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Microstructure of wing scales in butterfly species from Alagar Hills, Tamil Nadu, India

E. Joy Sharmila*, A. Joseph Thatheyus, S. Susaritha, M. Snega Priya, B. Archana and K. Rangesh#

PG & Research Department of Zoology, The American College, Madurai 625002,Tamil Nadu, India.
#Department of Marine and Coastal Studies, School of Energy, Environment and Natural Resource, Madurai Kamaraj University, Madurai 625021, Tamil Nadu, India.
Email: joysharmila@americancollege.edu.in

ABSTRACT: Study undertaken to find the structural arrangement of scales in similar coloured regions within butterfly wings of different species revealed their scale structure was architecturally different. The scales under scanning electron microscope showed difference among butterflies. Scales of *Papilio polymnestor* (Cramer) had high concentration of windows, *Pareronica ceylanica* (Felder) with beads and other species such as *Talicada nyseus* (Guerin Meneville), *Atraphaneura aristolochiae* (Fabricius), *Junonia heirta* (Fabricius), *Neptis hordonia* (Moore), *Acrae violae* (Fabricius) and *Danus chrysippus* (Linnaeus) had network and lamina. Tonality of colours showed differences in the arrangement of scales. © 2022 Association for Advancement of Entomology

KEYWORDS: Butterfly Scales, SEM, Nanostructure, Beads, Window, Lamina and Pillar

INTRODUCTION

Butterflies come under the order Lepidoptera that exhibit brilliant iridescence (Stavenga *et al*., 2008). Just like a pointillist painting the surface of the wing is a collection of coloured dots called scales. The scales of butterflies are detached easily when their wings are touched. Colours that appear in butterflies are diverse and serve different functions (Nobre *et al*., 2021). Even by using computer generated images, these colours are highly complex to reproduce. Colours are created in two different ways via pigments and nanostructures (Wu *et al*., 2012; Yoshidha *et al*., 2001; Zhu *et al*., 2009). The patterns on the wings enable butterflies to recognize their own species at a distance and differentiate between males and females. Many reports have shown two mechanisms for colour generation in butterfly wings (Tilley and Eliot, 2002). Pigmentary colours of organisms are produced by differential absorption of visible wavelength by pigment molecules (Fox, 1976). Majority of colour patterns in Lepidoptera correspond to pigments incorporated in scales or cuticle (Ghiradella, 1998). But the most beautiful tonalities of iridescent colour exhibited by some butterflies are generated by light interference in specialized scales (Vukusic *et al*., 2000). The structural colours of organisms are produced by physical interaction of light waves with biological nanostructures that vary in refractive index (Prum and Torres, 2003). Bright animal colours are some of the most intriguing and poorly understood natural phenomena that continue to preoccupy scientists working at all levels of scientific inquiry. Ghiradella (1998) carried out studies on butterfly scales patterning in insect cuticle and morphology of

* Author for correspondence

Andean butterfly population. It was found that vivid colours are caused by pepper pot nanostructures. Vertesy *et al.* (2006) observed the wing scale microstructure and nanostructure in butterflies and structural coloration of blue colour of three Lycaenids. Fiber optic spectrophotometry and 2D-fourier analysis have been used to investigate the physical mechanism of colour production in twelve Lepidoptera species of four families by Prum *et al.* (2006). Novel photo anode structures have been templated by using butterflies wing scale (Zhang *et al.*, 2009; Zhu *et al*., 2009). Studies have been carried out in butterflies on their diversity (Alturi *et al*., 2011; Devi *et al.*, 2021), and lifecycle (Appalanaidu and Venkataramana, 2010), but very limited studies have been carried out on the scales of butterflies which will have applications in nano material sector. Thus, the present work was carried out to investigate the structural pattern of scales pertaining to different colours on the wings of butterflies found in Alagar Hills, Tamil Nadu.

MATERIALS AND METHODS

Specimen collection and preparation

Samples of coloured butterfly wings were taken from the specimens collected from Alagar Hills (10°04' N; 78°12'E), Madurai, Tamil Nadu, India. Alagar hills is one of the reserve forest in Tamil Nadu which houses the richest diversity of butterflies (Sharmila *et al*., 2020). The scales studied were blue, red, yellow and orange belong to the Pieridae, Papilionidae, Lycaenidae and Nymphalidae families. The eight species are *Pareronica ceylanica* (Felder), *Papilio polymnestor* (Cramer), *Talicada nyseus* (Guerin Meneville), *Atraphaneura aristolochiae* (Fabricius), *Junonia heirta* (Fabricius), *Neptis hordonia* (Moore), *Acrae violae* (Fabricius) and *Danus chrysippus* (Linnaeus).

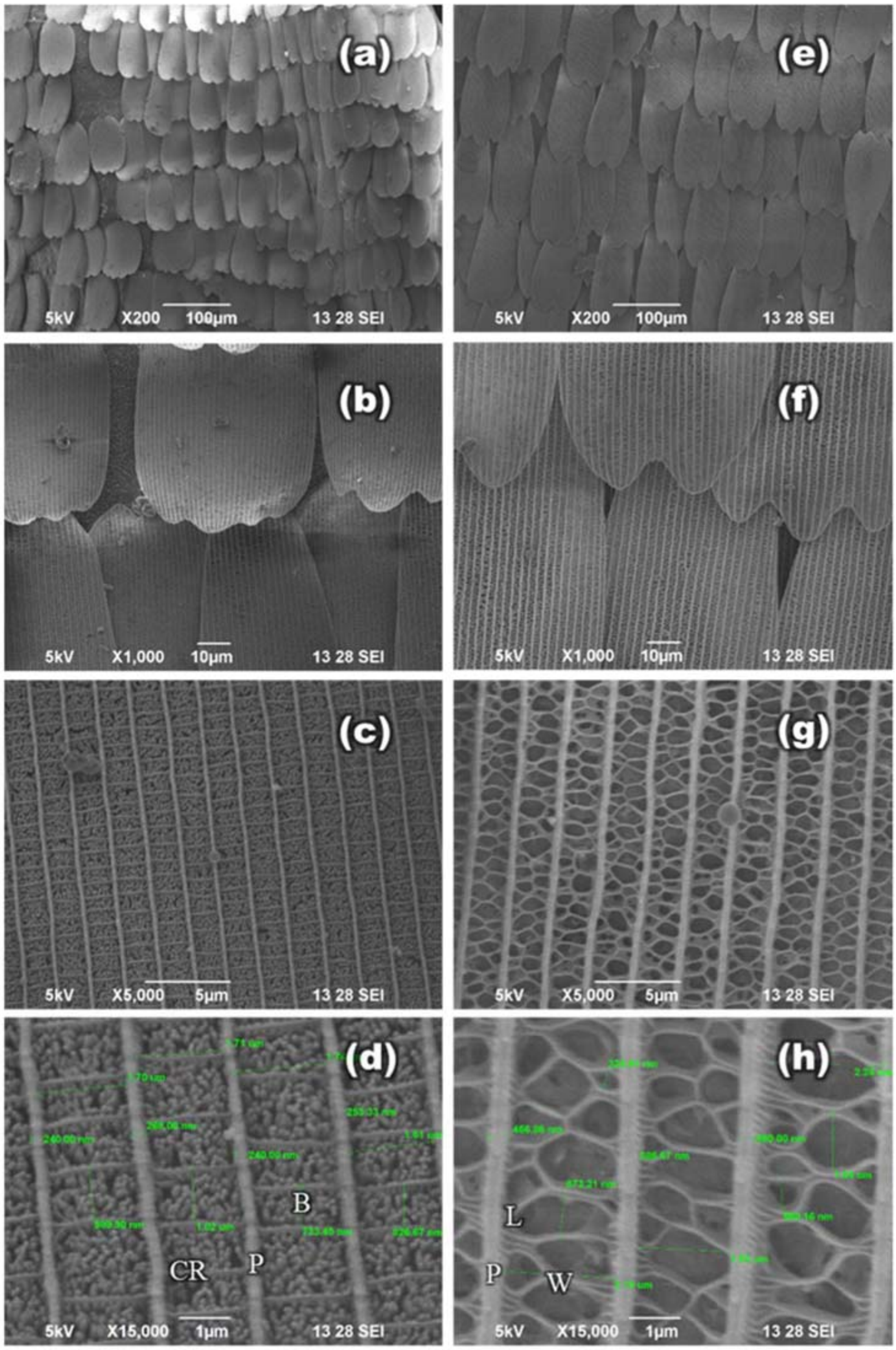
Scanning electron microscopy (SEM)

Dried specimens of butterflies were used to find out the qualitative approximation for the trends in different wing scales related to colour. The wing related to the specific colour was cut, stuck to microscope stubs and coated with iridium. The micro-configuration of scales was observed under the scanning electron microscope JSM – IT 300 L V (Prum *et al*., 2006).The butterfly scales examined include four colours from eight species of four Lepidoptera families. The microstructures were categorized into pillar, window, lamina and cross rib for determining the wings architecture. Scales were observed in different magnifications such as 1µm, 5µm, 10µm and 100µm.

RESULTS AND DISCUSSION

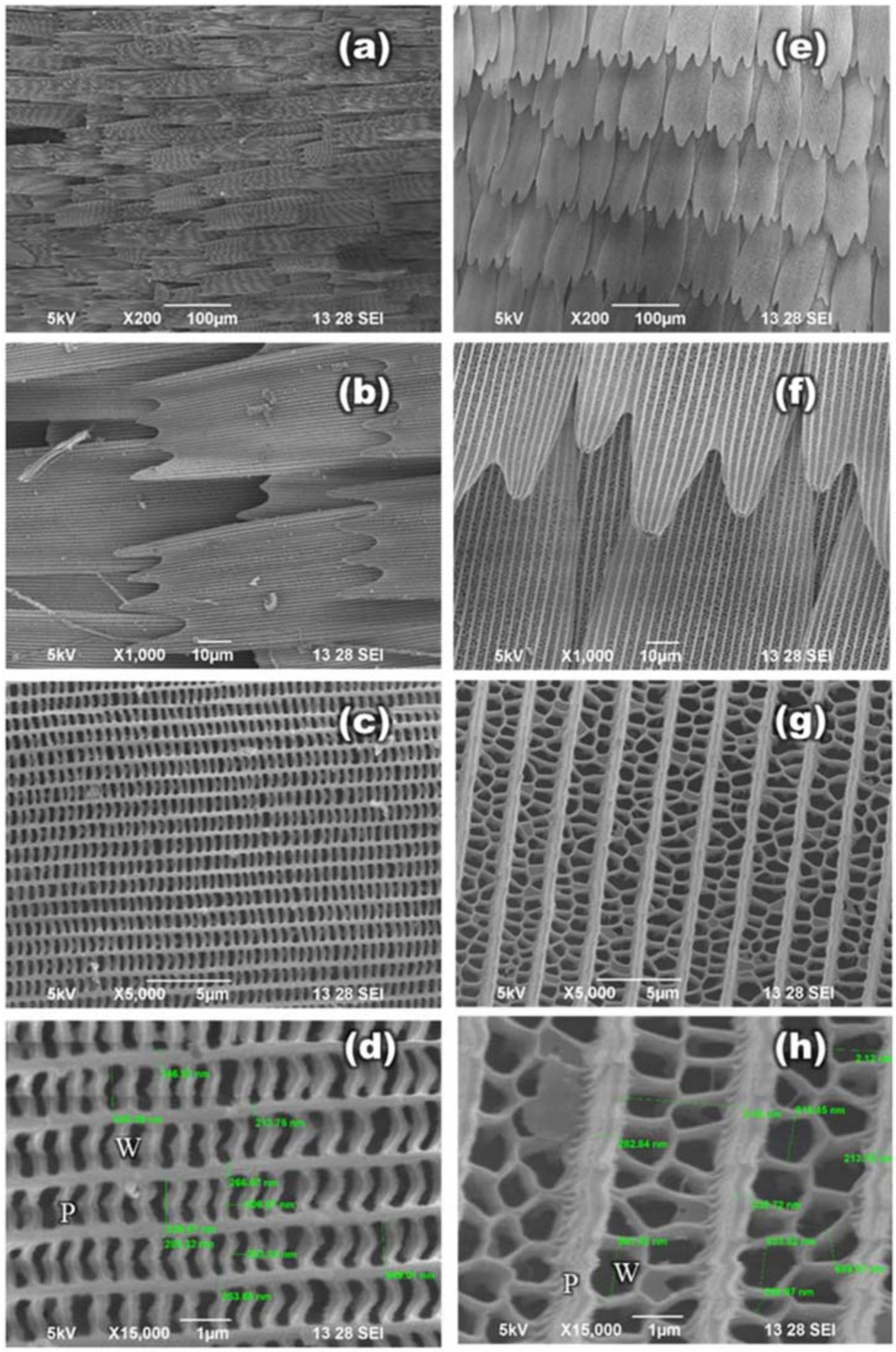
The present study highlights the diversity in the arrangement of scales for different colours in different families of butterflies. The SEM has helped in the investigation of butterfly wings not only in the scale shape but has helped to observe micro and nano-sized structures. *Pareronica ceylanica* and *Papilio polymnestor* with blue colour have scales that have grooves, but at higher magnification the pattern within the scales was different. The pillar of *P. ceylanica* were of the size 240-268nm and the distance of cross ribs ranged from 1.70 to1.74µm, and the cross ribs in between were filled with beads. The scales of *P. polymnestor* had pillars of the size 360-446nm and the distance of cross ribs ranged between 1.82 and 2.24µm, few open windows were found while some windows were filled with lamina. The structure between the cross ribs had network like arrangement (Fig. 1). *Talicada nyseus* and *A. aristolochiae* with red colour had teethed scales and the number of grooves vary between them. *T. nyseus* had pillars of the size 826-949nm and the distance between cross ribs ranged from 1.34 to 1.69µm, and showed open windows. In *A. aristolochiae* the pillar size ranged from 213-282nm and distance between pillars ranged between 2.12 and 2.34µm, and the open windows in a network like arrangement (Fig. 2).

In *J. heirta* and *N. hordonia* with yellow colour the scales look alike with grooves, but at higher magnification they showed slight difference. The pillars of *J. hierta* ranged from 248 to 333nm and the distance of cross ribs ranged from 824nm to1.07µm. *N. hordonia* pillars were in the range between 200 and 223nm and cross ribs ranged from 162 to 223nm. In *J. hierta,* the cross ribs were



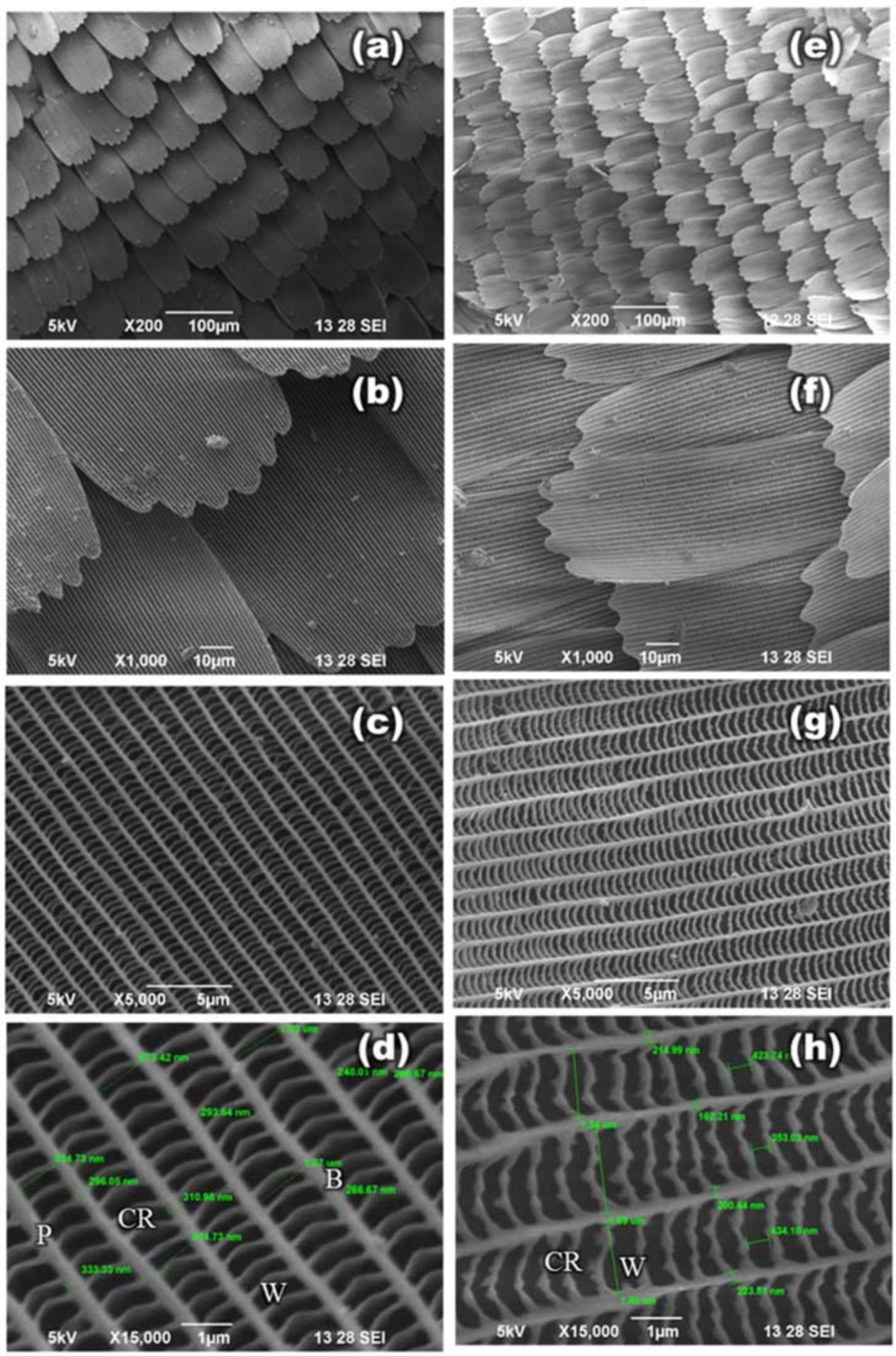
(a-d) Scales of *Pareronia ceylanica* ; (e-h) Scales of *Papilio polymenestor* P-Pillar; CR-Cross Rib; B-Beads; L-Lamina; W-Window

Fig. 1 Scanning electron micrograph of blue colour scales of *Pareronia ceylanica* and *Papilio polymenestor* butterflies



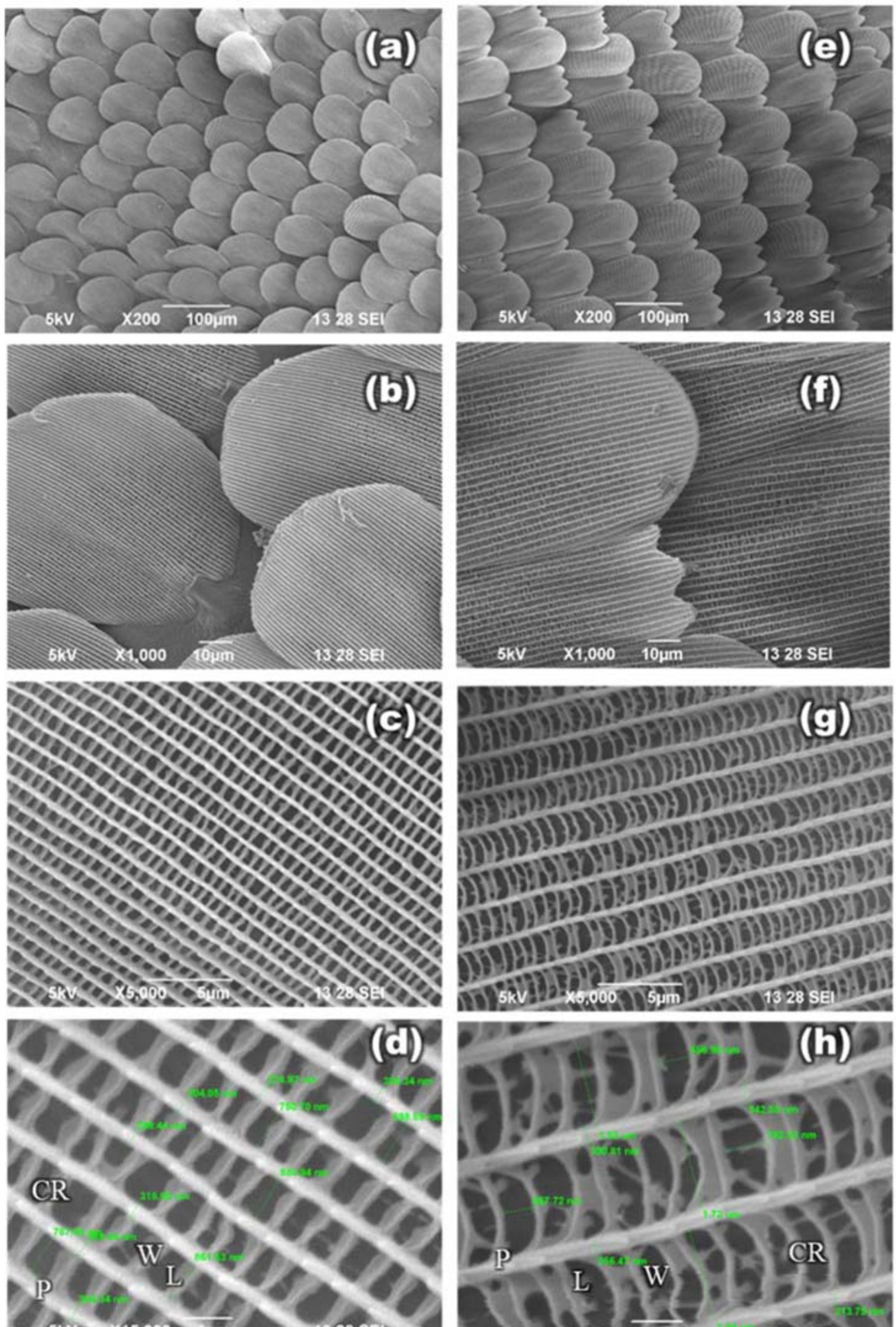
(a-d) Scales of *Talicada nyseus* ; (e-h) Scales of *Atraphaneura aristolochiae*. P- pillar; CR-Cross Rib; B-Beads; L-Lamina; W-Window

Fig. 2 Scanning electron micrograph of red colour scales of *Talicada nyseus* and *Atraphaneura aristolochiae* butterflies



(a-d) Scales of *Junonia hierta*; (e-h) Scales of *Neptis hordonia* P- Pillar; CR-Cross Rib; L-Lamina; W-Window

Fig. 3 Scanning electron micrograph of light yellow colour scales of *Junonia hierta* and *Neptis hordonia* butterflies



(a-d) Scales of *Acraea violae* ; (e-h) Scales of *Danaus chrysippus ;* P- Pillar; CR-Cross Rib; L-Lamina; W-Window

Fig. 4 Scanning electron micrograph of dark orange coloured scales of *Acraea violae* and *Danaus chrysippus* butterflies smooth and in *N. hordonia* they had slight projection and beaded appearance (Fig. 3). *A. violae* with orange colour at lower magnifications exhibit the scales with smooth round edge, while the pillars size ranged from 274 to 304nm and the cross ribs ranged from 750 to 898 nm with the scales of open windows. *D. chrysippus* showed two types of scales which have smooth edge and with grooves. The pillar size ranged from 214 to 232nm and cross ribs of the size from 1.72 to1.84µm. Most of the windows had lamina and cross veins between them (Fig. 4).

Scales in the under surface are smooth and the upper surface has parallel ridges. The spatial structure of a scale depends on the type of butterfly. Distribution of beads had been seen in Pierid scales. The beads effectively absorb short wavelength light and at the same time scatter long wavelength light (Moorehouse *et al.*, 2007; Parnell *et al.*, 2018). There was a slight similarity in the structure of scales of *J. heirta* and *N. hordonia* and for others there was a vast difference. Coloration in certain species of butterflies of subfamily Nymphalidae is due to species specific patterning of different coloured scales on their wings (Stavenga *et al.*, 2014). The scales in *Morpho agea* showed pine shaped structures (Yoshioka and Kinoshita 2003). In *Uranea fulgens* they were characterized by laminar nanostructures of air cavities within the body of scales (Vukusic *et al.*, 2000). In Marcedus Blue the upper surface of the scale is formed of high longitudinal ridges with open microcells (Balint *et al.*, 2004). The present study also highlights that even though the colours in species of butterflies look similar, the architectural plan of the scales was different. The morphology of the scales had thrown light on their structure and the structure was unique to each species and colour. The construction of scale structure is malleable that allowing extravagantly varying shapes (Thayer *et al.*, 2020). This study has limitations on explorations of different dimension of the nano-architecture however this investigation will add the biomimetic values from butterfly scales and promising application for nano-scale photonics in future.

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