Captive breeding of two insular populations of *Pachyrhynchus sarcitis* (Coleoptera: Curculionidae) from Lanyu and Babuyan Islands

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**A B S T R A C T**

Endemic species of oceanic islands are vulnerable due to their geographical isolation and small population sizes. For endangered island species, captive breeding program is an important strategy for conservation and sustainable management. *Pachyrhynchus sarcitis* is a flightless weevil decorated with colourful markings and distributed exclusively on several islets of Southeast Asia including Lanyu, Ludao, and Babuyan Islands. The life history of threatened *Pachyrhynchus* species of these islands was poorly known. This study reared *P. sarcitis* from Lanyu and Babuyan Islands for the first time in the laboratory using their host plant (*Leea guineensis*). The two island populations showed significant differences in instar numbers, in which the weevils from Lanyu Island had a higher instar number regardless the length of developmental duration. The adult body size of both sexes of the Babuyan population became smaller under laboratory condition; whereas Lanyu population in the laboratory did not show this trend. The differences in larval development might suggest local adaptation to the host plants or other life history associated characteristics which requires further research.

**Introduction**

Endemic species of oceanic islands are vulnerable due to geographic isolation, small population size and habitat destruction (Losos and Ricklefs, 2009). Therefore, island species may face higher risk of extinction than their mainland counterparts (Frankham, 1998; Kier et al., 2009). *Pachyrhynchus* weevils (Coleoptera: Curculionidae) are a group of insects decorated with astonishing colouration. They distributed on the Southeast Asian islands from the southern Ryukyus to New Guinea, and 90% of the species were endemic in the Philippines (Schultze, 1923). These weevils are flightless due to the fused elytra which may have limited their dispersal to the remote oceanic islands of Southeast Asia (Schultze, 1923). Although these colourful *Pachyrhynchus* weevils are popular among amateur collectors and researchers, current knowledge of their life history is poorly known, with only a limited number of studies on the mechanism of structural colours (Seago et al., 2009; Welch et al., 2007), biological function of colouration (Tseng et al., 2014), host plants (Hsu et al., 2017; Kayashima, 1940; Oshiro, 1991), secondary defense mechanism (Wang et al., 2018), taxonomy (Barševskis, 2016; Bollino and Sandel, 2015; Chen et al., 2017; Rukmane and Barševskis, 2016; Yoshitake, 2012) and biogeography (Tseng et al., 2018). The detailed information of larval development of *Pachyrhynchus* weevils is still lacking perhaps due to the difficulty of rearing them in the laboratory and the difficulty of finding larvae and eggs in the field (Hsu et al., 2017).

*Pachyrhynchus sarcitis sarcitis* (Behrens, 1887) was originally described from Calayan Island, which is one of the Babuyan Islands north of Luzon, the Philippines (Behrens, 1887; Schultze, 1923). This species is characterized for its glossy black body covered with pale green or pale flesh-coloured spots. In 1930, a population of this species having a black body decorated with green and blue spots, was described as a subspecies *P. sarcitis kotoensis* (Kôno, 1930) from Lanyu (Kôtošî) (Kôno, 1930) and Ludao (Green Island; (Starr and Wang, 1992)) off shore from southeast Taiwan (Fig. 1A). Although only two subspecies have been described from the three islands (*P. s. sarcitis* on Calayan, *P. s. kotoensis* on Lanyu and Ludao), a different *P. sarcitis* population was discovered from Babuyan Island during our expedition to Babuyan Islands. This *P. sarcitis* population has distinct glossy dark purplish colouration decorated with pale yellow spots (Fig. 1B). In geological history of islands, Lanyu, Ludao and Babuyan Islands are all belonged to
Taiwan-Luzon volcanic Arc. This volcanic arc is generated by the subduction of the South China Sea which collided with the eastern margin of the Eurasian Plate. These three islands did not connect with other islands in the glacial period. Despite of the colour difference, a preliminary molecular phylogeny suggested close affinity among these populations (Tseng et al., unpublished data).

Captive breeding of endangered species is a useful and practical solution in conservation biology (Frankham, 2008; Griffiths and Pavajeau, 2008; Seddon et al., 2007). Captive breeding can help restore the population in the wild and obtain the life history information in the process. The populations of \textit{P. sarcitis} endemic to Lanyu (47 km²) and Ludao (16 km²) Islands were listed as a “rare and valuable species” (Category II) by the Wildlife Conservation Act in Taiwan because of their small population sizes, heavy threats from tourism, collection pressure and habitat destruction (Chao et al., 2009). Under the serious threats of the ongoing habitat loss in these small islands, developing an effective rearing technique of \textit{Pachyrhynchus} weevils in the laboratory is urgent and necessary for the conservation and research application.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1}
\caption{Life stages of \textit{Pachyrhynchus sarcitis}. (A) Adult of \textit{Pachyrhynchus sarcitis kotoensis} from Lanyu, Taiwan; (B) adult of \textit{Pachyrhynchus sarcitis} from Babuyan Island, the Philippines; (C, D) larvae were fed on the stems of \textit{Leea guineensis} under laboratory conditions; (E) eggs of \textit{Pachyrhynchus sarcitis kotoensis} from Lanyu were covered by feces; (F) feces were removed from the egg of \textit{Pachyrhynchus sarcitis kotoensis}; (G) larva of \textit{Pachyrhynchus sarcitis kotoensis} from Lanyu Island; (H) pupa of \textit{Pachyrhynchus sarcitis kotoensis} from Lanyu Island in the pupal chamber; (I, J) newly emerged adults of \textit{Pachyrhynchus sarcitis kotoensis} in pupal chamber showing the faint colour spots. I and J are different individuals. The black bars represent the scales.}
\end{figure}
Makihara (Makihara, 1975) and Starr and Wang (Starr and Wang, 1992) recorded a Cissus species (Vitaceae) and Clematis gouriana (Ranunculaceae) as the adult food plants of P. s. kotoensis, respectively. From our own field observations, many P. s. kotoensis adults fed on leaves of Leea guineensis (Leeaceae) and L. philippinensis on Lanyu Island, however, the host plant of P. sarcitis from Babuyan Islands was unknown. This study reported for the first time a new rearing procedure for P. sarcitis in the laboratory. Weevil populations from Lanyu and Babuyan Islands were reared on the host plant (L. guineensis), and the larval development and adult body sizes were recorded and compared.

Material and methods

Adults of P. sarcitis were collected from Babuyan (19°32′58″N, 121°54′40″E) and Lanyu (22°44′44.57″N, 121°33′15.25″E) Islands, respectively. Because most individuals were discovered on L. guineensis and L. philippinensis, the leaves of L. guineensis were used to feed adult weevils in the laboratory. Three individuals from Babuyan Island and eight from Lanyu Island were kept separately in plastic cages (34 × 25 × 22.5 cm) placed in a growth chamber with 12:12 h (L:D) photoperiod and 25 °C, 75% relative humidity (RH) in an insectarium of Taipei Zoo, Taiwan. Eggs of the weevils were checked every day.

To observe the developmental stages of the larvae, fresh stems of L. guineensis (ca. 3 cm in diameter and 5 cm in length) were split vertically into two parts (Fig. 1C, D). Each egg was buried into a stem, and checked for their larval stages every day by the presence of molted cuticles and the changes of their head width. When the stems were consumed completely by the larvae, we provided them with a fresh stem until the larvae molted into pupal stages. The duration of pupal stage were recorded once the adult emerged from the pupa. When the pupae emerged to adults, the weevils stayed in the chamber several days before leaving. The number of days from the emergence to the adult leaving the pupal chamber was recorded as the duration of imago stage. All larvae were kept in the same growth chamber and maintained at 25 °C and 75% relative humidity.

The length and width of the eggs, head width of the larvae, and adult body size were measured to the nearest 0.1 mm using a digital caliper (Mitutoyo, Kanagawa, Japan). Instar number and size differences between the two populations were compared using the Welch two sample t-test followed by a false discovery rate (FDR) correction and Fisher’s exact test in R software (https://cran.r-project.org/), respectively. For the Lanyu population with a sufficient sample size (n = 70), Pearson’s product-moment correlation coefficient in R was used to examine whether there is a correlation between the durations from egg to emergence and adult sizes. Welch two sample t-test was used to examine whether there is size difference between wild and captive breeding individuals in each population.

Results

Larval development

Under laboratory conditions, weevils laid eggs on the wall of cages or on the leaves of the plants. The eggs were usually covered with feces (Fig. 1E, F). Eggs from the Lanyu population were 2.31–2.93 mm (mean ± SE = 2.73 ± 0.13) in length and 1.60–1.97 mm (1.83 ± 0.07) in width. Eggs from the Babuyan population were 2.54–3.03 mm (2.82 ± 0.12) in length, 1.72–2.05 mm (1.86 ± 0.08) in width. The mean duration of egg stage was 20.79 ± 2.34 days (between 10 and 24) in the Lanyu and 20.68 ± 1.94 days (between 17 and 24) in the Babuyan population (Tables 1, 2).

The two populations showed significant difference in instar numbers (p = .038, Fisher’s exact test). The Babuyan population had a lower number of instars from four (23%), five (68%), to six (9%). Although the majority of the Lanyu population had the same instars of four (5%), five (53%) and six (40%), they had a higher instar number and extended to seven (1%) and eight (1%) (Table 2; Fig. 2).

The first larval stage was 6–14 (9.55 ± 1.66) and 7–15 (9.32 ± 1.96) days in the Lanyu and Babuyan populations, respectively. In the second larval stage, larvae of Lanyu ranged from 9 to 28 days (15.02 ± 3.38), and larvae of Babuyan ranged from 12 to 20 days (15.45 ± 2.54). The durations of the third instar ranged from 13 to 29 (19.48 ± 3.16) and 15–33 days (22.59 ± 4.88) in the Lanyu and Babuyan populations, respectively. The difference of larval development had increased since the fourth (10–53 days, 24.73 ± 7.35), fifth (14–73 days, 35.72 ± 11.02) and sixth instar (13–63 days, 33.8 ± 11.60) of the Lanyu population, and the ranges of these instars were smaller (fourth instar: 15–46 days, 24.68 ± 7.87; fifth: 18–48 days, 34.65 ± 8.10; sixth: 39–43 days, 41.00 ± 2.83) in the Babuyan population.

For individuals with four instars, only the duration of the fourth instar was significantly different between two populations (50.26 ± 2.22 vs. 35.00 ± 8.34, p < .05), and the egg size and head width were similar (Table 1). There was no difference of the durations of the fifth instar between Lanyu and Babuyan individuals, but the head width of larvae from Lanyu in the fifth instar was significantly larger than that of larvae from Babuyan (2.95 ± 0.16 vs. 2.77 ± 0.15, p < .05). For the individuals with six instars, the duration of the third instar was longer (27 vs. 19.54 ± 3.67 days, p < .05), and the egg length (2.72 ± 0.13 vs. 2.89 ± 0.01, p < .05) and head width of the first instar larvae (1.14 ± 0.04 vs. 1.17 ± 0.01, p < .05) were larger in the Babuyan population, respectively. In the fifth instar, however, the larvae of Lanyu population became slightly larger than those of Babuyan population (2.80 ± 0.17 vs 2.73 ± 0.00, p < .05) (Table 1).

Adult body size

After emergence from pupal chamber, the blue or yellow colour markings started to appear during the first few minutes, and their bodies stayed soft until a few days later. Under laboratory conditions, the elytral length of adults in both sexes were significantly larger in Lanyu individuals (male: Lanyu, 9.80 ± 0.57 vs. Babuyan, 9.00 ± 0.50, p < .05; female: Lanyu, 10.51 ± 0.64 vs. Babuyan, 9.64 ± 0.57, p < .05), but the body width was similar in two populations (male: 6.25 ± 0.38 vs. 6.11 ± 0.41; female: 6.76 ± 0.46 vs. 6.84 ± 0.46). Compared to the wild populations, both captive breeding males and females became smaller in the Babuyan population (male: wild, n = 4, 10.48 ± 0.12 vs. CB, n = 15, 9.00 ± 0.50, p < .05; female: wild, n = 4, 11.17 ± 0.41 vs. CB, n = 14, 9.64 ± 0.57, p < .05), but not in the Lanyu population (male: wild, n = 8, 9.87 ± 0.67 vs. CB, n = 39, 9.80 ± 0.57; female: wild, n = 5, 10.68 ± 0.68 vs. CB, n = 31, 10.51 ± 0.64) (Fig. 3). In the Lanyu population, the duration between oviposition and adult emergence was from 123 to 227 days. Pearson’s product-moment correlation coefficient (r) between the body sizes of males and females and the durations between eggs and adults was 0.04–0.25 (p = .80 and 0.18), respectively, suggesting that the male and female adult sizes had not increased when the growth duration increased.

Discussion

This study reported the first successful rearing for Pachyrhynchus weevils using their host plants. Compared to captive breeding of P. infernalis, of which larvae fed on sweet potatoes or artificial agar-based food (Anbutsu et al., 2017), P. sarcitis fed on L. guineensis showed a higher survival rate (ca. 80–90% vs. 13.7%). This might represent the suboptimal food for P. infernalis or different reproductive strategies (eggs of P. sarcitis are larger than that of P. infernalis, and larger eggs may have a higher survival rate). The rearing technique developed in this study not only facilitates future research projects, but also provides crucial information for the conservation of this endangered species with narrow geographical distribution. It could effectively reduce the...
number of weevils needed from the wild population for research projects, and provides adequate number of individuals for releasing back to the islands after a careful assessment of conservation management programs.

Establishing an effective captive breeding technique is a critical first step in many research and conservation projects. One of the most famous examples is Heliconius butterfly (Mavárez et al., 2006; Melo et al., 2009). The speciation process and genetic control of wing colouration were revealed through captive breeding programs (Huber et al., 2015; Joron et al., 2006; Le Poul et al., 2014). Pachyrhynchus weevils display astonishing diversity of aposematic colouration and extremely high insular species diversity (Chen et al., 2017; Schultz, 1923; Tseng et al., 2014). Nevertheless, except for a few studies on the taxonomy and the structure and function of colours, relevant studies considering the ecology and evolution of their warning colouration, as well as the process of diversification in weevils (McKenna et al., 2009). Understanding the variation of larval development between island populations of P. sarcitis can facilitate initial recognition of potential host plant adaptation. Host shift to distantly related host plants during the process of inter-island diversification in Pachyrhynchus weevils may have provided the platform for speciation (Chen et al., 2017; Tseng et al., 2018). In this study, the adult body size of captive breeding individuals was similar to those of the wild population in Lanyu Island, but significantly smaller for the population from Babuyan Island. One probable explanation is the adaptation on local host plant species. On the other hand, the process of diversification in weevils (McKenna et al., 2009) facilitates the recognition of potential host plant adaptation. Host shift to distantly related host plants during the process of inter-island diversification in Pachyrhynchus weevils may have provided the platform for speciation (Chen et al., 2017; Tseng et al., 2018). In this study, the adult body size of captive breeding individuals was similar to those of the wild population in Lanyu Island, but significantly smaller for the population from Babuyan Island. One probable explanation is the adaptation on local host plant species. On the other hand, the process of diversification in weevils (McKenna et al., 2009). Understanding the variation of larval development between island populations of P. sarcitis can facilitate initial recognition of potential host plant adaptation. Host shift to distantly related host plants during the process of inter-island diversification in Pachyrhynchus weevils may have provided the platform for speciation (Chen et al., 2017; Tseng et al., 2018). In this study, the adult body size of captive breeding individuals was similar to those of the wild population in Lanyu Island, but significantly smaller for the population from Babuyan Island. One probable explanation is the adaptation on local host plant species.

### Table 1

<table>
<thead>
<tr>
<th>4-instar individuals</th>
<th>P</th>
<th>5-instar individuals</th>
<th>P</th>
<th>6-instar individuals</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lanyu (n = 4)</td>
<td>Babuyan (n = 5)</td>
<td>Lanyu (n = 46)</td>
<td>Babuyan (n = 15)</td>
<td>Lanyu (n = 35)</td>
<td>Babuyan (n = 2)</td>
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<tr>
<td>Egg length</td>
<td>2.73 ± 0.08</td>
<td>2.77 ± 0.09</td>
<td>N.S.</td>
<td>2.74 ± 0.13</td>
<td>2.83 ± 0.14</td>
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<td>Egg width</td>
<td>1.85 ± 0.05</td>
<td>1.87 ± 0.07</td>
<td>N.S.</td>
<td>1.84 ± 0.07</td>
<td>1.86 ± 0.09</td>
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<td>1st instar</td>
<td>1.14 ± 0.04</td>
<td>1.15 ± 0.02</td>
<td>N.S.</td>
<td>1.14 ± 0.04</td>
<td>1.12 ± 0.05</td>
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<td>2nd instar</td>
<td>1.56 ± 0.09</td>
<td>1.52 ± 0.06</td>
<td>N.S.</td>
<td>1.50 ± 0.07</td>
<td>1.49 ± 0.07</td>
</tr>
<tr>
<td>3rd instar</td>
<td>2.05 ± 0.08</td>
<td>2.03 ± 0.10</td>
<td>N.S.</td>
<td>1.95 ± 0.11</td>
<td>1.96 ± 0.13</td>
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<td>4th instar</td>
<td>2.59 ± 0.11</td>
<td>2.48 ± 0.10</td>
<td>N.S.</td>
<td>2.43 ± 0.14</td>
<td>2.39 ± 0.11</td>
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<td>5th instar</td>
<td>2.95 ± 0.16</td>
<td>2.77 ± 0.15</td>
<td>N.S.</td>
<td>2.80 ± 0.17</td>
<td>2.73 ± 0.00</td>
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<tr>
<td>6th instar</td>
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</table>

* p < .05.  
** p < .01.  
*** p < .001.

### Table 2

<table>
<thead>
<tr>
<th>4-instar individuals</th>
<th>P</th>
<th>5-instar individuals</th>
<th>P</th>
<th>6-instar individuals</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lanyu (n = 4)</td>
<td>Babuyan (n = 5)</td>
<td>Lanyu (n = 46)</td>
<td>Babuyan (n = 15)</td>
<td>Lanyu (n = 35)</td>
<td>Babuyan (n = 2)</td>
</tr>
<tr>
<td>Egg length</td>
<td>22.3 ± 1.5</td>
<td>21.4 ± 1.1</td>
<td>N.S.</td>
<td>20.6 ± 2.3</td>
<td>20.3 ± 2.1</td>
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<td>1st instar</td>
<td>8.8 ± 0.5</td>
<td>8.8 ± 1.3</td>
<td>N.S.</td>
<td>9.6 ± 1.8</td>
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<tr>
<td>2nd instar</td>
<td>15.8 ± 4.7</td>
<td>17.2 ± 2.6</td>
<td>N.S.</td>
<td>14.7 ± 3.0</td>
<td>15.0 ± 2.5</td>
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<td>3rd instar</td>
<td>22.5 ± 1.3</td>
<td>20.4 ± 3.3</td>
<td>N.S.</td>
<td>19.2 ± 2.7</td>
<td>22.7 ± 5.3</td>
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<td>4th instar</td>
<td>50.3 ± 2.2</td>
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<td>5th instar</td>
<td>39.6 ± 9.6</td>
<td>36.5 ± 6.5</td>
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<td>36.5 ± 6.5</td>
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<td>6th instar</td>
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<tr>
<td>Pupal stage</td>
<td>13.8 ± 2.1</td>
<td>14.8 ± 2.1</td>
<td>N.S.</td>
<td>16.0 ± 1.8</td>
<td>14.8 ± 1.5</td>
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<td>Imago</td>
<td>6.0 ± 1.4</td>
<td>4.8 ± 2.2</td>
<td>N.S.</td>
<td>7.0 ± 1.9</td>
<td>6.7 ± 1.5</td>
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<td>Total</td>
<td>133.3 ± 5.9</td>
<td>117.6 ± 6.9</td>
<td>N.S.</td>
<td>143.7 ± 10.3</td>
<td>141.1 ± 9.3</td>
</tr>
</tbody>
</table>

* p < .05.  
*** p < .001.

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Fig. 2. Number of larval stages in Pachyrhynchus sarcitis kotoensis of Lanyu Island and Pachyrhynchus sarcitis from Babuyan Island.
Lanyu, P. s. kotoensis is usually found on Leea plants. However, the host plant of P. sarcitis was not recorded on Babuyan Island. Since the host plant of Babuyan P. sarcitis was not confirmed, the decreased larval performance and body size might due to the low food utilization, low nutrition conversion efficiency, or proteinase inefficiency (Messina, 2004; Milanovic et al., 2016; Yang et al., 2008). The other potential factor that affect adult body size in Babuyan population might be environmental factors, such as ambient temperature which affects the duration and the growth rate of larva, and adult size in ectotherms (Anigillete Jr. et al., 2004; Antonatos et al., 2013; Ferro et al., 1985; Van der Have and De Jong, 1996). The environmental temperature in Lanyu (http://www.cwb.gov.tw/V7/climate/monthlyMean/Taiwan.tx.htm) is 2–4°C lower than in Babuyan (https://www.yr.no/place/philippines/cagayan%20valley/babuyan%20island/statistics.html) during the whole year, and the smaller body size might due to the unsuitable temperature. Genetic effect of founder population may also be a possible reason that effect the body size of adult insects due to lower genetic diversity (Berggren, 2005), and in our case, only few individuals were included in the breeding experiment, which may cause the size differences between captive-breeding and wild populations.

The species number of Pachyrhynchus weevils remains under-estimated, where new Pachyrhynchus species are being described almost every year (Barsevskis, 2016; Bollino and Sandel, 2015; Chen et al., 2017; Rukmane and Barisevskis, 2016; Yoshtake, 2012). Several studies of Pachyrhynchus weevils suggested divergent or convergent evolution in their colouration and marking patterns (Rukmane, 2016; Schultz, 1923; Yoshtake, 2017), and the colourations of these weevils may have evolved rapidly (Tseng et al., 2018). Therefore, species delimitation of Pachyrhynchus weevils based only on colourations is difficult and insufficient (Chen et al., 2017). The two insular populations of P. sarcitis have different colours and adult sizes under a common laboratory condition, which may reflect the host adaptation, genealogy, and other life history associated characteristics. These results also suggest that they may represent two cryptic species, and further studies are needed to examine the species boundary of P. sarcitis.

Declarations of interest

None.

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