Combined molecular and morphological data provide insights into the evolution and classification of Chilocorini ladybirds (Coleoptera: Coccinellidae)

WENJING LI1,2, PIOTR ŁACZYŃSKI3, HERMES E. ESCALONA4,5, JONAS EBERLE4, LIZHI HUO6, XIAOSHENG CHEN1, WEIDONG HUANG1, BINGXU CHEN2, DIRK AHRENS4, ADAM ŚLIPIŃSKI5, WIOLETTA TOMASZEWSKA3 and XINGMIN WANG1

1Engineering Technology Research Center of Agricultural Pest Biocontrol, South China Agricultural University, Guangzhou, China, 2Guangdong Provincial Key Laboratory of High Technology for Plant Protection, Plant Protection Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, China, 3Museum and Institute of Zoology, Polish Academy of Sciences, Warszawa, Poland, 4Zoological Research Museum Alexander Koenig, Bonn, Germany, 5Australian National Insect Collection, CSIRO, Canberra, Australia and 6Guangzhou Institute of Forestry and Landscape Architecture, Guangzhou, China

Abstract. Ladybirds of the cosmopolitan tribe Chilocorini prey mainly on coccids and include several important biocontrol agents. The phylogenetic relationships of Chilocorini are poorly known. In this paper, we provide a phylogenetic reconstruction of Chilocorini containing all 27 genera based on five molecular markers and 86 adult morphological characters. Morphological character states were mapped on the combined data tree from Bayesian inference to analyse morphological traits of each genus. Sixteen morphological characters were selected to reconstruct the ancestral states using maximum parsimony and maximum likelihood methods. Divergence times were estimated based on the relaxed molecular clock approach. Our results indicate that Chilocorini, excluding Chilocorellus Miyatake, is monophyletic and closely related to Plotinini. The crown group Chilocorini was estimated to date back to the Middle Cretaceous. Anisorcus Crotch, Egius Mulsant, Phaenochilus Weise and Simmondsius Ahmad & Ghani are synonymized here with Chilocorus Leach (syn.n.). The genus Chilocorellus is excluded from Chilocorini. The split of current genera was estimated to have occurred during the Middle Paleogene to Late Paleogene.

Introduction

The family Coccinellidae, more commonly known as ladybirds or ladybugs, contains c. 6000 species distributed worldwide. Many of them are well documented as predators of aphids and other important crop pests, such as whiteflies and scale insects (Ślipiński & Tomaszewska, 2010). However, the group also includes phytophagous and mycophagous clades (Giorgi et al., 2009). Coccinellidae is classified in the superfamily Coccinelloidea, separated from Cucujioidea by Robertson et al. (2015). The taxonomic status of this superfamily was also supported by the latest phylogenetic study of Coleoptera based on the analysis of 95 protein-coding genes in 373 beetle species (Zhang et al., 2018).

Chilocorini is a medium-sized group of rather distinctive ladybird beetles. They are usually smooth and shiny with domed elytra and expanded clypeus covering most of the antennal bases. Larvae usually have a nearly cylindrical and broadly fusiform body with dorsal and lateral surfaces covered with setose senti or prominent parascoli. This tribe was established by Mulsant (1846) and originally contained two European genera, Chilocorus Leach and Exochomus Redtenbacher. Chilocorini now includes 27 genera and more than 280 species (Łaczyński &
Tomaszewska, 2012; Li et al., 2017). The majority of Chilocorini species are coccidophagous (Giorgi et al., 2009), though some species can also prey on aphids (Ślipiński & Giorgi, 2006) and whiteflies (Giorgi & Vandenberg, 2012), all belonging to the sternorrhynchan Hemiptera. As natural predators of scale insects, Chilocorini belong to the most economically important group of Coccinellidae as they are potential biocontrol agents. For example, the well-known species *Chilocorus niger* (Fabricius) was first introduced deliberately from India to several localities in the Seychelles in 1939 where it then gradually spread worldwide due to its effectiveness in controlling scale insects (Booth, 1998).

The adults of Chilocorini have mostly bright external appearances and various colours and shapes (Figs 1, 2). They can be easily distinguished from other ladybirds by the following combination of morphological characters: (i) the habitus is short oval to rounded and hemispherical; (ii) the clypeus expands laterally and covers the bases of the antennae; (iii) the antenna is shorter than half the width of the head; (iv) the pronotal anterior angles are usually strongly produced anteriorly; and (v) the prosternal process is smooth and without carinae (Fig. 3).

The monophyly of Coccinellidae has been confirmed and the higher relationships analysed by a number of recent phylogenetic studies (Robertson et al., 2008, 2015; Giorgi et al., 2009; Aruggoda et al., 2010; Magro et al., 2010; Seago et al., 2011; Che et al., 2017). However, there is no consensus concerning the relationships between Chilocorini and other Coccinellidae. Traditional classifications (Sasaji, 1968; Kovář, 1996; Pang et al., 2004) recognized that the subfamily Chilocorinae contained the tribes Chilocorini, Platyaspini and Telsimiini. However, the putative close relationships among these groups were not supported by molecular phylogenetic analyses of the family Coccinellidae. For example, Chilocorini has since been determined to be a sister group to Platyaspini following analyses based on 18S rDNA and 28S rDNA (Giorgi et al., 2009), and to Telsimiini based on 16S rDNA analysis (Aruggoda et al., 2010). However, most recent phylogenetic studies support Chilocorini as being a sister group to Coccinellini (Magro et al., 2010; Seago et al., 2011; Robertson et al., 2015; Che et al., 2017; Escalona et al., 2017). Although these studies did not yield consistent results, they confirmed that the formerly recognized subfamily Chilocorinae did not constitute a monophyletic group.

Within Coccinellidae, to date the phylogeny of Microweiseinae, Epilachnini and Coccinellini have been the subject of recent comprehensive research (Escalona & Ślipiński, 2012; Szawaryn et al., 2015; Escalona et al., 2017), while the evolutionary history and systematics of Chilocorini remain poorly studied. Despite the inclusion of Chilocorini in the high-level relationships study of Coccinellidae, only relatively few taxa from this tribe were sampled in various studies (Giorgi et al., 2009; Aruggoda et al., 2010; Magro et al., 2010; Seago et al., 2011).

Here we present the first comprehensive study on the phylogenetic relationships among members of Chilocorini. We include representatives of all 27 Chilocorini genera worldwide. Integrated data from five DNA fragments and 86 morphological characters provide robust results on the relationships among these genera. The morphological characteristics are tracked on the phylogenetic tree based on the combined dataset, in order to find distinguishing characters for each genus. Based on these results we reconstruct the evolution of selected morphological traits and estimate divergence times of the tribe and its major lineages. Finally, a generic-level revision of Chilocorini based on these results is presented.

### Materials and methods

#### Taxon sampling

We obtained 82 samples of Coccinellidae, including 67 in-group species, representing all 27 known genera of Chilocorini, and 15 outgroup species, having been shown to have close or controversial relationships in prior research (i.e. Serangini, Aspidimerini, Telsimini, Platynaspini, Epilachnini, Coccidulini, Coccinellini and Plotinini) (Table S1) (Chapin, 1965; Ślipiński & Giorgi, 2006; Seago et al., 2011, Łączyński & Tomaszewska, 2012; Li et al., 2017).

The morphological and combined (morphology and molecules) analyses included all 82 taxa; the purely molecular analyses were based on a total of 61 samples, with 46 samples representing 17 genera of Chilocorini. Type species of 23 genera of Chilocorini were included in the analyses. Vouchers were deposited at the South China Agricultural University, China (SCAU), and the Australian National Insect Collection, Commonwealth Scientific and Industrial Research Organisation, Canberra, Australia (ANIC-CSIRO). Vouchers data and GenBank sequence accession numbers are given in Table S1.

#### Morphological data

The data matrix of 86 morphology characters for the 82 terminal taxa was assembled by NDE (Page, 2001) and exported into Nexus format for subsequent phylogenetic analysis. The list of morphological characters (Table S2) was developed from that of Łączyński (2013). All characters were treated as unordered and unweighted. The data matrix is presented in Table S3.

#### DNA sequencing and alignment

DNA was extracted from either thorax muscles or entire individuals previously preserved in 95% ethanol using Qiagen (Beijing, China) DNeasy Blood & Tissue Kit according to the manufacturer’s instructions. The primers used in this study are listed in Table S4. Polymerase chain reaction (Qiagen Multiplex PCR Kit) was performed in 25 μL volumes containing: 12 μL dNTP mix, 10 μL water, 1 μL of each primer, and 1 μL of template DNA per reaction. The two parts of carbamoyl-phosphate synthetase (CAD) and wingless (WGL) were amplified by performing nested PCR. Topoisomerase (TOPO) and cytochrome oxidase subunit I (COI) were amplified by performing nested touchdown and touchdown PCR, respectively. Detailed PCR
Fig. 1. Representatives of Chilocorini in their natural environments. (a) *Chilocorus* cacti, larvae, pupae and adults; (b) *Chilocorus* sp., pupa; (c) *Halmus chalybeus*, adult feeding on coccid; (d) *Orcus australiasiae*, adult; (e) *Phaenochilus metasternalis*, adult; (f) *Brumoides lineatus*, adult; (g) *Curinus coeruleus*, larva; (h) *Exochomus quadripustulatus*, adult and coccid.
conditions for nuclear and mitochondrial fragments are listed in Table S5. The products were purified with a DNA Purification Kit (Beyotime, Shanghai, China). DNA extraction and PCR experiments were performed in the molecular laboratories of the Engineering Technology Research Centre of Agricultural Pest Biocontrol (SCAU) or Zoological Research Museum Alexander Koenig (ZFMK). The sequencing was undertaken by Macrogen or the Beijing Genomics Institute.

All sequences were aligned using muscle (Edgar, 2004) as implemented in Geneious 7.1.4 (Biomatters; available from http://www.geneious.com/). All alignments were visually inspected for errors.

**Molecular data analyses**

Maximum likelihood (ML) and Bayesian inference (BI) were used for the molecular data analyses. We used PartitionFinder 1.1.1 (Lanfear et al., 2012) to assess a partition strategy and best-fitting models of nucleotide evolution, resulting in four partitions (two fragments of CAD were in the same partition) under the GTR+G+I model. Bayesian phylogenetic analysis was performed with MrBayes 3.2.6 (Ronquist et al., 2012). Tree search was conducted for $50 \times 10^6$ generations, using a random starting tree and two runs of three heated and one cold Markov chains (heating of 0.1). For each run, trees were sampled every 5000 generations and the first 25% of the sampled trees were discarded as burn-in. The remaining trees were then used to construct a consensus tree under the rule of adding all compatible groups to such a tree. Convergence was assessed for all parameters by evaluating stationarity of the Markov chain with Tracer 1.5 (Rambaut & Drummond, 2009) and by sampling of Bayesian tree topologies with AWTY (Wilgenbusch et al., 2004). The ML phylogenetic analysis was performed in Raxml 8.2.8 (Stamatakis, 2014). The GTR+G+I model was applied to a single-gene dataset or five-gene concatenated dataset. The tree search was conducted 100 times, using the rapid hill-climbing method and finally choosing the best tree found based on this likelihood. Branch
**Fig. 3.** *Chilocorus* cacti (Linnaeus). (a) body, ventral view; (b) pronotum and basal margin of elytra; (c) head; (d) mouthparts and prothorium; (e) antenna; (f) part of female genitalia showing sperm duct and spermatheca; (g) tarsal claws; (h) body, dorsal view.

© 2019 The Authors. *Systematic Entomology* published by John Wiley & Sons Ltd on behalf of Royal Entomological Society. 45, 447–463
supports were determined by 1000 replicates of nonparametric bootstrap.

**Morphological data analyses**

The morphological matrix was analysed under the maximum parsimony (MP) criterion implemented in TNT (Goloboff & Catalano, 2016). At first, the most parsimonious trees were searched using the parsimony ratchet (Nixon, 1999) under the following settings: ‘initial addsequences’ = 10 and ‘find minimum length’ = 100. Default settings were used for other parameters. Subsequently, implied weighting was used to reduce the effects of homoplasy. Other settings were unchanged. We repeated this analysis with various values of the concavity constant $K$, ranging from 3 to 15. Character changes were mapped on the implied weighting consensus tree using WINCLADA (Nixon, 2002).

**Combined data analyses**

The molecular and morphological matrices were manually concatenated using NOTEPAD++. The BI was performed on the combined matrix using MRBAYES 3.2.6 (Ronquist et al., 2012). The partition strategy and substitution model was consistent with the molecular data analysis. A gamma distribution model (Lewis, 2001) was used for the morphology partition. Posterior probabilities (PPs) > 0.95 were considered strongly supported (Huelsenbeck & Rannala, 2004). Subsequently, character states were mapped on this tree in WINCLADA.

**Ancestral character state reconstruction**

The ancestral character states of selected 16 morphological characters frequently used for previous tribal and generic classification of Chilocorini (Table S2) was performed using the Bayesian consensus tree as backbone, using the MP and ML methods as implemented in MESQUITE 3.1 (Maddison & Maddison, 2008). The Mk1 model was used to calculate the ML probabilities of the ancestral states.

**Estimation of divergence times**

Two fossils were conservatively selected to be used as calibration points (Table S6). Divergence times were estimated based on the relaxed molecular clock method with BEAUTI 1.8.2 (Drummond et al., 2012). The evolutionary models of nucleotide substitutions were the same as those used in the molecular phylogenetic tree inference. Calibrated nodes and the tribe Chilocorini were constrained to be monophyletic. A Yule prior was applied for the speciation process. The fossil calibrations were specified using a lognormal prior (mean = 30, log SD = 0.75) on the targeted clades. Four MCMC chains of $50 \times 10^6$ generations each were sampled every 5000 generations.

We collected 75% post burn-in samples and used TRACER 1.6 to check whether effective sample sizes were large enough for reliable parameter estimation.

**Results**

**Morphology**

Parsimony analysis under implied weighting with concavity constant $K$ set in the range 3−15 found few minimum length trees, mostly only one tree ($K = 6−13$) and rarely eight or nine trees ($K = 3−5$). All these trees show basically the same topology. The most parsimonious tree ($K = 8$) (tree length, $L = 585$ steps; consistency index, CI = 22; retention index, RI = 70; Fig. S1) shows an internal topology of Chilocorini (most relationships recovered) moderately concordant with those of the molecular and combined trees, particularly with respect to major clades. The results of the morphological phylogenetic analysis recovered Chilocorini as a monophyletic group (Chilocorini sensu n.) when excluding the genus Chilocorellus from it and as sister group to Telsimini + (Platynaspini + Aspidimerini). The topology within the tribe Chilocorini constructed only based on the morphological characters was more or less similar to the results obtained in the analysis of the combined dataset, but there was a significant conflict between results of both analyses concerning phylogenetic relationships outside the tribe Chilocorini.

**Molecular data**

The length of the concatenated alignment was 3496 bp composed as follows: two fragments of CAD (760 + 763 bp), TOPO (703 bp), wingless (444 bp) and COI (826 bp). The NCBI gene accession numbers are listed in Table S1. Single-gene phylogenetic analyses are presented in Figs S2.1−S2.5. The topology of the phylogenetic tree from BI was the same as the one from ML inference, differing in a few nodal supports [BI topology (Fig. 4); the result from the ML analysis (Fig. S3)]. In general, most nodes were well supported [PP > 0.95 or bootstrap support (BS) > 90] and both analyses supported the monophyly of Chilocorini sensu n. (PP/BS = 1.0/100) and Plotinini was recovered as a sister group to Chilocorini (PP/BS = 1.0/96).

**Combined dataset**

The combined data matrix consisted of 3496 base pairs and 86 morphological characters. The topology of the phylogenetic tree based on the combined dataset is consistent with molecular-data-only analysis. However, the support of some nodes was lower (Fig. S4). Although the phylogenetic trees inferred by molecular data alone resolved the relationships within Chilocorini with good support, the taxon sampling only covered 17 out of 27 currently known genera,
Fig. 4. Phylogeny of Chilocorini from Bayesian inference based on five genes. Numbers at nodes represent Bayesian posterior probabilities and bootstrap support. Synonymized genera are marked in yellow.
while the combined dataset included all known genera of Chilocorini. In addition, 86 morphological characters were successfully mapped to this tree, in order to find homologous and homoplasious traits for each genus, as discussed later (Fig. 5).

Most recovered relationships among the major groups of Chilocorini were supported by molecular data with high confidence (Fig. 4); by contrast, support values of some nodes in the combined data phylogenetic tree were lower (Fig. S4). Nevertheless, the tree resulting from combined dataset analysis is more informative about the generic relationships in the tribe, and the character mapping on this tree provided more objective morphological criteria for the generic classification.

The following further discussion is mainly based on the tree obtained from the BI of the combined data analysis (treated here as our preferred tree; Fig. 5) with references to the results from the molecular data analysis.

Evolution of morphological traits

The results from the ancestral state reconstruction of 16 morphological characters based on ML and MP approaches are congruent (Figs S5.1–S5.16). Thirteen characters were most likely present in the most recent common ancestor of Chilocorini. These characters include: antennal insertion hidden (dorsal view) (2:2) (Fig. S5.1); anterior clypeal margin emarginate medially, rounded or truncate laterally (3:2) (Fig. S5.2); clypeus expanded laterally (4:1) (Fig. S5.3); eyes with inner orbits parallel, the same distance along frons (8:0) (Fig. S5.4); antennal insertions broadly separated (broader than inner orbits of eyes) (11:0) (Fig. S5.6); mandible with one smooth apical tooth at apex (22:1) (Fig. S5.8); maxilla with cardo quadrate to weakly transverse reaching at most slightly outside of mouth cavity (25:0) (Fig. S5.9); surface of prosternal process smooth, without cariniae (45:2) (Fig. S5.11); elytral epipleuron surface smooth (59:0) (Fig. S5.12); parameres well developed, simple apically (76:1) (Fig. S5.14); spermatheca without membranous beak-like projection at apex (84:1) (Fig. S5.15); and sperm duct between the bursa copulatrix and spermatheca is composed of two or three parts of different diameters (85:1) (Fig. S5f). Eight other diagnostic but homoplastic characters are: antennal insertion hidden (2:2); anterior clypeal margin emarginate, rounded or truncate laterally (3:2); clypeus expanded laterally (4:1); eyes with inner orbits parallel, the same distance along frons (8:0); antennal insertions broadly separated (broader than inner eye orbits) (11:0); mandible with one smooth apical tooth at apex (22:1); surface of prosternal process smooth, without carinae (45:2) (Figs 3a–e, g. h). Chilocorini was recovered in our analyses as the sister group of Plotinini. The close relationship between Chilocorini, Telsimiini and Platynaspini was not supported as monophyletic (i.e. the former Chilocorinae).

The tribe was divided into two large clades (shown on the trees as clade A and clade B). Both clades are strongly supported in the molecular data analysis (in both cases PP/BS = 1.00/100). Combined data analysis supports clade A (PP = 0.9) which is supported by two morphological homoplastic characters: prosternal process expanded to apex (44:1) and spermatheca with membranous beak-like projection at apex. However, the combined data analysis only weakly supported the clade B (PP = 32) with no morphological character optimized on the combined tree as a synapomorphy for this clade.

Clade A genera

Renius Li & Wang (clade A1). This monotypic genus is known from China and India and was recovered as the sister taxon to the Chilocorinus group (clade A2) in molecular and combined analyses (PP/BS = 1.00/100, PP = 0.90). The monophyly of this genus is supported by two morphological autapomorphies – anterior clypeal margin distinctly projecting medially (3:3) and ovipositor with subtringular sclerotized construction between coxites (81:0) – and by the following homoplastic characters: the shape of antennal scape normal (about as long as broad, at most slightly asymmetrical) (14:0); terminal labial palpomere distinctly narrower than penultimate one (38:0); submarginal carina of pronotum present along whole pronotal base (40:2); prosternal process truncate at apex (43:1); and lateral margins of elytra distinctly explanate (56:2).

Chilocorus sensu n. (clade A2). The monophyly of this clade was supported by molecular and combined data analyses
Fig. 5. Bayesian inference consensus tree based on analysis of five gene markers and 86 morphological characters. Numbers of clades are included in cases of taxa used also in molecular analysis. On the branches: nonhomoplasious changes, homoplasious changes; the numbers above and below each circle correspond to the character number and character state, respectively. On the nodes: posterior probability (PP) = 1; PP ≥ 90; 70 ≤ PP < 90; otherwise, PP < 70. Synonymized genera are marked in yellow. Branch lengths are meaningless.
Fig. 6. Divergence time estimation based on five genes performed with BEAST. Numbers at nodes represent the median of node ages. Blue bars indicate 95% mean confidence interval (CI) of each node.
Evolution and classification of Chilocorini

The genus *Chilocorus* has been the largest genus in Chilocorini containing about 80 species that are widely distributed worldwide and morphologically relatively variable. The results of our analyses reject the monophyly of *Chilocorus*. Four other genera (*Phaenochilus, Anisorcus, Egius, Simmondsius*) were recovered as embedded in the clade of this genus. All together they form a single clade, which we refer to here as *Chilocorus sensu n.*

*Phaenochilus* is a small genus distributed in the Oriental region, currently containing 13 species. Three species were analysed in the molecular data, and the type species was also included in the combined data analyses. Our molecular and combined analyses recovered *Phaenochilus* as not a monophyletic group, even within the *Chilocorus* clade, e.g. the species *P. mikado* is more closely related to some species of *Chilocorus* and *Anisorcus* than to the remaining analysed *Phaenochilus* species.

*Anisorcus* consists of three species distributed only in the Australian region. Similarly to *Phaenochilus*, the monophyly of this genus was not supported by our molecular and combined data. Two analysed species of *Anisorcus* form a clade with two Australian *Chilocorus* species.

*Egius* and *Simmondsius* are two monotypic genera known from South America and Pakistan, respectively. No molecular data were available for these two genera. Although each of the two genera are quite well defined morphologically, they are deeply embedded in *Chilocorus*, although with weakly supported relations to *Chilocorus* species (PP = 0.53, PP = 0.48).

**Clade B genera**

*Endochilus* Weise. No molecular data were available for this genus known only from the Afrotropical region. *Endochilus* was recovered as the sister group to *Chapinaria* with moderate support (PP = 0.86). The monophyly of this genus was strongly supported in the combined data analysis (PP = 0.99). The diagnostic characters of *Endochilus* include one apomorphic character (epipleuron wide, more than 8x wider than metanepisternum (60:2) and six homoplastic characters: interocular distance less than 0.5x width of head (7:0); antenna with eight antennomeres (13:1); prosternal process broad, about 0.8–1.0x width of coxal diameter (41:1); lateral margins of elytra distinctly explanate (56:2); prosternal line of ventrite 1 parallel to hind margin of ventrite, at most scarcely recurved, incomplete (72:3); and hind margin of abdominal ventrite 5 in male emarginate medially (74:1).

*Chapinaria* Łaczyński & Tomaszewska, 2012. This monotypic genus known from the Afrotropical region was established by Łaczyński & Tomaszewska (2012) who separated it from *Endochilus*. In this study, no molecular data were obtained for *Chapinaria*. The only known species was included in the combined analysis, where it was recovered as the sister group to *Endochilus* (PP = 0.86). However, the position of *Chapinaria + Endochilus* in the tribe Chilocorini remains uncertain. It was recovered as a sister group to the remaining genera of the clade B, but with no statistical support (PP = 0.32). *Chapinaria* is a very distinctive genus defined by a number of morphological characters as follows: antenna with 11 antennomeres (13:4); terminal antennomere very small, embedded in penultimate antennomere (19:3); mandible with two smooth apical teeth (22:2); submarginal carina of pronotum absent (40:0); prosternum in front of coxa shorter than half length of coxal longitudinal diameter (46:2); lateral margins of elytra not explanate, not or hardly visible from above (56:0); epipleuron of elytra more or less horizontal (58:1); surface of elytra epipleuron with weak cavities for hind legs (59:1); epipleuron moderately wide, 3.0–6.0x wider than metanepisternum (60:1); outer edge of fore tibia angulate (61:1); tarsus three-segmented (65:1); male abdomen with five visible ventrites (70:0); abdominal postcoxal line recurved roundly but incomplete laterally (72:1); spermatoeca well developed with membranous beak-like projection at apex (84:2).

*Sicardiana* Łaczyński & Tomaszewska. No molecular data were available for this monotypic genus from New Guinea, the monophyly of which was supported by single apomorphy in the combined data analyses: prosternal process narrowed to apex (44:2) and by the following homoplasious characters: antennal club with at least one segment asymmetrical (14:0); terminal antennomere very small, embedded in penultimate antennomere (19:3); submarginal carina of pronotum absent (40:0); junction of meso- and metaventrite arcuate anteriorly (51:1); abdominal postcoxal lines recurved roundly but incomplete laterally (72:1); and penis guide on inner edge with additional process (78:0). The relationship of this genus to other genera of Chilocorini remains unclear. It was recovered as a sister group to the remaining groups in clade B (except *Chapinaria + Endochilus*), but with a low support value (PP = 0.27).

*Chujochilus* Sasaji (clade B1). This eastern Palearctic genus of three species is known from China and Japan. In our analyses it was strongly supported as a sister group to *Orcus* (PP/BS = 1.00/100, PP = 0.96). The monophyly of this genus is supported by the following morphological characters: inner orbits of eyes convergent, closer anteriorly than on vertex (8:3); head with ventral antennal grooves present in form of long and deep groove between eye and mouthparts (9:2); antennal scape as long as broad, at most slightly asymmetrical (14:0); terminal antennomere very small, embedded in penultimate antennomere (19:3); submarginal carina of pronotum absent (40:0); junction of meso- and metaventrite arcuate anteriorly (51:1); abdominal postcoxal lines recurved roundly but incomplete laterally (72:1); and penis guide on inner edge with additional process (78:0). The relationship of this genus to other genera of Chilocorini remains uncertain. It was recovered as a sister group to the remaining groups in clade B (except *Chapinaria + Endochilus*), but with a low support value (PP = 0.27).
tibia with two spurs (63:0); and apex of hind tibia with two spurs (64:0).

Orcus MULTANT (clade B2). Orcus is distributed in Australia and the Oriental region. Our molecular analysis included two species and this group was strongly supported (PP/BS = 1.00/100). The type species O. janthinus was added to the combined data analysis and the Orcus clade was also recovered as monophyletic, but this was only weakly supported (PP = 0.67); no apomorphic character was found for the group, either on the morphological only or the combined tree. In their review of Orcus, Łączyński & Tomaszewska (2012) indicated that Orcus is most similar to Halmus but can be distinguished from it by having distinctly emarginated eyes, nine-segmented (rarely eight-segmented) antenna, elytral epipleuron with usually deep foveae to receive femora and well-developed ovipositor. Our analyses, however, recovered Orcus as a sister group to Chijuochilus with strong support values and the following characters: antenna with ten antennomeres (13:2); terminal labial palpmere much narrower than penultimate one (< 0.7) (38:0); elytral epipleuron with weak (59:1) or deep cavities for hind legs (59:2); and abdominal postcoxal lines connected on intercoxal process (71:1).

Trichocorus Blackburn (clade B3). This Australian monotypic genus was recovered as a sister group to Hypoceras (clade B4) + Halmus (clade B5) in both the molecular and combined data analyses, with strong support values (PP/BS = 1.00/95, PP = 0.91), although any apomorphy for this relationship was not inferred on the combined tree. The monophyly of this genus was supported by four homoplastic characters: penultimate antennomere distinctly shorter than terminal antennomere (18:0); terminal maxillary palpmere shorter than broad, expanded apically (30:2); lateral margins of elytra not explanate, not or hardly visible from above (56:0); and abdominal postcoxal lines connected at intercoxal process (71:1).

Hypoceras CHAPUIS (clade B4). The molecular and combined data analyses strongly supported this Australian monotypic genus as a sister group to Halmus (clade B5) (PP/BS = 1.00/99, PP = 0.99). The monophyly of this genus was supported by the following diagnostic characters: pronotum without submarginal carina (40:0); prosternum in front of coxa shorter than half length of coxal longitudinal diameter (46:2); meso-metaventral process 0.7–0.8× wider than coxal diameter (50:1); junction of meso- and metaventrite arcuate anteriorly (51:1); postcoxal lines on metaventrite complete and distinctly recurved or straight (55:0); lateral margins of elytra distinctly explanate (56:2); sperm duct between bursa copulatrix and spermatheca of the same diameter along whole length (85:0).

Halmus MULTANT (clade B5). The molecular and combined data strongly supported the monophyly of this genus known from Australia and New Guinea (PP/BS = 1.00/99, PP = 1.00). Its diagnostic morphological characters are: antenna with seven antennomeres (13:0); antennal scape as long as broad, at most slightly asymmetrical (14:0); antennomere 3, 1.2–2.0× longer than antennomere 4 (16:1); and apex of terminal antennomere distinctly prominent (20:1). Sister relationships of Halmus + Hypoceras are supported by the following characters: pedicel distinctly narrower than scape (15:0); and terminal labial palpmere distinctly narrower than penultimate one (< 0.7).

Exochomus Redtenbacher (clade B6). Exochomus is the second largest genus in Chilocorini with c. 75 species distributed around the world (except for Australia, where only one introduced species is known). The monophyly of this clade was strongly supported (PP/BS = 1.00/100, PP = 1.00). Two diagnostic, morphological characters support it: lateral margins of elytra not explanate, not or hardly visible from above (56:0); and outer margin of elytra with distinct bead (57:0). Due to the very small and geographically limited sampling of Exochomus used in our analyses (three Palaearctic species), the results must be treated as preliminary and the taxonomy of this group warrants further study.

Our analyses revealed Exochomus as the sister to a larger clade within clade B. This group includes 12 genera and is well supported in all analyses (PP/BS = 1.00/100, PP = 0.94). Six of them from the Neotropical region (Arawana, Axion, Cladis, Curinus, Harpusas and Zagreus), and the other six distributed mainly in the Palaearctic, Oriental, Ethiopian and Australian regions (Brumoides, Brumus, Parexochomus, Priscibrumus, Xanthocorus). The whole group is supported by the following morphological, diagnostic characters: antenna with terminal antennomere very small, embedded in penultimate one (19:3); apex of mid- and hind tibiae with two spurs (63:0 and 64:0); postcoxal line on ventrite 1 recurved roundly and complete or recurved roundly but incomplete (72:0,1); and hind margin of ventrite 5 in male emarginate medially (74:1).

Xanthocorus Miyatake (clade B7). This small genus contains three species distributed only in China. The monophyly of Xanthocorus was strongly supported in combined data analyses (PP = 0.90) and is defined by single homoplastic character: pronotum lacking submarginal carina (40:0). Xanthocorus was recovered in our analysis as a sister group to the remaining genera of Exochomus clade, after the splitting of Exochomus.

Priscibrumus Kovář (clade B8). This genus consists of seven species known to occur in the western Palaearctic and Oriental regions. Only one species was available for our study. The morphological diagnostic characters supporting this genus based on our dataset are as follows: dorsum with setae at least on pronotum or elytra (0:1); prosternum in front of coxa longer than coxal longitudinal diameter at the same position (46:1); elytral epipleuron more or less horizontal (58:1); elytral epipleuron narrow, less than 2.5× wider than metanepisternum (60:0). Priscibrumus was recovered as a sister group to the remaining genera of Exochomus clade, after the splitting of Exochomus and Xanthocorus. However, this relationship was not strongly supported in either the molecular or combined analyses (PP/BS = 0.72/50, PP = 0.58).

© 2019 The Authors. Systematic Entomology published by John Wiley & Sons Ltd on behalf of Royal Entomological Society. 45, 447–463
Parexochomus Barovsky (clade B12). This genus is widely distributed in both the Palaearctic and Afrotropical regions. Three of the 11 species of this genus that were used in our study form a monophyletic clade (PP/BS = 1.00/1.00, PP = 1.00). Parexochomus is revealed as a sister group to Brumoides + Brumus clade. This relationship is supported by an antennal scape of normal shape, about as long as broad, and at most, slightly asymmetrical (14:0). The monophyly of Parexochomus, however, was not supported by any morphological character in our dataset.

Brumoides Chapin (clade B11). This genus is distributed worldwide and was recovered as a sister group to Brumus in the combined data (PP = 0.94) and both (Brumus + Brumoides) as a sister to Parexochomus in the combined analysis. The sister relationship between Brumus and Brumoides in the combined analysis is well supported by diagnostic morphological characters such as: elytral epipleuron narrow, < 2.5x wider than metanepisternum (60:0); pro-, mid- and hind tarsal claws in male single, smooth or swollen at base (66:0; 67:0; 68:0); and hind margin of ventrite 5 in male rounded or straight (74:0). The monophyly of Brumoides was strongly supported in molecular and combined data analyses (PP/BS = 1.00/100, PP = 0.99); and its diagnostic characters are as follows: antenna with eight antennomeres (13:1); elytral epipleuron more or less horizontal (58:1); hind legs (59:2). The monophyly of Harpusus was supported by two homoplastic characters: antenna with nine antennomeres (13:2); antennal scape as long as broad, and at most slightly asymmetrical (14:0).

Brumus Mulsant. No molecular data were available for this Palaearctic genus. The combined data analysis revealed a close relationship of Brumus with Brumoides with strong support value (PP = 0.95). The monophyly of the genus Brumus was strongly supported in the combined data analysis (PP = 0.99), and its diagnostic characters are as follows: prosternal process rounded apically (43:0) and meso- and metaventrite junction arcuate anteriorly (51:1).

Zagreus Mulsant. No molecular data were available for this Neotropical genus. The monophyly of Zagreus was strongly supported in the combined data analysis (PP = 0.99), and one morphological homoplastic character, antenna with eight antennomeres (13:1), is shown on the combined tree as diagnostic. Our analyses revealed Zagreus as sister to a clade comprising mainly Neotropical Chilocorini within a large clade B. Its sister position to (Cladis + (Harpalus + (Curinus + (Axion + Arawana)))) is also supported by two morphological characters [pronotum without submarginal carina (40:0) and junction of meso- and metaventrite arcuate anteriorly (51:1)], but with a weak support value in the combined analysis (PP = 0.47).

Cladis Mulsant. No molecular data were available for this Neotropical, monotypic genus. It was recovered as a sister group to (Harpalus + (Curinus + (Axion + Arawana))) in our combined data analysis (PP = 0.98). This clade containing five genera is well supported and defined by morphological apomorphy: parameres well developed, with triangular projection on outer margin (76:2). Cladis is distinctive from other genera of this group based on one homoplastic character: terminal maxillary palpomere shorter than wide, expanded apically (30:2).

Harpalus Mulsant. No molecular data were available for this Neotropical genus. It was recovered as the sister to (Curinus + (Axion + Arawana)) in combined data analyses with a strong support value (PP = 0.98), sharing the following homoplastic characters: terminal palpomere of labium distinctly narrower than penultimate palpomere (< 0.7) (38:0); prosternum in front of coxa longer than coxal longitudinal diameter at the same position (46:1); elytral epipleuron surface with deep cavities for hind legs (59:2). The monophyly of Harpusus was supported by two homoplastic characters: antenna with nine antennomeres (13:2); antennal scape as long as broad, and at most slightly asymmetrical (14:0).

Curinus Mulsant (clade B9). This genus contains only two species distributed in the Neotropical region. It was recovered in our molecular analysis as the sister group to Arawana with strong support (PP/BS = 1.00/100). In the combined data, Curinus was recovered as a sister group to Axion + Arawana (PP = 0.71), sharing two homoplastic characters: outer edge of foretibia angulate (61:1); and hind margin of ventrite 5 in male rounded or straight (74:0). The diagnostic characters for Curinus are prosternal process rounded apically (43:0) and postcoxal lines on metaventrite complete, distinctly recurved or straight (55:0).

Axion Mulsant. No molecular data were available for this Nearctic genus comprising two species and it was recovered as a sister group to Arawana in the combined data analysis. However, there was no statistical support (PP = 0.44) and no morphological synapomorphies to support this sister relationship. The diagnostic characters of Axion are as follows: eyes with inner orbits parallel, of the same distance along frons (8:0); and terminal maxillary palpomere about as long as wide, expanded apically (30:2).

Arawana Leng (clade B10). The molecular data strongly supported the monophyly of this genus distributed in the Nearctic and Neotropical regions (PP/BS = 1.00/100). In the combined data, the monophyly of Arawana is, however, weakly supported (PP = 0.64) and no morphological character was optimized on the combined tree, as being diagnostic for this genus.

Chilocorellus Miyatake. In both, the molecular and the combined data analyses, Chilocorellus was recovered distant from Chilocorini with numerous characters separating it, e.g. antennal insertion exposed (with at least part of the foramen visible from above) (2:0); eye with ocular canthus extending slightly into eye (6:1); inner orbits of eyes convergent, closer anteriorly than on vertex (8:3); penultimate antennomere distinctly shorter than terminal antennomere (18:0); maxillary palpomere 2 long (at least 3× as long as wide) (29:1). Our study recovered Chilocorellus as the sister to Plotiniini + Chilocorini with high and moderate support values (PP/BS = 0.92/65, PP = 0.93) in the molecular and combined data analyses, respectively.

© 2019 The Authors. Systematic Entomology published by John Wiley & Sons Ltd on behalf of Royal Entomological Society. 45, 447–463
Relationships among higher groups

The monophyly of Chilocorini was strongly supported (PP/BS = 1.00/100, PP = 1.00) in both molecular or combined data analyses. The former subfamily Chilocorinae was not recovered as a monophyletic group, and our analyses revealed Plotinini as the sister group to Chilocorini. This relationship is indicated for the first time here, as previous phylogenetic studies have placed Chilocorini as sister group to Coccinellini (Magro et al., 2010; Seago et al., 2011; Robertson et al., 2015; Che et al., 2017). However, in the analyses of Magro et al. (2010) and Che et al. (2017), no specimen of Plotinini was included, while Robertson et al. (2015) recovered Chilocorini + Coccinellini as the sister to a clade comprising Plotinini and Sticholotis. Meanwhile, the position of Plotinini within Coccinellidae was unclear in the results of Seago et al. (2011). The placement of Chilocorini as sister to Plotinini is strongly supported in molecular and combined analyses (PP/BS = 0.99/96, PP = 0.97); morphological characters to unite these groups include antenna with ten antennomeres (13:3) and coxites with styli indistinct, only two longer setae visible (82:0). Coccinellini formed a sister group to Chilocorellus + (Plotinini + Chilocorini) (PP/BS = 1.00/50, PP = 1.00).

The monophyly of most traditionally recognized genera of the tribe Chilocorini has been supported in this study. Chilocorellus was transferred to Chilocorini from Sticholotidini by Seago et al. (2011), as their phylogenetic analysis based on COI, COII, and 28S gene fragments strongly supported this genus as a sister group to Chilocorus. By contrast, our results based on morphological and molecular data show that Chilocorellus is not a part of the clade of Chilocorini. However, Chilocorellus + Coccinellini did form a sister group with Chilocorus based on the analysis of the mitochondrial gene COI alone (Fig. S4). Our analyses based on three nuclear genes and one mitochondrial one is inconsistent with the results of Seago et al. (2011). Furthermore, Chilocorellus is significantly different from members of Chilocorini in overall morphology, as stated earlier. Therefore, based on these results, we exclude Chilocorellus from the tribe Chilocorini sensu n.

The genus Axios was removed from Chilocorini by Seago et al. (2011) based on their analysis, which included only one specimen of this genus: Axios cocc123. However, it appeared that this specimen was misidentified. Our check of this sample revealed that it was actually Arawana scapularis. This species was also used in our analysis, and the results of the combined data analysis show Arawana as a sister group to Axios.

Chilocorus was considered by Chapin (1965) as a complex genus which should be divided. In a study of East Asian Chilocorini, Miyatake (1970) separated Chilocorus into seven groups. However, most of these groups were not supported in our analyses, and Chilocorus itself was not recovered as monophyletic. Indeed, it was rendered paraphyletic by Egius, Simmondsius, Phenochilus and Anisorcus. This clade is quite well supported in both molecular and combined data analyses (PP/BS = 1.00/50, PP = 0.84), therefore we propose to synonymize all five genera under the Chilocorus. The genus Rentius possessing a number of diagnostic morphological characters including autapomorphic features was recovered as a sister group to the Chilocorus clade, and forms, together with Chilocorus, a strongly supported monophyletic group (PP/BS = 1.00/100, PP = 0.90).

The taxonomic status of the genera Exochomus and Brumus has been controversial for a long time. Brumus was considered a junior synonym of Exochomus by Ślipiński & Giorgi (2006), while Kovář (1997) attributed the Palaearctic Exochomus species to Brumus. Our results indicated that the relationship between Exochomus and Brumus was not so close; Exochomus is the sister of the large clade containing various genera, including Brumus and Brumoides as a sister pair, but these genera were recovered with Parexochomus. As the sampling of Exochomus used in our analyses was very small and geographically limited only to the Palaearctic region, the taxonomic status of this genus warrants further study.

Ancestral state reconstruction

When Sasaji (1968) proposed the subfamily Chilocorinae, a clypeus expanded laterally (4:1) was considered as a strong character uniting Chilocorini, Platynaspini and Telsmini. Our ancestral state reconstruction analysis shows that this character occurred independently within these three tribes (Fig. S5.3). In the combined data analysis, the monophyly of Chilocorini was supported by one unambiguous apomorphic and eight homoplastic characters (Fig. 5). All these characters were present in the most recent common ancestor of Chilocorini according to our ancestral state reconstruction analyses (Figs S5.1–S5.6, S5.8, S5.11, S5.16). Two interesting character reversals are observed in Chilocorini, each involving a single monotopic genus: (i) the development of two smooth apical teeth in mandible in the genus Chaparinia; and (ii) the uniform diameter of the sperm duct between bursa copulatrix and spermatheca in Hypoceras. However, the most recent common ancestor and the remaining known species of Chilocorini have a single apical mandibular tooth (Fig. S5.8) and the sperm duct composed of two or three parts of different diameters (Fig. S5.16).

A temporal framework of Chilocorini evolution

Current studies indicate that the origin time of Coccinellidae divergence can be traced back to the early Cretaceous [c. 123 Ma (McKenna et al., 2015) or c. 144 Ma (Toussaint et al., 2017)]. Our results are closer to those of Toussaint et al. (2017), suggesting that the minimum age of Coccinellidae is 168 Ma. Our inferred divergence times for Chilocorini places its age as far back as the Middle Cretaceous (85.86 Ma). The divergence of the existing genera was recovered to be during the Middle to Late Paleogene.

Revised classification of Chilocorini

Based on the combined (molecular and morphological data) phylogenetic analysis conducted in this study (our preferred
tree, Fig. 5), the generic classification of the tribe Chilocorini was revised. A new classification of Chilocorini comprising 22 genera (four new synonyms and one genus exclusion) is proposed here.

Subfamily: Coccinellinae
Tribe: Chilocorini Mulsant, 1846: 28.

**Axion** Mulsant, 1850: 477.
Type species: *Coccinella tripustulata* DeGeer, 1775: 393.

**Arawana** Leng, 1908: 38.
Type species: *Exochomus arizonicus* Casey, 1899: 107.

**Brumoides** Chapin, 1965: 237.
Type species: *Coccinella sartalis* Fabricius, 1798: 78

**Brumus** Mulsant, 1850: 492.
Type species: *Coccinella octosignata* Gebler 1830: 225.

**Chapinaria** Łączyński & Tomaszewska, 2012: 2.
Type species: *Endochilus meridionalis* Sicard, 1929: 518.

**Chilocorus** Leach, 1815: 116.
Type species: *Coccinella cacti* Linnaeus, 1767: 584.

**Simmondsius** Ahmad & Ghani, 1966: 9. Type species: *Simmondsius pakistanensis* Ahmad & Ghani, 1966: 9. *syn.n.

**Cladis** Mulsant, 1850: 479.
Type species: *Coccinella nitidula* Fabricius, 1792: 286.

**Chujochilus** Sasaji, 2005: 61.
Type species: *Exochomus isensis* Kamiya, 1966: 80.

**Curinus** Mulsant, 1850: 472.
Type species: *Orcus (Curinus) coeruleus* Mulsant, 1850: 472.

**Endochilus** Weise, 1898: 119.
Type species: *Endochilus cavisfrons* Weise, 1898: 120.

**Exochomus** Redtenbacher, 1843: 11.
Type species: *Coccinella quadripustulata* Linnaeus, 1758: 367.

**Halmus** Mulsant, 1850: 471.
Type species: *Coccinella chalybea* Boisduval, 1835: 595.

**Harpasus** Mulsant, 1850: 473.
Type species: *Orcus (Harpasus) pallidilabris* Mulsant, 1850: 473.

**Hypoceras** Chapuis, 1876: 225.
Type species: *Hypoceras mulsanti* Chapuis, 1876: 226.

**Orcus** Mulsant, 1850: 465.
Type species: *Orcus janthinus* Mulsant, 1850: 466.

**Parexochomus** Barovsky, 1922: 292.
Type species: *Exochomus pubescens* Küster, 1848: 94.

**Priscibrumus** Kovář, 1997: 114.
Type species: *Exochomus punicipennis* Semenov, 1900: 684.

**Renius** Li & Wang, 2017: 122.
Type species: *Renius cornutus* Li & Wang, 2017: 124.

**Sicardiana** Łączyński & Tomaszewska, 2010: 196
Type species: *Sicardiana aureomarginata* Łączyński & Tomaszewska, 2010: 198.

**Trichorinus** Blackburn, 1892: 73.
Type species: *Trichorinus cinctus* Blackburn, 1892: 73.

**Xanthocorus** Miyatake, 1970: 312.
Type species: *Exochomus (Xanthocorus) nigromarginatus* Miyatake, 1970: 312.

**Zagreus** Mulsant, 1850: 488.
Type species: *Exochomus (Zagreus) bimaculosus* Mulsant, 1850: 488.

**Supporting Information**
Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1.** Strict consensus tree obtained using implied weighting in tnt with K = 8.

**Figures S2.1–S2.5.** Maximum likelihood trees based on analysis of single gene marker.

**Figure S3.** Maximum likelihood tree based on analysis of five gene markers.

**Figure S4.** Bayesian inference consensus tree based on analysis of five gene markers and 86 morphological characters.

**Figure S5.1–S5.16** Ancestral state reconstruction of 16 morphological characters based on parsimony and maximum likelihood. The topology is derived from the BI tree in Fig. 5.

**Table S1.** Taxa used in this study.

**Table S2.** List of morphological characters used in the morphological dataset.

**Table S3.** Morphological matrix.

**Table S4.** The primers used to amplify gene sequences.

**Table S5.** Amplification conditions used in PCR reaction.

**Table S6.** Fossils and corresponding references used in the node dating analyses implemented in BEAST.

**Acknowledgements**

WL would like to thank the Graduate School of South China Agricultural University (SCAU) for funding his research on the classification and phylogeny of Coccinellidae at the Zoological Research Museum Alexander Koenig (ZFMK) in Bonn, Germany. The authors also thank Silvia Fabrizi (ZFMK) for her help with morphological studies and use of the microscope; Claudia Etzbauer (ZFMK) for helping us to accomplish the task of gene amplification; and Michael Geiser for his kind help during WL’s visit to the Natural History Museum, London, U.K. The research was supported by the National Natural Science Foundation of China (31802003, 31501884), Science and Technology Planning Project of Guangdong Province (2017A020208060), and The Science and Technology Program of Guangzhou (201804020070, 151800033). The authors declare there are no conflicts of interest.
References

Abouheif, E. & Wray, G.A. (2002) Evolutionary lability of the regulatory gene network underlying the wing polyphenism in ants. Science, 297, 249–252.

Ahmad, R. & Ghani, M.A. (1966) A new genus and species of Chilocorina (Coleoptera: Coccinellidae) from Pakistan. Proceedings of the Royal Entomological Society of London (B), 35, 9–10.

Aragoda, A.G.B., Ren, S.X. & Qiu, B.L. (2010) Molecular Phylogeny of Ladybird Beetles (Coccinellidae: Coleoptera) Inferred from Mitochondrial 16S rDNA Sequences. Tropical Agricultural Research, 21, 209–217.

Barovsky, V. (1922) Revisio specierum palaearcticorum Coccinellinarum generis Exochomus Redd. Annaire du Musée Zoologique de l’[1]Académie des Sciences de Russie. 23, 289–303.

Blackburn, T. (1892) Further notes on Australian Coleoptera, with descriptions of new genera and species. XII. Transactions of the Royal Society of South Australia, 15, 207–261.

Boisduval, J.B.A. (1835) Voyage de Découvertes de l’Astrolabe. Exécuté par ordre du Roi, Pendant les Années 1826–1827–1828–1829, sous le Commandement de M. J. Dumont d’Urville. Faune Entomologique de l’Océan Pacifique, avec l’illustration des Insectes Nouveaux Recueillis Pendant le voyage, Deuxième Partie. Coleoptères et autres ordres. J. Tastu, Paris, viii + 716 pp.

Booth, R.G. (1998) A review of the species resembling Chilocorus nigrita (Coleoptera: Coccinellidae): potential agents for biological control. Bulletin of Entomological Research, 88, 361–367.

Casey, T.L. (1899) A revision of the American Coccinellidae. Journal of the New York Entomological Society, 7, 71–169.

Chapin, E.A. (1965) The genera of the Chilocorini (Coleoptera, Coccinellidae). Bulletin of the Museum of Comparative Zoology, Harvard University, 133, 227–271.

Chapuis, F. (1876) Famille des Erotyliens, des Endomychides et des Coccinellides. Histoire Naturelle des Insectes. Genera des Coleopteres ou exposé methodique et critique de tous les genres proposts jusqu’ici dans cet ordre d’insectes (ed. by T. Lacordaire and F. Chapuis), p. 424. Tome Douzieme. Librairie Encyclopedique de Roret, Paris.

Che, L.H., Zhang, S.Q., Li, Y., Liang, D., Pang, H., Ślipiński, A. & Zhang, P. (2017) Genome-wide Survey of Nuclear Protein-Coding Markers for Beetle Phylogenetics and Their Application in Resolving both Deep and Shallow-Level Divergences. Molecular Ecology Resources, 17, 1342–1358.

Crotch, G.R. (1874) (ed. by T. Lacordaire and F. Chapuis), p. 424. Tome Douzieme. Librairie Encyclopedique de Roret, Paris.

Crotch, G.R. (1874) A Revision of the Coleopterous Family Coccinellidae. E. W. Janson, London. 311p.

Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the beast 1.7. Molecular Biology and Evolution, 29, 1969–1973.

Edgar, R.C. (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics, 5, 113.

Escalona, H.E. & Ślipiński, A. (2012) Generic revision and phylogeny of Microweiseinae (Coleoptera: Coccinellidae). Systematic Entomology, 37, 125–171.

Escalona, H.E., Zwick, A., Li, H.S. et al. (2017) Molecular phylogeny reveals food plasticity in the evolution of true ladybird beetles (Coleoptera: Coccinellidae: Coccinellini). BMC Evolutionary Biology, 17, 151.

Fabricius, J.C. (1798) Supplementum Entomologiae Systematicae. Prof. et Storch, Hafniae, 572 pp.

Giori, J.A. & Vandenberg, N.J. (2012) Review of the lady beetle genus Phaenochilus Weise (Coleoptera: Coccinellidae: Chilocorini) with description of a new species from Thailand that preys on cyclad aulacaspis scale, Aulacaspis yasumatsui Takagi (Hemiptera: Sternorrhyncha: Diaspididae). Zootaxa, 3478, 239–255.

Giori, J.A., Vandenberg, N.J., McHugh, J.V. et al. (2009) The evolution of food preferences in Coccinellidae. Biological Control, 51, 215–231.

Goloboff, P. & Catalano, S.A. (2016) TNT version 1.5, including a full implementation of phylogenetic morphometrics. Cladistics, 32, 221–238.

Huelsniececk, I. & Rannala, B. (2004) Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. Systematic Biology, 53, 904–913.

Kamiya, H. (1966) On the Coccinellidae attacking the scale insects and mites in Japan and the Ryukyus. Mushi, 39, 69–95.

Kovář, I. (1996) Phylogeny. Ecology of Coccinellidae (ed. by I. Hodek and A. Honěk), pp. 19–31. Kluwer Academic Publishers, Dordrecht.

Kovář, I. (1997) Revision of the genera Branus Muls. and Exochomus Redd. (Coleoptera: Coccinellidae) of the Palaearctic region. Part I. Acta Entomologica Musei Nationalis Pragae, 44, 5–124.

Lanfear, R., Calcott, B., Ho, S.Y.W. & Guindon, S. (2012) Partition-finder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution, 29(6), 1695–1701.

Leach, W.E. (1815) In Brewster: Articles on Entomology. Edinburgh Encyclopaedia, 9, 57–172.

Leng, C.W. (1908) Notes on Coccinellidae. III. Journal of the New York Entomological Society, 16, 33–44.

Lewis, P.O. (2001) A likelihood approach to estimating phylogeny from discrete morphological character data. Systematic Biology, 50(6), 913–925.

Li, W.J., Huo, L.Z., Ahrens, D., Ren, S.X. & Wang, X.M. (2017) Renius cornutus, a new genus and species of Chilocorini from Tibet, China (Coleoptera, Coccinellidae). Zootaxa, 478, 121–128.

Linnaeus, C. (1758) Systema naturae per regna tria naturae, secundum classes, ordines, genera, species cum characteribus, differentiis, synonymis, locis. Tomus I. Editio Decima Reformata. Laurentii Salvii, Holmiae, 824 pp.

Linnaeus, C. (1767) Systema Naturae, per Regna Tria Naturae, secundum Classes, Ordines, Genera, Species, cum Characteribus, Differentiis, Synonymis, Locis. Tom I. Pars I. Editio Decima tertia, ad Editionem duodecimam reformatam Holmiensem. Vindobonae, Typis Ioannis Thomae nob. de Tratten, pp. 533–1364.

Łączyński, P. (2013) Morfologia, filogenesa i klasyfikacja rodzajów biedronek z plemienia Chilocorini (Coleoptera: Coccinellidae). PhD thesis, Muzeum i Instytut Zoologii, Polska Akademia Nauk, (in Polish; unpublished).

Łączyński, P. & Tomaszewska, W. (2010) Sicardiana aureomarginata, a new genus and new species of Chilocorini from New Guinea (Coleoptera: Coccinellidae). Annales Zoologici, 60, 195–202.

Łączyński, P. & Tomaszewska, W. (2012) Chapinaria, New Genus of Chilocorini for Endochilus meridionalis Sicard from Africa (Coleoptera: Coccinellidae). Annales Zoologici, 62, 1–9.

Maddison, W.P. & Maddison, D.R. (2008) Mesquite: A modular system for evolutionary analysis. Version 2.7 [WWW document]. URL http://mesquiteproject.org [accessed on September 2019].

Maggro, A., Lecompte, E., Magne, F., Hemptinne, J. & Crouau-Roy, B. (2010) Phylogeny of ladybirds (Coleoptera: Coccinellidae): are the subfamilies monophyletic? Molecular Phylogenetics and Evolution, 54, 833–848.

McKenna, D.D., Wild, A.L., Kanda, K., Bellamy, C.L., Beutel, R.G., Caterino, M.S. et al. (2015) The beetle tree of life reveals that Coleoptera survived end of Permian mass extinction to diversify during the cretaceous terrestrial revolution. Systematic Entomology, 40, 835–880.

Miayatake, M. (1970) The East-Asian coccinellid beetles preserved in the California Academy of Sciences. Tribe Chilocorini. Memoirs of the College of Agriculture, Ehime University, 14, 19–56.
Mulsant, E. (1846) Salci colles-Securipalpes. Histoire Naturelle des Coléoptères de France. Maison, Paris. xxiv + 280 pp.
Mulsant, E. (1850) Species des Coléoptères Trimères Securipalpes, Annales des Sciences Physiques et Naturelles, d’Agriculture et d’Industrie, de Lyon Septième Série, Vol. 2. Publiées par la Société Nationale d’Agriculture. etc., Lyon.

Nixon, K.C. (1999) The parsimony ratchet, a new method for rapid parsimony analysis. Cladistics, 15, 407–414.

Nixon, K.C. (2002) Winclada, Version 1.00.08. Published by the author, Ithaca, New York.

Page, R.D.M. (2001) NDE (NEXUS data editor for windows). Version 0.5.0 NDE [WWW document]. URL http://taxonomy.zoology.gla.ac.uk/rod/NDE/nde.html [accessed on 1 December 2016].

Pang, H., Ren, S.X., Zeng, T. & Pang, X.F. (2004) Phylogeny and their utilization of Coccinellidae in China. Science and Technology Press of Guangdong, Guangzhou, 168 pp. (In Chinese).

Rambaut, A. & Drummond, A.J. (2009) Tracer v. 1.5 [WWW document]. URL http://beast.bio.ed.ac.uk/Tracer [accessed on October 2016].

Redtenbacher, L. (1843) Tetamen Dispositionis Generum et Specierum Coleopterorum Pseudotrimeorum Archiducatus Austriae. Disert. Inaug, Vindobone, 32 pp.

Robertson, J.A., Ślipiński, A., Moulton, M. et al. (2015) Phylogeny and classification of Cucujoida and the recognition of a new superfAMILY Coccinellioidea (Coleoptera: Cucujiformia). Systematic Entomology, 40, 745–778.

Robertson, J.A., Whiting, M.F. & McHugh, J.V. (2008) Searching for natural lineages within the Cerylonid Series (Coleoptera: Cucujoida). Molecular Phylogenetics and Evolution, 46, 193–205.

Ronquist, F., Teslenko, M., Mark, P.V. et al. (2012) MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. Systematic Biology, 61, 539–542.

Sasaji, H. (1968) Phylogeny of the family Coccinellidae (Coleoptera). Etizenia, Occasional Publications of the Biological Laboratory, Fuku University, 35, 1–37.

Sasaji, H. (2005) Additional revision of the tribe Chilocorini (Coleoptera, Coccinellidae) of Japan, Elytra, Tokyo, 33, 61–68.

Seago, A.E., Giorgi, J.A., Li, J.H. & 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.

Ślipiński, A. & Giorgi, J.A. (2006) Revision of the Australian Coccinel- lidae (Coleoptera). PART 6. Tribe Chilocorini. Annales Zoologici, 56, 265–304.

Ślipiński, A. & Tomaszewska, W. (2010) Coccinellidae Latreille, 1802. Handbook of Zoology, Vol. 2: Coleoptera (ed. by R.A.B. Leschen, R.G. Beutel and J.F. Lawrence), pp. 454–472. Walter de Gruyter GmbH & Co. KG, Berlin/New York, NY.

Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 30, 1312–1313.

Szawaryn, K., Bocak, L., Ślipiński, A., Escalona, H.E. & Tomaszewska, W. (2015) Phylogeny and evolution of phytophagous ladybird beetles (Coleoptera: Coccinellidae: Epilachnini), with recognition of new genera. Systematic Entomology, 40, 547–569.

Timmermanns, M.J.T.N., Dodsworth, S., Culverwell, C.L. et al. (2010) Why barcode? High-throughput multiplex sequencing of mitochondrial genomes for molecular systematics. Nucleic Acids Research, 38, e197.

Ward, P.S. & Downie, D.A. (2005) The ant subfamily Pseudomyrmecineeae (Hymenoptera: Formicidae): phylogeny and evolution of big-eyed arboreal ants. Systematic Entomology, 30, 310–335.

Weise, J. (1895) Neue Coccinelliden, sowie Bemerkungen zu bekannten Arten. Annales de la Société Entomologique de Belgique, 39, 120–146.

Weise, J. (1898) Coccinelliden aus Kamerun. Deutsche Entomologische Zeitschrift, 97–125.

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

Wilgenbusch, J.C., Warren, D.L. & Swofford, D.L. (2004). AWTY: A system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference [WWW document]. URL http://ceb.csit.fsu.edu/awsy [accessed on March 2017].

Zhang, S.Q., Che, L.H., Li, Y., Liang, D., Pang, H., Ślipiński, A. & Zhang, P. (2018) Evolutionary history of Coleoptera revealed by extensive sampling of genes and species. Nature Communications, 9, 205.

Accepted 1 November 2019
First published online 10 December 2019