

Structure and Ultrastructure of the Egg Capsule of *Thermobia domestica* (Packard) (Insecta, Zygentoma)

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Eggs of *Thermobia domestica* (Packard) were collected from a laboratory culture. They were prepared for analysis in light and electron microscopes (TEM, SEM). A few hours after oviposition the egg capsule starts to tarnish and changes its colour to brown. Polygonic shapes on its surface can be seen. The egg capsule consists of a thin vitelline envelope and the chorion. The chorion consists of a one-layered endochorion and a three-layered exochorion. There are minor and major mushroom-like structures placed on the surface of the chorion. Their function is proposed. One micropyle is observed on the anterior pole of the egg. The micropylar opening is formed on the process of a follicular cell.

Key words: Zygentoma, egg capsule, chorion, vitelline envelope, micropyle.

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Insects egg capsules protect the oocyte and developing embryo (e.g. protection against loss of the water), while facilitating gas exchange, the access of sperm to the oocyte, and the hatching of the larva. Egg capsules are formed of substances synthesized and excreted by follicular cells (somatic cells accessory to the oocyte) or by the oocyte (BLAU & KAFATOS 1978; MARGARITIS 1985; REGIER & KAFATOS 1985). The egg capsule consists of a vitelline envelope and a chorion. The vitelline envelope does not have any regional differentiation and tightly adheres to the multi-layered chorion. The structure and chemical composition distinguishes the two fundamental layers of the insect chorion: exo- and endochorion (HINTON 1969). The external layer always possesses a varied structure formed by the follicular cell imprints. These may appear through the whole egg surface or only on certain regions (COBBEN 1968). Specialized regions, such as the micropyle allowing for entrance of the sperm, the aeropyle used for breathing, the hydropyle for water uptake, the attachment structures (discs, hooks, denticles), the operculum and the hatching line facilitating hatching of the larva, are usually formed on the insects egg capsules (BILIŃSKI & KLAG 1977; BILIŃSKI & SZKLARZEWICZ 1987; BILIŃSKI & JANKOWSKA 1987; BILIŃSKI & LARINK 1989; ROŚCISZEWSKA 1995, 1996; SIMICZYJEW 1995).

Eggs of insects belonging to Zygentoma are elongate and oval in *Lepisma saccharina* L., *Ctenolepisma lineata* Fabr., *Thermobia domestica* Pack. (SAHRHAGE 1953; SHAROV 1953; WOODLAND 1957; SZKLARZEWICZ 1989) or they may be flattened laterally, e.g. *Machilis alternata* Silvestri (HEYMONS & HEYMONS 1905). No structures on the egg capsule surface were described in this group, except for the micropyle defining the polarity of the egg. Egg capsule ultrastructure has not been described for these species. KLAG (1971) described the way in which hatching occurred. The most important is the surface on which the eggs lie. If it is smooth, the larva is not able to hatch, when it is rough, the egg is attached to it and hatching proceeds in a proper manner. In this paper, TEM and SEM are used to investigate structure and ultrastructure of the egg capsule.

Material and Methods

The eggs of *Thermobia domestica* were collected every 12-24 hours and reared under laboratory conditions (temperature of 37°C, humidity 60%) (KLAG 1971). The egg capsule structure was observed with an Olympus BX60 light microscope

using Nomarski optics and chlorazol E staining (CARAYON 1969).

Eggs were fixed with 2.5% glutaraldehyde in 0.1M phosphate buffer at pH 7.4 (2 h) and post-fixed with 2% OsO₄ (1.5 h). After dehydration in a graded series of alcohols (50, 70, 90, 95, and 100%, each for 15 min) and acetone (15 min) some eggs were embedded in an embedding mixture (Epon 812). The remaining eggs were prepared for a scanning electron microscope (dried at the critical point PELCO CPD 2, coated with gold in the PELCO SC-6 duster) and analysed with Tesla BS340 scanning electron microscope.

Semi- and ultrathin sections were cut with a Leica Ultracut UCT25 ultramicrotome. Semithin sections were stained with 1% methylene blue in 0.5% borax (DYKSTRA 1992), and also with the PAS method to locate polysaccharides, bromophenol blue (BPB) – polypeptides, and Sudan black B – lipids (LITWIN 1985). Semithin sections were observed with the OLYMPUS BX 60 light microscope.

Ultra-thin sections were stained with uranyl acetate and lead citrate (REYNOLDS 1963) and examined with a Hitachi H500 transmission electron microscope at 75kV.

Results

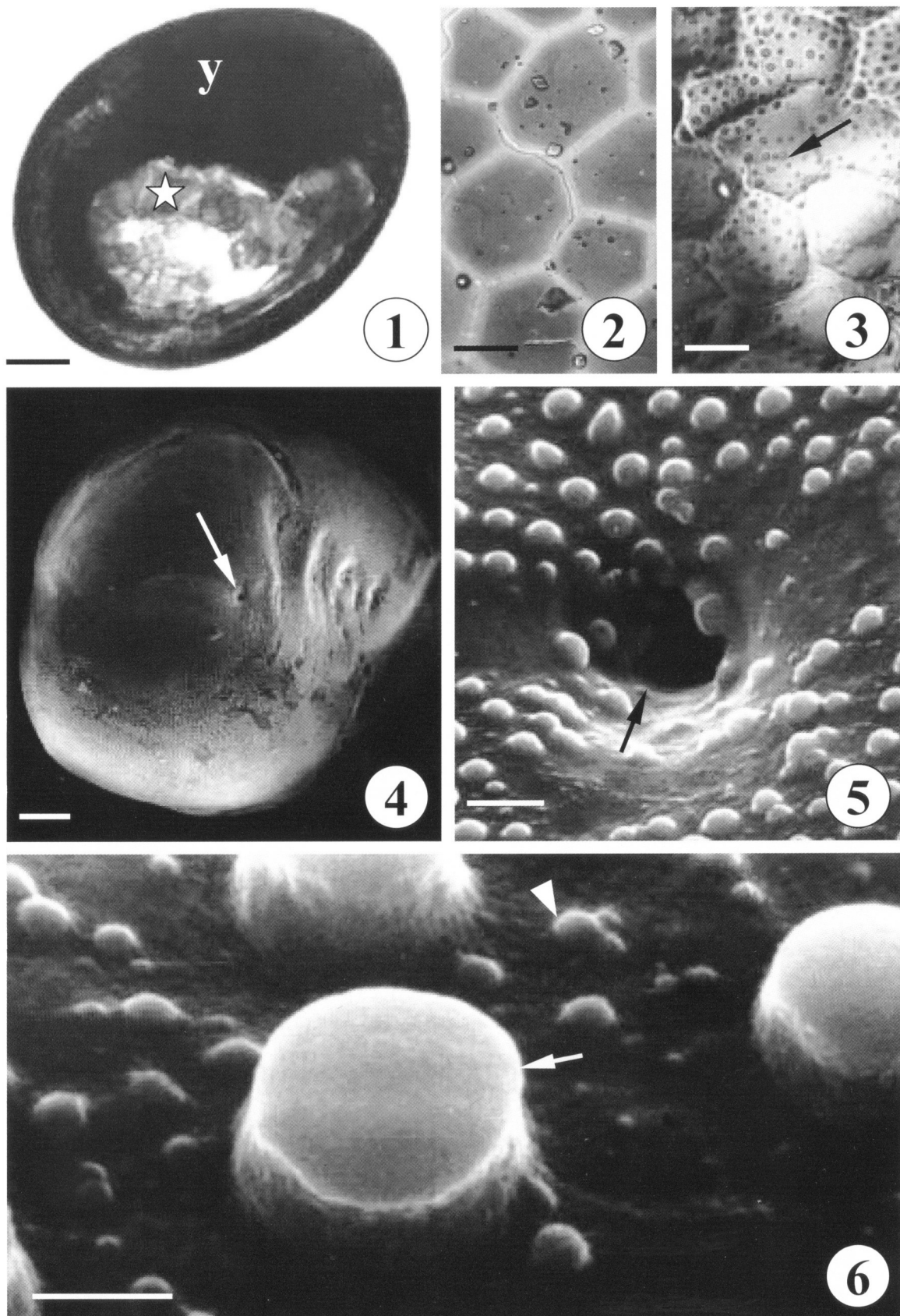
The eggs of *Thermobia domestica* are oval (Figs 1, 4), slightly flattened laterally, white and shiny, with a soft chorion. The average dimensions are 1.1 mm in length, 0.85 mm in width (in the widest place). A few hours after oviposition, the eggs become non-glossy and change colour into light brown. The egg capsule becomes harder and characteristic polygons become visible on its surface (Figs 2, 3). The capsule is thin and transparent which makes it possible to observe embryo development (Fig. 1). Major and minor mushroom-like structures are almost regularly arranged on the whole egg surface (Figs 3, 4, 6). Average dimensions of major structures reach 0.3 μ m at height and 0.4 μ m at width, and minor ones: 0.05 μ m at height and 0.08 μ m at width. The single micropylar opening occurs on the lateral side of the egg anterior pole (Figs 4, 5). During choriogenesis, this structure is formed on the process of the follicular cell (Fig. 8). Ooplasm neighbouring the micropylar opening has a specific structure (Fig. 9). The egg capsule of *Thermobia domestica* consists of a vitelline envelope and the chorion. The vitelline envelope is thin (0.01 μ m thick) and closely adheres to the chorion (Fig. 7). It shows a positive reaction for the presence of polysaccharides, peptides, and lipids.

The chorion consists of a three-layered exochorion and a single-layered endochorion which is 0.05 μ m thick. The endochorion is of high electron density, weakly PAS-positive, weakly BPB-positive and Sudan-negative. The internal layer of the exochorion is thin (0.01 μ m) and also has a high electron density. Similarly to the endochorion, it is weakly PAS and BPB-positive and Sudan-negative. The middle layer of the exochorion has a low electron density and is about 0.045 μ m thick (Fig. 7). It is PAS and BPB-positive and Sudan-negative. The external part of the exochorion is about 0.01 μ m thick and shows a high electron density. A positive reaction for polysaccharides, peptides, and lipids is visible there. Mushroom-like structures on the chorion surface are PAS and BPB-positive and Sudan-negative. These structures are surrounded by a 0.01 μ m thick layer, PAS and BPB-positive and of high electron density (Fig. 7). This layer is similar to the external layer of the exochorion, but shows a negative reaction for the presence of lipids. The results of the histochemical methods are presented in Table 1.

Discussion

The characteristic network of polygons sculpturing the whole chorion surface in the studied species is an imprint of the follicular cells. Similar structures were observed on the chorion surface in several insect species, e.g. *Leptotus marmoratus* Goeze (Heteroptera) (COBBEN 1968), *Perla marginata* Panzer (Plecoptera), and *Isoperla rivulorum* Pictet (Plecoptera) (ROŚCISZEWSKA 1991a, 1991b). On the eggs of many insect species, the follicular cell imprint is only on a part of the chorion. In *Hydrometra stagnorum* L. (Heteroptera) the major part of the egg lacks any marks left by the follicular cells; however, some may be found on its anterior pole (SIMICZYJEW 1994).

The major and minor mushroom-like structures, formed of polysaccharides and peptides, occur on the surface of *Thermobia domestica* eggs. Similar structures are observed in insects which lay their eggs in packets (Neuroptera, Hymenoptera). In this case their role is to protect an air-space around each egg in order to facilitate adequate gas exchange inside the packet (HINTON 1969). Mushroom-like structures are also present on the chorion of *Psorophora columbiae* (Dyar and Knab) (Diptera), where they serve a catching purpose (BOSWORTH *et al.* 1998), and also in the mosquito *Anopheles albitarsis* Lynch-Arribalzaga (Diptera: Culicidae) (MONNERAT *et al.* 1999). The authors suggest that mushroom-like structures on the ventral side of mosquito eggs form a specific "float", allowing the egg to survive on the surface



Figs 1-6. Fig. 1. 7-day-old embryo of *Thermobia domestica* seen through the thin egg capsule. Yolk (y), embryo (asterisk), Light microscope. Bar = 15 μm . Fig. 2. Characteristic network of polygons on the surface of the chorion. Light microscope, chlorazol E. Bar = 1.4 μm . Fig. 3. Characteristic polygons and mushroom-like structures (arrow) seen on the egg capsule surface. Light microscope, Nomarski optics. Bar = 1.5 μm . Fig. 4. Egg of *Thermobia domestica*. The micropylar opening (arrow) on the anterior pole of the egg, SEM. Bar = 8.5 μm . Fig. 5. Anterior part of the egg capsule with the micropylar opening (arrow), SEM. Bar = 4 μm . Fig. 6. The part of the chorion with smaller (arrowhead) and larger (arrow) mushroom-like structures, SEM. Bar = 1.2 μm .

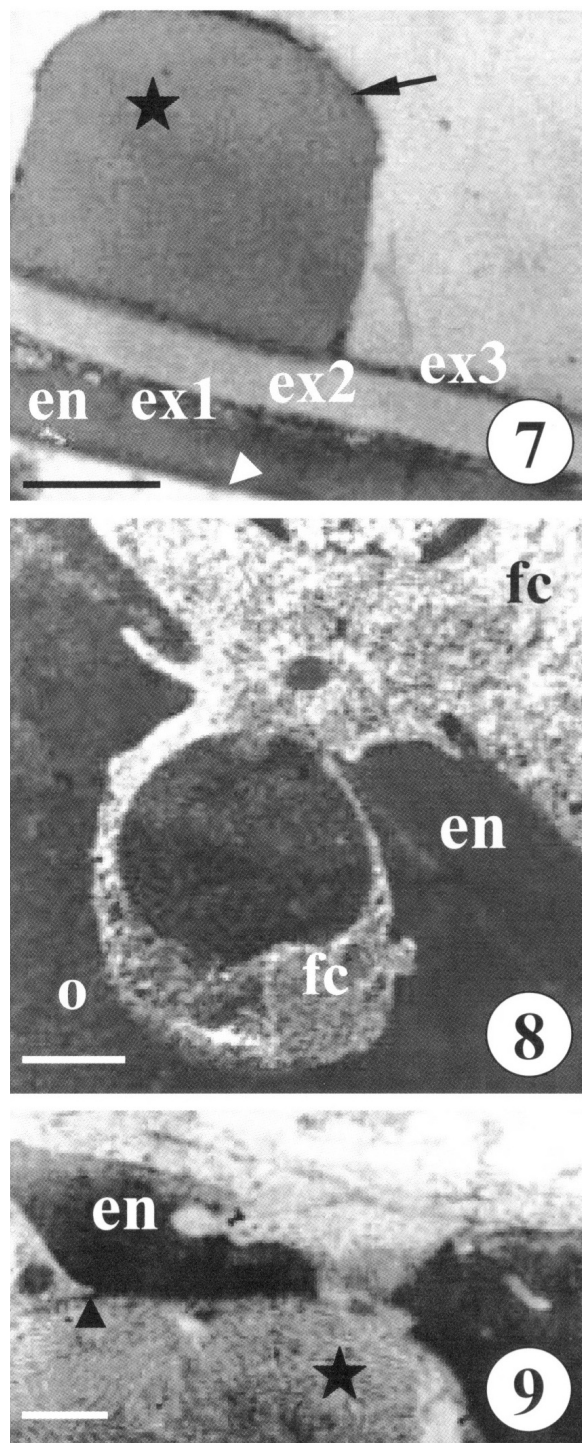


Fig. 7. A fragment of the longitudinal section of the chorion of *Thermobia domestica*: (arrowhead) vitelline envelope, (en) endochorion, (ex1) internal layer of exochorion, (ex2) intermediate layer of exochorion, (ex3) external layer of exochorion, (asterisk) mushroom-like structure, (arrow) layer of electron dense material covering the mushroom-like structure, TEM. Bar = 0.3 μ m. Fig. 8. The micropylar opening forming on the process of a follicular cell (fc). (en) endochorion, (o) oocyte, TEM. Bar = 1 μ m. Fig. 9. The specific structure (asterisk) located in the ooplasm in the neighbourhood of the micropyle at the anterior pole of the egg, (arrowhead) vitelline envelope, (en) endochorion, TEM. Bar = 0.3 μ m.

Table 1

The chemical compounds of the different parts of the egg capsule of *Thermobia domestica*

Part of the egg capsule	BPB	PAS	Sudan black B
Vitelline envelope	+++	+++	+
Endochorion	+	+	–
Internal layer of the exochorion	+	+	–
Middle layer of the exochorion	++	++	–
External layer of the exochorion	+++	+++	++
Mushroom-like structures	+++	+++	–
Thin layer surrounding the mushroom-like structures	+++	+++	–

– negative reaction

+ weak-positive reaction

++ medium-positive reaction

+++ strong-positive reaction

of the water. The egg surface in *Orthesia* (Hemiptera: Coccinea) is covered with spiral wax filaments (MARGARITIS & MAZZINI 1998; VOGELGESANG & SZKLARZEWCZ 2001). The function of these structures is not known. In *Heteromorbus nitidus* Templeton (Collembola) and *Hypogastrura succinea* Gisim (Collembola), the chorion surface possesses numerous granules (LARINK & BILINSKI 1989). In *Thermobia domestica* the mushroom-like structures probably take part in adhering the chorion on a rough surface. This facilitates hatching for larvae. The egg capsule of the studied species does not have the operculum and the hatching line. The embryo leaves the egg by breaking the capsule with an embryonic tooth („Eizahn”) located on the head (KLAG 1971). The observations (KLAG 1971) also showed that on a smooth surface the larva may break the egg capsule, but it is not capable of leaving it and in consequence dies. Except for mushroom-like structures, which may facilitate the fastening of the egg to the underlying surface, the surface of the egg capsule in *Thermobia domestica* does not possess any other attachment structures typical for other insect species. These structures may be developed in a varied manner, such as discs in *Perla marginata* (ROŚCISZEWSKA 1991a), attachment processes as in Mallophaga (ZAWADZKA *et al.* 1997), threads ending with disks as in some ephemeropterans (MAZZINI & GAINO 1988; POPRAWA & ROST 2001). No attachment structures were observed in some species laying eggs into water, such as *Leuctra autumnalis*

Aubert (Plecoptera) (POPRAWA *et al.* 2002) or *Protonemura intricata* Ris (Plecoptera) (ROŚCISZEWSKA 1996). They are equipped with adhesive layers, which fasten eggs to substrate and protect them against damage.

A micropylar apparatus may be located on the egg anterior pole, as for example in *Ischnura elegans* Van der Linden (SCHANZ 1965), on the egg posterior pole as in representatives of Isoptera (TRUCKENBRODT 1964), or on its lateral sides as in *Acheta domestica* L. (Diptera) (SAUER 1966). The number of micropylar openings is species-specific and it is believed that the evolution of the micropylar system has led to an increase in the number of micropylar openings. In *Heteromorus nitidus* and *Hypogastrura succinea* there are no micropylar openings (LARINK & BILIŃSKI 1989). In *Thermobia domestica* there is one micropylar opening, in Ephemeroptera 3-4 (MAZZINI & GAINO 1988), and in more advanced Heteroptera there are up to 70 openings (SOCHA 1988).

No specialized gas exchange structures, as in the egg capsule of *Thermobia domestica*, were observed in *Eosentomon* (Protura) and *Hypogastrura succinea* (LARINK & BILIŃSKI 1989). This suggests that in *Thermobia domestica*, similar to other described Zygentoma species, gas exchange occurs by diffusion through the egg capsule.

The egg capsule of the studied species consists of a vitelline envelope and a chorion comprised of an endo- and exochorion. The presence of lipids in the vitelline envelope of *Thermobia domestica* is closely connected to the function of this layer. Females lay eggs on the ground so the vitelline envelope protects developing embryos against desiccation.

The chorion layers differ in electron density, and also in their chemical composition. Both the endochorion and exochorion consist of polysaccharides and peptides. The external layer of the exochorion contains lipid components, not observed in the endochorion. The exochorion may, as the vitelline envelope, protect the embryo against excessive loss of water. This seems obvious because the embryos develop at a relatively high temperature and in a dry environment.

WOODLAND (1957) reports that the chorion in *Thermobia domestica* is smooth, does not have any processes, and is equipped with a single micropylar opening. However, our electron microscopy studies (TEM and SEM) showed the presence of mushroom-like structures on its surface, and the histochemical studies revealed its complex chemical composition. The presence of mushroom-like structures, which probably take part in fastening the egg to roughnesses of the substrate, explain why in laboratory conditions on the

smooth surface of Petri dishes, larvae cannot leave their egg capsules and die.

The results presented in this study add information on the structure of the egg capsule in Zygentoma insects and it may be concluded that even a non-complicated egg capsule adequately protects the embryo in its environment.

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References

- BILIŃSKI S. M., KLAG J. 1977. The oogenesis in *Acerentomon gallicum* Jonescu (Protura). An ultrastructure analysis of chorion formation. Acta Biol. Cracov., Ser. Zool. **20**: 101-106.
- BILIŃSKI S. M., LARINK O. 1989. Fine structure of the egg envelope and the supporting stalk in the dipluran *Campodea* (Apterygota, Campodidae). Int. J. Insect Morphol. Embryol. **18**: 199-204.
- BILIŃSKI S. M., JANKOWSKA W. 1987. Oogenesis in the bird louse *Eomenacanthus stramineus* (Insecta, Mallophaga). I. General description and structure of the egg capsule. Zool. Jb. Abt. Anat. **116**: 1-12.
- BILIŃSKI S. M., SZKLARZEWICZ T. 1987. Ultrastructural modifications of the follicular epithelium accompanying the onset of vitellogenesis in the whirligig beetle, *Gyrinus nator*. Cell Tissue Res. **249**: 209-214.
- BLAU H. N., KAFATOS F. C. 1978. Secretory kinetics in the follicular cells of silkmoths during eggshell formation. J. Cell Biol. **78**: 131-151.
- BOSWORTH A. B., MEOLA S. M., THOMPSON M., OLSON J. K. 1998. Chorionic morphology of eggs of the *Psorophora* *confinis* complex in the United States. II. Pre- and postdeposition studies of *Psorophora columbiae* (Dyar and Knab) eggs. J. Am. Mosquito Contr. **14**: 46-57.
- CARAYON J. 1969. Emploi du noir chlorazol en anatomie microscopique des insectes. Ann. Soc. Entomol. Fr. **5**: 179-193.
- COBBEN R. H. 1968. Evolutionary Trends in Heteroptera. Part I. Eggs, architecture of the shell, gross embryology and eclosion. Centre for Agricultural Publishing and Documentation, Wageningen.
- DYKSTRA M. J. 1992. Biological Electron Microscopy. Theory, techniques and troubleshooting. Plenum Press, New York & London. Pp. 171.
- HEYMONS R., HEYMONS H. 1905. Die Entwicklungsgeschichte von *Machilis*. Verh. Dt. Zool. Ges. **15**: 123-135.
- HINTON H. E. 1969. Respiratory systems of insect egg shells. Ann. Rev. Entomol. **14**: 343-369.
- KLAG J. 1971. The biology of *Thermobia domestica* (Pack.) (Thysanura) in the laboratory culture. Zeszyty Naukowe UJ. **17**: 7-28. (In Polish).
- LARINK O., BILIŃSKI S. M. 1989. Fine structure of the egg envelopes of one proturan and two collembolan genera (Apterygota). Int. J. Insect Morphol. Embryol. **18**: 39-45.
- LITWIN J. A. 1985. Light microscopic histochemistry on plastic sections. Progr. Histochem. Cytochem. **16**: 1-84.

- MARGARITIS L. H. 1985. Structure and physiology of the egg-shell. (In: Kerkut, G. A. and Gilbert, L. J. eds, Comprehensive Insect Physiology, Biochemistry and Pharmacology, vol. I. Embryogenesis and Reproduction. Pergamon, Oxford): 153-230.
- MARGARITIS L. H., MAZZINI M. 1998. Structure of the egg. (In: Microscopic Anatomy of Invertebrates: Insecta. Wiley-Liss Inc., vol. 11C) **39**: 995-1037.
- MAZZINI M., GAINO E. 1988. Oogenesis of the mayfly *Habrophlebia eldea*: synthesis of vitelline and chorion envelopes. Gamete Res. **21**: 439-450.
- MONNERAT A. T., SOARES M. J., PEREIRA LIMA J. B., ROSA-FREITAS M. G., VALLE D. 1999. *Anopheles albitarsis* eggs: ultrastructural analysis of chorion layers after permeabilization. J. Insect Physiol. **45**: 915-922.
- POPRAWA I., BARAN A., ROŚCISZEWSKA E. 2002. Structure of ovaries and formation of egg envelopes in the stonefly *Leuctra autumnalis* Aubert, 1948 (Plecoptera: Leuctridae). Ultrastructural studies. Folia biol. (Kraków) **50**: 29-38.
- POPRAWA I., ROST M. 2001. The structure of insects egg capsule and strategies of oviposition. Przegląd Zool. **XLV**: 7-20. (In Polish).
- REGIER J. C., KAFATOS F. C. 1985. Molecular aspects of chorion formation. (In: Kerkut G. A., Gilbert L. J. eds. Comprehensive Insect Physiology, Biochemistry and Pharmacology, vol. I. Embryogenesis and Reproduction. Pergamon, Oxford): 113-152.
- REYNOLDS E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. **17**: 208-212.
- ROŚCISZEWSKA E. 1991a. Ultrastructural i histochemical studies of the egg capsules of *Perla marginata* (Panzer, 1799) and *Dinocras cephalotes* (Curtis, 1827) (Plecoptera: Perlidae). Int. J. Insect Morphol. Embryol. **20**: 189-203.
- ROŚCISZEWSKA E. 1991b. Morphological changes developing after oviposition on egg capsule surface of *Isoperla rivulorum* (Plecoptera: Perlodidae). Zool. Jb. Anat. **121**: 253-258.
- ROŚCISZEWSKA E. 1995. Oogenesis of Stone Flies. Development of the follicular epithelium and formation of the egg-shell in ovaries of *Perla marginata* Panzer and *Perla pallida* Guérin (Plecoptera: Perlidae). Int. J. Insect Morphol. Embryol. **24**: 253-271.
- ROŚCISZEWSKA E. 1996. Egg capsule structure of the stonefly *Protonemura intricata* (Ris, 1902) (Plecoptera: Nemuridae). Acta Biol. Crac., Ser. Zool. **XXXVIII**: 41-49.
- SAHRHAGE D. 1953. Ökologische Untersuchungen an *Thermobia domestica* (Packard) und *Lepisma saccharina* L. Zeitschr. Wiss. Zool., Leipzig **157**: 77-168.
- SAUER H. W. 1966. Zeitraffer-Mikro-Film-Analyse Embryonaler Differenzierungsphasen von *Gryllus domesticus*. Zeit. Okol. Natur. **56**: 143-251.
- SCHANZ G. 1965. Entwicklungsvorgänge im Ei der Libelle *Ischnura elegans* und Experimente zur Frage ihrer Aktivierung. Eine Mikro-Zeitraffer-Film-Analyse. Inaugural-Dissertation Marburg/Lahn. 92.
- SHAROV A. G. 1953. Razvitye shchetinokhvostok (Thysanura, Apterygota) v svyazi s problemoi filogenii nasekomykh. Trudy Inst. Morf. Zhivotnykh. **8**: 63-127.
- SIMICZYJEW B. 1994. Egg morphology and chorion fine structure of *Hydrometra stagnorum* (Heteroptera). Zool. Pol. **39**: 79-86.
- SIMICZYJEW B. 1995. Insects egg shells – structure and formation. Przegląd Zool. **39**: 213-225. (In Polish).
- SOCHA R. 1988. Altered anterioposterior polarity of micropyle ring formation in the eggs of *Pyrrhocoris apterus* L. (Heteroptera: Pyrrhocoridae). Int. J. Insect Morphol. Embryol. **17**: 135-143.
- SZKLARZEWICZ T. 1989. Ultrastructural studies on the vitellogenesis of *Nicoletia phytophila* (Zygentoma, Nicoletidae). Zool. Jb. Abt. Anat. **118**: 483-491.
- TRUCKENBRODT W. 1964. Zytologische und Entwicklungsphysiologische Untersuchungen am Besanten und am Parthenogenetischen Ei von *Kaloterme flavicollis* Fabr. Zool. Jb. Anat. **81**: 359-434.
- VOGELGESANG M., SZKLARZEWICZ T. 2001. Formation and structure of egg capsules in scale insects (Hemiptera: Coccinea). I. Ortheziidae. Arthr. Struct. Dev. **30**: 63-68.
- WOODLAND J. T. 1957. A contribution to our knowledge of lepidematid development. J. Morphol. **101**: 523-577.
- ZAWADZKA M., JANKOWSKA W., BILIŃSKI Sz. M. 1997. Egg shells of mallophagans and anoplurans (Insecta: Phthiraptera): morphogenesis of specialized regions and the relation to F-actin cytoskeleton of follicular cells. Tissue Cell **29**: 1-9.