東南アジアにおけるマーブルゴビOxyeleotris marmorataの
ミトコンドリアDNAによる集団構造解析

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Population Structure of Marble Goby *Oxyeleotris marmorata* (Bleeker) in Southeast Asia Inferred from Mitochondrial DNA

Hou Chew Ha, Shigeharu Senoo, Kazunobu Tsunemoto, Yoshizumi Nakagawa, Shigeru Miyashita, Osamu Murata and Keitaro Kato

Abstract: To investigate the population structure of the marble goby *Oxyeleotris marmorata* in Southeast Asia, a total of eighty-five samples were collected from three regions (the mainland, the peninsula, and the islands) for mitochondrial DNA analysis. Sampling locations that were geographically close were pooled and treated as a single population. Fourteen haplotypes were detected among all the samples. Hap-5 was the most widespread haplotype among the six populations, comprising of 29.4% of all samples. Both the non-significant values of Tajima's $D$ and Fu's $F_S$ suggested that all populations were at equilibrium. Analysis of molecular variance (AMOVA) revealed significant differences among and within populations, and no variance was due to regional site ($F_{CT} = -0.1498, P > 0.05$). In pairwise comparisons of $F_{ST}$, Ayutthaya, Dong Nai and Sabah showed significant values between the all populations. The negative values of $F_{ST}$ showed that Sarawak, Indonesia, and West Malaysia are less genetically different. This suggests that the marble goby in Ayutthaya, Dong Nai and Sabah may be genetically differentiated populations compared to the other populations in Southeast Asia.

Key words: *Oxyeleotris marmorata*; Mitochondrial DNA; Control region; Population structure
Furthermore, newly hatched larvae take more than 40 days to grow into juveniles in captivity. Darwis et al. (2008) reported that marble goby juveniles are only 0.3 g in body weight and 3.5 cm in total length after 100 days of rearing in captivity. The slow growth from the juvenile stage to a commercial size is also another constraint on marble goby culture.

Studies on artificial seed production for the marble goby have been ongoing since the 1970's, however, the life cycle of the marble goby in nature is not clear (Senoo et al. 1992; Cheah et al. 1994). This species is widely distributed in Southeast Asia regions. Thus, a better understanding of its population structure would aid more effective and sustainable fisheries management. Molecular genetics has become a powerful tool to determine the levels of differentiation among populations. Examination of mitochondrial DNA (mtDNA) markers is now an established technique for elucidating population genetic structure. The genetic diversity present in a species is hierarchically structured. In addition to differences among individuals within any one population, there may be differences among populations within a given geographical region, differences among populations from different geographical regions, and differences among entire geographical regions.

The goal of this study was to determine the population structure of the marble goby in Southeast Asia using partial sequence analysis of the control region of the mtDNA. Study of the marble goby population structure will enhance the broodstock management for aquaculture seed production. Marble goby fins were sampled from three regions: the mainland (Thailand and Vietnam), the peninsula (West Malaysia), and the islands (Sabah and Sarawak in East Malaysia, and Indonesia). Partial mtDNA control regions were amplified, sequenced, and analyzed to determine the population structures among the regions.

**Materials and Methods**

**Fin samples**

In this study, the term of "population" is used to indicate a pool of close sampling locations of marble goby, according to their geographical distribution. Due to the difficulties of collecting samples, the small sample numbers from sampling locations that were geographically close were pooled and treated as a single population for the comparison between populations.

Eighty-five samples were collected from six populations from August 2009 until February 2010, among which two were from the mainland, one was from the peninsula, and three were

<table>
<thead>
<tr>
<th>SEA Regions</th>
<th>Populations</th>
<th>Sampling locations</th>
<th>Abbreviations</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mainland</td>
<td>Ayutthaya, Thailand</td>
<td>Ayutthaya</td>
<td>Ban</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Dong Nai, Vietnam</td>
<td>Dong Nai</td>
<td>Vt</td>
<td>7</td>
</tr>
<tr>
<td>Peninsula</td>
<td>West Malaysia</td>
<td>Ipoh</td>
<td>Ipo</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Melaka</td>
<td>Mek</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rantau</td>
<td>Rat</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kuala Selangor</td>
<td>PM</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kuala Terengganu</td>
<td>KT</td>
<td>2</td>
</tr>
<tr>
<td>Islands</td>
<td>Sabah, East Malaysia</td>
<td>Penampang</td>
<td>BP</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kimanis</td>
<td>SK</td>
<td>9</td>
</tr>
<tr>
<td>Sarawak, East Malaysia</td>
<td>Bintangor</td>
<td>Big</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kuching</td>
<td>Sa</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Niah</td>
<td>Nia</td>
<td>2</td>
</tr>
<tr>
<td>Indonesia</td>
<td>Samarinda, Kalimantan</td>
<td>Sam</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sukamandi, Java</td>
<td>Suk</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

In total eighty-five samples were collected from fourteen sampling locations in six populations.
Population Structure of Marble Goby 385

from the islands (Table 1). A small portion of the pectoral fin was cut from each individual and preserved in 90% ethanol for DNA examination.

**DNA extraction, amplification, and sequencing**

Fin samples were digested using proteinase K, followed by standard phenol-chloroform extraction. 3 M sodium acetate (pH5.2) was added, and the DNA was precipitated with 70% ethanol. The DNA samples were resuspended in 200 μl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and stored in a freezer at -20°C.

A pair of primers (forward primer 5'-CGGA GGTTAAAATCCTCCCT-3', reverse primers 5'-TAGGAACCAAATGCCAGGAATA-3') was designed to amplify the partial mtDNA control region using polymerase chain reaction (PCR). The forward primer is located inside the threonine tRNA gene, and the reverse primer is located inside the control region. PCR was performed using a thermal cycler, GENEAMP PCR SYSTEM9700 (Applied Biosystems, CA, USA) in 20 μl reaction volumes containing approximately 20 ng of DNA, with 0.5 unit Ex Taq (TaKaRa Bio Inc, Otsu, Japan), 1 × Ex Taq Buffer, 200 μM each dNTP, and 0.5 μM of primers. The PCR cycling conditions were 94°C for 5 min; 35 cycles at 94°C for 30 s, 52°C for 30 s, and 72°C for 90 s; followed by a final extension for 10 min at 72°C.

The PCR products were electrophoresed on a 1.5% agarose gel in 1×TAE buffer to check the yield. The amplified DNA was excised from the gel under irradiation UV rays, and extracted using a QIAQUICK Gel Extraction Kit (QIAGEN, Hilden, Germany), following the manufacturer’s instructions. The same PCR primers were also used for sequencing reactions using the DTCS Quick Start Kit (Beckman Coulter, CA, USA) according to the manufacturer's instructions. The nucleotide sequences were determined for both ends of the PCR products using a BECKMAN COULTER CEQ8000 automated sequencer (Beckman Coulter).

**Data analysis**

Nucleotide composition and number of variable sites were assessed using MEGA version 4 (Tamura et al. 2007). The aligned sequences were used to analyze the population structure and genetic variation using ARLEQUIN version 3.5 (CMPG, University of Berne; Excoffier et al. 2005). Genetic diversity in each population was measured as haplotypic diversity (Nei 1987) and nucleotide diversity (Tajima 1983). Heterozygous nucleotide sites can be estimated sufficiently with a sample size of ten (or even five) (Tajima 1983). Tajima’s D (Tajima 1989) and Fu’s F_S (Fu 1997) tests, as implemented in ARLEQUIN version 3.5, were used to evaluate the neutrality of the investigated sequences. To perform the test, homologous DNA sequences from at least three individuals were used for Tajima’s D test to compute a standardized measure of the total number of segregating sites and the average number of mutations between pairs. Fu’s F_S test detects an excess of mutation and it is more powerful in cases of population expansion. The level of genetic population differentiation was tested using analysis of molecular variance (AMOVA) as implemented in ARLEQUIN version 3.5 (Excoffier et al. 2005), using the genetic distance matrix to estimate the components of variance that are attributable to differences among populations and among individuals within populations. Populations were combined into three regions, as defined by geographical features (Fig. 1). Significance of variance components was tested by a nonparametric permutation procedure with 1,000 permutations (Excoffier et al. 1992). The pairwise fixation index (F_{ST}) was employed to check the genetic differentiation between populations. The correlation of genes of different individuals in the same population and the genetic differences among populations were also tested by the F_{ST}. For between population differences, if all haplotypes were identical, then F_{ST} equaled 0; if they were all different, then F_{ST} equaled 1. Thus, pairwise comparisons of F_{ST} values among populations can be considered as standardized distances between populations (Excoffier et al. 2005). A neighbor-joining tree of the haplotypes was constructed under the model of the Kimura 2-parameter using MEGA version 4, and evaluated with 1,000 bootstrap replicates.
Results

Sequence variation

A total of eighty-five 394 bp fragments were sequenced successfully from the six populations (Table 2). Among the examined sequences, the A/T base contents were significantly higher than the C/G base content (mean: A=35.4%, T=27.7%, C=21.3%, G=15.6%), which is consistent with the results of Brown et al. (1986), who found that the control region is an A-T rich region of the mtDNA. The sequences from Ayutthaya contained two variable sites, eighteen variable sites were found in West Malaysia, eleven variable sites were found in Sabah, thirteen variable sites were found in Sarawak, and seven variable sites were found in Indonesia. No variable sites were found in the samples from Dong Nai (Table 2).

Population structure and genetic diversity

Among all the samples, fourteen haplotypes were detected (GenBank accession numbers JF300363-JF300376); four haplotypes were found in the mainland, nine haplotypes in the

<table>
<thead>
<tr>
<th>Regions</th>
<th>Populations</th>
<th>Sample size</th>
<th>Number of haplotypes</th>
<th>Haplotypic diversity</th>
<th>Number of polymorphic sites</th>
<th>Nucleotide diversity</th>
<th>Tajima's D</th>
<th>Fu's Fs P-value</th>
<th>Tajima's D P-value</th>
<th>Fu's Fs P-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mainland</td>
<td>Ayutthaya</td>
<td>10</td>
<td>3</td>
<td>0.6222 ± 0.1383</td>
<td>2</td>
<td>0.0018 ± 0.0017</td>
<td>0.0189</td>
<td>0.662 ± 0.1561</td>
<td>0.313</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dong Nai</td>
<td>7</td>
<td>1</td>
<td>0.0000 ± 0.0000</td>
<td>0</td>
<td>0.0000 ± 0.0000</td>
<td>0.0000</td>
<td>N.A.</td>
<td>0.0000</td>
<td>N.A.</td>
<td>0.0000</td>
</tr>
<tr>
<td>Peninsula</td>
<td>West Malaysia</td>
<td>31</td>
<td>9</td>
<td>0.8021 ± 0.0474</td>
<td>18</td>
<td>0.0125 ± 0.0069</td>
<td>0.3953</td>
<td>0.687 ± 1.1834</td>
<td>0.739</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Islands</td>
<td>Sabah</td>
<td>18</td>
<td>4</td>
<td>0.6983 ± 0.0840</td>
<td>11</td>
<td>0.0099 ± 0.0058</td>
<td>0.8183</td>
<td>0.827 ± 4.0133</td>
<td>0.955</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sarawak</td>
<td>12</td>
<td>5</td>
<td>0.8333 ± 0.0691</td>
<td>13</td>
<td>0.0115 ± 0.0069</td>
<td>0.2368</td>
<td>0.657 ± 1.9066</td>
<td>0.831</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indonesia</td>
<td>7</td>
<td>3</td>
<td>0.5238 ± 0.2086</td>
<td>7</td>
<td>0.0080 ± 0.0054</td>
<td>0.5177</td>
<td>0.675 ± 2.3136</td>
<td>0.894</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>6</td>
<td>85</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

N.A., not available.  
No significant values of Tajima's D and Fu's F_2 were observed in the neutrality test, showing that all populations were in genetic equilibrium.
peninsula, and eight haplotypes in the islands (Table 3). Six haplotypes were shared between more than one population. Among the shared haplotypes, only Hap-5 was shared in four populations. Hap-5 comprised 29.4% of all samples. It was clear that Hap-5 was the most widespread haplotype among the populations and the most common among the control region sequences. Eight distinct haplotypes were found in one single population only. Ayutthaya, Sarawak, Sabah, and Indonesia each contained a distinct haplotype, while West Malaysia population had four distinct haplotypes.

The genetic diversity of all populations’ sequences is shown in Table 2. West Malaysia had the highest nucleotide diversity (0.0125). The highest haplotype diversity, 0.8333, was found in Sarawak. The zero values for haplotype and nucleotide diversity in Dong Nai resulted from no variable sites being detected in the sequences.

Tajima’s $D$ and Fu’s $F_s$ neutrality tests were performed to determine departures from neutrality in the sequence data. No significant deviations from neutrality were detected in any of the populations using both of neutrality tests ($P>0.1$).

Geographic differentiation

AMOVA produces estimates of variance components reflecting the correlation of haplotypic diversity at different levels of hierarchical divisions. AMOVA assessment of the difference among the regions generated a negative variance component ($-0.4070$) indicating that genetic structure was absent at this level (Table 4). $F$ statistics from AMOVA also revealed significant differences among and within populations. 67.32% of the variance was attributable to individual variation ($F_{ST}=0.3268$, $P<0.01$), 47.66% was attributable to populations ($F_{SC}=0.4145, P<0.01$), and no variance was due to regional site ($F_{CT}=-0.1498$).

The genetic differentiation among the six populations was significant, except for the pairwise $F_{ST}$ values between Sarawak-Indonesia.

**Table 3.** Number of fish from six populations of marble goby according to the haplotype distribution

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Ayutthaya</th>
<th>Dong Nai</th>
<th>West Malaysia</th>
<th>Sabah</th>
<th>Sarawak</th>
<th>Indonesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hap-1</td>
<td>6</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hap-2</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hap-3*</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Hap-4</td>
<td></td>
<td>9</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hap-5</td>
<td>7</td>
<td>11</td>
<td>3</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hap-6*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hap-7</td>
<td></td>
<td>8</td>
<td>3</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hap-8</td>
<td></td>
<td>4</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hap-9*</td>
<td></td>
<td></td>
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<tr>
<td>Hap-10*</td>
<td></td>
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<tr>
<td>Hap-11*</td>
<td></td>
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<tr>
<td>Hap-12*</td>
<td></td>
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<tr>
<td>Hap-13*</td>
<td></td>
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<tr>
<td>Hap-14*</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>7</td>
<td>31</td>
<td>18</td>
<td>12</td>
<td>7</td>
</tr>
</tbody>
</table>

$*$ Distinct haplotype.

Sample sizes of each population are shown at the bottom of the table.

**Table 4.** AMOVA of mtDNA control region nucleotide sequence data in six marble goby populations from three regions

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Sum of squares</th>
<th>Variance components</th>
<th>Percentage of variance</th>
<th>$F$ statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among regions (mainland, peninsula, islands)</td>
<td>2</td>
<td>26.40</td>
<td>-0.4070</td>
<td>-14.98</td>
<td>$F_{CT} = -0.1498$</td>
</tr>
<tr>
<td>Among populations, within regions</td>
<td>3</td>
<td>45.97</td>
<td>1.2950</td>
<td>47.66</td>
<td>$F_{SC} = 0.4145$ $*$</td>
</tr>
<tr>
<td>Within populations</td>
<td>79</td>
<td>144.52</td>
<td>1.8294</td>
<td>67.32</td>
<td>$F_{ST} = 0.3268$ $*$</td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>216.89</td>
<td>2.7174</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$*$ The significant level ($P < 0.05$) of the $F$ statistics, with 1,023 permutations.

AMOVA results showing the most molecular variance were observed among and within populations, but no variance was observed among the regions.
Table 5. Pairwise comparison $F_{ST}$ value of population differentiation (below diagonal), genetic distance within population (diagonal) and between populations (above diagonal)

<table>
<thead>
<tr>
<th></th>
<th>Ayutthaya</th>
<th>Dong Nai</th>
<th>West Malaysia</th>
<th>Sabah</th>
<th>Sarawak</th>
<th>Indonesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ayutthaya</td>
<td>0.0018</td>
<td>0.0207</td>
<td>0.0186</td>
<td>0.0174</td>
<td>0.0179</td>
<td>0.0177</td>
</tr>
<tr>
<td>Dong Nai</td>
<td>0.9470*</td>
<td>0.0000</td>
<td>0.0089</td>
<td>0.0157</td>
<td>0.0082</td>
<td>0.0074</td>
</tr>
<tr>
<td>West Malaysia</td>
<td>0.5168*</td>
<td>0.1495*</td>
<td>0.0127</td>
<td>0.0151</td>
<td>0.0120</td>
<td>0.0104</td>
</tr>
<tr>
<td>Sabah</td>
<td>0.6071*</td>
<td>0.5753*</td>
<td>0.2390*</td>
<td>0.0101</td>
<td>0.0139</td>
<td>0.0121</td>
</tr>
<tr>
<td>Sarawak</td>
<td>0.6023*</td>
<td>0.2033*</td>
<td>-0.0204</td>
<td>0.2178*</td>
<td>0.0117</td>
<td>0.0097</td>
</tr>
<tr>
<td>Indonesia</td>
<td>0.7475*</td>
<td>0.4500*</td>
<td>-0.0227</td>
<td>0.2352*</td>
<td>-0.0322</td>
<td>0.0081</td>
</tr>
</tbody>
</table>

*Significant level ($P<0.05$) of $F_{ST}$ value, with the sequential Bonferroni correction (Rice 1989).

Fig. 2. The evolutionary history of marble goby haplotypes was inferred using the Neighbor-Joining method (1,000 bootstraps). Numbers at nodes indicate the bootstrap values, and only values >50% are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method, and the units are the number of base substitutions per site. The abbreviations (refer to Table 1) and numbers in the brackets show the sampling locations and the number of samples, respectively.

Phylogenetic relationship

A neighbor-joining tree was constructed using the Kimura 2-parameter model. The tree indicated no obvious genealogy among the marble goby (Fig. 2). The topology of the neighbor-joining tree of marble goby haplotypes was shallow, and there were no significant genealogical branches or clusters of samples corresponding to sampling locations. Typically, haplotypes in a population were scattered throughout the tree, except for the Dong Nai population.

Discussion

Genetic diversity and structure

In this study, the genetic diversity, as expressed by haplotype and nucleotide diversities, is higher in the West Malaysia, Sarawak,
and Sabah populations compared to other populations (Table 2). This might be explained by
the smaller sample size in the other populations (Tajima 1983; Nei 1987): the seven samples
from Dong Nai (Vietnam) consisted of one haplotype only with no polymorphic sites in
the sequences. To further elucidate the genetic diversities of the marble goby, more samples
per site should be collected from different populations (Rand et al. 1994).

mtDNA is maternally inherited; samples with
distinct haplotypes in Ayutthaya, West Malaysia,
Sarawak, Sabah, and Indonesia probably repre-
sent the indigenous populations in those regions
(Table 3). These distinct haplotypes have no
introgression stemming from other populations,
which could be explained by the natural
geographic boundaries and different maternal
ancestors (Weiss et al. 2001). These distinct
haplotypes can be used as indicators or DNA
markers to identify the indigenous populations
of those regions. Genetic diversity should be
higher in the ancestral population compared to
a derived population (Savolainen et al.
2002).

West Malaysia has the highest number of haplo-
types (nine) and the highest nucleotide diversity
(0.0125) among the six populations, indicating
that the West Malaysia population probably rep-
resents ancestral population in Southeast Asia.
Hap-5 is the most common haplotype among the
fourteen haplotypes, and was present in 29.4% of
the samples. Even though Hap-5 can be found in
four different populations, it might derive from a
single ancestor according to the theory of matern-
al inheritance of mtDNA.

Tajima’s $D$ neutrality test for all the populations
indicated that the populations are in genetic equi-
librium (Kimura 1983). Fu’s $F_S$ test, which is pro-
posed to detect population growth, also did not
show significant values for any of the populations.
In fact, smaller sample sizes were used in this
study, and a reduction in the number of sig-
nificant deviations from neutrality are expected
(Simonsen et al. 1995; Wayne and Simonsen
1998). Small sample sizes preclude a reasonable
argument for neutrality if neutrality tests fail to be
significant. On the other hand, when neutrality is
significantly rejected, the small sample sizes may
provide cogent evidence of strong evolutionary
forces at work (Reinaldo et al. 2002). In future
studies, the demography of marble goby population
may be revealed by increasing the sample
number in each population, and sampling from
smaller scale regions.

In this study, we obtained negative variance
components in AMOVA at the region hierar-
chical level. The AMOVA methodology relies
on estimates of relationships between haplo-
types in the same populations relative to hap-
lotypes of different populations (Weir 1996).
Negative variance components can result from
very small, but positive estimates of genetic
structure indices from data (Weir 1996). This
implies that haplotypes are more related among
than within regions, and most of the variability
occurs among and within populations (Weir and
Cockerham 1984; Weir 1996).

**Phylogenetic relationship**

The neighbor-joining tree revealed that haplo-
types of marble goby were widespread in
Southeast Asia, except for the eight distinct haplo-
types (Fig. 2, Table 3). The complete life cycle
of the marble goby in nature has not been clearly
defined. However, according to Akihito et al.
(2000), there is a possibility that the worldwide
distribution of the gobiod fishes occurs by the
passive migrations of larvae via currents. Akihito
et al. (1984) reported that some freshwater gobio-
d species could have positively advanced into
continental inland areas, consequently changing
their life style from amphidromous to a fluvial
type. It is generally assumed that goboids arose
in freshwater, from a marine ancestor, and then
returned to marine habitats once or many times
(Allen 1989; Allen et al. 2002). These could be
the reasons for the widespread occurrence of
Hap-5 in Southeast Asia. However, further study
on the life cycle of marble goby in nature is
necessary to understand their habitat and physi-
ology to improve artificial seed production of
marble goby in hatcheries.

There is a probable explanation for the occur-
pence of the same haplotypes in different popu-
lations. During the last glacial period, the sea
level was lower and much of the archipelago
of Southeast Asia was terrestrial (Sundaland) (Harold 2000; Marwick 2009). During this period, freshwater fish, including marble goby, would have dispersed throughout the Sundaland via freshwater currents. In the Holocene, the Sunda Shelf, which includes southern Thailand, Peninsular Malaysia, Sumatra, Java, and Borneo, was flooded when thawing occurred, thus forming the current geographical features (Harold 2000; Marwick 2009). Thus, the marble goby had been separated and isolated on the archipelago, peninsula, and mainland respectively. This could explain the widespread distribution of the same haplotypes in these regions. The genetic similarity between West Malaysia, Sarawak, and Indonesia also supports this hypothesis (Table 5). Fauna from Vietnam and Cambodia include species that are widely distributed across the Indochina peninsula, and mainland respectively. This could explain the widespread distribution of the same assemblages would be affected by common Paleo-geographical events (Marwick 2009).

The results of the present study have important implications for the fisheries management of marble goby. This study revealed highly significant differences in the mtDNA control region of Ayutthaya, Dong Nai, and Sabah compared to other populations (by $F_{ST}$ values and genetic distances). This could be because these populations are geographically much farther from the other populations, and no gene flow existed among them. Our data showed that the populations of West Malaysia, Sarawak, and Indonesia were closely related. A more rigorous study using larger samples sizes and longer mtDNA fragments is recommended to help elucidate the genetic variation of these populations.

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References


Population Structure of Marble Goby


東南アジアにおけるマールゴビ Oxyeleotris marmorata のミトコンドリア DNA による集団構造解析

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東南アジアにおけるマールゴビ Oxyeleotris marmorata の集団構造を調べるために、ミトコンドリア DNA 解析用に85尾のサンプルを3地域（タイおよびベトナム、マレーシア半島、並びにジャワおよびボルネオ島）から収集し、近隣地から得られたサンプルをブールして1集団と仮定して解析した。全サンプルから14ハプロタイプが得られた。全ての集団において Tajima’s D および Fu’s Fs 値に有意差はなかったことから、遺伝的に平衡状態にあることが示唆された。分散分析（AMOVA）の結果、集団間および集団内に有意差がみられた。Ayutthaya, Dong Nai および Sabah は他のすべての集団との間に有意な Fs 値および高い遺伝的距離を示し、Ayutthaya, Dong Nai および Sabah のマールゴビが他の集団と比較してそれぞれ遺伝的に異なる集団であることが推察された。