Morphology and Development of *Tetrastichus* sp. (Hymenoptera: Eulophidae), Parasitizing Fallwebworm Pupae, *Hyphantria cunea* Drury (Lepidoptera: Arctiidae)

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Key Words:
Tetrastichus sp.
Fecundity
Sex ratio
Development
Hyphantria cunea
Eulophidae

The life history and development of *Tetrastichus* sp. parasitizing fallwebworm pupa in Korea were studied. The mean lifetime fecundity was 94.7 with a maximum of 125, and the portion of female progeny averaged 90.3%. A male and a female lived for an average of 19.8±4.5 and 21.7±4.2 days, respectively. The duration of development for each stage was as follows: egg, 2 d; the 1st instar larva, 2±1 d; the 2nd instar larva, 2±1 d; the 3rd instar larva, 3±1 d; the 4th instar larva, 6±2 d; and pupa, 8±2 d. Total developmental duration from hatching of the larva to adult emergence required 21.1±3.7 d, and females took 1-2 days more than males in the incubator (28℃, 70% RH, and 16:8 LD).

Fallwebworm, *Hyphantria cunea* Drury, is native to North America, and it was introduced accidently into Korea in 1958 (Kim and Lee, 1982). Since then, it has spread throughout the country rapidly and caused great economic damage mainly to roadside and garden trees. Although chemical control has been the major method for controlling the pest, natural predators also have played an important role in suppressing fallwebworm populations. Since the pest was introduced, some parasites and predators naturally occurring in Korea have adapted to the fallwebworm life system and those natural enemies currently are major factors that suppress fallwebworm populations below outbreak levels. This case is one successful example of an exotic species being controlled by indigenous natural enemies. The native natural enemies, both parasites and predators of fallwebworm, were scarab beetles, true bugs, tachinid flies, and parasitic wasps (Kim et al., 1967, 1968). Kim and Lee (1982) reported seven species of hymenopterous parasitoids and two species of tachinid flies reared from overwintering pupae of the fallwebworm. Parasitic Hymenoptera included four species of Ichneumonidae, one of Pteromalidae, one of Torymidae, and one of Chalcididae.

Recently we found another hymenopterous parasitoid emerging from the fallwebworm pupae collected in Sinsa-dong, Seoul, in feburary, 1997. The parasitic wasp was a species of the genus *Tetrastichus* belonging to Family Eulophidae. The species is a pupal endoparasitoid and is gregarious with a group of wasps developing and emerging from one host pupa.

The biology of several species of *Tetrastichus* has been investigated. Sharma et al. (1965) reported on the biology of *T. rhipiphorothripscidis*, a parasite of *Mallothrips indicus*. Streams and Fuester (1967) reported on the reproduction and developmental biology of *T. incertus*, which is a larval parasitoid of alfalfa weevil. Hamerski and Hall (1988) reported on the developmental times at different temperatures, sex ratio, and the rate of parasitization of *T. gallerucae*, an egg parasitoid of the elm leaf beetle. Some species of *Tetrastichus* had been introduced in a few countries as biocontrol agents: for example, in South Africa, *T. howardi* as a potential biocontrol agent of several stem borers; and in the U.S., *T. julis* for the control of the cereal leaf beetle and *T. incertus* to control from South Africa alfalfa weevil in 1960s.

This study of *Tetrastichus* is the first report on the biology of *Tetrastichus* sp. parasitizing *H. cunea* pupa in Korea. In this paper, we studied its longevity, fecundity, sex ratio, and developmental time, and described its morphological characteristics.

Materials and Methods

Rearing Tetrastichus sp. and maintaining host pupa

The rearing of the parasitoid and the host was conducted in laboratory under controlled temperatures. The controlled conditions were temperature of 27±

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2℃, 40-50% relative humidity, and 16 h light : 8 h dark. The parasitoids used were originally collected from fallwebworm pupae collected at Sinsa-dong, Seoul, in Febuary, 1997.

The parasite was identified only to the genus level as *Tetrastichus*, and we had taxonomical suggestions on the specimen from several experts in Eulophid taxonomy. Therefore we use *Tetrastichus* sp. as the name of the species in this report, and the taxonomical study is under investigation.[1)]

To maintain a *Tetrastichus* sp. colony, 5 or 6 pairs of adults were placed in plastic vials, and provided with a small amount of 10% diluted honey on the inner wall of the vial. One or two host pupae were also provided. Parasitized pupae were placed in the incubator at 28℃, 70% RH, and 16:8 LD.

Fallwebworm pupae were used as hosts for *Tetrastichus* sp. Some of the host pupae were reared in laboratory, and others were collected from field. The pupal sizes were so varied that it was necessary to select pupae of similar size, because some pupal parasitoids are known to be affected by their fecundities, sex ratios, and sex allocations depending on the host size and type (Quicke, 1997). Pupae about 1.1 cm in length were used.

Host pupae were kept at 4℃ to prevent them from developing further. Refrigerated host pupae, however, had to be used within two weeks from initial refrigeration because pupae kept for more than 21 d usually desiccated and chitinized, which was unfavorable to the *Tetrastichus* sp. or resulted in stunted parasites when parasitization was successful (Legner, 1979).

Development of Tetrastichus sp.

To observe the developmental biology of *Tetrastichus* sp., three to five mated females were transferred to each of 30 plastic vials with one host pupa. The parasitoids were allowed to oviposit for 24 h and then removed. The pupae in each vial were placed in the incubator. Total of 30 pupae were used. To observe how the parasite develops in host pupae, one parasitized pupa was dissected daily. The dissected pupa was washed with physiological saline solution to separate parasite stages (egg, larva, and pupa) from the host material. Among many parasites developing in a pupa, 20 were chosen randomly at each dissection and examined under a microscope.

A larva was measured in body length and the size of mandibles. The body length was measured from the head to the end of body (40x magnification). The mandible size was measured for maximum both in length and in width in order to distinguish instars (400x magnification).

Measuring the mandible size requires clearing the larval specimen. Pupa and adult were measured for body length from the vertex to the end of the last abdominal segment. Males and females were distinguishable from the pupal stage. Thus male and female pupae were measured in the same way as adults.

Fecundity, sex ratio, and longevity

Ten pairs of *Tetrastichus* sp. that were one day old after emergence were separated and each pair were placed into a plastic vial stoppered with a paper lid. They were provided with 10% diluted honey and an unparasitized *H. cunea* pupa every 24 h until the female died. After each 24 h period, the parasitized pupae were gathered and kept in the incubator until the parasite progeny emerged. The emerged progeny were counted and sex ratio (female: male) was calculated.

To investigate fecundity, another ten pairs of the wasp were used in the same way. The parasitized host pupae were dissected after 3 to 5 d from oviposition, and the larvae of the parasitoid were counted. Observations of longevity were conducted during the fecundity experiment. During the experiment, the date of death was recorded.

Results and Discussion

Description of stages and development

Total developmental duration of *Tetrastichus* sp. was 21.1±3.7 days in incubator conditions (28℃, 70 RH). Kfir et al. (1993) reared *T. howardi* on different hosts at various temperatures (20-32℃), and at 28℃, total developmental duration of *T. howardi* was 15.95±3.09 days. In *Tetrastichus* sp., it took almost 5 days longer than that of *T. howardi*. There is a possibility that the species may not be *T. howardi* but a different species.

Adult: This *Tetrastichus* sp. was similar to *T. howardi*, which has two setae on the submarginal vein (SMV), a dark fore coxa, and a distinct median carina in the dorsellum. However, this species has a pale fore coxa. This may represent variation or indicate the presence of a distinct species. Boucek (1988) synonymized *T. ayyari* and *T. israeli* with *T. howardi*. If it is eventually proved that there are more than one species under the name of *howardi*, one of these names may be valid. Further taxonomic study is necessary to identify this species and suggest whether it is a native species or an introduction.

The female (Fig. 1A) with metallic green highlights, is delicately reticulated in thorax, and has a dark brown abdomen. Antennae have 3 funicles and clava (3-segmented) are dark brown. There is a median line in the middle of the scutellum and 2 submedian lines in the scutum. Legs are yellowish or pale and tarsi are 4 segmented. Females vary from 1.63 to 2.02 mm in length (avg. 1.81 mm). The male is smaller (avg. 1.30 mm) and may be easily distinguished from the female by the swollen scapes (in lateral view) and clava (Fig. 1B), which are only dark brown at the antennae.

1) Voucher specimens for taxonomic purposes deposited with Natural History Museum at Dept. of Applied Biology, Dongguk University.

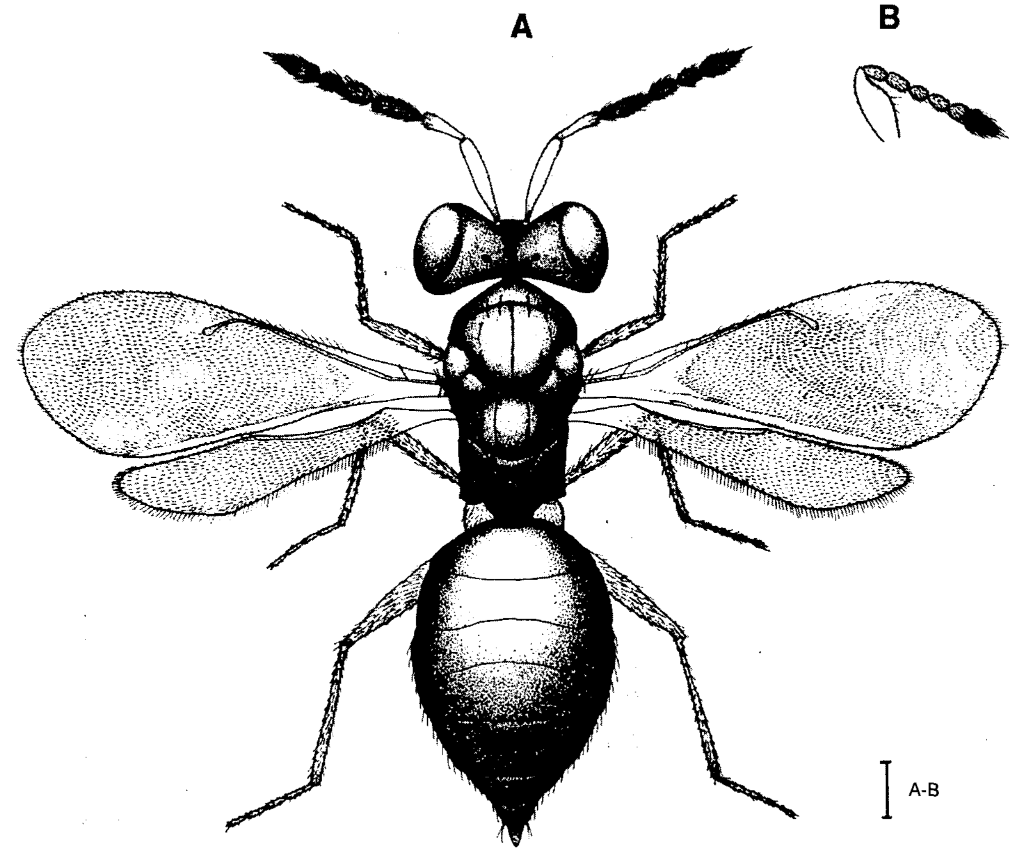

Fig. 1. *Tetrastichus* sp.; A, Adult female. B, Antenna of male in lateral view. Scale bar=0.15 mm.

Egg: The egg of *Tetrastichus* sp. is elongated and milk-white oval, measuring an average of 0.32 mm in length. An anterior region is tapered and transparent, and the central region is white and opaque (Fig. 2A). According to Gerling and Legner (1968), embryonic development takes place within the white middle section of the egg. Eclosion took place 2 days after oviposition at 28℃, while *T. incertus* takes 47-60 h to eclose at 26±1℃ (Streams and Fuester, 1967).

The 1st instar: The 1st-instar larva was first seen on the second day after oviposition. Body length averaged 1.18±0.15 mm (Table 1). Although the 1st instar larva has body segments, they were not clearly distinguishable. The opaque-white digestive tract and mid-gut, translucent mandibles, and tracheal system were visible. Spiracles of 1st-instar larva could be seen clearly only if body fluid was removed, but they did not seem to be developed completely. The tracheal system was simple. The mandibles averaged 9.86±1.11 µm in length and 8.56±0.99 µm in width (Table 2; Fig. 3). The mandibles of the 1st-instar larva were translucent, not particularly brown-colored at the tip yet. Development to the 2nd-instar larva required about 1 to 3 days.

The 2nd instar: The 2nd-instar larva could be found from the 4th to the 5th day after oviposition. It was large enough to be observed with the naked eye. At the 2nd-instar, larval structure became more complicated. There were 9 pairs of spiracles, one on each body segment from the 2nd through 10th. The tracheal system seemed to be more complicated when compared to the 1st-instar larva. The appearance of the 2nd-instar larva was generally cloudy and translucent, with the exception of a large, median, and ovoid area which was opaque white. The different color of this

Table 1. Measurement (mm, x±SD) of the egg, larva, pupa, and adult of *Tetrastichus* sp. reared from *Hyphantria cunea* pupa (n=20)

Egg		Larval instars				Pupa		Adult	
Length	Width	1st	2nd	3rd	4th	Female	Male	Female	Male
0.32±0	0.10±0.01	1.18±0.15	1.37±0.12	1.90±0.21	2.77±0.51	2.17±0.10	1.85±0.05	1.96±0.12	1.56±0.10

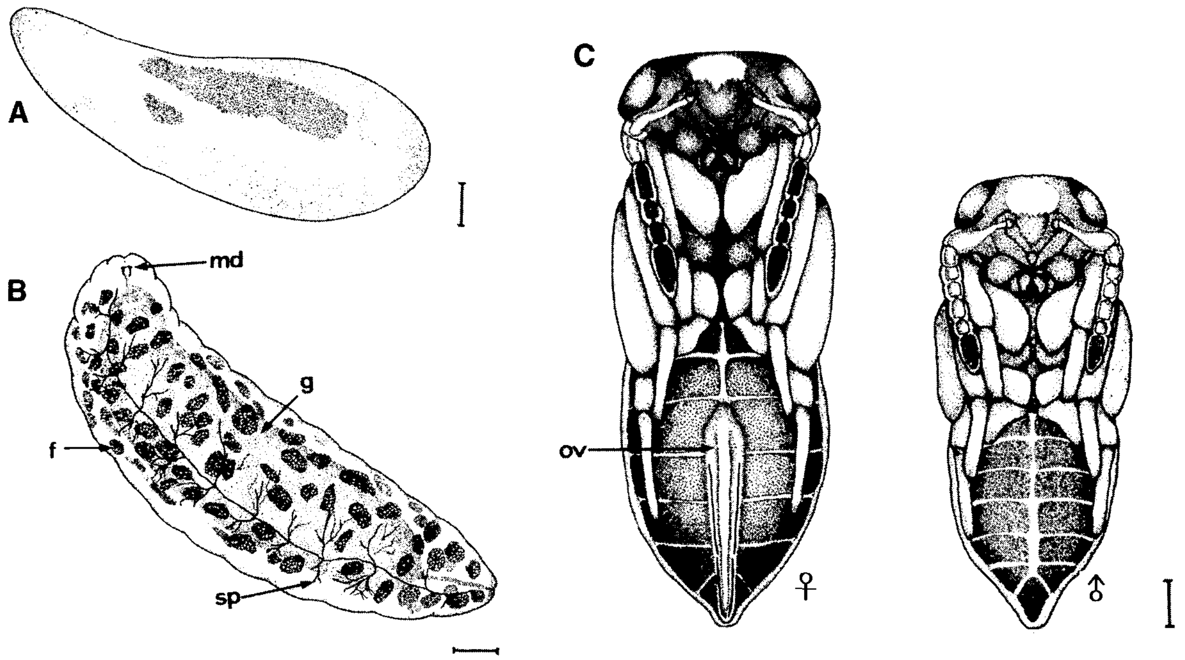

Fig. 2. The developmental stages of *Tetrastichus* sp. A, Newly deposited egg. B, The 4th-instar larva. C, Pupae. f, fat body. g, gut. md, mandible. ov, ovipositor, sp, spiracle. Scale bars=0.01 mm (A), 0.15 mm (C), and 0.20 mm (B).

area was due to the presence of whitish food in the alimentary tract. Body length averaged 1.37±0.12 mm (Table 1), and mandible size averaged 19.58±1.58 µm in length and 14.72±1.54 µm in width (Table 2; Fig. 3). The mandibles seemed to be sharper than those of the 1st-instar larva, but they were still translucent. Development to the 3rd-instar larva required 1 to 3 days.

The 3rd instar: The 3rd-instar larva was seen from the 3rd to the 6th day. It was likely to be a faint brown, but the body color seemed to depend on the amont of food the larvae had ingested and digested. The 3rd-instar larva closely resembled the 2nd except that the tips of mandibles which became brown, were no longer translucent, and were much larger. The body length averaged 1.90±0.21 mm (Table 1), and mandible size averaged 25.12±1.89 µm in length and 20.62±1.11 µm in width (Table 2; Fig. 3). Development to the 4th-instar larva required 2 to 4 days.

Fully developed 3rd-instars began to have small fat bodies distributed in the body region.

The 4th instar and prepupa: The 4th-instar larva was seen from the 5th to the 11th day. The fat bodies began to be observed in this stage. The fat bodies were distributed sparsely throughout the body (Fig. 2B). The mature 4th-instar averaged 2.77±0.51 mm in length, and the mandibles averaged 30.25±1.97 µm in length and 25.62±1.37 µm in width (Table 2). The mandibles were much bigger and the tips were much sharper and longer than those of the 3rd-instar larva (Fig. 3). The 4th-instar larva lasted 6±2 days.

The fully mature 4th-instar larva began to excrete brown meconium before it became a prepupa. From the prepupa stage, the body had three portions: head, thorax, and abdomen. The prepupa was milky white or yellowish, and slightly smaller than the 4th-instar larva. This period lasted approximately 2 days.

Table 2. Larval mandible size (µm, $\bar{x}\pm$SD) of *Tetrastichus* sp. reared from *Hyphantria cunea* pupa (n=20)

Mandible size	Measurement of the larval mandibles (Mean±SD)			
	1st instar	2nd instar	3rd instar	4th instar
Length	9.86±1.11	19.58±1.58	25.12±1.89	30.25±1.97
Width	8.55±0.99	14.72±1.54	20.62±1.11	25.62±1.37

Pupa: The body length of the female pupa averaged 2.17±0.10 mm, and 1.85±0.00 mm in the male (Table 1). At the beginning, the pupa was white or yellowish. As it developed, its sex was distinguishable. The male pupa was smaller than the female and had differences in its antennae and abdomen from the female. During development, only the antennal clava of the male pupa were dark brown, whereas the female had dark brown funicles and clava. The ovipositor could be seen on the middle abdomen of the female (Fig. 2C). From the 2nd day after pupation, the eyes of the pupa began to turn red and became darker thereafter. The abdomen, thorax, and part of the head became black on the 4th day. Thereafter, the antennal funicles began to turn black. This process occurred faster in the male than

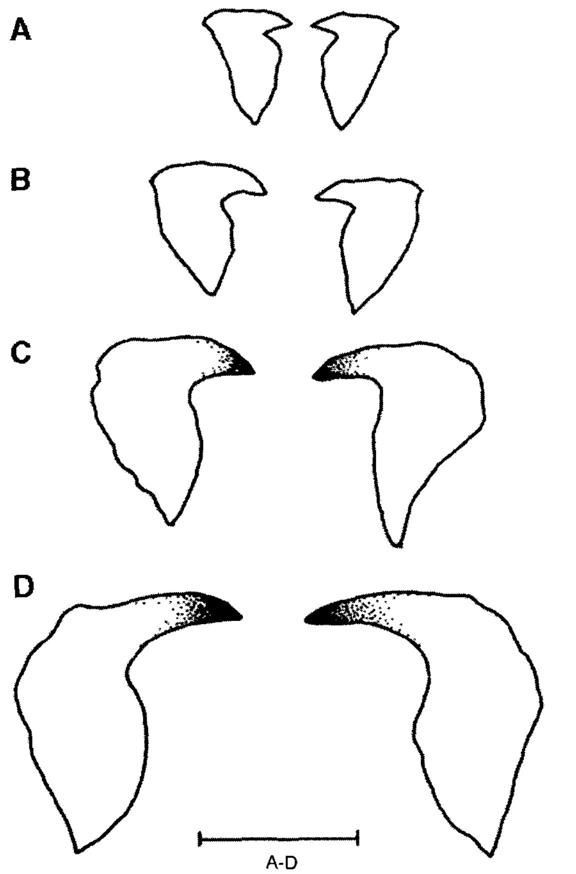

Fig. 3. Mandibles of larvae of *Tetrastichus* sp. A, 1st instar. B, 2nd instar. C, 3rd instar. D, 4th instar. Scale bar=0.03 mm.

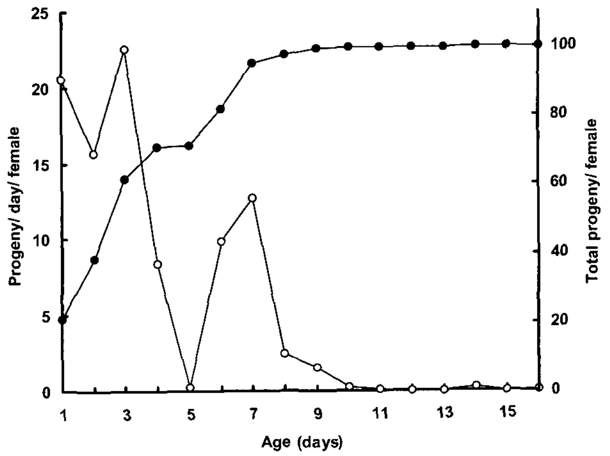

Fig. 4. Daily (○) and cumulative (●) fecundity of *Tetrastichus* sp. females.

the female. The pupal period lasted 6 to 10 days (Table 3).

Adult emergence: It was observed that the adult stayed about 1-2 days in the host pupa and then escaped through one or two emergence holes on the body wall of the host.

Fecundity, sex ratio, and longevity

A female of *Tetrastichus* sp. produced an average of 94.7±36.0 progeny during her lifetime (Table 4). The largest number of progeny was 125 and the smallest, 34. Kfir et al. (1993) reported that a female of *T. howardi* produced an average of 101 progeny. This was similar to the fecundity of *Tetrastichus* sp. However, there was a wide difference, compared with other species. Streams and Fuester (1967) reported that a female of *T. incertus* produced an average of 342.6 progeny. That was considerably greater than the reported fecundity of many other species of parasites.

Table 3. Developmental period (days) of *Tetrastichus* sp. from the egg to pupa (n=20)

Egg	Larval instars				Pupa	Adult
	1st	2nd	3rd	4th		
2	2±1	2±1	3±1	4±2	8±2	21.1±3.7

The oviposition period of *Tetrastichus* sp. lasted for an average of 7.4 days after adult emergence. Five females (out of 10) began to oviposit from the first day after emergence. More than four or five min were required for ovipositor insertion. The entire oviposition process was usually completed within 10-15 min. The results indicated that oviposition was concentrated at the first to the third times, and then, declined significantly. After the 9th day, females usually did not oviposit (Fig. 4), and it was observed that the abdomen of female became flat in the lateral view.

Additionally, a female consumed an average of 4 pupae (range, 1-7) for oviposition during a life time, even though just one host pupa was provided every 24 h.

The sex ratio of progeny (female: male) averaged 90.3±5.3% (range, 78-97%) (Table 4). Streams and Fuester (1967) reported that female progeny of *T. incertus* averaged 52.8%, and unmated females produced male progeny only. In the majority of parasitic hymenoptera, females lay a high proportion of female (fertilized) eggs immediately after mating, and later in life tend to lay increasing proportions of male eggs. This gradual (or in some cases, rapid) increase in male production could be due to the female running out of viable sperm (Quicke, 1997). However, in this test, a female was kept together with a male until the female died, and she probably could mate readily. That is probably why the sex ratio of progeny was slightly higher than in *T. incertus*.

Females and males of *Tetrastichus* sp. lived for an average of 21.7±4.2 and 19.8±4.5 days, respectively, under incubator conditions (Table 4). In a study on the biology of *T. incertus*, females and males lived for an average of 28.3 and 28.0 days, respectively, at 26±1℃ and 50±8% RH (Streams and Fuester, 1967).

Table 4. Longevity and reproduction ($\bar{x}\pm$SD) of *Tetrastichus* sp. reared in the pupae of *Hyphantria cunea*

Preoviposition period (days)	Oviposition period (days)	Mean no. of eggs laid/day	Total no. of eggs laid	Longevity (days)		Sex ratio
				F	M	
0.43±0.67[a]	7.66±4.97	5.91±7.97	94.67±35.95	21.7±4.2	19.8±4.5	90.3±5.3

[a] 10 replications supplied one fresh pupa every 24 h

T. howardi females lived for an average of 23.6 days and males 16.7 days (23±2℃ and 60±10%) (Kfir et al., 1992).

Leatemia et al. (1995) reported that the longevity of *Trichogramma minutum* (Trichogrammatidae) decreased significantly (44.7, 35.2, 27.3, and 17.1 days) with increasing temperatures (16, 20, 25, and 30℃). The temperature condition for this study was 28℃, which was somewhat higher than the condition in the studies of the other two *Tetrastichus* species. It appears that the longevity of *Tetrasichus* sp. was shorter due to the higher temperature conditions.

Acknowledgements

We thank Dr. John LaSalle, International Institute of Entomology, London, for valuable taxonomic suggestions and for sharing references on the parasitioid. Grateful thanks to Dr. Geon-hyung Lee, NPQS, Seoul and Dr. David W. Williams, USDA, USA, for valuable comments and assisting in the detailed reviews.

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[Received September 20, 1999; accepted October 25, 1999]