

Research article

urn:lsid:zoobank.org:pub:9BC36596-2248-4A84-B514-938054D32D57

Morphological and genetic analyses verify the occurrence of the butterfly *Graphium chironides* (Lepidoptera: Papilionidae) in Peninsular Malaysia and resolve the confusion on the validity of its subspecies *malayanum* Eliot, 1982Chooi-Khim PHON^{1,*.§}, Laurence G. KIRTON^{2,§} & Meng-Kiat KUAH³^{1,2}Entomology Branch, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia.³Lab-Ind Resource Pte. Ltd., 57–59 Jalan Adenium 2G/6, Pusat Perniagaan Adenium, 48300 Bandar Bukit Beruntung, Selangor, Malaysia.*Corresponding author: phonchooikhim@frim.gov.my²Email: kirton.frim@gmail.com³Email: kuahmk@gmail.com

§Joint first authors

¹urn:lsid:zoobank.org:author:4AC10597-7AA4-401B-8444-3F46E3F0EE44²urn:lsid:zoobank.org:author:40CFD67F-FEF4-4490-9C49-F17CE622DD7C³urn:lsid:zoobank.org:author:D65D5963-575B-4AEE-AB64-C4698FE86EC6

Abstract. *Graphium chironides malayanum* Eliot, 1982 was described as a taxon occurring sympatrically with *G. bathycles bathycloides* in Peninsular Malaysia. However, the validity of the subspecies has been questioned in a recent publication that was based on a study of DNA and morphology, implying that *G. c. malayanum* is a synonym of *G. b. bathycloides* and *G. chironides* is absent from the Peninsula. A re-examination of male wing morphology, genitalia and DNA shows that *G. c. malayanum* is a valid taxon distinguished from *G. b. bathycloides* by wider discal markings, a less falcate forewing, distinct differences in the arms of the harpe in the male genitalia and clearly divergent mtDNA COI genes. In the DNA analysis, *G. c. malayanum* formed a monophyletic clade closely related to *G. chironides* from China, and both were well-separated from the *G. b. bathycloides* clade. An examination of characters used in the previous study showed that the conclusions reached were due to misinterpretation of diagnostic characters, misidentification of specimens and the absence of *G. c. malayanum* among the specimens examined. When these characters were correctly interpreted, each specimen was readily assigned to the correct taxon. Diagnostic morphological characters are reclarified based on the current data.

Keywords. Papilionidae, *Graphium bathycles bathycloides*, *Graphium chironides malayanum*, morphology, DNA barcode.

Phon C.-K., Kirton L.G. & Kuah M.-K. 2024. Morphological and genetic analyses verify the occurrence of the butterfly *Graphium chironides* (Lepidoptera: Papilionidae) in Peninsular Malaysia and resolve the confusion on the validity of its subspecies *malayanum* Eliot, 1982. *European Journal of Taxonomy* 917: 94–121. <https://doi.org/10.5852/ejt.2024.917.2391>

Introduction

Graphium bathycles (Zinken, 1831) and *G. chironides* (Honrath, [1884]) are closely related species of swallowtail butterflies that are distinguished from other similar black-and-blue species in their genus by having the discal series of bluish spots on the hindwing well-divided by dark lines along the upper discocellular vein and the cubitus. In addition, the bluish discal spots in the hindwing cell and at the bases of spaces 7 and 8 form a broken or continuous, curved band.

Graphium bathycles is a Sundaic species, while *Graphium chironides* is primarily a continental species. The latter was long considered a subspecies of *G. bathycles*, following Rothschild (1895), until Saigusa *et al.* (1977) showed that *G. bathycles* and *G. chironides* (“*chiron*,” name preoccupied) have very different male genitalia and are sympatric in Peninsular Malaysia. The Peninsular Malaysian phenotype of *G. chironides*, which was later described as a new subspecies, *malayanum* Eliot, 1982, has a broader forewing bluish band than *G. b. bathycloides*, the Peninsular Malaysian race of *G. bathycles*.

A recent study on the DNA barcode sequences of thirteen species of *Graphium* Scopoli, 1777 and the morphology of these two taxa and their type specimens (Wilson *et al.* 2014) casts doubt on the validity of the taxon *malayanum* as a subspecies of *chironides* that can be differentiated from the taxon *bathycloides*. It suggests that the nominal taxon *malayanum* was based on doubtful taxonomic characters and, by implication, is a synonym of *bathycloides* (type locality: Peninsular Malaysia and Borneo). This would also imply that *G. chironides* is confined to the continent and does not occur in the Peninsula. The resulting uncertainty over the status of the taxon in Peninsular Malaysia is perhaps reflected in the choice of the nomenclatural combination “*Graphium bathycles malayanum*” for the holotype and one of the paratypes of *malayanum* in the Data Portal of the Natural History Museum, London (Natural History Museum 2023a, 2023b, respectively), which suggests that *malayanum* is an infraspecific taxon under *G. bathycles* rather than *G. chironides*.

As a result of these uncertainties, the joint first authors of the present study have in the past received several enquiries about the status of *G. chironides* in Peninsular Malaysia. Therefore, in this paper, we address the question of whether there are two separable phenotypes that correspond to two different species in the Peninsula using the morphology of the wings and genitalia, and genetic sequences.

Material and methods

To determine whether there are two phenotypes in the Peninsula, we measured wing characters, including those described by Eliot (1982). Principal components analysis (PCA) of key characters combined with bivariate ordination was used to identify differing phenotypes. Male genitalia and genetic sequences were used to associate phenotypes to known taxa outside the Peninsula. The interpretation of characters by Wilson *et al.* (2014) was examined, and the reliability of all measured wing characters was determined by critical quantitative analysis.

Specimens examined

We examined and measured 54 specimens of the *bathycloides-malayanum* group from the collections and repositories listed below, including all of the rarer broad-banded phenotypes. All specimens were males, which is the more commonly encountered sex because of its habit of puddling and patrolling.

Collections and repositories

Chong-Arshad = The joint private collection of Chow-Yang Chong and Sabri John Arshad
 FRIM = Entomological Reference Collection, Forest Research Institute Malaysia, Kepong
 Kirton = The joint private collection of Eric, Colin and Laurence Kirton
 Liew = The private collection of Nyok-Lin Liew

MNM	= Natural History Museum of the Malaysian National Museum, Department of Museums, Kuala Lumpur
MZUM	= Museum of Zoology, University of Malaya, Kuala Lumpur (abbreviated as UMKL in Wilson <i>et al.</i> 2014)
ZRC	= Zoological Reference Collection, Lee Kong Chian Natural History Museum, National University of Singapore

In addition, we examined photographs of types and their labels in the Data Portal of the Natural History Museum, London: a specimen labelled and identifiable as the holotype of *G. c. malayanum* with accession number BMNH(E)#149669 (Natural History Museum 2023a), and a specimen said to be a syntype of *G. b. bathycloides* with accession number BMNH(E)#149397 (Natural History Museum 2023c). These are likely to be the same photographs said to be provided by John Chainey of the Natural History Museum, London to Wilson *et al.* (2014), as they have the same accession numbers mentioned above. Two specimens figured in Wilson *et al.* (2014) that could not be traced in the MZUM and a specimen seen in the wild at Fraser’s Hill, Pahang by Michael Soh (Soh 2023) were also examined only from photographs.

Wing characters and measurements

Wing characters measured are listed and described in Table 1 and illustrated in Fig. 1. Several of the characters (nos. 1 to 5 and 8 to 13) are based on Eliot’s (Eliot 1982; Corbet & Pendlebury 1992), while two (nos. 6 and 14) were based on suggestions by C.Y. Chong (pers. com.) and five (nos. 7, and 15 to 18) were our own. Characters on which nos. 14 and 18 are based were originally pointed out by Wallace (1865) in his original description of *Graphium chironides* (“*Papilio chiron*”). We did not attempt to quantify the colour characters described by Eliot, as we deemed these to be inconsistent and affected by specimen age.

Specimens were usually photographed against a calibrated ruler (GEI International) with an error of less than ± 0.0083 mm per cm over a scale measurement length of 15.24 cm. When a different ruler was used, it was calibrated against the former wherever possible. The right wing was measured, or the left if the right was too damaged. Linear measurements were made using image analysis software (Digimizer® ver. 4.6.1.), while area measurements were made using Adobe Photoshop® ver. 6.0.1 with the aid of the magic wand tool. Where specimens were too damaged or could only be analysed based on available photographs that lacked a scale, measurements were restricted to ratio and angle characters that were independent of scale.

Ordination of phenotypes

Since the width of the blue spots on the forewing upperside was a key character used by Eliot (1982) in distinguishing *G. c. malayanum* from *G. b. bathycloides*, we used principal components analysis (PCA) of the correlation matrix in Minitab® ver. 19.2020.1 to reduce the individual widths of the blue spots in spaces 1b to 5 to a linear combination of these widths that represented the greatest variance among the specimens (component 1 of the PCA). Scores for principal component 1 were plotted against forewing length as a standardising variable for size, and against forewing angle because a less falcate forewing has been said to be a character of *Graphium chironides* (Wallace 1865). In addition, we plotted the width of the forewing spot in space 5 relative to the length of vein 5 (a ratio) against the forewing angle and included other specimens such as the types and specimens for which only images without a scale were available. Representative specimens from the natural groups (phenotypes) that could be seen in the scatter plots were examined for differences in their genitalia and DNA barcodes.

Genitalia

The genitalia of six males of each phenotype that was identified by the ordination procedure described above were dissected, examined, and matched with described taxa. Since a detailed comparison of the male genitalia of the two taxa, *G. c. malayanum* and *G. b. bathycloides*, has not previously been published, we have figured and described the genitalia in detail with an emphasis on key differences between the taxa and individual variation. An explanation of the methods used in imaging the genitalia is given in Supp. file 1 (Part A).

DNA barcodes

Genetic analysis was used to further investigate the phenotypes obtained by ordination and to validate the identities that were obtained by comparison of genitalia morphology. Mitochondrial DNA (mtDNA) from the cytochrome oxidase subunit I gene (COI) was used since ribosomal RNA (28S rRNA) did not adequately separate species of *Graphium* in Wilson *et al.* (2014). Two specimens of each phenotype that was identified by the ordination were sequenced. In addition, we used three Peninsular Malaysian specimen sequences from Wilson *et al.* (2014) and two available sequences of specimens from China.

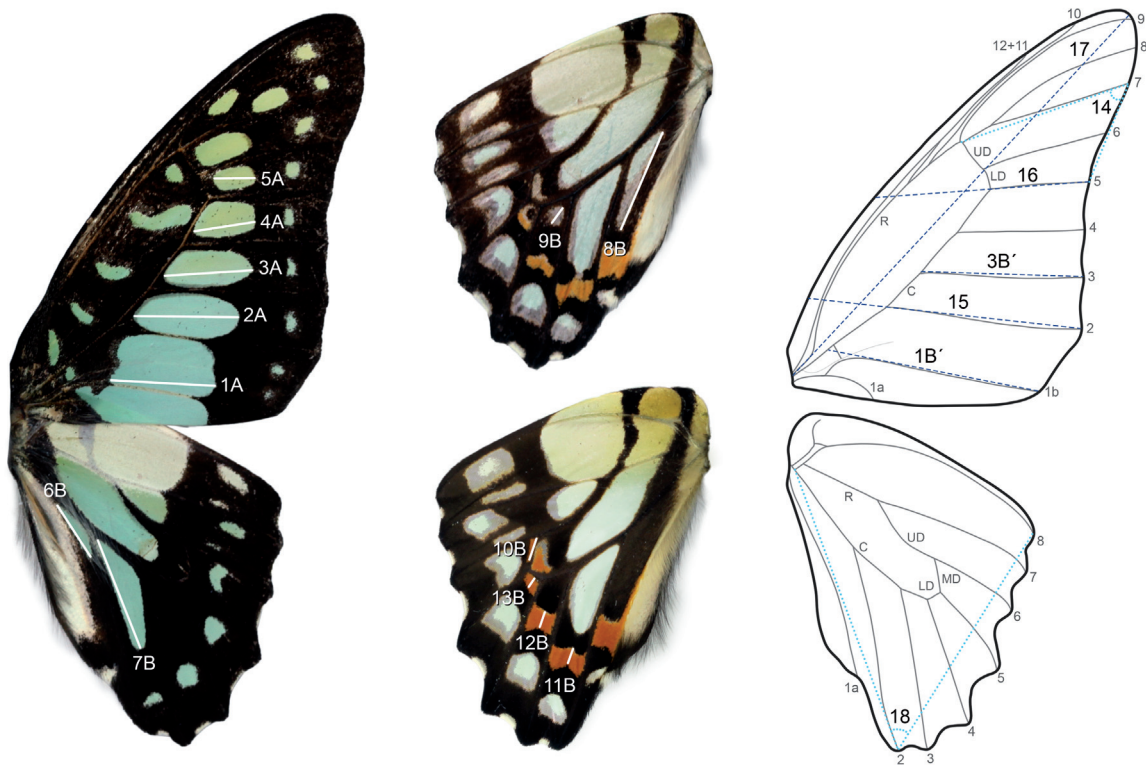


Fig. 1. Illustrations of characters measured on the right wings of specimens of *Graphium* Scopoli, 1777, as described and numbered in Table 1. Area measurements are not illustrated but were measured for the corresponding spots on which linear measurements are shown. Left: upperside wing spot measurements. Middle: underside hindwing spot measurements for two phenotypes. Right: wing dimension, vein and angle measurements (diagrammatic, showing wing outline, veins and vein numbers). Abbreviations: 1B' = straight-line length representing vein 1b as measured for calculation of ratio 1B; 3B' = example straight-line vein length as measured for calculation of ratio 3B; C = cubitus; R = radius; UD, MD and LD = upper, middle and lower discocellular veins, respectively.

Table 1 (continued on next page). Descriptions of wing characters measured. Characters are illustrated with their numerical codes in Fig. 1.

Type of character	No.	Character abbreviation	Character description [§]
Forewing upperside band	1A	UpFwSp1bBW	The width of the discal band between the innermost and outermost edges of the pale blue spot in space 1b, 2, 3, 4 and 5, respectively.
	2A	UpFwSp2BW	
	3A	UpFwSp3BW	
	4A	UpFwSp4BW	
	5A	UpFwSp5BW	
	1B	UpFwSp1bBWR	The ratio formed by dividing characters 1A–5A above by a straight-line length representing the vein beneath the spots: in 1B, the straight-line being from the cubitus along vein 1b bisecting the spots in spaces 1a and 1b and terminating at the end of vein 1b; in 2B–5B, the straight lines being from the origins to the ends of the respective veins.
	2B	UpFwSp2BWR	
	3B	UpFwSp3BWR	
	4B	UpFwSp4BWR	
	5B	UpFwSp5BWR	
Hindwing upperside bluish spots	6A	UpHwSp1bSA	Size (area) of the streak at the base of space 1b, if present.
	6B	UpHwSp1bSL	Length of the streak at the base of space 1b, if present, from its furthest basal extent to its most apical end.
	7A	UpHwSp2SA	Size (area) of the spot in the basal half of space 2.
	7B	UpHwSp2SL	Length of the spot in the basal half of space 2 from its furthest basal extent to its most apical end.
Hindwing underside bluish spots	8A	UnHwSp1bSA	Size (area) of the subdiscal streak in space 1b, if present.
	8B	UnHwSp1bSL	Length of the subdiscal streak in space 1b, if present, from its furthest basal extent to its most apical end, even if the streak is broken.
	9A	UnHwSp3SA	Size (area) of the spot at the base of space 3, if present.
	9B	UnHwSp3SL	Length of the spot at the base of space 3, if present, from its furthest basal extent to its most apical end.

Table 1 (continued). Descriptions of wing characters measured. Characters are illustrated with their numerical codes in Fig. 1.

Type of character	No.	Character abbreviation	Character description [§]
Hindwing underside orange spots *	10A	UnHwSp5SA	Size (area) of the spot at the base of space 5, if present.
	10B	UnHwSp5SL	Maximum length of the spot at the base of space 5, if present.
	11A	UnHwSp2OSA	Size (area) of the spots in spaces 2, 3 and 4, respectively.
	12A	UnHwSp3OSA	
	13A	UnHwSp4OSA	
	11B	UnHwSp2OSW	Width of the spots in spaces 2, 3 and 4, respectively, measured midway between and parallel to the bounding veins.
	12B	UnHwSp3OSW	
	13B	UnHwSp4OSW	
Wing shape and size	14	FwAng	Angle formed between two lines originating at the end of vein 7 on the forewing margin, one extending to the base of vein 7 at the cell-end and the other extending to the end of vein 5 on the forewing margin.
	15	FwWV2	The widths of the forewing between the costa and termen on straight lines that cross the bases of veins 2 and 5, respectively, and terminate at the end of the respective vein.
	16	FwWV5	
	17	FwL	Forewing length, measured from the base of the cell to the wing apex.
	18	HwAng	Angle formed between two lines originating at the end of vein 2 on the apical hindwing margin, one extending to the base of the cubitus and the other extending to the end of vein 8 on the wing margin.

[§] Units for measurements: mm (linear), mm² (area), degrees (angle). *Hindwing underside orange spot in space 1b omitted because the hindwing tends to fold near the dorsum, partially occluding the spot.

Graphium agamemnon agammemnon (Linnaeus, 1758) and *G. evemon eventus* (Fruhstorfer, 1908) were used as outgroups. All specimens were examined except those from China.

DNA was extracted from a single hindleg of each specimen using the QIAamp DNA Mini Kit (Qiagen 2016: 32–35, 53–54). The primer pair described by Wilson (2012), LepF1 and LepR1, was used to amplify a 658-bp fragment from the COI gene. PCR was performed on 3 µl of extracted DNA in a Q5[®] Hot Start High-Fidelity Master Mix (New England Biolabs, USA) following the thermocycles described by Wilson (2012). The resulting PCR products were then gel purified and sequenced in both forward and reverse directions.

Sequences were aligned using ClustalW with default parameters in MEGA ver. 11.0.11. Base pairs outside of the target 658-bp region were trimmed manually. A maximum-likelihood (ML) tree was constructed using IQ-TREE ver. 2.2.0 (Minh *et al.* 2020) in which ModelFinder (Kalyaanamoorthy *et al.* 2017) was applied to determine the best-fit model, and ultrafast bootstrap (Hoang *et al.* 2018) and an SH-like approximate likelihood ratio test (SH-aLRT) were used in the analysis. ModelFinder selected the model TIM2+F+I with the highest Bayesian Information Criterion (BIC) weight. The analysis produced one maximum likelihood tree and one consensus tree. Support values were determined from 1000 re-samplings.

Examination of characters used by Wilson *et al.*

Specimens used by Wilson *et al.* (2014) were re-examined as far as possible, and the characters they used were also examined in detail with reference to their illustrations and descriptions so as to determine reasons for their perceived ambiguity.

Separability of taxa based on each wing character

Quantified characters were compared to determine those that differ on average between taxa and those that can reliably separate them. Median, mean, inter-quartile range (IQR), $1.5 \times \text{IQR}$ and outliers were compared graphically between the taxa. Nonparametric multivariate tests (NPMV) in R ver. 4.2.0 were used to determine whether there were significant differences between taxa after grouping variables by type (structural characters and wing markings were tested separately). Where width and a width ratio were measured for the same wing marking, only width was tested to avoid redundancy. Similarly, where area and length or width were measured for the same wing marking, only area was tested. Based on the results, we clarify the diagnostic characters of the taxa concerned.

Results

Ordination of phenotypes

Component 1 of the PCA of widths of the forewing blue spots in spaces 1b to 5 represented 86.4% of the variance. Eigenvectors were 0.392, 0.464, 0.467, 0.459, and 0.449, respectively, indicating all spots but especially those from space 2–5 contributed in the same direction and in nearly the same magnitude to this component.

Scores of the first component of PCA were plotted against forewing length and forewing angle, and the resulting ordinations revealed two clusters, one comprising 11 specimens with markedly wider wing spots and the other comprising 43 specimens with narrower wing spots with respect to wing length and forewing angle (Fig. 2). The cluster with wider wing markings corresponded exactly with the cluster with a larger forewing angle or less falcate forewing shape.

Similar separation into two groups was observed when the ratio of the width of the forewing spot in space five to vein length was plotted against forewing angle (Fig. 3). Measurements made on the images of the holotype of *G. chironides malayanum* and a syntype of *G. bathycles bathycloides* enabled them to be included in the ordination, and they clustered with the wide- and narrow-spotted phenotypes, respectively (Fig. 3). Three specimens used by Wilson *et al.* (2014) that we were either unable to trace (KC0002 and KC0003) or that had broken wing apices (JJW0119) clustered with the narrow-spotted phenotype as did the other specimens they used (Fig. 3), while a specimen photographed in the wild (Soh 2023) clustered with the wide-spotted phenotype (Fig. 3). All measurements and specimen data are given in Supp. file 2 (Part A). Georeferencing of the collection localities from the data labels (excluding two imprecisely labelled specimens indicated in the supplement) showed that the two phenotypes occurred sympatrically and sometimes at the exact same location in Peninsular Malaysia (Fig. 4).

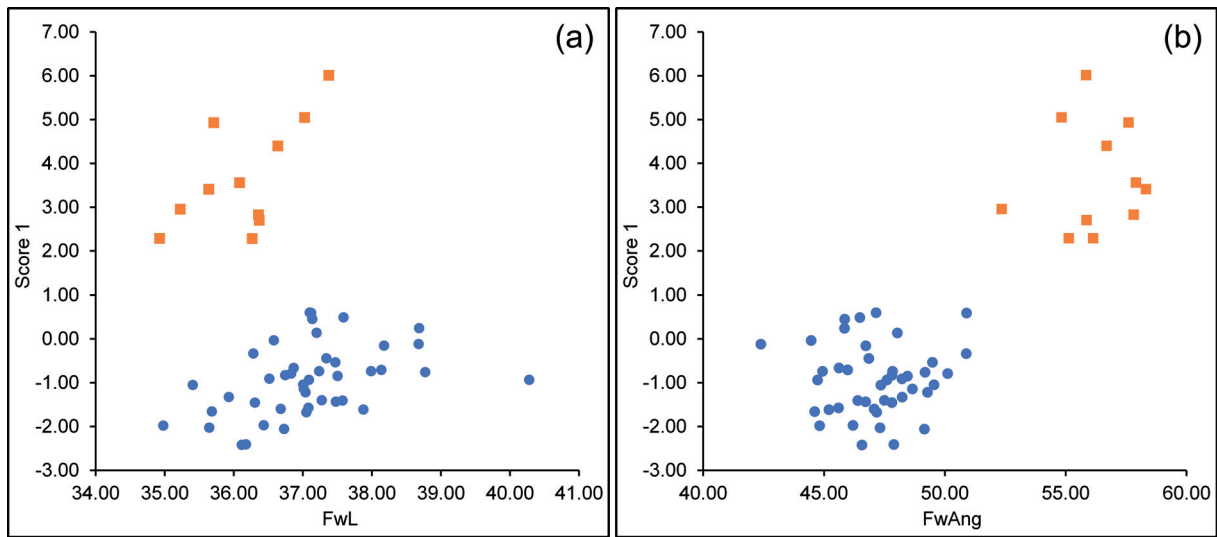


Fig. 2. Scatterplots of scores of PCA component 1 (a linear combination representing forewing band width) against (a) forewing length and (b) forewing angle. The two resulting widely-separated clusters are indicated by square and round markers, and two clusters correspond in specimens between graphs.

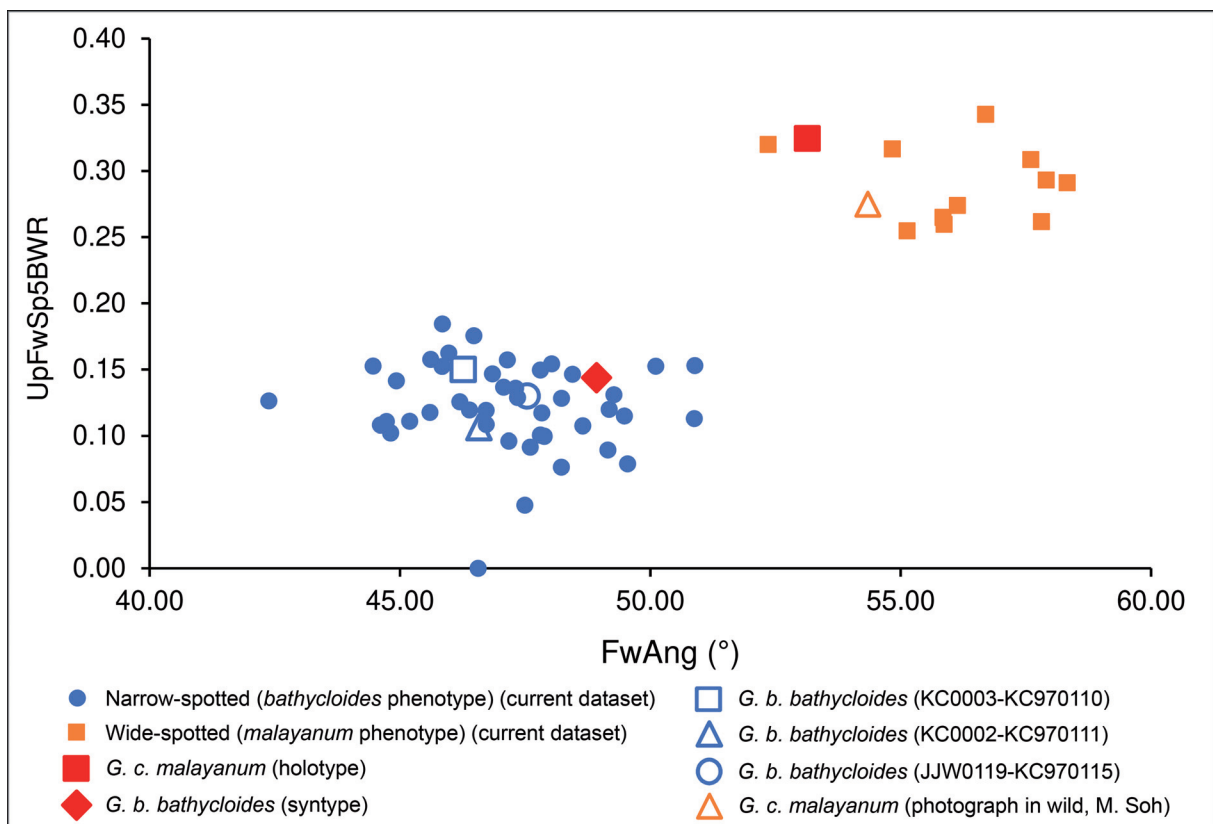


Fig. 3. Scatterplot of the width ratio of the forewing spot in space five against the forewing angle, for both the current dataset and specimens examined in images without an included scale.

Differences in the structure of the genitalia

When six representative specimens from each of the two clusters in the scatter plots (Figs 2–3) were dissected, clear differences could be seen between them, and they were readily recognisable as the two taxa, *G. bathyclades* and *G. chironides*. An overview of the lateral profile of the genitalia of the Peninsular Malaysian races of both taxa is shown in Fig. 5, with greater detail of the different parts of the genitalia shown in Fig. 6. The general structure of the genitalia of both taxa is discussed in Supp. file 1 (Part B). The genitalia differ between the two taxa mainly in the harpe, which is the highly sclerotised, armed structure in the middle of the inner surface of the valva (Fig. 5b, d). The harpe comprises three arms. One arm (hereafter referred to as the lower arm) is situated posteroventrally, and the arm on the opposite end of the harpe (upper arm) is situated anterodorsally, while the other arm (middle arm) is located between them (Figs 5, 6d, h).

Differences between the harpe of the two taxa are illustrated most clearly by images of the arms of the harpe shown in Fig. 7, which were taken at different angles. The harpe of the narrow-spotted phenotype

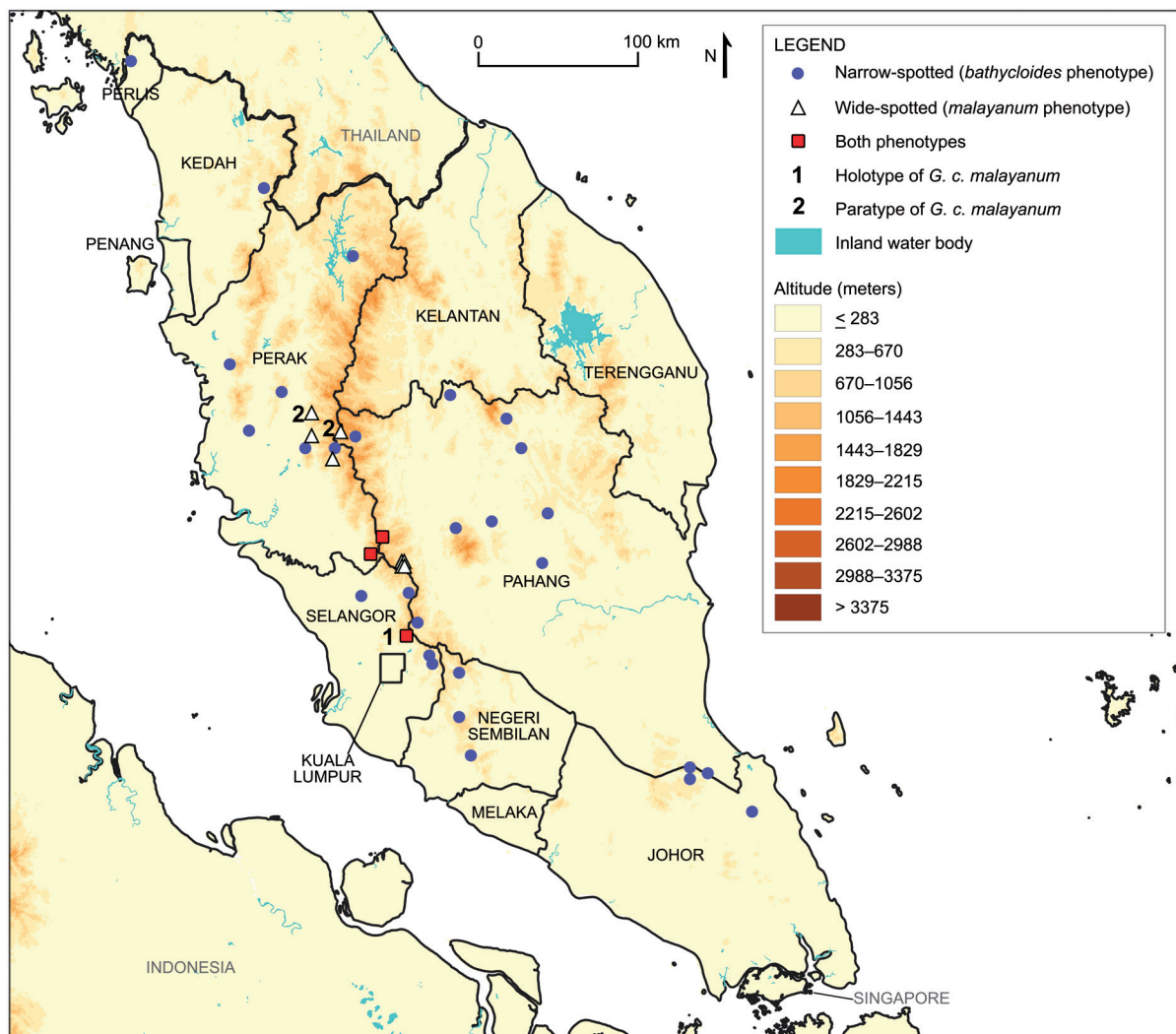


Fig. 4. Distribution of *G. c. malayanum* Eliot, 1982 (wide-spotted) and *G. b. bathycloides* (Honrath, [1884]) (narrow-spotted) phenotypes in Peninsular Malaysia based on georeferenceable locality data of specimens used in Fig. 3.

agrees with the illustrations of the harpe of *G. bathycles* in Saigusa *et al.* (1977) (locality unspecified), Eliot (1982) (Peninsular Malaysia) and Tsukada & Nishiyama (1982) (Sumatra) in having the middle arm close to the lower arm and joined to it at its base (Fig. 7c–d). This phenotype is therefore *G. b. bathycloides*, the Peninsular Malaysian subspecies of *G. bathycles*. The harpe of the wide-spotted phenotype agrees with the illustrations of *G. chironides* shown in Saigusa *et al.* (1977) (“*chiron*,” locality unspecified) and Racheli & Cotton (2009) (Fujian, China) in having the middle arm located almost midway between the upper and lower arms (Fig. 7h–i). This phenotype is, therefore, *G. c. malayanum*,

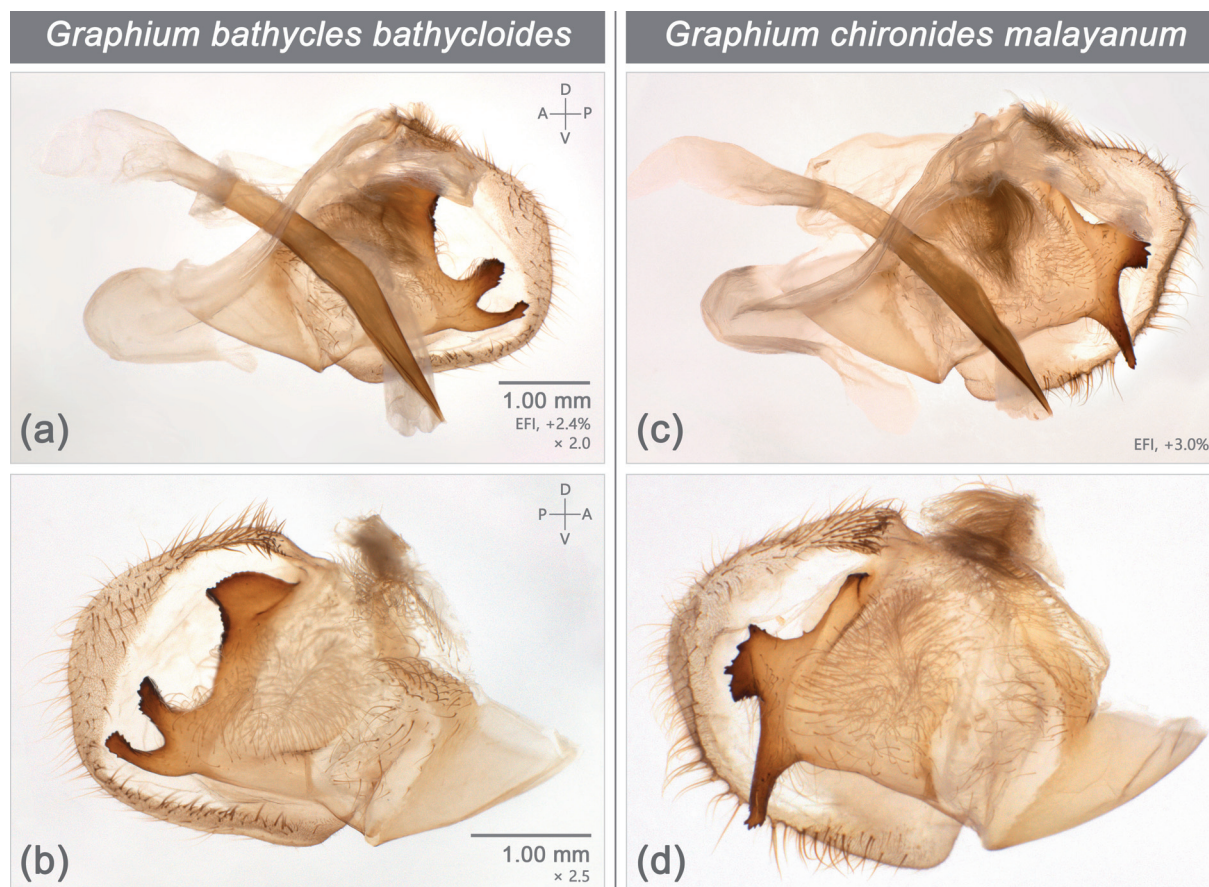


Fig. 5. Genitalia. **a–b.** *G. b. bathycloides* (Honrath, [1884]). **c–d.** *G. c. malayanum* Eliot, 1982. **a, c.** Left lateral view of genitalia with left valva removed and aedeagus angled posteroventrally to show harpe on right valva. **b, d.** Inner lateral view of dissected left valva with valvula and hairs that surround the aedeagus dissected and upturned to expose inner surface of valva. Conventions used are defined here for all subsequent genitalia figures. All images for each taxon are based on the same specimen, unless otherwise stated. Scales for images produced by EFI are based on the lowermost focal plane unless otherwise stated and show a compensation factor for measurements in the uppermost focal plane (positive percentage). Where the scale is not based on the lowermost focal plane, an additional compensation factor is provided for this focal plane (negative percentage). Magnifications shown are objective \times tube factor. Axes show the orientation of structures. Abbreviations: \angle = angle of inclination stated in degrees (with ‘+’ indicating upward tilt, and ‘-’ indicating downward tilt); A = antero/anterior; D = dorso/dorsal; I = inner; L = lateral; O = outer; P = postero/posterior; V = ventro/ventral. Where not otherwise indicated, all scales, magnifications, orientations and angles of inclination are identical between corresponding images on the same row.

the Peninsular Malaysian race of *G. chironides*, and the structure of its harpe also agrees with the illustration for *malayanum* in the original description by Eliot (1982).

The setae on the less sclerotised surfaces of the harpe are more numerous and denser in *G. b. bathycloides* (Fig. 7c–d) than in *G. c. malayanum* (Fig. 7h–i). There are also clear differences between the taxa in the shape and size of the arms. In *G. b. bathycloides* the upper arm of the harpe is trihedral, large, toothed, hump-like in anterolateral profile, and usually with a prominent pointed apex (Fig. 7c–e). In *G. c. malayanum*, the upper arm follows a similar trihedral pattern but it is very small and untoothed, being reduced almost to a tooth itself (Fig. 7h–j). The middle arm of the harpe is relatively narrow in *G. b. bathycloides* in lateral profile, expanding slightly and becoming highly sclerotised at its toothed distal end before tapering to a pointed apex (Fig. 7c–e). In contrast, the middle arm of *G. c. malayanum* is very broad, moderately sclerotised and somewhat quadrate in shape, with its distal margin bearing teeth and serrations of varying size that are most prominent on its dorsal and ventral apical corners (Fig. 7h–j). The lower arm of the harpe is directed posteriorly in *G. b. bathycloides* (Figs 5a–b, 7a–d) and is strongly incurved (Figs 6d, 7e). It may also be slightly expanded towards the apex (Fig. 7c). In *G. c. malayanum*, the lower arm is straighter and longer, and is slenderer towards the apex (Figs 5c–d, 7h–j). The most obvious difference is that it is less strongly incurved (Fig. 6h) than in *G. b. bathycloides* (Fig. 6d) and is

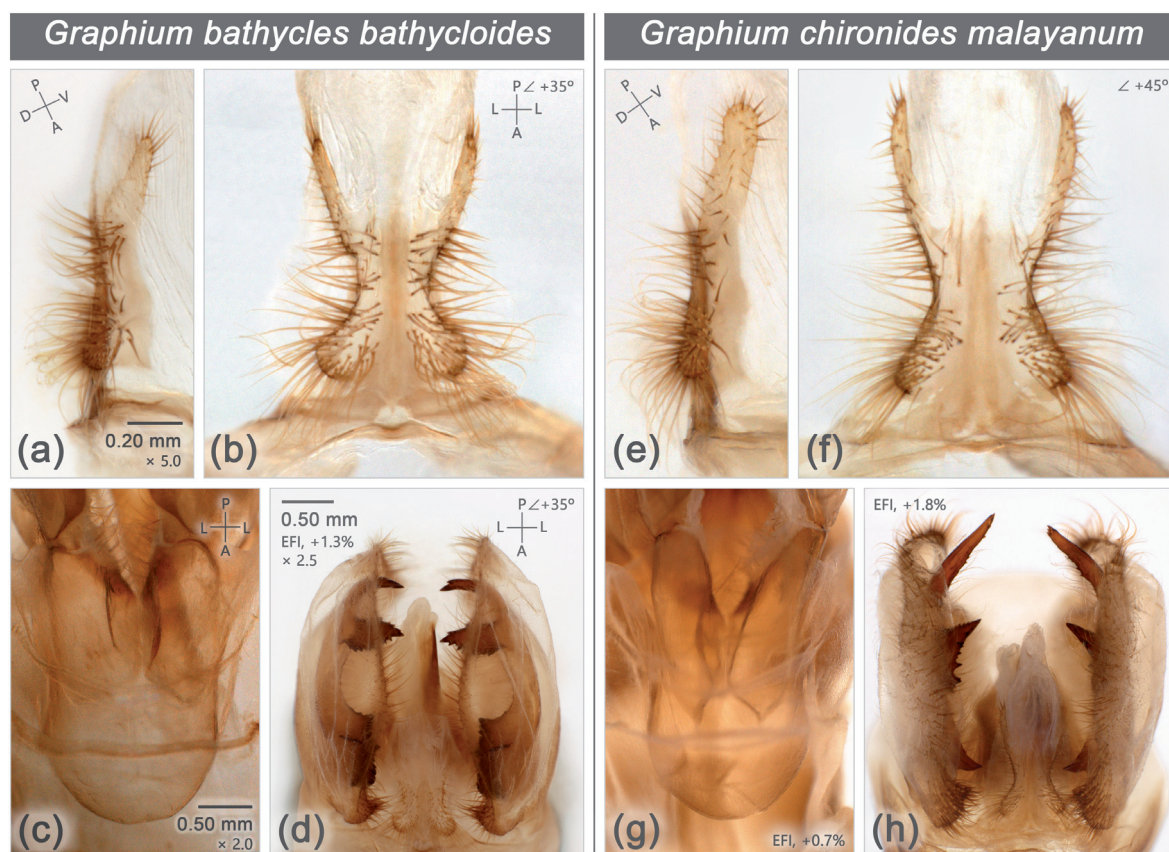


Fig. 6. Structure of the uncus, saccus and paired valvae. **a–d.** *G. b. bathycloides* (Honrath, [1884]). **e–h.** *G. c. malayanum* Eliot, 1982. **a, e.** Left lateral view of uncus. **b, f.** Posterodorsal view of uncus. **c, g.** Ventral view of saccus and sacculus (within the saccus) seen through the abdominal sternites. **d, h.** Posterodorsal view of entire genitalia showing paired valvae with the arms of the harpe curving inwards towards each other. Scales, magnifications, orientation axes and angles as explained in Fig. 5. Image (d) is not based on the same specimen of *G. b. bathycloides* as the other images.

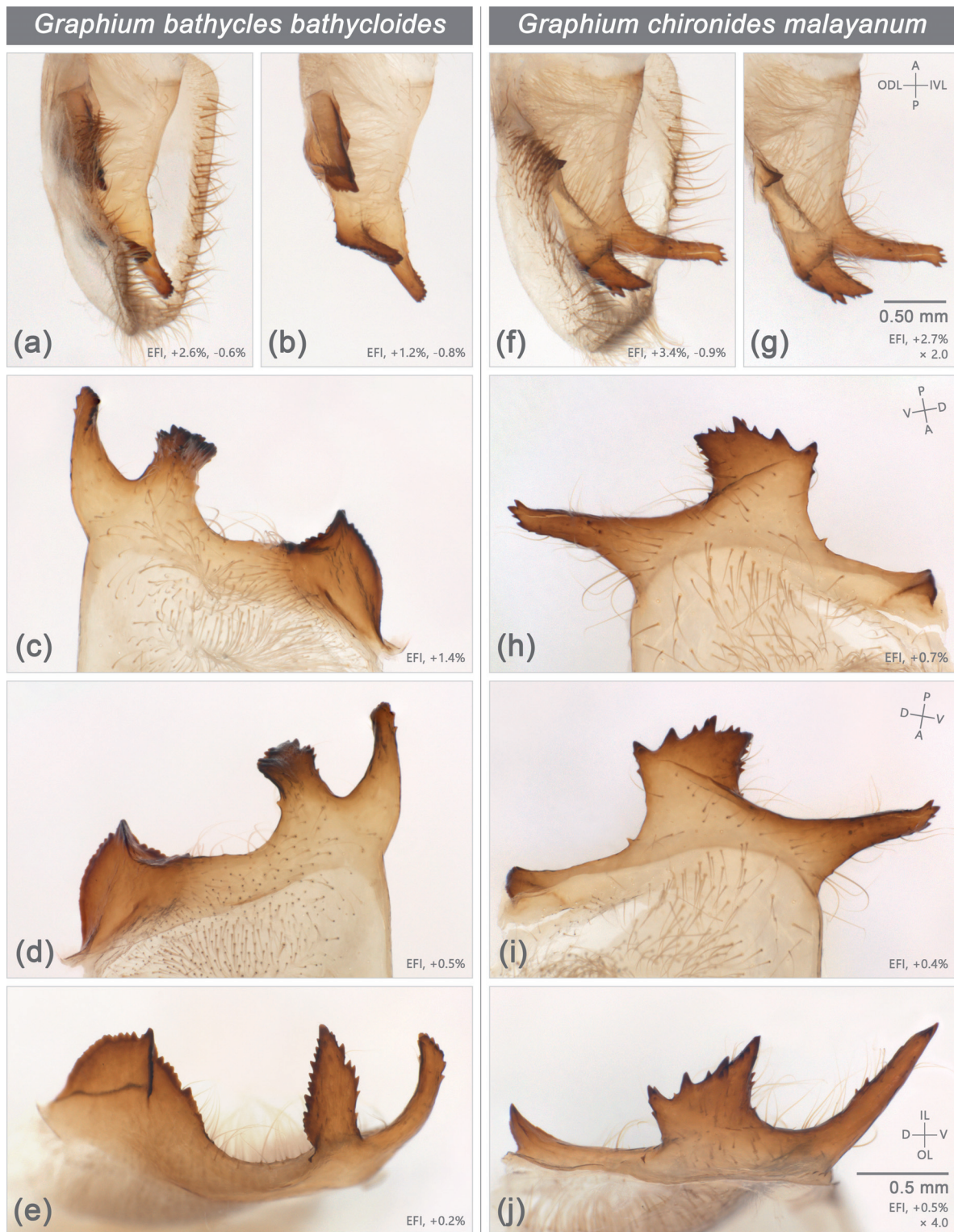


Fig. 7. Structure of the left harpe. **a–e.** *G. b. bathycloides* (Honrath, [1884]). **f–j.** *G. c. malayanum* Eliot, 1982. **a–b, f–g.** Inner dorsolateral view. **c, h.** Inner lateral view. **d, i.** Outer lateral view. **e, j.** Posterior view. The harpe is shown within the entire valva in images (a) and (f), and after dissection from the valva in all other images. Scales, magnifications and orientation axes as explained in Fig. 5. The scale shown in (g) applies to the tip of the ventral arm of the harpe (also for all images in the same row).

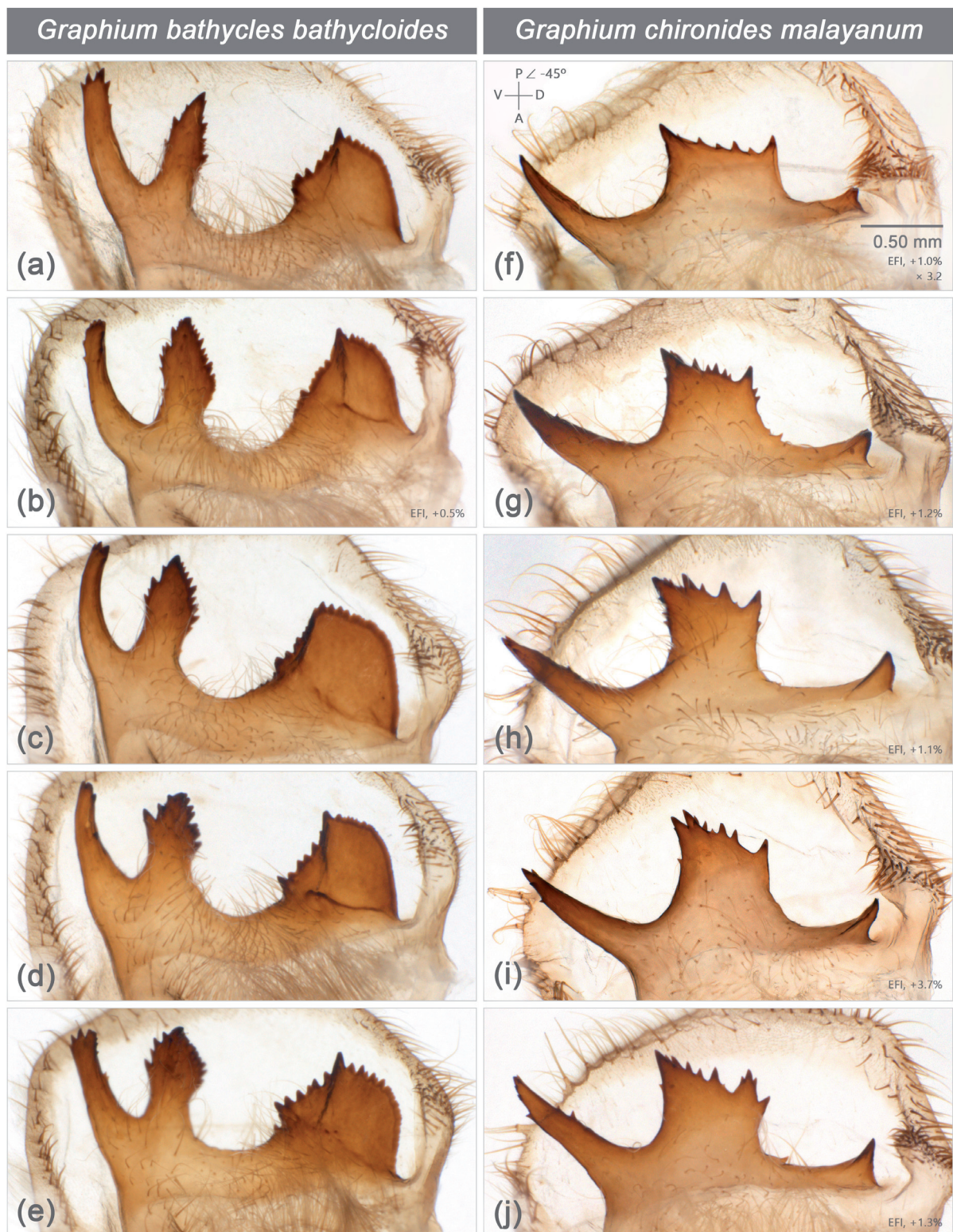


Fig. 8. Variation in the harpe of the left valva seen at an inner anterolateral angle of 45° in five specimens each. **a–e.** *G. b. bathycloides* (Honrath, [1884]). **f–j.** *G. c. malayanum* Eliot, 1982. Scales, magnification, orientation axis and angles as explained in Fig. 5.

directed posteroventrally (Fig. 7f–j) instead of posteriorly. Additional differences between the genitalia of the two taxa are given in Supp. file 1 (Part C).

Variation in the shape, size, and curvature of the arms of the harpe and their teeth occurs within the two taxa (Fig. 8). In *G. b. bathycloides*, the upper arm differs the most between specimens in width, shape, and size of serrations (Fig. 8a–e), which range from small (Fig. 8b) to large and tooth-like (Fig. 8e). In *G. c. malayanum*, the greatest variation occurs in the middle arm (Fig. 8f–j), which varies from being laterally wide and short (Fig. 8f) to being longer and narrower (Fig. 8h). The serrations and teeth on its apical margin vary from relatively small (Fig. 8f) to very large (Fig. 8h). The drawing by Eliot (1982), which is partly re-illustrated in Fig. 9, shows that the middle arm can be bifurcated at its apex. Although none of the specimens we dissected had a bifurcated middle arm, one specimen had a notch that formed a slight cleft on the apical margin, which might suggest a trace of a bifurcation (Fig. 8j).

Affinity of DNA barcodes

The gene analysis showed strong agreement between clades, ordination clusters and genitalia type. The ML tree separated the nine samples into two distinct clades (Fig. 10), with individuals from different clades separated by 18–23 base pairs and ML distances of 10.17–13.49%. The two clades corresponded with the two clusters in the ordination and the genitalia morphology of the two taxa, *G. bathycles* and *G. chironides* (Fig. 10). Specimens from China formed a subclade within the *G. chironides* clade, sister to the Peninsular Malaysian subclade (Fig. 10). Specimens from these two geographically distinct subclades were separated by 6–7 base-pair differences and ML distances of 3.21–3.78%, lending further support to the inferred identity of the *chironides* clade. Within the *G. bathycles* clade, specimens differed by at most one base pair. Similar topologies for the *bathycles-chironides* clade were obtained when our sequences were integrated into a re-analysis of the sequences used by Wilson *et al.* (2014), using both MP and ML (Supp. file 3). Support values for the *bathycles-chironides* node increased in the full ML tree.

Examination of characters used by Wilson *et al.*

An examination of the characters used by Wilson *et al.* (2014) reveals that the characters on the forewing upperside that differentiate *G. c. malayanum* from *G. b. bathycloides* were misinterpreted (Table 2, characters A and B). These characters that were explained by Eliot (1982) are the widths of the pale blue discal markings on the forewing upperside (character A) and in particular the spot in space 5 (Table 2, character B). The illustrations of Wilson *et al.* (2014), in which they circle the character they used as the widths of the markings, show that they misinterpreted it to be the small post basal bluish streak-like marking that is joined to the inner edge of the bluish discal spot in space 1a (Fig. 11). As a result, they scored most of their specimens as having broad markings as in *G. c. malayanum*, when the actual markings were narrower than in the photograph of the type of this taxon. The forewing upperside spot in space 5 (character B) is a single spot on the disc (Fig. 11). However, Wilson *et al.* (2014) again misinterpreted this character (Fig. 11), as they circled both the discal and submarginal spot in their illustrations. In one specimen (JJW0017), spots in space 6 instead of space 5 were circled. It is therefore uncertain what was actually measured or scored for this character. Although Eliot (1982) did not specifically state the width of the pale blue discal spot in space 5 in *G. b. bathycloides*, referring to it as “little more than a dot,” he specified a range for the spot width in *G. c. malayanum*. In the six specimens examined by Wilson *et al.* (2014), the widths were stated to be “around 1 mm wide in all.” Despite this being much smaller than Eliot’s stated range of 2.0–2.5 mm for *G. c. malayanum*, they scored all but one of their six specimens as having the character state of this taxon. Using the ratios of spot widths to vein lengths measured from the illustrations of Wilson *et al.* (2014) and the smallest vein lengths of both taxa in our samples, we calculate the maximum likely spot widths to be less than 1.5 mm wide in those of their specimens that we were unable to examine physically (Supp. file 2, Part B), while its width was 0.94 in the specimen we excluded due to broken wing apices.

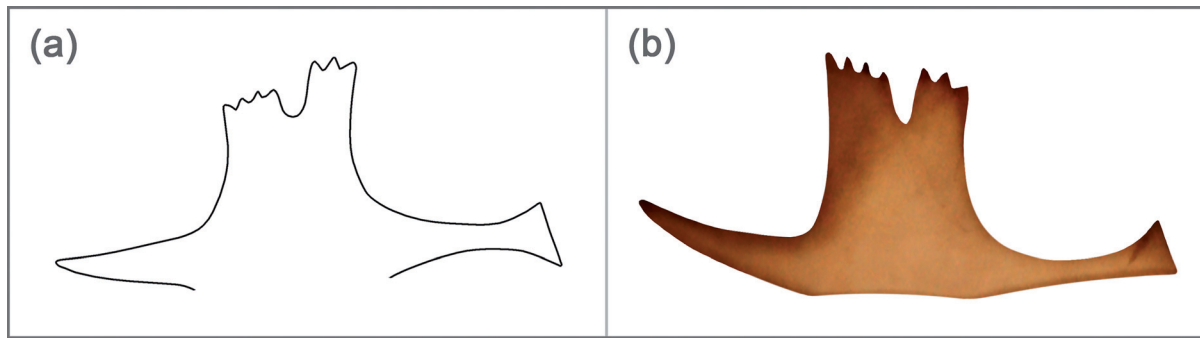


Fig. 9. Harpe of *G. c. malayanum* Eliot, 1982 based on the illustration by Eliot (1982). **a.** Outline of harpe in Eliot's original line drawing. **b.** An approximate predicted projection if the valva is viewed from the same angle as the illustrations in Fig. 8.

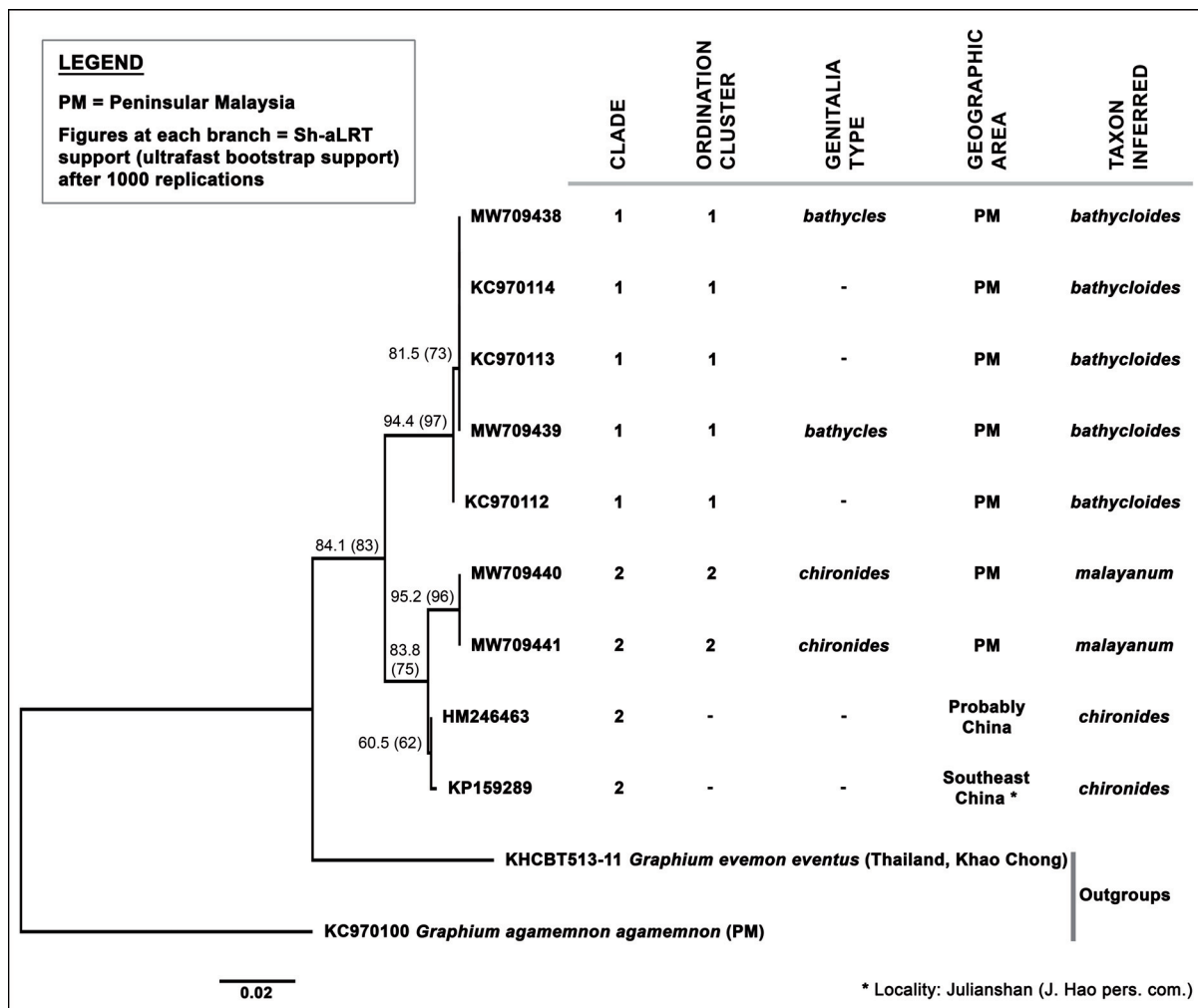


Fig. 10. Maximum likelihood tree for COI mtDNA barcodes in *G. bathycles bathycloides* (Honrath, [1884]) and two subspecies of *G. chironides* (Honrath, [1884]), i.e., subspecies *malayanum* Eliot, 1982 and the nominate subspecies *chironides* (Honrath, [1884]), with two other species of *Graphium* Scopoli, 1777 as outgroups.

Table 2. Characters for the differentiation of *G. c. malayanum* Eliot, 1982 (M) and *G. b. bathycloides* (Honrath, [1884]) (B) as given by Eliot (1982), and as interpreted by Wilson *et al.* (2014) for the six specimens of *Graphium* Scopoli, 1777 they examined, shown alongside the correct interpretation for the same specimens.

	Character defined by Eliot	Character state (Eliot)	Interpretation of character by Wilson <i>et al.</i>	Character state as interpreted by Wilson <i>et al.</i>	Correct interpretation of character state
Forewing upperside markings:					
A	Pale blue discal markings (five spots)	M: wider B: narrower	Misinterpreted as postbasal bluish streak in space 1a	Four wider; two narrower	All six narrower (than in the type of <i>malayanum</i>)
B	Discal spot in space 5	M: wider (2.0–2.5 mm) B: little more than a dot	Misinterpreted as discal and submarginal spots in space 5	Five wider; one little more than a dot (all around 1 mm wide)	All six narrow, being under 1.5 mm wide
Hindwing underside markings:					
C	Basal and subbasal spots in space 8	M: pale blue, faintly yellowish B: pale blue	Correctly interpreted	Five faintly yellowish; one pale blue	All six faintly yellowish
D	Postdiscal orange markings in spaces 1b to 4	M: orange-yellow, narrow B: richer orange, much wider	Colour and width of the spots combined as one character	Four orange-yellow, narrow; two richer orange, wider	Applying one state to two characters results in conflicts
E	Basal spot in space 3	M: prominent B: absent or vestigial	Correctly interpreted	Two prominent; four absent or vestigial	Two vestigial, four absent
F	Orange stria in space 5	M: absent B: present	Uncertain—question mark for all specimens	No character state specified	Five present; one absent
G	Pale blue streak (“window”) in space 1b	M: present, long B: virtually obliterated by black	Correctly interpreted	Three long; three obliterated	Three present, much shorter than in <i>malayanum</i> type

Besides misinterpreting the characters on the forewing upperside, Wilson *et al.* (2014) also misassigned some of the character states for the hindwing underside or were unable to understand the character (Table 2, characters C–G). They scored one of their specimens as having the basal and subbasal spots in space 8 pale blue when all are faintly yellowish (Table 2, character C). They applied the same character states to two characters, width and colour of the orange markings in spaces 1b to 4 (Table 2, character D), which resulted in the states conflicting with some of the characters. Thus, the two specimens they considered to have wide spots have spots that are no wider than in some of the others they figure but

appear to be scored as wide only because of their more orange spot colour. The two specimens that they scored as having prominent spots at the base of space 3 on the hindwing underside (Table 2, character E) have very small spots that are much smaller than in the photograph of the type of *G. c. malayanum* that they examined. They appear to have been uncertain what the additional stria in space 5 was (Table 2, character F), as they used a question mark for this character for the types and all their specimens. The stria is visible in all but one of their specimens and in the syntype of *G. b. bathycloides*. It is just barely visible in the holotype of *G. c. malayanum*. They scored the pale blue subdiscal streak (or window) in space 1b of the hindwing underside (Table 2, character G) as long in three specimens although the streaks are much shorter than in the type of *G. c. malayanum*.

Separability of taxa based on each wing character

Boxplots for the individual wing characters measured for *G. b. bathycloides* and *G. c. malayanum* are shown in Fig. 12 based on measurement data given in Supp. file 2 (Part A). Table 3 summarises the degree of overlap between measurement ranges of the two taxa and shows whether the medians differed significantly between them. The only characters that did not differ significantly between taxa in the NPMV tests in R were the area measurements of the hindwing upperside blue spot in space 2 (character #7A in Tables 1 and 3) and the hindwing underside orange spots in space 5 (#10A) and space 2 (#11A). Although the significant differences in other characters tested indicate differences in the population medians, their usefulness as diagnostic characters for the two taxa depends on the degree of overlap in their respective ranges.

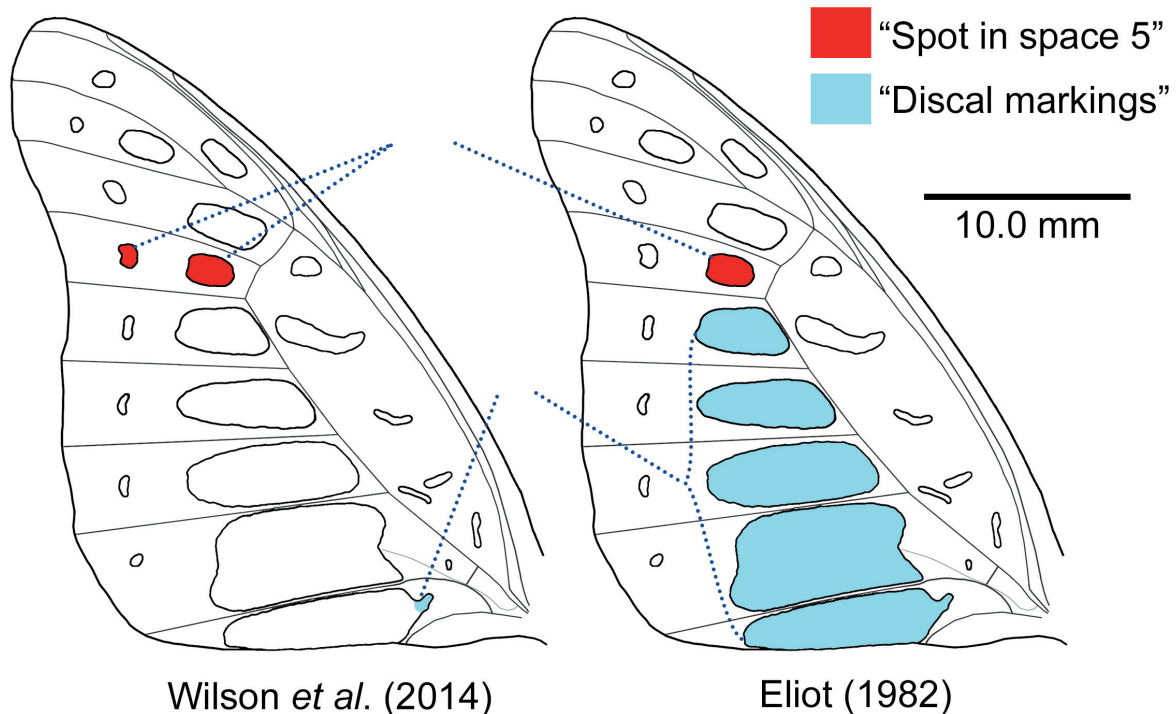


Fig. 11. Diagrammatic representation of the forewing upperside and its venation and markings in *G. c. malayanum* Eliot, 1982, showing characters mistakenly used by Wilson et al. (2014) (left) compared with the actual characters used by Eliot (1982) (right).

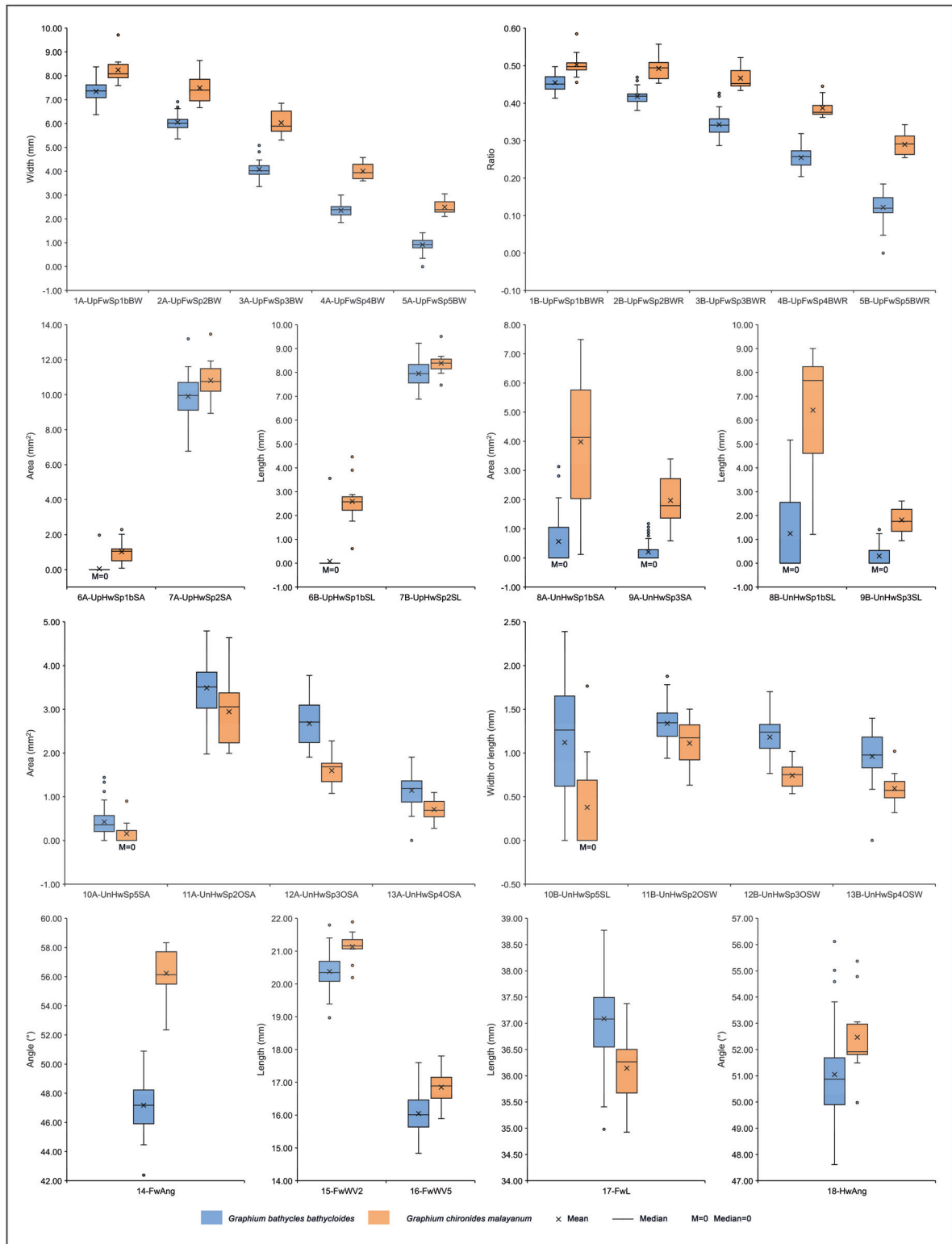


Fig. 12. Box and whisker plots for all wing characters measured for *G. b. bathycloides* (Honrath, [1884]) and *G. c. malayanum* Eliot, 1982. Bars are interquartile ranges (IQR) and whiskers represent $1.5 \times$ IQR, beyond which are outliers (point markers).

Table 3. Levels of usefulness of different characters in differentiating *G. c. malayanum* Eliot, 1982 from *G. b. bathycloides* (Honrath, [1884]) in Peninsular Malaysia, based on non-parametric multivariate (NPMV) test results and degree of overlap between measurement ranges.

Level of overlap in character	Level of usefulness	Character No.	Character code	NPMV test result
No overlap between ranges	Diagnostic	3A	UpFwSp3BW	+
		3B	UpFwSp3BWR	
		4A	UpFwSp4BW	+
		4B	UpFwSp4BWR	
		5A	UpFwSp5BW	+
		5B	UpFwSp5BWR	
		14	FwAng	+
One or more outliers in a taxon are within the whiskers or IQR of the other taxon or exceed its median	Usually reliable	2A	UpFwSp2BW	+
		2B	UpFwSp2BWR	
		6A	UpHwSp1bSA	+
		6B	UpHwSp1bSL	
A whisker of one taxon reaches the whisker or IQR of the other taxon	Sometimes usable	9A	UnHwSp3SA	+
		9B	UnHwSp3SL	
		8A	UnHwSp1bSA	+
		8B	UnHwSp1bSL	
		12A	UnHwSp3OSA	+
		12B	UnHwSp3OSW	
The median of one taxon is within the IQR or whiskers of the other taxon, or their IQRs overlap	Not useful	13A	UnHwSp4OSA	+
		13B	UnHwSp4OSW	
		1A	UpFwSp1bBW	+
		1B	UpFwSp1bBWR	
		15	FwWV2	+
		16	FwWV5	+
		17	FwL	+
		18	HwAng	+
		7A	UpHwSp2SA	–
		7B	UpHwSp2SL	
		10A	UnHwSp5SA	–
		10B	UnHwSp5SL	
11A	UnHwSp2OSA	–		
11B	UnHwSp2OSW			

NPMV test results: significant difference (+), no significant difference (–), not tested (blank).

The most diagnostic wing markings in which the range did not overlap between taxa were the widths of the blue spots in spaces 3 to 5 (#3A, 4A, and 5A) and their respective ratios with respect to vein length (#3B, 4B, and 5B). The only structural character that was diagnostic was the forewing angle (#14).

Two other characters, both on the upperside, were usually reliable, with only occasional outliers in one taxon infringing into the range of the other. They were the width and ratio of the forewing spot in space 2 (#2A and 2B) and the area and length of the hindwing spot in space 1b (#6A and 6B).

A few underside characters on the hindwing were sometimes usable in that they did not overlap between taxa very often. In these characters, the whisker of one taxon overlapped with the whisker or IQR of the other taxon but did not reach its median. They were the area measurements and lengths of the bluish spots in spaces 1b (#8A and 8B) and 3 (#9A and 9B) and the area and width of the orange spot in space 3 (#12A and 12B).

The remaining characters were not useful in separating the taxa because of frequent overlap in measurements between taxa, with the median of one taxon being encompassed by the whiskers or even IQR of the other taxon, or with their IQRs overlapping. They included the three characters that did not differ significantly between taxa.

Revised differential diagnoses for *G. c. malayanum* and *G. b. bathycloides*

On the basis of the analysis of wing characters and differences in genitalia, we provide the following improved diagnoses of the two taxa.

***Graphium chironides malayanum* (male)**

Wings (Fig. 13a): upperside forewing with the pale blue discal markings comparatively broad, especially in spaces 3 to 5, the spot in space 5 at least 2.0 mm wide at its widest extent. Spot width in space 4 at least 3.5 mm, and spot in space 3 exceeding 5.0 mm. Forewing apex less falcate and less pointed than in *G. b. bathycloides*. Hindwing upperside and underside usually with a bluish streak at base of space 1b. Hindwing underside pale blue spot at the base of space 3 always present, 1.0–2.5 mm long (but see measurements for *G. b. bathycloides*). Genitalia (Figs 5c–d, 6e–h, 7f–j, 8f–j): harpe with middle arm located about midway between the upper and lower arms. Upper arm of harpe very small, being reduced to almost a tooth. Middle arm of harpe very broad and somewhat quadrate with teeth and serrations on its distal margin. Lower arm of harpe relatively long, moderately incurved and directed posteroventrally, tapering to a point towards the apex.

***Graphium bathycles bathycloides* (male)**

Wings (Fig. 13b): upperside forewing with the pale blue discal markings comparatively narrow, the spot in space 5 not more than 1.5 mm wide at its widest extent and sometimes absent. Spot in space 4 not more than 3.0 mm wide, and the spot in space 3 rarely exceeding 5.0 mm in width. Forewing apex more falcate and more pointed than in *G. c. malayanum*. Hindwing upperside and underside usually without a bluish streak at base of space 1b, especially on the upperside. Hindwing underside pale blue spot at the base of space 3 sometimes absent, and when present usually relatively small, up to 1.5 mm long (but see measurements for *G. c. malayanum*). Genitalia (Figs 5a–b, 6a–d, 7a–e, 8a–e): harpe with middle arm located close to the lower arm and joined to its base. Upper arm of harpe large and hump-like in anterolateral profile, usually with a pointed apex and slightly serrated margins. Middle arm of harpe relatively narrow, expanding at its toothed distal end and tapering to a point. Lower arm of harpe relatively short, strongly incurved, and directed posteriorly, with a blunt and slightly serrated apex.

The differences described above apply well to *G. c. malayanum* and *G. b. bathycloides* from Peninsular Malaysia but not always to subspecies that occur outside this region. Illustrated specimens of the

nominate subspecies of *G. bathycles* from Java (Tsukada & Nishiyama 1982; Page & Treadaway 2014) resemble *G. c. malayanum* in its broad discal band. Throughout its range, however, *G. chironides* usually has a touch of blue in space 1b on the hindwing upperside, which is absent in *G. bathycles*. Specimens of continental subspecies of *G. chironides* illustrated by various authors (Saigusa *et al.* 1977; Chou 1994; Gu & Chen 1997; Osada *et al.* 1999; Monastyrskii 2007; Racheli & Cotton 2009; Kimura *et al.* 2011; Page & Treadaway 2014; Inayoshi 2023a) vary in forewing discal band width and many have a slightly narrower band than in subspecies *malayanum*, resembling that in *G. b. bathycloides*. However, the spot in space 5 is usually wider than in *G. b. bathycloides*, the veins across all the pale bands are often blackened, and there is sometimes a pale postdiscal spot in space 3 on the hindwing upperside.

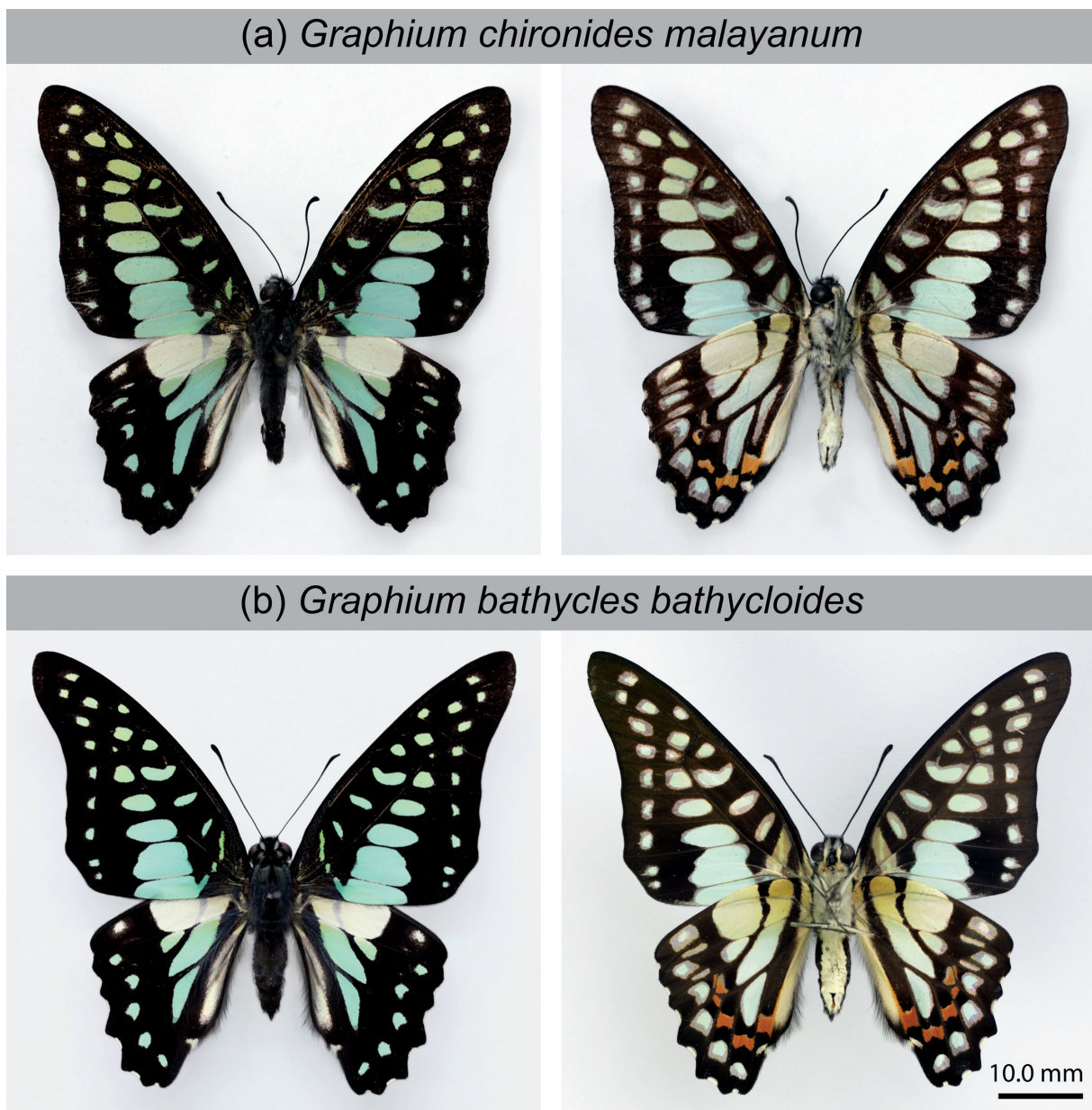


Fig. 13. Upperside (left) and underside (right) of the two taxa. **a.** *Graphium chironides malayanum* Eliot, 1982. **b.** *G. bathycles bathycloides* (Honrath, [1884]).

Where there is a need, dissection can be used to confirm identity. However, difficulties in identification based on wing markings should rarely arise if locality information is correct since, as far as is known, *G. bathycles* does not occur on the continent, its northernmost limits being the southern half of the Isthmus of Kra, and *G. chironides* does not occur in Java.

Revised differential diagnoses for *G. c. malayanum* as a subspecies

Graphium c. malayanum is the only non-continental subspecies of *G. chironides*. It has been recorded from southernmost Thailand (Inayoshi 2023b) to Peninsular Malaysia. Other subspecies (Page & Treadaway 2014) are *G. chironides chironides*, which occurs from northeast India to central and south China, *G. chironides tereus* (Fruhstorfer, [1908]), which occurs in Hainan, and *G. chironides clanis* Jordan, 1909, which occurs in Southeast China. A fifth nominal subspecies, *G. chironides punctatus* Page & Treadaway, 2014, said by its describers to occur in Thailand, Laos and Vietnam, is of uncertain validity as a subspecies because the characters used to diagnose it, such as an additional blue hindwing spot in space 3, can sometimes be found on specimens of subspecies *chironides* (e.g., Kehimkar 2008: 147, fig. 17UP) and may be absent in specimens from within the stated geographical range of *punctatus* (e.g., Ek-Amnuay 2012: pl. 33, P65, first row, right). Furthermore, the stated range of *punctatus* divides the range of the nominate subspecies, making the distribution of the latter somewhat disjunct.

In the continental subspecies, the forewing pale bluish discal band is very often crossed by black scaling along the upperside of veins 1b and 2, which may be so extensive as to form wide black borders between well-separated pale spots. The hindwing band is also usually crossed by black scaling that may be very prominent too. The forewing band is variable in width but is often narrower than in subspecies *malayanum*. Continental subspecies sometimes have a bluish white spot in space 3 at the cell-end on the hindwing upperside.

The following is a more accurate differential diagnosis for subspecies *malayanum* with respect to the continental subspecies of *G. chironides*, based on Eliot's (1982) original diagnosis and specimens we were able to examine: forewing upperside lacks black scaling along vein 1b on the pale bluish discal band, and usually also lacks black scaling across the band along vein 2, or the latter may be very narrowly black-scaled. Hindwing upperside lacks black scaling across the pale bluish band along vein 8, the basal part of vein 7 and the radius. Upperside pale discal forewing band wide. No bluish-white spot present in space 3 at the cell-end on the hindwing upperside.

Synonymies and corrections to literature

Only historical name changes and current corrections are shown in the brief synonymic lists below. For explanatory notes on the nomenclatural history of *G. chironides* and the stability of its name, see Supp. file 4 (Part A). Our rationale for rejecting the synonymy of *malayanum* with *chironides* suggested by Ek-Amnuay (2012) is explained further in Supp. file 4 (Part B). The errors in Saigusa *et al.* (1977) corrected below are a mix-up in the plates that do not appear to have affected the analyses and conclusions of the authors.

Graphium chironides (Honrath, [1884])

Papilio chiron Wallace, 1865: 66; type locality Assam, Sylhet; preoccupied by *Papilio chiron* Fabricius, 1775.

Papilio chiron var. *chironides* Honrath, 1884: 397, pl. 10 fig. 4; type locality Darjeeling, Sikkim.

Graphium clanis Jordan, 1909 – Eliot 1982: 180; replacement name.

Graphium chironides, syn. *chironicum* Eliot, 1982 – Eliot 1983: 283–284 (*chironicum* was the name proposed for the Indian subspecies in combination with *Graphium clanis*).

Graphium chironides malayanum Eliot, 1982

Graphium clanis malayanum Eliot, 1982: 180–181; genitalia illustrated, p. 181; type locality Selangor (Peninsular Malaysia).

Graphium bathycles bathycloides – Saigusa *et al.* 1977: fig. 37 mislabelled, recte *G. chironides malayanum* (upperside), likely the same specimen shown on underside in fig. 40 as “*chiron*” (i.e., *chironides*); see also correction to Saigusa *et al.* (1977) below.

Graphium chironides malayanum – Eliot 1983: 283–284. — Corbet & Pendlebury 1992: 75–76, 594, pl. 6 no. 2; 2020: 69, 492, pl. 10 no. 5, text and related couplets in identification key quote Corbet & Pendlebury (1992) verbatim.

Graphium chironides chironides – Ek-Amnuay 2012: 120; unjustified synonymy.

Graphium bathycles bathycloides (Honrath, [1884])

Graphium chiron – Saigusa *et al.* 1977: figs 39 and 41 mislabelled, recte *G. bathycles bathycloides*; fig. 39 (showing upperside) likely same specimen shown on underside in fig. 38 as “*bathycles*”; see also correction to Saigusa *et al.* (1977) above.

Habitat differences of taxa in Peninsular Malaysia

Graphium c. malayanum inhabits the foothills and mountains of Peninsular Malaysia and is more frequently encountered in the highlands than *G. b. bathycloides*. The latter occurs mainly in the lowlands and foothills and is not rare, but it is also occasionally encountered in the highlands. Relatively few specimens of *G. c. malayanum* are found in collections or in photographs taken in the wild in comparison to *G. b. bathycloides*, which suggests it is relatively scarce in the Peninsula. The recognition of the existence of this species in the Peninsula and its occurrence as a distinct subspecies has conservation importance.

Discussion

Based on our analysis of wing and genitalia morphology as well as genetic sequences, we affirm that *G. chironides* occurs in the Peninsula, and that the Peninsular Malaysian subspecies *G. chironides malayanum* is a good subspecies. Ordination of the width of the forewing band against forewing length and forewing angle showed the existence of two very distinct phenotypic clusters in specimens that we measured against a scale. Ordination of the width ratio of the forewing bluish spot in space 5 against forewing angle enabled inclusion of specimens from published figures, and similarly produced two clusters, with the holotype of *G. c. malayanum* and syntype of *G. bathycles bathycloides* clustering with the wide- and narrow-spotted phenotypes, respectively. Genitalia morphologies of representative specimens of each cluster were also distinctly different and clearly diagnostic. The cluster with broader forewing bands and a less falcate forewing was identifiable from published genitalia illustrations of specimens from other regions as *G. chironides*, and the other cluster was identifiable as *G. bathycles*. Although some variation in genitalia morphology occurred within the two taxa, they were readily distinguishable by major differences, especially in the location of the middle arm of the harpe. Genetic sequencing also confirmed the existence of two species in Peninsular Malaysia by virtue of two well-separated clades. The Peninsular Malaysian specimens that were identified as *G. chironides* formed a monophyletic clade with specimens of *G. chironides* from China, with which they formed a closely related sister group.

We determined from our analysis that all the specimens examined by Wilson *et al.* (2014) in the MZUM were *G. b. bathycloides*. Wilson *et al.* (2014) did also assign all their specimens as *G. b. bathycloides* in their paper despite some of the specimens they examined in the MZUM being misidentified as *G. c. malayanum* on their original specimen labels. They also rightly stated that the genitalia were similar to those of *G. b. bathycloides* and that there was a lack of specimens and sequence data. This should

have led to the conclusion that *G. c. malayanum* was absent in their sample. However, based on a morphological analysis, the taxa were deemed to have ambiguous and overlapping wing characters. The very similar DNA barcodes that were obtained due to the absence of *G. c. malayanum* in their sample contributed to the conclusion that the status of *G. chironides malayanum* is in question, implying that *G. b. bathycloides* and *G. c. malayanum* could be a single taxon as stated in the original dissertation (Karen-Chia 2014). However, this view is untenable and is a result of confusion over the morphology of the two taxa. As shown in the foregoing analysis of specimens occurring in the Peninsula, the two taxa are both morphologically and genetically distinct.

The wider width of the pale blue forewing discal band was the first and most diagnostic character of *G. c. malayanum* pointed out by Eliot (1982). In particular, the band's terminal spot in space 5 was emphasised, and its size range was stated. The pale blue discal spots that make up the band are the largest markings on the forewing except in space 5, where it is small (Fig. 11). However, Wilson *et al.* (2014) mistook a very small streak joined to the inner margin of the discal spot in space 1a as the band referred to by Eliot (1982), while in space 5 they appear to have used two spots instead of just the discal spot. In our data, the width ranges of the spots in spaces 4 and 5 that form part of the discal band were mutually exclusive for the two taxa. In fact, the differences in the width of these distal spots on the forewing of the two taxa are sufficiently large and reliable to enable the taxa to be differentiated by simple comparison without the need for measurement. Thus, we do not consider the two taxa "particularly hard to distinguish" or all the characters used to distinguish them "subtle" or "obscure". The magnitude of the differences in these diagnostic characters also makes it extremely unlikely that overlap in the ranges would occur had our sample size of *G. c. malayanum* been larger.

Other characters that we analysed were less diagnostic. Some were generally reliable, some were helpful to a limited extent, and others were not useful. It is common for a new taxon to be described from a limited number of samples. As more samples become available and the extent of variation within the taxon becomes better understood, character differences described by the original author are re-evaluated and refined by subsequent authors. This involves narrowing down the most diagnostic characters and sometimes identifying additional characters that might have been overlooked. However, the conclusion reached by Wilson *et al.* (2014) that *G. c. malayanum* has ambiguous and overlapping wing characters was not so much due to a lack of usefulness of some characters as it was to their misjudgement of most of the characters. In some cases, they misinterpreted the characters. In other cases, character states were misassigned. Occasionally, characters were not understood, or two characters that should have been evaluated separately were combined. However, we agree with Wilson *et al.* (2014) that colour characters can be subjective to apply, and that colours may fade in older specimens. Our observations were that these colour characters were not reliable, and therefore we did not attempt to use them. There were many specimens of *G. b. bathycloides* that had pale orange postdiscal markings in spaces 1b to 4 on the hindwing underside, and even more that have yellow-tinged basal and subbasal pale blue spots in space 8 on the hindwing underside.

In addition to the diagnostic characters mentioned by Eliot (1982), the less falcate forewing in *G. chironides* that was implied by Wallace (1865) is a good character, as the ranges for the angle that we measured to quantify this character in the two species were mutually exclusive. The less falcate forewing is also seen in a shorter forewing length and longer length of vein 2 and vein 5 on average in comparison to *G. b. bathycloides*. In other words, the forewing is shorter and wider in its proportions in *G. c. malayanum*, in addition to being less curved outwards at the apex.

Reliance on incorrectly identified specimens for gene analysis was the first of a sequence of problems in the work of Wilson *et al.* (2014). The misidentifications should have become apparent from the absence of clear genetic and genitalic differences. However, their inability to understand some of the

differentiating characters on the wings, including the most diagnostic characters of *malayanum*, and their misjudgements in the assigning of character states to their specimens in a few other characters, led them to the wrong conclusion that *malayanum* has ambiguous and overlapping wing characters and is therefore of doubtful status. Their problems with this taxon were further compounded by inherent variation in some of the characters originally proposed for its diagnosis. The confusion caused by their conclusions shows the importance of a good understanding of morphology as a basis for accurate identification, and the importance of both morphology and identification in the interpretation of gene data. The utility of DNA barcoding for the identification of taxa is lost if the reference specimens on which it is based are not correctly identified in the first place on the basis of their morphology. Mistakes in the recognition of morphological characters inevitably lead to mistakes in taxon identification and can subsequently lead to wrong inferences in the molecular phylogenies of taxa, or even cast doubt on the validity of genuine taxa like *G. c. malayanum*.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author responsibilities

The joint first authors were responsible for the morphological and taxonomic work in this article. DNA extraction and sequencing was the work of the third author, and its analysis was the joint work of the first-named and third authors.

Acknowledgements

The authors express their gratitude to the following museums and their curators and staff for access to physical specimens or digital images: H. Omar, A.A. Azidah, N. Mamat and S. Amni Bazilah of the University of Malaya Museum of Zoology, Kuala Lumpur; S. Farizawati and J. Mohd. Khairill of the Natural History Museum of the Malaysian National Museum, Kuala Lumpur; W.S. Hwang of the Lee Kong Chian Natural History Museum, Singapore. We are grateful to C.Y. Chong and J. Arshad, and to N.L. Liew, for the use of specimens from their personal collections. We are also thankful to C.Y. Chong for providing valuable advice, especially on the choice of characters, to A.M. Cotton for useful discussions and comments on the manuscript, and to K. Willmott and F.L. Condamine for reviewing an earlier version of the manuscript and suggesting improvements. We also thank C.Y. Chong, A.M. Cotton and S. Takagi for providing useful reference material, J. Hao of Anhui Normal University, Wuhu for locality information of his *G. chironides* sequence, and M. Soh for locality information of his field photograph of *G. c. malayanum*. This study was funded by the Economic Planning Unit of the Prime Minister's Department, Malaysia under the Twelfth Malaysia Plan project entitled "Documentation and Conservation of Biodiversity for the Well-Being of Forests and Sustainability of Natural Resources (Phase 2)" (project no. P23085100210003).

References

- Chou I. (ed.). 1994. *Monographia Rhopalocerorum Sinensium (Monograph of Chinese Butterflies)*. Vol. 1. Henan Scientific and Technological Publishing House, Zhengzhou. [In Chinese.]
- Corbet A.S. & Pendlebury H.M. 1992. *The Butterflies of the Malay Peninsula*. 4th Ed. Revised by J.N. Eliot. Malayan Nature Society, Kuala Lumpur.
- Corbet A.S. & Pendlebury H.M. 2020. *The Butterflies of the Malay Peninsula*. 5th Ed. Revised by G.M. Van der Poorten & N.E. Van der Poorten. Southdene, Kuala Lumpur.
- Ek-Amnuay P. 2012. *Butterflies of Thailand*. 2nd Ed. Amarin Printing and Publishing, Bangkok.

- Eliot J.N. 1982. On three swallowtail butterflies from Peninsular Malaysia. *Malayan Nature Journal* 35 (1–2): 179–182.
- Eliot J.N. 1983. *Graphium clanis* (Jordan): a correction. *Malayan Nature Journal* 36 (4): 283–284.
- Fabricius J.C. 1775. *Systema Entomologiae, sistens Insectorum Classes, Ordines, Genera, Species, adiectis Synonymis, Locis, Descriptionibus, Observationibus*. Korte, Flensburg and Leipzig [Flensburgi et Lipsiae]. <https://doi.org/10.5962/bhl.title.36510>
- Fruhstorfer H. 1908. Neue Papilio-rassen aus der *Eurypylus*-gruppe. *Entomologische Zeitschrift Stuttgart* 21 (37): 222. Available from <https://www.biodiversitylibrary.org/page/31581324> [accessed 3 Jan. 2023].
- Gu M.B. & Chen P.Z. 1997. *Butterflies in Hainan Island*. China Forestry Publishing House, Beijing. [In Chinese.]
- Hoang D.T., Chernomor O., von Haeseler A., Minh B.Q. & Vinh L.S. 2018. UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* 35 (2): 518–522. <https://doi.org/10.1093/molbev/msx281>
- Honrath E.G. 1884. Beiträge zur Kenntniss der Rhopalocera (2). *Berliner entomologische Zeitschrift* 28 (2): 395–398. <https://doi.org/10.1002/mmnd.18840280218>
- Inayoshi Y. 2023a. *Graphium chironides chironides*. A check list of butterflies in Indo-China, chiefly from Thailand, Laos and Vietnam. Available from <http://yutaka.it-n.jp/pap/13000001.html> [accessed 3 Jan. 2023].
- Inayoshi Y. 2023b. *Graphium chironides malayanum*. A check list of butterflies in Indo-China, chiefly from Thailand, Laos and Vietnam. Available from <http://yutaka.it-n.jp/pap/13000010.html> [accessed 3 Jan. 2023].
- Jordan K. 1909. Die Indo-Australischen Tagfalter. Papilionidae. In: Seitz A. (ed.) (1908–1911) *Die Gross-Schmetterlinge der Erde, eine systematische Bearbeitung der bis jetzt bekannten Gross-Schmetterlinge Band 9*: 11–109. Alfred Kernen, Stuttgart. <https://doi.org/10.5962/bhl.title.62014>
- Kalyaanamoorthy S., Minh B.Q., Wong T.K.F., von Haeseler A. & Jermin L.S. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 14: 587–589. <https://doi.org/10.1038/nmeth.4285>
- Karen-Chia H.-M. 2014. *Exploring the Diversity of Butterflies (Lepidoptera) at Different Elevations in Genting Highlands and the Validity of Graphium species in Peninsular Malaysia*. MSc thesis, University of Malaya, Kuala Lumpur, Malaysia. Available from <https://core.ac.uk/download/pdf/268876258.pdf> [accessed 3 Jan. 2023].
- Kehimkar I. 2008. *The Book of Indian Butterflies*. Bombay Natural History Society and Oxford University Press, Mumbai and Oxford.
- Kimura Y., Aoki T., Yamaguchi S., Uémura Y. & Saito T. 2011. *The Butterflies of Thailand – Based on Yunosuke Kimura Collection. Vol. 1*. Mokuyosha, Tokyo.
- 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 (5): 1530–1534. <https://doi.org/10.1093/molbev/msaa015>
- Monastyrskii A.L. 2007. *Butterflies of Vietnam, Papilionidae. Vol. 2*. Dolphin Media, Hanoi.
- Natural History Museum. 2023a. 149669. Dataset: Collection specimens. Natural History Museum Data Portal (data.nhm.ac.uk). Available from <https://data.nhm.ac.uk/dataset/collection-specimens/resource/05ff2255-c38a-40c9-b657-4ccb55ab2feb/record/840014> [accessed 22 Jul. 2023].

- Natural History Museum. 2023b. BMNH(E)149670. Dataset: Collection specimens. Natural History Museum Data Portal (data.nhm.ac.uk). Available from <https://data.nhm.ac.uk/dataset/collection-specimens/resource/05ff2255-c38a-40c9-b657-4ccb55ab2feb/record/840015> [accessed 22 Jul. 2023].
- Natural History Museum. 2023c. BMNH(E)149397. Dataset: Collection specimens. Natural History Museum Data Portal (data.nhm.ac.uk). Available from <https://data.nhm.ac.uk/dataset/collection-specimens/resource/05ff2255-c38a-40c9-b657-4ccb55ab2feb/record/839696> [accessed 22 Jul. 2023].
- Osada S., Uémura Y. & Uehara J. 1999. *An Illustrated Checklist of the Butterflies of Laos P.D.R.* Nishiyama Y. (ed.). Mokuyo-sha, Tokyo.
- Page M.G.P. & Treadaway C.G. 2014. Revisional notes on the *Arisbe eurypylus* species group (Lepidoptera: Papilionoidea: Papilionidae). *Stuttgarter Beiträge zur Naturkunde A, neue Serie* 7: 253–284.
- Qiagen. 2016. *QIAamp® DNA Mini Blood Mini Handbook, May 2016*. Qiagen, Germantown. Available from <https://www.qiagen.com/us/resources/resourcedetail?id=62a200d6-faf4-469b-b50f-2b59cf738962&lang=en> [accessed 22 Jul. 2023].
- Racheli T. & Cotton A.M. 2009. Papilionidae part 1, subfamily Papilioninae, tribes Leptocircini, Teinopalpini. In: Bozano G.C. (ed.) *Guide to the Butterflies of the Palearctic Region*. Omnes Artes, Milan.
- Rothschild W. 1895. A revision of the *Papilios* of the eastern hemisphere, exclusive of Africa. *Novitates Zoologicae* 2 (3): 167–463. Available from <https://www.biodiversitylibrary.org/page/3859442> [accessed 22 Jul. 2023].
- Saigusa T., Nakanishi A., Shima H. & Yata O. 1977. Phylogeny and biogeography of the subgenus *Graphium* Scopoli (Lepidoptera: Papilionidae, *Graphium*). *Acta Rhopalocerologica* 1: 2–32. [In Japanese.]
- Soh M. 2023. Only Butterflies! Veined Jay, (*Graphium chironides malayanum*), May 2018, Pahang, Malaysia. Available from <https://web.facebook.com/groups/butterflysm/permalink/2713360545352118/> [accessed 1 Dec. 2023].
- Tsukada E. & Nishiyama Y. 1982. *Butterflies of the South East Asian Islands. Vol. 1*. Plapac, Tokyo.
- Wallace A.R. 1865. On the phenomena of variation and geographical distribution as illustrated by the Papilionidae of the Malayan Region. *Transactions of the Linnean Society of London* 25 (1): 1–71. <https://doi.org/10.1111/j.1096-3642.1865.tb00178.x>
- Wilson J.J. 2012. DNA barcodes for insects. In: Kress W.J. & Erickson D.L. (eds) *DNA Barcodes: Methods and Protocols. Methods in Molecular Biology* 858: 17–46. Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-61779-591-6_3
- Wilson J.-J., Karen-Chia H.-M., Sing K.-W. & Sofian-Azirun M. 2014. Towards resolving the identities of the *Graphium* butterflies (Lepidoptera: Papilionidae) of Peninsular Malaysia. *Journal of Asia-Pacific Entomology* 17 (3): 333–338. <https://doi.org/10.1016/j.aspen.2014.02.007>
- Zinken J.L.T.F. 1831. Beitrag zur Insecten-Fauna von Java (1). *Nova Acta Physico-Medica, Academiae Caesareae Leopoldino-Carolinae, Naturae Curiosium* 15 (1): 129–194. Available from <https://archive.org/details/novaactaphysicom15183kais/page/n223/mode/2up> [accessed 3 Jan. 2023].

Manuscript received: 7 January 2022

Manuscript accepted: 4 August 2023

Published on: 9 January 2024

Topic editor: Tony Robillard

Section editor: Jurate de Prins

Desk editor: Pepe Fernández

Printed versions of all papers are also deposited in the libraries of the institutes that are members of the *EJT* consortium: Muséum national d'histoire naturelle, Paris, France; Meise Botanic Garden, Belgium; Royal Museum for Central Africa, Tervuren, Belgium; Royal Belgian Institute of Natural Sciences, Brussels, Belgium; Natural History Museum of Denmark, Copenhagen, Denmark; Naturalis Biodiversity Center, Leiden, the Netherlands; Museo Nacional de Ciencias Naturales-CSIC, Madrid, Spain; Leibniz Institute for the Analysis of Biodiversity Change, Bonn – Hamburg, Germany; National Museum of the Czech Republic, Prague, Czech Republic.

Supp. file 1. **A.** Methods used in the dissection and illustration of genitalia. **B.** General structure of the genitalia of *Graphium bathycles bathycloides* (Honrath, [1884]) and *Graphium chironides malayanum* Eliot, 1982. **C.** Additional differences between the genitalia of *G. b. bathycloides* and *G. c. malayanum*. <https://doi.org/10.5852/ejt.2024.917.2391.10541>

Supp. file 2. **A.** Specimen data and measurements. **B.** Predicted size limits of forewing spot in space 5 for specimens figured by Wilson *et al.* (2014) that we were unable to trace and measure physically. <https://doi.org/10.5852/ejt.2024.917.2391.10543>

Supp. file 3. Reanalysis of phylogenies in the genus *Graphium* Scopoli, 1777 based primarily on the data of Wilson *et al.* (2014). <https://doi.org/10.5852/ejt.2024.917.2391.10545>

Supp. file 4. **A.** Further notes on the nomenclatural history of *Graphium chironides* (Honrath, [1884]). **B.** Unjustified synonymy of *Graphium chironides malayanum* Eliot, 1982. <https://doi.org/10.5852/ejt.2024.917.2391.10547>

ZOBODAT - www.zobodat.at

Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

Zeitschrift/Journal: [European Journal of Taxonomy](#)

Jahr/Year: 2024

Band/Volume: [0917](#)

Autor(en)/Author(s): Phon Chooi-Khim, Kirton Laurence G., Kuah Meng-Kiat

Artikel/Article: [Morphological and genetic analyses verify the occurrence of the butterfly *Graphium chironides* \(Lepidoptera: Papilionidae\) in Peninsular Malaysia and resolve the confusion on the validity of its subspecies *malayanum* Eliot, 1982 94-121](#)