

Is the Gill Skeleton of Acorn Worms (Enteropneusta) Similar to the Gill Skeleton of Amphioxus (Cephalochordata)?

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Received April 23, 2020; revised April 30, 2020; accepted April 30, 2020

Abstract—The gill skeleton of the enteropneust *Saccoglossus mereschkowskii* consists of a series of tridents. The central prong of each trident bifurcates in its ventral end. The most anterior gill skeletal element has a simple horseshoe shape. Homologues of the elements of the enteropneust gill apparatus were found in the structure of the gill apparatus of Cephalochordata. The organization of the gill skeleton of Enteropneusta and Cephalochordata can be derived from the metameric horseshoe-shaped elements. The similarity of the structure of the gill skeleton of Enteropneusta and Cephalochordata contradicts a common “upside-down theory” of the origin of Chordata.

Keywords: gill slits, hemichordates, *Saccoglossus*, Branchiostoma, chordate upside-down theory, deuterosomes

DOI: 10.1134/S001249662005004X

Gill slits are unique organs occurring exclusively in two groups of animal kingdom, namely, hemichordates and chordate. Features of the skeleton structure and development of hemichordates are essential for understanding the origin of the chordate. However, no attempt has been made to date to compare gill skeleton structure of Hemichordata and Chordata. In his review, Ruppert [1] offers most elaborate comparison of organization of gills between hemichordates and lower chordates with respect to a shape of gill slits and gill pores, character of their development in ontogenesis, support of gill slits by collagenous skeletal elements (rods), occurrence of synapticles, presence of coelomic cavities in gill bars, mono- and multiciliated nature of endoderm lining the gill slits, and arrangement in it of glandular cells, blood-flow direction in gills, and occurrence in the latter of podocytes and neural elements. The cited review, however, is missing the comparison between shapes of the gill skeletal elements of hemichordates to lower chordates.

The goal of the present study was to reconstruct the organization of gill skeleton in hemichordate specimen of enteropneust *Saccoglossus mereschkowskii* and make an attempt to compare structure of gill skeleton in hemichordates and chordates.

Adult specimens *S. mereschkowskii* were collected from silty sediments at a depth of 4–10 m in the proximity to White Sea Biological Station of Moscow State University in the Kandalaksha Gulf of the White Sea.

Animal fragments were fixed in Bouin solution, dehydrated in increasing alcohol series, and embedded in paraplast blocks. A series of frontal sections of *S. mereschkowskii* 7 μm thick each was made for subsequent 3D reconstruction. The sections were stained with hematoxylin and captured using a Zeiss Axioplan 2 photomicroscope. Simplified schematic drawings presented here were based on 3D reconstruction in the Amira version 6.4.0 software.

Importantly, to avoid confusion, herewith the attribute “horseshoe-shaped” applies only to skeletal elements, while “U-shaped” describes gill slits exclusively.

Skeletal elements, supporting a shape of gill slits in pharynx of *Saccoglossus mereschkowskii*, are represented by thickenings of extracellular matrix in pharyngeal endoderm folds. Each skeletal element except for the first most anterior pair of skeletal elements has a shape of trident with prongs directed downward to the ventral side (Fig. 1). The first pair of skeletal elements has a horseshoe-like “two-pronged” shape with prongs of different lengths (Fig. 2a). The reconstruction shows clearly that the trident shape is due to fusion of such horseshoe-shaped elements in all other gill skeletal elements. As a result of this fusion, the central prong doubles, becomes stronger, elongates, and bifurcates at the end. Lateral, or marginal, prongs are simple and shortened (Fig. 2a).

Gill slits in pharyngeal endoderm of *S. mereschkowskii* are U-shaped and separated from each other by a so-called primary gill bars or septa (Fig. 1). Their U-shape is achieved due to partial secondary gill bars (or tongue bars) (Fig. 1). Both primary and secondary

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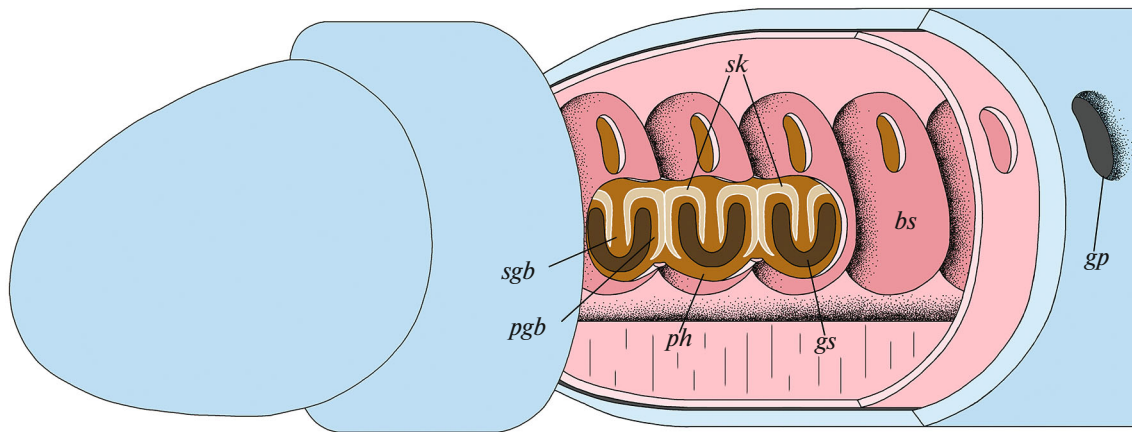


Fig. 1. Reconstruction of gills in Enteropneusta *Saccoglossus mereschkowskii*, demonstrating the organization of gill skeleton: (bs) branchial sac; (gp) gill pore; (gs) gill slit; (pgb) primary gill bar; (ph) pharyngeal endoderm; (sgb) secondary gill bar; (sk) skeletal elements.

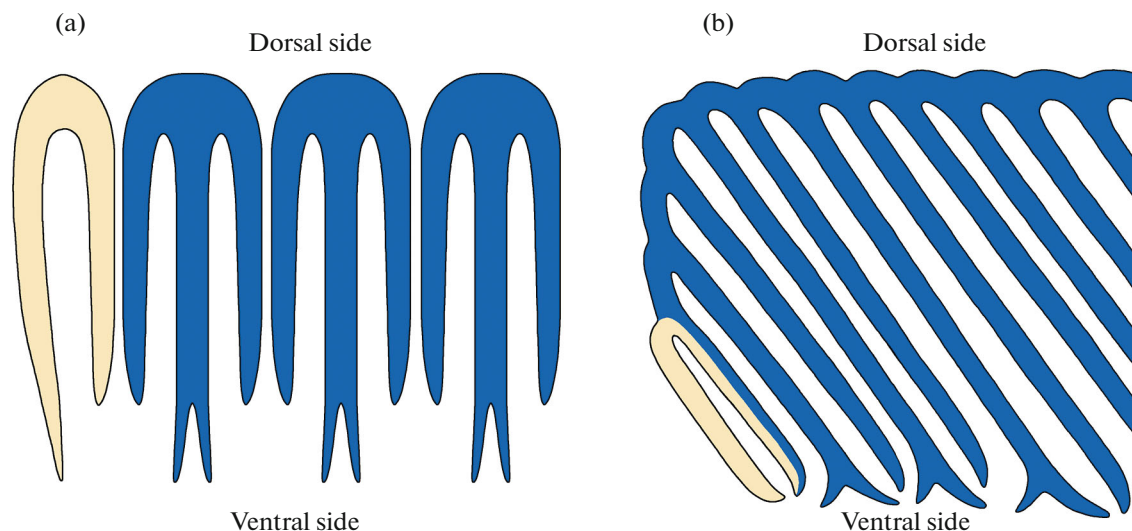


Fig. 2. Schematic of gill skeleton structure in (a) *Saccoglossus mereschkowskii* (our data) and (b) *Branchiostoma* (modified from [6], synapticles are not shown).

gill bars are supported by skeletal elements. The central prongs of the gill skeletal elements, which are bifurcated at the end, pass within the primary gill bars. Marginal prongs support the secondary gill bars. Each secondary gill bar includes one marginal prong from preceding skeletal element and one marginal prong from the succeeding skeletal element. Thus, each skeletal trident supports posterior half of one gill slit and anterior half of the adjacent gill slit (Fig. 1).

Simple unfused horseshoe-shaped skeletal elements of the first pair support secondary bar of the first gill slit by their posterior shorter prong, while their anterior elongated prong is merely anchored within pharyngeal endoderm fold.

All enteropneusts are characterized by trident shape of their gill skeleton elements. In Harrimaniidae and a number of species of Spengelidae, secondary gill bars hang down into gill slit, while the gill skeletal elements have a shape of simple tridents. In Ptychoderidae, gill slit openings are partitioned horizontally by synapticles running between the primary and secondary gill bars [2].

In other hemichordate class, Pterobranchia, the gill apparatus is very simplified in comparison with enteropneusts. The *Cephalodiscus* spp. has a single pair of the oval gill slits (not U-shaped) in the absence of secondary bars [3, 4]; gill pores are altogether absent in *Rhabdopleura* [5]. There are no skeletal structures to support gill slits in *Cephalodiscus*. Only branchial channels, which connect pharyngeal cavity with exter-

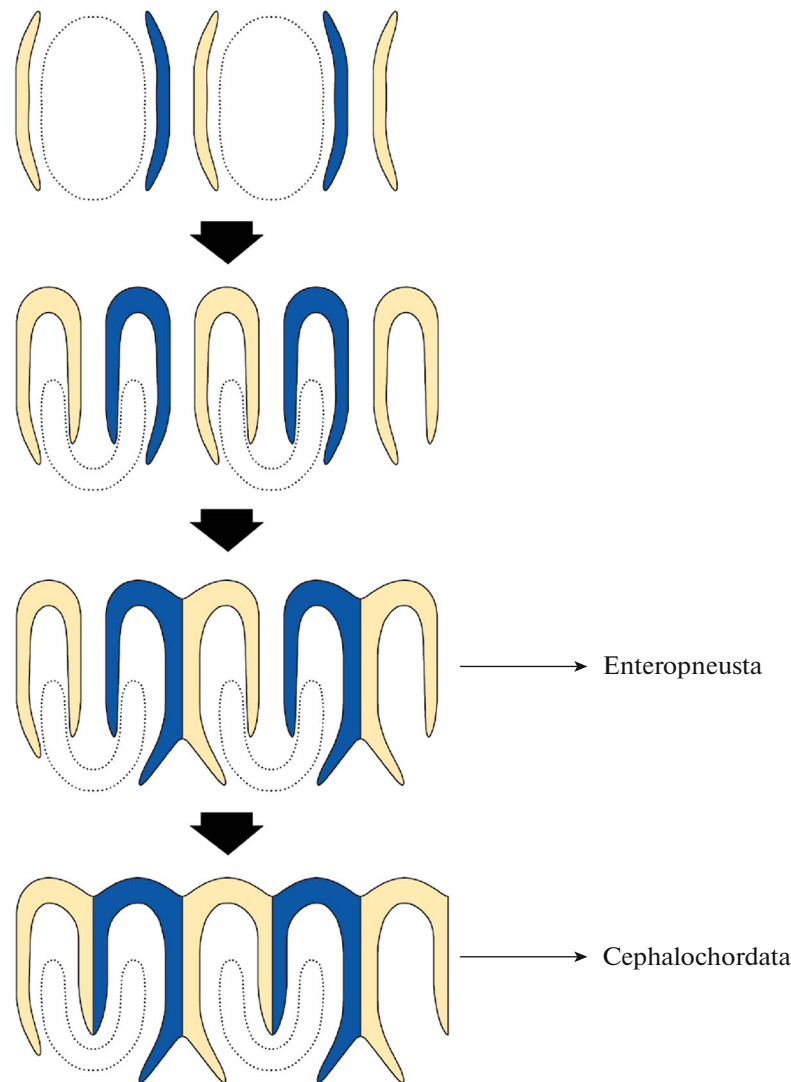


Fig. 3. Hypothetical schematic of gill skeleton evolution in Enteropneusta and Cephalochordata. Dotted lines show the gill slits.

nal environment, are lined with vacuolated cells [3], which appear to provide the necessary stiffness to the oval gill slit walls.

Enteropneusts and lower chordates, namely Cephalochordata, display apparent similarities of gill skeleton (Fig. 2). The gill skeletal elements of enteropneusts and amphioxus are also similar histologically and in chemical composition. In both cases, the gill skeletal elements are the thickenings of extracellular matrix, lacking cells and containing collagen and proteoglycans [7, 8]. In both Enteropneusta and Cephalochordata, the gill skeleton is composed of metameric horseshoe-shaped elements fused to one another (Fig. 2b). The ends of the horseshoe elements are directed toward the ventral side in both groups. In enteropneusts, horseshoe elements fuse in pairs to form tridents. As a result of such fusion, central elon-

gated prongs bifurcated at the end alternate with shortened unfused marginal prongs (Fig. 2a). In Amphioxus, all gill skeletal elements fuse to each other to form common lattice throughout the pharynx. At the same time, amphioxus shows similar alternation of bifurcated and simple non-bifurcated prongs (Fig. 2b). This suggests that this branchial lattice in amphioxus has evolved from the same variant preserved in the modern enteropneusts. The central prongs of the enteropneust trident (bifurcated at the end) correspond to the prongs of the amphioxus lattice with the similar bifurcations at the end. The alternating simple prongs of the amphioxus lattice correspond to unfused marginal prongs of the enteropneust gill skeleton (Fig. 3). Interestingly, the most anterior element of enteropneust gill skeleton preserved its horseshoe shape. This most anterior horseshoe-shaped element can be singled out easily in gill skeleton of amphioxus (Fig. 2).

Figure 3 shows a hypothetical schematic diagram of gill skeleton evolution in Enteropneusta and Cephalochordata.

The gill slits of enteropneusts appear as the oval openings (they are preserved in this form in Pterobranchia). During the ontogenesis, the gill slits become U-shaped due to the tongue bar, containing the unfused skeletal marginal prongs, grows into each slit from the dorsal side [9]. Similarly, the gill slits emerge as oval opening in amphioxus [10]. Developmentally, the gill slits acquire the U shape due to intergrowth of gill skeletal prongs, lacking bifurcation, from dorsal side. In amphioxus, these prongs continue the growth and partition a U-shaped gill slit into two separate slits.

The foregoing considerations are of some interest in regard to the “upside-down theory” of chordates. The modern variant of this hypothesis was proposed by one of the present authors [11]. The hypothesis was later supported by data on expression of homeobox genes [12]. Currently, the upside-down theory of chordates is regarded as one of the modern zoology paradigms [13].

If we consider chordates to be upside-down enteropneusts, then the open ends of the horseshoe-shaped elements of the gill skeleton should point in different directions. If they are directed ventrally in enteropneusts, they should point in dorsal direction in upside-down chordates. The same applies to the gill slits. Enteropneusts have open ends of the U-shaped gill slits directed dorsally. The open ends of the U-shaped gill slits are supposed to be directed ventrally in upside-down chordates. Both groups, however, feature open ends of the horseshoe-shaped skeletal elements with the ventral orientation and open ends of the U-shaped gill slits directed dorsally.

These facts contradict the upside-down theory of chordates. An attempt to continue giving steadfast support to treating the chordates as upside-down Enteropneusta on the basis that this theory is substantially supported by current data of developmental biology will result in situation where we have to accept that evolutionary fate of enteropneusts and chordates diverged at a stage when they had simple oval gill slits, after which the gill skeletons have been evolving in each group independently. In his review, Rupert arrives to the same conclusion based on comparison of other details in the gills structure of the deuterostomes [1].

ACKNOWLEDGMENTS

We are grateful to the anonymous reviewer of the manuscript for valuable corrections and assistance in achieving clarity of presentation of our ideas to readers.

FUNDING

Sampling of the material and its histological investigation were supported by the Russian Foundation for Basic Research, project no. 20-04-00909-a; 3D reconstruction and preparation of graphics and manuscript were supported by the Russian Science Foundation, project no. 18-74-10025.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest. All authors have read and approved the final version of the manuscript.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Translated by E. Kuznetsova