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1 **Ultrastructural research of spermiogenesis in two sponges, *Crellomima***
2 ***imparidens* and *Hymedesmia irregularis* (Demospongiae): new evidence of sperms with**
3 **acrosome in sponges**

4
5 **Research Highlights:**

- 6 1. The ultrastructure of sperm were studied in two marine demosponges, *Crellomima*
7 *imparidens* and *Hymedesmia irregularis* (order Poecilosclerida)
8 2. The bundles of microtubules arranged along the nucleus during spermiogenesis found in
9 both species.
10 3. Sperm nucleus have elongated and helical shape and twisted chromatin.
11 4. The spermatozoon of both species has an acrosome at apical part.
12

13
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22

23 **Abstract**

24 Details of spermatogenesis and sperm organization are often useful for reconstructing
25 the phylogeny of closely related taxa of invertebrates. Here, the spermiogenesis and the
26 ultrastructure of sperm were studied in two marine demosponges, *Crellomima imparidens*
27 and *Hymedesmia irregularis* (order Poecilosclerida). In *C. imparidens* and *H. irregularis*
28 we found bundles of microtubules arranged along the nucleus during spermiogenesis.
29 These bundles derived from the basal body of axoneme, reaching the apical pole of the
30 cell. In *C. imparidens* the microtubules surround the nucleus, forming the manchette. In *H.*
31 *irregularis* the microtubules pass along only one side of the cell periphery. During
32 spermiogenesis, the nucleus stretches and elongates. In both species the nucleus is twisted
33 into a spiral structure. We suppose that the manchette of microtubules could be responsible
34 for controlling the elongation and shaping of the sperm nucleus to a helical form and for
35 the twisting and/or condensation of chromatin in these sponges. The spermatozoon of both
36 species has an elongated shape. Its apical part has an acrosome, which is dome-shaped in
37 *C. imparidens* and flattened and lenticular in *H. irregularis*. The cytoplasm of the
38 spermatozoa contains some small mitochondria, and proximal and distal centrioles

1 arranged at an angle to each other. There is a small volume of residual cytoplasm with dark
2 glycogen-like granules. The axoneme of the spermatid and the flagellum of the sperm of
3 both sponges is located in the deep tunnel-like cytoplasmic depression. The comparison of
4 spermatozoa morphology of different species of the order Poecilosclerida demonstrates
5 that the knowledge of variation within genera and families can give valuable insights into
6 the significance of many characters proposed for phylogenetic studies of this order.

7
8 **Keywords:** spermiogenesis, sponges, acrosome, ultrastructure

9 10 **1. Introduction**

11
12 Electron microscopic observations of gametogenesis in sponges (Porifera) have
13 demonstrated a high morphological diversity of spermatozoa in these animals. Moreover,
14 all three morphological groups of metazoan sperm cells can be found in sponges from
15 different taxa: “primitive spermatozoa,” “derived or modified spermatozoa,” and “aberrant
16 spermatozoa” according to Reunov (2005). Accordingly, there are sponge species
17 possessing sperm with an acrosome, which was earlier considered as a trait of more highly
18 organized groups of animals (Afzelius, 1978; Berruti et al., 1978; Baccetti, 1984). The
19 presence of the acrosome in spermatozoa has been shown in the class Homoscleromorpha
20 (Gaino et al., 1986; Baccetti et al., 1986; Boury-Esnaul & Jamieson, 1999; Ereskovsky,
21 2010; Riesgo et al., 2007a), in one species of the class Calcarea (Nakamura et al., 1998)
22 and, finally, in some Demospongiae from the order Poecilosclerida: *Crambe crambe*
23 (Tripepi et al., 1984; Riesgo & Maldonado, 2009), *Lycopodina occidentalis* and *L.*
24 *hypogea* (Riesgo et al., 2007b; Ereskovsky et al. In prep.).

25 Ultrastructural studies of spermiogenesis and sperm morphology in different
26 invertebrates have provided many characters used as apomorphies as well as
27 morphological evidence used in taxonomy and phylogeny (for review see Jamieson et al.,
28 1995). Differences in sperm morphology and spermiogenesis have been found at high
29 taxonomic levels within various animal groups such as Turbellaria (Watson & Rhode,
30 1995), Cestoda (Ba & Marchand, 1995), Gastrotricha (Ferraguti & Balsamo, 1995),
31 Polychaeta (Jamieson & Rouse, 1989), Cnidaris (Hinsch, 1974), and Polyplacophora
32 (Buckland-Nicks, 2006).

33 However, sperm morphology has never been used in taxonomy and phylogeny of
34 sponges, mostly because spermatogenesis has been studied at the ultrastructural level only

1 in a few species from different sponge taxa. Most studies of spermatogenesis in sponges
2 have been made on representatives of their largest class, Demospongiae. Spermatogenesis
3 has been examined using TEM in 27 species from 10 orders of this class (Table 1). The
4 order Poecilosclerida has been studied best, with information on the ultrastructure of
5 spermatids or spermatozoa being available for 9 species (Table 1) (Efremova et al., 1987;
6 De Vos et al., 1990; Riesgo et al., 2007b; Riesgo & Maldonado, 2009; Pérez-Porro et al.,
7 2012; Vasconcellos et al., 2019; Ereskovsky et al., In prep).

8 The aim of this work was to perform an electron microscopic study of the late stages
9 of spermatogenesis—spermiogenesis and sperm formation—in two sponge species from
10 different families of the order Poecilosclerida: *Crellomima imparidens* and *Hymedesmia*
11 *irregularis*.

12 13 **2. Materials and methods**

14
15 Sponges *Crellomima imparidens* Rezvoj, 1925 (family Crellidae) and *Hymedesmia*
16 *irregularis* Lundbeck, 1910 (family Hymedesmiidae) (Fig. 1 a,b) were collected by
17 SCUBA diving from June to August 2006 and from August to September 2009 in the
18 Chupa Inlet located in the innermost part of the Kandalaksha Bay, the White Sea, at depths
19 of 7-12 m. Pieces of each specimen were fixed immediately after collection. For electron
20 microscopy the sponges were cut into cubes of about 1mm. These cubes were prefixed in
21 1% OsO₄ (Spi Supplies, West Chester) for 10 min and fixed in 2.5 % glutaraldehyde (Ted
22 Pella, Redding) in phosphate buffer at room temperature for 1 h. After fixation, sponge
23 samples were washed in the phosphate buffer and postfixed in 1% OsO₄ in phosphate
24 buffer for 1 h. After postfixation, the samples were kept in 5% HF solution for 3 hr at 4°C
25 in order to dissolve the spicules. Samples were dehydrated through a graded ethanol series
26 at RT and stored in 70% ethanol at 4°C.

27 For semithin sections and transmission electron microscopy, the specimens were
28 embedded in Araldite (Sigma-Aldrich) according to the manufacturer's instructions.
29 Semithin sections (1 µm) were cut on an Ultramicrotome PowerTome XL (RMC Boeckle-
30 ler, Tucson), and then stained with 1% toluidine blue–0.2% methylene blue mixture. They
31 were studied under a Leica DM5000B (Leica) and a Wild M20 microscope (Wild). Digital
32 photos were taken with a Leica DMLB microscope (Leica) using the Evolution LC color
33 photo capture system (Media Cybernetics, Rockville).

1 Ultrathin sections (60–80 nm) were cut with an Ultramicrotome PowerTome XL,
2 equipped with a Drukkert 45° diamond knife and contrasted with 4% aqueous uranyl
3 acetate and 0.4% lead citrate according to Reynolds (1963). Ultrathin sections were studied
4 under Jeol JEM-100B (Jeol, Akishima, Japan) transmission electron microscope.

5 6 **3. Results**

7 8 **3.1. Sperm cysts**

9 Spermatogenesis in both *Crellomima imparidens* and *Hymedesmia irregularis* occurs
10 in sperm cysts, temporary oval structures with a diameter of about 38-42 μm in *C.*
11 *imparidens* and 26-40 x 17-22 μm in *H. irregularis* (Fig. 2 a-d). Spermatogenesis within a
12 cyst is relatively synchronous. However, in different cysts of the same sponge
13 spermatocytes can be at different stages of spermatogenesis. The cyst wall is single-
14 layered, formed with flat follicular cells with long inwardly directed pseudopodia (Fig. 2
15 c). The cyst cells have a large spherical anucleolate nucleus (about 3.8 μm in diameter in
16 *C. imparidens* and 4 μm in *H. irregularis*) with islands of heterochromatin. The cytoplasm
17 contains numerous spherical heterophagosomes (from 0.5 to 2.5 μm) and electron
18 transparent vacuoles of different diameter. The cyst cells contact by simply overlying each
19 other.

20 21 **3.2. Spermiogenesis in *Crellomima imparidens***

22 Early spermatids are oval cells with a diameter about 2.9 μm in cross section (Figs. 2a,
23 3a). Cells in groups of four are often interconnected by cytoplasmic bridges (Fig. 3b).
24 Spermatids have a flagellum. The nuclei of early spermatids are oval with a diameter of
25 about 1.8 μm . In the cytoplasm there are rounded mitochondria (diameter about 1.8 μm)
26 and the Golgi apparatus, represented by flat cisterns located near the nucleus. These
27 cisterns remain in the residual cytoplasm in the course of sperm maturation. During
28 maturation chromatin in the nuclei of spermatids condenses. This process begins at the
29 periphery, while the central part of the nucleus remains lighter. The nuclei gradually
30 acquire an elongated cylindrical shape. The spermatids themselves also elongate, reaching
31 up to 6.3 μm in length. The residual cytoplasm accumulates in the basal part of spermatids,
32 which is discharged at the end of spermiogenesis.

1 As the sperm stretches, the nucleus is twisted into a spiral structure (Figs. 3 c,d; Fig. 4
2 a ; 5– scheme long.). The sperm nucleus is electron-dense, homogeneous, 4.5 μm long. It is
3 narrower in the proximal part than in the distal part (diameter, respectively, 0.5 and 0.23
4 μm). In the central part of the distal pole, the nucleus has a small funnel-shaped depression
5 up to 0.06 - 0.11 μm deep (Figs. 3 e, m-o; Fig. 4 a). Microtubules are uniformly arranged
6 around the nucleus, in parallel with it (Figs. 3e, f, g; 4 a, c-e; 5-scheme section). They
7 begin from the basal body of the axoneme (Fig. 3 e) and reach the apical part of the
8 nucleus.

9 There is one flagellum in the basal part of the sperm. It has a typical eukaryotic
10 axoneme (9 +2), which is located in the cytoplasmic depression with a depth of 1.1 μm
11 (Fig. 3 m). The basal body lies at the base of the axoneme (Figs. 3 n, o; 4a). An accessory
12 centriole is located at an angle to the basal body (Fig. 3 n). A short rootlet arises from the
13 terminal part of the basal body (Fig. 3 n) and extends to contact the basal part of the
14 nucleus.

15 In general, the sperm cytoplasm is fairly homogeneous. There are small mitochondria
16 and lipid droplets in its basal part and several cisterns derived from the Golgi apparatus
17 around the axoneme.

18 The acrosome forms in early spermatids. It is located in the immediate vicinity of the
19 Golgi apparatus. In the early stages, it is rounded, with a diameter of about 1.2 μm . In
20 more mature spermatids, the acrosome occupies an apical position, adjacent to the distal
21 part of the nucleus. It is dome-shaped, homogenous, with a diameter of 0.36 μm and a
22 height of 0.31 μm (Figs. 3 h-l, 4 a, b). However, it has two electron-transparent regions: a
23 lenticular region in the apical part and an annular groove in the middle part (Fig. 3 h-j).
24 Moreover, its basal part, which is adjacent to the nucleus, is filled with a darker electron-
25 dense material, while the opposite part, which faces the cytoplasmic membrane, is more
26 electron-transparent (Fig. 3 h-j). The acrosome has its own membrane.

27 Sperm are released from the cyst due to the disruption of cellular contacts of the sperm
28 cyst.

30 **3.3. Spermiogenesis in *Hymedesmia irregularis***

31 Early spermatids of *Hymedesmia irregularis* are oval monoflagellated cells, with an
32 elongated nucleus, the chromatin of which begins to condense (Figs. 2b; 6 a). Their
33 cytoplasm contains numerous small mitochondria, small electron-transparent vacuoles,
34 lipid droplets and several elongated, sinuous cisterns derived from the Golgi apparatus in

1 the distal part (Fig. 6 a). In the course of further development, the spermatid nucleus
2 lengthens and acquires a spiral shape. The main volume of the cytoplasm shifts distally and
3 eventually becomes the residual cytoplasm, which is discharged into the cyst.

4 At a more advanced stage the spermatids are elongated cells (about 4.5 μm long) with
5 an elongated spiral nucleus, 3.8 μm long and 0.55 μm in diameter (Fig. 6 b, c). Nuclear
6 chromatin is considerably condensed. In the proximal part, the nucleus has a small
7 depression, 0.08 μm deep (Fig. 6 d, e), where the acrosome is situated. The acrosome is
8 formed as a flattened lenticular structure above the proximal part of the condensed nucleus
9 of the spermatid (Fig. 6 d, e). At an early stage, it is surrounded by a bundle of
10 microtubules (Fig. 6 e). The acrosome has its own membrane (Fig. 6 d).

11 A bundle of microtubules stretches along the long axis of the cell, reaches its apical
12 part and passes along one side of the cell periphery (Fig. 6 f-h). The bundle starts from the
13 kinetid of the axoneme (Fig. 6 i). The flagellum has a typical eukaryotic axoneme (9 + 2)
14 immersed in a small (depth about 0.6 μm) invagination of the cytoplasm (Figs. 2 d; 6 f, i).
15 The basal part of the axoneme is associated with a basal body, which is situated next to the
16 accessory centriole (Fig. 6 i).

17 The main volume of the cytoplasm is concentrated in the distal third of the cell. It
18 contains numerous small mitochondria (Fig. 6 c, g, h). Several cisterns derived from the
19 Golgi apparatus are located near the nucleus and surround the axoneme. A scheme of
20 sperm structure in *H. irregularis* is shown in Fig. 7.

21 22 **4. Discussion**

23 In this work, we examined the structure of mature sperm and the process of
24 spermiogenesis in two poecilosclerid demosponges. The information obtained in our study
25 can be used to clarify several important points concerning the reproductive biology of
26 sponges. The development of the spermatozoa of *Crellomima imparidens* and *Hymedesmia*
27 *irregularis* is interesting in several respects.

28 29 **4.1. Spermiogenesis**

30 The last stages of spermiogenesis occur in the two poecilosclerids species studied here
31 according to a scheme, which is common for all animals with elongated spermatozoa:
32 chromatin condenses, the nucleus elongates, the acrosome forms in the apical part of the
33 sperm, the main volume of the cytoplasm (residual cytoplasm) shifts basally and is later
34 discharged, mitochondria concentrate in the basal part of the sperm. Finally, a

1 spermatozoid is formed.

2

3 **4.2. Nucleus elongation**

4 In *Crellomima imparidens* and *Hymedesmia irregularis* we found the bundles of
5 microtubules passing along the nucleus during spermiogenesis. These microtubules start
6 from the basal body of the axoneme and stretch along the entire nucleus, reaching the
7 apical pole of the cell, where the acrosome is formed. Perinuclear microtubules starting
8 from basal body have also been described, e.g., in spermatids of *Cryptochiton stelleri*
9 (Mollusca), where they extend anteriorly and encircle the nucleus forming a manchette
10 (Buckland-Nicks et al., 1990). Two other poecilosclerid sponges that have sperm with an
11 elongated nucleus, *Lycopodina occidentalis* and *Crambe crambe*, also have microtubules
12 arranged along the nucleus (Riesgo et al., 2007a; Riesgo & Maldonado, 2009). However,
13 no microtubules have been found in the spermatids of *Lycopodina hypogea*, which has a
14 long helical nucleus (Ereskovsky et al., in prep.).

15 In *C. imparidens* the microtubules surround the nucleus and are arranged along its
16 longer axis, similarly to the manchette microtubules in the sperms of some metazoans
17 (Kondo et al., 1988; Buckland-Nicks et al., 1990; Russell et al., 1991; Maretia, 1995). Such
18 manchette-like structures have never been reported in Porifera before. In *H. irregularis*,
19 unlike *C. imparidens*, the microtubules pass along only one side of the cell periphery. A
20 single bundle of microtubules was described in the sperm of passerine birds during
21 spermiogenesis (Kondo et al., 1988).

22 The manchette of microtubules is thought to be responsible for controlling the
23 elongation and shaping of the spherical sperm nucleus to a highly helical form and also for
24 the twisting and/or condensation of chromatin (Russell-Pinto et al., 1983; Lehti & Sironen
25 2016). This mechanism was described in different animals, for example, in some
26 gastropods (REF: Buckland-Nicks et al., 1983; Maxwell, 1983; Buckland-Nicks et al.,
27 1990), in oligochaetes (Webster & Richards, 1977; Lehti & Sironen, 2019), in passerine
28 birds (Kondo et al., 1988), and in mammals (Russell et al., 1991). Stretched and helical
29 spermatozoa are well known in different invertebrates such as Tubificidae (Oligochaeta)
30 (Erseus & Ferraguti, 1995), Gastrotricha (Ferraguti & Balsamo, 1995), Chelicerata
31 (Alberti, 1995), cephalopod molluscs (Giménez-Bonafé et al., 2002), etc. In sponges this
32 mechanism has been described in *Lycopodina occidentalis* and *Crambe crambe* (Riesgo et
33 al., 2007a; Riesgo & Maldonado, 2009).

34

4.3. Acrosome

Another remarkable feature of spermiogenesis of *Crellomima imparidens* and *Hymedesmia irregularis* is that both species have an acrosome. Sperm with an acrosome has long been thought to be the prerogative of eumetazoans. In Porifera this structure was described for the first time in homoscleromorph *Oscarella* in 1986 (Baccetti et al., 1986; Gaino et al., 1986). Later it was detected in homoscleromorphs *Pseudocorticium jarrei* and *Corticium candelabrum* (Boury-Esnault & Jamiesson, 1999; Riesgo et al., 2007b), a calcareous sponge *Sycon calcaravis* (Nakamura et al., 1998), and in poecilosclerid demosponges *Crambe crambe* and *Lycopodina hypogea* (Riesgo & Maldonado, 2009; Ereskovsky et al., in prep). The position of the acrosome in *L. hypogea* is typical of all spermatozoa with a dense elongated nucleus and the flagellum on the opposite side of acrosome. However, in *C. crambe*, which has V-shaped spermatozoa, the acrosome is situated close to the basal body at the functionally anterior cell pole.

Moreover, proacrosomal vesicles originating from the Golgi apparatus were described at the apical pole of cells forming during spermiogenesis in many demosponges (Table 1). At later stages of spermiogenesis, the Golgi cisterns disappear but in *Aplysilla rosea*, *Suberiles massa*, *Spongia officinalis*, *Lycopodina occidentalis*, *Petrosia ficiformis*, *Tedania ignis*, and *Geodia phlegraei* dense proacrosomal vesicles of Golgi origin persist near the nucleus of sperms (Tuzet et al., 1970a; Diaz & Connes, 1980; Gaino et al., 1984; Riesgo et al., 2007a; Maldonado & Riesgo 2009; Vasconcellos et al., 2019; Koutsouveli et al., 2020). Proacrosomal vesicles are known in the spermatozoa of many cnidarians (Hinsch, 1974; Franzen, 1996; Larkman & Carter, 1980). They are thought to be a rudimentary acrosome. Nevertheless, proacrosomal vesicles are often involved in fertilization of sea anemones, in which they form aggregations between the nucleus and the plasma membrane in the apical part of the spermatozoon and can be mistaken for the acrosome (Hinsch & Clark, 1973; Dewel & Clark, 1972).

Microtubular transportation of cell organelles and proacrosomal vesicles is well-known in various animals (Hayden et al., 1983; Kierszenbaum & Tres, 2004; Dunleavy et al., 2019). The assembly of the spermatid acrosome depends on the microtubule organization during spermiogenesis (Moreno et al., 2006). We could not trace the formation of acrosomes from the Golgi apparatus in *Crellomima imparidens* and *Hymedesmia irregularis* in detail, and the mechanism of transportation of proacrosomal vesicles remains unresolved. The microtubules in spermatids in the two sponges in our study reach the apical part of the cell where the acrosome is formed. We hypothesize that

1 the proacrosomal vesicles may be transported along the microtubules, which project all the
2 way to the tip of the sperm.

3 Thus, we suppose that the acrosome may have appeared more than once in the
4 evolution of the Porifera. Its origin is associated with the features of insemination and
5 fertilization and with the structure of the vitelline envelope and its penetrability for sperm
6 rather than with the taxonomic affiliation or phylogenetic position of the species.

7 Acrosomes of sponges are variable in shape. Within Homoscleromorpha, they are
8 cup-like in *Oscarella* (Baccetti et al., 1986; Gaino et al., 1986), lens-like in
9 *Pseudocortidium jarrei* (Boury-Esnault & Jamiesson, 1999), and C-shaped in *Cortidium*
10 *candelabrum* (Riesgo et al., 2007b). In the calcarean sponge *Sycon calcaravis* the
11 acrosome is conical, with an electron-dense axial rod in the center (Nakamura et al., 1998).
12 In poecilisclerid demosponges, this structure is also variable. In *Crambe crambe* the
13 acrosome is electron-dense and conical, with an electron-dense acrosomal rod located
14 between it and the nucleus (Riesgo & Maldonado, 2009); in *Lycopodina hypogea* the
15 acrosome is opaque and cone-shaped (Ereskovsky et al., in prep); in *Crellomima*
16 *imparidens* the acrosome is dome-shaped (this work), and in *Hymedesmia irregularis* the
17 acrosome is formed as a flattened lenticular structure (this work).

18 19 **4.4. Flagellum and basal apparatus**

20 As in all investigated demosponges, the flagellum in *Crellomima imparidens* and
21 *Hymedesmia irregularis* is already present in the spermatocyte. The axoneme of the
22 spermatid and the sperm flagellum of both investigated sponges have a typical eukaryotic
23 organization (9 + 2) and are located in a deep tunnel-like cytoplasmic depression (1.1 and
24 0.6 μm depth, correspondingly). It should be noted that in sponges there is a correlation
25 between the shape of sperm and the depth of the cytoplasmic depression (Table 1). In
26 elongated spermatozoa, the tunnel is usually quite deep (e.g., Riesgo et al., 2007a; Riesgo
27 & Maldonado, 2009; Vasconcellos et al., 2019; Ereskovsky et al., in prep.), while in
28 rounded spermatozoa it is either absent or very shallow (e.g. Diaz & Connes, 1980; Tuzet
29 et al., 1970a; Baccetti et al., 1986; Gaino et al., 1984). However, this tunnel, or
30 cytoplasmic depression, of the axoneme is absent in *Halichondria panicea*, which has long
31 (up to 6 μm) spermatozoa (Barthel & Detmer, 1990). A deep cytoplasmic depression of the
32 axoneme has been described in elongated sperms of other invertebrates such as the sea pen
33 *Pennatula aculeata* (Eckelbarger et al., 1998) and the polychaetes from the genera
34 *Capitella* and *Capitomastus* (Jamieson & Rose, 1989).

1 As it is typical of invertebrate sperm, the axoneme of the flagellum in both
2 investigated species arises from the basal body. Near this basal body an additional centriole
3 is situated at the angle in both species. It is interesting that an additional centriole was
4 detected only in two other poecilosclerid species, *Iophon proximum* and *I. piceum*
5 (Vasconcellos et al., 2019), out of the ten investigated in this respect. However, it was
6 described in spermatozoa of some sponges from other orders: Axinellida, Haplosclerida,
7 Spongillida, Suberitida, Dicytyoceratida, and Dendroceratida (Table 1) (Tuzet et al., 1970a,
8 b; Vacelet, 1979; Diaz & Connes, 1980; Efremova & Papkovskaya, 1980; Paulus, 1989;
9 Riesgo et al., 2008; Maldonado & Riesgo, 2009).

10 We found a short rootlet starting from a terminal part of the basal body and extending
11 to contact the nucleus in *C. imparidens*. No such structure was detected in *H. irregularis*.
12 In other sponges sperm axoneme rootlet has been described only in poecilosclerids *C.*
13 *crambe* and *I. piceum* (Riesgo & Maldonado, 2009; Vasconcellos et al., 2019).

15 **4.5. Phylogeny and Taxonomy**

16 According to the comparative morphological concept, there are three morphological
17 types of metazoan spermatozoa: “primitive spermatozoon”, “derived or modified
18 spermatozoon”, and “aberrant spermatozoon” (Franzen, 1956; Reunov, 2005). As pointed
19 out by Riesgo and Maldonado (2009), the spermatozoa of demosponges can be classified
20 as belonging to two structural types, “primitive” or “derived or modified”, depending on
21 the taxa. At the same time, it is sometimes difficult to attribute sperm of the studied species
22 of Demospongiae to one or another category from this classification. The same sperm often
23 has both “primitive” and “derived or modified” traits.

24 The sperms of *C. imparidens* and *H. irregularis* have such “derived” features as an
25 elongated shape with an elongated helical nucleus, an apical acrosome and manchette-like
26 microtubules. At the same time, they also possess “primitive” traits such as numerous
27 small mitochondria and a large volume of the cytoplasm.

28 Nevertheless, spermatozoa of poecilosclerids *L. occidentalis*, *L. hypogea*, *C. crambe*,
29 *C. imparidens* and *H. irregularis* are “modified or derived” rather than “primitive”, while
30 spermatozoa of some other species from this order (*Myxilla incrustans*, *Iophon piceum*, *I.*
31 *proximum*, *Hemimycale columella*, *Tedania ignis*), as well as those of other demosponges,
32 should be classified as “primitive” (sensu Reunov, 2005). **SCHEMES of Sperms?**

33 The organization of spermatozoa is considered as a sound taxonomic and phylogenetic
34 character (see Afzelius, 1979; Franzén, 1996; Jamiesson et al., 1995; Boury-Esnault &

1 Jamiesson, 1999). However, the presence of the acrosome in spermatozoa of
2 Poecilosclerida is at variance with this idea. For instance, *Lycopodina hypogea* (fam.
3 Cladorhizidae), *Crambe crambe* (fam. Crambeidae), *Crellomime imparidens* (fam.
4 Crellidae) and *Hymedesmia irregularis* (fam. Hymedesmiidae) have an acrosome;
5 *Lycopodina occidentalis* (fam. Cladorhizidae) and *Tedania ignis* (fam. Tedaniidae) have
6 proacrosomal vesicles, but other investigated sponges from the same order, *Myxilla*
7 *incrustans* (fam. Myxillidae), *Iophon piceum*, *I. proximum* (fam. Acarnidae), and
8 *Hemimycale columella* (fam. Hymedesmiidae), have none (Table 1). The acrosome is
9 likely to have originated several times in the course of the evolution. In order to verify this
10 hypothesis, thorough studies of the ultrastructural features of insemination in sponges are
11 necessary.

12

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23

24 **Author contributions**

25 AE collected and fixed the animals, interpret the results, write the manuscript, prepared all
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27 photographed the sections.

28

29 **Data availability statement**

30 The data that support the findings of this study are available from the corresponding author
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32

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34

1 **Figure legends**

2

3 **Figure 1.** *In situ* photographs of the investigated poecilosclerids. (a) *Crellomima*
4 *imparidens* ; (b) *Hymedesmia irregularis*. ca – aquiferous system canals; o – oscula.

5

6 **Figure 2.** TEM micrographs of spermatid cysts of investigated species. (a) The cyst with
7 early spermatids in *Crellomima imparidens*. (b) The cyst in *Hymedesmia irregularis*. (c)
8 The cyst with later spermatids and spermatozooids in *C. imparidens*. (d) The cyst with later
9 spermatids in *H. irregularis*. co – cytoplasm outgrowths of follicular cells; fc - follicular
10 cell; n – nucleus; ph – phagosomes; st - spermatids. Scale bars: a-c – 5µm; d – 2 µm.

11

12 **Figure 3.** TEM images of spermiogenesis in *Crellomima imparidens*. (a) Early spermatids
13 in the cyst. (b) Spermatids connected through cytoplasmic bridges (br). (c,d) Longitudinal
14 section of the spermatids at the later stages during cells stretching and the nuclei twisting.
15 (e) Longitudinal section of the basal part of sperm, showing the microtubules (arrowheads)
16 begin from the basal body of the axoneme and are uniformly extending along the nucleus.
17 (f, g) Cross-section of the sperms at the basal (f) and central (g) parts showing the
18 microtubules (arrowheads) manchette surrounding the nucleus. (h- j) Longitudinal sections
19 of the apical part of sperms with the acrosome. (k, l) Cross-sections of the acrosome. (m)
20 Cytoplasm depression at the base of sperm flagellum. (n, o) Kinetid of sperm axoneme
21 with basal body (bb), accessory centriole (ac) and the short rootlet (arrowhead). a –
22 acrosome; ac – accessory centriole; bb – basal body of sperm axoneme; f - flagellum; n –
23 nucleus; rc - residual cytoplasm. Scale bars: a – 5 µm; b – 2 µm; c-e – 1 µm; f - g – 0.5
24 µm; h – l – 0.5 µm; m – 2 µm; n – 1 µm; o – 2 µm.

25

26 **Figure 4.** Longitudinal (a) and corresponding cross-sections (b-f) of sperm in *Crellomima*
27 *imparidens*. a – acrosome; ac – accessory centriole; bb – basal body of sperm axoneme; mt
28 – microtubules; mtc – mitochondria; n - nucleus. Scale bars: a, b – 0.5µm.

29

30 **Figure 5.** Schematic drawing of *Crellomima imparidens* spermatozoon. (a) Longitudinal
31 section. (b) Cross and longitudinal section of the apical part of sperm. a – acrosome; ac –
32 accessory centriole; bb – basal body of sperm axoneme; f – flagellum; mt – microtubules;
33 mtc – mitochondria; n – nucleus.

34

35 **Figure 6.** Spermiogenesis in *Hymedesmia irregularis*. (a) Early spermatids of *Hymedesmia*
36 *irregularis*. (b, c) Spermatids at the late stages with an elongated spiral-bound nucleus (c).
37 (d, e) Apical part of the nuclei in late spermatids with small depression (arrowheads) and
38 an acrosome. (f-h) Longitudinal (f, g) and cross-sections of spermatids, showing the
39 bundles of microtubules stretches along the long axis of the cell. (i) Kinetid of sperm
40 axoneme with basal body (bb), accessory centriole (ac); the microtubule bundle starts from
41 the axoneme's kinetid (arrowheads). a – acrosome; f – flagellum; mt – microtubules; n –
42 nucleus rc – residual cytoplasm; st - spermatids. Scale bars: a, b, c, f, g, h, i – 1 µm; d, e –
43 0.5 µm.

44

45 **Figure 7.** Schematic drawing of *Hymedesmia irregularis* spermatozoon. (a) Longitudinal
46 section. (b) Cross and longitudinal section of the apical part of sperm. a – acrosome; ac –
47 accessory centriole; bb – basal body of sperm axoneme; f – flagellum; mt – microtubules;
48 mtc – mitochondria; n – nucleus.

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