



Original Article

## Bionomics of the apefly, *Spalgis epius* (Lepidoptera: Lycaenidae), predatory on the papaya mealybug, *Paracoccus marginatus* (Hemiptera: Pseudococcidae), in Thailand

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### Abstract

We investigated the life history, growth ratio and feeding potential of the hemipterophagous butterfly or the apefly, *Spalgis epius* (Westwood) (Lepidoptera: Lycaenidae: Miletinae), a promising augmentative biological control agent of the papaya mealybug, *Paracoccus marginatus* Williams & Granara de Willink (Hemiptera: Pseudococcidae), which is an invasive alien species detected in 2008 in Thailand. The investigation was carried out under laboratory conditions at 25-30°C and 40-60% RH using papaya mealybug reared on Thai pumpkin as its prey. The mean duration of the egg, larval and pupal stages were  $3.54 \pm 0.50$ ,  $12.01 \pm 2.40$ , and  $10.32 \pm 0.88$  days, respectively, and the mean duration from egg to adult emergence was  $25.87 \pm 3.78$  days. The geometric growth ratio of successive larval instars using the head capsule width as a parameter was 1.65 and conformed to Dyar's Law. As for the predatory potential of *S. epius*, the total number of papaya mealybug consumed during the larval stage were  $4,115.75 \pm 553.28$  eggs,  $281.25 \pm 45.08$  nymphs and  $77.50 \pm 16.52$  female adults. The instar-specific prey consumption from the first to fifth larval instars in the order of eggs, nymphs and female adults were  $170.00 \pm 26.32$ ,  $4.25 \pm 1.71$  and  $2.00 \pm 0.82$ ;  $654.00 \pm 67.97$ ,  $35.35 \pm 12.48$  and  $10.25 \pm 4.29$ ;  $1,376.75 \pm 130.95$ ,  $93.25 \pm 11.70$  and  $23.50 \pm 3.94$ ;  $1,426.50 \pm 252.93$ ,  $110.75 \pm 15.60$  and  $31.00 \pm 5.25$ ; and  $31.00 \pm 5.25$ ,  $37.75 \pm 3.59$  and  $10.75 \pm 2.22$ , respectively. These findings warrant further investigation into the amenability and suitability of the entomophagous apefly, *S. epius*, for as an integrated pest management strategy for the augmentative biological control of the papaya mealybug in Thailand.

**Keywords:** apefly, entomophagous butterfly, papaya mealybug, invasive alien species

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### 1. Introduction

The papaya mealybug, *Paracoccus marginatus* Williams & Granara de Willink (Hemiptera: Pseudococcidae) is native to Mexico and Costa Rica (Walker *et al.*, 2006). It was detected as an invasive alien insect species in Thailand in 2008 and has since become a serious pest on papaya (*Carica*

*papaya* L.), cassava (*Manihot esculenta* Crantz), plumeria (*Plumeria* spp.) and hibiscus (*Hibiscus rosa-sinensis* L.). In a preliminary survey of its natural enemies, larvae of the apefly, *Spalgis epius* (Westwood) (Lepidoptera: Lycaenidae, Miletinae), a hemipterophagous butterfly were found feeding on eggs, nymphs and adults of the papaya mealybug. *Spalgis epius* is a member of the entirely entomophagous lycaenid subfamily Miletinae, most species of which feed on Hemiptera (Pierce *et al.*, 2002). In a study on the biology of carnivorous lycaenid butterfly larvae in Thailand and the Philippines, Lohman and Samarita (2009) indicated that

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*S. epius* is known to occur in Thailand. The genus *Spalgis* is widespread and found in the Oriental, Australian, and Afrotropical regions. Ackery (1990) reported *Spalgis jacksoni* Stempffer, *Spalgis lemolea* Druce, *Spalgis pilos* Druce and *Spalgis tintinga* Boisdual from the Afrotropical region and suggested the potential for other members of the genus *Spalgis* in Africa as biological control agents.

Apefly larvae are hemipterophagous and are generally known to be mealybug predators. Their common name, the apefly, is derived from the appearance of the dorsal view of the pupa which resembles the face of a monkey, and thus its Thai vernacular name is *Phi Suea Dak Dae Hua Ling* (Lekagul *et al.*, 1977) which literally means "a butterfly whose pupa resembles a monkey head." *Spalgis epius* occurs widely in the Oriental region, including throughout Thailand (Godfrey 1930, 1932; Lekagul *et al.*, 1977; Pinratana, 1981; Ek-Amnuay, 2006). In Asia, *S. epius* is reported by various authors to be widely distributed in Bangladesh, India, Sri Lanka, Bhutan, China (Hainan and Yunnan provinces), Taiwan, Myanmar, Thailand, Laos, Vietnam, Malay Peninsula, Singapore, the Philippines and Indonesia (Corbet and Pendlebury, 1976; Dinesh *et al.*, 2010; Ek-Amnuay, 2006; Godfrey, 1930, 1932; Lekagul *et al.*, 1977; Lohman and Samarita, 2009; Pinratana, 1981).

In a study on the development, life history and behavior of *S. epius* on the citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), Dinesh *et al.* (2010) stated that although *S. epius* is an important predator of mealybugs in India, virtually no research has been conducted on its development and biology. Similarly, in Thailand, *S. epius* is mostly overlooked as an important mealybug predator. This is probably due to its strong resemblance to mealybugs. The body of the larva is always covered with a thick coating of white wax filaments adhering to its short stiff setae. Only with a very careful examination can it be distinguished from a mealybug. In a survey conducted between 2008-2009, we found that *S. epius* often feeds on the papaya mealybug, and therefore warrants further investigation as a potential predator for augmentative biological control of the papaya mealybug. The objective of this study is to document the bionomics, life history, growth ratio and predatory potential of *S. epius* as a biological control agent of the papaya mealybug in Thailand.

## 2. Materials and Methods

### 2.1 Laboratory culture of *P. marginatus*

Egg sacs and nymphs of *P. marginatus* infesting papaya (*Carica papaya* L.) under field conditions were collected by using a camel hair brush and used to establish a laboratory culture of *P. marginatus*. The culture of *P. marginatus* was maintained to provide prey for *S. epius* during the course of this investigation. They were reared on medium-sized Thai pumpkin fruits (*Cucurbita moschata* Duchesne) (ca. 20 cm diameter) as an artificial host. Before

use, the pumpkins were first thoroughly washed with tap water, soaked in a 5% solution of sodium hypochlorite bleach for 15 minutes, rinsed with tap water again and then air-dried. Each pumpkin was placed on two layers of square paper napkin on a disposable plastic plate. After inoculating with a few *P. marginatus* egg sacs and nymphs, they were kept on shelves fitted with two 60 cm 18W Sylvania fluorescence lamps and maintained at 12L:12D using a Panasonic TB178K electric switch timer. Three inoculated pumpkins were kept on each shelf. New pumpkins were provided and changed approximately once a month when they began to deteriorate and were no longer suitable for supporting mealybugs. The mealybug culture was maintained under laboratory conditions at 25-30°C and 40-60% RH.

### 2.2 Laboratory culture of *S. epius*

Pumpkins with moderate infestations of mealybugs were kept in a fine screen cage measuring 30x30x30 cm with a front sliding glass opening to maintain a laboratory culture of *S. epius*. In each cage, 20 field-collected larvae of *S. epius* were placed on the pumpkin and allowed to prey on the mealybugs to complete their development. Newly emerged adults of both sexes were kept in a fine mesh aluminum mating cage with removable panels on top and all four sides measuring 60x60x90 cm. In addition to the infested pumpkins, a vase with freshly cut lantana flowers was kept inside the cage and sprayed sparingly with water using an atomizer to provide water, and perches for the adults. The flowers were changed whenever necessary. The mating cage was then kept in an outdoor walk-in nylon screen cage measuring 1.80x1.80x1.80 m. As such, adult apeflies could mate and females could deposit their eggs on the mealybug-infested pumpkins. In this manner, it was possible to obtain freshly laid eggs and larvae of *S. epius* for life history and growth ratio observations and also as a supplementary source of larvae for our investigation on the apefly's predatory potential.

### 2.3 Description of stages of development of *S. epius*

We measured the length and width of adult *S. epius* specimens of both sexes, egg diameter, and the width and length of all five larval instars and pupa. All measured immature specimens were first preserved in 70% ethyl alcohol. For the measurement of the size of each developmental stage, 20 specimens were used.

### 2.4 Life history and growth ratio of *S. epius*

The incubation period of *S. epius* was determined by placing more than 50 newly laid eggs individually on a piece of grid-lined filter paper in a petri dish, so that after egg hatching only 50 newly hatched larvae were used for further observation. The incubation period of each egg was recorded until 50 newly hatched larvae were obtained. After that, each of the 50 newly hatched first instar larvae was kept indivi-

dually in a circular clear plastic container with a perforated cover, 8 cm in diameter and 5 cm tall. Each container was provided with a small cut piece of pumpkin infested with papaya mealybug as food for the larva. Each container was examined daily for any evidence of molting, including the presence of an exuvium and/or the head capsule. Observations continued until each larva either died or matured. Stage-specific mortality was recorded. The duration of each larval instar was recorded as the number of surviving individuals in that particular stage. This was used to calculate the mean duration of successive larval instars and pupal stage.

The head capsules cast off during each larval instar were preserved in 70% ethyl alcohol. These head capsules were used for measuring the head capsule width. The measurement was done using an ocular micrometer calibrated to the nearest mm. It was carried out to determine if the geometric growth ratio of *S. epius* larvae in successive larval instars would be in conformity with Dyar's Law (Dyar, 1890; Imms, 1957; Gordh and Headrick, 2001), using the Chi-square test as described in LeClerc *et al.* (1966) and Snedecor and Cochran (1989).

## 2.5 Predatory potential of *S. epius*

We employed two measures to determine the predatory potential of *S. epius* on *P. marginatus* under laboratory conditions: 1) daily prey consumption by *S. epius* larvae from hatching until pupation, and 2) larval stage-specific prey consumption. Both measures were simultaneously carried out in one experiment, but the data were recorded differently: one to obtain daily prey consumption, and another to obtain prey consumption during different larval stages.

We assessed the potential of *S. epius* as a papaya mealybug predator by quantifying prey consumption and prey preference using modified methods from Dinesh and Venkatesha (2010), who studied *S. epius* predation on the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), and the citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae).

To determine daily prey consumption, the eggs of *S. epius* laid on the mealybug masses were collected with a fine camel hair brush and kept individually in petri dishes to hatch. This experiment was replicated four times using a new larva in each replicate. In each replicate, the newly emerged larva was provided daily with a known number of *P. marginatus* eggs (extracted from the egg sacs), nymphs, and adult females until they reached the pupal stage, when prey consumption ceased. The remaining eggs, nymphs, and adult females of *P. marginatus* were then counted under a stereomicroscope so that the number of prey of each life stage could be tallied. The larval excreta and remaining prey were removed after counting them, and a new batch of prey was provided to the larvae every day. The assessment was carried out until the larvae reached the pupal stage, which could be from 9 to 12 days. Our detailed observations and record

keeping permitted us to count the number of prey consumed during each larval instar.

## 3. Results and Discussion

### 3.1 Description of stages of development of *S. epius*

Adults of *S. epius* are small butterflies with dark brown wings in males and grayish brown wings in females with a prominent somewhat square spot near the tip of the forewing.

The body length averaged  $10.63 \pm 0.48$  mm and ranged from 9.95-11.50 mm; the wingspan averaged  $20.83 \pm 1.44$  mm and ranged from 18.0-22.5 mm (Figure 1). Adult aepflies were normally found flying rapidly and fluttering in the vicinity of mealybug-infested host plants to deposit their eggs on the mealybug masses. Eggs were disk-shaped, and flat like a minute pumpkin. The newly laid eggs were somewhat greenish and changed in color to become whitish before hatching. Eggs were laid singly, being swiftly depositing on the mass of the mealybugs. After hatching from the eggs, the first instar larvae were found feeding mainly inside the egg sac, while the older larvae fed on eggs, nymphs, and adult females of *P. marginatus*. Larvae underwent five molts during the larval stage to reach the pupal stage (Figure 2). Morphologically, larvae of all five instars did not differ greatly except in size. The egg and body size of the immature stages of *S. epius* are given in Table 1. We found that *S. epius* underwent five larval instars and a pupal stage before becoming a winged adult. This agrees with Dinesh and Venkatesha (2010) and Dinesh *et al.* (2010) in Bangalore, India, where

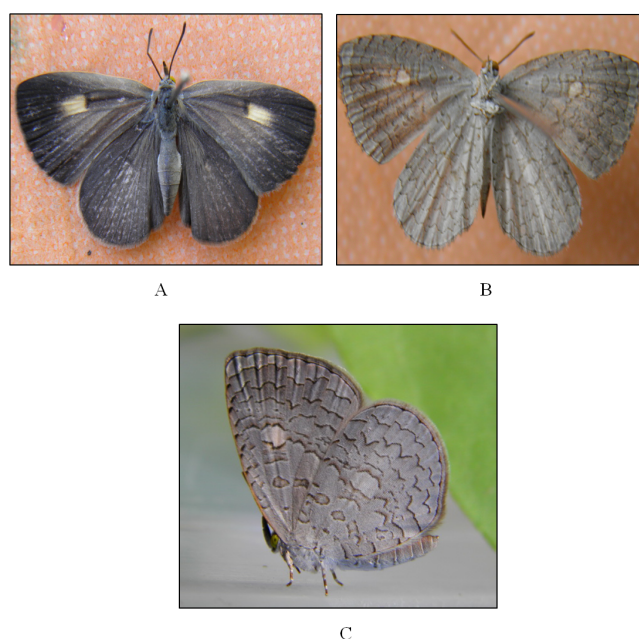


Figure 1. Adults of the aepfly, *Spalgis epius*. A. Dorsal view, B. Ventral view, and C. Lateral view.

*S. epius* was reared on the pink hibiscus mealybug, *M. hirsutus*, and the citrus mealybug, *P. citri*. Thangamalar *et al.* (2010) also reported that *S. epius* underwent five larval instars when reared on the papaya mealybug, *P. marginatus*, on mulberry in a study carried out in Coimbatore, India.

The head capsule was dark brown and the body was covered with setae and whitish wax, resembling the mealybug. After the fifth instar, the larva ceased feeding and turned dull black to form the pupa. The pupa was light brown dorso-laterally and somewhat grayish white ventrally (Figure 3). At this stage, the pattern on the anterior dorsal view of the pupa resembled the face of a rhesus monkey (Aitken, 1894), demonstrating why the species is known as the “apefly” in English and *Phi Suea Dak Dae Hua Ling* in Thai.

### 3.2 Life history and growth ratio of *S. epius*

The duration of each developmental stage of *S. epius* was measured under laboratory conditions with the temperature averaging  $28.5 \pm 1.7^\circ\text{C}$ , ranging from  $26.8$ – $30.4^\circ\text{C}$ , the relative humidity ranging from  $40.0$ – $70.0\%$ , and the photoperiod duration of 12L:12D, using papaya mealybugs reared



Figure 2. Larval stages of the apefly, *Spalgis epius*.



Figure 3. Monkey-faced pupae of the apefly, *Spalgis epius*.

Table 1. Body size of egg, different larval instars and pupa of *Spalgis epius* (n=20).

Stage of development (n=20)	Width (mm)		Length (mm)	
	Mean $\pm$ SD	Range	Mean $\pm$ SD	ange
Egg	0.47 $\pm$ 0.05	0.40-0.50		
Larva: 1st instar	0.61 $\pm$ 0.11	0.50-0.80	1.94 $\pm$ 0.11	1.07-2.10
2nd instar	1.72 $\pm$ 0.11	1.50-1.90	3.57 $\pm$ 0.16	3.20-3.90
3rd instar	3.80 $\pm$ 0.17	3.40-4.20	7.05 $\pm$ 0.32	6.40-7.50
4th instar	6.29 $\pm$ 0.32	5.70-6.90	11.74 $\pm$ 0.42	10.90-12.40
5th instar	5.08 $\pm$ 0.20	4.70-5.30	8.95 $\pm$ 0.43	8.30-9.70
Pupa	4.00 $\pm$ 0.22	3.60-4.40	7.01 $\pm$ 0.29	6.40-7.50

Table 2. Duration of developmental stages of *Spalgis epius* reared on *Paracoccus marginatus* under laboratory condition (n=34-50).

Stage of development	Number of specimens (n)	Duration (days)	
		Mean $\pm$ SD	Range
Egg	50	3.54 $\pm$ 0.50	3-4
Larva: 1st instar	47	2.43 $\pm$ 0.50	2-3
2nd instar	42	2.48 $\pm$ 0.51	2-3
3rd instar	39	2.26 $\pm$ 0.44	2-3
4th instar	36	3.28 $\pm$ 0.45	3-4
5th instar	36	1.56 $\pm$ 0.50	1-2
Total larval period	36	12.01 $\pm$ 2.40	9-1
Pupa	34	310.32 $\pm$ 0.88	8-1
Egg to adult	34	125.87 $\pm$ 3.78	21-30



on pumpkin as food. The incubation period was recorded as the elapsed time at which the first 50 eggs hatched from an egg sac. This experimental cohort was then used to calculate the duration of successive developmental stages. Sample size declined with each developmental stage due to innate natural mortality until only 23 adults emerged during a period of almost one month. The developmental period of each *S. epius* life stage is given in Table 2. While the mean duration from egg to adult emergence was  $25.87 \pm 3.78$  days, the mean duration of the egg stage, larval instar and pupal stages were  $3.54 \pm 0.50$ ,  $12.01 \pm 2.40$ , and  $10.32 \pm 0.88$  days, respectively. These developmental periods are somewhat longer than those reported by other investigators. Dinesh *et al.* (2010) reported  $23.8 \pm 1.5$  days as the duration from egg to adult whereas it was  $25.87 \pm 3.78$  days in this study. The combined duration of larval and pupal stages was reported as  $14.83 \pm 0.44$  days by Thangamalar *et al.* (2010), as  $19.8 \pm 1.39$  days in Dinesh *et al.* (2010), but was  $22.33 \pm 3.28$  days in this study. These differences could be due to different rearing regimes or perhaps to real biological differences in *S. epius* populations, but may be of no consequence to the utility of *S. epius* as a biological control agent.

The larvae of *S. epius* underwent five larval instars and a pupal stage to reach adulthood. Table 3 shows the geometric growth ratio of *S. epius* larvae in each larval instar. The mean width of the head capsule yielded a consecutive geometric progression during each stage ranging from 1.39 to 1.87, averaging 1.65. The calculated or theoretical head capsule width was thus computed from the average mean geometric progression of 1.65. In the comparison between the observed and theoretical head capsule widths using the chi-square test, the computed pooled chi-square value ( $\chi^2$ ) was 0.18,  $df = 3$ , with  $P\text{-value} > 0.99$ . Therefore, the difference or the discrepancy between the observed and theoretical head capsule widths was not significant and the geometric growth ratio of 1.65 of *S. epius* larvae conformed to Dyar's Law (Imms, 1957). We found no reports on the growth ratio of *S. epius* elsewhere to determine its development. Dyar (1890) observed the head capsule width of 28 species of Lepidoptera and found that it followed a regular geometric progression in successive instars. Gordh and Headrick (2001), how-

ever, argued that the law is a predictive tool for estimating instar number for some species, and has been extrapolated to other groups of insects, but subsequent investigations have shown that Dyar's Law and Przibram's Rule (Przibram and Megušar, 1912), which uses the doubling weight of the larvae, are not accurate predictors of instar number and are not generally valid as an allometric growth measurement.

### 3.4 Predatory potential of *S. epius*

The daily consumption of papaya mealybug by larvae of *S. epius* for 12 days is shown in Figure 4. The total numbers of prey consumed during the larval instar by the five larvae were  $4,115.75 \pm 553.28$  eggs,  $281.25 \pm 45.08$  nymphs and  $77.50 \pm 16.52$  adults (Table 4). After its fifth day as a larva, the instar-specific rate of mealy bug consumption more or less doubled. Nymphs were consumed more than adults from the third day onwards. We surmise that eggs were preferred over later developmental stages because they could not escape the rapacious butterfly larvae. The stage-wise prey consumption indicated that the older the larval stage the higher the number of prey consumed. The predatory potential of *S. epius* as a biological control agent of *P. marginatus* was evident from this investigation on prey consumption. Should these figures be extrapolated to known popula-

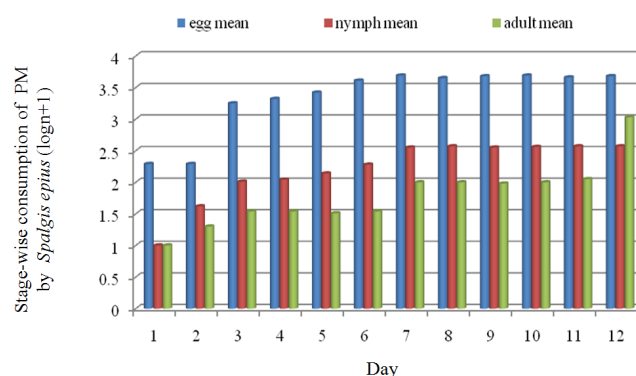


Figure 4. Daily consumption of *Paracoccus marginatus* by larvae of *Spalgis epius* under laboratory conditions.

Table 3. Geometric growth ratio of *Spalgis epius* larvae using head capsule width as a parameter (n=20).

Stage of development	Number of specimens (n)	Mean observed head capsule width (mm)	Geometric progression	Theoretical head capsule width (mm)	$\chi^2$
Larva:					
1st instar	47	0.24	$0.45/0.24 = 1.88$	0.24	0.000
2nd instar	42	0.45	$0.76/0.45 = 1.69$	$0.24 \times 1.6 = 0.40$	0.006
3rd instar	39	0.76	$1.23/0.76 = 1.6$	$0.40 \times 1.65 = 0.66$	0.152
4th instar	36	1.23	$21.72/1.23 = 1.40$	$0.66 \times 1.65 = 1.09$	0.018
5th instar	36	1.72		$1.09 \times 1.65 = 1.80$	0.004
		4.40	Mean geometric progression = 1.65	4.19	$\chi^2 = 0.18$ $P > 0.99$

Table 4. Larval stage-wise consumption of *Paracoccus marginatus* by larvae of *Spalgis epius* in different instars under laboratory condition.

Stage of development	Egg		Nymph		Adult	
	Mean±SD	Range	Mean±SD	Range	Mean ±SD	Range
Larva:						
1st instar	170.00±26.32	65-105	4.25±1.71	2-6	2.00±0.82	1-3
2nd instar	654.00±67.97	132-269	35.25±12.48	6-20	10.25±4.29	1-5
3rd instar	1,376.75±130.95	374-516	93.25±11.70	15-43	23.50±3.94	2-12
4th instar	1,426.50±252.93	369-596	110.75±15.60	31-45	31.00±5.25	9-12
5th instar	488.50±75.11	401-571	37.75±3.59	35-43	10.75±2.22	8-13
Total	4,115.75±553.28	65-596	281.25±45.08	2-45	77.50±16.52	1-13

tion densities of *S. epius*, its predatory potential as a biological control agent of *P. marginatus* would be considerable. These findings suggest that further investigation on the population dynamics of both the papaya mealybug and the predatory aphyllid is justifiable as a basis for exploiting *S. epius* for augmentative biological control of *P. marginatus*. An integrated pest management program of *P. marginatus* which is a recently detected invasive alien species requires immediate action. The use of *S. epius* and/or other resident natural enemies of *P. marginatus* already present in Thailand is an appropriate approach to augmentative biological control which avoids the potential risks of adverse ecological consequence of incompatibility inherent in the introduction or importation of natural enemies from abroad. Classical biological control of *P. marginatus* by introduction of its natural enemies consisted of *Acerophagus papayae* Noyes and Schauff, *Anagyrus loecki* Noyes and Menezes and *Pseudoleptomastix mexicana* Noyes and Schauff (Hymenoptera: Encyrtidae) from Mexico has been implemented in Florida, USA (Walker *et al.*, 2006), Guam (Meyerdirk *et al.*, 2004), Republic of Palau (Muniappan *et al.*, 2006), and Hawaii (Heu *et al.*, 2007). Initial attempt to carry out classical biological control of *P. marginatus* in Thailand through the introduction of these natural enemies from Mexico and Puerto Rico in 2009 failed to become materialized due to technical reasons (B. Napompeth, Pers. Comm.).

#### 4. Conclusions

As a recently detected and damaging invasive alien species (IAS) in Thailand since 2008, the papaya mealybug, *P. marginatus*, deserves an immediate and effective integrated pest management strategy to prevent it from becoming a devastating insect pest of economic importance in Thailand. In the absence of effective imported natural enemies, the use of the entomophagous aphyllid, *S. epius*, to control *P. marginatus* through an augmentative biological control program may be warranted. *Spalgis epius* occurs in all regions of Thailand and has a demonstrated capacity to destroy large numbers of *P. marginatus*. This investigation

on its life history, growth ratio and its predatory potential supports its amenability and suitability for further intensive utilization for augmentative biological control of the papaya mealybug in Thailand.

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