Phylogenetic relationships in the Neotropical bruchid genus *Acanthoscelides* (Bruchinae, Bruchidae, Coleoptera)

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ABSTRACT

Adaptation to host-plant defenses through key innovations is a driving force of evolution in phytophagous insects. Species of the neotropical bruchid genus *Acanthoscelides* Schilsky are known to be associated with specific host plants. The speciation processes involved in such specialization pattern that have produced these specific associations may reflect radiations linked to particular kinds of host plants. By studying host-plant associations in closely related bruchid species, we have shown that adaptation to a particular host-plant (e. g., with a certain type of secondary compounds) could generally lead to a radiation of bruchid species at the level of terminal branches. However, in some cases of recent host shifts, there is no congruence between genetic proximity of bruchid species, and taxonomic similarity of host plants. At deeper branches in the phylogeny, vicariance or long-distance colonization events seem to be responsible for genetic divergence between well-marked clades, rather than adaptation to host plants. Our study also suggests that the few species of *Acanthoscelides* described from the Old World, as well as Neotropical species feeding on Mimosoideae, are misclassified, and are more closely related to the sister genus *Bruchidius*.

INTRODUCTION

Secondary metabolites in plants are known to play an important role in defense against herbivores (*e. g.* Herms and Mattson 1992; McKey 1979). In legumes, the diversity of such compounds seems to be even larger than in other plant families, and new secondary metabolites continue to be discovered (see Hegnauer 1994; Hegnauer and Hegnauer 1996, 2001). Based on the tendency of related species to possess similar metabolites, several studies have addressed the use of secondary metabolites as chemical markers in legume taxonomy (e. g., Evans *et al.* 1994; Kite and Lewis 1994; Wink *et al.* 1995). For phytophagous insects, these compounds represent defenses to overcome. However, once an adaptation permitting this has appeared (e. g., by sequestration or detoxification of the toxic compound), the insect can also exploit chemically similar (and usually closely related) plant species. In two examples concerning legumes, *Macrosiphum albifrons* Essig 1911 is the only known species of aphid able to develop on the alkaloid-rich varieties of lupin (Wink and Römer 1986); and the bruchid *Caryedes brasiliensis* Thunberg 1816 develops on host plants whose seeds contain high concentrations of arginase and canavanine (Bleiler *et al.* 1988; Rosenthal 1990; Rosenthal and Janzen 1983). Adaptation to a secondary metabolite (or class of similar metabolites) characteristic of a group of closely related plant species may allow a lineage of phytophagous insects to radiate adaptively onto several host plants of this group (Ehrlich and Raven 1964).

Bruchid beetles, with about 1700 known species (Borowiec 1987), are one of the most interesting groups of phytophagous beetles. Larvae of bruchids feed only inside seeds during their development, and most species are associated with legumes. Bruchids have countered the

mechanical protection of hard-seeded angiosperm species and have subsequently been able to use hard seed coats as a shield for their developing larvae. This adaptation has constrained bruchids to specialize particularly on legumes, but has allowed them to undergo radiations in other hard-seeded and stone-fruited families (Borowiec 1987), such as Malvaceae *sensu lato* (see Bayer *et al.* 1999) and Arecaceae. *Acanthoscelides* Schilsky 1905 (Bruchinae, Bruchidae, Coleoptera) is the largest Neotropical bruchid genus (Johnson 1981). Currently, about 300 species have been described, and many still likely await discovery, especially in poorly studied parts of South America, such as Amazonia and southern South America (Kergoat *et al.* submitted). Most of the species described are oligophagous or monophagous. Among the species for which a host plant has been reliably identified (Johnson 1983, 1989, 1990), about one hundred species develop on Faboideae, 35 species on Mimosoideae, and 6 species on Caesalpinioideae. A minority of the described species feed on non-legumes, such as Malvaceae *sensu lato* [Malvoideae (30 species), Grewioideae (8 species), Byttnerioideae (2 species)], Onagraceae (1 species), Rhamnaceae (1 species) and Cistaceae (1 species). Using morphological and ecological criteria, Johnson (1983, 1990) defined 15 groups of species of *Acanthoscelides*, all neotropical. Finally, about nine Palearctic species apparently restricted to seeds of herbaceous species of the faboid tribe Galegeae, such as *Astragalus* spp., were treated as *Acanthoscelides* by Lukjanovitsch and Ter-Minassian (1957), but their status as members of *Acanthoscelides* has been questioned (Borowiec 1987). One of these species was placed in *Bruchidius* by Egorov and Ter-Minassian (1981), and four were placed in a new genus, *Palaeobruchidius*, by Egorov (1990).

Acanthoscelides represents a very good model to examine adaptive radiation of phytophagous insects in legumes, and other hard-seeded families. A recent study of *Bruchidius* Schilsky, the Old-World sister genus of *Acanthoscelides*, has shown the role played by key innovations in the adaptive radiation of several groups of *Bruchidius* species on closely related host plants (Kergoat *et al.* 2004). Another study focusing on European species of *Bruchidius* has demonstrated several cases of ecological specialization in some beetles that were able to feed only on specific host plant species (Jermy and Szentesi 2003). In the present study, we compare host plant associations of different, apparently monophyletic, groups of *Acanthoscelides* species. Toward this goal, we analyzed relationships in a sample of 26 species of *Acanthoscelides*, including mostly ones specialized on the faboid tribe Phaseoleae, using phylogenetic methods applied to mitochondrial gene sequences. Our goal was to test the role of host-plant identity in the radiation of *Acanthoscelides*. We also included some other Old World and New World Bruchinae as outgroups, to confirm the monophyly of *Acanthoscelides* and of groups of species within it, and to explore the status of the Palearctic species that have been treated as *Acanthoscelides*.

MATERIAL AND METHODS

Establishing species groups of Acanthoscelides *for studying evolution of host-plant association*

Morphological similarity in male genitalia (considered the morphological criterion the most indicative of evolutionary relationships in bruchids [Borowiec 1987]) is not always a rule within the fifteen species groups of neotropical *Acanthoscelides* recognized

by Johnson (1983, 1990). We therefore tried to determine which groups presented consistently similar male genitalia and thus were most likely to represent monophyletic groups. Based on illustrations by Johnson (1983, 1990), we examined for each species five qualitative traits of male genitalia that describe the characteristics of the virga (the ventral valve at the apex of the median lobe), the median lobe, and the lateral lobes:

(i) shape of the apical surface of the virga (rounded vs. sharp);

(ii) shape of the lateral edges of the virga (straight vs. concave vs. convex);

(iii) ratio between height and width of the virga (height smaller than half the base vs. height greater than half the base);

(iv) shape of sclerified parts in the lateral edge of the median lobe (straight vs. curved);

(v) proportion of length of lateral lobes fused to each other (less than 1/3 vs. between 1/3 and 2/3 vs. more than 2/3).

We also included a sixth variable corresponding to the biogeographic range of the species (distributed no further south than Panama [N] vs. distributed south of Panama [S] vs. distributed both north and south of Panama [N+S]). In organisms with limited dispersal, closely related species are expected to live in the same biogeographic region.

We considered only groups containing five or more species (N=10 groups). Thus, we examined the *aequalis*, *albopygus*, *blanchardi*, *flavescens*, *megacornis*, *mexicanus*, *obtectus*, *pertinax*, *puellus* and *quadridentatus* groups (Johnson 1983, 1990). We then constructed a multiple correspondence analysis, considering the species group as a supplementary variable, using SAS (1999). We then conducted a discriminant analysis based on the coordinates of each species in the best represented groups for the nine first dimensions using S-Plus (2001). In this analysis, we tested if well represented groups were

different from each other, by a discriminant analysis and by paired comparisons (Hotelling's T Squared for Differences in Means) using S-Plus (2001).

Sampling

Sampling of Bruchinae included 26 species of *Acanthoscelides*, four species of *Bruchidius*, *Merobruchus placidus*, and *Palaeoacanthoscelides gilvus.* As outgroups, we used *Zabrotes planifrons* Horn (subfamily Amblycerinae). Material available for this study was mostly dried, pin-mounted specimens from the personal collections of J. Romero Napoles, K. W. Anton, and C. D. Johnson, collected from 08 Nov. 1983 to 21 Aug. 2002. In addition to these specimens from collections, specimens of *Acanthoscelides obtectus*, *A. obvelatus, A. argillaceus* and *Merobruchus placidus* were collected in 2002 by N. Alvarez. Table 1 summarizes information on sampled specimens and associated host plants for all species discussed in this paper. Although we analyzed the phylogenetic position of 26 out of the 300 *Acanthoscelides* species thus far described, those species are well representative of the genus, when considering both the morphological groups and the plant families on which the larvae develop.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted using the DNeasy™ kit (Qiagen). Qiagen protocol for animal tissues was modified to increase yield, due to the fact that most of our dried specimens, some up to 20 years old, contained low amounts of DNA. In particular, the lysis steps lasted 24 hours instead of three; particular attention was given to tissue crushing; final elution lasted 2 hours rather than 1 minute, and was done in 30µL final volume instead of 100µL. PCR amplifications for

three mitochondrial genes – *cytb* (primers CB1 & CB2), *COI* (primers C1-J-2183 & TL2-N-3014), and *12S rRNA* (primers 12sai & 12sbi) – were performed (Simon *et al.* 1994). Final volume was 10 µL, and contained 1 to 5 µL of extracted DNA, 1 µL of 25 mM MgCl2, 0.1 µL of 10mM dNTPs, 1 µL of PCR buffer (Eurogentec), 1 unit of Taq DNA polymerase (Eurogentec Red Goldstar™), 0.5 µL of forward primer, and 0.5 µL of reverse primer. PCRs were performed separately for each primer pair on a PTC-200™ thermocycler using the following cycling conditions: initial denaturation at 92 °C (1 min 30 s); 30 to 40 cycles of 92 °C (30 s), annealing at 55°C (45 s), elongation at 72 °C (1 min 30 s); final elongation at 72 °C (10 min). Sequencing reactions were carried out using Applied Biosystems BygDye™ protocol. Products of the sequencing reactions were then analyzed on an ABI Prism 310 sequencer.

Phylogenetic analyses

Chromatograms were manually corrected using Chromas 2.23 (Technelysium Pty. Ltd., Helensvale, Australia) and further aligned using ClustalW 1.83 (Thompson *et al.* 1994). The phylogenetic signal of our data was tested by performing a likelihood mapping analysis, using TREE-PUZZLE 5.2 (Strimmer and Von Haeseler 1997). Parsimony analysis and maximum likelihood analysis were carried out on an Intel Pentium IV 2.4 Ghz processor. Parsimony analysis was performed using PAUP* 4.0b10 (Swofford 2003), whereas maximum likelihood analysis was achieved using both PAUP* 4.0b10 and PHYML 2.4.4 (Guindon and Gascuel 2003). All analyses were performed using heuristic search and tree-bisection-reconnection (TBR) branch-swapping-algorithm. For parsimony analysis, gaps were treated as a fifth character, and all characters were re-weighted on the basis of their rescaled consistency index (Farris 1989). Bootstrap

values were calculated on 1000 replicates. For maximum likelihood analysis, we used a general time reversible (GTR) model with eight evolutionary rate categories. Gamma shape parameter, proportion of invariable sites, base frequencies and probabilities of substitution were estimated through heuristic search. Bootstrap values were calculated both on 100 replicates using PAUP* 4.0b10, and on 1000 replicates using PhyML (much less time-consuming than PAUP). Likelihoods of constrained and non-constrained trees were compared with a Kishino-Hasegawa (RELL bootstrap) test, using PAUP* 4.0b10 (Swofford 2003).

Bayesian inferences were determined using MrBayes version 3.0b4 (Huelsenbeck and Ronquist 2001) on an Apple G5 1.8 Ghz. We used Modeltest 3.06 (Posada and Crandall 1998) to assess the best-fit substitution model, through hierarchical likelihood ratio tests. The asymptote of the fluctuating likelihood values of the Bayesian trees (or burnin period) was determined through preliminary runs. We ran four Metropolis-coupled chains in one run of 20000000 generations, and sampled one tree every 10000 once cycles after the burnin period had passed. The sampled trees were used to generate a majority rule tree showing all compatible partitions and the support for the nodes of this tree was given by posterior probability estimates for each clade.

Character tracing of host plant genera (or host plants tribes or subfamilies) corresponding to each studied bruchid species was carried out on the majority rule tree obtained through Bayesian methods, using MacClade 4.06 (Maddison and Maddison 2004) with DELTRAN optimization.

RESULTS

Multiple correspondence analysis and discriminant analysis of species groups of Acanthoscelides

The nine dimensions of the MCA on morphological and biogeographical characters explained respectively 19.89%, 15.34%, 13.17%, 11.61%, 10.40%, 8.94%, 8.11%, 7.23% and 5.31% (graphs not shown). The discriminant analysis using the coordinates of each species in the ten groups demonstrated highly significant differences among species groups (Hotelling-Lawley Trace: $p=6*10^{-15}$). Pairwise comparisons demonstrated that 33 of 45 pairs of these 10 groups were significantly discriminated (Table 2). Among these groups, five (*aequalis*, *albopygus*, *blanchardi*, *pertinax*, *puellus*) showed significant differences with seven or more other groups (*i. e.* each of these five groups was different from more than 75% of all other groups). The host-plant associations for species of these five groups are represented in Table 3. Each group appears to be associated with a different taxonomic group of host plants, except groups *aequalis* and *blanchardi*, whose species with known host plants (respectively 26 species in group *aequalis* and 6 species in group *blanchardi*) feed on Malvaceae *sensu lato*. The other groups are associated with different legume groups, all faboids, except for group *albopygus*, of which all species with known host plants (4) feed on the mimosoid tribe Mimoseae. In group *pertinax*, most of the species (9) develop on Desmodieae, the others developing on Phaseoleae (2), Amorpheae (1), and on Aeschynomeneae/Amorpheae/Desmodieae (1). In group *puellus*, most of the species feed on Phaseoleae (12), and the others feed on Indigofereae (4), on Galegeae/Loteae (1), and on

Phaseoleae/Millettieae (1). In addition, one species of this group feeds on species of the non-legume family Rhamnaceae.

Phylogenetic reconstruction

Since most of the specimens were collected several (up to 20) years before the study, and had been preserved dried in insect collections, DNA was in most cases considerably degraded. Therefore, we could not obtain usable sequences for *COI* and *Cytb*. However, we obtained very good results with primers 12sai and 12sbi, and we could therefore sequence 384 nucleotides for the *12s rRNA* gene, in all the studied species (see accession numbers in Table 1). Although the total number of analyzed nucleotides was lower than expected (since we obtained no results with *cytb* and *COI*), the phylogenetic signal of our sequence matrix was good, since 86% (29,2% + 28.1% + 28.7%) of the data set support resolved topologies in the likelihood mapping analysis (see Figure 1).

We reconstructed the consensus maximum parsimony phylogenetic tree with 1000 bootstraps after 5 hours of simulation (Figure 2a). Maximum-likelihood phylogenetic trees and bootstrap support values were obtained after 1126 hours of simulation using PAUP* (100 replicates) and after only 4 hours of simulation using PHYML (1000 replicates). Parameters estimated in the maximum likelihood analysis using PAUP* were as follows: Gamma = 0.404344, proportion of invariable sites = 0.163879. Bases frequencies were estimated as follows: A = 0.38002, C = 0.07098, G = 0.13872, T = 0.41028. Substitution probabilities were estimated as follows: A-C = 0.12602, A-G = 5.55604, A-T = 1.74627, C-G = $1.73*10^{-10}$, C-T = 2.76787. The same parameters were used in the PHYML analysis, producing the phylogenetic tree presented in Figure 2b. In this figure, bootstrap values obtained with both PAUP* and PHYML are represented on

each node (when at least one of the two values was greater than 20%). The optimal phylogenetic tree obtained with PAUP* is not shown.

We computed Bayesian inferences using the following prior probabilities parameters determined by Modeltest: GTR model of substitution, Gamma = 0.45, proportion of invariable sites = 0.1409. Bases frequencies were estimated as follows: A = 0.4349, C = 0.0497, G = 0.1151, T = 0.4002. Substitution probabilities were estimated as follows: A-C = 0.1347, A-G = 3.5899, A-T = 1.0650, C-G = 0.2239, C-T = 2.8091. The burnin period was estimated to 100000 cycles. One thousand and nine hundreds ninety trees were sampled and the majority rule tree with posterior probability estimates was reconstructed after 11 hours of simulation in total. The tree obtained by Bayesian inferences with corresponding posterior probabilities is represented in Figure 3.

Reconstructions obtained through maximum parsimony and maximum likelihood analysis were different (18 of 33 nodes in common using PHYML and 19 of 33 nodes in common using PAUP*). This discrepancy was particularly expressed at the level of intermediate nodes. Reconstructions obtained through Bayesian inferences led to a slightly higher similarity with other reconstructions, with 20 of 33 nodes in common both with maximum parsimony and maximum likelihood (using PHYML) reconstructions. The level of similarity reached 29 of 33 nodes in common when comparing Bayesian inferences reconstruction with the optimal maximum likelihood tree obtained using PAUP*.

Due to the higher similarity of the Bayesian inferences reconstruction with any other kinds of reconstructions, we tend to favor the phylogenetic tree obtained through Bayesian inferences rather than another.

The 32 Bruchinae species analyzed in this study are represented in two different clades: a first clade containing 22 of the 26 *Acanthoscelides* species studied, and a second containing all Palearctic species plus four Neotropical species, *Acanthoscelides macrophthalamus*, *A. oblongoguttatus*, *A. mexicanus* and *Merobruchus placidus*, all of them feeding on Mimosoideae. Globally, *Acanthoscelides* seems thus to be a "good" genus, with only the species feeding on Mimosoideae (i. e., *A. macrophthalamus*, *A. mexicanus* and *A. oblongoguttatus*) and the Old-World species *A. plagiatus* being misplaced, actually belonging to the *Bruchidius* clade (see Figure 4). Indeed, constraining the *Acanthoscelides* species feeding on Mimosoideae to cluster together with the other *Acanthoscelides* species (instead of branching in the *Bruchidius* clade) leads to a tree whose likelihood is significantly lower (Kishino-Hasegawa test, P = 0.0269). In the "true" *Acanthoscelides* (i. e., the 22 species branching together in a single clade), a strong tendency to radiation on similar host-plants is shown, particularly for species feeding on the two Phaseoleae, *Phaseolus* and *Rhynchosia*, on the Desmodieae *Desmodium*, and those on Malvaceae *sensu lato* (except *A. sanblas* [*megacornis* group], which develops on grewioid species and is unrelated to the other Malvaceae feeders) (see Figure 4). However, in some cases, there is evidence of recent host shifts, for example in the case of *A. puellus* (developing on *Calopogonium* sp.), a species closely related to *Desmodium* feeders.

Robustness (in terms of monophyly) of the morphological groups defined by Johnson (1983, 1990) was variable. Whereas species from groups *obtectus* (*A. obtectus*, *A. obvelatus* and *A. argillaceus*) and a*equalis* (*A. anoditus*, *A. guazumae* and *A. malvastrumicis*) clustered strictly together, groups *flavescens* (*A. flavescens* and *A. isla*), *pertinax* (*A. biustulus*, *A. cuernavaca*, *A. desmodicola*, *A. desmoditus*,

A. mazatlan, *A. stylifer*, and *A. zonensis*) and *puellus* (*A. clandestinus*, *A. palmasola*, *A. puellus*, *A. sanfordi* and *A. taboga*) were not monophyletic. Concerning groups *megacornis*, *mexicanus*, *mundulus* and *oblongoguttatus*, we were unable to test monophyly since we analyzed only one species per group.

DISCUSSION

Use of molecular techniques on pin-mounted dry specimens

Because of the poor preservation of DNA of the studied specimens, we were able to amplify and sequence a sufficiently long portion of only one of the genes tested, about 400 bp of the mitochondrial *12s rRNA*. To our knowledge, most molecular phylogenetic studies of insects have been done on fresh material or material conserved in alcohol (or acetone, or other fluids). This study suggests that when no fresh material is available, working with air-dried specimens may yield to good results, depending on the nature of the sequenced gene. The quality of the specimens we analyzed appears to be higher than expected by previous studies (e.g. Quicke *et al.* 1999), in which air-dried insects were considered as extremely poor sources of amplifiable DNA, oppositely to specimens preserved through other methods such as critical point drying or Hexamethylenedisilazane drying. The primer pair 12Sai & 12Sbi appears capable of annealing onto DNA present in very low concentrations, compared to the *CytB* and *COI* universal primers, with which we could not obtain clean sequences long enough to be informative. However, due to the fact that we were not able to sequence genes other than *12s rRNA*, bootstrap values of some internal nodes were relatively low, and results obtained by the different methods of reconstruction yielded to

relatively incongruent trees. Nevertheless, the good congruence between results obtained by Bayesian inferences and maximum likelihood (using PAUP*) argues for a good quality of our data.

Host-plant association

In each of the five groups (*aequalis*, *albopygus*, *blanchardi*, *pertinax*, and *puellus*) well defined on the basis of morphology of the male genitalia, there was a very strong tendency for species of the same group to be associated with closely related host plants. This tendency was especially marked for species of the groups whose species develop on Malvaceae (i. e., groups *aequalis* and *blanchardi*) and Mimosoideae (i. e., group *albopygus*). The tendency was less strongly marked for species of groups *puellus* and *pertinax*, which in addition were demonstrated by the phylogenetic analysis to be paraphyletic. On the basis of the phylogenetic tree obtained from *12s rRNA* sequences, the role of host plants in driving fine-scale patterns of radiation is generally confirmed. Four clades attest to radiation after adaptation to particular kinds of host plant. These are three *Acanthoscelides* species on *Phaseolus*, four species on *Rhynchosia*, four species on *Desmodium* and three species on Malvaceae. This result suggests that when a lineage of bruchids becomes adapted to a certain kind of host-plant, it may undergo evolutionary radiation onto other closely related plants. Adaptation to the particular secondary metabolites of a group of plants is a likely candidate for such a key innovation. However, such an adaptation can lead to host shifts, when genetically distant plants share similar secondary compounds. This could be the case in our study in which species feeding on Faboideae and species specialized on Malvaceae are phylogenetically close. The chemistry of seeds of Faboideae has been broadly studied for decades (Harborne *et al.* 1971; Bisby *et al.* 1994; Hegnauer 1994; Hegnauer

and Hegnauer 1996, 2001; Wink and Mohamed 2003), and species of most legume tribes seem to exhibit secondary compounds such as lectins or alpha-amylase inhibitors, that inhibit or reduce the digestive capability of seminivorous insects (Marshall and Lauda 1975; Chrispeels and Raikhel 1991; Giri and Kachole 1998; Melo *et al.* 1999; Wink and Mohamed 2003). Oppositely, very little is known on the chemistry of seeds of other hard-seeded families, such as Malvaceae. Nevertheless, digestive inhibitors, such as gossypol (Meisner *et al.* 1978) have also been identified in several Mesoamerican Malvoideae. For instance, high amounts of gossypol were detected in seeds from species of *Anoda* and *Hibiscus* (Sotelo *et al.* 2005). Circumventing digestive inhibitors in legumes may represent – for a given bruchid lineage – a pre-adaptation to overcome the action of other secondary compounds such as gossypol, and make possible a further radiation on Malvaceae. A fifth clade, the group with *A. macrophthalamus*, *A. oblongoguttatus* and *Merobruchus placidus* – the three species feeding on Mimosoideae – also demonstrate an association between phylogenetic proximity and host-plant categories. This particular case will be discussed later in this study.

Particular attention must be given to the proximity between the clade of species feeding on *Phaseolus* and the clade of species feeding on *Desmodium.* Although Phaseoleae and Desmodieae were long considered not particularly closely related, recent phylogenies indicate that the two tribes can be grouped in a monophyletic clade (Wink and Mohamed 2003). Our results suggest that this phylogenetic relatedness is probably accompanied by some chemical similarity constraining host-plant association in the *Acanthoscelides* on *Phaseolus* and *Desmodium*. This is a good example of how the evolutionary history of phytophagous insects can give insights on the

evolution of host plants. However, at least two cases of host shifts at terminal branches attests a more complex dynamics of speciation, since key innovations in herbivores may allow a lineage to colonize newly and chemically-different host plants.

Nature and origin of the genus Acanthoscelides

Our data reveal that *Acanthoscelides* is monophyletic, if the species on Mimosoideae and the Palearctic species questionably attached to the genus (e.g., *A. plagiatus* in this study) are removed. Our study shows that *A. plagiatus* should be placed in *Bruchidius* as previously argued by Borowiec (1987). We consider it highly likely that this result could be generalized to the other Palearctic species described or treated as *Acanthoscelides* by Lukjanovitsch and Ter-Minassian (1957).

The *Acanthoscelides* species specialized on Mimosoideae, along with the other Neotropical bruchid studied here (*Merobruchus placidus*) are clearly more closely related to the old world genus *Bruchidius* Schilsky (the sister genus of *Acanthoscelides*), than to the main *Acanthoscelides* clade.

The two main clades of Bruchinae studied here are therefore the *Bruchidius* clade (including the species discussed above that are incorrectly assigned to *Acanthoscelides*) and *Acanthoscelides* (with these species excluded), respectively. Since most of the species of the *Bruchidius* clade are from the Old World, and all the species of the *Acanthoscelides* clade are from the New World, this dichotomy could be explained by a Gondwanan vicariance origin 90 Mya, or more recently by the disconnection of the early Beringian Bridges between the Eastern Paleartic and the Western Nearctic (35 Mya) (Scotese 2004). However, the position of the small New World clade, represented by *A. oblongoguttatus*, *A. macrophthalamus* and *Merobruchus placidus*, along with *A. mexicanus*, all branching inside

the *Bruchidius* clade, could be explained either by vicariance events or by a more recent colonization to the New World by one or more members of the Old World *Bruchidius* clade.

***Gondwanan vicariance hypothesis for the origin of the New World species branching inside* Bruchidius**

Because several New World species branch in the *Bruchidius* clade, we cannot eliminate the hypothesis of a Gondwanan vicariance to explain this pattern. We could easily imagine that lineages of all species studied have a New World origin, and that a process of speciation anterior to the separation of Gondwana occurred between what represents now the main *Acanthoscelides* clade, and the clade with the other species of Bruchinae examined here. Subsequent to this divergence and the Gondwanan separation, ancestors of this latter clade could have engendered both Old World *Bruchidius* and species of the small New World clade incorrectly assigned to *Acanthoscelides*.

***Colonization hypothesis for the origin of the New World species branching inside* Bruchidius**

We could also imagine that *A. macrophthalamus, A. oblongoguttatus, A. mexicanus,* and *Merobruchus placidus* are descendants of one or more members of Paleotropical *Bruchidius* ancestors that colonized the New World. This colonization could have been effected by migrants issued from the *Bruchidius* clade posterior to the Gondwanian or Beringian separation, possibly developing on Mimosoideae, as is consistent with the fact that *A. oblongoguttatus, A. mexicanus, A. macrophthalamus* and *Merobruchus placidus* are the only New World species in our study that feed on Mimosoideae. Colonization of the New World unambiguously posterior to the breakup of Gondwana has been suggested, on the basis of molecular

evidence, for a rainforest tree with amphi-Atlantic distribution, *Symphonia globulifera* (Clusiaceae), which may have reached America through marine dispersal of trunks or roots (Dick *et al.* 2003), and for caviomorph rodents via “stepping stone” islands, and rafts carried by tropical rivers into the ocean (Huchon and Douzery 2001). For a more general review about oceanic dispersal, see de Queiroz (2005).

In the case of bruchids, several plausible hypotheses can be formulated. First, since most hurricanes that reach the Atlantic coast of America arise off the coast of Africa, bruchids could have been able to cross the ocean. Insects do occasionally disperse long distances, moved by storms. American Monarch butterflies (*Danaus plexippus*), for example, have been able to reach and establish colonies on the coast of western Europe, as well as on Pacific islands, probably through cyclonic winds or hurricanes (Zalucki and Clarke 2004). Besides, insects are known to be able to cover hundreds, or even thousands, of kilometers when they are carried away in ascending air currents (Compton 2001). However, taking into account the seminivorous biology of bruchids, transcontinental colonization by arrival on floating seeds, or seeds carried in rafts, could also be plausible. Seeds of several African species of legume trees have been found on the Atlantic and Caribbean coasts of America, among them species of *Cassia* or *Caesalpinia* (Gunn *et al.* 1976).

CONCLUSION

Despite the morphological and ecological diversity among species of the genus *Acanthoscelides* (long considered paraphyletic by several authors, e. g. Borowiec (1987), the majority of the species described

as *Acanthoscelides* constitute a monophyletic group. Exceptions to this are Palearctic species, and Neotropical species developing on Mimosoideae. Whereas deep nodes are the result of either geological vicariance or long-distance colonization, the role of host plant seems globally determinant in driving radiation in the terminal branches, although several host-shift processes have also been addressed. As suggested by Kergoat *et al.* (2004), chemical compounds could be the principal host-plant traits driving these radiations. Testing this hypothesis, already demonstrated in several other phytophagous groups of beetles (Becerra 2003; Termonia *et al.* 2002), will represent the next step of this study.

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ZUSAMMENFASSUNG

Phylogenie der neotropischen Gattung Acanthoscelides *(Bruchinae, Bruchidae, Coleoptera)*

Die Adaption an die Abwehrmechanismen ihrer Futterpflanzen ist eine der treibenden evolutionären Kräfte in phytophagen Insekten. Auch die Bruchiden im neotropischen Genus *Acanthoscelides* Schilsky weisen äußerst spezifische Assoziationen mit ihren Futterpflanzen auf. Diese Spezialisierung legt nahe, dass die darin involvierten Artbildungsprozesse evolutionäre Radiationen wiederspiegeln, die aufgrund der Bindung an bestimmte Futterpflanzen entstanden sind. In der vorliegenden Studie zeigen wir anhand der Assoziation nahe verwandter Bruchidae und ihrer Futterpflanzen, dass die Adaption an eine bestimmte Futterpflanze (z.B. jene, die einen gewissen Typ von sekundären Pflanzenstoffen ausscheiden) zur Radiation der Bruchiden an den terminalen Ästen der Phylogenie geführt haben könnte. Bei Fällen von rezentem Futterpflanzenwechsel fanden wir jedoch keine Übereinkunft zwischen dem Grad der genetischen Verwandschaft und der taxonomischen Ähnlichkeit der Futterpflanzen. An den tieferen Ästen der Phylogenie scheinen daher eher Vikarianz oder über größere geografische Distanzen hinweg erfolgende Kolonisationsvorgänge für die genetische Divergenz zwischen den Ästen des Stammbaumes verantwortlich zu sein, als die Bindung an bestimmte Futterpflanzen. Unsere Arbeit suggeriert, das die wenigen aus der Alten Welt beschriebenen Arten der Gattung *Acanthoscelides,* wie auch die neotropischen Schwesterarten an Mimosoideae, falsch klassifiziert wurden, und tatsächlich der Schwesterart *Bruchidius* näher stehen.

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Table 1.

Genus	Species	Author and year	Site of sampling	Samp. date	Collector	Host plant	Morph. group	accession
Zabrotes	*planifrons*	Horn 1885	Mex. Huautla	16/IV/2000	Figueroa de la R. I	ND	-	AY945992
Acanthoscelides	*anoditus*	Johnson 1983	Mex. Irapuato	16/X/2000	ND	*Anoda cristata* (*)	Aequalis	AY945996
Acanthoscelides	*argillaceus*	Sharp 1985	Mex. Playa Azul	01/II/2001	Aebi A	*Phaseolus lunatus*	Obtectus	AY945967
Acanthoscelides	*biustulus*	Fall 1910	Mex. Amealco	11/X/2002	Romero N. J	*Desmodium* sp. (*)	Pertinax	AY945968
Acanthoscelides	*clandestinus*	Motschoulsky 1874	Mex. C. Carmen	17/II/1996	Ramírez DR	*Vigna adenantha*	Puellus	AY945969
Acanthoscelides	*cuernavaca*	Johnson 1983	Mex. Huautla	4/II/2000	Romero N. J	*Desmodium* sp.(*)	Pertinax	AY945970
Acanthoscelides	*desmodicola*	Johnson 1983	Mex. Huautla	5/II/2000	Figueroa de la R. I	*Desmodium* sp. (*)	Pertinax	AY945971
Acanthoscelides	*desmoditus*	Johnson 1983	Ven. Barquisimeto	17/VII/1984	Johnson CD	*Desmodium tortuosum*	Pertinax	AY945972
Acanthoscelides	*flavescens*	Fahraeus 1839	Mex. El Maruqes	22/II/1998	Luna Cozar J	*Rhynchosia minima* (*)	Flavescens	AY945997
Acanthoscelides	*guazumae*	Johnson & Kingsolver 1971	Mex. Huautla	3/XI/1996	Romero N. J	*Guazuma tomentosa*	Aequalis	AY945974
Acanthoscelides	*isla*	Johnson 1983	Ecu. Guayaquil	3/VII/1984	Johnson CD	*Rhynchosia minima*	Flavescens	AY945975
Acanthoscelides	*macrophthalamus*	Schaeffer 1907	Vie. Saïgon	ND	Delobel H	*Leucanea leucocephala*	Mexicanus	AY945976
Acanthoscelides	*malvastrumicis*	Johnson 1983	Mex. El Cielo	28/VII/1998	Niño S & Hernández J	*Malvastrum americanum* (*)	Aequalis	AY945977
Acanthoscelides	*mazatlan*	Johnson 1983	Mex. Huautla	16/IV/2000	Romero N. J	*Desmodium* sp. (*)	Pertinax	AY945978
Acanthoscelides	*mexicanus*	Sharp 1885	Mex. Coxcatlan	15/XII/2002	Alvarez N & Ciao V	*Mimosa* sp.	Mexicanus	AY945979
Acanthoscelides	*mundulus*	Sharp 1885	Mex. Jalcomulco	18/II/1996	Romero N. J	*Nissolia fruticosa*	Mundulus	AY945980
Acanthoscelides	*oblongoguttatus*	Fahraeus 1839	Mex. Cotaxtla	28/VII/2000	Morse GE & Romero N. J	*Acacia cornigera*	Oblongoguttatus	AY945981
Acanthoscelides	*obtectus*	Say 1831	Mex. Tepoztlan	15/I/2002	Alvarez N & Aebi A	*Phaseolus vulgaris*	Obtectus	AY945998
Acanthoscelides	*obvelatus*	Bridwell 1942	Mex. Tepoztlan	15/I/2002	Alvarez N & Aebi A	*Phaseolus vulgaris*	Obtectus	AY945983
Acanthoscelides	*palmasola*	Johnson 1983	Mex. Tenabo	1/I/1979	Johnson CD	*Rhynchosia longeracemosa*	Puellus	AY945984
Acanthoscelides	*plagiatus*	Reiche & Saulcy 1857	Tur. Van Gölü	29/VI/1993	ND	*Astragalus* sp.	-	AY945999
Acanthoscelides	*puellus*	Sharp 1885	Nic. El Progreso	15/IV/1998	Maes JM	*Calopogonium mucumoides*	Puellus	AY946000
Acanthoscelides	*sanblas*	Johnson 1983	Mex. Cordoba	1/III/1996	Romero N. J	*Triumfetta lappula*	Megacornis	AY945986
Acanthoscelides	*sanfordi*	Johnson 1983	Mex. Huautla	4/XI/2000	Romero N. J	*Rhynchosia* sp.	Puellus	AY945987
Acanthoscelides	*stylifer*	Sharp 1885	Mex. Ixmiquilpan	21/VIII/2002	Romero N. J	*Desmodium* sp. (*)	Pertinax	AY945988
Acanthoscelides	*taboga*	Johnson 1983	Pan. Chepo	2/IV/1980	Johnson CD	*Calopogonium caeruleum*	Puellus	AY945989
Acanthoscelides	*zonensis*	Johnson1983	Col. Palmira	8/XI/1983	Johnson CD	*Teramnus uncinatus*	Pertinax	AY945990
Bruchidius	*foveolatus*	Gyllenhal 1833	Alg. Amouchas	02/VI/1986	Warchalowski A	*Cytisus* sp. (*)	-	AY946001
Bruchidius	*quinqueguttatus*	Olivier 1795	Tur. Anamurium	01/V/2001	Anton KW	*Vicia* sp. (*)	-	AY945961
Bruchidius	*raddianae*	Anton & Delobel 2003	Yem. Lahj	1/IX/2001	Sallam A	*Acacia tortilis*	-	AY625297
Bruchidius	*tuberculatus*	Hochhut 1847	Aze. Talysh	01/V/1993	Alexeevka V	Unknown Faboideae (*)	-	AY946002
Palaeoacanthoscelides	*gilvus*	Gyllenhal 1839	Tad. Oktynbrskaya	18/V/1991	Dangara S	Unknown Faboideae	-	AY946004
Merobruchus	*placidus*	Horn 1873	Mex. Coxcatlan	20/XII/2002	Alvarez N & Ciao V	*Acacia* sp.	-	AY945965

Table 2.

	albopygus	*blanchardi*	*flavescens*	*megacornis*	*mexicanus*	*obtectus*	*pertinax*	*puellus*	*quadridentatus*
aequalis	***	***	*	NS	*	*	***	***	NS
albopygus		**	**	***	NS	*	***	***	**
blanchardi			NS	***	**	*	***	***	***
flavescens				**	NS	NS	***	***	**
megacornis					NS	*	***	*	NS
mexicanus						NS	***	*	*
obtectus							NS	NS	*
pertinax								**	***
puellus									NS

Table 3.

Group	Species	Associated host-plants
Aequalis (aeq.)	*aequalis* (aeq.)	*Abutilon* (Mal.), *Pseudabutilon* (Mal.), *Wissadula* (Mal.)
	altocaura (aeq.)	?
	anoditus (aeq.)	*Anoda* (Mal.)
	apicalis (aeq.)	*Malachra* (Mal.)
	aragua (aeq.)	*Wissadula* (Mal.)
	bechyneorum (aeq.)	?
	bogot (aeq.)*a*	?
	bolivar (aeq.)	?
	brevipes (aeq.)	*Malvastrum* (Mal.), *Sida* (Mal.)
	colombiano (aeq.)	?
	coro (aeq.)	*Malvastrum* (Mal.), *Sida* (Mal.)
	elkinsae (aeq.)	*Hibiscus* (Mal.)
	falcon (aeq.)	*Abutilon* (Mal.)
	guaibacoa (aeq.)	*Abutilon* (Mal.)
	guazumae (aeq.)	*Guazuma* (Mal.)
	guerrero (aeq.)	*Herissantia* (Mal.), *Malvastrum* (Mal.)
	guiana (aeq.)	*Abutilon* (Mal.), *Hibiscus* (Mal.)
	herissantitus (aeq.)	*Herissantia* (Mal.), *Malvastrum* (Mal.)
	Johni (aeq.)	*Herissantia* (Mal.)
	Machiques (aeq.)	*Pavonia* (Mal.)
	malvastrumicis (aeq.)	*Malvastrum* (Mal.)
	malvitus (aeq.)	*Abutilon* (Mal.), *Malva* (Mal.)
	maturin (aeq.)	*Hibiscus* (Mal.)
	merida (aeq.)	*Abutilon* (Mal.)
	monagas (aeq.)	*Hibiscus* (Mal.)
	pyramididos (aeq.)	*Sida* (Mal.)
	Santarosa (aeq.)	*Herissantia* (Mal.)
	sleeperi (aeq.)	*Abutilon* (Mal.)
	subaequalis (aeq.)	*Abutilon* (Mal.)
	tepic (aeq.)	*Abutilon* (Mal.)
	univittatus (aeq.)	*Guazuma* (Mal.)
albopygus (alb.)	*albopygus* (alb.)	?
	buenaventura (alb.)	legume tree (Fab. Mim.)
	caripe (alb.)	?
	cesari (alb.)	legume tree (Fab. Mim.)
	elevatus (alb.)	?
	elvalle (alb.)	?
	lituratus (alb.)	?
	petalopygus (alb.)	*Acacia* (Fab. Mim.)
	sousai (alb.)	*Acacia* (Fab. Mim.)
	sublituratus (alb.)	?
	Tinalandia (alb.)	?
blanchardi (bla.)	*blanchardi* (bla.)	*Kosteletzkya* (Mal.)
	fryxelli (bla.)	*Kosteletzkya* (Mal.), *Malachra* (Mal.)
	hibiscicola (bla.)	*Hibiscus* (Mal.)
	orlandi (bla.)	*Kosteletzkya* (Mal.), *Malachra* (Mal.)
	pavoniestes (bla.)	*Pavonia* (Mal.)
	Santander (bla.)	?
	vexatus (bla.)	?
	wicki (bla.)	?

Table 3 (continued).

Group	Species	Associated host-plants
Pertinax (per.)	*argutus* (per.)	*Teramnus* (Fab. Phas.)
	biustulus (per.)	*Desmodium* (Fab. Des.)
	cuernavaca (per.)	*Desmodium* (Fab. Des.)
	desmodicola (per.)	*Desmodium* (Fab. Des.)
	desmoditus (per.)	*Desmodium* (Fab. Des.)
	howdenorum (per.)	*Desmodium* (Fab. Des.)
	Lichenicola (per.)	?
	mazatlan (per.)	*Desmodium* (Fab. Des.)
	oaxaca (per.)	?
	pedicularius (per.)	*Petalostemum* (Fab. Amor.)
	pertinax (per.)	*Aeschynomene* (Fab. Aesch.), *Desmodium* (Fab. Des.), *Dalea* (Fab. Amor.), *Stylosanthes* (Fab. Aesch.)
	Puelliopsis (per.)	*Desmodium* (Fab. Des.)
	schubertae (per.)	*Desmodium* (Fab. Des.)
	stylifer (per.)	*Desmodium* (Fab. Des.)
	zonensis (per.)	*Teramnus* (Fab. Phas.)
puellus (pue.)	*amabilis* (pue.)	*Rhynchosia* (Fab. Phas.)
	aureolus (pue.)	*Acmispon* (Fab. Lot.), *Astragalus* (Fab. Gal.), *Glycyrrhiza* (Fab. Gal.), *Hosackia* (Fab. Lot.), *Ottleya* (Fab. Lot.), *Oxytropis* (Fab. Gal.), *Syrmatium* (Fab. Lot.)
	barneby (pue.)	?
	barrocolorado (pue.)	?
	caroni (pue.)	*Indigofera* (Fab. Ind)
	chiapas (pue.)	?
	clandestinus (pue.)	*Phaseolus* (Fab. Phas.)
	colombia (pue.)	?
	dominicana (pue.)	*Calopogonium* (Fab. Phas.)
	donckieropsis (pue.)	?
	Fernandezi (pue.)	?
	griseolus (pue.)	*Calopogonium* (Fab. Phas.)
	guarico (pue.)	*Rhynchosia* (Fab. Phas.)
	indigoforestes (pue.)	*Indigofera* (Fab. Ind)
	jardin (pue.)	?
	Kingsolveri (pue.)	*Indigofera* (Fab. Ind)
	leisneri (pue.)	?
	luteus (pue.)	?
	Palmasola (pue.)	*Rhynchosia* (Fab. Phas.)
	prosopoides (pue.)	*Ziziphus* (Rha.)
	puellus (pue.)	*Calopogonium* (Fab. Phas.)
	rhynchosiestes (pue.)	*Rhynchosia* (Fab. Phas.)
	ruficoxis (pue.)	*Indigofera* (Fab. Ind)
	rufovittatus (pue.)	*Galactia* (Fab. Phas.), *Tephrosia* (Fab. Mill.)
	sanfordi (pue.)	*Pachyrhizus* (Fab. Phas.), *Rhynchosia* (Fab. Phas.)
	schaefferi (pue.)	?
	suaveolus (pue.)	*Vigna* (Fab. Phas.)
	surrufus (pue.)	*Rhynchosia* (Fab. Phas.)
	taboga (pue.)	*Calopogonium* (Fab. Phas.), *Pachyrhizus* (Fab. Phas.)
	yecora (pue.)	?

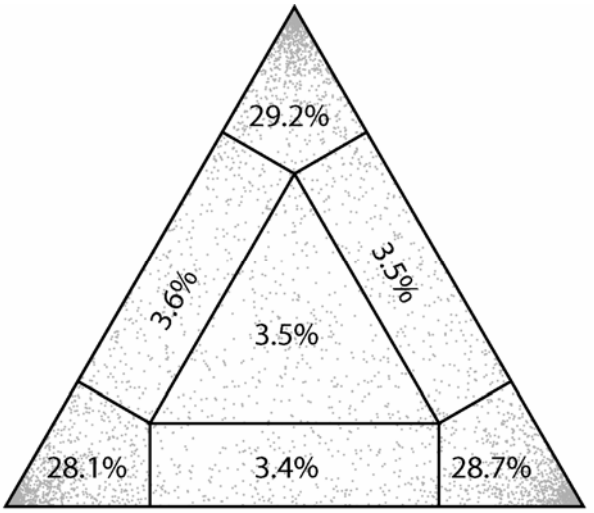

Figure 1

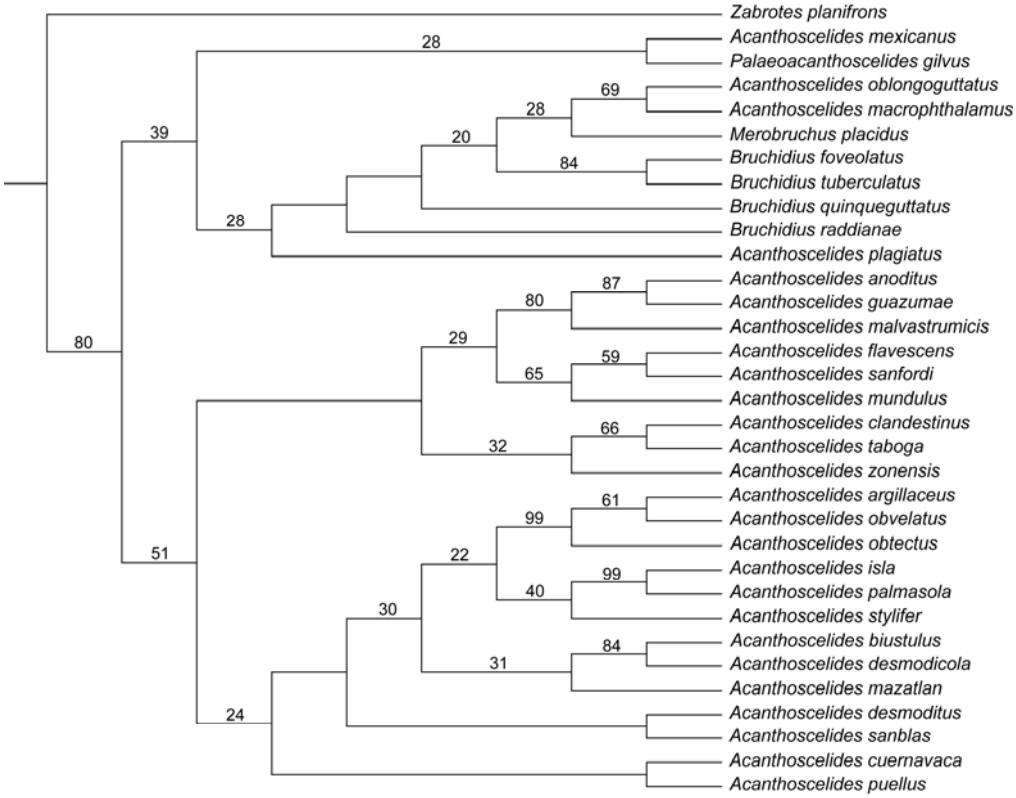

Figure 2 (a)

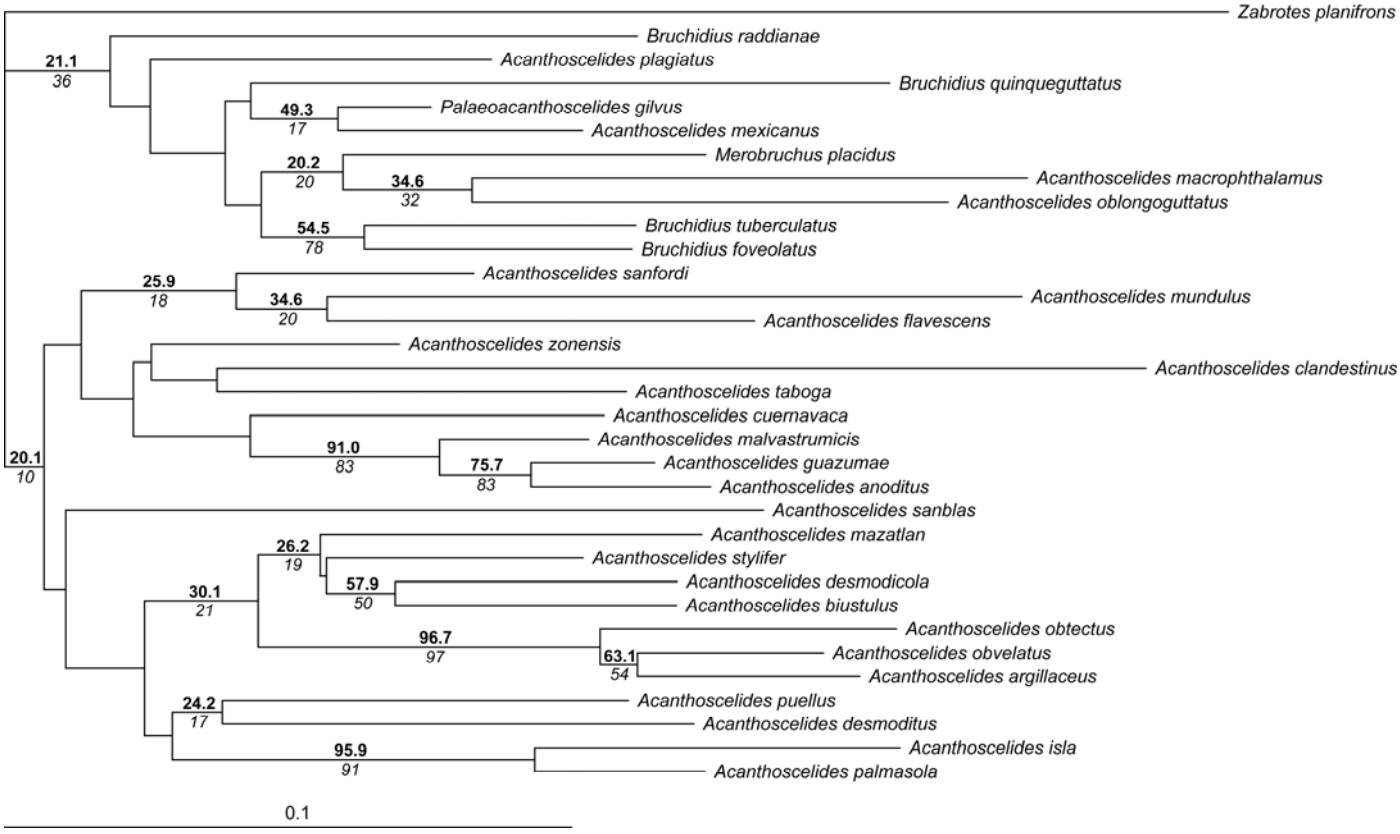

Figure 2 (b)

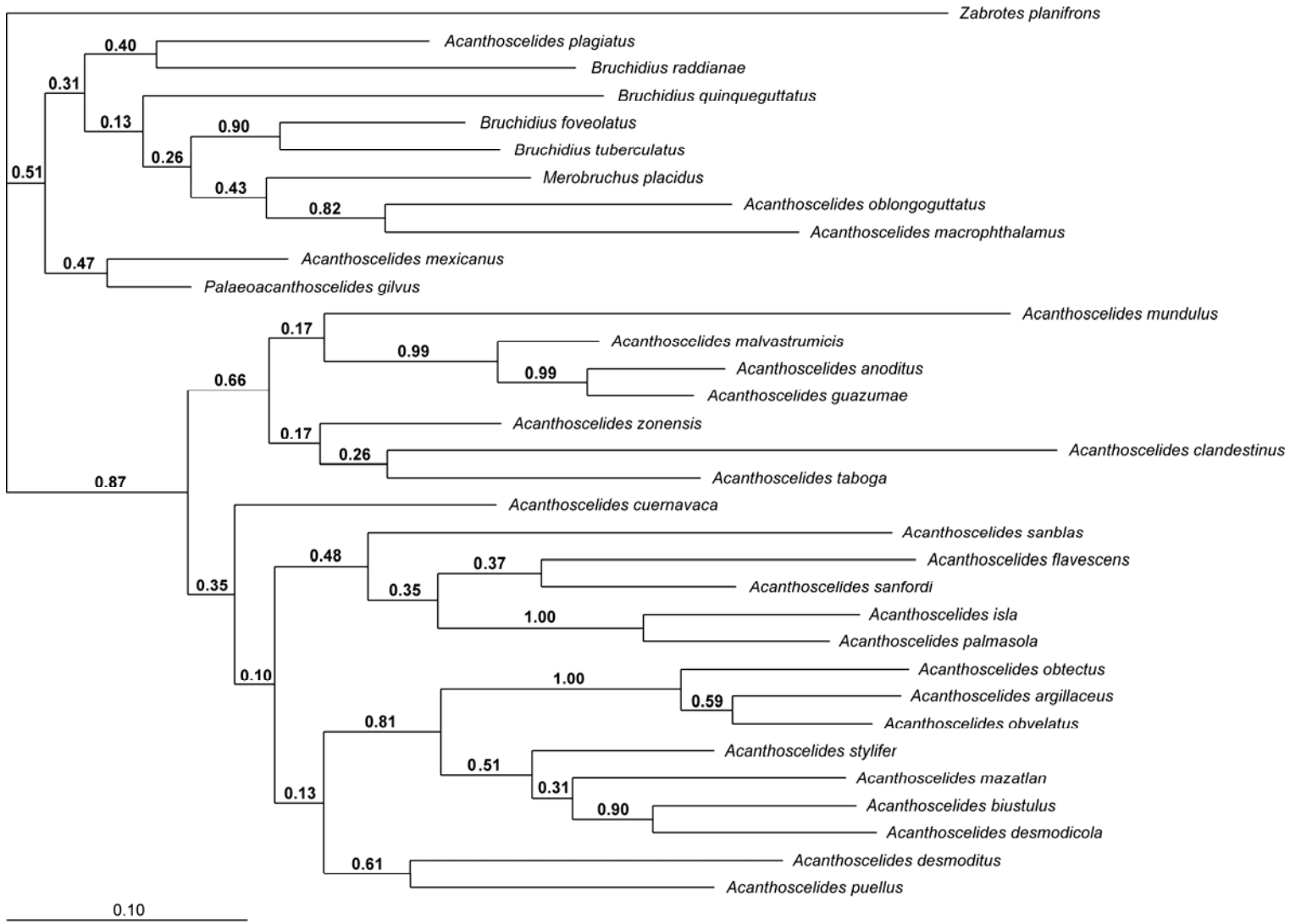
Zabrotes planifrons
0.40
Acanthoscelides plagiatus
Bruchidius raddianae
0.31
Bruchidius quinqueguttatus
0.13
0.90
Bruchidius foveolatus
Bruchidius tuberculatus
0.26
0.51
Merobruchus placidus
0.43
0.82
Acanthoscelides oblongoguttatus
Acanthoscelides macrophthalamus
0.47
Acanthoscelides mexicanus
Palaeoacanthoscelides gilvus
Acanthoscelides mundulus
0.17
Acanthoscelides malvastrumicis
0.99
0.99
Acanthoscelides anoditus
0.66
Acanthoscelides guazumae
Acanthoscelides zonensis
0.17
0.26
Acanthoscelides clandestinus
Acanthoscelides taboga
0.87
Acanthoscelides cuernavaca
Acanthoscelides sanblas
0.48
Acanthoscelides flavescens
0.37
0.35
Acanthoscelides sanfordi
0.35
1.00
Acanthoscelides isla
Acanthoscelides palmasola
0.10
Acanthoscelides obtectus
1.00
Acanthoscelides argillaceus
0.59
Acanthoscelides obvelatus
0.81
Acanthoscelides stylifer
0.51
Acanthoscelides mazatlan
0.31
0.13
0.90
Acanthoscelides biustulus
Acanthoscelides desmodicola
0.61
Acanthoscelides desmoditus
Acanthoscelides puellus
0.10

Figure 3

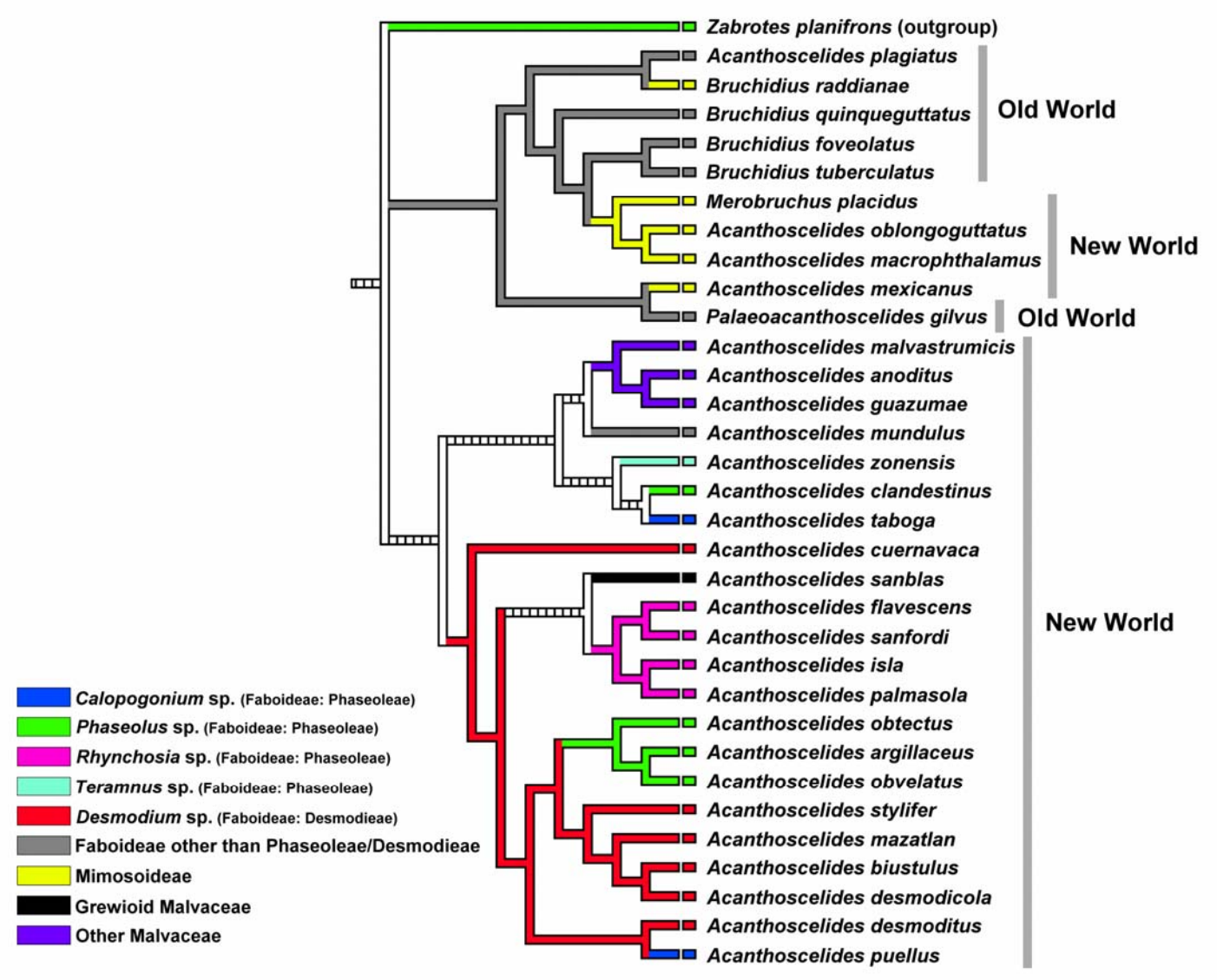
Zabrotes planifrons (outgroup)
Acanthoscelides plagiatus
Bruchidius raddianae
Bruchidius quinqueguttatus
Bruchidius foveolatus
Bruchidius tuberculatus
Old World
Merobruchus placidus
Acanthoscelides oblongoguttatus
Acanthoscelides macrophthalamus
Acanthoscelides mexicanus
New World
Palaeoacanthoscelides gilvus
Old World
Acanthoscelides malvastrumicis
Acanthoscelides anoditus
Acanthoscelides guazumae
Acanthoscelides mundulus
Acanthoscelides zonensis
Acanthoscelides clandestinus
Acanthoscelides taboga
Acanthoscelides cuernavaca
Acanthoscelides sanblas
Acanthoscelides flavescens
Acanthoscelides sanfordi
Acanthoscelides isla
Acanthoscelides palmasola
Acanthoscelides obtectus
Acanthoscelides argillaceus
Acanthoscelides obvelatus
Acanthoscelides stylifer
Acanthoscelides mazatlan
Acanthoscelides biustulus
Acanthoscelides desmodicola
Acanthoscelides desmoditus
Acanthoscelides puellus
New World
Calopogonium sp. (Faboideae: Phaseoleae)
Phaseolus sp. (Faboideae: Phaseoleae)
Rhynchosia sp. (Faboideae: Phaseoleae)
Teramnus sp. (Faboideae: Phaseoleae)
Desmodium sp. (Faboideae: Desmodieae)
Faboideae other than Phaseoleae/Desmodieae
Mimosoideae
Grewioid Malvaceae
Other Malvaceae

Figure 4

Legends

Table 1. List of sampled species, with information about the author, the site of sampling, the sampling date, the name of the collector, the host plant, the morphological group (in Neotropical *Acanthoscelides*, as defined by Johnson [1983, 1990], and the accession number corresponding to the *12s rRNA* sequence deposited in Genbank. Sampling countries were abbreviated as follows: Alg.=Algeria, Aze.=Azerbaijan, Col.=Colombia, Ecu.=Ecuador, Nic.=Nicaragua, Pan.=Panama, Tad.=Tadjikistan, Tur.=Turkey, Ven.=Venezuela, Vie.=Vietnam, Yem.=Yemen. An $^{(*)}$ indicates that collected species were obtained from unknown host plants, and that we assigned the host-plant most commonly associated with the species (from Johnson [1983, 1990] and Udayagari and Wadhi [1989]).

Table 2. Differences revealed by discriminant analysis between the species groups defined on morphological grounds. Pairs of groups were compared using Hotelling's T Squared statistics based on axis values of the multivariate correspondence analysis. ***: $p < 10^{-3}$; **: $p < 10^{-2}$; * : $p < 0.05$; NS : non significant. Groups in bold show significant differences from at least seven of the nine other groups.

Table 3. Host-plant associations for the species groups *aequalis* (*aeq.*), *albopygus* (*alb.*), *blanchardi* (*bla.*), *puellus* (*pue.*), and *pertinax* (*per.*). Names of host-plant groups were abbreviated as follows: Faboideae (Fab.), Aeschynomeneae (Aesch.), Amorpheae (Amor.), Desmodieae (Des.), Galegeae (Gal.), Indigofereae (Ind.), Loteae (Lot.), Millettieae (Mil.), Phaseoleae (Phas.), Mimosoideae (Mim.), Malvaceae *sensu lato* (Mal.), Rhamnaceae (Rha.).

Figure 1. Likelihood mapping analysis of the data set, represented as a triangle. Values at the corners indicate the percentages of well-resolved phylogenies for all possible quartets, and values at the central and lateral regions are percentages of unresolved phylogenies. The cumulatively percentage (86%) from the corner values indicates the presence of a good overall phylogenetic signal.

Figure 2. (a) Maximum parsimony consensus phylogenetic tree obtained after 1000 bootstraps from the re-weighted parsimony analysis (most parsimonious tree = 577 steps; rescaled consistency index = 0.2869). Numbers adjacent to nodes give bootstrap support values greater than 20% calculated for 1,000 replicates. **(b)** Optimal maximum likelihood phylogenetic tree obtained using PHYML (log[likelihood] = -3082.061831). Bootstrap support was determined using both PHYML (1000 replicates) and PAUP* (100 replicates), and is shown by numbers adjacent to nodes (PHYML values in bold; PAUP* values in italic). Bootstraps are shown only when for a given node, a value greater than 20% was determined either by PHYML or by PAUP*.

Figure 3. Phylogenetic tree obtained from the Bayesian inferences analysis. At each node, the number indicates the Bayesian posterior probabilities.

Figure 4. Consensus phylogenetic tree obtained from the Bayesian inferences analysis. On the cladogram is represented (with different branch colors) the host-plant genus – or tribe or subfamily – on which a considered bruchid species develops. On the right side of the tree is figured the biogeographic origin of the species (New World *vs.* Old World).

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