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Kinetid structure in larval and adult stages of the demosponges *Haliclona aquaeductus* (Haplosclerida) and *Halichondria panicea* (Suberitida)

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Abstract

At larval and adult stage of life cycle sponges (Porifera) possess different flagellated cells that play different roles in their body. Cells of larval epithelium serve as locomotory organs, while collar cells (choanocytes) of adults have a feeding function. Here, we describe for the first time the detailed structure of the flagellar apparatus (kinetid) in larval cells and choanocytes of the demosponges *Haliclona aquaeductus* and *Halichondria panicea*, and propose schemes of its organization based on longitudinal and transversal serial ultrathin sections. The kinetid of larvae has proved to be more complicated than that of choanocytes. It includes well-developed transverse and longitudinal cytoskeletons that strengthen the larval flagellum. The longitudinal skeleton in *H. aquaeductus* is represented by unusual structures previously understudied: large tubular rootlets made of oblique crossed fibers and connecting the kinetosome with mitochondria. We also found out the kinetid composition varies between different cell types of a highly structured parenchymella of *H. aquaeductus*. In simply organized parenchymella of *H. panicea* flagellated cells are identical and its kinetid includes a consistent additional centriole absent in choanocytes of adult sponges. We tried to evaluate the diversity of sponge larval kinetid organization based on literature data and found it variable within Haplosclerida and quite conservative within other sponge lineages.

Key words: Porifera, larvae, choanocytes, ultrastructure, kinetid

Introduction

Invertebrates have evolved wide variety of life cycles, among which the biphasic benthic-pelagic life cycle stands out. It realizes in alternation between a small motile larvae emerging from the egg and larger benthic adult, which is often sedentary or sessile. The appearance of dispersing larval stage in evolution was obviously a great step for development of diverse life forms known for today. Investigation of larvae can shed the light on the intricate evolutionary questions as they can retain fundamental ancestral features that allow us to look in depths of time. The larva of certain invertebrates display a core set of synapomorphic traits and gene expression patterns that are lacking in the adult form.

Sponges (Porifera) belong to one of the most ancient metazoan lineage that represents one of the earliest branches of the animal tree (Simion et al. 2017). Sponge larvae are regarded as sharing more traits and complexity with eumetazoans than adults (Maldonado 2004; Nielsen 2008). These facts induce to reflect who appeared first, the sponge larva or the adult. But this question is probably far from solution. Even though the sponge larva is an object of numerous thorough studies, many aspects of its biology remain largely obscure. For example, *Amphimedon queenslandica* Hooper and van Soest, 2006 is one of the most-studied sponge with deciphered genome and larval gene expression patterns, but we still cannot precisely identify all types of its larval cells (MahandLeys 2017). For more complete understanding of the sponge's life morphological studies of both stages are needed. The present work is intended to contribute the knowledge about sponge ultrastructure.

We focused on sponge flagellated cells, trying to reveal how elements of the flagellar apparatus, or the kinetid are arranged. It is worth to notice that larval flagellated cells serve as locomotory (or, rarely, sensory) organs, while the flagellum in adults' choanocytes participate in water pumping. Thus, flagella are essentially important for sponges on both stages. The kinetid is composed of three main parts: a free part, or the flagellum itself; a basal intracellular part containing normally two kinetosomes (the non-flagellated one is often called accessory centriole) with attached microtubular or fibrillar rootlets; and the transition zone connecting the free and basal parts, often containing phylogenetically important structures (Moestrup 1982, 2000; Andersen et al. 1991; Karpov 2000; Barr 2001). It has been shown that kinetid structure can be used in the taxonomy and phylogeny of unicellular eukaryotes (e. g., Yubuki and Leander 2013). In sponges, kinetid is also known as a very perspective morphological phylogenetic marker, since it includes evolutionary conservative structures along with variable ones (Pozdnyakov et al. 2017; 2018). Kinetid structure of choanocytes, collar cells of adults, can be successfully superimposed on phylogenetic tree of sponges based on molecular surveys (Pozdnyakov et al. 2018), while in sponge larva it is largely understudied.

The larval kinetid was first specially studied by Woolacott and Pinto (1995); the authors found it more complex and more diverse than that of choanocytes whose kinetid was not well studied that time (Pozdnyakov and Karpov 2013). Some components of the flagellar apparatus were properly illustrated in many papers (Boury-Esnault 1976; Nielsen 1987; Gallisier and Vacelet 1991; Woolacott 1993; Amano and Hori 1992, 1994, 1996, 2001; Ivanova 1997; Leys and Degnan 2001; Uriz et al. 2001; Boury-Esnault et al. 2003; Maldonado et al. 2003; Ereskovsky and Tokina 2004; Usher and Ereskovsky 2005; Gonobobleva 2007; Ereskovsky and Willenz 2008; Maldonado 2009; Lanna and Klatau 2012; Stephens et al. 2013). Due to these studies one can reveal some distinctions between kinetids of the adult and larva, trace common characters of their arrangement and compare sponges of different taxonomic position.

Phylum Porifera comprise classes Demospongiae, Calcarea, Homoscleromorpha, and Hexactinellida with over 9 000 extant species. The biggest class Demospongiae includes three subclasses: Verongimorpha, Keratosa and Heteroscleromorpha (Morrow and Cardenas 2015).

A special attention should be paid to the large and phylogenetically problematic order Haplosclerida that forms most basal branch at the phylogenetic tree of subclass Heteroscleromorpha (Morrow and Cardenas 2015). Kinetid structure of its representatives significantly differs. For example, Stephens et al. (2013) investigated both larva and adult of *Haliclona indistincta* (Bowerbank, 1866), and the provided illustrations of the kinetid are not in accordance with previously studied haplosclerids and even genus *Haliclona* (Woolacott and Pinto 1995; Amano and Hori 1996; Leys and Degnan 2001; Maldonado et al. 2003).

To contribute the assessment of the distribution of larval kinetid structure within polyphyletic genus *Haliclona* (Redmond et al. 2011), we have chosen *Haliclona aquaeductus* (Schmidt 1862) as an object of the current study.

Other sponge under consideration, the representative of the order Suberitida (Heteroscleromorpha), *Halichondria panicea* (Pallas 1766), is distantly related to *Haliclona aquaeductus* (Morrow and Cardenas 2015). The kinetid structure of flagellated cells of larvae in *H. panicea* is interesting: Woolacott and Pinto (1995) drawn an accessory centriole in kinetid scheme of the suberitids *Halichondria melanodocia* Laubenfels, 1936, *Halichondria coerulea* Bergquist, 1967 and *Hymeniacidon heliophila* (Wilson, 1911) (fig. 2 in their paper), while choanocytes of suberitids are supposed to be devoid of the permanent centriole (Pozdnyakov and Karpov 2016).

Kinetid of both larvae and adults of the studied species, *Haliclona aquaeductus* and *Halichondria panicea* were investigated on serial consecutive sections at TEM, the kinetid schemes were reconstructed and discussed. This research is aimed to enlarge our knowledge on the sponge flagellar apparatus and contribute to morphological basis for phylogenetic studies.

Materials and methods

Sponges with mature larvae were collected in the Kandalashsky Bay of the White Sea (WSBS MSU) in first couple of weeks of July 2017. Larvae were gathered via glass Pasteur pipette after the sponge had been put in the tank with stagnant water. Larvae and adults' pieces were fixed and treated for transmission electron microscopy according the following protocol. For prefixation, 1 mL of 1% osmium tetroxide in cacodylate buffer (0.1 mol l^{-1} , pH 7.4) was added to 3 mL of water containing a sponge fragment. Next, 4 mL of 4% glutaraldehyde in the same buffer was added and the sponge fragment was kept in this mixture for 15 min on ice in the dark. Then, the fixative mixture was replaced with 2% glutaraldehyde for 1 h on ice. Afterwards, samples were rinsed twice in the buffer and postfixated in 1% osmium tetroxide for 1 h at room temperature. The samples were then washed twice for 10 min in the same buffer, dehydrated in a graded ethanol series and embedded in Spurr resin. After polymerization, the resin blocks with sponge fragments were trimmed and treated with 10% hydrofluoric acid for 5 min to remove siliceous skeletal elements. Ultrathin sections (60 nm) were cut with a Leica EM UC6 ultramicrotome using a glass knife. The sections were double stained in uranyl acetate (15 min) and subsequently lead citrate (3 min) and were observed in a JEM 1400 and Morgagni 268 D electron microscopes equipped with an Olympus Veleta digital camera. For scanning electron microscopy fixed larvae were put in series of ethanol with increasing concentration of acetone dried in critical point and observed via Quanta 250 and Hitachi S-465A microscopes.

Several larvae and choanosomal pieces collected in different time were investigated for each species.

Descriptions follow the terminology used by Andersen et al. (1991) and Woolacott and Pinto (1995).

Results

Haliclona aqueductus

The mature larva of *H. aqueductus* is highly mobile ellipsoid parenchymella of nearly 500-700 μm length and 200-300 μm width. Three morphological zones can be distinguished in its body (fig. 1 a): 1) anterolateral zone covered with slightly spiral longitudinal rows of short (about 20 μm) cilia; 2) tuft, or narrow (5-7 cells) ring of long (about 110 μm) cilia combined in 'compound cilia' (fig. 1 b); 3) a non-flagellated pigmented posterior pole ringed by the tuft. The posterior pole includes pigmented dark-brown ring, which is well seen against the background of the pale larva.

The surface layer is formed by single-row ciliated cells of at least three types: anterolateral columnar cells, tuft cells and flask cells.

The most common cells are elongated narrow *columnar cells* (fig. 1 c). Their nuclei are basal and anucleolated, and the proximal part of flagellar axoneme is often immersed in the apical cytoplasm (fig. 1 d). Their flagellum has an axoneme with typical (9+2)+2 microtubular organization. In transitional zone the central microtubules are covered with prominent central sheath (fig. 2 e-g) that becomes barely distinguishable up from this zone (fig. 2 h). Nine spokes radiate from the central sheath to the transitional cylinder, an electron-dense structure constituted by two-layered membrane (fig. 2 e-g). Central microtubules start at the same level with transitional cylinder or somewhat lower, while the sheath starts at the same level with the cylinder. More proximal from the cylinder there is usually a heterogeneous electron-dense material (fig. 2 d). Thin transverse plate (fig. 3 b), sometimes slightly inflated at the center, marks the distal end of the kinetosome, having typical (9×3)+0 organization. Transversal cell skeleton is represented by lateral microtubules extending to the rootlets, mitochondria and cell membrane, and lateral arm (*sensu* Woolacott and Pinto 1995), which is constituted by microtubules associated with electron-dense material (fig. 2 a). The lateral arm is attached to microtubular organization center (MTOC) shaped as a basal foot. The distal part of the foot, bean-like cap, is attached to the kinetosome by several stalks (fig. 2 a, fig. 3 a). Nine transitional fibers (=alar sheets) attach the kinetosome to the plasmallema: each filament raises from a single triplet and connects it to the cell membrane via an electron-dense anchoring point (fig. 2 c, fig. 3 e). Longitudinal cytoskeleton is comprised of peculiar rootlets. They appear to be large hollow tubes of various diameter (probably contractive) with the surface looking crisscross-striated due to the opposite orientation of oblique fibers (fig. 3 c-g). Rootlets start from the kinetosome and extend toward the proximal part of the cell. Bunch of asymmetric mitochondria is closely associated with the rootlets, but direct contact was not observed (fig. 3 f, g).

Columnar cells of the tuft (*tuft cells*) bear a prominent single protrusion (fig. 4 b); axoneme of their flagellum is completely covered by the flagellar membrane. Unlike anterolateral columnar cells, these cells are often covered with glycocalyx (fig. 4b). Transitional zone arrangement coincides with that in previous cell type, but central microtubules start more distal than the transitional cylinder (fig. 4 b). The kinetosome bears an electron-dense element with unresolved structure, which is located proximal to the basal foot and, probably, represents an accessory MTOC (4 d-f).

In tufts of studied larvae, we observed a prominent tendency to rootlets reduction. Rootlets can be completely absent or as long as half or third of the long rootlets in anterolateral columnar cells. When rootlets are lacking, mitochondria are associated directly with the kinetosome (fig. 4 b, d, f).

Flagellated *flask cells* (fig. 5 a, b) that wedge between columnar cells are quite rare (about 10-20 per larva) cell type. These cells form the lobopodia throughout their entire length and has

somewhat irregular flask shape. The kinetid of flask cell has no rootlets (fig. 5 c). MTOC associated with the kinetosome looks like a dense mass of irregular shape (fig. 5 c). Bicorn-shaped parabasal nucleus has no direct link with the kinetosome, but is often associated with a centriole (fig. 5 f). The centriole is somewhat smaller than the kinetosome; its location in relation to the kinetosome is not standing. We failed to figure out whether this centriole is a constant element of the cell or match a certain state of cell cycle.

Choanocyte chamber of adult *H. aquaeductus* consists of choanocytes (fig. 6 a, b) of roundish shape. Its kinetid has transverse plate more prominent than in larval cells (fig. 7a). The kinetosome is associated with the Golgi apparatus by thin fibrillary rootlets (fig. 7 b), and sometimes the vesicles rest on the kinetosome (fig. 7 b). Its kinetosome serves as MTOC for radially arranged microtubules (fig. 7c, e), and basal foot is absent (fig. 7d).

Halichondria panicea

H. panicea develops a middle-size (about 300 μm) ellipsoid bright-yellow parenchymella (fig. 8 a) covered by loosely arranged identical flagellated cells (fig. 8b, c). Its flagellum emerges from a pit, and the pit's walls contain excretory granules (fig. 9 a).

Its larval kinetid is closely associated with pear-shaped nucleolated nucleus by thin rootlets projected from the distal part of kinetosome (fig. 9a). The Golgi apparatus lies on the rootlets, so does the accessory centriole (fig. 9 a, b). Mitochondria are scattered in the cytoplasm, mainly in distal half of the cell (fig. 9 d). In the transitional zone of the flagellum one can see the transitional cylinder (fig. 9 b). Central axonemal microtubules start at the level of the cylinder (fig. 9 b). Axial granule hangs on the filaments at the level of the plasma membrane (fig. 9 b). Nine transitional fibers attach the kinetosome to the cell membrane (fig. 9 b). Long (about 160-190 nm) cone basal foot with roundish cap gives rise to the transverse skeleton formed by lateral microtubules (fig. 9c).

Choanocyte chamber of adult *H. panicea* (fig. 10 a) is constituted by irregularly arranged flattened choanocytes that have kinetosome-nucleus connection. The Golgi apparatus lies close to the nucleus (fig. 10 b). The rootlets are much more short and rare than in larvae (fig. 10 b, c); the accessory centriole is absent. Basal foot is also shorter, and there is small additional MTOC (satellite) on the opposite side of the kinetosome (fig. 10 b-d). As in other sponges, the flagellum includes so-called 'dark zone' (Pozdnyakov and Karpov 2015, 2016; Pozdnyakov et al. 2018), which appears to be the poorly fixated area of the transitional cylinder and central sheath.

Discussion

Flagellated cells of larval epithelium in Haplosclerida (Amano and Hori 1996; Leys and Degnan 2002; Nakanishi et al. 2014) and Suberitida (Ereskovsky 2010) are known to be transdifferentiated during the metamorphosis and having no continuity between flagellum of the

larva and choanocytes. It is not surprising, therefore, that the kinetid structure in flagellated cells of *Haliclona aquaeductus* and *Halichondria panicea* displays a profound difference. The schemes of studied flagellar apparatuses of these species are presented in figures 11 and 12.

Kinetid comparison of larvae and adults of the two sponges from different orders reveals more complex flagellar structure in larval cells. Their flagellum is strengthened by transverse (lateral arm in *H. aquaeductus* and multiple microtubules in *H. panicea*) and longitudinal (well-developed fibrillar rootlets) cytoskeleton. Also, in *H. aquaeductus* the flagellum is immersed in apical part of columnar cell which probably defends its most fragile basic part (Blum 1971; Chakrabarti 1998). Kinetid structures of choanocytes are in accordance with those of closely related species (Pozdnyakov and Karpov 2015, 2016; Pozdnyakov et al. 2017).

Trying to figure out how variable the kinetid organization is among sponge larvae and whether or not it has phylogenetic relevance within certain groups, it is necessary to compare the obtained results with the already known data.

Haplosclerid larvae had been investigated in several studies, and some information on their kinetid structure is available. According to the literature data, kinetid structure is variable within the order Haplosclerida and even within the genus *Haliclona*, which is polyphyletic (Redmond et al. 2011). System of flagellar rootlets is apparently the most plastic element of the kinetid in these sponges.

Rootlets. The most peculiar feature found in the flagellar apparatus of *H. aquaeductus*, large tube fibrillar rootlets, is also recognizable on the photos of *Amphimedon queenslandica* (Leys and Degnan 2001, fig. 9c) and *Haliclona (Soestella) caerulea* (Hechtel, 1965) (Maldonado et al. 2003, fig. 3a-c; 5c) and clearly seen in *Haliclona tubifera* (George & Wilson, 1919) (Woolacott 1993, fig. 7; Woolacott and Pinto 1995, fig. 3; 10), though the kinetid scheme proposed in the last article does not reflect their three-dimensional tube organization. The layered structure of rootlets described by Woolacott and Pinto (1995) has not been revealed in *H. aquaeductus*. Unlike *H. tubifera*, the rootlets of *H. aquaeductus* are multiple (up to four) and have its criss-cross cortical pattern starting just from the kinetosome. Rootlets of other haplosclerids studied look like a bunch of filaments with closely associated mitochondria: *Haliclona* sp. 1 (Nielsen 1987), *Haliclona* sp. 2 (Amano and Hori 1994) and *Haliclona permollis* (Bowerbank, 1866) (accepted name – *Haliclona cinerea* (Grant, 1826)) (Amano and Hori 1996).

Basal foot structure also appears to be a variable morphotrait. It is quite similar in some haplosclerid larvae and presented by double (*H. caerulea* and, apparently, *Haliclona* sp. 2) or triple (*A. queenslandica*) foot connected with the kinetosome by bands (Amano and Hori 1994; Leys and Degnan 2001). In *H. tubifera* the foot is shown stalked, with roundish cap in

longitudinal section (as in *H. aquaeductus*), but without cross sections it is not possible to identify its real shape.

Lateral arm found in *H. aquaeductus* was also reported for *H. tubifera* by Woolacott and Pinto (1995). However, it is worth to note that the arm is well-seen on cross sections but not on longitudinal ones, and this kinetid element could be missed in previous studies devoted to other species.

A personal discussion deserves *Haliclonaindistincta* dealt by Stephens et al. (2013). Being clear haplosclerid according to the genetic studies (Stephens 2013), this sponge has larva that differs from larvae of other haplosclerid species by several features such as lacking of spicules, ciliary tuft and unciliated posterior pole. Its adults also have an unusual for haplosclerid morphotrait: choanocyte chambers directly contact with mesohyl, which matches topoecilosclerids rather than haplosclerids (Langenbruch and Jones 1990). Kinetid of both larva and adult also does not look like characteristic of Haplosclerida. It has an axial granule, typical of several sponge lineages but not haplosclerids (Pozdnyakov et al. 2018), and pear-shape nucleus in larval cells, which was noted in Suberitida (Woolacott 1990; Woolacott and Pinto 1995), Spongillida (Ivanova 1997), Poecilosclerida (Woolacott and Pinto 1995), Keratosa (Woolacott and Pinto 1995; Ereskovsky and Tokina 2004; Usher and Ereskovsky 2005; Gonobobleva 2007; Maldonado 2009) and calcareous sponges (Borojevic 1969; Amano and Hori 1992; Gallisian and Vacelet 1992; Amano and Hori 2001; Ereskovsky and Willenz 2008; Gallisian 1983; Lanna and Klatau 2012). It is likely that the nucleus of *H. indistincta* has a connection with the kinetid, which sometimes can be definitely detected only on series sections, as well as the accessory centriole. Flagellar rootlets of *H. indistincta* larva look like short tuft of filaments not associated with mitochondria, but adjoining the Golgi apparatus, and the kinetosome contains the axial granule. Such set of characters resembles the kinetid in Poecilosclerida and Suberitida (Woolacott and Pinto 1995; Uriz et al. 2001; Pozdnyakov and Karpov 2016), although the axial granule is also seen in larval cells of *Haliclonas* sp. (Nielsen 1987). Complex of the abovementioned features prompts to pay more attention to this species and make sure that the material of morphological studies matches to that of molecular genetic analyses.

In our study, three types of flagellated cells were detected in larvae of *H. aquaeductus*: 1) columnar cells, the main cell motors; 2) photosensitive tuft cells that detect light and serve as eyes (Leys and Degnan 2001; Maldonado et al. 2003); 3) flask cells that are probably in charge of settlement processes and apparently fulfill a sensory function (Nakanishi et al. 2015; Mah and Leys 2017). These cell types possess distinct kinetid components (fig. 11). Differences in the kinetid structure of different cells of a sponge larva were briefly discussed in few works. Kinetid structures of anterolateral cells and long-flagellated cells of the tuft were compared for *H.*

caerulea by Maldonado et al. (2003). The authors pointed out that central pair of microtubules of the short flagella start more distal than surrounding plasma membrane, while in long-flagellated tuft cells it rises at the level of the surrounding membrane, which corresponds to the scheme observed in *H. aquaeductus*. In both sponges there is also an additional MTOC-like structure under the basal foot in tuft cells. However, unlike *H. aquaeductus*, in *H. caerulea* rootlets of tuft cells are more developed than in common columnar cells. In *A. queenslandica* kinetid structure of long posterior flagella does not appear different from that of the short lateral cilia (Leys and Degnan 2001).

Flask cells were reported to be devoid of rootlets and additional centriole in *H. tubifera*. They possess round nucleus and kinetosome serving as MTOC, but series sections were not investigated (Woolacott 1993). In *H. aquaeductus* these cells also have no rootlets, but possess prominent MTOC of irregular shape and the centriole attached to the protrusion of the nucleus. The inconsistent position of the centriole and absence of bridges that usually connect the accessory centriole with the kinetosome allow us to doubt about its participation in the flagellar functioning.

It should be also noted that the third central microtubule in the transition zone of larval flagellum in *H. caerulea* (Maldonado et al. 2003; Maldonado 2004) actually can be interpreted as an element of the central sheath, which is thickened in the area of the central cylinder in *H. aquaeductus* (fig. 2 f1) and may give an appearance of an additional central tubule in this region.

Kinetid structure in Suberitida larvae is known from three species: *Halichondria melanodocia*, *H. coerulea*, *Hymeniacidon heliophila* (Woolacott and Pinto 1995). Its organization corresponds to the obtained scheme for *Halichondria panicea* and differs from the scheme of choanocyte kinetid proposed for Suberitida and Poecilosclerida (Pozdnyakov and Karpov 2016) primarily by the presence of the permanent additional centriole situated on the rootlets, quite far from the kinetosome. It should be noted that poecilosclerid larvae studied for today also have the accessory centriole (*Mycale cecilia* Laubenfels, 1936 (Woolacott and Pinto 1995), *Crambecrambe* (Schmidt, 1862) (Uriz et al. 2001), *Hymedesmia irregularis* Lundbeck, 1910 (Sokolova et al. in preparation)).

Thus, one can observe a significant diversity of sponge larval kinetid within the only one order of demosponges, Haplosclerida. This seems not surprising because this order is really large; it is considered as the sister clade with the rest of Heteroscleromorpha, the group which includes the majority of Demospongiae species (Morrow and Cardenas 2015). Given the relative consistency of the flagellar apparatus in adult sponges (Pozdnyakov et al. 2017), it is of interest to reveal the

patterns underlying such a diversity. Choanocytes of different demosponges function in the same way, while flagella of larval locomotory cells can realize diverse moving patterns (Maldonado 2006). It can be supposed that the kinetid structure correlates with locomotion patterns of larvae: the more complex the kinetid is, the more mobile and agile the larva is. However, larvae of *Haliclona aqueductus* possess a kinetid structure that shares few features with larvae of the dictyoceratid *Ircinia oros* (Schmidt, 1864) (Ereskovsky and Tokina 2004) having a similar body plan (tufted parenchymella with bare posterior pole).

One should note that it is the high-speed maneuverable larva of *H. aqueductus* that possess the stout rootlets, which serve actually as the pipelines leading to mitochondria and obviously effective machinery for energy transformation/transport/accumulation and usage. Probably, rootlet reduction in the cells of tuft reflects the low energy requirement of these cells (serving rather as a rule than as a motor). But the reverse trend in *H. caerulea* (Maldonado et al. 2003) does not correspond to this assumption. The biochemical processes taking place in the tube rootlets are a relevant theme for prospective studies.

At the same time, other sponge lineages display some consistency in larval kinetid structure, as can be inferred for today. Particularly, studied calcareous sponges (Borojevic 1969; Amano and Hori 1992; Gallisian and Vacelet 1992; Amano and Hori 2001; Ereskovsky and Willenz 2008; Gallisian 1983; Lanna and Klatau 2012) as well as homoscleromorphs (Boury-Esnault et al. 2003, Maldonado and Riesgo 2008) and eumetazoans (Nielsen 1987) possess apical nuclei linked to the kinetid in larval ciliated cells, accessory centriole and striated rootlets. Kinetids of Poecilosclerida *Crambecrambe* (Uriz et al. 2001), *Mycale cecilia* (Woolacott and Pinto 1995), *Hamigera hamigera* (Schmidt, 1862) (Boury-Esnault 1987) and *Hymedesmia irregularis* (Sokolova et al. in preparation) comprise a kinetosome which bears laminar rootlets and, apparently, accessory centriole (seen in *H. irregularis*, *C. crambe*, *M. cecilia*). The abovementioned kinetids of Suberitida (*Halichondria melanodocia*, *H. caerulea*, *Hymeniacidon heliophila* (Woolacott and Pinto 1995)) are also similar within the group. Spicule-less sponges *Halisarca dujardini* Johnston, 1842 (Gonobobleva 2007), *Ircinia oros* (Ereskovsky and Tokina 2004), *Aplysina aerophoba* (Nardo, 1833) (Maldonado 2009), *Aplysilla* sp. (Woolacott and Pinto 1995), *Cacospongia mollior* Schmidt, 1862 (Uriz et al. 2008), *Chondrilla australiensis* Carter, 1873 (Usher and Ereskovsky 2005), also possess the kinetid quite similar to that in calcareous and homoscleromorph sponges iterating the situation observed in choanocytes (Pozdnyakov et al. 2017); though unlike *Calcarea* larval kinetid of these sponges lack striated rootlets, they are shown present in embryos of *H. dujardini* (Gonobobleva 2007). It should be emphasized that Verongimorpha representatives were shown to possess the apical nucleus associated with the kinetid (*A. aerophoba*, *H. dujardini*, *C. australiensis*), while studied Keratosa have the basal nucleus placed far from the flagellum (*C. mollior*, *I. oros*, *Aplysilla* sp.,

Pleraplysillaspinifera(unpubl. data)). However, *Aplysillasp.* (Woolacott and Pinto 1995), is out of that line as its kinetid has MTOC shaped as lateral arms (as in some *Haliclona* representatives) and laminar rootlets (as in Poecilosclerida representatives), and *Halichondria moorei* Bergquist, 1961 (Evans 1977) possesses the basal nucleus. Also, large portion of illustrations have not enough resolution for resolving of fine structures and no series sections and scarce cross sections that allow us to detect precisely the configuration of the flagellar apparatus. Thus, any conclusions about phylogenetic signal of kinetid components should be made carefully and until more sponge larvae are studied, this issue awaits elucidation.

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Figure legends

Fig. 1 Larval stage of *Haliclona aquaeductus*. a – generalview of the larva (SEM), b – tuft consisting of the compound cilia (SEM), c – generalview of the anterolateral columnar cells (SEM), d - longitudinal section through the anterolateral columnar cells. Abbreviations: *sc* – short cilia, *lpc* – long posterior cilia, *pp* – posterior pole, *cc* – compound cilia, *fl*– flagellum, *n* – nucleus. Scale bars: a – 80 μm , b – 2 μm , c – 5 μm , d – 1.5 μm

Fig. 2 Distal part of the kinetid in *Haliclona aquaeductus* larval cells. a – transversalsection at the level of the basal foot, b-h – serial sections of the same cell. fl – section of another cell. Abbreviations: *bf* – basal foot, *st* – stalks of the foot, *la* - lateral arm, *tf*– transitional fibers, *mct* – microtubules, *ap* – anchoring points, *pd* – peripheral doublet, *cm* – central microtubules, *sh* – sheath, *tc* – transitional cylinder. Scale bar: 0.2 μm

Fig. 3Details of the kinetid structure in antero-lateral columnar cells of *Haliclona aquaeductus*larva. a, b – longitudinal sections through the kinetosome, c-e – longitudinal sections through the rootlets, f-g – cross sections of the rootletssurrounded by mitochondria (arrows indicate the rootlets). Abbreviations: *cm* – central microtubules, *tc* – transitional cylinder, *bf* – basal foot, *st* –stalks of the foot, *fl* – flagellum, *k* – kinetosome, *tp* – transverse plate, *mct*– microtubules, *rtl* – rootlets, *mtch* – mitochondrion, *rtl (ep)* – external part of rootlets, *rtl (ip)* – internal part of rootlets, *of* – oblique fibers, *ap* – anchoring points. Scale bars: a-g – 0.5 μm , c – 0.25 μm .

Fig. 4Tuft cells of *Haliclona aquaeductus* and their kinetid. a – common view of the cells, b-f – details of the kinetid structure. Abbreviations: *cc* – compound cilia, *tc* – transitional cylinder, *gl*

– glycocalyx, *cm* – central microtubules, *MTOC* – microtubule organizing center, *mtch* – mitochondria, *tp* – transverse plate, *rtl* – rootlet. Scale bars: a – 3 μm , b-h – 0.5 μm

Fig. 5 Flask cells of *Haliclona aquaeductus* and their kinetid. a, b – common view of the cells, c – kinetid appearance, d-h – serial longitudinal sections of the same cell. Abbreviations: *fl* – flagellum, *n* – nucleus, *mtch* – mitochondria, *ac* – accessory centriole, *cm* – central microtubules, *mct* – microtubules, *MTOC* – microtubule organizing center, *k* – kinetosome. Scale bars: a, b – 1 μm , c, d – 0.5 μm

Fig. 6 Flagellated cells of adult *Haliclona aquaeductus*. a – choanocyte chamber, b – choanocyte. Abbreviations: *fl* – flagellum, *mcv* – microvilli, *n* – nucleus. Scale bars: a – 10 μm , b – 1 μm

Fig. 7 Details of the choanocyte kinetid of *Haliclona aquaeductus*. a, b – longitudinal sections through the kinetosome, c – cross section of the apical part of choanocyte, d – serial cross sections of through the kinetid of the same cell, e – cross section through the kinetosome of another cell. Abbreviations: *tf* – transitional fiber, *tp* – transverse plate, *mct* – microtubules, *Ga* – Golgi apparatus, *mcv* – microvilli, *pd* – peripheral doublet of microtubules. Scale bars: a, b – 0.25 μm , c – 1 μm , d, e – 0.2 μm

Fig. 8 Larva of *Halichondria panicea*. a – common view of larva (SEM), b – surface of larva (SEM), c – longitudinal section of flagellated cell. Abbreviations: *fl* – flagellum, *n* – nucleus, *nu* – nucleolus. Scale bars: a – 100 μm , b – 2 μm , c – 1 μm .

Fig. 9 Details of kinetid structure in larva of *Halichondria panicea*. a, b – longitudinal sections through the kinetosome, c – cross section at the level of basal foot, d – cross section of a cell at the level of the Golgi apparatus. Abbreviations: *eg* – excretory granule, *ag* – axial granule, *bf* – basal foot, *mct* – microtubules, *rtl* – rootlets, *Ga* – Golgi apparatus, *n* – nucleus, *k* – kinetosome, *cm* – central microtubules, *tc* – transitional cylinder, *ac* – accessory centriole, *mtch* – mitochondria. Scale bars: 0.5 μm

Fig. 10 Choanocytes of the adult *Halichondria panicea*. a – choanocyte chamber, b-d apical part of the three choanocytes. Abbreviations: *mcv* – microvilli, *fl* – flagellum, *k* – kinetosome, *Ga* – Golgi apparatus, *n* – nucleus, *bf* – basal foot, *st* – satellite, *tf* – transitional fiber, *ag* – axial granule, *rtl* – rootlets. Scale bar: a – 2 μm , b-d – 0.5 μm

Fig. 11 Scheme of kinetid structures of *Haliclona aquaeductus*. a-b – antero-lateral columnar cells at longitudinal (a) and transversal (b) sections (lateral arm is not shown on the longitudinal section), c – flask cell, d – tuft cell, e – choanocyte. Abbreviations: *cm* – central microtubules, *sh* – sheath, *tc* – transitional cylinder, *tp* – transverse plate, *bf* – basal foot, *mct* – microtubules, *rtl* – rootlets, *rtl (ip)* – rootlet (internal part), *rtl (ep)* – rootlet (external part), *k* – kinetosome, *mtch* – mitochondria, *MTOC* – microtubule organizing center, *ac* – accessory centriole, *n* – nucleus, *Ga* – Golgi apparatus, *mcv* – microvilli

Fig. 12 Scheme of the kinetid found in flagellated cells of *Halichondria panicea*. Abbreviations:
n – nucleus, *nu* - nucleolus























