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MEDIAN EYE IN LARVAE OF *HYDRYPHANTES RUBER* (DE GEER, 1778) (ACARIFORMES: HYDRYPHANTIDAE)

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ABSTRACT: Median eye in larva of the water mite *Hydryphantes ruber* (De Geer, 1778) was investigated by means of light-optical and electron-microscopical (SEM and TEM) methods. The median eye is a single organ situated at the dorsal body surface under a slightly thickened and protruded cuticle in the middle of the dorsal shield. The median eye is a simply organized structure and is composed of small retinula cells with everted mostly isolated rhabdomeres looking to the cuticle. Each rhabdomere consists of irregularly arranged microvilli. The basal nuclear zones of these cells are occupied by electron-dense pigment granules. Two laterally situated nerves are composed of five axons each that suggest the paired origin of the median eye. Epidermal cells reach in lipid inclusions totally enclose the eye and are underlain by a flat basal lamina. Retinula cells contact to each other and to the epidermal cells by septate junctions. The larval median eye appears to be rather similar to median eye in adult mites of this species studied previously (Mischke 1981).

KEY WORDS: Parasitengona; water mites; photoreceptor organs; ultrastructure

INTRODUCTION

The presence of median eye is considered to be a primitive character among arachnids and in particular Acariformes (Mischke 1981; Alberti et al. 1991; Alberti and Coons 1999; Olomski 2012). Nevertheless, the median eye is distributed quite irregularly among families of acariform mites and mostly associated with the frontal body protuberance termed 'naso' (Grandjean 1958; Wachmann et al. 1974; Alberti 1975; Alberti and Coons 1999; Haupt and Coineau 2002). In many groups, the median eye is obviously reduced. Conversely, in fresh water mites the median eye is frequently present on anterior surface of the mite body (Mischke 1981; Olomski 2012). Moreover, R. Olomski showed that representatives of many families possess a paired median eye, whereas others demonstrate a single eye or the eye may be totally absent in derived groups. Based on these findings, R. Olomski (2012) has postulated that, in contrast with the main evolutionary trend in fusion of the median eyes in arachnids (Leonovich 2005), "the proposed division of the eye in the course of the evolution of the Euhydrachnidia can be regarded as evolutionary 'throwback'" (Olomski 2012, p. 455).

Hydryphantes ruber (De Geer, 1778), belonging to more primitive Hydrachnidia, is a single species among the highly diverse group of water mites, in which the ultrastructure of median eye is studied (Mischke 1981). This eye is organized simply consisting of several (up to five) receptor retinula cells with irregular everted rhabdomeres simultaneously possessing few pigment granules and of additional epidermal cells. There is no available morphological data on the median eyes in water mite larvae. It is considered that apart of the family Hydryphantidae, the unpaired median eye in larvae is not present (Sparing 1959). B.A. Vainstein (1980) has only mentioned a median eye in larvae of *H. ruber* (p. 107) referring to dark dots, whereas V. Prasad and D.R. Cook (1972), describing larval morphology, did not say anything about median eye in larvae of this species (p. 35).

Rearing water mites in the laboratory and based on the above mentioned reasons, we have obtained larvae of *H. ruber* and undertaken this study to clarify the ultrastructural organization of unpaired median eye in larvae of this water mite species.

MATERIALS AND METHODS

Mites, collecting site and laboratory observations

Adult mites (females) of *Hydryphantes ruber* (De Geer, 1778) were collected in May 2013 in the artificial lake Dubrovenskoye (54°47′N 31°56′E) — the widest part of the same river, the right tributary of the river Dnepr in the north vicinity of Smolensk city. The mites were placed separately in the same plastic 100 ml containers with pure bottled artesian water (certification of conformity N POCC RU.AE05.H02957, www.smolvoda.ru) distributed in Smolensk city. The containers were sterilized using a hot steam, and during usage they were kept un-tightly covered with a small Petri dish to allow the intake of air. Some time after capture, the mites produced egg masses (Fig. 1 A), from which larvae hatched later (Fig. 1 B). Live

specimens — adult mites, prelarvae and larvae — were examined and photographed with a Bresser Advance ICD dissecting microscope combined with a Levenhuk C NG digital camera.

Light-microscope observations

Active unfed larvae were placed on microscope slides in a drop of Faure-Berlese solution, covered with a cover-glass, dried up in a thermostat (60°C) for several hours and examined. These preparations were examined and photographed with a Leica DM LS-2 light-optical microscope equipped with a Leica EC-3 digital camera. Semi-thin sections of about 400 nm thick were stained with toluidine blue and examined with the same microscope.

Scanning Electron Microscope (SEM) examination

Unfed larvae, initially fixed in 70% ethyl-alcohol, were then passed through increased alcohol series and then treated with hexamethyldisilazane (HMDS) for 5–10 min for providing natural shape and size of the mite body as alternative method to critical point drying. Immediately after these procedures specimens were covered with a platinum layer in an Eiko IB-5 apparatus, and then examined with SEM Quanta 250 (FEI Company) at 10–20 kV.

Transmission Electron Microscope (TEM) examination

Larvae were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2–7.4) for several month, then washed in 0.2 M cacodylate buffer for 2 hours, postfixed in 2% osmium tetroxide in 0.1 M cacodylate buffer for 20 h, dehydrated in ethanol and acetone series, and finally embedded in an araldite mixture. Ultra-thin sections both in transverse and in sagittal planes to a mite body were made on a Leica UC-6 ultramicrotome and mounted on copper-rhodium grids with an oval hole provided with a formvar support. These sections, after staining with uranyl-acetate and lead citrate, were examined with TEM Morgagni 268-D (digital visualization) at 80 kV.

Confocal Laser Scanning Microscope (CLSM) observations

Larvae on microscope slides in Faure-Berlese solution were investigated for detection of chitin autofluorescence in a confocal laser scanning microscope Leica TSC SP 5 with an excitation wavelength of 488 nm.

All instrumental procedures were performed on the basis of the Centre of Collective Use "TAXON", Zoological Institute of the Russian Academy of Science, St.-Petersburg, Russia.

RESULTS

Median eye may be distinguished already in pharate larvae enclosed within the prelarval cuticle (Fig. 1 B). Observation of light-optical whole-mount preparations of unfed larvae reveals the median eye looking like single round spot located at the dorsal body surface (Fig. 1 C, D). This spot consists of small dense grains obviously representing pigment granules. The median eye is placed approximately at the middle of the weakly outlined triangular dorsal shield on the imaginary transverse line running between the paired lateral eyes expressed much more greatly (Figs. 1 D, 2 A). Importantly, that CLSM examination shows relatively moderate chitinization of the lateral eyes' corneae and, conversely, strong chitinization of the area of the dorsal shield including region of the median eye (Fig. 2 B–D) Semi-thin sections indicate the median eye as a small flattened pigmented organ situated just beneath a slightly convex cuticle of the dorsal shield (Fig. 2 E–F).

SEM study showed a conspicuous prominence at the middle of the dorsal shield that obviously manifests the presence of the median eye (Fig. 3 A–D). In this region, the surface of the dorsal shield is smooth with fine orifices of pore canals while surrounding body cuticle covered with tight epicuticular ridges which may come onto the shield from its margins (Fig. 3 D). Besides this prominence of the smooth cuticle, nothing indicates the availability of the median eye in these larvae from the external observation. In contrast to the median eye, the paired lateral eyes are expressed much stronger (Fig. 3 B–D) demonstrating not only double-convex corneae but also the developed pigment cup (see below). It is of interest that the anterior pair of the lateral eyes is developed greatly than the posterior one showing intensively protruding cornea (Fig. 3 C–D). The detailed observation of the lateral eyes is beyond the scope of the present work.

TEM study reveals that the median eye is a very small organ consisting of few compact sensitive/retinula cells arranged in one row under a sclerotized cuticle of the dorsal shield (Figs. 4 A–B, 5). The cuticle in this region, built of weakly lamellar electron-clear procuticle, is smooth but does not form cornea and is only slightly thick-

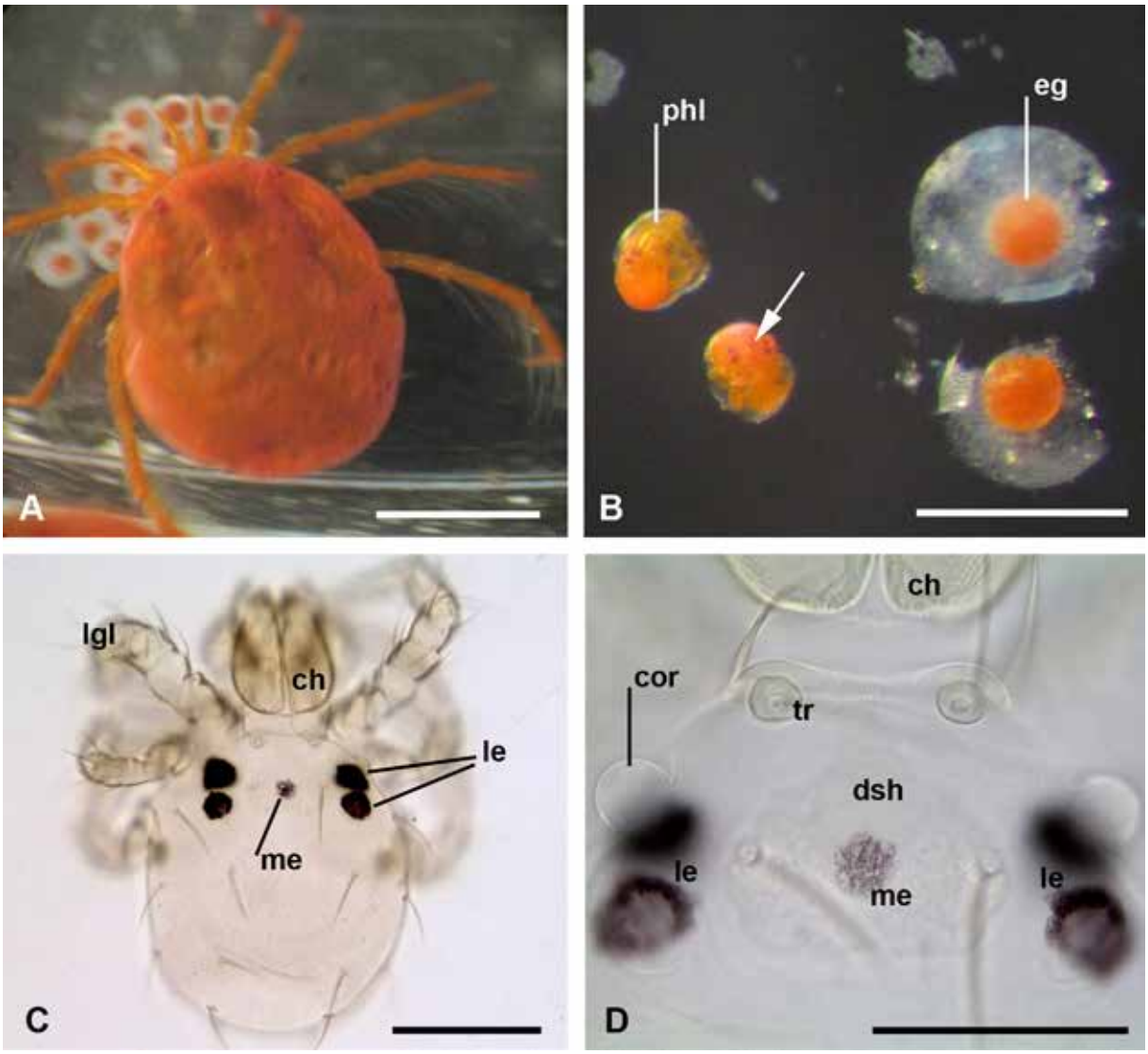

Fig. 1 (A–D). External view of the life stages (A–B) and light-optical identification of the median eye in larvae of *H. ruber* on whole mount preparations (C–D). A — Dorsal view of female and egg mass at its ventral body side. Scale bar — 1 mm. B — Separate eggs in an accompanied substance and pharate larvae still enclosed within the prelarva cuticle. *Arrow* indicates median eye. Scale bar — 0.5 mm. C — General view of larva showing the lateral and the median eyes. Scale bar — 100 µm. D — Region of the dorsal shield showing the intensively pigmented lateral eyes and the median eye with a lesser intensive pigmentation. Scale bar — 50 µm.

Abbreviations on Figures

bl — basal lamina; br — brain (synganglion); ca — capitular hemocelic; ch — chelicerae; cor — cornea; cut — cuticle; dsh — dorsal shield; ec — epidermal cell; eg — egg; hs — haemocoelic space; ic — infracapitulum; le — lateral eyes; lgI — leg I; ly — lysosome; m — mitochondrion; me — median eye; n — nucleus; nec — nucleus of epidermal cell; pc — pore canal; pg — pigment granules; phl — pharate larva; rc — retinula cell; rh — rhabdomere; sj — septate junction; sg — salivary gland; tr — trichobotrium.

ened (not more than on around 10%) and convex a little in comparison with the adjacent area of the shield (Fig. 4 A–B). The procuticle is pierced by wide pore canals (Fig. 4 D).

The median eye is totally enclosed within the epidermal tissue, which underlies the body cuticle (Figs. 4 A, 5). The single-layered epidermis is composed of flattened epidermal cells, which contain compact oval nuclei with large amount of heterochromatin applied to the nuclear envelope and numerous round electron-clear lipid inclusions (Fig. 4 A–C). These cells envelope retinula cells of the median eye by their thin processes and are not delimited from the latter by the basal lamina. Conversely, the basal lamina underlies the epidermis together with the median eye as a separate organ thus delimiting it from the underlying tissue — tightly opposed salivary glands (Fig. 4 A–C). Processes of the epidermal cells extending between the rhabdomeres and the cuticle of the dorsal

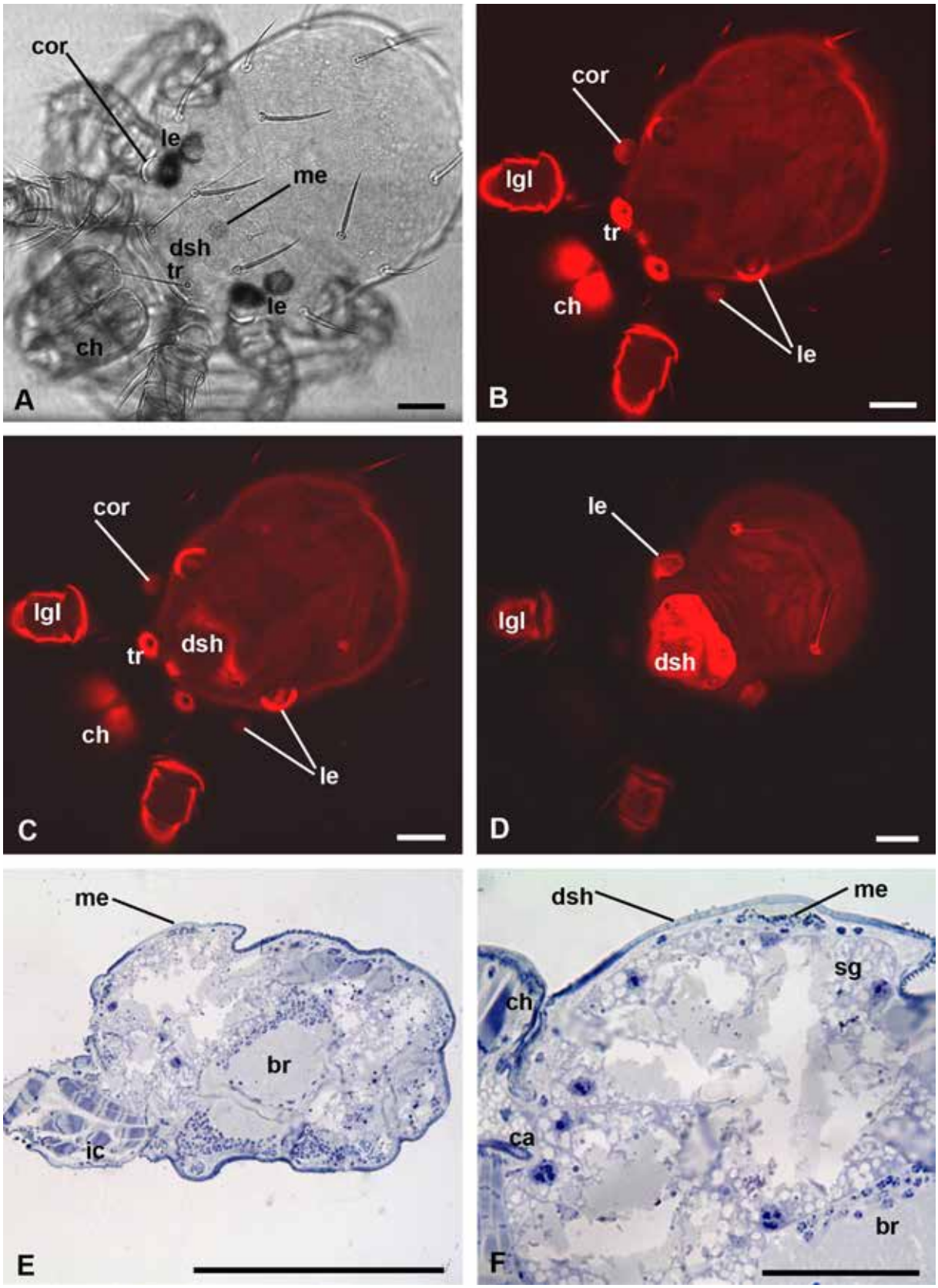

Fig. 2 (A–F). Confocal laser scanning microscopy (CLSM) (A–D) and sagittal semi-thin toluidine blue stained sections (E–F) of larvae *H. ruber*. A — Optical section of larva without laser inclusion on the level of median eye. Scale bar — 25 μm. B–D — Sequential optical sections of larva upwards from below showing fluorescence of the most chitinous places. Scale bar — 25 μm. E — General view of the larva longitudinal section showing the median eye situated at the dorsal body surface beneath the cuticle of the dorsal shield. The chelicerae are lost during sectioning. Scale bar — 100 μm. F — Antero-dorsal body region with the median eye placed just beneath the dorsal shield. Scale bar — 25 μm.

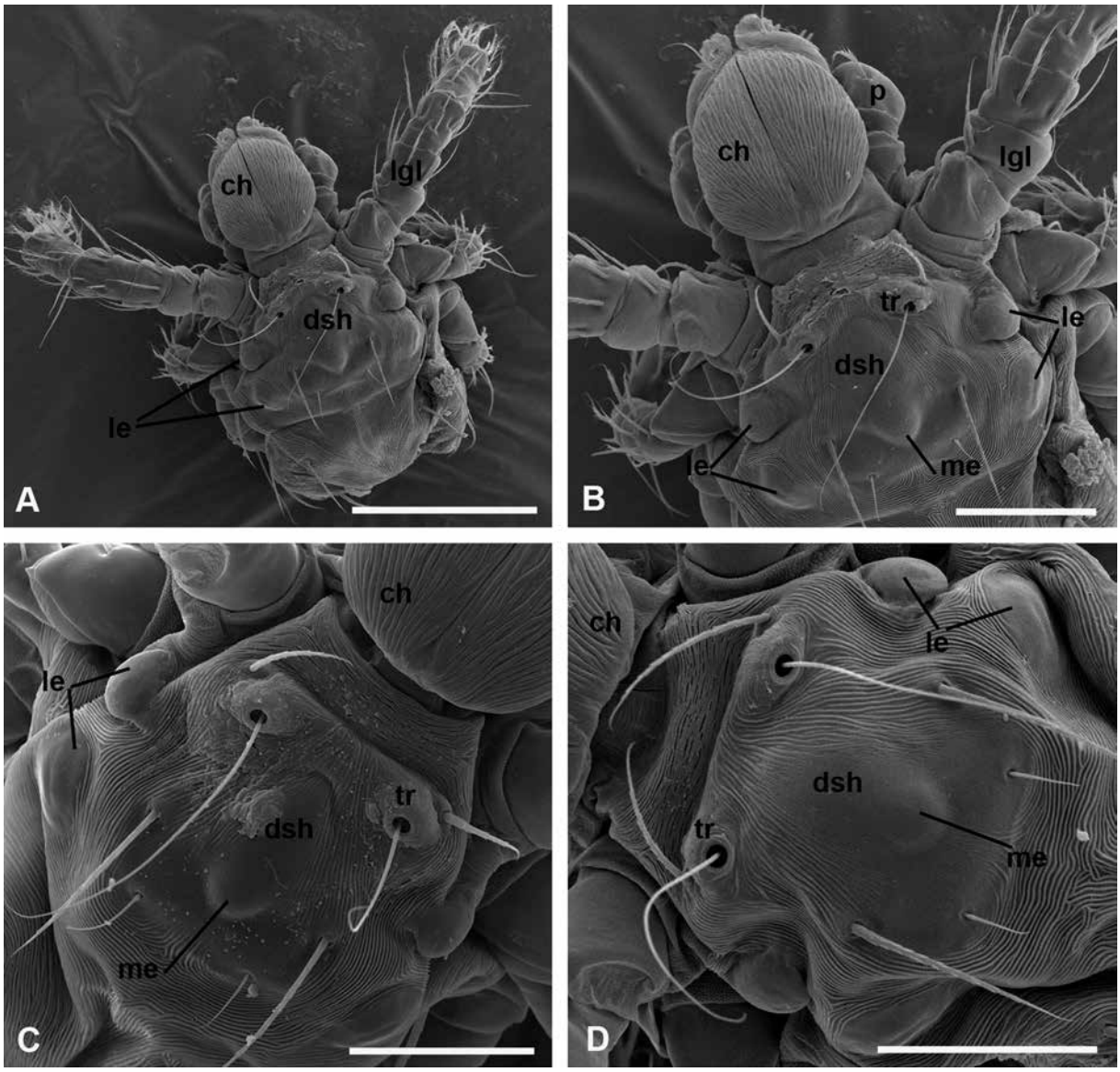

Fig. 3 (A–D). Scanning electron microscopy (SEM) of larvae *H.ruber*. A — General view of unfed larva from the antero-dorsal aspect. Scale bar — 100 μm. B — Frontal body region of the same larva with the higher magnification. Scale bar — 50 μm. C — Region of the dorsal shield indicating the lateral eyes' corneae and protuberance of the dorsal shield cituicle above the median eye. Scale bar — 40 μm. D — Dorsal shield with the accompanied structures. Scale bar — 40 μm.

shield are very slim (Fig. 4 D–E), whereas the processes extending between the retinula cells and the basal lamina may be relatively thick even containing the epidermal cell's nuclei (Fig. 4 C).

The retinula cells of the median eye are everted, i.e. their rhabdomeres look to the cuticle and the cell bodies occupy the basal position (Fig. 4 A–C, 5). The rhabdomeres of the sensitive cells do not form a united rhabdom and are more or less separated from those of the adjacent cells by a cell membrane (Fig. 4 C–D). Microvilli in each rhabdomere are tightly packed but arranged at different angle to the cuticle or even irregularly (Figs. 4 B–D, 5). The basal cell portions contain round electron-dense pigment granules scattered freely (Fig. 4 B–C) and irregularly shaped frequently elongated nuclei applied to the basal plasma membrane and showing large amount of heterochromatin mostly located along the nuclear envelope (Fig. 4 B). The elongated mitochondria with an electron-dense matrix, some variously shaped autophagic vacuoles/lysosomes and free ribosomes are also present in the cell bodies (Fig. 4 C). Rough endoplasmic reticulums as well as Golgi bodies are not present. Some membrane whorls of unknown origin may be also found in the basal cell zones. The basal plasma membrane remains flat. The lateral cell membrane is slightly wavy but

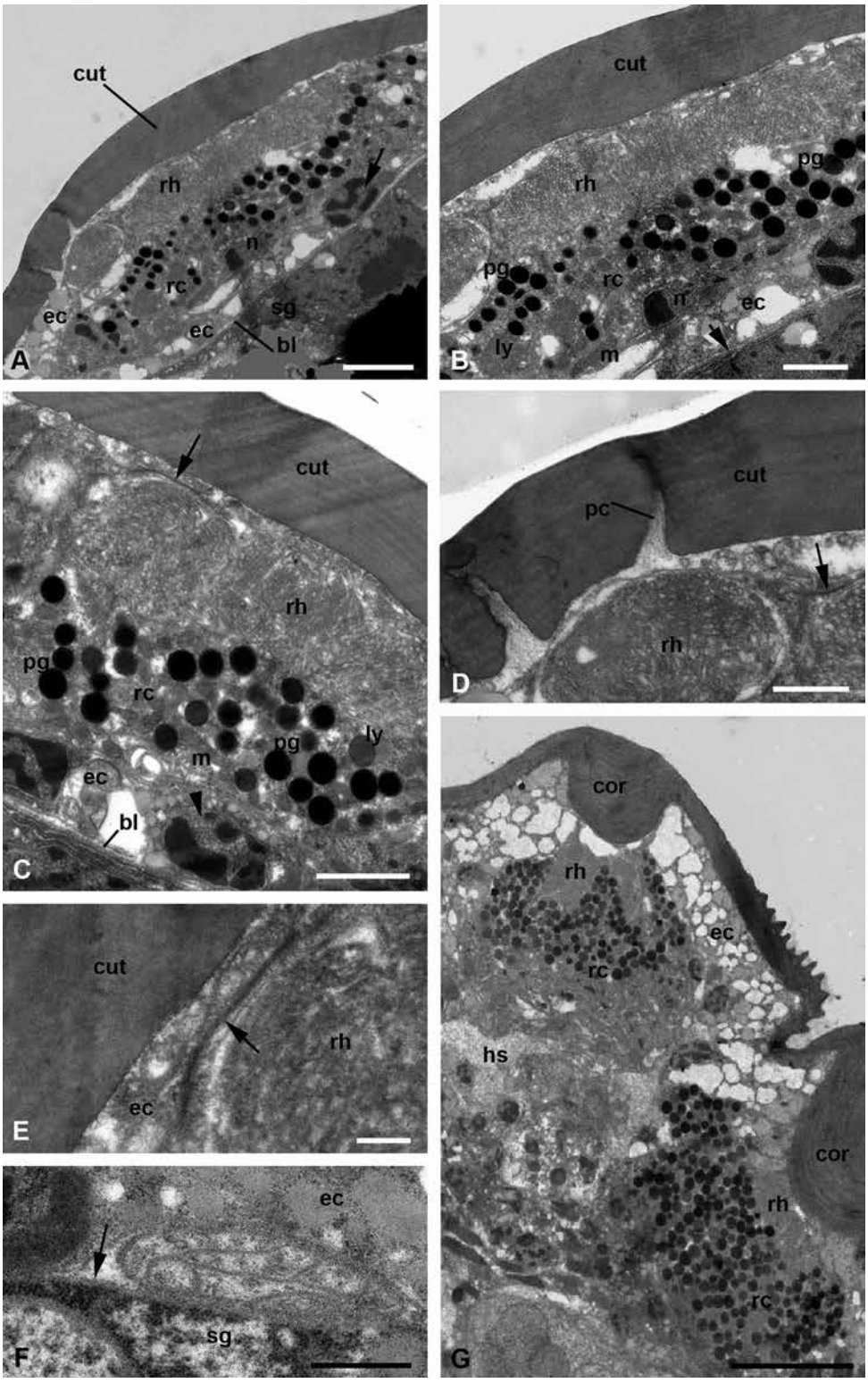

Fig. 4 (A–G). Transmission electron microscopy (TEM) of eyes of larvae *H. ruber*. A — General view of the median eye enclosed within epidermal cells, which are tightly adjoined to a salivary gland. Sagittal section along the middle line. *Arrow* indicates nucleus of epidermal cell. Scale bar — 2 μm. B — The same eye with the higher magnification. *Arrow* points to the basal lamina. Scale bar — 1 μm. C — Median eye composed of retinula cells with everted rhabdomeres facing the cuticle and the basal regions occupied by pigment granules. *Arrow* shows septate junction between the rhabdomeric plasma membrane and the process of epidermal cell entered between sensitive cells and cuticle. *Arrowhead* points to nucleus of epidermal cell. Scale bar — 1 μm. D — Isolated rhabdomere beneath the cuticle. Note wide pore canals. *Arrow* indicates septate junction between rhabdomere and the process of epidermal cell. Scale bar — 0.5 μm. E — Septate junction (*arrow*) between the process of epidermal cell tightly applied to the cuticle and rhabdomere. Scale bar — 0.2 μm. F — One of the paired nerve fiber composing of five axons on transverse section appressed between the epidermal cell and the salivary gland laterad to the median eye. *Arrow* points to the basal lamina. Scale bar — 0.5 μm. G — Lateral eyes on sagittal section with developed corneae. Scale bar — 5 μm.

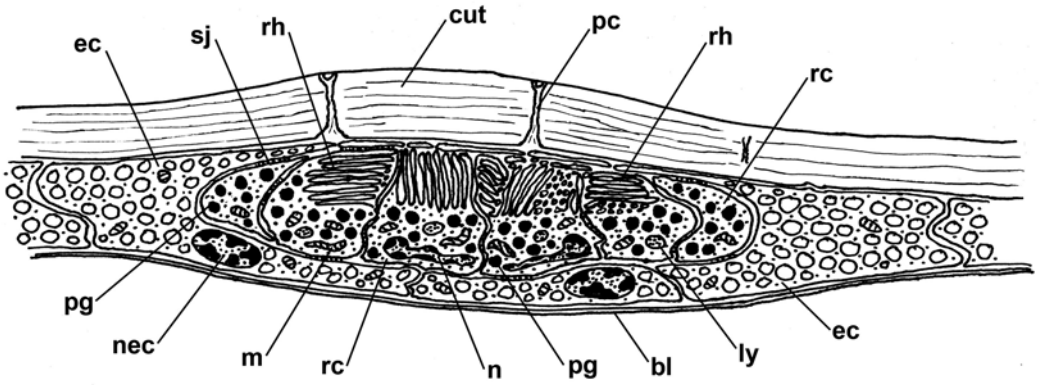

Fig. 5. Schematic drawing illustrating TEM organization of the median eye of *H. ruber* larva on sagittal section.

does not leave conspicuous extracellular spaces. The retinula cells contact to each other and to the surrounding epidermal cells by relatively long septate junctions (Fig. 4 C–E, 5). One pair of nerve fibers obviously composing of several axons (up to five) each may be apparently recognized on both sides of the median eye on cross sections (Fig. 4 F). No glial cells are present around this nerve, which is, however, surrounded by a relatively thick neural lamella.

In contrast with the median eye, the lateral eyes are composed of the greatly expressed retinula cells forming a large pigment cup protruded into the body cavity (Fig. 4 G). Nevertheless, both lateral eyes are organized principally identical with the median eye composing of one cell type possessing a basal region with nuclei and pigment granules and apically situated rhabdomeres with variously arranged microvilli. However, the lateral eyes demonstrate double-convex cornea developed greater in the first pair of eyes (Fig. 4 G).

DISCUSSION

The present investigation shows that the median eye of *H. ruber* larvae reveals practically the same organization as adult mites of this species (Mischke 1981). In both cases, only one type of everted retinula/sensitive cells are recognized, which possesses irregularly packed microvilli looking to the low developed cornea. Several pigment granules are placed in the basal nuclear region. Nevertheless, some substantial differences are present. In larvae, the epidermal cells containing lipid inclusions totally envelope retinula cells of the eye both from the dorsal and from the ventral sides. In contrast, in adult mites the epidermis around the eye is much lesser presented, and the retinula cells are immediately underlain by the basal lamina. In the apical eye region, as well, the sensitive cells directly contact with the cornea, whereas in larvae, conversely, rhabdomeres leave some clear space under the cuticle where processes of the epidermal cell are extending.

The most important difference in the organization of the median eye between larvae and adult mites is that in adults only one nerve composing of five axons is recognized (Mischke 1981), whereas in larvae two such nerves containing five axons each on both sides of the eye are found that may reflect the total number of sensitive cells. This number (10) appears to be in two times larger than in adults. It may suggest that in historical retrospection the median eye in larvae was a paired organ that corresponds with the general opinion on the paired origin of the median eye in the arachnids and its subsequent fusion in the course of evolution (Grandjean 1958; Foelix 1996; Alberti and Coons 1999) and contradicts with the idea of R. Olomski (2012) that in the Euhydrachnidia the originally unpaired madian eye has undergone division. However, in accordance with the data of R. Olomski (2012), the paired median eye is widely represented within the Euhydrachnidida. Probably, the larvae possessing two optical nerves recapitulate a particular ancient and simultaneously transitional character in the course from the paired to unpaired median eye in this species.

In comparison with the median eyes of other investigated acariform groups (Wachmann et al. 1974; Alberti et al. 1991; Alberti and Coon 1999; Haupt and Coineau 2002), the median eye in *H. ruber*, both in larvae and in adults (Mischke 1981) is organized rather simply and obviously regressed. There are no special pigment cells, which are typically present in the composition of the median eye in *Cyta latirostris* (Hermann, 1804)

(Bdellidae) (Alberti et al. 1991; Alberti and Coons 1999) and *Penthalodes ovalis* (Dugès, 1874) (Penthalodidae) (Haupt and Coineau 2012), particular sheath cells as in *Microcaeculus sabulicola* (Franz, 1952) (Caeculidae) (Wachmann et al. 1974; Alberti and Coons, 1999) and certain crystalline-like inclusions in the retinula cells as in *C. latirostris* (Alberti et al 1991; Alberti and Coons 1999). If in representatives of Caeculidae the median eye is paired (Wachmann et al. 1974), in bdellids (Alberti and Coons 1999), penthalodids (Haupt and Coineau 2012) and, probably, in endeostigmatids (Grandjean 1958) the median eye is an unpaired organ. These findings may suggest that the main evolutionary trend in Acariformes is fusion and progressive reduction of the median eye although in different groups a particular course of evolution may vary significantly.

The paired lateral eyes in larvae and adults of *H. ruber* are organized rather similar and principally identical to the median eye differing only in possessing a large pigment cup built of the basal portions of everted retinula cells with rhabdom, more regular than in the median eye, facing the double-convex corneal lens protruding above surrounding cuticle (Mischke 1981). In both cases, the epidermal cells underlying the cuticle in the region of the eye, tightly envelope retinula cells.

In contrast to the median eye, the lateral eyes in the studied acariform groups (Alberti and Coons 1999) and especially in the terrestrial Parasitengona (Leonovich and Shatrov 2002; Leonovich 2005) are organized much more complicated provided with particular pigments cells, corneagen cells situated just beneath the cornea and tapetum with crystalline-like inclusions. It was also found that if in larvae of *Platytrombidium fasciatum* (C.L. Koch, 1836) (Microtrombidiidae) rhabdom is everted, i.e., facing the cornea, in adult mites of this species rabdom, in contrast, inverted, facing tapetum (Leonovich and Shatrov 2002; Leonovich 2005) like in opilioacarid mite *Neocarus texanus* (Chamberlin at Mulaik, 1942) (Opilioacaridae) (Alberti et al. 1991).

Available data on the median eye in the Acari obviously indicate that these eyes are distributed quite irregularly among different groups (Alberti et al. 1991; Alberti and Coons 1999) and greatly reduced in the present historical period. At the same time, they retain certain primitive characters like everted rhabdom that is not typically found in more progressed organs and in more derived groups.

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