# Microscopic Anatomy and Fine Structure of the Skeleton-Heart-Kidney Complex in *Saccoglossus mereschkowskii* (Hemichordata, Enteropneusta): 1. Stalk Skeleton

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**Abstract**—This is the first paper of the series devoted to the microscopic anatomy and fine structure of the skeleton—heart—kidney complex in the acorn worm *Saccoglossus mereschkowskii* Wagner 1885. The skeleton of *S. mereschkowskii* consists of the unpaired anterior plate (lamina imparis) and two posterior horns (corni). The anterior plate bears a pair of lateral wings (alae laterales), the midventral keel (carina ventralis), the central fossa (fossa centralis) with the bordering ridge (crista circumflexa fossae centralis), two symmetrical supporting saucers (subiculi), and the conical rostrum (rostrum). The skeleton is an accretion (overgrowth) of the basal lamina between the epidermis and the endodermal epithelium of the buccal diverticulum (in the anterior part) and between the endodermal epithelia of the buccal diverticulum and the gut (in the posterior part) and consists of collagen fibers, mostly longitudinal. In all representatives of the Enteropneusta studied to date, the skeleton is a wishbone-like structure with the unpaired anterior plate and paired posterior horns, but its components widely vary in shape between species. In the family Harrimaniidae, the horns are long, and the anterior plate is rod-shaped. In the Ptychoderidae, the horns are short, and the anterior plate is rectangular. In the Spengelidae, the skeleton has an intermediate shape.

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# INTRODUCTION

The Enteropneusta are a relatively small group of marine invertebrates consisting of about 70 species according to recent data (Ruppert et al., 2004). These animals are of major interest to biologists, having been regarded as the nearest relatives of chordates since the time of Bateson (1886) and Schimkewitsch (1889). Today, however, many specialists consider them to be closer to echinoderms rather than to chordates (Tagawa et al., 1998; Peterson et al., 1999a, 1999b; Shoguchi et al., 1999; Cameron, 2000; Janies, 2001).

Current knowledge of microscopic anatomy and fine structure of the Enteropneusta are based mainly on classic studies performed in the late 19th—early 20th centuries. Against the background of numerous recent publications on the molecular biology of the Hemichordata, their morphological organization remains poorly known. Therefore, the analysis of microscopic anatomy and fine structure of the Hemichordata and Enteropneusta is a highly relevant task for zoologists.

The purpose of this study was to reconstruct the microscopic anatomy and fine structure of the skele-ton-heart-kidney complex in the hemichordate *Saccoglossus mereschkowskii*.

## MATERIAL AND METHODS

Adult acorn worms *S. mereschkowskii* were collected by divers from silty bottom sediments at depths of 4–10 m in the vicinity of the White Sea Biological Station, Moscow State University (village of Primorskii, Louhi district, Republic of Karelia), in Kandalaksha Bay of the White Sea in July 2005, 2006, and 2007. Studies on the skeleton–heart–kidney complex were performed using only the anterior body segment including the proboscis, collar, and a small anterior fragment of the trunk.

Specimens for histological analysis were fixed in Bouin's fluid and stored in 70% ethyl alcohol. After dehydration in ethyl alcohol solutions of increasing concentrations (without xylene), specimens were embedded in paraplast to prepare serial 7- $\mu$ m sections. On the whole, four complete series of sections of *S. mereschkowskii* anterior body end were prepared and analyzed under a light microscope. Characteristics of the corresponding four specimens are shown in the table.

Histological sections were photographed under a Zeiss Axioplan 2 photomicroscope (Carl Zeiss, Germany). Reconstructions of the skeleton, buccal diverticulum, and the whole skeleton—heart—kidney complex were made on the basis of serial sections, by making their proportional copies from plasticine sheets and superimposing them on each other to obtain

Speci- men no	Proboscis diameter, mm	Collar length, mm	Collar diameter, mm	Section plane
1	1.70	1.45	1.78	Transverse
2	1.10	1.56	1.20	Sagittal
3	1.24	1.30	1.35	Sagittal
4	1.05	0.96	1.02	Frontal

Characteristics of *S. mereschkowskii* specimens studied by histological methods

three-dimensional models of the above organs (the His method of reconstruction from projections). The models were used to make drawings of the organs.

For ultrastructural analysis, the specimens were placed into the fixative and cut into fragments 1.5- to 2-mm fragments, each including the posterior part of the proboscis, the whole collar, and a small anterior fragments of the trunk. These fragments were fixed in 2.5% glutaraldehyde solution in 0.1 M sodium cacodylate buffer (pH 7.35) with 0.1 M sucrose and postfixed in 1%  $OsO_4$  solution in 0.1 M sodium cacodylate buffer (pH 7.36) for 1 h.

For scanning electron microscopy (SEM), the postfixed fragments were dehydrated and dried in a critical point dryer. Their analysis was performed under a JSM-6380LA microscope. Two specimens of *S. mereschkowskii* were studied by this method.

The material for transmission electron microscopy (TEM) was embedded in Epon or Araldite resin. Ultrathin sections were contrasted with ammonium uranyl acetate and lead citrate and examined under a JEOM-1011 or JEM-100B microscope. Three specimens of *S. mereschkowskii* were analyzed in this way. Both SEM and TEM studies were performed at the Interdepartmental Laboratory of Electron Microscopy, Moscow State University.

## RESULTS

#### Microscopic Anatomy

The skeleton of *S. mereschkowskii* is a Y-shaped organ located in the stalk (Figs. 1, 2). It consists of the unpaired anterior plate (lamina imparis skeletale), which extends through the stalk to the proboscis, and two posterior horns (corni), which extend backward, to the collar region, on both sides of the gut (Figs. 2, 3).

In essence, the skeleton is an accretion (overgrowth) of the basal lamina that separates endodermal epithelia of the pharynx and buccal diverticulum in the zone of posterior horns and endodermal epithelium of the buccal diverticulum from ectodermal epithelium of the proboscis in the zone of the anterior plate (Figs. 1, 4). Its length in the largest specimen studied was 1.2 mm, with the horns being 0.7 mm long (i.e., about 55% of the total length).

The anterior part of lamina imparis consists of the cone-shaped rostrum with symmetrical supporting saucers (subiculi) on both sides (Figs. 3, 4e and f). The rostrum fits into the corresponding cavity in the buccal diverticulum (see below). Subiculi underlie paired lateral infolds of the proboscis epithelium and, on the other side, fit into depressions in the buccal diverticulum. The central fossa (fossa centralis) with the bordering ridge (crista circumflexa fossae centralis) is located on the ventral side of the skeleton, between the bases of subiculi (Figs. 3, 4d). Lateral wings (alae laterales) extend along the skeleton from the bases of subiculi to the narrowest part of the anterior plate (Figs. 3, 4c and d). The midventral keel (carina ventralis) begins at the posterior edge of fossa centralis and passes along the central axis of the anterior plate (Figs. 3a, 4b). The dorsal surface of the anterior plate, convex in cross section, has no specific relief elements (Figs. 3b, 4).

At the anterior end of the collar, the skeleton is divided into two symmetrically curved horns tapering toward their posterior ends, which extend to the middle of the collar region (Figs. 2, 3). The horns are flattened in cross section, with the plane of each horn forming an angle of about  $45^{\circ}$  with the dorsoventral plane of the animal body (Figs. 4a and b).

### Fine Structure

The skeleton of *S. mereschkowskii* is formed due to the overgrowth of the basal lamina separating two epithelia: the endodermal epithelia of the gut and of the buccal diverticulum (in the zone of horns) and the endodermal epithelium of the buccal diverticulum and the ectodermal epithelium of the proboscis (in the zone of unpaired anterior plate). Such basal laminae exist in all zones of contact between two epithelia: ectodermal and endodermal, ectodermal and mesodermal (Figs. 5, 6a and d), endodermal and mesodermal (Fig. 6b), or two mesodermal epithelia (Fig. 6c). However, the fine structure of the skeleton somewhat differs from that of ordinary basal laminae.

Basal laminae between neighboring epithelia in *S. mereschkowskii* are usually 0.8 to 1.4  $\mu$ m thick. In electron micrographs, they have a complex structure and basically consist of three layers.

Two of them are basal plates (about 70 nm thick) underlying each of the two epithelia. Cells are attached to the basal plates by means of hemidesmosomes, which are visualized as aggregations of electron-dense material adjoining the cytoplasmic membrane from the inside (Fig. 5b). The intermediate layer, usually 0.6 to 1.2  $\mu$ m thick, is the reticular matrix formed by collagen fibers about 20 nm in diameter, which show characteristic striation in some micrographs (Fig. 6a). As can be seen in transverse sections, collagen fibers are oriented at a large angle to the body axis, i.e., arranged in an annular pattern. In some cases, fibers in the reticular matrix appear to form two layers, with collagen fibrils of one layer extending at an angle to



Fig. 1. Sagittal section through the proximal part of proboscis and stalk of S. mereschkowskii. Scale bar 500 µm.

those of the other layer (Fig. 6). Processes of connective tissue cells can often be found within the reticular layer (Fig. 6a).

In addition to collagen fibers, blood lacunae can often be found in the reticular layer of the basal lamina. These lacunae are filled with large hemoglobin molecules, which are visualized in TEM preparations as dark particles arranged in regular parallel rows (Figs. 6a and b).

As noted above, *S. mereschkowskii* is an accretion (overgrowth) of the reticular matrix (Figs. 7a, 8) between endodermal epithelia of the gut and buccal diverticulum (in the zone of horns) and between the endodermal epithelium of the buccal diverticulum and the ectodermal epithelium of the proboscis. The basal plate of the gut epithelium is about 60 nm thick (Fig. 7b).

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The basal plate underlying epidermal cells of the proboscis epithelium is 70 nm thick (Fig. 6d). Epithelial cells adjoining either plate are attached to it with hemidesmosomes. The overgrown reticular matrix is filled with collagen fibrils, with their arrangement changing in different parts of this layer: fibrils adjoining the basal plates are oriented randomly, at different angles to the body axis, whereas those in the middle of the matrix extend mainly along this axis (Fig. 8a).

In addition, the reticular matrix of the skeleton contains numerous cavities, which are also oriented longitudinally. In some cases, cell processes passing through such cavities can be detected (Fig. 8). It may well be that these cavities are actually canals that previously contained the processes of cells producing substances for skeleton formation.



**Fig. 2.** Position of the skeleton (three-dimensional image) in the body of *S. mereschkowskii*: (a) ventral view; (b) lateral view, the ventral side on the right. Scale bar 500 μm.

## DISCUSSION

The skeletons of all known species of the Enteropneusta have a common pattern (Fig. 9) and consist of the unpaired anterior plate and paired posterior horns (Spengel, 1893; Van der Horst, 1939). The shape of the anterior plate markedly differs between representatives of different genera. Thus, the anterior plate is rectangular in *Balanoglossus aurantiacus* (Van der Horst, 1939); tapering off anteriorly, toward the bases of supporting saucers, in *Schizocardium peruvianum* (Spengel, 1893); rod-shaped, thickening posteriorly in *Saccoglossus caraibicus* (Van der Horst, 1939); and triangular, broadening anteriorly in *S. kowalevskii* (Spengel, 1893). The unpaired anterior plate of *S. mereschkowskii* is rod-shaped, thickening anteriorly.

In all known Enteropneusta, the unpaired anterior plate bears carina ventralis and alae laterales developed to different degrees. Its anterior part forms symmetrical subiculi, which strongly differ in shape even between closely related species. The rostrum in the form of a short projection has been described in almost all species except *S. kowalevskii* (Spengel, 1893). The rostrum of *S. mereschkowskii* is well developed and conspicuous, and terminal parts of subiculi are bent toward the posterior body end (in only one among all Enteropneusta species studied). The anterior plate of *Harrimania planktophilus* is elongated posteriorly, far into the collar zone, with the bifurcation point lying beyond its middle part (Cameron, 2000). In *S. mereschkowskii*, by contrast, its posterior end barely enters the most anterior part of this zone.

The horns of the skeleton are long, extending far to the collar zone, in most species of the families Spengelidae (Spengelia, Glandiceps) (Willey, 1897) and Harrimaniidae (Harrimania, Saccoglossus) (Spengel, 1893; Cameron, 2000). In S. mereschkowskii, they reach the middle of the collar zone. The horns of most species are symmetrically bent processes tapering posteriorly; only *B. aurantiacus* (Ptychoderidae) has horns with thickened posterior ends (Van der Horst, 1939). The horns of B. aurantiacus extend far apart from each other. Saccoglossus kowalevskii, S. caraibicus, and S. mereschkowskii (described here) have horns with distal ends bent ventrally and toward each other so that this part of the skeleton is lyre-shaped. Species of the genera Xenopleura (Harrimaniidae), Balanoglossus, Ptvchodera, and Glossobalanus (Ptvchoderidae) have skeletons with short horns (Cameron, 2000). The long skeleton of Saxipendium coronatum is similar to that characteristic of the family Spengelidae but differs from it in being slightly bent (Woodwick and Sensenbaugh, 1985). The skeleton of Glandiceps gingdaoensis



Fig. 3. The skeleton of S. mereschkowskii: (a) ventral view, (b) lateral view (at an angle). Scale bar 500 μm.

has long horns that extend far into the collar zone and reach the middle of its posterior half (Jianmey and Xinzheng, 2005).

Some other structural features of the skeleton in *S. mereschkowskii* have no counterparts in other species. It appears, however, that these features are not unique to this species, but the situation is explained by insufficient knowledge of skeletal morphology in other representatives of the Enteropneusta. Thus, the presence of fossa centralis is not indicated in the descriptions of most species, but the skeleton of *S. caraibicus* in the picture by Van der Horst (1939) has an oblong ventral depression that may well be a homolog of fossa centralis found in *S. mereschkowskii*.

Skeletons of three species studied in the family Harrimaniidae (*S. caraibicus, S. kowalewskii*, and *S. mereschkowskii*) are obviously similar in having lyre-shaped horns longer (at least, no shorter) than the unpaired anterior plate. In *Sch. peruvianum* (Spengelidae), the horns are straight and deviate from each

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other at an acute angle. In *B. aurantiacus* (Ptychoderidae), the unpaired anterior plate is broad, while the horns are relatively short and deviate from each other at an obtuse angle. It may well be that each family of the class Enteropneusta is characterized by a certain form of the skeleton (retaining its general pattern characteristic of the class as a whole), with its individual structural elements being species-specific. The structural diversity of the skeleton indicates that its morphological features may be of major significance for studies on the taxonomy of Enteropneusta.

The skeleton of all hemichordates is anchored to the collar with its posterior horns, with lamina imparis passing through the stalk and ending in the most proximal part of the proboscis. Therefore, the term "proboscis skeleton" widely used in the international literature is not quite correct. The wide anterior part of the skeleton (with subiculi and rostrum) indeed supports the base of the proboscis together with organs formed by soft tissues (first of all, the buccal diverticulum).



**Fig. 4.** Fragments of serial transverse sections through (a, b) the collar, (c) stalk, and (d–f) proboscis of *S. mereschkowskii* (from the posterior to the anterior body end). Scale bar 100  $\mu$ m.

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**Fig. 5.** (a) Fragment of the perihaemal coelom and (b) basal lamina between the dorsal nerve tube and perihaemal coelom of *S. mereschkowskii*. Arrows indicate basal plates of neighboring epithelia. TEM micrographs (JEM-100B). Scale bars: (a) 1  $\mu$ m, (b) 0.5  $\mu$ m.

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Fig. 6. Fine structure of basal laminae (a) between dorsal nerve tube and coelothelium of the collar coelom, (b) between epithelium of the buccal diverticulum and coelothelium of the proboscis coelom, (c) between coelothelia of the proboscis and pericardial coeloms, and (d) between epidermis and the proboscis coelom. Arrows indicate basal plates of neighboring epithelia. Transverse sections, TEM micrographs (JEM-100B). Scale bar  $0.5 \,\mu$ m.



Fig. 7. (a) The skeleton of *S. mereschkowskii* with attached cells and (b) basal lamina underlying epithelium in the proximal part of the buccal diverticulum. Arrows indicate basal plates. Transverse sections, TEM micrographs (JEM-100B). Scale bars: (a) 1  $\mu$ m, (b) 0.5  $\mu$ m.

However, the sturdiest part of the skeleton—the unpaired anterior plate—lies within the narrow stalk between the collar and proboscis, serving as the main structure reinforcing the stalk and preventing its breakage during animal movements within bottom sediments. Hence, the term "stalk skeleton" appears to be more adequate. Although the horns as the most posterior part of the skeleton are located in the collar region, their morphology is connected in a certain way with the structure of the proboscis, which is evidence for the structural unity of all skeletal elements. As a rule, species with a short spherical or egg-shaped proboscis (*Balanoglossus, Glossobalanus, Ptychodera, Schizocardium*)

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Fig. 8. Fine structure of the skeleton of *S. mereschkowskii*. Transverse sections, TEM micrographs (JEM-100B). Scale bars: (a) 1  $\mu$ m, (b) 0.1  $\mu$ m.

have a relatively small skeleton with short horns (shorter than the anterior plate) ending in the most anterior part of the collar, whereas the horns of species with a long, cone-shaped proboscis (*Glandiceps, Saccoglossus, Spengelia*) are longer than the anterior plate.

Histologically, the skeleton is an accretion (overgrowth) of basal lamina between two epithelia, i.e., a modification of the structure that in other body regions contains blood lacunae or consists of a thin layer of connective tissue matrix separating two epi-



**Fig. 9.** Skeletons of different enteropneusts: (a) *Balanoglossus aurantiacus* (from Van der Horst, 1939), (b) *Schizocardium peruvianum* (from Spengel, 1893), (c) *Saccoglossus caraibicus* (from Van der Horst, 1939), (d) *S. kowalevskii* (from Spengel, 1893), and (d) *S. mereschkowskii* (our study).

thelial sheets. Many specialists (e.g., Pardos and Benito, 1988) emphasize the close relationship between skeletal structures and blood in enteropneusts. Special histochemical studies confirm that the skeleton of these animals is formed of collagen (Pardos and Benito, 1982, 1988; Balser and Ruppert, 1990). In S. kowalevskii, collagen fibers in the skeleton are packed more densely in its peripheral parts than in the center, and processes of connective tissue cells can be found amid them (Benito and Pardos, 1997). The same is true of S. mereschkowskii, with collagen fibers and cell processes being oriented mainly in the anteroposterior direction. We have revealed cavities within its skeleton, which appear to be canals left by the processes of connective tissue cells. These cavities are also oriented anteroposteriorly. Similar cavities were described in the gill bars of Glossobalanus minutus, but their arrangement in these structures was random (Pardos and Benito, 1907).

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Abbreviations in figures: (bd) buccal diverticulum, (cl) collar, (clc) collar coelom, (hds) hemidesmosomes, (phr) pharynx, (ds) desmosomes, (clf) collagen fibers,  $(clf^*)$  collagen fibers lying at different angles to the body axis, (bld) blood (hemoglobin molecules), (gt) gut, (mtc) mitochondria, (dnt) dorsal nerve tube, (phc) perihaemal coelom, (bl) basal lamina, (lc) longitudinal cavities, (skl) skeleton, (st) stalk, (ctc) processes of connective tissue cells, (tr) trunk, (pb) proboscis, (emc) epithelial muscle cells, (ger) cisterns of granular endoplasmic reticulum; (alsk) alae laterales skeletale, (*cor*) corni, (*crc*) crista circumflexa fossae centralis, (*cv*) carina ventralis, (*fc*) fossa centralis, (*lisk*) lamina imparis skeletale, (*rsk*) rostrum skeletale, (*sub*) subiculi.

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