

## **Structural characterization of appendages of different butterfly species (Insecta: Lepidoptera): Review**

### **Abstract**

Insects form a very important and integral part of any ecosystem. Their contributions include agriculture, biodiversity, human health as well as conservation of natural resources. Butterflies belonging to this category falls under suborder of Lepidoptera called Rhopalocera. They have large and vividly coloured wings, which are used for both defence and communication. At rest, their wings are characterised. At rest, their wings are held together upright, which sets them off from all other insects. Butterflies are extremely important in pollination as most of them are important pollinating agent for many plant species, contributing to the enhancement of genetic diversity and stability within the ecosystem. However, their fascinating complex sensory systems go beyond their obvious beauty and importance in ecology. For instance, their legs are more than just organs of locomotion; they are actually very fine devices with sensilla. These sensilla function as receptors so that butterflies would be able to sense various aspects of their surroundings. This review article deals with different species of butterflies and the different sensory adaptations of these sensilla. By looking at these sensory structures, one can gather information about different behavioural patterns, ecological interactions and evolved tactics of an organism.

**Keywords:** Ecosystem, Insects, Lepidoptera, Pollinating agents, Rhopalocera, Sensilla.

### **Introduction**

Insects are very important due to their ecological role, effect on agriculture, diversity and social well- being. They are most prevalent fauna and found virtually everywhere. Number of insects is three times higher as compared to other animals. Some species are listed as pests and many species have not been exposed yet. Pests are plants or animals that harm people or cause them concern. It's a useless insect or another animal that spells things like food, livestock, crops etc. (Khan. ;2021). Lepidoptera is the second largest insect order consisting of moths and butterflies. It is among the most common insect orders

and consists of most easily recognisable insects in the world today (Lin; 2020). Lepidopteran insects exhibit numerous differences in their basic body forms, which have evolved to improve their chances of survival. Almost every class of Lepidopteran insects has a few types of membrane- forming wings. Lepidopteran insects, which include moths, butterflies and nearly all other insects are holometabolous which means they undergo through entire mutation. While mating, adult insects deposit their eggs on plants which serves that serves as their larval host. The first stage of larvae is referred as

caterpillars. They have cylindrical shaped body with properly evolved mouth parts. As soon as they mature, the first stage of larva grows into a pupa. Following the pupa's mutation, an adult insect with sexual development appears. In addition, female insect in many classes can produce between two hundred and six hundred eggs, but in other classes, the variety can produce thirty thousand eggs in a single day. Butterflies are often polymorphic, and many species make use of camouflage, mimicry to evade their predators (McClure & Melanie; 2019). Butterflies are also very important from an ecological perspective as pollinators, as they assist in plant reproduction and thereby the diversity of life forms within the ecosystem. While drinking nectar, butterflies cluster on flowers and carry pollen from one flower to another supplying a abundance of plants with reproductive services, many of which yield edible goods for the organisms. Butterflies also plays an important role in food web as well, as they are food for birds, reptiles and other predators creating an equilibrium in nature. Their prevalence as well as their number clearly are the indicators of health of ecosystem especially when there are changes in environment such as pollution or land degradation. Therefore, butterflies are important for ecosystem stability and biome variety.

Modern technologies like high- resolution imaging and genetic sequencing, continues to uncover hidden facts of Lepidopterans such as their navigation mechansim during migration and their role in nocturnal ecosystems. These findings demonstrate how these delicate insects are actually essential component of biodiversity, unveiling the complex relationships that connect every aspect of the natural world.

### Butterfly Body parts:

Body of butterflies is made up of three segments; head, thorax and abdomen.

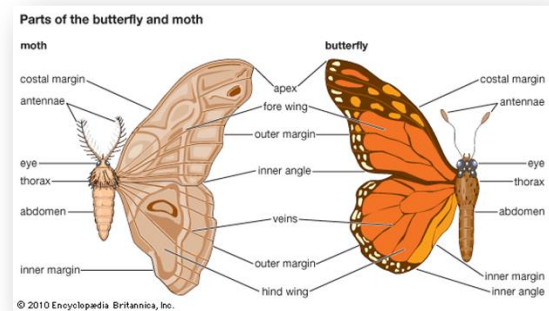


Fig 1: Body parts of Moth & Butterfly.

**Head:** The head is globular in size and top of head is called as vertex; the anterior portion is called as front. Large compound eyes are situated on each side of head between which antennae are present. Antennae help butterflies to find food or mate. The antennae of butterflies are always provided at the extremity with a club-shaped enlargement, and because of this clubbed form of the antennae the entire group are known as the Rhopalocera. The upper lip of butterfly is quite small and is called as labrum. Rudimentary mandibles are present on both sides of mouth. They have a long, straw- like structure known as proboscis, which is coiled under their head. The proboscis is used for sipping nectar and water. The length of proboscis varies; at its base and on either side are place what are known as the maxillary palpi, which are very small.

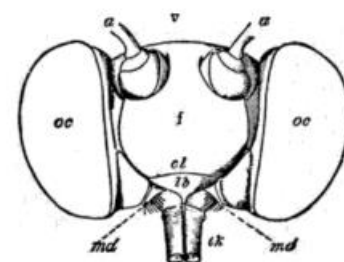


Fig 2: Head of milkweed butterfly: (v) vertex; (f) front; (cl) Clypeus; (lb) labrum; (md) mandibles; (tk) proboscis.

**Thorax:** The central segment of butterfly's body is referred to as thorax. It is somewhat flattened upon its upper surface. Thorax is made of three fused segments, the prothorax, mesothorax, and metathorax, each with a pair of legs. (Carde; 2009). Legs are attached in pairs to these subdivisions of thorax. Therefore sometimes anterior pairs of legs is called as prothoeacic legs, the second pair as mesothoracic legs, and the latter pair as the metathoracic legs. Anterior pair of wings are present on either side of mesothorax.

**Abdomen:** Normally it is made up of nine segments and in most butterflies it is shorter than hind wings. There are various appendages on the last segment which are mainly sexual in nature.

### Butterfly Legs

Butterflies have six legs arranged in three pairs. First part which is nearest to the body is called as coxa, with which a ring like piece trochanter is placed. Femur is attached with trochanter which is connected and forming an angle with tibia. To the tibia, tarsus is attached, the last segment of which bears the claws. These claws are very minute and blunt in butterflies and in moths they are strongly hooked. In some groups of butterflies anterior pair of legs is aborted or dwarfed, either in one or both sexes. It is useful in determining the location of species in their systematic order.

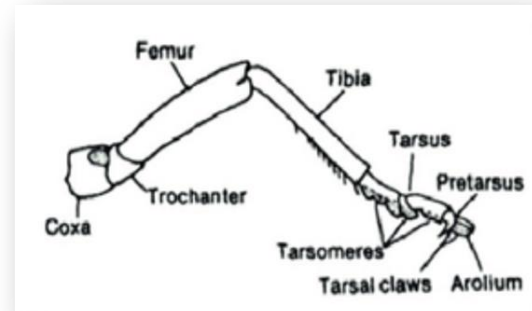


Fig 3: Generalize structure of Lepidoptera leg.

The morphology of the foreleg coxa, trochanter, and femur in butterflies is complex and three-dimensional, making it challenging to depict accurately on a two-dimensional printed page. Additionally, butterflies have hair-like structures on their legs called sensilla. Sensilla also plays important role in feeding. Members of the Diplura and Machilidae have two pairs of abdominal processes in each segment called styli (more laterally located) and eversible vesicles (more medially located) (Machida, 1981).

Lepidoptera also have olfactory organs on their feet, which aid the butterfly in "tasting" or "smelling" out its food. (Heppner, 2008.). In the larval form there are 3 pairs of true legs, with up to 11 pairs of abdominal legs (usually eight) and hooklets, called apical crochets (Cranston; 2004). In some basal moths, these prolegs may be on every segment of the body, while prolegs may be completely absent in other groups, which are more adapted to boring and living in sand. (Carde, 2009). In addition to the structural changes, limbs have also been evolving in number. Most adult insects have three pairs of walking legs, juveniles display variations in both thoracic and abdominal appendages, only some of which are used for walking (McGraw-Hill, 1935).

## Nymphalidae

In Nymphalidae forelegs in both sexes are vestigial and useless for walking. They have reduced forelegs which make them appear as if they have only have two pairs of legs. Therefore butterflies of this family are also called as “Four Footed Butterflies”. In many forms of these subfamilies, the forelegs are kept pressed against the underside of thorax and are often present in males (Bingham, 1905). Heliconian butterflies (Nymphalidae) have reduced prothoracic legs as compared to mesothoracic and metathoracic legs. They apparently have no function in males, but are used by females to drum on the host plant prior to oviposition (Renou 1983; Thiele *et al.*, 2016).

Remaining two pairs of legs in Nymphalidae are extremely hairy and resemble brushes, so they are also named as “Feather legged butterflies”.

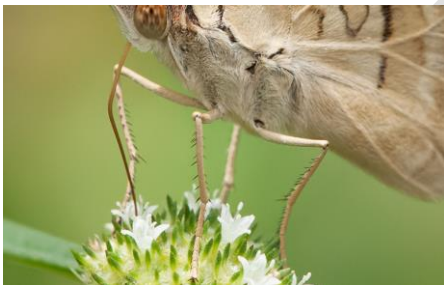


Fig 4: Sharp spiny hairs on legs of Blue Pansy.



Fig 5: *Heliconius melpomene* rudimentary forelegs highlighted.

Several members of nymphalidae are considered model organisms in evolutionary biology (Joron, *et al*; 2006).

## Papilionidae

All six legs are present and well developed in all species of Papilionidae family. They have longest legs (measured from coxa to tarsus) as compared to the species in other families. They have more muscular looking femur as compared to species of other families.



Fig 6: Long slender legs in Papilionidae species.

Tarsal segments are typically long. The body of a Papilionid butterfly is lifted high and some distance away from the food source when it puddles or uses its legs to grasp onto a flower while feeding. Majority of Papilionid species have tibia spurs and femur, tibia and tarsal segments may contain small spiny hairs which help in holding and gripping onto flowers as butterfly feeds. Butterfly's legs have noticable tarsal claws which help to hold on branches or leaves on which the butterfly is resting.



Fig 7: Lime butterfly uses its sharp tarsal claws to hold something while resting.

## Pieridae



Every member of Pieridae family has six completely grown and working legs.



Fig 8: All six developed legs in Pieridae.

Legs of many species typically match the color of butterfly's abdomen or wings. There is usually a darker longitudinal stripe throughout the length of the leg. The legs of butterflies in Pieridae are shorter than those of Papilionidae. The femur is coated with soft, short hairs. They also have tarsal claws and fine hairs on the tarsal area of leg to help it hold on to its perch while it stops to rest or while feeding. They use all six legs to hold or balance on a branch.

### **Riodinidae**

The Riodinidae is a large family of butterflies with a worldwide distribution. They are noted for extreme diversity in wing shape and pattern, which is perhaps the greatest among all families of the Papilionidae (true butterflies)(Harvey, Donald James; 1987). The Riodinids are unique because only female have six fully developed legs. In male specimens forelegs are diminished to hair like tufts similar to those like in Nymphalidae and these legs are not functional. Females generally hold their forelegs tightly against the front of the thorax and it appears like they have only four legs to stand on.

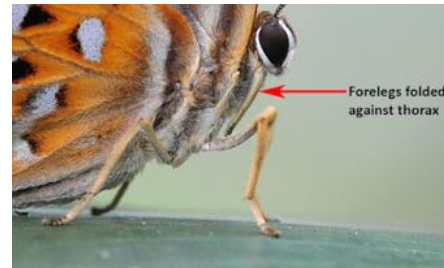


Fig 9: Folded forelegs in females

Their legs are short and thick with tarsal segments, especially at the tips close together, resulting in a banned look. Legs of Riodinids also seems to lack noticeable tibia spur that is found in other species of butterflies.

### **Lycaenidae**

Lycaenidae is the second largest family of butterflies after Nymphalidae (brush-footed butterflies) with over 6000 species worldwide (Pierce & Naomi; 2002). Members of Lycaenidae are also called Gossamer-winged butterflies. The diversity of leg structure among the numerous genera of Lycaenidae is truly remarkable. Lycaenid family also shows different leg pattern as compared to other families. Female Lycaenids have functional and completely segmented foretarsai, while nearly all male species feature fused tarsomeres. Males also lack foretarsal claws. However, in both genders foretarsai are used for walking. As a rare exception (70 out of roughly 4500 described species ;Bridges; 1988), segmented foretarsai with claws occur in males of a few Lycaenidae genera. The legs of several species of the Miletinae subfamily (Lycaenidae) is quite amazing like in Miletus species. The legs taper towards the joints and seem flattened.



Fig 10: Flat forelegs

The forelegs seem to be devoid of tarsal claws at least in one of the species, and the tarsal are wider towards extremities. The legs of other species in the Theclinae subfamily are short, stout and occasionally banded in black and white while some other species have six legs that seems like that they are wearing white stockings.



Fig 11: Six legged Theclinae with pure white legs

The tibia spur is also present in many species across the family. Tarsus is also covered with small spiny hairs which allow butterfly to hold on to its perch firmly.

### Hesperiidae

Skippers are group of butterflies that are placed in family Hesperiidae within the order Lepidoptera. Previously they were assigned to distinct superfamily Hesperioidea. They are named so because of their quick, fluttering flight movements. All six legs of skippers are completely developed. In many species femur is covered with thick hairs which gives the illusion of being fairly heavy and muscular.



Fig 12: Exceptionally hairy femur of some Hesperiidae species.

In some species the hairs extend to the next segment, tibia. The hairs on Tibia are thick and noticeable. In some species there is an additional pair of tibia spurs on the hind legs. The hind legs of certain unique species, such as the White Banded Flat, have undergone further evolution to develop a noticeable hair tuft that covers the tibia and extends beyond the leg.

### Leg sensilla in butterflies

A sensillum (plural- sensilla) is a sensory organ that emerges from cuticle of exoskeleton or sometimes rests inside or under it. Sensilla appear as small hairs over individuals's body. Inside each sensillum there are two or four sensory neurons. These neurons or receptors gather information about environment in which the arthropod resides (Alexander ;2007). Sensilla are specially shaped according to the type of information they are gathering. Certain similarities in the structure of sensilla in different arthropod groups can be the result of adaptations to specific environments. In holometabolous insects larval sensilla appear to be structurally quite advanced, and more complex than in adult.

### Campaniform Sensilla

Campaniform sensilla are a class of mechanoreceptors found in insects, which respond to local stress and strain within the animal's cuticle. Campaniform sensilla function as proprioceptors that detect

mechanical load as resistance to muscle contraction (Zill; 2012). Sensory feedback from campaniform sensilla is integrated in the control of posture and locomotion (Tuthill & Wilson; 2016). Each campaniform sensillum consists of flexible dome, which is embedded in a spongy socket within the cuticle and innervated by the dendrites of a single bipolar sensory. Campaniform sensilla are often oval-shaped with long axes. Campaniform sensilla are distributed across the body surface of many insects. The fruit fly *Drosophila melanogaster*, has over 680 sensilla (Dinges; 2020). Campaniform sensilla are located in regions where stress is likely to be high, including on the legs, antennae and wings (Dinges ; 2020)

### Campaniform sensilla on legs

On the legs, groups of campaniform sensilla are located close to the joints on all segments except for the coxa, with most sensilla located on the proximal trochanter (Harris; 2020). The number and location of sensilla on the legs varies little across individuals of the same species (Dinges; 2020) and homologous groups of sensilla can be found across species (Harris; 2020).

### Function of leg Campaniform sensilla

Campaniform sensilla on legs are activated during standing and walking. Their sensory feedback is thought to reinforce muscle activity during the stance phase (Zill; 2015) and to contribute to inter-leg coordination, much like sensory feedback from mammalian Golgi tendon organs (Pearson; 2008). Feedback from leg campaniform sensilla is also important for the control of kicking and jumping.

### Structure

A channel extending through the entire thickness of the cuticle, topped with a

dome- shaped cap, and lined with a material exhibiting distinct staining reactions compared to the surrounding cuticle. Usually, the cap membrane arches over the surface of the cuticle, but sometimes it can be recessed such that its peak lines p with the surface. Sensilla are present in clusters in specific pattern. The single sensory cell is large and has a distal extension on the underside of the rounded cap's centre by a highly refractive structure. In oval sensilla, this extension may also extend down the long axis. In some transmitted light configurations, this body makes the dome which seems to be a pore. Other modifies hypodermis calls are usually absent, however occasionally chitogenous cells appear.

### Monarch Butterfly

There are six legs on the monarch butterfly. The two forelegs, situated just behind the head, are so tiny that they must be seen under a microscope or magnifying lens. Three pairs of tiny, copper- colored needles are carried at the tips of each female foreleg.



Fig 13: Male and female forelegs.

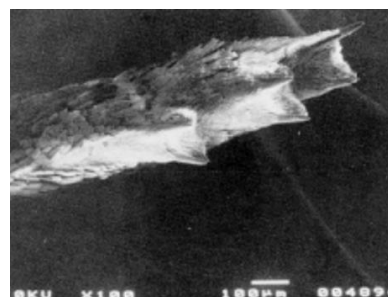


Fig 14: Six needles of female foreleg magnified by SEM.

The female assesses if the milkweed is suitable for depositing her eggs by tasting

and smelling the secretions. She uses the smell sensors on the tips of each antenna and the taste sensors on the bottom of the other four legs to ascertain this. Male forelegs are different in shape.

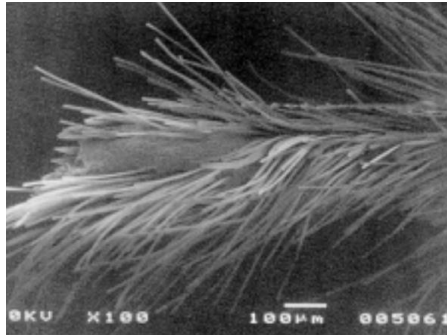


Fig 15: Tip of male foreleg (x 100) by SEM.

While mating, the male monarch utilizes its forelegs to stay balanced while his other two pairs of legs grip the female. He uses his forelegs to grasp a flower or other item.

The taste sensors on the ends of the two large pairs of legs on both sexes are 2000 times more sensitive to tasting sugars than the human taste sensors. The red smelling sensors on each of the antenna tips are about 5000 times more sensitive to smell than the human nose sensor.

Scales cover the dorsal and lateral surfaces of the lycaenid male foretarsus, but not the distal ventral surface, where many trichoid sensilla occur. A- type and B- type trichoid sensilla on lycaenid male forelegs as well as distinctive setae that also may be trichoid sensilla.

**1) A- type trichoid sensilla (spines):** Spines are stout trichoid sensilla, sometimes called “bristles”, that have fluted sides and occur primarily on the ventral surface of lycaenid foretarsus (Bates 1861). Similar spines are occur on ventral abdomen of some lycaenids and riodinids (Inoue and Kawazoe; 1966)

**2) B- type trichoid sensilla:** These sensilla are scattered over the dorsal, lateral and

ventral sides of the lycaenid male foretarsus. At magnifications below about 1000 times, B- type trichoid sensilla on male lycaenid fore-tarsi appear to be smooth-walled (Robbins 1987a) but at magnifications above 8000 times, they have a variable fine structure.

## Riodinidae

Unlike male lycaenid fore-tarsus, the male riodinid fore-tarsus is not used for walking (Bates; 1861), and is covered dorsally, laterally ad ventrally with elongate scales. The male riodinid pretarsus is like that of lycaenids in that it lacks claws. The occurrence of spines (A- type trichoid sensilla) on male fore-tarsai differs between lycaenids and riodinids. Male riodinids lack forelegs spines (Bates; 1861). Usually one or two “microtrichia” is present at tarsal tip and sometimes a few B- type trichoid sensilla. Many setae on the tarsus resemble B- type trichoid sensilla at lower magnifications, but at higher magnifications appears to be scales with longitudinal ridges ad scutes.

## *Styx infernalis*

The structure of the male *S. infernalis* foretarsus and pretarsus is similar to those of riodinids in some respects, and is unique in others. The *S. infernalis* male forelegs pretarsus lacks claws (Harvey; 1987). One foreleg had no spines (A- type trichoid sensilla) on the tarsus, whereas the other foreleg from same specimen had a spine on the second tarsomere. The fine structure of these trichoid sensilla is superficially more similar to that of ‘macrotrichia’ than to that of lycaenid and riodinid B- type trichoid sensilla.



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