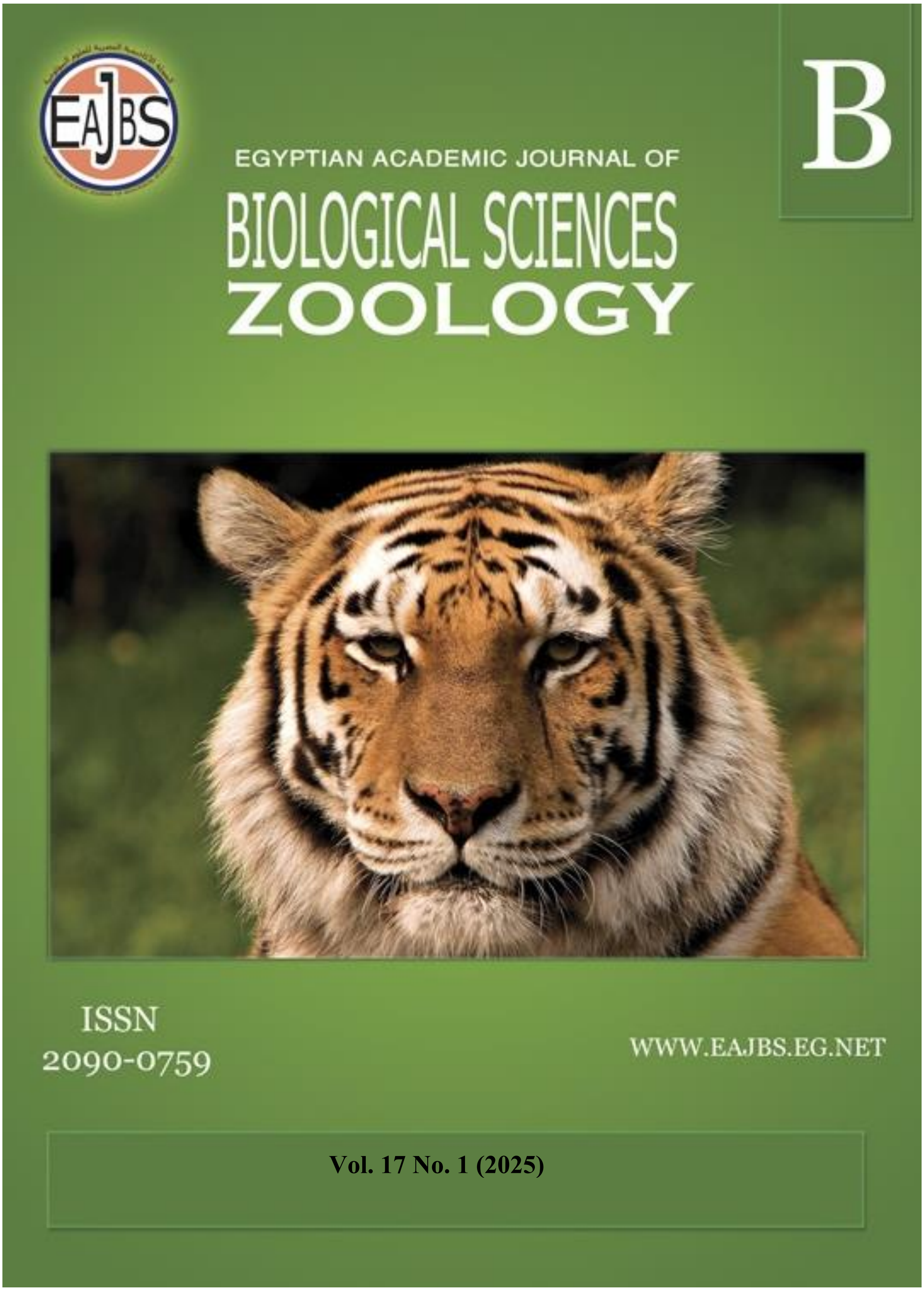

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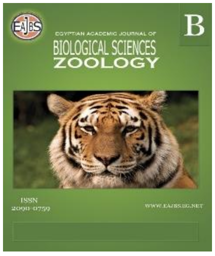

Microstructure of the Attaching Device of *Sitophilus oryzae* L. (Curculionidae: Coleoptera)

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ABSTRACT

Many insect species are supplied with specific attachment devices, with different morphological features according to the species and the function of the attachment device. It is observed that the rice weevil, *Sitophilus oryzae* L. efficiently climb on different types of substrates including vertical smooth surfaces. This weevil has strong attachment force regardless of the type of the substrate roughness. This probably results from the specific complex attachment device found on the tarsi. To understand the extraordinary ability of this weevil, scanning electron microscopy was conducted on the tarsai of the proleg, mesoleg and metaleg to explore the micro-structure of its attachment system that enable the weevil to move effectively even upward on not only the rough surfaces, but also on the smooth surfaces as well. The findings of this research reveal that *Sitophilus oryzae* weevils attach to rough surfaces using their strong claws. While, they attach to smooth surfaces by means of complex attachment system composed mainly of two groups of fringed attachment pads. These fringed attachment pads consist of tufts of setae projected forwardly with various sizes distributed at the central region of the pad._The attachment system used by *Sitophilus oryzae* was revealed through study of the tarsal surface using the scanning electron microscopy. This study may provide more declaration of this weevil behaviour and may be used as inspiration model to develop promising efficient climbing robots.

INTRODUCTION

Insects can survive in a diversity of ecosystems because of their capability to move and attach to different kinds of surfaces with the help of claws and hairy attachment systems on their legs. Substrate attachment is important for insect to occupy the niche with a variety of nature from rigid to soft surfaces. Consequently, diverse structures which enhance attachment are found among insects (Haas & Gorb 2004, Schnee *et al.*, 2019).

In particular, many species of insects possess diverse tarsal attachment systems optimized for attachment to a variety of natural substrates. These devices have specific shapes based on their function, so that they are characteristic of the species (Klann *et al.*, 2021). The legs of stored product pests play a role in the selection process of habitat for feeding and oviposition (Seada& Hamza, 2018).

The attachment systems recently attract research from the engineering and physical

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sciences as they may represent encouraging models for novel applications (Gorb, 2008). Though, several studies focused on the attachment of animals to rigid substrates, so far, there is a lack of detailed studies on attachment ability to soft surfaces. Understanding of the structures that help natural climbers may inspire the development of climbing robots and many relevant applications.

The idea of bio-inspired robots is to mimic nature, principally in the morphology and locomotion of living organisms; insects represent a source of inspiration. For example, the dung beetle used as a model due to its multifunctional capabilities; this insect uses its legs to walk fast and roll a ball in the same time (Thor et al., 2018). These robots may be could be used in several applications and areas, like the rescue and military operations (Wang *et al.*, 2018).

Among the stored-product insects *Sitophilus oryzae* L. (Curculionidae: Coleoptera) can be considered a major stored products pest that cause serious damages to several plant products during storage (Campbell 2012, Omar 2012). The adult weevil feed on corn, rice, barley, wheat, and other grains. Adults of *Sitophilus oryzae* are great climbers not only on the rough surfaces, but also on the smooth ones (Cline & Highland, 1976). However, the attachment system of this weevil is still not sufficiently studied and understood. To fill this gap, this research aimed to study the fine structure of the tarsai of this weevil using scanning electron microscopy.

MATERIALS AND METHODS

The Wheat Grains:

The wheat grains were stored for three weeks at -4 °C to eliminate any stages of weevil that may be found in the kernels.

Insects:

The adult insects of *Sitophilus oryzae* were placed into transparent plastic vials containing undamaged and disinfested wheat grains and covered with nylon mesh for proper ventilation; the population was maintained on wheat grains under ambient laboratory conditions in the dark and room temperature simulating stored seeds in warehouses. Living adult individuals of *Sitophilus oryzae* were put in plastic and glass vials to observe the climbing performance on smooth surfaces, and the contact area between legs and the substrate.

Scanning Electron Microscopy:

Each weevil was taken one by one, and its six appendages were gently removed with tweezers and dissecting scissors. The specimens were fixed in glutaraldehyde and then they were dehydrated in a graded ethanol series. Specimens were allowed to dry under room conditions. The legs were mounted on aluminum stubs by a double-sided sticky tape. Thereafter, the specimens were covered with 20 nm coating of 200 A° gold using a splutter coater. Finally, specimens were explored using JEOL JSM-5200 scanning electron microscope and micrographs were taken. The scanning electron microscopy was conducted at the Applied Center for Entomonematodes, Department of Zoology and Agricultural Nematology, Faculty of Agriculture, Cairo University.

RESULTS

General Morphology of the Leg:

The leg of *Sitophilus oryzae* consist of five segments; coxa, trochanter, femur, tibia and tarsi. The trochanter is reduced, subtriangular and articulated with the femur which is laterally flattened. Tibia is elongated and slender with sharp uncus arising apically on the external side. The tarsi consist of five articulated tarsomeres, the formula of tarsai is 5, 5, 5. They appear to be only four (pseudotetramerous) because tarsomere IV is minute and concealed between the lobes at the apex of tarsomere III (Fig.1). The first and the fifth tarsomeres are longer than the rest (Fig. 2B). Each leg carries a pair of strong claws on the end of the fifth tarsomere (Fig. 1), no pulvilli or arolium adhesion devices were observed (Fig. 1, 6).

Attachment Device:

The attachment device of *Sitophilus oryzae* consist of five components, all the three pairs of legs are equipped with the same attachment system. The first component of the attachment device is a strong uncus, which is an apical tooth at the side of tibial tip (Fig 2A, B), the uncus is found on all the legs. The second component of the attachment device is the two fringed attachment pads on the two lobes at the distal end of tarsomere III (Fig 3A, B), each pad carry a tuft of setae concentrated in the center of the pad and projecting forward to face the substrate (Fig 3C), helping the weevil to adhere to the surface of the substrate, the setae may have pointed or spinose tips (Fig.3C). The third component of the attachment device is two strong bristles on the lateral sides of tarsomeres I, II, III (Fig. 4), it is suggested that they act to enhance the attachment and to interlock with the substrate surface. The fourth component of the attachment device is two spines detected in the distal part of tarsomere V in all legs, the tip of spines are spinose (Fig 5), these spines are thought to enhance the attachment to smooth surfaces. The fifth component of the attachment device is the strong claws, the equal size claws are slender, elongated and apically pointed and curved to be adapted to walk on rough surfaces (Fig 6).

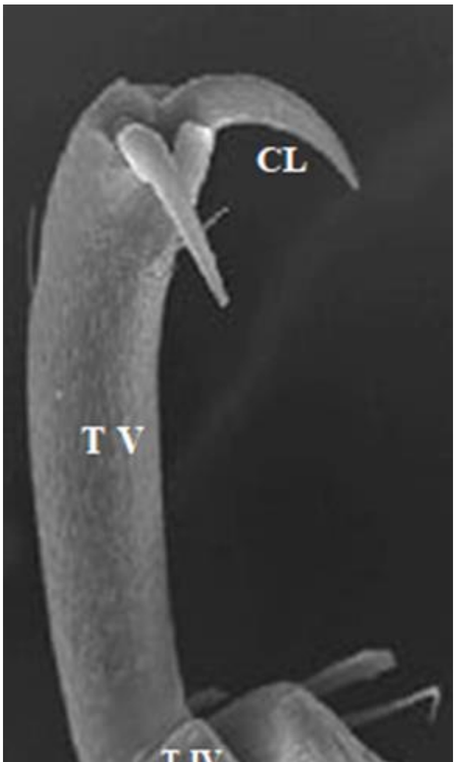

Fig. 1. (A) Scanning electron micrograph of tarsomeres IV concealed between the lobes tarsomere III of mesoleg of *Sitophilus oryzae* adult 1000x. CL, claw; T III, tarsomere III; T V, tarsomere V.

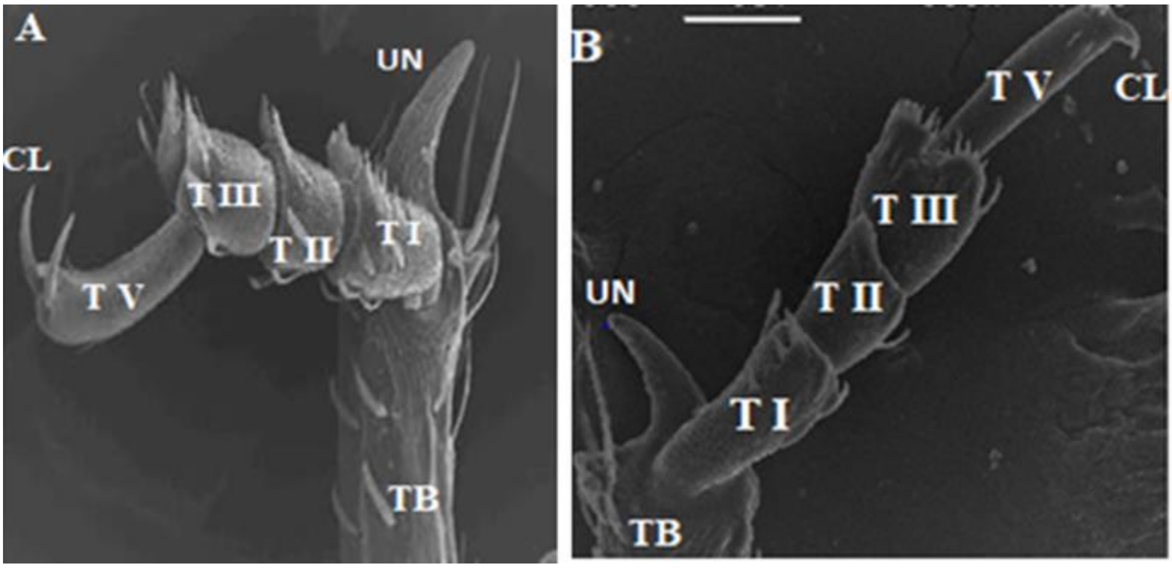

Fig. 2. (A) Scanning electron micrograph of latero-ventral view of metaleg of *Sitophilus oryzae* adult showing the general morphology of the leg 200x. (B) Ventral view of metaleg of *Sitophilus oryzae* adult 200x. CL, claw; T, tarsi; TB, tibia; T I, tarsomere I; T II, tarsomere II; T III, tarsomere III; T IV, tarsomere IV; T V, tarsomere V; UN, uncus.

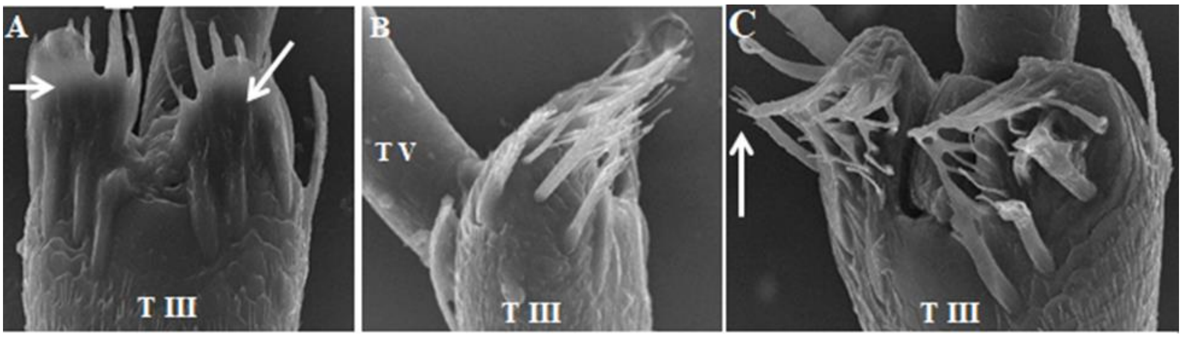

Fig. 3. (A) Scanning electron micrograph of ventral view of proleg of *Sitophilus oryzae* adult the attachment pads on tip of tarsomere III 1000x as indicated by arrows. (B) Lateral view of the attachment pads on tarsomere III of the proleg 750x. (C) Top lateral view of the attachment pads on tarsomere III of the mesoleg 1000x showing the setae may have pointed or spinose tips as indicated by arrow. T III, tarsomere III; T V, tarsomere V.

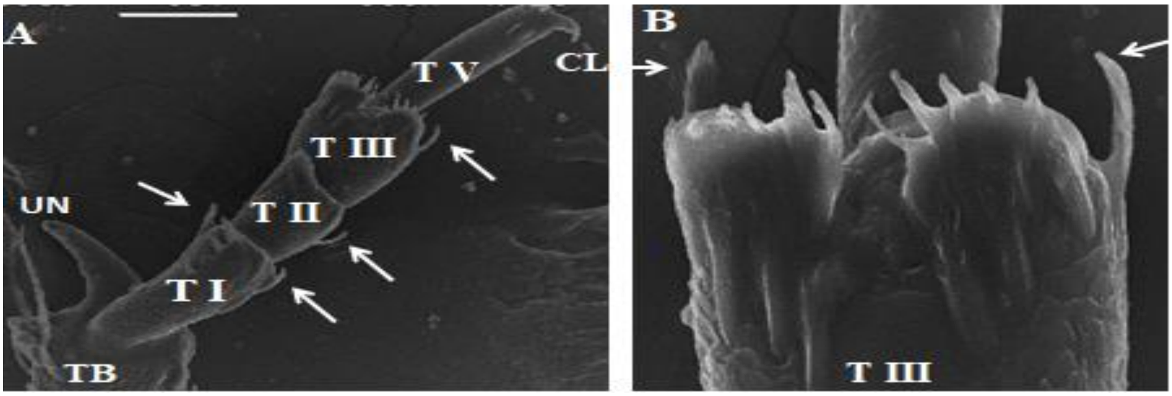

Fig. 4. (A) Scanning electron micrograph of ventral view of metaleg of *Sitophilus oryzae* adult 200x. (B) Tip of tarsomere III of metaleg 1000x. Arrows indicate the strong lateral bristles. CL, claw; TB, tibia; T I, tarsomere I; T II, tarsomere II; T III, tarsomere III; T V, tarsomere V; UN, uncus.

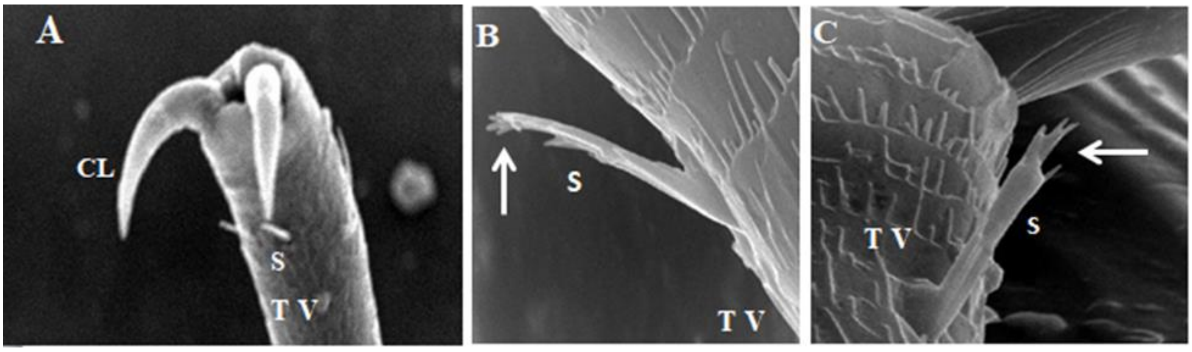

Fig. 5. (A) Scanning electron micrograph of distal part of tarsomere V of mesoleg of *Sitophilus oryzae* adult 750x. (B) Magnification of spine on the distal part of tarsomere V of proleg 3.500 x. (C) Magnification of spine on distal part of tarsomere V of metaleg 3.500 x showing spinose nature of the setae as indicated by arrows. CL, claw; S, spine; T V, tarsomere V.

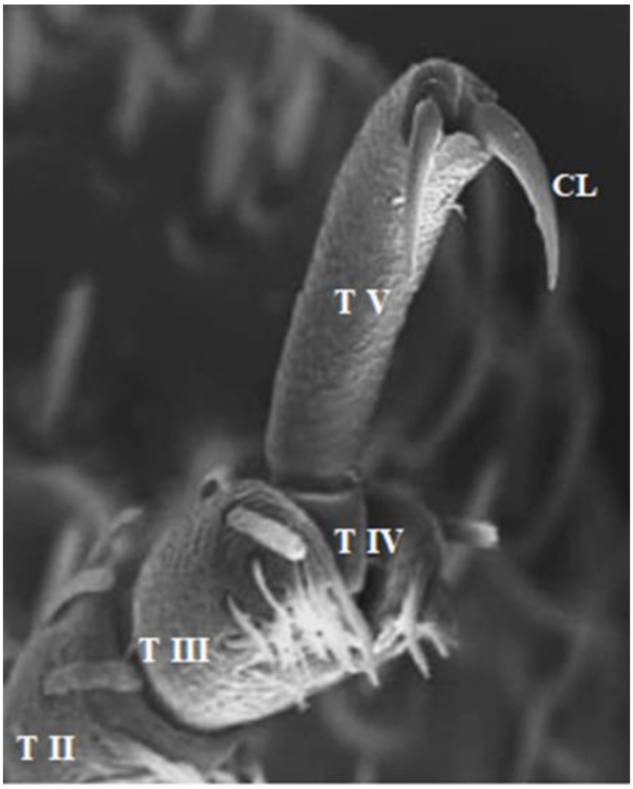

Fig. 6. Scanning electron micrograph of tarsi of mesoleg of *Sitophilus oryzae* adult 350x. CL, claw; T II, tarsomere II; T III, tarsomere III; T IV, tarsomere IV; T V, tarsomere V.

DISCUSSION

Attachment devices are functional systems used for temporary or permanent attachment of an insect to the substrates. Their morphology depends on biological function of the device (Gorb, 2008). Several insects bear attachment devices that enable strong attachment with different types of substrates (Federle, 2006). Attachment of insects to substrate plays an important role in the niche occupation and the selection process for feeding and oviposition (Seada& Hamza, 2018). Hairy pads or smooth pads are principally used on smooth surfaces, while claws are used to attach to rough substrates (Beutel & Gorb 2001). Claws are composed of chitinous sclerotised material that enhances stiffness of them (Salerno *et al.*, 2023). While, the elastic protein resilin causes elasticity and softness of attachment pads. Tarsal movements associated with attachment and detachment is recorded in many insects like flies (Moon *et al.*, 2012).

The success of attachment of insects to substrates depends on several factors such as the substrate irregularities and the attachment structures on the insect legs, such as claws and the adhesive pads or structures (Gorb 2008).

Adult of *Sitophilus oryzae* move efficiently on smooth surfaces (Gvozdenac *et al.* 2020). To achieve this extraordinary attachment to vertical surfaces, they use a special attachment system on their tarsal appendages. The leg morphology of *Sitophilus oryzae* was reported previously, but not based on Scanning microscopy and without a detailed description of the tarsal morphology that may explain the great climbing ability of this weevil. So, in this study tarsal attachment system on the legs was explored using scanning electron microscopy to explore the functional attachment device behind the efficient steadily climbing ability of *Sitophilus oryzae* on smooth and rough vertical surfaces. The tarsai were the focus of the study, because they were observed to be the contact position between the weevil and the substrate. According to our observations of the scanning electron micrographs, it was revealed that the attachment device of *Sitophilus oryzae* consist of five components similar on the three pairs of legs; a pair of strong lateral uncus at the tip of the tibia, a pair of attachment pads on the ventral surface of bilobed tip of tarsomere III with a tuft of attachment setae on each pad, two strong lateral bristles at the outer sides of the attachment pads, two spinose spines at ventral surface of the distal part of tarsomere V, and two strong apical curved claws at the tip of tarsomere V. It is suggested that the attachment to rough surfaces depends mainly on claws and uncus due to their hook-like structures that help the weevil to cling during moving on rough surfaces, with the help of the other attachment components. The claws are considered as the morphological adaptation to move on rough surfaces depending on the size and the claw tip diameter as reported by Dai *et al.*, 2002 While, it is suggested that the attachment to smooth surfaces depends mainly on the hairy attachment pads on the tarsi (Bullock & Federle, 2011; Vendl *et al.*, 2019), the lateral setae and the spines at the distal part of tarsomere V. No pullvilli or arulium were noticed, so, the fringed nature of the attachment setae and their projection forward to face the substrate surface play the major role in attachment to the smooth substrates.

To our knowledge, this is the first time the detailed structure of the attachment of *Sitophilus oryzae* is revealed. Such a study could help in further understanding of attachment mechanism to smooth surface and may become an inspiration for applications such as a model for developing bio-inspired climbing robots.

Conclusions

The findings of this study reveal details of the attachment system used by *Sitophilus oryzae* enabling efficient movement on both rough and smooth surfaces. This study provides the essential background for more understanding of the mechanism of attaching of this economically important weevil. The deep understanding in turn, forms a base for future studies on the behavior of this weevil and in other areas of applications such as developing efficient climbing robots.

Declarations:

Ethical Approval: This research was approved by ethics committee of Faculty of Science, Ain Shams University (SCI/ENTO/2023/9/4).

Competing interests: The author states that there are no competing interests to declare.

Author's Contributions: RA analyzed and interpreted the scanning micrographs and wrote the manuscript.

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Availability of Data and Materials: Not applicable.

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