## Original Article

# Bionomics of *Anopheles culicifacies* Sensu Lato in two Malaria Endemic Districts of Central Gujarat, India

***Rajendra K Baharia[1], Jaspreet Kaur[2], Ankita Sindhania[2], Gunjan Sharma[2], Harish C Srivastava[1], Chandra S Pant[1], Manju Rahi[3], Kamaraju Raghavendra[2], Om P Singh[2]**

[1]Indian Council of Medical Research (ICMR) - National Institute of Malaria Research, Field unit, Civil Hospital Nadiad, Gujarat, India
[2]Indian Council of Medical Research (ICMR) - National Institute of Malaria Research, Dwarka Secctor-8, New Delhi, India
[3]Indian Council of Medical Research (ICMR), Division of Epidemiology and Communicable Diseases, Ansari Nagar, New Delhi, India

***Corresponding author:** Dr Rajendra Kumar Baharia, E-mail: rajendrabaharia@gmail.com

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## Abstract

**Background:** Gujarat State has been witnessing large scale urbanization, in last two decades, resulting changes in local environment and microclimate may have also influenced the resting, feeding habits and development of *Anopheles culicifacies* sensu 1ato. Therefore, a systematic longitudinal study was undertaken to know the bionomics of *An. culicifacies* s.l. in present study.
**Methods:** The study was conducted in four sentinel villages in Kheda and Panchmahal Districts. The mosquitoes resting indoors and outdoors were collected in early morning hours, using mouth aspirator, pyrethrum space spray and light traps. Mosquito landing collections on human volunteers was carried out from dusk to dawn. Species composition, abundance, seasonal prevalence, resting behavior (Endophily and Exophily), sibling species composition, vector potential and insecticide susceptibility status of malaria vectors was studied.
**Results:** Six *Anopheles* species were collected, *An. subpictus* s.l. was the predominant species followed by *An. culicifacies* s.l., a known malaria vector was resting indoor and zoophagic behaviour. *Anopheles culicifacies*, sibling species B (89%) was found. The sporozoite rate (%) and entomological inoculation rate in Kheda was 2.33%, 3.09 per bite/ person/annum and they were 1.05% and 0.475 bite/person/annum in Panchmahal, respectively. *Anopheles culicifacies* s.l. was found possible resistance to alpha-cypermethrin.
**Conclusion:** *Anopheles culicifacies* s.l. showed endophillic, zoophagic behaviour and found possible resistance to alpha-cypermethrin. Early biting behaviour of *An. culicifacies* s.l. in this area is a cause of concern. Therefore, there is need for frequent monitoring and evaluation of vector control measures in order to achieve the elimination target of malaria in this area.

**Keywords:** Endophilic; Zoophagic; Alpha-cypermethrin; Susceptibility; Sibling species

## Introduction

Vector spatial selection is greatly influenced either due to species competition or changes in environmental factors such as temperature and lead to the local adaptation (1). It has been described that climate change will escalate the transmission of vector borne diseases. Several authors have claimed decline in certain areas or no change in disease prevalence due to overlooked ecological factors (2–3) and development in community (4–5). Of the 58 Anophelines in India, only six taxa are major malaria vectors with significant regional distribution (6). *Anopheles culicifacies* s.l. has been reported as the major malaria vector in the plains in Indian sub-continent. It has been incriminated as malaria vector from rural area of various states



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of India (7–11) including in Gujarat State (12). Various aspect of bionomics of *An. culicifacies* s.l. was studied in India (13). The abundance, prevalence, biting and vector potential of *An. culicifacies* s.l. in Gujarat State was studied in 1980s and 1990s (14–21). Cytotaxonomic examination of *An. culicifacies* showed five 5 sibling species named as A, B, C, D and E in various parts of the India (22–25). The species has shown differential sympatricity, resting and feeding behavior, insecticide susceptibility, vectorial capacity (12, 14, 17, 19, 21–22) and have indicated their differential role in transmission of malaria (10–11, 26–27). *Anopheles culicifacies* s.l. is primarily zoophagic in behavior and its impact on malaria transmission was assessed in Gujarat State (12–14). Resistance in *An. culicifacies* against three insecticides, used in public health programme for vector control namely DDT, Malathion and Diealdrin has been reported from Gujarat during 1960s and 1970s (28). Gujarat being a progressive state has witnessed rapid urbanization, industrial development, and construction of development projects and associated deforestation during last two decades in the state, resulting in changes in local climate and environment. Accordingly, change in microclimate may have influenced some of the bionomic attributes such as resting and feeding habits that may need renewed attention. Malaria is unstable in Gujarat State, western part of India. During last one-decade malaria has declined to annual parasite incidence (API) of 0.31 in 2019, at the verge of elimination (29). Therefore, a systematic longitudinal study was undertaken to know the bionomics of *An. culicifacies* s.l. in some districts of Gujarat State. The results will help in making evidence-based decision for suggesting effective vector control strategy and understand existing lacunae to control and eliminate malaria in similar ecological settings in Gujarat.

## Materials and Methods

### Study area

The study was undertaken in Kheda District (22°45' N, 72°45' E) and Panchmahal (Godhra) District (22°45′ N, 73°36′ E) located in central Gujarat (Fig.1). Based on malaria incidence, potential mosquito breeding sites and baseline entomological survey, four (2 riverine and 2 canal irrigated) villages in each district were included in the study in May-June 2017. The selected villages Angadi, Pali (Kheda), Juni Dhari, Vinzol (Godhara) are riverine whereas the villages such as Ravaliya, Muliyad (Kheda), Khazoori and Nandisar (Godhara) have perennial irrigation canal. The villages were approachable at ease by road with good condition throughout the year. Most of the houses are made up of brick with concrete roof; however, a few mud houses with tin or tile roof also exist. Major crops in this area are Paddy, Millet, Corn and Tobacco. The maximum and minimum external air temperature, relative humidity and rainfall in this area were obtained from Anand Agriculture University, field unit at Thasra, District of Kheda.

### Mosquito Collections

The indoor and outdoor resting, bait and trap collections were carried out at monthly frequency in sentinel villages following standard WHO methods (30). In each village, 6 human dwellings (HD) were fixed for indoor resting mosquito collection. Outdoor resting mosquitoes were collected from underneath of culverts, abandoned temples and bushes.

### Indoor Resting Mosquito Collection from Human Dwellings

Adult female *Anopheles* species resting indoors in fixed six human dwellings and two cattle sheds were collected with the help of flashlight and mouth aspirators in early hours of the day (0600 to 0800hrs) for 15 minutes in each of the fixed dwellings. After 15 minutes of hand catch, pyrethrum spray collection (PSC) was done from the same room. Mosquito collected through hand catch were kept in paper cups and PSC kept in Petri dishes lined with wet cotton and filter paper at the bottom and transported to the laboratory for processing. The total number of mosquitoes/species resting per structure was calculated as density (per man per hour density for HC) or numbers of mosquitoes/room (PSC).

### Outdoor Resting Mosquito Collection

Mosquitoes resting outdoors were collected with the help of flashlight and mouth aspirators during morning hours (0600 to 0800hrs) from available different outdoor sites in the village. The mosquitoes collected were kept in paper cup with wet cotton and transported to the laboratory for further processing. The number of mosquitoes/species collected was calculated as the density (man hour density).

### Human Landing Collection (HLC)

Hourly whole night collection of mosquitoes landing on human bait (volunteer) was carried out from dusk to dawn (1800 to 0600hrs). Care was taken to collect mosquitoes as soon as they land on the host to evade mosquito bites on volunteer. A paper cup fastened with netting was used for each hour of collection. The collected mosquitoes were brought to field laboratory identified to species and vector(s). Mosquitoes were processed for parity rates and the head and thorax was preserved for enzyme-linked immunosorbent assay (ELISA) test.

### Light-Trap Catch (by CDC Light Trap)

Two light-traps, one each in indoor and outdoor was used for mosquito collection. The indoor trap was placed 60cm above the ground in the sleeping unit of the household. For outdoor collections, traps were placed just outside the houses where people sit and sleep or in open away from the habitations in the village. Mosquitoes were collected in morning from the trap was used for estimating proportion of different species prevalent in the area. These collections expressed as numbers of mosquitoes per trap-night (number of mosquitoes/ trap).

### Processing of Mosquito Samples

Anopheline mosquitoes identified to species based on morphological characters using key (31–35), the physiological condition of stomach was determined such as unfed (UF), freshly fed (FF), half gravid (HG) and gravid (G), blood meal of *An. culicifacies* s.l. were collected on Whatman no. 1 filter paper for host source using gel diffusion technique to determine the feeding preference (Human blood Index), half gravid ovaries of *An. culicifacies* s.l. was collected and preserved for sibling species identification, the head and thorax of each specimen was preserved for sporozoite ELISA test and unfed females used for estimating parity.

### Susceptibility Status of Vectors to Different Insecticides

*Anopheles culicifacies* s.l. susceptibility status against pyrethroids was estimated in October 2017, January 2019 and March 2019, according to standard WHO method (36). Field collected mixed age population of *An. culicifacies* s.l. of villages Anghadi, Pali, Revaliya, and Vinzol were tested using WHO prescribed discriminatory concentration impregnated papers of malathion, deltamethrin and alpha-cypermethrin. A minimum of 75 mosquitoes (25 mosquito/replicate) for test and 50 (25/replicate) for control were used. The test was conducted in National Institute of Malaria Research (NIMR) laboratory maintained at 27±2 ºC temperature and 60–70% relative humidity during test period. Percent mortality was determined post 24 hrs of holding period after one-hour exposure from the total number of alive and dead mosquitoes in the replicates. If control mortality was greater than or equal to 5% and less than or equal to 20%, the value for exposure mortality was corrected by using the Abbott's formula (37).



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### Sibling Species Identification

Morphologically identified specimens stored in isopropanol were processed for *An. culicifacies* sibling species (38). Species A/D and species B/C/E were identified by two allele specific primers ACA (forward, sequence 5'-GCC GTC CCC ATA CAC TG-3') and ACB (reverse, sequence 5' CCG TAA TCC CGT GAT AAC TT-3'). PCR conditions were: one cycle of denaturation at 95 °C for 5min followed by 35 cycles of each of denaturation at 95 °C for 30s, annealing at 55 °C for 30s and extension at 72 °C for 60s, and final extension at 72 °C for 7min.

### Vector Incrimination

The head and thorax of *An. culicifacies* s.l. preserved were assayed for presence of sporozoite using ELISA (39–41). The specimens were tested for species specific circumsporozoite antigen (CSP) of Pf, Pv210 and Pv247 using the protocol as described by Akhatar et al. (41).

## Results

### Mosquito Abundance and Seasonal Prevalence

A total of 14056 mosquitoes were collected by all methods used of which, 73.94% (10389/14056) was *Anopheles* along with 25.46% *Culex* and 0.62% *Aedes*. Among anophelines *An. subpictus* s.l. (69.03%) species followed by *An. culicifacies* s.l. (17.93%), *An. annularis* (12.35 %), *An. stephensi* (1.02%), *An. fluviatalis* s.l. (0.16%) and *An. vagus* (0.0096%). *Anopheles culicifacies* s.l. was encountered from all habitats in both districts. From outdoor resting habitats *An. subpictus* s.l., *An. culicifacies* s.l. species and *Culex* spp were found.

### Indoor Resting Collections

### Human Dwelling

The indoor resting mosquito collections were made for 13 months (July 2017 to July 2018) from fixed Human dwellings in sentinel villages of Kheda and Panchmahal Districts. In both the districts, bimodal population increase of *An. culicifacies* s.l. densities were observed, a minor peak in March and a major peak in August. The indoor resting density in canal irrigated area was higher during most of the study period as compared to in riverine areas in both the districts. In post-monsoon months (December to March), indoor resting density was significantly high in both the areas of Panchmahal compared to Kheda (Fig. 2). During the transmission season, *An. culicifacies* s.l. density of Kheda irrigated versus Panchmahal riverine and Kheda riverine versus Panchmahal irrigated were significant ($p= 0.0092$, $t= 2.77$, $p< 0.05$ and $p= 0.0357$, $t= 2.0$, $p< 0.05$).

### Cattle Shed

Indoor resting sampling in cattle sheds revealed that the density of *An. culicifacies* s.l. was greater in cattle sheds than human dwellings in Kheda and Panchmahal. In riverine area of Kheda, *An. culicifacies* s.l. density was higher as compared to irrigated area throughout the study except in post monsoon period, whereas in Panchmahal, it was high in riverine area in all the seasons.

### Physiological Condition

It was observed that in indoor resting samples from riverine villages of Kheda District, the proportion of half-gravid + gravid *An. culicifacies* s.l. was higher compared to full fed and unfed in pre-monsoon, monsoon, and post-monsoon seasons. In canal-irrigated areas of this district, the proportion of fully fed mosquito was higher compared to half-gravid + gravid, and no unfed was encountered during pre-monsoon period. While in monsoon and post-monsoon period, canal-irrigated villages had higher proportion of half-gravid + gravid as compared to fully fed and unfed. The riverine and canal-irrigated villages of Panchmahal had higher proportion of half-gravid + gravid compared to fully fed. It indicated that



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large proportion of *An. culicifacies* s.l. bites in the evening and late evening and rests indoor in both the districts indicating endophillic behavior.

## Parous Rate

Altogether 1223 ovaries (Kheda 534 ovaries, Panchmahal 689 ovaries) of *An. culicifacies* s.l. were examined for parity. Overall, 30 % (200/689) were found to be parous in Panchmahal District throughout the study period while in Kheda District it was low in winter 13% (3/22) and summer 7% (1/14). *Anopheles culicifacies* s.l. parity varied in different months, ranging from 7% in May to 60% in July. In the transmission season, parity rate of Kheda and Panchmahal District were significant ($p= 0.0305$, $t= 3.9$, $p< 0.05$).

## Light Trap

In Kheda, the densities of *An. culicifacies* s.l. (3.08 per trap) and other mosquitoes (25.58 per trap) were higher in outdoor trap compared to indoor. In Panchmahal, the density of other mosquitoes (22.41 per trap) was 10-fold higher than *An. culicifacies* s.l. in both traps. In outdoor traps, the density was higher in Kheda compared to in Panchmahal (Figs. 3 and 4). *Anopheles culicifacies* s.l. density from the districts of Kheda and Panchmahal were significant with outdoor other mosquitoes' density ($p= 0.0030$, $t= 3.4$, $p< 0.05$ and $p= 0.0044$, $t= 3.18$, $p< 0.05$).

## Human Landing Collection (HLC)

The HLC yielded five anopheline species comprising of *An. culicifacies* s.l., a few *An. stephensi*, *An. annularis*, *An. fluviatilis* s.l., *An. vagus*, and a few specimens of *Aedes* spp and *Culex* spp. In general, the bite rate of *An. culicifacies* s.l. was greater in Kheda compared to Panchmahal in all seasons and found biting throughout the night in both districts. In Kheda, the mean biting rate of *An. culicifacies* (9.0 bite/bait/night) was high during post-monsoon period, September to October. Whereas biting activity of other mosquitoes was maximum during March-May, it was 20 times higher than *An. culicifacies* s.l. In both the districts, most of the landing mosquitoes were caught either in early night or in early morning. Thus, two peaks of biting activities were observed in each night, first between 20–21hrs and second at 02–04hrs in both districts (Fig.5). Human landing catch were significant in between Kheda and Panchmahal Districts ($p= 0.0292$, $t= 2.11$, $p< 0.05$).

## Host Blood Preference

A total of 577 (320, riverine and 257 irrigated area) blood meal samples of *An. culicifacies* s.l. were collected from sentinel villages in Kheda District. From villages in Panchmahal District altogether 573 (327 riverine and 246 irrigated villages) blood meal samples were assayed for blood meal sources. The anthropophilic index (AI) of *An. culicifacies* s.l. in Kheda District, in canal irrigated area was 2.33 % (6/257) and in riverine area none was positive for human blood (0/320). In Panchmahal District, AI was 1.62% (4/246) in canal irrigated area and 1.52% (5/327) in riverine area. Majority of blood meal of *An. culicifacies* s.l. were found bovine indicating its established zoophagic behavior in both the districts.

## Insecticide Susceptibility Status

The susceptibility status of *An. culicifacies* s.l. was assessed against Malathion, Deltamethrin, Permethrin and Alpha-cypermethrin using WHO discriminatory dose impregnated papers. It was observed that *An. culicifacies* s.l. has developed resistance against Deltamethrin (24 hrs mortality: 75%) and Malathion (24hrs mortality 70%), whereas it was found possible resistance to Alpha-cypermethrin with 24hrs mortality 95% in Kheda (Table 1). In Panchmahal District, *An. culicifacies* s.l. was possible resistance to pyrethroid Alpha-cypermethrin with 24hrs mortality 95%, but has developed resistance against Malathion (24hrs mortality 75%).

### Sibling Species Composition

Altogether 67 and 223 *An. culicifacies* s.l. from Kheda and Panchmahal, respectively were examined for sibling species identification. Its sibling species distribution pattern was found similar in both the study areas. The primers were specified for detection of the A/D and B/C/E sibling species complex. In Kheda, *An. culicifacies* species B (82%; 55/67) was predominant species followed by species A/D (12 %; 8/67), in Panchmahal B and A was 91% (203/ 223) and 8% (17/223), respectively (Table 2).

### Sporozoite and Entomological Inoculation Rates

In Kheda and Panchmahal Districts 916 and 900 *An. culicifacies* s.l. were analyzed for vector incrimination studies. In Kheda, 12 (*Plasmodium falciparum*: 7, *P. vivax*: 5) and in Panchmahal District, 04 (*P. falciparum*: 3, *P. vivax*:1) specimens were found positive for sporozoite. The sporozoite and biting rate in Kheda was 1.33% and 2.33 bite/bait/night and 0.45% and 1.05 bite/bait/night in Panchmahal, respectively. Entomological inoculation rate (EIR) in Kheda and Panchmahal was estimated to be 3.09 and 0.475 per bite/person/annum, the higher EIR in the Kheda may be the result of high biting rate of *An. culicifacies* s.l. (Table 3).

### Additional Data

Additional observations were made on cattle shed location, sleeping behaviour of inhabitants (indoors/outdoors) and livestock enumeration to assess any possible impact on the behaviour of vector and its transmission potential.

A total of 120 households were visited in the both districts to know the location of cattle shed in study villages. The maximum cattle sheds were adjacent to human dwelling in Kheda (60%) and Panchmahal (55%). The mix-dwellings (cattle and human habitations under same roof) were more than those of cattle shed away from human dwellings in both districts. In all the sentinel villages, livestock census was undertaken by NIMR staff to estimate the cattle human ratio in 2018. The buffalos were found as major livestock followed by cow and other animals (goat and chicken) in each village. The cattle to human ratio were low in both Panchmahal (0.19: 1) and Kheda (0.20: 1) districts. These indicate the greater risk of human vector contact in both districts and explain the observed epidemiological attributes sporozite rates and EIR.

The inhabitants of these dwellings were interviewed to know the sleeping behavior (indoor/outdoor) of villagers in different seasons. The behaviour of villagers was similar in all three seasons in both the districts. More than 60% inhabitants sleep indoors during winter and monsoon in both districts and > 50% sleep outdoors in summer.

**Table 1.** Susceptibility status of *Anopheles culicifacies* s.l. against different insecticides

| Insecticides (%) | Kheda | | Panchmahal | |
|---|---|---|---|---|
| | 1h Knockdown (%) | 24hrs mortality (%) | 1h Knockdown (%) | 24hrs mortality (%) |
| Control Group | 0.0 | 0.0 | 0.0 | 0.0 |
| Deltamethrin (0.05%) | 70 | 75 | 75 | 85 |
| Permethrin (0.75%) | 80 | 85 | 83 | 90 |
| Alpha cypermethrin (0.05%) | 90 | 95 | 90 | 95 |
| Malathion (5%) | 60 | 70 | 65 | 75 |

**Table 2.** Sibling species composition of the *Anopheles culicifacies* complex from July 2017 to July 2018

| District | n | Genotype (%) | | | |
|---|---|---|---|---|---|
| | | A/D | B/C/E | NA (Not amplified) | UK (Unknown) |
| **Kheda** | 67 | 08 (12) | 55 (82) | 04 | 00 |
| **Panchmahal** | 223 | 17 (8) | 203 (91) | 01 | 02 |
| **Grand Total** | 290 | 25 (9) | 258 (89) | 05 | 02 |

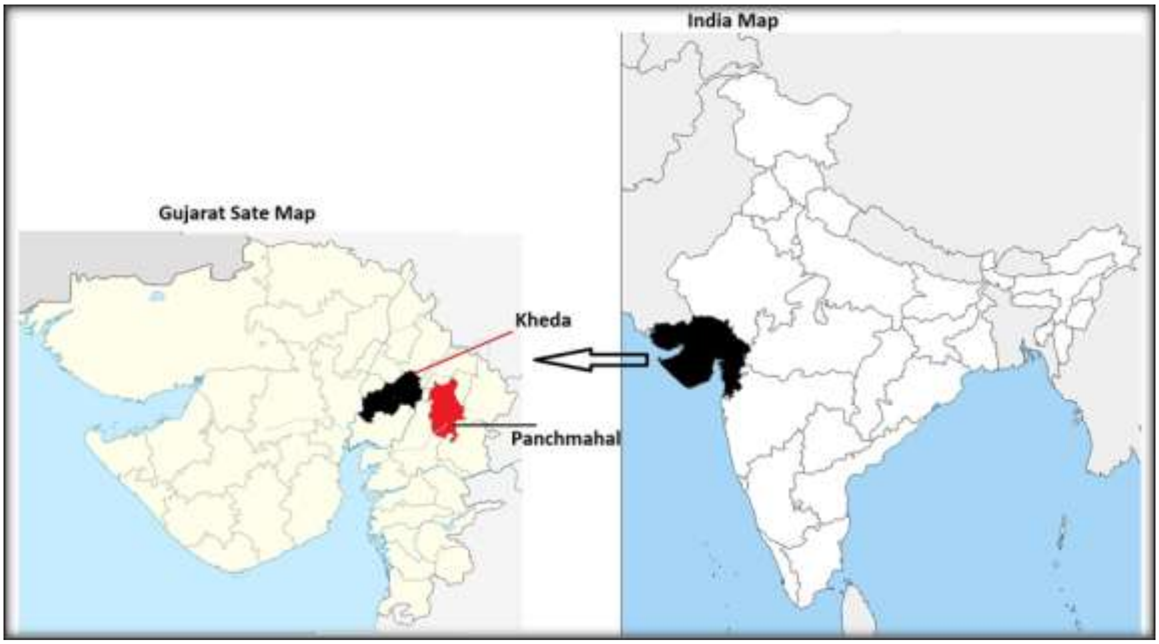


**Fig. 1.** Location of study districts of Kheda and Panchmahal in Gujarat State

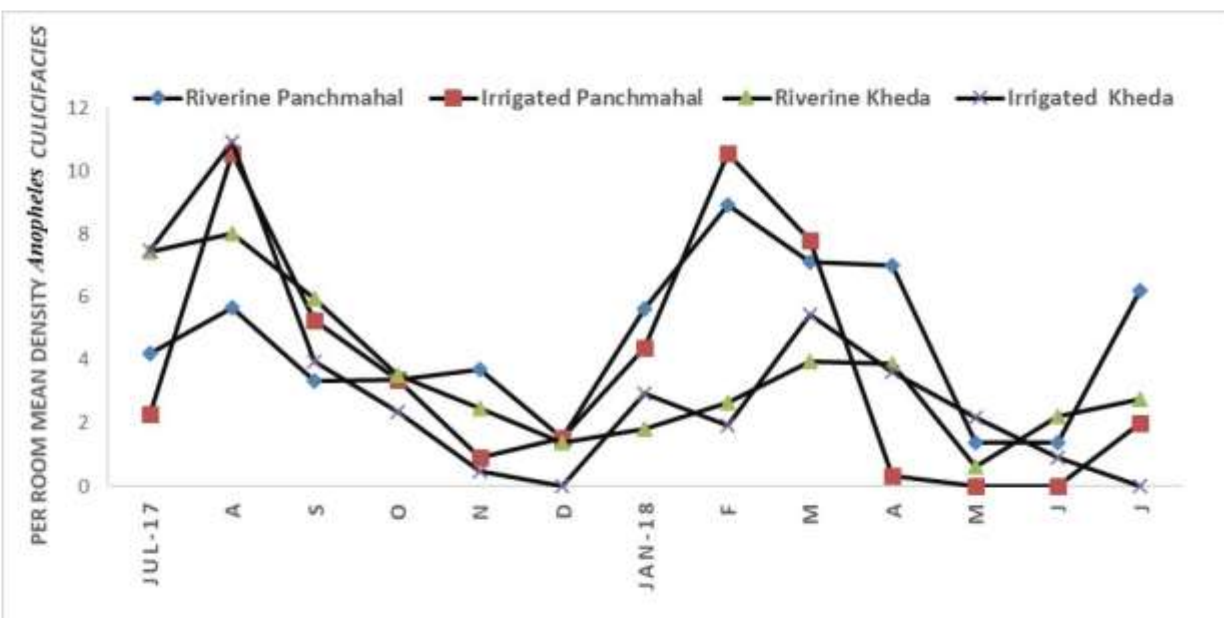


**Fig. 2.** Mean indoor densities of *Anopheles culicifacies* s.l. in Kheda and Panchmahal Districts from July 2017 to July 2018



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**Table 3.** Sporozoite rate, biting rate and entomological inoculation rate (EIR) of *Anopheles culicifacies* s.l. in study area from July 2017 to July 2018

| Districts | n | Pf +ve | Pv 210 +ve | Pv 245 +ve | Total +ve | Sporozoite rate % | Biting Rate | EIR |
|---|---|---|---|---|---|---|---|---|
| **Kheda** | 916 | 7 | 4 | 1 | 12 | 1.33 | 2.33 | 3.09 |
| **Panchmahal** | 900 | 3 | 1 | - | 4 | 0.45 | 1.05 | 0.472 |

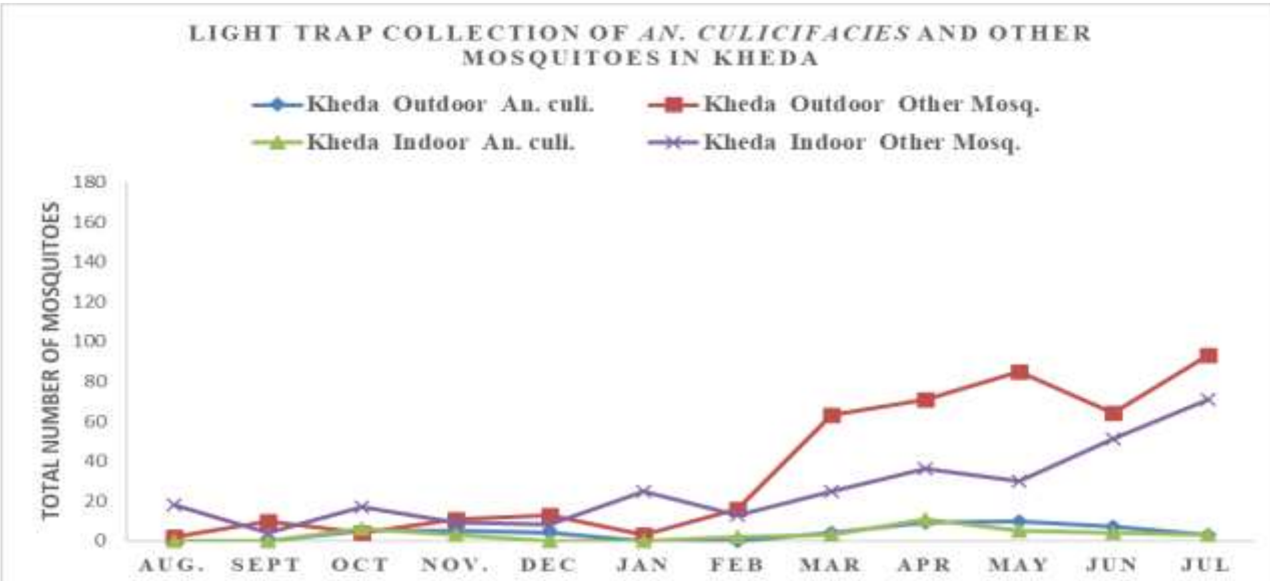


**Fig. 3.** *Anopheles culicifacies* s.l. and density of other mosquitoes per light trap indoors and outdoors in Kheda District from August 2017 to July 2018

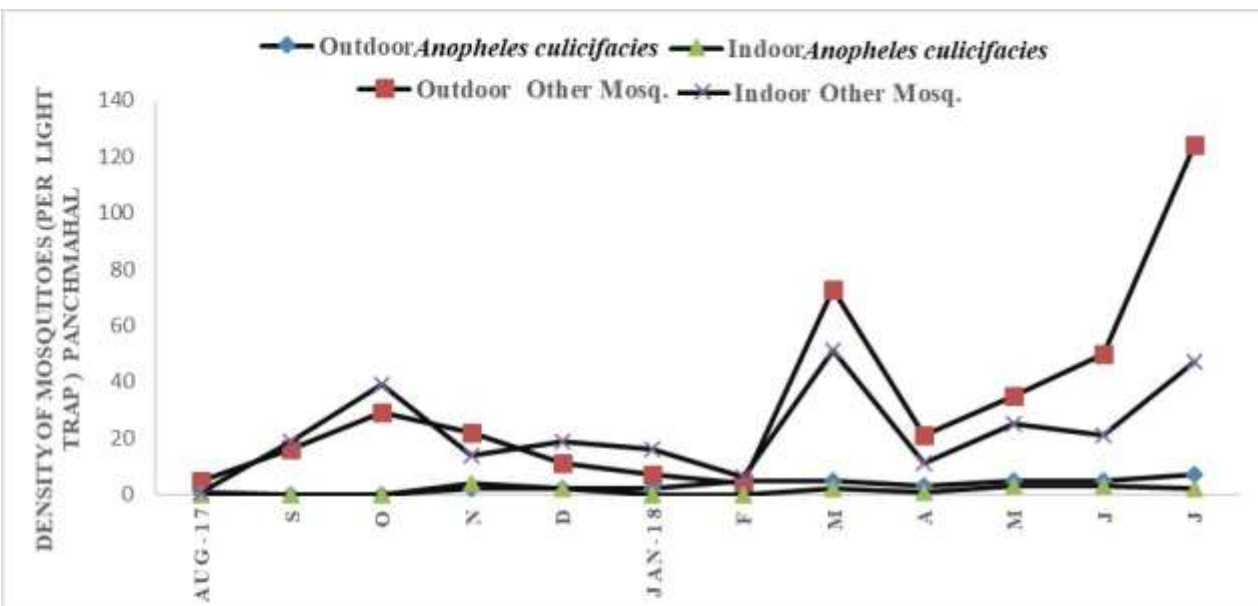


**Fig. 4.** The density of *Anopheles culicifacies* s.l. and other mosquitoes per light trap indoors and outdoors in the district of Panchmahal from August 2017 to July 2018



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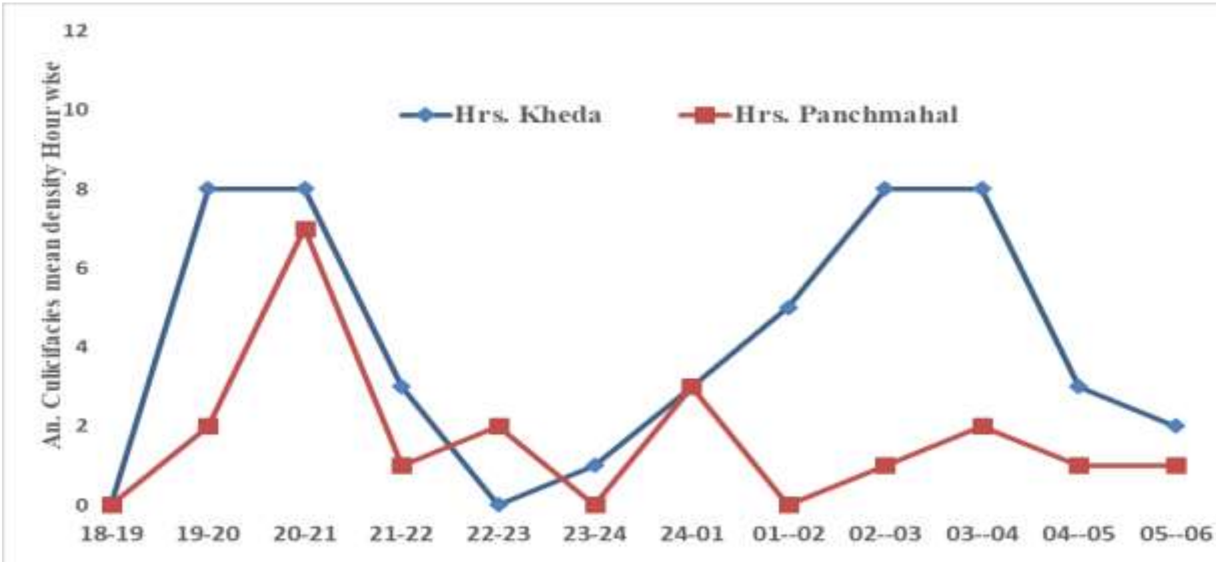


**Fig. 5.** Mosquito landing rates of *Anopheles culicifacies* s.l. on human baits from dusk to dawn from July 2017 to July 2018

## Discussion

The study was carried out in riverine and canal irrigated villages of Kheda and Panchmahal in July 2017 to July 2018, to update the information on bionomics of *An. culicifacies* s.1. in these districts of central Gujarat. In villages, the number of brick houses were more compared to mud houses, 50–60% of inhabitants reportedly slept indoors in all seasons. Such sleeping behaviour of inhabitants explains observed increased endophagic behaviour of mosquitoes. Using various collection methods, six *Anopheles* species were collected namely *An. culicifacies* s.1., *An. stephensi*, *An. fluviatilis* s.1., *An. subpictus* s.1., *An. annularis* and *An. vagus* which include three primary vectors and a secondary vector species (16–22).

In earlier studies in Kheda, varied prevalence of *An. culicifacies* s.l, *An. stephensi*, *An. annularis*, *An. subpictus* s.1., *An. aconitus*, *An. barbirostris*, *An. nigerrimus* and *An. tessellatus* was reported (16–22). Among anophelines, *An. subpictus* s.1. was predominant in human dwellings and cattle sheds. While observations from Jabalpur reported *An. culicifacies* s.1. as most predominant species followed by *An. Subpictus* s.1. (8). From outdoor habitats, *An. culicifacies* s.1., *An. subpictus* s.1. and *Culex* spp. could be captured, and similar observations were made in earlier studies conducted at Kheda District (14–22). The previous studies have reported large numbers of *An. culicifacies* resting in outdoor habitats in Gujarat (16, 42). On the contrary we found low outdoor resting densities of *An. culicifacies* s.l. throughout the study, probably due to non-availability of suitable resting habitats outdoors that were hot and humid due to changes in the local environment during last few decades. Hence, these observations indicate the need for suitable micro-climate niche for prevalence of mosquitoes either indoors or outdoors.

*Anopheles culicifacies* s.1. was prevalent throughout the year in both the districts in varying proportions. In our observations, the density of *An. culicifacies* s.1. started to build up in the month of February with the increase of temperature suitable for breeding and survival and reached to first peak in March thereafter it declined gradually by July, and with the onset of monsoons increase in density was observed with second peak in September and was similar to observations made in earlier reported studies in 1990s (17–18, 21), indicating no differences in seasonal prevalence in major vector, *An. culicifacies* s.l. in last few decades.

Light traps collections yielded *An. culicifacies* s.l., *An. stephensi*, *An. annularis*, *An. fluviatilis* s.1., *An. vagus*, *Aedes* and *Culex* in varied proportions. During post monsoon period, *An. culicifacies* s.1. density in trap were higher in both the districts. The density of other mosquito species in traps was nearly ten times more than the density of *An. culicifacies* s.l. which was like densities in studies in Madhya Pardesh (8). In study from Jabalpur, Madhya Pradesh, in light trap collection, *An. culicifacies* s.1., *An. fluviatilis*, *An. subpictus* s.1., *An. annularis*, *An. vagus*, *An. pallidus*, *An. splendidus*, *An. barbirostris* and *An. theobaldi* have been reported (8). In Bastar, Chattishgarh, in light trap catches, *An. culicifacies*, *An. subpict us* s.l., *An. vagus* and *An. annularis* were recorded (43).

In the Odisha State, *An. fluviatilis* s.l. and *An. culicifacies* s.l., the recognized primary vectors, and *An. aconitus*, *An. annularis*, *An. jeyporiensis*, *An. maculatus* and *An. varuna* the reputed secondary vectors of malaria in India (44).

However, significantly high number of mosquitoes were trapped in tribal villages of tribal Panchmahal District due to availability of large number of suitable mosquito breeding sites. Another possible reason could be low infrastructure development in these villages compared to villages in plain areas.

High proportion of fed and half-gravid + gravid *An. culicifacies* s.1. in indoor resting catches in human dwellings in riverine and irrigated villages in both the areas, exhibited endophilic and endophagic behaviour of *An. culicifacies* s.1. which was similar to observations in earlier reported studies in Kheda District in early 1990s (17) indicating no change in resting and feeding behaviour of mosquitoes inspite of regular use of insecticidal interventions and changes in housing.

The parous rate of *An. culicifacies* s.1. was nearly 30% in Panchmahal district in all the seasons. While, in Kheda District it was low in winter and summer and varied in different months ranging from the lowest in May to the highest in July. The prolonged ovarian cycle or multiple feeding during same cycle may be responsible for low parous rate in winter and summer. Another study from Kheda District reported overall parous rate of 38.2% during 1991–92 and 36.4% during 2000–2001 (14). An earlier study on seasonal prevalence of *An. culicifacies* s.1., estimated > 35% parous rate in Kheda District (22). The study clearly shows higher survivorship of *An. culicifacies* s.l. population in all the seasons in Panchmahal, owing to suitable climatic condition.

In both the districts, *An. culicifacies* sibling species ‘B’ was predominant but it was not involved in the malaria transmission in this area. Our study confirms the results of earlier study in central Gujarat (NIMR, unpublished report, 2009). The study conducted in 1990s in Kheda district also supports our results which reported > 60% sibling species B (14). These observations contrasted with the distribution in other districts. In Orissa an eastern part of India predominant species was *An. culicifacies* C (77.9%) followed by B (21.1%) and A (48%) (45–46). Earlier studies in Madhya Pradesh in central part of India also reported highest prevalence of *An. culicifacies* C (8, 47). In Chhattisgarh state, central India a study reported equal prevalence of sibling species B and C (48). In Uttar Pradesh, only *An. culicifacies* sibling species A and B were found with predominance of A (49). The results of the above reported studies are contradictory to our present study as in the eastern and central states of India, malaria is stable and most of the parts of state are hilly forested. *Anopheles culicifacies* A and C are known malaria vector in most part of the India (13, 50).

In Kheda District, the human biting activity of *An. culicifacies* s.1. was intense during post-monsoon (September to November) and



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Published Online: June 31, 2022

in Panchmahal (September and November) and low biting in summer in both districts. Similar seasonal shift in biting period was also reported in previous studies in early 1990s (17–18, 21). The biting occurred throughout the night with maximum biting in the first quarter and third quarter of night in villages of both districts, our observations agreed with previous reports from Gujarat (14, 18). The early night biting of *An. culicifacies* s.1. is concern in area where Long lasting insecticide nets (LLINs) is main component of vector control strategy.

The low anthropophagic index of *An. culicifacies* s.1. indicated high feeding preference to bovine blood which is like previously reported studies from Gujarat (12, 48, 51–53). anthropophagic index may be due to low cattle–human ration in Gujarat State (14). During summer, villagers were found sleeping outdoors close to cattle shed without protection against mosquito bites in both the districts. It has been observed by previous authors that indoor resting anopheline comes out at dusk, bite outdoor and enters indoor at dawn for resting (20). The present study shows that villagers having low cattle population are at greater risk of malaria transmission by *An. culicifacies* s.1 in these districts, despite zoophagic nature. This could be one of the reasons for the increased EIR in Kheda District.

*Anopheles culicifacies* s.1. was found possible resistance to alpha-cypermethrin in the both the districts which is also presently used for indoor residual spraying in this area for malaria vector control under the National Vector Borne Disease Control Programme. *Anopheles culicifacies* s.1. showed resistance to Malathion in both the districts. In Kheda district, it has developed resistance to Deltamethrin too. Another study from Kheda District reported resistance in *An. culicifacies* s.l. against DDT, Dieldrin and Malathion (14). Similarly, *An. culicifacies* s.1., was reported resistant to DDT, Malathion and Deltamethrin in Surat District of Gujarat in 2005–2006 (28). Extensive use of insecticides increased the resistance. Some of the point mutation in the voltage-gated sodium channel (NaV) was responsible for the resistance for the Pyrethroids and DDT insecticides (54). In the Indian subcontinent, the point mutation leading to Leu-to-Phe substitution in the voltage gated Na+ channel (VGSC) at residue 1014, a most common Knockdown resistance (kdr) mutation was reported in *An. culicifacies* s.l.-a major malaria vector which is responsible for resistance against DDT and Pyrethroids (55–57). The susceptibility of *An. culicifacies* s.1. status to insecticides reported multiple resistance including to Pyrethroids and could be a concern for vector control as the programme is still reliant on chemical insecticides mainly Pyrethroids. *Anopheles culicifacies* s.1. vector is resistant to multiple insecticides, and this underlines the need for development/use of new insecticides for management of insecticide resistance (58).

In present study, a low Anthropophilic index (AI) of *An. culicifacies* s.1. was recorded in both the districts revealing it's predominant zoophagic behaviour. Earlier studies carried out on host preference of *An. culicifacies* s.1. in India have recorded a wide range of variation in the anthropophily (13). However, there are evidence that feeding preference to human changes in different situations, a high AI in areas with high cattle population (14) or during epidemic period (59). Owing to zoophagic nature of *An. culicifacies* s.1., low sporozoite rate was recorded in both the districts. Earlier studies have observed varied degree of sporozoite rates in naturally infected *An. culicifacies* s.1. in India (12–14). Mean annual entomological inoculation rate was estimated to be low in both the districts. Similar observations were made from studies in central Gujarat have also estimated low EIR ranging 0.022 to 0.110 infective bite/person/annum in different periods (12, 14). In plain areas of Sundargarh District in Odisha where *An. culicifacies* s.1. plays main role in malaria transmission, EIR was estimated to 0.014 infective bites/ person/ night (44). Therefore, EIR estimates in our study indicate active malaria transmission in Kheda and Panchmahal Districts of Gujarat.

## Conclusion

For preparation of effective vector control strategy updated knowledge on vector biology and behavior is an essential requirement. Our present study generated important information on prevalence and behaviour of *An. culicifacies* s.l. and other attributes related to malaria transmission. The abundance, physiological conditions, and blood meal analysis of indoor resting *An. culicifacies* s.l. showed endophilic and endophagic behavior in both districts. In both the districts, its highest biting during monsoon and post monsoon coincides with malaria transmission season. We found *An. culicifacies* s.l. possible resistance to alpha-cypermethrin, currently used insecticide. Hence, indoor residual spray (IRS) with alpha-cypermethrin may be evaluated in other villages of the districts to control *An. culicifacies* s.l. in this area. Effectiveness of LLINs depends on site of use (indoor/outdoor) and on feeding time of biting hence early biting behaviour of *An. culicifacies* s.l. in this area is a cause of concern. Although, there have been changes in human housing and local ecology due to developmental activities in villages of both districts during last two decades in Gujarat, but our study clearly demonstrated no major change in behaviour of *An. culicifacies* s.l. Therefore, there is need for frequent monitoring and evaluation of vector control measures to achieve the elimination target of malaria in this area.

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## Ethical considerations

Institutional Ethical Committee approval was obtained vide letter no. ECR/NIMR/EC/2017/142 dated 21 June 2017.

A meeting with villagers was convened with the help of Sarpanch and other opinion leaders to apprise them about the purpose of the study. NIMR staff coordinated with the Primary Health Centre staffs, Medical Officer, Multipurpose health worker (MPHW) (male and female) and Accredited Social Health Activist (ASHA) to solicit the cooperation of the villagers during study. Informed consent of householders of selected sentinel sites for collection and of volunteer to be baits for landing mosquito collections were obtained prior to initiating studies.

## Conflict of interest statement

Authors declare that there is no conflict of interest.

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