

Review article.

Symbioses between Marine Nematodes and Sulfur-oxidizing Chemoautotrophic Bacteria

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Abstract

Over the course of evolutionary history nematodes and sulfur-oxidizing chemoautotrophic bacteria have formed symbiotic relationships in marine sediments on at least two independent occasions. Mouthless worms of the genus *Astomonema* have endosymbionts which live intracellularly or extracellularly in the gut rudiment of their host. The ultrastructure of the bacteria and the ecology of the symbiosis are strong indications for a chemoautotrophic nature of the microorganisms; the lack of feeding structures in the host suggests a nutritive dependence on the microbes. In the Stilbonematinae, bacteria cover the outside of the body in a characteristic, species-specific pattern. Ultrastructure, incorporation of inorganic carbon and the presence of RuBisCo and key enzymes of sulfide oxidation pathways show the bacteria to be sulfur-oxidizing chemolithoautotrophs. Stable isotope analysis suggests that the worms depend on the bacteria for their nutrition. In turn, they provide the microorganisms with sulfide and oxygen by repeatedly migrating through the chemocline in sheltered sediments.

Keywords: Symbiosis, nematodes, chemoautotrophic bacteria, meiofauna, sulfide system

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1. Marine Free-Living Nematodes in Sulfidic Habitats

Sulfidic habitats

Free-living nematodes are an important component of marine sub-oxic, anoxic and sulfidic benthic habitats (Giere, 1992). Together with gnathostomulids and selected taxa from the plathelminths (Acoela-Solenophylomorphidae: Crezee, 1976, Catenulida-Retronectidae: Sterrer and Rieger, 1974; Ott et al., 1982), gastrotrichs (Boaden, 1974) and ciliates (Fenchel, 1969) they constitute the thiobios (Boaden and Platt, 1973), the characteristic fauna of the sulfide system (Fenchel and Riedl, 1970).

Sulfidic habitats occur whenever an excess of organic material leads to an exhaustion of the available electron acceptors O₂, nitrate, ferric iron and manganese by bacterial respiration. Under these conditions sulfate-reducing bacteria utilize sulfate to oxidize organic compounds or hydrogen and consequently produce hydrogen sulfide (Jorgensen, 1989). In shallow water this is the case in ephemeral or permanent accumulations of macrophyte material, such as algae, seagrass leaves or mangrove peat. Sulfidic zones occasionally also occur in exposed, wave-beaten habitats, e.g. within mussel beds or among the roots of surf grass. Another sulfidic habitat is developed on the sea bottom below upwelling zones due to the input of pelagic primary production (Gallardo, 1977). Recently, Bernhard et al. (2000) described meiofauna from sulfidic sediments from the dysoxic Santa Barbara Basin.

The by far largest sulfidic habitat, however, is the sulfide system that underlies the oxic surface layer of all intertidal and subtidal porous sediments, with the exception of the narrow "high-energy windows" along exposed sandy beaches (Fenchel and Riedl, 1970). Most data on thiobiotic meiofauna come from studies of this vast and widely distributed system (Giere, 1993).

More exotic sulfidic habitats are found in connection with geologic processes where pore fluids are squeezed from sediments by tectonic forces, such as the formation of accretion wedges at subduction zones or the rising of salt diapirs through shelf sediments, and emanate as "cold seeps" (Sibuet and Olu, 1998). Some contain hydrogen sulfide in addition to hydrocarbons and some of the seeps are hypersaline (Paull and Neumann, 1987). From several of these seeps, meiofauna, including nematodes, has been described (Powell and Bright, 1981; Powell et al., 1983, 1986; Jensen 1985, 1986a-c; Montagna and Spies, 1985; Montagna et al., 1987, 1989; Shirayama and Ohta, 1990; Dando et al., 1991; Kamenev et al., 1993; Bernard and Fenchel, 1995; Olu et al., 1997; Buck and Barry, 1998).

In connection with seafloor spreading on mid-oceanic ridges, hot hydrothermal fluids bring high concentrations of geochemically produced sulfide to the surface of the sea bottom. In contrast to the extensive literature

already existing on the macrofauna, virtually nothing is known about the meiofauna, in particular the nematodes (Dinet et al., 1988; Shirayama, 1992; Vanreusel et al., 1997). The meiofauna of shallow water vents has received more attention (Meyl, 1954; Kamenev et al., 1993; Fricke et al., 1989; Thiermann et al., 1994, 1997, 2000; Bright et al., 2003).

Distribution

Hydrogen sulfide is an extremely toxic substance for animals. It interferes with aerobic metabolism by blocking cytochrome c oxidase. Animals therefore have to either avoid, tolerate or detoxify hydrogen sulfide (Vismann, 1991; Bagarinao, 1992). In those marine sediments showing a chemocline (redox potential discontinuity layer, RPD) that separates an oxic surface layer from an anoxic sulfidic deeper layer, meiofauna species usually show a distinct vertical zonation leading to a marked distinction between communities of the surface and deeper layers (Ott, 1972; Ott and Schiemer, 1973).

Nematodes are the metazoans that penetrate deepest into anoxic, sulfidic sediments thus making them likely candidates for symbioses with sulfur bacteria. Their metabolic level is closely correlated with oxygen availability in the environment, with low respiration rates and high tolerance for anoxic conditions in those species living in the RPD and below (Ott and Schiemer, 1973; Schiemer et al., 1990; Ott, 1995). Anoxic tolerance allows the animals to live under sulfidic conditions at least briefly. Detoxifying mechanisms are probably not very effective in such small animals (Powell, 1989), although intracellular metal sulfide inclusions have been suggested to be part of a detoxifying system (Nuss, 1984; Nicholas et al., 1987; Thiermann et al., 2000).

Several families of marine nematodes appear to be characteristic for dysoxic or even anoxic habitats, e.g. Linhomoeidae, Comesomatidae, Cyatholaimidae, Siphonolaimidae and most notably the Desmodoridae. Among the latter a monophyletic group of species classified as the subfamily Stilbonematinae exclusively occurs in sediments with low oxygen content, where it is occasionally the dominant nematode taxon (Ott and Novak, 1989). These animals are remarkable for being associated with symbiotic microbes which obligatorily cover their cuticle in a highly regular fashion. An even closer association exists between mouthless nematodes classified with the family Siphonolaimidae; they contain large endosymbiotic bacteria living in the gut rudiment (Ott et al., 1982; Giere et al., 1995). In all cases the symbiotic bacteria appear white in incident light due to elemental sulfur contained in membrane-bound vesicles and abundant reserve substances such as poly- β -hydroxy butyric acid (PHB) granules.

2. Symbioses with Chemoautotrophic Bacteria

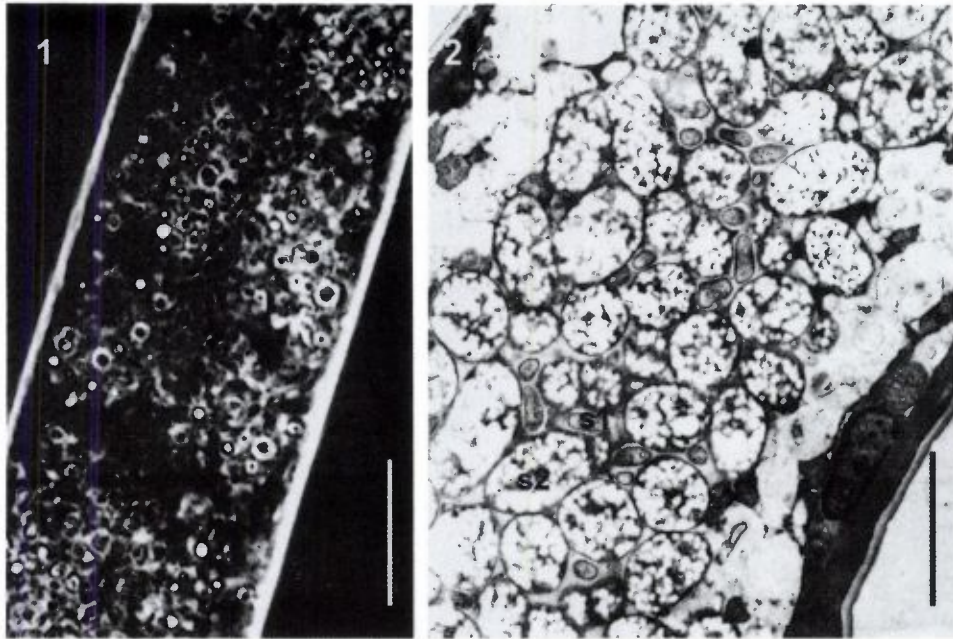
A variety of bacteria can oxidize sulfide or other reduced sulfur species to sulfur and finally sulfate and use the reaction energy for chemolithoautotrophic carbon fixation. In the environment such bacteria are confined to those narrow interfaces between oxic and sulfidic habitats where sulfide or thiosulfate co-exist with suitable electron acceptors such as oxygen or nitrate. Associating with large or motile animals can aid finding and bridging those interfaces. A number of symbioses with large macrofauna (bivalves, polychaetes, in particular the vestimentiferan tube worms) have been described from both shallow water and the deep sea (Fisher, 1996). For the meiofauna, symbioses with oligochaetes are well studied (Giere and Langheld, 1987; Giere, 1996). In thiotrophic nematodes, sulfur bacteria are found as both endo- and ectosymbionts.

Endosymbioses

Five species of mouthless nematodes containing endosymbiotic bacteria have been described from intertidal and subtidal sediments. They have been placed within the family Siphonolaimidae and comprise the genera *Astomonema* Ott, Rieger, Rieger, Enderes 1981 (4 species: *A. jenneri* Ott et al., 1981, *A. otti* and *A. obscura* Vidacovic and Boucher 1987, *A. southwardorum* Austen, Warwick and Ryan 1993) and *Parastomonema* Kito 1982 (1 species: *P. fijiense*). The type species *A. jenneri* is known to occur in sulfidic sediments, and the whitish color of the bacteria in incident light together with the similarity in ultrastructure suggest that they are sulfur bacteria. Buchholz and Lampadariou (2002) report a mouthless siphonolaimid which may belong to the genus *Astomonema* as the dominant nematode in a silty sediment in 70 m depth off Crete.

Astomonema jenneri (Fig. 1) has been collected in an intertidal mudflat on the North Carolina coast from the sediment surrounding the parchment-like tubes of an onuphid polychaete. The up to 16 mm long worms are extremely thin (length: width ratio up to 550:1). They live with their anterior end embedded in folds of the polychaete tube and the thin body extending into the surrounding, reduced sulfidic sediment. There is no mouth opening at the anterior end and the foregut (pharynx) is completely reduced. TEM sections show the anterior body region (70–90 μm), to be occupied by epidermis, gland and nerve cells. The gut rudiment is a solid strand of tissue without lumen. It extends throughout the body except for the tail.

Two types of microorganisms are present: small 0.1–0.5 μm long rods and 3 \times 1 μm elliptical cells. The former are found in low numbers at the beginning of the gut rudiment, while the latter occur in high numbers, are filled with dark globules or clear voids (presumably PHB granules) and contain large membrane-



Figs. 1-2. Intracellular endosymbiosis of *Astomonema jenneri*.

Figure 1. Light micrograph of anterior portion of gut rudiment with endosymbionts; scale bar 10 μm .

Figure 2. TEM micrograph of similar region with small rods (s1) and larger rods (s2); scale bar 5 μm .

bound vesicles (presumably sulfur storage) (Fig. 2). The microorganisms lie within the cytoplasm of the host cell as no surrounding cytoplasmic membrane has been found (Ott et al., 1982).

Mouthless animals have to rely on substances absorbed through their body wall. Although there is still no direct proof, there are several indications in this case for a thiotrophic/symbiotic way of life: the lack of specialization of the body surface for an uptake of organic molecules; the structural similarity of the microorganisms to thiotrophic symbionts in other invertebrates; the white color of the symbionts in freshly collected worms (which indicates storage of elemental sulfur) and, finally, the sulfidic nature of the habitat. There are no molecular data yet available from the microorganisms that would allow identification and clarification of their relationship to other symbiotic bacteria. It is unclear how the symbionts are acquired or transmitted.

Little is known about the biology or ecology of the remaining species. There are a number of differences between the species assigned to these two genera, which suggests that they may not be as closely related as has been previously thought. With regard to morphology, *Parastomonema fijiense* seems to be closest to *A. jenneri* (length, length/width ratio, cylindrical body shape, setose cephalic sense organs, shape of male spiculum and gubernaculum). The microorganisms are about twice as large as in *A. jenneri* judged from the illustrations (the numbers given in the text, however, are higher). Whether they are enclosed in a membrane-bound vacuole is not known. The coral sand in which this symbiosis has been found most probably contains sulfide even if it had a pure white appearance.

Astomonema otti and *A. southwardorum* (Fig. 1c) are in many respects very different from the above two species. They are smaller, fatter, their anterior end is conical, they have papillose cephalic sense organs, and the male copulatory apparatus is of different shape. Moreover, the microorganisms are considerably larger than in *A. jenneri* (about $10 \times 5 \mu\text{m}$) and lie – at least in *A. southwardorum* – in the lumen of the gut rudiment (Giere et al., 1995) (Fig. 1d). Incidentally, Vidakovic and Boucher (1987), Austen et al. (1993) and Giere et al. (1995) designate their species as "gutless" in the title, although in the text describe the position of the microorganisms as being in the gut. Both species are found in soft subtidal sediments that contain little free sulfide but that are underlain by sulfide-rich layers. Thus, the microorganisms may also be sulfide-oxidizing chemoautotrophs.

Ectosymbioses

All marine nematodes with sulfur-oxidizing chemoautotrophic ectosymbionts described so far belong to a group of closely related genera classified as the subfamily Stilbonematinae within the family Desmodoridae (Chromadoria, Adenophorea). At present, 8 genera with about 25 species are recognized. The group is monophyletic in possessing unique glandular sense organs (Bauer-Nebelsick et al., 1995) and forms a distinct clade within the Desmodoridae (Kampfer et al., 1998) based on the morphological characters and 18S rDNA sequences.

The first description of these worms was given by Greeff (1869), who considered the symbiotic bacteria of two *Eubostrichus* species to be part of the cuticular ornamentation. Later, Chitwood (1936) described them as fungal spores. Gerlach (1950) and Wieser (1959) thought them to be "blue-green algae" (cyanobacteria), a view that was taken also by Coull (1970) and Hopper and Cefalu (1973). Note that Cobb (1920) did not mention the ectosymbionts when describing the type species of the genera *Stilbonema*, *Laxus* and *Catanema*.

Because of the absence of both photosynthetic pigments and cellular structures, Ott et al. (1982) considered the symbionts to be sulfide-oxidizing bacteria as had been suggested by Powell et al. (1979). For the best studied example, the symbionts of *Laxus oneistus*, uptake of radioactively labeled bicarbonate (Schiemer et al., 1990) and the presence of the key enzyme for autotrophic carbon fixation (RuBisCo) (Polz et al., 1992) proved the autotrophic nature of the microorganisms. The discovery of ATP sulfurylase and sulfite oxydase (Polz et al., 1992) confirmed that the bacteria are sulfur-oxidizing chemoautotrophs, which is further supported by low $\delta^{13}\text{C}$ values (Ott et al., 1991) which are typical of many free-living and symbiotic thiotrophic microorganisms (Rau, 1981; Spiro et al., 1986). The sequence of 16s rDNA places them into the γ -proteobacteria (Polz et al., 1994).

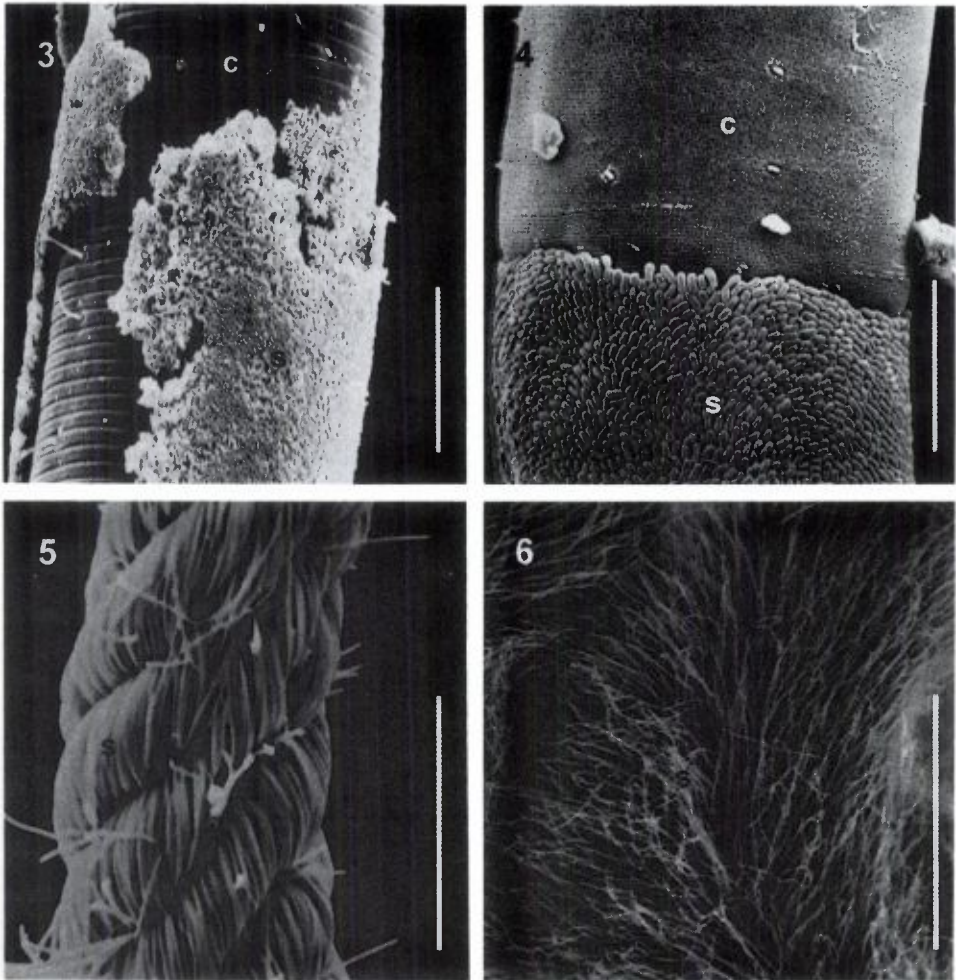
Types

Size, morphology and arrangement of the microorganisms vary between genera and even species of the host worms: In the genus *Stilbonema* the bacteria are small ($1.3 \times 0.6 \mu\text{m}$) cocci embedded in a gelatinous matrix. This gelatinous sheath covers the whole body of the worm except for the anterior end and the very tip of the tail. The bacteria are arranged in many (up to 10) layers and only microbes of the innermost layer are in contact with the worm's cuticle (Fig. 3). When subjected to mechanical stress the bacterial coat dissociates from the worm in large chunks. A similar condition is found in several species in the genus *Leptonemella*, although a number of not yet described species have differently shaped microorganisms (large rods with rounded or pointed ends, up to $40 \mu\text{m}$ long) arranged in a single layer on their cuticle.

In the genera *Laxus* and *Catanema* the rod-shaped microbes form a monolayer with the rods curiously standing on end and dividing longitudinally (Fig. 4). In both genera there are species (*Laxus oneistus* Ott et al., 1995 and a yet undescribed *Catanema* species from the Adriatic Sea) in which the microbial coat starts with a sharp onset some distance behind the anterior end of the animal (Fig. 4). At this level the diameter of the worm decreases by exactly the thickness of the bacteria layer so that the overall diameter of the animal does not increase.

In the genus *Robbea* two different arrangements of rods have been observed, one perpendicular to the body surface and one parallel to it.

The most complex arrangements are found in the genera *Eubostrichus* and *Adelphus*. Here the bacteria form non-septate filaments, attached to the worm's cuticle either with both or just one end. In many *Eubostrichus* species, such as *E. parasitiferus*, *E. topiarius*, and in *Adelphus rolandi* they are up to $45 \mu\text{m}$ long, crescent-shaped and attached to the host with both ends in a spiral pattern (Fig. 5). In *E. diana* the filaments are up to $120 \mu\text{m}$ long and attached with only one end, forming a fur-like coat that appears nicely groomed in live



Figs. 3–6. Ectosymbiosis of Stilbonematinae.

- Figure 3. SEM micrograph of *Stilbonema* sp. with multiple layers of coccoid symbionts (s) on the outside of the cuticle (c); scale bar 30 μ m.
- Figure 4. SEM micrograph of *Catanema* sp. with rod-shaped symbionts (s); note the sharp onset of bacterial occurrence and the concurrent decrease of worm's diameter; scale bar 10 μ m.
- Figure 5. SEM micrograph of *Eubostrichus parasitiferus* with curved non-septate filaments (s) attached with both ends to the host's cuticle forming a spiral pattern; scale bar 30 μ m.
- Figure 6. SEM micrograph of *E. diana*e with long, straight non-septate filaments (s) attached with one end to the host's cuticle; scale bar 100 μ m.

animals (Fig. 6). Whereas the crescent-shaped filaments contain up to 16 nucleoids (Polz et al., 1992), the long filaments in *E. dianae* show up to 50 when stained with DAPI (Ott, unpublished observations).

In addition to the above-mentioned symbionts of *Laxus oneistus* (Polz et al., 1994), Vanura (2001) identified γ -proteobacteria as the bacterial coat of a second species of *Laxus* (*L. cosmopolitus*), a yet undescribed *Catanema* species, *Stilbonema majum* and *Eubostrichus topiarius*. In the latter two species, as in *E. dianae* (Polz et al., 1999), however, several other bacterial phylotypes were identified by 16rDNA sequencing analysis. These bacteria belong to a variety of groups, such as cytophaga, caulobacters, α -, γ - and δ -proteobacteria. Some occur regularly, but in such low densities that they could not yet be localized using FISH. Most notable is the finding of sulfate-reducing bacteria in *E. dianae* (Polz et al., 1999). The role these bacteria play in the biology of the symbioses remains unclear.

Mutual benefits

Wieser (1959) noted the similarity between the microorganisms attached to the cuticle and those in the gut lumen of the worms and was the first to suggest that they may feed on their ectosymbionts. Further microscopical evidence, including TEM cross sections of the nematodes, was presented by Ott and Novak (1989) for *Eubostrichus dianae* and Ott et al. (1991) for *Stilbonema* sp. (Fig. 7). An analysis of $\delta^{13}\text{C}$ values showed low values (-25.9) for worms from which the bacteria were removed (Ott et al., 1991). This is close to the values for free-living sulfur bacteria and for the symbionts of bivalves from hydrothermal vents and subtidal sediments (Rau, 1981; Spiro et al., 1986). In contrast, particulate organic matter and non-symbiotic nematodes from the same habitat had $\delta^{13}\text{C}$ values of -10.5 to -10.2 . Since the isotopic composition of animals closely resembles that of their food, the similarity of the $\delta^{13}\text{C}$ values of the worms to the epibiotic bacteria (and the dissimilarity to ambient organic matter) is strong evidence for an important role of the symbiotic bacteria in the nutrition of the worms. In fact, it may safely be assumed that the worm diet consists almost exclusively of their ectosymbionts.

Although ingestion of the bacteria has never been observed, probably the worms graze on their symbiotic coat. The thin animals have a great ability to coil and can reach virtually any region of their body with their mouth with the exception of the immediate area behind the anterior end which – as mentioned above – is even free of symbionts in some species. A higher frequency of dividing symbiont cells can be observed around patches without bacteria; such patches may have been produced by recent grazing in those species with a single layer of symbionts (Polz et al., 1992), suggesting that grazing may have stimulated bacterial growth and division at the margin of the cleared patch.

In contrast to close relatives such as non-symbiotic desmodorids, the

Stilbonematinae have an extremely small buccal cavity, which is unarmed. The pharynx is weak and the terminal bulb – which is the main muscular pump in related species – is mostly glandular (Hoschitz et al., 2001). The pharynx muscles are better developed in the anterior corpus region, which in many species is enlarged and in the genera *Laxus*, *Catanema* and especially *Robbea* forms an anterior muscular bulb just behind the mouth (Gerlach, 1956; Platt and Zhang, 1982; Ott et al., 1995). Whether these anatomical differences are due to the symbiotic way of life is still unclear.

Several observations point to an additional role of the bacteria in sulfide detoxification of the worms. Although non-symbiotic nematodes from sulfidic sediments appear to have similar survival in 500 μM sulfide at ambient temperature (28°C), when exposed to high temperatures for three hours they have a LT_{50} of 40°C in the presence of sulfide, whereas without sulfide their LT_{50} is 42°C. In contrast, sulfide does not affect the temperature tolerance in Stilbonematinae ($\text{LT}_{50} = 42\text{--}42.5^\circ\text{C}$) (Ott, 1995). Hentschel et al. (1999) showed that freshly collected *Stilbonema majum* and *Laxus oneistus* have much lower internal sulfide and thiosulfate concentrations than non-symbiotic nematodes from sulfidic sediments, whereby *Stilbonema* with its thick coat of bacteria had the lowest values. Aposymbiotic *Stilbonema majum* died rapidly in 200 μM sulfide after their internal sulfide and thiosulfate concentrations increased significantly, whereas the same concentration did not affect the survival of symbiotic worms. It therefore appears that worms also benefit from the bacterial coat because it may provide an efficient barrier against sulfide poisoning.

The benefit for the bacteria seems to lie in their association with a motile host. Most chemolithoautotrophic sulfide oxidizers need oxygen as an electron acceptor. Oxygen co-occurs with sulfide only in narrow micro-zones, where sulfide is rapidly and spontaneously oxidized. Many of the sediments containing Stilbonematinae show no overlap between oxic and sulfidic sediment layers. The distribution pattern of Stilbonematinae in sediments with low sulfide content, a weak chemocline or patchy sulfide sources, together with experimental evidence, suggests that the worms migrate between the oxic sediment surface and sulfidic deeper layers or sulfidic patches (Ott et al., 1991; Berger, 1995) (Fig. 8). Measurements of the oxygen uptake of freshly collected worms and of those which had been kept under oxidized conditions for extended periods showed that the bacteria are able to store reduced sulfur species when available and oxidize these in the presence of oxygen (Schiemer et al., 1990) (Fig. 9). To a certain extent nitrate can be used as an electron acceptor in both nitrate respiration and denitrification when the symbionts possess internal stores of elemental sulfur and polythionates, which makes them appear white. This allows the worms to tap into the energy of high sulfide concentrations in deeper layers without being dependent on oxygen (Hentschel et al., 1999).

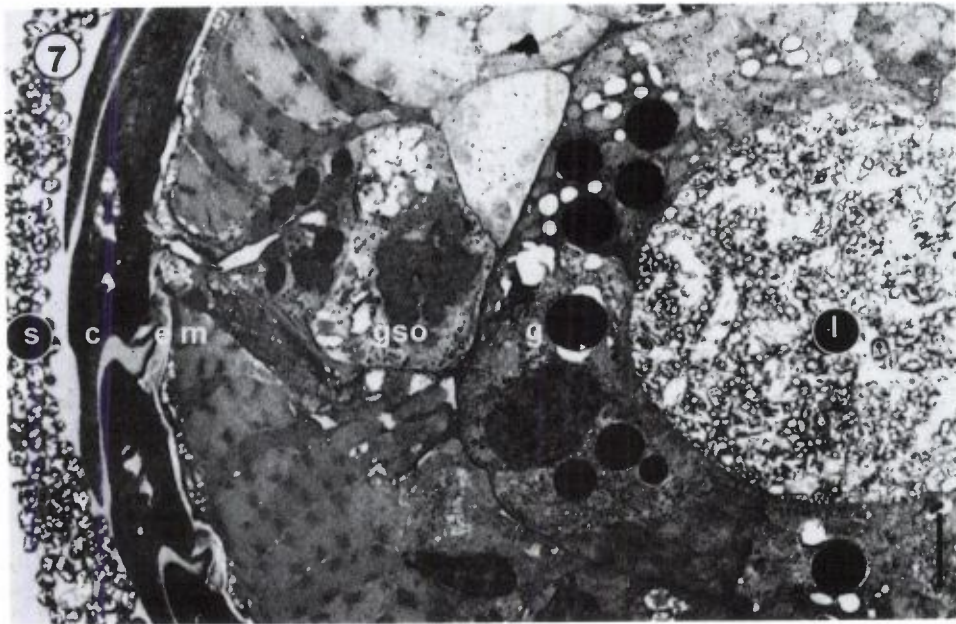


Figure 7. TEM micrograph of *Stilbonema* sp. with multiple layers of coccoid symbionts (s) above the host's cuticle (c); the epidermis (e), longitudinal muscles (m), glandular sense organ (gso), and the gut epithelium (g) are bacteria-free; note that the gut lumen (l) is filled with symbiont-like bacteria; scale bar 1 μ m.

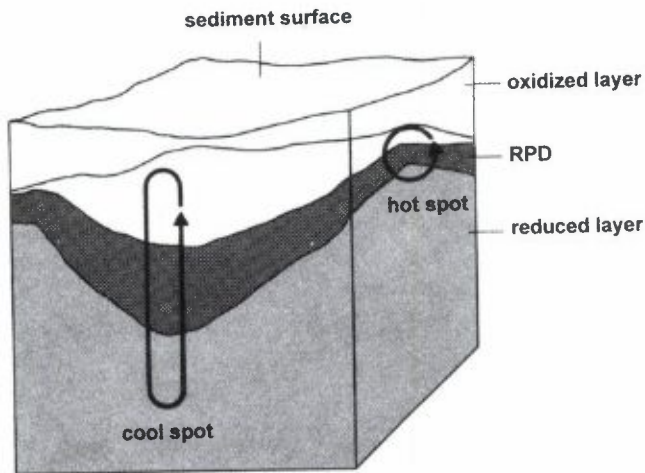


Figure 8. Model of migration pattern of Stilbonematinae between oxidized surface sediment layers and reduced sulfidic layers crossing the redox potential discontinuity (RPD). While at "hot spots" oxidized and sulfidic layers are in close vicinity and the worms migrate short distances, at "cool spots" the oxidized and sulfidic layers are more distant and the worms migrate larger distances (after Ott et al., 1991).

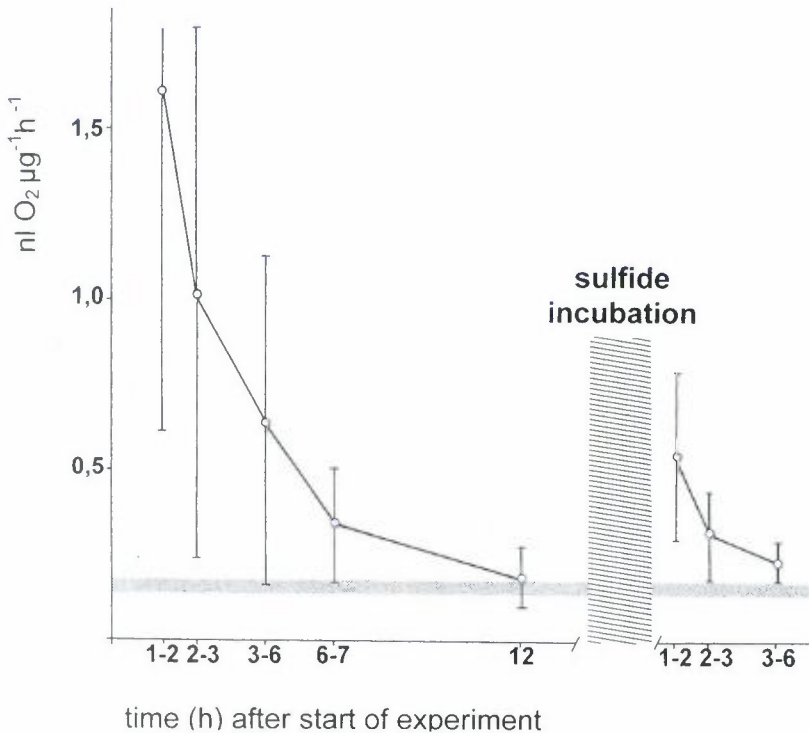


Figure 9. Oxygen consumption of *Laxus oneistus* and its symbionts kept under oxic conditions decreases within 12 hours due to oxidation of reduced sulfur species. Incubations in sulfide leads to a recharge of reduced sulfur in the bacteria and results in temporarily increased respiration rates. Worms were repeatedly washed in sulfide-free seawater before being introduced into the Cartesian diver respirometer (after Schiemer et al., 1990).

Partner acquisition, recognition, and loss

There is no evidence of vertical transmission from parents to offspring. Nevertheless, even very small (stage 1) juveniles of several species belonging to several genera have been observed to carry a complete microbial coat (Ott, unpublished observations). Furthermore, nematodes molt four times during their development into adults. Several stilbonematid specimens have been observed in the process of molting, leaving the symbionts behind when shedding the old cuticle (Wieser, 1959; Ott unpublished observations). Field collections rarely yield aposymbiotic animals. These facts point to an environmental transmission with a rapid colonization of newly hatched worms and rapid recolonization of recently molted worms. We have, however, so far been unable to observe this process experimentally (Henschel et al., 1999).

The fact that in at least two *Laxus* species (*L. oneistus*, *L. cosmopolitus*) and

one *Catanema* species the bacterial coat is composed exclusively of a single γ -proteobacteria phylotype (Polz, 1994; Vanura, 2001) suggests a very specific partners' recognition mechanism. Nussbaumer et al. (in press) showed that in the two *Laxus* species the bacteria specifically detach from the worms upon incubation in mannose and rhamnose. Moreover, labelled mannose-specific lectins stained the bacterial surface but not the worm cuticle and abundantly expressed mannose-binding lectins were affinity purified from the worms (Nussbaumer et al., in press; S. Bulgheresi, I. Schabussova, personal communication). Taken together these data suggest that the binding of particular worm lectins to bacterial mannose residues might play a crucial role in partners' adhesion.

We hypothesize that, after hatching or molt, worms may acquire their symbionts from the environment through lectin-sugar interactions. Once attached to the cuticle the microbes probably undergo changes in morphology and dividing mechanisms. The peculiar longitudinal division as seen in *Laxus* spp. and *Catanema* sp. has also been reported from the ectosymbionts of the ciliate *Kentrophoros* sp. (Fenchel and Finlay, 1989). In the *Eubostrichus* and *Adelphus* species the microorganisms apparently grow without dividing, forming large non-septate filaments. Whether and how the worms are responsible for this unusual behavior is still unknown. The worms may profit by an easier harvest of the large cells.

The extent to which the peculiar large glandular sense organs (gso) (Nebelsick et al., 1992), which are unique to the Stilbonematinae (Bauer-Nebelsick et al., 1995), are responsible for the formation and maintenance of the bacterial coat remains unclear. In *Laxus oneistus* and *Catanema* sp. the gso appear to produce a mucus layer that covers the cuticle of the anterior, symbiont-free part of the worm and extends posteriorly over the microbial coat. One possibility is an anti-microbial function in order to prevent opportunistic, "unwanted" microbes colonizing the body surface.

Both the worms and the microorganisms apparently grow very slowly. Worms have been kept alive in dishes without sediment for up to three weeks without showing marked changes except for a paling of the originally white symbionts. The difference in frequency of dividing cells (FDC) between the intact symbiotic coat and the margin of presumed feeding patches in *Laxus oneistus*, where FDC is 2.5 times higher (Polz et al., 1992), suggests the ability of quorum sensing by the microbes.

3. Evolution

Symbiosis is one of the driving forces in evolution. In order for a symbiosis to be formed the future partners have to co-occur in the same environment. This

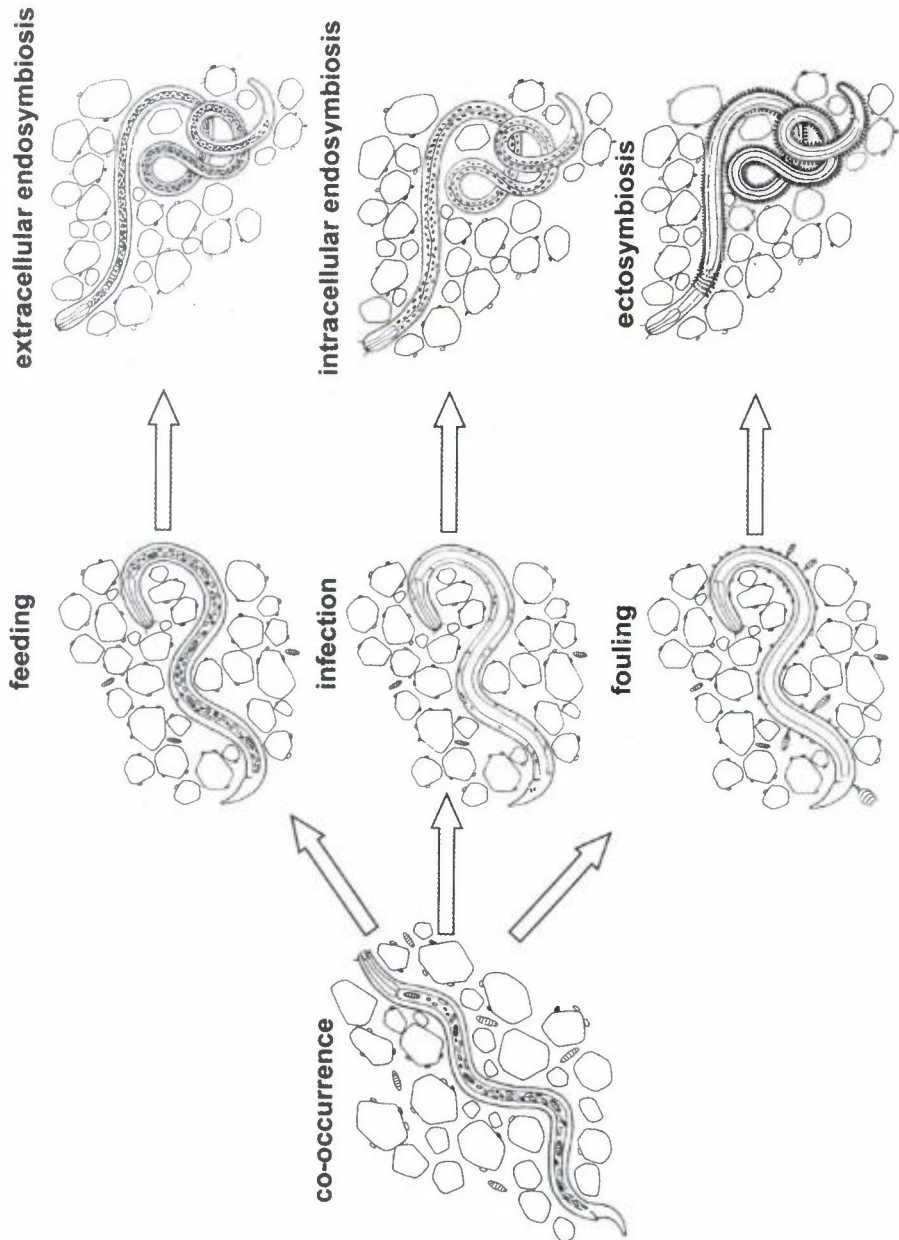


Figure 10. Model of evolution of symbiosis. Co-occurrence of putative host and symbiont in the same environment might lead to interactions such as a predator-prey relationship, a microbial infection, or microbial fouling. Escape from digestion might lead to an extracellular endosymbiosis in the lumen of the gut. Resistance of the host to parasitic infection might lead to an intracellular endosymbiosis. Unspecific microbial epigrowth might lead to an ectosymbiosis through specific recognition mechanisms (small circles: unspecific microbes; black dots: symbionts).

implies similar ecological preferences and tolerances – such as the ability to live in microoxic/sulfidic environments. This condition may lead to the next step – formation of an association in which both species increasingly depend on each other. This may be in the form that one species constitutes the substrate for settlement of another species, or that one is the prey of the other. In a final step the commensal/phoretic or episitic/parasitic relationships convert into mutualisms when the neutral or even negative interactions between partners are turned into positive ones (Fig. 10).

Endosymbiosis (feeding vs. infection)

Two pathways may be envisaged for the formation of an endosymbiosis: (1) the ancestor of the smaller (microbial) partner was once a food item of the ancestor of the larger (animal) partner which has escaped being digested. The microbial symbionts may then reside extracellularly in the lumen of a vestigial intestine or – in those animal exhibiting phagocytosis – intracellularly within intestinal cells confined in a membrane-bound vesicle. (2) Alternatively, the ancestors of the microbes parasitically infected the ancestors of the animals, which in turn resisted the pathogenic effects of the infection. In this case the symbiotic microbes should be intracellular, either contained in a vesicle or embedded in the cytoplasm.

In the genus *Astomonema* both situations can be found: in *A. southwardorum* the microbes lie extracellularly in the lumen of the gut rudiment (Giere et al., 1995), whereas in *A. jenneri* the microbes are intracellular, lying in the cytoplasm of the cells of the vestigial gut. Incidentally, in another member of the family Siphonolaimidae, *Siphonolaimus tubicen*, which has a normally developed pharynx and gut, bacteria have been observed colonizing the epidermal cells underlying the cuticle (Ott et al., 1982).

This is additional evidence for the above-mentioned difference between the various species assigned to *Astomonema* and *Parastomonema*. Whereas *A. jenneri* and *P. fijiense* appear to be true siphonolaimids, where the route to symbiosis may have been via infection, the remaining species are probably not closely related to the latter and may have acquired the symbionts through a trophic interaction. Due to the highly modified body plan in the animal partners of the endosymbioses, the reconstruction of the evolution and maintenance of the symbioses is difficult. In both *A. jenneri* and *A. southwardorum* juveniles have been observed with a mouth opening through which the uptake of symbionts from the environment could be accomplished.

Ectosymbiosis (from nuisance to benefit)

In the case of Stilbonematinae we are in a better position to reconstruct how

ectosymbioses evolved. Whereas the surface of most marine free-living nematodes is rarely fouled by bacteria, microalgae, or protozoa, the cuticle of members of several families within the Chromadoria frequently shows an overgrowth by a mixture of the above microorganisms. Many Epsilonematidae, virtually all Draconematidae and a large number of Desmodoridae frequently carry bacteria, diatoms and suctoria. This fouling community may occasionally be so conspicuous that the body of the carrier worm disappears beneath the dense overgrowth, e.g. in draconematids or in *Desmodora ovigera* (Ott, 1996). In *D. schulzi* a bacterial growth which regularly appears on the ventral side of females has been associated with brooding (Blome and Riemann, 1987); in other species the microbes may merely be a nuisance for the worm. The benefits for the microbes may go beyond simply having a substrate to grow on: on one hand excretions by the worm may be utilized by the bacteria, on the other hand the ability of the worm to move may guarantee a better supply of nutrients from the ambient pore water and ensure a certain position in the varying gradients within the sediment. Assuming that the fortuitous epigrowth is recruited from the community of free-living microbes – on which most nematodes presumably feed – it is likely that the worms graze the microbial lawn that covers their cuticle, maybe even in an attempt at grooming, although no direct evidence is available yet.

How has the highly specific symbiotic coating by one species of bacteria in the subfamily Stilbonematinae evolved from the mixed overgrowth found in Desmodoridae? Special preferences and behavioral patterns of the worm can be expected to exert a selective force on the composition of the microbial community on its cuticle. The distribution pattern and migration through the oxygen/sulfide gradients in sheltered sediments favor the growth of sulfide-oxidizing chemoautotrophic bacteria. Note that Stilbonematinae are rather rare in sediments with a stable chemocline showing a considerable overlap of oxygen and sulfide distribution. Here, a carrier that will eventually eat part of their production has no selective advantage for the bacteria. The highest density and diversity of symbiotic associations is found in sediments with a shifting, weakly or patchily developed RPD where there is a zone lacking both oxygen and sulfide. Under these conditions a carrier that ensures alternate visits to sulfidic and oxic layers is of great advantage, provided that the microbes can store sulfur in a reduced form. Nevertheless, a superior competitive ability of sulfur-oxidizing bacteria growing at optimal conditions could only explain the prevalence of those microbes, but not the highly ordered and monospecific symbiotic coat found in some representatives of the Stilbonematinae. The specificity seen in the microbial coat of animals belonging to different species and genera co-occurring in the same habitat, and the morphological and physiological differences to other Desmodoridae, show that the symbiosis has reached an appreciable degree of complexity, where

the worms are not just a moving substrate but exert a control over their epigrowth. On cuticle patches where the original surface coat has been disturbed, the occurrence of bacteria other than the symbionts or of suctorians (own unpublished observations), shows that opportunistic microorganisms have the potential to colonize the surface of the worm. This suggests an active role of the worm in maintaining its "kitchen garden". Similarly, the bacteria have evolved complex cell cycles including a terminal morphological differentiation in *Eubostrichus* and *Adelphus* species (Bright, 2002).

4. Conclusions and Perspectives

Thiotrophic symbioses between marine nematodes and chemoautotrophic bacteria apparently evolved independently several times: even in the closely related monophyletic Stilbonematinae, the symbionts appear on at least two different clades within the γ -division of the proteobacteria (Vanura, 2001). Although we still lack molecular data for both the symbionts and hosts in the *Astomonema* symbiosis, there are good reasons to believe that two independent pathways have led to the evolution of the mouthless worms. The lack of a mouth and a reduced digestive tract have been reported for the deep-sea genus *Rhaptothyreus* (Hope and Murphy, 1969; Hope, 1977; Riemann, 1993). Whether the rod-shaped bodies described from the interior of the worms are bacterial symbionts is unknown. The oxidized nature of the habitat in the sampled depth (1300–4500 m), however, makes a thiotrophic symbiosis unlikely.

With the exception of some specimens found in continental slope brine seeps (Jensen, 1985, 1986a–c) all nematodes with thiotrophic microbial symbionts are found in shelf sediments and they have not yet been reported from hot vents. The spatial and temporal scale of fluctuations in the sulfide and oxygen content near vents makes it unlikely that an association with a relatively slow carrier such as the nematodes could be advantageous. Here sulfur-oxidizing bacteria are either associated with rapidly moving animals such as the bresiliid shrimp *Rimicaris exoculata* or are contained within the bodies of large animals such as the Vestimentifera, which provide them with the necessary nutrients.

Unfortunately, all nematode species with thiotrophic symbionts have so far resisted cultivation. We know very little about their biology, especially how the partners in the symbiosis find and recognize each other, whether there is a vertical transmission from parents to offspring, whether the symbionts are acquired from the free-living bacterial community and, if so, where the free-living stages reside. Hopefully, molecular methods will help to solve these puzzles in the future.

Only a few cases of bacterial symbioses have been reported from parasitic or

terrestrial nematodes. Filarial nematodes regularly host rickettsial endobacteria of the genus *Wolbachia*. The bacteria reside in vacuoles contained in the lateral chords cells of the nematodes and in developing female reproductive cells. They are transmitted vertically via the eggs and they appear to contribute to parasite immune evasion. Endotoxins produced by the bacteria are suspected to be the principle cause of inflammatory filarial disease. Because antibiotics treatment finally results in nematode death, it may open a new way to treat filarial diseases (Bandi et al., 2001; Taylor and Hoerauf, 2001; Hoerauf et al., 2002). Similarly, bacteria of the genera *Photorhabdus* and *Xenorhabdus* live symbiotically in the gut of entomopathogenic nematodes but are pathogenic towards the insects that are infected by the nematode (Forst et al., 1997). Common molecular mechanisms such as quorum sensing and horizontal gene transfer are supposed to be shared by both symbiosis and pathogenesis (Hentschel et al., 2000; Goebel and Gross, 2001; Ochman and Moran, 2001).

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