

A multilocus phylogenetic framework of the tribe Aeromachini (Lepidoptera: HesperIIDae: HesperIIDinae), with implications for taxonomy and biogeography

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Abstract. The Oriental tribe Aeromachini (Lepidoptera: HesperIIDae: HesperIIDinae) is diverse and widespread, but its monophyly and circumscription remain unresolved. In this study, we inferred phylogenetic relationships within the tribe using two mitochondrial (*16S* and *COI-COII*) and two nuclear genes (*EF-1 α* and *Wingless*) from 71 samples representing all of the known genera of Aeromachini (s.l.). Phylogenetic topologies obtained from two model-based methods (maximum-likelihood and Bayesian inference) were largely congruent, showing that Aeromachini was monophyletic. Many genera within Aeromachini were polyphyletic, especially *Arnetta*, *Thoressa*, *Ampittia* and *Sovia*. Based on these results, we propose a new classification consisting of 14 genera, of which two are newly described: *Halpemorpha* **gen.n.** and *Praethoressa* **gen.n.** *Ampittia* and *Ochus* are restructured, with: (i) *Ampittia nana* and *Ampittia dalailama* moved to *Aeromachus*, (ii) new combinations proposed for the remaining *Ampittia* species, and (iii) a new combination, *Ampittia subvittatus* **comb.n.**, proposed. The Oriental members of the genus *Arnetta* belong to this tribe, but the African *Arnetta hyposticta* was found to be embedded into the outgroup and closely related to *Isoteinon*. We propose that *hyposticta* be reinstated in the genus *Galerga* Mabilie, along with two other African species (*fito* and *ellipsis*). *Thoressa* is polyphyletic, with three distinct lineages. The type species *Thoressa masoni* forms a clade closely related to the sister genera *Sebastonyma* and *Parasovia*. We thus describe *Praethoressa* **gen.n.** to include the species *varia* (formerly of *Thoressa*), and transfer the remaining *Thoressa* to *Pedesta*, which is confirmed as a valid genus. Although the relationships of some genera remained unsolved in our work, the present phylogenetic hypothesis will serve as a reference for further studies. A secondary calibration was used to estimate the divergence time, which indicated that the diversification of Aeromachini started in the late Eocene and early Oligocene (c. 34.22 Ma). Further, ancestral range estimates indicated that the common ancestor of Aeromachini originated in Southeast Asia. Diversification *in situ* and range expansion to adjacent areas have played essential roles in the evolution of Aeromachini, but founder-event speciation and vicariance also have likely been important factors in shaping the biogeographical history of this tribe.

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Introduction

Considering the limits to morphological classification methods, robust molecular phylogenies can contribute to the development of more stable classifications and provide better frameworks for recovering the patterns of diversification and historical biogeography. The family Hesperidae is well-represented in all zoogeographical regions, but the phylogeny of this family and other biological aspects, such as utilization of host plants, biogeography and adaptive evolution, are much better understood for the European and American lineages than for the existing Asian and African lineages. The classification of Hesperidae has been revised based on molecular analyses alone or in combination with morphological characters, but its higher-level classification is still uncertain (Warren *et al.*, 2008, 2009; Sahoo *et al.*, 2016). The largest subfamily Hesperinae has been shown to be monophyletic, but the tribe-level systematic associations of many genera within this subfamily remain ambiguous. The tribe Aeromachini (Hesperidae: Hesperinae) is currently treated as a monophyletic group within Hesperinae. However, tribal delimitation and generic inter-relationships are inconclusive and inadequately demonstrated at present. The tribe Aeromachini (Lepidoptera: Hesperidae: Hesperinae) is one of the most diverse lineages of Hesperinae. The tribe currently comprises approximately 130 described species in 12 genera, which are predominantly distributed in the Oriental Region, with some species in the Palaearctic Region and some *Ampittia* species distributed in Madagascar (Evans, 1937, 1949; Devyatkin, 1996; Warren *et al.*, 2009; Cock & Congdon, 2012; Yuan *et al.*, 2015a). Members of this tribe are small- to medium-sized, usually with yellowish to dark brown wing colour, and with a brand or stigma on the forewing of males. Vein CuA_2 of the forewing arises opposite to or beyond the origin of vein R, closer to CuA_1 than to M_3 . The antennae are less than half the length of the forewing costa (Evans, 1949; Eliot, 1992; Chou, 1998; Warren *et al.*, 2009; Yuan *et al.*, 2015a). Larval hostplants are generally from the grass family Poaceae (Inoué & Kawazoé, 1966; Hsu, 1999, 2015; Igarashi & Fukuda, 2000; Lyu & Chen, 2015), except that some species feed on Malvaceae (Saji & Valappil, 2017).

The tribe Aeromachini s.l. has been a taxonomically unresolved group due to the disputed generic and specific placements in this taxon and their uncertain taxonomic validity. It also has been called the *Astictopterus* group (Evans, 1949), *Halpe* group (Inoué & Kawazoé, 1966), *Ampittiini* and *Astictopterini* (Chou, 1994, 1998) or *Halpini* (Yuan *et al.*, 2015a). Tutt (1906) originally introduced *Aeromachinae* as a subfamily and *Aeromachidi* as a tribe for genera *Taractrocera* Butler, *Ampittia* Moore and *Aeromachus* Nicéville, although *Taractrocera* is currently placed in the tribe *Taractrocerini*. According to articles 23.3.1 and 34.1 of the Code of the International Commission on Zoological Nomenclature (ICZN, 1999), the designation *Aeromachini* is the valid one. Despite the variety of names and taxonomic designations applied to this taxon, the disagreements among authors are concerned mainly with the systematic positions of *Astictopterus* and *Arnetta*. The genus *Astictopterus* was successively placed into the *Astictopterus* group (Evans, 1949), *Isoteinon* group (Inoué & Kawazoé, 1966), *Ancistroides* group

(Eliot, 1992), *Astictopterini* (Chou, 1994, 1998) and *Ancistroidini* (Yuan *et al.*, 2015a). Moreover, Warren *et al.* (2008) showed that *Astictopterus* was closely related to *Kedestes*, and these two genera were closely associated with *Isoteinon*, suggesting that *Astictopterus* does not belong to *Aeromachini*. This result was reinforced by combined molecular and morphological data (Warren *et al.*, 2009). Evans (1949) treated the other poorly resolved genus *Arnetta* as a member of the *Astictopterus* group based mainly on the following morphological characters: veins Sc and R apart, apiculus obtuse or hooked, and the upperside of forewing with or without hyaline spots. He pointed out that the tuft on the dorsum and an obscure brand were present in males of only two species, *Arnetta atkinsoni* and *Arnetta verones*. Subsequently, Inoué & Kawazoé (1966) placed *Arnetta* in their *Halpe* group based on the porrect palpi of three Malaysian specimens of *Arnetta atkinsoni*. Eliot (1978, 1992) noted that *Arnetta* was probably not monophyletic and provisionally transferred it to the *Plastingia* group based primarily on the shape of wings and the slightly oblique direction of vein M_2 on the hindwing. Warren *et al.* (2009) agreed that *Arnetta* should not be included within *Aeromachini* and listed it as *incertae sedis* due to the absence of the 'Halpe brand'. However, there is no molecular evidence in support of most of these arrangements. Yuan *et al.* (2015a) noted that *Arnetta* resembled *Iambrix* and *Idmon* in some characters, but treated it as a member of *Isoteinonini*. Thus, the circumscription of the tribe *Aeromachini* is uncertain.

Although Warren *et al.* (2008) presented a revised classification of the family Hesperidae based on large-scale sampling of molecular data, the infrafamily phylogenetic relationships are still unresolved, particularly for the Old World taxa. Warren *et al.* (2008, 2009) included only four out of the 12 known genera of *Aeromachini* in their analysis, and concluded with strong phylogenetic support that the tribe is monophyletic and basal within Hesperinae. A recent study (Sahoo *et al.*, 2016) built on the findings of Warren *et al.* (2009) and corroborated the monophyly of *Aeromachini*, although without examining additional specimens. Yuan *et al.* (2015b) divided the Chinese Hesperidae into five subfamilies, but did not recover *Aeromachini* as a monophyletic group, perhaps because *Halpe*, *Pithauria* and *Aeromachus* were represented by only one species each. Thus, although some attempts have been made to improve the situation (Dodo *et al.*, 2008), phylogenetic relationships within the tribe *Aeromachini* are still poorly resolved.

Due to the scarce hesperiid fossil record (de Jong, 2016), little of the historical biogeography of this tribe is understood. Sahoo *et al.* (2017) reconstructed a time-calibrated phylogeny to explore what factors affected the diversification of skipper butterflies (Hesperidae) using previously estimated ages as a split range, and a fossil as a minimum stem age. Most recently, a more comprehensive time-calibrated phylogeny of butterflies (Papilionoidea) was proposed, which used 12 fossils and 10 host plant nodes for calibration (Chazot *et al.*, 2018). Such frameworks provide an opportunity to illuminate the history of different butterfly groups. The development of a similar framework for the *Aeromachini* provides useful information for the study of this group.

The present study aimed to: (i) propose an alternative hypothesis of generic relationships within the *Aeromachini* with additional taxon sampling and multi-locus mito-nuclear phylogenetic analyses; (ii) clarify taxonomic status of some genera listed as *incertae sedis* by Warren *et al.* (2008, 2009); and (iii) provide a preliminary hypothesis of the historical biogeography of this tribe.

Methods

Taxonomic sampling

We sampled all of the genera listed in the *Astictopterus* Group (Evans, 1949), and *Parasovia* as described later. We included the type species for each genus except for *Baracus vittatus*, *Onryza meiktia* and *Pithauria murdava*, in addition to as many species as possible from each genus. We sampled a total of 71 individuals belonging to 46 species in 13 genera as ingroup taxa, and 10 species from other subfamilies and related genera within the Hesperinae as outgroup taxa. We sampled and sequenced most of the individuals used in this study except for 24 sequences from eight samples obtained from Genbank. Table S1 gives a checklist of specimens with the collecting localities and voucher codes. We deposited the voucher specimens in the Insect Collection of the South China Agricultural University (SCAU), except for the Indian samples, which were deposited in the research collections of the National Centre for Biological Sciences (Bangalore, India).

DNA extraction, PCR amplification and sequencing

We extracted genomic DNA using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Germany) or HiPure Insect DNA Kit (Magen, China) from two or three legs of dried adult specimens or specimens preserved in pure molecular-grade ethanol following slightly modified protocols recommended by the manufacturers.

We sequenced four loci commonly used in butterfly phylogenies: mitochondrial *Cytochrome c oxidase subunit I and II (COI-COII)*, 931 bp) and *16S ribosomal RNA (16S rRNA)*, 591 bp), and nuclear *Elongation factor 1 alpha (EF-1α)*, 1066 bp) and *Wingless* (400 bp). We excluded 89 bp of *16S rRNA* from subsequent analyses due to ambiguous regions (see below). Table 1 lists the PCR primers.

We performed most PCR amplifications in 20 μL reactions containing 10 μL KOD PCR Buffer (Toyobo, Osaka, Japan), 4 μL 2× dNTPs, KOD FX, 0.5–1 μL of each primer and 1 μL of the DNA extract. The amplification cycle for *COI-COII* and *16S* was 2 min at 94 °C, and 35 or 40 cycles of 10 s at 98 °C, 0.5 or 1 min at 47 °C (*COI-COII*), or 48 °C (*16S*) and 1.5 min at 68 °C. For *EF-1α* and *Wingless*, the initial 94 °C at 2 min was followed by 35 or 40 cycles of 10 s at 98 °C, 55 °C (*EF-1α*) or 58 °C (*Wingless*) for 1 min and then 1.5 min at 68 °C. All amplification cycles included a final extension period of 68 °C for 10 min. We visualized the results by electrophoresis in 1%

agarose gel stained with ethidium bromide. We sequenced the PCR products after cloning into pMD 18-T vector (TAKARA) referring to Fan *et al.* (2016). We compared all of our sequences using blastn in GenBank to check for cross-contamination. Our new sequences are deposited in GenBank (Table S1).

Phylogenetic inference and divergence time estimate

We aligned all sequences, except the *16S* ribosomal RNA sequences, using Clustal W (Thompson *et al.*, 1997) implemented in MEGA 7.0 (Kumar *et al.*, 2016), with default parameters. For *16S rRNA*, which contained ambiguous regions of alignment representing hypervariable regions (HVRs) unlikely to evolve on a per-site nucleotide substitution basis, we aligned sequences online with MAFFT v7 (<http://mafft.cbrc.jp/alignment/software/>; Katoh & Standley, 2013) by applying a secondary structure model with a stem candidate length of 2 and a threshold of base pairing probability of 0.01. We identified and excluded the ambiguously aligned regions using GBLOCK v0.91b (Castresana, 2000; Talavera & Castresana, 2007), allowing for smaller final blocks, gap positions within the final blocks and less strict flanking positions. We treated gaps in all analyses as missing data.

We used PARTITIONFINDER v2.1.1 (Lanfear *et al.*, 2016) to select the optimal partitioning scheme under the Bayesian Information Criterion (BIC). We performed phylogenetic analyses using maximum-likelihood (ML) and Bayesian Inference (BI). We performed ML analyses using IQ-TREE 1.5 (Nguyen *et al.*, 2015) as implemented on the W-IQ-TREE web server (<http://iqtree.cibiv.univie.ac.at/>; Trifinopoulos *et al.*, 2016). We used the partition scheme produced by PARTITIONFINDER, but the best fitting model was selected using MODELFINDER (Kalyaanamoorthy *et al.*, 2017) implemented in IQ-Tree because it has more sequence evolution models than PARTITIONFINDER. We estimated models of substitution using *Auto* function with FreeRate heterogeneity. We evaluated the node support by 1000 ultrafast bootstrap replicates (UFBS) (Minh *et al.*, 2013). We also conducted standard ML analyses using RaxML (Stamatakis *et al.*, 2008) on CIPRES Science Gateway (Miller *et al.*, 2010) under the GTR+GAMMA model, using the partition scheme from the PARTITIONFINDER analysis. We performed bootstrapping with 1000 replicates under auto Majority Rule Criterion (autoMRE).

We used BEAST v2.4.8 (Bouckaert *et al.*, 2014) for BI to simultaneously investigate phylogenetic relationships and estimate the divergence times. Calibration data were taken from the divergence time estimate of butterflies (Papilionoidea) (Chazot *et al.*, 2018). The second calibration point was set as an age 28.67 Ma (95% highest posterior density, HPD: 35.94–22.84 Ma), to the timing of the split between *Halpemorphia* and *Pedesta*. We employed the partition scheme and models produced by PARTITIONFINDER. We applied the uncorrelated log-normal relaxed clock model and the Yule model as tree priors. The prior for the hyperparameter ‘uclMean’ was assigned as an exponential distribution of mean 10.0. We specified an Markov Chain Monte Carlo (MCMC) chain length of 100 million.

Table 1. Primers used for amplification and sequencing in this study.

Gene	Primer	Sequence (5'-3')	Reference
<i>EF-1α</i>	ef44	GCGYARCGYGGARCGTGGTATYAC	Monteiro & Pierce (2001)
	efrcM4	ACAGCVACKGTYTGCTCATRTC	Monteiro & Pierce (2001)
<i>Wingless</i>	LepWG1	GARTGYAARTGYCAYGGYATGTCTGG	Warren <i>et al.</i> (2008)
	LepWG2	ACTICGCARCACCARTGGAATGTRCA	Warren <i>et al.</i> (2008)
<i>COI-COII</i>	Gary	TAGGAATAATTTATGCMATAATAGC	Warren <i>et al.</i> (2008)
	Susan	TTGTTGTTCTAATARAATCG	Warren <i>et al.</i> (2008)
<i>COI</i>	K698	TACAATTTATCGCCTAAACTTCAGCC	Brower <i>et al.</i> (2006)
	Jane	TAAAATTACTCCTGTTAATCCTCC	Brower <i>et al.</i> (2006)
	Jerry	CAACAYTTATTTTGATTTT TTGG	Simon <i>et al.</i> (1994)
	Pat	ATCCATT ACA TAT AATCTGCCATA	Simon <i>et al.</i> (1994)
<i>16S rRNA</i>	LRJ12887	CCGGTTTGAGCTCAGATCA	Simon <i>et al.</i> (1994)
	LRN13398	CGCCTGTTTATCAAAAACAT	Simon <i>et al.</i> (1994)

Priors for all other parameters were kept at default values. The program TRACER v1.6 (Rambaut *et al.*, 2014) was used to check the convergence of posterior distributions and ensure all parameter estimates had ESS (effective sampled sizes) over 200. We summarized the trees in TREEANNOTATOR v2.4.8. The maximum clade credibility (MCC) tree was generated using TREEANNOTATOR v 2.4.8, after discarding 30% of the posterior sampled trees as burn-in. The MCC tree was annotated with median ages and 95% highest posterior density (HPD) intervals for node ages, and visualized with FIGTREE v1.4.0.

We estimated clade robustness by posterior probabilities (PP) of BI and ultrafast bootstrap values (UFBS, IQ-Tree) in ML. We deemed branch support to be strong when PP was 0.95–1 and UFBS was 95 or higher, moderate when PP was 0.90–0.95 and UFBS 90–95, and weak/low when PP was 0.50–0.90 and UFBS less than 90.

Ancestral range estimation

We pruned the MCC tree using the R (R Core Team, 2013) package APE (Paradis *et al.*, 2004) to obtain a phylogeny with only one tip per species of *Aeromachini* spp. We recognized and coded six distribution areas based on biogeographical realm and geotectonic plates, with some modifications according to the certain distributions of species, as follows: (A) Southeast Asia, including South China and Indochina, (B) the Indian Subcontinent, (C) the Southwest Mountains of China including the Himalayan–Hengduan Mountains and West Sichuan (SWC), (D) North China and the Russian Far East, (E) Japan and Sakhalin (JS), (F) the Malay Archipelago, and (G) the Philippines. Due to the main body of geological assemblages of the Malay Archipelago (excluding the Philippines, G) was similar to present-day conditions by *c.* 35 Ma (Hall, 2012, 2013) and that there were few endemic species in each area, the time slices methods were not applied in these analyses. The maximum number of ancestral areas was set to three, as no extant species occurs in more than three biogeographical regions.

Ancestral areas inferences without constraints were performed with the R package BioGeoBEARS (Matzke, 2014) for the tribe *Aeromachini*. We tested each of the implemented models

DEC, DIVALIKE and BAYAREALIKE, both with and without the additionally implementation of J (Matzke, 2013), which specifically donated the parameter representing the process of ‘jump dispersal’ or ‘long-distance dispersal’. The log-likelihood (LnL) and Akaike Information Criterion (AIC) were used to select and evaluate the best model.

Results

Dataset properties

The final dataset for the molecular phylogenetic analysis consisted of 81 taxa representing 56 species, of which 10 were outgroups, and representing all of the genera within the tribe *Aeromachini*. The resultant alignment of the four mito-nuclear genes consisted of 2899 bp, of which 1213 were variable and 917 parsimony-informative sites.

Phylogenetic inference

File S1 lists the best partitioning schemes and the optimal evolutionary models from PARTITIONFINDER for each of the partitioned datasets. File S2 lists the specific models for each partition matrix from IQ-TREE. IQ-TREE and RAXML runs produced similar topologies (Fig. 1 and File S3). We present below the results from IQ-TREE. The BI tree had congruent topologies to ML trees within *Aeromachini*, but some nodes were different among the outgroup taxa (File S4). Nodes supported strongly in ML analyses were always recovered with strong posterior probabilities, expect that the basal support was low (File S4). Both BI and ML analyses supported the monophyly of *Aeromachini sensu* Warren *et al.* (2008) (Fig. 1 and File S4). Within the tribe, *Baracus*, *Halpe* and *Pithauria* were each found to be monophyletic with strong support, whereas the remaining genera were either paraphyletic or polyphyletic.

The genus *Arnetta* was polyphyletic, which divided into two distantly related clades. The clade including two Asian species, *Arnetta atkinsoni* (the type species) and *Arnetta verones*, was

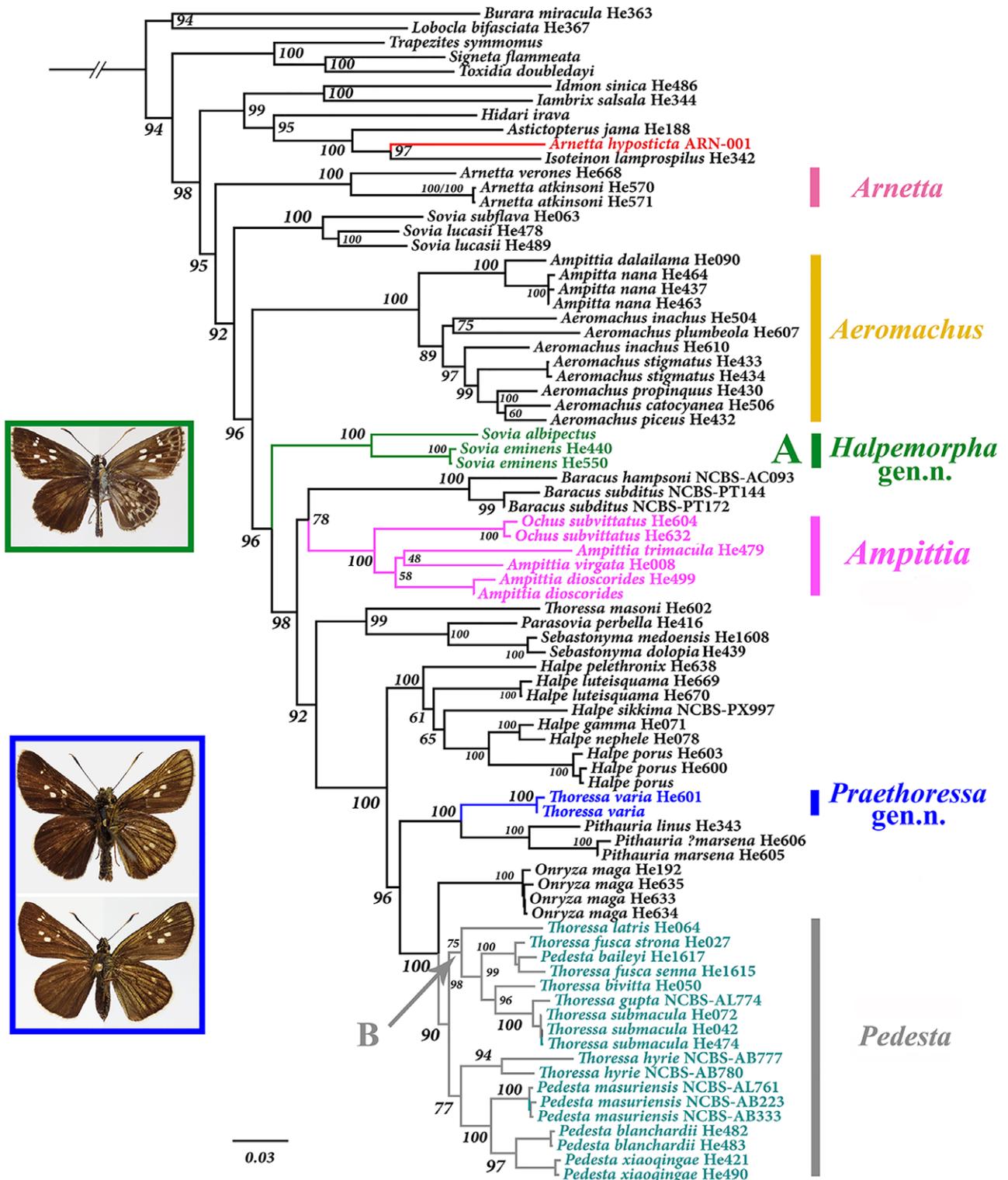


Fig. 1. Maximum-likelihood topology recovered from the IQ-TREE analysis of the combined dataset with the best-fit partitioning strategy. Ultrafast bootstrap support values (UFBS) are indicated at the nodes. Insect panels provide representative images for two new genera: upper row, *Halpemorpha gen.n.* and lower row, *Praethoressa, gen.n.* [Colour figure can be viewed at wileyonlinelibrary.com].

sister to the rest of the Aeromachini. *Arnetta hyposticta* from Madagascar showed a close relationship with *Isoteinon*, and the relation was strongly supported (UFBS = 97, PP = 0.99).

Sovia also was polyphyletic and divided into two unrelated clades: the *Sovia* clade and Clade A. The *Sovia* clade, including *S. lucasii* and *S. subflava*, was strongly supported (UFBS = 100, PP = 1). Clade A also was strongly supported as a monophyletic group (UFBS = 100, PP = 1), consisting of *S. albipectus* and *S. eminens*, and sister to the remaining taxa except the genera *Arnetta*, *Sovia* and *Aeromachus*. Accordingly, we describe this clade (Clade A) below as a new genus, *Halpemorpha* **gen.n.**

The genus *Ampittia* was not recovered as a monophyletic group. The clade including the type species *Ampittia dioscorides* clustered together with the monotypic genus *Ochus* with strong support (UFBS = 100, PP = 1), but the inter-relationships within this clade remained uncertain. The other subclade comprising two species, *Ampittia nana* and *A. dalailama*, clustered together with *Aeromachus* with strong support (UFBS = 100, PP = 1).

Although the monophyly of *Halpe* was strongly supported, the inter-relationships remained unresolved. Its three species (*H. porus*, *H. nephele* and *H. gamma*) formed a well-supported subclade. *Halpe pelethronix* and *H. luteisquama* were recovered as sister species in the BI tree, but this topology was not obtained in ML analyses. Species of *Thoressa* formed four distinct clades. The type species, *Thoressa masoni*, was the sister taxon to a clade composed of *Parasovia* and *Sebastonyma*, with strong support (UFBS = 99, PP = 1). Another representative of this genus, *Thoressa varia* from Japan, was placed as a sister group to *Pithauria* with strong support (UFBS = 100, PP = 1). We recognize and describe this clade as a new genus, *Praethoressa* **gen.n.** The remaining *Thoressa* species clustered together with species of *Pedesta* with moderate support in ML and strong support in BI (UFBS = 90, PP = 0.98). Two samples of *Thoressa hyrie* from India formed a weakly supported isolated subclade that was a sister clade to another subclade that included *Pedesta masuriensis*, *P. blanchardii* and *P. xiaoqingae*. *Pedesta baileyi* was nested within the Clade B that included five *Thoressa* species: *T. latris*, *T. fusca*, *T. bivitta*, *T. gupta* and *T. submacula*.

Divergence time and ancestral range estimation

We used TRACER v1.6 to check the BEAST log file, which showed that all parameters had ESS >300, and most were >1000. The MCC trees resulting from BEAST analyses generated congruent topologies with our phylogenetic analyses (as aforementioned). The divergence time estimates indicated that the diversification of the Aeromachini started in the late Eocene and early Oligocene (Fig. 2; median 34.22 Ma, 95% HPD: 38.47–30.49 Ma). More information on divergence times for each genus/species are given in File S3. Most extant lineages appeared during the Miocene–Pliocene (18.13–1.59 Ma). Ancestral area estimates indicated that the biogeographical model DEC+J was the best-fitting model, with the highest likelihood among all tested models (LnL = -147.70) (Table 2) based on the one tip per species chronogram (excluding outgroup). We thus present and discuss the results from this

analysis herein. The common ancestor of the Aeromachini was estimated to have originated in Southeast Asia (Fig. 3). Four founder-event speciation events were estimated during the mid-Miocene: *A. plumbeola*, and *H. luteisquama* colonized Philippines via long-distance dispersal at *c.* 11.14 and 11.56 Ma, respectively; *P. varia* colonized Japan and Sakhalin (JS) at *c.* 11.57 Ma; the genus *Baracus* was inferred to originate in the Indian Subcontinent and diversified there in the late Miocene (*c.* 7.74 Ma); and the genus *Pedesta* split into two lineages at *c.* 11.44 Ma – one of colonized and diversified in SWC at *c.* 9.95 Ma and the other diversified *in situ* and dispersed to adjacent areas. The most probable ancestral species of all other genera within Aeromachini likely occupied Southeast Asia and diversified in adjacent regions around the mid-Miocene.

Discussion

Systematics of Aeromachini

The dense and extensive taxon sampling that we used and the resultant molecular evidence confirmed that the tribe Aeromachini (*sensu* Warren *et al.*, 2008) is a monophyletic group. Relative to the previous analyses (Warren *et al.*, 2008, 2009; Yuan *et al.*, 2015b), the present study has resulted in considerable resolution at the genus level within this tribe. However, the statistical support for the stem of the tribe was not strong in our phylogenetic analyses, and the inter-relationships and monophyly of some genera still remained poorly resolved across all the analyses, and thus requires further investigation to resolve. On the basis of the phylogenetic results and morphological characteristics, we recognize 14 genera within Aeromachini, of which seven are largely consistent with traditionally recognized genera as already described or with little revision (*Baracus*, *Halpe*, *Pithauria*, *Onryza*, *Sebastonyma*, *Parasovia*, *Aeromachus*), two are proposed as new (*Halpemorpha* **gen.n.** and *Praethoressa* **gen.n.**) and the remaining five are inconsistent with previous generic concepts (*Arnetta*, *Ampittia*, *Sovia*, *Pedesta* and *Thoressa*). All of the taxonomic changes proposed in the present study are listed in File S5. Below we provide detailed taxonomic notes on the significant genera within the tribe Aeromachini, and describe in detail the two new genera.

Genus *Arnetta* Watson, 1893

The genus *Arnetta*, a small genus with seven species (three from Madagascar and four distributed from India to Borneo), has an ambiguous taxonomic status due to the paucity of genus-specific diagnostic characters. Evans (1937, 1949) defined the genus mainly based on the wing pattern and ignored the extensive differentiation in the morphology of the male genitalia among species. Due to its unclear morphological definition, subsequent researchers continued to change the taxonomic position of *Arnetta* based on some particular traits, which, according to our results, are not apomorphic characters of this genus. The African species *hyposticta* is not congeneric with two Asian species, *atkinsoni* and *verones*, especially with respect to their

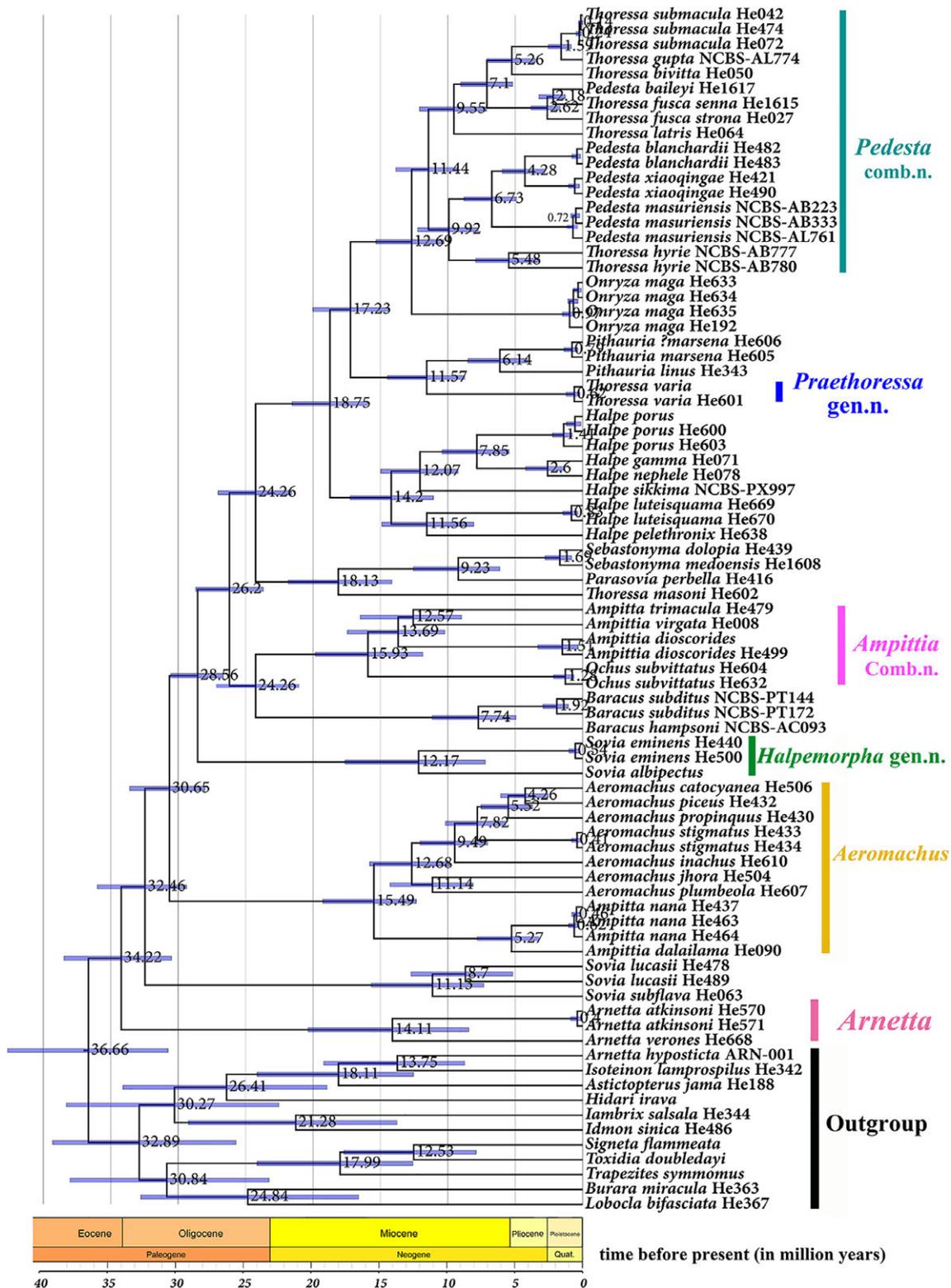


Fig. 2. The maximum clade credibility (MCC) tree resulting from the Bayesian analysis of the combined dataset in BEAST. The numbers at nodes indicate the mean age and blue bars represent 95% highest posterior density intervals for the node ages. [Colour figure can be viewed at wileyonlinelibrary.com].

Table 2. Comparison of the different biogeographical models with the results of the likelihood under each model, and evaluation with Akaike Information Criterion (AIC) for sample size, and the AIC weights.

	LnL	d	e	j	AIC	AIC_wt
DEC	-148.7	0.012	0.0005	0	301.5	0.48
DEC+J	-147.7	0.011	1.00E-12	0.0098	301.4	0.5
DIVALIKE	-152.5	0.013	2.00E-09	0	308.9	0.012
DIVALIKE+J	-152.4	0.013	1.00E-12	0.0027	310.8	0.0046
BAYAREALIKE	-159.2	0.011	0.044	0	322.5	1.30E-05
BAYAREALIKE+J	-155.9	0.0084	0.025	0.021	317.8	0.0001

male genitalia. Uncus is not separated and gnathos is stout in *hyposticta*, whereas uncus is deeply bifurcated at the tip and gnathos is slender and almost reaches the tip of uncus in the latter two species (Evans, 1937, 1949). Eliot (1978, 1992) realized that the genus was probably not a monophyletic group, but the lack of sufficient specimens and species for examination resulted in placing the genus in the *Plastingia* group. Our phylogenetic analyses indicated that these two Asian species of *Arnetta* belong to the Aeromachini, which is unlike the classification in previous studies (Warren *et al.*, 2009; Yuan *et al.*, 2015b), but partially agrees with Inoué & Kawazoe (1966). Another species in our phylogeny, *A. hyposticta* (the type species of *Galerga*), shows a close relationship with the genus *Isoteinon*, but there are strong morphological dissimilarities between the genera *Galerga* and *Isoteinon*.

Galerga (type species *Galerga hyposticta* Mabilie) was treated as a synonym of the genus *Arnetta* by Evans (1937). Lees *et al.* (2003) treated *Galerga* as a subgenus of *Arnetta*, and included two species, *Arnetta (Galerga) hyposticta* and *A. (G.) ellipsis*. Following Viette's (1956) suggestion, we here reinstate the genus *Galerga* as valid, and reinstate *Galerga hyposticta* Mabilie, **rest.stat.** Two other African species, *ellipsis* Saalmüller and *fito* Evans, are similar to *hyposticta* in term of the wing pattern and genitalia (Evans, 1937; Viette, 1956). Hence, we reinstate *ellipsis* and *fito* as valid species under *Galerga*, as *Galerga ellipsis* (Saalmüller), **rest.stat.** and *Galerga fito* (Evans, 1937), **rest.stat.** As noted by Evans (1949), two other Indian species (*Arnetta mercara* Evans and *Arnetta vindhiana* Moore) resemble *Astictopterus jama* in external features (also see Kunte *et al.*, 2017) but their male genitalia are closer to those of *Galerga* than to those of *Arnetta* and *Astictopterus jama*. Thus, it seems reasonable to place them in *Galerga*, although molecular phylogenetic evidence will be useful to confirm this hypothesis.

In summary, we retain only the species *verones* and *atkinsoni* in the genus *Arnetta*. The other five species previously assigned to this genus should be members of *Galerga*. The systematic positions of the species *mercara* and *vindhiana* need further research. Based on the molecular evidence, as well as morphological characters, we conclude that *Arnetta* is a subordinate taxon within Aeromachini.

Genus *Sovia* Evans, 1949

Evans (1949) recognized six species and several subspecies under *Sovia*. Subsequently, four new species were described and

a subspecies was elevated to species level (Devyatkin, 1996; Huang, 2003; Huang & Wu, 2003; Miyazaki & Saito, 2010; Xue *et al.*, 2015). To date, *Sovia* consists of 11 species that are exclusively distributed in South and Southeast Asia. Xue *et al.* (2015) classified *Sovia* into two species groups based on their distributional patterns as well as on morphological characters. In our phylogeny, the four *Sovia* species sampled were divided into two unrelated clades (*Sovia* clade and Clade A), which should be treated as two distinct genera. Consequently, we here recognize the clade with the type species, *S. lucasii*, along with *S. subflava*, as the genus *Sovia*, and the other clade (Clade A) as a new genus (discussed below). The genus *Sovia* is morphologically distinguishable by the following characters: one large spot across the cell on the forewing, and stigma of male located below the spot in space CuA₁; uncus is broad, as broad as tegument, obtuse apically; gnathos elongate, elbow-shaped, densely covered with small spines, and separated; valvae broad, ventrodistal process is irregularly semicircular or rectangular, covered with small teeth at apex or dorsal edge, and centrally with a robust inner, spined, sharp projection. The other five species, *S. separata* (Moore), *S. lii* Xue, *S. fangi* Huang & Wu, *S. grahami* (Evans) and *S. malta* Evans also belong to *Sovia* based on the morphological characteristics (Evans, 1949; Huang, 2003; Huang & Wu, 2003; Xue *et al.*, 2015).

Genera *Ampittia* Moore, 1881 and *Aeromachus* de Nicéville, 1890

According to our phylogenetic analyses, the genus *Ampittia* is a polyphyletic assemblage with the species analysed here separating into two phylogenetically distinct clades. The clade including *nana* and *dalailama* clustered with the genus *Aeromachus*, and the other clade was comprised of three species including the type species, *A. dioscorides*. The genus *Ochus* was nested within the second clade. The two species, *nana* and *dalailama*, traditionally assigned to *Ampittia*, share an unibifid uncus of the male genitalia with *Aeromachus* (the type species *Thanaos stigmatus* Moore), whereas species of *Ampittia* (the type species *Hesperia dioscorides* Fabricius) share bifid uncus (Fig. 4). Therefore, we agree with Wu & Hsu (2017) that these two species should be transfer to *Aeromachus*, as *Aeromachus nana* and *A. dalailama*. The monobasic genus *Ochus* (the type species *Cyclopides subvittatus* Moore) shares the following characters with *Ampittia*: male genitalia with uncus as

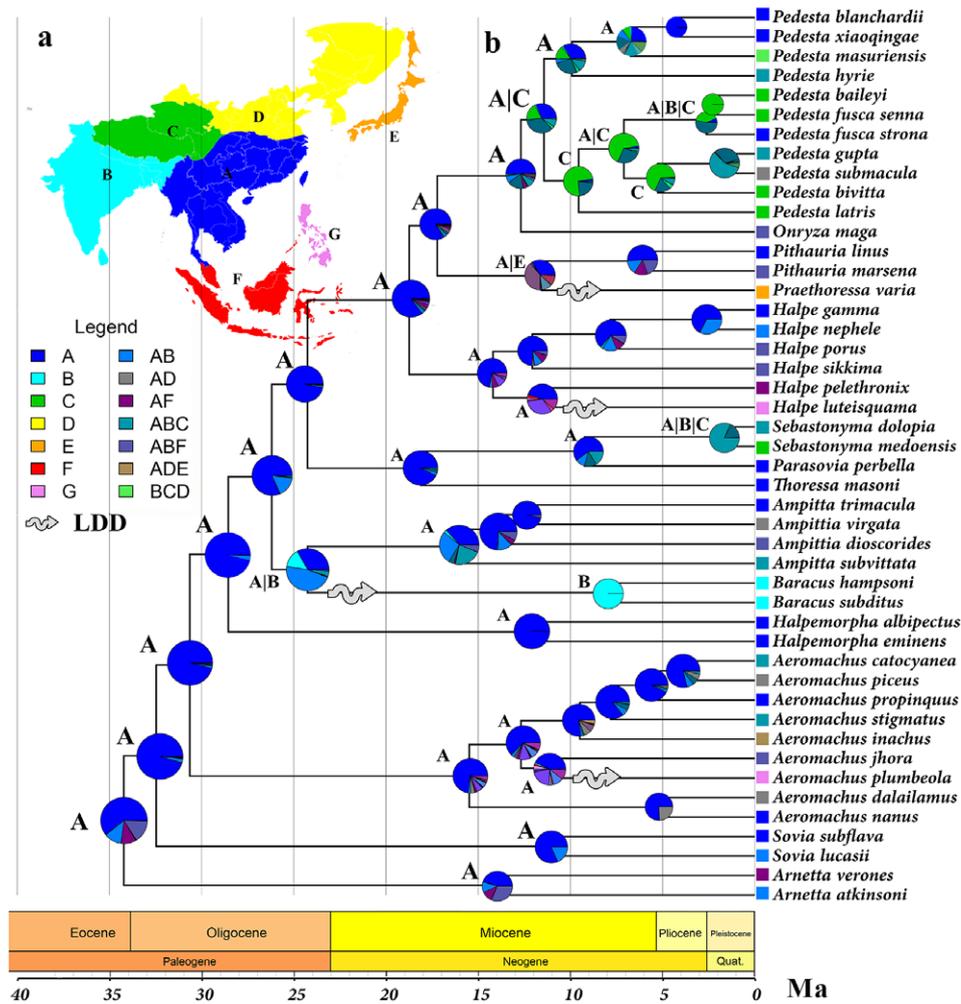


Fig. 3. Historical biogeography of the tribe Aeromachini butterflies using BioGeoBEARS. (a) Coloured boxes identify biogeographical regions: (A) Southeast Asia, including South China and Indochina, (B) Indian Subcontinent, (C) Southwest Mountains of China [including Himalayan–Hengduan Mountains and West Sichuan (SWC)], (D) North China and Russian Far East, (E) Japan and Sakhalin (JS), (F) Malay Archipelago and (G) Philippines. Species distributions with more than one area have combined letters for biogeographical regions and alternate colour boxes. (b) The ancestral areas of Aeromachini was inferred under DEC model with founder-event speciation (DEC+J). Pie charts showed relative probabilities of ancestral areas and the most probable ancestral areas were given at each pie chart. LDD, long-distance dispersal. Grey vertical lines refer to time intervals used in the analysis. [Colour figure can be viewed at wileyonlinelibrary.com.]

broad as tegumen and bifid apically (Fig. 4). Based on the phylogenetic analyses and morphological characteristics, *Ampittia* and *Ochus* should be merged into one. According to the rule of priority, *subvittatus* is herewith subsumed under *Ampittia*, as *Ampittia subvittatus* **comb.n.**

Currently, the genus *Aeromachus* comprises 17 species distributed in the Oriental and Palearctic Regions (Yuan *et al.*, 2015a; Kunte *et al.*, 2017; Wu & Hsu, 2017; present study), but the knowledge on the inter-relationships within *Aeromachus* is limited. Larsen & Congdon (2012) expected to establish a new genus for the African species of *Ampittia*. However, they concluded that the African species were congeneric with the Asian species using a combination of biological information and male genitalia characters as well as wing venation. We were unable to

sample the African taxa to settle this issue. According to our proposal, *Ampittia* now consists of three African species and four Asian species, whose inter-relationships are uncertain. Further studies are warranted to investigate the species-level relationships among and within *Aeromachus* and *Ampittia*.

Genus *Thoressa* Swinhoe, 1913

The genus *Thoressa* traditionally includes 25 species distributed in the Oriental and Palearctic Regions (Yuan *et al.*, 2015a). Morphologically, these species are complex and diverse. Our phylogenetic analyses clearly showed that the genus is a polyphyletic assemblage. The eight species examined here



Fig. 4. Genitalia of Aeromachini species (a–f and h, i, male; g, female). (a) *Aeromachus nana*; (b) *Aeromachus dalailama*; (c) *Aeromachus stigmatus*; (d) *Halpemorpha eminens* **comb.n.**; (e) *Sovia lucasii*; (f, g) *Praethoressa varia* **comb.n.**; (h) *Ampittia subvittatus* **comb.n.**; (i) *Ampittia dioscorides*. [Colour figure can be viewed at wileyonlinelibrary.com].

formed four distinct clades: *masoni* clade, *varia* clade, *hyrie* clade and Clade B (see below for details). The type species, *T. masoni*, shows a sister relationship with a pair of genera, *Parasovia* and *Sebastonyma*, with strong support values. This species differs morphologically from most of the representatives of the traditional concept of *Thoressa* in the following characters: typical ‘*Halpe* brand’ absent; uncus slightly concave distally; tegumen with short and angled shoulders; uncus narrower than tegumen; gnathos small, spoon-shaped and covered with spines, and separated distally; valvae symmetric with a complex and serrate footstalk (Evans, 1949; present study). It does, however, share the following morphological characteristics (Fig. 5): angled shoulders of tegumen, gnathos, symmetric valvae and yellow spots in spaces Rs-CuA₂ on hindwing upperside. *Thoressa masoni* Moore, along with the unsampled *T. decorata* Moore, *T. monastyrskiyi* Devyatkin and *T. honorei* de Nicéville are the only species within the genus (Evans,

1949; Devyatkin, 1996; Tsukiyama *et al.*, 1997). Therefore, we remove all but four species – *T. masoni* Moore (type species), *T. decorata* Moore, *T. monastyrskiyi* Devyatkin and *T. honorei* de Nicéville – from the genus. Among these four species, only *T. decorata* also shares the male ‘*Halpe* brand’ and distally bifid uncus with most of the traditionally recognized *Thoressa*. However, the above-mentioned morphological similarity is prominent, suggesting that these four species are members of the same genus. Further study is necessary to confirm this hypothesis.

Genus *Pedesta* Hemming, 1934

Pedesta is a replacement name for *Pedestes* Watson, which was pre-empted by *Pedestes* Gray by Hemming (1934). According to Evans (1949), the genus *Pedesta* morphologically resembles *Thoressa*, but can be separated from the latter by the following



Fig. 5. Genitalia of *Thoressa* spp. (j) *Thoressa masoni*; (k) *Thoressa monastyrskiyi*. [Colour figure can be viewed at wileyonlinelibrary.com].

characters: antennal apiculus blunt and obtuse, with nudum of 11 to 12 segments; male genitalia lateral processes absent. Actually, the nudum is often variable within taxa and not a good diagnostic character (de Jong, 1983; Huang & Zhan, 2004). Huang & Zhan (2004) treated *Pedesta* as a synonym of *Thoressa*, based on the nudum number, shape of the apiculus, and gradual process of change in lateral processes within these two genera. However, this arrangement is not appropriate because they did not examine both of the type species, *P. masuriensis* and *T. masoni*. In our phylogeny, six species of *Pedesta* including the type species *Isoteinon masuriensis* Moore and five species of traditionally recognized as *Thoressa* analysed here formed a strongly supported monophyletic group, which was widely separated from the *masoni* clade. Our morphological study showed that these species shared the following morphological characteristics: (i) males of most of the species have a stigma in space CuA_2 and 2A on the upper side of the forewing; (ii) gnathos developed, fused with each other, and distally armed with small spines; and (iii) valvae asymmetric (Fig. 6). For lateral processes of tegumen, we found that this taxon was different from that in the traditional concepts of *Pedesta* or *Thoressa*, changing gradually within all these species of the *Pedesta* clade (Fig. 6) as follows: absent (*P. masuriensis*), short and obtuse (*P. baileyi*), short and slightly pointed (*T. latris*), short and pointed (*P. gupta*, *P. fusca*), long, slender and pointed

(*P. submacula*). Therefore, the genus *Pedesta* recognized here includes all the traditionally assigned *Pedesta* species and some *Thoressa* species sharing the above morphological characters.

Implications for taxonomy and biogeography

Reconstructing the ancestral range of the tribe Aeromachini clearly pointed to Southeast Asia as its area of origin, from which it has subsequently expanded to the adjacent regions. Within this tribe, the common ancestors of most genera originated in Southeast Asia, which is characterized as *in situ* diversification, and then subsequently underwent a range expansion to neighbouring areas. Our phylogenetic analyses showed that the genus *Arnetta* as defined in this study is the basal lineage within the Aeromachini. Biogeographical analyses showed that the most probable ancestral range of *Arnetta* was in Southeast Asia, which indicated that *A. atkinsoni* originated in Southeast Asia and subsequently moved eastward and invaded the Indian Subcontinent. *Sovia* had a similar origination and dispersal pattern to that of *A. atkinsoni*. A founder-event speciation event was inferred to have occurred for *Baracus* during the late Miocene, indicating a direction of colonization from Southeast Asia to the Indian Subcontinent. As argued by Holloway (1974), de Jong (1983) and Kunte (2016), these

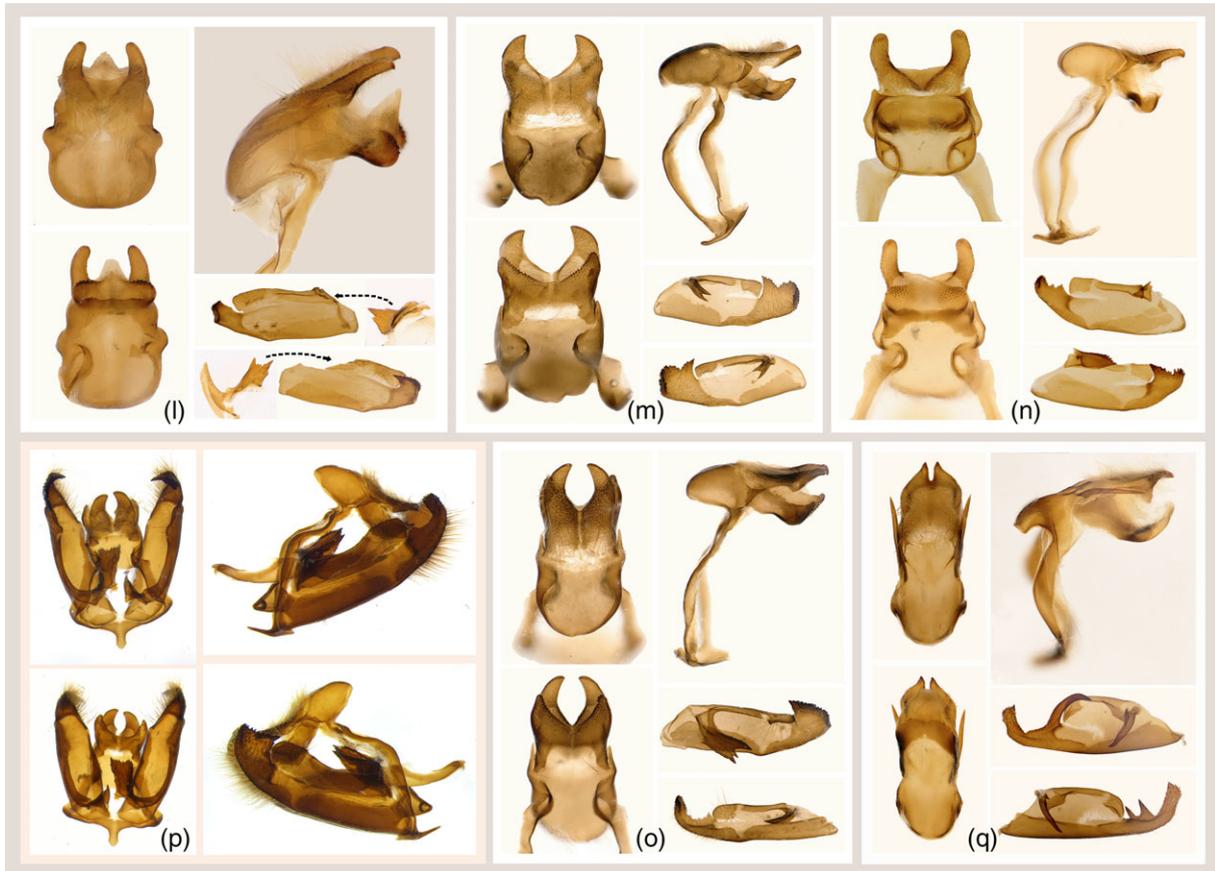


Fig. 6. Genitalia of *Pedesta* spp. (l) *Pedesta masuriensis masuriensis*; (m) *Pedesta baileyi*; (n) *Pedesta latris* **comb.n.**; (o) *Pedesta fusca senna* **comb.n.**; (p) *Pedesta gupta* **comb.n.**; (q) *Pedesta submacula* **comb.n.** [Colour figure can be viewed at wileyonlinelibrary.com].

butterflies should have originated after the collision of the Indian tectonic plate with Asia, which is in line with the results of our analyses. In addition, dispersal events of several taxa were dated during the mid-Miocene (*c.* 14–7 Ma) and coincided with the period when the Indian plate collided with Asia (*c.* 55 Ma; Hall, 2012). As mentioned above, two species (*A. plumbeola* and *H. lutsquama*) colonized the Philippines via long-distance dispersal, both during the mid-Miocene when the proto-Philippines Archipelago was probably connected (Hall, 2002). Three potential colonization routes into the Philippines have been outlined (Wu *et al.*, 2009; Esselstyn & Oliveros, 2010; Heaney *et al.*, 2012; Matuszak *et al.*, 2015; Kyriazis *et al.*, 2018, and references therein). The eastern route was from Borneo to Mindoro using Palawan as a stepping-stone, which may have been a land-bridge during the Pleistocene. The southern route was from Borneo to Mindanao via Sulu Island. A northern route was from mainland Asia and/or Taiwan to the Philippines. The northern hypothesis is only supported by long-distance dispersal, because of the distant isolation of Luzon and the Asian continent by the Bashi strait (Wu *et al.*, 2009). The ancestors of these two species were inferred to have come from Southeast Asia during the mid-Miocene, supporting the northern route. Another long-distance event was also inferred for *P. varia*

dispersing to the Japanese and Sakhalin Archipelago during the mid-Miocene (*c.* 11.57 Ma). Japan and Sakhalin have been an archipelago since the Miocene (25–15 Ma), when the Japanese Sea separated them from East Asia (Maruyama *et al.*, 1997; Isozaki *et al.*, 2010). The common ancestor of *Pedesta* likely had colonized Southeast Asia and SWC by the mid-Miocene and diversified there. During the mid-Miocene, the Southwest Mountains (including the Himalayan–Hengduan Mountains) were already high and undergoing extensive uplift (Clift, 2006; Wang *et al.*, 2012). Vicariance thus seems likely to have played a significant role in the diversification of *Pedesta*. Dramatic crustal deformation induced by the Indo-Asian collision contributed to the complicated landscapes in western China. Massive mountains and deeply carved valleys formed, which acted as barriers to expansion and resulted in speciation, that subsequently gave rise to two interesting lineages, of which one originated from Southeast Asia and another from SWC. Thus, the SWC lineage might have undergone an *in situ* adaptive radiation due to vicariance. This pattern also can be found in amphibians (Che *et al.*, 2010; Li *et al.*, 2013). On the basis of adult morphological and pupal characters, the African *Ampittia* species are congeneric to the Asian species, although only the Asian species were sampled in this study. The present *Ampittia* grouping suggests that there

was at least one trans-oceanic dispersal event from Southeast Asia to the Afrotropical Region, although the divergence time of this event was not clear. In summary, our findings indicated that the probable common ancestor of the *Aeromachini* came from Southeast Asia. Diversification *in situ* and range expansion to adjacent areas have played essential roles within the *Aeromachini*, but founder-event speciation and vicariance also have been important factors in shaping the diversification of this tribe. However, this pattern might be challenged if additional African, island, and SWC endemic species samples were included.

Evans (1937, 1949, 1951) treated Hesperidae of different geographical regions separately. He considered that many genera are distributed both in Africa and Asia, such as *Parnara*, *Pelopidas*, *Borbo*, *Isoteinon*, *Astictopterus*, *Celaenorrhius*, *Tagiades*, *Sarangesa*, *Caprona*, *Spialia*, *Ampittia*, *Kedestes* and *Gegenes*. Larsen (2005) first challenged Evans's Hesperidae classification. New taxonomic frameworks of Hesperidae have recently been proposed (Warren *et al.*, 2008, 2009; Sahoo *et al.*, 2016), although disagreements at the subfamily and tribe levels still exist. Recently, Fan *et al.* (2016) clarified the phylogeny of Baorini (Hesperidae: Hesperinae) and established a new genus for some of the African species traditionally assigned to *Borbo*. The widespread *Borbo* was separated into two distinct clades. The clade of four African *Borbo* (*holtzi*, *perobscura*, *gemella* and an unidentified species) was found to be the basal clade and sister to the rest of the tribe, implying that the probable ancestral area of Baorini may have been Africa. While investigating the phylogeny of the Old World Hesperidae, we were fortunate to be able to include two African *Astictopterus* samples in analyses. Surprisingly, the African *Astictopterus* clade is clearly far separated from the Asian clade, namely from the type species *Astictopterus jama* (Huang *et al.*, in preparation). Many African and Asia lineages were placed into one genus based on morphological characters, but the molecular phylogeny revealed that these lineages always show relative distant relationships. Examples of this phenomenon also are found in other lepidopteran groups and different zoogeographical regions, including nymphalid butterflies (e.g. Wahlberg & Freitas, 2007; Aduse-Poku *et al.*, 2016) and erebid moths (e.g. Zenker *et al.*, 2017). Such so-called congeneric taxa that occur in different biogeographical regions or on different tectonic plates are not always actually members of the same genus, as we 'traditionally' assumed. Therefore, taxonomic classification of such genera that occur across different biogeographical regions or geotectonic plates needs to be inspected more closely. These taxa, particularly the endemics, are critical in investigating historical biogeography, range expansions and adaptive radiations to shed light on how Earth's biodiversity has been shaped.

New taxonomic descriptions

***Halpemorpha* Huang, Fan & Chiba gen.n.**

<http://zoobank.org/urn:lsid:zoobank.org:act:EAEAA45E-8924-48E6-BC50-9353AC824F7C>.

Type species: *Sovia eminens* Devyatkin, 1996

Etymology

The name is derived from the genus *Halpe* with respect to the similarity in external features.

Diagnosis

Antenna almost equal to 2/3 forewing length. Labial palpus erect, densely covered with black scales dorsally and grey-white scales ventrally; the third segment short, porrect, and slightly protruding. Forewing upperside ground colour black; three white spots usually present in space R_3 - R_5 ; two cell spots separated. Underside almost same as upperside, but paler and with a row of obscure or distinct whitish submarginal stripes. Hindwing ground color black, upperside without spots, but underside with black, grey or white striped pattern. Typical 'Halpe' stigma located between spots in discal cell and CuA_1 .

Male genitalia (Fig. 4d)

Uncus narrower than tegumen, tongue-shaped, and bluntly cut apically; gnathos developed, densely covered with small spines; valvae broad and symmetric, and with a spined transtilla; aedeagus straight, nearly as long as valvae.

Remarks

We can easily distinguish the new genus from 'Sovia' by the following characters: cell spots separated; male stigma located between the spots in space CuA_1 and cell. In *Sovia*, cell spots conjoined and male stigma located below spot in space CuA_1 . The genus is also similar to *Parasovia* in wing pattern on the upperside, but markings on the underside of hindwing and male genitalia are completely different, namely socius absent, uncus unbifid and apically flat; whereas *Parasovia* has long and slender socius, and uncus apically blunt.

Based on the combination of characters of two separated cell spots and male genitalia (Evans, 1949; Devyatkin, 1996; Miyazaki & Saito, 2010; Xue *et al.*, 2015), we move two other 'Sovia' species, *hyrtacus* and *ueharai*, to the new genus. To date, the genus *Halpemorpha* comprises four species: *Halpemorpha eminens* **comb.n.**, *H. albipectus* **comb.n.**, *H. hyrtacus* **comb.n.**, and *H. ueharai* **comb.n.** The *Halpemorpha* are distributed from South China, through Vietnam and Myanmar, westward to the Western Ghats of SW India.

***Praethoressa* Huang, Chiba & Fan gen.n.**

<http://zoobank.org/urn:lsid:zoobank.org:act:E4E3D67A-CDC0-4876-9305-4DFAD4715BF6>.

Type species: *Pamphila varia* Murray, 1876

Etymology

The name is derived from false (Prae-) *Thoressa*, indicating the previous misplacement.

Diagnosis

Antennae slightly longer than 1/2 forewing length. Labial palpus with second segment stout and erect, covered with

ochreous hairs ventrally, third segment black and porrect. Wing underside with submarginal series of yellow spots separated by black veins; forewing with two cell spots or only lower one; hindwing underside usually with three yellowish white spots in spaces R_s , M_3 and CuA_1 . Male stigma below the cell spot.

Male genitalia (Fig. 4f)

Tegumen broad, nearly equal to uncus in length with long and slender lateral processes; uncus broad, flat and inwardly hollow, deeply bifid, U-shaped; gnathos well-developed, fused with each other, with spines ventrally; valvae symmetric; costa almost as long as sacculus; footstalk developed with small spines; ventrodistal process rectangular distally, upper edge with a long robust spine, and outer edge straight, covered with small spines; aedeagus straight.

Female genitalia (Fig. 4g)

Lamella antevaginalis boat-shaped, and lateral angle pointed; lamella postvaginalis plate-shaped, upper edge U-shaped medially, and overlapping lamella antevaginalis; ductus bursa short, membranous; corpus bursa oblong.

Remarks

The new genus externally resembles *Thoressa* s.s. and *Pedesta*, but it can be distinguished from both by the following characters: wing underside with submarginal series of yellow spots; uncus flat and inwardly hollow.

Both the molecular phylogeny and morphological characters support this new genus as distinct from all the other known genera. An endemic species from Taiwan, *horishana*, resembles *varia* in male genitalia and wing pattern so much that it is sometimes considered a subspecies of *varia* (Evans, 1949; Shirôzu, 1960; Tsukiyama *et al.*, 1997; Hsu, 2015; Wu & Hsu, 2017). Thus, we transfer this species to *Praethoressa*, as *Praethoressa horishana* **comb.n.** The genus *Praethoressa* extends its range northward to Sakhalin and the Kuril Islands, across Japan, and southernmost to Taiwan. On the other hand, the distribution of *Thoressa* (s.s.) (present study) extends further southward than that of *Praethoressa*, ranging northeastward to Vietnam, and southwestward to southern India. These distributional patterns are in line with our phylogenetic and morphological conclusions.

Supporting Information

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

Table S1. Information and GenBank accession numbers of the voucher specimens used in this study. *indicates that gene sequence was downloaded from NCBI.

File S1. The best-fit partition schemes and models of molecular evolution of *Aeromachini* data matrix. The best fitting model was selected by PARTITIONFINDER under BIC.

File S2. The models were selected by MODELFINDER implemented in IQ-Tree.

File S3. ML tree from RAxML under GTR+GAMMA with best-fit partitions.

File S4. The MCC trees from BEAST.

File S5. Taxonomic changes raised by this study; * denotes that the taxonomic changes were only based on morphological characters.

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