Molecules, morphology and *Mimeoma* scarabs: evolutionary and taxonomic implications for a palm-associated scarab group

MATTHEW R. MOORE1,2, CRISTIAN F. BEZA-BEZA1,3, DAVID A. WICKELL1, JAMES B. BECK1 and MARY L. JAMESON1

1Department of Biological Sciences, Wichita State University, Wichita, KS, U.S.A., 2Department of Entomology and Nematology, University of Florida, Gainesville, FL, U.S.A. and 3Department of Biological Sciences, University of Memphis, Memphis, TN, U.S.A.

Abstract. Cyclocephaline scarabs, the second largest tribe of rhinoceros beetles, are important pollinators of early-diverging angiosperm families in the tropics. The evolutionary history of cyclocephaline genera is poorly resolved and several genera are thought to be nonmonophyletic. We assess the monophyly of *Mimeoma* Casey, a group of Neotropical palm-feeding scarabs, and its relationship to *Cyclocephala* with a phylogenetic analysis of 2899bp of DNA sequence data and 18 morphological characters. All five species of *Mimeoma* were included in analyses along with species of *Cyclocephala* Dejean, *Dyscinetus* Harold and *Tomarus* Erichson as outgroup taxa. Nearly complete 28S, 12S and CO1 data were collected from 26 of 29 specimens, of which 16 samples were pinned, museum specimens. 28S data strongly support a nonmonophyletic *Mimeoma*; mitochondrial data (CO1 and 12S) suggest that *Mimeoma* species are nested within an apical clade of other *Cyclocephala* species; combined molecular and morphological data identify two strongly supported clades of *Mimeoma* species but do not support their sister relationship. Combined data show that *Mimeoma* species are nested within *Cyclocephala*, thus rendering *Cyclocephala* paraphyletic. *Mimeoma* is synonymized within *Cyclocephala* resulting in the following new combinations: *Cyclocephala acuta* Arrow n.comb., *Cyclocephala englemani* (Ratcliffe) n.comb., *Cyclocephala maculata* Burmeister n.comb., *Cyclocephala nigra* (Endrödi) n.comb. and *Cyclocephala signatooides* Höhne n.comb. Our results demonstrate that pinned, museum specimens can be used to obtain DNA sequence data (particularly high-copy gene regions) for evolutionary studies, and provide the first empirical support that host-plant associations within cyclocephaline scarab clades are conserved at the plant family-level.

Introduction

The Cyclocephalini (Coleoptera: Scarabaeidae: Dynastinae) is the second largest rhinoceros beetle tribe, containing 15 genera and nearly 500 described species concentrated in the New World tropics (Smith, 2006). Cyclocephalines are important economically and ecologically as both root pests and pollinators (Ratcliffe & Paulsen, 2008). Adult cyclocephaline beetles have been collected from the inflorescences of nearly 60 plant genera representing 17 families where they generally feed on floral parts and mate (Moore & Jameson, 2013). The group is most strongly associated with early-diverging angiosperm families and has been shown to contribute to pollination in the Annonaceae, Araceae, Arecaceae, Cyclanthaceae, Magnoliaceae and Nymphaeaceae (Cramer et al., 1975; Beach, 1982, 1984; Young, 1986, 1988; Gottsberger, 1989; Dieringer...
et al., 1999; Hirthe & Porembski, 2003; Gottsberger et al., 2012). Although cyclocephaline/angiosperm mutualisms may be best explained by pre-existing sensory biases towards floral volatile organic compounds, coevolutionary mechanisms could potentially explain cyclocephaline visitation of some Araceae genera (Schiestl & Dötterl, 2012).

The evolution of cyclocephaline floral associations can only be assessed in the context of a well-resolved cyclocephaline phylogeny. Few rigorous phylogenetic analyses have been performed on the tribe, and many aspects of its evolutionary history are poorly resolved. In particular, the monophyly of several genera, including Mimeoma Casey, Acrobolbia Ohaus, Ancognatha Ericson, Arriguttia Martinez, Aspidolea Bates and Cyclocephala Dejean are in doubt (Ratcliffe, 2003; Ratcliffe & Cave, 2006; Clark, 2011). We assess the monophyly of one of these problematic genera, Mimeoma, with a phylogenetic analysis of molecular and morphological data.

*Mimeoma* species are recorded from inflorescences of the palm genera Astrocaryum, Bactris and Socratea (Arecaceae) (Bullock, 1981; Beach, 1984; Ponchel, 2010; Moore & Jameson, 2013). Similar to several early-diverging angiosperm groups with which cyclocephaline beetles are associated, palm inflorescences warm above ambient temperature, causing floral scent compounds to be volatilized, possibly attracting floral visitors (Bullock, 1981; Beach, 1984). However, unlike the numerous ecological, behavioural and plant-pollinator studies that have examined the closely related genus *Cyclocephala* (Gottsberger, 1989; Dieringer et al., 1999; Hirthe & Porembski, 2003; Maia et al., 2012; Schiestl & Dötterl, 2012), no studies have been conducted of the plant–pollinator interactions of *Mimeoma* species. In particular, the speculation that the acute clypeal apex (diagnostic of some *Mimeoma* species) assists in some aspect of this floral association (B. Ratcliffe, personal communication) remains unevaluated. Whereas some cyclocephalines appear to visit inflorescences from several plant families, *Mimeoma* species appear to be exclusively associated with palm inflorescences (Moore & Jameson, 2013), thus making this a target group for examining host-plant associations within the Cyclocephalini.

*Mimeoma* includes five species that are distributed from central Mexico to northern South America, as well as one species in the West Indies (Endrödi, 1985). The subtriangular or acute clypeal apex and the weakly emarginate apex of the mentum serve to separate species of *Mimeoma* from *Cyclocephala* and *Ancognatha* (Endrödi, 1985; Ratcliffe, 2003). The genus was described by Casey (1915), at which time only *Cyclocephala maculata* Burmeister was included in the taxon. Casey (1915) characterized *Mimeoma* as having widely separated mesocoxae, a glorious venter, a poorly developed prosternal process, and by the ‘nature of the female sexual characters’ (discussed in Moore, 2012). *Mimeoma* remained monotypic until *M. acuta* (Arrow) and *M. signatoides* (Höhne) were transferred into the genus from *Cyclocephala* (Endrödi, 1966). Two more species were added later: *M. englemani* Ratcliffe from Panama and *M. nigra* Endrödi from the Dominican Republic (Ratcliffe, 1977; Endrödi, 1979). The larvae of *Mimeoma* species are undescribed, and characters that circumscribe the genus and separate it from other Cyclocephalini genera are vague. In particular, variation among *Mimeoma* species in the form of the clypeal apex (apically acute to narrowly parabolic) (Fig. 1B, C), causes overlap in diagnostic characters between *Cyclocephala* and *Mimeoma*. Further study is needed to determine whether genera such as *Mimeoma*, *Cyclocephala*, *Aspidolea*, and *Ancognatha* represent true clades or merely grades (Ratcliffe, 2003).

In order to examine monophyly of the genus *Mimeoma* and its relationship to *Cyclocephala* we conducted phylogenetic analyses incorporating both 28S/mitochondrial sequence data and morphological characters. The lack of freshly collected specimens of both *Mimeoma* and outgroup genera compelled us to modify existing molecular techniques/protocols for their use on dried museum specimens. Additionally, we investigated correlations between morphological traits of *Mimeoma* scarabs and floral associations with early-diverging angiosperms.

**Materials and methods**

**Taxa selection**

A total of 29 specimens representing 21 species were included. Seven *Mimeoma* specimens representing all five species were included: *M. maculata*, *M. signatoides*, *M. acuta*, *M. englemani* and *M. nigra*. Selection of outgroup taxa was based on two phylogenetic analyses that included cyclocephaline scarabs. A preliminary, unpublished phylogeny of the superfamily Scarabaeoidea based on 28S and 18S molecular sequence data provided evidence that the cyclocephaline genera *Dyscinetus* Harold and *Stenocrates* Burmeister were more closely related to some Pentodontini (Dynastinae) (*Euthela* Bates, *Oxygrylius* Casey and *Tomarus* Ericson) than to other cyclocephaline genera (*Acrobolbia*, *Ancognatha*, *Aspidolea* and *Cyclocephala*, in part), and demonstrated paralogy of both tribes (Team Scarab, UNSM 2006). Second, a morphological analysis of the Cyclocephalini supported *Dyscinetus* as an early diverging member of the Cyclocephalini (Clark, 2011; Breeschoten et al., 2013). In that analysis, *Cyclocephala* consisted of two sub-clades, one of which included two species of *Mimeoma* as apical members of ‘*Cyclocephala* sub-clade 1’ (Clark, 2011). Thus, to address monophyly of *Mimeoma* and its relationship to *Cyclocephala*, taxon sampling in the large genus *Cyclocephala* (~350 species) included several members of ‘sub-clade 1’ and other species that are morphologically similar to members of this group. Based on both of these phylogenetic analyses, we selected the pentodontine genus *Tomarus* and the cyclocephaline genera *Dyscinetus* and *Cyclocephala* as outgroup taxa.

*Mimeoma* species superficially resemble small species of *Ancognatha*, especially based on the narrowly parabolic clypeal apex (Endrödi, 1985; Ratcliffe, 2003; Ratcliffe & Cave, 2006). However, *Ancognatha* is clearly separated from *Mimeoma* as well as *Cyclocephala* by the following characteristics: dorsoventrally furrowed mentum (not shared with *Mimeoma* or *Cyclocephala*), a labrum that is ventrally inclined towards the venter of the clypeus [planar from dorsum to venter in *Cyclocephala* and *Mimeoma* (Bates, 1888; Ratcliffe & Cave, 2006)].
Fig. 1. Morphological characters for phylogenetic analysis. Clypeal shape of (A) Cyclocephala sexpunctata morphspecies 2, (B) Mimeoma maculata, (C) M. acuta and (D) Tomarus gibbosus. Mentum apex of (E) C. maffa, (F) C. amazona and (G) T. gibbosus. Male protibia (dorsal view) of (H) C. tutilina, (I) M. acuta, (J) D. morator and (K) T. gibbosus showing form of the external protibial teeth and enlarged (H–I) or simple (K) medial foreclaw. Lateral margin of metacoxa of (L) Dyscinetus morator and (M) M. englemani showing apicolateral margin of metacoxa perpendicular with respect to the venter of the metacoxa (L) or acutely angled with respect to the venter of the metacoxa (M). Male protibia, internolateral view, showing protibial spur (arrow) of (N) M. englemani and (O) D. morator.

© 2015 The Authors. Systematic Entomology published by John Wiley & Sons Ltd on behalf of Royal Entomological Society. 40, 891–900
male protibia with three teeth (bidentate in *Mimeoma* and bidentate or tridentate in *Cyclocephala*), maxillary teeth that are rudimentary and peg-like (with five or more well-developed teeth in *Cyclocephala* and *Mimeoma*), and membranous margin of the elytra in some species [a characteristic shared with anomaline scarabs (Scarabaeidae: Rutelinae: Anomalini), but lacking in *Mimeoma* and *Cyclocephala*] (personal observation by MRM). Taken together, these characteristics justify the exclusion of *Ancognatha* species from this analysis.

**Specimen acquisition**

Both high-quality specimens (preserved in 95% ethanol in the field) and dried, pinned specimens collected between 2 and 26 years ago (field-preserved with unknown methods) were used. Material was obtained primarily through loans of pinned specimens from museums and private collections (Table S1) or collected by M. R. Moore in 95% ethanol during a trip to Guatemala in July 2011 (deposited at Wichita State University).

**DNA extraction**

DNA was extracted from pinned and ethanol-preserved specimens based on a modified version of the protocol detailed in Tagliavia *et al.* (2011). Tissue was taken from thoracic muscle or by removing an entire leg along with the attached tissue from the coxal cavity. Samples taken from specimens preserved in ethanol were allowed to dry completely at 36°C for 1 h. Tissue samples and two steel ball bearings were placed in 1.5-mL wide-bottom tubes and shaken using the modified reciprocating saw shaker described in Alexander *et al.* (2007). Samples were shaken for 1 min or until tissue was reduced to a fine powder. Two hundred and forty microlitres of lysis buffer with detergent (5 mM guanidinium isothiocyanate, 1% sarkosyl, 20 mM EDTA, 25 mM sodium citrate pH 7) was added to each tube and the samples were incubated at 65–70°C for 2 h with vortexing every 20 min. DNA was extracted by adding 240 μL of a (25:24:1) phenol/chloroform/isoamyl alcohol mixture, followed by agitation in order to form an emulsion. The tubes were then centrifuged for 5 min at 13,000 g. The resulting aqueous phase was transferred to another 1.5-mL tube and diluted with four volumes of detergent-free lysis buffer. Samples were then acidified by adding 4 mM sodium acetate (1/10 of the original sample volume) and purified using glass fiber columns (GE Healthcare Illustra GFX PCR DNA and Gel Band Purification Kit, Pittsburgh, PA, U.S.A.) per manufacturer’s instructions.

**PCR protocols**

Two mitochondrial (cytochrome oxidase subunit I, ‘COI’; 12S small ribosomal subunit, ‘12S’) and one ribosomal (28S ribosomal RNA, ‘28S’) gene region were targeted for amplification. Due to the presumably low quality and/or quantity of the template DNA extracted from pinned specimens, we used a series of primers to amplify the entire COI and 28S gene regions (Table S2). Primers were designed or modified based on Dynastinae and Passalidae (Scarabaeoidea) sequence data available via GenBank.

COI was amplified using the primer sets C1-J-1751/TL2-N-3014, C1-J-1751/Nancy2, C1-J-1751/C1-N-2191, C1-J-2183/TL2-N-3014, C1-J-2183/C1-N-2659, C1-J-2183/Maryliz4 and C1-J-2441/TL2-N-3014 (Simon *et al*., 1994). An approximately 400-bp segment of the S′ section of 12S was amplified using the primer set 12S 2F/SR-N-14594 (Kambhampati & Smith, 1995; this study). 28S was amplified using the primer sets 28SF/28SR, 28SF/Yoshi, Squirtle/Peach and Bulbasaur/28SR (Whiting *et al*., 1997; Whiting, 2001; this study). Annealing temperatures and extension times for each primer set are provided in Table S3. Reactions (10 μL) included: 50 μM Tris-Cl pH 7.9, 16 mM ammonium sulfate, 0.025% Brij 58, 3.5 mM magnesium chloride, 1 μM each of forward and reverse primers, 250 μM each dNTP mixture, and 1 Unit of Klen Taq LA (DNA Polymerase Technology, Inc., St. Louis, MO). Surplus primers and dNTPs were removed using a standard Exonuclease 1/Shrimp Alkaline Phosphatase protocol, and amplicons were sequenced at the University of Chicago Comprehensive Cancer Center DNA Sequencing and Genotyping Facility.

**Molecular analyses**

Sequence contigs were assembled with CLC Main Workbench 6.7.1 (CLC bio, Aarhus, Denmark), aligned using the default settings of ClustalW (Larkin *et al*., 2007) and MUSCLE (Edgar, 2004) as implemented in MEGA 5.10 (Tamura *et al*., 2011), and subsequently verified by eye. Gaps were treated as missing data in all analyses. Preliminary parsimony bootstrap analyses (heuristic search, 1000 bootstrap replicates, each with 100 random addition replicates) of individual 12S, COI and 28S datasets were performed in PAUP* 4.0a125 (Swofford, 2002) in order to assess congruence among datasets. Minimal incongruence was observed between the 12S and COI datasets, and these were combined into a single mitochondrial dataset. The mitochondrial dataset was later combined with the 28S data into a single combined molecular dataset. Maximum parsimony bootstrap analyses were then performed on the mitochondrial, 28S, combined molecular and total evidence (including the morphological characters detailed below) datasets using the parameters described above.

The best-fitting model of sequence evolution (GTR + I + G) for the mitochondrial and 28S datasets was identified using the Akaike Information Criterion in Modeltest 3.06 (Posada & Crandall, 1998), and Bayesian Markov Chain Monte Carlo analyses were performed on each dataset in MrBayes 3.2.1 (Ronquist & Huelsenbeck, 2003). Bayesian analyses comprised four independent runs, each with four chains (one cold and three heated). Flat priors were used. Chains were run for 1 or 2 million generations, with trees sampled every 1000 generations. Convergence was evaluated by examining the
standard deviation of split frequencies among runs and by plotting the log-likelihood values from each run using Tracer 1.5 (Rambaut & Drummond, 2007). These diagnostics indicated that runs reached convergence within 5000–20 000 generations (depending on the dataset), and trees sampled during this period were excluded before obtaining clade posterior probabilities. Morphological data were analysed in MrBayes with the standard discrete morphology model based on Lewis (2001). Combined datasets were partitioned by gene region and morphology in MrBayes with their parameters unlinked and allowed to vary independently.

**Morphological data**

The morphological matrix included characters that are used for identification of genera and species in the Cyclocephalini (Ratcliffe, 2003; Ratcliffe & Cave, 2006). All observations of morphology were made using Leica M80 microscopes and LED light sources. Morphological data were scored in Mesquite 2.75 (Maddison & Maddison, 2011) and matrices were exported as NEXUS files for phylogenetic analyses. Images were captured with a Leica IC80 HD digital camera, processed using the Leica Application Suite v3.8.0, and further modified in Photoshop CS 4 v11.0.2.

**Character statements**

1. Pronotum with basal bead complete at middle (0) or incomplete at middle (1).
2. Pronotum with apicolateral portions glabrous or with minute setae (less than 0.5 adjacent puncture diameters long) (0) or with long setae (more than 1.5 adjacent puncture diameters long) (1).
3. Frons glabrous or with minute setae (less than 0.5 adjacent puncture diameters long) (0) or with long setae (more than 1.5 adjacent puncture diameters long) (1).
4. Frontoocular suture complete at middle (0) (Fig. 1A, C, D) or incomplete at middle (1) (Fig. 1B).
5. Frontoocular suture with raised, transverse carina (0) (Fig. 1D) or without raised, transverse carina (1) (Fig. 1A, C, D).
6. Clypeus apex rounded (0) (Fig. 1B, C), emarginate (1) (Fig. 1A), or truncate (2) (Fig. 1D).
7. Clypeus glabrous or with minute setae (less than 0.5 adjacent puncture diameters long) (0) or with long setae (more than 1.5 adjacent puncture diameters long) (1).

Some *Cyclocephala* species have a rugose clypeal apex. In these cases, puncture diameter was measured for punctures at the base of the clypeus near the frontoclypeal suture.
8. Clypeal apex without tubercle(s) (0) (Fig. 1A, B), with one apically produced tubercle (1) (Fig. 1C), or with two dorsally produced tubercles (2) (Fig. 1D).
9. Medial protarsal claw of males enlarged and swollen compared to lateral protarsal claw (0) (Fig. 1H–J) or simple, not enlarged or swollen compared to lateral protarsal claw (1) (Fig. 1K).
10. Apex of medial protarsal claw of males complete (0) or narrowly split with well-developed lateral ramus (1).
11. Protibia of males tridentate, basal tooth reduced and removed from apical two teeth (0) (Fig. 1H), bidentate, basal tooth greatly reduced (Fig. 1I) (1), tridentate, basal tooth not reduced and removed from apical two teeth (2) (Fig. 1K), or tridentate, basal tooth not reduced and teeth subequally spaced (3) (Fig. 1J).
12. Protibial spur straight to slightly decurved (0) (Fig. 1O) or strongly decurved (1) (Fig. 1N).
13. Mesocoxae contiguous (0) or not touching, widely separated (1).
14. Apicolateral margin of epipleuron glabrous or only with minute setae (less than 0.5 adjacent puncture diameters long) (0), with stout, spinose setae (approximately 1.0 adjacent puncture diameters long) (1), or with long, fine setae (more than 1.5 adjacent puncture diameters long) (2).

Spinose setae are distinctly conical throughout their length, with the setae continuously tapering from the base to the apex. Long, fine setae have a consistent diameter until the last fourth of their length, after which they taper to the apex.
15. Male parameres glabrous (0) or with setae (1).
16. Mentum with apex weakly emarginate (0) (Fig. 1E), moderately emarginate (1) (Fig. 1I) or truncate (2) (Fig. 1G).

Weakly emarginate was defined as an emargination that does not approach the insertion point of the labial palps on the mentum (Fig. 1E). Moderately emarginate was defined as having an emargination that is deep enough to nearly reach the insertion point of the labial palps on the mentum (Fig. 1F).
17. Lateral margin of metacoxa with longitudinal sulcus (0) (Fig. 1L) or without longitudinal sulcus (1) (Fig. 1M).
18. Apicolateral margin of metacoxae perpendicular to venter of metacoxa (0) (Fig. 1L) or acutely angled to venter of metacoxa (1) (Fig. 1M).

Specimens scored as state (1) have the apicolateral margin of the metacoxa acutely angled and tucked under the ventral plane of the metacoxa. This forms a distinct lip on the lateral margin of the metacoxa (Fig. 1M).

**Results**

**Total evidence analyses**

All four Bayesian runs converged near 50 000 generations. Trees sampled during the first 100 000 generations were conservatively discarded as burn-in. Trees sampled from the remaining 900 000 generations were used to generate a 50% majority-rule consensus tree of Bayesian posterior probabilities. Bayesian posterior probabilities and bootstrap support values are presented on the Bayesian 50% majority-rule consensus tree (Fig. 2).

The total evidence dataset exhibited 16 strongly supported (>0.95 PP and >75% BS) internal nodes. The monophyly of *Mimeoma* could not be rejected based on the total evidence topology. Two strongly supported *Mimeoma* clades (*M. signatoides* + *M. maculata* (1.0 PP/95 BS); *M. englemani* +
Fig. 2. Bayesian 50% majority-rule consensus phylogram (with average branch lengths) for the combined 28S, mitochondrial and morphological datasets. Bayesian posterior probabilities (>0.50) and parsimony bootstrap support values (>50%) appear above branches. *Mimeoma* exemplars appear in bold. Three highly supported (>0.95 PP/75% BS) clades are labelled as A, B and C. Coloured boxes directly to the right of terminal taxa indicate known host plant families (data from Moore & Jameson, 2013). Species without known hosts are indicated with a grey box and a question mark. Vertical bars highlight character state differences between clade B and clade C.

*M. acuta* (1.0 PP/100 BS) are identified, although their sister relationship is not supported. All five *Mimeoma* species are nested within a strongly supported clade that includes *Cylocephala stictica*, *C. amazona*, *C. multiplex*, *C. discolor*, *C. inca* and *C. aequatoria* (1.0 PP/84 BS).

**28S dataset**

28S data were obtained for all 29 specimens, although 21 specimens yielded partial 28S sequence data. The majority of this missing data are located within a 5′ 150-bp region for which a conserved primer for amplification and sequencing was not developed. The 28S alignment included 1165 characters. Of these, 161 (14%) were variable and 79 (7%) were parsimony-informative. Each of the four parallel Bayesian runs converged near 20000 generations, and trees sampled during the first 50000 generations were conservatively discarded as burn-in. Trees sampled from the remaining 950000 generations were used to generate a 50% majority-rule consensus tree.

© 2015 The Authors. *Systematic Entomology* published by John Wiley & Sons Ltd on behalf of Royal Entomological Society. 40, 891–900
are presented on the Bayesian 50% majority-rule consensus tree (Figure S1A).

The 28S topology exhibited eight strongly supported (>0.95 Bayesian posterior probability (PP) and >75% bootstrap support (BS)) internal nodes. Based on 28S data the monophyly of Mimeoma can be rejected. A strongly supported (0.98 PP/86 BS) clade contains Mimeoma maculata, M. acuta, M. englemani, M. signatoides and certain Cyclocephala species. Mimeoma nigra is not placed in this clade and the 28S data do not provide strong support for M. nigra’s overall position.

Mitochondrial dataset (CO1 and 12S)

12S data were obtained for 27 of 29 specimens, including all seven Mimeoma samples. At least partial CO1 data were obtained for all 29 specimens. The mitochondrial alignment (12S and CO1) included 1734 characters. Of these, 637 (37%) were variable and 491 (28%) were parsimony-informative. Each of the four parallel Bayesian runs converged near 15 000 generations, and trees sampled during the first 50 000 generations were conservatively discarded as burn-in. Trees sampled from the remaining 950 000 generations were used to generate a 50% majority-rule consensus tree. Bayesian posterior probabilities and bootstrap values are presented on the Bayesian 50% majority-rule consensus tree (Figure S1B).

The mitochondrial topology exhibited 11 strongly supported (>0.95 PP and >75% BS) internal nodes. The monophyly of Mimeoma could not be rejected based on the mitochondrial dataset. Mimeoma species were recovered in three clades (0.96 PP/-- BS, 0.98 PP/-- BS and 1.0/100) nested within a more inclusive clade of Cyclocephala species. There was no support for relationships between the three clades containing Mimeoma species and, in general, no support for relationships deeper in the tree. No strongly supported conflicts were identified between the 28S and mitochondrial topologies (Figure S1).

Combined molecular dataset

Bayesian runs converged near 10 000 generations. Trees sampled during the first 50 000 generations were conservatively discarded as burn-in. Trees sampled during the remaining 950 000 generations were used to generate a 50% majority-rule consensus tree. Bayesian posterior probabilities and bootstrap support values are presented on the Bayesian 50% majority-rule consensus phylogram (Figure S2).

The combined molecular dataset exhibited 15 strongly supported internal nodes (>0.95 PP and >75% BS). The monophyly of Mimeoma could not be rejected based the combined molecular dataset topology. Mimeoma maculata and M. signatoides form a strongly supported clade (1.0 PP/86 BS), as do M. englemani and M. acuta (1.0 PP/100 BS). The relationship between these two Mimeoma clades and M. nigra is unclear (Figure S2).

Morphological analyses

The morphological matrix included 18 characters, 15 (83%) of which were parsimony-informative (Table S4). Each of the four Bayesian runs converged near 50 000 generations, and trees sampled during the first 50 000 generations were conservatively discarded as burn-in. Trees sampled from the remaining 950 000 generations were used to generate a 50% majority-rule consensus tree of Bayesian posterior probabilities (Figure S3).

The morphological dataset exhibited one strongly supported (>0.95 PP and >75% BS) internal node. The monophyly of Mimeoma could not be rejected based the morphological topology. The seven Mimeoma specimens are part of a strongly supported clade (0.99 PP/95 BS) in the Bayesian 50% majority-rule consensus tree along with C. aequatoria, C. discolor, C. inca, C. amazona, C. multiplex and C. stictica (Figure S3).

Discussion

Is Mimeoma monophyletic?

Although strongly supported clades of Mimeoma species are identified by both mitochondrial and 28S data, neither dataset nor the combined analysis provides strong support for a clade comprising all Mimeoma specimens (Fig. 2, Figures S1–S3). The 28S topology strongly suggests that Mimeoma is not monophyletic (Figure S1A). Both mitochondrial and combined molecular datasets identify strongly supported sister relationships among Mimeoma species pairs, but cannot provide support for an overall Mimeoma clade (Figures S1B and S2). Morphological data (Figure S3) do not resolve sister relationships between Mimeoma species. The total evidence dataset recovered two strongly supported clades containing Mimeoma species, but the relationship between these two clades was not resolved (Fig. 2).

Relationships of Mimeoma and Cyclocephala

Combined data (Fig. 2) provide strong support for large-scale relationships among Mimeoma and Cyclocephala species. Mimeoma species are members of a strongly supported clade that includes Cyclocephala and Mimeoma (Clade A, the Cyclocephala clade). Cyclocephala maffia is sister to all Cyclocephala and Mimeoma species (Clade A). Two strongly supported sub-clades are included in the Cyclocephala clade (Clade A): Cyclocephala + Mimeoma (Clade B) and Cyclocephala (Clade C).

Mimeoma species are members of a strongly supported clade that also includes certain Cyclocephala species (Clade B) (Fig. 2). This clade includes C. amazona (the type species of Cyclocephala). The following morphological character states are present in all members of Clade B and are putative synapomorphic character states: pronotum with basal bead complete at middle [character 1:0], males with protibia bidentate (Fig. 11) [11:1], males with apex of enlarged medial protarsal claw complete [10:0], apicolateral margin of epipleuron with spinose © 2015 The Authors. Systematic Entomology published by John Wiley & Sons Ltd on behalf of Royal Entomological Society. 40, 891–900
setae [14:1], and mentum with apex moderately emarginate (nearly to the insertion of the labial palps on the mentum) (Fig. 1F) [16:1]. Several other character states are shared by most Clade B species, including the strongly decurved protibial spur (Fig. 1N) [12:1] (C. aequatoria with a straight protibial spur), a rounded clypeal apex [6:0] (C. stictica with clypeal apex emarginated), male parameres setose [15:1] (glabrous in C. aequatoria, C. inca and C. discolor), and the apicolateral portion of metacoxa acutely angled underneath the ventral portion (Fig. 1M) [18:1] (perpendicular in C. stictica). The subtriangular and acute clypeal apex (interpreted here as an apically produced tubercle, Fig. 1C) of Mimeoma, thought to be a diagnostic character for the genus (Ratcliffe, 2003; Ratcliffe & Cave, 2006), is shared only in M. englemani and M. acuta [8:1]. In contrast, M. signatoides, M. maculata and M. nigra lack a tubercle at the clypeal apex (Fig. 1B) [8:0].

All datasets consistently and strongly supported Cyclocephala Clade C (Fig. 2), a homogenous group that is sister to Cyclocephala + Mimeoma (Clade B) (Fig. 2). This group includes species with six well-developed maxillary teeth with apical teeth enlarged and is composed of Cyclocephala species that all share the following synapomorphous character states: pronotum with basal bead incomplete at middle [1:1], clypeal apex emarginate [6:1], males with apex of enlarged medial claw narrowly split [10:1], males with protibia tridentate, basal tooth reduced and removed from apical two teeth (Fig. 1H) [11:0], protibial spur straight or slightly decurved (Fig. 1O) [12:0], male parameres glabrous [15:0], and mentum with apex weakly emarginate [16:0].

Despite strong evidence of multiple clades within Cyclocephala (Fig. 2), the sampling of Cyclocephala species is not robust enough to warrant the application of generic or subgeneric status to Clade B, Clade C or Cyclocephala maffiata (Fig. 2). 28S (Figure S1A), morphological (Figure S3) and total evidence datasets (Fig. 2) identify Mimeoma species as members of a clade including Cyclocephala species (thus rendering Cyclocephala paraphyletic). Cyclocephala has nomenclatural priority over Mimeoma. Mimeoma is a new synonym of Cyclocephala and this results in the following new combinations: Cyclocephala acuta Arrow n.comb., Cyclocephala englemani (Ratcliffe) n.comb., Cyclocephala maculata Burmeister n.comb., Cyclocephala nigra (Endrödi) n.comb., and Cyclocephala signatoides Höhne n.comb.

Floral associations

Cyclocephaline species are generally oligophagous (multiple host genera within a family) or polyphagous (multiple host families) visitors of early-diverging angiosperm inflorescences where they pollinate, mate, and feed on floral parts, exudates and pollen (Moore & Jameson, 2013). However, cyclocephaline floral associations have never been analysed in the context of a well-resolved phylogeny of the scarab tribe, and it is currently unknown whether floral associations tend to be conserved across large clades or are evolutionarily labile. Our data allow for preliminary examination of floral associations within the Cyclocephala clade (Fig. 2, Clade A).

Our data suggest that host-plant associations among the Cyclocephala clade are conserved at the plant family level (Fig. 2). Cyclocephala species in Clade C are broadly associated with inflorescences of aroids (Araceae), whereas Cyclocephala + Mimeoma species in Clade A are broadly associated with palms (Arecales). More specifically, Cyclocephala species in Clade C have been recorded from the inflorescences of six Araceae genera (versus two Araceae genera for species in Clade B), whereas in contrast species in Clade B have been recorded from eight palm genera [versus one palm genus (Socratea) for species in Clade C]. These data suggest that host-plant association exhibits some level of evolutionary conservation among these species.

Implications for future studies

The combination of molecular and morphological data has been effective for phylogeny reconstruction at the subfamilial and tribal levels in Coleoptera (Seago et al., 2011; Robertson et al., 2012). However, our study combining molecular and morphological data focused on resolving relationships between more shallowly diverged groups (= generic-level), and many relationships among major clades were poorly supported when datasets were analysed separately (Fig. 2, Figures S1–S3).

Resolving these relationships at moderate phylogenetic depths and obtaining enhanced support for all portions of the topology will require additional data. Single copy nuclear gene regions (e.g. CAD, Wingless, PepCK) have been shown to be highly informative at a variety of phylogenetic depths within scarabaeoids, and more broadly within Coleoptera (Wild and Maddison, 2008; Dole et al., 2010). However, many of these loci require the use of highly degenerate primers and nested PCR protocols that may preclude their use with degraded DNAs that are typically recovered from museum material. Indeed, the primary challenge of this study was the lack of freshly collected material, as 16 of 29 samples for four of five ingroup taxa were only available as pinned, museum specimens. Our strategy involved obtaining sequences from small fragments of high-copy gene regions (28S, CO1 and 12S) and combining these with morphological data. Using a mixed sample of pinned and alcohol specimens, this was effective at resolving generic-level lineages in Cyclocephala. Similar procedures should be useful for phylogeny reconstruction at appropriate evolutionary timescales in other scarabaeoid groups that lack freshly collected material and may assist in unravelling an evolutionary framework for understanding associations with host plants, distributional patterns, behaviours and adaptations.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12139

Figure S1. Fifty percent majority-rule consensus trees of Bayesian posterior probabilities for the 28S (A) and mitochondrial (B) datasets. Bayesian posterior probabilities
 (>0.50) and parsimony bootstrap support values (≥50%) appear above branches. *Mimeoma* exemplars appear in bold.

**Figure S2.** Bayesian 50% majority-rule consensus phylogram (with average branch lengths) for the combined molecular dataset. Bayesian posterior probabilities (>0.50) and parsimony bootstrap support values (≥50%) appear above branches. *Mimeoma* exemplars appear in bold.

**Figure S3.** Bayesian 50% majority-rule consensus tree for the morphological dataset. Bayesian posterior probabilities (>0.50) and parsimony bootstrap support values (≥50%) appear above branches. *Mimeoma* exemplars appear in bold.

**Table S1.** Details regarding analysed specimens. Depository institution acronyms include: WICH [Wichita State University Collection, Wichita, KS (Mary Jameson)], MLJC (Mary Liz Jameson Collection, Wichita, KS), FSCA [Florida State Collection of Arthropods, Gainesville, FL (Paul Skelly)], SEMC (retained at WICH) [Snow Entomological Museum, University of Kansas, Lawrence, KS (Zach Falin and Jennifer Thomas)], UNSM [University of Nebraska State Museum, Lincoln, NE (Brett Ratcliffe and Matt Paulsen)], USNM [U.S. National Museum, Washington, D.C. (currently housed at the University of Nebraska State Museum for off-site enhancement) (Floyd Shockley and Dave Furth)]. VoucherE, ethanol-preserved specimen; voucherP, pinned specimen. Asterisk (*) indicates type species for the genus.

**Table S2.** Primers used to amplify 12S, CO1 and 28S gene regions.

**Table S3.** PCR cycling conditions.

**Table S4.** Morphological character state scores.

**Acknowledgements**

We thank the institutions and individuals who provided specimens for this research: Zach Falin and Jennifer Thomas (SEMC: Snow Entomological Museum Collection, University of Kansas, Lawrence, KS, U.S.A.), Brett Ratcliffe and M.J. Paulsen (UNSM: University of Nebraska State Museum, Lincoln, NE, U.S.A.), and Dave Furth and Floyd Shockley (USNM: United States National Museum, currently housed at UNSM). We also thank Oliver Keller (WICH) for his support and assistance during the development of this research, Dan Clark for cyclocephaline exemplar dissections, Brett Ratcliffe (UNSM) for identifications of some cyclocephaline specimens. We thank Brett Ratcliffe (UNSM) and Ron Cave (University of Florida) for their helpful comments and reviews of a draft of this manuscript. This research was supported by funds from Wichita State University. The authors declare no conflict of interest.

**References**

Alexander, P.J., Rajanikanth, G., Bacon, C.D. & Bailey, C.D. (2007) Recovery of plant DNA using a reciprocating saw and silica-based columns. Molecular Ecology, 7, 5–9.

Bates, H.W. (1888) Pectinocornia and Lamellicornia, Family Dynastidae. Biologia Centrali-Americana. Insecta, Coleoptera, Vol. 2, Part 2 (ed. by F.D. Godman and O. Salvin), pp. 296–342. R. H. Porter, London.

Beach, J.H. (1982) Beetle pollination of Cyclanthus bipartitus (Cyclanthaceae). American Journal of Botany, 69, 1074–1081.

Beach, J.H. (1984) The reproductive biology of the peach or “pejibaye” palm (*Bactris gasipaes*) and a wild congener (*B. porciflora*) in the Atlantic lowlands of Costa Rica. Principes, 28, 107–119.

Breeschoten, T., Clark, D.R. & Schilthuizen, M. (2013) Evolutionary patterns of asymmetric genitalia in the beetle tribe Cyclocephalini (Coleoptera: Scarabaeidae: Dynastinae). Contributions to Zoology, 82, 95–106.

Bullock, S.H. (1981) Notes on the phenology of inflorescences and pollination of some rain forest palms in Costa Rica. Principes, 25, 101–105.

Casey, T.L. (1915) A review of the American species of Rutelinae, Dynastinae, and Cetoniinae. Memoirs of the Coleoptera, 6, 1–394.

Clark, D.R. (2011) Phylogenetic analysis of the scarab beetle tribe Cyclocephalini (Coleoptera: Scarabaeidae: Dynastinae) based on adult morphological characters. Masters Thesis, Wichita State University, Wichita, Kansas.

Cramer, J.M., Meese, A.D.J. & Tuenissen, P.A. (1975) A note on the pollination of nocturnally flowering species of Nymphaea. Acta Botanica Neerlandica, 24, 489–490.

Dieringer, G., Cabrera, R.L., Lara, M., Loya, L. & Reyes-Castillo, P. (1999) Beetles pollination and floral thermogenicity in *Magnolia tamaulipana* (Magnoliaceae). International Journal of Plant Sciences, 160, 64–71.

Dole, S.A., Jordal, B.H. & Cognato, A.I. (2010) Polyphyly of *Xylosandrus* Reitter inferred from nuclear and mitochondrial genes (Coleoptera: Curculionidae: Scolytinae). Molecular Phylogenetics and Evolution, 54, 773–782.

Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research, 32, 1792–1797.

Endrödi, S. (1966) Monographie der Dynastinae (Coleoptera, Lamellicornia). I. Teil. Entomologische Abhandlungen, 33, 1–460.

Endrödi, S. (1979) Neue arten des Dynastinen Tribus Cyclocephalini (Coleoptera, Melolonthidae) aus Amerika. Annales Historico–Naturales Musei Nationalis Hungarici, 71, 215–218.

Endrödi, S. (1985) *The Dynastinae of the World*, 46 plates. Dr. W. Junk, Dordrecht.

Gottsberger, G. (1989) Beetle pollination and flowering rhythm of *Annona* sp. (Annonaceae) in Brazil. Entwicklungsgeschichte und Systematik der Pflanzen, 167, 165–187.

Gottsberger, G., Silberbauer-Gottsberger, I., Seymour, R.S. & Dötterl, S. (2012) Pollination ecology of *Magnolia olinii* may explain the overall large flower size of the genus. Flora – Morphology, Distribution, Functional Ecology of Plants, 207, 107–118.

Hirthe, G. & Porembski, S. (2003) Pollination of *Nymphaea lotus* (Nymphaeaceae) by rhinoceros beetles and bees in the northeastern Atlantic lowlands of Costa Rica. Systematic Entomology, 364, 5–9.

Kambhampati, S. & Smith, P.T. (1995) PCR primers for the amplification of four insect mitochondrial gene fragments. Insect Molecular Biology, 4, 233–236.

Larkin, M.A., Blackshields, G., Brown, N.P. et al. (2007) ClustalW and ClustalX version 2. Bioinformatics, 23, 2947–2948.

Lewis, P.O. (2001) A likelihood approach to estimating phylogeny from discrete morphological character data. Systematic Biology, 50, 913–925.

Maddison, W.P. & Maddison, D.R. (2011) Mesquite: A Modular System for Evolutionary Analysis. Version 2.75 [WWW document]. URL http://mesquiteproject.org [accessed on 20 January 2014].
Maia, A.C.D., Gibernau, M., Carvalho, A.T., Gonçalves, E.G. & Schlindwein, C. (2012) The cowl does not make the monk: scarab beetle pollination of the Neotropical aroid Taccarum ulei (Araceae, Spathicarpaceae). *Biological Journal of the Linnean Society*, **108**, 22–34. DOI: 10.1111/j.1095-8312.2012.01985.x.

Moore, M.R. (2012) A new female elytral character for the tribe Cyclocephalini (Coleoptera: Scarabaeidae: Dynastinae) and an observation of its possible function. *The Coleopterists Bulletin*, **66**, 200–202.

Moore, M.R. & Jameson, M.L. (2013) Floral associations of cyclocephaline scarab beetles. *Journal of Insect Science*, **13**, 1–43.

Ponchel, Y. (2010) *Note sur Cyclocephala virgo Dechambre, 1999 et mise point sur trios espèces de Dynastidae récemment décrites de Guyane (Coleoptera Dynastidae).* *L'Entomologiste*, **66**, 171–172.

Posada, D. & Crandall, K.A. (1998) MODEL TEST: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.

Rambaut, A. & Drummond, A.J. (2007) *Tracer v. 1.5.* Computer program and documentation distributed by the authors at [WWW document]. URL http://beast.bio.ed.ac.uk/Tracer. [accessed on 22 February 2014].

Ratcliffe, B.C. (1977) Four new species of Neotropical Cyclocephalini (Coleoptera: Scarabaeidae) and an observation of its possible function. *L'Entomologiste*, **66**, 200–202.

Ratcliffe, B.C. (2003) The dynastine scarab beetles of Costa Rica and Panama (Coleoptera: Scarabaeidae: Dynastinae). *Bulletin of the University of Nebraska State Museum*, **16**, 1–506.

Ratcliffe, B.C. & Cave, R.D. (2006) The dynastine scarab beetles of Honduras, Nicaragua, and El Salvador (Coleoptera: Scarabaeidae: Dynastinae). *Bulletin of the University of Nebraska State Museum*, **21**, 1–424.

Ratcliffe, B.C. & Paulsen, M.J. (2008) The scarabaeoid beetles of Nebraska. *Bulletin of the University of Nebraska State Museum*, **22**, 1–569.

Robertson, J.A., Ślipiński, A., Hiatt, K., Miller, K.B., Whiting, M.F. & McHugh, J.V. (2012) Molecules, morphology and minute hooded beetles: a phylogenetic study with implications for the evolution and classification of Corylophidae (Coleoptera: Cucujoidea). *Systematic Entomology*, **38**, 209–232.

Ronquist, F.H. & Huelsenbeck, J.P. (2003) MrBayes 3: bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Schiestl, F.P. & Dötterl, D. (2012) The evolution of floral scent and olfactory preferences in pollinators: coevolution or pre-existing bias? *Evolution*, **66**, 2042–2055. DOI: 10.1111/j.1558-5646.2012.01593.x.

Seago, A.E., Giorgi, J.A., Li, J. & Ślipiński, A. (2011) Phylogeny, classification and evolution of ladybird beetles (Coleoptera: Coccinellidae) based on simultaneous analysis of molecular and morphological data. *Molecular Phylogenetics and Evolution*, **60**, 137–151.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, **87**, 651–701.

Smith, A.B.T. (2006) A review of the family-group names for the superfamily Scarabaeoidea (Coleoptera) with corrections to nomenclature and a current classification. *The Coleopterists Bulletin*, **60**, 144–204.

Swofford, D.L. (2002) *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods*). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Tagliavia, M., Massa, B., Albanese, I. & La Farina, M. (2011) DNA extraction from Orthoptera museum specimens. *Analytical Letters*, **44**, 1058–1062.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) *MEGA5*: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**, 2731–2739.

Whiting, M.F. (2001) Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. *Zoologica Scripta*, **31**, 93–104.

Whiting, M.F., Carpenter, J.C., Wheeler, Q.D. & Wheeler, W.C. (1997) The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. *Systematic Biology*, **46**, 1–68.

Wild, A.L. & Maddison, D.R. (2008) Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. *Molecular Phylogenetics and Evolution*, **48**, 877–891.

Young, H.J. (1986) Beetle pollination of *Dieffenbachia longispatha* (Araceae). *American Journal of Botany*, **73**, 931–944.

Young, H.J. (1988) Neighborhood size in a beetle pollinated tropical aroid: effects of low density and asynchronous flowering. *Oecologia*, **76**, 461–466.

Accepted 30 June 2015

First published online 7 August 2015

© 2015 The Authors. *Systematic Entomology* published by John Wiley & Sons Ltd on behalf of Royal Entomological Society, **40**, 891–900