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Early Embryonic Development of *Tritneptis diprionis* (Chalcidoidea, Hymenoptera)*

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Synopsis

Early embryonic stages of *Tritneptis diprionis*, an ectoparasite of Diprionidae, are described. A freshly laid egg contains the oosome. The chromatin of the nuclei of the blastoderm cells shows polarity. The germ cells enter the embryo interior partly through blastoderm. The primary endoderm arises from primary vitellophages. The definitive mid gut epithelium is formed by the cells lying at the bottoms of stomodaeum and proctodaeum. The gastrulation is accomplished by the sinking of the middle plate of germ band.

Introduction

The genus *Tritnepsis* is one of the rarer ectoparasites of Diprionidae. In Polish fauna the genus is represented by two species: *T. klugii* and *T. diprionis*. So far, the embryonic development of any species of this genus was studied.

The present paper is a continuation of series of works on the embryonic development of hymenopterans — parasites of diprionid larvae (Kościelska, 1980, 1981, 1985).

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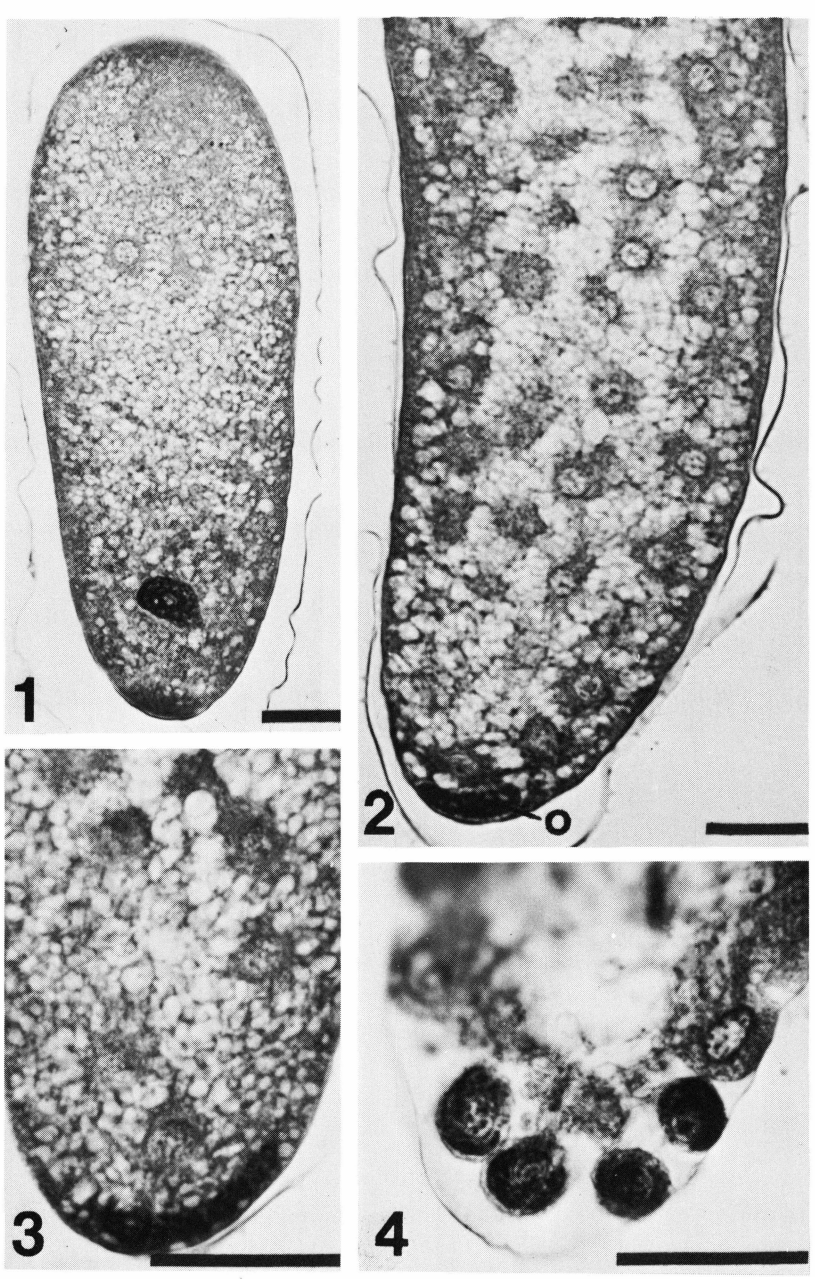


Fig. 1. Longitudinal section through the egg of *Tritneptis diprionis*. Posterior pole of the egg contains oosome. Scale: 25 μm.
Fig. 2. Formation of the syncytial blastoderm. Cleavage nuclei and oosome (o). Scale: 25 μm.
Fig. 3. Posterior pole of the egg. Oosome fragments visible. Scale: 25 μm.
Fig. 4. Germ cells. Scale: 25 μm.

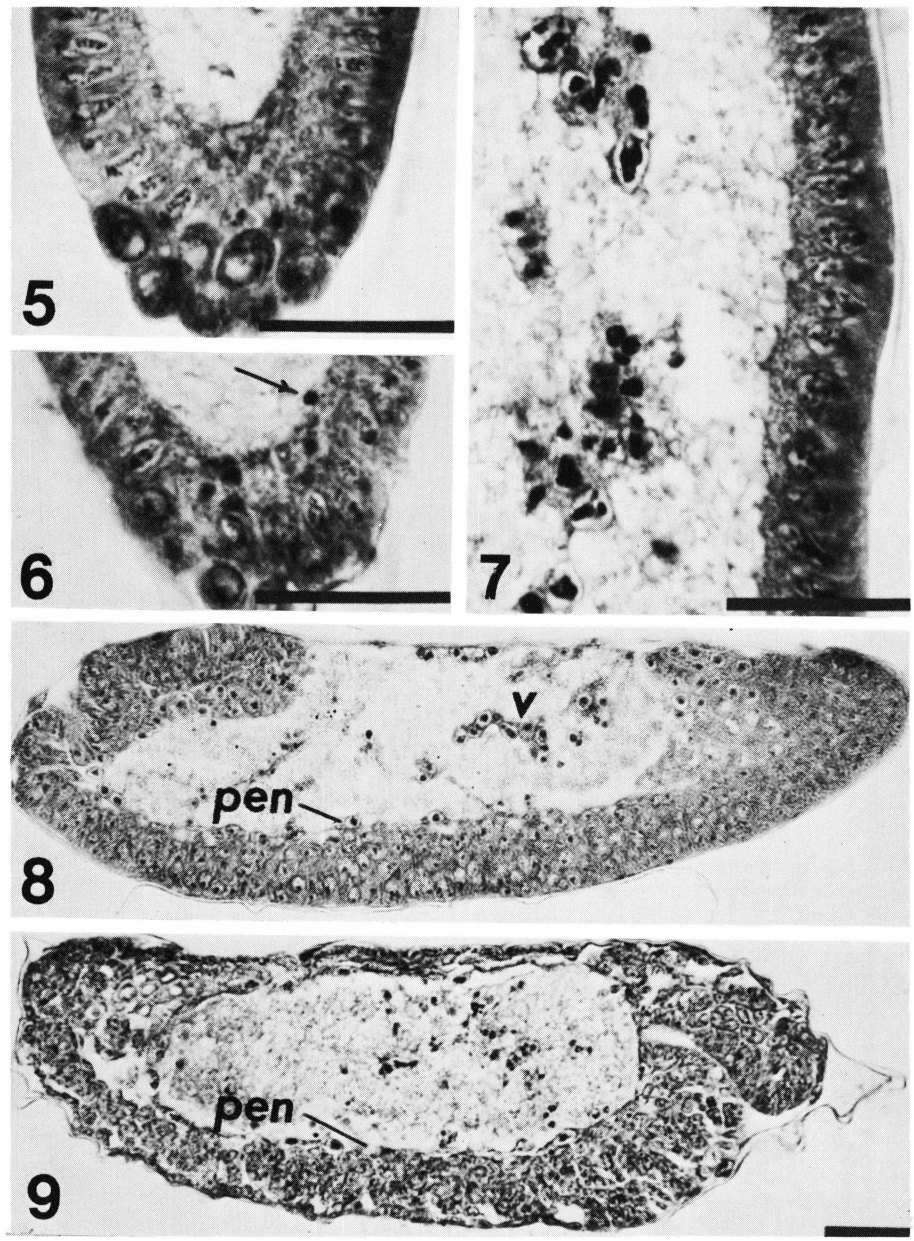


Fig. 5. Penetration of germ cells through the blastoderm. Chromatin polarization in the nuclei of the blastoderm cells. Scale: 25 μm.
Fig. 6. atin granule underneath the blastoderm cells. Scale: 25 μm
Fig. 7. Irregular accumulations of the vitellophages. Chromatin polarization in the nuclei of the blastoderm cells visible. Scale: 25 μm.
Figs. 8, 9. Formation of the primary epithelium of the mid gut. Longitudinal sections. pen, primary endoderm; v, vitellophages. Scale: 25 μm.

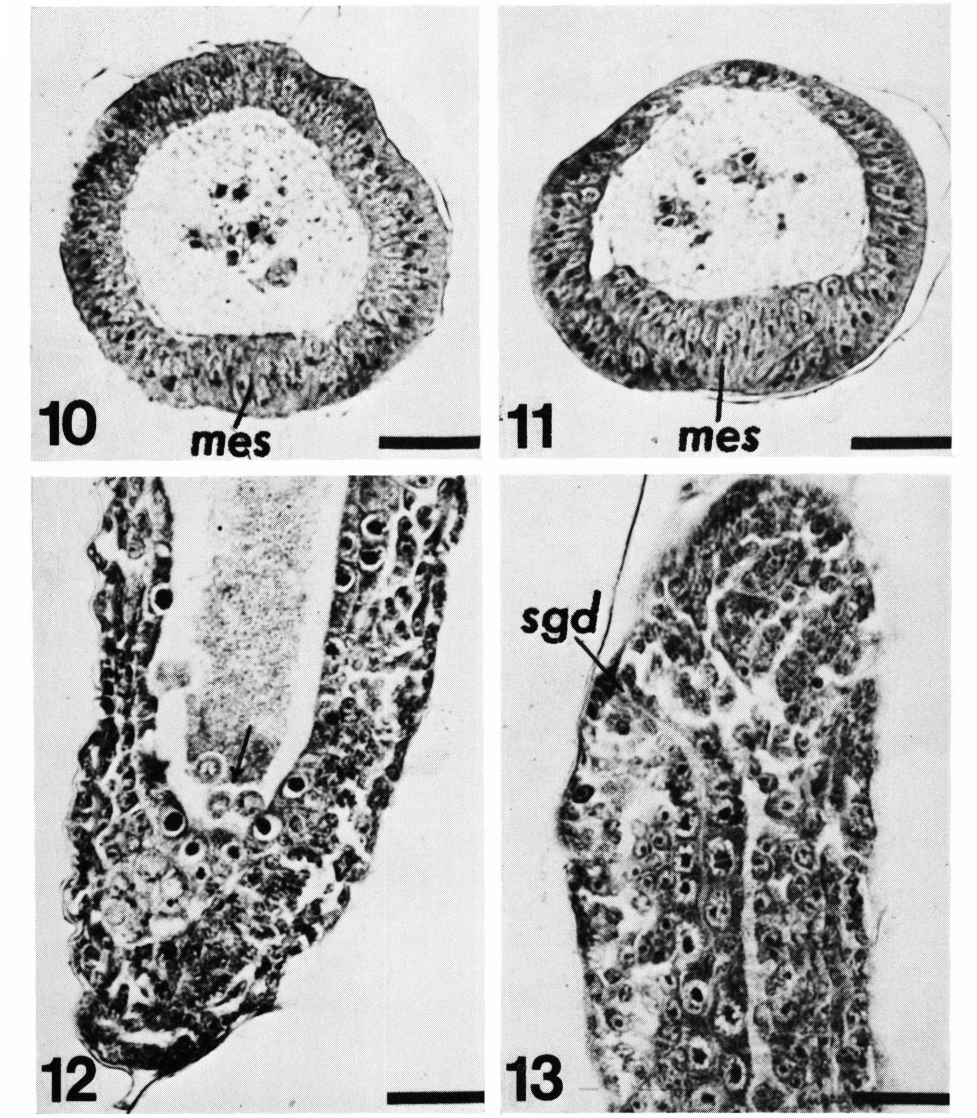
Material and Methods

The material used in the study was taken from a laboratory culture in which larvae (in cocoons) of *Diprion pini* and *D. frutetorum* were used as hosts of parasite. Larvae in cocoons had been collected in pine woods. This made it possible to obtain any number of *Tritneptis diprionis* eggs in various growth stages. After puncturing the chorion with a tungsten needle sharpened in molten sodium nitrite, the eggs were fixed for 10—20 min in Carnoy's fluid, and then embedded in paraffin. The sections, 5 μm thick, were stained in Delafield's haematoxylin and eosin.

Results

A freshly laid egg contains fine yolk granules evenly scattered in the ooplasm. In the posterior part of the egg there is an oval oosome (Fig. 1). During the cleavage the oosome migrates to the peripheral region of the posterior egg pole and there it undergoes fragmentation (Figs. 2, 3). Finally the material of the oosome gets into the germ cells. The germ cells get out of the embryo even before the definitive blastoderm is formed (Fig. 4). These processes take place within the first few hours after the egg-laying. Between the 10th and 20th hour of the embryonic development single germ cells penetrate through the blastoderm inside the embryo(Fig. 5). The chromatin of the blastoderm nuclei shows a polarization, being accumulated mostly in the apical portions of the nuclei (Fig. 5). Immediately below the blastoderm, most often on the posterior pole of the embryo, chromatin granules may occur, coming from the blastoderm nuclei (Fig. 6). At the stage of blastula a larger accumulation of yolk is localized in the anterior egg portion. In course of the further development the yolk distribution again becomes even. In an initial period of the blastoderm formation yolk nuclei (vitellophages) arrange in two rows parallel to the egg long axis. At the stage of the definitive blastoderm the vitellophages divide intensively and at the same time their linear arrangement becomes disturbed: irregular accumulations of several and sometimes more than ten vittelophages form (Fig. 7). Initially the division of the vitellophages is mitotic, then probably amitotic. After numerous divisions the primary vitellophages assume again their linear arrangement. A part of the vitellophages (*ca.* 30th hour of the development) get to the surface of the yolk to form the primary mid gut epithelium (Figs. 8, 9). In the posterior part of the primary and the definitive mid gut a few nuclei can be observed originating probably from the germ cells (Fig. 12). After the definitive mid gut epithelium has been formed, the primary vitellophages undergo a gradual degeneration.

Between the 20th and the 30th hour of the development gastrulation takes place, effected by the sinking of the middle plate (Figs. 10, 11). The process begins in the mid part of the germinal band and from there it proceeds to the posterior and anterior part of the embryo. The course of this process is rapid and is completed within a few hours. During the gastrulation also the only embryonic membrane — the serosa — forms. At about the 40th hour of the development, in the anterior part of the embryo on its ventral side salivary glands arise from the ectoderm, characterized by large, polyploid nuclei (Fig. 13). At that time also the definitive mid gut epithelium forms fro m a group of



Figs. 10, 11. Sequence of formation of the mesoderm (mes). Cross sections. Scales: 25 μm.

Fig. 12. Longitudinal section through the embryo. In the posterior part of the definitive mid gut the nuclei derived from the germ cells can be seen (arrow). Scale: 25 μ m.

Fig. 13. Formation of the salivary gland. Longitudinal section. sgd, salivary gland duct. Scale: 25 μm.

cells at the bottom of the stomo- and proctodaeum.

Discussion

In the embryonic development of *Tritneptis diprionis* an array of features occurs characteristic of the development of hymenopterans, especially the parasitic ones. The newly laid egg containing few and fine yolk granules is similar to that of *Monodontomerus dentipes* (Kościelska, 1981). During the cleavage the oosome fragments, like in other Hymenoptera, determine the germ cells (Beams and Kessel, 1974). Similarly, the penetration of the germ cells through the blastoderm to the inside of the embryo was observed in many parasitic hymenopterans (Bronskill, 1959, 1964; Ivanova-Kasas, 1950, 1952, 1958, 1960, 1961; Kościelska, 1980, 1985). Some of the germ cells penetrate into the posterior part of the primary mid gut and form there a small aggregation. Their role has not been explained. Most authors (see Bronskill, 1959, 1964) regard them as tertiary vitellophages which take part in digestion. Maybe in the case of *T. diprionis* these cells actually digest the remnants of the primary mid gut epithelium, like in *Pleolophus basizonus* (Kościelska, 1980). In *M. dentipes*, however, in which the primary vitellophages disappear early, these cells probably take part in the digestion of yolk (Kościelska, 1981).

A polarization of the chromatin in the blastoderm cells, resembling that of *T. diprionis*, was observed in *Dahlnominus fuscipennis* (Kościelska, 1985) and in *Eurytoma aciculata* (Ivanova-Kasas, 1958). The significance of this phenomenon has not been explained.

The process of the vitellophage divisions , at the stage of definitive blastoderm, was observed not only in Hymenoptera (Kościelska, 1980, 1985) but also in Diptera (Anderson, 1962). The greatest number of the vitellophages in the studied species occurs during the gastrulation, like in *D. fuscipennis* (Kościelska, 1985). The vitellophage division, initially mitotic and then probably amitotic was described also in the development of *P. basizonus* (Kościelska, 1980) and *D. fuscipennis* (Kościelska, 1985). In *T. diprionis*, like in *D. fuscipennis* the vitellophages are still present at the stage of the primary mid gut, in other species *e. g. M. dentipes* they disappear at earlier development stages (Kościelska, 1981). The primary mid gut epithelium in *T. diprionis* forms from the primary vitellophages, like in some other hymenopterans, both parasitic and non-parasitic (Carrière and Bürger, 1897; Strindberg, 1915; Błedowski and Kraińska, 1926; Dondua, 1953; Bronskill, 1959, 1964; Ivanova-Kasas, 1960; Kościelska, 1980, 1985).

The gastrulation in *T. diprionis* resembles this process in other studied species of hymenopterans parasitizing larvae of diprionids, *i. e.*, it is accomplished by sinking of the middle plate. Until not long ago it was supposed that (see Ivanova-Kasas, 1961) this type of gastrulation is characteristic of Aculeata and a few species of parasitic Hymenoptera (Bronskill, 1959, 1964; Ivanova-Kasas, 1961). It follows from the present paper and from the earlier studies (Kościelska, 1980, 1981, 1985) that the gastrulation effected by sinking of the middle plate is not a rare phenomenon in the development of the parasitic hymenopterans.

The chromatin granules observed below the blastoderm in *T. diprionis* were described also in other Hymenoptera. The problem was discussed in detail in the paper

on the development of *P. basizonus* (Kościelska, 1980). More recently granules of nuclear origin at the blastoderm stage were found by Tanaka (1985) in *Trichogramma chilonis*.

The salivary glands in *T. diprionis* are ectodermal origin, like in other parasitic hymenopterans (Bronskill, 1959, 1964; Kościelska, 1981, 1985). Also the definitive mid gut epithelium is formed, like in most insects, from the cells situated at the bottom of the stomo- and proctodaeum (Anderson, 1972).

In the development of *T. diprionis* there are some features characteristic of other hymenopterans parasitizing diprionids, *e. g.* formation of the primary and the definitive mid gut, and the type of gastrulation. On the other hand, these characters are typical of the development of other Hymenoptera, especially Aculeata. Thus it is another proof of the phylogenetic affinity of the parasitic hymenopterans and Aculeata.

References

Anderson, D. T., 1962. The embryology of *Dacus tryoni* (Frogg.) (Diptera, Trypetidae = Tephritidae). *J. Embryol. Exp. Morphol.* 10: 248-292.

————, 1972. The development of holometabolous insects. *In* S. J. Counce and C. H. Waddington (eds.), Developmental Systems: Insects, Vol. 1, 165-242. Academic Press, London, New York.

Beams, H. W. and R. G. Kessel, 1974. The problem of germ cell determinants. *Int. Rev. Cytol.* 39: 413-479.

Błedowski, R. and M. K. Kraińska, 1926. Die Entwicklung von *Banchus femoralis* Thoms. (Hymenoptera, Ichneumonidae). *Bibl. Univ. Liberae Pol.* 16: 3-50.

Bronskill, J. F., 1959. Embryology of *Pimpla turionellae* (L.) (Hymenoptera, Ichneumonidae). *Can. J. Zool.* 37: 655-688.

————, 1964. Embryogenesis of *Mesoleius tenthredinis* Morl. (Hymenoptera, Ichneumonidae). *Can. J. Zool.* 42: 439-453.

Carrière, J. and O. Bürger, 1897. Die Entwicklungsgeschichte der Mauerbiene (*Chalicodoma muraria* Fabr.) im Ei. *N. Acta Abh. Kaiserl. Leop. -Carol. Dtsch. Acad. Naturf.* 69: 253-419.

Dondua, A. K., 1953. K embrionalnomu razwitiju *Scolia quadripunctata* F. *Zool. Żur.* 32: 631-634.

Ivanova-Kasas, O. M., 1950. Prisposoblenije k parazitizmu w embrionalnom razwittii najezdnika *Prestwichia aquatica* (Hymenoptera). *Zool. Żur.* 29: 530-544.

————, 1952. Embrionalnoje razwitije najezdnika *Mestocharis miliaris* R. -Kors. (Hymenoptera, Chalcidoidea). *Entomol. Obozr.* 32: 160-166.

————, 1958. Biologija i embrionalnoje najezdnika *Mestocharis miliaris* R. -Kors. (Hymenoptera, Eurytomidae). *Entomol. Obozr.* 37: 5-23.

————, 1960. Embrionalnoje razwitije *Angitia vestigialis* Ratz. (Ichneumonidae) vnutrennego parazita pililszczika *Pontania capreae* L. *Entomol. Obozr.* 39: 284-295.

————, 1961. Essay on the Comparative Embryology of Hymenoptera (in Russian). Leningrad Univ. Publ.

Kościelska, M. K., 1980. Early developmental stages and germ layer differentiation in *Pleolophus basizonus* (Grav.) Townes (Ichneumonidae). *Zool. Pol.* 27: 547-564.

————, 1981. Early developmental stages of *Monodontomerus dentipes* (Dalman) (Hymenoptera, Chalcidoidea). *Zool. Pol.* 28: 469-482.

————, 1985. Early developmental stages and germ layer differentiation in *Dahlbominus fuscipennis* Zett. (Chalcidoidea, Hymenoptera). *Zool. Pol.* 32: 63-72.

Strindberg, H., 1915. Zur Kenntnis der Hymenopteren Entwicklung. *Z. Wiss. Zool.* 112: 1-47.

Tanaka, M., 1985. Early embryonic developement of parasitic wasp, *Trichogramma chilonis* (Hymenoptera, Trichogrammatidae). *In* H. Ando and K. Miya (eds.), Recent Advances in Insect Embryology in Japan, 171-179. Arthropod. Embryol. Soc. Jpn. (ISEBU Co. Ltd., Tsukuba).

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