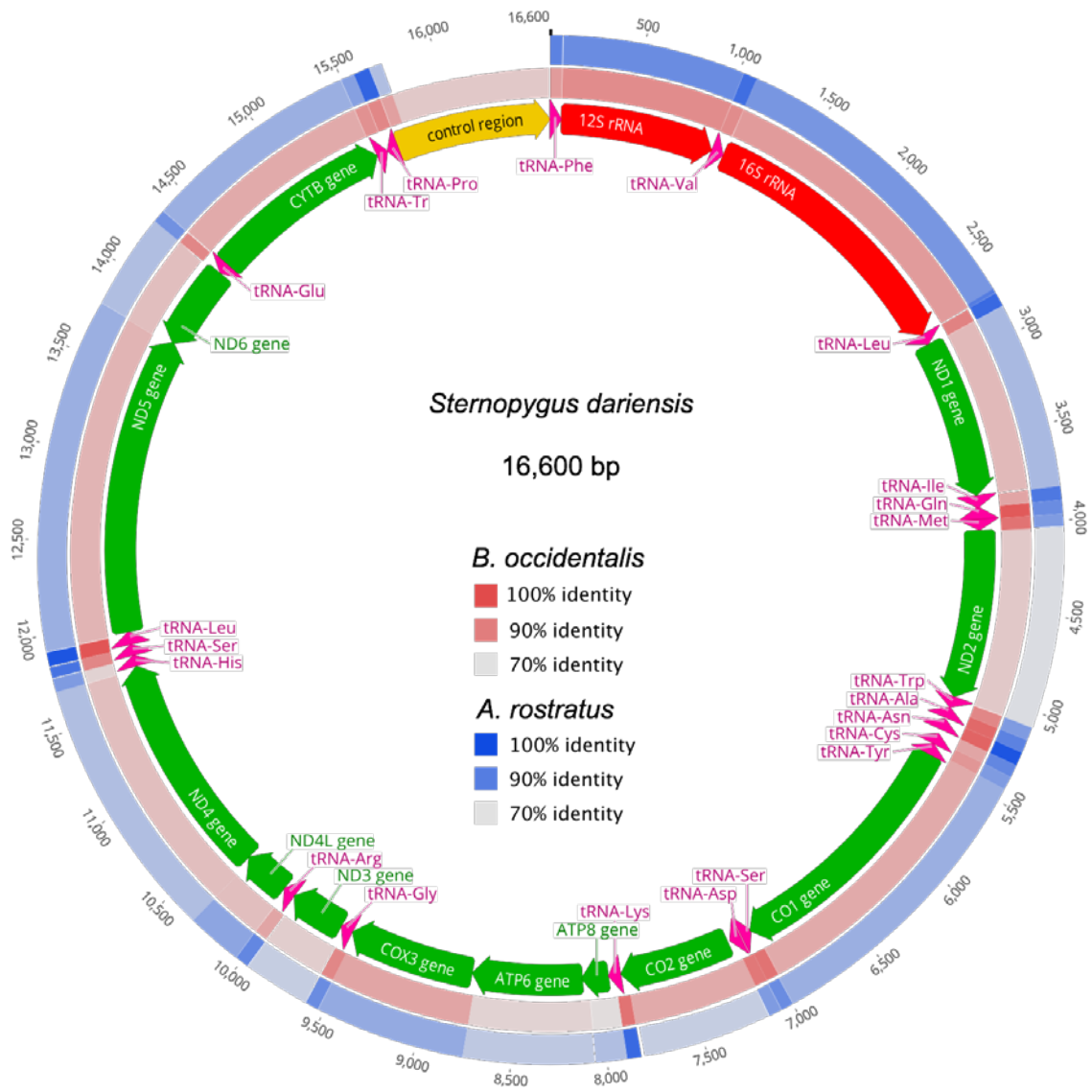


Fig. 2

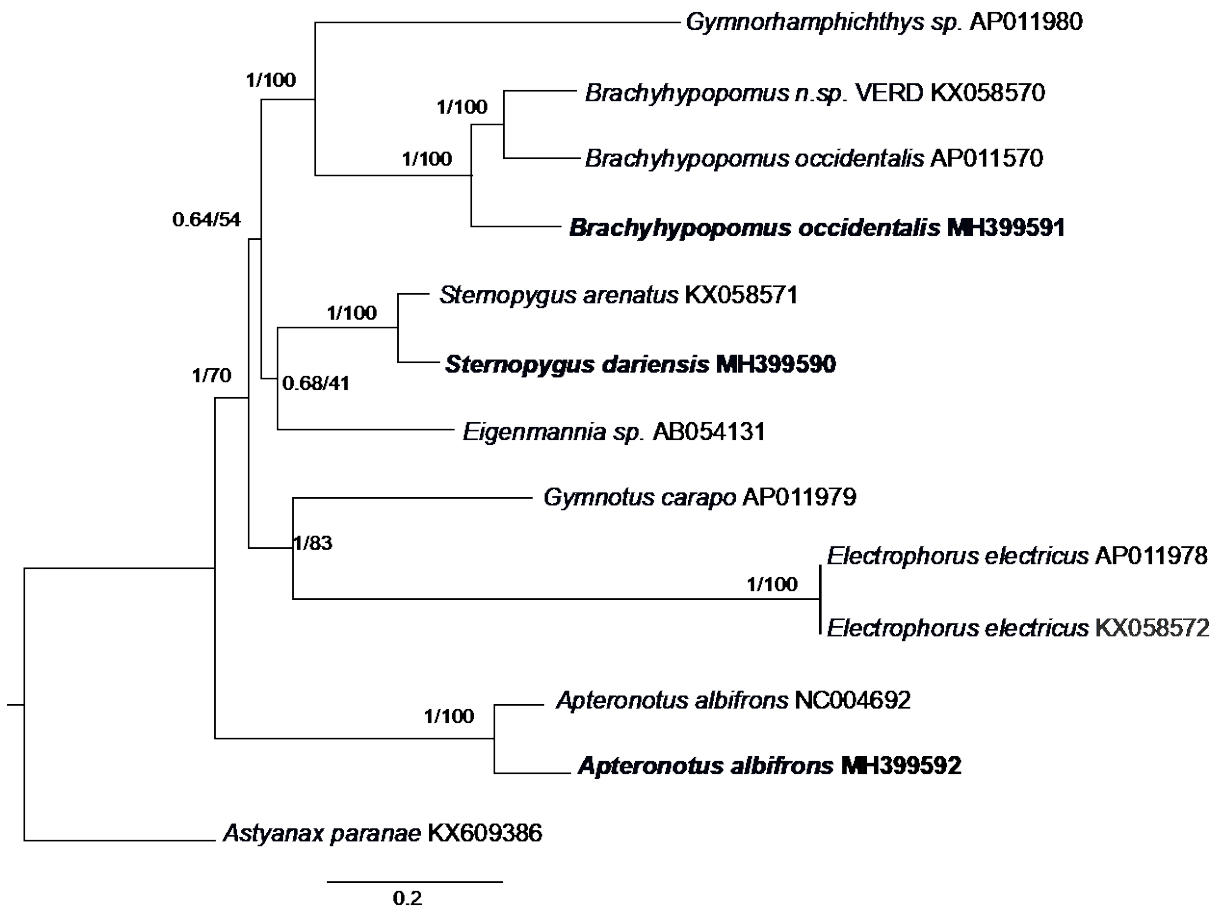
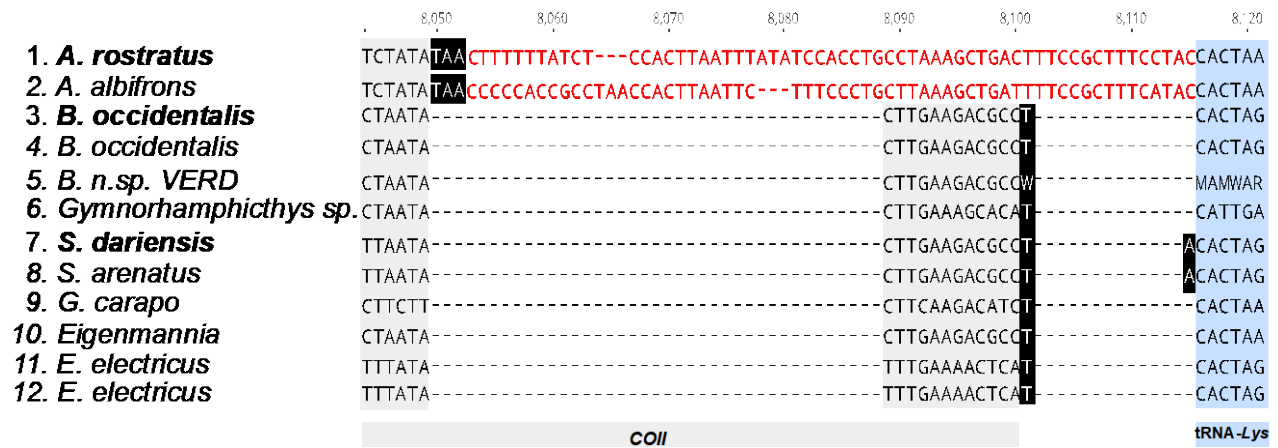


Fig. 3



*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<sup>Lys</sup> 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

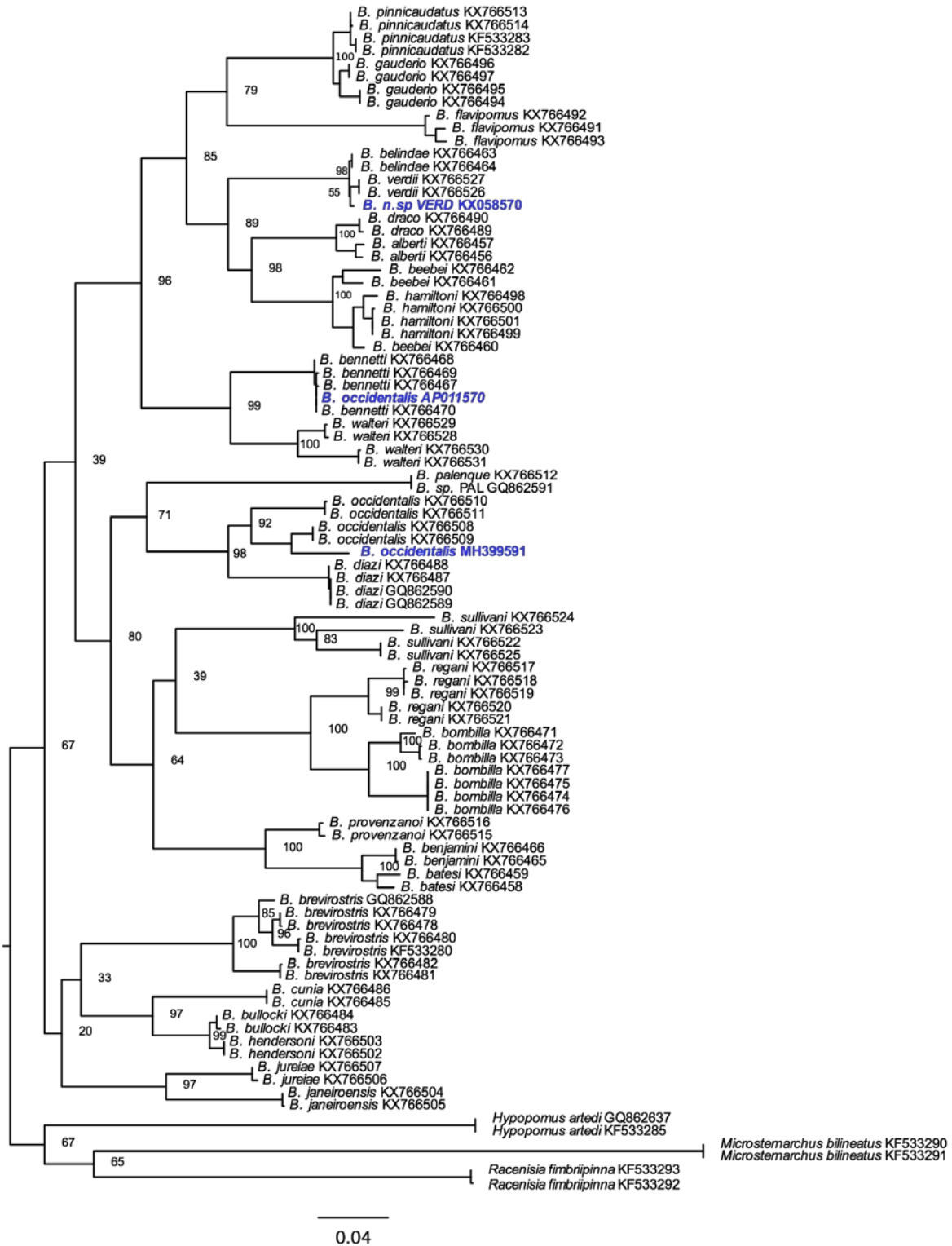


Figure S1. RAxML phylogenetic tree of 26 *Brachyhypopomus* species based on the *Cyt b* gene. Individuals in blue represent mitogenomes reported here (Genbank MH399591) and two additional sequences obtained from GenBank (AP011570, KX058570).