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Comparative study of wings of Butterflies and Moths by Scanning Electron Microscopy

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Abstract:

Scanning Electron Microscopy of the wing scales from specific locations in butterfly and moth species, influenced by their respective lifestyles, revealed distinct scale arrangements. The length of the scales in *Graphium agamemnon* measures 107 µm, whereas *Thyas coronata* exhibits longer scales at 208 µm. The width of *G. agamemnon* scales is 42.5 µm, whereas *T. coronata* has narrower scales, measuring 31.6 µm.

The spacing between two scales, a crucial characteristic for species differentiation, is 79.1 µm in *G. agamemnon* and slightly smaller at 63.2 µm in *T. coronata*. This variation in scale width contributes to the distinctive appearance of their wings. Additionally, the distance between trabeculae—microscopic structures on the scales—differs significantly. *G. agamemnon* exhibits a larger trabecular spacing of 1.46 µm, while *T. coronata* has a more compact arrangement with a distance of 0.5 µm.

These intricate measurements provide valuable insights into the morphological differences between *G. agamemnon* and *T. coronata*.

Keywords: Scanning Electron Microscopy (SEM) , Wing Scales , Butterfly Morphology

Introduction

Butterflies and moths both belong to the order **Lepidoptera**, sharing similarities in their winged structures. However, there are distinct differences in their flight characteristics. Butterflies generally have more slender bodies and larger wings, resulting in a graceful, fluttery flight. In contrast, moths have thicker bodies and smaller wings, contributing to a more erratic flight pattern. These differences are adaptations to their respective lifestyles, with butterflies being diurnal and moths primarily nocturnal.The surfaces of butterfly and moth wing scales are often covered with elaborate patterns, ridges, and other microscopic structures. These features play a crucial role in various biological functions, including camouflage, mimicry for predator avoidance, mate attraction, aposematism (warning coloration to indicate toxicity), and thermoregulation by trapping or reflecting sunlight (Ghiradella, 1998; Goodwyn et al., 2009).

Scanning electron microscopy (**SEM**) has revolutionized the study of butterfly and moth wing scales by revealing their intricate microstructures. Butterfly wings are adorned with tiny,

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www.ijasem.org

Vol 19, Issue 1, 2025

overlapping scales that contribute to their vibrant colors and complex patterns. Under SEM, these scales display a mesmerizing array of textures and designs, providing insights into their functional significance. Similarly, moth wing scales exhibit diverse patterns and textures under SEM, highlighting the sophistication of nature's design.

Graphium agamemnon, a butterfly species from the family **Papilionidae**, and Thyas coronata, a moth species from the family **Noctuidae**, provide an excellent comparison of wing scale structures. In this study, we examine the wing scales of *G. agamemnon* and *T. coronata* using SEM and discuss their microstructural differences.

Materials and method:

1. Collection of Specimens

- Butterflies and moths were collected from various habitats, including gardens, forests, and grasslands, using an insect net.
- Specimens were identified based on morphological characteristics using field guides and taxonomic keys. i. e. *G. agamemnon* (family Papilionoidae) and another, T. coronate (family Noctuidae),
- Collected specimens were euthanized using ethyl acetate and stored in airtight containers for further analysis.

2. Sample Preparation

- The wings of butterflies and moths were carefully detached using fine forceps under a stereomicroscope.
- The samples were air-dried at room temperature to remove moisture.
- For better visualization, scales from selected wing regions were gently removed using a fine brush.

3. Fixation and Coating

- Wings were mounted on aluminum stubs using double-sided conductive carbon tape.
- Specimens were coated with a thin layer of gold or platinum using a sputter coater to enhance conductivity and improve image resolution.

4. Scanning Electron Microscopy (SEM) Analysis

- Prepared wing samples were examined under a Scanning Electron Microscope JEOL6380A (SEM) at varying magnifications from the Department of Metallurgy, Visvesvaraya National Institute of Technology, Nagpur(VNIT).
- Observations focused on:
    - Scale structure and arrangement
    - Differences in microstructures between butterflies and moths

INTERNATIONAL JOURNAL OF APPLIED SCIENCE ENGINEERING AND MANAGEMENT

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www.ijasem.org

Vol 19, Issue 1, 2025

  - Presence of ridges, pits, and nanostructures affecting light reflection and coloration
- Images were captured for comparative analysis.

5. Data Analysis

- Structural variations between butterfly and moth wings were compared based on SEM images.
- Statistical analysis was performed to quantify differences in scale size, shape, and density.

Fig. a- Ventral wing of *G. agamemnon*

Fig. b- Dorsal wing of *T. coronata*

Observations:

1. **Scale Structure and Arrangement**
   - Butterfly wings exhibited broad, flattened scales arranged in overlapping rows, contributing to their vibrant coloration.(Fig. 9)
   - Moth wings displayed narrower, more elongated scales, often with a denser arrangement, providing a more cryptic appearance. (Fig.10)
2. **Surface Microstructures**
   - SEM images revealed intricate ridges and grooves on both butterfly and moth scales.
   - Butterfly scales showed well-defined parallel ridges (Fig. 3), whereas moth scales had a more irregular surface texture.(Fig.4)
3. **Scale Density and Overlapping Pattern**
   - Butterfly wings had relatively lower scale density, allowing for structural coloration due to light interference.(Fig.5)
   - Moth wings demonstrated higher scale density, which contributed to their powdery texture and enhanced camouflage (Fig. 6).
4. **Presence of Micropores and Nanostructures**
   - Micropores were observed on the scale surfaces of both butterflies and moths, likely aiding in temperature regulation. (Fig.11 &12)

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www.ijasem.org
Vol 19, Issue 1, 2025

- Moth wings exhibited more pronounced Nano-ridges (Fig. 7) which look like honey comb may contribute to their nocturnal adaptability by reducing light reflection.

5. **Structural Adaptations**
    - The SEM analysis confirmed that butterfly wings are adapted for diurnal activity with enhanced color display mechanisms.
    - Moth wings showed adaptations for nocturnal environments, including anti-reflective properties and increased scale density for better insulation.

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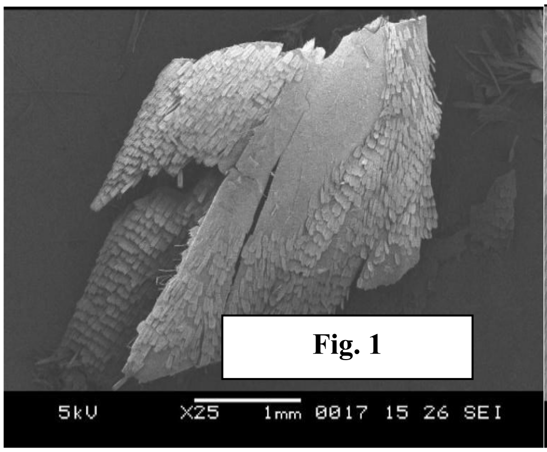


Fig. 1

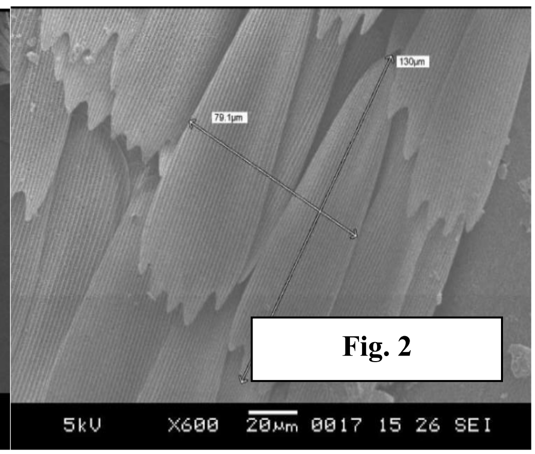
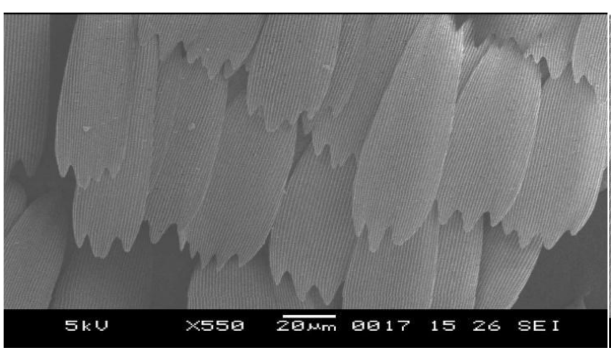


Fig. 2

Fig.1- showing ventral basal view of G. Agamemnon (butterfly) wing scales
Fig. 2- Closed view of flat, overlapping scales



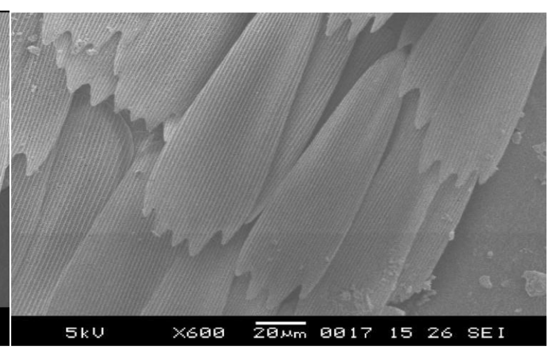


Fig. 3

Fig. 4

Fig.3- showing scales with numbers of ridges; **Fig. 4**- showing length and width of scale

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ISSN 2454-9940

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Vol 19, Issue 1, 2025

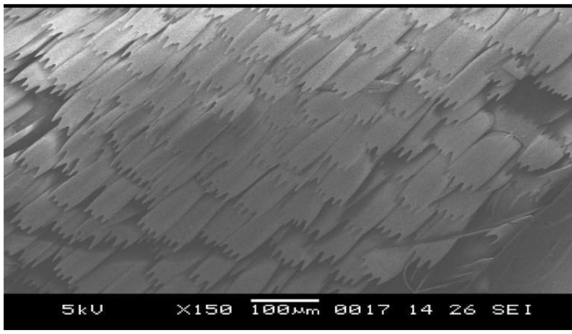

Fig. 5

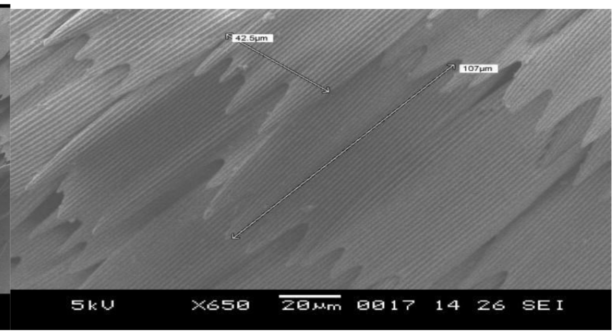

Fig. 6

Fig.5- Types of ridges on scales; Fig.6- length and width between scales

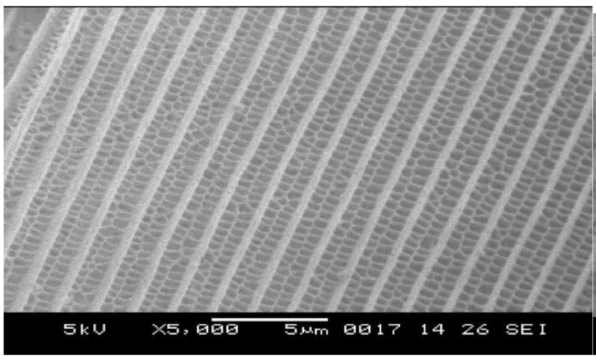

Fig. 7

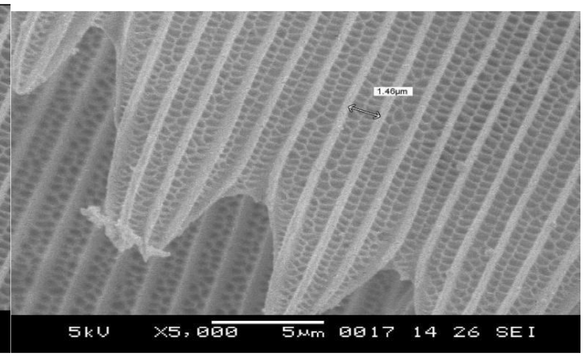

Fig.8

Fig. 7- Showing trabeculae, ribs, microribs which looks like honeycomb; Fig.8- distance between trabeculae

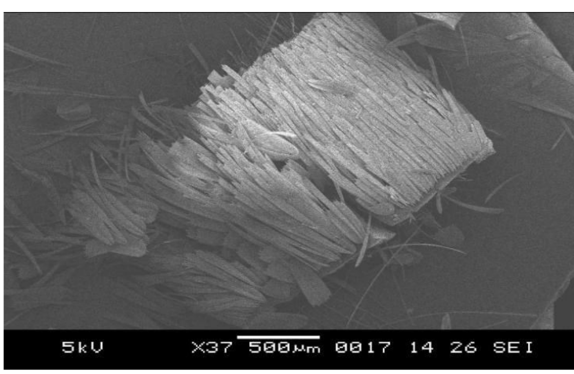

Fig. 9

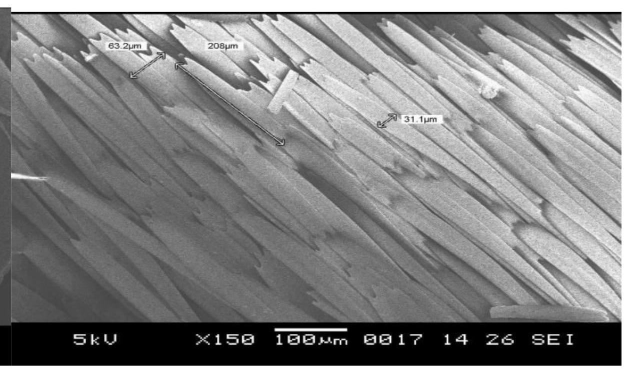

Fig. 10

Fig. 9- showing dorsal basal wing part of *T. coronata*; Fig.10- elongated and much overlapping scales

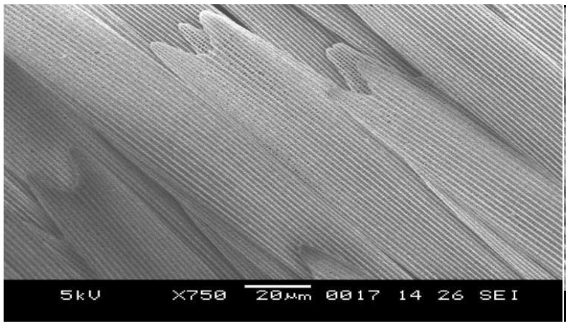

Fig.11

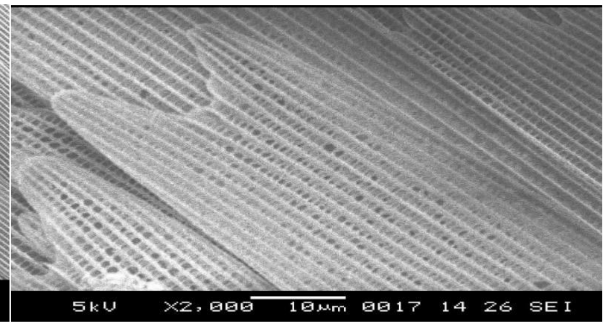

Fig.12

Fig.11- showing ribs on the scales; Fig.12- trabeculae

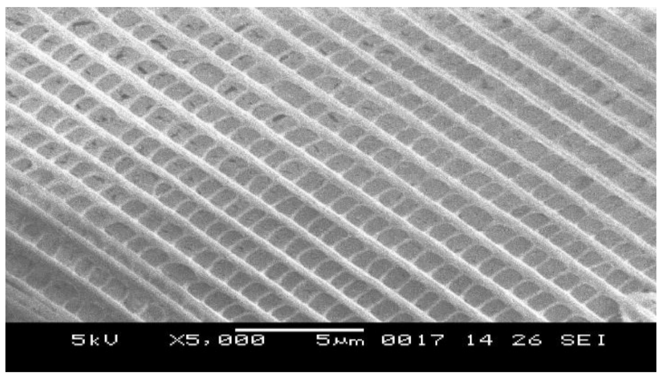

Fig.13

Fig.13- Trabeculae showing the cross ribs look like spider wick

Table 1. – After studied the Scanning electron microscopy of *Graphium agamemnon* and *Thyas coronate*, showing following features are-

Sr.No.	Parameter	*Graphium agamemnon*	*Thyas coronate*
1.	Length of scale	107 µm	208 µm
2.	Width of scale	42.5 µm	31.6 µm
3.	Width between two scale	79.1 µm	63.2 µm
4.	Distance between two trabeculae	1.46 µm	0.5µm

Results

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www.ijasem.org
Vol 19, Issue 1, 2025

The **comparative SEM analysis** of butterfly and moth wings revealed significant differences in **scale structure, density, microstructures, and functional adaptations**. These variations correspond to their distinct ecological roles and flight behaviors.

1. Scale Structure and Arrangement

- **Butterfly wings** exhibited **broad, flattened scales arranged in overlapping rows**, which enhance their vibrant coloration (Fig. 9).
- **Moth wings** had **narrower, elongated scales with a denser arrangement**, contributing to a more cryptic appearance suited for nocturnal camouflage (Fig. 10).

2. Surface Microstructures

- Both butterflies and moths displayed **intricate ridges and grooves** on their scales, as observed in SEM images.
- **Butterfly scales** featured **well-defined parallel ridges**, which may contribute to structural coloration (Fig. 3).
- **Moth scales** had a more **irregular surface texture**, likely aiding in stealth and reducing light reflection (Fig. 4).

3. Scale Density and Overlapping Pattern

- **Butterfly wings** had a **lower scale density**, allowing for **light interference effects** that enhance color vibrancy (Fig. 5).
- **Moth wings** demonstrated a **higher scale density**, contributing to a **powdery texture** and improved camouflage (Fig. 6).

4. Presence of Micropores and Nanostructures

- **Micropores** were identified on the scale surfaces of **both butterflies and moths**, suggesting a potential role in **temperature regulation** (Fig. 11 & 12).
- **Moth wings** exhibited **more pronounced nano-ridges** resembling a honeycomb structure, which may enhance **nocturnal adaptability by minimizing light reflection** (Fig. 7).

5. Structural Adaptations

- **Butterfly wings** are structurally optimized for **diurnal activity**, with **enhanced color display mechanisms** that aid in mate attraction and predator deterrence.
- **Moth wings** exhibit specialized adaptations for **nocturnal survival**, including **anti-reflective properties, increased scale density for insulation, and structural modifications to minimize light detection by predators**.

Discussion

ISSN 2454-9940
www.ijasem.org
Vol 19, Issue 1, 2025

In coloration, camouflage, thermoregulation, and survival strategies. The **comparative SEM analysis** of butterfly and moth wing scales provides valuable insights into their **structural, functional, and ecological adaptations**. The findings align with previous studies on Lepidopteran microstructures, reinforcing the role of scale morphology

1. Scale Structure and Arrangement

Butterfly and moth wing scales serve essential aerodynamic, thermoregulatory, and visual signaling functions. The **broad, flattened scales** of butterflies, arranged in overlapping rows, contribute to their **vivid coloration** (Fig. 9). This arrangement enhances **structural color production**, primarily through **light diffraction and interference** (Kinoshita & Yoshioka, 2005). In contrast, the **narrower, elongated scales** of moths, with a denser packing arrangement, contribute to **stealth and reduced light reflection**, making them less visible to nocturnal predators (Fig. 10) (Stavenga et al., 2018).

2. Surface Microstructures

The presence of **intricate ridges and grooves** on both butterfly and moth scales was confirmed through SEM imaging (Fig. 3, 4). However, **butterfly scales displayed well-defined parallel ridges**, aiding in **color production and light refraction**, which enhances their diurnal visibility (Giraldo & Stavenga, 2007). In contrast, **moth scales exhibited irregular textures**, which likely **reduce light reflection**, thereby aiding in **camouflage and predator evasion** (Fig. 4) (Berthier, 2007).

3. Scale Density and Overlapping Patterns

Butterflies exhibited a **lower scale density**, allowing **light interference to generate iridescent hues** (Fig.5). This aligns with the **structural coloration phenomenon** commonly observed in butterflies such as *Morpho* species, where microscopic ridges amplify specific wavelengths of light (Vukusic et al., 2001).

On the other hand, **moth wings had a higher scale density**, giving them a **powdery texture** that enhances **camouflage and thermoregulation** (Fig. 6). The dense arrangement of scales in nocturnal moths provides an **optical adaptation**, reducing **specular reflection** and making them less visible to predators in low-light conditions (Stavenga, 2006).

4. Presence of Microspores and Nanostructures

The presence of **micropores** on the scale surfaces of both butterflies and moths suggests a role in **temperature regulation and airflow control** (Fig. 11 & 12). These micropores may assist in dissipating heat efficiently, preventing overheating in diurnal species (Ghiradella, 1998).

Additionally, **moth scales exhibited pronounced nano-ridges** (Fig. 7), forming a **honeycomb-like structure** that helps to **minimize light reflection**—a critical adaptation for nocturnal species (Goodwyn et al., 2009). This nano-texturing effect, often termed **anti-reflective**

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www.ijasem.org

Vol 19, Issue 1, 2025

structuring, has been extensively studied in *Noctuidae* moths, where it significantly reduces visibility to predators such as bats and birds (Binetti et al., 2009).

5. Structural Adaptations to Ecological Niches

The results reinforce the theory that **butterfly wings are evolutionarily optimized for diurnal activity**, with **bright coloration mechanisms for mate attraction and predator deterrence**. Conversely, **moth wings are adapted for nocturnal survival**, incorporating **anti-reflective properties, increased scale density, and thermoregulatory adaptations**. The structural differences observed in SEM imaging confirm the **ecological divergence** between butterflies and moths, as reported in prior studies (Stavenga et al., 2018; Yoshida et al., 1996).

Conclusion

The **comparative SEM analysis** revealed **distinct microstructural adaptations** in butterfly and moth wing scales, emphasizing their **functional significance** in survival, camouflage, thermoregulation, and ecological specialization. Butterflies exhibit **broad, ridged scales** optimized for **coloration and light manipulation**, whereas moths possess **dense, Nano-textured scales** suited for **stealth and nocturnal adaptability**. These findings contribute to the broader understanding of **Lepidopteron wing morphology** and its role in **adaptive evolution**.

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ISSN 2454-9940

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Vol 19, Issue 1, 2025

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