Comparative ultrastructure of the spermatogenesis of three species of Poecilosclerida (Porifera, Demospongiae).

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1 Comparative ultrastructure of the spermatogenesis ofthree species of Poecilosclerida (Porifera,

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24 Abstract.

25   The spermatogenesis of Porifera is still relatively poorly understood. In the past, it was accepted

26   that all species presented a primitive-type spermatozoon, lacking special structures and

27   acrosome. Nonetheless, a very peculiar spermatogenesis resulting in V-shaped

28   spermatozoonwith acrosome was found in Poecilosclerida, calling into question the

29   reproductive and evolutionary aspects of Porifera.We investigated theultrastructure of the

30   spermatogenesis of three different species of Poecilosclerida (Porifera, Demospongiae):*Iophon*

31   *proximum, I. piceus* and *Tedania ignis*. In all three species,spermatogenesis

32   wassynchronizedwithin the spermatic cysts. *Iophon proximum*and *T. ignis*presented elongated

33   sperm cells, but in *I. piceus* their shape was globular, suggesting a primitive spermatozoon. A

34   structure resembling an acrosome was only found in *T.ignis*. Apparently, the type of

35   spermatozoid in poeciloscleridsis not dictated by the phylogeny nor the environment.

36   Nonetheless, we are proposing that the modified type of spermatozoon is the ancestral state in

37   Poecilosclerida.

39   Introduction

40   Spermatogenesis is the process that gives rise to the male (usually motile) gamete of

41   animals, i.e. the spermatozoon. In general, this process is characterized by the establishment of

42   the germ-line, successively followed by a proliferation of germ cells, production of spermatids

43   by meiosis and finally the differentiation of the mature spermatozoa (L'Hernault 2006; White-

44   Cooper et al. 2009). Spermatozoa are among the most specialized of all metazoan cells. Their

45   diverse and sometimes intricate morphologies are adapted to a primary function – the

46   transmission of their own genetic contents to an egg of the same species (White-Cooper et al.

47   2009).

48   Sponges (Porifera) lack special organs or systems (Bergquist 1978; Simpson 1984) and

49   their spermatogenesis occurs through the transdifferentiation of somatic cells into germ cells

50   usually in non specific regions of the animal body (Boury-Esnault and Jamieson 1999;

51   Ereskovsky 2010). In Porifera, spermatocytes can either derive from archaeocytes or from

52   choanocytes, but in most Demospongiae, spermatocytes are usually derived from choanocytes

53   (Maldonado and Riesgo 2008; Ereskovsky 2010). In this class, choanocytes will form

54   aggregates progressively bordered by a follicular layer of pinacocyte-like cells, forming the so-

55   called spermatic cysts (Reiswig 1983; Boury-Esnault and Jamieson 1999). Spermatogenesis in

56   sponges may either occur synchronously in all spermatic cysts of a given specimen, or in a

57   given population, or only within individual spermatic cysts, independently from each

58   other(Reiswig 1983). In general, as in other animals, sponges also present four

59   spermatogenicstages:the first stagebegins with spermatogonia, which suffer two consecutive

60   divisions resulting in primary spermatocytes and secondary spermatocytes, when the cells

61   remain interconnected by cytoplasmic bridges. During the third stage, these cells differentiate

62   into haploid spermatids.At the fourth stage, spermatozoa become mature(Riesgo and

63   Maldonado 2009; Ereskovsky 2010; Lanna and Klautau 2010).

64   Invertebrate spermatozoa can be divided into three types: (i) primitive, (ii) modified,

65   and (iii) aberrant, the first two types being the most common ones (Hodgson 1986; Reunov

66   2005).The main difference among these types are the position/presence of different

67   compartments of the cell, including acrosome, and the shape of the sperm.Although it has long

68   been assumed that Porifera presenteda primitive type of spermatozoa, today a great diversity in

69   their morphology and size is indubitable.Reiswig (1983) described at least eleven different

70   shapes of sperm cells in Porifera.In most species of Poecilosclerida (Demospongiae)

71   investigated so far, mature spermatozoa are elongated cells (Ereskovsky 2010). However,

72   adifferentform of spermatozoa wasrecently described for this lineage: a sophisticated V-shaped

73   form in *Crambe crambe*(Schmidt, 1862)(Riesgoand Maldonado 2009).

74   The order Poeciloscerida is one of the most speciose in the phylum Porifera, comprising

75   four suborders, 25 families, 142 genera and more than 2500 species (van Soest et al. 2018).

76   However, neither the order, nor many of the inner clades are monophyletic (Redmond et al.

77   2013). As occurs in many other lineages of sponges, phylogenies based on molecular markers

78   provides groups that have no morphological characteristics to support them, probably related to

79   the homoplasy of the main characters used in the taxonomy of Porifera (Redmond et al. 2013).

80   This lack of congruence between molecules and morphology are not an exclusivity of the

81   sponges, but in other groups of metazoans, the use of the ultrastructure of the sperm cells in the

82   phylogenies has helped to better understand the relationship among the clades (reviewed in

83   Tudge 2009).

84   Apart from showing different types of spermatozoa, there is no pattern for the origin of

85   germ line in Poecilosclerida. In *Mycale fistulifera* (Row, 1911) and in the carnivorous sponge

86   *Lycopodinaoccidentalis*(Lambe, 1893) spermatocytes were suggested to originate from

87   archaeocytes (Meroz and Ilan 1995; Riesgo et al. 2007).On the other hand, in

88   *Hemimycalecolumella*(Bowerbank, 1874) and *Crellaelegans* (Schmidt, 1862)spermatocytes

89   seem to originate from choanocytes (Pérez-Porro et al. 2012).In addition, it has been postulated

90   that the shape of the spermatozoon is influenced not only by the phylogenetic position of the

91   species, but also by the physiological and functional demands during dispersal of the gamete

92   and its subsequent fertilization process (Franzén 1956; Pitnick et al. 2009). In sponges, both the

3

93   functional and phylogenetic significance of the diverse morphologies remain poorly understood.

94   Expanding the number of species from different lineages of Poecilosclerida will help to

95   understand the evolution of the group and also the usefulness of the ultrastructure of sperm cells

96   to comprehend the phylogenetic relationship within this lineage of sponges. Therefore, in the

97   present study, we compared the ultrastructure of the spermatogenesis of three species distantly

98   related within the Poecilosclerida phylogeny (Vargas et al. 2015):*Tedania ignis*(Duchassaing &

99   Michelotti, 1864) (Fig. 1a), *Iophon proximum*(Ridley, 1881)(Fig. 1b), and *Iophon*

100 *piceus*(Vosmaer, 1881) (Fig. 1c), found in the tropical, temperate and polar zones of the world,

101 respectively.

102

103 Material and Methods

104   Tissue samples of *Tedaniaignis* (with ca. 5 cm^3) were collected at Porto da Barra,

105 Salvador, Bahia, Brazil (13º00'15.2"S 38º32'00.2" W) in October 2014, January and

106 November 2015, at 1-2 m depth. Specimens of*Iophonproximum*were collected in Comau Fjord,

107 Southern Chilean Patagonia (42°24'9.66"S – 72°25'14.01"W) in April 2004, at 20-25 m depth.

108 Five specimens of *Iophon piceus*were collected near the White Sea Marine Biological Station

109 «Belomorskaia» of St. Petersburg State University (Kandalaksha Bay, White Sea) (66°17′N,

110 33°39′E), at 16 m depth in July 2002.While *T.ignis* was collected by snorkeling, both species of

111 *Iophon*weresampled bySCUBA diving.

112   Specimens of *T. ignis*were processed for both light and electron microscopy. For light

113 microscopy (LM), specimens were fixed in a solution of saline formaline (4%) for 24 h and

114 processed for standard histology, as described in Lanna et al. (2018). For transmissionelectron

115 microscopy (TEM), fragments were fixed (for at least 24 h at 4 ºC) in a solution of 25%

116 glutaraldehyde, 0.2 M of sodium cacodylate (pH 7.0) and filtered seawater (1:4:5) or fixed in a

117 solution of 25% glutaraldehyde, 0.4M PBS and 0.34M NaCl (1:4:5).For scanning electron

118 microscopy (SEM), fixed fragments were further rinsed with filtered seawater, dehydrated

119 through a graded ethanol series, fractured in liquid nitrogen, thawed in 100% ethanol at ambient

120 temperature and dried by the critical point method from carbon dioxide before being mounted

121 onaluminum stubs, coated with gold using a sputter coater and observed with a JEOL (JSM-

122 6390LV)scanning electron microscope at 12 kV. For transmission electron microscopy (TEM),

123 fixed fragments were rinsed three times in 0.2M sodium cacodylate buffer and post-fixed in a

124 solution of 1% osmium tetroxide for 30 minutes at room temperature. Later, specimens were

125 dehydrated in a graded acetone series and embedded in Epon. Semi-thin sections (1 μm thick)

126 were stained with toluidine blue.Ultrathin sections (60-90 nm thick) double-stained with uranyl

127 acetate and lead citrate (Reynolds 1963)were observed with a JEOL (JEM-1230) transmission

128 electron microscope.

129   Specimens of both species of*Iophon* were also fixed for light and electron microscopy,

130 but using different protocols. *Iophonpiceus* specimens prepared for light microscopy were

131 immersed in a Bouin fixative. Vouchers were then dehydrated through an ethanol series,

132 immersed in a celloidin-castor oil mixture and then in chloroform before embedding in paraffin.

133 Thick sections (6 μm thick) were mounted on glass slides and stained with Mayer's

134 hematoxylin, and eosin.For electron microscopy, fragments of about 1 mm^3 were fixed in 2.5%

135 glutaraldehyde in phosphate buffer (pH 7.4) at room temperature for 1h. After fixation,

136 fragments were washed in phosphate buffer and postfixed in 1% osmium tetroxide in phosphate

137 buffer for 1 h. Samples were dehydrated through a graded ethanol series and embedded in Epon-

138 Araldite.

139   Vouchers of *Iophon proximum*(specimen RBINS-IG 32231-POR 8209 = MNRJ 8209)

140 were fixed when arriving at the surface in 4% glutaraldehyde in 0.2 M sodium cacodylate

141 buffer(pH 7.4) supplemented with 0.35 M sucrose and 0.1 M NaC1 to obtain a final osmotic

142 pressure of 1105 mOsM for 24 h at 4°C. Vouchers were then washed six times for 10 min in 0.2

143 M sodium cacodylate buffer (pH 7.4) and post fixed for 1 h in 1% osmium tetroxide in 0.2 M

144 sodium cacodylate and 0.3 M NaCl, dehydrated through a graded ethanol series and embedded

145 in ERL 4206 according to Spurr (1969).

146   For both *Iophon* species, sections were obtained with a diamond knife on a Leica

147 Ultracut UCT ultramicrotome. Semi-thin sections (1 μm thick) were dried onto slides and

148 stained with methylene blue-borax. For TEM, thin sections double-stained with uranyl acetate

149 and lead citrate (Reynolds 1963) and later observed with a Tecnai 10 transmission electron

150 microscope. For SEM, specimens were fractured in liquid nitrogen, critical-point-dried, sputter

151 coated with gold-palladium, and observed with a Philips (XL30 ESEM)scanning electron

152 microscope.

153

154 **Institutional abbreviations and acronyms**

155 MNRJ = Museu Nacional da Universidade Federal do Rio de Janeiro, Brazil; RBINS = Royal
156 Belgian Institute of Natural Sciences
157

158 **Results**

159 *Tedania ignis*

160   *Tedania ignis* is a simultaneous hermaphrodite and its spermatogenesis occurs all year

161 round (Lanna et al. 2018). Spermatic cysts were enveloped by a single layer of follicle cells

162   (Fig.2a-b), were rounded, and measured ca. 45µm in diameter(Fig.2b). They were spread in the

163   choanosome without any clear pattern of distribution. Different stages of maturation occurred in

164   the same individual, without synchronism between cysts, but the spermatic cells matured at the

165   same time within each cyst. Somatic cells were seldom observedinside the spermatic cysts

166   (Fig.2c).

167   The first stage of spermatogenesis was characterized by the presence of large cells

168   (3.6µm in diameter) with spherical shape, hyaline cytoplasm and a large nucleus (2.1 µm in

169   diameter). In this stage, the nucleus was rounded and its ratio to the cytoplasm was low. The

170   chromatin was partially condensed and spread evenly in the nucleus (Fig. 2d). The cytoplasm

171   presented a relatively well-developed Golgi apparatus and some spherical mitochondria (Fig.

172   2e).

173   The second stage of the spermatogenesiscomprised cells showinga volume reduction

174   (2.2µm in diameter) due to the division of the spermatogonia (Fig. 3a),which

175   apparentlyunderwent successive divisions toward primary and latersecondary spermatocytes.

176   Primary spermatocytes were rounded, with a hyaline cytoplasm filled with small clear vesicles,

177   a prominent Golgi apparatus and a rounded nucleus.The most important characteristic inthis

178   stage was the presence of synaptonemal complexes next to the nuclear envelope (Fig. 3b). In the

179   next stage (secondary spermatocyte), another roundof cell divisions occurred, but cytoplasmic

180   bridges kept the daughter cells connected. These cells were smaller (< 2.0 µm) than in the

181   previous stages, the nucleus ratio to the cytoplasm was ca. 1:1, and the cytoplasm presented

182   several small mitochondria. In this stage, the rounded nucleus started to condense, as some

183   heterochormatin spots were observed in different areas (Fig. 3d). Following the second division,

184   the secondary spermatocytes started to differentiateinto spermatids, still connected through

185   cytoplasmic bridges (Fig. 3c, f).However,dramatic changes occurred during this stage. The

186   volume of the cytoplasm started to decrease, probably by shedding clear vesicles into the lumen

187   of the spermatic cyst. Meanwhile, a flagellum started to emerge close to the nucleus, which was

188   still rounded (Fig. 3e). Later, while the condensation of chromatin progressed, the nucleus

189   became elongatedandan axoneme as well as some mitochondria were observednext to its basal

190   region (Fig. 3f). In this early stage, the spermatid could present a curved shape (Fig. 3c, f, h),

191   but when itfurther differentiated to become a sperm cell, the cytoplasm shedding induced the

192   elongation of the cell, withmitochondria still rounded and gathered in the posterior part of the

193   cell body, next to the flagellum insertion (Fig. 3g). In the late spermatid stage, the nucleus was

194   elongated, occupyingmost of the cytosol and an electron dense material,likely pre-acrosomal

195   vesicles, was present at the tip of the cell (between the plasma membrane and the apical portion

196   of the nucleus), resembling a pre-acrosomal complex (Fig. 3i). No fully mature spermatozoon

197   could be observed in electron microscopy despite the timing of the samples collections.

199 *Iophon proximum*

200   In April 2004, the investigated specimens of*Iophon proximum*where actively carrying

201 out their spermatogenesis. As in *T. ignis*(and most Demospongiae), spermatogenesis in *I.*

202 *proximum* took place in spermatic cysts. The shape of spermatic cysts were rounded, they

203 measured from 6 to 11 μmand it was covered by follicle cells. Spermatic cysts were located in

204 the choanosome of the sponges without any special localization (Fig. 4a). The follicle cells were

205 flattened (Fig. 4b),with a lentil-like shape next to the nuclear region. The outward and inward

206 surfaces of the follicle cells had numerous membrane protuberances (Fig. 4c). Maturation of the

207 spermatic cells was not synchronous at the individual level but was evident within each

208 spermatic cyst, encountered atdifferent stages of spermatogenesis, from primary spermatocyte to

209 mature spermatozoa.The early spermatic cysts development had already occurred when the

210 sponges were collectedand the spermatogonia origincould not be observed.

211   Primary spermatocytespresented numerous cytoplasmic inclusions and a flagellum. The

212 cytoplasm presented several mitochondria (0.25μm in diameter), which were round shaped with

213 well-defined laminar cristae. The Golgi apparatus was large(1.7μm long) with an irregular shape

214 and consisted of 3 to 4 cisternae. In addition, several small electron-clear vesicles were visible

215 in this stage (Fig. 4d). The flagella derived from large cytoplasmic outgrowthson one side of the

216 cell. The flagellar basal apparatus was composed of a long basal body (1.05 μm long, 0.2 μm in

217 diameter) to which an associated accessory centriole (0.71 μmlong, 0.2 μmin diameter) and alar

218 sheets were found. A rootlet was absent. The basal body was situated near the Golgi apparatus

219 (Fig. 4e-f). The cell shape of the primary spermatocyte was not regular(3.8 μm in diameter)and

220 a rounded nucleus (2.5 μm in diameter) was observed during this stage. During the prophase I,

221 thenucleus of the primary spermatocyte presented typical synaptonemal complexes (Fig. 4g).

222   The first meiotic division was not synchronous, primary spermatocytes in –prophase I

223 and secondary spermatocytesoccurred at the same time within each cyst (Fig. 4g-h). Bi-

224 nucleated secondary spermatocytes, deriving from incomplete cytoplasm division at the end of

225 the first meiotic divisionwere recognizable (Fig. 5a). The cytoplasm of the secondary

226 spermatocytes was similar to the previous stage, but the cells were slight smaller. The chromatin

227 formed electron-dense areas in the nucleus (1.4 μm in diameter). Bi-nucleated cells could be

228 observed after the second meiotic division, indicating the transition from secondary

229 spermatocyte to spermatid stages (Fig. 5a), along with the formation of lipid droplets (Fig. 5b).

230 The Golgi complex was not as long as in the previous stage, but consisted also of3 to 4 cisternae

231   (1.2 µm long). In this stage only,a cytoplasmic invagination, forming a pit in the plasma

232   membranearound the basal part of the flagellum was observed. Cytoplasmic bridges were still

233   observed connecting the spermatids (Fig. 5c).

234   Later, secondary spermatocytes evolved into spermatids, acquiring anelongatedshape

235   (3.4 µm long, 1.3 µm in diameter) and being characterized by chromatin at different stages of

236   condensationas well as a stretched nucleus andthe elongation and in eccentric position (2.4 µm

237   long, 0.9 µm in diameter). During this stage, pocket-like cytoplasmic invaginations arose around

238   the basal portion of the flagella (Fig. 5d-e).The spermiogenesiswas accompanied by the full

239   compaction of the nuclear content altogether with the elongation of the nucleus. In

240   addition,residual cytoplasm was shed at the basal sideof the cell or alongside the flagellum

241   reducing the overallvolume of the cell (Fig. 5f-6a).Spermatozoa were elongated (up to 4.2 µm

242   long,with a diameter of 1.25 µm in basal and 0.83 µm in apical portion of the cell),with a long

243   flagellumarising from a cytoplasmic invagination of variable depth(Fig. 6a-b). The anterior part

244   of the nucleus presenteda trapezoid shape in section. Mitochondria with constant spherical

245   shape and diameter (about 0.3 µm)occurred at the basal part of spermatozoa (Fig. 6b). No

246   particular orientation of spermatozoa within spermatocystswas noticeable.

247   *Iophon piceus*

248   *Iophon piceus* is a simultaneous hermaphrodite reproducing throughout the hydrological

249   summer, i.e. late April to early October (Ereskovsky 2000). The spermatogenesis of this species

250   was previously investigated in light and electron microscopy (Efremova et al. 1987) but the

251   samples collected in 2002 are providing more information about the ultrastructure of its

252   spermiogenesis, although only late stage of spermatic cysts were present (Fig. 7a).Spermatids

253   and spermatozoa were enveloped by flattened cellsof the spermatic cyst (Fig. 7a). Spermatids

254   were rounded, measuring ca. 1.4 µm in diameter. The residual cytoplasm presented many large

255   electron clear vesicles and some tubules (likely resulting from the degenerating endoplasmic

256   reticulum)specially found in the residual cytoplasmic bridges uniting two or more spermatids

257   (Fig. 7b-c). At this stage, the nucleus was already fully condensed and started to stretch out,as

258   well as the cell, which also gained an elongated shape. The volume of the cell decrease and

259   large electron clear vesicles started to appear in the cytoplasm. (Fig. 7d). A single amorphous

260   crystalline inclusion was then observed in different regions of the cell, but usually close to the

261   nucleus (Fig. 7e-h). The flagellum of the spermatid/spermatozoon was long and free of

262   ornamentation (Fig. 7a-b, e). Even though the insertion of the flagellum was not observed in an

263   invagination of the cell membrane, small microvilli expansions were found at the basis of the

264   flagellum in several sections (Fig. 7e-f, h-i). The accessory centriole was located close to the

265 nucleus (Fig. 7d, f) and the basal body was positioned at the posterior end of the cell. Coarse

266 microtubule fibers, supposedly related to the rootlet of the flagellum, spread from the basal

267 body toward the nucleus. The nucleus reached ca. 3 μm in length and presented sharp endings in

268 some sections, indicating a truncated tip of the cell (Fig. 7h).

269 Discussion

270 We are here describing the morphology of several steps of the spermatogenesis of three

271 species belonging to two different genera of Poecilosclerida. These species inhabit different

272 regions of the globe (from tropics to polar regions), but still present sperm morphology with

273 stark resemblance to each other and to other previous studied poecilosclerids. Sampling was not

274 spread over time for the threepoecilosclerids described here and spermatogenesis was already in

275 progress in all samples examined. Therefore, the cell lineage of the spermatic cellscould not be

276 determined. Nonetheless, based on the position, shape, and sizes of the spermatic cysts of the

277 three investigated species, we hypothesize that they also derive from choanocyte chambers, as

278 showed previously for other poecilosclerids (Pérez-Porro et al. 2012).Thespermatogonia of

279 *Tedaniaignis*(and also in *Iophon piceus*Efremova et al. 1987) seemed to lose their flagella

280 during the first divisionand to produce new ones at the later spermatid stage. However, in

281 *Iophonproximum*,as also occurs in the Homoscleromorpha investigated so far (Gaino et al.

282 1986; Riesgo et al. 2007b; Ereskovsky 2010) and some freshwater sponges (Paulus 1989), the

283 division ofspermatocytes occurred without the loss of the flagellum. This situation is in

284 contradiction with most Metazoa that have the flagellumappearing only at the late stages of

285 gametogenesis(Reunovand Hodgson 1994). Besides sponges, such situation was only observed

286 in bivalves (Reunovand Hodgson 1994).

287 In the present study, the spermatozoa of all three species showed an elongate shape

288 (about 5.8x0.7 μm in *T. ignis*;4.0 x0.9 μm in *I. piceus*;and 4.5x0.8 μm in *I. proximum*). The

289 morphology of their nucleus presented a striking similarity: in*T. ignis* as in the two *Iophon*

290 species it was cylindrical with a wavy surface and a trapezium-like anterior end.

291 Both*Iophon*speciesshared the presence of a shallow cytoplasmic invagination around the basal

292 part of theirsperm flagellum and an unusually elongated basal body (in *I. piceus* it was about 1.7

293 μm and in *I. proximum*1.1 μm). Within the order Poecilosclerida,former investigations in

294 electron microscopy were carried out on *Myxilla incrustans*(Johnston, 1842), *Iophon piceus*

295 (Efremova et al. 1987), *Lycopodinaoccidentalis*(Lambe, 1893) (Riesgo et al. 2007),*Crambe*

296 *crambe*(Maldonado and Riesgo 2008), *Hemimycalecolumella*(Pérez-Porroet al. 2012),

297 *Crellomimaimparidens* and*Hymedesmiairregularis*(Ereskovsky 2010). The ultrastructure of the

298 spermatozoa of all three speciesinvestigated herewas similar to that formerly observed for the

299   sperm of *I. piceus* (Efremova et al. 1987) and of *L. occidentalis*(Riesgo et al. 2007). In addition,

300   the presence of a shallow invagination in the flagellum insertion of *I. proximum*and *I. piceus* is

301   similar to that observed in the sperm cells of *H. columella* (Pérez-Porroet al. 2012).

302   Nevertheless, the very sophisticated spermatozoon observed in *C. crambe* (V-shaped) (Riesgo

303   and Maldonado 2009) was not observed in any of the three investigated species and is still a

304   peculiarity of this Mediterranean species.*Crambecrambe*is, apparently, in a sister-group of all

305   other poecilosclerids investigated so far (Hestetun et al. 2016). It could be, therefore, that this

306   sophisticated characteristic of the sperm of *C. crambe* is restricted to the lineage of the

307   Crambeidae, rather than widely spread in Poecilosclerida as a whole. We expect that new

308   investigations in other species of *Crambe* and of*Monanchora*, for example, are likely to answer

309   this question.

310   Elongated and V-shaped spermatozoa with elongated nucleus are considered as

311   'modified' and are widely regarded as a derived character in Metazoa (Reunov 2005). The co-

312   occurrence of 'primitive' and 'modified' spermatozoa is a situation established in Porifera prior

313   to the emergence of higher metazoans. The occurrence of 'modified' spermatozoa among

314   Porifera sharing many ultrastructural traits with those of higher metazoans could be explained

315   by multiple cases of convergent evolution (Riesgo and Maldonado 2009).The same explanation

316   might also be useful for the acrosome. Most spermatozoa of Porifera described until de end of

317   the 1990's lacked an acrosome (Boury-Esnault and Jamieson 1999) and it was commonly

318   admitted that they allwere of the 'primitive' type and were more basic than the primitive sperm

319   of the majority of the other groups of metazoans. However, acrosomes were revealed in

320   spermatozoa of Homoscleromorpha (Ereskovsky 2010), of two Calcarea species (Nakamura et

321   al. 1997; Lanna and Klautau 2010) and also in several Demospongiae, especially

322   Poecilosclerida (Riesgo et al. 2007; Riesgo and Maldonado 2009; Ereskovsky 2010).The

323   spermatozoa of *T.ignis* presented small vesicles on top of the nucleus, that are likely pre-

324   acrosomal vesicles,as in other Poecilosclerida(*L. occidentalis*, *C. crambe*, and

325   *Crellomimaimparidens*(Rezvoi, 1925))(Riesgo et al. 2007; Riesgo and Maldonado 2009;

326   Ereskovsky 2010). In contrast, in the spermatozoa of *I. piceus* and *I. proximum*these electron

327   dense granules were absent, as in *M. incrustans* (Efremova et al. 1987). Historically, the

328   presence of acrosome in Porifera brought the following question: is the acrosome a primitive

329   organelle that disappeared during evolution in most of the orders, or a new organelle

330   secondarily evolved in a few species? (Baccetti 1986). In the past, thesperm ultrastructure in

331   sponges supported the hypothesis presented by Afzelius(1972) that primitive spermatozoa may

332   have arisen twice, once in Porifera and another inall other metazoans. The sperm in Porifera

333   seems to have arisen primarily from choanocytes and has probably evolved independently of

334   sperm in Cnidaria and other groups(Franzén1996). However, it is now being suggested that the

335 spermatozoon is homologous in all Metazoa and that the absence of acrosome in most sponges

336 could be a derived condition (Boury-Esnaultand Jamieson 1999; Riesgo and Maldonado 2009).

337 Apparently, the general shape of the sperm might be related more to the fecundation physiology

338 of the animal, than being constrained by the phylogeny of the group.Although some

339 similaritiescould be observed amongst the three poecilosclerids investigated here, especially in

340 the elongated shape of the sperm cell, the morphology of the spermatozoa of these species still

341 presentdistinctive characteristics from each other.Expanding the investigations to other

342 poecilosclerids is mandatory if we are to understand whether the diversity of sperm cell

343 morphology is related to the phylogeny of the group or an adaptation to the fertilization process.

344 Nonetheless, till these new studies investigating a large variety of species in different lineages

345 of the group is carried out, we suggest that the "modified" sperm (elongated with a basal

346 flagellum) is the ancestral state in the lineage. Further modifications in the morphology of the

347 sperm, as the V-shaped ones of *C. crambe* (Riesgo and Maldonado 2009), are novelties within

348 Poecilosclerida. We conclude that the current data about spermatozoa ultrastructure in Porifera

349 as a whole (and in Poecilosclerida, particularly) is not sufficient to be used for taxonomy or

350 phylogeny as already proposed in other groups of metazoans, e.g.annelids, insects, birds (Tudge

351 2009). However, it indicates that the fertilization (which is largely unknown in Demospongiae,

352 Ereskovsky 2010) of these different species could be carried out in an apparently similar

353 pattern.

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470 Figure captions

471

472 Figure 1. In situ photographs of the investigated poecilosclerids: (a) *Tedania ignis*from Salvador

473 de Bahia, Brazil. (b) *Iophon proximum*from Comau Fjord, Chile. (c) *Iophon piceus* from White

474 Sea, Russia.

475

476 Figure 2. *Tedania ignis*: Spermatic cysts and spermatogonia. (**a**) Choanosome (ch)with different

477 spermatic cysts (sc) enveloped by a thin layer of follicle cells (cryofracture seen in SEM). (**b**)

478 Spermatic cyst (sc) with spermatic cells developing in synchrony(LM). (**c**) Spermatids (spd),

479 flagella (fl) and a large somatic cell (so) within a late stage spermatic cyst(TEM). (**d**)

480 Spermatogoniumand its large nucleus (n) with compacting chromatin and clear cytoplasm (cy)

481   (TEM). (**e**)Mitochondria (mi) and a Golgi apparatus (ga) in the cytoplasm of a spermatogonium.

482   Arrows indicate follicle cells.

483

484   Figure 3. *Tedania ignis*: Spermatocytes and spermatids. (**a**) Spermatogonia (sp) and primary

485   spermatocytes (sc1) in a spermatic cyst(mes – mesohyl). (**b**) Primary spermatocyte with a large

486   nucleus (n) in prophase I (arrows indicate synaptonemal complex) clear cytoplasm (cy) and

487   Golgi apparatus (ga). (**c**) Spermatic cyst withsecondary spermatocytes (sp2), spermatids (spd)

488   and spermatozoa (spz). (**d**) Secondary spermatocytes connected through cytoplasmic bridges

489   (cb) (mi – mitochondria; n – nucleus). (**e**) Secondary spermatocyte in a late stage of transition

490   towards spermatid, with compacting heterochromatin (hc) in the nucleus (n) (fl – flagellum; mi

491   – mitochondria). (**f**) Mid stage spermatid with cytoplasm containing many clear vesicles (cv).

492   An elongated nucleus (n) is above the axoneme (ax). (**g**) Spermatid in a late stage, with

493   stretched out nucleus (n) in the apical portion of the elongated cell.The base of the flagellum (fl)

494   appears in the cytoplasm. (**h**) Basal portion of the spermatid (neck) showing the flagellum (fl)

495   emerging from the cytoplasm, several mitochondria (mi)and clear vesicles (cv). (**i**) Tip of a late

496   stage spermatid showing the pre-acrosomal vesicles (arrow) (n – nucleus).

497

498   Figure 4. *Iophon proximum*: Spermatic cysts, spermatogonia, and primary spermatocytes. (**a**)

499   Spermatic cysts (sc) at different maturation stages in the choanosome. (**b**) Detail of a spermatic

500   cyst (sc),(fc - follicle cells; mes - mesohyl). (**c**) Detail of a follicle cell with membranous

501   protuberances (mb). (**d**) Spermatogonia with a large nucleus (n) andGolgi apparatus (ga). (**e**)

502   Primary spermatocyte showing basal body and accesory centriole (ac) of the flagellum (fl).(**f**)

503   Detail of the long basal body (bb) close to the Golgi apparatus (ga) and the accessory centriole

504   (ac). (**g**) Primary spermatocyte with nucleus (n) in prophase I (arrows indicate synaptonemal

505   complexes). (**h**) Secondary spermatocyte with a large nucleus (n) and compacting chromatin.

506   Mitochondria (mi) are abundant in all stages.

507

508   Figure 5. *Iophon proximum*: Secondary spermatocytes and spermatids of *I. proximum*. (**a**)

509   Secondary spermatocyte (spc2) soon after nuclear division. (**b**) Detail of a secondary

510   spermatocyte with a large nucleus. (**c**) Two secondary spermatocytes united by cytoplasmic

511   bridges (cb). (**d**) Spermatids with compacted and elongated nucleus and cytoplasm being shed.

512   Several mitochondria (mi) are located in the putative neck of the sperm cell and the flagellum

513   (fl) is emerging from a deep pit (arrows) in the cytoplasm. (**e**) Detail of the neck of the

514   spermatids with the flagellum insertion in the invagination of the cell (arrows). (**f**) Late

515   spermatid with stretched nucleus and residual cytoplasm (rc) being shed alongside the cell.(cy –

516   cytoplasm; mi – mitochondria; n – nucleus; spc1 – primary spermatocyte).

517

Figure 6. *Iophon proximum*: Spermatozoon. (**a**) Spermatozoon (spz) with long flagellum seen in
SEM. (**b**) TEM view of the spermatozoon showing compacted nucleus (n) with a trapezoid
apical shape (fl – flagellum; mi – mitochondria).

Figure 7.*Iophon piceus*: Spermatids. (**a**) Overview of a spermatic cyst (sc) with spermatids
(spd)(mes– mesohyl; fc – follicle cells). (**b**) Spermatids still connected by cytoplasmic bridges
(cb). (**c**) detail of the cytoplasmic bridge seen in b, with the presence of several microtubules.
(**d**) Spermatids containing important clear vesicles (cv). The centriole (ce) is inserted alongside
the compacted nucleus. (**e**) Late spermatid with globular shape. A crystaline amorphous
inclusion (ai) is located next to the compacted nucleus. The flagellum is inserted in the neck of
the cell attached to the basal body (bb). A fibrous rootlet (r) is connected to the nucleus. (**f**)
Detail of the flagellum (fl) insertion showing the basal body (bb) and the accessory centriole
(ac). (**g**) Amorphous inclusions are located in different regions of the cytoplasm. The tip of the
nucleus bears a trapezoid shape (arrow). (**h**) Elongated spermatid with long nucleus and
trapezoid apical shape (arrow). (**i**) Detail of the basal body (bb) and a microvilosity at the neck
of the spermatid. (ai – amorphous inclusion; fl – flagellum; mv – microvilosity; n – nucleus).

Figure 1

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Figure 2

Figure 3

Figure 4

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Figure 5

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Figure 6

Figure 7