# MORPHOMETRIC CHARACTERISATION OF THE GENUS *Mycalesis* BUTTERFLIES IN SRI LANKA

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## INTRODUCTION

Butterflies of the subtribe Mycalesina (family Nymphalidae, subfamily Satyrinae) are restricted to Africa, Indo-Australia and certain parts of temperate Asia (Kodandaramaiah *et al.*, 2010). The genus *Mycalesis* consisting of over 100 species is spread throughout the Australasian region (Kodandaramaiah *et al.*, 2010). The five species found in Sri Lanka are *M. rama, M. subdita, M. perseus, M. mineus*, and *M. patnia* of which the former two are endemic with *M. rama* categorized as endangered (Van der Poorten, 2012). Members of this subtribe inhabit forest edges, grasslands and savannahs preferring the early morning or late evening low light conditions to venture out into the open. As non-migratory species, these butterflies display polyphenism as a mechanism to survive the wet and dry seasonal changes in their habitats (Braby, 1994). In some species, polyphenism is accompanied with behavioral, reproductive and habitat changes. In Sri Lanka polyphenism has resulted in dry and wet seasonal forms as well as geographically variable morphological forms in the different climatic zones of the country (Ormiston, 1924). This makes identification of *Mycalesis* species somewhat difficult.

As the genus has diverse phenotypes that vary geographically and seasonally within species, this group shows strong potential for research that investigates scientific issues such as the general processes driving adaptation and evolution and impacts of climate change. In Sri Lanka, studies on seasonal polymorphism in butterflies have not received much attention. Though past literature has described the seasonal and geographical variation in morphology of *Mycalesis* species found in Sri Lanka, primarily based on wing coloration, size of dorsal forewing ocelli, width of the discal band and size of ocelli on the ventral surface, the information available appears to be incomplete and inconsistent between authors (Bingham 1905; Talbot, 1947, Woodhouse, 1950).

The objectives of this study, therefore, were to carry out a comprehensive morphometric analysis to characterize the *Mycalesis* species present in Sri Lanka and to determine the important morphological parameters that could be used in the identification of seasonal forms. This study was undertaken as part of an overall study which is investigating the evidence for local adaptation, if any, by focusing on the intra- and inter-specific genetic variation of Sri Lankan *Mycalesis* butterflies.

## METHODOLOGY

**Sampling:** Butterflies of the genus *Mycalesis* were sampled from 7 districts representing the three climatic zones in the island during 2012 and 2013. Site selection was based on previous sightings of the *Mycalesis* species. Adult butterfly specimens were captured from each location using nets and baited traps. GPS data were recorded from each location. Specimens were identified with standard taxonomic keys prior to preserving as voucher specimens. Genitalia were dissected and forelegs were cleaned of hair and scales before observation under a stereo microscope.

**Morphometric measurements:** 36 morphometric characters were measured for each butterfly to obtain a complete representation of the wing elements, genitalia and forelegs

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of the *Mycalesis* specimens. Fourteen wing characters were measured using a vernier caliper (0.05mm accuracy). Six genital and five foreleg characters were measured with a calibrated eyepiece micrometer (0.03mm accuracy). The colour of the proximal half of the discal cell in the dorsal forewing was measured from a 5x5 pixel area with ColorPic software (ICONICO). An ordinal scale was created to represent the range of colours. Each wing, genital and foreleg character of the butterfly was represented as an average relative size of the wing length from base to apex, total clasper length of genitalia and the total length of foreleg, respectively.

Of the 36 characters in the matrix, eight were initially selected for the morphometric analysis of variation between and within species. The characters selected for quantitative analysis included the diameter of dorsal forewing ocellus (DFWoc), forewing length from base to apex (BA), width of ventral forewing discal band (VFWwDis), colour of ventral forewing (VFWcol), diameter of ventral forewing 5$^{th}$ ocellus (VHW5oc), length of clasper (Lcl), length of dorsal process of clasper (LDcl) and length of trochanter of foreleg (LTr).

## RESULTS

A total of 80 specimens of four *Mycalesis* species (except *M. rama*) collected from the island were analysed for the selected eight parameters as shown in Table 1.

Table 1. Descriptive statistics of wing, genital and foreleg parameters of *Mycalesis* in Sri Lanka.

| Parameter | Average relative size (mm) ± SD (range) | | | |
|---|---|---|---|---|
| | *M. perseus* | *M. mineus* | *M. subdita* | *M. patnia* |
| DFWoc | 0.03 ± 0.03 (0.00 - 0.08) | 0.16±0.03 (0.10-0.21) | 0.16±0.02 (0.12-0.19) | 0.24±0.02 (0.21-0.27) |
| BA | 19.75±2.25 (13.15 -24.70) | 21.84±1.42 (19.20-23.75) | 21.43±0.97 (19.75-22.60) | 20.20±1.06 (18.05-23.05) |
| VFWwDis | 0.03 ± 0.01 0.01 - 0.06 | 0.05±0.01 0.03-0.07 | 0.04±0.01 (0.01-0.05) | 0.06±0.01 (0.04-0.08) |
| VHW5oc | 0.10±0.02 (0.04-0.14) | 0.12±0.01 (0.08-0.15) | 0.10±0.03 (0.05-0.12) | 0.07±0.01 (0.05-0.10) |
| Lcl | 0.58 ± 0.10 (0.33-0.72) | 0.45±0.18 (0.26-0.75) | 0.53±0.10 (0.41-0.65) | 0.90±0.01 (0.88-0.93) |
| LDcl | 0.18 ± 0.03 (0.12 - 0.23) | 0.07±0.01 (0.04-0.09) | 0.12±0.02 (0.10-0.14) | 0.10±0.01 (0.07-0.12) |
| LTr | 0.36 ± 0.03 (0.29 - 0.41) | 0.40±0.04 (0.37-0.56) | 0.39±0.05 (0.33-0.45) | 0.38±0.04 (0.31-0.54) |

All characters showed considerable individual variation among the specimens. Based on classification and regression tree (CRT) analysis, the highly variable characters between the species were DFWoc (100%), LDcl (70.3%) and Lcl (68.6%). The least variable of the characters were VHW5oc (2.4%) and BA (8%).

**Wing characters**: Wing length of *M. perseus* (92.86%) and *M. patina* (82.76%) was observed to be smaller (<20.9mm) and *M. mineus* (68.42%) and *M. subdita* (83.33%) were generally larger (>20.9mm). In majority of *M. mineus (*84.21%) and *M. subdita (*83.33%) specimens, the size of DFWoc was found to vary between 0.054-0.18mm. In comparison, 85.71% of *M. perseus* had a smaller diameter (<0.054mm) and *M. patnia* had the largest ocellus diameter (>0.18mm).

There was a gradual variation in VFWcol from very light brown to very dark brown in *M. perseus* individuals. *M. mineus* was also observed to vary from the light to the darkest colour scale. However, a higher proportion of specimens were found to be light brown in shade. The VFWcol of all captured specimens of *M. subdita* was very dark in colour and all *M. patina* butterflies were yellow brown in colour. Within the genus *M. patnia* was

easily distinguished with the presence of an ochreous yellow-orange triangular patch on the dorsal surface of the forewing extending from directly behind the large ocellus at CuA1 to midpoint of the discal cell. The ocellus itself is not symmetrical as in the other *Mycalesis* and consists of a thicker ochreous yellow semicircle.

**Genital characters**: The relative clasper length of most *M. perseus* (60.71%) was between 0.54 – 0.89mm with the remaining specimens having a length of <0.54mm. A higher proportion of *M. patnia* (82.76%) was shown to possess relatively longer claspers of >0.89 mm. Claspers of *M. patnia* are remarkably different from the other *Mycalesis* consisting of a thin, long clasper with a small rounded dorsal process. The distal process is absent and the ventral surface of the clasper has a row of thick hairs running its length to midpoint. The claspers of *M. perseus* genitalia were broader than in the others, with a well rounded dorsal process. This feature could be used as a distinguishing character for this species. The diameter of the clasper was narrowest in *M. mineus* among the four species.

**Foreleg characters**: The size of the foreleg was found to vary only slightly between species. However, the longest trochanters (>0.37mm) were possessed by *M. mineus* (84.21%), *M. subdita* (66.67%) and *M. patnia* (58.62%), indicating a general tendency for these three species to have a longer foreleg. *M. perseus* forelegs were among the smallest, with longer and thicker hair than the other three species.

**Phenotypic variations in wet and dry season forms:** Among the collected specimens there were wet and dry season forms as well as intermediate forms that could not be clearly classified into either a wet or dry category. These seasonal forms were not strictly confined to their respective dry or wet period of the year. Except *M. patnia*, the other three species showed seasonal polyphenism in the size of DFWoc and VFWwDis. These changes were most pronounced in *M. perseus*. Wet season forms were generally observed to possess a large prominent ocellus and a thick discal band. Particularly in *M. perseus*, the dorsal forewing ocellus was absent in some wet season forms. The typical dry season forms were observed to differ from the wet season form in having a smaller ocellus and a thinner discal band. In these dry season morphs, the outer rings of the dorsal forewing ocellus tend to disappear with the white center becoming more prominent.

## DISCUSSION

The preliminary data collected in this study indicate that *M. mineus* and *M. subdita* exhibit closer similarity in the size of forewing, dorsal forewing ocellus, ventral forewing discal band, clasper and foreleg, with majority of specimens in each species falling to the same size class for the respective character. Such morphological similarity confirms the close association emphasized by Bingham (1905) and Ormiston (1924) between *M. mineus* and *M. subdita* within the genus. Such similarity makes the identification of *M. subdita* and *M. mineus* difficult, compelling researchers to rely on other subjective parameters as the purplish tinge of the proximal edge of the ventral wings or the sharp edges of the DFWoc compared to the faded ones of *M. mineus*.

The patterns of seasonal phenotypic variations in ventral hindwing ocellus size and forewing length observed in this study are similar to those reported for tropical *Mycalesis* species studied from other parts of the world (Braby 1994). The larger size of dry season forms and their dull cryptic colourations that resemble the surrounding dry vegetation are believed to be mechanisms to minimize predation, as this is the period during which the female butterflies remain restive in reproductive diapause (Braby, 1994).

In the specimens examined so far in this study, no consistent seasonal changes were observed in the other selected morphological characters for analysis. We also did not observe consistent changes between wet and dry forms of *M. patina*. However, three of the five VHW ocelli appear to express a large white centre with thin black rings in dry season

forms. Preliminary results also indicate that variations in claspers, especially in length and of the distal process, may also be important, apart from the DFWoc and VHWwDis to differentiate between polyphenic morphs of *M. mineus*.

It has been suggested that many factors such as temperature, rainfall, humidity and photoperiod may play a role in determining the wet and dry phenotypes of adult butterflies under field conditions. Sri Lanka, lacking dramatic seasonal changes in temperature, conditions such as rainfall, humidity and altitude are likely to be more important in influencing phenotypic variations in adult butterflies. We intend to perform correlation analyses between climatic and geographical variables with the morphological data, once larger numbers of specimens are collected from each species, to identify which environmental cues govern phenotypic changes and to determine which morphological characters are under environmental control.

## CONCLUSIONS

This paper presents the preliminary morphometric data obtained for *Mycalesis* populations in Sri Lanka with respect to selected wing, genital and foreleg characters. These results demonstrate that there are phenotypic differences of importance concerning especially the relative size of the dorsal forewing ocelli and claspers of adult *Mycalesis* species. Furthermore, there appears to be characteristic phenotypic differences between wet and dry season forms of *Mycalesis*. Further studies are required to strengthen the analysis with larger sample sizes representing more locations covering the different elevations and climatic zones to determine the temporal and spatial phenotypic variations of species of *Mycalesis* populations in Sri Lanka.

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