

## **Preimaginal Phases and Development of *Spalangia gemina* and *Pachycrepoideus vindemiae* (Hymenoptera: Pteromalidae) on House Fly (Diptera: Muscidae)**

**Ubong TANGKAWANIT<sup>\*</sup>, Siripa KAEWKAMSAN and Nutcharee SIRI**

*Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand*

(\*Corresponding author's e-mail: [ubonta@kku.ac.th](mailto:ubonta@kku.ac.th))

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### **Abstract**

The development and morphology of immature phases of *Spalangia gemina* Boucek and *Pachycrepoideus vindemiae* Rondani are described from a laboratory reared culture maintained on the house fly *Musca domestica* L. (Diptera: Muscidae). Body width and length of pupal parasitoid were recorded. These 2 characteristics were determined for each larval instar and classified using cluster analysis in the SAS program. Developmental time from egg to adult of *S. gemina* and *P. vindemiae* was approximately 18 - 24 days and 15 - 18 days respectively at 25±3 °C and 45 - 65 % relative humidity. Eggs of *S. gemina* and *P. vindemiae* are hymenopteriform. Their incubation times are 24 - 48 h. The results show that the different numbers of larval instars in these parasitoids are well classified. The larvae of *S. gemina* has 3 instars with protuberances present on the last instar, whereas, *P. vindemiae* has 4 larval instars and the final instar lacks protuberances. The instars of *P. vindemiae* are morphologically similar except size and color of internal organs. Prepupae of both species are exarate and noticeably excrete meconium from the body. This information on preimaginal stages can be useful in the production and identification of parasitoids of the house fly.

**Keywords:** Pteromalidae, immature phase, parasitoids, Muscidae, *Musca domestica*

### **Introduction**

The house fly (*Musca domestica*) is a nuisance pest of livestock, poultry, and humans. They use human and animal waste products such as animal faeces, garbage and other decomposing organic materials for food and reproduction. Even though, house flies are not blood feeding insects, they are competent vectors of microbial and metazoan pathogens which are important for sanitation. They transmit intestinal worms or their eggs, and are potential vectors of pathogens of diarrhoea, gastroenteritis, typhoid, cholera, anthrax, eye inflammation and possibly tuberculosis [1]. High population densities of house flies in poultry units and cattle farms cause irritation and annoyance to animals and farmers. In poultry units, flies commonly develop in large numbers in manure and could reduce egg production [2]. House flies can produce high population densities at warm temperatures and are active during the day. From this reason, the house fly is one of the important pests in tropical regions [1].

Farmers in many countries employ insecticides such as cyromazine, 2,2-dichlorovinyl dimethyl phosphate, permethrin and dichlorvos to decrease house fly populations in housing areas and livestock units [3,4]. However, insecticides have disadvantages such as insecticide resistance in the house fly [3,4] and chemical contamination of animal products such as milk [3,4] and manure [5].

House fly parasitoids have been used as biological control agents for house fly and other filth flies on the livestock and pig farms of Norway [6] and Denmark [5]. Adult parasitoids deposit their eggs into

the pupal host. After egg hatching, parasitoid larvae feed on the pupal host's tissue and pupate on the host tissue. Finally, the pupal host dies and the parasitoids then emerge from the host cocoon. In Thailand, 14 filth fly parasitoids species has been reported [7]. However, they have not yet been used as biological control agents against Diptera.

*S. gemina* and *P. vindemiae* are 2 pupal parasitoids of the house fly and stable fly in Thailand [7], especially, in dairy farms in the northeastern region (Tangkawanit, unpublished data). These parasitoids may have the potential to control house flies and stable flies in animal farms. However, before using the insects as biological control agents, basic biological knowledge of the parasitoid is needed, in order to predict duration in the pupal host and enable species identification of the preimaginal phase. The preimaginal stages of some Pteromalid species have been reported, such as *S. endius* on house fly pupa [8], *S. cameroni* [9] and *P. vindemiae* on fruit flies (*Ceratitidis capitata*) [10]. However, the preimaginal stages of *S. gemina* and *P. vindemiae* on house fly have not been studied.

In this study, the developmental and characteristics of preimaginal stages including egg, larva, and pupa of *S. gemina* and *P. vindemiae* were investigated to identify both species in all preimaginal instars found in house fly pupae, without rearing them in a laboratory. Characteristics of each stage were clustered in order to support classification. Identification of the differences in biology and morphology of the 2 species will allow us to estimate parasitoid populations in a control area for house fly pupa without the need to examine emerged adult flies. It will also have useful applications for mass rearing methods and management of parasitoids.

## Materials and methods

### Insect rearing

Insect colonies were reared at the National Biological Control Research Center (Upper Northeastern Regional Center), Khon Kaen University, Thailand. House flies were maintained in a mesh cage 30×30×30 cm<sup>3</sup>. Adults were fed by food containing powdered milk and sugar (3:1). A Petri dish with moist tissue paper and fishmeal was provided in the cage as a site for oviposition. Egg batches were transferred to a container with a mixture of water, powdered milk, yeast and rice bran. After 3 - 5 days, food and larvae were transferred to a sieve container that allowed the mature larvae to move to dry areas underneath the sieve where they could develop to pupae in the same container. Pupae were transferred to a new cage for rearing the emerging adults and providing the hosts for parasitoids oviposition.

*S. gemina* and *P. vindemiae* were separately reared in plastic cages 21×10.5 cm<sup>2</sup> (radius×height) with ventilation and were fed with 10 % solution of honey soaked into a piece of wet blotting cotton, and were maintained at room temperature (25±3 °C) and 45 - 65 % relative humidity.

Pupae of house flies were used as hosts. One hundred pupae were provided to 20 pairs of the parasitoid for oviposition.

### Developmental and Morphological studies of preimaginal stages

Twenty pairs of each newly hatched parasitoid species were separately exposed in a plastic cage (5.5×5.5×2.5 cm<sup>3</sup>), containing 400 individuals of one-day old house fly pupae per cage (5 cages were prepared for the experiment). After 24 h of exposure, parasitoids were separated from the cage. Twenty fresh individuals of parasitized pupae were daily dissected using a small needle and photographed under the stereomicroscope. The size of pupal parasitoids was measured by ocular micrometer (n = 20). Morphological characteristics and developmental time of each immature stage of both parasitoid species were examined. To determine the underlying implicit grouping of the different instars with respect to larval size (mean of width and length), morphological characteristics (protuberance, color of alimentary tract, meconium) and some larval exuviae of *S. gemina* and *P. vindemiae* larvae, a cluster analysis was performed using the hierarchical clustering method. The statistical analysis was performed using the SAS package [11].

## Results and discussion

### Developmental and morphological studies of preimaginal stages

*S. gemina* and *P. vindimiae* are morphologically different and slightly different within each species, some larval instars were different from each other. Total developmental period from egg to adult of *S. gemina* was between 18 - 24 days (mean±SD = 18.22±0.96) whereas in *P. vindimiae* it was between 15 - 18 days (mean±SD = 16.95±0.59) (Table 1). The developmental stages are described below (Figures 1 and 2).

#### *Spalangia gemina*

##### Egg

The egg of *S. gemina* is hymenopteriform with an ovoid to cylindrical shape and pointed apically. Color depends on the developmental time. Newly laid eggs are transparent and turn white before hatching (Figure 1a). A black spot is present on the anterior and posterior ends. Egg size ranges between 0.30 - 0.38 mm (0.34±0.01 mm) in length and 0.08 - 0.10 mm (0.09±0.004 mm) in width. In all observed cases, 1 - 11 eggs (average 6.5) were deposited on a host and they hatched between 24 and 48 h after oviposition.

##### Larva

###### 1<sup>st</sup> Instar

Newly hatched larvae are vermiform shape with 13 segments. Small mandibles are present at the blunt end. The body is translucent, the internal part of the alimentary tract has a white color that is visible through the larval skin (Figure 1b). Body length is 0.24 - 0.44 mm (0.34±0.03 mm), body width is 0.10 - 0.16 mm (0.12±0.01 mm). The developmental time is approximately 1 day. Even though, more than one egg was found per host pupa, there was usually only one 1<sup>st</sup> instar larva per pupa, although exceptionally 2 hosts were found with 2 parasitoids larvae inside (10 %).

###### 2<sup>nd</sup> Instar

The morphological character of the 2<sup>nd</sup> instar larva is similar to the 1<sup>st</sup> instar but the middle part of the body part is broader and the alimentary tract turns to reddish brown color (Figure 1c). Body length is 0.32 - 0.70 mm (0.45±0.04 mm), body width is 0.12 - 0.30 mm (0.21±0.03 mm) (n = 20). The developmental time is approximately 1 - 2 days.

###### 3<sup>rd</sup> Instar

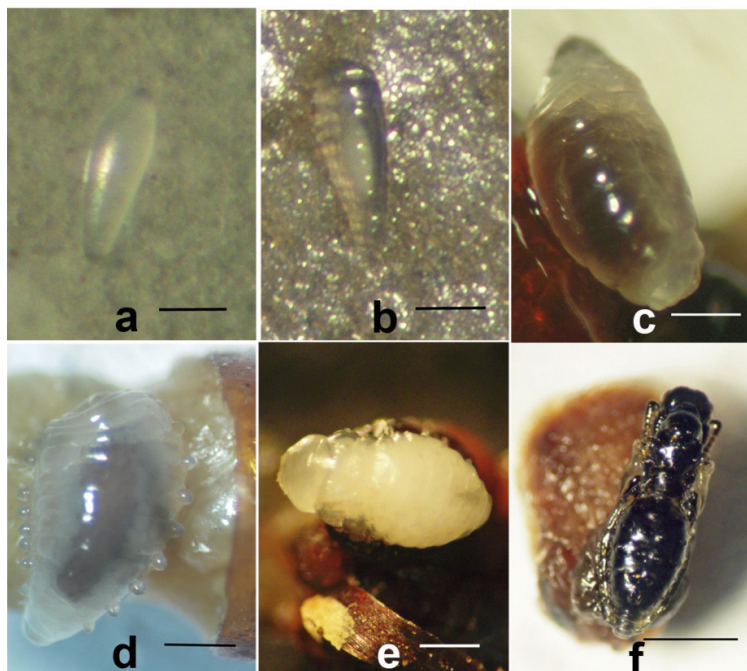
Protuberances are well developed on the 3<sup>rd</sup> thoracic and the 1<sup>st</sup> to 8<sup>th</sup> abdominal segments (Figure 1d). Body length is 0.44 - 3.00 mm (1.93±0.34 mm), body width is 0.22 - 1.50 mm (0.97±0.17 mm) (n = 20). The developmental time is approximately 3-8 days.

##### Prepupa

In this stage, the larva stops moving. Prepupal characters are similar to those of the 3<sup>rd</sup> instar larvae, Protuberances are present, but the body is elongated and white (Figure 1e). Meconium is excreted from the body. The body length of *S. gemina* is 1.90 - 3.00 mm (2.3±0.26 mm), body width is 0.60 - 1.45 mm (1.02±0.13 mm). The developmental time of the prepupal stage is approximately 2 - 4 days.

##### Pupa

The pupa is exarate. The early stage is white and turns to pale yellow or darker (Figure 1f). Fresh pupa usually has some remaining meconium at the abdominal end. After 3 days, red compound eyes and 3 ocelli are visible. After 5 days, the body become darker. Body length is 1.75 - 3.50 mm (2.79±0.19 mm), body width is 0.6 - 1.40 mm (0.97±0.06 mm). The developmental time is approximately 5 - 11 days.



**Figure 1** Preimaginal stages of *S. gemina*: a, egg; b, 1<sup>st</sup> instar larva; c, 2<sup>nd</sup> instar larva; d, 3<sup>rd</sup> instar larva; e, prepupa; f, pupa (last stage), scale bar = 0.1 mm.

### *Pachycrepoides vindemiae*

#### **Egg**

The egg of *P. vindemiae* is similar to *S. gemina*, but there are no black spots on the anterior and posterior ends. The egg size of *P. vindemiae* is smaller than *S. gemina*. Egg length and width are 0.20 - 0.24 mm (0.21±0.01 mm) and 0.08 - 0.09 mm (0.08±0.004 mm), respectively. Pupal hosts were found with 1 to 5 eggs (average 3.95). The incubation period is 24 - 48 h (**Figure 2a**).

#### **Larva**

##### **1<sup>st</sup> Instar**

This instar is similar to *S. gemina*. The larva is vermiform in shape with 13 segments. The body is translucent, the white internal part of the alimentary tract is visible through the larval skin (**Figure 2b**). Body length is 0.2 - 0.32 mm (0.24±0.02 mm), body width is 0.08 - 0.15 mm (0.09±0.01 mm). The developmental time is approximately 1 - 2 days. There is usually one 1<sup>st</sup> instar larva per pupa although one pupa was found with 2 parasitoids larvae (5 %).

##### **2<sup>nd</sup> Instar**

The morphology of the 2<sup>nd</sup> instar is similar to that of the 1<sup>st</sup> instar; the alimentary tract turns to reddish brown color (**Figure 2c**). Body length is 0.30 - 0.74 mm (0.40±0.08 mm), body width is 0.16 - 0.36 mm (0.22±0.04 mm). The developmental time is approximately 1 day.

##### **3<sup>rd</sup> Instar**

The protuberances that are present in *S. gemina* are absent in *P. vindemiae* (**Figure 2d**). Body shape is similar to the 2<sup>nd</sup> instar larvae but larger. Body length is 0.44 - 1.14 mm (0.83±0.15 mm), body width is 0.26 - 0.60 mm (0.46±0.08 mm). The developmental time is approximately 1 day.

#### 4<sup>th</sup> Instar

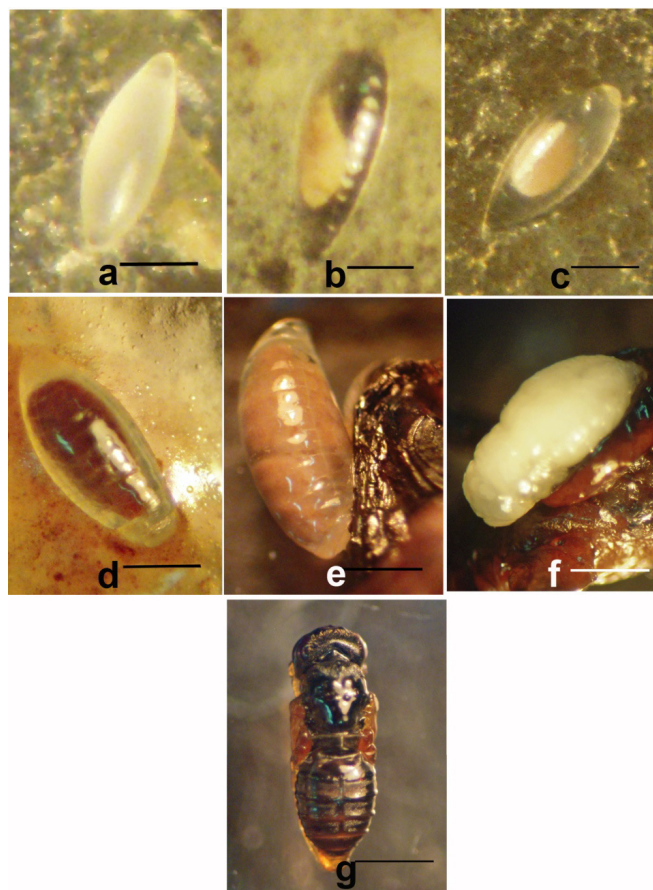
The 4<sup>th</sup> instar is bigger than the 3<sup>rd</sup> instar. Body length is 1.30 - 2.00 mm ( $1.62 \pm 0.16$  mm), body width is 0.50 - 0.90 mm ( $0.62 \pm 0.08$  mm). The body becomes shorter in the last stage of the instar (**Figure 2e**). The developmental time is approximately 3 - 4 days.

#### Prepupa

Protuberances are not present and the body color is white. The body shape is stout (**Figure 2f**). Meconium is excreted from the body, as found in *S. gemina*. The body length is 0.84 - 1.62 mm ( $1.27 \pm 0.19$  mm), body width is 0.44 - 0.82 mm ( $0.69 \pm 0.09$  mm). The developmental time of prepupal stage is approximately 1 - 3 days.

#### Pupa

The pupa is exarate. The early stage is white subsequently becoming pale orange and black. Newly transformed pupa usually have some remaining meconium at the abdominal end as in *S. gemina*. Red compound eyes and 5 ocelli are visible after 1 day. The thoracic segments become darker after the 4<sup>th</sup> day, becoming black after the 5<sup>th</sup> day (**Figure 2g**). Body length is 1.10 - 1.90 mm ( $1.60 \pm 0.21$  mm), body width is 0.36 - 0.80 mm ( $0.59 \pm 0.04$  mm). The developmental time is approximately 3 - 6 days. Males and females emerge on the same day.



**Figure 2** Preimaginal stages of *P. vindemiae*: a, egg; b, 1<sup>st</sup> instar larva; c, 2<sup>nd</sup> instar larva; d, 3<sup>rd</sup> instar larva; e, 4<sup>th</sup> instar larva; f, prepupa; g, pupa (last stage), scale bar = 0.1 mm.

**Table 1** Developmental time of immature stages of *S. gemina* and *P. vindemiae*.

Stages*	<i>S. gemina</i>		<i>P. vindemiae</i>	
	Range (days)	Mean ± SD	Range (days)	Mean ± SD
Egg	1-2	1.38 ± 0.48	1-2	1.45 ± 0.49
Larva				
1 <sup>st</sup> instar larva	0-1	2.60 ± 0.51	1-2	1.61 ± 0.51
2 <sup>nd</sup> instar larva	0-2	2.92 ± 0.65	0-1	1.57 ± 0.49
3 <sup>rd</sup> instar larva	3-8	4.75 ± 0.75	0-1	1.62 ± 0.48
4 <sup>th</sup> instar larva	-	-	0-4	2.08 ± 0.70
Prepupa	2-4	1.47 ± 0.63	0-2	2.06 ± 0.74
Pupa	5-11	8.03 ± 1.30	3-6	4.80 ± 0.82
Egg - Adult	18-24	21.17 ± 0.72	15-18	16.95 ± 0.59

\*n = 20

### Cluster analyses

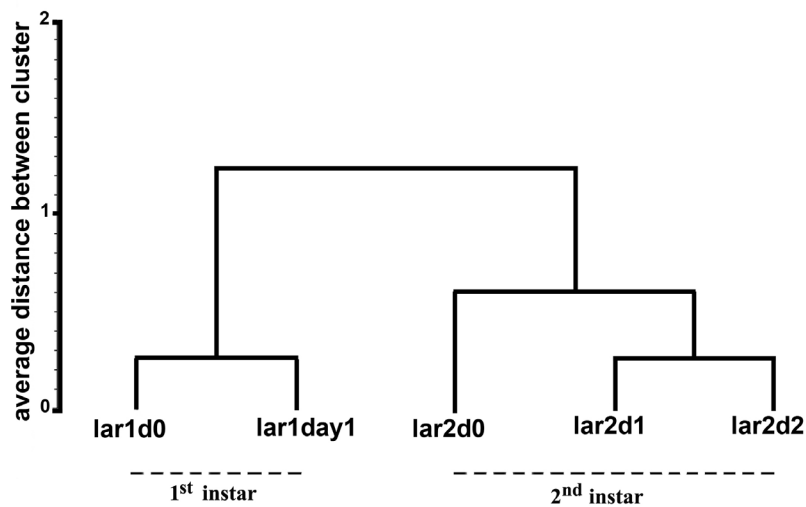
The characteristics of larval stages of house fly parasitoids are difficult to classify. Protuberances on third instar larvae of *S. gemina* can separate the last instar from the others [12]. However, identification based on first and second instars remains problematic. Therefore, the dendrograms were constructed using PROC cluster data (SAS Institute, 2001) using data of width and length (**Table 2**). The cluster analysis perfectly resolved 1<sup>st</sup> and 2<sup>nd</sup> instar larvae of *S. gemina* into 2 groups (**Figure 3**) with  $r^2 = 0.843$ .

The characteristics of all instars of *P. vindemiae* are similar. Characteristics for species recognition such as the protuberances found in *S. gemina* are absent in *P. vindemiae*. Therefore, all larval instars were clustered by size. The cluster analysis of larval instars of *P. vindemiae* revealed 2 main groups. Group I was divided into 2 subgroups. First instar larvae formed subgroup I, while second instar larvae formed subgroup II. Group II was also composed of 2 subgroups. Subgroup II-1 contained only the third instar larvae and subgroup II-2 was composed of the fourth instar larvae (**Table 2** and **Figure 4**).

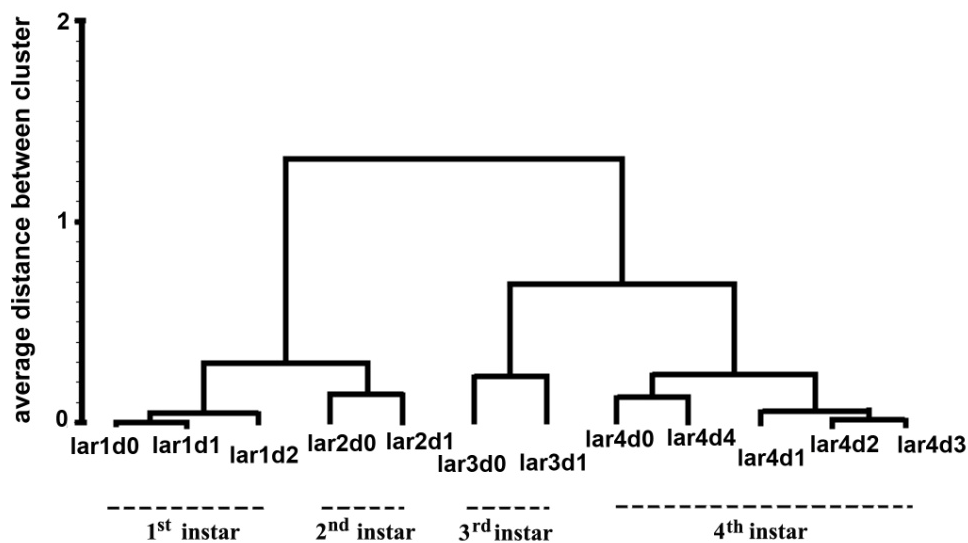
**Table 2** Developmental time of immature stages of *S. gemina* and *P. vindemiae*. L1 = 1<sup>st</sup> instar larva, L2 = 2<sup>nd</sup> instar larva, L3 = 3<sup>rd</sup> instar larva, L4 = 4<sup>th</sup> instar larva.

Species*	Stage and day	Width mean±SD	Range	Length mean±SD	Range
<i>S. gemina</i>	L1 day0	0.11±0.01	0.1-0.14	0.32±0.04	0.24±0.44
	L1 day1	0.13±0.02	0.1-0.16	0.35±0.04	0.26-0.44
	L2 day0	0.18±0.01	0.18-0.20	0.43±0.04	0.32-0.50
	L2 day1	0.23±0.04	0.12-0.30	0.47±0.06	0.34-0.70
	L2 day2	0.25±0.07	0.20-0.30	0.50±0.02	0.50-0.54
<i>P. vindemiae</i>	L1 day0	0.09±0.01	0.08-0.14	0.24±0.01	0.2±0.26
	L1 day1	0.09±0.01	0.08-0.14	0.24±0.02	0.2-0.32
	L1 day2	0.11±0.02	0.09-0.15	0.28±0.03	0.26-0.32
	L2 day0	0.21±0.03	0.16-0.30	0.38±0.06	0.30-0.62
	L2 day1	0.25±0.05	0.16-0.36	0.53±0.08	0.32-0.74
	L3 day0	0.43±0.07	0.26-0.64	0.91±0.15	0.44-1.14
	L3 day1	0.54±0.07	0.42-0.60	1.06±0.07	0.94-1.16
	L4 day0	0.64±0.07	0.50-0.80	1.45±0.16	1.00-1.74
	L4 day1	0.75±0.05	0.60-0.90	1.68±0.18	1.30-2.00
	L4 day2	0.78±0.04	0.60-0.84	1.70±0.16	1.36-1.96
	L4 day3	0.77±0.05	0.72-0.90	1.69±0.11	1.54-1.90
	L4 day4	0.68±0.10	0.60-0.80	1.58±0.16	1.40-1.70

\*n = 20



**Figure 3** Dendrogram based on width and length of *S. gemina* generated by cluster R-square method using PROC cluster data (SAS Institute, 2001). lar1d0 = 1<sup>st</sup> larval instar day 0, lar1d1 = 1<sup>st</sup> larval instar day 1, lar2d0 = 2<sup>nd</sup> larval instar day 0, lar2d1 = 2<sup>nd</sup> larval instar day 1, lar2d2 = 2<sup>nd</sup> larval instar day 2.



**Figure 4** Dendrogram based on width and length of *Pachycrepoideus vindemiae* generated by cluster R-square method using PROC cluster data (SAS Institute, 2001). lar1d0 = 1<sup>st</sup> larval instar day 0, lar1d1 = 1<sup>st</sup> larval instar day 1, lar1d2 = 1<sup>st</sup> larval instar day 2, lar2d0 = 2<sup>nd</sup> larval instar day 0, lar2d1 = 2<sup>nd</sup> larval instar day 1, lar3d0 = 3<sup>rd</sup> larval instar day 0, lar3d1 = 3<sup>rd</sup> larval instar day 1, lar4d0 = 4<sup>th</sup> larval instar day 0, lar4d1 = 4<sup>th</sup> larval instar day 1, lar4d2 = 4<sup>th</sup> larval instar day 2, lar4d3 = 4<sup>th</sup> larval instar day 3, lar4d4 = 4<sup>th</sup> larval instar day 4.

Our results indicate that eggs of *S. gemina* and *P. vindemiae* are hymenopteriform and are thus similar to *S. cameroni* [9] and *P. vindemiae* [10] on the fruit fly. The incubation periods of the 2 species on house flies are 24 - 48 h (at 25±3 °C) which are also similar to that of *P. vindemiae* on fruit flies (at 21 - 26 °C). However, there are differences in the incubation periods of *S. cameroni* on fruit flies (at 21 - 26 °C) and *S. endius* on house flies (at 26±2 °C) which are 36 - 48 h and 12 h, respectively.

Morphological characteristics of larvae in each stage are largely indistinguishable and of no value for species identification although the last instar of *S. gemina* and *P. vindemiae* can be separated by the presence or absence of protuberances as protuberances are developed on the last instar of *S. gemina*, but not on *P. vindemiae*. Cluster analysis is useful for classifying the larval instars in both species. Laval development of *S. gemina* and *P. vindemiae* are different. *S. gemina* has 3 larval instars whereas *P. vindemiae* has 4. *P. vindemiae* on fruit flies pupae reported by Tormos *et al.* [10] has 5 larval instars but the prepupa was not classified. However, the last instar has meconium which was present in prepupa in this study. Therefore, the prepupa in this study is equivalent to 5<sup>th</sup> instar of *P. vindemiae* on the fruit fly. The number of instar in the genus *Spalangia* is variable, some species have 3 larval instars, e.g. *S. muscidacum* [12] and *S. cameroni* [9], but some have 4, e.g. *S. nigar* [12]. First and second instar of *S. gemina* and *P. vindemiae* are similar. The third larval instar of *S. gemina* has protuberances on the 1<sup>st</sup> to 8<sup>th</sup> abdominal segments, a condition characteristic of mature larva of the genus *Spalangia* [12]. The third to the fourth instar larvae of *P. vindemiae* are without protuberances. Apart from *Spalangia*, some parasitoid larvae also have protuberances in the last instar such as those of Eucharitinae and Oraseminae [13]. Internal organs were visible through the larval skin. The color becomes white in the fourth instar larva. In prepupa, meconium is observed at the abdominal end. The prepupa shape of *P. vindemiae* is white and stout with no protuberances, whereas in *S. gemina* it is pink and elongated with protuberances on the abdominal segments. Like other hymenopteran species, pupae of these 2 species are exarate. The body color becomes darker in older pupae.

The developmental time of *S. gemina* is 18 - 24 days, which is longer than in *P. vindemiae* (15 - 18 days). This may be useful to predict the species within the house fly pupae. However, there are overlapping times during day 18<sup>th</sup> which may cause an error in prediction. Geden [14] reported that the developmental time of *S. gemina* at a temperature of 25 °C is 25 - 27 days. In this study, males and females emerged at the same time unlike *S. endius* in which males emergence first, followed by females 24 h later [8].

## Conclusions

A means to differentiate between the preimaginal stage of *S. gemina* and *P. vindemiae* is provided. Developmental time from egg to adult of *S. gemina* is longer than in *P. vindemiae*, 18 - 24 days and 15 - 18 days, respectively. Eggs of both species are hymenopteriform. Laval development of *S. gemina* and *P. vindemiae* is different; *S. gemina* has 3 larval instars whereas *P. vindemiae* has 4. Distinguishing between each larval stage is difficult but cluster analysis can classify the larval instars by size and protuberances. Pupae of the 2 species are exarate. Knowledge of this study will be useful for predicting the timing of parasitoid emergence in mass production and release for biological control, and for estimating the parasitoid population from house fly pupae after release.

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