The phylogeny of monkey beetles based on mitochondrial and ribosomal RNA genes (Coleoptera: Scarabaeidae: Hopliini)

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ARTICLE INFO
Article history:
Received 27 December 2010
Revised 5 April 2011
Accepted 18 April 2011
Available online 6 May 2011

Keywords:
Scarabaeidae
Melolonthinae
Classification
Activity patterns
Sexual dimorphism
Species diversification

ABSTRACT

Monkey beetles (Hopliini) are a large clade of flower and leaf feeding species within the Scarabaeidae (chafers) with greatest diversity in southern Africa. Their internal relationships and sister group affinities have not been studied with DNA methods. We used partial gene sequences for 28S rRNA, cytochrome oxidase I (cox1) and 16S rRNA (rrnL) for 158 species, representing most recognized subfamilies of Scarabaeidae, including 46 species of Hopliini. Combined analyses using maximum likelihood and Bayesian inference under the two preferred alignment parameters recovered the Hopliini as monophyletic. Hopliines were inserted at the base of a clade of Cetoniinae + Rutelinae + Dynastinae, being either recovered as their immediate sister group, or as part of an expanded set of basal branches that also includes the tribe Macroactylini which has been classified as part of the Melolonthini (may chafers). At the level of subtribes, we found Hopliina paraphyletic with respect to Pachycnemina which also includes the monophyletic clade of Heterochelina and Gymnolomina. Trait mapping under parsimony on the preferred tree resulted in inferences of three independent origins of sexual dimorphism, which coincided with shifts to 'flower-embedding' pollination. In contrast, night active taxa, which are general phyllophages as other pleurostict chafers, never show clear sexual dimorphism. South African lineages include several deep branching lineages. The exceptional morphological and phylogenetic diversity of the South African fauna may therefore be due to their antiquity, in addition to sexual selection in the day-active lineages. Phylogenetic studies of the endemic South African plant radiations have demonstrated the repeated evolutionary shift to beetle pollination, but it remains to be investigated if this is driven by the hopliine pollinators present in the bioregion or by a propensity of the local plant lineages favoring this pollination syndrome.

1. Introduction

Among the scarab beetles (Scarabaeidae), the tribe Hopliini, or monkey beetles, are a diverse group of phytophages comprising about 1200 species (Lacroix, 1998). They are widely distributed throughout the temperate and tropical regions, but missing from the Neotropics and Australia. Hopliines are particularly diverse in Southern Africa, which harbors up to half of the world's diversity of species and genera, as well as showing great morphological diversity, while levels of endemism are also very high in this region (Colville et al., 2002; Colville, 2006). Several species are generalist herbivores, while others exhibit close associations with flowers as mating sites and as a source of pollen on which most Hopliini feed as adults (Steiner, 1998; Colville et al., 2002; Krenn et al., 2005; Carrillo-Ruiz and Morón, 2006). Due to this life style they are important pollinators, and several groups of plants have developed specialized features to attract these beetles (Steiner, 1999; Colville et al., 2002; Goldblatt et al., 2005; Goldblatt and Manning, 2006; van Kleunen et al., 2007). Specifically, they have driven the evolution of particular flower types in several genera of Iridaceae: Hopliine-pollinated flowers are often very different in pigmentation pattern, tepal size and orientation, and floral symmetry from their closest relatives pollinated by bees, moths or hummingbirds (Goldblatt and Manning, 2006). These beetle pollinated flowers are typically radially symmetrical, bowl-shaped, and relatively large, and are thus able to accommodate two or more beetles. They often show 'beetle marks', which are prominent, contrast-rich markings on the vividly pigmented floral tepals (van Kleunen et al., 2007). In addition, pheromones contribute to species-specific aggregation and mate recognition in hopliine beetles (Zhang et al., 2003).

The unique features of the hopliines raise the question about their evolutionary origin. Without doubt they are members of
the ‘pleurostict’ scarab beetles, a huge radiation of almost exclusively herbivorous species, of which only a small proportion are flower feeding. However, the precise relationships of Hopliini with other major scarab lineages, and hence the first appearance of this specialized life style, remain unclear. Some authors considered Hopliini as a lineage that is phylogenetically separated from the main scarab clades of ‘rutelines’ and ‘melolonthines’ based on striking diagnostic features, specifically the single metatarsal claw that distinguishes them from all related groups (e.g. Mulsant, 1842; Reitter, 1902; Peringuey, 1902; Janssens, 1949; Medvedev, 1976; Iablokoff-Khnzorian, 1977). Others considered Hopliini as part of the Melolonthidae (Lacordaire, 1856), Melolonthinae (Gemminger and von Harold, 1869), Melolonthinae (e.g. Dalla-Torre, 1913; Evans, 2003; Smith, 2006), or Melolonthidae (Bates, 1888; Balthasar, 1963). Yet others placed the Hopliini within ‘Rutelidae’ (=Rutelinae) (Burmeister, 1844; Mulsant and Rey, 1871; Paulian, 1959; Paulian and Baraud, 1982). Alternatively, Lacroix (1998) conside-
ered Hopliini as an independent group at the family level, but he did not comment on its relationships with other groups of Scarabaeoidea. The discussion about the position of Hopliini among either rutelines or melolonthines focused on the retractable metatarsal claws found in both Hopliini and Rutelinae (Burmeister, 1844). Only Hopliini and some species of the distinct tribe Macrodactylini have a single claw, as opposed to two claws in all other groups (Katovic, 2008). The close relationship of the latter with Hopliini was also suggested in a study using various morphological charac-
ters (Carrillo-Ruiz and Morón, 2006). However, recent studies have shown that establishing the systematic position of Hopliini is com-
plicated because the Melolonthinae, the largest group of pleuro-
stit scarabs, has been found to be paraphyletic (Ahrens, 2006; Ahrens and Vogler, 2008). Consequently, inferring the position of Hopliini will require the resolution of basal relationships of pleu-
rostict chafers more widely, based on broad taxon sampling be-
ond what is currently available (e.g. Browne and Scholtz, 1998; Hunt et al., 2007).

In addition, the internal relationships of Hopliini are of interest for studying what drives the morphological, behavioral and ecologi-
cal diversity in this lineage (Midgeley, 1992; Colville et al., 2002; Colville, 2006). In particular, a phylogenetic framework is needed to explore the evolution of major morphological tendencies and life traits (e.g. feeding behavior, diel activity pattern, sexual dimor-
phism) and the underlying processes of trait selection (Midgeley, 1992) and niche partitioning (Picker and Midgeley, 1996). As a framework for investigating the evolution of species richness in the Hopliini and their mutual relations with flowering plants we study the phylogenetic relationships of the tribe using DNA se-
quences from one nuclear and two mitochondrial genes. We are specifically interested in the extent to which the pollinator guilds, as defined by Picker and Midgeley (1996), are phylogeneti-
cally related to lineages with different life styles and how the origin of flower feeding might be correlated with the occurrence of sexual dimorphism as a possible mechanism for increased diversification rates via sexual selection.

2. Material and methods

2.1. Taxon sampling, DNA extraction, and DNA sequencing

A representative sample of 46 species was collected from vari-
ous biogeographic regions with focus on South Africa and Mad-
a-gascar representing almost 20% of the 108 genera of Hopliini. Together, these regions harbor almost 70% of species and >80% of genera of hopliones in the world. Missing genera from elsewhere are likely to be phylogenetically close to those included in the study, e.g. Palearctic species not included resemble the large genus Hoplia. Other pleurostict chafers included here represent all princi-
pal lineages (subfamilies) of Scarabaeidae, plus two species each of Hybosoridae and Gephyridae, largely following Ahrens and Vogler (2008) but with the number of Sericini reduced (Supplementary Table S1). All trees were rooted with Hybosorus illigeri. DNA was extracted from thoracic leg muscle tissue using Promega WizardSV extraction plates. Following DNA extraction, beetles were dry mounted. Vouchers will be deposited at ZFMK Bonn.

Mitochondrial gene regions included cytochrome oxidase sub-
unit 1 (cox1) and 16S ribosomal RNA (rrnL) with the adjacent regions NAD dehydrogenase subunit 1 (nad1) and tRNA leucine (trnL), hereafter referred to as rrnL. PCR and sequencing was performed for various primers Pat and Jerry for cox1 and 16Sar and ND1A for rrnL (Simon et al., 1994). For a few specimens only a shorter fragment could be obtained using the primer pair 16Sar and 16SB2 (Simon et al., 1994). Nuclear 28S rRNA containing the variable domains D3–D6 was amplified using primers FF and DD (Monaghan et al., 2007). Sequencing was performed on both strands using BigDye v. 2.1 and an ABI3730 automated sequencer. Sequences were edited manually using Sequencher v. 4.8 (Gene-
codes Corp., Ann Arbor, MI, USA). GenBank accession numbers are given Supplementary Table S1.

2.2. Phylogenetic analysis using multiple alignment

Evolutionary dynamics of mitochondrial and nuclear rRNA genes differ (e.g. Vogler and Pearson, 1996; Terry and Whiting, 2005), and hence different alignment parameters and algorithms were applied to various partitions. As we used the new data for Hopliini in the context of an existing taxon sampling of pleurostict scarabs of Ahrens and Vogler (2008), the best parameters established in that study were applied here. The choice of combi-
nation of aligned gene matrices was made according to the preferred combinations established in Ahrens and Vogler (2008) based on congruence of phylogenetic signal in a hundred combinations of different primary alignments made from various alignment procedures and parameters with various software package.

Here we only used the two best performing combinations using MAFFT (vers. 5.8; Katoh et al., 2002, 2005) and MUSCLE vers. 3.6 (Edgar, 2004) for rrnL (both with default parameters), and Clustal X (Thompson et al., 1997) for 28S (optimized parameters gap opening cost 10 and gap extension cost 6.66). These alignments were combined for phylogenetic analysis together with cox1, referred to as AL1 (cox1 + rrnLMuscof + 28SChlatax) and AL2 (cox1 + rrnLMuscle + 28SChlatax).

The concatenated data matrices were subjected to parsimony tree searches using TNT (Goloboff et al., 2004) with 10 ratchet iterations, 10 cycles of tree drifting and three rounds of tree fusing for each of 200 random addition sequences. Gaps were treated as a fifth character state. Model-based phylogenetic analyses under Maximum Likelihood (ML) were performed in PhyML (Guindon and Gascuel, 2003) using a GTR model (as selected by Modeltest using AIC; Posada and Crandall, 1998; Akaike, 1974) with all parameters estimated from the data and four substitution rate cate-
gories. Bayesian analysis was conducted using parallel MrBayes 3.2 (Huelsenbeck and Ronquist, 2001), conducting MCMC runs (Yang and Rannala, 1997) after partitioning (Nylander et al., 2004; Brandley et al., 2005) the data for rrnL, 28S, and three codon position of cox1. Based on the preferred alignment combination, we used a GTR + I + G model as determined by Modeltest with five par-
titions referring to rrnL, 28S, and the first, second and third codon position of cox1. Tracer 1.3 (Rambaut and Drummond, 2003) was used to graphically determine stationarity and convergence of runs. Tree searches were conducted for 13 and 25 × 10⁶ genera-
tions, using a random starting tree and two runs of three heated
and one cold Markov chains (heating of 0.1). Chains were sampled every 1000 generations and 10⁶ generations were discarded as burn-in based on the average standard deviation of split frequencies as well as by plotting −ln L against generation time. Bayes factors (Kass and Raftery, 1995) were calculated for both the MAFFT and the MUSCLE alignments in order to determine significant differences in the marginal likelihood of initial unconstrained compared to analyses using a constraint topology for the monophyly of Hoplini with a single metatarsal claw.

3. Results

Sequences (n = 158) for three loci (cox1, rml, 28S) were aligned and concatenated to result in two alignments produced with MAFFT (AL1) and MUSCLE (AL2), to produce matrices of 2491 and 2487 nucleotide positions, respectively (Table 1). Parsimony ratchet searches on the combined matrices using gaps as 5th character state resulted in two overall shortest trees of 21,621 and 22,037 steps for alignment AL1 and AL2. These analyses performed poorly with regard to recovery and position of well established monophyletic groups (Supplementary Fig. S3), for example placing the dung beetles (Scarabaeinae) among the pleurostict clades under AL2, and the macrodactyline genus Plectris nested within the Hoplini in the AL1 alignment. Only the AL2 alignment recovered the Hoplini as monophyletic in the strict consensus, as sister to a clade that comprised Rutelinae and Dynastinae. The poor performance of parsimony was not improved when gaps were treated as missing data, resulting in two best trees of 20,625 steps (AL2) or in four best trees of 20,597 steps (AL1) (see Supplementary Fig. S3). While in AL2 dung beetles (Scarabaeinae) were nested again within pleurosticts as sister to the monophyletic Hoplini (Supplementary Fig. S3), the strict consensus tree of the AL1 was only poorly resolved but recovered most major clades including the Hoplini as monophyletic (tree not shown).

Model based analyses performed with PhyML and MrBayes separating functional data partitions produced trees that were similar for AL1 and AL2 (Tables 1 and 2). All analyses recovered the Hoplini as monophyletic (Fig. 1, node 1), and while posterior probabilities for this node was always 1 in the Bayesian analysis, bootstrap support for the Hoplini was low under ML (18%). The tree (Fig. 1) also provides an update on the phylogeny of Pleurosticta and Plectris. The Hoplini, however, seem to be particularly well supported mainly by the mtDNA since they did not appear monophyletic in model based searches (PhyML, MrBayes) on the 28S rRNA gene alone (Supplementary Fig. S4). At the basal node, a group of Southern Hemisphere melolonthines (node 5 in Fig. 1) was found sister to all other Pleurosticti. This group had been recognized in Ahrens and Vogler (2008) as a deeply separated lineage, as sister to Sericini + Abalaberi (node 6). The Macroaustelyti (nodes 2 and 3), the possible sister group of Hoplini (Carrillo-Ruiz and Morón, 2006), was not monophyletic. In the Bayesian analysis the three Isonomyi taxa species (node 3 in Fig. 1) were widely separated from Plectris, the fourth representative of this tribe in this study. In both PhyML trees Macroaustelyti were monophyletic but only under inclusion of the genus Diphycerus which has been treated as separate tribe since the work of Medvedev (1952) (see Smith, 2006). However, in all analyses both lineages of Macroaustelyti were closely associated to the cetonine–ruteline–dynastine clade (node 4). Several rutelines and dynastines added here resulted in a placement of the monophyletic Dynastinae either as sister to Anomalini in the Bayesian analysis, or as sister to Adoretini + Anomalini (node 2) or Adoretini (AL1) with PhyML (Supplementary Fig. S1). The Rutelini + Geniatini always diverged at the base of the dynastine–ruteline clade. The South African genus Isoplia which strongly resembles the Hoplini in external appearance, in particular the hairy species of Eriesthis, was always recovered as sister of Anomalini, albeit with low branch support (Fig. 1 and Supplementary Fig. S1). Hence, these similarities are due to convergence, as initially proposed by Peringsky (1902), who treated this group as a separate tribe (Isoplia) of the Rutelinae.

The critical question about the sister group of Hoplini remained incompletely resolved. The Bayesian analyses identified the macroaustelyti genus Plectris (node 2) as sister to the Hoplini, but the relevance of this finding is unclear because of the non-monophyly of the Macroaustelyti in these trees. Under ML, in the AL1 tree Macroaustelyti + Abalaberi were sister to the Hoplini

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison of alignments and tree scores under various tree search methods. All likelihood and Bayesian analyses were conducted with the GTR + I + G model. The last row shows the results from a Bayesian analysis constrained for monophyly of taxa with a single metatarsal claw.</th>
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<tr>
<td>Alignment length</td>
<td>AL1</td>
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<td>cox1</td>
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<tr>
<td>rml</td>
<td>884 bp</td>
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<td>28S rRNA</td>
<td>781 bp</td>
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<td>420</td>
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<tr>
<td>rml</td>
<td>451 (486)</td>
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<tr>
<td>28S rRNA</td>
<td>84 (100)</td>
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<td>Tree scores</td>
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<tr>
<td>MrBayes constr.</td>
<td>78166.27</td>
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Table 2 Comparison of tree topologies from the different analyses regarding the phylogenetic resolution for major clades. Topologies differing from the preferred tree are highlighted bold (M – monophyletic, P – paraphyletic; − under inclusion of Diphycerus; 7 – uncertain polytomy; § – Cetoniinae + Rutelinae [including Dynastinae]; § – including the tribes Automolii, Liparetriini, Scitalini, Maechidiini, Heteronycini).
(Supplementary Fig. S1) while in the AL2 tree Macroductylini was again sister (under inclusion of Pachydemini, i.e. the genus Buettickeria) to the cetonine–ruteline–dynastine clade which together were the sister of Hopliini.

Moving now to the relationships within Hopliini (Fig. 2), the tree topology was very similar among various model-based searches. We found the Hopliina, one of the currently recognized subtribes (Smith, 2006), paraphyletic with respect to the Pachycnemina (clade A in Fig. 2), as well other clades such as clade B (corresponding to Heterochelina Burmeister, 1844) and clade C, corresponding to the genus Gymnoloma (=Gymnolomina Burmeister, 1844). The latter two groups have already been synonymized with Hopliina (Smith, 2006). In all model-based analyses Hoplia was monophyletic, as sister to a clade of night active species from Madagascar (Amorphochelus, Michaeloplia) and South Africa (Congella, Microplus). Only five taxa changed positions slightly in the two Bayesian searches (Fig. 2). These were Scelophysa and Echyra which switched their positions close to the ‘Clania’-Eriesthis and the Hoplia-Congella clade. In AL1 Eriesthis was paraphyletic in the MrBayes and ML trees since Pachycnema and Lepithrix were nested between Eriesthis cf. rhodesiana and all other Eriesthis species, while in AL2 Eriesthis was monophyletic in a polytomy with Pachycnema and Lepithrix (Fig. 2, node A).

3.1. Evolutionary trends and biogeography

The analysis established a phylogenetic framework for investigating the evolution of Hopliini and its interaction with flowering plants. We found that, although the monophyly of Hopliini was well supported, the tree topology was not consistent with a potential key synapomorphy, the single metatarsal claw, contradicting Carrillo-Ruiz and Morón (2006) (see Fig. 2). According to their phylogenetic hypothesis, a group of ‘Hopliini’ with two metatarsal claws, which were not included in their study, were expected to be the sister of all single-clawed Hopliini. This concerns only two hopliine genera, Cylichnus Burmeister (1844) and Nanaga Peringuey (1902) that have been classified as Hopliini by Peringuey (1902). In our analysis the double-clawed genus Cylichnus was nested deeply within the single-clawed clade and never branched off at the base. Therefore, we performed the Bayesian MCMC constrained for the monophyly of hopliine taxa with single metatarsal claw and found the Bayes factors ($B_{10}$) for AL1 and AL2 (see Fig. 1. Majority-rule consensus-tree of the Pleurosticti from Bayesian analysis of the AL1 alignment. Posterior probabilities are given below the branches. Key nodes discussed in the text are marked by numbers 1–7.)
Table 1), i.e. the present data would not reject the hypothesis that the single metatarsal claw had a single origin. Reversals to a second claw may not be implausible because rudiments of it are evident in many species of *Hoplia*, indicating a labile trait.

Further morphological and ecological features were mapped on the preferred tree, to assess their role in clade diversification and in the evolution of the beetle-flower mutualism. Many taxa exhibit sexual dimorphism (gray shading in the left tree, Fig. 2), whereby males exhibit strongly developed hind legs and claws (Scelophysina, Pachycnemina) or bear strong spines on the hind legs and differ in the color of the pygidium (often the only part of the beetle that is visible when embedded in the flower bottom) from females (Heterochelina). Based on inferred character changes on the tree, sexual dimorphism has originated three times given the present taxon sampling, although only two nodes defining these transitions are strongly supported. The dimorphism is only found in day active flower visiting taxa (black branches in tree on the right of Fig. 2), and specifically only among ‘embedding’ pollinators that persist on a single flower large enough to harbor multiple individuals. In contrast, night active taxa, all of which are general phyllophages, never show clear sexual dimorphism. Due to the poorly supported basal relationships of Hopliini the ancestral state for diel activity pattern (day or night active) remains unresolved. Interestingly, sexual dimorphism is found predominantly in South African lineages although flower feeding may occur also in European and Asian Hopliini, while night activity occurs in lineages from Asia, South Africa and Madagascar.

4. Discussion

Using the dataset of Ahrens and Vogler (2008) as a backbone, and applying the same alignment and tree search procedures found to be preferable, we now modified the sampling scheme to
focus on the systematic position of Hopliini within the major herbivore scarab lineages. Taxon sampling of Hopliini was incomplete but likely included a large proportion of major clades, as the basis for a framework of phylogenetic relationships and major evolutionary trends within the tribe. The strategy for alignment was based on the notion that homology at the nucleotide level can be established, first, relative to a phylogenetic tree that recovers ‘known’ groups whose monophyly is not in doubt based on existing knowledge and, second, using an overall measure of character homology at the nucleotide level (taxonomic congruence and character congruence sensu Wheeler, 1995). Ahrens and Vogler (2008) combined both measures of congruence into a single value to select the best alignment. All alignments generated in this study were based on progressive methods of pairwise alignment following a guide tree, implemented initially in the Clustal software and in more recent methods that use an additional refinement step. The latter would be expected to improve homology statements over the simple Clustal procedure by realignment of several closely related sequences from the initial pairwise alignment, but this was not the case throughout, as apparently the theoretical improvements of the refinement do not necessarily provide an increase of homology across the entire data set, given the criteria used (e.g. the ensemble RI as a measure of total synapomorphy). This seems to be confirmed by recent studies showing that no single method was capable of producing high quality alignments for all types of data (Aniba et al., 2010). Here we accepted the preferred alignment parameters of the earlier study under the assumption that in the current data set new taxa are added to existing deep branches already present on the tree. Hence, by adding these taxa, homology assignment at the nucleotide level would mainly affect the sampling density of indels (and nucleotide changes) on the tree, but their variation is the result of the same mutational steps and therefore can be approximated by the same alignment parameters. While specific tests might be required to establish this more firmly, we accepted the preferred settings from the earlier study on the higher hierarchical level, not least to maintain the basal relationships found there (i.e. the outgroups for the Hopliini).

The test of homology was based on parsimony analysis, and unlike the current implementations of likelihood and Bayesian analyses, parsimony searches permit the use of indel variation for tree building by coding ‘gaps’ as a fifth character state. Parsimony analysis therefore is an important heuristic tool for assessing the data set. However, phylogenetic relationships inferred with this method were unsatisfactory in particular for deep nodes. Both types of model-based analyses performed better in this regard, indicating that the complex character variation is better described by these models. Problems with the tree are therefore mainly due to the difficulties with character reconstruction in the parsimony analysis, in particular affecting basal relationships, rather than the alignment. However, although the results from an unpartitioned likelihood analysis and a partitioned Bayesian analysis were largely consistent, both using the GTR model, differences among the two alignments still show the dominant effect of alignment choice on the tree topology and the difficulty of selecting one alignment over another.

With regard to the taxonomic conclusions, we confirm the consistent recovery of a monophyletic cetonine–ruteline–dysantine clade (node 4 in Fig. 1), and the association of Macroactylini and Hopliini with that clade. This is broadly congruent with the topology proposed by Carrillo-Ruiz and Morón (2006) based on a morphological character set. Whereas the evidence for the monophyly of Macroactylini and their phylogenetic position relative to other major groups including the Hopliini remains somewhat unclear, these findings resolve the reasons for the controversy about the placement of Hopliini in the past. In particular, the discussion about their position among either rutelines or melolonthines focused on the retractable metatarsal claws found in both Hopliini and Rutelinae (Burmeister, 1844) that are present also in some Macroactylini (Katovic, 2008). Given Burmeister’s (1844) classification grouping Hopliini within Rutelinae, this character might be a synapomorphy for the Rutelinae plus Hopliini, if the single-clawed genus Cylichnus represents the ancestral state for the Hopliini. The inferred trees (Fig. 1, Supplementary Fig. S1) contradict this possibility, although a topology required under this scenario that places Cylichnus as sister to all other Hopliini could not be excluded with high statistical support. Metatarsal claws are of high adaptive value for clinging to leaves and other plant parts (see Fig. 1) and seem to be subject to selection for reduction and asymmetry of both claws, leading to convergent evolution. The evolutionary plasticity of this trait is also evident in some species of Hoplia that only show a trace of a second metatarsal claw, with some species even showing intraspecific polymorphism.

Although the functional significance of the reduction or elimination of the second metatarsal claw is unclear, these changes are found not only in the obligatory pollen feeders. The latter show some apparent adaptations for pollination shared among all day-active and florileous species. These include dense and long pilosity of the body that makes hopliine beetles more effective pollen carrier even than bees (Mayer et al., 2006). In addition, hopliones often exhibit striking coloration conferred by setae that have been transformed to colored scales. These are likely to be relevant in mate recognition and also produce mimetic patterns, e.g. to resemble bumblebees. Finally, flower visiting species show great diversity in the morphology of mouth parts (Peringuey, 1902; Nel and Scholtz, 1990; Carrillo-Ruiz and Morón, 2006), including the presence of either toothed maxilla or untoothed to membranous maxilla. These appear to be correlated with functional aspects of the feeding on pollen vs. nectar, respectively, and may be important for partitioning the food resources. Detailed analysis of various character states and homology assignment of various mouth parts are required to corroborate the link of mouthpart morphology and feeding choice.

It is well known that morphological diversity of Hopliini is greatest in the South African lineages (Peringuey, 1902). This might be due to a greater lineage age of the flower dwelling hopliines in South Africa, or to evolutionary pressures that differ from other regions in the world. The latter hypothesis would predict that South African lineages are more diverse than their sister taxa elsewhere, but this is not the case for at least one clade composed of herbivoruous South African (Congella) and Malagasy taxa that is the sister to the species rich genus Hoplia (Fig. 2). On the contrary, the South African fauna appears to be a composite of several basal lineages whose greater clade age results in higher species richness overall compared to other regions. However, the support at basal nodes is weak and the reconstruction of ancestral geographic areas (South Africa vs. Asia/Holarctic) remains uncertain (Fig. 2). In addition, lineages associated with flower feeding may not have exhibited this life style ancestrally, as it requires co-radiation with plants. Studies of the evolution of pollinator syndromes in Iridaceae (genus Babiana; Schnitzler et al., in press) demonstrated that hopliine beetle pollination has arisen multiple times as a recently derived trait. Moreover, host data of Babiana species (Goldblatt and Manning, 2007) indicate that various lineages have recruited pollinators independently from different genera, arguing against ancestral relationships with pollinators or co-evolution. Vice versa, closely related lineages of pollinators are associated with several Babiana species, suggesting that these lineages of hopliines existed already before a shift to feeding on certain flowers. The age of lineages therefore seems to be less crucial for the flower–beetle mutualism and possible effects on diversification rate. It remains to be investigated if the repeated shifts to beetle pollination is...
driven by the pollinator environment or due to traits of the endemic plant radiations in the South African bioregion. On the other hand, given multiple origins of strong sexual dimorphism in lineages of Hopliini that are all very diverse, it is likely that traits involving sexual selection might have evolved independently from a specific beetle-flower mutualism. Already a shift to unspecific pollen feeding on flowers would be sufficient to drive the evolution of sexual dimorphism due to sexual selection, since the food resource (florescence) also becomes a site for species aggregation and mating where males compete for females (Midgeley, 1992). This scenario is consistent with the fact that many pollen feeding monkey beetles are encountered on a great variety of host plants. Which of the potential drivers of diversification in South African monkey beetles is the most crucial, needs to be investigated with a denser taxon and geographical sampling comparing the influence of sexual selection vs. geographical speciation on the diversification rates.

Acknowledgments

We are grateful to Silvia Fabrici for help with the laboratory analysis; to the A. Vogler labgroup for helpful discussions; to Gerhard Burmeister, Benjamin Isambert, Michael Kuhlmann, David Lees, Sergio Murzin, and Stefan Schmidt, for collecting specimens; and to James Harrison for help with access to literature. We thank the anonymous referees who helped to improve the final version of the manuscript. Laboratory analysis at the NHM was partly supported by NHM grant-in-aid. DA was supported by the German Science Foundation (DFG/ AH175/1-2 and AH/175/2-1).

Appendix A. Supplementary material


References


