Pakistan J. Zool., vol. 51(3), pp 871-885, 2019. DOI: http://dx.doi.org/10.17582/journal.pjz/2019.51.3.871.885

Complete Mitochondrial Genomes of Two Oriental Sweetlips, *Plectorhinchus orientalis* and *Plectorhinchus vittatus* (Perciformes: Haemulidae) with the Molecular Analysis on their Synonym Controversies

Rishen Liang*, Meng Zhou, Zhenxiang Lin, Guozhang Li, Yuan Chen, Xuan Lin and Zaohe Wu

College of Animal Technology, Zhongkai University of Agriculture and Engineering, Guangzhou, China

Article Information
Received 11 August 2017
Revised 10 October 2017
Accepted 13 November 2017
Available online 19 March 2019

Authors' Contribution
RL and ZW designed the research and wrote the manuscript. RL and MZ collected the samples. RL, ZL and GL carried out experiments. YC and LX analyzed sequencing data and experimental results.

Key words
Complete mitochondrial genome, Plectorhinchus orientalis, Plectorhinchus vittatus, Molecular phylogeny, Mitogenome.

ABSTRACT

Plectorhinchus orientalis and *Plectorhinchus vittatus* were two species of colorful coral reef sweetlips which distribute in the areas of Indo-Western Pacific. The two species have long been considered as a synonym (oriental sweetlips) in morphology. In order to investigate the validity of two species at the molecular level, complete mitochondrial genomes of the two species were first determined. The genomes were 16,546 bp (*P. orientalis*) and 16,545 bp (*P. vittatus*) in size, respectively, which both consisted of a typical structure of 13 protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes, and one noncoding control region. Genomic composition, organization and gene order were similar to that obtained in most vertebrates. By comparative analysis of the two genomes, 941 variable sites (5.69%) were found. Sequence divergences of 13 protein-coding genes, 2 rRNA genes and one control region which are commonly used as molecular markers between the two species ranged from 2.4% (12S rRNA) to 11.2% (ND6), all divergence values were larger than 2%. Great variations of Cyt b and COI between *P. orientalis* and *P. vittatus* were revealed to be 6.0% and 7.4%, respectively, which were largely greater than the threshold of species diagnosis divergence value of 2%. Inner individual divergence values of each species were less than 0.5%. In the molecular phylogenetic trees, the longer branch length also clearly distinguished the independent placement of the two species. These results revealed great genetic differences between *P. orientalis* and *P. vittatus*, and strongly suggested that they might be two distinct species and should not be placed as synonym.

INTRODUCTION

The vertebrate mitochondrial genome (mitogenome) is a small, double-stranded and circular DNA ranging in size from 15-20 kb. The structural gene organization is quite conserved within vertebrate mitogenome which contains 13 protein-coding genes, 2 ribosomal RNA genes (12S and 16S), 22 transfer RNA genes and one noncoding control region. In addition, mtDNA possesses two main non-coding regions involved in replication and transcription processes: the control region or D-loop and the light-strand replication origin or O_L (Clayton, 1992; Shadel and Clayton, 1997; Boore, 1999). The mitochondrial DNA has been extensively used as a marker for molecular evolution, genetic diversity studies and phylogenetic analyses due to its compact size, maternal inheritance, rapid evolutionary rate and lack of recombination (Inoue *et al*., 2001; Miya *et al*., 2003; Saitoh *et al*., 2006; Kawahara *et al*., 2008). In fish, the mitogenome was first reported in freshwater loach (*Crossostoma lacustre*) by Tzeng *et al*. (1992). At the time of writing, complete mitogenomes from more than 472 species in Perciformes are available in NCBI database.

Family Haemulidae belongs to the suborder Percodei of order Perciformes, which comprised two subfamilies: Plectorhynchinae (sweetlips) and Haemulinae (grunts). Sweetlips are globally marine fish and mainly found in the oceanic waters of tropical, subtropical water along the Indo-Western Pacific. Most colorful sweetlips represent a drastic change in their external coloration appearance from juvenile to adult, which makes difficulties in species identification. Two oriental sweetlips *Plectorhinchus orientalis* (Bloch, 1793) and *Plectorhinchus vittatus* (Linnaeus, 1758) are common colorful sweetlips and mainly distributed in the areas of Indo-Western Pacific. *P. vittatus*

* Corresponding author: cheetahliang@126.com
0030-9923/2019/0003-0871 $ 9.00/0

has been widely known as *P. orientalis* (Satapoomin and Randall 2000), and most studies suggested *P. orientalis* and *P. vittatus* refer to the same species: Oriental sweetlips (McKay, 1984, 2001; Randall and Lim, 2000; Gillibrand *et al*., 2007; Unsworth, 2010) and considered *P. orientalis* as synonym of *P. vittatus* (Randall and Johnson, 2000; McKay, 2001; Randall, 2005; Froese and Pauly, 2014). Randall and Johnson (2000) has resurrected *P. vittatus* (Linnaeus) as an older name. Recently, some studies have separated oriental sweetlips from the Indian Ocean and Pacific Ocean populations and considered *P. vittatus* as the Indian Ocean populations (Indian Ocean oriental sweetlips) and *P. orientalis* (oriental sweetlips) as the Pacific Ocean populations. Despite a large number of morphology-based hypotheses, no molecular studies have been examined on the two debated species. As mention above, mitochondrial DNA has become a very useful marker for species identification and phylogenetic studies. Thus, further studies of genetic characteristics of *P. orientalis* and *P. vittatus* based on multiple mitochondrial gene markers appear necessary to recover conclusive phylogenetic relationships of the two species and resolve the classification and identification problem.

In the present study, we first reported the complete mitogenome sequences of *P. orientalis* and *P. vittatus* and further compared their genome structure and organization, nucleotide variation and codon usage. We also reported the phylogenetic analysis using protein sequences from *P. orientalis*, *P. vittatus* and other Haemulidae species to clarify the interrelationship of the two species and investigate their evolutionary position within Haemulidae.

MATERIALS AND METHODS

Sweetlips samples collection and genomic DNA extraction

Sample of *P. orientalis* was collected in its natural habitats, the coral reef areas of Jiyin Island in Paracel Islands. Sample of *P. vittatus* was a vouchered specimens obtained from SAIAB (South African Institute for Aquatic Biodiversity). A small piece of fresh muscle was sampled and preserved in 95% ethanol and total genomic DNA was extracted using a Tissue Genomic DNA Extraction kit (Omega, USA) following the manufacturer's protocol and stored at -20°C. All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals, and protocols were approved by the Institutional Animal Care and Use Committee in Zhongkai University of Agriculture and Engineering, Guangdong, China.

Primer design and long-PCR amplification

The complete mitochondrial genomes of *P. orientalis* and *P. vittatus* were both amplified using long PCR technique (Miya and Nishida, 1999). Three sets of primers were designed from conservative regions of the genes for 16SrRNA , CO II and $tRNA^{(Leu)}$ to efficiently amplify the complete mitochondrial genome in three long-PCRs using EX Taq DNA polymerase (TaKaRa, Japan). All PCR products were purified using QIAquick Gel Extraction Kit (Qiagen, German).

The purified PCR products were then cloned into the pUCm-T cloning vectors (Invitrogen, USA) and sequenced according to the manufacturer's protocol by using T7 and M13R inner primers of pUCm-T vector. Primer walking method was applied for sequencing the DNA fragment. Each segment overlapped the next contig by 80–120 bp to ensure the accuracy of the sequence.

Sequence analysis

The primary DNA sequence data was characterized by BLAST tools at NCBI database (www.ncbi.nlm.nih.gov). Then the DNA sequences were edited and analyzed by the computer programs Vector NTI Suite 8 (Invitrogen, USA). The locations of the 13 protein-coding genes were determined by comparisons with nucleotide or amino acid sequences of other bony fish mitochondrial genomes. The 22 tRNA genes and their anticodon sequences were identified by their proposed cloverleaf secondary structures drawn by tRNAscan-SE software (Lowe and Eddy, 1997). The two ribosomal RNA (rRNA) genes and the control region were identified by sequence homology and proposed secondary structure (Gutell *et al*., 1993).

Phylogenetic analysis

To determine the phylogenetic position of *P. orientalis* and *P. vittatus* in the subfamily Plectorhynchinae, sequences of Cytb and COI genes of 21 ingroup species in Plectorhynchinae and 4 outgroup species in Haemulinae from GenBank were selected (Table I). individuals of certain species Besides, in order to examine the evolutionary status of Haemulidae of the phylogenetic relationships in Percoidei species, 12 protein-coding sequences of 42 species belonged to 25 families in the suborder Percoidei and one outgroup species Salarias fasciatus in suborder Blennioidei were also selected (Table II). Both datasets were aligned with Clustal X 1.85 (Thompson *et al*., 1994) with default gap penalties. The molecular phylogeny was analyzed by Maximum Likelihood (ML) and Bayesian Inference (BI) methods. The Modeltest 3.7 software (Posada and Crandall, 1998) was used as a guide to determine the best-fit evolutionary model under the Akaike information criterion (AIC) (Posada and Buckley, 2004) and the GTR (general time reversible) model was chosen in the data computing.

Table I.- Species used for Percoidei phylogenetic analysis, along with GenBank accession numbers and reference.

Families	Species	GenBank No.	References
Percoidei			
Serranidae	*Epinephelus coioides*	EU043376	Zhuang *et al*., 2009
	Anyperodon leucogrammicus	GQ131336	Unpublished
Carangidae	*Carangoides armatus*	AP004444	Miya *et al*., 2003
	Caranx melampygus	AP004445	Miya *et al*., 2003
	Seriola lalandi	AB517557	Iguchi *et al*., 2012
Latidae	*Lates calcarifer*	DQ010541	Lin *et al*., 2006
Toxotidae	*Toxotes chatareus*	AP006806	Yagishita *et al*., 2009
Haemulidae	*Diagramma pictum*	AP009167	Yamanoue *et al*., 2007
	Parapristipoma trilineatum	AP009168	Yamanoue *et al*., 2007
	Hapalogenys nigripinnis	HM754620	Liang *et al*., 2012
	Plectorhinchus orientalis		This study
	Plectorhinchus vittatus		This study
Centrarchidae	*Micropterus salmoides*	DQ536425	Broughton and Reneau, 2006
	Micropterus dolomieu	AB378750	Mukai and Sato, 2009
Emmelichthyidae	*Emmelichthys struhsakeri*	AP004446	Miya *et al*., 2003
Monodactylidae	*Monodactylus argenteus*	AP009169	Yamanoue *et al*., 2007
Oplegnathidae	*Oplegnathus fasciatus*	DQ872160	Oh *et al*., 2007
	Oplegnathus punctatus	AP011066	Yagishita *et al*., 2009
Kyphosidae	*Labracoglossa argentiventris*	AP011062	Yagishita *et al*., 2009
	Scorpis lineolata	AP011063	Yagishita *et al*., 2009
	Kyphosus cinerascens	AP011061	Yagishita *et al*., 2009
Acropomatidae	*Doederleinia berycoides*	AP009181	Yamanoue *et al*., 2007
Caesionidae	*Pterocaesio tile*	AP004447	Miya *et al*., 2003
Lutjanidae	*Lutjanus malabaricus*	FJ824741	Unpublished
	Lutjanus sebae	FJ824742	Unpublished
Terapontidae	*Rhynchopelates oxyrhynchus*	AP011064	Yagishita *et al*., 2009
Chaetodontidae	*Chaetodon auripes*	AP006004	Yamanoue *et al*., 2007
	Heniochus diphreutes	AP006005	Yamanoue *et al*., 2007
Sciaenidae	*Larimichthys polyactis*	GU586227	Cheng *et al*., 2012
	Sciaenops ocellatus	JQ286004	Cheng *et al*., 2012
Lethrinidae	*Lethrinus obsoletus*	AP009165	Yamanoue *et al*., 2007
	Monotaxis grandoculis	AP009166	Yamanoue *et al*., 2007
Kuhliidae	*Kuhlia mugil*	AP011065	Yagishita *et al*., 2009
Pomacanthidae	*Chaetodontoplus septentrionalis*	AP006007	Yamanoue *et al*., 2007
	Centropyge loricula	AP006006	Yamanoue *et al*., 2007
Sparidae	*Pagrus major*	AP002949	Miya *et al*., 2001
	Pagellus bogaraveo	AB305023	Ponce *et al*., 2008
	Acanthopagrus latus	EF506764	Xia *et al*., 2008
Centracanthidae	*Spicara maena*	AP009164	Yamanoue *et al*., 2007
Pseudochromidae	*Labracinus cyclophthalmus*	AP009125	Mabuchi *et al*., 2007
Moronidae	*Morone saxatilis*	HM447585	Unpublished
Lateolabracidae	*Lateolabrax japonicus*	JQ860109	Unpublished
Sillaginidae	*Sillago sihama*	JQ048935	Unpublished
Blennioidei			
Blenniidae	*Salarias fasciatus*	AP004451	Miya *et al*., 2003

Table II.- Species used for Haemulidae phylogenetic analysis, along with GenBank accession numbers of COI and Cyt b gene sequences.

Species	GenBank Accession No.	
	Cyt b	COI
Ingroup species		
Plectorhinchus albovittatus	JX042230	JX042260
Plectorhinchus chaetodonoides	JX042231-JX042234	JX042261-JX042264
Plectorhinchus chubbi	JX042235	JX042293
Plectorhinchus cinctus	JX042236-JX042238	JX042265-JX042267
Plectorhinchus diagrammus	JX042239-JX042241	JX042268-JX042270
Plectorhinchus flavomaculatus	JQ741559-JQ741561	JF494169-JF494171
Plectorhinchus gaterinus	JX042289-JX042290	JX042291-JX042292
Plectorhinchus gibbosus	JX042242-JX042243	JX042271-JX042272
Plectorhinchus lineatus	JX042244-JX042246	JX042273-JX042275
Plectorhinchus macrolepis	HQ676733	HQ676788
Plectorhinchus orientalis	JX042247-JX042249 JX042256	JX042276-JX042278 JX042285
Plectorhinchus picus	JX042250-JX042251	JX042279-JX042280
Plectorhinchus plagiodesmus	JX042252	JX042281
Plectorhinchus playfairi	JX042253	JX042282
Plectorhinchus polytaenia	JQ741565	JQ741334
Plectorhinchus schotaf	JX042254	JX042283
Plectorhinchus sordidus	JX042255	JX042284
Plectorhinchus vittatus	JX042257-JX042259	JX042286-JX042288
Diagramma pictum	JX042214-JX042217	JX042226-JX042229
Diagramma centurio	JX042212-JX042213	JX042224-JX042225
Parapristipoma trilineatum	JX042210-JX042211	JX042222-JX042223
Outgroup species		
Pomadasys maculatus	JX042209	JX042221
Haemulon aurolineatum	JX042208	JX042220
Anisotremus virginicus	JX042206	JX042218
Conodon nobilis	JX042207	JX042219

BI analysis was implemented in Mrbayes version 3.1.2 (Ronquist and Huelsenbeck, 2003), The MCMC (Markov Chain Monte Carlo) simulation was run for 10 million generations, with trees sampled and saved every 1000 generations (10,000 trees saved per run), and the bootstrap value of internal branch of the phylogenetic tree was supported by the posterior probabilities.

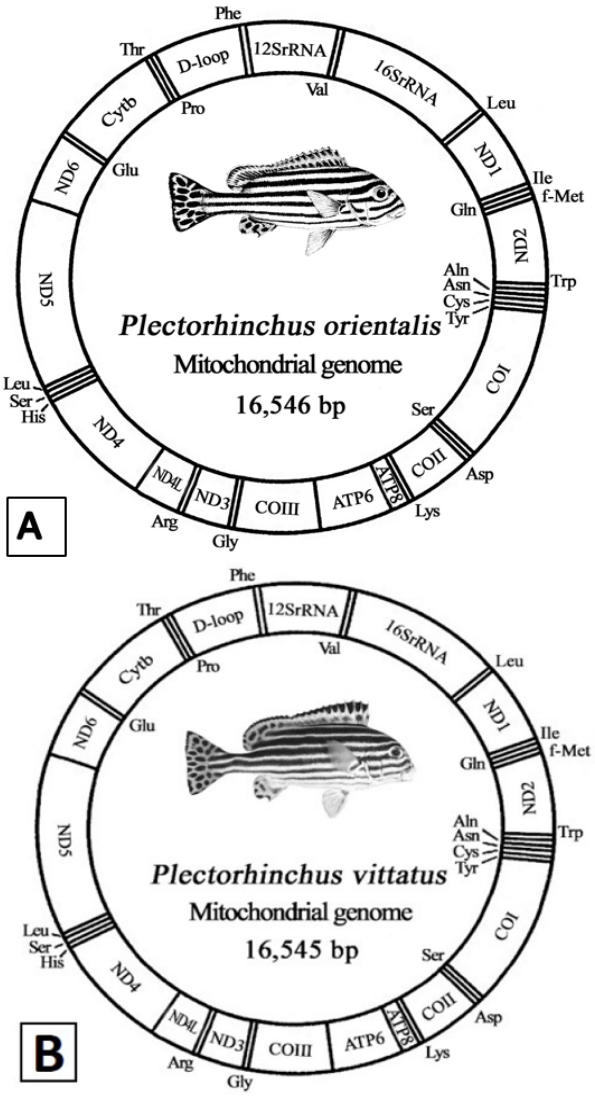

Fig. 1. Mitochondrial genome of *P. Orientalis* (A) and *P. Vittatus* (B).

RESULTS

Genome information

The complete mitochondrial genomes of *P. orientalis* and *P. vittatus* were sequenced as 16,546 bp and 16,545 bp in length, which have been submitted to GenBank database (Accession no. KP966562, KP976103). The structural organization and arrangement of both genomes consisted of 13 typical vertebrate protein-coding genes, 22 transfer RNA (tRNA) genes, 2 ribosomal RNA (rRNA) genes and one putative control region (Fig. 1A, B; Table III). Overall

base compositions of L-strand nucleotide sequences in the two mitochondrial genome were as follows, *P. orientalis*: A, 27.9%; G, 16.4%; T, 24.7%; and C, 31.0%, A+T=52.6%C+G=47.4%; *P. vittatus*: A, 28.0%; G, 16.4%; T, 24.7%; and C, 30.9%, A+T=52.7%C+G=47.3% (Table IV).

Table III.- Main charateristic of mitochondrial genome of *P. orientalis* and *P. vittatus.*

Gene	Strand	*P. orientalis*			*P. vittatus*			Codon	
		Position From-to	Nucleotide Size/bp	Intergenic nucleotide	Position From-to	Nucleotide Size/bp	Intergenic nucleotide	Start	Stop
$tRNA^{Phe}$	H	1-69	69	0	1-69	69	0		
12S rRNA	H	70-1020	951	0	70-1021	952	0		
$tRNA^{val}$	H	1021-1091	71	0	1022-1092	71	0		
16S rRNA	H	1092-2786	1695	0	1093-2783	1691	0		
$tRNA^{Leu(UUR)}$	H	2787-2860	74	0	2784-2857	74	0		
ND1	H	2861-3835	975	5	2858-3832	975	5	ATG/ ATG	TAA/TAG
$tRNA^{Ile}$	H	3841-3910	70	-1	3838-3907	70	-1		
$tRNA^{Gln}$	L	3910-3980	71	-1	3907-3977	71	-1		
$tRNA^{Met}$	H	3980-4049	70	0	3977-4046	70	0		
ND2	H	4050-5096	1047	0	4047-5093	1047	0	ATG/ ATG	TAA/TAA
$tRNA^{Trp}$	H	5097-5168	72	0	5094-5165	72	0		
$tRNA^{Ala}$	L	5169-5237	69	1	5166-5234	69	1		
$tRNA^{Asn}$	L	5239-5311	73	40	5236-5308	73	39		
$tRNA^{Cys}$	L	5352-5418	67	-1	5348-5415	68	-1		
$tRNA^{Tyr}$	L	5418-5488	71	1	5415-5486	72	1		
COI	H	5490-7049	1557	-13	5488-7050	1563	-13	GTG/GTG	AGG/AGG
$tRNA^{Ser(UCN)}$	L	7037-7107	71	3	7038-7108	71	3		
$tRNA^{Asp}$	H	7111-7182	72	3	7112-7183	72	4		
COII	H	7186-7876	691	0	7188-7878	691	0	ATG/ ATG	T--/ T--
$tRNA^{Lys}$	H	7877-7951	75	1	7879-7953	75	1		
ATPase8	H	7953-8120	168	-10	7955-8122	168	-10	ATG/ ATG	TAA/TAA
ATPase6	H	8111-8793	683	0	8113-8795	683	0	ATG/ATG	TA-/TA-
COIII	H	8794-9578	785	0	8796-9580	785	0	ATG/ATG	TA-/TA-
$tRNA^{Gly}$	H	9579-9650	72	0	9581-9652	72	0		
ND3	H	9651-9999	349	0	9653-10001	349	0	ATG/ ATG	T--/ T--
$tRNA^{Arg}$	H	10000-10068	69	0	10002-10070	69	0		
ND4L	H	10069-10365	297	-7	10071-10367	297	-7	ATG/ ATG	TAA/TAA
ND4	H	10359-11739	1381	0	10361-11741	1381	0	ATG/ ATG	T--/ T--
$tRNA^{His}$	H	11740-11808	69	0	11742-11810	69	0		
$tRNA^{Ser(AGY)}$	H	11809-11875	67	4	11811-11877	67	4		
$tRNA^{Leu(CUN)}$	H	11880-11952	73	0	11882-11954	73	0		
ND5	H	11953-13791	1839	-4	11955-13793	1839	-4	ATG/ ATG	TAG/TAA
ND6	L	13788-14309	522	0	13790-14311	522	0	ATG/ ATG	TAA/TAA
$tRNA^{Glu}$	L	14310-14378	69	4	14312-14380	69	4		
Cyt*b*	H	14383-15523	1141	0	14385-15525	1141	0	ATG/ ATG	T--/ T--
$tRNA^{Thr}$	H	15524-15595	72	33	15526-15597	72	29		
$tRNA^{Pro}$	L	15629-15698	70	0	15627-15696	70	0		
D-loop	H	15699-16546	848	0	15697-16545	849	0		

Table IV.- Base composition of mtDNA in *P. orientalis* and *P. vittatus*.

	P. orientalis						*P. vittatus*					
	T%	**C%**	**A%**	**G%**	**A+T%**	**C+G%**	**T%**	**C%**	**A%**	**G%**	**A+T%**	**C+G%**
Mitochondrial genome	24.7	31.0	27.9	16.4	52.6	47.4	24.7	30.9	28.0	16.4	52.7	47.3
13 protein-coding genes	26.5	32.4	25.2	15.9	51.7	48.3	26.5	32.3	25.4	15.8	51.9	48.1
1st	20.0	28.9	25.7	25.5	45.7	54.4	20.0	29.7	26.2	24.6	46.2	54.3
2nd	40.0	27.8	18.3	13.5	58.3	41.3	39.0	27.9	18.4	14.5	57.4	42.4
3rd	19.0	40.5	31.5	8.7	50.5	49.2	21.0	39.3	31.7	8.3	52.7	47.6
22 tRNAs	23.8	25.9	29.9	20.4	53.7	46.3	24.0	25.7	29.8	20.4	53.8	46.1
2 rRNAs	20.6	26.2	32.5	20.8	53.1	47.0	20.4	26.3	32.5	20.8	52.9	47.1
Control region	29.8	25.8	29.5	14.9	59.3	40.7	29.1	25.9	29.6	15.4	58.7	41.3

Protein-coding genes

The 13 protein-coding genes in the two sweetlips mtDNA were similar in length to their corresponding vertebrate counterparts. The total length of the protein-coding genes was 11,435 bp in *P. orientalis* and 11,441 bp in *P. vittatus* without introns, accounting for 69.12% and 69.15% of the complete genome. A6 bp differences of the protein-coding genes was found between the two species in gene COI, which was 1557 in *P. orientalis* and 1563 in *P. vittatus* (Table III). All protein-coding genes use ATG as a start codon except for COI, which started with GTG. A diverse pattern of codon usage was found within stop codons: six genes ended with TAA: ND1 (*P. orientalis*), ND2, ATPase8, ND4L, ND5 (*P. vittatus*) and ND6, two ended with TAG: ND1 (*P. vittatus*) and ND5 (*P. orientalis*), one ended with AGG: COI, the remaining genes had incomplete stop codons, TA (ATPase6, COIII) or T: COII, ND3, ND4 and Cyt *b*. Among the 13 protein-coding genes, three reading-frame overlaps were found: the pair of gene ATPase 8 and ATPase 6 overlapped by 10 nucleotides, ND4L and ND4 overlapped by 7 nucleotides, ND5 and ND6 overlapped by 4 nucleotides, no overlaps were between ATPase and COIII.

The base composition of the two sweetlips mitochondrial protein-coding genes is summarized in Table IV. The most frequent nucleotides at the first, second and third codon positions were C (28.9%, 29.7%), T (40.0%, 39.0%), C (40.5%, 39.3%) in *P. orientalis* and *P. vittatus*, respectively. The frequency of nucleotide G at the third codon position was relatively low (only 8.7%, 8.3%), showing a strong bias against G at this position. In addition, pyrimidines at the second codon position were over-represented (T+C=67.8%, 66.9%).

Codon usage in 13 protein-coding genes of the two sweetlips were analyzed and shown in Table V. The total number of codons for 20 amino acids were 3789 (*P. orientalis*) and 3791 (*P. vittatus*) excluding start and stop codons. The most frequent used amino acids are Leu (17.5%, 17.2%), followed by Ile (7.3%, 7.2%), Ser (6.4%, 6.5%), Gly (6.2%, 6.2%), and Val (5.7%, 5.5%). For amino acids with four-fold degenerate third positions, codon families ending in C were the most frequent, followed by codons ending in A. Among two-fold degenerate codons, C appeared to be used more than T in pyrimidine codon families, whereas purine codon families ended mostly with A.

Transfer RNA genes and ribosomal RNA genes

The two sweetlips mitochondrial genomes contain 22 tRNA genes, which are interspersed between rRNA and protein-coding genes, ranging from 67 ($tRNA^{Cys}$, $tRNA^{Ser(AGY)}$) to 76 ($tRNA^{Lys(UUR)}$) nucleotides in length (Table III). Two forms of tRNA-Leu (UUR and CUN) and tRNA-Ser (UCN-AGY) in the two sweetlips were identified. The anticodons of 22 tRNA genes were shown in Table III. Two ribosomal RNA genes, the small subunit (12S) and the large subunit (16S) of rRNAs were 951bp and 1,695 bp in *P. orientalis* and 952bp and 1,691 bp in *P. vittatus*, respectively. The two ribosomal RNA genes were located between the genes of tRNA-Phe and tRNA-Leu, separated by the gene tRNA-Val.

Noncoding sequence

The light strand replication origin (O_L) was comprised of 40 nucleotides in *P. orientalis*, and 39 nucleotides in *P. vittatus*, which could be folded into a stable stem-loop secondary structure containing 22 bp in the stem, 15 or 16 bp in the loop. The conserved motif of the two sweetlips were also 5'-GGCGG-3' found at the base of the stem within the tRNA-Cys gene. The control region was located between the $tRNA^{Pro}$ and $tRNA^{Phe}$ genes and determined to be 848 bp in *P. orientalis* and 849 bp in *P. vittatus*. The control regions of the two species were both heavily biased towards A+T nucleotides with A+T content 59.3% in *P. orientalis* and 58.7% in *P. vittatus* (Table III).

Table V.- Codon usage in *P. orientalis* and *P. vittatus* mitochondrial protein-coding genes.

Amino acid number	Codon	*P. orientalis*		*P. vittatus*		Amino acid number	Codon	*P. orientalis*		*P. vittatus*	
		n	(%)	n	(%)			n	(%)	n	(%)
Phe	TTT	68	1.8	68	1.8	Tyr	TAT	33	0.9	41	1.1
	TTC	164	4.3	145	3.8		TAC	85	2.2	80	2.1
Leu	TTA	52	1.4	47	1.2	Stop	TAA	0	0	7	0.2
	TTG	21	0.6	26	0.7		TAG	1	0	0	0
	CTT	133	3.5	136	3.6	His	CAT	27	0.7	25	0.7
	CTC	194	5.1	194	5.1		CAC	79	2.1	84	2.2
	CTA	194	5.1	190	5	Gln	CAA	87	2.3	86	2.3
	CTG	69	1.8	59	1.6		CAG	13	0.3	14	0.4
Ile	ATT	123	3.2	120	3.2	Asn	AAT	24	0.6	40	1.1
	ATC	157	4.1	153	4		AAC	97	2.6	80	2.1
	ATA	91	2.4	95	2.5	Lys	AAA	67	1.8	69	1.8
Met	ATG	59	1.6	64	1.7		AAG	7	0.2	3	0.1
Val	GTT	46	1.2	51	1.3	Asp	GAT	20	0.5	19	0.5
	GTC	79	2.1	67	1.8		GAC	56	1.5	55	1.5
	GTA	64	1.7	63	1.7	Glu	GAA	79	2.1	78	2.1
	GTG	27	0.7	25	0.7		CAG	19	0.5	16	0.4
Ser	TCT	46	1.2	46	1.2	Cys	TGT	4	0.1	7	0.2
	TCC	72	1.9	70	1.8		TGC	19	0.5	22	0.6
	TCA	62	1.6	61	1.6	Stop	TGA	97	2.6	96	2.5
	TCG	6	0.2	6	0.2	Trp	TGG	23	0.6	18	0.5
Pro	CCT	58	1.5	74	2	Arg	CGT	8	0.2	15	0.4
	CCC	110	2.9	112	3		CGC	19	0.5	23	0.6
	CCA	49	1.3	52	1.4		CGA	43	1.1	42	1.1
	CCG	8	0.2	10	0.3		CGG	5	0.1	11	0.3
Thr	ACT	44	1.2	46	1.2	Ser	AGT	12	0.3	10	0.3
	ACC	123	3.2	124	3.3		AGC	45	1.2	53	1.4
	ACA	125	3.3	118	3.1	Stop	AGA	0	0	5	0.1
	ACG	13	0.3	14	0.4		AGG	0	0	11	0.3
Ala	GCT	65	1.7	57	1.5	Gly	GGT	22	0.6	30	0.8
	GCC	147	3.9	148	3.9		GGC	89	2.3	80	2.1
	GCA	102	2.7	103	2.7		GGA	80	2.1	88	2.3
	GCG	25	0.7	15	0.4		GGG	46	1.2	37	1

The incomplete T/TA of the stop codon is not included.

Sequence comparison between P. orientalis *and* P. vittatus

The comparison analysis of 16 genes between *P. orientalis* and *P. vittatus* were showed in Table VI. The complete mitogenome of the two sweetlips were 16,546 bp (*P. orientalis*) and 16,545 bp (*P. vittatus*) in length, but there were 941 variable sites (5.69%) in the two mitogenomes, the sequence divergence value was revealed to be 6.5% based on the Kimura-2 Parameter model. The differences of 13 protein-coding genes, 2 ribosomal RNA genes and one control region which are commonly used as molecular markers between the two species were also analyzed. That showed great genetic differences between the two sweetlips existed in all 16 genes. The divergence values based on K-2-P model between *P. orientalis* and *P. vittatus* ranged from 2.4% (12S rRNA) to 11.2% (ND6) with nucleotide mutation ranged from 6 bp to 132 bp, all divergence values were larger than 2%, even in the two ribosomal RNA genes (12 and 16 rRNAs) which retained very low evolutionary rate, revealing a significant genetic differences between the two sweetlips at molecular level.

Table VI.- Comparison analysis of 16 gene sequences between *P. orientalis* and *P. vittatus*.

	Length (*P. orientalis*)	Length (*P. vittatus*)	Number of variable sites (bp)	Bases variation (%)	Sequence divergence (%)
D-loop	848	849	83	9.78	10.6
12S rRNA	951	952	22	2.31	2.4
16S rRNA	1695	1691	45	2.66	2.7
COI	1557	1563	94	6.03	6.4
COII	691	691	23	3.33	3.4
COIII	785	785	41	5.22	5.5
ND1	975	975	76	7.79	8.4
ND2	1047	1047	91	8.69	9.4
ND3	349	349	26	7.49	8
ND4L	297	297	12	4.04	4.2
ND4	1381	1381	109	7.89	8.5
ND5	1839	1839	132	7.18	7.8
ND6	522	522	53	10.15	11.2
Cyt*b*	1141	1141	82	5.69	7.7
ATPase6	683	683	40	5.86	6.2
ATPase8	168	168	6	3.57	3.7
Whole sequences	16546	16545	941	5.69	6.5

Phylogenetic analysis

Molecular phylogenetic trees of 21 sweetlips (*Plectorhynchinae*) were constructed using ML and BI methods based on combined Cyt b and COI sequences, the trees were well divided the sweetlilps into three groups with high bootstrap values and posterior probabilities (Fig. 2). Group one composed sweetlips with beautiful coloration pattern, group two and three composed sweetlips with simple patterns and dark appearances. Two sweetlips *P. orientalis* and *P. vittatus* were positioned in group one, clustering together as sister species and closely related to the cluster *P. lineatus* and *P. diagrammus* which also possessed black stripes along the body, indicating their closed relationships with each other. Another phylogenetic trees constructed from 42 Percoidei species of 25 families formed 5 large groups, Diagramma pictum, Parapristipoma trilineatum, *P. orientalis* and *P. vittatus* formed a monophyletic Haemulidae in group I, sister to the cluster Lethrinus obsoletus + Monotaxis grandoculis. The species Hapalogenys nigripinnis, which was a Haemulidae member morphologically, was separated from Haemulidae group. It was placed in group IV and clustered with Sillago sihama, revealing its distant relationship with Haemulidae species.

DISCUSSION

Similar organization of two mitogenomes with other vertebrates

The two mitogenomes both consisted of 13 typical vertebrate protein-coding genes, 22 transfer RNA (tRNA) genes, 2 ribosomal RNA (rRNA) genes and one putative control region, which were similar to other vertebrate mitogenomes. As in other vertebrate species (Miya *et al*., 2003; Oh *et al*., 2007; Ponce *et al*., 2008; Catanese *et al*., 2010), most genes were encoded on the H-strand except for one protein-coding gene ND6 and 8 tRNA genes (Gln, Ala, Asn, Cys, Tyr, Ser^{UCN}, Glu and Pro) which were encoded on the L-strand. As shown in Table IV, the contents of A+T of the two sweetlips were both higher than that of C+G, indicating a strong compositional base bias in the mitochondrial genome which was in accordance with the variation tendency of the A+T content in other vertebrates.

For 13 protein-coding genes (Table V), variances were observed in stop codons, which seemed to be a tendency in fish mitochondrial genomes (Miya and Nishida, 1999; Ponce *et al*., 2008; Catanese *et al*., 2010). The three reading-frame overlaps observed between protein coding genes showed different from the previously reported species that 1 nucleotide was overlapped between the two genes (Oh *et al*., 2007; Ponce *et al*., 2008; Catanese *et al*., 2010). As shown in Table IV, the base composition of the two sweetlips mitochondrial protein-coding genes showed a strong bias against G, which seemed due to the hydrophobic characteristic of the proteins (Naylor *et al*., 1995). These typical features of protein-coding genes were also found in most vertebrates (Oh *et al*., 2007; Catanese, 2010; Cheng *et al*., 2012a, b). These codon usage patterns in protein coding genes were in accordance with the overall bias against G, because G was the least common third position nucleotide in all codons except for glycine, in which G was more frequent than T. All these features were very similar to other bony fish (Miya *et al*., 2003; Ponce *et al*., 2008; Cheng *et al*., 2012a, b).

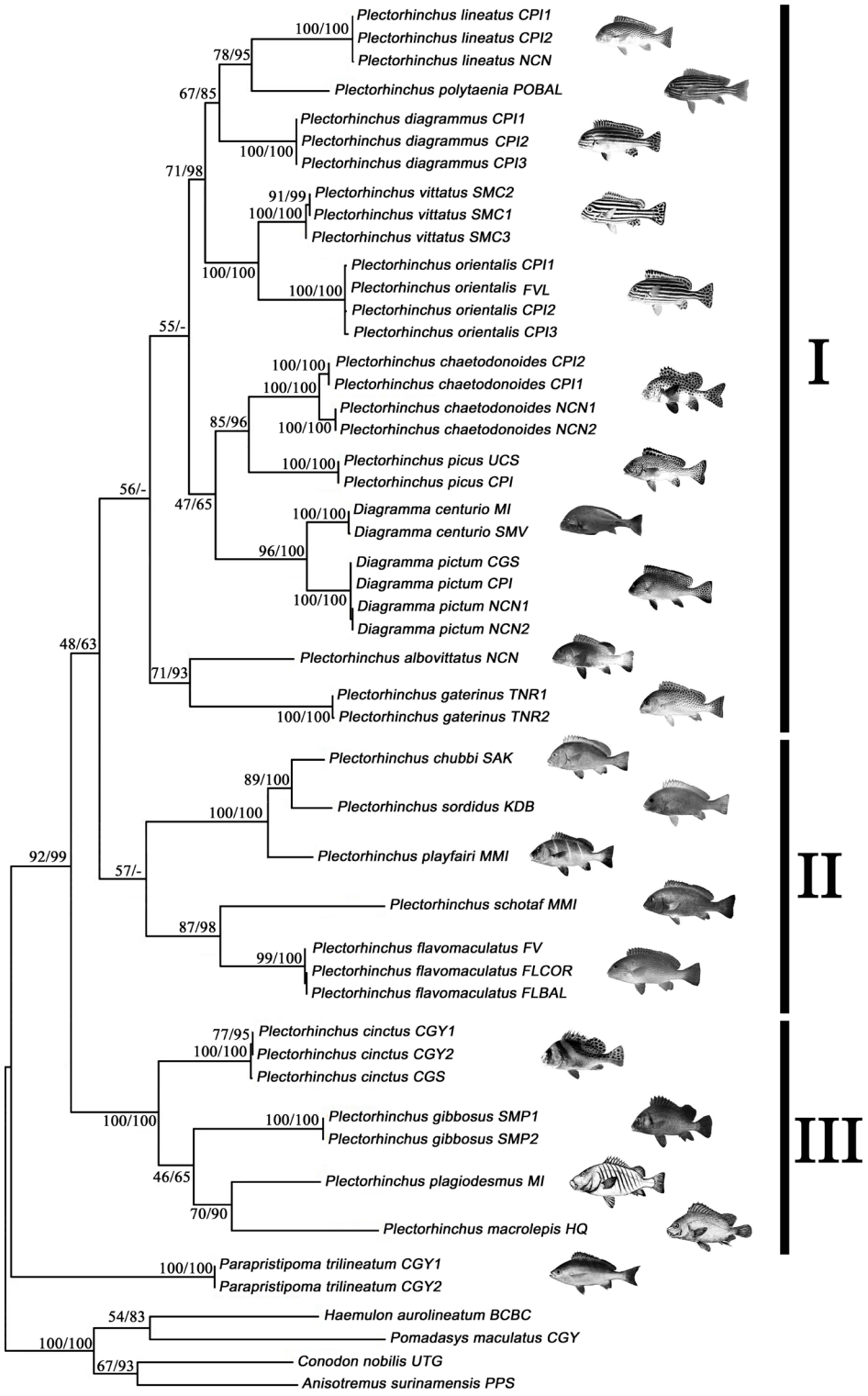

Fig. 2. Molecular phylogenetic trees of 21 Haemulidae species based on combined cyt b and COI dataset constructed by maximum likelihood (ML) and Bayesian inference (BI) methods. Numbers at the nodes are bootstrap values of ML and Bayesian posterior probabilities, respectively. Pictures of species were the Fishbase website provided by Randall JE, Cornish, A, FAO and website of eol.org

For tRNAs, They showed the typical arrangement in vertebrates. Most of them could be folded into the typical secondary structure-canonical cloverleaf, which were determined by the tRNAscan-SE software (Lowe and Eddy, 1997). The two ribosomal RNA genes were located between the genes of tRNA-Phe and tRNA-Leu, separated by the gene tRNA-Val, as in other vertebrates (Miya *et al*., 2001; Oh *et al*., 2007; Catanese *et al*., 2010). For noncoding sequences, the light strand replication origin (O_L) in *P.orientlais* and *P. vittatus* was positioned in a cluster of five tRNA genes known as the WANCY region between the $tRNA^{Asn}$ and $tRNA^{Cys}$ genes as most of the vertebrates.

The differences of mitogenomes between P. orientalis *and* P. vittatus

Avise and Walker (1999) revealed divergence values between species of vertebrates in Cyt b were ordinarily greater than 2%. Hebert (2003a) and (2003b) studied the COI sequences of 13320 species from 11 phyla and suggested that the threshold of divergence values in COI for species diagnosis was 2%. Intraspecific divergences were rarely greater than 2% and most were less than 1% (Hebert, 2003b; Avise, 2000). In our study, sequence divergences were revealed in COI and Cyt b between the two sweetlips were 6.4% and 7.7%, respectively, largely greater than the 2% value suggested by Hebert. The other 14 genes in Table VI were also larger than the threshold 2% value, even in the two highly conserved ribosomal RNA genes. In our previous studies, we had investigated the phylogenetic relationship of 17 sweetlips involved 3 individuals of *P. orientalis* and 3 individuals of *P. vittatus* based on Cyt b and COI genes (Liang *et al*., 2016). The result revealed that sequence divergences in each individual sample of *P. orientalis* and *P. vittatus* were all less than 0.5% in the two genes. The study also discovered that the divergence value between *P. orientalis* and *P. vittatus* was larger than some inter-sweetlips species values like *Plectorhinchus chubbi* vs *Plectorhinchus sordidus* (COI: 3.6%; Cytb: 3.4%), *Plectorhinchus chubby* vs *Plectorhinchus plafairi* (COI: 4.8%; Cytb: 5.0%) and *Plectorhinchus sordidus* vs *Plectorhinchus plafairi* (COI: 5.7%; Cytb: 4.5%). All these pieces of evidences indicated that great sequence variation and molecular differentiation were existed in the two sweetlips, suggested that they might be considered as two separate species.

Phylogenetic analysis of P. orientalis *and* P. vittatus

Phylogenetic trees constructed from 21 Haemulidae species based on the the combined sequences of Cyt b, COI were well divided the sweetlilps into three groups (Fig. 2). Group one composed sweetlips with beautiful coloration pattern, group two and three composed sweetlips with simple patterns and dark appearances, which was in accordance with traditional morphological classifications (McKay, 1984, 2001). The two sweetlips *P. orientalis* and *P. vittatus* were positioned in group one, clustering together as sister species and closely related to the cluster *P. lineatus* and *P. diagrammus*, which was consistent with the molecular phylogeny of *Haemulidae* species based on 16S rRNA and TMO-4C4. By morphological diagnosis, they all had black stripes and bands on their bodies. In the phylogenetic trees, the long branch clearly distinguished the separated evolutionary positions of the two orientalis sweetlips. Along with the great genetic differences result of sequence comparison analysis on 16 molecular marker genes above, we suggested that *P. orientalis* and *P. vittatus* might be placed as two distinct species and were not synonym.

The phylogenetic position of *Haemulidae* among the *Percoidei* was also examined in this study (Fig. 3). The tree revealed 4 *Haemulidae* species *Diagramma pictum*, *Parapristipoma trilineatum*, *P. orientalis* and *P. vittatus* formed a monophyletic group except for *Hapalogenys nigripinnis*, which was placed in an independent position out of the *Haemulidae* clusters. Such finding was not congruent with the traditional morphological studies (Shen, 1993; McKay, 2001; Nelson, 2006) but agreed with some other morphology and molecule-based studies which suggested that the genus *Hapalogenys* were removed from *Haemulidae* (Springer and Raasch, 1995; Leis and Carson-Ewart, 2000; Froese and Pauly, 2014). Recent phylogenetic analysis on the relationships of *Hapalogenys* and *Haemulidae* also revealed a distant relationship between them (Ren and Zhang, 2007; Sanciangco *et al*., 2011). Our result was in agreement with those molecular reports, indicating the fundamental status of *Hapalogenys* in the *Percoidei* may need to be redefined. The family *Haemulidae* was grouped with family *Lethrinidae* as sister lineage and was closely related to families *Lutjanidae*, *Caesionidae*, *Emmelichthyidae* and *Monodactylidae*. Traditionally, potential sister groups to Haemulidae as well as its placement among *Percoidei* were uncertain either in morphological or in molecular studies (Johnson, 1981; Sanciangco *et al*., 2011; Tavera *et al*., 2012). Recent studies on higher-level relationships of *percomorphs* and *acanthomorphs* have shown potential outgroups for *Haemulidae* on the basis of molecular characters but the relationships and placement of *Haemulidae* varied through time according to different authors (Dettai and Lecointre, 2005; Chen *et al*., 2007; Craig and Hastings, 2007; Smith and Craig, 2007; Li *et al*., 2009). The current studies on *Haemulidae* relationships by Tavera *et al*. (2012) have revealed *Sillaginidae* sister clade to

Haemulidae, but the support values were relatively low. Our molecular phylogenetic study on the placement of *Haemulidae* among *Percoidei* included the previously suggested potential *Haemulidae*-close families. The result recovered *Haemulidae* sister to *Lethrinidae* and was placed in a cluster comprised of families *Lutjanidae*, *Caesionidae*, *Emmelichthyidae* and *Monodactylida*e, which were consistent with the studies of Tavera *et al*. (2012). However, the *Percoidei* is the largest suborder of the *Perciformes* and the sequences of most *Percoidei* species are not available. A complete mitochondrial genomes are needed to be sequenced for further detailed analysis of the evolutionary relationships of *Haemulidae* within *Percoidei* species.

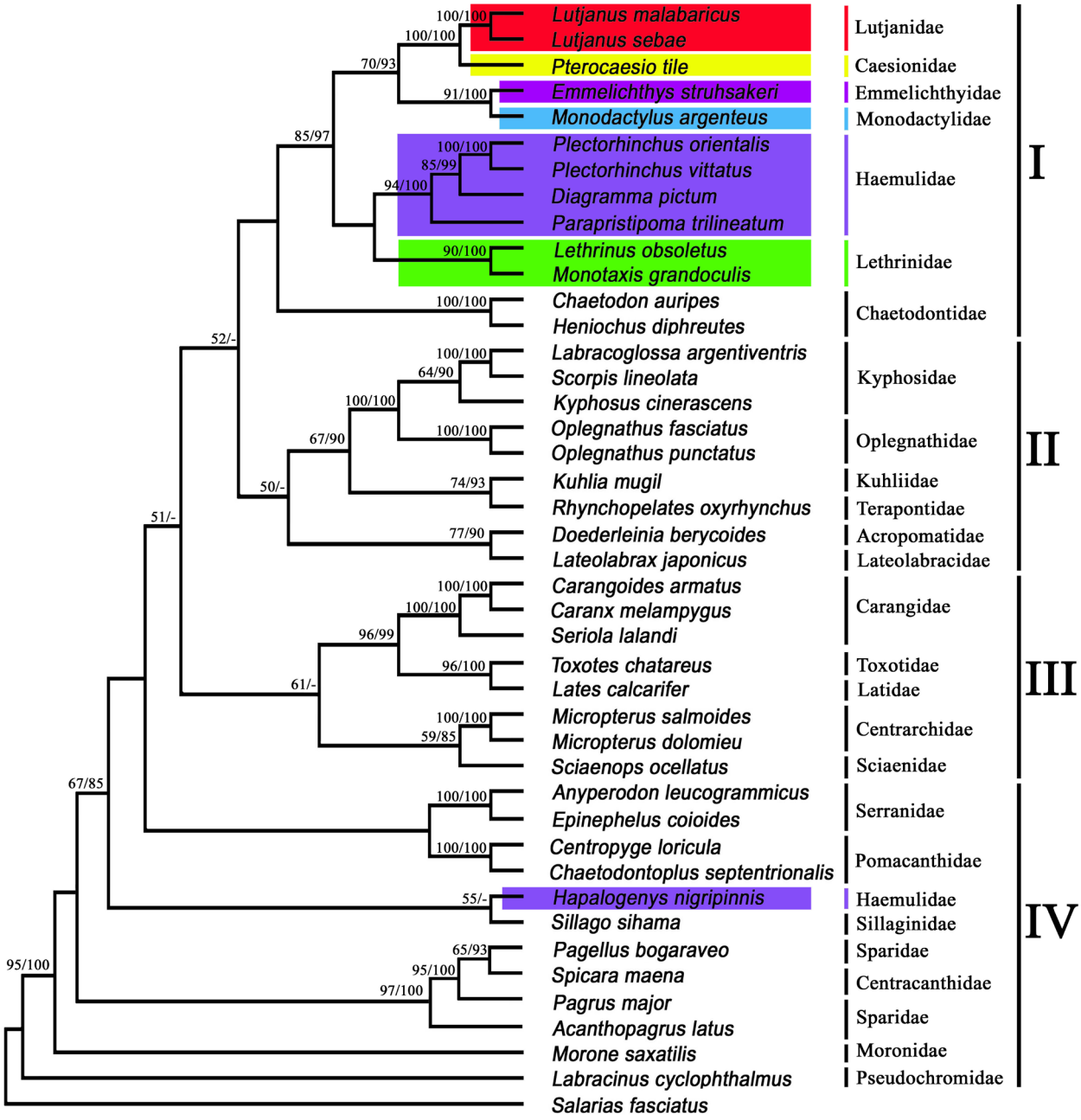

Fig. 3. Molecular phylogenetic trees of 42 Percoidei species based on 12 protein-coding genes (excluding the ND6 gene and the 3rd codon positions) constructed by maximum likelihood (ML) and Bayesian inference (BI) methods. Numbers at the nodes are bootstrap values of ML and Bayesian posterior probabilities, respectively

CONCLUSIONS

The mitochondrial genome of two synonyms debated species *P. orientalis* and *P. vittatus* were first determined in this study. The lengths of the two mitogenome are 16,546 bp and 16,545 bp, which both consist of 13 typical vertebrate protein-coding genes, 22 transfer RNA (tRNA) genes, 2 ribosomal RNA (rRNA) genes and one putative control region. Genomic composition, organization, and gene order of the two genomes are similar to that obtained in most vertebrates. All sequence divergences of 13 protein-coding genes, 2 rRNA genes and one control region which are commonly used as molecular markers between the two species are larger than the species diagnosis divergence value 2%. And divergence values of Cyt b (6.0%) and COI (7.4%) are also largely greater than 2%. Furthermore, molecular phylogenetic trees also clearly distinguish the independent position of the two species. These results above reveal great genetic differences between *P. orientalis* and *P. vittauts*, and strongly suggest that they are two distinct species and should not be placed as synonyms.

ACKNOWLEDGMENTS

The study was supported by the Natural Science Foundation of Guangdong Province, China (2016A030310236) and Foundation for Young Talents in Higher Education of Guangdong, China (2014KQNCX164). We wish to thank Bernard Mackenzie (SAIAB South African Institute for Aquatic Biodiversity, Grahamstown, South Africa) for his kind assistance in providing samples of *P. vittatus*. We would also like to thank the College of Animal Science in South China Agricultural University for their laboratory facilities and experimental support to this study.

Statement of conflict of interest

Authors declare that they have no competing interests.

REFERENCES

Avise, J.C. and Walker, D., 1999. Species realities and numbers in sexual vertebrates: perspectives from an asexually transmitted genome. *Proc. natl. Acad. Sci. USA*, **96**: 992-995. https://doi.org/10.1073/pnas.96.3.992

Avise, J.C., 2000. *Phylogeography. The history and formation of species*. Harvard University Press, Cambridge, MA.

Boore, J.L., 1999. Animal mitochondrial genomes. *Nucl. Acids Res.,* **27**: 1767-1780. https://doi.org/10.1093/nar/27.8.1767

Broughton, R.E. and Reneau, P.C., 2006. Spatial covariation of mutation and nonsynonymous substitution rates in vertebrate mitochondrial genomes. *Mol. Biol. Evol.,* **23**: 1516-1524. https://doi.org/10.1093/molbev/msl013

Catanese, G., Manchado, M. and Infante, C., 2010. Evolutionary relatedness of mackerels of the genus Scomber based on complete mitochondrial genomes: Strong support to the recognition of Atlantic *Scomber colias* and Pacific *Scomber japonicus* as distinct species. *Gene*, **452**: 35-43. https://doi.org/10.1016/j.gene.2009.12.004

Chen, W.J., Ruiz-Carus, R. and Ortí, G., 2007. Relationships among four genera of mojarras (Teleostei: Perciformes: Gerreidae) from the western Atlantic and their tentative placement among percomorph fishes. *J. Fish Biol*., **70**: 202-218. https://doi.org/10.1111/j.1095-8649.2007.01395.x

Cheng, Y.Z., Shi, G., Xu, T.J., Li, H.Y., Sun, Y.Y. and Wang, R.X., 2012a. Complete mitochondrial genome of the red drum, *Sciaenops ocellatus* (Perciformes, Sciaenidae): Absence of the typical conserved motif in the origin of the light-strand replication. *Mitochondrial DNA*, **23**: 126-128. https://doi.org/10.3109/19401736.2011.653807

Cheng, Y.Z., Wang, R.X., Xu, T.J. and Sun, Y.N., 2012b. The complete mitochondrial genome of the small yellow croaker and partitioned Bayesian analysis of Sciaenidae fish phylogeny. *Genet. Mol. Biol.,* **35**: 191-199. https://doi.org/10.1590/S1415-47572012005000006

Clayton, D.A., 1992. Transcription and replication of animal mitochondrial DNAs. *Int. Rev. Cytol.,* **141**: 217-232. https://doi.org/10.1016/S0074-7696(08)62067-7

Craig, M.T. and Hastings, P.A., 2007. A molecular phylogeny of the groupers of the subfamily Epinephelinae (Serranidae) with a revised classification of the Epinephelini. *Ichthyol. Res*., **54**: 1-17. https://doi.org/10.1007/s10228-006-0367-x

Dettai, A. and Lecointre, G., 2005. Further support for the clades obtained by multiple molecular phylogenies in the acanthomorph bush. *C. R. Biol.,* **328**: 674-689. https://doi.org/10.1016/j.crvi.2005.08.001

Froese, R. and Pauly, D. (eds.), 2014. *FishBase, version 03/2014*. World Wide Web Electronic Publication. Available at: www.fishbase.org

Gillibrand, C.J., Harris, A.R. and Mara, E., 2007. Inventory and spatial assemblage study of reef fish in the area of Andavadoaka, South-West Madagascar (Western Indian Ocean). *J. mar. Sci.,*

6: 183-197.

Gutell, R.R., Gray, M.W. and Schnare, M.N., 1993. A compilation of large subunit (23S and 23S-like) ribosomal RNA structures. *Nucl. Acids Res.,* **21**: 3055-3074. https://doi.org/10.1093/nar/21.13.3051

Hebert, P.D.N., Cywinska, A., Ball, S.L. and deWaard, J.R., 2003a. Biological identifications through DNA barcodes. *Proc. R. Soc. Lond. B*, **270**: 313-322. https://doi.org/10.1098/rspb.2002.2218

Hebert, P.D.N., Ratnasingham, S. and de Waard, J.R., 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. Lond. B,* **270**: 596-599. https://doi.org/10.1098/rsbl.2003.0025

Iguchi, J., Takashima, Y., Namikoshi, A. and Yamashita, M., 2012. Species identification method for marine products of seriola and related species. *Fish Sci.,* **78**: 179-206. https://doi.org/10.1007/s12562-011-0433-9

Inoue, J.G., Miya, M., Tsukamoto, K. and Nishida, M., 2001. A mitogenomic perspective on the basal teleostean phylogeny: resolving higher-level relationship with longer DNA sequences. *Mol. Phylogenet. Evol.,* **20**: 275-285. https://doi.org/10.1006/mpev.2001.0970

Johnson, G.D., 1981. The limits and relationships of Lutjanidae and associated families. In: *Bulletin of the Scripps Institution of Oceanography* (eds. C.S. Cox, E.S. Vincent, A. Fleminger and R.H. Rosenblatt). University of California Press, Berkeley, pp. 1-112.

Kawahara, R., Miya, M., Mabuchi, K., Lavoué, S., Inoue J.G., Satoh, T.P., Kawaguchi, A. and Nishida, M. 2008. Interrelationships of the 11 gasterosteiform families (sticklebacks, pipefishes, and their relatives): a new perspective based on whole mitogenome sequences from 75 higher teleosts. *Mol. Phylogenet. Evol.,* **46**: 224-236. https://doi.org/10.1016/j.ympev.2007.07.009

Leis, J.M. and Carson-Ewart, B.M., 2000. The larvae of Indo-Pacific coastal fishes. An identification guide to marine fish larvae. *Fauna Malesiana Handbooks 2*. E.J. Brill., Leiden, pp. 870.

Li, B., Dettaï, A., Cruaud, C., Couloux, A., Desoutter-Meniger, M. and Lecointre, G., 2009. RNF213, a new nuclear marker for acanthomorph phylogeny. *Mol. Phylogenet. Evol.*, **50**: 345-363. https://doi.org/10.1016/j.ympev.2008.11.013

Liu, Z.S., Song, N., Yanagimoto, T., Han, Z.Q., Shui, B.N. and Gao, T.X., 2017 Complete mitochondrial genome of three fish species (Perciformes: Amblyopinae): Genome description and phylogenetic relationships. *Pakistan J. Zool.,* **49**: 111-120. http://dx.doi.org/10.17582/journal.pjz/2017.49.1.111.120

Liang, R.S., Zheng, W.J., Zou, Q., Zeng Y.D., Zhu, S.H. and Zou, J.X., 2012. The complete mitochondrial genome of black grunt *Hapalogenys nigripinnis*. *Mitochondrial DNA*, **23**: 444-446. https://doi.org/10.3109/19401736.2012.710221

Liang, R.S., Wang, C., Zou, Q., Zhou, A.G. and Zhou, M., 2016. Molecular phylogenetic relationships of some common sweetlips Haemulidae Plectorhynchinae and the synonyms controversy of two *Plectorhinchus* species. *Mitochondrial DNA*, **27**: 2209-2214.

Lin, G., Lo, L.C., Zhu, Z.Y., Feng, F., Chou, R. and Yue, G.H., 2006. The complete mitochondrial genome sequence and characterization of single-nucleotide polymorphisms in the control region of the Asian seabass (*Lates calcarifer*). *Mar. Biotechnol.,* **8**: 71-79. https://doi.org/10.1007/s10126-005-5051-z

Lowe, T.M. and Eddy, S.R., 1997. tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. *Nucl. Acids Res.,* **25**: 955-964. https://doi.org/10.1093/nar/25.5.0955

Mabuchi, K., Miya, M., Azuma, Y. and Nishida, M., 2007. Independent evolution of the specialized pharyngeal jaw apparatus in cichlid and labrid fishes. *BMC Evol. Biol.,* **7**: 10. https://doi.org/10.1186/1471-2148-7-10

McKay, R.J., 1984. Haemulidae. In: *FAO species identification sheets for fishery purposes* (eds. W. Fischer and G Bianchi), Vol. 2. Western Indian Ocean (Fishing Area 51), FAO, Rome.

McKay, R.J., 2001. Haemulidae = Pomadasyidae. Grunts (also sweetlips, rubberlips, hotlips, and velvetchins). In: *The living marine resources of the Western Central Pacific* (eds. K.E. Carpenter and V.H. Niem), Volume 5. FAO, Rome, pp. 2961-2989.

Miya, M. and Nishida, M., 1999. Organization of the mitochondrial genome of a deep-sea fish, Gonostoma gracile (Teleostei: Stomiiformes): first example of transfer RNA gene rearrangements in bony fishes. *Mar. Biotechnol.,* **1**: 416-426. https://doi.org/10.1007/PL00011798

Miya, M., Kawaguchi, A. and Nishida, M., 2001. Mitogenomic exploration of higher teleostean phylogenies: A case study for moderate-scale evolutionary genomics with 38 newly determined complete mitochondrial DNA sequences. *Mol. Biol. Evol.*, **18**: 1993-2009. https://doi.org/10.1093/oxfordjournals.molbev.a003741

Miya, M., Takeshima, H., Endo, H., Ishiguro, N.B.,

Inoue, J.G., Mukai, T., Satoh, T.P., Yamaguchi, M., Kawaguchi, A., Mabuchi, K., Shirai, S.M. and Nishida, M., 2003. Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. *Mol. Phylogenet. Evol.,* **26**: 121-138. https://doi.org/10.1016/S1055-7903(02)00332-9

Mukai, T. and Sato, C., 2009. Complete mitochondrial DNA sequences of two haplotypes of the smallmouth bass, *Micropterus dolomieu*, collected from nonindigenous populations in Japan. *Ichthyol. Res*., **56**: 204-207. https://doi.org/10.1007/s10228-008-0074-x

Naylor, G.J., Collins, T.M. and Brown, W.M., 1995. Hydrophobicity and phylogeny. *Nature*, **373**: 565-566. https://doi.org/10.1038/373565b0

Nelson, J.S., 2006. *Fishes of the world*. John Wiley & Sons Inc., New York, pp. 368-369.

Oh, D.J., Kim, J.Y., Lee, J.A., Yoon, W.J., Park, S.Y. and Jung, Y.H., 2007. Complete mitochondrial genome of the rock bream *Oplegnathus fasciatus* (Perciformes, Oplegnathidae) with phylogenetic considerations. *Gene*, **392**: 174-180. https://doi.org/10.1016/j.gene.2006.12.007

Ponce, M., Infante, C., Jiménez-Cantizano, R.M., Péreza, L. and Manchadoa, M., 2008. Complete mitochondrial genome of the blackspot seabream, *Pagellus bogaraveo* (Perciformes: Sparidae), with high levels of length heteroplasmy in the WANCY region. *Gene*, **409**: 44-52. https://doi.org/10.1016/j.gene.2007.11.004

Posada, D. and Crandall, K., 1998. Modeltest: Testing the model of DNA substitution. *Bioinformatics*, **14**: 817-818. https://doi.org/10.1093/bioinformatics/14.9.817

Posada, D. and Buckley, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio tests. *Syst. Biol.,* **53**: 793-808. https://doi.org/10.1080/10635150490522304

Randall, J.E., 2005. *Reef and shore fishes of the South Pacific*. New Caledonia to Tahiti and the Pitcairn Islands. University of Hawaii Press, Honolulu, Hawaii, pp. 271.

Randall, J.E. and Johnson, J.W., 2000. *Perca lineata* and *P. vittata* established as valid species of Plectorhinchus (Perciformes: Haemulidae). *Mem. Queensl. Mus.,* **45**: 477-482.

Randall, J.E. and Lim, K.K.P., 2000. A checklist of the fishes of the South China Sea. *Raffles Bull. Zool. Suppl.,* **8**: 569-667.

Ren, G. and Zhang, Q., 2007. Phylogeny of haemulid with discussion on systematic position of the genus Hapalogenys. *Acta Zootaxon. Sin.,* **32**: 835-841.

Ronquist, F. and Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics,* **19**: 1572-1574. https://doi.org/10.1093/bioinformatics/btg180

Saitoh, K., Sado, T., Mayden, R.L., Hanzawa, N., Nakamura, K., Nishida, M. and Miya, M., 2006. Mitogenomic evolution and interrelationships of the cypriniformes (Actinopterygii: Ostariophysi): the first evidence toward resolution of higher-level relationships of the world's largest freshwater fish clade based on 59 whole mitogenome sequences. *J. mol. Evol*., **63**: 826-841. https://doi.org/10.1007/s00239-005-0293-y

Sanciangco, M.D., Rocha, L.A. and Carpenter, K.E., 2011. A molecular phylogeny of the Grunts (Perciformes: Haemulidae) inferred using mitochondrial and nuclear genes. *Zootaxa,* **2966**: 37-50.

Satapoomin, U. and Randall, J.E., 2000. Plectorhinchus macrospilus, a new species of thicklip (Perciformes: Haemulidae) from the Andaman Sea off southestern Thailand. *Phuket Mar. Biol. Cent. Res. Bull.,* **63**: 9-16.

Shadel, G.S. and Clayton, D.A., 1997. Mitochondrial DNA maintenance in vertebrates. *Annu. Rev. Biochem.,* **66**: 409-435. https://doi.org/10.1146/annurev.biochem.66.1.409

Shen, S.J., 1993. *Fishes of Taiwan*. National Taiwan University, Department of Zoology, Taipei, pp. 360-363.

Smith, W.L. and Craig, M.T., 2007. Casting the Percomorph Net Widely: The importance of broad taxonomic sampling in the search for the placement of Serranid and Percid fishes. *Copeia*, **1**: 35-55. https://doi.org/10.1643/0045-8511(2007)7[35:CTPNWT]2.0.CO;2

Springer, V.G. and Raasch, M.S., 1995. Fishes, angling, and finfish fisheries on stamps of the World. American Topical Association. *Fishes on Stamps Handbook*, **129**: 1-110.

Tavera, J., Acero, A., Balart, E.F. and Bernardi, G., 2012. Molecular phylogeny of grunts (Teleostei, Haemulidae), with an emphasis on the ecology, evolution, and speciation history of New World species. *BMC Evol. Biol*., **12**: 57. https://doi.org/10.1186/1471-2148-12-57

Thompson, J.D., Higgins, D.G. and Gibson, T.J., 1994. CLUSIAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties

and weight matrix choice. *Nucl. Acids Res.,* **22**: 4673-4680. https://doi.org/10.1093/nar/22.22.4673

Tzeng, C.S., Hui, C.F., Shen, S.C. and Huang, P.C., 1992. The complete nucleotide sequence of the Crossostoma lacustre mitochondrial genome: Conservation and variations among vertebrates. *Nucl. Acids Res.,* **20**: 4853-4858. https://doi.org/10.1093/nar/20.18.4853

Unsworth, R.K.F., 2010. Seagrass meadows of the Wakatobi National Park. In: *Marine conservation and research in the coral triangle* (eds. J. Clifton, R.K.F. Unsworth and D.J. Smith). The Wakatobi National Park. Nova Publishers, New York, pp. 101-126.

Xia, J.H., Xia, K.F. and Jiang, S.G., 2008. Complete mitochondrial DNA sequence of the yellowfin seabream *Acanthopagrus latus* and a genomic comparison among closely related sparid species. *Mitochondria DNA*, **19**: 385-393.

Yagishita, N., Miya, M., Yamanoue, Y., Shirai, S.M., Nakayama, K., Suzuki, N., Satoh, T.P., Mabuchi, K., Nishida, M. and Nakabo, T., 2009. Mitogenomic evaluation of the unique facial nerve pattern as a phylogenetic marker within the percifom fishes (Teleostei: Percomorpha). *Mol. Phylogenet. Evol.,* **53**: 258-266. https://doi.org/10.1016/j.ympev.2009.06.009

Yamanoue, Y., Miya, M., Matsuura, K., Yagishita, N., Mabuchi, K., Sakai, H., Katoh, M. and Nishida, M., 2007. Phylogenetic position of tetraodontiform fishes within the higherteleosts: Bayesian inferences based on 44 whole mitochondrial genome sequences. *Mol. Phylogenet. Evol.,* **45**: 89-101. https://doi.org/10.1016/j.ympev.2007.03.008

Zhuang. X., Ding, S.X., Wang, J., Wang, Y. and Su, Y.Q., 2009. A set of 16 consensus primer pairs amplifying the complete mitochondrial genomes of orange-spotted grouper (*Epinephelus coioides*) and Hong Kong grouper (*Epinephelus akaara*). *Mol. Ecol. Resour.,* **9**: 1551-1553. https://doi.org/10.1111/j.1755-0998.2009.02716.x