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Paddle cilia on the cephalic sensory organs (CSOs) of Opisthobranchia (Mollusca: Gastropoda) – genuine structures or artefacts?*

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Abstract. Paddle cilia are characterised by a curved axoneme at the distal end enclosed by the ciliary membrane. They have been described in numerous different marine invertebrates including one species of Opisthobranchia. There is still controversy about the nature of paddle cilia. Various considerations have been made concerning their function regarding them as genuine structures, whereas other authors claimed paddle cilia to be artefacts. The current study focuses on investigating paddle cilia on the cephalic sensory organs (CSOs) of different opisthobranch species in order to present more data and perhaps clarify the paddle cilia discussion. For this purpose specimens were fixed using two different methods. One method comprises an isoosmotic fixation solution which did not induce paddle cilia formation in bivalve larvae (SHORT & TAMM 1991). The other method utilises a hypoosmotic fixation solution. Using scanning electron microscopy paddle cilia can be detected in all investigated species. The abundance of paddle cilia was lower in the majority of specimens fixed with the isoosmotic solution which led us to conclude that paddle cilia are indeed artefacts.

Keywords. Scanning Electron Microscopy, isoosmotic fixation.

1. INTRODUCTION

Paddle cilia or discocilia were first described by TAMARIN et al. (1974) as cilia whose cylindrical stem curves back upon itself and forms a 360° loop which is completely enveloped by the ciliary membrane. Since then paddle cilia have been discovered in many different marine invertebrates (EHLERS & EHLERS 1978; HEIMLER 1978; BONE et al. 1982; MATERA & DAVIS 1982; CAMPOS & MANN 1988).

Various considerations were made concerning the function of this special structure. TAMARIN et al. (1974) proposed that paddle cilia function as microscopic spatulas for the application of adhesive plaque material to substrate surfaces. HEIMLER (1978) supposed that they are locomotory organs since the disc shaped heads can be interpreted as enlargements of the surface to improve the efficiency of the ciliary beat. Thus paddle cilia also influenced the nutrition of the larvae investigated in his study. CAMPOS & MANN (1988) detected paddle cilia on the velum of bivalve larvae of two different species. They regarded them as genuine structures and favoured a locomotory and/or chemosensory function although they were not able to prove this assumption, since the occurrence of paddle cilia did not enhance the rate of movement in the examined species (CAMPOS & MANN 1988).

So far, only one study deals with the presence of paddle cilia in Opisthobranchia. The investigation of *Pleurobranchaea californica* revealed that paddle cilia solely occurred in chemosensitive regions of this species (DAVIS & MATERA 1982). This fact led DAVIS & MATERA (1982) to the conclusion that paddle cilia are most likely chemoreceptors. The dilatations were expected to enlarge the membrane surface area, increasing the opportunity for interaction with chemical substances.

Other authors supposed paddle cilia to be artefacts resulting from osmotic stress, increased temperature, non-physiological conditions or fixation (EHLERS & EHLERS 1978; BONE et al. 1982; NIELSEN 1987; SHORT & TAMM 1991; DEINER et al. 1993).

SHORT & TAMM (1991) discovered that the occurrence of paddle cilia is associated with the osmolarity of the fixation solution. After the application of an isoosmotic fixation solution with 50% seawater no paddle cilia could be detected in their bivalve larvae, whereas other hypoosmotic fixation solutions induced the formation of paddle cilia. However, the occurrence of paddle cilia was always restricted to certain areas and never affected all cilia of the larvae. NIELSEN (1987) argued that different cilia - even on the same organism - are not equally sensitive to stress

and some cilia are indeed difficult to fix in a normal shape. DEINER et al. (1993) reported that paddle cilia induced by hypotonic solutions usually regain their normal appearance if specimens are returned to isotonic solutions.

The current study investigates the cilia on the cephalic sensory organs (CSOs) of different Opisthobranchia. CSOs are special structures in the head region of sea slugs, which are sensitive to several stimuli. The purpose of this investigation is to detect paddle cilia and clarify the question whether they are genuine structures or artefacts.

2. MATERIAL AND METHODS

Specimens of *Acteon tornatilis* (Linnaeus, 1758) (Acteonoidea), *Aeolidiella glauca* (Alder and Hancock, 1845) (Nudibranchia), *Aplysia punctata* Cuvier, 1803 (Anaspidea) and *Berthella plumula* (Montagu, 1803) (Pleurobranchoidea) were collected in the intertidal at Roscoff and Saint Michel-en-Grève (Brittany, France). *Haminoea hydatis* (Linnaeus, 1758) (Cephalaspidea) was obtained from a laboratory culture at the J.W.G.-University of Frankfurt.

We applied the isoosmotic fixation method of SHORT & TAMM (1991) and a hypoosmotic fixation solution in order to clarify the nature of paddle cilia on the cephalic sensory organs of different Opisthobranchia. The specimens were anaesthetised by an injection of 7% $MgCl_2$ in the foot and the CSOs were abscised. Two different methods have been applied: CSOs of investigated species were fixed in 2.5% glutaraldehyde, 1% paraformaldehyde in phosphate buffer, pH 7.2 – 7.4 at room temperature (method 1). The osmolarity of this solution was approximately 250 mOsmols less than the osmolarity of the isoosmotic fixation solution and the artificial seawater in which the specimens were cultivated (determined with the help of Knauer Semi-Micro Osmometer). Additional CSOs of *Acteon tornatilis*, *Aplysia punctata*, *Berthella plumula* and *Haminoea hydatis* were fixed in an isoosmotic solution after SHORT and TAMM (1991) containing 2.5% glutaraldehyde, 0.13 M NaCl and 50% seawater, pH 7.2 – 7.4 at room temperature (method 2). The investigated CSOs and number of

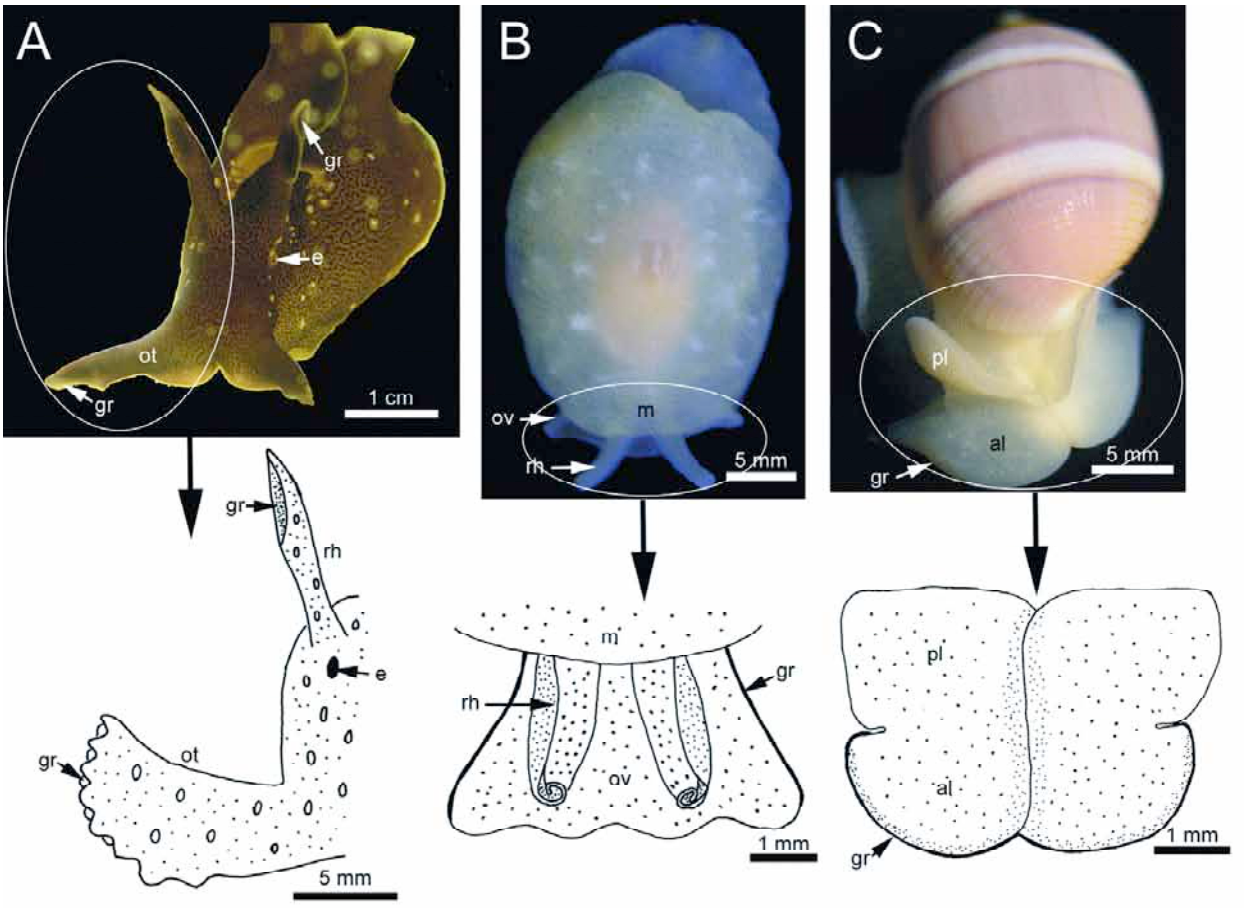

Fig. 1. Photographs of the investigated species and schematic drawings of their cephalic sensory organs (CSOs). **A** *Aplysia punctata.* **B** *Berthella plumula.* **C** *Acteon tornatilis*. al anterior lobe, e eye, gr groove, m mantle, ot oral tentacle, ov oral veil, pl posterior lobe, rh rhinophore.

replicates were as follows (method 1/ method 2): *Acteon tornatilis* head shield (4/1), *Aeolidiella glauca* rhinophore (2/-), oral tentacle (2/-), *Aplysia punctata* rhinophore (4/2), oral tentacle (4/2), *Berthella plumula* rhinophore (6/2), oral veil (3/1), *Haminoea hydatis* head shield (4/1), lip organ (4/2), Hancock´s organ (4/2).

For SEM, fixed CSOs were dehydrated through a graded acetone series, critical point dried (BAL-TEC, CPD 030), sputtered with gold (Sputter-Coater, Agar Scientific) and examined with a Hitachi S4500 SEM. Photographs were taken with DISS – Digital Image Scanning System (Point Electronic).

Sizes of cilia were determined by average over measurement of at least ten cilia.

3. RESULTS

The investigated species exhibit several different types of CSOs (Figs. 1 and 2), like rhinophore, oral tentacle, oral veil, head shield, lip organ and Hancock´s organ. The rhinophores and oral tentacles of *Aplysia punctata* (Fig. 1A) possess dark pigmented grooves. These grooves contain tufts of cilia. A total of four rhinophores fixed with method 1 were investigated. One of these exhibits paddle cilia (Fig. 3A), whereas the other reveal cilia without paddle shaped ends (Fig. 3B). The rhinophore exhibiting paddle cilia has a total length of 3 mm, whereas the other investigated rhinophores without paddle cilia are 5 mm long. The rhinophores fixed with method 2 show both cilia with and without paddle-shaped ends. The paddle cilia are always shorter (~12 µm) than the other cilia (~16 µm).

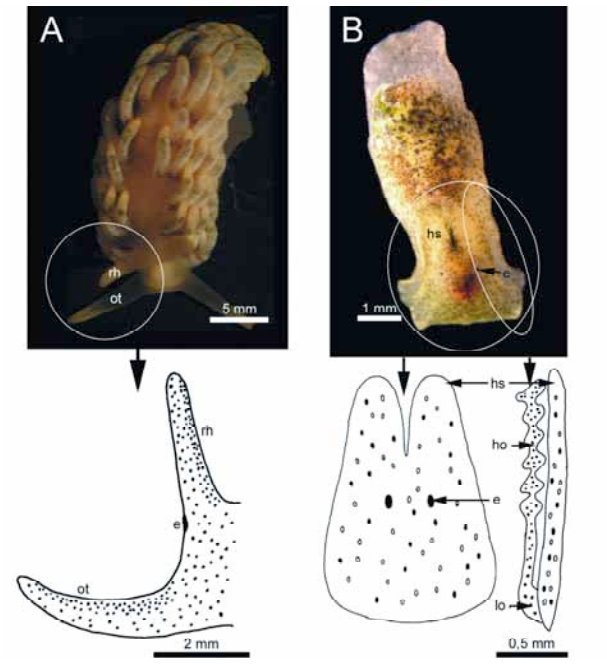

Fig. 2. Photographs of the investigated species and schematic drawings of their CSOs. **A** *Aeolidiella glauca.* **B** *Haminoea hydatis* – scheme: dorsal (left) and lateral (right) view of the CSOs. e eye, ho Hancock´s organ, hs head shield, lo lip organ, ot oral tentacle, rh rhinophore.

The oral tentacles fixed with method 1 reveal mainly paddle cilia (Fig. 3C) with a length of ~12 µm. Cilia without paddles are ~16 µm long (Fig. 3D). The oral tentacles fixed with method 2 possess mainly cilia without paddle-shaped ends (Fig. 3E), the fewer paddle cilia occur in single tufts.

Berthella plumula possesses rolled rhinophores and an oral veil with lateral grooves (Fig. 1B). Inside the rhinophores tufts of cilia are arranged regularly from the tip to the base of this CSO. These cilia have straight tips in rhinophores (Fig. 3F) fixed with method 1, whereas they reveal paddle-shaped ends in those preserved with method 2. Outside the rhinophores mainly paddle cilia are found (Fig. 3G) irrespective of fixation methods.

Inside the lateral grooves of the oral veils fixed with method 1 tufts of cilia with straight tips were found (Fig. 3H). On the margin of the grooves as well as on the dorsal and ventral side of the oral veil paddle cilia are predominant (Fig. 3I). The isoosmotically fixed oral veil also reveals paddle cilia inside the grooves.

The head shield of *Haminoea hydatis* possesses postero-lateral flattened appendages. The lip organ is located anteriorly beneath the head shield and continues into the Hancock´s organ. The arrangement of the three CSOs is shown in Fig. 2B. The lip organ reveals tufts of paddle cilia as well as tufts of cilia with straight tips and even both types of cilia in one tuft (Fig. 4A) after fixation with method 1. The Hancock´s organ also exhibits cilia with (Fig. 4B) and without paddle-shaped ends when fixed with method 1. In contrast to this, both CSOs showed no paddle cilia after fixation with method 2 (Fig. 4C and D). The head shield is densely covered by cilia on the dorsal side. No paddle cilia could be detected after application of both fixation methods. Nevertheless fixation with method 1 resulted in cilia whose tips are curved to the axoneme (Fig. 4E), whereas fixation with method 2 revealed cilia with straight tips (Fig. 4F).

The head shield of *Acteon tornatilis* is completely covered by cilia. It is divided into a pair of antero-lateral lobes and a pair of postero-lateral lobes (Fig. 1C). A groove runs along the front and lateral side of the anterior lobe. The specimens fixed with method 1 show numerous cilia with slightly, and some cilia with extremely, swollen ends in-

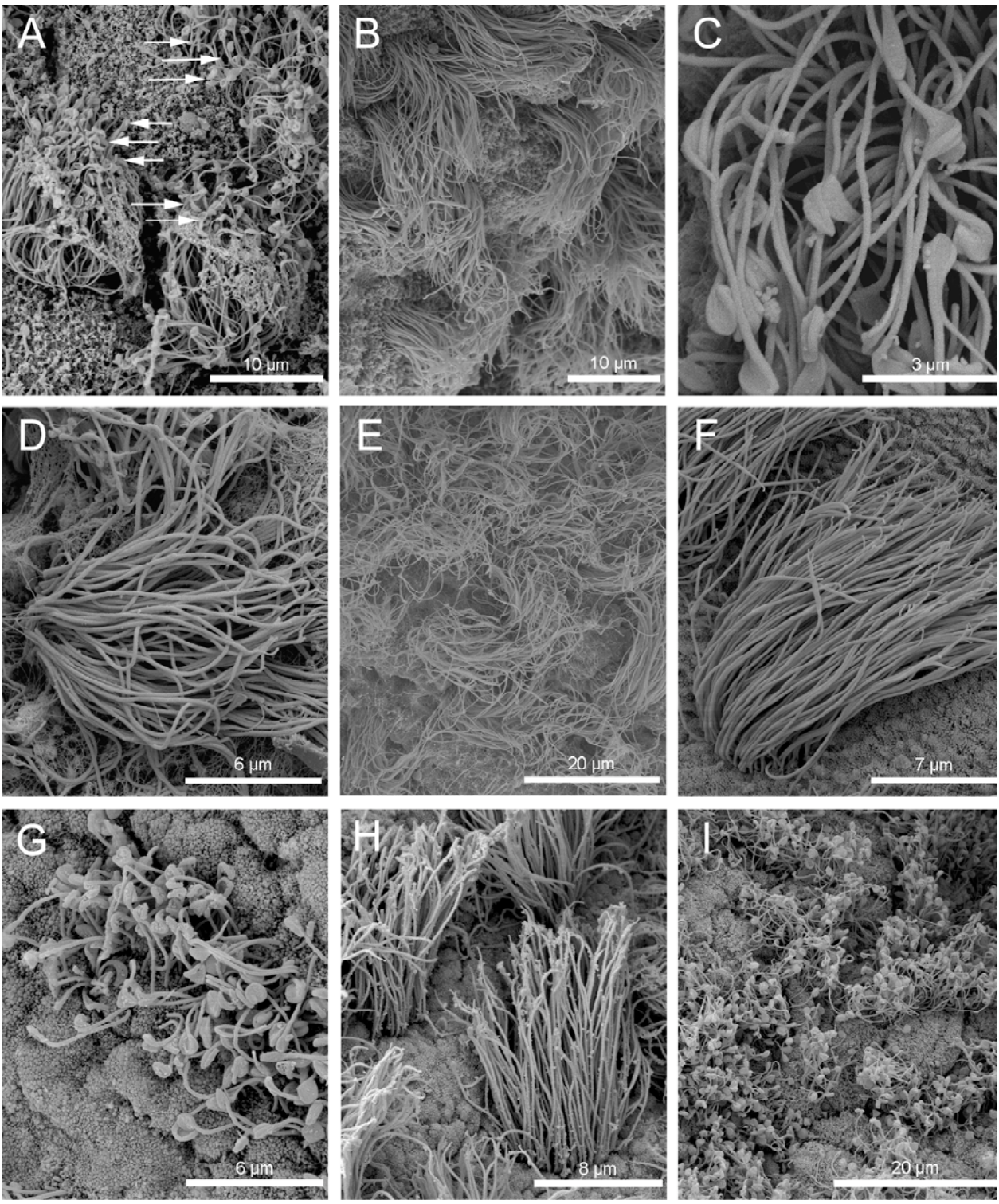

Fig. 3. Scanning electron microscopy of sensory regions of the CSOs of *Aplysia punctata* and *Berthella plumula* fixed with method 1 (except E). **A** rhinophore of *Aplysia punctata* – paddle cilia (arrows) inside the groove of a small individual. **B** rhinophore of *Aplysia punctata* – cilia inside the groove. **C** oral tentacle of *Aplysia punctata* – paddle cilia inside the groove. **D** oral tentacle of *Aplysia punctata* – tuft of cilia without paddle-shaped ends inside the groove. **E** oral tentacle of *Aplysia punctata* fixed with method 2 – cilia inside the groove. **F** rhinophore of *Berthella plumula* – cilia on the inside. **G** rhinophore of *Berthella plumula* – paddle cilia on the tip. **H** oral veil of *Berthella plumula* – cilia inside the lateral groove. **I** oral veil of *Berthella plumula* – paddle cilia on the outside, beneath the groove.

side the groove (Fig. 5A). In addition, clusters of paddle cilia could be detected in different parts of the groove (Fig. 5B). These paddle cilia are longer than the surrounding cilia without paddle-like ends. In the region of the mouth below the groove numerous paddle cilia are found (Fig. 5C). These paddle cilia could not be detected in the specimen that was fixed with the isoosmotic solution (method 2 / Fig. 5D). Moreover, no paddle cilia occur inside the groove of this specimen.

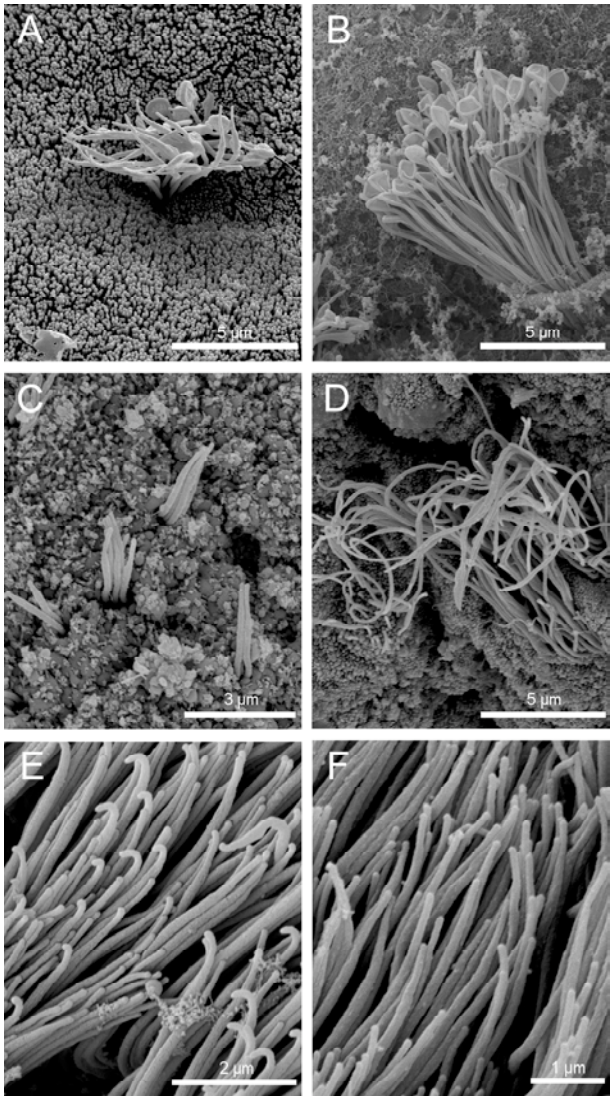

Fig. 4. Scanning electron microscopy of sensory regions of the CSOs of *Haminoea hydatis.* **A** lip organ fixed with method 1 – paddle cilia and cilia with straight tips in one tuft. **B** Hancock´s organ fixed with method 1 – tuft of paddle cilia. **C** lip organ fixed with method 2 – tuft of cilia with straight tips. **D** Hancock´s organ – tuft of cilia with straight tips. **E** head shield fixed with method 1 – cilia with curved tips. **F** head shield fixed with method 2 – cilia with straight tips.

The rhinophores and oral tentacles of *Aeolidiella glauca* are solid structures (Fig. 2A). Both were only fixed with method 1. Paddle-like structures could be detected on the tip of the rhinophores (Fig. 5E). Some of these paddle-like structures are not, as usually, found on the distal end of the cilia, but occur subapically. Paddle cilia are also present laterally on the oral tentacles (Fig. 5F). In addition, the cilia on the tip of the oral tentacles reveal swollen ends.

4. DISCUSSION

All investigated taxa and organs exhibit cilia with and without paddle-shaped ends. This fact might on the one hand support the assumption that paddle cilia are genuine structures. On the other hand it might be concluded that cilia with variable morphological features react differently to special fixation solutions.

The only specimen of *Aplysia punctata* exhibiting paddle cilia inside the groove of the rhinophore when fixed with method 1 was distinctly smaller than the other ones. This might possibly indicate that juveniles possess paddle cilia that get stretched during growth. It is also possible that this specimen was not handled with enough care which might be a reason for paddle cilia formation according to NIELSEN (1987). Thus the natural condition would be displayed by the straight tips. EHLERS & EHLERS (1978) also discovered that paddle cilia normally do not appear in the same quantity in all the individuals when several animals are fixed. Since paddle cilia are predominant inside the groove of the oral tentacle fixed with method 1, the cilia in this CSO seem to be more sensitive to paddle cilia formation than cilia in the rhinophore. The length of the cilia is equal in rhinophore and oral tentacle and cilia with straight tips are 4 µm longer than paddle cilia. This difference might be caused by rolling in of the tips of straight cilia resulting in paddle cilia formation. Thus paddle cilia would not be a genuine type of cilia but an artefact. The isoosmotic fixed specimens of both CSOs of *Aplysia punctata* reveal paddle cilia as well as cilia without paddle-like structures. This could indicate that paddle cilia are a distinct type of cilia. On the other side, it could also mean that SHORT & TAMM´S (1991) fixation method does not work as efficiently for opisthobranchs as it did for bivalves. The specimens could have also been exposed to another form of stress. Perhaps paddle cilia formation might even be induced by collecting or anaesthetisation of the animals. The findings for *Berthella plumula* are contrary to the results of SHORT & TAMM (1991), because isoosmotic fixation results in additional formation of paddle cilia, instead of decreasing the amount of these cilia. These results nevertheless reveal that cilia react differently to the application of variable fixation solutions, a fact

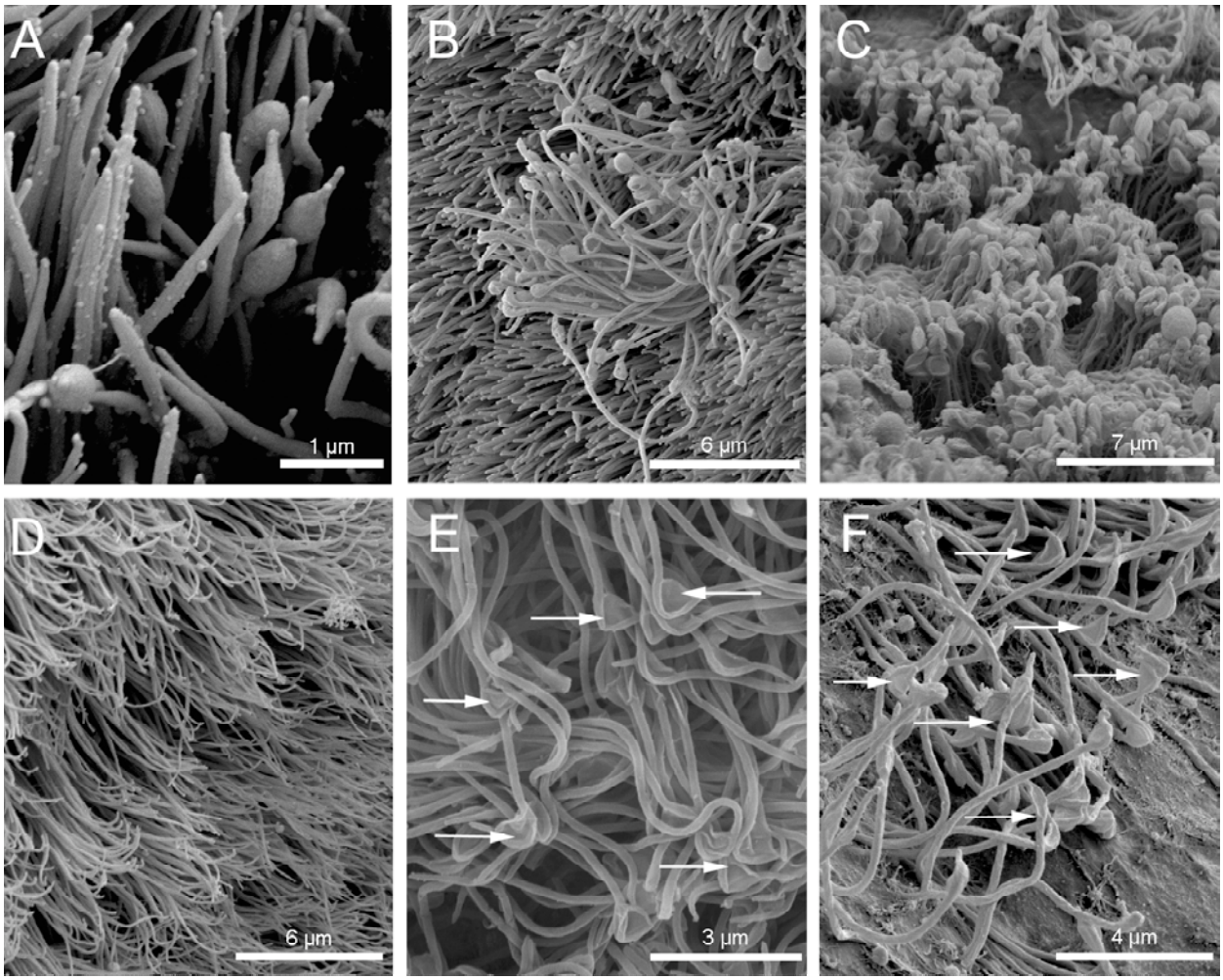

Fig. 5. Scanning electron microscopy of sensory regions of the CSOs of *Acteon tornatilis* and *Aeolidiella glauca* fixed with method 1 (except D). **A** *Acteon tornatilis* – cilia with dilated tips inside the groove. **B** *Acteon tornatilis* – tuft of paddle cilia inside the groove. **C** *Acteon tornatilis* **–** paddle cilia in the region of the mouth. **D** *Acteon tornatilis* – cilia in the region of the mouth. **E** rhinophore of *Aeolidiella glauca* – paddle cilia (arrows) on the tip. **F** oral tentacle of *Aeolidiella glauca* – paddle cilia (arrows) laterally.

that strengthens the assumption of paddle cilia being artefacts.

The tufts of paddle cilia found inside the groove of the head shield of *Acteon tornatilis* fixed with method 1 are longer than the surrounding cilia. BONE et al. (1982) investigated the nature of cilia in the endostyl of a tunicate. They argued that paddle cilia are artefacts and particularly prone to occur in long cilia. This seems to be true for *Acteon tornatilis*.

The lip and Hancock´s organ of *Haminoea hydatis* fixed with method 1 reveal cilia with and without paddle-like structures even in the same tuft. EHLERS & EHLERS (1978) described a similar situation for turbellarians. Not all the cilia of a given cell show paddle-like ends in these species, unmodified cilia also occur (EHLERS & EHLERS 1978). Morphological differences are apparently not the only reason for the formation of paddle cilia because it seems unlikely that cilia of one single tuft exhibit variable morphological features. Cilia with tips curved to the axonemes found on the hypoosmotically fixed head shield of *Haminoea hydatis* might be a developmental stage of paddle cilia since swelling of the ciliary membrane would lead to fully developed paddle cilia.

The fact that paddle cilia could not be detected on specimens of *Acteon tornatilis* and *Haminoea hydatis* fixed with method 2 corresponds to the findings of SHORT & TAMM (1991) and further indicates that paddle cilia are indeed artefacts.

The paddle-like structures found on the rhinophores and oral tentacles of *Aeolidiella glauca* fixed with method 1

are partly not located at the distal end of the cilia but are situated subapically. In addition numerous cilia with swollen ends were found on the oral tentacles. HEIMLER (1978) described these special forms as different types of paddle cilia. He distinguished three different types of paddle cilia. Type 1 has swollen, bulblike heads with a central straight axoneme. Type 2 paddle cilia are characterized by heads with a curved lateral axoneme and in type 3 paddle cilia the axoneme forms a loop. On the CSOs of *Aeolidiella glauca* type 1 and 2 paddle cilia could be detected whereas *Aplysia punctata* and *Berthella plumula* reveal type 3. Furthermore, HEIMLER (1978) supposed that these types represent different developmental stages of these cilia. Thus *Aeolidiella glauca* does not display the fully developed stage of paddle cilia.

MATERA & DAVIS (1982) investigated the distribution of paddle cilia on the opisthobranch *Pleurobranchaea californica*. Since paddle cilia only occurred in areas known to mediate chemoreception, they assumed them to be chemoreceptors. The current study shows that there are lots of other cilia with straight tips on the chemoreceptive structures of different Opisthobranchia, especially when using an isoosmotic fixation solution. This observation does not correspond to the conclusions of MATERA & DAVIS (1982), but indicates that paddle cilia might rather be artefacts.

EHLERS & EHLERS (1978) equally detected paddle-like structures only in sensory cells of the investigated Turbellaria. But they demonstrated that the number of paddles of sensory cilia increases in proportion to increasing osmolarity of the fixation solution and increasing temperature during fixation and concluded that paddle cilia are artefacts.

Altogether the current study enforces the assumption that paddle cilia are artefacts. Especially the results for *Acteon tornatilis* and *Haminoea hydatis* confirm that paddle cilia formation can be induced by the application of different fixation solutions. Nevertheless the results for *Aplysia punctata* and *Berthella plumula* leave the possibility that beyond being artefacts paddle cilia might occur naturally in a lesser amount. This speculation needs to be proven in living, unstressed animals.

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