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Life cycle of *Chetoneura shennonggongensis* (Diptera: Keroplatidae: Keroplatinae) from Jiangxi Province, China

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Abstract We report the first description of the biology of non-bioluminescent Keroplatidae from China. The life cycle of *Chetoneura shennonggongensis* is documented from laboratory culture and from field observations. The larval stage usually lasts 8–10 months. Large amounts of pupae are observed in late June, where they remain suspended horizontally or vertically from silk threads. The pupal suspending postures differ from other Keroplatidae species. Sexual differentiation is evident from the pupal stage. July is the peak time of adult emergence. Mating usually takes place immediately upon female emergence if adult males are available. The adult life span is short. Females live a maximum of 5 days and males 7 days. Egg development time varies from 20–30 days depending on environmental temperature. Larvae hang long sticky silk threads with a series of mucous droplets to capture prey. Some behaviors associated with snare construction and prey capture are also described.

Key words *Chetoneura shennonggongensis*, Chinese Keroplatidae, life cycle

Introduction

Chetoneura shennonggongensis is a recently described cave-dwelling species from Jiangxi Province, China (Amorim *et al.*, 2008). It belongs to the dipteran family Keroplatidae, subfamily Keroplatinae. The family Keroplatidae comprises approximately 950 species in about 87 genera, with worldwide distribution (Matile, 1990; Evenhuis, 2006). The Keroplatidae larvae construct long vertical, sticky threads used to capture small invertebrate prey. They prefer damp, dark places and are mostly found in caves, old mines, and native bushlands, especially along streams or underground water. Some of them glow, such as the famous glowworms in Australia and New Zealand (Baker & Merritt, 2003; Meyer-Rochow, 1990). Other species do not produce light, such as the South American species *Neoditomyia andina* and *N. columbiana* (Sturm, 1973) and *N. farri* (Stringer & Meyer-Rochow, 1996). *C. shennonggongensis* is a non-luminescent species. Members of this family are commonly known as fungus gnats.

Meyer-Rochow (2007) suggested that the Keroplatidae went through a gradual evolution from fungus-eating habits to the characteristic carnivorous life-style with sticky threads and photic lures to entrap prey. Much research has focused on the New Zealand and Australian species due to their tourist attention and therefore economic significance (Baker, 1999; Baker & Merritt, 2003). However, little is known about non-luminescent species from Oriental biogeographic regions, although they also play important roles in the evolution of the Keroplatidae. Studies of Chinese species may provide important clues for understanding evolutionary adaptation in this group.

Chetoneura shennonggongensis was first discovered in Shennong Gong, a show-cave in Jiangxi Province in 2005. To better understand the species we took the larvae from the field into an artificial cave, where we reared them from eggs to adults. The morphological characteristics of

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the adults led to the formal identification of this species (Amorim *et al.*, 2008). The only closely related species is from an early record of *Chetoneura cavernae*, in Malaya (Colless, 1962).

In this paper we provide the first detailed description of the life cycle of non-luminescent Keroplatidae from China, with major focus on *C. shennonggongensis*.

Materials and methods

Field observations and specimen collection

We observed *C. shennonggongensis* larvae, pupae and adults in a cave in Shennong Gong, Jiangxi Province from 2005–2007, in a series of six 5–10-day field trips. *C. shennonggongensis* larvae were collected from the cave during each of the observation trips. Larvae on the roof of the cave moved quickly and were collected by cutting the threads suspended from the substrate. Each larva was identified according to Amorim *et al.* (2008) and placed in a 1.5-mL plastic tube with damp cotton wool on the bottom to maintain humid conditions. Pinholes were drilled into the bottom of each tube. Tubes were inserted into 20 cm × 8 cm × 6 cm moist floral foam which was wrapped with black cloth. Larvae were transferred and reared in an artificial cave in Huazhong Agricultural University (HAU), Wuhan, China. The voucher specimens were deposited in the Plant Science & Technology Institute of HAU.

Maintenance of cultures

Chetoneura shennonggongensis larvae require high humidity for survival. We maintained the larvae 94% RH and temperature 18 ± 2°C. Individual larvae were reared separately in 10 cm long × 8 cm wide × 6 cm high moist floral foam which was hollowed inside. The floral foam was put in a porcelain container with 1/3 volume of water. A 20-cm piece of absorbent material was draped over the top, with one end in the water to act as a wick. Larvae were on the top of the floral foam (Fig. 1).

Larvae were fed 2–3 *Drosophila melanogaster* adults per week. The fruit flies were maintained in culture on an artificial diet (Peng *et al.*, 2006), and they were anesthetized with ether before placing them in the larval snares.

Egg laying

One male pupa and one female pupa were placed in the same crystal bottle with damp cotton wool for oviposition to count the number of eggs deposited by a female. Egg deposition by 30 pairs of adults was counted.

Fig. 1 *Chetoneura shennonggongensis* larval culture in an artificial cave. Larvae were reared individually in 6 cm × 8 cm × 10 cm moist floral foam supports placed in porcelain containers with 1/3 volume of water. Larvae were on the top of the foam supports with long sticky silk threads.

Body size

Vernier calipers (type 560, Sanfeng Medical Instrument Limited Company, Dongguan, China) were used to measure the body length and width of the larvae. Egg size and pupal size were measured under a Nikon dissecting microscope (type Fx-35, Nikon Limited Company, Dongguan, China). The shape, color and other characteristics of the egg and pupa were recorded. Body length, width and wing length of the adults were measured under the same dissecting microscope. Body length is defined as the length from the head apex to terminal end of the abdomen. Body width is defined as linear measure between the bases of the two forewings.

Behavioral observations

To observe nest-building behavior, the snares of a single larva were destroyed and the larva was left without snares in the floral foam. To observe predatory behavior, a fruit fly was anesthetized with ether and then glued onto the fishing line using a fine set of pliers. To determine whether larvae discriminated between living and dead prey, we glued a dead adult fruit fly and an anesthetized adult fruit fly to the fishing line, then observed food selected by the larvae. To test the possibility of cannibalism, two third instar larvae were placed in a floral foam without food for 1 month. We made regular observations for cannibalism. For each study, 10 larvae were observed.

Pupae with the floral foam were placed in a 100 cm long × 80 cm wide × 60 cm high cage to observe emergence. Ten pairs of adults (male : female = 1 : 1) were reared in the same cage to observe mating. Wet cotton was placed at the bottom of the cage to observe oviposition.

Results

Field observations

The microclimate in the cave was fairly constant, with low light conditions, cool (16–18°C) temperatures and high humidity (RH ∼94%). The larval period is the longest part of the *C. shennonggongensis* life cycle and larvae were seen on all field trips. Larvae prey traps were observed. Prey appeared to be abundant as small insects were seen entrapped in silk-like fishing lines. Pupae were abundant during late June and adult emergence peaked in July. In the cave, mating took place immediately adult females emerged if males were present.

Eggs

The *C. shennonggongensis* eggs were deposited separately onto substrate (moist cotton) rather than *en masse* (Fig. 2A). The eggs are spherical, 0.3–0.5 mm in diameter. Newly deposited eggs were cream colored, then turned to either light brown or orange-red within 3–4 h. The eggs turned black 7–8 h later. The eggs were sticky and adhered to the substrate. The eggs hatched after 25 ± 5 days at 18 ± 2°C and 96% ± 2% RH.

Larvae

There are five larval instars. First instar larvae were 3 ± 1 mm long and 0.33 mm wide ($n = 10$). First instars were transparent and difficult to see with unaided eyes (Fig. 2B). The first instar larval mortality was as high as 69% ($n = 10$). Living larvae commenced building nests and let down sticky threads approximately 15 days after hatching. The ability to build nests is maintained throughout the larval stages. The completed nests captured prey using the fishing lines. Larval movement was similar to the movement of an earthworm.

The larvae grew over a period of 8–10 months to reach a body length of 35 ± 5 mm ($n = 10$). At pupation the body length decreased greatly to approximately 10 ± 2 mm ($n = 10$).

Larval nest-building behavior

First instar larvae were placed on top of the floral foam. Two weeks later, the first instar larvae began to construct snares. Larval snares were composed of bracing cords that suspended the snare, the mucous tube in which the larva rested and fishing lines let down by the larva to capture prey. The larvae spent most of their time inside their mucous tubes, which they broke through to repair their snares, feed and turn around. Fishing lines consisted of silk-like threads with a series of sticky mucous droplets. The silk lines and mucous droplets were presumably produced by the larval salivary glands. The larvae pushed against the floral foam to lift the front part of their bodies into the air, possibly to search for a suitable nest site. They bent forward with a sudden darting movement and pulled the silk threads out to the labrum by moving their mandibles constantly. They deposited a drop of silk onto the roof of the floral foam, forming a fine thread that would act as a brace. This process was repeated, with the larvae moving forward into the droplets, until mucous and silk had passed down the length of the body and the hollow nest was complete. The formation of fishing lines was a continuous process. A nest could be built in 10–20 min (Fig. 2C).

A larva usually produced up to 50 strings with sticky droplets. These droplets varied in size, but their average diameter was 1 mm. The length of the vertical silk depended on the speed of the wind. If the wind speed was rapid, the silk would be tangled, apparently an unworkable situation for capturing prey. The length of the vertical silk was usually approximately 50–80 mm in the lab, but 100–300 mm in the field. The tensile strength of the silk was substantial, supporting 8 mg in the laboratory and 15 mg in the field, measured by weights of *Drosophila.*

Prey capture

The larvae were the only feeding stage in the life cycle. They used the sticky fishing lines to capture prey, usually

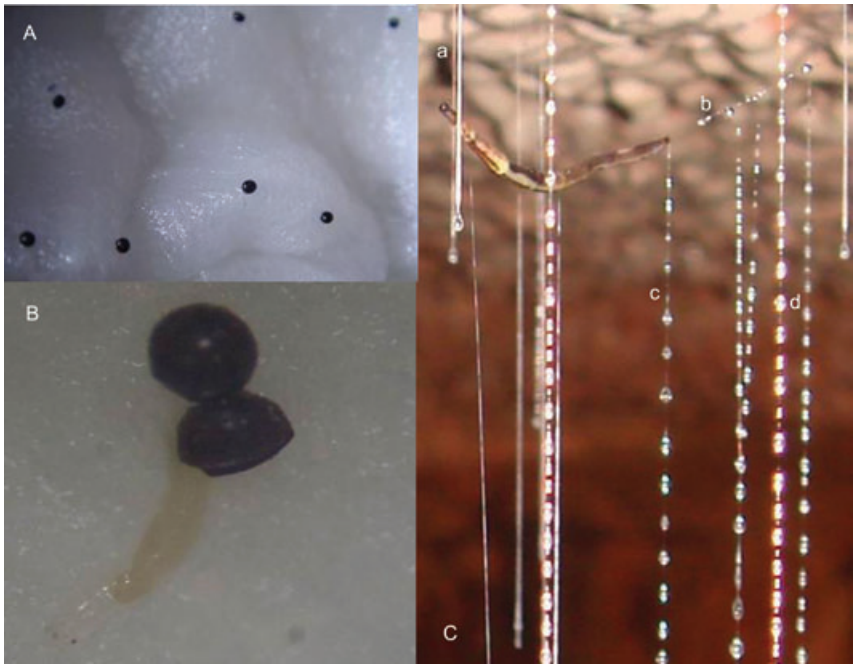

Fig. 2 Eggs and larvae of *Chetoneura shennonggongensis*. (**A**) The eggs are black in color and round in shape. There is a short distance between every two eggs. (**B**) An early first instar larva, which split the egg shell to hatch. The larva was transparent, 3 ± 1 mm long and 0.33 mm across. (**C**) The composition of the larval snares. a, indicates bracing cords for suspending the snare. b, shows the mucous tube larva lay inside for repairing the snares or for turning around. c, indicates silk-like threads. d, shows mucous droplets. c and d make up the fishing line deployed to capture prey and maintain humidity.

small flying insects. When the larvae sensed movement, they crawled down the appropriate line until only their posterior halves were left in the nest. Waves of the contractions traveling along their bodies allowed the larvae to haul up the line. The line was held by bristles on the body, while the larvae stretched down across the next two or three droplets. This process was repeated until the prey died or the larvae stopped. Finally the larvae hauled up the line again, pulled the prey into their mucous tubes, and began to eat. Larvae attached many bracing threads to the prey, blocking any chance of escape. Usually the larvae did not eat the entire *Drosophila* adult in a single feeding episode. They first aspirated the body fluids of the prey, leaving the remaining carcass. Five to six minutes later, they consumed the whole insect. If prey was less plentiful they would eat the whole insect immediately (Fig. 3A,B).

Cannibalisim

Two larvae were put in one floral foam without food for 1 month and were observed daily. Upon initial contact, they both moved back quickly. Ten minutes later, one moved again and began to fight with the other. Their snares were seriously damaged. Finally, the winner ate the loser entirely (Fig. 3C; $n = 10$ pairs).

Pupae

The larvae removed many of their fishing lines before pupatation. The larval exuvium was pushed to the posterior end of the pupa, where it remained attached to the posterior suspensory thread. The larvae suspended themselves vertically or horizontally by two long threads. One thread was attached to the thorax and the other to the distal end of abdomen (Fig. 4). The pupae were 10 ± 2 mm ($n = 30$) in length. Before pupation the larvae shrank and became opaque. The pupal stage lasted 8 ± 2 days at 18 ± 2°C and 96% ± 2% RH ($n = 30$).

Sexual differentiation first became evident in the pupal stage. The female was larger and stouter than the male, and it possessed two prominent papillae at the end of the abdomen. Two or three days before emergence, eggs became visible through the transparent pupal skin of the female.

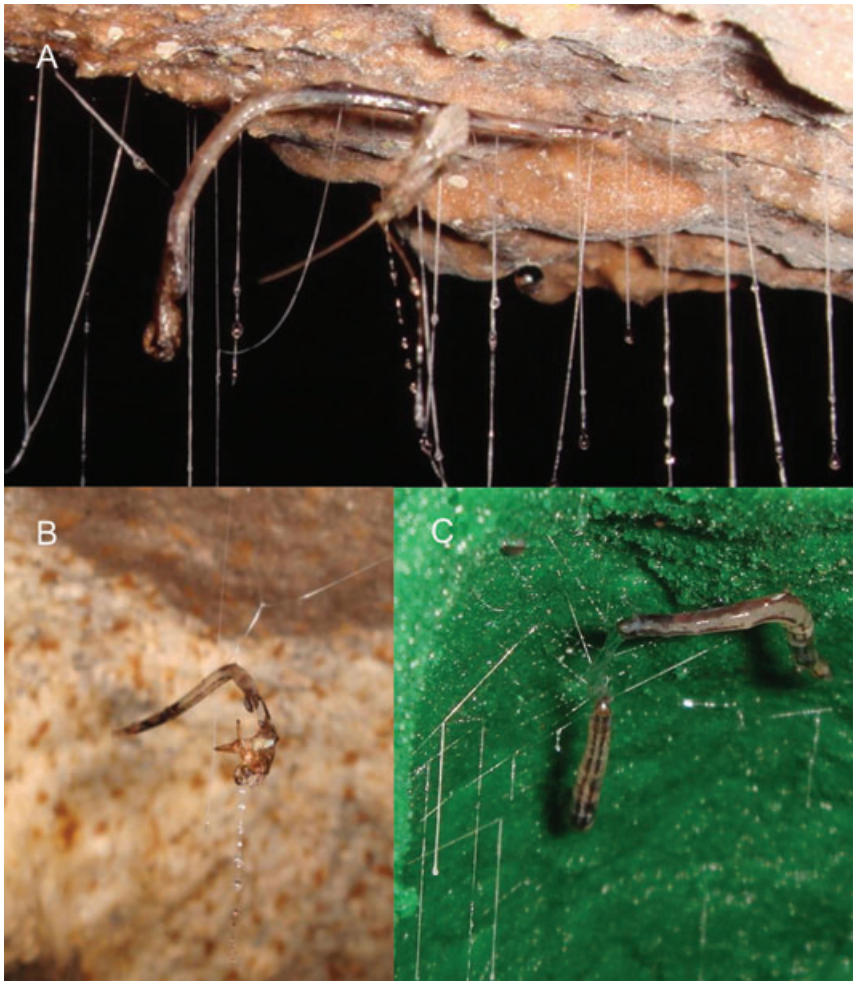

Fig. 3 Prey capture and cannibalism of *Chetoneura shennonggongensis*. (**A** and **B**) Larva preying on a moth in the field. When a moth got into the fishing lines, the larva crawled down until only its posterior half remained in the mucous tube, then hauled up the line and bit into the moth repeatedly. (**C**) Two larvae fighting mouth to mouth, for food or habitat. The winner invariably ate the loser.

Adults

The emergence began from the head, followed by the legs, the wings and finally the abdomen. The fly "escaped" from its pupal case by muscular contraction, expansion of the body and wriggling of the legs (Fig. 5). At emergence, the thorax of the adult was a creamy pink color with up to 10 black setae arranged irregularly. The abdomen was pale fawn and transparent. After 3 h, the color gradually intensified to dark brown with a dorsal, median fawn stripe on the thorax and lateral fawn stripes at the anterior end of each abdominal segment.

After emergence, the adults hung their heads down from the pupal case for nearly 24 h until dry, then turned through 180° and hung from the pupal case until the wings could support flight. This took approximately 3 h.

The adults are sexually dimorphic. The coloring was more strongly marked in males than females (Fig. 6). The female's abdomen was enlarged by visible eggs; the male's body was much smaller and narrower. Both genders have a short life span, 3–5 days for females and 5–7 days for males. Both male and female fungus gnats are very sluggish, although males are the more active fliers. They move only comparatively short distances, about 1–2 m per flight, and consequently are easily caught.

Mating often took place immediately upon female emergence when adult males were confined with females. The abdomen of the male lay to the side of the female's and the tip was turned to effect coitus. The wings of the female were folded while those of the male were widespread. Copulation lasted for more than 1 h. Males mated several times, while females mated only once.

Fig. 4 *Chetoneura shennonggongensis* pupae. (**A**) Larva preparing to pupate. Body length and the sticky threads both became shorter and the mucous droplets increased in size as the mature larva approached pupation. (**B**) Vertically hanging pupa in floral foam in the lab. This method facilitates eclosion. (**C**) Horizontally hanging pupa in the wild. There were two threads suspending the pupa. One thread was attached to the thorax and the other to the distal end of the abdomen.

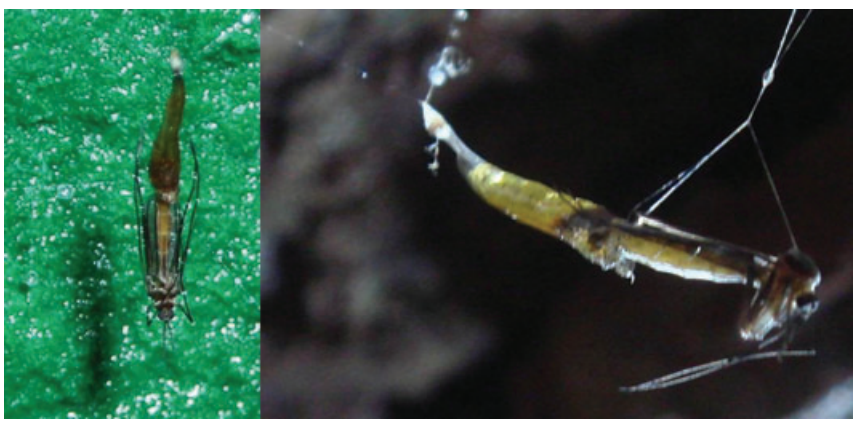

Fig. 5 *Chetoneura shennonggongensis* adult emergence. Emergence began from the head with muscular contractions and expansion of the body. The legs and wings came out first, and then the wriggling legs pulled the whole abdomen out of the case.

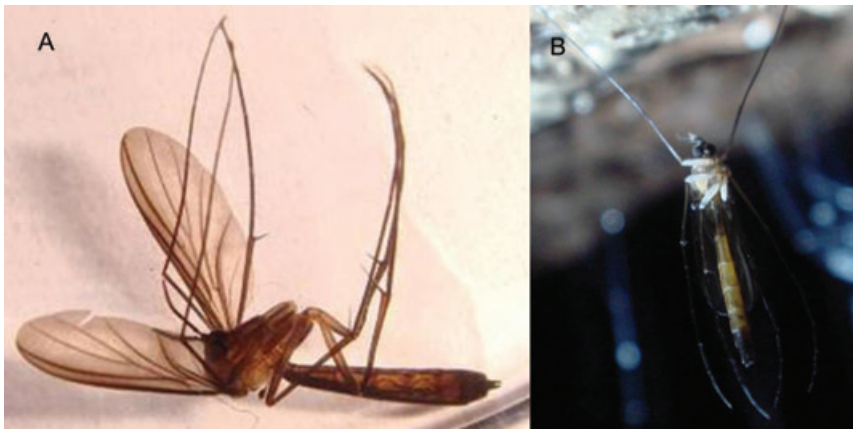

Fig. 6 *Chetoneura shennonggongensis* adult male and female. (**A**) Female adult with body lengths ranging from 7.5–10 mm and wing lengths 4.2–5.3 mm. The abdomen is enlarged due to developing eggs inside. The eggs are visible through the integument. (**B**) Male adult with body lengths ranging from 6.5 mm to 8.8 mm and the wing lengths ranged 4–4.7 mm. Compared to females, the male body was slim and narrow.

Eggs were deposited onto damp cotton wool. Females deposited approximately 85 eggs (range of 50–120 eggs/female, $n = 10$) (Fig. 2A). Adults apparently did not feed.

Discussion

Eggs

The egg stage of *C. shennonggongensis* lasted 20–30 days under laboratory conditions, similar to the eggs of *A. luminosa* in the cave (22–24 days at 13.7–15.6°C) (Richards, 1960). Egg development time for *A. flava* was 7–9 days at 23°C (Baker & Merritt, 2003). This may relate to the different environmental temperatures or reflect species differences. The average number of eggs laid by *C. shennonggongensis* was 50–120 in the laboratory, but the number is difficult to count in the wild. A fecund female deposited approximately 170 eggs in the wild (Richards, 1960). The eggs did not cling to each other. Cannibalism is common in glowworms, including *C. shennonggongensis*. The early instar larvae did not catch prey larger than themselves.

Larvae

The first instar larvae had a high mortality, possibly because they were so small. Alternatively, they may have been cannibalized. The larvae usually feed on living prey (Meyer-Rochow, 2007). However, in the laboratory we found that the larvae also eat fresh dead *Drosophila* if we kept the dead *Drosophila* trapped in their lines vibrating. Thus, we infer that the larvae were not selective, but will eat anything trapped in their lines as long as the vibrations can be sensed by them. Our anatomical study revealed two papillae at the end of the larval body. These papillae appear to contain sense organs responsible for detecting vibrations from the fishing lines.

Larvae are the only feeding stages. As seen in many insect orders, the larvae accumulate and store enough energy reserves to ensure adequate nutrition for pupae, adults and the following generation of eggs. The larvae continue eating captured prey until they begin deconstructing their fishing lines prior to pupation.

Observation of larval behavior revealed some similarities in snare maintenance and prey capture between *C. shennonggongensis* and other Keroplatids. First, they have the same behavior constructing fishing lines. They all produce the fishing lines with their salivary glands and use these fishing lines to capture prey (Harrison, 1966; Richards, 1960; Gatenby & Cotton, 1960; Meyer-Rochow, 1990, 2007; Broadley & Stringer, 2001). Second, *C. shennonggongensis* and most Keroplatids share the same predatory behavior. They all depend on fishing lines to capture prey except for a few species that live on fungi in Sweden (Meyer-Rochow, 2007).

Pupae

Before pupation, the larva removed the fishing lines and shortened the lines. We speculate this action helps to avoid the adult becoming entangled during emergence. There are two different hanging postures for pupation: horizontal and vertical hanging. Horizontal hanging appears to be safer than vertical hanging. It can prevent the

pupa from falling due to air movement. Pupae of *A. flava*, *A. richardsae* and *A. tasmaniensis* suspend themselves horizontally rather than vertically as is the case for *A. luminosa* (Richards, 1960); but Chinese species have both hanging postures. Posture selection appears to depend on the environmental conditions. The horizontal posture is more common in the laboratory than in the wild. We infer that if the environmental condition is suitable, such as mild wind and appropriate temperature, the larvae will prefer vertical hanging, because this hanging method facilitates emergence. However, if the environmental conditions are not ideal, the larvae will use the horizontal hanging posture.

Adults

The range of adult life spans and body sizes of *C. shennonggongensis* are similar to those of *A. flava*, but *A. luminosa* may have both longer life span and larger body size. The adults of *C. shennonggongensis* apparently do not feed; as seen with *Arachnocampa* adults, they too have vestigial mouthparts and are unable to feed (Meyer-Rochow & Waldvogel, 1979). The body sizes of New Zealand cave glowworms are larger than their epigean con-specifics, such as the glowworms living in the forest; this is attributed to an increased food supply within the cave systems (Richards, 1960; Broadley & Stringer, 2001). More suitable climatic factors within caves may also influence increased body size (Pugsley, 1983).

Males generally eclose earlier than females. Males often await female emergence to copulate. Two mating positions have been recorded (Richards, 1960). One position is most common and similar to our cave observations. The second type is rare. The two gnats mated vertically, tail to tail. The male hung head down clinging to the empty pupal case from which the female had just emerged, while the female clung to the limestone wall.

Egg laying may commence immediately after fertilization. The singular and spaced egg distribution may reduce cannibalism in the early instars as the larvae are territorial and readily feed on each other if crowded.

Adults generally have two purposes in life: reproduction and dispersal. Although the adults are weak fliers, we suppose their short flights help them extend their distribution within the cave environment.

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