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RESEARCH ARTICLE

A GANDER THROUGH THE POWDERY STUFF ENSHROUDING THE WINGS OF APE FLY *SPALGIS EPIUS*

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ABSTRACT

Background: *Spalgis epius,* the 'Ape Fly' is a small butterfly that belongs to the lycaenids or blues family. The powdery stuff sheathing their wings are called scales which are the main factor for wing coloration. Ape fly on appearance shows blue color on the dorsal wing area and ventral wing area shows ash color. The wing scales forms vivid or indistinct patterns and helps the organism to protect itself by camouflage, mimicry and warning. Wing scales perform a great role in the natural history of Lepidoptera. **Objective:** To examine the various types of wing scales, their morphology and dimensions (Length and Width) in *Spalgis epius.* **Methods:** The Standard method of Grodnitsky and Kozlov (1991) is used. Several hundreds of scales were scrapped off from the dorsal and ventral wing separately into a glass slide. The sample is fixed using xylene and studied under light microscope. The dimensions are measured with micrometry. **Results:** A wide variety of scales has been observed from dorsal and ventral wing area. A total of 175 scales were studied which include 88 varieties from dorsal side and 87 varieties from ventral side. On analyzing the blue coloured dorsal wing area several colored scales including dull brown, cream, pale black colored scales are identified. Many broad, flat and short sized scales are observed in this area. Here the majority of scales are devoid of dendations. Flat, thin, glassy type scales are also observed. For most of the scales, the abwing and adwing are broad. The length and width of the scales vary. Transverse lines in scales are not clearly seen. The blue color of dorsal wing is not due to blue scales instead it is because of structural colors formed when the light passes through the other colored scales. The dimensions of dorsal wing scales ranges between 82.74µ-110.32µ in length and 55.16µ-74.86µ in width. The ventral wing scales shows several long scales unlike the dorsal side. Several grayish white colored scales are identified. Long narrow scales are the major scales. In the ash colored ventral wing some reddish brown scales can be seen in the strigae. The scales are darker towards the upper lamina and colorless towards the lower lamina. Thin transparent glassy type scales are observed. The wing scale dimensions ranges between 90.62µ-130.02µ in length and 47.28µ-78.8µ in thickness. **Conclusion:** Studies focusing on butterfly wing scales are important as the scientific works based on the above is only minimum. A wide variety of scales are been observed in *Spalgis epius* with varying dimensions. Structural and coloration aspect of wing scales lead to the development of new ideas in science and technology.

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INTRODUCTION

Spalgis epius, the 'Ape fly' belongs to the lycaenids or blues family. Dorsal forwing and hindwing similar with blue colored appearance. Ventral wing is pale ash with very slender, short, brown strigae. The color forming powdery stuff present in the wings of butterfly's are called scales. The color patterns are due to tapestry of numerous small scales, each with a distinct color, which together create the species-characteristic appearance as in pointillist paintings (Nijhout, 1991).

Scales have been classified into three groups namely, hair –like or piliform, blade-like or lamellar and other variable forms (Scoble, 1995). The lamellae of scales are held apart by struts called trabaculae and contains pigments which give colour. The scales cling somewhat loosely to the wing and come off easily without harming the butterfly (Chapman, 1988; Scoble, 1995; Heppner, 2008).A butterfly wing scales varies in shape. The commonly seen shape includes scales with rounded terminus, scales with dentate or toothed terminus, small androconia or scent scales and hair- like scales. Three basic types of scales which are the characteristics of a butterfly wing of a species are the pigmentary scales, structural scales and

Androconia. Pigmentary scales are mostly flat. The pigment amount for the basic colours found in the butterfly wing-black, red and yellow. The juxta position of the various coloured scales and the amount of pigment they each contains, can create the illusion of additional colours such as orange, cream and green. Structural scales are larger than the pigmentary scales and often overlap pigmentary scales and are semitransparent so that the colors of the pigmentary scales can be seen through them.

Androconia are specialized scent scales that are found on male butterflies and usually exist as slightly raised dark streaks or patches on the forewings. Scales perform functions such as thermoregulation, aid in flight, coloration, mimicry and warning etc. A great deal of research work has been done by many scientists with butterfly, that relay on their diversity, wing coloration, wing pattern from different parts of the world including Indian subcontinent. Also, research is being proceeding in the molecular level also. The scientific works emphasizing on butterfly wing scales are less. However, findings on the nanostructural level, is one of the successful highlights of the present scenario. French (1997) studied on the pattern formation in color on butterfly wings.

Nijhout (2001) made an extensive analysis on the elements of butterfly wing patterns.Sekimura *et al*., (2002) investigated on the pigmentation of pattern formation in the butterfly wing of *Papiliodardanus* by numerical simulations of a reaction – diffusion model on a geometrically accurate wing domain. Stavenga (2004) studied on the wing –scale morphologies of the Pierid butterflies *Pieris rapae* (small white) and *Daliasnigrina* and the *Heliconiusmelpomene* are compared and related to the wing- reflectance spectra. Prum *et al*., (2006) investigated on anatomically diverse butterfly scales. In the study, it is revealed that all butterflies produce structural colours by coherent scattering.

Giraldo (2008) studied the pigmentation and structural properties of butterfly wing scales. Stavenga *et al*., (2009) studied on the imaging scatterometry of butterfly wing scales. Zhang *et al*., (2012) made an extensive analysis on morph genetic materials which is inspired from butterfly wing scales. Radadia (2012) investigated on the geometrical complexity in the wing pattern of the butterfly species of different taxa which was analysed for their fractal dimention, using mathematical models in Matlab. The analysis was restricted to a small number of butterfly species of Saurashtra region, Gujarat, India. Osotsi *et al*., (2020) 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 focus on the morphology, types, and dimensions of wing scales in *Spalgis epius.*

MATERIALS AND METHODS

Specimen Collection: *Spalgis epius* was collected 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). The bag is folded into the ring using iron wire. The meshes of the cloth is small that helps to hold even small butterflies that is captured. Then the specimen is pinned directly with a single pin through the body (thorax) and then it is fixed into the insect box.

Sample Preparation: The wing scales are isolated from the wing substrate as per the standard method of Grodnitsky (1991).

Several hundreds of scales were scrapped off from dorsal and ventral wing area of *Spalgis epius* separately into a glass slide. A clearing agent xylene is used to fix the specimen.After xylene has been evaporated, the samples were studied under the light microscope. The dimentions of the scales were measured using micrometry.Microscopic photography was used for taking the photomicrographs of the prepared scale sample.

Butterfly wing scale measurement: Dimensions of butterfly wing scales can be measured by the following process. 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 diameter and even the length of the wing scale can be determined.

RESULTS

Spalgis epius, the 'Ape fly' is a small butterfly that belongs to the lycaenids or blues family.Dorsal wing area is dull blackish blue while ventral wing area is ash colored with numerous, very slender, short, transverse dark brown strigae which are outwardly slenderly edged.

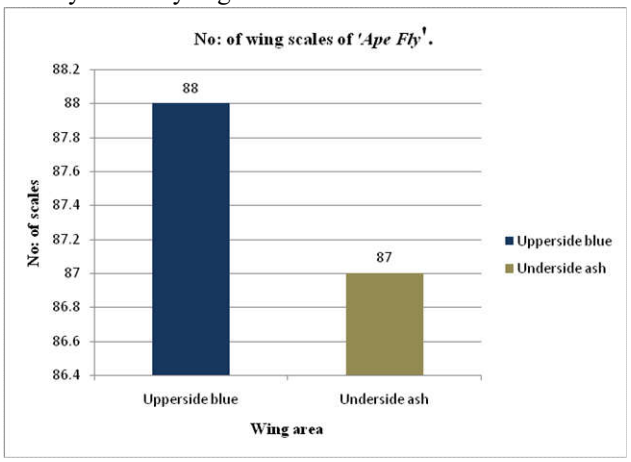

Figure 1. Shows number of wing scales in '*Ape fly*' on dorsal (upperside) and ventral (underside) wing area

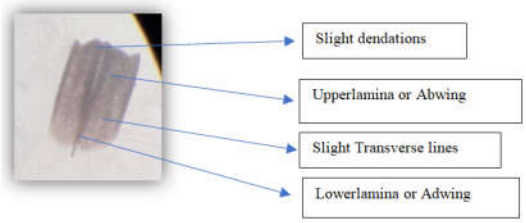

Figure 2. Structure of a scale

Wing scale description: The scale anatomy consists of an abwing or upper lamina, adwing or the lower lamina and a stalk. The abwing of some scales are highly dendated while some are devoid of dimensions (Figure 2).The scales enclosing dorsal and ventral wing area have distinct colors. The blue colored region of dorsalwing and ash colored region of ventral wing are isolated and studied.

A total of 175 scales were studied. Among them, 88 varieties of scales were studied from the dorsal (upperside) blue colored wing area and 87 varieties of scales were studied from the ventral (underside) ash colored wing area (Figure 1). On analyzing the blue colored region of dorsal wings several dull brown, cream, pale black colored scales are identified. Many broad and flat scales are observed that are short sized. The cream colored scales are so pale and majority of them are devoid of dendations or are reduced. It is a flat, thin glassy type scale. The body or blade of a typical scale consist of the upper lamina (abwing) and the lower lamina (adwing). For most of the scales, the abwing and adwing are broad. The length and thickness of each flat scales vary even if they are visually similar. Some scales are thicker towards there edge and thinner towards theinnerside. The dimensions of the scale ranges between 82.74µ-110.32µ in length and 55.16µ-74.86µ in thickness. Here the transverse lines that runs between the abwing and adwing cannot be clearly seen. The blue color of the upper side forewing is not due to the presence of blue scales as we guess, instead they are structural colors formed when the light passes through the pale black, cream and dull brown colored scales present in this region. The short, flat scales are more in number than the narrow- long scales. The underside wing scales shows several long scales unlike the upperside. Here several grayish white colored scales are identified. Long – narrow scales are the major scales. On focusing the underside, the majority are long scales. An odd scale is identified in this region. The reddish brown scales can be assumed as being the scale on the region of underside strigae. For the majority of scales the eitheredges are more darker than their inner side of the scale. Here some scales have double shades on their scales by having darker color towards the upper lamina or the abwing and colorless towards the adwing. Thin transparent or glassy type scales are also been identified in this area. The wing scale dimensions ranges between 90.62µ-130.02µ in length and 47.28µ-78.8µ in thickness.

DISCUSSION

Butterflies are brightly coloured beautiful creatures that plays an important role in nature's foodchain and a key factor for maintaining the ecological balance of the nature.The present study provides a basic idea about the scales and their colouration. The colors observed locally on the wing are also due to the degree of scale stacking. A wide variety of 175 scales were observed overall from the dorsal and ventral wing area. The blue colour of dorsal wing is due to scattering of light by the other basic colored scales such as dull brown ,cream, pale black colored scales. The nanostructural work of 'Stavenga' (2014) revealed that the green and blue colors are due to the structural colors that are created by the lower lamina, which acts as an optical thin film. Its reflectance spectrum, crucially determined by the lamina thickness, appears to be well tuned to the scales pigmentary spectram. The colored scales altogether create the specific pattern of wing color that represent a specific species. Some of the relevance of the study include butterfly wing scale pattern can be used for constructing solar panels. Osotsi *et al*., (2020) 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 study evoke an aesthetic sense in an individual and is often used in the textile industry to develop new designing patterns. Das *et al*., (2017) has studied on the potential of Biomimicry in the field of Textile Technology. In the result, it explains that the Butterflies are probably the most important aesthetic insect group, they possess a diverse coloration and wing patterns to camouflage, to attract other individuals with mating purposes and for thermoregulation and also they display aposematic colors to warn and confuse potential predators. These features have evolved to adapt and respond to the changing environment and to people they are the key component in their attractiveness. Structural coloration of the wing scales are 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) 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 jewellery and ornaments.

CONCLUSION

The present work is innovative as scientific literatures based on the above is only minimum. The study on the wing scales provides the basic idea about the various types of scales present in the butterfly wing that contribute to its wing color pattern. The shape, color and dimensions of the scales were noted in the study. The coloured scales all together form the specific colour pattern in the butterfly wing. The scales posses similar length and width within a species and differ only slightly. Scales are important for the butterfly as it helps the organism to protect itself from predators by camouflage and mimicry. The concept of butterfly wing scales provide wide range of application over the present scenario. Bioinspired sensors developed from the pattern of butterfly wing scales has much relevance today. Unfortunately, the butterfly species are facing threat as they are now becoming endangered. Climatic variations as well as the anthropogenic activities is the major problem faced by these creatures. Butterfly species are collected and preserved as specimens for scientific investigations. Habitat loss due to the human activities leads to the extinction of these valuable creatures.

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Conflict of Interest:

We declare that there is no conflict of interest regarding to the publication of this article.

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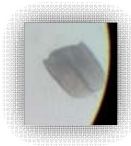
High Power View (40x)

1. Dorsal (Upperside) Wing Scales

 **1** Length:90.62µ Width:63.04µ	 **2** Length:94.56µ Width:70.92µ	 **3** Length:90.62µ Width:66.98µ	 **4** Length:90.62µ Width:70.92µ	 **5** Length:94.56µ Width:66.98µ	 **6** Length:90.62µ Width:70.92µ	 **7** Length:90.62µ Width:70.92µ
 **8** Length:90.62µ Width:74.86µ	 **9** Length:86.68µ Width:70.92µ	 **10** Length:86.68µ Width:70.92µ	 **11**	 **12** Length:94.56µ Width:66.98µ	 **13** Length:90.62µ Width:66.98µ	 **14** Length:98.5µ Width:94.86µ
 **15** Length:90.62µ Width:70.92µ	 **16** Length:90.62µ Width:66.98µ	 **17** Length:82.74µ Width:55.16µ	 **18** Length:78.8µ Width:63.04µ	 **19** Length:78.8µ Width:59.1µ	 **20** Length:78.8µ Width:63.04µ	 **21** Length:90.62µ Width:63.04µ
 **22** Length:86.68µ Width:55.16µ	 **23** Length:90.62µ Width:70.92µ	 **24** Length:90.62µ Width:66.98µ	 **25** Length:90.62µ Width:59.1µ	 **26** Length:86.68µ Width:63.04µ	 **27** Length:90.62µ Width:70.92µ	 **28** Length:90.62µ Width:66.98µ

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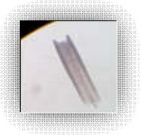
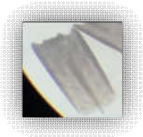
 29 Length:94.56µ Width:47.28µ	 30 Length:94.56µ Width:70.92µ	 31 Length:94.56µ Width:63.04µ	 32 Length:86.68µ Width:70.92µ	 33 Length:90.62µ Width:70.92µ	 34 Length:94.56µ Width:70.92µ	 35 Length:90.62µ Width:70.92µ
 36 Length:90.62µ Width:70.92µ	 37 Length:90.62µ Width:66.98µ	 38 Length:106.38µ Width:70.92µ	 39 Length:86.68µ Width:66.98µ	 40 Length:98.5µ Width:70.92µ	 41 Length:90.62µ Width:66.98µ	 42 Length:90.62µ Width:70.92µ
 43 Length:90.62µ Width:66.98µ	 44 Length:94.56µ Width:63.04µ	 45 Length:94.56µ Width:70.92µ	 46 Length:110.32µ Width:74.86µ	 47 Length:94.56µ Width:74.86µ	 48 Length:90.62µ Width:66.98µ	 49 Length:86.68µ Width:70.92µ
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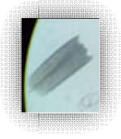
 57 Length:90.62µ Width:70.92µ	 58 Length:94.56µ Width:70.92µ	 59 Length:90.62µ Width:63.04µ	 60 Length:90.62µ Width:63.04µ	 61 Length:86.68µ Width:66.98µ	 62 Length:86.68µ Width:66.98µ	 63 Length:90.62µ Width:66.98µ
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 **71** Length:90.62µ Width:66.98µ	 **72** Length:90.62µ Width:66.98µ	 **73** Length:82.74µ Width:55.16µ	 **74** Length: 86.68µ Width:63.04µ	 **75** Length:90.62µ Width:70.92µ	 **76** Length:86.68 Width:66.98µ	 **77** Length:98.5µ Width:74.86µ
 **78** Length:90.64µ Width:70.92µ	 **79** Length:94.56µ Width: 66.88µ	 **80** Length:90.62µ Width:63.04µ	 **81** Length:86.68µ Width:66.88µ	 **82** Length:90.62µ Width:66.98µ	 **83** Length:90.62µ Width:55.16µ	 **84** length:86.68µ Width:66.98µ
 **85** Length:90.62µ Width:66.98µ	 **86** Length: 94.56µ Width:55.16µ	 **87** Length:94.56µ Width:70.92µ	 **88** Length:90.62µ Width:66.98µ			

Ventral (Underside) Wing Scales

 **89** Length:106.38µ Width:74.86µ Width:66.98µ	 **90** Length:110.32µ Width:66.98µ	 **91** Length:110.32µ Width:66.98µ	 **92** Length:114.26µ Width:63.04µ	 **93** Length:90.62µ Width:47.28µ	 **94** Length:98.5µ Width:66.98µ	 **95** Length:126.08µ Width:66.98µ
 **96** Length:98.5µ Width:70.92µ	 **97** Length:98.5µ Width:70.92µ	 **98** Length: 98.5µ Width:74.86µ	 **99** Length: 106.32µ Width: 78.8µ	 **100** Length:90.62µ Width:66.98µ	 **101** Length:90.62µ Width:74.86µ	 **102** Length:133.96µ Width:70.92µ
 **103** Length:118.2µ Width:74.86µ	 **104** Length: 90.62µ Width: 70.92µ	 **105** Length:94.56µ Width:70.92µ	 **106** Length:90.62µ Width:63.04µ	 **107** length:90.62µ Width:55.16µ	 **108** Length:110.32µ Width:63.04µ	 **109** Length:106.38µ Width:66.98µ

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 **110** Length: 94.56µ Width:63.04µ	 **111** Length:106.38µ Width: 66.98µ	 **112** Length:90.62µ Width:66.98µ	 **113** Length:106.38µ Width: 70.92µ	 **114** Length:110.32µ Width:66.98µ	 **115** Length: 114.26µ Width:70.92µ	 **116** Length:126.08µ Width:66.98µ
 **117** Length:118.2µ Width:74.86µ	 **118** Length:130.02µ Width:74.86µ	 **119** Length:94.56µ Width:63.04µ	 **120** Length:90.62µ Width:63.04µ	 **121** Length:94.56µ Width: 66.98µ	 **122** Length:90.62µ Width:66.98µ	 **123** Length:90.62µ Width:66.98µ
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 **145** Length:90.62µ Width: 66.98µ	 **146** Length:90.62µ Width:70.92µ	 **147** Length:90.62µ Width:66.98µ	 **148** Length:90.62µ Width:66.98µ	 **149** Length: 90.62µ Width: 66.98µ	**150** Length: 90.62µ Width:66.98µ	**151** Length:90.62µ Width: 66.98µ

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 **152** Length:86.68µ Width:66.98µ	 **153** Length:86.68µ Width:66.98µ	 **154** Length:90.62µ Width:66.98µ	 **155** Length:90.62µ Width: 66.98µ	 **156** Length:90.62µ Width:70.92µ	 **157** Length:94.56µ Width:55.6µ	 Le W
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 **166** Length:90.65µ Width: 70.92µ	 **167** Length:94.56µ Width:66.98µ	 **168** Length: 90.62µ Width: 74.86µ	 **169** Length:94.56µ Width:70.92µ	 **170** Length: 90.62µ Width:63.04µ	 **171** Length:90.62µ Width:66.98µ	 Le V
 **173** Length:126.08µ Width: 55.16µ	 **174** Length: 110.32µ Width: 70.92µ	 **175** Length: 118.2µ Width: 66.98µ				

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