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Amina Thaj
 Postgraduate, PG and
 Research department of
 Zoology, Sree Narayana
 College, Kollam, Kerala, India

Sheeba S
 Assistant Professor, PG and
 Research Department of
 Zoology, Sree Narayana
 College, Kollam, Kerala, India

Morphological characteristics of plate like setae encasing the wings of tiny grass blue “*Zizula hylax*”

Amina Thaj and Sheeba S

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Abstract

Background: Butterfly wings are covered by tiny, coloured plate like setae called scales. *Zizula hylax* the ‘tiny grass blue’ is a small sized butterfly which belongs to the ‘Lycaenids’ or blues family. It is characterised by the dull violet blue appearance on the dorsal wing area and, they are pale grey with multiple arcs of brown dashes, brown border termen surrounded by numerous cilia on their ventral wing area.

Objective: To investigate the wing scale morphology and length & width of scales using micrometry.

Methods: ‘*Zizula hylax*’ is collected using handheld insect net in which they are pinned into the insect box. The method of Grodnitsky and Kozlov (1991) [5] was followed. The scales were scrapped off from distinct regions of dorsal and ventral wing separately which is taken in different glass slides. The sample is fixed with xylene when evaporates is studied under light microscope. Photographs were taken to record the images which is viewed under the light microscope. Micrometry is used to measure the length and width of the scales.

Results: A total of 151 wing scales were analysed. 62 varieties of scales were studied from the dorsal blue coloured wing area and 89 varieties of scales were studied from the ventral ash coloured wing area. A typical scale consists of an abwing or upper lamina which constitute the upper portion of scale, an adwing or lower lamina which constitute the lower portion of the scale and a stalk. Several transverse lines run on the surface of the scales. Study on dorsal wing scales shows several dull black, pale yellow and dull brown coloured scales with flat and short size. Denudations of the scales are not well defined. The structural scales form the blue colour on the dorsal surface. The blue colour is formed when the light passes through the pale black, brown and yellow scales present in this region. The dimensions of the wing scales ranges between 74.86 μ -94.56 μ in length and 55.16 μ -74.86 μ in thickness. The ventral wing scales shows several long- narrow scales unlike the dorsal side. The ventral side of the ‘*Zizula hylax*’ is ash coloured and 89 scales were identified. All are transparent and glassy type white scales. Some flat white scales have also been seen. Here, the denudations are not yet clearly defined. The transverse lines can be seen clearly. The dimensions of the scale’s ranges between 82.74 μ -177.3 μ in length and 55.16 μ -74.86 μ in thickness.

Conclusion: The present study provides an idea about the different types of scales, their morphology and size mainly the length and width. Scales play an important role in the life of butterfly as it aids in flight, predator defence, thermoregulation, camouflage, mimicry, and coloration. Studies on wing scales possess many bioinspired applications also.

Keywords: Structural scales, light microscope, wing scales, micrometry, transverse lines

Introduction

The wings of butterflies are minutely scaled. These scales are responsible for the distinct colouration exhibited by the wings. The scales which are pigmented with melanins that give them blacks and browns, but blues, greens and reds and iridescence are usually created not by pigments but the microstructure of the scales. The structural coloration is the result of coherent scattering of light by the photonic crystal nature of the scales (Mason, 1927; Vukusic *et al.*, 2000; Prum *et al.*, 2006) [6, 16, 10]. Butterfly wing pattern are becoming the topic of choice for studies in evolution and development (French Constant, 2012) [2]. The colour patterns are due to a tapestry of numerous small scales, each with a distinct colour, which together create the species-characteristic appearance as in pointillist paintings (Nijhout, 1991) [8]. Scales have been classified into three groups namely, hair-like or piliform, blade-like or lamellar and other variable forms (Scoble, 1995) [11]. Three basic types of scales which are the characteristics of a butterfly wing of a species are the pigmentary scales, structural scales and Androconia. Scales play an important part in the natural history of Lepidoptera. Scales enable the development of vivid or indistinct patterns which helps the

Corresponding Author:
Amina Thaj
 Postgraduate, PG and
 Research department of
 Zoology, Sree Narayana
 College, Kollam, Kerala, India

organism to protect itself from predators by mimicry and warning. They also play a major and aid in flight of butterfly. Majority of the scientific works emphasizing majority the wings scales are done internationally. French (1997) [3] studied on the pattern formation in colour on butterfly wings. Nijhout (2001) [8] made an extensive analysis on the elements of butterfly wing patterns. Sekimura *et al.*, (2002) [12] investigated on the pigmentation of pattern formation in the butterfly wing of *Papilio dardanus* by numerical simulations of a reaction – diffusion model on a geometrically accurate wing domain. Stavenga (2004) [13] studied on the wing –scale morphologies of the Pierid butterflies *Pieris rapae* (small white) and *Dalians nigrina* and the *Heliconius melpomene* are compared and related to the wing- reflectance spectra. Prum *et al.*, (2006) [10] investigated on anatomically diverse butterfly scales. Giraldo (2008) [4] studied the pigmentation and structural properties of butterfly wing scales. Stavenga *et al.*, (2009) [15] studied on the imaging stereometry of butterfly wing scales. Zhang *et al.*, (2012) [17] made an extensive analysis on morph genetic materials which is inspired from butterfly wing scales. Stavenga *et al.*, (2014) [14] observed the coloration principles of nymph line butterflies- thin films, melanin, ommochromes and wing scale stacking. Das *et al.*, (2017) [1] has studied on the potential of Biomimicry in the field of Textile Technology. Osotsi *et al.*, (2020) [9] explored the butterfly wing architecture as bioinspired sensor and energy materials by replicating their unique micro/nanostructure light trapping mechanisms and selective responses to external stimuli. Thus, the present study emphasizes on the morphology and dimensions of wing scales in *Zizula hylax*.

Materials and Methods

Specimen Collection: The collection of '*Zizula hylax*' was using handheld insect net which consist of a steel ring (10 inch), a conical nylon bag (26 inch long), and a handle (17 inch long). After the collection, the specimen was pinned into the insect box.

Sample Preparation: The method of Grodnitsky and Kozlov (1991) [5] was followed. Several hundreds of scales were scrapped off from dorsal and ventral wing area of *Zizula hylax* separately into different glass slides. Xylene is used to fix the specimen. After xylene has been evaporated, the samples were studied under the light microscope. The length and width measurement of the scales were measured using micrometry. Photographs of the observed images were recorded.

Butterfly wing scale measurement: Dimensions of butterfly wing scales are measured after microscopic calibration. The number of divisions of stage micrometer that corresponds to a full scale on the ocular micrometer is recorded first. Since the measurement of each division of stage micrometer is known, the ocular micrometer divisions are appropriately converted to micrometers. Once the ocular micrometer is calibrated, dimensions of object measured.

$$\text{Calibration value (C)} = \frac{X}{Y} \times 10$$

Where, X = Number of divisions in the stage micrometer
Y = Number of divisions in the ocular micrometer
Value of one stage micrometer division = 0.01mm or 10 mm.

After microscopic calibration, the stage micrometer is removed and the slide having the prepared wing scales is placed on the stage and focused. Now the number of ocular divisions occupied by a single wing scale is counted. Then by multiplying this number of divisions with the calibration factor, the width and length of the wing scale are obtained.

Results and Discussion

Zizula hylax, the 'Tiny Grass Blue' is a small sized butterfly wandering over shrubs and bushes which belongs to the 'Lycaenids' or blues family. Dorsal forewing and hindwing possess dull violet blue iridescence. Underneath, they are pale grey with multiple arcs of brown dashes with brown bordered termen and is surrounded by numerous cilia.

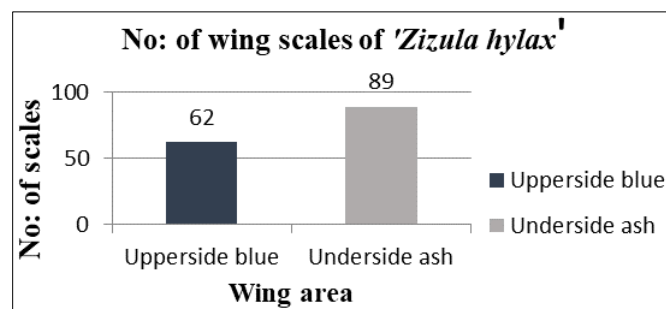


Fig 1: Shows number of wing scales in '*Zizula hylax*' on dorsal (upper side) and ventral (underside) area.

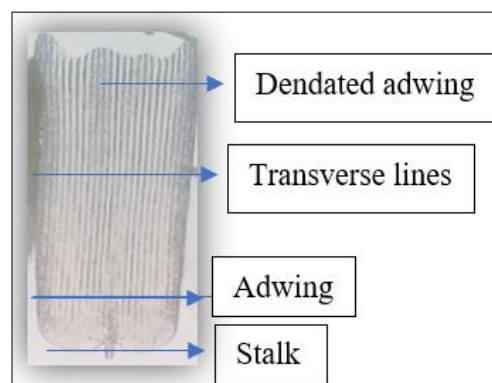


Fig 2: shows the structure of a single scale

Wing scale description

The body of a single scale consist of an awning, adding and stalk (Fig.2). The stalk helps the scales to stick on to the wings. A total of 151 scales were identified. Among them, 62 varieties of scales were from the dorsal blue coloured wing area and 89 varieties of scales were from the ventral ash coloured wing area (Fig.1). Several dull black, pale yellow and dull brown coloured scales constitute the dorsal wing area. Majority of the scales are flat and short sized. Here, the denudations of the scales are not well defined. Here the transverse lines can be clearly seen. The blue colour of the dorsal wing surface is not due to the blue scales instead, they are the colour formed when the light passes through the pale black, brown and yellow scales present in this region. The dimensions of the wing scales ranges between 74.86µ-94.56µ in length and 55.16µ-74.86µ in thickness. The ventral wing scales shows several long-narrow scales unlike the dorsal side. All are transparent and glassy type white scales. A few flat white scales have also been identified. Here, the denudations are not yet clearly defined. The transverse lines can be seen clearly. The





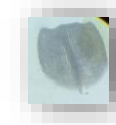






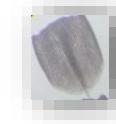














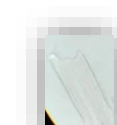
dimensions of the scale's ranges between 82.74 μ -177.3 μ in length and 55.16 μ -74.86 μ in thickness. Heat absorbing property of scales are used in constructing solar panel energy sensors. Osotsi *et al.*, (2020)^[9] explored the butterfly wing architecture as bioinspired sensor and energy materials by replicating their unique micro/nanostructure light trapping mechanisms and selective responses to external stimuli. The colored scales altogether create the specific pattern of wing color that represent a specific species. The coloration property of scales can be often used in the textile industry to develop new designing patterns. Das *et al.*, (2017)^[11] has studied on the potential of Biomimicry in the

field of Textile Technology. Development of more efficient light emitting diode inspired by the structural property of scales is one of the highlights of the present scenario. Structural coloration of the wing scales is an inspiring to nanotechnology research to produce paints that do not use toxic pigments and the development of new display technologies. Zhang *et al.*, (2012)^[17] made an extensive analysis on morph genetic materials which is inspired from butterfly wing scales. Butterfly wing color patterns are extensively used in art cafe's, modeling and even in designing jeweler and ornaments.

High power view (40x)

Out of 151 scales identified some are represented below:

Dorsal Wing Scales

						
1 Length:86.68 μ Width:66.98 μ	2 Length:82.74 μ Width:66.98 μ	3 Length:78.8 μ Width:59.1 μ	4 Length:74.86 μ Width:55.16 μ	5 Length:90.62 μ Width:70.92 μ	6 Length:86.68 μ Width:66.98 μ	7 Length:94.56 μ Width:70.92 μ
						
8 Length:90.62 μ Width:70.92 μ	9 Length:90.62 μ Width:70.92 μ	10 Length:82.74 μ Width:66.98 μ	11 Length:86.68 μ Width:66.98 μ	12 Length :90.62 μ Width:70.92 μ	13 Length:90.62 μ Width:66.98 μ	14 Length:90.62 μ Width:66.98 μ
1. Ventral wing scales						
						
1 Length:90.62 μ Width:66.98 μ	2 Length:98.5 μ Width:55.16 μ	3 Length:94.56 μ Width:70.92 μ	4 Length:94.56 μ Width :63.04 μ	5 Length:90.62 μ Width:66.98 μ	6 Length:94.56 μ Width:63.04 μ	7 Length:94.56 μ Width:63.04 μ
						
8 Length:94.56 μ Width:78.8 μ	9 Length:110.32 μ Width:66.98 μ	10 Length:90.62 μ Width:63.04 μ	11 Length:94.56 μ Width:59.1 μ	12 Length:98.5 μ Width:66.98 μ	13 Length:94.56 μ Width:66.98 μ	14 Length:90.62 μ Width:66.98 μ

Conclusion

The present innovative work provides an idea on the powdery stuff encasing the wings of *Zizula hylax*. Thus, the wing scales contribute a major role in enhancing the beauty of butterfly. Both pigmented scales and structural scales forms the colour patterns of butterfly wings.

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