

1 **Morphological features of the testis of freshwater blood flukes of the genus *Sanguinicola* Plehn, 1905 with**
2 **consideration of the testicular patterns in the Aporocotylidae**

3

4

5 Larisa G. Poddubnaya^{1*}, Alexander E. Zhokhov¹, Willy Hemmingsen², Ken MacKenzie³

6 ¹ I. D. Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, 152742 Borok, Yaroslavl

7 Province, Russia

8 ² Department of Arctic and Marine Biology, Faculty of Biosciences, Fisheries and Economics, University of Tromsø,

9 9037 Tromsø, Norway

10 ³ School of Biological Sciences (Zoology), University of Aberdeen, AB24, 2TZ, Aberdeen, Scotland

11

12 Corresponding author: E-mail address: poddubnaya@ibiw.ru (L. Poddubnaya)

13 Larisa G Poddubnaya, ORCID id: 0000-0003-0472-9846

14 Alexander E. Zhokhov, ORCID id: 0000-0002-4677-3311

15 Willy Hemmingsen, ORCID id: 0000-0003-0454-9634

16 Ken MacKenzie, ORCID id: 0000-0003-2742-5044

17

18

19

20

21 **Abstract**

22 This is the first detailed study assessing the morphological features of the testis and testicular sperm of members of
23 the freshwater blood flukes *Sanguinicola* sp. from *Leuciscus idus* (Cyprinidae) and for *S. volgensis* from *Pelecus*
24 *cultratus* (Cyprinidae), and the marine blood fluke *Aporocotyle simplex* from *Hippoglossoides platessoides*
25 (Pleuronectidae). The present study reports a unique feature in the distribution of germinal cellular components in
26 freshwater *Sanguinicola* sp., showing the presence of the individual spermatocytes or their clusters in the testicular
27 lobes, and the gathering of spermatid rosettes and spermatozoa within middle testicular lumen, which extends along
28 the entire length of the testis. In contrast, each testis of marine *A. simplex* contains the usual mixed distribution of
29 germ cells at various stages of development. The first TEM data on spermatozoon character of studied species has
30 shown, unusual for digenean sperm structure, the absence of cortical microtubules in sperm principal region.
31 Moreover, a variation in axoneme patterns is revealed in the studied aporocotylids, belonging to the different
32 aporocotylid lineages a 9 + 0 axonemal type observed for freshwater teleost-infective species, *Sanguinicola* sp. and *S.*
33 *volgensis*, and a 9 + '1' axonemal type revealed in spermatozoa of marine teleost-infective species *A. simplex*. The
34 results discussed with the published data on the digenean sperm structure and the testicular patterns in the
35 Aporocotylidae likely represent additional characteristics supporting the divergent evolutionary lineages of freshwater
36 and marine aporocotylids. We anticipate future morphological studies of the sperm structure in aporocotylids of three
37 lineages for an understanding of their phylogenetic relationships.

38

39 **Key words:** Testis Sperm pattern TEM *Sanguinicola* *Aporocotyle* Divergent evolutionary lineages

40

41

42

43

44

45 **Introduction**

46 Blood flukes of the digenean family Aporocotylidae Odhner, 1912 represent an ancient and taxonomically diverse
47 group, distinguished by their three distinct aporocotylid lineages, chondrichthyan-infective species, marine teleost-
48 infective species and fresh-water teleost-infective species (Cribb et al. 2017; Pérez-Ponce de León and Henández-
49 Mena 2019). The pattern of the testes is a commonly quoted taxonomic feature of the Aporocotylidae (Van der Land
50 1967; Thulin 1980; Smith 2002; Kamegai et al. 2002; Nolan and Cribb 2005, 2006; Bullard et al. 2006, 2008; Nolan
51 et al. 2016; Warren and Bullard 2019, 2021). The value of such a character is considered to be also important for the
52 identification of the species of the genus *Sanguinicola* Plehn, 1905, belonging to the freshwater teleost-infective
53 lineage (Ejsmont 1926; Schell 1974; Guidelli et al. 2002; Kirk and Levis 1993). The number of testes or the number
54 of bilateral lobes of a single testis have been identified as the characteristic traits for each *Sanguinicola* species.
55 However, in the species *S. inermis* Plehn 1905 different authors have revealed the presence of testes (Sommerville and
56 Iqbal 1991) or a single testis (Kirk and Levis 1993). For other *Sanguinicola* species, irregular-shaped testes have been
57 observed for *S. intermedia* Ejsmont, 1926 and *S. armata* Plehn, 1905 (see Ejsmont 1926), *S. lophophora* Erickson &
58 Wallace, 1959 and *S. argentinensis* Szidat, 1951 (see Erickson and Wallace 1959) and *S. davisii* Davis et al, 1961 (see
59 Davis et al. 1961). However, for freshwater *S. idahoensis* sp. n. (see Shell 1974) and *S. platyrhynchi* n. sp. (see
60 Guidelli et al. 2002) and for marine *S. maritimus* n. sp. (see Nolan and Cribb 2005), the presence of a single lobed
61 testis was noted. According to the diagnosis of the genus *Sanguinicola* by Smith (2002), the occurrence of a single
62 testis with deep bilateral lobes is a character of the species belonging to this genus.

63 The present study represents the first detailed transmission electron microscopical (TEM) observations on
64 the testicular structure of the freshwater teleost-infective lineage of aporocotylids, *Sanguinicola* sp., parasite of ides,
65 *Leuciscus idus* Linnaeus, 1758. Additionally, light microscopical observations on the testicular structure are made
66 for *S. volgensis* Rašin, 1929 infecting cyprinid freshwater sabres, *Pelecus cultratus* Linnaeus, 1758, and for
67 *Sanguinicola* sp. from ides. For comparison, we included the original ultrastructural data on the cytoarchitecture of
68 the testes for species of the marine teleost-infective lineage, *Aporocotyle simplex* Odhner, 1900 infecting the arteries
69 of the long rough dab *Hippoglossoides platessoides* Fabricius, 1780 (Norwegian Sea, Norway).

70

71 **Material and Methods**

72 Specimens of *Sanguinicola* sp. were collected from the bulbus arteriosus leading from the heart of naturally infected
73 ides *Leuciscus idus* (Cyprinidae) and *S. volgensis* from sabres, *Pelecus cultratus* (Cyprididae) in the Upper Volga
74 River Basin, Russia, during 2020 and 2021. Specimens of marine *Aporocotyle simplex* were obtained from branchial
75 arteries of the long rough dab *Hippoglossoides platessoides* trawled from the Norwegian Sea, Norway during 2017.

76 For light microscopy (LM), the specimens of *Sanguinicola* spp. of the abovementioned freshwater blood-
77 flukes were placed in 6.5% saline, killed in hot 10% buffered formalin, stored in 70% ethanol and stained with alum
78 carmine (*Sanguinicola* sp.) and Mayer's hematoxylin (*S. volgensis*), then dehydrated in ascending concentrations of
79 ethanol, cleared in dimethylphthalate and mounted in Canada balsam. Photomicrographs were taken with the aid of
80 a stereomicroscope system (Olympus CX 43) using phase-contrast and microimage analysis. The specimens of *A.*
81 *simplex* in the collection of the Natural History Museum, London, were examined under a Leica DM5000B light
82 microscope and photographed with a Leica BAC450C Camera using Leica Application Suite (v. 4.6) Software.

83 For transmission electron microscopy (TEM), live freshwater *Sanguinicola* sp. from ides and *S. volgensis*
84 from sabres, and marine *A. simplex* were fixed using 3% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.2)
85 for 10 days at 5°C, rinsed four times for 20 min periods in the same buffer and postfixed in 1% osmium tetroxide for
86 1h. Fixed specimens were dehydrated in a graded ethanol series, with a final change to absolute acetone and were
87 embedded in a mixture of Araldite and Epon. Ultrathin sections were stained with uranyl acetate and lead citrate,
88 and examined using a JEM 1011 microscope operating at 80 kV.

89

90 **Results**

91

92 Light microscopical (LM) observation of the testis of freshwater *Sanguinicola* sp. and *S. volgensis*

93

94 In the studied specimens of *Sanguinicola* sp., a large, irregular-shaped, lobular testis prolonged longitudinally along
95 the middle of the body from the posterior margin of the intestinal ceca to the ovary was observed (Fig. 1a – c). In
96 *Sanguinicola* sp. the testis is 244 – 518 (357) long and 88 – 132 (85) wide (Fig. 1a, b, d). In specimens stained with
97 carmine (Fig. 1a, b, d), numerous, closely packed, testicular lobes arranged in two rows relative to the middle region
98 of the testis may be seen. Figures 1d and 1f show the lumen of the middle testicular region to be filled with

99 spermatozoa. Additionally, in Figure 1f it can be clearly seen that spermatozoa are localised in the middle testicular
100 region. However, posterior to the testicular field and directed towards the ovary, spermatozoa are seen inside the
101 sperm duct (see Fig. 2h).

102 In *S. volgensis* the testis is 339 – 565 (442) long and 69 – 183 (105) wide (Fig. 1c). Visually, the testicular
103 lobes of *S. volgensis* are located more loosely than in *Sanguinicola* sp. (Fig. 1c). The different cellular compositions
104 of the testicular lobes and middle testicular region may be seen in Fig. 1e stained with Mayer's hematoxylin. The
105 lateral testicular lobes filled with dense cells (spermatocytes), and the rosettes of spermatids and spermatozoa in the
106 lumen of middle region may be distinguished (Fig. 1e).

107
108 TEM observation of the testis of freshwater *Sanguinicola* sp. from *Leuciscus idus*
109
110 TEM sections of the testis show irregular-shaped testicular lobes within the lumen in which spermatogonial cells are
111 present (Fig. 2a). Serial reconstructions of TEM sections revealed all testicular lobes to be combined into a middle
112 testicular lumen (Fig. 2d). The testis is covered by a rim of extracellular fibrous matrix (EM) about 0.15 µm in
113 thickness, which forms a continuous layer around the testis (Fig. 2b – d). Just beneath EM, a discontinuous layer of
114 circular muscle fibres is scattered (Fig. 2b, d). The perikarya of muscle cells with their sarcoplasmic processes
115 extend around the testis (Fig. 2d). Above, and in proximity to EM, the testicular wall consists of an extremely flat
116 cytoplasmic layer produced by somatic cells of the testis, which spreads over the entire surface of the testis (Fig. 2b,
117 c, h). The cytoplasmic matrix of this flat layer is electron-dense and contains a high concentration of free ribosomes
118 (Fig. 2b, c). Serially, in certain parts of this layer the larger cytoplasmic areas observed give rise to long, thin
119 cytoplasmic processes forwarded into each of the testicular lobes (Fig. 2c, h). Commonly, these processes of somatic
120 cells are intimately associated with spermatogonia and spermatocytes that occlude the lumen of each lateral
121 testicular lobe (Fig. 2c, h). Along the flat cytoplasmic layer of the lobes, small clusters with spermatozoa may be
122 enclosed by such cytoplasmic processes (Fig. 2b).

123 TEM observation revealed the difference in cellular content of various regions of the testis (Fig. 2a, d). As
124 a rule, spermatocytes dominated in the cavity of the lateral lobes of the testis. Initially, the clusters of spermatocytes
125 are intimately associated with cytoplasmic processes of somatic cells, but individual cells in these small clusters do

126 not appear to be connected by intercellular bridges (Fig. a, c, d, h). Furthermore, cellular bridges of spermatocytes
127 join the cytoplasmic compartments of adjacent cells, forming aggregates within testicular lateral lobes (Fig. 2d). The
128 lumen of the middle testicular region, extending along its entire length, is filled with spermatids, which are grouped
129 in a rosette cluster, and numerous spermatozoa (Fig. 2a, d). Each spermatid is connected by a cytoplasmic bridge to
130 a central cytophore of the spermatid cluster (Fig. 2d). Longitudinal and transverse sections along the length of the
131 sperm revealed that the spermatozoon of both species, *Sanguinicola* sp. and *S. volgensis*, exhibits two axonemes of 9
132 + 0 pattern, nucleus, mitochondria and the absence of cortical microtubules (Fig. 2e - g).

133 The single sperm duct (vas deferens) arises from the posterior middle region of the testis passing into the
134 two-winged ovary (Fig. 1j). In Figure 2j it is clearly visible that the duct epithelium is a prolongation of the middle
135 testicular area. The epithelium of the vas deferens is thin and cellular, possessing elongated epithelial nuclei and
136 luminal surface lamellae (Fig. 2j).

137

138 TEM observation of the testes of marine *Aporocotyle simplex* from *Hippoglossoides platessoides*

139

140 A large number of small, densely packed, variable in shape testes fill up the space between the caecal limbs and
141 genital atrium (Fig. 3a, c). Each testis is bounded by a finely fibrous layer of EM, which is supported by muscle
142 fibres with muscle cells and their sarcoplasmic processes (Fig. 3b, f). The cellular organization of each testis is
143 similar, containing a distribution of germ cells in various stages of development, clusters of spermatocytes, rosettes
144 of spermatids and numerous spermatozoa (Fig. 3d, f, g). The spermatogonia occupy a peripheral position in each
145 testis (Fig. 3g). The head region of the spermatozoon contains the nucleus, mitochondrion and two axonemes, which
146 are composed of nine peripheral doublets and central core of 9 + '1' pattern and with the cortical microtubules
147 absent (Fig. 3e). Loops of the sperm duct were observed close to the testes (Fig. 3g). The lumen of the duct is filled
148 with spermatozoa, the epithelial lining of which is underlaid by EM, muscle fibres with muscle cells (Fig. 3g). The
149 luminal surface of the sperm duct is ornamented with lamellae that extend into the duct lumen (Fig. 3g, insert).

150
151
152 **Discussion**
153
154 Taxonomic character of the testis of aporocotyloid digeneans genus *Sanguinicola*
155
156 The present study of the testis of two freshwater *Sanguinicola* species revealed the occurrence of a single testis in
157 these species to support previous LM investigations on freshwater blood flukes *S. idahoensis* by Shell (1974), *S.*
158 *inermis* by Kirk and Levis (1993), *S. platyrhynchi* by Guidelli et al. (2002) and marine *S. maritimus* by Nolan and
159 Cribb (2005). The present study is consistent with the taxonomic diagnosis of the genus *Sanguinicola* by Smith
160 (2002), according to which the testis has deep bilateral lobes. Our LM and TEM observations on *Sanguinicola*
161 species show that the bilateral lobes of the testis are highly irregular and branched in outline and it is thus difficult to
162 count the number of testicular lobes or the number of pairs of testes. The last mentioned taxonomic character was
163 used for descriptions of *S. intermedia* (10 pairs of testes) and *S. armata* (15 pairs of testes) (see Ejsmont 1926) as
164 well as for *S. lophophora* (17-18 pairs of testes) and *S. argentinensis* (10-20 pairs of testes) (see Erickson and
165 Wallace 1959). The number of testicular lobes in the testis was counted for the description of *S. idahoensis* (14 – 18
166 bilateral lobes) by Shell (1974) and *S. inermis* (14 – 15 bilateral lobes) by Kirk and Levis (1993). Based on our LM
167 and TEM study of *S. volgensis* and *Sanguinicola* sp., we propose to consider as taxonomic features the length and
168 width of the testis, taking into account the visible packing density of the testicular lobes in *Sanguinicola* species.
169 The testis of the studied *S. volgensis* is 339 – 585 (442) long and 69 – 183 (105) wide; the testis of *Sanguinicola* sp.
170 is 244 – 518 (357) long and 88 – 132 (85) wide; the testicular lobes of *Sanguinicola* sp. are closely packed together,
171 whereas in *S. volgensis* they are more loosely packed. Previously, the size of the testis has been reported for other
172 *Sanguinicola* species, *S. platyrhynchi* [1460 (1370 – 1530) long and 510 (500 – 530 wide)] (Guidelli et al. 2002); *S.*
173 *inermis* [132–181.5 long] (Kirk and Levis 1993); *S. idahoensis* [495 – 624 long and 170 – 187 wide] (Shell 1974)
174 and marine *S. maritimus* [632 – 889 (722) long and 167 – 302 (234) wide] (Nolan and Cribb 2005).
175
176 Comparative cytoarchitecture of the testis of *Sanguinicola* sp. and testes of *Aporocotyle simplex*

177
178 The present ultrastructural investigation of the testis of *Sanguinicola* sp., belonging to freshwater teleost-infective
179 species and testes of *A. simplex*, belonging to marine teleost-infective species of the Aporocotylidae, revealed some
180 original characters in testicular cytoarchitecture of *Sanguinicola* sp. compared to those in *A. simplex* with multiple
181 testes. First, we have presented for the first time the confluence of testicular lobes into the middle testicular lumen as
182 ultrastructural evidence of the presence of a single testis in *Sanguinicola* sp. Second, for the first time for parasitic
183 Platyhelminthes we report a unique feature in the distribution of germinal cellular components in the *Sanguinicola*
184 testis. We observed that within the lumen of each testicular lobe the individual spermatocytes or their clusters are a
185 dominant cellular component, intimately associated with thin cytoplasmic processes of somatic cells. However, the
186 middle testicular region, extending along the entire length of the testis, may be considered as a place of
187 spermiogenesis and sperm formation, because here the gathering of rosettes of spermatids and spermatozoa takes
188 place. It should also be emphasized that as shown in our TEM (for *Sanguinicola* sp.) and LM (for *S. volgensis*)
189 investigations, the middle testicular area is part of the testis and is not a sperm duct, following a sinuous course from
190 the anterior-most testicular lobes to the posterior end (see Davis et al., 1961; Shell 1974; Kirk and Levis 1993).
191 According to our LM and TEM study, the sperm duct of *Sanguinicola* sp. arises mid-line posterior to the testis as a
192 prolongation of the middle testicular area. TEM on the studied *Sanguinicola* sp. revealed that over the entire surface
193 of all lobes and middle lumen, the testicular wall consists of an extremely flat cytoplasmic layer produced by
194 somatic cells of the testis. This layer is surrounded by a continuous rim of extracellular fibrous matrix and muscle
195 fibres. The transition of the posterior extremity of the testis into a sperm duct is accompanied by morphological
196 modifications of the testicular wall to the duct epithelial lining, possessing elongated nuclei and luminal surface
197 lamellae.

198 It should be noted that both the testicular wall of the testes of marine *A. simplex* and the epithelial lining of
199 the sperm ducts have the same fine morphology as we observed for *Sanguinicola* sp. In contrast, as shown in the
200 present TEM study, each testis of marine *A. simplex* contains a mixed distribution of germ cells at various stages of
201 development, spermatogonia, spermatocytes, rosettes of spermatids and numerous spermatozoa into the testicular
202 lumen. Such cytoarchitecture of the testes has been observed in most digenean species belonging to different
203 taxonomic groups (see Swarnakar 2010; Bakhoun et al. 2017).

204
205 Ultrastructural characters of the spermatozoa of freshwater *Sanguinicola* and marine *A. simplex*
206
207 Besides testicular cytoarchitecture, we have TEM data on spermatozoon characters of three studied aporocotylics,
208 freshwater *Sanguinicola* sp. and *S. volgensis*, and marine *A. simplex*. In these three aporocotylic species, belonging
209 to two different aporocotylic lineages, the principal region of their spermatozoa (i.e. the region with nucleus and
210 mitochondrion) has two axonemes and the absence of cortical microtubules, underlying the sperm plasma
211 membrane. In addition, a previous observation on the aporocotylic, *A. spinosicanalis*, has shown the absence of
212 peripheral microtubules in the sperm sections of this species (see Justine 1995). Within the Digenea, cortical
213 microtubules located between the two axonemes have been observed in the majority of digenean species (Justine
214 1991, 2001; Bakhoum et al. 2017). Besides this pattern, digenean spermatozoa with one field of cortical
215 microtubules were noted in species belonging to the order Hemiuroidea (Bakhoum et al. 2017). Additional to the
216 aporocotylics, the absence of cortical microtubules has been recorded for spermatozoa of the didymozoid digenean
217 *Didymozoon* sp. (Justine and Mattei 1983; Bakhoum et al. 2017). It is also interesting to note that within
218 neodermatan flatworms the absence of cortical microtubules has been proposed for the spermatozoa of
219 monopisthocotylean monogeneans (Justine et al. 1985, 1993; Justine 1991, 2001). Our finding concerning the
220 absence of cortical microtubules in spermatozoa of studied aporocotylics, which is considered to be the most basal
221 group of the Digenea, should be considered as an important character for examining the phylogenetic relationships
222 between three distinct evolutionary lineages within the Aporocotylidae, the Aporocotylidae, Schistosomatidae and
223 Spirorchidae within the Schistosomatida.

224 In addition to the abovementioned characters of the sperm structure of aporocotylic digeneans, our
225 observation revealed a variation in axoneme patterns of the studied freshwater and marine aporocotylics. Along
226 most of the length of the spermatozoa of freshwater *Sanguinicola* sp. and *S. volgensis*, the 9 + 0 axonemal type is
227 observed. In contrast, the spermatozoa of *A. simplex* have the 9 + '1' axonemal type. Such variable ultrastructural
228 characters of the axonemal structure revealed in these aporocotylic species belonging to the different aporocotylic
229 lineages - marine teleost-infective *A. simplex* and freshwater teleost-infective *Sanguinicola* sp. and *S. volgensis* -
230 may provide additional evidence for the affiliation of the studied species to divergent evolutionary lineages. Before

231 the present study, in most digeneans the usual 9 + '1' axonemal configuration of their spermatozoa has been
232 recorded (see Bakhoum et al. 2017), with the exception of schistosomes (see Jamieson and Justine 2017) and
233 didymozoids (Justine and Mattei 1983). It was concluded that the sperm structure of schistosomes has a single
234 axoneme with a diffuse central structure (Justine et al. 1993), and in didymozoids, as in aporocotylids of the genus
235 *Sanguinicola*, two sperm axonemes show 9 + 0 configuration.

236

237 Testicular patterns in the Aporocotylidae

238

239 The species belonging to the family Aporocotylidae are characterized by significant diversity in the number and
240 shape of the testes in their different genera (see Smith 2002). According to Smith (2002), some genera have one
241 testis, which may be elongate, tubular or ovoid and irregularly lobed, or with deep bilateral lobes, bilateral wings or
242 branches, but the number of testes in other genera can vary from two to more 200 testes. Based on the available
243 literature and taking into account the morphological diversity and number of testes in aporocotylids, it is interesting
244 to trace such variations within each of the three aporocotylid lineages, chondrichthyan-infective species, marine
245 teleost-infective species and freshwater teleost-infective species.

246 The blood flukes infecting sharks, chimaeras and rays are characterized by a single large testis, occupying
247 the middle 1/3 of their body and localized immediately posterior to the intestinal bifurcation between two
248 intestinal caeca. This information was found for the genera *Hyperandrotrema* (see Smith 2002; Orélis-Ribeiro et al.
249 2013) and *Selachohemecus* (see Bullard et al. 2006) in sharks, for the genus *Chimaerohemecus* (see Van der Land,
250 1967; Kamegai et al. 2002) in chimaeras and for *Aetohemecus* (see (Warren and Bullard 2021) in rays. Furthermore,
251 the blood flukes infecting rays of the genera *Orchispirium* (see Bullard and Jensen 2008), *Electrovermis* (see
252 Warren and Bullard 2019), *Achorovermis* (see Warren et al. 2020) and *Homestios* (see Warren and Bullard 2021)
253 have a single, long testis, curving more 30 times with smooth testicular margins. Besides cartilaginous fishes,
254 gnathostomata of the order Acipenseriformes, for which we have knowledge on blood flukes for the genus
255 *Acipensericola*, are distinguished by a testicular column with 6 spheroid testes, each approximately equal in
256 diameter (see Bullard et al. 2008).

257 In marine teleost-infective aporocotylids, a single testis was reported for the following genera: *Skoulekia*
258 (see Alama-Bermejo et al. 2011), *Cardicola* (see Nolan and Cribb 2006, Bullard 2010, 2013; Palacios-Abella et al.
259 2021), *Braya* (see Nolan and Cribb 2006), *Primisanguis* (see Bullard et al. 2012), *Psettarium* (see Bullard and
260 Overstreet 2006), *Ankistromeces* (see Nolan and Cribb 2004), *Plehnella* (see Orélis-Ribeiro and Bullard 2015),
261 *Cladocaecum* and *Kritsky* (see Orélis-Ribeiro and Bullard 2016), *Elopicola* (see Orélis-Ribeiro et al. 2017). Smith
262 (2002) also noted the presence of one testis for the genera *Parasanguinicola*, *Pearsonellum*, *Deontacylex*,
263 *Cruoricola* and *Metaplebniella*. Furthermore, two testes have been reported for the following aporocotylid genera
264 *Neoparacardiocola* and *Paracardiocola* (see Smith 2002), *Paracardicoloides* (see Bullard 2014) and *Phthinomita*
265 (see Nolan et al. 2016). In the diagnosis of the family Aporocotylidae by Smith (2002), the presence of 5 testes was
266 recorded for the genus *Pseudocardicola*, 19-71 testes in the genus *Paradeontacylix*, 28-203 testes in the genus
267 *Aporcotyle* and more than 100 testes in the genus *Pletborchis*.

268 The present LM and TEM investigation, previous LM investigations and our abovementioned summarized
269 data on the testis of freshwater teleost-infective aporocotylids reported the occurrence of a single, lobed testis in
270 species belonging to the freshwater teleost-infective lineage. The presence of a single testis is also a characteristic
271 feature of the species of chondrichthyan-infective lineages, within which two distinct morphotypes of their testis has
272 been found a large compact testis and a long, tube-like testis, curving numerous times. The greatest variations in the
273 number and morphology of the testes may be observed among aporocotylid genera of the marine-teleost infective
274 lineage. Visible morphological testicular varieties among different lineages and different genera within the family
275 Aporocotylidae may testify to the ancient position of this digenean group and the significant taxonomical diversity
276 of the aporocotylids within this family.

277 Interestingly, in spirorchiid turtle blood flukes of the genera *Spirorchis* (see Jackson et al. 2016) and
278 *Enterohaematotrema* (see Dutton and Bullard 2020), and in aporocotylids of the genus *Acipensericola* from the
279 ‘primitive fish’, paddlefish, (Polyodontidae) (see Bullard et al. 2008) the same testicular anatomy may be
280 distinguished. The three abovementioned genera have a testicular column with a variable number of testes, i.e., 4 –
281 11 testes in *Spirorchis*; 2 testes in *Enterohaematotrema*, 6 testes in *Acipensericola*. Surprisingly, the testis lobes
282 (alternatively regarded as individual testes) of *Schistosoma* numbering 3 to 6 are also arranged in the testicular
283 column (see Jones et al. 2017).

284 The phylogenetic classification of the Digenea proposed by Olson et al. 2003 and Pérez-Ponce de León and
285 Hernández-Mena (2019) show the family Aporocotylidae as paraphyletic. The recognition of three basally distinct
286 lineages of aporocotylids by Cribb et al. (2017) allows important new inferences with respect to aporocotylid
287 evolution. According to Cribb et al (2017, p. 890) ‘such a pattern would be consistent with an origin for the
288 Aporocotylidae (and thus the Schistosomatoidea as a whole) before the separation of chondrichthyan and
289 actinopterygian fish lineages, which may have occurred approximately 415 million years ago’. The importance of
290 morphological and molecular research on blood flukes infecting basal fishes (Chondrichthyes, Acipenseriformes,
291 Elopiformes and Siluriformes) for an understanding of their evolutionary origins was noted by Oréllis-Ribeiro et al.
292 (2014). For this purpose, we anticipate future morphological studies of the testes and the structure of spermatozoa,
293 showing a wide structural variety, in a wide range of aporocotylid species belonging to their three basally distinct
294 lineages, and this is especially important for chondrichthyan-infecting species.

295

296 **Acknowledgements** The authors would like to thank the staff of the RV ‘Johan Ruud’, belonging to Tromsø
297 University (Norway), for their help with fish capture in June, 2017. We are also grateful to Master’s students, Ben
298 Hall and Jonathon McFall (University of Aberdeen, Scotland) for their help with dissection of the fishes and the
299 collection of parasites at sea. Our thanks are due to the staff of the Centre of Electron Microscopy, I.D. Papanin
300 Institute for the Biology of Inland Waters (Borok, Russia) for technical assistance in TEM investigations. We
301 acknowledge Dr. David Gibson (UK) for providing us with LM photo of *Aporocotyle simplex*.

302

303 **Declarations**

304

305 **Funding**

306 The results were obtained within the Russian Foundation for Basic Research, Research project No. 20-04-00086
307 'Evolutionary studies on a basal group of trematodes: detailed morphological and molecular genetic analyses of
308 freshwater aporocotylids, with an evaluation of their phylogenetic relationships with marine members of the family'.

309 **Conflicts of interest**

310 The authors declare no competing of interests.

311 **Availability of data and material**

312 Not applicable

313 **Authors' contributions**

314 Larisa G. Poddubnaya performed scanning and transmission electron microscopy, prepared figures, wrote
315 manuscript, approved the final draft

316 Alexander E. Zhokhov performed light microscopy of freshwater species

317 Willy Hemmingsen performed light microscopy of marine species, text review

318 Ken MacKenzie analyzed data, text review, approved the final draft

319 **Ethics approval**

320 Applicable national and/or institutional guidelines for the care and use of animals were followed in all procedures
321 performed.

322 **Consent to participate**

323 Consent was obtained from all authors to participate in this work.

324 **Consent for publication**

325 The authors declare that they have all seen and approved the final version of this manuscript
326
327

328 **References**

- 329
- 330 Alama-Bermejo G, Montero FE, Raga JA, Holzer AS (2011) *Skoulekia meningialis* n. gen., n. sp. (Digenea:
331 Aporocotylidae Odhner, 1912) a parasite surrounding the brain of the Mediterranean common two-banded
332 seabream *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817) (Teleostei: Sparidae): Description, molecular
333 phylogeny, habitat and pathology. *Parasitol Int* 60:34–44. doi:10.1016/j.parint.2010.10.001
- 334 Bakhom AJS, Miquel J, Ndiaye PI, Justine J-L, Falchi A, Bá CT, Marchand B, Quilichini Y (2017) Advances in
335 spermatological characters in the Digenea: review and proposal of spermatozoa models and their
336 phylogenetic importance. *Adv Parasitol* 98:111–165. <https://doi.org/10.1016/bs.apar.2017.04.001>

337 Bullard SA (2010) A new species of *Cadicola* Short, 1953 (Digenea: Aporocotylidae) from the heart and branchial
338 vessels of two surfperches (Perciformes: Embiotocidae) in the Eastern Pacific ocean off California. J
339 Parasitol 96:382–388. doi: 10.1645/GF-2325.1

340 Bullard SA (2013) *Cardicola langeli* sp.n (Digenea: Aporocotylidae) from heart of sheepshead, *Archosargus*
341 *probatocephalus* (Actinopterygii: Sparidae) in the Gulf of Mexico, with an undated list of hosts, infection
342 sites and localities for *Cardicola* spp. Folia Parasitol 60:17–27.

343 Bullard SA (2014) Blood flukes (Digenea:Aporocotylidae) of elopomorphs: Emendation of *Paracardicoloides*,
344 supplemental observations of *Paracardicoloides yamagutii*, and a new genus and species from ladyfish,
345 *Elops saurus*, (Elopiformes: Elopidae) in the Gulf of Mexico. J. Parasitol 100:305–316. doi: 10.1645/13-
346 391.1

347 Bullard SA, Jensen K (2008) Blood flukes (Digenea: Aporocotylidae) of stingrays (Myliobatiformes: Dasytidae):
348 *Orchispirium heterovitellarium* from *Himantura imbricate* in the Bay of Bengal and a new genus and
349 species of Aporocotylidae from *Dasyatis sabina* in the Northern gulf of Mexico. J Parasitol 94:1113–1321.

350 Bullard SA, Overstreet RM (2006) *Psettarium anthicum* sp. n. (Digenea: Sanguinicolidae) from
351 the heart of cobia *Rachycentron canadum* (Rachycentridae) in the northern Gulf of Mexico. Folia Parasitol
352 53:117–124.

353 Bullard SA, Overstreet RM, Carlson JK (2006) *Selachohemecus benzin*.sp. (Digenea: Sanguinicolidae) from blacktip
354 shark *Carcharhinus limbatus* (Carcharhinidae) in the northern Gulf of Mexico. SystParasitol 63:143–154.
355 doi 10.1007/s11230-005-9010-x

356 Bullard SA, Snyder SD, Jensen K, Overstreet RM (2008) New genus and species of Aporocotylidae (Digenea) from
357 a basal Actinopterygian, the american paddlefish, *Polyodon spathula*, (Acipenseriformes: Polyodontidae)
358 from the Mississippi delta. J Parasitol 94:487–495.

359 Bullard SA, Williams EH, Bunkley-Williams L (2012) New genus and species of fish blood fluke (Digenea:
360 Aporocotylidae Odhner, 1912) from stoplight parrotfish, *Sparisoma viride* (Bonnaterre, 1788), (Labridae:
361 Scarinae) in the Caribbean sea. J Parasitol 98:1139–1143. doi: 10.1645/GE-3099.1

362 Cribb TH, Chick RC, O'Connor W, O'Connor S, Johnson D, Sewell KB, Cutmore SC (2017) Evidence that blood
363 flukes (Trematoda: Aporocotylidae) of chondrichthyans infect bivalves as intermediate hosts: indications of

364 an ancient diversification of the Schistosomatoidea. Int J Parasitol 47:885e891.
365 <https://doi.org/10.1016/j.ijpara.2017.05.008>

366 Davis G, Hoffman L, Surberet EW (1961) Notes on *Sanguinicola davisii* (Trematoda: Sanguinicolidae) in the Gills
367 of Trout. J Parasitol 47:512–514.

368 Dutton HR, Bullard SA (2020) A new species and emendation of the seldom reported *Enterohaematotrema* Mehra,
369 1940 (Digenea: Schistosomatoidea), including a revised phylogenetic hypothesis for turtle blood flukes.
370 Syst Parasitol 97:335–345. <https://doi.org/10.1007/s11230-020-09920-w>

371 Ejsmont L (1926) Morphologische, systematische und entwicklungsgeschichtliche Untersuchungen an Arten des
372 Genus *Sanguinicola* Plehn. Bull Int L'Acad Polan Sci et Let. Cl Sci Math Nat, Ser B:877–966.

373 Erickson DG, Wallace FG (1959) Studies on blood flukes of the genus *Sanguinicola*. J Parasitol 45:310e322.
374 <https://doi.org/10.2307/3274507>

375 Jackson RR, Oréllis-Ribeiro R, Halanych KM, Arias CR, Bullard SA (2016) A new species of *Spirorchis*
376 MacCallum, 1918 (Digenea: Schistosomatoidea) and *Spirorchis* cf. *scripta* from chicken turtle, *Deirochelys*
377 *reticularia* (Emydidae), with an emendation and molecular phylogeny of *Spirorchis*. Folia Parasitol
378 63:041.doi: 10.14411/fp.2016.041

379 Jamieson BGM, Justine J-L (2017) Spermatozoa, Spermiogenesis and Fertilization in *Schistosoma*. In: Jamieson
380 BGM (ed) *Schistosoma: Biology, Pathology and Control*, Chapter 14. Taylor & Francis Group, CRC Press,
381 pp 300–317.

382 Jones MK, Jamieson BGM, Justine J-L (2017) Reproductive system of *Schistosoma*. In: Jamieson BGM (ed)
383 *Schistosoma: Biology, Pathology and Control*, Chapter 13. Taylor & Francis Group, CRC Press, pp 280–
384 299.

385 Justine J-L (2001) Spermatozoa as phylogenetic characters for the Platyhelminthes. In: Littlewood DTJ, Bray RA
386 (eds) *Interrelationships of the Platyhelminthes*. Taylor and Francis, London, pp 231–238.

387 Justine J-L (1991) Phylogeny of parasitic Platyhelminthes: a critical study of synapomorphies proposed on the basis
388 of the ultrastructure of spermiogenesis and spermatozoa. Can J Zool 69:1421–1440.
389 <https://doi.org/10.1139/z91-203>

390 Justine J-L (1995) Spermatozoal ultrastructure and phylogeny in the parasitic Platyhelminthes. In: Jamieson BGM,
391 Ausio J, Justine JL (eds) Advances in spermatozoa phylogeny and taxonomy. Mém Mus NatnHist Nat
392 166:55–86.

393 Justine J-L, Mattei X (1983) A spermatozoon with two 9 + 0 axonemes in a parasitic flatworm, *Didymozone*
394 (Digenea: Didymozoidae). J Submicrosc Cytol 15:1101–1105.

395 Justine J-L, Lambert A, Mattei X (1985) Spermatozoon ultrastructure and phylogenetic relationships in the
396 monogeneans (Platyhelminthes). Int J Parasitol 15:601–608.

397 Justine J-L, Afzelius A, Malmberg GG, Mattei X (1993) Ultrastructure of spermiogenesis in *Acanthocotyle* and
398 *Mysinidocotyle* (Platyhelminthes, Monogenea, Acanthocotylidae). Acta Zool 4:119-126.

399 Guidelli GM, Isaac A, Pavanelli GC (2002) *Sanguinicola platyrhynchi* n.sp. (Digenea: Sanguinicolidae) parasite of
400 visceral cavity of *Hemisorubim platyrhynchos* (Valenciennes, 1840) (Pisces: Pimelodidae) from the
401 floodplain of the upper Paraná River, Brazil. Braz J Biol 62:801–806.

402 Kamegai S, Machida M, Kuramochi T (2002) The blood flukes from deep-sea fishes of Suruga Bay, Japan. Bull
403 Natn Sci Mus, Ser A 28:29–34.

404 Kirk RS, Lewis JW (1993) The life-cycle and morphology of *Sanguinicola inermis* Plehn, 1905 (Digenea:
405 Sanguinicolidae). Syst. Parasitol 25:125e133. <https://doi.org/10.1007/bf00009982>

406 Nolan MJ, Cribb TH (2004) *Ankistromece mariae* n. g., n. sp. (Digenea: Sanguinicolidae) from
407 *Meuschenia freycineti* (Monacanthidae) off Tasmania. Syst Parasitol 57:151–157.

408 Nolan MJ, Cribb TH (2005) *Sanguinicola maritimus* n. sp. (Digenea: Sanguinicolidae) from Labridae (Teleostei:
409 Perciformes) of southern Australian waters. Syst Parasitol 61:99–106. doi:10.1007/s11230-005-3153-7

410 Nolan MJ, Cribb TH (2006) *Cardicola* Short, 1953 and *Braya* n. gen. (Digenea: Sanguinicolidae) from five families
411 of tropical Indo-Pacific fishes. Zootaxa 1265:1–80. www.mapress.com/zootaxa/

412 Nolan MJ, Cantacessi C, Cutmore SC, Cribb TH, Miller TL (2016) High-intensity cardiac infections of *Phthinomita*
413 *heinigeriae* n. sp. (Digenea: Aporocotylidae) in the orangeline cardinalfish, *Taeniamia fucata* (Cantor), off
414 Heron Island on the Great Barrier Reef. Parasitol Int 65, 371–377.
415 <http://dx.doi.org/10.1016/j.parint.2016.05.006>

416 Odhner T (1900) *Aporocotyle simplex* n.g.n.sp., ein neuer Typus von ektoparasitischen Trematoden. Centralbl.
417 Bakteriolog., I. Abt. 27 (2), 62–66.

418 Olson PD, Cribb TH, Tkach VV, Bray RA, Littlewood DTJ (2003) Phylogeny and classification of the Digenea
419 (Platyhelminthes: Trematoda). Int J Parasitol 33:733–755. [https://doi.org/10.1016/s0020-7519\(03\)00049-3](https://doi.org/10.1016/s0020-7519(03)00049-3)

420 Oréris-Ribeiro R, Arias CR, Halanych KM, Cribb TH, Bullard SA (2014) Diversity and ancestry of flatworms
421 infecting blood of nontetrapod craniates “fishes”. Adv Parasitol 85:1–62.
422 <http://dx.doi.org/10.1016/B978-0-12-800182-0.00001-5>

423 Oréris-Ribeiro R, Bullard SA (2015) Blood flukes (Digenea: Aporocotylidae) infecting body cavity of South
424 American catfishes (Siluriformes: Pimelodidae): two new species from rivers in Bolivia, Guyana and Peru
425 with a re-assessment of *Plehnella* Szidat, 1951. Folia Parasitol 62:050.
426 <https://doi.org/10.14411/fp.2015.050>

427 Oréris-Ribeiro R, Bullard SA (2016) Two new genera of fish blood flukes (Digenea: Aporocotylidae) from catfishes
428 in the Peruvian Amazon. J Parasitol 102:357–368. doi:10.1645/15-905

429 Oréris-Ribeiro R, Halanych KM, Dang BT, Bakenhaster MD, Arias CR, Bullard SA (2017) Two new species of
430 *Elopicola* (Digenea: Aporocotylidae) from hawaiian ladyfish, *Elops hawaiiensis* (Eastern Sea) and atlantic
431 tarpon, *Megalops atlanticus* (Gulf of Mexico) with a comment on monophyly of elopomorph blood flukes.
432 Parasitol Int 66:305–318. <http://dx.doi.org/10.1016/j.parint.2017.02.008>

433 Oréris-Ribeiro R, Ruiz CF, Curran SS, Bullard SA (2013) Blood flukes (Digenea: Aporocotylidae) of epipelagic
434 lamniforms: redescription of *Hyperandrotrema cetorhini* from basking shark (*Cetorhinus maximus*) and
435 description of a new congener from shortfin mako shark (*Isurus oxyrinchus*) off Alabama. J Parasitol
436 99:835–846. doi: 10.1645/12-125.1

437 Palacios-Abella J, Montero FE, Merella P, Mele S, Raga JA, Répullés-Albelda A (2021) *Cardicola mediterraneus* n.
438 sp. (Trematoda, Aporocotylidae): a new species infecting the gilthead seabream, *Sparus aurata* L., from the
439 Western Mediterranean Sea. Parasitol Res 120:1949–1963. <https://doi.org/10.1007/s00436-021-07143-6>

440 Pérez-Ponce de León G, Hernández-Mena DI (2019) Testing the higher-level phylogenetic classification of Digenea
441 (Platyhelminthes, Trematoda) based on nuclear rDNA sequences before entering the age of the ‘next-
442 generation’ Tree of Life. J Helminthol 93:60e276. <https://doi.org/10.1017/s0022149x19000191>

443 Rašin K (1929) *Janickia volgensis* n. gen. sp., krevni motolice z ryby *Pelecus cultratus* (L.). Biol Spisky Acad Vet,
444 8:111–123.

445 Schell SC (1974) The life history of *Sanguinicola idahoensis* sp. (Trematoda: Sanguinicolidae), a blood parasite of
446 steelhead trout, *Salmo gairdneri* Richardson. J Parasitol 60:561–566.

447 Smith JW (2002) Family Sanguinicolidae von Graff, 1907. In: Gibson DI, Jones A, Bray RA (eds.). Keys to the
448 Trematoda, Vol. 1. CABI Publishing and The Natural History Museum, Wallingford, UK, pp 433–452.

449 Swarnakar G (2010) Ultrastructural study on spermiogenesis in rumen amphistome *Orthocoelium scolicoelium*
450 (Trematoda: Digenea), a parasite of *Bubalus bubalis* in Udaipur. The Bioscan 5:347–353.

451 Sommerville C, Iqbal NAM (1991) The process of infection, migration, growth and development of *Sanguinicola*
452 *inermis* Plehn, 1905 (Digenea: Sanguinicolidae) in carp, *Cyprinus carpio* L. J Fish Diseases 14:211–219.

453 Thulin J (1980) A redescription of the fish blood-fluke *Aporocotyle simplex* Odhner, 1900 (Digenea,
454 Sanguinicolidae) with comments on its biology. Sarsia 65:35–48.
455 <https://doi.org/10.1080/00364827.1980.10431470>

456 Warren MB, Bakenhaster MD, Scharer RM, Poulakis GR, Bullard SA (2020) A new genus and species of fish blood
457 fluke, *Achorovermis testisinosus* gen. et sp. n. (Digenea: Aporocotylidae), infecting critically endangered
458 smalltooth sawfish, *Pristis pectinata* (Rhinopristiformes: Pristidae), in the Gulf of Mexico. Folia Parasitol
459 67:009. doi: 10.14411/fp.2020.009

460 Warren MB, Bullard SA (2019) First elucidation of a blood fluke (*Electrovermis zappum* n.gen., n. sp.) life cycle
461 including a chondrichthyan or bivalve. Int J Parasitol: Parasites and Wildlife 10:170–183.
462 <https://doi.org/10.1016/j.ijppaw.2019.06.008>

463 Warren MB, Bullard SA (2021) Fish blood flukes (Digenea: Aporocotylidae) from Indonesia: Two new genera and
464 species infecting the banded eagle ray, *Aetomylaeus nichofii* (Bloch and Schneider, 1801) Capapé and
465 Desoutter, 1979 (Myliobatiformes: Myliobatidae) from Borneo. Int J Parasitol 15:43–50.
466 <https://doi.org/10.1016/j.ijppaw.2021.02.002>

467 Van der Land J (1967) A new blood fluke (Trematoda) from *Chimaera monstrosa* L. Proceedings K. Nederlandse
468 Akademie van Wetenschappen, Section C, Biological and Medical Sciences 70: 110–120.

469

470 **Figure captions**

471

472 **Fig. 1** Light microscope (LM) study of testis in freshwater *Sanguinicola* sp. from ides, *Leuciscus idus* and *S.*
473 *volgensis* from sabres, *Pelecus cultratus*. **a, b** Dorsal view of testis of *Sanguinicola* sp., note numerous closely
474 parked testicular lobes arranged in two rows relative to the middle testicular lumen; alume carmine, phase-contrast.
475 **c** Dorsal view of testis of *S. volgensis*, note loosely parked testicular lobes; Mayer's hematoxylin. **d** Ventral view of
476 middle testicular region of *Sanguinicola* sp., note spermatozoa within lumen; alume carmine. **e** Ventral view of
477 testis of *S. volgensis*, note dense cells (spermatocytes) within lumen of testicular lobes and rosettes of spermatids
478 and spermatozoa within middle testicular lumen; Mayer's hematoxylin. **f** Ventral view of *Sanguinicola* sp. showing
479 spermatozoa within middle testicular lumen pass within sperm duct, which is above ovary. *ic* intestinal ceca, *mtl*
480 middle testicular lumen, *ov* ovary, *rs* rosette spermatids, *s* spermatozoa, *sd* sperm duct, *tl* testicular lobes.

481

482 **Fig. 2.** TEM study of the testis of freshwater *Sanguinicola* sp. from ides, *Leuciscus idus*. **a** Transverse section from
483 testis, note testicular lobes filled with spermatocytes and the middle testicular lumen filled with spermatozoa and
484 rosette of spermatids. **b** Testicular lobe border showing EM layer and circular muscle fibres, note flat cytoplasmic
485 layer above EM giving rise to cytoplasmic processes which enclosed spermatozoa. **c** Portion of testicular lobe, note
486 EM, flat cytoplasmic layer and thin cytoplasmic processes close to spermatocytes. **d** Transverse section showing
487 testicular lobes combined into middle testicular lumen, note a vitelline duct close to middle lumen of the testis. **e, f**
488 Transverse sections of the principal region of spermatozoa from testis of *Sanguinicola* sp. (**e**) and *S. volgensis* (**f**)
489 showing two axonemes of 9 + '0' pattern and the absence of cortical microtubules. **g** Longitudinal section of sperm
490 axoneme of *S. volgensis*, note the absence of axonemal central core. **h** Section of the portion of testicular lobe
491 showing the processes of flat cytoplasm intimately associated with spermatocytes. **j** Section showing distal portion
492 of the middle testicular area directly continuing into sperm duct. *ax* axoneme of spermatozoa, *cm* circular muscle
493 fibres, *em* extracellular fibrous matrix, *cst* cluster of spermatocytes, *fc* flat cytoplasmic layer, *ll* lumen of lobe, *m*
494 mitochondrion, *mc* muscle cell, *mtl* middle testicular lumen, *n* nucleus, *pfc* process of flat cytoplasm, *s*

495 spermatozoa, *sd* sperm duct, *sl* surface lamellae, *sm* spermatids, *st* spermatocytes, *tl* testicular lobe, *vc* vitelline
496 cell, *vd* vitelline duct.

497

498 **Fig. 3.** TEM study of testes of marine *Aporocotyle simplex* from *Hippoglossoides platessoides*. **a** LM view of
499 densely packed testes between the caecal limbs. **b** Border of the testis bounded by thick EM layer and muscle
500 fibres. **c** TEM view of the testes, note thin EM field between testes. **d** Rosette of spermatids and gathering of
501 spermatozoa within testicular lumen. **e** Transverse sections of spermatozoa, note axonemes of 9 + '1' pattern. **f**
502 Extracellular area between testes, note extracellular matrix, muscle cell and muscle fibres. **g** Loop of the sperm duct
503 filled with sperm, EM matrix and muscle cells around duct, note adjoining testis with various germ cells, **insert**
504 Epithelial duct wall with surface lamellae. *ax* axoneme of spermatozoon, *cc* central core of sperm axomeme, *cl*
505 caecal limb, *em* extracellular fibrous matrix, *ew* epithelial wall of sperm duct, *cst* cluster of spermatocytes, *mc*
506 muscle cell, *mf* muscle fibres, *n* nucleus, *s* spermatozoa, *sd* sperm duct, *sl* surface lamellae, *sm* spermatids, *st*
507 spermatocytes, *ts* testis.