

**BREEDING HABITATS OF *PLATYNECTES* SP. NOV. (COLEOPTERA: DYTISCIDAE),
PREDATORY TO *STEGOMYIA ALBOPICTA* AND *STEGOMYIA AEGYPTI* IN THE
RUBBER PLANTATIONS OF KERALA, INDIA.**

¹Adil Bashir, ²Saima Syed, ³N. Pradeep Kumar, ⁴Sheikh Afaq Gowhar

¹Department of Zoology, Govt. Degree College for Women Pulwama, Jammu and Kashmir, India.

²Department of Zoology, Govt. Degree College for Women Pulwama, Jammu and Kashmir, India.

³Vector Control Research Centre (ICMR), Puducherry.

⁴College of Public Health and Health informatics, University of Ha'il KSA.

*Corresponding Author: Dr. Adil Bashir

Department of zoology, Govt. Degree College for Women Pulwama, Jammu and Kashmir, India.

Article Received on 08/03/2020

Article Revised on 29/03/2020

Article Accepted on 19/04/2020

ABSTRACT

The breeding habitats of *Platynectes* sp. nov. was found in the rubber plantations located at foothills in the Western Ghats of Kerala. *Platynectes* beetle was also found to be prevalent in (Latex Collection Containers) LCCs, the key breeding habitats of *Stegomyia* mosquitoes in the rubber plantations. Rubber plantations are criss-crossed by streams which are maintained for irrigation purpose of the plantations. The natural habitat of the *Platynectes* sp. nov. was found to be these irrigation streams/pools. During the monsoon season these streams and pools have abundant of fresh water. Different variety of aquatic plants were abundantly growing in these streams and *Epiphyllum angulier* was found to be the commonest aquatic plant in these streams. Mostly the beetles were hiding under the leaf of these plants. Streams and pools have cool and high oxygenated water, during the monsoon season in the rubber plantations of Kerala. *Platynectes* beetles were found in both the pools and streams during the months, April to October (pre-monsoon and monsoon seasons). However in summer season these beetles were found to be less prevalent in the streams and pools. *Platynectes* beetles invade the rubber latex collection containers fixed to the trunks of the rubber plantations. The beetles flew from its original breeding sites of streams and pools to LCCs for feeding *St. albopicta* larvae present in these LCC cups. The beetles were found prevalent in LCCs from May to December months.

KEYWORDS: *Platynectes* sp. nov, *Stegomyia albopicta*, *Stegomyia aegypti*, Breeding habitats, Rubber Plantations of Kerala.

INTRODUCTION

Chikungunya virus (CHIKV), is transmitted to humans through *Stegomyia* mosquitoes. Kerala was the worst affected State in Indian 2007 with a total of 55.8% of the reported Chikungunya fever cases in the country from the State (NVBDCP, India). Almost all the districts of Kerala were affected with the infection during 2006-2007. The factors attributed to this outbreak were the crucial mutation "A226" acquired by the virus in 2007 and the abundance of the vector species *Stegomyia albopicta* in the region.^[1] *St. aegypti* and *St. albopicta*, the vectors for CHIKV were widely distributed and abundant during the pre and post monsoon season. The prevailing climate, terrain and agricultural practices in these districts were conducive for the breeding of *St. albopicta*/*St. aegypti*, the vector of Chikungunya and Dengue in Kerala. The worst affected region was the mid-highland regions which harbour the vast rubber plantation sectors in the State. The hilly and semi-forested districts of Kottayam and Pathanamthitta were the most affected districts during 2007 epidemic, which

constitutes the major portion in the rubber plantation sectors of Kerala. About 63.0% of people living in the rubber plantation areas (Kerala contributes 80.0% of the rubber production in India), was afflicted with this disease^[2], *St. albopicta* acted as main vector species.^[1]

Incidence of Dengue fever, another mosquito borne arbo-viral disease is also on an increasing trend in Kerala.^[3] *St. albopicta* (Skuse) 1894 (Diptera: Culicidae), the predominant *Stegomyia* species prevalent in Kerala was recorded as the vector species of both these arbo-viral diseases.^[1, 4] Innumerable Discarded Latex Collection containers (DLCCs), unused Fixed Latex Collection Containers (FLCCs), and tree holes, leaf axils of pine-apple plants and fallen leaves of areca-nut tree etc. were recorded the key breeding habitats of this species in rubber plantation sectors in Kerala.^[2, 5]

Dengue a vector – borne and emerging infectious disease is estimated to affect 50-100 million individuals each year in tropical and subtropical areas.^[6, 7] Demographic

and societal changes, such as population growth, unplanned urbanization, microevolution of the virus, climatic change and modern transportation, have greatly contributed to the increased incidence and geographical spread of Dengue virus infection in recent decades and distributed among about 120 countries globally. Dengue was first recognized in the 1950s during its epidemic in the Philippines and Thailand.^[8] Dengue is caused by four serologically distinct types of Dengue virus. DENV-1, DENV-2, DENV-3 and DENV-4, belonging to the family *Flaviviridae*, genus *Flavivirus*. The viruses are transmitted to human beings through the bite of *Stegomyia* mosquitoes such as *St. aegypti* and *St. albopicta* which are considered as primary and secondary vectors of Dengue respectively.^[8] The incidence of Dengue fever has increased significantly around the world in recent decades. About half of the global population lives under the risk of infection of this arboviral disease.^[9] *St. albopicta* is becoming an increasingly important vector of Dengue because of its rapidly changing global distribution^[10], and it is generally believed to be a less efficient vector of arboviruses than *St. aegypti*. *St. albopicta* is highly adaptive and therefore can survive even in cooler temperate regions. Its spread to new areas is due to its tolerance to temperatures below freezing, hibernation and ability to shelter in microhabitats.^[8] *St. albopicta* is an aggressive day-biting species known in different parts of the world including South East Asia^[11], and Southern China.^[12] Worldwide trade in second hand tires transported to various places, which often contain water could be an ideal place for eggs and larvae of these mosquitoes, has been a key factor in the large-scale conquest of *St. Albopicta* in new areas which easily adapts to new environments, even in a temperate climate. This expansion is creating new opportunities for viruses to circulate in new areas, becoming a common cause of epidemics in *St. aegypti*-free countries from Hawaii to Mauritius. However *St. albopicta* is considered as an inefficient vector of Dengue because not well adapted to urban domestic environments and is less anthropophilic than *St. aegypti*. The Asian tiger mosquito (*St. albopicta*) belongs to the East and Southeast Asia, where it originally lived at the edges of forests, breeding in tree hole, containers and other natural reservoir.^[10]

The entire population of India is under the risk of Dengue. In India the first case of Dengue has been reported from Kolkata (previously Calcutta) during 1963. The number of Dengue cases had been an increasing trend since 2001 and the maximum number of suspected cases (28055) has been recorded during 2010. In Kerala Dengue fever was first recorded in Kottayam District in 1997.^[13] This district continues to contribute the maximum number of cases of Dengue fever in the State, next to Trivandrum District in the south Kerala every year. Topographically, Kottayam District is semi-forested region with hills and hillocks located at the foothills of the Western Ghats. This district is the abode of rubber plantations in the country and grows rubber in

about 60% of its net sown area under agriculture. *St. albopicta* the vector of Dengue fever, is abundant throughout the plantation belt of Kerala, including Kottayam District.^[2] All the four Dengue serotype are prevalent in the State, however, Dengue 2 and 3 are the major serotypes prevalent in Kerala State.^[3]

Rubber plantations in the region could be classified as immature ones and mature ones based on the age and productivity of the trees, broadly. Latex collection is carried out by placing hemispherical plastic containers of either 450ml/600ml/900ml capacity, attached to the bark of the mature trees about 7 years old. A small portion of bark of the tree is removed in a horizontal oblique manner by a sharp knife in an interval of 2-5 days and the latex oozing out is collected into the containers fitted to the tree. Also rain guards are fitted to the latex yielding section of the bark to prevent rainwater interfering with the tapping process and collection of latex. The latex oozing out of the scar is removed about 3 hours after making the cuts in the bark. The container is kept in the tree itself after latex collection in upright position to collect the residual latex.

Platynectes beetles are predatory insects inhabiting streams and riverine swamps as well as in irrigation ditches^[14]. They also inhabit springs and streams in foothill and lower mountain rainforests. Exposed streams and pools in peat and grasslands are occupied at higher altitudes. The known altitudinal range is from 300 to 2000 m.^[15] *Platynectes* sp. nov. were recorded from the key breeding habitats of *St. albopicta*, in fixed latex collection containers and discarded latex collection containers from rubber plantation area of Aimcompu, Kottayam District (09°46.77' N; 76°41.113' E). Field surveys in other two study areas also recorded the natural occurrence of this beetle species in Pampady (09°32.457' N; 76°38.952' E) in Kottayam District and Chethackal (09°26.241' N 76°48.440' E) in Pathanamthitta District. The prevalence, density and predatory role of the *Platynectes* beetles in the rubber plantations affects the density of immatures of *St. albopicta* in the latex collection containers of rubber plantations of Kerala.

MATERIALS AND METHODS

An exploratory survey was carried out to find the natural habitats of the beetle in the rubber plantations, showed that the beetles were abundant in fresh water pools and streams amidst the plantations (**Fig 1 and 2**). Surveys were carried out on fortnightly intervals in these natural habitats by taking dips separated by about 10m for studying the population of the beetles from these ponds. Different tools were used for the surveys. Two types of dippers and enamel trays were used for the survey of beetles in the natural habitats, long enamel dipper with '5' feet ladle length with capacity of 1 litre and small enamel dipper with '2' feet ladle length capacity of half a litre capacity.^[16] Long enamel dipper were used in the stream sides with water depth more than '2' metres and

small enamel dip were used in the pools with water depth less than '2' meters. Beetles were collected from pools along with the water by using these dippers. After collecting from the pools and streams by using the dippers, these beetles were transferred to the enamel trays with length of '1' X '1' and '2' X '2' ft along with water. The beetles were observed in the enamel trays and poured into the containers with capacity of 500 ml of water and were transported to the laboratory.^[16, 17]

As these beetles invade in latex collection containers (LCCs), both fixed latex collection containers (FLCCs) and discarded latex collection containers (DLCCs), which are 3-5 meters above the ground level, containing various stages (first to fourth instars) of the mosquito larvae, acts as key breeding habitats of *St. albopicta* in the rubber plantations. Surveys were carried out in the 3 study areas in Kerala State, for a period of one year to understand its population parameters.

Population density of beetles in the latex collection containers was evaluated once in fortnightly in all the three study areas, Aimcompu, Chethackal and Pampady. Fixed latex collection containers and discarded latex collection containers in rubber plantations were surveyed to find out the beetle density. The number of wet

containers which are positive for beetles in the study areas were observed. The whole content of water from positive FLCCs and DLCCs were transferred to the enamel trays for observing the density of the beetles in each containers.^[16] Enamel trays and pipettes were used for the study of population density of the beetles in the rubber plantations.

The study was continued for a year covering 200 rubber trees were covered in each village, once in a fortnight. These were selected in 4 radials from a fixed point, covering about 50 trees on a radial.^[18] After completing the first row up to 50 trees (latex collection containers), second row of trees was surveyed in the opposite direction of the first row. The distance in all the four directions was same, as each rubber tree is fixed at same distance from each other. The trees were marked during the observation every fortnightly. Next fortnight survey, the density of the *Platynectes* sp. nov. were observed in the next field area which were not surveyed in the previous survey. The total area covered using this sampling procedure was about 20 ha of rubber plantations in each village. These three villages were selected as these were worst affected by Chikungunya outbreak during 2007 and they represented both large and small scale rubber plantations.



Fig. 1. Fresh water pool in the study area Aimcompu in the rubber plantations of Kerala.



Fig. 2. Fresh water pool in the study area Chethackal in the rubber plantations of Kerala.

RESULTS AND DISCUSSION

Natural habitats of *Platynectes* sp. nov.

Platynectes beetle was found to be prevalent in LCCs, the key breeding habitats of *Stegomyia* mosquitoes in the rubber plantations. Rubber plantations are criss-crossed by streams which are maintained for irrigation purpose of the plantations. The natural habitat of the *Platynectes* sp. nov. was found to be these irrigation streams/pools. During the monsoon season these streams and pools have abundant of fresh water. Different variety of aquatic plants were abundantly growing in these streams and *Epiphyllum angulier* was found to be the commonest aquatic plant in these streams. Mostly the beetles were hiding under the leafs of these plants. Streams and pools have cool and high oxygenated water, during the monsoon season in the rubber plantations of Kerala. *Platynectes* beetles were found in both the pools and streams during the months, April to October (pre-monsoon and monsoon seasons). However in summer season these beetles were found to be less prevalent in the streams and pools.

Platynectes beetles invade the rubber latex collection containers fixed to the trunks of the rubber plantations. The beetles flew from its original breeding sites of streams and pools to LCCs for feeding *St. albopicta* larvae present in these LCC cups. The beetles were found prevalent in LCCs from May to December months.

Prevalence of *Platynectes* sp. nov. in the rubber plantations

Seasonal distribution

The seasonality of *Platynectes* beetles in the rubber plantations were studied in all the three study areas. It was found that the prevalence rate of *Platynectes* beetles in the LCCs vary through different months, ranging from 1.0- 49.62 percentage (%) in the study areas. Highest prevalence of beetles in the latex collection containers were recorded from Aimcompu village (49.62%), during August month with a rainfall of 464.2 mm (peak monsoon season yearly). The average highest prevalence rate of beetles (22.68%) from all the study areas was recorded during the month September in the southwest monsoon season. The highest and maximum prevalence rate was recorded during the monsoon season. Beetles were not found prevalent in the containers from January to April (summer season) in LCCs in all the three study areas. The re-occupation of beetles in containers commenced during the month of May pre monsoon season, when intermittent rainfall ensued prior to the onset of monsoon season. The minimum prevalence rate of beetles in the latex collection containers recorded during the month of June in all the study areas, as shown in **Table 1**. However the average minimum prevalence rate of beetles in LCCs recorded during the month of May (May month is the transition between summer and monsoon seasons).

The prevalence (%) of wet containers (which supports the prolific breeding of *St. albopicta* in the rubber plantations) in Aimcompu ranged from 3 to 60 %, Chethackal area contributed 1 to 89 % of wet containers

and Pampady area contributed 2 to 70 % of wet containers through different months. The prevalence (%) of *St. albopicta* pupae in the LCCs ranged from 1-5 %, in all the three study areas.

Temporal Distribution

The prevalence of beetles increases with the increase of rainfall in the pre monsoon and monsoon seasons. In Kerala, pre monsoon showers starts from April–May months which contributes 25-30% of rainfall, southwest monsoon contributes nearly half 50% of rain and northeast monsoon contributes 20% annually. The prevalence of the beetles starts form pre monsoon showers and reaches to maximum during southwest monsoon and again declines to minimum during northeast monsoon in the breeding habitats of *St. albopicta* LCCs. This indicates that the prevalence of

Platynectes beetles increases with the increase of rainfall in the rubber plantation sectors. The prevalence of beetles in the latex collection containers through different months in the rubber plantation sectors is given in Fig. 3, Fig. 4 and Fig. 5. Beetles were found in Aimcompu, Chethackal and Pampady areas during the months, May to November, June to October and June to December respectively. From June to September southwest monsoon is active with an average rainfall 1434.5 mm (annual rainfall 2911 mm) and relative humidity 63.89-87.92 in the Kerala State. During this time period we recorded the highest prevalence (%) of beetles in the all three study areas (Table 1). Prevalence of *Platynectes* beetles recorded from Pampady area during pre-monsoon of month May and from Aimcompu area post monsoon month of December.

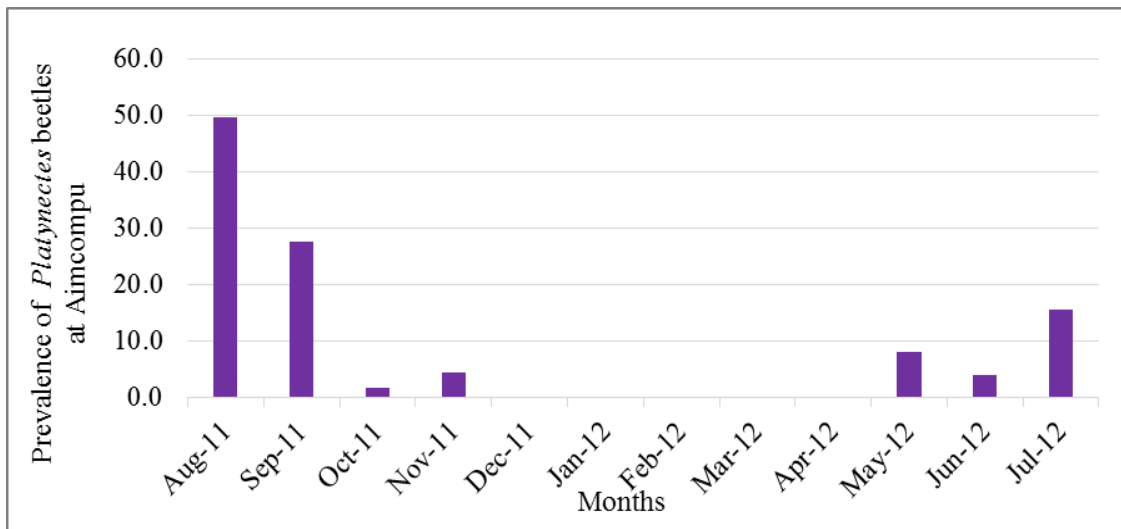


Fig. 3. Prevalence of *Platynectes* beetles at Aimcompu study area through different months.

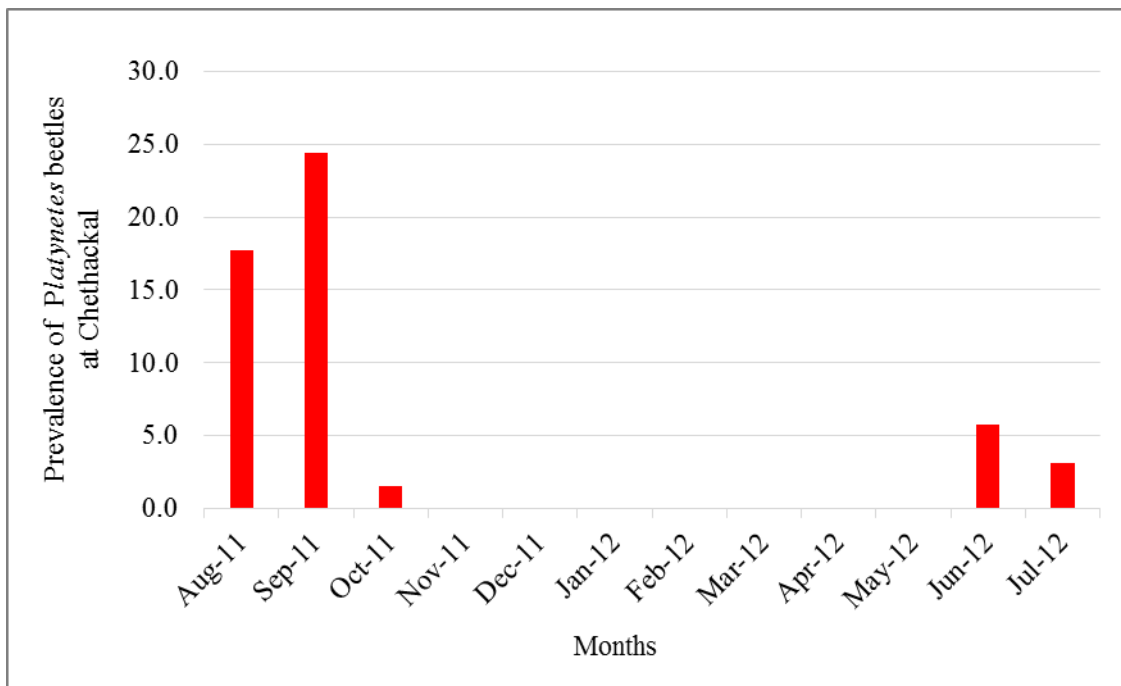


Fig. 4. Prevalence of *Platynectes* beetles at Chethackal study area through different months.

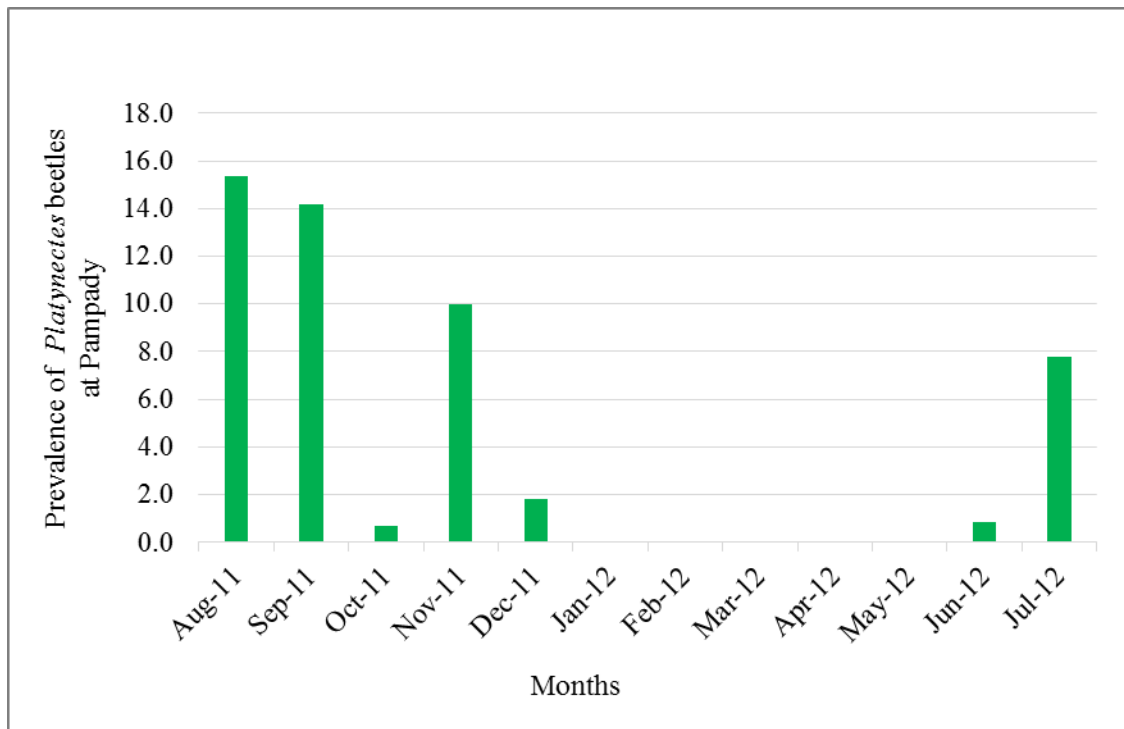


Fig. 5. Prevalence of *Platynectes* beetles at Pampady study area through different months.

Density of *Platynectes* sp. nov. in the rubber plantations

Seasonal distribution

The seasonal density of *Platynectes* beetles in the rubber plantations was studied in all the three areas. *Platynectes* beetles were found in the latex collection containers in the rubber plantations and the population density of the beetles per latex collection container was found to be maximum during the south-west monsoon season which contributes 50% rainfall annually rubber plantations of Kerala. It was found that the maximum density of the beetles per latex collection container vary in different months, ranging from 1.0-3.8 per latex collection container in all the study areas. Maximum density was recorded at Aimcompu (3.8) during the month of September. The average density of *Platynectes* beetles per LCC was found (2.34) from all study areas, during the monsoon season. Beetles were not found in LCCs from January to April which contributes only 272 mm of rainfall (annual rainfall 2991 mm). The minimum density of beetles per latex collection container was recorded in the study areas, Aimcompu and Chethackal from months of November and October respectively (northeast monsoon which contributes 20% of rainfall) as shown in **Table 2**. However minimum density of beetles per LCC in study area Pampady was recorded from months of June, October and December (during these months 1 beetle per LCC). During pre-monsoon month of May and post monsoon month of December, density of *Platynectes* beetles were recorded from Pampady and Aimcompu area. The beetles were found predatory on *St. albopicta* larvae abundant in the rubber plantations.

Temporal Distribution

The density of *Platynectes* beetles per latex collection container increases with the increase of rainfall in the monsoon seasons, starts from pre monsoon and reaches to maximum during southwest monsoon and again declines to minimum during northeast monsoon. The highest density of the beetles recorded in the all three study areas in the rubber plantation sectors of Kerala (**Table 2**) during June to September (southwest monsoon with an average rainfall 1434.5 mm (annual rainfall 2911 mm)). The population density of *Platynectes* beetles per LCC in all the three study areas through different months in the key breeding habitats of *St. albopicta* in the rubber plantations sectors of Kerala is shown in **Fig. 6, Fig. 7 and Fig. 8**. The density of beetles in the LCCs were found during the months of May to November, June to October and June to December in Aimcompu, Chethackal and Pampady respectively.

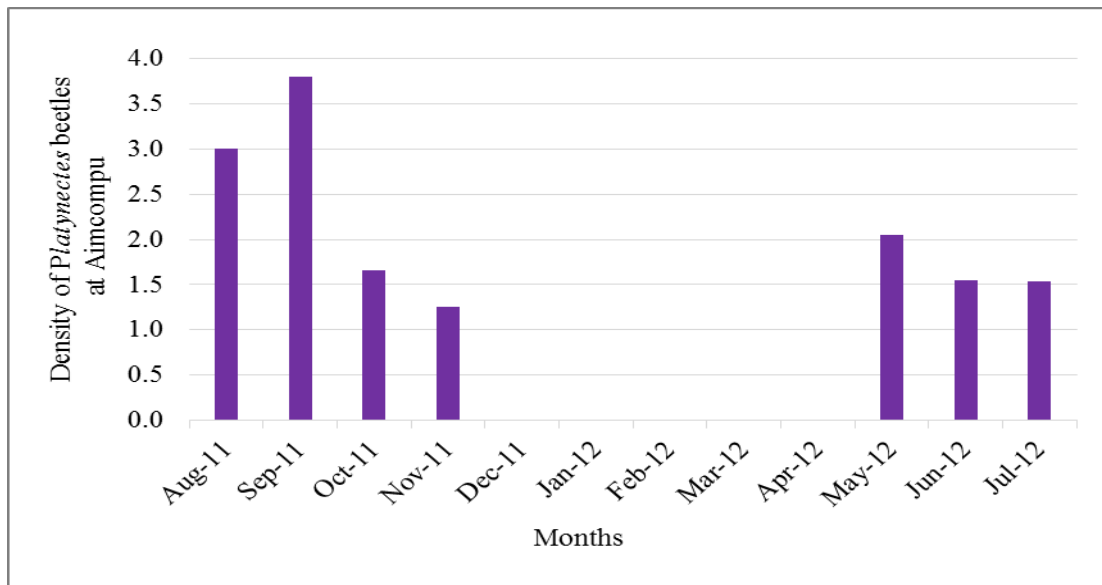


Fig. 6. Density of *Platynectes* beetles at Aimcompu study area through different months.

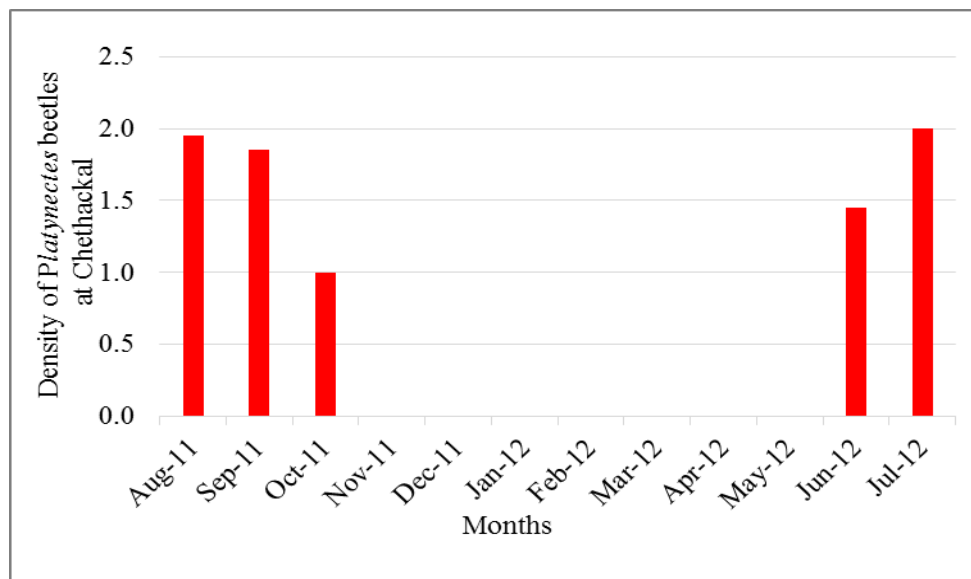


Fig. 7. Density of *Platynectes* beetles at Chethackal study area through different months.

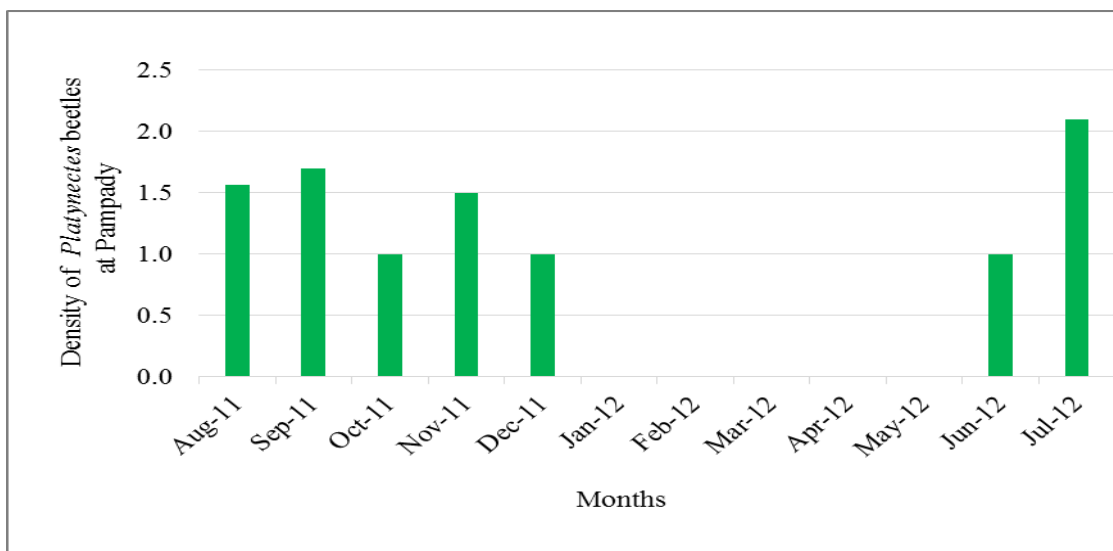


Fig. 8. Density of *Platynectes* beetles at Pampady study area through different months.

Table 1. Prevalence of *Platynectes* beetles in rubber plantation areas of Kerala through different months.

Months	Prevalence of <i>Platynectes</i> beetles (%)			
	Aimcompu	Chethackal	Pampady	Average
Aug-2011	49.62	17.76	15.38	22.63
Sep-2011	27.48	24.39	14.18	22.68
Oct-2011	1.76	1.50	0.68	11.06
Nov-2011	4.39	0	10.00	1.92
Dec- 2011	0	0	1.81	1.75
Jan- 2012	0	0	0	0
Feb- 2012	0	0	0	0
Mar-2012	0	0	0	0
Apr-2012	0	0	0	0
May-2012	8	0	0	1.23
Jun-2012	4.05	5.76	0.83	2.66
Jul- 2012	15.59	3.12	7.80	14.28

Table 2. Density of *Platynectes* beetles in rubber plantation areas of Kerala through different months.

Months	Density of <i>Platynectes</i> beetles per positive container			
	Aimcompu	Chethackal	Pampady	Average
Aug - 2011	3.00	1.95	1.57	2.27
Sep - 2011	3.80	1.85	1.70	2.34
Oct - 2011	1.66	1.00	1.00	1.40
Nov - 2011	1.25	0	1.50	1.60
Dec - 2011	0	0	1.00	1.00
Jan - 2012	0	0	0	0
Feb - 2012	0	0	0	0
Mar - 2012	0	0	0	0
Apr - 2012	0	0	0	0
May - 2012	2.05	0	0	2.05
Jun - 2012	1.55	1.45	1.00	1.47
Jul - 2012	1.54	2.00	2.10	1.75

Presence of suitable habitats like streams, fresh water pools either permanent or temporary in the hot spot of biodiversity rich area rubber plantations semi forested areas may be one clue to evolution of Dytiscidae beetle fauna in this region. Also the presence of different monsoon seasons southwest and northeast with annually rainfall 2991 in the rubber plantation sectors of Kerala State may be the indication to the evolution of this *Platynetes* species in the hot spot of biodiversity regions. The habits of *St. albopictus* is also prevalent in the semi-forested and forested areas. Due the same type of habits like fresh water pools and fresh water LCCs provides suitable relation between predators and pry. The

conservation of these natural habitats in the rubber plantation sectors may open new avenues for further research, and conservation of this new species of Dytiscid beetle in these semi forested areas, located at the foot hills of Western Ghats (also known as hot spot of biodiversity, UNESCO).

The natural prevalence of *Platynectes* beetles recorded in the key breeding habitats of *St. albopicta* in the rubber plantations of Kerala during the fortnightly surveys in the study areas Aimcompu, Chethackal and Pampady is shown as **Table 3.**,**Table 4.**and**Table 5.** respectively.

Table 3. Natural prevalence of *Platynectes* beetles in the study area Aimcompu throughout one year.

Months	Number of wet container (LCCs)	Number of containers (LCCs) with beetles	Total number of beetles in containers (LCCs)
Aug-2011	135	67	201
Sept-2011	131	36	137
Oct-2011	240	6	10
Nov-2011	91	4	5
Dec-2011	0	0	0
Jan- 2012	15	0	0
Feb- 2012	0	0	0
Mar-2012	195	0	0

Apr- 2012	0	0	0
May-2012	75	6	15
June-2012	222	9	14
July-2012	109	16	23

Table 4. Natural prevalence of *Platynectes* beetles in the study area Chethackal throughout one year.

Months	Number of wet container (LCCs)	Number of containers (LCCs) with beetles	Total number of beetles in containers (LCCs)
Aug-2011	356	63	123
Sept-2011	335	81	150
Oct-2011	200	3	3
Nov-2011	0	0	0
Dec-2011	2	0	0
Jan- 2012	0	0	0
Feb- 2012	0	0	0
Mar-2012	0	0	0
Apr- 2012	0	0	0
May-2012	0	0	0
June-2012	156	9	13
July -2012	160	40	67

Table 5. Natural prevalence of *Platynectes* beetles in the study area Pampady throughout one year.

Months	Number of wet container (LCCs)	Number of containers (LCCs) with beetles	Total number of beetles in containers (LCCs)
Aug-2011	260	40	63
Sept-2011	141	20	34
Oct-2011	232	1	1
Nov-2011	20	2	3
Dec-2011	155	1	1
Jan- 2012	8	0	0
Feb- 2012	280	0	0
Mar-2012	80	0	0
Apr- 2012	264	0	0
May-2012	18	0	0
June-2012	218	1	1
July-2012	165	6	13

CONCLUSION

A new species of *Platynectes* beetle (Family: Dytiscidae), predatory to *Stegomyia albopicta* was recorded in the present study, prevalent in the fresh water pools and streams amidst rubber plantations of Kerala State. These beetles migrated from their original breeding sites (water pools and streams), to the Latex Collection Containers (LCCs) fitted to the rubber trees, the key breeding habitats *St. albopicta* and fed voraciously on larvae of these mosquitoes during rainy season. The beetle population was found abundant in LCCs from May to December months and it indeed played a significant role in the control of the vector population in the rubber plantations of Kerala. The density of *Platynectes* beetles ranged from 1.0-3.8 in the study areas and average density was recorded to be 2.34 per latex collection container. The population density was found to be maximum during September (southwest monsoon season) in the LCCs.

The present study clearly indicated that when beetle density was high, the vector population density was low and vice versa. The peak density of the *St. albopicta* was recorded, when *Platynectes* beetles were not found in the LCCs. A negative linear correlation ($r = - 0.589$, $p = 0.048$) between *Platynectes* beetle density and *St. albopicta* density was recorded in the rubber plantation sectors of Kerala. Natural prevalence rate (22.68%) of the beetles was found to be maximum during September (southwest monsoon season) in LCCs and varied from 1.0- 49.62 in all the study areas. Highest prevalence of the beetle was recorded in Aimcompu village (49.62%) in the plantation sector. Beetles were not found prevalent in the LCCs from January to April. The re-occupation of beetle in containers commenced during the month of May, when intermittent rainfall ensued prior to the onset of monsoon season.

ACKNOWLEDGEMENT. The authors are grateful to Mr. P. M. Ajithlal, Vector Control Research Centre Field

Station, Kottayam Kerala, for technical and field assistance rendered.

REFERENCES

1. Kumar NP, Joseph R, Kamaraj T, Jambulingam P. (2008). A226V mutation in virus during the 2007 chikungunya outbreak in Kerala India. *J. Gen Virol.*, 89: 1945-1948.
2. Kumar NP, Suresh A, Vanamail P, Sabesan S, Krishnamoorthy K, Mathew J, Jose VT., Jambulingam P. (2011). Chikungunya virus outbreak in Kerala, India, 2007. A sero-prevalence study. *Memórias do Instituto Oswaldo Cruz* 106: 912-916.
3. Kumar NP, Jayakumar RP, George K, Kamaraj T, Krishnamoorthy K, Sabesan S, Jambulingam P. (2013). Genetic characterization of dengue viruses prevalent in Kerala State. *J. Med. Microbiol.*, 62: 545-552.
4. Thenmozhi V, Hiriyani JG, Tewari SC. (2007) Natural vertical transmission of dengue virus in *Aedes albopictus* (Diptera: Culicidae) in Kerala, a southern Indian state. *Jpn J Infect. Dis.* 60: 245-249.
5. Sumodan PK. (2003). Potential of rubber plantations as a breeding source for *Aedes albopictus* in Kerala India. *Dengue Bull.*, 27: 197-198.
6. Gubler DJ. (2006) Dengue/dengue haemorrhagic fever: history and current status. In *New Treatment Strategies for Dengue and Other Flaviviral Diseases*, Novartis Foundation Symposium 277, pp. 3–16.
7. Halstead SB. (2007) Dengue. *The Lancet* 370, 1644–1652.
8. WHO (2015). Dengue and severe dengue. Fact sheet N^o 117, updated 2015. www.who.int/mediacentre/factsheets/fs117/en/
9. WHO (2009) *Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control*. Geneva: World Health Organization, pp. 1-17.
10. Russell PK, Gould DJ, Yuill TM, Nisalak A, Winter PE. (1969). Recovery of dengue-4 viruses from mosquito vectors and patients during an epidemic of dengue haemorrhagic fever. *Am. J. Trop. Med. Hyg.*, 18: 580-583.
11. Qui F, Zhang H, Shao L Li X, Luo H, Yu Y. (1981). Studies on the rapid detection of dengue virus antigen by immunofluorescence and radioimmunoassay. *Chin Med J*, 94: 653-658.
12. Rezza G. (2012) *Aedes albopictus* and the re-emergence of Dengue. *BMC Public Health* 12:72. <http://www.biomedcentral.com/1471-2458/12/72>.
13. Kalra N L, Prasittisuk C. (2004). Sporadic prevalence of DF/DHF in the Nilgiri and Cardamom hills of Western Ghats in South India: is it a seeding from sylvatic dengue cycle – a hypothesis. *Dengue Bull.*, 28: 44–50.
14. Balke M, Jach MA., Hendrich L. (2002). Latest version, order Coleoptera, Department of Entomology. The Natural History Museum, Cromwell Road, London SW7 5BD- 15/02/2002, pp. 1-42.
15. Hendrich L, Balke B. (2000). The Genus *Platynectes* Regimbart in the Moluccas (Indonesia) taxonomy faunistics and zoogeography. *Koleopterol. Rundschau*, 70: 37-52.
16. Servic MW. (1976). Mosquito ecology—field sampling methods. Elsevier Applied Science Publishers Ltd., London, pp. 43-62.
17. Nilsson AN, Soderberg H. (1996). Abundance and species richness patterns of diving beetles (Coleoptera, Dytiscidae) from exposed and protected sites in 98 northern Swedish lakes. *Hydrobiologia*, 321: 83-88.
18. Southwood TRE. (1978). *Ecological methods with particular reference to the study of insect population*, second edition, University Printing House Cambridge, pp. 1-218.