INTRODUCTION

The District Ganjam is located 19.4° North Latitude to 20.17° North Latitude and 84.7° East Longitude to 85.12° 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 with 18 Urban Bodies under 3212 villages in the district. 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 five 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 entomological forceps and transferred to the test tube. Outdoor 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 D3 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$_3$A: 5’ GAC CCG TCT TGA AAC ACG GA 3’
Reverse D$_3$B: 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’-GACCGTGCTTGAAACACGGA-3’ and D3 reverse 5’TCCGAGGAACCAGCCTACTA-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.
4. An. (An.) argyropus (Swellengrebel), 1914
7. An. (An.) barianensis James, 1911.
10. An. (An.) culciformis Cogill, 1903.
17. An. (An.) peditaeniatus (Leicester), 1908.
22. An. (An.) umbrosus (Theobald), 1903

Subgenus Cellia Theobald
28. An. (C.) elegane (James), 1903.
29. An. (C.) fluviatitis James, 1902
31. An. (C.) jeyporiensis James, 1902.
32. An. (C.) karvari (James), 1902.
34. An. (C.) maculates Theobald, 1901.
35. An. (C.) majidi Young & Majid, 1928.
37. An. (C.) moghulensis Christophers, 1924.
38. An. (C.) multicolor Cambouliu, 1902.
39. An. (C.) nivipes (Theobald), 1903.
40. An. (C.) pallidus Theobald, 1901.
42. An. (C.) pseudojamesi Strickland & Chowdhury, 1927.
44. An. (C.) pulcherrimus Theobald, 1902.
46. An. (C.) stephensi Liston, 1901.
47. An. (C.) subpictus Grassi, 1899.
48. An. (C.) sundicus (Rodewald), 1925.
49. An. (C.) tessellates Theobald, 1901.
50. An. (C.) theobaldi Giles, 1901.
52. An. (C.) vagus Doentiz, 1902.
54. An. (C.) willmori (James), 1903.

Species Groups of Indian Anopheles:

Subgenus Anopheles
Aitkenii group: aitei, bengalensis, insulaeformum, 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)

91. Gigan 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. nigerinus* 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

1949. 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* Grasi, 1899

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

20. *An. pseudojamesi* Strickland and Chowdury, 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 subgenus, (i) **Cellia** (ii) **Anopheles** 11 Groups and 7 complexes. The malarious complex and groups like Funestus Group; *An. culicifacies*, Annularis complex; *An. Annulais* found abundantly in coastal blocks of Ganjam whereas the Maculates 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*  

2006. 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, Brahmanpadar, Ganjam.
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

Characteristics:
Bionomics: Gopalpur, Baharapadar Ganjam
Feeding time: Evening
Medical and Economic Importance: Common
Host: Human, Livestock

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

Classification:
Genus Anopheles
Subgenus Cellia
Species pallidus

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

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

Characteristics:
Bionomics: Gopalpur, 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

Characteristics:
Bionomics: Baharapadar, Nuagaon, 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

Characteristics:
Bionomics: Baharapadar, Ganjam
Feeding time: Evening
Medical and Economic Importance: Common
Host: Human, Livestock

2005. The Subgenus Cellia is monophyletic. It belongs to Funestus Group (Garros et al., 2005b) and Culicifacies subgroup (Garros et al., 2005b).
Characteristics:
Bionomics: Baharapadar, Ganjam
Feeding time: Evening
Medical and Economic Importance: 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, Baharapadar 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 (Ratanaritkul 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 Subgenera *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:

380bp →

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. balbacinis was taken as a reference sequence and blasted in NCBI it shows 94% similarity score with 98% Query coverage with Anopheles sinensis NCBI accession number is AY376321. The blast result of 505 bp 28S rRNA Sequence of An. Vagusa shows 99% similarity score with 67% Query coverage with Anopheles varuna NCBI accession number is EU570062 at the same time 279 bp 28S rRNA Sequence of An. Lindeyai 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. tesselatus was taken as a reference sequence and blasted in NCBI it shows 99% similarity score with 94% Query coverage with Anopheles tesselatus 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 with 99% Query coverage with Anopheles jeyporienis 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. insulceflorum 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.

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

<table>
<thead>
<tr>
<th>Anopheles Species or Group</th>
<th>1937-1942 Reported by Senior-White &amp; Adhikari (1939) and/or Covel &amp; Singh (1942)</th>
<th>1995-1996 Reported by Dash et al.</th>
<th>2010-11 detected during present survey.</th>
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<td><em>annandelei</em> Prasad, 1918</td>
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<td><em>moughulensis</em> Christophers, 1924</td>
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<td>An. <em>vagus</em> Doenitz, 1902</td>
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<td><em>aconitus</em> Doenitz, 1902</td>
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The phylogeny tree is based on COII gene sequence of Anopheles generated by using Neighbor Joining method using the Tamura-Nei model of Mega 6 software showed that the Anopheles 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 (Subbarao, 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 Ganjam 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 species (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 taxa of anophelines of Studied area.

ACKNOWLEDGEMENT

I am thankful to the Director, Zoological Survey of India, Kolkata, Officer-in-Charge, EBRC, Gopalpur-on-Sea and the Director, R.M.R.C., Bhubaneswar for their benign cooperation and gesture of goodwill in course of making of this work.

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file:///E:/Molecular%20studies/GIS,%20Ganjam.htm

Manuscript Received : 14th August, 2012; Accepted : 10th June, 2014.
PLATE I

Characteristics of Species *balabacensis*

- 4 banded Palpi
- White spot at tibiotarsal joint of hind leg
- Pre-sector dark mark of vein 1(R1) not reaching up to the distal end of humeral dark mark on the costa

Characteristics of Species *elegans*

- 4 banded Palpi
- Length of Proboscis longer than fore femur

Characteristics of Species *tessellates*

- 4 banded Palpi, Speckling in legs
- Narrowly banded hind leg tarsomeres
PLATE II

Characteristics 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

Characteristics 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
Characteristics 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

Characteristics 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

Characteristics of Species *Pallidus*

*Anopheles pallidus*

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

Apex of Hind tarsomere without any pale band

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

Characteristics 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
Characteristics of Species *aconitus*

Intervening dark band on the palpi very small

Apical half of the proboscis light yellow

Characteristics of Species *majidi*

Intervening dark band on the palpi very small

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

Characteristics of Species *minimus*

*Anopheles (C) minimus*

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

Apical and sub-apical pale band equal

Inner costa interrupted can be seen at least in one wing
PLATE VII

Characteristics 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

Characteristics of Species karwari

Four banded palpi

Legs without speckling and 5th tarsomere of hind leg pale
PLATE VIII

Characteristics of Species *annandalei*

Anopheles annandalei

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

Small pale bands at the joints of Palpi

Wing with sub costal pale spot absent on costa

Characteristics of Species *interruptus*

Anopheles annandalei

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

Small pale bands at the joints of Palpi

Wing with sub costal pale spot absent on costa
PLATE IX

Characteristics of Species *gigas*

- Pale ring on dorsal side towards the apex of mid leg femur absent
- Pale spot on vein 6 present

Characteristics of Species *nigerimus*

- Four banded palpi (tip of the palpi pale)
- Size of basal dark mark on wing vein 5(Cu) long

Characteristics of Species *nitidus*

- Four banded palpi (tip of the palpi pale)
- Size of basal dark mark on wing vein 5(Cu) small
PLATE X

Characteristics 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

Characteristics of Species *culiciformis*

*Anopheles culiciformis*

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

*Palpi smaller than proboscis*

Scales on the head completely dark and broad in size