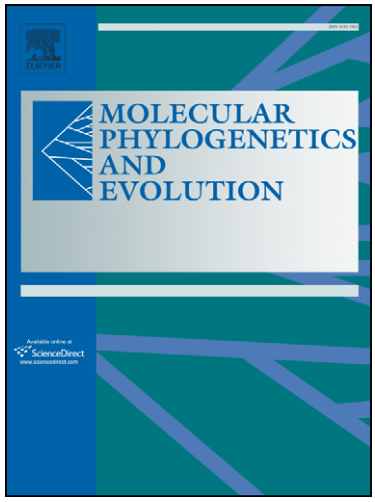

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Molecular phylogeny and historical biogeography of the Holarctic wetland leaf beetle of the genus *Plateumaris*

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Abstract

Leaf beetles of the genus *Plateumaris* inhabit wetlands across the temperate zone of the Holarctic region. To explore the phylogeographic relationships among North American, East Asian, and European members of this genus and the origin of the species endemic to Japan, we studied the molecular phylogeny of 20 of the 27 species in this genus using partial sequences of mitochondrial cytochrome oxidase subunit I (COI) and the 16S and nuclear 28S rRNA genes. The molecular phylogeny revealed that three species endemic to Europe are monophyletic and sister to the remaining 11 North American and six Asian species. Within the latter clade, North American and Asian species did not show reciprocal monophyly. Dispersal–vicariance analysis and divergence time estimation revealed that the European and North America–Asian lineages diverged during the Eocene. Moreover, subsequent differentiation occurred repeatedly between North American and Asian species, which was facilitated by three dispersal events from North America to Asia and one in the opposite direction during the late Eocene through the late Miocene. Two Japanese endemics originated from different divergence events; one differentiated from the mainland lineage after differentiation from the North American lineage, whereas the other showed a deep coalescence from the North American lineage with no present-day sister species on the East Asian mainland. This study of extant insects provides molecular phylogenetic evidence for ancient vicariance between Europe and East Asia-North America, and for more recent (but pre-Pleistocene) faunal exchanges between East Asia and North America.

Keywords: 28S rRNA; Chrysomelidae; Dispersal; Divergence time; Mitochondrial gene; Vicariance.

1. Introduction

Historical biogeography has suggested that the present biota in the Holarctic region has been affected by large-scale dispersal and vicariance events across Eurasia and North America (e.g., Wen, 1999; Sanmartín et al., 2001; Tiffney and Manchester, 2001). To reconstruct precise, testable hypotheses for such dispersal–vicariance events, a reliable phylogenetic analysis is essential, and molecular phylogenetic approaches including divergence time estimation are increasingly being used in historical biogeography. For the Holarctic biota, detailed molecular phylogenetic analyses of individual groups have been attempted on some plants and mammals (e.g., Johnson et al., 2006; Nie et al., 2006; Spalik and Downie, 2006), while few such studies have been performed on insects.

Here we focus on the wetland leaf beetle genus *Plateumaris* of the subfamily Donaciinae (Coleoptera, Chrysomelidae). This genus is distributed in North America and Eurasia with 27 known species, 17 of which occur in North America, three in Europe, five in East Asia, and two in Asia and Europe (Askevold, 1991; Hayashi, 2001). Its distribution patterns make it useful in the study of dispersal and vicariance histories of organisms across the Holarctic region. Askevold (1991) published a comprehensive account of the evolutionary history of the genus based on morphology, fossils, and biogeography. In particular, the morphological cladistics of *Plateumaris* (Fig. 1) provides a phylogenetic hypothesis that can be tested with molecular data. Recently, Sota and Hayashi (2007) reconstructed the historical biogeography of five *Plateumaris* species within the Japanese islands using molecular phylogeographic analysis. In Japan, *Plateumaris* consists of two endemic species that may be closely related to North American species based on morphology (Askevold, 1991), and three species common to the Asian mainland. Thus, to fully understand the evolutionary process of local species assemblages, an analysis of larger geographic distributions is needed to resolve the phylogenetic origin and biogeographical events of local species assemblages. It is therefore reasonable to extend the molecular phylogenetic analysis to the entire distribution range of *Plateumaris* including Europe, Asia, and North America to understand the present-day distribution and diversity of the genus *Plateumaris* across the Holarctic region.

In this study, we reconstructed species relationships among 17 of 27 species of the

genus *Plateumaris*. Based on the phylogenetic tree, we estimated dispersal and vicariance events involved in the divergence process of *Plateumaris* as well as the timing of these events.

2. Materials and methods

2.1. Sampling

Samples of all ten Palaearctic species were collected from Japan, Russia, Mongolia, Czechia (the Czech Republic), and Slovakia, and 10 of 17 Nearctic species from Canada (Table 1). Field collection of adult beetles was conducted in May through July. Collected beetles were preserved in 99% ethanol. Six *Donacia* species and two *Neohaemonia* species of the subfamily Donaciinae comprised the outgroup.

2.2. DNA extraction, PCR, and sequencing

Total genomic DNA was extracted from thoracic muscles using an AquaPure Genomic DNA Kit (Bio-Rad Laboratories, Hercules, CA). Partial sequences of the mitochondrial cytochrome oxidase subunit I (COI) and 16S rRNA gene were PCR-amplified using the following primers: COS2183N (forward), 5'-CAR CAY YTA TTY TGR TTY TTY GG-3' and COA3107 (reverse), 5'-TC TAT TAR DGG DGA DGC DCT ATC TTG-3'; 16Sar (forward), 5'-CGC CTG TTT AAC AAA AAC AT-3' and 16Sbr (reverse), 5'-CTC CGG TTT GAA CTC AGA TCA) (Sota and Hayashi, 2004). In addition, two parts of the nuclear 28S rRNA gene were PCR-amplified using the primer sets 28S-01 (5'-GAC TAC CCC CTG AAT TTA AGC AT-3')/28SR-01 (5'-GAC TCC TTG GTC CGT GTT TCA AG-3') (Kim et al., 2000) and 28v-5' (AAG GTA GCC AAA TGC CTC ATC)/28jj-3' (AGA CTC CTT GGT CCG TGT TTC AAG AC) (Palimbi, 1996). Purified PCR products were used in a dye terminator cycle-sequencing reaction using an ABI PRISM BigDye Terminator Cycle Sequencing FS Ready Reaction Kit, and the products were electrophoresed on an ABI 377 sequencer (Applied Biosystems, Foster City, CA). Sequence data were deposited in GenBank (accession numbers: COI, EF532502-532546; 16S, EF532547-EF532591; 28S, EF532412-EF532501).

2.3. Phylogenetic analysis

DNA sequences were aligned using the multiple-sequence alignment program Clustal X version 1.83 (Chenna et al., 2003) and later refined manually. Alignment of the 16S and 28S sequences required gaps. Two ambiguously aligned regions of 16S sequences (8 and 11 bp) were eliminated in the phylogenetic analyses, whereas all six gap regions in 28S data were unambiguously aligned and retained in the phylogenetic analyses.

Phylogenetic trees were reconstructed by the maximum-likelihood (ML), Bayesian, and parsimony methods, for mitochondrial and nuclear data. The substitution model and parameters used in the ML analysis were obtained by Modeltest version 3.7 (Posada and Crandall, 1998) using the Akaike Information Criterion (AIC). PAUP* version 4.0b10 (Swofford, 2002) was used for the ML tree search. The search was heuristic, using ten random addition analyses with tree bisection-reconnection (TBR) branch-swapping with the MulTrees option activated. A Bayesian approach was implemented in MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001). The substitution model was selected by Modeltest as above. Two runs of one million Markov Chain Monte Carlo (MCMC) analyses were performed with trees sampled every 100th generation. Burn-in time was set at 100,000 generations after graphical inspection of parameter convergence. Parsimony analyses were made using PAUP*. Tree search was heuristic, using 100 random addition analyses with TBR branch-swapping and the MulTrees option. Gaps were treated as a new state. Bootstrap values of nodes were obtained by 1,000 replications using heuristic searching and 100 random addition analyses with TBR branch-swapping as above. Simultaneous analyses of combined mitochondrial and nuclear data were performed as in the separate analyses except that two partitions with different substitution models were used in the Bayesian analysis.

2.4. Dispersal and vicariance analysis

To reconstruct the dispersal-vicariance history for *Plateumaris* in the northern hemisphere, a dispersal-vicariance analysis proposed by Ronquist (1997) was performed using the program DIVA version 1.1 (Ronquist, 1996). The distribution ranges of species were classified into North America, Asia, and Europe. Default optimization with no cost for vicariance and a cost of one for dispersal and extinction

was applied to a data set created with the ML tree resulting from the combined data set.

2.5. Divergence time estimation

To determine the times of dispersal and vicariance events in *Plateumaris*, node ages were estimated for a tree resulting from ML analysis of combined data. The ML tree was converted to an ultrametric tree using a Bayesian approach (Thorne and Kishino, 2002). Two *Neohaemonia* species were treated as outgroup taxa; ingroup taxa consisted of all *Plateumaris* and *Donacia* species used in this study. According to the protocol, branch lengths of each of the mitochondrial and nuclear partitions were obtained using Baseml and Estbranches programs implemented in PAML version 3.14 (Yang, 1997) with the F84+Γ substitution model (Felsenstein, 1984). The program Multidivtime (Thorne and Kishino, 2002) was then run for a Bayesian analysis of divergence time with two gene partitions. Each MCMC analysis consisted of 100,000 burn-in cycles and one million post burn-in cycles with sampling at every 100th tree. We conducted two independent Markov chain Monte Carlo (MCMC) analyses to confirm convergence based on the similarity of the estimated divergence times.

To set constraints on the node ages and priors of multidivtime parameters, we referred to the fossil record. Because donaciine fossils have been found only after the Paleocene, whereas other chrysomelids have been found in the Mesozoic (Santiago-Blay, 1994), we assumed the origin of the donaciine genera after 65 mya. Accordingly, we set the a priori time between tip and root, rttm, equal to 6.5 (unit time, 10 my) with its standard deviation (rttmsd = 0.5 × rttm). The prior rate of evolution (rtrate) was set to approximate median branch length of the combined mitochondrial and nuclear data (= 0.15) divided by rttm, with rtratesd = 0.5 × rtrate.

Askevold (1990) redescribed the fossil species *Plateumaris primaeva* from the Early Oligocene in Colorado, USA. This fossil is similar to *Plateumaris nitida* and its morphological allies (*P. nitida* group), which is one of the most derived groups in Askevold's (1991) cladogram (see Fig. 1a). Askevold (1990) stated, "The possibility that *P. primaeva* is the same species as *P. nitida* cannot be ruled out, but this is difficult to rationalize… most, if not all, species groups of *Plateumaris* existed prior to 30 million years ago." Since the resemblance of external characters is not always reliable evidence of phylogenetic relationships and because we found that the *P. nitida* group is

not monophyletic in the molecular phylogeny, we initially adopted the second part of Askevold's (1990) statement and set the node for all North American species at more than 30 mya. Since this assignment may be too conservative, we also used an alternative setting of >30 mya at the node to coalescence all *P. nitida* group species (*P. nitida*, *P. frosti*, *P. sericea*, *P. shirahatai*). Among Japanese fossils, the oldest *P. constricticollis* was found from the Late Pleistocene (2.6 mya), and the ancestral node of this species was set at more than 2.6 mya. In addition, colonization in Japan by *P. sericea* occurred 0.8-0.5 mya (Hayashi, 2004; Sota and Hayashi 2007); this time interval was set to the ancestral node for Japanese and mainland populations. There are many Quaternary fossils of *Plateumaris* (reviewed by Santiago-Blay, 1994), but they were not useful in our calibration. For *Donacia*, Askevold (1990) described *D. wightoni* from the Paleocene (58 mya) in Alberta, Canada. The ancestral node for *Donacia* was therefore set at more than 58 mya.

3. Results

3.1. Phylogeny

Although there is a large difference in the substitution rate between mitochondrial and nuclear 28S genes, separate phylogenetic analyses for these data revealed largely similar topologies. For mitochondrial data with 1248 characters (726 constant), the log-likelihood (ln *L*) score was –11487.5 in the ML analysis, and the harmonic mean of ln *L* was –11567.36 in the Bayesian analysis. Parsimony analysis resulted in a single shortest tree of 2195 steps (consistency index excluding uninformative characters [CI] =0.34; retention index [RI] =0.48). For nuclear 28S data with 1587 characters (1435 constant), ML and Bayesian ln *L* scores were –3781.2 and –4004.0 (harmonic mean), respectively; parsimony analysis (with gaps treated as a new state) resulted in 110 short trees of 261 steps (CI=0.68; RI=0.89). In both the mithochondrial and the nuclear trees (Fig. 2), there were two major clades, one with three European endemic species (*P. rustica*, *P. consimilis*, *P. braccata*; clade A; Fig. 2) and the other with North American and Asian species (clade B). Second, in the latter clades, three Asian species formed a clade with *P. rufa* (clade C); in its sister clade with nine North American and four Asian species (clade D), *P. germari*, *P. akiensis*, and *P. pusilla* diverged directly from the

ancestral node D. Within clade D, clade E included three Asian species, of which *P. sericea* and *P. shirahatai* were derived sister species.

The combined data set of all genes consisted of 2835 characters. The ln *L* scores were –15920.6 in the ML analysis and –15589.7 in the Bayesian analysis (harmonic mean). The parsimony analysis resulted in a single shortest tree of 2401 steps (CI=0.36; RI=0.52). The three methods resulted in similar topologies except a few differences within clade D. The ML tree (Fig. 3) as well as trees from the parsimony and Bayesian analyses did not support the phylogenetic hypothesis based on morphology (Fig. 1) except for four sister species relationships: *P. consimilis – P. braccata*, *P. fulvipes – P. roscida*, *P. nitida – P. frosti*, and *P. sericea – P. shirahatai*.

3.2. Dispersal-vicariance events and divergence time

The dispersal-vicariance analysis under the ML tree topology (Fig. 3) resulted in an optimization with six dispersal events following the initial vicariance of *Plateumaris* between North America-Asia and Europe, and the subsequent vicariance between North America and Asia (Fig. 4). Dispersal occurred three times from North America to Asia, once from Asia to North America (*P. rufa*), and twice from Asia to Europe (*P. sericea*, *P. weisei*).

The estimated divergence time under the initial node constraint (>30 mya at node of clade B) revealed that the genera *Plateumaris* and *Donacia* diverged 72 mya (95% credible interval: 87 – 61 Ma), during the late Cretaceous (*Donacia* is not shown in Fig. 4), and the ancestral node for all *Plateumaris* was about 43 mya (58-33 mya; Fig. 4). The vicariance between North America-Asia and Europe occurred during the Eocene. Within the North America-Asian lineage (clade B), clade C diverged at 24 mya (32-18 mya), and clade D at 31 mya (42-24 mya), both probably during the Oligocene. Within clade C, the North American species *P. rufa* diverged from Asian species around 21 mya (30-15 mya). Within clade D, three Asian lineages, *P. akiensis*, *P. roscida*, and *P. shirahatai* + *P. sericea*, diverged from North American lineages about 29 mya (40-22 mya), 15 mya (24-9 mya), and 7 mya (12-3 mya), respectively, during the Oligocene and Miocene.

Under the alternative node constraint (the coalescent time of all *P. nitida* group species at the node of clade E > 30 mya), the estimated divergence times were older by

10 my maximally (Fig. 4). However, the vicariance between North America–Asia and Europe remained an Eocene event, and the overall results remained similar to those under the initial time constraints.

4. Discussion

4.1. Phylogeny of Plateumaris

The present study provides a comprehensive account of the phylogeny and historical biogeography of the genus *Plateumaris* in the Holarctic region based on molecular phylogenetic approaches. The species relationships derived from the molecular phylogeny were inconsistent with those based on morphology (Fig. 1[a]; Askevold, 1991). The morphological cladistics of Askevold (1991) were based mainly on ovipositor, pygidium, and external characters (35 characters), which showed restricted variation, and a relatively small number (six) of genital (endophallus) characters. The genital characters may have been preferable phylogenetic markers. In fact, in our molecular phylogeny, most of these characters showed homoplastic changes (ten of 12 ovipositor, six of seven pygidium, and 14 of 15 external characters), whereas only two of six genital characters did. Askevold (1991) used specific assumptions on character change to obtain the parsimony tree shown in Fig. 1(a), and without those assumptions a parsimony analysis would result in the consensus tree in Fig. 1(b). The species groups in Fig. 1(a) are not recovered in the latter tree, indicating that the morphological hypothesis has not been robust. Nonetheless, four sister relationships are recovered in both trees and also in the molecular phylogeny, representing robust hypotheses.

We found two unexpected discrepancies between molecular and morphological analyses. Although one East Asian species, *P. akiensis*, has been considered to be close to *P. constricticollis* (in clade C) because of morphological similarity and distribution (both are endemic to Japan), *P. akiensis* was a member of clade D. Instead, the Eurasian species *P. weisei* belonged to clade C although it is close to North American species in the morphological cladogram.

In interpreting our molecular phylogeny of *Plateumaris*, caution is needed because of the lack of seven North American taxa in the present study. *Plateumaris diversa* in

the *P. rufa* group is distributed in eastern North America. Inclusion of this species in the molecular phylogeny may improve our understanding of the origin of clade C and the Japanese endemics (*P. akiensis*, *P. constricticollis*) because *P. diversa* is close to these species as well as to *P. rufa*. Similarly, *P. robusta*, which is a sister to *P. pusilla* in the morphological cladogram, may be a candidate for species related to *P. akiensis*. Two unsampled species of the *P. shoemakeri* group in western North America (*P. neomexicana*, *P. dubia*) are important in estimating dispersal-vicariance within North America. Three eastern North American species in the *P. nitida* group that were not sampled (*P. schaefferi*, *P. balli*, *P. notmani*) are also important in estimating dispersal-vicariance within North America and the origin of Eurasian species *P. shirahatai* and *P. sericea*. There is no doubt that a more robust hypothesis can be proposed once all missing taxa are included in the molecular analysis.

4.2. Historical biogeography

Diversification of the Polyphaga, including the Chrysomelidae, is likely associated with the diversification of angiosperms during the Cretaceous (Farrell, 1998). Fossils of some Chrysomelidae have been discovered from the Jurassic, but donaciine fossils appeared only after the Cenozoic (Askevold 1990; Santiago-Blay, 1991). However, Wilf et al. (2000) found fossil hispine damage on Zingibrales, despite the absence of insect body fossils, and proposed that many chrysomelid clades evolved well before the terminal Cretaceous. In our divergence time estimation, the differentiation between *Donacia* and *Plateumaris* occurred in the latest part of the Late Cretaceous. This estimate may be rather conservative. Based on vicariance biogeography, Askevold (1991) assumed that donaciine lineages must have diverged well in advance of the late Cretaceous, and that any exchange or vicariance among lineages of Donaciinae must have occurred before the Miocene. The latter may be warranted by the absence of Holarctic species in the Donaciinae (Askevold, 1991), and our age estimation generally supports this reasoning. Askevold (1991) further discussed the biogeography of *Plateumaris* based on a morphological cladogram (Fig. 1). However, the validity of his inference is questionable because the morphological cladogram is not fully supported by our molecular phylogeny.

North America harbors more extant species of *Plateumaris* than Eurasia and hence

is likely to be the origin of this genus. However, based on our molecular phylogeny, we only could determine that ancestral *Plateumaris* were once spread over the Holarctic and divided into two lineages by the Atlantic Ocean. The differentiation of the European lineage is fairly ancient, during the mid-Paleogene, suggesting a dispersal event between North America-Asia and Europe. This dispersal event may have involved a trans-Atlantic land bridge such as the Thulean Bridge in the early Eocene (Sanmartín et al., 2001). The presence of the Turgai Strait from the late Paleocene until the end of the Eocene would have facilitated the differentiation of the European lineage from lineages in North America and Asia. Migration of *Plateumaris* between North America and East Asia likely involved the Beringian Land Bridge. Migration of terrestrial biota via the Beringian Land Bridge could have occurred from the early Paleocene until its closure in the late Miocene or early Pliocene, between 7.4 and 4.8 mya (Tiffney and Manchester, 2001). Although the bridge was available during Pleistocene glaciations, the tree-less steppe (tundra) connecting the two continents was an unlikely dispersal route for donaciine beetles. Dispersal and vicariance of terrestrial biota have been estimated to occur from the Oligocene to the Pliocene, mostly in the mid-Miocene through the late Pliocene, for flowering plants (reviewed by Wen, 1999) and in the late Miocene and the late Pliocene for felid mammals (Johnson et al., 2006) based on molecular phylogenies.

A comprehensive account of historical biogeography in Holactic animals has been presented by Sanmartín et al. (2001). In this analysis, most trans-Atlantic distributions were common in the early to mid-Tertiary (70-20 mya), which probably resulted in disjunctions, whereas trans-Beringian distributions were rare in that period. Sanmartín et al. (2001) included *Plateumaris* in their analysis and determined dispersal-vicariance within the genus after 150 mya based on the morphological cladogram and biogeographical inference of Askevold (1991). They used the *nitida* and *shoemakeri* species groups and assumed their vicariance by the Mid-Continental-Seaway at 150 mya. However, because these species groupings have not been reproduced by our molecular phylogeny, the analysis of Sanmartín et al. (2001) requires revision. Because few historical biogeographical analyses have been conducted on holarctic animal groups using molecular data (e.g., Johnson et al., 2006), meta-analyses (e.g., Sanmartín et al., 2001) should be repeated after case studies of different animal groups have accumulated sufficient new information.

5. Conclusion

This study revealed that dispersal-vicariance events between North America and Europe and between North America and East Asia throughout the Paleogene and Neogene resulted in the present holarctic distribution of *Plateumaris* leaf beetles. Molecular phylogenetics should focus on groups with wide distributions across the Holarctic region when testing ancient faunal exchanges between Europe and North America, the role of the Turgai Strait as a geographic barrier between Europe and Asia, and the trans-Beringian faunal exchange between Asia and North America. .

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References

Askevold, I.S., 1990. Classification of Tertiary fossil Donaciinae of North America and their implications about evolution of Donaciinae (Coleoptera: Chrysomelidae). Can. J. Zool. 68, 2135-2145.

Askevold, I.S., 1991. Classification, reconstructed phylogeny, and geographic history of the New World members of *Plateumaris* Thomson, 1859 (Coleoptera: Chrysomelidae: Donaciinae). Mem. Entomol. Soc. Canada 157, 5-175.

Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T. J., Higgins, D. G., Thompson, J. D., 2003. Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res. 31, 497-500.

Farrell, B.D., 1998. “Inordinate fondness” explained: why are there so many beetles? Science 281, 555-559.

Felsenstein, J., 1984. Distance methods for inferring phylogenies: a justification. Evolution 38, 16-24.

Hayashi, M. 2001. Taxonomic notes on *Plateumaris amurensis* Weise and *Plateumaris*

weisei (Duvivier) (Coleoptera: Chrysomelidae: Donaciinae). Entomol. Rev. Japan 56, 1-8.

Hayashi, M. 2004. Faunal changes in Donaciinae during the Quaternary in central Japan (Coleoptera, Chrysomelidae). In: Jolivet, P., Santiago-Blay, J.A., Schmitt, M. (Ed.) New development in the biology of Chrysomelidae, SPB Academic Publishing, The Hague, pp. 263-274.

Huelsenbeck, J. P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754-755.

Johnson, W.E., Eizirik, E., Pecon-Slattery, J., Murphy, W.J., Antunes, A., Teeling, E., O'Brien, S.J., 2006. The late Miocene radiation of modern Felidae: a genetic assessment. Science 311: 73-77.

Kim, C-G., Zhou, H-Z., Imura, Y., Tominaga, O., Su, Z-H., Osawa, S. 2000. Pattern of morphological diversification in the *Leptocarabus* ground beetles Coleoptera, Carabidae as deduced from mitochondrial ND5 gene and nuclear 28S rDNA sequences. Mol. Biol. Evol. 17, 137-145.

Nie, Z-L., Sun, H., Li, H., Wen, J., 2006., Intercontinental biogeography of subfamily Orontioideae (*Symplocarpus*, *Lysichiton*, and *Orontium*) of Araceae in eastern Asia and North America. Mol. Phylogenet. Evol. 40, 155-165.

Palumbi, S. R., 1996. Nucleic acids II: the polymerase chain reaction. In: Hillis, D. M., Moritz, C., Mable, B. K. (Eds.), Molecular systematics, 2nd ed. Sinauer, Sunderland, Massachusetts, pp. 205-247.

Posada, D., Crandall, K. A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817-818.

Ronquist, F. 1997. Dispersal-vicariance analysis: A new approach to the quantification of historical biogeography. *Syst. Biol.* 46:195-203.

Ronquist, F. 1996. DIVA version 1.1. Computer program and manual available by anonymous FTP from Uppsala University (ftp.uu.se or ftp.systbot.uu.se)

Sanmartín, I., Enghoff, H., Ronquist, F., 2001. Patterns of animal dispersal, vicariance and diversification in the Holarctic. Biol. J. Linn. Soc. 73: 345-390.

Santiago-Blay, J.A., 1994. Paleontology of leaf beetles. In: Jolivet, P.H., Cox, M.L., Petitpiere, E. (Ed.) Novel aspect of the biology of Chrysomelidae, Kluwer, Hague, pp. 1-68.

Sota, T., Hayashi, M., 2004. A molecular phylogenetic analysis of the genus *Donacia* (Coleoptera, Chrysomelidae) in Japan based on mitochondrial gene sequences. In: Jolivet, P. J., Santiago-Blay, A., Schmitt, M. (Eds.), New developments on the biology of Chrysomelidae. SPB Academic Publishing, The Hague, pp 105-116.

Sota, T., Hayashi, M., 2007. Comparative historical biogeography of *Plateumaris* leaf beetles (Coleoptera: Chrysomelidae) in Japan: interplay between fossil and molecular data. J. Biogeogr. (in press)

Spalik, K., Downie, S. R., 2006. The evolutionary history of *Sium* sensu lato (Apiaceae): dispersal, vicariance, and domestication as inferred from ITS rDNA phylogeny. Am. J. Bot. 93, 747-761.

Swofford, D. L., 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4.0. Sinauer Associates, Sunderland, Massachusetts.

Thorne, J.L., Kishino, H., 2002. Divergence time and evolutionary rate estimation with multilocus data. Syst. Biol. 51, 689-702.

Tiffney, B. H., Manchester, S. R., 2001. The use of geological and paleontological evidence in evaluating plant phylogeographic hypotheses in the northern hemisphere Tertiary. Int. J. Plant. Sci. 162, S3-S17.

Wen, J. 1999. Evolution of eastern Asian and eastern North American disjunct distributions in flowering plants. Annu. Rev. Ecol. Syst. 30, 421-455.

Wilf, P., Labandeira, C.C., Kress, W.J., Staines, C.L., Windsor, D.M., Allen, A.L., Johnson, K.R., 2000. Timing the radiations of leaf beetles: hispines on gingers from latest Cretaceous to recent. Science 289, 291-294.

Yang, Z., 1997. PAML: a program package for phylogenetic analysis by maximum likelihood. CABIOS (Comp. Appl. Biosci.) 13, 555-556.

Table 1

List of samples used in the molecular phylogenetic study.

Species	Locality (collector, year)	Code
P. braccata (Scopoli)	Dalesice, nr. Ml. Boleslav, Czechia (Bocák, 2006)	1421
	Dalesice, nr. Ml. Boleslav, Czechia (Bocák, 2006)	1429
P. consimilis (Schrank)	Nova Sedlica, Slovakia (Bolm, 2005)	1407
	Namest na Hane, Moravia, Czechia (Bocák, 2006)	1413
P. rustica (Kunze)	Namest na Hane, Moravia, Czechia (Bocák, 2006)	1417
	Namest na Hane, Moravia, Czechia (Bocák, 2006)	1418
P. rufa (Say)	Dufferin Co., Ontario, Canada (Sota, 2006)	1384
	Wellington Co., Ontario, Canada (Sota, 2006)	1455
P. akiensis Tominaga and Katsura	Hiroshima, Honshu, Japan (Sota, 2001)	641
P. constricticollis Jacoby	Nanae, Hokkaido, Japan (Sota, 2005)	1008
	Aichi, Honshu, Japan (Sota, 2005)	969
	Fukushima, Honshu, Japan (Yagi, ?)	482
P. pusilla (Say)	Winnipeg, Manitoba, Canada (Hayashi, 2006)	1467
	Wellington/Dufferin Co., Ontario, Canada (Sota, 2006)	1495
P. aurifera (LeConte)	Winnipeg, Manitoba, Canada (Sota, 2006)	1406
P. flavipes (Kirby)	Winnipeg, Manitoba, Canada (Sota, 2006)	1371
P. germari (Mannerheim)	Winnipeg, Manitoba, Canada (Hayashi, 2006)	1465
P. weisei (Duvivier)	Higashikawa, Hokkaido, Japan (Sota, 2001)	667
	Terelj, Mongolia (Hayashi, 2004)	802
	Khasan, Primorsky, Russia (Hayashi, 2002)	249
P. amurensis Weise	Kaimanovka, Primorsky, Russia (Nagahata, 2003)	289
P. fulvipes (Lacordaire)	Winnipeg, Manitoba, Canada (Sota, 2006)	1452
	Wellington/Dufferin Co., Ontario, Canada (Sota, 2006)	1454
P. roscida Weise	Kaimanovka, Primorsky, Russia (Nagahata, 2003)	672
P. shoemakeri (Schaeffer)	Winnipeg, Manitoba, Canada (Hayashi, 2006)	1471
	Dufferin Co., Ontario, Canada (Sota, 2006)	1386
P. metallica (Ahrens)	Dufferin Co., Ontario, Canada (Hayashi, 2006)	1459
P. nitida (Germer)	Wellington Co., Ontario, Canada (Sota, 2006)	1380
	Winnipeg, Manitoba, Canada (Sota, 2006)	1461
P. frosti (Shaeffer)	Winnipeg, Manitoba, Canada (Hayashi, 2006)	1463

P. sericea (Linnaeus)	Shimane, Honshu, Japan (Hayshi, 2005)	923
	Kamikawa, Hokkaido, Japan (Sota, 2001)	1028
	Namest na Hane, Moravia, Czechia (Bocák, 2006)	1415
	Terelj, Mongolia (Hayashi, 2004)	801
P. shirahatai Kimoto	Bolshoy Kamen, Primorsky, Russia (Hayashi, 2004)	820
	Higashikawa, Hokkaido, Japan (Sota, 2001)	1015
	Terelj, Mongolia (Hayashi, 2004)	797
Outgroup		
Neohaemonia minnesotensis Askevold	Wellington/Dufferin Co., Ontario, Canada (Sota, 2006)	1403
N. nigricornis (Kirby)	Winnipeg, Manitoba, Canada (Sota, 2006)	1405
Donacia cazieri Marx	Winnipeg, Manitoba, Canada (Sota, 2006)	1389
D. biimpressa Melsheimer	Winnipeg, Manitoba, Canada (Sota, 2006)	1387
D. distincta Leconte	Winnipeg, Manitoba, Canada (Sota, 2006)	1391
D. semicuprea Panzer	Moravicany, Moravia, Czechia (Bocák, 2006)	1441
D. clavipes Fabricius	Dalesice, nr. Ml. Boleslav, Czechia (Bocák, 2006)	1425
D. provostii Fairmaire	Atsuma, Hokkaido, Japan (Sota, 2006)	1445

Legends for figures

Fig. 1. (a) Cladogram of species in the genus *Plateumaris* based on parsimony analysis of 41 morphological characters with distribution ranges and species-group designations (adapted from Fig. 270 of Askevold, 1991). An additional species, *P. amurensis*, is not included. Species with asterisks were not used in the present study. (b) Reanalysis of morphological data (Table 2 of Askevold, 1991) with additional data for *P. amurensis* (character states were assessed by M. H.). The tree is the consensus of the nine shortest trees (CI=0.65; RI=0.83) resulting from successive heuristic searches using character re-weighting according to the rescaled CI; each search used ten random addition analyses of TBR branch-swapping with the MulTree option. Unlike Askevold's (1991) analysis, polymorphic states are treated as missing, and recoding for possible reversal was not used. Bootstrap percentages (when >50%) based on 1000 replications are provided above branches.

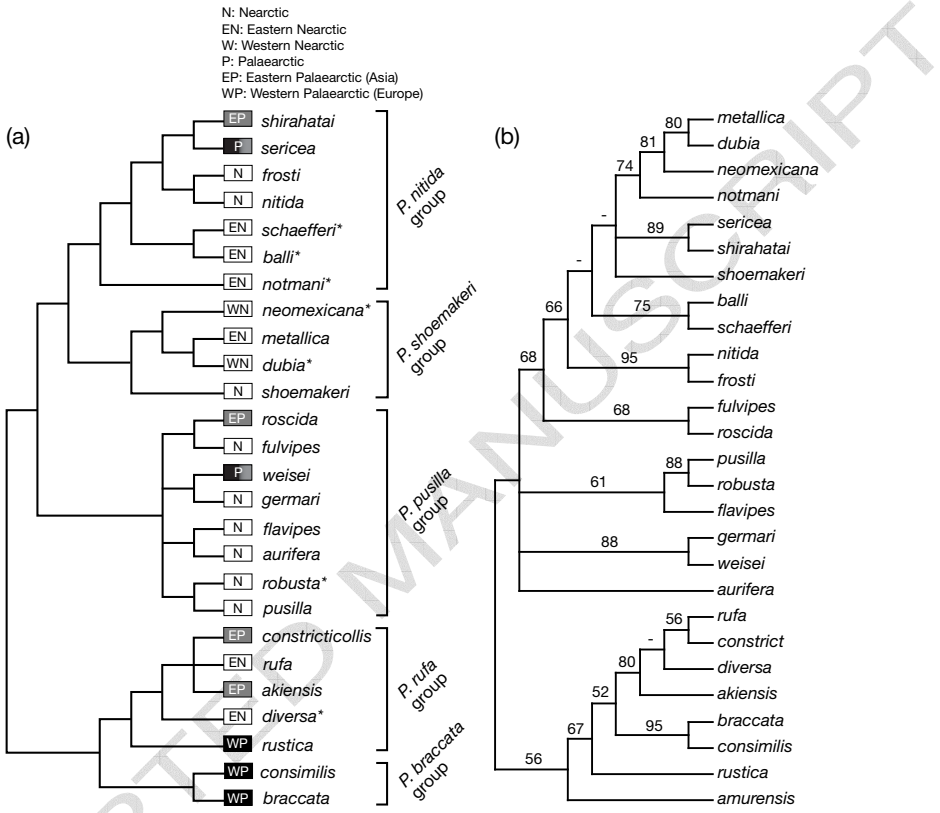
Fig. 2. ML trees resulting from mitochondrial COI+16S and nuclear 28S data. Bayesian posterior probabilities (%) followed by bootstrap percentages in parsimony analysis are shown for each branch. A + indicates that the node is recovered by the analysis, but the percentage is <50%, whereas a – indicates that the node is not recovered by the analysis. A-E represent major clades mentioned in the text.

Fig. 3. ML trees resulting from simultaneous analysis of the combined data for mitochondrial and nuclear genes. See legend of Fig. 2 for further explanation.

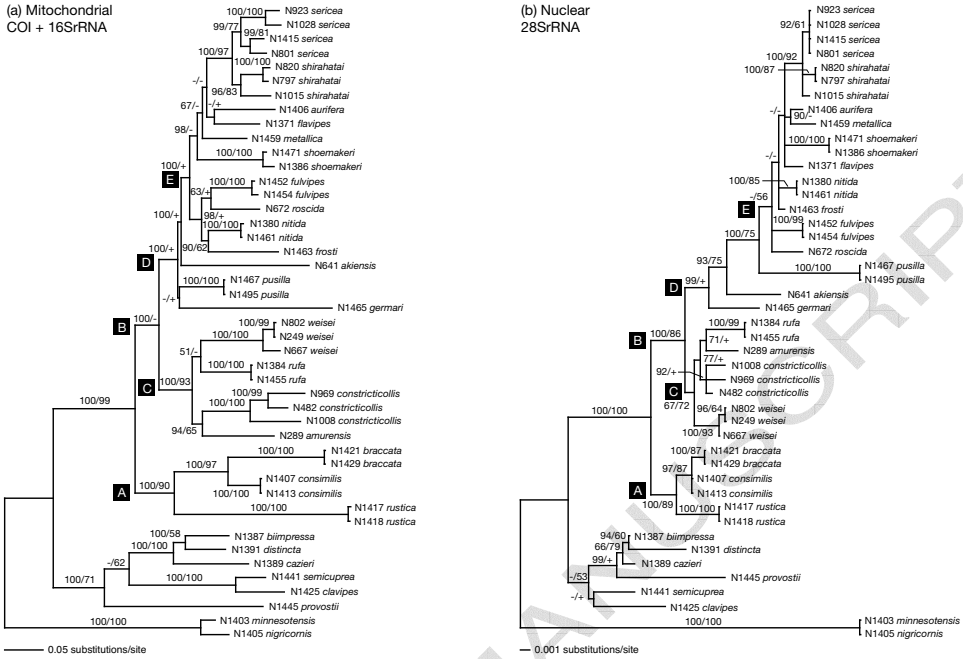
Fig. 4. Divergence times of *Plateumaris* lineages with reconstructed ancestral distribution ranges. The tree is an ultrametric tree resulting from Multidivtime analysis. Constrained nodes are indicated by open circles with the constraints used. Grey bars show 95% credible intervals of estimated ages. Star marks and dotted lines are divergence times and their 95% credible intervals under the alternative constraint (>30 mya at the node of clade E, instead of the node of clade B). The Multidivtime analysis used two *Neohaemonia* species as an outgroup and six *Donacia* species as part of the ingroup taxa. These are absent from this figure. At each node, the ancestral range estimated by DIVA analysis is indicated. At the bottom, information on land connection

and disconnection is shown for North America, Europe, and East Asia.

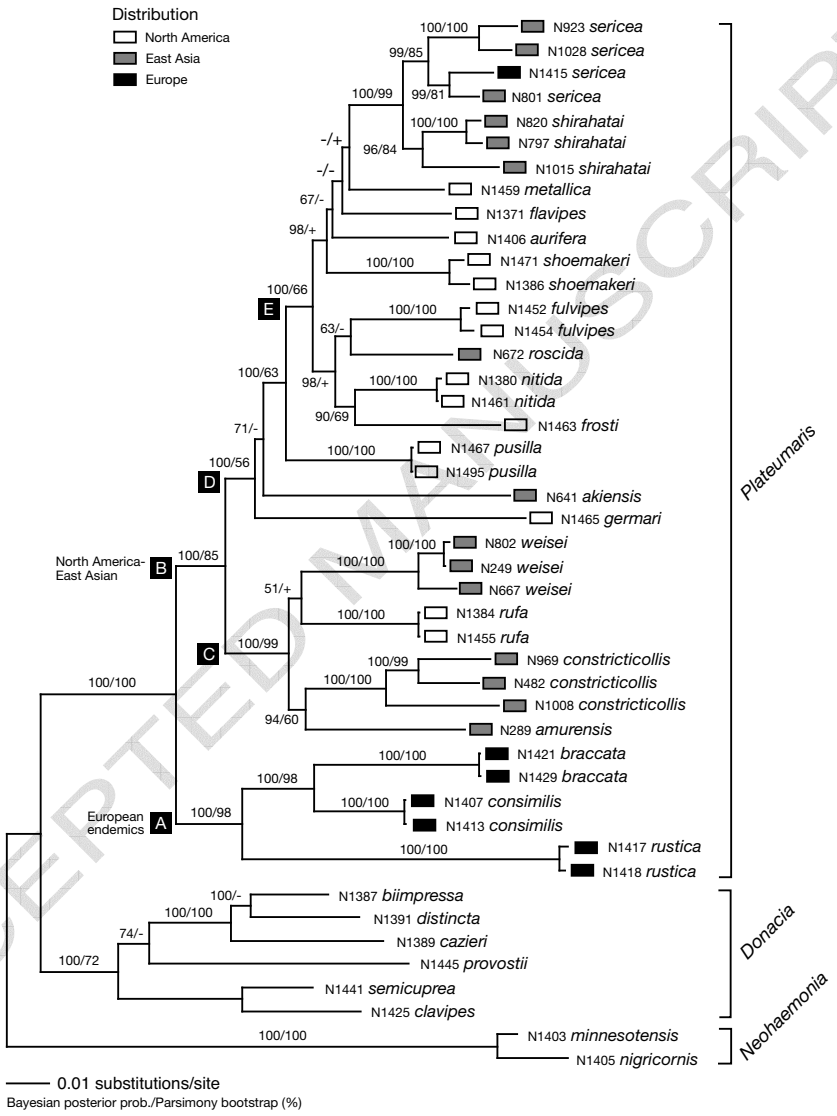
Sota et al. Fig. 1



Sota et al. Fig. 2



Sota et al. Fig. 3



Sota et al. Fig. 4