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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF GENUS *ANOPHELES* (DIPTERA: CULICIDAE) OF GANJAM DISTRICT ORISSA, INDIA

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INTRODUCTION

The District Ganjam is located 19.4^0 North Latitude to 20.17^{0}North Latitude and 84.7^0 East Longitude to 85.12^{0}East Longitude spread over the geographical area of 8070.60 square km. Ganjam district is broadly divided into two divisions, the coastal plains on the east and hill and table lands on the west. There are 22 blocks with18 Urban Bodies under 3212 villages in the district (file:///E:/Molecular%20studies/GIS,%20Ganjam.htm). The district is surrounded by Khurda, Phulbani, Gajapati and Nayagarh districts in four different directions. The Eastern Ghats run along the western side of the district. The extreme north east is occupied by a portion of the famous Chilika Lake. The district is characterized by hot temperature all through the year, particularly in the coastal regions with higher humidity. The human malarial transmission is very high in this district.

The application of molecular approaches to systematic studies ranging from sub-generic and species level is helping to address various questions such as anopheline phylogenetics and biogeography, nature of species boundaries and the forces that have structured genetic variation within species.

In India, 58 Anopheles have been described, six of which have been implicated to be main malaria vectors, namely *An. culicifacies, An. dirus, An. fluviatilis, An. minimus, An. sundaicus* and *An. stephensi*. Besides, some vectors of local importance, termed as secondary vectors viz. *An. philippinensis-nivipes, An. varuna, An. annularis* and *An. jeyporiensis*. The six recognized primary malaria vectors in India, all except *An. stephensi* are species complexes. The five species complexes are Culicifacies complex, Fluvitilis complex, Minimus complex, Dirus complex and Sundaicus complex. There are growing evidences that the members of species complexes differ significantly in biological characteristics that are vital for malaria control such as vectorial potential, host-preference, resting behaviour and response to insecticides.

Twenty two species has been reported from the Jeypore hills of Koraput district of Orissa by Gunasekaran *et al.,* (1989) which is the nearest district to the Ganjam district, Orissa. However Rajavel (2005) reported only 8 species from the same place. The relatively higher humidity and the stagnant water bodies of Ganjam district provide a good breeding ground for the mosquitoes throughout the year especially in coastal areas. Thus diversity of the species is remarkably high in this area. During the past three decades, due to various changes in the ecological conditions by extensive deforestation, frequent cyclones and extensive use of insecticides. More number of malaria cases have been recorded, however there are no study on mosquitoes.

There are a few sporadic studies on the distribution of the fauna Dash *et al.* (1984) Nagpal and Sharma (1983), Rajavel (2005). It is likely

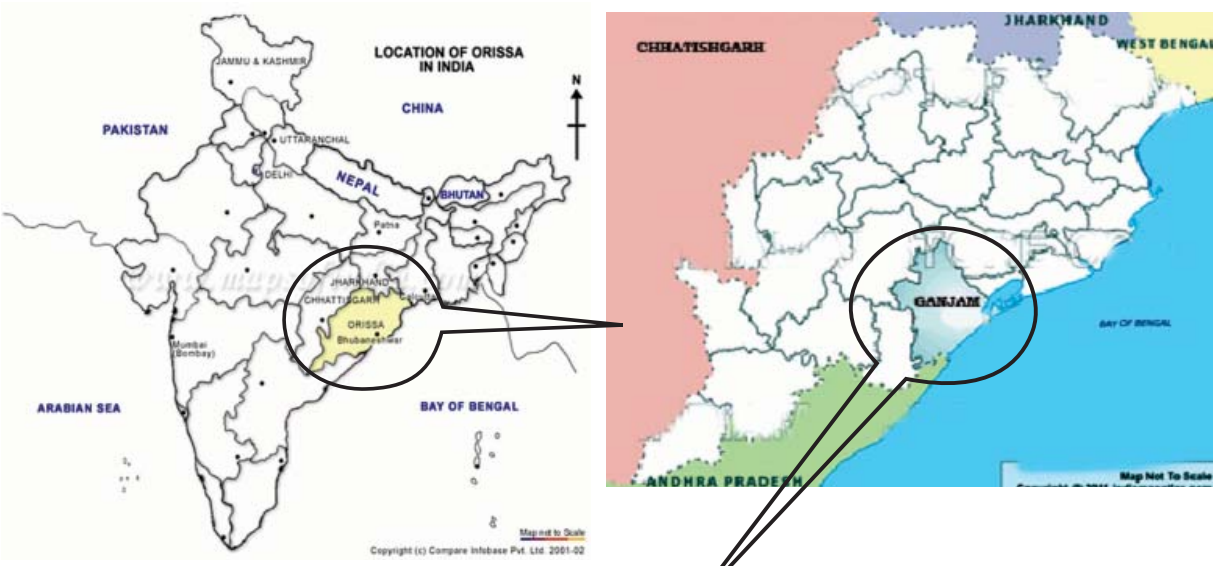
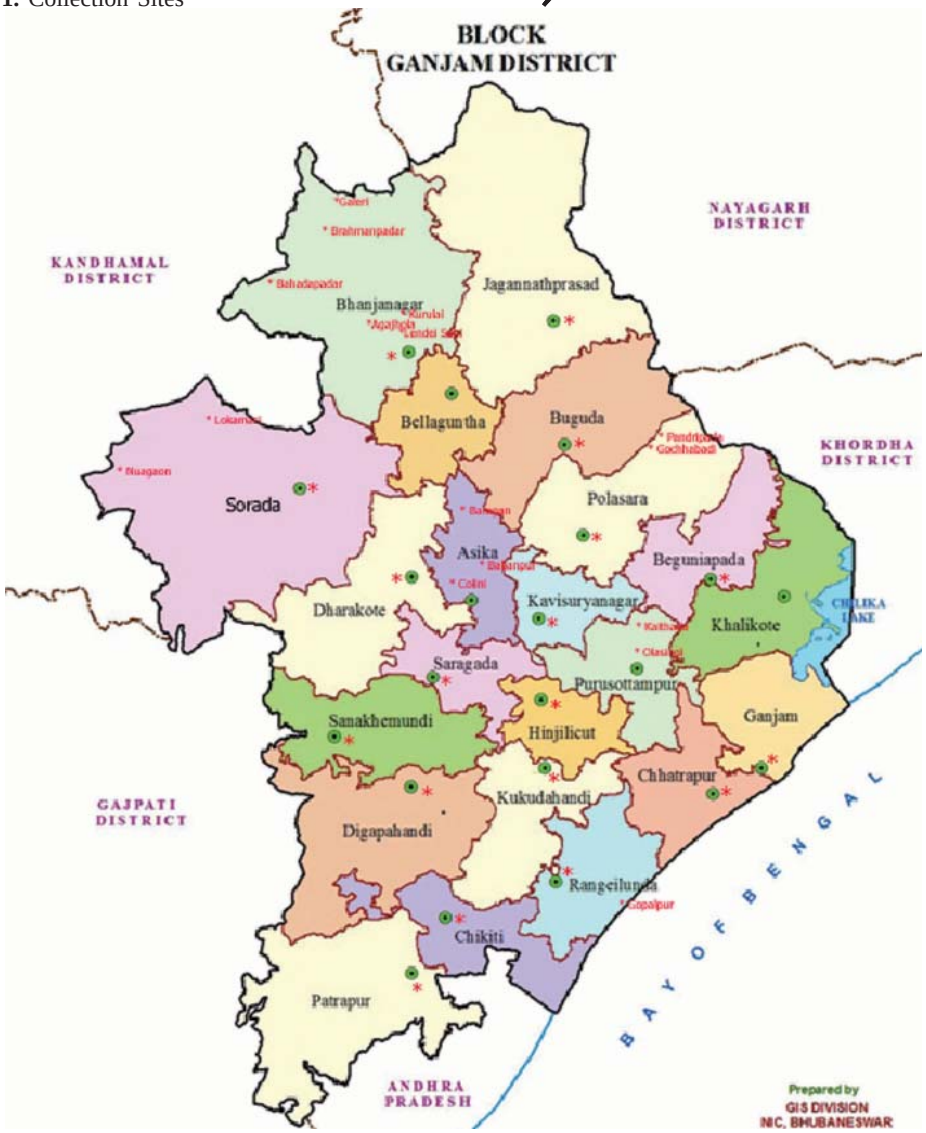


Fig. 1. Collection Sites



that some of the previously reported species would have disappeared or reappeared. This phenomenon has already been observed in other states like Meghalaya, Madhya Pradesh, Chhatisgarh, Delhi [Rajagopal (1976), Kalra (1978), Jambulingam, P. (2005)]. The application of molecular approaches to systematic problems ranging from sub-generic relationships to relationships at and below the species level is helping to address the phylogenetics and biogeography of *Anopheles*, the nature of species boundaries (Jaroslaw and Nora (2003). In the present study, 24 species of *Anopheles* are described on morphological basis and the samples processed for sequencing.

MATERIALS AND METHOD

Mosquito collections were undertaken in sixteen villages of 22 blocks of Ganjam District during the year 2009-2010. The villages are *viz.*, Kurulai, Agajhola, Lendei sahi, Colini, Baragan, Babanpur, Lokamari, Nuagaon, Sorada, Baharapadar, Galeri, Brahmanpadar, Olasinghi, Kaithada, Pandripada, Gochabadi and all other block headquarters. (Fig. 1). Adult mosquitoes that were resting indoor, outdoor, biting man and cattle were collected by suction tube; spray sheet, cdc-light trap collections used during dawn and dusk. The indoor resting mosquitoes collected from both human dwelling and cattle sheds. The total catch collection made by spraying pyrethrum in a close room to knock down the mosquitoes then the mosquitoes which were collected from a white bed sheet by the entomological forceps and transferred to the test tube. Out door resting adult mosquitoes were collected by netting from shrubs near cowsheds, paddy fields, littoral forest etc. The mosquito samples were identified by using the key of (Christophers, 1933, Barraud, 1934 and Rao, 1984, Nagpal *et. al.*, 2005). The species are distinguished on the basis of characters of adult females.

MOLECULAR STUDY

DNA isolation: The DNA extraction from single adult mosquito was performed as per modified phenol chloroform method (Coen *et al.,* 1982).

Polymerase Chain Reaction (PCR)

Standardization of PCR amplification of the D_3 region

The D_3 domain of 28S rDNA region was amplified by PCR using universal primers with slight modification with annealing temperature 50°C.

Sequence of universal primers

Forward D_3A: 5’ GAC CCG TCT TGA AAC ACG GA 3’

Reverse D_3B: 5’ TCG GAA GGA CCA GCT ACT A 3’

AGAROSE GEL ELECTROPHORESIS

The amplified products were separated by electrophoresis through 1.5% agarose gel in 1X TBE buffer pH 8 (Sambrook *et al.,* 1989) visualized and photographed using gel documentation system (Bio rad USA) after staining with ethidium bromide.

DNA SEQUENCING

Automated sequencing DNA

In automated DNA sequencing method the dideoxy nucleotides not the primers are tagged with different coloured fluorescent dyes, thus all four reactions occur in the same tube and are separated in the same lane on the gel. As each labeled DNA fragment passes a detector at the bottom of the gel, the colour is recorded and the pattern of colours representing each nucleotide in the sequence.

Molecular characterization of the collected samples is under investigation using molecular biology techniques (Wilkerson *et al.*, 1993). The D3 forward 5’-GACCCGTCTTGAAACACGGA-3’ and D3 reverse 5’TCGGAAGGAACCAGCTACTA-3’primers were used to amplify the D3 region of 28S rDNA (Litvaitis *et al*., 1994) seven members of Funestus group of Myzomyia and Annularis group of Neocellia series.

Sequencing of the D3 fragment

Nucleotide alignment of the D3 region for the three species of the *An. annularis* group is shown in Figure 2. The D3 sequences are present in the large subunit 28S of rDNA.

Performing a BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) using the *Anopheles* mosquitoes nucleotide sequence.

Data: The Indian Anopheline mosquito species broadly fall into two categories *viz.* (i) subgenus *Anopheline* (ii) subgenus *Cellia*.

TAXONOMIC LIST

Subgenus ***Anopheles*** Meigen

1. *Anopheles (Anopheles) ahomi* Chowdhury, 1929.
2. *An. (An.) aitkenii* James, 1903.
3. *An. (An.) annandalei* Prasad, 1918.
4. *An. (An.) argyropus* (Swellengrebel), 1914
5. *An. (An.)barbirostris* Vander Wulp, 1884.
6. *An. (An.) barbumbrosus* Strickland & Chowdhury, 1927.
7. *An. (An.) barianensis* James, 1911.
8. *An. (An.) bengalensis* Puri, 1930.
9. *An. (An.) crawfordi* Reid, 1953.
10. *An. (An.) culciformis* Cogill, 1903.
11. *An. (An.) gigas* Giles, 1901.
12. *An. (An.) insulaeflorum* (Swellengrebel& Swellengrebel de Graaf), 1919.
13. An. (An.) interruptus Puri, 1929.
14. *An. (An.) lindesayi* Giles, 1900.
15. *An. (An.) nigerrimus* Giles, 1900.
16. *An. (An.) nitidus* Harrison, Scanlon & Reid, 1973.
17. *An. (An.) peditaeniatus* (Leicester), 1908.
18. *An. (An.) pinjaurensis* Barraud, 1932.
19. *An. (An.) roperi* Reid, 1950.
20. *An. (An.) sinensis* Weidemann, 1828.
21. *An. (An.) sintoni* Puri, 1929.
22. *An. (An.) umbrosus* (Theobald), 1903
23. *An. (C.) aconitus* Doenitz, 1902.

Subgenus ***Cellia*** Theobald

24. *An. (C.) annularis* Vander Wulp, 1884.
25. *An. (C.) culicifacies* Giles, 1901.
26. *An. (C.) dirus Peyton* & Harrison, 1979.
27. *An. (C.) dithali Patton,* 1905.
28. *An. (C.) elegane* (James), 1903.
29. *An. (C.) fluviatitis* James, 1902
30. *An. (C.) jamesii* Theobald, 1901.
31. *An. (C.) jeyporiensis* James, 1902.
32. *An. (C.) karwari* (James), 1902.
33. *An. (C.) kochi* Doenitz, 1901.
34. *An. (C.) maculates* Theobald, 1901.
35. *An. (C.) majidi* Young & Majid, 1928.
36. *An. (C.) minimus* Theobald, 1901.
37. *An. (C.) moghulensis* Christophers, 1924.
38. *An. (C.) multicolor* Cambouliu, 1902.
39. *An. (C.) nivipes* (Theobald), 1903.
40. *An. (C.) pallidus* Theobald, 1901.
41. *An. (C.) philippinensis* Ludlow, 1902.
42. *An. (C.) pseudojamesi* Strickland & Chowdhury, 1927.
43. *An. (C.) pseudwillmori* Theobald, 1910.
44. *An. (C.) pulcherrimus* Theobald, 1902.
45. *An.* (C.) splendidus Koidzumi, 1920.
46. *An. (C.) stephensi* Liston, 1901.
47. *An. (C.) subpictus* Grassi, 1899.
48. *An. (C.) sundaicus* (Rodenwaldt), 1925.
49. *An. (C.) tessellates* Theobald, 1901.
50. *An. (C.) theobaldi* Giles, 1901.
51. *An. (C.) turkhudi* Liston, 1901.
52. *An. (C.) vagus* Doentiz, 1902.
53. *An. (C.) varuna* Iyengar, 1924.
54. *An. (C.) willmori* (James), 1903.

Species Groups of Indian *Anopheles*:

Subgenus ***Anapheles***

Aitkenii group: *aitkeii, bengalensis, insulaeflorum, pnnjaurensis*.

Asiaticus group: *annandalei.*

Barbirostris group: *ahomi, barbirostris, barbumbrosus*.

Barianensis group: *barianensis.*

Culiciformis group: *culiciformis.*

Hyrcanus group: *argyropus, crawfordi, nigerrimus, nitidus, peditaeniatus, sinensis*.

Lindesayi group: *gigas, lindesayi*. (These two species could not be segregated as a separate group in the key).

Subgenus ***Cellia***

Umbrosus group: *roperi, umbrosus*. (This subgenus is not divided into groups in the key, although some of the species can be arranged into groups).

Annularis group: *annularis, nivipes, pallidus philippinensis*.

Maculates group: *maculates, pseudowillmori, willmori*.

Minimus group: *aconitus, minimus, varuna*.

RESULTS AND DISCUSSION

24 species of anopheles are identified from the studied area. The lists are given below:

TAXONOMIC LIST OF ANOPHELES MOSQUITOES OF GANJAM DISTRICT, ODISHA (MORPHOLOGY)

Class INSECTA

Order DIPTERA

Family CULICIDAE

Genus ***Anopheles***

Subgenus ***Anopheles***

1968. Asiaticus Group (Reid)

1. *An.* ***annandelei*** Prasad, 1918

Interruptus Subgroup (Rattanarithikul *et al.*, 2006b)

2. *An.* ***interruptus*** Puri, 1929

1961. Lindesayi Group (Reid & Knight)

1991. Gigas Complex (Harrison *et al.*)

3. *An.* ***gigas*** Giles, 1901

1961. Culiciformis Group (Reid & Knight)

4. *An.* ***culiciformis*** Cogill, 1903

1953. Hyrcanus Group (Reid)

5. *An.* ***sinensis*** Weidemann, 1828

1972. Nigerrimus Subgroup (Harrison)

6. *An.* ***nigerimus*** Giles, 1900

7. *An.* ***nitidus*** Harrison, Scanlon and Reid, 1973

Subgenus ***Cellia***

1968. Annularis Group (Reid)

1999. Annularis Complex (Atrie *et al.*)

8. *An.* ***annularis*** Van der Wulp

9. *An.* ***pallidus*** Theobald, 1901

1949. Leucosphyrus Group (Reid)

Leucosphyrus complex (Sallum *et al.*, 2005a)

10. *An.* ***balabacensis*** Baisas, 1936

Dirus complex (Sallum *et al.*, 2005b)

11. *An.* ***elegans*** (James), 1903

Funestus Group (Garros *et. al.*, 2005b)

Culicifacies subgroup (Garros *et al.*, 2005b).

12. *An.* ***culicifacies*** Giles,1901

13. *An.* ***karwari*** (James), 1902

1987. Maculates Group (Rattanarithikul & Green)

Maculates subgroup (Rattanarithikul *et al.*, 2006b)

14. *An. maculates* Theobald 1901

15. *An. pseudowillmori* Theobald 1910

2003. Minimus Subgroup (Chen *et al.*)

1990. Minimus Complex (Green *et al.*)

16. *An. minimus* Theobald, 1901

***Neocellia* Series *(*Christophers, 1924a)**

17. *An.* ***moughulensis*** Christophers, 1924

18. *An.* ***vagus*** Doenitz, 1902

19. *An.* ***subpictus*** Grassi, 1899

Jamesii Group (Rattanarithikul *et al.*, 2006b)

20. *An.* ***pseudojamesi*** Strickland and Chowdhury, 1927

Myzomyia Series (Christophers, 1924a)

21. *An.* ***majidi*** Young and Majid, 1928

Tessellatus Group (Rattanarithikul *et al.*, 2006b)

22. *An.* ***tessellates*** Theobald, 1901

Funestus Group (Garros *et al.*, 2005b)

Aconitus Subgroup (Chen *et al.*, 2003)

23. An. ***varuna*** Iyengar, 1924

24. *An.* ***aconitus*** Doenitz, 1902

The morphological characterization has been done by dataset provided by Nagpal *et al.*, 2005. The species found are grouped under two sub genus, (i) *Cellia* (ii) *Anopheles* 11 Groups and 7 complexes. The malarius complex and groups like Funestus Group; *An. culicifacies*, Annularis complex; *An. Annulais* found abundantly in coastal blocks of Ganjam whereas the Maculatus Group;

An. maculates, Minimus complex; *An.minimus* found abundantly at the Western part of Ganjam district.

1. Ganjam Mosquito Species: ***Anopheles (C.) balabacensis*** Baisas, 1936

Classification:

Genus ***Anopheles***

Subgenus ***Cellia***

Species ***balabacensis***

1949. The Subgenus *Cellia* includes Leucosphyrus Group (Reid) and Leucosphyrus complex (Sallum *et al.*, 2005a)

Characteristics:

Bionomics: Baharapadar, Galleri, Ganjam

Feeding time: Evening

Medical and Economic Importance: *Common*

Host: Human, Livestock

2. Ganjam Mosquito Species: ***Anopheles (C.) elegans*** (James), 1903

Classification:

Genus ***Anopheles***

Subgenus ***Cellia***

Species ***elegans***

1949. The Subgenus *Cellia* comprises Leucosphyrus Group (Reid) and Dirus complex (Sallum *et .al.*, 2005b)

Characteristics:

Bionomics: Baharapadar, Ganjam

Feeding time: Evening

Medical and Economic Importance: *Common*

Host: Human, Livestock

3. Ganjam Mosquito Species: ***Anopheles (C.) tessellates*** Theobald, 1901

Classification:

Genus ***Anopheles***

Subgenus ***Cellia***

Species ***tessellates***

2006. The Subgenus *Cellia* includes Tessellatus Group (Rattanarithikul *et al.*, 2006b). (www.mosquito-taxonomic-inventory.info/)

Characteristics:

Bionomics: Baharapadar, Brahmanpadar, Ganjam.

Feeding time: Evening

Medical and Economic Importance: Common

Host: Human, Livestock

4. Ganjam Mosquito Species: ***Anopheles (C.) pseudojamesi*** Strickland and Chowdhury, 1927

Classification:

Genus ***Anopheles***

Subgenus ***Cellia***

Species ***pseudojamesi***

2006. The Subgenus *Cellia* belongs to Jamesii Group (Rattanarithikul *et al.*, 2006b). (www.mosquito-taxonomic-inventory.info/)

Characteristics:

Bionomics: Baharapadar, Ganjam

Feeding time: Evening

Medical and Economic Importance: Common

Host: Human, Livestock

5. Ganjam Mosquito Species: ***Anopheles (C) maculates*** Theobald 1901

Classification:

Genus ***Anopheles***

Subgenus ***Cellia***

Species ***maculates***

1987. The Subgenus *Cellia* belongs to maculates Group (Rattanarithikul & Green) and maculates subgroup (Rattanarithikul *et al.*, 2006b). (www.mosquito-taxonomic-inventory.info/)

Characteristics:

Bionomics: Sorada,Galeri, Gopalpur Ganjam

Feeding time: Evening

Medical and Economic Importance: Common

Host: Human, Livestock

6. Ganjam Mosquito Species: ***Anopheles (C) pseudowillmori*** Theobald 1910

Classification:

Genus ***Anopheles***

Subgenus ***Cellia***

Species ***pseudowillmori***

1987. The Subgenus *Cellia* belongs to maculates Group (Rattanarithikul & Green).

Characteristics:

Bionomics: Baharapadar, Ganjam

Feeding time: Evening

Medical and Economic Importance: Common

Host: Human, Livestock

7. Ganjam Mosquito Species: ***Anopheles (C) annularis*** Van der Wulp 1884

Classification:

Genus ***Anopheles***

Subgenus ***Cellia***

Species ***annularis***

1968. The Subgenus *Cellia* belongs to Annularis Group (Reid) and Annularis Complex (Atrie *et al.,* 1999).

Characteristics:

Bionomics: Gopalpur, Baharapadar Ganjam

Feeding time: Evening

Medical and Economic Importance: Common

Host: Human, Livestock8. Ganjam

8. Ganjam Mosquito Species: ***Anopheles (C) pallidus*** Theobald 1901

Classification:

Genus ***Anopheles***

Subgenus ***Cellia***

Species ***pallidus***

1968. The Subgenus *Cellia* belongs to Annularis Group (Reid) and Annularis Complex (Atrie *et al.,* 1999).

Characteristics:

Bionomics: Brahmanpadar, Ganjam

Feeding time: Evening

Medical and Economic Importance: Common

Host: Human, Livestock

9. Ganjam Mosquito Species: ***Anopheles (C) culicifacies*** Giles 1901

Classification:

Genus ***Anopheles***

Subgenus ***Cellia***

Species ***culicifacies***

2005. The Subgenus *Cellia* is monophyletic. It belongs to Funestus Group (Garros *et. al.*, 2005b) and Culicifacies subgroup (Garros *et. al.,* 2005b).

Characteristics:

Bionomics: Gopalpur, Ganjam

Feeding time: Evening

Medical and Economic Importance: Common

Host: Human, Livestock

10. Ganjam Mosquito Species: ***Anopheles (C) moghulensis*** Christophers, 1924

Classification:

Genus ***Anopheles***

Subgenus ***Cellia***

Species ***moghulensis***

1924. The Subgenus *Cellia* is monophyletic. It belongs to *Neocellia Series* (Christophers, 1924a)

Characteristics:

Bionomics: Baharapadar, Nuagaon, Ganjam

Feeding time: Evening

Medical and Economic Importance: Common

Host: Human, Livestock

11. Ganjam Mosquito Species: ***Anopheles (C) subpictatus*** Grassi 1899

Classification:

Genus ***Anopheles***

Subgenus ***Cellia***

Species ***subpictatus***

1924. The Subgenus *Cellia* belongs to *Neocellia Series* (Christophers, 1924a)

Characteristics:

Bionomics: Baharapadar, Ganjam

Feeding time: Evening

Medical and Economic Importance: Common

Host: Human, Livestock

12. Ganjam Mosquito Species: ***Anopheles (C) vagus*** Iyengar, 1924

Classification:

Genus ***Anopheles***

Subgenus ***Cellia***

Species ***vagus***

1924. The Subgenus *Cellia* is monophyletic. It belongs to *Neocellia Series* **(Christophers, 1924a)**

Characteristics:

Bionomics: Baharapadar, Ganjam

Feeding time: Evening

*Medical and Economic Importanc*e: Common

Host: Human, Livestock

13. Ganjam Mosquito Species: ***Anopheles (C) aconitus*** Doenitz, 1902

Classification:

Genus ***Anopheles***

Subgenus ***Cellia***

Species ***aconitus***

2003. The Subgenus *Cellia* belongs to Aconitus Subgroup (Chen *et al*., 2003)

Characteristics:

Bionomics: Baharapadar, Brahmanpadar, Gopalpur Ganjam

Feeding time: Evening

Medical and Economic Importance: Common

Host: Human, Livestock

14. Ganjam Mosquito Species:*Anopheles (C) majidi* Young and majid,1928

Classification: Genus :*Anopheles*

Subgenus: *Cellia*

Species: *majidi*

1924. The Subgenus *Cellia* belongs to Myzomyia Series (Christophers, 1924a)

Characteristics:

Bionomics: Nuagaon, Gopalpur Ganjam

Feeding time: Evening

Medical and Economic Importance: Common

Host: Human, Livestock

15. Ganjam Mosquito Species: ***Anopheles (C) minimus*** Theobald, 1901

Classification:

Genus ***Anopheles***

Subgenus ***Cellia***

Species ***minimus***

2003. The Subgenus *Cellia* belongs to Minimus Subgroup (Chen *et al*.,) and Minimus Complex (Green *et al*., 1990)

Characteristics:

Bionomics: Baharapadar, Galeri, Brahmanpadar, Ganjam

Feeding time: Evening

Medical and Economic Importance: Common

Host: Human, Livestock.

16. Ganjam Mosquito Species: ***Anopheles (C) varuna*** Iyengar, 1924

Classification:

Genus ***Anopheles***

Subgenus ***Cellia***

Species ***varuna***

2003. The Subgenus *Cellia* belongs to Funestus Group (Garros *et al*., 2005b) and Aconitus Subgroup (Chen *et al*.,)

Characteristics:

Bionomics: Lokamari, Gopalpur, Ganjam

Feeding time: Evening

Medical and Economic Importance: Common

Host: Human, Livestock

17. Ganjam Mosquito Species: ***Anopheles (C) karwari*** (James), 1902

Classification:

Genus ***Anopheles***

Subgenus ***Cellia***

Species ***karwari***

1924. The Subgenus *Cellia* belongs to Neocellia Series (Christophers, 1924a)

Characteristics:

Bionomics: Nua gaon,Baharpadar Ganjam

Feeding time: Evening

Medical and Economic Importance: Common

Host: Human, Livestock

18. Ganjam Mosquito Species: ***Anopheles annandalei*** Prashad,1918

Classification:

Genus ***Anopheles***

Subgenus ***Anopheles***

Species ***annandalei***

1968. The Subgenus *Cellia* belongs to Asiaticus Group (Reid,)

Characteristics:

Bionomics: Brahmanpadar, Ganjam

Feeding time: Evening

Medical and Economic Importance: Common

Host: Human,Livestock

19. Ganjam Mosquito Species: ***Anopheles interruptus*** Puri, 1929

Classification:

Genus ***Anopheles***

Subgenus ***Anopheles***

Species ***interruptus***

1968. The Subgenus *Cellia* belongs to Asiaticus Group (Reid) and Interruptus Subgroup (Rattanarithikul *et al*., 2006b)

Characteristics:

Bionomics: Brahmanpadar, Ganjam

Feeding time: Evening, Night

Medical and Economic Importance: Common

Host: Human, Livestock

20. Ganjam Mosquito Species: ***Anopheles gigas*** Giles, 1901

Classification:

Genus ***Anopheles***

Subgenus ***Anopheles***

Species ***gigas***

1961. The Subgenus *Cellia* belongs to Lindesayi Group (Reid & Knight)and Gigas Complex (Harrison *et al*., 1991)

Characteristics:

Bionomics: Sorada, Ganjam

Feeding time: Evening

Medical and Economic Importance: Common

Host: Human,Livestock

21. Ganjam Mosquito Species: ***Anopheles nigerimus*** Giles, 1900

Classification:

Genus ***Anopheles***

Subgenus ***Anopheles***

Species ***nigerimus***

1953. The Subgenus *Cellia* belongs to Hyrcanus Group (Reid) and Nigerrimus Subgroup (Harrison, 1972)

Characteristics:

Bionomic: Brahmanpadar, Ganjam

Feeding time: Evening

Medical and Economic Importance: Common

Host: Human, Livestock

22. Ganjam Mosquito Species: ***Anopheles nitidus*** Harrison, Scanlon and Reid, 1973

Classification:

Genus ***Anopheles***

Subgenus ***Anopheles***

Species ***nitidus***

1953. The Subgenus *Cellia* belongs to Hyrcanus Group (Reid) and Nigerrimus Subgroup (Harrison, 1972)

Characteristics:

Bionomic: Brahmanpadar, Ganjam

Feeding time: Evening

Medical and Economic Importance: Common

Host: Human, Livestock

23. Ganjam Mosquito Species: ***Anopheles sinensis*** Wiedemann,1828

Classification:

Genus ***Anopheles***

Subgenus ***Anopheles***

Species ***sinensis***

1953. The Subgenera *Cellia* belongs to Hyrcanus Group (Reid)

Characteristics:

Bionomic: Brahmanpadar, Ganjam

Feeding time: Evening, Night

Medical and Economic Importance: Common

Host: Human, Livestock

24. Ganjam Mosquito Species: ***Anopheles culiciformis*** Cogill, 1903

Classification:

Genus ***Anopheles***

Subgenus **Anopheles**

Species ***culiciformis***

1961. The Subgenus *Cellia* belongs to Culiciformis Group (Reid & Knight)

Characteristics:

Bionomic: Brahmanpadar, Ganjam

Feeding time: Evening, Night

Medical and Economic Importance: Common

Host: Human, Livestock

The anopheline diversity especially the subgenus *Anopheles* has been increased in comparison to Anopheles species recorded during the following Periods [1939 (Senior White & Adhikari), 1942 (Covell & Singh), 1983 (Nagpal & Sharma) and 2000 (Dash *et al.*,)] in the coastal district Ganjam (Table. 3).

Molecular study:

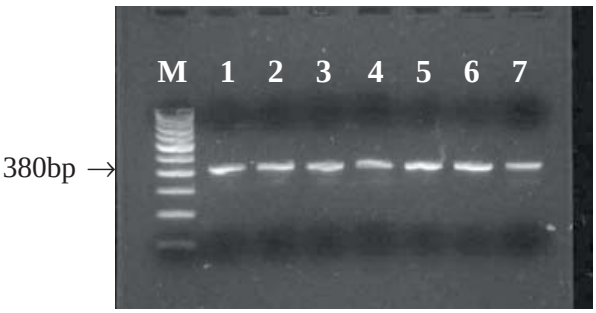


Fig. 2. Lanes 1: *An. annularis* species; lanes 2: *An. pallidus* species; lanes 3: *An. culicifacies*; lanes 4: *An. subpictus* species; lanes 5: *An. vagus* species; lanes 6: *An. varuna* species; lanes 7: *An. aconitus* species. Lane M, 100-bp DNA ladder, lanes 1–7 showed common 380-bp product from the D3 domain of 28S rDNA of members of the Myzomyia and Neocellia series. Ethidium bromide-stained agarose gel-electrophoresis D3 PCR products of members of Funestus group of Myzomyia and Annularis group of Neocellia series.

Sequencing analysis was done to identify the species collected from villages of Ganjam District. After sequencing the samples were matched with the data present in the genbank and they were matched. The following results are obtained in the sequencing.

The 309 bp 28S rRNA Sequence of *An. balbacensis* was taken as a reference sequence and blasted in NCBI it shows 94% similarity score with 98% Query coverage with *Anopheles tessellatus* isolate FAts2 NCBI accession number is FJ159601. Similarly when 295 bp 28S rRNA Sequence of *An. interruptus* was taken as a reference sequence and blasted in NCBI it shows 83% similarity score with 37% Query coverage with *Anopheles hyrcanus* NCBI accession number is AY376903. It indicates very poor homology of this sequence is available in the database till date. It concludes that it may be a new species and to further characterize it whole genome sequencing is essential. The 149 bp 28S rRNA Sequence of *An. Bengalensis* was taken as a reference sequence and blasted in NCBI it shows 95% similarity score with 67% Query coverage with *Anopheles sinensis* NCBI accession number is AY376321. The blast result of 505 bp 28S rRNA Sequence of *An. Varuna* shows 99% similarity score with 62% Query coverage with *Anopheles vagus* NCBI accession number is EU570062 at the same time 279 bp 28S rRNA Sequence of *An. Lindesayi* shows 98% similarity score with 93% Query coverage with *Anopheles culicifacies* isolate FAcu1 NCBI accession number is FJ159604. When 313 bp 28S rRNA Sequence of *An. tessellatus* was taken as a reference sequence and blasted in NCBI it shows 99% similarity score with 94% Query coverage with *Anopheles tessellatus* isolate FAts2 NCBI accession number is FJ159601. The 313 bp 28S rRNA Sequence of *An. Subpictus* was taken as a reference sequence and blasted in NCBI it shows 77% similarity score with 92% Query coverage with *Anopheles albimanus* NCBI accession number is L78065 whereas with *Anopheles lesteri* NCBI accession number is AY376317 shows 92% similarity score and 80% Query coverage. When 306 bp 28S rRNA Sequence of *An. Vagus* was taken as a reference sequence and blasted in NCBI it shows 78% similarity score and 99% Query coverage with *Anopheles jeyporiensis* NCBI accession number is AJ512724.1 whereas with *Anopheles lesteri* NCBI accession number is AY376317 shows 96% similarity score and 80% Query coverage. When 345 bp 28S rRNA Sequence of *An. insulaeflorum* was taken as a reference sequence and blasted in NCBI it shows 100% similarity score and 100% Query coverage with *Anopheles ulicifacies* isolate FAcu1 NCBI accession number is FJ159604.

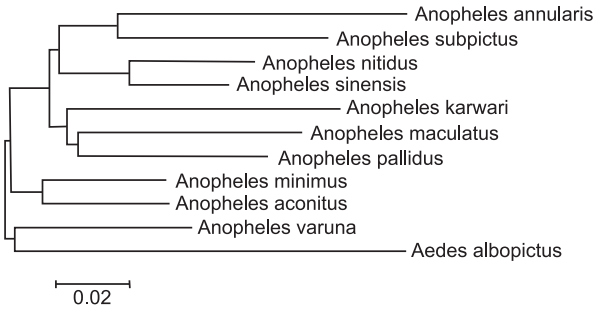


Fig. The analysis of sequence Phylogenetic tree of COII gene of *Anophelines* collected from Ganjam, Odisha.

Table 2. *Anopheles* mosquito Diversity of Ganjam District.

No.	Blocks of Ganjam	*An. annandelei*	*An. interruptus*	*An. gigas*	*An. culiciformis*	*An. sinensis*	*An. nitidus*	*An. nigerimus*	*An. subpictatus*	*An. annularis*	*An. pallidus*	*An. balabacensis*	*An. elegans*	*An. culicifacies*	*An. karwari*	*An. maculates*	*An. pseudowillmori*	*An. minimus*	*An. moughulensis*	*An. vagus*	*An. pseudojamesi*	*An. majidi*	*An. tessellates*	*An. varuna*	*An. aconitus*
1	Chatrapur	+	+						+	+														+	+
2	Ganjam	+	+					+	+	+														+	+
3	Khalikote	+	+				+		+	+														+	+
4	Beguniapada	+		+		+	+		+	+									·						
5	Polasara	+	+				+	+		+		+								+	+				+
6	Purrushottampur	+	+			+	+	+		+					+					+				+	
7	Kabisurya Nagar								+	+															
8	Hinjli								+	+										+					+
9	Rangeilunda									+				+		+						+		+	+
10	Kukudakhandi								+	+															+
11	Sanakhemundi	+								+	+														
12	Digapahandi	+								+					+			+		+					
13	Chikti						+	+		+								+							
14	Patrapur	+					+			+										+			+		
15	Bhanjanagar	+	+		+	+	+	+	+	+	+	+	+		+		+	+	+	+	+		+		+
16	Belaguntha									+										+					
17	Jaganathprasad									+										+					+
18	Buguda			+	+					+					+										
19	Dharakot									+															+
20	Aska									+															
21	Sorada			+						+					+	+			+			+		+	
22	Sheragada									+														+	

Table 3. Anopheles fauna of coastal Orissa:Surveys Separated by over Half a Century.

Anopheles Species or Group	**1937-1942 Reported by Senior-White& Adhikari(1939) and/or Covel & Singh(1942)**	**1995-1996 Reported by Dash et.al.**	**2010-11 detected during present survey.**
annandelei Prasad,1918	-	-	+
interruptus Puri,1929.	-	-	+
gigas Giles, 1901	-	-	+
culiciformis Cogill,1903	-	-	+
sinensis Weidemann,1828	-	-	+
nigerimus Giles, 1900	+	-	+
nitidus Harrison, Scanlon and Reid, 1973	-	-	+
annularis Van der Wulp	+	+	+
pallidus Theobald, 1901	+	+	+
balabacensis Baisas, 1936	-	-	+
elegans (James), 1903	-	-	+
culicifacies Giles,1901	+	+	+
karwari (James),1902	+	+	+
maculates Theobald 1901	+	-	+
pseudowillmori Theobald 1910	-	-	+
minimus Theobald,1901	+	-	+
moughulensis Christophers, 1924	-	-	+
An. vagus Doenitz,1902	+	+	+
subpictus Grassi, 1899	+	+	+
pseudojamesi Strickland and Chowdhury, 1927	-	-	+
majidi Young and Majid, 1928	-	-	+
tessellates Theobald, 1901.	-	+	+
varuna Iyengar, 1924	-	+	+
aconitus Doenitz, 1902	+	+	+

The Phylogeny tree is based on COII gene sequence of *Anophelines* generated by using Neighbor Joining method using the Tamura-Nei model of Mega 6 software showed that the *Anophelines* from Odisha were clustered into 1 groups. COII gene sequence of Ae. *albopictus* was taken as outgroup.

Homology data of D3 domain of 28S ribosomal RNA gene sequences of ten *Anopheles sp.* revealed that the species identification results both at morphological and molecular level not substantiates each other. The sequence revealed one species named *An. listeri* which is having no distributional record from Indian subcontinent although it is a member of the Hyrcanus complex found in South-east Asea and morphologically similar to species *An. sinensis* (Subarao,2007;Reid,1953) found in this sub-continent. Hence it was concluded that 28S ribosomal RNA gene sequencing is not sufficient for species level identification and whole genome sequencing of the same has to be done further for confirmation at species level.

The anopheline fauna was surveyed in the Koraput district which is an adjacent district to Ganjam District of Orissa by Gunasekaran et al. 1989.The district is known for malaria and consists of anophelines belonging to 22 species and two varieties. Later Rajavel et al. in the year 2004 surveyed the Jeypore Hills of Koraput District and identified only 8 species of *Anopheles.* The malarious anopheline complex of Gnajam district never highlighted although this district is badly affected by Malaria. It does not need to be emphasized that revisionary studies on the taxonomy of the anopheline fauna of the country are urgently required (Das *et al.,* 1990). During the past two or three decades numerous studies on the various groups of anophelines such as Anopheles annularis, culicifacies, hyrcanus, maculatus, subpictus, etc have been made leading to many changes in the nomenclature of the anopheline specie s(Subbarao, 2007). An important step in the assessment of the disease potential of an insect is the rapid separation and identification is needed therefore the present study holds significant reasons in studying the taxas of anophelines of Studied area.

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PLATE I

Chacteristics of Species ***balabacensis***

4 banded Palpi

White spot at tibiotersal joint of hind leg

Pre-sector dark mark of vein 1(R1) not reaching upto the distal end of humeral dark mark on the costa

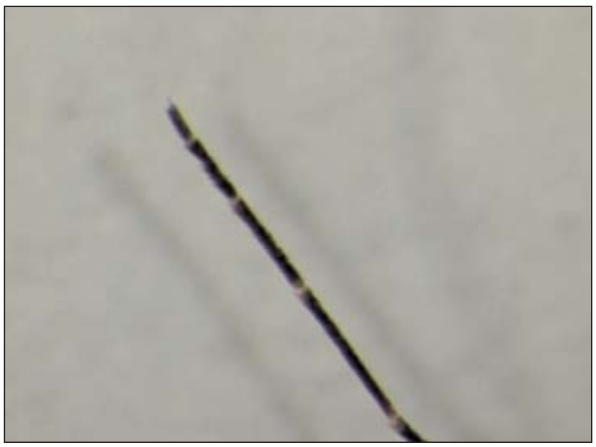
Chacteristics of Species ***elegans***

4 banded Palpi

Length of Proboscis longer than fore femur

Chacteristics of Species ***tessellates***

4 banded Palpi, Speckling in legs



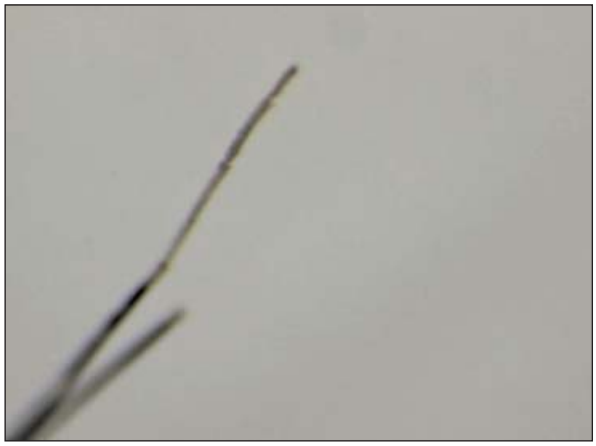
Narrowly banded hind leg tarsomeres

PLATE II

Chacteristics of Species ***Pseudojamesi***

Apical Pale band on Palpi nearly equal to the pre-apical dark band

Area at the bifurcation of wing vein 5 (Cu) dark and inner-costa interrupted



Hind tarsomeres 5,4,3 completely pale

Chacteristics of Species ***maculates***

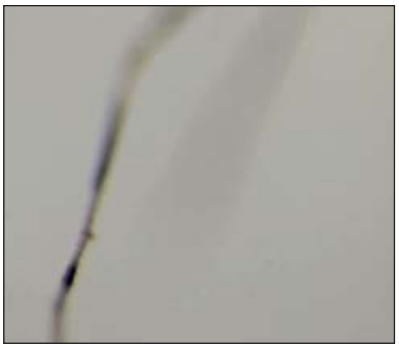
Apical Pale band on Palpi nearly equal to the Sub-apical Pale band

Dark band at 4th tarsomeres of hind leg

PLATE III

Chacteristics of Species ***pseudowillmori***

Apical Pale band on Palpi nearly equal to the Sub-apical Pale band



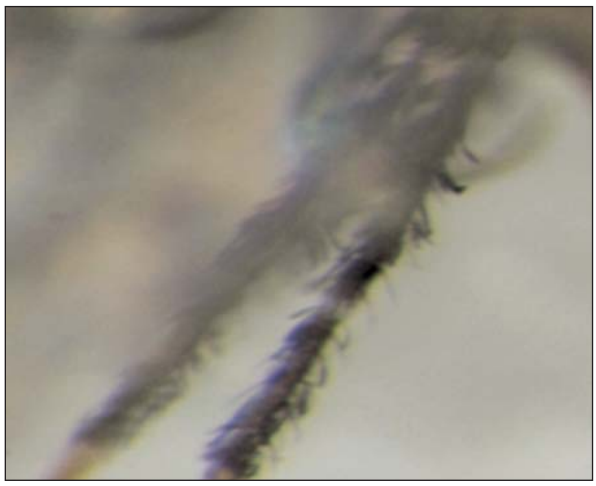
Dark band at 4th tarsomeres of hind leg

Abdomen without any broad golden scales

Chacteristics of Species ***annularis***

Anopheles annularis

Hind leg tarsomeres 5, 4, 3 completely pale



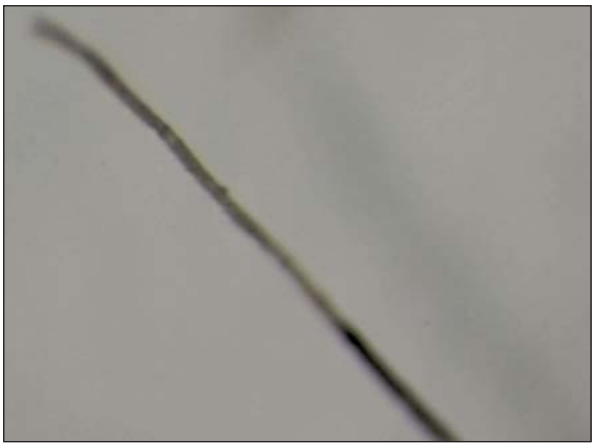
Apical Pale band on Palpi nearly equal to the pre-apical dark band

Area at the bifurcation of Wing vein 5(Cu) Dark

PLATE IV

Chacteristics of Species ***Pallidus***

Anopheles pallidus



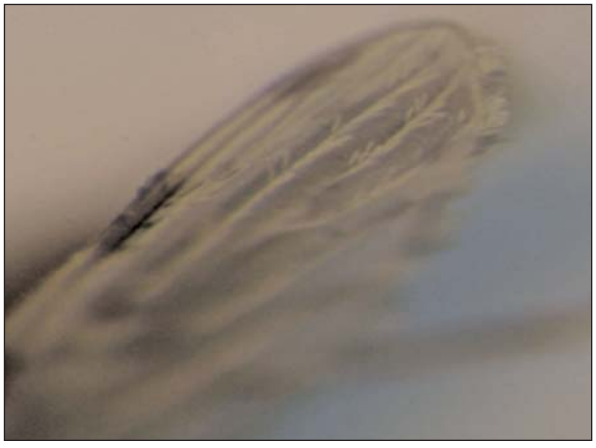
Apex of Hind tarsomere without any pale band

Apical Pale band on Palpi nearly equal to the pre-apical dark band

Area at the bifurcation of Wing vein 5(Cu) Pale

Chacteristics of Species ***culicifacies***

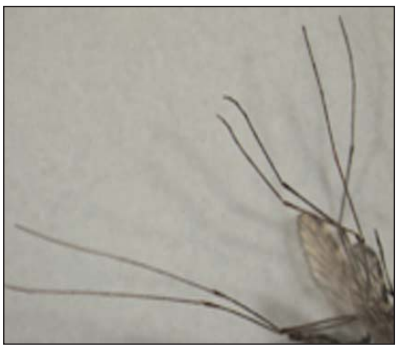
Pre-apical dark band ¼ of the Apical Pale band on Palpi



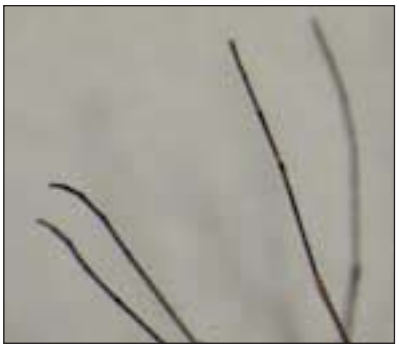
Fringe spot on vein 3(R4+5) absent

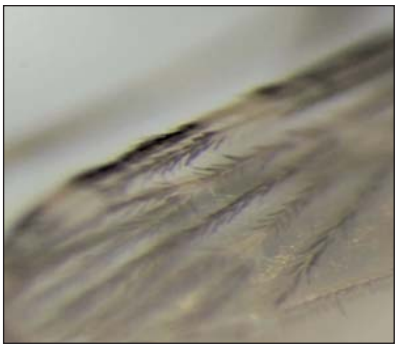
PLATE V

Chacteristics of Species ***moghulensis***



Band on fore leg tarsomeres very small



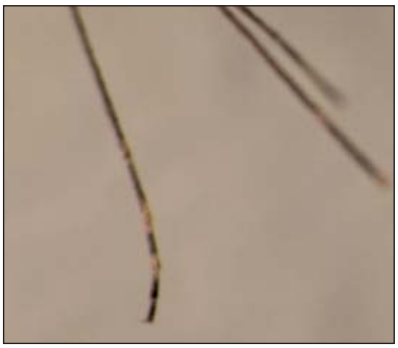


Distance of the anterior forked cell from the base of the costa compared to that of posterior forked cell more

Chacteristics of Species ***subpictatus***

Anopheles (C) *subpictatus*

Apical Pale band on Palpi nearly equal to the pre-apical dark band

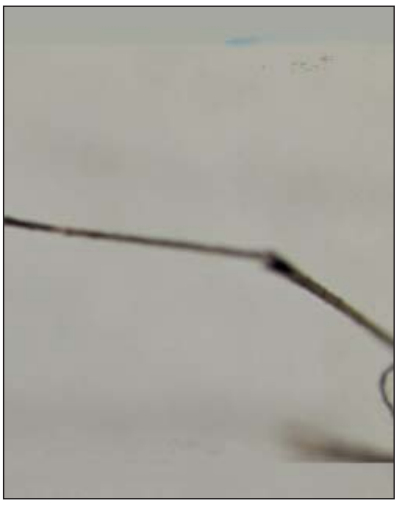


Bands on fore leg tarsomeres broad

Chacteristics of Species ***vagus***

Anopheles (C) *vagus*

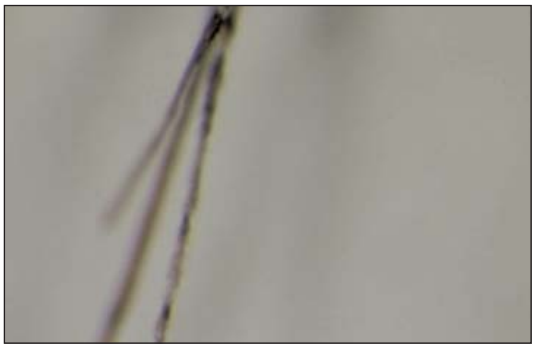
Pre-apical dark band ¼ or 1/5 of Apical Pale band on Palpi



Bands on fore leg tarsomeres broad

PLATE VI

Chacteristics of Species ***aconitus***

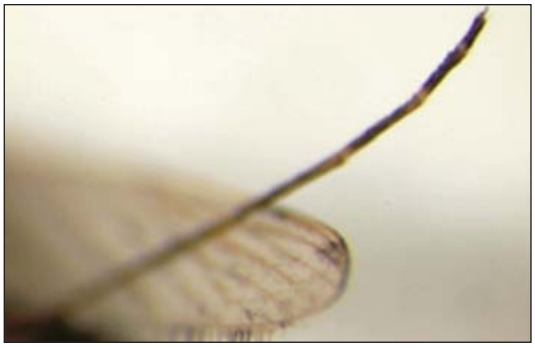


Intervening dark band on the palpi very small

Apical half of the proboscis light yellow

Chacteristics of Species ***majidi***

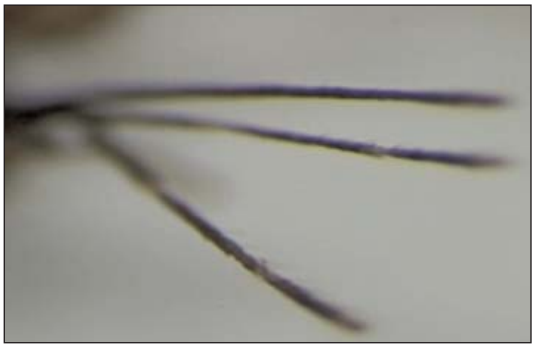
Intervening dark band on the palpi very small



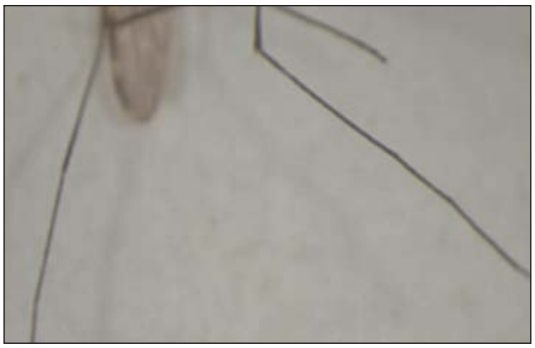
Tip of hind leg tarsomere and bands on legs pale and bands present

Chacteristics of Species ***minimus***

Anopheles (C) *minimus*



Apical and sub-apical pale band equal

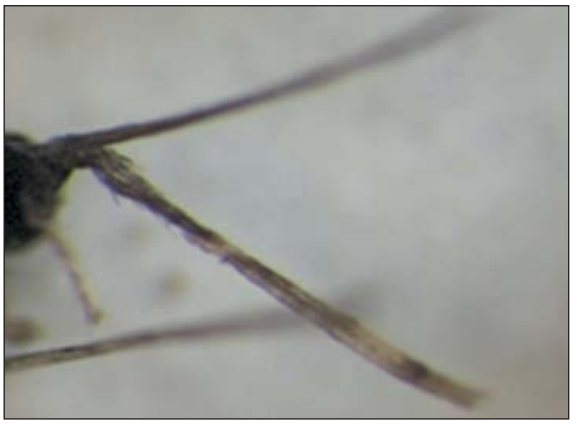


Tip of hind leg tarsomere and bands on leg black and bands absent

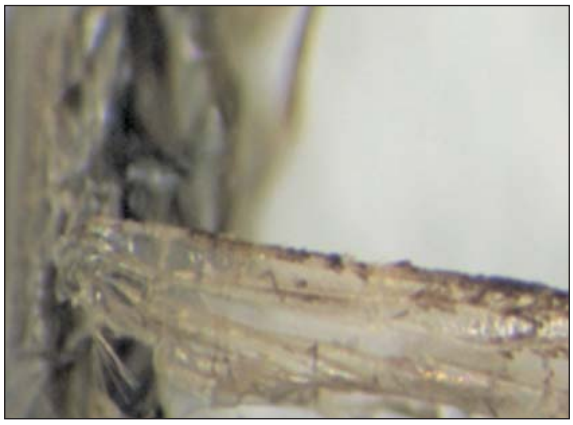
Inner costa interrupted can be seen at least in one wing

PLATE VII

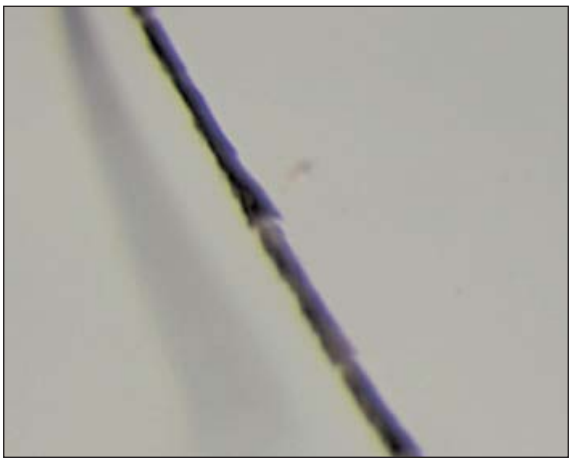
Chacteristics of Species ***varuna***



Apical pale band equal to pre-epical dark band



Innercosta Dark



Tip of hind leg tarsomere and bands on legs black and bands absent

Chacteristics of Species ***karwari***

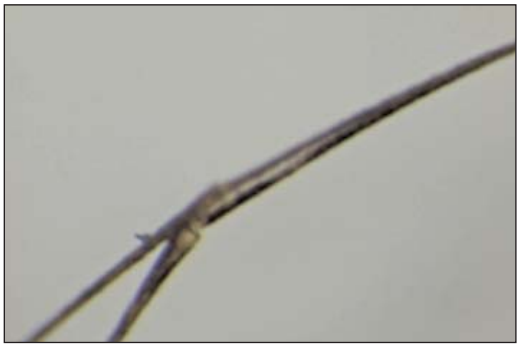
Four banded palpi

Legs without speckling and 5th tarsomere of hind leg pale

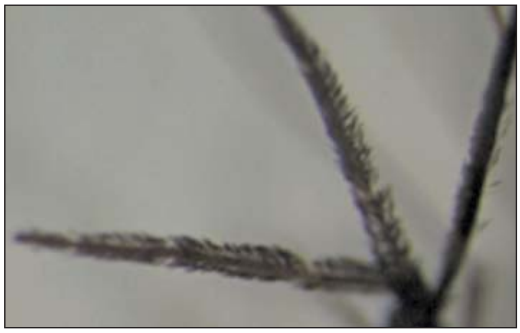
PLATE VIII

Chacteristics of Species ***annandalei***

Anopheles annandalei



A tuft of pale and black scales towards apex of hind femur present



Small bpale bands at the joints of Palpi

Wing with sub costal pale spot absent on costa

Chacteristics of Species ***interruptus***

Anopheles annandalei

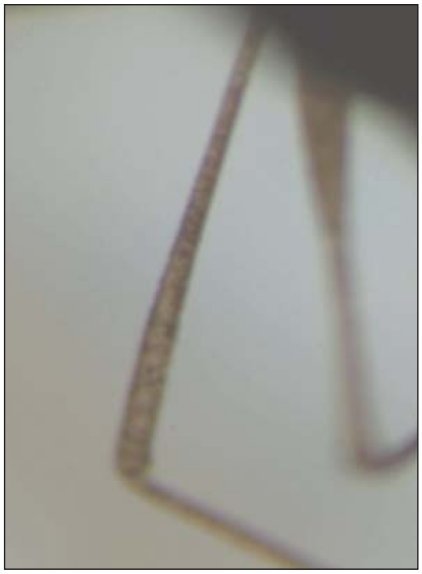
A tuft of pale and black scales towards apex of hind femur present

Small bpale bands at the joints of Palpi

Wing with sub costal pale spot absent on costa

PLATE IX

Chacteristics of Species ***gigas***

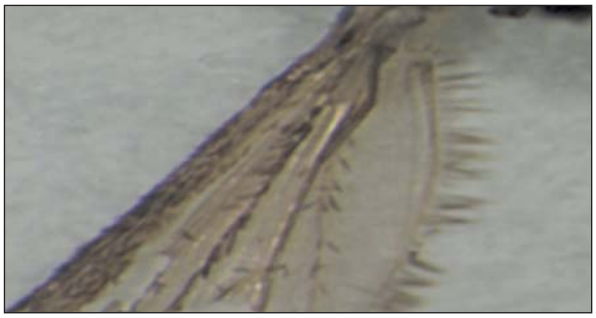


Pale ring on dorsal side towards the apex of mid leg femur absent

Pale spot on vein 6 present

Chacteristics of Species ***nigerimus***

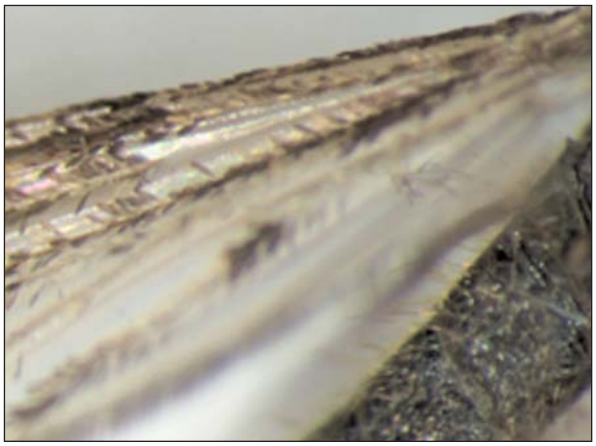
Four banded palpi (tip of the palpi pale)



Size of basal dark mark on wing vein 5(Cu) long

Chacteristics of Species ***nitidus***

Four banded palpi (tip of the palpi pale)

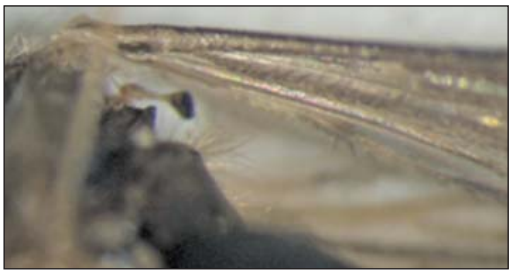


Size of basal dark mark on wing vein 5(Cu) small

PLATE X

Chacteristics of Species ***sinensis***

Four banded palpi (tip of the palpi pale)



Pale scale on inner costa and fringe spot on vein 5.2(cu2) abset

Size of pale bands on hind leg tarsomeres very small

Chacteristics of Species ***culiciformis***

Anopheles culiciformis

Palpi smaller than proboscis

Costa and sub-costa including vein 1(R1) completely dark

Scales on the head completely dark and broad in size