



Original/Investigación animal

Morphologic features of *Sancassania berlesei* (Acari: Astigmata: Acaridae), a common mite of stored products in China

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Abstract

Objective: *Sancassania berlesei* (*S. berlesei*) is the leading threatening mite in breeding industry of *Eupolyphaga sinensis* and public health.

Methods: Living specimens of *S. berlesei* were obtained from the surface of *Eupolyphaga sinensis* and purified with double-distilled water. The egg, larva, nymph, hypopus, adult male and female of *S. berlesei* were screened and picked out under microscope.

Results: Morphological variations of *S. berlesei*, including its legs, setae, external genitalia and accessories, are clearly identified under SEM. The larva has three pairs of legs, with no leaf-like setae, yet its coxal rod is well-developed. By nymph stage, four pairs of legs and the fourth dorsal seta arise, whereas the genital area looks still under-developed. At hypopus, the claws and tarsules appear well-built, and leaf-like setae, setae of tibia and setae of genu are seen. The sucker plate totally contains nine suckers and four shell-like dimplings in which there are symmetric distributions with 1 pair of central suckers, 2 pairs of side suckers and 1 pair of anterior suckers, respectively. One pear-like posterior sucker is located at the back of sucker plate. All suckers are smooth except for anterior sucker with radial stripe. The genital sense organ of adults exhibits itself with cordiform external aspect and typical ossification texture; whereas the male is dissimilar with the female regarding seta number on the genital sense organ.

Conclusion: Description of the morphological structure in great detail for *S. berlesei* tends to supply the important information for the taxonomy and further study.

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Key words: *Sancassania berlesei*. Morphology. Hypopus. Sucker. Scanning electron microscopy (SEM).

LAS CARACTERÍSTICAS MORFOLÓGICAS DE SANCASSANIA BERLESEI (ACARI: ASTIGMATA: ACARIDAE), UN POCO DE PRODUCTOS ALMACENADOS EN CHINA

Resumen

Objetivo: *Sancassania berlesei* (*S. berlesei*) es el líder en la industria de cría amenaza mite *Eupolyphaga sinensis* y la salud pública.

Métodos: los especímenes vivos de *S. berlesei* fueron obtenidos a partir de la superficie de *Eupolyphaga sinensis* y purificado con agua bidestilada. El huevo, larva, ninfa, hypopus, macho adulto y la hembra de *S. berlesei* fueron evaluados y elegidos bajo microscopio.

Resultados: las variaciones morfológicas de *S. berlesei*, incluidas sus piernas, setas, genitales externos y accesorios, están claramente identificadas en la SEM. La larva tiene tres pares de patas, sin hojas como setas, pero su coxal Rod es desarrollada. Por la etapa de ninfa, cuatro pares de patas y el cuarto dorsal seta surgen, mientras que la zona genital se ve aún en desarrollo. En hypopus, las garras y tarsules aparecen bien construido, y la hoja como setas, setas de tibia y setas de Genu son vistos. La ventosa plato totalmente contiene nueve retoños y cuatro Shell como dimplings en que hay distribuciones simétricas con 1 par de mamones, 2 pares de lado anterior de ventosas y 1 par de tontos, respectivamente. Una pera como posterior ventosa está ubicado en la espalda de Sucker plato. Todos los tontos son suaves excepto anterior Sucker con franja radial. El órgano de sentido genital adultos exhibe con cordiforme aspecto externo y osificación típica textura; mientras que el macho es diferente con la hembra respecto a seta numero en el sentido de órganos genitales.

Conclusión: descripción de la estructura morfológica en gran detalle para *S. berlesei* tiende a suministrar la información importante para la taxonomía y más estudio.

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Palabras clave: *Sancassania berlesei*. Morfología. Hypopus. Mamón. Microscopía electrónica de barrido (meh).

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Introduction

Caloglyphus berlesei (*C. berlesei*; Michael, 1903), also termed as *Sancassania berlesei* (*S. berlesei*), belongs to the subclass Acari of the class Acaridae, suborder Acaridida under the order Acariformes, genus *Caloglyphus* of Acaridae. It lives favorably in high temperature (30-35 °C) humid areas [relative humidity (75%-80%)] and occurs in Asian, European and North American countries¹⁻⁴. *S. berlesei* can cause wider harm to the stored products as well as human health, and its infestation was described in occurrence in external auditory meatus and urine samples of patients^{5,6} and associated with the sheep reared outdoors⁷ and poultry yard⁸. Breeding *Eupolyphaga sinensis* is popular in north Anhui of China, where *S. berlesei* is also occurred seriously, which causes heavy economic loss of the local breeding industry⁹.

The life cycle or biological trait of *S. berlesei* has been widely investigated⁹⁻¹¹, yet its morphology is unclear. Understanding the morphological structures of *S. berlesei* in details is helpful to identify this species from its similar counterparts. In this study, we aimed to the external structures of *S. berlesei* in more details and benefited to clarify the morphological characterization of this species in systematics.

Materials and methods

The *S. berlesei* samples were collected in March 2012 from the bed feeds in a breeding farm (33.52°N/115.47°E) of *Eupolyphaga sinensis* in north Anhui Province, China. Living specimens obtained from the external surface of *Eupolyphaga sinensis* were cleansed with double-distilled water, picked out the egg, larva, nymph, hypopus, adult male and female under the microscope. Then, the living mites were anesthetized with ether, positioned on the conductive double-sided tape and fixed with extremely fine pins. By critical-point drying, the specimen was observed under SEM (JEOL, JSM-490LA, Japan) and the high-resolution images were taken.

Results

Egg stage

The dimensions of the eggs are approximately 154.2µm (L) and 104.8 µm (W). The egg is typically elliptical in shape, semitransparent, and offwhite in color. The egg shell is smooth, and any punctuates are absent on the exposed surface of the egg (Plate I-1). A larva is occasionally seen under the translucent surface of the egg in light microscope.

Larva stage

Three-legged larvae were hatched from eggs, and there is no apparent sexual dimorphism in the larval stage, the dimension of the larva varies between 261.5µm long and 150.6 µm wide (Fig. 1-2). On the dorsal surface of the larval, only three pairs of setae (d1-d3) and one pair of lateral postanal bristle (lp) are present, and d4 is absent (Fig. 1-3). The idiosoma is born three pairs of legs without leaf-like setae, and each leg has 5 free segments, namely coxa, femur, genu, tibia and tarsus. The tarsus, the most distal segment, is slender and elongated. The Claparède's organ (cr) on the propodosoma is well-developed. The external genitalia appear immature. Only one pair of anal setae (pa) is found on the opisthosoma (Fig. 1-4).

Protonymph stage

The protonymph is approximately 321.8µm by 144.5µm, colorless and bears four pairs of legs. The coxal rod is almost invisible. Although the gonopore (Gp) looks still immature, yet the genital sensillum begins visible. In addition, three pairs of anal setae (a1, a2, a3) are seen (Fig. 1-5). The fourth dorsal seta (d4), antero-lateral seta (la), posterolateral seta (lp) and internal humeral seta (hi) arise at the hysterosoma, which are not totally as long as those of adults (Fig. 1-6).

Hypopus stage I

The hypopus is approximately 300.2µm long and 204.1µm wide. The idiosoma is dark brown in color, arciform in shape, and entirely smooth. The propodosoma is triangularly shaped and curved forward into round-narrow tip with internal verticals (iv) born on the anterior margin of the apex (Fig. 1-7). The two pairs of scapular setae (sc) are short and arranged in arc manner. The hysterosoma is four to five times as long as the propodosoma and bears fine setae (Fig. 1-8). The ossific genital operculum lays the upper margin of the sucker plate. The longitudinal aperture of gonopore is rested in the centre of the genital operculum, with one pair of suckers and one pair of bristles arising at the opposite site of the genital aperture. The sucker plate takes a single sucker, four pairs of suckers, and two pairs of shell-like microbumps. One pair of central suckers, two pairs of lateral suckers and one pair of anterior suckers are symmetrically distributed. A posterior sucker behind the sucker plate presents with pear-like shape. Totally, the suckers look smooth except for the anterior suckers displaying in aspects by radial striation running from marginal circumference (Fig. 1-9). Each claw and tarsule is well-built. The first and the second tarsus are enclosed by leaf-like incurvated frondose setae (f). The first so-



Fig. 1.— SEM observation on external morphology of *Sancassania berleseii* (Acari: Astigmata: Acaridae) in distinct stages 1: Egg of *Caloglyphus berleseii*; 2: Ventral view of the larva; 3: Dorsal view of the larva; 4: Lateral view of the larva; 5: Vntral view of the protonymph; 6: Dorsal view of the protonymph; 7: Ventral view of deutonymph (hypopus); 8: Dorsal view of deutonymph (hypopus); 9: Sucker plate of deutonymph (hypopus); 10: Dorsal view of propodosoma of deutonymph (hypopus); 11: Ventral view of the gnathosoma of adult male; 12: Dorsal view of the adult male.

Cr: coxal rod; d (d1, d2, d3, d4): dorsal setae; la: lateral anterior bristle; lp: lateral postanal bristle; sci: internal scapular setae; sce: external scapular setae; vi: internal verticals; pa: post anal; Gp: gonopore; a (a1, a2, a3, a4): anal seta; vi: internal verticals; hi: Internal shoulder; ω : solenidia; gt: setae of tibia; mg: setae of genu; The abbreviations in Figure 1are consistent with Figure 2.

lenidia (ω 1) in tarsulus I is longer than that of tarsulus II and the ω 1 in each tarsule is a little wider than its coxal base. The setae of tibia (gt) on leg I and II and setae of genu (mg) are entirely aculeate and shorter than that of ω 1 (Fig. 1-10).

Adult male

The adult male *S. berleseii* measures 670.2 μ m by 300.4 μ m. Its cuticle is colorless and brightly smooth and its appendage looks light brownish. In a damp se-

ting, the idiosoma exhibits itself with rod-shape and expands most at the site of the tarsi III and IV. The slender gnathosoma grows thinner at its terminal and divides into two operative chelicerae, in which a pair of palps arranges inversely triangularly with furcated terminal and sensillum, and a pair of setae is seen on the ventral side of the gnathosoma (Fig. 1-11). The entire dorsal setae on the idiosoma appear totally smooth and widen at their bases. External verticals (ve), small and short, arise in between the propodosoma plate and its lateral margin. Two pairs of the scapular setae are measured equally as the length, whereas external scapular setae (sce) are three to four times longer than the internal ones (sci). The supra-coxal setae (ps) are apparent and smooth. On dorsal hysterosoma, the dorsal seta is shorter than d_1 , and the d_2 is two to three times longer than d_1 , whereas the anterolateral seta (la) and the internal humeral seta (hi) are one and half to twice longer than d_1 . The dorsal setae d_3 and d_4 as well as posterolateral setae appear longer, particularly, the d_4 goes a lot beyond the end of the idiosoma (Fig. 1-12). The Grandijean's organ rests at the junction of the base of gnathosoma and lateral dorsal-ventral idiosoma, with separate right one and left one and spine-like process surface (Fig. 2-1). On the ventral hysterosoma, postanal seta (pa1) is the shortest, and pa3 measures three to five times longer than pa2. Round anal sucker (as) is apparent, bearing one pair of anal setae (preanal seta, pra; postanal seta, pa) (Fig. 2-2, -3). Appendage I and II look slender and the anterior tarsule is well-built, with solenidia (ω) at the extreme end of each tarsulus. The setae (a, b, c, and d) are arranged contralaterally at the solenidia, which the end is a hook-like claw. The median short seta (f) and setae of tarsus (ra) are falciform and expanded by leaf-shape at the tip. Individual falciform hook is seen at the upper end of each appendage (Fig. 2-4). The number of sensillums and setae at the 3rd and 4th appendage are typically reduced compared to that at the 1st and 2nd appendage. One pair of ambulacra (aa) arise from the extreme upper end of the 4th appendage (Fig. 2-5). Male genital sense organ, unfolding cordiform external aspect and typical ossification texture, consists of the right and the left genial valve (gv) with single seta (i) born contralaterally. The inner margin of the genial valve looks concavo-convex (Fig. 2-6)

Adult female

The adult female of *S. berlesei* measures 832.1 μ m by 413.4 μ m. Although the idiosoma is colorless and has smooth and bright cuticle, yet its body is larger than male one and shaped more round, and its dorsal setae are not so long as those of the adult male, with d_4 shorter than d_3 , whereas the antero-lateral setae (la) and posterolateral setae (lp) are in equal length. The lateral humeral setae (hi) are shorter, and arrangement of external scapular setae (sce), internal scapular setae

(sci) and internal verticals (iv) is similar to the adult male (Fig. 2-7). The appendage is somewhat light brownish. Genital sensillum sits in between the third leg and ventral side, and the entire genital region wears heart-shaped figure. The gonopore (Gp) of adult female has a longitudinal aperture, giving saphoid aspect of the two genital valves and forming the tocostome at the divided distal end. Three pairs of genital setae (f, h, i) occur around the tocostome (Fig. 2-8,-9). Six pairs of smaller anal setae (a) arise circumferentially from the anus, in which two pairs occur antero-bilaterally at and four pairs posteriorly around the anus (Fig. 2-10). The chelicerae of adult female appear shorter than that of male one (Fig. 2-11), while the gnathosoma resembles the adult male (Fig. 2-12).

Discussion

Sancassania berlesei (Michael, 1903), also termed as *Caloglyphus berlesei*, belongs to the subclass Acari of the class Arachnida, suborder Acaridida under the order of Acariformes, family of Acaridae, genus of *Caloglyphus*. To date, eight species of *Caloglyphus* have been described, in which *S. berlesei* is often found on damp, mouldy stored products such as cereals, flour, linseeds, garlic, mushroom, cookies, medicinal herbs, medicinal insects in storage and flax due to its biological nature inclining to live in hot and damp conditions¹². It is a free-living species, widely distributed in China, Germany, U.K. and other countries, and important infestation of house environment and storages.

Eupolyphaga sinensis Walker is an important medicinal ingredient and valuable insect origin of certain health products. Unfavorably, its breeding environment is liable to infestation of *S. berlesei*, a pest degrading the insect quality besides its yield. In order to control the pest infestation of *Eupolyphaga sinensis* Walker for improving the quality and output, it is necessary to identify the parasitic species that actually affects the insect breeding, especially the morphology of the pest, for which is of great significance to the classification of harmful mites and targeted controlling of them, and ideally to eliminate such pests to a certain degree.

The frequent approach to taxonomy of mites is direct observation of them, including *S. berlesei*, under microscope for their morphological identification. This technique is involved in simple slide preparation and unnecessary complex instruments. However, it has certain disadvantages for its precision limitation from instrument used and difficulty in detecting the ultra-structure of the subject observed. That is resolved currently through introduction of available SEM to this field¹³⁻¹⁶. In our study, we applied the SEM to a thorough live examination of the ultra-structure of *S. berlesei*, especially of the cuticle structure, seta arrangement, suckers, genital sense organ and anal region in compliance with its egg, larva, nymphs in various life cycle, and adult males and females, and systematically described the morpho-

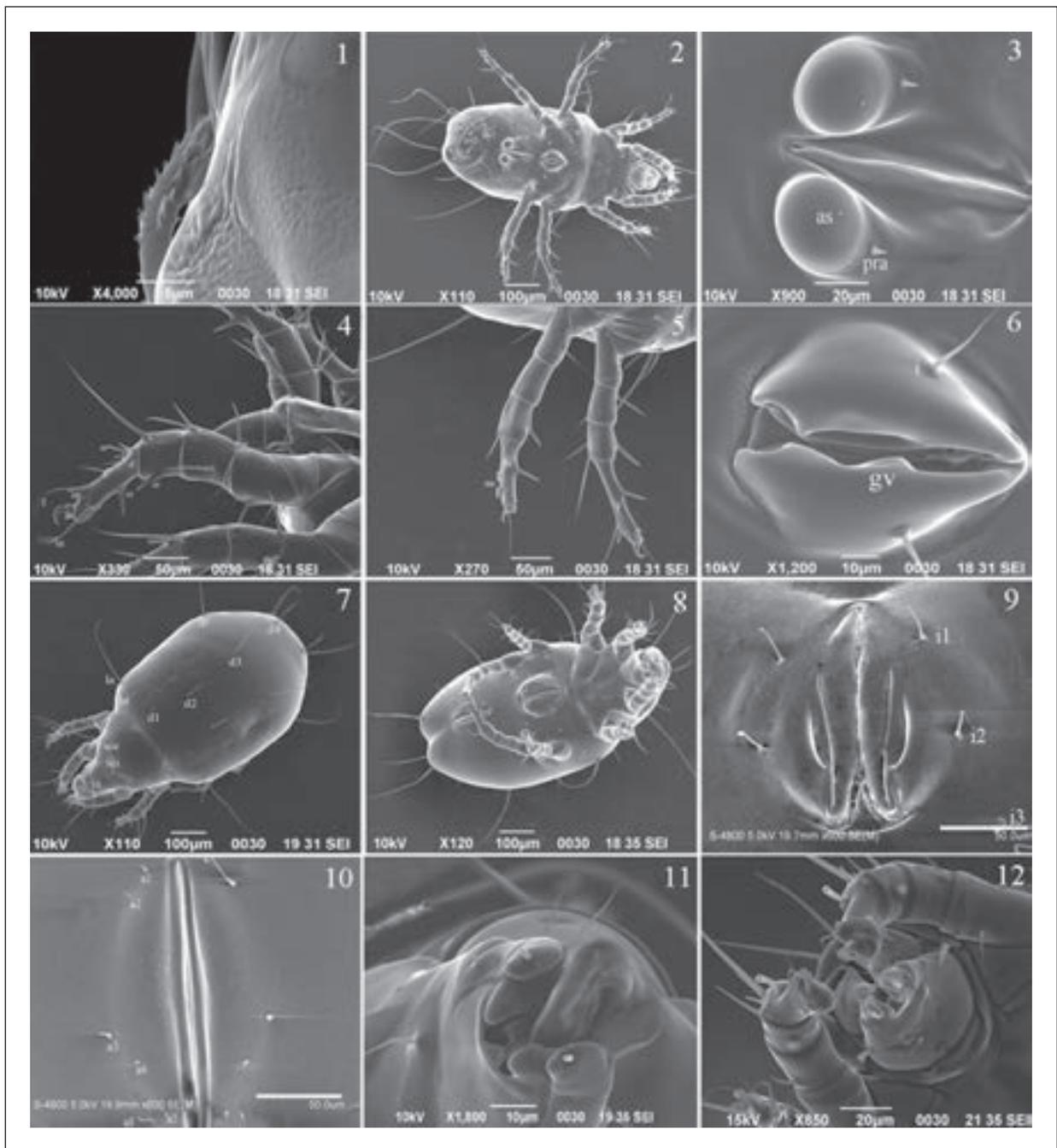


Fig. 2.— SEM observation on external morphology of *Sancassania berlesei* (Acari: Astigmata: Acaridae) in various stages .1: Grandjean's organ; 2: Ventral view of the adult male; 3: Anal area of the adult male; 4: Flank view of the 1st and 2nd appendix masculina of adult male; 5: Flank view of the 3rd and 4th appendix masculina of adult male; 6: Genital area of adult male; 7: Dorsal view of the adult female; 8: Ventral view of the adult female; 9: Genital area of the adult female; 10: Anal area of the adult female; 11: Chelicera of the adult female; 12: Gnathosoma of the adult female; as: Anal sucker; pra: Preanal seta; aa: Ambulacra; gv: Genital valve.

logy of this species in combination with previous light microscopic findings. Live observation of *S. berlesei* in its life cycle by SEM suggested that: 1) the legs varied from three pairs absent of leaf-like setae (larvae) to four pairs in protonymph stage, and by the deutonymph stage, the four pairs of legs, claws and tarsules became well-developed, with occurring of leaf-like setae, setae of tibia and setae of genu; 2) the setae in typical larva

experienced morphologically from occurrence of three pairs of dorsal setae (absence of d4) and posterolateral setae (lp) to arising of four pairs of dorsal setae, d4 and posterolateral setae (lp) in protonymph stage, and dorsal terminal seta, cup-shaped sucker and tarsus IV with serrated edge in deutonymph stage. The setae were further developed and extended in adult; and 3) the morphological change for the external genitalia and its acces-

sories were involved in immature external genital organ and only one pair of anal seta in larva stage, occurring of genital sense organ, genital seta and one pair of setae of post-anal and pre-anal in protonymph, typically ossific genital plate with one pair of suckers and one pair of setae bilaterally surrounding the gonopore in deutonymph, and the genital sense organ became more ossific and heart-shaped towards adult stage. As for the number of setae on the genital sense organ, the adult male differs from the female counterpart. The former has only one pair of setae, whereas the latter bears three pairs of genital setae. Under the SEM, the morphological aspects of *S. berlesei* were exposed completely and straightforward, exhibiting itself with subtle color (especially the viridescently fluorescent ω_1), shining appearance and characterization, suggesting that this is an effective approach to identification of the morphology of this mite species. In addition, our definition on the number of suckers and the ultra-structure in hypopus are inconsistent with the previous studies¹², and this discrepancy remains further determined.

In summary, we have successfully observed the morphological changes and described the characteristics of *S. berlesei* among its diverse life cycle. This will undoubtedly supply the concerned information for the classification and further study of *S. berlesei* as well as basis for controlling of this pest. Regrettably, we failed to identify the penis of the male and copulatory pouch for the female except for the genital valves in adult male seen under the SEM. The likely explanation for this result may be that the copulatory organ is instinctively protected; only probably displaying itself when mating. Accordingly, necessary investigation on its physiological process still remains.

Competing interests

The authors declare that they have no competing interests.

Statement

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Authors' contributions

Wei Guo identified the specimens, performed them for electron and light microscopy and carried out the SEM observations, organized the photo gallery and drafted the manuscript. Chaopin Li conceived the study, collected and identified the samples, and helped to draft the manuscript. Yuxin Jiang participated in the design of the study, collected and identified the samples, and Lianping He reviewed the manuscript. All authors read and approved the final version of the manuscript.

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