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Molecular phylogeny of reed beetles (Col., Chrysomelidae, Donaciinae): The signature of ecological specialization and geographical isolation

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ABSTRACT

The Donaciinae consist of approximately 165 species predominantly occurring in the northern hemisphere. We analysed mitochondrial and nuclear DNA (COI, EF-1α) of 46 species to investigate their phylogeny and to discuss general topics in the context of insect herbivory (generalists *versus* specialists, ecological speciation). Phylogenetic reconstructions from various methodical approaches yielded very similar results. Clades corresponding to the traditional tribes/genera were recovered. Within the genus *Donacia*, species groups with characteristic host plant preference were identified. Estimated divergence times are discussed on the background of geological events. The origin of the Donaciinae is dated to 75–100 million years before present, after which they quickly diversified into the main groups. An initial split of those groups occurred in the Palaeocene. In the Eocene and Oligocene, major lineages specialized on certain host plants, where they radiated in the Miocene. This radiation was enforced by geographic isolation brought about by the final separation of America and Europe, after which there arose continental lineages within three larger species groups. In their evolution based on ecological specialization with a recently superimposed geographic isolation, the Donaciinae follow a pattern of specialists arising from generalists. Host plant shifts show that such a specialization is not necessarily an 'evolutionary dead-end'.

1. Introduction

Insect herbivory has long attracted attention from biologists of various disciplines. While applied issues arise from the pest status of herbivore species and the resulting economic loss, the diversity of insect herbivores itself was and is the subject of innumerable studies. Recent years saw an increase in estimates of species numbers, which were largely based on surveys of tropical biodiversity (Erwin, 1982)—much of which can be attributed to herbivores so far unknown. While an estimate of 5–10 million species (Ødegaard, 2000; Novotny et al., 2002) discloses the tremendous taxonomic mission to be completed, evolutionary biologists set out to find general rules to explain the success of herbivores (e.g., Novotny et al., 2006): Herbivory arose more than 50 times independently among insects, and the herbivore clades are more speciose than their immediate sister groups (Mitter et al., 1988). The diversification rates are higher in herbivores (Funk et al., 2002).

One key to understanding the evolutionary success of herbivores seems to be the generally high degree of specialization (Ward et al., 2003; Morse and Farrell, 2005). The sheer magnitude of the resource "plant kingdom" can support a large number of consumers. However, due to resource partitioning among specialists we do not simply face few, individual rich species, but a large number of taxa. While the majority of herbivores is oligophagous, many are even strictly monophagous (Strong et al., 1984; Farrell and Mitter, 1993). This pattern has elicited the formulation of theories on the origin and the evolutionary benefits of host specialization (Futuyma and Moreno, 1988; Jaenike, 1990; Kawecki, 1994; Whitlock, 1996). One conceptual framework embraces the interplay between generalization and specialization. Evidently, specialists develop more frequently from generalists than *vice versa* (Nosil, 2002; Morse and Farrell, 2005), leading to the question: Does a narrowed host spectrum set an end to any further development? This would be an evolutionary dead-end (Kelley and Farrell, 1998), where the insect is 'trapped' on its host: Specialization would lead to a loss of genetic variation and hence of the ability to use alternate hosts or to react to environmental changes. Janz et al. (2001), Termonia et al. (2001) and Yotoko et al. (2005) showed that this dead-end is not inevitable (further reviewed by Nosil, 2002). Instead, there are examples of secondary diversification or host shifts (e.g., Radtkey and Singer, 1995).

However, host shifts by phytophagous insects are not arbitrary. Transition to a closely related host plant is not a host shift in the sense of escaping the trap of specialization. The new host usually resembles the former one in its bouquet of secondary compounds. Host shifts to distantly related plants often involve new hosts containing similar key substances (Mitter et al., 1991; Bernays and

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Chapman, 1994; Menken and Roessingh, 1998; Termonia et al., 2001; Becerra, 1997; Kergoat et al., 2005b). Many insect adaptations involve the detoxification of noxious substances (Rosenthal and Berenbaum, 1991). The broad spectrum of secondary compounds with toxic or feeding deterrent effect on most enemies gave rise to the idea of continuous evolution of new such substances as plant defence. Herbivores, on the other hand, overcome this line of defence by developing a mechanism of avoidance, detoxification, insusceptibility or even sequestration for their own purposes (Futuyma and Keese, 1992; Ode, 2006; Després et al., 2007). The result is a scenario of adaptation and counter-adaptation (co-evolution), which can lead to speciation on both the plant and the herbivore side, resulting in parallel phylogenies. At this point, the phenomenon herbivory gains importance beyond merely the high diversity as statically observed: It offers an explanation in terms of an underlying process, namely sympatric speciation due to ecological specialization, which had long been controversial. The idea is that speciation as a consequence of ecological specialization can occur in sympatry *via* the formation of host races of the herbivores (Strong et al., 1984; Farrell and Mitter, 1990; Bush, 1994; Schluter, 1998; Berlocher and Feder, 2002; Rundle and Nosil, 2005; Stireman et al., 2005). It should be noted, however, that the diversification in question is not necessarily co-evolution. Instead, sequential evolution without direct interaction can yield similar pictures and the general term 'parallel cladogenesis' (Futuyma and Keese, 1992) should be used. The classical scenario of allopatric speciation (Bush, 1975) can also be accompanied by a host shift, especially if areas beyond the range of the former host are being colonised. In this case, the relative importance of ecological and geographical factors, respectively, is difficult to reveal.

In order to assess the influence of geography and ecology on diversification, we chose the reed beetles (Donaciinae Kirby, 1837), a subfamily of the leaf beetles (Chrysomelidae), for a molecular phylogenetic analysis. Donaciinae occur in the northern hemisphere, in North Eastern Australia and in Africa, with northern temperate zones as the centres of their diversity. The total number of species is about 165, the largest genera are *Donacia* Fabricius, 1775 (approx. 100 species) and *Plateumaris* Thomson, 1859 (26 species, Askevold, 1990b). This intermediate group size allows for an appreciable pattern of differentiation, and still renders possible the coverage of all major lineages and a significant proportion of all members. The Donaciinae arose early in the evolution of the Chrysomelidae, and the most relevant events of geographic isolation affecting their distribution are the major rearrangements of land masses on a geological time scale. Fossil specimens date back to the Palaeocene and are remarkably similar to extant forms, suggesting a longer history of the group.

Reed beetles are a morphologically and ecologically well-defined group. The larvae are aquatic and adults live and feed on various species of reed plants *sensu lato* (from grasses in wet marshes to floating-leaf plants and submerged macrophytes). Most species are oligo- or monophagous, but a single host plant species can be shared by various beetle species. The notion by Jolivet (1988), according to which the Donaciinae are polyphagous and simply use any plant occurring in their wetland habitat, appears simplified. Hence their ecological differentiation (host plant preference) can form the basis of an ecological analysis with the reconstruction of similarities by descent.

Although far from fully resolved, the taxonomy of reed beetles is in a comparatively good state. There are identification keys and species accounts for many regions (Freude et al., 1966; Mohr, 1985). Recent work by Askevold led to a revision of the genera of the world (Askevold, 1990b, 1991a) and of the genera *Plateumaris* (Askevold, 1991b) and *Neohaemonia* Székessy, 1941 (Askevold, 1988) in the Nearctic Region. Molecular data are only available for a limited set of species from Japan (Sota and Hayashi, 2004, 2007) and for the genus *Plateumaris* world wide (Sota et al., 2008). Several attempts were made to subdivide the larger genera *Donacia* and *Plateumaris* into subgenera or at least morphological/ecological species groups, which shows that the lineages required for a detailed analysis indeed exist.

The aims of this paper are (a) to reconstruct a molecular phylogeny of Donaciinae and to add molecular data to cases where classical taxonomic work was inconclusive, (b) to relate phylogenetic results to ecological data of host plant use, (c) to combine the phylogeny with a scenario of changes in the distribution of land masses on a geological time scale, and finally (d) to contrast the effects of ecological specialization and geographic isolation.

2. Material and methods

2.1. Sampling, DNA extraction, PCR, sequencing

Fourty-six species of Donaciine beetles could be included into this study. For each species usually two to four individuals were analysed. For some specimens the target DNA sequences could not be amplified and additional beetles of the species were used to complement the data set (130 individuals; Table 1). For *Macroplea japana* and *Donaciasta goeckei* only data for COI could be obtained (a short fragment only in the case of *M. japana*), but the species were still included due to their taxonomic and faunistic importance. DNA was extracted with the Qiagen DNeasy tissue kit following the protocol for animal tissue (with volumes adjusted to the small amounts of tissue involved). Usually two legs of a beetle were used. We analysed the second half of the gene coding for Cytochrome *c* oxidase subunit I (COI) by using the primers C1-J-2183 and TL-N-3014 (Simon et al., 1994), and a partial sequence of the gene for elongation factor-1α (EF-1α) using the following primers: EF1a-SN 5′-TGGGAAAAGGYYCCTTCAAATATGC-3′ and EFA1106neu 5′-GTATATCCATTGGAAATTTGACCNGGRTG-3′. Newly designed internal primers for smaller sections were used in cases where the DNA was of poorer quality (sequences available from the authors). The PCR procedure for COI was as follows: initial denaturation for 5 min at 95 °C; 35 cycles of 30 s at 95 °C, 40 s at 47 or 48 °C, 1 min at 72 °C; final elongation for 5 min at 72 °C. For EF-1α, the cycles were 45 s at 95 °C, 1 min at 47 °C and 1 min at 72 °C. Cycle sequencing in both directions was carried out either with the Big Dye Terminator kit (Applied Biosystems) or the Thermo Sequenase Primer Cycle Sequencing Kit (Amersham Biosciences). The respective products were sequenced on an ABI 377 or Licor 4200L automated sequencer. The raw sequencing data were edited using Sequencher version 4.5, build 1416 (Gene Codes Corp.). Alignment of the COI data was accomplished visually. Sequences for EF-1α were first aligned by using Clustal W version 1.83, and obvious inconsistencies were subsequently corrected by hand. Corrections were indicated where ambiguities caused the software to invoke gaps and where a subset of the sequences was isolated without adequate insertion of gaps in the intron section. DNA sequences were deposited in GenBank (Accession Numbers EU880600–EU880721 and EU880723–EU880819).

2.2. Phylogenetic analysis

Homogeneity of base frequencies among sequences was confirmed using DAMBE 4.5.33 (Xia and Xie, 2001Xia and Xie 2001). Sequences containing too many missing data had to be omitted from some calculations, e.g., for *Macroplea japana* only 209 bases of COI were available and for other specimens this section was missing entirely, rendering the calculation of genetic distances impossible. This results in slightly different numbers of taxa

Table 1
List of specimens

Genus	Species	ID-#	Site	Country	Collectors
Donacia	*bicolor* Zschach, 1788	149, 150	Lough Ree	Ireland	G. Kölsch, E. Meichßner
Donacia	*biimpressa* Melsheimer, 1847	225	Tay River, Perth, ON	Canada	M. Bartsch, G. Kölsch
Donacia	*caerulea* Olivier, 1795	161	W Selkirk Prov. Park, ON	Canada	M. Bartsch, G. Kölsch
Donacia	*caerulea* Olivier, 1795	162	Pine Creek, 10 mi S Battle Creek, MI	USA	M. Bartsch, G. Kölsch
Donacia	*cazieri* Marx, 1957	191	Munising Tourist Park, MI	USA	M. Bartsch, G. Kölsch
Donacia	*cincticornis* Newman, 1838	163	Little Silver Lake, ON	Canada	M. Bartsch, G. Kölsch
Donacia	*cincticornis* Newman, 1838	164, 219, 226	Stone Lake, La Porte, MI	USA	M. Bartsch, G. Kölsch
Donacia	*cinerea* Herbst, 1783	104, 105, 116, 117	Langenhorn, Schleswig–Holstein	Germany	T. Behrends, G. Kölsch
Donacia	*clavipes* Fabricius, 1792	112–115, 118	Langenhorn, Schleswig–Holstein	Germany	T. Behrends, G. Kölsch
Donacia	*confluenta* Say, 1827	192, 193	Pine Creek, 10 mi S Battle Creek, MI	USA	M. Bartsch, G. Kölsch
Donacia	*confluenta* Say, 1827	208	Tay River, Perth, ON	Canada	M. Bartsch, G. Kölsch
Donacia	*confluenta* Say, 1827	209	ditch along Mitchell Lake Rd., MI	USA	M. Bartsch, G. Kölsch
Donacia	*crassipes* Fabricius, 1795	151, 152	Grand Canal/Barrow Line, Vicarstown	Ireland	G. Kölsch, E. Meichßner
Donacia	*dentata* Hoppe, 1795	157, 158	River Arlau, Schleswig–Holstein	Germany	G. Kölsch, R. Suikat
Donacia	*distincta* LeConte, 1851	165	Cranmoor W Nekoosa, WI	USA	M. Bartsch, G. Kölsch
Donacia	*distincta* LeConte, 1851	166	Embarass River, 4 mi S Biwawik, MN	USA	M. Bartsch, G. Kölsch
Donacia	*fulgens* LeConte, 1851	167, 168	Mulvihill Lake, Parc Gatineau, QC	Canada	M. Bartsch, G. Kölsch
Donacia	*fulgens* LeConte, 1851	169	small lake near Lake City, MI	USA	M. Bartsch, G. Kölsch
Donacia	*fulgens* LeConte, 1851	190	Mulvihill Lake, Parc Gatineau, QC	Canada	M. Bartsch, G. Kölsch
Donacia	*hirticollis* Kirby, 1837	194, 195	small lake near Lake City, MI	USA	M. Bartsch, G. Kölsch
Donacia	*impressa* Paykull, 1799	153, 154	Grand Canal/Barrow Line, Vicarstown	Ireland	G. Kölsch, E. Meichßner
Donacia	*marginata* Hoppe, 1795	132, 133	Ravnsoe	Denmark	G. Kölsch, E. Meichßner
Donacia	*marginata* Hoppe, 1795	136, 137	Rosenfelder See, Schleswig–Holstein	Germany	G. Kölsch
Donacia	*palmata* Olivier, 1795	196	small lake near Lake City, MI	USA	M. Bartsch, G. Kölsch
Donacia	*palmata* Olivier, 1795	200	Madoc, ON	Canada	M. Bartsch, G. Kölsch
Donacia	*piscatrix* Lacordaire, 1845	170	shallow lake 3 km W Whitefish, S Hwy 55, ON	Canada	M. Bartsch, G. Kölsch
Donacia	*piscatrix* Lacordaire, 1845	171	shallow lake E Tacoma Lake, MI	USA	M. Bartsch, G. Kölsch
Donacia	*porosicollis* Lacordaire, 1845	172, 173	Bohall Lake, Itasca State Park, MN	USA	M. Bartsch, G. Kölsch
Donacia	*porosicollis* Lacordaire, 1845	220	northern part of Itasca State Park, MN	USA	M. Bartsch, G. Kölsch
Donacia	*porosicollis* Lacordaire, 1845	221	Bohall Lake, Itasca State Park, MN	USA	M. Bartsch, G. Kölsch
Donacia	*proxima* Kirby, 1837	174	lake E Hwy 71 near Itasca State Parc, MN	USA	M. Bartsch, G. Kölsch
Donacia	*semicuprea* Panzer, 1796	134, 135	Ravnsø	Denmark	G. Kölsch, E. Meichßner
Donacia	*semicuprea* Panzer, 1797	138, 139	Rosenfelder See, Schleswig–Holstein	Germany	G. Kölsch
Donacia	*simplex* Fabricius, 1775	119–121, 144	Postsee, Schleswig–Holstein	Germany	T. Behrends, G. Kölsch
Donacia	*sparganii* Ahrens, 1810	159, 160	River Arlau, Schleswig–Holstein	Germany	G. Kölsch, R. Suikat
Donacia	*subtilis* Kunze, 1818	197, 198	Kalamazoo River, 4 mi E Douglas, MI	USA	M. Bartsch, G. Kölsch
Donacia	*subtilis* Kunze, 1818	199	Fawn River, 1 mi E Constantine, MI	USA	M. Bartsch, G. Kölsch
Donacia	*thalassina* Germar, 1811	102, 103	Langenhorn, Schleswig–Holstein	Germany	T. Behrends, G. Kölsch
Donacia	*thalassina* Germar, 1811	130, 131	Knudsø	Denmark	G. Kölsch, E. Meichßner
Donacia	*tomentosa* Ahrens, 1810	216, 217	Postsee, Schleswig–Holstein	Germany	G. Kölsch, R. Suikat
Donacia	*versicolorea* (Brahm, 1719)	100, 101, 110, 111	Langenhorn, Schleswig–Holstein	Germany	T. Behrends, G. Kölsch
Donacia	*vulgaris* Zschach, 1788	106–109	Langenhorn, Schleswig–Holstein	Germany	T. Behrends, G. Kölsch
Donaciasta	*goeckei* Monrós, 1958	215	D'Nyala Nature Reserve	Rep. of South Africa	E. Grobbelaar & I.H. Rong
Macroplea	*appendiculata* (Panzer, 1794)	17–20	Ravnsø	Denmark	G. Kölsch, V. Mahler, E. Meichßner
Macroplea	*japana* (Jacoby, 1885)	85, 90	Heilongjiang site 3/4	People's Rep. of China	G. R. Buckingham, Chen Zhiqun
Macroplea	*mutica* (Fabricius, 1792)	61–64	Fehmarn	Germany	G. Kölsch, E. Meichßner, R. Suikat
Macroplea	*pubipennis* (Reuter, 1875)	35–38	Kirkkonummi	Finland	O. Biström
Neohaemonia	*melsheimeri* (Lacordaire, 1845)	201, 213	Beaver Lake, Itasca State Park, MN	USA	M. Bartsch, G. Kölsch
Neohaemonia	*melsheimeri* (Lacordaire, 1845)	222	Otter Creek, Lombardy, ON	Canada	M. Bartsch, G. Kölsch
Neohaemonia	*minnesotensis* Askevold, 1988	203	Fish Lake, Anoka County, MN	USA	M. Bartsch, G. Kölsch
Neohaemonia	*minnesotensis* Askevold, 1988	204	near Taylor Lake, Parc Gatineau, QC	Canada	M. Bartsch, G. Kölsch
Neohaemonia	*minnesotensis* Askevold, 1988	214	Cranmoor W Nekoosa, WI	USA	M. Bartsch, G. Kölsch
Neohaemonia	*nigricornis* (Kirby, 1837)	176	County Route 4 Biwawik / Duluth, MN	USA	M. Bartsch, G. Kölsch
Neohaemonia	*nigricornis* (Kirby, 1837)	177	reservoir betw. Westport and Maberly, ON	Canada	M. Bartsch, G. Kölsch
Plateumaris	*flavipes* (Kirby, 1837)	178, 179	SW part of Itasca State Park, MN	USA	M. Bartsch, G. Kölsch
Plateumaris	*frosti* (Schaeffer, 1925)	180	Wanoka Lake, WI	USA	M. Bartsch, G. Kölsch
Plateumaris	*frosti* (Schaeffer, 1925)	181	Soldier Lake, MI	USA	M. Bartsch, G. Kölsch
Plateumaris	*fulvipes* (Lacordaire, 1845)	182	Embarass River, 4 mi S Biwawik, MN	USA	M. Bartsch, G. Kölsch
Plateumaris	*fulvipes* (Lacordaire, 1845)	183	near Taylor Lake, Parc Gatineau, QC	Canada	M. Bartsch, G. Kölsch
Plateumaris	*metallica* (Ahrens, 1810)	205, 206	near Taylor Lake, Parc Gatineau, QC	Canada	M. Bartsch, G. Kölsch
Plateumaris	*nitida* (Germar, 1811)	184	near Taylor Lake, Parc Gatineau, QC	Canada	M. Bartsch, G. Kölsch
Plateumaris	*nitida* (Germar, 1811)	185	115 crossing the Middle Branch River, MI	USA	M. Bartsch, G. Kölsch
Plateumaris	*pusilla* (Say, 1827)	186	S Esquagama Lake, County Rd. 525, MN	USA	M. Bartsch, G. Kölsch
Plateumaris	*pusilla* (Say, 1827)	187	small river SW Richmond, ON	Canada	M. Bartsch, G. Kölsch
Plateumaris	*rustica* (Kunze, 1818)	122–129	Lebrade, Schleswig–Holstein	Germany	G. Kölsch
Plateumaris	*sericea* (L., 1758)	218	Duvenstedt, Schleswig–Holstein	Germany	R. Suikat

Table 1 (*continued*)

Genus	Species	ID-#	Site	Country	Collectors
Plateumaris	*sericea* (L., 1758)	140–143	Fockbek	Germany	Th. Behrends
Plateumaris	*sericea* (L., 1758)	155	Royal Canal, Coolnahay	Ireland	G. Kölsch, E. Meichßner
Plateumaris	*sericea* (L., 1758)	156	Lake Windermere	England	G. Kölsch, E. Meichßner
Plateumaris	*shoemakeri* (Schaeffer, 1925)	188	shallow lake 3 km W Whitefish, S Hwy 55, ON	Canada	M. Bartsch, G. Kölsch
Plateumaris	*shoemakeri* (Schaeffer, 1925)	189	Tay River, Perth, ON	Canada	M. Bartsch, G. Kölsch

included for the different datasets (descriptive statistics calculated in MEGA 3.1, Kumar et al., 2004: Table 2). The data for COI and EF-1α were analysed separately and together (total evidence), with and without third codon positions and the EF-1α-intron. Phylogenetic analyses were carried out in PAUP* 4.0b10 (Swofford, 1998) (maximum parsimony, maximum likelihood) and MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) with settings as follows (default settings were used for all options not mentioned). Maximum parsimony (MP): Heuristic search using the TBR algorithm, random addition of sequences with 1000 replicates, gaps treated as fifth character state, accelerated transformation of character states (ACCTRAN), number of trees in memory ('maxtrees') automatically increased; bootstrap analyses using fast stepwise addition with 1000 replicates. —Maximum likelihood (ML): substitution model GTR+I+G (selected in Modeltest 3.7, Posada and Crandall, 1998, for all separate and combined data sets by the likelihood ratio test and in most cases also the AIC), with base frequencies, shape parameter and proportion of invariable sites user defined (Modeltest); heuristic search using the TBR algorithm, random addition of taxa with ten replicates. —MrBayes: substitution model as for maximum likelihood (but without specification of base frequencies and the shape parameter of the gamma-distribution, as recommended by Ronquist et al., 2005), 3,000,000 generations, samplefreq = 100, burnin = 7500 samples. Standard procedures were used to ensure a sufficient number of generations and length of the burnin (graphical presentation of the log likelihood values using the sump-command, PSRF convergence diagnostic). Five independent analyses at each of the following temperatures were run to ensure consistency of the results: 0.05, 0.1, 0.2 (default), 0.3, 0.5. For the combined analysis of both genes a mixed model was used by unlinking the parameters across the partitions. In any case, individual parameters were allowed for each of the three codon positions and the EF-1α intron.

Two curculionid beetles and one chrysomelid were chosen as outgroup species: *Diocalandra frumenti* (GenBank accession no. COI: AY131104, EF-1α: AY131133), *Hylocurus femineus* (COI: AF187108, EF-1α: AF186678) and *Crioceris duodecimpunctata* (COI: Dobler and Krobbach unpublished, EU880722EF-1α: this study).

The homogeneity of the rates of molecular evolution in the different lineages was tested in a relative rate test by using the software GRate (Müller, 2002). The taxonomic units defined for pair wise comparisons were the five groups described in the Results section. Additional units were the isolated taxa (*D. hirticollis* + *D. tomentosa*), *D. sparganii*, *D. caerulea* and *Donaciasta goeckei*. The Haemoniini were split into the genera *Neohaemonia* and *Macroplea*. Likelihood parameters were estimated from a neighbor joining tree under the GTR + I + G model.

Partitioned Bremer support (PBS) calculated in TreeRot 3 (Sorensen and Franzosa, 2007) for the maximum parsimony tree was used to assess the congruence between the phylogenetic reconstructions based on different data partitions (two partitions equivalent to the two genes). A negative and a positive PBS for a node stemming from the two partitions can be taken as a sign of conflict (Lambkin et al., 2002). For the same purpose, the partition homogeneity test implemented in PAUP* (incongruence length difference test, ILD-test) was used with 1000 random addition sequence replicates.

Phylogenetic hypotheses were tested using the Shimodaira-Hasegawa-test (SH-test) in PAUP* with the RELL approximation and 1000 replicates.

2.3. Estimation of divergence times

Divergence times were calculated by using two software products: In BEAST 1.4.7 (Drummond and Rambaut, 2007), monophyletic groups corresponding to the clades resulting from the phylogenetic analyses were predefined (using the accessory software BEAUti 1.4.7). Analyses without topological constraints gave inacceptable results. Substitution rates for the two partitions (genes) and the three codon positions were unlinked to be estimated independently. A 'GTR + gamma + invariant sites' model of sequence evolution was assumed. The 'relaxed clock' rate variation model (Drummond et al., 2006) was used with lognormal distribution of rates and Yule tree priors. The MCMC chain was 20,000,000 iterations long and parameters were sampled every 1000th generation. In each run, the age of one taxon was fixed (see below) with a normal distribution of the prior probability (standard deviation = 1). All scenarios were run two or three times to assess variation in the results. From triplicate runs the arithmetic mean and standard deviation was calculated (Table 4, columns A and E). The logged trees were summarized using TreeAnnotator 1.4.7 and displayed in FigTree 1.1.2.

For comparison, the software r8s 1.70 (Sanderson, 2003) was used. Calculations were made for the complete data set using the method of nonparametric rate smoothing with the Powell algorithm. They were based on trees stemming from maximum likelihood and Bayesian phylogenetic reconstruction. Several approaches were followed for an absolute calibration of the phylogeny:

Table 2
Descriptive statistics for the DNA sequences analysed, calculated with pair wise deletion (in brackets: complete deletion) in case of missing data

	No. of taxa	Sequence length	Variable sites:		Informative sites:		Ratio transitions/transversions	Average *p*-distance	% A	% C	% G	% T	% A+T
			N	Proportion (in % of total)	*N*	Proportion (in % of total)							
COI	45	830	406	48.9	329	39.6	0.97 (1.20)	0.147	32.1	15.70	13.7	38.5	70.6
EF1a—Exon	43	896	283	31.6	183	20.4	1.82 (2.12)	0.074	30	19.1	23	27.9	57.9
EF1a—Intron	43	236	165	69.9	146	61.9	0.88 (/)	0.263	27.8	12	13.4	46.8	74.6
EF1a—total	43	1132	448	39.6	329	29.1	1.51 (2.12)	0.090	29.6	18.1	21.7	30.1	59.7
COI + EF1a	45	1962	854	43.5	658	33.5	1.08 (1.20)	0.121	30.8	17	17.9	34.3	65.1

2.3.1. Fossil calibration

The oldest fossils of Donaciinae stem from the Tertiary North America (Askevold, 1990a). *Donacia wightoni* Askevold, 1990 was recovered from a formation approximately 58 million years (Mya) old. It resembles extant species of the water lilies-group we define below (Askevold, 1990a). Therefore, the age of 58 Mya was assigned to the split of a group we term 'modern Donacia', which gives rise to the stem form of the water lilies-group. Since it cannot be ruled out that this morphological type represents a more ancestral state of the genus *Donacia*, in a second scenario 58 Mya were assigned to the basal split of the genus *Donacia*. This leads to a more conservative estimate of divergence times. The second fossil treated by Askevold (1990a) is the 30 Mya old *Plateumaris primaeva* (Wickham, 1912), which resembles *Plateumaris nitida* strikingly. We use this fossil to evaluate the estimations obtained by other calibrations. (Calculation of divergence times assuming 30 Mya as the age of the radiation in *Plateumaris* led to unrealistically high divergence times for the basal branches of the tree, due to the general problem with considerable extrapolations into the past.)

2.3.2. Appearance contemporarily with host plants

One explanation for the diversity of phytophagous beetles is the adaptation to specific host plants, which may have occurred soon after the appearance of the hosts. The lineages leading to the modern host plants of Donaciinae have existed since approximately 100 Mya before present (BP): The in evolutionary terms old angiosperms Nymphaeaceae (Doyle, 1998) are 120 Mya old (Magallon and Sanderson 2001). The Poales evolved in wet habitats (Linder and Rudall, 2005) with the relevant lineages appearing 90–100 Mya BP (Bremer, 2002): Typhaceae, Sparganiaceae, Cyperaceae, Juncaceae. Therefore, in the second set of calculations an age of 100 Mya is assumed for the basal split of the Donaciinae.

2.3.3. Calibration based on Gómez-Zurita et al. (2007)

This publication dates the origin of Chrysomelidae to 73–79 Mya BP and that of the Donaciinae to about 60 Mya (taken from their Fig. 2). The latter date was used in another set of calculations for the basal split of the Donaciinae.

An extra approach for the establishment of divergence times was the usage of a molecular clock. An evolutionary rate of 2.3% sequence divergence per one million years (Brower, 1994) was assumed for the COI data. Genetic distances were calculated in PAUP* (Swofford, 1998) under the GTR+I+G model of sequence evolution. The maximum distance between members of a given clade was used to calculate the age of the segregating node.

Host plant use was recorded during the collection of beetles and summarized from the literature (Deibel, 1911; Marx, 1957; Freude et al., 1966; Parry, 1979; Wilcox, 1979; Borowiec, 1984; Klausnitzer, 1984; Lopatin, 1984; Mohr, 1985; Askevold, 1987, 1988, 1991b; Koch, 1992; Downie and Arnett, 1996; Menzies and Cox, 1996; Nilsson, 1996; Foster, 2001; Bienkowski and Orlova-Bienkowskaja, 2003). Home ranges of the species were characterized as North American, European or Eurasian (i.e., with a distribution extending far east) based on data in Goecke (1960) and Borowiec (1984).

3. Results

3.1. General aspects

The COI sequence chosen for analysis is 830 base pairs long and covers the 3′-end of the gene (the last base included is the first one belonging to the leucine-tRNA, in which the primer sequence is located). The alignment was unambiguous, since no indels occurred. The EF-1α sequence yielded an alignment of 1132 base pairs in length, starting at position 2232 of the *Drosophila* F1-copy of the EF-1α gene (GenBank Accession No. X06869). It contains one intron of 236 base pairs, located between positions 583 and 820 of the alignment (corresponding to position 2808/2809 of the *Drosophila* sequence and to 753/754 as termed in the review by Djernæs and Damgaard (2006). The presence of the intron inferred from the high degree of variation could be verified by comparison with a sequence of mRNA obtained *via* RT-PCR for *Donacia crassipes* (not shown). The intron was never found in specimens of the genus *Plateumaris*. The EF-1α intron shows the highest proportion of variable and parsimony informative sites, followed by COI (Table 2). Excluding the outgroup from the data set decreases the overall degree of variation particularly in the EF-1α exon (not shown). This considerable contribution of the outgroup to the degree of variation in EF-1α is a hint at the power of this gene to resolve deeper phylogenetic nodes than COI (Hughes and Vogler, 2004; Jordal et al., 2004).

The AT-content of the COI sequence and the intron exceeds 70%, while the value for the EF-1α exon is 57.8%. This is in accordance with published data for the respective gene (Brady and Danforth, 2004; Jin et al., 2005; Kölsch et al., 2006, and references therein). The overall ratio of transitions over transversions is smaller in COI than in EF-1α. This reflects the lower variation in the latter, which brings about less transitional saturation.

3.2. Phylogenetic reconstruction

All individual sequences for a given species (as identified using morphological characters) clustered closely together (one exceptional case in the genus *Plateumaris* is discussed below). Since intraspecific variation was very low, individual sequences could be concatenated into species consensus sequences, which contained hardly any ambiguities. Phylogenetic analyses of individuals (not shown) and species yielded identical results.

All data partitions, combinations and alterations of the data sets yielded very similar results, irrespective of the analytical method employed. In particular, omission of third codon positions or the EF-1α intron did not alter the picture. The most severe problem was a limited resolution of the basal parts of the trees, especially in the COI data (Figs. 1 and 2). Three main groups traditionally identified are supported as monophyletic units: the genera *Donacia* and *Plateumaris* (Askevold, 1990b, erected the tribes Donaciini and Plateumarini) and the tribe Haemoniini Chen, 1941, with the latter comprising the genera *Neohaemonia* and *Macroplea*. Either the basis of the tree remains polytomous or *Plateumaris* is the sister group of (*Donacia*+Haemoniini). However, a monophyly of *Plateumaris* and *Donacia* is not significantly less likely (SH-test, $p > 0.05$), neither for the combined data nor the EF-1α data. The relationships within each of the three taxonomic units are more stable and clearer:

Genus *Donacia* (for the presentation of the results, we use only one genus name *Donacia sensu lato*; the system of subgenera, some of which were meanwhile risen to full genus status, is discussed below): *Donacia tomentosa* and *D. hirticollis* are grouped together at the root of *Donacia*. Sometimes this common ancestry is dissolved into polytomy (Fig. 1c) or the species pair assumes an ill-defined position (COI data). In any case, the two species are assigned a long history independent from each other.

There are five well-defined species group within *Donacia*, which occur in all reconstructions (Figs. 1 and 2):

(1) With the *clavipes*-group, four European/Eurasian species form—according to the EF-1α data and the combined data set—the sister group to all other *Donacia* (except for *D. hirti-*

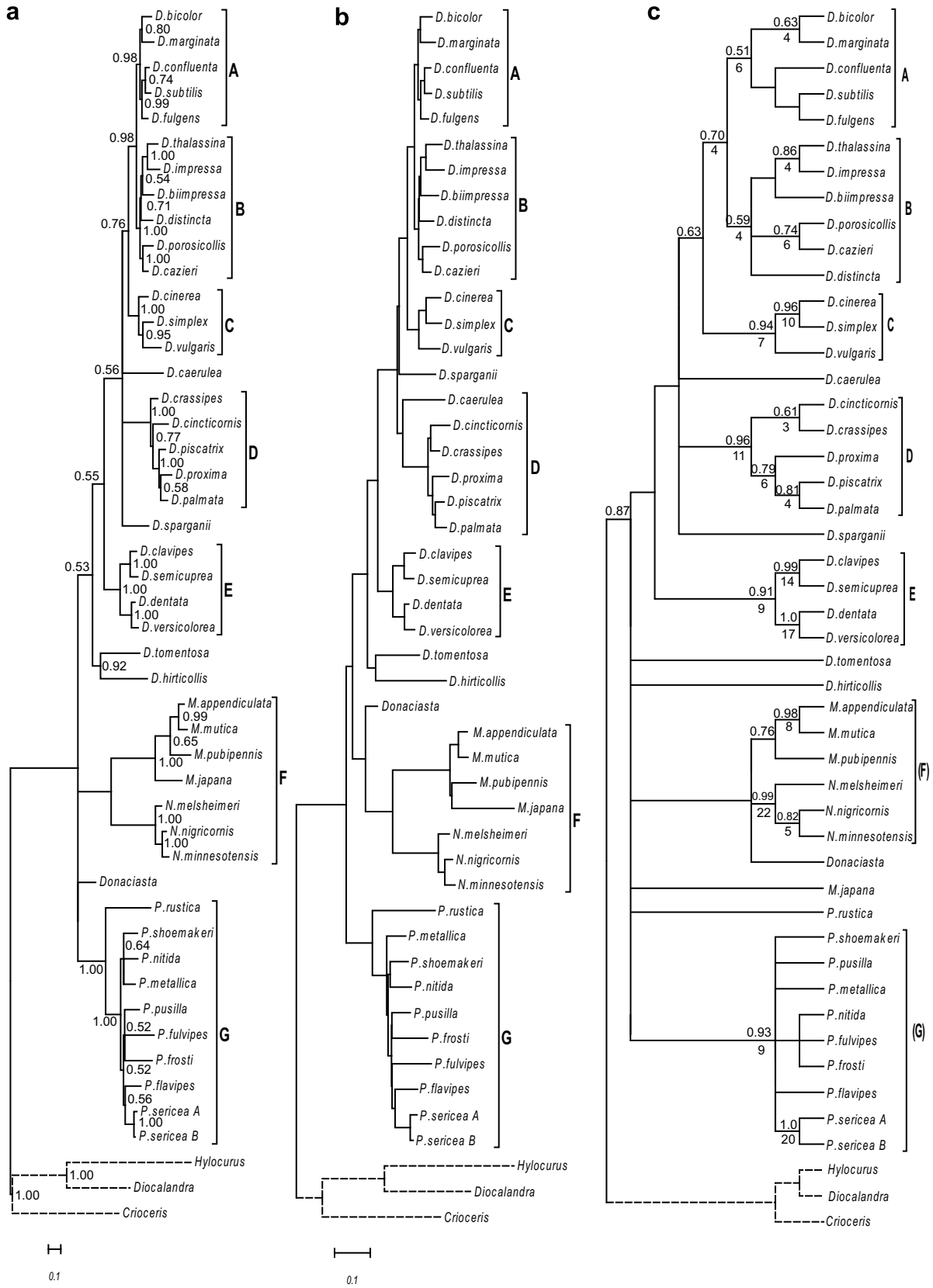

Fig. 1. Phylogenetic trees based on simultaneous analyses of both genes. (a) Phylogram based on the Bayesian approach; the posterior probabilities of nodes are shown; scale: no of substitutions per site. (b) Phylogram based on maximum likelihood (−ln likelihood = 20449.89392); scale: no of substitutions per site. (c) Cladogram based on maximum parsimony (strict consensus of 16 equally parsimonious trees, 4042 steps, consistency index = 0.2933, retention index = 0.4978); bootstrap values (1000 replicates) are shown above the branches, Bremer support values (if greater than 2) below the branches. Seven major groups (A–G; see Fig. 2) further discussed in the text are identified.

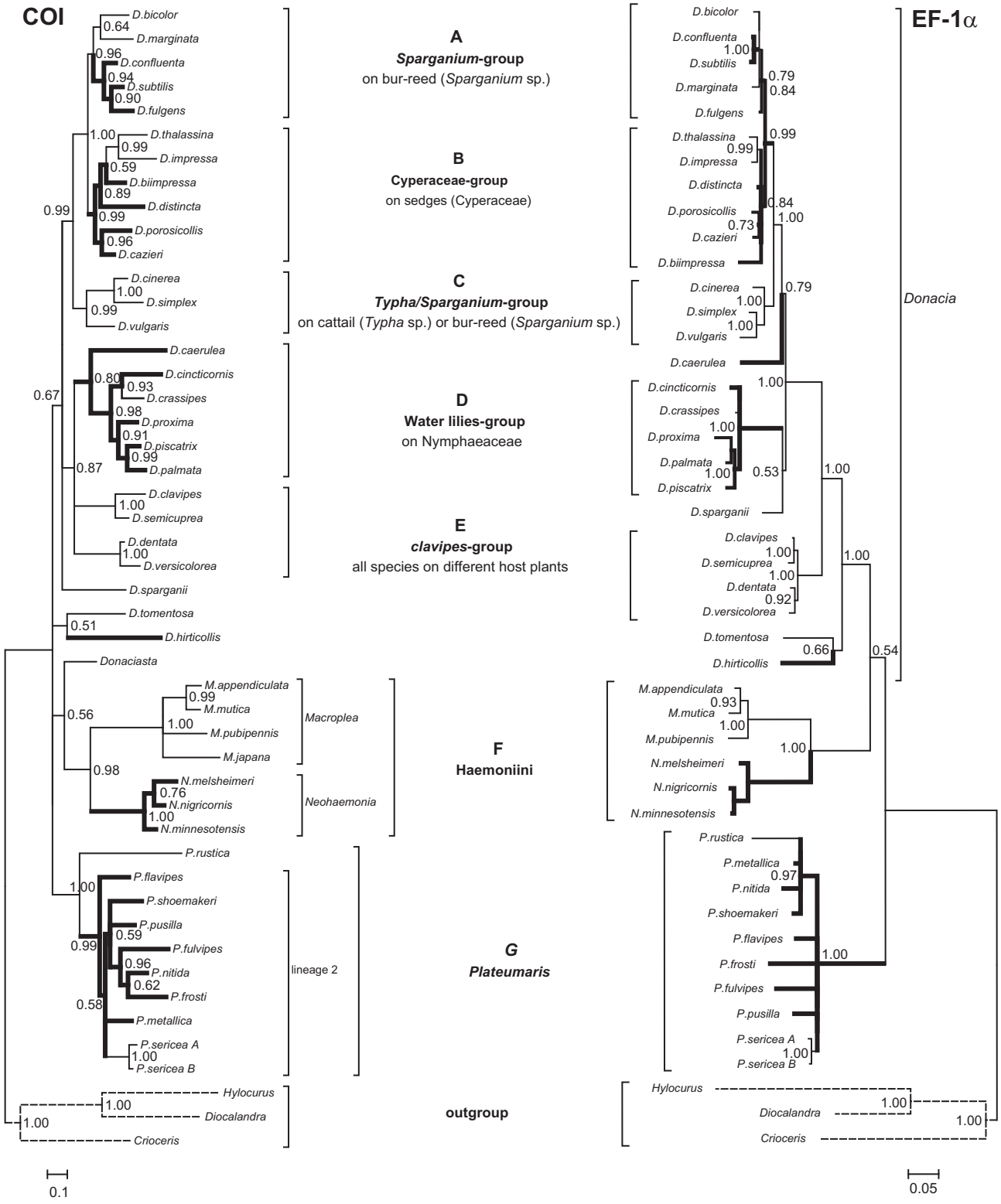

Fig. 2. Phylogenetic trees based on Bayesian analyses; left panel, data for COI only; right panel, data for EF-1α only. The posterior probabilities of nodes are shown; scale: no of substitutions per site. Branches of Nearctic taxa are printed in bold. Seven major groups (A–G) further discussed in the text are identified, and host plant associations are given for the species groups within the genus *Donacia* (groups A–E).

collis and *D. tomentosa*). The COI data point at a closer relationship with the water lilies-group (see below). Within this group, the two species pairs *clavipes* + *semicuprea* and *versicolorea* + *dentata* are well-supported.

(2) Water lilies-group. Five species with similar habits and morphology occur on water lilies and other floating leaf plants. They are very active in sunny weather, have elongate hind legs with clavate femora and prominent femoral teeth. This

well-defined group comprises one Palaearctic species (D. crassipes), which is either sister species to its Nearctic relatives or clusters together with *D. cincticornis* (Fig. 1b and 2).

(3) *Typha*/*Sparganium*-group: Three European species form a sister group to the two remaining groups. *D. cinerea*, *D. vulgaris* and *D. simplex* occur on *Typha* sp. and *Sparganium* sp.

(4) *Sparganium*-group: Two European and three American species live on *Sparganium* sp. The three American representatives are morphologically extremely similar and identification of members of this "*subtilis*-group" relies on male genitalia (Askevold, 1987).

(5) Cyperaceae-group: In this cluster of six species, the two European ones are more closely related to each other than to the American group members. Ecologically, we deal with beetles occurring on various genera of Cyperaceae (sedges; genus *Carex* and others) or occasionally Juncaceae (rushes).

Both *D. caerulea* and *D. sparganii* take isolated positions, which vary slightly between methods of reconstruction, in proximity to the *clavipes*-group and the water lilies-group.

With the water lilies-, the *Sparganium*- and the Cyperaceae-groups there are three indisputable units in the phylogeny of *Donacia sensu lato*, which each contain ecologically similar species from both Europe and America (Fig. 2).

Tribus Haemoniini: The two genera *Neohaemonia* and *Macroplea* Samouelle, 1819 are each monophyletic. An affiliation of *Donaciasta goeckei* to the Haemoniini is indicated (Bayesian analysis of COI, maximum likelihood analysis of COI and total evidence), but remains uncertain probably because of the lack of data for EF-1α. In the Bayesian analyses of the combined data set, this species is positioned at varying places within the genus *Donacia*. However, this affiliation is not significantly more or less likely than the one with Haemoniini (SH-test, $p > 0.05$). *Neohaemonia nigricornis* and *N. minnesotensis* are the sister group to *N. melsheimeri*. *Macroplea japana* branches off early within its genus, *M. pubipennis* is intermediate, and *M. mutica* and *M. appendiculata* are sister species (see Kölsch et al., 2006).

In the genus *Plateumaris*, there is a deep split between what Askevold (1991b) termed lineages 1 and 2. *Plateumaris rustica* is our only representative of the lineage 1. The associations within lineage 2 are not consistent. For *P. sericea*, two different consensus sequences were included, which stem from specimens with divergent male genital morphology. This will be dealt with in a separate account in the light of the discussion about a split of *P.sericea* into two species; *P. sericea* and *P. discolor*, (Kölsch in preparation).

The first approach to potential conflict between data partitions is a detailed analysis of the partitioned Bremer support (PBS; Table 3). For nodes with contradicting results (positive and negative value paired), the most parsimonious trees of both partitions were consulted again: In seven cases the nominally negative PBS occurred in spite of the fact that the branch in question occurred in the tree. Seven apparent conflicts were cases where one data partition yielded only a polytomy. The only two hard conflicts concerned the relationships within trios of species. The ILD-test formally indicates significant differences between the phylogenetic signals in the COI- and EF-1α data sets ($p \leqslant 0.01$).

For 41% of the pair wise comparisons between major lineages within the Donaciinae the mutation rates were significantly different according to the relative rate test conducted with GRate. *Donaciasta goeckei* deviated from almost all other rates. This is interpreted as an artefact due to a lack of data for EF-1α for this taxon. Likewise, the relative rate test routinely requested in r8s rejected the null hypothesis of equal rates in all lineages. As a consequence, for the estimation of divergence times methods not relying on a molecular clock had to be employed.

Table 3
Partitioned Bremer support for the maximum parsimony tree based on the complete data set (cf. Fig. 1c)

Members of clade	PBS:COI	PBS:EF-1a	BS	
Donaciinae	3.4	−1.4	2	a
Donacia (sensu lato)	0.9	0.1	1	
Plateumaris lineage 2	9.4	−0.4	9	a
P.frosti, P.nitida, P.fulvipes	5.4	−4.4	1	b
P.sericea A/B	19.4	0.6	20	
clavipes-group	−2.9	11.9	9	b
D.clavipes, D.semicuprea	8.4	5.7	14	
D.versicolorea, D.dentata	16.4	0.6	17	
modern' *Donacia*	0.9	0.1	1	
Water lilies-group	13.4	−2.4	11	a
D.piscatrix, D.palmata, D.proxima	3.9	2.2	6	
palmata-group	4.8	−0.8	4	c
D.crassipes, D.cincticornis	3.9	−0.9	3	b
Typha+Sparganium stem clade	1.4	−0.4	1	a
Typha/Sparganium-group	11.4	−4.4	7	a
D.cinerea, D.simplex	15.1	−5.1	10	c
(*Sparganium*-group + Cyperaceae-group)	6.4	−2.4	4	b
Sparganium-group	3.4	2.6	6	
Sparganium-group, Europe	4.7	−0.7	4	b
subtilis-group	1.4	0.6	2	
D.fulgens, D.subtilis	0.8	1.2	2	
Cyperaceae-group	0.4	3.6	4	
D.thalassina, D.impressa, D.biimpressa	−1.6	2.6	1	b
D.thalassina, D.impressa	3.4	0.6	4	
D.porosicollis, D.cazieri	5.3	0.7	6	
Haemoniini + *Donaciasta*	3.4	−2.4	1	a
M.appendiculata, M.mutica, M.pubipennis	2.4	−1.4	1	a
M.appendiculata, M.mutica	8.8	−0.8	8	a
N.nigricornis, N.minnesotensis, N.melsheimeri	14.9	7.1	22	
N.nigricornis, N.minnesotensis	−0.7	5.7	5	b

a, node in fact exists in phylogenetic trees resulting from *both* separate analyses
b, polytomy occurs in phylogeny from one data partition
c, true conflict.

3.3. Divergence times

The divergence times obtained by using different data partitions yield that the more recent splits are dated to considerably younger ages when EF-1α-data are used instead of COI-data (not shown). The complete data set usually yields intermediate values. Due to this 'smoothing' effect, and because it would be impossible to decide which partition gave the most realistic results, only the total evidence calculations are further considered. Generally, the estimations based on Bayesian inference of the phylogeny yield lower divergence times than maximum likelihood (Table 4, columns C/D, G/H; only the branch leading to the Haemoniini deviates from this rule). Results from analyses in BEAST and r8s are comparable (columns B:C/D, F:G/H). Any further treatment of the divergence times is postponed to the Discussion, because description and evaluation of the results can hardly be separated.

4. Discussion

4.1. Methodical aspects

In spite of the obvious agreement between the phylogenetic reconstructions based on the different data partitions (including the total evidence approach), the formal indications of conflict have to be discussed. Both the calculation of the partitioned Bremer support in TreeRot and the ILD-test were based on the strict consensus tree of 16 equally parsimonious trees, which contained several polytomies and inconsistencies not shared by other trees (including the respective trees for the separate data partitions). This may have contributed to the overall picture of incongruence. Similar settings may have contributed to critical evaluation of both methods in the past (Lambkin et al., 2002; Goldman et al., 2000;

Table 4
Divergence times (ages of selected nodes of the phylogenetic tree, in million years before present) calculated using the programs BEAST and r8s

	Assumption:	*Donacia* = 58 Mya	Donaciinae = 75 Mya			'Modern' *Donacia* = 58 Mya	Donaciinae = 100 Mya			Molecular clock: COI
	Software used: Method of phylogenetic reconstruction (r8s only):	BEAST mean±SD	BEAST	r8s ML	r8s Bayes	BEAST mean±SD	BEAST	r8s ML	r8s Bayes	model: GTR+I+G
Group split ...:	... Giving rise to:	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Donaciinae	Haemoniini, *Donacia*, *Plateumaris*	75.7 ± 3.3	**76.1**	**75**	**75**	155.4 ± 9.3	**101.3**	**100**	**100**	53.8
Haemoniini	*Macroplea, Neohaemonia*	45.1 ± 4.7	39.9	45.9	49.8	93.0 ± 14.9	60.0	61.2	66.3	25.3
Macroplea	*Macroplea* spp.	25.7 ± 2.0	16.9	19.0	21.1	51.9 ± 6.7	35.9	25.4	28.2	12.5
Macroplea Europe	*M. mutica, M. appendiculata*	7.9 ± 1.4	3.8	11.6	5.2	17.6 ± 3.1	9.1	15.5	6.9	3.5
Neohaemonia	*Neohaemonia* spp.	14.2 ± 2.1	7.3	11.0	9.8	20.1 ± 1.8	13.4	14.7	13.0	5.0
N. nigr./N. minn.	*N. nigricornis, N. minnesotensis*	7.4 ± 1.3	7.3	6.3	4.3	10.8 ± 2.7	5.5	8.4	5.8	0.2
Donacia	*clavipes*-group and others, *D. tomentosa* and *D. hirticollis* excluded	**57.1 ± 1.9**	57.9	59.3	62.3	102.5 ± 16.9	67.2	79.1	83.1	32.0
clavipes-group	2 species pairs	18.2 ± 2.9	23.5	32.4	29.7	40.8 ± 6.5	22.2	43.2	39.6	12.2
species pair 1	*D. versicolorea, D. dentata*	8.0 ± 2.9	7.7	13.6	9.7	14.5 ± 8.9	7.3	21.2	13.0	3.2
species pair 2	*D. semicuprea, D. clavipes*	7.5 ± 1.1	7.5	15.9	12.6	17.4 ± 5.3	6.0	18.2	16.8	6.0
'modern' *Donacia*	Water lilies-group and others	33.8 ± 4.0	36.2	36.3	35.9	**58.7 ± 0.3**	40.7	48.4	47.9	19.7
Cyperaceae-group		15.7 ± 2.7	11.5	17.1	13.5	26.0 ± 4.0	18.1	22.8	18.0	11.9
	Cyperaceae-group Europe	14.1 ± 1.3	10.1	15.2	11.1	23.5 ± 4.1	15.8	20.3	10.2	11.9
Cyperaceae-group Europe	*D. thalassina, D. impressa*	11.0 ± 1.1	9.0	11.5	7.6	15.7 ± 4.7	13.7	15.3	10.2	10.1
Sparganium-group		12.5 ± 1.7	11.1	17.0	13.1	20.4 ± 1.8	15.8	22.7	17.4	7.8
Sparganium-group Europe	*D. marginata, D. bicolor*	9.8 ± 2.0	10.7	14.4	11.0	18.3 ± 2.8	13.0	19.2	14.6	6.3
subtilis-group		7.8 ± 1.3	7.0	13.5	10.4	12.4 ± 5.9	8.9	18.0	13.8	4.5
	D. confluenta, D. subtilis	4.7 ± 1.1	5.0	8.5	5.8	6.8 ± 2.3	6.8	11.3	7.7	3.1
Typha/Sparganium-group		16.4 ± 2.8	10.4	18.0	14.4	25.1 ± 4.1	14.9	24.0	19.2	9.7
Water lilies-group		16.5 ± 3.5	14.8	14.2	12.5	29.4 ± 4.5	16.5	18.9	16.6	9.6
	D. crassipes/other group members	16.5 ± 3.5	14.8	12	12.5	29.4 ± 4.5	16.5	16	16.6	5.9
palmata species group	*D. piscatrix, D. palmata*	6.6 ± 1.9	4.9	8.6	5.2	12.7 ± 4.2	7.9	11.5	6.9	4.3
Plateumaris	*Plateumaris* lineages 1 and 2	47.4 ± 9.3	36.1	52.7	47.3	83.0 ± 12.9	68.0	70.3	63.0	46.6
Plateumaris lineage 2	*Plateumaris* spp.	19.2 ± 1.9	20.7	36.6	29.4	44.7 ± 5.6	30	48.8	39.1	18.2
P. sericea	*P. sericea* A and B	3.0 ± 0.7	3.0	7.1	6.2	6.6 ± 3.3	3.8	9.5	8.3	1.8

In the scenarios presented, different taxa were assigned a fixed age (printed in bold). Columns A and E give the arithmetic mean and standard deviation of three independent analyses. Calculations in r8s (columns C, D, G, H) were based on two different phylogenetic trees (Bayesian and maximum likelihood, ML, see Fig. 1a and b). Shaded lines represent splits that lead to separate Nearctic and Palaearctic lineages within species groups of the genus *Donacia*.

Barker and Lutzoni, 2002; Darlu and Lecointre, 2002; Sota et al., 2005). As corroborated by the graphical display (Fig. 1 and 2), all conflicts can be traced to either methodical artefacts or lack of resolution, or they represent minor rearrangements of terminal taxa within the species groups. The partitioned Bremer support shows that all relevant nodes are supported by both partitions within the combined data set. Missing data were a problem particularly with specimens that originally had not been preserved for molecular studies (*M. japana*, *Donaciasta goeckei*). In all other cases at least substantial parts of the sequences required were obtained. We decided to use all available data in the analysis, following the rationale of Hughes and Vogler (2004).

Analysis of elongation factor-1α always requires particular alertness with respect to multiple copies (Danforth and Ji, 1998; Hedin and Maddison, 2001; Jordal, 2002; Djernæs and Damgaard, 2006). The lack of an intron in all sequences for *Plateumaris* might have hinted at an amplification of a second, no-intron copy in this genus. However, since we never obtained the longer copy (containing the intron), we assumed that this was not the case (Hughes and Vogler, 2004 obtained both copies in a similar case, thus ensuring two paralogs). Generally, intron loss is not too uncommon (Jordal, 2002; Wada et al., 2002; Jin et al., 2005), with intron gain occurring merely three times more often than a loss (Brady and Danforth, 2004). We preliminarily handle this as a case of intron loss, although it requires closer examination. The relevant question is, if a potential inclusion of a paralogous sequence would affect the conclusions of the present work. We do not think so: There are indications that both copies are functional, which means that none is free to mutate at an excessive rate (Jordal, 2002). The inclusion of a different gene copy for *Plateumaris* would have positioned this genus far away from the other ones in the tree, because those additional copies are phylogenetically old and would have accumulated mutations. In contrast to this prediction, the genus *Plateumaris* is not isolated on the tree to a particular extent in the separate analysis of the EF-1α data (Fig. 2). In a similar case (Cruickshank et al., 2001) the authors likewise proceeded with their analysis.

4.2. General phylogeny

The monophyletic origin of Donaciinae cannot be challenged by our data. Although some phylogenetic reconstructions show a basal polytomy, this is merely interpreted as a lack of resolution. Donaciinae are too well defined by their morphology and biology (e.g., by the unique aquatic larvae). Our data do not shed light on the early evolution of reed beetles. While Borowiec (1984) regards *Plateumaris* as derived, Chen (1941) and Askevold (1990a, 1991b) position that genus at the basis, as the sister group of all other Donaciinae. The molecular data support a derived status of *Donacia sensu lato*, but the Haemoniini are not more clearly allied to them than the Plateumarini. The controversy might reflect the real situation quite well: The key innovation of the Donaciinae, namely the evolution of aquatic larvae, enabled them to quickly colonize the hitherto underexploited adaptive zone (Miller and Crespi, 2003; Mitter et al., 1988) of semiaquatic habitats. Such a rapid radiation on newly colonised hosts is not unusual (Strong et al., 1984) and did not leave behind in the genomes the footprints of a gradual evolution with ideally dichotomous speciation (cf. Shavit et al., 2007; Whitfield and Kjer, 2008). An explicit outgroup position of *Neohaemonia* (Sota et al., 2008) should be treated with care in future analyses.

4.3. Species groups in the genus Donacia

Using the name *Donacia sensu lato* as an inclusive term is justified by the fact that all relevant species form a monophyletic taxon. However, potential subdivisions have to be discussed. Based on our molecular analysis, the relevance of the genus *Donaciella* as defined by Askevold (1990b) (four species, three of which were included in our study) is challenged by the isolated position of its member *D. tomentosa*, which is grouped together with *D. hirticollis*. This might be a case of long branch attraction, but removal of *D. hirticollis* still leaves *D. tomentosa* in the position as before, and *vice versa* ("outgroup extraction" as reviewed by Bergsten (2005). Therefore, the affiliation of the two species and in particular a split of the genus *Donaciella* is supported. The latter had been erected to accommodate several species with dorsal pubescence. This does not seem to be a meaningful character (cf. Bienkowski and Orlova-Bienkowskaja, 2003), although the present affiliation of *D. tomentosa* and *D. hirticollis* again links two dorsally pubescent species.

The "*clavipes*-group" so far only has Eurasian members. Further subdivision results in two species pairs, which are morphologically dissimilar. *D. clavipes* and *D. semicuprea* both live on Poaceae (*Phragmites* and *Glyceria*, respectively) and they are strikingly similar with respect to their general habits (slow movements, hardly reacting to disturbance, reluctant to fly). The difference in body size (*D. clavipes* is one of the largest *Donacia*-species, *D. semicuprea* one of the smallest ones) seems plausible given the generally high intraspecific size variation of Donaciinae, which renders possible disruptive selection due to environmental factors. With the new classification proposed by us, *D. clavipes* has to be removed from the genus *Donaciella* as defined by Askevold (1990b) (cf. Bienkowski and Orlova-Bienkowskaja, 2003). *D. versicolorea* and *D. dentata* share a dark coloration with some iridescence. Their general morphological similarity is reflected by the fact that in identification keys they are separated only in the final step. They both utilize host plants unusual for *Donacia* (*Potamogeton natans* and *Sagittaria*, respectively).

Although we only included five out of fifteen species, there is no doubt that our "water lilies-group" is identical to what Askevold (1990b) classified as the subgenus *Donacia*. Our phylogenetic reconstruction supports the existence of a *palmata*-group (Askevold, 1988) by grouping *D. palmata* and *D. piscatrix* together.

Exactly the same affiliation of the three species as in our "*Typha*/*Sparganium*-group" was found by Bienkowski and Orlova-Bienkowskaja (2003) on the basis of adult and larval characters. According to their findings and our molecular data, *D. cinerea* is the second Eurasian species to be removed from the genus *Donaciella*. This results in a monotypic genus *Donaciella* (consisting only of *D. tomentosa*). *D. vulgaris* and *D. simplex* traditionally appear together in identification keys.

A more recent evolutionary step in *Donacia* is the formation of two sister groups with divergent host plant preference. One branch specialized on *Sparganium*, while the other one diversified on Cyperaceae (sedges). The restriction to *Sparganium* could be seen as a narrowing of the host spectrum compared to a common ancestor they share with the *Typha*/*Sparganium*-group. The *Sparganium*-group is divided into a European and an American part. The two European species are discernible, whereas the American species are very similar (with the females of *D. subtilis* and *D. confluenta* remaining unidentifiable, Askevold, 1987). Based on morphology, Askevold (1987) placed four European species in the *D. subtilis*-group or a sister group to it: *D. marginata*, *D. bicolor*, *D. thalassina* and *D. impressa*. For the two latter ones, the molecular data support the sister group option: *Donacia thalassina* and *D. impressa* form the European branch of the Cyperaceae-group, in which the American *D. cazieri* and *D. porosicollis* are firmly defined sister species.

The two species *incertae sedis* in our phylogenetic tree are *D. caerulea* and *D. sparganii*. At first sight it appears unusual that *D. sparganii* is not included in the *Sparganium*-group. However, this species is ecologically distinct. It colonizes lotic habitats, where it lives on floating *Sparganium* leaves, which reach the water surface rather late in the season.

4.4. The tribe Haemoniini

The scenario of a rapid initial radiation of reed beetles implies that even the highly specialised life style of the Haemoniini arose early in evolution: *Neohaemonia* lives in the floating leaves zone, deliberately entering the water, and the genus *Macroplea* lives under water without ever reaching the surface. Selection may have favoured an exploitation of the aquatic environment and the advantages associated with it: oviposition out of reach of insect parasitoids (many Donaciinae oviposit under water, where only few parasitoids can reach), stable environmental conditions, ample high quality food (plants with less structural elements). Pre-adaptations seen in other species could further develop as demanded by life under water. For example, the dense pubescence of the body became the plastron of *Macroplea*. The distal tarsomere became elongated to expose the prominent tarsal claws for attachment, while the tarsal adhesive pads—useless as they are under water—were reduced. This tendency is already visible in the genus *Donaciasta* (own obs.), which was positioned close to the Haemoniini also by Askevold (1990b).

Concerning the relationships of the five species within the genus *Neohaemonia*, Askevold (1988) hypothesized a common ancestor for *N. flohri*, *N. melsheimeri* and *N. nigricornis* and a sister group consisting of *N. flagellata* and *N. minnesotensis*. Our COI data support this classification, while the EF-1α data join *N. minnesotensis* and *N. nigricornis*, with *N. melsheimeri* as their sister taxon.

A more detailed account of the genus *Macroplea* is found in an earlier paper Kölsch et al. (2006), which provides new data on the distribution of the genus and molecular evidence for the species status of *M. japana* (cf. Hayashi and Shiyake, 2001).

4.5. Divergence times

We used different approaches to dating the major steps in the evolution of the Donaciinae (Table 4). In one scenario, we followed Gomez-Zurita et al. (2007), who postulated a relatively recent origin of chrysomelid beetles in general (73–79 Mya BP) and Donaciinae in particular (approximately 60 Mya). The ages of the two fossils Donaciinae mentioned above (Askevold, 1990a) are not compatible with this. The 58 million years old *Donacia wightoni* is characterised by Askevold as relatively derived, resembling members of the water lilies-group. However, the equivalent positions in the phylogenetic tree (the splits of *Donacia* in general or the 'modern' *Donacia*, i.e. *Donacia* excluding the *clavipes*-group) are estimated to be considerably younger than 50 (or even 40) million years in this scenario (not shown). This would be too young to appear in Palaeocene deposits. Furthermore, this figure raises conflict with an Eocene fossil resembling *Donacia crassipes* (discussed in Askevold, 1991b) and the modern-looking fossil *Plateumaris primaeva* (30 Mya, Askevold, 1990a). The molecular clock approach (column I, Table 4) does not alleviate this conflict but exacerbates it. The accumulation of 2.3% sequence divergence per one million years leaves all nodes even younger than under the assumption of 60 Mya BP for the basal split considered above, and the critical ages of the 'fossil-bearing' branches are not attained.

The limits of a realistic range for the age of the Donaciinae as a whole can be marked by the two scenarios that use the age of the fossil *D. wightoni* (58 Mya for the split of *Donacia* or the 'modern' *Donacia*, columns A and E, Table 4). In particular in the latter case it becomes obvious that by fixing the age of a recent node within the tree the extrapolation into the past leads to relatively old ages assigned to deeper nodes. In addition, the estimates are affected by greater uncertainty (standard deviation in columns A and E). To circumvent this problem, equivalent scenarios with fixed ages of the first split of the Donaciinae were searched. It turns out that the fossil-based approaches largely match calculations with the age of the first split of the Donaciinae fixed to 75 Mya BP (columns B–D) and to 100 Mya BP (columns F–H), respectively. The latter scenario is equivalent to the evolution of the Donaciinae contemporarily with their host plants since 100 Mya BP. To be more exact, with this assumption the fossilization of *D. wightoni* (58 Mya BP) occurred between the splits of the entire genus *Donacia* and the 'modern' *Donacia*, respectively. At the same time, the radiation of the *Plateumaris*-lineage two took place contemporarily with (column F) or slightly earlier than (columns G, H) the formation of the sediments that preserved the *P. primaeva* specimen (30 Mya BP), which is possible, since fossils give only a minimum age (c.f. Kergoat et al., 2005a).

To summarize: The first diversification of reed beetles leading to the major extant lineages took place between 75 and 100 Mya BP. This estimate is the best reconciliation of the fossil record with the molecular data that is possible at the moment. This is in agreement with the estimate by Sota et al. (2008) for the divergence of the genera *Plateumaris* and *Donacia* (72 Mya; 61–87 Mya). As outlined above, the radiation of Donaciinae most probably took place soon after the evolution of a stem group with aquatic larvae. Therefore, this time estimate is equivalent to dating the origin of the Donaciinae *per se*. The lower (=younger) limit of this interval coincides with the origin of the Chrysomelidae as a whole according to Gomez-Zurita et al. (2007). While our estimate would shift the origin of the Chrysomelidae back towards the Mid-Cretaceous, it does not challenge the main statement of the authors, namely that beetle radiation took place independently of (i.e. later than) host plant diversification. The Donaciinae colonised aquatic habitats with existing plant species and repeatedly switched to long-existing host plant lineages (see below).

4.6. Biogeographic aspects

For an analysis of Donaciine evolution and their present distribution (Goecke, 1960; Borowiec, 1984), five major geological phenomena are relevant (Strauch, 1970; Raven and Axelrod, 1974; McKenna, 1975, 1983; Eldholm and Thiede, 1980; Hamilton, 1983; Smith et al., 1994; Sanmartín et al., 2001; Fig. 3a and c):

(a) North America was longitudinally divided into a western and an eastern part until the end of the Cretaceous (65 Mya BP) by the Mid-continental Seaway (MCS).
(b) Western Europe and North America were connected *via* the so-called Thule Bridge (Greenland–Iceland–Faeroes; TB). Usually the end of this bridge is dated to between 50 and 35 Mya BP, which, however, will be discussed below.
(c) A northerly bridge across the North Atlantic existed between northern Scandinavia, Greenland and Canada (DeGeer Bridge, dGB). Generally more suited for cold adapted organisms, this connection ceased to exist by the end of the Eocene (38 Mya BP).
(d) Europe and Asia were long divided by an epicontinental sea east of the Ural, the Turgai Strait (TS), until 30 Mya BP.
(e) The land bridge between Asia and North America across the Bering Strait initially existed until the end of the Eocene (35 Mya BP), was re-established in the late Miocene (14–10 Mya BP) and flooded again in the Pliocene (probably 5 Mya BP or even earlier, Marincovich and Gladenkov, 1999).

One crucial point for the interpretation of the phylogeography of reed beetles is the exact timing of the break up of the North Atlantic bridges. Generally, the North Atlantic was more suitable for the dispersal of temperate zone organisms than the Bering Strait, because the latter was positioned at higher latitude than the DeGeer Bridge and than it is today (the North Pole was closer to it by 7° during mid-Eocene due to a shift in the Earth's rotational

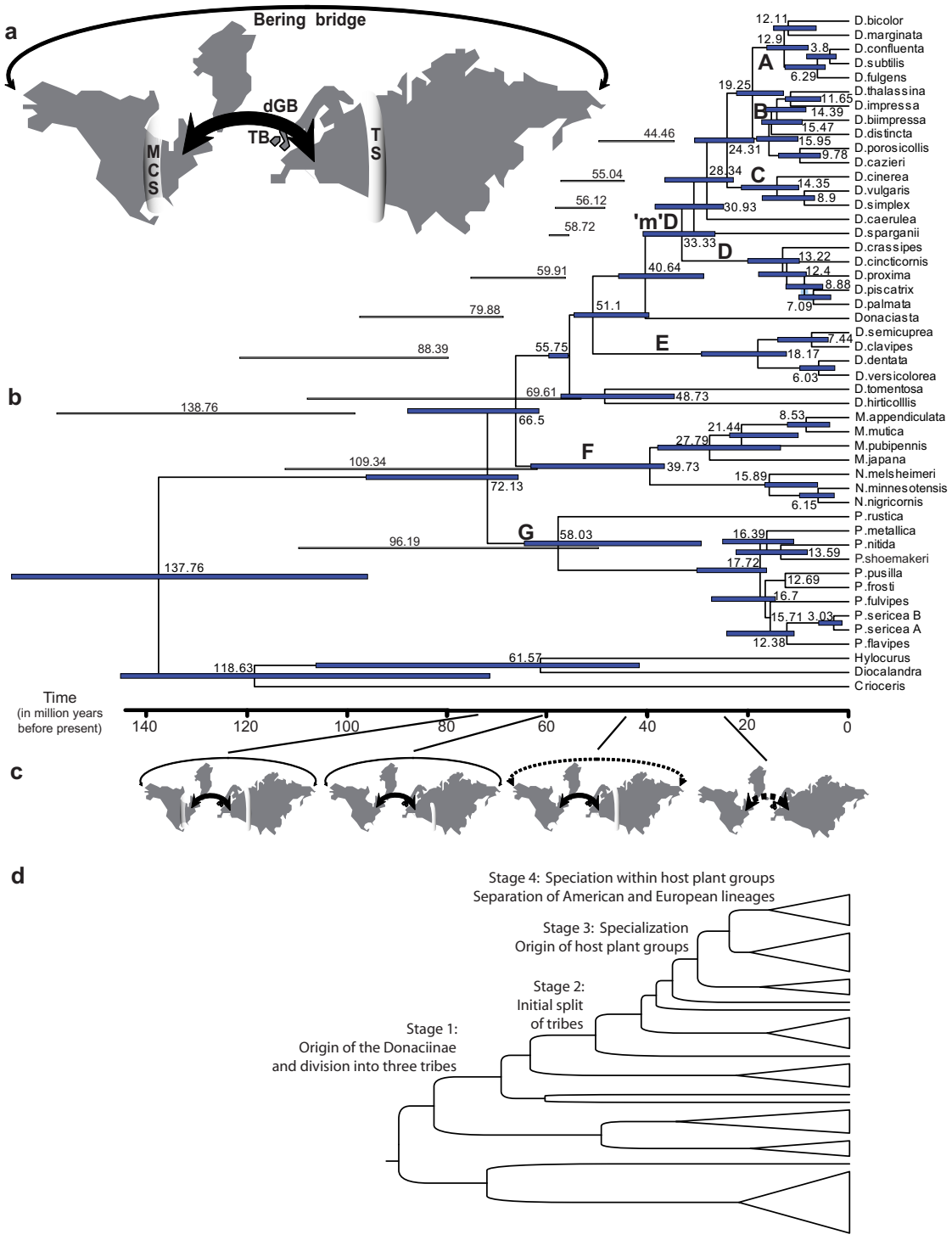

Fig. 3. The phylogeography of the Donaciinae: biogeographic background and divergence times. (a) Land masses of the Northern Hemisphere with land bridges connecting the continents and straits dividing them (dGB deGeer Bridge, TB Thule Bridge, MCS Mid-continental Seaway, TS Turgai Strait; straits and bridges did not necessarily exist at the same time). (b) Divergence times calculated in BEAST; horizontal bars in the tree give the 95% credible interval for the age of a node (given by the number next to the bar, in million years before present), when the age of the genus *Donacia* is fixed at 58 Mya. As long as the readability was not compromised, the results of an alternative scenario are given by horizontal lines at the same levels offset to the left (clade 'm'D = 'modern' *Donacia* assumed to be 58 Mya old). Data presented here are based on a single estimate and deviate from the values listed in Table 4 (= mean of three independent estimates). The letters A–G refer to the major groups as in Fig. 1 and 2. (c) Alterations in the separation and connection of the continents in geological times as relevant to the evolution of the Donaciinae, resulting from the presence/absence of the structures explained in a). To facilitate identification, the shape of the continents is depicted in their present day form (as in a). Hatched lines indicate diminishing or restricted interconnection (see text). (d) Schematic representation of the evolution of the Donaciinae, drawn to the time scale in the centre of the figure (the outgroup was removed from the tree). In this abstraction, all single steps are summarized into four major stages.

axis; Cox, 1974; Raven and Axelrod, 1974). The figure given above for the end of the Thule Bridge (50 Mya) can be found throughout the literature, but there are indications that animal dispersal *via* a North Atlantic bridges was possible until much later:

Strauch (1970) provides ample evidence of continued faunal exchange. Vogt (1972) is convinced that "there was essentially no open water before the Miocene". Concerning the Thule Bridge, Eldholm and Thiede (1980) state that "the main ridge platform

... did not sink below sea level before the Middle Miocene" and that the "highest areas of the ridge ... appear to have been submerged only in the Pliocene". Hamilton (1983) adopts this view by citing their paper. Matthews (1980) compares the Miocene conditions along the Thule Bridge to a "picket fence sinking into the water, with the last remaining islands (the picket points) disappearing in the late Miocene or early Pliocene time". McKenna (1983) states that "a continuous and habitable land connection ... may still have been intact above sea level as late as the Miocene". He postulates that "the Eocene climate of the areas where North America was connected to other northern land masses was generally warm but seasonal"—so obviously he considers a land bridge during the Eocene, which is considerably later than the 50 Mya BP in question. The climate on Earth was mild throughout the Oligocene, culminating in the Miocene climate optimum at around 16 Mya BP (Flower and Kennett, 1994), which would have favoured continued animal migrations in spite of the fact that the connection between Greenland and Canada was relatively far to the North. Enghoff (1995), referring to Smith et al. (1994), describes a "more or less substantial trans-Atlantic bridge ... from 70/60 to 20/12 mya".

To summarize, we consider a faunal exchange along a land bridge or a chain of islands possible until well into the Miocene (20–25 Mya BP). In addition to these geological arguments, active or passive long-distance dispersal in the absence of a land bridge is more likely to have occurred in Donaciinae than in other groups, leading to prolonged admixture of genetic material. Many species in this group are excellent flyers, and a combination of deliberately crossing water bodies and wind drift would enable them to cover considerable distances over water. Eggs, larvae or adults could raft on/in floating plant material. Finally, especially eggs are good examples of items that can be transported passively by migrating waterfowl (cf. Figuerola and Green, 2002; Green and Sanchez, 2006; Frisch et al., 2007).

4.7. The complete scenario

An integration of the evolutionary and geological timetables outlined above yields a general phylogeographic picture for the Donaciinae (Fig. 3): This beetle subfamily originated and soon thereafter formed three tribes in the Upper Cretaceous, i.e. at a time when the land masses of the northern hemisphere formed Asiamerica and Euramerica. The diversification of the major groups in the Palaeocene (55–65 Mya BP) coincided with a maximum interconnection of continents on the northern hemisphere: The Mid-continental Seaway in North America had retreated, and the Turgai Strait was temporarily passable (dry) at its northern and southern end (Smith et al., 1994; or a Turgai Bridge connected Asia to Fennoscandia in the early Eocene: McKenna, 1983). This diversification continued during the Eocene (36–54 Mya BP), initially with the Turgai Strait fully re-established (until 30 Mya BP), but intensified in the warm and seasonal climate during the Oligocene (25–35 Mya BP) and early Miocene (until approximately 20 Mya BP): On the one hand, speciation took place on host plants that had been used before (*Sparganium*, Nymphaeaceae in *Donacia*; Cyperaceae in *Plateumaris*; Haemoniini on Nymphaeaceae and submerged macrophytes), although the coarse picture of host plant use (genus level and above) might obscure specialization events. On the other hand, new host plants were colonised: *Donacia* radiated on the Cyperaceae, hitherto monopolized by the genus *Plateumaris*, and the long existing *clavipes*-group split up into four species on four different hosts. Most interestingly, the major clades (water lilies-group, *Sparganium*-group and Cyperaceae-group) each branched into a Nearctic and a Palearctic species assembly. It is worth emphasizing that a partition according to geographical origin took place that late, well within a final phase of ecological diversification coinciding with specialization. This can be regarded as a case of classical allopatric speciation due to large scale geographic isolation. The faunal exchange between North America and Europe must finally have ceased in the first half of the Miocene.

4.8. Early Donaciine evolution and the places of origin

This phylogeographic scenario still does not establish the location of the Donaciinae cradle. Nowadays the genera *Donacia* and *Plateumaris* have Holarctic distributions, which is not informative in this respect. Borowiec (1984) invokes a 'holarctic origin' in the centre of the northern continent, from where the present America was colonised. This would explain the present pattern of a predominantly north-eastern occurrence of reed beetles in North America.

For the genus *Donacia*, the pattern of distribution areas in the Palaearctic is peculiar: Western species generally have wide ranges, because they spread eastward after the Pleistocene (Borowiec, 1984), while Eastern Asian species remained more regionally distributed. This is in contrast to a generalised biogeographic pattern (Sanmartín et al., 2001: frequent range expansion of Eastern Palaearctic species, relative stasis in the Western Palaearctic; c. f. Ribera et al., 2004).

The biogeography of the Haemoniini and the African genus *Donaciasta* raises some problems (what follows refines the treatment by Kölsch et al., 2006). With *Macroplea* as a Eurasian genus and *Neohaemonia* being restricted to America, we apparently encounter a perfect case of vicariance. However, Medvedev (1977), fully aware of the problems arising, described a species of *Neohaemonia* from Mongolia. The type specimen is not available, but Askevold (1988) suggested the future erection of a genus of its own to accommodate this taxon. Apart from those nomenclatural problems, there seems to exist a species in Mongolia (Central Asia in general?) belonging to the Haemoniini, which might mirror the faunal affiliation between Eastern Asia and North America going back to the phase of Asiamerica (this would be the only sign of biogeographic affiliation between America and Asia for the Donaciinae, which is otherwise evident, especially from plants: Schmidt, 1946; Guo, 1999; Wen, 1999). The Haemoniini split up into *Neohaemonia* and *Macroplea* in the Palaeocene, when the ancient continents Asiamerica and Euramerica ceased to exist. Geographically isolated in North America, *Neohaemonia* retained morphological and biological characters of the (Asiamerican) ancestor.

For the genus *Macroplea*, ecological specialization can be the basis for its evolution from a *Neohaemonia*-like ancestor. The candidate key plant group for this step are the Potamogetonaceae. This family comprises species with floating leaves, present day hosts for *Neohaemonia*. Other representatives grow entirely submerged, being fed upon by *Macroplea* spp. Janssen and Bremer (2004) date the origin of the Potamogetonaceae to 47 Mya BP (split from Zosteraceae), but the division from the Ruppiaceae (also a host plant) occurred as early as 65 Mya BP. Hence, the potential host plants were available at the time proposed for the branching off of the genus *Macroplea*. The two early separated species, *M. japana* and *M. pubipennis* (Kölsch et al., 2006), are restricted to or occur predominantly in Asia. As the Turgai Strait recovered to completely divide Europe and Asia, geographic isolation may have promoted speciation 30–40 Mya BP (Table 4; origin of *M. mutica*?), and finally *M. appendiculata* arose in the West. The latter speciation event is dated to 8–15 Mya BP in the present paper. The dating by Kölsch et al. (2006) (2.5 Mya BP) was based on a molecular clock approach (3.5 Mya in the present study), and it should be mentioned that the calculation of divergence time based on the Bayesian tree yielded as little as 6.9 Mya BP (with the assumption of 100 Mya BP for the basal split of the Donaciinae). Later the western *Macroplea* species re-colonised Siberia and East Asia, much like

other Donaciinae (*M. mutica* was described from China, Kölsch et al., 2006; *M. appendiculata* was described from Lake Baikal, Dubeschko, 1973, but not from further east). This rapid range expansion is reflected by the occurrence of identical mitochondrial DNA haplotypes of *M. mutica* in Europe and China (Kölsch et al., 2006).

How does the genus *Donaciasta* fit into this picture? Borowiec (1984) regards this genus as most primitive, but Askevold (1990b) reconstructs a derived status together with the Haemoniini. Our molecular data also indicate an affiliation with the Haemoniini. Borowiec (1984) favours an African origin of *Donaciasta* (and hence all Donaciinae). This however, would lead to a problematic palaeo-biogeographic scenario: Origin of the Donaciinae at least 150 Mya BP, before the split between Laurasia and Gondwana; restriction to the African part of Gondwana in spite of prolonged connection with South America; 150 Mya of evolutionary stasis (*Donaciasta* is the only African genus), while in other parts of the world the reed beetles proliferated. Instead, attention should be paid to the allocation of the former *Donacia assama* to *Donaciasta* by Askevold (1990b). This species occurs in the East Indian province Assam, China and North Vietnam. A relatively recent (Miocene?) advent of *Donaciasta* from Asia when Africa approached this continent and before the aridification of North-eastern Africa is a plausible concept, particularly because low sea levels in the late Early Miocene "... allowed extensive faunal interchange between both Africa and Eurasia ..." (Janis, 1993). The geological basis for this was the "*Gomphotherium* Landbridge" emerging in the late early Miocene (Harzhauser et al., 2007). Presently the Indian subcontinent is largely devoid of Donaciine species, and the occurrence of *Donaciasta* in the east can be explained by relatively recent colonization.

The genus *Plateumaris* has hardly been mentioned so far in the discussion, because both old and new phylogenetic patterns are weak. We clearly follow Askevold (1991b) in his argument for full generic status: the group is monophyletic and well-differentiated from *Donacia* by molecular data (cf. Sota and Hayashi, 2007). It cannot be of recent origin (Pliocene; Borowiec, 1984), but constitutes an old taxon (the oldest one according to Askevold, 1991b and Chen, 1941). A subgeneric classification (e.g., *Juliusiana* + *Plateumaris*; cf. Freude et al., 1966) was abandoned by Askevold (1991b). He speaks of two lineages (one of which is represented only by the isolated species *P. rustica* in our analysis) consisting of species groups. As already stated by Askevold (1991b), the host plants do not justify an ecological characterization of species groups either, due to a lack of specificity and consistency. There are some parallels with the more comprehensive study by Sota et al. (2008) (e.g., the association of *P. nitida*, *P. frosti* and *P. fulvipes* we find in the mitochondrial data set), but species relationships are too inconsistent for a detailed discussion. Their analysis reveals that *Plateumaris* apparently differs from *Donacia* fundamentally in their phylogeography: The former genus has only few European or Eurasian species and the dispersal/vicariance-analysis yields a closer connection between Asia and America than for *Donacia* in the present study (relatively recent splits between American and European species). Inclusion of truly Asian members of the genus *Donacia* into an analysis is desirable to examine this more closely.

4.9. Generalists versus specialists

Almost all species included in the present study have to be categorized as oligophagous or even monophagous specialists (Fig. 2; exception: *D. hirticollis*).

The ancestral state of host plant affiliation in Donaciinae or *Donacia* cannot be reconstructed. All deep splits give rise to groups with divergent associations. This problem is closely linked to the question of the ancestral group within the subfamily. If it is *Plateumaris*, the affiliation with sedges seems probable. The same holds true for the question if the ancestor was a generalist or a specialist. The generalist *D. hirticollis* is assigned a rather isolated early origin within *Donacia*, but the strictly monophagous *D. tomentosa* (on *Butomus*) is immediately beside it. The wide spectrum of hosts found in the *clavipes*-group could be taken as a sign in favour of a generalist-first-situation, from which four different specialists evolved. The lack of generalists shows that a scenario, in which generalists develop from specialists (see Introduction), is not realized. This implies the next question: Was specialization a dead-end for Donaciinae? Clearly not: In the tribus Haemoniini, the initial restriction to plants with floating leaves was the springboard to submerged macrophytes. Another example of 'escape from the trap' comes from the *Donacia* species occurring on *Typha* and/or *Sparganium* (species groups A–C, Fig. 2). Their most likely ancestral host plant is *Sparganium*. In spite of that, a group of species diversified on Cyperaceae, a host plant family formerly monopolized by *Plateumaris*. Nowadays both genera occur syntopically on sedges.

Given that in other species groups there is no such obvious host shift, does that mean that those groups ran into the 'dead end', a situation where lack of adaptability leads to greater risk of extinction? The similarity of fossils to extant species (Askevold, 1990b) indicates a low extinction rate. And it is questionable if 'dead-end' is a correct term to describe the successful story of how the Donaciinae have colonised the reed beds of the temperate zones and beyond. Partitioning of sufficiently stable resources allows for persistence in terms of both evolutionary timescales and individual lifetime. In this respect the fringes of water bodies are exceptionally stable habitats: either the conditions are stabilised/reset on a regular basis (by flooding and erosion) or successional stages slowly advance, with a given habitat being gradually relocated. This predictable availability would have favoured specialization and thereby speciation (Bernays and Chapman, 1994).

4.10. Parallel evolution

In the general picture of the evolution of phytophagous insects, the rise of the flowering plants obviously contributed to beetle species richness (Farrell, 1998). But the fact that the diversification of seed plants and herbivores occurred contemporaneously in the Tertiary (Farrell, 1998) can not be taken as evidence of co-evolution *sensu stricto*. Lopez-Vaamonde et al. (2006) categorized phylogenies of hosts and their associated insects into three patterns (strict co-evolution, sequential speciation of host and herbivore and the "delayed colonization hypothesis" with an appreciable time lag between speciation processes).

In the Donaciinae, the dominant pattern is that species flocks formed on closely related plant species, which is congruent with the fast colonization hypothesis. In the literature, evidence of strict co-evolution is scarce (Strong et al., 1984; Termonia et al., 2001); examples can be found in Farrell and Mitter (1990, 1993). Most cases are similar to the situation in the Donaciinae (host fidelity on the level of genera or families, most probably sequential radiation; Futuyma and McCafferty, 1990; Miller and Wenzel, 1995; Silvain and Delobel, 1998; Swigonova and Kjer, 2004; Kergoat et al., 2005a; Auger-Rozenberg et al., 2006; Lopez-Vaamonde et al., 2006).

The shift from *Sparganium* to Cyperaceae mentioned above is not the only case where a new host plant taxon was occupied by the Donaciinae. Under water, *Macroplea* does not only feed on *Potamogeton* spp. and related species (*Ruppia*, larvae also on *Zostera*; for a reconstruction of their relationship see Janssen and Bremer, 2004), but also on the more distantly related genera *Myriophyllum* and *Ranunculus*. In this case, host plant colonization was not driven by biochemical similarity but by shared aquatic habitat: *Myriophyllum* contains considerable amounts of polyphenols, which inhibit insect growth (Choi et al., 2002). Since both *Myriophyllum* and

the Cyperaceae have existed long before they were used by *Macroplea* and *Donacia*, respectively, we deal with cases of delayed colonization *sensu* Lopez-Vaamonde et al. (2006). The same holds true for the occurrence of the water lilies-group on the Nymphaeaceae, which originated well before the Donaciinae (120 *vs.* <100 Mya BP).

4.11. Ecological specialization versus geographical isolation

As outlined in the introduction, ecological speciation represents a generally accepted concept of how species can arise. In the Donaciinae, ecological specialization on different host plants in different habitats was most likely involved in the speciation process both in the early phase of adaptive radiation as the Donaciinae split up into genera and later in the formation of the species groups.

The contribution of geographic isolation is apparent in the more recent speciation events, which led to the establishment of Nearctic and Palaearctic lineages in three larger species groups (Cyperaceae-group, water lilies-group, *Sparganium*-group; Fig. 2). The co-occurrence of clades from two continents in relatively terminal position on a phylogenetic tree is not unusual (e.g., Moran et al., 1999; Cognato and Sperling, 2000; Dobler and Müller, 2000; Hughes and Vogler, 2004; Martinez-Navarro et al., 2005; Sachet et al., 2006). Explicit statements concerning the timing of the split between North American and Eurasian clades centre around 35–50 Mya BP (Gomez-Zurita, 2004, *Timarcha*: 47 Mya; Ribera et al., 2004, *Agabus*: 39 Mya; Dumont et al., 2005, *Calopteryx*: 35 Mya, assuming a dispersal *via* the Bering Bridge; Kergoat et al., 2005a Bruchidae: 40–50 Mya). As most authors, we correlate this cladogenesis to the final separation of the continents in the northern hemisphere (20–25 Mya BP), which superimposed the effect of geographic isolation on a pre-existing pattern of ecological specialization.

Our study of the reed beetles demonstrates how an adaptive radiation following a key adaptation (the aquatic larvae) led to the complete colonisation of a new habitat with considerable ecological and phenotypic diversity (as predicted, Gavrilets and Vose, 2005) in the only seemingly homogenous habitat of wetlands. The picture revealed by our analysis goes far beyond the pattern sometimes implied in the past (Jolivet, 1988), according to which we simply deal with a polyphagous group the members of which feed on whatever they encounter in the habitat colonised. The species groups defined are a starting point for future efforts to establish a subgeneric classification particularly for the genus *Donacia*.

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References

Askevold, I.S., 1987. The identity of *Donacia* cuprea Kirby, 1837, and *Donacia quadricollis* Say, 1827, with a taxonomic revision of members of the *Donacia subtilis* Kunze-group (Coleoptera: Chrysomelidae: Donaciinae). Can. Entomol. 119, 629–645.

Askevold, I.S., 1988. The genus *Neohaemonia* Székessy in North America (Coleoptera: Chrysomelidae: Donaciinae): Systematics, reconstructed phylogeny, and geographic history. Trans. Am. Entomol. Soc. 113, 361–430.

Askevold, I.S., 1990a. 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., 1990b. Reconstructed phylogeny and reclassification of the genera of Donaciinae (Coleoptera: Chrysomelidae). Quaestiones Entomologicae 26, 601–664.

Askevold, I.S., 1991a. An annotated list of nearctic Donaciinae (Coleoptera: Chrysomelidae): The generic classification and type specimens of the new world species. Psyche 98, 165–192.

Askevold, I.S., 1991b. Classification, reconstructed phylogeny, and geographic history of the New World members of *Plateumaris* Thomson, 1859 (Coleoptera: Chrysomelidae: Donaciinae). Mem. Entomol. Soc. Canada 157, 1–175.

Auger-Rozenberg, M.-A., Kerdelhue, C., Magnoux, E., Turgeon, J., Rasplus, J.-Y., Roques, A., 2006. Molecular phylogeny and evolution of host–plant use in conifer seed chalcids in the genus *Megastigmus* (Hymenoptera: Torymidae). Syst. Entomol. 31, 47–64.

Barker, F.K., Lutzoni, F.M., 2002. The utility of the incongruence length difference test. Syst. Biol. 51, 625–637.

Becerra, J.X., 1997. Insects on plants: macroevolutionary chemical trends in host use. Science 276, 253–256.

Bergsten, J., 2005. A review of long-branch attraction. Cladistics 21, 163–193.

Berlocher, S.H., Feder, J.L., 2002. Sympatric speciation in phytophagous insects: Moving beyond controversy? Annu. Rev. Entomol. 47.

Bernays, E., Chapman, R.F., 1994. Host–Plant Selection by Phytophagous Insects. Chapman & Hall, New York..

Bienkowski, A.O., Orlova-Bienkowskaja, M.J., 2003. On the systematics of the subgenus *Donaciella* Reitter, 1920 of the genus *Donacia* Fabricius, 1775, with description of the larva of *Donacia tomentosa* Ahrens, 1810 (Coleoptera: Chrysomelidae: Donaciinae). Genus 14, 59–68.

Borowiec, L., 1984. Zoogeographical study on Donaciinae of the world (Coleoptera, Chrysomelidae). Pol. Pismo Entomol. 53, 433–518.

Brady, S.G., Danforth, B.N., 2004. Recent intron gain in elongation factor-1α of colletid bees (Hymenoptera: Colletidae). Mol. Biol. Evol. 21, 691–696.

Bremer, K., 2002. Gondwanan evolution of the grass alliance of families (Poales). Evolution 56, 1374–1387.

Brower, A.V.Z., 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proc. Nat. Acad. Sci. Am. 91, 6491–6495.

Bush, G.L., 1975. Modes of animal speciation. Annu. Rev. Ecol. Syst. 6, 339–364.

Bush, G.L., 1994. Sympatric speciation in animals: new wine in old bottles. Trends Ecol. Evol. 9, 285–288.

Chen, S.H., 1941. Notes on Donaciine beetles. Sinensia 12, 1–17.

Choi, C., Bareiss, C., Walenciak, O., Gross, E.M., 2002. Impact of polyphenols on growth of the aquatic herbivore *Acentria ephemerella*. J. Chem. Ecol. 27, 2245–2256.

Cognato, A.I., Sperling, F.A.H., 2000. Phylogeny of *Ips* DeGeer species (Coleoptera: Scolytidae) inferred from mitochondrial cytochrome oxidase I DNA sequence. Mol. Phylogenet. Evol. 14, 445–460.

Cox, B.C., 1974. Vertebrate palaeodistributional patterns and continental drift. J. Biogeogr. 1, 75–94.

Cruickshank, R.H., Johnson, K.P., Smith, V.S., Adams, R.J., Clayton, D.H., Page, R.D.M., 2001. Phylogenetic analysis of partial sequences of elongation factor 1α identifies major groups of lice (Insecta: Phthiraptera). Mol. Phylogenet. Evol. 19, 202–215.

Danforth, B., Ji, S., 1998. Elongation factor-1 alpha occurs as two copies in bees: implications for phylogenetic analysis of EF-1 alpha sequences in insects. Mol. Biol. Evol. 15, 225–235.

Darlu, P., Lecointre, G., 2002. When does the incongruence length difference test fail? Mol. Biol. Evol. 19, 432–437.

Deibel, J., 1911. Beiträge zur Kenntnis von *Donacia* und *Macroplea* unter besonderer Berücksichtigung der Atmung. Zool. Jb., Anat. Ont. Tiere 31, 107–160.

Després, L., David, J.-P., Gallet, C., 2007. The evolutionary ecology of insect resistance to plant chemicals. Trends Ecol. Evol. 22, 298–307.

Djernæs, M., Damgaard, J., 2006. Exon-intron structure, paralogy and sequenced regions of elongation factor-1 alpha in Hexapoda. Arthropod Syst. Phylogen. 64, 45–52.

Dobler, S., Müller, J.K., 2000. Resolving phylogeny at the family level by mitochondrial cytochrome oxidase sequences: phylogeny of carrion beetles (Coleoptera, Silphidae). Mol. Phylogenet. Evol. 15, 390–402.

Downie, N.M., Arnett Jr, R.H., 1996. The Beetles of Northeastern North America. The Sandhill Crane Press, Gainsville, FL.

Doyle, J.A., 1998. Phylogeny of vascular plants. Annu. Rev. Ecol. Syst. 29, 567–599.

Drummond, A., Rambaut, A., 2007. BEAST: bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.

Drummond, A., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biology 4, e88.

Dubeschko, L.N., 1973. Listoedy primorskovo chrebta na baikale. In: Kulik, S.A. (Ed.), Fauna i Ekologija Nasekomych Vostocnoj Sibiri i Dal'nego Vostoka. University of Irkutsk, Irkutsk, pp. 152–170.

Dumont, H.J., Vanfleteren, J.R., De Jonckheere, J.F., Weekers, P.H.H., 2005. Phylogenetic relationships, divergence time estimation, and global biogeographic patterns of Calopterygoid damselflies (Odonata, Zygoptera) inferred from ribosomal DNA sequences. Syst. Biol. 54, 347–362.
Eldholm, O., Thiede, J., 1980. Cenozoic continental separation between Europe and Greenland. Palaeogeogr. Palaeoclimatol. Palaeoecol. 30, 243–259.
Enghoff, H., 1995. Historical biogeography of the Holarctic: area relationships, ancestral areas, and dispersal of non-marine animals. Cladistics 11, 223–263.
Erwin, T.L., 1982. Tropical forests: their richness in Coleoptera and other arthropod species. Coleopt. Bull. 36, 74–75.
Farrell, B.D., 1998. "Inordinate fondness" explained: why are there so many beetles? Science 281, 555–559.
Farrell, B., Mitter, C., 1990. Phylogenesis of insect/plant interactions: have *Phyllobrotica* leaf beetles (Chrysomelidae) and the Lamiales diversified in parallel? Evolution 44, 1389–1403.
Farrell, B.D., Mitter, C., 1993. Phylogenetic determinants of insect/plant community diversity. In: Ricklefs, R.E., Schluter, D. (Eds.), Species Diversity: Historical and Geographical Perspectives. University of Chicago Press, Chicago, pp. 253–266.
Figuerola, J., Green, A.J., 2002. Dispersal by aquatic organisms by waterbirds: a review of past research and priorities for future studies. Freshw. Biol. 47, 483–494.
Flower, B.P., Kennett, J.P., 1994. The middle Miocene climatic transition: east Antarctic ice sheet development, deep ocean circulation and global carbon cycling. Palaeogeogr. Palaeoclimatol. Palaeoecol. 108, 537–555.
Foster, G.N., 2001. Atlas of Scottish water beetles. Scottish Natural Heritage.
Freude, H., Harde, K.W., Lohse, G.A., 1966. Die Käfer Mitteleuropas. Goecke & Evers, Krefeld.
Frisch, D., Green, A.J., Figuerola, J., 2007. High dispersal capacity of a broad spectrum of aquatic invertebrates via waterbirds. Aqua. Sci. 69, 568–574.
Funk, D.J., Filchak, K.E., Feder, J.E., 2002. Herbivorous insects: model systems for the comparative study of speciation ecology. Genetica 116, 251–267.
Futuyma, D.J., Keese, M.C., 1992. Evolution and coevolution of plants and phytophagous arthropods. In: Rosenthal, G.A., Berenbaum, M.R. (Eds.), Herbivores. Their Interactions with Secondary Plant Metabolites. Academic Press, San Diego, pp. 440–475.
Futuyma, D.J., McCafferty, S.S., 1990. Phylogeny and the evolution of host plant associations in the leaf beetle genus *Ophraella* (Coleoptera, Chrysomelidae). Evolution 44, 1885–1905.
Futuyma, D.J., Moreno, G., 1988. The evolution of ecological specialization. Annu. Rev. Ecol. Syst. 19, 207–233.
Gavrilets, S., Vose, A., 2005. Dynamic patterns of adaptive radiation. Proc. Nat. Acad. Sci. USA 102, 18040–18045.
Goecke, H., 1960. Monographie der Schilfkäfer III. Die Gattungen und Arten der Donaciinae (Col. Chrys.) und ihre Verbreitung. Entomol. Blätter 56, 1–19.
Goldman, N., Anderson, J.P., Rodrigo, A.G., 2000. Likelihood-based tests of topologies in phylogenetics. Syst. Biol. 49, 652–670.
Gomez-Zurita, J., 2004. Molecular systematics and time-scale for the evolution of *Timarcha*, a leaf-beetle genus with a disjunct Holarctic distribution. Mol. Phylogenet. Evol. 32, 647–665.
Gomez-Zurita, J., Hunt, T., Kopliku, F., Vogler, A.P., 2007. Recalibrated tree of leaf beetles (Chrysomelidae) indicates independent diversification of angiosperms and their insect herbivores. PLoS ONE 2, e360.
Green, A.J., Sanchez, M.I., 2006. Passive internal dispersal of insect larvae by migratory birds. Biol. Lett. 2, 55–57.
Guo, Q., 1999. Ecological comparisons between Eastern Asia and North America: historical and geographical perspectives. J. Biogeogr. 26, 199–206.
Hamilton, W., 1983. Cretaceous and cenozoic history of the northern continents. Ann. Miss. Bot. Gar. 70, 440–458.
Harzhauser, M., Kroh, A., Mandic, O., Piller, W.E., Göhlich, U., Reuter, M., Berning, B., 2007. Biographic responses to geodynamics: a key study all around the Oligo-Miocene Tethyan Seaway. Zool. Anz. 246, 241–256.
Hayashi, M., Shiyake, S., 2001. The identity and distribution of *Macroplea japana* (Jacoby) (Coleoptera, Chrysomelidae, Donaciinae). Bull. Osaka Mus. Nat. Hist. 55, 15–22.
Hedin, M.C., Maddison, W.P., 2001. Phylogenetic utility and evidence for multiple copies of elongation factor-1α in the spider genus *Habronattus* (Araneae: Salticidae). Mol. Biol. Evol. 18, 1512–1521.
Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.
Hughes, J., Vogler, A.P., 2004. The phylogeny of acorn weevils (genus *Curculio*) from mitochondrial and nuclear DNA sequences: the problem of incomplete data. Mol. Phylogenet. Evol. 32, 601–615.
Jaenike, J., 1990. Host specialization in phytophagous insects. Annu. Rev. Ecol. Syst. 21, 243–273.
Janis, C.M., 1993. Tertiary mammal evolution in the context of changing climates, vegetation, and tectonic events. Annu. Rev. Ecol. Syst. 24, 467–500.
Janssen, T., Bremer, K., 2004. The age of major monocot groups inferred from 800+ rbcL sequences. Bot. J. Linn. Soc. 146, 385–398.
Janz, N., Nyblom, K., Nylin, S., 2001. Evolutionary dynamics of host–plant specialization: a case study of the tribe Nymphalini. Evolution 55, 783–796.
Jin, S., Hu, G.A., Qian, Y.H., Zhang, L., Zhang, J., Qiu, G., Zeng, Q.T., Gui, J.F., 2005. Identification of one intron loss and phylogenetic evolution of Dfak gene in the *Drosophila melanogaster* species group. Genetica 125, 223–230.
Jolivet, P., 1988. Food habits and food selection of Chrysomelidae. Bionomic and evolutionary perspectives. In: Jolivet, P., Petitpierre, E., Hsiao, T.H. (Eds.), Biology of Chrysomelidae. Kluwer Academic Publishers., Dordrecht, pp. 1–24.
Jordal, B.H., 2002. Elongation Factor 1 α resolves the monophyly of the haplodiploid ambrosia beetles Xyleborini (Coleoptera: Curculionidae). Insect Mol. Biol. 11, 453–465.
Jordal, B.H., Kirkendall, L.R., Harkestad, K., 2004. Phylogeny of a Macaronesian radiation: host–plant use and possible cryptic speciation in *Liparthrum* bark beetles. Mol. Phylogenet. Evol. 31, 554–571.
Kawecki, T., 1994. Accumulation of deleterious mutations and the evolutionary cost of being a generalist. Am. Nat. 144, 833–838.
Kelley, S.T., Farrell, B.D., 1998. Is specialization a dead end? The phylogeny of host use in *Dendroctonus* bark beetles (Scolytidae). Evolution 52, 1731–1743.
Kergoat, G.J., Alvarez, N., Hossaert-McKey, M., Faure, N., Silvain, J.-F., 2005a. Parallels in the evolution of the two largest New and Old World seed-beetle genera (Coleoptera, Bruchidae). Mol. Ecol. 14, 4003–4021.
Kergoat, G.J., Delobel, A., Fediere, G., Ru, B.L., Silvain, J.-F., 2005b. Both host–plant phylogeny and chemistry have shaped the African seed-beetle radiation. Mol. Phylogenet. Evol. 35, 602–611.
Klausnitzer, B., 1984. Käfer im und am Wasser. Neue Brehm-Bücherei, Wittenberg Lutherstadt.
Koch, K., 1992. Die Käfer Mitteleuropas, vol. E3. Ökologie. Goecke & Evers, Krefeld.
Kölsch, G., Biström, O., Pedersen, B.V., 2006. Species delimitation in the leaf beetle genus *Macroplea* (Coleoptera, Chrysomelidae) based on mitochondrial DNA, and phylogeographic considerations. Insect Syst. Evol. 37, 467–479.
Kumar, S., Tamura, K., Nei, K., 2004. MEGA3: integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Briefings Bioinform. 5, 150–163.
Lambkin, C.L., Lee, M.S.Y., Winterton, S.L., Yeates, D.K., 2002. Partitioned Bremer support and multiple trees. Cladistics 18, 436–444.
Linder, H.P., Rudall, P.J., 2005. Evolutionary history of Poales. Annu. Rev. Ecol. Evol. Syst. 36, 107–124.
Lopatin, I.K., 1984. Leaf beetles (Chrysomelidae) of Central Asia and Kazakhstan. Oxonian Press, New Delhi Calcutta.
Lopez-Vaamonde, C., Wikstrom, N., Labandeira, C., Godfray, H.C.J., Goodman, S.J., Cook, J.M., 2006. Fossil–calibrated molecular phylogenies reveal that leaf-mining moths radiated millions of years after their host plants. J. Evol. Biol. 19, 1314–1326.
Marincovich, L., Gladenkov, A.Y., 1999. Evidence for an early opening of the Bering Strait. Nature 397, 149–151.
Martinez-Navarro, E.M., Galian, J., Serrano, J., 2005. Phylogeny and molecular evolution of the tribe Harpalini (Coleoptera, Carabidae) inferred from mitochondrial cytochrome-oxidase I. Mol. Phylogenet. Evol. 35, 127–146.
Marx, E.J.F., 1957. A review of the subgenus *Donacia* in the western hemisphere (Coleoptera, Donaciidae). Bull. Am. Mus. Nat. Hist. 112, 191–278.
Matthews Jr, J.V., 1980. Tertiary land bridges and their climate: backdrop for development of the present Canadian insect fauna. Can. Entomol. 112, 1089–1103.
McKenna, M.C., 1975. Fossil mammals and early Eocene North Atlantic land continuity. Ann. Miss. Bot. Gar. 62, 335–353.
McKenna, M.C., 1983. Holarctic landmass rearrangement, cosmic events, and cenozoic terrestrial organisms. Ann. Miss. Bot. Gar. 70, 459–489.
Medvedev, L.N., 1977. (Discovery of a member of the American genus *Neohaemonia* Szek. (Coleoptera, Chrysomelidae) in Mongolia) (in Russian). Doklady Akademii Nauk SSSR 236, 488–490.
Menken, S.B.J., Roessingh, P., 1998. Evolution of insect-plant associations. In: Howard, D.J., Berlocher, S.H. (Eds.), Endless Forms. Species and Speciation. Oxford University Press, New York, Oxford, pp. 145–156.
Menzies, I.S., Cox, M.L., 1996. Notes on the natural history, distribution and identification of British reed beetles. Br. J. Entomol. Nat. Hist. 9, 137–162.
Miller, D.G., Crespi, B., 2003. The evolution of inquilinism, host–plant use and mitochondrial substitution rates in *Tamalia* gall aphids. J. Evol. Biol. 16, 731–743.
Miller, J.S., Wenzel, J.W., 1995. Ecological characters and phylogeny. Annu. Rev. Entomol. 40, 389–415.
Mitter, C., Farrell, B.D., Wiegmann, B., 1988. The phylogenetic study of adaptive zones: has phytophagy promoted insect diversification? Am. Nat. 132, 107–128.
Mitter, C., Farrell, B.D., Futuyma, D.J., 1991. Phylogenetic studies of insect-plant interactions: insights into the genesis of diversity. Trends Ecol. Evol. 6, 290–293.
Mohr, K.-H., 1985. Beiträge zur Insektenfauna der DDR: Coleoptera—Chrysomelidae: Donaciinae, Orsodacninae, Criocerinae, Clythrinae. Beitr. Entomol. 35, 219–262.
Moran, N.A., Kaplan, M.E., Gelsey, M.J., Murphy, T.G., Scholes, E.A., 1999. Phylogenetics and evolution of the aphid genus *Uroleucon* based on mitochondrial and nuclear DNA sequences. Syst. Entomol. 24, 85–93.
Morse, G.E., Farrell, B.D., 2005. Interspecific phylogeography of the *Stator limbatus* species complex: the geographic context of speciation and specialization. Mol. Phylogenet. Evol. 36, 201–213.
Müller, K., 2002. GRate—relative rate tests for taxon groups using general DNA substitution models in PAUP*. Program distributed by the author, Bonn, http://www.nees.uni-bonn.de/downloads/GRate.
Nilsson, A.N., 1996. Coleoptera Chrysomelidae Donaciinae, Water lily beetles. In: Nilsson, A.N. (Ed.), Aquatic Insects of North Europe. Apollo Books, Stenstrup, pp. 209–216.
Nosil, P., 2002. Transition rates between specialization and generalization in phytophagous insects. Evolution 56, 1701–1706.
Novotny, V., Basset, Y., Miller, S.E., Weiblen, G.D., Bremer, B., Cizek, L., Drozd, P., 2002. Low host specificity of herbivorous insects in a tropical forest. Nature 416, 841–844.

Novotny, V., Drozd, P., Miller, S.E., Kulfan, M., Janda, M., Basset, Y., Weiblen, G.D., 2006. Why are there so many species of herbivorous insects in tropical rainforests? Science 313, 1115–1118.
Ode, P.J., 2006. Plant chemistry and natural enemy fitness: effects on herbivore and natural enemy interactions. Annu. Rev. Entomol. 51, 163–185.
Ødegaard, F., 2000. How many species of arthropods? Erwin's estimate revised. Biol. J. Linn. Soc. 71, 583–597.
Parry, J.A., 1979. Notes on the Donaciinae (Col: Chrysomelidae), with a list of recent East Kentish localities known to the author. Entomologist's Rec., 323–326.
Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinform. 14, 817–818.
Radtkey, R.R., Singer, M.C., 1995. Repeated reversals of host-preference evolution in a specialist insect herbivore. Evolution 49, 351–359.
Raven, P.H., Axelrod, D.I., 1974. Angiosperm biogeography and past continental movements. Ann. Miss. Bot. Gar. 61, 539–673.
Ribera, I., Nilsson, A.N., Vogler, A.P., 2004. Phylogeny and historical biogeography of Agabinae diving beetles (Coleoptera) inferred from mitochondrial DNA sequences. Mol. Phylogenet. Evol. 30, 545–562.
Ronquist, F., Huelsenbeck, J.P., van der Mark, P., 2005. MrBayes 3.1 Manual, draft 5/26/2005.
Rosenthal, G.A., Berenbaum, M.R., 1991. Herbivores: Their Interactions with Secondary Plant Metabolites. Academic Press, San Diego.
Rundle, H.D., Nosil, P., 2005. Ecological speciation. Ecol. Lett. 8, 336–352.
Sachet, J.-M., Roques, A., Despres, L., 2006. Linking patterns and processes of species diversification in the cone flies *Strobilomyia* (Diptera: Anthomyiidae). Mol. Phylogenet. Evol. 41, 606–621.
Sanderson, M.J., 2003. r8s; inferring absolute rates of evolution and divergence times in the absence of a molecular clock. Bioinformatics 19, 301–302.
Sanmartín, I., Enghoff, H., Ronquist, F., 2001. Patterns of animal dispersal, vicariance and diversification in the Holarctic. Biol. J. Linn. Soc. 73, 345–390.
Schluter, D., 1998. Ecological causes of speciation. In: Howard, D.J., Berlocher, S.H. (Eds.), Endless Forms. Species and Speciation. Oxford University Press, New York, Oxford, pp. 114–129.
Schmidt, K.P., 1946. On the zoogeography of the Holarctic region. Copeia 3, 144–152.
Shavit, L., Penny, D., Hendy, M.D., Holland, B.R., 2007. The problem of rooting rapid radiations. Mol. Biol. Evol. 24, 2400–2411.
Silvain, J.F., Delobel, A., 1998. Phylogeny of West African *Caryedon* (Coleoptera: Bruchidae): Congruence between molecular and morphological data. Mol. Phylogenet. Evol. 9, 533–541.
Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87, 651–701.
Smith, A.G., Smith, D.G., Funnell, B.M., 1994. Atlas of Mesozoic and Cenozoic Coastlines. Cambridge University Press, Cambridge.
Sorensen, M.D., Franzosa, E.A., 2007. TreeRot, version 3. Boston University, Boston, MA.
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., Santiago-Blay, J.A., Schmitt, M. (Eds.), New Developments in 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. 34, 977–993.
Sota, T., Takami, Y., Monteith, G.B., Moore, B.P., 2005. Phylogeny and character evolution of endemic Australian carabid beetles of the genus *Pamborus* based on mitochondrial and nuclear gene sequences. Mol. Phylogenet. Evol. 36, 391–404.
Sota, T., Bocak, L., Hayashi, M., 2008. Molecular phylogeny and historical biogeography of the Holarctic wetland leaf beetle of the genus *Plateumaris*. Mol. Phylogenet. Evol. 46, 183–192.
Stireman III, J.O., Nason, J.D., Herard, S.B., 2005. Host-associated genetic differentiation in phytophagous insects: general phenomenon or isolated exceptions? Evidence from a goldenrod-insect community. Evolution 59, 2573–2587.
Strauch, F., 1970. Die Thule-Landbrücke als Wanderweg und Faunenscheide zwischen Atlantik und Skandik im Tertiär. Int. J. Earth Sci. 60, 381–417.
Strong, D.R., Lawton, J.H., Southwood, R., 1984. Insects on Plants: Community Patterns and Mechanisms. Blackwell, Oxford.
Swigonova, Z., Kjer, K.M., 2004. Phylogeny and host–plant association in the leaf beetle genus *Trirhabda* LeConte (Coleoptera: Chrysomelidae). Mol. Phylogenet. Evol. 32, 358–374.
Swofford, D.L., 1998. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Sinauer Ass, Sunderland, Massachusetts.
Termonia, A., Hsiao, T.H., Pasteels, J.M., Milinkovitch, M.C., 2001. Feeding specialization and host-derived chemical defense in Chrysomeline leaf beetles did not lead to an evolutionary dead end. Proc. Nat. Acad. Sci. USA 98, 3909–3914.
Vogt, P.R., 1972. The Faeroe–Iceland–Greenland aseismic ridge and the Western Boundary Undercurrent. Nature 239, 79–81.
Wada, H., Kobayashi, M., Sato, R., Satoh, N., Miyasaka, H., Shirayama, Y., 2002. Dynamic insertion–deletion of introns in deuterostome EF-1α genes. J. Mol. Evol. 54, 118–128.
Ward, L.K., Hackshaw, A., Clarke, R.T., 2003. Do food-plant preferences of modern families of phytophagous insects and mites reflect past evolution with plants? Biol. J. Linn. Soc. 78, 51–83.
Wen, J., 1999. Evolution of Eastern Asian and Eastern North American disjunct distributions in flowering plants. Annu. Rev. Ecol. Syst. 30, 421–455.
Whitfield, J.B., Kjer, K.M., 2008. Ancient rapid radiations of insects: challenges for phylogenetic analysis. Annu. Rev. Entomol. 53, 449–472.
Whitlock, M.C., 1996. The Red Queen beats the jack-of-all-trades: the limitations on the evolution of phenotypic plasticity and niche breadth. Am. Nat. 148, 65–77.
Wilcox, J.A., 1979. Leaf Beetle Host Plants in Northeastern North America. World Natural History Publications, Kinderhook, NY.
Xia, X., Xie, Z., 2001. DAMBE: Data analysis in molecular biology and evolution. J. Hered. 92, 371–373.
Yotoko, K.S.C., Prado, P.I., Russo, C.A.M., Solferini, V.N., 2005. Testing the trend towards specialization in herbivore—host plant associations using a molecular phylogeny of *Tomoplagia* (Diptera: Tephritidae). Mol. Phylogenet. Evol. 35, 701–711.