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SCANNING ELECTRON MICROSCOPIC STUDIES ON THE STRUCTURAL CHARACTERIZATION OF WING SCALES OF BUTTERFLIES OF KARANTHAMALAI RESERVE FOREST, TAMIL NADU, INDIA

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Abstract– Six species of butterflies namely *Jamides celeno, Junonia orithya, Neptis hylas, Delias eucharis, Papilio demoleus* and *Eurema blanda* were selected from the Karanthamalai reserve forest and their wing scales were observed using scanning electron microscopy (SEM). The SEM images revealed the presence of thousands of scales, and their size was observed to be species-specific. The scales are organized in alternate rows, as is typical for Lepidoptera wings, with a long set of scales covering and concealing a shorter set of ground scales. SEM photography of the wings (dorsal side) of selected butterflies showed a direct comparison of the structure of cover and base scales which was observed to be normal in most cases but *Eurema blanda* shows the presence of overlapping scales and size of longitudinal ridges is about 1.66 µm; cross rib varies from 1.23 to 1.47 µm. The diameter of the ridges in *Neptis hylas* scales varies from 1.19 to 1.39 µm. In *Junonia orithya,* the interspace between cross rib ranges from 0.45 to 0.87 µm. Differences between the results for the six species appear to fit their morphological and architectural differences.

INTRODUCTION

The butterflies are known as Winged Wonders rightly depicted by their beautiful colour, size, texture and shape. Their beauty is due to the unique feature of the wings. The main function of the wings is to help in flight motion which shows the complexity of each family. The complexity of the wing scales varies in size, venation and elasticity (Combes and Daniel, 2003), and nature of flight or orientation of flight (Betts and Wootton, 1988; Wootton, 1981, 1992). Apart from their role in locomotion, wings have been involved in secondary functions including camouflage, visual and acoustic signaling, thermoregulation, and structural protection. The wing structure, shape, contour and texture are damaged due to ecological stress, biochemical changes and pollution (DeVries *et al.* 2010; Johansson *et al.* 2020; Suárez-Tovar and Sarmiento, 2016). The present study focused on the structural characterization of the wing scales of six species of butterflies in the Karanthamalai Reserve Forest using Scanning electron microscopy.

MATERIALS AND METHODS

Study Site

The present study was carried out in the Karanthamalai Reserve Forest (KMRF) of Dindigul district, Tamil Nadu, India. The total geographical area of Karanthamalai Reserve Forest is 8016.6 hectares (80.16 sq. km) and the total forest area of the Dindigul Reserve Forest is 80173.485 ha (www.forest.tn.gov.in), which is 9.99 % of the total reserve forest area of the district (Dharumarajan *et al.,* 2015). The study area is corresponding to dry forest with local variations in topography.

Identification of Butterfly species

The KMRF was observed with the highest population of butterflies during the study. The selected butterflies were identified by the key characters provided by Kunte (2000) and Mukherjee *et al.* (2015). Six species namely *Jamides celeno, Junonia orithya, Neptis hylas, Delias eucharis, Papilio demoleus and Eurema blanda* were selected from KMRF based on their wing colour (Blue, White and Yellow) pattern to analyze the microstructural and morphological differences between the taxa.

Wing Scale Structure Characterization (Scanning Electron Microscopic Studies)

Wing scale structures of the selected butterfly species were imaged by scanning electron microscopy (SEM) to study the typical structure of the wing scales in different magnifications. SEM allows studying the superficial structure as well as the inner structure of single scales. In this study, six different species from four families were examined for the typical structural details which are shown in Table 1. Butterfly wings are cut into small squares using a razor blade and mounted with carbon tape to aluminum stubs. In addition, wing scales were brushed off with a small paint brush and mounted with carbon tape onto aluminum stubs. All their stubs were coated with a layer of 20 nm gold to increase sample conductivity. All SEM images were photographed usingVEGA3-TESCAN Scanning Electron Microscope at Gandhigram Rural Institute (GRI), Dindigul.

RESULTS

Six different species were selected from KMRF and are identified as *Jamides celeno, Junonia orithya, Neptis hylas, Delias eucharis, Papilio demoleus and Eurema blanda* based on their morphology (Plate 1). The wing scale microstructure of the selected butterfly species were studied using SEM photomicrography. The SEM images of butterfly wing scales are depicted in Plates 2–7. Table 1 presents the wing micro structure details of the selected butterflies. SEM microscopic images show dense and overlapping scales in *Ja.celeno* and *D.eucharis* wings. *Ja. celeno* and *Ju. orithya*, both are dominantly having blue colour on their wings. The cover scales are noted to partly overlap the ground scales as depicted in Plate 2 (2490 X). The upper lamina of the scales has longitudinal ridges. Single scale in a 25000 X magnification shows the ridges and ribs of the upper lamina as well as trabeculae which connect the upper and lower laminae. In *Ja.celeno* wing scales are with wide space between the ridges as compared

Table 1. Butterfly species chosen for the study and their Wing architecture

S. No	Scientific Name	Common Name	Family	Structure betweencross ribs	Distance between pillars (µm)	Beads	Margin terminaus	Windows	Wing colour
1.	*Jamides celeno*	Common cerulean	Lycaenidae	Network	1.53-1.78	Absent	Dentated	Absent	Blue
2.	*Junonia orithya*	Blue pansy	Nymphalidae	Few connected with trabeculae	2.10-2.25	Present	Wavy	Open, filled with beads	Blue
3.	*Neptis hylas*	Common sailor	Nymphalidae	Connected with trabeculae	1.19-1.39	Absent	Highly serrated	Open, with lamina	White
4.	*Delias eucharis*	Common jezebel	Pieridae	Fabry–Perot cavity occupied by oval shaped beads	1.44-1.64	Absent	Wavy	Open	White
5.	*Papilio demoleus*	Lime yellow butterfly	Papilionidae	Scaly	1.19-2.35	Absent	Wavy	Absent	Yellow
6.	*Eurema blanda*	Three spot grass yellow	Pieridae	Large beads on the sides of cross ribs and pillar	1.39-1.66	Present	Highly wavy	Open, filled with beads	Yellow

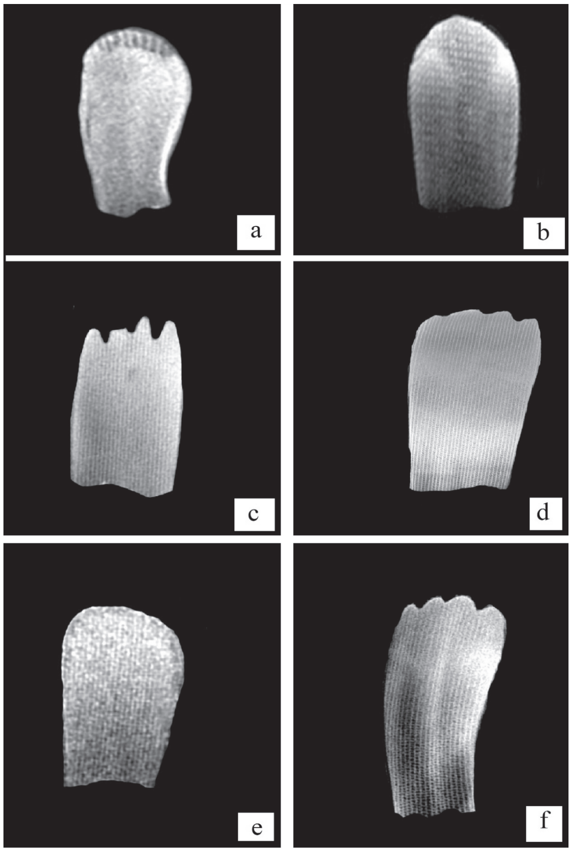
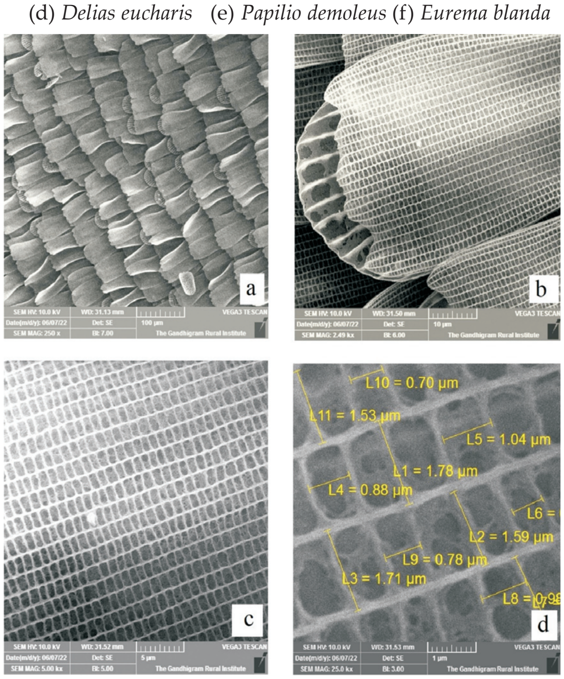


Plate 1. Morphological diversity in scales of selected butterfly species in the Karanthamalai Reserve forest

(a) *Jamaides celeno* (b) *Junonia orithya* (c) *Neptis hylas* (d) *Delias eucharis* (e) *Papilio demoleus* (f) *Eurema blanda*

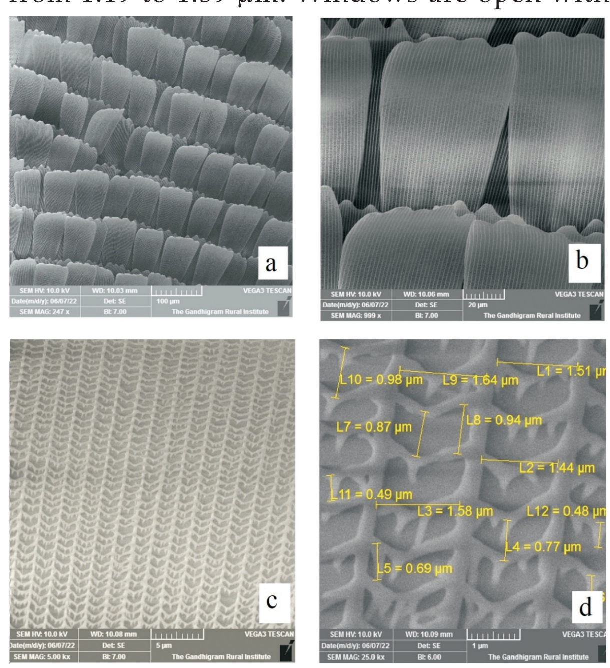


a) 250x b) 2490x c) 5000x d) 25000x

Plate 2. SEM images of the wing scales of *Jamides celeno*

with the other scales and monolayered cuticles are arranged on the ridge. SEM photograph of dorsal wings of *Ja. celeno* at higher magnification shows the size of the pillar cells ranging from 1.53 to 1.78 µm and the distance between the cross ribs ranged from 0.70 to 1.04 µm. Windows and beads adjacent to the crossribs are absent. Terminal margin areas are dentate. Distance between the pillars are 1.53 to 1.78 µm. Plate 3 shows the scanning electron micrograph of *Ju.orithya* wing scales at different magnifications. The wings studded with scales were partly overlapping as tiles on a roof; scales were flat with longitudinal ridges on the upper lamina while the presences of longitudinal ridges among the laminae are connected by cross ribs. At higher magnification *J. orithya* shows the interspace between cross rib ranging from 0.45 to 0.87 µm and the width of the cross ribs ranged from 1.44 to 1.64 µm. Windows are open and are filled with beads. Terminal margin areas are wavy. Distances between the pillars are 2.10-2.25 µm.

Plate 4 and 5 exhibit the scale structure of white colour winged butterflies *N. hylas* and *D. eucharis* respectively. The SEM micrograph of *N. hylas* scales had a vein observed near the upper right corner. The scales are noted to be curved where others are almost at exhibiting the slit top tails. The flat layers in the areas are enclosed by the ridges and the ribs. The diameter of the ridges in *N. hylas* scales vary from 1.19 to 1.39 µm. Windows are open with

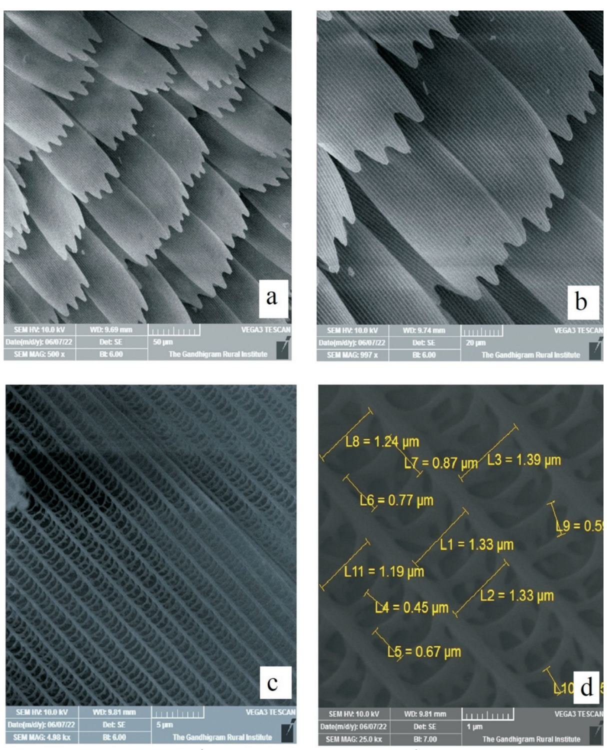


a) 247x b) 999x c) 5000x d) 25000x

Plate 3. SEM images of the wing scales of *Junonia orithya*

lamina. Terminal margin areas are highly serrated. Distance between the pillars are 1.19-1.39 µm. A dense mass of scales were observed for *D.eucharis*; SEM photograph revealed micrometer-scale features that resemble shingles on a roof, and an even finer nanometer-scale features: parallel grooves on the surface of the shingles. Between the wing lamellae, there exists a reticular porous network structure, which is made by an organic material called chitin. The reticular porous network or "window-like structure" is with an average pore size around 1 µm. At the same time wings of *D. eucharis* were noted to have reduction in Fabry–Perot cavity which was occupied by oval shaped beads. Bend ribs were observed in *D.eucharis*. Each scale has numerous parallel lines formed by the overlapping of Y-shaped structures. Numerous parallel ladders that resemble crossribs join two parallel lines (pillars). Each crossribs wall is covered with numerous thick beads in the form of spindles (Plate 2.c). At higher magnification the size between the cross ribs ranges from 2.10 to 2.23 µm. The width of the cross ribs is about 0.37 µm. The maximum distance between the cross ribs is 0.88 µm.

Plate 5 and 6 divulge the scale structure of white colour winged butterflies *Papilio demoleus* and *Eurema blanda* wings respectively. The scales of *P. demoleus* are arranged in a shingle-like structure. Two types of scales which are alternating in position were observed in *P. demoleus* and a scale with blunt end and peripheral or slight splits. The size of the



a) 500x b) 997x c) 4980x d) 25000x

Plate 4. SEM images of the wing scales of *Neptis hylas*

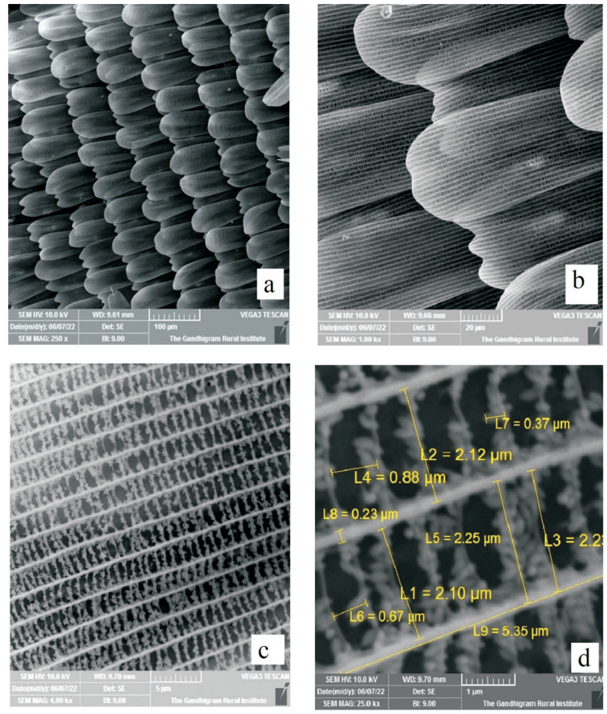


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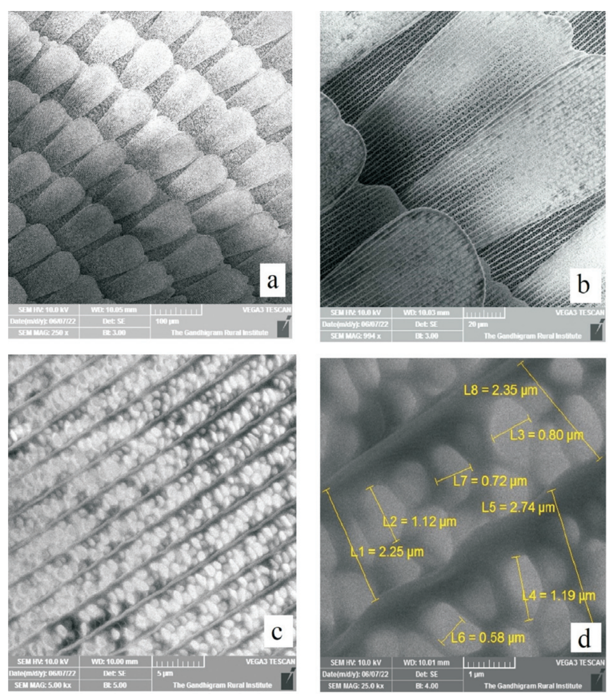
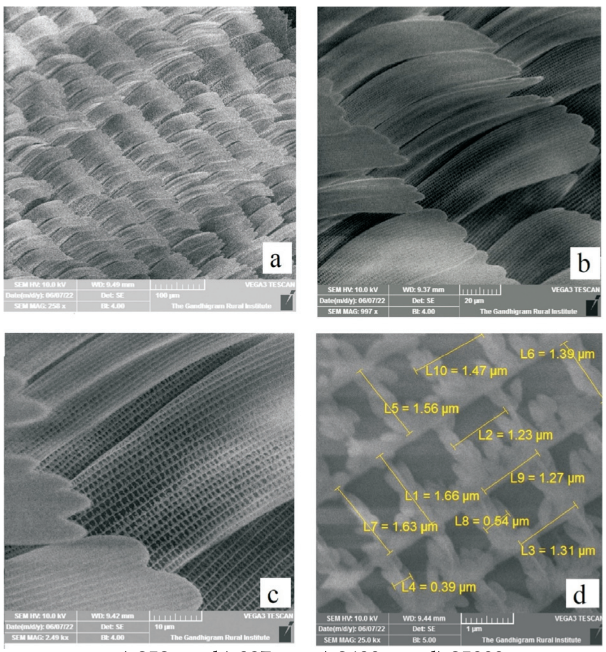
Plate 5. SEM images of the wing scales of *Delias eucharis*



a) 250x b) 994x c) 5000x d) 25000x

Plate 6. SEM images of the wing scales of *Papilio demoleus*

cross ribs is 2.25 to 2.35 µm. Windows and beads are absent. Terminal margin area are wavy. Distances between the pillars are 1.19-2.35 µm and the structures between the cross ribs are scaly. The scales of *E. blanda* are overlapping with the scales one another and the size of longitudinal ridges was about 1.66 µm; cross rib varies from 1.23 to 1.47 µm. Windows are open and are filled with beads. Terminal margin areas are highly wavy. Distance between the pillars is 1.39 to 1.66 µm.



a) 258x b) 997x c) 2490x d) 25000x

Plate 7. SEM images of the wing scales of *Eurema blanda*

DISCUSSION

Butterflies are well-known to people for their aesthetic values. The attractive bright colours and architectures on the wings are due to the nanostructures. These nanostructures are integrated into a complex structure of scales that densely cover the wings. There are several types of scales that cover a butterfly's wing. Viewed under SEM, they are of varying shapes and sizes. The majority have the function of building up a colourful pattern and are of two types. The pigmented scales are variously coloured, each scale bearing only a single colour. The browns and blacks are caused by pigments called melanins, while the yellows, oranges, and reds are coloured by pteridine pigments (Smetacek, 2000). Presently, blue, white and yellow-colour winged butterflies are selected to study their microstructure of wings. The nanostructures in the scales assist the butterflies in aerodynamics, self-cleaning, thermoregulation, and camouflage (Köchling *et al.*, 2020). Butterfly wings are so delicate that getting dirt or moisture on them makes it hard to fly. The colour and patterns on wings makes sexual dimorphism and uniqueness among species. So they have to keep their wings bright and visible in order to reproduce (Le *et al.,* 2019). Wing scales adapt to different environmental conditions influenced by factors such as time, speed, foraging, calling, finding places for spawning and avoiding predators (Breuker *et al.,* 2007).

Investigation on the structure of scales can be focused on the following four levels, and each level has its own characters. The first level is the customary dimension which can be seen by naked eyes. The scales look like powder and may fall easily when being touched. There are some setae structures on the scales. The second level can be observed under common optical microscope. In this level, many regular scales array in certain sequence which can be observed. Each single scale shape can be seen clearly (Wu *et al.,* 2007).

Sharmila *et al.* (2022) studied the structural arrangement of scales in similar coloured regions within butterfly wings of different species and reports that their scale structure is architecturally different. The scales under SEM showed differenced among butterflies and scales of *P.polymnestor* had high concentration of windows, *Ju.heirta* and *N. hordonia* had network and lamina. According to their report, *N. hylus*, *Ju.celeno* and *Ju.orithya* scales are having small beads on the sides of cross ribs, network and curved striation between cross ribs. Vukusic *et al.* (2000a, 2000b and 2001) and Lawrance *et al.* (2002) have studied the optical attributes of physical colours in butterfly species belonging to the family Nymphalidae and Papilionidae producing blue and green reflections. The size of the butterflies' scales changes from individuals to individuals and even among the same individual, scales can be different in size (Vértesy *et al.,* 2004).

Evidently, the selected butterflies wings are made up of thousands of scales and are organized in alternate rows, with a long set of cover scales covering and concealing a shorter set of ground scales. A scale's upper surface is made up of a grid of elevated longitudinal quasiparallel lamellae ridges that are spaced roughly 2.5 µ apart and run the length of the scale. A net-like reticulum latticework made of tiny tubes fills the area between adjacent lamellae. SEM images of the wing dorsum of selected butterflies show a direct comparison of structure of cover and base scales (Plates 2-7) which was observed to be normal in most cases but *E.blanda* shows the presence of overlapping scales. The self-cleaning property of butterfly wing surface is the result of the anisotropic microstructures and the energy barrier difference in different directions. Due to the overlapping scales, the directional vertical gibbosities and the nano-protuberances, the energy may be less due to the presence of protuberance as observed in earlier studies by Sun *et al.,* (2009).

Wing pattern is successful in establishing the correlation in adaptation and adaptive change. Both changes in morphology of wings and the pattern of flight are closely associated with the genetic material. The spacing in between the ribs observed among yellow *E.blanda* are chitinous membranes, nourished and supported by tubular veins. These veins also function in exchange of oxygen. Further, venation has aerodynamic importance, plays specific role in flight system, and adapts to different surroundings (Mishra *et al.,* 2007).

Transparency in the butterfly wing was a genetically evolved character. Efficiency at transmitting light is largely determined by clear wing microstructure (scale shape, insertion, colouration, dimensions, and density) and macrostructure (clear wing area, species size, or wing area). Microstructural traits, scale density and dimensions, are tightly linked in their evolution, with different constraints according to scale shape, insertion, and colouration. Transparency appears highly relevant for concealment, with size-dependent variations. Links between transparency and latitude are consistent with an ecological relevance of transparency in thermoregulation. Altogether, our results shed new light on the physical and ecological processes driving the evolution of transparency on land and underline that transparency is a more complex colouration strategy (Gomez *et al.,* 2021).

CONCLUSION

The scanning electron microscopic studies show overlapping scales in *Ja. celeno* and *D.eucharis* wings. At the same time *Ju.orithya* wings are studded with scales and were partly overlapping as tiles. Dense mass of scales were noted among *D. eucharis* and *P.demoleus* wings are arranged like Shingle. Only *D.eucharis* wing scales were noted to have reduction in Fabry–Perot cavity which was occupied by oval shaped beads. Others were noted to be normal cross ribs joining with two parallel lines.

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