Molecular Ecology (2005) **14**, 4003–4021 doi: 10.1111/j.1365-294X.2005.02702.x

Parallels in the evolution of the two largest New and Old World seed-beetle genera (Coleoptera, Bruchidae)

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Abstract

This study provides the first phylogenetic analysis of a large sample of the two largest genera of seed-beetles, *Acanthoscelides* Schilsky and *Bruchidius* Schilsky, which mostly feed on legumes (Fabaceae). The goal of this study was to investigate evolutionary patterns in relation to biogeography and host-plant associations. We used three mitochondrial molecular markers and parsimony and Bayesian inference methods to reconstruct the phylogeny of 76 species. In addition, we critically reviewed host-plant records in the literature for these two bruchid genera. Our results demonstrated the existence of two major clades, one New World and one largely Old World, which generally correspond to the two genera. Yet, current classification of several species is erroneous, so that both genera as currently defined are paraphyletic. We highlighted a strong trend toward specialization (with high taxonomic conservatism in host-plant use) exhibited by the two studied genera. However, we showed the existence of several host shifts during the evolution of this group of bruchids. Our phylogenetic hypotheses and our evaluation of host-plant associations both suggest that the two genera have undergone parallel evolution, as they have independently colonized similar host plants in their respective areas of distribution. Our estimation of divergence times indicated a more ancient origin for bruchids than that suggested by the fossil records. Interestingly, the suggested timing of diversification is consistent with the hypothesis of a radiation that could have occurred contemporaneously with the diversification of their legume hosts.

Keywords: adaptive radiation, Bruchidae, host-plant associations, key innovation, parallel evolution, phytophagous insects

Received 12 May 2005; revision accepted 11 July 2005

Introduction

The evolution of the species-rich superfamily Chrysomeloidea is a fascinating example of insect radiation on angiosperms. During their diversification, they successfully used almost all flowering plant parts as larval food resources (Johnson 1981). In Curculionoidea, the sister group of Chrysomeloidea, a similar trend is observed (Anderson 1995; Marvaldi *et al*. 2002), and many authors have introduced the concept of adaptive radiation (Simpson 1953; Schluter 2000) to explain the enhanced rate of diversification of phytophagous Coleoptera on angiosperms (Mitter *et al*. 1988; Farrell 1998; Marvaldi *et al*. 2002; Farrell & Sequeira 2004). According to these authors this diversification is likely to be linked either to the frequent availability of new ecological niches (for which the insect is pre-adapted) or to the development of 'key innovations' (Simpson 1953), which enable the use of new food resources (in effect, creating new potential niches). In the co-evolutionary model of Ehrlich & Raven (1964), evolutionary novelties enable insects to circumvent the defences of plants, particularly toxic secondary compounds. The insects able to bypass these defences are then likely to undergo a successful diversification on these hitherto unexploited

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resources. Adaptation to chemical defences that then constrain host-plant use can account for two major patterns in phytophagous insect host-plant associations. First, the strong trend of taxonomic conservatism in host use observed among endopterygote insects can be explained by the fact that related plants often share similar chemical defences (Kergoat *et al*. 2005). Second, convergent similarity in host-plant chemistry can explain host shifts among taxonomically unrelated plants observed in groups such as Chrysomelidae (Becerra 1997; Termonia *et al*. 2001).

The study of the evolutionary processes driving the diversification of Chrysomeloidea has been particularly intensive in the seed-beetles and is well summarized in Johnson's (1990a) review. Recent changes in taxonomy and thus nomenclature seem to favour the use of the subfamily name Bruchinae rather than Bruchidae (C. D. Johnson, personal communication), but for convenience we have retained the name Bruchidae in this study. Bruchids constitute a homogeneous group of about 1700 described species in about 60 genera (Southgate 1979; Johnson 1994), most of which are associated with the family Fabaceae (Johnson 1981). Most bruchid species are specialized on a narrow range of host plants and their larval stages develop exclusively inside seeds (Borowiec 1987). This adaptation has led bruchids to specialize on legumes, but has likely allowed them to undergo numerous parallel radiations on other taxa with hard-coated seeds. Moreover, the family Bruchidae is of particular interest as it contains several pest species of economic importance with worldwide distributions (Delobel & Tran 1993). Bruchid taxonomy, biology and ecology have been intensively investigated by many authors (e.g. Johnson 1981; Borowiec 1987), but only recently have molecular phylogenetic methods been applied to evolutionary studies of this group (Silvain & Delobel 1998; Kergoat *et al*. 2004, 2005).

In this study, we have focused on the two largest seed-beetle genera, *Acanthoscelides* and *Bruchidius*, which represent about half of the known bruchid species. As currently circumscribed, *Acanthoscelides* is restricted to the New World and *Bruchidius* to the Old World. Working with distinct bruchid groups that have radiated independently in different geographical areas will allow us to test hypotheses concerning adaptive radiation in the sense of Schluter (2000) who defines it as 'the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage'. Indeed, these two largest bruchid genera are relevant models to examine general patterns in adaptive radiation of specialized phytophagous insects, especially concerning the role of host-plant association.

We will also study possible parallel patterns between these two genera by comparing the diversification they have undergone on a large number of host-plant species (Johnson 1981; Borowiec 1987) in their respective areas of distribution in which they seem to occupy similar ecological niches (Borowiec 1987). Our approach is based on the hypothesis that each of the two genera constitutes a distinct monophyletic group. However, the two genera are morphologically very similar and can only be distinguished by the number of femoral spines on the hind leg. Nonetheless, this usually diagnostic character fails to separate some *Bruchidius* species from *Acanthoscelides* species (A. Delobel, personal communication). This observation could be explained if the genera as currently circumscribed were in some respects artificial. Considering the lack of clear synapomorphies supporting the monophyly of each, several authors have considered these genera as paraphyletic (Johnson 1981; Borowiec 1987; A. Delobel, personal communication). Therefore, we decided to test the monophyly of the two genera.

To test these phylogenetic hypotheses and examine the radiation of these bruchids, we investigated a large sample of *Acanthoscelides* and *Bruchidius* species, and other representatives of tribe Acanthoscelidini, by using sequences for three different mitochondrial genes (12S rRNA, cytochrome *b*, and cytochrome *c* oxidase subunit I).

Materials and methods

Taxon sampling and DNA sequencing

The 76 species analysed, their geographical provenance, collectors and host-records are listed in Table 1. Although the species included in this study comprise fewer than 20% of known species for the two genera, our sample can be considered as representative of the diversity of host-plant associations. For instance, we have sampled species associated with 16 of the 19 tribes of the family Fabaceae that are known to be larval hosts of *Acanthoscelides* and *Bruchidius*. Sampled individuals were generally obtained by rearing larvae from seeds collected in the field, with the exception of two species as presented in Table 1. As outgroups, in addition to 11 other species of the tribe Acanthoscelidini, a representative of the supposed primitive subfamily Pachymerinae (Borowiec 1987) was used. Both dry and ethanol-preserved specimens were used for DNA extractions (one individual was sequenced per species). With the exception of the *Pachymerus cardo* specimen (for which we only used hind legs), whole specimens were used to obtain total DNA by using QIAGEN extraction columns. Partial sequences for the three mitochondrial genes were amplified using primers listed by Simon *et al*. (1994) and by Monteiro & Pierce (2001). Standard cycling conditions were 5 min at 94 °C followed by 35–40 cycles of 1 min at 94 °C, 1 min at 45–50 °C (depending on the primers used), 1 min at 72 °C, and a final step at 72 °C for 10 min. Partial sequences for the 12S rRNA gene were successfully amplified for all specimens, but for some dry specimens it was not possible to obtain

Table 1 Material examined in this study

Taxon	Locality*	Collector	Host-plant†		GenBank Accession No.		
					12S rRNA	Cyt *b*	COI
Subfamily Bruchinae Latreille, 1802							
Tribe Acanthoscelidini Bridwell, 1946							
Acanthoscelides Schilsky, 1905							
anoditus Johnson, 1983	Mex. El Copal	N Alvarez	*Anoda cristata*	Malvoideae	AY945966	none	none
argillaceus (Sharp, 1885)	Mex. Playa azul	A Aebi	*Phaseolus lunatus*	Fab. Phaseoleae	AY945967	none	AY947513
biustulus (Fall, 1910)	Mex. Amealco	J Romero N	*Desmodium* sp.	Fab. Desmodieae	AY945968	none	none
clandestinus (Motschulsky, 1874)	Mex. C. Carmen	R Ramírez D	*Vigna adenantha*	Fab. Phaseoleae	AY945969	none	none
cuernavaca Johnson, 1983	Mex. S. Huautla	J Romero N	*Desmodium* sp.	Fab. Desmodieae	AY945970	none	AY947514
desmodicola Johnson, 1983	Mex. S. Huautla	I Figueroa R	*Desmodium* sp.	Fab. Desmodieae	AY945971	none	AY947515
desmoditus Johnson, 1983	Ven. Barquisimeto	CD Johnson	*Desmodium tortuosum*	Fab. Desmodieae	AY945972	none	AY947516
flavescens (Fähraeus, 1839)	Mex. Presa Diablo	J Luna Cozar	*Rhynchosia minima*	Fab. Phaseoleae	AY945973	none	none
guazumae Johnson & Kingsolver, 1971	Mex. S. Huautla	J Romero N	*Guazuma tomentosa*	Malvoideae	AY945974	none	none
isla Johnson, 1983	Ecu. Guayaquil	CD Johnson	*Rhynchosia minima*	Fab. Phaseoleae	AY945975	none	none
macrophthalmus (Schaeffer, 1907)	Vie. Saïgon (I)	H Delobel	*Leucaena leucocephala*	Fab. Mimoseae	AY945976	none	AY947517
malvastrumicis (Johnson, 1983)	Mex. El Cielo	S Niño	*Malvastrum americanum*	Malvoideae	AY945977	none	none
mazatlan (Johnson, 1983)	Mex. S. Huautla	J Romero N	*Desmodium* sp.	Fab. Desmodieae	AY945978	none	none
mexicanus (Sharp, 1885)	Mex. Coxcatlan	N Alvarez	*Mimosa* sp.	Fab. Mimoseae	AY945979	none	AY947518
mundulus (Sharp, 1885)	Mex. Jalcomulco	J Romero N	*Nissolia fruticosa*	Fab. Aeschynomeneae	AY945980	none	none
oblongoguttatus (Fähraeus, 1839)	Mex. Cotaxtla	J Romero N	*Acacia cornigera*	Fab. Acacieae	AY945981	none	none
obtectus (Say, 1831)	Egy. Giza (I)	G Fédière	*Phaseolus vulgaris*	Fab. Phaseoleae	AY945982	AY947505	AY947519
obvelatus Bridwell, 1942	Mex. Tepoztlan	N Alvarez	*Phaseolus vulgaris*	Fab. Phaseoleae	AY945983	none	AY947520
palmasola Johnson, 1983	Mex. Tenabo	CD Johnson	*Rhynchosia longeracemosa*	Fab. Phaseoleae	AY945984	none	none
puellus (Sharp, 1885)	Nic. Mombacho	JM Maes	*Calopogonium mucunoides*	Fab. Phaseoleae	AY945985	none	none
sanblas Johnson, 1983	Mex. Córdoba	J Romero N	*Triumfetta lappula*	Tilioideae	AY945986	none	none
sanfordi Johnson, 1983	Mex. S. Huautla	J Romero N	*Pachyrhizus erosus*	Fab. Phaseoleae	AY945987	none	AY947521
stylifer (Sharp, 1885)	Mex. Ixmiquilpan	J Romero N	*Desmodium* sp.	Fab. Desmodieae	AY945988	AY947506	AY947522
taboga Johnson, 1983	Pan. Chepo	CD Johnson	*Calopogonium caeruleum*	Fab. Phaseoleae	AY945989	none	none
zonensis Johnson, 1983	Col. Palmira	CD Johnson	*Teramnus uncinatus*	Fab. Phaseoleae	AY945990	none	none
Algarobius Bridwell, 1946							
prosopis (LeConte, 1858)	Egy. El Tur (I)	G Fédière	*Prosopis glandulosa*	Fab. Mimoseae	AY945964	AY947503	AY947511

Table 1 *Continued*

Taxon	Locality*	Collector	Host-plant†		GenBank Accession No. 12S rRNA	Cyt *b*	COI
Bruchidius Schilsky, 1905							
auratopubens Decelle, *in litt.*	Sen. Fatick	M Sembène	*Faidherbia albida*	Fab. Ingeae	AY625282	AY625429	AY625379
aurivillii (Blanc, 1889)	Sen. Fleuve	M Sembène	*Acacia tortilis*	Fab. Acacieae	AY625283	AY625430	AY625380
bimaculatus (Olivier, 1795)	Fra. Corse	A Delobel	*Medicago marina*	Fab. Trifolieae	AY390640	AY390672	AY390704
bernardi Delobel & Anton, 2004	Ita. Basilicata	A Delobel	*Astragalus depressus*	Fab. Galegeae	AY945957	none	none
cadei Decelle, *in litt.*	Sen. Thies	H Delobel	*Faidherbia albida*	Fab. Ingeae	AY625284	AY625431	AY625381
campylacanthae Decelle, *in litt.*	Sen. Thies	H Delobel	*Acacia polyacantha*	Fab. Acacieae	AY625285	AY625432	AY625382
caninus (Kraatz, 1869)	Fra. Corse	A Delobel	*Astragalus hamosus*	Fab. Galegeae	AY390641	AY390673	AY390705
centromaculatus (Allard, 1868)	Sen. Fleuve	MT Gueye	*Acacia nilotica*	Fab. Acacieae	AY625287	AY625434	AY625384
chloroticus (Dalm., 1833)	Sen. Fatick	H Delobel	*Sesbania pachycarpa*	Fab. Robinieae	AY625286	AY625433	AY625383
dichrostachydis Delobel & Anton, 2003	Sen. Fatick	H Delobel	*Dichrostachys cinerea*	Fab. Mimoseae	AY625288	AY625435	AY625385
dispar (Gyllenhall, 1833)	Fra. Montfuron	A Delobel	*Trifolium repens*	Fab. Trifolieae	AY945958	none	AY947507
dialii Decelle, 1973	Sen. Ziguinchor	A Delobel	*Dialium guineense*	Fab. Cassieae	AY625289	none	none
elnairensis (Pic, 1921)	Ken. Kabarnet	B Le Rü	*Acacia dolichocephala*	Fab. Acacieae	AY625290	none	AY625386
fulvicornis (Motschulsky, 1874)	Fra. Corse	A Delobel	*Trifolium vesiculosum*	Fab. Trifolieae	AY390644	AY380676	AY390708
grandemaculatus (Pic, 1933)	Ken. Tsavo	B Le Rü	*Acacia nilotica*	Fab. Acacieae	AY945959	none	AY947508
incarnatus (Boheman, 1833)	Egy. Baharîya (I)	G Fédière	*Vicia faba*	Fab. Vicieae	AY625292	AY625437	AY625388
lineatopygus (Pic, 1924)	Sen. Louga	H Delobel	*Indigofera tinctoria*	Fab. Indigofereae	AY625293	AY625438	AY625389
lividimanus (Gyllenhall, 1833)	Fra. Monsols	A Delobel	*Cytisus scorparius*	Fab. Cytiseae	AY390645	AY390677	AY390709
marginalis (Fabricius, 1776)	Fra. Montfuron	A Delobel	*Astrag. monspessulanus*	Fab. Galegeae	AY390646	AY390678	AY390710
nanus (Germar, 1824)	Ita. Basilicata	A Delobel	*Medicago orbicularis*	Fab. Trifolieae	AY390647	AY390679	AY390711
niokolobaensis (Decelle, 1969)	Sen. Thies	H Delobel	*Tephrosia bracteolata*	Fab. Milletieae	AY625294	AY625439	AY625390
pauper (Boheman, 1829)	Fra. Corse	A Delobel	*Ornithopus compressus*	Fab. Loteae	AY390648	AY390680	AY390712
picipes (Germar, 1824)	Fra. Corse	A Delobel	*Trifolium angustifolium*	Fab. Trifolieae	AY390649	AY390681	AY390713
poecilus (Germar, 1824)	Ita. Basilicata	A Delobel	*Astrag. contortuplicatus*	Fab. Galegeae	AY945960	AY947501	AY947509
pusillus (Germar, 1924)	Fra. Gard	A Delobel	*Hippocrepis emerus*	Fab. Loteae	AY390650	AY390682	AY390714
pygidiopictus Decelle, *in litt.*	Sen. Louga	M Sembène	*Faidherbia albida*	Fab. Ingeae	AY625295	AY624440	AY625391
pygmaeus (Boheman, 1833)	Fra. Corse	A Delobel	*Trifolium angustifolium*	Fab. Trifolieae	AY390651	AY390683	AY390715
quinqueguttatus (Olivier, 1795)	Tur.	K-W Anton	*Lupinus* sp.‡	Fab. Cytiseae	AY945961	none	none
raddianae Anton & Delobel, 2003	Sen. Fleuve	M Sembène	*Acacia tortilis*	Fab. Acacieae	AY625297	AY625442	AY625393
rubicundus (Fahraeus, 1839)	Ken. Taveta	B Le Rü	*Acacia laeta*	Fab. Acacieae	AY625298	AY625443	AY625394
rubiginosus (Desbrochers, 1869)	Fra.	A Delobel	*Lupinus* sp.	Fab. Cytiseae	AY945962	AY947502	AY947510
seminarius (Linnaeus, 1767)	Fra. Séderon	A Delobel	*Lotus maritimus*	Fab. Loteae	AY390652	AY390684	AY390716
sericatus (Germar, 1824)	Fra. Corse	A Delobel	*Trifolium angustifolium*	Fab. Trifolieae	AY390653	AY390685	AY390717
submaculatus (Fahraeus, 1839)	Sen. Fleuve	M Sembène	*Acacia senegal*	Fab. Acacieae	AY625301	AY625446	AY625397
trifolii (Motschulsky, 1874)	Egy. Baharîya (I)	G Fédière	*Trifolium alexandrinum*	Fab. Trifolieae	AY509806	AY509809	AY509812
tuberculatus (Hochhuth, 1847)	Kyr.	K-W Anton	(not based on rearing)	Fab.	AY945963	none	none
uberatus (Fahraeus, 1895)	Sen. Fleuve	MT Gueye	*Acacia nilotica*	Fab. Acacieae	AY625302	AY625447	AY625398
varipictus (Motschulsky, 1874)	Fra. Corse	A Delobel	*Medicago murex*	Fab. Trifolieae	AY390657	AY390689	AY390720
villosus (Fabricius, 1792)	Fra. Saclas	A Delobel	*Laburnum anagyroides*	Fab. Cytiseae	AY390655	AY390687	AY390719
varius (Olivier, 1795)	Fra. Corse	A Delobel	*Trifolium angustifolium*	Fab. Trifolieae	AY390656	AY390688	none

Table 1 *Continued*

Taxon	Locality*	Collector	Host-plant†		GenBank Accession No. 12S rRNA	Cyt *b*	COI
Callosobruchus Pic, 1902							
chinensis (Linnaeus, 1758)	Egy. Giza (I)	G Fédière	*Cajanus cajan*	Fab. Phaseoleae	AY625319	AY625465	AY625416
maculatus (Fabricius, 1775)	Vie. Saïgon (I)	H Delobel	*Vigna unguiculata*	Fab. Phaseoleae	AY625320	AY625466	AY625417
phaseoli (Gyllenhall, 1833)	Egy. Baharîya (I)	G Fédière	*Lablab purpureus*	Fab. Phaseoleae	AY625321	AY625467	AY625418
subinnotatus (Pic, 1914)	Sen. Cap Vert (I)	H Delobel	*Vigna subterranea*	Fab. Phaseoleae	AY625322	AY625468	AY625419
Conicobruchus Decelle, 1951							
strangulatus (Fahraeus, 1839)	Sen. Cap Vert	H Delobel	*Crotalaria podocarpa*	Fab. Crotalarieae	AY625323	AY625469	AY625420
Decellebruchus Borowiec, 1987							
atrolineatus (Pic, 1921)	Sen. Cap Vert	H Delobel	*Vigna unguiculata*	Fab. Phaseoleae	AY625324	AY625470	AY625421
Gibbobruchus Pic, 1913 sp.	French Guyana	G Couturier	(unknown Cercidae)	Fab. Cercideae	AY625331	AY625477	AY625428
Merobruchus Bridwell, 1946							
placidus (Horn, 1873)	Mex. Coxcatlan	N Alvarez	*Leucaena leucocephala*	Fab. Mimoseae	AY945965	AY947504	AY947512
Tuberculobruchus Decelle, 1951							
albizziarum (Decelle, 1958)	Sen. Cap Vert (I)	H Delobel	*Albizia lebbeck*	Fab. Ingeae	AY625325	AY635471	AY625422
natalensis (Pic, 1903)	Sen. Thies	H Delobel	*Acacia sieberiana*	Fab. Acacieae	AY625327	AY625473	AY625424
Subfamily Pachymerinae Bridwell, 1929							
Tribe Pachymerini Bridwell, 1929							
Pachymerus Thunberg, 1805							
cardo (Fahraeus, 1839)	French Guyana (I)	G Couturier	*Elaeis guineensis*	Arecaceae	AY390636	AY390668	AY390700

*Colombia (Col.), Ecuador (Ecu.), Egypt (Egy.), France (Fra.), Italy (Ita.), Kenya (Ken.), Kyrgistan (Kyr.), Mexico (Mex.), Nicaragua (Nic.), Panama (Pan.), Senegal (Sen.), Turkey (Tur.), Venezuela (Ven.), Vietnam (Vie); (I) introduced host plant.

†Host-plant systematics were abbreviated as follows: Fabaceae (Fab.), Malvaceae (Mal.).

‡With high probability (A. Delobel, personal communication).

polymerase chain reaction (PCR) products for the two other genes. PCR products were purified by using QIAGEN purification kits. Both strands of the PCR products were sequenced by the Sanger dideoxy method and sequence data were obtained by analysing samples on ABI 373 and ABI 3100 automated sequencers (Applied Biosystems). The new sequences generated in this study were deposited in GenBank (see Table 1 for Accession nos). Specimens corresponding to this study are kept in the collection of the Institut de Recherche pour le Développement (IRD) (MNHN, Paris, France) and in the Centre d'Ecologie Fonctionnelle et Evolutive (CEFE) (Montpellier, France).

Phylogenetic analyses

The alignment of coding sequences (cytochrome *b* and cytochrome *c* oxidase subunit I fragments) was trivial, as no gap event was detected. Minor differences in the length of 12S rRNA sequences were observed, and their alignments were performed by using CLUSTAL_X (Thompson *et al*. 1997) with default settings. After alignment, the combined sequence data set was 2216 bp in length: (i) the sequenced cytochrome *b* region contained 782 characters, 305 of which were parsimony informative; (ii) the sequenced cytochrome *c* oxidase I region contained 1018 characters, 375 of which were parsimony informative; (iii) the sequenced 12s rRNA region contained 416 characters, 171 of which were parsimony informative (gaps were treated as a fifth character). To estimate phylogenetic relationships among taxa, we carried out parsimony analyses, using PAUP* version 4.0b10 (Swofford 2003), and Bayesian inferences, using MRBAYES version 3.0b4 (Huelsenbeck & Ronquist 2001).

All parsimony analyses were performed using heuristic search option [tree-bisection–reconnection (TBR), random sequence addition, MaxTrees: 500] with 1000 random-addition replicates. We first conducted separate analyses of the three data sets. Congruence between the data sets for the three gene was assessed by using the incongruence length difference (ILD) test (Farris *et al*. 1994), as implemented in PAUP*, with 1000 replicates and all invariant characters excluded (Cunningham 1997). Although several sequences were missing for some specimens (only the 12s rRNA data set was complete) we have analysed the combined data set following Wiens (1998), who demonstrated, by performing multiple analyses of simulated data sets, that the addition of an incomplete data set to a data set complete for other markers is more likely to increase than decrease the phylogenetic accuracy. The robustness of topologies was assessed by bootstrap procedures (1000 replicates) and the estimation of decay indexes (Bremer 1994) using TREEROT 2.0 (Sorenson 1999). Preliminary analyses were also conducted to estimate partitioned Bremer support (PBS) values (Baker & DeSalle 1997) for each data set (with TREEROT). However, the incompleteness of the cytochrome *b* and cytochrome *c* oxidase I data sets resulted in an underestimation of PBS values. As a result, decay indexes and summed PBS values exhibited major discrepancies (e.g. summed PBS values were negative for some nodes) so that we have chosen to present only decay indexes on the tree corresponding to the combined data set analysis under parsimony.

For Bayesian inference, we performed partitioned Bayesian analyses (Jordal & Hewitt 2004; Nylander *et al*. 2004) on the combined data set. Under this approach, the computational efficiency of the Bayesian Markov chain Monte Carlo (MCMC) method allows the use of more realistic and complex evolutionary models for each data partition (Nylander *et al*. 2004). For each defined partition (one partition per gene sequenced), the best-fit substitution model was determined by MODELTEST 3.06 (Posada & Crandall 1998) through hierarchical likelihood-ratio tests (LRTs). We subsequently used four Metropolis-coupled chains with incremental heating in four distinct runs of 2 000 000 generations. Distinct parameters were estimated for each partition defined under the appropriate best-fit substitution models previously determined. During the run process, trees were saved to a file every 100 generations (20 000 trees were thus saved at the end of each MCMC run). Overall, model likelihood scores were further plotted against generations of the chains in order to determine the burn-in period. The results were presented in the form of a 50% majority-rule consensus tree (in which trees corresponding to the burn-in period were discarded) and the support for the nodes of this tree was given by posterior probability estimates for each clade. In addition, the branch lengths of this topology were estimated through maximum likelihood by using the best-fit substitution model determined by MODELTEST.

Character optimizations and hypothesis testing

To study the evolutionary history of several characters of interest, we used the program MESQUITE 1.05 (Maddison & Maddison 2004), which allows the reconstruction of ancestral character states under maximum likelihood. In comparison with parsimony optimizations the likelihood reconstruction method has the advantage of providing, for each node, a probability estimate for each ancestral character state (Jordal & Hewitt 2004). This state assignment maximizes the probability of arriving at the observed states in the terminal taxa (for a given model of evolution) and it allows the states at all other nodes to vary (Maddison & Maddison 2004). Two independent optimizations were performed using likelihood reconstruction: (i) character optimization of host-plant associations at the plant subfamily level; (ii) character optimization of host-plant associations at the plant tribal level (for the legume feeders only). Owing to the high level of host-plant specificity of the sampled

bruchid species (all the species studied were associated with only one botanical family, subfamily and tribe) the character coding of host-plant associations was facilitated and no polymorphic characters were included in the analyses. Since the results of character optimizations are highly dependent on the robustness of the phylogenetic hypotheses available, we chose to only perform character optimizations on the tree resulting from the Bayesian analyses.

To test several competing evolutionary hypotheses we generally used a likelihood-based statistical test, the SH test (Shimodaira & Hasegawa 1999; Goldman *et al*. 2000), as implemented in PAUP* (RELL method; 1000 replicates). This test was used to see if the difference between an optimal tree (resulting from an unconstrained analysis under likelihood) and a constrained tree (e.g. a tree in which some groups of species were constrained to be monophyletic) was significant. SH tests were used to test hypotheses regarding biogeography and evolution of host-plant associations and to evaluate the monophyly of the genera *Acanthoscelides* and *Bruchidius*. In addition, Wilcoxon signed rank tests (Templeton 1983), as implemented in PAUP*, were conducted to test the possible paraphyly of both genera under parsimony.

Estimation of divergence times

To estimate ages of nodes we first tested the hypothesis of a molecular clock for the combined data set by implementing an LRT that compares the likelihood score of the Bayesian phylogenetic hypothesis with (L_1) and without (L_0) the molecular clock enforced (the best-fit model of evolution selected by MODELTEST was used). As the LRT rejected the hypothesis of overall rate homogeneity [$-2(\ln L_1 - \ln L_0) = 188.68$, d.f. = 74, $P = 95.081$], we further estimated an ultrametric tree using the nonparametric rate smoothing (NPRS) method of Sanderson (1997), as implemented in TREEEDIT (Rambault & Charleston 2002; http://evolve.zoo.ox.ac.uk). This penalized likelihood method is appropriate for data sets that depart from a molecular clock as it smoothes the rapidity of rate change among lineages (in the same way as smoothing techniques in regression analyses). For the timescale estimation, we used the standard rate estimate [2% per million years (Myr)] for mitochondrial DNA evolution in arthropods (Brower 1994). In addition, standard errors for estimates of node age were calculated for seven nodes (corresponding to possible vicariant events) by using the bootstrap resampling method under PAUP* (no swapping option; topological constraints enforced; 100 replicates). For each of the 100 resulting trees a distribution of the variation in node height was created and used to obtain the standard error for the estimate of node age.

Furthermore, to test the adaptive radiation hypothesis, we calculated rates of diversification ($r_{0.0}$ and $r_{0.9}$) of both genera as a whole by using Magallon & Sanderson's (2001) equation for a crown group age. Estimates of branch lengths were also used to investigate this hypothesis.

Evaluation of host-plant associations

Associations were determined both by sampling seeds of potential host plants in the field with subsequent monitoring of adult emergences (e.g. Janzen 1980; Gillon *et al*. 1992; Silvain & Delobel 1998; Jermy & Szentesi 2003; Kergoat *et al*. 2004, 2005), and by critical examination of the available literature. A summary of known host-plant associations (based on literature and field data) for the genera *Acanthoscelides* and *Bruchidius* is provided in Table 2. Host-plant records for *Acanthoscelides* were intensively and rigorously investigated by Johnson (e.g. see Johnson 1989). He published major studies on species of North and Central America (Johnson 1970, 1983) and of northern South America (Johnson 1990b). However, there is still a gap in knowledge concerning southern South American species. For *Bruchidius*, many studies on specific groups of species and local and regional faunas are available (e.g. Hoffmann 1945; Lukjanovich & Ter-Minassian 1957; Arora 1977), but must be used cautiously if they are not explicitly based on rearing. A major review on the family Bruchidae (Udayagiri & Wadhi 1989) that includes extensive host-plant information was used as a major source of information for *Bruchidius* species, but in a very conservative way, so as to avoid many unreliable and unverified records (Delobel & Delobel 2003; Jermy & Szentesi 2003). Indeed, numerous host-plant records in this study were doubtful, and names for many bruchids and host-plants were outdated. Therefore, we have only included questionable host-plant records if they have been reported by at least two independent sources. Data from 12 other studies were also included in our evaluation of host-plant associations (Janzen 1980; Borowiec 1988; Johnson 1990c; Morimoto 1990; Gillon *et al*. 1992; Delobel & Tran 1993; Johnson & Siemens 1995; Anton 1998; Anton & Delobel 2003; Delobel & Anton 2003; Delobel & Delobel 2003; Delobel *et al*. 2004). Supplementary information on host-plant biogeography and specific host-use in some bruchid taxa were also provided. The International Legume Database and Information Services (www.ildis.org) database was used to update host-plant names from the literature and as a source of information on host-plant biogeography and species richness.

Results

Phylogenetic hypotheses

Separate analyses performed under parsimony yielded poorly resolved topologies (not shown). The ILD test was

Table 2 Host-plant use for the *Acanthoscelides* and *Bruchidius** genera

Host-plant systematics	Host-plant genus†	Host-plant distribution (by genus)	Estimated number of bruchid species‡	
			Acanthoscelides spp.	*Bruchidius* spp.
Family Apiaceae			0	1
Family Cistaceae			1	3
Family Fabaceae				
Subfamily Caesalpinioideae				
Tribe Caesalpinieae	*Delonix* (3)	Old World	0	1
	Hoffmannseggia (26)	New World–Old World	1	0
Tribe Cassieae	*Apuleia* (1)	New World	3	0
	Cassia (73)	New World–Old World	1	1
	Dialium (30)	Old World	0	3
	Senna (234)	New World–Old World	5	4
Subfamily Mimosoideae				
Tribe Acacieae	*Acacia* (431)	New World–Old World	3	45
Tribe Ingeae	*Albizia* (70)	New World–Old World	0	22
	Faidherbia (1)	Old World	0	4
Tribe Mimoseae	*Desmanthus* (24)	New World	5	0
	Dichrostachys (5)	Old World	0	3
	Leucaena (25)	New World	4	0
	Mimosa (523)	New World–Old World	19	1
	Piptadenia (32)	New World	2	0
	Prosopis (45)	New World–Old World	1	1 (I)
Tribe Parkieae	*Parkia* (31)	New World–Old World	7	0
Subfamily Papilionoideae				
Tribe Aeschynomeneae	*Aeschynomene* (170)	New World–Old World	4	2
	Chaetocalyx (13)	New World	1	0
	Nissolia (14)	New World	2	0
	Stylonsanthes (41)	New World–Old World	1	0
Tribe Amorpheae	*Amorpha* (16)	New World	3	0
	Dalea (170)	New World	7	0
	Errazurizia (4)	New World	1	0
	Parryella (1)	New World	1	0
Tribe Cicereae	*Cicer* (31)	Old World	1 (I)	2
Tribe Cytiseae	*Adenocarpus* (15)	Old World	0	2
	Argyrocytisus (1)	Old World	0	1
	Calicotome (4)	Old World	0	2
	Cytisophyllum (1)	Old World	0	2
	Cytisus (62)	Old World	0	8
	Genista (114)	Old World	0	4
	Laburnum (2)	Old World	0	4
	Lupinus (463)	New World–Old World	0	2
	Petteria (1)	Old World	0	2
	Spartium (1)	Old World	0	4
Tribe Desmodieae	*Desmodium* (268)	New World–Old World	13	7
	Lespedeza (29)	New World–Old World	1	1
	Pseudarthria (5)	Old World	0	1
Tribe Galegeae	*Astragalus* (1399)	New World–Old World	7	13
	Alhagi (4)	Old World	0	3
	Galega (6)	Old World	0	1
	Glycyrrhiza (17)	New World–Old World	2	5
	Halimodendron (1)	Old World	0	1
	Oxytropis (173)	New World–Old World	1	2
	Sphaerophysa (2)	Old World	0	1

Table 2 *Continued*

Host-plant systematics	Host-plant genus†	Host-plant distribution (by genus)	Estimated number of bruchid species‡	
			Acanthoscelides spp.	*Bruchidius* spp.
Tribe Hedysareae	*Hedysarum* (69)	New World–Old World	1	2
	Onobrychis (117)	Old World	0	7
Tribe Indigofereae	*Indigofera* (554)	New World–Old World	5	11
Tribe Loteae	*Acmispon* (8)	New World	1	0
	Anthyllis (22)	Old World	0	2
	Coronilla (9)	Old World	0	3
	Dorycnium (8)	Old World	0	1
	Hippocrepis (29)	Old World	0	1
	Hosackia (11)	New World	1	0
	Hymenocarpos (1)	Old World	0	1
	Lotus (116)	Old World	0	2
	Ornithopus (6)	New World–Old World	0	2
	Ottleya (12)	New World	1	0
	Scorpiurus (3)	Old World	0	1
	Securigera (13)	Old World	0	3
	Syrmatium (14)	New World	1	0
Tribe Milletieae	*Tephrosia* (307)	New World–Old World	3	3
Tribe Phaseoleae	*Cajanus* (17)	New World	1 (I)	0
	Calopogonium (9)	New World	6	0
	Eriosema (146)	New World–Old World	2	0
	Flemingia (13)	New World	1 (I)	0
	Galactia (98)	New World	2	0
	Lablab (2)	Old World	1 (I)	2
	Macroptilium (14)	New World	1	0
	Pachyrhizus (4)	New World	2	0
	Phaseolus (36)	New World–Old World	4	0
	Rynchosia (221)	New World–Old World	12	0
	Teramnus (8)	New World–Old World	2	1
	Vigna (86)	New World–Old World	5	1
Tribe Robinieae	*Sesbania* (52)	New World–Old World	2	6
Tribe Trifolieae	*Medicago* (74)	Old World	0	5
	Trifolium (227)	New World–Old World	3	10
	Trigonella (72)	Old World	0	2
Tribe Vicieae	*Lathyrus* (127)	New World–Old World	1 (I)	1
	Lens (4)	Old World	1 (I)	2
	Pisum (3)	Old World	1 (I)	3
	Vicia (193)	New World–Old World	1 (I)	5
			1	0
Family Lythraceae				
Family Malvaceae				
Subfamily Malvoideae			31	0
Subfamily Sterculioideae			2	0
Subfamily Tilioideae			7	0
Family Onagraceae			1	0
Family Rhamnaceae			1	0
Family Verbenaceae			1	0

*Including *Decellebruchus* spp. and *Tuberculobruchus* spp.
†The estimated numbers of species for each plant genus are given in parentheses.
‡(I): Introduced host plant. Although the corresponding plant genus originates from the other area of distribution (New World or Old World), a bruchid species has managed to develop upon it.

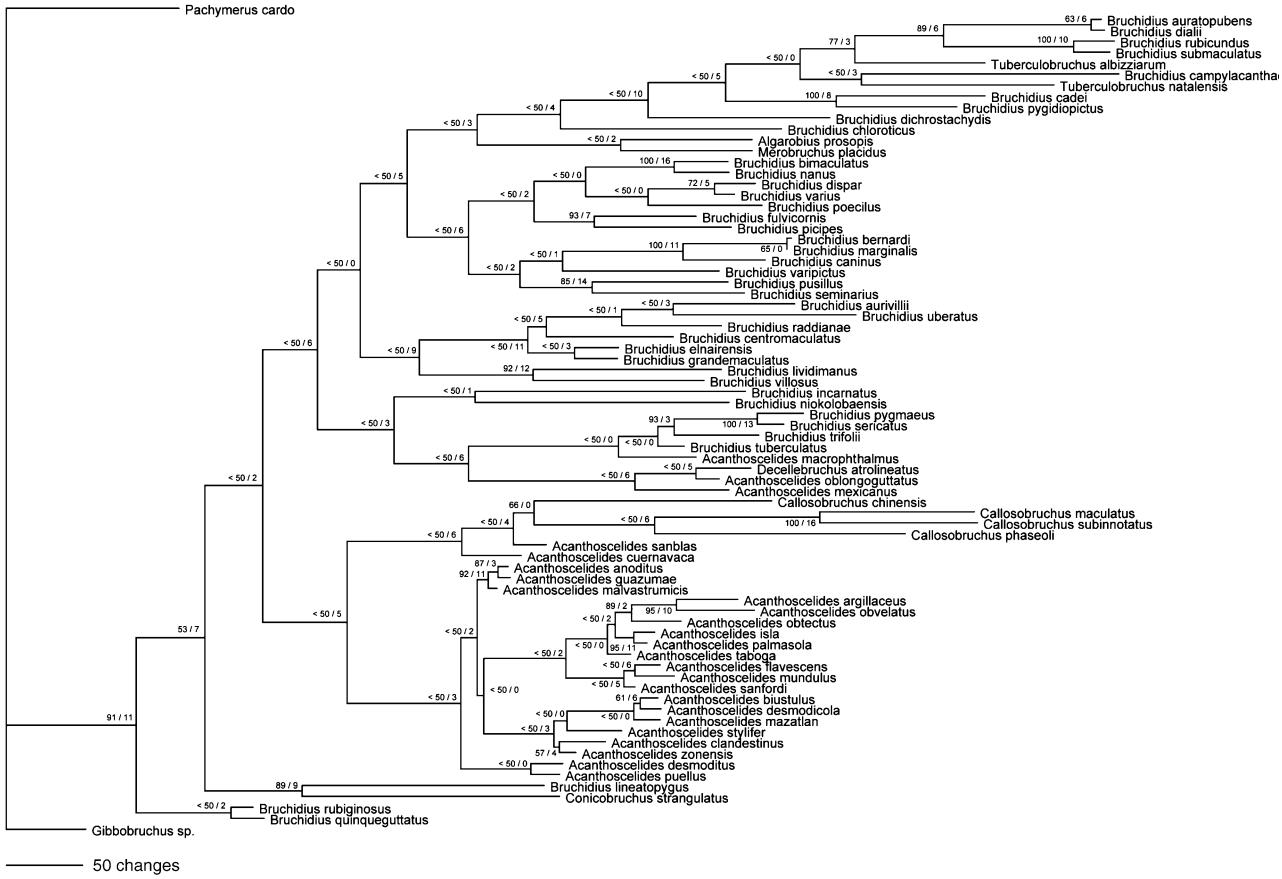

Fig. 1 Most-parsimonious tree (7375 steps; CI = 0.236; RI = 0.360) from the unweighted parsimony analysis of the combined data set (heuristic search option with 1000 random-addition replicates). Numbers adjacent to nodes give bootstrap support values greater than 50% calculated for 1000 replicates and secondly decay indexes.

not significant ($P = 0.674$) and thus supported the combination of the data sets for the three genes. The analysis of the combined data set yielded a single most-parsimonious tree (7375 steps; CI = 0.236; RI = 0.360), which is presented in Fig. 1. Overall, this topology is not well supported, as only 20 of 73 nodes are supported by bootstrap values greater than 70% and only 29 of 73 nodes are supported by decay indexes greater than 5.

For Bayesian analyses, the same model of evolution was selected by the LRTs for the three defined partitions, namely the general time reversible (GTR) model with a proportion of invariable sites and a gamma distribution. After four distinct runs of 2 000 000 generations, a burn-in period of 100 000 generations was identified, by plotting graphically likelihood values for each generation. The 1000 trees corresponding to this burn-in period were subsequently not retained in the 50% majority-rule consensus trees. Three of the four analyses yielded the same consensus trees (Fig. 2) but one of the four runs resulted in another topology, which only differs by the position of one species (not shown; SH test not significant). Basal and terminal nodes are well supported in this phylogenetic hypothesis but some internal nodes are only weakly supported (less than 50%), probably because of the missing sequences of the dried specimens.

Both *Acanthoscelides* and *Bruchidius* appear paraphyletic in all analyses, in agreement with the views of several authors (Johnson 1981; Borowiec 1987; A. Delobel, personal communication). Within *Acanthoscelides*, two clades are recovered under Bayesian inference, whereas parsimony analyses suggested a more scattered pattern. Within *Bruchidius*, the majority of the sampled species are included in a major clade that also includes other representatives from the tribe Acanthoscelidini. In all analyses, the genus *Tuberculobruchus* appears paraphyletic, as the two sampled species of *Tuberculobruchus* are included within a clade of morphologically similar *Bruchidius* species, which present similar genitalia (Kergoat & Silvain 2004). Interestingly, one *Bruchidius* species, *Bruchidius lineatopygus*, is found grouped, with strong support (posterior probability of 100%; bootstrap of 89%; decay index of 9), with the sole studied representative of the genus *Conicobruchus* in a very basal position. Although Bayesian and parsimony analyses recovered similar relationships to a large extent, some

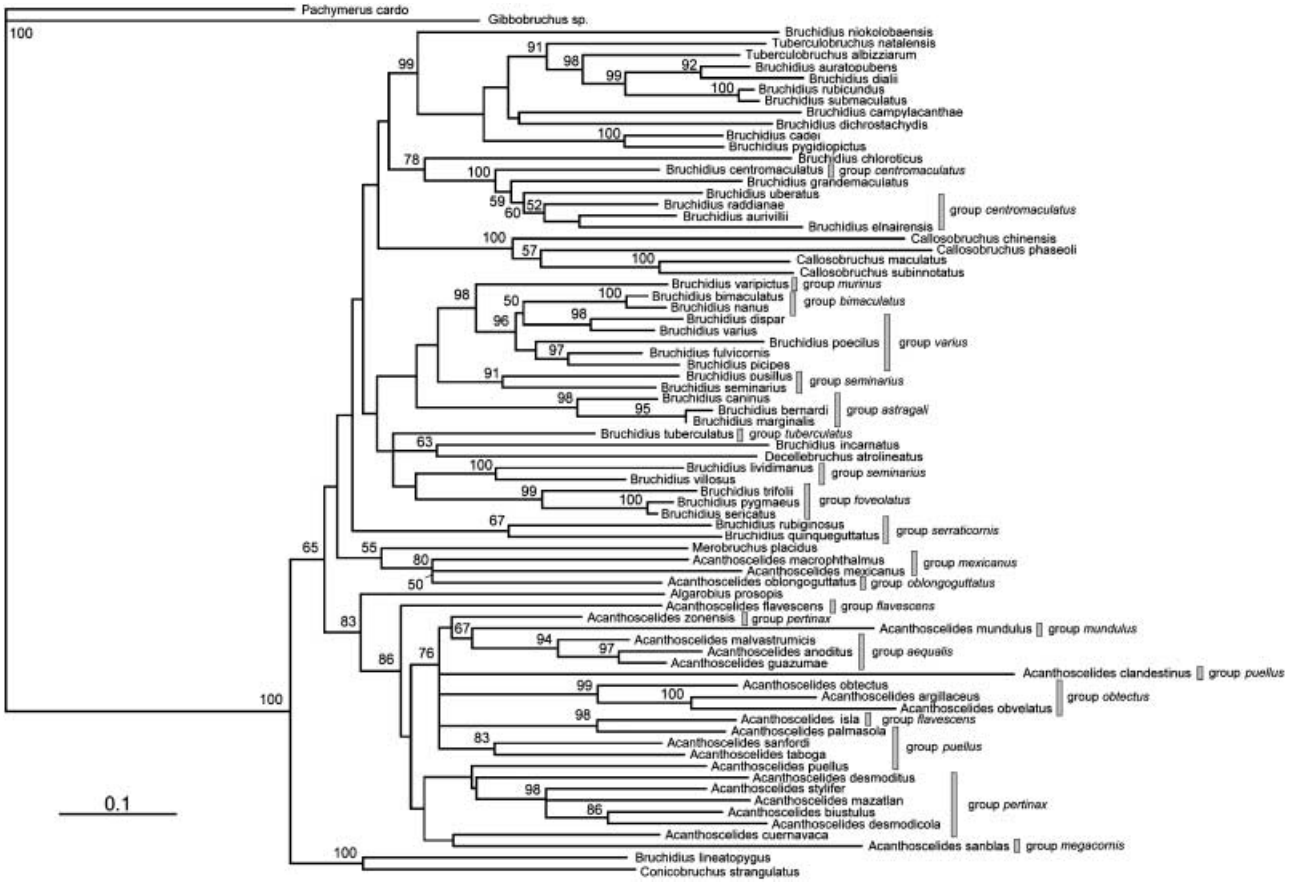

Fig. 2 Single tree from the partitioned Bayesian inference analysis of the combined data set (topology identical to the 50% majority-rule consensus tree). Branch lengths were estimated through maximum likelihood by using the general time reversible (GTR) model with a proportion of invariable sites and a gamma distribution. Numbers adjacent to nodes give Bayesian posterior probabilities of nodes greater than 50%. In addition, on the right, extant taxonomic groups are figured.

major discrepancies were found between them, as indicated by a significant SH test ($P = 0.016$). For instance, under Bayesian inference, *Decellebruchus atrolineatus* is related to a *Bruchidius* species whereas it is related to an *Acanthoscelides* species under parsimony. In comparison with the tree obtained with Bayesian analyses, the phylogenetic hypothesis from the combined analysis under parsimony appears less resolved, as fewer nodes are well supported. The parsimony tree also shows greater conflicts with the systematic propositions (i.e. taxonomic groups) based on morphological data (see Table 3). It thus appears that the results of Bayesian analyses provide a clearer view of the phylogeny of the tribe Acanthoscelidini, and a clearer circumscription of the genera *Acanthoscelides* and *Bruchidius*, than do parsimony analyses.

Character optimization and hypothesis testing

In Fig. 3, character histories of host-plant preferences are mapped onto a mirror-image cladogram. On the left cladogram, the character history of host-plant preferences at the subfamily level indicates a fairly high conservative pattern of host-plant use (phylogenetically related insects are generally associated with phylogenetically related host plants). Our results show two unambiguous independent host shifts onto Malvaceae (each time to a distinct subfamily) for New World species of *Acanthoscelides*. Regarding the species associated with the Fabaceae, only five unambiguous independent host shifts have occurred at the subfamily level. Interestingly, the latter events have always involved a shift from the subfamily Papilionoideae toward subfamilies Caesalpinioideae or Mimosoideae. Thus, the ancestral host plants for the lineage represented by the sampled species of *Acanthoscelides* and *Bruchidius* appear to have been members of the subfamily Papilionoideae. The latter assumption is strongly supported by a probability of 97% under likelihood ancestral state reconstruction. On the right cladogram, the character history of host-plant preferences at the tribe level (for the studied legume feeders) is illustrated and suggests a more dynamic pattern. This is well illustrated

Table 3 Host-plant preference for existing *Acanthoscelides* and *Bruchidius* taxonomic groups

Taxonomic groups*	References†	Host-plant preference‡	
(*Acanthoscelides*)			
aequalis	Johnson 1983	subfamily Malvoideae	(exclusively)
albopygus	Johnson 1983	tribe Acacieae	(mainly)
blanchardi	Johnson 1983	subfamily Malvoideae	(mainly)
chiricahuae	Johnson 1983	tribe Mimoseae	(exclusively)
flavescens	Johnson 1983	tribe Phaseoleae	(mainly)
megacornis	Johnson 1983	subfamily Tilioideae	(mainly)
mexicanus	Johnson 1983	tribe Mimoseae	(mainly)
mundulus	Johnson 1983	tribe Aeschynomeneae	(exclusively)
obtectus	Johnson 1983	tribe Phaseoleae	(exclusively)
pertinax	Johnson 1983	tribe Desmodieae	(mainly)
puellus	Johnson 1983	tribe Phaseoleae	(mainly)
quadridentatus	Johnson 1983	tribe Mimoseae	(mainly)
(*Bruchidius*)			
astragali	Borowiec 1988; Delobel *et al*. 2004	tribe Galegeae	(exclusively)
bimaculatus	Borowiec 1988	tribe Trifolieae	(exclusively)
centromaculatus	Anton & Delobel 2003	tribe Acacieae	(exclusively)
foveolatus	Borowiec 1988	tribe Trifolieae	(mainly)
glycyrrhizae	Borowiec 1988	tribe Galegeae	(exclusively)
murinus	Borowiec 1988	tribe Trifolieae	(exclusively)
seminarius	Borowiec 1988; Anton 1998	various tribes	
serraticornis	K.-W. Anton, personal communication	tribe Cytiseae	(mainly)
tibialis	Borowiec 1988	tribe Trifolieae	(exclusively)
unicolor	Borowiec 1988; A. Delobel, personal communication	tribe Hedysareae	(mainly)
varius	Borowiec 1988	tribe Trifolieae	(mainly)

*Only taxonomic groups with more than one species and those for which host plants are known for at least two species were studied.
†Sources used to circumscribe the taxonomic groups.
‡(mainly) Indicates that more than 50% of the species were associated with a specific plant group; host-plant data were missing for about 20% of the species belonging to the studied taxonomic groups.

for the species associated with the tribe Phaseoleae, in which character optimization indicates three independent colonization events but also three secondary losses of plant use. Several gain and loss events have also occurred for the species associated with tribes Desmodieae and Trifolieae, but to a lesser extent.

In addition to the previous results, the phylogenetic framework allows investigation of the biogeographical patterns of the sampled species. Most of the sampled species group into two large clades, each of which is restricted either to the New World or the Old World (see Fig. 4). The first clade includes *Algarobius prosopis* and all *Acanthoscelides* species with the exception of the *Acanthoscelides* species associated with Mimosoideae that in both parsimony and Bayesian inference are separated from the main clade of *Acanthoscelides*. The second clade groups all *Bruchidius* species (with the exception of *Bruchidius lineatopygus*), but also the sampled members of genera *Callosobruchus*, *Decellebruchus* and *Tuberculobruchus*. The results of the character optimization of the geographical distribution of species under maximum likelihood suggest that the observed pattern for the Old World species results from two distinct vicariant events (see Fig. 4). However, the hypothesis of a single vicariant event cannot be excluded, as the result of a SH test in which Old World species are constrained to form a monophyletic group is not significant ($P = 0.165$).

Regarding the hypothesis of the monophyly of genera *Acanthoscelides* and *Bruchidius*, both SH and Wilcoxon signed rank tests were significant for the genus *Bruchidius* ($P = 0.001$ and $P < 0.0001$, respectively). For the genus *Acanthoscelides*, unlike the Wilcoxon signed rank test ($P = 0.0472$) the SH test was not significant ($P = 0.105$). As a consequence, although the paraphyly of this genus is strongly suggested, the hypothesis of monophyly for this genus cannot be totally excluded.

Estimation of divergence times

We present in Fig. 4 a timescaled NPRS ultrametric tree estimated under TREEEDIT, using the topology estimated through Bayesian analyses and initial branch lengths estimated under maximum likelihood (Ribera *et al*. 2004). Our dating indicates an older origin (~70 Myr) for bruchids

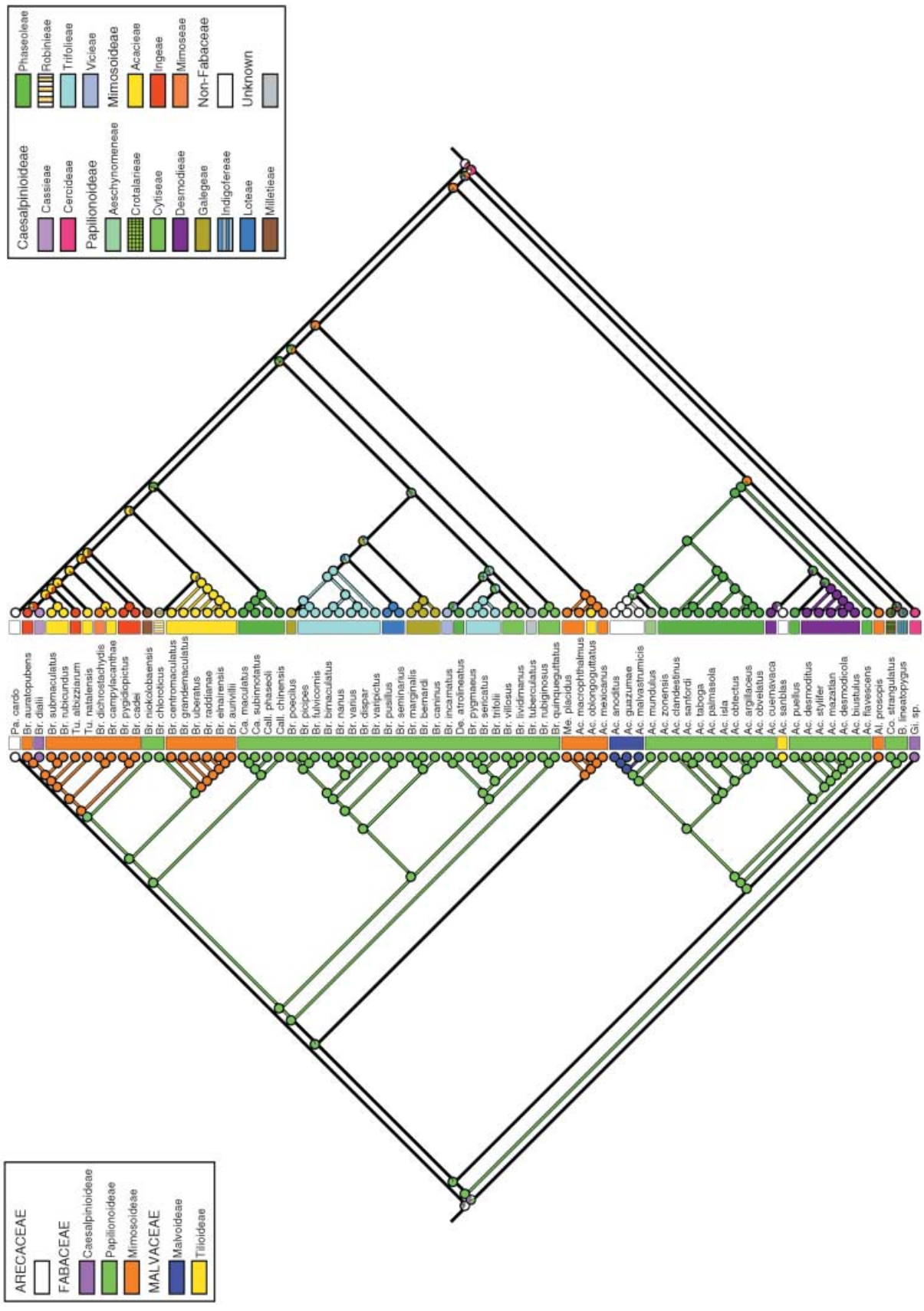

Fig. 3 Mirror image of the 50% majority-rule consensus tree from the partitioned Bayesian inference analysis of the combined data set. On the left cladogram the character history of host-plant preferences at the plant subfamily level is figured. On the right cladogram the character history of host-plant preferences at the plant tribe level is figured. Ancestral character states were reconstructed under MESQUITE using likelihood reconstruction (probabilities of character states are figured at the nodes with coloured pie diagrams). Detailed legends for both character optimizations are provided on the top of the figure. In addition, between trees vertical bars with the corresponding patterns show the actual host-plant associations.

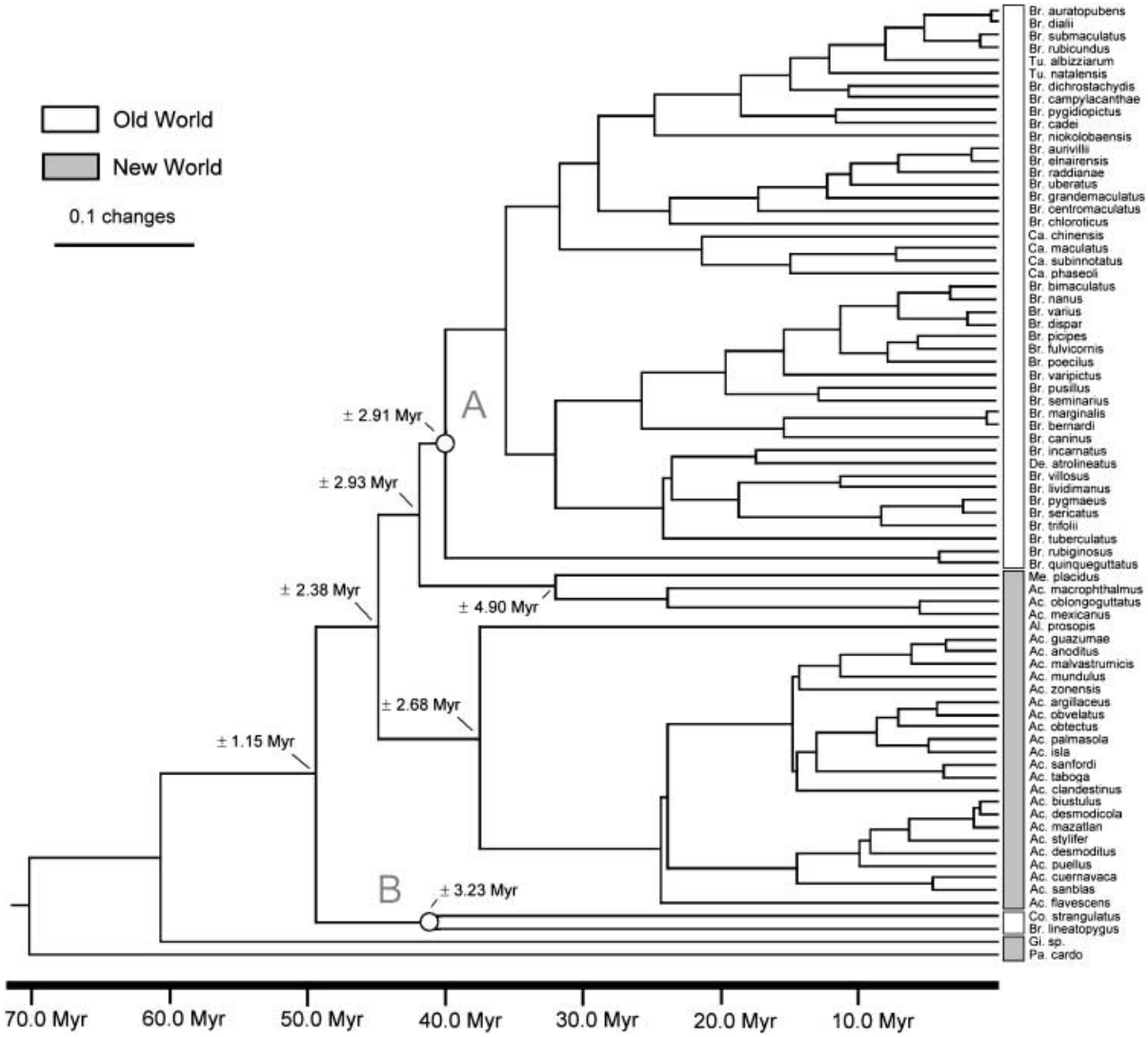

Fig. 4 Time-calibrated NPRS ultrametric tree estimated under TREEEDIT, using the topology estimated through partitioned Bayesian inference analyses and initial branch length estimates obtained by using maximum likelihood. Standard errors for estimates of node age are figured for seven important nodes. On the right vertical bars show the distribution of the species. Hypothetical vicariant events are also figured (A and B).

than suggested by the extant fossil record, as the oldest known bruchid fossils (assigned to tribe Pachymerinae) only date from the early Eocene some 50 million years ago (Ma) (Archibald & Mathewes 2000). Our dating is consistent with the estimation of bruchid divergence times from previous molecular studies that also suggest a late Cretaceous–early Tertiary origin (Farrell 1998; Farrell & Sequeira 2004). This older origin is particularly interesting from an evolutionary perspective, as it suggests that the diversification of seed-beetles could have occurred nearly at the same time as that of their legume host plants (Herendeen *et al*. 1992).

On the basis of a crown group age estimate of 49.5 Myr, and on an estimated diversity of 600 species (for *Acanthoscelides* plus *Bruchidius*), we estimated a maximum diversification rate ($r_{0.0}$) of 0.050 net speciation events per Myr in the absence of extinction and a minimum rate ($r_{0.9}$) of 0.035 net speciation events per Myr under a high relative extinction rate.

Patterns of host-plant associations in Acanthoscelides *and* Bruchidius

Our results indicate that the two genera share a similar pattern in their host-plant use. Host-plant records indicate that most species of *Acanthoscelides* and *Bruchidius* are specialists that feed on a limited number of host plants. At the plant family level, each of the *Acanthoscelides* and *Bruchidius* species samples exhibited a strong specificity, feeding exclusively on seeds of species from a single plant

family. They are also mostly associated with the family Fabaceae, like most bruchids. This trend is stronger for the genus *Bruchidius*, in which only four species are known to feed outside the Fabaceae. For *Acanthoscelides*, the known host range is wider as valid host-plant records are known for six other plant families, most frequently Malvaceae.

Regarding species associated with the family Fabaceae, a closer look at the subfamily level reveals once again a high degree of specificity for species of both genera. In both, each species feeds exclusively on a single subfamily, with the exception of *Acanthoscelides compressicornis*, which is known to feed on both *Desmanthus* (Mimosoideae) and *Hoffmanseggia* (Caesalpinioideae). Fewer than 10 species of *Acanthoscelides* and *Bruchidius* are known to feed on members of the subfamily Caesalpinioideae, whereas dozens of species are associated with subfamilies Mimosoideae or Papilionoideae. For these latter subfamilies, important differences in host use pattern are noticeable. Indeed *Acanthoscelides* and *Bruchidius* associated with Mimosoideae predominantly feed on three genera, namely *Acacia*, *Albizia* and *Mimosa*, whereas species associated with Papilionoideae do not exhibit such strong preferences and are associated with 66 distinct genera of host plants.

At the tribal level, the previously observed taxonomic conservatism in host-plant use is still evident, and most species feed on plant species belonging to a single botanical tribe. A total of 14 papilionoid tribes are exploited by *Acanthoscelides* and *Bruchidius* species, and 12 of these 14 tribes are used by both genera. Careful examination of the pattern of host-plant distribution shows that when a host-plant genus occurs in both the New World and Old World, it is generally attacked by both *Bruchidius* (Old World) and *Acanthoscelides* (New World). This finding is consistent with the suggestion of Borowiec (1987) that the two genera seem to occupy similar ecological niches in their respective areas of distribution.

Discussion

Phylogeny and taxonomy

Although this study focuses on only 76 species, our results provide valuable information on bruchid phylogeny and taxonomy. Our molecular analyses support the paraphyly of genera *Bruchidius* and *Tuberculobruchus* and, to a large extent, that of *Acanthoscelides* (given the results of the SH test). Species of *Callosobruchus* and *Decellebruchus* are found within the clade that groups the majority of *Bruchidius* species. Members of these two genera are morphologically very similar to certain *Bruchidius* species that are closely related to them in our analyses. They are distinguishable only by the structure of the hind femur (for *Callosobruchus*) and of the antenna in males (for *Decellebruchus*). All these results suggest the utility of either redefining a larger genus *Bruchidius* or further splitting this genus into smaller monophyletic genera. The status of the basal *Bruchidius lineatopygus* is also questionable and we advocate its inclusion within a larger redefined genus *Conicobruchus*. Regarding *Acanthoscelides* species, two groups seem to be well differentiated. Interestingly, the position of *Merobruchus placidus* as the sister taxon of one of the two clades of *Acanthoscelides* is consistent with morphological evidence (J. Romero, personal communication). Further studies should investigate larger samples of *Acanthoscelides* to clarify relationships within the genus. The boundaries of some extant taxonomic groups should also be investigated in view of our results. For example, the inclusion of *Bruchidius grandemaculatus* and *Bruchidius uberatus* within the group *centromaculatus* should be studied closely from a morphological point of view.

Evolution of host-plant use and role of host-plant chemistry

Our mapping of host-plant preferences onto bruchid phylogeny underlines the strong taxonomic conservatism of host use, which is shared by both of the genera studied. Within the species specialized on Fabaceae, only a few species, such as *Acanthoscelides compressicornis*, are known to develop on host plants belonging to more than one tribe or subfamily. For these species, the extant patterns of host-plant use may be better explained by an expansion of host-plant range (C. D. Johnson, personal communication). The majority of species are specialized on a given host-plant genus or tribe and most host shifts have occurred between related host plants. However, a few host shifts between distantly related plants, followed by successful diversifications, have occurred in the evolutionary history of the two genera. This quite dynamic pattern underlines the fact that there is no evidence of co-speciation processes between either *Acanthoscelides* or *Bruchidius* and their respective host plants. Thus, for our sample, at least two major host shifts between distinct plant families, and five major host shifts between distinct subfamilies (for the Fabaceae) are revealed. Moreover, character optimizations of plant families and subfamilies (for the Fabaceae) suggest that the subfamily Papilionoideae is the ancestral host-plant group of *Acanthoscelides* and *Bruchidius*, at least for the species we sampled. Nonetheless, hasty generalization of this finding is unwarranted, since our sample represents only a subset of the known species of these two bruchid genera.

The integration of reliable host-plant records for a large number of species of *Acanthoscelides* and *Bruchidius* permits discussion of the evolution of host-plant use at a larger scale. In view of our evaluation of host-plant associations, the trend suggested by the mapping of host-plant prefer-

ences onto bruchid phylogeny is likely to fit with all *Acanthoscelides* and *Bruchidius* as well. Indeed, in both genera, related species belonging to the same taxonomic group are generally associated with host plants of the same tribe (if they develop on Fabaceae) or family (if they develop on hosts other than the Fabaceae) as shown in Table 3. We can thus assume that conservatism in host use and specialization has strongly influenced the evolution of the two genera. Our molecular analyses also suggest that a total of at least four lineages of *Acanthoscelides* and *Bruchidius* have radiated independently on various host-plant groups. Yet, our sampling certainly does not encompass the full extent of these successful diversifications, particularly for the clade that is represented only by *Bruchidius lineatopygus* and *Conicobruchus strangulatus*. These independent radiations have occurred on similar host plants (belonging to the same taxonomic groups) in the New World and the Old World and thus suggest that *Acanthoscelides* and *Bruchidius* have undergone a parallel evolution in their history, successfully colonizing the same niches (i.e. new food resources) in their respective areas of distribution. The occurrence of multiple independent host shifts between distinct plant families, and between distinct subfamilies (for the Fabaceae), is suggested not only by our character optimization but also by many host-plant records from the literature. We can suppose that these host shifts between distantly related host plants have played an important role in the successful diversification of these seed-beetles.

Host-plant chemistry is likely to be important in the evolution of host-plant use. Many secondary compounds (alkaloids, nonprotein amino acids, cyanogenic glycosides, lectins, proteinase inhibitors) are frequently found in seeds, and their toxicity against bruchids has been demonstrated by previous studies (Janzen *et al*. 1977; Janzen 1981; Birch *et al*. 1986; Gatehouse *et al*. 1990). Consequently the strong specificity and taxonomic conservatism in host use exhibited by most bruchids suggests that they are somewhat constrained to feed on chemically similar host plants. However, since host-plant chemistry is generally correlated with host-plant phylogeny, we can hardly exclude other factors that may constrain bruchid evolution (but see Kergoat *et al*. 2005). Insects feeding inside plant organs must be finely adapted to the morphology, the physiology and the chemistry of the host, and generally tend to be more constrained in host-plant choice than are externally feeding phytophagous insects (Bucheli *et al*. 2002; Farrell & Sequeira 2004). Furthermore, our results also suggest that some species have undergone host shifts to chemically dissimilar host plants (Wink & Mohamed 2003) (e.g. between plants belonging to the subfamilies Papilionoideae and Mimosoideae), a phenomenon that likely has involved the development of 'key innovations' in the form of detoxification mechanisms. Members of both *Acanthoscelides* and *Bruchidius* have independently developed abilities to feed on many toxic seeds. A striking example is the adaptation of many *Acanthoscelides* and *Bruchidius* species to the nonprotein amino acid L-canavanine, widespread in Papilionoideae (Bell *et al*. 1978). The mechanisms of detoxification of canavanine and other compounds (e.g. proteinase inhibitors) are now well understood (Bleiler *et al*. 1988; Rosenthal 1990; Oliveira *et al*. 2002) and suggest either widespread pre-adaptation to many toxic compounds or the independent evolution of similar 'key innovations' at several times.

Biogeography

Our analyses point to the existence of two large clades, one strictly New World, the other Old World in distribution. Using the timescale presented in Fig. 4, we can suppose that the separation between Old World and New World species occurred early in the history of the tribe Acanthoscelidini, some 50–40 Ma. The suggested early Tertiary period of divergence is consistent with biogeographical evidences (Sanmartin *et al*. 2001), which indicate that the observed basal splits could be accounted by two distinct vicariant events. First, the closure of the Tulean land bridge which connected Europe and North America (this land bridge was definitively closed some 50 Ma). Second, the progressive closure of the early Beringian Bridge which connected the eastern Palearctic with the western Nearctic. Yet, prior to his closure some 35 Ma (Sanmartin *et al*. 2001), the early Beringian Bridge was under the influence of major climate changes (from warm climates to colder ones) that have certainly posed intermittent barriers to the migration of non-cold-tolerant organisms such as seed-beetles. Interestingly our timing excludes the alternative hypothesis of an older vicariant event, found in other Chrysomeloidea (Becerra 2003), namely the breakup of West Gondwana (between Africa and South America) some 100 Ma.

Evidence for adaptive radiation in Acanthoscelides *and* Bruchidius*?*

Schluter (2000) has linked the concept of adaptive radiation to the following four major features: common ancestry, trait utility, phenotype–environment correlation and rapid speciation. At least two of these features are exhibited by the bruchids we have studied. Common ancestry is assessed by our phylogenetic analyses, whereas trait utility is supported by the multiple suggested 'key innovations' that are involved in detoxifying many toxic seed compounds (Bleiler *et al*. 1988; Rosenthal 1990; Oliveira *et al*. 2002). In the absence of strong morphological differentiation within bruchids, it is difficult to demonstrate a phenotype–environment correlation, even if the latter seems to be quite intuitive when examining larval structures involved in seed boring. The hypothesis of rapid speciation

is not supported by our estimated diversification rates that are significantly lower than the rates observed in fast-diversifying clades of organism (Magallon & Sanderson 2001). The hypothesis of rapid speciation is also not fully supported by our estimation of divergence times (Fig. 4) as branch lengths between ancestors are not short on the average. Yet, more precise information on more recent vicariant events are required to better constrain the ages of nodes under NPRS optimization to render our conclusions more precise.

Conclusions

This study has produced the first phylogenetic reconstruction for a large sample of the two largest genera of seed-beetles, allowing investigation of their evolutionary patterns. Despite some limitations due to sample size, we have clarified phylogenetic relationships within the tribe Acanthoscelidini and circumscribed more clearly the genera *Acanthoscelides* and *Bruchidius*. We have also shown a strong trend towards taxonomic conservatism in host use, despite the occurrence of several host shifts during the evolution of these two genera. Our phylogenetic analysis and our evaluation of host-plant associations both suggest that the two genera have undergone parallel evolution, as they have independently colonized similar host plants, and probably developed similar mechanisms of detoxification, in their respective areas of distribution. The more ancient than previously thought origin of seed-beetles that is suggested by our estimation of divergence times is consistent with the hypothesis of a radiation which could have occurred contemporaneously with the diversification of their legume host plants. However, adaptive radiation cannot yet be fully demonstrated because we lack sufficient evidence of rapid speciation.

Acknowledgements

This study is dedicated to the memory of Clarence Dan Johnson (northern Arizona University, Flagstaff, USA) who died on 28 March 2005. Clarence Dan Johnson made numerous studies on the co-evolution of plants and insects and was one of the greatest specialists on systematics, ecology and behaviour of seed-beetles. We are particularly grateful to him for many helpful comments on an earlier version of the manuscript. We are also indebted to Alex Delobel (IRD, Paris, France), Doyle McKey (CEFE-CNRS, Montpellier, France), Paul Sunnucks (Monash University, Australia) and two anonymous reviewers for valuable comments and corrections on the manuscript. This work would not have been possible without the help of all the collectors (listed in Table 1) who provide inestimable specimens.

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Gaël Kergoat's interest are centred on studying the evolutionary interactions between bruchids and their host-plants. Nadir Alvarez also works on legume/bruchid interactions, focusing on the Neotropical genus *Acanthoscelides*. Martine Hossaert-McKey is studying the evolutionary ecology of several plant/insect systems. Nathalie Faure and Jean-François Silvain are investigating the ecological factors influencing the evolution of phytophagous insects associated with cultivated tropical crops.
