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Arif Mehmood
Department of Entomology,
Faculty of Crop and Food
Sciences, PMAS Arid Agriculture
University, Rawalpindi. 46000.
Punjab, Pakistan

Muhammad Naeem
Department of Entomology,
Faculty of Crop and Food
Sciences, PMAS Arid Agriculture
University, Rawalpindi. 46000.
Punjab, Pakistan

Imran Bodlah
Department of Entomology,
Faculty of Crop and Food
Sciences, PMAS Arid Agriculture
University, Rawalpindi. 46000.
Punjab, Pakistan

Ata-ul-Mohsin
Department of Entomology,
Faculty of Crop and Food
Sciences, PMAS Arid Agriculture
University, Rawalpindi. 46000.
Punjab, Pakistan

Systematics of *Anopheles* and *Armigerus* (Culicidae: Diptera) mosquitoes in the Pothwar Region, Punjab, Pakistan

Arif Mehmood, Muhammad Naeem, Imran Bodlah, and Ata-ul-Mohsin

Abstract

Mosquitoes are widely distributed in tropical and sub-tropical areas of the world. In Pakistan they are found in almost all the parts but their fauna is unexplored in many areas of the country. In this study, the *Anopheles* and *Armigerus* mosquitoes of Pothwar region has been explored. Different sampling points were made in the study area which covered breeding places of mosquitoes including houses, park, graveyard, scrape yard, animal shed, standing water and forest area. Study was conducted from 2014-16. A total of eight mosquito species: six from *Anophlese* and two belonging to *Armigerus* were recorded from Pothwar Region, Punjab, Pakistan of which *Armigerus kuchingensis* Edwards, 1915 is recorded for the first time from Pakistan. Main identification characters supported with micrographs and GPS positions of all recorded species have been provided. Field samples were preserved in the Biosystematics Laboratory, Department of Entomology, Pir Mehr Ali Shah- Arid Agriculture University Rawalpindi (PMAS-AAUR).

Keywords: Taxonomic keys, *Armigerus*, *Anopheles*, Pothwar, Punjab, Pakistan

1. Introduction

Among arthropods, mosquitoes are the most dangerous for humans. They are the vector of many lethal diseases to humans e.g., dengue, malaria, yellow fever etc. They belong to the order Diptera, suborder Nematocera and family Culicidae. There are three subfamilies of the family Culicidae including Anopheline, Culicinae and Toxorhynchites (Service, 2008). These two winged flies are distributed worldwide in different types of habitats. Till date, almost 3500 species of mosquitoes have been discovered (Khan *et al.*, 2015)^[8, 9]. Recently, four species of subfamily Anophlinae have been reported from Murree (Qasim *et al.*, 2014)^[11]. *Armigerus subalbatus* have been reported from Peshawar (Naz *et al.*, 2014)^[10]. Four species of *Anoplese* mosquitoes were reported from Swat (Illahi and Suleman, 2013)^[7]. Six species belonging to *Anoplese* and one from *Armigerus* were reported from Malakand District (Ali *et al.*, 2013)^[1]. Five species belonging to *Anoplese* genus and one from *Armigerus* were reported from Narowal (Rasool *et al.*, 2015)^[12].

Thirty four anopheline mosquitoes were reported from Bangladesh. Three *Anophlese* species were reported from Iran (Banafshi *et al.*, 2013)^[2].

Keeping in view the importance of mosquitoes as a vector of diseases and the unexplored fauna, this study was planned to fulfill the research gap.

2. Materials and Methods

The study was conducted in four Districts of Pothwar Region including Rawalpindi, Attock, Jhelum and Chakwal from 2014-16. The sampling sites were visited for the survey on the bases of different habitats where mosquitoes commonly prefer to breed and live. The sampling sites include human dwellings, parks, forest areas, scrapyard, animal sheds and standing water. In these sites, there were also some microhabitats including tree holes, earthen pots, discarded receptacles, standing water, vegetation, old tires, sewerage drains etc. Immature stages were collected from standing water at specified sampling points with the help of standard larval dipper. The immature stages of mosquitoes were reared for obtaining the adults.

Correspondence
Arif Mehmood
Department of Entomology,
Faculty of Crop and Food
Sciences, PMAS Arid Agriculture
University, Rawalpindi. 46000.
Punjab, Pakistan

Adults of mosquitoes were collected from the specified sampling points with the help of aerial net, aspirator and dry CO₂ trap. For indoor collection aspirator was used. Human and animal landing mosquitoes were captured with the help of aspirator. From open places the adults of mosquitoes were collected with the help of aerial net. Aerial net was swept in the vicinity of mosquitoes, the captured mosquitoes were killed using cyanide killing jar. The collected specimens were mounted on entomological pins. Pinned specimens were tagged, labeled and preserved in boxes. Naphthalene bolls and coopex powder was used for preservation. The preserved specimens were identified with the help of Swift microscope using different available taxonomic keys including The Fauna of British India (Barraud PJ, 1934), Mosquito (Diptera: Culicidae) of Murree Hills, Punjab, Pakistan (Qasim et al., 2014) and A Study of Mosquito Fauna of District Upper Dir, Khyber Pakhtunkhwa Pakistan (Khan et al., 2015). During the survey, different equipment were used. Temperature and humidity was measured with the help of thermo hygrometer. pH was measured with the help of pH meter. Altitude and latitude of the location were measured with the help of GPS device (etrex Garmin)

3. Results and Discussion

3.1 Key to Subfamilies of Family Culicidae

1. Palpi and proboscis equal scutellum almost in rounded form..... Subfamily Anophelinae
2. Palpi are shorter than the proboscis, scutellum trilobed..... Subfamily Culicinae

3.2 Subfamily Anophelinae

Anophelinae consist of three genera including *Anopheles*, *Chagasia* and *Bironella*. Palpi of both male and female are equal in length with respect to proboscis.

3.2.1 Genus *Anopheles* Meigen, 1818

Subfamily Anophelinae is widely distributed. Genus *Anopheles* Meigen belongs to this subfamily. The prominent character is wing spots. The sitting position is very specific, making an angle. In females, proboscis and palpi are equal in length. Palpi are swollen at end in males.

3.2.1.1 Subgenus *Cellia* Theobald, 1902

Wing contains four pale spots that are present in costal region instead of humeral and anal region.



Fig 3.1: Wings have four pale spots

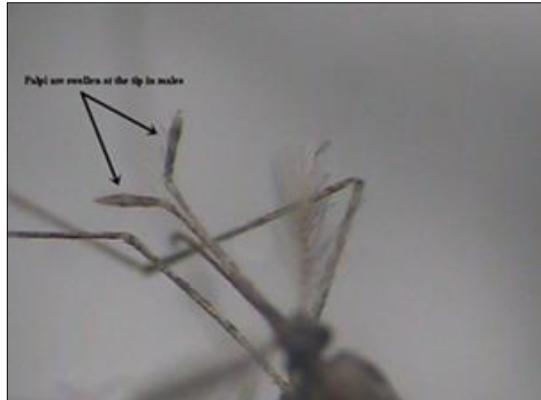


Fig 3.2: Palpi are swollen at the tip in males

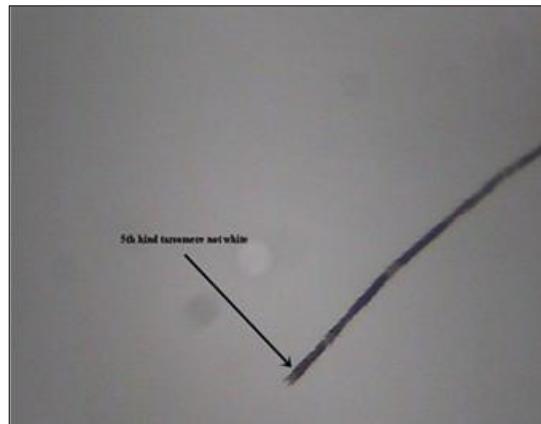


Fig 3.3: 5th hind tarsomere not white (*Anopheles stephensi*)



Fig 3.4: 5th, 4th and 3rd hind tarsomere completely white



Fig 3.5: 5th vein dark near fork

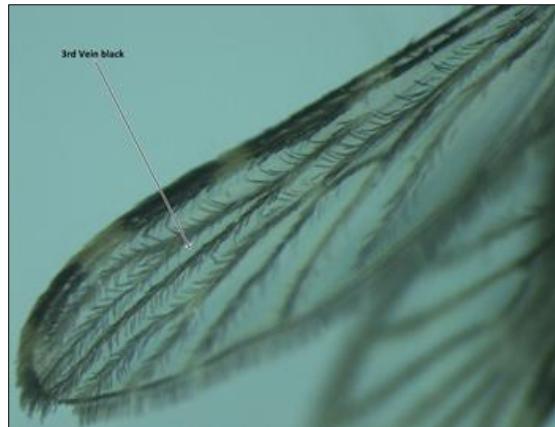


Fig 3.6: 3rd vein black



Fig 3.10: Femora and tibia not spotted

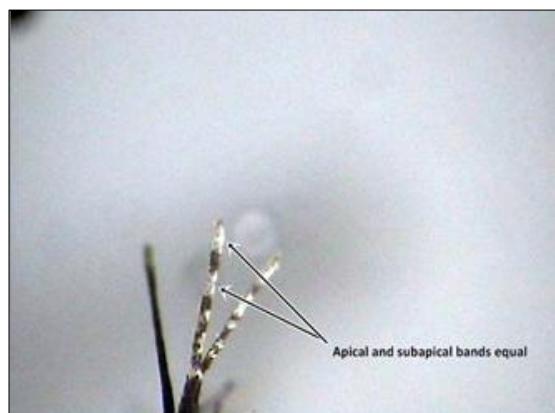


Fig 3.7: Apical and pale apical bands equal



Fig 3.11: Palpi with four bands



Fig 3.8: Sub apical band expanded than

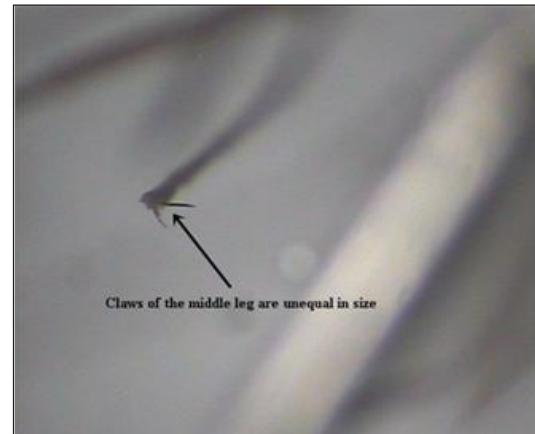


Fig 3.12: Claws on middle leg unequal in size



Fig 3.9: Femora and tibia spotted



Fig 3.13: Variously marked on 3rd to 6th abdominal sternites (*Armigeres kuchingensis*)



Fig 3.14: Claws of the middle leg are of same size (*Armigeres kuchingensis*)

3.2.1.1.1 *Anopheles annularis* Van der Wulp, 1884

Synonyms: *Anopheles jamesi* Liston, 1901; *Anopheles fuliginosus* Giles, 1901; *Anopheles jamesi* James, 1902.

White scales are present. White scales are present on two to sixth flagellum segments. Palpi are pale scaled and pale apical bands are present on it. Mesonotum is black and covered with oval shape scales. Two dark colored scales are present on thorax. Spiracular setae are present and about six upper mesepimeral bristles are present. Costal base is dark with subcostal pale area. Vein five is dark at the region of branch (Fig. 3.5). Wings are spotted. Front femor is swollen. Middle femora are pale, tibia dark and front tarsus is banded. Hind tarsal segment 3rd, 4th and 5th are white (Fig. 3.4). Dark scales present on abdominal tergites 6th, 7th and 8th. Some pale scales also present on 8th segment. Cerci contain dark scales.

Material Examined

Park, houses, standing water (Attock): 72.3562E, 33.7739N, 72.3536E, 33.7732N, 72.3595E, 33.7633N, Elev: 1145 ft, 13-IV-2014, 11-V-2014, 12-VII-2014, 4♂, 4♀. Animal Shed, houses (Jhelum): 73.432712E, 32.5512420N, 73.4330152E, 32.5518418N, Elev: 728 ft, 8-V-2015, 11-VI-2015, 12-VII-2015, 15-VIII-2015, 4♂, 3♀.

Comments

Published description given by Barraud (1934)^[3] and Bina pani das *et al.*, (1990)^[6] indicates that main distinct character of *Anopheles annularis* is wing vein five is dark and 3rd, 4th and 5th hind tarsomere white.

3.2.1.1.2 *Anopheles culicifacies* Giles, 1901

Synonyms: *Anopheles punjabensis* James, 1911; *Anopheles listonii* Giles, 1901.

Scales are present. Vertical setae are yellow and forming a row of about five hairs. Scales on antennae are absent or rudimentary. Terminal segment of palpi is very short. Dark area is present between apical and subapical bands. Propleural setae are present. Pale scales are present on mesonotum with dark median area. Pleura are devoid of scales, two spiracular hairs and thirteen upper mesepimeral bristles. Vein third is completely black (Fig. 3.3). In some species it is pale in middle. Fore femora is not swollen. Femora near the base is little pale beneath. Tibia are dark colored. Tarsi are not banded (Fig. 3.6). Abdominal scales are absent and also no scales on cerci.

Material Examined

Scrapyard, Animal Shed (Attock): 72.3512E, 33.7706N, 72.3536E, 33.7732N, Elev: 1145 ft, 13-VII-2014, 11-VIII-2014, 11-IX-2014, 14-XI-2014, 19♂, 21♀. Animal shed, houses, standing water (Jhelum): 73.432712E, 32.5512420N, 73.4330152E, 32.5518418N, 73.4339134E, 32.5510423N, Elev: 728 ft, 24-V-2015, 12-VI-2015, 14-VII-2015, 11♂.

Comments

Published description given by Barraud (1934)^[3] and Das *et al.*, (1990)^[6] indicates that third vein of wing is black in *Anopheles culicifacies* that is main distinct character.

3.2.1.1.3 *Anopheles stephensi* Liston, 1901

Synonyms: *Neocelha intermedia* Rothwell, 1907; *Anopheles metaboles* Theobald, 1902; *An. folquei* Mello, 1918

Pale scales are present on eye margins. Male palpi are swollen at tip and are equal in length to proboscis. Small pale scales present on pedicel. Two equal pale bands are present on palpi in females (Fig. 3.7). Apical part of pronotum contains pale scales. Mesothorax is covered with pale scales and lateral area is dark colored. Pleurae are devoid of scales. Upper mesepemiron contains five to six hairs and spiracular area with two to three hairs. Four or more black spots present on the costal region. Colored scales are present on wings. Femur and tibia contain white spots (Fig. 3.9). Fore femur is swollen basally. First tarsal segment contain spot. Hind femor and tibia are pale colored. Narrow scales are present on 2nd, 3rd and 8th tergites and are also covered with dark spots. Cerci are covered with scales.

Material Examined

Animal shed, forest area, standing water (Attock): 72.3536E, 33.7732N, 72.3595E, 33.7633N, Elev: 1145 ft, 15-III-2014, 10-IV-2014, 14-V-2014, 24-VI-2014, 12-VII-2014, 10-VII-2015, 11-IX-2015, 16-X-2015, 10-XI-2015, 20♂, 10♀. Animal shed, forest area, standing water (Rawalpindi, Islamabad): 73.1334E, 33.2517N, 74.1335E, 32.2015N, Elev: 1667, 7517 ft, 2-IV-2014, 10-V-2014, 6♂, 6♀. Animal shed, houses (Chakwal): 72.86619E, 32.92070N, 72.5170235E, 32.590690N, Elev: 1634 ft, 10-IV-2015, 12-V-2015, 11-VIII-2015, 13-IX-2015, 6♂, 4♀.

Comments

Published description given by Barraud (1934)^[3] and Becker *et al.*, (2010) indicates that main distinct character of *Anopheles stephensi* is speckled femur and tibia.

3.2.1.1.4 *Anopheles fluviatilis* James, 1902

Synonyms: *Anopheles listonii* Liston, 1901

Antennae have no scales. Palpi straight, cylindrical and thin. Palpi have dark ring on the sub apical part that is wider than pale apical bands. Mesonotum pale scaled. Base of costa is dark colored and third vein is pale scaled. Femor and tibiae are dark in color and not pale from beneath (Fig. 3.10). Abdominal scales completely absent. No scales on cerci.

Material Examined

Animal shed, standing water (Attock): 72.3536E, 33.7732N, 72.3595E, 33.7633N, Elev: 1145 ft, 18-VII-2014, 24-VIII-2014, 13-IX-2015, 13♂, 5♀.

Comments

Published description given by Barraud (1934)^[3] and Das *et*

al., (1990) [6] indicates that main distinguished character of *Anopheles fluviatilis* is femora and tibia are not speckled.

3.2.1.5 *Anopheles tessellatus* Theobald, 1901

Synonym: *Anopheles punctulatus* Don, 1901

Scales are present with well-marked vertical white colored area. Four pale bands on palpi that include two narrow black bands (Fig. 3.11). Apical segments of palpi long. Dark scales on thorax. Propleural hairs present on thorax. Pleurae dark colored and spiracular hairs absent. Wings have spots. Wing characters almost resemble with *Anopheles leucosphyrus*. Spots present on femora and tibiae. Hind tarsomere five not white. Abdomen is dark colored and has hairs. Cerci have no scales.

Material Examined

Animal shed, Park (Attock): 72.3536E, 33.7732N, 72.3562E, 33.7739N, Elev: 1145 ft, 14-VI-2014, 3♂, 2♀. Animal sheds, Houses, standing water (Jhelum): 73.432712E, 32.5512420N, 73.4330152E, 32.5518418N, 73.4339134E, 32.5510423N, Elev: 728 ft, 10-V-2015, 15-VI-2015, 11-VII-2015, 13-VIII-2015, 7♂, 2♀.

Comments

Published description given by Barraud (1934) [3] and Das *et al.* (1990) [6] indicates that in *Anopheles tessellatus* was found in Burma, India, Pakistan, Srilanka, China and Peninsula. *Anopheles tessellatus* feed on buffalo blood.

3.2.1.6 *Anopheles splendidus* Koidzumi, 1920

Synonym: *Anopheles sinensis* Theobald, 1901

Scales are present with well-marked vertical white colored area. First flagellum segment is white scaled. Palpi is moderately thickened and have two equal broad bands (Fig. 3.7). Thorax has no scales. Mesonotum dark but white in middle. Pleurae dark colored have white setae and no scales. Nine upper mesepimeral bristles and one to six spiracular hairs present. Costal base is dark and subcostal region is pale. Vein two is scaled. Wings are spotted. Wing characters almost resemble with *Anopheles annularis*. Femor and tibiae have round and spherical white spots. Hind tarsal segments are white. Abdomen is dark colored. 8th abdominal segment is pale scaled dorsally. Cerci contain black scales.

Material Examined

Animal shed, houses (Chakwal): 72.86619E, 32.92070N, 72.5170235E, 32.590690N, Elev: 1634, 10-IV-2015, 11-V-2014, 18-VIII-2014, 12-IX-2014, 8♂, 10♀.

Comments

Published description given by Barraud (1934) [3] and Das *et al.*, (1990) [6] indicates that in *Anopheles splendidus* third, fourth and fifth abdominal segments are white in color.

3.3 Subfamily Culicinae

Subfamily culicinae contains fifteen genera. Most members are brown silver and metallic colored.

Key to Genera of Culicinae

2. Proboscis turn downward *Armigeres*

3.3.2 Genus *Armigeres* Theobald, 1901

Proboscis is bended downward. Large size mosquitoes have resemblance with *Aedes*. Male palpi are equal to proboscis.

Female abdomen taper toward apex.

3.3.2.1 Subgenus *Armigeres* Theobald, 1901

Proboscis is bended downward. Postspiracular area has bristles and scales. Lower mesepimeral have a single bristle. Palpi of female are only one third length of proboscis.

Key to the Adult Speci1. Wide black apical bands present on 3rd to 6th abdominal sternites (Fig. 3.13). Claws of the middle leg unequal in size (Fig. 3.12).....

Armigeres obturbans

-Variously marked on 3rd to 6th abdominal sternites. Claws of the middle leg are of same size (Fig. 3.14)..... *Armigeres kuchingensis*

3.3.2.1.1 *Armigeres (Armigeres) obturbans* Walk, 1860

Synonyms: *Armigeres panalectoros* Giles, 1902; *Culex subalbatus* Coquillett, 1898; *Culex ventralis* Walker, 1861. White scales are present on the vertex and remaining area contains flat blackish blue scales. Creamy white scales are present near eye margins. Proboscis and the palpi are brownish black in colour. Dorsal side of the thorax covered with the narrow dark brown scales. Bristles and the scales are present just behind the anterior spiracle. Wings have dark scales. Brownish black is the colour of legs. Hind femur have white colour from outside up to knee. Legs are pale in colour when seen from back side. Abdomen have brownish black colour on the dorsal side and contain white markings on the lateral sides. 3rd and 4th segments have white basal band while having black bands apically.

Material Examined

Forest area, park, standing water (Attock): 72.3512E, 33.7706N, 72.3595E, 33.7633N, Elev: 1145 ft, 17-V-2014, 19-VII-2014, 13-VIII-2014, 7♂, 9♀. Forest area, park, standing water (Rawalpindi, Islamabad): 74.1335E, 32.2015N, 73.0801900E, 33.5731300N, 73.1334E, 33.2517N, Elev: 1667, 7517 ft, 17-V-2014, 13-VII-2014, 12-VIII-2014, 11♂, 12♀. Parks, standing water (Chakwal): 73.1334E, 33.2517N, Elev: 1634 ft, 11-VII-2015, 13-VIII-2015, 10-IX-2015, 12-X-2015, 7♂, 7♀. Forest area, park, standing water (Jhelum): 73.4550160E, 32.5520428N, 73.4337158E, 32.5522434N, 73.4339134E, 32.5510423N, Elev: 728 ft, 10-I-2016, 12-II-2016, 11-III-2016, 15-IV-2015, 17-V-2015, 11-VI-2015, 9-VII-2015, 10-VIII-2015, 11-IX-2015, 10-X-2015, 12-XI-2015, 10♂, 7♀.

Comments

Specimens match with the published description given by Barraud (1934) [3]. *Armigeres obturbans* have unequal size of the claws of the middle leg as a major identification character.

3.3.2.1.2 *Armigeres (Armigeres) kuchingensis* Edwards, 1915

White broad scales are present on the vertex and remaining area carry flat blackish blue scales. Proboscis and the palpi are brownish black in colour. Dorsal side of the thorax covered with the narrow dark brown scales. Mesothorax contains white scales. Wings have few dark scales. Colour of legs is brownish. Hind femur have pale colour from outside up to knee. Legs are pale in colour when seen from back side. Abdomen have brownish colour on the dorsal and ventral sides and contain white scales on the ventral sides.

Material Examined

Houses, forest area, park, standing water (Chakwal): 72.5224956E, 32.5432972N, 72.5112265E, 32.566686N, 73.1334E, 33.2517N, Elev: 1634 ft, 17-VII-2015, 19-VIII-2015, 12-IX-2015, 11♂, 12♀. Forest area, park, standing water (Jhelum): 73.4550160E, 32.5520428N, 73.4337158E, 32.5522434N, 73.4339134E, 32.5510423N, Elev: 728 ft, 17-I-2016, 11-II-2016, 13-III-2016, 12-IV-2015, 13-V-2015, 11-VI-2015, 10-VII-2015, 11-VIII-2015, 16-IX-2015, 10-X-2015, 9-XI-2015, 11♂, 12♀.

Comments

Specimens match with the published description given by Barraud (1934) [3]. *Armigeres kuchingensis* have same size of the claws of the middle leg as a major identification character. This is reported from Pakistan for the first time.

Acknowledgments

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