ABSTRACT. – We present a molecular phylogeny of Chaetodon derived from the analysis of sequences from mitochondrial cytochrome (cyt) b and 12S rRNA genes for 41 ingroup Chaetodon species. One species of Chelmon and one species of Prognathodes were used as outgroups to root the molecular trees. Two different computer software packages were utilized, namely, MEGA 2 and PHYLIP version 3.6a2. Multiple methods such as neighbor-joining (NJ), minimum evolution (ME), maximum likelihood (ML) and maximum parsimony (MP) were used. Cytochrome b sequence data provided greater resolution than 12S rRNA. Of the nine Chaetodon subgenera sampled, all were in the monophyletic group, but their phylogenetic relationships differed significantly from those inferred from morphological characters. Lepidochaetodon was the basal subgenus followed by Exornator and others. Molecular data support that Corallochaetodon and Citharoedus are sister groups, as suggested by morphological analysis. We found no geographical pattern of speciation events in the Indo-West Pacific and speciation occurred throughout the Indo-West Pacific as a result of diverse mechanisms. The evolutionary patterns of species pairs or species groups were more complex than previous envisioned as evidence from estimated divergences between species pairs suggests that the speciation events have occurred many times.

KEY WORDS. – Indo-West Pacific, speciation, biodiversity, Chaetodon.
many reports of what appears to be closely related species pairs of the genus *Chaetodon* (Allen, 1979). The species pairs reported seem to have evolved relatively recently judging by their similarity in morphology, especially in color pattern. Randall (1998) lists seven pairs of geminate species of *Chaetodon* in his article of zoogeographical pattern of Indo-Pacific shorefishes. Here, we generate a molecular phylogeny inferred from the analysis of mitochondrial DNA (mtDNA) cytochrome *b* and 12S rRNA genes for *Chaetodon*. Our aim is to examine the mode, geography and timing of speciation in *Chaetodon*. In this study, we present a molecular phylogeny that includes all the 41 recognized taxonomic species in the genus. We combine the results with detailed distribution data to examine the speciation mechanism in the IWP region.

**MATERIALS AND METHODS**

Live fish for the experiments were obtained from field collections around Taiwan and aquariums. A total of 142 specimens, representing 41 species and 11 subgenera of the genus *Chaetodon* were included in this study. There were at least 2 (range = 2 - 8) individuals representing each species of the genus *Chaetodon*. We used 2 other butterflyfishes, *Chelmon rostratus* and *Prognathodes aculeatus* as outgroups to root our phylogenetic analysis. All of the specimens were used for partial mtDNA cytochrome (*cty*) *b* and 12S rRNA sequencing. The species used and their sample sizes are listed in Table 1.

Approximately 0.5 g of muscle tissue was obtained from each specimen and was homogenized in 2.0 ml of cold grinding buffer (0.2 M NaCl, 0.05M EDTA, pH 8.0). Tissue previously stored in 95% ethanol was soaked in 5.0 ml of grinding buffer on ice for approximately 1 hour before grinding. High molecular weight DNA was isolated by proteinase K/phenol extraction procedures (Duda & Palumbi, 1999). The *cty* *b* gene was amplified with primers *cty F* (5'-'ATGGCCACGCT TAGTAAAC-3') and *cty R* (5'-AAGAACGTATCCTACAAAG-3'). The 12S rRNA gene was amplified with primers 12SF (5'- TACGTAAAAC-3') and cyt *b* and 12S rRNA sequence by using neighbor-joining (NJ), minimum evolution (ME), maximum likelihood (ML) and maximum parsimony (MP) methods. Parsimony analyses were done by considering the TSs and TVs equally and using an *a priori* weighting scheme in which a TS / TV weight of 1:2 was employed. NJ and ME trees were generated according to pairwise distance obtained by the Kimura's two-parameter model (Kimura, 1980). The ML tree was constructed according to Tamura & Nei's substitution model (Tamura & Nei, 1993), which recovers slightly biased branch lengths of lineages (Håstad & Björklund, 1998). The analyses were performed in MEGA2 (Kumar et al., 2001) and Phylogeny Inference Package (PHYLIP) version 3.6a2 (Felsenstein, 1993) programme packages. We tested the confidence of reconstructed clades by bootstrapping (Felsenstein, 1985) with 1,000 replicates using unweighted characters. The nodes with bootstrap values greater than 70, as a rule of thumb, were significantly supported with 95 % probability (Hillis & Bull, 1993).

A well-documented evolutionary rate is needed for estimating divergence between species. The rate was estimated to be 2% per million years for the mtDNA *cty b* gene (Johns & Avise, 1998). In this study, using this evolutionary rate, the divergence times of *Chaetodon* species were estimated based on the average of the number of nucleotide substitutions (K) among lineages by removing sequences that deviated from a molecular clock model. Estimates of divergence times between species were then calculated using penalized likelihood in PHYLIP, as implemented in the r8s program. A 95% confidence interval was calculated for two relevant nodes using an algorithm included in r8s with a cut-off value of 4 (Sanderson, 2002). At least one dated reference point was needed to calibrate the output from r8s program into actual age estimates. The calibration point should have a date assigned, which is based on other data such as fossil information. However, we could not identify fossils for *Chaetodon* and the penalized likelihood analysis was therefore performed on a dataset representing divergence between lineages. In our analysis, we used the other two butterflyfishes (*Chelmon rostratus* and *Prognathodes aculeatus*) to root the tree and used the divergence time of the overall species estimated above based on a molecular clock, as a calibration point. The maximum likelihood-based PHYLIP version 3.6a2 (Felsenstein, 1993) was used to estimate branch lengths with the general time reversible model. The best tree recovered was input to r8s. An unconstrained penalized likelihood analysis (Sanderson, 2002) was conducted with the Powell algorithm (Chen et al., 1998).
Table 1. The subgenera list of the genus *Chaetodon* and the species number of each subgenus from Blum (1988), sample size and collection source used in this study. The values in parentheses represent the number of each subgenera used in this study.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Species Number</th>
<th>Sample Size</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order Perciformes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family Chaetodontidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genus <em>Chaetodon</em></td>
<td>84 (42)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subgenus <em>Chaetodon</em></td>
<td>7 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subgenus <em>Rabdophorus</em></td>
<td>26 (15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. mesoleucos</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. rafflesii</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. collare</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. flavirostris</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. auriga</td>
<td>6</td>
<td></td>
<td>North Taiwan</td>
</tr>
<tr>
<td>C. decussatus</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. vagabundus</td>
<td>8</td>
<td></td>
<td>Taiwan</td>
</tr>
<tr>
<td>C. ephippium</td>
<td>5</td>
<td></td>
<td>North Taiwan</td>
</tr>
<tr>
<td>C. uileniensis</td>
<td>2</td>
<td></td>
<td>Taiwan</td>
</tr>
<tr>
<td>C. lineolatus</td>
<td>5</td>
<td></td>
<td>Taiwan</td>
</tr>
<tr>
<td>C. oxycephalus</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. lunulatus</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. melannotus</td>
<td>5</td>
<td></td>
<td>Taiwan</td>
</tr>
<tr>
<td>C. ocellarcaudus</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. semilarvatus</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>Subgenus <em>Rooops</em></td>
<td>6 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. tinker</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>Subgenus <em>Exornator</em></td>
<td>22 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. citrinellus</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. quadrimaculatus</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. argentatus</td>
<td>5</td>
<td></td>
<td>Taiwan</td>
</tr>
<tr>
<td>C. xanthorus</td>
<td>5</td>
<td></td>
<td>Taiwan</td>
</tr>
<tr>
<td>C. madagascariensis</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. paucifasciatus</td>
<td>5</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. punctatofasciatus</td>
<td>5</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. guttatissimus</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. pelewensis</td>
<td>5</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>Subgenus <em>Lepidochaetodon</em></td>
<td>3 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. unimaculatus</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. kleinii</td>
<td>3</td>
<td></td>
<td>Taiwan</td>
</tr>
<tr>
<td>C. trichrous</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>Subgenus <em>Megaprotodon</em></td>
<td>2 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. trifasciatus</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>Subgenus <em>Gonochaetodon</em></td>
<td>3 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. baronessa</td>
<td>3</td>
<td></td>
<td>Taiwan</td>
</tr>
<tr>
<td>C. larvatus</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>Subgenus <em>Tetrachaetodon</em></td>
<td>4 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. plebeius</td>
<td>3</td>
<td></td>
<td>Taiwan</td>
</tr>
<tr>
<td>C. speculum</td>
<td>3</td>
<td></td>
<td>Taiwan</td>
</tr>
<tr>
<td>Subgenus <em>Discochaetodon</em></td>
<td>4 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. aureofasciatus</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. octofasciatus</td>
<td>5</td>
<td></td>
<td>Taiwan</td>
</tr>
<tr>
<td>C. rainfordi</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>Subgenus <em>Corallochaetodon</em></td>
<td>4 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. austriacus</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. trifasciatus</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. lunulatus</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>Subgenus <em>Citharoedus</em></td>
<td>3 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. meyeri</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. ornatissimus</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
<tr>
<td>C. reticulatus</td>
<td>3</td>
<td></td>
<td>Aquarium</td>
</tr>
</tbody>
</table>
RESULTS

Among a total of 670 bp of aligned nucleotide sequences (324 bp of cyt b, 346 bp of 12S rRNA), 233 sites were variable, with 219 being phylogenetically informative (121 bp of cyt b, 98 bp of 12S rRNA). For cyt b, the average frequencies of nucleotides for all taxa are as follows: A = 23.8%; C = 28.1%; G = 17.3%; T = 30.8%. This partial cyt b gene of the genus Chaetodon is A + T biased (54.6%) and the average TS / TV ratio across all pairwise sequence comparisons in the data set was 3.2. This level of transition bias of cyt b is within the range of biases previously reported for other vertebrates and serves as a basis for the transition / transversion weighting ratios used in phylogenetic reconstruction. For 12S rRNA, the average frequencies of nucleotides for all taxa are as follows: A = 30.2%; C = 25.9%; G = 22.2%; T = 21.7%. This partial 12S rRNA gene of the genus Chaetodon is A + T biased (51.9%) and the average TS / TV ratio across all pairwise sequence comparisons in the data set was 2.3. The average TS / TV ratio suggests that variations at most sites for cyt b and 12S rRNA have not reached saturation. Third codon positions of cyt b and 12S rRNA sequences were retained in all analyses.

The phylogenetic trees were reconstructed by four methods (MP, ME, NJ and ML) for cyt b, 12S rRNA and combined cyt b + 12S rRNA genes. The MP, ME and NJ trees have similar topology. The data fully supports the monophyletic grouping of all the individuals representing the same species, except for the C. punctatofasciatus species group (Fig. 1). Hybridization between closely-related Chaetodon species has long been suspected. For example, McMillan et al. (1999) proposed overwhelming evidence from behavioural, genetic and phenotypic between C. pelewensis and C. punctatofasciatus, making it impossible to justify their continued species-level status.

Figure 1 represents reconstructions of the phylogeny of the genus Chaetodon inferred from 12S rRNA (Fig. 2A), cyt b (Fig. 2B) and combined trees (Fig. 2C) by the NJ method (MP and ME trees are not shown). However, trees obtained from three runs of the 12S rRNA and cyt b data were not identical and showed large differences in topology in the form of poorly supported branches. According to the phylogenetic trees inferred from 12S rRNA, the subgenera Rabdophorus and Corallochaetodon are polyphyletic groups while the others are poorly supported. Nevertheless, the trees obtained by analyses of cyt b sequences and combined cyt b + 12S rRNA were very similar. The subclade, including Lepidochaetodon, Corallochaetodon and Citharoedus, was the most basal subgenus. Based on cyt b and combined trees, all the nine Chaetodon subgenera sampled belonged to a monophyletic group. However, their phylogenetic relationships differed significantly from those inferred from morphological characters. Based on the ML phylogenetic tree (Fig. 3), Lepidochaetodon is the basal subgenus followed by Exornator and others. Molecular data support that Corallochaetodon and Citharoedus were sister groups. However, this data set could not resolve the relationships among subgenera within the genus Chaetodon, as the relationships among the subgenera are not well supported by bootstrapping.

In this study, we examined 11 possible species pairs of the genus Chaetodon (Table 2), as proposed by previous literature (Burgess, 1978; Steene, 1978; Allen, 1979; Blum, 1989; Kuiter, 1995; Allen et al., 1998). Among these species pairs, four pairs are sympatrically distributed, three pairs are parapatrically distributed and the remaining four pairs are allopatrically distributed (Table 2). The geographical distributions of Chaetodon species are shown together within their phylogenetic relationships in Figure 5. The pairwise sequence divergences of the sympatric (average = 5.3%) and parapatric (average = 4.3%) distribution species pairs are higher than the allopatric distribution (average = 1.5%). We calculated the separation times for these grouped species pairs. Separation times have been implied by using a divergence rate of 2% per million years (Johns & Avise, 1998) for this data set. The separation time between sympatric pairs will be approximately 1.5 to 4.0 million years ago (mya) and similar to the time between parapatric species pairs. Separation times between allopatrically distributed species pairs ranged from 0.5 to 1.0 mya (Table 2). Consequently, based on the speciation time of the species pairs of allopatric distribution being more recent than that of the sympatrically- and parapatrically distributed species pairs, the phylogeography of coral reef fishes in the IWP may have experienced vicariance. In this study, the relative rate tests via pairwise comparisons of mtDNA sequences (using Chelmon rostratus as an outgroup) revealed that most lineages were not consistent with the molecular clock.

DISCUSSION

Before reconstructing the phylogenetic relationships, it is necessary to choose an applicable molecular tool. In this study, we use cyt b, 12S rRNA and combined trees as phylogenetic trees to confer the evolution within the genus Chaetodon. The topologies of the cyt b and combined trees...
Fig. 2. Neighbor-joining tree of the genus Chaetodon inferred from the DNA sequence variations of A) 12S rRNA, B) cyt b, C) combined 12S rRNA and cyt b. Numbers at nodes indicate the bootstrap values estimated from 1,000 replications.

Table 2. The pairwise divergence values of possible species pair examined in this study. Genetic distances calculations were made by the Kimura’s two-parameter model. Standard errors were also indicated.

<table>
<thead>
<tr>
<th>Species Pair</th>
<th>Distribution Pattern</th>
<th>Reference</th>
<th>Pairwise Divergence</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. oxycephalus / C. lineolatus</td>
<td>Sympatric</td>
<td>Burgess, 1978, Allen, 1979</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>C. vagabundus / C. decussatus</td>
<td>Sympatric</td>
<td>Burgess, 1978, Allen, 1979</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>C. melanotus / C. ocelliaudus</td>
<td>Sympatric</td>
<td>Burgess, 1978, Allen, 1979</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>C. xanthurus / C. argentatus</td>
<td>Sympatric</td>
<td>Blum, 1988</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>C. meyeri / C. ornatissimus</td>
<td>Parapatric</td>
<td>Allen et al., 1998</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>C. pelevensis / C. punctatofasciatus</td>
<td>Parapatric</td>
<td>Steen, 1978</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>C. lunulatus / C. trifasciatus</td>
<td>Parapatric</td>
<td>Kuiter, 1995</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>C. xanthurus / C. madagascariensis</td>
<td>Allopatric</td>
<td>Allen et al., 1998</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>C. kleinii / C. trichrous</td>
<td>Allopatric</td>
<td>Allen et al., 1998</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>C. guttatissimus / C. punctatofasciatus</td>
<td>Allopatric</td>
<td>Blum, 1988</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>C. paucifasciatus / C. madagascariensis</td>
<td>Allopatric</td>
<td>Blum, 1988</td>
<td>0.01 ± 0.01</td>
</tr>
</tbody>
</table>

were very similar but the 12S rRNA topology differed significantly. The 12S rRNA tree showed that some subgenera of Chaetodon were polyphyletic groups and it also showed that the genus Chaetodon was not a monophyletic group. 12S rRNA provided considerably less resolution with poor nodal supports, relatively shorter internal branch lengths and it is unlikely that the gene will provide a cost-effective marker in further resolving the Chaetodon phylogeny. Furthermore, the secondary structure of RNA is strongly conserved over long time periods and natural selection is acting to maintain a structure that is essential for the function of these molecules (Nicholas et al., 2001). We conclude that the 12S rRNA phylogenetic tree of Chaetodon was not applicable to investigate the evolution of Chaetodon. Some studies (e.g. Rosenberg, 2002; Dettman et al., 2003; Slatkin & Pollack, 2006) on the concordance of gene trees and species trees have indicated that the correct species tree may be inferred if several unlinked genes are studied. Therefore, we used the combined phylogenetic tree (Fig. 3) to investigate the evolution in geminate species pairs and species groups of Chaetodon.

There are many studies about the Chaetodon phylogeny based on morphology and molecular data (e.g., Blum, 1988; Smith et al., 2003; Littlewood et al., 2004). In the absence of members of the subgenera Roaops and Chaetodon, the subgenus Lepidochaetodon was the most basal clade and confirmed the close relationship between C. kleinii and C. trichrous, as discussed by Blum (1988). The close relationship between Lepidochaetodon and Exornator is also well supported by morphological analyses (Blum, 1988; Smith et al., 2003). The sister group relationship between the monophyletic subgenera Corallochaetodon and Citharoedus was confirmed with molecular data in our study and Littlewood et al.’s hypothesis (2004). The resolution of Tetrachaetodon and Discochaetodon as sister taxa were also supported by morphological data (Littlewood et al., 2004). According to the previous studies described above, different analysis with different samples yielded different topologies. Therefore, wider taxonomic sampling remains preferable. Consequently, we would like to perform a re-analysis of Chaetodon phylogeny in the future.
The Indo-Pacific region is a place where speciation may have occurred because of a barrier to the East-West dispersal of marine fishes. This is a result of the lowering of sea-levels during glacial periods. Within the Indo-West Pacific, vicariance has been invoked to explain pairs of sister species in the Indian and Pacific Oceans (McMillan & Palumbi, 1995; Williams & Benzie, 1998; Benzie, 1999). However, the number, location and timing of these vicariance events in the Indian and Pacific Oceans were unknown.

Blum (1989) infers 12 barriers based on the distributions of Chaetodon species complexes and suggests that these barriers may be sufficient to isolate the species (Fig. 4). These barriers might influence the dispersal and speciation mode of Chaetodon. In this study, most geminate species pairs were distributed on each side of these barriers. We suggest that these barriers are vicariance events that disturbed the dispersal of the Chaetodon species. We suggest that the fall in sea levels associated with glacial maxima resulted in a massive loss of shallow inner reefs and lagoons. It may have exposed large areas of continental shelf and reduced reef habitats to narrow fringes, or, may even have increased it in some areas by exposing submerged islands. Thus, habitat loss and increased/reduced circulation have all contributed to the isolation between the adjacent regions. There is evidence that climatic oscillations during the late Pliocene and Pleistocene have been important causes of marine speciation, especially in the Indo-West Pacific (Palumbi, 1997; Benzie, 1999). Molecular phylogenetic studies of small, closely related clades also show that sister species diverged in the last one to five million years (McCartney et al., 2000; Williams, 2000; Lessios et al., 2001; Harrison, 2004). In Chaetodon, divergence times between species pairs were mostly 0.5 to 5 mya (Table 2). However, we could not estimate the evolutionary rates for Chaetodon by molecular tools as the Tajima’s relative rates test (Tajima, 1989) rejected clock-like behaviour.

The previous study on the rate of control-region evolution in Pacific butterflyfishes also supports that the evolutionary rate

Fig. 3. A combined molecular phylogenetic analysis of Chaetodon from a maximum likelihood (ML) analysis of 12S rRNA and cyt b sequences.

Fig. 4. Barriers (C – K) inferred from the distribution of species within complexes by Blum (1989).
Fig. 5. Distributions and phylogenetic relationships based on the combined tree of the Chaetodon species A) C. auriga spp. group, B) C. lineolatus complex, C) Discochaetodon subgenus, D) Corallochaetodon subgenus, E) Citharoedus subgenus, F) C. punctatofasciatus spp. group, G) Rhombochaetodon spp. group, H) Lepidochaetodon subgenus.
may be different between clades and the evolutionary rate of
the mitochondrial D-loop of Chaetodon is rapid (McMillan & Palumbi, 1997). Hence, we could not confirm that the times
estimated were valid. However, in terms of the 12 barriers
(Fig. 4; Blum, 1989), we find different barriers affect different
species. For example, barrier F limits the distribution of C.
baronessa and C. unimaculatus while barriers G, H and I do
not. On the other hand, the distribution of C. speculum was
limited by barriers F, G, H and I. According to these results,
we suggest that barrier F formed before C. speculum, C.
baronessa and C. unimaculatus speciated and barriers G, H
and I were formed before C. speculum speciated but after C.
baronessa and C. unimaculatus speciated and dispersed.
These results also suggest that lowering of sea levels during
Pleistocene glaciations may have occurred many times for
different barriers and speciations.

Cases of allopatric sister species in which their ranges are
strongly asymmetric in size and separated by wide areas of
inhospitable habitats are examples of ‘classical founder
speciation’ (Paulay & Meyer, 2002). Endemic species on
islands are likely examples of founder speciation. In the Indo-
West Pacific, these include C. austriacus, C. madagascariensis, C. paucifasciatus and C. trichrous (Fig.
5D, G & H). In the Indo-West Pacific, the geography of
speciation events has been repeatedly discussed in
explanations of the focus of diversity in the East Indies
Triangle between the Philippines, Indonesia and New Guinea
(Palumbi, 1997). According to the center-of-origin
hypothesis, species originate within this central region and
then expand their range outwards (Briggs, 1999, 2000, 2003).
In contrast, the central region can be viewed as a ‘center of
accumulation’ for species that originated at the periphery of
the Indo-West Pacific (Jokiel & Martinelli, 1992). These two
hypotheses make different predictions about the location of
recently-formed species and their direction of spread.

In Chaetodon, we have found evidence of speciation in both
the Central Indo-West Pacific and at its periphery. A number of
narrowly-endemic species were distributed around the
periphery of the Indo-West Pacific, but not all the species
can be attributed to recent speciation events. Some species
(C. plelewensis, Fig. 5F; C. trichrous, Fig. 5H) are terminal
branches of the phylogeny and are therefore the products of
relatively recent divergence, as predicted by the center-of-
accumulation hypothesis. However, the others are relatively
basal branches of the phylogeny (C. auriga, Fig. 5A; C.
lineolatus, Fig. 5B; C. lamulatus, Fig. 5D; C. unimaculatus,
Fig. 5H) and represent ancient speciation events or even
peripheral relics (as predicted by the center-of-origin idea).
We have no evidence of range expansion by peripheral species
toward the center of the Indo-West Pacific. Endemic species
are also present in the Central region of Indo-West Pacific
and again, some are relatively basal (C. xanthurus, Fig. 5G)
as predicted by the center-of-origin hypothesis, whereas
others are terminal (C. octofasciatus, Fig. 5C). In summary,
evidence from Chaetodon adds to that from other studies and
reviews (Palumbi, 1996, 1997; Palumbi et al., 1997; Palumbi, 1997; Bellwood & Wainwright, 2002; Bernardi et al., 2004;
Williams & Reid, 2004) that suggest that speciation occurred
throughout the Indo-West Pacific, as a result of diverse
mechanisms.

The processes in evolutionary history are not only speciations,
but also extinctions. In the absence of a good fossil record,
there is no direct evidence of extinction in the genus
Chaetodon. In general, post-Miocene extinction is believed
to have been lower in the Indo-West Pacific than in the other
regions of the marine tropics (Vermeij, 1989; Palumbi, 1997;
Meyer, 2003). Pliocene extinctions were most severe in the
Western Atlantic and Eastern Pacific where 32% and 15% of
genera went extinct, respectively, compared to none in the
Indo-West Pacific (Vermeij, 1989).

However, at least one pattern within the subgenus Exornator
(C. fremblii spp. group.) does imply extinction. There is great
geographical distance between C. fremblii in Hawaii and its
sister clade, C. blackburnii, in East-South Africa and the
distance is too far to be explained by dispersal. Another
similar pattern happens within the subgenus Chaetodon. The
great distance between C. marleyi in East-South Africa and
its closely-related species in Western Atlantic also implies
extinction. According to these two patterns described above,
the distance between the two sister species are too great for
dispersal. Thus, we suggest that there were extinction events
that happened in the genus Chaetodon.

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