Molecular and morphological evidence for the identity of two nominal species of Astegopteryx (Hemiptera, Aphididae, Hormaphidinae)

Qiang Li¹, Jiamin Yao¹, Lingda Zeng¹, Xiaolan Lin¹, Xiaolei Huang¹

¹ State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou 350002, China

Corresponding author: Xiaolei Huang (huangxl@fafu.edu.cn)

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Abstract

The morphology of many insect species is usually influenced by environmental factors and therefore high phenotypic variation exists even within a species. This causes difficulty and uncertainty in species taxonomy, which can be remedied by using molecular data and integrative taxonomy. Astegopteryx bambusae and A. bambucifoliae are currently regarded as two closely related aphid species with similar bamboo hosts and overlapping distributions in the oriental region. However, in practice it is hard to distinguish between them. By incorporating molecular data from four mitochondrial and nuclear genes as well as morphological information from an extensive collection of live specimens, the present study indicates that A. bambucifoliae is a junior synonym of A. bambusae. The data also indicate that large-scale geographic patterns of population differentiation may exist within this species.

Keywords

DNA barcoding; Hormaphidinae; integrative taxonomy; species delimitation

Introduction

For many insect groups, morphology is influenced by environmental factors. For example, aphids are a plant-feeding group with extremely high phenotypic plasticity across space and time, which can be influenced by different factors such as host plant (Wool
and Hales 1997; Margaritopoulos et al. 2000), associated ant species (Yao 2012), climate and temperature (Blackman and Eastop 1994), as well as geography (Madjdzadeh and Mehrparvar 2009). In traditional insect taxonomy, species identification depends heavily on specimen morphology, and many species are first described based on only a small number of samples (Winston 1999; Eastop and Blackman 2005). However, for species with high intraspecific morphological variation, small samples from restricted areas and times cannot represent the complete range of morphological variation. This can cause difficulty and uncertainty in species delimitation, so that synonymies inevitably occur in taxonomy (Eastop and Blackman 2005; Meier 2017). Fortunately, new types of data yielded by new technologies such as DNA barcoding (Hebert et al. 2003; Foottit et al. 2008) and integrative taxonomic practices (Schlick-Steiner et al. 2010) can help to solve these problems and improve the quality and efficiency of taxonomy (Turčinavičienė and Rakauskas 2009; Jensen et al. 2010; Heethoff et al. 2011).

The genus *Astegopteryx* is an oriental aphid group with more than twenty species, and is the largest genus in the tribe Cerataphidini (Hemiptera, Aphididae, Hormaphidinae) (Blackman and Eastop 2018; Favret 2018). Some species of *Astegopteryx* have host alternation between their primary host plants, *Styrax* (Styracaceae) trees, on which they form multiple-cavity galls, and secondary host plants, mainly bamboos and palms (Kurosu and Aoki 1991; Aoki and Kurosu 2010; Huang et al. 2012; Blackman and Eastop 2018). However, many species can live exclusively on their secondary host plants with parthenogenetic reproduction (Blackman and Eastop 2018) and display variable morphology (Noordam 1991; Stern et al. 1997). In the taxonomic history of this genus, due to morphological variation between generations on different host plants (e.g. primary and secondary hosts) and even within generations (Aoki and Kurosu 2010), as well as species description on the basis of limited sampling, many synonyms have been created (Blackman and Eastop 1994; Favret 2018). Two currently valid species, *A. bambusae* (Buckton, 1893) and *A. bambucifoliae* (Takahashi, 1921), occur simultaneously on similar bamboo hosts and have overlapping distributions in the oriental region (Noordam 1991; Blackman and Eastop 1994; Qiao et al. 2018). These species have been distinguished mainly by differences in color and appearance in life, as well as some differences in morphology of antennae and wax glands in mounted specimens (Blackman and Eastop 1994). *Astegopteryx bambusae* was originally described as *Oregma bambusae* by Buckton (1893) based on samples on *Bambusa arundinacea* in Dehra Dun, India, with the erection of the genus *Oregma*, now a junior synonym of *Astegopteryx* (Buckton 1893; Blackman and Eastop 2018). The original description of the oval-shaped apterous viviparous female was obscure and simple when judged by today’s criteria. Moreover, the description as “color greenish brown, more or less mottled with black” in Buckton (1893) may have been based on dead specimens (Blackman and Eastop 2018). Takahashi (1921) originally described *A. bambucifoliae* (as *Oregma bambucifoliae*) attacking *Bambusa* spp. in Taiwan Island, with yellowish or fresh green body and a distinct character, “a pair of longitudinal dark green patches on the dorsum, which are often interrupted at mid-length” (Takahashi 1921). Later other morphological characters observed in mounted specimens such as the morphology of the wax glands were introduced to distinguish these two species.
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(Noordam 1991; Qiao et al. 2018). For example, in the key to species of Astegopteryx of Qiao et al. (2018), wax cells tightly connected or not, and wax cells discernible or not, were used to separate these two species. However, in practice it is still hard to distinguish them due to overlap of morphological characters of different populations. We also observed many times in the field that the occurrence of wax and dark green patches varied across populations in both *A. bambusae* and *A. bambucifoliae*. This indicates that the stability of proposed morphological diagnostic characters for these two species with similar habitats and times of occurrence is uncertain (Blackman and Eastop 2018), leading to doubts about their validity. Further detailed study including wider sampling is necessary to understand more about the morphological variation in both species, and molecular data analysis is crucial to clarify any distinction between them. In addition, considering that the mounting process of aphid slides may discard some useful morphological information, we think that the appearance of live specimens is helpful to understand morphological variation within or between species.

In the present study, based on an extensive sampling effort in subtropical China as well as molecular data from four mitochondrial and nuclear gene markers (cytochrome c oxidase subunit I, COI; cytochrome b, Cytb; tRNA/COII; elongation factor-1α, EF-1α), we aimed to show the spatial and temporal morphological diversity of both species, and test the validity of the two species by integrating the molecular and morphological data.

**Materials and methods**

**Sampling**

We did extensive field collections in subtropical China (including Fujian, Guangdong, Hainan, Guangxi, Yunnan provinces, ca. 18°15’–27°19’N, 100°15’–120°12’E) from 2015 to 2017. During the field work, photographs of live individuals were taken for all samples using a digital camera (Cannon EOS 7D plus Canon EF 100mm f/2.8L Macro IS USM Lens). Collected specimens were preserved in 95% ethanol and stored at -20 °C for further molecular experiments. The voucher specimens were stored at the Fujian Agriculture and Forestry University. For the final analyses, 37 specimens were chosen to represent the diversity of geography and time as clearly as possible. In accordance with the original descriptions of the two nominal species (Buckton 1893; Takahashi 1921) and other references (Noordam 1991; Blackman and Eastop 2018; Qiao et al. 2018), sixteen samples with an obvious pair of longitudinal dark green patches on the dorsum and relatively narrower body shape were tentatively identified as *A. bambucifoliae*, while 21 samples with relatively broader pear-shaped body and more wax were tentatively determined as *A. bambusae*. Based on current knowledge about the species relationships among this genus and related groups from previous literature (Aoki and Kurosu 1995; Stern et al. 1997; Blackman and Eastop 2018), two specimens of the closely-related but distinct species *A. formosana* were used as outgroups for phylogenetic tree reconstruction. Detailed specimen information including host plant, collection locality, voucher number, and GenBank accession number are shown in Table 1.
### Table 1. Samples used in this study, with collection information and GenBank accession numbers.

| Species (putative designation) | Host plant | Location | Voucher number | Accession number |
|-------------------------------|------------|----------|----------------|------------------|
| *Astegopteryx bambucifoliae*  | bamboo     | Fujian, Fuzhou | HL20160326_4  | MH821567         |
|                              | bamboo     | Fujian, Fuzhou | HL20160326_5  | MH821568         |
| *A. bambusae*                | bamboo     | Fujian, Fuzhou | HL20160409_11 | MH821537         |
|                              | bamboo     | Fujian, Fuzhou | HL20160417_7  | MH821538         |
| *A. bambucifoliae*           | bamboo     | Fujian, Fuzhou | HL20160512_1  | MH821539 MK028307 MK028325 MK372350 |
|                              | bamboo     | Fujian, Fuzhou | HL20161127_3  | MH821542         |
|                              | bamboo     | Fujian, Fuzhou | HL20161127_4  | MH821543 MK028308 MK028331 MK372351 |
|                              | bamboo     | Fujian, Fuzhou | HL20161228_18 | MH821544         |
|                              | bamboo     | Guangdong, Shenzhen | HL20170205_7 | MH821545 MK028309 MK028332 MK372352 |
|                              | bamboo     | Guangdong, Shenzhen | HL20170205_8 | MH821546         |
|                              | bamboo     | Fujian, Fuding  | HL20170403_10 | MH821549 MK028310 MK028333 MK372353 |
|                              | bamboo     | Fujian, Fuzhou | HL20170409_2  | MH821551         |
| *A. bambusae*                | bamboo     | Fujian, Fuding  | HL20170409_3  | MH821554 MK028311 MK372354 |
|                              | bamboo     | Fujian, Fuzhou | HL20170419_4  | MH821556         |
|                              | bamboo     | Fujian, Fuzhou | HL20170926_23 | MH821559 MK028312 MK028334 MK372355 |
|                              | bamboo     | Guangxi, Chongzuo | HL20171102_15 | MH821571 MK028313 MK372356 |
|                              | bamboo     | Fujian, Fuzhou | HL20150416_14 | MH821562         |
|                              | bamboo     | Fujian, Fuzhou | HL20150510_2  | MH821570         |
|                              | bamboo     | Fujian, Fuzhou | HL20150530_4  | MH821561         |
| *A. bambucifoliae*           | bamboo     | Fujian, Xiamen  | HL20160131_8  | MH821563 MK028314 MK028335 MK372357 |
|                              | bamboo     | Hainan, Sanya   | HL20160217_1  | MH821565 MK028315 MK372358 |
|                              | bamboo     | Fujian, Fuzhou | HL20160308_1  | MH821566         |
| *A. bambusae*                | bamboo     | Fujian, Fuzhou | HL20160412_5  | MH821569 MK028316 MK028336 MK372359 |
|                              | bamboo     | Fujian, Ninning | HL20161004_1  | MH821540 MK028317 MK028337 MK372360 |
|                              | bamboo     | Guangdong, Shenzhen | HL20170205_9 | MH821548 MK028318 MK028338 MK372361 |
|                              | bamboo     | Fujian, Fuzhou | HL20170226_3  | MH821560         |
|                              | bamboo     | Fujian, Fuzhou | HL20170318_3  | MH821547         |
|                              | bamboo     | Fujian, Fuding  | HL20170403_13 | MH821550         |
| *A. formosana*               | bamboo     | Fujian, Fuzhou | HL20170409_4  | MH821555         |
|                              | bamboo     | Fujian, Fuzhou | HL20170606_8  | MH821557         |
| *A. bambusae*                | bamboo     | Yunnan, Kunming | HL20170806_1  | MH821558 MK028319 MK372362 |
|                              | bamboo     | Guangxi, Chongzuo | HL20171103_22 | MH821572         |
| *A. bambucifoliae*           | bamboo     | Yunnan, Kunming | HL20171108_6  | MH821573 MK028320 MK028326 MK372363 |
| *A. bambusae*                | bamboo     | Yunnan, Kunming | HL20171108_7  | MH821574         |
| *A. formosana*               | bamboo     | Yunnan, Kunming | HL20171111_3  | MH821576 MK028321 MK028327 MK372364 |
|                              | bamboo     | Yunnan, Dali   | HL20171126_6  | MH821577         |
| *A. bambusae*                | bamboo     | Yunnan, Dali   | HL20171126_7  | MH821578 MK028322 MK028328 |
| *A. bambucifoliae*           | bamboo     | Guangxi, Chongzuo | HL20171102_16 | MH821579 MK028323 MK028329 |
| *A. bambusae*                | bamboo     | Guangxi, Chongzuo | HL20171103_19 | MH821582 MK028324 MK028330 MK372365 |
| *A. bambucifoliae*           | bamboo     | Guangxi | ZMIOZ13322 | JN032708 DQ493848 |
| *A. bambusae*                | bamboo     | India, Karnataka | ORP-2010-61  | HQ112196         |
| *Bambusa tulda*              | bamboo     | Guangxi | ZMIOZ14592 | JX282768 JX282692 JX282849 |
| *Bambusa tulda*              | bamboo     | India, Karnataka | KBRIIHR-172  | JX051408         |
| *Bambusa tulda*              | bamboo     | India, Karnataka | KBRIIHR-149  | JX051385         |
| *Bambusa tulda*              | bamboo     | India, Karnataka | KBRIIHR-148  | JX051384         |
| *Bambusa tulda*              | bamboo     | India, Karnataka | KBRIIHR-147  | JX051383         |
| *Bambusa tulda*              | bamboo     | India, Karnataka | KBRIIHR-146  | JX051382         |
| *A. bambucifoliae*           | Poaceae    | Taiwan, Puli   | L27324        |
| *A. formosana*               | Poaceae    | Taiwan, Sun Moon Lake | L27326       |

* indicates the sequences downloaded from the GenBank.
DNA extraction, PCR, and sequencing

We used DNeasy Blood & Tissue Kit (QIAGEN, GERMANY) to extract total genomic DNA from one individual per sample. The primers LepF (5’-ATTCAACCATCATAAAGATATTGG-3’) and LepR (5’-TAAACTTCTGGATGTCCAACAAAATCA-3’) (Footit et al. 2008) were used to amplify COI barcode region. The primers for amplification of Cytb were CP1 (5’-GATGATGAAATTTTGGATC-3’) and CP2 (5’-CTATGCAATACTCCCTC-3’) (Harry et al. 1998). EF-1α sequences were amplified based on EF3 (5’-GACGTGAACGTGGTATCAC-3’) and EF2 (5’-ATGGAGCAGTGTGGCAATCCAA-3’) (Palumbi 1996; von Dohlen et al. 2002). tRNA/COII sequences were amplified based on mt2793+ (5’-ATACTCGACGTTATTCA-3’) and mt3660- (5’-CCACAAATTTCTGAACATTGACCA) (Stern 1994). The PCR was performed in 30 μl reaction volumes: 20 μl ddH2O, 3 μl 10X buffer, 2.4 μl dNTP, 0.6 μl forward and reverse primer (10 μM), 0.4 μl of Taq DNA polymerase (5U/μl) and 3 μl of template DNA. All polymerase chain reactions included an initial denaturation step for 5 min at 95 °C and final extension step for 10 min at 72 °C. The cycling conditions of COI included 35 cycles of denaturation at 94 °C for 20s, annealing at 50 °C for 30s and extension at 72 °C for 2 min. The cycling conditions for Cytb were: 35 cycles of 1 min at 92 °C, 1.5 min at 48 °C and 1 min at 72 °C. The thermal setup for EF-1α was: 35 cycles of 30s at 95 °C, 1 min at 51 °C and 1 min at 72 °C. The cycling conditions for tRNA/COII were 34 cycles of 30s at 95 °C, 1 min at 54 °C and 1 min at 72 °C. Detection of the PCR products was performed on a 1% agarose gel. The eligible products were bidirectionally sequenced using the same PCR primer pairs by Sangon Biotech (Shanghai).

Sequence and phylogenetic analyses

Thirty-nine COI sequences were successfully obtained from the 37 ingroup samples and two A. formosana outgroups. In addition, eight COI sequences including one of A. bambucifoliae and seven of A. bambusae were downloaded from GenBank (accession numbers: JN032708, HQ112196, JX282768, JX051408, JX051385, JX051384, JX051383 and JX051382) for further phylogenetic analyses (Table 1). Based on the topology of the COI tree, sixteen ingroup samples were selected for Cytb, tRNA/COII, and EF-1α amplification. Finally, a total of 16 Cytb sequences, 12 EF-1α sequences and 15 tRNA/COII sequences were successfully generated. We downloaded several Cytb (accession number: JX282692) and EF-1α (accession numbers: DQ493848, JX282849) sequences of both species from the GenBank. Furthermore, as A. bambucifoliae was originally described from Taiwan, we downloaded two tRNA/COII sequences L27324 (A. bambucifoliae) and L27326 (A. formosana), which were obtained from Taiwanese samples from GenBank to test the relationships between them and our sequences (Table 1). For all the sequences obtained
in this study, the raw forward and reverse sequences were corrected based on the chromatograms and assembled using BioEdit software (Hall 1999). Subsequently, the sequences were aligned by MAFFT (Kazutaka and Standley 2013) and trimmed to the same length with BioEdit. For the EF-1α sequences, the introns were removed according to the GT-AG rule and the cDNA region of a *Schizaphis graminum* reference sequence (GenBank accession number AF068479), and the coding regions of EF-1α were used in further phylogenetic analyses.

The Kimura 2-parameter (K2P) model (Kimura 1980) were used to calculate pairwise distances among nucleotide sequences in MEGA 7.0 (Kumar et al. 2016). The optimal nucleotide substitution models were determined based on Akaike Information Criterion (AIC) by using jMODELTEST 2.1.7 (Darriba et al. 2012) for COI (GTR+I), Cytb (GTR), EF-1α (HKY+I) and tRNA/COII (GTR). For each marker, different phylogenetic reconstruction methods (Neighbor-joining, NJ; Maximum likelihood, ML; Bayesian inference, BI) were used to estimate the topologies. MEGA 7.0 was used to build the NJ trees based on the K2P model and 1,000 bootstrap replicates. Based on the estimated models, the ML trees were estimated in RAxML (Silvestro and Michalak 2012) with the settings of ML+ rapid bootstrap, and nodal support was calculated by 1000 replicates. The Bayesian analyses were performed with MrBayes 3.2.6 (Ronquist et al. 2012). Two million generations Markov Chain Monte Carlo (MCMC) were run and sampled every 100 generations, and the first 25% of trees were discarded as burn-in to acquire posterior probability values (PP). The phylogenetic trees were represented and edited using the online tool iTOL (Letunic and Bork 2016).

The haplotype network analysis of COI sequences was also implemented to illustrate the population genetic structure in space based on geographic groups. The COI sequences were imported into DNAsp 5.0 (Librado and Rozas 2009) to analyze the haplotype composition. Then the median-joining network of the haplotypes was computed by using NETWORK 5.0.0.3 (Bandelt et al. 1999) based on default settings.

**Results**

**Sequence characters**

Forty-seven COI sequences were aligned to a final length of 556 bp, which included 527 conserved sites, 29 variable sites, and 24 parsimony-informative sites. The nucleotide composition of COI alignment displayed a strong bias toward A+T content (T: 42.6%, C: 12.7%, A: 36.2% and G: 8.5%). The 718 bp long Cytb alignment with 19 sequences included 689 conserved sites, 29 variable sites, and 28 parsimony-informative sites. The nucleotide composition of Cytb alignment was 44.8% T, 12.3% C, 34.2% A, and 8.7% G. After the introns were excluded, sixteen EF-1α sequences were trimmed to a 785 bp long alignment with 769 conserved sites, 16 variable sites, and 13 parsimony-informative sites. The nucleotide composition was 26.2% T, 20.9% C, 27.8% A, and 25.1% G. The tRNA/COII alignment had 626bp with 595 conserved
sites, 31 variable sites and 25 parsimony-informative sites. The nucleotide composition of tRNA/COII alignment was 41.0% T, 11.1% C, 41.1% A, and 6.8% G.

Genetic distances and phylogenetic analyses

The intraspecific and interspecific K2P genetic distances among the samples are shown in Table 2. The maximum genetic distances (1.46%) were between some Indian samples and the other samples. Basically, the COI sequences were able to contribute more informative sites to understand the population structure.

In general, different reconstruction approaches yielded similar phylogenetic trees for the same marker (Figure 1, Suppl. materials 1, 2). Phylogenetic trees showed that all four genes failed to support the monophyly of both *A. bambucifoliae* and *A. bambusae*. Samples of these two species were dispersed in different clades of the phylogenetic trees. Based on the COI tree with more samples (Figure 1), some well-supported clades were distinct. All the samples from the Yunnan and Guizhou plateau of southwestern China as well as all the Indian samples clustered into separate clades. These samples were all morphologically identified as *A. bambusae*. There was also a separate clade including many samples of both morphologically identified species and from different localities of southeastern and southern China, but with low genetic distances.

The network analysis of the COI haplotypes (Figure 2) indicated that all the Indian samples, assigned as haplotypes H6 and H7, were linked together and showed greatest differentiation from the other haplotypes. The samples from southwestern China, including almost all samples from Yunnan and Guizhou Plateau and some from Guangxi, were of haplotype H5. Haplotype H1 with most samples included almost all samples from Fujian in southeastern China. The other samples from southeastern and southern China (Fujian, Guangdong, Hainan) were assigned to several other haplotypes, i.e., H8, H9, H2, H3, H4.

Phylogenetic pattern of morphological variation

The photographs of live specimens that we took during the field work in different localities and at different times indicated the spatial and temporal diversity of all samples (Figure 3). When these photographs were compared with the phylogenetic tree (Figure 1), it was apparent that some key morphological diagnostic characters used to distinguish both species, such as the wax types and the green patches, have no distinct phylogenetic pattern. For example, within the separate clade including many samples of both morphologically identified species from different localities of southeastern China (Figure 3[1–17]), the appearance of these samples based on wax layout and green patches varied greatly, whereas their genetic distances were very low. Moreover, although the samples from Yunnan Plateau with identical COI sequence (Figure 3[21–24]) had relatively similar green patches and were collected at similar times (November 2017), their wax density and distribution were clearly different.
Figure 1. The Neighbor-joining (NJ) trees based on COI (A), Cytb (B), EF-1α (C), tRNA/COII (D), and the combined data of all four genes (E). The ingroup specimens are printed in bold and the bootstrap values higher than 50 are indicated. The sequences are named as putative species name plus specimen voucher number.
Table 2. Genetic distances among *Astegopteryx bambucifoliae* and *A. bambusae* samples based on COI, Cytb, EF-1α, and tRNA/COII sequences.

| Genetic distance | Species                     | Gene  | Range (%) | Mean (%) |
|------------------|-----------------------------|-------|-----------|----------|
|                  | *Astegopteryx bambucifoliae* | COI   | 0–0.91    | 0.15     |
|                  |                             | Cytb  | 0         | 0        |
|                  |                             | EF-1α | 0–0.26    | 0.13     |
|                  |                             | tRNA/COII | 0–0.48 | 0.12     |
| Intraspecific    |                             | COI   | 0–1.46    | 0.56     |
|                  |                             | Cytb  | 0–0.28    | 0.11     |
|                  |                             | EF-1α | 0–0.38    | 0.19     |
|                  |                             | tRNA/COII | 0–1.46 | 0.61     |
|                  | *Astegopteryx bambusae*     | COI   | 0–1.46    | 0.38     |
|                  |                             | Cytb  | 0–0.28    | 0.08     |
|                  |                             | EF-1α | 0–0.38    | 0.14     |
|                  |                             | tRNA/COII | 0–1.46 | 0.38     |
| Interspecific    | *Astegopteryx bambucifoliae* & *Astegopteryx bambusae* | COI   | 0–1.46    | 0.38     |
|                  |                             | EF-1α | 0–0.38    | 0.14     |
|                  |                             | tRNA/COII | 0–1.46 | 0.38     |

Figure 2. Haplotype networks based on COI sequences. The circles represent different haplotypes, while different colors correspond to the geographical origins of samples and sizes represent relative numbers of sequences (H_1: 23; H_2: 1; H_3: 2; H_4: 3; H_5: 7; H_6: 3; H_7: 3; H_8: 1; H_9: 1; H_10: 1). The short line segments indicate mutated positions between haplotypes.

Discussion

Species descriptions based on limited samples are often unable to represent the whole picture of morphological variation within the species, making it likely that some names will subsequently be synonymised (Winston 1999; Eastop and Blackman 2005). A review of the relevant literature and the results of our present study indicate that *A. bambusae* and *A. bambucifoliae* should be such a case. Based on the molecular data from extensive sampling, our results show that relatively low genetic distances of four genes exist among all samples of both morphologically identified species. In previous DNA barcoding studies of aphids (Footitt et al. 2009; Zhu et al. 2017), 2% has been used as a
threshold value of COI genetic distances for species delimitation. This threshold has also been proposed for other insect groups (Hajibabaei et al. 2006; Zahiri et al. 2014). In the present study, the maximum and mean COI genetic distances (1.46% and 0.56%, respectively; Table 2) among all samples from southern China to India do not reach the 2% threshold value to define separate species. Moreover, no matter what phylogenetic methods were used, the monophyly of neither of the morphologically identified Astegopteryx species has been supported by the phylogenetic trees based on any of the four genes. Although all ingroup samples form one well-supported clade, several inner clades with dispersed samples of both species have been less supported with lower bootstrap values. Thus, the molecular data indicate that all samples belong to a single species.

Our study also provides information on the taxonomic significance of variations in appearance in life. Results show that there is no distinct phylogenetic pattern for key diagnostic characters such as green patches on the dorsum and distribution of wax. The high spatial and temporal morphological diversity among all samples used in the present study support our and other colleagues' speculation (Blackman and Eastop 2018) that the stability of these proposed morphological discriminants for the two Astegopteryx species is uncertain. The distinct character of a pair of longitudinal dark green patches often interrupted at mid-length on the dorsum of live specimens was proposed by Takahashi (1921) to distinguish A. bambucifoliae. However, this character has been described as “uninterrupted longitudinal markings on dorsum” by other taxonomists (Joshi and Porrani 2007), indicating that this character cannot be a stable diagnostic character at species level. Wax gland plates occur widely in the subfamily Hormaphidinae, which Astegopteryx belongs to, and have a variety of shapes and sizes as well as complex arrangements (Chen and Qiao 2012). Previous studies showed that characters related to wax gland plates even change ontogenetically, for example, wax gland plates may be present in nymphs and embryos but absent in adults (Shaposhnikov and Gabrid 1987). Considering aphids are producing honeydew and Cerataphidini aphids often live as large colonies in wet subtropical regions (Noordam 1991; Huang et al. 2012; Blackman and Eastop 2018; Qiao et al. 2018), the wax probably has a functional role to protect aphids from possible contamination of honeydew, rain, natural enemies, and other environmental factors (Pope 1983; Heie 1987; Smith 1999; Pike et al. 2002; Moss et al. 2006). Such a functional character may not necessarily be phylogenetically informative for species delimitation, as the appearance and arrangement of wax cells may be easily affected by environmental changes. This is shown by the high wax variation among all samples showed in the present study.

**Figure 3.** Photographs of live specimens showing high morphological variation among samples. Based on specimen voucher number, these photographs correspond to the following sequences in the phylogenetic trees; 1 HL20170205_7 2 HL20170606_8 3 HL20170409_2 4 HL20170403_13 5 HL20170226_3 6 HL20150416_14 7 HL20160417_7 8 HL20161004_1 9 HL20161228_18 10 HL20150530_4 11 HL20160326_4 12 HL20170403_10 13 HL20160131_8 14 HL20160512_1 15 HL20170318_3 16 HL20170419_4 17 HL20170926_23 18 HL20160217_1 19 HLzd20171102_15 20 HLzd20171103_22 21 HLzd20171108_6 22 HLzd20171108_7 23 HLzd20171111_3 24 HLzd20171126_6 25 HL20170205_8 26 HL20170806_1 27 HL20160412_5 28 HLzd20171102_16.
By integrating the molecular data and morphological information, our results indicate that *A. bambusae* and *A. bambucifoliae* should be regarded as a single species with high intraspecific morphological variation. Based on the history of the two species, we place *A. bambucifoliae* (Takahashi 1921) as a junior synonym of *A. bambusae* (Buckton 1893). Considering the results of our study, as well as published descriptions (Buckton 1893; Takahashi 1921; Noordam 1991; Qiao et al. 2018), it seems that large-scale geographic patterns of population differentiation may exist within the species. For example, the Indian samples we cited seem more genetically divergent. Noordam (1991) reviewed the Javanese *Astegopteryx* species, in which several species originally described by van der Goot (1917) were considered as color varieties of *A. bambusae*. However, based on the color plates (Pl. 1–5) of live specimens in Noordam (1991), the patterns of green bands and wax distribution of those color varieties are quite different from our photographed specimens. This may raise the question of whether the treatment in Noordam (1991) is appropriate. Therefore, future investigation is needed to resolve the identity of populations in Southeast Asia. In addition, considering this species has previously been recorded with facultative host alternation between primary host *Styrax* and secondary host bamboos in Taiwan (Aoki and Kurosu 2010), it will be interesting to have some molecular work done in future on populations from *Styrax*.

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**References**

Aoki S, Kurosu U (1995) Valid name for the aphid *Astegopteryx* “insularis” (sensu van der Goot 1917), with notes on its biology (Homoptera, Aphidoidea). Japanese Journal of Systematic Entomology 1: 151–152.

Aoki S, Kurosu U (2010) A review of the biology of Cerataphidini (Hemiptera, Aphididae, Hormaphidinae), focusing mainly on their life cycles, gall formation, and soldiers. Psyche: A Journal of Entomology 2010. https://doi.org/10.1155/2010/380351

Bandelt H-J, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Molecular Biology & Evolution 16: 37–48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Blackman RL, Eastop VF (1994) Aphids on the world’s trees: an identification and information guide. 1994. CAB International, Wallingford, United Kingdom.
Molecular and morphological evidence for the identity of two nominal species...

Blackman RL, Eastop VF (2018) Aphids on the World’s Plants: An online identification and information guide. Published online at http://www.aphidsonworldsplants.info [Accessed 25 October 2018]

Buckton GB (1893) Notes on Indian aphids. Indian Museum Notes 3: 87–88.

Chen J, Qiao GX (2012) Wax Gland Plates in Hormaphidinae (Hemiptera: Aphididae): Morphological Diversity and Evolution. Entomological News 122: 27–45. https://doi.org/10.3157/021.122.0104

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772. https://doi.org/10.1038/nmeth.2109

Eastop VF, Blackman RL (2005) Some new synonyms in Aphididae (Hemiptera: Sternorrhyncha). Zootaxa 1089: 1–36. https://doi.org/10.11646/zootaxa.1089.1.1

Favret C (2018) Aphid Species File. Version 5.0/5.0. http://Aphid.SpeciesFile.org [Accessed 25 October 2018]

Foottit RG, Maw HE, von Dohlen CD, Hebert PD (2008) Species identification of aphids (Insecta: Hemiptera: Aphididae) through DNA barcodes. Molecular Ecology Resources 8: 1189–1201. https://doi.org/10.1111/j.1755-0998.2008.02297.x

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Harry M, Solignac M, Lachaise D (1998) Molecular evidence for parallel evolution of adaptive syndromes in fig-breeding Lissocephala (Drosophilidae). Molecular Phylogenetics & Evolution 9: 542–551. https://doi.org/10.1006/mpev.1998.0508

Hebert PDN, Cywinska A, Ball SL, Dewaard JR (2003) Biological identification through DNA barcodes. Proceedings of the Royal Society of London Series B, Biological Sciences 270: 313–321. https://doi.org/10.1098/rspb.2002.2218

Heie OE (1987) Paleontology and phylogeny. In: Minks AK, Harrewijn P (Eds) Aphids: their biology, natural enemies and control. World crop pests, vol. 2A. Elsevier, Amsterdam, 367–391.

Huang XL, Xiang Yu JG, Ren SS, Zhang RL, Zhang YP, Qiao GX (2012) Molecular phylogeny and divergence times of Hormaphidinae (Hemiptera: Aphididae) indicate Late Cretaceous tribal diversification. Zoological Journal of the Linnean Society 165: 73–87. https://doi.org/10.1111/j.1096-3642.2011.00795.x

Jensen AS, Miller GL, Carmichael A (2010) Host Range and Biology of Uroleucon (Lambersi sius) erigeronense (Thomas 1878), and its Synonymy with Uroleucon (Lambersius) escalantii
(Knowlton) 1928 (Hemiptera: Aphididae). Proceedings of the Entomological Society of Washington 112: 239–245. https://doi.org/10.4289/0013-8797-112.2.239
Joshi S, Poorani J (2007) Aphids of Karnataka. http://www.nbair.res.in/Aphids/index.php [Accessed 25 October 2018]
Kazutaka K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology & Evolution 30: 772–780. https://doi.org/10.1093/molbev/msr010
Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120. https://doi.org/10.1007/BF01731581
Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054
Kurosu U, Aoki S (1991) The Gall Formation, Defenders and Life Cycle of the Subtropical Aphid Astegopteryx bambucifoliae (Homoptera). Japanese Journal of Entomology 59: 375–388.
Letunic I, Bork P (2016) Interactive tree of life (iTOl) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Research 44: W242–W245. https://doi.org/10.1093/nar/gkw290
Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452. https://doi.org/10.1093/bioinformatics/btp187
Madjdzadeh SM, Mehrparvar M (2009) Morphological discrimination of geographical populations of Macrosiphoniella sanborni (Gillette, 1908) (Hem.: Aphididae) in Iran. Northwestern Journal of Zoology 5: 338–348.
Margaritopoulos JT, Tsitsipis JA, Zintzaras E, Blackman RL (2000) Host-correlated morphological variation of Myzus persicae (Hemiptera: Aphididae) populations in Greece. Bulletin of Entomological Research 90: 233–244. https://doi.org/10.1017/S0007485300000353
Meier R (2017) Citation of taxonomic publications: the why, when, what and what not. Systematic Entomology 42: 301–304. https://doi.org/10.1111/syen.12215
Moss R, Jackson RR, Pollard SD (2006) Mask of wax: Secretions of wax conceal aphids from detection by spider’s eyes. New Zealand Journal of Zoology 33: 215–220. https://doi.org/10.1080/03014223.2006.9518448
Noordam D (1991) Hormaphidinae from Java (Homoptera: Aphididae). Zoologische Verhandelingen 270: 1–525.
Palumbi SR (1996) Nucleic acids II : The polymerase chain reaction. Molecular Systematics: 205–247.
Pike N, Richard D, Foster W, Mahadevan L (2002) How Aphids Lose Their Marbles. Proceedings of the Royal Society Biological Sciences 269: 1211–1215. https://doi.org/10.1098/rspb.2002.1999
Pope RD (1983) Some aphid waxes, their form and function (Homoptera: Aphididae). Journal of Natural History 17: 489–506. https://doi.org/10.1080/00222938300770431
Qiao G, Jiang L, Chen J, Zhang G, Zhong T (2018) Hemiptera: Hormaphididae, Phloeomyzidae. Fauna Sinica Insecta 60: 1–414. [+ 8 pls]
Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic in-
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Supplementary material 1

The Maximum likelihood (ML) trees based on COI (A), Cytb (B), EF-1α (C), tRNA/COII (D) and the combined data of all four genes (E)
Authors: Qiang Li, Jiamin Yao, Lingda Zeng, Xiaolan Lin, Xiaolei Huang
Data type: molecular data
Explanation note: The ingroup specimens are printed in bold and the bootstrap values over 50 are indicated. The sequences are named as species name plus specimen voucher number.
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Link: https://doi.org/10.3897/zookeys.833.30592.suppl1

Supplementary material 2

The Bayesian trees based on COI (A), Cytb (B), EF-1α (C), tRNA/COII (D) and the combined data of all four genes (E)
Authors: Qiang Li, Jiamin Yao, Lingda Zeng, Xiaolan Lin, Xiaolei Huang
Data type: molecular data
Explanation note: The ingroup specimens are printed in bold and the posterior probabilities over 90% are indicated. The sequences are named as species name plus specimen voucher number.
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