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A DNA barcode reference library of Neuroptera (Insecta, Neuropterida) from Beijing

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Abstract

Neuroptera (lacewings) is one of the ancient holometabolous insect groups, but some extant species stand as important natural enemies for biological control. As the capital city of China, Beijing has a rich fauna of Neuroptera, previously with 47 species recorded and sorted in 32 genera of seven families. In this study, DNA barcoding based on sequences of COI gene fragments is used to discriminate lacewing species from Beijing. 217 DNA barcode sequences belonging to 49 species were successfully obtained. The COI barcode data worked well for identification of almost all lacewing species herein examined except *Pseudomallada prasinus* (Burmeister), in which cryptic species may exist. Twenty species of Neuroptera are newly recorded from Beijing. Besides, Nothochrysinae is first recorded from Beijing. *Chrysopidia ciliata* (Wesmael) and *Drepanepteryx algida* (Erichson) are first recorded from China.

Keywords

China, cytochrome c oxidase subunit I, mitochondrial DNA, lacewings, taxonomy

Introduction

Neuroptera (lacewings) is the most species-rich order of the superorder Neuropterida. Hitherto, there are about 6000 described species worldwide in 16 families (Engel et al. 2018; Winterton et al. 2018). Adult lacewings in general are delicate insects, having two pairs of membranous wings with highly reticulate venation, while the lacewing

larvae are characterized by the specialized mandibles and maxillae that are combined into a pair of sucking jaws. The common groups of Neuroptera consist of Chrysopidae (green lacewings), Hemerobiidae (brown lacewings), Myrmeleontidae (antlions), and Coniopterygidae (dusty lacewings), while the other lacewing families each comprises much fewer species and some of these families (e.g., Nevrorthidae, Rhachiberothidae, Ithonidae, Psychopsidae) have much narrower distributions. However, the diversification of Neuroptera in morphology as well as in biology is remarkable (Aspöck et al. 2012; Engel et al. 2018).

Because of the predatory feeding habits, some lacewing species, e.g., the species of Chrysopidae, Hemerobiidae, and Coniopterygidae, are economically important and have been used for the biocontrol of agricultural pest insects (Goolsby et al. 2000; McEwen et al. 2001; Sato and Takada 2004; Bezerra et al. 2006; Abdrabou 2008; Vidya et al. 2010; Messelink et al. 2016). However, the species identification of these lacewing groups is not easy to handle, particularly for people who are not the specialists of Neuroptera, because there are many morphologically similar species, which require examination of detail morphological characters, such as marking patterns on body and genitalia. Moreover, for some species-rich groups, such as Chrysopidae, the taxonomy still requires comprehensive revision (Henry and Wells 2010; Henry et al. 2013, 2014; Duelli et al. 2016; Dai et al. 2017).

DNA barcoding has become the most popular approach for the species identification and the assignment of specimens throughout all life stages to described species (Hebert et al. 2003a, b). In animals, including insects, an app. 660 base pair (bp) fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene has been chosen as standardized barcode marker (Hebert et al. 2003a, b). As a molecular marker for efficient species identification, DNA barcoding with COI yields excellent results across a broad spectrum of insects, and even reveals unknown cryptic species diversity of certain groups (Smith et al. 2006; Burns et al. 2008; Huemer et al. 2014; Song et al. 2018). Besides, DNA barcoding based on COI with the Automatic Barcode Gap Discovery and the Bayesian Poisson Tree Processes model was also used to separate some new antlion species (Pantaleoni and Badano 2012; Badano et al. 2016). Notwithstanding, there is still limited number of works on DNA barcoding of Neuroptera (Morales and Freitas 2010; Morinière et al. 2014; Choi et al. 2015; Price et al. 2015).

Beijing, as the capital city of China, is located at northern China and surrounded by Hebei Province, belonging to the eastern Palaearctic region. To the west of Beijing is Mt. Xishan, forming the eastern flank of the Taihang Mountains range, which runs north-south up the spine of Hebei province. Mt. Xishan covers nearly all of Fangshan and Mentougou Districts west of the city. The mountains north of Beijing including Mt. Wulingshan, Mt. Jundushan, and Mt. Fenghuanling all belong to the Yanshan range, which runs east-west, across northern Hebei Province. Climate of Beijing is typical humid continental monsoon climate with hot and rainy summers, cold and dry winters. The majority flora of Beijing is temperate deciduous forest. Despite high-speed increase of economic development and population, relatively well-

preserved natural environment still remains in Beijing, particularly in the aforementioned mountainous areas.

Concerning Neuroptera, Beijing has relatively rich fauna of lacewing species, currently with 47 species recorded based on the recently published catalogue of the Chinese Neuropterida (Oswald 2018; Yang et al. 2018). Remarkably, the lacewing fauna of Beijing appears still not to be thoroughly explored considering recent findings of new species from this area (Zhao et al. 2013; Zhang et al. 2014).

Here we present a preliminary DNA barcode library for the lacewing species from Beijing. A total of 217 barcode sequences were amplified, and this dataset comprises the barcodes of 49 species (including seven undetermined species). Twenty species are newly recorded from Beijing, and two of them are first recorded from China (Figures 2–4; Suppl. material 1–3: Figures S1–3). An updated checklist of species of Neuroptera from Beijing is provided (Suppl. material 6: File S2).

Material and methods

Sampling of specimens

The lacewing specimens herein studied were collected between 2013 and 2017 using sweeping net and light trap. The collecting areas mainly comprise the Xiaolongmen Forestry Park, Mentougou District, northwestern Beijing, the Wulingshan National Nature Reserve that is located across Miyun District in northeastern Beijing and Xinglong County in Hebei Province, an organic orchard in Wangjiayuan Village, Changping District, northern Beijing, and the Olympic Forest Park, Chaoyang District in the metropolitan area of Beijing. The specimens were preserved in ethanol (95%) and identified based on the morphological characteristics using the keys to the species (Aspöck et al. 1980; Liu 2003; Yang et al. 2005; Zhao 2016; Wang et al. 2018). The number of specimens per species ranged from 1 to 26. All specimens herein studied are deposited in the Entomological Museum of China Agricultural University (CAU), Beijing, China.

DNA extraction

Total genomic DNA was isolated from mid legs using the TIANamp Genomic DNA Kit (TIANGEN Inc., Beijing, China) according to the manufacturer's instructions. The barcoding fragments of COI were amplified by Polymerase chain reactions (PCR). The reaction was conducted in a final volume of 25 μL consisting of 14.5 μL of ddH_2O, 1 μL (10 μM) of each of the primers, 2 μL of dNTP, 0.5 μL of polymerase and 1 μL DNA template (~30 ng). For Chrysopidae, the COI gene fragments were amplified with specific primers, i.e., COIa–F (5'–TACAATTTATCGCCTAAACTTCAGCC–3') and COIa–R (5'–CCCGGTAAAATTAAAATATAAACTTC–3') because the univer-

sal primers (i.e., LCO1490 and HCO2198; see Folmer et al. 1994) did not work well for this group in our study. For the other groups, the COI gene fragments were amplified with the aforementioned universal primers, i.e., LCO1490 (5'–GGTCAACAAATCATAAAGATATTGG–3') and HCO2198 (5'–TAAACTTCAGGGTGACCAAAAAATCA–3'). The PCR amplifications were run under the following conditions: initial denaturation at 95 °C for a half minute, followed by 40 cycles of 10 seconds at 95 °C, 50 seconds at 47 °C, and 2 minutes at 65 °C; a final extension phase of 65 °C for 10 minutes. The PCR products were subjected to electrophoresis in 1% agarose gel and stained with GoldView (1ng/mL) to confirm amplification. Amplicons were sequenced bidirectionally, using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on an ABI 3730XL Genetic Analyzer (PE Applied Biosystems, San Francisco, California, USA).

Data analysis

The final consensus COI sequences were obtained after overlapping both forward and reverse sequences by ContigExpress. All sequence data are deposited in GenBank (see Accession number in File S1). All sequences were aligned using Clustal W (Thompson et al. 1994) and analyzed using a neighbor-joining cluster analysis (NJ; Saitou and Nei. 1987) based on the Kimura-2-Parameter (K2P; Kimura 1980) distances with MEGA v. 5.0 (Tamura et al. 2011). The consequence of NJ tree was explored the Newick tree file and subsequently modified with FigTree v1.4.3. (http://tree.bio.ed.ac.uk/software/figtree/, Andrew 2006). Nucleotide composition and the K2P distances between and within species were also calculated by MEGA v. 5.0. Additional species-delimitation methods were also included in our study, i.e., the Automatic Barcode Gap Discovery (ABGD; Puillandre et al. 2012) and the Bayesian Poisson Tree Processes model (bPTP; Zhang et al. 2013). ABGD is an automatic procedure that sorts the sequences into hypothetical species based on the threshold of pairwise genetic distances. The ABGD analyses were performed on the web interface (http://wwwabi.snv.jussieu.fr/public/abgd/). The K2P distance was selected for the datasets, and other parameters were set to default except the default values of steps=50 and relative gap width (X)=0.5. bPTP is an updated version of the original PTP with bayesian posterior probability, providing more accurate results, maximal likelihood solution and bayesian supported solution, for species delimitation i.e., bPTP_ML and bPTP_BS. For the bPTP analyses, the ML trees were constructed using RAxML v8.2.10 under the GTRGAMMA evolutionary model and performed on the bPTP web server (http://species.h-its.org/), with 0.25 burn-in and 500,000 MCMC generations. To test the reliability of results, each run was checked for convergence by visualizing the likelihood plot. The COI sequence of *Lepicerus inaequalis* (Coleoptera: Lepiceridae; GenBank: KJ871320) and *Nebria formosana* (Coleoptera: Carabidae; GenBank: KT306091) were selected as outgroups because of the close relationship between Coleoptera and Neuropterida (Misof et al. 2014).

Results

The present study generated 217 sequences of 639 bp each, with an average nucleotide composition of 39.5% thymine (T), 15.8% cytosine (C), 28.4% adenine (A), and 16.3% guanine (G). Base frequencies analysis revealed low GC-contents (average: 31.1%) for the barcode fragment. The above COI barcode sequences were found to belong to 49 species of Neuroptera. A full list of these species and their collecting information are presented in the Suppl. material 5: File S1. A threshold of the COI genetic distance ≥ 2% was applied for a rough differentiation between intraspecific and interspecific distances based on Hebert et al. (2003b). Intraspecific distances ranged from zero to 2.7% (see *Pseudomallada prasinus* (Burmeister, 1839); Suppl. material 8: Table S2. Interspecific distances ranged between 2.9% (see species of *Pseudomallada*) and 25.3% (see *Semidalis aleyrodiformis* (Stephens, 1836) and *Coniopteryx plagiotropa* Liu & Yang, 1997; Suppl. material 7: Table S1). The number of recovered clusters (= 49), each of which can be clearly separated from all neighboring species (Figure 1), is identical to the number of species identified based on morphological characters, suggesting that the species in question can be identified unambiguously by DNA barcoding.

Coniopterygidae

Seven species of Coniopterygidae from Beijing were studied, including two species newly recorded from Beijing, i.e., *Conwentzia sinica* Yang, 1974 and *Semidalis bicornis* Liu & Yang, 1993, and two undetermined species of *Coniopteryx* with a minimum mean distance 10.9% (Suppl. material 7: Table S1). *Semidalis aleyrodiformis* and *Coniopteryx plagiotropa* possess a maximum mean distance 23.3%. Results of species delimitation based on ABGD and bPTP_ML are congruent with our identification based on morphology (Figure 5A). However, bPTP_BS divided *Semidalis aleyrdiformis* into five Molecular Operational Taxonomic Units (MOTUs; n = 5) with low posterial probabilities (< 60%). It is probably overestimated because the intraspecific variation within the specimens of *Semidalis aleyrodiformis* is 0.

Chrysopidae

The present analysis resulted in 18 species of Chrysopidae from Beijing. Three of them could not be identified to species. Among them, there are 10 species newly recorded from Beijing, including *Chrysopa intima* McLachlan, 1893, *Chrysoperla furcifera* (Okamoto, 1914), *Chrysopidia ciliata* (Wesmael, 1841), *Mallada flavimaculus* Yang & Yang, 1991, *Pseudomallada cognatellus* (Okamoto, 1914), *Pseudomallada prasinus* (Burmeister, 1839), *Pseudomallada qinlingensis* (Yang & Yang, 1989), *Nineta grandis* Navás, 1915, *Nineta shaanxiensis* Yang & Yang, 1989 and *Nothochrysa sinica* Yang, 1986. Fur-

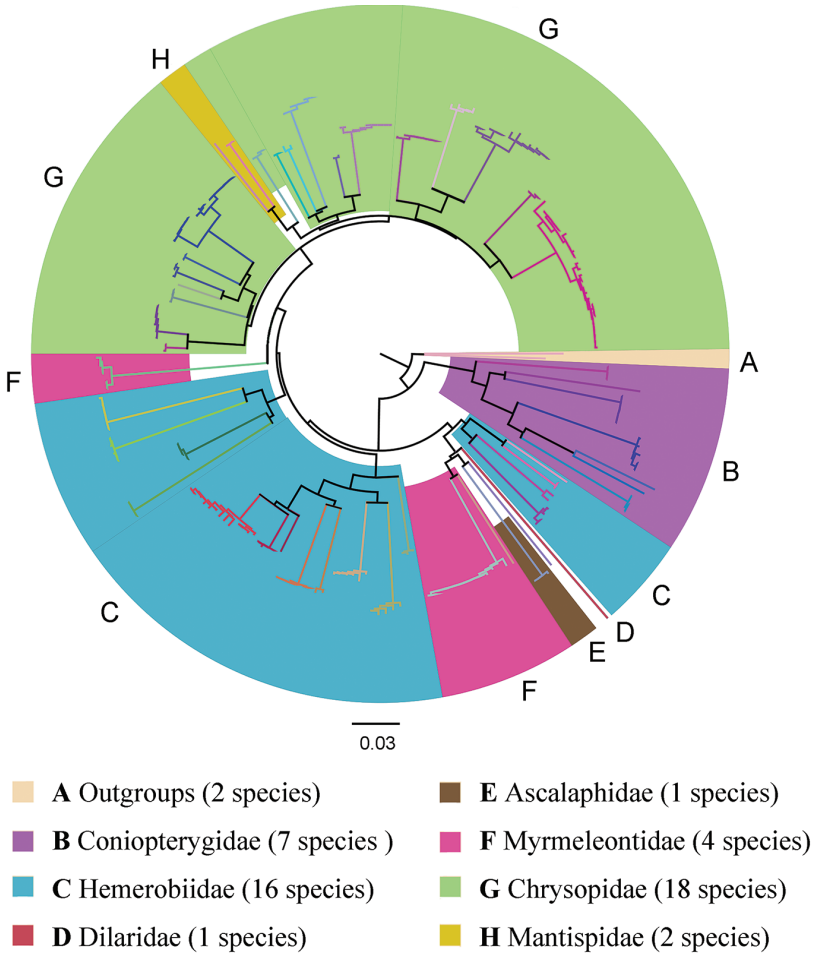

Figure 1. Neighbor-joining tree based on the COI sequence dataset of the lacewing species from Beijing. Different color of clades represents different species.

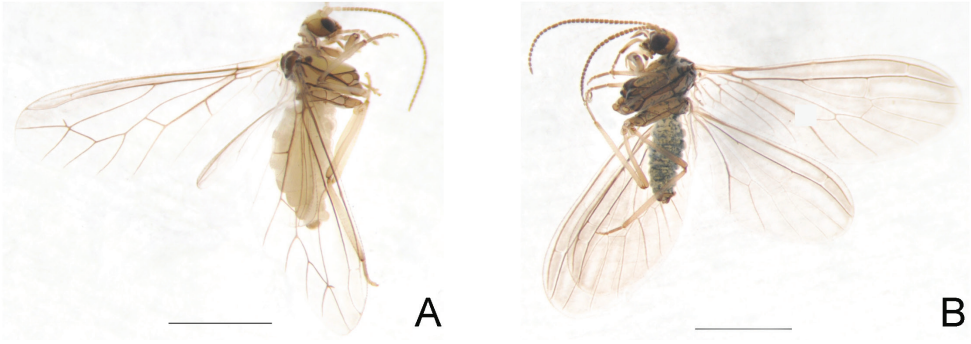

Figure 2. Habitus photographs of species of Coniopterygidae newly recorded from Beijing. **A** *Conwentzia sinica* Yang, 1974 **B** *Semidalis bicornis* Liu & Yang, 1993. Scale bar: 1 mm.

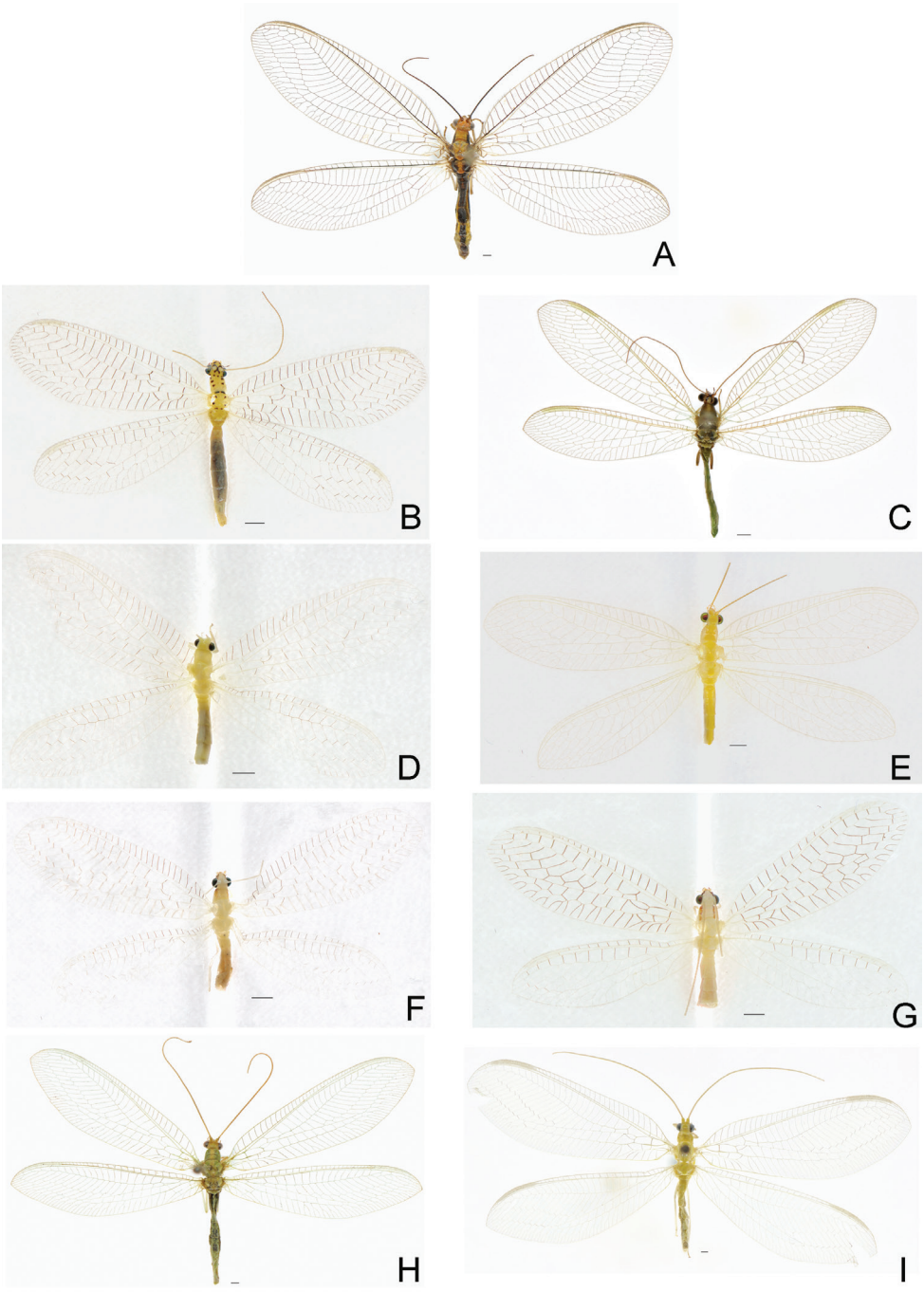

Figure 3. Habitus photographs of species of Chrysopidae newly recorded from Beijing. **A** *Nothochrysa sinica* Yang, 1986 **B** *Chrysopa intima* McLachlan, 1893 **C** *Chrysoperla furcifera* (Okamoto, 1914) **D** *Chrysopidia ciliata* (Wesmael, 1841) **E** *Mallada flavimaculus* Yang & Yang, 1991 **F** *Pseudomallada cognatellus* (Okamoto, 1914) **G** *Pseudomallada qinlingensis* (Yang & Yang, 1989) **H** *Nineta grandis* Navás, 1915 **I** *Nineta shaanxiensis* Yang & Yang, 1989. Scale bar: 1 mm.

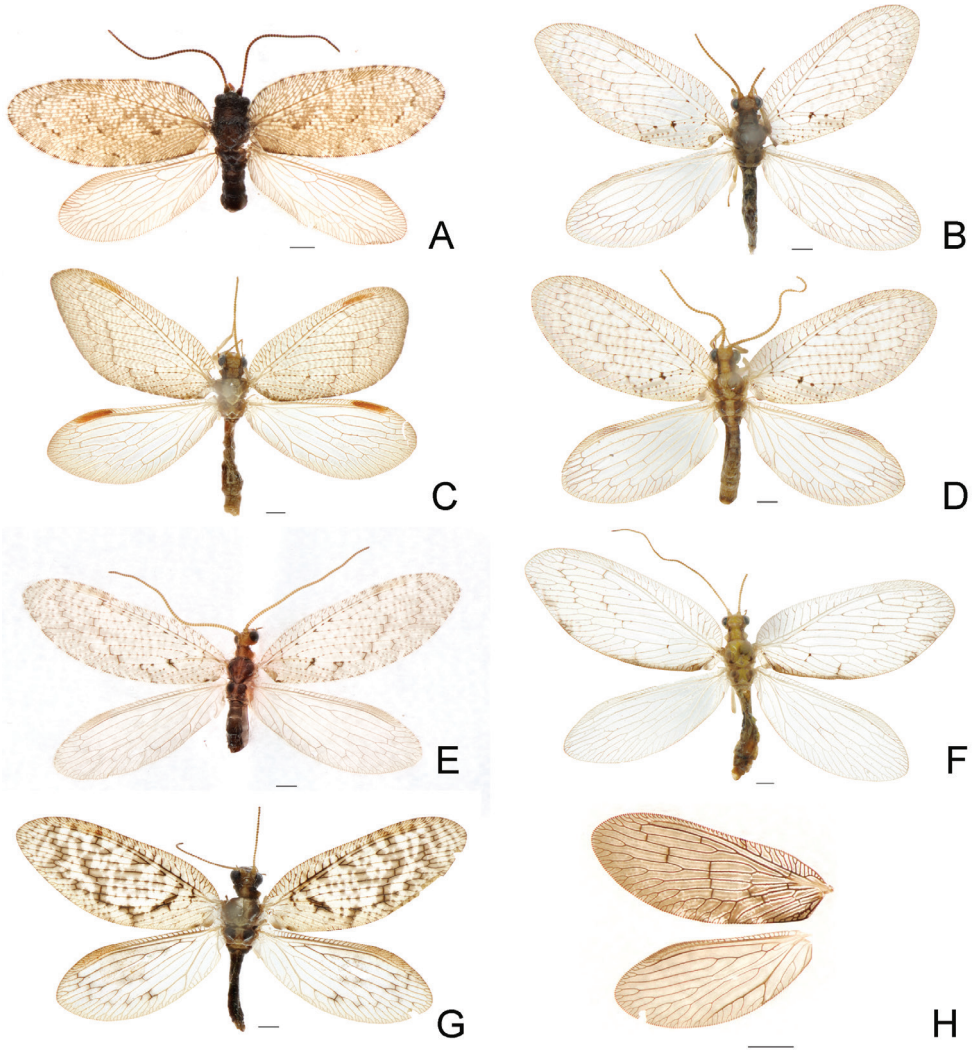

Figure 4. Habitus photographs of species of Hemerobiidae newly recorded from Beijing. **A** *Drepanepteryx algida* (Erichson, 1851) **B** *Hemerobius bispinus* Banks, 1940 **C** *Hemerobius exoterus* Navás, 1936 **D** *Hemerobius humulinus* Linnaeus, 1758 **E** *Hemerobius japonicus* Nakahara, 1915 **F** *Hemerobius marginatus* (Stephens, 1836) **G** *Hemerobius subtriangulus* Yang, 1987 **H** *Sympherobius manchuricus* Nakahara, 1960. Scale bar: 1 mm.

thermore, *Nothochrysa sinica* represents the first record of the subfamily Nothochrysinae from Beijing, while *Chrysopidia ciliata* is first recorded from China.

For testing the present identification, we also compare the barcode sequences of several green lacewing species [i.e., BINS: ACF7085 (*Chrysopa formosa*); AAB0373 (*Chrysoperla nipponensis*); AAJ3493 (*Chrysopidia ciliata*); ABU9179, ACF9046 (*Pseudomallada prasinus*); GenBank: KJ592516 (*Chrysopa pallens*)] obtained from the Barcoding of Life Data systems (BOLD, http://www.barcodinglife.org/) and the National

Center Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/) by using a neighbor-joining cluster analysis based on the K2P distances with MEGA v. 5.0. Most of these sequences were respectively clustered with those of same species herein sequenced, verifying our identification (Suppl. material 4: Figure S4). However, in *P. prasinus* specimens from Europe and from Beijing are clearly assigned into two clades. A similar result concerning *P. prasinus* from Europe and East Asia was also found in a phylogenetic analysis of *Pseudomallada* combining morphology, life-history traits, and nuclear DNA sequences (Duelli et al. 2017).

Among the green lacewing species herein studied, the bPTP_ML and bPTP_BS analyse resulted in 21 and 20 MOTUs, respectively (Figure 5B). Notably, bPTP_ML divided *P. cognatellus* (Okamoto, 1914) into two MOTUs (n=3) while the intraspecific distance is 0. Both solutions of the bPTP divided *Chrysopa pallens* (Rambur, 1838) into two MOTUs (n=12), but the intraspecific divergence is relatively lower (1.1%). Furthermore, the bPTP species delimitation sorted *P. prasinus* into two MOTUs (i.e., types A and B). Meanwhile, *P. prasinus* of high intraspecific divergence (2.7%) was detected using K2P distance analysis (Suppl. material 8: Table S2). We carefully differentiated the morphological characters between these two types, and we found difference of color patterns on every segment of maxillary and labial palps. Those palps in type A are almost entirely black except for joints that are yellow, but in type B they are largely yellow except for the terminal segments and several joints that are black. Besides, the number of blackish markings on pronotum is different between types A and B. Type A has only one pair of blackish markings on the middle of pronotum, while type B possesses three pairs of additional blackish markings on the lateral margins of pronotum beside the medial pair of markings. Moreover, the apex of male sternum 9 in type A is narrowed distad, while in type B it is broader and subquadrate in lateral view. Nevertheless, no morphological difference was detected concerning the shape of the complex of gonocoxites, gonapophyses, and gonostyli 9 as well as the gonocoxites 10 (Figure 6). Thus, cryptic species may exist in *P. prasinus*, as mentioned in Duelli et al. (2017). The ABGD analysis resulted in 17 MOTUs, within which two species (i.e., *Pseudomallada* sp. 2 and sp. 3) were assigned into a same species.

Hemerobiidae

The study resulted in 16 species of Hemerobiidae from Beijing although two species of them are undetermined. Eight species are newly recorded from Beijing, i.e., *Drepanepteryx algida* (Erichson, 1851), *Hemerobius bispinus* Banks, 1940, *Hemerobius exoterus* Navás, 1936, *Hemerobius humulinus* Linnaeus, 1758, *Hemerobius japonicus* Nakahara, 1915, *Hemerobius marginatus* (Stephens, 1836), *Hemerobius subtriangulus* Yang, 1987 and *Sympherobius manchuricus* Nakahara, 1960. Seven species of them, except *D. algida*, were recorded from Beijing in an unpublished doctoral thesis (Zhao 2016) but not listed with their distribution from Beijing in Yang et al. (2018). *Drepanepteryx algida* is also first recorded from China.

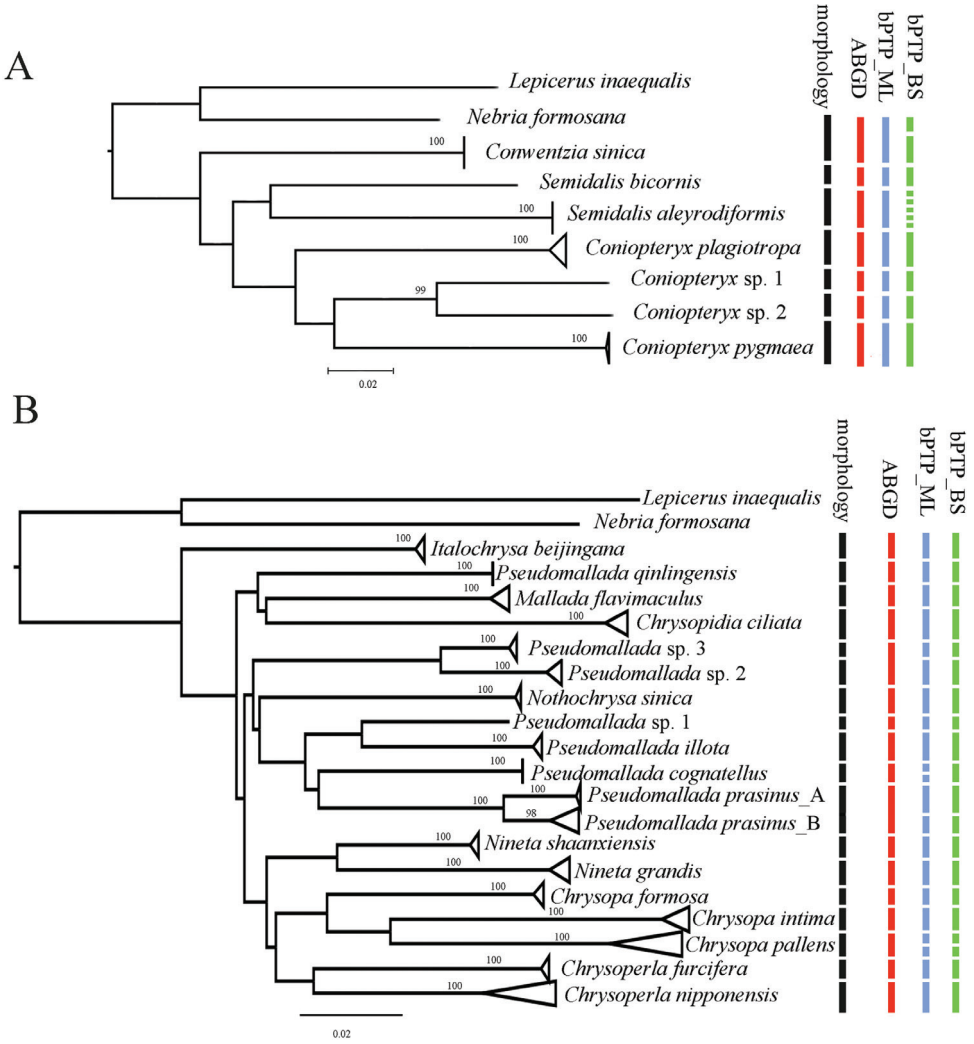

Figure 5. Neighbor-joining tree and result of molecular species delimitation based on COI barcodes. **A** Coniopterygidae **B** Chrysopidae **C** Hemerobiidae **D** Myrmeleontidae and Ascalaphidae. The terminal nodes in the tree are collapsed for each morphological species, the width of triangles shows the sequence divergence. Only bootstrap supports (1,000 replicates) > 0.95 are labelled.

Hemerobius humulinus and *Hemerobius japonicus* possess a minimum mean interspecific distance 5.3% (Suppl. material 9: Table S3). Meanwhile, *Hemerobius japonicus* and *Neuronema* sp. 1 demonstrate a maximum mean interspecific distance 21.8%. Results of species delimitation based on ABGD and bPTP_ML are consistent with our identification based on morphology. But in bPTP_BS analysis, all samples were sorted into 17 MOTUs, because *Neuronema* sp. 1 was divided into two MOTUs (n = 3; Figure 5C) even without any intraspecific divergence.

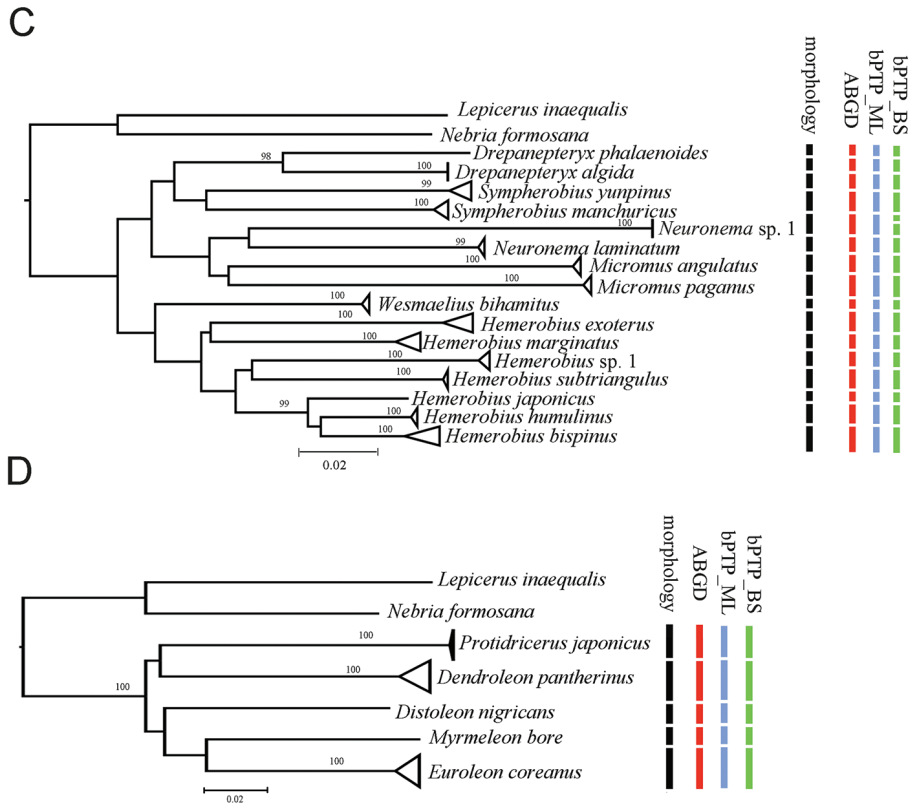

Figure 5. Continued.

Mantispidae and Dilaridae

The study obtained COI barcodes from two species of Mantispidae, i.e., *Eumantispa harmandi* (Navás, 1909) and *Mantispa styriaca* (Poda, 1761), and from one species of Dilaridae (*Dilar hastatus* Zhang, Liu, H. Aspöck & U. Aspöck, 2014; Wang et al. 2017).

Myrmeleontidae and Ascalaphidae

Four species of Myrmeleontidae, i.e., *Dendroleon pantherinus* (Fabricius, 1787), *Distoleon nigricans* (Matsumura, 1905), *Euroleon coreanus* (Okamoto, 1926) and *Myrmeleon bore* (Tjeder, 1941) and one species of Ascalaphidae [*Protidricerus japonicus* (McLachlan, 1891)] from Beijing were studied. The consequence of two species delimitation methods is consistent with our identification based on morphology.

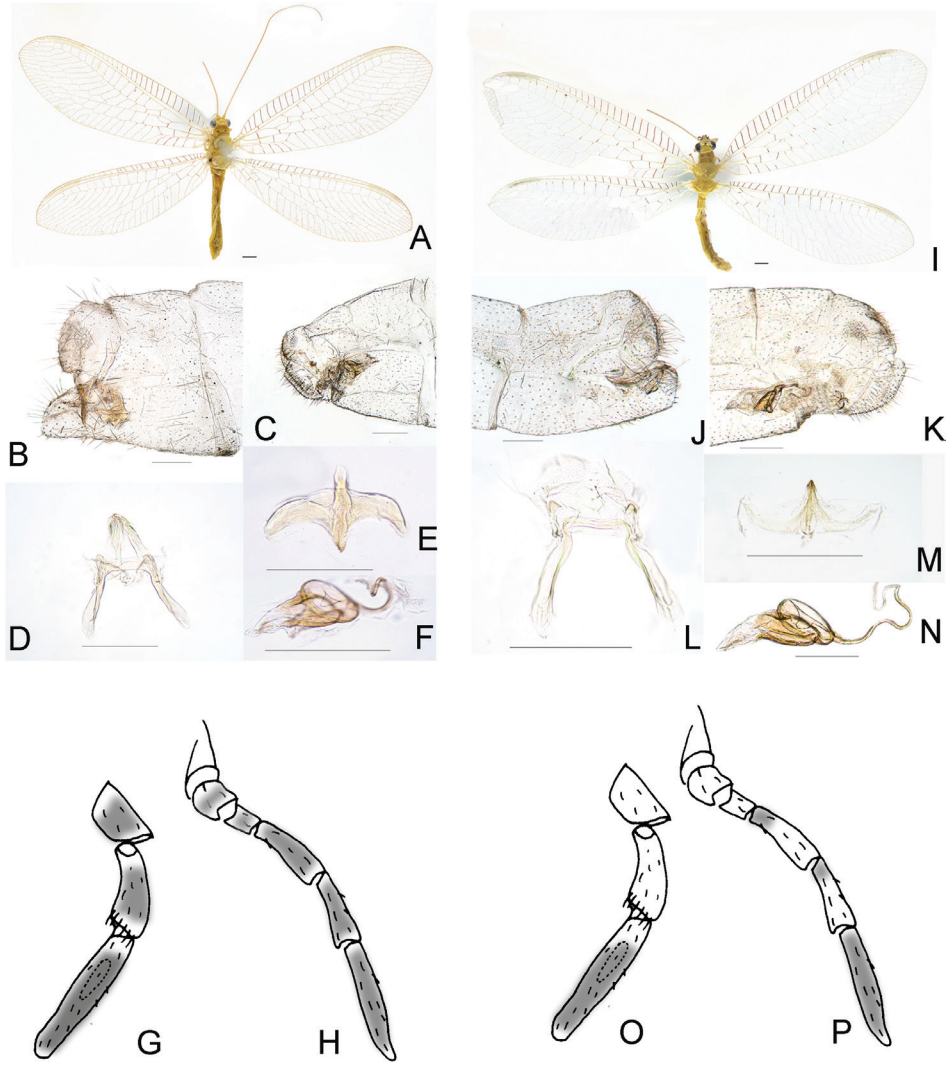

Figure 6. Photographs of habitus and genitalia of *Pseudomallada prasinus* (Burmeister, 1839). Type **A** (**A–H**); type **B** (**I–P**); photographs of habitus (**A, I**); apex of abdomen in male (**B, J**); apex of abdomen in female (**C, K**); the complex of gonocoxites, gonapophyses and gonostyli 9, dorsal view (**D, L**); gonocoxites 10, dorsal view (**E, M**); spermatheca, lateral view (**F, N**); labial palps (**G, O**); maxillary palps (**H, P**). Scale bar: 1mm (**A, I**); 0.25 mm (**B–F, J–N**).

Discussion

Within the past few years, DNA sequence-based approaches have become more and more popular for the assessment of biodiversity and identification of species, in particular where the traditional morphology-based identification is hard to apply (Taberlet et al. 2012). However, COI gene is known to be affected by several biases and is considered to better utilized in combination with, at least, other independent genes, but also with morphological, geographical or ecological data to clearly

delimit species (Will et al. 2005; Ahrens et al. 2007; Padial et al. 2010; Hajibabaei et al. 2011).

The present DNA barcode library of Neuroptera from Beijing stands an important step not only for the molecular identification of lacewing species from Beijing but also for the future construction of DNA barcode database of Neuroptera from China. In light of obvious gap between intraspecific and interspecific genetic distance, the present COI barcode data allow unambiguous identification of almost all lacewing species from Beijing herein examined. Nevertheless, it should be noted that some other methods we tested for species delimitation (i.e., ABGD and bPTP) based on present barcode data may result in some problematic identification (see above results on *Semidalis aleyrodiformis*, *Pseudomallada* spp., and *Neuronema* sp. 1)

According to the updated catalogue of Neuroptera from China (Yang et al. 2018), 7 families, 12 subfamilies, 32 genera, and 47 species were recorded from Beijing. Here, Neuroptera from Beijing are composed of 7 families, 13 subfamilies, 37 genera, and 67 species (Suppl. material 6: File S2, excluding unidentified species).

Beijing is located at the eastern Palaearctic region. Among the 67 lacewing species from Beijing, 30 species (44.8% of total species) are distributed only from the Palaearctic region, while the remaining 37 species (55.2% of total species) occur in both Palaearctic and Oriental regions. The species of Chrysopidae and Hemerobiidae account for a great proportion (38.2% and 34.0% respectively) of Neuroptera in this study. They also represent substantial species numbers based on the checklist of Neuroptera from Beijing (28.4% and 25.4% respectively). Due to lack of specimens, species of Aleuropteryginae and many tribes of Myrmeleontidae were not studied here, but will be supplemented in our dataset in near future.

Conclusions

Our study provided the first DNA barcode library of Neuroptera from Beijing, including 49 species (73% of all lacewing species recorded in Beijing). It is clearly indicated that the use of DNA barcodes for the identification of lacewing species is promising. The present dataset will be the first step toward the DNA barcoding of Chinese Neuroptera. It is also useful for the identification of immature stages and/or females of the lacewing species from Beijing. In future study, the DNA barcoding could be applied for comparison and assessment of lacewing species diversity and its dynamic change among different types of ecosystems and regions in Beijing for understanding the effect of urbanization on this important insect group.

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Supplementary material 1

Figure S1. Photographs of male genitalia of species of Coniopterygidae newly recorded from Beijing

Authors: Pan Yi, Pei Yu, Jingyi Liu, Huan Xu, Xingyue Liu

Data type: multimedia

Explanation note: A. *Conwentzia sinica* Yang, 1974; B. *Semidalis bicornis* Liu & Yang, 1993. Scale bar 0.5 mm.

Link: https://doi.org/10.3897/zookeys.807.29430.suppl1

Supplementary material 2

Figure S2. Photographs of male genitalia of species of Chrysopidae newly recorded from Beijing

Authors: Pan Yi, Pei Yu, Jingyi Liu, Huan Xu, Xingyue Liu

Data type: multimedia

Explanation note: A. *Nothochrysa sinica* Yang, 1986; B. *Chrysopa intima* McLachlan, 1893; C. *Chrysoperla furcifera* (Okamoto, 1914); D. *Chrysopidia ciliata* (Wesmael, 1841); E. *Pseudomallada cognatellus* (Okamoto, 1914); F. *Pseudomallada qinlingensis* (Yang & Yang, 1989); G. *Nineta grandis* Navás, 1915. Scale bar: 0.5 mm.

Link: https://doi.org/10.3897/zookeys.807.29430.suppl2

Supplementary material 3

Figure S3. Photographs of male genitalia of species of Hemerobiidae newly recorded from Beijing

Authors: Pan Yi, Pei Yu, Jingyi Liu, Huan Xu, Xingyue Liu

Data type: multimedia

Explanation note: A. *Drepanepteryx algida* (Erichson, 1851); B. *Hemerobius bispinus* Banks, 1940; C. *Hemerobius exoterus* Navás, 1936; D. *Hemerobius humulinus* Linnaeus, 1758; E. *Hemerobius japonicus* Nakahara, 1915; F. *Hemerobius marginatus* (Stephens, 1836); G. *Hemerobius subtriangulus* Yang, 1987; H. *Sympherobius manchuricus* Nakahara, 1960. Scale bar: 0.5 mm.

Link: https://doi.org/10.3897/zookeys.807.29430.suppl3

Supplementary material 4

Figure S4. Neighbor-joining tree based on the COI sequence dataset of Chrysopidae
Authors: Pan Yi, Pei Yu, Jingyi Liu, Huan Xu, Xingyue Liu
Data type: phylogenetic tree
Explanation note: Neighbor-joining tree based on the COI sequence dataset of Chrysopidae. Only bootstrap supports (1,000 replicates) > 0.95 are labelled.

Link: https://doi.org/10.3897/zookeys.807.29430.suppl4

Supplementary material 5

File S1. List of all specimens used in this study, including GenBank accession numbers
Authors: Pan Yi, Pei Yu, Jingyi Liu, Huan Xu, Xingyue Liu
Data type: molecular data

Link: https://doi.org/10.3897/zookeys.807.29430.suppl5

Supplementary material 6

File S2. Checklist of the species of Neuroptera from Beijing
Authors: Pan Yi, Pei Yu, Jingyi Liu, Huan Xu, Xingyue Liu
Data type: species data

Link: https://doi.org/10.3897/zookeys.807.29430.suppl6

Supplementary material 7

Table S1. Intraspecific and interspecific divergence of Coniopterygidae based on COI barcode sequences (%)

Authors: Pan Yi, Pei Yu, Jingyi Liu, Huan Xu, Xingyue Liu

Data type: molecular data

Explanation note: The range of interspecific distance = means interspecific distance ± standard error. N/A indicates intraspecific distance not available because only one specimen was sequenced.

Link: https://doi.org/10.3897/zookeys.807.29430.suppl7

Supplementary material 8

Table S2. Intraspecific and interspecific divergence of Chrysopidae based on COI barcode sequences (%)

Authors: Pan Yi, Pei Yu, Jingyi Liu, Huan Xu, Xingyue Liu

Data type: molecualr data

Explanation note: The range of interspecific distance = mean interspecific distance ± standard error. N/A indicates intraspecific distance not available because only one specimen was sequenced.

Link: https://doi.org/10.3897/zookeys.807.29430.suppl8

Supplementary material 9

Table S3. Intraspecific and interspecific divergence of Hemerobiidae based on COI barcode sequences (%)

Authors: Pan Yi, Pei Yu, Jingyi Liu, Huan Xu, Xingyue Liu

Data type: molecular data

Explanation note: Tthe range of interspecific distance = mean interspecific distance ± standard error. N/A indicates intraspecific distance not available because only one specimen was sequenced.

Link: https://doi.org/10.3897/zookeys.807.29430.suppl9

Supplementary material 10

Table S4. Intraspecific and interspecific divergence of Myrmeleontidae and Ascalaphidae based on COI barcode sequences (%)

Authors: Pan Yi, Pei Yu, Jingyi Liu, Huan Xu, Xingyue Liu

Data type: molecular data

Explanation note: The range of interspecific distance = mean interspecific distance ± standard error. N/A indicates intraspecific distance not available because only one specimen was sequenced.

Link: https://doi.org/10.3897/zookeys.807.29430.suppl10