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Original Article

Development, reproduction and longevity of *Aprostocetus* sp. (Hymenoptera: Eulophidae), an egg parasitoid of the Brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae)

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ABSTRACT

Aprostocetus sp. (Hymenoptera: Eulophidae) is an egg parasitoid of *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae). This parasitoid was surveyed and collected from paddy fields in Nonthaburi and Pathum Thani provinces, Thailand. *Aprostocetus* sp. was reared on *N. lugens* eggs under laboratory conditions at the National Biological Control Research Center, Kasetsart University, Bangkok, Thailand. The development time, reproductive performance and adult longevity of *Aprostocetus* sp. were investigated. The development time of *Aprostocetus* sp. took 12–19 d; the mean development time of female and male wasps was 15.4 ± 1.3 and 14.8 ± 1.3 d, respectively. The mean daily offspring production was 3.5 ± 0.6 wasps; total offspring production was 74.7 ± 33 wasps; and the sex ratio (female to male) was 1.8:1. The longevity of adult female wasps fed with honey and without honey was 5.3 ± 0.88 and 1.9 ± 0.14 d, respectively. The longevity of adult male wasps fed with honey and without honey was 1.9 ± 1.2 and 1.4 ± 0.5 d, respectively. The longevity was significantly ($p < 0.05$) different between food sources for both females and males.

Introduction

The Brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) is the most serious rice insect pest worldwide with severe outbreaks in many countries of Asia (Dyck and Thomas, 1979; Pathak and Khan, 1994) including Thailand (Escalada et al., 2012; Sriratanasak et al., 2011).

N. lugens feeds on rice stems directly and it can transmit viral diseases to rice including rice grassy stunt or rice ragged stunt disease (Dale, 1994). Symptoms of rice infestation by *N. lugens* include: 1) fewer panicles and grains; 2) lower percentages of ripened grains and gram weight; and 3) high-yield loss from “hopperburn” (Sogawa and Cheng, 1979).

Controlling *N. lugens* by integrated pest management includes cultural control (Oka, 1979), biological control (Chiu, 1979), chemical control (Heinrichs, 1979) and varietal resistance (Khush, 1979; Pathak and Khush, 1979; Wei et al., 2009), which might be the best choice for *N. lugens* control (Plantwise Knowledge Bank, 2014) though an insecticidal control method has been most common. However, repeated application of insecticides can cause *N. lugens* to develop resistance to insecticides with additional outbreaks (Basanth et al., 2013) while natural enemies were also damaged from insecticidal usage.

Natural enemies in paddy fields have a role in controlling insect pests of rice. Many natural enemies of *N. lugens* are always present in paddy fields especially *Anagrus* spp. (Hymenoptera: Mymaridae) and *Oligosita* spp. (Hymenoptera: Trichogrammatidae), which are dominant parasitoids of *N. lugens* (Chiu, 1979; Yasumatsu et al., 1983; Watanabe et al., 1992; Gurr et al., 2011). Important predators include *Cyrtorhinus lividipennis* Reuter (Hemiptera: Miridae) and *Pardosa psuedoannulata* (Araneae: Lycosidae) (Heong et al., 1992; Ooi and Waage, 1994; Lu et al., 2006).

The eulophid wasp *Aprostocetus* sp. (Hymenoptera: Eulophidae) is an egg parasitoid of *N. lugens* that is always found in paddy fields but often it has been overlooked. Little information concerning its biology and behavior is available. Thus, new information concerning *Aprostocetus* sp. must be collected to enhance its use in biological control programs in paddy fields.

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Materials and methods

Experimental preparation

Rice KDML 105 and RD7 (susceptible varieties) were planted under natural light in a greenhouse. KDML 105 was used for mass-rearing *N. lugens* and the RD7 variety was used for the development time and reproductive performance tests. Rice plants were grown every week and rice aged 21–40 d was used for the tests.

N. lugens was collected from paddy fields in Nonthaburi province, Thailand. Nymphs and adults were kept in cages and brought to a greenhouse for mass rearing. Gravid females of *N. lugens* were released in cages containing rice plants. These females produced eggs used to mass rear the parasitoid. Host egg cages were provided every week.

The egg parasitoid *Aprostocetus* sp. was surveyed and collected from paddy rice fields in Nonthaburi and Pathum Thani provinces, Thailand using KDML 105 rice plants with *N. lugens* eggs as oviposition bait. Unparasitized and parasitized *N. lugens* eggs were brought to a laboratory at the National Biological Control Research Center at Kasetsart University, Bangkok, Thailand. Unparasitized and parasitized *N. lugens* eggs were placed in cages. Parasitoids were kept after emergence from host eggs and subsequently moved to new cages which contained rice plants with *N. lugens* eggs. Maintenance of *Aprostocetus* sp. was continued under laboratory room conditions.

Study on development of *Aprostocetus* sp.

Three brachypterous, gravid females of *N. lugens* were placed inside test tubes (2.5 cm diameter and 15 cm long) containing rice plants for 24 h. This allowed *N. lugens* to lay eggs for 24 h before the release of *Aprostocetus* sp. One female (Fig. 1A) and one male (Fig. 1B) of *Aprostocetus* sp. were released into the test tubes to initiate the parasitism of *N. lugens* eggs. Parasitoids were removed from the test tubes after 24 h. Parasitized egg masses were observed under a microscope every day until all adult parasitoids had emerged. The test tubes of parasitized *N. lugens* were observed about 5 times daily (100 tubes in total). The development time was recorded.

Reproductive performance

Three brachypterous, gravid females of *N. lugens* were released into test tubes (2.5 cm diameter and 15 cm long) containing rice plants for 24 h. This allowed *N. lugens* to lay eggs for 24 h before the release of *Aprostocetus* sp. Male and female adults of *Aprostocetus* sp. were released in the test tubes with host eggs during the first day. The female parasitoid was moved to new host eggs tube every day until the female parasitoid died. This promoted the parasitism of *N. lugens* eggs. Emerged parasitoids were counted and the sex ratio was calculated.

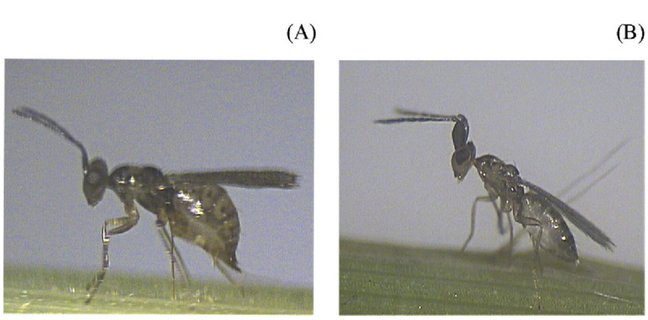

Fig. 1. Adults of the parasitoid *Aprostocetus* sp.: (A) female (17×); (B) male (20×).

Longevity

After *Aprostocetus* sp. emerged from host eggs, the male and female adults were fed with honey while some were not fed honey. The survival time of each female and male was recorded.

Statistical analysis

Data were analyzed using Student's t test in the IBM SPSS Statistics software (Version 20; Armonk, NY, USA) for comparison treatments among longevity tests.

Results

Development time

After 2 d of parasitism, a black scar appeared on the operculum of parasitized eggs of *N. lugens* and the parasitized eggs became brownish. After 3 d of parasitism, *Aprostocetus* sp. larvae were visible inside *N. lugens* eggs (Fig. 2A).

After 4 d of parasitism (parasitoid release), the parasitoid larvae hatched from the host eggs (Fig. 2B). The larvae fed internally on the host egg contents, then proceeded to feed externally on nearby host eggs until the parasitoid larvae pupated. The last larval stage of the parasitoid became orange-red; some larvae became orange 5 d after parasitism and the body was short (Fig. 2C). Emerged larvae required 2–4 d before pupation. One larval parasitoid emerged from one host egg.

Then, 6 d following parasitism (pupae aged 1–2 d), the body appeared translucent or transparent (Fig. 2D); a red eye-spot appeared during the third day. After 6–7 d, the body became dark with an enlarged scape in male antennae, and the female ovipositor became visible (Fig. 2E). Adults of *Aprostocetus* sp. emerged 13–16 d after parasitism (Fig. 2F).

The pupal stage of *Aprostocetus* sp. required about 7 d (6–15 d after parasitism). The total development time of *Aprostocetus* sp. (from oviposition to adult emergence) required about 13–16 d (Table 1).

Reproductive performance

Aprostocetus sp. began ovipositing eggs during the first day following emergence. Female longevity was 21.4 ± 8.4 d and the oviposition period was 20.7 ± 8.2 d.

One female adult of *Aprostocetus* sp. produced an average of 3.5 ± 0.6 offspring per day resulting in total offspring of 74.7 ± 33 per female wasp. The proportion of females to males was 1.8:1. The development of *Aprostocetus* sp. from egg to adult required 12–19 d (Fig. 3); female and male development averaged 15.4 ± 1.3 d and 14.8 ± 1.3 d, respectively.

Longevity of adults, *Aprostocetus* sp.

The longevity of female and male adult *Aprostocetus* sp. fed with honey was significantly longer than individuals deprived of honey. The mean longevity of females fed with honey and without honey was 5.3 ± 0.88 d and 1.9 ± 0.14 d, respectively. The mean longevity for males fed with honey and without honey was 1.9 ± 0.18 d and 1.4 ± 0.08 d, respectively (Table 2).

Discussion

Aprostocetus sp. is a solitary egg parasitoid because one progeny was produced from one parasitized egg of *N. lugens*.

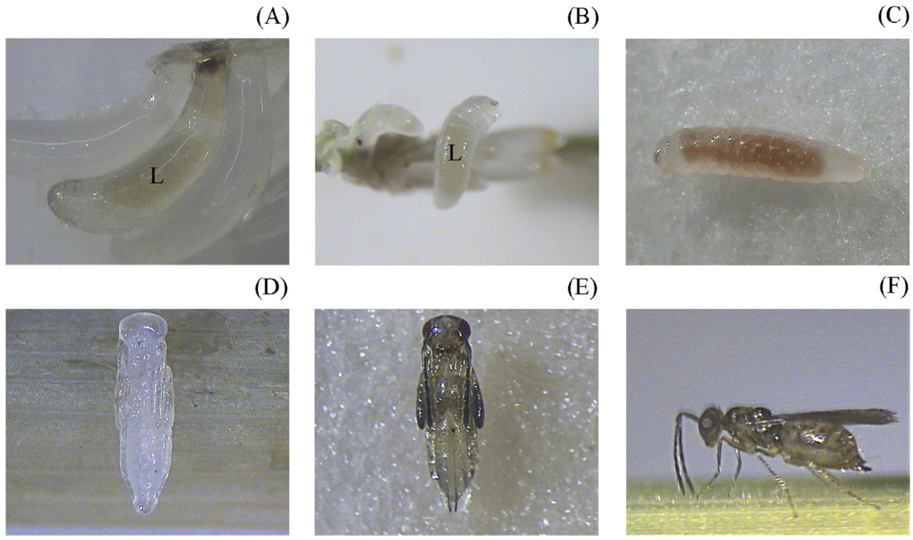

Fig. 2. Development of *Aprostocetus* sp.: (A) L = young larva of *Aprostocetus* sp. visible inside the egg of *Nilaparvata lugens* (3 d after parasitism, magnification 60×); (B) L = emerged-larva (4 d after parasitism, magnification ×20); (C) emerged larva (6 d after parasitism, magnification 20×); (D) pupa (aged 1 d, magnification 20×); (E) female pupa (aged 6 d, magnification 20×); (F) female adult (magnification 20×).

Table 1
Developmental stages of *Aprostocetus* sp. on the eggs of *Nilaparvata lugens* (Stål), n = number of *Aprostocetus* sp.

Days after parasitism	Stage	n	Characteristics
1–3	–	24	Parasitoids develop inside *N. lugens* eggs
4–9	Larva	48	First and second day larvae: larval body translucent to yellowish with 11 segments, mandible visible
			Third day larvae: yellow-orange body
			Fourth day larvae: orange-red body; short body with slow movement
6–15	Pupa	77	First and second day pupae: body translucent.
			Third day pupae: red eye and appendages visible
			Fourth to fifth day pupae: translucent bodies become yellowish and mandibles visible
			Sixth to seventh day pupae: bodies become dark, antennal sexual dimorphism apparent Ovipositor becomes apparent
13–16	Adult	47	Emergence.

The Aprostocetus sp. female adult examined the host eggs before depositing her eggs. Examining the host egg is a common characteristic of all egg parasitoids including Eulophidae (Ahmed, 2008).

Aprostocetus sp. female adults that host-fed on host eggs lived an average of 21.4 ± 8.4 d longer than females fed on honey only (average 5.3 ± 0.88 days) indicating that feeding results in increased longevity of female adults. It was concluded that providing host eggs can be an advantage to mass rearing the parasitoid in the laboratory. Feeding of *Aprostocetus* sp. females might be necessary for egg production, as generally, females need nutrient for egg production (Ahmed, 2008). Female and male adult

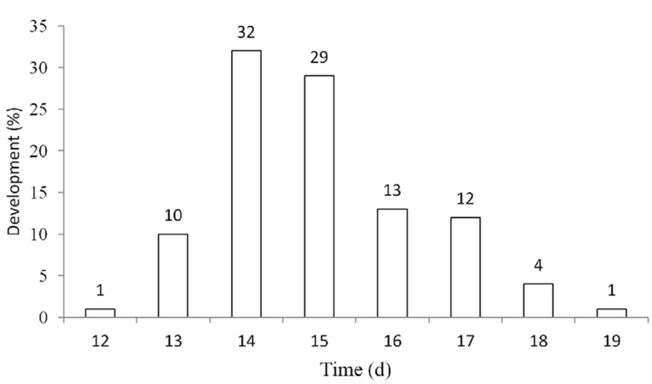

Fig. 3. Percentage of adult progeny emerging 12–19 d after parasitism.

Table 2
Longevity of female and male adults of *Aprostocetus* sp. fed honey and deprived of honey, n = number of *Aprostocetus* sp.

	Longevity (d)			
	Females		Males	
	With honey	Without honey	With honey	Without honey
	(n = 48)	(n = 42)	(n = 50)	(n = 50)
Range	1–37	1–4	1–8	1–3
Mean[a] ± SE	5.3 ± 0.88	1.9 ± 0.14	1.9 ± 0.18	1.4 ± 0.08

[a] Means are significantly different between food sources for both females and males at $p < 0.05$.

parasitoids kept under room conditions in a laboratory (25 ± 2 °C) without host eggs but fed honey lived longer than wasps deprived of food.

Aprostocetus sp. females produced progeny throughout their lifetime and lived an average of 20.7 ± 8.2 d.

Aprostocetus sp. larvae fed externally on *N. lugens* eggs for 5 d until pupation. Many *N. lugens* eggs were consumed by each larval parasitoid (number of host eggs consumed was not recorded), so *Aprostocetus* sp. larvae showed predatory behavior. This characteristic of *Aprostocetus* sp. larvae in the current study agreed with Jacas et al. (2005) who reported that *Aprostocetus vaquitarum* Wolcott (Hymenoptera: Eulophidae) was a predator of *Diaprepes abbreviates* (Linnaeus) (Coleoptera: Curculionidae). *Aprostocetus* sp. was a predator, like other species of this genus. One adult female of

Aprostocetus sp. can produce an average of 74.7 ± 33 offspring during her lifetime. These results suggest improved efficiency in the control of *N. lugens*. The numbers of larval instars were not investigated, but Lee et al. (2010) and Llácer et al. (2005) reported that tetrastichine Eulophidae display 3–4 larval instars.

Male adults of *Aprostocetus* sp. emerged (14.8 ± 1.3 d) before female adults (15.4 ± 1.3 d) in common with other parasitoids and this is presumably an advantage for mating immediately following female emergence (Macdonald and Caveney, 2004).

Aprostocetus sp. is an egg parasitoid of *N. lugens*. Previous reports about this parasitoid did not mention the Brown planthopper as a host. Pathak and Khan (1994) and Gurr et al. (2011) reported that *Ootetrastichus* and *Tetrastichus* were egg parasitoids of *N. lugens* and *Sogatella furcellata* (Horvoth) (Hemiptera: Delphacidae) and distributed in many countries including Thailand. Vungsilabutr et al. (2002) and Sriratanasak et al. (2007) reported that *Tetrastichus* spp. (Eulophidae) was an important ectoparasitoid that consumed *N. lugens* eggs. Furthermore, Nacro and Nénon (2009) and Heinrichs and Barrion (2004) reported that *Aprostocetus procerae* Risbec (Hymenoptera: Eulophidae) was a solitary pupal parasitoid of the rice gall midge, *Orseolia oryzivora* Harris and Gagné (Diptera: Cecidomyiidae). Sriratanasak et al. (2007) also reported that *Aprostocetus* sp. attacked pupae of the rice gall midge, *Orseolia orysae* (Wood–Mason) (Diptera: Cecidomyiidae).

The number of parasitized eggs or eggs that were consumed by *Aprostocetus* sp. were not counted in the current study, so this should be investigated because it would assist in forecasting mortality of the Brown planthopper, *N. lugens* in paddy fields.

The identification of the parasitoid, *Aprostocetus* sp. was provided by Dr. Charuwat Taekul, a taxonomist from the Entomology & Zoology Group, Plant Protection Research and Development Office, Department of Agriculture, Ministry of Agriculture and Cooperative, Bangkok, Thailand. Voucher specimens were deposited at National Biological Control Research Center, Kasetsart University, Bangkok, Thailand.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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