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The Morphology of the Venom Apparatus and Histology of Venom Gland of *Pimpla turionellae* (L.) (Hym; Ichneumonidae) Females

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Abstract: The morphology of the parts of the venom apparatus and the histology of venom glands of the idiobiont solitary pupal endoparasitoid *Pimpla turionellae* (L.) were examined under microscope. There are two valvula 1, one valvula 2, two valvula 3, two valvifer 1, two valvifer 2, a Dufour's gland and a venom gland in the venom apparatus of *P. turionellae.* These structures were described and their characteristics were determined. Several venom gland tubules are attached to the reservoir at the top of the venom gland of *P. turionellae.* These tubules consist of spherical and oval cells of various sizes arranged around the lumen. The venom is poured into reservoir through the lumen. The reservoir part of the venom gland is quite wide and possesses a thin wall which consists of an irregular muscle layer, chitin and ectodermal cells that form chitin. More gland cells are present in the region of the venom gland opening into the tubules. The duct of the venom gland does not possess a muscle layer, and it is in the form of a canal supported by chitinous layers.

Key Words: Parasitoid, *Pimpla turionellae,* Venom gland, Morphology, Histology.

Pimpla turionellae (L.) (Hym; Ichneumonidae) Dişilerinin Zehir Aparatının Morfolojisi ve Zehir Bezinin Histolojisi

Özet: İdiobiont soliter pupal endoparazitoit olan *Pimpla turionellae* (L.)'nin zehir aparat kısımlarının morfolojisi ve zehir bezinin histolojisi mikroskobik olarak incelenmiştir. *P. turionellae*'nin zehir aparatında iki tane valvula 1, bir valvula 2, iki tane valvula 3, iki tane valvifer 1, iki tane valvifer 2, Dufour bezi ve zehir bezi bulunmaktadır. Bu yapıların şekilleri çizilmiş ve özellikleri verilmiştir. *P. turionellae* zehir bezinde çok sayıda salgı yapan tüpçükler hazneye üsten bağlanmıştır. Bu tüpçükler bir lümen etrafında sıralanan farklı büyüklükteki yuvarlak ve ovalimsi hücrelerden oluşmuştur. Zehir lümenle hazneye boşaltılır. Zehir bezinin hazne kısmı oldukça geniş olup çeperi düzensiz bir kas tabakası ile kitin ve kitin meydana getiren ekdodermal hücrelerden meydana gelmiştir. Zehir bezinin tüplere açılan bölgede daha fazla bez hücresi bulunur. Zehir bezinin taşıma kanalı ise kas tabakası bulundurmaz ve kitinli tabakalar tarafından güçlendirilmiş bir kanal şeklindedir.

Anahtar Sözcükler: Parazitoit, *Pimpla turionellae,* Zehir bezi, Morfoloji, Histoloji.

Introduction

The clinical events caused by certain hymenopterous species stinging their preys or hosts in order to make them passive, and by others stinging humans, have led scientists to examine the stinging and venom apparatuses of these insects. The first studies on this subject were done by Reaumur (1) and Swammerdam (2). Studies on the morphology of the venom apparatus in Hymenoptera are mainly concerned with the species of the genera *Apis* and *Vespa*, which are of greater economic and medical significance. The venom apparatus has a complex structure that varies not only among families but also among genera or even species (3-9). Compared with morphological studies, there are fewer on the ultrastructure of venom secretory cells and the histology of venom glands. The ultrastructure of the venom secreting gland cells of the venom glands have been studied on various Hymenopteran species (1, 10-13). It has been stated that their basic structures are similar, although there are some differences (12). Examining the venom glands of *Microbracon hebetor* and *Philanthus trigangulum* structurally and histochemically, Van Marle (14) proposed that there were structural and histochemical differences between the venom glands of these species.

As yet there have been no studies on the morphological properties of the apparatus parts and the histologic structure of venom gland of the pupal

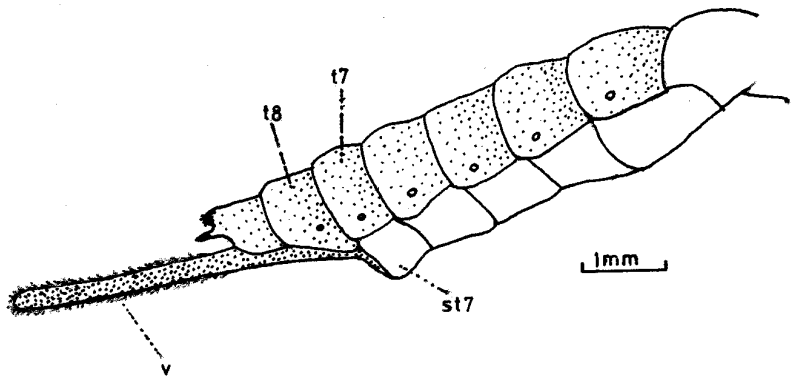

Figure 1. Lateral view of the free ovipositor of *P. turionellae.* st 7 sternum No.7; t 7 and t 8, tergum Nos. 7, 8; v, valvula (ovipositor).

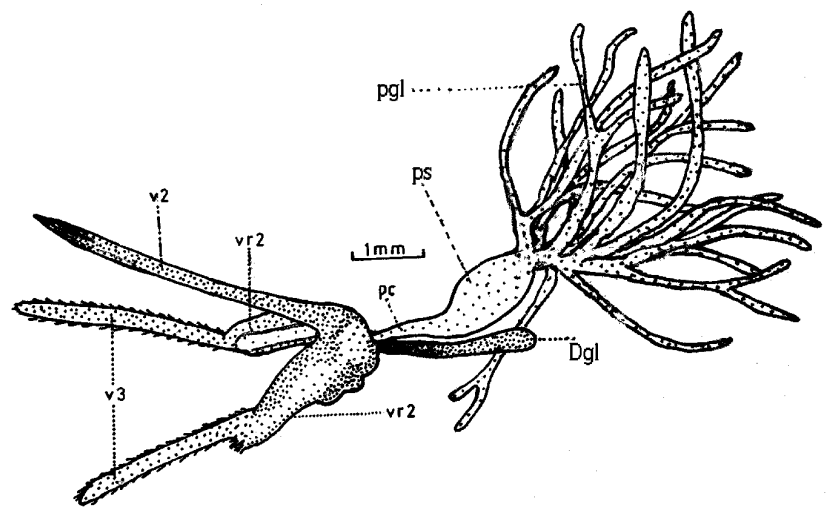

Figure 2. Venom and Dufour gland of the sting apparatus of *P. turionellae.* v2, v3, valvula Nos. 2, 3; vr 1, valvifer No. 1; Dgl, Dufour gland tubules; ps, venom reservoir; pgl, venom gland tubules; pc, venom duct.

endoparasitoid *P. turionellae,* which can be a good biological agent in biological control, except for electron microscope studies on the ultrastructure of the secreting cells in venom gland tubules (12) and studies on the essential chemical groups of venom (15). Therefore, the purpose of the present study was to examine the morphological properties of the parts of venom apparatus, and the histological structure of entire venom gland of *P. turionellae.*

Materials and Methods

The study began with the preparation of the stock cultures of the host, *Galleria mellonella,* and of the parasitoid *P. turionellae.* The methods indicated previously (15) were used in forming the stock cultures of host and parasitoid.

A venom apparatus from a 15- to 20-day-old mature female parasite taken from a parasitoid culture with the aid of the dissection needles was examined morphologically in the physiological water after stained by 0.01% concentrated methylene blue. The drawings were made by a Nikon, stereoscopic microscope with the aid of camera lucida.

Venom glands taken from 15- to 20-day-old mature parasite females by dissection were fixed in 10% concentrated formalin for histological studies. After dehydration and embedding, serial sections were cut and stained with Hematoxylin-Eosin. The sections were labelled and kept for drying, and later investigated under the Zeiss Axiophot microscope and photographed.

Results

Morphology: The venom apparatus of *P. turionellae* is situated at the end of abdomen with a completely free short ovipositor. One part of the apparatus is located at the abdomen, while another part is located on the ventrals of the eighth, ninth and tenth segments. Another part is positioned as a unique structure in an elongated position at the end of the abdomen (Fig. 1). The valvula 2 (ovipositor) in the venom apparatus is related with the

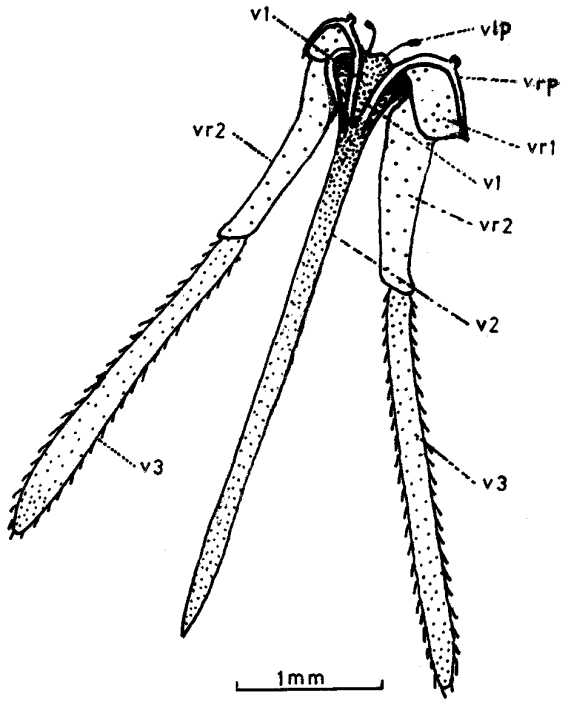

Figure 3. General plan of *P. turionellae* sting apparatus. v1, 2, 3, valvula Nos. 1, 2, 3; vr1, 2, valvifer Nos 1, 2; vrp, valvifer processus; vlp, ventral processus.

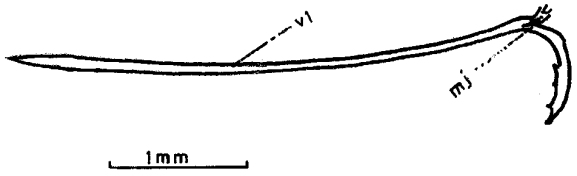

Figure 4. General figure of valvulo 1 of *P. turionellae*. v 1, lancet (First valvula); mj, muscle junction.

sternum and tergum of the seventh abdominal segment. Valvifers are connected to each other and to the eighth and ninth segments. The valvulas 3 are completely free and used like hands during oviposition (Fig. 2). In addition, valvula 3 has hairs the function of which is not clear yet. There are an ovary canal, a venom canal and two valvula 1 in valvula 2. The release of venom and the laying of eggs occur through these structures. The valvifers, venom gland, Dufour's gland and ovaries are connected at the thickened basal of valvula 2 (Fig. 3). Some of the valvula 1 in valvula 2 are arched and extend out at the basal of valvula 2 in order to set up a relation with valvifers. The muscle layer connected to the upper midsection of the curved surface of valvula 1 plays a role in the activities of the valvulae (Fig. 4). In addition, there are two ventral extensions among valvula 1 (Fig. 2). Valvifer 2 is the smallest valvifer and it is connected to the valvula axis, valvifer 2 and eighth abdominal segments.

Histology: Complex in structure, the venom glands of *P. turionellae* consist of a reservoir, a venom duct and venom gland tubules (Fig. 2, and 5). These tubules are connected to the reservoir at the upper side. The venom-secreting tubules consist of cells each having an oval or a circular view of different size (Fig. 6 and 7). These cells are arranged around a lumen, the size of which varies according to the number of cells that surround it (Fig. 5, 6 and 7). The venom gland cells consist of vacuoles of different size. The secretion of the venom gland cells in the venom-producing tubules is evacuated with the lumens of the tubules to the venom reservoir (Fig. 7). There are a few venom gland cells at the region opening to the venom gland tubules in the reservoir (Fig. 8).

The reservoir of venom gland is extremely wide (Fig. 5 and 8) and its wall consists of an irregular muscle layer

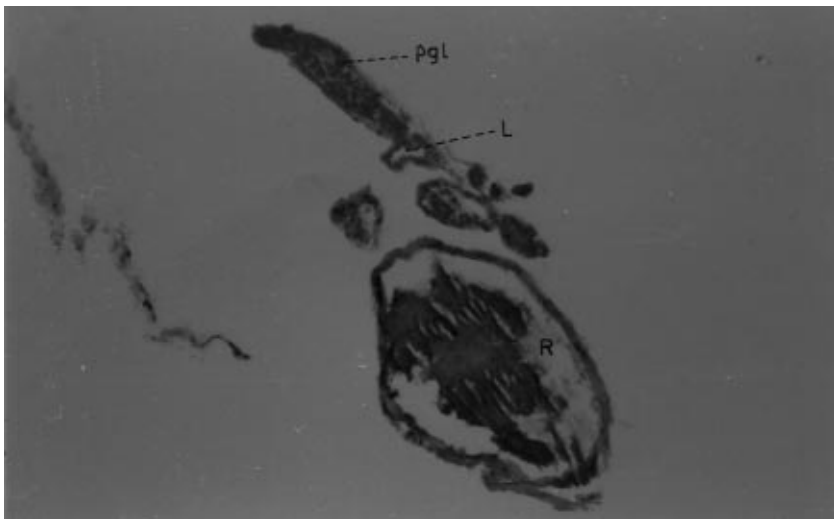

Figure 5. Microscopic view of cross-section from venom reservoir and venom gland tubules of *P. turionellae* X400. Pgl, venom gland tube; L, lumen of the gland; R, reservoir.

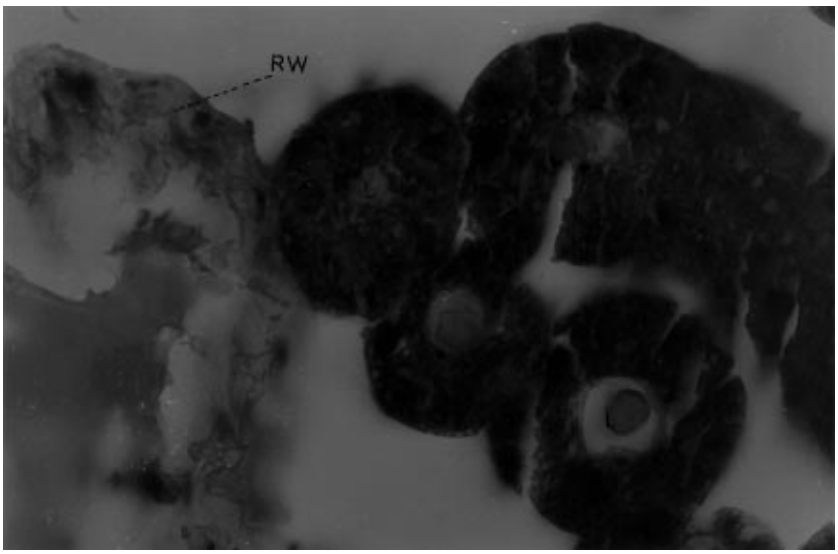

Figure 6. Reservoir wall and venom gland tubes of *P. turionellae* showing different types of cells present in a cross-section X2000. RW, reservoir wall; L, lumen of the venom tube.

Figure 7. The microscopic view of the lumen (venom transporting canal) of venom gland tubules and reservoir of *P. turionellae* X800. L, lumen (Venom transporting canal); R, reservoir.

and ectodermal cells (Fig. 6 and 8). The venom reservoir consists of two parts: one where the duct of the venom glands debouches, the wall of which, apart from chitin and ectodermal cells, is lined with gland cells (Fig. 8); the wall of the other part of the venom reservoir consists only of chitin and chitin-producing ectodermal cells. The venom duct of the venom gland has the appearance of a canal that is supported by irregular chitin layers and has no muscle layer.

Discussion

The completely free position of the venom apparatus of *P. turionellae* at the end of abdomen with a short ovipositor indicates that *P. turionellae* may be a primitive species, as asserted by Oeser (16). This structure, with part of the venom apparatus in the abdomen, another part on the eighth, ninth, tenth segments ventrally and another part independent (Fig. 1), resembles the structure seen in most Symphyta and Terebranta (3), whereas the venom apparatus of Aculeata is inserted in the abdomen (3). The structure of the venom apparatus of *P. turionellae,* except for the venom gland and Dufour's dufour gland, which resemble the general structure given for the venom apparatus of Hymenoptera (3). There are only important differences in the structure of valvifer 1. The general structure of the venom apparatus also resembles that given for *Nasonia vitripennis.* The structure of valvula 2 resembles for the most part the structure given for *N. vitripennis.* Despite the differences in the structure, the position of valvifer 2 is similar to its position in other species (3, 9). Valvula 3 is longer than

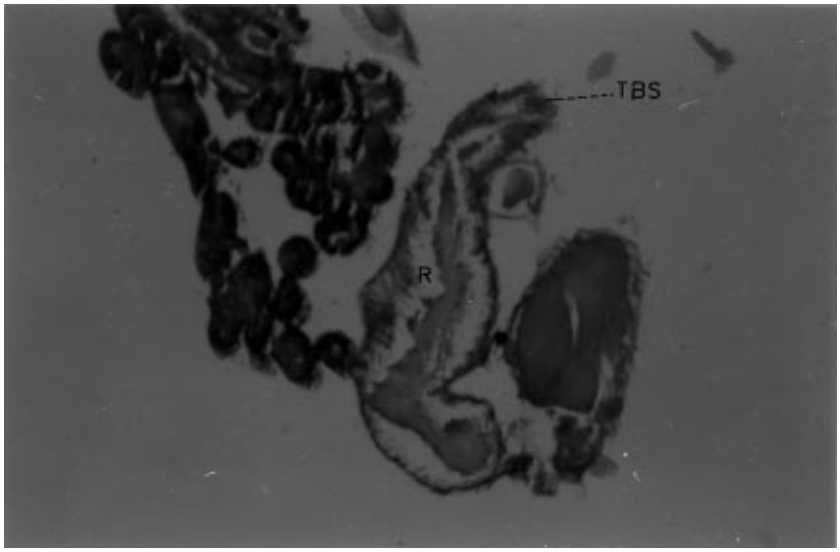

Figure 8. A cross-section from venom gland tubules and reservoir of *P. turionellae* X400. R, reservoir; TBS, tubule binding site.

the others and it also has sensory cells. Although some secretory glands other than the venom gland and Dufour's gland have been determined in different groups of Hymenoptera (3), *P. turionellae* has only venom and Dufour's glands. In terms of internal structure and function, valvula 2 has some similarities to the structure and function given for Hymenoptera (3). The existence of two valvula 1 and structures with the functions of secretion of venom and laying of egg are the common properties. As is the case in *P. turionellae,* the basal of the valvulas 2 is thickened, and the venom gland, Dufour's gland and ovaries are tied to this section. The structure of valvula 1 (Fig. 4) resembles the structure described by Oeser (16), but the connection point of muscle layer in the valvula 1 of *P. turionellae* has some differences. It is thought that this muscle layer is used in the drilling operation performed by valvula 1. The morphology of the venom gland of *P. turionellae* resembles both the structure of the species of Ichneumonidae defined in advance (4, 8) and the second type of structure of venom gland given for Braconidae (7, 10). This supports the idea that there may be a continuity between Ichneumonids and Braconids (4). There is a thin-walled reservoir in the *P. turionellae* venom gland, and this reservoir has a small muscle layer. The venom secreting tubules consist of cells lined up around a lumen that are connected to the ovipositor by a venom transporting canal. All this indicates that the venom gland of *P. turionellae* resembles the second type of venom gland given for Braconids (10). The morphological structure of the venom gland of *P. turionellae* is thought to be useful for systematic and phylogenetic studies. These results may contribute much to studies on the evolution of the venom gland in Hymenoptera.

The histological structure of the venom gland of *P. turionellae* resembles that of *Philanthus triangulum* (14) with the presence of venom secretory cells around a lumen of different size and shape (Fig. 5 and 6) similar to the venom gland tubules of *P. triangulum.* There are differences only in the connection of venom secretory tubules to the reservoir. The gland cells of both species have vacuoles of different sizes. Both *P. turionellae* and *P. triangulum* reservoirs have a reservoir wall with an irregular muscle layer, chitin and chitin-producing ectodermal cells. The *Microbrocon hebetor* venom gland consists of a reservoir among eight venom secretory tubules connected below, and it is surrounded by a strong muscle layer (14). The venom-secretion organelles of the three species are alike, and there are microvilli on the side facing the lumen. This occurs in the lumen of venom gland and it continues through the venom transporting canal and ending at the reservoir (8). A similar system is defined in Vespa (17), Apis (18) and Nasonia species (12). The venom reservoir of *P. turionellae* having more glands at the region opening to venom tubules in proportion to other regions was determined in *P. triangulum* as well (Fig. 8). The absence of a muscle layer in the structure of the venom duct of the venom gland and its reinforcement by a chitinous structure resembles the structure previously described (3). The morphological and histological similarities seen between the venom glands of *P. turionellae* and *P. triangulum* indicate that there may be a phylogenetic relation between these two species.

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