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ULTRASTRUCTURAL LOCALISATION OF ACID PHOSPHATASE IN THE HEMOCYTES OF AGROTIS IPSILON (HUFN.) (LEPIDOPTERA: NOCTUIDAE)

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SUMMARY

In this study, acid phosphatase activity has been demonstrated by Gomori techique in the last instar larvae of *Agrotis ipsilon*. In the cytoplasm of prohemocytes, no evidence of acid phosphotase activity was found. Ultracytochemical localization of acid phosphatase were observed particularly in the plasmatocytes and granular cells of *A. ipsilon*, In the cytoplasm of granular cells, several lysosome-like vacuole series which include varing electro-dense acid phosphatase reaction products appear. Spherule cells and oenocytoids have the vaculoes a few in number indicating at least weakly acid phosphatase reaction products.

INTRODUCTION

The morphology of the hemocytes of *Agrotis ipsilon*, has studied in previous work (Ayvalı, 1989). Their ultrastructural characters was pointed out by light and electron microscope. According to this earlier study, five type of hemocytes were identified: prohemocytes, plasmatocytes, granulocytes (granular cells), spherule cells and oenocytoids.

A lepidopteran insect, *Antherae pernyi* were examined cytochemically (Beaulaton and Monpyssin, 1976, 1977). In this species, granules contents of granulocytes were tested. The contents of this granules have given a positive reaction to the periodic acidethiocarbonidrazide-silver proteinat method. This reaction has indicated the existence of muco-or-glycoproteins in the granules. Some histochemical observations were made on the hemocyte of *Galleria mellonella* by light microscope (Ashhurst and Richards, 1964).

Neuwirth (1973) also studied acid phosphatase, acidic mucosubstances and polypenoloxidase ultracytochemically on the hemocytes of *G. mellonella*. A cytochemical study of the interaction between latex particles and hemocytes of. *G. mellonella* of which plasmatocytes have the phagosomes including acid phosphatase positive was made. The monolayer culture of *Pieris brassica* hemocytes were observed and it was found that acid mucopolysaccharides are existed aorund the spheru-

le cells (Ratcliffe, 1975). Akai and Sato (1973) have suggested that spherules of the spherule cells in *Bombyx mori* are sources of some blood proteins. They also have demonstrated that the oenocytoids of the some species include tyrosinase.

On the hemocytes of *Locusta migratoria*, some histochemical analysis were reported by Costin (1975). Scharrer (1972) in the plasmatocytes of cockroaches and Crossley (1975) in *Calliphora erythrocephala* were identified the presence of the proteolytic marker enzymes, acid phosphatase.

In this preliminary study, ultrastructurally localization of acid phosphataes in the hemocytes of larval stage of *A. ipsilon* were observed qualitatively. Later study will consider qualitative and quantitative treatments during the life cycle of this lepidopteran insect to reavel of activities of hemocytes.

MATERIALS AND METHODS

The larvae of *A. ipsilon* wree collected from the field of different part of Anatolia, and reared in laboratory conditions (Harris et. al 1962; Levine at. al., 1982). In this study last larvae were used. Blood was obtained by cutting proleg, and first fixed in cold 2,5 % glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2 for two hours. And then the suspension was centrifuged at 2500 rpm for 10 minutes. The pellet was rinsed in several changes of buffer solutions with 5 % sucrose. For acid phosphatase reactions, metal salt precipitation tecniques were used (Simina and Barendsen, 1980). The hemocyte pellet was incubated in Gomori medium and Barka and Anderson's medium (Hayat, 1973). Incubation was for one hour at 37° C. Controls were incubated without substrate (Na-B-glycerophosphate) or NaF was added to incubation medium. Following incubation, pellet was rinsed and postfixed in 1 % osmium tetroxid in cacodylate buffer. After washing the pellet in several changes, dehydrated in graded aceton and were embeded in Araldite. Thin sectiones were not stained and examined with AEI Corinth 275 electron microscope.

RESULTS

The acid phosphatase (Acph) activity was observed in all types of hemocytes of *A. ipsilon*. In the plasmotocytes and granular cells. There are Acph reacting positive vacuoles that indicate lysosome-like vesicles.

Prohemocytes, normally do not include vacuoles or they can be found a few in number. It might be found by chance. Because this kind of haemocytes has been expected not to be active

In the spindly-shaped plasmatocytes, one or more lysosome like bodies can be observed (Fig. 1). Different kinds of the plasmatocytes such as vermiform or filopodia, show a weakly Acph activity in their cytoplasm. Some unstructed and small granules of plasmotocytes show strongly enzyme activity. Generally, this kind of granules are not more than 3–4 and can be interrupted as primary lysosomal granules. In the cytoplasm of plasmatocytes may be found large vacuoles in which strong Acph activities were demonstrated (Fig. 2). These large vacuoles have involved membranous debris and myelinated structures (Fig. 3). In the circulating hemolymph discharged or protruted membrane fragments which also show Acph were observed (Fig. 4). Acph distributed in the cisternae of rough endoplasmic reticulum of plasmatocytes has been seen in Fig. 5.

The granular cells have more abundant vacuoles showing positive Acph reactions. These lysosome-like vacuoles were clearly seen and easily differentiated from the other granules (Fig. 6). In the same time, lysosome-like vacuoles having not any Acph positive reaction were also observed. It is thought that, these of bodies could be inactive during the treatments (Fig. 7).

In the cytoplasm of granular cells, there were large Acph positive vacuoles in which the reaction products accumulated heterogenously. Some time two lysosome like vacuoles may fuse with others (Fig. 8). The heterogenously accumulated reaction products may indicate that lysosomal vacuole include the membrane fragments or tissue debris which may be originated from different sources. In the granular cells of *A. ipsilon*, Acph positive unstructed types of granules were also well identified (Fig. 9). The members of lysosome series which indicated Acph activity were appeared most probably in aged granular cells. In cytoplasmic matrix of spherule cells, Acph positive small vacuoles could be weakly observed (Fig. 10). The other type of haemocytes, oenocytoids may include Acph positive lysosomal vacuoles a few in number. This kind of vacuoles was observed in the cytoplasmic matrix where the free ribosomes and the other organelles are more consantrated (Fig. 11).

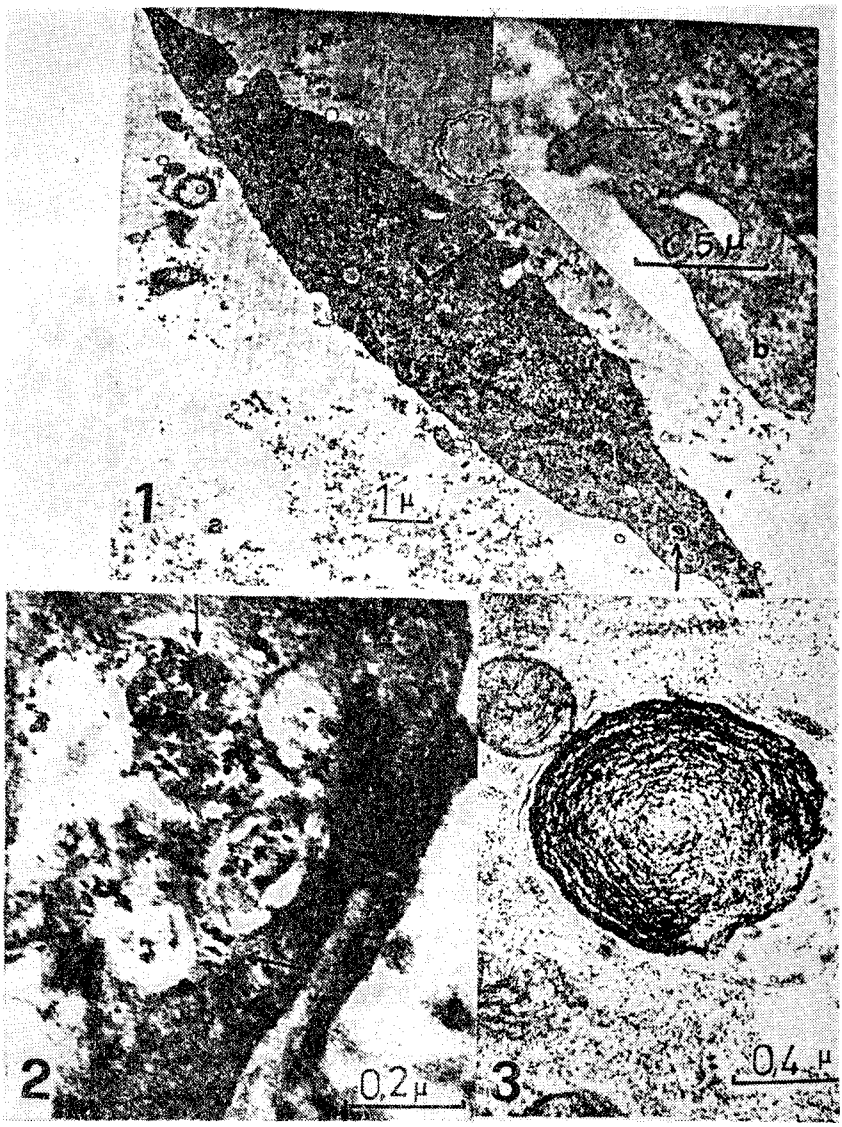

Fig. 1– Plasmatocytes: Acph reaction products (arrow). b*j* Magnificated area from a.

Fig. 2– Large vacuoles in which strong Acph positive in plasmatocyte.

Fig. 3– Acph positive in myelinated structures.

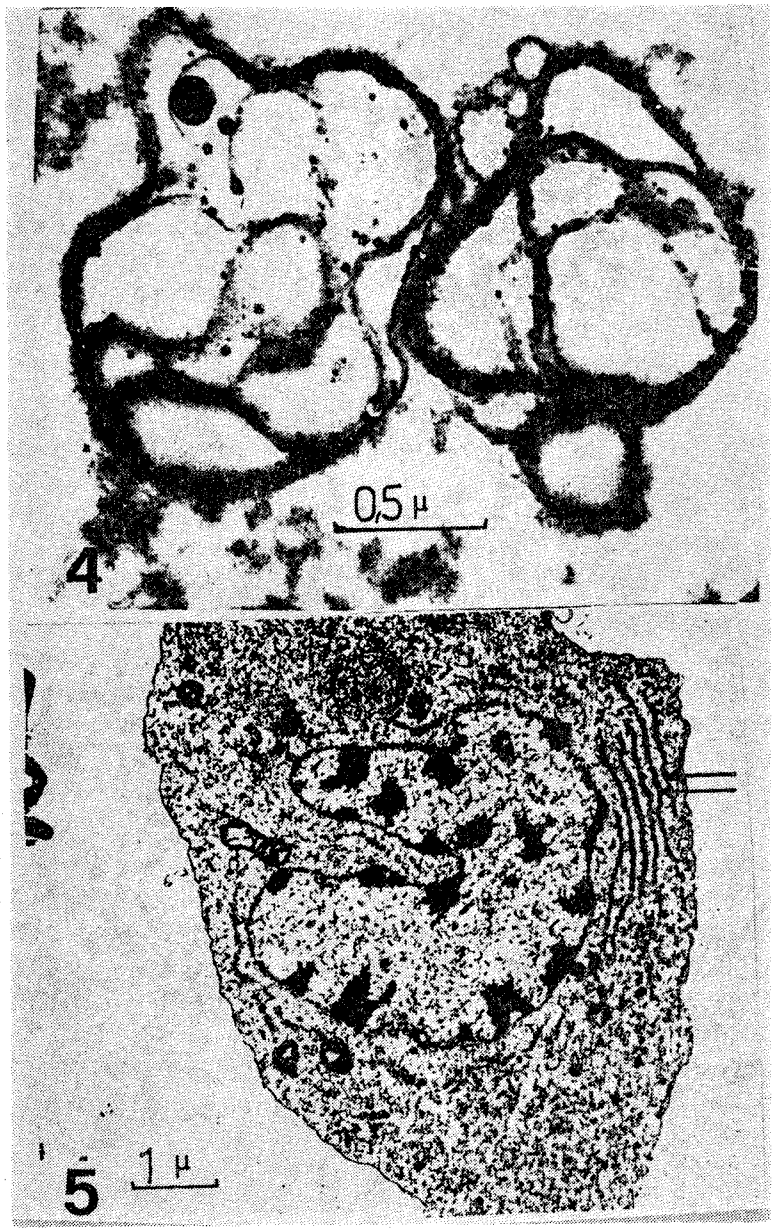

Fig. 4– Discharged membran fragments with Acph positive.
Fig. 5– Acph in the cisternae rough endoplasmic reticulum of plasmatocyte.

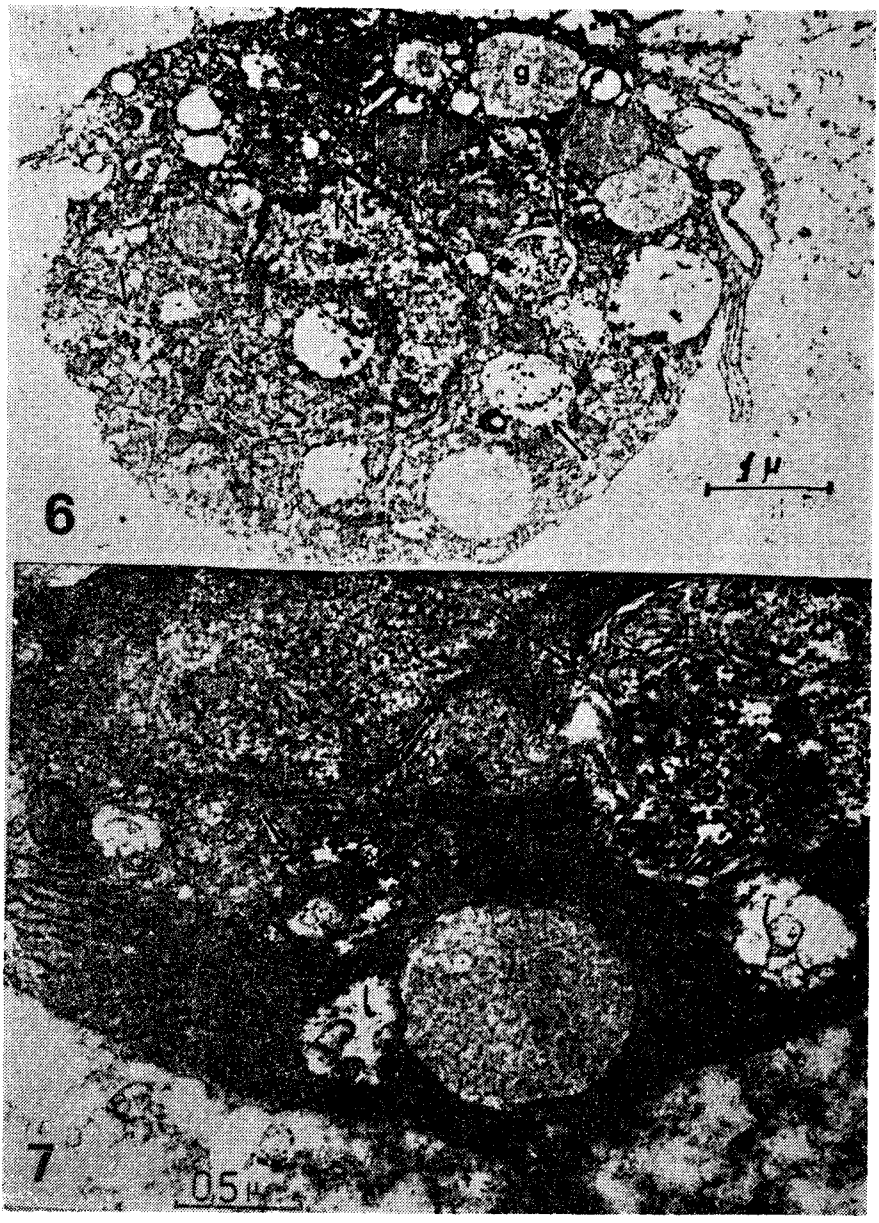

Fig. 6– Acph positive vacuoles in granulare cell. (arrow). N: Nucleus, g: granules.
Fig. 7– Acph positive vacuoles (arrow) and negative lysosomes-like (l) vacuoles in granular cell.

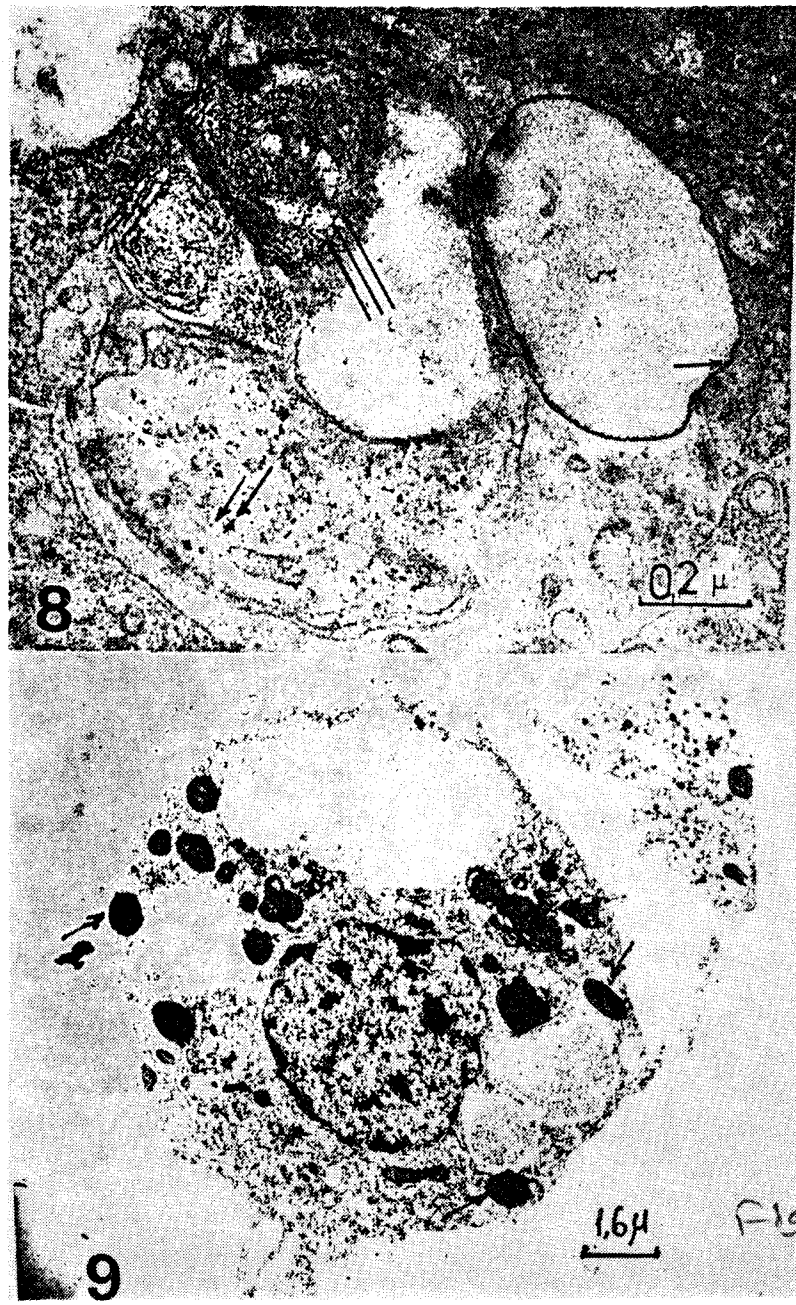

Fig. 8– Acph positive reaction prioducts accumulated heterogenously (arrows) and fused vacuoles.

Fig. 9– Acph positive unstructured granules in granular cell (arrows).

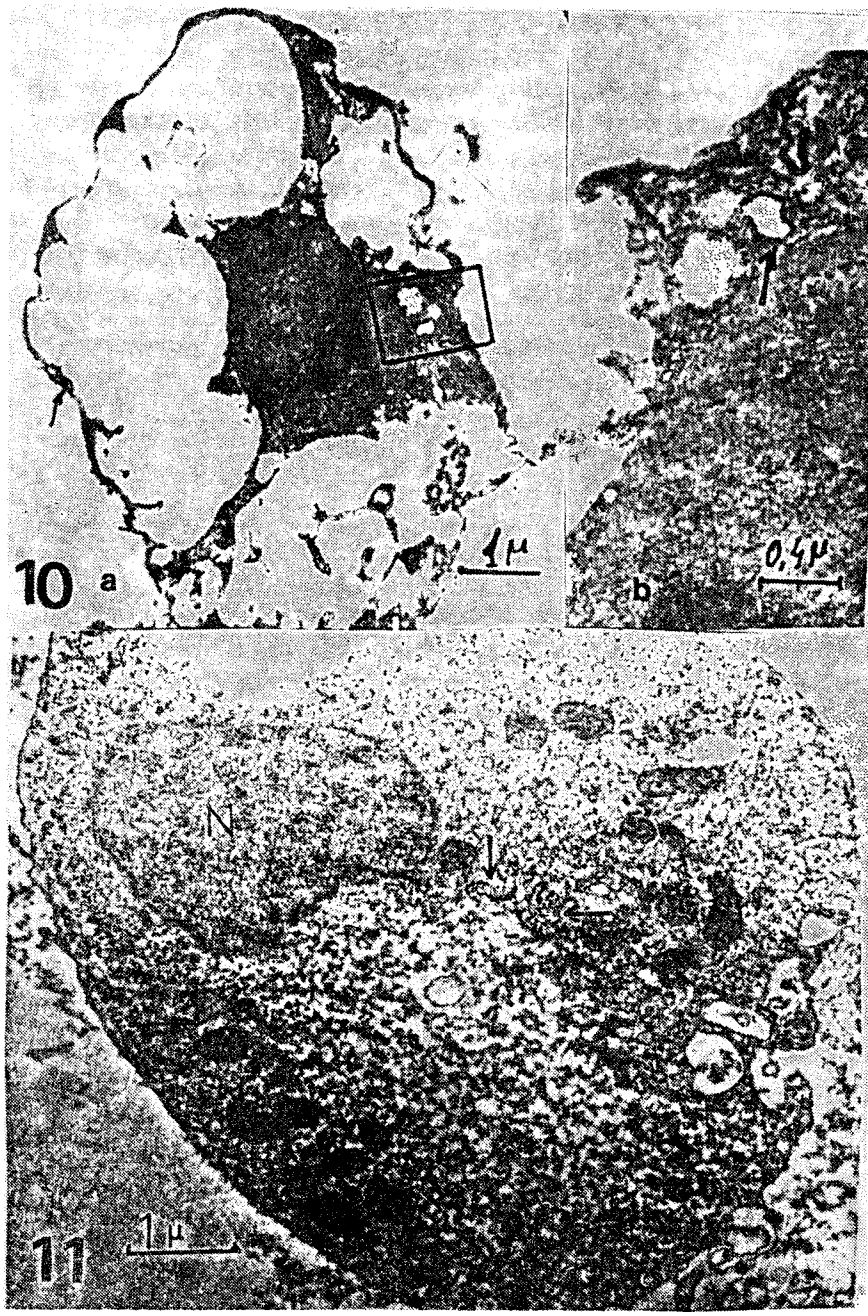

Fig. 10– Acph positive of small vacuole (arrow) in spherule cell(a) and magnificated area from a (b).

Fig. 11– Acph positive vacuoles (arrows) in oenocytoid.

DISCUSSION

In the circulating hemolymph, the activities of hemocytes could not be expected in same cytophysiological conditions. The cytophysological activities of these cells may be physiologically different according to developmental stages of insect, invasion by parasitic organisms and starmation etc. Acid phasphatases localization of ultrastructtructural level have been studied in the plasmatocytes of *G. mellonella* by Neuwirth (1973). Rowley and Ratcliffe (1979).

Neuwirth (1973) have not found Acph reaction in the plasmatocyte but observed only in the lysosomal vacuoles of granular cells. Rowley and Ratcliffe have reported Acph localization in the primary lysosomes and phagoromen of plasmatocytes of some Lepidopteron species. In the plasmacytes and particularly granular cells of *A. ipsilon* acph reaction positives vacuoles were clearly observed. Acph activities have been reported by Crossley (1975) in the lysosomal vacuoles of the granular hemocytes of *Calliphora erythrocephala*

Neuwirth (1973) have reported that there was no evidence of Acph activity for spherule cells and oenocytoids of *G. mellonella*. In this paper weakly Acph reaction products have been shown in small vacuoles of spherule cells and oenocytoid of *A. ipsilon*. Although these last two hemocytes have not phagocytic function, but in normally ultrastructural study of Acph reaction of spherule cells and oenocytoids of *A. ipsilon* were observed.

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