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Immunohistochemical investigations of the interstitial cnidarian *Halammohydra octopodides* (Hydrozoa)

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Abstract

We describe immunohistochemical staining of *Halammohydra octopodides* (Hydrozoa, Cnidaria) with antibodies against RFamides, LWamides, serotonin, acetylated α-tubulin, tyronsinated α-tubulin and β-tubulin, in combination with markers of the musculature (phalloidin) and the nuclei (DAPI). Immunoreactivity against RFamides, LWamides and β-tubulin shows elements of the nervous system, in particular a solid nerve ring in the aboral cone, basal to the tentacle rings. Further nervous structures include diverse neurites extending into the tentacles and a trunk plexus with differential expression of RFamides and LWamides; a condensation of neurites is also present around the mouth opening. The three tubulin markers stain the epidermal cilia and conspicuous neuronal fibers in the neck region. Further staining (internal cilia, nervous structures) is different among these markers.

Introduction

Few cnidarian species have invaded the meiofaunal habitat, also known as the interstitial system (Clausen 1971, Thiel 1988), among them species from the genus *Halammohydra* are the most well known. *Halammohydra* was described by Remane (1927) with two species from the Western Baltic Sea and Helgoland in the Central North Sea. Since then, 10 species have been described from several locations (Thiel 1988, Bouillon et al. 2006). Knowledge on the body organization of *Halammohydra* species is based one some histological investigations (e.g. Remane 1927, Swedmark & Teissier 1967) and few TEM (transmission electron microscopy) investigations dealing with the cnidocysts (Clausen 1991) and the spermatozoa (Ehlers 1993).

Halammohydra species have a slender trunk region with a broad mouth opening at one end (Fig. 1A,C,D, 2B). The term "trunk" is used here in a neutral sense and should not imply homology to the trunk in polyps. Instead, an interpretation as an enlarged manubrium is also possible (see below). Within this trunk are the gastric cavity and the gonads. The trunk narrows in a neck region, leading to the aboral cone, which contains an adhesive organ and from which statocysts and two rings of tentacles originate (Figs. 1A,B, 2A,C,D). The adhesive organ is composed of an epithelially lined cavity opening with an apical pore (Fig. 1B) and thereby marking the aboral pole

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of the animal. Remane's (1927) original assumption that the lumina of gastric cavity and adhesive organ were connected proved to be incorrect. Several authors observed that animals may attach to sand grains with their aboral pole, so this structure was assumed to be an adhesive organ. The tentacles can vary in number and length, but always two alternating rings are present. These rings are called aboral (furthest away from the mouth opening) and subaboral ring. Statocysts occur alternating with the subaboral tentacles, their number usually corresponds to the number of subaboral tentacles. Within the aboral cone, on the basis of the tentacles, is a nerve ring, from which fine neurites run towards the statocysts and into the tentacles (Swedmark & Teissier 1967).

It is quite clear that the invasion of the interstitial habitat in cnidarians is a secondary phenomenon which is connected to several changes in body organization. Species of *Halammohydra* show characters reminiscent of the polyp stage (general body form), of the medusa stage (statocysts) and from larval stages (complete external ciliation). To better understand the adaptations of *Halammohydra* to the interstitial system it is important to understand the structure on each organ system. Here we have investigated the nervous system of the species *H. octopodides* Remane, 1927.

Textbook knowledge on the cnidarian nervous system is often generalized as a simple nerve net (a plexus) without components of "higher organization". However, there are numerous examples of concentrations or condensations of neurons within this plexus, forming either neurite bundles or ganglion-like concentrations (see e. g. Satterlie 2002, Garm et al. 2007, Koizumi 2007, Schmidt-Rhaesa 2007, Watanabe et al. 2009). Medusae generally have a more complex nervous system than polyps, including one or two nerve rings in the margin of the umbrella basal to the tentacles and concentrations of neurons associated with sensory structures (e. g., rhopalia), further concentrations may also be present. Polyps generally have fewer concentrations in their plexus, but polyps from some species show a nerve ring around the mouth opening (see e. g. Koizumi et al. 1992, Koizumi 2007).

Immunohistochemical investigations with a targeted staining of neurogenic substances (either transmitters or cytoskeletal molecules) have been established to reconstruct the nervous system architecture in different animals including cnidarians (Grimmelikhuijzen & Westfall 1995). Neuropepides appear to be more common transmitters in the cnidarian nervous system than biogenic amines (Grimmelikhuijzen et al. 1991). It was our aim to use a set of different markers to create a more inclusive picture of the nervous system of *Halammohydra octopodides*. We used markers against two neuropeptides (RFamides, LWamides), serotonin and cytoskeletal structures (tubulin as acetylated α-tubulin, thyronsinated α-tubulin, β-tubulin) in combination with markers of the musculature (phalloidin) and the nuclei (DAPI).

Material and Methods

Specimens of *Halammohydra octopodides* Remane, 1927 were extracted from coarse sand collected in Hörnum (Southern tip of the island Sylt, North Sea). Specimens were extracted from the sediment with the seawater-ice method (Uhlig 1964, Higgins & Thiel 1988). A total of 129 specimens were prepared for immunohistochemical investigations. Specimens were sorted out under a stereomicroscope, anesthesized with 7 % $MgCl_2$-solution, fixed in 4 % paraformaldehyde (PFA) in 0.1 M phosphate buffered saline (PBS) over night at 4 °C, then washed and transferred to PBS with sodium nitrate (NaN_3).

Prior to immunostaining, the samples were washed three times in 0.1 M PBS and pre-treated with preincubation buffer (PIB; 0.1 M PBS, 0.5 % Triton X-100 [Sigma], 6 % goat serum and 0.5 % BSA [bovine serum albumin]) overnight at 3 °C. The preparations were incubated for 22–25 h in a solution of the primary antibody in PIB at 4 °C. The following antibodies were used: anti-RFamide (ImmunoStar 41020202, polyclonal) 1:800, anti-LWamide (received from Thomas Leitz, University Kaiserslautern; polyclonal) 1:400, anti 5-HT (Sigma S5545, polyclonal) 1:800, anti-β-tubulin (Sigma T5293, monoclonal) 1:200 or 1:400, anti-acetylated α-tubulin (Sigma T6793, monoclonal) 1:200 and anti-tyrosinated α-tubulin (Sigma T9028, monoclonal) diluted 1:200. After incubation, samples were rinsed three times in 0.1 M PBS. This was followed by incubation with the secondary antibody solution diluted 1:100 in PIB at 3 °C overnight or at room temperature for 2.5 h. Secondary antibodies used were either anti-rabbit immunoglobulin conjugated with the cyanine dye Cy5 (Jackson) or TRITC (Tetramethylrhodamine isothiocyanate) (Sigma) and anti-mouse immu-

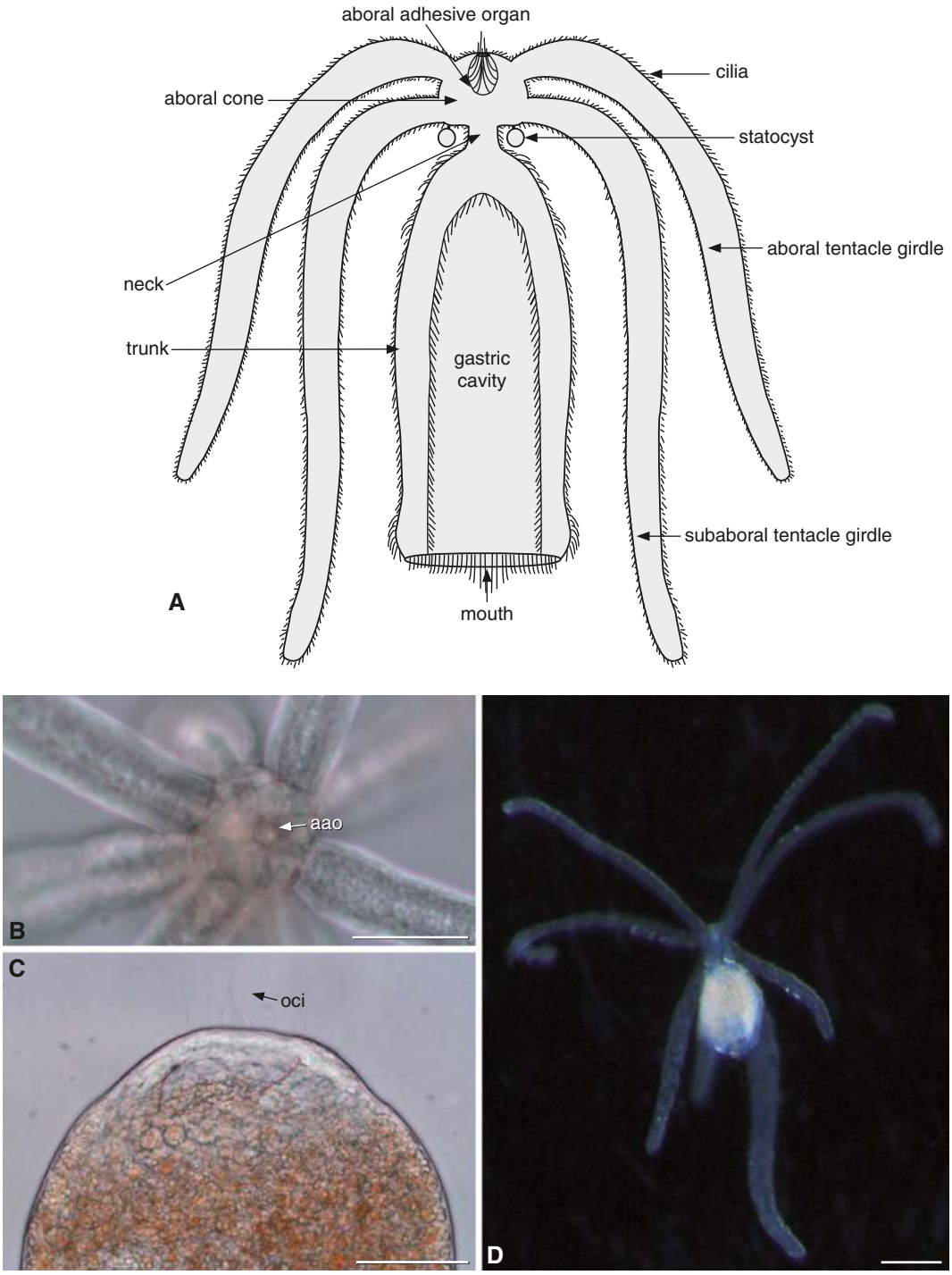


Fig. 1. *Halammohydra octopodides*. **A.** Schematical overview. **B–D.** Light microscopical micrographs. **B.** View on the aboral side, showing opening of adhesive organ (**aao**). **C.** View on the oral side with oral cilia (**oci**). **D.** Entire animal. Scale bars: B, 10 µm; C, 20 µm; D, 40 µm.

noglobulin conjugated with Dylight 488 (Sigma) or TRITC (Sigma). Additionally, counterstaining with TRITC-conjugated phalloidin (Sigma) and DAPI (4',6-Diamidino-2-phenylindol) (Sigma) was done. A 1:25 solution of 3.8 µM phalloidin was used.

The staining was terminated by rinsing again several times in 0.1 M PBS. In the first wash step 0.5 µl (1 mg/ml Aqua dest.) of DAPI were added to 200 µl PBS.

The microscopic investigation took place on a confocal laser scanning microscope Leica TCS SPE. The post-processing of the data and the projections with greater focal depth were made by using LCS software (Leica). Negative controls (staining without primary antibody) revealed no unspecific labelling.

Some specimens were prepared for Scanning Electron Microscopy (SEM) by dehydration in an increasing ethanol series, critical point drying and and sputter-coating with gold. Obervation took place with a LEO SEM 1524 under 10 kV. Digital images were taken. Further specimens were prepared for Transmission Electron Microscopy (TEM). Specimens were fixed in 2.5 % glutaraldehyde in sodium-cacodylate buffer (pH 7.25) for one hour at room temperature, postfixed with OsO_4 and embedded in LR-White. Sections of 70 nm were cut with an ultramicrotome, stained with uranyl-acetate (2.5 %) and lead-citrate (0.4 %) and investigated in a Zeiss EM 902A. Digital images were taken.

Results

The body organization of specimens investigated by us corresponds to previous descriptions of *Halammohydra octopodides*. The body is composed of a large trunk (manubrium) with apical mouth opening and an aboral cone with the aboral adhesive organ, tentacles and statocysts (Fig. 1A-D, 2A,B). Both regions are connected through a narrow neck region (Fig. 1A, 2D). The epidermal cells of the entire body are ciliated (Fig. 2A-G). Around the mouth opening, cilia are longer (Fig. 1A,C).

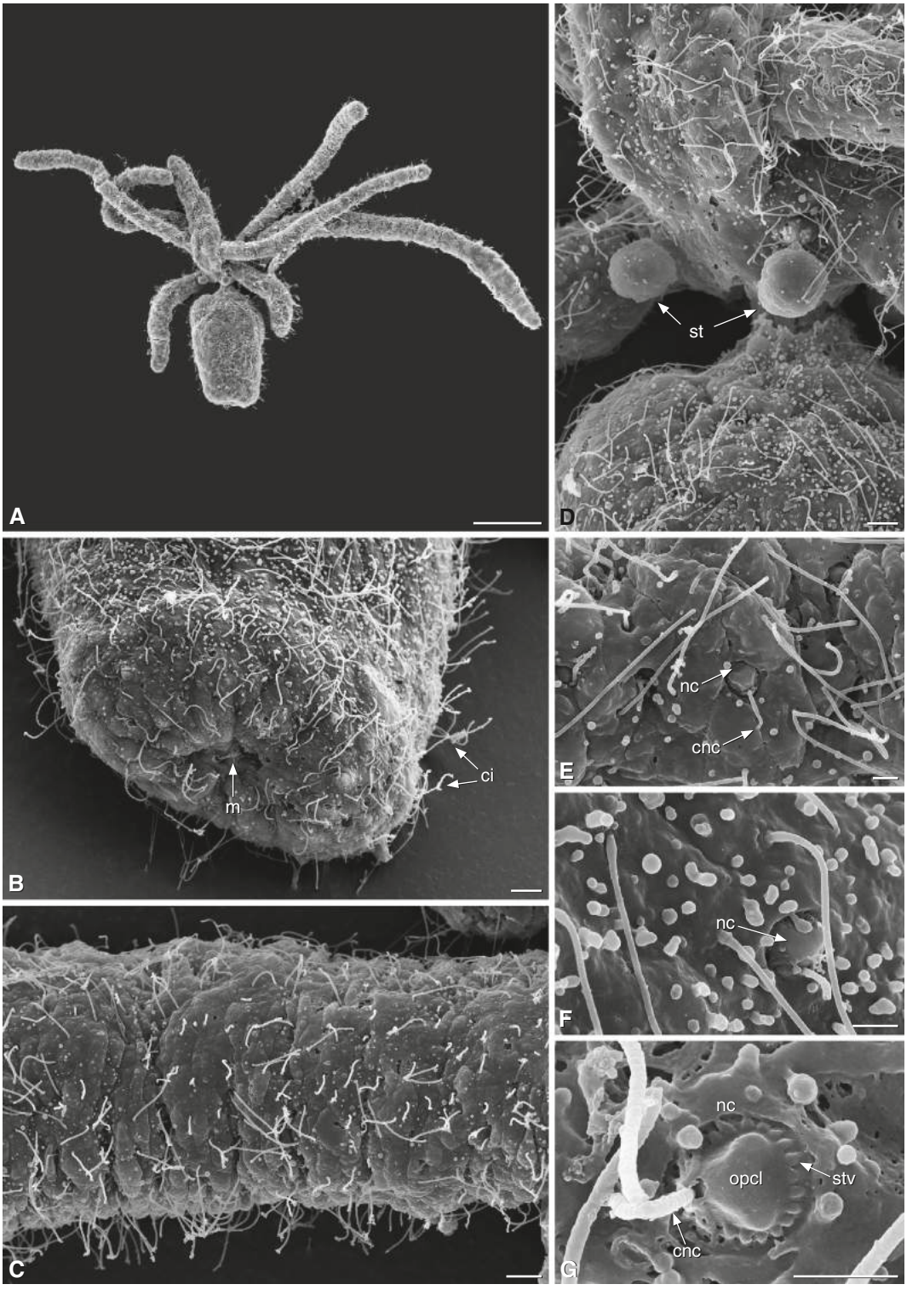
With the exception of 5-HT (serotonin), all markers created a signal which is described in the following. A positive signal is indicated with the abbreviation "-IR" (immunoreactive, immunoreactivity).

RFamide-like immunoreactivity. RFamide-IR is present in the entire animal in a particular pattern (Fig. 3A–D). There is a strong ring-like arrangement of RFamide-IR neurites at the base of the tentacles (nr = nerve ring; Fig. 3A,B). From this nerve ring several neurites run into each tentacle (Fig. 3B). Most such neurites are short and only reach the proximal region of the tentacle (ptn = proximal tentacle neurite; Fig. 3A,B), while one neurite runs into the distal tentacle tip (ltn = longitudinal tentacle neurite; Fig. 3A,C–E). RFamide-IR somata are present along the length of each tentacle in more or less regular distances (Fig. 3A,D,E).

From the nerve ring, 5-6 longitudinal neurites run along the trunk to the mouth opening (Fig. 3A,C,D). Towards the mouth opening, they enter a dense plexus which is present in the oral quarter of the trunk (Fig. 3A,C,G). The extension of this oral plexus differs among specimens, in some it is broader, in some it is smaller (e.g. Fig. 3D,H). In the specimens with the smaller plexus, up to 20 immunoreactive structures, probably somata after co-staining with DAPI, can be identified (Fig. 3H,I).

LWamide-like immunoreactivity. LWamide-IR is present in the entire animal in a particular pattern that is in part different from RFamide-IR (Fig. 4A). There is a strong nerve ring at the tentacle base (Fig. 4A–D), from which neurites run into the tentacles. One neurite, the longitudinal tentacle neurite (ltn), runs into the distal tip of the tentacle. It originates from the fusion of two proximal neurites, named proximal tentacle loop (ptl) (Fig. 4A–C). No somata were observed along the tentacle neurites (Fig. 4F). A strong, short neurite (ptn_{LW} = proximal tentacle neurite) runs into four of the tentacles and ends in an LWamide-IR soma (Fig. 4B,C, 5B–D,F). DAPI staining reveals the nucleus inside this soma (Fig. 5D). Further LWamide-IR somata can be found close

Fig. 2. *Halammohydra octopodides*, SEM. **A.** Entire animal. **B.** View on oral side with mouth opening (**m**). **C.** Tentacle with ciliation. **D.** Neck region and statocysts (**st**). **E–G.** Cilia and nematocytes on the surface. Abbreviations: **ci**, cilia; **cnc**, cnidocil; **m**, mouth; **nc**, nematocyst; **oplc**, operculum; **st**, statocyst; **stv**, stereovilli. Scale bars: A, 40 µm; B–D, 3 µm; E–G, 1 µm. ▷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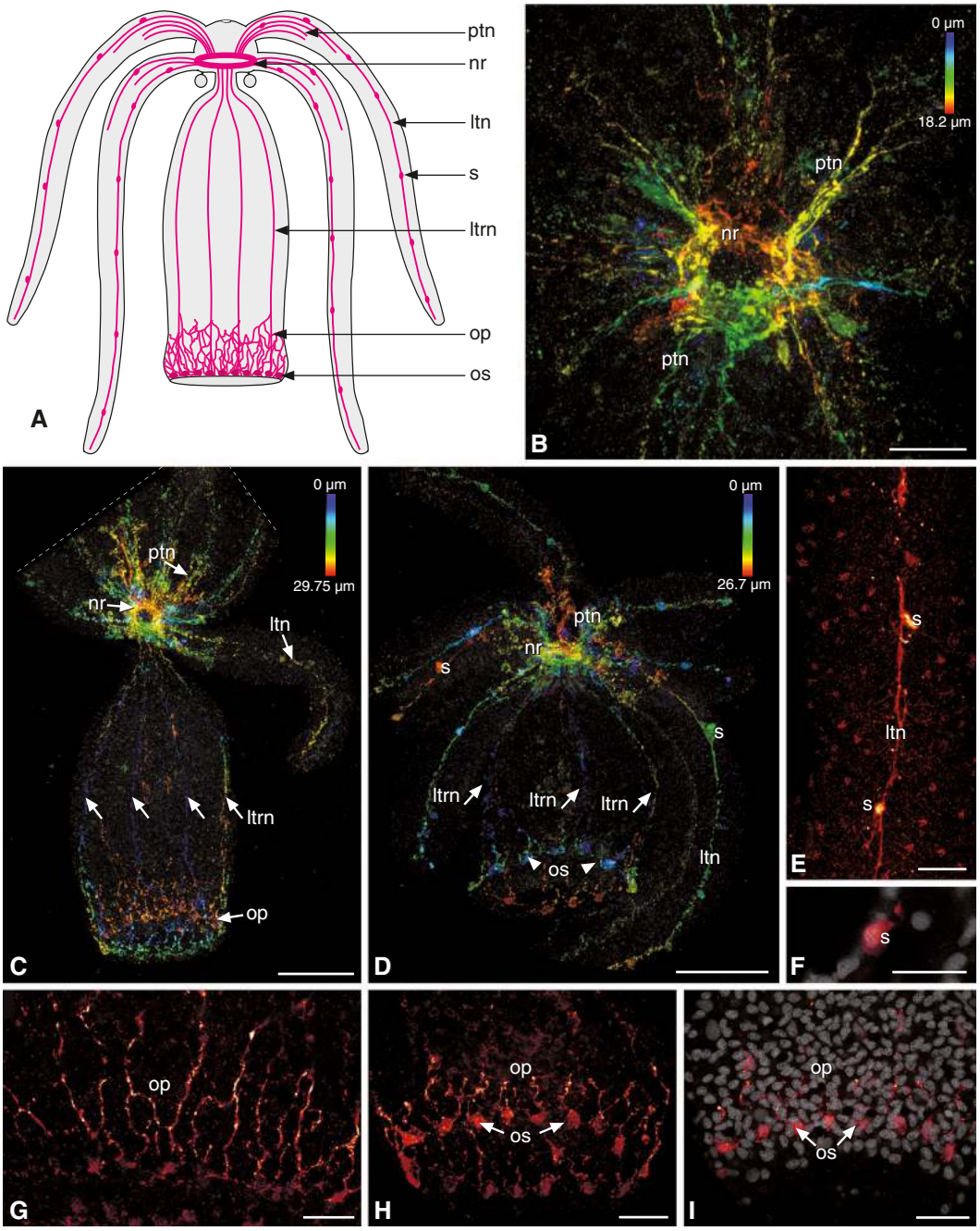


Fig. 3. *Halammohydra octopodides*, RFamide-IR. **A.** Schematical overview, RFamide-IR in pink. **B.** Nerve ring (**nr**) with emerging tentacle neurites, among them the proximal tentacle neurites (**ptn**). **C, D.** Overview of two specimens, showing nerve ring (**nr**), proximal (**ptn**) and longitudinal tentacle neurites (**ltn**), the longitudinal trunk neurites (**ltrn**, arrows) and the oral plexus (**op**) with few oral somata (**os**, arrowhead in D). Somata along the longitudinal tentacle neurite are labelled "**s**". **E.** Longitudinal tentacle neurite with somata (**s**). **F.** Demonstration of nucleus in a soma. **G–I.** Oral plexus (**op**) of different specimens with oral somata (**os**). B–D, colour-coded by depth (cco-projections). E, H, maximum projection; F, I, double labelling of RFamides (red) and nuclei (white); G, single optical section. Scale bars: B, E, G–I, 20 µm; C, D, 50 µm; F, 15 µm.

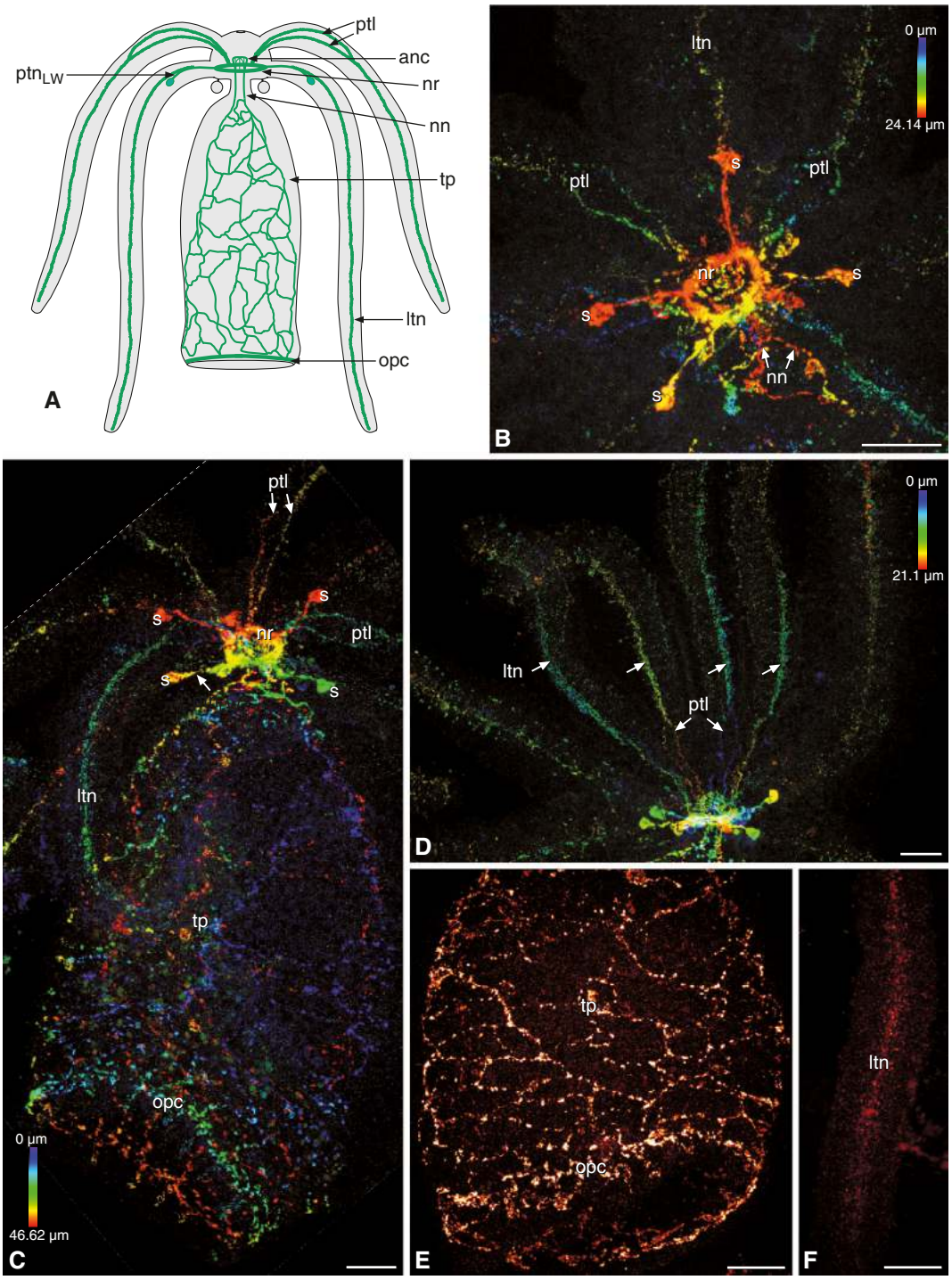


Fig. 4. *Halammohydra octopodides*, LWamide-IR. **A.** Schematical overview, LWamide-IR in turquoise. **B, D.** Nerve ring (**nr**) with emerging tentacle neurites, the proximal tentacle loops (**ptl**), the longitudinal tentacle neurites (**ltn**), short neurites running to somata (**s**) and neck neurites (**nn**). **C.** Overview showing structures as in B and trunk plexus (**tp**) with oral condensation (**opc**). **E.** Trunk plexus with oral condensation. **F.** Longitudinal tentacle neurite (**ltn**). B–D, colour-coded by depth (cco-projections); E, F, maximum projection. Scale bars: B–F, 20 µm.

to the nerve ring, probably close to the origin of the tentacle neurites (Fig. 5B,D, asterisks). The exact location of the four tentacles, into which the proximal tentacle neurites run, is not clear in most specimens, but in some specimens it appears that these belong to the subaboral ring of tentacles (Fig. 5E).

Towards the trunk, short neurites (nn = neck neurites) run from the nerve ring region along the neck region and merge into a trunk plexus (tp) (Fig. 4A,C, 5A–C). The neck neurites do not fuse with the nerve ring, but run through it and turn on the aboral side where they form a kind of dome (Fig. 5A,C). The trunk plexus covers the entire trunk (Fig. 4A,E). Around the mouth opening there is a stronger condensation in the trunk plexus, called here the oral plexus condensation (opc) (Fig. 4A,C,E).

Tyrosinated α-tubulin immunoreactivity. There is tyrosinated α-tubulin-IR in the cilia, as an unspecific signal in the tentacles and in the neck region. There is no obvious staining (or no sufficiently strong signal) of the neuronal cytoskeleton. The entire external surface of *H. octopodides* is covered by cilia, as well as the entire gastric cavity (Fig. 6A). In the region of the mouth opening, cilia are longer than the ones in other body regions (Fig. 6A,F). Within the tentacles, there are dot-like regions with tyrosinated α-tubulin-IR (Fig. 6H), these regions can not be assigned to a particular structure.

Very conspicuous are filamentous structures lining the neck region. They originate in the basal region of the aboral cap, run parallel to the longitudinal axis of the trunk/neck through the neck region and spread in the adjacent region of the trunk for about 30 µm (Fig. 6B,C,E). These filaments can also be observed on TEM sections which reveal that they are situated in elongated cells or cell processes of the ectoderm (Fig. 6D,G,I). No neurovesicles were observed in these cells.

Acetylated α-tubulin immunoreactivity. The acetylated α-tubulin-IR resembles the tyrosinated α-tubulin-IR, but differs in intensity and shows additional structures. There also is a strong signal in cilia of the external surface and in the gastric cavity (Fig. 7A,C, see also 7G,H for TEM section of gastric cilia). Additional cilia (aci = aboral cilia) are stained within the adhesive organ in the aboral cap (Fig. 7A,D,I see also 7J for TEM section of aboral cilia). They partly emerge through the apical opening of the adhesive organ (Fig. 7D). The neck filaments also have an acetylated α-tubulin-IR, but this is distinctly weaker than the tyrosinated α-tubulin-IR (Fig. 7B). Additionally, the filaments do not spead into the trunk, but end at the base of the neck.

There is also signal associated with the statocysts; this is in the form of short fibres basal to statocyst attachment (Fig. 7B,E,F) and within the stalk and in the form of two broader, dotlike signal close to the nuclei within the statocyst (Fig. 7E,F).

β-tubulin immunoreactivity. The β-tubulin-IR resembles the IR of the two α-tubulins in some respects, but shows additional structures. β-Tubulin is the only tubulin that was stained in nervous structures.

There is β-tubulin-IR in the cilia of the external surface, but not in those of the gastric cavity (Fig. 8A,C). The neck fibres also show β-tubulin-IR (Fig. 8B–E,G,H), the intensity is between that of acetylated α-tubulin-IR and tyrosinated α-tubulin-IR.

The nerve ring at the base of the tentacles shows strong β-tubulin-IR, as well as some tentacle neurites (Fig. 8A–D,F). There are few fine neurites running into the proximal region of the tentacles (Fig. 8A,B,D) and one stronger tentacle neurite (ltn = longitudinal tentacle neurite) runs up to the distal tentacle tip (Fig. 8A,C,F). The signal associated with the statocysts is comparable to the signal of acetylated α-tubulin, with signal basal to the stalk, in the stalk and close to nuclei within the statocyst itself (Fig. 8E,H).

Discussion

The five markers used in this study each stain a different subset of structures and together form a picture of the general architecture of the nervous system and some further structures of *Halammohydra octopodides*.

The nervous system of *Halammohydra octopodides*. There is a nerve ring at the tentacle base in the aboral cap from which several neurites run into the tentacles. Most of these are short and end in the proximal region of the tentacle, but there is one larger and longer neurite reaching the distal tentacle tip. There is signal within the

statocysts, but the exact pattern of this signal is not easy to interpret (see below). The nerve ring is also connected by neurites running through the neck region to the trunk plexus. This is at least evident with RFamide-IR, while with LWamide-IR the neck neurites seem to be not connected with the nerve ring or their connection remains unclear. Within the trunk, there is a plexus, but this shows a differentiated signal. In the upper trunk (= aboral half), there is an LWamide-IR plexus and longitudinal RFamide-IR neurites. It can not be decided whether the RFamide-IR neurites run within the LWamide-IR plexus or are separate. In the lower trunk (= oral half), both markers stain a plexus, but with different densities of neurites. Around the mouth opening, there is a condensation of neurites, but this is clearly recognizable as a denser region in the plexus and is therefore not considered as a nerve ring here.

Signal in the statocysts. *Halammohydra* species possess statocysts classified as lithostyles (Horridge 1969, Singla 1975). Each statocyst is composed of a statolith completely surrounded by cells, which is connected by a stalk to the aboral cap. The cells are concentrated in the basis of the statolith and Remane (1927) has described fibres running from the stalk into the endoderm tissue. The signal observed with immunohistochemistry may indicate innervation of the statocyst, but it is not exactly clear what kind of structures is labelled. This accounts in particular to the two dotlike signals within the statocyst, which can not be interpreted and no connection of the statocysts to the remaining nervous system was observed.

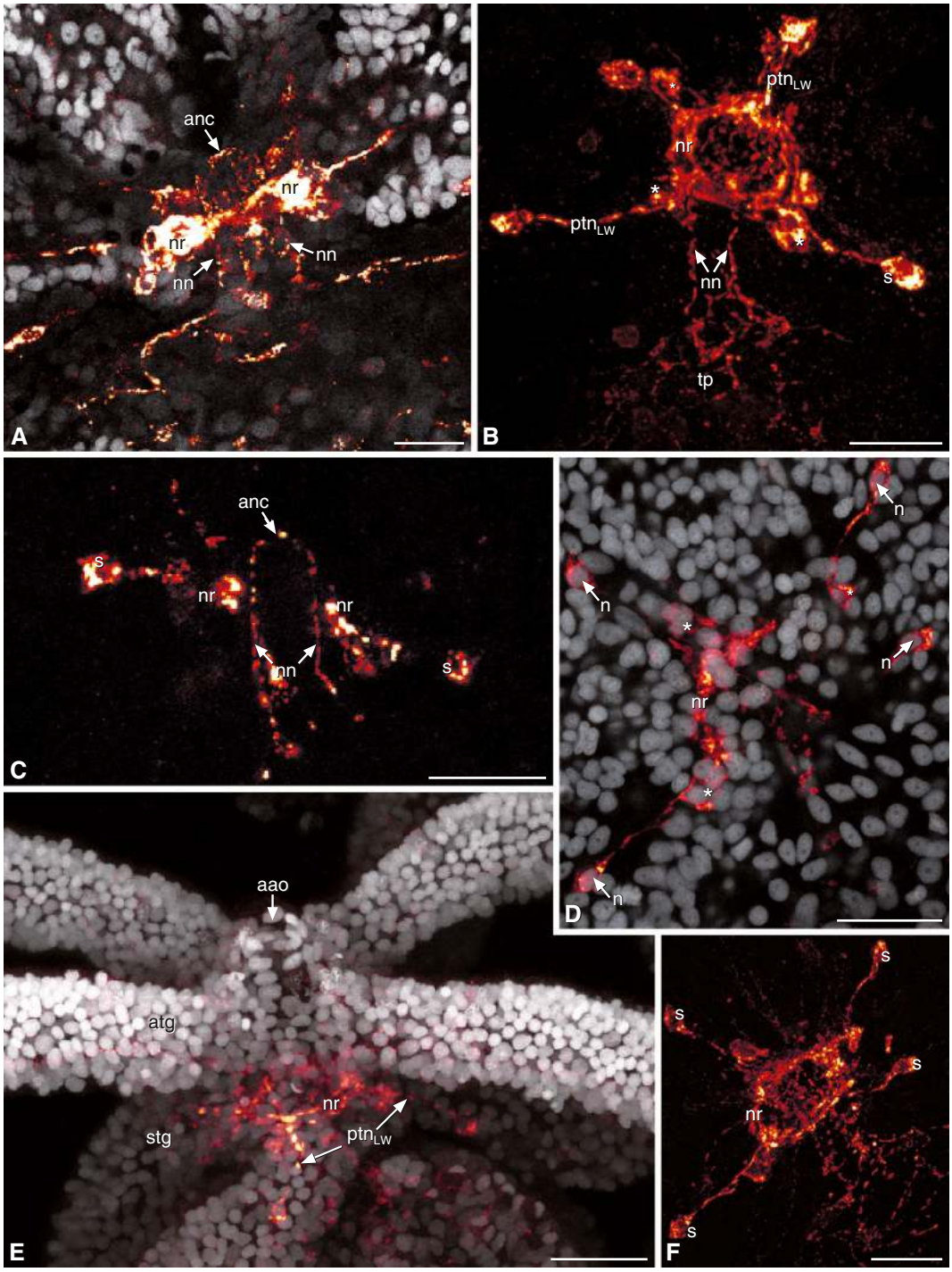
The neck fibres. All three tubulin markers stain fibres in the neck region between trunk and aboral cap. In comparison with ultrastructure, it is evident that these fibres are really elaborations of the tubulin cytoskeleton and do not belong to neurites. Such fibres have not been reported before, but their function in the narrow neck region is clear. The interstitial system is a dynamic habitat, in which sand grains may be moved by wave action, therefore body rigidity is an important factor.

Comment on the markers used. Cnidarians use a number of different transmitters, but in general, neuropeptides appear to be more common transmitters in the cnidarian nervous system than biogenic amines (Grimmelikhuijzen et al. 1991, Grimmelikhuijzen & Westfall 1995). This could be confirmed in our investigation through the positive immunoreactivity to LWamides and RFamides. Neuropeptides have been stained in cnidarians before and were used as the main markers for the nervous system (see e. g. Gajewski et al. 1996, Schmich et al. 1998, Watanabe et al. 2009).

Data on serotonin (Mathias et al. 1960, Welsh 1960, Wood & Lenz 1964, Westfall et al. 2000, Kass-Simon & Pierobon 2007) in cnidarians show a much more restricted and specialized distribution of this transmitter compared to neuropeptides. The failure to detect serotonin as a neural marker in our investigation can have different reasons, from methodological problems to the absence or very limited presence in the nervous system, but it tentatively supports the assumption that serotonin is not an important transmitter in cnidarians.

Tubulin has become a good marker for the nervous system in some bilaterian animals (see Rothe & Schmidt-Rhaesa 2009, 2010 as examples), but in cnidarian species, data and successful staining with tubulin are quite restricted (Agosti & Stidwill 1991, Gröger & Schmid 2000, Girosi et al. 2005, Zega et al. 2007, Nakanishi et al. 2009). In this context, our differential staining with three different forms of tubulin is very interesting. At least in *Halammohydra octopodides* it seems to be the case that the tubulin present in a subset of axons is β-tubulin, while acetylated α-tubulin and tyrosinated α-tubulin are not present in neurites. Interesting, however, is also the differential signal in cilia, with gastrodermal cilia being stained only by the two α-tubulins and the cilia in the adhesive

Fig. 5. *Halammohydra octopodides*, LWamide-IR, aboral cap. **A, C.** Single images with lateral view on nerve ring (**nr**) showing how neck neurites (**nn**) run through the nerve ring and terminate in an aboral neurite cap (**anc**). **B, F.** Nerve ring with proximal tentacle neurites ($\mathbf{ptn_{LW}}$) running to a soma (**s**). **D.** Structures as in F with additional labelling of nuclei (**n**), showing presence of nucleus in the somata of $\mathbf{ptn_{LW}}$. **E.** Location of nerve ring between aboral (**atg**) and subaboral (**stg**) tentacle girdle. Proximal tentale neurites ($\mathbf{ptn_{LW}}$) run into subaboral tentacles. Abbreviations: **aao**, apical adhesive organ; **tp**, trunk plexus; * in B, D, somata along nerve ring. B, C, single optical section; B, D–F, maximum projection; A, D, E, double labelling of LWamides and nuclei (white). Scale bars: A, B, 15 µm; C–F, 20 µm. ▷▷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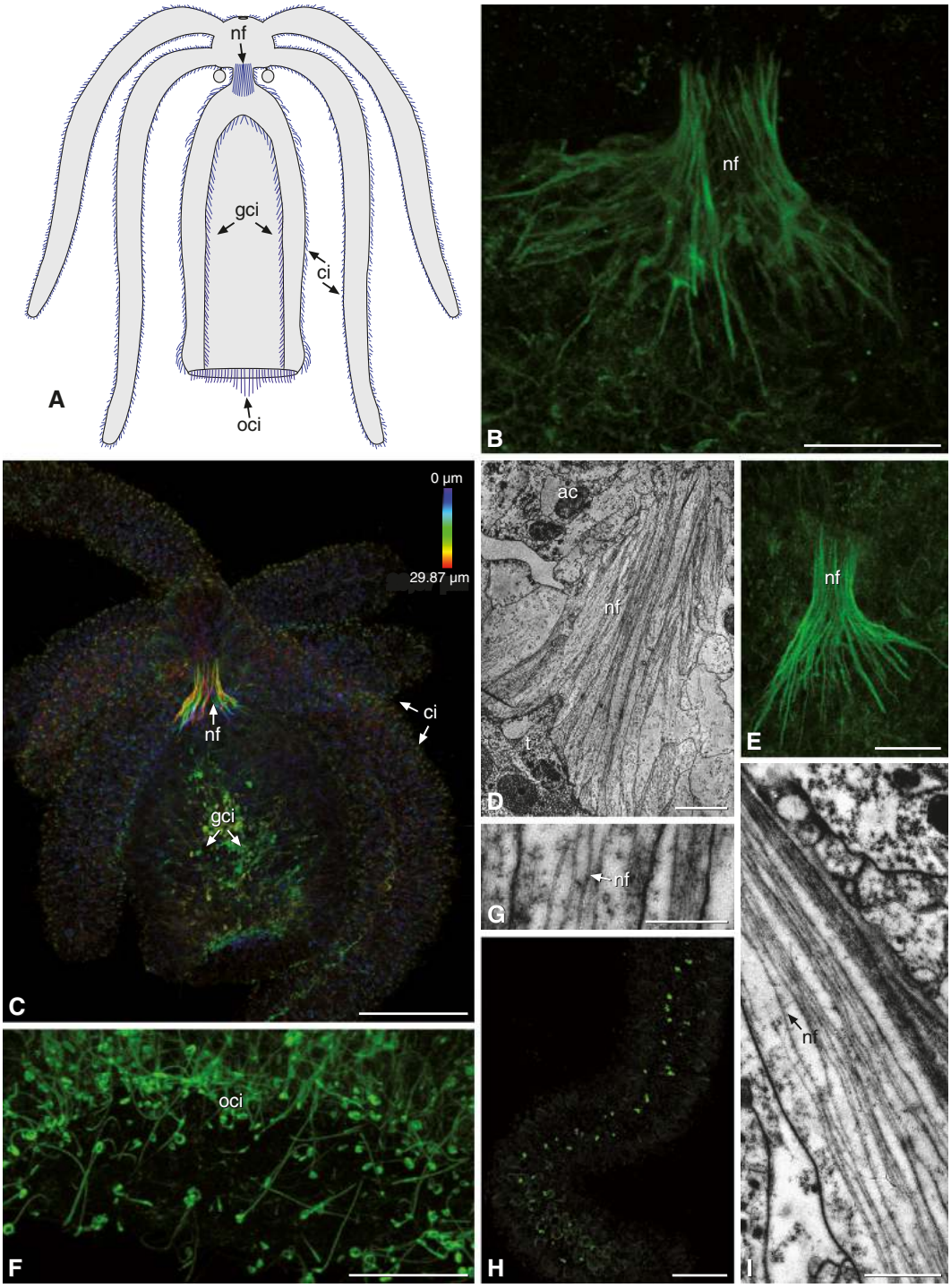
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organ being stained only by acetylated α-tubulin. The exact roles of the different forms of tubulin, especially their posttranslational modifications, are far from being known (Fukushima et al. 2009). Their differential staining certainly reflects their differential presence within the nerve cells and differential staining of cilia.

Comparison with other cnidaria – the nerve ring. The nervous system of many cnidarian species includes, besides a plexus, condensations in the form of neurite bundles or nerve rings (see e. g. Mackie 1973, 1989; Satterlie 1979; Anderson & Schwab 1981; Skogh et al. 2006; Garm et al. 2006, 2007; Watanabe et al. 2009). For comparison we want to concentrate here on the nerve ring of *Halammohydra octopodides*. Nerve rings are observed in some polyps (see e. g. Matsuno & Kageyama 1984 for *Pelmatohydra robusta*, Koizumi et al. 1992 for *Hydra oligactis*). It is not directly at the tentacle base, but shifted slightly towards the mouth, but the nerve ring seems to play an important role in tentacle function as has been shown by Koizumi (2007). Nerve rings are also present in medusae, here one or two such rings are located in the umbrella basal to the attachment of the tentacles and statocysts (Werner et al. 1976, Chapman 1978, Singla 1978a,b, Garm et al. 2007). This position corresponds to the position of the nerve ring in *Halamohydra octopodides*. This fits to the interpretation of *Halammohydra* as a modified medusa (see e. g. Remane 1927).

Halamohydra octopodides does also possess a ring-like concentration of neurites around the mouth opening, but this can not be regarded as a ring, but as a local condensation in the trunk plexus around the mouth.

Comparison with other hydrozoan species: the plexus. The entire trunk of *Halammohydra octopodides* is covered by a plexus, but the LWamide-IR and the RFamide-IR are different within this plexus. Such a differential presence of different neuropeptides, including LWamides and RFamides, has also been observed in the trunk of *Hydra magnipapillata* (Koizumi et al. 2004). A plexus is absent in the tentacles of *H. octopodides*, where only individual neurites are present. In most cnidarian species the plexus is also present in tentacles (see e. g. Westfall & Elliott 2002). This may be a consequence of miniaturization of *Halammohydra* in adaptation to the interstitial environment.

Acknowledgements

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◁◁ **Fig. 6.** *Halammohydra octopodides*, tyrosinated α-tubulin-IR. **A.** Schematical overview, tyrosinated α-tubulin-IR in blue. **B, E.** Neck fibres (**nf**). **C.** Overview showing external ciliation (**ci**) and gastric cilia (**gci**) as well as the neck fibres (**nf**). **D, G, I.** TEM images of neck fibres. **ac**, (aboral cone) and **t** (trunk) mark the orientation of section in D. **F.** Cilia around the mouth opening (**oci** = oral cilia). **H.** Dotlike signal in the tentacles. B, C, E, F, maximum projection; C, colour-coded by depth (cco-projections); D, G, I, TEM sections; H, single optical section. Scale bars: B, E, F, H, 20 µm; C, 50 µm; D, 5 µm; G, 0.5 µm; I, 1 µm.

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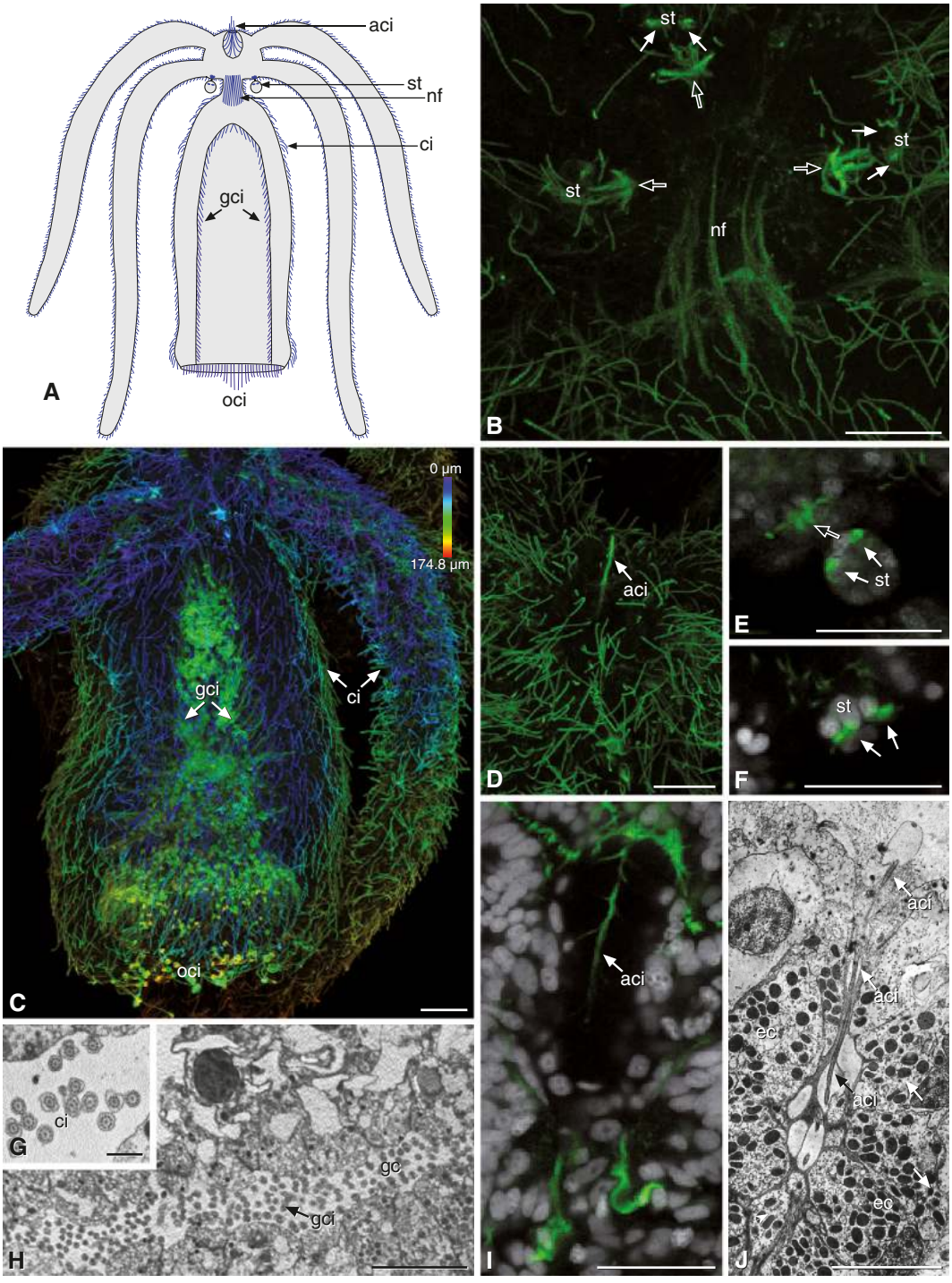
Fig. 7. *Halammohydra octopodides*, acetylated α-tubulin-IR. **A.** Schematical overview, acetylated α-tubulin-IR in ▷▷ blue. **B.** Neck fibres (**nf**) and signal leading to statocysts (**st**): signal at statocyst base (open arrow) and dotlike signal within statocyst (arrows). **C.** Overview showing external (**ci**) and gastric ciliation (**gci**); **oci**, oral cilia. **D.** Aboral cilia (**aci**) emerge from aboral opening of the adhesive organ. **E, F.** Signal in the statocysts (see B) with additional labelling of nuclei (white). **G, H.** Cilia (**gci**) in gastric cavity (**gc**). **I.** Aboral cilia (**aci**) within aboral cap. **J.** TEM section corresponding to I. B-D, maximum projection; C, colour-coded by depth (cco-projections). E, F, I, double labelling of acetylated α-tubulin and nuclei (white). G, H, J, TEM sections. Scale bars: B, E, F, 15 µm; C, D, I, 20 µm; G, 1 µm; H, J, 5 µm.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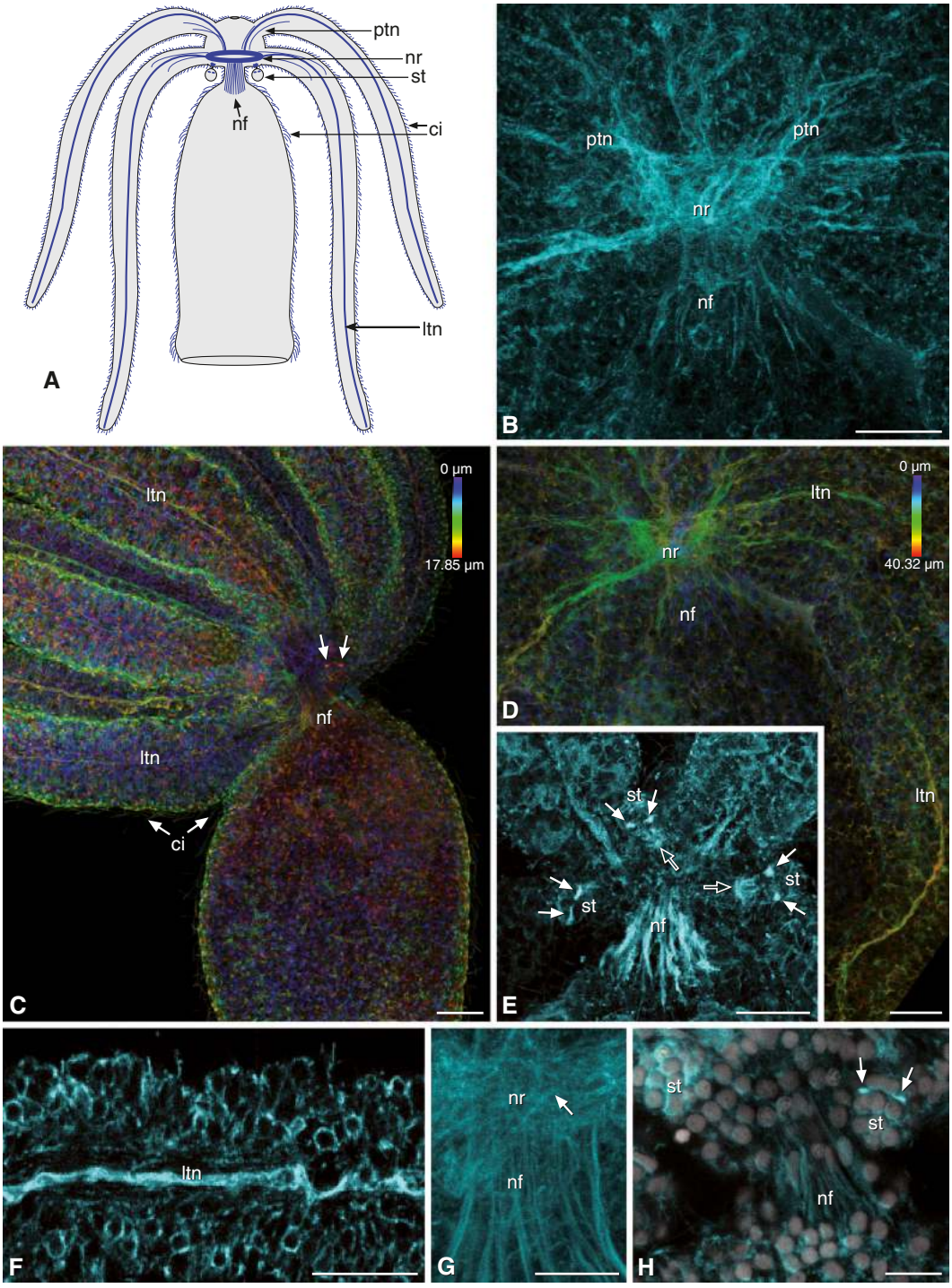
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◁◁ **Fig. 8.** *Halammohydra octopodides*, β-tubulin-IR. **A.** Schematical overview, β-tubulin-IR in blue. **B, D.** Nerve ring (**nr**), longitudinal (**ltn**) and proximal (**ptn**) tentacle neurites are labelled as well as neck fibres (**nf**). **C.** Overview showing external (**ci**), but no gastric cilia. **E.** Signal at statocyst base (open arrow) and dotlike signal within statocyst (arrows). **F.** Longitudinal tentacle neurite (**ltn**). **G.** Detail of the neck fibres (**nf**) in relation to the nerve ring (**nr**). **H.** Double labelling β-tubulin and nuclei (white) showing neck fibres (**nf**) and spatial position of the statocyst signal (arrows close to **st**). B–E, G, maximum projections; C, D, colour-coded by depth (cco-projections); F, single image; H, double labelling of β-tubulin and nuclei (white). Scale bars: B–F, 20 µm; G–H, 10 µm.