A New Chemosensory System in the Chiton Larva (Mollusca: Polyplacophora): Fine-structure and Immunocytochemistry

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Introduction

Marine pelagic larvae of most invertebrate taxa bear sensory systems like the apical organ in the pretrochal area, which have become emphasised in a number of recent investigations with modern techniques (TEM, immuncytochemistry) (e.g. Sensenbaugh & Franzén, 1987; Kempf et al., 1997; Page & Parries, 2000). Although the ontogeny of the primitive molluscs Polyplacophora has been studied by several authors, fine-structural investigations (eg. Rosen et al. 1979; Bartolomaeus 1989, 1992) on the subject are scarce and immunocytochemical methods have not yet been applied. In the course of a general study on larval features of polyplacophorans we encountered a new sensory system in the pretrochal area of three species representing three different families.

Materials and Methods

Larvae and juveniles were either obtained by maintaining cultures after spawning of adults in the laboratory (*Chiton olivaceus* Spengler, 1797, *Mopalia muscosa* (Gould, 1846)) or by hatching of metamorphic competent larvae of the brooding *Lepidochitona* aff. *corrugata* (Reeve, 1848). Semithin sectioning and electron microscopy followed routine procedures.
For immunocytochemistry larvae and juveniles of *Mopalia muscosa* were fixed with 4% paraformaldehyde in 0.1M phosphatebuffer (=PBS) with 10% sucrose added. Specimens were blocked in 6% goat serum (= block serum) prior to antibody incubation. Anti-FMRF-amide and anti-serotonin, both raised in rabbit were used as primary antibodies. Secondary antibodies were goat anti rabbit immunglobulin G conjugated with rhodamin-(TRITC) or oregon green-(FITC). Analyses were done on a Leica DM IRBE microscope supplied with a confocal laser scanning unit.

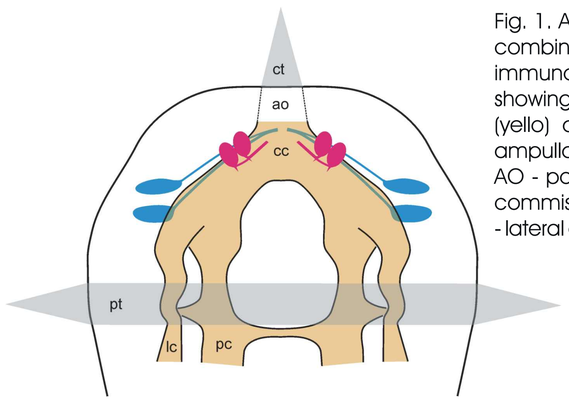


Fig. 1. Anterior portion of the larva (diagrammic - combined from serial section reconstruction and immunocytochemistry in *Mopalia muscosa*.) showing the main cords of the nervous system (yello) and the ventral (blue) and dorsal (red) ampullary cells with innervation; Abbreviations: AO - position of the apical organ, CC - cerebral commissure, CT - ciliary tuft of the apical organ, LC - lateral cord, PC - pedal cord, PT - prototroch.

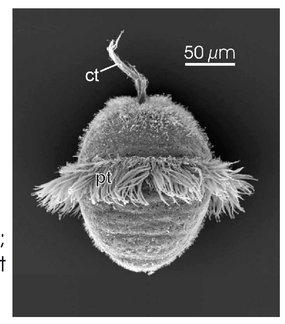


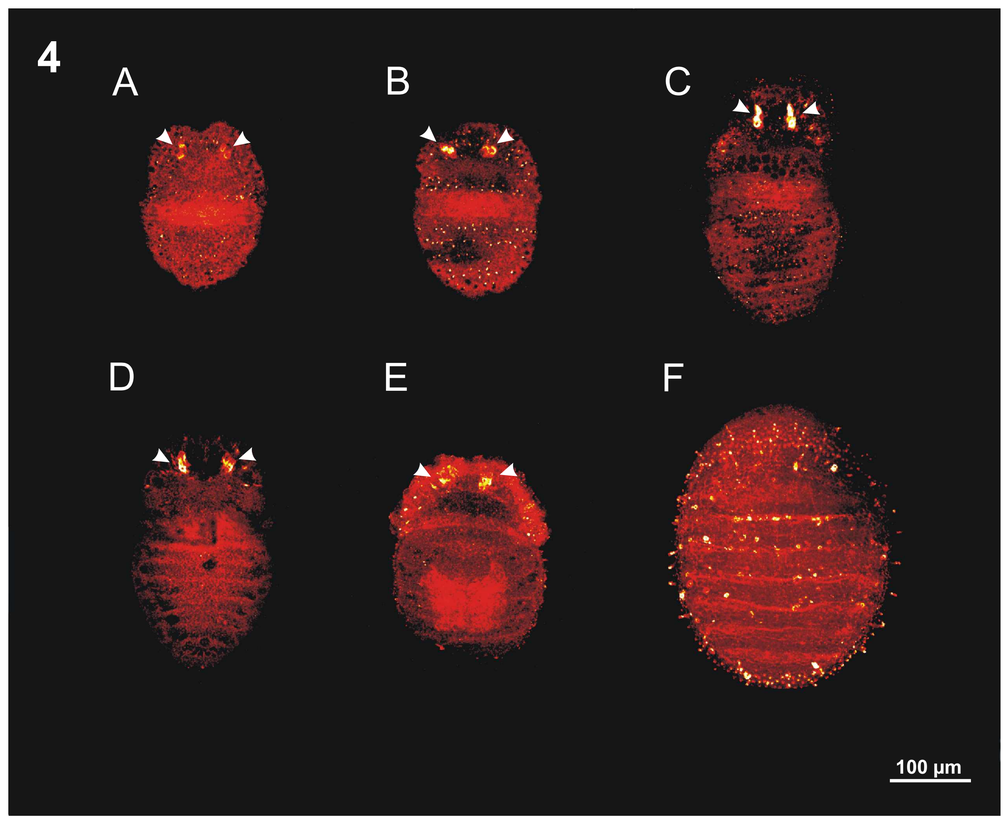
Fig. 2. *Mopalia muscosa*. 74 hpf; SEM; dorsal view; Abbreviations: ct - ciliary tuft, pt - prototroch.

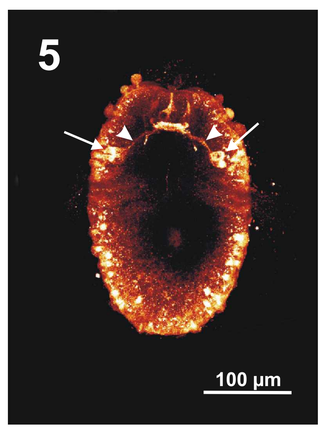
RESULTS

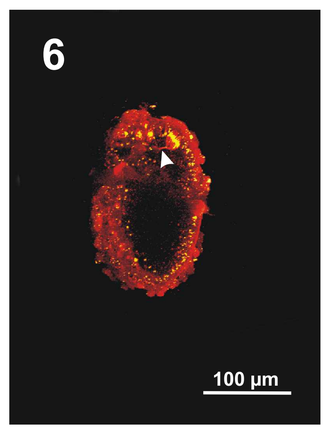
General
The new sensory system is very similar in the three species investigated. In general the system consists of one dorsal and one ventral pair, each consisting of two so-called ampullary cells lying in the pretrochal epithelium (Figs. 1, 6) of the chiton larva (Fig. 2). The ventral ones lie more laterally than the dorsal ones. The ampullary system disappears at metamorphosis (Fig. 4F).

TEM
Each ampullary cell is mainly characterized by a prominent vacuole (ampulla), which occupies the distal half of the cell. It communicates with the external environment via a minute pore (Fig. 3B). The ampulla contains numerous spirally arranged cilia emerging from the lateral walls. While the plane of the ciliary spiral arrangement is horizontal in *M. muscosa*, it is oblique to vertical in *L.* aff. *corrugata* (Fig. 3D) and *Ch. olivaceus*. Along their length the cilia show modifications in microtubular pattern from regular 9x2+2 to various reduction stages leading to a decreasing diameter and irregular contour in cross section of the cilium (Fig. 3E). In the basal area the ampulla also contains dense material of uncertain nature. (Fig. 3B,D). The nucleus lies basally near the descending axon (Fig. 3B).

Immunocytochemistry
The cytoplasm of the ampullary cells is heavily stained by anti-FMRF-amide (Figs. 4A-E, 5-7). In addition, the dorsal ones show weak reactivity against serotonin. All ampullary cells are innervated by anti-FMRF-amide positive axons emerging anteriorly from the cerebral commissure (Figs. 5, 6).







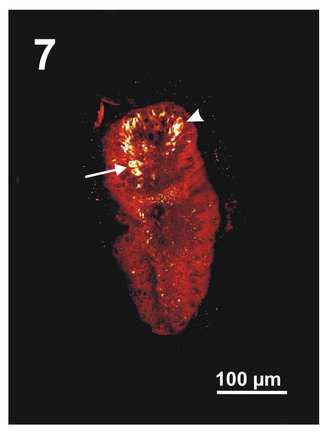
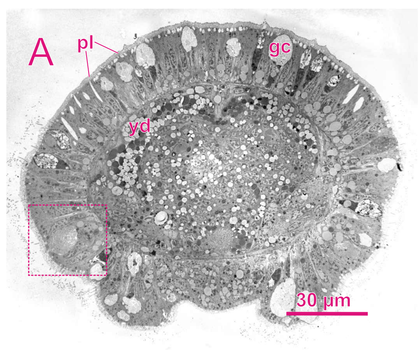
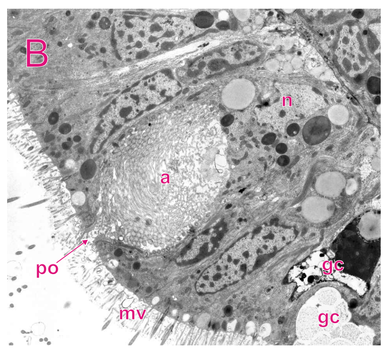
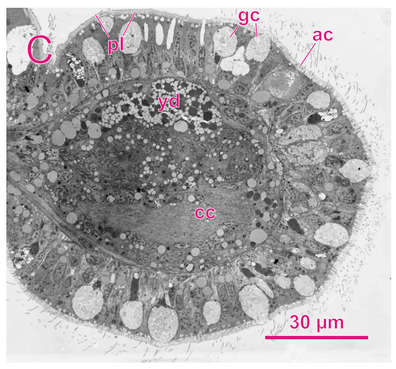
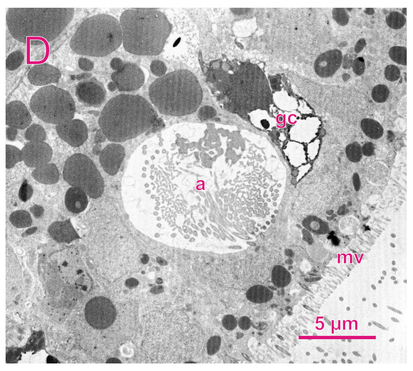


Fig. 4. *Mopalia muscosa*. Anti FMRF-immunoractivity during of the dorsal portion during development; dorsal ampullary cells are marked by arrow heads; A. Larva, 58 hours post-fertilization (= hpf). B. Larva 82, hpf; C. Larva, 134 hpf; D. Larva, 214 hpf; E. Metamorphic stage, 282 hpf; F. Juvenile, 306 hpf, primordia of shell plates are marked by asterisks.
Fig. 5. *Mopalia muscosa*. 67 hpf; Anti FMRF-immunoractivity of the ventral ampullary cells (Arrows) with innervation (arrow heads).
Fig. 6. *Mopalia muscosa*. 74 hpf; dorsal, slightely turned to show the innervation (arrow head) of the dorsal ampullary cells.
Fig. 7. *Mopalia muscosa*. 202 hpf; lateral view; showing both ventral and dorsal (arrow head) ampullary cells (arrow).









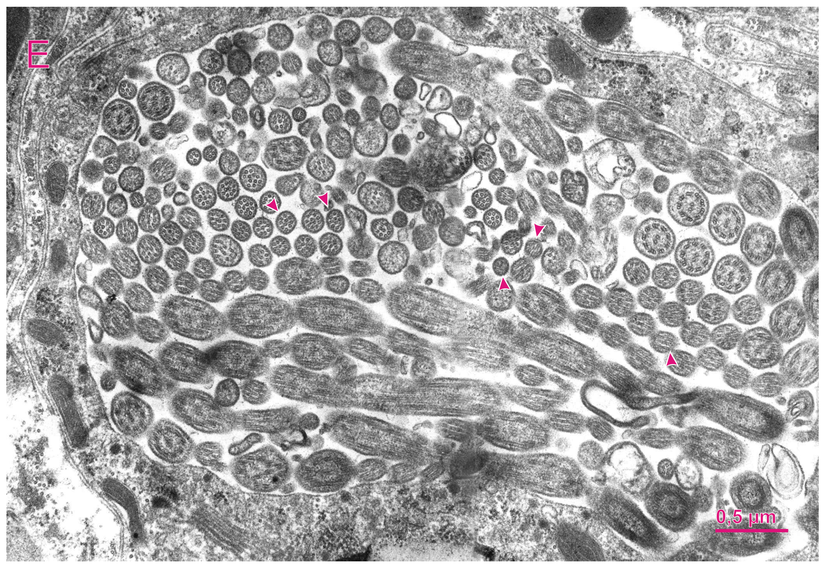


Fig. 3. TEM section through larvae. Abbreviations: a - ampulla, gc - glandular cells, m - mitochondrium, mv - microvillar border, n - nucleus of the ampullary cell, pl - primordial cuticula of shell plate I, po - porus of the ampulla, yd - yolk droplets. A. *Mopalia muscosa*. Larva 240 hpf, cross section through pretrochal area showing the position of the ampllary cells (in rectangle); B. *Mopalia muscosa*. Detail of Fig. 1 showing the ventral ampullary cell. C. *Mopalia muscosa*. Larva240 hpf, anterior is to the right, longitudinal section through pretrochal area with dorsal ampullary cell. D. *Lepidochitona* aff. *corrugata*. Hatching metamorphic larva; cross section through ventral ampullary cell. E. *Chiton olivaceus*. 94 hpf; longitudinal section, details of ventral ampullary cell showing various microtubular reduction stages of cilia (examples indicated by arrow heads).

DISCUSSION

Cytological aspects
The most remarkable feature of the ampullary cilia is their irregular microtubular pattern. In particular the elongated tips of these cilia are peculiar. The differences regarding the orientation of the ciliary spiral suggest that the direction of the respective signal is of minor importance (see below).

Homology relations
The highly specific fine-structure of the ampullary cells provides strong evidence for a cytological homology with other ampullary cells reported in various organs throughout the Mollusca and other spiralians such as the osphradia of vetigatropods, lips and rhinophores of cephalopods, and the so-called cephalic sense organs of gastropod and bivalve veligers and polychaete trochophores. A possible homology at the organ level the cephalic sensory organs is contradicted by: (1) In contrast to the arrangement in bivalve, gastropod and polychaete larvae, the chiton ampullary system is a double-paired structure; (2) Location of the chiton ampullary system differs from ampullary cells of bivalve and gastropod larvae, where they are generally integrated in the apical complex. (4) Ampullary cells are entirely lacking in the apical complex of the primitively lecithotrophic larvae of the patellogastropod *Patella caerulea* (pers. obs.), of the scaphopod *Antalis vulgaris* (pers. obs.), and of the protobranch bivalve *Acila castrensis* (Zardus & Morse, 1998). Therefore, the ampullary system as a whole is considered as a synapomorphy for at least chitonid Polyplacophora.

Functional significance
The sensory nature of the organ is supported by the presence of basally located axonal processes, the irregular pattern of microtubules and lack of ciliary roots, the staining characteristics by anti-FMRF-amide and (less significant) by anti-serotonine. Evidence for chemoreceptory function includes: (1) The location of the sensory cilia within an ampulla and their contact with the external environment via a very narrow opening contradict a mechanoreceptory role. (2) All other ampullary cells in molluscs are known or believed to be chemoreceptors. (3) No evidence suggests a photoreceptive role. (4) The varying orientation of the ciliary spirals between species contradicts an orientated signal. (5) The strong staining of the cytoplasm by anti-FMRF-amide and the weak signal against serotonin corresponds to other anti-FMRF-amide reactive cells found in molluscan chemoreceptors (eg. Suzuki et al., 1997). The specific function of the ampullary system is obviously correlated with larval biology because of its reduction at metamorphosis.

REFERENCES

Bartolomaeus, T. 1989 Larvale Nierenorgane bei *Lepidochiton cinereus* (Polyplacophora) und *Aeolidia papillosa* (Gastropoda). Zoomorphology. 108: 297-307.
Bartolomaeus, T. 1992 Ultrastructure of the photoreceptor in the larvae of *Lepidochiton cinereus* (Mollusca, Polyplacophora) and *Lacuna divariacta* (Mollusca, Gastropoda). Microfauna Marina. 7: 215-236.
Kempf, S.C., Page, L.R. & Pires, A. 1997 Development of serotonin-like immunoreactivity in the embryos and larvae of nudibranch mollusks with emphasis on the structure and possible function of the apical sensory organ. Journal of Comparative Neurology. 386: 507-528.
Page, L.R. & Parries, S.C. 2000 Comparative study of the apical ganglion in planctotrophic caenogastropod larvae: ultrastructure and immunoreactivity to serotonin. Journal of Comparative Neurology. 418: 383-401.
Rosen, M.D., Stasek, C.R. & Hermans, C.O. 1979. The ultrastructure and evolutionary significance of the ocelli in the larva of *Katharina tunicata* (Mollusca, Polyplacophora). Veliger. 22: 173-178.
Suzuki, H., Kimura, T., Sekiguchi, T. & Mizukami, A. 1997 FMRF amide-like-immunoreactive primary sensory neurons in the olfactory system of the terrestrial mollusc, *Limax marginatus*. Cell and Tissue Research. 289: 339-345
Sensenbaugh, T. & Franzén, A. 1987 Fine structural observations of the apical organ in the larva of *Polygordius* (Annelida; Polychaeta). Scanning Microscopy. 1: 181-189.
Zardus, J.D. & Morse, P.M. 1998 Embryogenesis, morphology and ultrastructure of the pericalymma larva of *Acila castrensis* (Bivalvia: Protobranchia: Nuculoidea). Invertebrate Biology. 117: 21-244.