Localization of digestion activities in polyps of *Nausithoe planulophora* and *Thecoscyphus zibrowii* (Coronatae, Scyphozoa, Cnidaria)

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ABSTRACT: Coronate polyps are unique among cnidarians in having a complete peridermal tube, a ring canal, and four radial canals or pores at their oral region. Moreover, most of these species possess tooth whorls that narrow the gastric cavity considerably. Using fluorescence-labeled prey, it was demonstrated that the ring canal is not involved in digestion or redistribution of nutrients but possibly serves as a hydrostatic flex point for the fast retraction of the tentacle crown into the exoskeleton. The tooth whorls considerably affect the localization of digestion activities by blocking the passage of large prey fragments. Hence, endocytosis in a coronate species with tooth whorls occurred around the uppermost tooth whorl whereas, in a species lacking tooth whorls endocytosis occurred at the basal end. To meet the high nutrient demands of the basal region, nutrients must be redistributed in the species with tooth whorls. The extra energy required for this redistribution could be an important disadvantage of tooth whorls.

INTRODUCTION

The taxon Coronatae (Scyphozoa, Cnidaria) includes environmentally (Mauchline & Harvey, 1983; Larson, 1990; Fossaa, 1992; Alvarez-Cadena & Segura-Puertas, 1993) and medically (Black et al., 1994; Wong et al., 1994) important species. Moreover, this taxon is of phylogenetic interest since the close similarities between Coronatae polyps and the extinct Byroniida – early Cambrian to Permian (Bischoff, 1989) – suggest an early divergence of Coronatae during the evolution of the Scyphozoa (Werner, 1966, 1967, 1984; Bischoff, 1978, 1989; Van Iten, 1991, 1992a, 1992b; Van Iten & Cox, 1992; Schuchert, 1993; Jerre, 1994). Despite this presumably early divergence, coronate polyps have a comparably complex gastric cavity. The presence of a ring canal in the oral region (Figs 1–4; Chapman & Werner, 1972) is unique among cnidarian polyps. Moreover, in most coronate polyps the gastric cavity is narrowed by one or more tooth whorls (consisting of 1–16 teeth) of the chitinous periderm tube (Fig. 6; Jarms, 1991). These rings of internal processes serve for stabilisation of the tube and it has been postulated that these tooth whorls protect the highly regenerative basal part of the polyp against predators (Jarms, 1990).
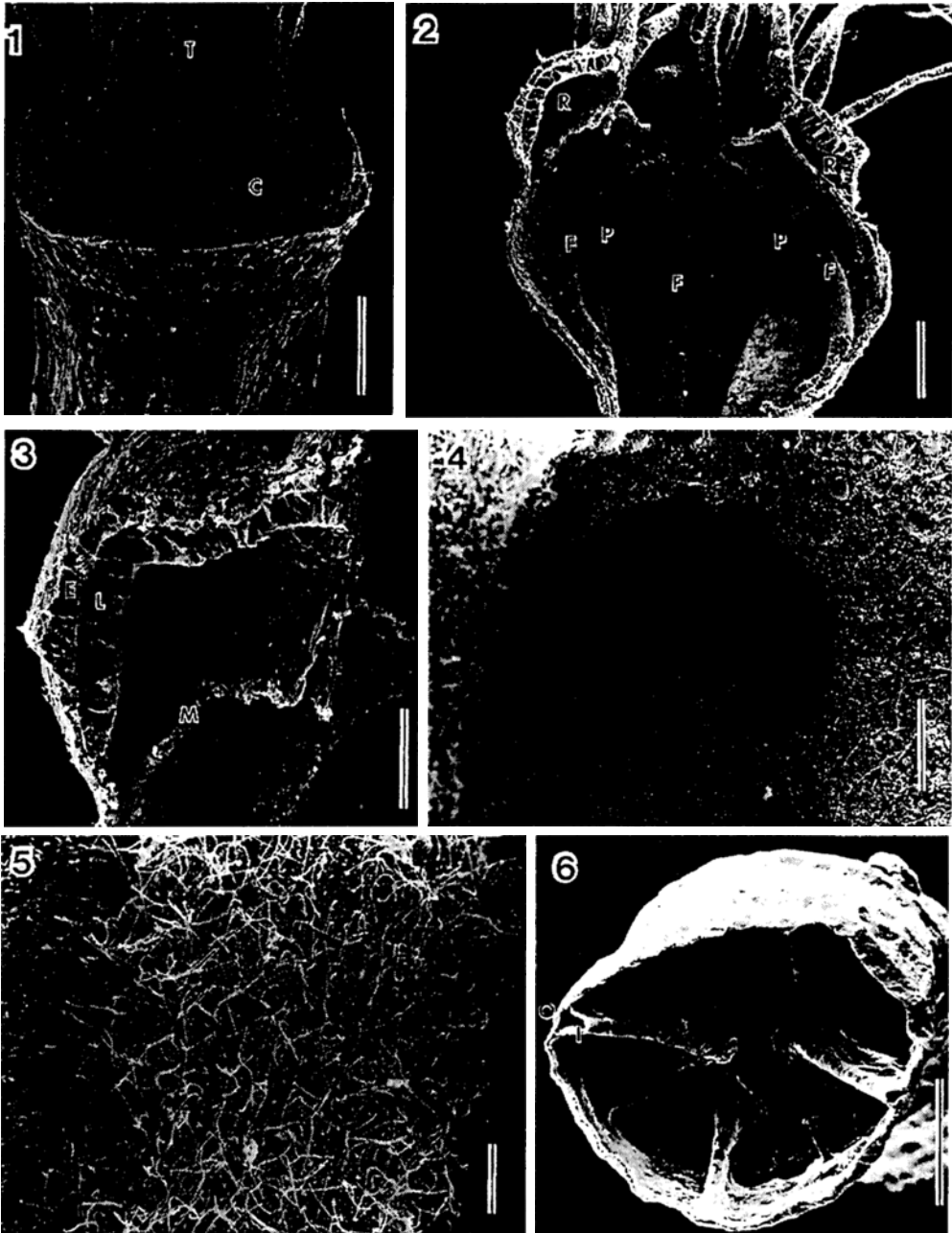
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Fig. 1. *Nausithoe planulophora.* SEM micrograph showing morphology of the external oral region after removal of the periderm (Scale = 200 μm). C: capitulum, T: tentacles

Fig. 2. *N. planulophora.* Internal morphology of the oral region (one quadrant removed, scale = 200 μm). F: gastric septum, P: perradial pore of the ring canal, R: ring canal

Both unique features of coronate polyps (apical canal system and tooth whorls) could affect prey digestion and the distribution of nutrients. The ring canal might facilitate the nutrient transport to the tentacle crown and the capitulum where glandular cells secrete the outer peridermal layer (Chapman & Werner, 1972; Werner, 1984). The tooth whorls could impair the typical basal transport of prey and food fragments normally occurring in coronate and other scyphozoan polyps (Chapman, 1973). To investigate these potential impacts, we observed the digestion of fluorescence-labeled prey in two coronate species, one with tooth whorls (*Nausithoe planulophora* (Werner, 1971)) and another without tooth whorls (*Thecoscyphus zibrowii,* Werner, 1984).

MATERIALS AND METHODS

Specimens of *Nausithoe planulophora* were collected in submarine caves in the Mediterranean Sea near Marseille, France, in 1969. Since then they have been in continuous culture (Werner, 1971). *Thecoscyphus zibrowii* was detected among material from submarine caves in the Gulf of Sorrent in 1975 (Werner, 1984) and is also in culture since then.

Coronate polyps were cultivated in glass dishes in filtered natural seawater (salinity 30–35) at 21 °C in the dark. They were fed once a week with harpacticoid copepods *(Tisbe holothuriae)*. This prey was cultivated in seawater at ambient temperature and fed once a week with cooked, dried, and minced mussels *Mytilus edulis* (Jarms, 1988).

To investigate digestive activities, covalently fluorescence-labeled prey was used. The fluorophore, Texas Red was used because its spectroscopic characteristics are rather insensitive to environmental influences (i.e. the pH) and its long-wavelength emission maximum at 620 nm can be spectrally separated from endogenous fluorescence (around 500 nm) of the species used. To label the prey, a few crystals Texas Red sulfonyl chloride (Molecular Probes) dissolved in 10 µl dimethylformamide were added to some 50 juvenile *T. holothuriae* suspended in 1 ml seawater. After incubation for 30 min at ambient temperature in the dark, the labeled prey was washed three times with seawater and incubated for 30 min in fresh seawater to remove any non-reacted or inactivated dye. Fluorescence-labeled prey survived at least six days indicating supravital staining. For the feeding experiments, single freshly labeled prey was fed to a coronate polyp. Because of the more translucent periderm we used young specimens from 1.8 mm up to 3.2 mm of both coronate species. Prey capture, ingestion, digestion,

Fig. 3. *N. planulophora.* Enlargement of the ring canal detailing the vacuolarized nature of the endodermis (scale = 100 µm). E: epidermis, L: lateral endodermis of the ring canal, M: medial endodermis of the ring canal

Fig. 4. *N. planulophora.* Enlargement of the perradial pore which connects the ring canal with the rest of the gastric cavity (scale = 30 µm)

Fig. 5. *N. planulophora.* Enlargement of the gastric septum illustrating its dense ciliation (scale = 10 µm)

Fig. 6. *N. planulophora.* View into the periderm tube detailing the uppermost tooth whorl (scale = 200 µm). O: outer periderm layer, I: inner periderm layer
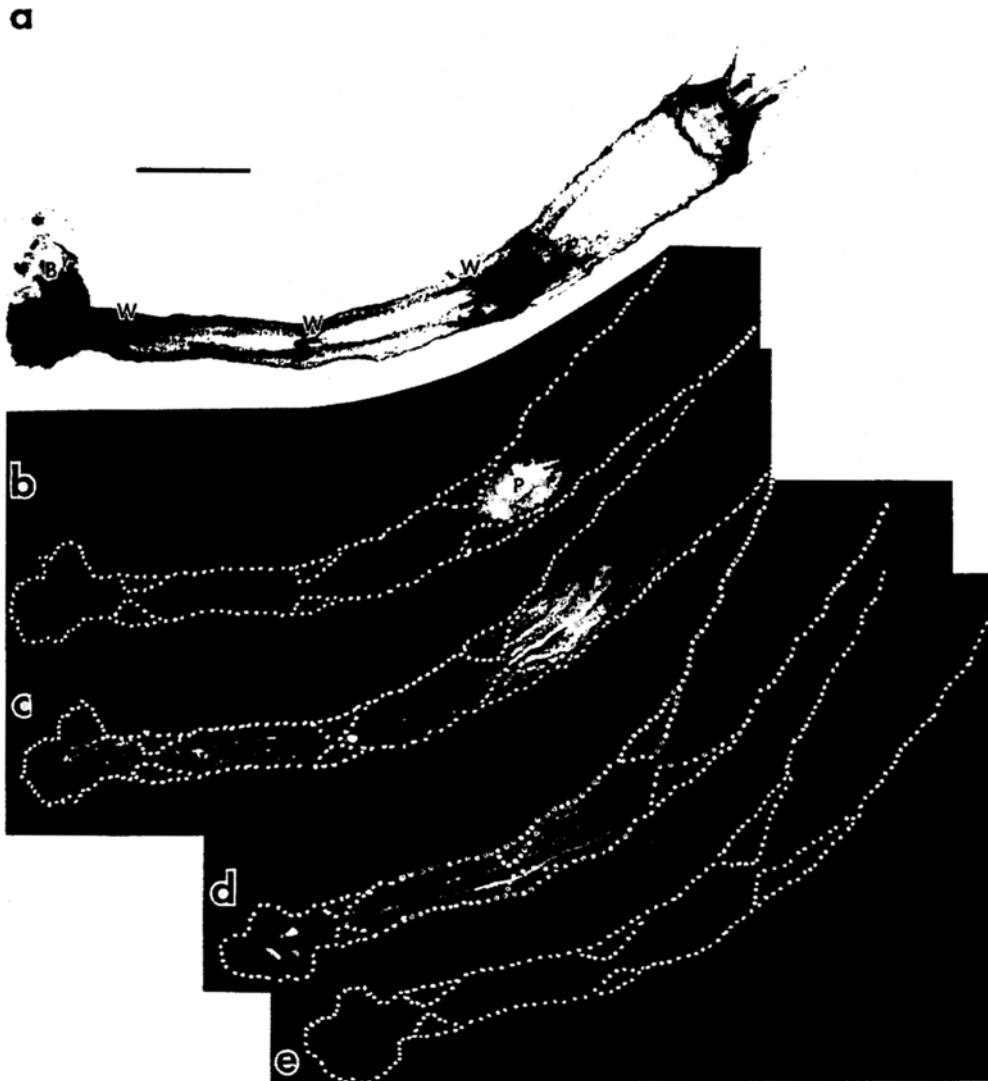
Fig. 7. *N. planulophora*. Digestion of Texas Red-labeled copepod, *Tisbe holothuriae*. Bright field micrograph, 1 h after feeding (a) and fluorescence micrographs 1 h (b), 4 h (c), 1 week (d), and 3 weeks (e) after feeding (scale = 500 μm). B: basal disk, P: prey, T: tentacles, W: tooth whorl

and egestion were observed for ten replicates of each species using a fluorescence microscope (Zeiss Universal with MC 63 camera).

For scanning electron microscopy, polyps were anaesthetized with 330 mM $MgCl_2$, fixed with 2.5% glutaraldehyde in 80% sea water, and critical point dried.

RESULTS

The thickened oral end of the coronate polyp is called a capitulum and contains the ring canal (Figs 1, 2, 3; Chapman & Werner, 1972; Werner, 1984) that opens to the rest of the gastric cavity through 4 perradial pores (Figs 2, 4). The lateral endodermis of the ring canal consists of highly vacuolarized cells (Fig. 3). The endodermis of the ring canal and the pores bear only a few cilia, while other endodermal regions (especially the gastral septa) are densely ciliated (Fig. 5) as reported earlier for the Coronatae (Chapman, 1973) and other Scyphozoa (Blanquet & Wetzel, 1975; Hentschel & Hündgen, 1980).

The gastric cavity of *Nausithoe planulophora* is narrowed by several tooth whorls which contain up to 16 teeth (Figs 6, 7). These tooth whorls mechanically block the passage of particles larger than about 50 μm which could impair the transport of large food fragments in the gastric cavity. To investigate this, we compared the digestion of fluorescence-labeled food in *N. planulophora* (Fig. 7) with the digestion in *Thecoscyphus zibrowii* which lacks tooth whorls (Fig. 8).
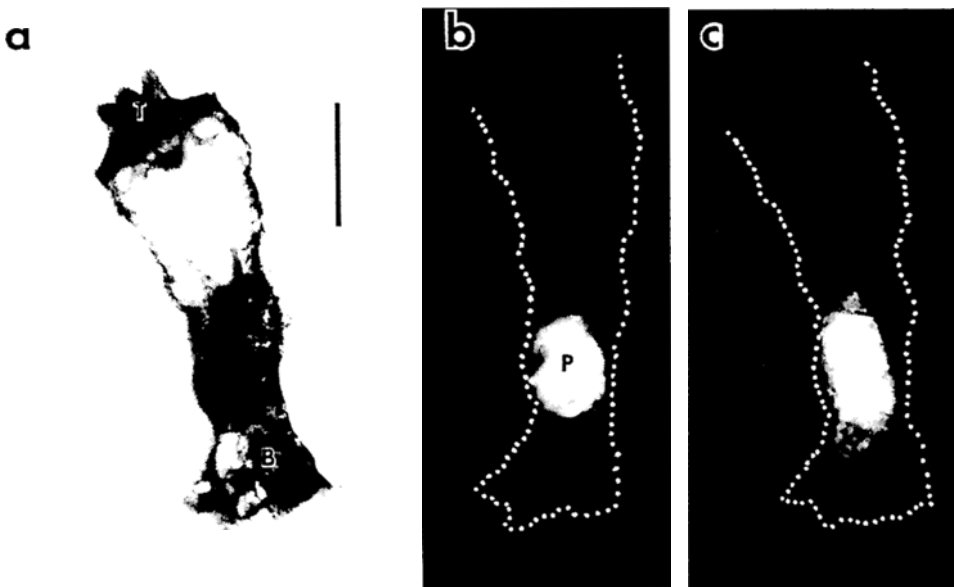

Fig. 8. *Thecoscyphus zibrowii.* Digestion of Texas Red-labeled copepod, *Tisbe holothuriae.* Bright field micrograph, 1 h after feeding (a) and fluorescence micrographs 1 h (b) and 4 h (c) after feeding (scale = 500 μm). B: basal disk, P: prey, T: tentacles

After capture by *N. planulophora* copepod prey survived inside the gastric cavity for up to 30 min. After death the prey was transported within 15 min down to the uppermost tooth whorl which mechanically blocked further basal transport (Fig. 7a, b). This transport was active, since it occurred also in upside down-orientated polyps. The prey was extracellularly digested over the next several hours as indicated by the appearance of small (diameter <40 µm) fluorescent food fragments some of which were transported along the gastral septa through the tooth whorls to the basal region with a velocity in the range of 30 µm min⁻¹. However, most fragments were unable to pass beyond the first tooth whorl that was clogged by the slowly digesting prey exoskeleton. During digestion, small traces of solubilised food fragments were uniformly distributed within the gastric cavity except the ring canal where no fluorescence was detected. Parallel to the extracellular digestion, single cells of the polyp became highly fluorescent indicating the endocytosis of food fragments. Most endocytosis took place in the region close to the uppermost tooth whorl. After the completion of extracellular digestion, indigestible material (mainly chitinous parts of the copepod exoskeleton) was egested and the predominant endocytosis near the uppermost tooth whorl became especially evident (Fig. 7c). During the following days, some redistribution of the fluorescence occurred towards the basal region while the total fluorescence yield steadily decreased (Fig. 7d). After three weeks, the weak fluorescence was mostly restricted to the basal disk (Fig. 7e). No fluorescence could be detected within the ring canal at any stage.

In contrast, *T. zibrowii* polyps killed copepod prey immediately upon contact. After ingestion, the prey was transported within 15–30 min to the aboral end of the polyp (Fig. 8a, b). Extracellularly digested food fragments were mostly absorbed in the basal region while only small traces of solubilized food fragments were uniformly distributed within the gastric cavity except in the ring canal where no fluorescence was detected. During the subsequent days, almost all of the fluorescent label remained in the basal region, but a small fraction was redistributed towards the oral end. However, no fluorescence was detected in the ring canal at any stage.

DISCUSSION AND CONCLUSIONS

Nearly nothing is known about the natural diet of the mainly deep-sea or cave dwelling coronate polyps. But since we sometimes have found copepods in the gastric cavity of preserved specimens we think copepods are at least partly within the normal prey spectrum. In culture *Tisbe* serves as a good and sufficient food. Protozoans always present in the culture glass dishes cause no reaction if touching tentacles and never become ingested. So the results by using *Tisbe* can be called representative.

In this study, the localization of digestive activities in two coronate polyps was investigated with covalently fluorescence-labeled prey. In both polyps, the fluorophore remained largely confined to a small fraction of the gastric cavity throughout extracellular digestion and endocytosis which indicates that both processes were highly localized. This is consistent with results recently obtained for another scyphozoan polyp *(Aurelia aurita)* where enzyme secretion, extracellular digestion, and endocytosis are all confined to a small region of contact between the prey and the polyp's gastrodermis (Bumann et al. in preparation).

In the two coronate species investigated, this localized digestion led to an entirely different localization of digestive activities depending on the absence or presence of tooth whorls. In *Thecoscyphus zibrowii* the prey was transported to the basal (aboral) region immediately after ingestion and digested there. In *Nausithoe planulophora*, however, tooth whorls mechanically block the passage of particles larger than about 50 µm. Larger prey could thus be transported only down to the uppermost tooth whorl where most digestion and endocytosis took place. To identify possible consequences of these differently localized digestive activities, one needs to consider where nutrients are used. Coronate polyps regenerate a whole new polyp after proliferation by terminal strobilation from the remaining small basal residue (Werner, 1984; Jarms, 1988). Since this regeneration is the main synthesis effort in coronate polyps, the basal region probably has the highest nutrient demand, although some nutrients are used by the capitulum to synthesize the outer layer of the chitinous periderm (Chapman & Werner, 1972). In contrast, the middle region of the polyps is not likely to have elevated metabolic activities.

In *T. zibrowii*, food is transported to the nutrient demanding basal region by ciliary currents prior to digestion and endocytosis. *N. planulophora* has similar ciliary currents but they mostly fail to transport food fragments beyond the uppermost tooth whorl.

Accordingly, endocytosis takes place principally in the middle of the polyp where nutrient demands are low. Both the capitulum and the basal disk are several mm away from this region. Hence, considerable redistribution of nutrients must occur after endocytosis. The observed redistribution of the fluorescent label could reflect this, although it is unclear how long after endocytosis the fluorophore remains covalently bound to nutrients. The redistribution of nutrients in *N. planulophora* could be mediated by amoebozytes as has been shown for other cnidarians (Van Praet, 1980; Werner, 1984).

In both species, the gastral septa produce ciliary currents with probably similar energy requirements. However, *N. planulophora* additionally needs a considerable redistribution of nutrients after endocytosis. The extra energy for this redistribution is an inevitable consequence of the presence of tooth whorls and could partially compensate the benefits of tooth whorls from protection against predation. This argument is supported by the fact that some Coronatae with low predation risk like colonial species or with a strong short tube as *T. zibrowii* do not posses tooth whorls (Jarms, 1988; Uriz et al., 1992).

In both coronate polyps neither soluble molecules nor particles entered the ring canal during digestion. This indicates that at least during the several hours of digestion the fluid of the ring canal was physically separated from the gastric cavity. Redistribution of nutrients after endocytosis also did not involve the ring canal. Little fluid transport into the ring canal is expected from the scarce ciliation. Moreover, vacuolarized cells, as found in the lateral endodermis of the ring canal, are usually not involved in food processing in cnidarians (Lesh-Laurie & Suchy, 1991). Thus both physiological and cytological evidence do not support the proposed function of the ring canal to facilitate distribution of nutrients to and within the capitulum (Werner, 1984).

The vacuolarized highly turgescent cells of the ring canal could mechanically stabilize the extended tentacle crown (Chapman & Werner, 1972; Werner, 1984) similar to their role in tentacle reextension (Lesh-Laurie & Suchy, 1991). Possibly the ring canal assists in this stabilization by acting as a hydrostatic device. The distinct separation of

fluids in the ring canal from the rest of the gastric cavity observed here supports this hypothesis. More importantly, together with the margin of the periderm tube this separation of fluids suggests that the ring canal could provide a hydrostatic flex point for the very fast retraction of the entire tentacle crown into the gastric cavity as a defense mechanism or after prey contact that is only observed in coronates. The muscles used for this retraction run through the medial wall of the ring canal (Chapman & Werner, 1972), in agreement with this putative mechanism. Indeed, some hydrostatic device is needed, since hard parts apart from the uppermost rim of the exoskeleton are not involved and the thin epithelia of coronate polyps are unlikely to provide the necessary counter forces for such a fast bending. Further experiments to test this hypothesis are currently under way.

In conclusion, the results indicate a highly heterogeneous function of different regions of the gastric cavity of coronate polyps. This is remarkable given the simple homogeneous histology of the gastrodermis (Chapman & Werner, 1972) and the presumably early divergence during the evolution of the Cnidaria.

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