Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 6, No. 3, p. 359-372, 2015
http://www.innspub.net

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RESEARCH PAPER
OPEN ACCESS

A review on morphological characterization, variation and distribution pattern of *Eurema* butterflies of Peninsular Malaysia

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Article published on March 28, 2015

Key words: *Eurema*, Morphology, Characterization, Variation, Distribution.

Abstract

Seven species of *Eurema* butterflies comprised of 263 adult individuals collected from various sampling sites of Peninsular Malaysia were identified and diagnosed based on their morphological characteristics and variation pattern. The result from diagnosis revealed that the main morphological characteristics that differentiated between the members of *Eurema* butterflies are the number of cell spots in discoidal cell and the pattern of brown apical patch, both located on the underside of the forewing. *E. sari*, *E. blanda* and *E. tilaha* are morphologically distinct and easily identified. Species of *Eurema* butterflies showed no variations, except for *E. hecabe* that had variable patterns of forewing black apical border in several individuals, which corresponded to the altitudinal changes of their sampling sites. The distribution of the genus *Eurema* in Peninsular Malaysia is also discussed based on the recorded field sampling data. The record shows that all six species with the exception of *E. tilaha* which was excluded from this study were evenly distributed across all sampling areas and can be found at most part throughout the Peninsular Malaysia with species of *E. andersonii* has the most consistent distribution pattern in all four different areas of sampling. This study also suggested that the most common species of the genus *Eurema* in Malaysia is species of *E. blanda* while *E. tilaha* was reported as the rare species.

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Introduction

The butterflies of the genus *Eurema* are classified under family Pieridae and typically recognized by the bright to pale yellow or lemon coloured wings which are bordered with black margin on the apical site of forewings. It is comparatively small in size and commonly found fluttering around the bushes or roadside. The *Eurema* butterflies in Malaysia were discovered by Yata in 1989. Since their discovery, there are nine species recorded in Malaysia which were identified using taxonomic keys developed by Colbert and Pendlebury (1992) that relied on the morphological characteristics of butterfly's wings including the pattern, structure and colouration.

Even though this genus was developed well taxonomic keys, however, the *Eurema* is still one of the most burdensome species for taxonomists in doing identification and classification due to its cryptic species complexes. Cryptic species complexes are defined as assemblages of closely related species that have been classified as one broadly delimited species due to the difficulty of identifying the species on the basis of visible phenotype (Collins and Paskewitz, 1996; Bickford *et al.*, 2006). Such species complexes present a worst case challenge for the use of morphological characteristics in species identification. Species identification is difficult due to high intra-specific variation of form and colour (Heim, 2003). This problem sometimes may leads to the misidentification of the species.

Moreover, the condition of the studied samples used at that time must be taken into consideration. Some morphological characteristics such as wing structure, colour and size are really affected by the habitat preference including elevation, climate change, development, habitat changes or destruction, and so on to adapt with the changed environment. Other factors also can be caused by the condition of the specimens that affected by the changes in the developmental temperature or photoperiod of the juvenile stages and also by other geographical related factors that include clinical genetic differences (Jones, 1992; Yata, 1989). All of these factors sometimes, make the use of wings morphology is not a preferred way for a very accurate in differentiation of the species.

Apart from that, all the existing morphological characteristics of various butterflies have certain connections with the survival environment and the natural selection. Within a long period, in the long-term struggle between butterflies and predators, the morphological characteristics that only benefit to survival are usually preserved, whereas, non benefit characteristics will lost. The loss of several characteristics that might be important in species classification thus will promote the burdensome to future taxonomist in species classification studies. So that, the revision on the recent status of butterflies is needed to ensure the reliability of taxonomic keys developed in early days to be used in current species identification.

The present study aims to characterize all observed morphological characteristics of members within the genus *Eurema* and to identify any diagnostic morphological characteristic in wing patterns, structures and colouration possessed by each *Eurema* species which deemed suitable for their quick identification. Moreover, any variation in wing patterns that found within the species members also will be discussed. The study also will describe the distribution pattern of *Eurema* butterflies in Peninsular Malaysia through the analysis of sampling records.

Materials and methods

Sampling sites

Samples were collected at various sites comprising of four main areas which are North, West, South and East of Peninsular Malaysia. The Northern area includes sampling sites within the states of Kedah, mainland of Pulau Pinang, western side of Kelantan until the centre part of Perak. The Western area includes the southern part of Perak down into Selangor and Kuala Lumpur. The Eastern area comprises of the states of Kelantan, Terengganu and Pahang, while the Southern area consists of the Negeri Sembilan, Melaka and Johor states. Samplings to obtain as many individuals as possible were conducted at several sites that were chosen to maximize the geological and ecological coverage of the area (Fig. 1) since the distribution and abundance of *Eurema* species is almost unpredictable and irregular. Sampling was done around the forest reserves and recreational forests since these types of forests are highly undisturbed. The vegetations here are mostly abundant and provide many host-plants for butterflies as well as flowering plants. Sampling also was conducted along the periphery of the forests since the studied butterflies can be found abundantly in opened areas especially those with direct exposure to sunlight, such as along the roadsides, river banks and bushes.

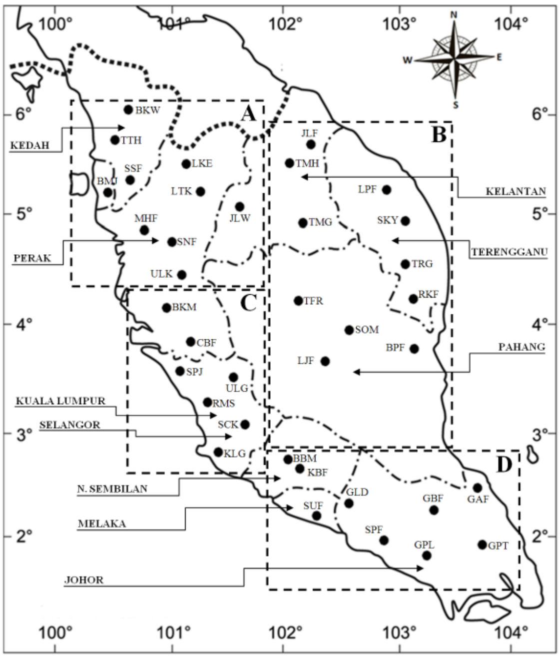

Fig. 1. The geographical sites where the samplings have been conducted in Peninsular Malaysia. **A** - Northern area; **B** - Eastern area; **C** - Western area; **D** - Southern area. Black dots indicate the distribution of various sampling sites in this study. Triple letters represent the site code. The details of site code, sampling location name and GPS coordination are given in Table 1.

Besides that, the occurrence of the studied butterflies at particular sites also was determined by looking on the presence of their eggs and pupas that inhabit the natural host-plants. Collection of butterflies in recreational forests were done only during the weekdays when number of visitors are low and disturbance of the forest environment is minimal, thus ensuring efficient collection of samples.

Sample collection and storage

Sampling is preferably refrained when weather is rainy or relatively cloudy as the butterflies are only active during hot sunny days. The time for sampling ranged dawn until just before dusk because butterflies are only active at day time, although some species can be collected at any hour. Opportunistic sampling strategy was employed to capture as many available butterflies that were sighted, without any quantitative method of sampling. Method of butterfly collection is based on Orr (2003). Butterflies were caught by using insect sweep net throughout the sampling sites. Caught butterfly was grasped by its body and stunned by pinching the thorax before it can be removed from the net.

Then, the sample was placed temporarily in the insect envelope by folding the wings together above the body to avoid damaging their fragile scaly wings. The antennae were laid parallel along the diagonal fold of the envelope so that it is less likely to break when the sample is later removed from the envelope. All the information of collected sample such as locality, date, time and collector's name were written on the opened flap of the envelope for recording purposes.

After that, collected sample needs to be preserved before it can be stored permanently. The best way to preserve butterfly is by pinning. Pinning will retain the normal appearance of butterfly especially for the use in morphological study. The forewings were spread forward until the dorsum of inner margin is perpendicular to the thorax part and were held in place by tightening with paper strips and pins. The hindwings were spread backward until the position is in right angle normally about 45° towards the abdomen and also fixed with paper strips and pins. The antenna was arranged in position parallel to the costa of forewing and held by cross-pin. The abdomen also was crossed-pin to hold it in a central horizontal position.

Table 1. Sampling location area in Peninsular Malaysia (Northern, Western, Eastern and Southern) with details of sampling site name, site code and GPS coordination.

Area	Site Code	Sampling site name	GPS Coordination
Northern	LKE	Tanjung Kala Recreational Forest, Grik, Perak	N 5°61', E 101°08'
	LTK	Lata Kekabu Recreational Forest, Lenggong, Perak	N 5°34', E 100°24'
	MHF	Maxwell Hill Forest Reserve, Taiping, Perak	-
	SNE	Sungai Nyior Recreational Forest, Taiping, Perak	N 5°35', E 100°72'
	SSF	Sungai Sedim Recreational Forest, Kulim, Kedah	N 5°97', E 100°43'
	TTH	Titi Hayun Recreational Forest, Gurun, Kedah	N 5°48', E 100°05'
	BKW	Bukit Wang Recreational Forest, Jitra, Kedah	-
	BMJ	Bukit Mertajam Recreational Forest, Pulau Pinang	N 4°67', E 100°.04'
	ULK	Ulu Kinta Forest Reserve, Ipoh, Perak	N 5°23', E 101°.88'
	JLW	Jelawat Recreational Forest, Jeli, Kelantan	-
Western	ULG	PPLUM, Ulu Gombak, Gombak, Selangor	N 3°17', E 101°46'
	RMS	Raja Musa Forest Reserve, Kuala Selangor, Selangor	N 3°24', E 101°20'
	CBF	Changkat Baharu Forest Reserve, Tanjung Malim, Perak	-
	KLG	Bukit Cherakah Forest Reserve, Shah Alam, Selangor	N 3°06', E 101°30'
	BKM	Bikam Forest Reserve, Sungkai, Perak	N 3°58', E 101°14'
	SPJ	Sungai Panjang Forest Reserve, Sabak Bernam, Selangor	-
	SCK	Sungai Congkak Forest, Hulu Langat, Selangor	-
Eastern	TMH	Lata Hujan Forest Reserve, Tanah Merah, Kelantan	-
	TRG	Bumbung Raja Forest Reserve, Dungun, Terengganu	-
	LPF	Lata Payung Forest Reserve, Setiu, Terengganu	-
	JLF	Jeram Linang Forest Reserve, Pasir Puteh, Kelantan	-
	TMG	Temangan Forest Reserve, Kuala Krai, Kelantan	-
	RKF	Rasau Kerteh Forest Reserve, Kemaman, Terengganu	N 4°34', E 103°17'
	BPF	Bukit Pelindung Recreational Forest, Kuantan, Pahang	-
	SOM	Som Forest Reserve, Jerantut, Pahang	N 3°59', E 102°16'
	TFR	Terenggun Forest Reserve, Kuala Lipis, Pahang	N 4°10', E 101°59'
	LJF	Lata Jarum Recreational Forest, Raub, Pahang	N 3°56', E 102°01'
	SKY	Sekayu Forest Reserve, Marang, Terengganu	-
Southern	BBM	Berembun Forest Reserve, Negeri Sembilan	-
	KBF	Kenaboi Forest Reserve, Jelebu, Negeri Sembilan	N 3°07', E 102°03'
	SUF	Sungai Udang Recreational Forest, Ayer Keroh, Melaka	N 2°18', E 102°08'
	SPF	Soga Perdana Recreational Forest, Batu Pahat, Johor	N 1°49', E 102°58'
	GLD	Gunung Ledang Forest Reserve, Muar, Johor	N 2°22', E 102°38'
	GAF	Gunung Arong Forest Reserve, Mersing, Johor	N 2°34', E 103°46'
	GBF	Gunung Belumut Forest Reserve, Kluang, Johor	N 2°02', E 103°34'
	GPL	Gunung Pulai Forest Reserve, Kulai, Johor	N 1°37', E 103°33'
	GPT	Gunung Panti Forest Reserve, Kota Tinggi, Johor	N 1°49', E 103°51'

The pinned butterfly then will be dried up to remove the excess water body content. When drying was completed, sample can be removed from the setting board with great care as the sample is now more brittle and easily to damage. The cross-pins were removed from the setting board first followed by the pins holding the paper strips. The sample then was transferred into the insect storage box to be stored as permanent voucher specimens. A label must have proper information consists of species name, date and site of collection and collector's name. All the voucher specimens later were stored in the Museum of Zoology, University of Malaya.

Species identification and diagnosis

The diagnosis of morphological characteristics for species characterization was done along with the species identification. The diagnosis of morphological characteristics involved in the characterization of the species was related to the characteristics used in the taxonomic keys derived by Colbert and Pendlebury (1992). All the characteristics were mostly the observable structure and pattern on the upperside and underside of both forewing and hindwing (Fig. 2).

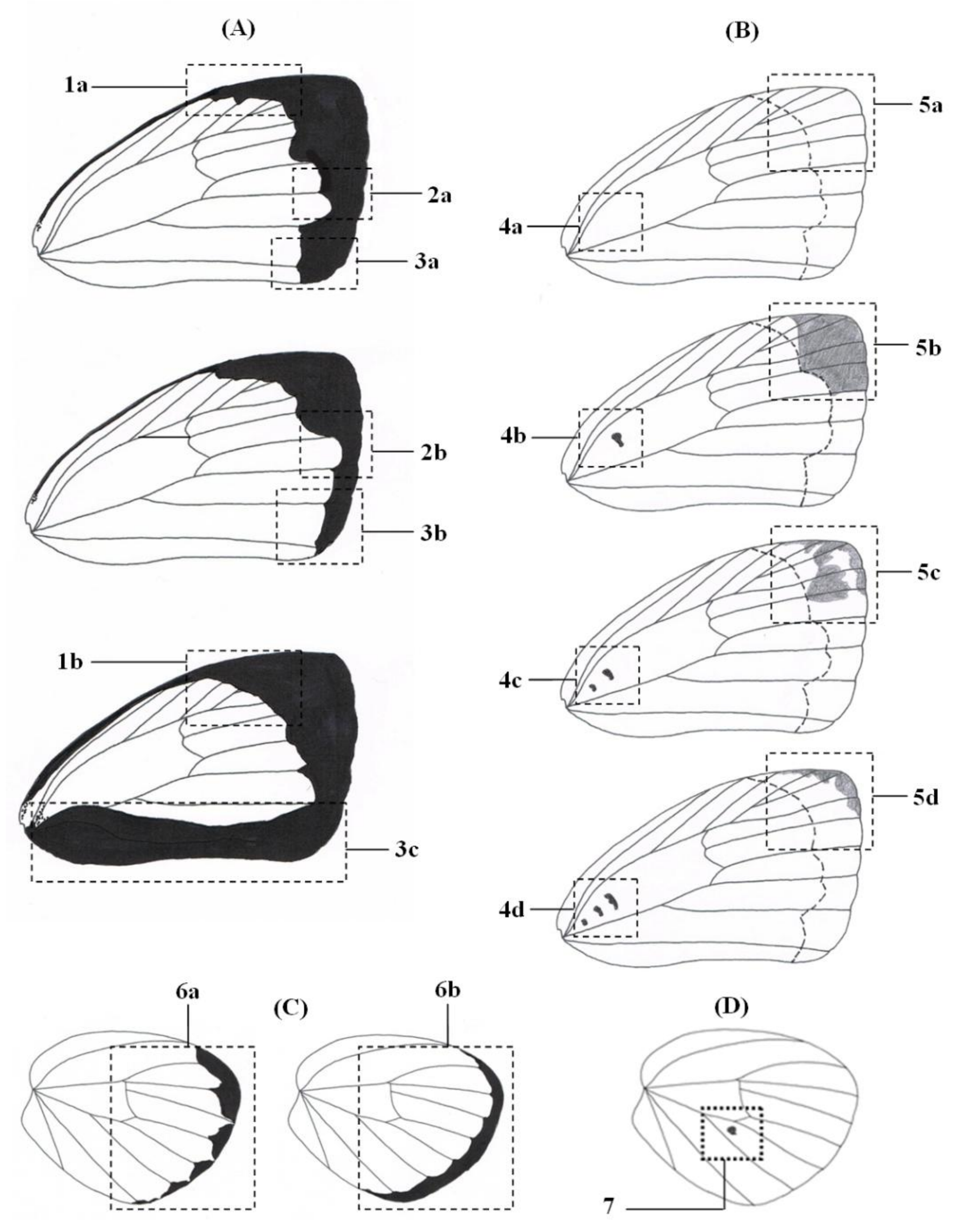

Fig. 2. Morphological characteristics used in species identification and characterization. **(A)** - Forewing upperside; **(B)** - Forewing underside; **(C)** - Hindwing upperside; **(D)** - Hindwing underside. **1**- Pattern of inner edge of apical border: **(1a)** excavated, **(1b)** uniform; **2**- Space of inner edge of apical border deeply excavated: **(2a)** second space, **(2b)** third space; **3**- Pattern of basal part of black apical border: **(3a)** broad, **(3b)** reduced, **(3c)** broaden and continued along basal line; **4**- Number of cell spot in discoidal cell: **(4a)** none, **(4b)** one, **(4c)** two, **(4d)** three; **5**- Pattern of brown marking patch: **(5a)** totally disappeared, **(5b)** Large dark brown, **(5c)** small brown patch, **(5d)** small brown patch along the tip of the wing; **6**- Pattern of black distal border: **(6a)** reduced to spot-like vein dots, **(6b)** continuous form a streak. **7** – Presence of basal spot in space seven.

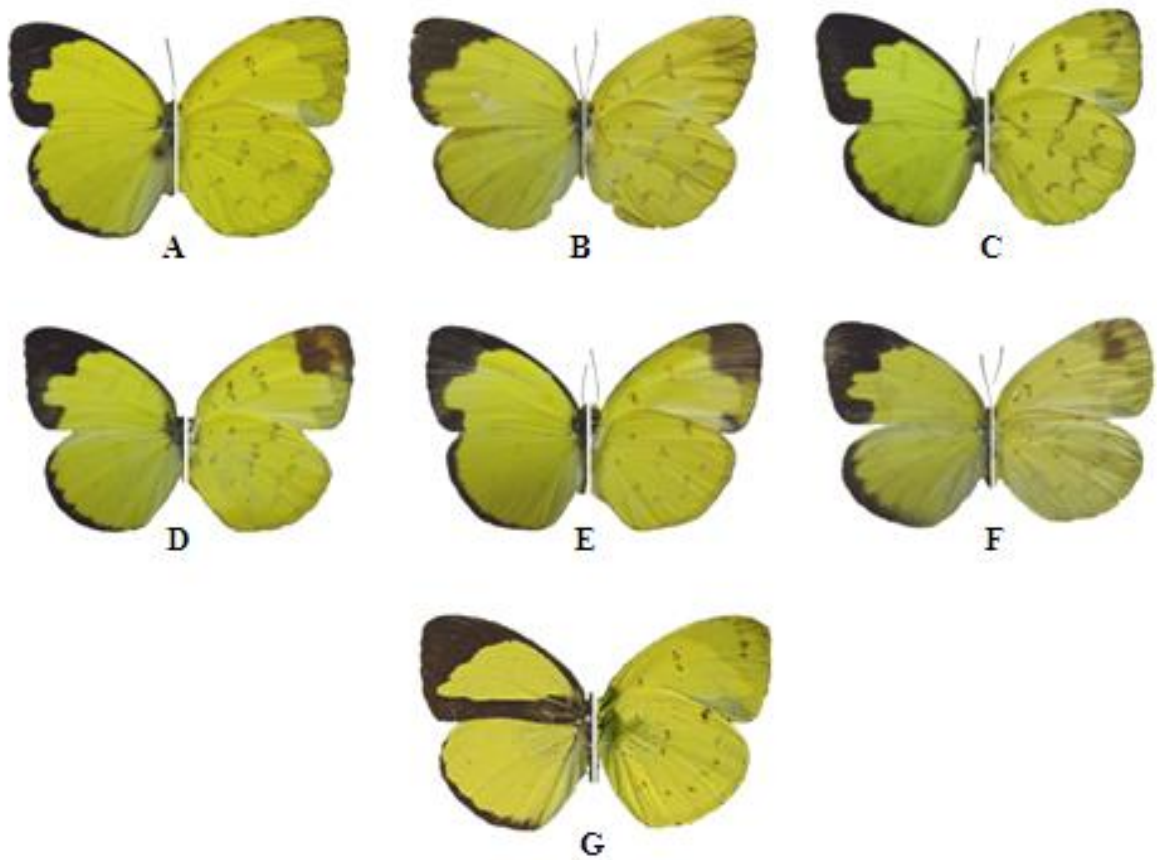

Fig. 3. Adult butterflies of the genus *Eurema* in Malaysia. Fig.s show the upperside of wings (left) and underside of wings (right). **A** – *Eurema hecabe contubernalis*; **B** – *Eurema blanda snelli*; **C** – *Eurema andersonii andersonii*; **D** – *Eurema simulatrix tecmessa;* **E** - *Eurema sari sodalis*; **F** – *Eurema ada iona*; **G** – *Eurema tilaha nicevillei*. Specimens were not scale into the actual size.

On the upperside of the forewing, the diagnosis involves observing the patterns of black apical border, including the space of the inner edge of black apical border excavated the most and also size of black apical border at the basal part. These patterns vary among *Eurema* species which can be reduce, broad or continuous along the basal line of the forewing. On the underside of the forewing, the diagnosis involves the observation on the pattern of brown apical patch and number of cell spots. Some species can easily being identified by the presence of large dark-brown patch and some species only have small and tiny brown patch while in some species, the brown patch is totally absent. The number of cell spots also was examined.

On the upperside of the hindwing, diagnosis was focused on the pattern of black distal border which can be serrated or dotted in shape while on the underside of the hindwing, the diagnosis was done by observing on the presence and intensity of the basal spot located on the space seven. Except for the damaged samples, the identification and diagnosis were carried up on the left side of both forewing and hindwing of each sample.

Results

From the study, the two most important morphological characteristics initially used for the species identification of *Eurema* butterflies were the number of cell spot in discoidal cell and the pattern of brown apical patch, both found on the underside of the forewing. The three most easily identified species were *E. blanda, E. sari*, and *E. tilaha*. *E. blanda* is the only species that has three cell spots while *E. sari* is the only species that has a large and entirely darken brown patch. In other hand, *E. tilaha* can easily being identified because of its unique pattern of black apical border at basal part.

For other remaining species, further identification was based on the pattern of black apical border located on the upperside of the forewing. Specimens later were subsequently confirmed to its respective species based on the pattern of black distal margin on the upperside of the hindwing and the presence of basal spot on the underside of the hindwing. Diagnosis of morphological characteristics however reveals that respective species can easily recognizable based on their own distinctive characteristics.

Morphological characterization of Eurema species

(A) *Eurema hecabe contubernalis* (Linnaeus, 1758)

Forewing underside has two cell spots in discoidal cell. Brown apical patch is only present as small tiny patches around the wing edge. Black apical border of forewing upperside in space 1a and 1b is at right angles to dorsum or sloping towards base. Inner edge of black apical border is more deeply excavated in space two than in space three. Basal part of black apical border is broad. Hindwing underside has no basal spot at the base of space seven. Black distal margin of hindwing upperside is broad and usually moderately zig-zag in shaped. Upperside of wings' ground colour is bright yellow and paler on the underside of wings.

(B) *Eurema blanda snelli* (Boisduval, 1836)

Forewing underside has three cell spots in discoidal cell. Brown apical patch is totally absent. Black apical border of forewing upperside is reduced towards the basal part. Inner edge of black apical border is more deeply excavated in space two than in space three. Hindwing underside has faint small basal spot at the base of space seven. Black distal margin of hindwing upperside is diffused together forming a narrow stripe. Upperside of wings' ground colour is bright yellow and paler on the underside of wings.

(C) *Eurema andersonii andersonii* (Moore, 1906)

Forewing underside has one cell spot in discoidal cell similar to those of *E. sari*. Brown apical patch only appears as small and faint patches. Inner edge of black apical border of forewing upperside is more deeply excavated in space three than in space two. Black apical border inclined slightly in space 1a and 1b towards basal part. Hindwing underside has no basal spot at the base of space seven. Black distal margin of hindwing upperside is broad and zig-zag in shape. Upperside of wings' ground colour is bright yellow and paler on the underside of wings.

(D) *Eurema simulatrix tecmessa* (Staudinger, 1891)

Forewing underside has two cell spots in discoidal cell similar to those of *E. hecabe* and *E. ada*. Has a large cleft dark brown apical patch but not entirely darkened. Black apical border of forewing upperside at basal part is broad but slightly inclined. Inner edge of black apical border is more deeply excavated in space two than in space three. Black apical border inner margin in space 1a and 1b diffused towards basal part. Has a well defined basal spot at the base of space seven of hindwing underside that differentiates it from the other species. Black distal margin of hindwing upperside is in modulated zig-zag shaped. Upperside of wing's ground colour is bright yellow and paler on the underside of wings.

(E) *Eurema sari sodalist* (Horsfield, 1829)

Forewing underside has one cell spot in discoidal cell. Brown apical patch is very large, quadrate form and entirely darken with reddish brown in coloured that differentiate it from the other species of *Eurema*. Black apical border of forewing upperside is broad towards basal part and its inner edge is more deeply excavated in space two than in space three. Hindwing underside has no basal spot at the base of space seven. Black distal margin of hindwing underside is broad with its margin diffused together forming a stripe. Upperside of wing's ground colour is bright yellow and paler on the underside of wings.

(F) *Eurema ada iona* (Distant and Pryer, 1887)
Forewing underside has two cell spots in discoidal cell. Brown apical patch is faint and almost unmarked. Black apical border of forewing upperside is almost equally excavated in second and third spaces but seen to be deeper in third space. Hindwing underside has no basal spot at the base of space seven. Black distal margin of hindwing upperside is broad as compared to other species and in modulated zig-zag shaped. Upperside of wing's pale greenish yellow in colour.

(G) *Eurema tilaha nicevelli* (Horsfield, 1829)
Forewing underside has one cell spot in discoidal cell. Brown apical patch only appears as small and faint patches. Black apical border of forewing upperside at basal part is broad and continuous along the basal line. Hindwing underside has no basal spot at the base of space seven. Black distal margin of hindwing upperside is broad and slightly broadened towards the tornus. Upperside of wing's ground colour is bright lemon-yellow and paler on the underside of wings.

Discussion

Morphological characterization

From the study, the number of cell spots in discoidal cell located on the forewing underside is the most important morphological characteristic in the identification of *Eurema* species. This characteristic was widely used in the identification of *Eurema* and other butterfly species (e.g.: Yata, 1989; Colbert and Pendlebury, 1992; Jeratthitikul *et al.*, 2002). In butterflies, the cell spots occur in exactly the same location in all individuals of a particular species. More importantly, a spot can be traced from species to species within a genus and often from genus to genus within a family. The elements that constitute the wing pattern of butterflies are an anatomical system that is as organized and diverse as the vertebrate skeleton and the body segmentation and tagmatization of arthropods (Nijhout, 2001).

The presence of cell spots on the underside of the forewing within genus *Eurema* was fixed for all species and therefore regarded as the best morphological characteristic for species identification. However, in the genus *Eurema*, the number of cell spots was not species specific. Several species share the same numbers of cell spots and therefore, additional morphological characteristics were needed to identify and classify the species. Diagnosis of morphological characteristics shows that all species except *E. blanda* and *E. tilaha* were morphologically similar in terms of the pattern of black apical border of forewing upperside. Besides that, the wing colouration also resembled each other which may lead to species misidentification. However, the present study found that each species examined have their own unique characteristics that makes them easily to be recognized as respective species.

The diagnosis found that *E. sari* is easily identified from the pattern of brown apical patch located on the underside of the forewing which is larger and entirely darker than other *Eurema* species. Although *E. simulatrix* appears similar to *E. andersonii* in terms of the wing colouration and pattern of black apical border of forewing upperside, observations of the underside of the hindwing under the dissecting microscope revealed that *E. simulatrix* have a well defined black spot located at the base of space seven which was absent in other species.

In contrast, *E. andersonii* is easily distinguished by the pattern of black apical border on of forewing upperside in which the space of inner edge is deeply excavated in space three rather than in space two as found in other species. Moreover, quick identification of *E. blanda* can be accomplished based on two unique morphological characteristics; only this species has three cell spots in discoidal cell located on the underside of the forewing and also the reduced pattern of black apical border towards basal part of the forewing upperside.

Apart from wing pattern, the shape of the forewing can also be employed to identify *E. ada,* as it is the only species reported to have an apical rounded forewing. Furthermore *E. ada* was is the smallest Malayan *Eurema* species (Colbert and Pendlebury, 1992). Despite being reported as the most variable species, all specimens of *E. hecabe* in the present study consistently have two cell spots in discoidal cell and the black apical border of forewing upperside is more excavated in space 1a and 1b. Moreover, *E. tilaha* exhibits unique pattern of black apical border which is broad and continuous along the basal line from apical wing towards the articulation part on the upperside of forewing that differs from the normal pattern possessed by other species.

Furthermore, in this revision study, the identification of the species by using the morphological characteristics as described was well supported by the molecular data. We also have done the preliminary study on phylogeny of *Eurema* species in Malaysia by using mitochondrial DNA *COI* and ribosomal DNA *28S* molecular data to clarify the relationship status among the species. From the data, each species that was identified morphologically has forming the monophyletic group that represents their respective species. This suggested that, the use of the described morphological characteristics for identification of *Eurema* species are relevant and can be made confidently.

Variation within species

In this study, the only species among the genus *Eurema* to have variable wing pattern was species of *E. hecabe* which was also noted by previous study (Narita *et al.,* 2007). This species is also of interest to the systematic of *Eurema* because it features many unusual characteristics compared with other *Eurema* species. The species was reported to exhibit the morphological variation such as the pattern of black apical border, wing marking pattern on the underside of forewing as well as seasonal and geographic variations. Because of these variations, numerous subspecies have been described by previous researchers. Examples are *E. hecabe mandarina* from Japanese mainland (Narita *et al.,* 2007) and *E. hecabe hobsoni* from Taiwan (Yata, 1995). However, close inspection by Yata (1989) revealed that almost of all these variations are clinal ones according to latitude. Based on this finding, he proposed an integration of numerous subspecies into a single subspecies named as *E. hecabe hecabe*.

The variation that occurs in species of *E. hecabe* in this study is the structure of the black apical border located on upperside of the forewing. The study found that the variation pattern was similar to that species of *E. blanda* in which their pattern of black apical border had reduced towards the basal part instead of having the broad pattern as in the most members of *Eurema*. Yata (1989) found that in species of *E. hecabe*, the black apical border of forewing upperside and the brown patch marking on the underside of the forewing also show a continuous variation with the altitude. These factors probably support the existence of variation that found in species *E. hecabe* because the variant species that were recorded in this study have been collected in the Maxwell Hill Forest which has the elevation up to ~1200m. The high level altitude maybe has caused the species of *E. hecabe* to have variation in black apical border of forewing upperside in order to adapt with the ambient temperature or natural selection.

Other than that, the species members of the genus *Eurema* were reported to exhibit seasonal polyphenism (Gullan and Cranston, 2005) in which the structure of black apical border on the upperside of forewing shows the different pattern towards the seasonal changes. However, from the study, the other species of *Eurema* besides species of *E. hecabe* do not show any variation black apical border pattern. Malaysia is located on equatorial part which is being hot and humid throughout the year. Thus, the pattern of black apical border in *Eurema* species in Malaysia is not affected by the seasonal changes and can be said to be consistent through the seasons.

Moreover, most of the members of the genus *Eurema* show phenotypic variations in both body size and colour patterns (Braby, 2000; Jones, 1992; Pinratana, 1983; Yata, 1989), as is often found in other pierid butterflies. In this study, the variation on wing colour pattern can be seen on the species of *E. ada*. The ground colour of the upperside of the wings of several individuals varies from deep yellow to milky white. Variations in behavior and habitat selection are the reasons for butterflies that vary in thermally relevant morphological characteristics such as wing colouration (Van Dyck *et al.*, 1997). Body size variation occurs on almost all of the studied species. These variations may be caused by changes in developmental temperature or photoperiod during the juvenile stages, and also by other geographical related factors that presumably include clinal genetic differences (Jones, 1992; Yata, 1989).

Insect assemblages are thought to be structured by competition, with most of the insects found in medium-sized classes. Thus, the size of a particular insect is governed by its living habits and its feeding guild, in which competition with similar insects has forced some to evolve a larger or smaller body size. In addition, in insects there are examples where body size within a taxonomic group increases with altitude. These patterns often have a genetic basis, but there is a very common pattern also where insects grow phenotypically larger when reared in colder conditions. This pattern is suggested to have some effect on an individual butterfly's ability to maintain the body temperature (Heinrich, 1986; Gilcristh, 1990). The body size of an insect individual can also be determined by its genes and by the environment in which it grows. Temperature, crowding, food quantity, and food quality are examples of environmental factors that affect the insect's size specifically butterflies. Other than that, body size also can be affected by the seasonal variation or cause by the dependent environmental factor such as the availability of host plants.

Table 2. Summary of morphological characteristics of seven *Eurema* species derived from morphological diagnosis study.

Species	Forewing												Hindwing					
	Upperside					Underside							Upperside			Underside		
	Pattern of basal part of apical black border. **Br**= Broad; **Rd**= Reduced; **Cn**= Continuous			**Space of inner egde black border deeply excavated.** **2**= 2nd space; **3**= 3rd space		**Number of cell spots in discoidal cell**			**Pattern of brown apical patch.** **Am**= Almost absence; **Sm**= Small/few/faint; **Lg**= Large but not entirely darken; **Dk**= Large and entirely darken;				**Pattern of black distal margin.** **Bd**= Broad; **Nr**= Narrow; **Zz**= Zigzag form			**Basal spot at the base of space seven.** **Wd**= Well defined; **Ft**= Small and faint; **Ab**= Absence		
	Br	**Rd**	**Cn**	**2**	**3**	**1**	**2**	**3**	**Am**	**Sm**	**Lg**	**Dk**	**Bd**	**Nr**	**Zz**	**Wd**	**Ft**	**Ab**
E. hecabe (n=39)	•			•			•			•			•		•			•
E. ada (n=32)	•			•			•			•			•		•			•
E. sari (n=39)	•			•		•						•		•				•
E. simulatrix (n=37)	•			•			•				•				•	•		
E. andersonii (n=56)	•				•	•				•			•		•			•
E. blanda (n=58)		•		•				•	•					•			•	
E. tilaha (n=2)			•	•			•		•				•					•

Table 3. Number of individuals and species used in the study according to the sampling areas in Peninsular Malaysia. **N** - Northern area; **W** - Western area; **E** - Eastern area; **S** - Southern area.

Species	Number of individual				TOTAL INDIVIDUAL
	N	W	E	S	
E. blanda	42	6	4	6	58
E. hecabe	3	9	12	15	39
E. simulatrix	1	5	16	15	37
E. andersonii	15	11	10	20	56
E. sari	16	10	11	2	39
E. ada	4	5	11	12	32
TOTAL	81	46	64	70	261

* 2 samples of *E. tilaha* were taken from the Museum of Zoology, University of Malaya and were excluded in this part of study.

Distribution pattern

From this study, the highest numbers of individual collected from the sampling sites was species of *E. blanda* which contribute 22.2% (58 individuals) from the total individuals of the studied specimens (Table 2) while the lowest numbers of individual collected was species of *E. ada* with only 32 individuals (12.3%). Generally, the distribution pattern of butterflies of the genus *Eurema* in Peninsular Malaysia were divided into four areas which are North, East, West and South (Fig. 1).

Northern area shows the highest numbers of individual with 75 individuals (29%) collected from this area. In contrast, Western area shows the lowest numbers of individual with only 48 individuals (18%) collected from this area. The relationship between butterflies and their distribution involving all four stages of the life cycle and their food habit which indirectly govern their abundance (Hussain *et al.* 2011). Many reports show that availability of food plant and larval host plants play a major role in distribution and abundance pattern (Southwood, 1975). The distribution and abundance also were noted to be high when there was an increase in vegetation and floral density.

Therefore, the pattern of high numbers of individuals collected from Northern area can be related with several geographical factors. Northern area consist part of Kedah, Pulau Pinang and Perak. These states are known to possess many recreational forests where the butterflies are mostly like to inhabit this kind of habitat due to presence of flowering plant as well as host-plants. Apart from depending mainly on the host-plants for foods and egg-laying, butterflies also will be attracted to the flowering plants for nectaring behaviour. Like most other butterflies, *Eurema* species are nectar-feeders as adults. They will feed from wide range of flowers and tends to prefer different flowers species (Kitching, 1998). Butterflies are abundant when the flower density is high as they could maximize the net rate of energy intake per unit time (Choudhary *et al.*, 2002).

Besides that, recreational forest provides more open spaces as compared to deep forest. Pierid or small size butterflies were known to have basking behaviour in which they will search the area with direct sunlight or sometimes seen to fluttering around the lower plants that expose to sunlight. Hence a direct correlation was observed for the abundance of the butterflies with floral density and intensity of light and larval host plant. The availability of butterflies in open area also gave high chance for capturing them easily rather than in deep forest where trees can restrict the usage of sweep net.

The Southern and Eastern areas however also show high numbers of collected individuals respectively because these areas also possessed high numbers of undisturbed forests which provide many natural habitats for the butterflies. But, since the samplings in these areas were mostly conducted at forest reserve rather than recreational forest, this suggests why the numbers of collected individuals still low as compared to Northern area.

In contrast, Western area has the lowest numbers of collected individuals because this area consisting of developed states such as Selangor, Kuala Lumpur and Melaka. In developed areas, the presence of natural habitats is very limited and was reduced by the developing activities. Most of the natural forests were surrounded by the urban areas such as cities or residences causing the habitat fragmentation. Habitat fragmentation leads to a loss of habitat, a reduction of patch size and an increase of patch isolation (Andrèn, 1994; Fahrig, 2003). Thus, the chance to get high number of studied species is restricted due to the lost of host-plants of particular species.

Moreover, less number of individuals collected from several sampling sites, for example around Pahang and Kelantan particularly because of the raining season during the sampling period. The occurrence of butterfly in particular area mainly depends on the environmental condition of that area such as weather, climate and temperature. According to Orr (2003), sampling is not preferred to relatively cloudy and rainy weather as the butterflies only active during the hot sunny days. As its natural behaviour, butterfly will avoid the wet condition as their fragile scaly wing might damage when exposed to the water. Instead, they will find the area with direct expose to the sunlight for basking activity. From the distribution map (Fig. 4), all six species were distributed at all sampling areas and can be found at most part throughout the Peninsular Malaysia. In term of species abundance, North area was dominated mainly by the species of *E. blanda* with number of individuals is 42 (51.9%) (Table 2). The less species collected in this area was species of *E. simulatrix*. Moreover, both South and West areas were dominated by the species of *E. andersonii* with percentage of 28.8 % and 23.9 % respectively. In contrast, East area was mostly dominated by the species of *E. simulatrix* (25%).

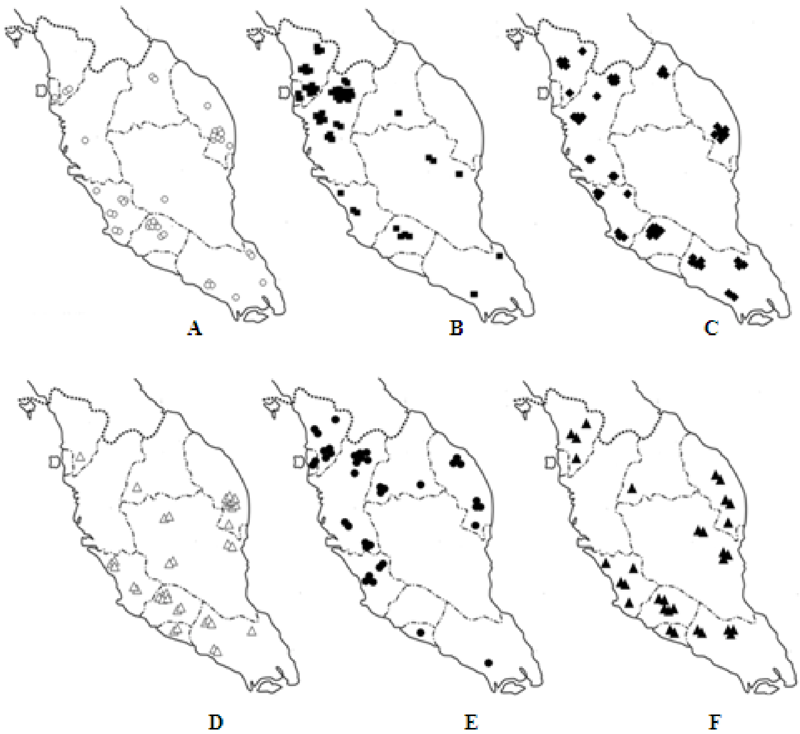

Fig. 4. Distribution map of the genus *Eurema* in Peninsular Malaysia: **A** - *Eurema hecabe* (○); **B** - *Eurema blanda* (■); **C** - *Eurema andersonii* (♦); **D** - *Eurema simulatrix* (Δ); **E** - *Eurema sari* (●); **F** - *Eurema ada* (▲).

In general, the distribution of all six species of the genus *Eurema* in Peninsular Malaysia is suggested having a uniform pattern. The chance of getting all of six species as described in this study is high even though sampling was focused on only one area. In addition, the total abundance and composition of species demonstrated the variations in species number at different study areas. However, the distribution of several species in four sampling areas was not consistent particularly for species of *E. blanda* in which most of the individuals collected were recorded to inhabit mainly the North area. *E. andersonii* in other hands shows the most consistent distribution pattern throughout all four sampling areas. This species only shows a few differences in their number of individuals collected between the different areas. From the study, species of *E. blanda* was suggested as the most common species of the genus *Eurema* in Malaysia due to highest number of individuals collected while species of *E. andersonii* has the most consistent distribution pattern.

Although, six species of the genus *Eurema* were found during this study, but some reported species from the previous study by Colbert and Pendlebury (1992) were not found. The species are species of *E. lacteola*, *E. brigitta* and *E. tilaha*. All these three species were considered as rare or least commonly encountered species by this study because of their difficulty to capture during field sampling. Common species have more individuals compared to the rare species due to their ability to survive in the existing environmental conditions (Shelton and Edward, 1983) and their ability to tolerate with the wide range of habitats (Hodgkin and Watson, 1958; Suhling *et al.,* 2004).

Conclusion

In conclusion, the identification of *Eurema* species based on morphological characteristics can be made confidently. Each species possesses its own unique characteristics that can aid in their identification, whereby *E. tilaha* was the easiest species to be recognized. Variation that occurs in *E. hecabe* appeared to correspond with the altitudinal changes by adaptation to the surrounding environment. The distribution of *Eurema* butterflies in Peninsular Malaysia was said to have uniform pattern with the most common species was species of *E. hecabe*.

Acknowledgement

A lot of appreciation to my supervisors, Prof. Dato' Dr. Mohd Sofian Azirun and Dr. Mohammed Rizman Idid, staffs of Institute of Biological Sciences (ISB), University of Malaya, my friends, parents and my families for the helps, supports and ideas contribution. Do not forget to the research fund, IPPP Grant (PV085-2011A) University of Malaya, Kuala Lumpur, Malaysia.

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