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Development, biology, and life table parameters of the predatory species, *Clitostethus brachylobus* Peng, Ren & Pang 1998 (Coleoptera: Coccinellidae), when fed on the whitefly, *Bemisia tabaci* (Genn.)

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Abstract

The predatory species, *Clitostethus brachylobus* Peng, Ren & Pang 1998 (Coleoptera: Coccinellidae), native to China, has been reported as a predator of the whitefly species, *Bemisia tabaci* (Genn.). Present study describes the development and biological characteristics of *C. brachylobus*. Developmental periods of different immature stages showed significant differences, when fed on different life stages of *B. tabaci*. Prey consumption capacity was reduced by the increase in prey age. Female longevity was 193.5 days, whereas fecundity was 154.70 eggs/female. Net reproductive rate was 53.60, whereas the mean generation time was 102.64 days. The daily adult survival rates gradually decreased 120 h post-adult emergence.

Keywords: *Clitostethus brachylobus*, *Bemisia tabaci*, Life table parameters

Background

The whitefly species, *Bemisia tabaci* (Genn.), is well known as an economic pest on crops, vegetables, and fruits. It prevails across whole China with a tendency for annual outbreaks (Qiu et al. 2011). *B. tabaci* exists in 31 provinces or municipalities of China, causing economic losses to different crops (Qiu et al. 2011). Agrochemicals are mostly used for whitefly management throughout the world. They affect natural environment in many harmful ways (Liang et al. 2012; He et al. 2013). This has necessitated requiring alternate control measures and applications of biocontrol agents, as safe options for environmentally sustainable whitefly management (Hernandez et al. 2013). Previously, many workers have successfully used coccinellid predators (*Serangium parcesetosum* Sicard, *Nephaspis oculatus* Blatchley, *Delphastus catalinae* Leconte, and *Axinoscymnus cardilobus* Ren and Pang) for whitefly management (Liu and Stansly 1999; Gerling et al. 2001; Simmons and Legaspi 2004; Huang et al. 2006; Huang et al. 2008).

Different species of tribe *Clitostethus* have been proved as specialized predators of whiteflies under laboratory and field conditions (Yazdani and Zarabi 2011). *C. brachylobus* was identified, on the basis of its adult morphology by Poprawski et al. (1998), as a new species and an indigenous potential whitefly predator in China. To date, majority of information regarding predatory potential of this beetle has been restricted to their feeding capacity. Very little information is available on the morphology of immature stages, biology, and life history of *C. brachylobus*. Biological studies and life table variables can be used to determine the predatory potential as an insect predator in terms of a detailed analysis of age-specific

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deaths in the population (Luck et al. 1988). When data are available on insect fecundity and age-specific mortality, the influence of the predator can be shown based on population growth rate (Sang et al. 2018).

The present study was designed to explain the morphological characteristics of different life stages of *C. brachylobus* and to record the growth parameters, reproduction, life table, and functional response of *C. brachylobus*, when fed on various stages of *B. tabaci*.

Materials and methods

Insect cultures

C. brachylobus and *B. tabaci* from a greenhouse colony were maintained on *Hibiscus rosa-sinensis* L. plants at Provincial Key Laboratory of Biopesticides, South China Agricultural University, Guangzhou, following the methods of Huang et al. (2008).

Morphology of different life stages of *C. brachylobus*

Description and illustration of all *C. brachylobus* developmental stages were based on fresh specimens collected from the Xishuang Banana National Nature Reserve, Yunnan Province, China. A stereoscope (Stereo Discovery V20) was used to describe the external morphology. Photos were taken, using a digital camera (AxioCam HRc) that was attached to the dissecting microscope. Axio Vision Rel Apps. 4.8 was used to capture camera images. Measurements were carried out, using a micrometer connected to a microscope for dissection. In this paper, the use of morphological terminology follows that of Ślipiński (2007) and Ślipiński and Tomaszewska (2010).

Life history and predatory potentials of *C. brachylobus* immatures

Adult beetles collected from the stock culture in the greenhouse were fed on *B. tabaci* and maintained on *H. rosa-sinensis* plants for egg oviposition following Huang et al. (2006). Leaves bearing beetle eggs less than 12 h old were then confined in clear plastic Petri dishes (9 cm diameter) lined with a moist filter paper (8 cm diameter) to prevent desiccation of the eggs. Beetle eggs were divided into two batches, and each batch was divided into three replicates of 50 eggs. The eggs were closely monitored for hatching. The newly hatched neonates were isolated in a plastic Petri dish containing a 10–15-cm^2 leaf disc of *H. rosa-sinensis* bearing *B. tabaci* and incubated at 25 ± 2 °C, 75 ± 5% RH, and 16:8 (light: dark) photoperiod. The neonates in two batches mentioned above maintained on individual leaf disc were then exposed to two different diet treatments namely eggs and assorted immature for consumption. The number of eggs and immatures consumed was recorded daily. The different developmental stages to adult emergence of the beetle and the survival rates of the immatures were also recorded.

Longevity and fecundity

Twenty-four pairs of newly emerged adults less than 12 h old divided into four groups in two treatments, with two replicates in each treatment, were provided with whitefly eggs and immatures on *H. rosa-sinensis* leaf in Petri dishes, respectively. The daily number of eggs laid, the number of adults surviving each day, and the longevity of the adults were recorded. The sex was determined by dissecting dead adults.

Life table analysis

Collected data (on survival and fecundity of *C. brachylobus*) was used to estimate different life table parameters of *C. brachylobus* feeding on *B. tabaci.* The life table calculation was carried out by using the following formula (Mutlu and Sertkaya 2016):

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$$

where x is the age of *C. brachylobus* in days, l_x implies the age-specific survival at time x, and m_x is the number of eggs (sex ratio dependent) laid by a single female each day. The data was analyzed based on theory of the age-stage, two-sex life table (Chi 1988) and using the program TWOSEX-MSChart (Chi 2015).

Data analysis

Developmental period, survivorship percentage, ovipositional period, and fecundity per female were analyzed, using analysis of variance and Tukey's HSD test, to derive mean values when the *F* value was lower than 0.05 (SAS 1988).

Results and discussion

Morphology of *Clitostethus brachylobus*

Eggs

The eggs are appearing reticulate oval, white, elongate chorion except for ventral surface. After hatching, the reticulation becomes more distinct. Sometimes in tandem with whitefly eggs, eggs laid singly on the leaf surface (Fig. 1a).

Larval instars

They are fusiform, elongated, white, and widest around the metathorax. Morphological characteristics were not distinctly different for each instance, except for the body size. Head is broader than long, widest near to ocelli, and every side of the head has 3 ocelli; the mandible is apically flat, and maxillary palpus has 3 palpomers and palpiper; there are 2–3 pairs of small collar setae above

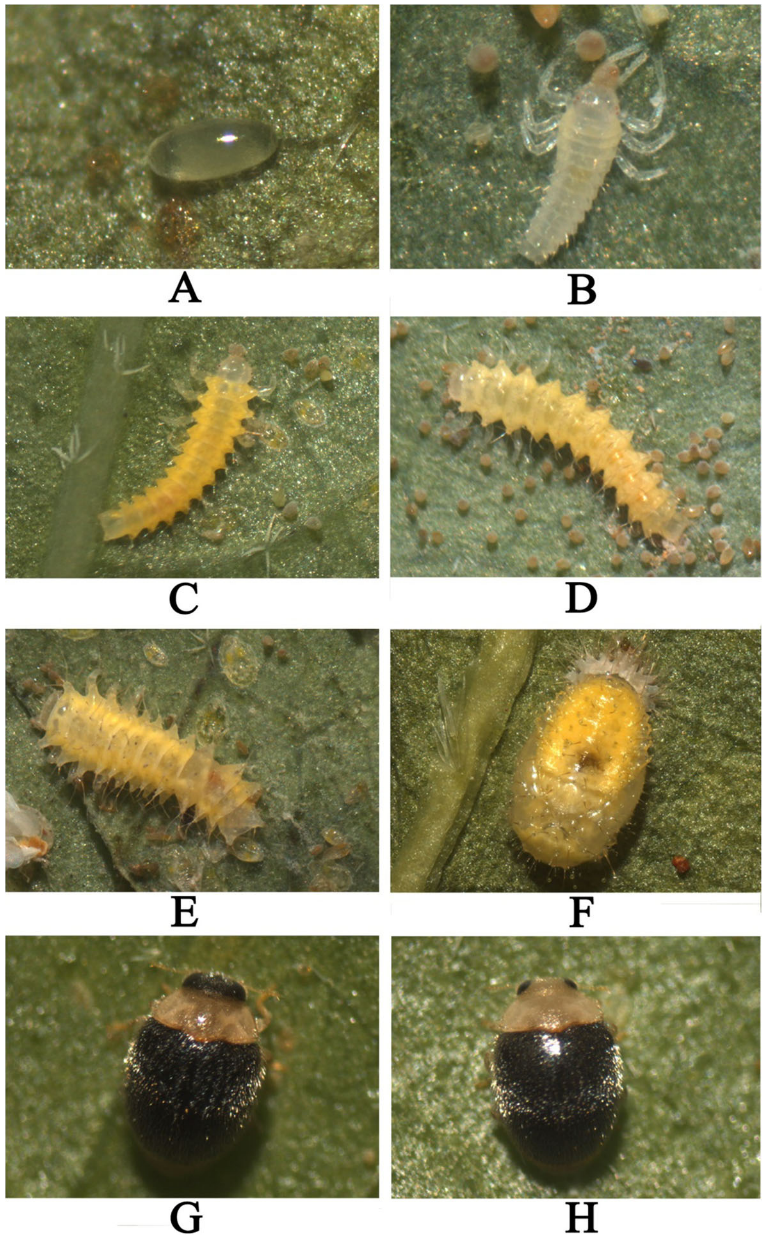

Fig. 1 Life stages of *C. brachylobus*. **a** Egg. **b** First larval instar. **c** Second larval instar. **d** Third larval instar. **e** Fourth larval instar. **f** Pupa. **g** Adult female. **h** Adult male

the mouthpieces on the front. Antenna comprised of 3 antennomers. The second antennomers are longer and more slender than the first one, with a minute spine-like sensory process and many sensory acute processes on the terminal side, and 1 long hair process; the third indistinct antennomers have a broad, acute conical sensory process. Both segments of the body are wider than the long. The pronotum has 2 pairs of elongated setae close to the front margin, 3 pairs along the rear margin, and 1 pair in the middle; there are 6–10 pairs of chalazae on each side of the dorsolateral region. The mesothorax is as wide as the metathorax, and the metanotum has 1 pair of elongated main setae next to midline and 1 pair on each side of submedium area; there are 8–10 dorsolateral chalazae. Setae are on mesonotum-like metanotum. Abdominal tergites I–VIII are with strumae, each bearing a small chalazae, 70–10, and a few long, slender collars. Ninth segment tergite is with rear edge truncate to slightly emarginated; there are 2 pairs of elongated setae next to midline, 2 pairs along rear edge, 4 pairs of chalazae on each side's dorsolateral region, and 1 pair of collar setae on each side's posterolateral area. There is

Table 1 Developmental period (days) of *C. brachylobus* immatures feeding on immatures of *B. tabaci* (days)

B. tabaci	***C. brachylobus***							
	Egg	**1st instar**	**2nd instar**	**3rd instar**	**4th instar**	**Larva**	**Pupa**	**Egg-adult**
Eggs	4.41 ± 0.17a	1.43 ± 0.11d	1.79 ± 0.08c	1.84 ± 0.09c	3.92 ± 0.13b	8.98 ± 0.04b	5.02 ± 0.13a	18.41 ± 0.14b
1st nymph	4.43 ± 0.17a	2.14 ± 0.09a	2.31 ± 0.11a	2.54 ± 0.12a	4.20 ± 0.13a	11.19 ± 0.06a	4.54 ± 0.08ab	20.16 ± 0.21a
2nd nymph	4.43 ± 0.16a	2.11 ± 0.10a	2.17 ± 0.10b	2.08 ± 0.11b	4.02 ± 0.17b	10.38 ± 0.07ab	3.97 ± 0.09b	18.78 ± 0.34b
3rd nymph	4.43 ± 0.17a	1.90 ± 0.12b	1.42 ± 0.06d	1.48 ± 0.08de	3.23 ± 0.12c	8.03 ± 0.10c	3.77 ± 0.14b	16.23 ± 0.17cd
4th nymph	4.42 ± 0.11a	1.67 ± 0.07c	1.31 ± 0.09e	1.29 ± 0.09e	3.16 ± 0.11c	7.43 ± 0.07d	3.69 ± 0.11b	15.54 ± 0.19d
Mixed nymph	4.43 ± 0.21a	1.88 ± 0.10b	1.42 ± 0.12d	1.56 ± 0.07d	3.10 ± 0.10c	7.96 ± 0.09c	3.76 ± 0.19b	16.15 ± 0.24cd
Pupa	4.43 ± 0.18a	1.93 ± 0.08b	1.48 ± 0.13d	1.54 ± 0.17d	2.98 ± 0.12d	7.93 ± 0.08c	4.98 ± 0.21a	17.34 ± 0.21c
F, d.f, *P*	15.23, 6, 0.17	23.21, 6, < 0.0001	19.64, 6, < 0.0001	12.72, 6, < 0.0001	18.23, 6, < 0.0001	32.41, 6, < 0.0001	25.91, 6, < 0.0001	43.62, 6, < 0.0001

Means (± SE) in the same column followed by different letters are significantly different from each other (Tukey's $P < 0.05$)

Table 2 Average prey consumption (mean ± SE) by *C. barchylobus* immatures

B. tabaci	***C. brachylobus***				
	1st instar	**2nd instar**	**3rd instar**	**4th instar**	**Total larval consumption**
Eggs	56.68 ± 12.26a	108.86 ± 25.27a	163.53 ± 29.54a	169.29 ± 13.50a	498.36 ± 80.57a
1st nymph	32.34 ± 13.84b	62.12 ± 28.52b	93.31 ± 13.34b	104.47 ± 18.94b	292.24 ± 94.64b
2nd nymph	30.92 ± 3.30b	59.38 ± 6.79b	89.20 ± 7.94b	97.70 ± 12.47b	277.20 ± 30.50b
3rd nymph	13.17 ± 1.72c	25.30 ± 3.54c	38.00 ± 4.14c	50.81 ± 9.01c	127.28 ± 18.41c
4th nymph	11.82 ± 2.93c	22.70 ± 6.04c	34.10 ± 7.06c	47.50 ± 5.10c	116.12 ± 21.13c
Pupa	8.60 ± 1.51c	12.50 ± 2.55d	16.20 ± 4.58d	21.60 ± 7.79d	58.80 ± 16.43d
F, d.f, *P*	41.32, 5, < 0.0001	64.91, 5, < 0.0001	42.17, 5, < 0.0001	31.82, 5, < 0.0001	52.73, 5, < 0.0001

Means (± SE) in the same column followed by different letters are significantly different from each other (Tukey's $P < 0.05$)

one extremely long chalaza on each side of the metathorax to abdominal segments I–VIII. The legs moderately elongate with few setae; the tibia is lacking terminal setae.

Growing larval head (capsule) width and body length are well associated although the head (capsule) width is less variable than the length of the body.

There were 4 distinct larval instars found. The neonate is thin, and the body gets bigger and whiter with each molt. When looking for prey, larvae move slowly, stop periodically, and move the body from side to side using uropodes in the caudal segment for processing on the surface of the leaf. Debris also builds up on the body from whiteflies, including eggshells, wax and exuviae, and exuviae from the previous larval installation.

Pupa

Newly formed pupa white had long dark setae. A tiny, clear droplet of liquid was seen adhering to the tip of each seta when the relative humidity was high. Pupa covered by whitefly debris, attached to the exuvia of the previous instar at posterior side.

Developmental periods of *C. brachylobus*

The developmental period of *C. brachylobus* immatures stages, when fed on *B. tabaci* immatures, differed significantly (Table 1). The developmental period of the 1st larval instar of *C. brachylobus* differed significantly, when fed on different life stages of *B. tabaci* (F = 23.21; d.f = 6; P < 0.001). The longest developmental period of the 1st larval instar of *C. brachylobus* (2.14 ± 0.09 days) was observed, while feeding on the 1st instar of *B. tabaci* nymphs, whereas the shortest developmental period (1.43 ± 0.11 days) was recorded, while feeding on *B. tabaci* eggs (Table 1).

The developmental period of the 2nd larval instar of *C. brachylobus* differed significantly while feeding on different life stages of *B. tabaci* (F = 19.64; d.f = 6; P < 0.001). The longest developmental period of the 2nd larval instar of *C. brachylobus* (2.31 ± 0.11 days) was observed, while feeding on the 1st instar of *B. tabaci* nymphs, whereas the shortest developmental period (1.31 ± 0.09 days) was observed, while feeding on the 4th nymphal instar of *B. tabaci* (Table 1).

The developmental period of the 3rd larval instar of *C. brachylobus* differed significantly, while feeding on different life stages of *B. tabaci* (F = 19.64; d.f = 6; P < 0.001). The longest developmental period (2.54 ± 0.12 days) for the 3rd larval instar of *C. brachylobus* was observed, while feeding on the 1st instar of *B. tabaci* nymphs, whereas the shortest developmental period (1.29 ± 0.09 days) was observed, while feeding on the 4th instar nymphs of *B. tabaci* (Table 1).

The total developmental period (egg-adult) differed significantly, while feeding on different life stages of *B. tabaci* (F = 43.62; d.f = 6; P < 0.001). Total larval developmental period was longest (11.9 ± 0.06 days), when *C. brachylobus* larvae were fed on the 1st instar of *B. tabaci* nymphs, whereas the shortest period (7.43 ± 0.07 days) was observed when larvae were fed on the 4th instar of *B. tabaci* nymphs (Table 1).

Obtained results regarding the developmental period of *C. brachylobus* feeding on *B. tabaci* immatures were different from the findings of Yazdani and Zarabi (2009). They showed that developmental periods of *Clitostethus arcuatus* fed on eggs; 1st, 2nd, 3rd, and 4th nymphal instars; and pupa of *Trialeurodicus vaporarium*

Table 3 Average prey consumption (mean ± S.E) by *C. barchylobus* adults

B. tabaci	***C. brachylobus* adults**	
	Female	**Male**
Eggs	165.08 ± 47.80a	147.24 ± 48.40a
1st nymph	84.29 ± 15.30b	83.60 ± 10.50b
2nd nymph	55.38 ± 17.01c	52.86 ± 13.71c
3rd nymph	22.22 ± 6.14d	14.48 ± 1.93d
4th nymph	13.24 ± 2.21e	12.50 ± 2.70d
Pupa	9.89 ± 1.51f	6.28 ± 1.55d
F, d.f, *P*	31.71, 5, < 0.0001	43.65, 5, < 0.0001

Means (± SE) in the same column followed by different letters are significantly different from each other (Tukey's $P < 0.05$)

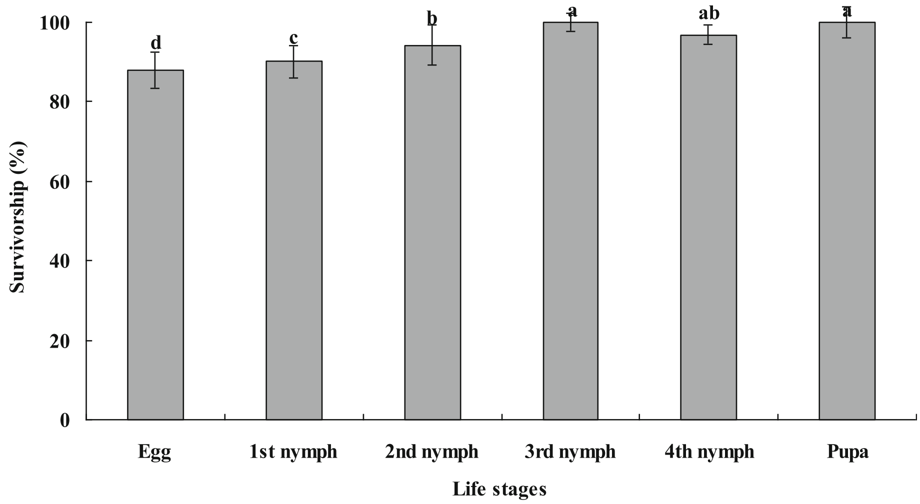

Fig. 2 Percent survival of *Clitostethus brachylobus* immatures. Bars marked by different letters are significantly different from each other (Tukey's $P < 0.05$)

(Hemiptera: Aleyrodidae) were 2.75, 4.75, 7.25, 9.5, and 4.25 days, respectively. Similar results have also been obtained by Yazdani and Zarabi (2011) for *C. arcuatus* feeding on ash whitefly, *Siphoninus phillyreae*. The said variations in results might be related to different predator or prey species.

Prey consumption of *C. brachylobus* immatures and adults

The average prey consumption by *C. brachylobus* immatures varied significantly among different treatments (Table 2). Total prey consumption by *C. brachylobus* larvae was highest (498.36 ± 80.57 individuals) when *B. tabaci* eggs were supplied as food, and the lowest (58.80 ± 16.43 individuals) when *C. brachylobus* larvae were fed on *B. tabaci* pupae. The consumption of *B. tabaci* eggs by *C. brachylobus* larval instars was 7–10 times higher than the consumption of pupae (Table 2).

The total prey consumption by *C. brachylobus* adults decreased as the whitefly immature grew in size. The *C. brachylobus* female consumed 165.08 ± 47.80 eggs, 84.29 ± 15.30 1st instar nymphs, 55.38 ± 17.01 2nd instar nymphs, 22.22 ± 6.14 3rd instar nymphs, 13.24 ± 2.21 4th instar nymphs, and 9.89 ± 1.51 pupae when different *B. tabaci* stages were supplied as food (Table 3).

C. brachylobus larvae consumed more eggs than other life stages of *B. tabaci*, and whitefly egg consumption by females was higher than males. The present study reveals that *C. brachylobus* shares many characteristics, a preference for whitefly eggs over nymphs, with *N. oculatus* and *Delphastus pusillus* (Hoelmer et al. 1993; Liu and Stansly 1999). This was similar with the results of Bathon and Pietrzik (1986). The differences in prey consumption may be related to the characteristics of the beetle population which can vary depending on the insect prey (Yazdani and Zarabi 2011).

Survival of *C. brachylobus* immatures

Percent survivorship of *C. brachylobus* immatures differed significantly among different treatments (F = 32.17; $d.f$ = 5; $P < 0.001$). Highest percent survivorship (100%) was observed at 3rd instar larva and pupa whereas the lowest survival percentage was observed at eggs, having an average survival of 88%. The survivorship for 3rd instar larva and pupa was statistically at par with each other (Fig. 2). These results are different from the findings of Huang et al. (2006) who showed that diet of eggs or nymphs of *B. tabaci* does not influence survivorship of *Axinoscymnus cardilobus* larvae; however, second instars proved to be an exception as their survival rate was significantly higher on a diet of eggs. The

Table 4 Biology and life table parameters of *C. brachylobus* feeding on *B. tabaci*

Parameters	Mean ± SE**
Adult longevity female (days)	193.50 ± 19.80
Adult longevity male (days)	175.40 ± 17.72
Pre-ovipositional period (days)	20.30 ± 1.64
Female to male ratio	0.50
Average number of eggs laid/female	154.70 ± 17.81
Net reproductive rate (R_0)	53.60
Mean generation time (T)	102.64
Intrinsic rate of increase (r_m)	0.0381
Finite rate of increase (λ)	1.0395

**± S.E stands for standard error of means based on five replicates

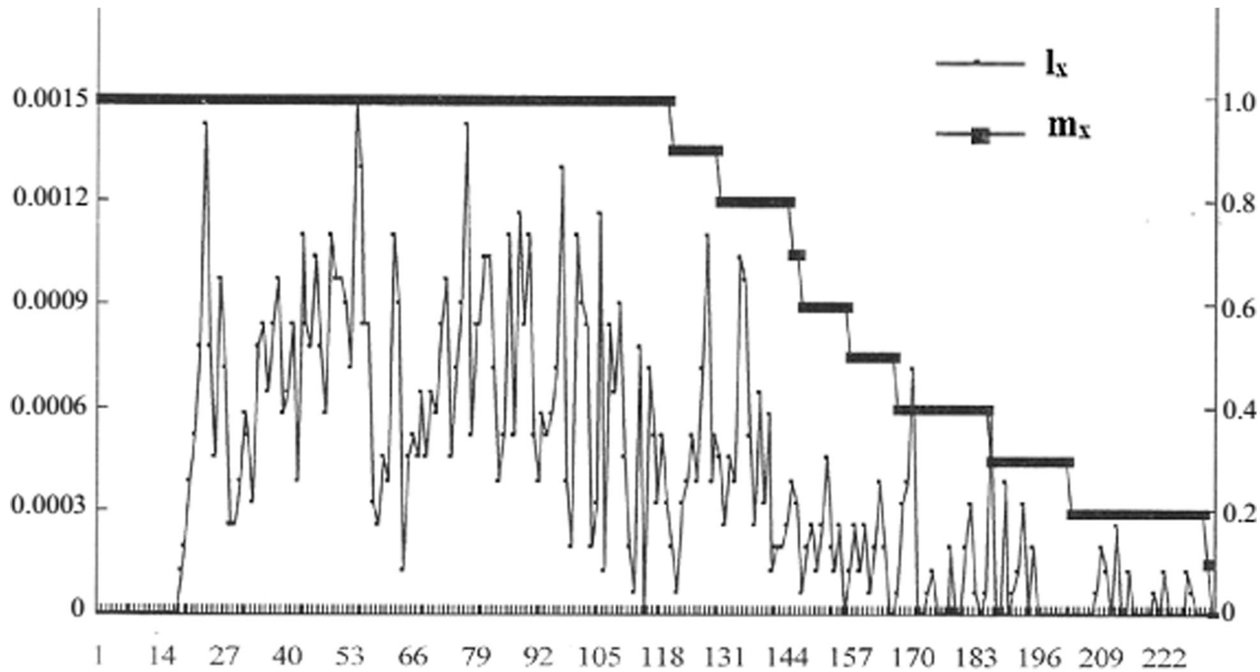

Fig. 3 Trends of age-specific survival rate (l_x) and age-specific fecundity (m_x) of *C. brachylobus* adult on whitefly immatures

differences in survival may be related to the characteristics of the beetle population which can vary depending on the insect prey (Huang et al. 2006).

Life history of *C. brachylobus*

Biological characteristics of *C. brachylobus* are presented in Table 4 and Fig. 3. The adult beetle ovipositional and daily survival rate data showed that survival rates steadily dropped from the 120th day of adult emergence. The line shows a triangular pattern for egg laying period (Fig. 3). The average female longevity was 193.5 days whereas the pre-ovipositional period of *C. brachylobus* female was 20.30. The average female fecundity was 154.70 eggs/female. The net reproductive rate (R_0) calculated through the life table analysis was 53.60, whereas the mean generation time (T) was 102.64 days (Table 4).

The average longevity of *C. brachylobus* adults (male and female) observed was statistically at par with Mesbah (2000) who reported male and female longevity of 192.2 and 207 days, respectively, of *C. arcuatus* fed on *B. tabaci*. Obtained findings generally agree with other data of Mesbah (2001), who found females lived longer than males.

Life table parameters of *C. brachylobus* were different from those observed for *C. arcuatus* by Yazdani and Samih (2012). It is well known that decrease in intrinsic rate of increase can result in large changes in pest population. Obtained results suggest the release of large numbers of *C. brachylobus* for whitefly management to obtain information on development *C. brachylobus* population in a specific period of time.

Conclusion

C. brachylobus showed an efficient prey consumption against *B. tabaci* suggesting the usage of *C. brachylobus* in integrated *B. tabaci* management programs. However, further work on the potential of *C. brachylobus* under natural conditions and studies on the compatibility of this ladybird beetle with other biocontrol agents are still required.

Abbreviations

Ø: Diameter; R_0: Net reproductive rate; T: Mean generation time; r_m: Intrinsic rate of increase; λ: Finite rate of increase

Authors' contributions

W.X.M. analyzed the data and wrote the paper. D.H. and D.C.L performed the experiments and data curation and took the pictures. S.A. designed the study and revised the final draft. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

The data and material used during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

We agree to all concerned regulations.

Consent for publication
We agree to publish this scientific paper in the EJBPC.

Competing interests
The authors declare no conflict of interest.

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