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Research Note

Bdelloid rotifer, *Philodina* species in the breeding containers of *Aedes aegypti* and *Aedes albopictus*

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Abstract. The vector mosquitoes of dengue and chikungunya fever, *Aedes aegypti* and *Aedes albopictus* have adapted to feed on humans and undergo larval and pupal development in natural and artificial freshwater collections. Although several studies reported, still, much information is required to understand the successful survival of *Aedes* mosquitoes in small temporary containers. In an investigation conducted in the chikungunya affected areas of Kerala state, India, the presence of Bdelloid rotifer, *Philodina* in 95% of breeding habitats of *Ae. aegypti* and *Ae. albopictus* was recorded. The role of *Philodina* in the breeding containers was investigated. It was found that while in control the number of *Philodina* was found increasing in the water sample during the study period of seven days, the number found decreased in the containers with larvae of *Aedes*. The gut content analysis also confirmed the presence of the rotating wheel, corona of *Philodina* in some of the specimen suggests its role as major larval food.

The vector mosquitoes of dengue and chikungunya fever, *Aedes aegypti* and *Aedes albopictus* have adapted to feed on humans and undergo larval and pupal development in natural (e.g. rock pools, tree holes, leaf axils) and artificial (e.g. water tanks, blocked drains, decorative pots and discarded tyres and food/beverage containers) freshwater collections in the urban and peri-urban environment (Ramasamy *et al*., 2011). The thriving mosquito larvae feeds on micro-organisms such as bacteria, cyanobacteria, fungi, protozoans, unicellular algae and diatoms which are present with or without detritus particles (Pumpuni *et al*., 1996, Thiery *et al*., 1991; Donmez *et al*., 1999; Ponnusamy *et al*., 2008). But still, much information is required to understand the successful survival of *Aedes* mosquitoes in small and temporary water collections. An investigation was carried out during the chikungunya outbreak of 2007 in Kerala State of India, to analyze the microbial diversity in both natural and artificial water collections in which *Ae. aegypti* and *Ae. albopictus* were breeding. Among the total 63 water samples collected, 36 were from Velanod, Nemum and Vizhinjam areas of Thiruvananthapuram district and 27 were from 9 villages belongs to three PHC *viz*. Puduserry, Vadagarapathy and Pudur of Palaghat District, Kerala State, India. The collected samples were brought to, and maintained at Centre for Research in Medical Entomology (CRME), Madurai, India for analysis. The major containers from where samples were collected included cement cisterns, plastic containers, discarded tires, grinding stones, plastic pots, metal containers etc. A total of 33 out of 36 samples from Thiruvananthapuram district

and all the 27 samples from Palaghat district were found positive for *Philodina* sp. (positivity 95.2%).

Placed in the class Bdelloidea (phylum Rotifera), which is Greek for leech, members of the genus *Philodina* includes some of the smallest metazoans ranging from 0.1 to 1 mm in length, most are microscopic, lives in freshwater, and free swimming. With two anterior rotating wheel organs referred to as coronas, *Philodina* can move like leeches or inchworms, extending and contracting as they crawl over aquatic plants and detritus. The same individuals can contract, extend their coronas, can create water current to bring the prey towards rostrum. This tiny animal superficially resembles ciliate protozoans in size and behavior but is multicellular, with about 1000 cells each (Melone & Ricci 1995). Although a rich microbial diversity was observed (Table 1), the presence of *Philodina* in almost all the containers (95.2%) made us to investigate its role further.

An experiment was undertaken to determine the role of *Philodina* in the breeding containers of *Aedes* mosquitoes. In a two sets of three (3+3) 500 ml glass beaker, 250 ml of water samples which was collected from the breeding places of *Ae. aegypti* and *Ae. albopictus*, were taken and initial load of *Philodina* sp was noted as day 1. Then 25 fourth instar larvae of *Ae. albopictus* was placed in each beakers of first set and named set A, similarly, *Ae. aegypti* was placed in set B and reared under laboratory conditions (28±2ºC, 70–80% R.H.). Next day, the larvae were removed and the number of *Philodina* was calculated and a fresh set of fourth instar larvae was added in each beaker and the experiment was continued for a total of seven days. A third set of water samples with *Philodina* was maintained in three beakers as control, in which the larvae were not added. The counting of *Philodina* was carried out as suggested by Wallace & Ricci (2002) by taking 1 ml of thoroughly mixed water sample and centrifuging it at 1000 rpm for ten minutes. The supernatant was discarded and the pellet was mixed with 100 µl of normal saline. This sample was then charged in a Neubauer counting chamber (hemocytometer) and the number of *Philodina* was counted at 10× objective of light microscope. The mean number of *Philodina* was calculated in each set (A, B and control) and a graph was drawn using Microsoft Excel (Fig. 2). The removed larvae were dissected and the gut contents were observed under 10× and 40× objectives of light microscope for the presence of *Philodina*.

Table 1. The microbial diversity observed in different containers

S. No.	Organism	No. containers positive
1	Gram positive bacteria	63
2	Gram Negative bacteria	23
3	Yeast	13
4	Molds	05
5	Paramecium species	06
6	Amoeba species	03
7	Diatoms species	46
8	Oscillatoria species	15
9	Chroococcus species	12
10	Merismopdia species	09
11	Chlamidomonas species	02
12	Spirulina species	04
13	Philodina species	60

There was sequential reduction in the number of *Philodina* noted every day in the samples after the release of *Aedes* larvae (Fig. 2). The consumption of *Philodia* was found almost equal for *Ae. aegypti* and *Ae. albopictus*. Although there were no intact *Philodina* found in the gut contents, the fragments of rotating wheel of *Philodina* (coronas) were found in four specimens. In contrast, the number of *Philodina* found slightly increased everyday in control when compared with the 1st day.

The present study suggests the role of *Philodina* sp. as larval food in the breeding habitats of *Ae. aegypti* and *Ae. albopictus*. As the *Philodina* feeds on bacteria, yeasts, diatoms, protozoans and cyanobacteria, it might be acting as a bio-mass magnifier of larval food. The magnified larval food might easily be consumed by the larvae particularly of 4th instar. In literatures, the class Bdelloidea is known for consisting of entirely females reproduced by apomixis, in which diploid

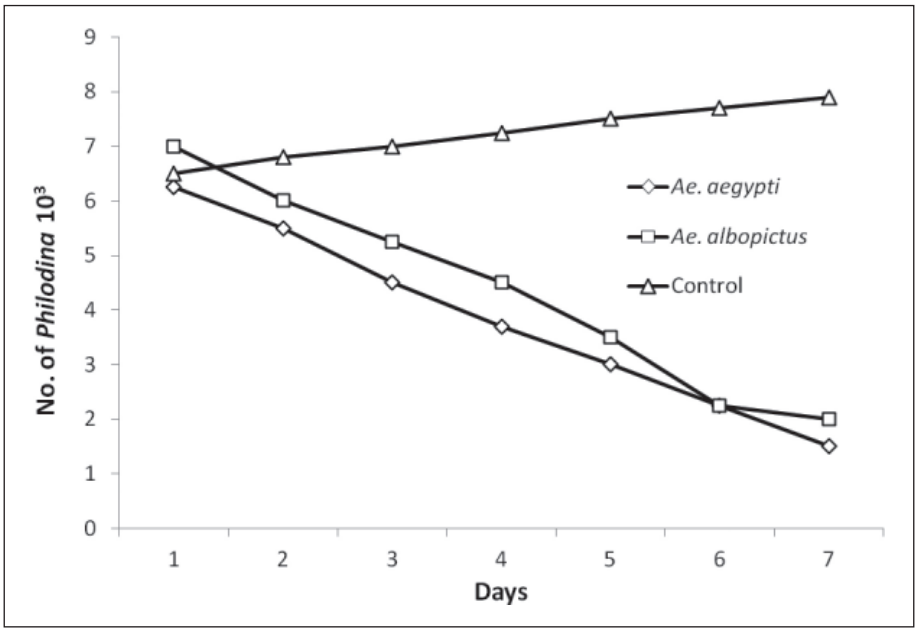

Figure 2. Number of *Philodina* reduced each day after the introduction of larvae of both *Ae. albopictus* and *Ae. aegypti* and in control, the number of *Philodina* was increased without the addition of *Aedes* larvae

eggs are produced by mitotic division, develop parthenogenetically into females (Wallace & Snell, 1991; Fontaneto *et al*., 2007; Gross, 2007), have existed for at least 35 to 40 million years while the diversity of their gene sequences suggests they are more than twice that age (Welch & Meselson, 2000). The success of the bdelloids seems to contradict the population genetic theory and empirical evidence, which show that asexual animals have shorter evolutionary life spans and less ability to diversify than do sexual organisms (Birky, 2004) hence they have been named "evolutionary scandals" by Smith (1986), as they survived and speciated in the absence of sex. The ubiquitous presence of rotifers in freshwater environments, particularly in temporary pools where the ability to survive desiccation offers a selective advantage for its survival (Martlet, 2003) and the survival of *Aedes* larvae.

The co-existence and probably having predator-prey relationship with *Aedes*, Bdelloid rotifer gets still more importance, mainly for the reason that, they adapted to survive episodes of desiccation ranging from days to years (Ricci *et al*., 2008), encountered in their characteristic habitats and that the damage incurred in such episodes includes DNA breakage that is repaired upon rehydration (Orstan, 1995; Ricci, 2001, Van Doninck *et al*., 2009). Under dehydrated condition, Bdelloid rotifers can be transported by wind in a form of anhydrobiotic state and will resume activity when the habitat is suitable again (Orstan, 1998, Van Doninck *et al*., 2009). Hence if a dried container is filled with water, the already existing anhydrobiotic form or transported by wind, Bdelloid rotifer could be the first organisms to resume at (Lapinski & Tunnacliffe, 2003) and act as an immediate source of food for the larvae. At the same time, since Bdelloid rotifer resist the desiccation, survives in a dormant state through entire dry period and resume activity once water filled the container, and reproduce parthenogenetically without male, it could be a hiding source for the arboviruses and passed to the progeny and reach the mosquitoes when it feeds on.

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