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Embryonic Molt Numbers in Hemimetabolous and Holometabolous Insects

Число эмбриональных линек у насекомых с неполным (Hemimetabola) и полным (Holometabola) превращением

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KEY WORDS: metamorphosis, Hemimetabola, Holometabola, pronymph, embryonic development, cuticle, ontogenesis.

КЛЮЧЕВЫЕ СЛОВА: метаморфоз, Hemimetabola, Holometabola, пронимфа, эмбриональное развитие, кутикула, онтогенез

ABSTRACT. Embryonic molts reflect free-living ancestral stages and facilitate the study of embryonization, the compressing of post-embryonic stages into the egg. In Hemimetabola, three cuticles are formed during embryonic development. Cuticles 1 and 2 are purely embryonic, while cuticle 3, also formed *in ovum*, becomes the first-instar nymphal cuticle. Holometabolism involves a single embryonic cuticle, same as the first-instar cuticle.

РЕЗЮМЕ. Изучение эмбриональных линек, отражающих особенности свободноживущих предковых стадий, даёт ключ к пониманию процессов эмбрионизации, т.е. прохождения отдельных стадий постэмбрионального развития под защитой яйцевых оболочек. У Hemimetabola в процессе эмбрионального развития кутикулярный покров формируется трижды. При этом первая и вторая кутикула являются исключительно эмбриональными, а третья, несмотря на то, что также формируется *in ovum*, представляет собой покровы нимфы первого возраста. У Holometabola формируется только одна эмбриональная кутикула, которая одновременно является кутикулой личинки первого возраста.

Introduction

Insect ontogeny studies are driven by the quest for the origins of the incomplete vs. complete metamorphosis that goes back to the Antiquity and by the desire to correlate the developmental stages between hemi- and holometabolous insects [Yezhikov, 1929; Chaika, 2003].

In the winged insects (Pterygota), the various developmental stages differ greatly, which means their postembryonic development involves metamorphosis. Based on how radical such metamorphosis is, two major groups are recognized among the Pterygota: Hemimetabola (with incomplete metamorphosis) and Holometabola (complete metamorphosis) [Ivanova-Kazas, 1981].

In the promising model developed by Truman and Riddiford [1999], the holometabolous larva is homologous to the hemimetabolous pronymph (pre-larva) that may show various degrees of embryonization and, therefore, various free-living times. The hemimetabolous nymph should be thus considered homologous to the holometabolous pupal stage. This model is supported by the findings of developmental biology, metamorphosis patterns and the presence of a pronymphal stage in Hemimetabola.

The degree of embryonization in a taxon may be established based on the outcome of embryogenesis and the presence of embryonic cuticles/molts [Polivanova, 1979]. We studied embryonic molting and integuments in the embryo and free-living stages of representative members of Holometabola and Hemimetabola.

Materials and Methods

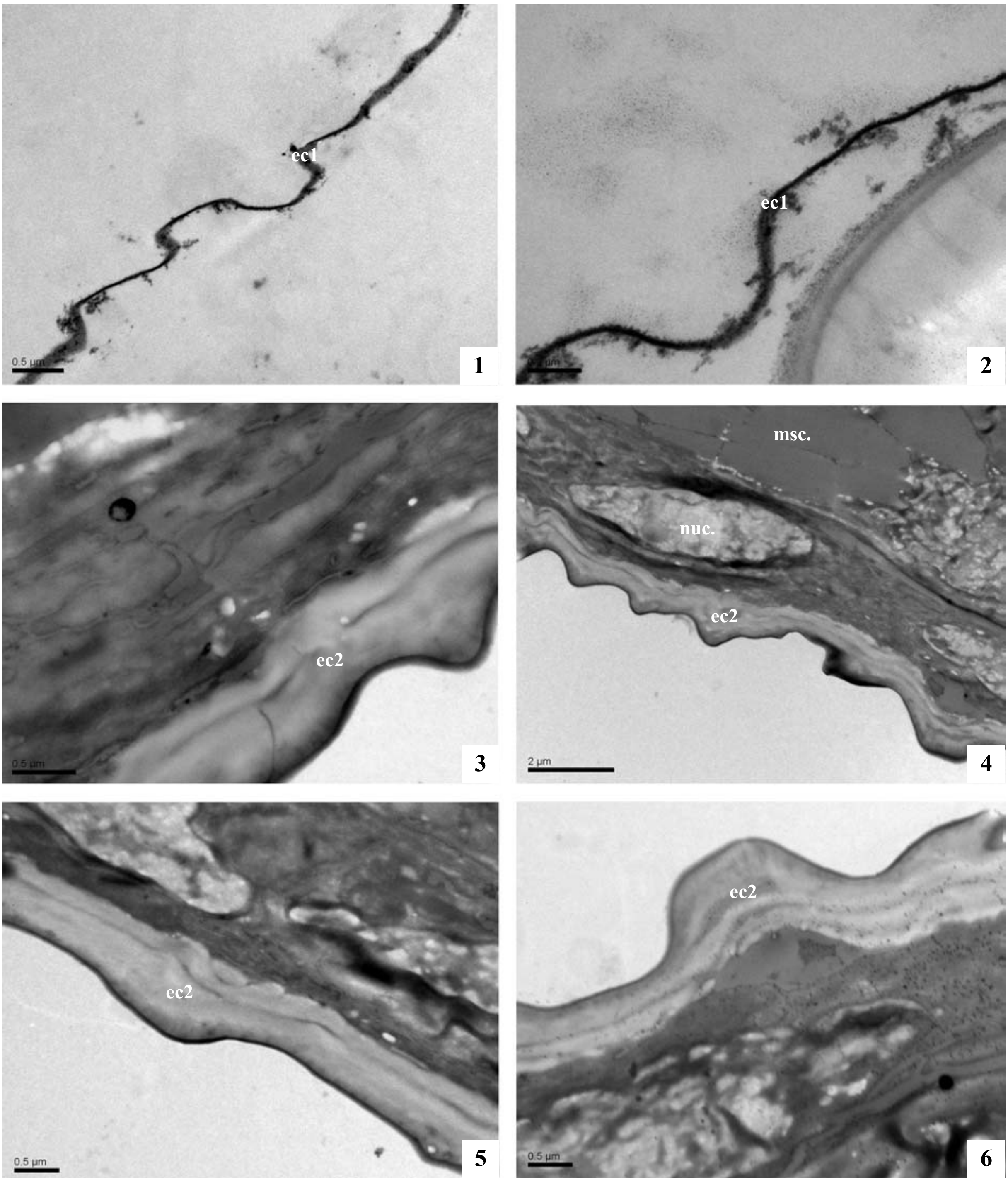
Two species, one hemimetabolous and one holometabolous, were studied: (i) *Locusta migratoria* L. (Hemimetabola: Orthoptera: Acrididae), the migratory locust, whose life cycle includes a vermiform larva representing a pronymphal stage; and (ii) *Lucilia caesar* L. (Holometabola: Diptera: Calliphoridae), the greenbottle fly — an advanced, less embryonized insect.

For electron microscopy, the samples were kept in 2.5% glutaraldehyde in phosphate buffer (pH 7.3) for 4 hours, then in 2% osmium tetroxide for 2 hours, then dehydrated in a battery of EtOH concentrations. The samples were opacified in saturated uranyl acetate in 70% EtOH and, after complete dehydration, embedded in Epon following the standard protocol. Ultrathin slices stained using the Reynolds technique were examined under JEM-1011 transmission electronic microscope with Gatan ES800W digital cell.

Results

Locusta migratoria

The locust produces three cuticles in the course of embryonic development: embryonic cuticle 1 (EC1), embryonic cuticle 2 (EC2), and the third cuticle that eventually becomes the first-instar integument.

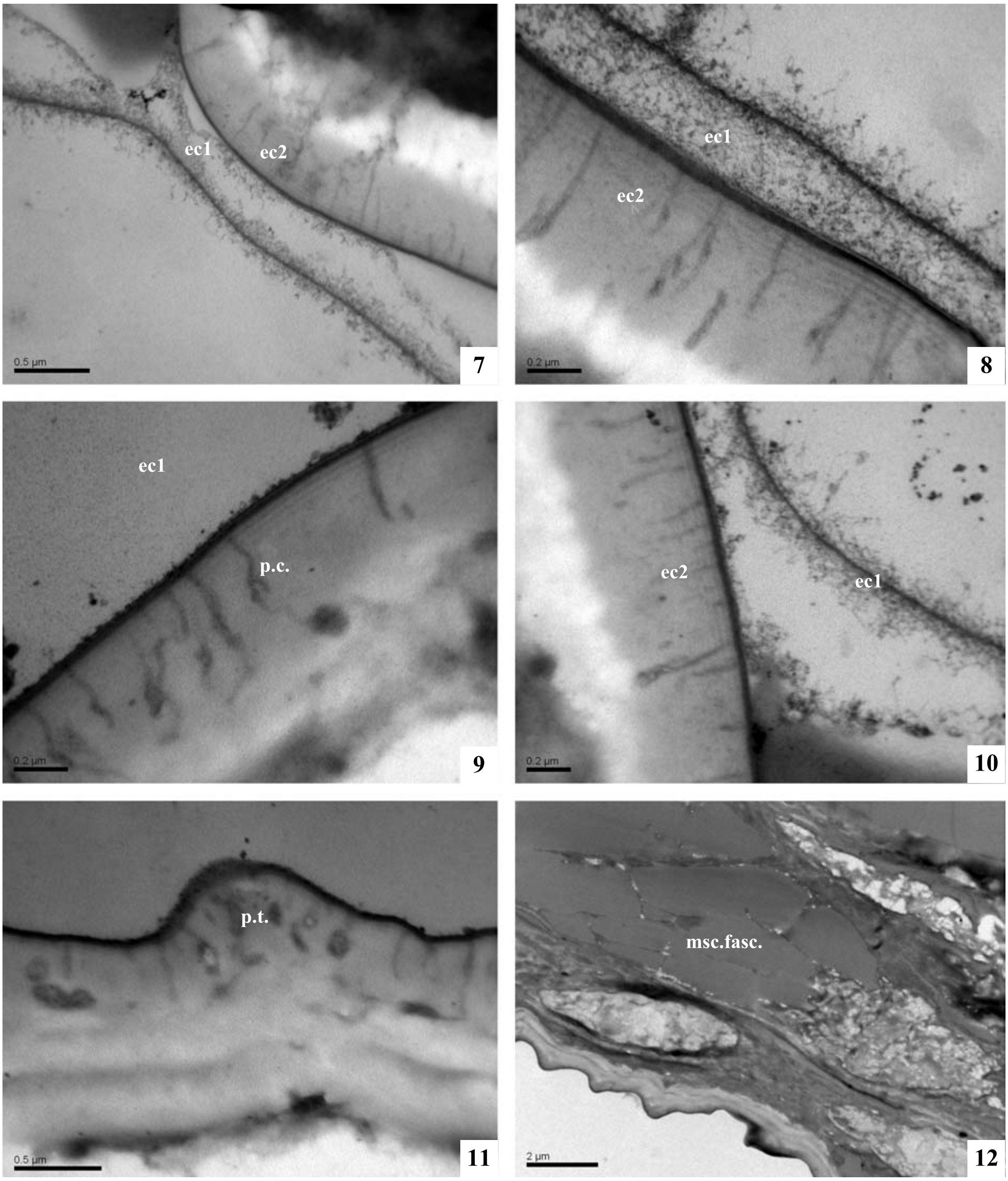


Figs 1–6. Embryonic cuticles of *Locusta migratoria*: 1–2 — first embryonic cuticle; 3–6 — second embryonic cuticle.

Рис. 1–6. Эмбриональные кутикулы саранчи *Locusta migratoria*: 1–2 — первая эмбриональная кутикула; 3–6 — вторая эмбриональная кутикула.

The EC1 appears at the limb formation stage as a 0.02 to 0.03 μm thick single-layer high electron density homogeneous cuticle (Fig. 1). The high electron density suggests its epicuticular nature — most likely, the cuticuline layer. The EC1 shows no aciculae or other outgrowths (Fig. 2) and disappears after the EC2 is fully formed.

During the EC2 formation, the embryonic integument is distinct and consists of three layers: the hypoderm, the basal membrane, and the cuticle. Individual hypodermal cells are barely distinguishable, but membrane structures in the cytoplasm are distinct (Fig. 3). The cells are short, slightly over 2 μm maximum height (Fig. 4), and contain

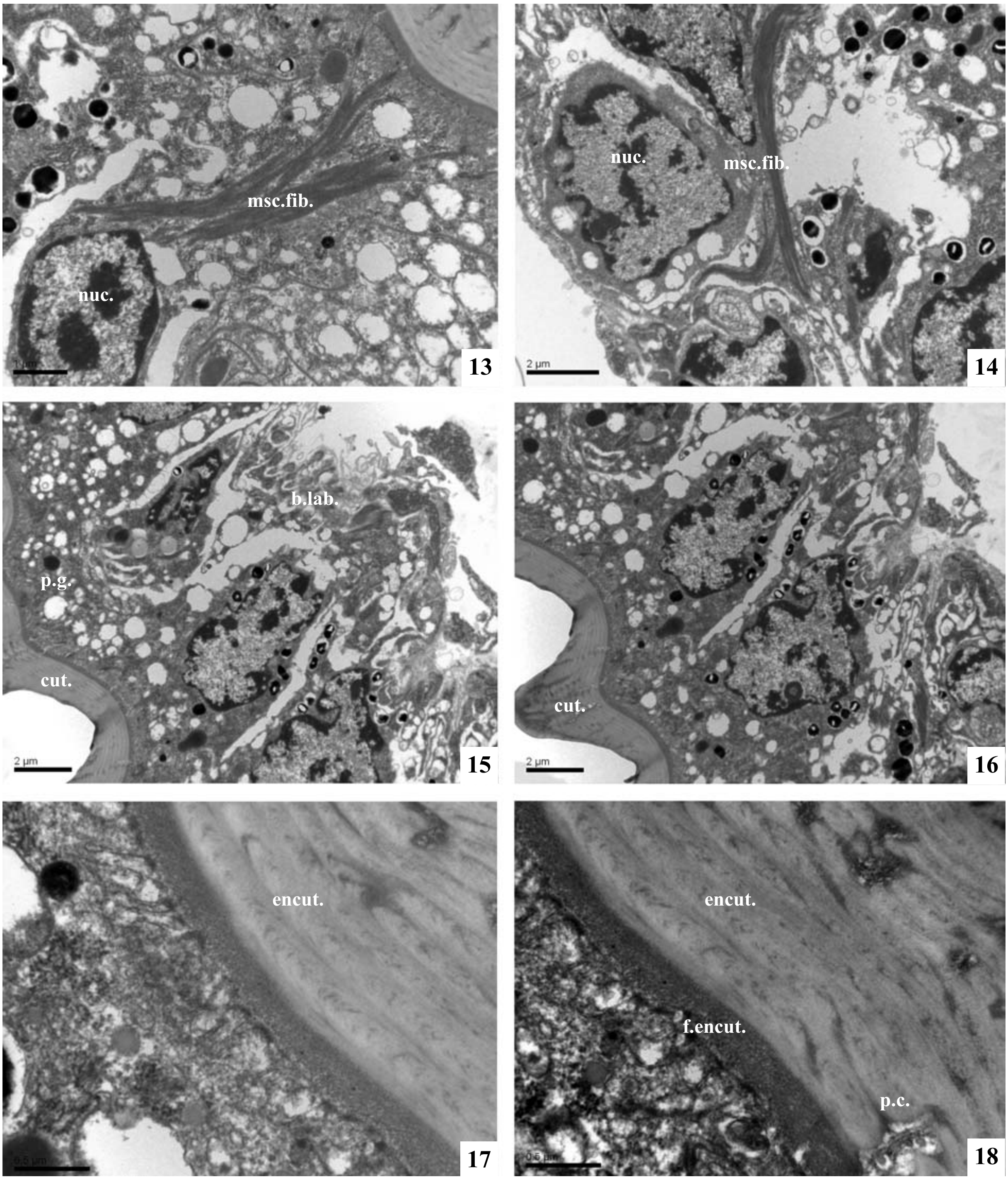


Figs 7–12. Second embryonic cuticle of *Locusta migratoria*: 7–8 — formation of the second embryonic cuticle; 9–11 — pore canals; 12 – muscle bundles.

Рис. 7–12. Вторая эмбриональная кутикула саранчи *Locusta migratoria*: 7–8 — формирование второй эмбриональной кутикулы; 9–11 — поровые каналы; 12 — пучки мышц.

large oblong nuclei up to 4 μm long. The cuticle is 0.8–1.5 μm thick, with two clearly distinguishable layers (Fig. 5). The outer, higher electron density layer is about 0.1 μm thick and is presumably homologous to the adult epicuticle. The second layer, less electron dense and up to 1.5 μm thick in some areas, shows several alternating higher/lower density sublayers and can be interpreted as procuticle (Figs 6–8). Pore canals, best visible in its outer part, are a typical feature of the procuticle (Figs 9–11). The integument has muscles attached to it (Fig. 12).

Once the embryonic development is completed, a vermiform larva (=pronymph) hatches.



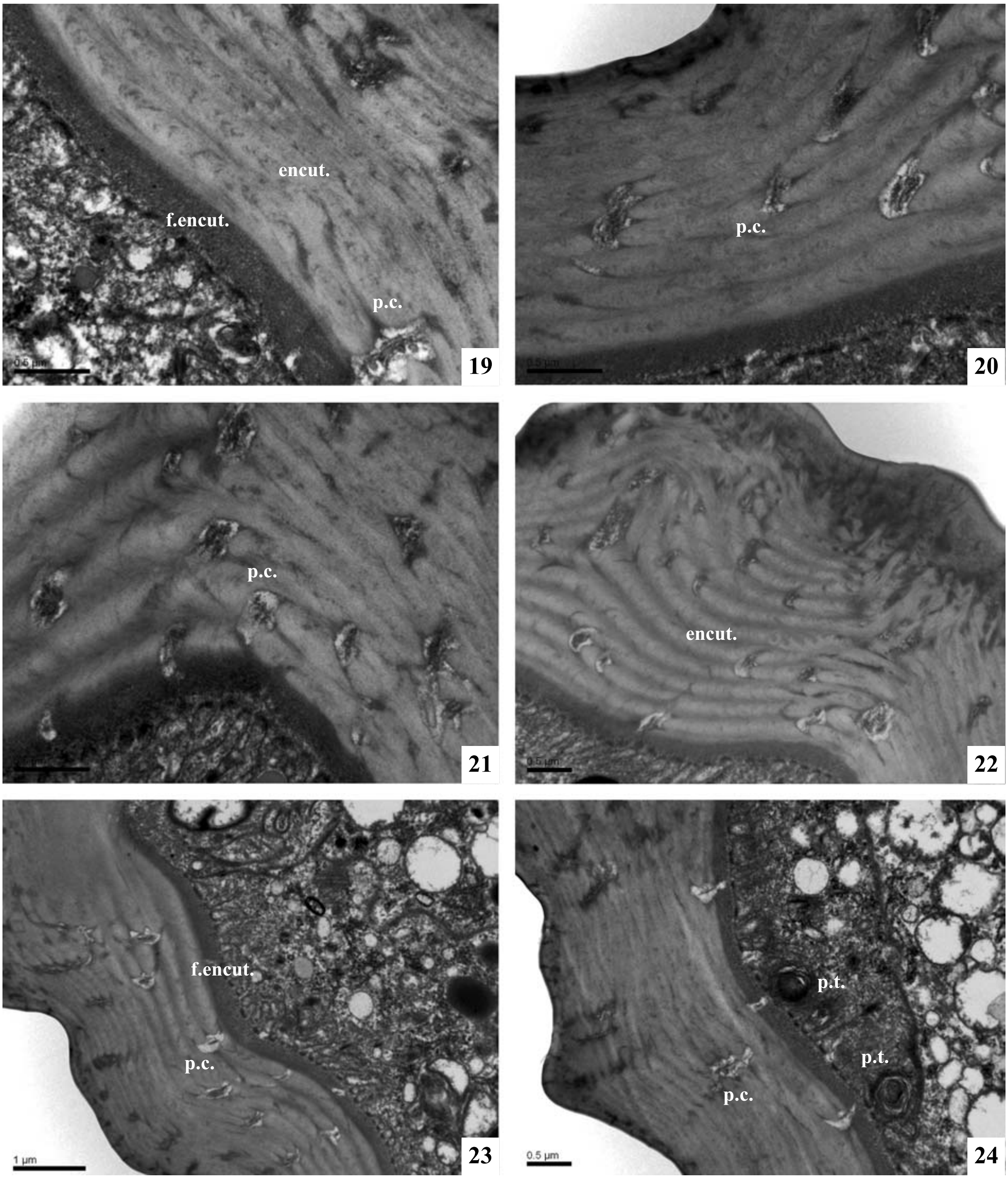
Figs 13–18. Structure of integument of instar I nymph of *Locusta migratoria*: 13–14 — ultrastructure of hypodermal cells; 15–16 — ultrastructure of integument; 17–18 — ultrastructure of endocuticle.

Рис. 13–18. Строение покрова нимфы I возраста саранчи *Locusta migratoria*: 13–14 — ультраструктура гиподермальных клеток; 15–16 — ультраструктура покрова; 17–18 — ультраструктура эндокутикулы.

Notably, the larva makes it to the surface fully covered with embryonic cuticle sculptured on the inside in a pattern matching (as a mould) the pattern of the underlying first-instar nymphal cuticle.

The vermiform phase lasts 2 to 5 minutes only; once in the open, the larva loses its embryonic cuticle ('intermediate molt') and turns into a first-instar nymph.

Unlike the embryonic cuticle, the first-instar cuticle shows a more elaborate pattern of subpentagonal cells, each with 2–5 small tubercles in the center. The entire body surface is covered with sensory organs (sensilla). The thoracic integument consists of an epithelial layer, a



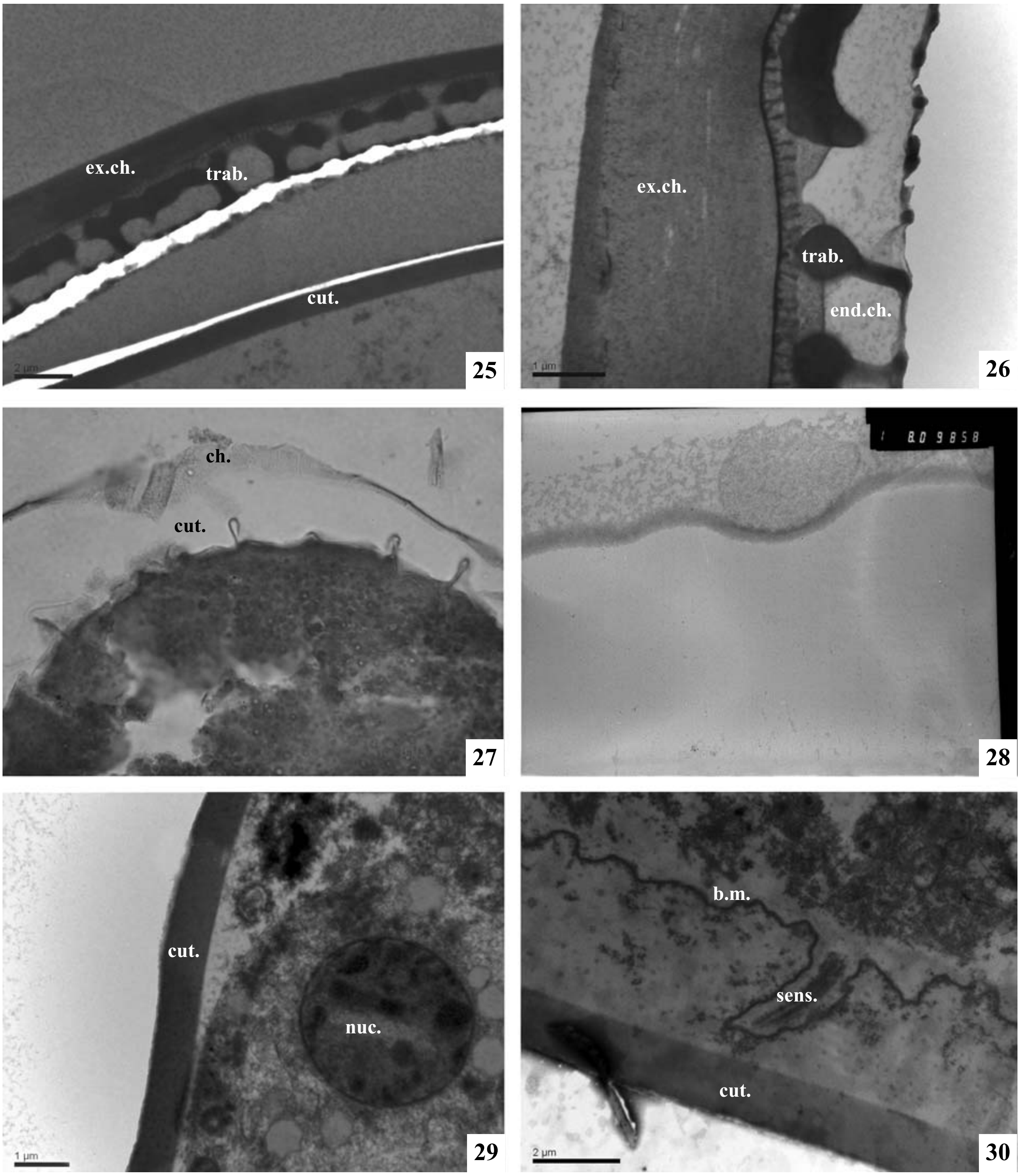
Figs 19–24. Structure of integument of instar I nymph of *Locusta migratoria*: 19–22 — ultrastructure of cuticle; 23–24 — ultrastructure of cuticle and apical part of hypodermal cells.

Рис. 19–24. Строение покрова нимфы I возраста саранчи *Locusta migratoria*: 19–22 — ультраструктура кутикулы; 23–24 — ультраструктура кутикулы и апикального отдела гиподермальных клеток.

basal membrane, and the cuticle. The epithelial cell, ca. 8 μm high, harbors a chromatin-containing nucleus located usually at the bottom (sometimes centrally or apically).

Besides the usual mitochondria, microtubules, vacuoles, and the granular endoplasmic reticulum, the cytoplasm contains high electron density globular granules (0.4–0.5 μm) and lower-density granules of similar or larger size. In the apical area, the hypodermal cells feature lysosome-like subglobular membranous elements.

Some cells contain long fibers running from the base to apex (Figs 13–14). Pore canals containing high-density rods strongly suggest that these are probably tonofibrillae connecting muscles with the cuticle.



Figs 25–30. Ultrastructure of coatings of *Lucilia caesar*: 25–26 — ultrastructure of chorion; 27 — cross slice of an egg; 28 — forming cuticle; 29 — formed cuticle; 30 — formation of sensilla.

Рис. 25–30. Ультраструктура покровов *Lucilia caesar*: 25–26 — ультраструктура хориона; 27 — поперечный срез яйца; 28 — формирующаяся кутикула; 29 — сформированная кутикула; 30 — формирование сенсиллы.

Cell apices and bases feature irregularly located projections or invaginations of cell membrane. Such invaginations are often well developed at the cell base and form the basal labyrinth (Figs 15–16).

Secretory cells scattered among the hypodermal cells are easily recognizable by their rather rounded shape and basal parts protruding beyond the hypodermal layer. Their cytoplasm is rich in inclusions ca. 2 μm in diameter. The 0.2 μm thick basal membrane appears homogenous, fine-grained, and of medium electron density.

The nymphal cuticle varies in thickness from 2 to 5 μm and has two easily visible layers, epicuticle and procuticle. The epicuticle is more electron dense, appears homogeneous and is 0.1–0.2 μm thick or up to 0.5 μm where associated with cuticular projections. Procuticle (Figs 17–19) has a layered structure, with nine layers in average, and conspicuous irregularly scattered pore canals, many with high-density muscle attachment rods (Figs 20–22).

Typical of this stage is a thin (0.2–0.3 μm) prominent granular layer between the bottom of the procuticle and the apical epithelial microvilli that appears on electron micrographs homogeneous and much darker than the procuticle (Figs 23–24). In areas where new cuticle layers are being formed, this layer appears transformed into a typical endocuticle with the helicoid lamellar arrangement. These areas can be seen only where the procuticle has been fully formed and the material is used to produce new procuticular layers, not the epicuticle. The tips of the microvilli touching this layer are high electron density — perhaps because this is where the cuticular material gets released, since there is no such material between the microvilli where the membranes show regular density. The procuticular layer appears at this stage not fully formed and thus has to be still growing.

Lucilia caesar

In *Lucilia caesar*, the 3.2 μm thick chorion is subdivided into a denser exochorion, a less dense endochorion, and clearly visible trabeculae (Figs 25–26). Fixed preparations show a 3.6 μm average gap between the embryo and the chorion (Fig. 27). A single embryonic cuticle is formed to eventually become the first-instar cuticle (Fig. 28). As the embryo develops, this cuticle changes. In freshly laid eggs, the cuticle is 0.25 μm thick, with no visible layers; the hypoderm, still underdeveloped, looks like a higher electron density (compared to other tissues) cell layer underlying the cuticle; 12 hours thereafter, the embryo is covered with a thin 0.8 μm cuticle (Fig. 29) that thickens as the embryo grows to reach 1.25 μm 24 hours after oviposition, at which point the basal membrane is clearly visible and closely underlies the hypoderm, with 3.2 μm gap between it and the embryonic cuticle. The cuticle has pore canals. At this stage, the sensory elements emerge at the surface of the cuticle (Fig. 30).

Discussion

In the Hemimetabola, three embryonic cuticles are produced, of which the third one becomes the cuticle of the first-instar nymph. The three cuticles differ greatly in structure. The thin single-layer EC1 can be considered epicuticle based both on its high electron density and the extensive electron microscopic studies suggest-

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ABBREVIATION

b.lab. — basal labyrinth
b.m. — basal membrane
cut. — cuticle
ec1 — first embryonic cuticle
ec2 — second embryonic cuticle
ec3 — third embryonic cuticle
msc. — muscles
msc.fasc. — muscular fascicles
msc.fib. — muscle fibrillae
p.g. — pigment granules
p.c. — pore canal
p.t. — pore tubes
sens. — sensilla
r.p.c. — rod of pore canal
trab. — trabecules
f.encut. — forming endocuticle
ch. — chorion
ex.ch. — exochorion
encut. — endocuticle
end.ch. — endochorion
nuc. — nucleus

УСЛОВНЫЕ ОБОЗНАЧЕНИЯ

b.lab. — базальный лабиринт
b.m. — базальная мембрана
cut. — кутикула
ec1 — первая эмбриональная кутикула
ec2 — вторая эмбриональная кутикула
ec3 — третья эмбриональная кутикула
msc. — мышцы
msc.fasc. — пучки мышц
msc.fib. — мышечные фибриллы
p.g. — пигментный гранулы
p.c. — поровые каналы
p.t. — поровые трубки
sens. — сенсиллы
r.p.ch. — стержень порового канала
trab. — трабекулы
f.encut. — формирующаяся эндокутикула
ch. — хорион
ex.ch. — экзохорион
encut. — эндокутикула
end.ch. — эндохорион
nuc. — ядро

ing that chitin-containing procuticle always exhibits lower electron density.

Unlike EC1, the EC2 consists of two distinct layers, homologous to the adult epicuticle and procuticle, respectively. The procuticle is further divided into sublayers and is pierced by numerous pore canals. Notably, the EC2 integument has muscles attached to it — the connection that does not occur before the EC2 had been fully formed. This is especially significant, since it is the EC2-stage embryo that is arguably homologous to the embryonized free-living ancestral larva.

The third embryonic cuticle, *i.e*. the larval cuticle, is about twice as thick as the EC2 and is also bi-layered, consisting of an epicuticle of higher electron density and a laminated (ca. nine-layered) procuticle. The procuticle is characterized by prominent pore canals with high electron density rods for muscle attachment. Unlike EC2, the third embryonic cuticle produces sensory cuticular elements of both mechano- and chemoreceptors.

How does the number of embryonic cuticles compare in Hemimetabola vs. Holometabola? This would require to find out how many embryonic cuticles the holometabolans have.

In *Lucilia caesar*, a higher dipteran, a single embryonic cuticle is formed to become the first-instar cuticle. As the embryo develops, the cuticle grows thicker, more elaborate, and produces the cuticular parts of the sensilla.

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