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Phylogenetic relationships of twenty *Gymnothorax* species based on cytochrome *b* sequence data

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ABSTRACT. To study the phylogenetic relationships of the genus *Gymnothorax* (moray eels) distributed in South China Sea, polymerase chain reactions were performed, and the amplification products were sequenced by cloning into the PMD18T-vector (TaKaRa). The entire gene sequences encoding cytochrome *b* (1140 bp) for 16 *Gymnothorax* (*G. flavimarginatus*, *G. meleagris*, *G. undulates*, *G. reticularis*, *G. reevesi*, *G. melanospilus*, *G. rueppeliae*, *G. javanicus*, *G. chilospilus*, *G. pseudothyrsoideus*, *G. fimbriatus*, *G. hepaticus*, *G. berndti*, *G. curostus*, *G. favagineus*, and *G. margaritophorus*) were obtained. Four additional *Gymnothorax* sequences from GenBank were also included. The nucleotide composition, genetic distances, and base substitution saturation analysis were calculated using the MEGA 5.0 Software. Phylogenetic analysis was performed using maximum-parsimony, maximum-likelihood (ML), and neighbor-joining (NJ). The results were as follows: 1) base-substitution saturation analysis suggested that both in third codon positions, and the full-length cytochrome *b* data

set, Ts are not saturated, but Tv substitutions may be saturated, 2) the genus *Gymnothorax*, native to the South China Sea, is divided into four distinct clades, with two clades in the NJ and ML trees, and 3) according to our experimental data, G. *melanospilus* (Bleeker, 1855) and G. *favagineus* (Bloch and Schneider, 1801) are the same species.

Key words: *Gymnothorax*; Mitochondrial DNA; Cytochrome *b*; Phylogenetic relationship

INTRODUCTION

Moray eels (*Gymnothorax*), belonging to the family Muraenidae, are widely distributed in the world's tropical and semi-tropical oceans. The family Muraenidae is one of the most abundant and widespread of all eel families. It is estimated to contain 200 species or more belonging to 15 genera. The genus *Gymnothorax* (moray eels) contains approximately 120 species worldwide (Tesch, 1977; Castle, 1994; Paxton and Eschmeyer, 1994; Kuiter, 1996). *Gymnothorax* have no pectoral fins or pelvic fins and are found in all tropical and temperate oceans around the world. Their streamlined bodies are adapted for hunting within the crevices of the reef (Lowe-McConnell, 1987; Bohlke and Chaplin, 1993; Randall, 1996). In recent years, *Gymnothorax* have become the subject of many investigations. For example, Gilbert et al. (2005) estimated the density and biomass of moray eels (Muraenidae). In another study, Ling reported a decrease in the Sr/Ca ratios in the otoliths of *Gymnothorax reticularis* during metamorphosis (Ling et al., 2005). In addition, Tamburrini et al. (2001) studied the structure/function relationships of the brown moray (*Gymnothorax unicolor*) hemoglobin system. However, there is still debate on the number of *Gymnothorax* species that inhabit the South and East China Seas. For example, Cheng and Zheng (1987) proposed that there are 20 *Gymnothorax* species in both South and East China Seas. However, Shen (1993) maintains that there are 27 *Gymnothorax* species. Furthermore, major criteria for the identification of *Gymnothorax*, which include ratios of morphological measurements, coloration pattern, variegated markings, and dentition, are vague because most *Gymnothorax* species are similar in morphology. In addition, their phylogenetic relationships have not been well established. Further studies are needed to resolve these problems.

Throughout the last decade, molecular phylogenetic techniques have led to important changes in the way we understand the evolution of marine life. The number of species for which appropriate sequence information is available has been increasing rapidly, and the methods used have become more sophisticated (Avise, 1994; Stepien and Kocher, 1997). Mitochondrial DNA (mt DNA) can be a powerful molecular marker for reconstructing evolutionary lineages for animals (Avise, 1994). The gene encoding cytochrome *b* is the most widely used gene for phylogenetic research, and has been the most prevalent source of sequence data. In recent years, the nucleotide sequence of the cytochrome *b* gene has been widely used in fish taxonomy studies (Song et al., 1998; Thomas et al., 2000; Harris et al., 2002; Bernardi et al., 2003; Wu et al., 2014).

In this paper, we describe a fresh attempt to assess the phylogenetic relationships among different species of the genus *Gymnothorax* via sequence analysis of mtDNA cytochrome *b* genes sequences. Our objectives were 1) to clarify the taxonomic status of different moray eels in the genus *Gymnothorax* of the family Muraenidae and to 2) investigate the status of the moray eels' idioplasm resources for the South China Sea.

MATERIAL AND METHODS

Sample collection

A total of 23 species (20 in-group and 3 out-group) were used for the study to evaluate phylogenetic relationships within the genus *Gymnothorax*. The cytochrome *b* genes of four species were obtained from GenBank. Other specimens were collected from fish markets in Haikou and Sanya, and from fishing vessels that fished near Xisha and Nansha Island in the South China Sea. Species were initially identified morphologically and identification was confirmed upon being deposited in the lab. Two or three individuals from each species were used for the sequence analysis. The specimens were frozen immediately after being dissected and later kept at -80°C.

DNA extraction, amplification, and sequencing

The crude DNA samples used in the study were prepared from the muscle or tail fin tissue (Kocher et al., 1989) of 16 *Gymnothorax* species (Table 1). About 0.1 g tissue was homogenized in 1 mL digestion buffer (10 mM Tris-HCl, pH 8.0, 2 mM EDTA, 10 mM NaCl, 1% SDS, 10 mg/mL dithiothreitol, 0.5 mg/mL Proteinase K) and incubated at 37°C for 8 to 16 h, followed by a standard phenol and chloroform extraction. The DNA samples were preserved in 50 μL TE buffer.

Table 1. Sources of sequence data and samples.

Species	Location	Cytochrome *b* GenBank accession No.
G. kidako	Japan	AP002976
G. flavimarginatus	The South China Sea	EU085360
G. meleagris	The South China Sea	EU085361
G. undulates	The South China Sea	EU085362
G. reticularis	The South China Sea	EU085363
G. reevesi	The South China Sea	EU085364
G. melanospilus	The South China Sea	EU085365
G. rueppeliae	The South China Sea	EU085366
G. javanicus	The South China Sea	EU085367
G. chilospilus	The South China Sea	EU085368
G. pseudothyrsoideus	The South China Sea	EU085369
G. fimbriatus	The South China Sea	EU085370
G. hepaticus	The South China Sea	EU085371
G. berndti	The South China Sea	EU085372
G. curostus	The South China Sea	EU085373
G. favagineus	The South China Sea	EU085374
G. margaritophorus	The South China Sea	EU170376
G. polygonius	Spain	DQ197954
G. unicolor	Spain	DQ197955
G. maderensis	Spain	DQ197953

Sequences for *G. kidako*, *G. polygonius*, *G. unicolor*, and *G. maderensis* were obtained from GenBank.

A total of 4 PCR primers for cytochrome *b* genes were designed using the Primer Premier 5.0 Software based on the *Gymnothorax kidako* mitochondrial genome sequence (Inoue et al., 2003). Primer pairs GYM-Cytb-sen1, GYM-Cytb-ant1, GYM-Cytb-sen2, and GYM-Cytb-ant2 were used to amplify the full-length sequences of the genes encoding cytochrome *b*. The sequences of those primers are as follows: 5'-CCAGGACCAATGATACGAAA-3' (GYM-

Cytb-sen1); 5'-AAATTAAGCTACTAGGGCGC-3' (GYM-Cytb-ant1); 5'-CCTTTAATGGCA AACCTACG-3' (GYM-Cytb-sen2); 5'-TAGGGCGCTATCAGTCAAGT-3' (GYM-Cytb-ant2). PCR amplifications were performed using Nested PCR: the first PCR was performed using the total DNA as the template and the second PCR was performed using the products of the first PCR as the template.

PCR amplifications for cytochrome *b* were performed in a mixture containing, in a final volume of 25 µL: 16.2 µL ddH_2O, 1 µL crude DNA as the template, 2.5 µL dNTP, 2.5 µL *Ex Taq* buffer containing $MgCl_2$, 1.3 µL of each primer, 0.2 µL *Ex Taq* DNA polymerase (TaKaRa Biotechnology Co., Ltd, Dalian, China). All amplifications were performed using the following PCR profile: a preliminary denaturation at 94°C for 4 min followed by 35 cycles consisting of 94°C for 30 s, 50°C for 30 s, 72°C for 2 min, and a final extension at 72°C for 8 min. The amplification products were separated by electrophoresis through a 1.5% agarose gel, and the correct-size bands were cut out and purified using the Sangon Gel Extraction Kit, and then cloned into the PMD18T-vector (TaKaRa) and sequenced using a 3770 DNA Sequencer (ABI PRISM) according to the manufacturer instructions. These sequences for the gene encoding cytochrome *b* for the 16 *Gymnothorax* species were submitted to GenBank, and the series of accession numbers are listed in Table 1.

Sequence alignment and phylogenetic analyses

The cytochrome *b* gene sequences were aligned manually as they are the same length (1140 bp). The aligned DNA sequences were analyzed using the statistical analysis system in the MEGA program (Tamura et al., 2011). To calculate the nucleotide base composition bias and the Ts/Tv ratio, base substitution saturation analysis was performed on the gene sequence encoding cytochrome *b*.

Phylogenetic trees were constructed using three methods of the maximum- parsimony (MP), maximum-likelihood (ML), and neighbor-joining (NJ) analyses implemented in PAUP*, version 4.0b10 (Swofford, 2002). Maximum parsimony tree searches were performed in PAUP* using the heuristic search option, with 100 random-addition sequences, tree-bisection-reconnection branch swapping, and collapsing zero-length branches. The reliability of nodes was assessed using 1000 bootstrap replicates (Felsenstein, 1985), and in each case the heuristic search was limited to a maximum of 10,000 saved trees. Consistency and retention indices (CI and RI, respectively) were generated using the computer program PAUP* 4.0b10 for the parsimony tree. Skewness values (Hillis and Huelsenbeck, 1992) were estimated from random samples of 10,000 trees generated by PAUP* 4.0b10. *Anguilla marmorata*, *A. japonica*, and *Ophisurus macrorhynchos*, which were obtained from GenBank, were used as an out group for the analysis.

The maximum-likelihood analyses (heuristic search with random addition and ten repetitions, TBR branch swapping, starting branch length using Rogers-Swofford method, and multi-trees on; Rogers and Swofford, 1999) were run using the GTR (general-time-reversible model; Goldman and Yang, 1994). The neighbor-joining (NJ) trees were generated using Kimura 2-parameter (Kimura, 1980) model by PAUP* 4.0b10.

RESULTS

Sequence data

All the gene sequences encoding cytochrome *b* for the 16 *Gymnothorax* species

aligned well, and were deposited in GenBank **(**Table 1). In this study, 20 cytochrome *b* encoding sequences were analyzed (4 cytochrome *b* sequences were obtained from GenBank; the accession numbers are listed in Table 1). Mean base composition showed an anti-G bias (Table 2 and Figure 1), which is characteristic for this mitochondrial gene (Cantatore et al., 1994; Briolay et al., 1998). The low G content (mean: 15.4%) and the almost identical A, C, and T contents (mean: 25.7, 28.7, and 30.2%, respectively) were similar to those in previous studies on Cyprinidae and Cichlids fish cytochrome *b* sequences (Briolay et al., 1998; Martin and Bermingham, 1998; Perdices et al., 2004). The A and T content (55.9%) was higher than that of G and C (44.1%). The total A, T, C, and G content in the first codon showed little difference, and no nucleotide compositional bias was found. The T content in the second codon was much higher than that of the other three bases (41.1%); but the G content in the third codon was much lower than that of A, T, and C (8.8%) (Figure 1). This pattern was commonly observed in Zebrafish (Xiang et al., 2004).

Table 2. Nucleotide composition comparison for the 20 species of *Gymnothorax* studied.

Species	Percentage (%)				Total	Percentage (%)				Pos#1
	A	T	C	G		A-1	T-1	C-1	G-1	
G. kidako	25.7	30.9	28.6	14.8	1140	24.5	24.5	26.3	24.7	380
G. fimbriatus	25.6	31.1	27.9	15.4	1140	24.5	24.7	25.8	25.0	380
G. meleagris	27.1	27.9	30.7	14.3	1140	24.5	22.6	27.6	25.3	380
G. undulates	26.0	29.6	29.4	15.1	1140	24.5	25.3	25.0	25.3	380
G. reticularis	25.5	31.1	27.8	15.5	1140	24.5	24.7	25.8	25.0	380
G. pseudothyrsoideus	25.4	31.4	27.6	15.5	1140	24.7	24.7	25.8	24.7	380
G. chilospilus	25.7	31.1	27.8	15.4	1140	24.5	24.7	25.8	25.0	380
G. reevesi	25.4	30.9	28.1	15.6	1140	24.2	24.5	25.8	25.5	380
G. melanospilus	25.5	29.1	29.5	15.9	1140	25.5	25.5	24.7	24.2	380
G. flavimarginatus	25.4	31.1	27.8	15.6	1140	24.5	24.7	25.8	25.0	380
G. rueppeliae	25.6	31.1	27.9	15.4	1140	24.7	24.7	25.8	24.7	380
G. margaritophorus	25.7	29.6	29.6	15.2	1140	24.5	25.3	25.0	25.3	380
G. javanicus	25.5	31.1	27.8	15.5	1140	24.5	24.5	26.1	25.0	380
G. hepaticus	26.4	29.3	29.1	15.2	1140	24.7	24.7	25.5	25.0	380
G. berndti	25.5	29.5	29.7	15.3	1140	24.5	25.3	25.0	25.3	380
G. curostus	25.7	31.0	27.9	15.4	1140	25.0	25.8	24.7	24.5	380
G. favagineus	25.5	29.2	29.5	15.8	1140	25.0	25.8	24.7	24.5	380
G. polygonius	31.2	28.1	26.1	14.6	1140	24.2	26.3	24.2	25.3	380
G. maderensis	29.7	28.1	26.4	15.8	1140	26.1	24.2	23.9	25.8	380
G. unicolor	28.8	30.4	24.4	16.4	1140	24.5	25.8	23.9	25.8	380
Overall	30.2	28.7	25.7	15.4	1140	24.8	25.6	24.5	25.1	380

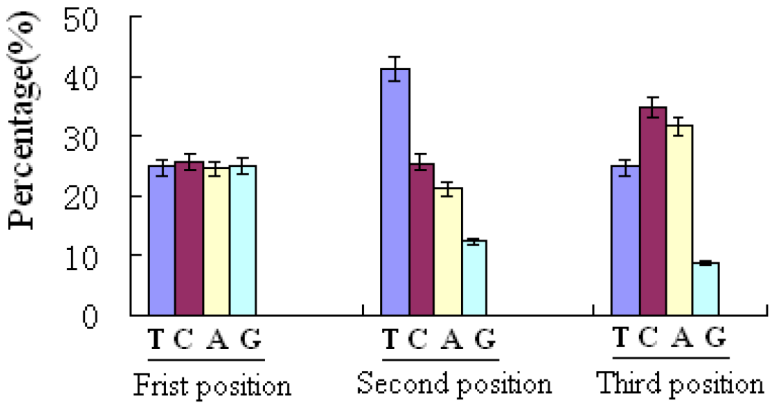

Figure 1. Mean nucleotide base composition of 20 *Gymnothorax* species (moray eels) at the three codon positions.

Among these 20 cytochrome *b* encoding gene sequences, 433 (37.9%) variables were observed, and 354 are parsimony-informative sites. More than 83.7% of all informative sites are third codon position substitutions. This observation was consistent with the high evolution rate of the third codon position. This pattern of nucleotide substitutions in *Gymnothorax* species is very similar to that observed for sharks (Martin, 1995). The transition (Ts)/transversion (Tv) rate ratios were $k_1 = 17.427$ (purines) and $k_2 = 9.951$ (pyrimidines). The rate of Ts substitutions was much higher than the rate of Tv substitutions, and the overall Ts/Tv bias was 2.3 (*R*) (Tamura et al., 2011).

To assess the pattern of variation at the third codon positions and the full-length cytochrome *b* data set, the number of Ts and Tv were plotted against the uncorrected p-distance, for all pairwise comparisons. This analysis showed that in both the third codon positions and the full-length cytochrome *b* data set, Ts are not saturated and instead show a straight-line trend, but Tv substitutions may be saturated, because the Tv trend plateaued when the uncorrected p-distance reached 0.25 in the graph (Figure 2).

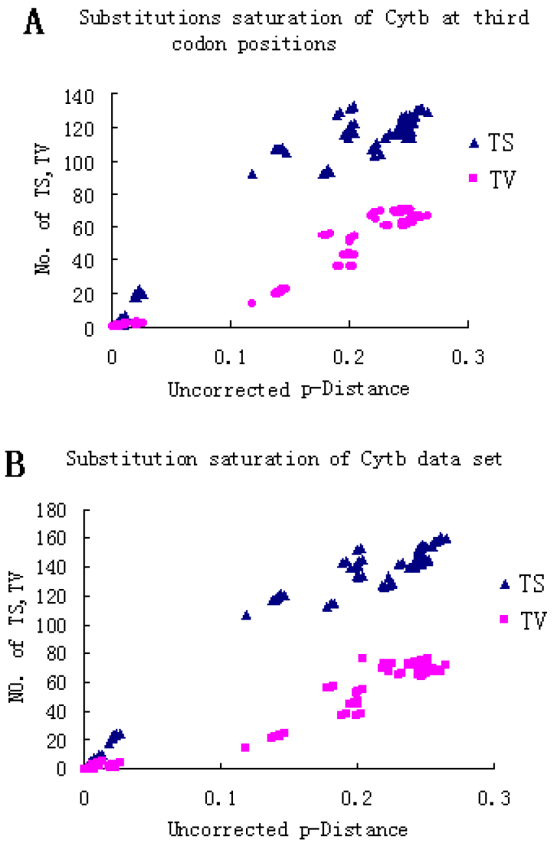

Figure 2. Substitution pattern at the third codon positions (**A**) and full length sequence (**B**) of the gene encoding cytochrome *b*. The number of transition (Ts) and transversion (Tv) substitutions is plotted against the uncorrected p-distance considering all sites. Each point represents a pairwise comparison among species. Both in the third codon positions, and the full-length cytochrome *b* data set, Ts are not saturated, but Tv substitutions may be saturated. The comparisons did not include outgroup taxa.

A summary of the genetic distances among *G. kidako* (from Japan), 3 *Gymnothorax* species (from Spain), and 16 species inhabiting the South China Sea is shown in Table 3. The average difference in base composition for the 16 species from the South China Sea, and *G. Kidako* (from Japan), is 15.0%, which is lower than that of the 3 species of *Gymnothorax* (22.4%) from Spain. This result suggests that the evolutionary rate of the *Gymnothorax* in the western Pacific, including Japan and the Southeast China Sea, is lower than that of the *Gymnothorax* (22.4%) from Spain. However, it is strange that the pairwise distance data analysis revealed only small genetic differences among *G. fimbriatus*, *G. reticularis*, *G. chilospilus*, *G. rueppeliae*, *G. javanicus*, *G. reevesi*, and *G. flavimarginatus* (0.4-0.8%, Table 3). These species exhibit great morphological differences (coloration patterns or variegated markings), which further confirms that the evolutionary rate of *Gymnothorax* from the western Pacific is lower.

Table 3. Summary of the mean pairwise distance (%) of sequences encoding cytochrome *b* among the 20 species of *Gymnothorax* analyzed.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1																				
2	14.2																			
3	13.8	0.40																		
4	20.0	22.7	22.4																	
5	20.3	22.5	22.1	16.9																
6	13.7	0.40	0.10	22.2	22.0															
7	13.5	2.20	2.00	22.0	22.2	1.90														
8	14.1	0.70	0.40	22.6	22.3	0.40	2.20													
9	13.8	1.10	0.80	22.1	21.8	0.70	2.20	1.10												
10	21.5	23.0	22.7	18.6	19.6	22.6	22.5	22.7	22.5											
11	14.2	0.70	0.40	22.6	22.4	0.40	2.20	0.70	1.10	23.0										
12	20.1	22.2	21.8	16.7	1.80	21.7	22.0	22.1	21.8	18.9	22.1									
13	13.9	0.50	0.30	22.5	22.2	0.20	2.10	0.50	0.90	22.8	0.40	22.0								
14	20.5	24.1	23.7	11.8	18.9	23.6	22.9	24.0	23.4	19.7	24.0	19.0	23.8							
15	20.6	22.6	22.2	17.1	2.10	22.1	22.5	22.5	22.2	19.2	22.5	0.40	22.3	19.2						
16	14.3	1.00	0.70	23.0	22.7	0.60	2.50	1.00	1.30	23.3	0.80	22.4	0.60	24.3	22.8					
17	21.3	23.0	22.7	18.3	19.3	22.6	22.7	22.7	22.6	0.50	23.0	18.7	22.8	19.4	18.9	23.3				
18	23.5	24.0	23.6	21.2	23.3	23.4	23.2	23.6	23.8	22.9	23.8	23.2	23.7	20.9	23.5	24.2	22.6			
19	10.4	14.6	14.3	19.9	21.8	14.2	13.7	14.6	14.3	22.2	14.4	22.2	14.4	20.7	22.7	14.8	21.9	22.5		
20	23.9	24.1	23.8	22.4	22.3	23.7	23.3	24.0	24.0	22.4	24.1	23.2	23.9	22.8	23.6	24.4	22.2	20.8	23.9	

1 = *G. kidako*; 2 = *G. fimbriatus*; 3 = *G. flavimarginatus*; 4 = *G. meleagris*; 5 = *G. undulates*; 6 = *G. reticularis*; 7 = *G. pseudothyrsoideus*; 8 = *G. chilospilus*; 9 = *G. reevesi*; 10 = *G. melanospilus*; 11 = *G. rueppeliae*; 12 = *G. margaritophorus*; 13 = *G. javanicus*; 14 = *G. hepaticus*; 15 = *G. berndti*; 16 = *G. curostus*; 17 = *G. favagineus*; 18 = *G. unicolor*; 19 = *G. polygonius*; 20 = *G. maderensis*.

Molecular phylogeny

The left-skew (g1 = -0.54, $p = 0.01$) for the random trees indicates that the cytochrome *b* data matrix contains a phylogenetic signal. A single most-parsimonious tree (length = 910, CI excluding uninformative characters = 0.56, retention index = 0.72) was obtained by setting the cytochrome *b* sequence data under unweighted parsimony (Figure 3).

The tree generated from MP analysis revealed a similar topology (Figure 3) to that from the ML and NJ methods, but with a few differences. Only bootstrap values higher than 50% are displayed. Overall, the phylogenetic trees obtained using MP methods divide the species into five clades (Figure 3). Group I consists of *G. flavimarginatus*, *G. chilospilus*, *G. reticularis*, *G. fimbriatus*, *G. javanicus*, *G. rueppeliae*, *G. curostus*, *G. reevesi*, *G. pseudothyrsoideus*, *G. kidako* (from Japan), and *G. polygonius* (from Spain). Strong bootstrap values (100%) exist for the formation of this clade. The internal branch of group I is also very clear. Interestingly, the Japanese species *G. kidako* is grouped with the Spanish species *G. polygonius* (bootstrap values = 65%), and the other species of *Gymnothorax* within group

I formed a cluster, supported by a high bootstrap value (100%), in which *G. javanicus*, *G. rueppeliae*, and *G. curostus* are a separate branch (bootstrap values = 66%). Group I then puts *G. flavimarginatus*, *G. chilospilus*, *G. reticularis*, and *G. fimbriatus* into a larger cluster (bootstrap value = 91%). Furthermore, *G. pseudothyrsoideus* and *G. reevesi* are formed into two separate internal branches within group I (bootstrap values = 99% and 100%).

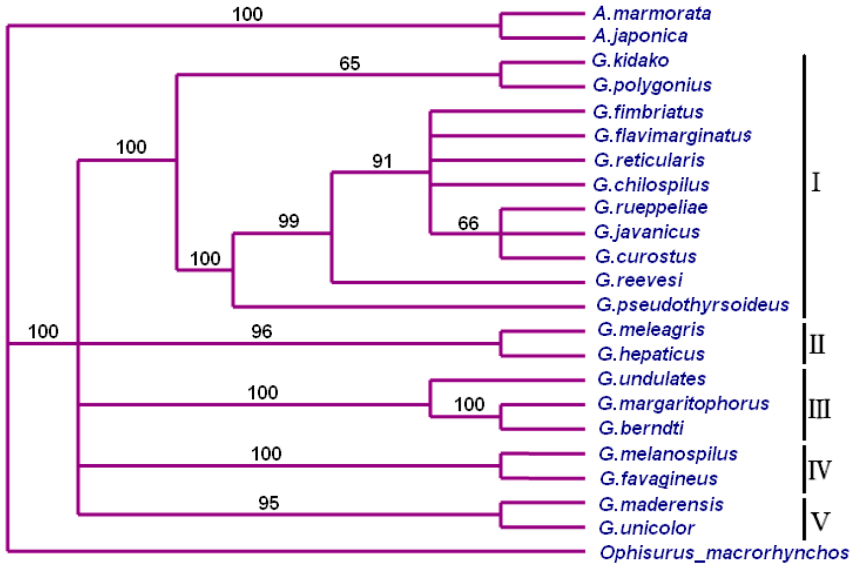

Figure 3. Most-parsimonious tree (L = 910 steps, CI = 0.56, RI = 0.72) resulting from the unweighted analysis of the cytochrome *b* sequences. Numbers at the nodes represent bootstrap values (%) with 1000 replicates. Only bootstrap values >50% are shown.

Group II contains *G. meleagris* and *G. hepatious* (bootstrap value = 96%). Group III is composed of *G. margaritophorus*, *G. berndti*, and *G. undulates*, which reveals a close genetic relationship between them (bootstrap value = 100%). Group IV consists of *G. melanospilus* and *G. favagineus* (bootstrap value = 100%), and Group V contains *G. unicolor* and *G. maderensis*, which originate from Spain (bootstrap value = 95%).

The NJ analysis produced a tree topology identical to that of the ML analysis (-ln L, unconstrained = 4707.71777). Thus, we combined the trees from both methods into a single tree represented by the NJ tree (Figure 4). The NJ tree divides the species into three clades. Group I consists of *G. flavimarginatus*, *G. chilospilus*, *G. reticularis*, *G. fimbriatus*, *G. javanicus*, *G. rueppeliae*, *G. curostus*, *G. reevesi*, *G. pseudothyrsoideus*, *G. kidako*, and *G. polygonius*, which is consistent with group I in the MP tree. Strong bootstrap values exist for the formation of this clade (bootstrap values = 100%). For the other clades, however, are a few differences between the MP tree and the NJ tree. Group II contains *G. margaritophorus*, *G. berndti*, *G. undulates*, *G. meleagris*, *G. hepatious*, *G. melanospilus*, and *G. favagineus* for the NJ tree, which reveals the close genetic relationship between them. The internal branch of group II is also very clear. It consists of two subgroups, which are divided by an internal node. The subgroup IIA includes *G. margaritophorus*, *G. berndti*, *G. undulates*, *G. meleagris*, and *G. hepatious*; *G. melanospilus* and *G. favagineus* are gathered into subgroup IIB. However,

the topology of these species for the MP tree is divided into three clades (group II, group III, and group IV). The two Spanish species (*G. unicolor* and *G. maderensis*) formed a separate evolutionary branch (group III), which is also consistent with the MP tree (group V).

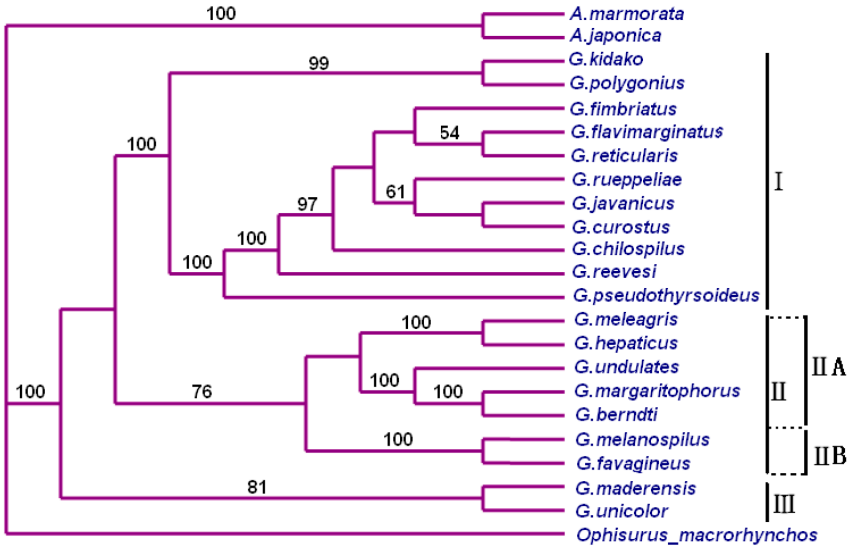

Figure 4. Neighbor-joining tree depicting relationships for 20 *Gymnothorax* species (moray eels) based upon gene sequences encoding cytochrome *b*. This tree resulted from the maximum likelihood analysis (PAUP* 4.0b10) following the GTR (general-time-reversible model; Goldman and Yang, 1994). Numbers indicate bootstrap values of 10 replicates. The ML tree (-in L = 4707.71777) was highly congruent and identified the same topology. Values at branches are NJ bootstrap estimates, based on 1000 replicates. Only bootstrap values >50% are shown.

DISCUSSION

Moray eels were given the genus name *Gymnothorax* by Bloch in 1795 (Cheng and Zheng, 1987). Conventionally, coloration pattern, variegated markings, and dentition are used as the major morphological features for species identification (Cheng and Zheng, 1987). However, because a moray eel's color patterns/markings vary during different growth stages, and morphological features of different species often overlap, it is very difficult to apply these morphological identification methods accurately. For this reason, researchers have argued for many years about the richness of *Gymnothorax* species within the same geological area. For example, Cheng and Zheng (1987) believe there are 20 *Gymnothorax* species in the South and East China Seas. Shen (1993) however, maintains that there are 27 *Gymnothorax* species. In our study, in the South China Sea alone, we found 16 *Gymnothorax* species. There may be two reasons for this: 1) certain species have become extinct, or came close to extinction, due to the deterioration of the marine environment in the recent years; 2) Some species may only reside in the East China Sea, which we did not sample. Concerning the distribution of fish resources, *G. undulates* is the most abundant species of *Gymnothorax* in the South China Sea. Additionally, *G. fimbriatus*, *G. flavimarginatus*, *G. meleagris*, and *G. favagineus* are also the abundant species.

In the present study, we constructed a phylogenetic tree based on 16 *Gymnothorax* species that inhabit the South China Sea, and combined this data with three species of *Gymnothorax* originating from Spain as well as a Japanese species. Our phylogenetic analysis indicates that the cytochrome *b* data matrix contains a phylogenetic signal. Base substitution saturation analysis suggested that for both third codon positions, and the full-length cytochrome *b* data set, Ts are not saturated, but Tv substitutions may be saturated.

Our phylogenetic tree showed that the genus *Gymnothorax* is divided into five distinct clades for the MP tree, and three clades for the NJ tree. The adult *G. melanospilus* and *G. favagineus* are morphologically similar, except that the shape and the spacing of spots on the body surface vary. Shih-chieh Shen argue that *G. melanospilus* and *G. favagineus* are the same species (Shen, 1993), and named it *G. favagineus,* but Cheng and Zheng had proposed that they are two different species (Cheng and Zheng, 1987); they were also divided into two species in FishBase. Based on our experimental data, the pairwise distance between *G. melanospilus* and *G. favagineus* is only 0.005, thus, we share Shih-chieh Shen's view in that these are the same species (Shen, 1993). To further resolve the issue, more genes should be sequenced, both mitochondrial and nuclear.

In this study, we conducted a preliminary investigation into the resource distribution of *Gymnothorax* (moray eels) in the South China Sea. We collected a total of 16 *Gymnothorax* species, as mentioned above. The phylogenetic relationships between those 16 *Gymnothorax* species were clarified according to the complete gene sequences encoding the mitochondrial protein cytochrome *b*.

Conflicts of interest

The authors declare no conflict of interest.

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