When insects help to resolve plant phylogeny: evidence for a paraphyletic genus *Acacia* from the systematics and host-plant range of their seed-predators

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In this study we use an indirect method to address the issue of the systematics of the large and economically important genus *Acacia* (Leguminosae, Mimosoideae, Acacieae). We propose the use of host-preference data in closely related insect species as a potentially useful tool to investigate host systematic issues, especially when other approaches yield inconsistent results. We have examined the evolution of host-plant use of a highly specialized group of seed-feeders who predate *Acacia* — the seed-beetles (Coleoptera, Chrysomelidae, Bruchinae). First, the evolution of host-plant preferences in a large clade of *Bruchidius* species was investigated using molecular phylogenetics and character optimization methods. Second, the scope of our study was enlarged by critically reviewing the host-plant records of all bruchine genera associated with *Acacia*. Both morphological and molecular data were used to define relevant insect clades, for which comparisons of host-plant range were performed. Interestingly, the analyses of host-plant preferences from 163 seed-beetle species recovered similar patterns of host-plant associations in the distinct clades which develop within *Acacia* seeds. Our results clearly support the hypothesis of *Acacia* being a paraphyletic genus and provide useful insights with reference to the systematics of the whole subfamily as well. This study should also be of interest to those involved in the numerous biological control programs which either already use or aim to use seed-beetles as auxiliary species to limit the propagation of several invasive legume tree species.
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Introduction

Within the economically important family Leguminosae, members of the large genus *Acacia* (Mimosoideae, Acacieae) have for many years provided the subject-matter of numerous studies with regard to their ecological and economic importance. Since its description in 1754 by P. Miller, the nomenclatural history of *Acacia* has been a complex one, with numerous controversial works and proposals (reviewed in Maslin *et al*. 2003). Recently, the Nomenclature Section of the 17th International Botanical Congress (ICB) ratified the retypification of the genus, following the proposal of Orchard & Maslin (2003) to change the type of *Acacia* from *A. scorpioides* (syn. *A. nilotica*) to *A. pennivernis* (see Maslin 2005 and Seigler *et al*. 2006 for a review of the nomenclatural implications).

Most *Acacia* species are characterized by free filaments of the stamens (Bentham 1842, 1875; but see Vassal 1981); however, as stressed by Miller & Bayer (2003), their positioning as a natural group is not supported by any synapomorphic characters. While the subdivision of the genus into smaller genera has been repeatedly proposed by many authors in the past (see in particular the contribution of Pedley 1986), it is only recently that new insights have been obtained following the plethora of phylogenetic studies using molecular markers (e.g. Clarke *et al*. 2000; Robinson & Harris 2000; Luckow *et al*. 2003; Miller & Bayer 2003; Miller *et al*. 2003).

Table 1 Main Acacia classification systems.

Bentham (1875) (1 genus, 6 series)	Vassal (1981) (1 genus, 3 subgenera)	Pedley (1986) (3 genera)	Maslin *et al*. (2003) (5 genera)
Gen. *Acacia*	Gen. *Acacia* Subg. *Phyllodineae*	Gen. *Racosperma*	Gen. *Acacia*
Ser. *Botrycephalae*			
Ser. *Phyllodineae*			
Ser. *Pulchellae*			
Ser. *Vulgares*	Subg. *Aculeiferum* Sec. *Monacanthea*	Gen. *Senegalia*	Gen. *Senegalia*
	Sec. *Aculeiferum*	Sec. *Senegalia*	
	Acacia coulteri group	*Acacia coulteri* group	Gen. *Mariosousa***
Ser. *Filicinae*	Sec. *Filicinae*	Sec. *Filicinae*	Gen. *Acaciella*
Ser. *Gummiferae*	Subg. *Acacia*	Gen. *Acacia*	Gen. *Vachellia*

*Formerly genus 'X' in Maslin *et al*. (2003).

As emphasized by Maslin *et al*. (2003), these studies indicate that the genus is paraphyletic and also stress the need of dividing the 1350 *Acacia* species into at least five distinct genera. In this paper we have followed the classification proposed by Maslin *et al*. (2003), in which *Acacia sensu lato* is treated as comprising multiple genera (see Table 1). Although the paraphyletic status of the genus is now widely acknowledged, numerous questions remain unanswered due to the inconsistency of the tree topologies obtained in various studies. For instance, the issue of the respective phylogenetic positions of the various *Acacia s.l.* lineages within subfamily Mimosoideae is still debated, with several molecular studies (Luckow *et al*. 2003; Miller & Bayer 2003; Miller *et al*. 2003) suggesting that the genus *Vachellia* (syn. *Acacia* subgen. *Acacia* in Vassal's classification) is more closely related to plants belonging to the tribe Mimoseae, while the four other *Acacia* genera are clustered with members of the Ingeae tribe. In this context it seems appropriate to provide additional insights into *Acacia s.l.* systematics by using other sources of information.

Phytophagous insect species are mostly oligophagous or monophagous and usually feed on a restricted number of host-plant taxa (Strong *et al*. 1984; Futuyma & Moreno 1988; Jaenike 1990; Ward & Spalding 1993; Bernays & Chapman 1994; Funk *et al*. 2002; but see also Novotny *et al*. 2002). Most of them also specialize in specific host-plant tissues like stems or leaves (Bernays & Chapman 1994). The latter trend, towards trophic specialization, is even more marked in insect groups which feed on internal tissues (e.g. roots, seeds or stems; Gaston *et al*. 1992; Anderson 1995; Marvaldi *et al*. 2002). Interestingly, numerous studies (e.g. Mitter *et al*. 1991; Farrell 1998) have revealed that these restricted feeding habits are often conserved over long time periods, resulting in a evolutionary pattern which is commonly referred as 'taxonomic conservatism in host-plant use'. In the latter pattern, closely related insects generally feed on closely related host-plants. In some past studies (Eastop 1979; Johnson 1980), the

analysis of the host-plant preferences of groups of specialized phytophagous insects has been proposed as a relevant means of investigating the systematics of their host-plants. The argument is that these predators are host-specific, enabling us to trace the evolutionary trajectories of their hosts fairly closely. Here we propose investigating the systematics of the genus *Acacia* by using evidence from the systematics and host-plant range of some of its seed-predators.

Numerous guilds of seed-predators develop within the seeds of most *Acacia s.l.* species. The specialized seed-beetles (Coleoptera, Chrysomelidae, Bruchinae) are especially noteworthy, because members of seven bruchine genera (*Acanthoscelides, Bruchidius, Caryedon, Merobruchus, Mimosestes, Pseudopachymerina* and *Stator*) are known to develop in *Acacia s.l.* seeds (Borowiec 1987). Previous studies have also demonstrated that bruchines generally exhibit a strong level of taxonomic conservatism in host-plant use (Silvain & Delobel 1998; Kergoat *et al*. 2004, 2005a, 2005b; Tuda *et al*. 2006).

In this paper we hypothesize that the study of host-plant associations in these bruchines may provide meaningful insights into *Acacia s.l.* systematics. We test this hypothesis by first investigating in depth the phylogenetic relationships of a large Palaeotropical clade of *Bruchidius* species associated with an exceptionally diverse set of Mimosoideae species belonging in particular to the tribe Ingeae and to the genera *Senegalia* and *Vachellia*, whose phylogenetic positions among Mimosoideae are unresolved. We use the resulting molecular phylogeny to discuss the evolution of host-plant preferences in this group by employing maximum likelihood optimization methods. Second, we enlarge the scope of our study by critically reviewing the host-plant records for all the bruchine genera which are known to develop within *Acacia s.l.* seeds. For six genera, we have defined relevant insect clades whose host-plant ranges were analysed. Finally, in the seed-feeding groups studied, we discuss the observed patterns of host-plant association with reference to extant phylogenetic

Table 2 Taxon sampling. *Abbreviations*: FG, French Guyana; In., India; Ja., Japan; Ke., Kenya; Se., Senegal; Ta., Taiwan; Th., Thailand.

Genus/Species	Reared from	GenBank Accession No.			
		12S	Cyt *b*	COI	28S
Bruchidius					
albizziarum (Decelle)	*Albizia lebbeck* (Se.)	AY625325	AY635471	AY625422	AY625372
auratopubens Decelle, in litt.	*Faidherbia albida* (Se.)	AY625282	AY625429	AY625379	AY625332
aureus Arora	*Albizia lebbeck* (In.)	DQ524346	DQ524340	DQ524356	DQ524352
babaulti (Pic)	*Acacia amythethophylla* (Ke.)	AY625326	AY625472	AY625423	AY625373
badjii Delobel	*Dialium guineense* (Se.)	AY625289	none	none	none
cadei Decelle, in litt.	*Faidherbia albida* (Se.)	AY625284	AY625431	AY625381	AY625334
campylacanthae Dec., in litt.	*Acacia polyacantha* (Se.)	AY625285	AY625432	AY625382	AY625335
chloroticus (Dalm.)	*Sesbania pachycarpa* (Se.)	AY625286	AY625433	AY625383	AY625336
dichrostachydis Del. & Ant.	*Dichrostachys cinerea* (Se.)	AY625288	AY625435	AY625385	AY625338
flavovirens Arora	*Albizia chinensis* (Th.)	DQ524347	DQ524341	DQ524357	none
lerui Delobel	*Delonix elata* (Ke.)	AY625312	AY625457	AY625408	AY625358
lineolatus Arora	*Albizia chinensis* (Th.)	DQ524348	none	DQ524358	none
natalensis (Pic)	*Acacia sieberiana* (Se)	AY625327	AY625473	AY625424	AY625374
pygidiopictus Decelle, in litt.	*Faidherbia albida* (Se.)	AY625295	AY625440	AY625391	AY625343
quadrisignatus (Fahraeus)	*Acacia ataxacantha* (Ke.)	AY625296	AY625441	AY625392	AY625344
rubicundus (Fahraeus)	*Acacia laeta* (Ke.)	AY625298	AY625443	AY625394	AY625346
saundersi (Jekel)	*Albizia lebbeck* (Th.)	DQ524349	DQ524342	DQ524359	DQ524353
silaceus (Fahraeus)	*Acacia macrostachya* (Se.)	AY625328	AY625474	AY625425	AY625375
sinaitus (K. Daniel)	*Acacia tortilis* (Se.)	AY625329	AY625475	AY625426	AY625376
sparsemaculatus Pic	*Albizia lebbeck* (Th.)	DQ524350	DQ524343	DQ524360	DQ524354
submaculatus (Fahraeus)	*Acacia senegal* (Se.)	AY625301	AY625446	AY625397	AY625348
subuniformis (Pic)	*Acacia ataxacantha* (Ke.)	AY625330	AY625476	AY947519	AY625377
terrenus (Sharp)	*Acacia confusa* (Ta.)	DQ524351	DQ524344	DQ524361	DQ524355
urbanus (Sharp)	*Acacia catechu* (Ja.)	none	DQ524345	DQ524362	none
sp. KE01	*Faidherbia albida* (Ke.)	AY625303	AY625448	AY625399	AY625350
sp. KE02	*Acacia brevispica* (Ke.)	AY625304	AY625449	AY625400	AY625351
sp. KE04	*Acacia etbaica* (Ke.)	AY625306	AY625451	AY625402	AY625353
sp. KE05	*Acacia nilotica* (Ke.)	AY625307	AY625452	AY625403	AY625354
sp. KE07	*Acacia oerfota* (Ke.)	AY625309	AY625454	AY625405	AY625356
sp. KE08	*Acacia zanzibarica* (Ke.)	AY625310	AY625455	AY625406	none
sp. KE09	*Albizia versicolor* (Ke.)	AY625311	AY625456	AY625407	AY625357
Gibbobruchus sp.	unknown Cercidae (FG)	AY625331	AY625477	AY625428	none
Pachymerus cardo (Fahraeus)	*Elaeis guineensis* (FG)	AY390636	AY390700	AY390668	AY625378

hypotheses for the subfamily Mimosoideae in order to help clarify the puzzling issues of *Acacia s.l.* systematics.

Materials and methods

Phylogenetic analyses – Evolution of host-plant association

Thirty *Bruchidius* species belonging to a monophyletic group (Kergoat & Silvain 2004; Kergoat *et al.* 2005b), herein referred to as 'clade I' and three outgroups (i.e. *Bruchidius chloroticus*, *Gibbobruchus* sp. and *Pachymerus cardo*) were sampled (see Table 2). In comparison with a previous study (Kergoat *et al.* 2005b), our sampling is much more representative of the diversity of this Palaeotropical group, as it encompasses Asian species. The use of more extensive sampling is noteworthy because it provides firmer ground on which to discuss the evolution of the whole group.

Sequences were obtained for four genes: mitochondrial 12 s rRNA (12S), cytochrome *b* (Cyt b) cytochrome *c* oxidase subunit I (COI), and nuclear 28s rDNA (28S) (see Kergoat *et al.* 2004, 2005b for a list of primers and PCR cycling conditions). Since the sequences of ribosomal genes (i.e. 12S and 28S) presented some variations in length, their alignment was performed using ClustalX (Thompson *et al.* 1997) with default option settings. Manual correction of small misalignments was conducted in Seaview (Galtier *et al.* 1996). The resulting combined data set (2944 bp in length) was deposited in TreeBase under accession number SN2846.

As a first approach to reconstructing the molecular phylogenies of the sampled *Bruchidius* species, we carried out unweighted maximum parsimony (MP) analyses using PAUP* ver. 4.0b10 (Swofford 2003). Under MP, we performed heuristic searches by using tree-bisection-reconnection (TBR) branch swapping, 1000 random-addition replicates, and a MaxTrees value of 1000. To test the congruence of the phylogenetic information carried out by the four genes, the incongruence length difference test (ILD; Farris *et al.* 1994) was used, with all invariant characters excluded (Cunningham 1997). Since the result of

the ILD test was not significant ($P > 0.05$), we chose to perform an analysis of the combined data set. Relative support of nodes for MP analyses was assessed by nonparametric bootstrap (Felsenstein 1985) procedures, with 1000 pseudoreplicates of 1000 random-addition replicates used. Nodes supported by bootstrap values ≥ 70% were considered to be strongly supported (Hillis & Bull 1993).

We then conducted Bayesian inference (BI) analyses using MrBayes ver. 3.1.1 (Huelsenbeck & Ronquist 2001). We carried out partitioned analyses of the combined data set to increase the fit of the evolutionary models with the data (Yang 1996; Nylander *et al.* 2004; Brandley *et al.* 2005). For each gene (one partition per gene was used), the best-fit model of evolution was determined by using the Akaike information criterion (AIC) as implemented in Modeltest ver. 3.06 (Posada & Crandall 1998). Two independent BI runs were conducted to identify whether convergence of clade posterior probabilities (CPP) had been reached (Huelsenbeck *et al.* 2002). For each run, four incrementally heated Markov chains of 2×10^6 generations were used, with random starting trees, default priors and trees sampled every 100 generations (branch lengths were also saved). A burn-in period of 1×10^5 generations was defined for all BI runs and stationarity was assessed graphically. Support of nodes for BI analyses was provided by CPP estimates. Only clades with posterior probabilities ≥ 90% were considered as well supported in BI analyses, in agreement with recent studies which have suggested that CPPs are less conservative than nonparametric bootstrap values (Alfaro *et al.* 2003; Erixon *et al.* 2003).

The evolution of host-plant association in *Bruchidius* species belonging to clade I was investigated by using maximum likelihood (ML) reconstructions. These were preferred over MP reconstructions because they allow the assessment of uncertainty in ancestral trait reconstruction and because they can take into account branch length information (Schluter *et al.* 1997; Pagel 1999). We used Multistate ver. 0.8 (Pagel 2003) to carry out local optimizations; a simpler model (in which forward and backward rates were constrained to be equal) was used to avoid over-parameterization issues (Pagel 1999). We considered that the support of one state over another (at a given node) was significant if the difference between their log-likelihoods was greater than or equal to 2.0 (Schluter *et al.* 1997; Pagel 1999).

In an initial attempt to categorize host-plant information, we used the following character states: (i) tribe Ingeae; (ii) genus *Senegalia* (syn. *Acacia* subg. *Aculeiferum*); (iii) genus *Acacia* (syn. *Acacia* subg. *Phyllodineae*); (iv) genus *Vachellia* (syn. *Acacia* subg. *Acacia*); (v) tribe Mimoseae; (vi) subfamily Caesalpinioideae; (vii) other legumes. However, it appeared that some species in our data-set were able to develop on plants from the tribe Ingeae and on plants belonging to either the genus *Senegalia* or the genus *Acacia*. To deal with these

multiple associations in our optimizations, we chose to consider only the most-represented host-plant tribe or genera in the coding of the corresponding character states. Consequently, one of the seven character states (i.e. genus *Acacia*) was discarded in our analyses, and only six were retained to study the evolution of host-plant association in this clade. While being not entirely satisfactory, this treatment of data was preferable to alternative methods which generally produce their own bias (Lopez-Vaamonde *et al.* 2003). In addition, phylogenetic structure in host-plant associations was assessed using the permutation tail probability test (PTP; Faith & Cranston 1991), as implemented in PAUP*. Within-character randomization was only applied to the ingroup taxa (i.e. *Bruchidius* clade I species) to avoid misleading PTP scores (Trueman 1996).

Host-plant records
Host-plant data were carefully reviewed and doubtful records discarded from the analysis (following Kergoat *et al.* 2005a). All the names from the literature were also checked by using the International Legume Database and Information Services database (ILDIS; http://www.ildis.org), and updated when necessary. Data based on the rearing of adults from seeds collected in the field were also preferred. Thanks to the rigorous fieldwork of several reputable entomologists such as L.J. Bottimer, C.D. Johnson and J.M. Kingsolver, reliable host-plant records were available for all studied New World genera (*Acanthoscelides*, *Merobruchus*, *Mimosestes*, *Pseudopachymerina* and *Stator*). This was also the case for the Old World genus *Bruchidius*, for which considerable sampling in the field has recently been performed in Africa (see Kergoat *et al.* 2005b) and Asia (this study). In addition, reliable host-plant records for this genus were found in the literature (e.g. see Arora 1977, 1980; Gillon *et al.* 1992). By contrast, and as stressed by the recent review of Johnson *et al.* (2004), reliable host-plant records were missing for the genus *Caryedon*. Although some trends toward taxonomic conservatism in host-plant use are discernible in this genus (e.g. all members of the species-group *interstinctus* appear to feed exclusively on *Vachellia* species), we have decided not to take this genus into account in our study.

For each bruchine study group, preference for a given subset of host-plants was assessed by using Fisher's exact test. Expected proportions (based on the host-plant records from all studied species) were compared with the observed proportions in each study group to test whether any difference of proportions was significant (see Table 3).

Definition of study groups
While considerable morphological and molecular evidence supports the monophyletic status of genera *Merobruchus*, *Mimosestes*, *Pseudopachymerina* and *Stator* (Borowiec 1987;

Table 3 Fisher's exact test.

Group	Host-plant group tested	Expected proportion	Observed proportion	P (5%)
Acanthoscelides (n = 36)	genus *Vachellia*	44/119	3/33	ns
	tribe Mimoseae	34/129	23/13	*
	tribe Parkieae	7/154	7/29	*
	subfam. Caesalp.	10/153	3/33	ns
Bruchidius clade I (n = 53)	genus *Acacia*	4/159	1/52	ns
	genus *Senegalia*	30/133	16/37	ns
	genus *Vachellia*	44/119	7/46	ns
	tribe Ingeae	56/107	27/26	*
	tribe Mimoseae	34/129	4/49	ns
	subfam. Caesalp.	10/153	4/49	ns
Bruchidius clade II (n = 16)	genus *Vachellia*	44/119	15/1	*
	tribe Mimoseae	34/129	1/15	ns
Merobruchus (n = 21)	genus *Acacia*	4/159	2/19	ns
	genus *Senegalia*	30/133	5/16	ns
	tribe Ingeae	56/107	17/4	*
	tribe Mimoseae	34/129	1/20	ns
Mimosestes (n = 16)	genus *Senegalia*	30/133	1/15	ns
	genus *Vachellia*	44/119	13/3	*
	tribe Ingeae	56/107	1/15	ns
	tribe Mimoseae	34/129	3/15	ns
	subfam. Caesalp.	10/153	3/16	ns
Pseudopachymerina (n = 1)	genus *Vachellia*	44/119	1/0	ns
Stator (n = 20)	genus *Acacia*	4/159	1/19	ns
	genus *Senegalia*	30/133	8/12	*
	genus *Vachellia*	44/119	5/15	ns
	tribe Ingeae	56/107	12/8	*
	tribe Mimoseae	34/129	4/16	ns
	subfam. Caesalp.	10/153	1/19	ns

*Number of species for each studied bruchine group in parentheses.

Silvain & Delobel 1998; Johnson *et al.* 2004; Morse & Farrell 2005; Katoh, *pers. comm.*), it appears that two genera (*Acanthoscelides* and *Bruchidius*) are likely paraphyletic (Borowiec 1987; Kergoat & Silvain 2004; Kergoat *et al.* 2005a; Alvarez *et al.* 2006). For these two large genera (which both comprise over 250 species) we have chosen to take into account only those clades associated with Mimosoideae. Within *Bruchidius* all mimosoid feeders belong to two well-defined clades (here referred to as clades I and II; see also the pruned tree in Fig. 1), whose monophyletic status is strongly supported by both molecular (Kergoat & Silvain 2004; Kergoat *et al.* 2005b) and morphological evidence (Anton & Delobel 2003; Kergoat & Silvain 2004). With regard to *Acanthoscelides*, almost all mimosoid feeders are found in five morphologically well-supported taxonomic groups (Johnson 1983, 1990). Although no comprehensive molecular studies have hitherto been made on a large sampling of these species, recent molecular phylogenetic reconstructions have nonetheless suggested that mimosoid feeder species constitute a

monophyletic group (Kergoat *et al.* 2005a; Alvarez *et al.* 2006).

Results and discussion

### *Phylogenetic analyses – evolution of host-plant association*

For *Bruchidius* clade I, we present the phylogenetic hypothesis resulting from the BI analyses (Fig. 1). Overall, this topology is well-supported, with a mean CPP value of 84.2%. The BI tree is largely compatible with the two equiparsimonious trees (length of 3609 steps) recovered under MP, and the topology differences observed among the two approaches (i.e. BI and MP) have no impact on the following discussion.

Evolutionary conservatism in host-plant associations among clade I species is suggested by the result of the PTP test ($P < 0.0001$) which indicates a significant (nonrandom) phylogenetic structure. This result is also supported by the ML reconstructions of ancestral character states which reveal a statistically supported trend toward taxonomic conservatism in two clades associated, respectively, with members of tribe Ingeae and of genus *Vachellia* (see Fig. 1). With the exception of two terminal nodes, the ML optimizations do not recover other statistically supported ancestral character states.

However, a close examination of the probabilities associated with each ancestral character state for all nodes (data not illustrated) indicate that two of them (tribe Ingeae and genus *Senegalia*) account for the majority of the observed proportions. Along with the fact that some species are able to feed on Ingeae and on members of *Senegalia*, this finding suggests a quite dynamic evolutionary pattern in which host-shifts between Ingeae and *Senegalia* are also facilitated because the two groups share the same main toxic compound (i.e. the nonprotein amino acid albizziine; see Kergoat *et al.* 2005b). By contrast, *Vachellia* spp. are well characterized by the sequestration in their seeds of the nonprotein amino acid *N*-acetyldjenkolic acid (Seigler 2003; Or & Ward 2004). All of these elements provide grounds for supporting the hypothesis of a close relationship between the two groups as well, suggesting that *Senegalia* is less closely related to *Vachellia* than to Ingeae.

Host-plant range of the mimosoid feeder bruchines

Host-plant records from 163 species were analysed (see supplementary material for a complete list of host-plant records). It is interesting to note that records of species feeding on the species-rich genus *Acacia* (for which nearly 1000 species are known) were exceptionally scarce (only 4 of the 163 studied species are associated with *Acacia*). The most likely explanation of this surprising observation can be found in the very poor knowledge of the Australian bruchine fauna (Borowiec 1987), as most of the known *Acacia* species occur in Australia. At the species level, the studied bruchines exhibit a strong trend toward specialization: nearly half of the species
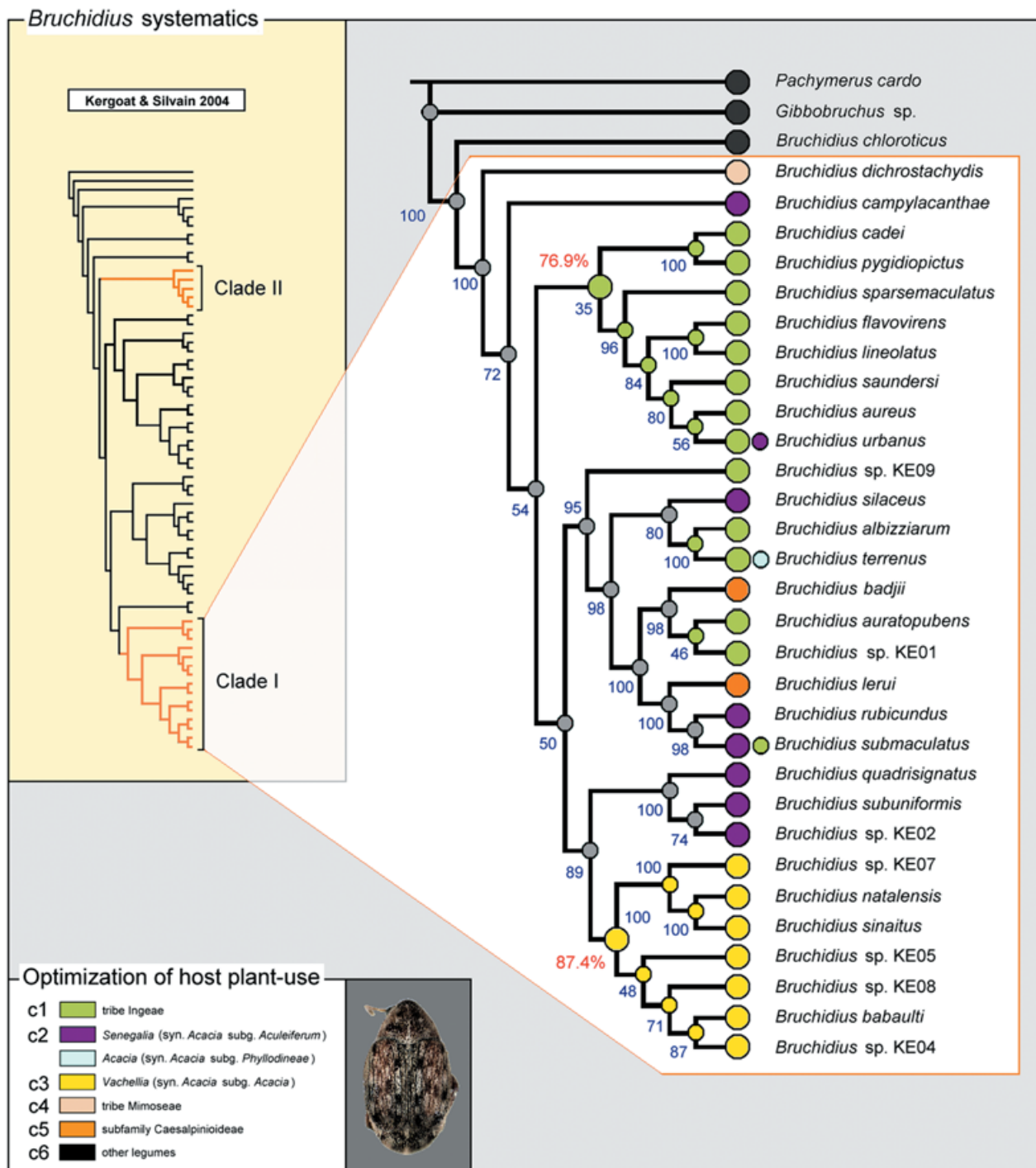
Fig. 1 Molecular systematics and optimization of host-plant use in *Bruchidius* clade I species. On the left, the pruned tree indicates the respective positions of both *Bruchidius* clade I and clade II species in the study of Kergoat & Silvain (2004). On the right, the phylogenetic relationships of clade I species are presented using the topology obtained under BI, with numbers at nodes indicating CPP values. Optimization of host-plant use was performed under Multistate using both the topology and branch lengths of BI analyses. Significantly supported character states at nodes are indicated by the various coloured circles (see the figure caption for details on host-plant groups) while their absence is indicated by uncoloured (grey) circles . The probabilities of the majority character states of two nodes are also listed in red. For illustrative purposes, a photograph of a *Bruchidius dichrostachydis* specimen is also provided.

(47.23%) are strictly monophagous, while the vast majority are oligophagous, mostly feeding on plants either from the same genus (82.82%) or tribe (88.34%).

The host-plant ranges of each group of mimosoid feeder are schematized in Fig. 2 (with the exception of the mono-specific genus *Pseudopachymerina*, which only feeds on three species of *Vachellia*). As indicated in the figure caption, multiple users of the same host-plant species by several members of a given group is indicated by the various proportions illustrated in the pie charts.

Several observations can be made from analysis of the host-range of the studied seed-beetles. First, a statistically significant bias in host-plant preferences was recovered in six of the seven studied groups of mimosoid feeder bruchines ($P < 0.05$, Fisher's exact test; see Fig. 2 for details); thus suggesting a far from random pattern of host-use in the mimosoid feeder groups. In *Acanthoscelides*, *Bruchidius* clade II and *Mimosestes* species, a similar pattern is recovered, as they are preferentially associated with one of the five recently accepted *Acacia* genera (namely *Vachellia*). In addition, they are found on Caesalpinioideae (members of tribes Cassieae and Caesalpinieae only) and on Mimosoideae (members of tribes Mimoseae and Parkieae).

By contrast, species of *Merobruchus* feed on a completely different set of host-plants, as they develop on the four other *Acacia* genera (*Acacia*, *Acaciella*, *Mariosousa* and *Senegalia*) and on members of the tribe Ingeae (Mimosoideae). Finally, in *Bruchidius* clade I and genus *Stator*, species of seed-beetles are able to feed on a larger set of host-plants which includes both *Vachellia* and other *Acacia s.l.* genera. For these two clades, molecular phylogenies permit discussion in greater depth of the evolution of host-plant range. With regard to *Stator*, the study by Morse & Farrell (2005) has revealed complex patterns of host-plant associations that are not only influenced by the relatedness of host-plants, but also by the oviposition behaviours of the females. Interestingly, their study indicates that the species that are only associated with *Vachellia* are closely related, thus suggesting that host-shifts to *Vachellia* are quite conservative. For both *Stator* (with the exception of the most polyphagous species such as *Stator pruininus*) and *Bruchidius* clade I species it thus appears that feeding on *Vachellia* and feeding on other *Acacia s.l.* genera (plus Ingeae) are generally mutually exclusive.

The above information is especially useful when considering (see Fig. 2) the various competitive phylogenetic hypotheses from five recent molecular studies on Mimosoideae (Clarke *et al.* 2000; Robinson & Harris 2000; Miller & Bayer 2003; Miller *et al.* 2003; Luckow *et al.* 2003). Two main patterns are recovered in the studied mimosoid feeder groups. In the first, the vast majority of species feed on *Vachellia* but also on members of tribes Cassieae, Caesalpinioideae, Mimoseae and Parkieae. In the second, the vast majority of species feed on other *Acacia*
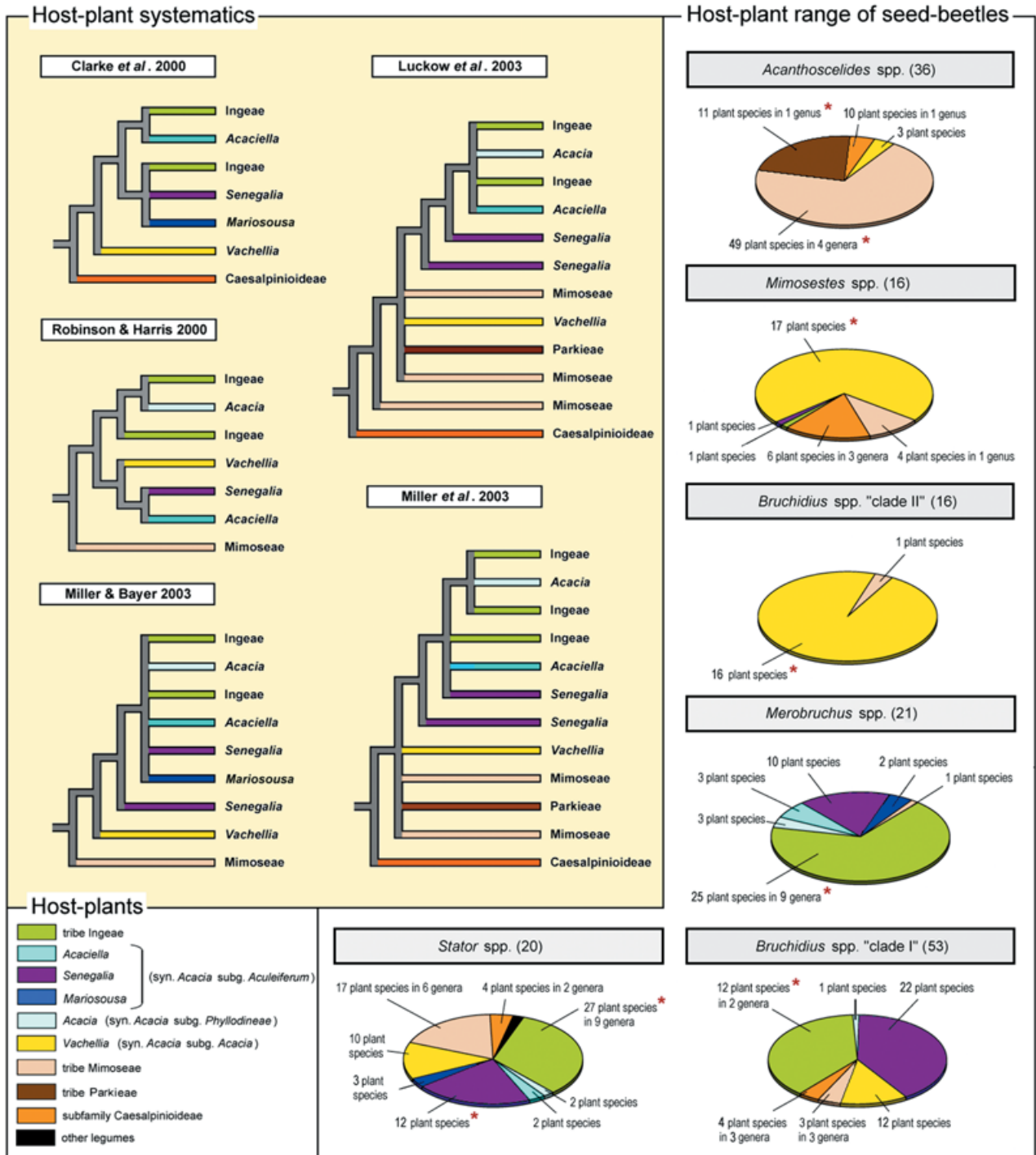
s.l. genera but also on members of the tribe Ingeae. Interestingly, the two patterns of host-plant associations are mutually exclusive, with only a few exceptions to the rule (e.g. some polyphagous *Stator* species or the two *Bruchidius* species associated with Caesalpinioideae).

Comparison of the above patterns with the existing plant phylogenies suggests a strong correspondence between the host-plant phylogenies and the host-range of the mimosoid feeders, as some groups of beetles are either associated with basal species of Mimosoideae (i.e. *Vachellia* and members of tribes Mimoseae, Parkieae) and Caesalpinioideae (tribes Cassieae and Caesalpinieae only) or with more derived species of Mimosoideae (i.e. genera *Acacia*, *Acaciella*, *Mariosousa*, *Senegalia* and members of the tribe Ingeae). The host-plant ranges of the seed-beetles do not overlap, thus suggesting that *Vachellia* spp. are not related to other species of *Acacia s.l.*, in agreement with the hypothesis of a paraphyletic genus *Acacia s.l.*

In summary, our results are consistent with those of the phylogenetic studies which suggest that *Vachellia* species are more closely related to basal mimosoid tribes, while other *Acacia s.l.* species are more closely related to the derived tribe Ingeae. They also suggest that basal mimosoids are more closely related to the tribes Cassieae and Caesalpinieae of the Caesalpinioideae, in agreement with previous studies on legume systematics (Käss & Wink 1996; Doyle *et al.* 1997; Bruneau *et al.* 2001). Since numerous species of seed-beetles are actually used in various biological control programs (e.g. see Fowler *et al.* 2000; Radford *et al.* 2001; van Klinken 2004), we stress the importance of carefully assessing the risks of possible host-shifts toward nontarget native plants, especially when dealing with invasive *Acacia s.l.* species. For instance, plants of the tribe Ingeae should be included in host-range tests of any biological control programs directed against *Senegalia* species. Since the pattern of host-plant association is quite conservative in bruchines, we also suggest studying the host-ranges of the related species of any seed-beetle species to be used in a biological control program.

Fig. 2 Host-plant systematics and known host-plant range of the seed-beetle genera associated with *Acacia* species. On the left, various phylogenetic hypotheses from five recent molecular studies on Mimosoideae are illustrated using pruned trees. On the right, the known host-plant range of each genus (or subset of a given genus in the case of *Acanthoscelides* and *Bruchidius* spp.) is summarized using a pie chart. Multiple uses of the same host-plant species by several members of a given genus (or a subset of a genus) were summed up to draw the various proportions which are depicted in the pie diagrams. For each host-plant category (e.g. tribe Ingeae or genus *Vachellia*) the known number of plant species (and also the number of genera when considering a suprageneric category) predated by a given insect group is also provided. Asterisks indicate statistically supported preferences for given subsets of host plant (using Fisher's exact test). Finally, the number of species analysed for each insect group is given in parentheses.

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