Clarification of the Phylogenetic Framework of the Tribe Baorini (Lepidoptera: Hesperiidae: Hesperiinae) Inferred from Multiple Gene Sequences

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Abstract

Members of the skipper tribe Baorini generally resemble each other and are characterized by dark brown wings with hyaline white spots. These shared characteristics have caused difficulties with revealing the relationships among genera and species in the group, and some conflicting taxonomic views remain unresolved. The present study aims to infer a more comprehensive phylogeny of the tribe using molecular data, to test the monophyly of the tribe as well as the genera it includes in order to clarify their taxonomic status, and finally to revise the current classification of the group. In order to reconstruct a phylogenetic tree, the mitochondrial COI-COII and 16S genes as well as the nuclear EF-1α and 28S genes were analyzed using parsimony, maximum likelihood, and Bayesian inference. The analysis included 67 specimens of 41 species, and we confirmed the monophyly of Baorini, and revealed that 14 genera are well supported. The genus Borbo is separated into three clades: Borbo, Pseudoborbo, and Larsenia gen. nov. We confirmed that Polytremis is polyphyletic and separated into three genera: Polytremis, Zinaida, and Zenonoida gen. nov., and also confirmed that the genus Prusiana is a member of the tribe. Relationships among some genera were strongly supported. For example, Zenonia and Zenonoida were found to be sister taxa, closely related to Zinaida and Iton, while Pelopidas and Baoris were also found to cluster together.

Introduction

The family Hesperiidae, commonly known as skippers or skipper butterflies, comprises approximately 4000 species belonging to 540 genera worldwide [1] and is defined by the following unique morphological character states: an "eye ring", a wide head, an area of small and specialized scales on the upper side of the hindwing base, and a large thorax, resulting in the mesoscutellum overhanging the metanotum [2]. These unique character states support
monophyly of the family [2–4], which has also been supported by molecular data [5]. The higher classification of Hesperiidae had long remained unchanged until Warren et al. challenged it with phylogenetic scheme [2, 5]. The traditional framework of six subfamilies was rearranged into seven: Coeliadinae, Euschemoninae, Eudaminae, Pyrginae, Heteropterinae, Trapezitinae, and Hesperinae. Moreover, they reinterpreted Evans’ [6] genus groups as tribes, and furthermore the subfamily Hesperinae was subdivided into six tribes, and the tribe Baorini was proposed [5]. Monophyly of the tribe was strongly supported by both a study using only molecular data [5] as well as another that combined molecular and morphological data [2].

The tribe Baorini was originally introduced as Baorina, one of the subfamilies of Hesperidae [sic] [7]. The subfamily level designation was also used by Bell [8] in which Baoris Moore, 1881, Caltoris Swinhoe, 1893, Chapra Moore, 1881, and Iton de Nicéville, 1895 were included. Evans [6] placed these genera in his Gegenes group. Subsequently, Eloit named it the Pelopidas group [9, 10], and Chou employed the tribe name Gegenini [11, 12]. According to Code article 23.3.1 and 34.1 of International Commission on Zoological Nomenclature [13], the designation Baorini is more appropriate than Gegenini.

Most members of the group resemble each other and have dark brown wings and hyaline white spots. Mainly due to this simple wing pattern, researchers have struggled to determine which species were related and should be assigned to the same genus. In the renowned Die Gross Schmetterlinge der Erde, all members, except for Gegenes, were placed in Parnara [14–16]. Simultaneously, Zenonia zeno was considered a species of Padraona, whose markings are orange and yellow. It is worth noting that Mabille described the genus Polytremis in 1904 [17], but this description was not reflected in a later publication in 1909 [14]. Evans [18, 19] also initially published contradictory definitions of the taxonomy within the group. Initially, he assigned almost all species to Baoris, except for some that were placed in the genera Iton and Gegenes, even though all of the major genera had been previously described. Evans worked extensively on the group until he finally settled on eight genera [6].

In most current taxonomic studies, the six genera mentioned above as well as two African allies, Zenonia and Brusa, are treated as members of the same group, regardless of the name used. However, Prusiana, Pseudoborbo, and Zinaida are exceptions and further explanation clarifying why they are distinct is necessary.

Although Evans recognized that the genitalia of the genus Prusiana were the same as the Gegenes group, he still treated the genus as a member of his Taractrocera group and placed it after the genus Cephrnes [6]. De Jong considered Prusiana to be a rather enigmatic group due to its unclear relationship to other genera [20]. Maruyama, regarded the difference in genitalia morphology to be an important taxonomic character, and moved the genus into the Pelopidas group [21], which is currently generally followed in classification schemes [2, 22].

Hesperia bevani Moore, 1878 was assigned previously to various genera, such as Baoris [23–26], Parnara [14, 16, 27–38], Caltoris [39, 40], or Pelopidas [19]. Since Evans described the genus Borbo [6], this species is usually placed in this genus [10, 22, 41–44]. Subsequently, Lee described the genus Pseudoborbo based on the adult and immature morphological characters of Borbo bevani and then reclassified this species as his monotypic genus [45]. Some subsequent authors, however, did not support Lee’s arrangement and considered the genus Pseudoborbo to be a synonym of Borbo [2, 5, 46, 47], while others followed Lee’s classification [1, 11, 12, 48–52].

The genus Zinaida was described by Evans with Parnara nascens Leech, 1893 as its type species. In addition to the type species, Z. theca Evans, 1937 was described and Pamphila caerulescens Mabille, 1876 and Pamphila mencia Moore, 1877 were also included in the genus [19]. Without any explanation, however Evans treated Zinaida as a synonym of the genus Polytremis.
Mabille, 1904 [6]. Subsequent authors also followed this classification scheme [1, 10–12, 41, 42, 52].

Few phylogenetic analyses involving the tribe Baorini have been published. Dodo et al. analyzed mitochondrial ND5 and COI of Japanese skippers, and concluded that the genera *Pelopidas* and *Parnara* were monophyletic groups [53], which we have confirmed in this study. Warren et al. investigated the phylogenetic relationships of subfamilies and the circumscription of tribes of the family Hesperiidae based on molecular data [5]. Baorini included only four species belonging to three genera—*Pelopidas*, *Iton*, and *Polytremis*—and it was concluded that the monophyly of the Baorine clade was strongly supported. Warren et al. used 49 morphological characters and molecular data to revise the classification of the family Hesperiidae and confirmed the robust monophyly of the tribe Baorini [2], although, only the above three genera were included. A molecular phylogenetic study of Chinese skippers, which sampled only six species across three genera (*Parnara*, *Pelopidas*, and *Polytremis*), provided evidence that the tribe is monophyletic [54].

Jiang et al. constructed a phylogeny of the genus *Polytremis* from China using one mitochondrial and two nuclear derived genes and claimed that the monophyly of the genus was supported [55]. Yuan et al. analyzed three mitochondrial genes of three species from China, but could not confirm these findings [54]. Our results also contradict the conclusions made by Jiang et al. [55].

The objectives of the present study were to infer a more comprehensive phylogeny of the tribe Baorini using molecular data, to test the monophyly of the tribe Baorini, to clarify the taxonomic status of multiple genera, and to revise the current classification within this tribe if necessary. A well-resolved phylogeny of the tribe Baorini will enhance the understanding of the evolution and biology among species within this group.

**Materials and Methods**

**Taxon sampling**

Samples were obtained from all major genera in the tribe Baorini except for *Brusa*. When possible, the type species was included and multiple species were chosen in controversial genera to correctly clarify taxonomic status. In total, 67 specimens representing 41 species across 11 genera of the tribe Baorini were selected as ingroup taxa. Specifically, we included the genus *Pseudoborbo*, which has been considered a synonym of *Borbo* by some authors; *Prusiana*, which was considered a member of Taractrocera group [6]; and *Polytremis nascens*, the type species of the genus *Zinaida*, believed to be a synonym of *Polytremis*. An additional six species, including single representatives from two genera of the Taractrocerini tribe, *Taractrocera* and *Telicota*, as well as the genera *Aeromachus*, *Ampittia*, *Daimio*, and *Tagiades* were used as outgroups to assess the status of the genus *Prusiana* and the stability of basal relationships among ingroup lineages. Voucher specimens representing all sampled species were deposited in the Insect Collection of the South China Agricultural University (SCAU). Specimen information and location data are presented in Table 1.

**DNA extraction, PCR amplification, and sequencing**

Total genomic DNA was extracted from the thorax of specimens preserved in ethanol, or from one to three legs of dried specimens. The tissues were macerated in 500 μL Proteinase K solution (10 mM Tris HCl, 10 mM EDTA, 150 mM NaCl, and 0.5 mg/mL proteinase K), and incubated at 55°C for 2–3 h. The resulting solution was extracted once with phenol saturated with TE buffer (10 mM Tri-HCl [pH 8.0] and 1 mM EDTA), once with phenol/chloroform (1:1), and once with chloroform/isoamyl alcohol (24:1). The total DNA was precipitated by adding
| Taxon | Locality | Voucher | The type species | GenBank Accession Nos. |
|-------|----------|---------|------------------|-----------------------|
|       |          |         |                  | **16S** | **COI-COII** | **28S** | **EF-1a** |
| Parnara guttata (Bremer & Grey, 1853) 1 | China: Guangdong, Yingde | He001 | ● | JX971164 | JX989082 | JX989114 | KX151612 |
| Parnara guttata (Bremer & Grey, 1853) 2 | China: Guangdong, Yingde | He003 | ● | JX971165 | JX989083 | JX989115 | KX151613 |
| Parnara ganga Evans, 1937 1 | China: Hainan, Jianfengling | He028 | | JX971166 | JX989084 | JX989116 | KX151609 |
| Parnara ganga Evans, 1937 2 | China: Hainan, Jianfengling | He029 | | JX971167 | JX989085 | JX989117 | KX151610 |
| Parnara ganga Evans, 1937 3 | China: Hainan, Jianfengling | He030 | | JX971168 | JX989086 | JX989118 | KX151611 |
| Parnara bada (Moore, 1878) | China: Guangxi, Maoershan | He012 | | JX971169 | JX989087 | JX989119 | KX151608 |
| Polytremis lubricans (Herrich-Schäffer, 1869) 1 | China: Guangdong, Nanling | He095 | ● | JX971170 | JX989088 | JX989120 | KX151619 |
| Polytremis lubricans (Herrich-Schäffer, 1869) 2 | China: Hainan, Jianfengling | He160 | ● | JX971171 | JX989089 | JX989121 | KX151620 |
| Polytremis lubricans (Herrich-Schäffer, 1869) 3 | Malaysia: Perak,Kinta Highland | He549 | ● | KX151512 | KX151572 | - | KX151621 |
| Polytremis lubricans (Herrich-Schäffer, 1869) 4 | Malaysia, Perak,Kinta Highland | He550 | ● | KX151513 | - | KX151545 | KX151622 |
| Polytremis lubricans (Herrich-Schäffer, 1869) 5 | Malaysia, Perak,Kinta Highland | He551 | ● | KX151514 | KX151573 | KX151546 | KX151623 |
| Polytremis caerulescens (Mabille, 1876) | China: Sichuan, Luding, Moxi | He087 | | JX971172 | JX989090 | JX989122 | KX151616 |
| Polytremis zina zina (Evans, 1932) | China: Guangdong, Nanling | He037 | | JX971173 | JX989091 | JX989123 | KX151631 |
| Polytremis zina taiwana Murayama, 1981 | Taiwan | He545 | | KX151519 | KX151578 | KX151551 | KX151632 |
| Polytremis theca theca (Evans, 1937) | China: Shaanxi, Qinling | He503 | | KX151518 | KX151577 | KX151550 | KX151630 |
| Polytremis theca fukia Evans, 1940 | China: Guangdong, Nanling | He009 | | JX971174 | JX989092 | JX989124 | KX151629 |
| Polytremis suprema Sugiyama, 1999 | China:Guangdong, Nanling | He070 | | JX971175 | JX989093 | JX989125 | KX151628 |
| Polytremis nascens (Leech, 1893) | China: Sichuan, Baoxing | He100 | ● | JX971176 | JX989094 | JX989126 | KX151626 |
| Polytremis gotama Sugiyama, 1999 | China: Yunnan, luguhu | He010 | | JX971177 | - | JX989127 | - |
| Polytremis mencia (Moore, 1878) | China: Sichuan, Hailuogou | He502 | | KX151516 | KX151575 | KX151548 | KX151625 |
| Polytremis matsuii Sugiyama, 1999 | Malaysia: Kumamoto | He392 | | KX151517 | KX151576 | KX151549 | KX151627 |
| Polytremis discreta (Elwes & Edwards, 1897) 1 | Vietnam: Dac Lae, Chu Yang Sin | He447 | | KX151506 | - | KX151539 | - |
| Polytremis discreta (Elwes & Edwards, 1897) 2 | China: Sichuan, Hanjuan | He448 | | KX151507 | - | KX151540 | - |
| Polytremis discreta (Elwes & Edwards, 1897) 3 | China: Sichuan, Yaan | He481 | | KX151508 | KX151570 | KX151541 | KX151617 |
| Polytremis eltola (Hewitson, 1869) 1 | China: Hunan, Mangshan | He104 | | KX151509 | - | KX151542 | - |
| Polytremis eltola (Hewitson, 1869) 2 | Vietnam: Dac Lae, Chu Yang Sin | He446 | | KX151510 | - | KX151543 | - |
| Polytremis eltola (Hewitson, 1869) 3 | China: Hunan, Mangshan | He509 | | KX151511 | KX151571 | KX151544 | KX151618 |
| Borbo borbonica (Boisduval, 1833) | Kenya: Embu | JS064 | ● | KX151490 | KX151557 | KX151525 | - |
| Borbo cinnara (Wallace, 1866) 1 | China: Guangdong, Nanling | He017 | | JX971178 | JX989095 | JX989128 | KX151587 |
| Borbo cinnara (Wallace, 1866) 2 | China: Hainan, Jianfengling | He017 | | JX971179 | JX989096 | JX989129 | KX151588 |
| Borbo fatuellus (Hopffer, 1855) 1 | Liberia: Nimba mountains | Tok17 | | KX151492 | KX151559 | KX151527 | - |
| Borbo fatuellus (Hopffer, 1855) 2 | Liberia: Nimba mountains | VA35 | | KX151491 | KX151558 | KX151526 | KX151589 |
| Borbo gemella (Mabille, 1884) | Liberia: Nimba mountains | GA13 | | KX151493 | KX151560 | KX151528 | KX151590 |
| Borbo holtzi (Plötz,1883) 1 | Liberia: Nimba mountains | VA24 | | KX151494 | KX151561 | - | KX151591 |

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Table 1. (Continued)

| Taxon                           | Locality                  | Voucher     | The type species | GenBank Accession Nos. |
|---------------------------------|---------------------------|-------------|------------------|------------------------|
|                                 |                           |             |                  |                        |
| **Borbo holtzi** (Plötz, 1883) 2| Liberia: Nimba mountains  | VA40        |                  | KX151495 KX151562 - - |
| **Borbo perobscura** (Druce, 1912) | Liberia: Nimba mountains | GA8         |                  | KX151496 KX151563 KX151529 - |
| **Borbo ratek** (Boisduval, 1833) 1| Madagascar               | JS071       |                  | KX151497 KX151564 KX151530 KX151592 |
| **Borbo ratek** (Boisduval, 1833) 2| Madagascar               | Szs-BOR-004 |                  | KX151498 KX151565 KX151531 KX151593 |
| **Borbo ratek** (Boisduval, 1833) 3| Madagascar               | Szs-BOR-006 |                  | KX151499 - KX151532 KX151594 |
| **Borbo sp.**                   | Liberia: Nimba mountains  | GA19        |                  | KX151500 KX151566 KX151533 KX151595 |
| **Pseudoborbo bevari** (Moore, 1878) | China: Guangdong, Yingde | He018       |                  | JX971180 JX989097 JX989130 KX151633 |
| **Pelopidas mathias** (Fabricius, 1798) | China: Fujian          | He194       |                  | JX971181 JX989098 JX989131 KX151615 |
| **Pelopidas agna** (Moore, 1866) | China: Hainan, Jianfengling | He013       |                  | JX971182 JX989099 JX989132 KX151614 |
| **Pelopidas thrax** Hübner, 1821* | Ghana: Ashanti Region    | -           |                  | EU364491* - EU364286* |
| **Caltoris bromus** (Leech, 1893) 1| China: Guangdong, Yingde | He002       |                  | JX971183 JX989100 JX989133 KX151596 |
| **Caltoris bromus** (Leech, 1893) 2| China: Guangxi, Maosheran | He024       |                  | JX971184 JX989101 JX989134 KX151597 |
| **Caltoris bromus** (Leech, 1893) 3| China: Guangxi, Maosheran | He025       |                  | JX971185 JX989102 JX989135 KX151598 |
| **Caltoris bromus** (Leech, 1893) 4| China: Hainan, Jianfengling | He032       |                  | JX971186 JX989103 JX989136 KX151599 |
| **Caltoris cahira** (Moore, 1878) 1| China: Guangxi, Maosheran | He022       |                  | JX971187 JX989104 JX989137 KX151601 |
| **Caltoris cahira** (Moore, 1878) 2| China: Guangxi, Maosheran | He023       |                  | JX971188 JX989105 JX989138 KX151602 |
| **Caltoris kumara** (Moore, 1878) | Java: Mt. Pagoberan       | He540       |                  | KX151504 KX151569 KX151537 KX151605 |
| **Caltoris malaya** (Evans, 1926) | Malaysia: Perak           | He541       |                  | KX151505 - KX151538 KX151606 |
| **Caltoris brunnea** (Snellen, 1876) | Java: Mt. Pagoberan       | He542       |                  | KX151501 KX151567 KX151534 KX151600 |
| **Caltoris cormasa** (Hewitson, 1876) 1| Java: Mt. Pagoberan       | He543       |                  | KX151502 - KX151535 KX151603 |
| **Caltoris cormasa** (Hewitson, 1876) 2| Malaysia: Perak           | He544       |                  | KX151503 KX151568 KX151536 KX151604 |
| **Baoris farri** (Moore, 1878) 1 | China: Hainan, Jianfengling | He091       |                  | JX971189 JX989106 JX989139 KX151584 |
| **Baoris farri** (Moore, 1878) 2 | China: Guangxi, Maosheran | He049       |                  | JX971190 JX989107 JX989140 - |
| **Baoris penicillata** (Moore, 1881) | China: Hainan, Yinggeling | He112       |                  | JX971191 JX989108 JX989141 KX151586 |
| **Baoris leechii** (Elwes & Edwards, 1897) | China: Guangdong, Nanling | He093       |                  | KX151489 - KX151524 KX151585 |
| **Iton semamora** (Moore, 1866) | Indonesia: Sumatra        | He239       |                  | JX971192 JX989109 JX989142 KX151607 |
| **Iton watsonii** (de Nicéville, 1890) | Thailand: Chiang Mai     | -           |                  | EU364490* - EU364285* |
| **Gegenes nostrodamus** (Fabricius, 1793) | Morocco: Marrakech       | He240       |                  | JX971193 - JX989143 - |
| **Prusiana prusias** (Frusthorer, 1911) 1 | Philippines: Leyte        | He241       |                  | JX971194 - JX989144 - |
| **Prusiana prusias** (Frusthorer, 1911) 2 | Philippines: C. Palawan  | He393       |                  | KX151520 KX151579 KX151552 KX151634 |
| **Zenonia zeno** (Trimen, 1864) 1 | Cameroon: N. Cameroon     | Szs-ZEN-001 |                  | KX151521 KX151580 KX151553 KX151635 |
| **Zenonia zeno** (Trimen, 1864) 2 | Kenya: Nairobi            | Szs-ZEN-002 |                  | KX151581 KX151554 - |
| **Aeromachus stigmatus** (Moore, 1878) | China: Yunnan, Hutiaoxia  | He434       |                  | KX151522 KX151582 KX151555 KX151636 |
| **Ampititia virgata** Leech, 1890 | China: Guangdong, Nanling | He008       |                  | KX151523 KX151583 KX151556 KX151637 |
| **Telicota augias** | China: Guangdong, Yingde | He082       |                  | JX971195 JX989110 JX989145 KX151638 |
| **Potanthus trachala** | China: Guangxi, Guiling  | He346       |                  | JX971196 JX989111 JX989146 KX151639 |

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twice the volume of 100% ethanol and one-tenth the volume of 3 M sodium acetate to the supernatant, washed with 70% ethanol, dried, and then dissolved in 80–100 μL TE buffer.

DNA from *Pseudoborbo bevani*, *Itön semamora*, *Prusiana kuehni*, and *Zenonia zeno* specimens was extracted from legs using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer’s protocol for animal tissue.

Four target regions were amplified by PCR using the primers listed in Table 2. PCR reactions were performed in 25 μL volumes containing 2.5 μL 10×PCR buffer (2.0 mM MgCl2), 2 μL dNTPs (containing 2.5 mM of each dNTP), 1 μL of each primer (10 pmol/μL), 1 μL of template DNA, and 1.25 units Taq DNA polymerase (Takara Inc, Shiga, Japan). The amplification cycle was 95°C for 5 min, and for the 16S and 28S genes was followed by 35 cycles of 94°C for 30 sec, then 47°C (16S) or 50°C (28S) for 30 sec and 72°C for 1.5 min. For the COI-COII and EF-1α, the initial 95°C at 5 min was followed by 35 cycles of 94°C for 1 min, 46°C (COI-COII) or 55°C (EF-1α) for 1 min and 72°C for 2 min. All amplification cycles included a final extension period of 72°C for 7 min. Successful amplification was verified using agarose gel electrophoresis.

PCR products were purified with a Gel DNA purification kit (Takara Inc), and were directly sequenced with the same primers listed in Table 2, or cloned and then sequenced. For cloning, the purified PCR products were cloned into the pMD18-T vector (Takara Inc) using *Escherichia coli* TG-1 as the host. At least three positive clones were selected for sequencing to correct for PCR errors. Sequencing was performed using the ABI 3730 automated sequencer. DNA sequences were assembled and edited with SeqManII in the DNASTAR package (DNASTAR Inc, Wisconsin, USA) and checked manually. All sequences were deposited in GenBank, and the accession numbers for each sequence are listed in Table 1.

### Data analyses

Alignments of the rRNA gene sequences were conducted with MAFFT (version 7) using separate gene partitions (16S and 28S) via the online sever (http://mafft.cbrc.jp/alignment/server/). We used the Q-INS-I strategy, which accounts for the secondary structure of the RNA and
small data sets (with less than 200 sequences), and '1PAM/κ = 2', which is recommended for aligning closely related DNA sequences and the offset was set at 0.1 when large gaps were not expected based on preliminary analyses [59–61]. Both the COI-COII (only one 3-bp gap) and EF-1α sequences were aligned using the Clustal X [62] with the default settings. All base frequencies and molecular character statistics were calculated using MEGA 6.0 [63]. Homogeneity of the base frequencies across taxa was tested using the Chi-square test implemented in PAUP* 4.0b10 [64]. The incongruence length difference (ILD) test [65] in PAUP* was conducted to evaluate the congruence of mitochondrial (COI-II and 16S) and nuclear (EF-1α and 28S) markers and determine if they could be analyzed together. Only taxa with sequence information for all four target regions were included in this analysis. Saturation for each gene and for the codon positions of COI, COII, and EF-1α were assessed using the substitution saturation test [66, 67] in the program DAMBE [68].

Phylogenetic trees were constructed using the maximum parsimony (MP), maximum Likelihood (ML), and Bayesian inference (BI) methods. MP analyses were conducted using TNT version 1.1 [69] with the following options: parsimony-informative characters were unordered and equally weighted, gaps were treated as missing data, searches heuristic using a “driven search” until the minimum length was hit ten times by means of a combination of TreeFusion, Ratchet, Tree Drifting, and Sectorial searches under default parameters [70]. Branch support was assessed using the bootstrap test [71] with 1000 replicates.

Prior to ML and BI analyses, the best-fit model of nucleotide substitution was selected using jModeltest 2.1.7 [72] for each gene region (COI (GTR+I+G), tRNAleu (HKY+I), COII (GTR+I+G), 16S (GTR+I+G), EF-1α (SYM+I+G), and 28S (GTR+I+G)), and by codon position for COI, COII and EF-1α (seven partitions: 1st+2nd (GTR+I+G) and 3rd codon positions (GTR+I+G) of the mitochondrial protein coding genes COI and COII together, same for the nuclear gene EF-1α (positions 1+2): SYM+I+G, position 3: GTR+I+G), the mitochondrial RNA genes tRNAleu and 16S, and also the nuclear 28S gene) under the Akaike Information Criterion [73].

ML analyses were carried out using RAxML version 8 [74] on a concatenated data set of all genes, with 1000 rapid bootstraps using both GTR+G and GTR+I+G. The topologies of the trees were consistent, and support values for the clades only differed slightly. Here, we have only presented the results from the analysis using the GTR+G model. Bayesian analyses were conducted using MrBayes 3.2.2 [75] using the best-fit model determined using the two above-mentioned schemes. Four simultaneous chains were run for 5×10^6 generations, and trees were sampled every 100 generations with the first 25% of sampled trees discarded as burn-in. The convergence of the analyses was determined with the program Tracer v1.6 [76] and Bayesian posterior probabilities were used to evaluate branch support. Both MrBayes and RAXML runs were carried out on the online CIPRES Science Gateway resource [77].

Bootstrap support values (BP, for MP; BS, for ML) and posterior probabilities (PP for BI) were used to assess the robustness of the results. In order to discuss the results, we have delimited the support values as strongly, moderately, and weakly supported. In the MP and ML analyses, we regard clades with bootstrap values of 69 and below to be weakly supported, 70–89 to be moderately supported, and 90 and above to be strongly supported. In the BI analyses, we considered clades with posterior probabilities of 0.79 and below to be weakly supported, those with probabilities of 0.80–0.94 to be moderately supported, and those with probabilities of 0.95 and above to be strongly supported.

Nomenclature Acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are
available under that Code from the electronic edition of this article. This published work and
the nomenclatural acts it contains have been registered in ZooBank, the online registration sys-
tem for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associ-
ated information viewed through any standard web browser by appending the LSID to the
prefix"http://zoobank.org/". The LSID for this publication is: urn:lsid:zoobank.org:pub:
89BFF498-46F3-4007-87A9-F826290724C7. The electronic edition of this work was published
in a journal with an ISSN, and has been archived and is available from the following digital
repositories: PubMed Central, LOCKSS.

Results
Sequence Characteristics
From a total of 71 samples, we obtained 60, 70, 58, and 69 sequences for
COI-COII, 16S, EF-1\(\alpha\) and 28S sequences, respectively. In addition, we included an additional four sequences from
two species from GenBank (Table 1).

The COI-COII (929 bp) region was composed of 703 bp of the
COI gene, the entire 70 bp
of the intervening
tRNAleu (including one 3-bp gap since Pelopidas agna has a three-base-
pair insertion), and 156 bp of the COII gene. Due to several small indels in some species,
the 16S and 28S sequence lengths varied between 512–520 bp and 825–840 bp, respectively.
In total, the alignment of the four regions consisted of a total of 3380 bp (929, 531, 1066,
and 854 bp of the COI-COII, 16S, EF-1\(\alpha\) and 28S genes, respectively), of which 975 positions
were variable, and 747 were parsimony-informative. We failed to obtain sequences for
some specimens, and the missing data were designated as a ‘?’ in the alignment. Within
the ingroup, average base composition was T = 30.4%, C = 21.1%, A = 28.8%, and G = 19.7%.
The Chi-square test revealed no significant base composition heterogeneity across samples
employed (df = 150, \(P = 1.00\)). For all three codon positions of COII and EF-1\(\alpha\) as well as for
the three regions tRNAleu, 16S, and 28S, the value of the substitution saturation index (\(I_{ss}\))
was much smaller than the critical value (\(I_{ss. c}\)), assuming either a symmetrical topology or
an asymmetrical topology. These results show that these data subsets are unlikely to have
reached saturation. For COI, only the third codon position reveals that \(I_{ss}\) is larger than \(I_{ss. c}\)
assuming an asymmetrical topology. Therefore, there is little substitution saturation in our
sequence data.

The ILD test revealed no significant incongruence between the two data sets (mtDNA
COI-II and 16S vs. rDNA EF-1\(\alpha\) and 28S, \(P = 0.19\)), indicating that the sequences could be
combined in the phylogenetic reconstruction.

Phylogenetic analyses
The three concatenated analyses (BI, ML, and MP) revealed similar topologies, differing mainly
in branch support (Fig 1, S1 Fig); however, the monophyly of the tribe Baorini is strongly sup-
ported in all methods (PP = 1.00, BS = 100, BP = 100). Within the tribe, although support for
some basal clades is low, the monophyly of the seven traditionally established genera (Parnara,
Pelopidas, Baoris, Caltoris, Prusiana, Iton, and Zenonia) is strongly supported in all phyloge-
netic analyses. On the other hand, contrary to conventional taxonomy, the genera Borbo and
Polytremis are not monophyletic. Members of Borbo did not form a cluster, but instead formed
three clades—Clade A, the Borbo clade, and the Pseudoborbo clade (which only included the
species P. bevani, which was previously placed within Borbo). Clade A is a strongly supported
monophyletic group (PP = 1.00, BS = 100, BP = 100) that consists of the following species: B.
sp., B. gemella, B. holtzi, and B. perobscura and is, by this analysis, sister to the other remaining
Baorini. We designated the clade to have a new genus status, Larsenia gen. nov. The genus
Fig 1. Majority-rule consensus tree from the Bayesian analysis (BI) of the concatenated COI-COII, 16S, EF-1α, and 28S sequences. Values at nodes represent the posterior probabilities (PP) of BI and the bootstrap support (BS) values of the maximum likelihood (ML) analysis, respectively. Asterisks indicate branches supported 100% by both PP and BS. “#” indicates that PP = 100. Colors highlight recognized genera.

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*Pseudoborbo* has a controversial taxonomic status and according to all of the methods is sister to *Pelopidas* and *Baoris*, which is moderately supported in BI analysis (PP = 0.80). We determined that *Pelopidas* is sister to *Baoris* (PP = 1.00, BS = 100, BP = 97). The genus *Borbo*, excluding *Larsenia* and *Pseudoborbo*, was moderately supported in the BI and ML analyses (PP = 0.83, BS = 70). For the genus *Polytremis*, all members analyzed here except for *P. lubricans*, together with the genus *Zenonia*, formed a strongly supported monophyletic clade (PP = 1.00, BS = 96, BP = 91), which is sister to the genus *Iton* (PP = 1.00, BS = 85, BP = 65). Within the clade, *P. eltola* and *P. discreta* formed a strongly supported monophyletic group (PP = 1.00, BS = 100, BP = 100), which is sister to the genus *Zenonia*, with moderate support (PP = 0.93, BS = 70, BP = 66). We recognized the *P. eltola* and *P. discreta* clade to have a new genus status. Other species of *Polytremis* sensu Evans [6] including *P. nascens* (the type species of *Zinaida*) appeared to form a monophyletic group with strong support (PP = 1.00, BS = 95, BP = 81). *P. lubricans*, the type species of *Polytremis*, formed a separate clade from other *Polytremis* sensu Evans [6] species. Consequently, we propose that the genus *Zinaida* Evans, 1937 be reinstated. Based on highly supported monophyly of these genera, together with morphological characters, herein we have designated the following fourteen clades as genera: *Larsenia* gen. nov., *Parnara*, *Gegenes*, *Borbo*, *Pelopidas*, *Baoris*, *Caltoris*, *Pseudoborbo*, *Polytremis*, *Prusiana*, *Iton*, *Zenonia*, *Zenonoida* gen. nov., and *Zinaida*.

**Discussion**

Although the basal relationships within Baorini were poorly resolved, proximal clades were strongly supported across all analyses. Of the 14 major lineages we defined here as genera, eight (*Parnara*, *Gegenes*, *Pelopidas*, *Baoris*, *Caltoris*, *Prusiana*, *Iton*, and *Zenonia*) are concordant with traditionally established genera, while the others are inconsistent with the previously described genera.

**Larsenia** Chiba, Fan & Sáfián gen. nov.

urn:lsid:zoobank.org:act:E3CA9226-4199-48BC-9D92-7A1A3F293E49

Type species. *Hesperia holtzi* Plötz, 1883 Male [78]. Diagnosis. Length of antennae less than half that of costa, with apiculus small and bent. Third segment of palpi short and bent slightly forward. New genus differing from other genera of tribe Baorini by harboring bifid uncus and developed socius.

Etymology. The genus is named after the late Dr. Torben Larsen, the leading expert on African butterfly taxonomy, who was a member of this project. He passed away suddenly in May 2015 and therefore did not see the final results of this research; with respect, we would like to name the new genus after him.

In our analyses, four species currently treated as members of the genus *Borbo*, namely *B. gemella*, *B. perobscura*, *B. holtzi*, and an unidentified species formed a distinct group that is basal and sister to the rest of Baorini. Based on these results, we established *Larsenia* as a new genus. Before describing *Borbo*, Evans [19] divided brown skippers into *Baoris* and *Pelopidas*. The three species above were all assigned to *Pelopidas*. After describing *Borbo*, he divided members into two groups: one with smooth mid-tibia and the other with spined mid-tibia [79]. Both *B. perobscura* and *B. holtzi* have spined mid-tibia but not *B. gemella*. These three species are autapomorphic with respect to their male genitalia, with developed socius. Although it is beyond the scope of this study, a detailed description of the new genus is in preparation pending further research determining which members of the African *Borbo* that were not included in this study should be assigned to the new genus.
**Pseudoborbo** Lee, 1966 confirmed status

Our morphological study shows that the type species of both genera are greatly different in wing venation and male genitalia. Specifically regarding wing venation (Fig 2A and 2B) on the forewing, the origin of M₃ is branched midway between M₂ and Cuₐ₁, while on the hindwing, the origin of vein Cuₐ₁ is distinctly closer to M₃ than to Cuₐ₂ in **Pseudoborbo**. Simultaneously, on the forewing, the origin of the vein M₃ is distinctly closer to M₂ than to Cuₐ₁, and on the hindwing, the origin of vein Cuₐ₁ is branched midway between M₂ and Cuₐ₂ in **Borbo**. In the male genitalia (Fig 3A and 3B) of **Pseudoborbo**, the uncus not separated at tip, while the gnathos is developed and nearly reaches the tip of uncus; the valva lacks transilla, and the aedeagus is characterized by a thick, long spine and an uneven cornuti. However, in **Borbo**, the uncus is bifid and bent ventrally at the tip, the gnathos is far from reaching to tip of uncus, the valva harbors transilla, and the aedeagus is simple without cornuti. Eight species of traditional **Borbo**, including the type species *Hesperia borbonica* Boisduval, 1833, as well as the type and sole species of **Pseudoborbo**, were analyzed in our molecular study. The results revealed that **Pseudoborbo bevani** is located separately from the two clades of the other members of **Borbo**. The relationship of *P. bevani* to the sister clades *Pelopidas* and *Baoris* is closer than its relationship to **Borbo**. Morphologically, **Pseudoborbo** is also much more similar to *Pelopidas* and *Baoris*, especially with regard to the male genitalia.

Based on molecular evidence as well as morphological characters, we propose that the genus **Pseudoborbo** Lee, 1966 is valid.

**Borbo** Evans, 1949

Currently, the genus **Borbo** consists of five Indo-Australian and 18 African species [22]. These species vary extensively in the morphology of the male genitalia, and, therefore, it is necessary to divide them into several groups according to their characteristic genitalia structures [78]. Our analyses clearly indicate that the eight species analyzed here are polyphyletic. Although **Borbo**, excluding Clade A and **Pseudoborbo**, forms a moderately supported clade, the relationship among the three sublineages (*B. cinnara*, *B. borbonica*, and *B. fatuellus*+*B. ratek*) is...
unclear. We did find that *B. fatuellus* is sister to *B. ratek* and each sublineage differs according to male genitalia morphology. Evans [19] determined that *Baoris* included *B. ratek* and *B. fatuellus* and *Pelopidas* included *P. borbonica*. Again, mid-tibial characteristics do not appear to be informative, since *B. ratek* and *B. fatuellus* have smooth mid-tibia while *P. borbonica* has a spined mid-tibia. However, since the sample size is not sufficient and the support for the *Borbo* clade is relatively low (PP = 0.83, BS = 70), additional species sampling and gene sequencing are necessary to resolve the phylogeny of *Borbo* in the future.

**Prusiana Evans, 1937 confirmed status**

*Prusiana*, a small genus with only three species, is obviously a monophyletic group with a synapomorphy in which the males have a brand at the base of the space M₁ on the hindwing [6, 22]. Nevertheless, the taxonomic position of *Prusiana* has been controversial, as mentioned above. Based on morphology rather than molecular evidence, Warren *et al.* included *Prusiana* in *Baorini* [2]. The molecular phylogeny presented here clearly indicates that *Prusiana* is a member of *Baorini* and that its sister-group relation to *Catoris* is weakly supported in the BI phylogeny (PP = 0.79, BS = 38).
Polytremis Mabille, 1904

In our present analyses (Fig 1, S1 Fig), twelve species of *Polytremis*, sensu Evans [6], were not determined to be a monophyletic group but were split into three strongly supported and very distant clades, of which the clade with the type species *P. lubricans* harbors five representative individuals from China and Malaysia. Therefore, we now recognize *Polytremis* Mabille, 1904 to be a monotypic genus (type species *Goniloba lubricans* Herrich-Schäffer). Morphologically, the genus is distinguishable based on the male genitalia (where the lateral process of the uncus, which is divided and horn-like, is clearly separated at its base (Fig 3E)) and the female genitalia (with sclerotized finger-like projections between the anterior and posterior lamella (Fig 3F)).

Zinaida Evans, 1937 reinstated status

Our morphological study shows that *Zinaida* is quite different from *Polytremis* in wing venation and genitalia. Unique characteristics in wing venation in *Zinaida* (Fig 2C) include the forewing, in which the origin of R1 follows that of CuA2 and is located nearly midway between CuA1 and CuA2, and the hindwing, in which the origin of Rs is before that of CuA2. However, in *Polytremis* (Fig 2D), the origin of vein R1 is opposite CuA2 and the origin of Rs is opposite CuA2. In addition, males of most species have a stigma in space CuA2 on the upper side of the forewing, and in *Polytremis* males, the hindwing expanded at middle A, basal M3, CuA1, and CuA2. The male genitalia (Fig 3C and 3E) in *Zinaida* are unique since the uncus is V-shaped, projects at the left and right and is attached at its base, while the gnathos is straight and has an attached uncus. In *Polytremis*, the uncus is completely separated, and the gnathos is elbow-shaped and located far from the uncus.

Of the 18 species included in *Polytremis* sensu Evans [6], 12 species, including the type species of both *Polytremis* and *Zinaida*, were analyzed in our study. Three clades were defined using all methods. One clade consisted of five individuals of *P. lubricans; P. discreta* and *P. eltola* and formed a strongly supported clade (PP = 1.00, BS = 100, BP = 100), which is sister to *Zenonia* with moderate support (PP = 0.93, BS = 70, BP = 66). The other samples, including *P. nascens*, formed a strongly supported monophyletic group. Our study thus suggests that the monophyly of *Polytremis* presented by Evans should be rejected and the genus *Zinaida* reinstated. Our result contradicts that of Jiang et al. [55]. In their analysis, the monophyly of the genus *Polytremis* is weekly supported in ML analysis (BS = 52 on the concatenated data; and BS = 73 on COI sequence), even though they claim that the monophyly is strongly supported. On the other hand, the clade including *P. lubricans, P. eltola*, and *P. discreta* is strongly supported (BS = 99 for both the COI sequence and combined data set). The DNA markers and samples (ingroup and outgroup) selected are essentially why the results are different. First, they used one mitochondrial gene COI (490 bp) and two nuclear genes (the D3 region of 28S rRNA gene and the V4 and V7 regions of the 18S rRNA gene, in total 1048 bp). The trees derived from the separate analyses of COI as well as the concatenated sequences (COI+rDNA) have roughly similar topologies; however, we determined that the COI gene contributed more to the phylogenetic signal, and combined analyses yielded lower resolution. This is because the two slowly evolving rDNA genes are usually used in higher taxonomic levels studies [80, 81]. Additionally, different genes are phylogenetically informative at various taxonomic levels [82]. Therefore, choosing suitable genetic markers is a key element in reconstructing improved molecular phylogenies. We chose COI-COII and 16S rRNA from mitochondrial DNA, rDNA EF-1α, and 28S rRNA as molecular markers. All of these markers have been previously used successfully to elucidate the relationships among many groups within the Lepidoptera, including at the levels of genera, tribe, and subfamily [5, 57,
Second, 15 Chinese species were used as the ingroup and four Baorine genera as the outgroup. Despite the relatively large number of samples included in the ingroup, the result of molecular phylogeny analysis is not ideal due to the unsuitable outgroup. Since relationships among genera in Baorini are unclear and Polytremis is a morphologically diverse group, all available genera should be included as the outgroup in analyses instead of only four. Our study included nearly all the major genera within Baorini all over the world. In order to test previous analyses, our study included 12 species, allowing for a broad representation of lineages within Polytremis, and containing more than three individuals for P. lubricans, P. eltola, and P. discreta. Although our species sampling is less extensive than in previous studies, the present trees (Fig 1, S1 Fig) are better resolved than those from Jiang et al. [55] and reveal that Polytremis sensu Evans [6] is not a monophyletic group, P. eltola as sister group to P. discreta rather than to P. lubricans.

Zenonoida Fan and Chiba gen. nov.

urn:lsid:zoobank.org:act:8CA5AEF0-E81D-4F74-8CA1-F62C407A5FBA

Type species. Hesperia eltola Hewitson, 1869 (Male). Diagnosis. New genus superficially similar to Polytremis Mabille, 1904 and Zinaida Evans, 1937, though distinguishable from other two genera as follows: palpi characterized by short third segment, stout and barely protruding; forewing cell spots conjoined or upper cell spot absent. Uncus with central-basal area membranous; gnathos elbow-shaped, sclerotized except for a narrow distal membranous band.

Etymology. The scientific name, Zenonoida is derived from the genus Zenonia since the new genus is significantly similar to Zenonia with respect to the male genitalia.

In our analyses, P. eltola and P. discreta were assigned to Polytremis sensu Evans [6], which is distantly located from both Polytremis and Zinaida. Thus, we describe Zenonoida as a new genus, and move P. eltola and P. discreta from Polytremis sensu Evans [6] to the new genus: Z. eltola comb. nov., Z. discreta comb. nov.

Supporting Information

S1 Fig. Strict consensus cladogram of the 5 equally parsimonious trees (length 3456, consistency index 0.402, retention index 0.679) inferred in TNT analysis of the concatenated COI-COII, 16S, EF-1α and 28S sequences. The numbers indicate bootstrap values. Colors highlight recognized genera.

(TIF)

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Author Contributions

Conceived and designed the experiments: XF MW. Performed the experiments: XF ZH WF. Analyzed the data: XF ZH. Contributed reagents/materials/analysis tools: HC ZH MW SS. Wrote the paper: XF HC.
References

1. Bridges CA. Catalogue of the Family-Group, Genus-Group and Species-Group Names of the Hesperiidae (Lepidoptera) of the World. Urbana: Published by Author; 1994.

2. Warren AD, Ogawa JR, Brower AVZ. Revised classification of the family Hesperiidae (Lepidoptera: Hesperioidea) based on combined molecular and morphological data. Syst Entomol. 2008; 34: 467–525.

3. de Jong R, Vane-Wright RI, Ackery PR. The higher classification of butterflies (Lepidoptera); problems and prospects. Entomol Scan. 1996; 27: 65–101.

4. Ackery PR, de Jong R, Vane-Wright RI. The butterflies: Hedyloidea, Hesperioidea and Papilionoidea. In: Kristensen NP, editor. Lepidoptera, Moths and Butterflies. 1. Evolution, Systematics, and Biogeography. Handbook of Zoology. 4 (35), Lepidoptera. Berlin: de Gruyter; 1999. pp. 263–300.

5. Warren AD, Ogawa JR, Brower AVZ. Phylogenetic relationships of subfamilies and circumscription of tribes in the family Hesperiidae (Lepidoptera: Hesperioidea). Cladistics. 2008; 24: 642–676.

6. Evans WH, A Catalogue of the Hesperiidae from Europe, Asia, and Australia in the British Museum (Natural History). London: The British Museum; 1949.

7. Doherty W. A list of butterflies taken in Kumaon. J Asiatic Soc Beng. 1886; 55: 103–140.

8. Bell TR. The common butterflies of the plains of India (including those met with in the hill stations of the Bombay presidency). J Bombay Nat Hist Soc. 1920–1927; 27: 26–32 (part 26, 1920), 211–227 (part 27, 1920), 431–447 (part 27, 1921), 778–793 (part 28, 1921); 28: 429–455 (part 29, 1923), 703–717 (part 30, 1923), 921–946 (part 31, 1923), 30: 132–150 (part 32, 1925), 285–305 (part 33, 1925), 561–586 (part 34, 1925), 822–837 (part 35, 1925); 31: 323–351 (part 36, 1926), 655–686 (part 37, 1926), 951–974 (part 38, 1927).

9. Eliot JN. [descriptions and revisions] In: Corbet AS, Pendlebury HM. The Butterflies of the Malay Peninsula, 3rd edn. Kuala Lumpur: Malayan Nature Society; 1978.

10. Eliot JN. [descriptions and revisions] In: Corbet A.S., Pendlebury H.M. The Butterflies of the Malay Peninsula, 4rd edn. Kuala Lumpur: Malayan Nature Society; 1992.

11. Chou I. (Ed.) Monographia Rhopalocerorum Sinensium. Henan, China: Henan Scientific and Technological Publishing House; 1994.

12. Chou I. Classification and Identification of Chinese Butterflies. Henan, China: Henan Scientific and Technological Publishing House; 1998.

13. International Commission on Zoological Nomenclature (ICZN). International Code of Zoological Nomenclature, 4th edn. London: The International Trust for Zoological Nomenclature; 1999.

14. Mabille P. Die palaearctica Tagfalter. Grypocera. In: Seitz A, editor. Grossschmetterlinge der Erde 1. 1909. pp. 329–354.

15. Aurivillius C. Die afrikanischen Tagfalter. In: Seitz A, editor. Grossschmetterlinge der Erde 13. 1925. pp. 11–613.

16. Seitz A. Die Indo-Australischen Tagfalter. Grypocera. Grossschmetterlinge der Erde 9. Stuttgart: Alfred Kernen; 1927.

17. Mabille P. Lepidoptera Rhopalocera. Family Hesperiidae. Gen Insecorum. 1903–1904; 17a: 1–78 (1903); 17b: 79–142 (1904); 17c: 143–182(1904); 17d: 183–210 (pls. 1904).

18. Evans WH. The identification of Indian butterflies. Second edition revised. Madras: Bombay Nat Hist Soc. 1932.

19. Evans WH. A catalogue of the Arican Hesperiidae. London: The British Museum; 1937.

20. de Jong R. Some aspects of the biogeography of the Hesperiidae (Lepidoptera, Rhopalocera) of Sulawesi. In: Knight WJ, Holloway JD, editors. Insects and the Rain Forests of South East Asia (Wallacea). R Entomol Soc Lond. 1990; 35–42.

21. Maruyama K. Butterflies of Borneo 2(2), Hesperiidae. Tokyo: Tobishima Corporation; 1991.

22. de Jong R, Treadaway CG. Hesperiidae of Philippines Islands. In: Bauer E, Frankenbach T, editors. Butterflies of the World, Supplement 15. Keltern: Goecke & Elvers; 2007. pp. 1–72.

23. Watson EY. A proposed classification of the Hesperiidae, with a revision of the genera. Proc zool Soc Lond. 1893; 1: 1–132.

24. Watson EY. A key to the Asiatic genera of the Hesperiidae. J Bombay Nat Hist Soc. 1895; 9(4): 411–437.

25. Aitkin EH, Comber E. A list of Butterflies of Konkan. J Bombay Nat Hist Soc. 1903; 15: 42–55.

26. Evans WH. Lepidoptera-Rhopalocera obtained by Mme J. Wisser-Hooff of the Hague (Holland) during an exploration of the unknown country in the western Karakorum, NW India 1925. Tijdschr Entomol. 1927; 70: 158–162.
27. de Nicéville L. Descriptions of some new Indian Rhopalocera. J Asia Soc Bengal, Part 2–Nat Sci. 1885; 54: 117–124.
28. de Nicéville L. On new and little-known butterflies from the Indian region, with description of three new genera of Hesperiidae. J. Bombay nat Hist Soc. 1890; 5(3): 199–225.
29. Swinhoe C. On the Lepidoptera of Bombay and the Deccan. Proc zool Soc Lond. 1885; 53(2): 287–307.
30. Swinhoe C. On the Lepidoptera of Mhow, in Central India. Zoological Society of London. 1886.
31. Moore F. On the lepidopterous insects of Bengal. Proc zool Soc Lond. 1866; 1865(3): 755–822.
32. Elwes HJ. On butterflies collected by Mr. W. Doherty in the Naga and Karen Hills and in Perak, Part II. Proc zool Soc Lond. 1892; 1892(4): 617–664.
33. Davidson J, Aitken E H. Notes on the larvae and pupae of some butterflies of the Bombay Presidency. J. Bombay Nat. Hist. Soc. 1890; 5: 260–278, 349–375.
34. Watson EY. Hesperiidae indicae. Vest and Co. Madras. 1891.
35. Betham JA. The butterflies of the Central Provinces. Part VI. (Hesperiidae). J Bombay nat Hist Soc. 1893; (4): 425–429.
36. Elwes HJ, Edwards J. A revision of the Oriental Hesperiidae. Trans zool Soc Lond. 1897; 14(4): 101–324.
37. Hannyngton F. The butterflies of Kumaon. J Bombay Nat Hist Soc. 1910; 20: 130–142.
38. Evans WH. A List of the Butterflies of the Painsi Hills with descriptions of two new species. J. Bombay Nat Hist Soc. 1910; 20(2): 380–390.
39. Swinhoe C. A list of the Lepidoptera of the Khasia Hills. Part I. Trans. ent. Soc. Lond. 1893; 41(3): 267–330.
40. Swinhoe C. In: Moore F. Lepidoptera Indica. London: L. Reeve & Co; 1912–1913.
41. Shirōzu T. Butterflies of Formosa in Colour. Osaka: Hoikusha; 1960.
42. Pinratana A, Eliot JN. Butterflies in Thailand. Vol. 5. Hesperiidae. Bangkok: Viratham Press; 1985.
43. Bascombe MJ, Johnston G, Bascombe FS. The butterflies of Hong Kong. Great Britain: Harcourt Brace & Company; 1999.
44. Chiba H. Sipper of Hainan (Lepidoptera: Hesperiidae). Report on Insect Inventory Project in Tropical Asia (TAIIV). 2008; 334–344.
45. Lee CL. On the Chinese species of Borbo Evans (Lep. Hesperiidae). Acta Zool Sinica. 1966; 18: 221–231.
46. Parsons MJ. The Butterflies of Papua New Guinea. Their Systematics and Biology. London: Academic Press; 1999.
47. Vane-Wright RI, de Jong R. The butterflies of Sulawesi: annotated checklist for a critical island fauna. Zool Verh. 2003; 343: 1–267.
48. Lee CL, Zhu BY. Atlas of Chinese butterflies. Shanghai: Shanghai Far East Publishers; 1992.
49. Osada S, Umura Y, Uehara J. An Illustrated Checklist of the Butterflies of Laos P D R. Tokyo: Mokuyo-sha; 1999.
50. Braby MF. Butterflies of Australia: Their Identification, Biology and Distribution. Melbourne: CSIRO Publishing; 2000.
51. Huang BK. Fauna of Insects in Fujian Province of China. Vol. 4. Fuzhou: Fujian Scientific and Technological Publishing House; 2001.
52. Yuan F, Yuan XQ, Xue GX. Fauna Sinica (Insecta: Lepidoptera: Hesperiidae). China: Science Press; 2015.
53. Dodo YT, Saigusa T, Chiba H, Nishiyama T, Ishii M, Yagi T, et al. Molecular phylogeny of Japanese skippers (Lepidoptera, Hesperioidea) based on mitochondrial ND5 and COI gene sequences. Trans Lep Soc Jap. 2008; 59: 29–41.
54. Yuan XQ, Gao K, Yuan F, Wang P, Zhang YL. Phylogenetic relationships of subfamilies in the family Hesperiidae (Lepidoptera: Hesperioidea) from China. Scientific reports. 2015; 5: 11140. doi:10.1038/srep11140
55. Jiang W, Zhu JQ, Xiao CS, Li Y, Yang Y, Yu WD. Molecular Phylogeny of the Butterfly Genus Polytmis (Hesperiidae, Hesperini, Baorini) in China. PLOS One. 2013; 8(12): e84098. doi:10.1371/journal.pone.0084098 PMID: 24391893
56. Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flock P. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann Entomol Soc Am. 1994; 87: 651–701.
57. Monteiro A, Pierce NE. Phylogeny of Bicyclus (Lepidoptera: Nymphalidae) Inferred from COI, COII, and EF-1α Gene Sequences. Mol Phy Evol. 2001; 1–18.

58. Kim CG, Zhou HZ, Imura Y, Tominaga O, Su ZH, Osawa S. Pattern of morphological diversification of the Leptocarabas ground beetles as deduced from mitochondrial NDS gene and nuclear 28S rDNA sequences. Mol Biol Evol. 2000; 17: 137–145. PMID: 10666713

59. Katoh K, Kuma K, Toh H, Miyata T. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res. 2005; 33(2): 511–8. PMID: 15661851

60. Katoh K, Misawa K, Kuma K, Miyata T. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 2002; 30(14): 3059–66. PMID: 12136088

61. Katoh K, Toh H. Recent developments in the MAFFT multiple sequence alignment program. Brief Bioinform. 2008; 9: 286–298. doi: 10.1093/bib/bbn013 PMID: 18372315

62. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 1997; 24: 4876–4882.

63. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013; 30: 2725–2729. doi: 10.1093/molbev/mst197 PMID: 24132122

64. Swofford DL. PAUP * 4.0: Phylogenetic Analysis Using Parsimony and Other Methods (Software). Sunderland, Massachusetts: Sinauer Associates; 2003.

65. Farris JS, Källersjö M, Kluge AG, Bult C. Testing significance of incongruence. Cladistics. 1995; 10: 315–319.

66. Xia X, Xie Z, Salemi M, Chen L, Wang Y. An index of substitution saturation and its application. Mol Phylogenet Evol. 2003; 26: 1–7. PMID: 12470932

67. Xia X, Lemey P. Assessing substitution saturation with DAMBE. In: Lemey P, Salemi M, Vandamme AM, editors. The Phylogenetic Handbook: A Practical Approach to DNA and Protein Phylogeny, second ed. Cambridge: Cambridge University Press; 2009. pp. 615–630.

68. Xia X, Xie Z. DAMBE: data analysis in molecular biology and evolution. J Hered. 2001; 92: 371–373. PMID: 11535656

69. Goloboff PA, Farris JS, Nixon KC. TNT, a free program for phylogenetic analysis. Cladistics. 2008; 24: 774–786.

70. Goloboff PA. Analyzing large data sets in reasonable times: solutions for composite optima. Cladistics. 1999; 15: 415–428.

71. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 1985; 39: 783–791.

72. Darriba D, Taboada GL, Doalla R, Posada D. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods. 2012; 9(8): 772.

73. Akaike H. A new look at the statistical model identification. IEEE Trans. Automat Control. 1974; 19: 716–723.

74. Stamatakis A. RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. Bioinformatics. 2014; 10:

75. Ronquist F, Teslenko M, van der Mark P, Ayres D L, Darling A, Hohna S, et al. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. Syst Biol. 2012; 61: 539–542. doi: 10.1093/sysbio/sys029 PMID: 22357727

76. Rambaut A, Suchard M A, Xie D, Drummond A J. Tracer v1.6. Available from http://beast.bio.ed.ac.uk/Tracer. 2014.

77. Miller MA, Pfeiffer W, Schwartz T. Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees. In: SC10 Workshop on Gateway Computing Environments (GCE10). 2010.

78. Larsen TB. Two new species in the African skipper genera Borbo (Hesperiinae, Baorini) and Platyliceshes (Hesperiinae, incertae sedis). Trop Lepid Res. 2013; 23(2): 92–98.

79. Evans WH. Revisonal notes on African Hesperiidae. Ann Mag Nat hist. 1946; (11)13: 641–648.

80. Caterino MS, Cho S, Sperling FAH. The current state of insect molecular systematics: a thriving tower of Babel. Annu Rev of Entomol. 2000; 45: 1–54.

81. Gillespie JJ, Munro JB, Herat JM, Yoder MJ, Owen AK, Carmichael AE. A Secondary Structural Model of the 28S rRNA Expansion Segments D2 and D3 for Chalcidoid Wasps (Hymenoptera: Chalcidoidea). Mol Biol Evol. 2005; 22(7): 1593–608. PMID: 15843598

82. Wahlberg N, Zimmermann M. Pattern of phylogenetic relationships among members of the tribe Melitaen (Lepidoptera: Nymphalidae) inferred from mitochondrial DNA sequences. Cladistics. 2000; 16: 347–363.
83. Niehuis O, Yen SH, Naumann CM, Misof B. Higher phylogeny of zygaenid moths (Insecta: Lepidoptera) inferred from nuclear and mitochondrial sequence data and the evolution of larval cuticular cavities for chemical defence. Mol Phylogenet Evol. 2006; 39: 812–829. PMID: 16483803

84. Nazari V, Zakharov EV, Sperling FAH. Phylogeny, historical biogeography, and taxonomic ranking of Pamassiniinae (Lepidoptera, Papilionidae) based on morphology and seven genes. Mol Phylogenet Evol. 2007; 42: 131–156. PMID: 16919972

85. Kim MI, Wan XL, Kim MJ, Jeong HC, Ahn NH, Kim KG, et al. Phylogenetic Relationships of True Butterflies (Lepidoptera: Papilionoidea) Inferred from COI,16S rRNA and EF-1α Sequences. Mol Cell. 2010; 30: 409–425.

86. Zaspel JM, Weller SJ, Wardwell CT, Zahir R, Wahlberg N. Phylogeny and Evolution of Pharmaco-phagy in Tiger Moths (Lepidoptera: Erebidae: Arctiinae). PLoS One. 2014; 9: e101975. doi: 10.1371/journal.pone.0101975 PMID: 25036028

87. Brower AVZ, Freitas AVL, Lee MM, Silva-Brandão KL, Whinnett A, Willmott KR. Phylogenetic relationships among the Ithomiini (Lepidoptera: Nymphalidae) inferred from one mitochondrial and two nuclear gene regions. Syst Entomol. 2006; 31: 288–301.

88. Lee S, Brown RL. Phylogenetic relationships of Holarctic Teleiodini (Lepidoptera: Gelechiidae) based on analysis of morphological and molecular data. Syst Entomol. 2008; 33: 595–612.

89. Wang HS, Wahlberg N, Holloway J D, Bergsten J, Fan XL, Daniel H, et al. Molecular phylogeny of Lymantriinae (Lepidoptera, Noctuoidea, Erebidae) inferred from eight gene regions. Cladistics. 2015; 1–14.