Molecular Phylogeny and Biogeography of the Grass Lizards Genus Takydromus (Reptilia: Lacertidae) of East Asia

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INTRODUCTION

The East Asian grass lizard genus Takydromus Daudin in the family Lacertidae is distributed in the Oriental and Palearctic regions with 16 or 17 species currently recognized (Arnold, 1997). Takydromus spp. are frequently found in grasslands, but some species prefer dense bush or forest environments (Ziegler et al., 1998; Ziegler and Bischoff, 1999). Most Takydromus spp. have slender bodies and extra-long tails (e.g., with a tail length usually over 300% of snout–vent length (SVL) in T. sexlineatus, and nearly 420% of SVL in T. sauteri). In some species, the ability to curl their tails helps them climb and move in dense vegetation (Arnold, 1997).

The systematic status of the genus Takydromus is controversial traditionally. Early taxonomists split several Takydromus spp. into the genera Apeltonotus and Platyplacopus (Boulenger, 1917). Apeltonotus, represented by T. dorsalis, was characterized by smaller dorsal scales. Later, the extremely rare A. sylvaticus with similar characters was described from Fujian Province, southeastern China (Zhao and Adler, 1993). Platyplacopus was characterized by the distinctive digit structure observed on T. kuehnei, with lamellae beneath the compressed toes. T. intermedius and Apeltonotus species were later transferred to Platyplacopus based on their differentiated toes (Pope, 1935). However, this character varies considerably among Platyplacopus and remaining Takydromus species (Arnold, 1997). Arnold (1989) proposed the union of these two genera, but later suggested a revision of Takydromus into the subgenera Takydromus and Platyplacopus based on phylogenetic analyses of morphological characters (Arnold, 1997). The subgenus Platyplacopus included T. sauteri, T. maragdinus, T. toyamai, and the former Platyplacopus species. The remaining species were placed in the subgenus Takydromus.

Despite the controversial phylogeny, one of the most spectacular features of grass lizards is the high percentage of endemism. Half of Takydromus spp. are endemic to islands aligned along the Pacific coast of the East Asian continent. These islands include several groups: mainland Japan, the Ryukyu Archipelago, and Taiwan (Fig. 1). T. tachydromoides is found on mainland Japan, with T. smaragdinus in the Amami and Okinawa group, T. toyamai in the Miyako group, and T. dorsalis in the Yaeyama group, and four species are endemic to Taiwan. T. hsuehshanensis is found only in the Central Mountain Range of Taiwan at over 2000 m. T. sauteri and T. stejnegeri are distributed on the east-
Molecular Phylogeny and Biogeography of Takydromus

Takydromus species were collected, and the complete mitochondrial 12S rRNA gene was sequenced. We used mtDNA sequence data to (i) construct a phylogenetic relationship of Takydromus and test the hypothesis of Arnold’s morphological phylogeny and the validity of two subgenera, (ii) evaluate alternative models of the biogeographic history of Takydromus on eastern Asian islands, and (iii) discuss general implications for the historical biogeography of Eastern Asia.

Materials and Methods

Sample Collection

During 1997–1999, we obtained 13 Takydromus species (Table 1). T. dorsalis was provided by Professor Hidetoshi Ota (Ryukyu University), and tissues of T. amurensis were provided by Dr. Szu-lung Chen (Kyoto University). We collected the remaining 11 species and these are now preserved in the Department of Biology, National Taiwan Normal University. Except for T. toyamai and T. intermedius, at least two individuals were sequenced in each species. Specimens of Eremius argus in our collection were used as the outgroup.

Genomic DNA was extracted from muscle tissue by a standard phenol/chloroform protocol. A complete mitochondrial 12S ribosomal RNA (rRNA) gene and flanking valine tRNA and 16S rRNA genes were amplified as a single fragment with “vertebrate-universal” primers, PL: 5'-AGTCTGCTCAAAAAAGATTAATTGA-3' and PH: 5'-TCTTGGTCGAAACCTCAGTTA-3', designed by Wang et al. (2000). Double-stranded PCRs were performed in 50-µl reaction volumes with the following thermal cycles: 1 cycle at 94°C (3 min); 35 cycles at 94°C (30 s), 55°C (40 s), and 72°C (70 s); and 1 cycle at 72°C (10 min). PCR products were run on 2% low-melt (65°C) NuSieve GTG agarose (BMA BioProducts) in 1 × TAE running buffer. The target fragment was gel-isolated and purified following the protocol for the Viogene PCR fragment purification system (Viogene). The purified PCR products were subsequently used as the template in the direct DNA sequencing reaction. Both strands were sequenced with the BigDye Terminator Sequencing Kit (PE Applied Biosystems) with the same primers used for PCR amplification. In addition, two internal primers, PL2: 5'-ACAAACTAGGATTAGATACCC-3' and PH2: 5'-AGTCTGCTCAAAAAAGATTAATTGA-3', were designed to facilitate complete sequencing. Sequencing products were run on an ABI 377 automated sequence analyzer (PE Corp.). The sequences obtained in this study have been submitted to GenBank under accession numbers listed in Table 1.

Sequence Alignment, Secondary Structure, and Phylogenetic Analyses

Sequences were initially assembled with the Pileup option in the GCG package (Wisconsin Package Ver-
tion 10.1, Genetics Computer Group, Madison, WI) and then checked and adjusted to the extent possible to confirm with secondary structure models (Neefs et al., 1993; De Peer et al., 1994; Springer et al., 1995; Hickson et al., 1996; Richards and Moore, 1996). The secondary structure of Takydromus 12S rRNA was constructed manually, and loops and stems were determined in all species. To investigate whether transitions and transversions in the Takydromus 12S rRNA gene may be saturated, the pairwise substitution rate, defined as numbers of transitions or transversions divided by the length of comparison, was plotted against pairwise Tamura–Nei distances (Tamura and Nei, 1993).

Phylogenetic analyses were performed with PAUP* 4.0b6 (Swofford, 2001). The 12S rRNA sequences of T. sexlineatus collected from Vietnam and another nine Eurasian lacertid species (Fu, 2000) were retrieved from GenBank for analyses (Table 1). The loop sequence at the 49th stem (44 bp in length) was excluded from further analyses due to its ambiguous alignment.

Maximum-parsimony (MP) analyses were performed with heuristic searches with 100 random additions of sequences to search for the most-parsimonious trees.
Bootstrap with 10,000 pseudo replicates and a heuristic search were used to examine the robustness of cladades in the resulting trees. Neighbor-joining (NJ) analysis based on Tamura–Nei distances (Tamura and Nei, 1993) was performed and corrections were made for unequal base frequencies and different transition and transversion rates. The robustness of NJ phylogenies was assessed by the 10,000-bootstrap option. For maximum-likelihood (ML) analysis, the best-fit model of substitution and the parameter estimates used for tree construction were chosen by performing hierarchical likelihood ratio tests (Huelesenbeck and Crandall, 1998). Likelihood-ratio tests indicated that the models D5 and no clock, with (n − 2) degrees of freedom where n is the number of sequences (Muse and Weir, 1992), was also performed by TREE-PUZZLE 5.0 (Schmidt et al., 2000) to determine whether there was a statistical difference in evolutionary rates among cladades. For comparing the alternative hypotheses of vicariant or dispersal speciation models, Kishino–Hasegawa test (Kishino and Hasegawa, 1989) and Templeton test (Templeton, 1983) were preformed in PAUP 4.0b6.

**RESULTS**

Sequence Characteristics

We obtained 32 haplotypes from the 40 Takydromus individuals and 2 haplotypes from E. argus. All haplotypes were submitted to GenBank (Table 1). The lengths of Takydromus 12S rRNA ranged from 949 (T. sauteri) to 958 bp (T. hsuehshanensis). Intraspecific length polymorphisms were observed in T. amurensis, T. tachydromoides, T. smaragdinus, and T. formosanus, with insertions or deletions of 1–3 bp. The 12S rRNA of E. argus was 949 or 950 bp in length, with a 1-bp insertion/deletion between the two haplotypes. Intraspecific sequence variation ranged from 0 to 0.57% (T. stejnegeri), and interspecific divergence ranged from 2.36% (T. stejnegeri vs T. septentrionalis) to 15.90% (T. stejnegeri vs T. sexlineatus from Vietnam) (Table 2). Comparisons between outgroups and ingroups ranged from 11.62% (Psammodromus algirus vs T. dorsalis) to 21.23% (Psammodromus algirus vs T. sexlineatus from Vietnam).

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<th>SAU</th>
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<th>DOR</th>
<th>TAC</th>
<th>AMU</th>
<th>SEP</th>
<th>STE</th>
<th>TOY</th>
<th>HSU</th>
<th>FOR</th>
<th>KUE</th>
<th>SEXHK</th>
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</table>

Note. SMA, T. smaragdinus; SAU, T. sauteri; INT, T. intermedium; DOR, T. dorsalis; TAC, T. tachydromoides; AMU, T. amurensis; SEP, T. septentrionalis; STE, T. stejnegeri; TOY, T. toyamai; HSU, T. hsuehshanensis; FOR, T. formosanus; KUE, T. kuehnei; SEXHK, T. sexlineatus from Hong Kong; SEXVN, T. sexlineatus from Vietnam (Fu, 2000).
Phylogenetic Analyses

Because intraspecific polymorphisms were much lower than interspecific divergences in Takydromus, we applied the consensus sequence of the 12S rRNA gene of each species for phylogenetic analyses. We obtained 14 ingroup OTUs (13 species in our collections plus T. sexlineatus from Fu (2000)) and 12 outgroup OTUs (E. argus plus 11 species retrieved from GenBank). Ambiguous regions with poor alignment consisting of 44 characters were eliminated from phylogenetic analyses. In the remaining 946 characters, 425 (44.93%) were variable, and 300 (31.72%) of the variable characters were parsimony informative. Parsimony analysis revealed a single MP tree (Fig. 3) with a tree length of 1496, a consistency index (CI) of 0.4519, a homoplasy index (HI) of 0.5481, a retention index (RI) of 0.5036, and a rescaled consistency index of 0.2276. The tree topology strongly supports Takydromus as a monophyletic group with 100% bootstrap support. Five internal nodes revealed high topology confidence with bootstrap values greater than 90%; (T. kuehnei, T. sexlineatus VN), (T. intermedius, T. dorsalis), (T. hsuehshanensis, T. formosanus), (T. toyamai, T. septentrionalis, T. sten negeri), and the combination of the last two clades. Three nodes revealed marginal significance (70% < BPs < 90%), including the grouping of (T. sauteri, (T. dorsalis, T. intermedius)), and two internal branches indicating the assemblage of lower groups. Most nodes among outgroup genera revealed bootstrap values lower than 50% (not shown), which might be the major reason for the low CI and RI values. This phenomenon reinforced our previous prediction on the poor resolution ability of the 12S rRNA gene for intergeneric analyses in lacertids.

Although the arrangement of outgroup species differed, neighbor-joining analysis produced an ingroup topology identical to that of the MP tree as shown in Fig. 3. Four nodes retained high bootstrap probabilities (>90%), and five nodes revealed marginal significance (70–90%). The monophyly of Takydromus remained 100% supported. ML analysis was based on the results of the Modeltest likelihood ratio test (Table 3), suggesting the application of Tamura–Nei’s Model (Tamura and Nei, 1993) and the general time-reversible option. It revealed an ingroup topology nearly identical to those of the MP and NJ trees (ln likelihood = 7699.27). Most nodes remained fairly well supported with quartet-puzzling steps between 65 and 90%. The only difference was the poor resolution among T. tachydromoides, T. amurensis, and the neighboring five-species monophyletic group. This node was later solved in another quartet-puzzling test with 1000 replicates in TREE-PUZZLE 5.0. T. amurensis was clustered with the monophyletic five-species group in this test (74%), showing the relationship of (T. tachydromoides, (T. amurensis, five species)). This result was identical to the previous MP and NJ tree topologies on this node.

According to the consistency of different tree criteria, we used this gene tree to stand for the possible speciation tree. Thirteen nodes (from a to m) and three major species groups were defined for further discussion (Fig. 2).
3). Group A contained T. kuehnei and T. sexlineatus, Group B contained T. smaragdinus, T. sauteri, T. dorsalis, and T. intermedius, and Group C contained the remaining species with two subgroups further defined. Subgroup C1 contained T. hsuehshenensis and T. formosanus, and Subgroup C2 contained T. septentrionalis, T. stejnegeri, and T. toyamai. It was controversial whether we should include T. smaragdinus in Group B since Groups A and C were monophyletic but Group B was not. However, we preferred this grouping based on the following reasons. First, T. smaragdinus is the most basal and ancient species compared to other Group B and C species. According to our deduction (to be described below), Group B is obviously an earlier-evolved clade compared to Group C. Under such a situation, we think it is reasonable to connect T. smaragdinus to the serial events of Group B speciation. Second, although the tree topology indicated a (T. smaragdinus, Group B, Group C) relationship, the bootstrap values on related nodes ("d" and "e") are less supported. These bootstrap values are lower because of the existence of another possible relationship, a (T. smaragdinus, Group C, Group B, Group C) relationship. The probability of clustering T. smaragdinus to Group C species is comparatively much lower. Such relationships, while not shown in the decisive tree topology, indicate a much closer relationship of T. smaragdinus to Group B species than to Group C species.

Molecular Clock Test

To apply the 12S tree topology to an assessment of speciation events of Takydromus on islands of eastern Asia, a maximum-likelihood ratio test was performed to examine the molecular clock hypothesis for Groups B and C. We excluded Group A species in this analysis because (1) T. sexlineatus from Vietnam is a long-branch species and (2), being distributed mainly in southern Asia, they are not directly related to specia-


**TABLE 3**

<table>
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<tr>
<th>Null hypothesis</th>
<th>Null Model</th>
<th>Alternative Model</th>
<th>$-\ln L_0$</th>
<th>$-\ln L_1$</th>
<th>df</th>
<th>P</th>
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</thead>
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<td>H$_0$: J C</td>
<td>H$_1$: F81*</td>
<td>8951.49</td>
<td>8898.10</td>
<td>3</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>Transition = transversion</td>
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<td>H$_1$: HKY*</td>
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<td>&lt;0.000001</td>
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<td>H$_1$: TrN¢</td>
<td>8610.31</td>
<td>8585.10</td>
<td>1</td>
<td>&lt;0.000001</td>
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<td>Equal transversion rates</td>
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<td>H$_1$: TIM¢</td>
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<td>H$_1$: TrNG¢</td>
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<td>7695.25</td>
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<td>&lt;0.000001</td>
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<td>H$_1$: TrNGf</td>
<td>7695.25</td>
<td>7687.40</td>
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<td>0.000075</td>
</tr>
<tr>
<td>*Molecular clock likelihood ratio test</td>
<td>H$_0$: with clock</td>
<td>H$_1$: without clock</td>
<td>3556.12</td>
<td>3561.78</td>
<td>10</td>
<td>0.3331</td>
</tr>
</tbody>
</table>

Note: The underlined model indicates the final suggestion.
* Likelihood ratio test (Muse and Weir, 1992) for examining whether a molecular clock exists among ingroup species. Only Takydromus species (but excluding T. kuehnei and T. sexlineatus from Vietnam) joined the latter analysis.
* Unequal base frequencies: G = 0.1232, A = 0.3895, T = 0.2604, C = 0.2269.
¢ Unequal ratio between transition and transversion.
¢ Equal transversion rates: R[A-T] = 0.6150.
f Gamma distribution shape parameter: G = 1.0000.
f Proportion of invariable sites: I = 0.3757.

**DISCUSSION**

Molecular Phylogeny of Takydromus

Our data do not support the division of Takydromus into the subgenera Platyplacopus and Takydromus (Arnold, 1997). The bootstrap values clustering this genus (node a in Fig. 3) are 100% in both the MP and the NJ trees and 95% in the ML quartet-puzzling test. Species of the subgenus Platyplacopus and the subgenus Takydromus (denoted as "+" and "*" in Fig. 3, respectively) were mixed in the phylogenetic tree. The highly probable clustering of (T. toyamai, T. stejnegeri, T. septentrionalis) revealed an assemblage of the subgenus Takydromus and Platyplacopus species while the clustering of the (T. kuehnei, T. sexlineatus) group revealed another. Because neither of these subgenera was monophyletic based on molecular phylogeny, we are not convinced of the subgeneric definitions.

The phylogenetic status of T. sexlineatus is worthy of note. The DNA sequence of a single T. sexlineatus specimen collected from Vietnam (Fu, 2000) was clustered with T. kuehnei (Taiwan) with very high bootstrap values (100% in NJ, 99% in the MP tree) and the same species collected from Hong Kong revealed an unexpected high genetic distance (0.1236) to the Vietnam specimen (Table 2). This value is much higher than most of the other interspecific pairings in this genus. Even for the two tightly clustered species of (T. kuehnei and T. sexlineatus VN), the genetic distance is still quite high (0.0819). Apparently these organisms belong to distinctly different species, and one of them either is misidentified or belongs to a new species. T. sexlineatus is the most problematic Takydromus species with the widest distribution throughout southern China, Vietnam, Laos, Cambodia, Thailand, Burma, Assam, the northern Malay peninsula, Borneo, Java, and Sumatra (Arnold, 1997). Two subspecies are recognized: T. s. sexlineatus Daudin 1802 and T. s. ocellatus Guerin-Meneville 1829. Specimens from Vietnam and southern China are usually regarded as T. s. ocellatus, and this subspecific name was still applied recently both in Hong Kong and in Vietnam faunal lists (Karsen et al., 1986; Bogadek and Lau, 1997; Ziegler et al., 1998, 1999). However, the type locality of this subspecies (described as T. ocellatus) was lost and controversial and considered to be either from southern China or from Vietnam (Zhao and Adler, 1993). Such a situation causes confusion in distinguishing Takydromus species in both regions.

Tropical Takydromus species require further investigations. T. wolteri has been reported from Vietnam, but is usually regarded a Palearctic species; the occurrence of this species in Vietnam is doubtful (Ziegler et al., 1998, 1999). Instead, a new subspecies, T. kuehnei vietnamensis (Ziegler and Bischoff, 1999), and a new species, T. hani (Chou et al., 2001), were recently described in Vietnam. Fu's specimen is probably a misidentification of one of these two species, or perhaps one of our specimens belongs to another cryptic species of T. sexlineatus. These findings suggest that the biodiversity of Takydromus fauna is probably more diverse than indicated by our present observations. We suspect that more cryptic Takydromus species in tropical regions will be discovered as additional field studies or
molecular studies are performed, especially for those widely distributed or morphologically variable species.

Biogeography of Takydromus on East Asian Islands

Most Takydromus biodiversity occurs on islands of eastern Asia. Located at the margin between the Eurasian and the Philippine Sea plates, these islands have experienced tremendous geological alterations since their formation around 10 million years ago. Sea level alteration caused by glaciations changed the pattern of connections among island groups. The process of alteration in land connections could be either deduced from the offshore topography or referred from paleogeographic models (e.g., Kimura, 2000). According to recent information, most offshore regions under the East China Sea would dry up during glaciations, causing the appearance of a larger continental land area (Fig. 4C). In some cases, more severe glaciations would provide the opportunity for a connection between the Southern Ryukyus (including the Miyako Group and the Yaeyama Group) and the continent of Asia via the land area extended from northeastern Taiwan (Fig. 4B). In the most severe glaciations, as the most rare geological cases, the Central Ryukyus would connect to the Asian continent through a peninsular-like land bridge (Fig. 4A). When sea levels rose again, this peninsula would sequentially break up. The Okinawa-Amami Group was isolated first, then the Miyako-Yaeyama Group, and finally the division between the Okinawa and the Amami Groups, between the Miyako and the Yaeyama Groups, and between Taiwan and mainland Asia (Figs. 4A–4D). Such repetitive and reversible processes must have been repeated several times since the formation of these islands in the Miocene.

In most cases, terrestrial animal species with poor oversea dispersal abilities could only disperse by dry lands. The peninsular-like land bridge during glaciations offered an excellent but the only opportunity to disperse and colonize from mainland Asia to the Ryukyus. However, when the glaciations ended, oceanic isolation would occur, and vicariant events began. If vicariant speciation is the major mechanism for the formation of island-endemic species in eastern Asia, the biogeography of terrestrial species should be tightly related to the sequential separation of island groups. As an idealized model, the terrestrial phylogeny should reveal a relationship of ((Amami, Okinawa), ((Miyako, Yaeyama), (Taiwan, China))), reflecting the sequential vicariant process. An UPGMA clustering of the similarity index for reptile fauna on these islands showed the existence of this tendency (Ota, 2000). Taxonomic studies for certain reptile lineages also suggested the affinity between closely related island groups (Hikida and Ota, 1997; Hikida and Motokawa, 1999).

The phylogeny of Takydromus species on these islands indeed supports this evolutionary model: a vicariant speciation process was observed in Group B and Group C2 species. Group B species revealed the relationship of (T. smaragdinus, (T. sauteri, (T. dorsalis, T. intermedius))). The hypothetical model for the speciation process is proposed in Fig. 5. T. smaragdinus, endemic to the Okinawa-Amami Group, is the
most basal and ancient species of Groups B and C, but with a closer phylogenetic relationship to Group B species. It is now distributed only on the farthest and most isolated islands. We deduced that ancient Takydromus species reached their modern distributions in Okinawa through a land connection (Fig. 5A). When the land bridge submerged, the Okinawa–Amami Group was isolated and caused the separation between ancient T. smaragdinus and other species (node d in Fig. 3; Fig. 5B). Mapping the distribution of Group B species reveals the biogeographical relationship of (Okinawa, (Taiwan, (Yaeyama, China))). In our hypotheses, we proposed the speciation of T. sauteri in eastern Taiwan as an independent allopatric speciation event, which caused a mismatch in the biogeographic model. The Central Mountain Range in Taiwan (the dashed line in Fig. 5) formed a major geographic barrier between this species and the others. It could be either a vicariance event (uplift of the mountains caused the isolation) or a dispersal event (lizards crossed the barrier and entered eastern Taiwan) (Fig. 5C), depending on which event (speciation versus geological alteration) occurred earlier. The remaining species would follow the relationship of (Okinawa, (Yaeyama, China)) which shows a partial match to the idealized phylogenetic relationships.

The phylogeny of Group C2 species (T. toyamai, (T. stejnegeri, T. septentrionalis)) also fits the vicariant hypothesis reflecting the biogeographic relationship of (Miyako, (Taiwan, China)) (Fig. 6). Speciation processes following the sequential breakup of land connections were also observed in this clade. Here we noticed that the interspecific genetic distance among C2 species range between 0.024 and 0.041, only half of that among Group B species (0.054–0.088). If species in these two groups evolved at similar rates, the speciation of C2 species must have occurred later than that of Group B species. In other words, the colonization, separations, and speciation of Group C2 species should have been caused by later geological events. Such a multicolonization hypothesis has been proposed in population genetic studies on the Indian rice frog (Rana...
limnocharis) distributed on East Asian islands (Toda et al., 1997, 1998; Toda, 1999).

Two proposals account for the absence of Group C2 in the Okinawa–Amami Group. First, the connection of continental Asia to Central Ryukyus is a very uncommon geological event; thus, Group C species have never obtained opportunities to reach this region. Second, niche competition may exist among older and younger Takydromus lineages. It is worthy of notice that different Takydromus species never coexist in the islands of Ryukyus. Principles of island biogeography (MacArthur and Wilson, 1966) could be applied to explain those “lost lineages,” e.g., the lack of Group C species in the Yaeyama Group or the lack of Group B species in the Miyako Group and western Taiwan. Competition might have caused frequent extinction events and the coexistence of Takydromus species on these islands because of their smaller terrestrial surfaces. The effects of niche competition must be extremely severe on tiny islets like those of the Miyako or the Yaeyama Groups.

Another example of allopatric speciation in north-eastern Asia explains the speciation of T. tachydromoides from other Group C species. It is reasonable to deduce that the oceanic separation between Japan and Korea led to the isolation and speciation of T. tachydromoides. The mean genetic distance between T. tachydromoides and other Group C species is 0.096, very similar to the genetic distance between T. smaragdinus and other species (0.100). We infer that these two species were isolated in a similar geological period. This radiation pattern offers another explanation for the comparatively low bootstrap values on nodes d, e, and h, which revealed poor resolution for solving the relationship among T. smaragdinus, T. tachydromoides, and other species.

Instead of vicariance, speciation in the Ryukyus may be caused by dispersals. For example, skinks of the genus Eumeces in eastern Asia, with a probably better ability of oversea dispersal, revealed the hypothetical speciation mechanisms of both vicariance and dispersals (Hikida, 1993; Hikida and Motokawa, 1999). If dispersals have occurred in the Takydromus lineages, the
phylogenetic relationship of Group B should be (T.intermedius, (T. sauteri, T. dorsalis, T. smaragdinus)), and for Group C2 the relationship should be (T. septentrionalis, (T. stejnegeri, T. toyamai)). However, a dispersal speciation model on the islands of eastern Asia was rejected by both the Kishino-Hasegawa test (P = 0.0036) and the Templeton-Wilcoxon signed-rank test (P = 0.0066), with the tree length of the alternative phylogenetic tree being 15 steps longer than that of the original MP tree. We deduced that vicariance should be the main mechanism for the speciation of island Takydromus.

Although time estimation by molecular clocks is controversial, estimating the approximate time of divergence can provide a crude pattern to help us understand the formation process of the fauna on the islands of eastern Asia. Taxon-specific clocks are usually reliable in local time, local range, or specific taxonomic levels. Because the model test on temperate and subtropical Takydromus species did not reject the molecular clock (Table 3), we assume that they evolved at a similar rate. The crucial point in this estimation is the separation of T. smaragdinus, the most isolated species basal to Group B and C members. If we could obtain the timing of the separation between T. smaragdinus and other species, the age of each node on the phylogenetic tree could be deduced.

Paleogeography of eastern Asian islands in the Pleistocene has been proposed by Kizaki and Oshiro (1980) and Kimura (2000). According to their hypotheses, the last connection of Okinawa Island to mainland Asia was in the early Pleistocene, at around 1.0–1.3 MYA. Since the possibility of overseas dispersal is excluded in island Takydromus, this should be the lower limit of the time scale for T. smaragdinus to separate from other species. The genetic distances between T. smaragdinus and other temperate and subtropical species range between 0.081 and 0.110 (Table 2), with a mean of 0.100. Therefore, the evolutionary rate of Takydromus 12S rRNA gene is about 0.100/MY between pairs of lineages. The interspecific genetic distances among Group B species range between 0.088 (T. smaragdinus vs other species) and 0.054 (T. dorsalis vs T. intermedius) (Fig. 5). Under the estimation of the molecular clock, we could restrict the speciation time of Group B species to 0.88–0.54 MYA. Similarly, the genetic distances among Group C2 species range between 0.041 (T. toyamai vs other species) and 0.024 (T. stejnegeri vs T. septentrionalis) (Fig. 6), indicating the speciation time of Group C2 from 0.41 to 0.24 MYA. According to Kimura’s hypothesis, the latter period is close to another severe sea level change that occurred in the late Pleistocene. Estimation of the speciation time scale reinforces our multicolonization hypothesis: colonization occurred at least twice, and two series of vicariant events have occurred in the evolutionary history of Takydromus. Under this hypothesis, it is obvious that Group B species should have existed and occupied localities similar to modern distributions when Group C2 species arrived (Fig. 6A).

However, if the time scale employed from the above estimation is corrected, the evolutionary rate of Takydromus 12S rRNA gene (0.1/MY/pair) is approximately five times higher than the common evolutionary rate of vertebrate mitochondrial genomes (0.02/MY/pair) (Brown et al., 1979; Avise, 2000). A possible explanation for this is the acceleration in the evolutionary rate for these lizards, since Squamata mitochondrial genes have been found to evolve at various rates in different taxonomic units according to our recent comparisons among several local organisms (S.-M. Lin, unpublished data). Nevertheless, the evolutionary rate of the Lacertidae 12S rRNA gene could be crudely estimated by referring to the research on the biogeography of this family (Fu, 1998; Harris et al., 1998). According to their inferences on the branching process of lacertid lineages, an evolutionary rate of 0.01–0.03/MY/pair could be obtained. Inference on the biogeography of varanid lizards (Reptilia: Varanidae) offered a similar but even slower evolutionary rate for this gene (Fuller et al., 1998). Under such circumstance, the unexpected rate bias of the Takydromus 12S rRNA gene seems to be less probable.

The alternative explanation indicates that T. smaragdinus should have a separation time scale longer than 1.0–1.3 MY. Instead of the previous Quaternary-origin model, a Tertiary-origin model seems to be more reasonable. Based on the highly divergent fauna and the deep branch length obtained from molecular evidence from several endemic organisms in Okinawa (Takeda and Ota, 1996; Toda et al., 1999), Hikida and Ota (1997) inferred a longer isolation time scale of the Okinawa-Amami Group. Their hypothesis assumed that the early Pleistocene land bridge did not connect to the Okinawa Group; thus, these islands had been completely isolated since the early Pliocene (Hikida and Ota, 1997; Hikida and Motokawa, 1999). Based on this model, the isolation of T. smaragdinus is about 8 MY. This separation time scale implies a 0.0125/MY/pair evolutionary rate in Takydromus, closer to the previous estimations on Squamata 12S rRNA genes. Under the estimation of this hypothesis, the speciation events of Group B species occurred from 7.0 to 4.3 MYA, while Group C2 species evolved during 3.3 to 1.9 MYA. The colonization events and serial vicariant speciation were still observed to have occurred twice. Our multicolonization hypothesis is still supported in this model, but their speciation age is advanced to the Tertiary. This time scale is also supported by the research on other endemic vertebrates in the Central Ryukyus, such as vipers or rodents (Toda et al. 1999; Suzuki et al., 2000).

Another possibility that did not reject the Tertiary-origin model is that the later geological events did not have a significant influence on the already-existing
species. Island Takydromus species may have evolved and existed at their modern positions since the Tertiary, with later glaciations making few alterations in their current distributions. Although hypothetical intraspecific competition and extinction may have occurred during their secondary contact, it did not influence the major distribution pattern of Takydromus species in the eastern Asian islands.

Applications of different models provide different explanations for the speciation of T. sauteri. The Central Mountain Range in Taiwan is the main barrier isolating this species. The mean genetic distance between T. sauteri and its sister taxa is 0.072, with a 0.72-MY separation age in the Quaternary-origin model and a 5.8-MY age in the Tertiary-origin model. The sudden separation age in the Quaternary-origin model and a and its sister taxa is 0.072, with a 0.72-MY ing this species. The mean genetic distance between Mountain Range in Taiwan is the main barrier isolating this species. Island

In conclusion, the purpose of this paper is to try to resolve the phylogeny of eastern Asian Takydromus and propose a hypothetical speciation process of this taxon deduced from geological inferences. Vicariant speciation events were deduced according to the match between molecular phylogeny and paleogeography, which is influenced by the alterations in sea levels during glaciations. Because different Takydromus lineages reveal different scales for their interspecific genetic distances, a multicolonization hypothesis was proposed under the application of a molecular clock. Alternative models for timing the occurrence of speciation events were discussed and the Tertiary-origin model seems to be more probable than the Quaternary-origin model. We do not expect simple models to resolve the complete course of speciation events that occurred on these islands, but to provide a crude and reasonable scenario to explain the richness of faunal endemism in this region. The speciation process and phylogeography of organisms on eastern Asian Islands are highlighted, especially for comparisons between different taxonomic units.

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