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A NEW FINDING OF INTRACELLULAR BACTERIAL SYMBIONTS IN THE NERILLIDAE (ANNELIDA: POLYCHAETA)

Alexander B. TZETLIN and Michail V. SAPHONOV

Department of Invertebrate Zoology
and The White Sea Biological Station,
Biological Faculty, Moscow State University
Moscow, 119899, Russia

ABSTRACT. Symbiotic bacteria were found in the epidermal cells of two representatives of meiofaunal group Nerillidae (Polychaeta Annelida). The bacteria inhabit highly specialized epidermal cells, bacteriocytes of *Trochonerilla mobilis* (from the marine aquaria in Moscow) and *Micronerilla brevis* from the subtidal of the White Sea. These bacteriocytes lack nuclei, and contain large vacuoles with numerous bacteria. Vacuoles are penetrated by irregular cytoplasmic extensions. The presence of residual bodies of lyzosomes in the bacteriocytes is the evidence of intracellular digestion (endocytosis) of bacteria. Energy nature of the symbiotic relations between bacteria and hosts in both species is assumed. The discovery of bacteria in adults, juveniles, and embryos and the presence of highly specialized bacteriocytes observed in both species give the reason to suggest that the symbiosis is obligate. In addition, *Micronerilla brevis* has symbiotic gramm-negative bacteria in the basicuticular zone. Bacterial cells in the cuticle are similar to intraepidermal ones. Whether these two different types of bacteria observed in the epidermis and cuticle of *Micronerilla* are two different species or two different life cycle stages was not determined. This is the first finding of symbiotic bacteria in Nerillida.

Key words: bacterial symbionts, bacteriocytes, Nerillidae, Annelida.

Introduction

Symbiotic bacteria associated with annelids have been found in a variety of taxa and body locations. Chemoautotrophic or methanotrophic bacteria are located in the cuticle of phallodrilins (Oligochaeta), on the body surface of alvinellids (Polychaeta), and in the trophosoma of pogonophorans (probably closely related to Annelida) (Giere 1981, 1985, Gail et al. 1987, Fisher and Childress 1992). Bacteria have also been found under the cuticle of the interstitial worm *Jennaria pulchra* (Rieger and Rieger 1991).

Less is known about intracellular epidermal symbionts of annelids and their possible role in the biology of hosts. There are some data, obtained with the light microscope, on the existence of symbiotic bacteria in the epidermal cells of leeches and oligochaetes (Büchner 1953). Douglass and Jones (1991) described parasitic bacteria inhabiting the epidermal cells of spionids and producing large lesions on the body surface. Bacterial colonization and endocytosis in highly specialized epidermal cells were not previously observed in the Annelida. The present paper reports new finding of symbiotic bacteria in two genera of Nerillidae.

Material and methods

Micronerilla brevis, a new species of the genus *Micronerilla* (Saphonov and Tzetlin in press), is a small interstitial animal inhabiting shell and gravel substrates subtidally. All material (about 100 specimens) have been extracted from sediment collected using SCUBA within 5.0 km of Velikaya Salma Strait (Kandalaksha Bay, the White Sea, 66°35′ N, 33°10′ E), in depths from 10 to 25 m. Adult animals were studied using light microscopic observations, and 5 specimens were studied with transmission electron microscopy (TEM).

Trochonerilla mobilis Saphonov and Tzetlin 1991 was found in the gravel bottom in one of large sea-tanks (volume 3000 L) of the Moscow Zoo in 1988. Since that time a culture of these animals has been kept in two sea-tanks (volume 70 L) in the Department of Invertebrate Zoology of Moscow State University. In all tanks the artificial sea-water (S — 35‰, T — near 20° C) is used. The animals inhabit thick layer of gravel (5-8

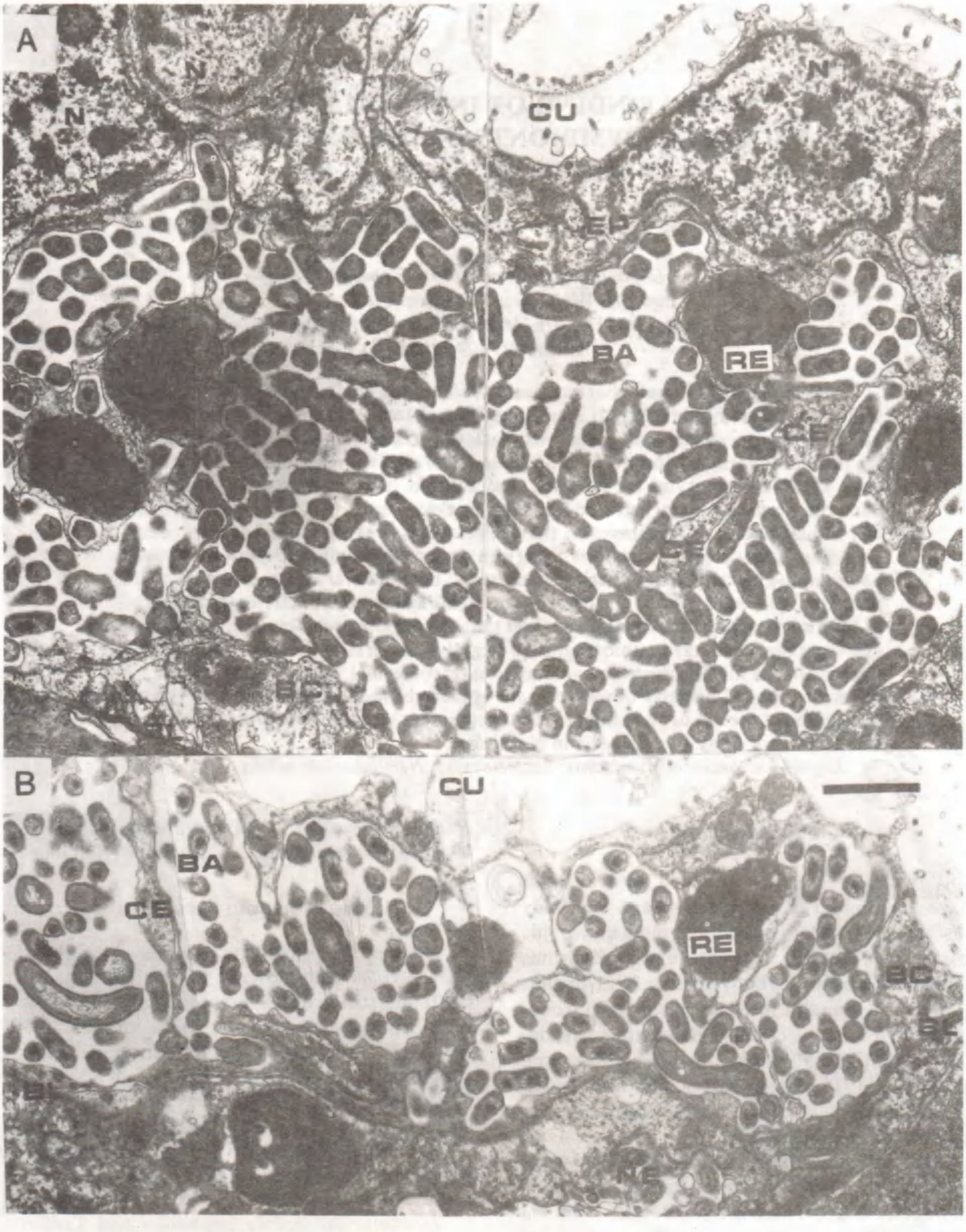

Fig. 1. Bacteria in the epidermal bacteriocytes. A — *Micronerilla brevis*. Detail of a transversal section through the middle part of the body, dorsal side. B — *Trochonerilla mobilis*. Detail of a transversal section through the middle part of the body, dorsal side. Scale: A, B — 1.0 µm. Abbreviations: non-specialized small epidermal cell — EP; bacteria — BA; bacteriocyte — BC; basal lamina — BL; cytoplasmic extension — CE; coelomic space — CO; cuticle — CU; longitudinal muscle cell — LM; nucleus — N; residual body — RE.

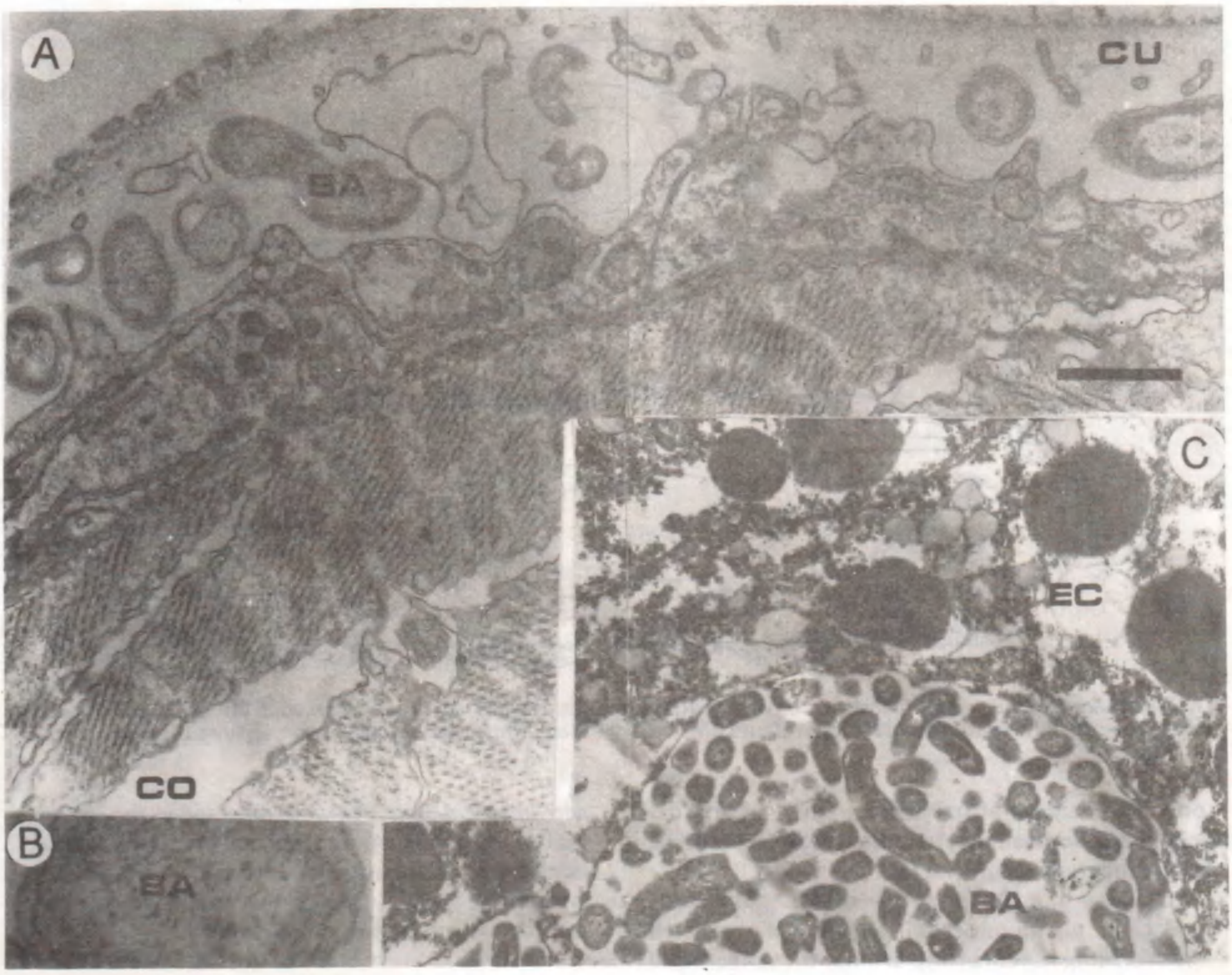

Fig. 2. *Micronerilla brevis*. A — Bacteria in the cuticle, detail of a transversal section, lateral side of the body. B — Detail of a bacterial cell. *Trochonerilla mobilis*. C — bacteria in the ectodermal cell of embryo from the egg cocoon (TEM micrographs). Scale: A — 0.5 µm, B — 0.15 µm, C — 1.0 µm. Abbreviations as in the Fig. 1.

cm) on the bottom of aquaria. Size of gravel grains is 4-6 mm. Animals feed on bacteria and diatoms covering the surface of gravel grains (Saphonov and Tzetlin 1991). Different stages of the life-cycle of this species were studied with TEM: adults, juveniles and embryos in the egg-cocoon. The geographical origin of *T. mobilis* is unknown (Saphonov and Tzetlin 1991).

For fixation, animals were kept for a few hours in Petri-dishes with clean sea-water, then fixed with 2.5% glutaraldehyde, buffered with 0.2 M Na-cacodylat buffer (pH 7.2-7.4) with 0.131 g sucrose per 1 mL of solution (1 hr) and postfixed with 1% osmium-tetraoxide with the same buffer. For TEM, fixed specimens were dehydrated in the ethanol series and acetone and embedded in Epon. Semi-thin sections produced with glass knives on a Dupont Ultracut microtome were stained with 1% toluidine blue. Ultrathin sections for TEM were obtained on a LKB-3 Ultracut microtome with diamond knife, stained in lead citrate and uranylacetate and then examined with a JEM-100b transmission electron microscope.

Results

Micronerilla brevis

There are two types of bacteria associated with this form: type 1 were present in specialized epidermal cells, here termed bacteriocytes (Fig. 1A). The bacteria were 1.0 µm long and ca. 0.25 µm across, with a gram-negative cell wall, and were restricted to large vacuoles here termed bacteriophorous vacuoles (BV) (Fig. 1A). Bacteriocytes occupy about 40% of the body surface. The membrane of the BV is typical and displays no additional structures (such as blebs, etc.). The BV were abundantly penetrated by irregular cytoplasmic extensions. The bacteria are surrounded by the membrane of BV and cytoplasm of the bacteriocyte can be seen on the border of the extensions (Fig. 1A), possibly reflecting different stages of endocytosis. In addition, cytoplasmic extensions containing electron-dense bodies about 1 µm in diameter with myelin-like structures were found (Fig. 1A). In the cytoplasm of the bacteriocytes

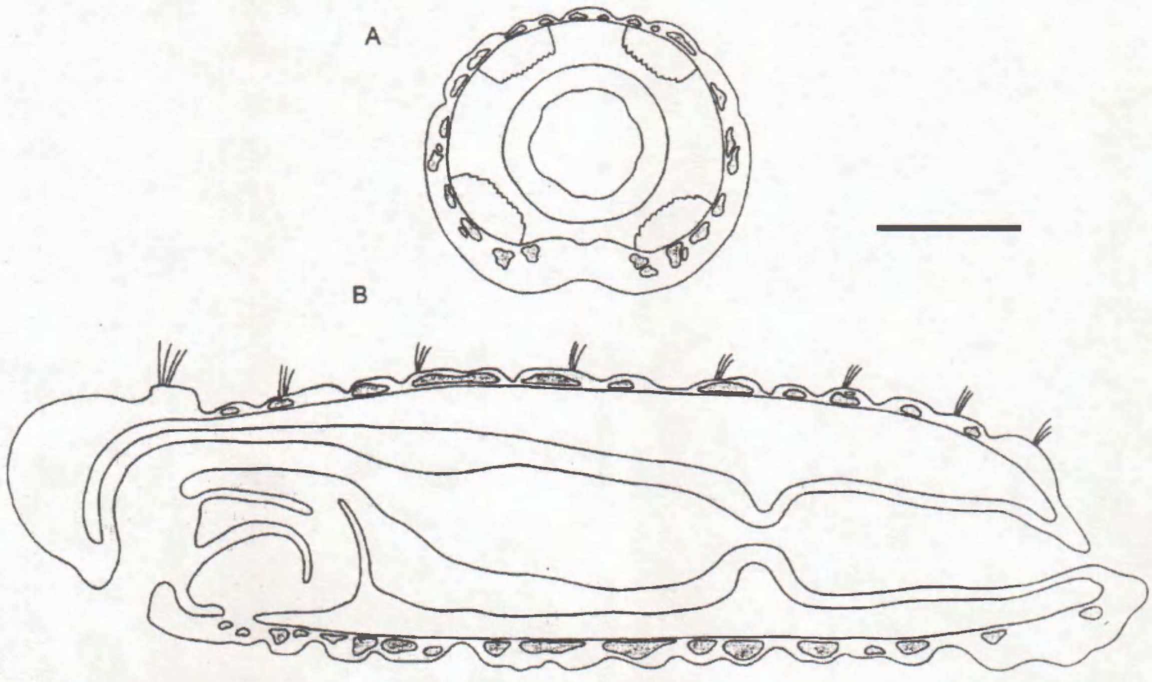

Fig. 3. *Trochonerilla mobilis*. Distribution of the bacteriophorous vacuoles: A. Transverse section through the middle part of the body. B. Parasagittal section, sheme after TEM reconstruction. More dense lining — bacteriophorous vacuoles. Scale — 50 µm.

only a few small mitochondria, and a poorly developed endoplasmic reticulum were observed. All bacteriocytes are anucleate.

Micronerilla brevis has several types of epidermal cells: glandular cells, sensory cells, ciliated cells forming the cilia rings around the setigers and the neurotroch; bacteriocytes; and small non-specialized epidermal cells about 5 to 7 µm long and located close to the bacteriocytes. Bacteria type 2 (Fig. 2A, B) found in the basal layer of the cuticle of epidermal cells are almost of the same size as intraepithelial ones (0.9-0.8 µm × 0.25-0.3 µm) and have gram-negative cell wall. Whether the two types of bacteria, from the epidermis and cuticle represent distinct species or two different life cycles stages remains unclear.

Trochonerilla mobilis

Morphology and localization of bacteria in the epidermal cells (bacteriocytes) of *Trochonerilla mobilis* are similar to those of *Micronerilla brevis* (Fig. 3A, B). The bacteria were observed in large vacuoles of anucleate bacteriocytes (Fig. 1B). Small non-specialized epidermal cells were also present near the bacteriocytes. The distribution of bacteriocytes is shown in Fig. 3A, B. Cytoplasmic extensions, penetrating BV, are less developed in comparison to those in *Micronerilla brevis*, but basically very similar to the latter and also contain electron-dense residual bodies with myelin-like structures (Fig. 1B). Bacteriocytes in *T. mobilis* cover 30-40% of the body surface, like in *Micronerilla brevis*. Bacteria were detected in the epidermal cells of all studied stages of the life-cycle: adults, juveniles and even embryos within the egg-cocoons (Fig. 2C). Bacteria were not observed in oocytes within bodies of adult nerillids.

Discussion

The finding of symbiotic bacteria in the epidermal cells of nerillids extends the list of bacterial symbionts found in the Annelida, being the first record of symbiotic bacteria in the order Nerillida. Both *M. brevis* and *T. mobilis* have the very specialized bacteriocytes with large bacteriophorous vacuoles and without nuclei. Small cells close to the bacteriocytes of *T. mobilis* and *M. brevis* are probably involved in the bacteriocyte differentiation. BV are very large and contain numerous cytoplasmic extensions penetrating the inner space of the vacuoles. The extensions harbor electron-dense bodies ca. 1 µm in diameter with myelin-like structures. According to de Burgh and Singla (1984), and Fisher and Childress (1992), such particles in the cytoplasm of bacteriocytes are the residual bodies of the lysosomes, and their presence suggests that epidermal cells exhibit intracellular digestion (endocytosis) of bacteria. This implies the energy nature of the symbiosis between bacteria and nerillids in both cases. All studied specimens of *M. brevis* and *T. mobilis* had symbiotic bacteria in the epidermal cells. The discovery of bacteria in adults, juveniles, and embryos and the presence of highly specialized bacteriocytes in both species suggest that the symbiosis (in the broad sense of this term) is obligatory.

Pathogenic bacteria causing epidermal lesions in spionids *Polydora nuchalis* and *Scolelepis maculata* were found in large oval vacuoles of the epidermal cells (Douglass and Jones 1991). In this case the vacuole membrane bears a system of blebs with no evidence of endocytosis. Chemoautotrophic bacterial symbionts in the epidermal cells have been found in the gills of several bivalves and limpets from hydrothermal vents (Southward 1986, de Burgh and Singla 1984, Galchenko et al. 1988, Fisher 1990, Fisher and Childress 1992). In those cases the proximal part of the gill bacteriocytes contained numerous small spherical vacuoles with a few bacteria, the basal parts holding nuclei, lysosomes and residual bodies. As our data show, both species, *T. mobilis* and *M. brevis*, inhabit biotopes with sufficient oxygen. We have no data on the physiology of symbionts, in particular on their oxygen demands. Possibly they are not chemoautotrophic (as in the phallodrilins or Vestimentifera) but utilize dissolved organic matter from the pore water.

In those few nerillids studied with TEM: *Nerilla antennata, N. jouinae, Nerillidium troglochaetoides* and *Mesonerilla intermedia* (Fransen 1983, Purschke 1985, Tzetlin et al. 1992), bacteria were not found. The question remains whether Nerillidae without symbiotic bacteria differ in their life style from the above-described species.

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Находка внутриклеточных бактериальных симбионтов у Nerillidae (Annelida: Polychaeta).

А.Б. Цетлин, М.В. Сафонов

Кафедра зоологии беспозвоночных и Беломорская Биологическая Станция, Биологический факультет, Московский государственный университет им. М.В.Ломоносова, Москва, 119899, Россия

РЕЗЮМЕ. Симбиотические бактерии были найдены в эпидермальных клетках двух представителей семейства Nerillidae (*Polychaeta Annelida*), все представители которого относятся к мейобентосу и обитают в интерстициали или в поверхностном слое мягких осадков. Бактерии обнаружены в сильно специализированных эпидермальных клетках — бактериоцитах — у *Trochonerilla mobilis* (из морского аквариума в Москве) и *Micronerilla brevis* из сублиторали Белого моря. Зрелые бактериоциты у обоих видов нериллид не имеют ядер и содержат крупные вакуоли с многочисленными бактериями. Эти вакуоли пронизаны цитоплазматическими выростами, в которых у обоих видов обнаружены миелиноподобные структуры, являющиеся резидиальными телами лизосом. Наличие резидиальных тел лизосом в цитоплазме бактериоцитов свидетельствует о внутриклеточном переваривании бактерий (эндоцитозе). Это позволяет предполагать энергетическую природу симбиотических отношений между бактериями и хозяевами у обоих видов. Наличие бактерий у всех исследованных экземпляров взрослых, ювенилей и эмбрионов, а также высокоспециализированные бактериоциты, обнаруженные в эпидермисе у обоих видов, дают основание предполагать, что симбиоз бактерий и червей носит облигатный характер. Помимо этого, у *Micronerilla brevis* обнаружены симбиотические бактерии в базальном слое кутикулы. Бактериальные клетки в кутикуле по строению сходны с таковыми в бактериоцитах. Являются ли эти два типа бактерий различными видами, или же это две различные стадии жизненного цикла бактерий — не выяснено. Симбиотические бактерии впервые найдены у Nerillidae. К настоящему времени на электронно-микроскопическом уровне изучено еще несколько видов семейства, которые, насколько это известно, также как *T. mobilis* и *M. brevis* обитают в хорошо аэрированных интрестициях гравия и ракуши, однако не имеют бактериальных симбионтов.