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Araştırma Makalesi / Research Article

Revealing Taxonomic Characters Through Wing Microstructures: A SEM Analysis Across Coleoptera, Diptera, Lepidoptera, and Odonata

Kanat Mikro Yapılarıyla Taksonomik Karakterlerin Ortaya Konması: Coleoptera, Diptera, Lepidoptera ve Odonata Üzerinde SEM Analizi

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Abstract

Objective: In this study, wing structures of insect specimens belonging to the orders Coleoptera, Diptera, Lepidoptera, and Odonata collected from Türkiye were examined in detail using Scanning Electron Microscopy (SEM). The main aim of the project is to contribute a novel perspective to classical taxonomy by identifying new microstructural diagnostic characters observable through SEM.

Materials-Methods: The analysis focused specifically on different wing types—elytra, halteres, scaled (pulsed), and membranous wings—which are significant evolutionary adaptations in the animal kingdom, marking the emergence of flight. Comparative morphological analyses were conducted across the four major insect orders, and SEM images were supplemented with stereomicroscope photographs. A comprehensive literature review was also performed to assess the novelty of the approach.

Results: The study revealed order-specific wing structures and uncovered previously undocumented microstructures. These findings provide new diagnostic characters that have potential importance in distinguishing morphologically similar species.

Conclusions: Overall, this SEM-based investigation offers a detailed insight into wing morphology and holds potential to enhance taxonomic resolution within entomological research by revealing novel microstructural characters.

Key words: Insect Wing Microstructure, Comparative Morphology, Diagnostic Characters, Scanning Electron Microscopy (SEM), Taxonomic Differentiation

Öz

Amaç: Bu çalışmada; Türkiye'den toplanan Coleoptera, Diptera, Lepidoptera ve Odonata takımlarına ait böcek örneklerinin kanat yapıları, Taramalı Elektron Mikroskobu (SEM) kullanılarak ayrıntılı bir şekilde incelenmiştir. Projenin temel amacı, SEM aracılığıyla gözlemlenebilen yeni mikroyapısal tanısal karakterleri tanımlayarak klasik taksonomiye özgün bir perspektif kazandırmaktır.

Gereç ve Yöntem: Analizler; hayvanlar aleminde uçuşun ortaya çıkışını simgeleyen ve önemli evrimsel adaptasyonlar olan elitra, halter, pullu ve zarsı kanat tipleri üzerine yoğunlaşmıştır. Dört büyük böcek takımı arasında karşılaştırmalı morfolojik analizler yürütülmüş; SEM görüntüleri stereomikroskop fotoğraflarıyla desteklenmiştir. Ayrıca, yaklaşımın özgünlüğünü değerlendirmek amacıyla kapsamlı bir literatür taraması gerçekleştirilmiştir.

Bulgular: Çalışma sonucunda takımlara özgü kanat yapıları ortaya konmuş ve daha önce belgelenmemiş mikroyapılar tespit edilmiştir. Bu bulgular, morfolojik olarak birbirine benzeyen türlerin ayırt edilmesinde potansiyel öneme sahip yeni tanısal karakterler sunmaktadır.

Sonuç: Genel olarak, SEM tabanlı bu inceleme kanat morfolojisine dair ayrıntılı bir bakış açısı sunmakta ve yeni mikroyapısal karakterleri açığa çıkararak entomolojik araştırmalarda taksonomik çözünürlüğü artırma potansiyeli taşımaktadır.

Anahtar Kelimeler: Böcek Kanat Mikroyapısı, Karşılaştırmalı Morfoloji, Tanısal Karakterler, Taramalı Elektron Mikroskobu (SEM), Taksonomik Ayırım

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INTRODUCTION

Located between Europe and Asia, Türkiye is a peninsula spanning 779,000 km² and surrounded on three sides by seas with distinctly different characteristics. Positioned as a bridge between three continents (Europe, Asia, and Africa), Türkiye encompasses diverse climatic zones within short distances. In addition to its varied climate, changes in elevation also contribute to environmental diversity. This geographical complexity has not only contributed to the diversification of species within Türkiye but has also resulted in significant biological differentiation among continents. The presence of ecological and geographical barriers has led to evolutionary divergence among many organisms. Especially during and after the glacial periods, these barriers largely prevented biological dispersal, promoting population differentiation and limiting the spread of species[1].

Approximately 70% of all described animal species on Earth belong to the Hexapoda superclass, which has led to the current era being referred to as the "Age of Insects." Insects have adapted to virtually every ecosystem except for the deep sea, upper atmosphere, and polar ice caps. One of the primary factors enabling this wide ecological adaptability is the evolution of wings[2].

Flight first evolved in insects, and the advantages of flying—such as movement, foraging, mate searching, and escaping unfavorable conditions—have allowed insects to spread across a wide range of ecosystems[2].

When discussing flight, Diptera (flies) often come to mind due to their unique adaptation in which the second pair of wings has evolved into halteres. These structures help maintain balance during flight, allowing flies to hover, travel long distances with minimal fatigue, and perform agile maneuvers. In fact, Diptera is the only group of known organisms capable of flying backward[2].

As the second-largest order within Insecta, Diptera has diversified into various ecosystems, with many families having adapted to live in close interaction with humans. While many Dipteran families feed on nectar, others have evolved to consume blood or decaying organic matter [2].

Wings, which may have originally evolved to facilitate respiration, later developed into flight structures that have been crucial to insect success[2]. In Diptera, flight has become an advanced and highly adapted behavior. Unlike other insect groups, Diptera has a reduced hindwing pair transformed into halteres, which play a vital role in balance and maneuverability during flight. Additionally, variations in haltere structure, wing shape, and especially wing venation are evident among Dipteran families. The venation subdivides the wing into cells, providing strength during flight. Factors such as flight speed, environmental conditions, habitat, and feeding behavior influence these venation patterns. For instance, members of the family Asilidae (Insecta: Diptera), which are predatory on other Diptera, possess wing venation suited for rapid flight and large halteres for balance during sharp maneuvers. Halteres play a major role in maintaining equilibrium during flight in Diptera[2].

Butterflies belong to the order Lepidoptera within the class Insecta[3]. Approximately 19,000 butterfly species have been described worldwide[4]. There are about 60 species in the UK and Ireland, 850 species across the USA and Canada, and around 500 species in all of Europe[3]. Türkiye, situated at the intersection of important biogeographical regions and characterized by diverse climates and vegetation, hosts rich habitats and species diversity, including many endemic species. A total of 413 butterfly species are known from Türkiye[3], including 45 endemic species and 21 near-endemic species that primarily occur within Türkiye's borders[5,6].

Due to their visibility, ecological importance, and aesthetic appeal, butterflies are one of the best-known insect groups. They are also widely used as model organisms in studies of speciation, community ecology, biogeography, climate change, and insect-plant interactions[3]. With their vivid colors, short lifespans, diurnal activity, flight ability, and harmlessness, they are among the few insect groups generally appreciated by humans[7].

Wing characteristics are critical in classifying insects at the ordinal level. Orders such as Diptera (two-winged), Orthoptera (straight-

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winged), and Lepidoptera (scale-winged) reflect this classification criterion[8]. Among insects, scale-covered wings are a unique feature of butterflies. The arrangement of these scales gives rise to species-specific colors and patterns[9]. Around 14,000 butterfly species can be identified based solely on wing color patterns[10]. Some species even have eye-like markings on their wings for predator deterrence. However, considering the estimated 150,000 butterfly species globally, wing patterns alone are insufficient for species-level identification[8]. Odonata, commonly known in Turkish as “su bakireleri,” “yusufçuklar,” or “helikopter böcekleri” (dragonflies and damselflies), were initially named “Libelle” by Linnaeus in 1758. In 1792, Fabricius established the order Odonata, grouping these species accordingly[11].
Odonates possess chewing-type mouthparts and are predatory insects. Their compound eyes are well-developed, and their antennae are short and bristle-like. Wings are long, narrow, membranous, and typically transparent, with a prominent pterostigma. Their legs are adapted for grasping prey. They are hemimetabolous, with aquatic nymphs feeding on small aquatic animals. As they consume other insects such as Diptera, Coleoptera, Hymenoptera, and Lepidoptera, they are considered beneficial insects. Adults undergo diapause during winter[12].
They possess two pairs of wings arising from the mesothorax and metathorax. Unlike many insects, their wings do not fold over the body. In resting position, wings are either held laterally (Anisoptera) or extended backward alongside the abdomen (Zygoptera). While generally transparent, they may also exhibit iridescent colors, which often differ between sexes[11]. In Zygoptera, the forewings and hindwings are similar in shape, whereas in Anisoptera, the hindwings are broader at the base and attached to the thorax via a petiole-like structure.
Their flight is highly characteristic—typically horizontal. With a wingbeat frequency of up to 30 beats per second, their flight is virtually silent. They can hover and even fly backward over water surfaces. When disturbed, they can take off abruptly and leap several meters through flight [11].

2. MATERIAL AND METHOD

2.1. Selection phase of materials to be used:

The species to be studied was selected from habitats with different vegetation and altitudes and from regions with different geographical and climatic characteristics, usually by sweeping using a net or by direct observation.

2.2. Sample Preparation Using a Stereomicroscope

In this study, elytra, halteres, scaled wings, and membranous wings were used from specimens belonging to the orders Coleoptera, Diptera, Lepidoptera, and Odonata, respectively, housed in the Gazi University Zoology Museum. Specimens were selected from the families Coccinellidae and Cleridae (Coleoptera), one specimen from the family Tipulidae (Diptera), a species from the family Libellulidae (Odonata), and specimens from the family Pyralidae (Lepidoptera).In some cases, the wings were removed directly from pinned specimens, while in others, they were separated from detached samples. The wings obtained from the specimens were first rinsed with water under a stereomicroscope and then placed in 70% ethanol for cleaning. The processed wing structures from the four major insect orders were either mounted on small individual cards or kept as separate samples. During the examination process, Olympus SZX7 and Leica Z-16 APO stereomicroscopes were used.

2.3. Sample preparation stage for SEM Electron Microscope

For scanning electron microscopy examinations, samples fixed in glutaraldehyde will be washed in sodium phosphate buffer. Then, they were passed through an increasing series of ethanol (70%, 80%, 90% and 100%) and dehydration steps were performed.
After dehydration, the samples were placed on standard aluminum SEM studs to which previously prepared double-sided tapes were glued (Fig. 1). After this, all samples were imaged on the JEOL JSM 6060 SEM at 5kV and 10kV at Hacettepe University HUNITEK Center after gold coating using a Polaron SC 502 Sputter Coater.

Figure 1. Coated samples before SEM examination

2.4. Interpretation of SEM photographs and taxonomic evaluation

While the elytra, halteres, scaled, and membranous wings of specimens from the orders Coleoptera, Diptera, Lepidoptera, and Odonata, respectively, revealed limited morphological differences under a stereomicroscope, their ultrastructural features could be observed much more clearly using a Scanning Electron Microscope (SEM). In this study, these diagnostic wing structures—elytra, halteres, scaled, and membranous wings—were examined and their fine structures described in detail. The ultrastructural details revealed through SEM allowed the identification of additional morphological characters not visible under light microscopy. These findings suggest that significant taxonomic characters may be uncovered through SEM analysis. While traditional morphological features often enable the differentiation of species, in some cases, such features may be ambiguous or insufficient for reliable identification. The detailed microstructural information obtained via SEM can provide supplementary diagnostic features, enhance identification accuracy, and support species verification.

RESULTS

In the order Coleoptera, the *elytra* are thickened and hardened structures. Their primary function is to protect the membranous hindwings, which are delicate and unsuitable for direct exposure. The venation is often faint or completely absent. Elytra typically possess a rigid composition and represent a distinctive characteristic unique to Coleoptera. While they may cover the entire body in many species, in some groups, the terminal portion of the abdomen remains exposed.

The specimens examined in this study belong to the Cleridae and Coccinellidae families, and in both cases, the elytra fully cover the body. Using Scanning Electron Microscopy (SEM), microscopic surface structures such as protrusions, sclerite boundaries, and sensilla patterns were observed. These features can serve as diagnostic characteristics for species within the Coleoptera order (Fig 2A-2D; Fig.3A-3F).

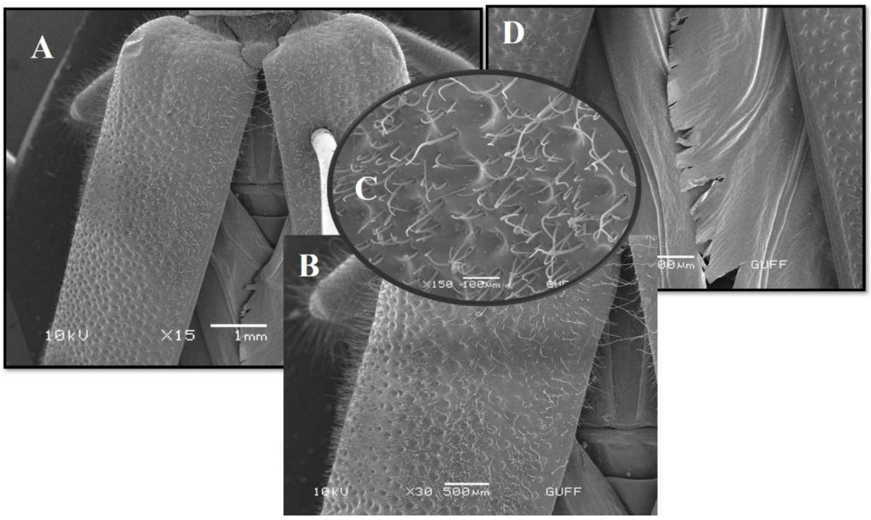

Figure 2. The specimen belonging to the family Cleridae of the order Coleoptera. A–B. Elytral structure; C. Sensilla structures on the surface of the elytra; D. Membranous wings visible between the elytra.

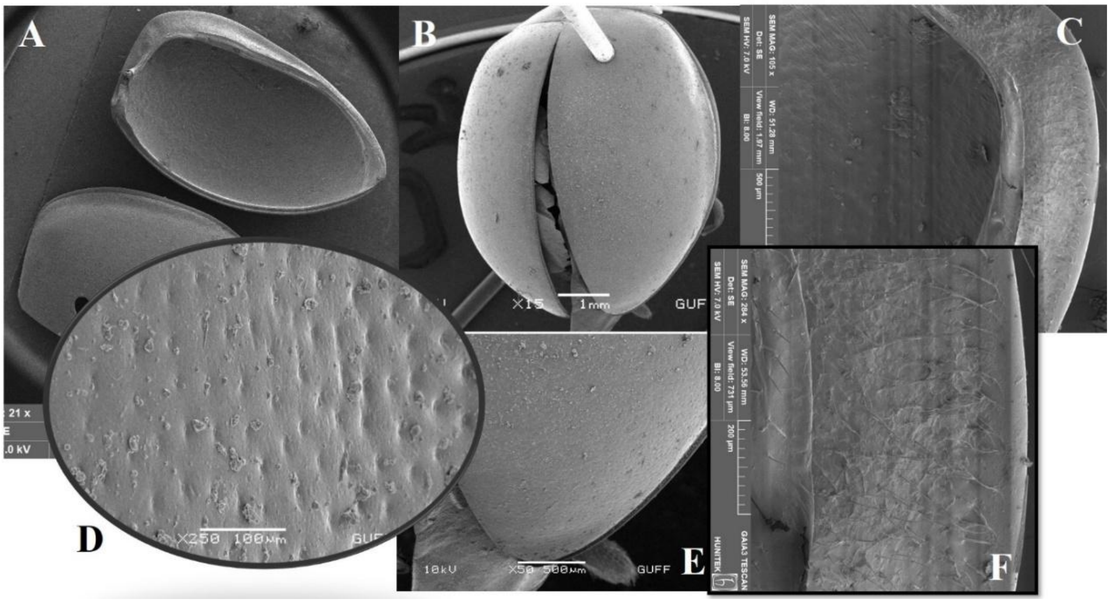

Figure 3. Species belonging to the family Coccinellidae (order Coleoptera): **A–B.** Elytral structure; **C.** Inner surface of the elytra; **D.** General view of the elytral surface; **E.** Outer surface of the elytra; **F.** Sensilla structures located on the inner, stripe-shaped region of the elytral surface.

In the Diptera order, the first pair of wings is membranous, while the second pair has gradually reduced over time and transformed into structures known as halteres (Fig. 4A-4C). These halteres play a crucial role in maintaining balance during flight and enhance sensitivity during changes in direction. They are small, stalked, and knob-shaped structures derived from the hindwings

The presence of halteres is a fundamental diagnostic feature of the Diptera order. The specimen examined in this study belongs to the Tipulidae family. On the surface of the halteres, the variety and density of sensilla (sensory structures), along with the length and thickness of the stalk, may serve as distinguishing characteristics between species (Fig. 5A-5D).

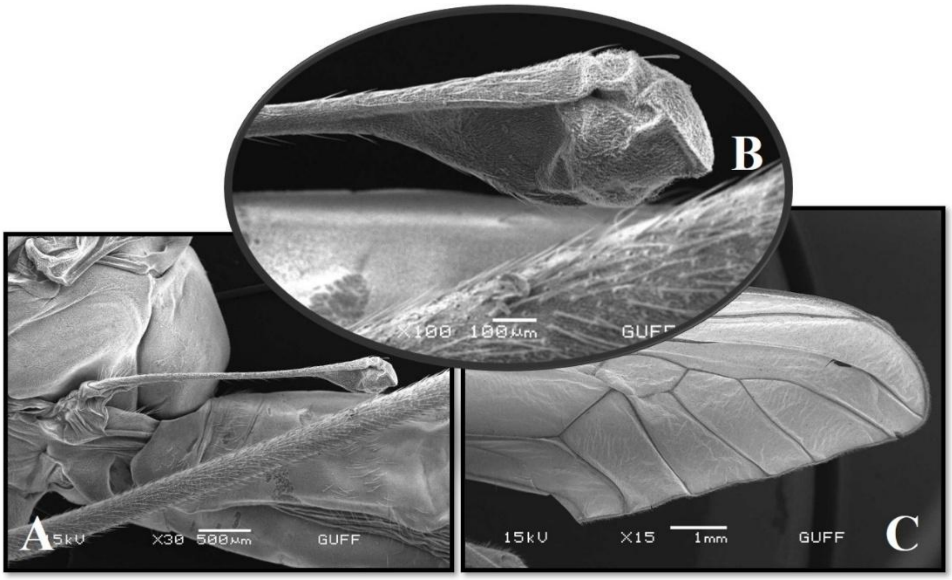

Figure 4. Observed in a species belonging to the order Diptera: A–B. Haltere structure; C. Membranous wing.

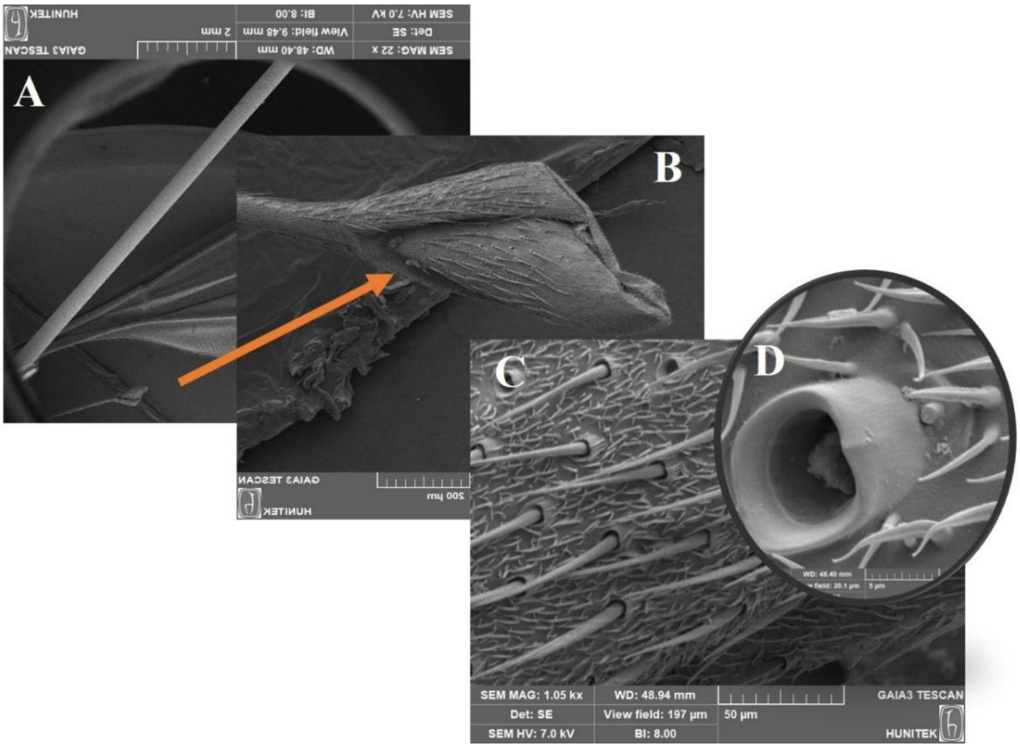

Figure 5. Observed in a species belonging to the order Diptera: **A–B.** Haltere structure; **C-D**. Sensilla structures located on the haltere surface.

The Lepidoptera order is characterized by wings covered with microscopic scales. These scaly wings serve multiple functions, including coloration, camouflage, aposematic signaling, and species identification. The forewings and hindwings are interconnected, facilitating coordinated movement during flight. Due to the dense layering of scales, wing surface structures could not be visualized under Scanning Electron Microscopy (SEM) in this study.

The wing surface is entirely covered with overlapping scales, which may exhibit pigmented or structural coloration. The presence of scales is a unique feature of Lepidoptera and is not observed in any other insect order. Features such as scale morphology, scale-to-scale connections, and microscopic hair-like structures on the scales vary among species and can be used as diagnostic criteria in SEM-based studies (Fig 6A-6C; Fig.7A-7C).

Figure 6. Lepidopteran specimen: **A.** Overall wing morphology; **B.** Scale-covered wing surface; **C.** Scales of varying morphology observed on the wing surface and margins.

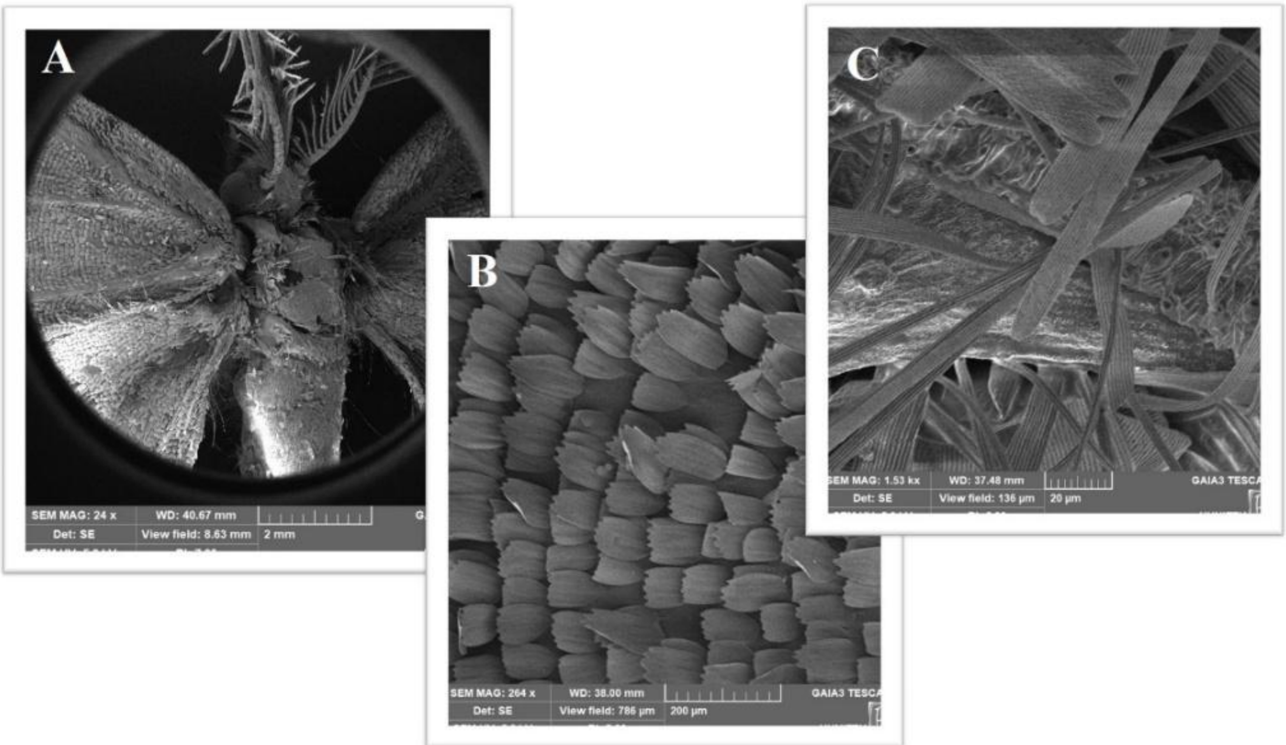

Figure 7. Lepidopteran specimen: **A.** Overall wing morphology; **B.** Scale-covered wing surface; **C.** Scale structures located on the body surface.

In the Odonata order, both the forewings and hindwings are membranous and capable of moving independently (Fig 8). This independent wing movement enables advanced flight abilities such as hovering, flying backwards, and enhanced maneuverability. The wing venation is highly pronounced, and the reinforced veins contribute to structural stability during flight.

A distinct feature of the wing is the pterostigma, a pigmented cell typically located near the distal edge of the wing. Microscopic structures on the membrane surface, interveinal connections, and microstructures surrounding the pterostigma exhibit variation among species and may serve as diagnostic features in comparative studies (Fig. 9).

Figure 8. General view of an Odonata specimen showing the head, thorax, abdomen, and wing arrangement.

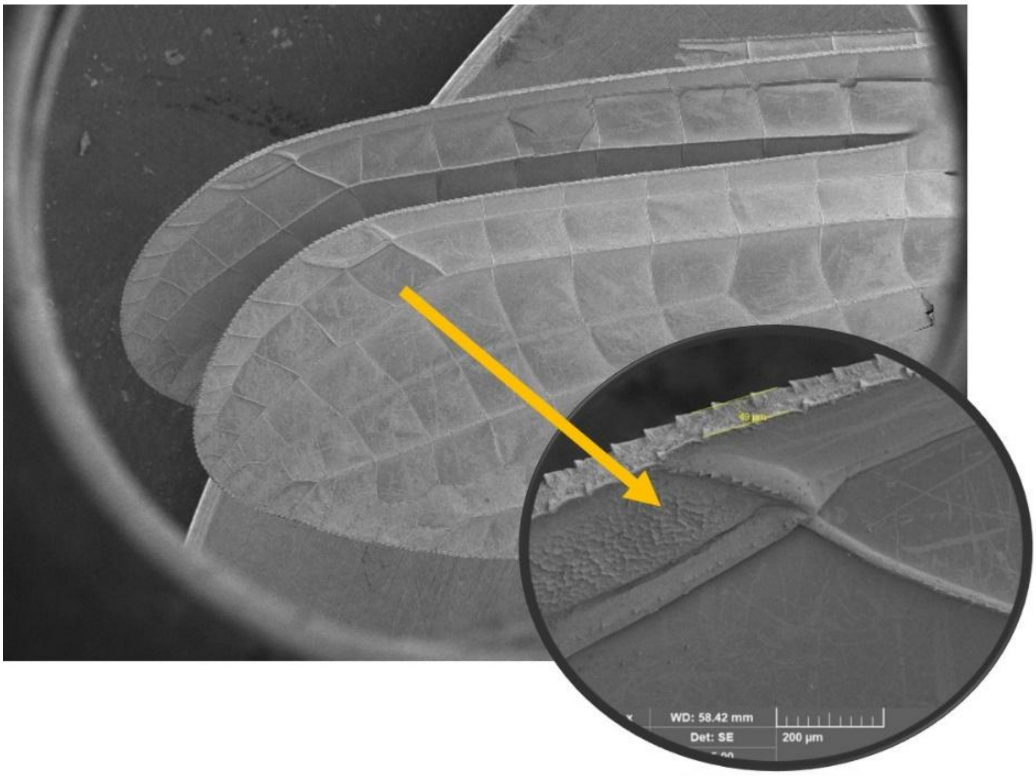

Figure 9. Detailed view of the pterostigma located near the distal region of the wing in an Odonata specimen

Table 1. Comparative Analysis of Wing Morphology in Coleoptera, Diptera, Lepidoptera, and Odonata

Features / Orders	**Coleoptera (Elytra)**	**Diptera (Halteres)**	**Lepidoptera (Scaly Wings)**	**Odonata (Membranous Wings)**
Number of Wings	2 pairs	1 active pair + 1 pair of halteres	2 pairs	2 pairs
Wing Type	Hardened forewings (elytra)	Hindwings modified into halteres	Membranous wings with scales	Membranous wings (independently movable)
Function	Protection; not directly used in flight	Provide balance during flight	Flight + coloration + camouflage	High maneuverability and balance during flight
Foldability	Elytra close; hindwings are foldable	Halteres are fixed	Foldable	Not foldable (especially in Anisoptera)
Distinctive Feature	Hard, pigmented shell-like wings	Stalked, knobbed balance organs	Wings covered with scales; species-specific color patterns	Transparent membranous wings with distinct venation
Morphological Details (SEM)	Microscopic protrusions, surface texture	High density of sensilla, surface protrusions	Scale structures and attachment mechanisms	Pterostigma structure, membrane/vein connection details

DISCUSSION

In summary, the comparative analysis of wing morphology across the insect orders Coleoptera, Diptera, Lepidoptera, and Odonata reveals distinct structural adaptations shaped by their ecological roles and flight strategies. Coleoptera possess hardened *elytra* that serve primarily as protective covers, with minimal flight involvement, marking a unique adaptation among insects. In contrast, Diptera exhibit a highly specialized reduction of the hindwings into *halteres*, functioning as gyroscopic stabilizers during flight—a defining feature of the order. Lepidoptera are distinguished by their densely scaled wings, which not only aid in flight but also contribute to coloration, camouflage, and species recognition. These scales are taxonomically exclusive to this group. Finally, Odonata exhibit independently movable membranous wings with prominent venation, supporting superior flight agility, including hovering and reverse flight. The presence of *pterostigma* and detailed interveinal microstructures further contribute to species-level differentiation. SEM observations underline that each order possesses unique wing surface features—such as sensilla, scale morphology, or vein configuration—that serve as valuable taxonomic characters. This diversity in wing structure highlights the evolutionary divergence and ecological specialization of insect flight mechanisms.

CONCLUSION

Scanning Electron Microscopy (SEM) has proven to be a powerful tool in revealing fine-scale morphological details of insect wing structures that are often invisible under light microscopy. In this comparative study of Coleoptera, Diptera, Lepidoptera, and Odonata, SEM imaging allowed for the identification of microstructures such as surface protrusions, sensilla, scale morphology, vein connections, and pterostigma-associated features. These subtle yet distinct characteristics carry significant taxonomic value, offering additional diagnostic traits that can aid in species-level identification and phylogenetic differentiation. For instance, the microscopic sculpturing and sensilla patterns on the elytra of Coleoptera, the sensilla density and stalk morphology on Dipteran halteres, the overlapping pigment- or structure-colored scales in Lepidoptera, and the interveinal connections and pterostigma structures in Odonata each provide unique markers for distinguishing taxa. Thus, SEM not only enhances our understanding of functional morphology but also enriches the taxonomic resolution by uncovering consistent, species-specific micro-morphological features across diverse insect orders.

RECOMMENDATIONS

Future studies should be conducted on a broader range of specimens, incorporating species from diverse families to enable more comprehensive and comparative analyses. Data obtained through Scanning Electron Microscopy (SEM) should be integrated with traditional morphological approaches and further supported by molecular datasets to enhance the resolution of phylogenetic relationships. The application of digital morphometric techniques and automated image analysis systems is recommended to quantify microscale structures, thereby increasing the objectivity and reproducibility of species identification. Additionally, the establishment of reference image databases for educational and diagnostic purposes would facilitate standardization in taxonomic studies and improve accessibility to high-resolution SEM data across the scientific community.

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