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Morphological variations in natural population of malaria vectors in Koraput district, Odisha State, India

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Abstract

Koraput district of Odisha State; hyper endemic for *falciparum* malaria, is rich in diverse Anopheline fauna. Morphological variations in different anophelines were reported during 1990 in this area. To understand the role of different anophelines in transmitting malaria parasites, proper identification of anopheline vectors and the possible variations in their morphology is necessary. For this study *Anopheles* mosquitoes were collected from random villages of Koraput district from January 2019 to December 2019 and were observed for morphological variations in different malaria vectors and non-vector species from the type form. Variations were recorded in 66 anophelines belonging to seven species including two primary vectors (*Anopheles culicifacies* and *An. fluviatilis*). Thirteen types of morphological variations have been observed; nine variations in palpi, three in wings and one in hind tarsi. These morphological variations observed in different *Anopheles* species could be helpful for proper species identification, where alternative molecular identification of species is not possible.

Keywords: *Anopheles*, Koraput, morphological variation, vector, Odisha

1. Introduction

The mosquito species belonging to the genus *Anopheles* play a pivotal role for transmission of malaria in human [1]. Approximately, 424 anopheline species have been identified morphologically, out of which only about 70 species are considered to be the main vectors of malaria in the world [1]. The anopheline fauna in India has been classified into 51 species and 7 sub-species or varieties [2, 3]. The major malaria vectors across India are *Anopheles culicifacies*, *An. dirus*, *An. fluviatilis*, *An. minimus*, *An. sunaicus* and *An. Stephensi* [4]. There are, however, many locally important secondary vectors viz. *An. philippinensis-nivipes*, *An. varuna*, *An. annularis*, *An. aconitus* and *An. jeyporiensis* present, in addition to the six major vectors [4].

Vector control remains the most effective means for combating malaria and is critical for elimination. To implement effective vector control, one needs to know which vector species are present in an area of interest. Therefore, proper identification of anopheline vectors is necessary [5]. At present, the molecular species-diagnostic PCR method is a valuable technique for identifying the *Anopheles* species [6]. However, the traditional morphological identification of species is convenient in order to achieve the goal within limited resources and is realistic for regular use in the field [7]. Morphological characters that are commonly used to identify the adult *Anopheles* species are mainly confined to scale and colour pattern of wings, legs, palpi and proboscis [8]. But, certain individual adult females may be difficult to identify due to overlapping variations (ornamentation of palpi, tarsi of hind legs and the wings) with other members of the species and sometimes leads to incorrect morphological identification of the species [9]. It is also observed that the incorrect morphological identification of mosquitoes can negatively impact further investigation [7]. Further, the identification of the morphological variations of species is required to understand the doubtful species complex of vectors which is important for disease transmission dynamics and vector control.

Odisha State of India has been highly malarious, since several decades and here multiple vectors play role in disease transmission [3, 10]. Koraput district in this State is home for the most efficient malaria vectors and it has been reported during 1989 that 22 anopheline species

with two varieties are present in this area [3]. Later, Rajavel *et al.* in the year 2004 surveyed the Jeypore Hills of Koraput district and identified 28 species of *Anopheles* including two varieties [11]. The information on morphological variations of anophelines was generated by Gunasekaran *et al.*, in Odisha State during 1990 [12]. Since then, no study on morphological variations of anophelines has been undertaken in the State. The phenotypic variations in anophelines may be the result of environmental stress and prolonged use of insecticides [12]. During the last three decades, major changes in the vector control interventions has occurred such as introduction of synthetic pyrethroids for indoor residual spraying (IRS) and use of long-lasting insecticidal nets (LLINs) [10]. Several environmental changes i.e., deforestation, changes in temperature, rainfall and humidity have also been observed [13, 14]. As all these factors could influence mosquito diversity, it is therefore necessary to give special emphasis on morphological variations of anopheline fauna with special reference to malaria vectors. Hence, here an attempt was made to observe the morphological variations of some of the anopheline mosquitoes in Koraput district of Odisha State.

2. Materials and methods

The study was carried out in 27 villages of Koraput district. The factors such as past malaria history, availability of breeding sites, an established agricultural community and feasibility of field operations to collect relevant data were also considered while selecting the study villages. Mosquitoes were collected from January to December 2019. Two methods were used for mosquito collections i.e., light traps in cattle sheds throughout the night and hand catches during early hours (06.00-08.00 h) from human dwellings and the cattle sheds. The collected mosquitoes were brought to the laboratory, anaesthetised with chloroform and identified using the taxonomic key of Christophers [8]. The morphological variations (deviated from the type form) of different malaria vectors and non-vector species such as ornamentation of palpi, wing, tarsi of hind leg were observed under binocular compound microscope and the images were captured. The morphologically variant specimens were preserved in the air tight glass vials with creosote for future reference. All the glass vials were secured in a fumigated insect box. The data on locality, source, species name and variations were recorded.

3. Results and Discussion

A total of 2543 anophelines comprising 18 species were collected. This included *An. fluviatilis* and *An. culicifacies*, the recognized primary vectors of malaria and *An. aconitus*, *An. annularis*, *An. jeyporiensis*, *An. maculatus* and *An. varuna*, the known malaria vectors of secondary importance in India. The most abundant mosquito species was *An. jeyporiensis* (33.5%) followed by *An. nigerrimus* (14.1%), *An. splendidus* (10.7%), *An. maculatus* (7.6%), *An. jamesi* (7.1%), *An. tessellatus* (6.0%), *An. culicifacies* (3.0%) and *An. fluviatilis* (2.1%). Among the 2543 mosquitoes examined, variations were recorded in 66 anophelines belonging to two primary vector species (*An. culicifacies* and *An. fluviatilis*), three secondary vectors (*An. jeyporiensis*, *An. annularis*, *An. aconitus*) and two non-vectors (*An. splendidus* and *An. jamesi*). In total, 13 types of morphological variations have

been recorded; nine variations in palpi, three in wings and one in hind tarsi. The details of the type form characteristics and the morphological variations of the respective species are given in Table 1.

The morphological variations were observed more in *An. jeyporiensis* (n=34) followed by *An. annularis* (n=10), *An. splendidus* (n=8), *An. jamesi* (n=3), *An. culicifacies* (n=4), *An. fluviatilis* (n=5), and *An. aconitus* (n=2) (Table 1). In 3 specimens of *An. fluviatilis*, third vein of wing was black and in 2 specimens, a patch of black scales was present in the apical pale band of the palpi (Fig.1). In *An. culicifacies*, a small black band on apical band of the palpi was recorded in 4 specimens (Fig. 2). In *An. jeyporiensis*, the variations observed were un-interrupted wing costa (n=18), palpi having apical pale band with a patch of extra dark scale (n=12), unequal palpal length (n=3) and apical pale band of palpi abnormally bigger than the type form (n=1) (Fig. 3). During the survey, *An. annularis* var. *nagpori* (with an extra dark band in the apical segment of the palpi) and *An. annularis* var. *adiei* (with an extra dark band on the hind tarsi) were collected. The presence of *An. annularis* varieties *nagpori* and *adiei* were earlier found in inlands of Odisha State [15, 16]. In addition, melanic variations present in both *nagpori* and *adiei* were also found together in one specimen of *An. annularis*, a feature which was also recorded earlier during 1989 in Koraput district [12]. Further, in the current survey, another variation in the wing was, veins of 2nd, 3rd, 4th & 5th were covered extensively with black scales in a few specimens of *An. annularis* (Fig. 4), which was not recorded earlier. In two specimens of *An. aconitus*, the intervening dark band between apical and subapical pale band of the palpi was absent (Fig. 5). In case of *An. splendidus*, the variations were noticed in the palpi; the banding pattern of the palpi was found to be irregular (Fig. 6). Out of two variant specimens of *An. jamesi*, the length of two palpi was unequal in one specimen and in other specimen, extra dark scales were present in the apical pale band of the palpi (Fig. 7).

There are some reports wherein vectors have been misidentified owing to the overlapping morphological characteristics in the closely related species [9, 17, 18, 19]. Only a few reports are available on morphological variation in Indian anophelines [12]. *An. culicifacies* and *An. fluviatilis* are the two potential malaria vectors in the study area [10, 13]. Morphological variations in these two vector species observed in the current study assumes significance as they may confuse a researcher with regard to the identification of the specimen. Some of these variations were earlier recorded by Gunasekaran *et al.*, in 1990 [12]. However, in the current study, the variation observed in the palpi of *An. culicifacies* was a new finding.

An. jeyporiensis is a predominant mosquito species in Jeypore hills and it served as a local vector during the pre-DDT era [20, 21]. Morphologically, it is a well differentiated species although having some characters similar to *An. fluviatilis*. It can be differentiated from *An. fluviatilis* by the presence of double interrupted costa. But, in the current study, many specimens of *An. jeyporiensis* had uninterrupted costa similar to *An. fluviatilis*. These specimens were carefully examined for other key characters of *An. jeyporiensis* for confirmation. Being aware of this variation, would prevent from misidentification of this species as *An. fluviatilis*.

Table 1: Morphological variations in different Anophelines collected from Koraput district

Sl. no.	Species	No. of specimens collected	Variation in body part	Description of variation from the type form	Type form characteristics described by Christophers, 1933 ⁸
1	<i>An. fluviatilis</i> James	2	Palpi	Apical pale band with a patch of black scales on tip of the palpi	<i>Palpi:</i> Apical and subapical pale bands intervening by a dark band which is usually 4-5 times bigger than subapical pale band. <i>Wing:</i> Uninterrupted costa; third vein extensively pale, Fringe spot absent in 6 th vein <i>Leg:</i> Femora, tibia and tarsi uniformly dark
2	<i>An. fluviatilis</i> James	3	Wing	Third vein completely dark.	
3	<i>An. culicifacies</i> Giles	4	Palpi	Apical pale band with a small dark band on tip of the palpi	<i>Palpi:</i> Apical and subapical pale bands intervening by a dark band usually 4-5 times bigger than apical pale band. <i>Wing:</i> Base of costa with one interruption; 3 rd vein completely dark; fringe spot usually at veins 4.2 and 5.1
4	<i>An. jeyporiensis</i> James	3	Palpi	One of the palpi (left side) shorter in length	<i>Palpi:</i> Apical and subapical pale bands intervening by a dark band which is usually 4-5 times bigger than subapical pale band and as long as or slightly longer than apical pale band. <i>Wing:</i> Base of costa having two interruptions, Fringe spot present in all veins, 3 rd vein is pale with dark band in base and apex. <i>Leg:</i> Uniformly dark with femora, tibia and hind tarsi banded.
5	<i>An. jeyporiensis</i> James	1	Palpi	One of the palpi (left side) shorter in length and a patch of dark scales in the longer palpi	
6	<i>An. jeyporiensis</i> James	5	Palpi	Apical pale band with a patch of dark scale on tip of the palpi	
7	<i>An. jeyporiensis</i> James	1	Palpi	Apical pale band abnormally bigger than the type form	
8	<i>An. jeyporiensis</i> James	2	Palpi, Wing	One of the palpi (left side) shorter in length, Base of costa uninterrupted	
9	<i>An. jeyporiensis</i> James	6	Palpi, Wing	Apical pale band with a patch of dark scales on tip of the palpi and base of costa uninterrupted	
10	<i>An. jeyporiensis</i> James	16	Wing	Base of costa uninterrupted	
11	<i>An. annularis</i> (<i>nagpori</i>) Van der Wulp	4	Palpi	Apical pale band with an extra narrow dark band in the middle of the palpi (var. <i>nagpori</i>)	<i>Palpi:</i> Apical band is usually broad and pale <i>Wing:</i> Base of costa mainly dark. Usually with patches of pale scales in veins 2-6. <i>Leg:</i> Hind tarsi 3, 4, 5 segments completely white and 2 nd segment 1/8 th of its length apically white
12	<i>An. annularis</i> Van der Wulp	3	Wing	2 nd , 3 rd , 4 th , & 5 th veins extensively covered with dark scales	
13	<i>An. annularis</i> (<i>adiei</i>) Van der Wulp	2	Leg	Hind tarsi with an extra black band present basally in 3 rd tarsal segment (var. <i>adiei</i>)	
14	<i>An. annularis</i> (<i>adiei</i>) Van der Wulp	1	Palpi, Leg	Apical pale band with an extra narrow dark band in the middle (var. <i>nagpori</i>). Hind tarsi with an extra black band present basally in 3 rd tarsal segment (var. <i>adiei</i>)	
15	<i>An. aconitus</i>	2	Palpi	Apical and subapical intervening dark band absent in the palpi	<i>Palpi:</i> A narrow intervening dark band between apical and subapical pale bands <i>Wing:</i> Base of costa uninterrupted. Fringe spot present in all veins.
16	<i>An. splendidus</i> Koidzumi	1	Palpi	Subapical pale band extended towards the base	<i>Palpi:</i> A narrow intervening dark band between apical and subapical pale bands. White spots on segment 3 of palpi <i>Leg:</i> Femora and tibia with pale spots.
17	<i>An. splendidus</i> Koidzumi	1	Palpi	Tip of the palpi with dark scales	
18	<i>An. splendidus</i> Koidzumi	2	Palpi	In palpi, intervening dark band between apical and subapical band is broader	
19	<i>An. splendidus</i> Koidzumi	4	Palpi	Intervening dark band between apical and subapical bands is broader and the subapical pale band is shorter	
20	<i>An. jamesi</i> Theobald	2	Palpi	An extra black band in the middle of the apical pale band of the palpi	<i>Palpi:</i> Apical pale band is broad followed by a dark band of equal length.
21	<i>An. jamesi</i> Theobald	1	Palpi	One of the palpi (left side) shorter in length	<i>Wing:</i> 5 th vein extensively pale. Fringe spot present in all veins. <i>Leg:</i> Femora and tibia with pale spots

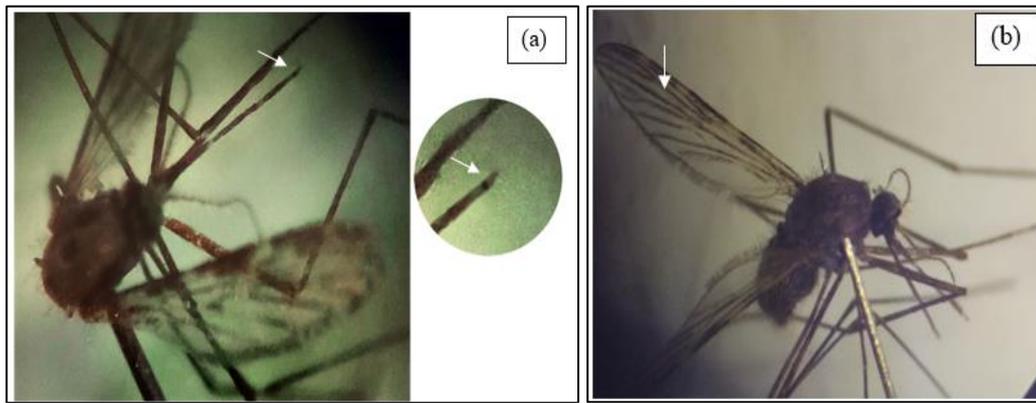


Fig 1: Morphological variation observed in *An. fluviatilis* (a) apical pale band with a patch of black scales on tip of the palpi (b) third vein completely dark



Fig 2: Apical pale band with a small dark band on tip of the palpi in *An. culicifacies*



Fig 3: Morphological variation observed in *An. jeyporiensis* (a) base of costa uninterrupted (b) one palpi (left side) shorter in length with an extra patch of dark scales on tip of the palpi (c) apical pale band bigger (d) one palpi (left side) shorter in length

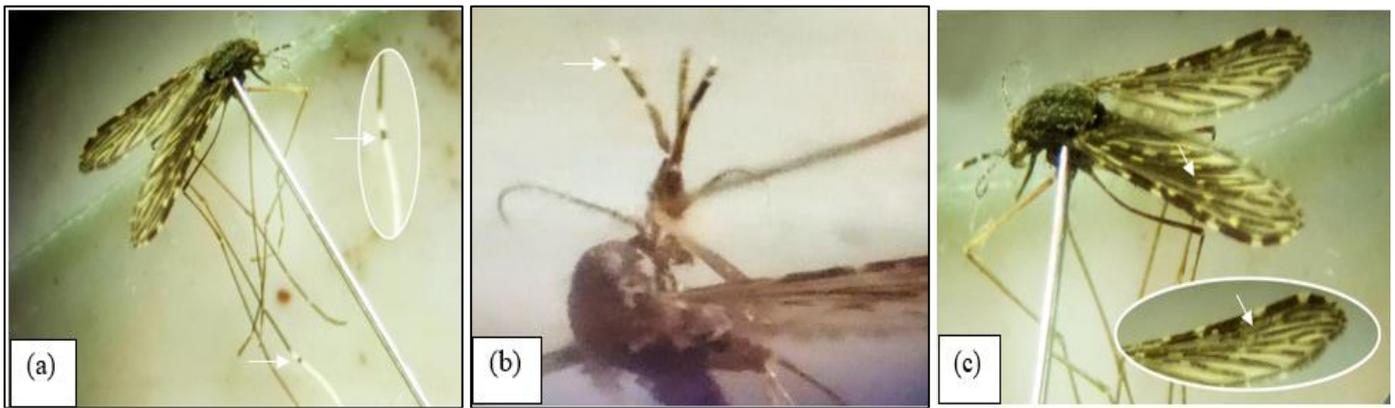


Fig 4: Morphological variation observed in *An. annularis* (a) hind tarsi with an extra black band present in 3rd tarsal segment of *An. annularis* (var. *adieii*) (b) apical pale band with an extra narrow dark band in the middle of the palpi of *An. annularis* (var. *nagpori*) (c) 2nd, 3rd, 4th & 5th vein extensively covered with dark scales



Fig 5: Apical and subapical intervening dark band absent in the palpi of *An. Aconitus*



Fig 6: Morphological variation observed in *An. splendidus* (a) subapical pale band extended towards the base (b) tip of the palpi with dark scales (c) In palpi, intervening dark band between apical and subapical bands is broader (d) palpal white and black banding pattern is irregular

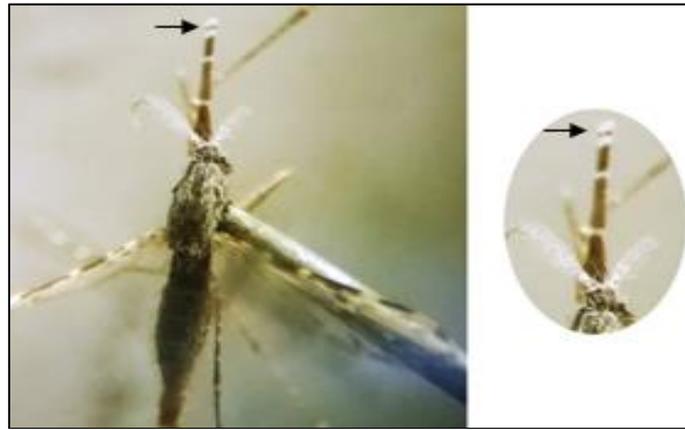


Fig 7: An extra black band in the middle of the apical pale band of the palpi of *An. jamesi*

4. Conclusion

The morphological variations identified in different *Anopheles* species in this study would be useful for proper species identification in settings where alternative molecular identification facility is not possible.

5. Acknowledgments

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