

Phylogenetic relationships of *Eurema* butterflies from Peninsular Malaysia inferred from CO1 and 28S gene sequences with emphasis on *Eurema hecabe*

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Abstract. The phylogenetic relationships among species of the genus *Eurema* from Peninsular Malaysia were reconstructed using nucleotide sequences of mitochondrial CO1 (307 bp) and nuclear ribosomal 28S DNA (471 bp). A total of twenty-eight sequences generated through PCR amplification for each gene region were used to construct the Maximum Likelihood (ML) and Maximum Parsimony (MP) phylogenetic trees. The separate taxonomic grouping of the genus *Eurema* and the genus *Gandaca*, and their close association is tested here together with sequences of other pierid butterflies obtained from GenBank. All trees reveal a strongly supported monophyletic group of *Eurema* conspecifics and well-resolved interspecific genetic distances, indicating the usefulness of the genetic markers in local species identification. The combined phylogenetic analyses of CO1 and 28S genes strongly supports a close relationship of *E. hecabe* with *E. blanda*, while *E. andersonii* is recovered as a sister taxon to *E. ada*.

Key words. *Eurema*, CO1, 28S, relationships, phylogenetic, Peninsular Malaysia

INTRODUCTION

The butterflies of the genus *Eurema* are classified under family Pieridae and typically recognised by the bright to pale lemon yellow coloured ground wings, bordered with black margin on the apical side of both forewings (Corbet & Pendlebury, 1992). Since their discovery by Yata in 1989, nine species have been recorded in Peninsular Malaysia which can be identified using taxonomic keys developed by Corbet & Pendlebury (1992). However, despite the availability of well-developed taxonomic keys for this genus in Malaysia, members of *Eurema* butterfly are notoriously difficult to identify due to their close morphological resemblance (Mal et al., 2014). This factor has limited the use of morphological characteristics for accurate species identification.

There have been several conflicts about the morphological classification and taxonomic position of *Eurema* species as shown by studies done in Malaysia (Corbet & Pendlebury,

1992) and Thailand (Jeratthitikul et al., 2009). Both studies have created competing arguments on the number of species, taxonomic position, and nomination of numerous subspecies. The disparities between the studies are resulting from the different selection of morphological characters as their main species identification criteria. Although both studies were conducted in different countries, Thailand, Malaysia, and also Singapore, are all located within the same geographical region. Hence the use of different classification keys should be revised and a single most appropriate key established eventually.

Concerning the systematics of *Eurema*, the taxonomic position of *Eurema hecabe* Linnaeus, 1758 is of particular interest because it was reported to exhibit several morphological variations of the black apical border pattern, and wing marking pattern on forewing (underside). These patterns were reported to differ seasonally and geographically (Yata, 1989; Corbet & Pendlebury, 1992; Jeratthitikul et al., 2009), and also by elevation (Azrizal-Wahid et al., 2015). For these reasons, identification and relationship status of *E. hecabe* among its congeners are disputable. Moreover, *E. hecabe* is the most widely distributed species and has highly variable wing markings, resulting in frequent misidentifications (Ek-Amnuay et al., 2007).

Despite the morphological description of *E. hecabe* having been revised (Yata, 1994; Kato & Yata, 2005; Jeratthitikul et al., 2009), its status remains unconfirmed. Although most subspecies of *E. hecabe* basically have two cell spots on forewing underside, Khan & Sahito (2012) found that the number of cell spots could vary from two to one to none when reared under different environmental conditions. In fact,

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