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Utility of male mosquito hypopygium in species identification

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ABSTRACT
Hypopygium is modified ninth abdominal segment present in the majority of insect groups. The male hypopygial structure of the mosquito genera could afford excellent mean of specific identification and might have immense importance in the classification. In the present study male mosquito specimens representing seven different genera namely, *Anopheles, Stegomyia, Culex, Mansonia, Hulecoeteomyia, Armigeres* and *Coquillettidia* were collected from field using CDC miniature light traps and hand collection using aspirators and flash torch. Male mosquitoes were separated, identified based on morphological characters, dissected and examined to record the variability in shape and size of hypopygium among the genera for further confirmation. Based on the difference in the characters of hypopygial structure the collected specimens were identified as *Anopheles stephensi, Stegomyia aegypti, Stegomyia albopicta, Stegomyia annandalei, Culex malayi, Culex gelidus, Mansonia uniformis, Armigeres subalbatus, Hulecoeteomyia chrysolineata* and *Coquillettidia crassipes*.

Keywords: Hypopygium, Male mosquito, Dissection, Identification.

1. Introduction
Mosquitoes (Diptera: Culicidae) have eminence medical importance and are among the most intensively studied insects across the world. Most of the taxonomic keys for mosquito identification are based on the morphological characters of female or larvae, while only a few use male mosquitoes and mosquito eggs for differentiating mosquito species [1]. Males of many insect species can be successfully identified using specific keys for female provided the specimens are in good condition. However, this approach has its own limitation that field collected mosquito may get damaged during transportation and storage. The male hypopygium can be used with more reliability in such circumstances as this is less susceptible to damage as compared to the scales and bristles which are used in morpho-taxonomical identification. These structures can also be used as morphological markers for the sibling and new species identification.

Female mosquitoes spread many diseases and are considered more important in epidemiological perspective in many countries. The male mosquitoes do not feed on animal blood and are not capable in disease transmission. However male mosquitoes have their own importance and their presence in the human habitat suggests that the breeding is continuing in the nearby areas. Further, their density and coexistence with female mosquitoes indicate that the reproductive activity and egg laying is increased in the nearby available suitable habitats. The modified structure beyond the eighth abdominal segment in mosquito is hypopygium, which differs considerably among the different genera. In the present study male mosquitoes corresponding to seven different genera were collected from Sonitpur district of Assam, India and identified on the basis of hypopygium. The specific hypopygial characters of mosquitoes of each genus have been noted so as to be used as morphological markers in the identification of species.

2. Methodology
2.1 Preparation of male hypopygium
Male mosquitoes were separated from the females and identified on the basis of morphological characters were placed for half an hour in a covered jar with some damp wool.

Last few segments of the abdomen were sniped off under the dissecting microscope and the specimens were kept in 90% alcohol for 15 minutes in order to prevent the hypopygium from floating during the subsequent stages of preparation (Fig. 1). The hypopygium was transferred to a test tube containing 10% potassium hydroxide (KOH) and slowly heated over a bunsen burner to nearly the boiling point [1]. The hypopygium was washed with a few drops of distilled water in a spot plate for 2-5 minutes. Mounted directly from water in Hoyer's medium [2]. The slides were examined under the Leica stereozoom microscope with 100 X magnification (Fig. 2).

Fig 1: Male mosquito terminal abdominal segment constituting hypopygium (HY)

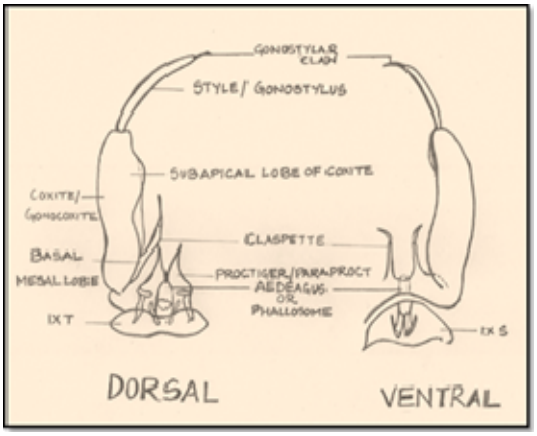

Fig 2: Diagrammatic representation of male hypopygium revealing minor details

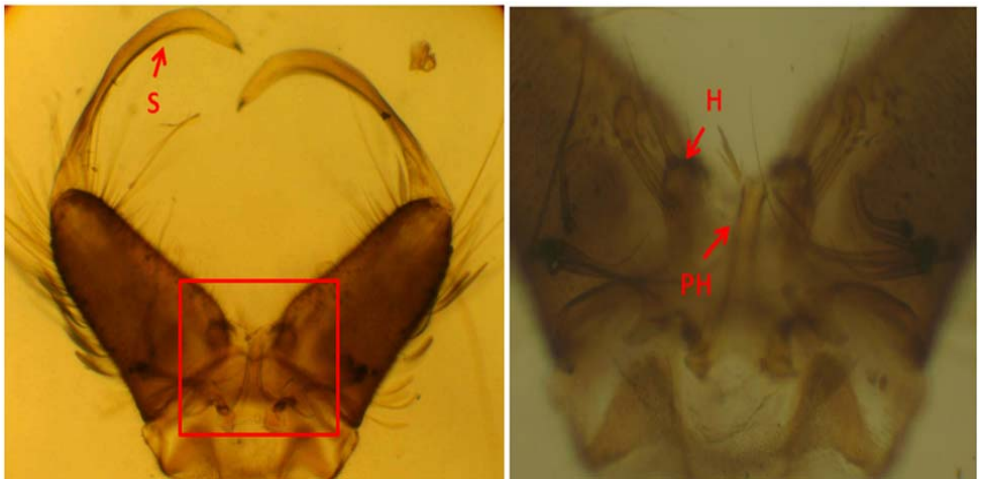

Fig 3: *Anopheles stephensi*

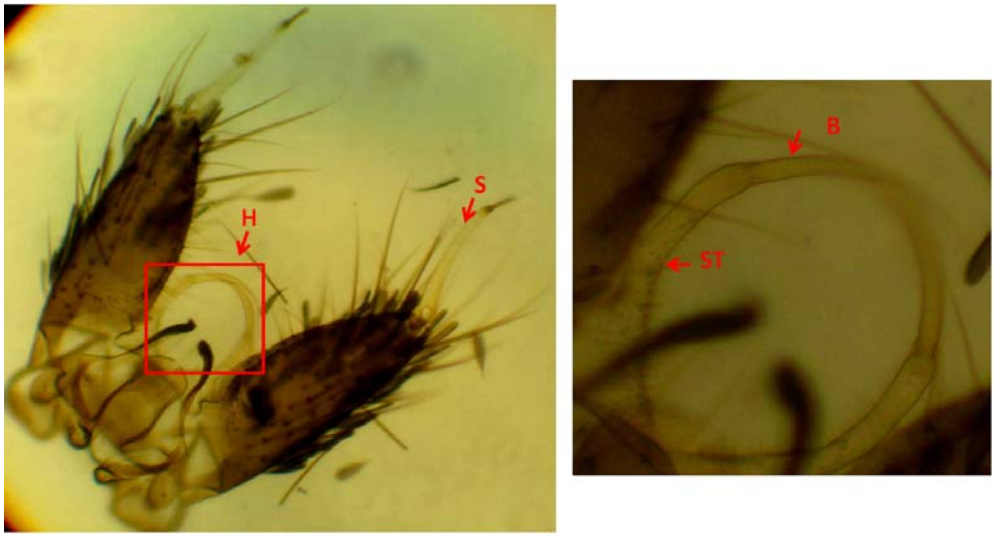

Fig 4: *Hulecoeteomyia crysolineata*

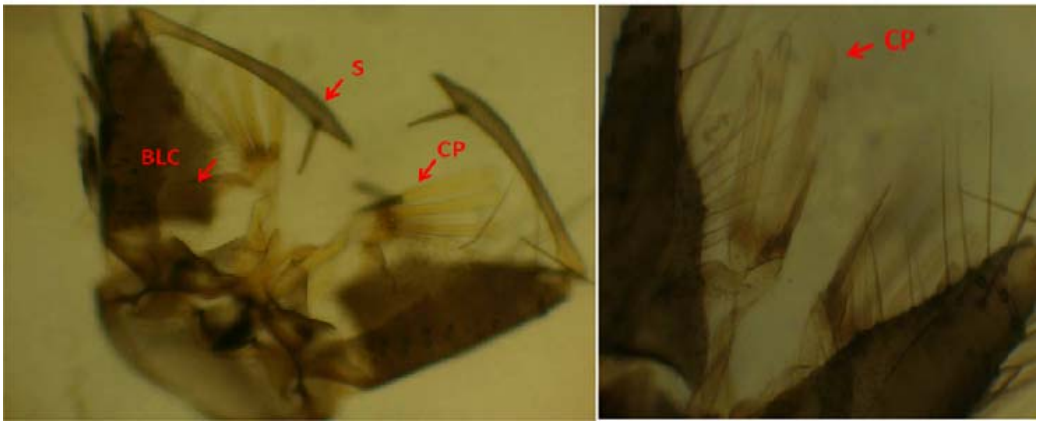

Fig 5: *Stegomyia annandalei*

Fig 6: *Stegomyia aegypti*

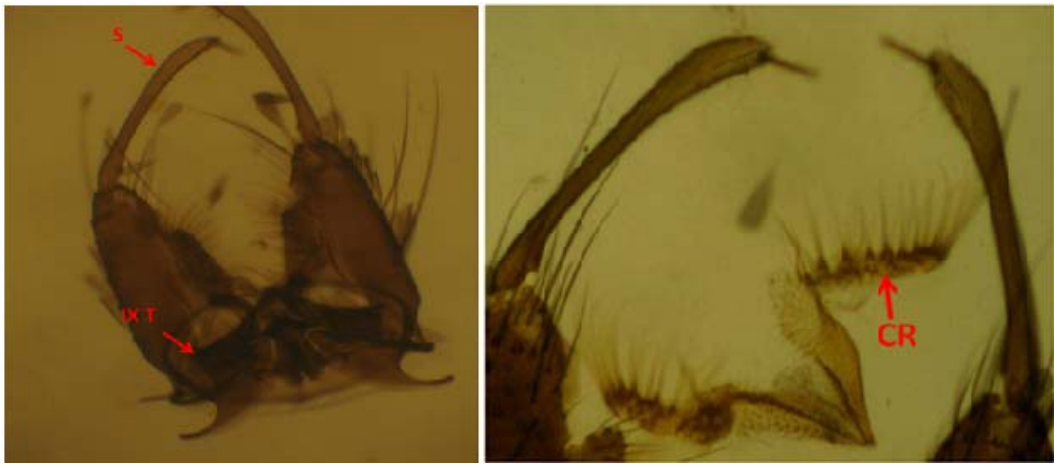

Fig 7: *Stegomyia albopicta*

Fig 8: *Culex gelidus*

Fig 9: *Culex malayi*

Fig 10: *Armigeres subalbatus*

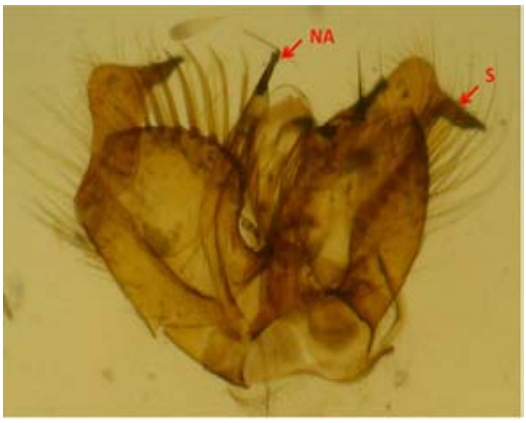

Fig 11: *Mansonia uniformis*

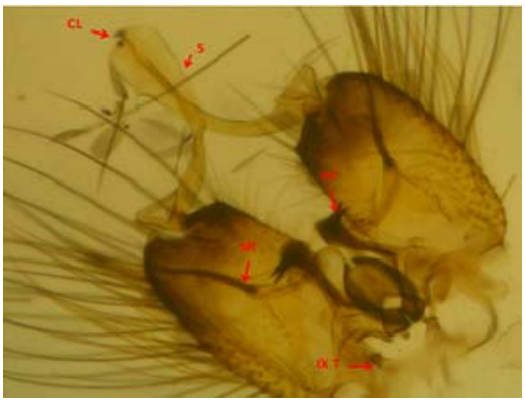

Fig 12: *Coquillettidia crassipes*

Abbreviation: S – Style, H- Harpago, PH- Phallosome, BLC- Basal lobe of coxite, T- Teeth, CL- Claw, SR- Sclerotized rod, PT- Phallosome teeth, IX T- Ninth tergite, LA- Lateral appendage, CR- Crown, SALC- Sub apical lobe of coxite, ST –Stem, B- Blade, NA- Notched appendage, P- Paraproct, VR- Ventral arm, CP- Clubbed processes

3. Results

Description of hypopygial structure of male mosquitoes:

A. Genus- *Anopheles*

1. *Anopheles stephensi* (Fig. 3)

1. Style is very long and arcuate with a small, terminal spur like appendage.
2. Harpago with apical hair somewhat longer than club.
3. Phallosome is 1/2 or 1/3 of coxite with 5-6 leaflets on each side. All leaflets broad, blade like with marked serrations along straighter edge.

B. Genus- *Hulecoeteomyia*

1. *Hulecoeteomyia chrysolineata* (Fig. 4)

1. Blade of harpago curved, tapering, little longer than stem.
2. Style with terminal appendage a little less than 1/3 of its length.

C. Genus - *Stegomyia*

1. *Stegomyia annandalei* (Fig. 5)

1. Style with a fairly long appendage attached some little distance from tip.
2. Basal lobe /process of coxite with three long clubbed processes and shorter thick hairs.

2. *Stegomyia aegypti* (Fig. 6)

1. Ninth tergite with strong lateral lobes, without median lobe.
2. Claspettes absent; basistyle less than twice as long as wide.
3. Paraproct has a well-developed ventral arm.

3. *Stegomyia albopicta* (Fig. 7)

1. Style spoon shaped with appendage arising near tip.
2. Crown of the paraproct has series of hairs arises from the single plane.
3. Ninth tergite with strong median lobe and weak lateral lobes.

D. Genus - *Culex*

1. *Culex gelidus* (Fig. 8)

1. Basal arm of paraproct long.
2. Style expanded in middle, abruptly narrowed at tip with a small terminal appendage.
3. Ventral cornu of lateral plate of phallosome with a pointed tip directed dorsally, median process with 2 teeth pointing downwards, lateral process well developed, external process rounded.

2. *Culex malayi* (Fig. 9)

1. Style is divided into two arms.

2. Sub apical lobe of coxite with a very large leaflet and several other processes.

E. Genus - *Armigeres*

1. *Armigeres subalbatus* (Fig. 10)

1. Basal lobe of coxite with 2 flattened curved spines.
2. Style slightly curved with about 18 teeth.

F. Genus - *Mansonia*

1. *Mansonia uniformis* (Fig. 11)

1. Style wide with pointed tip.
2. A large process arising from base of coxite on inner side, ending in an appendage which is notched at tip.

G. Genus - *Coquillettidia*

1. *Coquillettidia crassipes* (Fig. 12)

1. Coxite with numerous long strong setae on outer surface.
2. Stylar claw short, pointed and well sclerotized.
3. Mesal face of basistyle with a long strongly sclerotized blunt tip rod reaching near the apex of coxite.
4. Ninth tergite with wider interlobar space, lobes prominent with few weak setae.
5. Paraproct with 2 strong teeth and a few small denticles at apex.

4. Discussion

Approximately 113 genera including 3, 531 species of mosquito have been reported worldwide [3]. Mosquitoes are most extensively studied insect group still there taxonomic knowledge is far from complete. Some of mosquito species are vectors of medically important pathogens responsible for causing malaria, dengue, filaria and Japanese encephalitis (JE). Species identification constitutes the first step in surveillance and control of mosquito born diseases. Some of the mosquito vectors of public health importance present with high degree of morphological similarity at adult stage therefore are difficult to identify based solely on examination of adult females using the available morphological keys [4]. Many of the morphological characters such as, colouration of thorax, scales on the legs etc. are unreliable and may be lost during the transport and storage, while, many distinguishable characters are interpreted differently by different authors [5]. There are many common taxonomic characters which are ignored and not favoured for the identification purpose. Previous study has described the presence of two folds in cubital area the cubito-marginal ridge and the pre-cubital furrow of culicid wing. The distribution of these rather obscure structures provides a reliable diagnosis of anophelinae, toxorhynchitinae, and culicinae [6]. Previous studies conducted in Thailand have illustratively explained the morphological characters, geographical distribution, biology and medical significance of many mosquito species belonging to different subgenera and have laid down the frame work for taxonomical studies for each stage of the mosquito development [7, 8, 9]. However these studies have not emphasised the taxonomical importance of some little known mosquito genera such as, *Mansonia*, *Coquillettidia*, *Armigeres*, *Hulecoeteomyia* which have recently emerged as important genera in public health.

Unlike the female mosquitoes, the male mosquitoes feed on plant nectar and have no role in transmission of disease. However male mosquito could be helpful as indirect indicators for understanding mosquito population, breeding ecology and reproduction activity. Collection and presence of male of a particular species is an indication of pending emergence and coexistence of female mosquitoes in that area [10]. Among many mosquito species morphological differences are more prominent in male hypopygial than in female and larval characters. *Aedes atlanticus* and *Aedes tormentor* are difficult to distinguish based on morphological characters, but can be easily separated on the basis of male genitalia [1]. Male genitalia have been found to be very useful in separating Indiana representatives of the *Aedes stimulans* group; *Ae. stimulans*, *Ae. fitchii*, *Ae. excrucians*, and *Ae. flavescens*, where female characters are sometimes indistinct [1]. The male hypopygial, specific structure associated with the eighth, ninth and tenth abdominal segment differ considerably between different genera and species. Since it is less vulnerable to damage, therefore it can be used as a reliable morphological tool in mosquito identification even in damaged specimens.

5. Acknowledgement

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6. Conflict of interests

None

7. References

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