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Araştırma Makalesi / Research Article

SEM Analysis of Hymenoptera (*Apis sp.*) and Diptera (*Tipula sp.*): Revealing Hidden Morphological Traits

Hymenoptera (*Apis sp.*) ve Diptera (*Tipula sp.*) Türlerinin SEM Analizi: Gizli Morfolojik Özelliklerin Ortaya Çıkarılması

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Öz
Amaç: Bu çalışmanın temel amacı, ekolojik ve sistematik açıdan önemli olan iki böcek taksonu—Apis sp. (Hymenoptera) ve Tipula sp. (Diptera)—arasında ayrıntılı karşılaştırmalı morfolojik ve ultra-yapısal analiz yapmaktır. Her iki takson da zengin entomolojik çeşitliliği ile bilinen Türkiye'den toplanmıştır. Taramalı elektron mikroskobu (SEM) kullanılarak, genel vücut morfolojisi, baş kapsülü, antenler, bileşik gözler, ağız yapısı, bacaklar ve kanatlar gibi çeşitli anatomik bölgelerin ince ölçekli yapısal özellikleri ilk kez belgelenmeyi amaçlamaktadır. Yüksek çözünürlüklü görüntüleme ve karşılaştırmalı analiz yoluyla, türlerin daha doğru tanımlanmasına katkı sağlayabilecek taksonomik açıdan bilgilendirici karakterlerin ortaya konması, takımlar arası morfolojik farklılıkların vurgulanması ve polinasyon ile hareket gibi ekolojik işlevlerle ilişkili yapısal adaptasyonların anlaşılmasının geliştirilmesi hedeflenmektedir.
Materyal ve Yöntem: Vücut morfolojisi ve diğer yapılar SEM kullanılarak incelenmiştir. Çalışmada SEM ve stereo mikroskop ile çekilmiş fotoğraflar da yer almaktadır.
Bulgular: Yapılan analizde baş, antenler, ağız parçaları, gözler, bacaklar, kanatlar ve genel vücut morfolojisi gibi temel anatomik yapılarda önemli farklılıklar ortaya çıktı.
Sonuç: SEM incelemesi ağız ve baş yapısında türlerin birbirinden ayrılmasına olanak tanıyabilecek farklı karakterlerin ortaya çıkmasına neden olabilir. Bu yapıların detaylı incelenmesi, yeni tanısal karakterlerin elde edilmesi açısından çok önemlidir. Bu yeni tanısal karakterler, özellikle birbirine benzer türlerin ayrımında olmak üzere, türler arasındaki farkları ve önemli özellikleri belirlemek için kullanılacaktır.
Anahtar Kelimeler: *Apis sp*., Karşılaştırmalı morfoloji, Tanısal karakterler, SEM Analizi, *Tipula sp*.

Abstract
Objective: The primary aim of this study is to perform a detailed comparative morphological and ultrastructural analysis of two insect taxa—*Apis* sp. (Hymenoptera) and *Tipula* sp. (Diptera)—which are ecologically and systematically significant representatives of their respective orders. Both taxa were collected from Türkiye, a region known for its rich entomological diversity. By utilizing Scanning Electron Microscopy (SEM), this research aims to document, for the first time, fine-scale structural characteristics of various anatomical regions, including the general body morphology, head capsule, antennae, compound eyes, mouthparts, legs, and wings. Through high-resolution imaging and comparative analysis, the study seeks to reveal taxonomically informative characters that may contribute to more accurate species identification, highlight interordinal morphological divergences, and improve understanding of structural adaptations concerning ecological functions such as pollination and locomotion.
Materials-Methods: Scanning electron microscope (SEM) examined body morphology and other structures. Photographs taken with SEM and a stereo microscope are also included in the study.
Results: The analysis revealed significant differences in key anatomical structures such as the head, antennae, mouthparts, eyes, legs, wings, and general body morphology.
Conclusions: In addition, SEM microscopy examination may lead to the emergence of different characters in the mouth and head structure that will allow species to be separated from each other. A detailed investigation of these structures is critical to obtain new diagnostic characters. They can be used to determine the differences and essential characters among species, especially in separating similar species.

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Key Words: *Apis sp*., Comparative morphology, Diagnostic characters, SEM Analysis, *Tipula sp*.

INTRODUCTION

Considering that there are approximately 1,800,000 species worldwide, with around 1,000,000 of these being insects, insect biodiversity plays a crucial role in ecosystems due to the high number of species and their reproductive/population density. In the biosphere, insects hold significant ecological roles both in the food chain and through their activities. These small organisms fulfill various environmental functions, ranging from decomposers in nature to primary pollinators of plants. Approximately two-thirds of nearly 300,000 flowering plant species rely directly or indirectly on insects for pollination, and insects often disperse their seeds[1].

Scientifically, bees belong to the order Hymenoptera, superfamily Apoidea, and the group Apiformes.[2] They are distinguished from another group within Apoidea, Sphesiformes, by their branched and plume-like body hairs, as well as the expansion of the first segment of the hind tarsus, which is wider than the subsequent segments. Additionally, the proboscis of the Apiformes group is longer than that of Sphesiformes.[3] Bees classified within the genus Apis Linnaeus, belonging to the tribe Apini of the family Apidae, are commonly called honey bees. The genus Apis is distributed across the Palearctic region—from southern Norway to the Pacific coasts of Russia, as well as throughout Asia and Africa. Today, Apis mellifera has achieved a worldwide distribution due to human intervention.[4]

Honey bees provide substantial economic value through their production of honey and beeswax. Other bee-derived products, such as propolis, royal jelly, pollen, and venom, possess significant pharmacological importance. Moreover, honey is considered a functional food due to its content of vitamins, minerals, organic acids, flavonoids, phenolic acids, amino acids, and enzymes, making it easily digestible, nutritious, and protective and therapeutic properties against various diseases.5 More importantly, bees facilitate pollination in flowering plants, enabling fertilization, fruit, and seed formation. Due to this ability, bees are regarded as the most effective pollinators for numerous plant species.[4]

McGregor (1976), who conducted pioneering research on plant pollination and authored a seminal book on the subject, noted decades ago that 30% of human food originates from plants requiring bee pollination.[6] Delaplane and Mayer (2000) highlighted that 90% of human food 7 comes from 82 cultivated plant species, of which bees pollinate 63 species (77%).[4] With the global human population steadily increasing, food demand is rising parallelly. Pollinators, particularly bees, enhance plant production and thus contribute significantly to agriculture, supporting the continuation of plant life in ecosystems and positively affecting biological diversity.[8]

Within the order Diptera, suborder Nematocera, the family Tipulidae encompasses 4,286 known species and subspecies worldwide, with 1,335 species/subspecies recorded in the Palearctic region.[9] Tipulids predominantly inhabit moist and shaded areas such as grasslands along streams, heathlands, and forests during spring and summer. Their large bodies, long legs, and clumsy flight make tipulids easily recognizable.[10] Known commonly as crane flies or meadow mosquitoes, tipulids can be differentiated from other Tipuloidea families by their wing venation, the length of the last palp segment, and the absence of ocelli.[11]

Body size generally ranges between 7 and 35 mm, though some tipulid species reach up to 64 mm, and particular tropical species exceed 100 mm in length.[10] Both larvae and adults serve as essential food sources for various organisms. For instance, in New York State, 91 bird species are known to feed on tipulids.12 Except for some species with restricted ranges, tipulids are distributed worldwide except in desert and polar regions.[10] Species found in temperate zones are typically univoltine, although many are bivoltine. Some Tipula species exhibit a two-year life cycle (semivoltine), while Tipula carinifrons may have a 4–5 year life cycle (merovoltine).[10]

The first record of Tipulidae fauna in Turkey is Tipula (Lunatipula) nigdeensis, described from Niğde[13] Subsequently, Mannheims and Theowald (1980) reported a total of 33 species from Turkey11, including 17 new species. Later, Theischinger described 42 tipulid species [10, 14, 15]. To date, 132 Tipula species have been recorded in Turkey, of which 55 are endemic.[16]

Although research on tipulids and bees has intensified recently, no comparative studies exist between these two genera. Therefore, this study aims to compare Apis and Tipula species' morphological similarities and differences through scanning electron microscopy (SEM) images, thereby significantly contributing to the literature and guiding future studies.

This research examined morphological differences between bees and flies using SEM microscopy. A species from the genus Tipula within the order Diptera was selected alongside a species from the genus Apis, which holds a critical ecological role as a pollinator within the order Hymenoptera. Despite numerous individual studies on tipulids and bees, no comparative research exists, making the detailed examination of their morphological features via SEM microscopy a valuable contribution to the field

MATERIAL AND METHOD

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.

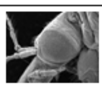
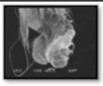
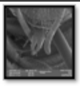
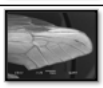
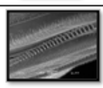
Sample Preparation Using a Stereomicroscope: This study used specimens of *Apis* sp. from the order Hymenoptera and *Tipula* sp. from the order Diptera, housed in the Zoology Museum of Gazi University. The specimens were mounted intact using standard museum techniques. For cleaning, the samples were first rinsed with water under a stereomicroscope and then preserved in 70% ethanol. Specimens from both genera were processed for detailed examination of the head, eyes, antennae, wings, legs, and overall body morphology. During the study, Olympus SZX7 and Leica Z-16 APO stereomicroscopes were utilized.

Sample preparation stage for SEM: Samples fixed in glutaraldehyde will be washed in sodium phosphate buffer for scanning electron microscopy examinations. 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 (Figure 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).

Figure 1. Coated samples before SEM examination

Interpretation of SEM photographs and taxonomic evaluation (Table 1): While the head, eyes, antennae, wings, legs, and general body morphology of Tipula sp. and Apis sp. show limited differences under a stereomicroscope, their ultrastructural features can be observed more clearly using SEM. Morphologically distinct structures, such as the antennae, wings, legs, and head, important taxonomic characters for distinguishing between the two orders, were examined and described in detail through SEM imaging. The fine structural details revealed by SEM may lead to the identification of novel distinguishing characters. Morphological characteristics are often crucial for differentiating between species; however, in some cases, these features may be unclear or insufficient for accurate species delimitation. In such instances, high-resolution ultrastructural details obtained through SEM can provide additional diagnostic information, facilitating identification and supporting accurate species confirmation.

Table 1. Morphological differentiation between *Apis* (Hymenoptera) and *Tipula* (Diptera) species

		Tipula sp. (Diptera)	*Apis sp.* (Hymenoptera)	
Number of Antennal Segments	Typically 13–16 segments; long and filiform antennae.			12 segments in females, 13 in males; elbowed (geniculate), shorter and thicker.
Eye Structure	Large compound eyes located laterally; lacks ocelli (simple eyes).			Large compound eyes; also possesses three ocelli (simple eyes) on the top of the head.
Mouthparts	Weak, adapted for lapping; feeding capabilities are limited.			Strong proboscis adapted for nectar intake; efficient feeding structures.
Wing Structure	One pair of large wings; hindwings are reduced to halteres (balancing organs)			Two pairs of membranous wings; hindwings linked to forewings by hamuli (hooks).
Leg Structure	Extremely long and slender legs, often exceeding body length.			Shorter, more robust legs; hind legs have pollen baskets (corbiculae).
Body Hair	Sparse or absent; not specialized for pollen transport.			Densely covered with branched, feather-like hairs adapted for carrying pollen.
Body Segmentation	Head, thorax, and abdomen are weakly joined and visibly distinct.			Head, thorax, and abdomen are compact and more integrated.
Wing Venation	Wing venation is dense and prominent; important for species identification.			Wing venation is simpler; wings are joined by hamuli.

RESULTS

When comparing the morphology of Tipula sp. (crane fly) from the order Diptera and Apis sp. (honey bee) from the order Hymenoptera, significant differences are observed in structures such as antennae, wings, legs, and body hair-features that are taxonomically and functionally relevant. These two insect groups have evolved under different ecological pressures, which is reflected in their anatomical adaptations.

Antennae are one of the key distinguishing features. In Tipula sp., the antennae are typically long, slender, and composed of 13–16 segments with a filiform structure, suited for mechanosensory functions. In contrast, Apis sp. exhibits elbowed (geniculate) antennae with 12 or 13 segments (depending on the sex), which are shorter and thicker, providing enhanced olfactory capabilities essential for detecting floral cues and pheromones.

Wing structure also marks a significant difference. Tipula sp. has a single pair of large wings, while the hindwings are reduced to small, knob-like halteres that function as gyroscopic stabilizers during flight. On the other hand, Apis sp. possesses two pairs of membranous wings, with the hindwings coupled to the forewings via small hooks called hamuli, enabling synchronized wing movement and more efficient flight, essential for long-distance foraging.

Leg morphology varies considerably. Crane flies have extremely long and slender legs that often exceed their body length, making them appear fragile and contributing to their clumsy flight. Honey bees, however, have shorter, more robust legs, particularly the hind legs of worker bees, which feature specialized structures such as corbiculae (pollen baskets) used for collecting and transporting pollen.

Another notable difference lies in the body hair. Tipula sp. has either sparse or simple unbranched hairs, with no specialization for pollen transport. In contrast, Apis sp. is covered in dense, branched (plumose) hairs that are highly effective in trapping and carrying pollen grains, reflecting their ecological role as primary pollinators.

Finally, mouthpart structure is adapted to their ecological niches. Tipula sp. exhibits weak, lapping-type mouthparts that are often non-functional in adults. In contrast, Apis sp. possesses a well-developed proboscis capable of extracting nectar from deep within flowers, highlighting its specialization in foraging and pollination

DISCUSSION

In summary, the morphological differences between Tipula sp. and Apis sp. are closely tied to their ecological functions and evolutionary pathways. While crane flies are primarily detritivores or non-feeding as adults with limited ecological interaction beyond their role as prey, honey bees are highly specialized pollinators with complex social behavior and significant agricultural and environmental value. These differences are crucial for taxonomy and understanding the broader roles of these insects in their respective ecosystems.

CONCLUSION

In this study, the comparative morphological and ultrastructural characteristics of Apis sp. (Hymenoptera) and Tipula sp. (Diptera) were examined in detail using both stereomicroscopy and scanning electron microscopy (SEM). The analysis revealed significant differences in key anatomical structures such as the head, antennae, mouthparts, eyes, legs, wings, and general body morphology. These findings highlight the distinct adaptations of each taxon to their ecological roles, particularly the specialized pollination-related structures in Apis sp. The use of SEM allowed for the observation of fine structural details that are not discernible under a stereomicroscope, and these may contribute to the identification of novel diagnostic characters valid in taxonomic differentiation. As no prior comparative SEM-based morphological study has been conducted on representatives of Diptera and Hymenoptera in Türkiye, this research provides a valuable foundation for future systematic and taxonomic studies. It contributes to the understanding of interordinal morphological diversity among insects.

RECOMMENDATIONS

Future studies should expand the taxonomic scope by including multiple species from different families within the Diptera and Hymenoptera orders to better understand the variability and evolutionary significance of observed morphological traits. Additionally, integrating SEM-based morphological data with molecular phylogenetic analyses could offer a more comprehensive framework for resolving taxonomic ambiguities and refining classification systems. The application of quantitative morphometric techniques and machine learning-based image analysis may also enhance objectivity in character evaluation and species delimitation. Finally, establishing a standardized image database comprising high-resolution SEM micrographs of diagnostic structures would be a valuable reference tool for taxonomists, ecologists, and entomologists working on insect systematics and biodiversity in Türkiye and beyond.

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