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Relevance of female genitalic attributes in species identification of Culicinae

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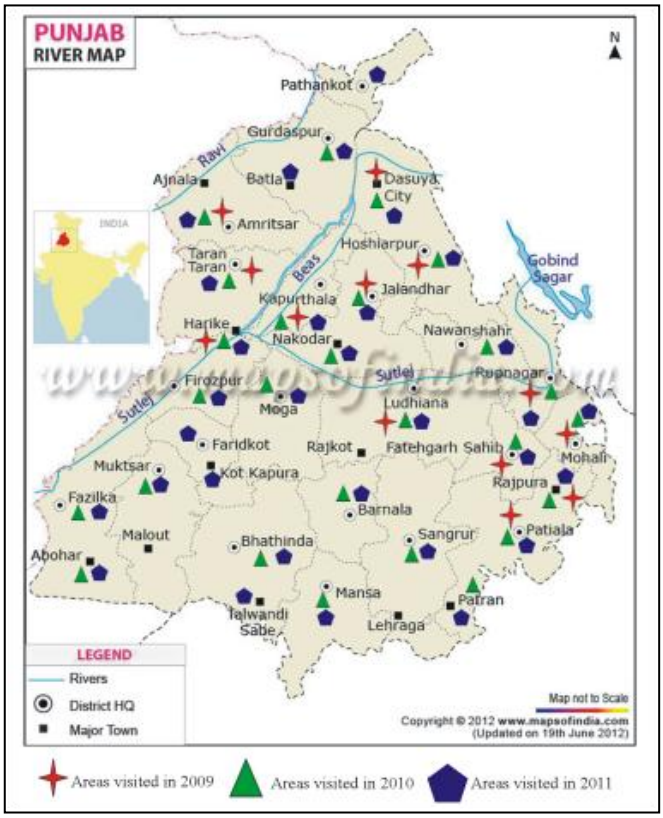
Abstract
The genitalia of female mosquitoes exhibits outstanding morphological variations which are of generic significance. A number of important taxonomic attributes have been studied in the genitalia of seven species viz., *Christopharsiomyia thomsoni* (Theobald), *Coquillettidia crassipes* (van der Wulp), *Culex* (*Eumelanomyia*) *brevipalpi* (Giles), *Fredwardius vittatus* (Bigot), *Mucidus scatophagoides* (Theobald), *Neomelaniconion lineatopenne* (Ludlow) and *Verrallina* (*Neomacleaya*) *indica* (Theobald) collected during survey cum collection tours from 2009-2011. The attributes like tergum IX, insula, lower and upper vaginal lips, spermathecal eminence, spermathecal capsules, cercus and post genital plate have been illustrated and discussed in detailed.

Keywords: Female genitalia, mosquitoes, identification

1. Introduction
Mosquitoes (Diptera: Culicidae) have eminence medical importance and are among the most intensively studied across the world. Identification of various mosquito species is still very difficult because of the occurrence of various sibling species and species complexes. Moreover, the external features, particularly such as scales and hairs are removable or losable, and often become unsuitable for differentiating a number of closely related species. Due to this, most of the authors have given importance to male genitalic attributes and all the known keys are based on larvae and male genitalia. However, recent taxonomic studies on family Culicidae have revealed additional and new taxonomic attributes on various immature stages, cibarium, mouth parts and genitalia by various workers (Chen [1]; Sirivanakarn [2]; Khalin [3]; Boza and Vargas [4]; Seenivasagam *et al*. [5]; Reinert [6]; Kirti *et al* [7-9]; Kirti and Shipali [10]; Kaur and Kirti [11]). But the female genitalia has been neglected and usually not considered important for species identification. In these cases, investigation on the internal structures of female genitalia becomes necessary since the field collections comprises usually of female specimens. In the present study emphasis has been given on female genitalia of seven species of different genera collected from Punjab. Some of the female genitalic attributes are highly significant and will prove useful for identification of species.

2. Materials and methods
2.1 Area
Punjab is located in northwestern India, and has an area of 50,362 km^2. It is located at the latitude 30° 4' North and longitude 75° 5' East. It is bounded on the west by Pakistan, north by Jammu and Kashmir, northeast by Himachal Pradesh and south by Haryana and Rajasthan. Most of the area of this state comprises of fertile plains, alluvial plain with three rivers and an extensive canal system for irrigation. A belt of undulating Lower Shivalik hills extends along the northeastern part of the state. Its average elevation is 300 meters above sea level, with a range from 180 meters in the southwest to more than 500 meters around the northeast border. The southwest of the state is semi-arid, eventually merging into the Thar Desert. Punjab is divided into three major regions, i.e. Malwa, Majha and Doaba and it covers as many as 23 districts (Map).



2.2 Collection and preservation of adult

Collection cum survey tours were conducted throughout the state of Punjab from 2009-2011 during the following months.

- Summer (April to June), when temperature typically rises as high as 43 ^{0}C.
- Monsoon season (July to September), when a majority of rainfall occurs.
- Winter (December to February), when temperatures typically falls as low as 4 ^{0}C.

There is a transitional period between winter and summer in March and early April, as well as a transitional season between monsoon and winter in October and November.

Adult mosquitoes were collected with the help of oral aspirators and insect collecting hand nets from gardens, cattle sheds, hay stacks, nurseries, forest and human shelters. Various details such as date, locality and source of the collection were noted down at the collection site itself. On bringing the collection to the laboratory, adults were killed with ethyl acetate. They were then preserved for taxonomic studies. The mosquito was fixed from the ventral surface of thorax on the pointed end of a wedge of thick paper, using mounting glue. The paper wedge was supported with the help of a paper pin. A slip with complete collection data written on it, was attached to the pin under each specimen. Mounted mosquitoes were kept in collection boxes. Naphthalene balls were kept in collection boxes for preserving the mosquitoes from any insect or fungal attack. The adults reared in the laboratory, were also preserved in the same way. All the mosquitoes were examined individually under the binocular microscope. The number of males and females of each species were counted.

2.3 Preparation of slides of genitalia (♂,♀)

For the slides preparation, the fixed mosquito was removed from the wedge of the paper. It was placed on a slide and the last 2-3 segments were cut with the help of a fine needle. The procedure given by Silverly and Shroyer [12] was followed with slight modifications for making genitalia slides. The cut segments were kept in boiling 10% KOH for 20-25 minutes. The material was then given 5-6 water washings in fresh water followed by dehydration in different grades of alcohol for 5 minutes each. It was then kept in xylene, minimum for 1 hour, for proper clearance. The genital material was then mounted in DPX and studied under research microscope. For naming of various parts of female genitalia Sirivanakarn [13]; Huang [14]; Reinert [15] were followed.

2.4 Identification of adult mosquitoes

The culicine and other anopheline species were separated by using standard taxonomic keys Sirivanakarn [13]; Barraud [16]; Huang [17]; Reuben *et al* [18]; Reinert *et al* [19].

2.5 Photography

2.5.1 Slides: Genitalia slides (whole mount) were photographed at 10X and 40X magnification under radical trinocular microscope.

2.5.2 Adults: Adult mosquitoes were photographed under Stereo Zoom binocular Microscope (Radical- RXLr-5) fitted with digital camera (Procan 1.3 with Tsview software) attached to computer.

3. Results

***Christophersiomyia thomsoni* (Theobald, 1905) (Fig. 1-8)**

Theobald, 1905, *J. Eco. Bio*., 1:18

Cerci short and broad with setae; post genital lobe with shallow notch, moderately long setae distally present and small setae on basal area; IX tergum bilobed; upper vaginal lip sclerite moderately; 3 spermatheca, one larger than the other two.

***Coquillettidia* (*Coquillettidia*) *crassipes* (Van der Wulp, 1881) (Fig. 9-14)**

Van der Wulp, 1881, *Bijd. Fauna Mid*. Sumatra, Dipt. p. 9

Cerci short, incurved, tips blunt; postgenital lobe bearing an inconspicuous notch in middle, setosed with 2 paris of setae pointing anteriorly on either side of mid line and 8-10 pair of setae pointing inwards; tergum IX band like, with a very inconspicuous median notch, furnished with 5-6 long setae on either side of notch; insula covered with sparse setae; sigma not differentiated; well sclerotized spermatothecal eminence visible; 3 spermatothecae present, one larger than others.

***Culex (Eumelanomyia) brevipalpi* (Giles, 1902) (Fig. 15-23)**

Giles, 1902, Handbook 2nd ed. p 384

Cerci short, rounded apically with several short and weak setae restricted to lateral surface; postgenital lobe broad with rounded caudal margin, apical half with a lateral row of 5-6 bristles; tergum IX band like and subapically setosed with 5-6 setae on each side; insula bearing a median group of 8-9 setae; sigma membranous; vaginal sclerite dark, U-shaped; three spematothecae present, the biggest one in the centre and other two of medium size.

***Fredwardsius vittatus* (Bigot, 1861) (Fig. 24-29)**

Bigot, 1861, *Ann. Soc. Ent*., France, 1:227.

Cerci long, slender with microsetae on lateral margins; VIII Sternum with deep notch at middle, IX tergum with well developed lateral lobes; post genital plate narrow and small with long setae on distal side and microsetae on basal side; upper and lower vaginal lips less sclerite; 3 spermatheca, one slightly larger than the other two.

***Mucidus* (*Mucidus*) *scatophagoides* Theobald, 1901 (Fig.30-37)**

Theobald, 1901, *Monog. Culic*., 1 : 277.

Cerci very long; postgenital lobe slightly emarginate at apex, furnished with a long seta and a moderate sized seta on either side of mid line besides numerous microsetae; tergum IX 2 winged, each bearing 8 papillated hairs; upper vaginal lip moderately chitinized; insula broader than long and bearing 4 long setae; 3 spermatothecae present.

***Neomelaniconion lineatopenne* (Ludlow, 1905) (Fig. 38-43)**

Ludlow, 1905, *Can. Entomol*., 37 : 133.

Cerci very long; subcylindrical, tapered at apex; postgenital lobe rounded with median apical emargination, apicoventral aspect setosed with 7-8 long and numerous microsetae on either side of mid line; tergum IX like narrow band with 2 prominent lobes, each bearing 7 setae apicodorsally; insula pilose; sigma flattened; upper vaginal lip slightly sclerotized with convex apices; 3 spermatothecae present.

***Verrallina (Neomacleaya) indica* (Theobald, 1907) (Fig. 44-52)**

Theobald, 1907, *Mongo. Culic*., 4:238

Cerci triangular in outline, moderately long, broad at base, apex acute, with numerous broad scales scattered over dorsal surface; post genital lobe short, narrow, apex with moderately deep median indentation, lateral lobes well developed, each lobe setosed with 3-5 setae; insula ill-defined; lower and upper vaginal lips wide and sclerotized; spermathecal eminence heavily pigmented, large, deep, ovoid in dorsal outline, long spicules with lateral projection attached to cephalic area; combined spermathecal eminence, upper vaginal lip and upper vaginal sclerite covering nearly entire upper vaginal wall with heavily pigmented structures; 3 spermathecae present.

4. Discussion

Mosquitoes are medically most important insects because they act as vectors of many serious diseases to mankind. The characters based on male and female genitalia have been the most reliable in species identification. A major contribution in studying and updating the status of many genera and subgenera of subfamily Culicinae using the female genitalic attributes has been done by Reinert [20-29]. The present seven species have been studied by earlier workers like Sirivanakarn [13]; Huang [14, 17]; Barraud [16] but for the first time the detail description of female genitalia have been supplemented with photographs.

Coquillettidia (Coquillettidia) crassipes (Van der Wulp)

Fig.9 Adult

Fig. 10 Thorax (Dorsal view)

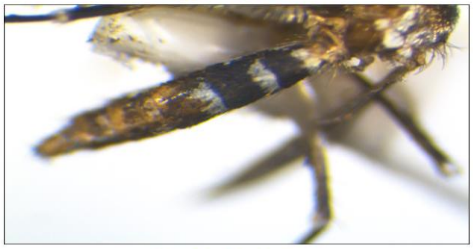


Fig.11 Abdomen

Fig. 12 Legs

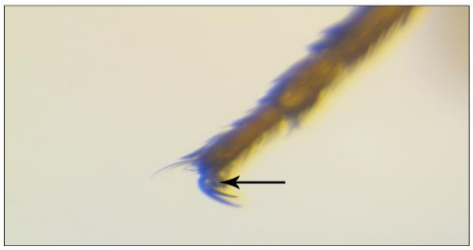


Fig.13 Hindleg showing pulvillus

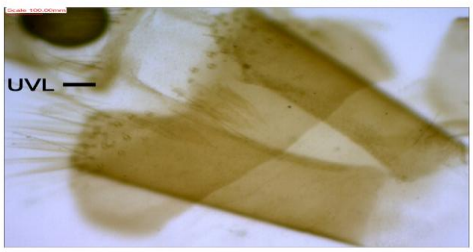


Fig.15 Female Terminalia

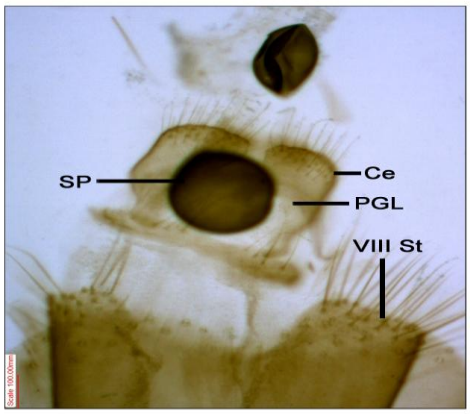


Fig.14 Female Genitalia

Culex (*Eumelanomyia*) *brevipalpis* (Giles)

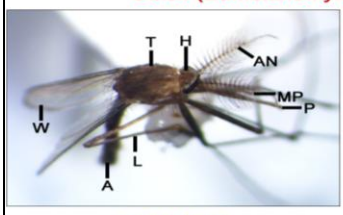


Fig.16 Adult

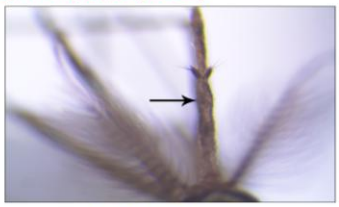


Fig.17 Proboscis

Fig.18 Thorax (Dorsal view)

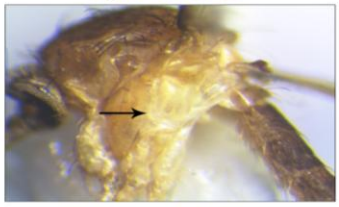


Fig.19 Thorax (Lateral view)

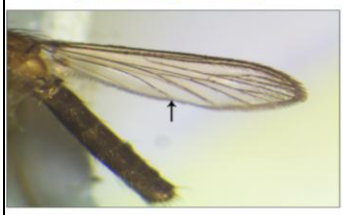


Fig.20 Abdomen and Wing

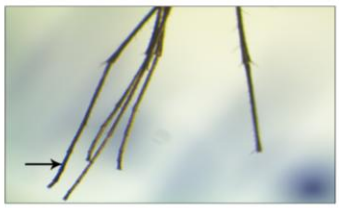


Fig.21 Legs

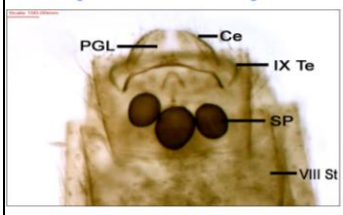


Fig.22 Female Genitalia

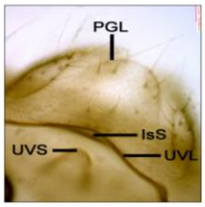


Fig.23 Female Terminalia

Mucidus (*Mucidus*) *scatophagoides* Theobald

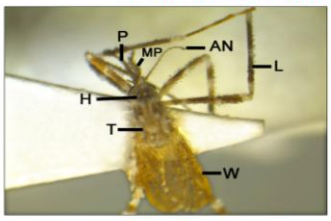


Fig.30 Adult

Fig.31 Thorax (Dorsal view)

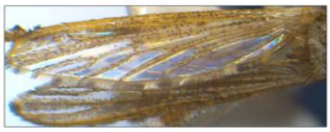


Fig.32 Wing

Fig.33 Thorax (Lateral view)

Fig.34 Legs

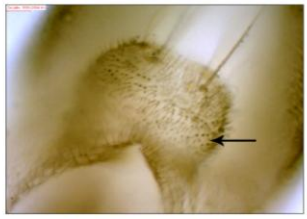


Fig.36 Postgenital Lobe

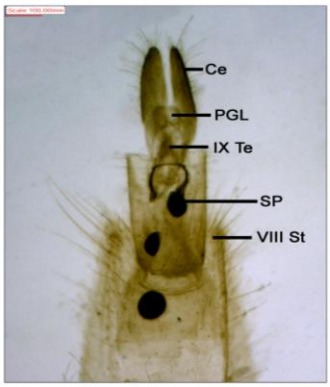


Fig.35 Female Genitalia

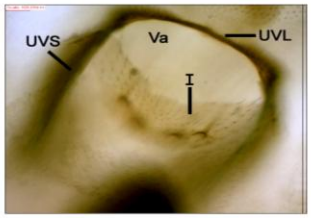


Fig.37 Female Terminalia

Fredwardius vittatus (Bigot)

Fig. 24 Adult

Fig. 25 Thorax (Dorsal View)

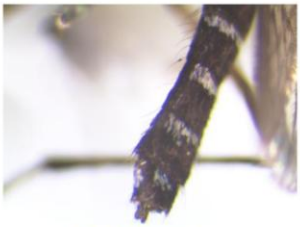


Fig. 26 Abdomen

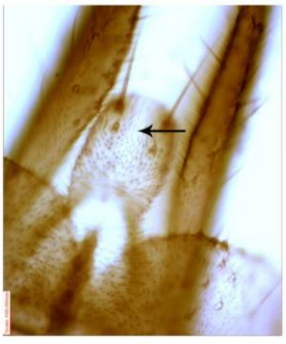


Fig. 28 Postgenital Lobe

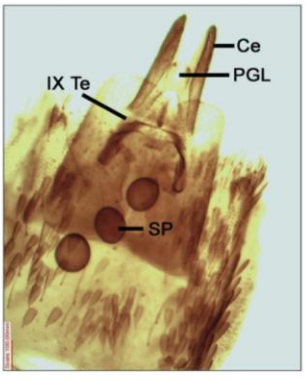


Fig. 27 Female Genitalia

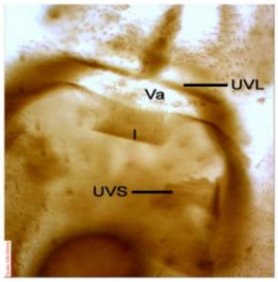


Fig. 29 Female Terminalia

Neomelaniconion lineatopenne (Ludlow)

Fig. 38 Adult

Fig. 39 Thorax (Dorsal

Fig. 40 Thorax (Lateral View)

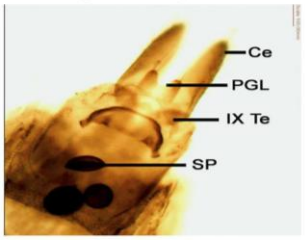


Fig. 41 Female Genitalia

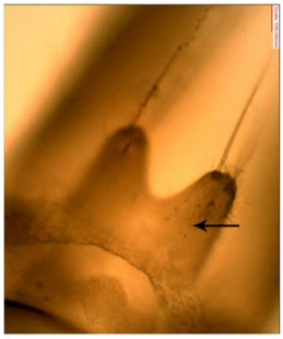


Fig. 42 Postgenital Lobe

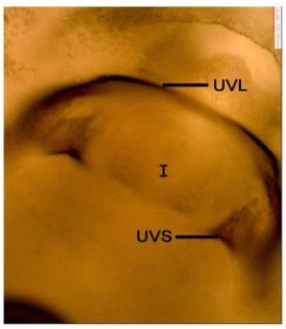
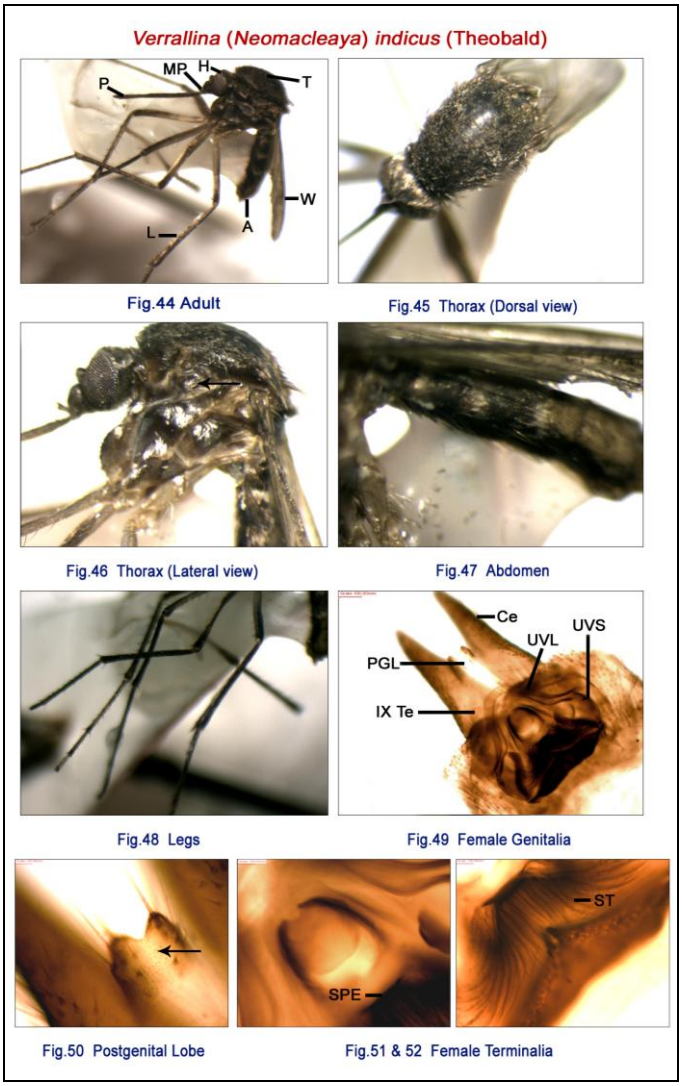


Fig. 43 Female Terminalia



5. Conclusion

The female genitalia usually possesses few features with which closely related species can be separated; however, the genitalia exhibit good characters for distinguishing species groups, subgenera and genera Reinert [30]. The characters like sternum VIII, tergum IX, insula, upper and lower vaginal lips, spermathecal eminence, spermathecal capsules, postgenital lobe and cercus are some of the reliable taxonomic attributes which can be safely used to identify different genera of mosquitoes.

6. Abbreviations

A : Abdomen; AGDB : Accessory gland duct base; AN : Antenna; BWB : Basal white band; Ce : Cercus; H: Head; I: Insula; IsS: Insular setae; IX- Te: Ninth tergum; L: Leg; MP: Maxillary palpi; P: Proboscis; PGL: Post genital lobe; SP: Spermatotheca; SPE: Spermathecal eminence; ST: Seta; T: Thorax; UVL: Upper vaginal lip; UVS: Upper vaginal sclerite; Va: Vagina; VIII St: Eighth sternum; W: Wing.

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