Korean J. Ichthyol. 19(4), 274~285, 2007

Species Identification and Molecular Phylogenetic Position of Korean Damselfishes (Pomacentridae: Chrominae) Based on DNA Bioinformation

Jeong Rack Koh* and Yung Chul Park[1]

Fisheries Resources Restoration Development and Management Center,
National Fisheries Research & Development Institute (NFRDI), Gijang-gun, Busan, Korea
[1]Research Institute of EcoScience, Ewha Womans University,
Seodaemun-gu Daehyun-dong 11-1, Seoul 120-750, Korea

The subfamily Chrominae of damselfishes (Teleostei: Pomacentridae) includes the genus *Chromis* and *Dascyllus*. They are found throughout the tropical oceans and form a major component of coral reef communities. There are 5 species of the Chrominae currently recognized in Korea. This study was conducted to infer phylogenetic position of two Korean *Chromis* species and one *Dascyllus* species within general category of their each genus in worldwide level. This study also includes one species of Japanese *Dascyllus*. In the phylogenetic analysis, the Japanese *D. aruanus* grouped with *D. aruanus* previously reported from French Polynesia. Korean *Chromis fumea* grouped with Australian *C. nitida* and the *p*-distance value between the two species is relatively very low (0.047). Korean *C. notatus* grouped together with *C. flavomaculata* (New Caledonia). In the sequence analysis of some Korean and Japanese damselfishes, there was no sequence variation between *D. melanurus* (Jeju, Korea) and *D. melanurus* (Indo-Pacific), but the sequences of the two populations were different in only one nucleotide sites from that of *D. melanurus* in Indonesian Archipelago. The sequences of *Dascyllus aruanus* (Japan) were different in two nucleotide sites from it in French Polynesia. There were high difference between the sequences of two Korean species, *Chromis fumea* and Korean *C. notatus*. The variations among mitochondrial cytochrome *b* sequences indicate that the gene sequence could be used as DNA barcode for identification of local populations of *D. aruaus* and *D. melanurus* as well as species level.

Key words : damselfishes, Chrominae, *Chromis, Dascyllus, Chromis notatus, Chromis fumea*, Pomacentridae

Introduction

Coral reef fishes are well known for their high species diversity. On many reef habitats, different families or guilds are represented by as many as 20～50 different species of the wrasse (Labridae), groupers (Serranidae) and dam-selfishes (Pomacentridae). Many species of these families seem to be similar in appearance to each other and frequently there is considerable apparent overlap in general habitats and foods (Randall, 1967; Hobson, 1974).

Of the coral reef fishes, damselfishes (Teleostei: Pomacentridae) are a diverse and widespread family. They are found throughout the tropical oceans and form a major component of coral reef communities (Allen, 1975), but some species are also found in temperate regions (Allen, 1989).

*Corresponding author: jrkoh0702@hanmail.net

Since most of the pomacentrid species live in coral reefs and tidal zones, their habitats are usually located in shallow water (within 20 m below the water surface) of near shore, though several species live in deeper zones than 100 m (Allen, 1975).

Pomacentridae is currently recognized as a member of the perciform suborder Labroidei (Nelson, 1994) including the families Cichlidae, Embiotocidae, and Labridae (Kaufman and Liem, 1982; Stiassny and Jensen, 1987), but the monophyly of this suborder has been questioned by recent molecular studies (Rosen and Patterson, 1990; Johnson, 1993; Streelman and Karl, 1997). There are approximately 350 pomacentrid species recognized in worldwide and they belong to 29 genera of the following four subfamilies: Amphiprioninae, Chrominae, Lepidozyginae, and Pomacentrinae (Allen, 1975, 1991). The majority of pomacentrid diversity is dependant on two subfa-milies of the Chrominae (over 80 species) and Pomacentrinae (over 200 species).

The species relationships within the subfamily Chrominae have ever been inferred in the recent studies conduced to understand phylogenetic relationship of pomacentrid fishes (Tang, 2001; Jang-Liaw *et al.*, 2002; Quenouille *et al.*, 2004). The subfamily Chrominae includes four genera of *Chromis, Dascyllus, Acanthochromis* and *Mecaenichthys,* and its monophyly has been supported to date based on morphological characters (Allen, 1975). Since the recent analyses of the molecular dataset (Tang, 2001; Jang-Liaw *et al.*, 2002; Quenouille *et al.*, 2004) indicated that the genera *Mecaenichthys* and *Acanthochromis,* two putative members of Chrominae, were posited within the subfamily Pomacentrinae, their monophyly appeared to be doubt. Within the subfamily Chrominae, there was strong support for the monophyly of the clades of *Chromis* and *Dascyllus,* and the genus *Chromis* within the clade was found to be paraphyletic relative to the genus *Dascyllus*.

In Korea, the phylogenetic relationship of Korean Chrominae has been poorly known to date. In Korea, 16 species of Pomacentridae have been described to date, 5 species of them belong to the subfamily Chrominae (*C. notatus, C. analis,* and *C. fumea* of the genus *Chromis* and *D. trimaculatus* and *D. melanurus* of the genus *Dascyllys*) (Koh *et al.*, 1997; Kim *et al.*, 2005). Recent accumulation of molecular data provides an opportunity to infer phylogenetic relationship of Korean Chrominae in a broader view, extending beyond regional fauna.

Aim of this study is to infer the phylogenetic position of three Chrominae species of two genera (*C. notatus, C. fumea* and *D. melanurus*) and one species of Japanese *Dascyllus*. We sequenced mitochondrial cytochrome *b* sequences from the Chrominae species, and to infer the phylogenetic position of Korean species in world wide point of view we combined our sequences with ones previously reported from various regions. National consortium for construction of DNA barcode system has been increasing importance in the identification of organism (Larson, 2007). DNA barcode is short sequences that could be used for identification of organisms in various levels like individuals, populations and species. We discuss utility of cytochrome *b* sequences as DNA barcode for identification of local populations and species.

Materials and Methods

1. Taxon collection and DNA extraction

We collected two species of the genus *Chromis* (*C. fumea* and *C. notatus*) and one species of the genus *Dascyllus* (*D. melanurus*) from the costal water of Jeju-do (island) (Table 1). Two individuals from each species were used for DNA extractions. Tissues for DNA extractions were severed from bodies of samples preserved in 100% ethanol, and then were dried on a heating block several times to remove excess ethanol. The genomic DNA was extracted using the chloroform with some modifications of the methods in Park *et al.* (2004). After vortexing for 1 min, body tissues were washed in 1.5 mL microcentrifuge tube containing 500 μL of cold (4˚C) 5% citric acid. After centrifugation at 8,000 rpm for 5 min, the tissues were homogenized with a pair of dissecting scissor in 1.5 mL microcentrifuge tube containing 500 μL of lysis buffer (100 mM Tris-HCl, 5 mM EDTA, 0.2% SDS, 200 mM NaCl) and 50 μL of proteinase K. The mixtures were digested overnight at 56˚C. Total genomic DNA was extracted with an equal volume of chloroform, precipitated with an equal volume of isoprophyl alcohol (isopropanol), and dissolved in 40 μL of distillized water.

2. DNA amplification, purification and sequencing

The fragments of mitochondrial cytochrome *b*

sequences were amplified using polymerase Chain Reaction (PCR). Primer sequences for the amplifications of the gene sequence are as follows: GULDG (5′-tgacttgaaraaccaycgttg-3′) (Palumbi, 1996) and H16460 (5′-cgaycttcggattacaagaccg-3′) (Quenouille *et al.*, 2004). The polymerase chain reaction was carried out in 20 μL volumes, using 2.0 μL of 1 to 10 dilution of genomic DNA as template. The PCR reactions included 2.0 μL of 5 mM each primer, 2.0 μL of dNTPs at 10 mM, 2.0 μL of 25 mM $MgCl_2$, 2.0 μL of 10X buffer and 1 units of Taq Polymerase. The temperature profile for amplifying the cytochrome *b* sequences was as follows: a denaturation at 94°C for 10 min, and 35 cycles of 94°C for 30s, 48～52°C for 40s, and 72°C for 40s followed by a final extension of 72°C for 2 min. PCR products were purified using UltraClean PCR Clean-up Kit (MO BIO Laboratories, Inc.), and sequenced in Bionics in Korea (http://www. bionicsro.co.kr/).

3. Data analysis

Sequences were aligned using the Clustal X program package (Thompson *et al.*, 1997) and manually edited using SeAl 1.0a (Rambaut, 1996). We sequenced the size of 517 bp in *Dascyllus melanurus* and *D. aruanus* and 642 in two *Chromis* species, and the sequences of the Korean taxa were aligned together with 37 previously

Table 1. Species used in this study, with collection locality, GenBank accession numbers for sequences and references

Species	Collection locality	Accession No.	References
*Dascyllus aruanus**	Japan	EU267222	Current study
D. aruanus	French Polynesia	AF119399	Bernardi and Crane, 1999
*D. melanurus**	Jeju Island, Korea	EU267221	Current study
D. melanurus	Indonesian Archipelago	AF119395	Bernardi and Crane, 1999
D. melanurus	Indo-Pacific	AF097924	Elliott *et al.*, 1999
D. flavicaudus	French Polynesia	AF119394	Bernardi and Crane, 1999
D. albisella	Kona, Hawaii	AF119396	Bernardi and Crane, 1999
D. carneus	Indian Ocean	AF119397	Bernardi and Crane, 1999
D. marginatus	Red Sea	AF119398	Bernardi and Crane, 1999
D. reticulatus	Western Pacific	AF119400	Bernardi and Crane, 1999
D. trimaculatus	French Polynesia	AF119393	Bernardi and Crane, 1999
D. trimaculatus	Maldives	AY208545	Quenouille *et al.*, 2004
*Chromis fumea**	Jeju Island, Korea	EU267219	Current study
*C. notatus**	Jeju Island, Korea	EU267220	Current study
C. agilis	New Caledonia	AY208522	Quenouille *et al.*, 2004
C. amboinensis	New Caledonia	AY208523	Quenouille *et al.*, 2004
C. atrilobata	Panama	AY208524	Quenouille *et al.*, 2004
C. atripectoralis	Australia	AY208525	Quenouille *et al.*, 2004
C. atripes	New Caledonia	AY208526	Quenouille *et al.*, 2004
C. chromis	France	AY208527	Quenouille *et al.*, 2004
C. chromis	Orbetello, Italy	AF119392	Bernardi and Crane, 1999
C. chrysura	New Caledonia	AY208528	Quenouille *et al.*, 2004
C. cyanea	Jamaica	AY208529	Quenouille *et al.*, 2004
C. flavomaculata	New Caledonia	AY208530	Quenouille *et al.*, 2004
C. iomelas	French Polynesia	AY208531	Quenouille *et al.*, 2004
C. margaritifer	New Caledonia	AY208532	Quenouille *et al.*, 2004
C. multilineata	Jamaica	AY208533	Quenouille *et al.*, 2004
C. nitida	Australia	AY208534	Quenouille *et al.*, 2004
C. retrofasciata	New Caledonia	AY208535	Quenouille *et al.*, 2004
C. viridis	Japan	AY208536	Quenouille *et al.*, 2004
C. weberi	Australia	AY208537	Quenouille *et al.*, 2004
C. xanthopterygia	Gulf of Oman	AY208538	Quenouille *et al.*, 2004
C. caudalis	Republic of Palau	AY289557	Johns and Somero, 2004
C. punctipinnis	California, USA	AY289559	Johns and Somero, 2004
C. xanthochira	Republic of Palau	AY289561	Johns and Somero, 2004
Abudefduf sordidus	Ascension Isl.	AY208556	Quenouille *et al.*, 2004
A. taurus	Panama	AY208559	Quenouille *et al.*, 2004

*Species marked with an asterisk are to be sequenced for this study.

published sequences of the Chrominae from other regions (Table 1). The sequence alignment of 37 individuals including two outgroup species generated a dataset of 642 bp in length.

We used maximum parsimony (MP) and NJ analyses and Bayesian analysis, to infer the phylogenetic positions of the Korean Chrominae within the subfamily Chrominae in worldwide. We performed MP and NJ analyses implemented in PAUP4.0* version beta 10 (Swofford, 2002) Bayesian analysis was performed by using MrBayes version 3.0b4 (Huelsenbeck and Ronquist, 2001). Neighbor-joining analyses was also conducted for calculating pairwise genetic distance (*p*-distance). The *p*-distances were corrected using the model obtained from the model test. NJ trees were produced under the model obtained from the model test, and topological reliability of the NJ trees was explored using 1,000 pseudoreplicates.

In MP analyses, topological space was explored using a heuristic search, with 10 random sequence additions holding 5 trees at each step, and with TBR branch swapping. Bootstrap support was calculated using 1,000 pseudoreplicates with the same searching protocol as for the heuristic search.

We determined the most appropriate substitutional model using a series of hierarchical likelihood ratio tests implemented with Model Test 3.06 (Posada and Crandall, 1998). Bayesian analysis was performed to estimate under the best-fit model determined by Model test. In the analysis of the dataset using Mr. Bayes v. 3.04b, Monte Carlo Markov chains were run for 200,000 generations. Trees were sampled every 100 generations where the Markov chain reached stationarity, leaving 5,000 trees for analysis.

Results

1. Phylogenetic analyses

The MP and Bayesian analyses were conducted for the combined dataset of the two generea. The tree topologies did not differ in both the analyses (Fig. 1 and 2). For the dataset, Model test 3.06 identified a likelihood setting from best-fit model <GTR+I+G>. Likelihood details identified by the Model test are the following: Base=0.2763 (A), 0.3821 (C), 0.1241 (G), 0.21740 (T); Nst=6 (GTR); Rmat=0.7682, 8.5588, 1.0136, 0.3319, 8.7650; Rates=gamma (shape=1.9147); Pinvar=0.5991. We used the MrBayes default settings to establish the initial heating values for four Markov chains, and default settings were also used to initially parameterize the GTR model. The four differentially heated Markov chains were initiated from random trees, run simultaneously, and were sampled every 100 cycles. Preliminary runs were performed to monitor the fluctuating value of the likelihoods of the Bayesian trees, and we found that stationarity was consistently observed before 5,000 generations for the dataset. The Markov chain analyses used to infer Pomacentridae phylogenies were run for 200,000 cycles for the dataset. All sampled trees preceding stationarity were discarded ("burnin"=5,000), and the remaining tree samples were used to generate a 50% majority rule consensus tree (=1,501 trees). The posterior probability of each

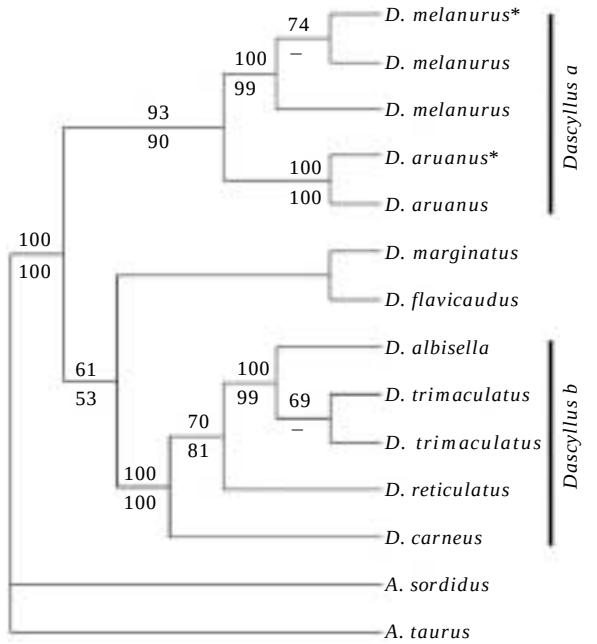

Fig. 1. The tree topologies of the genus *Dascyllus* derived from MP and NJ analyses. Since the topologies of MP and NJ trees are similar, only MP trees are shown. Numbers above/below branches indicate MP/NJ bootstrap values, respectively. In the MP analysis of the genus *Dascyllus*, a most single parsimonious tree was produced (Tree length=344, CI: 0.695, RI: 0.756, RC: 0.525, HI: 0.305). Of 642 characters, 454 characters were constant, and 141 characters were parsimony-informative characters. The species marked with asterisk (*) were sequenced in this study.

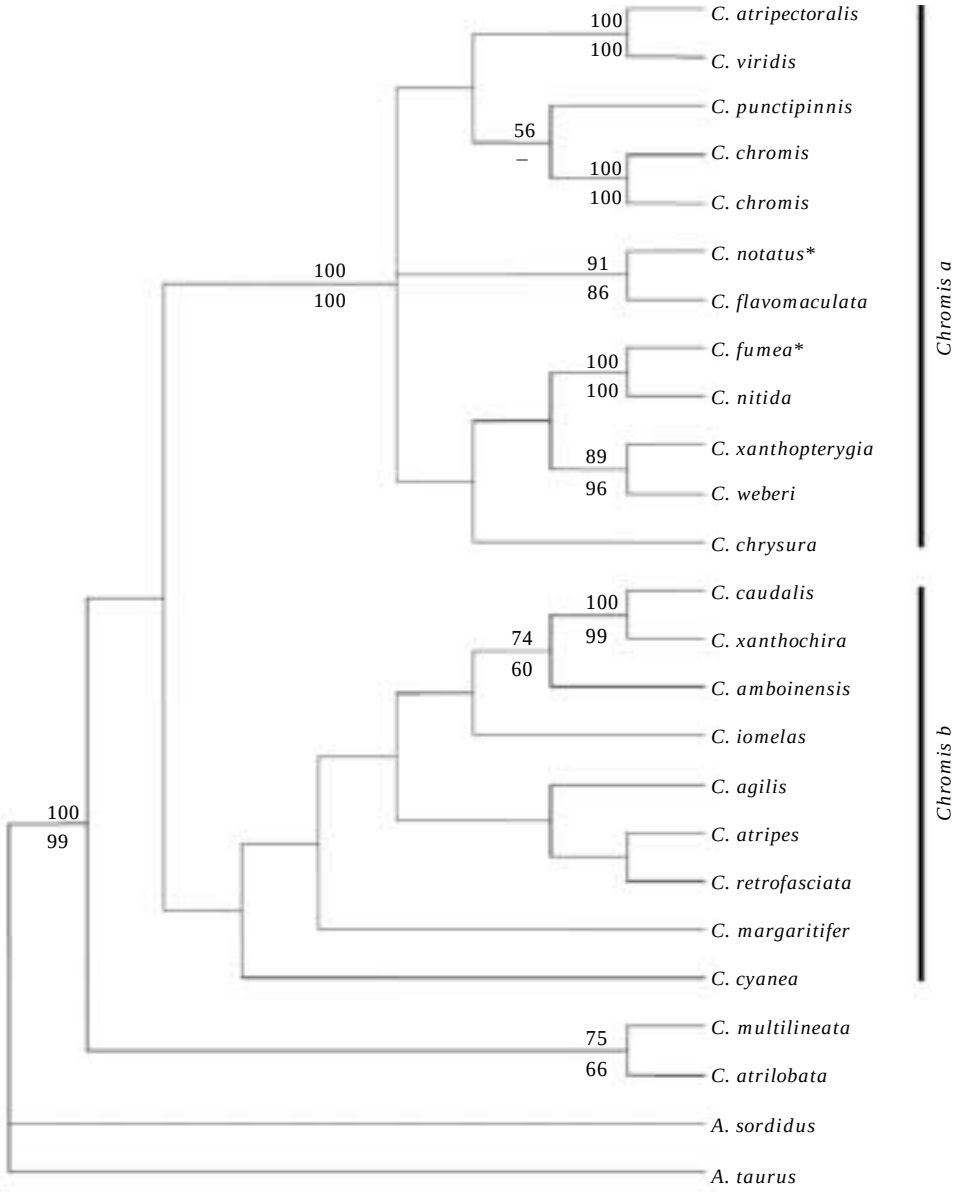

Fig. 2. The tree topologies of the genus *Chromis* derived from MP and NJ analyses. Since the topologies of MP and NJ trees are similar, only MP trees are shown in each genus. Numbers above/below branches indicate MP/NJ bootstrap values, respectively. The MP analysis of the genus *Chromis* produced stric consensus of three equally most-parsimonious trees resulting from an equally weighted analysis of the cytochrome b sequences (Tree length=884; CI: 0.399, RI: 0.516, RC: 0.206, HI: 0.601). In MP analysis, 405 of 642 characters were constant, and number of parsimony-informative characters was 206. The species marked with asterisk (*) were sequenced in this study.

clade is provided by the percentage of trees identifying the clade and these are true probabilities given the assumptions of the GTR model. The 95% credibility intervals (CI) for the nucleotide substitution were calculated from the trees sampled after the Markov chain analysis reached stationarity.

2. Phylogenetic position of Korean *Dascyllus* species

Species of the genus *Dascyllus* grouped together well by the MP and NJ analyses (100%/ 100% in MP and NJ, respectively), but within the group, the species formed two main clades (*Dascyllus a* and *b*: Fig. 1). *Dascyllus melanurus* formed a sister to *D. aruanus* clades with high supporting

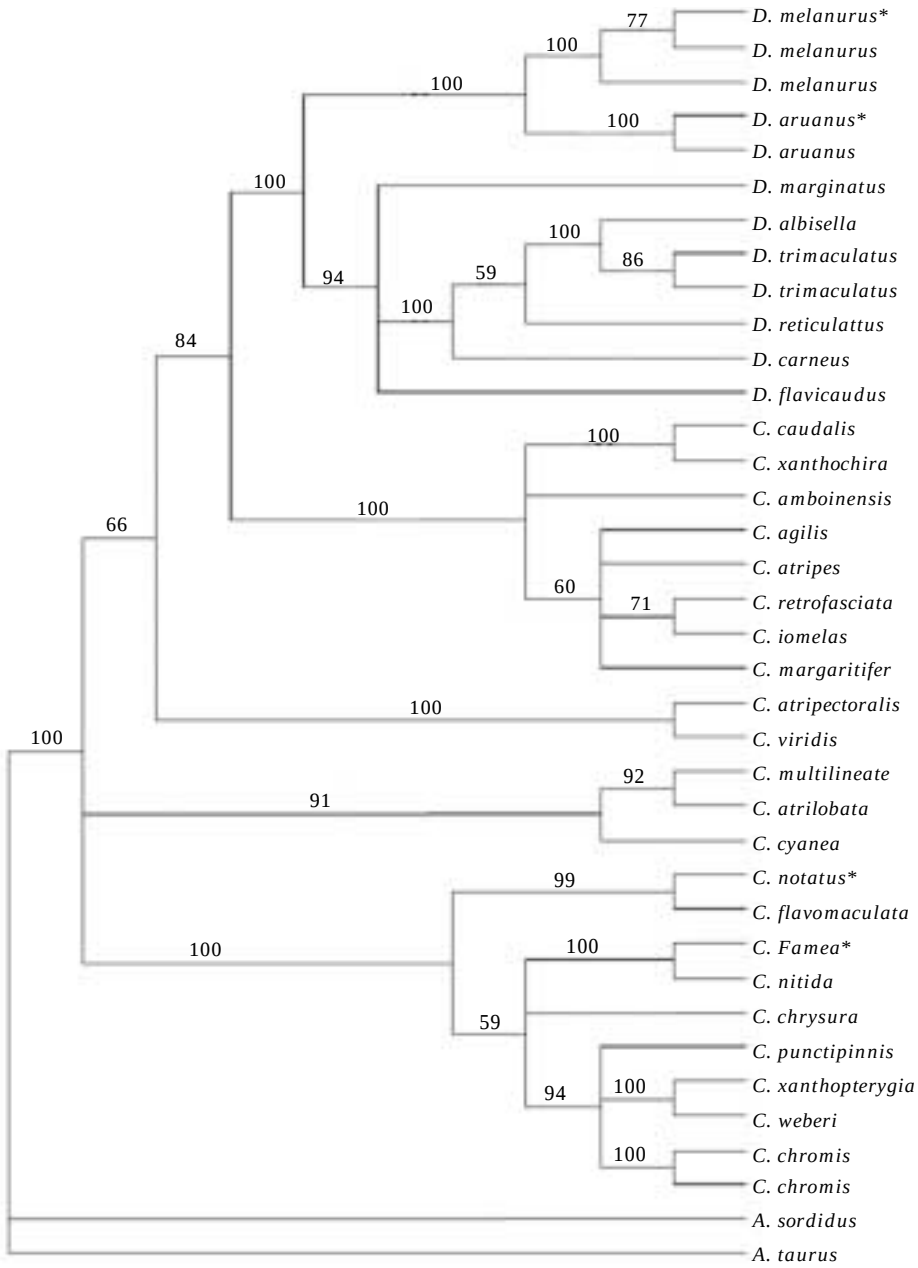

Fig. 3. The 50% majority-rule consensus tree from the Bayesian analysis of the dataset of the cytochrome *b* sequences representing *Chromis* and *Dascyllus*. The Bayesian tree was derived from the consensus topology of 1,500 trees sampled from Bayesian analysis assuming GTR model. All sampled trees preceding stationarity were discarded ("burnin"=5,000), and the remaining tree samples were used to generate a 50% majority rule consensus tree (=1,501 trees). The posterior probability of each clade is provided by the percentage of trees identifying the clade and these are true probabilities given the assumptions of the GTR model (Huelsenbeck and Ronquist, 2001). Numbers on the branches indicate posterior probabilities (PPs) from a consensus a consensus tree of all post burn-in topologies visited by the Markov chain. The species marked with an asterisk (*) were sequenced in this study.

value (93%). The Korean *D. melanurus* grouped well with *D. melanurus* (Indonesian Archipelago). Sequence analysis revealed that there is no sequence variation between the two individuals. Japanese *D. aruanus*, which was newly added in this study, also grouped with *D. aruanus* from French Polynesia.

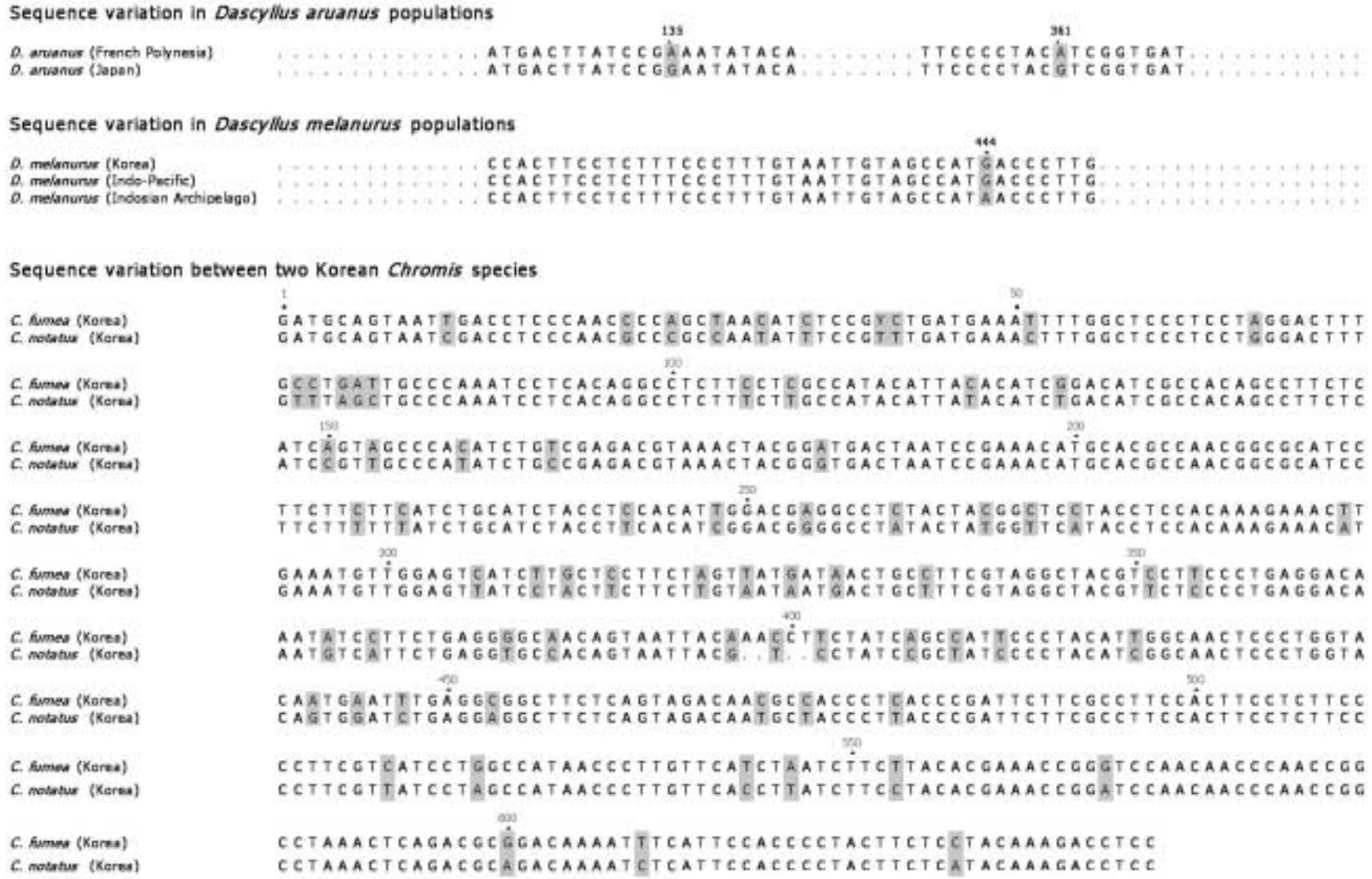

Fig. 4. Nucleotide variation in sequence alignments of *Dascyllus aruanus* individuals, *Dascyllus melanurus* individuals and two Korean *Chromis* species, *C. fumea* (Jeju) and *C. notatus* (Jeju).

Table 2. Pairwise genetic distance among the genus *Chromis* species

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	*C. atripectoralis*	–																								
2	*C. caudalis*	0.351	–																							
3	*C. multilineata*	0.305	0.280	–																						
4	*C. cyanea*	0.284	0.232	0.166	–																					
5	*C. amboinensis*	0.321	0.076	0.273	0.248	–																				
6	*C. notatus*	0.296	0.306	0.212	0.204	0.306	–																			
7	*C. fumea*	0.241	0.253	0.217	0.211	0.288	0.175	–																		
8	*C. agilis*	0.387	0.126	0.334	0.247	0.091	0.293	0.286	–																	
9	*C. atripes*	0.339	0.110	0.282	0.268	0.095	0.322	0.309	0.092	–																
10	*C. chrysura*	0.269	0.263	0.206	0.172	0.265	0.126	0.118	0.260	0.255	–															
11	*C. punctipinnis*	0.357	0.404	0.325	0.309	0.417	0.189	0.296	0.451	0.480	0.216	–														
12	*C. retrofasciata*	0.304	0.155	0.323	0.307	0.127	0.383	0.303	0.129	0.106	0.290	0.493	–													
13	*C. iomelas*	0.358	0.115	0.300	0.256	0.095	0.337	0.323	0.115	0.107	0.282	0.467	0.118	–												
14	*C. nitida*	0.297	0.333	0.275	0.241	0.384	0.199	0.047	0.336	0.373	0.161	0.314	0.335	0.370	–											
15	*C. xanthopterygia*	0.309	0.389	0.270	0.246	0.425	0.214	0.170	0.372	0.422	0.170	0.226	0.372	0.372	0.188	–										
16	*C. margaritifer*	0.328	0.130	0.316	0.284	0.133	0.288	0.262	0.115	0.123	0.259	0.431	0.127	0.117	0.306	0.403	–									
17	*C. atrilobata*	0.268	0.258	0.116	0.176	0.245	0.182	0.212	0.297	0.282	0.161	0.298	0.274	0.270	0.269	0.234	0.266	–								
18	*C. chromis*	0.328	0.385	0.291	0.246	0.358	0.235	0.240	0.333	0.344	0.185	0.216	0.387	0.387	0.303	0.221	0.340	0.272	–							
19	*C. weberi*	0.264	0.330	0.221	0.223	0.368	0.184	0.148	0.328	0.332	0.141	0.177	0.373	0.351	0.176	0.073	0.322	0.209	0.177	–						
20	*C. chromis*	0.314	0.385	0.285	0.244	0.360	0.230	0.215	0.326	0.350	0.190	0.216	0.393	0.398	0.273	0.213	0.354	0.281	0.014	0.171	–					
21	*C. viridis*	0.130	0.348	0.296	0.268	0.354	0.268	0.262	0.380	0.339	0.265	0.337	0.310	0.353	0.330	0.362	0.326	0.232	0.274	0.301	0.275	–				
22	*C. flavomaculata*	0.245	0.281	0.168	0.194	0.298	0.072	0.154	0.338	0.319	0.115	0.193	0.346	0.342	0.193	0.191	0.283	0.157	0.202	0.158	0.193	0.246	–			
23	*C. xanthochira*	0.362	0.009	0.291	0.226	0.078	0.322	0.277	0.135	0.121	0.282	0.422	0.160	0.126	0.361	0.407	0.134	0.251	0.409	0.348	0.409	0.374	0.299	–		
24	*A. sordidus*	0.387	0.493	0.258	0.365	0.406	0.298	0.363	0.458	0.375	0.267	0.330	0.477	0.451	0.404	0.472	0.480	0.262	0.363	0.384	0.339	0.392	0.253	0.520	–	
25	*A. taurus*	0.443	0.448	0.278	0.319	0.430	0.304	0.345	0.410	0.392	0.272	0.342	0.419	0.382	0.372	0.348	0.439	0.283	0.350	0.341	0.356	0.430	0.273	0.480	0.197	–

Thep-distances values were corrected using the likelihood model (GTR model) obtained from the model test.

3. Phylogenetic position of Korean *Chromis* species

The MP and NJ analyses showed that the genus *Chromis* is paraphyletic (Fig. 2) and the two-main clades occurred (*Chromis a* and *b*: Fig. 2). Korean *Chromis* species belong to *Chromis b* group. Some terminal branches were supported by high bootstrap values, but most of the branches were collapsed. Korean *C. fumea* grouped with *C. nitida*, supported by high bootstrap values (100%/100%). The *p*-distance value between the two species is relatively very low (0.047) (Table 2). Korean *C. notatus* and New Caledonian *C. flavomaculata* also grouped together, with relatively high supporting values as well.

Phylogenetic relationship was inferred from the combined dataset of the genus *Dascyllus* and *Chromis* by the Bayesian analysis (Fig. 3). The Bayesian analysis based on the combined dataset derived a little higher supporting values of each node than the previous analysis. The monophyly of *Dascyllus* was supported strongly (BP=100%) and two main clades also still occurred within the genus. The genus *Dascyllus* formed a sister clade to *Chromis b* group except *C. cyanea*. As in MP and NJ analyses, *C. notatus* was a sister to *C. flavomaculata* (BP=99%) and *C. fumea* was a sister to *C. nitida* (BP=100).

4. Cytochrome *b* sequence-based species identification

Sequence analysis was conducted for utility of cytochrome *b* as DNA barcode at eastern Asian local population level or species level (Fig. 4). There was no sequence variation between *D. melanurus* (Jeju, Korea) and *D. melanurus* (Indo-Pacific), but the sequences of the two populations were different in only one nucleotide sites from that of *D. melanurus* in Indonesian Archipelago. According to sequence identity matrix, the sequence identity between *D. aruanus* individuals of Korea and Indonesian Archipelago was 0.998 (sequence length=517 nucleotides).

The sequences of *Dascyllus aruanus* (Japan) were different in two nucleotide sites from it in French Polynesia. The sequence identity between *D. aruanus* individuals of the two localities was 0.996 (sequence length=593 nucleotides).

There was high variation between the sequences of two Korean species, *Chromis fumea* and Korean *C. notatus*. The sequence identity of the two species was 0.888 (sequence length=643 nucleotides).

Discussion

This study was conducted to investigate phylogenetic position of two Korean *Chromis* species and one *Dascyllus* species within general category of their each genus. Monophyly of the subfamily Chrominae was not supported in the previous studies based on molecular datasets. For example, the genus *Acanthochromis* (*A. polyacanthus*) formed a sister clade to the genus *Neoglyphidodon* (Pomacentrinae) (Jang-Liaw *et al.*, 2002; Quenouille *et al.*, 2004), or *Neoglyphidodon* and *Amblyglyphidodon* (Pomacentrinae) (Jang-Liaw *et al.*, 2002). Phylogenetic position of *Acanthochromis* was placed in ingroup of *Chrysiptera* (Pomacentrinae) (Jang-Liaw *et al.*, 2002; Quenouille *et al.*, 2004). In case of *Mecaenichthys*, its phylogenetic position was more basal than the other genera of the subfamily Chrominae, and placed within the subfamily Pomacentrinae as well (Jang-Liaw *et al.*, 2002). This study includes all of the published sequences of the genus *Chromis* and genus *Dascyllus*, with the exception of the two genera above.

The damselfish genus *Dascyllus* (Pomacentridae) comprises 10 species restricted to the coral reefs in the Indo-West Pacific (Randall and Allen, 1977; Randall and Randall, 2001), and seven species of which occur at islands of Oceania. Our MP and NJ analyses showed well-grouping of the genus *Dascyllus* species, but within the group, the species formed two main clades of *Dascyllus a* and *b* (Fig. 1). *Dascyllus aruanus* is widespread throughout the Indo-West Pacific, while *D. melanurus* has a range embedded in the *D. aruanus* range, within the Indonesian Archipelago and south to the Great Barrier Reef. Thus, the grouping of the two species is in agreement with the overlapping habitat distribution of the two species (Planes *et al.*, 1993).

In Korea, two species of *Dascyllus* (*D. trimaculatus* and *D. melanurus*) have been reported to date (Koh *et al.*, 1997; Kim *et al.*, 2005). The Korean *D. melanurus* used in this study grouped well with *D. melanurus* (Indonesian Archipelago). Japanese *D. aruanus*, which was newly added in this study, also grouped with *D. aruanus* previously reported from French Polynesia (Bernardi and Crane, 1999). *Dascyllus trimaculatus*, *D. albisella* and *D. reticulates*, which have

adjacent ranges (Godwin, 1995; Bernardi and Crane, 1999), grouped together and is a sister group to *D. carneus*.

There are about 80 *Chromis* species in worldwide (Allen, 1991), 23 individual of 22 *Chromis* species were used in this study (Table 1). In Korea, three *Chromis* species (*C. notatus*, *C. analis*, and *C. fumea*) occur in the sea near Jeju Island (Koh *et al.*, 1997; Kim *et al.*, 2005). Of the two Korean species used in this study, *C. fumea* grouped with *C. nitida* and *C. notatus* grouped with *C. flavomaculata*.

Interestingly, *p*-distance value between *C. xanthochira* and *C. caudalis* was very low (0.009), and the *p*-value was even lower than that (0.014) between individuals of *C. chromis* (Table 2). Thus, the taxonomic review appears to be required between the two species. Most of the branches were collapsed in the analysis of the combined dataset of the genus *Chromis* (Fig. 2), probably because the dataset includes only 25% of total species comprising the genus *Chromis*.

We investigated the utility of cytochrome *b* sequences as DNA barcode at eastern Asian local population level or species level. DNA barcode is a new technique for organism identification at various levels, like individuals, local populations and species. It uses a short DNA sequence from a standardized and agreed-upon position in the genome as a molecular diagnostic for organism identification with quick and easy methods. The variations among mitochondrial cytochrome *b* sequences indicate that the gene sequence could be used as DNA barcode for identification of local populations as well as species level. Sequence size from nucleotide site 100 to 500 was recommended for detection of Japanese *D. aruanus* and Korean *D. melanurus* (Fig. 4).

Acknowledgements

This work was supported by NFRDI (National Fisheries Research and Development Institute). The work was also supported by Ewha Womans University Research Grant 2007.

References

Kim, I.S., Y. Choi, C.L. Lee, Y.J. Lee and B.J. Kim. 2005. Illustrated book of Korean fishes. Kyo Hak Pub. Co., Seoul. pp. 615 (In Korean).

Allen, G.R. 1975. Damselfishes of the South Seas. T. F. H. Publications, Neptune City, NJ.

Allen, G.R. 1991. Damselfishes of the World. Mergus Publishers, Germany.

Bernardi, G. and N.L. Crane. 1999. Molecular phylogeny of the Humbug Damselfishes (*Dascyllus*, Pomacentridae) inferred from mtDNA sequences. J. Fish Biol., 54 : 1210～1217.

Elliott, J.K., S.C. Lougheed, B. Bateman, L.K. McPhee and P.T. Boag. 1999. Molecular phylogenetic evidence for the evolution of specialization in anemonefishes. Proc. R. Soc., Lond., 266 : 677～685.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol., 17 : 368～376.

Godwin, J. 1995. Phylogenetic and habitat influences on the mating system structure in the humbug damselfishes (*Dascyllus*, Pomacentridae). Bull. Marine Sci., 57 : 637～652.

Hasegawa, M., H. Kishino and T. Yano. 1985. Dating the humanape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol., 22 : 160～174.

Hobson, E.S. 1974. Feeding relationships of teleostean fishes on coral reefs in Kona. Hawaii Fish Bull., 72 : 915～1031.

Huelsenbeck, J.P. and F. Ronquist. 2001. MRBAYES. Bayesian inference of phylogeny. Bioinformatics, 17 : 754～755.

Jang-Liaw, N.H., K.L. Tang, C.F. Hui and K.T. Shao. 2002. Molecular phylogeny of 48 species of damselfishes (Perciformes: Pomacentridae) using 12S mtDNA sequences. Mol. Phylogenet. Evol., 25 : 445～454.

Johns, G.C and G.N. Somero. 2004. Evolutionary Convergence in Adaptation of Proteins to Temperature: A4-Lactate Dehydrogenases of Pacific Damselfishes (*Chromis* spp.) Mol. Biol. Evol., 21 : 314～320.

Johnson, G.D. 1993. Percomorph phylogeny: progress and problems. Bull. Mar. Sci., 52 : 3～28.

Jukes, T.H. and C.R. Cantor. 1969. Evolution of protein molecules. In: Munro HM ed. Mammalian protein metabolism. New York: Academic Press, 21～132.

Kaufman, L.S. and K.F. Liem. 1982. Fishes of the suborder Labroidei (Pisces: Perciformes): phylogeny, ecology, and evolutionary significance. Breviora, 472 : 1～19.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol., 16 : 111～120.

Koh, J.R., J.G. Myung and Y.U. Kim. 1997. Morphological study on the fishes of the Family Pomacentridae. I. A taxonomical revision of the Family Pomacentridae (Pisces; Perciformes) from Korea. Korean J. Syst. Zoo., 13 : 173～192.

Larson, B.M.H. 2007. DNA barcoding: the social frontier. Frontiers in Ecology and the Environment, 5 : 8～437.

Nelson, J.S. 1994. Fishes of the World, third ed. Wiley, New York.

Palumbi, S.R. 1996. Nucleic acids II: the polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK ed. Molecular Systematics. MA: Sinauer Associates, 205～247.

Park, Y.C., K. Maekawa, T. Matsumoto, R. Santoni and

J.C. Choe. 2004. Molecular phylogeny and biogeography of the Korean woodroaches *Cryptocercus* spp. Mol. Phylogenet. Evol., 30 : 450～464.

Quenouille, B., E. Bermingham and S. Planes. 2004. Molecular systematics of the damselfishes (Teleostei: Pomacentridae): Bayesian phylogenetic analyses of mitochondrial and nuclear DNA sequences. Mol. Phylogenet. Evol., 31 : 66～88.

Randall, J.E. 1967. Food habits of reef fishes of the West Indies. Stud. Trop. Oceanog., 5 : 655～847.

Planes, S., F. Bonhomme and R. Galzin. 1993. Genetic structure of *Dascyllus aruanus* populations in French Polynesia. Marine Biol., 117 : 665～674.

Rosen, D.E. and C. Patterson. 1990. On Muller's and Cuvier's concepts of pharyngognath and labyrinth fishes and the classification of percomorph fishes, with an atlas of percomporph dorsal gill arches. Am. Mus. Nov., 2983 : 1～57.

Posada, D. and K.A. Crandall. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics, 14 : 817～818.

Rambaut, A. 1996. Se-Al: Sequence Alignment Editor. Available at http://evolve.zoo.ox.ac.uk/

Randall, H.A. and G.R. Allen. 1977. A revision of the damselfish genus *Dascyllus* (Pomacentridae) with description of a new species. Res. Austral. Mus., 31 : 349～385.

Randall, J.E. and H.A. Randall. 2001, *Dascyllus auripinnis*, a New Pomacentrid Fish from Atolls of the Central Pacific Ocean. Zool. Stud., 40 : 61～67.

Stiassny, M.L.J. and J.S. Jensen. 1987. Labroid interrelationships revisited: morphological complexity, key innovations, and the study of comparative diversity. Bull. Mus. Comp. Zool., 151 : 269～319.

Streelman, J.T. and S.A. Karl. 1997. Reconstructing labroid evolution with singlecopy nuclear DNA. Proc. R. Soc. Lond., B 264 : 1011～1020.

Swofford, D.L. 2002. PAUP*, Phylogenetic Analysis Using Parsimony (*and other Methods), Version 4. Sinauer Associates, Sunderland, MA.

Tang, K.L. 2001. Phylogenetic relationships among damselfishes (Teleostei: Pomacentridae) as determined by mitochondrial DNA data. Copeia, 3 : 591～601.

Tompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin and D.G. Higgins. 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res., 25 : 4879～4882.

Yang, Z., N. Goldman and A. Friday. 1994. Comparison of models for nucleotide substitution used in maximum-likelihood phylogenetic estimation. Mol. Biol. Evol., 11 : 316～324.

Received : October 1, 2007
Accepted : November 28, 2007

DNA 생물정보를 이용한 한국산 자리돔과 어류의 분류 및 분자계통학적 위치

고 정 락*, 박 영 철[1]

국립수산과학원, [1]이화여자대학교

자리돔과(Teleostei: Pomacentridae) 어류는 열대 및 아열대 해역에 널리 분포하고 있으며 특히 산호해역의 해양생물 군락을 이루는 주요구성 어류이다. 현재 우리나라에는 본과에 5종이 분포하는 것으로 알려져 있는데 본 논문에서는 genus *Chromis*와 *Dascyllus* 2속을 대상으로 분자계통학적 위치를 규명하는데 그 목적이 있다. 분자계통분석에서 일본산 *D. aruanus*는 계통도상에서 폴리네시아산 *D. aruanus*와 함께 유집되었고, 우리나라의 *Chromis fumea*는 호주의 *C. nitida*와 함께 유집되었으며, 두 종간의 유전적 거리를 표시하는 *P* 값은 0.047로 상대적으로 매우 낮았다. 우리나라의 *C. notatus*는 뉴칼레도니아산 *C. flavomaculata*와 함께 유집되었는데, 특히 제주의 *D. melanurus*와 인도-태평양의 *D. melanurus* 사이에는 유전자 염기서열의 차이가 전혀 관찰되지 않았다. 그러나 이 두 지역의 *D. melanurs*와 인도네시아의 *D. melanurus*간에는 한 개의 염기서열 차이가 있었다. 일본산 *Dascyllus aruanus*의 염기서열은 폴리네시아산 *Dascyllus aruanus*와 2개의 염기서열 차이가 있었고, 한국산 *Chromis fumea*와 *C. notatus* 사이에는 다소 많은 수의 염기서열 차이가 있었다. 본 논문에서 도입한 미토콘드리아 cytochrome DNA의 염기서열 정보 및 분석은 이 유전자가 종 수준에서 뿐만 아니라 지역집단의 구분 및 확인을 위한 DNA 바코드로서 이용될 수 있음을 보여준다.