BODY EXTENSION TYPES OF *TETHYA WILHELMA*: CELLULAR ORGANISATION AND THEIR LOCOMOTORY FUNCTION

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ABSTRACT

Like some other species of the genus, *Tethya wilhelma* shows locomotion. The maximum speed was measured with 2 mm·h⁻¹. The rounded white body produces prominent extensions. Three types of extensions can be distinguished: type I produces buds (asexual reproduction), type II extensions (scout extensions) spread to all directions and may attach to the substrate and type III (guide extensions) play a role as guiding structures for movement. They develop from attached type II extensions, which extend at a maximum rate of 5 mm·h⁻¹. The main cell type inside type II and III extensions are actinocytes, amoebocytes, multipolar and spherulous cells as well as pinacocytes. Actinocytes are the fastest cell types, displaying a measured speed of 400 μm·h⁻¹. A preliminary hypothetical movement model for *T. wilhelma* is presented.

KEY WORDS

Tethya wilhelma, locomotion, body extensions, actinocytes, cell movement.

INTRODUCTION

Tethya wilhelma (Demospongiae, Hadromerida, Tethyidae) has recently been found in an aquarium habitat and described subsequently (SARÀ *et al.,* 2001). The typically rounded white body is highly capable of contraction and locomotion. This behavior has been previously reported for other *Tethya* species (FISHELSON, 1981; BOND & HARRIS, 1988; BOND, 1992). Like the other moving species of the genus, *T. wilhelma* produces long body extensions which are stabilized by bundles of anisostrongyles. These are the elongation of radial megasclere bundles originating from the choanosome core and protruding through the highly lacunar cortex (for general *Tethya* morphology see SARÀ, 2002). The extensions are able to attach to the surrounding substrate (Figs 1, 2). Their elongation was described as a sliding telescope mechanism and it was experimentally excluded that contractions of the extensions are the effectors of body movement, since no forces are created by the attached extensions to small substrate particles (BOND & HARRIS, 1988; BOND, 1992). The mechanism for movement of *Tethya* species is still unresolved. Here we report on the three extension types that are formed by *T. wilhelma*, the general cellular morphology of two of this types, the cell movements that are involved and present a preliminary hypothetical movement model. The nomenclature of BOURY-ESNAULT & RÜTZLER (1997) is used for cell types.

MATERIAL AND METHODS

Specimens of *T. wilhelma* were collected in the type habitat (aquarium of the zoological garden "Wilhelma", Stuttgart, Germany) and kept for months in artificial seawater in aquariums in our laboratory.

For morphological investigations we removed single extensions using micro scissors and performed immuno-fluorescent cytoskeleton staining. The extensions were fixed in 1 % paraformaldehyde. After repeated washing in PBS they were permeabilised in aceton:methanol 1:1 at -20° C for 20 minutes, washed 3x in PBS and incubated for 20 min in blocking solution (BS; 1 % BSA in PBS). Primary mouse-anti-β-tubulin-antibody (Sigma T4026) where incubated for 2 hours in BS at room temperature (RT). After 3x washing in BS, secondary Cy3-sheep-anti-mouse-antibodies (Sigma C2181) were incubated for 1 hour at RT and washed again in PBS. Samples were examined using a confocal laserscanning microscope (Zeiss LSM 410).

For digital *in vivo* time-lapse microscopy of cell behaviour, extensions were removed and transferred to micro observation chambers (two coverslips, silicon spacer frame; volume 1.6 ml). Samples were examined on an inverted microscope (Zeiss Axiovert 200) equipped with an digital camera (Zeiss Axiocam HRc) using differential interference contrast (DIC).

RESULTS

Tethya wilhelma possesses three types of body extensions which are visualised in figure 1. Their properties and biological functions are given in Tab. I. Type I extensions have reproductive functions (release of buds) and are not investigated in detail here. The role of type II and III extensions in movement is discussed below.

The maximum speed of *T. wilhelma* on a glass plate was recorded with 2 mm·h^{-1}. Under aquarium conditions, when attached to natural substrate, it is significantly slower (Fig. 2). Locomotion is not a permanent process. The specimens might just remain at a certain place, without moving. In this case they have reduced activity in formation of extension. In many cases movement could be induced by spreading sand on the specimens.
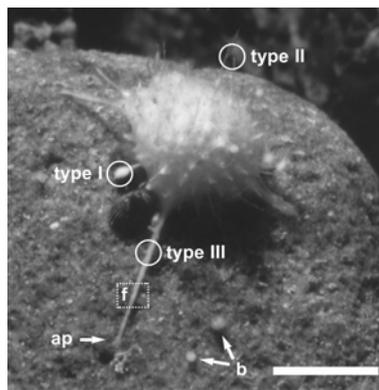

Fig. 1. *Tethya wilhelma* and its body extension types: Budding extensions (type I), scout extensions (type II) and guide extensions (type III) with attachment pad (ap; compare figure 5). Released buds (b) are attached to the substrate. Outlined square (f) represent an area shown in detail in figures 3 and 4. Bar = 10 mm.

Tab. I. Properties of *T. wilhelma* extension types.

Extension	Type I	Type II	Type III
Descriptive name	Budding extension	Scout extension	Guide extension
Assumed function	Bud formation (asexual reproduction)	Attachment to surrounding substrate	Leading structure during movement
Length	~5 - 10 mm	Max. ~40 - 50 mm	Max. ~40 - 50 mm
Diameter	Stalk: ~50 - 80 μm Bud: ~500 - 1000 μm	~50 - 100 μm	~100 - 250 μm
Profile	Round	Round	Flattened round
Appearance	White	Whitish opaque	White
Cell content	High	Low	High
Elongation speed	Not determined	Max. 5 mm·h^{-1}	None (attached)
Melting back	Very rare	Frequently when not attached	Rarely
Average number of parallel strongyles	Stalk: 3 - 8	3 - 5	5 - 12
Attachment	Bud attaches	By oligocellular pad	By multicellular pad
Comment	Many cells migrate along the stalk into the bud	Can transform to type III or rarely to type I	Thickened at the base, many cells migrate into the extension
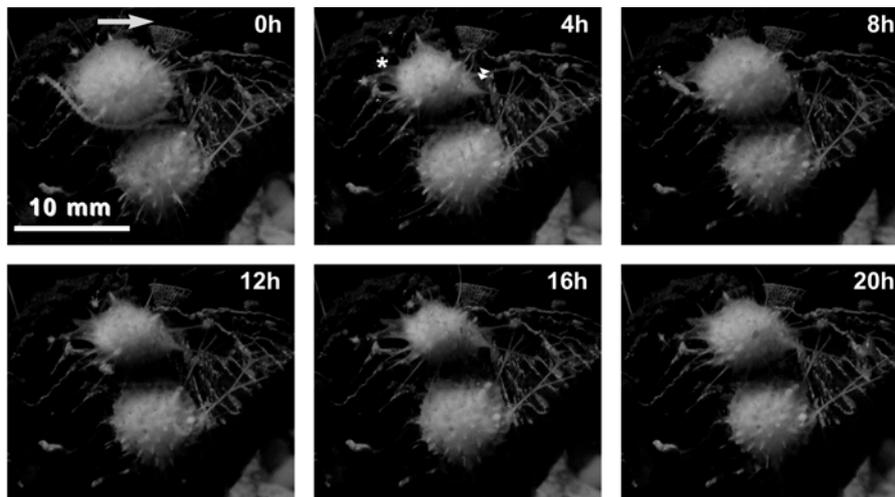
Fig. 2. Contracting and slowly moving specimen of *Tethya wilhelma*. Direction is indicated by an arrow. Guide extension (double arrowhead) and attachment remnants (asterisk) are changing in morphology. Maximum recorded speed was 2 mm per hour (on a glass plate). Contraction may reduce body diameter by around 40 % as demonstrated by the upper animal in comparison of 0 h and 4 h.
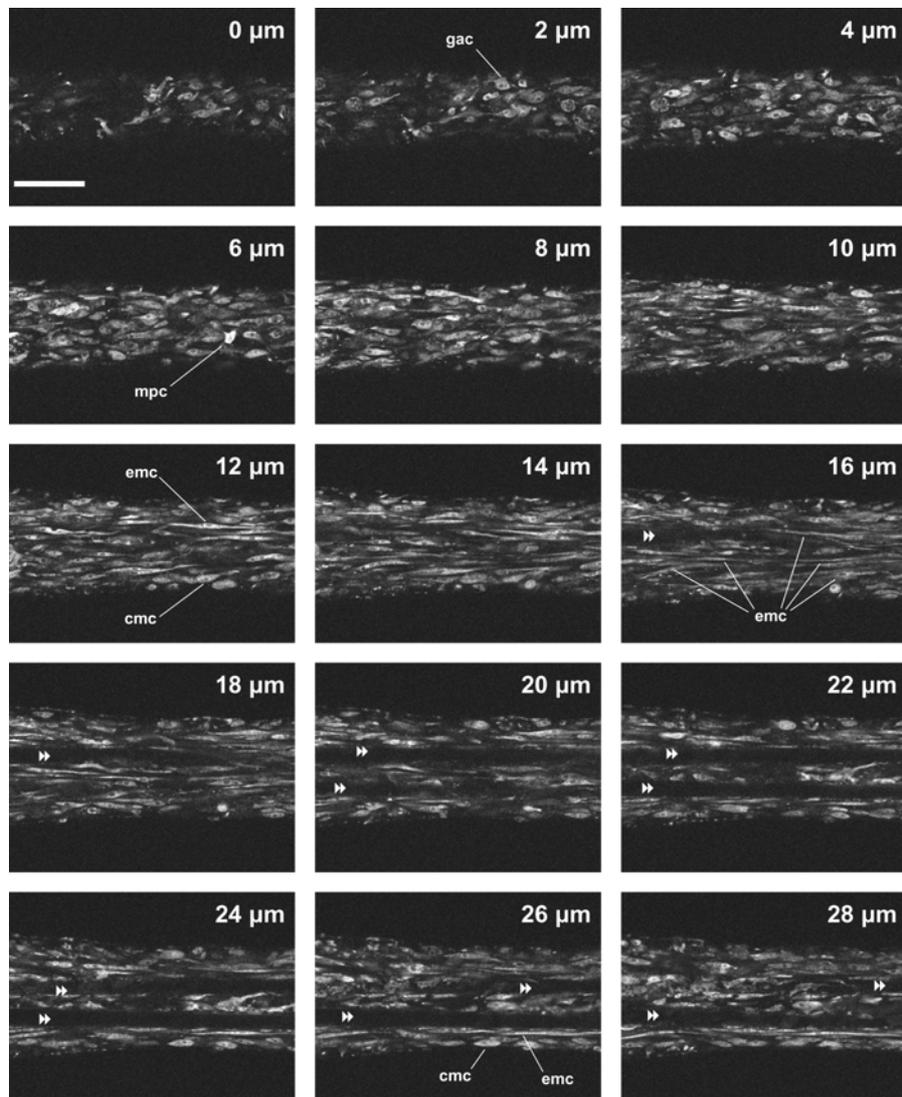
Fig. 3. Confocal LSM z-sections of a type II extension, projecting from left to right, anti-tubulin stained. Granulated archeocytes (gac) surround the core of megascleres (double arrowheads). Actinocytes (myocytes) in extended (emc) and contracted (cmc) form are the main cell types between the megascleres. In-between, there are multipolar cells (mpc).
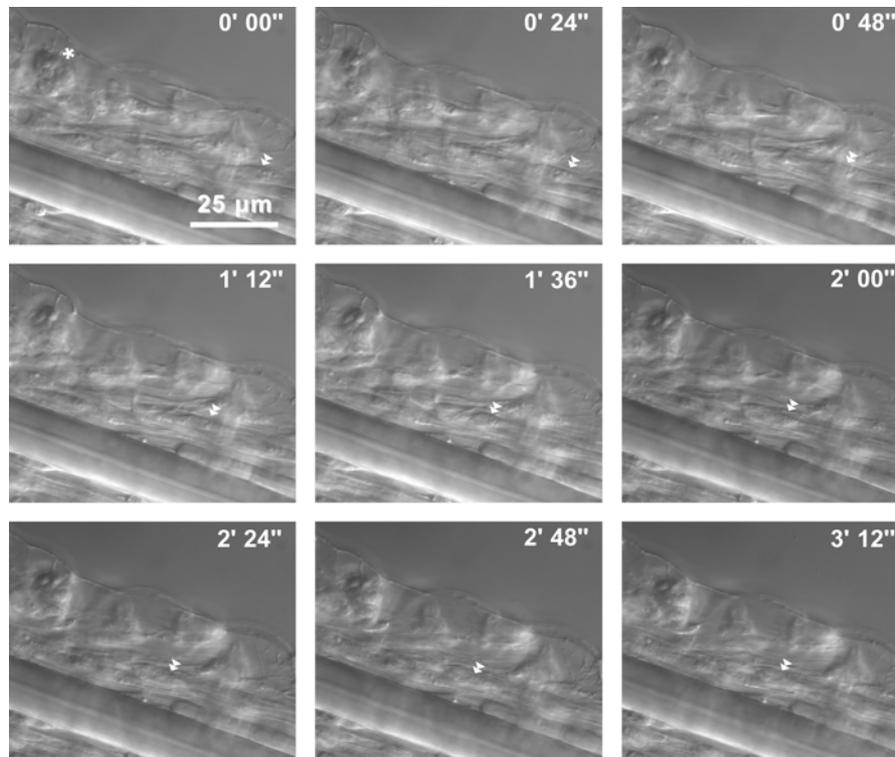
Fig. 4. Time-lapse of an actinocyte (double arrowhead) moving inside a type II extension for approximately 20 μm within 3 minutes. A granulated cell (asterisk) is slowly rotating and moving forward. Pinacocytes cover the surface.

The morphology of type II extensions is represented in confocal z-sections in Fig. 3. The core of the extensions is dominated by the presence of the strongyles which lay more or less in parallel. They are prominently surrounded by 50 - 200 μm long fusiform actinocytes (myocytes). The outer layers are dominated by amoebocytes, spherulous cells and multipolar cells. Using DIC lophocytes can also be detected (not shown here). Pinacocytes cover the surface (Fig. 4).

All three extension types are of enormous plasticity. Using digital *in vivo* time-lapse microscopy movement can be observed for all the cell types. The fastest cells we have observed are actinocytes which moved at a speed of 400 μm·h^{-1} (Fig. 4). The movement is amoeboid, though the slender, fusiform cell shape hardly changes. It seems that the main cell body with the nucleus is moving forward inside the cell, by cytoskeleton remodelling which is typical for amoeboid movements. The sliding telescope mechanism described by BOND & HARRIS (1988) could be confirmed by time-lapse microscopy. The main effectors for strongyle movement seem to be actinocytes. First video sequences demonstrate movement of the scleres by these cells, but detailed investigations are under process.

In contrast to many cell types, spherulous cells show no amoeboid movement. They rotate and move very slowly. From our observation it can not be excluded that they are transported passively by other cells.

DISCUSSION AND CONCLUSIONS

Tethya wilhelma moves with a speed of up to 2 mm·h^{-1} and is able to elongate its extensions with up to 5 mm·h^{-1}. The fastest cell movement was recorded with 400 μm·h^{-1}. At present, this values are among the fastest measured in sponges.

T. wilhelma forms many type II extensions in all directions. When they attach they might transform to type III by a strong migration of cells into the extension. Transformation from to type III only happens to few type II extensions. We have not observed more than one type III extension per sponge at a time. The control and coordination mechanisms for extension formation and transformation are not understood yet.

If a type III extension is formed, the sponge starts to locomote into this direction. BOND & HARRIS (1988) proposed a 'quasiamoeboid, crawling locomotion' for *Tethya*, but the strong involvement of the extensions was not taken into account, since there was no physical force measurable. Our refined hypothetical movement model includes the extensions. For their formation, *Tethya* uses a lot of energy (mainly for cell movement). Type II extensions are formed to sense the environment. If they attach they might transform to type III, if not, they will melt back. The sponge body is then moved towards and on the type III extension, which have the function of a guiding structure. We mainly expect a rearrangement of the cells of the cortex at the base of the type II extension. These local cell movements carry the sponge body forward, comparable to a conveyer mechanism. Therefore, type III extensions do not provide physical forces for locomotion, but signals to communicate the movement vector for the basal cells involved in sponge movement. This model resembles the movement of cultured fibroblasts or *Clathrina* cells (GAINO & MAGNINO, 1994). Filoopodia are formed, which spread over the substrate. Eventually they initiate the formation of lamellipodia, which are involved in cell migration. In a unicellular system, the driving forces are inherent in the cytoskeleton. In *T. wilhelma*, the driving forces reside in the basal cells which use the type III extensions as conductors. In contrast to a unicellular system, *T. wilhelma* is more limited in movement, since mineral skeleton is highly organized (NICKEL & BECKMANN, 2003) and not as variable as a cytoskeleton. The signalling mechanisms implied by our model are yet unknown, but currently under investigation. Considerations of JONES (1962), PAVANS DE CECCATTY (1974) and MACKIE (1990) will have to be taken into account again.

ACKNOWLEDGEMENTS

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