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# Population Structure of *Aegialites* Beetles (Coleoptera, Salpingidae) on the Coasts of Hokkaido, Northern Japan

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We conducted a molecular phylogeographic analysis of *Aegialites* beetles sampled on the coasts of Hokkaido, northern Japan, using a partial sequence (553 bp) of the mitochondrial ND2 gene. The beetles are flightless and are adapted to the supralittoral zone of rocky shores. In all, 249 individuals from 17 populations were included in the molecular analysis. Haplotypes of the *Aegialites* beetles studied comprised two distinct lineages with different distributional ranges: one (lineage A) on the coasts of Hokkaido facing the Sea of Japan and the Sea of Okhotsk, and the other (lineage B) mostly on the Pacific coast and on the Shiretoko Peninsula in northeastern Hokkaido. These haplotype lineages clearly indicate two groups of populations, one comprising lineage A haplotypes (group A) and the other lineage B haplotypes (group B). Only one population, at Taisei facing the Sea of Japan on the Oshima Peninsula, contained a mixture of haplotypes from the two lineages. Genetic divergence among populations was larger in group B than in group A. Despite the unidimensional nature of the beetles' habitat, no correlation was found between the degree of genetic differentiation and geographic distance in either group of populations. Population genetic parameters suggested that the two lineages originated in the Pliocene, and that the populations in group A underwent a rapid increase in population size, whereas those in group B did not. We present a scenario for the allopatric divergence of the two lineages.

**Key words:** AMOVA,  $d_A$ ,  $F_{ST}$ , flightless beetle, supralittoral zone

## INTRODUCTION

Seashores are a distinct habitat comprising the boundary between the land and the sea. For this reason, the distribution of animals adapted to seashores can be regarded as unidimensional, and analyses of population structure should yield more straightforward results than for organisms distributed in two or three dimensions. A number of studies have examined genetic variation in conspecific populations of intertidal or supralittoral animals (Johnson and Black, 1991; Matthaëis et al., 1998; Nohara, 1999; Piertney and Carvalho, 1994; Zaslavskaya and Pudovkin, 2005), but there have been few studies on the population structure of insects adapted to seashores.

Beetles of the genus *Aegialites* (Salpingidae) are flightless, a condition apparently associated with their habitat in spaces between rocks and in rock crevices in the supralittoral zone on rocky shores. They have been recorded from a wide range of northern Pacific coasts, including Sakhalin

Island, northern Japan, the Kurile Islands, and the Aleutian Islands and mainland Alaska, and also from the Persian Gulf (Zerche, 2004). In Japan, only one species, *A. stejnegeri* Linell, 1898, is known from fragmentary records: from Setana, Shakotan, and Rumoi on Hokkaido (Ôhara et al., 1989; Hinakura, 1990) and from Shimokita on Honshu (Araki, 1994). Due to their restriction to rocky shores, *Aegialites* beetles offer an opportunity to study the population structure of insects with a fragmented, unidimensional distribution. Here we present the results of a molecular phylogeographic analysis of *Aegialites* beetles sampled along the coasts of Hokkaido, northern Japan.

## MATERIAL AND METHODS

### Insect material

*Aegialites* beetles are small (approximately 3–4 mm in body length), with reduced hind wings, and are thought to feed on young algae on rock surfaces (Sugihara, 1938). Sampling was conducted from April 2006 to August 2007 at various sites on the coasts of Hokkaido Island and on three small adjacent islands, Rebun, Rishiri, and Daikoku. We lightly sprayed commercial insecticide (Earth Jet; Earth Chemical) into rock crevices, collected beetles as they escaped the crevices, and immediately put the specimens into 99.5% ethanol for subsequent DNA extraction. We sequenced part

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of the mitochondrial NADH dehydrogenase subunit 2 (ND2) gene to analyze the genetic structure of the beetle populations.

#### Molecular markers, PCR amplification, and DNA sequencing

We used the method of Boom et al. (1990) to extract total DNA. To amplify the ND2 gene, we designed primers ND2-sub2.0f (5'-GGCACTATTGTAGCTATTTC-3') and ND2-sub9.0r (5'-CTCAT-TCTCCAAAATAAACT-3'). PCR thermal cycling conditions were 94°C for 7 min; 35 cycles of 94°C for 45 sec, 45°C for 90 sec, and 72°C 2 min; and 72°C for 7 min. We determined nucleotide sequences by direct sequencing using a Big Dye Terminator Kit ver. 3.1 and an ABI 3100 Avant automated sequencer (Applied Biosystems, Foster City, CA). The amplification primers also served as the sequencing primers.

#### Analyses of haplotype relationships

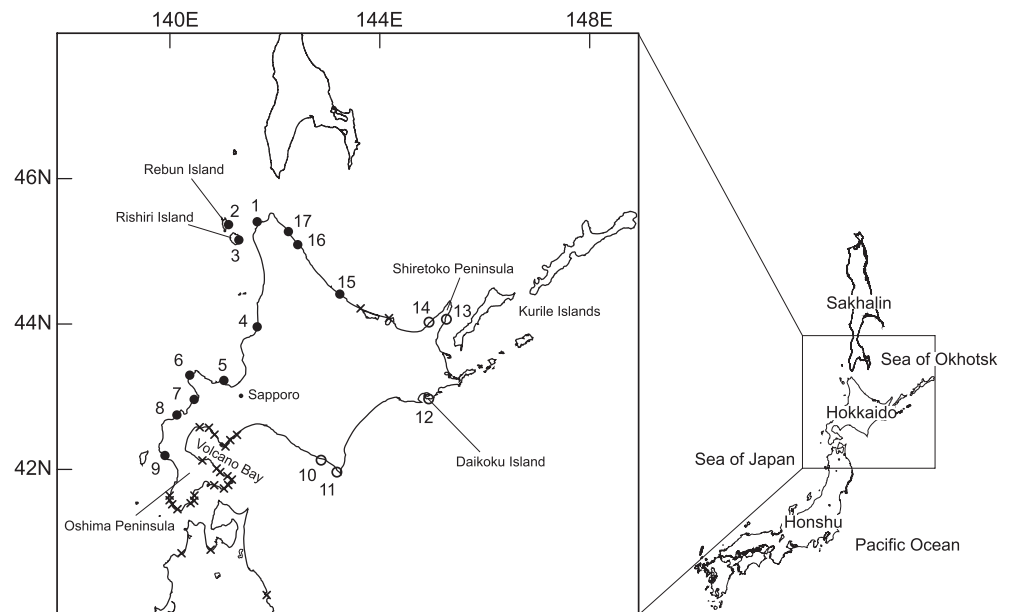
We used MEGA 4 software (Tamura et al., 2007) to align nucleotide sequences, DAMBE 4.5 (Xia and Xie, 2001) to search for haplotype variation, and NETWORK 4.5.1.0 (<http://www.fluxus-engineering.com>) to construct a median-joining (MJ) network (Bandelt et al., 1999) to describe haplotype relationships.

#### Population genetic analyses

We conducted population genetic analyses using sequences from the 14 populations for which more than 10 individuals each were available. To estimate the extent of genetic diversity in each population, we calculated the haplotype diversity ( $h$ ) (Nei, 1987) and nucleotide diversity ( $\pi$ ) (Nei, 1987) with ARLEQUIN 3.11 software (Excoffier et al., 2005). We calculated Tajima's  $D$  (Tajima, 1989a, b) and Fu's  $F_S$  statistics (Fu, 1997) to infer the extent of population expansion, also with ARLEQUIN 3.11. To estimate the extent of divergence between populations, we calculated the net nucleotide substitutions between populations ( $d_A$ ) (Nei, 1987) with MEGA4. Finally, to estimate the relative degree of genetic differentiation, we calculated pairwise  $F_{ST}$  values among populations using ARLEQUIN 3.11, and tested the significance of  $F_{ST}$  values with 1000 permutations in ARLEQUIN 3.11. We also performed an analysis of molecular variance (AMOVA; Excoffier et al., 1992) with ARLEQUIN 3.11 to examine the genetic structure of populations.

We used the Mantel test

(Mantel, 1967; ARLEQUIN 3.11) to examine the correlation between the degree of genetic differentiation ( $d_A$  and population pairwise  $F_{ST}$ ) and geographic distance. We obtained geographic distances between sites on the coasts of mainland Hokkaido by measuring the distance along the coast between two sites on a 1:900,000 scale map using Map Information Open Network (Mapion) (<http://www.mapion.co.jp/>). We calculated the distance between adjacent island populations and populations on Hokkaido as the distance between an island population and the nearest population on the coast of Hokkaido plus the distance along the coast from this



**Fig. 1.** Distribution of *Aegialites* beetles on the coasts of Hokkaido. Sites where beetles were collected (numbered as in Table 1): 1, Wakkanai; 2, Rebun; 3, Rishiri; 4, Rumoi; 5, Shukutsu; 6, Kamui; 7, Iwanai; 8, Shimamaki; 9, Taisei; 10, Samani; 11, Erimo; 12, Daikoku; 13, Rausu; 14, Shiretoko; 15, Oumu; 16, Esashi; 17, Hamatonbetsu. Populations were sorted according to haplotype lineage: solid circles, group A; open circles, group B. Crosses indicate sites where no *Aegialites* beetles were collected despite a seemingly suitable habitat.

**Table 1.** List of the 17 study populations of *Aegialites* beetles and the distribution of the 35 haplotypes detected among them. The site numbers correspond to those on the map in Fig. 1. Haplotypes in lineage A are in regular font; haplotypes in lineage B are in italics. The five populations included in group B are shaded; the other populations comprise group A. Note that the Taisei population contained one lineage-B individual.

| No. | Sampling site | Latitude     | Longitude     | Haplotype (number of individuals)                     |
|-----|---------------|--------------|---------------|---|
| 1   | Wakkanai      | N45°18'40.3" | E141°36'52.6" | Hap10 (19)  |
| 2   | Rebun         | N45°24'27.1" | E140°59'29.1" | Hap06 (1), Hap10 (18), Hap24 (1), Hap25 (1)           |
| 3   | Rishiri       | N45°14'55.2" | E141°13'10.7" | Hap10 (12), Hap17 (1), Hap19 (1)                      |
| 4   | Rumoi         | N43°56'50.1" | E141°37'50.2" | Hap03 (17)  |
| 5   | Shukutsu      | N43°14'10.4" | E141°00'58.4" | Hap08 (5), Hap10 (3)                                  |
| 6   | Kamui         | N43°19'47.6" | E140°22'00.5" | Hap01 (4), Hap02 (1), Hap07 (1), Hap10 (2), Hap18 (2) |
| 7   | Iwanai        | N42°57'41.0" | E140°27'43.8" | Hap09 (1), Hap10 (2), Hap12 (17), Hap13 (1)           |
| 8   | Shimamaki     | N42°44'53.9" | E140°08'00.0" | Hap04 (15), Hap14 (3), Hap15 (1), Hap20 (1)           |
| 9   | Taisei        | N42°09'56.0" | E139°54'20.4" | Hap05 (1), Hap10 (15), Hap16 (4), Hap35 (1)           |
| 10  | Samani        | N41°59'59.5" | E143°09'31.4" | Hap34 (16)  |
| 11  | Erimo         | N41°56'52.7" | E143°13'02.2" | Hap30 (7), Hap33 (1), Hap34 (8), Hap35 (4)            |
| 12  | Daikoku       | N42°57'29.7" | E144°52'17.9" | Hap31 (11), Hap32 (4)                                 |
| 13  | Rausu         | N44°02'10.8" | E145°13'01.7" | Hap27 (9), Hap28 (2), Hap29 (3)                       |
| 14  | Shiretoko     | N44°02'21.7" | E144°56'03.1" | Hap26 (1)   |
| 15  | Oumu          | N44°37'27.5" | E142°55'54.9" | Hap10 (17), Hap11 (1)                                 |
| 16  | Esashi        | N44°57'41.7" | E142°35'00.4" | Hap22 (8), Hap23 (3)                                  |
| 17  | Hamatonbetsu  | N45°03'33.0" | E142°29'37.0" | Hap10 (2), Hap21 (1)                                  |

nearest mainland population to the mainland population being considered.

## RESULTS

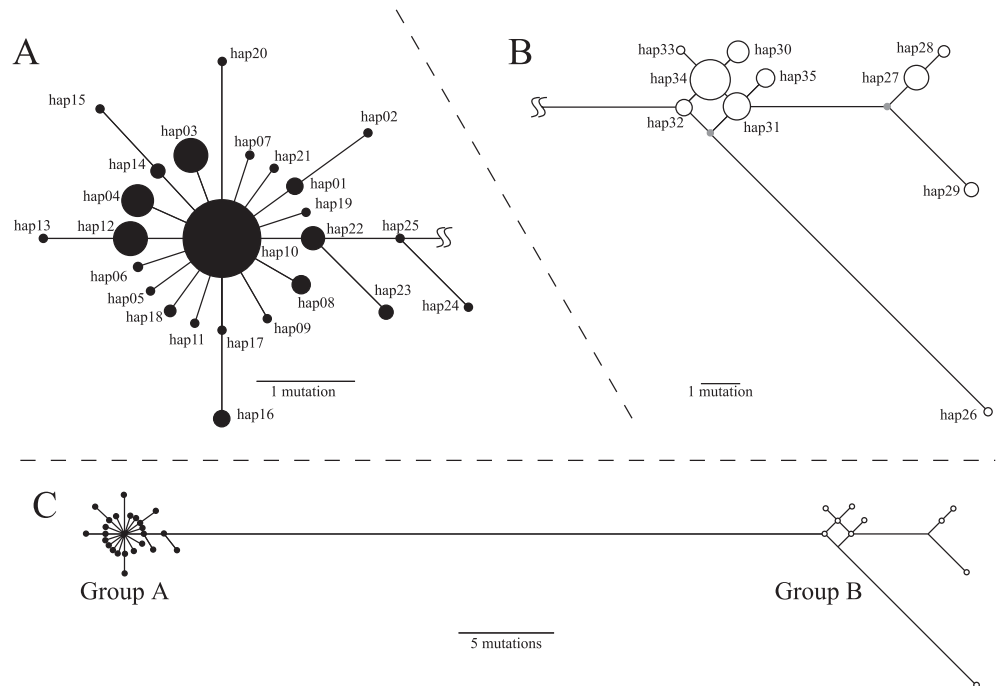
### Distribution of *Aegialites* beetles on the coasts of Hokkaido

In total, we collected 270 *Aegialites* beetles at 17 of 44 sites investigated; the 17 sites included 14 sites on mainland Hokkaido and one site each on three adjacent islands (Rishiri, Rebun, and Daikoku) (Fig. 1; Table 1). We identified all the beetles as *A. stejnegeri*, on the basis of external characters. The *Aegialites* habitats we observed were either type 2A (exposed wave-cut platforms in bedrock, mud, or clay) or type 6A (gravel beaches), according to the definitions of the Environmental Sensitivity Index Map (Geological Survey of Hokkaido, 2006). *Aegialites* beetles were widespread on rocky shores on Hokkaido, except in southern Hokkaido in the southern and middle parts of the Oshima Peninsula and in the areas facing or adjacent to Volcano Bay. In these latter areas, we found no *Aegialites* beetles, even though we found and frequently checked rocky shores seemingly suitable for *Aegialites*. We also attempted sampling at three sites in the most northern part of Honshu, but found no *Aegialites* beetles (Fig. 1).

### Haplotypes and haplotype relationships

We obtained nucleotide sequences of part of the mitochondrial ND2 coding region (553 bp long) for 249 *Aegialites* beetles from 17 sites and identified 35 haplotypes (Table 1). These sequences have been deposited in databases (DDBJ/EMBL/GenBank) under accession numbers AB546745–AB546779. A sequence alignment detected 67 variable sites, including 54 synonymous substitutions. Among the 35 haplotypes,

32 were unique to single sampling sites, two (haplotypes 34 and 35) were shared by two populations, and one (haplotype 10) was shared by nine populations (Table 1). In a haplotype network constructed with the MJ method (Fig. 2), two lineages differentiated by 35 nucleotide substitutions were evident, and are here designated as lineages A (haplotypes 1–25) and B (haplotypes 26–35) (Table 1). Lineage A showed a star topology, with haplotype 10 occupying the



**Fig. 2.** Median-joining (MJ) network for the ND2 haplotypes detected in *Aegialites* beetles on Hokkaido. **(A)** Lineage A. **(B)** Lineage B. **(C)** All haplotypes. In (A) and (B), each circle represents a haplotype, with the size of the circle proportional to the frequency of the haplotype among all populations. Gray dots in (B) are null haplotypes. Line lengths between circles are proportional to the number of mutational differences. Note that the scale bars for the number of mutational differences are different among (A), (B), and (C).

**Table 2.** Haplotype diversity ( $h$ ), nucleotide diversity ( $\pi$ ), Tajima's  $D$ , and Fu's  $F_S$  for 14 populations of *Aegialites* beetles on the coasts of Hokkaido. \*Excluding the unique lineage-B haplotype from Taisei.

| Population     | Haplotype diversity (SD) | Nucleotide diversity (SD) | Tajima's $D$ ( $p$ -value) | Fu's $F_S$ ( $p$ -value) |
|----------------|--------------------------|---------------------------|----------------------------|--------------------------|
| Wakkanai       | 0.0000 (0.0000)          | 0.000000 (0.000000)       | 0.0000 (1.0000)            | N.A.                     |
| Rebun          | 0.2714 (0.1242)          | 0.000999 (0.000953)       | -1.4339 (0.0710)           | -1.2956 (0.0630)         |
| Rishiri        | 0.2747 (0.1484)          | 0.000517 (0.000655)       | -1.4807 (0.0570)           | -1.4753 (0.0210)         |
| Rumoi          | 0.0000 (0.0000)          | 0.000000 (0.000000)       | 0.0000 (1.0000)            | N.A.                     |
| Kamui          | 0.8222 (0.0969)          | 0.002692 (0.002002)       | -0.6319 (0.3130)           | -1.2843 (0.0900)         |
| Iwanai         | 0.3476 (0.1276)          | 0.000809 (0.000835)       | -1.2167 (0.0720)           | -1.7701 (0.0340)         |
| Shimamaki      | 0.4316 (0.1262)          | 0.001923 (0.001482)       | -1.1835 (0.1150)           | 0.1336 (0.5240)          |
| Taisei         | 0.4714 (0.1161)          | 0.008025 (0.004601)       | -2.4102 (0.0000)           | 5.1324 (0.9750)          |
| Taisei*        | 0.4158 (0.1157)          | 0.001399 (0.001190)       | -0.22717 (0.3970)          | 0.6520 (0.5960)          |
| Samani         | 0.0000 (0.0000)          | 0.000000 (0.000000)       | 0.0000 (1.0000)            | N.A.                     |
| Erimo          | 0.7105 (0.0541)          | 0.001656 (0.001335)       | 0.2217 (0.6570)            | -0.2105 (0.3860)         |
| Daikoku        | 0.4190 (0.1132)          | 0.000758 (0.000818)       | 0.7421 (0.8440)            | 0.9086 (0.5390)          |
| Rausu          | 0.5604 (0.1245)          | 0.014069 (0.007827)       | 2.2416 (0.9970)            | 8.7695 (0.9990)          |
| Oumu           | 0.1111 (0.0964)          | 0.000201 (0.000381)       | -1.1647 (0.1610)           | -0.7943 (0.0990)         |
| Esashi         | 0.4364 (0.1333)          | 0.000789 (0.000861)       | 0.6714 (0.8330)            | 0.7785 (0.4950)          |
| GroupA pooled  | 0.7330 (0.0324)          | 0.00279 (0.00186)         | -2.5391 (0.0003)           | -16.5339 (0.0000)        |
| GroupA pooled* | 0.7300 (0.0326)          | 0.00204 (0.00148)         | -2.0226 (0.0088)           | -20.1113 (0.0000)        |
| GroupB pooled  | 0.8117 (0.0328)          | 0.00602 (0.00347)         | -0.9674 (0.1780)           | 0.6327 (0.6740)          |

**Table 3.** Pairwise  $F_{ST}$  (below diagonal) and  $d_A$  (above diagonal) values among 14 populations of *Aegialites* beetles on the coasts of Hokkaido. Significant  $F_{ST}$  values ( $p < 0.05$ ) are in bold font.

|           | Wakkanai     | Rebun        | Rishiri      | Rumoi        | Kamui        | Iwanai       | Shimamaki    | Taisei       | Samani       | Erimo        | Daikoku      | Rausu        | Oumu         | Esashi |
|-----------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------|
| Wakkanai  | –            | 0.001        | 0.000        | 0.002        | 0.002        | 0.002        | 0.002        | 0.005        | 0.080        | 0.080        | 0.083        | 0.087        | 0.000        | 0.002  |
| Rebun     | 0.043        | –            | 0.001        | 0.002        | 0.002        | 0.002        | 0.003        | 0.005        | 0.080        | 0.080        | 0.083        | 0.087        | 0.001        | 0.002  |
| Rishiri   | 0.066        | –0.029       | –            | 0.002        | 0.002        | 0.002        | 0.002        | 0.005        | 0.080        | 0.080        | 0.083        | 0.087        | 0.000        | 0.003  |
| Rumoi     | <b>1.000</b> | <b>0.851</b> | <b>0.875</b> | –            | 0.004        | 0.004        | 0.004        | 0.007        | 0.083        | 0.082        | 0.085        | 0.089        | 0.002        | 0.004  |
| Kamui     | <b>0.599</b> | <b>0.401</b> | <b>0.366</b> | <b>0.672</b> | –            | 0.002        | 0.004        | 0.006        | 0.082        | 0.082        | 0.084        | 0.087        | 0.002        | 0.004  |
| Iwanai    | <b>0.800</b> | <b>0.663</b> | <b>0.655</b> | <b>0.810</b> | <b>0.452</b> | –            | 0.004        | 0.006        | 0.083        | 0.082        | 0.085        | 0.085        | 0.002        | 0.004  |
| Shimamaki | <b>0.780</b> | <b>0.650</b> | <b>0.637</b> | <b>0.770</b> | <b>0.408</b> | <b>0.611</b> | –            | 0.007        | 0.083        | 0.083        | 0.085        | 0.089        | 0.002        | 0.004  |
| Taisei    | <b>0.164</b> | 0.042        | 0.030        | <b>0.744</b> | <b>0.275</b> | <b>0.561</b> | <b>0.548</b> | –            | 0.077        | 0.077        | 0.08         | 0.084        | 0.005        | 0.007  |
| Samani    | <b>1.000</b> | <b>0.848</b> | <b>0.871</b> | <b>1.000</b> | <b>0.662</b> | <b>0.806</b> | <b>0.764</b> | <b>0.738</b> | –            | 0.001        | 0.003        | 0.011        | 0.080        | 0.079  |
| Erimo     | <b>0.638</b> | <b>0.512</b> | <b>0.485</b> | <b>0.625</b> | <b>0.241</b> | <b>0.473</b> | <b>0.429</b> | <b>0.405</b> | <b>0.377</b> | –            | 0.004        | 0.012        | 0.080        | 0.078  |
| Daikoku   | <b>0.812</b> | <b>0.664</b> | <b>0.651</b> | <b>0.802</b> | <b>0.401</b> | <b>0.621</b> | <b>0.574</b> | <b>0.552</b> | <b>0.796</b> | <b>0.424</b> | –            | 0.012        | 0.083        | 0.081  |
| Rausu     | <b>0.755</b> | <b>0.604</b> | <b>0.582</b> | <b>0.742</b> | <b>0.319</b> | <b>0.560</b> | <b>0.511</b> | <b>0.489</b> | <b>0.735</b> | <b>0.358</b> | <b>0.512</b> | –            | 0.087        | 0.085  |
| Oumu      | 0.003        | –0.007       | –0.006       | <b>0.943</b> | <b>0.503</b> | <b>0.740</b> | <b>0.722</b> | 0.096        | <b>0.941</b> | <b>0.579</b> | <b>0.746</b> | <b>0.686</b> | –            | 0.002  |
| Esashi    | <b>0.835</b> | <b>0.666</b> | <b>0.652</b> | <b>0.825</b> | <b>0.376</b> | <b>0.618</b> | <b>0.566</b> | <b>0.543</b> | <b>0.819</b> | <b>0.406</b> | <b>0.573</b> | <b>0.497</b> | <b>0.760</b> | –      |

center; lineage B did not show a star topology. The  $d_A$  value between the two lineages was 0.083.

The geographic distributions of the two lineages were nearly mutually exclusive (Fig. 1). Haplotypes of lineage A occurred in all nine populations facing the Sea of Japan and in three populations facing the Sea of Okhotsk. In contrast, haplotypes of lineage B occurred in three populations facing the Pacific and two populations on the Shiretoko Peninsula. The populations were thus clearly separable by haplotypes. Hereafter, we refer to populations as members of either group A (composed of lineage A haplotypes) or group B (lineage B haplotypes) (Table 1). The only exception was Taisei, situated to the south on the Sea of Japan, where 20 of 21 sequences were lineage A haplotypes but one (haplotype 35) belonged to lineage B (Table 1). We treated the Taisei population as belonging to group A.

#### Genetic diversity within populations and within groups

As shown in Table 2, both the haplotype diversity and nucleotide diversity values were zero for the Wakkanai, Rumoi, and Samani populations. In contrast, the values were very large for Taisei and Rausu. Tajima's  $D$  values and Fu's  $F_S$  values were not significant, with the exception of Tajima's  $D$  for Taisei when the unique individual with a lineage B haplotype was included (Table 2). When we excluded this individual, Tajima's  $D$  value for the Taisei population was also not significant. Nucleotide diversity was 0.002 for group A and 0.006 for group B (Table 2). Both Tajima's  $D$  and Fu's  $F_S$  values were negative and significant ( $p < 0.01$  for both statistics) only for group A.

#### Genetic differentiation between groups and between populations

Pairwise  $F_{ST}$  values between populations (Table 3) were not significant ( $p < 0.05$ ) for seven combinations of pairs among group A populations. For the other combinations, including all inter-group combinations,  $F_{ST}$  values were significant. The genetic distance ( $d_A$ ) was 0.077–0.089 among populations between the two groups, but 0.000–0.007 among populations within each group. The results were similar when the unique lineage-B individual was excluded from the Taisei population, although the differences among pop-

**Table 4.** Results of Mantel tests for correlations between geographical distance and genetic parameters, performed for two groups of *Aegialites* beetles detected on the coasts of Hokkaido. \*Excluding the unique lineage-B haplotype from Taisei.

| Parameter | Category | Correlation coefficient | Determination (%) | $p$ -value |
|-----------|----------|-------------------------|-------------------|------------|
| $F_{ST}$  | GroupA   | 0.009293                | 0.000086          | 0.416      |
|           | GroupA*  | –0.003379               | 0.000011          | 0.481      |
|           | GroupB   | 0.297837                | 0.088707          | 0.286      |
| $d_A$     | GroupA   | 0.244161                | 0.059614          | 0.033      |
|           | GroupA*  | 0.079723                | 0.006356          | 0.263      |
|           | GroupB   | 0.778708                | 0.606387          | 0.08       |

ulations of group A were smaller, and the differences between the two groups were larger (data not shown) than those in Table 3.

The results of Mantel tests for correlations between geographic distance and  $F_{ST}$  or  $d_A$  values are shown in Table 4, separately for the two groups. There was no significant correlation between geographic distance and  $F_{ST}$  for either group. The correlation between geographic distance and  $d_A$  was significant only for group A, and only when the unique lineage-B individual was included in the Taisei population.

AMOVA showed that 15.58% of the variation was attributable to differences between the groups, 49.16% to differences among populations within the groups, and 35.26% to differences within populations. Average fixation indices were 0.1558 for  $\Phi_{CT}$  ( $p = 0.0098$ ), 0.5823 for  $\Phi_{SC}$  ( $p = 0.000$ ), and 0.6475 for  $\Phi_{ST}$  ( $p = 0.000$ ). We obtained similar results for AMOVA when we excluded the unique lineage-B individual from the Taisei population (data not shown).

## DISCUSSION

#### Distribution pattern

This study shows *Aegialites* beetles to be widespread on rocky shores around Hokkaido, except in the southwestern area around the Oshima Peninsula and Volcano Bay. However, since there is an isolated record of *A. stejnegeri* occurring on northern Honshu Island, south of Hokkaido Island (Araki, 1994), the apparent absence of *Aegialites* beetles in southwestern Hokkaido needs further confirmation.

*Aegialites stejnegeri* on Hokkaido comprises two genetically distinct lineages with nearly allopatric distributional

ranges. The two lineages do not overlap geographically on the coast of the Sea of Okhotsk, where they are separated by sandy seashores in the southeastern part. In southern Hokkaido, the two lineages are separated by the Oshima Peninsula, the only exception being the Taisei population (locality 9, Fig. 1) on the Sea of Japan. At Taisei, one lineage-B individual (haplotype 35) occurred along with 20 individuals of lineage A. As a result, the nucleotide and haplotype diversities were larger for the Taisei population than for other populations except that at Rausu (Table 2). The two lineages thus appear to occur sympatrically at Taisei; however, this is uncertain due to the ambiguity of the identity of the unique lineage-B individual at Taisei. Since Taisei is geographically distant from the nearest lineage-B population detected, and we treated many samples simultaneously, the possibility of contamination cannot be ruled out. If this individual really represented a contaminant from lineage B (e.g., due to a labeling or laboratory error), then the segregation of the two lineages on the coasts of Hokkaido is complete.

Throughout its range, lineage A is exposed to the warm Tsushima current flowing north along the Sea of Japan coast, with a sub-current flowing southeast along the coast of the Sea of Okhotsk, whereas lineage B is exposed to the cool Oyashio current flowing southwestward along the Pacific coast. Our results suggest that the distribution of the two lineages of *Aegialites* beetles observed on the coasts of Hokkaido may be related to climatic factors or the flow direction of the sea currents. Unfortunately, however, no information is available on climatic adaptations or the mode of dispersal of the *Aegialites* beetles.

### Population structures

The population genetic analysis provides some information on the history of the two *Aegialites* lineages. First, the two lineages are considerably genetically diverged ( $d_A = 0.083$ ), and we estimated the divergence time to be 3.61 Ma (mid Pliocene), assuming a substitution rate of 2.3% per million years for mitochondrial DNA (Brower, 1994). Second, significantly negative values of Fu's  $F_S$  and Tajima's  $D$  indicated that group A experienced a rapid increase in population size. Since the populations within group A are nearly as diversified as those in group B, the haplotypes in lineage A would have differentiated in a short period, expanded in range, and become fixed locally. Although group B is slightly more diverse than group A, there was no sign of a rapid expansion in population size. It is noteworthy that the nucleotide and haplotype diversities were higher for the Rausu population, situated close to the most western of the Kurile Islands (Fig. 1), than for the other populations (Table 2). In addition, the only individual sampled at Shiretoko, another site close to the Kuriles (Fig. 1), had a unique haplotype (No 26) that was considerably diverged from the other haplotypes (Nos. 27–35) in lineage B (Fig. 2). These results suggest that group B originated from multiple sources, i.e., is composed of the descendants of migrants of more than one group from the east.

When we excluded the unique lineage-B individual from the Taisei sample, we found no correlation between the degree of genetic divergence and geographic distance for populations in either lineage, despite the unidimensional nature of their distributions. Our study included samples

from neighboring islands as well as from localities separated by long, sandy shores apparently unsuitable for persistent habitation by *Aegialites* beetles. However, genetic divergences between populations separated by these potential dispersal barriers were similar to those between populations seemingly not separated by such barriers (Table 3). Although pairwise  $F_{ST}$  values were not significant among some populations of group A, it may have resulted from the rapid range expansion of lineage A rather than current gene flow between the populations, since the low  $F_{ST}$  values were caused by sharing of large proportions of putatively ancestral haplotype 10 (Fig. 2). In other words, some factors other than the straits and sandy shores block current gene flow among populations as effectively as these potential barriers. At present, it is unknown how *Aegialites* beetles disperse. To understand the factors that promoted genetic divergence among populations of the respective lineages, we need to more accurately evaluate the extent of gene flow between populations using samples collected at a finer geographic scale. In any case, the lack of a correlation between geographic and genetic distance is also consistent with our interpretation of the history of the two lineages (rapid population increase and range expansion for group A; multiple origins for group B) and suggests that these historical events occurred relatively recently.

### Possible allopatric divergence of the two lineages

During Pliocene and Pleistocene climatic oscillations, the sea level around Japan fluctuated drastically. The Sea of Japan was isolated from the Pacific to various extents during glacial maxima in the Pleistocene due to recessions in sea level (Ohshima, 1991). The condition of shores, i.e., whether or not they were suitable for *Aegialites* beetles, would have also varied. Taking these geohistorical changes into account, we propose here that allopatric divergence is a possible scenario for the evolutionary history of the *Aegialites* beetles around northern Japan. We postulate the following sequence of events: (1) an ancestral *Aegialites* population occupied coastal regions of the NW Pacific; (2) part of the ancestral population became isolated on the shore of the Sea of Japan sometime in the Pliocene; (3) this isolated population on the Sea of Japan (lineage A) subsequently diverged from the Pacific population (lineage B); (4) the range of lineage A shrank during the last glacial maximum, ca. 20,000 years ago (Clark et al., 2009); and (5) lineage A rapidly expanded in range (including dispersal to the coast of the Sea of Okhotsk) during the post-glacial period, due to the opening of straits to the northern Sea of Japan. This scenario is consistent with the present distribution patterns and genetic structures of the two lineages on Hokkaido, although its validity must be tested by further studies with adequate sampling in the areas adjacent to Hokkaido, namely, Sakhalin and the Kuriles.

Interestingly, in northern Japan the two lineages of *Aegialites* beetles are very similar in distribution to two lineages of the intertidal rock-dwelling periwinkle *Littorina sitkana* (Nohara, 1999). In the latter species, one lineage inhabits the shores of the Sea of Japan and the Sea of Okhotsk, and the other inhabits Pacific coasts. Although the *Aegialites* and *Littorina* lineages differ somewhat in the details of their distributions, especially around the Shiretoko

and Oshima Peninsulas, the similar distributional patterns suggest that some common factors have resulted in nearly allopatric distributions in animals adapted to rocky coastal environments in northern Japan. Nohara (1999) suggested that the current allopatric distributions of the two lineages of *Littorina sitkana* in northern Japan are the consequence of geographic isolation of part of the ancestral population during the last glacial period, an explanation also applicable to the distribution pattern of *Aegialites* beetles here reported.

### Taxonomic account

The large genetic difference and different demographic histories suggest that the two lineages may represent two different species, although we identified both as *A. stejnegeri* on the basis of external morphological characters. Lineage A may represent a species distributed on Hokkaido and Sakhalin, and lineage B another species distributed on Hokkaido and the Kuriles. Until recently, *A. stejnegeri* was the only species of *Aegialites* described from areas adjacent to Japan, with two subspecies (*A. s. stejnegeri* Linell on Tyulenii Island off Sakhalin, and *A. s. sugiharai* [Kôno, 1938] in the southern Kuriles) (Kôno, 1936, 1938; Sugihara, 1938). The two subspecies putatively differ in body color and the widest position of the pronotum, although we found it difficult to identify our specimens to subspecies using these characters. Zerche (2004) recently described several *Aegialites* species from the Kuriles. Further studies, including a detailed comparison with the species that Zerche described, will be necessary to determine the taxonomic status of the two lineages of *Aegialites* beetles we detected.

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