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Full Length Research Paper

Genetic and phylogenetic analysis of ten Gobiidae species in China based on amplified fragment length polymorphism (AFLP) analysis

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To study the genetic and phylogenetic relationship of gobioid fishes in China, the representatives of 10 gobioid fishes from 2 subfamilies in China were examined by amplified fragment length polymorphism (AFLP) analysis. We established 220 AFLP bands for 45 individuals from the 10 species, and the percentage of polymorphic bands was 100%. The percentage of polymorphic loci within species ranged from 3.61 to 58.56%. *Chaeturichthys stigmatias* showed the greatest percentage of polymorphic loci (58.56%), the highest Nei's genetic diversity (0.2421 ± 0.2190) and Shannon's information index (0.3506 ± 0.3092), while *Pterogobius zacalles* showed the lowest percentage polymorphic loci (3.61%), the lowest Nei's genetic diversity (0.0150 ± 0.0778) and lowest Shannon's information index (0.0219 ± 0.1136). The topology of UPGMA tree showed that the individuals from the same species clustered together and the 10 species formed two major clades. One clade consisted *Cryptocentrus filifer*, *P. zacalles*, *Tridentiger trigonocephalus*, *Chaeturichthys hexanema*, *C. stigmatias*, *Acanthogobius flavimanus* and *Synechogobius ommaturus*, and the other clade consisted *Odontamblyopus rubicundus*, *Trypauchen vagina* and *Ctenotrypauchen microcephalus*. The results agreed with the traditional taxonomy of the morphological characters. AFLP fingerprints were successfully used to study the phylogenetic relationship of the gobioid fishes and it identified species origins of morphologically similar taxa.

Key words: Phylogenetic, amplified fragment length polymorphism (AFLP), gobiidae, Amblyopinae, gobiinae.

INTRODUCTION

In recent studies, molecular markers have been proved to be effective tools to estimate phylogenetic relationships at various taxonomic levels (Miya and Nishida, 1996, 2000; Forey et al., 1996). Among different types of molecular markers, amplified fragment length polymorphism (AFLP) analysis is a PCR-based, multi-locus fingerprinting technique that combines the strengths and overcomes the weaknesses of the RFLP and RAPD methods (Vos et al., 1995), and it is highly efficient for the detection of genetic variations. It allows fast and efficient generation of a large amount of genetic data. Until now, AFLP has been successfully used in phylogenetic studies of plants (Wolfe and Liston 1998; Caicedo et al., 1999; Xu and Sun, 2001; Zhang et al., 2001), animals (Buntjer et al., 2002; Dasmahapatra et al., 2009) and fishes (Zhang and Liu, 2006; Yang et al., 2010) because of its capability to simultaneously screen large numbers of polymorphic loci, high reproducibility and relative cost effectiveness.

The suborder Gobioidei, the largest group in Perciforms, includes about 2211 species which belong to 270 genera of 9 families (Nelson, 2006). Most Gobioidei are benthic and widely distributed throughout the tropical, subtropical and temperate waters. The gobioid fishes are generally small and rarely exceed 50 cm. The smallest of all the fishes is found among them (Winterbottom and Emery, 1981). The classification for suborder Gobioidei is confused and it has been grouped into two, six, eight or nine families (Miller, 1973; Hoese, 1984; Hoese and Gill, 1993; Nelson, 1994; Thacker, 2000). The current

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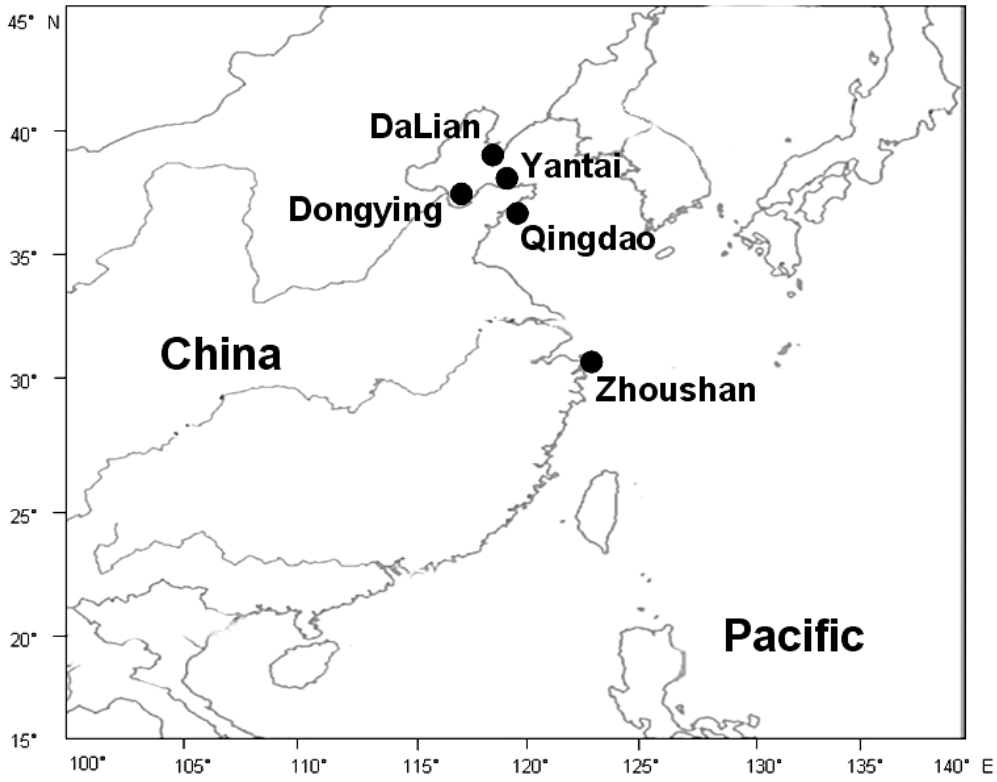

Figure 1. Map showing sample locations of the 10 Gobiidae species around China coast.

classification of gobioid fishes reflects phylogenetic uncertainty because gobioid fishes are not only small but also morphologically reduced when compared with other perciforms species (Thacker, 2000). The large number of species, small size and different morphological characters make it difficult to identify the gobioid fishes. With the development of molecular markers, some fragments of mitochondrial DNA have been successfully used to analyze the phylogenetic relationship among gobioid fishes. For example, cytochrome *b* genes were used to establish the phylogenetic relationship of 28 gobioid fishes (Akihito et al., 2000), and complete sequences of the mitochondrial ND1, ND2 and COI were analyzed for phylogenetic relationships of 8 families (Thacker, 2003) and 27 gobioid genera (Thacker and Hardman, 2005).

China is one of the regions with higher diversities in Gobioid fishes over the world and has 307 species of 106 genera which occupy 13.9% of the total 2211 species because the complex physical geographical structures of China can provide suitable habitats for gobioid fishes (Wu and Zhong, 2008). However, studies on gobioid fishes in China mainly focus on resource surveys and some simple descriptions of species due to the small size, low numbers and lack of economic value. Until now, there are no phylogenetic studies on gobioid fishes in China. In the present study, representatives of 10 species of two families from China: *Odontamblyopus rubicundus*, *Trypauchen vagina*, *Ctenotrypauchen microcephalus*, *Cryptocentrus filifer*, *Pterogobius zacalles*, *Tridentiger trigonocephalus*, *Chaeturichthys hexanema*, *Chaeturichthys stigmatias*, *Acanthogobius flavimanus* and *Synechogobius ommaturus*, were examined by AFLP analysis. The results of the study suggested phylogenetic relationship of these 10 species and provided useful information for the classification of the suborder Gobioidei. The results of the AFLP validated the taxonomy position of genus *Tridentiger* and the relationship between *C. hexanema* and *C. stigmatias*, which could be confirmed by morphological characters.

MATERIALS AND METHODS

Fish samples

A total of 45 specimens were collected along the China inshore waters during 2007, including 3 species from Amblyopinae (*O. rubicundus*, *T. vagina* and *C. microcephalus*) and 7 species from Gobiinae (*C. filifer*, *P. zacalles*, *T. trigonocephalus*, *C. hexanema*, *C. stigmatias*, *A. flavimanus* and *S. ommaturus*) (Figure 1 and Table 1). The samples were provided by resource surveys, and all the individuals were identified based on morphological characteristics. A piece of muscle tissue was obtained from each individual and

Table 1. Sampling information of gobioid fishes including sampling sites, date of collection and sample size.

Species	ID	Sampling site	Date of collection	Sample size
C. filifer	Cfi	Yantai, China	October 2007	6
P. zacalles	Pza	Yantai, China	October 2007	2
T. trigonocephalus	Ttr	Qingdao, China	September 2007	3
C. hexanema	Che	Yantai, China	October 2007	3
C. Microcephalus	Cmi	Dongying, China	May 2007	7
C. stigmatias	Cst	Yantai, China	October 2007	5
A. flavimanus	Afl	Yantai, China	October 2007	5
S. ommaturus	Som	Dalian,China	September 2007	7
O. rubicundus	Oru	Dongying, China	May 2007	2
T. vagina	Tva	Zhoushan, China	September 2007	5

Table 2. Adaptors and primer combinations sequences used in the present study.

Primer		Sequence
Adapters	EcoRI-adapter	5'-CTCGTAGACTGCGTACC-3' 5'-AATTGGTACGCAGTCTAC-3'
	MseI-adapter	5'-GACGTGAGTCCTGAG-3' 5'-TACTCAGGACTCAT-3'
Pre-amplification primer	EcoRI	5'-GACTGCGTACCAATTC-3'
	MseI	5'-GATGAGTCCTGAGTAA-3'
Selective amplification primer	EACG/MCTC	5'-GACTGCGTACCAATTCAGG-3' 5'-GATGAGTCCTGAGTAACTC-3'
	EAGG/MCTG	5'-GACTGCGTACCAATTCAGG-3' 5'-GATGAGTCCTGAGTAACTG-3'
	EACC/MCAT	5'-GACTGCGTACCAATTCACC-3' 5'-GATGAGTCCTGAGTAACAT-3'
	EAGG/MCAT	5'-GACTGCGTACCAATTCAGG-3' 5'-GATGAGTCCTGAGTAACAT-3'
	EACG/MCTG	5'-GACTGCGTACCAATTCACG-3' 5'-GATGAGTCCTGAGTAACTG-3'

was preserved in 95% ethanol or was frozen for DNA extraction.

Genomic DNA extraction and AFLP method

Genomic DNA was isolated from muscle tissue by proteinase K digestion followed by a standard phenol-chloroform extraction. Procedures of AFLP were essentially based on Vos et al. (1995) and Wang et al. (2000). About 100 ng genomic DNA was digested with 1 unit of *Eco*R I and *Mse* I (NEB) at 37℃ for 6 h. Double-stranded adapters were ligated to the restriction fragments at 20℃ overnight after adding 1 µl 10 × ligation buffer, 5 pmol E*co*R I adapter (E*co*R I-1/E*co*R I-2; Table 2), 50 pmol *Mse* I adapter (*Mse*I-1/*Mse*I-2; Table 2), 0.3 unit of T4 DNA ligase (Promega) with a final volume of 10 µl. Pre-amplification PCR reaction was conducted using an Eppendorf thermocycler (Mastercycler 5334) with a pair of primers containing a single selective nucleotide. Amplification was performed at an annealing temperature of 53℃ for 30 s. The 20 µl PCR product mixture was diluted 10-fold with distilled water and was used as the templates for the subsequent selective PCR amplification. The selective amplifications were carried out in 20 µl PCR reaction volume containing 1 µl productions of pre-amplifications, 1 × PCR reaction buffer, 150 µM of each dNTP, 30 ng of each selective primer, and 0.5 unit of Taq DNA polymerase on a gradient thermal cycler (Mastercycler 5334) with a touchdown cycling profile of nine cycles of 30 s at 94℃, 30 s at 65℃ (-1℃ at each cycle), and 30 s at 72℃ followed by the cycling profile of 28 cycles of 30 s at 94℃, 30 s at 56℃, and 1 min at 72℃. The final step was a prolonged extension of 7 min at 72℃. PCR products were run on 6.0% denaturing polyacrylamide gel electrophoresis (PAGE) for 2.5 h at 50℃ on the Sequi-Gen GT Sequencing Cell (Bio-Rad, USA), and finally were detected using the silver staining technique modified from Merril et al. (1979). Sequences of AFLP adapters and primers are listed in Table 2. Five primer combinations (EACG/MCTC, EAGG/MCTG, EACC/MCAT, EAGG/MCAT and EACG/MCTG) were chosen for AFLP analysis (Table 2).

Data analysis

Clear and unambiguous bands in length ranging from 50 to 1200 bp were considered as usable. AFLP bands were scored for presence (1) or absence (0) excluding the smeared and weak ones by visual inspection, and were transformed into 0/1 binary character matrix.

Table 3. Number of loci, number of polymorphic loci, percentage of polymorphic loci and several genetic diversity indices.

ID	Number of locus	Observed number of allele	Effective number of allele	Number of polymorphic locus	percentage of polymorphic locus (%)	Nei's gene diversity	Shannon's information index
Cfi	104	1.3654±0.4839	1.2616±0.3741	38	36.54	0.1477±0.2044	0.2151±0.2932
Pza	83	1.0361±0.1878	1.0256±0.1328	3	3.61	0.0150±0.0778	0.0219±0.1136
Ttr	76	1.0921±0.2911	1.0532±0.1832	7	9.21	0.0326±0.1061	0.0493±0.1583
Che	92	1.4783±0.5023	1.3817±0.4341	44	47.83	0.2068±0.2252	0.2969±0.3184
Cmi	82	1.5610±0.4993	1.3834±0.3962	46	56.10	0.2177±0.2119	0.3195±0.3016
Cst	111	1.5856±0.4949	1.4386±0.4207	65	58.56	0.2421±0.2190	0.3506±0.3092
Afl	93	1.5484±0.5004	1.3704±0.3874	51	54.84	0.2126±0.2085	0.3130±0.2987
Som	97	1.3093±0.4646	1.2178±0.3760	30	30.93	0.1195±0.1956	0.1745±0.2774
Oru	67	1.1940±0.3984	1.1372±0.2817	13	19.40	0.0804±0.1650	0.1173±0.2409
Tva	80	1.1250±0.3328	1.0750±0.2250	10	12.50	0.0442±0.1252	0.0663±0.1833

Percentages of polymorphic loci, observed number of alleles, effective number of alleles, Nei's genetic diversity and Shannon diversity index were calculated by POPGENE (Yeh et al., 1999). The 0/1 matrix was converted into a distance matrix using the program RESTDIST in the PHYLIP v3.6a3 Inference Package (Felsenstein, 1995). An UPGMA tree was constructed using the software MEGA 2 (Kumar et al., 2001) based on the matrix from RESTDIST and bootstrapped for 500 replicates in FreeTree (Hampl et al., 2001).

RESULTS

The study established AFLP fingerprints for 45 individuals from 10 species. A total of 220 bands were identified by 5 AFLP primer combinations among the 10 species, of which 220 bands were polymorphic, and the percentage of polymorphic bands was 100% (Table 3). The number of loci amplified per primer pair combination varied from 34 to 58, and the number of polymorphic loci ranged from 34 to 58, with an average of 44. The proportion of polymorphic loci for each primer pair combination was 100% (Table 4). The percentage of polymorphic loci within the species ranged from 3.61to 58.56% (Table 3). The values of observed number of alleles were from 1.0361 ± 0.1878 (*P. zacalles*) to 1.5856 ± 0.4949 (*C. stigmatias*) and effective number of alleles was from 1.0256 ± 0.1328 (*P. zacalles*) to 1.4386 ± 0.4207 (*C. stigmatias*) (Table 3). Assuming Hardy-Weinberg equilibrium, species *C. stigmatias* showed the greatest percent polymorphic loci (58.56%), the highest gene diversity (0.2421 ± 0.2190) and Shannon's diversity index (0.3506 ± 0.3092), while species *P. zacalles* showed the lowest percentage of polymorphic loci (3.61%), the lowest gene diversity (0.0150 ± 0.0778) and Shannon's diversity index (0.0219 ± 0.1136) (Table 3).

The average intra-specific genetic distances ranged from 0.0024 (between individuals of *T. trigonocephalus*) to 0.0767 (between individuals of *C. stigmatias*). The average inter-specific distances ranged from 0.0576 (between *A. flavimanus* and *C. stigmatias*) to 0.2747 (between *C. stigmatias* and *C. Microcephalus*). The UPGMA tree for the 45 individuals was constructed based on the genetic distances from PHYLIP. Individuals from each species clustered together and the 10 species formed two major clades. One clade consisted *C. filifer*, *P. zacalles*, *T. trigonocephalus*, *C. hexanema*, *C. stigmatias*, *A. flavimanus* and *S. ommaturus*, and the other clade consisted *O. rubicundus*, *T. vagina* and *C. microcephalus* (Figure 2).

DISCUSSION

AFLP, a highly reproducible DNA fingerprinting technique, is useful in a variety of evolutionary and taxonomic genetic studies (Vos et al., 1995; Mueller and Wolfenbarger, 1999; Dragoo et al., 2003; Techaprasan et al., 2008; Gwo et al., 2008). In comparison with RAPD, AFLP is more sensitive in the identification of alleles and determination of their polymorphism because of the use of polyacrylamide gel electrophoresis for separation of PCR products (Koeleman et al., 1998; Barker et al., 1999; Yan et al., 1999). Earlier studies revealed that AFLP could detect higher level genetic divergence than mitochondrial DNA sequences (Despres et al., 2003). The comparatively high resolution and sensitivity can provide large number of markers and enhance the efficiency in

Table 4. Number of bands generated by five primer combinations.

Parameter	EACG/MCTC	EAGG/MCTG	EACC/MCAT	EAGG/MCAT	EACG/MCTG	Total
Number of loci	58	41	47	34	40	220
Number of polymorphic loci	58	41	47	34	40	220
Proportion of polymorphic loci	100	100	100	100	100	100

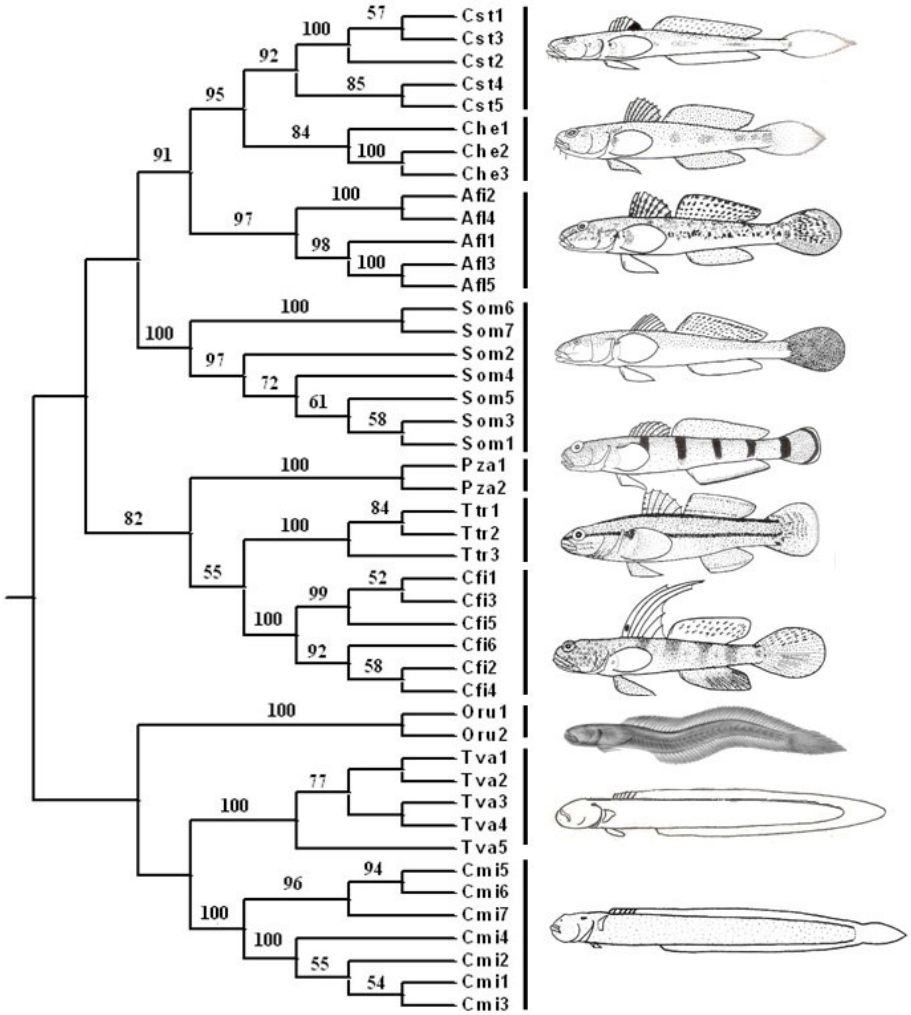

Figure 2. UPGMA phenogram of the 45 individuals of the 10 Gobiidae species based on genetic distances.

detecting polymorphic loci. In this study, the percentage of polymorphic bands in 5 primers for these 10 species was 100% and the genetic variations among the species were very high. The 10 species in the study belonged to 2 different subfamilies, and the distant affinity of inter-species may lead to high percentage of polymorphic lociand genetic variations. However, the percentage of polymorphic for each species ranged from 3.61 to 58.56%, showing variation in levels of intraspecific diversity. Given the small number of individuals and different sampling size for each species, it was reasonable that the results of the genetic diversity varied from each other.

The UPGMA tree supported the morphological evidences that there were two large clades for the 10 species; Amblyopinae clade and Gobiinae clade. Gobioid fishes of subfamily Amblyopinae are commonly referred to “eel gobioid fishes” or “worm gobioid fishes” because of their elongate bodies with a continuous dorsal fin (Murdy and Shibukawa, 2001). Dorsal and anal fins of this subfamily are connected to caudal fin, and both dorsal fins are united by membrane. Therefore, there are

distinct differences between these two subfamilies.

Within the larger clade Gobiinae, seven species were divided into two subclades, one group including *P. zacalles*, *T. trigonocephalus* and *C. filifer* and the other including *C. stigmatias*, *C. hexanema*, *A. flavimanus* and *S. ommaturus*. In comparison with earlier studies, Wu and Zhong, (2008) supported that *C. hexanema* belonged to a new genus *Amblychaeturichthys* while the this studies showed that relationship between the species *C. stigmatias* and *C. hexanema* was closer (Wu and Zhong, 2008; Zhu and Wu, 1963). Therefore, it is difficult to come to a conclusion that these two species belonged to different genus. The genetic distance between *S. ommaturus* and *A. flavimanus* was lower than that of *A. flavimanus* and *C. hexanema*, and then the results of this study did not support *S. ommaturus* as a member of the genus *Acanthogobius*. The four species, *C. stigmatias*, *C. hexanema*, *A. flavimanus* and *S. ommaturus* were clustered into one clade, which indicated that the relationships among them were closer. The morphological character evidences concurred with this grouping. However, earlier morphological studies showed that *T. trigonocephalus* were isolated from Gobiinae (Wu, 1987) but this study did not support this point. The genus *Tridentiger* was distinguished by the morphological character of the outer trilobed teeth in both jaws and should be considered to be a single subfamily in Gobiidae (Zhu and Wu, 1963). This study supported the Wu and Zhong, (2008) standpoint that *T. trigonocephalus* was sister to the *C. filifer* and should be one branch of the subfamily Gobiinae (Wu et al., 2008). Within the clade Amblyopinae, three species: *O. rubicundus*, *T. vagina* and *C. microcephalus* were divided into two clades. *T. vagina* was sister to *C. microcephalus*, and then clustered with *O. rubicundus*, which corresponded to the traditional taxonomy of the morphological characters.

In conclusion, AFLP was successfully used for studying the phylogenetic relationship among the gobioid fishes and it identified species origins of the morphologically similar taxa due to its high sensitive identification. However, there was no sufficient number of species and individuals for each species and therefore more samples should be provided to improve the reliability of the phylogenetic trees. It is necessary to increase numbers of individuals and use different molecular markers to assess and verify the results of AFLP analysis.

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REFERENCES

Akihito IA, Kobayashi T, Ikeo K, Imanishi T, Ono H, Umehara Y, Hamamatsu C, Sugiyama K, Ikeda Y, Sakamoto K, Fumihito A, Ohno S, Gojobori T (2000). Evolutionary aspects of gobioid fishes based upon a phylogenetic analysis of mitochondrial cytochrome *b* genes. Gene, 259: 5-15.

Barker JHA, Matthes M, Arnold GM, Edwards KJ, Ahman I, Larsson S, Karp A (1999). Characterization of genetic diversity in potential biomass willows (*Salix* spp.) by RAPD and AFLP analyses. Genome, 42: 173-183.

Buntjer JB, Otsen M, Nijman IJ, Kuiper MTR, Lenstra JA (2002). Phylogeny of bovine species based on AFLP fingerprinting. Heredity, 88: 46-51.

Caicedo AL, Gaitan E, Duque MC, Chica OT, Debouck DG, Tohme J (1999). AFLP fingerprinting of *Phaseolus lunatus* L. & related wild species from South America. Crop Sci. 39: 1497-1507.

Dragoo JW, Salazar-Bravo J, Layne LJ, Yates TL (2003). Relationships within the *Calomys callosus* species group based on amplified fragment length polymorphisms. Biochem. Syst. Ecol. 31: 703-713.

Dasmahapatra KK, Hoffman JI, Amos W (2009). Pinniped phylogenetic relationships inferred using AFLP markers. Heredity, 103: 168-177

Felsenstein J (1995). PHYLIP, Phylogenetic Inference Package. University of Washington, Seattle, USA.

Forey PL, Littlewood DTJ, Ritchie P, Meyer A (1996). Interrelationships of elopomorph fishes. Interrelationships of Fishes. Academic Press, San Diego, pp. 175-191.

Gwo JC, Hsu TH, Lin KH, Chou YC (2008). Genetic relationship among four subspecies of cherry salmon (*Oncorhynchus masou*) inferred using AFLP. Mol. Phyl. Evol. 48: 776-781.

Hampl V, Pavlicek A, Flegr J (2001). Construction and bootstrap analysis of DNA fingerprinting-based phylogenetic trees with the freeware program FreeTree: application to trichomonad parasites. Int. J. Syst. Evol. Microbiol. 3: 731-735.

Hoese DF (1984). Gobioidei: relationships. *In*: Moser HG (Ed.), Ontogeny and Systematics of Fishes. Spec. Pub. Am. Soc. Ichthyol. Herpetol. No.1. Allen Press, Lawrence, Kansas, pp. 588-591.

Hoese DF, Gill AC (1993). Phylogenetic relationships of eleotrid fishes (Perciformes: Gobioidei). Bull. Mar. Sci. 52: 415-440

Koeleman JGM, Stoof J, Biesmans DJ (1998). Comparison of amplified ribosomal DNA restriction analysis, random amplified polymorphic DNA analysis, and AFLP fingerprinting for identification of *Acinetobacter* genomic species and typing of *Acinetobacter baumannii*. J. Clin. Microbiol. 36: 2522-2529

Kumar S, Tamura K, Jakobsen IB, Nei M (2001). MEGA2: molecular evolutionary genetics analysis software. Bioinformatics, 17: 1244-1245

Merril CR, Switzer RC, Van Keuren ML (1979). Trace polypeptides in cellular extracts and human body fluid detected by two-dimensional electrophoresis and a highly sensitive silver stain. Proc. Natl. Acad. Sci. 76: 4335-4339

Miller PJ (1973). The osteology and adaptive features of *Rhyacichthys aspro* (Teleostei: Gobioidei) and the classification of gobioid fishes. J. Zool. Lond. 171: 397-434

Miya M, Nishida M (1996). Molecular phylogenetic perspective on the evolution of the deep-sea fish genus *Cyclothone* (Stomiiformes: Gonostomatidae). Ichthyol. Res. 43: 375-398

Miya M, Nishida M (2000). Molecular systematics of the deep-sea fish genus *Gonostoma* (Stomiiformes: Gonostomatidae): two paraphyletic clades and resurrection of Sigmops. Copeia, 2: 378-389.

Mueller UG, Wolfenbarger LL (1999). AFLP genotyping and fingerprinting. Trends Ecol. Evol. 14: 389-394.

Murdy EO, Shibukawa K (2001). A revision of the gobiid fish genus *Odontamblyopus*. (Gobiidae: Amblyopinae). Ichthyol. Res. 48: 31-43.

Nelson JS (1994). Fishes of the World. John Wiley & Sons Inc., New York. 3rd edition, p. 600.

Nelson JS (2006). Fishes of the world. New York. 4d edition, p. 601.

Techaprasan J, Klinbunga S, Jenjittikul T (2008). Genetic relationships and species authentication of *Boesenbergia* (Zingiberaceae) in Thailand based on AFLP and SSCP analyses. Biochem. Syst. Ecol., 36: 408-416.

Thacker CE (2000). Phylogeny of the wormfishes (Teleostei: Gobioidei: Microdesmidae). Copeia, 4: 940-957.

Thacker CE (2003). Molecular phylogeny of the gobioid fishes (Teleostei: Perciformes: Gobioidei). Mol. Phyl. Evol., 26: 354-368

Thacker CE, Hardman MA (2005). Molecular phylogeny of basal gobioid

fishes: Rhyacichthyidae, Odontobutidae, Xenisthmidae, Eleotridae (Teleostei: Perciformes: Gobioidei). Mol. Phyl. Evol. 37: 858-871.
Vos P, Hogers R, Bleeker M, Reijans M, Van De Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995). AFLP: a new technique for DNA fingerprinting. Nucleic. Acids. Res. 23: 4407-4414.
Wang ZY, Jayasankar P, Khoo SK (2000). AFLP fingerprinting reveals genetic variability in common carp stocks from Indonesia. Asian Fish. Sci. 13: 139-147.
Winterbottom R, Emery AR (1981). A new genus and two new species of gobiid fishes (Perciformes) from the Chagos Archipelago, Central Indian Ocean. Environ. Biol. Fish. 6: 139-149.
Wolfe AD, Liston A (1998). Contributions of PCR based methods to plant systematics and evolutionary biology. *In*: Soltis DE, Soltis PS, Doyle JJ eds. Molecular Systematics of Plants II: DNA Sequencing. Kluwer, Dordrecht, pp. 43-86.
Wu HL (1987). Systematic Synopsis of Chinese Fishes. Cheng Q T, Zheng B S eds. Science Press, Beijing. pp. 429-436. (In Chinese).
Wu HL, Zhong JS (2008). Fauna Sinica, Ostichthyes, Perciformes (V), Gobioidei. Science Press, Beijing. (In Chinese).
Xu F, Sun M (2001). Comparative analysis of phylogenetic relationships of grain amaranths and their wild relatives (Amaranthus; Amaranthaceae) using internal transcribed spacer, amplified fragment length polymorphism, and double-primer fluorescent intersimple sequence repeat markers. Mol. Phyl. Evol. 21:372-387
Yan G, Romero-Severson J, Walton M, Chadee DD, Severson DW (1999). Population genetics of the yellow fever mosquito in Trinidad: comparisons of amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) markers. Mol. Ecol. 8: 951-963
Yang QL, Han ZQ, Sun DR, Xie SG, Lin LS, Gao TX (2010). Genetics and phylogeny of genus *Coilia* in China based on AFLP markers. Chin. J. Oceanol. Limnol. 28: 795-801.
Yeh FC, Yang R, Boyle T (1999). POPGENE. Version 1.31. Microsoft Window-based Freeware for Population Genetic Analysis, University of Alberta. Edmonton, AB, Canada.
Zhang JB, Liu X (2006). The phylogenetic relationship of the family Lutjanidae based on analyses of AFLP and mitochondrial 12S rRNA sequences. Chin. Sci. Bull. 51: 143-148
Zhang LB, Comes HP, Kadereit JW (2001). Phylogeny and quaternary history of the European montane/alpine endemic Soldanella (Primulaceae) based on ITS and AFLP variation. Am. J. Bot. 88: 2331-2345.
Zhu YD, Wu HL (1963). Fishes of East China Sea. Chu YT (ed.), Pisces: Gobiidae. Science Press, Beijing. pp. 433-455. (In Chinese).