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Variation in Mitochondrial DNA Sequences of Black Porgy, *Acanthopagrus schlegeli*, in the Coastal Waters of Taiwan

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Chuen-Tan Jean, Sin-Che Lee, Che-Tsung Chen and Cho-Fat Hui (1998) Variation in mitochondrial DNA sequences of black porgy, *Acanthopagrus schlegeli*, in the coastal waters of Taiwan. *Zoological Studies* **37**(1): 22-30. Mitochondrial DNA sequences that include the 5' end of the D-loop region, the 3' end of the D-loop region, the tRNAPhe gene, and the 5' end of the 12S rRNA gene were determined from 49 specimens of *Acanthopagrus schlegeli* and 1 specimen of *A. australis* collected from 7 locations along the coastal waters of Taiwan and Matsu off the coast of mainland China, and from an aquaculture pond. Among 33 haplotypes identified from the 49 mtDNA sequences, there were 32 variable sites, most of which occurred in the 5' end of the D-loop region. Pairwise sequence distances among haplotypes, using the Tamura-Nei model, range between 0.0014 and 0.0127. The phylogenetic tree constructed by use of the unweighted pair-group method with arithmetic average shows neither significant genealogical branches nor geographic clusters. All of the bootstrap confidence levels resulting from 1000 bootstrap tests are below 50%. Furthermore, the sequence-statistics test reveals little genetic differentiation. Apparently, wild black porgies in the coastal waters of Taiwan and Matsu off the coast of mainland China, belong to a single population. There is no significant genetic differentiation between the wild and pond-cultivated populations.

Key words: *Acanthopagrus schlegeli*, mtDNA, Genetic structure.

Black porgy *Acanthopagrus schlegeli* is distributed in the West Pacific coasts from Japan and Korea to the East China Sea and Taiwan. It is an important food fish and a target species of recreational fisheries in estuarine and coastal waters of Taiwan. It is also a highly valued cultivated fish species in Taiwan. In addition to *A. schlegeli*, there are 3 other closely related species (*A. australis, A. berda*, and *A. latus*); altogether they comprise the so-called *A. schlegeli* species complex. In coastal waters of Taiwan, *A. schlegeli* is the most abundant species among the 4 species. Having close resemblance in external features, their easily confused species status can be discriminated by isozyme electrophoresis (Jean et al. 1995b) and mtDNA sequencing (Jean et al. 1995a). Due to the drastic decrease in wild populations, there is an urgent need to resurrect these stocks through conservation, management, and mariculture. Defining the systematic status and population structure of a species is a basic prerequisite for making informed decisions regarding rational exploitation and management, as well as for correct interpretation of ecological investigations (Ferguson and Mason 1981).

Due to maternal inheritance and a relatively faster evolutionary rate, mitochondrial DNAs (mtDNA) have been used to provide insights into population genetic structure, gene flow, hybridization, biogeography, and phylogenetic relationships of various animals (Avise et al. 1986, Moritz et al. 1987, Bartlett and Davidson 1991, Bowers et al. 1994, Sang et al. 1994, Taylor and Dodson 1994, Jean et al. 1995a).

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To provide an overview of genetic structure of natural populations and to understand genetic variation between wild and cultivated populations of *A. schlegeli*, in addition to previous electrophoretic analysis of allozymes (Jean et al. 1996), we conducted a nucleotide sequence analysis of mtDNAs.

MATERIALS AND METHODS

Sample collection and DNA extraction

A total of 49 specimens of adult *Acanthopagrus schlegeli* was used in the study. They included 43 specimens of wild fish collected at 5 localities (Fulung, 7; Tanshui, 6; Wuchi, 6; Taihsi, 6; and Tungkang, 6) around the coasts of Taiwan, Penghu Islands (6) in the Taiwan Strait, and an island Matsu (6) off the coast of mainland China. Moreover, 6 specimens of cultivated fish obtained from Tainan, Taiwan (Fig. 1) were included in the analyses. One specimen of *A. australis* (Aa) collected from Fulung was used as an outgroup for phylogenetic analyses. The specimens were iced or frozen immediately after capture and later kept at -75 °C. For each specimen, crude DNA was extracted from 100 mg of skeletal muscle by the method described by Kocher et al. (1989).

Amplification and sequencing of mtDNA

Two segments of mtDNA that include the 5' end of the D-loop region and from the 3' end of the D-loop region to the 12S rRNA gene were chosen for sequencing analysis. Primers PT and PU designed as those described in Jean et al. (1995a) were used for amplification and sequencing (Fig. 2). The processes of amplification and sequencing of mtDNA followed those described by Jean et al. (1995a).

Sequence analysis

Mitochondrial DNA sequences of 49 *A. schlegeli* and 1 *A. australis* were aligned using the Pileup program of the GCG software package (Genetic Computer Group, Version 7.0; Devereux et al. 1991), and then compared with their mtDNA sequences published by Jean et al. (1995a) to verify the boundaries of the genes.

Estimates of within-population genetic variation were obtained for each of the 8 samples in the form of haplotype (h) and nucleotide (π) diversities (Nei 1987: eqns. 8.4 and 10.5, respectively).

Pairwise sequence divergences between mtDNA haplotypes were calculated with the Tamura-Nei model, then the resulting distances were clustered using the UPGMA (unweighted pair-group method with arithmetic average) and a bootstrap with 1000 replications was run to test the confidence of the topology of this phylogenetic tree by using the MEGA software package (Molecular Evolutionary Genetics Analysis, Version 1.01; Kumar et al. 1993)

Geographic subdivision of the population was detected using K-statistics (Hudson et al. 1992). K values range from 0 to 1; 0 indicates no population subdivision, and 1 indicates complete population subdivision. Test of the neutral mutation hypothesis was conducted using the statistical method developed by Tajima (1989).

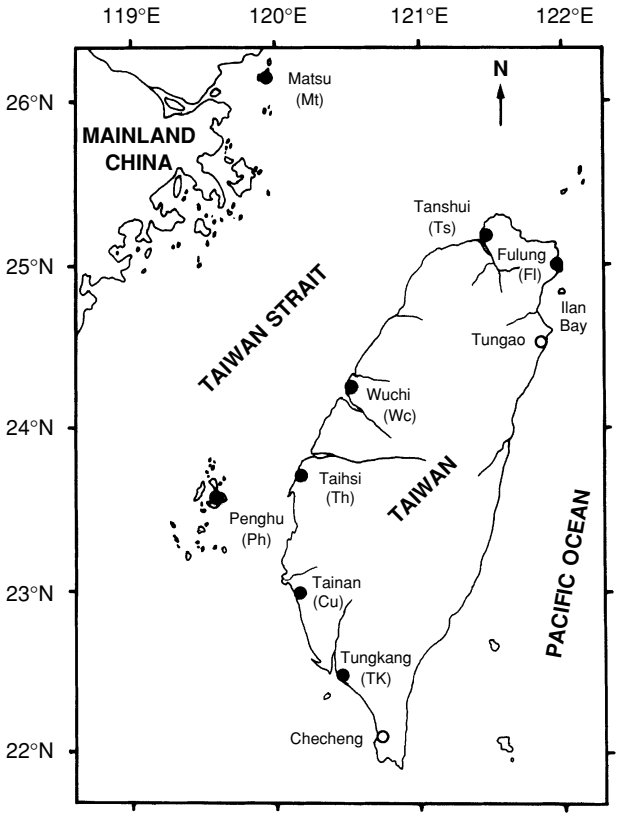

Fig. 1. Map of Taiwan and adjacent mainland China showing sampling localities (solid circles) of *Acanthopagrus schlegeli*. Open circles delimit the distribution of *A. schlegeli* in coastal waters of Taiwan. Natural population of *A. schlegeli* are distributed from Tungao northward, along the north and west coasts and then southward to Checheng near the southernmost tip of Taiwan.

RESULTS

Sequence variation

The sequence lengths of the 2 segments determined were 404 bp for the 1st segment that constituted the 5' end of the D-loop region, and 314 bp for the 2nd segment which included 195 bp of the 3' end of the D-loop region, 71 bp of the $tRNA^{Phe}$ gene, and 48 bp of the 5' end of the 12S $rRNA^{Phe}$ gene. The sequences in Figure 3 are 727, rather than 718, nucleotides in length because of 9 gaps introduced into all *Acanthopagrus schlegeli* sequences in order to align them with the *A. australis* sequence.

Thirty-two variable sites were found in the 718 bp sequences. Among them, 30 variable sites were at the 5' end of the D-loop region (Fig. 3A) and 2 variable sites at the 3' end of the D-loop region (Fig. 3B). All of the $tRNA^{Phe}$ gene and the 5' end of the 12S rRNA gene sequences were identical. It is obvious that most of the variable sites were distributed in the 5' end of the D-loop region. All sequence variations were due to single-nucleotide transitions except for 1 single-nucleotide transversion (variable site 9) and 1 single-nucleotide deletion (variable site 31) (Fig. 3; Table 1).

Thirty-three different haplotypes were found among 49 individuals (Table 2). Most of the haplotypes were unique to particular individuals. The exceptions were haplotypes Ts1 and Wc3 in 7 and 3 individuals, respectively, and 8 other haplotypes (Mt2, Fl2, Wc1, Th1, Th4, Th5, Ph4, and Tk1) each in 2 individuals (Table 2).

Measures of within-population variation, as determined by haplotype and nucleotide diversities are presented in Table 3. The lowest haplotype and nucleotide diversity values (0.5455 and 0.0084, respectively) for the cultivated population reflect the occurrence of a higher ratio of shared haplotypes. The overall haplotype and nucleotide diversities were estimated to be 0.9628 and 0.002 75, respectively.

Phylogenetic tree

The pairwise sequence distances among the 33 haplotypes using the Tamura-Nei model ranged from 0.0014 to 0.0127. The phylogenetic tree of 33 haplotypes constructed using Tamura-Nei pairwise sequence distances by UPGMA is shown in Figure 4. From this tree, neither obvious genealogical branches among the 33 haplotypes, nor clusters that correspond to specific sampling localities can be observed. All of the bootstrap confidence level (BCL) values resulting from 1000 bootstrap replications were below 50%. This verifies that there are no specific clusters among the 33 haplotypes.

Test of geographic subdivision

The results of the K statistics show that the weighted average of average number of pairwise sequences differences from within each locality (K = 0.0051; K = 0.0056 when the cultivated sample was excluded) was nearly identical to the average number of pairwise sequence differences regardless of their locality (K = 0.0056; K = 0.0061 when the cultivated sample was excluded). The value of K (0.0829; K = 0.0805 when the cultivated sample was excluded) approaches 0, which is expected under the null hypothesis; the null hypothesis is, thereby, not rejected. From the results of this statistical test, it is reasonable to infer that the 7 wild

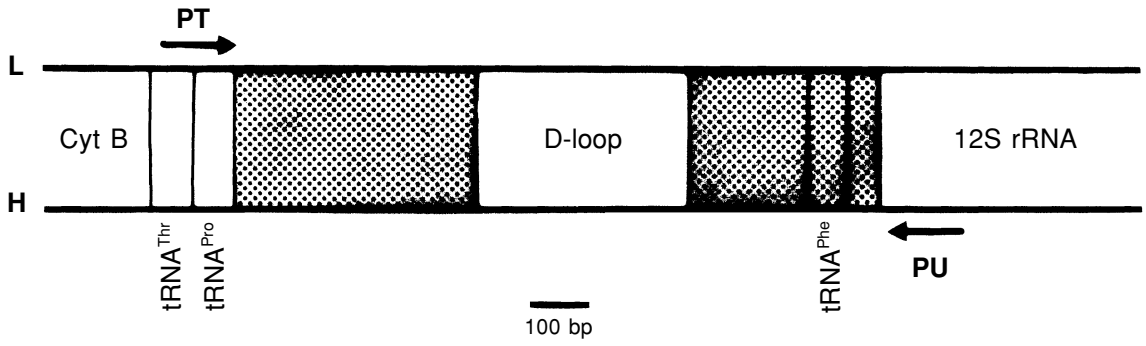

Fig. 2. Schematic diagram of the amplified and sequenced region (indicated by dots) of mtDNA in *Acanthopagrus schlegeli*, and the positions of primers PT (5'-CTTACTATCAACTCCCAAAGC-3') and PU (5'-GGGCATTCTCACGGGGATGCG-3') are indicated by arrows.

samples of black porgy belong to an identical population, and that genetic diversity between wild and cultivated samples is not obvious.

Test of neutral mutation

To test the neutral mutation hypothesis, Tajima (1989) developed a statistical method using the relationship between the number of segregation sites (Kimura 1983) and the average number of nucleotide differences (Tajima 1983) . Applying the Tajima statistical method to the D-loop segments of the mtDNA sequences of the 49 sequences, we found that the Tajima statistic value D (-1.5125) was not significantly different from 0, so we can conclude that the DNA variation of the black porgy mtDNA sequences might be influenced by neutral mutation.

DISCUSSION

It has been known that the 5' and 3' end segments of the D-loop region are highly variable in both base and length, and the central segment is conservative for the 5 species of sparid fishes (Jean et al. 1995b), as well as for many species of other vertebrates (Brown et al. 1986, Saccone et

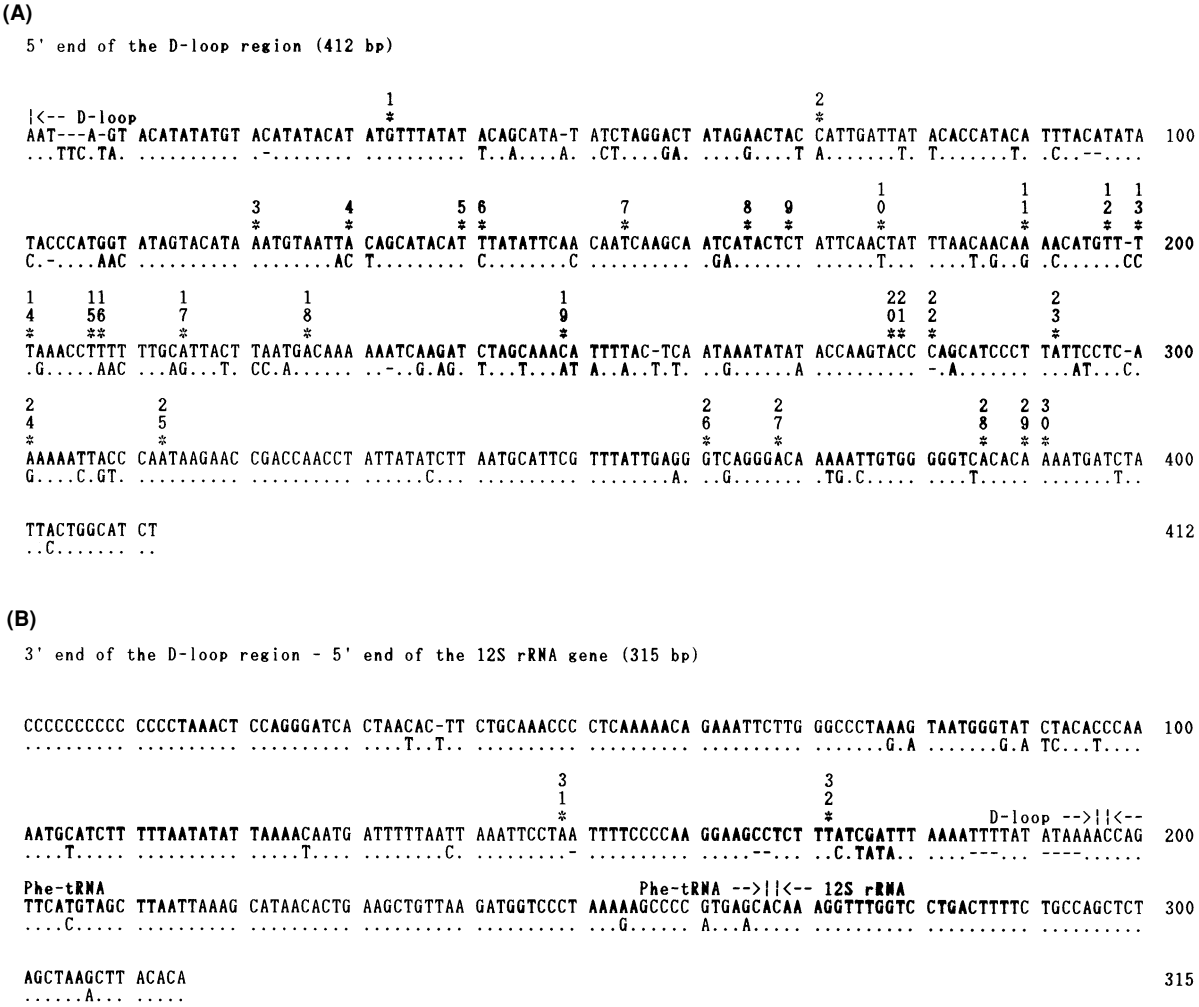

Fig. 3. Nucleotide sequences (L strand) of (A) the 5' end of the D-loop region and (B) the 3' end of the D-loop region to the 5' end of the 12S rRNA gene in mtDNA of 1 *Acanthopagrus schlegeli*, Mt1 (upper) and 1 *A. australis*, Aa (lower). Asterisks and numbers (shown vertically) mark the 32 variable positions among the 33 haplotypes determined. Dots represent identical bases, and dashes represent gaps.

al.1987, Tzeng et al. 1992). The neutral theory of molecular evolution predicts that regions of genome that evolve at high rates, as revealed by interspecific DNA sequence comparisons, will also exhibit high levels of polymorphism within a species (Hudson et al. 1987). Therefore, it is reasonable to choose the 5' and 3' end segments of the D-loop region for studies of sequence variation and population genetic structure. The results of this study show that the 5' end segment of the D-loop region is more variable than the 3' end segment of the D-loop region, and that the $tRNA^{Phe}$ gene and the 5' end of the 12S rRNA gene are highly conservative.

The phylogenetic tree constructed by UPGMA shows neither significant genealogical branches nor geographic clusters among the 33 haplotypes (Fig.4). The statistic value K (0.0829) is also not significant to reject the null hypothesis. These results suggest that the 43 black porgies collected from 7 localities around Taiwan, and Matsu off the coast of mainland China, belong to a single population; also the genetic divergence between the wild samples and the cultivated sample is not sig-

Table 1. Thirty-two variable sites among the 33 mtDNA haplotypes (718 bp) of *Acanthopagrus schlegeli*

	Variable sites																														
										1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3	3
Haplotype	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2
Mt1	A	C	A	A	T	T	T	T	C	C	A	T	C	C	C	C	A	A	T	A	C	T	G	A	A	G	A	A	A	A	A	T
Mt2	.	.	.	G	.	.	.	.	.	.	.	.	T	.	.	T	.	.	C	.	T	C	.	.	.	A	.	.	.	.	–	.
Mt3	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	G	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.
Mt4	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	T	.	.	C	.	.	C	.	.	G	A	G	.	.	.	.	.
Mt5	.	.	.	.	.	.	.	.	G	.	.	.	T	.	.	.	G	.	C	.	.	C	.	.	.	A	.	.	.	.	.	.
Mt6	.	.	G	.	C	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.
Fl1	G	.	.	.	.	.	.	.	.	.	.	.	T	T	T	T	.	.	C	.	.	C	A	.	.	G	.	.	.	.	.	.
Fl2	.	.	.	.	.	.	.	.	G	.	.	.	T	.	.	.	.	.	C	.	.	C	A	.	.	A	.	.	.	.	.	.
Fl3	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	G	.	C	.	.	C	A	.	.	.	.	.	.	.	.	.
Fl4	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	C	A	.	.	.	.	.	.	.	.	.
Fl5	G	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	A	.	.	.	.	.	.	.	.	.
Fl6	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	G	.	C	.	.	C	A	.	.	.	.	.	.	.	.	.
Ts1	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	C	.	.	.	A	.	.	.	.	.	.
Ts2	.	.	.	.	.	C	.	.	.	.	.	.	T	.	.	T	.	.	C	.	.	C	.	G	.	A	.	.	.	.	.	.
Ts3	.	.	.	.	.	.	.	.	.	.	.	.	T	.	T	T	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.
Ts4	.	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.
Ts5	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	C	.	.	.	A	.	.	.	.	.	C
Ts6	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	C	.	.	.	A	.	.	.	.	.	.
Wc1	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	C	G	.	C	.	.	.	A	.	.	.	.	.	.
Wc2	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.
Wc3	.	.	G	.	.	.	.	C	.	.	.	.	T	.	.	.	.	.	C	.	.	C	.	.	.	A	.	.	.	.	.	.
Th1	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	G	.	C	.	.	.	A	.	.	.	.	.	.
Th2	.	.	.	.	.	.	.	.	.	T	.	.	T	.	.	.	.	.	C	G	.	C	.	.	.	A	.	.	.	.	.	.
Th3	G	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.
Th4	G	.	.	.	.	.	.	.	.	.	.	.	T	T	T	T	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.
Th5	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	G	.	C	.	.	C	.	.	.	A	.	.	.	.	.	.
Ph1	.	.	.	.	.	.	.	.	G	.	.	.	T	.	.	.	G	.	C	.	.	C	.	.	.	A	.	G	.	.	.	.
Ph2	G	.	.	.	.	.	.	.	.	.	.	.	T	T	T	T	.	.	C	.	.	C	.	.	.	A	.	.	.	.	.	.
Ph3	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	T	C	.	.	.	A	.	.	.	.	.	.
Ph4	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	G	.	C	.	.	.	A	.	.	G	.	.	.
Tk1	G	.	.	.	.	.	.	.	.	.	.	C	T	.	.	.	.	.	C	.	.	C	.	.	.	A	.	.	.	.	.	.
Cu1	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Cu2	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.

Positions of the variable sites correspond to positions shown vertically in Fig. 3. Dots indicate identical bases with the haplotype Mt1 sequence. A dash indicates a single base deletion.

nificant. This inference is consistent with that made from electrophoretic analysis of allozymes (Jean et al. 1996).

The reasons why black porgies from localities around Taiwan and far remote Matsu off the coast of mainland China belong to a single population may be explained by geographic changes, dispersal ability, and water currents. The estimation of divergence time from a common ancestor from electrophoretic data of allozymes using Nei's formula (Ferguson 1980) for *Acanthopagrus schlegeli* and its sister taxa shows it would have been some 1.0-1.9 Ma(Jean et al. 1995a). It is well documented that Taiwan was once part of mainland China, and separated for the 1st time about 0.2-0.4 Ma. Taiwan was then separated and connected intermittently with mainland China several times until the most recent separation that occurred around 16 000 BP (Lin 1966). Along the coasts of Taiwan, *A. schlegeli* is distributed from Tungao in northeastern Taiwan, along the north and west

Table 3. Haplotype ($\hat{h}$) and nucleotide ($\hat{\pi}$) diversities for 8 samples of *Acanthopagrus schlegeli*. *N*, sample size

Samples	*N*	Haplotype diversity ($\hat{h}$)	Nucleotide diversity$^{\wedge}$ ($\hat{\pi}$)
Matsu	6	0.9091	0.00399
Fulung	7	0.8792	0.00381
Tanshui	6	0.9091	0.00232
Wuchi	6	0.8485	0.00274
Taihsi	6	0.8485	0.00232
Penghu	6	0.9091	0.00269
Tungkang	6	0.7879	0.00316
Cultivated	6	0.5455	0.00084
Overall		0.9628	0.00275

Table 2. Distribution of *Acanthopagrus schlegeli* haplotypes defined in Table 1

Haplotype	Matsu	Fulung	Tanshui	Wuchi	Taihsi	Penghu	Tungkang	Cultivated
Mt1	1							
Mt2	1			1				
Mt3	1							
Mt4	1							
Mt5	1							
Mt6	1							
F11		1						
F12		2						
F13		1						
F14		1						
F15		1						
F16		1						
Ts1			1	1		1		4
Ts2			1					
Ts3			1					
Ts4			1					
Ts5			1					
Ts6			1					
Wc1				2				
Wc2				1				
Wc3				1			2	
Th1					2			
Th2					1			
Th3					1			
Th4					1		1	
Th5					1	1		
Ph1						1		
Ph2						1		
Ph3						1		
Ph4						1	1	
Tk1							2	
Cu1								1
Cu2								1

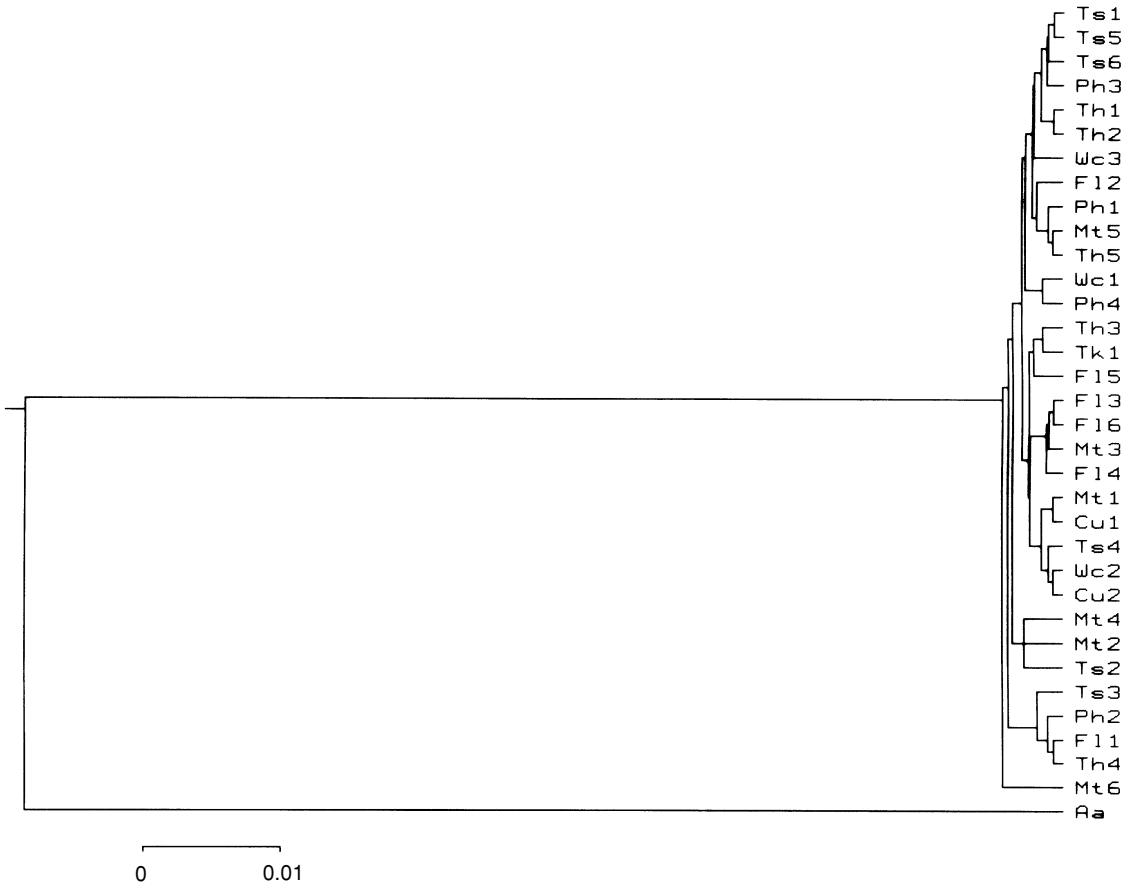

Fig. 4. Phylogenetic tree relating 33 haplotypes observed among 49 *Acanthopagrus schlegeli* mtDNA sequences. *A. australis* (Aa) is used as an outgroup.

coasts and south to Checheng near the southernmost tip of Taiwan. *A. schlegeli* is an euryhaline, inshore demersal fish with preferred habitats of sand-muddy substrates near harbors or bays with sparsely distributed rocks. The breeding season of this species extends from January to April, with the laying of pelagic eggs. After hatching, the larvae drift on the surface for about 20 d while being dispersed widely by currents. Three main currents (the Kuroshio current, the China coastal current, and the Taiwan coastal current) are encountered in the waters surrounding Taiwan during this period (Fan and Yu 1981,Wang and Chern 1989). According to the NOAA-AVHRR satellite imageries (Lin et al. 1994), the China coastal current intrudes into the eastern portion of the Taiwan Strait, then extends to Ilan bay and Tungao bay along the north coast, and extends to Checheng along the west coast in the autumn and winter. The boundary of the China coastal current coincides with the distribution of *A. schlegeli*. Therefore, the interaction of these currents results in dynamic gene flow in *A. schlegeli*, and the genetic differentiation between localities in Taiwan and Matsu off the coast of mainland China can be expected to be very slight.

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臺灣沿岸海域黑鯛粒線體 DNA 序列之變異

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本研究比較分析採自福隆、淡水、梧棲、臺西、東港、澎湖及馬祖等七個地區之 43 尾野生黑鯛及 6 尾養殖黑鯛粒線體 DNA 之 D-loop 區 5' 端段、 D-loop 區 3' 端段、 $tRNA^{Phe}$ 基因、及 12S rRNA 基因 5' 端段共 718 bp 之序列，以探討其序列之變異及其族群遺傳結構。

49 尾黑鯛之粒線體 DNA 序列中，共發現 33 種基因型，其差異在於 32 個位置發生變異，其中 30 個發生在 D-loop 區 5' 端段，另 2 個發生在 D-loop 區 3' 端段；而且除了 1 個變異位置係顛換(transversion)及另一變異位置係缺失(deletion)之外，其餘 30 個變異位置均係轉換(transition)。兩兩基因型間之 Tamura-Nei 序列距離為 0.0014-0.0127，依據序列距離使用 UPGMA 聚類方法繪出之基因型親緣關係樹並無明顯之系譜分支(genealogical branch)及地區標本群聚之情形，重複 1000 次 bootstrap test 所獲得親緣關係樹各分叉之 bootstrap confidence level (BCL) 值均小於 50%。表示地理族群間分化程度之 K 值，在包括地區野生黑鯛及養殖黑鯛之 8 個樣本間為 0.0829，而 7 個地區野生樣本間則為 0.0805。這些結果顯示台灣及馬祖 7 個地區野生黑鯛間之遺傳分化輕微，應屬於同一族群；而野生黑鯛與養殖黑鯛間之遺傳分化亦不顯著。 Tajima 之測驗中性突變假說之統計值 D 為 -1.5125，顯示黑鯛粒線體 DNA 序列之變異係中性突變(neutral mutation)所致。

關鍵詞：黑鯛，粒線體去氧核醣核酸，遺傳結構。

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