

# Peculiarities of Reproduction and Gamete Structure of the Shipworm *Zachsia zenkewitschi* (Bivalvia; Teredinidae)

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= Abstract =

배벌레, *Zachsia zenkewitschi* (Bivalvia; Teredinidae)의 생식 특성과 배우체 구조

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자웅이체인 배벌레(shipworm), *Zachsia zenkewitschi*의 생식에 관하여 연구를 진행하였다. 자성 생식기관은 하나의 난소와 소형의 웅성을 안고 있는 한 쌍의 외투막 주머니로 구성되어 있었다. 소형 웅성의 정소는 내장 신경절 주위에 위치하였다. 성숙된 알의 지름은 60  $\mu\text{m}$ 이고 난황과 젤리막으로 싸여 있었다. *Zachsia zenkewitschi*의 정자는 약 2.6  $\mu\text{m}$ 의 원추상 두부, 0.5  $\mu\text{m}$ 의 침체, 직경 약 0.9  $\mu\text{m}$ 의 중부로 되어 있었다.

Keywords : Shipworm, Structure, Reproduction, Bivalvia, Teredinidae, *Zachsia*

## INTRODUCTION

Detailed research on marine shipworms started in the 1930's, and revealed that three species of molluscs belonging to the family Teredinidae inhabit the seas of Far East of Russia (Roch, 1934). Two of these species, *Teredo navalis* Linnaeus, 1758 and *Bankia setacea* Tryon, 1863, are well known and widespread, living in the wood carried to the sea (Turner and Johnson, 1971). In contrast, the third species *Zachsia zenkewitschi* Bulatoff and Rjabtschikoff, 1933, living inside rhizomes of the

marine grass, was until recently the least investigated shipworm species (Bulatoff and Rjabtschikoff, 1933; Rjabtschikoff, 1957; Turner, 1966).

Reproduction follows different patterns in these three species: *T. navalis* has a hermaphroditic gonad, incubates his brood in early stages of development in the branchial cavity, and releases larvae in the veliger stage into the sea water (Turner and Johnson, 1971); *B. setacea* is a gonohoric mollusc; releases eggs and sperm into the water, where fertilization and subsequent planktonic development occur (Turner, 1971). Studies of *Zachsia*

*zenkewitschi* life cycle indicated that dwarf males were present (Turner and Yakovlev, 1983). *Zachsia zenkewitschi* males are almost one hundred times smaller than females and inhabit special cavities of the body. The ecological peculiarities, development and anatomy of dwarf males were investigated (Turner *et al.*, 1983; Yakovlev and Malakhov, 1987), but no detailed description of the reproductive pattern and gamete structure was made. We have tried to fill this gap in the knowledge of this case of extreme sexual dimorphism among molluscs.

### MATERIALS AND METHODS

*Zachsia zenkewitschi* Bulatoff and Rjabtschikoff 1933 was collected at the Marine Biological Station

of the Institute of Marine Biology, located in Vostok Bay (Peter the Great Bay; Sea of Japan). The females were extracted from the roots of the sea grass *Phyllospadix iwatensis* Makino. (Fam. Potamogetonaceae, Class Monocotyledoneae) from a depth of 1 - 2 m (Fig. 1). Parts of females body with gonads and whole males extracted from females under a microscope were fixed in a Bouin liquid, diluted 1:1 with sea water.

For electron microscopy we have cut the portion of female body with lateral pockets containing males. They were fixed at 20°C for 15 min in cacodylate buffered 2.5% glutaraldehyde (pH 7.2) with 4% sucrose added and postfixed for 30 - 60 min with 1% OsO<sub>4</sub> in the same buffer. The specimens were dehydrated in alcohol and embedded

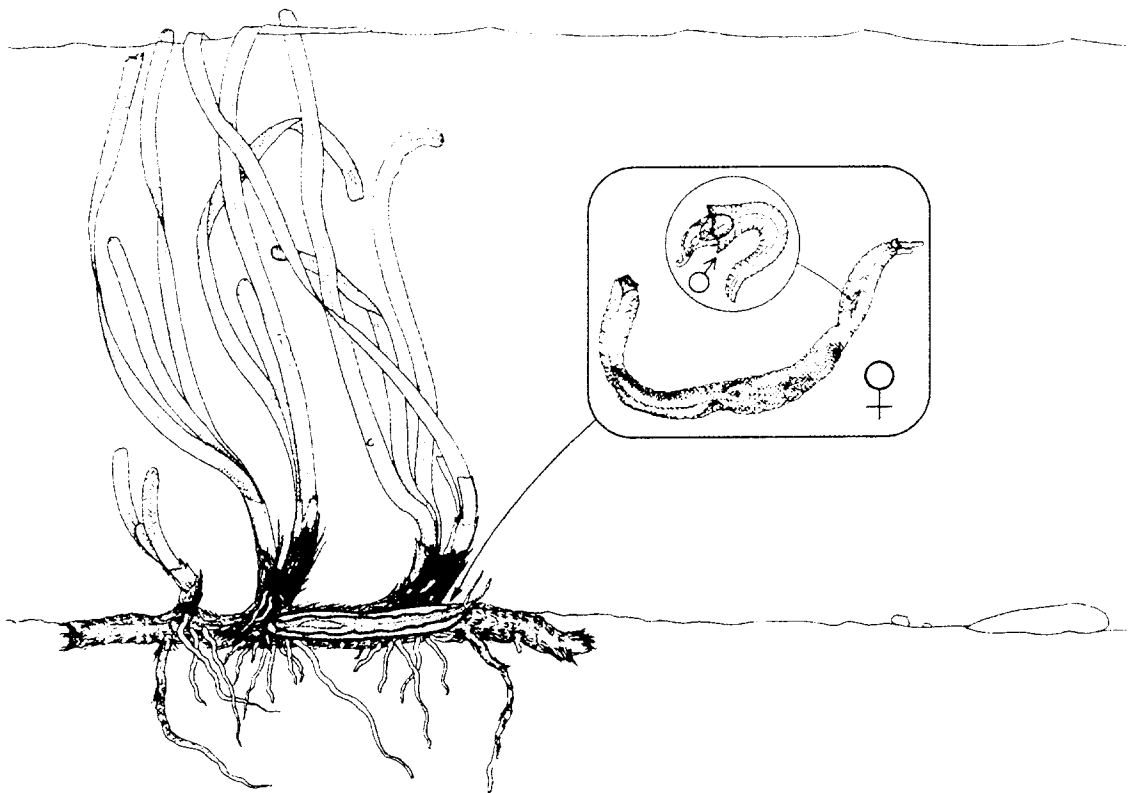


Fig. 1. The sea grass *Phyllospadix iwatensis* with *Zachsia zenkewitschi* inside the rhizon.

in araldite. The specimens were cut with a Reichert ultratome, and observed with a Jeol 100 SX electron microscope.

## RESULTS

### 1. Female reproductive system

The whitish ovary of *Zachsisia zenkewitschi* occupies about 1/5 of the length of the female body. It begins behind the shell and is localised under the kidney and the pericardial cavity, above the caecum up to the anterior gills edge (Fig.2). At maximum development the ovary intrudes among the visceral organs to reach the mantle. It is an acinal system (100 - 200 mm in diameter) containing the sexual cells. Interacinal tissue is insignificantly developed even after the periods of reproduction. The gonad opens to the epibranchial cavity by means of two gonopores situated in the posterior part of the body under the visceral ganglia. These nipple-shaped apertures are covered by a unilayered cubic epithelium, whereas the gonoduct is covered by a ciliated epithelium and is very short.

A couple of mantle pockets with dwarf males are also related to the reproductive organs. The dwarf males develop when the planktonic pediveliger larvae move into these pockets (Fig. 3). The pockets are layered by a flattened epithelium, and their diameter and length depends on number of males inhabiting them. As a rule, males densely fill the whole cavity of the pockets, but their amount may vary from 0 to 105 per each female.

In the reproductive cycle of *Z. zenkewitschi* female four stages could be recognised: prespawning, spawning, postspawning, and growth stage. The prespawning stage may start at any time from the end of May and to the first half of summer with a peak between the end of June and the beginning of July, and lasts fifteen days. The ovary at this time has the greatest volume. Acini are closely packed, and the interacinal tissue is poorly developed. Late oocytes are localized near the ovary wall at this

stage, whereas the mature eggs, recognisable by their granular cytoplasm, are localised in the acinal lumen.

Spawning in *Z. zenkewitschi* occurs 2 - 5 times in summer from May to the end of September. The eggs get to the gill cavity after spawning. There, fertilization and subsequent embryonic development occur. The diameter of mature eggs is about 60 mm. They have very thin egg envelopes: the plasma membrane is covered by a vitelline coat and a jelly coat.

The postspawning stage lasts from September to November. The gonad has its minimal size in this period. The amount of acini and their diameter are reduced. From November this stage gradually passes in the growth stage, the longest in *Z. zenkewitschi* sexual cycle. During winter and early spring the acini continue to develop.

The peculiarities of the reproductive cycle of female *Z. zenkewitschi* are connected with larval brooding in the gill cavity. The "brooding volume" of the epibranchial cavity has a limited size not allowing to realise the entire female reproductive potential at once, thus the repeated spawning.

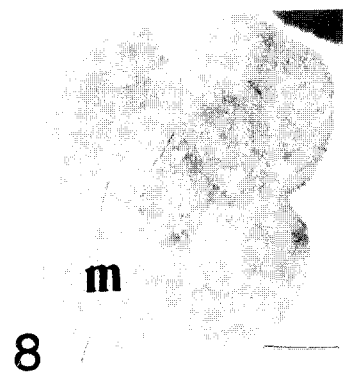
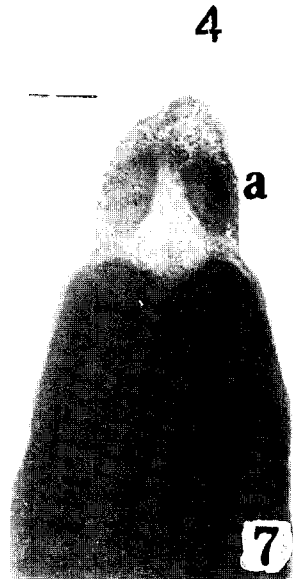
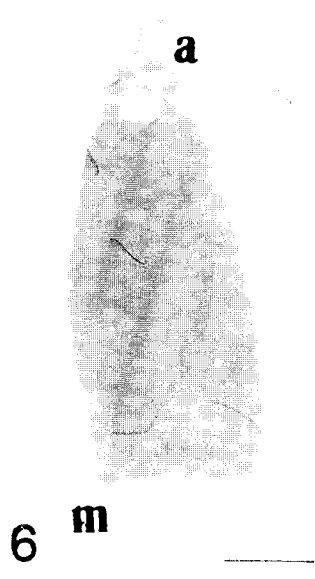
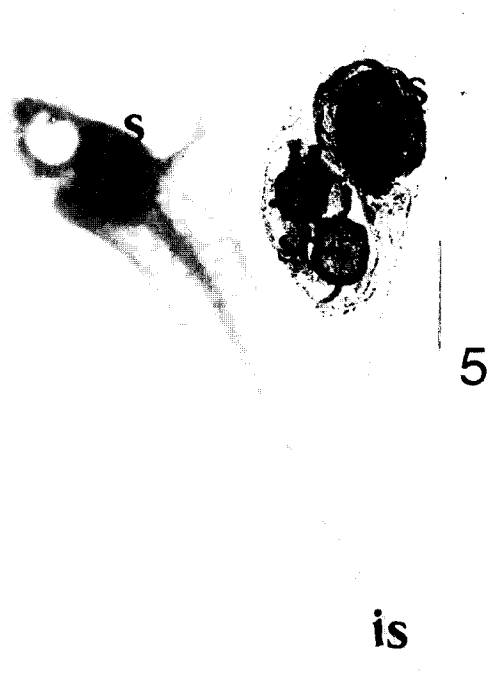
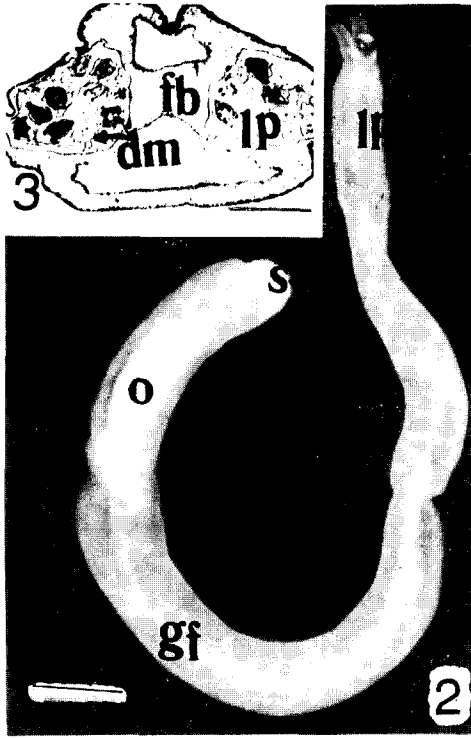
### 2. Male reproductive system

The unpaired testis of dwarf males is situated along the posterior wall of the visceral ganglia (Figs. 4, 5). The testis arises as a small group of cells near the visceral ganglia. In May the testis is represented by one acinus only with group of spermatocytes and solitary spermatogonia. At the end of spring, spermatids and spermatozoa can be found. In June the gametogenetic activity leads up to the end of spermiogenesis. A significant number of spermatids localizes near the acinal wall. In the centre of the testis, there are numerous spermatozoa.

The spermatozoa of *Z. zenkewitschi* have a lengthened conical head approximately 2.6 mm long, a midpiece with a diameter of approximately 0.9 mm and a long tail (Figs. 6, 7, 9). The four spherical mitochondria are located in the midpiece and surround the two centrioles, oriented at 90 degrees

to each other (Figs. 8, 9). The distal centriole, surrounded by a pericentriolar apparatus, gives origin to the flagellum. The sperm head is crowned

by an acrosome about 0.5  $\mu$ m long, consisting of two components: an apical acrosomal vesicle and periacrosomal granular material. The ratio of the



acrosome length to the head length is 0.23.

Four or five sperm bundles with a diameter of 100  $\mu$ m are present in dwarf male testis before spawning. They represent a special kind of spermatozeugma. They are shed during spawning through a very short excurrent siphon into the sea water filling the female lateral pockets. The sperm bundles are dissociated into the sea water very quickly (2 - 4 sec), and start moving immediately. The bundles periphery consists of sperm heads closely packed to each other, whereas the internal space is filled with sperm flagella. Within each female, there are males at different maturation stages: with mature sperm bundles, or with spermatocytes, or empty. The absence of strict synchronisation in the reproductive condition of males is apparently connected with the repeated spawning in females. Probably only some of the males take part to each spawning. We can not exclude the possibility that females are inseminated by extraneous males, "belonging" to other females; however, *Z. zenkewitschi* females are rarely encountered close each to other.

Sperm bundles are shed from the lateral mantle pockets via sea water into a space between the inhalant and the exhalant siphons of female. The inhalant siphon sucks the sperm with a current of water to the epibranchial cavity, where the spermatozoa pass through the gill filaments and

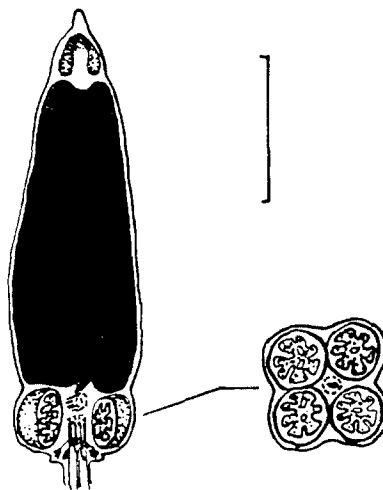


Fig. 9. Diagrammatic presentation of spermatozoon of *Z. zenkewitschi*.  
Bar - 1 mm.

fertilises the eggs there.

## DISCUSSION

The development and function of the sexual apparatus, as well as gamete formation, are affected by the external factors of environment and the internal condition of the organism. In his useful review Chieffi (1984) reminds us that as early as 1784 Spallanzani noted a periodicity in animal

Fig. 2. *Z. zenkewitschi* female observed by light microscopy. gf - gill filaments; lp - lateral pockets with dwarf males; o - ovary; s - shell. Bar - 0.5 cm.

Fig. 3. Transverse section of the female *Z. zenkewitschi* in the region of lateral pockets. dm - dwarf males; fb - female body; lp - lateral pockets. Bar - 1 mm.

Fig. 4. Dwarf male of *Z. zenkewitschi* observed by light microscopy. is - incurrent siphon; s - shell; sb - sperm balls. Bar - 100  $\mu$ m

Fig. 5. Anterior part of the mature dwarf male of *Z. zenkewitschi*. s - shell; sb - sperm balls. Bar - 100  $\mu$ m.

Fig. 6. Longitudinal section of the sperm head of *Z. zenkewitschi*. a - acrosome; m - mitochondrion. Bar - 0.5  $\mu$ m.

Fig. 7. Longitudinal section of the anterior part of the spermatozoon *Z. zenkewitschi*. a - acrosome; Bar - 0.2  $\mu$ m.

Fig. 8. Transverse section of the midpiece region of spermatozoon. m - mitochondrion. Bar - 0.2  $\mu$ m.

reproduction. Any unusual manifestation of the animal reproduction, especially adaptive significance of sexuality, have since attracted the attention of biologists (Csermely, 1984; Sara, 1984; Bremermann 1985). The problem of evolution of sexual conditions in the animal world is one of the fascinating chapters of general zoology.

It is a common knowledge that most of gonochoric molluscs has external. However, in some species, the males shed sperm into water from which it is sucked in the mantle cavity of female by the inhalant siphon. The eggs are shed also in the mantle cavity, and are localised among the gill filaments where fertilization occurs. Such type of insemination is named external-internal (Gilyarov, 1958; Jamieson, 1987).

The fertilization in teredinids may occur in several ways (Popham, 1974, 1975a,b; Turner, 1984, 1988; Turner *et al.*, 1986):

1) Eggs and sperm are released in sea water, where fertilization takes place. This is a typical external insemination.

2) Fertilization takes place in the epibranchial cavity, where sperm come from water through the inhalant siphon. This is an external-internal insemination or "ent aquatic fertilization" after Jamieson (1987). Because of the large densities of ship worms in the wood, sperm can get to the inhalant female siphon directly from the exhalant siphon of male.

As a variant of external - internal insemination the insemination in *Psiloteredo megotara*, living in floating wood, has sperm bundles, with an opaque coat. The female inhalant siphon takes them. *Teredora malleolus*, also living inside floating wood, extrudes a tooth paste-like cord of eggs that contains sperm.

3) *Z. zenkewitschi* has a specific kind of insemination. The sperm of dwarf males passes from the lateral mantle pockets to the water between the siphons, from which it is sucked by the inhalant siphon. It could be reasonable to expect

that *Z. zenkewitschi* has a purely internal insemination with modified spermatozoa (Franzen, 1956). However, this is not the case. Insemination of *Z. zenkewitschi* is a typical external - internal one and the sperm structure of *Z. zenkewitschi* corresponds to a sperm type, which is characteristic for all Teredinidae (Popham, 1974, 1975a). Their spermatozoa have a small conic head, crowned by an acrosome, a truncated cone nucleus, and mid-piece with four mitochondria and two centrioles.

According to Popham (1974, 1975a), the ship-worms with external insemination have a sperm cell with a large acrosome with an actin axial rod, ejected during the acrosomal reaction. On the contrary, *T. navalis*, *B. rochi*, and *B. australis*, which have an external-internal insemination, have a smaller acrosome, and the axial rod is reduced. The ratio of length of the acrosome to the length of the whole head in species with external insemination is 0.32 - 0.43, and in species with external-internal insemination it is 0.21 - 0.25. Such a ratio is in *Z. zenkewitschi* to 0.23.

The ultrastructure of *Z. zenkewitschi* sperm is similar to that of all members of the family Teredinidae. This confirms a conclusion, made by us earlier (Drozdov and Kas'yanov, 1985), that each family of bivalve molluscs has a specific sperm morphology. Within the family, there are minor differences in acrosomal structure. *Z. zenkewitschi* sperm size is close to that of species with external-internal insemination, but nevertheless they are the smallest among investigated species, and the axial rod is lacking.

So the permanence of sperm in the external environment is reduced to a minimum, and this results in a simplification of the structure of the sperm acrosomal apparatus.

### Summary

The reproduction of *Zachsisia zenkewitschi* - a shipworm with extreme sexual dimorphism was

studied. The female reproductive organs are formed by an ovary and a couple of mantle pockets containing dwarf males. The unpaired testis of dwarf males is situated near the visceral ganglia. Mature eggs are about 60  $\mu\text{m}$  in diameter and are covered by vitelline and jelly coats. The sperm of *Z. zenkewitschi* have a lengthened conical head approximately 2.6  $\mu\text{m}$  long, an acrosome about 0.5  $\mu\text{m}$  long, a midpiece with a diameter of approximately 0.9  $\mu\text{m}$ . Sperm bundles are shed into a space between the incurrent and the excurrent siphons of female. The fertilization takes place in the epibranchial cavity.

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