Tsukiyamaia, a new genus of the tribe Baorini (Lepidoptera, Hesperiidae, Hesperiinae)

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Abstract
Skippers of the tribe Baorini are evidently a monophyletic group in the subfamily Hesperiinae. In this study, a new Baorini member Tsukiyamaia albimacula gen. n. et sp. n. is described from north Myanmar, southwest China and north Vietnam. Despite its peculiar and striking wing-pattern, this new genus has some important characters of Baorini, such as a broad and bifid uncus and a well-developed gnathos. Based on an analysis of male genitalia and the molecular phylogenies inferred from both mitochondrial and nuclear genes (28 taxa, total aligned length: 2968 bp), it is proposed that the genus Tsukiyamaia is closely related to the genus Polytremis, which has high species diversity in China. This study not only describes a new skipper but also highlights that Tsukiyamaia is important in clarifying phylogenetic relationship of Polytremis and its allies.

Keywords
Polytremis, new species, cox1, cox2, EF-1a.
Introduction

Phylogenetic relationships and higher classifications of Hesperiidae at tribal level were primarily settled by Warren et al. (2008, 2009) based on morphological and molecular evidence. In this phylogenetic framework, the tribe Baorini is a well-supported monophyletic group belonging to the subfamily Hesperiinae (Warren et al. 2008). This tribe was established by Doherty (1886) as Baorinae and is currently composed of eleven genera: Brusa Evans, 1937, Zenonia Evans, 1935, Gegenes Hübner, 1819, Parnara Moore, 1881, Borbo Evans, 1949, Pelopidas Walker, 1870, Polytremis Mabille, 1904, Baoris Moore, 1881, Caltoris Swinhoe, 1893, Iton de Nicéville, 1895 and Prusiana Evans, 1937 (Warren et al. 2009) and 99 valid species (Evans 1937, Evans 1949, Chiba and Eliot 1991, Koiwaya 1996, Tsukiyama et al. 1997, Huang 1999, Sugiyama 1999, Devyatkin and Monastyrskii 2002, Huang 2003, Vane-Wright and de Jong 2003, Yuan et al. 2010, Zhu et al. 2012). Two genera, Brusa and Zenonia are endemic to the Ethiopian region, and the other nine genera are mainly Indo-Australian and south Palaeartic (Mediterranean and Manchurian). Evans (1937, 1949) completed the most recent revision of the world’s fauna of Hesperiidae, and arranged phenotypically similar genera into informal groups in his systematics. However, phylogenetic relationship of genera within the group is not clear. The above-mentioned genera were classified in the Gegenes-group, except for Prusiana which was treated as a genus in the Taractrocera-group (Evans 1949). Subsequently, the Pelopidas-group (Eliot 1978) and Gegenini (Chou 1994) were proposed based on the Malaysian and Chinese faunas respectively.

The members of the tribe Baorini are brown with small semi-hyaline white spots, except for two genera, Zenonia and Prusiana, which have extensive orange markings resembling those of Taractrocerini (Warren et al. 2009). Warren et al. (2009) stated that the male genitalia were distinctive in Evans’ Gegenes-group: a relatively broad, bifid uncus, a well-developed gnathos, and the harpe terminating in an upward-pointing, serrate hook.

Recently most of newly described Baorini taxa were discovered in the range from the south boundary of Himalayas to South China (Koiwaya 1996, Tsukiyama et al. 1997, Huang 1999, Sugiyama 1999, Huang 2003, Yuan et al. 2010, Zhu et al. 2012), where species richness and endemism are obviously higher than in other regions in East Asia (Chiba 2009). Some male specimens of an undescribed species were obtained from Myanmar, which were of uncertain taxonomic position due to only male characters. Subsequently, a female and some male specimens were added from southwest China and Vietnam, and molecular phylogenies based on mitochondrial and nuclear genes were inferred. This investigation suggests that this new species belongs to a new genus of the tribe Baorini, which is sister to Polytremis.
**Methods**

**Sampling**

For morphological comparison, eight male and one female specimens of this new taxon were examined. For inferring phylogenetic relationships of tribe Baorini to investigate the position of the new genus, 28 species were sampled (Table 1). A total of seven out of eleven genera in Evan’s Gegenes-group were sampled and they are all distributed with *Tsukiyamaia* in Indo-Australian and the south Palaearctic region. Data of ten taxa were obtained from previous studies (Warren et al. 2008, 2009; Table 1).

**Morphological procedures**

We employed the standard method in Lepidoptera research to examine the male and female genitalia as well as other morphological characters of *Tsukiyamaia* (Zhu et al. 2012). The terminology for wing patterns followed Evans (1949) and for genitalia Shirózu (1960) and Ehrlich (1958).

The holotype and one female paratype of the new taxon were deposited in Department of Biology, Shanghai Normal University, China. One male paratype was deposited in the private collection of Jia-Qi Wang. The other paratype from China is in the collection of Kadoorie Conservation China, Kadoorie Farm and Botanic Garden, Hong Kong. The rest of the paratypes are in Hiroshi Tsukiyama’s collection (Chiba-pref., Japan).

**Molecular procedures**

Genomic DNA was extracted from the thoracic or leg tissue via using the Purgene DNA Isolation kit (Gentra Systems, Minnesota, USA), following the manufacturer protocol. The primers used for amplifying the mitochondrial cytochrome c oxidase I and II (cox1 and cox2) and nuclear elongation factor 1 alpha (EF-1α) genes were adopted from previous studies (Caterino and Sperling 1999; Kandul et al. 2004; Simonsen et al. 2010; Lu et al. 2009). Each PCR reaction was carried out in a final volume of 30 µL with 0.32 µM dNTP, 1.5 mM MgCl2, 0.2 µM of each primer, 1X Taq buffer, 1U Taq DNA polymerase, and finally added dH2O up to 30 µL. The PCR program was setting as 2 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 50–55 °C, and 1–2 min at 72 °C. The final elongation step was continued for 7 min at 72 °C, and stopped at 4 °C. The PCR products were checked on 1.0 % agarose gels in 1X TBE buffer to ensure the PCR fragments were correctly amplified. DNA sequences were obtained by an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Molecular sequences of the *cox1-cox2* and *EF-1α* genes were checked and assembled into contiguous arrays using Sequencher 4.8 (GeneCode, Boston, USA). After
primer regions were cropped, the sequence dataset was aligned according to amino sequence similarity with the default settings by MUSCLE (Edgar 2004) in MEGA 5.1 software package (Tamura et al. 2011). Missing data and ambiguities were designated to IUPAC codes, and all the sequences were submitted to GenBank (Assission No. KT240144-KT240179; Table 1)

To evaluate species differentiation among Baorini skippers, genetic distance between species was calculated via MEGA 5.1. Pairwise distance with Kimura-2-parameter (Kimura and Ohta 1972) was performed, and bootstrap method was used to estimate its variance. For reconstructing phylogenies, two methods were used: Bayesian

### Table 1. List of the skippers used in this study.

| Name                      | Voucher | Locality | Accession number       | Reference          |
|---------------------------|---------|----------|------------------------|--------------------|
| Calpodes ethlius          | 144-ADW |          | EU364494 EU364289      | Warren et al. 2008 |
| Dubiella belpa            | 458-ADW |          | EU364051 EU364249      | Warren et al. 2008 |
| Ochlodes bouddha          | H1-0635 | Taiwan   | KT240162 KT240144      | this study         |
| Pyrrhopogopsis crates     | 64-ADW  |          | EU364503 EU364298      | Warren et al. 2008 |
| Saliama esperi            | 514-ADW |          | EU364501 EU364296      | Warren et al. 2008 |
| Suastus gremius           | H1-1548 | Taiwan   | KT240163 KT240145      | this study         |
| Synapte silius            | 634-ADW |          | EU364431 EU364226      | Warren et al. 2008 |
| Talides sinois            | 512-ADW |          | EU364457 EU364252      | Warren et al. 2008 |
| Thracides phidon          | 451-ADW |          | EU364502 EU364297      | Warren et al. 2008 |
| Udaspes folus             | H1-1546 | Taiwan   | KT240164 KT240146      | this study         |
| Baoris farri              | H1-0260 | Sichuan, China | KT240165 KT240147 | this study         |
| Brobo cinnara             | H1-0684 | Fujian, China | KT240166 KT240148 | this study         |
| Caltoris bromus           | H1-1645 | Taiwan   | KT240167 KT240149      | this study         |
| Caltoris cabina           | H1-1644 | Taiwan   | KT240168 KT240150      | this study         |
| Iton watsonii             | 600-MCZ |          | EU364490 EU364285      | Warren et al. 2008 |
| Parnara guttata           | H1-1008 | Sichuan, China | KT240169 KT240151 | this study         |
| Pelopidas conjuncta       | H1-1565 | Taiwan   | KT240170 KT240152      | this study         |
| Pelopidas mathias         | H1-0617 | Taiwan   | KT240171 KT240153      | this study         |
| Pelopidas thrax           | 570-ADW |          | EU364492 EU364287      | Warren et al. 2008 |
| Polytremis gotama         | H1-1019 | Yunnan, China | KT240172 KT240154 | this study         |
| Polytremis kinatizana     | H1-1437 | Taiwan   | KT240173 KT240155      | this study         |
| Polytremis lubricans      | H1-0052 | Taiwan   | KT240174 KT240156      | this study         |
| Polytremis matsuii        | H1-0982 | Sichuan, China | KT240175 KT240157 | this study         |
| Polytremis nascens        | H1-0321 | Sichuan, China | KT240176 KT240158 | this study         |
| Polytremis pellucida      | 234-ADW |          | EU364493 EU364288      | Warren et al. 2008 |
| Polytremis zina           | H1-0607 | Taiwan   | KT240177 KT240159      | this study         |
| Pseudobrobo bevani        | H1-0888 | Yunnan, China | KT240178 KT240160 | this study         |
| Tsukiyamaia albimacula    | H1-1661 | Yunnan, China | KT240179 KT240161 | this study         |
inference (BI) was carried out by using MrBayes v. 3.2.1 (Ronquist et al. 2012), and Maximum Likelihood (ML) was performed in RAxML Pthreads-based SSE3 version 7.4.2 (Ott et al. 2007; Stamatakis 2006). In BI method, the substitution model was set to GTR+Γ (GTR: General Time Reversible; Γ: gamma distribution), and the taxa *Udaspes folus* was set as functional outgroup for investigate genus relationship among Baorini based on the latest phylogenetic relationship of skippers (Warren et al. 2009). To evaluate the effect of different partition strategies, four different datasets were executed: (1) no partition (combined dataset); (2) two gene region partitions (mitochondrial and nuclear genes); (3) four gene partitions (*cox1*, tRNA-Leu, *cox2*, and *EF-1α*), and (4) both gene and codon partition (ten partitions). Each partition matrix has its independent substitution model if partition was setting. Each dataset has run with six chains (five heated and one cold) for one million generations and sampled trees every 100 generations. The log-likelihood scores were plotted against generation time, and then burn-in the first 25% trees and the remaining trees were used for representing the posterior probability if the stationarity was reached. In ML method, datasets were processed with the non-default settings as follows: substitution model was set to GTR-GAMMA. Outgroup and the four partition datasets were set as the BI method. The node support values of ML topology were evaluated by 1000 bootstrap (BS) replicates with ten additional searches per replicate to improve the confidence of each bootstrap search.

**Results**

**Morphological systematics**

*Tsukiyamaia* gen. n.

Type species. *Tsukiyamaia albimacula* sp. n.; designated by monotypy.

Description. Antennae: 9.5–10 mm in length, half-length of forewing, nudum 13–14 on apiculus; Labial palpi: Second segment stout and erect, with brown hairs dorsally and yellowish hairs ventrally; third segment short, pointed and erect. Legs: middle tibiae unspined. Wing-shape: Forewing 19–20 mm in length, triangular in shape; costa about 1.4 times as long as dorsum, approximately straight, weakly arched on anterior half; apex angulated; termen lightly curved on anterior half; inner dorsum almost straight. Hindwing nearly triangular in shape; costa slightly longer than dorsum, obviously arched; termen curved on anterior half; tornus concave; dorsum almost straight.

**Wing venation** (Fig. 5). Forewing: vein 2A very short not reaching dorsum; vein Cu₂ arising before the origin of vein R, and slightly nearer the origin of Vein M₃ than to base; Vein M₂ obviously closer to Vein M₃ than to Vein M₁ at origin; cell longer than half the wing length. Hindwing: Vein Cu₁ arising beyond the origin of Vein M₁;
Figures 1–5. *Tsukiyamaia albimacula*. 1 holotype, ♂, upperside 2 holotype, ♂, underside 3 paratype, ♀, upperside 4 paratype, ♀, underside 5 wing venation. Scale bar: 10 mm.
Vein Cu₁ arising beyond the origin of Vein Rs; Vein M₂ absent. Discocellular veins on both wings obviou.

**Wing markings** (Figs 1–4). Without stigma or secondary sexual characters; forewing with semi-hyaline spots in spaces Cu₂, M₃, M₂, R₃, R₄, R₅ and cell; hindwing upperside with a cigar-shaped spot in space M₂, underside centrally with a large white marking restricted from vein 2A to vein Rs.

**Male genitalia** (Figs 6–10). Tegumen swollen; uncus U-shaped bifurcated; gnathos bifurcated, slightly turned inside at tip and outwardly spined; valva approximately rectangle; dorsal process of harpe well produced; ventral process of harpe weakly protruded; phallus deeply bifid distally, well protruded and heavily spined outwardly; cornuti absent; manica membranous; juxta U-shaped.

**Etymology.** The generic name is derived from Hiroshi Tsukiyama, whose outstanding contribution to the taxonomy of Hesperidae is noteworthy.

**Tsukiyamaia albimacula** sp. n.

http://zoobank.org/6ED6C0E1-0571-4536-BF4B-B00F857B19FE

**Description.** Antennæ 9.5–10 mm in length, about 1/2 the length of forewing, black brown except club gray dorsally and grayish yellow ventrally; nudum 13–14 on apiculus. Palpi erect, with brown hairs dorsally and yellowish hairs ventrally. Thorax and abdomen covered with brown hairs. Forewing 19–20 mm in length. Both wings ground color black brown at each sides, with white spots and marking; costal area of forewing and entire hindwing covered with brown scales underside; cilia of both wings brown. Upperside forewing: three apical spots in spaces R₃–R₅, arranged linear; one discal spot present at the middle of the space M₂; in space M₃, a reduced spot present in the holotype, and absent in two paratypes; cell spots conjoined as trapezium-shaped, which also conjoined with the Cu₁ spot. Underside forewing markings same as upperside. Upperside hindwing: only with a cigar-shaped spot in space M₁. Underside hindwing: Discal area with a very large, rectangle white marking extending from vein Rs to the middle of space Cu₁. Inward margin smooth, upward to the end of the discal cell. Outward margin lightly serrated, and evidently elongated in space M₁.

**Male genitalia** (Figs 6–10). Tegumen swollen; uncus U-shaped, bifurcated dorsally, pointed at tip laterally; gnathos bifurcated, longer and wider than uncus, slightly turned inside at tip and outwardly spined; saccus short, pointed distally; valva approximately rectangle; ampulla slightly elongate upward, harpe dorsally with a long and straight elongated process and ventrally with a relatively short and small process, outward margin concave and covered with dense hairs; costa smooth dorsally, sacculus concave ventrally; phallus 1.4 times as long as valva; subzonal about 1.3 times as the length of suprational, distally deeply bifid as two protruded processes, equal in length and heavily spined outwardly; without cornuti; manica membranous; juxta U-shaped.

**Female genitalia** (Figs 11–12). Papilla analis nearly rectangle, covered with hairs on the surface; apophysis posterioris slender and short; Lamella postvaginalis oblong
Figures 6–10. Male genitalia of *Tsukiyamaia albimacula*. 6 lateral view of ring 7 dorsal view of tegumen 8 outer view of left valva 9 ventral view of phallus 10 lateral view of phallus. Scale bar: 1 mm.

Figures 11–12. Female genitalia of *Tsukiyamaia albimacula*. 11 ventral view 12 lateral view. Scale bar: 1 mm.

with outer margin arched; lamella antevaginalis with triangular parts laterally, slightly sclerotized; ductus bursae short, wide as ostium bursae, strongly sclerotized; bursa copulatrix oval, membranous with no signum.
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HOLOTYPE ♂: Phutao, Kachin, N. of MYANMAR, ~1000m, 09-VI-2000, Male genitalia examined by H. Chiba, #HC030511.

PARATYPES: 1♂, the same locality as the holotype, 29-V-2000.; 1♂ ditto, 08-VI-2000.; 1♂ Panglan, ~700m, Kachin, N. of MYANMAR, 02-IX-2002. 1♂ ditto, 04-IX-2002. 1♂ ditto, 05-IX-2002, 1♂ ditto, 29-IX-2002, 1♂ Mt. Fan Shi Pang, ~1800m, N. VIETNAM, IV-2002. 1♂ Baopo, Dulongjiang, Yunnan, CHINA, 1500m, 29-V-2011, Jia-Qi Wang leg.; 1♀, Maku, Dulongjiang, Yunnan, CHINA, 1900m, 03-VI-2009, Jian-Qing Zhu, leg; 1♂ CHINA, Yunnan, Tengchong, Gaoligongshan National Nature Reserve, Zhengding, 2200m, 26-IV-2014, LO Yik Fui Philip coll. (YFL140055).

Voltinism. Judging from the collecting data, the species is expected to be multi-voltine.

Distribution (Fig. 13). China (Yunnan), Myanmar (Kachin), and Vietnam (Mt. Fan Shi Pang).

Biology. Tsukiyamaia prefers open habitats, such as open field on the hillside, farmland and heavily disturbed shrub land. It is active near the ground and stream under strong sunlight. The female frequents flowers and the male performs padding behavior.

Etymology. The species is named for its large white marking on underside of the hindwing.
**Diagnosis.** In appearance, *Tsukiyamaia* is peculiar in Baorini with a large white marking in the center of the hindwing underside. The male genitalia of *Tsukiyamaia* can be separated from those of Baorini genera by the uncus lacking a pair of basal processes, and the harpe dorsally with a long and straight elongated process and ventrally with a relatively short and small process.

**Molecular information**

**Sequence information**

The gene length used in this study included *cox1* (1531bp), tRNA-Leu (71 bp), *cox2* (141 bp), and *EF-1α* (1225 bp) genes. Pairwise distance based on mitochondrial sequences showed that the smallest one between *Tsukiyamaia albimacula* and *Polytremis matsuii* was 6.8% (Table 2). If it was compared with other *Polytremis* skippers, it ranged from 7.2 to 10.6%. Whereas comparing to other genera, it ranged from 7.6% (*Iton watsonii*) to 12.8% (*Dubiella belpa*).

**Molecular phylogenies**

The total of eight topologies, inferred by four partitioning datasets and by two tree-reconstructing methods, have similar phylogenetic relationships (summarized in Fig. 14, Appendix: S1–S3). All the Baorini members are grouped together and *Parnara guttata* is the most primitive taxa. Although the genus-level relationships within Baorini are still unresolved, *Tsukiyamaia* is sister to *Polytremis* members with high support value. In addition, our Baorini topology also indicated that the genus *Polytremis* might not be a monophyletic group, and more taxa-sampling is needed for further phylogenetic studies.

**Discussion**

Although the monophyly of the tribe Baorini is well-supported by the molecular data, no synapomorphic character in external morphology have been found (Warren et al. 2009). Characters are either shared by most but not all the members of the tribe, or shared by members of other tribes.

Evans (1949) merely gives diagnostic difference between his Gegenes- group (= Baorini) and Taractrocera-group (= Taractrocerini), which is the wing color. The former is brown while the latter is yellow or orange. As mentioned in the introduction, it is not applicable for *Prusiana*, which Evans (1949) considered a member of Taractrocera-group, nor the African *Zenonia* as well as the new genus. The outstanding
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Figure 14. Bayesian phylogeny of the tribe Baorini based on four gene-partitioned dataset. The numbers above or below the branches are the ML bootstrap value / BI posterior probability.

Coloration of *Tsukiyamaia* may imply that there exist some unknown adaptive advantages driving the evolution of the peculiar marking with the slightest resemblance to its allies.

Eliot (1978) claims that the “internal veinlet entering the cell from just above the origin of vein 3 on the forewing” is the character shared by members of his Pelopidas-group but not the Taractrocera-group of genera. However, he only illustrated the wing venation of *Caltoris tulsi*, which apparently shows the veinlet. Figures of wing venation in Bascombe et al. (1999) suggest that the veinlet can be observed clearly only in *Caltoris bromus*, recognizable in *Borbo cinnara*, *Pelopidas conjunctus*, and *Polytremis lubricans*, absent in *Parnara guttata* and *Baoris farri*. We could not recognize the veinlet in the wing venation of *Tsukiyamaia*.

If the key for separation of genera in Evans (1949) or Eliot (1978) is applied, *Tsukiyamaia* is assigned to *Polytremis*, which is consent to the phylogeny based on molecular data.
**Table 2.** Pairwise distance based on the substitution model of Kimura 2-parameter and *cox1 – cox2* sequences (aligned length 1743 bp). The dash symbol means the overlap sequence is below 50 bp, the value is excluded.

| Species                        | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  |
|-------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| *Udaspes folus*               | 0.106 |     |     |     |     |     |     |     |     |     |     |     |     |     |
| *Suastus gremius*             | 0.122 | 0.101 |     |     |     |     |     |     |     |     |     |     |     |     |
| *Synapte silius*              | 0.123 | 0.116 | 0.113 |     |     |     |     |     |     |     |     |     |     |     |
| *Thracides phidon*            | 0.137 | 0.105 | 0.116 | 0.130 |     |     |     |     |     |     |     |     |     |     |
| *Pyrrhopygopsis crates*       | 0.121 | 0.065 | 0.097 | 0.109 | 0.118 |     |     |     |     |     |     |     |     |     |
| *Talides sinois*              | 0.124 | 0.104 | 0.095 | 0.113 | 0.116 | 0.097 |     |     |     |     |     |     |     |     |
| *Ochlodes bouddha*            | 0.132 | 0.111 | 0.112 | 0.105 | 0.133 | 0.118 | 0.096 |     |     |     |     |     |     |     |
| *Dalodes ethlius*             | 0.120 | 0.067 | 0.094 | 0.107 | 0.087 | 0.096 | 0.107 |     |     |     |     |     |     |     |
| *Saliana esperi*              | 0.118 | 0.080 | 0.101 | 0.100 | 0.124 | 0.095 | 0.088 | 0.118 | 0.071 |     |     |     |     |     |
| *Parnara guttata*             | 0.131 | 0.121 |     |     |     |     | 0.123 | 0.13 | 0.110 |     |     |     |     |     |
| *Baoris farri*                | 0.134 | 0.124 |     |     |     |     |     | 0.113 | 0.13 | 0.110 |     |     |     |     |
| *Pelopidas mathias*           | 0.121 | 0.091 | 0.115 | 0.112 | 0.118 | 0.100 | 0.100 | 0.121 | 0.098 | 0.096 | -   | -   | 0.060 |     |
| *Pelopidas thax*              | 0.123 | 0.111 | 0.103 | 0.104 | 0.117 | 0.104 | 0.103 | 0.116 | 0.114 | 0.115 | 0.094 | 0.086 | 0.059 | 0.059 |
| *Pelopidas conjuncta*         | 0.122 | 0.109 |     |     |     |     | 0.117 | 0.13 |     | 0.112 | 0.096 | 0.092 | -   |     |
| *Brobo cinnara*               | 0.113 | 0.112 | 0.106 | 0.100 | 0.110 | 0.089 | 0.093 | 0.117 | 0.101 | 0.102 | -   | -   | 0.074 | 0.084 |
| *Caltoris cabira*             | 0.126 | 0.098 | 0.111 | 0.115 | 0.127 | 0.109 | 0.106 | 0.117 | 0.101 | 0.101 | 0.116 | 0.117 | 0.101 | 0.100 |
| *Caltoris bromus*             | 0.125 | 0.113 | 0.116 | 0.113 | 0.129 | 0.105 | 0.114 | 0.119 | 0.096 | 0.103 | 0.127 | 0.137 | 0.110 | 0.095 |
| *Pseudobrobo bevani*          | 0.126 | 0.099 |     |     |     |     | 0.123 | 0.13 |     | 0.114 | 0.113 | 0.102 | -   |     |
| *Polytremis lucibrans*        | 0.137 | 0.133 | 0.097 | 0.116 | 0.135 | 0.126 | 0.124 | 0.151 | 0.106 | 0.107 | 0.131 | 0.117 | 0.104 | 0.122 |
| *Polytremis matsuii*          | 0.127 | 0.129 |     |     |     |     | 0.105 | 0.13 |     | 0.089 | 0.100 | 0.091 | -   |     |
| *Polytremis kirizana*         | 0.123 | 0.116 | 0.107 | 0.103 | 0.128 | 0.101 | 0.103 | 0.115 | 0.109 | 0.102 | 0.104 | 0.107 | 0.094 | 0.092 |
| *Polytremis nascens*          | 0.116 | 0.115 |     |     |     |     | 0.113 | 0.13 |     | 0.105 | 0.100 | 0.092 | 0.000 |     |
| *Polytremis gotama*           | 0.107 | 0.115 |     |     |     |     | 0.104 | 0.13 |     | 0.101 | 0.083 | 0.086 | 0.000 |     |
| *Polytremis zena*             | 0.116 | 0.115 | 0.108 | 0.123 | 0.142 | 0.100 | 0.107 | 0.117 | 0.092 | 0.109 | 0.093 | 0.091 | 0.082 | 0.089 |
| *Polytremis bellucida*        | 0.117 | 0.124 | 0.115 | 0.116 | 0.123 | 0.096 | 0.107 | 0.116 | 0.104 | 0.108 | 0.099 | 0.095 | 0.083 | 0.089 |
| *Tsukiyamaia albimacula*      | 0.120 | 0.119 | 0.114 | 0.121 | 0.123 | 0.114 | 0.108 | 0.128 | 0.121 | 0.109 | 0.105 | 0.098 | 0.087 | 0.085 |
### Table 2. Continued.

| # | Specie                                      | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 |
|---|---------------------------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 1 | Udaspes folus                               |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 2 | Synapte siliu                               |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 3 | Thrachides phidon                           |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 4 | Pyrrophygopsis crates                       |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 5 | Talides sinoi                               |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 6 | Ochlopes bouddha                            |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 7 | Eufala evelata                              |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 8 | Calyptorhiza edlaevis                       |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 9 | Calyptorhiza nebulae                        |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 10| Calyptorhiza pauli                          |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 11| Calyptorhiza sp.                            |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 12| Calyptorhiza univiridella                   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 13| Calyptorhiza vittata                        |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 14| Calyptorhiza sp.                            |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 15| Calyptorhiza sp.                            |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 16| Calyptorhiza sp.                            |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 17| Calyptorhiza sp.                            |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 18| Calyptorhiza sp.                            |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 19| Calyptorhiza sp.                            |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 20| Calyptorhiza sp.                            |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 21| Calyptorhiza sp.                            |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 22| Calyptorhiza sp.                            |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 23| Calyptorhiza sp.                            |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 24| Calyptorhiza sp.                            |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 25| Calyptorhiza sp.                            |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 26| Calyptorhiza sp.                            |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 27| Calyptorhiza sp.                            |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 28| Calyptorhiza sp.                            |    |    |    |    |    |    |    |    |    |    |    |    |    |
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Appendix

Figure S1. Bayesian phylogeny of the tribe Baorini based on no partitioned dataset. The numbers above or below the branches are the ML bootstrap value / BI posterior probability.
**Figure S2.** Bayesian phylogeny of the tribe Baorini based on two partitioned dataset. The numbers above or below the branches are the ML bootstrap value / BI posterior probability.
Figure S3. Bayesian phylogeny of the tribe Baorini based on codon partitioned dataset. The numbers above or below the branches are the ML bootstrap value / BI posterior probability.