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MORPHOLOGICAL AND SCANNING ELECTRON MICROSCOPIC (SEM) STUDIES OF THE PUPAE OF *Wohlfahrtia magnifica*

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ABSTRACT

Bactrian camel vaginal myiasis was caused by the larvae of *Wohlfahrtia magnifica*, and the development process of *W. magnifica* includes 3 metamorphic stages: larvae, pupae and flies. The morphological characteristics of the pupae stage of *W. magnifica* were observed by ultradepth imager and scanning electron microscopy (SEM). Through observation, the pupae eclosion process was divided into 3 stages, the 1st stage was from 1-4 days, its interior structure showing white and sticky; the 2nd stage was from 5-9 days, the head, wings, feet and abdomen of the pupa were forming and present milky white; the 3rd stage was from 10-12 days, the organs were developed and formed, and all coloured. Scanning electron microscopy (SEM) showed that the 1st, 2nd and 3rd segments of the head antennae were distinct in the 3rd stage of pupae, the 1st segment like a goat horn, the 2nd segment was trapezoidal, and the 3rd segment was rectangular with 2 aristate the outside. The antennae are covered with a large number of bristle, and the structure of bristle was flat and folded. Claw pads and claws were clearly observed on the feet, and there were a large number of bristle on its surface. The interval between the concave parts of the shell of *W. magnifica* pupae was uniform, but have not forming seven thoracic segments similar to that of the larval stage. The pupa shell was dark brown with evenly spaced segments and toughness, and not easily cut by sharp blades. Flies emerged from 13 - 15 days later. In this paper, we describe the morphological structure of pupae in its 3 development stages, which provided a biological data for the prevention and control of *W. magnifica* myiasis.

Key words: Morphology, scanning electron microscopy, ultradepth imager, vaginal myiasis, *Wohlfahrtia magnifica,* pupae

Adult *W. magnifica* feeds on plant juice, its larval stage is parasite of warm-blooded vertebrates causing myiasis (Bartel Huhe *et al,* 1994; 1995; Valentin *et al,* 1997). The mature larva is the 3rd instar leaves the host automatically, falls off to the ground, quickly burrows into the soil and metamorphoses into pupa after 1-3 days. The pupae becomes imago in the dry season after about 20 days (Wangchao, 2019) (Fig 1).

The larva of *W. magnifica* causes vaginal myiasis in Bactrian camels of Mongolia. The disease starts in early June and spontaneously stops in early October. (Schumann *et al,* 1976). Pirali Kheirabadi *et al* (2014) reported that *Wohlfahrtia* caused myiasis in Iranian camels. Xiwen *et al* (2019) reported the morphological characteristics of the larvae of Bactrian camels with vaginal myiasis.

Studies have found that the eclosion rate of pupa increases with the increase of temperature in a certain range. The high temperature and low rainfall in summer and autumn in Alxa region of China were very suitable for the survival and reproduction of *W. magnifica* (Xiwen, 2018). The incidence of this disease in camel population in Alxa region of China was 20%-30%, and the mortality rate was about 2% (Demtu Er *et al,* 2012). At present, there are only few reports on the morphological structure and living habits of the larvae, pupae and flies of *W. magnifica*. In this paper, the morphological and scanning electron microscopic (SEM) studies of the

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developing pupa of *W. magnifica* collected from vaginal lesions of Bactrian camels were done.

Materials and Methods

Sample collection

The 200 3rd instar larvae (10-20 mm) were collected from vaginal lesions of Bactrian camels. In the natural environment, the 3rd instar larvae were placed in plastic tanks with a diameter of 3.5 cm and a height of 5 cm (2/3 of the soft sand from the local shade, and ventilation ports were set around them. Five 3rd instar larvae were placed in each plastic tank). After the 3rd instar larvae drilled into the sand, the cans were covered with gauze and placed in shaded place. After 2 to 3 days, the 3rd instar larvae became wrapped in brown shell and became pupae, which was regarded as the beginning of pupae emergence. In order to observe the morphological changes of pupae during eclosion, pupae samples (10-15 pupae samples) were randomly taken out every day, fixed in 70% alcohol and marked the date.

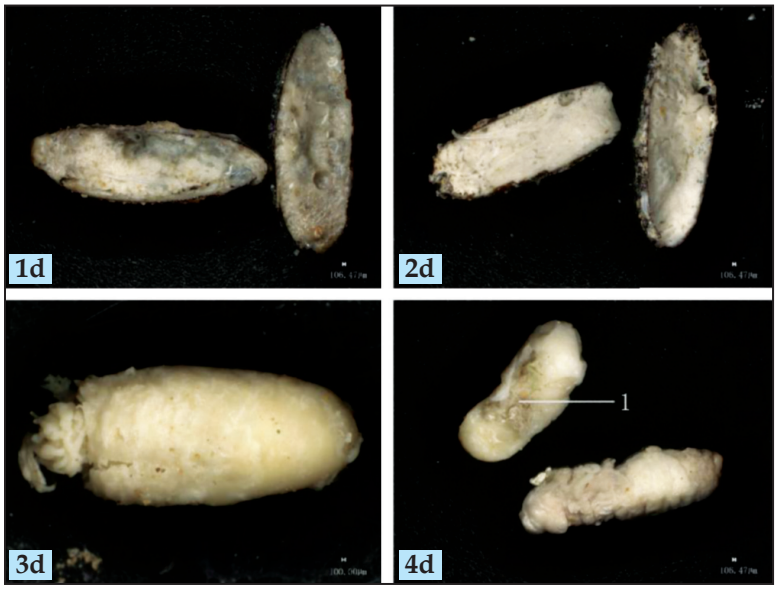

Fig 1. Developmental morphology from day 1 to day 4 of the pupa stage of *Wohlfahrtia magnifica*: 1 - Concave-convex structure.

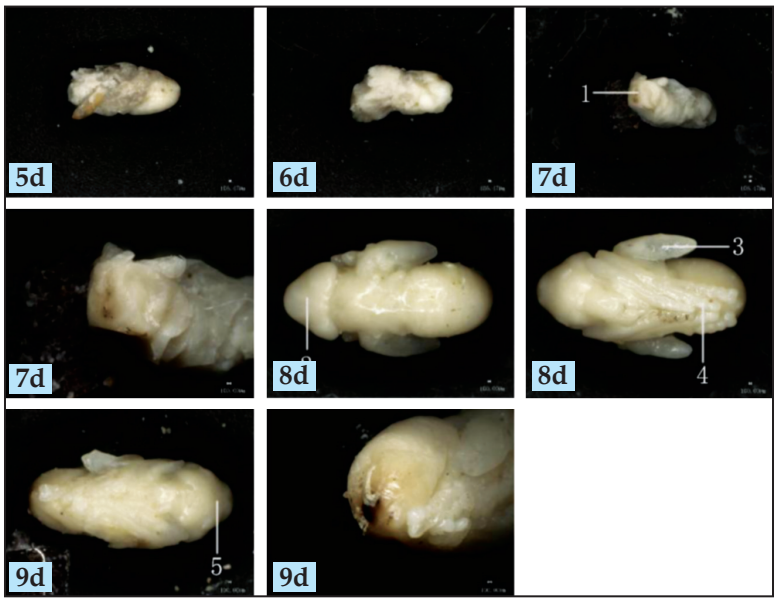

Fig 2. Developmental morphology from day 5 to day 9 of the pupa stage of *Wohlfahrtia magnifica*: 1 - anal foramen; 2 - head; 3 - wings; 4 - foot; 5 - tail.

Ultra depth imager sample preparation

Pupae samples were taken out from 70% alcohol every day during pupae eclosion. The morphological changes of pupae during eclosion were observed after the alcohol on the surface of samples completely volatilised using a KEYENCE-1000 Ultra Depth Imager.

Electron microscope sample preparation

Prior to examination, the pupae stored in 70% alcohol were cut and fixed for 12h with 2.5% glutaraldehyde solution. The fixative solution was replaced once during this period, then rinsed them six times with the phosphoric acid buffer and dehydration was carried out step by step with 55, 75 and 95% alcohol. Then, samples were dried on the critical point dryer and coated, with platinum coating on the conductive layer. Examination was done under the HITACHI-4800 Scanning Electron Microscope.

Results and Discussion

Observation results under Ultra depth imager

The 1st day of anatomical observation showed that the pupal content was a white viscous liquid, similar to egg white, without fixed shape, and could not peel off its interior with brown shell (Fig 1, 1d); the 2nd day of anatomical observation showed that the interior was white soft solid (Fig 1, 2d); the 3rd day of anatomical observation showed that the interior was white oval solid, which could peel off its interior with outer shell completely (Fig 1, 3d); and the 4th day of anatomical observation

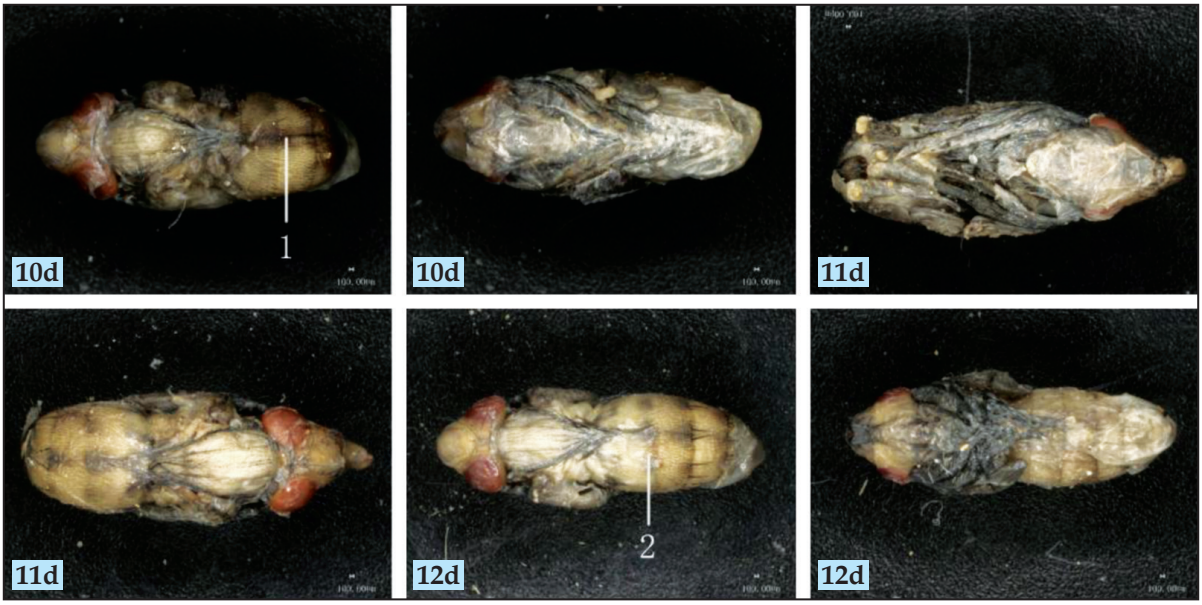

Fig 3. Developmental morphology from day 10 to day 12 of the pupa stage of *Wohlfahrtia magnifica:* 1 - mid-abdominal bristle; 2 - lateral abdominal bristle.

Fig 4. The morphological structure of the pupa stage of *Wohlfahrtia magnifica*. 1 - pupa shell; 2 - adult flies in early emergence.

showed that the interior began to have uneven structure (Fig 1, 4d).

On the 5th and 6th days anatomical observation, there was no obvious changes in the interior (Fig 2, 5d, 6d), and brown anal foramen appeared on the 7th day (Fig 2, 7d). On the 8th and 9th days, the head, wings, feet and tail of pupae had been formed, and the organs had no changes colour, all showing milky white (Fig 2, 8d, 9d).

On the 10th day of pupal development, a transparent film wrapped its interior, the colour of the head, eyes, wings, feet and abdomen had changed obviously, the colour of the black spots on the abdomen had deepened gradually, and the bristles appeared on middle and lateral margins of the abdomen; the wing chambers and veins had not been fully formed, and the wings were in a contraction state (Fig 3).

Visual observation results

The 3rd instar larvae falling to the ground became pupa. It was observed that the pupal shell of *W. magnifica* was dark brown with evenly spaced segments, and did not form 7 thoracic segments similar to the larval stage. The pupa shell was strong flexible, and difficult to cut with sharp blades, and not easily to be damaged from outside (Fig 4).

Observation results under electron scanning microscope

On the 10th day of eclosion, the antennae of the head was formed. The 1st, 2nd and 3rd segments of the antennae were clearly distinguised. The 1st segment started at the head and the 3rd is at the tip of antenna. The 1st section was curved like a goat horn at the top of the antenna, the 2nd section was trapezoidal in the middle of the antenna, the 3rd section was rectangular at the bottom of the antenna, and the 3rd parts were closely connected. There were 2 aristate on the outside of the 3rd section. A large number of bristles were found in the 1st, 2nd and 3rd segments of the antennae (Fig 5).

The eyes of the *W. magnifica* were composed of 2 smooth hemispheres by naked eye observation. The fly could not have rotated around the eyes like human eyes. It needed to observe the surrounding environment through the rotation of body and head.

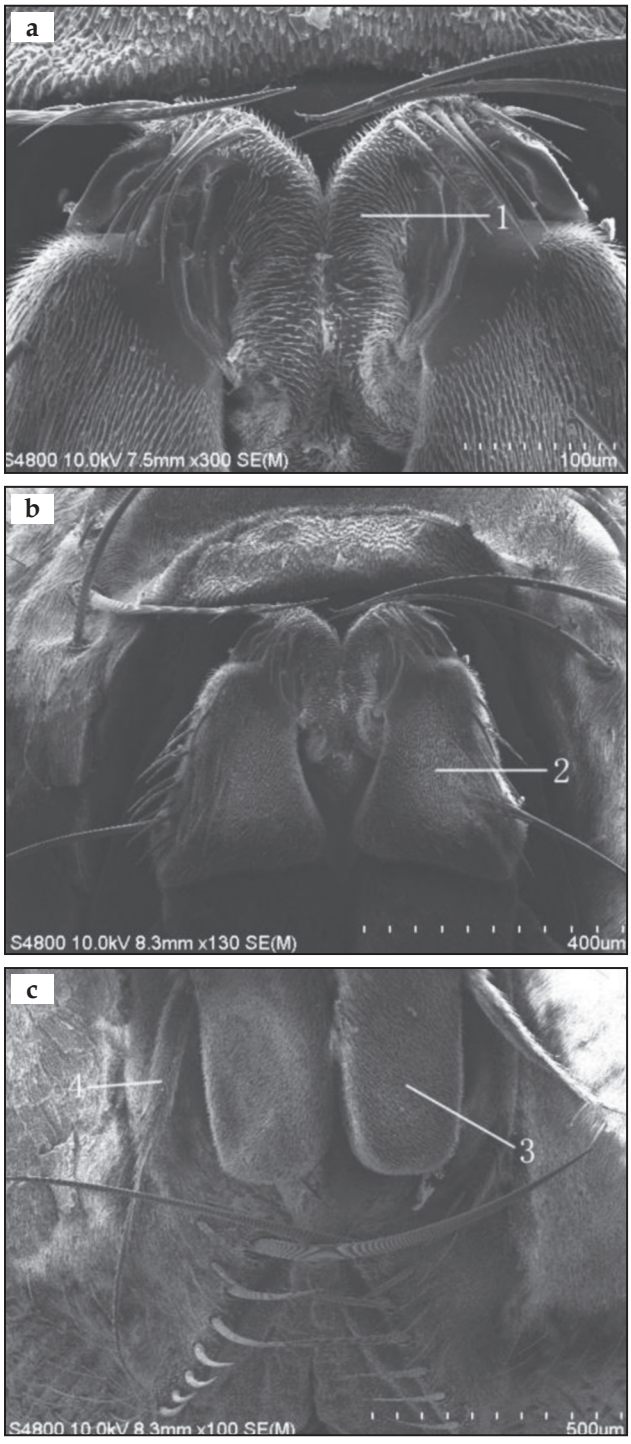

Fig 5. Electron microscopic observation of the head antennae of the pupa stage of *Wohlfahrtia magnifica*a: 1-first antennae; b: 2-antennae 2nd section; c: 3-antenna 3rd section, 4-aristate.

The outer membrane of the eyes was connected with the head. Under the electron microscope, it was found that the eyes of *W. magnifica* were composed of many small quadrangles, each of which was a small eye (Fig 6).

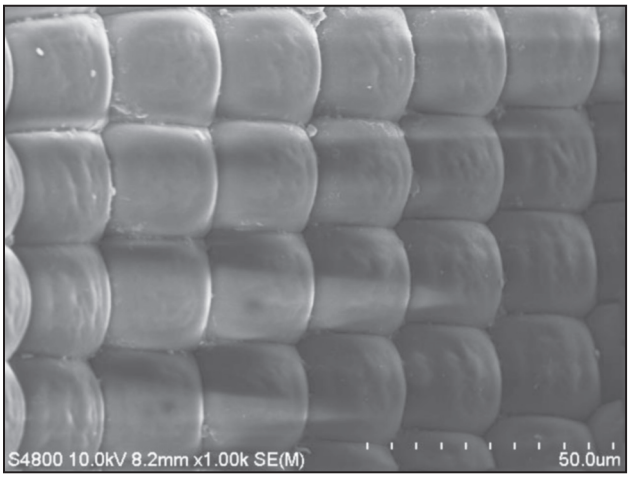

Fig 6. Electron microscopic observation of compound eyes in pupa stage of *Wohlfahrtia magnifica*.

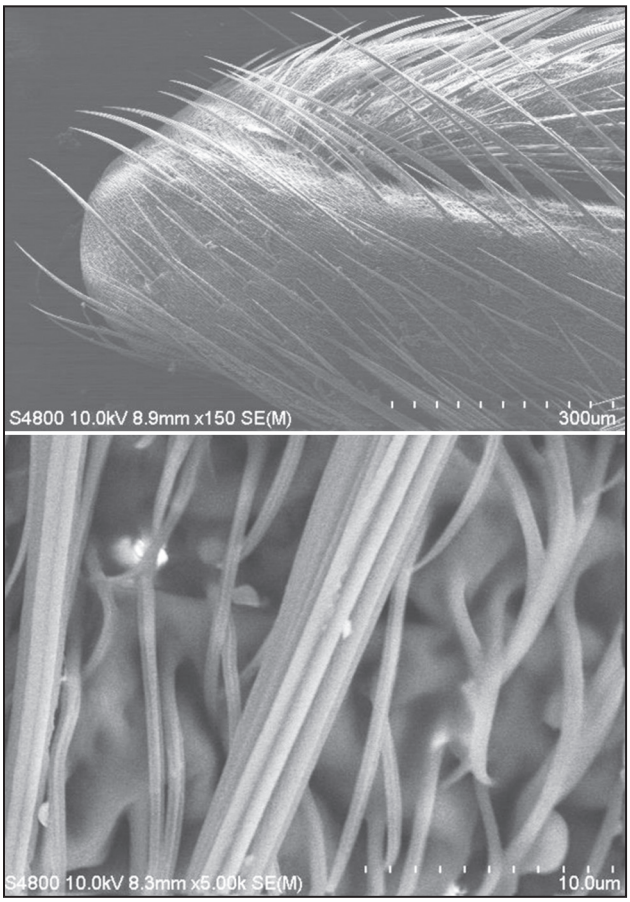

Fig 7. Electron microscopic observation of mane in pupa stage of *Wohlfahrtia magnifica*.

The structure of the bristles was found to be cylindrical needle-like with magnification of 150 times, and it was found flat and folded with 5000 times magnification (Fig 7).

Anatomical observation on the 12th day of emergence of *W. magnifica* showed that the foot had

Fig 8. Observation of foot in the pupal stage of *Wohlfahrtia magnifica*: 1-claw pad; 2-claw.

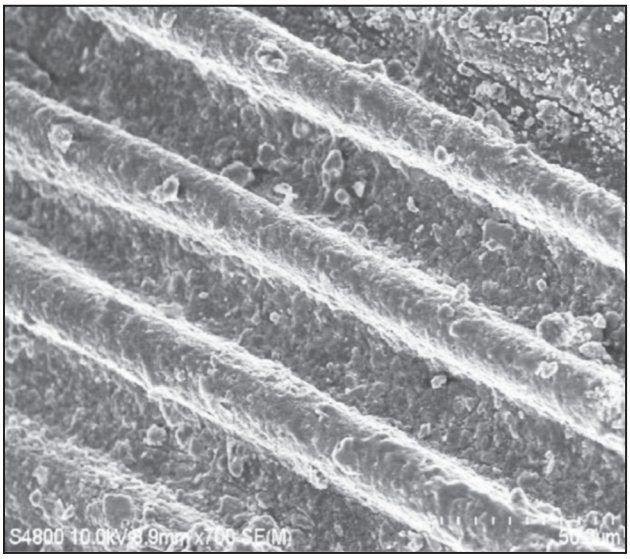

Fig 9. Electron microscope of the pupal shell of *Wohlfahrtia magnifica*.

developed and formed. The pads and claws could be seen clearly under the electron microscope, and a large number of manes were covered around them. Its claws were curved and sharp, making *W. magnifica* easy for landing in different places (Fig 8).

Under electron microscopy, the pits of the pupal shell were evenly spaced and its surface was not smooth. In the course of anatomy, the pupal shell was tough and not easily cut by sharp blades (Fig 9).

The *W. magnifica* was widely distributed in the Mongolian plateau, causing myiasis of the livestock. It is considered one of the harmful parasite in Bactrian camels causing losses to the camel industry (Guofan, 1958).

Miaomiao *et al (*2019) results showed that the pupal completes its development between 12~15 days and the development process of pupa could be divided into prepupa, 1st pupal phase, 2nd pupal phase, 3rd pupal phase before adult emergence according to the morphological characteristics. In this experiment, the pupal stage of *W. magnifica* was observed with the ultra-depth imager and it was divided into 3 stages: in the 1st stage, the pupa with white and viscous content from 1-4 days; in the 2nd stage, the organs of the imago begin to take shape from 5-9 days, and the shape of head, wings, foot and abdomen could clearly be observed under the ordinary microscope; in the 3rd stage, the internal body of the developing fly has been formed with from 10-12 days and each part was mature and coloured. From 13-15 days, the pupa developed into a fly.

The antennae of pupae head and the bristles on the antennae may be related to the odours. The antennae of adult mosquitoes carry numerous sensory structures called sensilla, which are the physical sites of odour detection (Hatem *et al,* 2018). The structure of the bristle on the antennae was flat and folded, which can increase its surface area and efficiency for receiving chemical signals from the surrounding environment. The feet pads were well developed, and its claws were curved and sharp at the end, which was convenient for the imago to land, and increases its foraging range and living space. The compound eye of *W. magnifica* was composed of many small quadrilateral eyes, which was convenient to search for hosts, food, spouses, etc, and can be used for species identification (Rafinejad *et al,* 2014). In the process of eclosion, the pigmentation of organs was carried out after the formation of organs, which may be related to the change of shell permeability and oxidation. The pupa's shell was flexible and not easily damaged by the outside, and shell surface has concave and convex segmented structure, which plays a protective role in the development of pupa during eclosion.

Acknowledgements

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