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NODING IN *Cyprideis torosa* AND ITS CAUSES

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Abstract
The ostracod *Cyprideis torosa* develops nodes on its calcified carapace in brackish waters and is therefore widely used in paleontology as a marker for lower saline waters. The formation of nodes in lower saline waters was investigated with SEM and TEM analyses and micro-cryoscopic measurements of the inner osmoregulation. Noding is considered a failure in osmoregulation, which causes the pillars connecting the inner and outer epidermal cell layer to rupture during the molting process. As a result, the still flexible shell develops protrusions, which then persist as nodes after subsequent calcification.

Key words: *Cyprideis torosa*, nodes, osmoregulation, histology

INTRODUCTION

Ostracods are small crustaceans characterized by a calcified shell, which encloses the body and appendages. Normally they vary in size between 0.4 mm to 1.5 mm, but animals are also known from 0.08 to 70 mm. They grow by a molting process involving the already shelled nauplius and eight stages up to the adult.

Cyprideis torosa (Jones) is a member of the brackish biocenoses in European coastal waters and is often very abundant in hypo- as well as in hyperhaline waters. In marine waters of very low salinity (<5 ‰) it develops nodes at certain places on the shell (Sandberg 1964, Vesper 1972). The presence of nodes is used widely in paleontology as an indicator for brackish water environments.

The origin of these nodal structures remained unsolved for a long time (Müller 1912, Fassbinder 1912, Triebel 1941, Sandberg 1964, Hartmann 1964, Jørgensen 1970, Vesper 1972, Kilenyi 1972, van Harten 2000). Based on SEM and TEM analyses of the nodes as well as micro-cryoscopic measurements of the inner osmoregulation, Keyser (in press) found that the main cause of the noding in low saline waters in *C. torosa* is a failure in osmoregulation capacities during molting. The present paper combines the results of noding peculiarities with osmoregulation results in *C. torosa*.

MATERIALS AND METHODS

Specimens of *C. torosa* were collected on the Schlei, a narrow bay of the Baltic Sea with salinities ranging from 1.5‰ to 15‰ (Fig. 1). A hand net with 180 µm mesh was used and the animals picked one by one with a pipette under a stereomicroscope. These animals were kept alive in small bowls containing water of different salinities at 12 to 15°C with 14 hours of light, a weekly change of water and no extra food.

Living animals were observed using a stereomicroscope in order to determine the optimum developmental status for fixation. They were fixed in 70% ethanol, dissected with needles and embedded and stained in polyvinyl-lactophenol with Orange-G. Some animals were macerated by heating in KOH (20%) prior to dissection and then embedded. Others were put in clove oil for a fortnight to make them translucent and then observed under the microscope.

Specimens used for sections in the light microscope (LM) and in the transmission electron microscope (TEM) were treated in basically the same way. They were fixed in 2.5% glutardialdehyde in 0.05 mM phosphate-buffer containing 5% sucrose. Then they were washed three times in buffer with sucrose, post fixed with 2% osmium tetraoxide in the same buffer, washed again three times and decalcified in 0.1M EDTA for 12 hours. After that they were dehydrated in a graded row of acetone before being embedded in Spurr's resin (Spurr 1969) and cured for 24 hours at 60 °C.

Semi-thin and ultra-thin sections were cut at the Reichert Ultracut E Ultramicrotome. Semi-thin sections were stained with toluidine blue and pyronine after Holstein & Wulfhenkel (1971). Ultra-thin sections were stained with uranyl acetate (Stemper, Ward, 1964) and lead citrate (Reynolds 1963). Photographs were made on a Leitz Dialux for LM and on a Zeiss EM 902 for TEM.

Specimens for the scanning electron microscope (SEM) were fixed in 2.5% glutardialdehyde in phosphate buffer, dehydrated in a graded acetone series and dried (critical-point) with CO_2 in a Balzers CPD. They were sputtered in a Gea-004 S manufactured in Graz, Austria, and viewed under a CamScan SEM DV 4 and a Leo 1525. Cryofixed animals were sectioned at –150°C on the Reichert Ultracut E Ultramicrotome equipped with the FC4 Cryostage. The animals were dehydrated at –80°C in a cooler in 100 % acetone for 4 days, and then warmed up to room temperature and critical point dried.

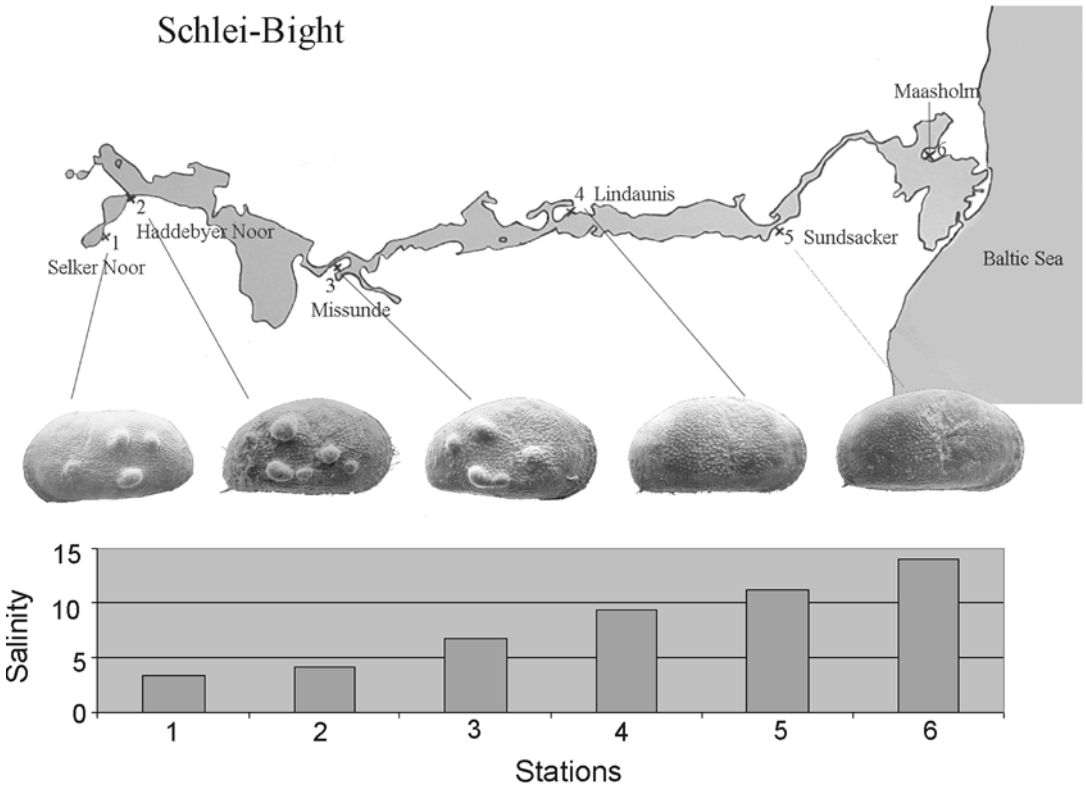

Fig. 1. The Schlei-Bight in northern Germany showing the difference in ornamentation of *C. torosa* in different salinities.

Cultured ostracods were able to adjust to different dilutions of marine water within ten days. Osmolarity of *C. torosa* was then measured by taking an aliquot of the hemolymph with a minute glass capillary inserted into the shell duplicature of the ostracod. This aliquot was frozen to –17 °C and then warmed under microscopic observation until the last crystals thawed. This temperature was measured and the osmolarity was calculated from the freezing point depression of the hemolymph (Aladin *et al*. 1986).

RESULTS

The distribution of *C. torosa* throughout the Schlei (Fig. 1) shows that nodded specimens increased with decreasing salinity. When noding occurs, it is often asymmetrical, i.e. one side of the shell develops larger nodes than the other (Fig. 2). SEM investigations show that the nodes occur at distinct places on the lateral side of the carapace. It is remarkable that these places are always found outside the part of the shell, which is connected to the body itself, the so-called isthmus (Fig. 4).

The nodes are normally rounded protrusions but can enlarge in some instances to balloon-like structures even having a terraced appearance. SEM studies of the surface of the nodes in *C. torosa* show that their basic ornament does not differ from that of the adjacent shell areas. However, the floor of the ornamental meshes has ripple-like striae (Fig. 3). It is interesting to note, that the cell boundaries of the epidermal cells, normally not detectable on the calcified shell are clearly detectable on the nodes. Also here stretching of these structures is obvious. The sieve pores on the nodes are only slightly distorted not stretched.

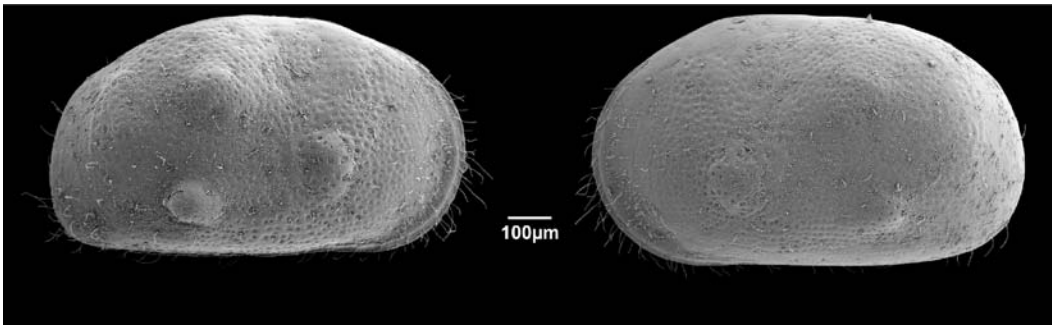

Fig. 2. Left and right valve of same specimen of *C. torosa* in low saline waters showing the difference in nodding.

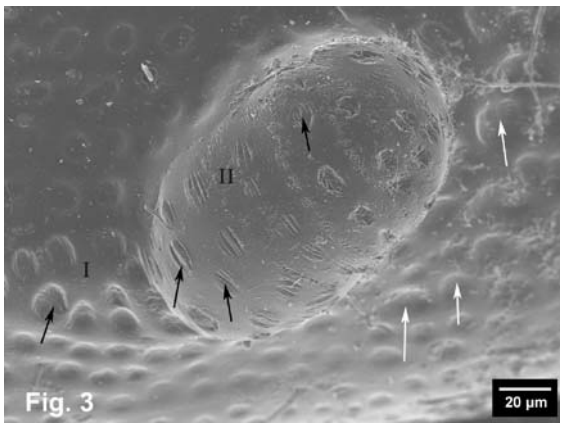

Fig. 3. Node of *C. torosa* in 3.5‰ salinity showing signs of stretching and deflation on the node (black arrows) compared to the smooth surface in adjacent areas (white arrows). Note the different elevation levels in II and I.

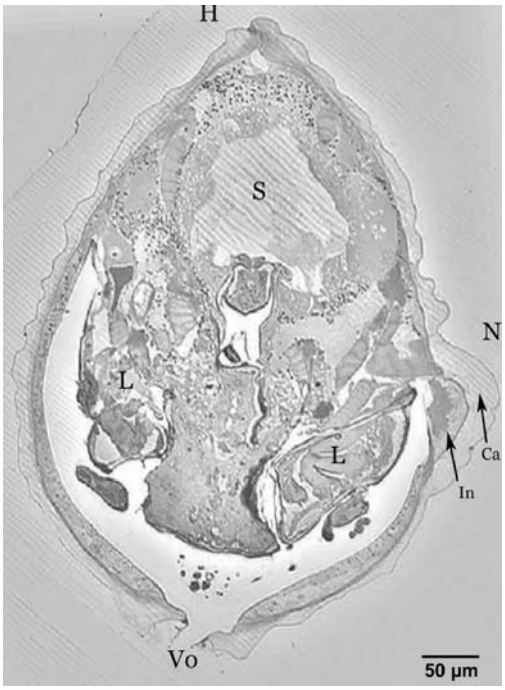

Fig. 4. Cross section of *C. torosa* juvenile (A-4) showing a node (N) and thickening of the outer epidermal cells (In), the calcified part of the shell (Ca), the area of the hinge (H), the ventral opening (Vo) the stomach (S) and sections of the limbs (L).

The inner surface of the calcified part of a nodded shell reveals only a cavity where the node is. In cross sections and in fractions of the animal the normally thin layer of outer epidermal cells (Fig. 5 OE) underlining the cuticle is somewhat thicker than usual and fills the hollow of the node completely. Sometimes even the inner epidermal cell layer bordering the inner lamella of the shell is thicker than normal (Fig. 4). Therefore, looking on the shell of a living specimen from the inside a hollow or indentation on the inner lining of the shell is not detectable.

With decreasing salinity of the ambient water the inner epidermal layer on the inside of the carapace develops some special features. At first cells can be seen with enlarged mitochondria with only few cristae. At salinities below 6‰ others replace these cells with many small and dense looking mitochondria accompanied by a typical apical labyrinthine structure (Fig. 6). The inner lamella is in this case often covered with bacteria, sometimes leaving no free space on the surface of this inner cuticle (Fig. 6). The epidermal layer bordering the outer calcified layer shows no alterations.

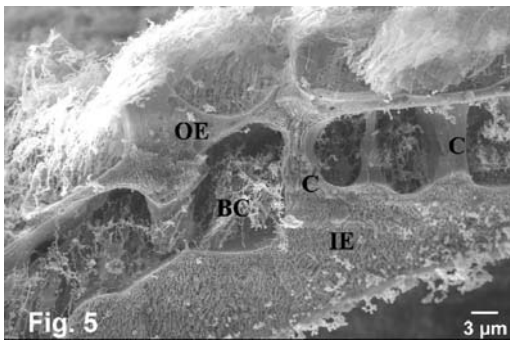

Fig. 5. SEM fracture of the epidermal cells of *C. torosa* showing the body cavity (BC), the inner epidermal cell layer, (IE) the outer epidermal layer (OE) and the connections between both cell layers (C) which can rupture during molting.

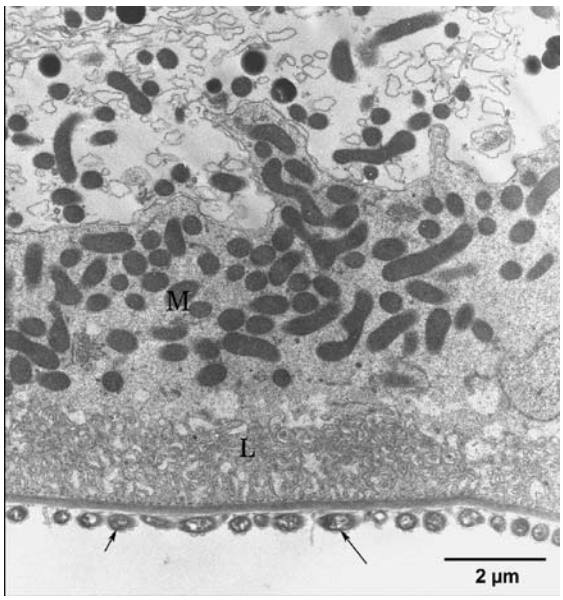

Fig. 6. TEM section of a smooth *C. torosa* in 4.0‰ showing the inner epidermal layer. Note the bacterial layer (arrows) on the outer side of the inner cuticle, the apical labyrinthine structure (L) and the small mitochondria (M).

Measurements of the salt concentration of the hemolymph of *C. torosa* kept at different salinities of ambient waters show that it is hyperosmotic in ambient waters below 6‰, while the animal reacts isoosmotically at higher salinities (Fig. 7). The concentration of the hemolymph in hypoosmotic conditions is about 200 mosm, which is comparable to about 6-‰ salinity (Table 1).

DISCUSSION

Nodded specimens of *C. torosa* are found at all salinities throughout the Schlei, however, the number of Nodded ani-

Table 1

Osmolarity of the hemolymph in *C. torosa*

Water, mosm	Hemolymph, mosm	Salinity water, ‰
Lowrest surviving limit, 14		0.5
31	180	0.9
72	184	2.1
134	209	4.5
150	220	4.7
202	225	6.3
263	266	8.3
356	359	11.3
490	500	15.9
591	596	18.8
708	703	22
825	824	26
925	928	30
978	982	30
1208	1203	38
1398	1406	44
Highest surviving limit, 1505		48

mals increases markedly below 6‰ (Vesper 1972), and is more common in younger stages (Fig. 8).

SEM and TEM investigations confirm that nodes develop at distinct places on the lateral side of the carapace (Sandberg 1964, Kilenyi 1972). However, it is important to note that these places are always located outside the part of the shell, which is connected to the body itself, *i.e*. outside of the so-called isthmus (Fig. 4). Here the outer and inner epidermal layers are running parallel to each other and are stabilized by cell-to-cell connections bridging the body cavity which is extended into the shells maintaining the circulation of the hemolymph (Fig. 5).

The surface ornamentation of the nodes resembles a balloon, which has been blown up and then released. The carapace shows signs of stretching on the nodes and the floor of the pits is striated. In order for this to happen, the normally hard and calcified cuticle must have been flexible when the node first appeared. Molting is the only period in the life of an ostracod when the cuticle is soft and flexible. Therefore, the development of a node would have to take place during molting or very shortly after the shedding of the old carapace. During the molting process there is also a strong internal force, which inflates the newly formed cuticle prior to the rupture of the old carapace. Therefore, we believe that the development of nodes is a malfunction during the enlargement of the new outer cuticle. This means that the nodation is not a normal procedure and is harmful to the animal.

Nevertheless, nodal deformation is typical for *Cyprideis* living in low saline waters (< 4.5‰). However, Schornikov (1973) reported nodded *Cyprideis* also from salinities of about 96‰. Taking all these mentioned factors into consideration, one is forced to conclude that the problem lies within the animal and the external situation only triggers the formation of the nodes. The most reasonable assumption is that a breakdown in the osmolarity control inside the animal is the cause of such a malformation.

Data on the osmolarity of several ostracods from a wide range of habitats has been provided by Aladin (1996). In the case of *C. torosa* he was able to show that normal osmolarity is of about 200 mosm in lower saline water (<6‰). Our own results on *Cyprideis* from the Baltic Sea in Germany support this finding (Table 1). Histological data (Fig. 6) also show that in order to survive in these low saline waters the epidermal layer in *Cyprideis* changes by developing new cells with an apical labyrinth and many small mitochondria. This transformation is regarded as evidence that the animal has problems with osmoregulation.

Observations on *Heterocypris salina* (Keyser *et al.* in press) show that in very low saline or freshwater ostracods usually lower the osmolarity of the hemolymph in order to in-

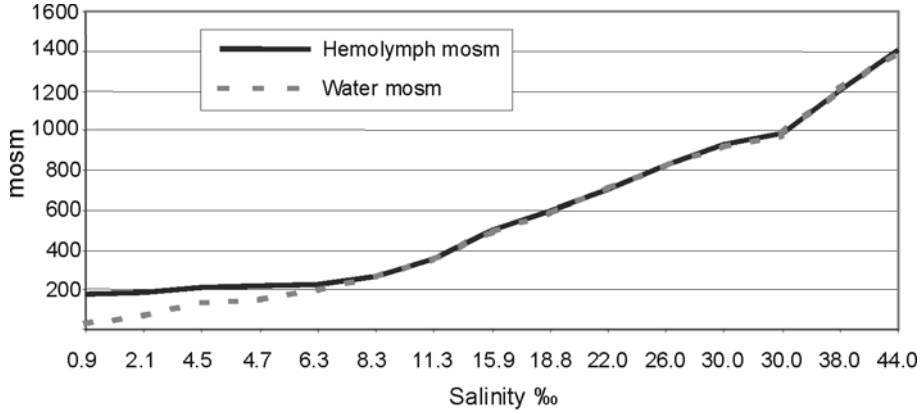

Fig. 7. Osmoregulation capacity in *C. torosa* in low saline to marine salinities.

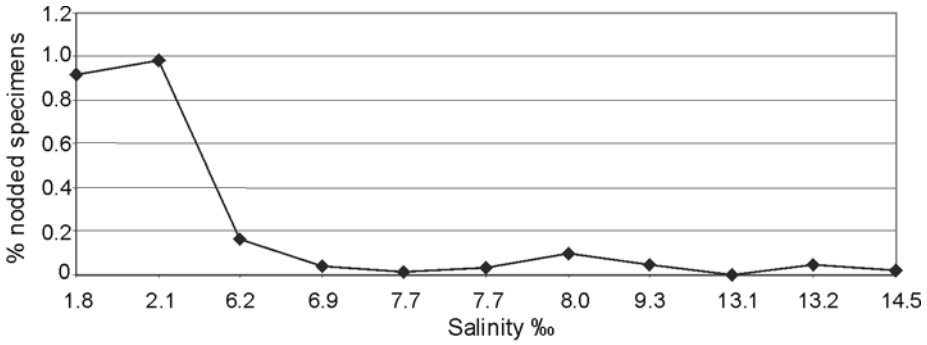

Fig. 8. Occurrence of nodded specimens of *C. torosa* in the Schlei Bight, Baltic Sea (adapted from Vesper 1972).

crease the volume of the body fluid during the molting process. It is supposed that *Cyprideis* in low saline waters reacts the same way.

Bearing in mind that *Cyprideis* is living here at salinity near to the border of its distribution, we assume that adjusting the osmolarity of its hemolymph during molting process is problematical. First, a certain amount of water has to be absorbed by the animal in order to increase its volume. But at the same time this intake must not dilute the inner salt concentration below a critical level, otherwise the cells and inner metabolism will be harmed. Clearly, animals living in less favorable environments (<6 ‰) could have difficulties achieving a balance across the newly formed cuticle during the molting process. If the internal pressure becomes too great, some of the connections holding the cells of the inner and outer epidermal layers together could tear apart. As a result the comparatively thin outer epidermal cell layer covered already with the thin flexible cuticle bulges out at these sites. The fact that sieve plates on the nodes are only slightly distorted suggests that these structures act as stabilizing elements, due to the different reaction of the sensory cells present at these areas.

After the old cuticle is ripped apart and the larger animal stretches out, the internal pressure of the hemolymph falls and the new cuticle is still flexible enough to retract slightly. However, like an over stretched balloon, it cannot revert to its original size. Ripple-like structures remain on the surface the node thus showing that its volume was once larger than at present. Cells of the outer epidermis, which have lost the stabilizing connections to the inner epidermal layer, contract forming a compact area beneath the node (Fig. 4). With the ongoing calcification these nodes are stabilized and are characteristic features of *C. torosa* living in low saline water.

The often-asymmetrical appearance of the nodes on the left and on the right valves of a single individual of *C. torosa* (Triebel 1941, Vesper 1972) can be explained by the molting behavior of the animal. During the inflation process the animal normally lies on one side of the shell. Therefore, additional force has to be applied to inflate the side carrying the weight of the animal. This, in turn, leads to lower and smaller nodes on the side facing the sediment and higher, larger nodes on the side facing the water.

Terraces of different heights on the nodes (Fig. 3) can also be explained by this view of the noding process. If one part of the epidermal connections (Fig. 5) tears it is very likely that other connections will not be able to withstand the additional force transmitted to them and rupture too. So the first part of the node expands the most, while later ruptures nearby, which are not subject to the initial pressure, are less elevated.

These results explain many observations connected to the formation and appearance of nodes in *C. torosa* in brackish waters. But nothing can be said at this time about the development of nodes in highly saline waters as the osmotic regulation during molting of animals living in these waters is not known.

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