

PROOF COVER SHEET

Author(s): I-Shiung Chen, Chiao-Ru Liao, and Kwang-Tsao Shao

Article title: The complete mitochondrial genome of Taiwanese barbel steed, *Hemibarbus schlegelii* (Günther) (Teleostei: Cyprinidae)

Article no: GDNA_A_855914

Enclosures: 1) Query sheet
2) Article proofs

Dear Author,

Please check these proofs carefully. It is the responsibility of the corresponding author to check against the original manuscript and approve or amend these proofs. A second proof is not normally provided. Informa Healthcare cannot be held responsible for uncorrected errors, even if introduced during the composition process. The journal reserves the right to charge for excessive author alterations, or for changes requested after the proofing stage has concluded.

The following queries have arisen during the editing of your manuscript and are marked in the margins of the proofs. Unless advised otherwise, submit all corrections using the CATS online correction form. Once you have added all your corrections, please ensure you press the “Submit All Corrections” button.

Please review the table of contributors below and confirm that the first and last names are structured correctly and that the authors are listed in the correct order of contribution.

Contrib. No.	Prefix	Given name(s)	Surname	Suffix
1		I-Shiung	Chen	
2		Chiao-Ru	Liao	
3		Kwang-Tsao	Shao	



AUTHOR QUERIES

Q1: Please update Chen & Wu (2013); Chen et al. (2013a); Chen et al. (2013b); and Han et al. (2013) if now available.

MITOGENOME ANNOUNCEMENT

The complete mitochondrial genome of Taiwanese barbel steed, *Hemibarbus schlegelii* (Günther) (Teleostei: Cyprinidae)I-Shiung Chen^{1*}, Chiao-Ru Liao^{1*}, and Kwang-Tsao Shao²¹Institute of Marine Biology, National Taiwan Ocean University, Keelung, Taiwan, ROC and ²Biodiversity Research Center, Academia Sinica, Taipei, Taiwan, ROC**Abstract**

In this study, the complete mitogenome sequence of cyprinid fish, Taiwanese barbel steed, *Hemibarbus schlegelii* (Günther, 1868) (Teleostei: Cyprinidae) has been amplified and sequenced by employing long polymerase chain reaction method. The mitogenome, consisting of 16,612 bp, had the typical vertebrate mitochondrial gene arrangement, including 13 protein-coding genes, 22 transfer RNAs, 2 ribosomal RNAs genes and a non-coding control region (CR). CR of 933 bp length is located between *tRNA^{Pro}* and *tRNA^{Phe}*. The overall base composition of *Hemibarbus schlegelii* is 29.9% for A, 27.2% for C, 25.9% for T and 17.0% for G, with a slight AT bias of 55.8%. The complete mitogenome may provide rather essential and important DNA molecular data for further phylogenetic analysis for not only congeneric species but also higher different taxa of Cyprinid fishes.

Keywords*Hemibarbus schlegelii*, mitogenome, Taiwanese barbel steed**History**

Received 30 September 2013

Accepted 1 October 2013

Published online ■■■

The cyprinoid fishes are highly diverse group in freshwater habitats of Eastern Asia. The fishes of freshwater cyprinid genus *Hemibarbus* Bleeker inhabit in river basins from Japan, Korea, Taiwan, China southward to Vietnam (Chen & Fang, 1999; Yue, 1995). The Taiwanese barbel steed, *Hemibarbus schlegelii* (Günther, 1868) was collected from northern Taiwan which had been overlooked as *H. labeo* (Pallas) morphologically for a long time. A fin tissue was sampled and preserved in 95% ethanol. Total DNA was extracted from the fish tissue using the High Pure DNA Extraction Kit (Roche Applied Sciences, Penzberg, Germany). The mitogenome of Taiwanese barbel steed, *Hemibarbus schlegelii* was amplified in its entirety using a long PCR technique (Miya & Nishida, 1999) with 16 pairs of primers designed in accordance with equivalent mitochondrial genome sequences of over 30 species of Cypriniformes (Chen, 2013; Chen et al., 2013a; Han et al., 2011; Li et al., 2012; Huang et al., 2012; Wang et al., 2008, 2011; Yang et al., 2012).

The complete mitochondrial genome of *Hemibarbus schlegelii* was found to be similar to that of most other vertebrates

(Chen, 2013; Chen & Wu, 2013; Chiang et al., 2013). It was amplified and sequenced to be 16,612 bp in size (GenBank accession number: KF 703442), including 13 typical vertebrate protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes and a control region (Table 1).

All genes were encoded on H-strand with exception of one protein-coding gene (*ND6*) and eight tRNA genes (*tRNA^{Gln}*, *tRNA^{Ala}*, *tRNA^{Asn}*, *tRNA^{Cys}*, *tRNA^{Tyr}*, *tRNA^{Ser(UCN)}*, *tRNA^{Glu}* and *tRNA^{Pro}*). The overall base composition is 29.9% for A, 27.2% for C, 25.9% for T and 17.0% for G, with a higher AT content of 55.8%

All the 13 mitochondrial protein-coding genes share the start codon ATG, except for *CO I* (GTG start codon). It also important to note that three of 13 protein-coding genes is inferred to termination with an incomplete stop codon T– (*CO II* and *Cyt B*) and TA– (*CO III*); five of them share typical termination codon TAA (*ND1*, *CO I*, *ATPase 8*, *ATPase 6* and *ND4L*); five of them share typical termination codon TAG (*ND2*, *ND3*, *ND4*, *ND5* and *ND6*). Many cyprinoid fishes also used such incomplete codon structure as a signal to halt the process of protein translation (Ai et al., 2013; Chen, 2013; Chen et al., 2011; Huang et al., 2012; Li et al., 2012; Tseng, 2013;). The longest one is *ND5* gene (1383 bp) in all protein coding genes, whereas the shortest is *ATPase 8* gene (165 bp). The CR (D-loop) is 933 bp in length, and is located between the *tRNA^{Pro}* and *tRNA^{Phe}* genes. The two ribosomal RNA genes, *12S rRNA* gene (960 bp) and *16S rRNA* gene (1690 bp), are located between *tRNA^{Phe}* and *tRNA^{Leu(UUR)}* and separated by *tRNA^{Val}*. We expect that the present result would elucidate the further phylogenetic approach among related group in Cyprinidae as well as the phylogenetic approach among different *Hemibarbus* species in Eastern Asia.

*These authors contributed equally to this work.

Correspondence: I-Shiung Chen, Institute of Marine Biology, National Taiwan Ocean University, Keelung 202, Taiwan, ROC. Tel: 886 24622192 (5305). Fax: 886 224633152. E-mail: isc@ntou.edu.tw

133 Table 1. Organization of the mitogenome in *Hemibarbus schlegelii*.

Gene/element	From	To	length	Start codon	Stop codon	Anticodon	Inetrgenic nucleotides*	Strand
<i>tRNA-Phe</i>	1	69	69			GAA	0	H
<i>12S rRNA</i>	70	1029	960				0	H
<i>tRNA-Val</i>	1030	1101	72			TAC	0	H
<i>16S rRNA</i>	1102	2791	1690				0	H
<i>tRNA-Leu 1 (UAA)</i>	2792	2867	76			TAA	1	H
<i>ND1</i>	2869	3843	975	ATG	TAA		4	H
<i>tRNA-Ile</i>	3847	3919	72			GAT	-2	H
<i>tRNA-Gln</i>	3918	3988	71			TTG	2	L
<i>tRNA-Met</i>	3990	4058	69			CAT	0	H
<i>ND2</i>	4059	5105	1047	ATG	TAG		-2	H
<i>tRNA-Trp</i>	5104	5174	71			TCA	2	H
<i>tRNA-Ala</i>	5177	5245	69			TGC	1	L
<i>tRNA-Asn</i>	5247	5319	73			GTT	32	L
<i>tRNA-Cys</i>	5352	5419	68			GCA	1	L
<i>tRNA-Tyr</i>	5421	5491	71			GTA	1	L
<i>CO 1</i>	5493	7043	1551	GTG	TAA		1	H
<i>tRNA-Ser 1 (UGA)</i>	7045	7113	69			TGA	4	L
<i>tRNA-Asp</i>	7118	7189	72			GTC	13	H
<i>CO 2</i>	7203	7893	691	ATG	T-		0	H
<i>tRNA-Lys</i>	7894	7969	76			TTT	1	H
<i>ATP8</i>	7971	8135	165	ATG	TAA		-7	H
<i>ATP6</i>	8129	8812	684	ATG	TAA		-1	H
<i>CO 3</i>	8812	9596	785	ATG	TA-		-1	H
<i>tRNA-Gly</i>	9596	9668	73			TCC	0	H
<i>ND3</i>	9669	10,019	351	ATG	TAG		-2	H
<i>tRNA-Arg</i>	10,018	10,087	70			TCG	0	H
<i>ND4L</i>	10,088	10,384	297	ATG	TAA		-7	H
<i>ND4</i>	10,378	11,760	1383	ATG	TAG		-1	H
<i>tRNA-His</i>	11,760	11,828	69			GTG	0	H
<i>tRNA-Ser 2 (GCU)</i>	11,829	11,897	69			GCT	1	H
<i>tRNA-Leu 2 (UAG)</i>	11,899	11,971	73			TAG	0	H
<i>ND5</i>	11,972	13,807	1836	ATG	TAG		-4	H
<i>ND6</i>	13,804	14,325	522	ATG	TAG		0	L
<i>tRNA-Glu</i>	14,326	14,394	69			TTC	4	L
<i>Cyt b</i>	14,399	15,539	1141	ATG	T-		0	H
<i>tRNA-Thr</i>	15,540	15,611	72			TGT	0	H
<i>tRNA-Pro</i>	15,612	15,679	68			TGG	0	L
Control region (D-loop)	15,680	16,612	933				0	-

*Intergenic nucleotides: negative number indicates that adjacent genes overlap.

Declaration of interest

The authors declare no competing materials in the preparation and execution of this manuscript. The authors are responsible for the content and writing of this article. The corresponding author wishes to thank for the grant support for aquatic biodiversity research from CMBB, NTU. The current work was also supported from the Taiwan-Russia international project grants of National Science Council, Taipei for the corresponding author.

References

Ai W, Chen X, Xiang D, Chen S, Chen Y. (2013). Complete mitochondrial genome of *Acrossocheilus wenchowensis* (Cyprinidae, Barbinae). *Mitochondrial DNA* 24:249-51.

Chen IS. (2013). The complete mitochondrial genome of Chinese sucker *Myxocyprinus asiaticus* (Cypriniformes, Catostomidae). *Mitochondrial DNA* 24:680-2.

Chen IS, Fang LS. (1999). The freshwater and estuarine fishes of Taiwan. Pingtung: The Preparatory Office of National Museum of Marine Biology & Aquarium.

Chen IS, Wu JH. (2013). The complete mitochondrial genome of variegated lizardfish *Synodus variegatus* (Perciformes, Synodontidae). *Mitochondrial DNA*. doi: 10.3109/19401736.2013.777744.

Chen IS, Han M, Wang CL, Shen CN. (2013). The complete mitochondrial genome of rainbow barbel *Acrossocheilus barbodon* (Nichols and Pope) (Teleostei, Cyprinidae, Barbinae). *Mitochondrial DNA*. doi: 10.3109/19401736.2013.810495.

Chen IS, Liao CR, Shao YT. (2013). The complete mitochondrial genome of Hainanese barbel steed, *Hemibarbus medius*

Yue (Teleostei: Cyprinidae). *Mitochondrial DNA*. doi: 10.3109/19401736.2013.845765.

Chiang TY, Chen IS, Lin HD, Chang WB, Ju YM. (2013). Complete mitochondrial genome of *Sicyopterus japonicus* (Perciformes, Gobiidae). *Mitochondrial DNA* 24:191-3.

Günther A. (1868). *Catalogue of the fishes in the British Museum*. Vol. 7. London: Taylor and Francis.

Han M, Wang CL, Shen CN, Chen IS. (2013). The complete mitochondrial genome of half-spined barbel *Acrossocheilus hemispinus* (Nichols) (Teleostei, Cyprinidae, Barbinae). *Mitochondrial DNA*. doi: 10.3109/19401736.2013.815172.

He S, Liang XF, Chu WY, Chen DX. (2012). Complete mitochondrial genome of the blind cave barbel *Sinocyclocheilus furcodorsalis* (Cypriniformes: Cyprinidae). *Mitochondrial DNA* 23:429-31.

Huang Y, Zhao GQ, Peng ZG. (2012). Mitochondrial genome of *Onychostoma lini* (Teleostei, Cypriniformes). *Mitochondrial DNA* 23:173-5.

Li P, Xu D, Peng Z, Zhang Y. (2012). The complete mitochondrial genome of the spotted steed, *Hemibarbus maculatus* (Teleostei: Cypriniformes). *Mitochondrial DNA* 23:34-6.

Miya M, Nishida M. (1999). Organization of the mitochondrial genome of a deep-sea fish, *Gonostoma gracile* (Teleostei: Stomiiformes): First example of transfer RNA gene arrangements in bony fish. *Mar Biotechnol* 1:416-26.

Tseng DY. (2013). Complete mitochondrial genome of *Onychostoma barbata* (Cypriniformes, Cyprinidae). *Mitochondrial DNA* 24:120-2.

Wang C, Chen Q, Lu G, Xu J, Yang Q, Li S. (2008). Complete mitochondrial genome of the grass carp (Teleostei: Cyprinidae: Gobiioninae). *Gene* 424:96-101.

199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264

265	Wang J, Li P, Peng Z. (2011). The complete mitochondrial genome of	<i>acathopterus</i> (Cypriniformes, Cyprinidae). Mitochondrial DNA 24:	331
266	Chinese rare minnow, <i>Gobiopterus rarus</i> (Teleostei: Cypriniformes).	108–10.	332
267	Mitochondrial DNA 22:178–80.	Yue PQ. (1995). A revision of the cyprinid fishes of the genus	333
268	Yang JQ, Cheng HL, Wu CY, Tsai KC, Chiang HC, Wang CW, Lin HD.	<i>Hemibarbus</i> in China (Cypriniformes: Cyprinidae). Acta Zootax Sini	334
269	(2012). Complete mitochondrial genome of <i>Scaphiodonichthys</i>	20:116–23.	335
270			336
271			337
272			338
273			339
274			340
275			341
276			342
277			343
278			344
279			345
280			346
281			347
282			348
283			349
284			350
285			351
286			352
287			353
288			354
289			355
290			356
291			357
292			358
293			359
294			360
295			361
296			362
297			363
298			364
299			365
300			366
301			367
302			368
303			369
304			370
305			371
306			372
307			373
308			374
309			375
310			376
311			377
312			378
313			379
314			380
315			381
316			382
317			383
318			384
319			385
320			386
321			387
322			388
323			389
324			390
325			391
326			392
327			393
328			394
329			395
330			396