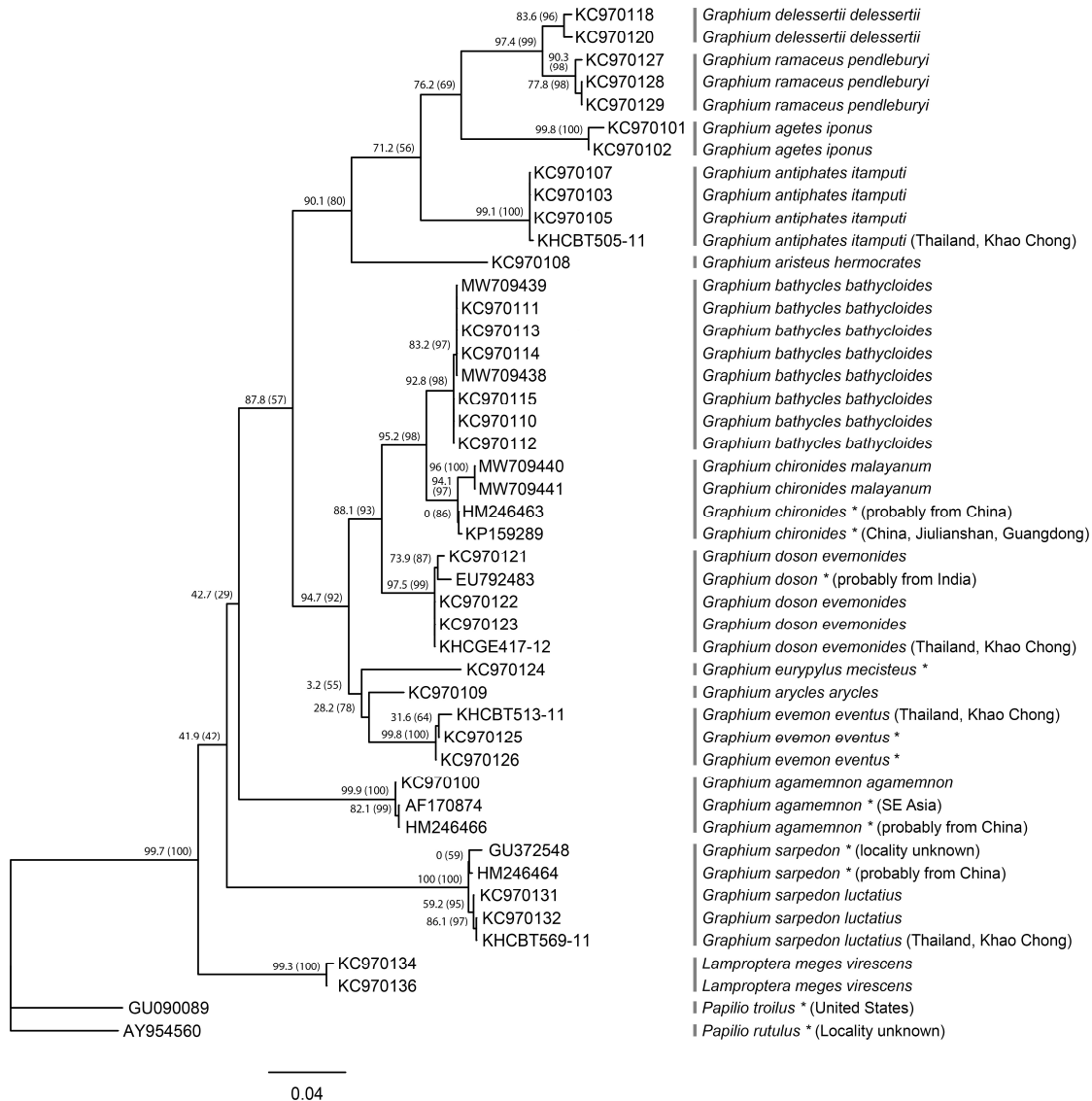


Reanalysis of phylogenies in the genus *Graphium* Scopoli, 1777  
based primarily on the data of Wilson *et al.* (2014)

Maximum Likelihood Tree



Notes:

Shows the original maximum likelihood tree for COI mtDNA in *Graphium* and *Lamproptera meges*, with two species of *Papilio* as outgroups, obtained using the same methods and program settings stated in the main text.

The model GTR+F+I+R2 was selected using ModelFinder.

The analysis produced one ML original tree and one bootstrap consensus tree. The original tree is shown here.

Numbers beside nodes are percentages of SH-aLRT support and ultrafast bootstrap support (in parentheses).

Dataset includes 41 of the 50 sequences used by Wilson *et al.* (2014). Only 56 base-pairs were held in common by all 50 of the original sequences. To avoid difficulties in resolving nodes (Min & Hickey 2007), the dataset was reduced to 41 specimens with complete 658-bp sequences.

Dataset includes an additional two *G. c. malayanum* and two *G. b. bathycloides* from this study, and a single *G. chironides* from China (GenBank).

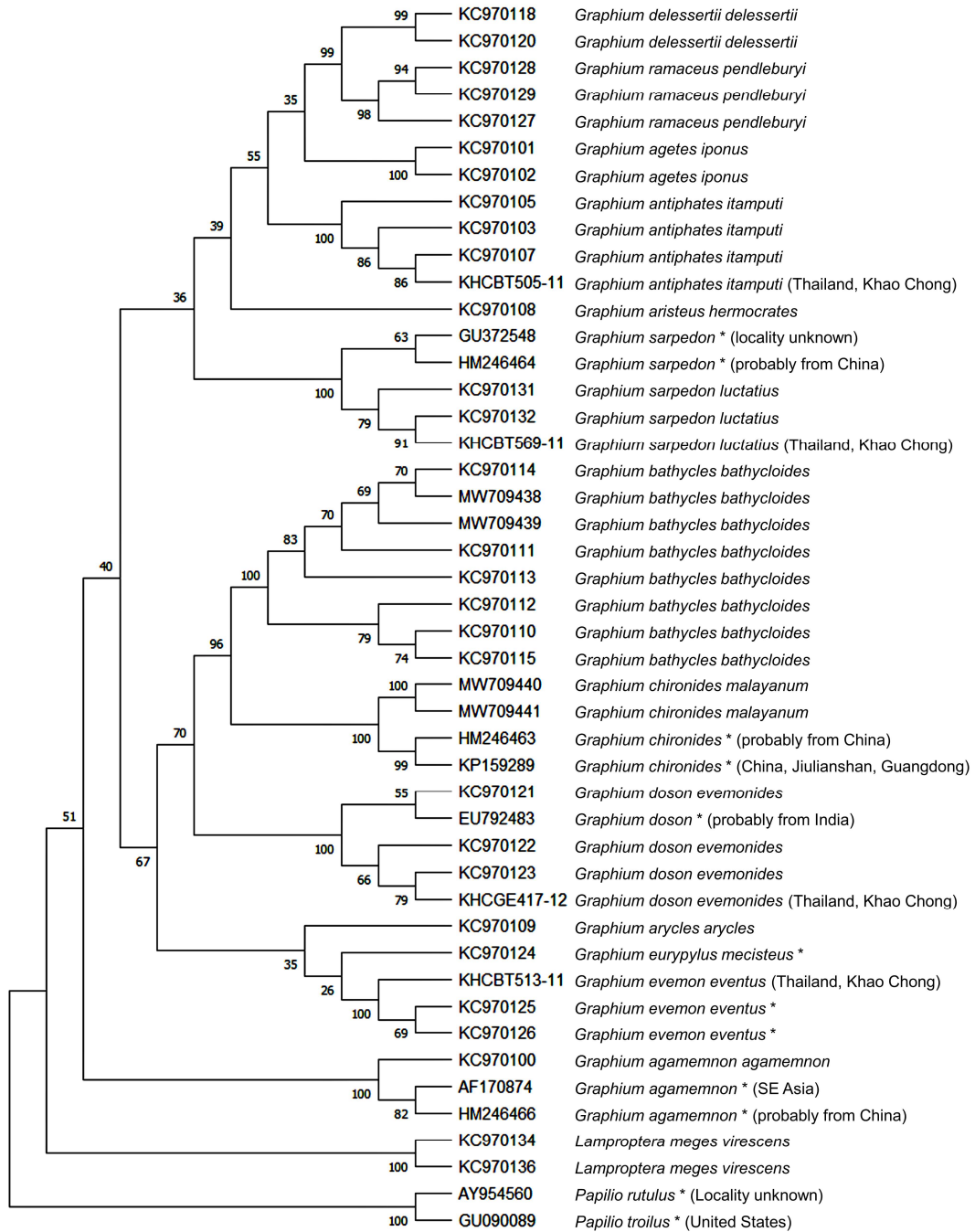
Where not otherwise stated, specimens are from Peninsular Malaysia.

Asterisks indicate specimens for which identities could not be verified from images or physical specimens. Where possible, identifications have been verified by examining the actual specimens or photographs of the specimens published in BOLD Systems. Peninsular Malaysian specimens of *G. eurypylus mecisteus* and *G. evemon eventus* could not be traced in the MZUM or identified from photographs of their uppersides in BOLD Systems.

The subspecies name *itamputi* is used here in combination with *G. antiphates* for ease of comparison with the trees figured in Wilson *et al.* (2014). The correct subspecies name is arguably *alcibiades* (Cotton 2018).

The accession number KHCGE417-11 (Wilson *et al.* 2014) for *Graphium doson* is a typological error and is corrected here to KHCGE417-12, as the former does not exist in GenBank or BOLD Systems, but the latter does and is the same species.

## Maximum Parsimony Tree



### Notes:

The COI mtDNA maximum parsimony tree above shows the consensus tree for the same sequence data shown in the maximum likelihood tree, for the purpose of comparison with the tree shown by Wilson *et al.* (2014).

See notes section under the ML tree for an explanation of the dataset, conventions used and notes on specimens.

Default program settings were applied in generating the tree if the settings were not mentioned in Wilson *et al.* (2014).

Numbers beside branches are bootstrap percentages.

The five most parsimonious trees produced by the analysis from 1000 bootstrap replications had three topologies. Differences were primarily in the position of *G. aristeus* on the tree, which was unstable and supported by low bootstrap values.

All five MP trees as well as the consensus tree differed in topology from the tree obtained by Wilson *et al.* (2014), which is due to the exclusion of short and incomplete sequences in our analysis. An analysis of the original 50 sequences used by Wilson *et al.* (2014), after partial deletion of alignment gaps and missing information using the default site-coverage cut-off point of 95% and with default settings in Mega 5.2, produced a consensus tree of similar topology to theirs but resulted in the retention of only 283-bp.

In the ML tree, *G. sarpedon* formed the earliest branch in *Graphium*, but with low support. This was followed by the *G. agamemnon* branch, which was also weakly supported. *Graphium bathycles* and *G. chironides* formed a sister clade with high support. The MP tree differed from the ML tree most noticeably in that *G. sarpedon* branched off much later in the tree.

The topology of the ML tree agrees to some extent with the phylogenies inferred by Saigusa *et al.* (1977, 1982) from wing morphology, genitalia and biogeography of species in the subgenus *Graphium*. They recognised the monophyly of the subgenus *Graphium* and considered it to comprise three groups: the *agamemnon* group, the *sarpedon* group and the *eurypylus* group, with the *agamemnon* group forming the earliest branch and the *sarpedon* and *eurypylus* groups diverging later and forming sister groups. Within the *eurypylus* group they recognised the *bathycles*-complex, comprising *bathycles*, *chiron* and *leechi*, and the *eurypylus*-complex, comprising *doson*, *procles*, *evemon* and *eurypylus*. Racheli & Cotton (2009) recognised four Asian lineages of the genus *Graphium*, treating them as subgenera (*Pathysa*, *Paranticopsis*, *Pazala* and *Graphium*) for convenience. The ML tree we obtained, although lacking taxa in the subgenus *Pazala*, broadly agrees with these subdivisions, except that the *G. sarpedon* lineage branched off earlier than all the other species of *Graphium*, including those in the subgenera *Pathysa* and *Paranticopsis*. In contrast, in an analysis of 28S rRNA and mitochondrial NADH dehydrogenase subunit 5 (ND5) genes by Makita *et al.* (2003), the *eurypylus* clade, for which they sequenced the taxa *evemon*, *doson*, *chiron*, *bathycles* and *leechi*, formed an early-branching clade with *G. phidias* (“*G. akikoe*”), rendering the species traditionally placed in the subgenus *Graphium* paraphyletic.

A broader and more in-depth study of species in the genus *Graphium* that incorporates a greater number of species and considers their wing patterns, structural morphology and genes, using multiple genes or entire genomes, would be necessary to fully resolve the relationships of the species within the genus.

#### **Additional references**

- Cotton A.M. 2018. Confirmation of the true identity of *Papilio alcibiades* Fabricius, 1787 (Lepidoptera: Papilionidae). *Butterflies* 79: 11–17.
- Makita H., Shinkawa T., Kondo K., Xing L. & Nakazawa T. 2003. Phylogeny of the *Graphium* butterflies inferred from nuclear 28S rDNA and mitochondrial ND5 gene sequences. *Transactions of the Lepidopterological Society of Japan* 54 (2): 91–110.
- Min X.J. & Hickey D.A. 2007. Assessing the effect of varying sequence length on DNA barcoding of fungi. *Molecular Ecology Notes* 7 (3): 365–373.
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