





https://doi.org/10.11646/zootaxa.5154.2.6

http://zoobank.org/urn:lsid:zoobank.org:pub:54BCF5D5-C08E-4B8B-91B0-D05EACA38816

Graphium septentrionicolus Page & Treadaway, 2013 (Lepidoptera: Papilionidae) is a distinct species

ADAM M. COTTON¹, TENZING DOLECK^{2,3}, XIN ZHANG⁴, YUTAKA INAYOSHI⁵, DAVID J. LOHMAN^{2,3,6*} & SHAO-JI HU^{7,8*}

¹86/2 Moo 5, Tambon Nong Kwai, Hang Dong, Chiang Mai, Thailand. ¹⁰ https://orcid.org/0000-0002-4852-903X ²Biology Department, City College of New York, City University of New York, 160 Convent Ave., New York, New York, 10031, United States. https://orcid.org/0000-0003-1323-2444 ³Biology Ph.D. Program, Graduate Center, City University of New York, 365 Fifth Ave. New York, New York, 10016, United States

⁴Kunming Youning Biotech Co., Ltd, Kunming, 650031, China. ⁶ https://orcid.org/0000-0002-4638-3997

⁵Sritana Condominium 2, 96/173, Huay Kaeo Rd., T. Suthep, A. Muang, Chiang Mai, Thailand

https://orcid.org/0000-0002-2017-2868

⁶Entomology Section, National Museum of Natural History, Manila, 1000, Philippines

⁷Institute of International Rivers and Eco-security, Yunnan University, Kunming, 650500, China

⁸Yunnan Key Laboratory of International Rivers and Transboundary Eco-security, Yunnan University, Kunming, 650500, China

*Corresponding authors. 🖃 dlohman@ccny.cuny.edu; 🐵 https://orcid.org/0000-0002-0689-2906 (DJL); 🔄 shaojihu@hotmail.com; https://orcid.org/0000-0002-1615-9601 (SJH)

Abstract

After molecular and morphological analyses, the taxon septentrionicolus Page & Treadaway, 2013 is shown to be a distinct species, and Graphium adonarensis (Rothschild, 1896) is placed as conspecific with Graphium sarpedon (Linnaeus, 1758). Graphium huangshanensis Wu & Ma, 2016 syn. nov. is synonymised with G. septentrionicolus.

Key words: adonarensis, China, India, Laos, Myanmar, sarpedon complex, Sunda Islands, Vietnam

Introduction

Page & Treadaway (2013) revised the taxa closely related to Graphium sarpedon on the basis of morphological characters, splitting them into eight species compared to the two species recognised by Tsukada & Nishiyama (1980), which are referred to here as the Graphium sarpedon complex. Members of the G. sarpedon complex (Papilionidae: Papilioninae: Leptocircini) are widely distributed throughout tropical and subtropical Asia, Australia, and Melanesia (Tsukada & Nishiyama 1980). Males are frequently encountered mud puddling (e.g., Tea et al. 2020), and both sexes can be seen nectaring at a variety of different flowers. However, research on the G. sarpedon complex is complicated by disagreement over species limits in this geographically widespread group (Page & Treadaway 2013).

Papilio sarpedon Linnaeus, 1758 is the type species of the genus Graphium Scopoli, 1777 in which it is now placed. Saigusa et al. (1982) included 9 species in the sarpedon group, and Hancock (1983) considered there to be 11 species in the group. These comprised several species that are obviously morphologically distinct from G. sarpedon, which is tailless with a blue-green discal band bisecting a black wing. The complex of species closely related to G. sarpedon is mostly allopatric, ranging from South and East Asia to the Solomon Islands and Australia.

In their revision, Page & Treadaway (2013) named six new taxa including septentrionicolus, which they described as a mainland Asian subspecies of their Sundaic island species Graphium adonarensis (Rothschild, 1896). They separated *adonarensis* from the very similar G. sarpedon based on perceived differences in genitalia. The holotype of septentrionicolus Page & Treadaway, 2013 came from 'Nowgang, Assam, India' and sixteen paratypes were also designated, twelve from Meghalaya, NE India, and four from Fujian and Hubei, eastern China. All type specimens were collected between April and June according to the data provided, thus no wet season specimens

Accepted by H. Chiba: 11 May 2022; published: 14 Jun. 2022

Licensed under Creative Commons Attribution-N.C. 4.0 International https://creativecommons.org/licenses/by-nc/4.0/

were included. *Graphium adonarensis septentrionicolus* is sympatric with *G. sarpedon* in mainland Asia; the only other co-occurrence of two species in the *Graphium sarpedon* complex occurs on Sulawesi.

During morphological studies on specimens in the *Graphium sarpedon* complex, the present authors discovered that the genitalia of *septentrionicolus* are distinct from those of *sarpedon* and *adonarensis*, which are almost identical to each other. Molecular phylogenetic analysis confirmed that *septentrionicolus* is not closely related to *sarpedon* or *adonarensis*. The status of taxa in the *G. sarpedon* complex other than the relationship between *G. sarpedon*, *adonarensis* and *septentrionicolus* is not evaluated in this study. Females of *septentrionicolus* could not be examined since no specimens were available for study. A single female illustrated by Wu & Ma (2016), is very similar in appearance to the male.

Unlike dry season specimens, which are relatively easy to separate from *G. sarpedon* by their much broader forewing band and small green patch at the apex of hindwing cell M_3 (occasionally absent on the upperside), specimens of *G. septentrionicolus* from the wet season were initially identified by their genitalia only after molecular phylogenetic analysis demonstrated their distinctiveness. These specimens were almost identical to sympatric *G. sarpedon* in general appearance, so morphological characters to separate the two consistently were evaluated.

Materials and methods

Morphological comparisons

Specimens were spread for examination, with the anal folds on their hindwings opened to expose androconia. Spread specimens were photographed using a digital camera over a medium grey background. Photos were adjusted using Adobe Photoshop CS (Adobe, USA). For comparison between taxa, the lengths of forewings were measured to 0.5 mm precision. Average forewing lengths were calculated, and the standard deviations were also calculated when $n \ge$ 3. To compare the male androconia, spread specimens were observed under a Nikon SMZ1500 stereoscope (Nikon, Japan). Photographs of magnified androconia were taken with a Nikon DMX1200 digital camera (Nikon, Japan) mounted on the stereoscope.

To examine male genitalia, the abdomen was removed from the specimen and placed into a 1.5 mL microcentrifuge tube; 1 mL water was added to the tube to rehydrate the tissue at 50° C for 30 min, then 1 mL 10% sodium hydroxide solution was used to digest soft tissue at 70° C for 1 h. The treated abdomens were neutralised with 2% acetic acid and then dissected in a water-filled Petri dish under the stereoscope to remove residual tissues, scales, and hair. The genitalia were then transferred to 80% glycerol for 12 h to render them transparent. Photographs were taken with a Nikon DMX1200 digital camera (Nikon, Japan) mounted on a Nikon SMZ1500 stereoscope (Nikon, Japan) and automatically stacked using Helicon Focus 7.5.8 (Helicon Software, USA). After observation and photography, all parts of the genitalia were fixed on a glue card using water soluble polyvinyl acetate and pinned with the specimen. No female specimens were available for genitalia examination. Terminology of the male genitalia structures follows Smith & Vane-Wright (2001).

Molecular phylogenetic analysis

Adult specimens were sampled in the field and air-dried without pinning. To obtain a robust phylogenetic relationship for the two focal species, five currently recognised species in the *G. sarpedon* complex, namely *G. choredon*, *isander, anthedon, milon*, and *monticolus*, were included in the molecular phylogenetic analysis (Page & Treadaway 2013). DNA was extracted from leg or abdominal tissue using a modified OmniPrep extraction protocol (gbiosciences.com). Up to 13 genetic loci (Table S1) were captured from 26 samples via anchored hybrid enrichment using the BUTTERFLY2.0 probe kit of Kawahara *et al.* (2018) prior to sequencing on the Illumina HiSeq or NextSeq platforms. These included partial sequences of one mitochondrial locus, cytochrome c oxidase I (COI, 657 bp), and 12 nuclear loci, acetoacetyl-CoA thiolase (AACT, 1020 bp), carbamoyl-phosphate synthetase 2/aspartate transcarbamylase/dihydroorotase (CAD, 1856 bp), catalase (CAT, 1293 bp), dopa-decarboxylase (DDC, 714 bp), elongation factor 1 alpha (EF-1a, 1050 bp), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 609 bp), hairy cell leukemia protein 1 (HCL, 633 bp), isocitrate dehydrogenase (IDH, 708 bp), cytosolic malate dehydrogenase (MDH, 717 bp), ribosomal protein S2 (RpS2, 473 bp), ribosomal protein S5 (RpS5, 555 bp), wingless (wg, 240 bp). Hybrid capture and sequencing were performed at RAPiD Genomics (Gainesville, FL). Quality control and bioinformatic processing of Illumina reads followed the protocols of Breinholt *et al.* (2018). The DNA barcoding fragment of COI was amplified via PCR from an additional 22 samples prior to Sanger sequencing using previously published protocols and primers (Hu *et al.* 2018).

Sanger and Illumina sequenced COI were combined, and all loci were aligned using MUSCLE (Edgar 2004) implemented in AliView 1.26 (Larsson 2014) before concatenation with SequenceMatrix 1.8 (Vaidya *et al.* 2011).

IQ-TREE 2.0 (Minh *et al.* 2020) was used to infer a maximum likelihood (ML) tree. ModelFinder Plus (Kalyaanamoorthy *et al.* 2017) implemented in IQ-TREE (MFP+MERGE) found 15 partitions using the corrected Akaike Information Criterion (AICc). These partitions were used in ML and subsequent Bayesian analysis (Table S2). Branch support on the most likely tree was assessed with 1000 ultrafast bootstraps (Hoang *et al.* 2017) implementing the bootstrap nearest neighbor interchange (-B 1000 -bnni) and 1000 replicates of the Shimodaira-Hasegawa approximate likelihood ratio test (-alrt 1000) (Shimodaira & Hasegawa 1999).

BEAST 1.10.4 (Suchard *et al.* 2018) was used to infer a Bayesian inference (BI) tree and infer divergence times. ModelFinder designated each codon position of COI as a separate partition; these were linked into a single mtDNA clock. All other partitions, which were wholly nuclear loci, were linked as a second nDNA clock. Each clock was assigned a separate uncorrelated relaxed clock lognormal prior. All tree priors were linked and assigned a Yule Process prior. Substitution models for each partition were designated as described in Table S2. Two nodes from the genus-level phylogeny of Kawahara *et al.* (2022) were used to calibrate the tree. We included three outgroup taxa in our analyses, which were related to the ingroup as follows: (*Eurytides marcellus, G. agamemnon (G. sandawanum (G. sarpedon* complex))). A normally distributed root calibration was used for the node between *Eurytides marcellus* and everything else (median = 34.5 my; 29.66–39.67 my 95% HPD; Stdev 2.1), and second normally distributed calibration was used for the node between *G. agamemnon* and *G. sandawanum* + *G. sarpedon* complex (median age = 18.28; 14.89.66-22.64 my 95% HPD; Stdev 1.5). The MCMC analysis was run for 200 million generations sampling every 15,000 generations. Tracer 1.7.1 was used to confirm that all parameters had an ESS value over 200. TreeAnnotator 1.10.4 was then used to infer a maximum clade credibility (MCC) tree after a 25% burn-in.

Results

Molecular phylogeny

The *Graphium sarpedon* species complex seems to have diverged from the common ancestor shared with its sister taxon *G. sandawanum* around 10 mya (\pm 4 Ma 95% HPD; Figures 1, S1), and subsequent species-level diversification took place within the late Neogene throughout tropical and subtropical Asia. Island-endemic taxa from the Indo-Australian Archipelago separated by Page & Treadaway (2013) as *Graphium adonarensis* (marked with an asterisk) were polyphyletic with high BI posterior probability support and ML bootstrap support (Figures 1, S1, S2). The monophyly of *Graphium septentrionicolus* (PP = 1, UFboot = 100, alrt = 100) and its non-sister relationship to *G. sarpedon* were strongly supported in all trees (Figures 1, S1, S2).

The topologies of putative species within the BI and ML trees (Figure 1) were nearly identical with a single exception. *Graphium monticolus* was sister to *G. milon* in the ML tree (Figure S1) with weak support (UFboot = 63.4, alrt = 48), whereas *G. milon* was weakly supported as sister to (*G. isander* + *G. choredon*) in the BI tree (Figure S2, PP = 0.61); *Graphium monticolus* was sister to this trio, again with weak support (PP = 0.5).

Morphological differences between G. septentrionicolus (Figure 2) and sarpedon (Figure 3)

First generation (dry season) specimens of *G. septentrionicolus* (forewing length 40–45 mm, mean = 42.6 ± 1.5 mm, n = 11) can easily be separated from *G. sarpedon* on wing pattern alone. In *septentrionicolus*, the forewing blue-green discal band is much broader than that of *sarpedon*, especially in the lower portion of the forewing. On the forewing underside the rather faint narrow submarginal band is longer than in *sarpedon*, reaching about half-way up the forewing from the tornus, whereas in *sarpedon* it is short or absent. On the hindwing the blue-green discal band normally extends into the apex of hindwing cell M₃ at least on the underside.

Wet season specimens of *G. septentrionicolus* were only initially identified by genitalic structure as they lack the broader discal band and blue-green extension into the apex of hindwing cell M_3 . These specimens are distinctly larger (forewing length 46–53 mm, mean = 49.2 ± 1.9 mm, n = 14) than wet season specimens of *G. sarpedon* from the same localities (forewing length 40–46 mm, mean = 43.1 ± 1.4 mm, n = 13). Specimens of all generations of *G. sarpedon* are approximately the same size, whereas there is a distinct size difference between dry and wet season



FIGURE 1. A phylogenetic tree of the *Graphium sarpedon* species complex reconstructed with BEAST showing that *Graphium septentrionicolus* is not closely related to *G. sarpedon*. Branch support for this BEAST chronogram is indicated with bullseyes at each important node. Bayesian inference posterior probability support values are indicated with a ring of colour surrounding a circle that indicates maximum likelihood ultrafast bootstrap support. See graphic in upper left corner for explanation of support values associated with each colour. Tip labels indicate species and subspecies (if applicable), collection locality formatted as two-letter ISO country codes with island or province (if applicable), followed by specimen ID. An asterisk indicates taxa placed in *Graphium adonarensis* by Page & Treadaway (2013).

generations of *G. septentrionicolus*. The forewing underside of *G. septentrionicolus* retains the better developed, rather faint narrow submarginal band, reaching about half-way up the forewing from the tornus, which in *sarpedon* is only usually clearly indicated near the tornus. The hindwing margin is distinctly more scalloped than in *sarpedon*, but the most definitive characters are found in the hindwing anal androconial fold.



FIGURE 2. Specimens of *G. septentrionicolus*. A (dsf.) and B (wsf.): Metok, S. Tibet, China; C: Yingjiang, western Yunnan, China (dsf.); D: Fugong, northwestern Yunnan, China (wsf.); E: northern Sagaing, Myanmar (dsf.); F: northern Kachin State, Myanmar (wsf.); G: Sam Neua, northeastern Laos (dsf.); H: Ha Giang, northern Vietnam (wsf.) (above upperside; below underside; dsf. = dry season form, wsf. = wet season form). Scale bar = 10 mm.

The hindwing androconial fold of species in the *G. sarpedon* complex consists of elongate hair-like scales originating from the base of the anal fold and a patch of modified androconial scales running down the middle of the fold surrounded by normal scales lining the edges and inner margin of the fold. The anal fold of *septentrionicolus* has pale yellowish-brown elongate hair scales, which are whitish in *sarpedon* and *adonarensis*, and the normal scales immediately below the lower tip of the androconial scales are dark brown in *septentrionicolus* as opposed to white in *sarpedon* (Figure 4). Moreover, the range of normal scales in *septentrionicolus* terminates near the tip of the modified androconial scales, while it extends further beyond and surrounds the androconial scales in *sarpedon* and *adonarensis* (Figure 4). These differences in the anal fold are also found in first generation specimens. When observed with a stereoscope, the normal scales neighbouring the modified androconial scales are short and round in *septentrionicolus*, while these scales are long and thin in *sarpedon* and *adonarensis* (Figure 5).

Description of male genitalia of G. septentrionicolus (Figure 6)

Male genitalia of fourteen specimens were dissected. Ring slightly wavy in upper half; saccus large; socii almost parallel, broad at base and slightly narrowed towards bifid inwardly curved, distinctly pointed, tips. Valve rounded with dorsal margin arched and ventral margin smooth in basal half and serrated in distal half, tip of valve shallowly concave; dorsal terminal harpe long and thin, with large, serrated lobe arising near base; dorsal medial harpe finger-like, located below base of the dorsal terminal harpe. Aedeagus long and straight, with funnel-shaped base and pointed tip. Juxta broad shield-shaped with hairy membrane on both sides.



FIGURE 3. Specimens of *G. sarpedon*. A–C: ssp. *sirkari*: A: Sagaing, Myanmar; B: Kachin State, Myanmar; C: Yingjiang, western Yunnan, China; D and E: ssp. *luctatius*: D: Mengla, southern Yunnan, China; E: Sam Neua, northeast Laos; F–N: ssp. *sarpedon*: F: Ha Giang, northern Vietnam; G and H: Kunming, central Yunnan, China; I: Guiyang, Guizhou, China; J: Guangzhou, Guangdong, China; K: Lingshui, Hainan, China; L: Xiangtan, Hunan, China; M: Deqing, Zhejiang, China; N: Fengxian, Shanghai, China (I to N © Jian-Qing Zhu, Shanghai, China); O: ssp. *adonarensis*, Flores Is., Indonesia; P: ssp. *toxopei*, Lombok Is., Indonesia (above upperside; below underside). Scale bar = 10 mm.



FIGURE 4. Close up of androconial folds of A: *septentrionicolus* (Myanmar), B: *sarpedon* (Laos), C: *adonarensis* (Indonesia); scale bar = 10 mm. The red arrow points to the difference in scales lining the tip of the scent scales.



FIGURE 5. Magnified scales near the lower tip of the androconia of A: *septentrionicolus* (China), B: *sarpedon* (China), C: *adonarensis* (Indonesia); scale bar = 1 mm. The red arrow points to the short and round scales in *septentrionicolus*, which are long and thin in *sarpedon*.

Comparison of genitalia of *G. septentrionicolus* (Figure 6), *G. sarpedon* (Figure 7) and *adonarensis* (Figure 8) **Valve:** In *G. septentrionicolus*, dorsal margin of valve less arched than that in *sarpedon* and *adonarensis*; dorsal terminal harpe of *septentrionicolus* with large, serrated lobe rising near base, while this lobe is absent in *sarpedon* and *adonarensis*. The valves of *sarpedon* and *adonarensis* are very similar, dorsal margin of *sarpedon* valve less arched than that in *adonarensis*; dorsal terminal harpe with small teeth near base in *sarpedon* (Figure 9). The apparent difference between the illustrations of the *sarpedon* and *adonarensis* valves in Page & Treadaway (2013: figs. 34 & 35) is due to the angle of observation.

Socii: In *G. septentrionicolus*, socii short, almost parallel and only slightly narrowed towards bifid in-bent pointed tips, in *adonarensis*, socii narrow at base but broader and widely diverging medially but parallel towards tip. Socii of *sarpedon* and *adonarensis* similar, those of *sarpedon* short, straight and bifid from middle section; in *adonarensis* socii narrow at base, broader and widely diverging medially but parallel towards tip.



FIGURE 6. Male genitalia of *G. septentrionicolus* (Yingjiang, western Yunnan, China). All: the whole male genitalia with left valve removed, R.: lateral view of ring, Ae.: aedeagus, Ju.: juxta, TSU: tegumen, socii, and uncus; a: dorsal terminal harpe, b: dorsal medial harpe, c: tip of socii; scale bar = 1 mm.



FIGURE 7. Male genitalia of *G. sarpedon sarpedon* (Kunming, central Yunnan, China); structures as those in Figure 6; scale bar = 1 mm.



FIGURE 8. Male genitalia of *G. sarpedon adonarensis* (Flores, East Nusa Tenggara, Indonesia); structures as those in Figure 6; scale bar = 1 mm.



FIGURE 9. Comparison of valves of: A: *G. septentrionicolus* (Yingjiang, western Yunnan, China); B: ditto (Metok, southern Tibet, China); C: *G. sarpedon sarpedon* (Kunming, central Yunnan, China); D: ditto (Yingjiang, western Yunnan, China); E: *G. sarpedon adonarensis* (Flores, East Nusa Tenggara, Indonesia); F: *G. sarpedon toxopei* (Lombok, Indonesia); scale bar = 1 mm.

Discussion

It is clear from both molecular and morphological analysis that *Graphium septentrionicolus* is not the sister taxon of *G. adonarensis*, which was separated by Page & Treadaway (2013) from *G. sarpedon*. The current study shows that *Graphium septentrionicolus* **stat. nov.** is a distinct species, the ancestor of which diverged before most species of the *sarpedon* complex.

The insular taxa placed in *Graphium adonarensis* by Page and Treadaway (2013) are conspecific with *Graphi-um sarpedon* (**stat. rev.**). These comprise subspecies *agusyantoei* Page & Treadaway, 2013 from Sumatra, *phy-risoides* Page & Treadaway, 2013 from Banka and Belitung, *rufofervidus* (Fruhstorfer, 1898) from Nias, *phyris* (Jordan, 1937) from Pagai and Sipora, *lycianus* (Toxopeus, 1951) from Java, *rufocellularis* (Fruhstorfer, 1905) from Bawean, *hundertmarki* Page & Treadaway, 2013 from Bali, *toxopei* Page & Treadaway, 2013 from Lombok, and *adonarensis* (Rothschild, 1896) from Adonara and nearby islands in the Lesser Sundas. The subspecies of *Graphium adonarensis* described by Page & Treadaway had previously all been placed in *Graphium sarpedon* (*s. l.*) and were separated from existing subspecies in that publication, so these are not treated as new combinations. Page and Treadaway (2013) presented three phenetic results. Based on the above-mentioned phylogenetic position of *G. adonarensis* and its morphological characters, especially the valve of male genitalia (lack of the large, serrated lobe near the dorsal terminal harpe), the present study concludes that *adonarensis* is only a subspecies of *G. sarpedon* rather than a distinct species.

Wu & Ma (2016) described *Graphium huangshanensis* as a new species in their book on butterflies of Anhui Province, China. The specimens illustrated (p. 29, figs. 77–81) are identical to *G. septentrionicolus*, and *Graphium huangshanensis* **syn. nov.** is hereby synonymised with *Graphium septentrionicolus*. It is not surprising that *G. septentrionicolus* is present on Mt. Huang Shan, southern Anhui Province, since Page & Treadaway (2013) included three specimens from Fujian and one from Shennogija [sic, = Shennongjia], W. Hubei, in the paratype series.

During this study, specimens of *Graphium septentrionicolus* were recorded from northeastern Laos and Ha Giang, Vietnam, as well as many from northern Myanmar and various localities in China. Recently, Kotaro Saito shared photos of two males from a mountain in Kon Tum Province, central Vietnam, and a confirmatory genitalia preparation from one of the specimens. This locality in Vietnam is currently the southernmost record of *Graphium septentrionicolus*.

Early stages of *Graphium "sarpedon*" from southern China have been illustrated, and the food plants have been recorded as *Cinnamomum camphora* and *C. burmanni* (Lauraceae) (Zhu *et al.* 2019). However, it is unclear whether these host records are from *G. sarpedon sarpedon* or *G. septentrionicolus* since the two species co-occur in southern China and were previously not recognized as distinct. Host records from Guangzhou, Hong Kong, and Taiwan mostly list Lauraceae, so it is likely that *G. septentrionicolus* larvae also feed on host plants in family Lauraceae (Hill *et al.* 1978; Chen *et al.* 2017; Hsu *et al.* 2018; Hong 2020).

Acknowledgements

Field work and lab work by SJH was funded by grant 41761011 from the NSFC Programme of China and grant SDZXWJZ01013 from the Biodiversity Conservation Programme of the Ministry of Ecology and Environment, China (China-BON Butterflies). Field work by DJL was funded by grant WW-227R-17 from the National Geographic Society, and lab work was funded by grant DEB-1541557 from the US National Science Foundation to DJL.

Hui-Hong Zhang (Yunnan University, Kunming, China) is thanked for assistance in obtaining specimens and Jian-Qing Zhu (Shanghai Zoological Park, Shanghai, China)_shared photos of specimens from S. and E. China. Ko-taro Saito (Tokyo, Japan) provided photos of specimens from C. Vietnam. Wolfgang Nässig and Massimo Terragni (Senckenburg Museum, Frankfurt am Main, Germany) are thanked for providing information about type specimens. Tasnim Jackson and Y-Lan Nguyen are thanked for their assistance in the laboratory.

References

- Breinholt, J.W., Earl, C., Lemmon, A.R., Lemmon, E.M., Xiao, L. & Kawahara, A.Y. (2018) Resolving relationships among the megadiverse butterflies and moths with a novel pipeline for anchored phylogenomics. *Systematic Biology*, 67, 78–93. https://doi.org/10.1093/sysbio/syx048
- Chen, X.C., Yang, J. & Liu, G. (2017) *Field Guide to Butterflies in Guangzhou*. Guangdong Science & Technology Publishing House, Guangzhou, 292 pp.
- Edgar, R.C. (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32, 1792–1797.

https://doi.org/10.2460/ajvr.69.1.82

- Fruhstorfer, H. (1898) Neue Papilioformen aus dem Indo-malayischen Peloponnes. Entomologische Zeitschrift, 11, 189–190.
- Fruhstorfer, H. (1903) Verzeichnis der in Tonkin, Annam und Siam gesammelten Papilioniden und Besprechung verwandter Formen. *Berliner Entomologische Zeitschrift*, 47, 167–234.
- Fruhstorfer, H. (1905) Neue Rhopaloceren. Insekten-Börse, 22, 36.
- Hancock, D.L. (1983) Classification of the Papilionidae (Lepidoptera): a phylogenetic approach. Smithersia, 2, 1-48.
- Hill, D.S., Johnston, G. & Bascombe, M.J. (1978) Annotated checklist of Hong Kong butterflies. *Memoirs of the Hong Kong Natural History Society*, 11, 1–62.
- Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q. & Vinh, L.S. (2017) UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, 35, 518–522. https://doi.org/10.1093/molbev/msx281
- Hong, Y. (2020) Host Plants for Taiwan's Butterflies. Owl Publishing House Co., LTD., Taipei, 328 pp.
- Hsu, Y.F., Huang, C.L. & Liang, J.Y. (2018) *Butterfly Fauna of Taiwan. Papilionidae. Vol. I.* Council of Agriculture Executive Yuan Forestry Bureau, Taipei, 239 pp.
- Hu, S.J., Cotton, A.M., Condamine, F.L., Duan, K., Wang, R.J., Hsu, Y.F., Zhang, X. & Cao, J. (2018) Revision of *Pazala* Moore, 1888: the *Graphium (Pazala) mandarinus* (Oberthür, 1879) group, with treatments of known taxa and descriptions of new species and new subspecies (Lepidoptera: Papilionidae). *Zootaxa*, 4441 (3), 401–446. https://doi.org/10.11646/zootaxa.4441.3.1
- Jordan, H.E.K. (1937) Spolia Mentawiensia: Papilionidae. Novitates Zoologicae, 40, 316–322.
- Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A. & Jermiin, L.S. (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods*, 14, 587–589. https://doi.org/10.1038/nmeth.4285
- Kawahara, A.Y., Breinholt, J.W., Espeland, M., Storer, C., Plotkin, D., Dexter, K.M., Toussaint, E.F.A., St Laurent, R.A., Brehm, G., Vargas, S., Forero, D., Pierce, N.E. & Lohman, D.J. (2018) Phylogenetics of moth-like butterflies (Papilionoidea: Hedylidae) based on a new 13-locus target capture probe set. *Molecular Phylogenetics and Evolution*, 127, 600–605. https://doi.org/10.1016/j.ympev.2018.06.002
- Kawahara, A.Y., Storer, C., Carvalho, A.P.S., Plotkin, D.M., Condamine, F.L., Braga, M.P., Ellis, E.A., Laurent, R.A.S., Barve, V., Cai, L., Earl, C., Frandsen, P.B., Li, X., Owens, H.L., Valencia-Montoya, W.A., Aduse-Poku, K., Toussaint, E.F.A., Dexter, K.M., Doleck, T., Markee, A., Messcher, R., Nguyen, Y.-L., Badon, J.A.T., Benítez, H.A., Braby, M.F., Buenavente, P.A.C., Chan, W.-P., Collins, S.C., Childers, R.A.R., Dankowicz, E., Eastwood, R., Fric, Z.F., Gott, R.J., Hall, J.P.W., Hallwachs, W., Hardy, N.B., Sipe, R.L.H., Heath, A., Hinolan, J.D., Homziak, N.T., Hsu, Y.-F., Inayoshi, Y., Itliong, M.G.A., Janzen, D.H., Kitching, I.J., Kunte, K., Lamas, G., Landis, M.J., Larsen, E.A., Larsen, T.B., Leong, J.V., Lukhtanov, V., Maier, C.A., Martinez, J.I., Martins, D.J., Maruyama, K., Maunsell, S.C., Mega, N.O., Monastyrskii, A., Morais, A.B.B., Müller, C.J., Naive, M.A.K., Nielsen, G., Padrón, P.S., Peggie, D., Romanowski, H.P., Sáfián, S., Saito, M., Schröder, S., Shirey, V., Soltis, D., Soltis, P., Sourakov, A., Talavera, G., Vila, R., Vlasanek, P., Wang, H., Warren, A.D., Willmott, K.R., Yago, M., Jetz, W., Jarzyna, M.A., Breinholt, J.W., Espeland, M., Ries, L., Guralnick, R.P., Pierce, N.E. & Lohman, D.J. (2022) Evolution and diversification dynamics of butterflies. bioRxiv:2022.2005.2017.491528.
- Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–120. https://doi.org/10.1007/BF01731581
- Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35, 1547–1549. https://doi.org/10.1093/molbev/msy096
- Larsson, A. (2014) AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics, 30, 3276-

3278.

https://doi.org/10.1093/bioinformatics/btu531

Linnaeus, C. (1758) Systema Naturae per Regna Tria Naturae, Secundum Classes, Ordines, Genera, Species, cum Characteribus, Differentiis, Symonymis, Locis (Editio 10). Tom. I. Pars II. Impensis Direct. Laurentii Salvii, Stockholm, 826 pp.

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A. & Lanfear, R. (2020) IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, 37, 1530–1534.

https://doi.org/10.1093/molbev/msaa015

- Mutanen, M., Kivelä, S.M., Vos, R.A., Doorenweerd, C., Ratnasingham, S., Hausmann, A., Huemer, P., Dincă, V., van Nieukerken, E.J., Lopez-Vaamonde, C., Vila, R., Aarvik, L., Decaëns, T., Efetov, K.A., Hebert, P.D.N., Johnsen, A., Karsholt O., Pentinsaari, M., Rougerie, R., Segerer, A., Tarmann, G., Zahiri, R. & Godfray, H.C.J. (2016) Species-level para- and polyphyly in DNA barcode gene trees: strong operational bias in European Lepidoptera. *Systematic Biology*, 65, 1024–1040. https://doi.org/10.1093/sysbio/syw044
- Page, M.G.P. & Treadaway, C.G. (2013) Speciation in *Graphium sarpedon* (Linnaeus) and allies (Lepidoptera: Rhopalocera: Papilionidae). *Stuttgarter Beiträge zur Naturkunde A*, Neue Serie, 6, 223–246.
- Rothschild, W. (1896) New Lepidoptera. Novitates Zoologicae, 3 (3), 322-328.
- Saigusa, T., Nakanishi, A., Shima, H. & Yata, O. (1982) Phylogeny and geographical distribution of the swallow-tail subgenus *Graphium* (Lepidoptera: Papilionidae). *Entomologia Generalis*, 8, 59–69.
- Scopoli, J.A. (1777) *Introductio ad historiam naturalem sistens genera lapidum, plantarum, et animalium*. Gerle, Prague, x + 506 pp.
- Shimodaira, H. & Hasegawa, M. (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution*, 16, 1114–1114.
- Smith, C.R. & Vane-Wright, R.I. (2001) A review of the Afrotropical species of the genus *Graphium* (Lepidoptera: Rhopalocera: Papilionidae). *Bulletin of the British Museum (Natural History) Entomology*, 70, 503–719.
- Suchard, M.A., Lemey, P., Baele, G., Ayres, D.L., Drummond, A.J. & Rambaut, A. (2018) Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution*, 4, vey016. https://doi.org/10.1093/ve/vey016
- Tea, Y.K., Wei, J.S.W., Gan, C.W. & Lohman, D.J. (2020) Beachgoing butterflies: Marine puddling on black sand beaches of Tangkoko Batuangus Nature Reserve, North Sulawesi. *Journal of the Lepidopterists' Society*, 74, 127–131. https://doi.org/10.18473/lepi.74i2.a8
- Toxopeus, L.J. (1951) On the collecting localities of some Linnaean types (Lepidoptera, Rhopalocera). Idea, 8, 53-74.
- Tsukada, E. & Nishiyama, Y. (1980) Butterflies of the South East Asian Islands. Part I. Papilionidae. Plapac Co., Ltd., Tokyo, 446 pp.
- Vaidya, G., Lohman, D.J. & Meier, R. (2011) SequenceMatrix: a user-friendly gene concatenation tool for assembling phylogenetic character matrices. *Cladistics*, 27, 171–180. https://doi.org/10.1111/j.1096-0031.2010.00329.x
- Wu, S.J. & Ma, X.Y. (2016) *Records of Butterflies in Anhui Province*. Anhui Science and Technology Publishing House, Hefei, 233 pp.
- Zhu, J.Q., Gu, Y., Chen, Z.B. & Chen, J.L. (2019) *The Life Histories of Chinese Butterflies*. Chongqing University Press, Chongqing, 616 pp.

Supplementary Figure Captions

FIGURE S1. BEAST Chronogram with all outgroups used for age calibration.

Branch support posterior probabilities < 0.5 have been removed. Bars at nodes represent 95% HPD intervals for age estimation. https://doi.org/10.5281/zenodo.6322723

FIGURE S2. IQ-TREE maximum likelihood phylogeny with Ultrafast Bootstrap / Shimodaira-Hasegawa approximate likelihood ration test branch support values. Low support values have been removed from some nodes and some values have been repositioned for clarity. https://doi.org/10.5281/zenodo.6322723

Supplementary Table Captions

TABLE S1. Collection information for specimens sequenced in this study. I/O = Ingroup or Outgroup; Voucher institutions: AMC: Adam Cotton Collection; ANIC: Australian National Insect Collection; CCNY: City College of New York;

DNPTH: Department of National Parks, Plant and Animal Conservation, Thailand; LKCNHM: Lee Kong Chian Natural History Museum, Singapore (formerly RMBR); MCP: Modern College, Pune, India; MCZ: Museum of Comparative Zoology, Harvard University; MGCL: McGuire Center for Lepidoptera and Biodiversity; NMNH: National Museum of Natural History, USA; YNU: Yunnan University. https://doi.org/10.5281/zenodo.6322723

TABLE S2. DNA sequence data partitions and substitution models selected by ModelFinder Plus and implemented in IQ-TREE and BEAST analyses. https://doi.org/10.5281/zenodo.6322723

APPENDIX 1. List of *Graphium sarpedon* group specimens examined in the morphological component of this study.

Names of depositories are given in alphabetical order, with institutions listed after private collections, and are abbreviated as follows: AMC: collection of Adam M. Cotton (Chiang Mai, Thailand); KS: collection of Kotaro Saito (Tokyo, Japan); SJH: collection of Shao-Ji Hu (Kunming, China); ZHJ: collection of Zhuo-Heng Jiang (Kunming, China); WWM: collection of Wei-Wei Mao (Shanghai, China); JQZ: collection of Jian-Qing Zhu (Shanghai, China); XZ: collection of Xin Zhang (Kunming, China); KIZ: Kunming Institute of Zoology, Chinese Academy of Sciences (Kunming, Yunnan); SFU: collection of Southwest Forest University (Kunming, China); SMF: collection of Senckenburg Museum (Frankfurt am Main, Germany).

Graphium septentrionicolus Page & Treadaway, 2013 stat. nov.

INDIA: 1⁽²⁾, HOLOTYPE [illustration], Nowgang, Assam, 1964-IV-14, C. G. Treadaway *leg*. [SMF].

MYANMAR: 1Å, Hkasi (1,050 m), Tarung Hka River, N. Sagaing State, 2007-VII-2, Prasobsuk Sukkit *leg*. [AMC]; 1Å, same locality, 2008-IV-22, Prasobsuk Sukkit *leg*. [AMC]; 1Å, same locality, 2008-IV-25, Prasobsuk Sukkit *leg*. [AMC]; 6ÅÅ, between Pangmandim & Gawai, Mayhka River, N. Kachin State, 1996-VII-20-27, Prasobsuk Sukkit *leg*. [AMC]; 1Å, Dasu Long, east of Renam, N.E. Kachin State, 1996-VI-21, Prasobsuk Sukkit *leg*. [AMC]; 1Å, same locality, 2005-V-23, Prasobsuk Sukkit *leg*. [AMC]; 1Å, same locality, 2005-VII-27, Prasobsuk Sukkit *leg*. [AMC]; 1Å, same locality, 2006-VII-15, Prasobsuk Sukkit *leg*. [AMC]; 1Å, same locality, 2007-VI-3, Prasobsuk Sukkit *leg*. [AMC]; 1Å, same locality, 2008-IV-26, Prasobsuk Sukkit *leg*. [AMC]; 1Å, same locality, 2008-VII-10, Prasobsuk Sukkit *leg*. [AMC]; 1Å, Mairudam, N.E. Kachin State, 2005-VIII-1, Prasobsuk Sukkit *leg*. [AMC]; 1Å, Mairudam, N.E. Kachin State, 2005-VIII-1, Prasobsuk Sukkit *leg*. [AMC]; 1Å, Mairudam, N.E. Kachin State, 2005-VIII-1, Prasobsuk Sukkit *leg*. [AMC]; 1Å, Mairudam, N.E. Kachin State, 2005-VIII-1, Prasobsuk Sukkit *leg*. [AMC]; 1Å, Mairudam, N.E. Kachin State, 2005-VIII-1, Prasobsuk Sukkit *leg*. [AMC]; 1Å, Mairudam, N.E. Kachin State, 2005-VIII-1, Prasobsuk Sukkit *leg*. [AMC]; 1Å, Mairudam, N.E. Kachin State, 2005-VIII-1, Prasobsuk Sukkit *leg*. [AMC].

CHINA: 1Å, Duoxiongla valley, Metok, S. Tibet, 2012-VII–22-VIII-10, Yang Yang *leg*. [AMC]; 5ÅÅ, Beibeng, Metok, S. Tibet, 2016-IV–23-V-1, Lu Ji *leg*. [AMC]; 2ÅÅ, Tongbiguan (1,000 m), Yingjiang, Yunnan, 2017-III-26; Wei-Zong Yang *leg*. [SJH]; 1Å, Fugong, N.W. Yunnan, 2006-VIII-13, Prasobsuk Sukkit *leg*. [AMC].

LAOS: 13, Phou Phan, Sam Neua, Houaphanh Province, 2005-III-7, Khamboun Sengheuangsomphou leg. [AMC].

VIETNAM: 1♂, Sapa, Lao Cai Province, 2007-V, local catcher *leg*. [AMC]; 2♂♂, Ngoc Linh (2,400 m), Kon Tum Province, 2021-IV, local catcher *leg*. [KS].

Graphium sarpedon sirkari Page & Treadaway, 2013

INDIA: 13, HOLOTYPE [illustration], Shillong, Khasi Hills, Assam, N. India, April–June 1968, leg. S. K. Sirkar. [SMF].

MYANMAR: 1Å, Hkasi (1,050 m), Tarung Hka River, N. Sagaing State, 2007-VII-14, Prasobsuk Sukkit *leg*. [AMC]; 1Å, same locality, 2007-VIII-4, Prasobsuk Sukkit *leg*. [AMC]; 1Å, same locality, 2008-VII-25, Prasobsuk Sukkit *leg*. [AMC]; 1Å, between Pangmandim & Gawai, Mayhka River, N. Kachin State, 1996-IX-14, Prasobsuk Sukkit *leg*. [AMC]; 1Å, Chudu Razi Hills, east of Kawnglanghpu, N.E. Kachin State, 2004-VI-24, Prasobsuk Sukkit *leg*. [AMC]; 2ÅÅ, same locality, 2005-VI-26, Prasobsuk Sukkit *leg*. [AMC]; 1Å, same locality, 2006-VII-17, Prasobsuk Sukkit *leg*. [AMC]; 2ÅÅ, same locality, 2007-VII-5, Prasobsuk Sukkit *leg*. [AMC]; 1Å, same locality, 2008-VI-24, Prasobsuk Sukkit *leg*. [AMC].

CHINA: 13, Fugong, N.W. Yunnan, 2012-VII-17, Prasobsuk Sukkit leg. [AMC].

Graphium sarpedon sarpedon (Linnaeus, 1758)

VIETNAM: 4♂♂, Dong Van, Ha Giang, 2006-V, local catcher *leg*. [AMC]; 1♂, same locality, 2008-VII, local catcher *leg*. [AMC].

Graphium sarpedon luctatius (Fruhstorfer, 1907)

CHINA: 1♀, Lushui, Yunnan, 1995-IX, A. Nakanishi *leg.* [KIZ]; 1♂, 1♀, Zhangfeng, Longchuan, Yunnan, 1981-X-12, Chang-Lian Li leg. [KIZ]; 13, Mangdong, Yingjiang, Yunnan, 1981-X-8, Shan-Yi Zhou leg. [KIZ]; 13, Tongbiguan (1,000 m), Yingjiang, Yunnan, 2017-III-26, Wei-Zong Yang leg. [SJH]; 13, Nongdao, Ruili, Yunnan, 1981-X-19, Chang-Lian Li leg. [KIZ]; 1♂, Manghai, Mangshi, Yunnan, 1985-VII-27, Chang-Lian Li leg. [KIZ]; 1♂, Daxue Shan, Yongde, Yunnan, 2015-VIII-25, Wei-Wei Mao leg. [WWM]; 1♂, 1♀, Cangyuan, Yunnan, 1981-VI-25, Chang-Lian Li & Da-Zhi Dong leg. [KIZ]; 2♂♂, Shuangjiang, Yunnan, 1981-VII-1-2, Da-Zhi Dong leg. [KIZ]; 13, Mengsuo, Ximeng, Yunnan, 1981-VI-21, Da-Zhi Dong leg. [KIZ]; 1♂, Jinggu, Yunnan, 1981-VI-25, Yun-Zhen Wang leg. [KIZ]; 1♀, the same locality, 1981-VII-13, Chang-Lian Li leg. [KIZ]; 1♂, Simao, Pu'er, Yunnan, 1981-V-17, Chang-Lian Li leg. [KIZ]; 1♂, Simao, Pu'er, Yunnan, 1990-XI-21, Su Lin leg. [KIZ]; 3 ご ご, Mangao, Menghai, Yunnan, 1989-X-9, Da-Zhi Dong leg. [KIZ]; 5 ご ご, Menghai, Yunnan, 1989-X-10–12, Da-Zhi Dong leg. [KIZ]; 1♂, Jinghong, Yunnan, 1974-VI-29, Yun-Xing Gan leg. [KIZ]; 1♀, the same locality, 1979-VIII-4, Da-Zhi Dong leg. [KIZ]; 13, the same locality, 1981-V-30, Da-Zhi Dong leg. [KIZ]; 13, the same locality, 1981-VI-2, Chang-Lian Li leg. [KIZ]; 13, Manzhuang, Mengla, Yunnan, 1978-VII-30, Yun-Xing Gan leg. [KIZ]; 433, Menglun (545 m), Mengla, Yunnan, 2014-IV-5, Shao-Ji Hu leg. [SJH]; 13, the same locality, 2012-X, local catcher leg. [SJH]; 13, Da Longha (770 m), Mengla, Yunnan, 2014-IV-6, Shao-Ji Hu leg. [SJH]; 13, Shangyong (780 m), Mengla, Yunnan, 2014-IV-6, Shao-Ji Hu leg. [SJH]; 1♂, 1♀, Ma'andi (680 m), Jinping, Yunnan, 2014-V-10, Quan Zeng *leg*. [SFU]; 7♂♂, 2♀♀, the same locality, 2013-VII-25-28, Quan Zeng leg. [SFU].

LAOS: 233, Phou Phan, Sam Neua, Houaphanh Province, 2005-IV-4–6, Khamboun Sengheuangsomphou *leg*. [AMC]; 233, same locality, 2006-V-2–5, Khamboun Sengheuangsomphou *leg*. [AMC].

Graphium sarpedon toxopei Page & Treadaway, 2013 stat. rev.

INDONESIA: 1Å, **HOLOTYPE** [illustration], Lombok Is., 1997-XII, local catcher *leg*. [SMF]; 1Å, Lombok Is., 2004-IX, local catcher *leg*. [SJH]; 1Å, Lombok Is., 2004-IX, local catcher *leg*. [AMC]; 2ÅÅ, Lombok Is., 2005-I, local catcher *leg*. [AMC].

Graphium sarpedon adonarensis (Rothschild, 1896) stat. rev.

INDONESIA: 1, **HOLOTYPE**, Adonara Is. 1891-IX, W. Doherty *leg*. [BMNH]; 1, Sumbawa Is., 2000-X, local catcher *leg*. [AMC]; 1, Sumbawa Is., 2003-I, local catcher *leg*. [AMC]; 1, Flores Is., 2004-II, local catcher *leg*. [SJH]; 2, Flores Is., 2005-V, local catcher *leg*. [AMC]; 1, Flores Is., 2005-XI, local catcher *leg*. [AMC]; 1, Alor Is., 2004-III, local catcher *leg*. [AMC]; 1, Alor Is., 2004-III, local catcher *leg*. [AMC]; 1, Alor Is., 2006-VII, local catcher *leg*. [AMC].