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Phylogeographic analysis of character displacement in feeding phenotypes of snail-feeding *Acoptolabrus* ground beetles

Running head: Phylogeography of character displacement

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Abstract

Ecological character displacement predicts that interspecific resource competition results in greater trait divergence between species in sympatry than in allopatry. Yet other processes such as ecological sorting result in the same pattern of trait variation. In this study, we characterize character displacement in 8 species of snail-feeding *Acoptolabrus* ground beetles in the Far East. *Acoptolabrus* exhibit divergent feeding phenotypes, including species with a slender forebody for intruding large shells and species with stout heads and mandibles for crushing small shells. The pattern of character displacement in feeding phenotypes was confirmed by multivariate analysis of body dimensions. Molecular phylogenetic analysis, divergent time estimation, and biogeographical analysis revealed that sympatry and phenotypic divergence occurred multiple times during the Pleistocene, and virtually simultaneously within each geographical area. Comparative analysis revealed that the evolution of feeding phenotypes best fit a selective model with three adaptive optima, corresponding to the three cases of sympatry with a congener. Repeated coincidences of sympatry and adaptive differentiation in feeding phenotypes suggest causal relationships, although the precise order of events was difficult to discriminate. This study provides insight into the spatio-temporal dynamics of interspecific interactions and adaptive phenotypic diversification.

Keywords

body size, *Carabus*, Carabidae, macrocephalism, Ornstein-Uhlenbeck process, stenocephalism, sympatry, resource competition

Introduction

Allopatric differentiation and subsequent geographical range expansion of closely related species can lead to secondary contact and competition for resources in sympatry (Brown and Wilson 1956). In the context of resource competition, natural selection may favor exploitation of less competitive resources and disfavor exploitation of resources that overlap with those of competitors. As a result, traits associated with resource exploitation can be expected to diverge in areas of sympatry; this process is referred to as ecological character displacement (Brown and Wilson 1956, Schluter 2000, Pfennig and Pfennig 2009, Stuart and Losos 2013). Ecological character displacement is often and initially recognized as a geographical pattern of trait variation, i.e., greater trait divergence between species in sympatry than in allopatry. However, multiple processes other than adaptation to interspecific resource competition can result in the same pattern (Stuart et al. 2017). In particular, it has often been difficult to distinguish between evolutionary trait divergence in response to competition between sympatric species (i.e., size adjustment) and ecological sorting in which only differently sized species can colonize an area of sympatry (i.e., size assortment) (Losos 1990, Miles and Dunham 1996, Giannasi et al. 2000, Moen and Wiens 2008).

The historical background is important for understanding the ecological and evolutionary processes of character displacement. Several criteria have been established to demonstrate ecological character displacement (Schluter and McPhail 1992, Stuart and Losos 2013, Stuart et al. 2017). From a historical point of view, size adjustment is inferred when trait diversification occurred after the occurrence of sympatry; while size assortment is inferred when diversification preceded the occurrence of sympatry (see Fig. 2 in Losos 1990). Additionally, ecological character displacement requires that the pattern of divergence in sympatry cannot be explained by stochastic processes. This can be tested by comparative phylogenetic analyses that discriminate between neutral and selective processes of trait evolution, which can detect shifts in selective regimes in response to distributional changes (Hansen et al. 2000, Butler and King 2004). Thus, phylogenetic, biogeographic, and comparative analyses can reveal the historical processes contributing to character displacement.

Feeding phenotypes have played pivotal roles in species diversification and coexistence (Goldschmidt 1994, Satoh et al. 2003, Grant and Grant 2014), and have provided key examples of character displacement in the literature (Grant and Grant 2006, Martin and Pfennig 2011). Organisms with low dispersal ability appear to be ideal for testing historical process of character displacement because it may be feasible to track changes in geographical ranges and thus selective regimes (i.e., sympatry or allopatry) (e.g., island *Anolis* lizards, Losos, 1990). Accordingly, flightless ground beetles with divergent feeding phenotypes provide a good opportunity for elucidating the historical process of character displacement. Snail-feeding ground beetles in the genus *Carabus* (sensu lato) (Insecta, Coleoptera, Carabidae) exhibit two phenotypes for land snail predation (Sturani 1962). Beetles with stenocephalism show a slender forebody that is able to intrude shells. Beetles with macrocephalism show a stout forebody, especially in the head and mandibles, which is able to crush shells. In *Damaster blaptoides* Koller 1836*,* distributed in the Japanese Archipelago (Konuma and Chiba 2007, Konuma et al. 2011, 2013ab, 2014), stenocephalic and macrocephalic phenotypes occur as geographical races. Differentiation of these phenotypes has a genetic basis (Konuma et al. 2013a, 2014). Morphological differentiation is linked to behavioral differentiation. Individuals from stenocephalic and macrocephalic populations are likely to attack snails via intrusion and biting, respectively, indicating a trade-off between the two functions (Konuma and Chiba 2007). This differentiation appears to be promoted by selection against intermediate phenotypes (Konuma et al. 2013b). Geographical variation in the shape of the forebody is associated with geographical variation in prey size (Konuma et al. 2010). Stenocephalism may be favored when large snails are common because the large entrance of the shells facilitates the intrusion of slender bodies, while macrocephalism may be selected when small snails are common because small shells are easy to crush (Konuma and Chiba 2007). These patterns suggest that the differentiation of stenocephalic and macrocephalic phenotypes depends on the distribution of resources. Interspecific resource competition is also a determinant of the distribution of resources and thus the fitness of stenocephalic and macrocephalic phenotypes. However, the contribution of interspecific resource competition to the differentiation of feeding phenotypes in snail-feeding ground beetles has not been evaluated, partly because the model species *D. blaptoides* is an exclusively snail-feeding species across most of its distributional range.

Snail-feeding *Acoptolabrus* Morawitz 1886 ground beetles are closely related to *D. blaptoides* and provide an opportunity to examine the effect of resource competition on the differentiation of feeding phenotypes. *Acoptolabrus* includes species with stenocephalism and macrocephalism. Unlike *D. blaptoides*, pairs of species co-occur in three regions (Fig. 1). Interestingly, at least two sympatric species pairs exhibit conspicuous divergence towards stenocephalism and macrocephalism (Ishikawa and Deuve 1982, Ishikawa and Kim 1983). Other species occurring as the only *Acoptolabrus* species in their ranges show intermediate to slightly stenocephalic phenotypes. This pattern of species distribution and trait diversification allows us to examine the contributions of species contact and resource competition to the differentiation of feeding phenotypes and to examine the difference in selective regimes between areas with and without sympatric species. Since *Acoptolabrus* species exploit common resources (i.e., land snails), more intense interspecific resource competition in sympatric species than in species that occur singly is expected to lead to greater divergence in feeding phenotypes.

In this study, we evaluated the hypothesis that resource competition and resultant ecological character displacement contribute to the diversification of feeding phenotypes in *Acoptolabrus* ground beetles. To test this, we first estimated phylogenetic relationships among species and evaluated changes in distributions by biogeographical analyses. We quantified the body size and shape of all species to evaluate whether patterns of divergence in feeding phenotypes are consistent with character displacement. Then, we examined changes in distribution, i.e., between less competitive (without sympatric congener) and more competitive (with sympatric congener) environments, to examine whether sympatric species experienced selective regimes favoring divergent feeding phenotypes. Based on these results, we discuss trait differentiation via interspecific resource competition and ecological character displacement from a phylogeographic perspective.

Materials and Methods

Organisms and study area

This study analyzed 8 species of *Acoptolabrus*, a subgenus of *Carabus* Linnaeus sensu lato, which is closely related to *Damaster* (Hauser 1921, Ishikawa 1986, Deuve 2004, Deuve et al. 2012). These species are distributed in the Far East including the Korean Peninsula [*A. leechi* (Bates 1888), *A. mirabilissimus* (Ishikawa et Deuve 1982) and *A. changeonleei* (Ishikawa et Kim 1983) (only in Mt. Jirisan)], Primorsky region of the eastern coastal regions of China and Russia [*A. constricticollis* (Kraatz 1886) and *A. schrencki* (Motschulsky 1860)], Sakhalin Island in Russia (*A. lopatini* Morawitz 1886), and Hokkaido Island in Japan [*A. gehinii* (Fairmaire 1876) and *A. munakatai* (Ishikawa 1968)] (Fig. 1). *Acoptolaburs mirabilissimus* and *A. changeonleei* are apparently macrocephalic (Ishikawa and Deuve 1982, Ishikawa and Kim 1983), while other species show intermediate conditions or a varied tendency of stenocephalism (Fig. 1). Of these, three pairs (i.e., *A. constricticollis* and *A. schrencki*, *A. leechi* and *A. mirabilissimus*, and *A. leechi* and *A. changeonleei*) are partially or entirely sympatric. Note that *A. leechi* is sympatric with two species, *A. mirabilissimus* and *A. changeonleei*. Hereafter, *A. leechi* populations in the Korean Peninsula and on Mt. Jirisan are treated as different taxa, unless otherwise indicated, for two reasons: (1) these populations have been recognized as different subspecies (Kwon and Lee 1984, Deuve 1990), with ranges separated by at least about 200 km between Mt. Sobaeksan and Mt. Jirisan; and (2) the populations may have experienced different selective regimes by coexisting with different congeneric species. *Acoptolabrus gehinii* and *A. munakatai* occur in parapatry in Hokkaido Island, and *A. lopatini* is the only *Acoptolabrus* species on Sakhalin Island. In addition, *D. blaptoides*, *Coptolabrus jankowski* Oberthür 1883 and *C. smaragdinus* (Fischer 1823) were included as outgroups. The study area is located at the easternmost part of the Palaearctic, where deciduous broad-leaved or coniferous forsts dominate (e.g., Park *et al*. 2017). Topography of the area has been completed after Miocene (Parfenov *et al*. 2009).

Our samples consisted of dried specimens (mostly collected before 1990s, stored in the R. Ishikawa Collection at the university museum in the University of Tokyo, Japan) used for morphological analysis, and newly collected samples used for DNA analysis as well as morphological analysis. The samples were collected from multiple sites in the species ranges (Fig. 1, Table S1) using pitfall traps. All the species collected by the authors were not protected by laws in respective countries at the time of collection (Table S1). Collected beetles were dissected and fresh muscles or gonads were preserved in absolute ethanol for DNA extraction. The body was pinned and dried for morphological analyses. Since the ranges of sympatric species pairs were not fully overlapping, they may occur singly in peripheries (Fig. 1). Our samples were distributed in both peripheral as well as central (i.e., sympatric) areas, pooled, and treated as representatives of the species. This is because *Acoptolabrus* species are rare in most localities and sample sizes tended to be insufficient for population-level analyses.

DNA extraction and sequencing

Phylogenetic analyses were based on 86 individuals corresponding to eight species of *Acoptolabrus* (Table S1), including retrieved data from GenBank for 17 individuals with at least one gene sequence and 69 newly sequenced individuals. Sample sizes for *A. leechi* and *A. lopatini* were relatively low, so the mid-tarsi of dried specimens stored in the R. Ishikawa Collection were also used for DNA extraction (Table S1).

Total DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer’s instruction. Two mitochondrial gene regions [cytochrome oxidase subunit I, *COI* (814-bp); and NADH dehydrogenase subunit 5, *ND5* (1083-bp)] and three nuclear gene regions [phosphoenolpyruvate carboxykinase, *PepCK* (623-bp); wingless, *Wg* (440-bp); and 28S rRNA, *28S* (852-881-bp)] were sequenced using primers shown in Table S2. A dye terminator cycle sequencing reaction was performed for the direct sequencing of the PCR products using the ABI PRISM BigDye Terminator Cycle Sequencing FS Ready Reaction Kit, followed by electrophoresis on an ABI3130 genetic analyzer (Applied

Biosystems, Foster City, CA, USA). The sequence data have been deposited in DNA Data Bank of Japan (accession numbers shown in Table S1). DNA sequences were aligned using MAFFT (Kato et al. 2002) implemented in Seqotron (Fourment and Holmes 2016). No gaps were required for any of the genes.

Phylogenetic analysis

The maximum likelihood (ML) gene tree for individual genes were used to check for shared sequences suggesting introgressive hybridization between sympatric species, which hinders proper inference of species relationships in *Carabus* beetles (Sota and Vogler 2001). Optimal substitution models for the dataset, including outgroups, were selected based on the Akaike information criterion (AIC) calculated using jModeltest ver. 2.1.3 (Darriba et al. 2012). The GTR+I+G substitution model was used for *COI*, *ND5*, *PepCK* and *Wg*, and GTR+I was used for *28S,* for rapid bootstrap analyses with 1,000 replicates using RAxML ver. 8.2.12 (Stamatakis 2014).

A concatenated data set of the five gene alignments was obtained (9 ingroup taxa and 3 outgroup species, total 3740 bp). Mitochondrial gene sequences for *A. leechi*, *A. mirabilissimus,* and *A. changeonleei* were removed because mitochondrial introgression was suspected (Fig. S1). For two samples of *A. lopatini* with only *28S* data, *ND5* data for another specimen were combined to reduce the impact of missing data. The GTR+I+G substitution model was used for each gene partition. Analyses were performed based on the partition scheme related to codon position in protein-coding genes: *COI, ND5, PepCK,* and *Wg*. Phylogenetic relationships were inferred by rapid bootstrap analyses with 1,000 replicates using RAxML.

For further examination of the possible effects of introgressive hybridization and incomplete lineage sorting, we ran multispecies coalescent analyses based on the data sets including and excluding the problematic sequences using BEAST 2.6.2 (Bouckaert et al. 2014). The data consisted of only in-group taxa. For substitution models, we used TrN+I for *COI*, HKY+I for *ND5*, K80+I for *PepCK* and *Wg*, and TIM1+I for *28S* following the results of model selection with jModeltest based on the minimal Bayesian Information Criterion. A Markov Chain Monte Carlo analysis was performed with a hundred million generations and sampling every 50,000 generations based on the log-normal relaxed clock model with a rate of 1.77% My^{-1} for *COI* (Papadopoulou et al. 2010, see also below). Convergence was confirmed using TRACER 1.7.1 (Rambaut et al. 2018). Following removal of 10% burn-in, the sampled posterior trees were summarized to generate a maximum clade credibility tree and to calculate the mean ages and 95% highest posterior density intervals using TreeAnnotator.

Divergence time estimation

Divergence times were estimated using BEAST 2.6.2 based on the log-normal relaxed clock model with a rate of 1.77% My^{-1} for *COI* (Papadopoulou et al. 2010). This rate was slightly faster than a recent estimate for *Carabus* species (Andújar et al. 2012) and may underestimate the node ages. Authentic fossil records of Carabinae are limited and not readily used for calibrating nodes in our divergence time estimation (Andújar et al. 2012, Sota et al. 2020). The data consisted of only in-group taxa and the topology was fixed to the ML tree. Substitution models were same as described above. A Markov Chain Monte Carlo analysis was performed with ten million generations and sampling every 1,000 generations. Convergence was confirmed using TRACER. Following removal of 10% burn-in using TreeAnnotator, the sampled posterior trees were summarized to generate a maximum clade credibility tree and to calculate the mean ages and 95% highest posterior density intervals.

Biogeographical analysis

A biogeographical analysis was performed based on the time-calibrated tree pruned to include eight species, treating *A. leechi* (populations in Mt. Jirisan and in other areas of the Korean Peninsula) as a single species. This analysis allowed one species to occupy multiple areas. The results of an analysis based on nine taxa (including two separate *A. leechi* populations) were consistent with those of the analysis based on eight taxa, but involved greater uncertainty. Based on the distributional data in our collection and in the literature (Breuning 1932-1937, Ishikawa 1991, Deuve 2004), the eight species were coded as present or absent in five discrete areas: Hokkaido Island, Sakhalin Island, Primorsky, Korean Peninsula, and Mt. Jirisan.

Ancestral range reconstruction was performed using BioGeoBEARS (Matzke 2013) implemented in RASP version 4.2 (Yu et al., 2015). Maximum likelihood analyses were performed under six different biogeographical models, dispersal-extinction cladogenesis (DEC, Ree and Smith 2008), likelihood-based dispersal-vicariance analysis (Ronquist 1997) (DIVALIKE), likelihood-based model in the program BayArea (Landis et al. 2013) (BAYAREALIKE), and models with an additional parameter for jump dispersal (i.e., founder event speciation) (DEC+J, DIVALIKE+J, and BAYAREALIKE+J). The fit of each model to the data was compared, based on the Akaike information criterion corrected for small sample size (AICc). We did not constrain possible combinations of the areas or dispersal rates between the areas, given that (1) the topography of the study area was stable during the diversification of this group of insects (Parfenov et al. 2009, see also divergence time estimation results), and because (2) *Acoptolabrus* species adapted to cool environments, as in Hokkaido Island and Korean Peninsula, inhabit only higher mountains, so that their dispersal and colonization may be facilitated in the glacial periods that occurred in this time scale (i.e., the mid to late Quaternary).

Comparative analysis of morphological traits

To evaluate morphological divergence among taxa, vertical and horizontal distances of the head (VH and HH), prothorax (VP and HP), and elytra (VE and HE) of adult beetles (Konuma and Chiba 2007) were measured to the nearest 0.01 mm on digital images of dried specimens using ImageJ ver. 1.49m (Schneider et al. 2012). Sample sizes for each species and sex were shown in Table S3. Briefly, measurements were obtained from 27 individuals of *A. gehinii*, 20 of *A. munakatai*, 7 of *A. lopatini*, 4 of *A. schrencki*, 14 of *A. constricticollis*, 10 of *A. leechi* from the Korean Peninsula, 8 of *A. leechi* from Mt. Jirisan, 26 of *A. mirabilissimus* and 12 of *A. changeonleei*. Of these, six samples of *A. leechi* from Mt. Jirisan were measured based on photographs in Imura and Kezuka (1992). These specimens consisted of samples for DNA analysis as well as specimens stored in the authors' collections.

To capture overall morphological variations, a principal component analysis was performed based on the correlation matrix for six measurements. PC1 (73.6%) represented overall body size, as indicated by all the positive loadings, and PC2 (14.5%) represented the stoutness of the forebody, as indicated by the positive loadings of head and pronotum widths and negative loadings of other traits (Table S4). Thus, PC1 and PC2 scores were used as indicators of body size and shape, respectively (Fig. S2). Morphological differences between taxa and sex were examined by general linear models (GLMs) using PC1 or PC2 scores as the dependent variable and taxa and sex as independent variables. Least square means and standard errors of body size and shape were calculated for each taxon to adjust for differences between sexes. Differences in least square means of body size and shape between taxa were examined by Tukey–Kramer tests.

Character displacement can be detected as greater trait divergence between sympatric species pairs than between pairwise combinations of allopatric taxa (Brown and Wilson 1956, Schluter 2000, Pfennig and Pfennig 2009, Stuart and Losos 2013). We examined this expectation by comparing species differences in body size and shape between sympatric and allopatric pairs of species. First, differences in the least square means of PC1 and PC2 scores were calculated for all pairwise taxa (N = 36 based on nine taxa). Then, these species pairs were classified as sympatric or not. GLMs were constructed with species difference as the dependent variable and distribution (sympatric or not) and divergence time (estimated from the time-calibrated molecular phylogenetic tree) as independent variables. Since species differences were not statistically independent, randomization tests with 10,000 pseudoreplicates were performed using the *PermTest* function in the package *pgirmess* 1.6.9 (Giraudoux et al. 2018) in R. Other statistical analyses of morphological variation were performed using JMP ver. 8 (SAS Institute, 2009).

Sympatric species under resource competition may experience different selective regimes from those of species without competitors. Specifically, character displacement as a result of resource competition may be driven by divergent selection with multiple optimal trait values. Additionally, resource competition may limit the amount of available resources and select for smaller body and trait sizes. To examine these expectations, we investigated the mode of the evolution of body size and shape by comparing model fits to the data using AIC, including (1) Brownian motion model

(BM), (2) Ornstein–Uhlenbeck (OU) model with a single global optimum (OU1), (3) OU model with two optima, "sympatric" and "single" (OU2-1), (4) OU model with two optima, "sympatric stout" and others (OU2-2), (5) OU model with two optima, "sympatric slender" and others (OU2-3), (6) OU model with three optima, "single," "sympatric stout," and "sympatric slender" (OU3). Under BM, traits evolve only along a random walk; accordingly, BM was regarded as a null model without the effect of selection. The OU models describe a random walk with stationary peak(s), such that trait values have a tendency to be attracted to optimal value(s) (Hansen, 1997; Butler & King, 2004). The selective regimes in internal branches depend on whether taxa are sympatric with a congener or not (OU2-1, 2-2, 2-3, and 3), and these were determined based on the ancestral range reconstruction. These evolutionary models were fit using the package *ouch* ver. 2.14-1 (King 2019) in R.

Results

Phylogenetic analysis

For *ND5*, 1069 of 1083 bp were used after trimming 7 bp of noncoding sequence and 7 bp of the 5' terminus of the phe-tRNA gene. For *28S*, a 794-bp region that was consistently obtained across samples was used. The ML tree based on the concatenated data set showed that *Acoptolabrus* is monophyletic with moderate support and contained two well-supported major lineages, lineage I and II (Fig. 2). Lineage I consisted of five species, which were found in Primorsky (*A. schrencki* and *A. constricticollis*), the Korean Peninsula (*A. leechi* and *A. mirabilissimus*), and Mt. Jirisan (*A. leechi* and *A. changeonleei*). Lineage II consisted of three species, which were found in Sakhalin Island (*A. lopatini*) and Hokkaido Island (*A. gehinii* and *A. munakatai*). The monophyly of species was supported by moderate-to-high bootstrap values except for *A. gehinii* with no bootstrap support (i.e., < 50%). *Acoptolaburs constricticollis* was divided into two well-supported lineages distributed in northeastern and southwestern regions of Primorsky. Two macrocephalic species, *A. mirabilissimus* and *A. changeonleei*, were sister taxa, although support for relationships within lineage I was relatively low. *Acoptolabrus mirabilissimus* tended to involve long branches in comparison to other species.

Multispecies coalescent analyses showed further evidence of mitochondrial introgression among three species in the Korean Peninsula, *A. leechi*, *A. mirabilissimus* and *A. changeonleei* (Fig. S3). Multispecies coalescent tree based on all the five genes indicated that divergence times between the three species were very short, and two species sympatric in Mt. Jirisan, *A. leechi* and *A. changeonleei*, were strongly inferred as sister taxa, probably due to shared mitochondrial gene sequences among the species. The divergence times in older nodes were also shorter in this tree, probably due to overestimation of molecular clock rates in nuclear genes via shared mithochondrial sequences among the species. By contrast, removing mitochondrial gene sequences from the three species, such patterns disappeared. The phylogenetic relationships among the three species were still ambiguous, as in paraphyly of *A. leechi*, possibly due to a limited number of genes (i.e., 3 nuclear genes). The phylogenetic relationships among species other than the three Korean species were same among the two multispecies coalescent trees and the concatenated tree (Fig. 2), suggesting little effect of incomplete lineage sorting. Since the multispecies coalescent tree tended to exhibit lower branch supports than the concatenated tree, the concatenated tree was used for subsequent analyses.

Divergence time

The estimated divergence time between lineages I and II was 1.55 million years ago (Ma) (Fig. 3). In lineage I, the estimated divergence time between species in Primorsky and those in the Korean Peninsula and Mt. Jirisan was 0.97 Ma, with subsequent speciation within the two lineages between 0.79 and 0.61 Ma. The differentiation in *A. leechi* between Mt. Jirisan (sample no. 46*) and Mt. Sobaeksan (45*) populations, which were the most geographically and phylogenetically closely related, was estimated to have occurred 0.08 Ma. In lineage II, the estimated divergence time between *A. lopatini* in Sakhalin Island and two species in Hokkaido Island was 0.69 Ma. In Hokkaido Island, the speciation of *A. gehinii* and *A. munakatai* occurred 0.33 Ma.

Historical biogeography

DIVALIKE was selected as the best-fit biogeographical model with a model weight of 63% (Table 1). The second best model was DIVALIKE+J (23%), which was marginally non-significantly worse than the DIVALIKE model (ΔAICc = 2.04, likelihood ratio test, $\chi^2_1 = 3.58$, $P = 0.058$). Estimated parameters for these two models indicated that the distributions could be explained (mostly) by dispersal, with a marginal effect of founder event speciation, but not extinction (Table 1). Other models revealed poorer fits to the data (ΔAICc > 3.60, model weight < 10%). Hereafter, we refer to the historical species distributions based on the DIVALIKE model results.

Ancestral range reconstruction based on the DIVALIKE model revealed that pairs of sympatric stenocephalic and macrocephalic species attained speciation within each area (Fig. 4). The common ancestor of *A. leechi* (stenocephalic), *A. mirabilissimus* (macrocephalic)*,* and *A. changeonleei* (macrocephalic) occupied either the Korean Peninsula or Mt. Jirisan (Fig. 4D). Speciation between stenocephalic *A. leechi* and the common ancestor of the two macrocephalic species occurred within this region (Fig. 4D, E). The ancestral range of the common ancestor of the two macrocephalic species was estimated to be the region spanning both the Korean Peninsula and Mt. Jirisan, followed by allopatric speciation between *A. mirabilissimus* and *A. changeonleei* (Fig. 4E).

The sympatric sister species *A. schrencki* (moderately macrocephalic) and *A. constricticollis* (stenocephalic) shared a common ancestral area (Primorsky, Fig. 4C). Lineage II from Sakhalin and Hokkaido islands diverged in allopatry to form *A. lopatini*, *A. gehinii,* and *A. munakatai* (Fig. 4F, G). The ancestral ranges of higher nodes were uncertain (Fig. 4A, B).

Morphological evolution

Body size (PC1) differed significantly among taxa and was larger in females (species, $F_{8,109} = 26.1$, $P < 0.0001$; sex, $F_{1,109} = 98.7$, $P < 0.0001$) (Fig. 5A). Body size did not differ significantly between two parapatric species in Hokkaido Island or between two sympatric species in Mt. Jirisan (Tukey–Kramer test, $P > 0.05$). Body sizes of *A. leechi* and *A. schrencki* were significantly greater than those of sympatric *A. mirabilissimus* and *A. constricticollis* in the Korean Peninsula and Primorsky, respectively ($P < 0.05$).

Body shape (PC2) also differed significantly among taxa but not between sexes (species, $F_{8,109} = 93.4$, $P < 0.0001$; sex, $F_{1,109} = 0.39$, $P = 0.53$) (Fig. 5B). *Acoptolabrus mirabilissimus* and *A. changeonleei* had the stoutest forebodies (i.e., macrocephalism). *Acoptolabrus lopatini* and *A. schrencki* had moderate values. The remaining species possessed relatively slender forebodies (i.e., stenocephalism). Body shape differed significantly in all three sympatric species pairs (Tukey–Kramer test, $P < 0.05$), while it did not differ between parapatric species in Hokkaido Island ($P > 0.05$).

Interspecific variations in body size (PC1) were not explained by distribution or divergence time (GLM with randomization test: distribution, $\beta = -0.021 \pm 1.126$, $P = 0.85$; divergence time, $\beta = 0.558 \pm 0.734$, $P = 0.45$) (Fig. 5C). By contrast, body shape (PC2) exhibited significantly greater divergence in sympatry (distribution, $\beta = 0.818 \pm 0.413$, $P = 0.045$; divergence time, $\beta = 0.017 \pm 0.229$, $P = 0.95$) (Fig. 5D). Thus, the pattern of interspecific variation in body shape was consistent with that expected from character displacement.

Based on the present sympatry and shared ancestral range between species (Fig. 4), we assumed the hypothetical selective regimes on tree branches for OU models (Fig. 6). The best-fit model for body size was OU2-1 with two optima (Table 2). This model predicted that sympatric congeners would be subjected to selection for smaller bodies than those of singly distributed species. The second best fitting model was OU1 (ΔAIC = 0.94), with ΔAIC values for comparisons with other models of >1.29. The best-fit model for body shape was OU3 with three optima for singly distributed species and sympatric (paired) species (Table 2). This model predicted that sympatric species exhibited divergence towards stout and slender bodies, whereas singly distributed species maintained intermediate phenotypes. The second best fitting model was OU2-2 (ΔAIC = 1.07); other models showed poorer fits (ΔAIC > 4.10).

Discussion

Interspecific divergence in *Acoptolabrus* feeding phenotypes corresponded to the expected geographical pattern for character displacement. The forebody shapes differed more substantially in sympatric species pairs than in others (Fig. 5D). The evolution of body shapes was not consistent with a random process but supported in a model where a selective process with multiple adaptive optima related to the presence of a congener (Table 2). Phylogenetic and biogeographic analyses indicated that species in sympatry with divergent feeding phenotypes were closely related and diverged around the present sympatric area (Fig. 4), particularly *A. constricticollis* and *A. schrencki* in Primorsky (Fig. 4C), and *A. leechi* and the common ancestor of the two macrocephalic species (*A. mirabilissimus* and *A. changeonleei*) in the Korean Peninsula and Mt. Jirisan (Fig. 4D). These results indicate that sympatry and adaptive differentiation of feeding phenotypes repeatedly coincided, suggesting their causal relationships. These results also indicate that the adaptive divergence of feeding phenotypes and range overlap occurred virtually simultaneously within each geographical area. However, because we cannot track changes in distribution within each area, it is difficult to determine whether phenotypic divergence preceded (size assortment) or followed (size adjustment, i.e., ecological character displacement) the occurrence of sympatry. The ML tree based on the concatenated dataset still includes weakly supported nodes (Fig. 2) and should, therefore, be interpreted with caution; further analyses based on larger data sets are needed.

Understanding the mode of speciation may provide insight into the historical process of the observed character displacement. From the geographic point of view of speciation, allopatric speciation due to geographical isolation explains the diversification of flightless ground beetles in general (Ishikawa 1991) and may explain patterns of evolution within *Acoptolabrus*. Species in *Acoptolabrus* assigned to lineage II are relatively young (Fig. 3), and speciation occurred between populations in nearby geographic areas divided by the sea (between Sakhalin and Hokkaido islands) and by the lowland along rivers (within Hokkaido Island) (Figs. 1 and 4). These species exhibit only slight divergence in body shape and do not yet occur in sympatry (Figs. 1 and 5).

Given a similar mode of speciation may also be the case in the relatively old *Acoptolabrus* species assigned to lineage I, allopatric speciation within respective distributions without phenotypic differentiation could be followed by secondary contact with resource competition and divergence in feeding phenotypes (i.e., size adjustment). Otherwise, allopatric speciation in lineage I may have been accompanied by the ecological differentiation of feeding phenotypes in response to the spatial heterogeneity of available resources (i.e., ecological speciation; Nosil 2012). If so, phenotypic differentiation may have preceded sympatry (i.e., size assortment). Geographical differentiation of feeding phenotypes in *D. blaptoides* may be consistent with the latter process, although populations with divergent feeding phenotypes have not yet come into contact (Konuma et al. 2010).

Stenocephalic and macrocephalic species in sympatry at Mt. Jirisan may experience both the processes of size adjustment and size assortment. The divergence time between two sister macrocephalic species (0.65 Ma between *A. changeonleei* in Mt. Jirisan and *A. mirabilissimus* in the rest of the Korean Peninsula; Fig. 3) was far longer than that between two lineages of stenocephalic *A. leechi* (0.08 Ma between populations in Mt. Jirisan and in Mt. Sobaeksan; Fig. 3). This suggests that the timing of the colonization of Mt. Jirisan by macrocephalic and stenocephalic species differed. It is possible that a macrocephalic ancestor first colonized Mt. Jirisan, followed by the divergence of *A. changeonleei* and the more recent colonization by the stenocephalic *A. leechi* (i.e., size assortment). If so, *A. changeonleei* may have lacked a competitive congener in Mt. Jirisan for a period of time. Such variation in the period of sympatry with a competitor may influence the evolution of macrocephalism (i.e., size adjustment). The degree of macrocephalism was significantly weaker in *A. changeonleei*, with a possible period without a congener than in *A. mirabilissimu*s with consistent overlap with a congener (Figs. 4 and 5). Otherwise, *A. leechi* might have continuously occupied both the Korean Peninsula and Mt. Jirisan just after its divergence (Fig. 4D), with relatively frequent gene flow between populations in the two areas. If this is the case, variation in macrocephalism may be due to factors other than the different durations in sympatry. Fine-scale phylogeographic analyses within respective geographic areas are necessary to reveal the relative timing of speciation, changes in distribution, and phenotypic differentiation, thereby improving our understanding of the historical process of character displacement in *Acoptolabrus*.

To demonstrate ecological character displacement or to conclude that resource competition resulted in character displacement, six criteria have been proposed (Schluter and McPhail 1992, Stuart and Losos 2013, Stuart et al. 2017). In the snail-feeding ground beetles including *Acoptolabrus*, at least three of the six criteria were met. First, our comparative analysis indicated that the evolution of feeding phenotypes (i.e., body shape) was better explained by selective processes than by chance. Second, the genetic basis of divergent feeding phenotypes has been confirmed, albeit in the closely related species *D. blaptoides* (Konuma et al. 2013a, 2014). Third, the divergent feeding phenotypes are related to competitive interactions between species with similar life histories (i.e., spring breeders) and shared food resources (i.e., land snails) (Karasawa 1988, Kim and Lee 1992). Our comparative analysis indicated that species overlapping with a congener experienced stronger selection for a smaller body size than that of species distributed singly (Table 2), providing indirect evidence for stronger resource competition between sympatric species. Note that *D. blaptoides* exhibits a latitudinal cline in body size, probably reflecting climatic adaptation (Konuma et al. 2010). By contrast, *A. lopatini* had the largest body size (Fig. 5A), although it was distributed in a northern area (Fig. 1), suggesting that body size evolution in

Acoptolabrus has been influenced by processes other than climatic adaptation.

To satisfy the fourth criterion, independent evidence for resource competition is needed. Experimental studies may be useful to examine whether populations with similar phenotypes exhibit stronger competition for food resources (Germain et al. 2018). Fifth, clear evidence for size adjustment (and against size assortment) is still lacking. To examine this criterion, macrocephalic *A. mirabilissimus* may be useful because it is distributed across the Korean Peninsula and partly overlaps with stenocephalic *A. leechi* (Fig. 1), allowing a comparison between allopatric and sympatric populations based on a fine-scale phylogeographic analysis within species. Sixth, environmental conditions should be evaluated. *Acoptolabrus* species inhabit a relatively wide latitudinal range with various geographical features (continental coast, peninsula, and islands, Fig. 1), where climatic conditions vary. The distribution of food resources may also vary among areas (Konuma et al. 2010). Additionally, the snail-feeding *Carabus* species belonging to the subgenera *Coptolabrus*, *Megodontus,* and *Damaster* co-occur with *Acoptolabrus* species on the Korean Peninsula (including Mt. Jirisan), Sakhalin and Hokkaido islands, and Hokkaido Island, respectively. However, these competitors tend to have life histories different from those of *Acoptolabrus* species (autumn breeding, Karasawa 1992, Kim and Lee 1992), suggesting that competition for food resources with species belonging to different subgenera may be relatively weak compared to competition between *Acoptolabrus* species. In addition to resource competition, reproductive interference could also contribute to character displacement because hybridization between sympatric species was detected as mitochondrial introgression (Fig. S2) (see also Imura 1994). Body size and genital morphology are important determinants of reproductive interference between ground beetle species in contact zones (Sota and Kubota 1998, Sota et al. 2000, Okuzaki et al.

2010), but we did not detect character displacement in body size (Fig. 5C), and interspecific variation in genital morphology is relatively low in *Acoptolabrus* (Ishikawa 1986).

In conclusion, we described a novel case of character displacement in the feeding phenotypes of snail-feeding ground beetle species and characterized the process in a biogeographical context. Resource competition between sympatric species can lead to the divergence of feeding phenotypes, but analysis of phenotypic variation within species, as well as evaluation of resource competition and environmental condition are warranted to confirm that all criteria for ecological character displacement are met. This study offers an interesting case to study the biogeography of character displacement, providing insight into the spatio-temporal dynamics of interspecific interactions and their effect on adaptive phenotypic diversification.

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Table 1. Comparison of biogeographical models. Best-fit model is in boldface.

Model	Dispersal	Extinction	Jump dispersal	N of parameters	LnL	AICc	ΔAICc	Model weight
DEC	0.092	0.058	–	2	-16.41	39.21	6.22	0.028
DEC+J	0.008	0.000	0.140	3	-12.30	36.59	3.60	0.100
DIVALIKE	**0.075**	**0.000**	–	**2**	**-13.30**	**32.99**	**0.00**	**0.630**
DIVALIKE+J	0.039	0.000	0.061	3	-11.51	35.01	2.02	0.230
BAYAREALIKE	0.064	0.900	–	2	-18.56	43.51	10.52	0.003
BAYAREALIKE+J	0.033	0.000	0.160	3	-14.66	41.31	8.32	0.010

Table 2. Comparison of trait evolution models. Best fit models are in boldface.

	θ	α	σ^2	AIC	ΔAIC	Optima
Body size (PC1)						
BM	0.73	–	7.61	42.74	1.29	
OU1	–	123.62	825.14	42.39	0.94	0.46
OU2-1	**–**	**5.82**	**29.18**	**41.45**	**0.00**	**Single=1.87, sympatric=-0.45**
OU2-2	–	232.22	1376.83	43.32	1.87	Sympatric stout=-0.40, others=0.90
OU2-3	–	5.94	39.52	44.01	2.56	Sympatric slender=-0.29, others=0.70
OU3	–	5.96	29.81	43.44	1.99	Single=1.86, sympatric stout=-0.51, sympatric slender=-0.35
Body shape (PC2)						
BM	0.01	–	1.07	25.01	7.89	
OU1	–	2.64	3.19	25.99	8.87	0.04
OU2-1	–	2.92	3.38	27.70	10.58	Single=-0.18, sympatric=0.25
OU2-2	–	3.79	1.48	18.19	1.07	Sympatric stout=1.13, others=-0.49
OU2-3	–	1.24	0.84	21.22	4.10	Sympatric slender=-2.61, others=0.41
OU3	**–**	**6.73**	**1.80**	**17.12**	**0.00**	**Single=-0.21, sympatric stout=0.93, sympatric slender=-0.86**

Table S1. Summary of samples included in molecular phylogenetic analysis. Sample numbers with an asterisk indicate old and dried specimens.

Sample No.	Locality	Year of collection	CO1	ND5	PepCk	Wg	28S	Reference
Acoptolabrus gehinii								
1	Furano, Hokkaido Is., Japan	2004	LC553397	LC567159	LC567452	LC567519	–	Present study
2	Furano, Hokkaido Is., Japan	2004	LC553398	LC567160	LC567453	LC567520	–	Present study
3	Furano, Hokkaido Is., Japan	2004	LC553399	LC567161	LC567454	LC567521	LC567588	Present study
4	Furano, Hokkaido Is., Japan	2004	LC553400	LC567162	LC567455	LC567522	LC567589	Present study
5	Hidaka, Hokkaido Is., Japan	2010	LC553401	LC567163	LC567456	LC567523	–	Present study
6	Hidaka, Hokkaido Is., Japan	2010	LC553402	LC567164	LC567457	LC567524	LC567590	Present study
7	Hidaka, Hokkaido Is., Japan	2010	LC553403	LC567165	LC567458	LC567525	–	Present study
8	Kushiro, Hokkaido Is., Japan	–	–	AB041085	–	–	–	Tominaga et al. (2000)
9	Tomakomai, Hokkaido Is., Japan	–	–	AB041086	–	–	–	Tominaga et al. (2000)
10	Higashigawa, Hokkaido Is., Japan	–	–	AB041087	–	–	–	Tominaga et al. (2000)
11	Hokkaido Is., Japan	–	–	D50339	–	–	–	Su et al. (1996)
12	Hokkaido Is., Japan	–	–	D50429	–	–	–	Su et al. (1996)
13	Chitose, Hokkaido Is., Japan	–	–	–	AY183527	AY183606	–	Sota and Ishikawa (2004)
A. munakatai								
14	Mt. Daisengendake, Hokkaido Is., Japan	2004	LC553404	LC567166	LC567459	LC567526	LC567591	Present study
15	Mt. Daisengendake, Hokkaido Is., Japan	2004	LC553405	LC567167	LC567460	LC567527	LC567592	Present study
16	Mt. Daisengendake, Hokkaido Is., Japan	2004	LC553406	LC567168	LC567461	LC567528	LC567593	Present study
17	Mt. Daisengendake, Hokkaido Is., Japan	2004	LC553407	LC567169	LC567462	LC567529	LC567594	Present study
18	Mt. Daisengendake, Hokkaido Is., Japan	2004	LC553408	LC567170	LC567463	LC567530	LC567595	Present study
19	Mt. Daisengendake, Hokkaido Is., Japan	2004	LC553409	LC567171	LC567464	LC567531	LC567596	Present study
20	Shirakami, Hokkaido Is., Japan	–	–	AB041083	–	–	–	Tominaga et al. (2000)

21	Otobetake, Hokkaido Is., Japan	–	–	AB041084	–	–	–	Tominaga et al. (2000)
A. lopatini								
24*	Sakhalin Is., Russia	1970	–	–	–	–	LC567597	Present study
25*	Sakhalin Is., Russia	1991	–	–	–	–	LC567598	Present study
26	Yuzino-Sakhalinsk, Sakhalin Is., Russia	–	–	AB041082	–	–	–	Tominaga et al. (2000)
A. schrencki								
27	Primorsky, Russia	2005	LC553410	LC567172	–	LC567532	–	Present study
28	Primorsky, Russia	2005	LC553411	LC567173	LC567465	LC567533	LC567599	Present study
29	Primorsky, Russia	–	–	AB041080	–	–	–	Tominaga et al. (2000)
30	Liaoning, China	2005	LC553412	LC567174	LC567466	LC567534	LC567600	Present study
31	Liaoning, China	2005	LC553413	LC567175	LC567467	LC567535	LC567601	Present study
32	Vysokogornyi, Russia	–	–	AB041081	–	–	–	Tominaga et al. (2000)
A. constricticollis								
33	Ussurisky, Russia	2005	LC553414	LC567176	LC567468	LC567536	LC567602	Present study
34	Ussurisky, Russia	2005	LC553415	LC567177	LC567469	LC567537	LC567603	Present study
35	Ussurisky, Russia	2005	LC553416	LC567178	LC567470	LC567538	LC567604	Present study
36	Ussurisky, Russia	2005	LC553417	LC567179	LC567471	LC567539	LC567605	Present study
37	Primorsky, Russia	–	–	AB050731	–	–	–	Su et al. (2001)
38	Liaoning, China	–	–	AB050674	–	–	–	Su et al. (2001)
39	Liaoning, China	–	–	AB050730	–	–	–	Su et al. (2001)
A. leechi								
40	Mt. Soraksan, Korea	2002	LC553418	LC567180	LC567472	LC567540	LC567606	Present study
41	Mt. Soraksan, Korea	2002	LC553419	LC567181	LC567473	LC567541	LC567607	Present study
42	Mt. Odaesan, Korea	2002	LC553420	LC567182	LC567474	LC567542	LC567608	Present study
43	Mt. Odaesan, Korea	2002	–	AB041079	–	–	–	Tominaga et al. (2000)
44*	Mt. Taebaeksan, Korea	–	–	–	–	LC567543	–	Present study

45*	Mt. Sobaeksan, Korea	1990	–	–	–	LC567544	–	Present study
46*	Mt. Jirisan, Korea	1985	–	–	–	LC567545	–	Present study
47	Mt. Jirisan, Korea	–	–	AB050683	–	–	–	Su et al. (2001)
A. mirabilissimus								
48	Mt. Hyangnobong, Korea	2002	LC553421	LC567183	LC567477	LC567546	LC567609	Present study
49	Mt. Hyangnobong, Korea	2002	LC553422	LC567184	LC567478	LC567547	LC567610	Present study
50	Mt. Hyangnobong, Korea	2002	LC553423	LC567185	LC567479	LC567548	–	Present study
51	Mt. Hyangnobong, Korea	2002	LC553424	LC567186	LC567480	LC567549	LC567611	Present study
52	Mt. Hyangnobong, Korea	2002	LC553425	LC567187	LC567481	LC567550	LC567612	Present study
53	Mt. Hyangnobong, Korea	2002	LC553426	LC567188	LC567482	LC567551	–	Present study
54	Mt. Hyangnobong, Korea	2002	LC553427	LC567189	LC567483	LC567552	–	Present study
55	Mt. Soraksan, Korea	2002	LC553428	LC567190	LC567484	LC567553	LC567613	Present study
56	Mt. Soraksan, Korea	2002	LC553429	LC567191	LC567485	LC567554	LC567614	Present study
57	Chuncheon, Korea	2002	LC553430	LC567192	LC567486	LC567555	LC567615	Present study
58	Chuncheon, Korea	2002	LC553431	LC567193	LC567487	LC567556	LC567616	Present study
59	Chuncheon, Korea	2002	LC553432	LC567194	LC567488	LC567557	LC567617	Present study
60	Chuncheon, Korea	2002	LC553433	LC567195	LC567489	LC567558	LC567618	Present study
61	Chuncheon, Korea	2002	LC553434	LC567196	LC567490	LC567559	LC567619	Present study
62	Mt. Odaesan, Korea	2002	LC553435	LC567197	LC567491	LC567560	LC567620	Present study
63	Mt. Odaesan, Korea	2002	LC553436	LC567198	LC567492	LC567561	LC567621	Present study
64	Mt. Odaesan, Korea	2002	LC553437	LC567199	LC567493	LC567562	LC567622	Present study
65	Mt. Odaesan, Korea	2002	LC553438	LC567200	LC567494	LC567563	LC567623	Present study
66	Mt. Odaesan, Korea	2002	LC553439	LC567201	LC567495	LC567564	LC567624	Present study
67	Mt. Odaesan, Korea	2002	LC553440	LC567202	LC567496	LC567565	LC567625	Present study
68	Mt. Odaesan, Korea	–	–	AB050684	–	–	–	Su et al. (2001)
69	Mt. Taebaeksan, Korea	2002	LC553441	LC567203	LC567497	LC567566	LC567626	Present study

70	Mt. Sobaeksan, Korea	2002	LC553442	LC567204	LC567498	LC567567	LC567627	Present study
71	Mt. Chiaksan, Korea	2002	LC553443	LC567205	LC567499	LC567568	LC567628	Present study
72	Mt. Obongsan, Korea	2002	LC553444	LC567206	LC567500	LC567569	LC567629	Present study
73	Mt. Deogyusan, Korea	2003	LC553445	LC567207	LC567501	LC567570	LC567630	Present study
74	Mt. Deogyusan, Korea	2003	LC553446	LC567208	LC567502	LC567571	LC567631	Present study
75	Mt. Deogyusan, Korea	2003	LC553447	LC567209	LC567503	LC567572	LC567632	Present study
76	Mt. Deogyusan, Korea	2003	LC553448	LC567210	LC567504	LC567573	LC567633	Present study
77	Mt. Deogyusan, Korea	2003	LC553449	LC567211	LC567505	LC567574	LC567634	Present study
78	Mt. Deogyusan, Korea	2003	LC553450	LC567212	LC567506	LC567575	LC567635	Present study
79	Mt. Deogyusan, Korea	2003	LC553451	LC567213	LC567507	LC567576	LC567636	Present study
80	Mt. Deogyusan, Korea	2003	LC553452	LC567214	LC567508	LC567577	LC567637	Present study
81	Mt. Deogyusan, Korea	2003	LC553453	LC567215	LC567509	LC567578	–	Present study
82	Mt. Deogyusan, Korea	2003	LC553454	LC567216	LC567510	LC567579	LC567638	Present study
83	Mt. Deogyusan, Korea	2003	LC553455	LC567217	LC567511	LC567580	LC567639	Present study
A. changeonleei								
84	Mt. Jirisan, Korea	2003	LC553456	LC567218	LC567512	LC567581	LC567640	Present study
85	Mt. Jirisan, Korea	2003	LC553457	LC567219	LC567513	LC567582	LC567641	Present study
86	Mt. Jirisan, Korea	2003	LC553458	LC567220	LC567514	LC567583	LC567642	Present study
Outgroups								
Damaster blaptoides	Hidaka, Hokkaido Is., Japan	2010	LC553459	LC567221	LC567515	LC567584	LC567643	Present study
D. blaptoides	Suzuka, Mie, Japan	2010	LC553460	LC567222	LC567516	LC567585	LC567644	Present study
Coptolabrus jankowski	Mt. Jirisan, Korea	2003	LC553461	LC567223	LC567517	LC567586	LC567645	Present study
C. smaragdinus	Mt. Jirisan, Korea	2003	LC553462	LC567224	LC567518	LC567587	LC567646	Present study

Table S2. Characteristics of the PCR primers used in this study

	Size (bp)	Direction	Sequence	Reference
COI				
C1-J-2195	24	forward	TTGATTTTTTGGTCATCCAGAAGT	Simon et al. (1994)
TL-2N-3014	25	reverse	TCCAATGCACTAATCTGCCATATTA	Simon et al. (1994)
COI 411f	20	internal f	TCTATAGGGGCCGTATTTGC	Present study
COI 452r	25	internal r	CCTGTAAATAAAGGGAATCATTGAA	Present study
ND5				
ND5 v1.06-1	20	forward	CCTGTTTCTGCTTTAGTTCA	Su et al. (1996)
ND5 v1.04-4	22	reverse	GTCATACTCTAAATATAAGCTA	Su et al. (1996)
ND5 Ezo-2	20	internal f	TTCATCTTTTAACTCATGCA	Su et al. (1998)
ND5 AO-3	21	internal r	ATATTCATTTCAACCTTGATC	Su et al. (1998)
ND5 411f	20	internal f	CGGGTTTTATTCTAAGGATT	Present study
ND5 490r	20	internal r	ACTGTTAACCCCGTAGAAAT	Present study
PepCk				
CARPEK1	20	forward	GCCATGATGACACCAACACT	Sota & Vogler (2001)
CARPEK3	20	reverse	GACGTGGAAGATCTTGGGCA	Sota & Vogler (2001)
PEK 207f	20	internal f	TCTGGGAAGGAATGGAAGAC	Present study
PEK 312r	20	internal r	CTGGAGAACAGAACCGTGAA	Present study
Wingless				
CARWL1	20	forward	ATGTCTGGCACCTGCACCGT	Sota & Vogler (2001)
CARWL2	22	reverse	CAAGCGCACCGTTCCACAACGA	Sota & Vogler (2001)
Wg113f	20	internal f	GGYGGTAACAATGCACACAG	Present study
Wg166f	29	internal f	AACAGTATCCAYAGYAATCACGCAAACAC	Present study
Wg242r	23	internal r	TGTTCYGGATTGAATGGTTTCAA	Present study
28S				
28S01	23	forward	GACTACCCCCTGAATTTAAGCAT	Kim et al. (2000)
28SR01	23	reverse	GACTCCTTGGTCCGTGTTTCAAG	Kim et al. (2000)
28S intF	28	internal f	AACAAGTACCGTGAGGGAAAGTTGAAAA	Present study
28S intR	21	internal r	AGGCATTGATTGAATCTCCCC	Present study

Table S3. Body dimensions of *Acoptolabrus* species. Mean ± s.d. (mm) is shown.

Species	N	Head length (VH)	Head width (HH)	Prothorax length (VP)	Prothorax width (HP)	Elytra length (VE)	Elytra width (HE)
A. gehinii male	15	4.75 ± 0.49	3.75 ± 0.21	4.65 ± 0.31	5.21 ± 0.32	16.13 ± 0.87	8.92 ± 0.56
A. gehinii female	12	4.88 ± 0.23	3.68 ± 0.15	4.80 ± 0.32	5.37 ± 0.30	17.89 ± 0.71	9.93 ± 0.45
A. munakatai male	10	4.92 ± 0.30	3.54 ± 0.17	4.73 ± 0.29	5.08 ± 0.20	16.22 ± 0.82	9.12 ± 0.52
A. munakatai female	10	5.59 ± 0.38	3.94 ± 0.24	5.15 ± 0.25	5.75 ± 0.33	19.24 ± 0.92	10.97 ± 0.64
A. lopatini male	4	5.22 ± 0.19	4.34 ± 0.20	5.76 ± 0.20	6.63 ± 0.30	19.91 ± 1.01	11.17 ± 0.52
A. lopatini female	3	5.63 ± 0.15	4.54 ± 0.21	6.01 ± 0.28	6.98 ± 0.09	22.87 ± 1.28	13.11 ± 0.93
A. schrencki male	2	4.60 ± 0.29	3.78 ± 0.16	4.46 ± 0.01	5.33 ± 0.42	15.57 ± 0.72	8.60 ± 0.57
A. schrencki female	2	5.31 ± 0.72	4.22 ± 0.35	5.15 ± 0.36	6.21 ± 0.76	18.79 ± 1.21	10.78 ± 0.61
A. constricticollis male	8	4.48 ± 0.35	3.47 ± 0.11	4.42 ± 0.29	4.56 ± 0.23	15.11 ± 0.77	8.04 ± 0.31
A. constricticollis female	6	4.85 ± 0.32	3.66 ± 0.18	4.75 ± 0.26	4.85 ± 0.30	17.22 ± 0.93	9.11 ± 0.48
A. leechi (Korean Penn.) male	6	5.23 ± 0.36	3.80 ± 0.09	5.12 ± 0.21	5.03 ± 0.09	17.06 ± 0.76	9.47 ± 0.37
A. leechi (Korean Penn.) female	4	5.95 ± 0.37	4.08 ± 0.28	5.65 ± 0.50	5.42 ± 0.40	20.08 ± 1.86	11.16 ± 0.86
A. leechi (Mt. Jirisan) male	4	4.93 ± 0.22	3.55 ± 0.06	4.98 ± 0.14	4.59 ± 0.07	15.71 ± 0.18	9.17 ± 0.16
A. leechi (Mt. Jirisan) female	4	5.19 ± 0.12	3.87 ± 0.07	5.32 ± 0.20	4.89 ± 0.17	18.46 ± 0.52	10.82 ± 0.23
A. mirabilissimus male	10	4.52 ± 0.26	4.09 ± 0.16	4.16 ± 0.15	4.99 ± 0.26	14.72 ± 1.06	8.37 ± 0.41
A. mirabilissimus female	16	4.90 ± 0.34	4.33 ± 0.22	4.30 ± 0.29	5.25 ± 0.36	16.51 ± 0.95	9.32 ± 0.57
A. changeonleei male	5	4.70 ± 0.23	4.09 ± 0.12	4.32 ± 0.20	4.76 ± 0.18	15.51 ± 0.47	8.54 ± 0.29
A. changeonleei female	7	5.13 ± 0.39	4.29 ± 0.12	4.52 ± 0.18	5.16 ± 0.17	17.65 ± 1.17	9.55 ± 0.37

Table S4. Principal component analysis based on six body dimensions for *Acoptolabrus* species

	Eigenvalue	Contribution (%)	Cumulative contribution (%)	Eigenvector					
				VH	VP	VE	HH	HP	HE
PC1	4.42	73.64	73.64	0.41	0.41	0.45	0.28	0.41	0.46
PC2	0.87	14.53	88.16	-0.11	-0.44	-0.13	0.85	0.23	-0.09
PC3	0.37	6.11	94.28	0.74	-0.01	-0.11	0.22	-0.61	-0.12
PC4	0.16	2.72	97.00	0.21	0.47	-0.54	0.01	0.45	-0.49
PC5	0.14	2.27	99.27	-0.47	0.65	-0.02	0.39	-0.45	0.03
PC6	0.04	0.73	100.00	-0.02	0.03	0.69	0.04	0.01	-0.73

VH, head length; VP, pronotum length; VE, elytra length; HH, head width; HP, pronotum width; HE, Elytra width

Figure legends

Fig. 1. Distribution of *Acoptolabrus* species in the Far East. Points indicate localities of DNA samples. Areas surrounded by dotted lines are species distributions estimated from other specimens and the literature. Mt. Jirisan is shown by a triangle, where *A. changeonleei* and an isolated population of *A. leechi* are distributed.

Fig. 2. Maximum likelihood tree of *Acoptolabrus* species based on the concatenated data set consisting of five gene sequences. Bootstrap values (>50%) are shown on branches. Scale bar represents 0.03 substitutions per site. Range overlap status and feeding phenotypes as well as distributions are shown in the right-hand columns.

Fig. 3. Time-calibrated tree of *Acoptolabrus* species. Node bars indicate 95% credible limits of node heights. Range overlap status and feeding phenotypes as well as distributions are shown in the right-hand columns.

Fig. 4. Ancestral range reconstruction of *Acoptolabrus* species based on the time-calibrated tree and DIVALIKE model. Letters on the tree (A–G) refer to branching events. Letters with dashed lines on the maps (A, B, E, and F) refer to vicariance events. Letters within an area on the maps (C, D, and G) indicate speciation within the area. The black area in the pie graphs indicates low probability (<10%).

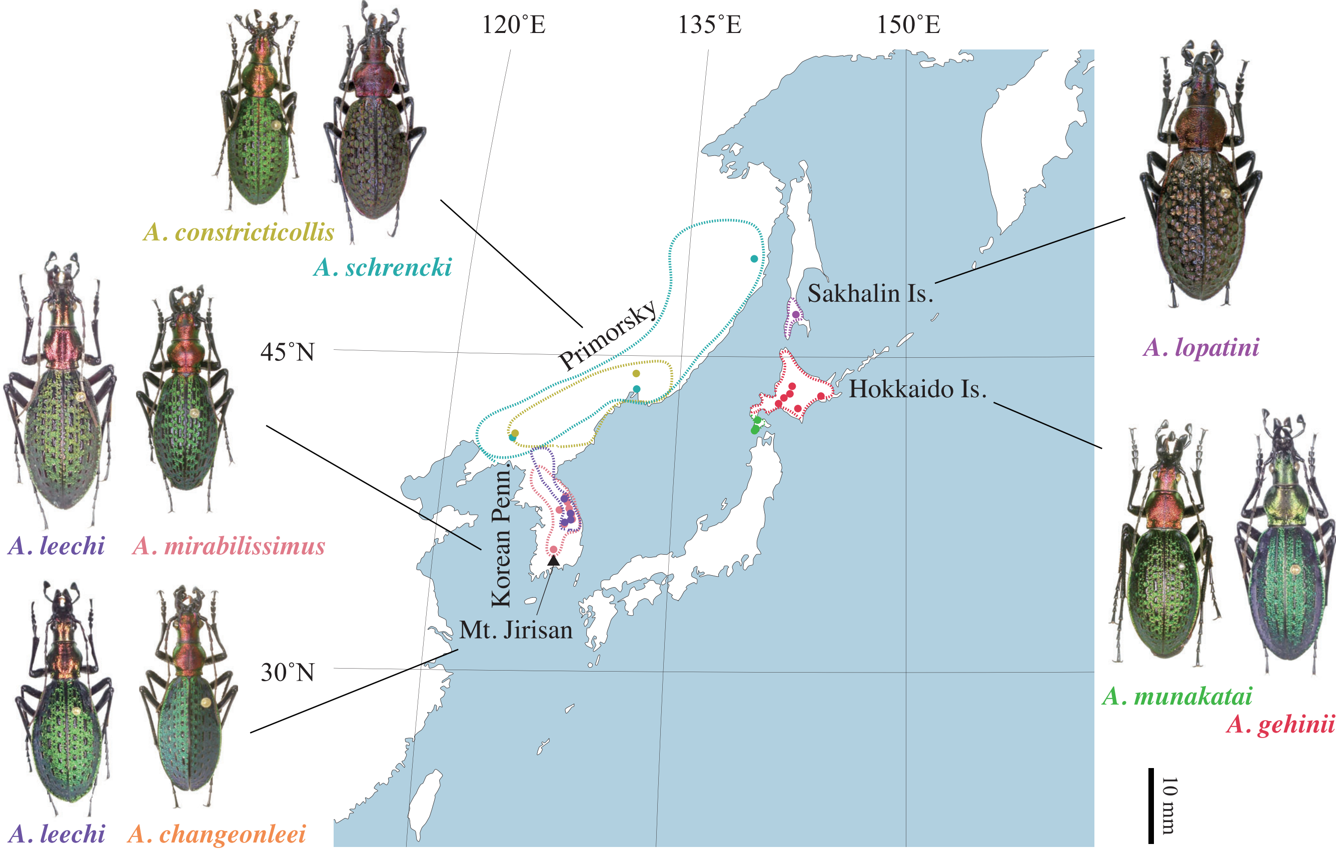
Fig. 5. Variation in the body size and shape of *Acoptolabrus* species in relation to distributional type and area: least square means (LSMs) of body size (A) and shape (B) in each species, and LSMs of body size difference (C) and body shape difference (D) between species. Different letters indicate significant differences, as determined by the Tukey–Kramer test ($P < 0.05$). *$P < 0.05$, NS $P > 0.05$.

Fig. 6. Hypothetical models of the selective regimes assumed in comparative analyses.

Fig. S1. Maximum likelihood trees of *Acoptolabrus* species based on individual gene alignments (mitochondrial *COI* and *ND5* and nuclear *PepCk, Wg,* and *28S*). Bootstrap values (>50%) are shown on branches. Three sympatric species on the Korean Peninsula and Mt. Jirisan (*A. leechi*, *A. mirabilissimus,* and *A. changeonleei*) shared some *COI* and *ND5* haplotypes, suggesting mitochondrial introgression. We removed mitochondrial sequence data for these three species from the concatenated data set. Two parapatric species in Hokkaido Island (*A. gehinii* and *A. munakatai*) also shared one sequence each of nuclear *Wg* and *28S*. *A. gehinii* was paraphyletic in mitochondrial *COI* and *ND5* trees, involving the *A. munakatai* lineage. Since divergence between these two species was relatively shallow, and there was no indication of current mitochondrial introgression, the shared nuclear sequences and paraphyly in mitochondrial trees may be results of incomplete lineage sorting and/or peripatric speciation of *A. munakatai* and *A. gehinii*. These sequences were retained in the concatenated data set.

Fig. S2. Plot of principal component scores for *Acoptolabrus* species. PC1 and PC2 explain body size and body shape, respectively.

Fig. S3. Multispecies coalescent trees of *Acoptlabrus* species based on the all the 5 gene sequences (upper) and on the dataset excluding two mitochondrial genes from three Korean species (*A. mirabilissimus*, *A. leechi* and *A. changeonleei*) (lower). Node bars indicate 95% credible limits of node heights. Posterior probabilities are shown on branches.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135˚E
150˚E
45˚N
30˚N
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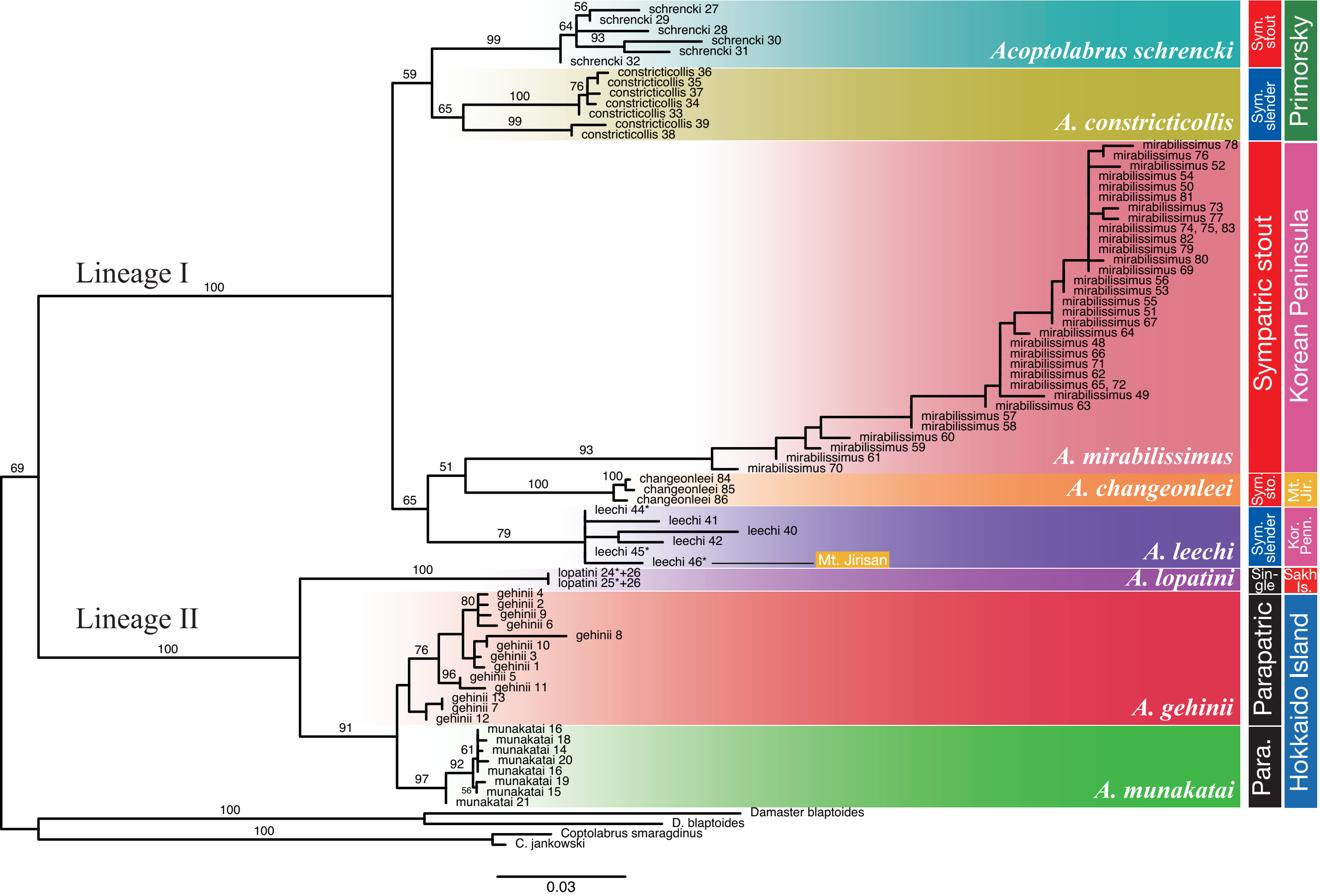
A. schrencki
Primorsky
Sakhalin Is.
A. lopatini
Hokkaido Is.
A. leechi
A. mirabilissimus
Korean Penn.
Mt. Jirisan
A. leechi
A. changeonleei
A. munakatai
A. gehinii
10 mm


56
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schrencki 29
64
schrencki 28
93
schrencki 30
99
schrencki 31
schrencki 32
Acoptolabrus schrencki
Sym. stout
Primorsky
59
constricticollis 36
constricticollis 35
76
constricticollis 37
100
constricticollis 34
65
constricticollis 33
99
constricticollis 39
constricticollis 38
A. constricticollis
Sym. slender
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mirabilissimus 76
mirabilissimus 52
mirabilissimus 54
mirabilissimus 50
mirabilissimus 81
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mirabilissimus 79
mirabilissimus 80
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Lineage I
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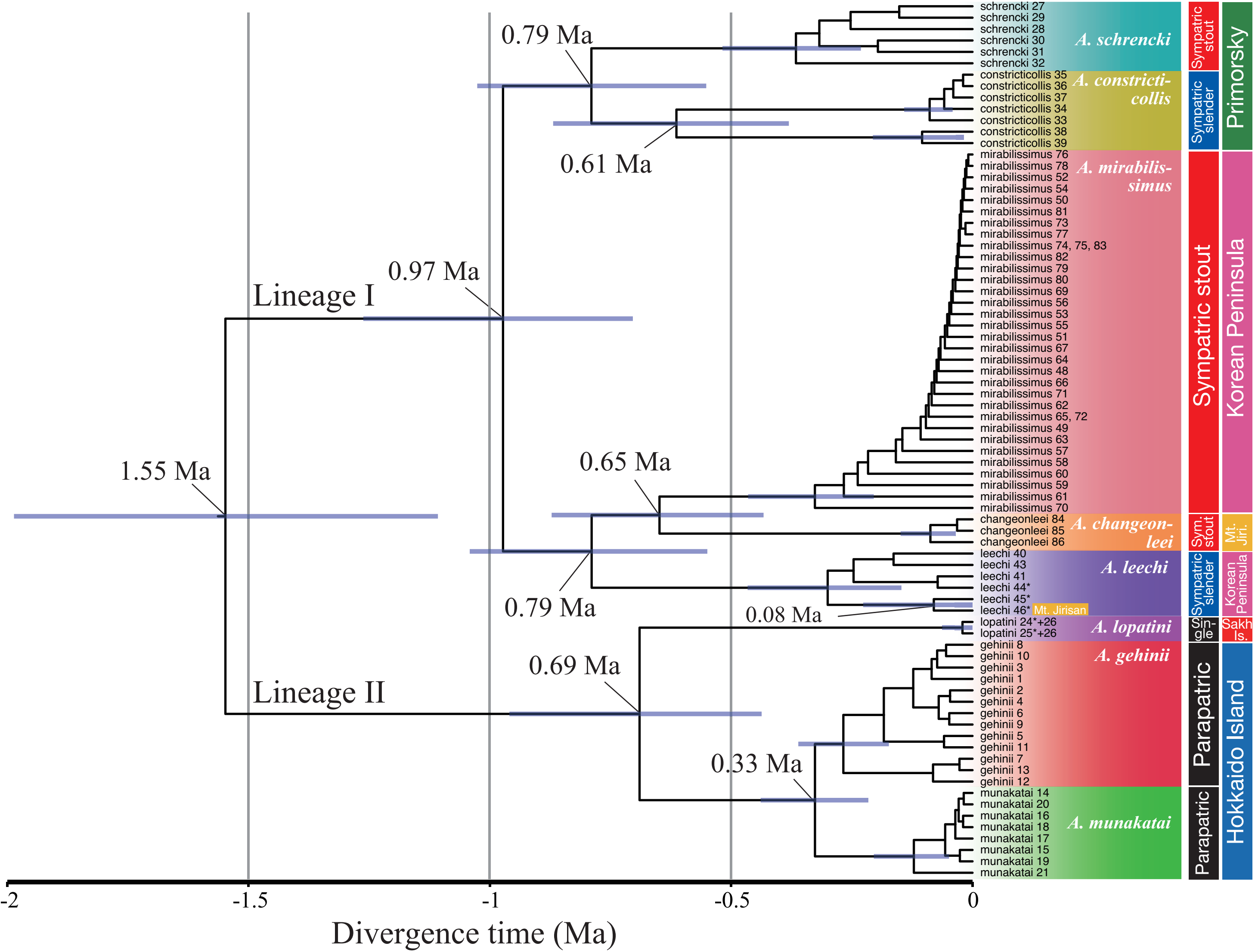
mirabilissimus 63
mirabilissimus 57
mirabilissimus 58
mirabilissimus 60
mirabilissimus 59
93
mirabilissimus 61
51
mirabilissimus 70
A. mirabilissimus
Sympatric stout
Korean Peninsula
69
100
changeonleei 84
100
changeonleei 85
65
changeonleei 86
A. changeonleei
Sym. sto.
Mt. Jir.
leechi 44*
leechi 41
leechi 40
79
leechi 42
leechi 45*
leechi 46*
Mt. Jirisan
A. leechi
Sym. slender
Kor. Penn.
100
lopatini 24*+26
lopatini 25*+26
A. lopatini
Sin-gle
Sakh Is.
80
gehinii 4
gehinii 2
gehinii 9
Lineage II
gehinii 6
gehinii 8
76
gehinii 10
gehinii 3
100
gehinii 1
96
gehinii 5
gehinii 11
gehinii 13
gehinii 7
gehinii 12
A. gehinii
Parapatric
Hokkaido Island
91
munakatai 16
munakatai 18
61
munakatai 14
munakatai 20
92
munakatai 16
97
munakatai 19
56
munakatai 15
munakatai 21
A. munakatai
Para.
100
Damaster blaptoides
D. blaptoides
100
Coptolabrus smaragdinus
C. jankowski
0.03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Lineage II
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schrencki 29
schrencki 28
schrencki 30
schrencki 31
schrencki 32
A. schrencki
constricticollis 35
constricticollis 36
constricticollis 37
constricticollis 34
constricticollis 33
constricticollis 38
constricticollis 39
A. constricticollis
mirabilissimus 76
mirabilissimus 78
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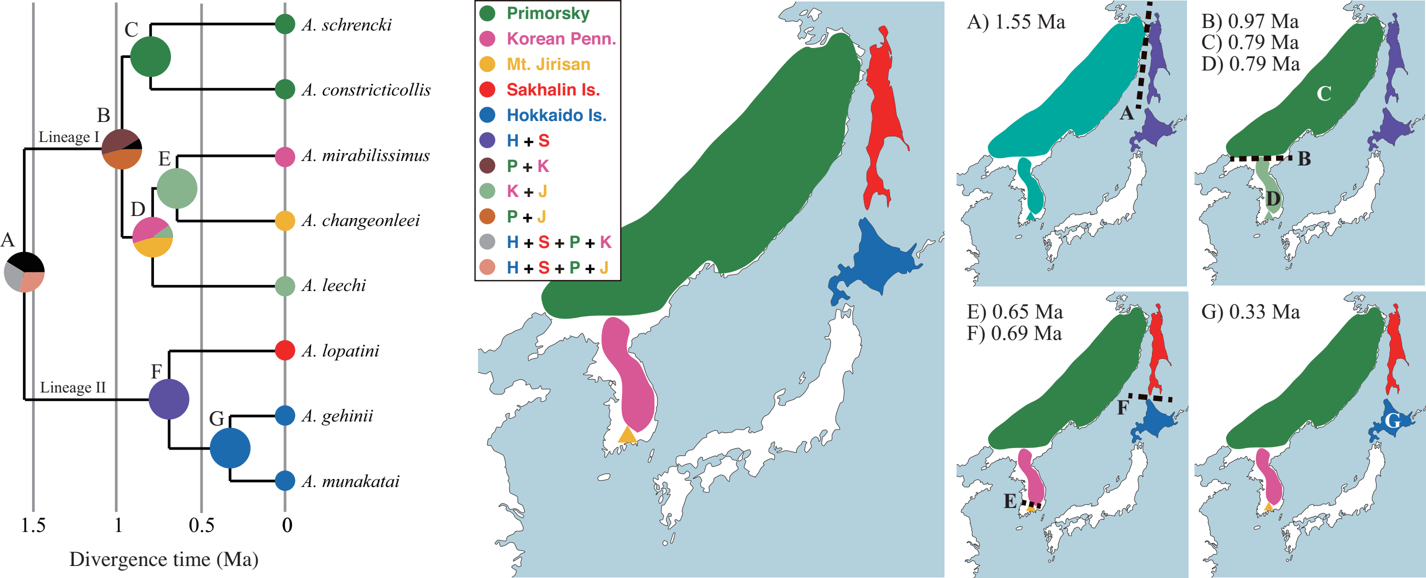
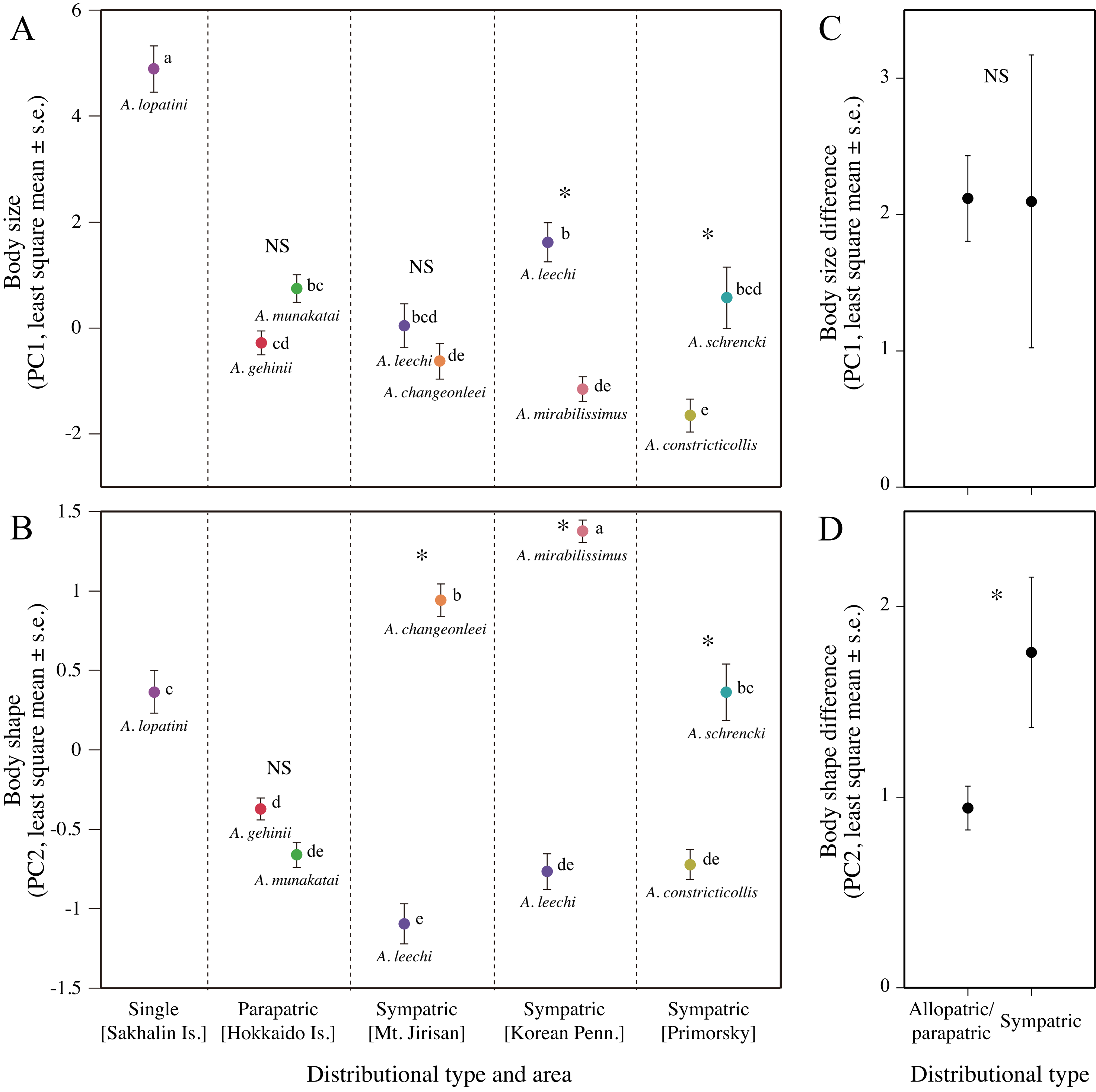
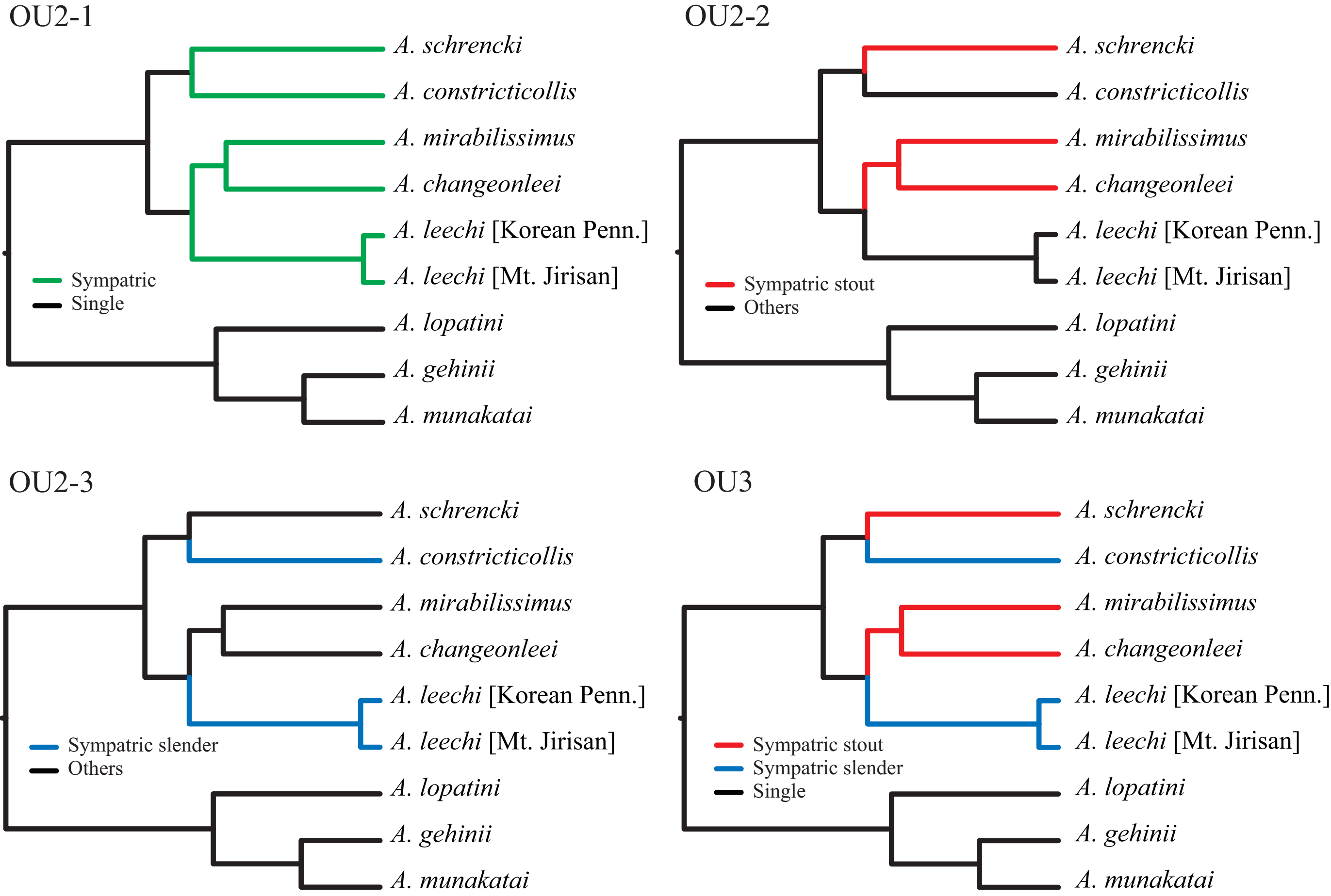
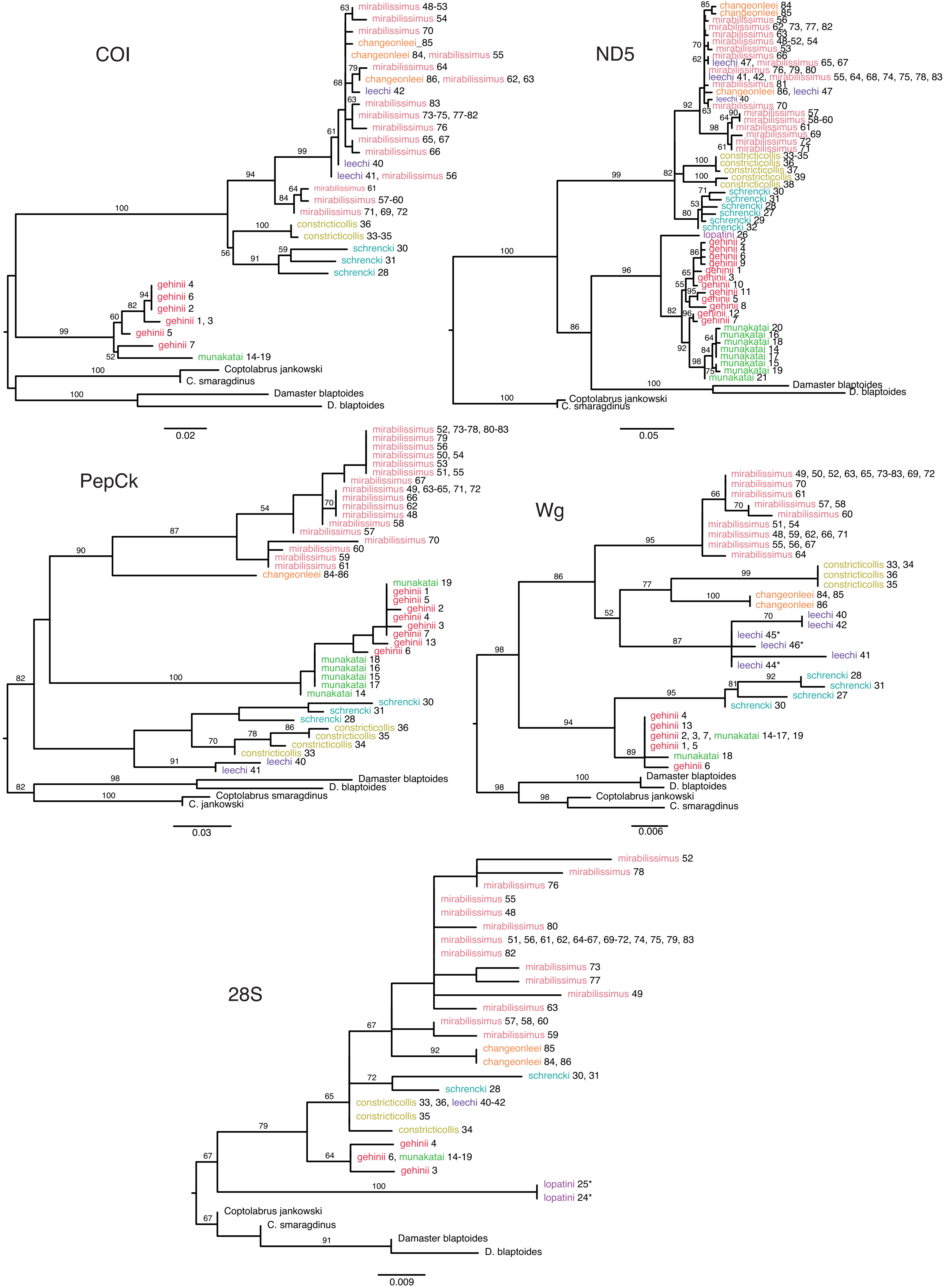
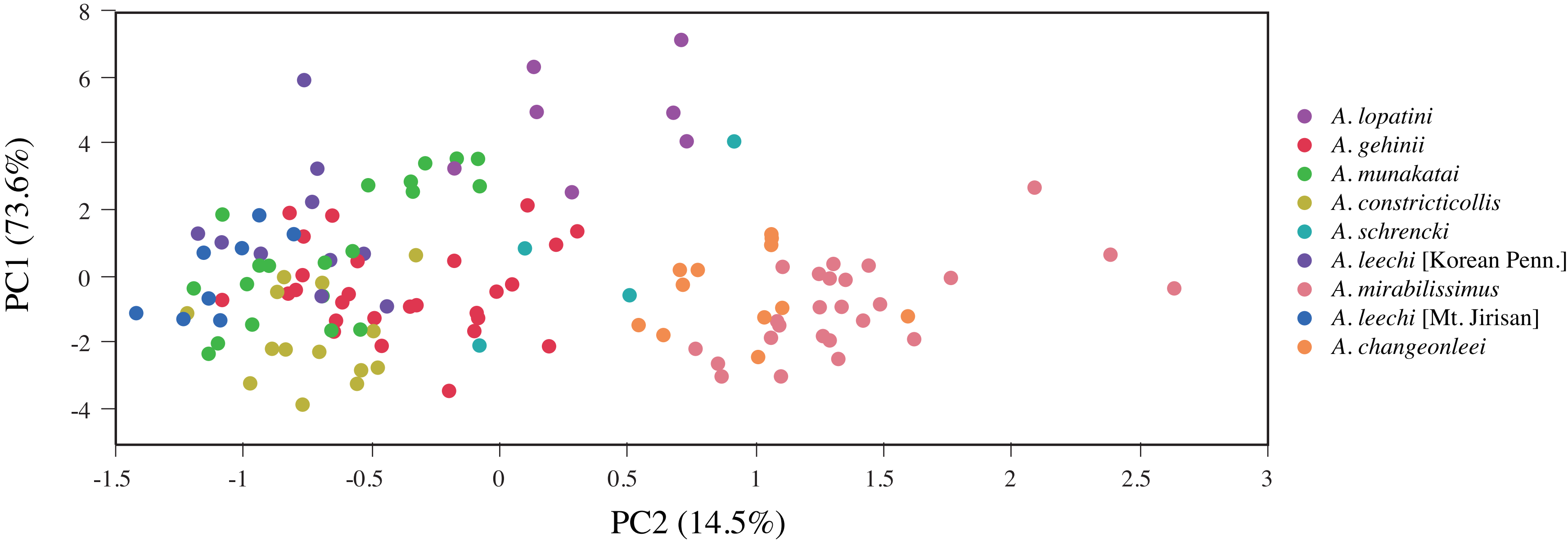
A. mirabilissimus
changeonleei 84
changeonleei 85
changeonleei 86
A. changeonleei
leechi 40
leechi 43
leechi 41
leechi 44*
leechi 45*
leechi 46*
Mt. Jirisan
A. leechi
lopatini 24*+26
lopatini 25*+26
A. lopatini
gehinii 8
gehinii 10
gehinii 3
gehinii 1
gehinii 2
gehinii 4
gehinii 6
gehinii 9
gehinii 5
gehinii 11
gehinii 7
gehinii 13
gehinii 12
A. gehinii
munakatai 14
munakatai 20
munakatai 16
munakatai 18
munakatai 17
munakatai 15
munakatai 19
munakatai 21
A. munakatai
Sympatric stout
Sympatric slender
Primorsky
Sympatric stout
Korean Peninsula
Sym. stout
Mt. Jiri.
Sympatric slender
Korean Peninsula
Single
Sakh Is.
Parapatric
Parapatric
Hokkaido Island
-2
-1.5
-1
-0.5
0
Divergence time (Ma)

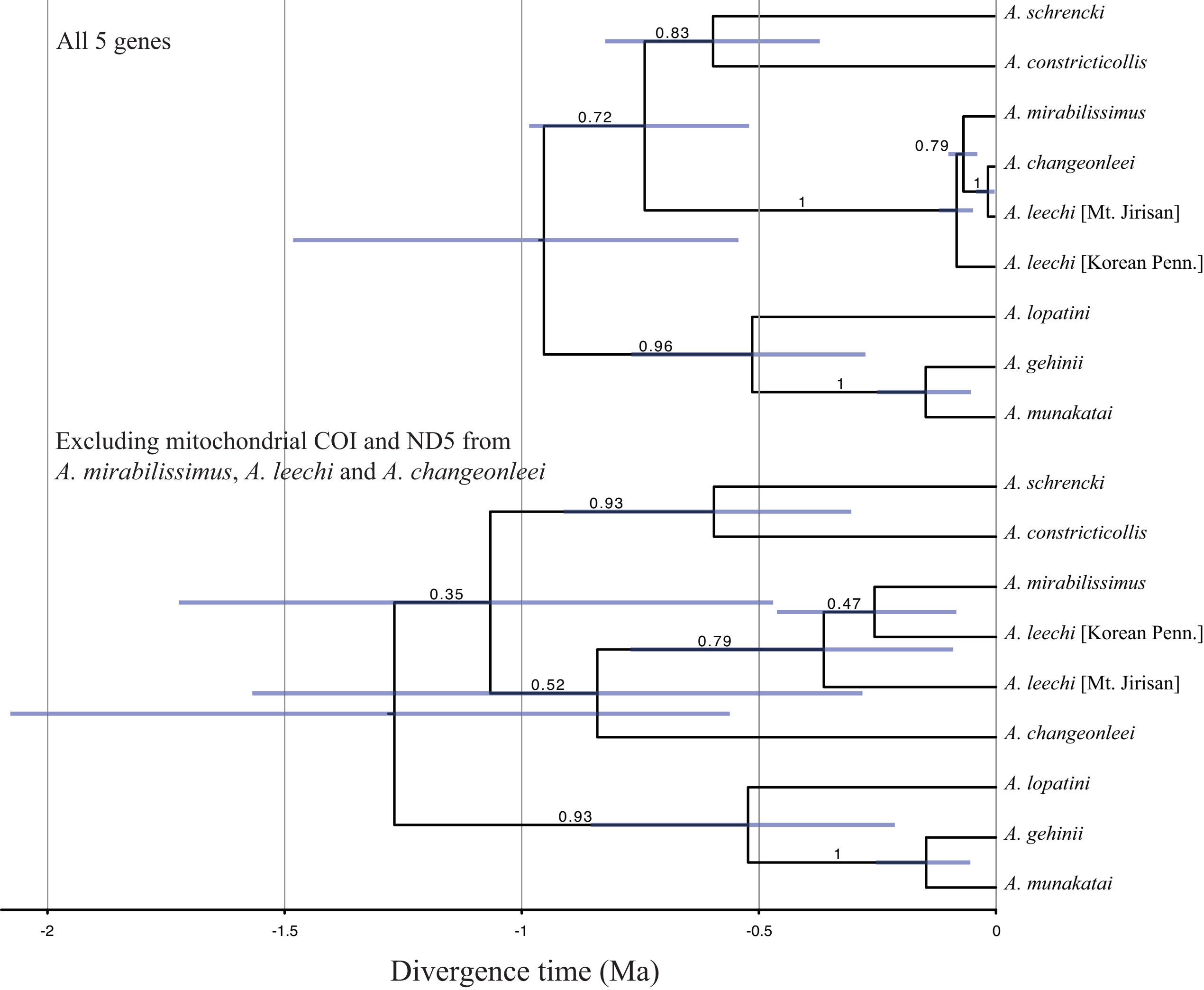

A
B
C
D
E
F
G
Lineage I
Lineage II
A. schrencki
A. constricticollis
A. mirabilissimus
A. changeonleei
A. leechi
A. lopatini
A. gehinii
A. munakatai
1.5
1
0.5
0
Divergence time (Ma)
Primorsky
Korean Penn.
Mt. Jirisan
Sakhalin Is.
Hokkaido Is.
H + S
P + K
K + J
P + J
H + S + P + K
H + S + P + J
A) 1.55 Ma
B) 0.97 Ma
C) 0.79 Ma
D) 0.79 Ma
E) 0.65 Ma
F) 0.69 Ma
G) 0.33 Ma
A
B
C
D
E
F
G


A
B
C
D
Body size
(PC1, least square mean ± s.e.)
Body shape
(PC2, least square mean ± s.e.)
Body size difference
(PC1, least square mean ± s.e.)
Body shape difference
(PC2, least square mean ± s.e.)
6
4
2
0
-2
1.5
1
0.5
0
-0.5
-1
-1.5
3
2
1
0
2
1
0
a
A. lopatini
NS
bc
A. munakatai
cd
A. gehinii
NS
bcd
A. leechi
de
A. changeonleei
*
b
A. leechi
de
A. mirabilissimus
*
bcd
A. schrencki
e
A. constricticollis
NS
c
A. lopatini
NS
d
A. gehinii
de
A. munakatai
*
b
A. changeonleei
e
A. leechi
*
a
A. mirabilissimus
de
A. leechi
*
bc
A. schrencki
de
A. constricticollis
*
Single
[Sakhalin Is.]
Parapatric
[Hokkaido Is.]
Sympatric
[Mt. Jirisan]
Sympatric
[Korean Penn.]
Sympatric
[Primorsky]
Distributional type and area
Allopatric/
parapatric
Sympatric
Distributional type


OU2-1
A. schrencki
A. constricticollis
A. mirabilissimus
A. changeonleei
A. leechi [Korean Penn.]
A. leechi [Mt. Jirisan]
A. lopatini
A. gehinii
A. munakatai
Sympatric
Single
OU2-2
A. schrencki
A. constricticollis
A. mirabilissimus
A. changeonleei
A. leechi [Korean Penn.]
A. leechi [Mt. Jirisan]
A. lopatini
A. gehinii
A. munakatai
Sympatric stout
Others
OU2-3
A. schrencki
A. constricticollis
A. mirabilissimus
A. changeonleei
A. leechi [Korean Penn.]
A. leechi [Mt. Jirisan]
A. lopatini
A. gehinii
A. munakatai
Sympatric slender
Others
OU3
A. schrencki
A. constricticollis
A. mirabilissimus
A. changeonleei
A. leechi [Korean Penn.]
A. leechi [Mt. Jirisan]
A. lopatini
A. gehinii
A. munakatai
Sympatric stout
Sympatric slender
Single


COI
mirabilissimus 48-53
mirabilissimus 54
mirabilissimus 70
changeonleei_85
changeonleei 84, mirabilissimus 55
mirabilissimus 64
changeonleei 86, mirabilissimus 62, 63
leechi 42
mirabilissimus 83
mirabilissimus 73-75, 77-82
mirabilissimus 76
mirabilissimus 65, 67
mirabilissimus 66
leechi 40
leechi 41, mirabilissimus 56
mirabilissimus 61
mirabilissimus 57-60
mirabilissimus 71, 69, 72
constricticollis 36
constricticollis 33-35
schrencki 30
schrencki 31
schrencki 28
gehinii 4
gehinii 6
gehinii 2
gehinii 1, 3
gehinii 5
gehinii 7
munakatai 14-19
Coptolabrus jankowski
C. smaragdinus
Damaster blaptoides
D. blaptoides
0.02
ND5
changeonleei 84
changeonleei 85
mirabilissimus 56
mirabilissimus 62, 73, 77, 82
mirabilissimus 63
mirabilissimus 48-52, 54
mirabilissimus 53
mirabilissimus 66
leechi 47, mirabilissimus 65, 67
mirabilissimus 76, 79, 80
leechi 41, 42, mirabilissimus 55, 64, 68, 74, 75, 78, 83
mirabilissimus 81
changeonleei 86, leechi 47
leechi 40
mirabilissimus 70
mirabilissimus 57
mirabilissimus 58-60
mirabilissimus 61
mirabilissimus 69
mirabilissimus 72
mirabilissimus 71
constricticollis 33-35
constricticollis 36
constricticollis 37
constricticollis 39
constricticollis 38
schrencki 30
schrencki 31
schrencki 28
schrencki 27
schrencki 29
schrencki 32
lopatini 26
gehinii 2
gehinii 4
gehinii 6
gehinii 9
gehinii 1
gehinii 3
gehinii 10
gehinii 11
gehinii 5
gehinii 8
gehinii 12
gehinii 7
munakatai 20
munakatai 16
munakatai 18
munakatai 14
munakatai 17
munakatai 15
munakatai 19
munakatai 21
Damaster blaptoides
D. blaptoides
Coptolabrus jankowski
C. smaragdinus
0.05
PepCk
mirabilissimus 52, 73-78, 80-83
mirabilissimus 79
mirabilissimus 56
mirabilissimus 50, 54
mirabilissimus 53
mirabilissimus 51, 55
mirabilissimus 67
mirabilissimus 49, 63-65, 71, 72
mirabilissimus 66
mirabilissimus 62
mirabilissimus 48
mirabilissimus 58
mirabilissimus 57
mirabilissimus 70
mirabilissimus 60
mirabilissimus 59
mirabilissimus 61
changeonleei 84-86
munakatai 19
gehinii 1
gehinii 5
gehinii 2
gehinii 4
gehinii 3
gehinii 7
gehinii 13
gehinii 6
munakatai 18
munakatai 16
munakatai 15
munakatai 17
munakatai 14
schrencki 30
schrencki 31
schrencki 28
constricticollis 36
constricticollis 35
constricticollis 34
constricticollis 33
leechi 40
leechi 41
Damaster blaptoides
D. blaptoides
Coptolabrus smaragdinus
C. jankowski
0.03
Wg
mirabilissimus 49, 50, 52, 63, 65, 73-83, 69, 72
mirabilissimus 70
mirabilissimus 61
mirabilissimus 57, 58
mirabilissimus 60
mirabilissimus 51, 54
mirabilissimus 48, 59, 62, 66, 71
mirabilissimus 55, 56, 67
mirabilissimus 64
constricticollis 33, 34
constricticollis 36
constricticollis 35
changeonleei 84, 85
changeonleei 86
leechi 40
leechi 42
leechi 45*
leechi 46*
leechi 41
leechi 44*
schrencki 28
schrencki 31
schrencki 27
schrencki 30
gehinii 4
gehinii 13
gehinii 2, 3, 7, munakatai 14-17, 19
gehinii 1, 5
munakatai 18
gehinii 6
Damaster blaptoides
D. blaptoides
Coptolabrus jankowski
C. smaragdinus
0.006
28S
mirabilissimus 52
mirabilissimus 78
mirabilissimus 76
mirabilissimus 55
mirabilissimus 48
mirabilissimus 80
mirabilissimus 51, 56, 61, 62, 64-67, 69-72, 74, 75, 79, 83
mirabilissimus 82
mirabilissimus 73
mirabilissimus 77
mirabilissimus 49
mirabilissimus 63
mirabilissimus 57, 58, 60
mirabilissimus 59
changeonleei 85
changeonleei 84, 86
schrencki 30, 31
schrencki 28
constricticollis 33, 36, leechi 40-42
constricticollis 35
constricticollis 34
gehinii 4
gehinii 6, munakatai 14-19
gehinii 3
lopatini 25*
lopatini 24*
Coptolabrus jankowski
C. smaragdinus
Damaster blaptoides
D. blaptoides
0.009


8
6
4
2
0
-2
-4
PC1 (73.6%)
-1.5
-1
-0.5
0
0.5
1
1.5
2
2.5
3
PC2 (14.5%)
● *A. lopatini*
● *A. gehinii*
● *A. munakatai*
● *A. constricticollis*
● *A. schrencki*
● *A. leechi* [Korean Penn.]
● *A. mirabilissimus*
● *A. leechi* [Mt. Jirisan]
● *A. changeonleei*


All 5 genes
0.83
0.72
0.79
1
1
0.96
1
A. schrencki
A. constricticollis
A. mirabilissimus
A. changeonleei
A. leechi [Mt. Jirisan]
A. leechi [Korean Penn.]
A. lopatini
A. gehinii
A. munakatai
Excluding mitochondrial COI and ND5 from
A. mirabilissimus, A. leechi and A. changeonleei
0.93
0.35
0.47
0.79
0.52
0.93
1
A. schrencki
A. constricticollis
A. mirabilissimus
A. leechi [Korean Penn.]
A. leechi [Mt. Jirisan]
A. changeonleei
A. lopatini
A. gehinii
A. munakatai
-2
-1.5
-1
-0.5
0
Divergence time (Ma)