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Embryonic Development and Eclosion Season of New Species *Berastagia* (Lepidoptera: Pyralidae) from Taiwan

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Abstract

This paper describes a new species of the snout moth *Berastagia tainanica* sp. nov. (Lepidoptera: Pyralidae) from Taiwan. From 2009 to 2016, a biology study was conducted on population dynamics and embryonic development. Spring season is the peak of the eclosion of overwintering larvae or pupae. The average longevity of adult was 14.8 ± 6.2 days (N = 174), the average number of eggs laid was 259 ± 3 eggs/moth (N = 2), the hatching rate of eggs was 95.4% (N = 262), and the average hatching time of eggs was 99.6 ± 18.6 hours (N = 68). The average body length of males was 5.64 mm ± 0.91 mm (N = 30), and the average body length of females was 6.28 mm ± 0.84 mm (N = 30). This finding indicates that female snout moths are larger than males (Global R = 0.058, P = 0.012). The snout moth eclosion rate was 16.9 moths/100 pods in the first year (2010/2011, N = 2,224 pods) and 10.9 moths/100 pods in the second year (2014/2015, N = 6,382 pods). The pod borer rate was 31.8% (N = 707) and the seed borer rate was 41.2% (N = 3,628) in the first year, whereas the pod borer rate was 76.2% (N = 6,382) in the second year.

Keywords

Phycitinae, New Species, Embryonic Development, Eclosion Season, Borer Damage Rate, Taiwan

1. Introduction

1.1. Plant Pests of *Pterocarpus* spp.

There are many kinds of snout moths living here. Some species are morphologi-

cally similar in external features and reproductive organs to other families of Lepidoptera. Therefore, the characteristics of life history also become a reference for classification [1] [2]. Examining how small insect populations grow quickly from a few insects to huge populations is crucial for population ecology studies and agricultural pest control. A leafhopper *Singapora nigropunctata* (Cicadellidae), which has 10 instars in its nymph stage that it then retains (**Figure 1**) [3], and a snout moth *Berastagia* (Phycitinae) have been documented. Both insects are pest insects that live on Burmese Rosewoods (*Pterocarpus indicus*) and Philippine Padauks (*P. vidalianus*). The tiny leafhopper primarily sucks the sap of the leaves [3], but the larvae of the snout moth primarily feed covertly, most often by boring the tender stems, pods, or seeds of *Pterocarpus* spp.

Host plants of the snout moth *Berastagia* at our surveyed sites comprised Philippine Padauks and Burmese Rosewoods. These host plants are distributed throughout Australia, Indonesia, Malaysia, Myanmar, Papua New Guinea, the Philippines, the Solomon Islands, and other tropical and subtropical regions [3].

In spring, *Pterocarpus* spp. begin to sprout, and the larvae of tiny leafhoppers and snout moths hatch on them. On the 14th day after hatching, the tenth molt occurs, at which time the nymphs become adults (**Figure 1**). Moths lay eggs near tree new shoots (**Figure 2** and **Figure 3**), and after hatching, the larvae burrow

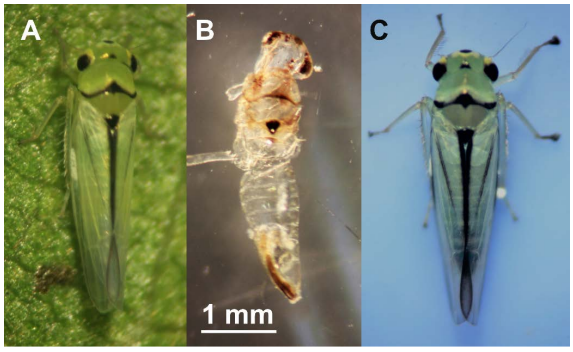

Figure 1. Immature stages of tiny leafhopper *Singapora nigropunctata*. (A) Ninth instar. (B) Molt of ninth instar. (C) Tenth instar. (Lin *et al*., 2019).

Figure 2. Snout moths *Berastagia tainanica* (Phycitinae) from *Pterocarpus vidalianus*. (A) Male. (B) Female.

into the tender stems and begin the first stage of the life cycle (**Figure 4**). Based on its life history characteristics, we exclude leafrollers (Tortricidae). These tiny leafhoppers and snout moths cause rosewoods to lose their leaves and inhibit the growth of side shoots, and consequently, the wood is not straight and has reduced its economic value. How and why these pests affect the trees thusly are crucial questions in ecology.

1.2. Taxonomic Status and Distribution of *Berastagia*

Berastagia is a monotypic snout moth genus described by Roesler and Küppers (1979) [4]. *Berastagia* includes the single species, *Berastagia dissolutella* (=*Nephopteryx dissolutella* (Snellen, 1880)) [5], which has brown forewings and translucent white hindwings and is found in China [6], New Guinea, and Australia [5].

This study investigated the biological performance, fecundity, eclosion (emergence of larvae from egg or adult from pupae), and lifespan of adult snout moth *Berastagia*.

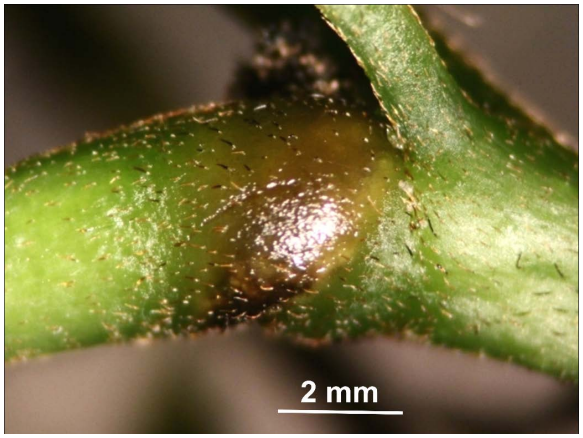

Figure 3. Egg of the snout moth *Berastagia tainanica* (Phycitinae) collected from *Pterocarpus vidalianu*s.

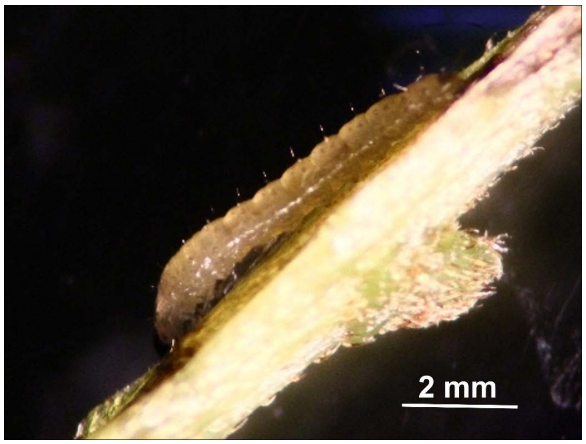

Figure 4. Larvae of the snout moth *Berastagia tainanica* (Phycitinae) collected from *Pterocarpus vidalianu*s.

2. Materials and Methods

2.1. Study Site

The weather in southern Taiwan is humid in summer, with occasional typhoons and torrential rains, whereas the winters are dry with little to no precipitation. The campus of Kun-Shan University (KSU, 23˚00'00"N, 120˚14'30"E) was chosen as the site of this study because it has several Burmese Rosewoods and Philippine Padauks [3]. At the research site, the growth peak for the sprouts of rosewoods occurs in January and February, with most rosewoods blooming in April and May. Dry pods hang on the trees' dry winter leaves.

2.2. Population Fluctuation of the Snout Moth

The fluctuation of the population density of the snout moth was evaluated at the KSU laboratory in the first year from January 16, 2010, to July 2, 2011, and in the second year from December 17, 2014, to May 31, 2015. In total, 2,224 and 6,382 pods of Philippine Padauks were collected in the first and second year, respectively, and each 100 pods were packed in a medium-sized nylon mesh bag (45 cm × 30 cm) and placed on a well-ventilated ledge outside the laboratory. Eclosioned snout moths were collected daily.

In the first year, Philippine Padauk pods were cut open in July to observe the number of end-instar larvae mouthparts, which remain in the pods (**Figure 5**). These larvae mouthparts can help in calculating the number of hatched snout moths and the respective pod and seed borer rates.

2.3. Survival Rates of Snout Moth Pupae and Adults

From October 27, 2009, to December 23, 2009, a total of 123 snout moth larvae were collected from the Philippine Padauk pods and reared in separate compartments (6-well acrylic tissue culture dish), and they were fed tender pods during rearing. Larvae pupation and pupal emergence were observed daily (**Figure 6** and **Figure 7**). In addition, we estimated the survival time and survival

Figure 5. Last molting of the snout moth *Berastagia tainanica* (Phycitinae) collected from *Pterocarpus vidalianu*s pod.

Figure 6. Male snout moth (*Berastagia tainanica* (Phycitinae)) larva.

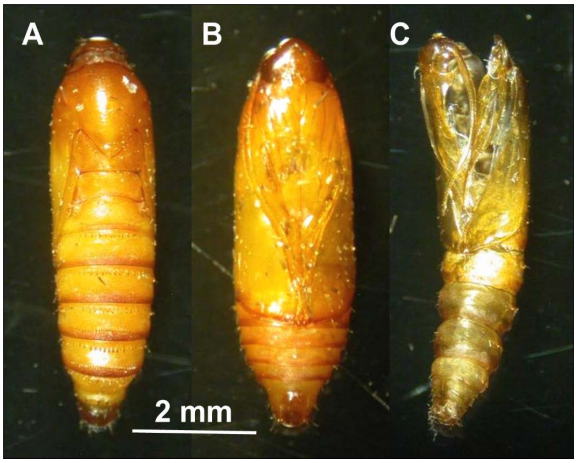

Figure 7. Snout moth *Berastagia* (Phycitinae) pupae. (A) Dorsal view; (B) ventral view; (C) pupa shell.

rate of adult snout moths. The room temperature (˚C) and relative humidity (%) of the laboratory were recorded during the study period.

2.4. Survival Time of Pupae and Adults

From October 27, 2009, to December 23, 2009, a total of 123 larvae of *B. tainanica* were collected from the KSU site and reared in separate compartments (2 pupae and 2 eclosion pupa shells were recorded on December 9, 2009, during collection). The pupation, eclosion, and survival of the adult snout moths were observed daily.

2.5. Embryonic Development of Snout Moth

After feathering, the adult snout moths were reared with 2% sugar water, the number of days of survival were observed and recorded, and the number of eggs laid was estimated by pairing. The rearing bottle was a transparent plastic bottle with a height of 15 cm, a width of 12 cm, and a width of 8 cm at the mouth; the

mouth of the bottle was changed to a breathable mesh (Figure 8). Male and female snout moths were mixed, and they were allowed to mate and lay eggs for embryonic development experiments.

The eggs were glued to the inner surface of the transparent plastic bottle, and the surface of the eggs had a metallic luster after drying. The embryonic development process could be clearly viewed from the outside. Subsequently, embryo development was recorded once an hour by using a USB digital microscope (UPG620, UPMODT). The hatching time of the eggs was estimated (hours), and the survival time of adults (including subadult stage) was also estimated (days) after eclosion [7].

The stages of embryonic development of the snout moth were divided into S1-S8. The characteristics of each phase are as follows: (S1) round vesicular blastoderm, (S2) embryo belt center depression, (S3) single red eye, (S4) pair of red eyes, (S5) pairs of black eyes and brown jaws, (S6) black head and neck, (S7) formation of the digestive tract and yolk left on the upper part of the mandible, and (S8) disappearance of the yolk and overfilling of the intestines, after which larvae hatched from eggs.

In order to make the holotype and paratype specimens, from December 2022 to February 2023, dry pods from red sandalwood trees were collected in KSU and placed on the edge of the house to wait for the moths to emerge. A total of 15 moths were collected until February 14. In total, 15 adults samples were freezing, and 1 pupa and 10 larvae samples were archived in the National Taiwan Museum (MTN), Taipei.

Color images of specimens were captured by using a stereo-zoom microscope (SZ-60, Olympus) and Nikon COOLPIX 4500 Camera. The male genitalia were photographed using a microscope (Eclipse 50i, Nikon). Measurement accuracy of body length, width, and each part of each specimen is within 0.01 millimeter.

The data recorded were analyzed using subroutines featured in the statistical

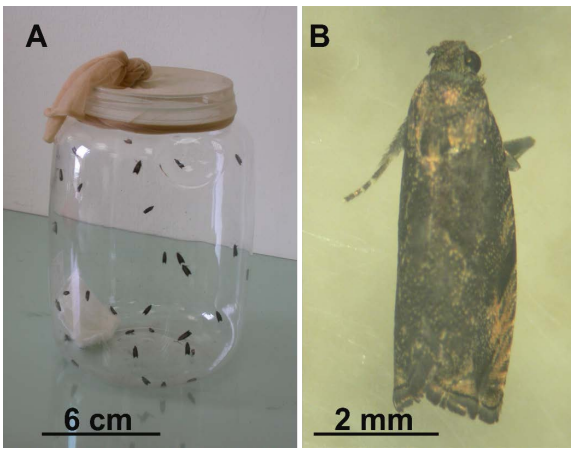

Figure 8. Laboratory setting for observation and recording of embryonic development of *Berastagia* (Phycitinae). (A) Plastic feeding bottle; (B) dorsal view of adult male.

analysis programs, RESEMBLANCE and SIMPER (PRIMER v.6, PRIMER-E, Plymouth, UK) [8]. Significance was indicated at $P < 0.05$. The data are presented as the mean ± standard deviation.

3. Results

3.1. Genus *Berastagia* from Taiwan

From January 2009 to July 2015 in Tainan City, 251 specimens *Berastagia* (Pyralidae) were identified. The characteristics of the moth include the following: 1) beak is developed, exposed, and visible externally (**Figure 9(A)**), 2) the base of the male antennae does not have a notch or scale clusters (**Figure 9(B)**), (3) the base of the male external genitalia with long scale hair bundles (**Figure 10**), and male valvae are long (**Figure 11**). There are 11 veins in the forewing and 10 veins in the hindwing. Both the forewing and the hindwing have M2 and M3 veins and are separated (**Figure 12**). According to Ren's (2006) "A systematic study on subfamily Phycitinae of Southern China (Lepidoptera: Pyralidae)," taxonomically [6], *Berastagia* is reclassified under Cryptoblani Roesler, 1968, and the genus *Berastagia* Roesler and Kuppers, 1979, which is also a newly recorded genus in Taiwan and can be clearly distinguished from *B. dissolutella* (Snellen, 1880).

3.2. *Berastagia tainanica* sp. Nov. Weng and Ueng, 2023 (Figure 2, Figure 6, Figure 7, Figures 9-13)

1) Type Material

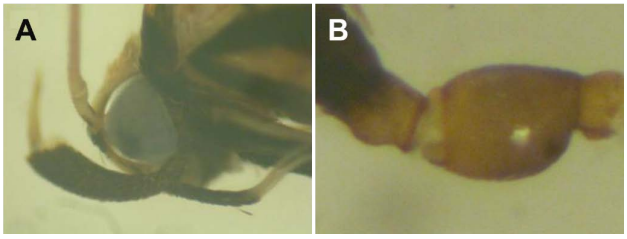

Figure 9. Ventral view of male *Berastagia tainanica* sp. nov. (A) Beak; (B) base of antennae.

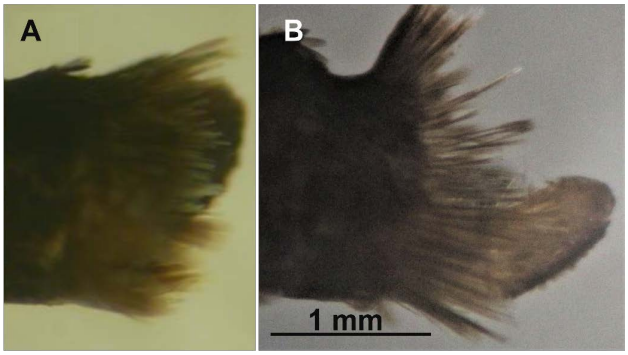

Figure 10. Left ventral view of male *Berastagia tainanica* sp. nov. (A) Base of external genitalia clavicular; (B) Valvae (remove some hairs).

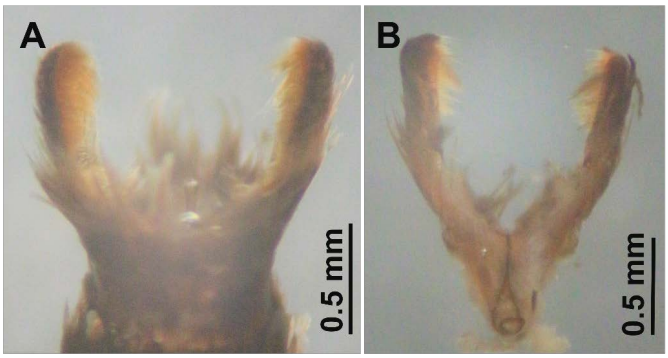

Figure 11. Adult male valvae of *Berastagia tainanica* sp. nov. ((A) and (B)) Ventral view, (A) base of external genitalia clavicular, (B) ventral view vinculum, juxta, and valvae.

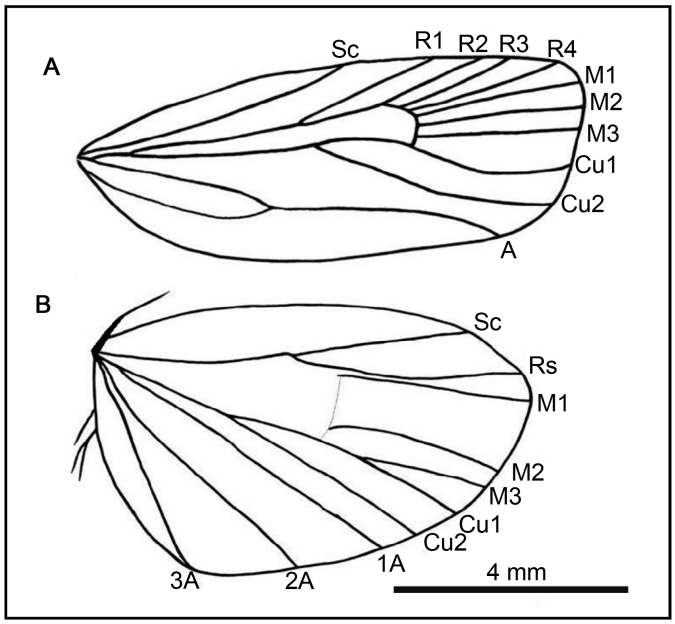

Figure 12. Wing of *Berastagia tainanica* sp. nov. (A) Forewing; (B) hindwing.

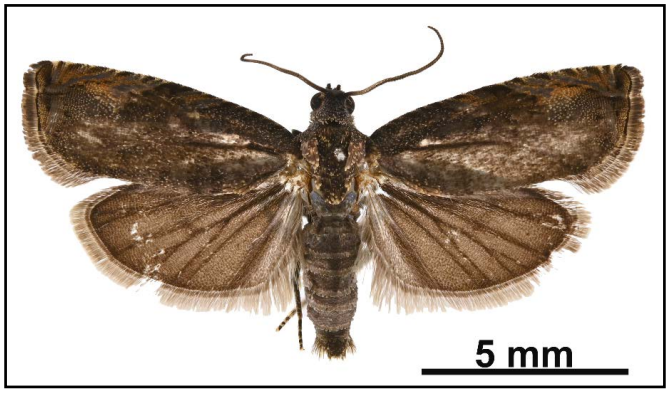

Figure 13. Dorsal view of male *Berastagia tainanica* sp. nov. Weng and Ueng, 2023, collected in February 14, 2023 (holotype, (Cat. No. TMIN3840)).

Holotype: One male snout moth specimen (Cat. No. TMIN3840) was collected from the campus of KSU, Tainan City, by Yih-Tsong Ueng on February 14, 2023 (Figure 13).

Paratypes: One male and one female snout moth (Cat. No. TMIN3840) were collected from the study site on the same date as the holotype by Yih-Tsong Ueng. The male is dark gray, and the female is dark brown (Figure 2, Figure 8(B), Figure 13).

2) Male

The average male body length was 5.64 mm ± 0.91 mm, the average head width was 0.95 mm ± 0.14 mm, and the average chest width was 1.39 mm ± 0.30 mm (N = 30). The valvae of male *B. tainanica* (Figure 11) was slender than that of *B. dissolutella* (Ren, 2006: Plate I Figure 6) [6] [9].

3) Female

The average female body length was 6.28 mm ± 0.84 mm, the average head width was 0.98 mm ± 0.14 mm, and the average chest width was 1.67 mm ± 0.20 mm (N = 30). Females were slightly larger than males, and significant differences between the sexes were observed (Global R = 0.058, P = 0.012).

4) Ecology

Forest.

5) Distribution

Southern Taiwan.

3.3. Eclosion Season of Snout Moth *Berastagia tainanica*

In the first year, 2,224 and 6,382 pods of Philippine Padauks were collected. In total, 376 snout moths *B. tainanica* were eclosion from pods (16.9 moths/100pods). The last feathered moth hatched on May 21, and six other species, which eclosion, were spotted borers. Early spring is the eclosion peak for overwintering larvae or pupae (Figure 14), and the maximum number of eclosions in 1 day was 22 (occurring on February 15). The average population dynamic curve (n = 7) is shown in Figure 15. A total of 707 Philippine Padauks' pods were eaten by the snout moth larvae (31.8%). Subsequently, these 1886 pods were cut open and inspected one by one (including 46 pods without seeds). We found one live larva, seven dead larvae, and five pupae in these pods. A total of 1495 (41.2%) of 3628 seeds were eaten by the snout moth larvae.

In the second year, 6,382 pods of Philippine Padauks were collected. In total, 697 snout moths of *B. tainanica* was closion from pods (10.9 moths/100 pods). The last feathered moth hatched on May 19. Late spring is the eclosion peak for overwintering larvae or pupae (Figure 14). The average population dynamic curve (n = 7) is shown in Figure 15. A total of 4,862 (76.2%) of 6,382 pods were eaten by the snout moth larvae.

3.4. Pupal Stage Period

From October 27, 2009, to December 23, 2009, the average pupal period of the

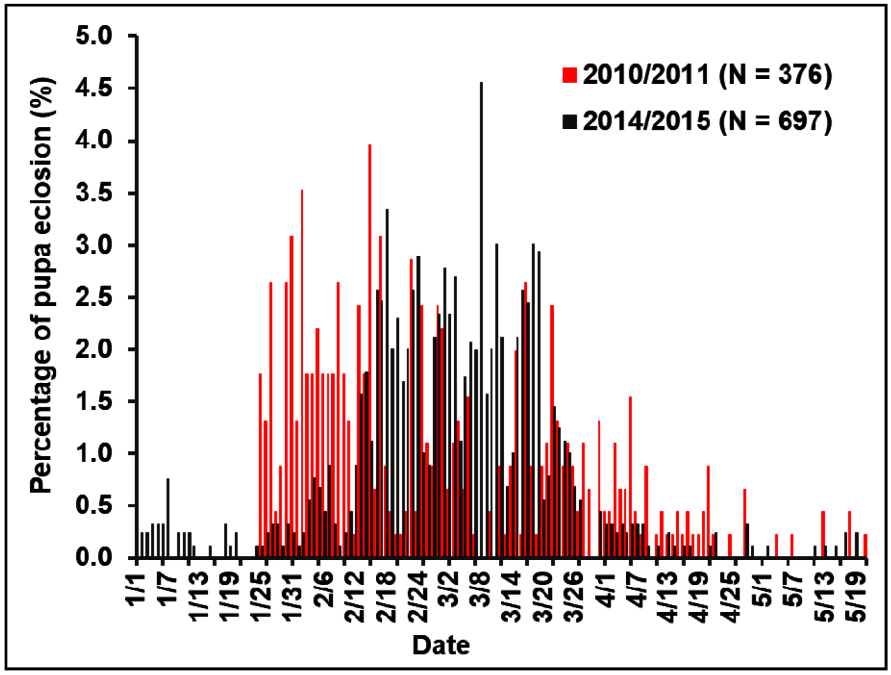

Figure 14. Changes in daily eclosion number of overwintering snout moth (*Berastagia tainanica*) larvae or pupae.

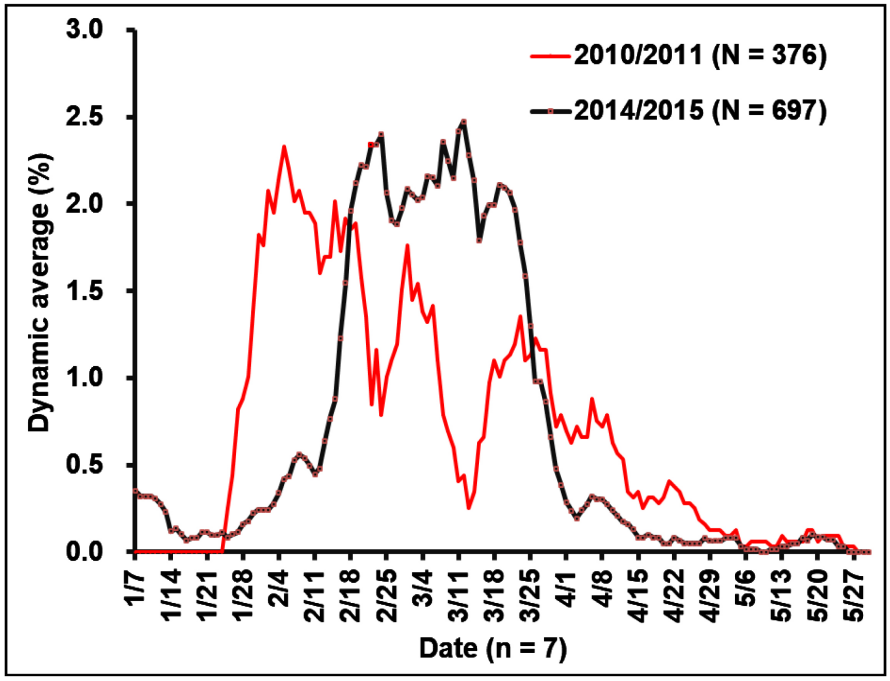

Figure 15. Time series change (n = 7) of the eclosion number of overwintering snout moth (*Berastagia tainanica*) larvae or pupae.

snout moth was 10.1 ± 1.6 days (N = 123), and the male-to-female sex ratio of adult snout moths is 1:1.1. Among these adult snout moths, 56 were females, 62 were males, and 5 died. Due to immaturity, these five moths died after eclosion. Male larvae with two black spots on the back of the abdomen on the eighth seg-

ment (Figure 6) were not included in the calculation.

3.5. Longevity, Fertility, and Development of Snout Moth Embryos

From February 3, 2010, to March 19, 2010, in a laboratory with a room temperature of 26.0˚C ± 2.5˚C and relative humidity of 62.9% ± 6.3%, the longevity, fertility, and development of embryos of the snout moth were observed. The average longevity of the adult snout moth *B. tainanica* was 14.8 ± 6.2 days (N = 174, range: 2 to 33 days). The survival curve is shown in Figure 16.

The fertility of each female was approximately 259 ± 3 eggs/moth (N = 2). The hatching rate of eggs was 95.4% (N = 262), and the average hatching time of eggs was 99.6 ± 18.6 hours (N = 68). The duration of the embryo development stages (S1–S8) of the snout moth were 7.9 ± 2.6, 2.0 ± 0.7, 30.4 ± 10.4, 36.1 ± 17.4, 7.8 ± 4.3, 4.4 ± 2.4, 5.9 ± 2.7, and 7.8 ± 6.8 hours (N = 68) (Figure 17).

4. Discussion

The gelatinous substance on the surface of newly laid eggs turned into an opaque film with metallic luster after drying (Figure 16(Ev)) [10], which affected observations when using a dissecting microscope. During the research process of our study, using the transparent ventral surface of eggs without damaging the developing embryo (Figure 16) and analyzed the development time of each stage, which was the first report on the development of the egg embryo [10] [11]. The total hatching time of egg to larva was 99.6, 121, and 175 hours for the snout moth *B. tainanica*, the greater wax moth (*Galleria mellonella*) [11], and the fairy moth (*Nemophora albiantennella*) [12], respectively. The embryonic development

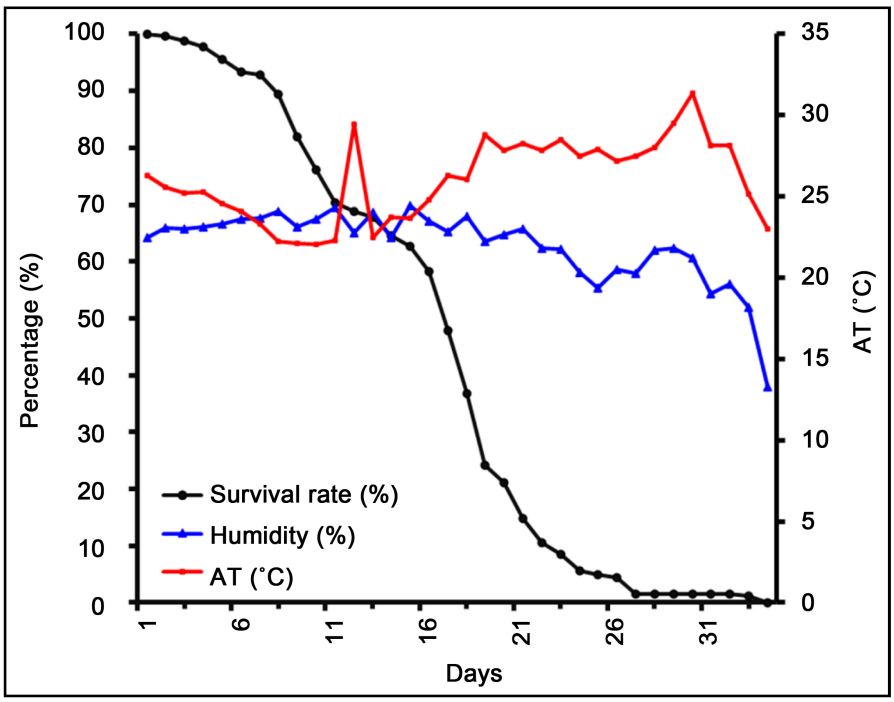

Figure 16. Survival curve of adult *Berastagia tainanica* (N = 174).

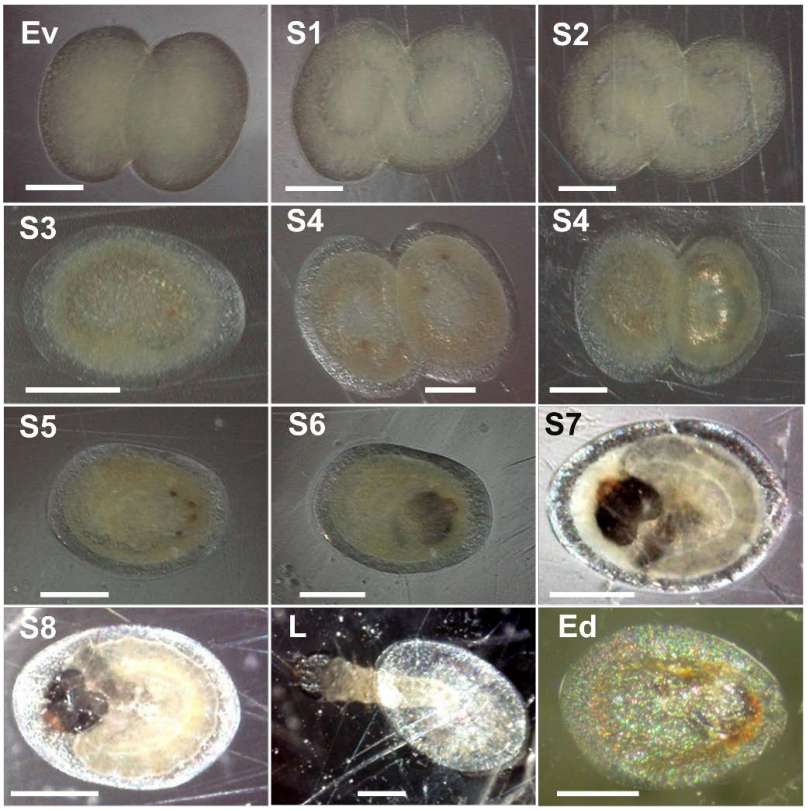

Figure 17. Images of each embryonic development period of the *Berastagia tainanica*. S1-S9, Ev, and L ventral view; Ed: dorsal view. Ev: two newly laid eggs; S1: round vesicular blastoderm, S2: embryo belt center depression, S3: single red eye, S4: pair red eyes, S5: pairs of black eyes and brown jaws, S6: black head and neck, S7: formation of the digestive tract and yolk left on the upper part of the mandible, S8: yolk disappearance and intestines full, L: broken egg hatching, Ed: dorsal of hatched shell. Bar, 1 mm.

period of the snout moth *B. tainanica* life cycle was relatively short. These findings indicate that the snout moth *B. tainanica* may rapidly expand, by more than 2 generations in 1 year, its population into other areas.

The potential fecundity of the snout moth (259 eggs per female) in this study was comparable to that of the spinach moth, *Hymenia recurvalis (*250 eggs per female) [13]. The eclosion rate of the snout moth *B. tainanica* was higher than that of the tropical warehouse moth *Cadra cautella*, and the lifespan of the adult snout moth was longer than that of the tropical warehouse moth [14].

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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