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Morphology and biology of litter-inhabiting *Buchananiella indica* Muraleedharan (Hemiptera: Anthocoridae)

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ABSTRACT: The life history, biological parameters and predatory potential of litter- inhabiting *Buchananiella indica* Muraleedharan (Hemptera: Anthocoridae) have been investigated. The adult, egg and immature stages of this anthocorid are described with live images. *Buchananiella indica* is amenable to continuous rearing on eggs of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) and the anthocorid was continuously reared for 20 generations. Fertility studies indicated that the reproductive rate is 12.6 and the finite rate of increase 1.08.

KEYWORDS: Anthocorid, biology, *Buchananiella indica,* diagnostic characters, predatory potential, rearing

INTRODUCTION

Muraleedharan and Ananthakrishnan (1974) described some species of anthocorids including *Buchananiella* from India. *Buchananiella* Reuter 1884 is a relatively small taxon coming under the tribe Dufouriellini consisting of ten described species (Yamada and Yasunaga, 2009). The members of this taxon are distributed in the tropics and sub-tropics, except for *Buchananiella continua* (White), which is a pantropical species, quite widely reported in western Palaearctic zone (Kirby, 1999). *Buchananiella indica* Muraleedharan was described based on the material collected in Garo Hills, Meghalaya (Muraleedharan, 1977). In India, the known *Buchananiella* include *B. indica*, *B. garoensis* Muraleedharan, *B. crassicornis* Carayon, *B. carayoni* Muraleedharan and Ananthakrishnan, and *B. pseudococci pseudococci* Wagner. In general, little information is available on the biology and ecology of species of *Buchananiella.*

In the present paper, we present the morphological characteristics of the egg, immature and adult stages of *B. indica. Buchananiella* is sometimes confused with other taxa such as *Amphiareus. Buchananiella indica* and *Amphiareus constrictus* (Stål) were the two litter inhabiting anthocorids which were most commonly recorded and both were amenable to rearing. Hence, we have also reported some distinguishing characters which can be used to separate the two. Different species of *Buchananiella* generally inhabit either leaf litter or decaying plant material. However, Yamada *et al.* (2008) recorded *Buchananiella pseudococci pseudococci* (Wagner) (as *Cardiastethus pseudococci pseudococci* - transferred to *B. pseudococci pseudococci* by Ghahari *et al.,* 2009) from Kerala as a predator of coconut black-headed

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caterpillar *Opisina arenosella* Walker (Lepidoptera: Xylorictidae), a serious pest of coconut in Kerala and Karnataka, India. The authors could record *B. pseudococci pseudococci* from mango inflorescence associated with unidentified mites and thrips in Karnataka. The current study was undertaken to understand whether *B. indica* could be amenable to rearing so that its predatory role, if any, could further be ascertained. We succeeded in rearing *B. indica* continuously for more than 20 generations in the laboratory and through this paper information is provided on its biology, life stages, feeding potential, fertility table parameters and rearing protocol.

MATERIALS AND METHODS

Populations (4 adult females, 3 adult males) of *B. indica* were obtained from dry *Crossandra* (*C. infundibuliformis* Lamiales: Acanthaceae) flowers in Bangalore. These adults were released into ventilated plastic containers (adult containers, 7.5 cm height x 8 cm diameter) provided with UV irradiated *Corcyra cephalonica* Stainton eggs as feed at the rate of 8 to 10 eggs per adult per day and cotton fibres to prevent cannibalism. *C. cephalonica* eggs used as feed were UV irradiated to prevent hatching of the eggs. Mated females laid eggs either on the walls or at the base of the containers or in between cotton fibres, eggs were glued to the oviposition substrates. Eggs were collected and placed in hatching containers (ventilated plastic containers; 7.5 cm height x 8 cm diameter). When nymphs hatched, UV irradiated *C. cephalonica* eggs were provided at the rate of 5 to 8 eggs per nymph per day till they formed adults.

The experimental set up for biological studies was initiated with twenty pairs of adult *B. indica*, each pair maintained as a replication. *Corcyra cephalonica* eggs were provided as prey and data recorded on total longevity of the adults and number of eggs laid by the female in each replication. Ten sets of freshly laid eggs were placed in separate ventilated containers (6.5 cm h x 2.5 cm diameter) as replications and observations recorded on the developmental durations (incubation, nymphal and total developmental period). The number of nymphs hatching out of the eggs placed in each replication and number of nymphs surviving to adult stage was recorded, from which per cent hatching and per cent nymphal survival were calculated. For comparative studies, eggs and nymphs of the *A. constrictus* were collected from the live insect repository at NBAIR (National Accession No. NBAII-MP-ANT-11), where it is being continuously maintained (original culture was obtained from dry sugarcane leaves in Mandya, Karnataka). Morphometric measurements of all stages of *B. indica* and eggs and nymphs of *A. constrictus* were obtained using the images of live materials (n = 20 for each stage) using Olympus Microscope SZX 16 and Cell sens software. Descriptions of the nymphal and adult stages are based on the dorsal view. Scanning Electron Microscopic studies were also done to study the eggs of *B. indica* and *A. constrictus* using Zeiss EVOMA 10 Scanning Electron Microscope at 20.00 KV and 122 pa between 276 x and 600 x. Biological studies were carried out in the laboratory at 26±3°C; 60±10% RH and 13: 11 L: D.

Ten nymphs and ten adults were maintained as replications to study the predatory potential. Five UV irradiated eggs of *C. cephalonica* were provided daily for each nymph till five days of nymphal period, beyond which each day ten eggs were provided for each mature nymph till it moulted into an adult. Ten eggs were provided daily for each adult till its mortality. Preliminary studies were conducted to distinguish between the fed and unfed eggs, based on which the eggs which appeared flattened or crushed were considered as fed. The number of eggs fed by one nymph / one adult in one day was recorded throughout the nymphal and adult stages, respectively, from which the total predatory potential was calculated.

Fertility table studies were conducted based on the data obtained on the day-wise fecundity of *B. indica.* This experiment was conducted with four replications and with five pairs of adults in each replication. The adults utilised in this experiment were from the culture which was laboratory reared over 15 generations. Adults were provided with *C. cephalonica* eggs as food and the number of eggs laid in each replication was recorded daily. Mortality of adult female in each replication was also recorded. The freshly hatched neonate nymphs were provided with *C. cephalonica* eggs as food and when they became adults, they were sexed to arrive at the female progeny produced by each female per day. The age specific survival (l_x) and age specific fecundity (m_x) at each pivotal age x were worked out for the entire reproductive period. The number of individuals alive at age x as a fraction of 1 was recorded as l_x and the number of female offspring produced per female at age interval x as m_x. Utilising these, the fertility table parameters were calculated based on the methods following Birch (1948) and Andrewartha and Birch (1954).

Net reproductive rate (R_0) = $\Sigma l_x m_x$; approximate duration of a generation (T_c) = $\Sigma_x m_x/R_0$; approximate intrinsic rate of increase (r_c) = $\log_e R_0/ T_c$; precise intrinsic rate of increase (r_m) = $e^{-r_m} \times l_x m_x = 1$; net generation time (T) = $\log_e R_0/ r_m$; finite rate of increase (λ) = anti $\log_e r_m$; weekly multiplication of the population (r_w) = $(_e rm)^7$; hypothetical F_2 females = $(R_0)^2$; doubling Time (DT) = $\log_e 2/r_m$; weekly multiplication rate (WMR)= $(\lambda)^7$. All the experiments were conducted in the laboratory (temperature: 26±5°C; humidity: 60±10%).

RESULTS

Buchananiella indica could be reared continuously in the laboratory and UV irradiated *C. cephalonica* eggs were used as prey. The morphology, biological parameters, predatory potential and morphometrics of *B. indica* are provided in this paper (Table 1, Fig 1, 2 and 3).

Eggs: Eggs are laid loose by the adult females, eggs glued to the substrates, slightly bent at the opercular region (Fig 1B) The eggs measure 512-556 µm in length and 213-230 µm wide; operculum diameter 112 – 120 µm (n=20). Freshly laid eggs milky white with faint orange markings and mature eggs yellowish. Besides the bright red markings of the eye spots and abdominal scent glands of the embryo, concentrated red patches at the anterior and posterior ends of eggs with the central region appearing as a transparent transverse band (Fig 1B). Mean incubation period 4.2 days and mean per cent hatching 99.3. The egg surface covered with raised dots, evident in Electron Micrographs (Fig 1J). Operculum circular, central region slightly convex, covered with sharp edged engraved reticulations, the reticulations well defined in the central region in contrast to the faint reticulations in the periphery (Fig 3A).

First instar nymph: The neonate nymph is reddish, except the first tergite, which appears as a transparent white band in mid dorsal region of the nymph. The abdominal scent glands present as red spots (Fig 1C). The antennae and legs whitish. The length, greatest thoracic width and greatest abdominal widths 722 - 776, 178 – 205 and 205 - 242 µm, respectively. The first-instar nymphal duration 4 (range 2 to 5) days.

Second instar nymph: Reddish, abdomen orangish red, one pair of long bristles at the apex of the abdomen. The head and the pro, meso and metanotum reddish (Fig 1D). Whitish first two tergites prominent in contrast to the deep red thoracic region and posterior abdominal tergites. The abdominal scent glands present as red marks as in the first instar nymph. The mean length 918 - 1051 µm and the greatest thoracic and abdominal widths 200 - 239 and 289 - 380 µm, respectively. The mean duration of the second instar nymph 3.3 (range 2 to 5) days.

Third instar nymph: The mean duration of this instar 2 to 4 days. Wing buds evident at this stage. The femur and the apex of the first antennal segments darker. The spines on the antennae, legs, head, thorax and abdominal regions and one pair of long bristles at the apex of the abdomen clearly visible (Fig 1E). As in the previous instars, the initial tergites of the abdominal region whitish in clear contrast to the red colour of the remaining parts of the nymphal body. The red marks of the abdominal glands not distinct, appear to merge with the red colour of the posterior abdominal tergites. The mean length 1248 to 1482, thoracic width 240 to 326 and abdominal width 432 to 586 µm, respectively.

Fourth instar nymph: Head yellowish red, pronotum and the region beneath the wing buds deep red, tergites other than the first two deep red. The other characters as in the previous instar. Wing pads more developed, with the scutellum clearly visible between the base of the wing pads (Fig 1F). Length, greatest thoracic width and greatest abdominal width 1611 to 1729, 340 to 396 and 547 to 642 µm, respectively. This instar lasts for 2 to 4 days (mean 2.6 days).

Fifth instar nymph: The mean duration of this instar is 5.1 days (range 4 to 6 days). The general colour and structure as in the previous instar. Wing pads fully developed, extend beyond the thoracic region (Fig 1G). Length 1731 to 2228, thoracic width 458 to 541 and abdominal width 640 to 783 µm, respectively.

The total nymphal duration was recorded as 16 days and total developmental duration 20 days. Percent nymphal survival was 93-100. The predatory potential of the nymphs on *C. cephalonica* eggs was 2.6 per day per nymph and 29.8 eggs throughout its nymphal period.

Adult: The adults blackish. Apical portions of first and second antennal segments deep blackish brown, base of the last antennal segment dark. Last segment of the rostrum curved and pointed (Fig 2E). Legs yellowish brown. The last abdominal sternites in male consists of asymmetrical pygophore with the evenly straight and short paramere visible (Fig 2D). The adult female larger than the male. In female, a tiny opening (omphalus) present in medioventral part of abdominal sternite VII. One-third of the second and third antennal segments from the apex darker in contrast to the pale yellow base in female; more than three quarter of the second and third antennal segments dark in male (Fig 2A and B). The length, thoracic and abdominal widths of male 1734 – 1889, 630 – 664 and 647 - 713 µm, respectively, the corresponding figures in female 2064 - 2297, 716 - 739 and 817 - 845 µm, respectively. Males lived for 10 - 89 days and females for 21 - 52 days. One female could lay 38 to 50 eggs and 32 to 55% were female progeny. The predatory potential of one adult male was 134 eggs and 137 eggs in female; while in one day an adult could feed on 2 to 3 eggs.

Comparison between *Buchananiella indica* and *Amphiareus constrictus*

The general shape and size of the egg of *B. indica* (Table 1) almost similar to that of *A. constrictus*. Operculum diameter, length and greatest width of *A. constrictus* egg - 111.3, 530.3 and 213.9 µm, respectively. The eggs and nymphs of *B. indica* differ from those of *A. constrictus* in the following combination of characters (Fig: 3). 1) In EM image of *B. indica* egg, the central region of operculum strongly convex, covered with well defined sharp angled reticulations in the central region and faint reticulations in the peripheral region, no furrow separating the operculum from the well defined narrow chorionic rim (*vs* in *A. constrictus,* central region slightly convex, uniform well defined follicular cells throughout the opercular region, furrow present separating the operculum from the broad chorionic rim) (Fig 3A and B). 3) In LM image, egg reddish with a transverse transparent band in the central region (corresponding to the first two whitish abdominal tergites of the embryo) (*vs* egg dark yellowish with the eyes and abdominal scent glands of the embryo appearing as red marks) (Fig 3C and D). 4) The thoracic region and the posterior abdominal tergites of the nymph dark red, the first two pale abdominal tergites appearing as a transverse transparent band (*vs* nymphal abdomen dark yellow with scent glands appearing as dark spots) (Fig 3E and F). 5) Last segment of rostrum curved (*vs* not curved) (Fig 3G and H).

Fertility table: The fertility parameters of *B. indica* are depicted in Table 2. With a generation time of 31 days, the reproductive rate of *B. indica* was 12.6 and the intrinsic and finite rates of increase were 0.08 and 1.08, respectively.

Fig 4 presents the age-specific survival and fecundity of *B. indica*. The immature stage occupied 20 days. The first mortality occurred on the 22nd day from adult emergence and 100% mortality was recorded on day 40. Egg laying was recorded two days after emergence and female

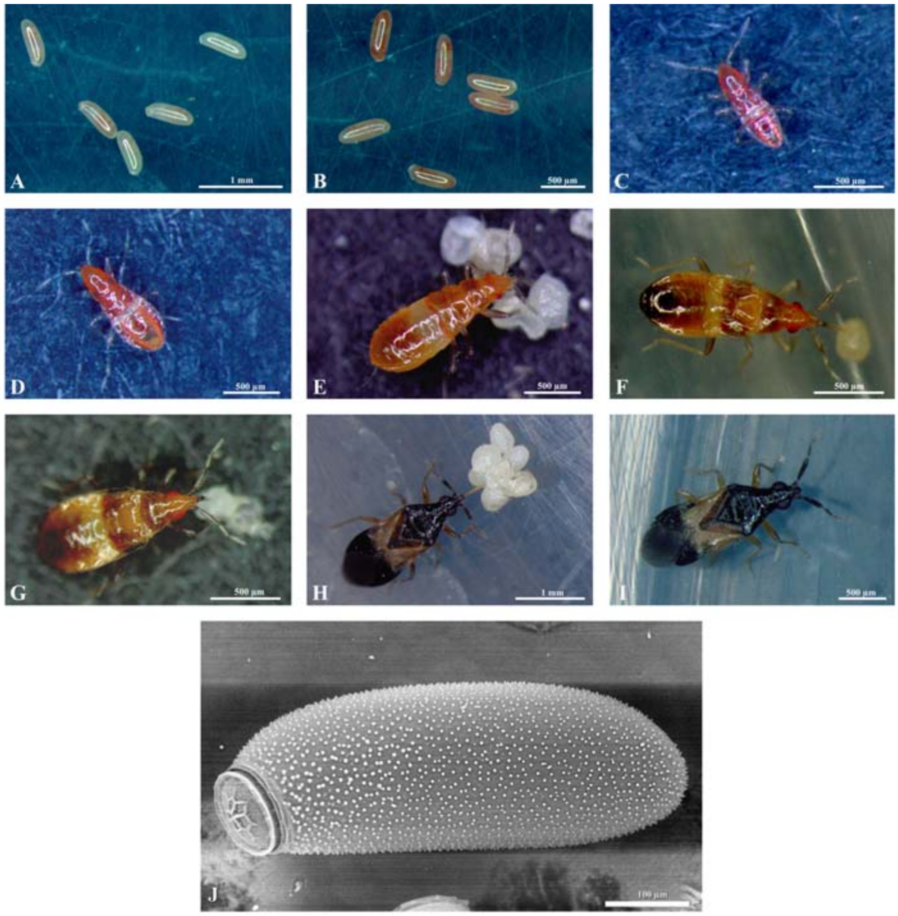

Fig 1. Life stages of *Buchananiella indica:* Fresh eggs (A), Mature eggs (B), First instar nymph (C), Second instar nymph (D), Third instar nymph (E), Fourth instar nymph (F), Fifth instar nymph (G), Adult female (H), Adult male (I), SEM image of egg (J)

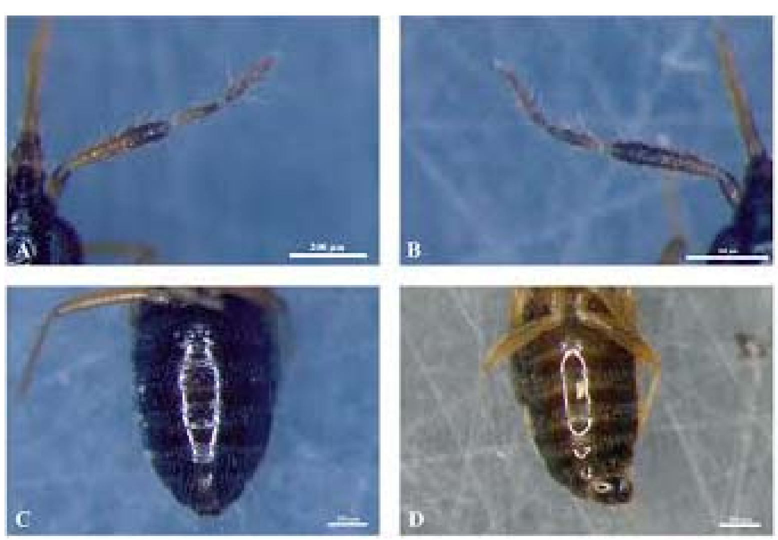

Fig 2. Antennal and abdominal characters of *Buchananiella indica :* Female antenna (A), Male antenna (B), Female abdomen (C), Male abdomen (D)

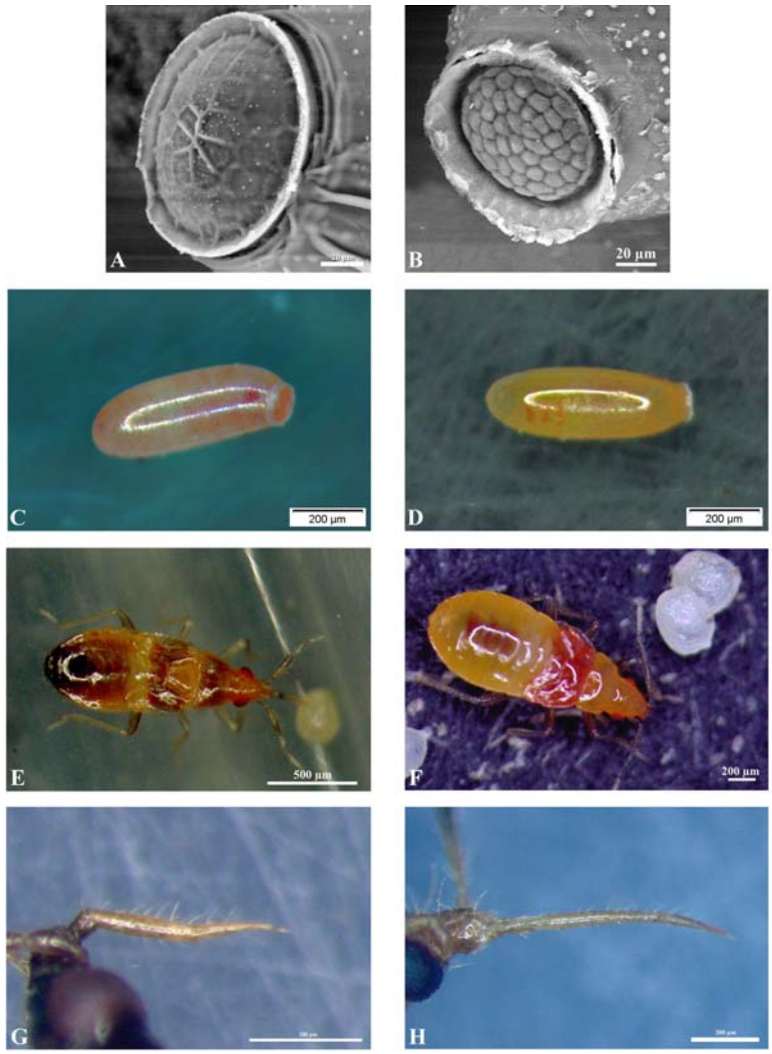

Fig 3. Characters to differentiate between *Buchananiella indica* and *Amphiareus constrictus* SEM image of egg operculum: *B. indica* (A), *A. constrictus* (B) LM image of mature egg: *B. indica* (C), *A. constrictus* (D) Mature nymph: *B. indica* (E), *A. constrictus* (F) Rostrum: *B. indica* (G), *A. constrictus* (H)

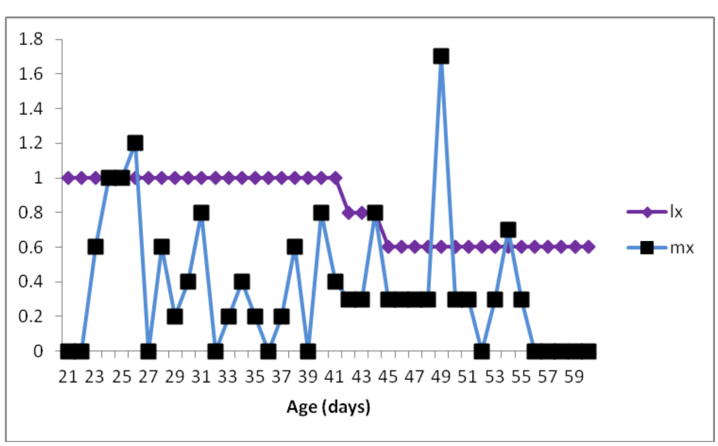

Fig 4. Age specific survival (lx) and age specific fecundity of *Buchananiella indica* (0 to 20 days – immature stages; 21 to 60 days - adult stage)

Table 1 Biological parameters and morphometrics of *Buchananiella indica*

Parameters		Duration (days) Mean±SE	Morphometrics Mean±SE		
Egg and incubation period		**Incubation period (days)**	**Operculum diameter (ìm)**	**Greatest length (ìm)**	**Greatest width (ìm)**
		4.2±0.1	116.4±0.4	523.1±8.6	218.9±1.5
Per cent hatching		99.3±0.7			
Nymphal instars		**Developmental period (days)**	**Total length (ìm)**	**Greatest Thoracic width(ìm)**	**Greatest Abdominal width (ìm)**
1st instar		4.0±0.4	736.7±8.8	188.0±4.2	226.6±5.2
2nd instar		3.3±0.4	976.2±31.3	220.2±10.6	331.9±23.7
3rd instar		3.0±0.3	1368.5±36.2	284.6±12.4	487.3±23.1
4th instar		2.6±0.4	1664.1±25.8	372.8±10.5	587.8±22.1
5th instar		5.1±0.3	1934.8±50.9	494.5±9.0	711.4±17.8
Total nymphal duration (days)	**Male**	15.9±0.3			
	Female	15.8±0.2			
Total developmental duration (days)	**Male**	20.1±0.3			
	Female	20.0±0.2			
Adult male			1830.4±18.1	642.8±3.9	676.1±7.3
Adult female			2171.0±28.3	724.7±2.5	827.7±3.0
Per cent nymphal survival to adult stage		98.2±1.8			
Male longevity (days)		35.9±10.5			
Female longevity (days)		34.6±5.6			
Mean Fecundity (number of eggs / female)		43.0±3.6			
Per cent females		42.8±4.7			
Predatory potential (on *C. cephalonica* eggs)					
Mean number of eggs consumed (Mean±SE)					
A) a nymph in one day		2.6±0.3			
Throughout the nymphal duration		29.8±3.0			
B) an adult male in one day		2.4±0.2			
Throughout its life time		133.8±29.6			
C) an adult female in one day		2.8±1.0			
Throughout its life time		136.7±53.8			

Table 2 Fertlity parameters of *Buchananiella indica*

R_0	T_c	r_c	r_m	T	λ	DT (days)	Hypo. F_2♀ s	WMR
12.6	35.89	0.07	0.08	31.09	1.08	8.66	158.76	1.71

progeny produced by one female per day ranged from 0 to 1.7, peak female progeny production was recorded when the parent female was 49 days old and ceased when it was 56 days old (Fig 4).

R_0: Net reproductive rate; T_c: Approximate duration of a generation; r_c: Approximate intrinsic rate of increase; r_m: Precise intrinsic rate of increase; T: Net generation time; λ: Finite rate of increase; Hypo.: Hypothetical; DT: doubling time; WMR: Weekly multiplication rate

Rearing protocol

The nymphs and adults can be reared in plastic containers (7.5cm h x 8cm dia) with a ventilated lid. Based on the predatory potential studies, it was concluded that *C. cephalonica* eggs are to be provided as food for nymphs and adults at the rate of five eggs per nymph / adult per day. In a container holding 50 adults, every three days, 0.05 cc of eggs are to be provided as food. Cotton strands are to be placed in the containers in order prevent cannibalism, they also serve as oviposition substrates. Eggs can be collected on alternate days and placed in a separate ventilated container (7.5cm h x 8cm dia) for hatching. Based on the oviposition pattern, egg harvesting can be initiated two days after emergence and can be continued for one month. Following this procedure, *B. indica* could be reared continuously for 20 generations. In a production system for *B. indica,* utilising 1 cc of *C. cephalonica* eggs (approximately 15000 to 17000 eggs), 97 nymphs/adults (with 43% females) could be reared. From a container holding 50 adults, 1100 eggs / nymphs can be harvested.

DISCUSSION

In this study, we report sexual dimorphism not only in the abdominal characters (which is common in Anthocoridae), but also in the antennal characters of *B. indica. Buchananiella* is sometimes confused with *Amphiareus* and we generally collect *B. indica* and *A. constrictus* from leaf litter and decaying plant materials and both were observed to be amenable to rearing. The taxonomic characters of adult stages are reported by Muraleedharan (1977), Yamada (2008) and Yamada and Hirowatari (2003). However, the egg and nymphal characters reported in this paper can be used to differentiate the immature stages of *B. indica* and *A. constrictus* in the rearing units and thus to maintain pure cultures.

Male genitalia characters are generally used for identification in family Anthocoridae. However, the general shape and size of the eggs and the structure of the chorial surface, operculum and flange are considered as important characters in egg systematics (Cobben, 1968; Carpintero, 2002). Information on the structure of eggs and nymphs of a few Anthocoridae is available (Southwood, 1956; Sands, 1957; Muraleedharan and Ananthakrishnan, 1978; Schuldiner-Harpaz and Coll, 2012). Anthocoridae, such as species of *Orius* and *Anthocoris* and *Blaptostethus pallescens* Poppius, *Carayanocoris indicus* Muraleedharan and *Xylocoris afer* (Reuter) insert eggs into plant tissue, with only the opercular region visible. However, Anthocoridae such as *Xylocoris flavipes* (Reuter), *Amphiareus constrictus* (Stål), *B. indica, Cardiastethus exiguus* Poppius and *Cardiastethus affinis* Poppius lay their eggs in either exposed or concealed substrates (e.g. beneath bark, in leaf litter, among decaying plant materials). Such egg laying behaviour enables us to examine the whole egg structure. SEMicrographs of eggs of four species of *Orius* were used to determine the species based on the structure of the operculum and the follicular cells along the outer ring of the operculum (Schuldiner-Harpaz and Coll, 2012). In the current study, the egg and nymphal characters of *B. indica* are described which can serve as additional characters for a non-destructive identification process, when either adult females or immature stages are collected from the field.

This study highlights an easy rearing protocol enabling the laboratory rearing of *B. indica* on *C. cephalonica* eggs. The fact that *B. indica* does not require a plant substrate for egg-laying, makes the rearing procedure simple and user-friendly.

No precise information is available on the prey range of *Buchananiella* In Thailand, *B. crassicornis* Carayon, *B. atrata* Yamada and Hirowatari and *B. pericarti* Yamada and Yasunaga were recorded from dry leaf materials (Yamada and Yasunaga, 2009). *B. crassicornis* and *B. leptocephala* Yamada and Hirowatari were recorded in Japan from dry banana leaves and dry leaves of broad-leaved trees, respectively (Yamada and Hirowatari, 2005). However, *B. pseudococci pseudococci* was recorded as a predator of *O. arenosella* (Yamada *et al.,* 2008). It is interesting to note that *A. constrictus*, a litter inhabiting species, was recently recorded as a predator of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Queiroz *et al.,* 2015).

The reproductive rate and intrinsic rate of increase can be used to estimate the growth rate of the population of a parasitoid / predator, which in turn can be used to optimise a mass rearing protocol. The fertility table parameters of *B. indica* is comparable to those of potential anthocorid predators *viz. Orius majusculus* (Reuter)*, Orius laevigatus* (Fieber) and *Orius tantillus* (Motsch.), which are also highly amenable to rearing on eggs of alternate laboratory hosts (Tommasini *et al.,* 2004; Ballal *et al.,* 2012). Based on the above and considering the fact that *B. indica* could be successfully reared for more than 20 generations, it would be worth investigating if this anthocorid has a functional role as a potential predator of field crop pests and storage pests. This study can form a prelude to future studies on prey preferences, dispersal and predatory attributes of this and other related species of anthocorids in different regions and agro-climatic conditions.

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