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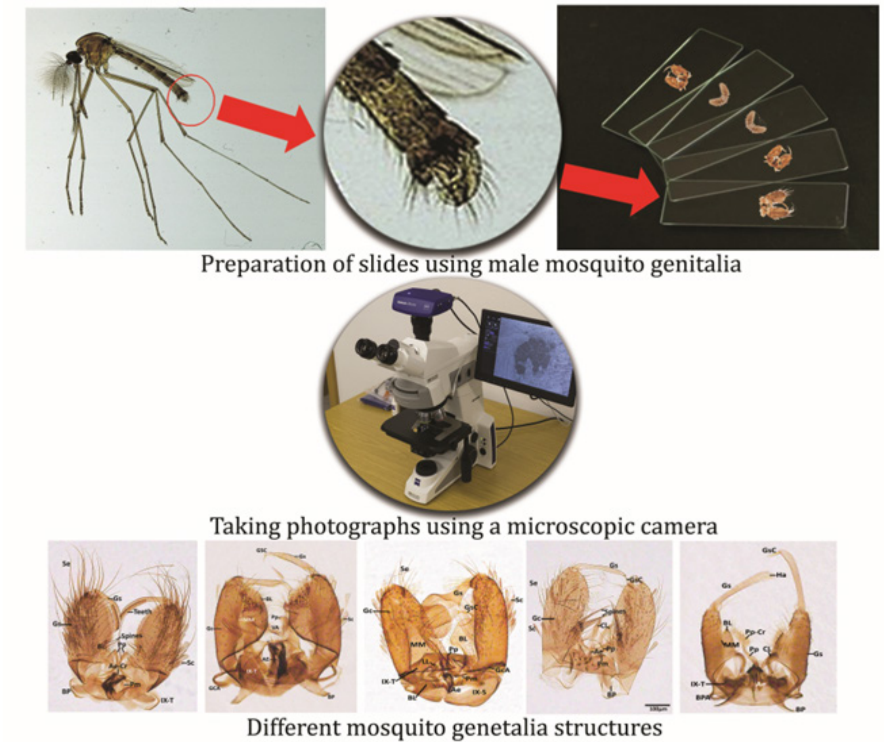
Morphological variations in the male genitalia of seven mosquito genera (Diptera: Culicidae), Sri Lanka

G.N.P.V. Anuradha, W.A.P.P. de Silva, W.G. D. Chathuranga, S.H.P.P. Karunaratne and T.C. Weeraratne*



Highlights

- Structural variations of the male genitalia of seven genera and 15 mosquito species.
- Species and generic specific features were identified.
- Male genitalia structures can be integrated into mosquito identification.

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RESEARCH ARTICLE

Morphological variations in male genitalia of seven mosquito genera (Diptera: Culicidae) in Kandy, Sri Lanka

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Abstract: Disease-causing vector mosquitoes are prevailing throughout Sri Lanka, hence proper identification of these mosquitoes is crucial in implementing effective mosquito control approaches. The use of taxonomic keys based on external morphological characters of adults and larvae, is the common identification method. Due to the high diversity, abundance and presence of species complexes, proper identification using external morphology is challenging. The molecular approach is well-known but a costly method of mosquito identification in the country. This study was proposed to identify the variations in the male genital anatomy of mosquitoes from the Kandy District, Sri Lanka, to be used in mosquito identification. Monthly samplings were done in Peradeniya, Hantana, and Halgolla areas in Kandy, Sri Lanka. Male mosquitoes were identified using standard taxonomic keys. Male genitalia of these mosquito species were slide mounted. Photographs of the genital structures were taken. Male mosquitoes representing six genera (*Aedes, Anopheles, Armigeres, Coquilletidia, Culex* and *Orthopodomyial*) and 15 species were identified based on their external morphological features. Generic and species-specific features of the male genitalia of the studied mosquitoes were primarily seen in the gonocoxite, gonostylus, claspette, and phallosome. Species-specific features identified in the genitalia of mosquitoes are more reliable in accurately identifying male mosquitoes.

Keywords: Mosquito taxonomy; Claspette; Gonostylus; Gonocoxite; Phallosome

INTRODUCTION

Mosquitoes are an important group of insects that belong to the Order Diptera, Family Culicidae, with over 3,500 mosquito species being identified and described worldwide (Wilkerson *et al.,* 2015). According to recent reports, about 159 mosquito species belonging to 19 genera are present in Sri Lanka, and many of these species exist as species complexes (Amerasinghe *et al*., 1987; Gunathilaka 2018). This is one of the most abundant groups of insects widespread throughout the country, and many act as vectors of life-threatening diseases such as dengue, chikungunya malaria, lymphatic filariasis and Japanese encephalitis (JE) (Herath *et al*., 1988; Peiris *et al*., 1993; Amerasinghe *et al*., 2002). Recent studies have reported the presence of ornithophilic and frog-biting mosquitoes, potentially vectors of emerging infectious diseases in humans and wildlife (Chathuranga *et al*., 2018; Chathuranga *et al*., 2021; de Silva *et al.,* 2020). Accurate and precise identification of these mosquito species and siblings of species complexes is crucial for managing and controlling mosquito-borne diseases.

The morphological taxonomy of mosquitoes is mainly based on identification keys that have been developed considering the external morphology of adults and fourth-instar larvae (Amerasinghe *et al.,*1989). Phenotypic plasticity, difficulty in distinguishing morphologically similar species and members of species complexes, and inability to identify damaged specimens due to loss of taxonomic features (scales, setae *etc*.) are notable drawbacks associated with morphology-based taxonomy of mosquitoes (Calle *et al*., 2002; Jorger & Schrodl 2013). Differences in the number of eggshell ridges and cytogenetic approaches have also been used to identify anopheline species complexes such as *An. culicifacies* (Surendran *et al*., 2013), *An. subpictus* (Surendran *et al*., 2010) and *An. gambiae* (Marrelli *et al*., 2006). Surendran *et al.,* (2013) detected four sibling species (A, B, C and D) of the Subpictus complex in Sri Lanka based on the number of egg ridges. However, molecular taxonomy-based phylogenetic analysis of these samples has revealed the presence of only two genetically distinct species (species A and species B) instead of four in Sri Lanka. Moreover, the molecular characterization of *An. subpictus* has shown that some morphologically identified *An. subpictus* B are members of Sundaicus complex but not of Subpictus complex (Surendran *et al.,* 2010). Therefore, morphological identification alone is not the best approach to accurately identify mosquitoes. Although some mosquito species in the country have been identified using DNA barcoding which is considered to be a relatively precise method (Surendran *et al*., 2013; Weeraratne *et al.,* 2017; Weeraratne *et al.,* 2018), due to the high cost involved and the specific requirements needed, this may not be the best approach for a field entomologist in a developing country. Also, the barcode used should be appropriate since the species detection and identification depend on how conserved the marker barcode is and the discriminatory power of the nucleotide variation. Therefore, the importance of economically feasible but reliable, morphology-based approaches can never be underestimated in species identification. Combining classic microscope-based

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identification with barcoding approaches will undoubtedly enhance the knowledge of taxonomy.

Species-level identification is further assured with the species-specific structural organization of the genital structures of mosquitoes. As far as the genitalia are intact, any physically damaged specimen could be recognized using this method (Yadav *et al*., 2014). Njabo *et al.,* (2009) have stated that the identification of closely related species of Culicidae could be only examined through male genitalia and have studied males to identify cryptic species, which could be used as a clue in identifying the females. Many other previous studies have reported the importance of male mosquito genitalia in mosquito identification and have successfully used male genital structure in identifying new mosquito species and sibling species, where the adults were indistinguishable according to their external morphology (Hara 1959; Silvery & Shroyer 1974; Song & Wenzel 2008; Kaur 2014; Yadav *et al*., 2014; Hall *et al.,* 2015; Kaur & Kirti 2017; Sallum *et al.,* 2020). Variations in male genital structures have been used to distinguish subspecies of *Cu. pipiens* complex (Dehghan *et al*., 2011; Diez *et al*., 2012; Wu *et al*., 2014) and genitalia features were promising features in distinguishing *Culex bidens* and *Cu. interfor* (Laurito *et al*., 2017). According to the literature, male genitalia is the only feature that could be used to distinguish sister species *Aedes atlanticus* and *Ae. tormentor*, and identifying four members belonging to *Aedes stiumulans* complex (Silvery & Shroyer 1974).

The male genitalia are formed by modifications in the IXth and Xth segments of the mosquito abdomen. The IXth tergem usually has lateral lobes bearing a various number of setae. Medium lobe is present in some species. Gonocoxite is the largest unit of male genitalia which articulate with the IXth sternum and is covered with scales and the varying number of setae having different lengths, widths and shapes. The gonocoxite might be with or without lobes. These lobes are named based on the position of the gonocoxite i.e., basal lobe, apical lobe, subapical lobe. Apically on the gonocoxite is the gonostylus which also shows species-specific variations. Gonostylar claw is present apically or subapically on the gonostylus and it is present in various shapes. Phallosome is the structure made up of aedeagus (median structure of genitalia that act as the intromittent organ), parameres and basal pieces. Paraproct or the Xth sternite, which is a part of the proctigar (formed by tergem X, cerci and paraprocts) also shows species-specific variations. Some species has a membranous structure called claspette attached to the internal surface of the gonocoxite (Becker *et al.,* 2010; Sallum *et al.,* 2020).

Climatic conditions in tropical countries support higher diversity, abundance and distribution of mosquitoes. There are records of species complexes and sibling species in many genera of Sri Lankan mosquitoes (Surendran *et al.,* 2000; Surendran *et al.,* 2010; Surendran *et al*., 2013; Weeraratne *et al*., 2018). Identification of these mosquitoes has been mainly made by morphological keys, as mentioned above, and many more species and sibling species of complexes in the country are yet to be ratified. To our understanding, identification efforts have not been attempted using the genital structures of Sri Lankan mosquito species. As mounted specimens of male genitalia are long-lasting and cost-effective, this would be a worthwhile addition to mosquito research in the country. In this study, we examined and described species-specific characteristics of the male genitalia of 15 mosquito species belonging to seven genera in Sri Lanka.

MATERIALS AND METHODS

Sample collection

Mosquitoes were collected from three selected localities in the Kandy District, Sri Lanka (7. 255010 N, 80. 601082 E). Two localities were near human settlements [Peradeniya University Park (7.2597 N, 80.5974 E) and Gampola (7.1652 N, 80.5734 E)] while the two other areas were forested habitats [Halgolla Forest Reserve (7.3066 N, 80.5222 E) and Hantana Forest Reserve (7.2497 N, 80.6131 E)]. The study localities (Fig. 1) belong to the wet zone of Sri Lanka and the mean annual temperature and the rainfall of the study localities are 23.5 °C and 2,266 mm respectively.

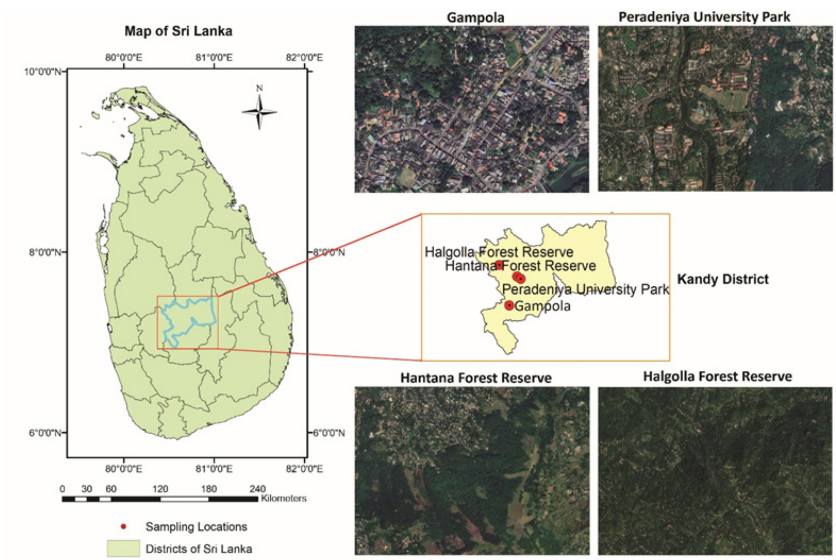

Figure 1: Map showing the four mosquito sampling sites in Kandy district, Sri Lanka.

Adult mosquitoes were collected from 1600 hours to 1900 hours using handheld mechanical aspirators (Hausherr's machine works, USA), sweep nets, CDC miniature light traps (BioQuip products, Rancho Dominguez, CA, USA) and UV traps (BioQuip products, Rancho Dominguez, CA, USA). Larvae were collected directly from their breeding sites. Collected specimens were brought to the laboratory and blood-fed female mosquitoes were kept for 48-72 hours until blood is digested. Larvae were allowed to emerge into adults in the laboratory. Male mosquitoes were separated and used for morphological identification and genitalia mounting.

Mosquito identification

Adult mosquito specimens were identified into generic/ species level using standard morphology based taxonomic keys (Barraud 1934; Bakeaud 1934; Amerasinghe, 1990. Amerasinghe 1995a; Amerasinghe 1995b; Darsie and Samanidou-Voyadjoglou 1997). Males of fifteen mosquito species were identified from the collection.

Male genitalia mounting

Male genitalia of morphologically identified adult males were slide mounted (Maximum of three specimens per species based on the availability) following the methodology described by Rattanarithikul (1982). The abdomens of male mosquitoes were simmered in 10% KOH solution for 20 – 30 min and then soaked in 70% ethanol to rinse off excess KOH. Specimens were soaked in glacial acetic acid for 2 – 3 min and clove oil for 10 min. Male genitalia were pulled out of the abdomen under a light microscope and slide-mounted on Canada balsam. Already developed taxonomic keys based on male genitalia and individual descriptions of genitalia were used in identification (Silvery & Shroyer 1974; Kaur 2014; Wu *et al*., 2014; Yadav *et al*., 2014; Kaur & Kirti 2017; Sallum, *et al.,* 2020). Images were captured using a Digital Microscopic Imager (cellSens 2.1, Germany) fitted to a stereo microscope under the magnification of x400.

RESULTS AND DISCUSSION

Morphological keys are still the most feasible and widely used approach for mosquito identification. This method has many challenges, as mosquitoes are very fragile insects and often get damaged during sampling. However, even in most damaged specimens, genitalia parts are well preserved as these structures are located internally. Compared to molecular identification, the male genitalia mounting method is cost-effective. This has been a precise method in identifying some of the closely related mosquito species and sibling species of mosquito complexes (Silvery & Shroyer 1974; Dehghan *et al*., 2011; Diez *et al*., 2012; Wu *et al*., 2014; Laurito *et al.,* 2017).

Results provide generic and species-specific characteristics of the male genitalia of 15 Sri Lankan mosquito species coming under six genera [(*Aedes aegypti* (Linnaeus), *Ae. albopictus* (Skuse), *Ae. greenii* (Theobald), *Ae. vittatus* (Bigot), *Anopheles elegans* (James), *An. varuna* (Iyengar), *An. maculatus* (Theobald), *An. jamesii* (Theobald), *Armigeres subalbatus* (Coquillett), *Ar. aurolineatus* (Leicester), *Coquilletidia crassipes* (Van der Wulp), *Culex quinquefasciatus* (Say), *Cu. uniformis* (Theobald),

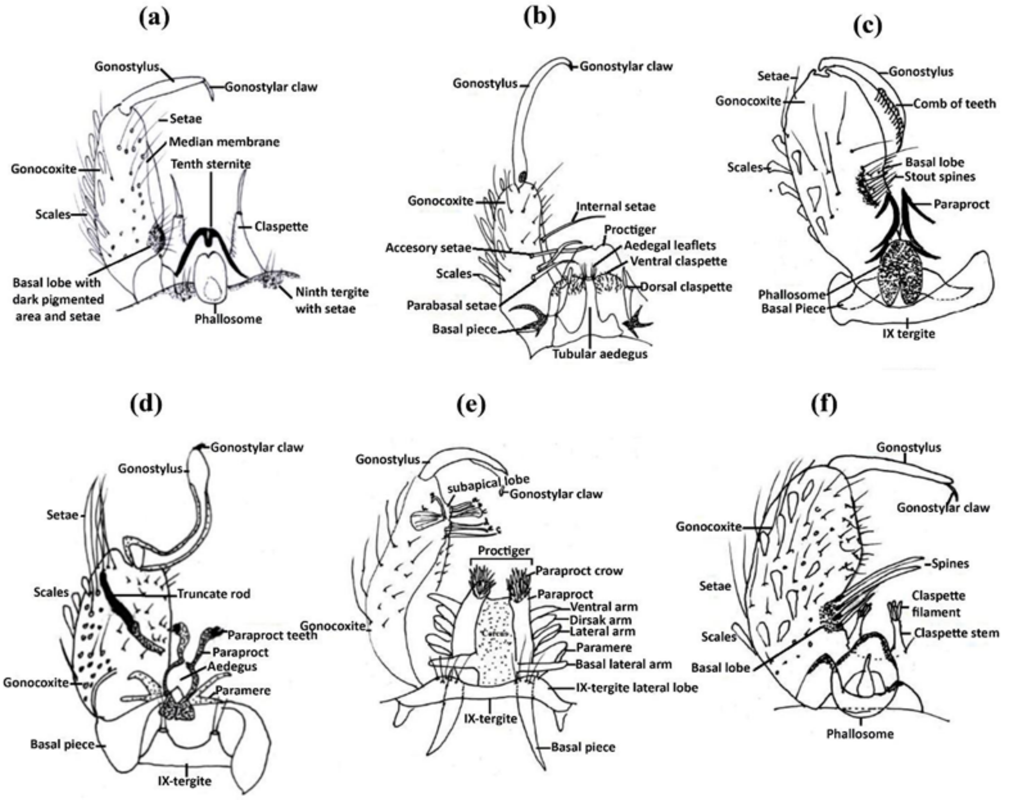

Figure 2: General structure of male genitalia (dorsal view) of the genera a) *Aedes*, b) *Anopheles* c) *Armigeres* d) *Coquilletidia* e) *Culex and* f) *Orthopodomyia* (adapted from Silvery & Shroyer 1974).

Orthopodomyia anopheloides (Giles) and *Or. flavithorax* (Barraud)]. Except for the four *Anopheles* species that belong to the Subfamily Anophelinae all the others were members of the subfamily Culicinae. According to the available literature, the male genitalia of only five species reported during this study has been described earlier in detail (Siverly & Shroyer 1974; Yadav *et al*., 2014; Kaur & Kirti, 2017).

Genus *Aedes*

Figure 2a shows the general structure of the male genitalia of the genus *Aedes*. Variations were observed in size, and the shape of the gonocoxite and gonostylus of the four species. Globular-shaped gonocoxite of *Ae. aegypti* is wide (length less than twice the width), while the gonocoxite of other species are narrower and conical in shape (Figure 3a). Gonocoxite of *Ae. greenii* is covered with a significant number of broad scales and setae (Figure 5a), unlike in the other three species. Length of the gonostylus of *Ae. albopictus* is almost equal to the length of its gonocoxite while the gonostylus of others were shorter than the gonocoxite (Figure 4a). The most conspicuous and unique features of *Ae. vittatus* were its gonostylus and gonostylar claw. The gonostylus is narrow at the base and greatly expands distally (the width of the distal end is more than four times the width at the base) and is covered with rows of numerous spicules. The long gonostylar claw joints ventrally by a short projection onto the base of the expanded area of the gonostylus of this species (Figure 6a, b).

The claspettes of *Ae. albopictus* is unbranched and lack a claspette filament (Figure 4b). The unique claspettes of *Ae. greenii* has a moderately long ventral columnar stem covered with tiny spicules. These spicules terminate with a large, flattened leaf-like filament bearing three short slender setae near the broader area. A narrow lateral arm curved dorsally extends from the base of this stem and bears several setae along the margin in addition to the broadly flattened distal setae (about 5 – 7) (Figure 5c). The structure of the male genitalia of *Ae. greenii* is similar to the description given by Reinert (2003), except for the absence of broadly flattened distal setae on the lateral arm in the samples of this study. Both *Ae. aegypti* and *Ae. vittatus* lack claspettes.

The paraproct of *Ae. aegypti* has well-developed ventral arms, while that of *Ae. albopictus* is a unique structure having a rounded apex crowned with a series of hairs. Paraproct of both *Ae. greenii* and *Ae. vitattus* is narrow and heavily pigmented, while the pointed beak-like apex of the former species has two apical teeth (Figure 5a), while the latter has only a single apical tooth (Figure 6a). The IXth tergite of the male genitalia of *Ae. albopictus* has a conspicuous, thick, broad, horn-like median lobe and weak bilobed lateral lobes (Figure 4b). Although Kaur (2014) has reported variations in the shape of median and lateral lobes of the IXth tergite in *Ae. albopictus*, such variations were not observed in the specimens examined during this study. The other three species have strong, well-developed lateral lobes but no median lobe (Figure 3, 5, 6).

Features of the male genitalia of *Ae. albopictus* (Kaur 2014; Yadav *et al.,* 2014), *Ae. aegypti* (Siverly & Shroyer 1974; Kaur 2014) was similar to the previous descriptions. Among the four *Aedes* species identified during this study *Ae. aegypti* is the primary vector of dengue while *Ae. albopictus* is the secondary vector of the disease.

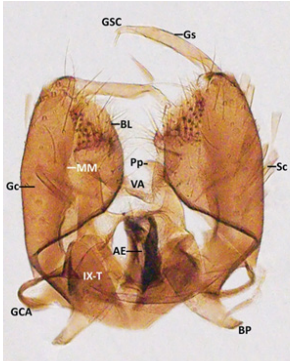

Figure 3: Dorsal view of *Aedes aegypti* male genitalia *(AE- Aedegus, BL- Basal lobe, BP- Basal piece, Gc- Gonocoxite, GCA- Gonocoxal apodeme, Gs- Gonostylus, GSC- Gonostylar claw, MM- Mesal membrane, Pp- Paraproct, Sc- Scales, VA- Ventral arm, IX-T- Ninth tergite)*

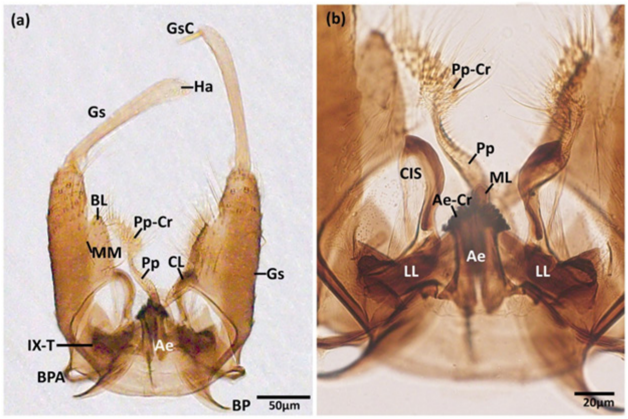

Figure 4: *Aedes albopictus* male genitalia a) dorsal view of full genitalia b) median structures *(Ae- Aedegus, Ae-Cr- Crown of aedegus, BL- Basal lobe, BP- Basal piece, Gc- Gonocoxite, GCA- Gonocoxal apodeme, Gs- Gonostylus, GsC- Gonostylar claw, LL- Lateral lobe, ML- Median lobe, Cl- Claspette, ClS- Claspette stem, MM- Mesal membrane, Pm- Paramere, Pp- Paraproct, Pp-Cr- Paraproct crow, Sc- Scales, IX-S- Ninth sternite, IX-T- Ninth tergite).*

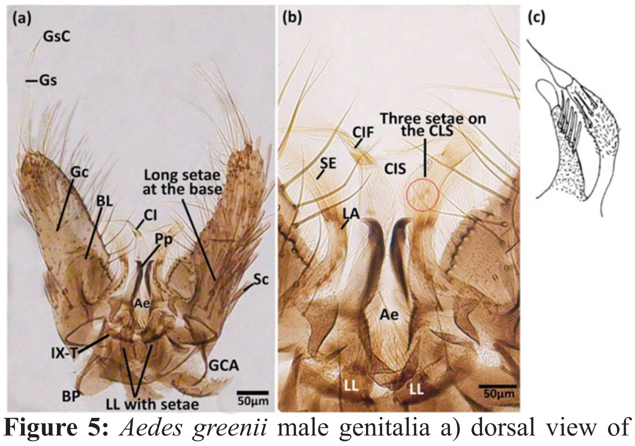

Figure 5: *Aedes greenii* male genitalia a) dorsal view of full genitalia b) median structures showing the claspettes c) line diagram showing the structure of claspettes *(Ae- Aedegus, Ae-Cr- Crown of aedegus, BL- Basal lobe, BP- Basal piece, Gc- Gonocoxite, GCA- Gonocoxal apodeme, Gs- Gonostylus, GsC- Gonostylar claw, LA- Lateral arm,*

LL- Lateral lobe, ML- Median lobe, Cl- Claspette, ClF- Claspette filament, ClS- Claspette stem, MM- Mesal membrane, Pm- Paramere, Pp- Paraproct, Pp-Cr- Paraproct crow, Sc- Scales, Se- Setae, IX-S- Ninth sternite, IX-T- Ninth tergite).

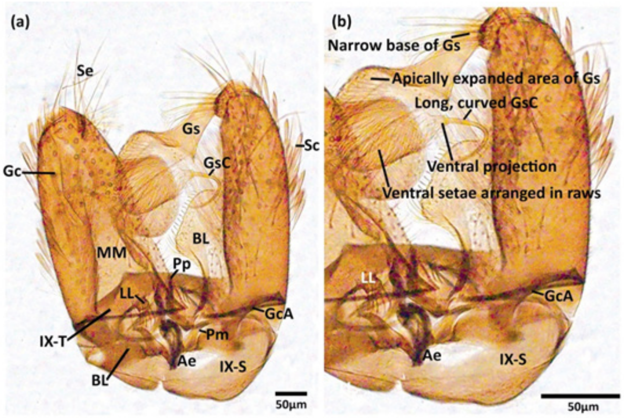

Figure 6: *Aedes vittatus* male genitalia a) dorsal view of full genitalia b) gonosylus *(Ae- Aedegus, BL- Basal lobe, BP- Basal piece, Gc- Gonocoxite, GcA- Gonocoxal apodeme, Gs- Gonostylus, GsC- Gonostylar claw, LL- Lateral lobe, MM- Mesal membrane, Pm- Paramere, Pp- Paraproct, Sc- Scales, Se- Setae, IX-S- Ninth sternite, IX-T- Ninth tergite).*

Genus *Anopheles*

Anopheles is an important mosquito genus as many *Anopheles* species are vectors of deadly diseases, including malaria (Cohuet *et al*., 2010; Manguin *et al*., 2013). Currently accepted sub-generic classification of *Anopheles* is based primarily on the number and positions of parabasal, internal and accessory setae on the gonocoxite of the male genitalia since it was introduced (Harbach 2012). A comprehensive study on the male genitalia of South American Anophelines has been published recently (Sallum *et al.,* 2020). Siverly & Shroyer (1974) also reported the details of the male genitalia of a few species of this genus. In our study we describe the male genitalia of four anopheline species (*i.e. Anopheles elegans*, *An. jamesii*, *An. maculatus* and *An. varuna*). Long tubular aedegus where the length is at least four times long as the width, presence of more than one aedegal leaflets of different shapes, absence of basal and subapical lobes at the gonocoxite and, presence of one to three stout parabasal seate (placed dorsobasally) and internal setae inserted on the ventral side of the gonocoxite are the most prominent characters of anophelines. Most of those previously described genital characters are seen in the four studied organisms (Figure 2b). Membranous two-lobed claspettes and IXth tergite with two lateral lobes without a median lobe are characteristic to *An. jamesii*, *An. elegans*, *An. maculatus* and *An. varuna*. The male genital structures of these four species showed species-specific variations, mainly in the gonocoxite (position and number of specialized setae), claspettes and aedegal leaflets.

Aedegus of *An. maculatus* is relatively shorter and, broader at the base than the aedeagus of the other three anopheline species. The shape of gonocoxite and, the number and shape of aedegal leaflets (three broad and short leaflets on each side) of *An. maculatus* (Figure 7a) and *An. jamesii* (Figure 8a) is similar. A single, short parabasal setae and a straight internal seta just below the apex of gonocoxite are characterized as *An. maculatus* while two parabasal setae and a straight, relatively long internal seta placed on the one-third position from the base of the gonocoxite are characteristics of *An. jamesii*. Ventral claspettes of both these species form an apical lobe, with a strong apical seta mounted on a small projection and two long sub-apical setae. The dorsal claspette of both species has an apical bulb-shaped seta. However, *An. maculatus* had a single long sub-apical seta where *An. jamesii* has more than one sub-apical setae on the dorsal claspette (Figure 7b and 8b).

Gonocoxite and the claspettes of *An. elegans* (Figure 9a) and *An. vaurna* (Figure 10a) share similar shape and structure respectively. However, these two species could be easily distinguished by the shape and structure of aedegal leaflets. *Anopheles elegans* has more than four leaf shaped aedegal leaflets on each side that gradually decreases in size (Figure 9b) while *An. varuna* has four slender aedegal leaflets on each side and sub-apical leaflets positioned almost parallel to its longitudinal axis (Figure 10a). Gonocoxite of *An. elegans* bears three parabasal setae directly inserted on to the surface, one accessory seta just above the parabasal setae and internal setae just above the accessory setae (figure 9a). Ventral claspette of both species are apically broad and has two long apical setae mounted on short projections. Dorsal claspettes are with two apically spatulate, closely approximated club-like setae (Figure 9b, 9c and 10a). Unlike in *An. jamesii* and *An. maculatus* with a prominent proctiger covered with numerous setae, the proctiger of these two species is not prominent.

Genus *Armigeres*

Armigeres is a common mosquito species in Sri Lanka and is known well for its biting nuisance behaviour. Some of the species belonging to this genus are also known as vectors of filarial worms (Aliota *et al*., 2010; Boonserm *et al*., 2019). *Armigeres subalbatus* is the most common species and, *Ar. aureolineatus*, *Ar. magnus* and *Ar. omissus* are the other three species reported from Sri Lanka (Amerasinghe & Munasinghe 1994). This is one of the less studied mosquito genera in the country. Identifying these mosquitoes at the species level is challenging due to the lack of species-level taxonomic keys. The male genitalia of the two *Armigeres* species examined during this study showed clear species-specific variations indicating the applicability of use of male genitalia in species identification.

The presence of closely set scythe-shaped teeth along the apical half of the gonostylus and the absence of gonostylar claw are the main distinguishing features of the male genitalia of this genus (Figure 2c). *Armigeres subalbatus* and *Ar. aurolineatus* can be easily distinguished by comparing the relative density of setae on the gonocoxite. *Armigeres subalbatus* is characterized by dense, long, curved setae on the ventral and lateral surfaces where *Ar. aurolineatus* have relatively fewer setae. The basal lobe of *Ar. aurolineatus* extends along the entire inner surface, while the basal lobe of *Ar. subalbatus* extends only to the base (Figure 11a, b). The gonocoxite of both species are large and the basal lobes contain relatively short, flat two spines and fewer short setae. The number of teeth along the

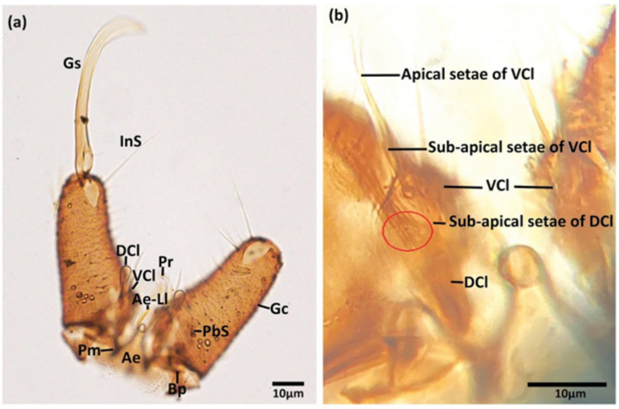

Figure 7: *Anopheles maculatus* male genitalia a) dorsal view of full genitalia b) ventral claspettes *(Ae- Aedegus, Ae-Ll- aedegal leaflets, BP- Basal piece, DCl- Dorsal claspette, Gc- Gonocoxite, Gs- Gonostylus, GsC- Gonostylar claw, In- Internal setae, VCl- Ventral claspette, PbS- Parabasal setae, Pm- Paramere, Pt-Protiger).*

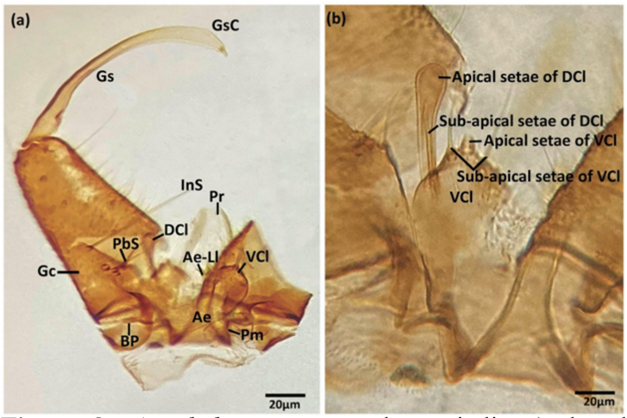

Figure 8: *Anopheles jamesii* male genitalia a) dorsal view of full genitalia b) claspettes *(Ae- Aedegus, Ae-Ll- aedegal leaflets, BP- Basal piece, DCl- Dorsal claspette, Gc- Gonocoxite, Gs- Gonostylus, GsC- Gonostylar claw, In- Internal setae, VCl- Ventral claspette, PbS- Parabasal setae, Pm- Paramere, Pt-Protiger).*

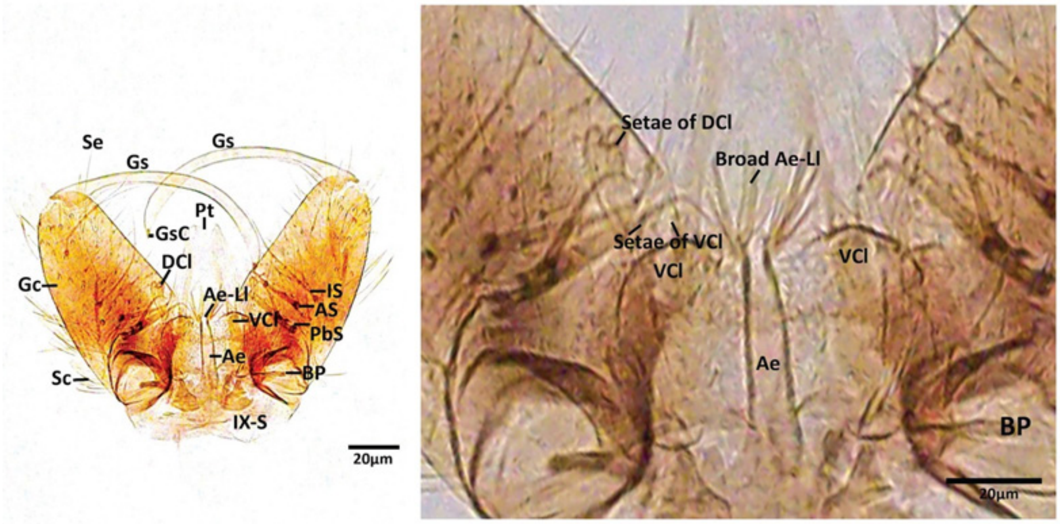

Figure 9: *Anopheles elegans* male genitalia a) dorsal view of full genitalia b) aedegus *(AS- Accessory setae, Ae- Aedegus, Ae-Ll- aedegal leaflets, BP- Basal piece, DCl- Dorsal claspette, Gc- Gonocoxite, Gs- Gonostylus, GsC- Gonostylar claw, In- Internal setae, VCl- Ventral claspette, PbS- Parabasal setae, Pm- Paramere, Pt-Protiger, Se- Setae, Sc- Scales, IX-S- Ninth sternite).*

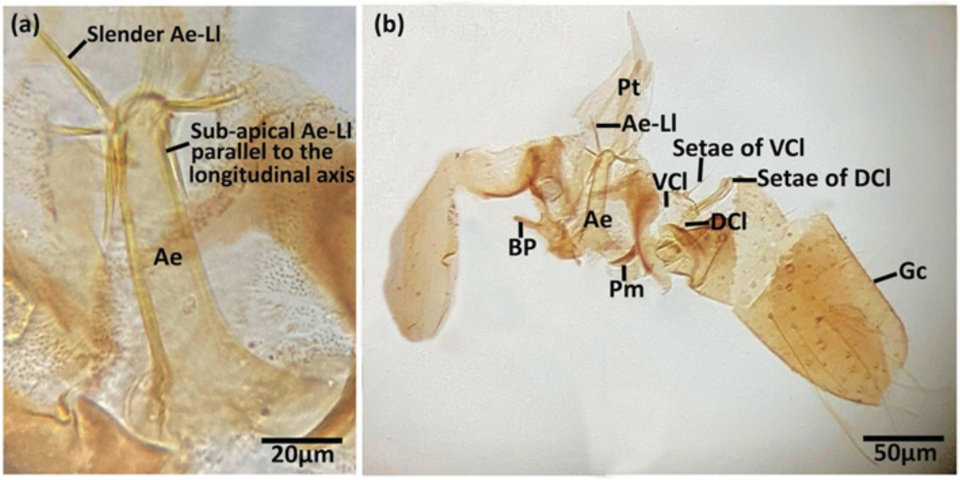

Figure 10: *Anopheles varuna* male genitalia a) Aedegus b) dorsal view of full genitalia *(Ae- Aedegus, Ae-Ll- aedegal leaflets, BP- Basal piece, DCl- Dorsal claspette, Gc- Gonocoxite, VCl- Ventral claspette, Pm- Paramere, Pt-Protiger).*

inner margin of the gonostylus of both species varied from 16-18, and the gonostylus is shorter than the gonocoxite and strongly curved inwards. Sub-triangular paraproct with broad outward curved apical teeth, IXth tergite with less prominent lobes, short phallosome and absence of claspettes are the other shared features of the genitalia of these two species.

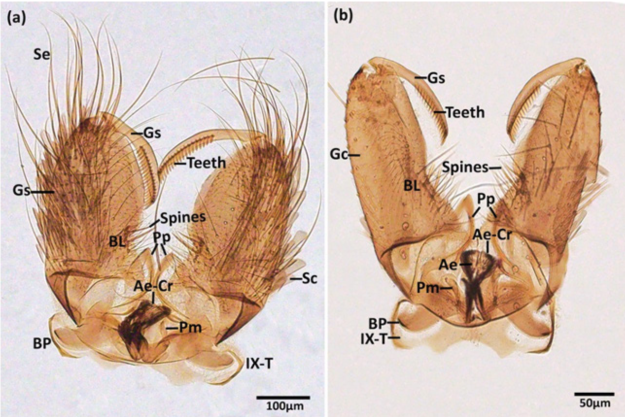

Figure 11: Dorsal view of a) *Armigerus subalbatus* and b) *Armigeres aurolineatus* male genitalia *(Ae- Aedegus, Ae-Cr- aedegal crown, BL-Basal lobe, BP- Basal piece, Gc- Gonocoxite, Gs- Gonostylus, GsC- Gonostylar claw, Pm- Paramere, Pp-paraproct, Sc-Scales, Se- Setae, IX-T- Ninth tergite).*

Genus *Coquilletidia*

Genus *Coquilletidia* is a rare mosquito genus in Sri Lanka. The long, strongly sclerotized blunt tip rod, starting on the mesal surface of the gonocoxite reaching near the apex, is unique to this genus (Silverly & Shroyer 1674). Other shared characteristics are the gonocoxite without stout spines at the base and long non-tubular aedegus (less than four times as long as the width).

In this study, we identified, *Coquilletidia crassipes* and described the genitalia of this species. Some studies reported the presence of Sporozoites of *Plasmodium* sp. in this mosquito species (Telford *et al*., 1997; Njabo *et al*., 2009), suggesting the potential vector status of malaria parasites. The species-specific features of the male genitalia of *Co. crassipes* reported during this study are consistent with previous reports by Yadav *et al.,* (2014). The gonocoxite of *Co. crassipes* is short, broad and globular in shape (width is more than half of the length) with a stout truncate rod as described above and with long cluster of bristles dorsally near the apex. The gonostylus and gonocoxite are equal in length. The gonostylus is swollen at the apex and curved outwards with a short, pointed and well-sclerotized gononostylar claw at the apex. The strongly sclerotized paraproct has a swollen apex with two strong teeth and a narrow stalk. IXth tergite has prominent lateral lobes, each containing two long setae and with a wide interlobar space (median lobe is absent) (Figure 12b).

Genus *Culex*

The genus *Culex* is the next most important mosquito genus, which includes many vectors of infectious diseases such as Filariasis (De & Chandra 1994; Murugan *et al.,* 2015), West Nile virus (Baqar *et al*., 1993; Kent *et al.,* 2010; Reisen *et al*., 2014), St. Louis encephalitis (Bailey *et al*., 1978; Reisen *et al*., 1993) and, avian malaria (Chathuranga *et al.,* 2020). In Sri Lanka, *Cu. quinquefasciatus*, a species examined during this study, is the transmitting agent of nematode *Wuchereria bancrofti,* the causative agent of lymphatic filariasis (Jayasekera *et al*., 1991). Rudolf *et al.,* (2013) and Dehghan *et al.,* (2016) have stated that the morphological identification of *Culex* mosquitoes is challenging due to the presence of sibling species.

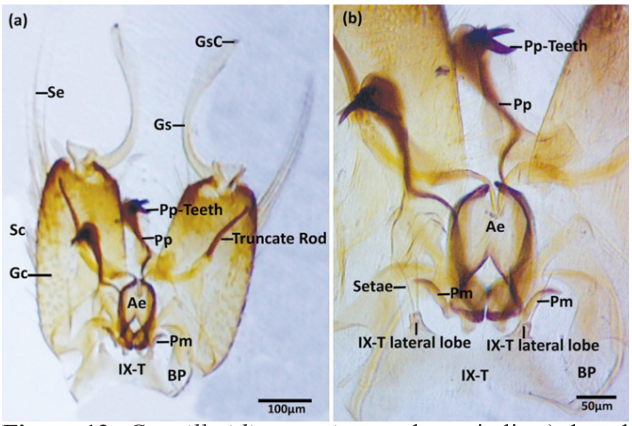

Figure 12: *Coquilletidia crassipes* male genitalia a) dorsal view full genitalia b) IX tergite *(Ae- Aedegus, BP- Basal piece, Gc- Gonocoxite, Gs- Gonostylus, GsC- Gonostylar claw, Pm- Paramere, Pp-paraproct, IX-T- Ninth tergite)*.

As described in previous literature, the genitalia of *Culex* mosquitoes are characterized by the unique structure of the phallosome. Non-tubular phallosome is nearly four times long as the width. The crowned apex of the prominent paraproct has numerous dense short setae, spines or a comb of teeth or a combination of all. Also, the gonocoxite has characteristic subapical lobe-bearing spines, filaments, or both. In general, their gonostylus is much shorter than the gonocoxite (less than half the length of the gonocoxite) and lack claspettes (Figure 2e).

This is the first study which describes the male genitalia of *Cu. uniformis*. The structure of *Cu. quinquefasciatus* male genitalia is consistent with the previous descriptions by Dehghan *et al.,* (2016) and Harbach (2012). Gonocoxite tapering beyond the subapical lobe, which bears eight setae with varying shape and length; having moderate to long, curved setae dorsally and laterally on the gonocoxite; phallosome plates connected by a narrow, sclerotized subapical bridge and, IXth tergite with two relatively narrow lobes each bearing four or more straight, long setae are features common to the male genitalia of both *Cu. uniformis* and *Cu. quinquifaciatus* (Figure 13a, 14a). However, these two *Culex* species showed significant species-specific variations. The most prominent differences between these two species were observed in the structure and shape of the ventral and dorsal arms of the phallosome (Figure 15). Six laterally directed triangular projections along the length of the dorsal arm of phallosome are unique to *Cu. uniformis* (Figure 14c, 15). The outer margin of the gonostylus of *Cu. uniformis* contains short setae (figure 15b) without a gonostylar claw. Instead, the gonostylus of *Cu. quinquefasciatus* contains a subapical claw but without any setae. Another major difference is seen at the basal lateral arm of the paraproct. In *Cu. quinquefasciatus*, the basal lateral arm is shorter than the paraproct (figure 13a, c), while in *Cu. uniformis* both the basal lateral arm and the paraproct have the same length (Figure 13b, 14c).

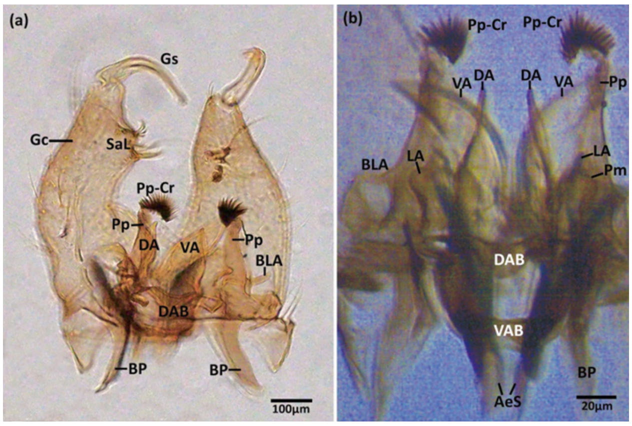

Figure 13: Male genitalia of *Culex quinquefasciatus* a) dorsal view of full genitalia b) ventral view median structures *(Ae- Aedegus, AeS- Aedegal sclerite, BP- Basal piece, BLA- Basal lateral arm, DA- Dorsal arm, DAB- Dosal aedegal bridge, Gc- Gonocoxite, Gs- Gonostylus, GsC- Gonostylar claw, LA- Lateral arm, Pm- Paramere, Pp-paraproct, Pp-Cr-Paraproct crow, SaL- Subapical lobe, Sc-Scales, Se- Setae, VA- Ventral arm, VAB- Ventral aedegal bridge).*

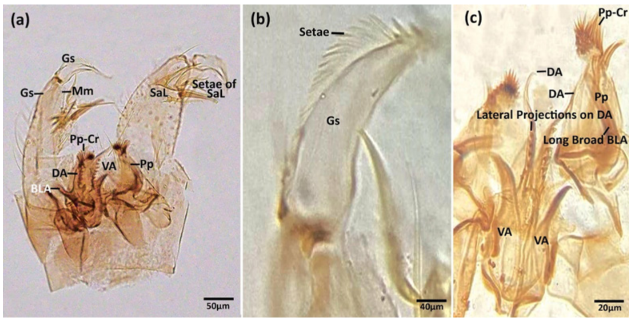

Figure 14: Male genitalia of *Culex uniformis* a) Dorsal view of full genitalia b) gonostylus c) median structures *(Ae- Aedegus, BLA- Basal lateral arm, DA- Dorsal arm, Gc- Gonocoxite, Gs- Gonostylus, Pp-paraproct, Pp-Cr-Paraproct crow, SaL- Subapical lobe, VA- Ventral arm).*

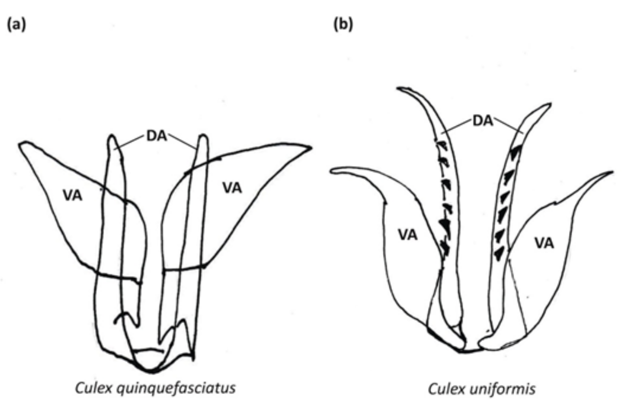

Figure 15: Comparison of ventral and dorsal arms of phallosome plates of *Culex quinquefasciatus* and *Cu. Uniformis.*

Genus *Orthopodomyia*

The genus *Orthopodomyia* includes the common bird-biting mosquitoes in Sri Lanka (Chathuranga, *et al.,* 2018) and the male genitalia of two species [*Or. anopheloides* (Figure 17a) and *Or. flavithorax* (Figure 18a)] are described in this study. The most distinguishing feature of the genus *Orthopodomyia* male genitalia is the long, flattened and blunt spines (2-4) on the basal lobe of gonocoxite (Figure 2f). However, unlike all the other mosquito species that showed species-specific features, the genitalia of the two *Orthopodomyia* species are mostly similar. The distinguishing feature is the number of spines present at the basal lobe (three spines in *Or. anopheloides* and four in *Or. flavithorax*). The length of the four spines of *Or. flavithorax* gradually decreases (Figure 16b, 17a).

Presence of a large number of long setae and scales especially on the lateral face and short setae on the mesal face of the gonocoxite; a spiniform and long gonostylar claw; none-tubular long phallosome; poorly developed paraproct which is not sclerotized and presence of claspettes (figure 2f) are other features common to both species. Further, the presence of short hair-like setae from the middle to the distal end of the gonostylus (Figure 16b,17b) and claspettes with a broad stem with strong setae laterally and a filament with blunt 3-5 finger-like projections at the apex (Figure 18c) were unique to these two species.

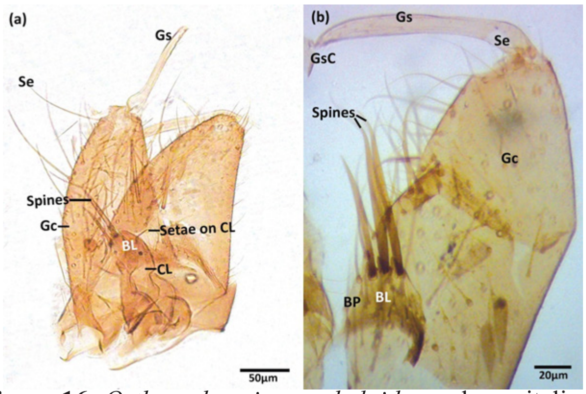

Figure 16: *Orthopodomyia anopheloides* male genitalia a) dorsal view of full genitalia b) gonocoxite and gonoxstylus *(BL- Basal lobe, Cl- Claspette, Gc- Gonocoxite, Gs- Gonostylus, GsC- Gonostylar claw, Se- Seta).*

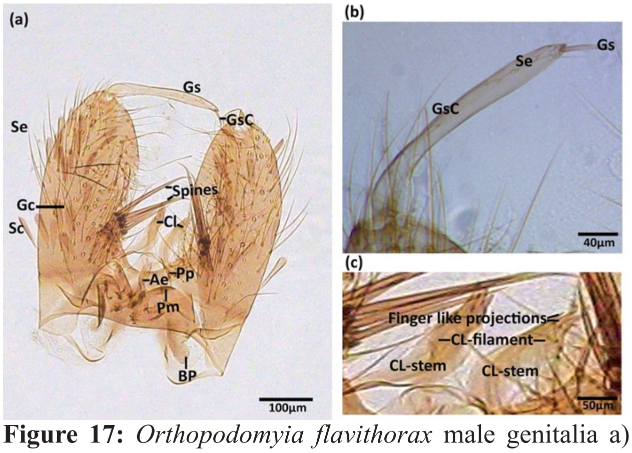

Figure 17: *Orthopodomyia flavithorax* male genitalia a) dorsal view of full genitalia b) gonostylus c) claspette *(Ae- Aedegus, BL- Basal lobe, BP- Basal piece, Cl- Claspette, Gc- Gonocoxite, Gs- Gonostylus, GsC- Gonostylar claw, Pm- Paramere, Pp- Paraproct, Se- Setae, Sc- Scales).*

All the mosquitoes of each genus except Aedes showed a unique feature that could be easily used in identifying the genus. Scythe-shaped teeth along the apical half of the gonostylus in *Armigeres*, special setae (parabasal, internal and accessory setae) on the basal lobe in anophelines, presence of paraproct crown in *Culex*, stout blunt tip truncate rod of *Coqulletidia*, long and flat spines on the basal lobe in *Orthopodomyia* are these generic-specific features (Figure 2b-f). A combination of characters is, however, required in identifying the male genitalia of the genus *Aedes* (Figure 2a). The generic and species features of male genitalia we identified from this study are primarily consistent with the previous descriptions in the literature (Siverly & Shroyer 1974; Reinert 2003; Yadav *et al*., 2014; Kaur & Kirti 2017). The most conspicuous species-specific feature/s that could be used to distinguish each mosquito species of the current study is presented in Table 1.

Although male genital structures could be used effectively in mosquito identification, time consumption in preparing mounted slides, lack of taxonomic features in damaged specimens, and the skills and expert knowledge required are the challenges. Sampling of male mosquitoes is also very challenging as most of the available mosquito traps attract females more than males. Future work is needed to identify the specific features of the male genitalia of sibling species and species complexes in Sri Lanka. More mosquito species are also needed to be examined to develop a comprehensive pictorial key.

Table 1: The most conspicuous species specific feature/s of male genitalia of mosquito species identified in the current study.

Species	Species specific feature
Ae. aegypti	Globular-shaped gonocoxite and paraproct with well-developed ventral arms, no claspettes
Ae. albopictus	Paraproct with rounded apex crowned with series of hairs and IXth tergite with horn-like median lobe and weak lateral lobes
Ae. greenii	Claspettes with ventral columnar stem covered with tiny spicules that terminate with a large, flattened leaf-like filament bearing three short slender setae
Ae. vittatus	Gonostylus narrow at the base and greatly expands distally and, the longer gonostylar claw joint ventrally by a short projection onto the base of the expanded area of the gonostylus
An. elegans	More than four aedegal leaflets on each side that gradually decreases in size
An. jamesii	Two parabasal setae and a straight, relatively long internal seta placed on the one-third position from the base of the gonocoxite
An. maculatus	A single, short parabasal setae and a straight internal seta just below the apex of gonocoxite
An. varuna	Four slender aedegal leaflets on each side and sub-apical leaflets positioned almost parallel to its longitudinal axis
Ar. aurolineatus	Gonocoxite with relatively fewer setae and 16-18 teeth along the inner margin of the gonostylus
Ar. subalbatus	Gonocoxite is densely covered with long, curved setae and 16-18 teeth along the inner margin of the gonostylus
Co. crassipes	*Gonocoxite with* stout truncate rod and the long cluster of bristles near the apex.
Cu. quinquefasciatus	Gonostylus containing a subapical claw without any setae and basal lateral arm and the paraproct equal in length
Cu. uniformis	Six laterally directed triangular projections along the length of the dorsal arm of phallosome
Or. anopheloides	Three spines present at the basal lobe of the gonocoxite
Or. flavithorax	Four spines present at the basal lobe of the gonocoxite

CONCLUSION

The study provides important information about the male genitalia of fifteen species belonging to six important mosquito genera (*Aedes, Anopheles, Armigeres, Coquilletidia, Culex* and *Orthopodomyia*) in Sri Lanka. According to our knowledge, this is the first study that describes the male genitalia of *Anopheles elegans, An. jamesii*, *An. maculatus An. varuna, Armigeres subalbatus, Ar. aurolineatus, Coquilletidia crassipes, Culex uniformis, Orthopodomyia anopheloides* and *Or. flavithorax*. The described male genital features can be integrated into the current common practices (i.e. morphological and molecular identification approaches) to make more accurate decisions in mosquito identification.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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