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Comparative Morphology of Male and Female Genitalia and Its Role in Reproductive Behaviour of Biting Midges (Diptera: Ceratopogonidae) from the Gangetic Plains of Uttar Pradesh, India

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Abstract

Biting midges belonging to the family Ceratopogonidae represent a diverse group of dipteran insects widely distributed across tropical and temperate regions. Their genital morphology plays a crucial role in species identification, reproductive isolation, and mating success. The present study investigates the comparative morphology of male and female genitalia in selected species of biting midges collected from the Gangetic plains of Uttar Pradesh, India. Specimens were collected using light traps and sweep nets from different aquatic and semi-aquatic habitats. Morphological examination was conducted under a compound microscope after appropriate slide preparation. Distinct differences were observed in the genital structures of males and females, particularly in the aedeagus, parameres, and gonocoxites in males and spermathecae in females. These morphological variations are essential for species-level taxonomy and influence mating behaviour and reproductive success. The findings contribute to the understanding of reproductive biology and taxonomy of Ceratopogonidae and highlight the significance of genital morphology in evolutionary adaptation and species recognition.

Keywords: Biting midges, Ceratopogonidae, genital morphology, reproductive behaviour, Diptera taxonomy

1. Introduction

The family Ceratopogonidae (Order: Diptera), commonly known as biting midges, comprises a diverse group of minute flies distributed throughout tropical, subtropical, and temperate regions of the world. These insects are of considerable medical, veterinary, and ecological importance because several species act as vectors of pathogens affecting humans and domestic animals. Members of genera such as *Culicoides* and *Forcipomyia* are known to transmit viruses, protozoans, and filarial nematodes responsible for diseases including bluetongue, African horse sickness, and other arboviral infections in livestock. Due to their economic and epidemiological significance, Ceratopogonidae have attracted increasing attention in the fields of entomology, vector biology, and taxonomy.

Accurate identification of species within Ceratopogonidae is often difficult because external morphological characters are highly variable and sometimes influenced by environmental conditions. Consequently, genital morphology has become one of the most reliable taxonomic criteria for

distinguishing closely related species and subfamilies. In Diptera, the male and female genital structures exhibit species-specific patterns with relatively low environmental variation, making them important diagnostic tools in systematic studies.

The male genitalia of Ceratopogonidae consist primarily of tergite IX, sternite IX, gonocoxites, gonostyli, parameres, and aedeagus, while the female genitalia include spermathecae, spermathecal ducts, cerci, postgenital plate, accessory glands, and ovipositor structures. Variations in the shape, size, degree of sclerotization, and arrangement of these structures are useful not only for taxonomic differentiation but also for understanding reproductive adaptations and evolutionary relationships among species.

Genital morphology is closely associated with mating behaviour and reproductive success in insects. During copulation, structural compatibility between male and female genitalia ensures proper alignment, sperm transfer, and fertilization. In biting midges, mating may occur in swarms, on water surfaces, or in sheltered habitats, and males often exhibit rotational movements of the abdomen to achieve successful copulation. Such behavioural adaptations are strongly influenced by the structure and flexibility of genital organs. Female spermathecae further play a crucial role in sperm storage and controlled fertilization during oviposition.

Despite the ecological and veterinary importance of Ceratopogonidae, comparatively little information is available on the detailed genital morphology and reproductive behaviour of species occurring in the Gangetic Plains of Uttar Pradesh, India. The region provides favourable environmental conditions such as high humidity, wetlands, agricultural fields, and riverine ecosystems that support the breeding and diversity of biting midges. However, comprehensive comparative studies on male and female genital structures of different subfamilies from this region remain limited.

Therefore, the present study was undertaken to investigate the comparative morphology of male and female genitalia in different subfamilies of Ceratopogonidae occurring in the Gangetic Plains of Uttar Pradesh and to analyze the role of these genital structures in reproductive behaviour, mating orientation, and species identification. The study also aims to contribute toward the development of reliable taxonomic characters for accurate identification of biting midges of medical and veterinary importance.

2. Objectives of the Study

1. To study the morphological characteristics of male genitalia in selected species of biting midges.
2. To examine the structural features of female reproductive organs.
3. To compare the genital morphology between male and female individuals.
4. To analyze the role of genital structures in mating behaviour and reproductive success.
5. To contribute to the taxonomic identification of Ceratopogonidae species in the Gangetic plains of Uttar Pradesh.

3. Materials and Methods

The proposed work was carried out by following methods given below.

(i) Collection Techniques and Source of Material

The imagoes of biting midges were collected by using aspirator. Swarm forming species of biting midges were collected by Diptera sweeping net. An UV-light trap method was used for collecting a variety of species in the field during night.

Virgin males and females were obtained from pupae collected from the field. Pupae were washed from sand sample by magnesium sulphate floating method and were rinsed thoroughly and then separated according to sex before being placed in sealed plastic dishes containing a shallow layer of damp sand.

The emergence of adults was induced by placing them in high temperature (30–35°C) in incubator. The conspecific male and female individuals were transferred into 3×1 inch glass vial containing an inclined strip of filter paper and a small wad of cotton wool saturated with 10% sucrose solution.

(ii) Transport of Living Material

Pupae were transported to the laboratory in small sand containers under moist conditions. Adult specimens were maintained in glass vials and rearing cages for further observation on genital morphology and reproductive behaviour.

(iii) Preservation of Material

Collected specimens were preserved in 70% ethanol. Male and female genitalia were dissected carefully under stereo binocular microscope and mounted on slides for detailed study.

(iv) Morphological Studies

Detailed studies of genitalia were carried out under compound microscope. Structures such as tergite IX, sternite IX, gonocoxite, gonostylus, parameres, aedeagus, spermathecae, cerci and postgenital plate were examined in different subfamilies of Ceratopogonidae.

(v) Observation of Reproductive Behaviour

Mating behaviour of biting midges was observed under laboratory conditions. Different mating positions such as face-to-face, end-to-end and 90° back position were recorded during copulation.

(vi) Dissection and Examination:

Male and female were first examined in the 70% ethanol and important character were noted down. The wing, genitalia, legs and antenna were dissected out for the identification of the spp.

The genitalia placed in 10% lactic acid for cleaning. This material was transfered on the glass bell slide which contains a drop of glycerine. Larvae were dissected under stereoscopic binocular and head, abdomen etc were passed through successive bath in 2 propanol, 2 pronal layered over cedenwood oil, cedenwood oil and finally Canada balsam. Pupa was mounted in the Canada balsam. Before dehydration pupa was cleared in 2 propanol. It was then transferred in clove oil and finally mounted in Canada balsam for permanent preparation.
was dehydrated by the Method developed by School of Entomology.

(vii) Measurement and Statistics:

Measurement were taken with the measuring micrometer for all stages, statistics were provided such as range mean and standard deviation.

(viii) Photography:

To enhance the discription the desirable event of the reproduction behaviour were photographed with the help of Nikon Photographic Instrument.

(ix) Terms for structure and illustration:

For adult and larvae saether's 1981 Terminologies were followed. With 1989 terminology were used for imagoes with some exceptions.

4. Results

The examination of collected specimens revealed clear differences in genital morphology between male and female biting midges and revealed significant variation in the genital structures among different subfamilies of Ceratopogonidae. And.

Male Genitalia in Ceratopogonidae

The male genitalia in ceratopogonidae consist of several important structures collectively known as the male terminalia. The basic components include:, Tergite IX (Epandrium), Sternite IX (Hypandrium), Gonocoxite (Basimere), Gonostylus (Distimere). Parameres, Aedeagus (median phallic organ). Male genitalia showed modifications in tergite IX, gonocoxite, gonostylus and aedeagus.

Comparative Morphology of different types of genitalia of sub families:—

The ceratopogonidae is divided into four sub families, Dasyheleinae, Leptoconopinae, Forcipomyiinae and Ceratopogoninae. We can identify the differentiation between them with the help of genitalia. The genitalia is the most important character to differentiate the sub families. Different type of genitalia found in the different type of subfamily. The male genitalia are differentiated by the following characters and modification of genitalia is described here.

1-Dasyheleinae:

In male genitalia of this sub family the tergite IX is may rectangular, triangular, simple and rounded to oval shape. It have 4–16 setae. Apicolateral process is present or may be absent but when it present tubular, lobe like bifurcated with at least single seta distally. Gonocoxite distally usually simple tubular anterolateral margin with setal patch. Setae is arranged in two to three rows. Gonostylus is most prob. single in some case it is bifurcated slender or thick bare or setose, distally pointed or broad apex. Paramere asymmetrical, paramere usually divided, division of paramere usually leaf like broad tubular thin. Aedeagus usually triangular. In Dasyheleinae, tergite IX was generally rectangular or triangular with several setae and gonostylus displayed variable forms including slender or bifurcated types.

2-Leptoconopinae:

In the male genitalia, anal anal tergal band H shaped. Gonocoxite cylindrical, setose. Gonostylus setose with apical megaseta. Leptoconopinae:The tergite IX is generally well developed and strongly sclerotized.The gonocoxite is cylindrical and moderately elongated.Gonocoxite bears several setae on its surface.The gonostylus is slender and movable.Gonostylus is usually setose and curved inward.A distinct apical megaseta is present at the tip of gonostylus.The parameres are generally simple and symmetrical.The aedeagus is narrow and well sclerotized. In Leptoconopinae, the gonocoxite was cylindrical and longer than the gonostylus, while the gonostylus possessed an apical megaseta.
These genital characters are important for the identification of species in this subfamily.

3-Ceratopogoninae:

In male abdomen slender IX tergum plate like with or without apicolateral process.IX sternum usually narrow, in some sp. broad with deep caudomedian excavation.Gonocoxite lobules elongated, broad and setose.Gonostylus usually broad based narrow distally end with or without megaseta and usually bare.

In Ceratopogoninae, tergite IX appeared plate-like and the gonocoxite was elongated and setose is important for the identification of species in this subfamily.

4-Forcipomyiinae:

Male abdomen slender and terminalia well developed.Tergite IX usually broad and sclerotized.Gonocoxite elongated and cylindrical.Gonocoxite generally bears several setae on its surface.Gonostylus slender and curved inward.Gonostylus usually narrow distally and pointed at apex.Parameres generally symmetrical and simple in structure.Aedeagus narrow and well developed.Male genitalia are important for species identification.The structure of gonocoxite and gonostylus is a key diagnostic character..

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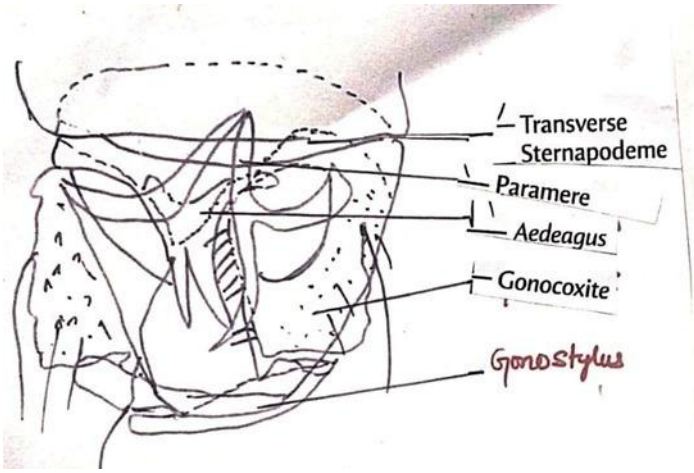

Figure- 1 male genitalia Dasyheleinae

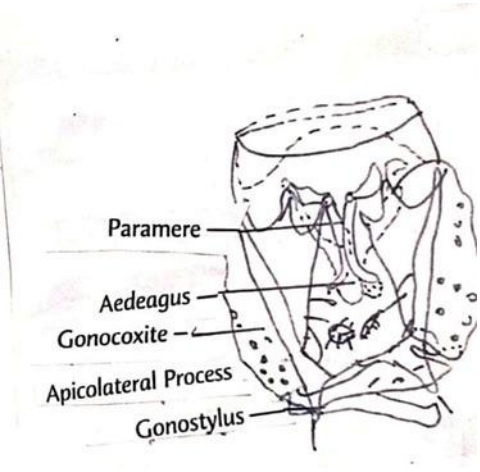

Figure- 2 male genitalia Leptoconopinae:

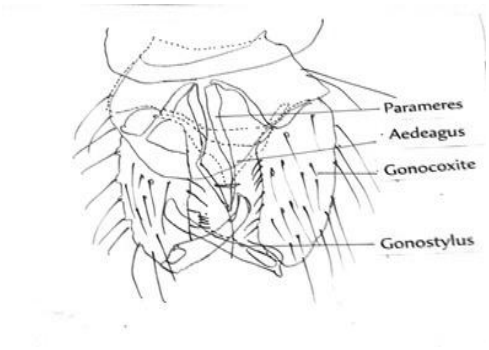

Figure- 3 male genitalia Ceratopogoninae

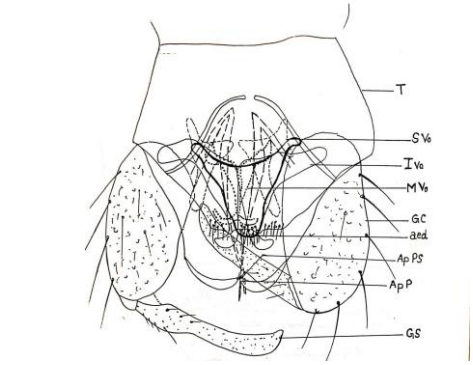

Figure- 3 male genitalia Forcipomyiinae

Female Genitalia

The female reproductive structures collectively form the ovipositor, which is involved in egg deposition.Important structures include:Genital chamber (vagina),Spermatheca (sperm storage organ),Accessory glands,Cerci,Postgenital plate.The spermatheca stores sperm after mating, allowing fertilization of eggs during oviposition. Female genitalia also showed considerable variation, particularly in the structure and number of spermathecae, shape of cerci and development of the ovipositor. These morphological differences were useful for distinguishing subfamilies and species. These structures function together to grasp the female during copulation and transfer sperm into the female reproductive system Structural Modification of Genitalia in Subfamilies

1. **Dasyheleinae**: Female genitalia show variations in the shape of tergite IX, which may be rectangular, triangular, or oval.,Gonostylus may be single, bifurcated, or trifurcated.Parameres are often asymmetrical and leaf-like.Female genitalia usually contain a single spermatheca with a poorly developed neck. Figure-1

2. Leptoconopinae:Female genitalia are characterized by an H-shaped anal tergal band.The gonocoxite is cylindrical and setose, while the gonostylus contains an apical megaseta.\Females possess 2–3 spermathecae, with well-developed spermathecal necks.

3. Ceratopogoninae: Female tergite IX is plate-like, sometimes with an apicolateral process.Gonocoxite is elongated and setose, and gonostylus narrows distally.Females may have 1–3 spermathecae, and the postgenital plate is well developed.

4. Forcipomyiinae: Females usually possess two spermathecae, which are oval or spherical.Cerci are short and narrow, and the postgenital plate is well developed.

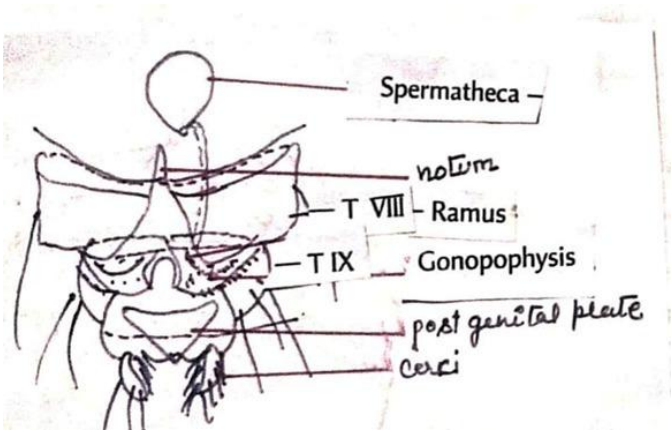

Figure-1 Female genitalia Dasyheleina**e**

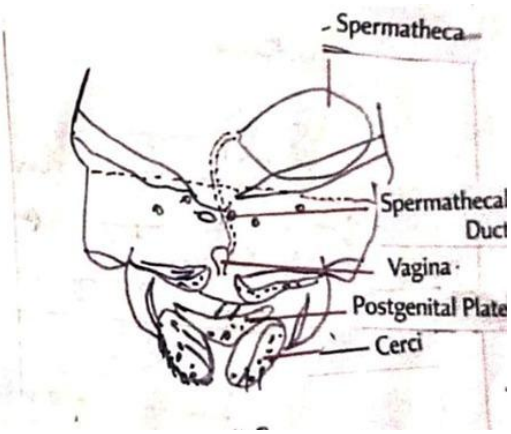

Figure-2 Female genitalia Leptoconopinae:

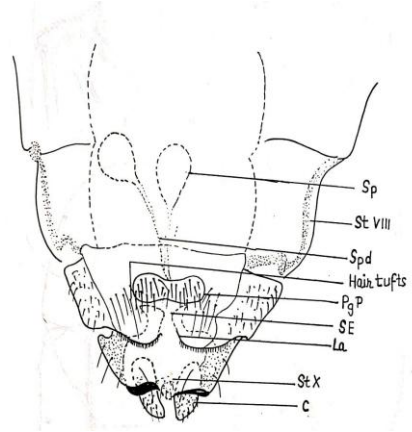

Figure- 3 Female genitalia Ceratopogoninae

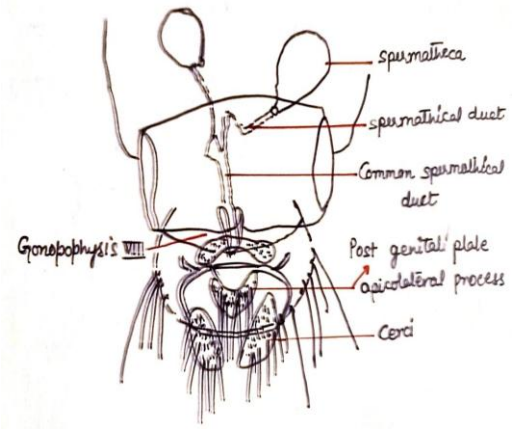

Figure-4 Female genitalia Forcipomyiinae

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5. Mating Behaviour in different spp.:

Mating in Ceratopogonidae occurs in three main situations:Swarm mating,Mating on water surface,Pair mating on sheltered surfaces

Types of Mating Orientation:Three main mating orientations have been observed:Face-to-face position,End-to-end position,90° back position

Male genitalia may undergo flexion and rotation (90°–360°) to achieve successful copulation.

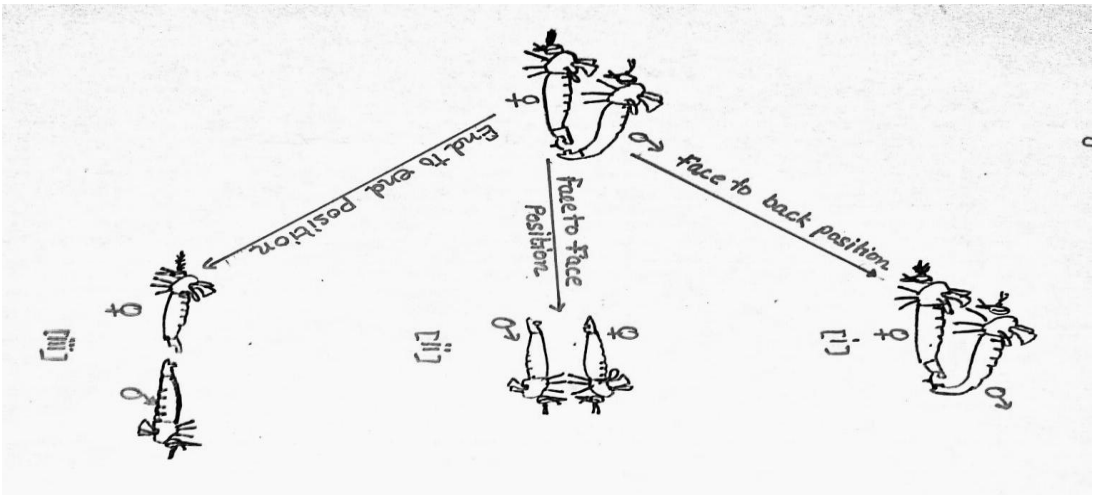

FIGURE-Three types of Mating Orientation

Mating behaviour in biting midges involves a sequence of interactions between male and female individuals. Males approach females and initiate copulation by grasping the female using legs and genital structures. Proper mechanical compatibility between genital organs allows successful insertion of the aedeagus and transfer of sperm to the female spermathecae. These specialized structures ensure species-specific mating and prevent hybridization.

Discussion

As in all **Nematocera**, the abdomen of the fly is primitively composed of 11 segments. The terminal part of the abdomen consists the rudiments of segment 11, namely a pair of cerci and the anus. These structure of segment eleven jointly called **proctiger**. In case of male genitalia the eight main elements constitute the basic pattern, where Tergite IX (epandrium) and Sternite IX (hypandrium) form the basal **genital ring**. The present investigation highlights that in **Ceratopogonidae**, the genital morphology is a highly stable and complex character, serving as the primary tool for taxonomic differentiation and species-level identification. As observed in all Nematocera, the abdominal terminalia consists of segments IX to XI, where structural modifications directly facilitate successful reproductive maneuvers.

1. Male Genital Complexity and Adaptations

The male genitalia follow a basic pattern consisting of eight main elements. A significant structural feature observed is the pair of **gonopods**, comprising the basal **gonocoxite** and a distal **gonostylus**, which arise postero-laterally on sternite IX. Following **Saether (1980)**, the paraphallic processes (parameres) are termed as **Volsella**. In the genus *Forcipomyia*, these are further categorized as superior, inferior, and median volsella based on their specific orientation. These structures serve as accessory supports for the **aedeagus**, protecting it when not in use and directing it during sperm transfer.

2. Female Reproductive Morphology

The female genitalia, spanning segments VIII to XI, feature the **spermathecae**—heavily sclerotized and differentiated organs for sperm storage. The study confirms that the number of spermathecae is a vital phylogenetic marker. While the presence of three spermathecae is considered **plesiomorphic** (primitive), the reduction to one, as seen in *F. mananthrai*, represents an **apomorphic** (derived) state.

3. Impact on Reproductive Behaviour

The structural modifications are intricately linked to mating strategies:

- **Swarming and Flexion:** Most species of *Forcipomyia* form swarms over specific markers. Species like *F. dibiyapurensis* and *F. barkhai* exhibit specific **swarm-marker** preferences (vegetation vs. stones). During these swarms, the male abdomen undergoes **flexion** (ventroflexed, dorsoflexed) or **rotation** up to 180° to achieve the necessary orientation for coupling.
- **Mating Orientation:** The transition from mating on the substratum (primitive) to completing the entire process in flight (advanced) marks a significant evolutionary shift and advancement in the reproductive biology of biting midges.

Conclusion

The specificity of the **aedeagal apex** (which can be simple, branched, or three-branched) and the configuration of **parameres** are found to be the most reliable characters for isolating closely related species. These genital modifications ensure reproductive isolation and are the crux of evolutionary adaptation within the **Ceratopogonidae** family.

7. Conclusion

The present study on the biting midges (**Ceratopogonidae**) of the Gangetic Plains of Uttar Pradesh provides critical insights into the complex relationship between genital morphology and reproductive biology. The investigation leads to the following major conclusions:

1. **Taxonomic Reliability:** Genital structures, particularly the **aedeagal apex**, **parameres (volsella)**, and the number of **spermathecae**, remain the most stable and reliable morphotaxonomic features. Unlike external body characteristics, these structures show minimal environmental variation, making them indispensable for the accurate identification of closely related species within the genera *Culicoides* and *Forcipomyia*.

2. **Reproductive Isolation:** The high degree of specificity in the male aedeagus (whether simple, branched, or tripartite) and the structural compatibility with female genital openings act as a "lock-and-key" mechanism. This ensures reproductive isolation between sympatric species, maintaining the genetic integrity of the populations in the Gangetic region.

3. **Evolutionary Adaptations:** The study confirms significant evolutionary shifts in mating behaviour. The transition from **plesiomorphic** traits (three spermathecae and mating on the substratum) to **apomorphic** traits (reduced spermathecae and aerial copulation) highlights the evolutionary advancement of these insects. The ability of the male terminalia to undergo **rotation (up to 180°)** and **flexion** is a vital adaptation for achieving successful coupling during flight.

4. **Ecological Significance:** The specific preference for **swarm markers** (vegetation, stones, or debris) at varying heights indicates a sophisticated level of habitat utilization and species-specific niche partitioning. In summary, the structural modifications in the genitalia of biting midges are not merely anatomical features but are fundamental to their evolutionary success, reproductive strategies, and species recognition. These findings contribute significantly to the entomological database of Uttar Pradesh and provide a robust framework for future studies in vector biology and the management of midge-borne diseases.

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