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DNA barcodes and citizen science provoke a diversity reappraisal for the “ring” butterflies of Peninsular Malaysia (*Ypthima*: Satyrinae: Nymphalidae: Lepidoptera)

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Draft

Abstract

The “rings” belonging to the genus *Ypthima* are amongst the most common butterflies in Peninsular Malaysia. However, the species can be difficult to tell apart, with keys relying on minor and often non-discrete ring characters found on the hindwing. Seven species have been reported from Peninsular Malaysia but this is thought to be an underestimate of diversity. DNA barcodes of 165 individuals, and wing and genital morphology, were examined to reappraise species diversity of this genus in Peninsular Malaysia. DNA barcodes collected during citizen science projects – School Butterfly Project and Peninsular Malaysia Butterfly Count – recently conducted in Peninsular Malaysia were included. The new DNA barcodes formed six groups with different Barcode Index Numbers (BINs) representing four species reported in Peninsular Malaysia. When combined with public DNA barcodes from the Barcode Of Life Datasystems several taxonomic issues arose. We consider the taxon - *Y. newboldi* - formerly treated as a subspecies of *Y. baldus*, as a distinct species. DNA barcodes also supported an earlier suggestion that *Y. nebulosa* is a synonym under *Y. horsfieldii humei*. Two *Ypthima* BINs comprising DNA barcodes collected during citizen science projects did not correspond to any species previously reported in Peninsular Malaysia.

Key words: COI, DNA barcodes, Satyrinae, taxonomy, *Ypthima*

Introduction

Butterflies (Lepidoptera: Papilionoidea) have important ecosystem roles, related to their trophic position and provision of pollination services (Moron et al. 2009). In addition, butterflies are considered sensitive indicators for assessing the condition, and changes in condition, of an ecosystem (Bonebrake et al. 2010; Cleary 2004). Their colored wings are highly visually conspicuous and have made them popular with professional scientists and citizen scientists alike. Consequently there is an abundance of recorded observations and historical collections (Kwon et al. 2010), including for the biodiversity hotspot of Peninsular Malaysia (Wilson et al. 2013).

In “The butterflies of the Malay Peninsula” (Fourth Edition) Corbet and Pendlebury (1992) recorded 1031 species from 284 genera, with butterfly species more or less evenly distributed throughout the peninsula. The relatively large-sized species from the Nymphalidae (e.g., the “tigers” and “crows”) are well-known, but the subfamily Satyrinae, containing the medium-sized “browns”, is comparatively poorly understood with little taxonomic attention (reflecting the situation for the group globally; Freitas and Brown 2004; Freitas 2004; Murray and Prowell 2005; Peña et al. 2006). The “rings” belong to the satyrid genus, *Ypthima* Hübner, 1818, which has a worldwide distribution and includes 126 species (Savelle 2015). *Ypthima* wings are dull brown, possess a large ocellus on the forewing, which reaches its greatest development on the underside, and have a submarginal series of yellow-ringed black ocelli on the underside of the hindwing (Corbet and Pendlebury 1978). In Peninsular Malaysia, seven species of *Ypthima* Hübner, 1818, one with two subspecies, were recorded in the latest checklist of Corbet and Pendlebury (1992): *Ypthima baldus newboldi* Distant, 1882, *Y. dohertyi mossmani* Eliot, 1967, *Y. fasciata torone* Fruhstorfer, 1911, *Y. horsfieldii humei*

Moore, 1884, *Y. huebneri* Kirby, 1871, *Y. pandocus corticaria* Butler, 1879, *Y. pandocus tahanensis* Pendlebury, 1933, *Y. savara tonkiniana* Fruhstorfer, 1908.

The classic work of Corbet and Pendlebury (1992) identified *Y. pandocus corticaria* and *Y. huebneri* as candidates for the title of the commonest butterfly in Peninsular Malaysia, and suggested that the eight taxa currently recorded for the peninsula could be significantly increased through “patient searching among the swarms of common and ubiquitous species which excite little interest in most collectors” (an opinion also recently re-expressed by Khew 2010). Elliot, in Corbet and Pendlebury (1992) predicted that some *Ypthima* species such as *Y. watsoni* (Moore, 1893), *Y. philomela* (Linnaeus, 1763), *Y. lisandra* (Cramer, 1780), *Y. iarba* de Nicéville, 1895 and *Y. nebulosa* Aoki and Uémura, 1982, found in Borneo, Java, Sumatra or Burma, were potentially present in Peninsular Malaysia. Furthermore, several new species of *Ypthima* have been described recently from other regions in Asia (e.g., Vietnam – Uémura and Monastyrskiy 2000; India - Singh 2007). *Ypthima* are an important group from an environmental monitoring perspective, as they are particularly abundant in urban and disturbed areas (Sing et al. 2015) and are common amongst low grasses so are present in Malaise trap samples (Yee 2014). Certain *Ypthima* species have also been used as models for ecology and evolution studies (see Noriyuki et al. 2010; Noriyuki et al. 2011). However, the species can be difficult to tell apart, with keys relying on minor and often non-discrete ring characters, the different series of ocelli found on the underside of hindwing, which are often damaged on specimens, and male genitalia (Corbet and Pendlebury 1992).

We recently initiated two citizen science projects in Peninsular Malaysia using butterflies as models to engage the general public, and especially schoolchildren, with wildlife and the impacts of global warming (Jisming-See et al. 2014; Jisming-See et al. 2015; Wilson et al.

2015). In both projects participants were asked to collect non-lethal samples (legs) of butterflies for identification through DNA barcoding (Floyd et al. 2009). Our first citizen science project, the School Butterfly Project, involves schoolchildren from five different schools across Peninsular Malaysia and we have received 338 butterfly legs so far. The Peninsular Malaysia Butterfly Count was conducted for the first time on Butterfly Education and Awareness Day – June 6th 2015 and we received 220 butterfly legs from the participants. Due to their abundance *Ypthima* species are likely to be well-represented amongst the butterfly legs collected during citizen science projects.

The objective of this study was to assemble cytochrome oxidase subunit I (COI) mtDNA barcodes (Hebert et al. 2003; Wilson 2012) and examine the wing and genital morphology of numerous individuals of *Ypthima* collected across Peninsular Malaysia to reappraise the species diversity of *Ypthima* in this region.

Materials and Methods

(i) Sampling

Ypthima butterflies were sampled from locations across Peninsular Malaysia. Specimen records are available in the public Barcode Of Life Datasystems (BOLD; Ratnasingham & Hebert 2013) dataset: The ring butterflies of Peninsular Malaysia [DS-YRBPM]. Most butterflies were caught in hand-nets, but several specimens were collected from a Malaise trap set at Rimba Ilmu Botanic Garden, University of Malaya, Kuala Lumpur. A single leg from each of the 128 collected butterflies was removed with sterile forceps for DNA extraction. The upperside and underside of pinned and spread butterflies were photographed using a macro lens. An additional 37 samples of *Ypthima* (as determined a-posteriori through

DNA barcoding, and strict tree-based assignment; Wilson et al. 2011) were received from participants in the School Butterfly Project (Jisming-See et al. 2014; Jisming-See et al. 2015) and the first Peninsular Malaysia Butterfly Count (Wilson et al. 2015).

(ii) DNA barcoding

Genomic DNA was extracted from butterfly legs using a modified alkaline lysis method whereby legs were digested in 17.5µl alkaline buffer for 20 minutes before adding 32.5µl of neutralization buffer (following Ivanova et al. 2009). The DNA extracts were diluted 1/10 in ddH₂O prior to PCR. DNA extracts were used for COI DNA barcoding following standard methods with the primer pair HCO2198 and LCO1490 (see Wilson 2012). PCR amplification was performed in a 26µl volume containing 12.5µl of premix Hotstart premix (Lucigen), 11µl of ddH₂O, 0.25µl of each primer and 2µl of diluted DNA. The thermocycle profile was 120sec at 98°C followed by 40 cycles for 60sec at 98°C, 60sec at 40°C, 90sec at 72°C, and a final extension step for 7min at 72°C. PCR products were visualized on a 1.5% agarose gel. PCR products were sequenced in one direction by a local company (MyTACG Bioscience). Products producing low quality chromatograms were re-sequenced and if necessary PCR was repeated. Chromatograms were edited with CodonCode Aligner (CodonCode Corp) and BioEdit (Hall 1999) following steps outlined in Wilson (2012). The COI DNA barcodes, together with collection locality information, were submitted to BOLD. Sequences are automatically translated upon upload to BOLD providing a further quality control measure; those DNA barcodes containing stop codons on initial upload were further checked and edited before re-upload.

(iii) Morphology

Based on neighbor-joining analysis of the COI DNA barcodes (using p-distance and default parameters in MEGA6; Tamura et al. 2013), 14 specimens were selected from among four distinct clusters for genitalia dissection. The tip of the abdomen was soaked in 10% KOH for one week prior to dissection of genitalia and observation under a light microscope.

(iv) Taxonomic analysis

Barcode Index Numbers (BINs) are Molecular Operational Taxonomic Units produced by Refined Single Linkage analysis of DNA barcodes across BOLD and have been shown to correspond closely with traditional species limits characterized by morphology (Ratnasingham and Hebert 2013; Hausmann et al. 2013). DNA barcodes, of certain length (500bp for new BINs and 300bp for existing BINs) and quality (maximum proportion of Ns), are assigned to BINs automatically upon upload to BOLD. 77% of our newly sequenced DNA barcodes were assigned BINs upon upload to BOLD and the remainder were unambiguously allocated to BINs based on their position on a phylogenetic tree (see below). BOLD BINs are presented below in the form - [BOLD:XXXXXXX] - which are searchable from - http://www.boldsystems.org/index.php/Public_BarcodeIndexNumber_Home. Our newly assembled DNA barcode dataset was combined with 125 *Ypthima* DNA barcodes which were publicly available on BOLD (based on a search for “*Ypthima*” under “Taxonomy”) together with two species (*Mycalesis mineus* and *M. jarnardana*) from a closely related genus as outgroups. This allowed us to interpret our taxonomic concepts and usage of Linnaean nomenclature in comparison to use by other BOLD users. The combined aligned matrix was analyzed using maximum likelihood (ML) in MEGA6 (Tamura et al. 2013). We first tested for the best fitting substitution model (in MEGA6 based on BIC), which was TN93+I, and then used this model, the Subtree-Pruning-Regrafting-FAST tree searching option, and otherwise default settings to search for ML trees. The “best” ML tree

was tested for “reliability” using 1000 neighbor-joining (p-distance) bootstrap pseudoreplicates. We used the “diagnostic” morphological characters reported for *Ypthima* species to provisionally assign Linnaean names to the BINs. We further determined if morphological characters were consistent for all members of BINs and levels of overlap between taxa.

Results and Discussion

Based on the maximum likelihood tree the newly sequenced specimens (165) formed six distinct groups corresponding to four (of seven) *Ypthima* species known from Peninsular Malaysia (Fig. 1). These six groups coincided with six BINs generated by BOLD (Fig. 1; Table 1). However, when combined with the public *Ypthima* DNA barcodes from BOLD several taxonomic issues became apparent and these are discussed below. When including the DNA barcodes from Wilson et al. (2013), DNA barcodes are now available on BOLD for six *Ypthima* taxa previously reported from Peninsular Malaysia: *Ypthima newboldi* (= *baldus newboldi*) [BOLD:AAN9479], *Y. fasciata torone* [BOLD:AAJ9576], *Y. horsfieldii humei* [BOLD:AAO0345], *Y. huebneri* [BOLD:AAZ4966], *Y. pandocus corticaria* [BOLD:ACA4198], *Y. savara tonkiniana* (the sequence is too short to be assigned a BIN). The two missing “known” taxa (one species and one subspecies) are *Y. dohertyi mossmani*, a very rare species that is known only from the Cameron Highlands, Pahang, and *Y. pandocus tahanensis* found only above 1700 m at Gunong Tahan in Taman Negara National Forest (Corbet and Pendlebury 1992). The correspondence of two additional clusters (BINs) of DNA barcodes from Peninsular Malaysia to any Linnaean species names remains undetermined but potential candidates are discussed below.

Ypthima “BALDUS” group

Ypthima baldus (Fabricius, 1775) was described as *Papilio baldus* based on a specimen from “India” although most likely the type locality is China (Savelle 2015). The analysis of the newly generated DNA barcodes and the public *Ypthima* DNA barcodes produced seven putative species (BINs) with one or more of their component DNA barcodes labeled as *Ypthima baldus* (Fig. 1). This large section of the tree (the “BALDUS group”) also included six other BINs, three of which contained only unnamed DNA barcodes, one comprising a single DNA barcode labelled *Y. avanta*, one with DNA barcodes labelled *Y. fasciata*, and one with DNA barcodes labelled *Y. horsfieldii humei* or *Y. nebulosa*. Interestingly, on the “Lepidoptera and some other life forms” website (Savelle 2015), the *philomela* species-group equivalent to our “BALDUS group” comprises 14 named species. Equating these 14 traditional species names with the 13 “BALDUS group” BINs presents an interesting taxonomic challenge.

(i) BALDUS 1/ NEWBOLDI [BOLD:AAN9479]

The largest BIN (BALDUS 1) consisted of 60 DNA barcodes from this study, one *Y. baldus newboldi* from Peninsular Malaysia from Wilson et al. (2013), two DNA barcodes labelled *Y. baldus* from Thailand, and two labelled *Y. baldus baldus* from Vietnam. *Y. baldus newboldi* is known from Peninsular Malaysia, Singapore and southern Thailand (Corbet and Pendlebury 1992). The subspecies “*newboldi*” has not been reported from Vietnam, although in Vietnam “*baldus*” shows notable variation (Monastyrskii and Holloway 2013). Interestingly, according to the GenBank records, these specimens came from both the far south (Phú Quốc) and north (Bắc Yên) of Vietnam. Given the presence of the specimens from Peninsular Malaysia and southern Thailand, we retain the name *newboldi* for this BIN. Considering the divergence from other *Y. baldus* DNA barcodes we suggest the BIN represents a distinct

species. *Ypthima newboldi* was described as a distinct species by Distant (1882) based on specimens from Perak, Malaysia (the type of *Y. newboldi* is not in the BMNH collection and the location is unknown; personal communication – John Chainey, Natural History Museum, London), but was subsequently synonymized under *Y. marshalli* Butler, 1882, by Elwes and Edwards (1893), before being treated as a subspecies of *Y. baldus* (Corbet and Pendlebury 1956). *Ypthima marshalli* was itself removed from synonymy under *baldus* on the basis of genitalic differences (Rose and Sharma 1999). Most of the specimens in the BALDUS 1 BIN, that we could examine, matched the morphological description from Corbet and Pendlebury (1992) – the underside hindwing has larger ocelli in space 2 and 3 which are almost always contiguous, and the ocellus in space 5 is always larger than that in space 6 (Fig. 2). However, two specimens from Peninsular Malaysia had ocelli of equal size in space 5 and space 6 matching the characters of *Y. horsfieldii humei* (Fig. 2).

(ii) BALDUS 2/ HUMEI/ NEBULOSA [BOLD:AAO0345] and BALDUS 3 [BOLD:ACB4115]

Ypthima horsfieldii Moore, 1884, was described from Java (Moore 1884), has a range encompassing Java, Peninsular Malaysia and Borneo, and currently contains two subspecies (Savelle 2015). *Y. humei* Elwes and Edwards, 1893, was initially described as a distinct species (or form of *Y. singala* Felder, 1868, a close relative of *Y. thora* Moore, 1880 = *Y. avanta* Moore, 1875) in a speculative note on a damaged specimen from southern Burma (Myanmar) by Elwes and Edwards (1893) (the type is in the BMNH and photographs have been examined by the authors). *Y. nebulosa* Aoki and Uémura, 1982, was described from Sumatra and has a range including Thailand, Laos, Vietnam and Peninsular Malaysia (Shima 1988). Eliot (in Eliot and Pinratana 1988) synonymized *Y. nebulosa* under *Y. horsfieldii humei*. To add further confusion, Uémura synonymized the type *humei* under *Y. baldus*

newboldi while retaining *Y. nebulosa* as a distinct species (Uémura and Monastyrskii 2004). As there are no DNA barcodes available for *Y. horsfieldii horsfieldii* it is difficult to comment on the species status of *humei*, but the DNA barcodes (and wing characters including specimens from Thailand identified by Pitoon Kongnoo) suggest Eliot (1988) was correct in his synonymization with *Y. nebulosa*. The close relationship between *Y. newboldi* (BALDUS 1) and *Y. horsfieldii humei* seen in the COI analysis was also supported by the similar male genitalia of both species (Fig. 2). The sister to *Y. horsfieldii humei* on the tree was BALDUS 3, a singleton DNA barcode from India without a specific epithet (from Gaikwad et al. 2012).

(iii) BALDUS 4/ ZODINA [BOLD:AAF4871] and BALDUS 5/ ZODINA [BOLD:AAF4872]

Another group of *Y. baldus* labelled DNA barcodes, which fell as sister to *Y. horsfieldii humei* (+BALDUS 3) DNA barcodes (Fig. 1), contained two BINs: *Y. baldus zodina* Fruhstorfer, 1911, from Taiwan (BALDUS 4), and one distinct haplotype (BALDUS 5) comprising three DNA barcodes: *Y. baldus zodina* from Hong Kong (although the subspecies has not previously been reported from there), *Y. baldus baldus* from the Ryuku Islands, southern Japan (800 km from Taiwan) and a *Y. baldus*, subspecies not specified, from Anhui, China. A presumably morphologically distinct *Ypthima* from Hong Kong has been described (*Y. baldus scota*, Fruhstorfer 1911) but is considered a junior subjective synonym of *Y. baldus baldus* (Beccaloni et al. 2011). The genetic distinctiveness of the *Y. baldus zodina* BIN from Taiwan (BALDUS 4) suggests this taxon, although not originally described as a distinct species, could deserve species status. The identity of the sister BIN (BALDUS 5) remains in question, but it may represent a divergent clade of *Y. zodina* or another distinct species.

(iv) BALDUS 6/ FASCIATA [BOLD:AAJ9576], BALDUS 7 [BOLD:ACX2349] and BALDUS 8 [BOLD:ACW8027]

Three DNA barcodes generated from legs collected during the Peninsular Malaysia Butterfly Count and the School Butterfly Project, formed two distinct BINs nested within the “BALDUS group”. One BIN (BALDUS 7) contained only a single DNA barcode collected from Penang Island. This singleton was sister, but almost 4% distant to a cluster comprising the other two DNA barcodes, one collected from the same location in Penang Island and another from the adjacent mainland region of Kedah. The correspondence of these taxa to *Ypthima* species unrecorded from the peninsula, but suspected, by Corbet and Pendlebury (1992), to be present (*Y. watsoni*, *Y. philomela*, *Y. lisandra*, *Y. iarba*) or indeed, any other described *Ypthima* species not yet represented in BOLD remains unknown. *Y. iarba*, part of the *sakra* species-group (Shima 1988), and *Y. watsoni*, part of the *motschulskyi* species-group (Shima 1988) with a close affinity to *Y. pandocus* (Corbet and Pendlebury 1992), can potentially be discounted as these “unknown” species clearly fall within the “BALDUS”/*philomela* species-group. *Y. philomela* (photograph of type specimen in the Linnean Society of London collection examined) and *Y. lisandra* (the type of *Y. lisandra* is thought to be in the collection of Naturalis Biodiversity Center, Leiden but could not be located by the curator and may be missing; personal communication - Eulàlia Gassó Miracle,) remain as potential candidates although the status of these names and taxa are also contentious (see below). Nonetheless the presence of these DNA barcodes in BOLD opens the possibility that connections to traditionally described taxa can be readily established as the DNA barcode reference library grows.

The two unknown *Ypthima* clusters (BALDUS 7 and BALDUS 8) collected by the citizen scientists fell as sister to *Y. fasciata torone* (BALDUS 6). Hewitson (1865) remarked that *Y.*

fasciata “bears great resemblance to *Y. baldus*” so it is unsurprising that the species fell within the “BALDUS group”.

(v) BALDUS 9 [BOLD:ACB5303], BALDUS 10 [BOLD:AAF4870] and BALDUS 11/ AVANTA [BOLD:AAN8843]

The BALDUS 9 cluster comprised DNA barcodes from India labeled as *Y. baldus* with no subspecies specified. There are at least four species of *Ypthima* known from India, besides those already recorded on BOLD. A GenBank record from Central Sulawesi, Indonesia is sister to the large BIN comprising the “BALDUS” clusters described above. A single DNA barcode labelled as *Y. avanta* (BALDUS 11) from Pakistan is sister to BALDUS 10+large BALDUS clade. *Y. avanta* is sometimes recognized as a subspecies under *Y. lisandra* (Uémura and Monastyrkii 2004).

(vi) BALDUS 12/ ZODIA [BOLD:AAC7872] and BALDUS 13 [BOLD:ACQ0516]

A sub-tree, sister to the rest of the “BALDUS group”, comprised two BINs from East Asia. Several DNA barcodes labelled as *Y. baldus* from Korea clustered with *Y. zodia* Butler, 1871, from China (BALDUS 12). A further BIN (BALDUS 13) of *Y. baldus* labelled DNA barcodes, comprising DNA barcodes from Japan was sister to BALDUS 12.

***Ypthima huebneri* group**

Ypthima huebneri Kirby, 1871, has a range extending from Northwest India, south to Singapore, and east to Yunnan (Savelle 2015). The name *Ypthima hübneri* Kirby, 1871, was a replacement name for *Ypthima philomela* Hübner, 1813 (type locality: Burma), a junior homonym of *Papilio philomela* Linnaeus, 1763 (type locality: Java). *Ypthima ceylonica*

Hewitson, 1865, was described from Sri Lanka (Hewitson 1865). Elwes and Edwards (1893) remarked of *Y. ceylonica*: “[Taylor (1888) suggested that *Y. ceylonica* Hewitson, 1865] is merely a local form of *Hübneri*, but the form of the clasps entirely negates any such assumption”. However, *Y. huebneri* appeared as a subspecies of *Y. ceylonica* in the second and third editions of Corbet and Pendlebury’s checklist (i.e., as *Y. ceylonica huebneri*; 1956; 1978) although it was listed as a distinct species in the fourth edition (Corbet and Pendlebury 1992). The phylogenetic analysis of the newly generated DNA barcodes and the public *Ypthima* DNA barcodes on BOLD produced two putative species with one or more of their component DNA barcodes labelled as *Y. huebneri* (Fig. 1) and an unnamed cluster nested between these two.

The first cluster (HUEBNERI 1 [BOLD:ACB5638]) consisted of four DNA barcodes from India, labelled as *Y. huebneri*. The second cluster (HUEBNERI 2/ CEYLONICA [BOLD:ACP7771], sister to HUEBNERI 1, consisted of a single DNA barcode from India, labelled as *Y. ceylonica*. The third cluster (HUEBNERI 3 [BOLD:AAN8844]) contained two *Ypthima* DNA barcodes from Pakistan, with no specific epithet. This third cluster fell as sister to the fourth cluster (HUEBNERI 4 [BOLD:AAZ4966]), which contained *Y. huebneri* labelled DNA barcodes from Vietnam and Peninsular Malaysia. HUEBNERI 4 was the largest HUEBNERI cluster consisting of 94 DNA barcodes. The members of this cluster mostly matched with the morphological description of *Y. huebneri* given in Corbet and Pendlebury (1992) – an ocellus in space 2 and 3 on the underside of the hindwing. However, there were two specimens with no ocellus in space 3 (Fig. 2).

Ypthima pandocus corticaria

The subspecies *Ypthima pandocus corticaria* Butler, 1879, has a range extending from Sumatra to Peninsular Malaysia and was described from Malacca (Savelle 2015). Our examined specimens from Peninsular Malaysia matched with the morphological description of Butler (1879): “.... wings below white, densely reticulated with brown, subparallel lines, primaries with the ocellus brighter than above, secondaries with three ocelli, one at apex, and two placed at anal angle, the lower one small and irregular” (see Fig. 2).

The phylogenetic analysis, albeit based on a short mitochondrial sequence (see Wilson 2010), of 10 newly generated DNA barcodes from Peninsular Malaysia placed the species as sister to *Y. multistriata* Butler, 1883 and *Y. motschulskyi* Bremer and Grey, 1853. Butler (1883) described *Y. multistriata* as allied to *Ypthima narenda* Kollar, 1844 and *Y. corticaria* Butler, 1879, intermediate in size between the two species. Additionally, Hewitson (1865) reported *Y. motschulskyi* as “very near” to *Y. pandocus* Moore, 1858, but differing on the upperside, with the ocellus of the forewing slightly divided, and in having only one ocellus on the hindwing.

Conclusions

Elliot, in Corbet and Pendlebury (1992), speculated that the species diversity of *Ypthima* butterflies in Peninsular Malaysia was significantly underestimated. We examined 165 specimens of *Ypthima* collected at 25 locations across the peninsula and recognized six species, of seven, recorded in Corbet and Pendlebury’s (1992) checklist. We also found evidence for two previously unrecorded species of undetermined correspondence to any Linnaean species names based on samples provided by citizen scientists. Through taking advantage of “eyes and ears” everywhere, citizen science can provide an effective way to find

rare or new species, track invasions and detect species declines (Dickinson et al. 2012). In Peninsular Malaysia a citizen scientist has already been responsible for the discovery of a new species, *Semachrysa jade*, a green lacewing (see Winterton et al. 2012). The fate and status of the two “known” *Ypthima* taxa, unsampled in our study and not represented in the Museum of Zoology, University of Malaya, remains in question. The Cameron Highlands where the rare taxon, *Y. dohertyi mossmani*, was reported from, has been developed as an agricultural center since Corbet and Pendlebury’s time in Peninsular Malaysia, and insecticide is widely used by local farmers (Mazlan and Mumford 2005). Consequently, the species diversity of *Ypthima* in Peninsular Malaysia has increased very slightly, moving from seven species in Corbet and Pendlebury’s (1992) checklist to eight BINs on BOLD in 2016.

When looking at *Ypthima* diversity, and the current taxonomic treatment of the genus, at a global scale, made possible through the DNA barcode records on BOLD, a pattern of rampant crypticism, or at the least, confused taxonomy, becomes apparent. Sequencing of type specimens can help shed light on such problems (Wilson et al. 2010), but is not possible when the type is lost (e.g., *Y. newboldi* and *Y. lisandra*). The example here of *Ypthima* is just another case demonstrating the role of DNA barcodes, and particularly databases like BOLD, allowing “online quantum contributions” (Maddison et al. 2012) for advancing taxonomic progress and efficient biodiversity communication.

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TABLE

Table 1: DNA barcode statistics for the *Ypthima* species of Peninsular Malaysia

Taxa	New DNA barcodes from this study	BIN	Maximum K2P distance within BIN (%)	Minimum K2P distance to nearest BIN (%)
<i>Ypthima newboldi</i> BALDUS 1	55	AAN9479	2.09	2.09
<i>Ypthima horsfieldii humei</i> BALDUS 2	6	AAO0345	0.54	3.82
<i>Ypthima fasciata</i> BALDUS 6	0	AAJ9576	N/A	6.61
BALDUS 7	1	ACX2349	N/A	3.85
BALDUS 8	2	ACW8027	0	3.85
<i>Ypthima huebneri</i> HUEBNERI 4	91	AAZ4966	2.34	3.24
<i>Ypthima pandocus corticaria</i>	10	ACA4198	1.3	4.16
<i>Ypthima savara tonkiniana</i>	0	N/A	N/A	4.6

FIGURE CAPTIONS

Fig. 1: Maximum likelihood tree of COI DNA barcodes of *Ypthima* available on BOLD and newly sequenced specimens from Peninsular Malaysia. Thick branches bound the nodes with bootstrap support values ≥ 98 . A more detailed tree is available as Fig. S1.

Fig. 2: The ring patterns of *Ypthima* species known from Peninsular Malaysia based on examined specimens deposited in the Museum of Zoology, University of Malaya, photographs in Corbet and Pendlebury (1992, 1978) and the type specimen of *Y. horsfieldii humei*. The image on the top is the upperside and below is the underside. Circles filled with black represent ocelli found on all examined specimens and dashed circles filled with white represent ocelli present on some specimens but not present on others. The male genitalia of specimens from Museum of Zoology, University of Malaya, where available, are also illustrated (also available as Fig. S2). Some potential species in Peninsular Malaysia are also illustrated showing the characters of the type specimen of *Y. philomela* (based on photograph from the Linnean society of London and *Y. lisandra* based on the painting in Cramer, 1780).

Maximum likelihood tree of COI DNA barcodes of *Ypthima* available on BOLD and newly sequenced specimens from Peninsular Malaysia. Branches with bootstrap support values ≥ 98 are shown in bold. A more detailed tree is available as Fig. S1.
172x227mm (300 x 300 DPI)

The ring patterns of *Ypthima* species known from Peninsular Malaysia based on examined specimens deposited in the Museum of Zoology, University of Malaya, photographs in Corbet and Pendlebury (1992, 1978) and the type specimen of *Y. horsfieldii humei*. The image on top is the upperside and below is the underside. Circles filled with black represent ocelli found on all examined specimens and dashed circles filled with white represent ocelli present on some specimens but not present on others. The male genitalia of specimens from Museum of Zoology, University of Malaya, where available, are also illustrated. Some potential species in Peninsular Malaysia are also illustrated showing the characters of the type specimen of *Y. philomela* (based on photograph from the Linnean society of London) and *Y. lisandra* (based on the painting of the type specimen in Cramer, 1780).

172x158mm (300 x 300 DPI)

Fig. S1. Maximum likelihood tree of COI DNA barcodes of *Ypthima* available on BOLD and newly sequenced specimens from Peninsular Malaysia. Thick branches bound the nodes with bootstrap support values ≥ 98 .

Fig. S2. The male genitalia of *Ypthima* species found in Peninsular Malaysia represented by specimens from Museum of Zoology, University of Malaya.

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The male genitalia of Ypthima species found in Peninsular Malaysia represented by specimens from Museum of Zoology, University of Malaya.
3522x1010mm (72 x 72 DPI)

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