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Evangelin G

School of Entomology and Centre
for Natural Resources
Management (SECNARM)
P.G. & Research Department of
Advanced Zoology and
Biotechnology, Loyola College,
Chennai 600 034, India

Bertrand Horne

School of Entomology and Centre
for Natural Resources
Management (SECNARM)
P.G. & Research Department of
Advanced Zoology and
Biotechnology, Loyola College,
Chennai 600 034, India

Marlin Jino

School of Entomology and Centre
for Natural Resources
Management (SECNARM)
P.G. & Research Department of
Advanced Zoology and
Biotechnology, Loyola College,
Chennai 600 034, India

John William S

School of Entomology and Centre
for Natural Resources
Management (SECNARM)
P.G. & Research Department of
Advanced Zoology and
Biotechnology, Loyola College,
Chennai 600 034, India

Correspondence:**Evangelin G**

School of Entomology and
Centre for Natural Resources
Management (SECNARM)
P.G. & Research Department of
Advanced Zoology and
Biotechnology, Loyola College,
Chennai 600 034, India

Feeding behaviour of *Antilochus coquebertii* (Hemiptera: Pyrrhocoridae) and its systematic positioning

Evangelin G, Bertrand Horne, Marlin Jino and John William S

Abstract

During the recent past, due to unfavourable climatic conditions in combination with unscientific agricultural practices there has been an exponential increase in pest population. The rampant pest population and the need to produce more yield in limited acreage of land has pressurized the farmers into extensively using harmful chemical insecticides and pesticides. This will undoubtedly cause severe and lasting damage to the land and the crops produced therein and eventually to human health. In order to ensure sustainability in agriculture, we need to move to other alternative means of pest management such as the use of botanicals and predatory insects to control pests. One such predatory insect is *Antilochus coquebertii*. It is imperative to study the feeding behaviour of *A. coquebertii* in order to implement them as efficient biological control agents in integrated pest management programmes.

Keywords: *Antilochus coquebertii*, feeding behaviour, *Pyrrhocoridae*, COI, predator

1. Introduction

Antilochus coquebertii Fabr belongs to the family Pyrrhocoridae. They are ground living insects which interestingly exhibit a strong predacious lifestyle. Though most members of Pyrrhocoridae are found to feed on oil seed and plant sap ^[1], *Dindymus* and *Antilochus* are the only two genera that show predatory behaviour ^[2]. Moore, 2001 ^[3], Zaidi, 1985 ^[4] have recorded the predatory habit of *Antilochus* species and their main prey species are other Pyrrhocoridan members though they occasionally prey on other smaller insects.

Their occurrence is widespread in India as they are seen to feed predominantly on the cotton stainers of the genus *Dysdercus* and other members of the Pyrrhocoridan family. Due to its aggressive predatory lifestyle on pests of cotton which cause huge economic losses to the farming community, they can be used in the control of this pest population. The biology, stage preference and functional response of *A. coquebertii* on *D. cingulatus* have been studied by Muthupandi *et al.*, 2014 ^[5]. It is seen that the predator exhibits type II functional response as the predator's potential to kill the prey steadily increases with increase in prey population in the initial stages but plateaus off in the later stages. Hence, studying the feeding behaviour of predatory insects is essential in order to understand their capacity in pest control and also in using the predator to subdue and control the pest population in integrated pest management programmes. Knowledge about the feeding method and efficiency of an insect will greatly aid in understanding their position and effects in the ecosystem. There is an urgent need to understand the predator-prey behaviour in order to determine the suitability of the predator for use in IPM programme. The predator-prey relationship in insects is a complex and specialized phenomenon that has the potential to bring down and collapse entire ecosystems. In the complete absence of a predator, there is an absolute increase in pest population that will completely destroy the crop they infest. If the prey population is on the decrease, the predator could move on to other insects present in the nearby area and destroy beneficial insects too. The predator exercises enough power to drive a pest species to extinction ^[6,7]. Thus studying the feeding behaviour of a predator will enable us to understand their relationship with the prey and manipulate it for our benefit. In this study, an attempt was made to observe and distinguish the different stages in the feeding mechanism of the predator *A. coquebertii*. The systematic positioning of this predator has also been studied based on the available COI sequences in NCBI.

2. Methodology

2.1. Collection and Rearing of *Antilochus conquebertii*

Adults and nymphs of *A. conquebertii* (Hemiptera: Pyrrhocoridae) were collected from the Adyar Theosophical Society, (13.0102 °N, 80.2703 °E) from March to May, 2014. Also known as the Huddleston gardens, it lies on the south bank of the Adyar River and is spread over 260 acres of greenery in the heart of Chennai city. They were reared on the first to fourth instars of *Iphita limbata* in plastic containers (20.5 cm x 16.5 cm) under laboratory conditions at a temperature of 30 ± 2 °C, relative humidity of $75 \pm 5\%$ and photoperiod of 12 ± 1 hr. Their feeding behavior was studied.

2.2 Systemic positioning using COI marker gene

The available mitochondrial Cytochrome Oxidase subunit I sequences of Pyrrhocorid species were obtained from NCBI and subjected to phylogenetic analysis to understand the systematic positioning of *A. conquebertii*. The COI gene of the following species was considered. *D. evanescens*, *D. poecilus*, *D. koenigii*, *D. koenigii(1)*, *Odontopus sp.*, *Odontopus nigricornis*, *Pyrrhocoris sinuaticollis*, *P. apterus*, *Pyrrhopleus posthumus*, *I. limbata* and *A. conquebertii* (Table 01). The COI sequences were subjected to pair wise distance analyses with a gap opening penalty of 15 and a gap extension penalty of 6.66 and Maximum Parsimony, Maximum Likelihood and Neighbour Joining trees were constructed using MEGA 6.0 software and studied.

In order to estimate the evolutionary distances between sequences, analyses were conducted using the maximum composite likelihood model [8]. The present analysis involved 11 COI nucleotide sequences. Codon positions included were 1st+2nd+3rd+Non coding. All positions containing gaps and missing data were eliminated. There were a total of 374 positions in the final dataset [9].

3. Results and discussion

3.1. Feeding behaviour

The feeding behaviour of *A. conquebertii* was studied and was broken down into three distinct steps which have been discussed below.

3.1.1. Identification of the prey

Prey identification was done using the visual receptors and the antennae. The predator was seen cleaning the antennae very often especially before prey capture for this purpose and also just after a feeding session. The predator was keenly aware of any movement around it. In case of any small movements in its immediate surroundings, it remained perfectly still and slowly moved its antenna to ascertain the presence of prey. Such antennal and visual cues in combination with their innate instinct also greatly help in prey identification. *Antilochus* also walks long distances searching for prey. During such a scenario, if the prey happens to collide with the predator, the prey is immediately captured.

3.1.2. Pouncing on the prey

Immediately after locating the prey, *A. conquebertii* pounces on the prey and subdues the prey with its body weight. If it happens to pounce on a prey which is nearly equal in size or larger than itself, the struggle to subdue the prey lasts for even 40 minutes. *Antilochus* grabs the prey with the help of the forelegs and pierces the head or neck region of the prey with its proboscis. This process lasts until the prey loses enough strength to escape or dies. The stylet penetration of the predator is quite powerful, causing pain and bringing about the immediate death of the prey. Barker, 2004 [10] has stated that

the movement of the proboscis in combination with stylet penetration in *Dindymus pulcher* is powerful enough to pierce through the gastropod shell. While *A. conquebertii* does not hesitate to pounce on a prey much larger than itself, at times the sheer size of the prey might help the prey in untangling itself from the predators grip and facilitates its escape. Unlike the predatory reduviidae, *Antilochus* does not seem to drag the prey to a corner before feeding [11].

3.1.3. Feeding

After successful capture, the predator positioned itself over the prey in a position comfortable enough to insert its stylet and starts feeding by sucking the bodily juices of the prey. The stylet being four segmented, bends at the edges while being inserted giving a zig zag appearance. This formation of the stylet moved ever so slightly up and down during feeding giving the impression of the stylet being compressed and relaxed. The feeding session lasts for upto an hour. In case of disturbances around, the predator looked up to check for potential signs of danger without completely removing the stylet from the point of insertion on the prey. At the end of a complete feeding session, the prey looked like a see through thin plastic sheet of what it used to be.

Even though *Antilochus* resembles reduviidae and nabids in their predatory habits in making sudden and powerful lunges as soon as it comes in contact with the prey, its rate of success with regard to prey capture is small. This is due to various facts including capturing a prey too big for the predator to subdue and also the relatively longer amount of time taken for controlling the prey wherein the increase of prey escape is higher.

3.2. Phylogenetic analysis

Maximum Parsimony, Maximum Likelihood and Neighbour Joining yielded very similar phylogenetic trees and hence only the tree generated by maximum parsimony is depicted (Fig j). The pairwise distances between the sequences are given in table 02. The overall mean distance is 3.785. The maximum parsimony tree shows that all the sequences considered were broadly grouped into three clusters.

P. sinuaticollis and *P. posthumus* were seen to be sister groups while *P. apterus* was in a separate clade and more closely related to *I. limbata*. Even though *P. sinuaticollis* and *P. apterus* belong to the same genus, the reason they are not closely related could be attributed to geographical isolation since *P. sinuaticollis* is from Korea and *P. apterus* from Germany.

Among the three species considered from the genus *Dysdercus*, *D. evanescens* from India was closely related with the *Odontopus sp.* clade. *D. koenigii* from India was in a sister group relationship to *I. limbata*, also from India. And *D. poecilus* from China was seen to exhibit greater affinity to *A. conquebertii* from India. The varying relationships that different species belonging to the genus *Dysdercus* exhibit with other Pyrrhocoridan members rather than with other species from its own genus gives a clear picture as to how they have evolved over the years and the role of geographical isolation in genetic variation.

It is interesting to note that *A. conquebertii* and *D. poecilus* have evolved from the same common ancestor considering that the former is highly predacious whereas the latter is known for its destructive phytophagous feeding that result in huge economic losses. Though cannibalism has been recorded in specific cases in Pyrrhocoridae [12, 13], Schaefer, 1997 [14] has stated that the cannibalistic trait could have evolved secondarily to their phytophagous character. Opportunistic

predatory behaviour wherein a phytophagous insect is seen to predate on dead individuals of their own species during times when availability of food is low has been observed in many members of Pyrrhocoridae including *I. limbata* (authors' observation), *D. sidae sidae* and *D. Versicolor* [15]. This mode

of switching the type of feeding is found to be extremely useful in helping the insect tide over hostile environmental conditions. A clearer picture of the origin the predatory trait in *Antilochus* can be understood with further phylogenetic studies including *Dindymus*.

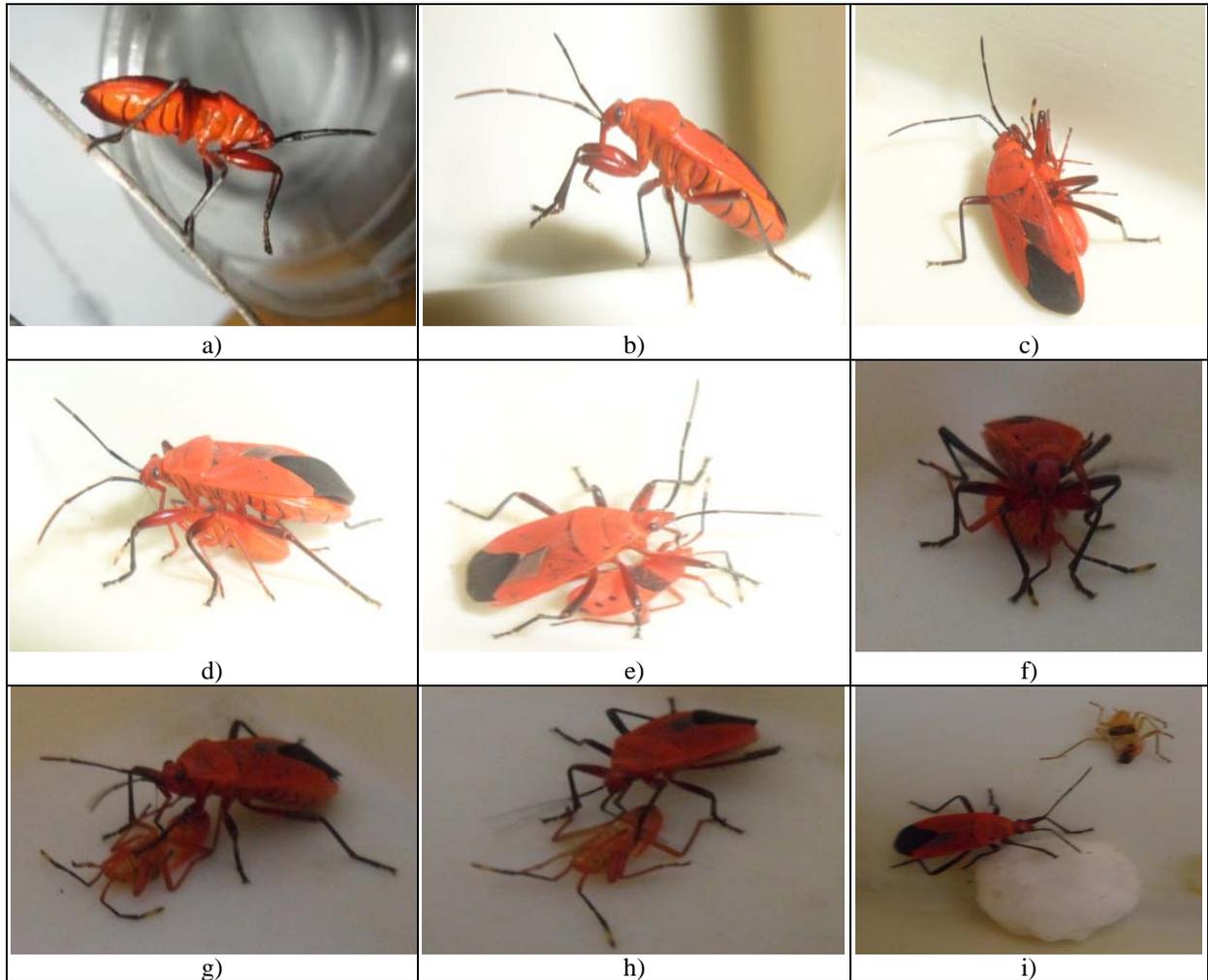


Fig: a), b) *A. conquibertii* cleaning the antennae using the forelegs Fig c) Predator pouncing on the prey *I. limbata* and holds it with the forelegs Fig d) Pinning the prey to the ground and inserting the stylet Fig e) Stylet insertion is seen at the base of the head region Fig f) Predator feeding on the prey Fig g) The zig zag movement of the stylet Fig h) Bodily juices being sucked out leaving a clear plastic sheet of what the prey used to be Fig i) Predator sucking water out of a cotton ball immediately after a complete session of feeding

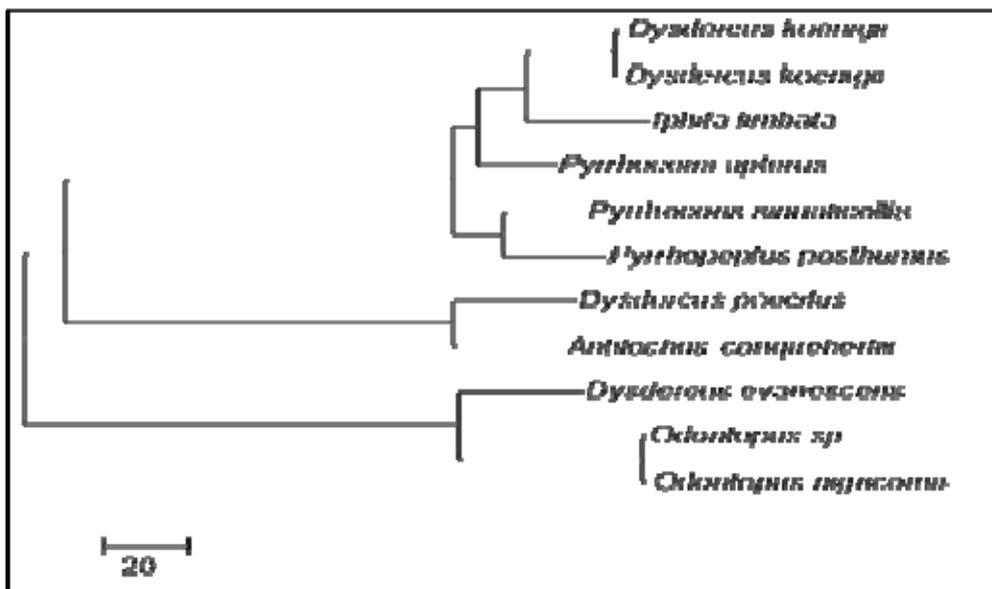


Fig j: Maximum Parsimony tree generated based on COI gene.

Table 01: Mitochondrial DNA sequences of the Pyrrhocoridan species considered for Phylogenetic analysis

Family	Species	Locality	GenBank Accession No.	Molecular marker	Feeding habit	References
Pyrrhocoridae	<i>Dysdercus poecilus</i>	China	AY627337.1	COI	Phytophagous	Kohno and Bui Thi, 2005 ^[16] ; Kohno, 2001 ^[17]
Pyrrhocoridae	<i>Dysdercus koenigii</i>	India	GQ306227.1	COI	Phytophagous	Saxena, 1963 ^[18]
Pyrrhocoridae	<i>Dysdercus koenigii</i>	India	KJ459924.1	COI	Phytophagous	Saxena, 1963 ^[18]
Pyrrhocoridae	<i>Odontopus sp.</i>	India	GU247509.1	COI	Phytophagous	Ambika and Selvisabhanayakam, 2012 ^[19]
Pyrrhocoridae	<i>Odontopus nigricornis</i>	India	GU247507.1	COI	Phytophagous	Rani and Madhavendra, 1995 ^[20]
Pyrrhocoridae	<i>Dysdercus evanescens</i>	India	GU247501.1	COI	Phytophagous	Maria <i>et al.</i> , 2002 ^[21]
Pyrrhocoridae	<i>Pyrrhocoris sinuaticollis</i>	Korea	GQ292283.1	COI	Phytophagous	Sakashita <i>et al.</i> , 1998 ^[22]
Pyrrhocoridae	<i>Antilochus conquebertii</i>	China	AY627351.1	COI	Predatory, Saprophytic	Kohno, 2003 ^[23] ; Muthupandi <i>et al.</i> , 2014 ^[5]
Pyrrhocoridae	<i>Pyrrhoplepus posthumus</i>	India	KJ408788.1	COI	Unknown	
Pyrrhocoridae	<i>Iphita limbata</i>	India	KJ459923.1	COI	Phytophagous, saprophytic	Gomathy <i>et al.</i> , 2014 ^[24]
Pyrrhocoridae	<i>Pyrrhocoris apterus</i>	Germany	KM021457.1	COI	Phytophagous	Marta <i>et al.</i> , 2011 ^[25]

Table 02: Pairwise distances of the COI sequences

	1	2	3	4	5	6	7	8	9	10	11
1											
2	4.029										
3	5.447	7.176									
4	5.446	7.176	0.008								
5	5.530	5.789	0.258	0.263							
6	4.085	0.184	7.052	7.052	6.479						
7	0.198	3.951	5.707	5.707	5.617	3.867					
8	4.208	0.192	7.570	7.570	6.698	0.136	3.899				
9	4.679	0.182	6.702	6.701	5.438	0.217	4.335	0.219			
10	4.029	0.003	7.176	7.175	5.789	0.180	3.951	0.188	0.178		
11	4.398	0.160	7.176	7.176	5.974	0.152	4.197	0.159	0.205	0.164	

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