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Behavior and Extra-Oral Digestion of the Wasp *Sclerodermus guani* (Hymenoptera: Bethylidae) Parasitizing

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Abstract:

For many decades, the parasitic behavior of wasp *Sclerodermus guani* (Hymenoptera: Bethylidae), a typical generalist gregarious ectoparasitoid, was described as paralyzing the hosts by stinging with venom for egg-laying, and then their offspring larvae after hatching feed on hosts' hemolymph for the development until emergence. However, this paper reported a new mechanism of the wasp to parasitize the host borer *Monochamus alternatus* (Coleoptera: Cerambycidae). Observation on their parasitic behavior and their offspring growth discovered that the wasp needs 6 steps to complete its parasitic process, i.e. host-biting and-fighting, host-subduing and-nursing, host-liquefying and-feeding, wasps' berried and oviposition, larval sucking and developing, cocooning and wasps' emerging. Host-biting and host-liquefying are the key steps which would evolve an amazing array of mechanisms to create a liquid nutrient reservoir inside the borer body cavity for the parasitoid larvae's sucking and developing. This mechanism suggests that *S.guani* is a species of ectoparasitoid

wasp, with the characteristic of extra-oral digestion; the nature of subduing is to fully liquefy the borer internal material and help their offspring smoothly sucking the liquid nutrient from the host for development.

Keywords: *Sclerodermus guani*, Bethylidae, *Monochamus alternatus,* Cerambycidae Parasitize, Extra-oral Digestion, Suck, Berried, Fecundity

Significance Statement: My study overturns the previous understanding of wasp Bethylidae's parasitic behavior, which had been long described as using aculeate to sting the host and inject venom. My paper presents a new mechanism: the wasp uses its mandibles to bite and subdue the host, liquefying the host through extra-oral digestion to prepare for its egg-lying.

INTRODUCTION

Scleroderma guani Xiao et Wu (Hymenoptera: Bethylidae), one of the generalist gregarious ectoparasitoid (50), belonging to the genus, Scleroderma of over 71 species and to the family, Bethylidae of more than 2000 species (39, 43), be found in the last century 70s' (71), has been used in China as the major insect natural enemy to control various wood borer pests, including *Monochamus alternatus* Hope (17, 19, 31, 45, 69, 72). In prior studies researchers have explained the parasitic mechanism (7-9, 12, 42, 59, 65-66). The venom in the ectoparasitoid wasp is considered the key factor that produced some parasitic behavior (25-27, 35). The wasp sting with its aculeate and inject venom (13, 32-33, 37, 58) to paralyze their hosts; then to lay eggs on integument (10-11); and then the larvae feed on hemolymph of host for development until new wasp emergence.

I discovered a novel mechanism: the wasp uses its mandibles to tightly bite and subdue the host rather than using venom to paralyze. After subdued, the host's internal solid mass was liquefying and its rigid cuticle was softening gradually, suggesting *S. guani* has the characteristics of extra-oral digestion. Its aculeate could only be used as a defense against the host's counterattacking. Once the aculeate was used, the wasp is about to die.

Materials and methods

Host and parasitoid colony maintenance

Over 100 borers, *M.alternatus* were collected from the wood chopped from Masson pine, *Pinus massoniana* or Black pine, *P. thunbergii,* in the Beilen District, Ningbo City, Zhenjing Province, China were prepared for the experiment. The borer was required uniform in size, fully mature in instar (the 4th instar), healthy and vigor, no damage in body. Each borer weight was measured by electronic scales (JA50003N, China) and size of prothorax width and the body length were measured by Vernier caliper (ZL0.01-125mm, MC, Chine). Each borer was placed individually into a glass tube (10mm in diameter×50mm in length). The tube mouth was tightly covered with cotton plug. About 500 Female parasitoid wasps *S. guani* (following wasp) were reared in the laboratory of Ningbo Forest Pest and Quarantine Station. They were placed in 5 glass test tubes. All the colonies of the borers and the wasps were maintained at 8-10℃ environment condition.

The borer exposure to the wasp

Tubes of borers and wasps were moved to a super-clean-worktable (SKJH – 1109, China). One wasp was picked and placed into the tube of the borer, after that the tube mouth were covered with cotton plug again, followed by from 2 to 10 wasps to one borer in a tube, 5 duplicates respectively, and then these tubes were numbered No.i-j (i=from 1 to 10, represent the number of the parasitoid wasps or the number of group; j=from 1 to 5, represent the number of the duplicates). These exposures were finished at 16:00 pm, Apr. 8, 2011. Another 5 tubes at the rate of 5 wasps per borer were conducted and numbered No.55-1~5 at 9:00 am, Apr. 22, 2011.

Observation on behavior and extra-oral digestion

These tubes of 11groups consisting of 55 borers and 300 wasps were moved to a room for observation on the wasp*s*' parasitizing behavior and process in 18-28°C room temperature and 65-85% relative humidity. The key behavior or characteristics was recorded by the digital camera (EX-P505 CASIO COPUTER CO., LTD, JAPAN). From 16:00 pm, Apr. 8, 2011 to Apr. 25, 2011, groups No.i-j and No.55-j were observed on behavior between the wasps and bores such as *borer biting and fighting,-subduing and nursing*; *borer liquefying and feeding, wasps' berried and oviposition; host sucking and parasitoid offspring developing till cocooning and emerging*; *the factors of the success and failure of wasps parasitizing* each other day. From 9:00 am, Apr. 22, 2011, the groups No.55-j was further observed every hour in details for the number of the bite-prints on the borers' surface under a stereomicroscope (Stemi2000 – C). During the period, another 4 borers were took from a wasp rearing lab and dissected into cross-section which represent the 3 different stages of biting, subdued and berried. These borers were first stored at -19°C refrigerator for solidifying and then were took out and transferred to the microscope slide, on which a abdominal cross-section was cut off by a scalpel. The cross-section state was observed and recorded as the solid, slurry and liquid state under a stereomicroscope. The apparatuses of the mandible, the salivary gland and the aculeate were simultaneously removed from the wasps and observed and recorded morphologically by camera under a stereomicroscope. The key factor led to parasitizing success or failure was observed and recorded in detail, so the remains of the borers were dissected and weighed for measurement of the consumed interior material of No.1-j, No.2-j, No.4-j, and No.8-j, which was calculated by the formula:

Extra-oral digestion rate≈Consumed rate = $(W1\text{-}W2)/\ W1\times100\%$;

$W1$ – the weight of the fresh borer;

$W2$ – the weight of the borer remains after parasitized and sucked.

Data analysis and statistics

One-way analysis of variance (ANOVA) and least significant difference (LSD) test were used to compare differences among these data of the site and frequency of wasps attacking, the number of eggs, larvae, pupates and newly wasps. All statistical analyses for this study were performed using *DPS* data processing (62). *Excel* was used to produce the graph and fit trend equations.

Results and analysis

Borer-biting and -fighting

Manner of wasp's fighting to borer. When the wasp meets the borer, a long and complex battle would occur. The wasp attacks the borer mainly by biting with its mandibles, rather than using its aculeate to sting. The borer also uses its mandibles to fight back, struggling to escape by twisting, rolling, contracting, spreading, bending and pressing. Biting would last from a few minutes to several hours (Photo 1 a, b) repeatedly until the borer is subdued, leaving many reddish-brown bite marks on the surface of the borer's body (photo 1 c). The wasp would not bite too deeply, so the wound could shrink without oozing hemolymph. Aculeate and venom have been considered as wasps' most effective weapon to subdue their host (12, 40-42, 48, 64, 68). However, this study shows that *S. guani's* aculeate and venom does not play an important role to subdue the borer. During the fighting, the aculeate can be seen moving frequently, but it is only "sting-like" touching on the surface to stimulate the borer, not a real inserting into the borer. However, when the borer attacks back, the wasp will insert its aculeate and inject venom into the borer. But unfortunately, the aculeate could only be used once. After the inserting the wasp will die (Photo 1e) in 12 hours, left a pinhole (Photo 1d) on the borer's surface and extracted a long aculeate (1.77mm long) out of the wasp tail end (Photo 1f).

Wasp's biting site and times. The average of bite marks per borer was bitten over 40 times or each wasp bit the borer over 8 times before the borer was subdued, differences were not significant (df = 4, 19; F = 0.175; P = 0.947). Wasps prefer to bite on the lateral side rather than the dorsal or ventral side of the borer. The five borers had 80 mandible-prints on lateral right, 75 on lateral left; 29 on dorsal, and 25 on ventral, in total, differences were significant (df = 3, 95; F = 5.031; P = 0.0028). Mandible-prints on different segments from head to abdomen distal were counted (Fig. 1 a) to the mean of 6.20 ± 0.245 in prothorax, 5.20 ± 1.357 in the 1st segment of abdomen, 3.0 ± 0.447 in the 4th segment of abdomen, respectively. On rest of segments of the borer few mandible-prints were distributed, 1-2.5 prints in 12 segments; less than 1 prints including 0 in 9 segments. Differences were significant (df = 23,119; F = 4.8530; P = 0.0001) among the segments.

Borer-subduing and nursing

Subduing means the borer gradually loses the resistance to the attack of the wasps. In this subduing, the borer's behavior could be divided into 3 stages: vigorous, the state the borer still has the energy to fight back; quasi-subdued, the state the borer is still alive but has few strength to fight back; subdued, the state the borer has been thoroughly subdued, and its body becoming soft and straight, bulging slightly (photo 2 a). In order to parasite on the borer, the wasp should control the status of the host to make sure whether the host is alive or dead. Wasps want to take a long time to subdue the borer slowly. If the host is dead during the subduing, it no longer has the value for parasitism. Thus, subduing for parasitism would be a slow and long process. The wasps would decide when to attack and when to rest, as well as how much effort it would invest into the battle. The wasps' behavior could be divided into 5 stages: *Combating*; *Adjustment; Maintaining; Re-biting; Judgment,* which was as similar as the reporters what other researchers had done (46).

According to the observation above, both borer-biting and -subduing behaviors exhibited four characteristics: 1) attacking by turns - not all of the wasps participated the attack concurrently; 2) site selecting for biting - the wasps preferred to bite at the segments from the 1^{st} thoracic to the 1^{th}~6^{th} abdomen of the borer, they seldom attacked other parts of the borer; 3) attacking based on reaction - if the borer stayed still, the wasp seldom attacked, it only bite the active parts of the borer; 4) subduing slowly - the wasps preferred to take a long time subdue the borer slowly.

Borer-subduing lasted for only 48 hours in No.55-3 as minimum; and lasted 79 hours in No.55-2 (Figure 2 a) as maximum. The average subduing time for No.55-j is 63.5 hours. The number of vigorous borers was inversely proportional to time, difference is significant with the time (df = 9, 59; F=3.645; P = 0.0015); and the number of subdued borers is proportional to subduing time, difference is significant with the time (d*f* = 9, 59; F = 3.278; P = 0.0037).The two equations intersect at the point $y = 2.5$, $x = 48$h, which means after fighting

48 hours, 40% borers were subdued, 20% was quasi-subdued, and 40% were still vigorous. Borer-subduing time was closely related to the number of wasps: the fewer the wasps are, the longer the subduing lasted (Figure 2 b). It took an average of 150 hours for the wasps in No1-j groups to subdue the hosts, yet the average subduing time for No.10-j groups was 60 hours. The number of wasps was inversely proportional to subduing time, difference is significant among the different number of wasps ($df = 9, 49$; $F = 4.398$; $P = 0.0005$).

Nursing began after the borer was subdued. There are two major nursing behaviors: self-grooming and borer-cleaning. Self-grooming would be conducted by the wasps first. The wasp grooms its antennas with its forelegs from base to tip repeatedly, following by grooming its mouthparts with its forelegs repeatedly. Then it cleans its forelegs with its mid-legs repeatedly, and uses hind-legs to clean its mid-legs repeatedly. Then the wasp slightly tilts its abdomen upward, grooming the end of abdomen with its hind-legs repeatedly. The wasp also grooms its legs crossly. After that, the wasp would conduct borer-cleaning. It cleans the subdued borer with its mouthparts to remove anything attached on the borer's surface, such as silks, hairs, threads, particles. It repeats the behavior to keep the borer clean and tidy (photo 2 a). The two nursing behaviors were conducted alternatively by the wasp, in order to provide a safe and sanitary environment to protect the subdued borer from insect pathogens.

Feeding and detecting

Before the wasp's berried, it need food for mature by absorbing liquid nutrition from the subdued borer rather then hemolymph from the living borer (1, 3, 5, 51). This behavior has another important function that wasp could detect the liquid nutrition capacity contained inside of the borer which would be used to support their offspring for development and judge whether to keep on parasitizing (52). During the feeding and detecting, the liquid nutrient capacity inside of the borer would increase, and the integument outer of the borer would be softened gradually. The wasp would immediately stop the parasitic process when it finds the borer's nutritional conditions were changed and no longer be suitable for the growth of their offspring. 3 liquefied stages of the cross-dissection of the borer's internal substance (Photo 2a, b, c, d) proved that behavioral process. In these 3 stages of combating, nursing and berried, the cross-dissection photos showed that the internal substance status of the borer was correspond to 1) liquefying-start, the thick solid mass around digestive tract was not liquefied much, and solid mass can be seen (Photo 2b); 2) quasi-liquefying, the solid mass was liquefied mostly as slurry (Photo 2c); 3) liquefied status, the solid mass disappeared completely, a plenty of liquid filled the borer body cavity, the tiny windpipes (Photo 2d) buried in the solid body was invisible previously, and now could be seen clearly. Meanwhile, the wasp also probes the "body wall" of the borer, because rigid exoskeleton is not suitable for feeding. Thus, the wasps must detect the softening degree of the exoskeleton prior to parasitizing. The fact that the borer's body was liquefied suggests something was pumped into borer body which could dissolve the internal tissues and organs by the extra-oral digestion mechanism (24).

The wasp's mouthpart structure and feeding mechanism also provides evidence for extra-oral digestion. The hook-shape mandibles have 3 small teeth and grooves attached to (photo 2 e). The mandibles are used to clamp the borer, but cannot be used to shred tissues from the borer. When its mandibles clamp the borer, some juice would oozing from the wound and be sucked by the wasp through grooves. When its mandibles loosen, the wound would close to avoid outflow of the juice (14, 49). In the wasp's mouthparts, there are big salivary glands (Photo 2 f). Thus, saliva can be pumped into the borer's body during the biting and diffuses rapidly to its whole body when the borer resists strongly. Wasp's long-time biting fits the characteristics of extra-oral digestion (4, 14, 15, 18). The subdued borer expresses the following symptoms: many mandible-prints on the surface (photo 1 c), a long dying period, irreversible subduing process, stiffened body, a large liquid reservoir inside the body, and slightly swollen, softened and glossy exoskeleton (photo 2 a).

Berried and egg-laying

Berried process starts when the wasps sucked certain nutrient solution (53). After berried, the wasps would select some sites among segments of the borer for eggs-laying. Wasp's abdomen swelling characterizes the berried process. When the wasps were berried, they spent much time, ca. 5 days to ingest more food for their ovary. Their abdomen expanded fully in the late feeding period. Their transparent yellow intervals between abdominal segments can be seen obviously (photos 3 a).

Behavior of egg-laying was a sequence of activities of sites selecting and cleaning and egg-laying. The wasp would first use its mandibles or cauda to clean or rub the egg-laying sits, which lasting for 10 seconds. Then the wasp bit the borer surface tightly, standing on its 6 legs steadily (photo 3 b) for 10 seconds. The wasp curled its abdomen from far and near, “sector scanning” area quickly for 15 seconds. It then stretched the aculeate repeatedly. Finally, the aculeate tip touched the borer’s cuticle (but not piercing) for 5 seconds. At this moment the wasp was in catatonic immobility with abdominal end swelling, arch-form, up its head, extruding an egg slowly, for 2 seconds. Once the egg was laid, the wasp put aculeate back and turned itself around immediately; checking the egg’s condition with its antenna and cleaning around the egg with its mouthparts to ensure the egg was firm and safe, for 4 seconds. Then, the wasp started to groom itself for 10 seconds, completing the 1st egg-laid. All egg-lying process lasts for 56 seconds (photo 3 a b c). After that, the wasp would leave and continue egg-laying. Eggs-laying could last for 3-6 days. If the wasps cannot find sites on the borer for egg-laying, they will suspend the process. When the fecundity was large, few eggs that deposited on the improper site were eaten by the wasp.

Sites of egg-laying are selected by the wasps carefully (2, 20). The berried wasp crawling on the borer would prefer the site where the exoskeleton is softened and the interior is liquefied to lay egg. Firstly eggs-lying were only distributed in a range of the 3rd-6th abdominal segments of the host. Then the range of eggs-laying sites also expended when fecundity increased (Fig.3 a), which showed 1 wasp laid 57 eggs on 6 segments, 2 wasps laid 60 eggs on 9 segments, 3 wasps laid 98 eggs on 11 segments, including thoracic segments, 4 wasps laid 106 eggs on 12 segments except for the head of borer.

Distribution of eggs-laid from head to last segment during the whole process (Fig.3 b) showed the wasps’ preference of egg-laying site clearly (2, 20, 40-42), difference was significant among the segments (df = 12, 51; F = 11.668; P = 0.0001). Whereas, the fecundity distributed on the 3rd, 4th, 5th, 6th abdominal segments were 14.00±2.65, 11.75±1.38, 12.50±0.87, 13.00±0.71 eggs, respectively, difference was not significant among the 4 segments (Fig. 3 c). The egg-laying sites selected were corresponded to borer-biting sites, i.e. the priority sites of wasp biting were same as the preferred sites of wasp egg-laying.The fecundity of wasp laid was equivalent to the number of eggs-loaded on the integument of the borer because the wasp did not lay extra eggs in other place. The borers with the maximum and minimum of eggs-loaded were selected from the groups in No.i-j (exclude 0 egg) to draw 2 curves of equation (fig.4 a). The two equations are quite different. The first is relatively simple, suggesting that successful egg-laying have same factors that the inside substance of the host was completely liquefied. The complexity of the second equation suggests that unsuccessful egg-laying have various factors interfering egg-laying and subduing, resulting in partially liquefying of the borer's internal tissues and un-softened integument. Thus, according to different conditions, the wasps would increase or reduce egg-laying amount exactly.

The average of eggs-loaded per borer was determined by the number of wasps obviously (Fig. 4 b), Difference was extremely significant among No.i-j (df = 9, 41; F = 6.077; P = 0.0001).

The average of eggs-laying per wasp was subjected to the average of eggs-loaded per borer so that fecundity of each wasp was inverse proportion to the number of wasps (Fig. 4 b), difference was great significant (df = 9, 41; F = 4.207; P = 0.0012).

The egg laying process lasted for 3-8 days in No.1-j, No.2-j and No.4-j. The three equations can be drawn according to the egg-lying process and number of wasps (Fig.4 c), that suggest the time of egg-laying was prolonged when wasps number increased (Fig. 4 c).The most important feature of wasps' eggs-laying is the wasp could actively control and adjust amount and process of egg-laying according to the nutrition status of the host. For example, in No.1-5, eggs were not seen on May 4. On May 5, 37 eggs were suddenly laid on the borer. However, in the next day, only 3 eggs were laid, while the number increased again to 14 eggs on May 7. In 3 days, a total of 54 eggs were laid but no regularity could be found. It is clear now that the wasps were capable of precisely judging and controlling egg-laying because the wasps continue to probe the nutrition status of the bore during egg-laying. If the nutrition is enough, it lays more eggs, *vice versa*.

Sucking and growing

After the egg hatching, the larvae began sucking liquid nutrients from the host for development. The larva can be divided into 6 stages (Photo 4 a) based on size and form. The 1st stage larva is egg-form and 0.3mm long, and an offset (Photo 4 b) can be seen at the cephalic region of c.a. 1/6 larval body length; larva in the 2^{ed} stage is pear-form and 0.4mm long; in the 3^{ir} stage is pyriform and 0.6mm long; in the 4th stage is pyriform and 0.8-1.0mm long; in the 5th stage is sacciform and 1.5-2.5mm long; in the 6th stage is vermiform and 3.0mm long and its segment-like starts emerging on the larval body (Photo 4 a). Larva in 1st-4th stage has no cauda and frass hole, but in the 5th and 6th stages it has caude and frass-hole-like at the last segment. No molting was seen during the larval development so larva is probably only one instar. Changes in shape, size, and color of larva depend on the feeding process. At 2^{ed} and 3^{ir} stages, the larva body inflated like a balloon, and there were several whitish, round spots scattered on the transparent wall (Photo 4 f); At 4th and 5th stages, the larvae body dilated continuously, and the color of larval body turned into brown, the same color of the host borer. Larva has a very fine pairs of mandibles at mouthparts, and its cephalic region can move back and forth. When its cephalic moved back, the sucker-like was formed around the mouthparts by the cephalic ring dilator muscles (Photo 4 d). When it starts to suck, larvae bends its mouthpart down to contact and anchor the epidermis (Photo 4 c) with its mandibles without shredding the host's tissue. It then moved back the mouthparts until the sucker attached to the epidermis closely, and erecting its body. The sucker generated a negative pressure for sucking (Photo 4 f g). Thus, the food larva took must be liquefied completely before it can be sucked. During the sucking, the liquefied nutrient could be seen being pumped forth and back in midgut (Photo 4 f). The larva haed would be caved into but not to puncture the epidermis of the borer. Once punctured, the host's body fluid (not hemolymph) would flow out, and the larvae cannot continue parasitizing. Sometimes larvae fell off the borer body, and the wasp would help the larvae re-hook the host's body for feeding at the original site or at another suitable location. After the larvae finished sucking, many circle pits (Photo 4 d h) were left on the surface of host body. The larvae can only suck in liquid nutrient liquefied by the wasp in extra-oral digestion to ingest and grow directly without digestion and excretion. But the larvae in the 6th stage maybe ingest the borer cuticles completely and make a final excretion (liquid) before pupation, leaving a stain on the extremity of cocoon.

Cocooning and emerging

Larvae in the 6th stage were carried by the wasps to a place away from the remains of borer, beginning spinning for cocoons. After the cocoon is made, larvae begin to pupate in the cocoon. Pupating duration from spinning silk to emerging of new wasp lasts for 11-18 days, averaging 14.7 days. Pupa inside the cocoon becomes adult, which bite through the cocoon to emerge. The emerging period lasts for ca. 13 days. From the exposure of wasp to borer to emergence of new wasps (from April 8 to early June), it lasted for 60 days, in which 39 in 50 borers produced 5087 new wasps, accounting for 78% of all borers with an average of 130.4±73.06 wasps per borer. 11 borers did not output new wasps with failed parasitizing, which means 41 wasps did not produced its offspring.

Mechanism to success was the both borer-subdued and borer-sucking. The borer-subdued means the beginning of liquefying internal solid materials of the borer into liquid nutrient reservoir which can be used by the wasp offspring (Fig. 6 a). The borer-sucking means the beginning of wasp offspring growth and development by feeding the liquid nutrients of the borer. The capacity of the liquid nutrient reservoir of the borer (as capacity of eggs-allowing) would restrict the fecundity of wasp laid; the capacity of eggs-allowing was embodied by the number of eggs-loaded on the borer which also limited the fecundity of wasp laid. So, there was a balance as one rises and another falls among the 3 patterns (Fig. 6 b). The fecundity of wasp laid depends on the capacity of eggs-allowing or the number of eggs-loaded. When information of the increase of the number of eggs-loaded along with the decrease of the capacity of eggs-allowing was obtained by the wasps, the wasp would reduce the number of eggs-laying. So, a ethogram (Fig. 6 b) illustrated these balanced relationships: ⑴wasps established a liquid nutrient reservoir inside the borer by using extra-oral digestion; ⑵wasps by feeding obtained the information of the capacity of eggs-allowing; ⑶the capacity of eggs-allowing was embodied by the number of eggs-loaded; ⑷at same time, the information of the number of eggs-loaded and the capacity of eggs-allowing was delivered; ⑸the wasp received the information and made the decision to continue or stop egg-laying; ⑹the capacity of eggs-allowing decreased with the number of eggs-loaded increasing,; ⑺based on the information, the wasp adjust at once to decrease or suspend the eggs-laying (Fig. 6 b). It is the special mechanisms that wasps adjust actual fecundity according to the borer cases of number of eggs-loaded and capacity of eggs-allowing in order to reach maximum utilizing of host nutrients. The pattern of the balance is "number of eggs-loaded≤capacity of eggs-allowing".

Extra-oral digestion rate after larvae sucking on liquid nutrition from the host borer until left its dried or wetted remains can be showed as the remains of the cross-section of the borer (photo8). During the fighting, the remains was harder, oval-form, a mass of solid materials visible, which indicate that the bore body has not been liquefied (Photo5 a); after wasp suspending the egg-laying behavior, the cross-section shape is oval, the solid materials is thinner, which show the borer body has been partially liquefied (Photo5 b); after larvae sucking, the cross-section is crescent-moon-shape, solid materials becomes very thinner, which indicate the most of the internal materials of the borer has been taking away in liquid nutrition by the larvae sucking (photo5 c); after larvae spinning silk, solid materials has disappeared, the integument of dorsal and ventral of the borer has been in one, which indicated that the liquid nutrient has been sucking to dry completely by the larvae (Photo 8 d), the cross-section is bow-shaped. According to these results after wasp's extra-oral digestion, the *Extra-oral digestion rate* is also the quantitative calculation by using the formula: Extra-oral digestion rate≈ (W1-W2) / W1×100%.The results were the sequence of 54.17±3.53%, 72.12±5.81%, 89.45±2.52%, 97.38±0.76% for No1-j, No2-j, No4-j, No8-j, respectively, which showed extra-oral digestion rate is proportional to the number of wasps. Difference was very significant (df=3, 14; F=34.076; P=0.0001). This result indicates that when the number of wasps was more than 4, the solid materials in the borer captive can be fully liquefied by extra-oral digestion mechanism of the wasps; when less than 4, it can be partially liquefied (Fig.6).

Mechanism to failure was the borer has not been liquefied. The wasps were forced to suspend the parasitic process on the borer. 41 failure wasps were divided into 3 categories by the shape after parasitism abandoned to 11 borers. First are the common wasps that its abdomen remains as same as that of fighting state i.e. the abdomen is not berried-like; there were16 wasps into the categories, accounting for 30.77%. Second are the post-berried wasps that its abdomen inflated obviously in the berried firstly then contract as the wasps stop parasitizing, going back to the normal state; there were 15 wasps went through the process from berried to shrink its abdomen, accounting for 28.85%. Third are the eggs-laying wasps, which go from eggs-laying to stop eggs-laying to contract its abdomen; there were 10 wasps laid 340 eggs, an average of 34 eggs per wasp later, the wasp stopped laying eggs, accounting for 19.23% wasps. 11 failure borers also can be divided into 3 categories in corresponds with 3 wasp categories after stopping parasitic behavior. First is dry-shrank state that the borer was bitten to die, and its internal materials was not be liquefied a bit, gradually shrank into a dried body with black or brown-hued (Photo 6 b, d, f), all of 6 borers in No1-1, No2-2, No3-4, No3-5, No6-3, No7-2 were turned into these states, accounting for 46.15%. Second is the insect-pathogen-infected borer. Even though the wasps have some anti-infected materials to prevent the borer from infected by insect pathogen, when the number of wasps were less than 3, the borer was apt to be infected (photo 6 a), such as “white fungi” *Beauveria spp-infected*. Third is the wet-deflated borer. This state occurred in the integument of the borer was bitted to rupture, resulting in the borer body fluid leaked (photo 6 e), which were 3 borers for No2-5, No3-2, No5-3, accounting for 23.08%. The larvae can not continue to suck liquid nutrients on the wet-deflated borer for development.

When parasitism is failure, the parasitoid-host systems are as 6 states (Table 1).

First is the “dry-shrank borer + common wasp” which is representative of 4 interactions in No2-2, No3-4, No3-5, No7-2 that the borers were all bit to death but abandoned by wasps (Photo 6 b d f), in which 2 borers in No2-2, No7-2 were too small to provide enough nutrition to be abandoned. For stance, the size of prothorax width × body length was 5.5mm×16mm and 4.5mm×15mm for No2-2, No7-2 respectively.

Second is the "white fungi borer + common wasps " Which is the system in No1-3 tube that was subdued in good condition, but the wasp give up parasitizing because the borer was infected by white mycelium *Beauveria spp*.(Photo 6 a).

Third is the " white fungi borer + berried wasp" which is the system in No8-3 that was subdued in good condition on April 17, and wasps are going to be berried and its abdomen expanded gradually. But the wasp finally gives up parasitizing and contracted its abdomen back to the normal state the day after the borer was infected by *Beauveria spp* on April 30.

Fourth is the "dry-shrank borer +berried wasp" (Photo 6 d).

Fifth is "dry-shrank borer + eggs-laying wasps" which is the system in No5-3 tube that the borer was subdued well, and the wasps were going to lay eggs. A total of 204 eggs were laid finally, an average of 41 eggs per wasp, on May 9 inspection; but on May 11, the borer was seen gradually shrinking, the wasps hurry up to stop eggs-laying and to eat most of the eggs-laid (Photo 9 c).

Six is the "wet-shrank borer + eggs-laying wasps" which is the system in No2-5 and No3-1 tubes that the borer were subdued well. A total of 136 eggs were laid, an average of 27 eggs per wasp, on May 9 inspection; to May 11, some eggs hatched; on May 13, some new larvae had been seen sucking food (Table 1); and then the liquid nutrient in the borer cavity flowed over the borer integument because its wall was rupture and wet-deflated; at same time a large number of new larvae died (photo 6 e).

Discussion and Review

Based upon the studies above, there was no existence of traditional parasitic behavior for *S.guani* with aculeate paralyzing the host borer. *S.guani* must go through 6 processes of borer-biting and -fighting, borer-subdued and –nursing, borer-feeding and –detecting, eggs-berried and -laying, borer-sucking and offspring development, cocooning and new-wasps emerging before the wasp complete the tasks for parasitizing the host. And the core activities can be summarized as that of borer-biting, extra-oral digestion and egg-laying, which expressed the *S.guani* have the characteristic of extra-oral digestion never found and reported before.

The extra-oral digestion mechanism" can better explain *S.guani* parasitic behavior, though it was beyond our mind. As you know the host borer has the wall of hard exoskeleton (21-22) which prevent wasp larvae from biting tightly with tiny mandibles for feeding. So the *S.guani* must have a set of highly efficient feeding mode to overcome the obstacle. In order to suck the nutrients inside of borer, *S.guani* must take the extra-oral digestive strategy which can meet the nutrition requirement for the larval growth and development. When the interior contents of the borer were sucked completely, the integument was still intact. The *S.guani* wasp behavior by biting at the integument and pumping special digestive enzymes into the inside of the host for liquefying and digesting of solid mass suitable for sucking was correspond with classical theory of extra-oral digestion described and summarized by entomologists (23-24, 36). Extra-oral digestion constitute the chemical pretreatment of food that enhances its nutrient quality or accessibility, which is widely distributed in Insecta (23-24, 36), such as Hemiptera, Coleoptera, Diptera, Neuroptera and so on (24). This paper present that, based on the *S.guani* feeding characteristics, especially the larvae can only sucking liquid nutrient without excretion habits, the Hymenoptera, at least many species, also belong to the insects of extra-oral digestion.

Hymenoptera parasitoids have not been considered to be extra-oral digestive insects. But in recent years, it has also been reported Hymenoptera wasp have similar habits of extra-oral digestion (6, 34, 47, 63, 67). Its larval salivary glands and digestive enzymes has been dissected and tested suggesting that these species of parasitic wasp with extra-oral digestive function, such as Phospholipase and hyaluronidase of Eupelmus orientalis (29), Trypsin-and chymotrypsin-like of Eulophus Pennicornis (29-30), Rhaconotus roslinensis (44). Nakamatsu and Tanaka (2003b) found that host weight decreased after *Pseudaletia separata* host was parasitized by *Euplectrus separatae* wasp, which suggest that the body material of the host was digested by the extra-oral and in following year, the enzymes Trypsin-like substance from salivary gland was determined to further illustrate the habits of extra-oral digestion (55-56); large secretory cells were there besides the salivary glands and mandibles of an egg parasitoid larvae *Trichogramma brassicae* (47), followed by digestive enzymes from salivary glands was confirmed (69), which suggest that this *T.brassicae* may be of extra-oral mechanism. These parasitoid mouthpart structures with extra-oral digestion function have been more researched (4, 14-15, 18). In fact, in the Hymenoptera, ecto- or endoparasitoids, as long as that hindgut is degraded and no excretion may have extra-oral digestion mechanism. Of course, many endoparasitoids during parasitic period, some teratocytes can secrete hydrolytic enzyme to aid in digestion, such as collagenase, carboxylesterase (28, 38, 54, 57, 60-61), and but it can not fully replace the extra-oral digestion of these parasitoids (16, 23). These studies on *S. guins* extra-oral digestion, especially, next, in testing of *S.guins* digestive enzymes pumped into the host in their fighting will further confirm the function of extra-oral digestion to regulate physiological and biochemical mechanism of the host, which will open a door led to Hymenoptera extra-oral digestion behavior and mechanisms understudied deeply and widely.

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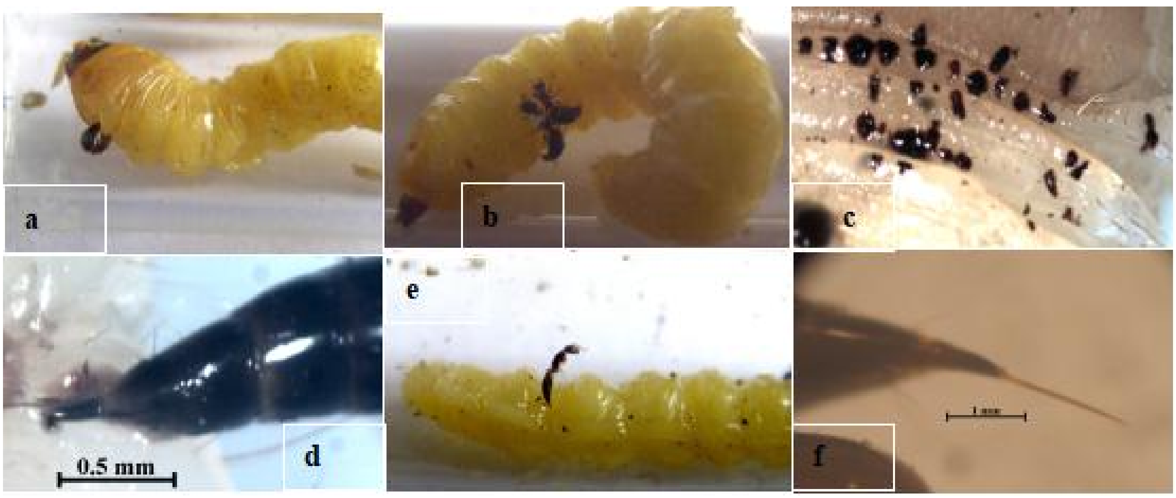

Photo1 of wasp exposure to borer showed biting borer and borer counterattacking.

(a, b) showing wasp bit and used aculeate to stimulate and the borer counterattacked. (c) showing many mandible-prints left on the surface of the borer. (d) showing a pinpoint hole and wasp died after piercing. (e) showing wasp died of piercing. (f) showing aculeate length longer than that of fake stinging as wasp piercing the borer.

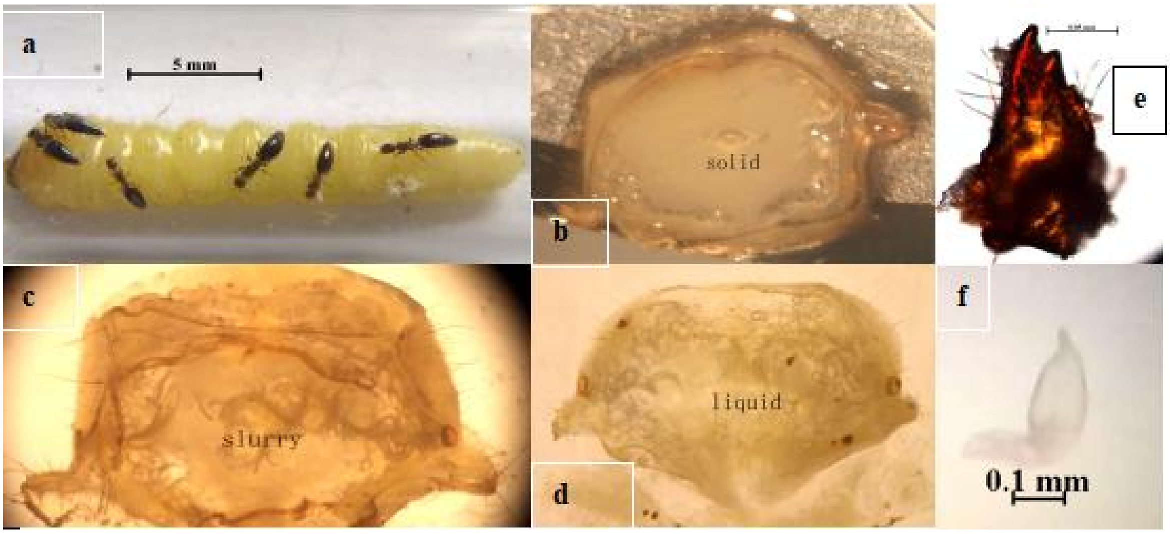

Photo2 of wasp nursing subdued borer showed shape subdued borer and its interior materials liquefying process.

(a) showing wasp was nursing subdued borer. (b)cross-section view of just subdued borer showed interior mass of borer was still solid.(c)cross-section view after wasp nursing showed interior mass of borer was collapsed and slurry. (d)cross-section view before wasp egg-laying showed interior mass of borer was liquefied.(e)showing wasp mandible.(f)showing the wasp salivary gland.

Photo3 showing wasp berried and laying eggs.

(a)wasp berried; (b)wasp preparing to lay egg; (c)wasp be laying egg and laid eggs

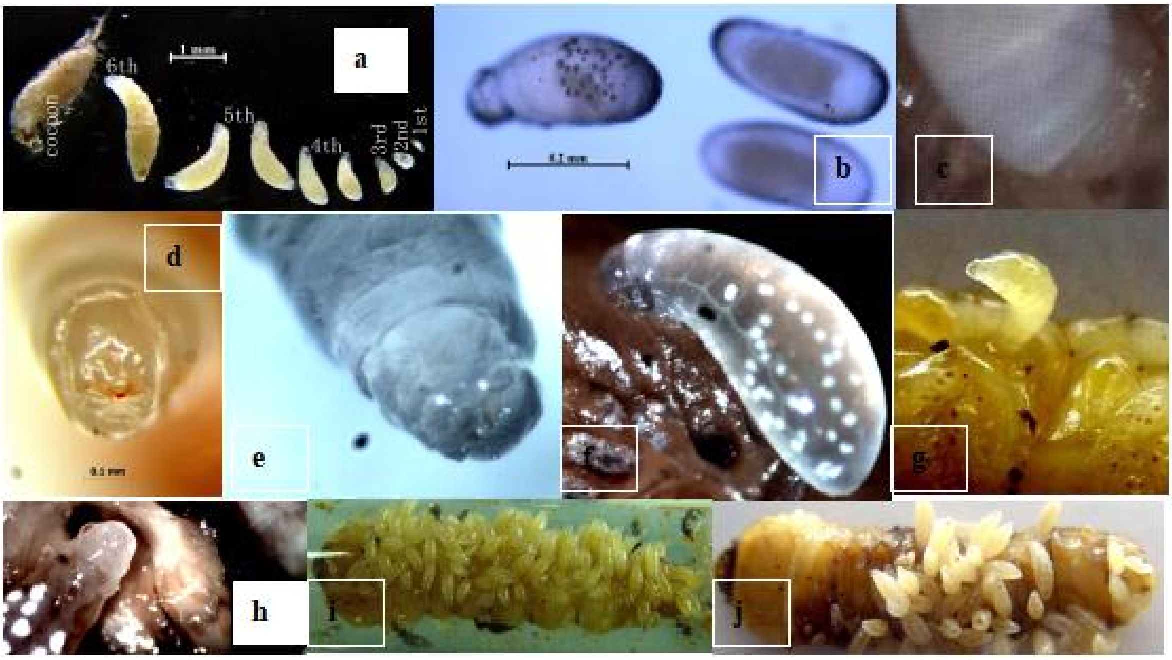

Photo4 showed larva sucking and growing.

(a)6 stages of larva developing; (b)shape of egg and larva in first stage; (c)the 1st larva access to integument of the borer with its mandibles;(d)the sucker of the larva; (e)larva extruding its head and exhibiting its mandibles; (f)a dent hole left in borer integument by larva feeding and the shape of the larva in 3rd stage; (g)process of lava in 4th,5th stage feeding and its head deepened into the borer but not break the integument;(h)the 6th larva stop feeding and leave the borer; (i, j)a large or mid number of larvae sucking liquid nutrients from the borer instead of hemolymph.

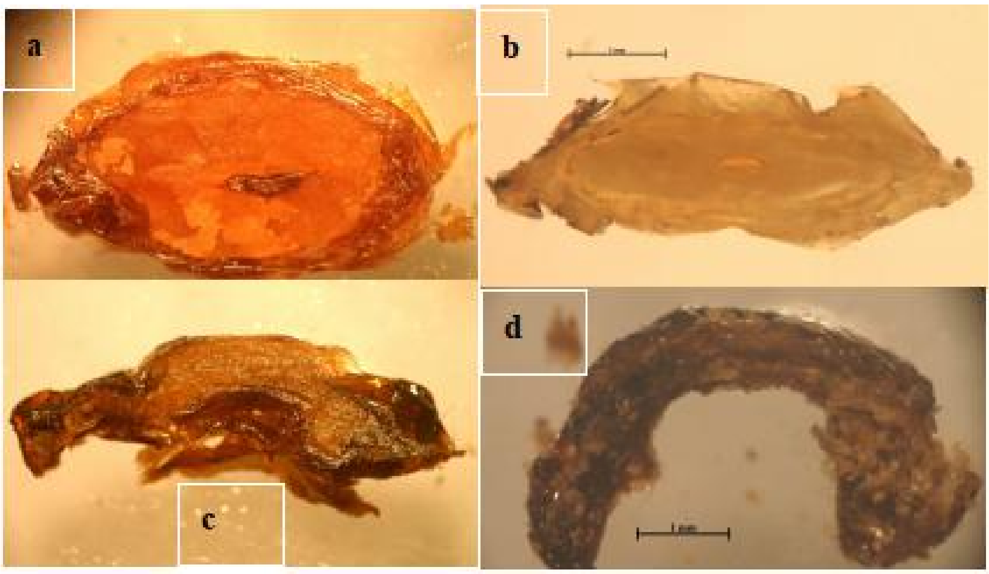

Photo5 showed change of the borer interior materials after sucked.

(a) a solid materials of the borer that means it was un-liquefied a bit after fighting stage;(b)a thin materials of the borer that means it was partially liquefied and sucked by a few of larvae; (c)a thinner materials of the borer that means it was much liquefied and sucked by a number of larvae; (d)non materials left in the interior cavity of the borer but the integuments that means it was liquefied and sucked completely.

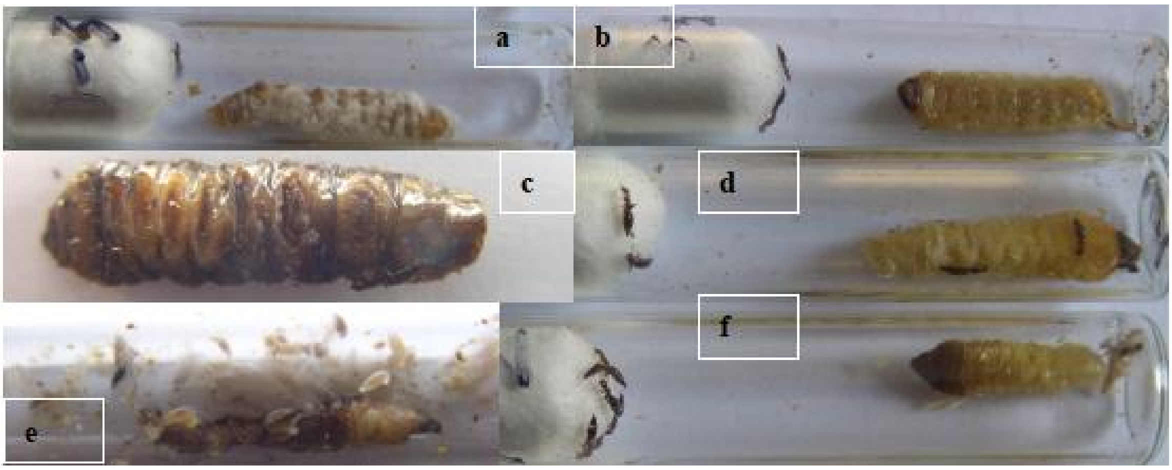

Photo6 showed parasitic failure state for wasp to the borer.

(a) borer infected by *Beauveria spp;* (b)a dry-shrank borer after bit by wasp; (c)a dry-shrank borer after eggs laid by wasp; (d)a dry shrank borer after wasp berried; (e)a wet-deflated borer after the offspring larvae were sucking and broken the borer body wall; (f)a dry-shrank borer after wasp judged the borer too small to provide sufficient nutrition and give up parasitizing.

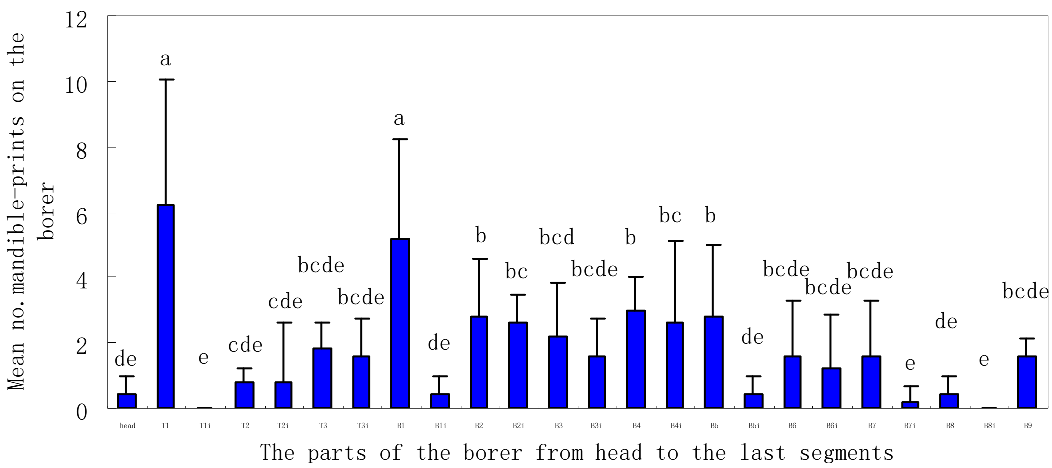

Fig.1. showed No. mandible-prints distributed on the borer longtitudinally.

Mandible-prints distributed on the parts of the borer from head to the last segment, column followed by different letters were significantly different by ANOVA (df=23, 119; F=4.853; P=0.0001).

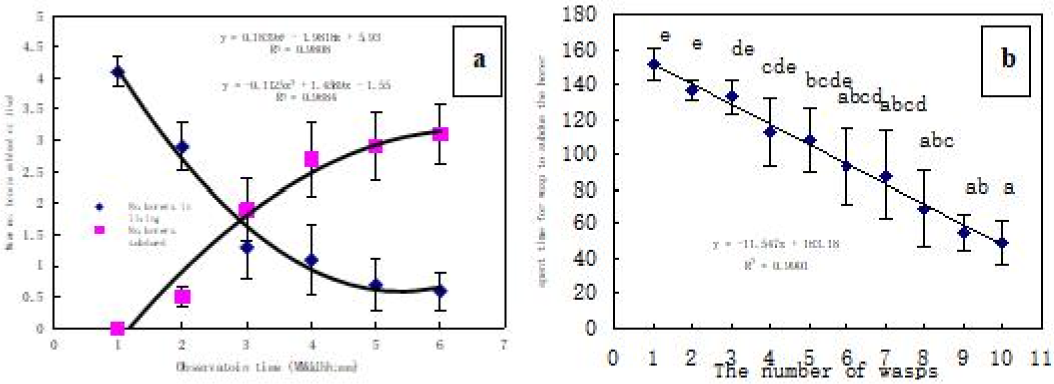

Fig.2. Spent time of the borer subdued has the relationship to the number of wasps.

(a) showing the lived borer decreased and subdued borer increased gradually with time for fighting went on (0-040816:00; 1-040915:30; 2-041009:30; 3-041015:30; 4-041114:30; 5-041312:00; 6-041413:30); (b) showing the relationship has the inverse proportion between spent time of the borer subdued and number of wasps, mean hours followed by differet letters were singnificantly different by ANOVA (df=9, 49; F=4.398; P=0.0005).

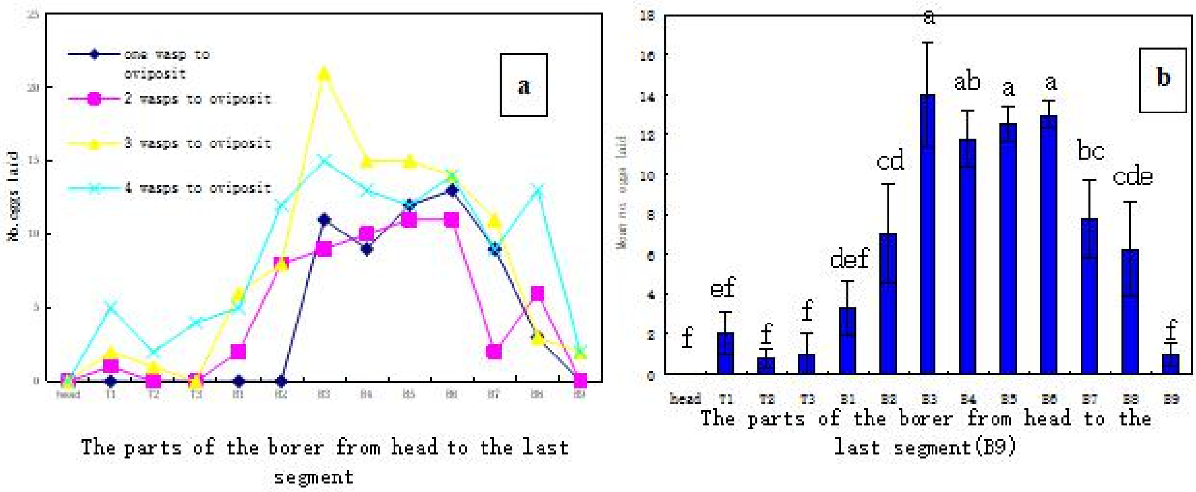

Fig.3. Wasp selecting sites for laying eggs and no. eggs laid on the parts of the borer.

(a) showing the range of borer segments of eggs-laying determined by the number of wasps laying eggs; (b) showing mean no. eggs laid on the parts of the borer, the column followed by different letters were significantly different by ANOVA (df=12,51; F=11.668; P=0.0001).

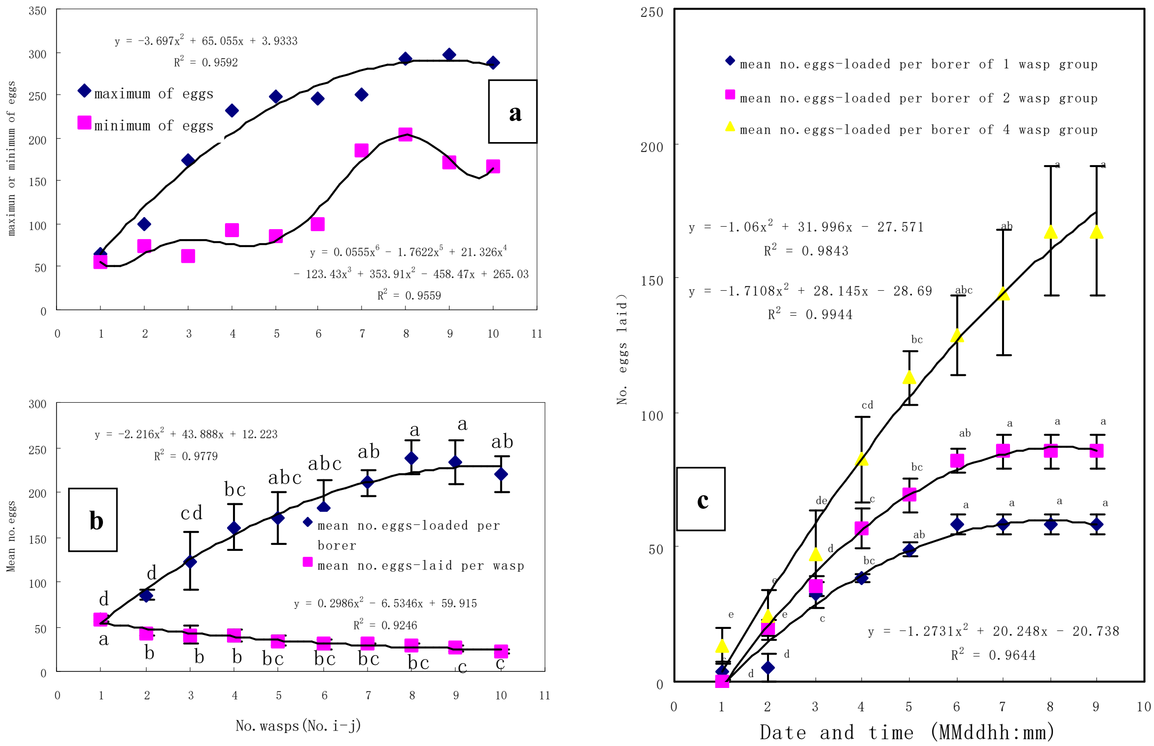

Fig.4. Maximum or minimum of eggs-loaded on the borer (a) and mean no. eggs-loaded per borer or mean no. eggs-laid per wasp (b) and spent time reached maximum of eggs-loaded on borer (c).

(a) showing maximum or ninimum of eggs-loaded on the borer, the maximum of eggs simply determinded by the equation (y=f (x^2); R^2=0.9592), wherars the minimum of eggs complexly determinded by the equation (y=f (x^6); R^2=0.9559); (b) showing mean no.eggs-loaded on the borer determinded by the equation (y=f (x^2); R^2=0.9779) and mean no.eggs-laid per wasp determinded by the equation (y=f (x^2); R^2=0.9246); (c) showing the spent time reached maximum of eggs, 1 wasp group for 5days (y=f (x^2); R^2=0.9644), 2 wasp group for 6 days (y=f (x^2); R^2=0.9944), 4 wasp group for 7 days (y=f (x^2); R^2=0.9843), (1, Mey3 14:00; 2, May4 15:00; 3, May5 16:00; 4, May6 14:00; 5, May7 13:00; 6, May8 15:00; 7, May9 15:00; 8, May10 16:00; 9, May11 13:00).

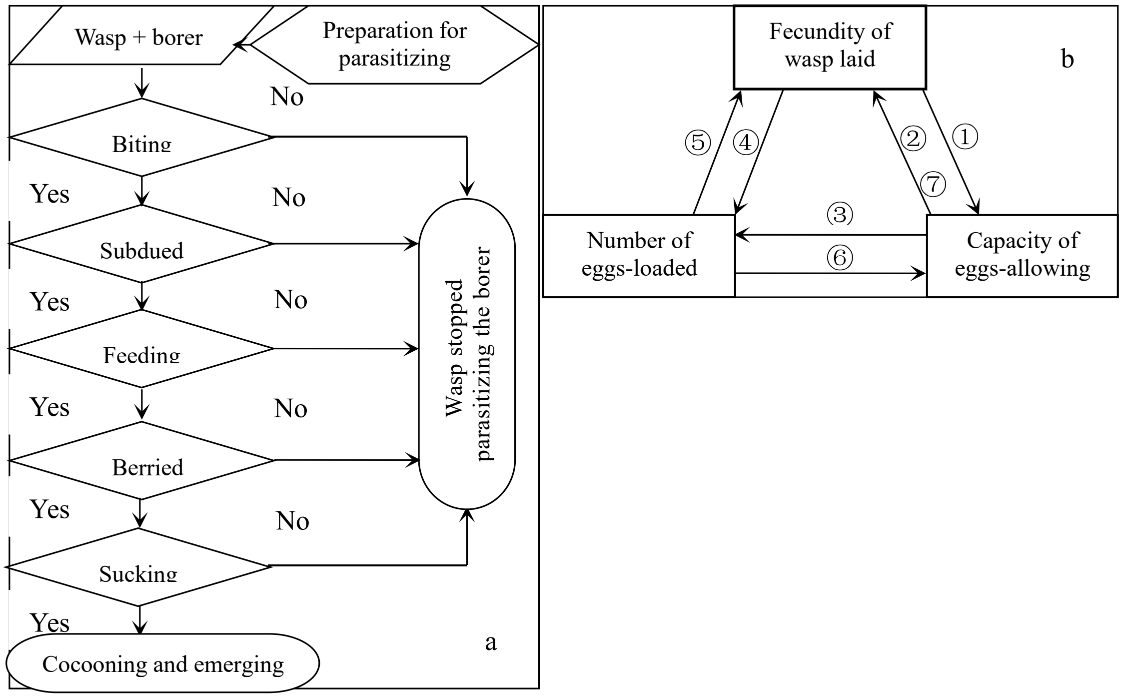

Fig.5. The program (a) and ethogram (b) of S. guani wasp parasitizing M. alternatus borer.

(a)showing each step to either success i.e. Yes or failure i.e. No; (b) showing the wasp using the extra-oral digestion mechanism built a liquid reservoir inside of the borer and made decision of fecundity based on between the reservoir of eggs-allowing and number of eggs loaded on the surface of the borer.

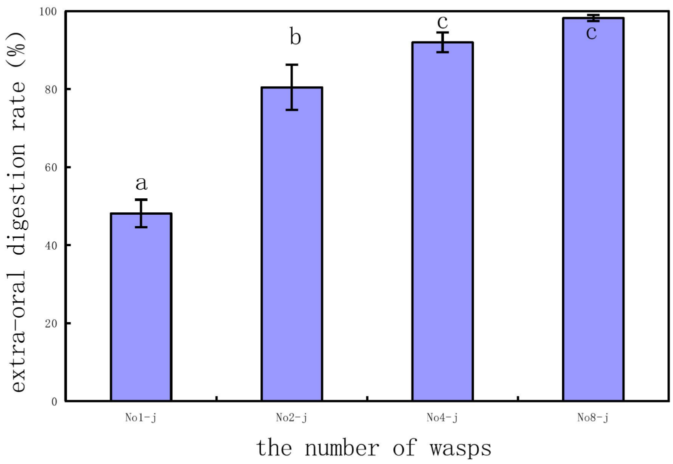

Fig.6. The relation between the number of wasps and the rate of extra-oral digestion.

Means followed by different letters in the groups were significantly different by ANOVA (df=3, 14; F=34.067; P=0.0001)

Table1. States of the parasitoid wasp and the host borer after S.guani wasp stop parasitizing the borer in Ningbo China 2011

No. i-j	Size of borer body length×width(mm)	States of wasp	States of borer	offspring no. eggs	offspring no. larvae	Process described briefly
1-1	22×5.5	berried	Dry shrank	0	0	Borer subdued on 21Apr;slightly shrank on 23 Apr;wasp berried on 30 Apr; dry-shrank borer on 2 May; wasp stop berried on 5 May
1-3	24×6.5	fighting	White mycelium	0	0	Borer body turned red on 17 Apr; infected by *Beauveria spp*.on 19 Apr; all turned white on 21 Apr
2-2	16×5.5	fighting	Dry shrank	0	0	Borer subdued on 9 Apr; yellow dry-shrank on 17 Apr
2-5	28×7.0	larvae	Wet	74	0	Borer subdued well on 17 Apr; wasp berried on 19 Apr; oviposit on 5 May;

			shrank			larvae sucking on 13 May; but the borer body wall broken and liquid nutriens outflow; and wet-shrank on 16 May
3-1	24×6.5	larvae	Wet shrank	62	32	Borer subdued well on 19 Apr; wasp berried on 21 Apr; oviposited on 6 May; larvae sucking on 13 May; but the borer body wall broken and liquid nutriens outflow; and wet-shrank on 20 May
3-4	20×6.0	fighting	Dry shrank	0	0	Borer subdued on 9 Apr; dry-shrank brown on 17 Apr
3-5	24×7.0	fighting	Dry shrank	0	0	Borer subdued on 9 Apr; dry-shrank brown on 17 Apr
5-3	26×6.5	egg laid	Dry shrank	204	0	Wasp berried on 23 Apr; oviposited on 5 May; borer dry-shrank on 9 May; wasp stop laying egg on 11 May
6-3	20×7.0	berried	Dry shrank	0	0	Borer subdued well on 17 Apr; slightly shrank on 19 Apr； Wasp berried on 23 Apr; borer dry-shrank on 30 Apr; wasp stop berried on 5 May
7-2	15×4.5	fighting	Dry shrank	0	0	Borer subdued on 9 Apr; dry-shrank brown on 10 Apr
8-3	27×6.5	berried	White mycelium	0	0	Borer subdued well on 19 Apr; asp berried on 21 Apr; borer infected by *Beauveria spp*. on 30 Apr; wasp stop berried on 9 May

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