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A comparative study of prevalence and spatial distribution of major *Anopheline* vector fauna in a hyper- and a hypo-malaria endemic district of Odisha, India with special reference to onset of first wet season

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Abstract. The state's retrospective findings indicate the incidence of malaria deaths, which is more during the onset of first monsoon season. Based upon this fact, our objective was to study the distribution pattern of major *Anopheles* vectors in two endemic districts viz. Kalahandi and Cuttack of Odisha, India that differ significantly according to malariogenic stratification, under the impact of first monsoon shower. A comparative study of vector abundance, predominance, their habitats, resting site preference, impact of abiotic rhythm (light/dark period) on vector distribution, gonotrophic status as well as sporozoite rate was established in a high and a low endemic district of Odisha, when the degree of malaria transmission elevates to its peak level *Anopheles culicifacies* was found to be predominant in hyper-endemic Kalahandi district while *Anopheles subpictus* was abundant in hypo-endemic Cuttack district. Both primary vector *An. culicifacies* and the secondary vector *An. subpictus* mostly prefer CS (Cattle shed) compared to HD (Human dwelling) in Kalahandi district where as there was slight shifting of resting habitat of *An. culicifacies* from CS to HD in Cuttack district. *Anopheles culicifacies* prefers to rest on wall besides objects in Kalahandi while no such site preference was observed in Cuttack district. On the other hand, distribution of *An. subpictus* was highly influenced by the daily rhythm of light/dark cycle (i.e. day and night) in Cuttack. The gonotrophic condition revealed the high tendency of the predominant vectors towards endophilic resting. The sporozoite rate was 0.66% in Kalahandi and nil in Cuttack district. The proper monitoring of vector prevalence and distribution, at least during the peak transmission period can avert a perpetuated upsurge in malaria.

INTRODUCTION

In India, malaria is a major public health problem causing high morbidity and mortality (Sharma 1998). About 36% of malaria cases in India are confined to Odisha, an eastern coastal state. The state lies between latitude 17° 78' N to 22° 73' N and longitude 81° 37' E to 87° 53' E which contributes 4.87% of total area of India (Odisha Wikipedia, 2016). The geography and diverse climate of Odisha provide a suitable environment for malaria vector and its parasite (Rao *et al.*, 2015). It has ranked as the second deadliest state in the country with 80 deaths in 2015 and the figure was 77 in the year 2016 (National vector Borne Disease Control programme, Odisha). In the last two years, the mortality graph continued in an upward manner, peaking in the months of June and July, the arrival of first monsoon season in the state. Out of the total 30 districts, the southern and western districts of Odisha are highly endemic for malaria (Sahu *et al.*, 2013). The disease is confined to all districts but the distribution is uneven. Based on the annual parasite index (API), ten districts reported API less than two (coastal and sub coastal) whereas in other districts, the API was ≥ 2 (District report 2016). Despite all control strategies, malaria continues to persist even in these coastal districts.

Therefore, a comparative study of vector distribution and their abundance was carried out to prevent the outbreak of malaria even in a low endemic region. The spatial distribution of *Anopheline* fauna should significantly contribute to the design of malaria control strategies. Several factors play important roles in the distribution and bionomics of *Anopheles* species among which climate factors are the major determinants (Martens *et al.*, 1995) In Odisha, there have been limited comparative studies conducted for understanding the vector distribution pattern during high transmission period.

A preliminary attempt was made to determine the status of the *Anopheline* mosquitoes in a high endemic and a low endemic region of Odisha, when the degree of malaria transmission rises to its peak level. The transmission rises during the arrival of rainy season, hence the study was conducted in June–July 2017.The two districts Kalahandi (API>20) and Cuttack (API<1) was taken into consideration for this study purpose.

Kalahandi district covering an area of 7920 km^2, is situated in south western region of Odisha between latitude 19º 3' N to 21º 5' N and longitude 82º 30' E to 83º 74' E mostly hilly, mountainous and covered with dense forest having low in human indices. Government data shows that the deaths due to malaria are increasing in Kalahandi since 2008. The district is highly endemic for malaria, richer in two major vector species viz. *Anopheles fluviatilis* James and *Anopheles culicifacies* Giles*s.l.* (Sahu *et al.*, 2017; Sharma *et al.*, 2004). The later one is resistant to common insecticides such as DDT, malathion but to some extent is susceptible to synthetic pyrethroid, deltamethrin (Sahu *et al.*, 2015; Sahu *et al.*, 2014).

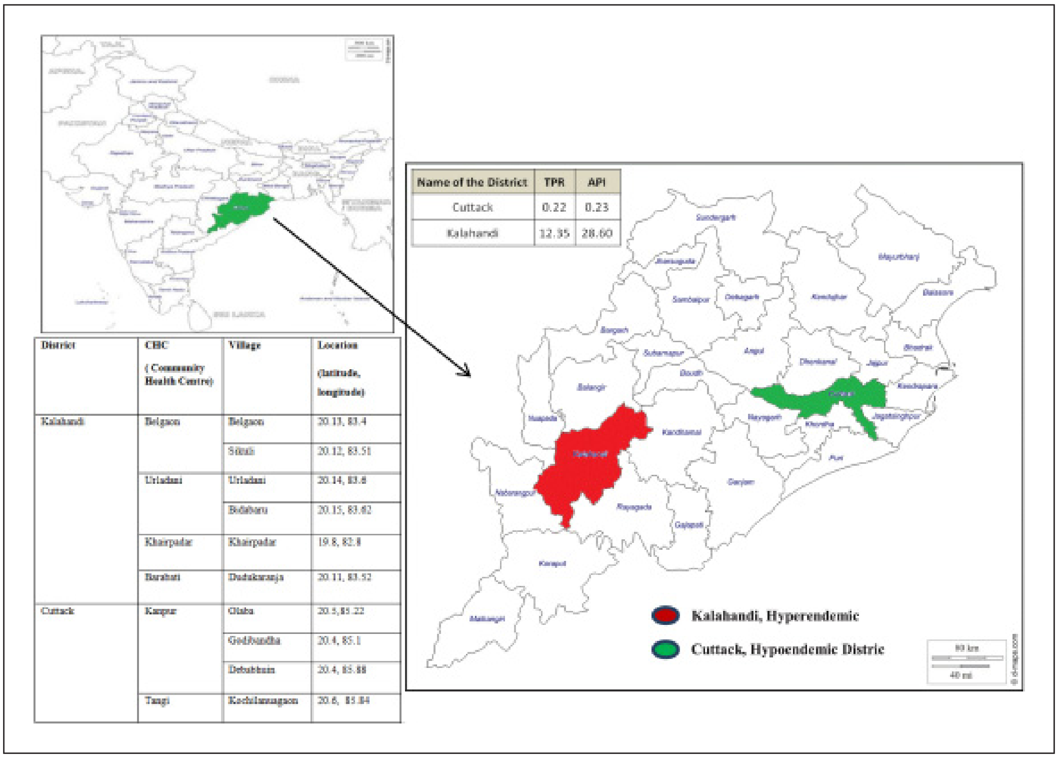
The other sub coastal district, Cuttack (former capital of Odisha) is located at 20º 31' 23'' N latitude and 85º 49' 60'' E longitude covering an area of 3932 km^2 with a tropical climate. In Cuttack district, Athgarh, Tigria, Narsinghpur and Badamba are major malaria prone areas. In the last five years only a few number of malaria cases have been reported from these areas (having average API<1), but still, these are the risk zones vulnerable to vector habitats. A few sibling species of *Anopheles culicifacies* Giles *s.l.* and *Anopheles subpictus* Grassi *s.l* have been reported in these coastal and sub coastal areas (Tripathy *et al.*, 2010; Kumari *et al.*, 2013). The species *Anopheles culicifacies* was found to be susceptible to synthetic pyrethroids in Cuttack district (Raghavendra *et al.*, 2014).

Our study was carried out during the actual peak transmission period after analysing the state's retrospective data for malaria endemicity in the two districts, Kalahandi and Cuttack with a purpose to assess the spatial distribution pattern of *Anopheles* fauna under the impact of arrival of first monsoon in the State.

MATERIALS AND METHODS

Study Area and mosquitoes collection

Kalahandi and Cuttack are the two districts of Odisha confined to south-western and eastern region of the state, respectively (Figure 1). Adult *Anopheles* mosquitoes were collected from six different villages of Kalahandi district and four different villages from Cuttack district in June-July 2017 when average temperature and rainfall were 34-38°C, 211mm and 31-33°C, 208mm in Kalahandi and Cuttack district, respectively. Due to a wide coverage area, six villages were selected from Kalahandi district and only four from Cuttack district. A total of 18 houses from each village were randomly selected. The indoor resting mosquito collection was carried out twice per day in each village during morning (6:30-8:30 AM) and evening (6:30-8:30 PM) hours from different biotopes like cattle sheds (CS) & human dwellings (HD). Majority of the houses were made of mud walls and thatched roofs with cattle shed either close to or somehow



District	CHC (Community Health Centre)	Village	Location (latitude, longitude)
Kalahandi	Belgaon	Belgaon	20.13, 83.4
		Siltali	20.12, 83.51
	Urladani	Urladani	20.14, 83.6
		Bidabaru	20.15, 83.62
	Khairpadar	Khairpadar	19.8, 82.8
	Barabati	Dadakaranja	20.11, 83.52
Cuttack	Kanpur	Olaba	20.5, 85.22
		Godibandha	20.4, 85.1
		Debabhuin	20.4, 85.88
	Tangi	Kochilanagaon	20.6, 85.84

Name of the District	TPR	API
Cuttack	0.22	0.23
Kalahandi	12.35	28.60

Figure 1. Map showing the study area, Kalahandi and Cuttack district of Odisha and their Annual Parasite index (API) & Total Positive Rate (TPR) [District Report 2016].

distant from their human habitant in these two districts. Mosquitoes were collected for 15 minutes from each habitat using an oral aspirator and a flash light. Equal time was spent for collection of mosquitoes from different habitat (CS/HD) and resting sites such as wall, roof, objects (clothes, umbrella, bags, boxes, chair, benches etc) & floor of each house/site. All the mosquito samples were labelled and brought to the laboratory for identification and molecular processing. Besides this prospective approach, a retro-spective data analysis on the density and distribution of *Anopheles* fauna in response to different seasonal phases was also carried out in Kalahandi and Cuttack district.

Morphological identification

The adult *Anopheles* mosquitoes were identified taxonomically following the key developed by Barraud (1934). A key for morphological identification of *Anopheles* species developed by Entomological division of RMRC (Regional Medical Research Centre), Bhubaneswar was also followed during the study. The gonotrophic stages of the female mosquitoes were also classified by the external examination of their abdominal conditions. The mosquito samples were then preserved in isopropanol and kept at -20°C for further processing.

Molecular work

Each individual mosquito was dissected into two parts, head thorax and abdominal part and kept in two separate 1 ml tubes. The genomic DNA was isolated from the corresponding body parts following the protocol developed by Barik *et al.* (2013). The polymerase chain reaction (PCR) for *P. falciparum* infection was performed following the procedure of Rath *et al.* (2015).

Data Analysis

The density of mosquitoes was expressed as the number of female *Anopheles* mosquitoes collected per man hour. One-way ANOVA was used to compare the mean density of total

Anopheles species as well as predominant *Anopheline* fauna in Kalahandi and Cuttack districts. The two-way ANOVA was performed to analyse the significant variation in distribution of major *Anopheles* species i.e. *An. culicifacies* in response to daily abiotic rhythms i.e. during day (light) and night (dark) period and different resting sites. Tukey's HSD post hoc test was performed to justify the variation of species within different resting sites. Two sample t-tests was used to compare the habitat preference of two vector species and also the quantitative distribution of *An. subpictus* species between two districts in relation to the abiotic factor (light/ dark period). Microsoft Excel 2007 was used for of all sets of statistical analysis.

RESULTS

Retrospective analysis of vector density in Kalahandi and Cuttack districts

On the basis of malariogenic stratification, the two districts (Kalahandi and Cuttack) differ from each other in relation to the API (Annual Parasite Index). According to the data reported last year (from Entomological division of RMRC), the mean density of *Anopheles* vector was found to be increasing with the arrival of rainy season and was at its peak during the end of season in both districts (Figure 2). However, the vector density was higher in Kalahandi in comparison to Cuttack. Further study was conducted to evaluate the differential pattern and distribution site of major *Anopheles* species in these districts.

Distribution pattern of *Anopheline* fauna in two districts

A total of 204 (7 distinct species) and 73 (belonging to 4 species) female *Anopheles* mosquitoes were collected indoors from different villages of Kalahandi and Cuttack district, respectively. *Anopheles culicifacies* was found to be predominant in almost all villages (Figure 3) in Kalahandi district whereas in Cuttack district apart from the primary vector, *An. culicifacies* another secondary vector, *An. subpictus* was found in majority.

One-way analysis of variance revealed that there was no significant difference between the mean densities (per man hour) of total *Anopheles* species in these two districts. However, some observable difference was found between the mean density of *An. culicifacies* and *An. subpictus* species (Figure 4) between these districts. The mean density (mean ± SE) of *An. culicifacies* was 4.1±1.09 and 1.5±0.53 whereas for *An. subpictus*, the density was 0.414±0.12 and 2.62±0.6 in Kalahandi and Cuttack district, respectively. One-way ANOVA result for the primary vector *An. culicifacies* ($F=9.03$, $P=0.023$) and the secondary vector, *An. subpictus* ($F=6.6$, $P=0.042$) in Kalahandi and Cuttack districts showed that there was a significant difference ($P<0.05$) between their mean vector density in these districts. The mean density for other vector species did not vary significantly. Accordingly, the two major vectors, *An. culicifacies* and *An. subpictus* were taken into consideration for further analysis.

Resting Habitat of *An. culicifacies* and *An. subpictus* (Human dwelling vs. Cattle shed)

The proportion of *An. culicifacies* collected from HD (Human dwelling) and CS (Cattle shed) was 0.08±0.05 and 0.91±0.05, respectively in Kalahandi, and 0.6±0.23 and 0.39±0.23, respectively in Cuttack. On the other hand, the proportion of *An. subpictus* from HD and CS was 0.21±0.07 and 0.78±0.07, respectively in Kalahandi, and 0.38±0.14 and0.61±0.14, respectively in Cuttack. The primary vector, *An. culicifacies* as well as the secondary vector, *An. subpictus* mostly preferred CS compared to HD in Kalahandi district (Figure 5). The t-test revealed a significant difference between the resting habitats of *An. culicifacies* and *An. subpictus* ($P<0.05$) in Kalahandi district but no such significant variation was observed between resting habitats of these two species in Cuttack district. However, there was some observable difference found in the resting habitat of *An. culicifacies* in Cuttack district, where the proportion was more in HD than CS.

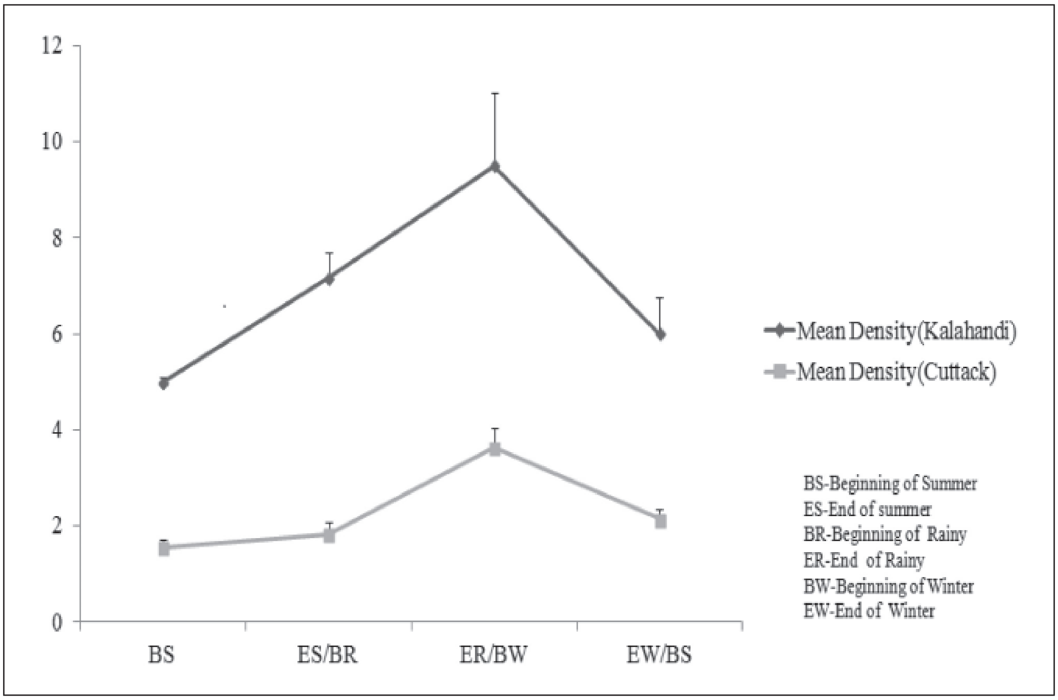


Figure 2. Mean density of total *Anopheline* species in Kalahandi and Cuttack district. (Entomological data from RMRC 2016). Error bar represents standard error of means.

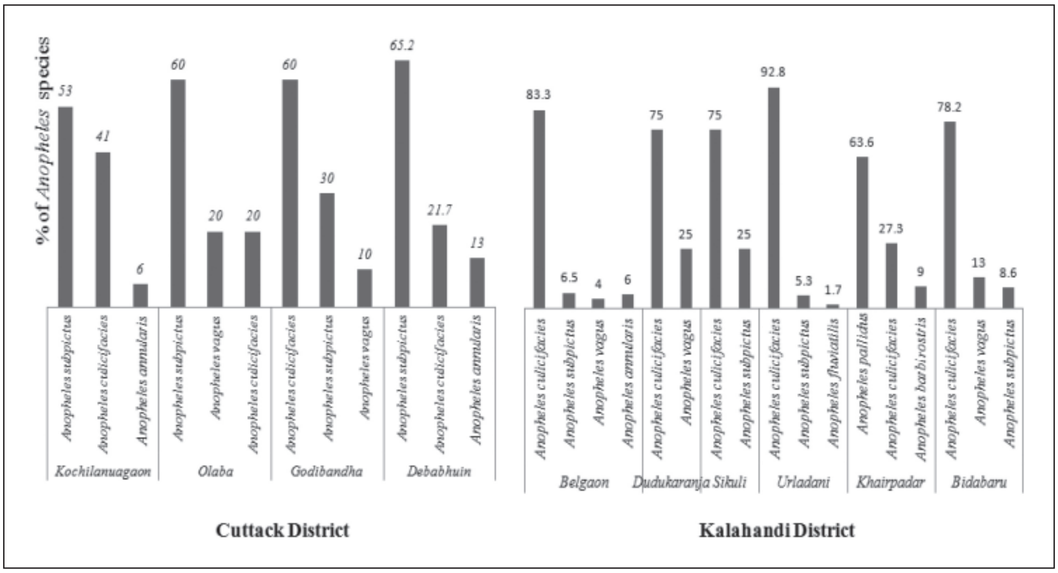


Figure 3. Major *Anopheles* species (%) distribution in different villages of Kalahandi and Cuttack district (June-July 2017).

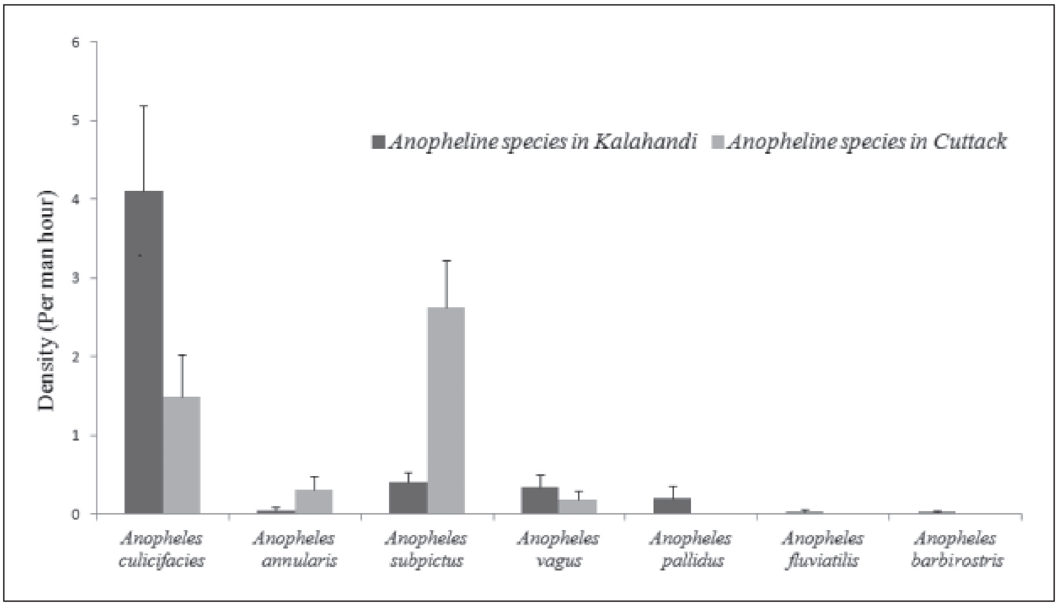


Figure 4. Per Man-hour Density (MHD) of *Anopheline* species of Kalahandi and Cuttack district during first onset of monsoon (June-July 2017). Error bar represents standard error of means.

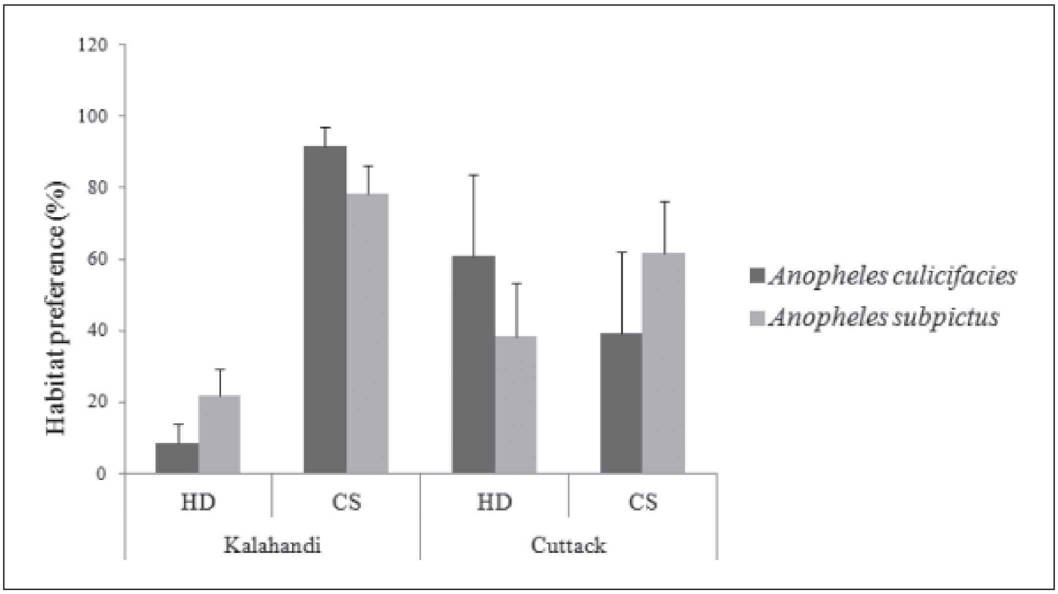


Figure 5. Resting habitat preference of primary vector *Anopheles culicifacies* and secondary vector *Anopheles subpictus* (%) in Kalahandi and Cuttack district. Error bar represents standard error of the proportion. HD-Human dwelling, CS-Cattle shed.

Impact of resting sites and abiotic factor light/dark on distribution of *An. culicifacies* and *An. subpictus*

The mean number of *An. culicifacies* differential resting preference showed that wall and objects were the most preferred resting sites in Kalahandi district (Figure 6) whereas no such site preference was observed in Cuttack. There was no strong evidence on the effect of light and dark period (mosquito collection was done after sunrise as well as after sunset i.e. in presence and absence of natural light) on vector species in both the districts (Figure 7). The two-way analysis of variance revealed that there was no significant effect of daily abiotic rhythm light/dark as well as the two way interaction of light/dark and resting sites on distribution of *An. culicifacies* in both districts (Table 1). However, the density variations of *An. culicifacies* in different resting sites were significant (F=15.71, P<0.00001) only in Kalahandi district. Further, a post hoc Tukey's HSD test was performed to justify the

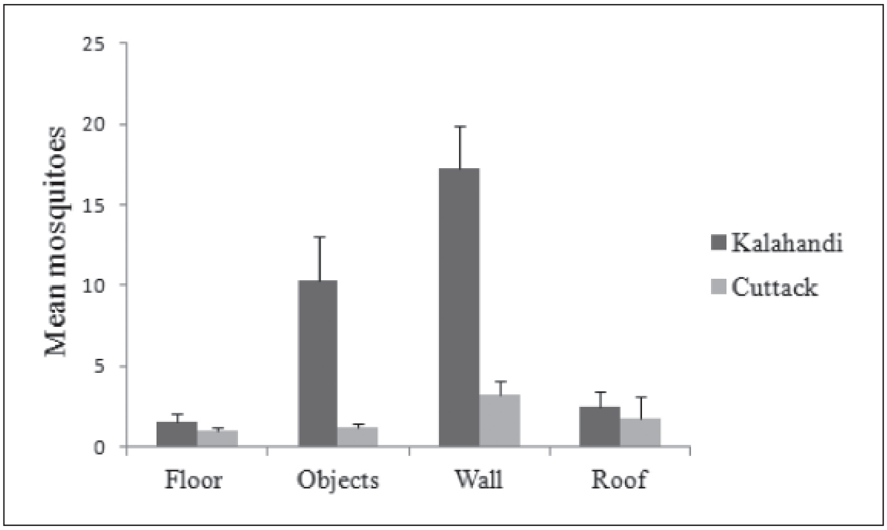


Figure 6. Resting behaviour of primary vector *Anopheles culicifacies* in Kalahandi and Cuttack district. Error bar represents standard error of means.

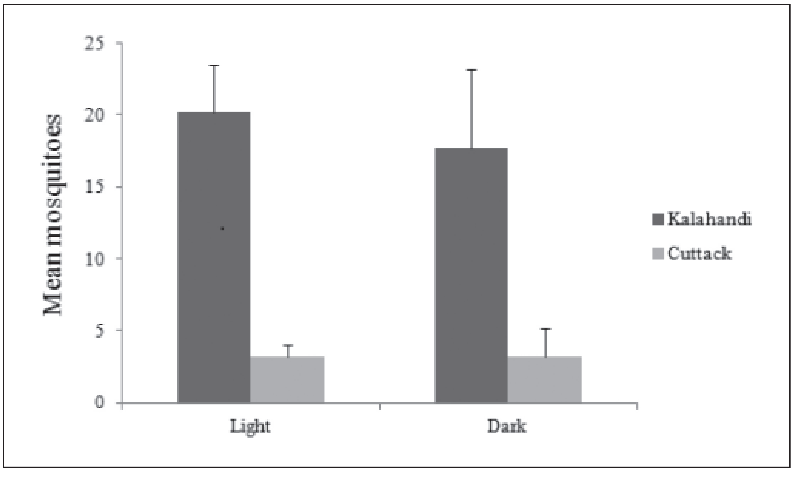


Figure 7. Impact of light and dark on *Anopheles culicifacies in* Kalahandi and Cuttack district. Error bar represents standarderror of means.

Table 1. Two-way ANOVA for effect of light/dark period and resting sites on number of *An. culicifacies* in Kalahandi and Cuttack district

Two way ANOVA for effect of light/dark and resting sites on number of *An. culicifacies* in Kalahandi district						
Effect	SS (Sum square)	Df-Degree of freedom	MSS (Mean Sum Square)	F value	P value	Significance
1st factor (light/dark)	2.88	1	2.88	0.21	0.65	NS
2nd factor (resting sites)	641.1	3	213.7	15.71	< 0.00001	S
1st x 2nd factor	17.12	3	5.7	0.41	0.74	NS
Error	326.9	24	13.6	x	x	x
Total	988	31	x	x	x	x

Two way ANOVA for effect of light/dark and resting sites on number of *An. culicifacies* in Cuttack district						
Effect	SS (Sum square)	Df-Degree of freedom	MSS (Mean Sum Square)	F value	P value	Significance
1st factor (light/dark)	0.96	1	0.96	0.56	0.46	NS
2nd factor (resting sites)	9.2	3	3.06	1.7	0.19	NS
1st x 2nd factor	1.84	3	0.6	0.35	0.77	NS
Error	42.69	24	1.7	x	x	x
Total	54.6	31	x	x	x	x

S - Significant, NS - Not Significant, x - Not Available.

Table 2. Tukey's HSD post hoc test between resting sites of *Anopheles culicifacies* in Kalahandi district

HSD 0.05=5.09	M1 (Floor) 0.625	M2 (Object) 5.1	M3 (Wall) 11.875	M4 (Roof) 1.25
M1 (Floor)	0	NS	S	NS
M2 (Object)		0	S	NS
M3 (Wall)			0	S
M4 (Roof)				0

S - Significant, NS - Not Significant.

difference (P<0.05) between the different resting sites (Table 2). There was no significant difference found between resting sites such as between floor-roof, object-roof and floor-object. The significant difference was highest in between floor and wall.

In case of *An. subpictus*, there was no significant difference between different resting sites. However, there was some impact of abiotic condition (light/dark) on species distribution in these two districts (Figure 8). The two sample t-test result (Table 3) showed that in Cuttack district, there was significant difference in the quantitative distribution of *An. subpictus* in response to light and dark whereas no such difference was observed in Kalahandi district.

Gonotrophic condition

The gonotropic condition of different *Anopheles* species collected from indoor resting population in Kalahandi and Cuttack was depicted in Table 4. The gravid/semi gravid appearance of abdomen indicate the resting stage, while those fully fed and

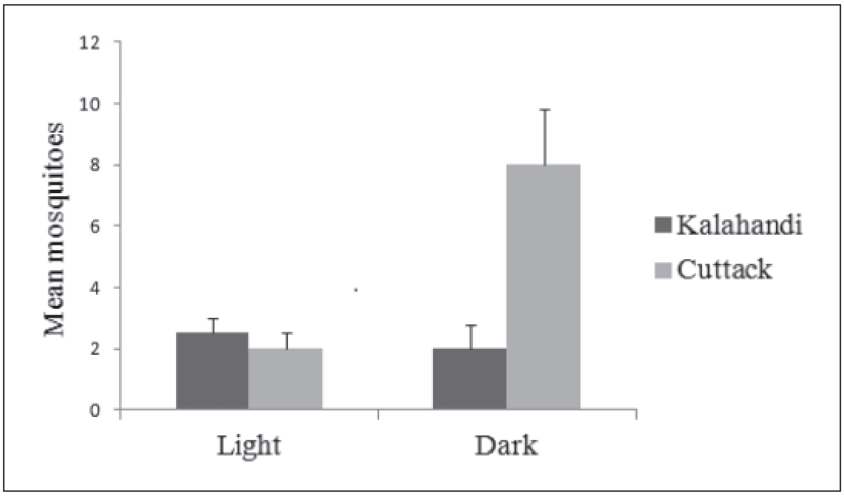


Figure 8. Impact of light and dark period on *Anopheles subpictus in* Kalahandi and Cuttack district. Error bar represents standard error of means.

Table 3. Two sample t-test result to study the effect of light and dark period on distribution of *Anopheles subpictus* in Kalahandi and Cuttack district

t-test for effect of light/dark on number of *An. subpictus*			
District	t-value	P value	Significance
Kalahandi	1	0.35	NS
Cuttack	3.13	0.02	S

S - Significant, NS - Not Significant.

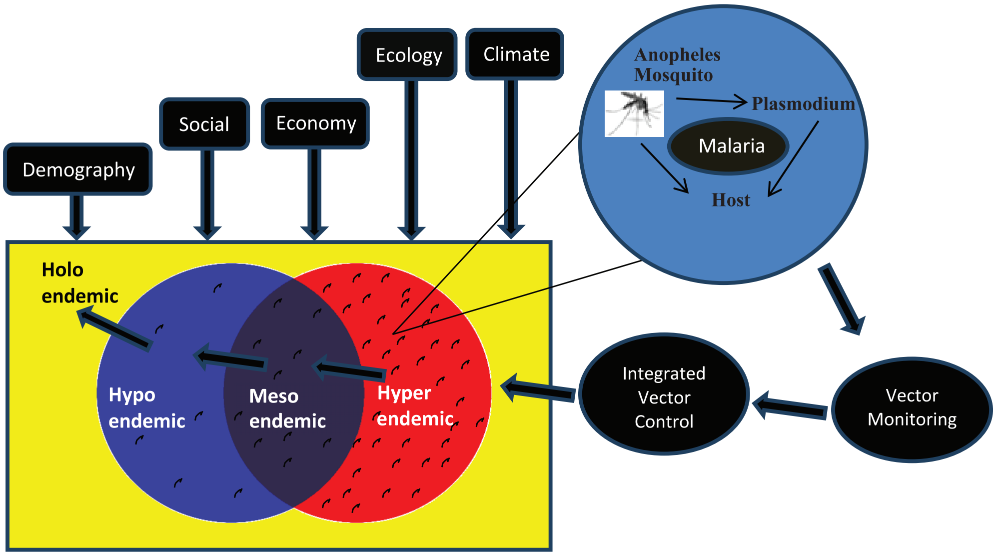
Table 4. Gonotrophic condition of different *Anopheline s*pecies (%) in Kalahandi and Cuttack district

Gonotrophic Condition of Different *Anopheline* species (%)									
Sl no	*Anopheles* species	KALAHANDI				CUTTACK			
		G	HG	FF	UF	G	HG	FF	UF
1	*Anopheles culicifacies*	22.5	77.1	0	0.4	5.2	63.1	31.5	0
2	*Anopheles subpictus*	30.7	53.8	13.5	2.0	33.3	66.7	0	0
3	*Anopheles fluviatilis*	50	50	0	0	NA	NA	NA	NA
4	*Anopheles vagus*	33.3	65	1.6	0	33.3	66.7	0	0
5	*Anopheles annularis*	100	0	0	0	50	50	0	0
6	*Anopheles palidus*	70	22.5	0	7.5	NA	NA	NA	NA
7	*Anopheles barbirostris*	100	0	0	0	NA	NA	NA	NA

G - Gravid, HG - Half/Semi Gravid, FF - Fully fed, UF - Un fed, NA - Not Available.

unfed guts are of seeking stage. *Anopheles culicifacies* having gravid and semi gravid abdominal condition constituted 22.5 and 77.1% of total resting population whereas the percentage of fully fed and unfed group was 0 and 0.4% respectively in Kalahandi district. The percentage of gravid, semi gravid, fully fed and unfed was 5.2, 63.1, 31.5 and 0%,

Graphical Presentation:
A comparative study of prevalence and spatial distribution of major *Anopheline* vector fauna in a hyper and a hypo malaria endemic district of Odisha with special reference to onset of first wet season.



respectively for *An. culicifacies* in Cuttack district. *Anopheles subpictus* having gravid, semi gravid, fully fed and unfed abdominal condition constituted 30.7, 53.8, 13.5 and 2%, respectively, in Kalahandi district, and 33.3, 66.7, 0, 0%, respectively, in Cuttack district.

Infection rate

Out of the total indoor collection, the sporozoite rate was found to be 0.66% in Kalahandi district for *An. culicifacies* whereas none of the species was found positive for sporozoite in Cuttack district.

DISCUSSION

Malaria is a vector-borne disease and endemic especially in tropical and subtropical ecosystems (Sutherst *et al.*, 2004) and the endemicity of which changes with change in ecological, climatic, and socio developmental conditions (Patza *et al.*, 2000). India accounts for approximately two-thirds of all confirmed malaria cases in the Southeast Asia regions (WHO, 2011). The state, Odisha is high malaria transmission zone compared to other states of India (Das *et al.*, 2006). The epidemicity of malaria is preventable by regular monitoring and screening of vector mosquito's occurrence, their distribution and bionomics of an area during the active transmission period.

The present study was undertaken in two districts of Odisha which significantly differ according to the malariogenic stratification. The study was carried out during the first arrival of monsoon shower that creates breeding ground for all sorts of mosquitoes including *Anopheles* species, the malaria vector. In Odisha, the major primary vector species include *An. fluviatilis*, *An. culicifacies* and *An. minimus* whereas the secondary vectors viz. *An. annularis*, *An. subpictus*, *An. varuna* are found in majority (Sharma *et al.*, 2015; Sahu *et al.*, 2011; Dash *et al.*, 2014). In the present study *An. culicifacies* was found to be predominant in majority of villages in Kalahandi district. However, fewer *An. fluviatilis* mosquitoes were identified during that period although they are the predominant primary vectors of malaria in southern districts (Sahu *et al.*, 2008). All the five sibling species (A, B, C, D, E) of the Culicifacies Complex are prevalent in Odisha with the predominance of species B while the species E is found to be an efficient vector (Das *et al.*, 2013). Among the siblings of the Fluviatilis Complex, species S is predominant in hilly regions of Odisha (Gunasekaran *et al.*, 2005) and are more abundant during post monsoon period. During the study period, the secondary vector, *An. subpictus* was found to be predominant in Cuttack district beside *An. culicifacies*. Earlier findings showed that the coastal districts of Odisha were richer in *An. annularis*, *An. varuna*, *An. acotinus* (Nagpal *et al.*, 1983). The sibling species B of *An. subpictus* infected with malaria sporozoites was reported in eastern region of Odisha (Kumari *et al.*, 2013). Besides *An. subpictus*, another secondary vector such as *An. annularis* was also found in Cuttack district. These secondary vectors might contribute to malaria transmission in these areas although the infection rate is relatively low as compared to the primary vectors.

During the study period, all mosquitoes were collected indoors only. As the villages were surrounded by open field and dense forest, the mosquito species were spreading widely instead of being confined to a particular area, hence it was very difficult for outdoor collection. Almost all members of *An. culicifacies* and *An. subpictus* are predominantly zoophlic mainly resting in cattle shed (Waite *et al.*, 2017; Sinka *et al.*, 2011). In our collections, *An. culicifacies* was found in greater densities in cattle sheds (CS) rather than human dwelling (HD) in Kalahandi. However, in Cuttack there was slight shifting in resting habitat from CS to HD. It is probably that the species B is altering its behaviour, becoming more anthropophlic making their ecology similar to species E (Barik *et al.*, 2009). For the secondary vector *An. subpictus*, the preference was always CS in both districts. The availability of eaves and crevices of thatched cattle sheds and unavailability of proper lighting provide a suitable environment for vector species and easy availability of blood hosts without any repellent pressure. Targeting these sites provide a cost effective and efficient vector control strategy by increasing mortality rate in the zoophilic cycle.

Looking at the resting sites in more detail, in Kalahandi for *An. culicifacies* the highest resting preference was wall followed by objects in both HD and CS. In Cuttack district there was no such resting site preference but an indication that they preferred to rest on walls. *Anopheles culicifacies* generally prefers roof as well as apex of wall. However, the changed resting behaviour might be due to avoidance mechanism for insecticide sprayable surfaces in houses such as roof or might be exposure of light because of gap between roof and walls. The objects particularly in dark coloured were more preferred ones as it provides excellent hideouts. Although, the walls were also sprayed with insecticides, repeated mud plastering might have reduced the residual efficacy. Hence more vectors were collected from different regions of the wall indicating insufficient implementation of indoor residual spraying over the years. Therefore, for effective vector control proper qualitative indoor insecticidal spray as well as use of insecticides in rotation could prevent broad spectrum resistant mechanism (Bhatt *et al.*, 2012). Recently a team from the entomological department, RMRC, Bhubaneswar has been actively engaged in Kalahandi for phase-III evaluation of deltamethrin 62.5SC-PE long lasting indoor residual spraying against *An. culicifacies*, at the same time educating the village community about the purpose of spray.

This study indicated that under different environmental condition *Anopheles* species distribution varies. Among them is the daily abiotic rhythm i.e. day (light) and night (dark). As during our study period mosquitoes were collected in morning (i.e. in the presence of light) and after sundown (i.e. in dark), there might be some impact of the light/dark period on species abundance and distribution. There was no effect of this abiotic factor on distribution of *An. culicifacies* in both the districts but there was a strong impact of light/dark on the secondary vector, *An. subpictus*. It was found that the vector was more prevalent in dark in Cuttack but no such variation was in Kalahandi. As these were found in majority in Cuttack district, photic effect might change the indoor resting habitat to outdoor. *Anopheline* mosquitoes are predominantly nocturnal and were more abundant during night or darkness (Paramasivan *et al.*, 2015). Also as a crepuscular feeder, they are active mostly during dawn and dusk and the hotter temperature of the day i.e. light provides a microclimate that is unsuitable for them (Sheppard *et al.*, 2017). So light as a modulator of vector distribution provides novel vector control strategies based on interfering host-vector interaction. Hitherto, the comprehensive study provides a characteristic distribution structure of predominant vector species in two different districts.

The examination of gonotrophic condition of *Anopheles* species, which is based on the ratio of abdominal condition (G+ SG/FF + UF) of the major vector species in between the two districts, suggests *An. culicifacies* was highly endophilic in Kalahandi compared to Cuttack district. Conversely the secondary vector, *An. subpictus* appeared more endophilic in Cuttack district. As majority of collected species in these two districts showed a high proportion of gravid/semi gravid compared to unfed/full fed abdominal status, they tended to be more endophilic for resting. The endophilicity of these vectors primarily depend on house structure, environmental conditions as well as demographic composition. Therefore, interventions that effectively target those vectors also can efficiently control high malaria transmission.

Finally, when considering the sporozoite rate during the study period, it was 0.66% in Kalahandi where as in Cuttack it was nil. The high infectivity of *An. culicifacies* in Kalahandi could be due to the absent of any effective vector control measures during the study period Although, the transmission was high in Kalahandi during that period, the lower infection rate might be due to unavailability of data from outdoor collection. The alternative reason might be also due to quantitatively low number of *An. fluviatilis* species that generally contribute maximum proportion to the overall sporozoite rate as compared to *An. culicifacies* (Mohanty *et al.*, 2007). Therefore, the infection rate governs decisions regarding vector control as well as therapeutic management.

The overall study provides an update on differential distribution of major *Anopheline* species in these two districts in relation to areas, resting habitats, resting sites, abiotic factor, gonotrophic condition, sporozoite rate, etc. during the onset of first monsoon. The ecology and climatic conditions in these two districts are the major factors for this variable spatio-temporal vector distribution pattern. These findings provide a baseline for evidence based planning and implementation of malaria control strategies.

Although, the study was conducted in a very short time period with less quantitative collection, but it provides an overall qualitative structure of vector distribution in these two districts which are significantly differ from each other in relation to the API (Annual Parasite Index). Economic conditions and awareness plays a very important role in epidemiological situation of malaria. The poor socioeconomic status is the major contributor to the high endemicity of Kalahandi district as compared to Cuttack, the former capital of Odisha which on other hand socially, commercially and economically more forward district. Moreover, the dependence of patients comparatively more on private health care rather than PHCs (Primary Health Centres) may also contribute to low API in those areas. In oppose to this, lack of proper communication, good health care infrastructure, public awareness and implementation of research programs to the inaccessible malaria endemic areas are the major causes for that high API. Therefore, there is a need to set up entomological department in each district to monitor the vector abundance and distribution and incrimination at least during the peak transmission period which could act as an alarming system for taking proper control measures. The establishment of the monitoring body might convert the hyper-endemic region to hypo-endemic and the later one to holo-endemic within a couple of years.

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