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# Genetic Population Structure of the Japanese Grass Lizard, *Takydromus tachydromoides* (Reptilia: Squamata), Inferred from Mitochondrial Cytochrome b Variations

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**Abstract: We investigated the genetic population structure of the Japanese grass lizard, *Takydromus tachydromoides*, based on polymerase chain reaction—restriction fragment length polymorphisms (PCR-RFLPs) and DNA sequence analyses of the mitochondrial cytochrome b gene. The PCR-RFLP analysis of 163 specimens collected from the main islands of Japan and the adjacent islands identified a total of 25 mitochondrial haplotypes (mitotypes). The phylogeny of the mitotypes revealed that Osumi and Tokara Island populations were remarkably diverged from all other populations on the main islands of Japan. Furthermore, several regional groups were also recognized at the western part of Japan, namely mitotype groups in Kyushu, those in the western Honshu, and those in the central Honshu and Shikoku. In contrast, little genetic diversity was observed throughout eastern Japan. The regional genetic differentiation and recent range expansion of this species are considered to be associated with past geological events and climate changes.**

Key words: Phylogeography; Mitochondrial DNA; Ryukyu Archipelago; Japanese grass lizard

## Introduction

The Japanese grass lizard, *Takydromus tachydromoides* (Lacertidae) is one of the most abundant reptiles in Japan. This species has a slender body with an extremely long tail, and is found typically in grasslands on Hokkaido, Honshu, Shikoku, Kyushu, their adjacent islands, and a part of Tokara Islands. Among *Takydromus* species, only this species

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is distributed widely in the main islands of Japan, while other species are found in the East Asian mainland and offshore islands (Hikida, 2002).

Twenty-two *Takydromus* species have been described so far (Utez and Hošek, 2015), and a half of them are endemic to the East Asian islands, ranging from the main islands of Japan to Taiwan (e.g., *T. smaragdinus* in the central Ryukyu Islands, *T. dorsalis* in the Yaeyama Islands, *T. toyamai* in Miyako Islands, and *T. formosanus* in Taiwan) (Lue and Lin, 2008). Their phylogenetic relationships and biogeography have been well studied based on morphological characters (Arnold, 1997) and molecular data (Lin et al., 2002; Ota et al., 2002). These studies suggested that a series of vicariances promoted allopatric speciation of these lizards among the islands. However, they focused mainly on interspecific relationships, and thus little is known about the genetic variation within *T. tachydromoides*.

With regard to the geographic variation of *T. tachydromoides*, neither morphological variation nor subspecific delimitation has been reported to date. Ota et al. (2002) included nine samples of this species, collected from the main islands of Japan and northern part of the Ryukyu Islands, in their molecular phylogenetic analysis of 12S and 16S rRNA genes. Although they found that *T. tachydromoides* is monophyletic with an intraspecific genetic structure, their study did not analyze populations from the northern part of Japan or discuss the phylogeography of the species. In addition, a recent genetic study identified two cryptic species within *T. formosanus* in Taiwan, which showed few morphological differences (Lue and Lin, 2008). Because *T. tachydromoides* has a wide distribution range along the main islands of Japan, more comprehensive analysis is required to investigate its genetic variation.

In this study, we assessed the genetic diversity and intraspecific phylogenetic relationships of *T. tachydromoides* using mitochondrial cytochrome b sequence data from samples covering almost its entire distribution range. Based on the inferred phylogeny, we discuss the regional genetic differentiation and historical biogeography of the species. The phylogeographic patterns of this species throughout its entire range will provide important information on the geographical divergence and historical gene flow among regional populations of Japanese reptiles, and the formation of herpetofauna in Japan.

## Materials and Methods

### *Sample collection*

We used 163 individuals of *Takydromus tachydromoides* collected from 53 different populations along the Japanese Archipelago (Fig. 1). We also collected four *Takydromus* species, *T. amurensis* at Vladivostok (site 55), *T. dorsalis* (unknown locality, provided by S. Sengoku), *T. smaragdinus* at Akashima (site 54) and *T. wolteri* at Vladivostok (site 55), and used them as outgroups for phylogenetic analyses.

### *DNA extraction and PCR amplification*

Total DNA was extracted from muscle tissue by proteinase K digestion, phenol/chloroform extraction and isopropanol precipitation. Each DNA sample was dissolved in TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0). The segment (1130 bp) containing the part of mitochondrial cytochrome b gene and $tRNA^{Thr}$ was amplified by PCR using the primers, L14990t (5'-CATCCAACATCT CTGCTTGATGAAA-3') modified based on the L14990 primer (Helm-Bychowki and Cracraft, 1993), and H16065 (5'-GGAGTCTT CAGTCTCTGGTTTACAAGAC-3') (Helm-Bychowki and Cracraft, 1993). Because these primers were not effective for two samples, we used other primer pairs, YK-L (5'-TTTTGG CTCCCTATTAGGTA-3') and YK-H (5'-CA GTGCTTTGAACTTAAGCT-3') for the Suwanosejima sample (site 53) of *T. tachydromoides*, and SK-L (5'-AACTTTGGATCA TTACTGGG-3') and H16065 for the *T. dorsalis* sample. PCR amplification was performed in a 25 µl volume containing

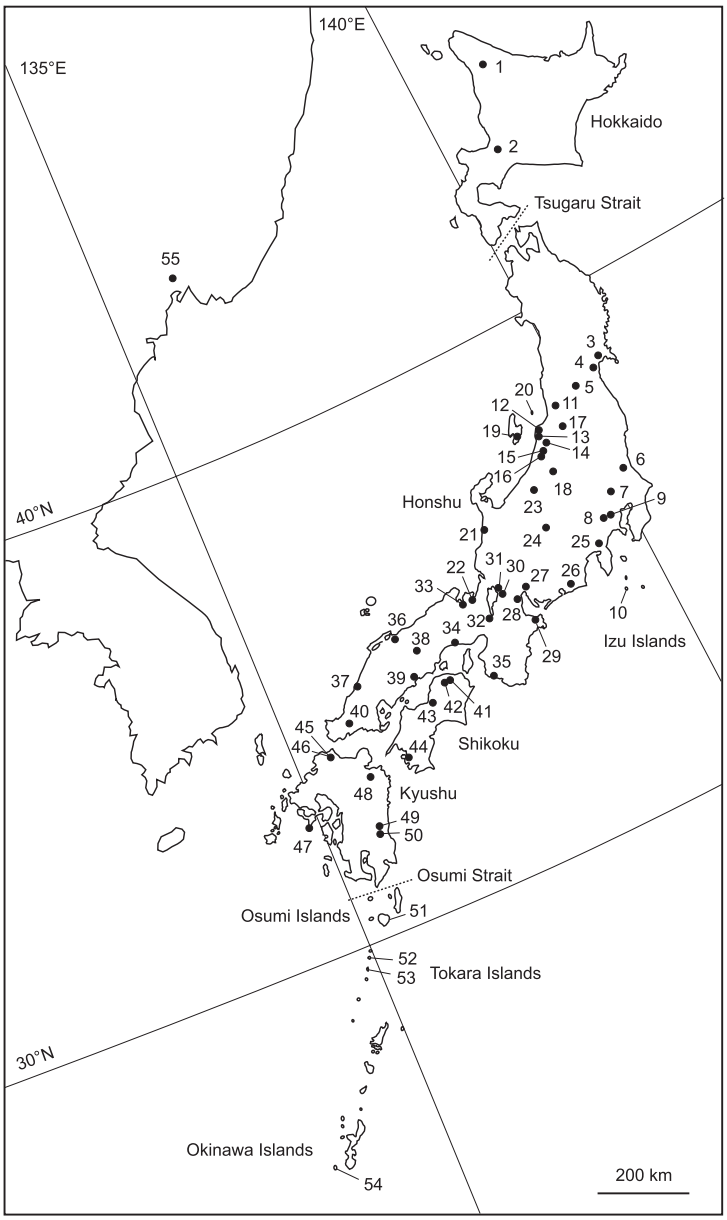


FIG. 1. Collection sites of *Takydromus tachydromoides*. The numbers refer to the locations listed in Table 3.

0.2 mM of dNTPs, 0.25 μM of each primer, 1 μl of template DNA (below 100 ng), 1 × Ex Taq Buffer and 0.6 units of Ex Taq polymerase (Takara Bio, Otsu, Japan). PCR reactions were performed under the following conditions: 96C for 2 min for initial denaturation, 35 cycles of amplification (96C for 30 sec, 43–60C for 30 sec and 72C for 2 min) and 3 min for the final extension at 72C.

*Identifying mtDNA haplotypes using PCR-RFLP analysis*

Amplified cytochrome b segments of all the samples were digested with six restriction

enzymes (Alu I, Hae III, Hha I, Mbo I, Msp I, and Rsa I), in accordance with the suppliers' instructions. The digested restricted fragments were separated by electrophoresis using 6% polyacrylamide gels. Digested fragments were visualized and photographed on an ultraviolet transilluminator after ethidium bromide staining. Mitochondrial DNA haplotypes (mitotypes) were assigned based on the combination of RFLP patterns.

*Sequencing and phylogenetic analysis*

To infer the phylogenetic relationships of the *T. tachydromoides* mitotypes, we sequenced the 1046 bp of the mitochondrial genome containing the cytochrome b gene for 30 individuals representing all the mitotypes and the outgroups. Direct sequencing of the PCR products was performed using an ABI PRISM 310 Genetic Analyzer with the BigDye Terminator Cycle Sequencing FS Ready Reaction Kits (Applied Biosystems, Foster City, CA, USA), following the supplier's instructions. Both strands were sequenced with the same primers used for PCR amplification and several internal primers. The nucleotide sequence data reported in this paper was deposited in the DDBJ/EMBL/GenBank (Accession numbers LC066048–LC066081).

Multiple alignments of the cytochrome b sequences were done using the CLUSTAL X version 1.81 (Thompson et al., 1997) with the default settings, and the DNA alignment was edited using MEGA version 6 (Tamura et al., 2013). The phylogenetic relationships were analyzed by Bayesian inference (BI) and neighbor-joining (NJ) methods. The BI analysis was performed based on Markov Chain Monte Carlo (MCMC) using MrBayes v3.2.5 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) with the GTR+I+G model selected by a hierarchical likelihood ratio test (hLRT) implemented in MrModeltest v2.3 (Nylander, 2004). Two independent runs of four Markov chains were conducted for 10 million generations each, sampling a tree every 100 generations. After checking the parameter estimates and convergence without discordance between the two independent MCMC runs using Tracer v1.6 (Rambaut and Drummond, 2009), we discarded the first 25,000 trees as burn-in for each run, and calculated a consensus topology and Bayesian posterior probabilities (PP) for the remaining 150,002 trees of combined sample from the two runs. The NJ method was applied on the basis of a pairwise matrix of distances from Kimura's two-parameter model (Kimura, 1980) with 1000 bootstrap replications (BP) (Felsenstein, 1985).

## Results

*PCR-RFLP analysis*

Four to nine cleavage patterns were detected with each of the six restriction enzymes (Table 1). A total of 25 mitotypes were observed from composite cleavage patterns (Table 2). The mitotypes in each locality are shown in Table 3. While most populations had only one mitotype, some populations (sites 30, 39, 45, 46, 50 and 52) had more than one mitotypes. Fifteen mitotypes were found at just one site, seven were shared by two sites, and three were shared by more than two sites.

*Sequencing and phylogenetic analysis*

The 1046-bp region containing an almost entire cytochrome b gene was successfully sequenced for all individuals without ambiguity. No gap was required in the alignment. Intraspecific sequence divergences based on pairwise uncorrected p-distance ranged from 0 to 0.098. Distances between outgroups and ingroups ranged from 0.163 to 0.224.

The BI and NJ analyses of sequence data set resulted in essentially the same topology. Therefore, only the BI phylogeny is shown in Fig. 2, with Bayesian posterior probabilities and bootstrap values from NJ analysis. The tree indicates that 25 mitotypes identified in *T. tachydromoides* can be divided into five major clades (A–E), with remarkable interclade divergences. Monophyly of each clade was supported by 0.99–1.0 PP and 69–100% BP. Clade E was further divided into three

Table 1. Fragment patterns and molecular size (bp) generated by six restriction enzymes of an amplified segment (1130 bp) of the cytochrome b gene.

| Enzyme | Fragment pattern (bp) | | | | | | | Enzyme | Fragment pattern (bp) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alu I | | | | | | | | Mbo I | | | | | | |
| A | 504 | 219 | 114 | 94 | 84 | 69 | 46 | A | 584 | 546 | | | | |
| B | 504 | 219 | 183 | 94 | 84 | 46 | | B | 615 | 515 | | | | |
| C | 687 | 303 | 94 | 46 | | | | C | 546 | 528 | 56 | | | |
| D | 425 | 262 | 219 | 94 | 84 | 46 | | D | 1074 | 56 | | | | |
| E | 687 | 219 | 94 | 84 | 46 | | | E | 584 | 348 | 198 | | | |
| F | 488 | 295 | 219 | 82 | 46 | | | F | 782 | 348 | | | | |
| G | 783 | 301 | 46 | | | | | G | 1067 | 63 | | | | |
| | | | | | | | | H | 1130 | | | | | |
| Hae III | | | | | | | | | | | | | | |
| A | 423 | 226 | 148 | 147 | 131 | 55 | | Msp I | | | | | | |
| B | 423 | 226 | 202 | 148 | 131 | | | A | 901 | 229 | | | | |
| C | 423 | 226 | 148 | 147 | 125 | 55 | 6 | B | 1130 | | | | | |
| D | 423 | 153 | 148 | 147 | 131 | 73 | 55 | C | 727 | 229 | 174 | | | |
| E | 571 | 153 | 147 | 131 | 73 | 55 | | D | 687 | 443 | | | | |
| F | 325 | 224 | 150 | 147 | 131 | 98 | 55 | | | | | | | |
| | | | | | | | | Rsa I | | | | | | |
| Hha I | | | | | | | | A | 521 | 333 | 148 | 128 | | |
| A | 508 | 435 | 187 | | | | | B | 649 | 333 | 148 | | | |
| B | 508 | 258 | 187 | 177 | | | | C | 599 | 255 | 148 | 128 | | |
| C | 508 | 435 | 129 | 58 | | | | D | 333 | 266 | 255 | 148 | 128 | |
| D | 687 | 258 | 185 | | | | | E | 333 | 266 | 250 | 148 | 128 | 5 |
| | | | | | | | | F | 488 | 333 | 148 | 128 | 33 | |
| | | | | | | | | G | 521 | 481 | 128 | | | |
| | | | | | | | | H | 481 | 266 | 240 | 128 | 15 | |
| | | | | | | | | I | 849 | 146 | 130 | 5 | | |

subclades (E1–E3) supported by 0.98–1.0 PP and 88–100% BP.

The present result strongly suggests monophyly of *T. tachydromoides* with 1.0 PP and 100% BP. As for phylogenetic relationships among clades, Clade A split at the basal portion, and the remainder with 0.99 PP and 100% BP was divided into several branches, four clades (B–E) and three subclades (E1–E3). These clades and subclades were likely to have diverged within a short period of evolutionary time, as evidenced by short branches between the nodes.

*Geographic distribution of mitotypes*

Figure 3 shows the geographic distribution of mitotypes of five clades and three subclades making up the BI tree. The distribution patterns of each clade and subclade demonstrated strong geographical associations. Mitotypes in Clade A were distributed only in the Yakushima Island (in the Osumi Islands) (site 51) and the Tokara Islands (sites 52 and 53). Mitotypes of Clade B were distributed in Kyushu, mitotypes of Clade C were in the western part of Honshu and in the northern part of Kyushu, and mitotypes of Clade D were around the central part of Honshu and northern Shikoku. Mitotype mt8 in Subclade E1 was found only in Tsushima (site 44), and mitotypes in Subclade E2 were observed in Takahama, Maizuru and Himeji (sites 22, 33 and 34). Mitotypes in E3 were widely distributed from the eastern part of Honshu to the Hokkaido.

TABLE 2. Mitotypes, composite fragment patterns, number of sites in which the mitotype was found, and number of samples (N) posessing each mitotype of *Takydromus tachydromoides*. Enzyme order is the following: Alu I, Hae III, Hha I, Mbo I, Msp I, and Rsa I.

| Mitotype | Composite | Number of sites | N |
|---|---|---|---|
| mt1 | AAAAAA | 24 | 54 |
| mt2 | BAAAAA | 1 | 2 |
| mt3 | AAAABB | 1 | 5 |
| mt4 | AAAABA | 1 | 1 |
| mt5 | BBAAAF | 1 | 1 |
| mt6 | BAAABE | 1 | 1 |
| mt7 | BAAAAE | 2 | 2 |
| mt8 | DBABAD | 1 | 1 |
| mt9 | BBAAAC | 3 | 5 |
| mt10 | CBAAAD | 2 | 3 |
| mt11 | BBAAAA | 2 | 15 |
| mt12 | EBAAAG | 2 | 3 |
| mt13 | BBBCAG | 1 | 4 |
| mt14 | BBADAG | 1 | 1 |
| mt15 | BCAAAD | 5 | 21 |
| mt16 | BAAACD | 2 | 3 |
| mt17 | CDAEAD | 1 | 5 |
| mt18 | EDCFAD | 1 | 2 |
| mt19 | EDAEAD | 2 | 18 |
| mt20 | EDAEAH | 1 | 5 |
| mt21 | EEAEAD | 1 | 1 |
| mt22 | EDAEAA | 1 | 1 |
| mt23 | FBDGDI | 2 | 2 |
| mt24 | FBDGBI | 1 | 2 |
| mt25 | GFDHDI | 1 | 5 |
| Total | | 61 | 163 |

## DISCUSSION

Our analyses based on samples collected from almost the entire distributional range of *Takydromus tachydromoides* demonstrate strong concordance between the phylogenetic history of this species and its current geographical distribution. In particular, mitotypes in the Osumi and Tokara Islands (Clade A), the southernmost of its distribution range, showed a remarkable genetic differentiation (the average uncorrected p-distance, 0.089) from the remaining mitotypes in the main islands of Japan (Clades B–E). This phylogeographic pattern was consistent with the previous phylogenetic study of *Takydromus* species using 12S and 16S rRNA genes (Ota et al., 2002), suggesting a long geographic isolation of the Osumi-Tokara populations separated from other populations by the Osumi Strait.

By contrast, lower genetic divergence was observed between samples across the strait in other reptiles. In the Japanese skink *Plestiodon japonicus*, a small genetic distance (mean Nei's D=0.069) was observed between populations of the Osumi Islands and Kyushu in the allozymic study (Motokawa and Hikida, 2003), and this was confirmed by the study using mitochondrial cytochrome b variations (mean p-distance, 0.041) (Okamoto and Hikida, 2009). Furthermore, in the case of the East Asian natricine snake, *Rhabdophis tigrinus*, lower sequence divergence (p-distance, 0.001) of cytochrome b haplotypes was observed between Tanegashima Island (one of the Osumi Islands) and Kyushu (Takeuchi et al., 2012), suggesting a more recent dispersal between the islands. Taken together, these findings suggest that *T. tachydromoides* had older colonization history across the Osumi Strait than other reptiles. Thus, the gene flow as well as taxonomic relationship between the Osumi-Tokara populations and others should be investigated more intensively by further analyses because no detailed morphological comparisons have been conducted for this species.

In the main islands of Japan, the second largest divergence (mean p-distance, 0.054) was observed between the mitotypes in Kyushu (Clade B) and those in Honshu and Shikoku (Clades C–E). The similar allopatric distribution between Kyushu and other main islands was reported also in *Rhabdophis tigrinus* (Takeuchi et al., 2012), suggesting a common vicariance event between Kyushu and the Honshu-Shikoku landmass in these reptile species. Several regional groups were also recognized on the western part of Japan in *T. tachydromoides* (e.g., Clade C mitotypes in the western Honshu, and Clade D mitotypes

TABLE 3. Mitotype distributions in *Takydromus tachydromoides*. Sample numbers correspond to those in Fig. 1.

| Sapmles | | N | mt1 | mt2 | mt3 | mt4 | mt5 | mt6 | mt7 | mt8 | mt9 | mt10 | mt11 | mt12 | mt13 | mt14 | mt15 | mt16 | mt17 | mt18 | mt19 | mt20 | mt21 | mt22 | mt23 | mt24 | mt25 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Nakagawa, Hokkaido Pref. | 2 | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 2. | Sapporo, Hokkaido Pref. | 1 | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 3. | Naruse, Miyagi Pref. | 1 | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 4. | Rifu, Miyagi Pref. | 1 | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 5. | Yamabe, Yamagata Pref. | 5 | 5 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 6. | Mito, Ibaraki Pref. | 6 | 6 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 7. | Kasukabe, Saitama Pref. | 2 | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 8. | Hachioji, Tokyo Pref. | 3 | 3 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 9. | Koganei, Tokyo Pref. | 1 | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 10. | Kozushima, Tokyo Pref. | 2 | — | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 11. | Asahi, Niigata Pref. | 1 | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 12. | Niigata, Niigata Pref. | 8 | 8 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 13. | Maki, Niigata Pref. | 1 | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 14. | Shitada, Niigata Pref. | 1 | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 15. | Nagaoka, Niigata Pref. | 1 | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 16. | Ojiya, Niigata Pref. | 2 | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 17. | Kamikawa, Niigata Pref. | 2 | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 18. | Yuzawa, Niigata Pref. | 1 | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 19. | Hamochi, Niigata Pref. | 5 | 5 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 20. | Awashima, Niigata Pref. | 1 | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 21. | Kanazawa, Ishikawa Pref. | 1 | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 22. | Takahama, Fukui Pref. | 1 | — | — | — | — | — | — | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 23. | Shinano, Nagano Pref. | 1 | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 24. | Chino, Nagano Pref. | 2 | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 25. | Mishima, Shizuoka Pref. | 4 | 4 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 26. | Hamakita, Shizuoka Pref. | 5 | — | — | 5 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 27. | Kasugai, Aichi Pref. | 1 | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 28. | Yokkaichi, Mie Pref. | 1 | — | — | — | — | — | — | — | — | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 29. | Ise, Mie Pref. | 1 | — | — | — | — | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 30. | Maibara, Shiga Pref. | 3 | — | — | — | 1 | — | — | — | — | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 31. | Nagahama, Shiga Pref. | 2 | — | — | — | — | — | — | — | — | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 32. | Otsu, Shiga Pref. | 1 | — | — | — | — | — | — | — | — | — | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 33. | Maizuru, Kyoto Pref. | 1 | — | — | — | — | — | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 34. | Himeji, Hyogo Pref. | 1 | — | — | — | — | — | — | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 35. | Inami, Wakayama Pref. | 2 | — | — | — | — | — | — | — | — | — | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 36. | Yodoe, Tottori Pref. | 5 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 5 | — | — | — | — | — | — | — | — | — | — |
| 37. | Hamada, Shimane Pref. | 8 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 8 | — | — | — | — | — | — | — | — | — | — |
| 38. | Ochiai, Okayama Pref. | 2 | — | — | — | — | — | — | — | — | — | — | — | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 39. | Fukuyama, Hiroshima Pref. | 5 | — | — | — | — | — | — | — | — | — | — | — | — | 4 | 1 | — | — | — | — | — | — | — | — | — | — | — |
| 40. | Yamaguchi, Yamaguchi Pref. | 4 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 4 | — | — | — | — | — | — | — | — | — | — |
| 41. | Okawa, Kagawa Pref. | 3 | — | — | — | — | — | — | — | — | — | — | 3 | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 42. | Miki, Kagawa Pref. | 12 | — | — | — | — | — | — | — | — | — | — | 12 | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 43. | Kawanoe, Ehime Pref. | 1 | — | — | — | — | — | — | — | — | — | — | — | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 44. | Tsushima, Ehime Pref. | 1 | — | — | — | — | — | — | — | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 45. | Kitakyushu1, Fukuoka Pref. | 3 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 1 | 2 | — | — | — | — | — | — | — | — | — |
| 46. | Kitakyushu2, Fukuoka Pref. | 4 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 3 | 1 | — | — | — | — | — | — | — | — | — |
| 47. | Sanwa, Nagasaki Pref. | 5 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 5 | — | — | — | — | — | — | — | — |
| 48. | Hazama, Oita Pref. | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 2 | — | — | — | — | — | — | — |
| 49. | Miyazaki, Miyazaki Pref. | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 2 | — | — | — | — | — | — |
| 50. | Kiyotake, Miyazaki Pref. | 23 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 16 | 5 | 1 | 1 | — | — | — |
| 51. | Yakushima, Kagoshima Pref. | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 1 | — | — |
| 52. | Nakanoshima, Kagoshima Pref. | 3 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 1 | 2 | — |
| 53. | Suwanosejima, Kagoshima Pref. | 5 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 5 |

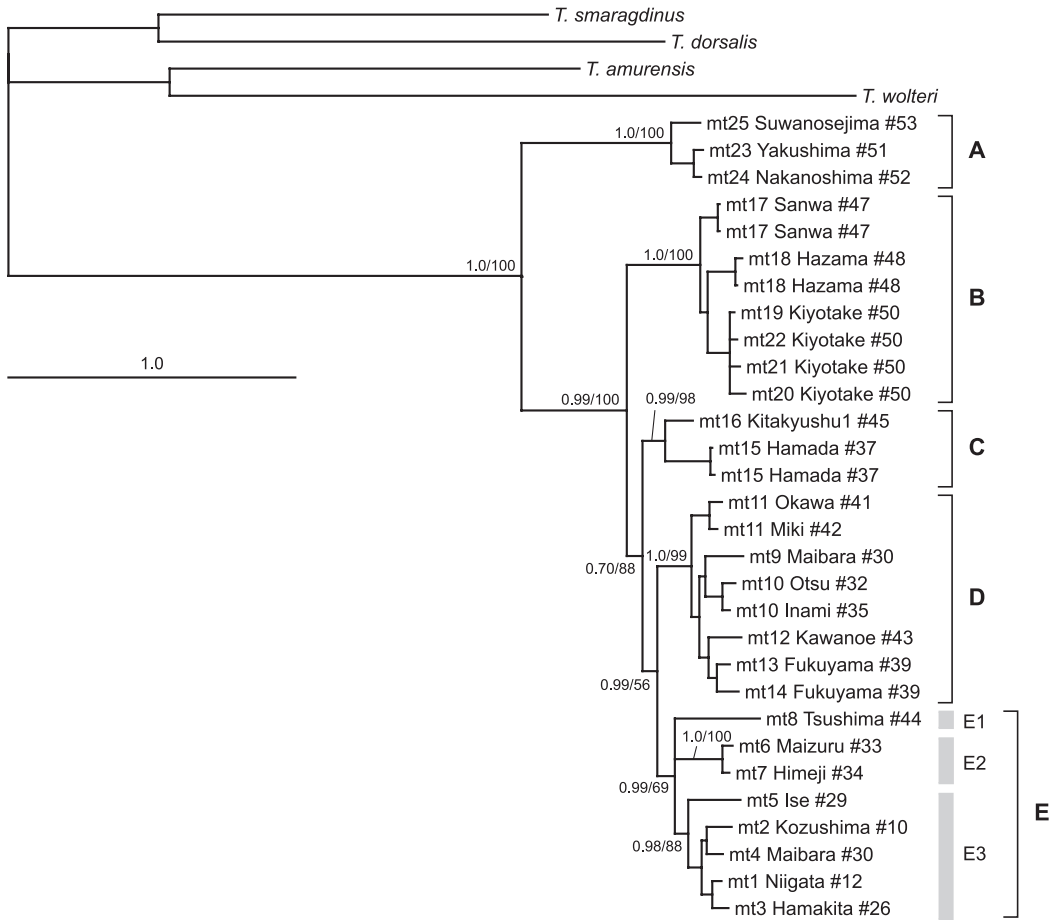


FIG. 2. A phylogenetic tree inferred from Bayesian analysis based on cytochrome b sequences of *Takydromus tachydromoides*. Mitotype, site name, and site number corresponding to Fig. 1 are indicated at each tip. Clade labels are described in the text. The numbers at nodes correspond to Bayesian posterior probabilities on the left and NJ bootstrap proportions on the right (only those at the major clades were shown).

in the central Honshu and Shikoku). However, this pattern is not common among those previously known for reptiles. No clear regional differentiation was observed within *Plestiodon japonicus*, which was widely distributed in the western part of Japan (Okamoto and Hikida, 2009, 2012). In *Rhabdophis tigrinus*, two genetically divergent mitochondrial groups were distributed sympatrically in a wide area of western Japan (Takeuchi et al., 2012). These findings suggest that the isolation of the regional populations at the western Japan in *T. tachydromoides* has been sustained over a long period during which other reptiles maintained gene flow between those regions. Such variations in population structure among species may be associated with differences in their dispersal abilities or local extinctions.

In addition, *T. tachydromoides* showed little genetic diversity in a wide area of the eastern Japan, as shown by the large distribution range of the Subclade E3 mitotypes. Particularly, the mitotype mt1 in Subclade E3 was found dominantly and exclusively in the northeastern Honshu and Hokkaido, suggest-

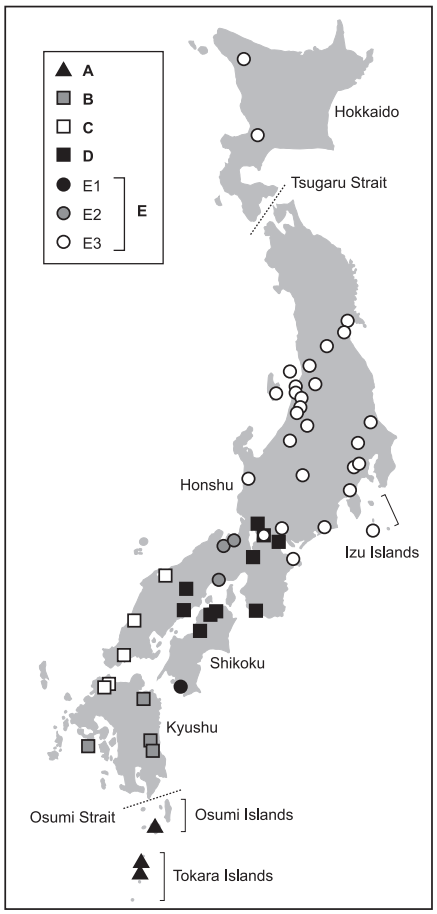


Fig. 3. Geographic distribution of mitotype groups. Grouping of mitotypes corresponds to the clades inferred from the phylogenetic analysis shown in Fig. 2. Intra-populational polymorphism is indicated by the merged combination of different symbols.

ing a recent range expansion in the northern Japan. Thus, the dispersal to the northern Honshu and crossing the Tsugaru Strait to Hokkaido may have progressed rapidly by recent climate change after the latest glaciation. A similar low genetic diversity between the Honshu and Hokkaido populations was reported in another reptile, *Plestiodon finitimus*, using allozymic and mtDNA analyses (Motokawa and Hikida 2003; Okamoto and Hikida, 2009; Okamoto and Hikida, 2012), suggesting the similar colonization history in the northern Japan.

The subclade E3 mitotypes were also observed in the Izu Islands. The mitotype mt2 specific to Kozushima (Site 10 in Fig. 1, one of the Izu Islands) was closely related to the remaining mitotypes, mt1, mt3 and mt4, in Subclade E3, with low genetic divergences (p-distance, 0.012–0.016). Around the Izu islands, different phylogeographic patterns have been shown between reptile species. Kuriyama et al. (2011) suggested the recent multiple colonization from the mainland to the Izu Islands within the past 0.25–0.58 million years ago (Ma) in the four-lined ratsnake, *Elaphe quadrivirgata*. On the other hand, the older colonization history (3–7.6 Ma) has been suggested in *Plestiodon latiscutatus* (Okamoto et al., 2006; Brandley et al., 2011, 2014). The low genetic divergence in *T. tachydromoides* suggests its recent colonization of the Izu Islands, although further extensive study around this region is required.

In conclusion, we clarified the phylogeographic pattern of *T. tachydromoides* based on mitochondrial cytochrome b variations. The largest intraspecific divergence of the Osumi-Tokara Island populations is not common among other reptile species because they show lower genetic divergences, suggesting that *T. tachydromoides* has an old colonization history across the Osumi Strait. This study also demonstrated the regional differentiation at the western Japan, and the low genetic diversity at the eastern Japan. These patterns suggest that the regional populations at the western region have been isolated for a long time by some barriers to gene flow, while those at the eastern region have maintained gene flow until recently, even between the mainland and adjacent islands. Finally, postglacial northward range expansion and overseas dispersal in the northern part of the mainland was suggested.

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