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Kinetid structure of choanoflagellates and choanocytes of sponges does not support their close relationship

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Summary

Any discussion of the origin of Metazoa during the last 150 years and particularly in the recent years when the sister relationship of choanoflagellates and Metazoa was unambiguously shown by molecular phylogeny refers to the similarity of sponge choanocytes to choanoflagellates. These two types of collared radially symmetric cells are superficially similar with respect to the presence of microvilli around a single flagellum and flat mitochondrial cristae which are common for many eukaryotes. But a comparison of the most informative structure having a stable phylogenetic signal, the flagellar apparatus or kinetid, has been neglected. The kinetid is well studied in choanoflagellates and is rather uniform, but in choanocytes this structure has been investigated in the last five years and is represented by several types of kinetids. Here we review the kinetid structure in choanocytes of Porifera to establish the most conservative type of kinetid in this phylum. The detailed comparison of this kinetid with the flagellar apparatus of choanoflagellates demonstrates their fundamental difference in many respects. The choanocyte kinetid contains more features which can be considered plesiomorphic for the opisthokonts than the kinetid of the choanoflagellates. Therefore, the hypothesis about the origin of Porifera, and consequently of all Metazoa directly from choanoflagellate-like unicellular organism is not confirmed by their kinetid structure.

Key words: origin of Metazoa, kinetid, ultrastructure, choanoflagellate, choanocyte, Porifera

Introduction

Choanocytes, flagellated cells of adult sponges, having a flagellum surrounded by the collar were first described by James-Clark (1867). They line the so called choanocyte chambers and serve as feeding cells in sponges. In his description, James-Clark noted their close morphological similarity to choanoflagellates, or collar flagellates, a group of heterotrophic flagellates with a single flagellum also surrounded by a collar (James-Clark, 1866). They are sedentary single or colonial and live in freshwater and marine habitats. The choanoflagellates are the only protozoa with a collar and are similar to choanocytes in morphology and mode of nutrition. As a result, James-Clark (1867) proposed that sponges should be considered as colonial protozoa.

His assumption did not persist for long. By the middle of the 19th century, sponges were considered to be the multicellular animals (Leuckart, 1854, cit. ex Leadbeater, 2015; Haeckel, 1866; Sollas, 1886; Delage, 1892), but the similarity between choanocytes and choanoflagellates determined the views on the origin and evolution of Porifera and multicellular animals in general. In the middle of the 19th century two views on the origin of sponges were proposed: Haeckel accepted one ancestor for the Metazoa including sponges (Haeckel, 1874) whilst Sollas suggested their independent origin (Sollas, 1886). All subsequent views on the position of sponges in the animal kingdom until the end of the 20th century were based on one or other of these views (Tuzet, 1963; Bergquist, 1978; Salvini-Plawen, 1978; Seravin, 1986). The majority of authors agreed with the evolutionary origin and deriving of sponges and all Metazoa from colonial choanoflagellates (Ivanov, 1968; Tuzet, 1963; Bergquist, 1978; Salvini-Plawen, 1978). The morphological similarity of choanocytes and choanoflagellates at the level of light and partly electron microscopy, based predominantly on their radial symmetry and apical collar around a single flagellum, was confirmed and highlighted many times (Tuzet, 1963; Salvini-Plawen, 1978; Simpson, 1984; Maldonado, 2004; Leadbeater, 2015).

Since the time when comparative molecular methods became available for the phylogeny of Metazoa (Wainright et al., 1993), the choanoflagellates were found to be a sister group to the Metazoa which led to the suggestion that both groups had evolved from a common ancestor. Since this first publication, the sister relationship between

the choanoflagellates and the Metazoa has now been confirmed on many occasions (Cavalier-Smith, 1996; Borchiellini et al., 1998, 2001; Leadbeater and Kelly, 2001; Medina et al., 2003; King et al., 2008; Philippe et al., 2009; Shalchian-Tabrizi, 2008; Taylor et al., 2007; Wörheide et al. 2012; Pisani et al., 2015; Torruella et al., 2015; Simion et al., 2017).

Do choanoflagellates represent the image of a metazoan ancestor?

In 2003 the genes responsible for cell adhesion and cellular signaling that allow cells of multicellular animals to interact with the extracellular matrix and with each other, were found in choanoflagellates (King, 2003). It became clear that metazoan multicellularity arose by modification of genes controlling cytological mechanisms already present in unicellular organisms (Ruiz-Trillo et al., 2007; King et al., 2008). Further the genes encoding neuropeptides (Fairclough et al., 2013), sphingoglycolipid metabolism regulators (Burkhardt, 2015), cadherins (Nichols, 2012), and signaling ability with the participation of Ca²⁺ ions (Cai, 2008) have been found in choanoflagellates. These findings further supported the hypothesis that the metazoan ancestor was similar to choanoflagellates. The phylogeny of multicellular animals with an origin from a choanoflagellate-like ancestor has been elaborated in the recent reviews (Nielsen, 2012; Cavalier-Smith, 2016; Brunet and King, 2017).

However, homologues of these specific metazoan genes proved to be the feature not only of choanoflagellates. Gene sets encoding some of the cellular systems and processes of Metazoa have been found in other unicellular representatives of both opisthokont subdivisions, the Holozoa (includes Metazoa and several protistan lineages) and the Holomycota (includes Fungi and two protistan lineages) (Sebe-Pedros et al., 2010, 2013). The homologues of proteins involved in the nervous activity of Metazoa were known for fungi earlier (Schulze et al., 1994). Moreover, individual components of their gene sets have also been found in Amoebozoa, the supergroup sister to Opisthokonta, and in one of the deepest lineages of the latter supercluster, the Apusozoa (Sebe-Pedros et al., 2010).

The choanoflagellates also do not occupy the first place in terms of the quantity of metazoan genes possessed. Some genes of multicellular animals

found in the lower representatives of Opisthokonta are absent in choanoflagellates (Sebe-Pedros et al., 2010, 2013, 2016). Thus, the choanoflagellates look more like simplified organisms that have apparently lost some of the genes present in a metazoan ancestor (O'Malley et al., 2016). Such an idea has already been expressed by Maldonado (2004) from the morphological and common biological points of view.

Thus, the latest molecular data are not in agreement with the hypothesis of the origin of Metazoa from a choanoflagellate-like ancestor (Fairclough et al., 2010; Sebe-Pedros et al., 2016; Hehenberger et al., 2017). Mah et al. (2014) came to the conclusion about the absence of homology of choanocytes and choanoflagellates on the basis of a difference in collar-flagellum interaction of *Spongilla lacustris* and *Monosiga brevicollis*.

Therefore, the unicellular ancestor of Metazoa might not be a choanoflagellate-like organism, but similar to some other group of Opisthokonta that perhaps has a complex life cycle and alternation of generations (Mikhailov et al., 2009; Sebe-Pedros et al., 2017).

Why we need the detailed study of kinetids?

Superficial similarity of choanocyte and choanoflagellate cell structure does not convince us of the homology of all systems of the cell besides the microvilli. It has been shown in many reviews on many eukaryotic groups/lineages that the flagellar apparatus (kinetid) is the most informative structure with clear phylogenetic signals that characterize modern branches on the global molecular phylogenetic tree (see for ref.: Yubuki and Leander, 2013). For instance, the star-like structure in the flagellar transition zone and crossed microtubular roots characterize the green algae and land plants (Moestrup, 1978; Melkonian, 1982, 1984; O'Kelly and Floyd, 1983), the transition helix in the transition zone and tripartite tubular mastigonemes are the characters of all stramenopiles (Patterson, 1989; Anderson et al., 1991; Karpov, 2000; Moestrup, 2000), a set of three roots (transversal and postciliary microtubular bands and a kinetodesmal filament) strongly describe the kinetid of ciliates (Seravin and Gerassimova, 1978; Lynn, 1981, 2016). In this case, to reconstruct the cell structure of the ancestral organism of the lineage Metazoa+Choanoflagellata we need to compare carefully the kinetid structure of sponge choanocytes and choanoflagellates.

The choanoflagellates are well studied in this respect and it has been shown in many papers that the single celled and colonial, sedentary and swimming, marine and freshwater, naked and thecate or loricate – all of them have very conservative internal cell structure and flagellar apparatus (Laval, 1971; Leadbeater, 1972, 1977, 1983, 1991, 2015; Leadbeater and Morton, 1974; Hibberd, 1975; Karpov, 1981, 1982, 1985; 2016; Zhukov and Karpov, 1985; Karpov and Leadbeater, 1998; Karpov and Zhukov, 2000; Leadbeater and Thomsen, 2000; Wylezich et al., 2012). The choanoflagellates have a highly conservative and unique kinetid structure: long transition zone with central filament, microtubular bands (roots) radiating from a dense circle (MTOC) around a kinetosome. In some species this circle splits into separate foci which produce radiating microtubular roots. A fibrillar bridge connects the kinetosome to the orthogonal centriole which produces a thin fibrillar root to the Golgi apparatus. There is no obvious connection of the kinetid to the nucleus, although in the lysing cell a kinetid retains connection to the nucleus (Karpov and Leadbeater, 1998).

In contrast to this unified cell structure in choanoflagellates a situation with sponge choanocytes is different. Superficially both types of collared cells are similar to each other (Brill, 1973; Amano and Hori, 1996; 2001; Maldonado, 2004; Gonobobleva and Maldonado, 2009), which has been explored in many reviews (see ref.: Brunet and King, 2017). But already in the first detailed description of a choanocyte kinetid we found that it is fundamentally different from that of choanoflagellates (Karpov and Efremova, 1994). Moreover, our further special kinetid studies (Pozdnyakov and Karpov, 2013, 2015, 2016; Pozdnyakov et al., 2017a, 2017b) have shown several types of kinetid structure in choanocytes that characterize major phylogenetic branches, Calcarea, Homoscleromorpha and Demospongiae, of the tree of Porifera (Figs 1-7, Table 1). Thus, we have to consider which kinetid characters are common for sponge choanocytes as a whole, and to propose the most appropriate description of conservative kinetid features for Porifera.

Here we present the main types of choanocyte kinetids in sponges, suggest a conservative kinetid type for each class of Porifera (excluding highly specialized Hexactinellida), propose plesiomorphic kinetid characters of sponges in general and, at last, compare this kinetid structure with that of choanoflagellates.

Kinetid diversity in choanocytes of sponges

The cell structure of choanocytes is more variable than in choanoflagellates as a nucleus can occupy an apical or basal position in a choanocyte, that is it may or may not be connected to the kinetid. Mitochondria have flat cristae and the dictyosome of the Golgi apparatus lies near the kinetid.

The kinetid itself also has some common features for all studied choanocytes: the flagellum has a normal arrangement of microtubules (9+2) in the axoneme and does not contain any additional structures like a paraxial rod. The longitudinal flagellar wings can be found in some species, but this is rare, and mostly a flagellum is smooth. The proximal ends of central microtubules of the axoneme are usually submerged in the electron dense area, thus, the structures inside this region (if they exist) are not resolved.

CLASS HOMOSCLEROMORPHA

In the class *Homoscleromorpha*, that is divided into two branches corresponding to the families *Oscarellidae* and *Plakinidae* (Wörheide et al., 2012), we found two types of kinetids (Pozdnyakov et al., 2017a, 2017b; Sokolova et al., 2017).

“CORTICIUM” TYPE (FIG. 1; TABLE 1)

Choanocytes have an apical nucleus with anterior projection which is connected to the kinetosome by a fibrillar root. The kinetosome has a basal foot ending with a round dense head which serves as a MTOC for single lateral microtubules. A centriole (non-flagellar kinetosome) is present and lies orthogonal to the kinetosome. The central microtubules start at some distance from the kinetosome, a transition plate, or septa is absent, and instead a prominent axial granule is visible at the kinetosome distal end (Pozdnyakov et al., 2017a, 2017b; Sokolova et al., 2017).

We call this kinetid type the “Corticium”, since such kinetid structure was first noted in the choanocyte of *Corticium candelabrum* (*Plakinidae*) by Boury-Esnault and co-workers (1984). The characters of this type were found in four species of *Oscarella* (*Oscarellidae*): *O. kamchatkensis*, *O. balibaloi*, *O. nicolae*, and *O. pearsei* and in *Plakina trilopha* (*Plakinidae*) (Pozdnyakov et al., 2017a, 2017b; Sokolova et al., 2017; Ereskovsky et al., 2017).

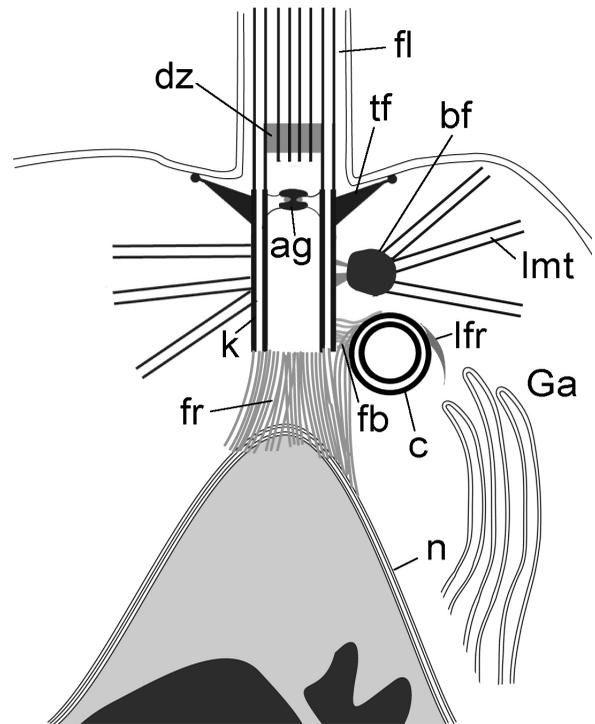


Fig. 1. The general scheme of the “Corticium” type kinetid. *Abbreviations:* ag- axial granule; bf – basal foot; c – centriole; dz – electron-dense zone; fb – fibrillar bridge; fl – flagellum; fr - fibrillar root; Ga - Golgi apparatus; k - kinetosome; lfr – lateral fibrillar root; lmt - lateral microtubules; n – nucleus; tf - transition fiber.

“OSCARILLA” TYPE (FIG. 2; TABLE 1)

Choanocytes of this type have a basal nucleus which is not connected to the kinetosome. The kinetosome has no fibrillar roots. All the other kinetid characters coincide with those of the “Corticium” type. This type of kinetid was called “Oscarella”, since it was described in the majority of the *Oscarella* species: *O. lobularis*, *O. tuberculata*, *O. bergenensis*, *O. carmela*, *O. rubra*, *O. viridis* and *O. microlobata* (Pozdnyakov et al., 2017b; Sokolova et al., 2017).

In spite of only having limited data on the choanocyte kinetid structure in the class *Homoscleromorpha*, we can make some preliminary assumptions based on the occurrence of the selected types of kinetid on the *Homoscleromorpha* phylogenetic tree (Gazave et al., 2013; Wörheide et al., 2012). Since the “Corticium” kinetid type was noted in two genera of *Plakinidae* and in

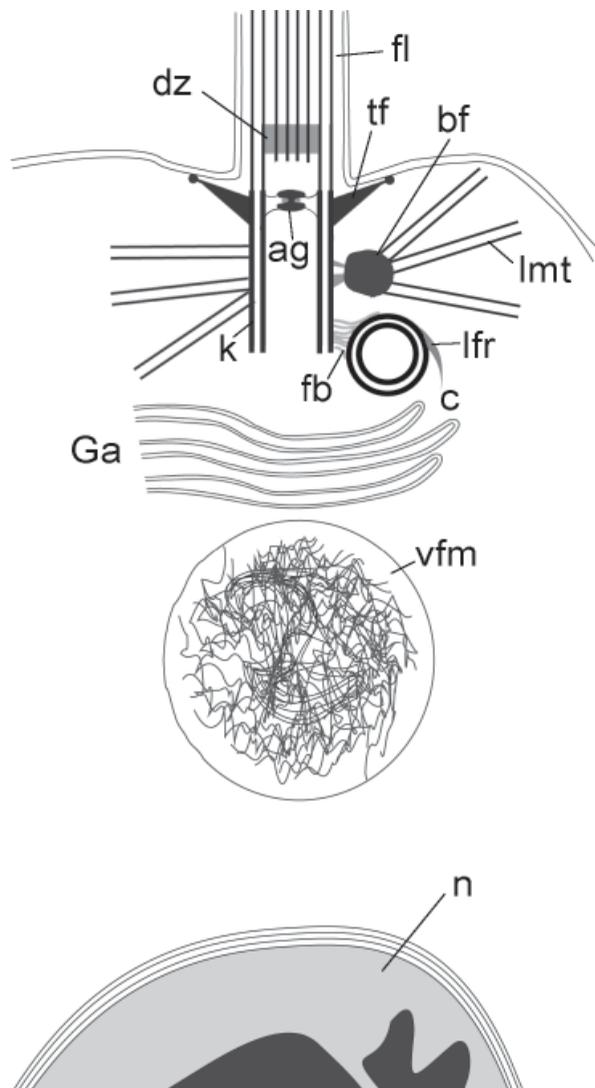


Fig. 2. The general scheme of the “Oscarella” type kinetid. Abbreviations: vfm – vacuole, other abbreviations as for Fig. 1.

Oscarella (Oscarellidae), but “Oscarella” type was noted in species of *Oscarella* only, one can hypothesize that the “Corticium” type is nearer to ancestral type of Homoscleromorpha (Fig. 5). The “Oscarella” type in such case arose from the “Corticium” by migration of the nucleus to the basal position and thereby becoming disconnected from the kinetosome (Pozdnyakov et al., 2017a, 2017b; Sokolova et al., 2017).

CLASS CALCAREA

In the choanocytes of calcarean sponges two types of kinetid corresponding to two subclasses

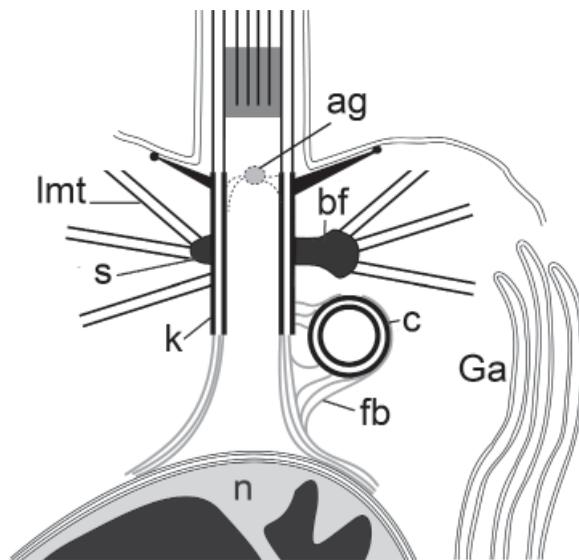


Fig. 3. The general scheme of the “Sycon” type kinetid. Abbreviations as for Fig. 1.

the Calcaronea and Calcinea have been described (Pozdnyakov and Karpov, 2013; Pozdnyakov et al., 2017a, 2017b; Sokolova et al., 2017).

“SYCON” TYPE (FIG. 3; TABLE 1)

Calcaroneans are represented by sponges of the genus *Sycon* in our research and a kinetid structure of one of its species was studied (Pozdnyakov and Karpov, 2013). The kinetosome connects to an apically located nucleus by the fibrillar root. It has a large basal foot and two small satellites producing solitary microtubules. A centriole is present and located at right angles to the kinetosome. A transverse plate is absent and an axial granule is probably present in the lumen of the kinetosome top.

“SOLENEISCUS” TYPE (FIG. 4; TABLE 1)

Another kinetid type was noted in *Soleneiscus* sp. the representative of the subclass Calcinea (Pozdnyakov et al., 2017a, 2017b; Sokolova et al., 2017). Its description is based on the data of Amano and Hori (2001). This kinetid type differs from the “Sycon” type by the lack of a connection between the kinetosome and nucleus as the latter has a basal position in the cell. Thus, the fibrillar root is absent, and unlike in *Sycon*, the Golgi apparatus locates under the kinetosome. Other characters of this kinetid type are not resolved in the authors’ illustration (Fig. 19 in Amano and Hori, 2001)

Table 1. Comparison of kinetid characters in sponge choanocytes and choanoflagellates

Characters Taxa	Kinetid type	Transition zone structures	Centriole presence/location	MTOCs	Microtubular roots	Fibrillar root presence/origin	Kinetosome-nucleus connection	References
Homoscleromorpha	« <i>Corticium</i> »	Axial granule	+/orthogonal	basal foot+ (satellite?)	Microtubular singlets	+/kinetosome	+	Boury-Esnault et al., 1984; Pozdnyakov et al., 2017b; Sokolova et al., 2017
	« <i>Oscarella</i> »	Axial granule	+/orthogonal	basal foot+ (satellite?)	Microtubular singlets	–	–	Pozdnyakov et al., 2017b; Sokolova et al., 2017
	« <i>Sycon</i> »	Axial granule, coil fiber	+/orthogonal	basal foot+ satellite	Microtubular singlets	+/kinetosome	+	Pozdnyakov and Karpov, 2013; Pozdnyakov et al., 2017b; Sokolova et al., 2017
	« <i>Soleniscus</i> »	Axial granule	+/orthogonal	basal foot+ (satellite?)	Microtubular singlets	– (?)	–	Amano and Hori, 2001; Pozdnyakov et al., 2017b; Sokolova et al., 2017
Demospongiae	« <i>Halisarca</i> »	Axial granule, cylinder	+/acute angle	basal foot+ satellite	Microtubular singlets	+/kinetosome	+	Gonobobleva and Maldonado, 2009; Pozdnyakov et al., 2017b; Sokolova et al., 2017
	« <i>Halichondria</i> »	Axial granule	–	basal foot+ satellite	Microtubular singlets	+/kinetosome	+	Pozdnyakov and Karpov, 2016; Pozdnyakov et al., 2017b; Sokolova et al., 2017
	« <i>Ephydatia</i> »	Transverse plate with axosome, coil fiber	–	Dispersed circle around kinetosome	Microtubular singlets	+/kinetosome	–	Sokolova et al., 2017
Porifera	«Ancestral» kinetid	Axial granule	+/orthogonal	basal foot+ satellite	Microtubular singlets	+/kinetosome	+	Pozdnyakov et al., 2017b; Sokolova et al., 2017
Choanoflagellata		Transverse plate with axosome, central filament	+/orthogonal	Dense circle/several foci around kinetosome	Microtubular bands	+/centriole	–	Karpov and Leadbeater, 1998; Leadbeater, 2015; Karpov, 2016

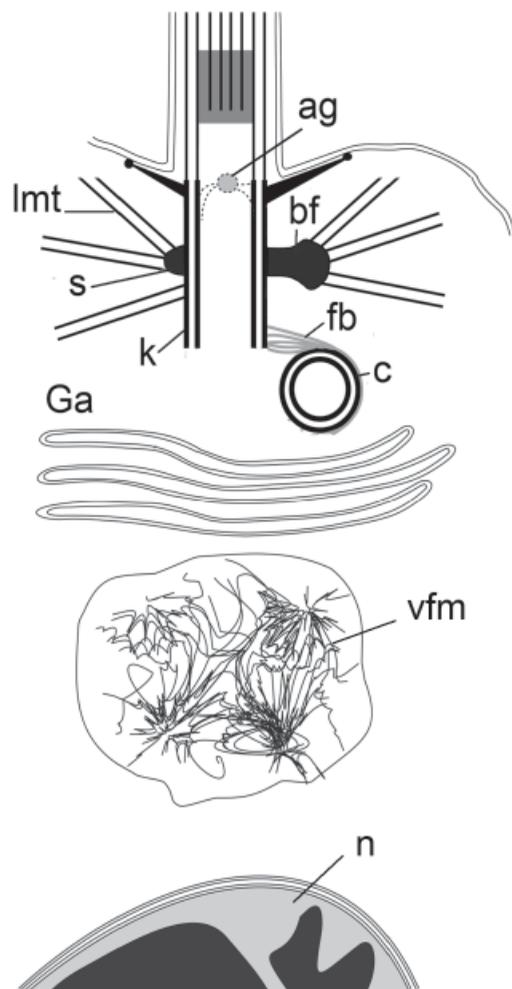


Fig. 4. The general scheme of the “Soleneiscus” type kinetid. *Abbreviations* as for Figs 1-3.

while we can assume by analogy the presence of an axial granule and a basal foot as the MTOCs of kinetosome also.

Choanocytes with “Sycon” and “Soleneiscus” kinetid types differ from each other by the apical or basal position of the nucleus respectively – the features that Minchin (1896) and Bidder (1898) used to establish subclasses Calcinea and Calcaronea at the end of the 19th century which are still accepted in the 21st century by morphological (Manuel et al., 2002) and molecular phylogenetic data (Manuel et al., 2003; Wörheide et al., 2012). As the position of the nucleus obviously defines the type of kinetid we can assume that the “Sycon” kinetid (or at least its main features) is characteristic for all representatives of subclass Calcaronea with an apical nucleus in choanocytes, and the “Soleneiscus” kinetid (or its main elements) characterizing the subclass Calcinea

with a basal nucleus in choanocytes (Pozdnyakov et al., 2017a, 2017b; Sokolova et al., 2017).

In the case of the class Calcarea we do not see the arguments to answer the question: which type of kinetid is closer to the ancestral kinetid of class Calcarea? However, including in the analysis the kinetid types of the sister class Homoscleromorpha we can see that the “Corticium” type almost coincides with the “Sycon” type (Fig. 5). In fact, at the level of the poriferan phylogeny as a whole, it is convenient to describe them as a single type, the “Sycon-Corticium” kinetid. Therefore, the “Sycon-Corticium” type should be the closest to the ancestral choanocyte kinetid of the Calcarea-Homoscleromorpha branch (Fig. 5).

Thus, in each sister branch, the Homoscleromorpha and the Calcarea, kinetid transformation occurred independently due to the nucleus migration to the basal position and the loss of nuclear-kinetosome connection.

CLASS DEMOSPONGIAE

This class of sponges contains the majority of species and is most diverged. Three kinetid types have been found in Demospongiae (Pozdnyakov and Karpov, 2015, 2016; Pozdnyakov et al., 2017a, 2017b; Sokolova et al., 2017).

“HALISARCA” TYPE (FIG. 6; TABLE 1)

Choanocytes with an apical nucleus have a kinetosome connected to the nucleus by a fibrillar root (Pozdnyakov and Karpov, 2016). The MTOCs are represented by a prominent basal foot and a small satellite located on the opposite side of the kinetosome; a centriole locates at acute angle to the kinetosome and is interconnected with the fibrillar bridge. A flagellar transition zone contains an axial granule, a transverse plate is absent.

This type of kinetid was found firstly by Gonobobleva and Maldonado (2009) in *Halisarca dujardini*. An analysis of the literature data suggests the presence of a similar kinetid type in the sponges *Dysidea avara* (Dictyoceratida) (Turon et al., 1997) and *Aplysina aerophoba* (Verongida) (Maldonado, 2009) also (Pozdnyakov and Karpov, 2016; Pozdnyakov et al., 2017a). We detected the same type of kinetid in the sponge *Lamellodysidea* sp. (Dictyoceratida) (Sokolova et al., 2017).

Thus, this type of kinetid is the feature of the different sponges belonging to the closely related subclasses “Keratosa” and “Verongimorpha”,

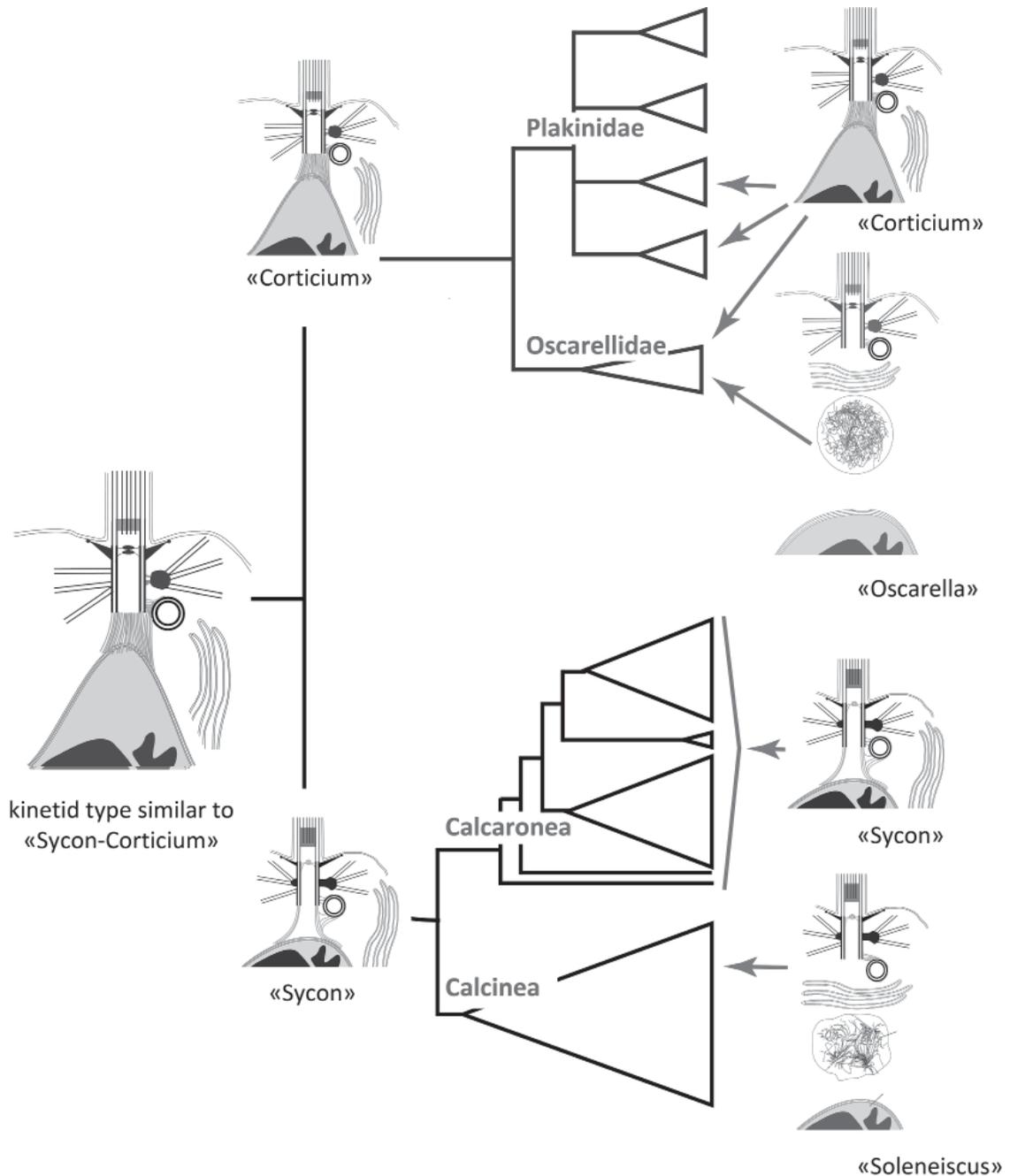


Fig. 5. Distribution of kinetid types on the phylogenetic tree of Calcarea + Homoscleromorpha (after: Worheide et al., 2012, modified) and proposed images of ancestral kinetids.

which form a monophyletic branch - one of two main demospongian branches (Morrow and Cárdenas, 2015) (Fig. 9).

“HALICHONDRIA” TYPE (FIG. 7; TABLE 1)

Representatives of the orders Suberitida (*Halichondria* sp.), Poecilosclerida (*Crellomima imparidens*) (Pozdnyakov and Karpov, 2016), and

possibly some related orders (Pozdnyakov et al., 2017a,b; Sokolova et al., 2017) are characterized by a kinetid similar to the “Halisarca” type, differing from it by the complete absence of a centriole (Pozdnyakov and Karpov, 2016). This kinetid is called the type “Halichondria” because of the first description of a representative of this genus (Pozdnyakov and Karpov, 2016).

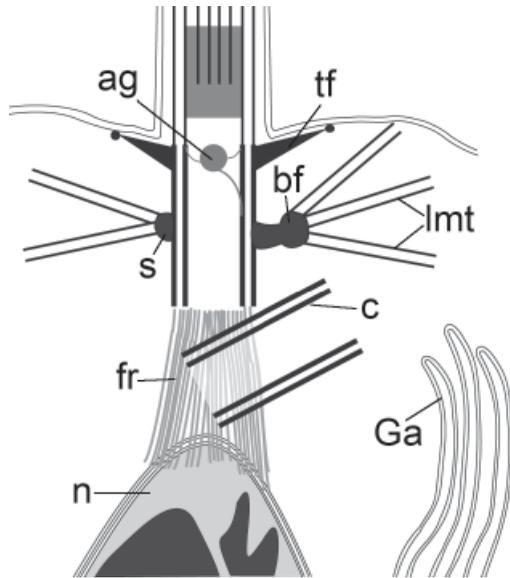


Fig. 6. The general scheme of the “Halisarca” type kinetid. *Abbreviations* as for Figs 1-3.

“EPHYDATIA” TYPE (FIG. 8; TABLE 1)

This type of kinetid is rather distinct. The choanocyte possesses a basal nucleus, thus, a kinetosome-nucleus connection is absent, but the kinetosome has a short fibrillar root ending in cytoplasm. The Golgi apparatus is located between the kinetosome and the nucleus. MTOCs represented by separate satellites of various sizes are distributed on the surface of kinetosome. In some species the satellites form an irregular ring around the kinetosome. The centriole is absent. The transition zone contains a transverse plate with an axosome and a coil fiber (Pozdnyakov et al., 2017a, 2017b; Sokolova et al., 2017).

This kinetid type was found in *Ephydatia fluvialtilis* (Spongillida) and *Haliclona* sp. (Haplosclerida) (Karpov and Efremova, 1994; Pozdnyakov and Karpov, 2015). The same kinetid type is visible in the illustrations of *Haliclona indistincta* (Stephens et al., 2013) and *H. cinerea* (Amano and Hori, 1996) choanocytes (Pozdnyakov et al., 2017a).

The distribution of “Halisarca” and “Halichondria” kinetid types on the phylogenetic tree of Demospongiae (Fig. 9) suggests that the “Halisarca” type is nearer to the ancestral form of Demospongiae, because *Halisarca* belongs to the more basal branch, and a centriole presence is the plesiomorphic character for Metazoa (Pozdnyakov et al., 2017a, 2017b; Sokolova et al., 2017).

The “Ephydatia” type of kinetid located in the crown of the demospongian phylogenetic tree differs

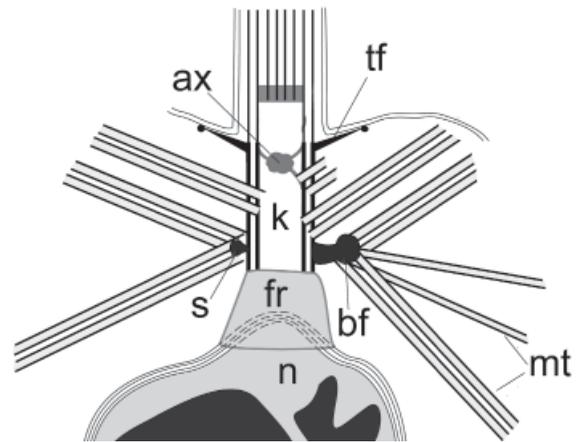


Fig. 7. The general scheme of the “Halichondria” type kinetid. *Abbreviations* as for Figs 1-3.

fundamentally from the “Halisarca” type and is obviously secondarily transformed (Fig. 9).

Among all types of choanocyte kinetid in the class Demospongiae, the “Halisarca” type is similar to the “Sycon-Corticium” type differing only in the angle of the centriole position in relation to the kinetosome. All other characters of these kinetid types (the nucleus-kinetosome connection, the main MTOC in the form of the basal foot, and a transition zone with an axial granule) are practically identical.

This comparison leads to the conclusion that the “Sycon-Corticium” type and the “Halisarca” type originated from a single ancestral kinetid, and the latter arose due to a rotation of the centriole. Thus, we have the mutual confirmation for the “Halisarca”, as well as for the “Sycon-Corticium” type on their similarity to the ancestral kinetids of their classes (Fig. 10).

PLESIOMORPHIC KINETID CHARACTERS IN PORIFERA

The kinetids of Homoscleromorpha + Calcarea and Demospongiae differ from each other only in the centriole orientation (Fig. 10), therefore, we have to decide which position of centriole is more common to be a plesiomorphic character. A review of the kinetid structures in various unicellular organisms of Holozoa: Choanoflagellata (Karpov, 2016) and Ichtyosporea (Pekkarinen et al., 2003) shows the centriole orthogonal to the kinetosome. Two centrioles in the cells of the higher Metazoa are orthogonal to each other also (Westheide and Rieger, 2007). Thus, a common and probably more

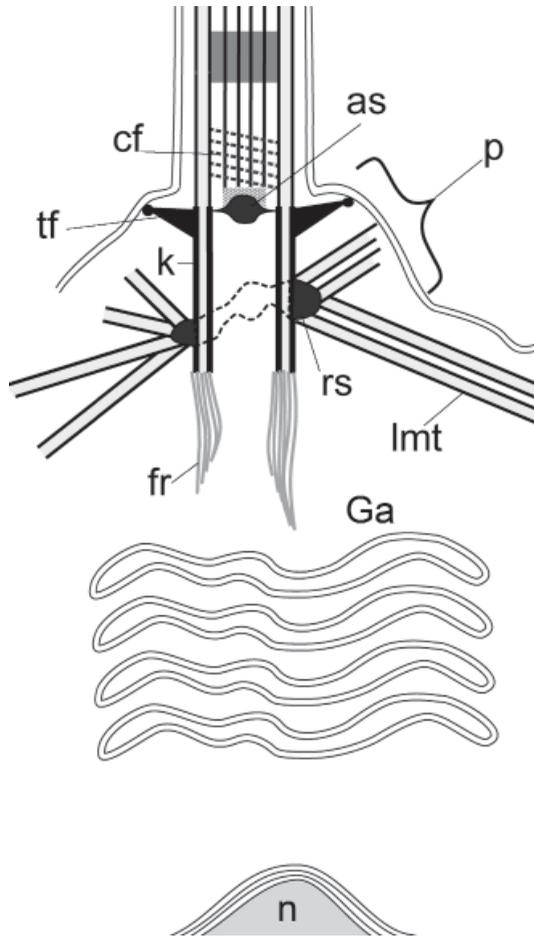


Fig. 8. The general scheme of the “Ephydatia” type kinetid. *Abbreviations:* as - axosome; cf - coil fiber; p - protrusion at the base of flagellum; rs - ring of satellites; other abbreviations as for Fig. 1.

ancient orientation of the centriole is orthogonal as in *Sycon* sp. and *Corticium candelabrum* (Fig. 10).

The choanocyte structure proposed to be nearest to the common ancestor of Porifera coincides with the “*Sycon-Corticium*” type, and its main elements are depicted in the scheme shown in Figs 10, 11A and 12A (Pozdnyakov et al., 2017a, 2017b; Sokolova et al., 2017). A centriole is connected to the kinetosome by a fibrillar bridge, the kinetosome has satellites and a basal foot initiating the lateral microtubular singlets, a kinetosome-nucleus fibrillar connection is present, and an axial granule is also present in the transition zone.

KINETID OF SPONGE CHOANOCYTE VS. KINETID OF CHOANOFLAGELLATES (FIGS 11, 12; TABLE 1)

Having an image of a kinetid with plesiomorphic and also most conservative characters for Porifera

we can compare it with the choanoflagellate kinetid and check: 1) the long-standing idea that sponge choanocytes are nearly identical to the cells of choanoflagellates, and 2) the suggestion of a direct origin of Metazoa from a choanoflagellate-like ancestor.

Similar characters

- developed transition fibers
- centriole orthogonal and connected to kinetosome by a fibrillar bridge

Different characters

A) choanoflagellates have, choanocytes have not:

- central filament in transition zone (unique structure for eukaryotes)
- transverse plate is always present
- radial microtubular roots organized in bands
- electron dense ring or foci as MTOCs
- centriole produces fibrillar root to Golgi apparatus

B) choanocytes have, choanoflagellates have not:

- axial granule instead of transverse plate
- kinetosome with fibrillar root to the nucleus
- typical metazoan MTOCs (foot and satellites)
- dark region in flagellar transition zone

Such common characters of the choanoflagellates and choanocyte kinetids as strong transition fibers and a full-length centriole orthogonal to the kinetosome are plesiomorphic for opisthokonts (Barr, 1981; Pekkarinen et al., 2003; Westheide and Rieger, 2008), and are not unique morphological features of the choanocytes and choanoflagellates (Karpov, 2016). The structure of the kinetid as a single complex does not give any basis for the assertion that the choanoflagellate was an ancestor of Metazoa and the choanocytes retained the structure of such ancestral cell. The results of this comparison do not allow us to decide which of the descendants underwent a bigger evolutionary transformation on the way from the common ancestor to the Choanoflagellata and the Metazoa.

However, the accumulated evidence provides a basis for some assumptions.

First, there are sufficiently convincing arguments that the connection of the nucleus with the flagellar apparatus is the original state for eukaryotes, as it occurs in many distantly related flagellates and is associated with the proposed origin of the eukaryotic flagellum from centrioles of the centrosome (Yubuki

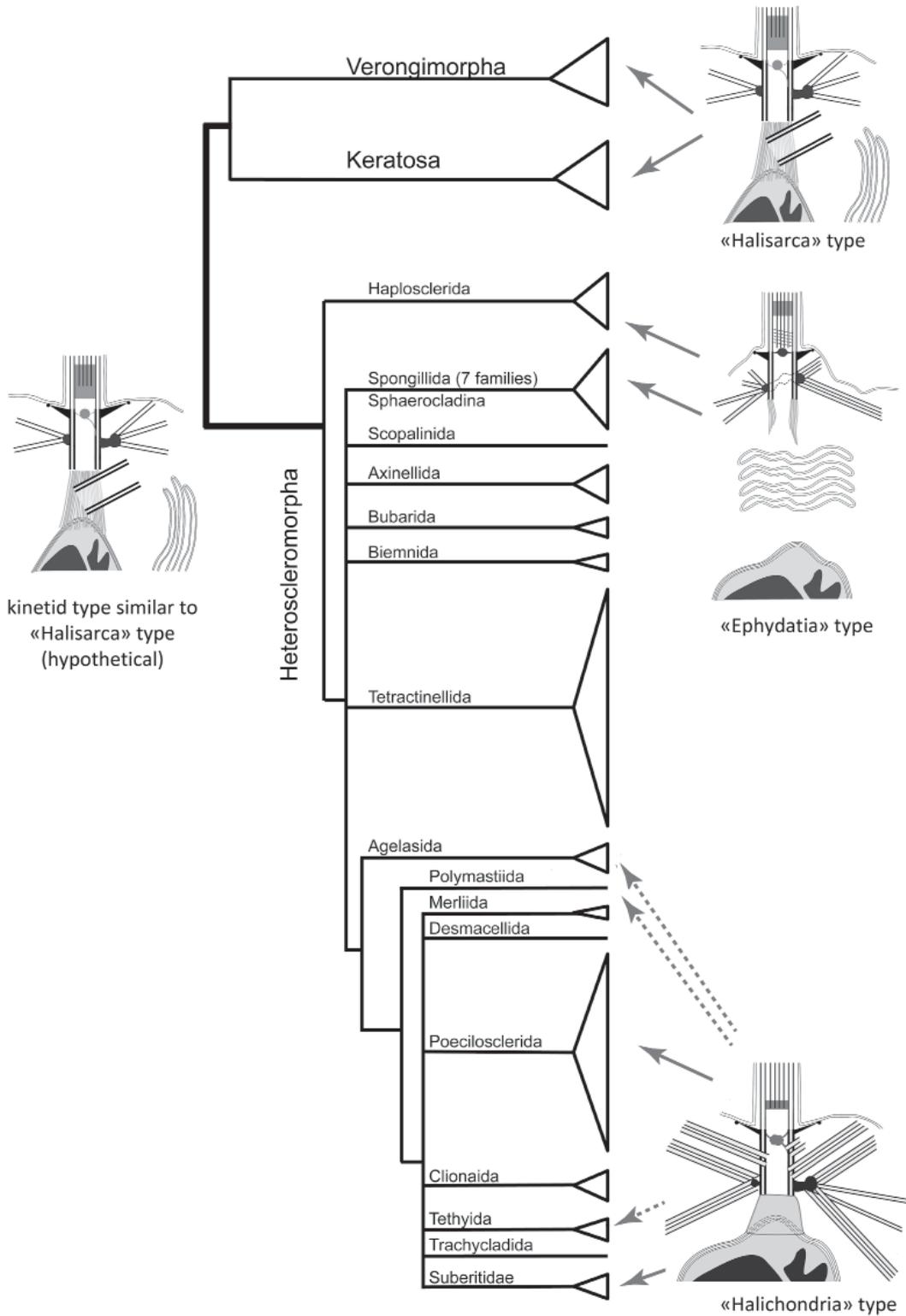


Fig. 9. Distribution of kinetid types on the phylogenetic tree of Demospongiae (after: Morrow and Cardenas, 2015, modified). Dashed arrows show taxa which presumably have this kinetid type.

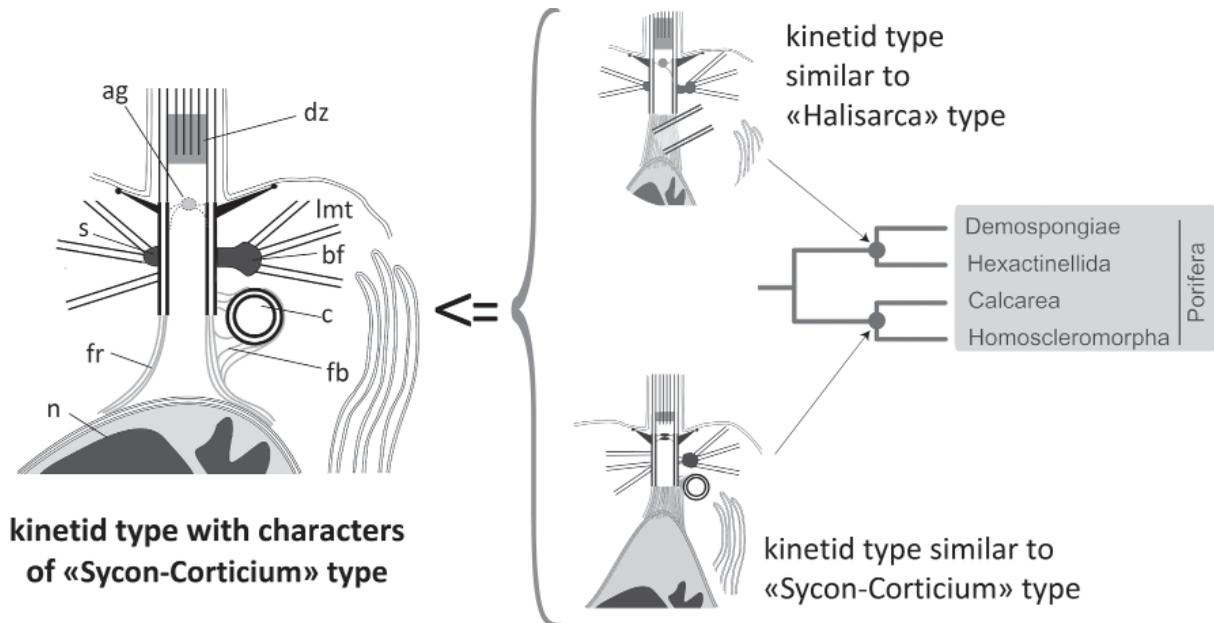


Fig. 10. Comparison of proposed ancestral kinetids of main poriferan branches and reconstruction of kinetid with plesiomorphic characters (phylogenetic tree after: Worheide et al., 2012; modified). Abbreviations as for previous figures.

and Leander, 2013). From this point of view the choanocyte bears more primitive features than the choanoflagellate. An indirect confirmation of this opinion is the evolutionary tendency to break the nucleus-kinetosome connection in case of sponges (Pozdnyakov et al., 2017a, 2017b; Sokolova et al., 2017). Perhaps the same trend was also realized in the evolution towards the modern choanoflagellates.

Second, it seems that the choanocyte kinetid contains more features that can be considered primitive for the opisthokonts than the kinetid of the choanoflagellate cell. Therefore, the hypothesis about the origin of sponges, and consequently of all Metazoa from choanoflagellate-like unicellular organism directly is not confirmed at the level of ultrastructure. Both Choanoflagellata and Metazoa have apparently evolved from an ancestor whose cellular structure was different from the structures of its descendants, and, perhaps, the choanoflagellate cell is more transformed than the choanocyte, in comparison with the cell of the common ancestor.

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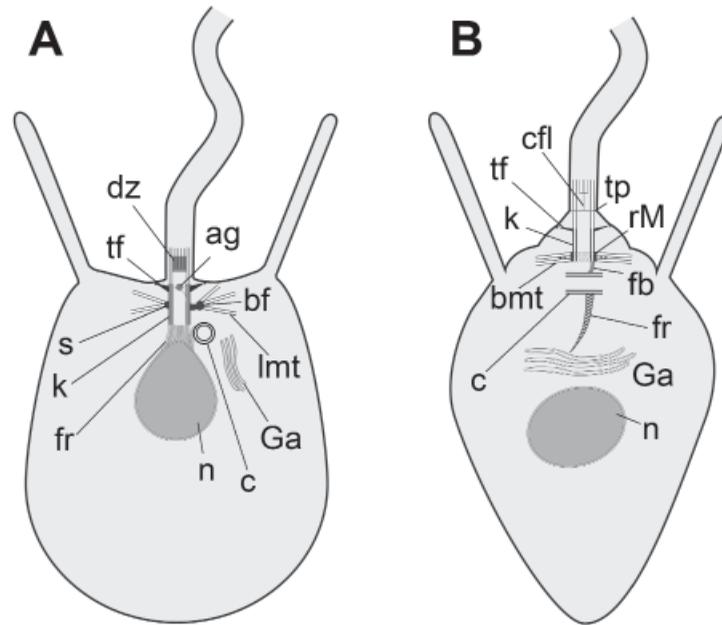


Fig. 12. Cell organization of choanocyte (A) and choanoflagellate (B). Details of flagellar apparatus are magnified relatively to cell dimensions. *Abbreviations* as for previous figures.

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