

## Short Note

# Ultrastructural peculiarities in the eggshell of *Binema* sp. (Oxyuridomorpha: Thelastomatoidea: Chitwoodiellidae)

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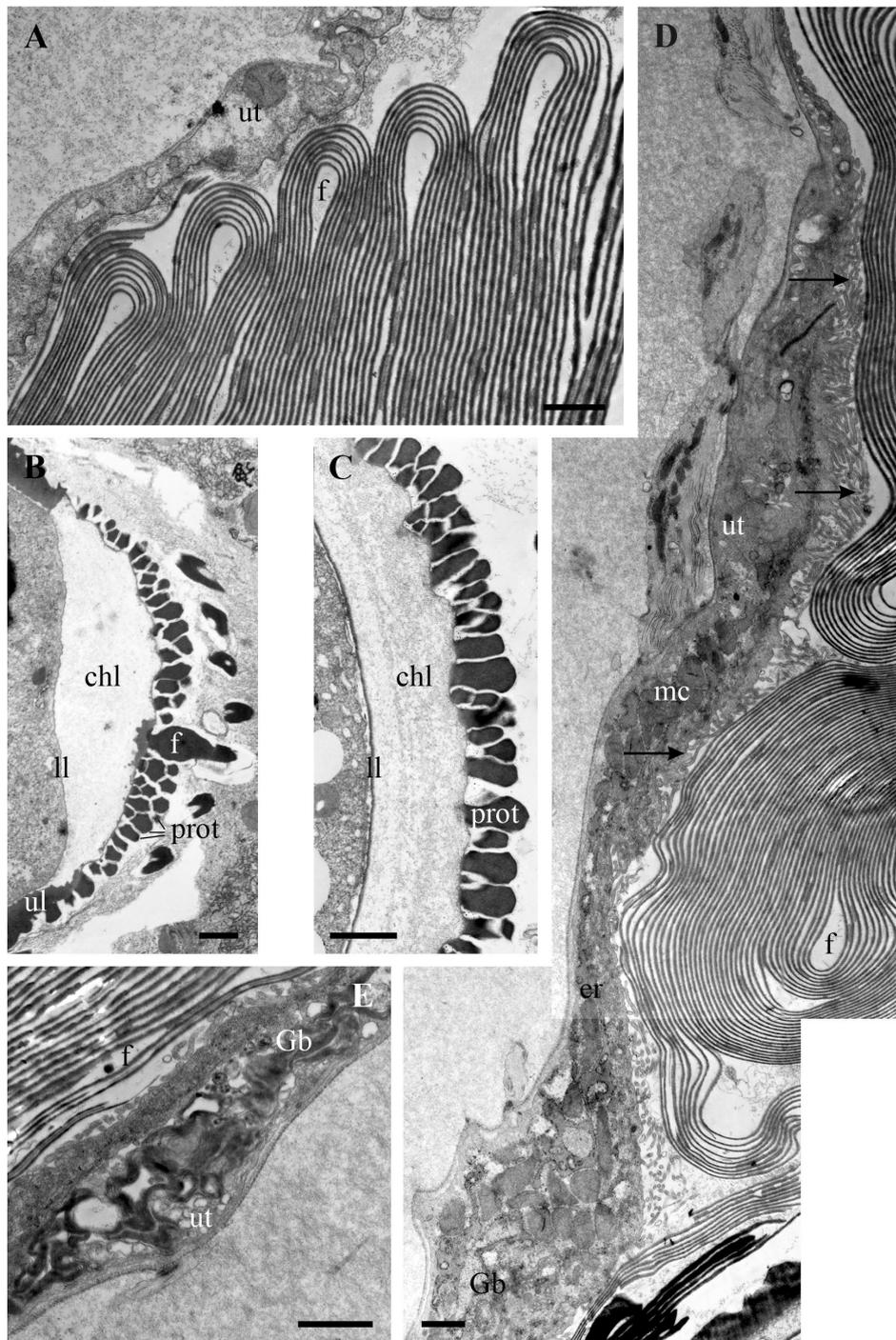
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The nematode genus *Binema* Travassos, 1925 is one of the nine chitwoodiellid (Oxyuridomorpha, Chitwoodiellidae) genera inhabiting the hindgut of mole-crickets (Gryllotalpidae), which are considered to be a specific host for these nematodes (Adamson, 1989; Adamson & Van Waerebeke, 1992). Two morphologically distinct forms of this genus were found in the mole-crickets of the Russian Far East. One of these forms was identified as a cosmopolitan-distributed species *Binema ornata* Travassos, 1925. Another form was characterised by a unique type of the eggshell agglomeration as a new one (formal description could not be prepared in the absence of males). Eggs of *Binema* sp. in uteri are arranged pairwise, where they closely adjoin each other due to the interweaving of lamellar parts of the filaments originating from their polar regions (Pham Van Luc & Spiridonov, 1988). The eggshell external morphology of *Binema* sp. has been studied using scanning electron microscopy (Pham Van Luc & Spiridonov, 1988; Spiridonov & Zograf, 2007). These observations demonstrated changes in the egg sculpture during the shell formation. At the early stage of formation, the eggshell surface is covered with tightly connected projections of irregular shape, forming a rugose appearance, and partially wrapped into a very delicate film, which represents a continuation of polar filaments (*ca* 0.5 µm in diam. at base). The fully formed eggshell is characterised by 0.13–0.17 µm diameter pits scattered throughout the surface and giving an ‘orange-peel’ texture; the polar filaments consist of roundish basal parts (~ 1.1–1.4 µm in diam.), cylindrical middle parts bound into the tight bundle of fibres and distal lamellar parts packed into regular stacks (Spiridonov & Zograf, 2007). It was shown with the use of a hanging drop technique

that at the poles of the egg these filaments straighten to form fan-shaped structures and adhere to the substrate after the completion of embryonic development (Pham Van Luc & Spiridonov, 1988). These unusual morphological features of eggs in *Binema* sp. need more explanations by study of their internal structure. In the present study, the eggshell ultrastructure of *Binema* sp. has been observed by transmission electron microscopy (TEM) to elucidate the processes of shell formation in these nematodes.

Adult specimens of *Gryllotalpa africana* Palisot de Beauvois, 1805 were collected in the neighbourhood of the “Vostok” Marine Biological Station of the A.V. Zhirmunsky National Scientific Center of Marine Biology FEB RAS (Nakhodka district, Primorye) in July 2008. Insects were dissected to recover the hindgut, which then was transferred into 0.9% saline and ruptured by needles to release nematodes. The nematodes were pre-fixed in glutaraldehyde for 1 h and then each female was cut at head and tail regions to obtain a piece containing ovary and uterus. The specimens were fixed overnight at 4°C in 2.5% glutaraldehyde in 0.05 M cacodylate buffer (pH = 7.4) with 5.4 mg ml<sup>-1</sup> NaCl and 0.25 mg ml<sup>-1</sup> MgCl<sub>2</sub> and post-fixed in 2% osmium tetroxide in the same buffer containing 4.5 mg ml<sup>-1</sup> NaCl. Post-fixation was followed by *en bloc* staining for 2 h in 1% solution of uranyl acetate in distilled water and then the specimens were dehydrated in ethanol followed by isopropanol series and embedded in Spurr resin (Sigma-Aldrich®, USA). Thin sections were cut with a Leica EM UC6 ultramicrotome (Germany), stained with lead citrate and then examined with a JEOL JEM 100S (Japan) and Zeiss LIBRA 120 (Germany) electron microscopes.



**Fig. 1.** Ultrastructure of the eggshell and the uterine wall of *Binema* sp., TEM. A: Accordion-like arrangement of filaments (f). B: Longitudinal section demonstrating modification of the eggshell ultrastructure at polar area: there is a thickening of the chitinous layer (chl) and thinning of the uterine layer (ul), which has polar filaments (f) anchoring within the chitinous layer and numerous electron-opaque protrusions (prot). C: The same as 1B at higher magnification. D: The cytoplasm of epithelial cells forming the uterine wall (ut) is filled with ribosomes, mitochondria (mc), cisterns of rough endoplasmic reticulum (er) and Golgi bodies (Gb). Black arrows show finger-shaped outgrowths on the inner surface of the uterine wall. E: The cell of the uterine wall (ut) containing the myelin-like cytoplasm surrounded by Golgi body (Gb). Abbreviations: chl – chitinous layer; er – rough endoplasmic reticulum; f – polar filaments; Gb – Golgi body; ll – lipid layer; mc – mitochondria; prot – protrusions; ul – uterine layer; ut – uterine wall. Scale bars: A, D and E = 1  $\mu$ m; B = 0.3  $\mu$ m; C = 0.5  $\mu$ m.

The formation of the eggshell in *Binema* sp. begins immediately after the fertilisation and soon at the zygote stage it is possible to distinguish tiny filaments at the poles. Moving through uterine tubes, the egg takes the elliptic shape; its shell and then the surface filaments thicken considerably. At the early stages of embryonic development, filaments are suspended in the fluid that fills uterine tubes. Finally, two eggs are tightly united with interwoven lamellar parts of the polar filaments of the shell (Pham Van Luc & Spiridonov, 1988). In free poles of both eggs, filaments of the first (anterior) egg are distributed along the shell, whereas filaments of the second (posterior) egg are folded like an accordion (Fig. 1A). In the ultrastructure of oxyuridomorph eggshells Wharton (1980) distinguished five layers: the three endogenous layers, such as lipid, chitinous and vitelline, and the two exogenous layers, the internal and external uterine layers.

Only three layers were clearly detected in approx. 3 µm thick *Binema* sp. eggshell: single uterine, chitinous and lipid layers. The lipid layer is a thin, osmiophilic inner layer, which underlies the chitinous layer (Fig. 1B). The chitinous layer has a similar ultrastructure to those reported earlier in mice pinworms, *Aspicularis tetraptera* (Nitzsch, 1821) and *Syphacia obvelata* (Rudolphi, 1802), and cockroach thelastomatids, *Hammerschmidtella diesingi* (Hammerschmidt, 1838) and *Blatticola blattae* (Graeffe, 1860) (Wharton 1979a, b, c, d; Guzeeva & Spiridonov, 2012). The outermost layer, which is identified as the uterine one, on the TEM sections is composed of electron-dense material (Fig. 1B). Material with a similar appearance to the outer layer forms the polar filaments and opercular part of the shell. Beneath these structures forming the uterine layer, the chitinous layer is about two times thicker compared to lateral sides of the eggshell. The basal parts of polar filaments are attached to a very thin electron-dense layer – a continuation of the uterine layer covering the main part of the eggshell; however, this layer cannot be defined as a separate layer on the rest of the shell. The polar filaments anchor within the chitinous layer forming electron-dense extensions beneath the uterine layer. Protrusions between the basal parts of filaments present electron-opaque structures on the outer surface of the uterine layer (Fig. 1C).

The uterine wall is formed with flattened epithelial cells with prominent secretory activity (Fig. 1D). The majority of the cell volume is occupied by vast cisterns of the rough endoplasmic reticulum.

There are also areas of the myelin-like cytoplasm surrounded by the Golgi body (Fig. 1E), while the rest of the cytoplasm is filled with free ribosomes and mitochondria. The surface of the cells facing towards the eggs forms numerous finger-shaped outgrowths, which extend to the forming eggshell and fill the space between their folds.

The eggshells of the free-living nematodes do not have any exogenous (uterine) layers (Wharton, 1980). Such layers appear in several evolutionary lines of nematodes leading to the acquisition of parasitism. In some parasitic nematodes, different types of novel superficial structures are distinguished, such as polar byssi, filaments, opercula *etc.* Byssi comprising unbranched or branched polar cords forming tassel-like structures on eggs are known for some species of mermithid nematodes of the genus *Mermis* Dujardin, 1842 (*Dorylaimia*, Mermithidae). Filaments can be polar (as in some spiruromorph and oxyuridomorph nematodes) or equatorial (as in *Pseudonymus* Diesing, 1857, an oxyuridomorph from aquatic hydrophilid beetles). These superficial structures cause the clumping of eggs with each other and facilitate entangling in aquatic or terrestrial vegetation to prevent eggs settling and loss in the sediment and, as a result, ensuring the massive infection of hosts (Christenson, 1950; Wharton, 1983). According to Wharton (1983), the filaments of nematode eggshells are secreted by the uterine cells similar to 1 or 2 uterine layers, of which they become a part. An operculum is another modification of one or both polar regions of the nematode eggshell. For instance, in *A. tetraptera* the uterine and chitinous layers are modified over the whole of the opercular region, whereas in *S. obvelata* this modification is delimited by the opercular groove only (Wharton, 1979b, d).

An impressive diversity of complicated ultrastructural adaptations was described for Oxyuridomorpha nematodes of the superfamilies Oxyuroidea and Thelastomatoidea (Wharton, 1983). Other previously undescribed ultrastructural features are characteristic for Chitwoodiellidae nematodes, a group of unresolved phylogenetic affiliation within Oxyuridomorpha. Such specific peculiarities emphasise the independent position of chitwoodiellids from two other superfamilies of Oxyuridomorpha.

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