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Morphology and Distribution of Thermo- Hygro-Ir- and Chemo-Receptors for Females of Family Diaspididae (Hemiptera: Sternorrhyncha)

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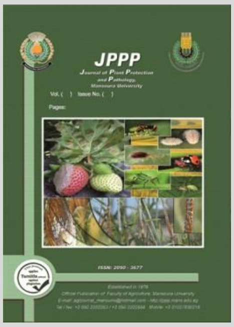

ABSTRACT

The comparative study of the female body structures of many species of Diaspididae has been done using Leica microsystems. Several types of sensilla were described for three species namely: *Parlatoria zizyphii*, *Lepidosaphes ficus* and *Lepidosaphes beckii*. Four thermo-hygro-sensory species as sensing organs for temperature and humidity, namely plachodea in *lepidosaphes beckii*, sensilla basiconica and styloconica in *Parlatoria zizyphii*, sensilla caeloconica in *lepidosaphes ficus* and tuft organ in *lepidosaphes ficus*; One type of gustatory receptor is a styloconica in *Parlatoria zizyphii*; infrared receptors (IR), also in *Parlatoria zizyphii*; olfactory organs such as sensilla placodea in *Parlatoria zizyphii.* Measurements of sense organs in different species were done using ocular micrometer. Also, dimension measurements of each sensillum of temporary and permanent females mounts were done. According to these comparative studies, the general regulation of sensilla was assumed to be Diaspididae. Eight sensory phenotypes were identified, with an attempt to use these data at the systemic level. The results of this study provide an important basis for linking morphological characteristics of sensory organs to insect behavior and should stimulate the development of effective quasi-chemistry-based control strategies against species belonging to the diaspididae.

Keywords: Diaspididae , IR, Thermo-hygro-receptors, gustatory receptors, olfactory receptors.

INTRODUCTION

Insects are particularly interesting models of flexible sensory control. Although early descriptions often depicted insects as simple robots, "depriving them of any part of intelligence", careful observation since then has revealed that insects' locomotor patterns are, in fact, remarkably adaptable (Kirby and Spence,1822) .

Hemipterans, distinguished by their piercing, sucking mouthparts, constitute one of the most diverse and economically important insect orders (Weintraub and Beanland, 2006). Within this group, Sternorrhyncha (aphids, psyllids, scale insects, and whiteflies) play a very important ecological and economic role as plant pests and virus vectors (Eastop, 1977, Hühnlein *et al.*, 2016).

Insects regulate their body temperatures through a process known as thermoregulation. Historically, insects have been categorized as thermophiles (animals whose body temperatures vary and depend on their environment) rather than isotherms (animals that maintain a constant internal body temperature regardless of external influences). The ability of insects and other animals to maintain a consistent temperature (either above or below ambient temperature), at least in portion of their bodies, by physiological or behavioural mechanisms is known as temperature regulation, or thermoregulation (Heinrich and Bernd, 1993). Some insects are endotherms (animals that can produce heat internally by biochemical processes), whereas others are ectotherms (animals whose primary source of heat is from their environment). Because they are not all uniformly endothermic, these endothermic insects are best classified as heterothermic. When heat is produced, different body sections maintain distinct body temperatures. For instance, before taking flight, mites develop heat in their thorax, but their abdomen is kept relatively chilly(Heinrich and Bernd, 1981).

Insect survival is mostly impacted by moisture because it changes their water content. It might not be hazardous to be exposed to dry or wet circumstances if humidity can be kept within specific bounds. By looking for an appropriate environment, insects are able to maintain a constant water balance. Experiments in numerous species have shown that responses to moisture selection depend on the presence of future sensitivities to moisture. This sensory is visible from the outside as tiny ice wedges that protrude from the antennae's surface or are set into crevices. It has two different kinds of moisture receptor cells that react to variations in humidity in opposing ways. Dry air causes a drop in the discharge rate of one type whereas wet air increases it. They were referred to as "wet" cells. The second type of cells, referred to as "dry" cells, have a higher discharge rate in dry air and a lower discharge rate in moist air. The "cold" heat-receptive cell type and both types of moisture-receptive cells coexist in the same way (Altner *et al*.,1983; Altner and Loftus,1985; Steinbrecht, 1999; Tichy and Loftus, 1996; Yokohari, 1999; Tichy and Gingl, 2001).

Different substances in environment can be detected by insects. Olfactory receptors may detect these substances as scents (smells) when they are present in gaseous form (in relatively low concentrations). They are regarded as tastes by gustatory receptors when they are present in solid or liquid form (often at higher concentrations). While the sense of smell typically entails the detection of substances in gaseous or airborne form, the sense of taste typically entails

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direct contact with the substrate (contact chemoreceptor) (remote chemoreceptor). (Agriculture and Life Sciences at NC State) https://genent.cals.ncsu.edu/

The exoskeleton, endogenous morphology and anatomy, and biological and behavioural aspects of insect reproductive biology are among the characteristics of adult males and females in diaspidids that have been discussed in various publications (Bustshik, 1958; Ghauri, 1962; Borchsenius, 1965; Borchsenius, 1966; El-Minshawy and Osman, 1973; Davidson and Miller, 1977; Nada and Mohammad, 1984; Rosen, 1990; Hamdy, 2020). However, little is known about the sensations experienced by female diaspidids, despite the fact that male diaspidids' senses are involved in sexual activity and female diaspidids are drawn to them for mating. Therefore, according to Procelli (1995), claimed that in order to receive the male emasculation deity and complete the insemination process, female bodies are supposed to have sensitive organs.

The aim of this study is to examine and define the different types of thermo- hygro-IR- and Chemo sense organs on the different parts of *Parlatoria zizyphii* , *Lepidosaphes ficus, Lepidosaphes beckii* bodies.

MATERIALS AND METHODES

a. Mounting slides for adult females for identification procedure

The methods used to distinguish between the several species of diaspidids depend on the adult females' taxonomic traits. Adult female materials were prepared by both temporary and permanent mountain slides. For preparing temporary mounts, the scale covers of females were removed using a small needle, then the female soft body was placed on glass slide that having droplets of Hoyer media, then covered with a glass lid, and then being dried on a hot plate for a few hours. Adult females were first boiled in 10% caustic potash solution in an aqueous solution for 15–30 minutes, then overnight in chloral phenol (1:1 chloral hydrate:phenol), before the material was stained with 1%. Basic fuchsine, washed in and dried in ascending chain ethyl alcohol, Carbolexylene cleaning, Canda Balsam fixation on a glass slide, and 40°C oven drying follow.

b. Leica Photomicroscope

This study made used theof optical microscopy from Leica Microsystems. With a Leica microscopy camera, which boasts quick reaction times, excellent pixel resolution, and sharp contrast, it can analyzse multidimensional dynamic processes in living cells. https://www.leicamicrosystems.com/products/light-microscopes.

With a 100x magnification, the various sensory organs of the researched insects were completely clear (high resolution), allowing for easy description, measurement, and identification of their sensory functional roles.

a. Morphometric sensilla

An ocular micrometre was used to measure the sensilla of various species. Additionally, measurements of each species were taken from the females of both temporary and permanent mounts.

RESULTS AND DISCUSSIONS

The first sensory organs or foci on the female armoured scales were the prosoma, which symbolises the merger of the head region, and the postoma, which symbolises the connection of the end of the thorax and belly. The morphological properties of female armoured scales were studied at a high magnification level of 100-fold. There were found to be clusters of organelles and structures that resembled other insects' sense organs. The functional roles of these organs in past studies have not been elucidated, and they have not been explained by an unexpected repeat interpretation. Microscopy revealed that mechanical and chemical sensory organs were a set of foci and organs that resembled the sensory organs found in other insect species.

These insects, which are members of the Diaspididae, have a set of sensilla that have been identified; these sensilla have been enumerated in various parts of the insects' bodies and linked to sensilla in other insect species based on their location, related morphological traits, and determination of their functional significance.

a. Plachodea without pore as thermohygro receptor and mechanoreceptors

They were found in similarity in the prominent side lobes of Lepidosaphes beckii in the form of transparent circular discs as thermohygro receptors and mechanoreceptors , surrounded by a group of conical sensory bristles as the type of trichodea , these sensilla feel the environment surrounding the insect body as movement of wind or predators and sensation of heat and humidity (Figure 1) .They are with dimensions of 0.093 ± 0.031 µm dimeter (Table 1).

The presence of a pair of cells of the sensillum may give it a dual function. One of the two sensory cells may act as a mechanical receptor, and the other neuron acts as a receptor for heat and moisture, as shown in Figure1, where by tracking the sensory organ, it was found a pair of nerve extensions that lead to two neurons.

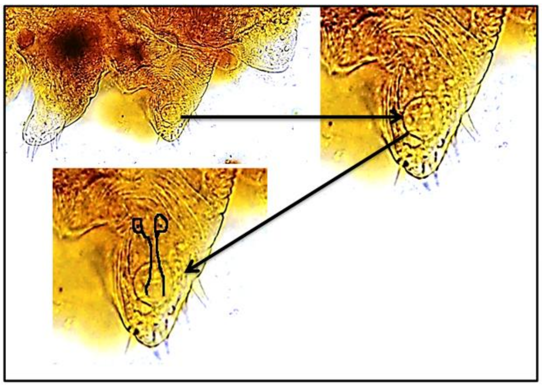

Fig. 1. Plachodea as thermo-hygroreceptors sensilla in *Lepidosaphes beckii*

b. Sensilla Basiconica as thermohygroreceptors

Microscopic examination of the bodies of the female insect *P. zizyphii*, revealed that a pair of sensory organ have cone shape with flattened top of type sensilla basiconica, with dimensions of 0. 049 ± 0.02 µm width and 0.053 ± 0.011 µm length (Table 1). Each of them is located on both sides of the outlet of the styloid of the mouth parts and near the base of the tentorium and located 14.5±0.46 µm away from it (figure 2). It looks like a sensory organ near the mouth parts as shown in

Figure 8. It is possible that it senses the temperature and humidity of the atmosphere and the juice in the leaf of the plant. (Nabil *et al*., 2019) and (Abd-Rabou and Farag, 2014) studied on seasonal fluctuations of *P. ziziphi* who concluded that a directly proportional was found between temperature and the predicted population of *P. ziziphi* ,which could provide a valuable tool in monitoring, managing and controlling pests spread in the coming future .

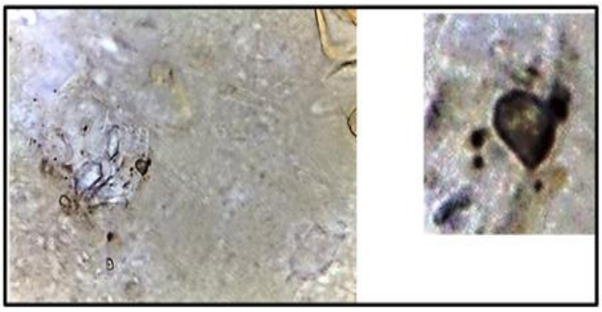

Fig. 2. Sensilla basiconica as Thermo-hygroreceptors sensilla in *Parlatoria zizyphii*

c. Styloconica as thermohygroreceptors and gustatory receptors

During tracing the sensory organs in *P. zizyphii* as in (Figure 3,a), it is found on the edges on both sides of the prosoma and the bottom of the atrial appendage on both sides four sensory organs combined at a distance of 13 µm away from the base of mouth parts and they are all of the styloconica type. The dimensions of one organ were 0.033 ± 0.031 µm in length and 0.064 ± 0.011 µm in width (Table 1).

Here the member of S. styloconica in *P. zizyphi*, as shown in (Figure 3,b), appeared in the form of ten domes with a single transverse opening in the shape of a crescent in the space between the bases of the styloid mandible or the arms of the tentorium. The dimensions of one of them are 0.29 ± 0.12 µm in width and 0.09 ± 0.02 µm in length .This organs are used by the insect to taste the plant juice before feeding (Table 1).

In addition, next to the first pair of stomata on both sides of the mouthparts, four members of S. styloconica are found in the form of domes with one round orifice (Figure 3,c). The dimension of the organ was 0.038 ± 0.013 µm diameter (Table 1 and Figure3). These observed pores were not mentioned in previous researches as one of the sense organs, but they were identified as they responsible for the waxy secretion of the waxy cover (Takagi, 1990). However, it is not possible for a waxy secretion to occur in the path of the respiratory stomata, where it blocks the stomata and it is more precisely that its role as a sensory organ responsible for taste, especially which the openings responsible for the waxy secretion are on both surfaces of female bodies. This finding needs more studies to prove it.

d. Sensilla Caeloconica as thermohygroreceptors

Devices made of Sensillum coeloconica have the ability to absorb heat and moisture. The cuticular organ of sensation is a poreless, mushroom-shaped protrusion that is concentrically positioned within a shallow cuticular depression. The edge can have pores. Three or four receptor cells are possible. Unbranched sensory cilia on three receptor cells are filled with tightly packed microtubules that extend deep into the dermal apparatus and entirely fill its lumen. One of the three ramifications is "wet," another is "dry," and the third is "temperature." (Coeloconic literally translates as "peg in a hole"). Beekeeping research. And this is what we discover, which is consistent with Lepidosaphes ficus: at the end of the prosoma region on both sides, four sensory organs of the type Caeloconica S. were discovered, as shown in Figure 4, as it was thought that they play a part in determining the hibernation period by sensing the low temperatures in the surrounding environment, as it occurs in hibernation of species that belong to the genus Lepidosaphes. In their 1995 research of the Lepidosaphes ficus life cycle, Carrillo *et al*., 1995 discovered that this species was monovoltine, with hibernation taking place in the egg stage. The sensory organ has dimensions of 0.063 0.031 m and 0.044 0.011 m (Table 1). The thermo-hygroreception sense nerve. They are rigid, peg-like structures that are encased in the cuticle's hollow. All members of the Corixidae family have these sensilla, according to Agnieszka *et al*. (2020), but only one of them has a pore at the tip. They also have a nonporous surface.

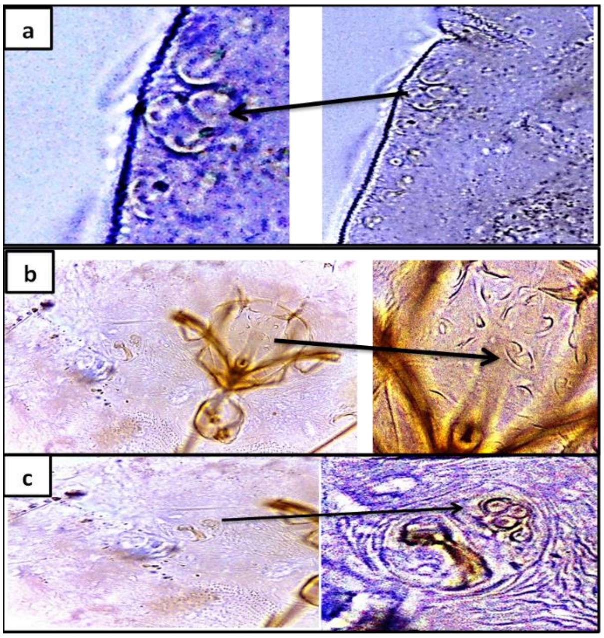

Fig. 3. Different sensilla styloconica as thermo-hygroreceptors sensilla in *Parlatoria zizyphii* a, on the edges of both sides of the prosoma; b, between the bases of the styloid mandible; c, both sides of the mouthparts as gustatory receptors .

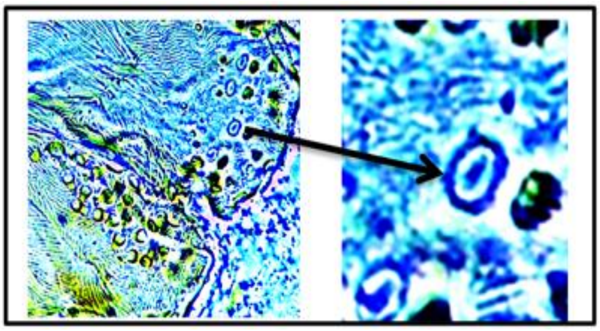

Fig. 4. Sensilla caeloconica thermo-hygroreceptors sensilla in *Lepidosaphes ficus*

e. Tuft organ as thermohygroreceptors

A pair of tuft members at the end of the pygidium region is found at both sides in *L. ficus* as shown in Figure 5 where three cavities are found, inside each of them a pair of finger appendages on both sides, next to the second lobe at the end of the pygidium outward the position of the horns. The anal area of ordinary insects, as for its role, is to sense the heat and humidity of the female at the posterior region, where the place of laying eggs to feel the appropriate temperature where the female expects to laid eggs. These organs are very similar to the Tuft organs at the end of the antenna of the human louse *Pediculus humanus*. Insaurralde *et al.* (2019) identified Two tuft organs, one located at the dorso-lateral side of F2 and the other at the dorso-lateral side of F3 .Each tuft organ consist of a deep and circular pit (3.54 ± 0.21 µm diameter) from which six pegs emerged and each with a mean length of 3.31 ± 0.18 µm

Similarly, the same six number of finger appendages were found in the current study at the end of the *L. ficus* and emerge from three deep oval cavities (Figure 5). The dimensions of the first cavity, which is the largest of them, are in-between the other two cavities (1.44 ± 0.21 µm in width and 2.56±0.42 µm in length). The second cavity on the left was 0.57 ± 0.11 µm in width and 1.76±0.35 µm length. The third one on the right is closed to second cavity in dimensions of 0.49 ± 0.12 µm in width and 1.46±0.32 µm in length (Table 1).

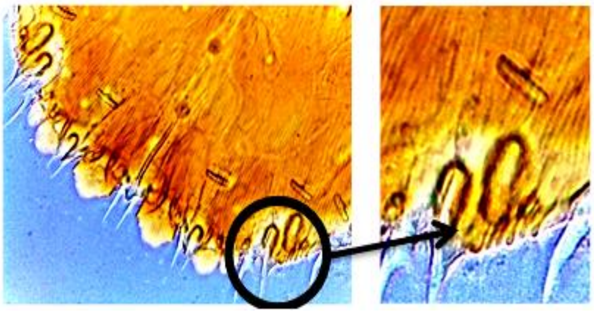

Fig .5. Tuft organs as Thermo-hygroreceptors sensilla in *Lepidosaphes ficus*

f. Infrared receptors (IR) as mechanoreceptors

According to technical definitions, thermal infrared (IR) detectors are sensors that identify incident IR by the resulting rise in temperature of a detector target. Many various techniques, such as changes in electrical conductivity, capacitance, and resistance, can be used to quantify temperature increases. Some species, like snakes and insects, utilise this detecting technique (Helmut *et al*., 2012).

Under the bases of the mouthparts on both sides of *P. zizyphii*, there are 10–12 rings. Infrared sensors are layered in large numbers on each ring, with counts between (25-35). It appears that females can sense the temperature of the ideal feeding locations and the ideal moment to lay eggs (Figure, 6). Mean dimensions of the sensory organ were 0.063 ± 0.021 µm in length and 0.041 ± 0.029 µm in width (Table 1).

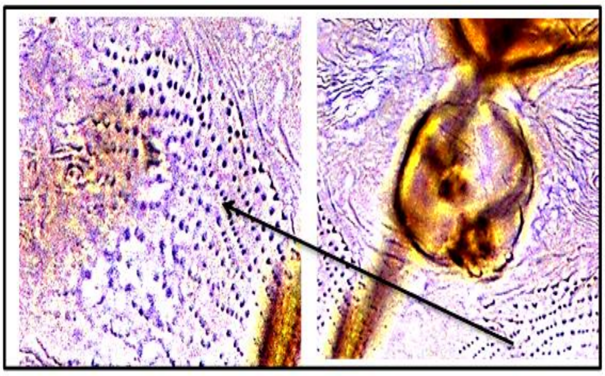

Fig .6. View into an IR sensilla of a *Parlatoria zizyphii* .

Table 1. Morphometry (length and width) of sense organs present on females bodies of some species belonging to diaspididae.

Species diaspid	Species sense organs	Name of sense organs	Length		Width/ Diameter	
			Range	Mean ± SE	Range	Mean ± SE
Parlatoria zizyphii	Thermo-hygro receptors	Basiconica	0.042- 0.064	0.053 ± 0.011	0.047- 0.05	0. 049 ± 0.02
	Thermo-hygro receptors	styloconica	0.002- 0.064	0.033 ± 0.031	0.053-0.075	0.064 ± 0.011
	Gustatory receptors	Styloconica1	0.07-0.11	0.09 ± 0.02	0.17-0.41	0.29 ± 0.12
		Styloconica2	------	-----	0.026-0.013	0.038 ± 0.013
	Infrared receptors	Infrared receptors	0.061-0.065	0.063 ± 0.021	0.39-0. 43	0.041 ± 0.029
	Olfactory organs	Sensilla placodea	------	-------	0.49-0. 53	0.51± 0.11
Lepidosaphes ficus	Thermo-hygro receptors	Tuft organ (cavity1)	2.13- 2.95	2.56± 0.42	1.23-1.64	1.44 ± 0.21
		Tuft organ (cavity2)	1.41- 1.76	1.76±0.35	0.46-0.68	0.57 ± 0.11
		Tuft organ (cavity3)	1.14- 1.78	1.46±0.32	0.37- 0.51	0.49 ± 0.12
	Thermo-hygro receptors	Caeloconica	0.34 – 0.92	0.063 ± 0.031	0.033- 0.055	0.044 ± 0.011
Lepidosaphes beckii	Thermo-hygro receptors	Plachodea			0.062-0.14	0.093 ± 0.031

SE, Standard error

It is consistent with the findings of Schmitz *et al.* (2007) who discovered that IR receptors are housed in extra-antennal sensory organs, which can be located on the thorax or on the abdomen, that infrared (IR) radiation is present below the mouth parts in *Parlatoria zizyphii. Melanophila acuminata*, a pyrophilous beetle, has infrared receptors and the sensory neurons that go along with them that are descended from mechanoreceptors.

It might be because these insects require the use of infrared (IR) radiation sensing to determine whether their environment is suitable for their biological tolerance. It differs with the findings of Schmitz *et al.* (2007) who claimed that fire-loving (pyrophilous) insects depend on forest fires for their reproduction since it has a chance to leave the unsuitable environment that it despises, aside from forgoing egg-laying. These insects approach active fires and swarm into the burned area right after a fire. These insects have specific smoke and infrared (IR) radiation sensors for long-range navigation toward a fire as well as for short-range orientation on a freshly burned region, whereas the antennae are home to the olfactory receptors for smoke. Iron biomineralization has also been observed in honeybees (*Apis mellifera*), which forms the basis for magnetoreception. showed earlier that superparamagnetic magnetite was present in the iron granules produced by honeybees, and they agreed with the theory that external magnetic fields might cause the superparamagnetic particles to expand or contract in a way that is specific to their orientation, transmitting the signal via the cytoskeleton (Hsu and Li, 1994).

Here, we find that armed scale insects may have (IR) to help them find the appropriate time for laying eggs, while in fire-loving insects, their guide is by infrared rays to reach them.

g. Sensilla placodea as olfactory receptors

Insect olfaction is the ability of chemical receptors to detect and identify volatile substances, which enables insects to forage for food, avoid predators, find mates (through pheromones), and choose homes for laying eggs. It is therefore the most significant feeling for insects (Carraher *et al.*, 2015). Insects should ideally time their most significant behaviours, which depends on their fragrance and when they smell Gadenne et al. (2016). For instance, many insect species depend on scent to find food sources and hunt for prey; this is true of the sensory organs of smell used by Diaspididae.

At the end bodies of *P. zizyphi* females and according to microscopic examination there were openings known as Perivulvular pores and Prevalvular pores. Their functional role has been described as the possibility of secreting a substance to prevent egg agglomeration during egg laying (Takagi,1990). On the other hand, according to its morphological shape, it is a sensory organ responsible for smelling as placodea. This organ consists of 4 groups around the genital opening (fig. 7). The two groups before the genital opening included 5-6 sensory organ and the two groups next to the genital opening include 9-11 sensory organ. The organ was a plate that includes 9-11 opening with mean dimension of the sensory organ of 0. 51 ± 0. 11 µm diameter.

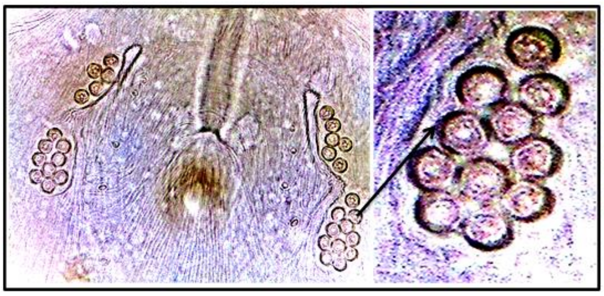

Fig .7. Sensilla placodea as olfactory sense organs in *Parlatoria zizyphi*

According to Onagbola *et al.* (2008); Li *et al.*, (2020); and Visser, (1986), Diaspididae may be able to accurately detect and recognize various odors from their environment, which influences insect behaviour such as host choice and egg-laying decisions. In addition, Pratibha and Renee (2017) suggested that the insect ovipositor is an olfactory responsive organ to volatiles and carbon dioxide in gaseous form. This phenomenon is demonstrated in parasitic wasps connected to *Ficus racemosa*

However, ovipositors have far fewer and smaller sensilla compared with antennae; this may explain an almost complete absence of studies on electrophysiology of ovipositors. The few conducted studies have been mostly recorded as gustatory sensilla (Rice, 1977; Crnjar *et al.*, 1989; Van Lenteren *et al.*, 2007; but see Klinner *et al.*, 2016).

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مورفولوجيا وتوزيع المستقبلات الحراره و الرطوبه و الأشعة تحت الحمراء والكيميائية لإناث عائلة Diaspididae (Hemiptera: Sternorrhyncha)

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الملخص

تمت دراسة بنية الجسم لإناث العديد من أنواع فصيله Diaspidiae باستخدام أنظمة لايكا الدقيقة. تم وصف عدة أنواع من الحسية لثلاثة أنواع هي *Parlatoria zizyphii* و *Lepidosaphes ficus* و *Lepidosaphes beckii*. حيث وجد اربعه أنواع من أجهزة استشعار الحرارة والرطوبة وهي Plachodea في *lepidosaphes beckii* وعضو Basiconica و عضو styloconica في *Parlatoria zizyphii* و عضو Caeloconica في *lepidosaphes ficus* وعضو Tuft organ في *lepidosaphes ficus* ؛ وجدنا نوع واحد من المستقبلات الذوقية هو styloconica في *Parlatoria zizyphii* ؛ وكذلك مستقبلات الأشعة تحت الحمراء (IR) في *Parlatoria zizyphii* ؛ و أعضاء حاسة الشم مثل Sensilla placodea في *Parlatoria zizyphii* ، أجريت قياسات للأعضاء الحسيه في الأنواع المختلفة باستخدام ميكرومتر العين. أيضا ، تم قياس أبعاد كل محس لجميع الإناث بعد اجراء تحميلات مؤقتة ودائمة للعينات. وفقا لهذه الدراسات تم تحديد ثمانية أنماط ظاهرية حسية ، مع محاولة استخدام هذه البيانات على المستوى التصنيفي.

توفر نتائج هذه الدراسة أساسا مهما لربط الخصائص المورفولوجية للأعضاء الحسية بسلوك الحشرات ، وينبغي أن تحفز تطوير استراتيجيات مكافحة فعالة شبه كيميائية ضد الأنواع التي تنتمي إلى الدياسبيدات.