A study of external morphological changes and the development time towards further understanding the biology of *Elenchus yasumatsui* Kifune & Hirashima (Strepsiptera: Elenchidae) male

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ABSTRACT: *Elenchus yasumatsui* Kifune & Hirashima (Strepsiptera: Elenchidae) is an endoparasitoid of a key rice pest, the brown planthopper *Nilaparvata lugens* (Stål). There are few biological studies available on this species because of difficulties in making observations. Thus, the aims of this study were to investigate the morphological changes and the development periods of the *E. yasumatsui* male. Parasitized *N. lugens* by *E. yasumatsui* males and females were collected from rice fields and transferred to the laboratory as pairs in mating tubes. After the emerging of triungulins, 250 uninfected *N. lugens* were placed as hosts for 24 h and kept separately. From daily dissections, it was observed that the triungulins had elongated and flattened bodies with distinct appendages. From 2nd to 4th instar, their shape became maggot-like with an increase in size and slight changes in morphology. The pupae had sac covering its body with a dark brown pupal cap. Results also revealed that 99 triungulins, from 542, were able to parasitize and develop inside the hosts. The periods of time taken to develop into 1st instar ($n = 59$), 2nd instar ($n = 1$), 3rd instar ($n = 23$), 4th instar ($n = 11$), pupae ($n = 4$), and adult ($n = 1$) were 2–6, 4, 4–7, 4–7, 6–7, and 14 days, respectively. The critical information derived from one survivor of *E. yasumatsui* adult showed that the larval growth period was 10 days and the pupation time since the pupal cap extruding from the host abdomen was 3 days. During this period the adult male lasted 4.1 h. These data can be used to elucidate the actual biology of *E. yasumatsui* for enhancing the success of biological control in *N. lugens* populations.

KEYWORDS: Strepsiptera, *Elenchus yasumatsui*, brown planthopper, morphological change, insect development, endoparasite

INTRODUCTION

Rice is the principal staple food (of national security) for more than half of the world's population [1, 2]. However, losses of the harvest caused by pests remain a key constraint to achieving high rice yields [3]. The brown planthopper (BPH) *Nilaparvata lugens* (Stål) is a major biotic constraint of rice production as it can attack at all stages of rice growth [4]. Elimination of *N. lugens* infestations is difficult, depending on many factors including climate, huge increase in fertilizer application, abuse of pesticides, changes in rice varieties, and intensive cultivation [5]. Insecticides are commonly used to control the *N. lugens*, but reports showed that the pest has rapidly evolved an insecticide resistance that enhances its ability to overcome or avoid the toxic effect of insecticides [6–8]. Furthermore, excessive use of insecticides has resulted in several environmental problems including the destruction of natural enemy populations [9]. Several natural enemies are found in the rice fields and play an important role in suppressing pest populations,

especially parasitoid groups with high level host specificity [10]. The rice planthoppers are attacked by a large range of parasitoids from Diptera, Hymenoptera, and Strepsiptera [11]. *Elenchus yasumatsui* Kifune & Hirashima (family Elenchidae, order Strepsiptera) is known as an important endoparasitoid of the rice planthoppers [12, 13]. There are 2 main species that act as biocontrol agents against the *N. lugens* including *E. yasumatsui* and *E. japonicus* [14]. However, only *E. yasumatsui* has been reported to parasitize the *N. lugens* in Thailand [15]. Moreover, it can parasitize and feed on both *N. lugens* nymphs and adults [16, 17]. All species in Strepsiptera have unique and complex life cycles due to sexual dimorphism [18]. The male of *E. yasumatsui* displays from first to fourth instar, pupa, and adult; whereas the female does not have pupal stage and stays in the host for her entire life [14, 19]. After mating, the parenting female produces the free-living first instars which are called triungulins and releases them to seek new rice planthopper hosts [20]. Even though data on biology and ecology of this endoparasitoid species are useful for manipulating the

efficacy of biological control of the rice planthoppers, most of the reports are taken from field investigation and surveys on the seasonal abundance and incidence of parasitism in delphacid hosts [16, 21–26] and there are few reports on their biology [12, 20, 27]. Till now, the biology, especially the immature stages of *E. yasumatsui*, is still poorly understood. This is probably because the *E. yasumatsui* mostly spends time developing itself inside the host and only two free-living stages, the first larva-instar and later an adult male, could be found outside the host [17], leading to difficulties in collecting specimens and requirements on the dissection of the host and, hence, identifying the larval stage of this endoparasitoid species [28]. In addition, little experimental work has been conducted on how *E. yasumatsui* changes in morphology during its development inside the hosts. Against this background, the aims of this research were to gain insights about the morphological changes and development period of *E. yasumatsui* under laboratory conditions with emphases and details on the male. Our results on the study of the biology of *E. yasumatsui* could provide improvements of crop protection and long-term sustainability control of the *N. lugens*.

MATERIALS AND METHODS

Stock cultures of *Nilaparvata lugens*

The unparasitized *N. lugens* were randomly collected from a rice field in Suphan Buri province and reared on *Oryza sativa* var. TN1 in plastic tubes until the emergence of the next generation. Only third and fifth instar stages of *N. lugens* were used as the host of *E. yasumatsui* in the experiments. All experiments were conducted from August 2019 to May 2020.

Investigation of external morphological changes of *E. yasumatsui* male

The *N. lugens* were collected from rice paddy fields in Suphan Buri, Chai Nat, Phra Nakhon Si Ayutthaya, Nakhon Nayok, Prachin Buri, and Chachoengsao provinces with various techniques including sweeping, sucking with D-vac, and using aspirators, and taken to the laboratory. The parasitized *N. lugens* by *E. yasumatsui* males and females were chosen and transferred as pairs in a mating tube (2.5$\times$20 cm test tube sealed with parafilm) containing the rice plant (*O. sativa* var. TN1) in the laboratory under uncontrolled environmental conditions: temperature varied from 23.3 to 30.93 °C (28.6 ± 1.33 °C) and relative humidity ranged from 51.57 to 81.77% (70.3 ± 6.09%). Sex separation in *E. yasumatsui* was considered from the features of a hole and lid made by *E. yasumatsui* on its host abdominal wall (Fig. 1).

All mating tubes were checked daily under a microscope until the triungulins emerged outside the host. Assuming that parasitization occured on *N. lugens* within 24 h, 250 uninfected hosts at third and
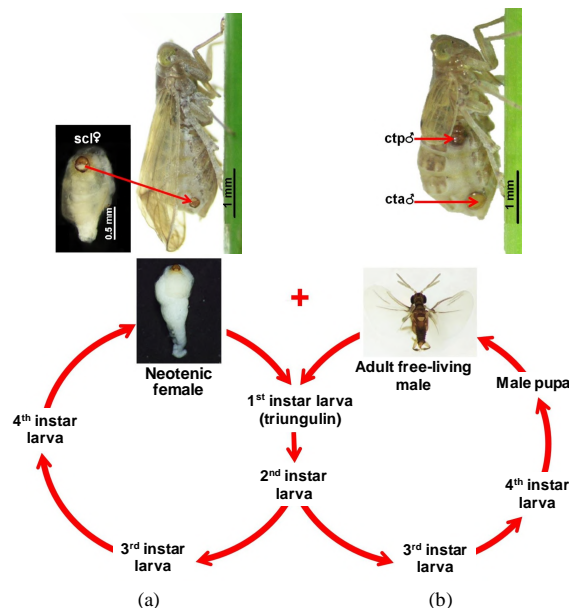
Fig. 1 The life cycles and differences of the lids on the abdominal body wall of parasitized *N. lugens* developed by female and male of *E. yasumatsui*: (a), the *E. yasumatsui* female develops the half-open lid with a semi-circle shape (scl♀); and (b), a convex lid created by the *E. yasumatsui* male is appeared in the pupal stage (ctp♂). Once the adult of *E. yasumatsui* flies away from its host, the lid is come off and an exit hole is emerged with a circular shape (cta♂).

fifth instars from the stock cultures were placed in each mating tube for 24 h. Then, the *N. lugens* were reared individually in a new tube containing the rice plants. All development patterns of *E. yasumatsui* in each life stage were photographed and measured for size using the stereomicroscope SZX16 with a digital camera DP22 (Oylumpus, Japan). The frame rate and resolution were 26.22 frames/s and 2 megapixel, respectively.

Measurement of the time to complete each stage of *E. yasumatsui* male

To provide an important biological data for applying in biological control work in the future, individual records of *E. yasumatsui* males in the previous section were used to analyze the development time required to complete larval, pupal, and adult stages. Simultaneously, the parasitized *N. lugens* were reared until the external symptom of an opening hole on the abdominal wall was recorded. The emerged *E. yasumatsui* males were continually reared in a tube with the host to observe the development time in the adult stage. It was noted that a sudden death to the host samples once they were dissected to examine the life stage and development pattern of the *E. yasumatsui*. Consequently, the duration for completing the larval, pupal,

and adult stages were assessed when the adult male of *E. yasumatsui* flying away from its host.

RESULTS

Expression of laminins' cognate receptors using RNA-seq data from PCAWG and TCGA

The results showed that most *E. yasumatsui* females ($n = 48$) had developed an opening hole on theirs hosts before their arrival to the laboratory. From our observations, 20 of 48 female parents had already mated and released triungulins since they were in the paddy field because the ovaries of these female parents were in macerated conditions, and only 2 female parents had constructed the open pore on the abdominal body wall of *N. lugens* during the period of rearing in the laboratory. The remaining 26 female parents were reared in the mating tube together with the male parents. However, the time-to-emergence in adult females mismatched with the timing of adult males. Thus, from collecting insects in the fields, we gained 542 triungulins hatched and moved outside from only 3 female parents (Fig. 2). After the triungulins' emergence outside their hosts, these female parents of *E. yasumatsui* were observed by dissecting the hosts. Interestingly, the dissection showed that 71 unemerged triungulins were unable to escape from two female parent's brood canals (Fig. 2b,c). The results revealed that the time durations that all triungulins spent in order to leave the mother and the host were from 1.92 to 2.4 h. After the emergence of 542 triungulins from their mothers' brood canals, 750 uninfected nymphs of *N. lugens* from the stock cultures were placed in the mating tubes for 24 h for exposure to the triungulins. From daily dissecting the hosts, the results highlighted that only 20.11% of the triungulins ($n = 109$) were able to seek and parasitize new hosts, while the remaining 79.89% ($n = 433$) died in the mating tube. Details of the growth pattern in each stage were as follows.

The external morphological changes of *E. yasumatsui* male

For the first instar larvae, 59 triungulins were observed from dissecting *N. lugens* (Fig. 3). Their body lengths were from 0.11 to 0.284 mm. The mean body lengths of triungulins on days 1–3 and days 4–6 were 0.1659 ± 0.0409 mm and 0.135 ± 0.0186 mm, respectively. For external morphology, the triungulin had an elongated and flattened body. Its head was unsegmented and slightly forward curving with two prominent eye spots on the head capsule. The triungulin had three pairs of well-developed legs on the thoracic region. In the caudal region, there were 2 pairs of tails including a lateral caudal setae and a long medial caudal setae. From video observations, it was found that one of the triungulins used its caudal filaments to spring onto a host. The body color of the

triungulins gradually changed from light to dark brown before developing to the next stage.

Fig. 4 shows the sequential developmental stages from the second to the fourth instars. In daily efforts of the *N. lugens* dissection, only one male *E. yasumatsui* in the second instar was found. The body of the second instar male was 0.294 mm long and 0.105 mm wide, the width of its head was 0.0578 mm, and the body wall was clear with one layer and without leg appendages. A total of 23 third instar larvae of *E. yasumatsui* males were observed from dissection. The ranges of the body lengths, the body widths, and the head widths were 0.241–1.444 mm, 0.078–0.528 mm, and 0.043–0.223 mm, respectively. They had more slender and elongated body shapes than those of the second instar with whitish bodies. For the next developmental stage, a total of 11 male larvae in the fourth instar were found from dissecting *N. lugens*. The body lengths were from 0.463 to 1.036 mm, while the widths of their bodies and heads were from 0.185 to 0.505 mm and 0.135 to 0.379 mm, respectively. They had a whitish body color. Towards the end of this stage, new body wall layers were developed covering its body. This development pattern might be involved with the development of the next pupal stage when a closed sac and lid would be formed.

Fig. 5 displays development patterns in the pupal stage after parasitizing the host for 6 and 7 days. From observations on four pupae, their body lengths varied from 0.923 to 1.407 mm, while the widths of their bodies and heads were from 0.331 to 0.4551 mm and 0.16 to 0.203 mm, respectively. The body was covered with sac and the head with dark brown cephalothecas (caps of the puparium). The ranges of lengths and widths of the cephalothecas were 0.256–0.383 mm and 0.279–0.344 mm, respectively. In this stage, their heads, thoraxes, and abdomens were developed. Their compound eyes resembled raspberries. Moreover, their mouths and legs could be clearly seen, though these organs were not fully developed. When they nearly entered the adult stage, their heads emerged from outside of the abdomen host.

At the adult stage, their body lengths were 1.043 mm, while the widths of their bodies and heads were 0.348 and 0.168 mm, respectively. As for the external morphology, the adult male body color was brown with two large berry-like eyes on the head. The antennae had 4 segments with a length of 0.571 mm. The third segment was Y-shaped, and the length of its lateral flabellum was 0.301 mm. The third segment was shorter than the fourth segment with the length of 0.168 and 0.302 mm, respectively (Fig. 6a,b)).

In the metathorax region, it was found that the postscutellum was large. The scutellum was shorter than the postnotum with a length of 0.049 and
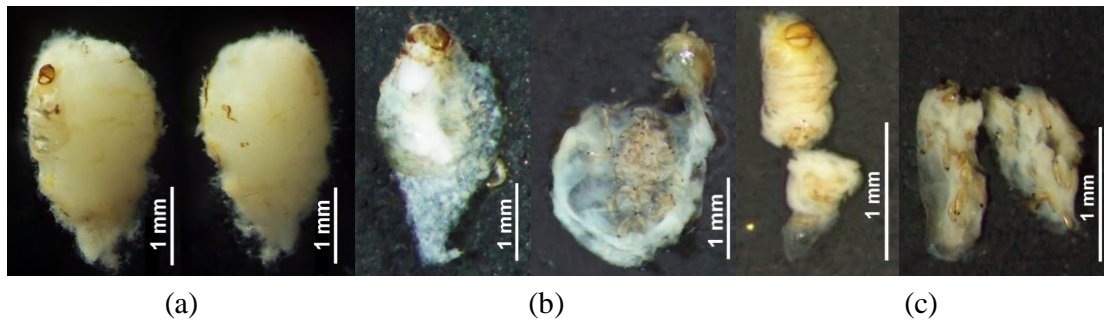
Fig. 2 The morphological feature of 3 female parents *E. yasumatsui* is in macerated conditions after triungulins clawed off: (a), the triungulins move out from their mother through brood canal opening to find new hosts; and (b, c), some unemerged triungulins remaining in the brood canal of their two female parents are found.
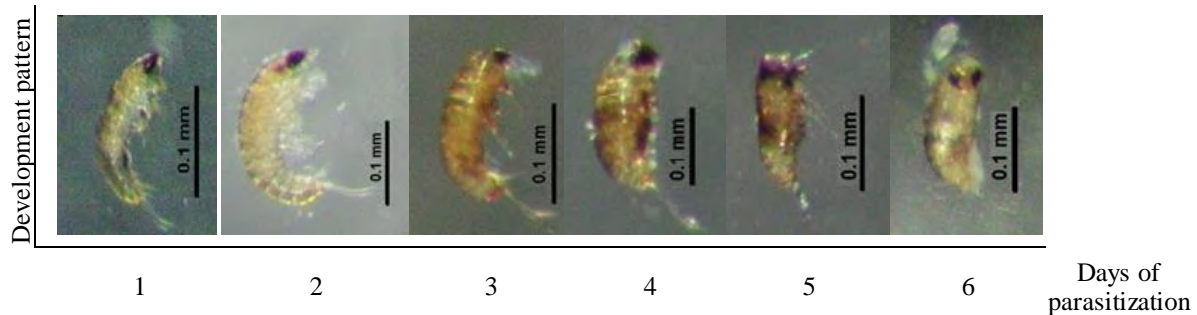
Fig. 3 Development patterns of the triungulins after 1–6 days of parasitization, lateral views.
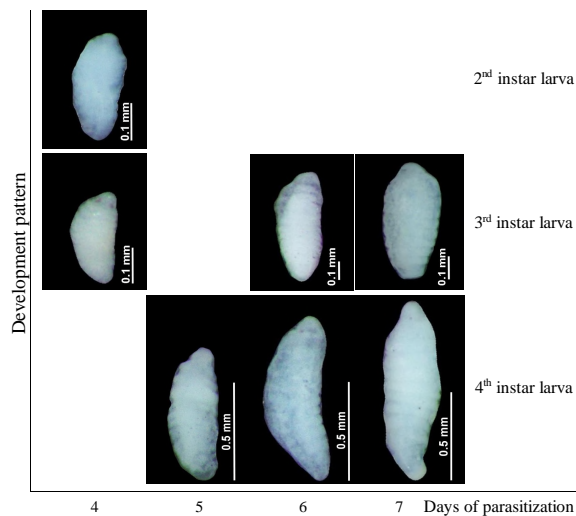
Fig. 4 The morphological features of nymphal stage in *E. yasumatsui* from second to fourth.

0.166 mm and a width of 0.13 and 0.185 mm, respectively. The postlumbium was large and of a semicircular shape (Fig. 6c). There were 2 pairs of wings. Only the hindwings (0.910 mm long and 0.695 mm wide) were used for flying. By contrast, the forewings were reduced to what are known as pseudohalteres.

Time period to complete each developmental stage of *E. yasumatsui* male

From a total of 99 *E. yasumatsui* males, the number of dissected hosts containing *E. yasumatsui* males in 1st, 2nd, 3rd, 4th, pupal, and adult stages were 59, 1, 23, 11, 4, and 1, respectively. Fig. 7a displays the time taken to develop in each stage by *E. yasumatsui* males. The results showed that a maximum time taken by *E. yasumatsui* males to develop in the first instar stage was 6 days after parasitization, whilst the *E. yasumatsui* male was still in the second instar after it had parasitized the host for 4 days. The time for males entering the third and the fourth instar stages ranged from 4 to 7 days. For the pupal stage, four pupae discovered after parasitizing *N. lugens* ranged from 6 to 7 days. There was only one male adult that we could discover in this experiment after parasitizing *N. lugens* for 14 days. Interestingly, data from our experiment showed that the total period from first instar to pupal stages of this one only adult male lasted for 13 days. The adult male emerged and moved out from the host to find its mate, and its life span was 4.1 h. The results also showed that the cap of the puparium protruded from the abdomen of the host for 3 days in both *E. yasumatsui* males, one grown in the laboratory and the other collected from the field before the adult male flying away from the hosts (Fig. 7b). However, the adult period of the *E. yasumatsui* males collected
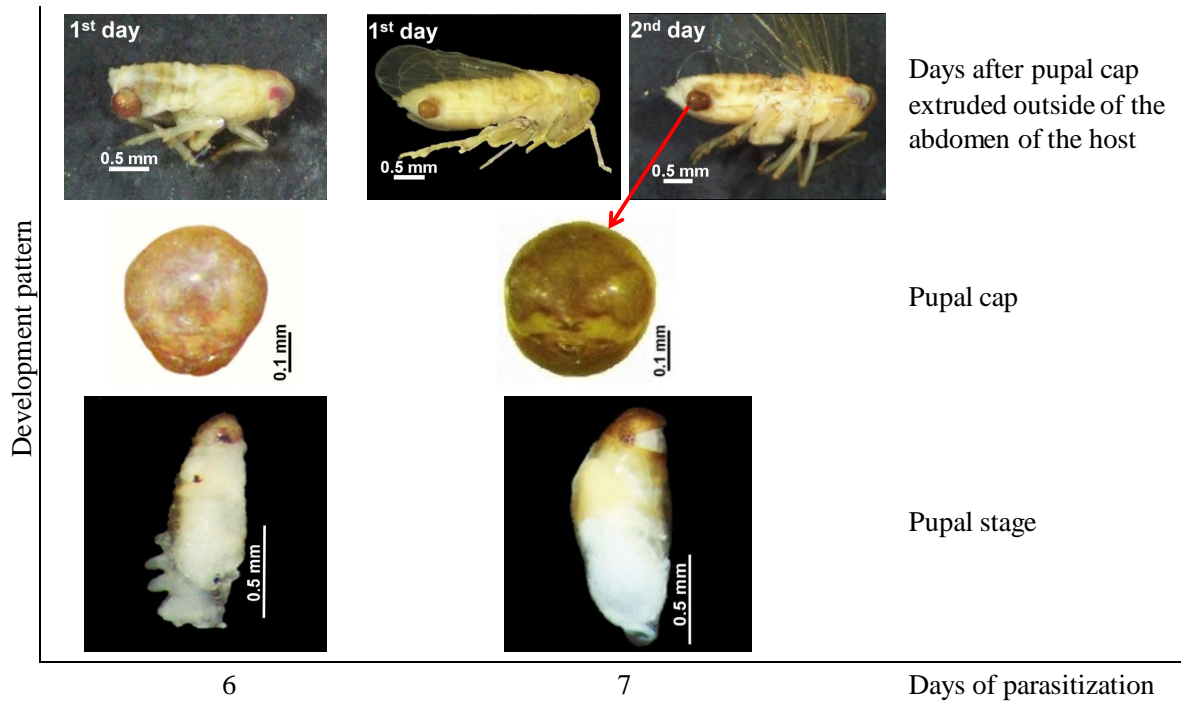
Fig. 5 Lateral view of *E. yasumatsui* pupa after 6–7 days of parasitization and top view of cephalotheca that *E. yasumatsui* male developed in pupal stage. The cephalotheca is clearly visible with convex and oval shape.
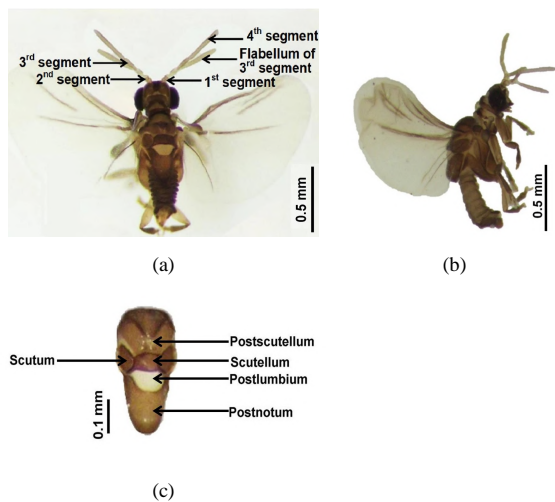

Fig. 6 A free-flying *E. yasumatsui* male after 14 days of parasitization: (a), dorsal view of antenna; (b), lateral view; and (c), dorsal view of metathorax.

from the field and reared in the laboratory was less than 5 h (1.35–4.1 h). All key results on external morphological changes and development times of the *E. yasumatsui* males were summarized in Table S1. Important issues for each life stage were discussed in detail in subsequent sections.

DISCUSSION

E. yasumatsui is one of the important natural enemies and a strong force in the suppression of nymphs and adults of the two rice planthoppers, *N. lugens* and *Sogatella furcifera* (Horvath) [29]. However, the most extensive studies on *E. yasumatsui* have been carried out on survey data to estimate the abundance and understand the morphology of the adult stage. The understanding is important because it has a tiny size and spends nearly its entire life in the host [30]. Before discussing the development of the *E. yasumatsui* male, it is worthwhile to first consider how successful the establishment and maintenance of the culture collection has been. It was found that the chances for success in establishing cultures of *E. yasumatsui* were low. Firstly, some of the *N. lugens* that were parasitized by pupae of *E. yasumatsui* were also infected by fungi, and most of them grow near the pupal cap. This could have a major impact on growth, development and fitness of not only the host, but also the endoparasitoids. In addition, combined parasitism by *E. yasumatsui* and dryinid wasps (*Pseudogonatopus hospes*) was found on the field-collected population of *N. lugens*. It was reported that the survival of dryinids was higher than the *E. yasumatsui* and that the larva of *E. yasumatsui* seemed to get eaten by the larva of dryinids when they parasitized on the same host [26]. From our observations on emerged triungulins from 3 field collected *N. lugens* females, there was a high possibility
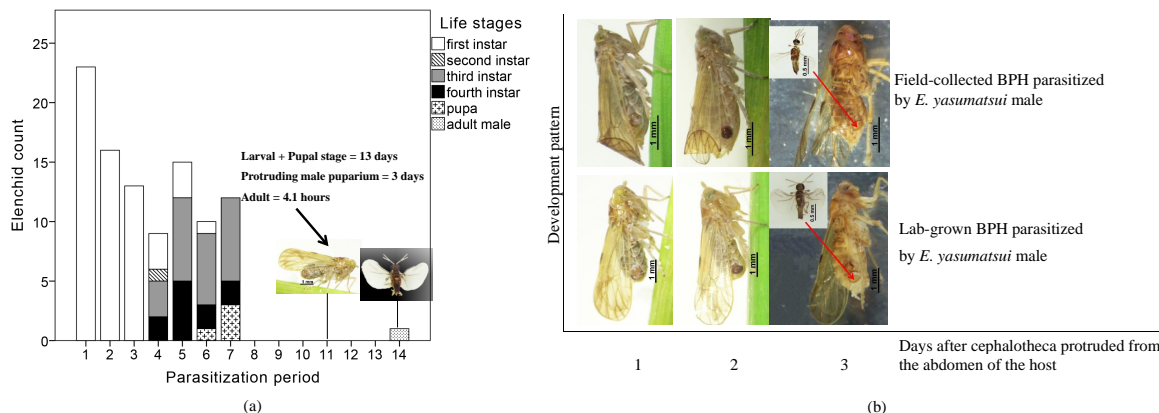
Fig. 7 Development pattern of *E. yasumatsui* male: (a), the time taken to develop in each stage of the *E. yasumatsui* males; and (b), the time period and changing patterns of cephalotheca feature in pupal stage comparing between the filed-collected and the lab-grown *E. yasumatsui* males.

that these 3 female parents of *E. yasumatsui* had been mated already in the rice field before being collected and taken back to the laboratory. However, too little is currently known about the strategies and mating behavior of E. yasumatsui. From this study, we obtained fertility data from an actual emerged triungulin count which amounted to 542 triungulins per three adult females with approximately 181 triungulins produced per *E. yasumatsui* female. Our fertility results of this species were different from what reported by Lewvanich and Wongsiri [30], who claimed that one *E. yasumatsui* female could produce 500–2000 triungulin per lifetime without experimental data or evidence to support their conclusions. It is worth noting that data on the actual fecundity, that can be used to explain the physiological potential of the *E. yasumatsui* female to produce total triungulins in her lifespan, are still not available. However, our study revealed that there were some unemerged triungulins remaining in the mother's brood canal under laboratory conditions. Besides, the ability of the triungulins to emerge from the mother's brood under natural conditions is still a puzzle.

Regarding the external morphological changes from larval to adult stages, the sexes of *E. yasumatsui* could be separated after molting to the third instar; therefore, the detailed development patterns for the first and the second instar larvae were similar for both sexes. It is also noted that *E. yasumatsui* larvae could only be discovered by dissection of the host and leading to a sudden death for *E. yasumatsui*. Therefore, there was no resultant data on the exact development time for each life stage. However, dissection of parasitized *N. lugens* could reveal the possible time periods of development of each life stage. As there has been no published paper on the development and the growth pattern of *E. yasumatsui*, the changes in external morphology of *E. yasumatsui* will be mainly compared

with *E. japonicus* due to its geographical distribution being located in Asia [13, 31]. Discussed details are as follows.

The external morphology of the triungulin was C-shaped and light brown in color. The present study revealed that the triungulin of *E. yasumatsui* had body lengths ranging from 0.11 to 0.284 mm, with a mean body length of 0.16 mm. Our results were similar to the findings of Gu et al [32] in *E. japonicus*. They reported that the body length of the triungulins of *E. japonicus* was 0.2 mm. A critical finding from this study was that a total of 433 triungulins, which were not able to parasitize the host, could survive outside the host for no more than 22 h (ranging from 19 to 22 h), which agreed with what reported by Gu et al [32] and Maeta et al [33] that triungulins of *E. japonicus* could survive for only 15–24 h. Another interesting result from our study was that the triungulins spent about 2.16 h (ranging from 1.55 to 2.24 h) to move out from the mother's brood canal. On the other hand, the emergence of triungulins in *E. japonicus* continued to occur for 12 h [33]. However, the biology and natural history of the host-seeking primary larva are still poorly known [19]. About the second stage of larval development, our findings taken from one sample suggested that the *E. yasumatsui* was at this stage inside the host after 4 days of parasitization. The following two life stages, the third and the fourth instar, developed inside the *N. lugens* in the same time periods ranging from 4 to 7 days after parasitization. For the development pattern from the second to the fourth instar, their shapes were maggot-like with an increase in size and a slight change in morphology. However, the developmental process in such minor morphological changes was complex [19, 34].

In the pupal stage, the *E. yasumatsui* males went through a series of distinct pupal form changes be-

tween the first and the second days into this stage. The puparium, which is inside the host, has a soft texture, but the pupal cap that extruds outside the host is thick and sclerotized. From our observations, the cephalotheca became dark brown two days after extrusion. Cook [19] pointed out that the pupal cap has a taxonomic value; and from this structure alone, specimens can often be identified, at least to the generic level. The results of the dissection of the host revealed that the four *E. yasumatsui* males began entering the pupal stage after parasitizing the host for 6 days, together with the protrusion of the pupal cap. It is noted that the time required to complete the larval and pupal stage is still unknown. However, information derived from the only emerged *E. yasumatsui* male highlighted that the cephalotheca extruded from the abdomen of the host on day 11 after parasitization, and the adult flew away from the host on day 14. Thus, it could be hypothesized that the time periods taken to complete larval and pupal stages were 10 and 3 days, respectively. By comparison, Gu et al [32] reported that the development periods in larval (from the second to the fourth instars) and pupal stages of *E. japonicus* lasted 10–11 and 2–4 days, respectively. Meanwhile, Maeta et al [33] found that the larval period was 8–9 days, and the time taken from the cephalotheca extruding from the abdomen of the host to the emergence of the adult in male *E. japonicus* was 3.7–4.5 days.

In the adult stage, we discovered one male after parasitizing *N. lugens* for 14 days, which was a similar result of 13 days in *E. japonicus* reported by Maeta et al [33]. Furthermore, the adult *E. yasumatsui* in our study stayed alive for 4.1 h after leaving its host, The adult males in Strepsiptera are typically short-lived and have merely 4–6 h to find a female [14] while the adult male active flight reported for *E. japonicus* was 0.5–3.3 h [33]. In addition, our results on the development time from larval to adult stages of *E. yasumatsui* corresponded to the reports of Gu et al [32] and Maeta et al [33] in *E. japonicus*. Another important conclusion from the present study is that the individual development time of the larva, as well as the pupa, showed variations in time taken from the molting stage to the next stage in *E. yasumatsui*. This may be because the larva and the pupa of *E. yasumatsui* males develop inside the host and thus live in a complex biochemical milieu. Therefore, this endoparasitoid species might be highly susceptible to very small changes in the quality of the host's internal environment. Consequently, the development of *E. yasumatsui* is closely coordinated with the development of their hosts [35]. Likewise, the environmental factors, especially the temperature, play an important role in controlling the development and the reproduction of the insects [36], whereas more study on how the *E. yasumatsui*'s host system reacts to changes in temperature is needed. One limitation of our study was that the development periods and patterns were gained from 99 *E. yasumatsui* males. In conclusion, the duration to reach pupae from larval stage varies drastically among individuals in *E. yasumatsui*. Our results from the second instar to the adult stages were not complete due to the few *E. yasumatsui* males available for the experiments; however, our data comprise the most complete morphological characteristics of this endoparasitoid species which could help fill the existing gaps of information on the larval and pupal stages. Our findings also raise a lot of issues for further researches including the lifecycle and the biology of *E. yasumatsui* female, the relationship between *E. yasumatsui* and its host in terms of abundance and distribution, and genotypes and responses to environmental factors. These data might allow us to find the culture methodology and suitable conditions for enhancing the chance of releasing *E. yasumatsui* into outbreak areas, especially in the irrigated rice fields of the central plains and the lower northern region of Thailand, which may contribute towards the successful control of the *N. lugens* in the future.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874. 2022.074.

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Appendix A. Supplementary data

Table S1 External morphological changes and development times of 99 *E. yasumatsui* males by using *N. lugens* as the host. The experiments were conducted in the laboratory under uncontrolled environmental conditions from August 2019 to May 2020.

Life stage	External morphological change		Development time
	Body size	External shape	
1st instar larva (triungulin) ($n = 59$)	0.11–0.284 mm for body lengths	Light brown body color, elongate and flatten body shape for early (day 1–3) and late development (day 4–6)	1–6 days
2nd instar larva ($n = 1$)	0.294 and 0.105 mm for body length and width	Clear body wall with one layer and without leg appendages	4 days
3rd instar larva ($n = 23$)	0.241–1.444 mm and 0.078–0.528 mm for body lengths and widths	Slender and elongate body shape with whitish body color	4–7 days
4th instar larva ($n = 11$)	0.463–1.036 mm and 0.185–0.505 mm for body lengths and widths	Whitish body color with new developed body wall layers covered its body at the late of this stage	4–7 days
Pupa ($n = 4$)	0.923–1.407 mm and 0.331–0.4551 mm for body lengths and widths 0.256–0.383 mm and 0.279–0.344 mm for pupal caps	Well-developed head, thorax, abdomen, and incompletely developed mouth and legs	6–7 days
Adult ($n = 1$)	1.043 mm and 0.348 mm for body length and width	Brown body color, large berry-like eyes, 4-segmented antenna, 2 pairs of wings with reduced forewings	14 days