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Variation in Mitochondrial DNA and Phylogenetic Relationships of Fishes of the Subfamily Sparinae (Perciformes: Sparidae) in the Coastal Waters of Taiwan

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Chuen-Tan Jean, Cho-Fat Hui, Sin-Che Lee and Che-Tsung Chen (1995) Variation in mitochondrial DNA and phylogenetic relationships of fishes of the subfamily Sparinae (Perciformes: Sparidae) in the coastal waters of Taiwan. *Zoological Studies* **34**(4): 270-280. Mitochondrial DNA (mtDNA) sequences that included the non-coding D-loop region, the $tRNA^{Phe}$ gene, and a part of the 12S rRNA gene from 60 individuals belonging to 5 Sparinae species were sequenced to document the mtDNA sequence variations as well as to elucidate the phylogenetic relationships of these species. The intraspecific and interspecific comparisons of aligned sequences demonstrate that the $tRNA^{Phe}$ gene and the 12S rRNA gene are highly conserved. The left and right domains of the D-loop region contain a higher A content and a lower G content, and are highly variable in both sequences and lengths. The central domain of the D-loop region contains a lower A content and a higher G content, and is more conserved than the other two domains. The conserved sequence elements TAS, CSB-2, and CSB-3, which have been reported previously from other vertebrates, are maintained in Sparinid fishes. The intraspecific pairwise sequence distances using the Tamura-Nei model ranged between 0.0021-0.0130 in *Acanthopagrus australis*, 0.0000-0.0115 in *A. berda*, 0.0014-0.0209 in *A. latus*, 0.0014-0.0093 in *A. schlegeli*, and 0.0000-0.0056 in *Sparus sarba*. The interspecific pairwise sequence distances using the Tamura-Nei model, ranged from 0.1498 to 0.1914 between species of *Acanthopagrus*, and from 0.2386 to 0.2708 between species of the genera *Acanthopagrus* and *Sparus*. The phylogenetic tree constructed by UPGMA based on the mtDNA sequence data shows the same topology as that based on allozyme electrophoresis data, but it is different from that based on morphometric data.

Key words: mtDNA, Sequence variation, Interspecific relationships, Sparinae.

Fishes of the subfamily Sparinae, commonly known as porgies, are important commercial species for food consumption and recreational fisheries in estuarine and coastal waters of Taiwan. They also have become pond-cultivated species after the proven success of artificial mass propagation of fingerlings (Lin and Yen 1980, Lin et al. 1988, Leu et al. 1991).

In Taiwan, the subfamily Sparinae contains two genera and five species. The genus *Acanthopagrus* includes four nominal species, namely *Acanthopagrus schlegeli*, *A. latus*, *A. berda*, and *A. australis*. The genus *Sparus* includes only one species, *Sparus sarba* (Lee 1983, Jean and Lee 1992). Among these five species, *A. berda* and *S. sarba* are the most widely distributed species, extending from southern Japan, Southeast Asia, Australia, the Indian Ocean, and the Red Sea to southeastern Africa. *A. latus* occurs in southern Japan, Southeast Asia, Australia, the Indian Ocean, and the Red Sea to northeastern Africa. *A. schlegeli* is found around Japan, South Korea, China, with its southernmost extension to Taiwan. The distribution of *A. australis* ranges from northern Australia to southern Japan (Okinawa) (Hayashi 1993).

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Recently, porgy resources in Taiwan have decreased drastically due to overfishing, pollution, and utilization of illegal fishing techniques such as poisoning and electric shock. Thus, there is a need to recover these resources through conservation, sound resource management, and mariculturing. However, before the practices of conservation, management, and mariculture can be put to good use, better knowledge of the phylogenetic relationships and population structure of these fishes is necessary. Previously, in order to understand the phylogenetic relationships of fishes of the subfamily Sparinae, we conducted a traditional morphometric study and an electrophoretic analysis of allozymes (Jean et al. 1992 1995). Molecular and chromosomal features usually confirm classification based on morphological characters (Patterson et al. 1993). General morphology usually reflects a part of the genotype, and thus it permits reliable conclusions concerning relationships. However, when discrepancies occur between a classification that is based on morphology and one that is not, additional sets of taxonomic characters must be used.

In recent years, due to their maternal inheritance and relatively faster evolutionary rate, mitochondrial DNAs (mtDNA) have been used to provide insights into population 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, Yang et al. 1994). In this paper, a nucleotide sequence analysis of mtDNA that includes the D-loop region, the $tRNA^{Phe}$ gene, and a part of the 12S rRNA gene was used to document the mtDNA sequence variations as well as to elucidate the phylogenetic relationships among species of the subfamily Sparinae.

MATERIALS AND METHODS

Sample collection and DNA extraction

A total of 60 full grown specimens, 12 specimens for each of 5 species, were collected from Fulung, Tanshui, Wuchi, Taihsi, and Penghu between September 1991 and December 1991 (Fig. 1). Specimens were caught by handline, longline, or spear. Specimens were iced or frozen immediately after capture and later kept at $-75°C$ until DNA extraction. Crude DNA was extracted from 0.1 g of skeletal muscle as described by Kocher et al. (1989).

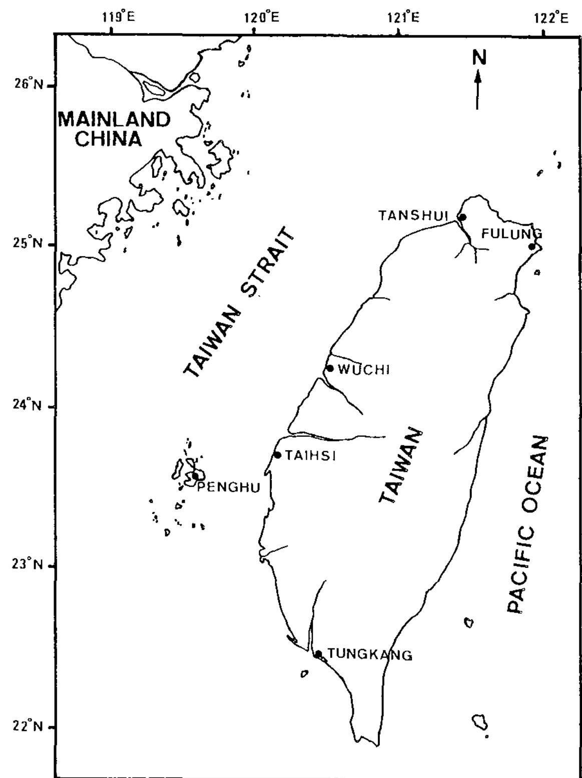

Fig. 1. Map of Taiwan showing sampling locations.

Amplification and sequencing of mtDNA

A segment of mtDNA that included the D-loop region, the $tRNA^{Phe}$ gene, and a part of the 12S rRNA gene was chosen for sequencing analysis in the present study. Primers P1, PB, PT, and PU were used for amplification and sequencing; and primers PW and PX were used only for sequencing (Fig. 2). The nucleotide positions of primers P1 and PB correspond to nucleotides 16280-16304 and 1388-1407, respectively, of the mtDNA sequences of an indigenous oriental stream loach, *Crossostoma lacustre* (Tzeng et al. 1992). This pair of primers amplified a DNA segment of about 1.7 kb. Primers PT and PU were designed according to the nucleotide sequence of black porgy *Acanthopagrus schlegeli* obtained using primers P1 and PB. Primers PT and PU amplified a DNA segment of about 1.1 kb. Primers PW and PX were designed according to nucleotide sequences of black porgy obtained using primers PT and PU.

Double-stranded polymerase chain reaction (PCR) was performed in 100 μl reaction volume

containing 10 μl of 10X reaction buffer (10 mM Tris-HCl, pH9.0; 50 mM KCl; 15 mM $MgCl_2$; 0.1% (w/v) gelatin; 1% Triton X-100), 0.4 μM each of the light and heavy strand primers, 0.2 μM each of dNTP, 0.5 μl of DNA extract, and 2 units of Super-Taq polymerase (HT Biotechnology LTD). PCR amplification was carried out in a Perkin Elmer-Cetus thermal cycler using the following cycling parameters: 35 cycles of denaturation at 94°C for 40 s, primer annealing at 55°C for 40 s, and extension at 72°C for 1.5 min or 2 min.

The double-stranded DNA products were purified using electroelution (Sambrook et al. 1989). The electroeluted DNA was extracted by phenol/chloroform and precipitated by ethanol. The vacuum-dried DNA was resuspended into 20 μl of double-distilled H_2O and used as the sequencing template.

DNA sequencing was performed by the dideoxynucleotide chain termination method (Sanger et al. 1977) using CircumVent Thermal Cycle Dideoxy DNA Sequencing Kit (New England Biolabs Inc.), with [α ^{35}S]-dATP as a label. The DNA sequencing reaction was carried out using the following cycling parameters: 20 cycles of denaturation at 95°C for 30 s, primer annealing at 55°C for 30 s, and extension at 72°C for 30 s. The products of the sequencing reactions were electrophoresed in a 6% polyacrylamide/7 M urea gel. Then, the gel was fixed, dried, and autoradiographed on X-ray film for 24-96 hr.

Sequence analysis

Mitochondrial DNA sequences of 12 specimens for each species that included a part of the tRNA^{Pro}gene, the D-loop region, the tRNA^{Phe} gene, and a part of the 12S rRNA gene 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 the published mtDNA sequences of other fishes to verify the boundaries of the genes. mtDNA sequences that included the D-loop region, the tRNA^{Phe} gene, and a part of the 12S rRNA gene were used for analysis. When performing interspecific analysis, a consensus sequence of each species was aligned.

Aligned sequences were analyzed using the MEGA software package (Molecular Evolutionary Genetics Analysis, version 1.01; Kumar et al. 1993) for (1) calculating the mean nucleotide composition of each species, (2) calculating the intra- and interspecific frequency distribution of the numbers of variable sites, (3) calculating the interspecific pairwise differences in numbers of transitions and transversions, (4) calculating the intra- and interspecific pairwise sequence distances using the Tamura-Nei model, and (5) constructing a phylogenetic tree by UPGMA (unweighted pair-group method with arithmetic average) using the values of the interspecific pairwise sequence distances from (4).

RESULTS

DNA sequence and length variations

Twelve sequences of each species from the tRNA^{Pro} gene to the 12S rRNA gene were aligned and compared with those of the Atlantic cod *Gadus morhua* (Johansen et al. 1990), white sturgeon *Acipenser transmontanus* (Buroker et al. 1990),

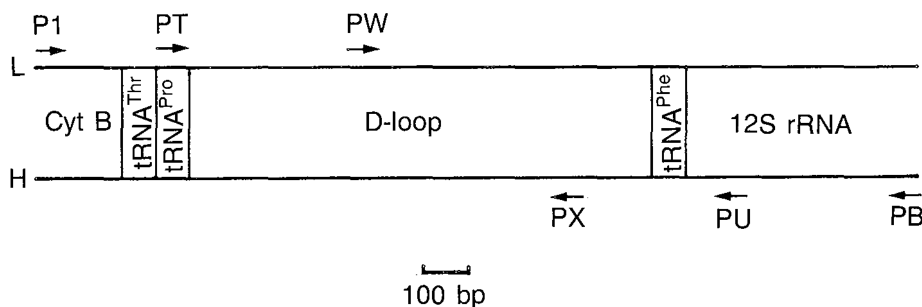

Fig. 2. Schematic diagram of the amplified and sequenced region of mtDNA in the 5 species of the subfamily Sparinae, and the locations of 6 primers, P1 (5'-CTAACATGAATTGGGGGTCAACCAG-3'), PT (5'-CTTACTATCAACTCCCAAAGC-3'), PW (5'-CAGG-GACAAAAATTGTGGGGG-3'), PX (5'-TGCAGAAGTGTTAGTGATCCC-3'), PU (5'-GGGCATTCTCACGGGGATGCG-3') and PB (5'-AGTGGGGTATCTAATCCCAG-3'), indicated by arrows.

rainbow trout *Oncorhynchus mykiss* (Digby et al. 1992, Shedlock et al. 1992), Taiwan stream loach *Crossostoma lacustre* (Tzeng et al. 1992), and Japanese eel *Anguilla japonica* (Sang et al. 1994). The DNA sequences appear to include a part of the $tRNA^{Pro}$ gene, the complete D-loop region, the complete $tRNA^{Phe}$ gene, and a part of the 12S rRNA gene. In this study, the D-loop region, the $tRNA^{Phe}$ gene and a part of the 12S rRNA gene were chosen for analysis. The lengths of these segments show some differences within each species as well as among the five studied species. In *Acanthopagrus australis*, the lengths of these segments are 932-948 bp for the D-loop region, 71 bp for the $tRNA^{Phe}$ gene, and 401 bp for a part of the 12S rRNA gene. The length variations in the D-loop region are due to a 1-bp deletion in five individuals and a 15-bp segment tandemly repeated once in three individuals. In *A. berda*, the lengths of these segments are 931-938 bp for the D-loop region, 71 bp for the $tRNA^{Phe}$ gene, and 400 bp for a part of the 12S rRNA gene. The length variations in the D-loop region are due to a 7-bp deletion in six individuals. In *A. latus*, the lengths of these segments are 942-1003 bp for the D-loop region, 71 bp for the $tRNA^{Phe}$ gene, and 401 bp for a part of the 12S rRNA gene. The length variations in the D-loop region are due to a 1-bp deletion in one individual and a 20-bp segment tandemly repeated 3 times in another individual. In *A. schlegeli*, the lengths of these segments are 940 bp for the D-loop region, 71 bp for the $tRNA^{Phe}$ gene, and 406 bp for a part of the 12S rRNA gene. In *S. sarba*, the lengths of these segments are 971-973 bp for the D-loop region, 71 bp for the $tRNA^{Phe}$ gene, and 398 bp for a part of the 12S rRNA gene. The length variation in the D-loop region is due to a 2-bp insertion in one individual. We reduced these tandem repeats to a single repeat unit for the construction of consensus sequence for each species, and they are aligned and given in Fig. 3.

In the vertebrate sequences analyzed, the 5′ end of the D-loop L-strand contains the proposed termination signals for D-loop DNA synthesis. The 3′ end of the D-loop L-strand contains a number of functionally important elements that include the origin of heavy strand replication (OH), sites of initiation of transcription for the heavy and light strands (HSP and LSP), and three phylogenetically conserved sequence blocks (CSB-1, 2, and 3) located in the vicinity of the OH (Doda et al. 1981, Walberg and Clayton 1981, Roe et al. 1985, Brown et al. 1986, Shedlock et al. 1992). In sparid fishes, several pentanucleotide sequences, TACAT, are located near the 5′ end of the D-loop L-strand (Fig. 3). This sequence is also present in the termination associated sequence (TAS) of *Xenopus laevis* and has been suggested to serve as a recognition signal for the arrest of the H-strand synthesis (Doda et al. 1981). Two conserved sequences AAACCCCCCCCCCCCCC and TGCAAACCCCTC-AAAAACA are located near the 3′ end of the D-loop L-strand (Fig. 3) which are similar to the CSB-2 and CSB-3 sequences previously reported in mammals and *Xenopus laevis* (Walberg and Clayton 1981).

Nucleotide composition

The mean nucleotide composition of sequences for the D-loop region, the $tRNA^{Phe}$ gene, and a part of the 12S rRNA gene among the 5 species (12 specimens for each species) are presented in Table 1. In the D-loop region, the A content (29.9%-33.4%) and the T content (31.1%-32.3%) are obviously higher than the C content (21.0%-22.8%) and the G content (13.8%-16.2%) which is consistent with previous findings that the D-loop is an A-T rich region of the mitochondrial genome (Brown et al. 1986, Saccone et al. 1987). In the $tRNA^{Phe}$ gene and 12S rRNA gene, the A contents of 33.8%-36.6% and 29.4%-30.8%, respectively, are higher than those of T, C, and G. Generally, in the segment of mtDNA analyzed, the most represented base is A, followed by T>C>G.

Intraspecific and interspecific variations

Among the 12 specimens of *Acanthopagrus australis*, there were 31 variable sites that include 30 base substitutions and 1 base deletion. Pairwise sequence distances within this species ranged from 0.0021 to 0.0130. Among the 12 specimens of *A. berda*, there were also 31 variable sites that include 24 base substitutions and one 7-base deletion. Pairwise sequence distances within this species ranged from 0.0000 to 0.0115. Among the 12 specimens of *A. latus*, there were 52 variable sites that include 51 base substitutions and 1 base deletion. Pairwise sequence distances within this species ranged from 0.0014 to 0.0209. Among the 12 specimens of *A. schlegeli*, there were 28 variable sites, all of which are base substitutions. Pairwise sequence distances within this species ranged from 0.0014 to 0.0093. Among the 12 specimens of *Sparus sarba*, there were only 12 variable sites that include 10 base substitu-

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    |<-- D-loop                                                     *****       *** **
AA  AATTTCAT-- ---------- ---------- ---------- ATACATATAT GT-GATATAC ATATATCTAT ATTCAACATA ATACTTAGGG ATATAGGACT  100
AB  .-------.. .......... .......... .......... G......... ..AT.AT... ...C...... .CC....... C..T.....A C.....C...
AL  ..C.....GT CTTAT..... .......... .......... G........C ..AT...... ......T... .AA...T... T..T-....T ..........
AS  ...A----.. .......... .......... .......... G......... ..AC...... ......T... ..A..G.... -..TC....A C.....A...
SS  .CCAC...AT ATACATATGT AAATGCCCGC ACGGCCATTT .C.T...... A.AT.GT... ......A... ..AGGCGCAT ...TG..AT. ..G.......
                                                                   ####### ########

                         ** ***                                                         **** *
AA  ATAATTGATT TTTCACCATA TATCTATATA CA---CCATA ACATAGTACA TAA------- --ATGTAATA CTAACATACA TCTATATTCA CCAATCAAGC  200
AB  ....C..... A.AG...... C......... TGTAC....T .T........ ..C....... .......T.. AC.G...... .T........ ....C.....
AL  .C........ A.A..-.... C......... TGTACAT... TTCAT...T. ...CACATAT ATT....... AC.G...T.. .......... ...TA.....
AS  .CC....... A.A....... C..T..C... T.TAC....G GT........ .......... .........T AC.G...... .T........ A.........
SS  .CCTA..G.A C..T.....T ACC.C..... ACCATA...T .TGGTT..A. ..C....ACT CT.....T.. TC.C....A. .TA..G..A. ....A....A
                    @@@@ @@@@ @@@@@@@@@@ @@

AA  AAGAATACTC TATTCAATTA TTTAATAGCA GATCATGTTC CCGAACCCAA CTTGAGTTAT TCCAAGACAA AAATCGAAGT TTAGTAAAAT -ATTAACTTT  300
AB  ...G...TAA ......G.C. .....C.A.. .CAA.....- T.A......C T....A.... CTTG...... .....T.G.A CCTAG.T..C ..G.G.A...
AL  ...G.C.T.. ...C...G.. ....CC.... -.AAG..A.. ..A...TACC T.A..A.GT. .AAGGACA.. T.C..T.G.A CCTAGT...C TT..G.A..C
AS  ..TC...... .......C.. .....C.A.. A.A......- T.A.....CT T...CA...C .TA.T..... ...ATC...A .CTAGC...C ....TTAC.C
SS  ..TG-...AA A......... ....TC.C.. ACCATCCAAT GGATGG.T.G .AATGAA.TA ....GTC.TT ..CA.A.GAC C..TC.C..C TG.CTTA.--

AA  AATGAATATA AACCAAGTAC CAACATCCCT TATATCTCCA GAAAACTGTC CAATAAGAAC CGACCAAC-C TATTATATCT TAATGCATTC GTTTATTGAA  400
AB  ...A...... C........T TT.GCA.T.C .GGT..CT.. A..G....C. .......... ......T.A. ..C....... G.......A. ..........
AL  ...A...... C........T .C.GCATT.C .GGTC.CT.. A..C.T.AC. .......... ...T....T. -......... G......... ..........
AS  ...A...... T......... .C.GCAT..C .TGT..CT.. A....T.AC. .......... .......... .......... .......... .........G
SS  ...A...... T.......CT .C.GCA...C .GC.A.C.AC TT.C.GGA.G ..G....... .......... .G.G..T... .T........ ..........

AA  GGTGAGGGAC AATGACTGTG GGGGTTACAC AAAATGATTT ATTCCTGGCA TCTGGTTCCT ATTTCAGGGC CATTAAATGG TACCATTTCC ACTAACTTTC  500
AB  ........G. ..AA.T.... .....C.... .TCG...... .......... .......... .......... ......CA.C --.T.A.... ..C...A...
AL  ...C...... ..GA.T.... .....C.... ........C. ...A...... .......... .......... ......TA.C .-TG.G.... .........T
AS  .A.C...... ..AA.T.... .....C.... ........C. ...A...... .......... .......... ...C...AA. --.TTAA.A. ...G..G...
SS  ........G. ..GT.T...A .....AG.T. ........C. ...A...... .......... .......... ......TA.C .-TT.G.... .........T

AA  ATCGACGCTT GCATAAGTTA ATGGTGTCAA CCAGATGGCG AGATAACCCC CCATGCCGGG CGTTCTCTCC ACAGGTGCCA GGG--TGCTT TTTCCTTTTT  600
AB  .......... .......... ......G.G. ..G....... ....G..T.. .......... .......... .......TT. ...GTAT..C .C.TT.....
AL  .......... .......... ......G... .......... .......... ........A. ......T... .......... A.-.A.T... ....TC..C.
AS  .......... .......... ......G.G. T......... ....G..T.. .......... .C........ ........A. ...GT-TT.. ...TA...C.
SS  .......... .......... ......G... .......... .......... .......... ......T... .........- -.AGA.T... ....TC..C.

AA  TAGCTTTTCA TGGTCATTTC ACAGTGCACT CCGAAGTCCT TGAACTAAGG TGGTACTATC ACCCCGCACG -AGGCAATGA TGATGAGATA TTTTAAGACT  700
AB  ....C...G. .......... .........G T.....CTTG AT........ ........AT C.......AT C.A.A.TAA. .A...GATGT ..GA....T.
AL  ........G. .......... .........G .T........ CAG....... ........AT .......... ...TA..AT. ..TG..AG.. ...A...G..
AS  ........G. .......... .........G TT........ GT........ ........AT .T........ C.A.G...A. ..T...AG.. ...A......
SS  ........G. .......... .........G .T........ CAG....... .........A TA...CGCAC G..TA..AT. ..TG..AG.. ...A...G..

AA  TTCATTTTTG AACTACATAA CTGATTTCAT GAGCATAATA TGTGATTTTT TCCCCTAAAA ATTTATATAT GAACCCCTTT GGTTTTTGGT CGTTAAACCC  800
AB  .......A.. ..T.G..... .......... .......... .......... .........T. T..ATATATC ..C....C.. .......... ..........
AL  .......A.. G.TA...C.. T......... .........G .......... .......... T..ATATATC ..C....C.. .......... ..........
AS  .AA.C..A.. ..T.G..... .....C.... .......... .......... .......... T.GATATATC ..C....C.. .......... ..........
SS  .......A.. G.TA..TC.. T......... .........G .......... .......... T..ATATATC ..C....C.. .......... ..........
                                                                                                 |<--

AA  CCCCCCCCCC CTAAACTCCA GGGATCACTA ATACTTTCTG CAAACCCCTC AAAAACAGAA ATTCTTGGG- CCCTAGAATA ATGGGGAATC ACATCCAAAA  900
AB  .......... .......... ......CT.. ....-..... G......... .......... .....G.... T...G.GC.. ..A......T ..........
AL  .......... ......C... .A........ ....-..... .......... .G........ .........C .......G.G ..A..T.TAT ..G.....TG
AS  .......... .......... .......... .C..-..... .......... .......... .......... .....A.G.. .....T.TCT ...C......
SS  .......... .......... ....C.T... .C.....-.. T......... .G.....G. G...G..... ....G..G.. ..A......T .T........
    CSB-2    -->|                          |<--     CSB-3     -->|

                                                                                        D-loop -->||<--
AA  TGTATCTTTT TAATATATTA AAATAATGAT TTTTAACTAA ATTCCTA-TT TTCCCCAAGG AAGTCTTTCT TAT-ATT-AA AA---TATAA ----CCAGTT  1000
AB  ..C....... .......... ...C...... ....-.T... .......T.. ...T...... ......A.T. CTCA...TT. ..CTT..C.T ACA.ATC...
AL  ATGCCTC... .......... ...CCG.C.. ..A.T.ACT. .G.TTCTA.A ..T.....TT C.AAT...TA C.CACGA.-- --...----- ....AT..C.
AS  ..C....... .......... ...C...... ......T... .......A.. .......... ...C..C.T. ATCG...T.. ..TTT....T AAAA......
SS  ..C....... ......TAA. ...A.TCTT. AACC..A.TT ..A..CTT.. .C....C.A. .G.CTG.CT. .TGGGC.T.C GTGC.----- ....AACTG.
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   Phe-tRNA                                                    Phe-tRNA -->||<-- 12S rRNA
AA CACGTAGCTT AATTAAAGCA TAACACTGAA GCTGTTAAGA TGGTCCCTAA AGAGCCCCAT GAACACAAAG GTTTGGTCCT GACTTTTCTG CCAGCTCTAG 1100
AB .......... ..C....... .......... .......... ......G... .A......G. .G........ .......... .........A ..........
AL .......... .......... .....T.... ..C....... ........G. .A..A...G. A.G....... .......... .......... ..........
AS ..T....... .......... .......... .......... .......... .A......G. ..G....... .......... .......... ..........
SS TG........ .......... .G........ .....C.... .......... .A.A....GC A.G....... .......... .......... ......T...

AA CTAAACTTAC ACATGCAAGT ATCCGCATCC CCGTGAGAAT GCCCTGTAGT TCCCTGCCCG GAAACAAGGA GCCGGTATCA GGCACATTTA ATTTAGCCCA 1200
AB ...G...... .......... .......... .......... ....C..... .T........ .......... .......... ......C... ..........
AL ...G.T.... .......... .......... .......... .......... .......... .......... ..T....... .......... ..........
AS ....G..... .......... .......... .......... .......... .....A.... AG........ ..T....... .......CC. ..C.......
SS .......... .......... ......GC.. .......... .....A.C.G .T..-..... .......... ..T....... ......-C.. .ACA......

AA CGACACCTTG CTCAGCCACA CCCTCAAGGG TACTCAGCAG TGATAAACAT TG----ACAC ATAAGTGAAA ACTTGACTCA GTCAAAGCTA AGA-AGGGCC 1300
AB ....G..... .........G .......... .......... .......... .......... .......... .......... ..T....... .TT.......
AL T......... ..A....... ...C...... .......... .......... .......T.. .......... .......... ....G..... ..T.......
AS .......... ..A....... .......... .......... .......... ..ATAT...T .......T.. .......... .C........ .A.C......
SS ....G..... .......... .......... .......... ...C.T.... .......T.. .......... G......... ..T....... ..-.......

AA GGTAAAACTC GTGCCAGCCA CCGCGGTTAT ACGGGAGGCC CAAGTTGTTA GAAGTCGGCG TAAAGGGTGG TTAAGAACAA GACTAAGATT AAAGCCGAAC 1400
AB .......... .......... .......... ....T..... .......... .......... .......... .......... A.T...-... ..........
AL .......... .......... .......... .......... ........C. .......... .......... .......... ......A... ..........
AS .......... .......... .......... .......... .......C.. .G........ .......... .......... A.....A... ....T.....
SS .......... .......... .......... .....T.... ........C. .......... .......... ......G... ACT....... ..........

                                                                  12S rRNA -->
AA ATCTTCCGAG CTGTTATACG CATCCGAAGA TAAGAAGCTC AACTGCGAAA GTAGCTTTAT ATATTCCGAA T                                1471
AB .......A.. .......... ...T...... .......... ..A....... .......... ......T... .
AL .......... .......... .........G .......... ....A..... .......... .C....T... A
AS .......... .......... .........G .......... ..A....... .......... ..C...T... .
SS .C.....AGA .C........ T........G C......... ..T.A..... .......... ..T...T... .
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Fig. 3. mtDNA sequences from the D-loop region to the 12S rRNA gene (L-strand, consensus sequences of 12 specimens for each species) for 5 species of the subfamily Sparinae. AA, *Acanthopagrus australis*; AB, *A. berda*; AL, *A. latus*; AS, *A. schlegeli*; SS, *Sparus sarba*. A dot indicates identical nucleotides, a dash indicates a gap, * indicates TAS-like sequences, # indicates a segment tandemly repeated once in 3 specimens of *A. australis*, @ indicates a segment tandemly repeated 3 times in 1 specimen of *A. latus*, and CSB-2 and CSB-3 indicate conserved sequence blocks.

tions and one 2-base insertion. Pairwise sequence distances within this species ranged from 0.0000 to 0.0056. The frequency distribution of the numbers of variable sites shows that most of the variable sites occur in the D-loop region, especially between nucleotides 1-300 and 500-700.

Among the 5 consensus sequences of the 5 species of the subfamily Sparinae, there were 568 variable sites. The frequency distribution of the numbers of variable sites shows that the $tRNA^{Phe}$ gene and the 12S rRNA gene are relatively conserved and the D-loop region is highly evolutionary, especially in the left and right domains of the D-loop (Fig. 4). The pairwise sequence difference in number of transitions and transversions of the 5 consensus sequences are presented in Table 2. The ratio of transitions to transversions ranged from 1.110:1 to 1.429:1 (mean 1.280:1) among the four species of the genus *Acanthopagrus*; and from 0.647:1 to 0.944:1 (mean 0.827:1) between *Sparus sarba* and species of the genus *Acanthopagrus*.

Phylogenetic tree

The interspecific pairwise sequence distances using the Tamura-Nei model ranged from 0.1498 to 0.1914 between species of the genus *Acanthopagrus*, and from 0.2386 to 0.2708 between species of the genera *Acanthopagrus* and *Sparus* (Table 3). The phylogenetic tree constructed using data from Table 3 by UPGMA is shown in Figure 5. From this molecular tree, the 5 species are clustered into two distinct groups, one group includes *A. australis*, *A. berda*, *A. latus*, and *A. schlegeli* (genus *Acanthopagrus*); the other includes only *Sparus sarba* (genus *Sparus*). Within the *Acanthopagrus*, *A. australis* is closest to *A. schlegeli*, followed by *A. berda*, and then *A. latus*.

DISCUSSION

The gene arrangement from Cytochrome b, tRNA^{Thr}, tRNA^{Pro}, D-loop, tRNA^{Phe}, to 12S rRNA in the mitochondrial genome of sparid fishes is consistent with that in most vertebrates (Tzeng et al. 1992).

The lengths of the D-loop region vary broadly in different organisms, from 121 bp in the sea urchin, *Strongylocentrotus purpuratus* (Jacobs et

Table 1. Lengths and mean nucleotide composition of sequences from the D-loop region to the 12S rRNA gene in mtDNA of the 5 species of the subfamily Sparinae

Species[a]	Region	Nucleotides						
		A%	T%	C%	G%	A+T%	C+G%	Length[b](bp)
AA	D-loop	32.9	32.3	21.0	13.9	65.2	34.9	932-933
	tRNA^{Phe}	35.2	22.5	23.9	18.3	57.7	42.2	71
	12S rRNA	30.4	22.2	24.9	22.4	52.6	47.3	401
	Total	32.3	28.9	22.2	16.5	61.2	38.7	1404-1405
AB	D-loop	31.5	31.6	21.6	15.2	63.1	36.8	931-938
	tRNA^{Phe}	33.8	22.5	22.5	21.1	56.3	43.6	71
	12S rRNA	30.5	23.8	24.0	21.8	54.3	45.8	400
	Total	31.4	28.9	22.3	17.4	60.3	39.7	1402-1409
AL	D-loop	32.0	31.9	21.6	14.5	63.9	36.1	942-943
	tRNA^{Phe}	36.6	22.5	21.1	19.7	59.1	40.8	71
	12S rRNA	.30.4	22.7	24.2	22.7	53.1	46.9	401
	Total	31.8	28.8	22.3	17.1	60.6	39.4	1414-1415
AS	D-loop	33.4	31.4	21.4	13.8	64.8	35.2	940
	tRNA^{Phe}	33.8	23.9	22.5	19.7	57.7	42.2	71
	12S rRNA	30.8	22.4	24.9	21.9	53.2	46.8	406
	Total	32.7	28.4	22.5	16.4	61.1	38.9	1417
SS	D-loop	29.9	31.1	22.8	16.2	61.0	39.0	971-973
	tRNA^{Phe}	35.2	21.1	23.9	19.7	56.3	43.6	71
	12S rRNA	29.4	22.1	25.4	23.1	51.5	48.5	398
	Total	30.0	28.1	23.6	18.3	58.1	41.9	1440-1442

[a]AA, *Acanthopagrus australis*; AB, *A. berda*; AL, *A. latus*; AS, *A. schlegeli*; SS, *Sparus sarba*.
[b]Only one copy of the tandemly repeated sequence was included in the length of the D-loop region.

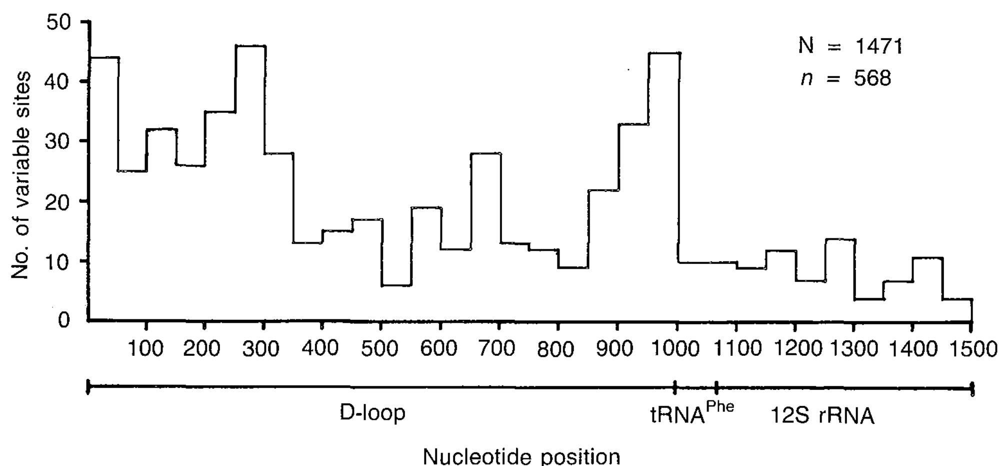

Fig. 4. Interspecific frequency distribution of the numbers of variable sites for sequences from the D-loop region to the 12S rRNA gene among the 5 species in the subfamily Sparinae. N, length of sequence; *n*, total number of variable sites.

Table 2. Pairwise difference of sequences (consensus of 12 specimens for each species, 1471 bp) from the D-loop region to the 12S rRNA gene in numbers of transitions (above the diagonal) and transversions (below the diagonal) among the 5 species of the subfamily Sparinae

Species[a]	AA	AB	AL	AS	SS
AA	—	113	123	110	144
AB	88	—	121	112	152
AL	109	109	—	120	112
AS	77	78	93	—	142
SS	162	161	173	172	—

[a]AA, *Acanthopagrus australis*; AB, *A. berda*; AL, *A. latus*; AS, *A. schlegeli*; SS, *Sparus sarba*.
[b]Mean ratio of transitions: transversions = 1.280:1 (1.110:1-1.429:1) among the four species of genus *Acanthopagrus*.
[c]Mean ratio of transitions: transversions = 0.827:1 (0.647:1-0.944:1) between *Sparus sarba* and the four species of the genus *Acanthopagrus*.

Table 3. Pairwise sequence distances (above the diagonal) and standard errors (below the diagonal) of sequences (consensus of 12 specimens each species, 1471 bp) from the D-loop region to the 12S rRNA gene among 5 species of the subfamily Sparinae using the Tamura-Nei model

Species	AA	AB	AL	AS	SS
AA	—	0.1626	0.1914	0.1498	0.2631
AB	0.0117	—	0.1901	0.1515	0.2708
AL	0.0128	0.0128	—	0.1741	0.2386
AS	0.0112	0.0112	0.0122	—	0.2703
SS	0.0155	0.0159	0.0143	0.0158	—

AA, *Acanthopagrus australis*; AB, *A. berda*; AL, *A. latus*; AS, *A. schlegeli*; SS, *Sparus sarba*.

al. 1988), to 2134 bp in the frog, *Xenopus laevis* (Roe et al. 1985). Among fishes examined, the lengths of the D-loop region ranged from 841 bp in the goby, *Rhinogobius giurinus* (Chen 1994), to 1003 bp in rainbow trout *Oncorhynchus mykiss* (Digby et al. 1992). In the subfamily Sparinae, the lengths of the D-loop region are similar within the genus *Acanthopagrus*, but significantly different between the genera *Acanthopagrus* and *Sparus*. The length variations among sparid fishes mainly occur near the 5′ and 3′ ends of the D-loop region which is consistent with the observation that the left and right domains of the D-loop region are highly variable in length (Saccone et al. 1987).

Tandem repeat sequences in the D-loop region have been found in a variety of animals, such as the sea scallop, *Placopecten magellanicus* (La Roch et al. 1990), *Drosophila* (Solignac et al. 1986), cricket (Rand and Harrison 1989), evening bat (Wilkinson and Chapman 1991), lizard (Moritz and Brown 1986), Taipei treefrog *Rhacophorus taipeianus* (Yang et al. 1994), American shad *Alosa sapidissima* (Bentzen et al. 1988), and white sturgeon *Acipenser transmontanus* (Buroker et al. 1990). In sparid fishes, occurrence of tandem repeats in the D-loop region are not common. Among the 60 individuals, tandem repeats occurred only in 3 *A. australis* individuals with a 15-bp segment tandemly repeated once and 1 *A. latus* individual with a 20-bp segment tandemly repeated 3 times.

The D-loop region can be divided into three domains on the basis of the interspecific frequency distribution of the numbers of variable sites (Fig. 4). The left (L) domain (1-350 bp) that is adjacent to the $tRNA^{Pro}$ gene has the highest A content and the lowest G content with high sequence divergence. The central (C) domain (350-650 bp) has the lowest A content and the highest G content with highly conserved sequences. The right (R) domain (650 bp ~) that is adjacent to the $tRNA^{Phe}$ gene also has high A content and low G content with high sequence divergence. These D-loop characteristics in the sparid fish are consistent with observations for other vertebrates (Brown et al. 1986, Saccone et al. 1987).

The phylogenetic tree relating the five species of sparid fishes constructed by UPGMA based on the mtDNA sequence data, shows the same topology with that based on allozyme electrophoresis data (Jean et al. 1995). It shows similar clustering with that based on morphometric data at the genus level for *Acanthopagrus* and *Sparus*, while the interspecific relationships within the genus *Acanthopagrus* are not in good agreement. The phylogenetic tree, constructed from the morphometric data, groups *A. australis* and *A. berda* together, while the other group consists of *A. latus* and *A. schlegeli* (Jean et al. 1992). The phylogenetic trees constructed from mtDNA sequence data and allozyme electrophoresis data show that *A. latus* is well separated from the other three species, *A. berda*, *A. australis*, and *A. schlegeli* (Fig. 5). This disagreement shows that different interpretations on interspecific relationships may exist when using different characters.

There has been considerable debate over whether molecular or morphological features are inherently better sources of information for estimating phylogeny (Patterson 1987). Most

systematists recognize that morphological and molecular approaches each have distinct advantages (Shoshani 1986). Furthermore, systematists from each camp are able to address questions and problems that cannot be addressed by systematists from the other (Hillis 1987). Comparative studies also have shown that morphological change and molecular divergence are quite independent, responding to different evolutionary pressures and following different rules (Wilson et al. 1974 1977). However, for the practicing systematist, the real concerns are whether the characters examined have a clear and independent genetic basis, whether the characters exhibit variations appropriate to the question posed, and whether the data are collected and analyzed with a proper approach (Moritz and Hillis 1990).

Based on the genetic basis of characters examined in our studies, morphological characters involve certain non-genetic variations that can be better correlated with more immediate environmental factors, and the expression of allozymes also can be affected by the environment, while DNA sequence data are nearly or completely free of nonheritable variations. In general, however, biomolecular data are confounded less by environmental influences than morphological data (Hillis 1987). Therefore, in the present instance, we believe that molecular characters may be more appropriate than morphometric characters.

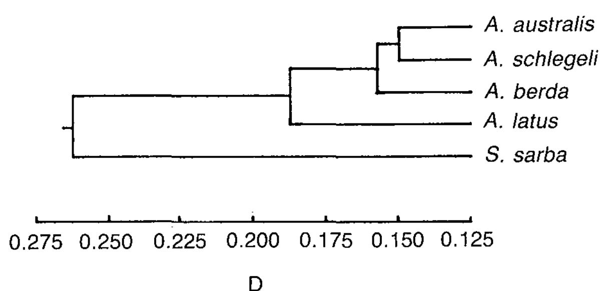

Fig. 5. Phylogenetic tree of 5 species of the subfamily Sparinae based on the Tamura-Nei sequence distances (D) using the UPGMA clustering method.

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臺灣沿岸海域鯛亞科魚類粒線體DNA核苷酸序列之變異及種間親緣關係

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本研究比較分析澳洲黑鯛(*Acanthopagrus australis*)、灰鰭鯛(*A. berda*)、黃鰭鯛(*A. latus*)、黑鯛(*A. schlegeli*)及黃錫鯛(*Sparus sarba*)各12尾標本粒線體DNA之D-loop區、$tRNA^{Phe}$基因及部分12S rRNA基因之核苷酸序列，以探討其核苷酸序列之變異及闡明其種間親緣關係。

種內及種間核苷酸序列之排序、比較結果，D-loop區、$tRNA^{Phe}$基因及部分12S rRNA基因核苷酸序列之長度，在澳洲黑鯛分別爲932-948 bp、71 bp及401 bp；在灰鰭鯛爲931-938 bp、71 bp及400 bp；在黃鰭鯛爲942-1003 bp、71 bp及401 bp；在黑鯛爲940 bp、71 bp及406 bp；在黃錫鯛爲971-973 bp、71 bp及398 bp。$tRNA^{Phe}$基因及12S rRNA基因相當保守，變異很小，D-loop區之左、右區段含有較高比例之腺嘌呤(A, adenine)及較低比例之鳥糞嘌呤(G, guanine)，且具有較大之序列及長度變異；而D-loop區之中央區段則含有較低比例之腺嘌呤及較高比例之鳥糞嘌呤，且較左、右區段保守，變異較小。出現在其它脊椎動物D-loop區之保守序列(conserved sequence elements) TAS、CSB-2及CSB-3亦出現在鯛亞科魚類之D-loop區。

種內兩兩標本間之Tamura-Nei序列距離，澳洲黑鯛爲0.0021-0.0130，灰鰭鯛爲0.0000-0.0115，黃鰭鯛爲0.0014-0.0209，黑鯛爲0.0014-0.0093，黃錫鯛爲0.0000-0.0056；而種間之Tamura-Nei序列距離在黑鯛屬內爲0.1498-0.1914、在黑鯛屬與鯛屬間則爲0.2386-0.2708。依據種間序列距離使用UPGMA聚類方去繪出之系統樹顯示，鯛亞科五種魚明顯分成二群，一群包括澳洲黑鯛、灰鰭鯛、黃鰭鯛及黑鯛，即黑鯛屬；另一群僅有黃錫鯛，即鯛屬。而黑鯛屬四種魚之親緣關係則澳洲黑鯛與黑鯛最接近，其次依序爲灰鰭鯛、黃鰭鯛。此系統樹與使用同功異構酶電泳法所獲者一致，但與形態測定方法所獲者不一致。

關鍵詞：粒線體, 序列變異, 種間關係, 鯛亞科。

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