

# Arms versus Brachioles: Morphogenetic Basis of Similarity and Differences in Food-gathering Appendages of Pelmatozoan Echinoderms

S. V. Rozhnov

*Borissiak Paleontological Institute, Russian Academy of Sciences, Profsoyuznaya ul. 123, Moscow, 117997 Russia*

*e-mail: rozhnov@paleo.ru*

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**Abstract**—The similarity in the skeleton model of the brachiolar food-gathering system of Blastozoa and the arm system of Crinozoa, including the apical growth with enantomorphous displacement of skeletal elements, is explained by the primary organizing role of the radial ambulacral canals, which have the same branching model for ambulacral tentacles. The difference in the positions of brachioles and arms relative to the theca (exothecal and endothecal) is associated with the formation of the primary ambulacral tentacles directly on the body surface of the majority of Blastozoa, particularly, the closed vestibular cavity of crinoids. The supporting skeleton of brachioles arose as a branch of the plates covering the floor of the ambulacrum, if they were present, or formed similarly as a new formation outside the theca. The supporting skeleton of arms, brachials, developed as a result of the serial growth of plates positioned radially at the boundary of the aboral skeleton and tegmen formed due to the appearance of the vestibulum. The hypothesis of the inductive role of hydrocoel and its radial ambulacral appendages, which organize the arrangement of skeletal elements in the morphogenesis of echinoderms, enables the refinement of the principle of skeleton division into the axial and extraxial parts. The axial skeleton has a developmental model formed under the control of the radial ambulacral canals. Remaining skeleton is extraxial, subdivided into the symmetrized part arranged under direct or indirect organizing effect of the hydrocoel and unregulated, nonsymmetrized part, which is not connected initially with the influence of the hydrocoel.

**Keywords:** Echinodermata, Pelmatozoa, Crinoidea, ambulacral system, axial skeleton, extraxial skeleton, embryonic induction, arms, brachioles

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## INTRODUCTION

The echinoderms are one of a few faunal groups, evolutionary history of which in many respects and at different taxonomic levels is distinctly traced in the geological time from the very origin in the Cambrian. On the other hand, echinoderms, primarily echinoids, are a favorite experimental object of embryologists and developmental biologists. In echinoids, many ontogenetic details, including features of arrangement and expression of many genes have been revealed. Some features are known for members of other extant echinoderm classes (David and Mooi, 2014). The task of paleontologists is to apply these data to comprehension of evolutionary development of echinoderms, which display extremely wide morphological and taxonomic diversity of fossil forms. Although this task is usually connected with the opportunity of studying the skeleton preserved in fossils, it requires reconstruction of certain important features of the soft body. Paleontologists frequently fail to bear in mind that the skeleton is only a part of the organism, while the evolutionary process involves the organism as a whole. However,

geneticists usually overlook that the organism evolves as a whole system of successive developmental stages rather than a set of genes.

The present study considers developmental features of the skeletal parts of pelmatozoan echinoderms which are closely connected with the ambulacral system organizing their symmetry and determining growth features of particular appendages involved in food-gathering functions. These are the arms of crinoids and brachioles of other pelmatozoan echinoderms and also the “arm” of solutes (stalked echinoderms initially lacking radial symmetry), which shows some resemblance to the arms and brachioles and is important for understanding their morphogenesis.

Arms and brachioles can be very similar in morphology, but sharply differ in the relation to the theca (Fig. 1). It is generally believed that arms are endothecal appendages, which enclose the internal cavity of the theca (Fig. 1a), while brachioles are exothecal appendages, which deviate from the thecal surface (Fig. 1b) and lack elements of the thecal cavity (Ubahgs, 1978). Sprinkle (1973) developed this idea,

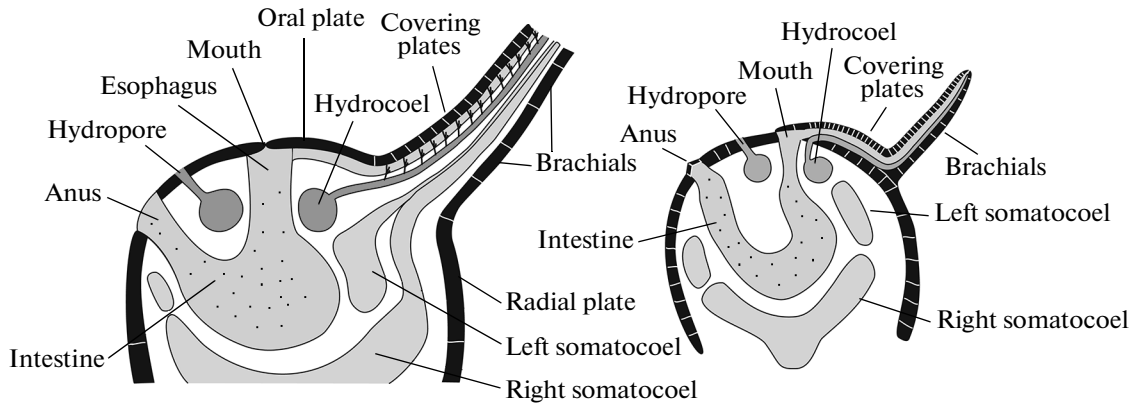


Fig. 1. Schemes of (a) endothecal structure of arms and (b) exothecal brachioles.

proposing that brachioles lacked radial ambulacral canals. On this basis, he divided all stalked echinoderms into two groups, Crinozoa only including the crinoid class and Blastozoa comprising all other former pelmatozoan echinoderms. Thus, the difference between food-gathering skeletal appendages, arms and brachioles, were the major criterion for the establishment of the two subphyla and rejection of the united subphylum of pelmatozoan echinoderms. Therefore, the analysis of morphological similarity and differences between two types of food-gathering appendages and features of their morphogenesis is crucial for the reconstruction of phylogeny and systematics of echinoderms and also provides a new insight into the extraxial theory of homology of the echinoderm skeleton developed by David and Mooi (1998).

#### MORPHOLOGY AND DEVELOPMENTAL FEATURES OF ARMS IN CRINOIDS

The internal soft parts of the arm are largely covered by the skeleton of the oral and aboral parts (Fig. 2). The aboral skeleton of the arm is its support formed of the brachial plates identical in size, arranged in one or two rows, and connected with each other by muscular and (or) ligamentous bands, which provide them with mobility. From the right or left side of each brachial, a nonbranching pinnule sometimes deviate; it is a reduced copy of the main branch of the arm. On the oral side, arms and pinnules are covered with two or more series of covering plates, among which there are two medial rows of ambulacra contacting with each other through a zigzag suture. Between the rows of ambulacra and brachial margins, one or two series of adambulacral plates are sometimes located. The ambulacral and adambulacral plates are located on the soft appendages lapetts on the margins of the ambulacral groove, which are able to open rising the covering plates and opening the food-gathering

groove located under them and coated with ciliated epithelium.

Just under the food-gathering groove, the arm contains the radial ambulacral canal, which gives rise to branches of the radial canal extending into pinnules, alternating on the right and left sides, with the step on each side usual equal to the length of two brachials (Fig. 3a). In pinnules, also alternating on the right and left sides, triads of ambulacral tentacles deviate, with the step as in covering ambulacral plates (Fig. 3b). Each triad consists of long, intermediate, and short tentacles. The long tentacle catches food particles with the use of sticky mucus; the intermediate, and short tentacles combine them in a bolus and transport into the food-gathering groove, where the ciliated epithelium moves it to the mouth (Meyer, 1982).

The arm of living sea lilies is a complex organ which includes derivatives of all of three coeloms, the nervous system, strong muscles, skeleton, and gonads (Fig. 2). This complex system of arm organs is easily to represent as an outgrowth of the theca along with parts of internal organs. Therefore, the arms of crinoids are named endothecal appendages (Ubaghs, 1978). Attention is drawn to the fact that, in cross section of the arm of *Antedon*, the food-gathering groove and ambulacral canal occupy a small volume, whereas its main part is occupied by strong muscles. It is possible that many Paleozoic crinoids lacked muscles in arms or they were very weak.

Although the radial ambulacral canal occupies a small part of the total volume of soft tissues of the arm, it is the major part of this organ, because if it were absent, the arm would not function as a food-gathering organ. The triads of tentacles alternately deviating from the radial canal on the right and left capture food particles with the help of mucus, form a bolus, and transfer it into the food-gathering groove leading to the mouth. The ciliated epithelium transports the bolus to the mouth, through which it gets into the digestive system. It becomes evident that the main role in the food-gathering system belongs to tentacles of

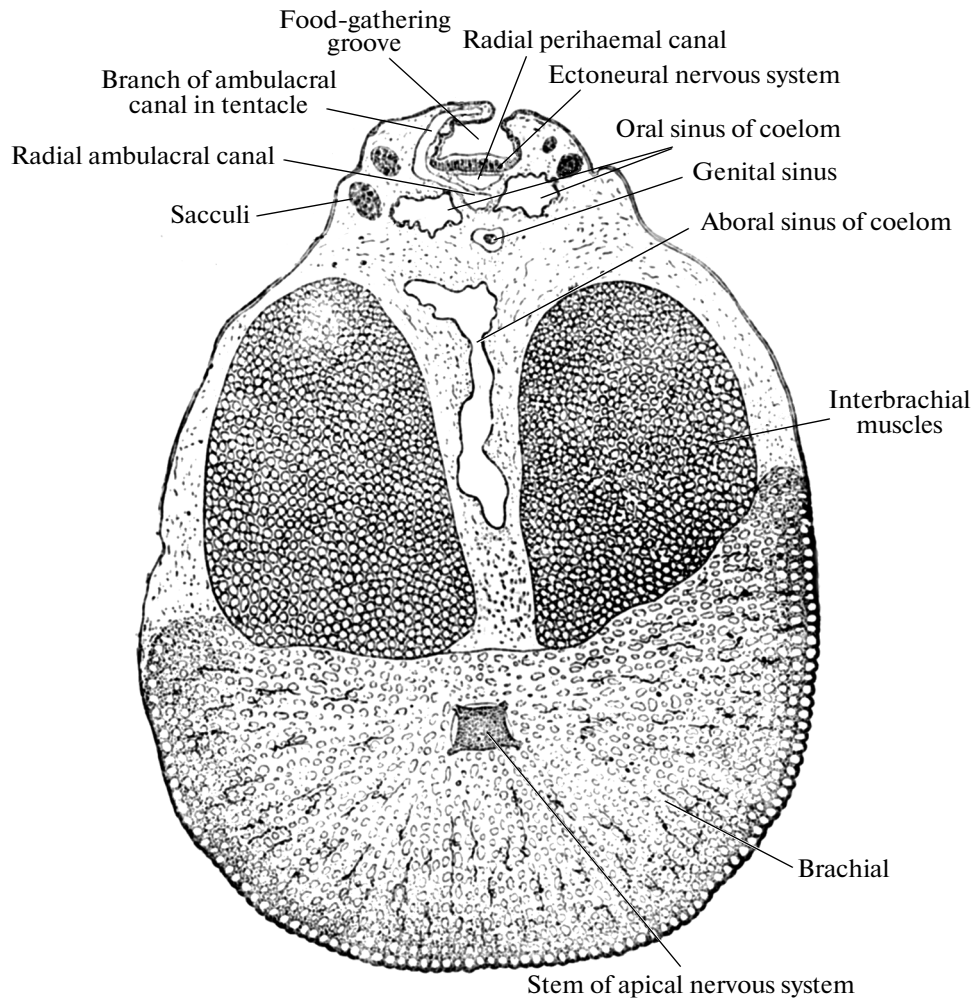
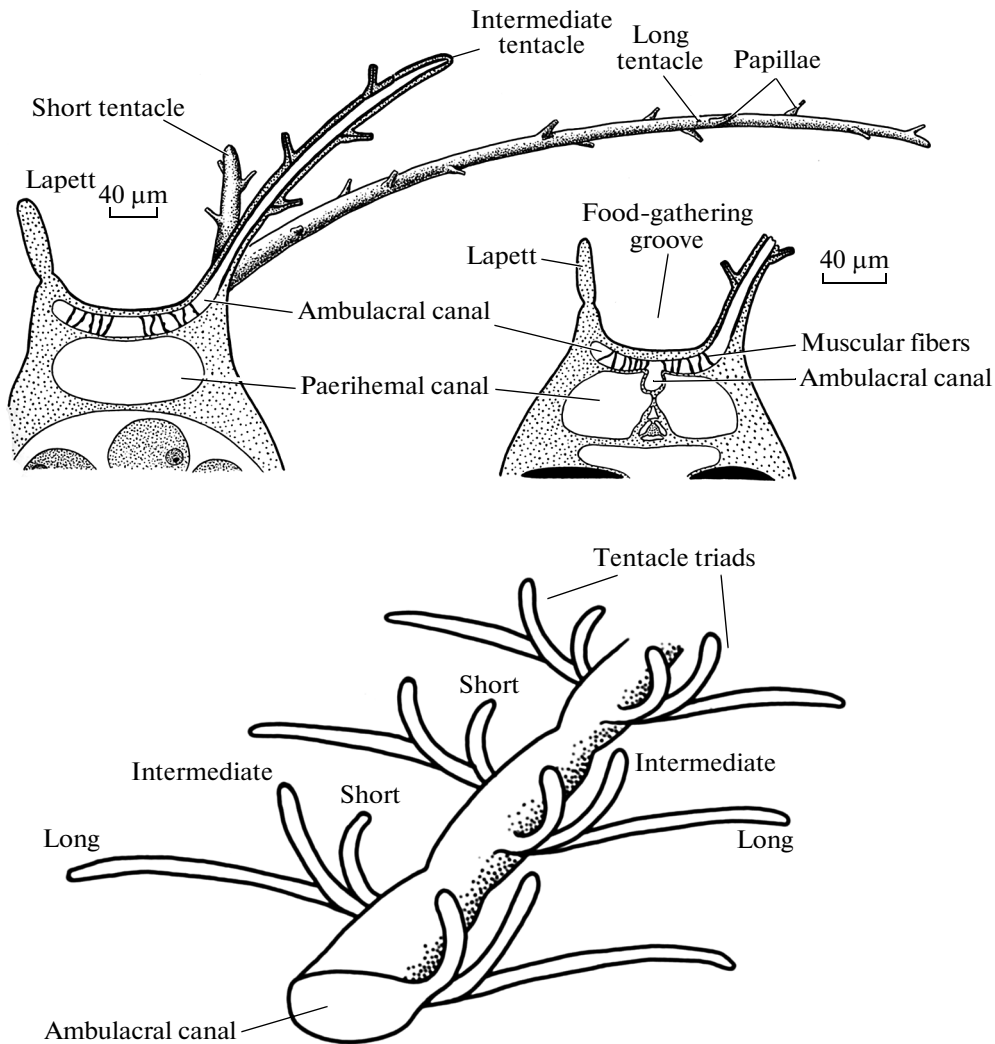


Fig. 2. Cross section of the arm of the Recent crinoid *Heliometra glacialis* (after Ivanov et al., 1946).

the ambulacral canal, while the food-gathering groove only moves the bolus to the mouth, although it undoubtedly plays an important role. In the absence of tentacles of the ambulacral system, it cannot function, since it cannot capture efficiently food particles in the water current. This trivial conclusion is important for subsequent consideration of the morphology and functions of brachioles, which are characteristic of other pelmatozoan echinoderms and food-gathering grooves of some carpozoan echinoderms.

In living crinoids, the food-gathering groove, along with the radial ambulacral canal, extending from arms along the tegmen, come directly to the mouth, where the radial ambulacral canal passes to the ring ambulacral canal. Although the food-gathering groove is usually covered from above by covering ambulacral plates, passing radially between interradial plates of the tegmen, it usually lacks special plates covering its bottom. The formation of the radial canal and primary ambulacral tentacles in crinoids is closely connected with the development of the vestibulum and described in

many crinoids in original works and reviews (Barrois, 1888; Gislén, 1924; Hyman, 1955; Ivanova-Kazas, 1978; Engle, 2013; Amemiya et al., 2016). Based on these descriptions, the following developmental features of radial canals, food-gathering grooves, and other peristomal structures of living crinoids are recognized. In parallel with the development of radial tentacles of the hydrocoel, the vestibulum is formed; initially, it is a small invagination on the ventral side of the larva, which is located in *Antedon* between the second and third ciliated rings. Even during the pelagic life, this invagination becomes deeper and wider, involving the ventral part of the third ciliated ring. The lateral margins of the vestibulum converge and then are fused rearwards. The fusion is completed soon after attachment of the larva and the vestibulum becomes a closed flattened cavity with a thickened internal wall, bottom, thinner external wall, and vault (Fig. 4a). Soon after this, an invagination is formed on the bottom of the vestibulum and grows towards the invagination of the intestine rudiment, passing through the forming ring of the hydrocoel, which is still horseshoe-

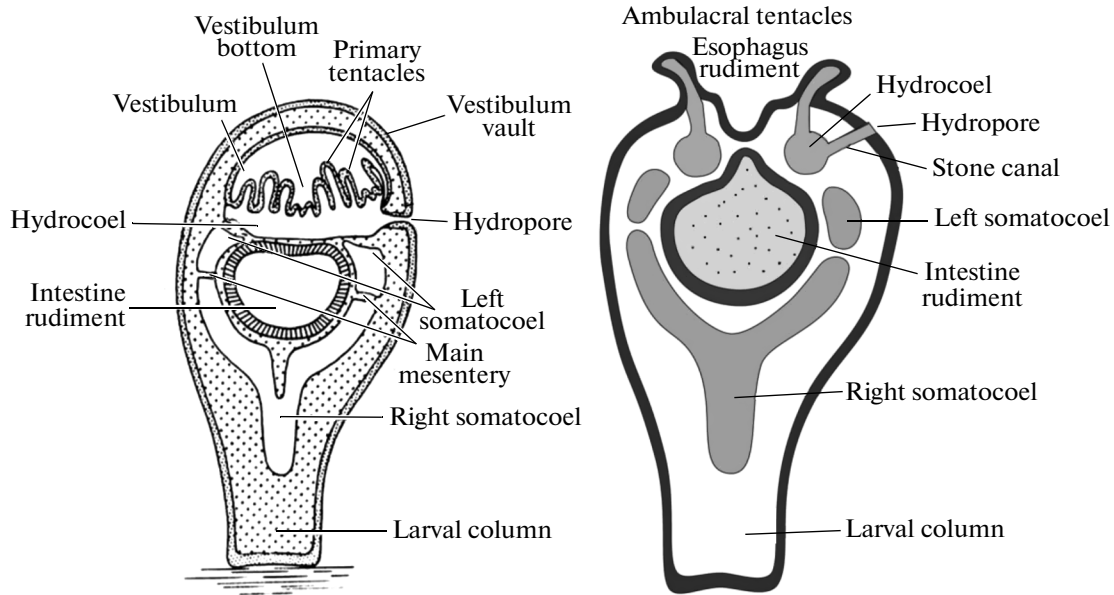


**Fig. 3.** The radial ambulacral canal and tentacle triads in the crinoid *Antedon*: (a) pinnule and (b) arm; and the scheme of their relation (after Nichols, 1966).

shaped at this stage. The outgrowth of the vestibulum bottom becomes fused with the intestine outgrowth to form ectodermal esophagus, around which the horseshoe of the hydrocoel becomes closed forming the ambulacral ring. Simultaneously, the entire complex of these rudimentary organs turns, so that the vestibulum comes to the upper (former posterior) end of the larva. The vertical mesentery separating the right and left somatocoel has a horizontal position; the left somatocoel becomes oral one; and the right somatocoel, which is now positioned under the left one, occupies an aboral position. As a result, a new antero-posterior body axis inverted relative to that of larva is formed; this probably could have resulted in the opposite order of location of the anterior *Hox* genes (Rozhnov, 2012a).

Five lobes are formed on the hydrocoel; each is divided into three diverticula, which protrude the ves-

tibulum bottom before them to form five groups of three tentacles enclosed in the vestibular cavity (Fig. 4a). On the sides of each of these tentacle triads, the intermediate of which is positioned radially, a tentacle appears, so that the triad turns into pentad and the total number of tentacles becomes 25. At a later stage, only five of these 25 are retained, occupying the radial positions. Others disappear. Approximately at the same time, the vestibulum vault is divided by five radial fractures into five lobes, each of which gives rise to an oral plate of the skeleton. Oral lobes are displaced apart and tentacles occupy external positions. Arm rudiments in the shape of radial appendages appear between the oral lobes along the calycine margin. Extensions of the oral and aboral coeloms, along with five remaining primary radial tentacles developing in radial ambulacral canals, grow into them. Prior to the formation of arm anlagen, radial skeletal plates



**Fig. 4.** Scheme of the formation the primary ambulacral tentacles in the vestibulum of crinoids (after Thompson, 1865) (a) and reconstruction of their appearance directly on the larval surface in the peristomal region of blastozoan echinoderms (b).

and the first brachials as their extensions appear between oral plates of the skeleton. They support developing arms.

The arms grow with the growth of the skeleton supporting them and oral lobes, along with oral plates, are reduced. The food-gathering grooves covered by ciliated epithelium and leading to the mouth appear above the radial canals. These radially positioned grooves divide the oral part of the calyx, tegmen, into five interradial segments. The above description of ontogenetic development of the oral region of crinoids gives evidence that the tegmen of extant crinoids is a derivative of the vestibulum bottom, the external part of which (vestibulum vault), after division by fractures into five lobes and opening, is reduced along with the oral plates.

However, in many Paleozoic crinoids and some extant taxa, the oral plates are not reduced; instead, they independent or along with other plates form a vault, under which food-gathering grooves and radial ambulacral canals pass. These grooves extend to the mouth either under the closed vault of oral plates, as, for example, in Paleozoic *Haplocrinites* and *Pisocrinus*, or pass between oral plates covered from above by covering plates, as in the living crinoid *Hyocrinus*. Under the grooves, skeleton is usually absent. However, there are some exceptions, for example, hybocrinids and protocrinids.

In hybocrinids, the food-gathering groove passes to the mouth above the tegmen, resting on a special skeleton of double-row plates covering the ambulacrum bottom and the radial ambulacral canal extends to the ambulacral ring under the tegmen. Beginning close to the mouth, the food-gathering groove is connected to

the radial ambulacral canal into a united arm near its beginning, at the point where it deviates from the theca between the aboral calyx and tegmen. This is distinct, for example, in Ordovician *Hybocystites*, as was indicated by Sprinkle (1973). In protocrinids, the food-gathering groove rests directly on special plates covering the ambulacrum bottom not only in the tegmen, but also in arms with their skeleton formed of single-row brachial plates (Guensburg et al., 2010). As indicated in this work, there are pores between these pores. These pores possibly provided passage for simple ambulacral tentacles of these crinoids. Other function is difficult to imagine for them. Therefore, it is proposed here that the ambulacral canal passed under the plates covering the ambulacrum bottom, instead of above them, as was figured by Guensburg et al. (2010, text-fig 6). The cavity between these plates and brachials probably contained not only a radial ambulacral canal, but also appendages of the right and left coeloms and other systems of organs.

## COMPARATIVE MORPHOLOGY OF ARMS AND BRACHIOLES

### *Brachioles*

Brachioles deviate from the food-gathering groove, which extends on the surface of the thecal skeleton. At the point of deviation of each brachiole, there is an articular facet for it; the food-gathering groove coming to it extends further along the brachiole. Brachioles are almost always double-row, composed of brachiolar plates alternating on the right and left sides to form a canal supporting the food-gathering groove covered from above by covering plates, which are also double-

row, sometimes with additional series of plates between the axial series and groove edges.

Based on the absence in all blastozoan echinoderms of a special opening providing passage for the ambulacral canal and its connection with the ambulacral groove, Sprinkle (1973) proposed that their food-gathering grooves and brachioles lacked a radial ambulacral canal. In his opinion, this assumption is also supported by the fact that the food-gathering canal is very shallow. However, judging from the arm structure in living crinoids, most of the arm is occupied by muscles. Therefore, in the absence of muscles in typical brachioles, the ratios of the diameters of supporting skeleton, food-gathering groove, and radial ambulacral canals in arms and brachioles are approximately equal.

In any event, blastozoan echinoderms have a madreporite, which is evidence that they have an ambulacral ring, which should give rise to the radial canals forming tentacles. These tentacles should come out. The only point where the primary tentacles of blastozoan echinoderms could have passed outside is the nearest vicinity of the mouth close to the beginning of food-gathering grooves. If this is the case, there is no reason to believe that they could not pass farther into food-gathering grooves. Moreover, the presence of tentacles with the ambulacral canal inside the food-gathering groove is evidenced by morphofunctional analysis.

Actually, if an ambulacral canal is absent, tentacles deviating from it and participating in capturing food particles are also absent. Thus, it is only possible to propose that food-gathering canals provided passage for water with food particles, which could be filtered near the mouth, using, for example, peristomal tentacles. However, in this case, there would have been an apparatus for water withdrawal after filtering, whereas such an apparatus is absent. Therefore, this variant is inappropriate from the morphofunctional point of view.

Another variant is connected with filling the food-gathering canal with mucus, which passively sticks food particles from marine water. Then, mucus, along with stuck food particles, is transported to the mouth by cilia. Such a system would have required continuous secretion of mucus by tissues of the food-gathering groove and, hence, inflow of respective nutrients to these tissues and development for this purpose of a strong circulatory system. Such a mode is also extremely inefficient.

Therefore, brachioles cannot function without the radial canal with its tentacles capturing food particles from marine water. Therefore, there is no reason to propose that brachioles lack a valuable radial ambulacral canal. To connect it with the food-gathering groove, blastozoan echinoderms have the only place just around the mouth. This reasoning leads to the conclusion that the radial ambulacral canal of blastozoan echinoderms could only come onto the thecal surface directly near the mouth, in the peristomal

region and then extended along the external surface of the theca and passed into brachioles.

The brachiolar food-gathering system is comparable in complexity (branching, presence of pinnulation) to the arm system of crinoids (Sprinkle et al., 2011). Therefore, the growth and vital activity of the brachiolar system requires not only radial ambulacral canals, but also at least one nervous system and coelomic and blood canals supplying soft parts of brachioles and also a special mesenchymal tissue for the formation of the skeleton. The thin longitudinal canals extending along the bottom of the brachiole groove skeleton of some Blastozoa corroborate this conclusion. Therefore, we should search for the explanation of similarity and differences between the brachiole system of blastozoan echinoderms and the arm system of crinoids in their morphogeneses rather than in the presence or absence of particular elements of soft tissues.

#### *Comparison of Arms and Brachioles*

Arms and brachioles may be rather similar in morphology. Both can be single-row or double-row, branching or nonbranching, possess pinnules or not. However, the accents in morphological features and frequencies or variants in the morphological spectrum are different; the characters usual for crinoid arms are unusual for the brachiolar food-gathering system. For example, pinnulation is characteristic of many crinoids and only rarely occurs in blastozoan echinoderms.

The differences between them are primarily four major features: (1) The radial ambulacral canal of brachioles is external relative to the thecal skeleton throughout its extent, because it passes outside the theca along its surface (Fig. 1b). In arms, it is internal, since it passes under the tegmen up to the point of entering the arm. As follows from the arm structure in Ordovician protocrinids, the radial canal in arms could initially have been internal, located under the plates covering the ambulacrum bottom. (2) Brachioles did not contain gonads and extensions of the sex cords, whereas the gonad of crinoids was inside the arms or pinnules, although in Paleozoic crinoids, it could have been located in a certain other place, probably, in the anal sack tightly connected in origin with an arm branch C. This difference is evidenced by the presence of a special gonopore in blastozoan echinoderms and the absence of this element in crinoids. (3) The initial double-row pattern of the supporting skeleton of brachioles contrasts with the initial single-row pattern of arms in crinoids. (4) The mean diameter of the food-gathering groove of crinoids is usually considerably larger, almost by an order of magnitude than that of crinoids. This is probably connected with the adaptation for feeding on smaller food particles.

The similarity in the morphology of arms and brachioles is possibly accounted for by the same growth model, which is based on the initial organizing role of

the radial ambulacral canal, and the differences are attributed to ontogenetic features of the ambulacral canal, vestibulum, and tegmen relative to each other and to the development of the thecal skeleton, primarily in time. The displacement of gonad from the thecal cavity into arms could have been caused by the displacement of gametes from the axial complex into arms, through which they passed outside, and later they became to complete maturation their.

The morphofunctional link of the radial ambulacral canal and food-gathering groove occurred at an early stage of evolutionary development of echinoderms and was probably determined by the dominant role and developmental model of the radial ambulacral canal. In other words, all initial features of arms in crinoids and brachioles in other pelmatozoan echinoderms, including the development of skeleton, were organized by the developmental model of the radial ambulacral canal, which in terms of developmental biology can be designated as the embryonic inductor or the organizer inducing the development of arms. Let us consider this topic in more detail.

#### PHENOMENON OF EMBRYONIC INDUCTION

Embryonic induction is rather thoroughly investigated in amphibians, beginning from classical works by Spemann, and considered in detail in many recent reviews and handbooks on developmental biology (Belousov 1993; Gilbert, 2010; Wolpert and Tickle, 2011). However, this phenomenon is known in a number of simpler organized animals, for example, coelenterates (Kraus, 2011; Mayorova et al., 2015). In these works, embryonic induction is defined as the effect of one embryonic structure on the other responding structure; as a result, the latter changes its morphogenetic trend and differentiation. Spemann revealed this process in amphibians, but used the terms organization and organizer instead of induction and inductor named the organizer (or organizational center) (Gilbert, 2010). As various experiences of inductor transplantation have shown, only embryonic material having competence rather than any other tissue responds to inductor. Competence means the ability of embryonic material to respond to various influences by the change in presumptive destiny.

Among inductive interactions, two major modes are recognized, i.e., instructive and permissive (Belousov, 1993; Gilbert, 2010). In the case of instructive induction, the signal of inducing tissue is necessary for activation of a respective gene in the responding area, without which it does not undergo suitable differentiation. In the case of permissive interaction, responding tissue is ready to differentiate and only waits for a signal from environment to initiate appropriate differentiation of its cells (Gilbert, 2010).

It is plausible that, in historical development, induction was initially instructive and its role at this

stage was probably organization of coordinated and successive development of parts of the organism or anlagen of organs, which have properties of modularity. Therefore, it is possible to propose that, initially, the development of radial canals of the hydrocoel dictated its own model of development to the structures surrounding it, primarily to the supporting skeleton, and determined many features of their development. For this purpose tissues of the developing supporting skeleton should have a respective competence, that is, ability to respond correctly to certain signals. This competence at early developmental stages could have been in various parts of the skeleton that supported the ambulacral system, as is evidence by the case of extraordinary growth of the food-gathering groove onto the skeleton regions unusual for it, for example, onto the stem in some hybocrinids and paracrinoideans. Further development of the skeleton could have been more autonomous and the directive induction was replaced by permissive one. This was probably the reason why, in the development of many living animals, as Belousov (1993, p. 203) marked, "most of the induction processes in the course of organogeneses are regarded as permissive induction, i.e., such that the inductor acts as a trigger of differentiation that was previously predetermined by the character of induced tissue." Based on the assumption of the organizing role in the development of skeleton of the radial ambulacral canal, it is possible to reveal similar and different features in the models and opportunities of growth and evolutionary development of arms and brachioles and also to substantiate the model of transition from the brachiolar food-gathering system to crinoid system, from brachioles to arms.

#### COMPARISON OF THE GROWTH MODELS FOR THE RADIAL AMBULACRAL CANAL, SKELETON OF ARMS, AND BRACHIOLES

The radial ambulacral canal at crinoids has an apical growth zone of epidermal cells. From this zone, triads of tentacles branch off, alternating on the right and left sides. Close to each triad tentacles, the margin of the food-gathering groove gives rise to folds of epithelial tissue, lapetts, which bearing a covering ambulacral plate (and sometimes an adambulacral plate). Thus, the arrangement of covering ambulacral plates corresponds to the positions of tentacle triads and developmental model of the radial canal. Simultaneously, single-row brachials appear, each having several, three or four, tentacle triads.

In the Recent crinoid *Antedon*, pinnules appear beginning from the seventh or eighth brachial. Then, pinnules appear on more proximal brachials. This order of the appearance of pinnules in the proximal part of arms suggests that some tentacles, one of distal ones of each brachial, can develop into pinnules. This assumption is also supported by the later development of specialized pinnules on the tegmen. Arm branching

may be terminal, by division of the growth zone into two or by the development of already formed pinnules into a true arm branch having new pinnules.

In crinoids, as in other echinoderms, growth and branching features of the radial ambulacral canals can be regarded as the model organizing the development of the axial skeleton and, then, some parts of the initially extraxial skeleton. The uniform model for the growth of radial canals of the ambulacral system and axial skeleton appears distinct, as the skeleton morphology of many echinoderm groups is analyzed. For example, the covering plates of pelmatozoan echinoderms, as ambulacral plates of echinoids, appear not merely apically, but alternating to the right and left of its plane of symmetry. Therefore, in such a double-row ambulacrum, plates alternate, so that the centers of plates of one row are positioned opposite the sutures of plates of the other row. As the plates appear following this model, all of them gradually increase in size with the growth of the animal. This growth model is the same as in the radial ambulacral canal of crinoids and echinoids, i.e., the terminal tentacle grows and gives rise to ambulacral feet alternating on the right and left sides. This growth model is termed the model of apical serial enantomorphous monopodial branching, because ambulacral feet and, especially, tentacles are branches of the radial canal, from the morphogenetic point of view. This was probably the initial branching type of radial canals, which initially induced this terminal growth model of the skeleton surrounding them, which prevailed, although was not unique to metameric skeletal structures of echinoderms. Subsequently, the growth of the axial skeleton could become less dependent on the inductive influence of the radial ambulacral canal. This is evidenced, for example, by the earlier development of the ambulacral plates than the ambulacral canal in echinoids and the appearance of brachials earlier than ambulacral tentacles grow into them in living crinoids. The radial ambulacral canals of echinoderms diverge from the ambulacral ring and extend along the skeleton above or under it. Extending on the external skeleton surface, they form a food-gathering groove with covering plates. The groove bottom may have a special skeleton, which is a part of the total skeleton, or extend on the surface of a nonspecialized skeleton, sometimes forming an additional skeleton on its surface, as, for example, in some paracrinoids and hybocrinid crinoids. In blastozoan echinoderms, the radial ambulacral canal is able to come out of the theca, since it usually lacks terminal plates restricting its growth, and branch, inducing the development of food-gathering appendages deviating from the main stem, the brachioles, composed of double-row extension of plates covering the ambulacrum bottom within the theca or organizing the formation of the main brachiolariae *de novo*.

In brachioles, the formation of covering plates and brachiolariae of the supporting skeleton follows the same pattern, but on different scale; covering plates

probably correspond to tentacle triads deviating from the radial canal, while brachiolariae are from three to seven times larger. Nevertheless, in the “arm” of solutes and some eocrinoids, supporting and covering plates are identical in size (Rozhnov, 2015, text-fig. 2, pls. 1, 2). In single-row arms of crinoids, the growth model of brachials seems somewhat different, simply terminal without enantiomorphism. However, in the presence of pinnules, enantiomorphism in their arrangement becomes evident.

For comparison of the brachiolar system and crinoid arm system, it is necessary to compare the morphology and development of the tegmen in crinoids with the system of peristomal plates of the theca in blastozoan echinoderms. As shown above, the formation of tegmen in living crinoids is usually connected with the bottom of closed vestibulum and its vault is formed by the lobes with oral plates, which are either reduced or form a skeletonized tegmen, which is particularly characteristic of Paleozoic crinoids. Just because of the closure of the vestibulum and appearance of the vestibular cavity, the radial ambulacral canals of crinoids are initially located inside the theca and the vault boundaries of the vestibular cavity are the tegmen boundaries in adult crinoids. At the beginning of the formation of the body plan of crinoids, ambulacral tentacles, which develop and come out at the edge of the vestibular cavity, at the boundary between the tegmen and calyx, probably “forced” other internal organs to follow it. Simultaneously, the growing tentacles, along with the ambulacral canals inside, induced the serial apical growth of the future radial plates of the calyx at the boundary with the tegmen.

It is highly probable that, in blastozoan echinoderms, the radial ambulacral canals developed in a somewhat different way, rather than inside a closed cavity similar to the vestibular cavity of crinoids. Therefore, the reconstruction of development of the peristomal part of the blastozoan larva of echinoderms is a key problem for substantiation of an opportunity of transformation of the exothecal brachiolar system of blastozoan echinoderms into endothecal crinoid arms.

Isolation and displacement of the peristomal part from the anterior end to the posterior (upper) end of larva of blastozoan echinoderms is evidenced by the existence in many of them of an aberrant regularly curved theca formed because of a delay in elevation (Rozhnov, 1998, 2002, 2012a). It is evident that this isolation involved at least the appearance in place of the future mouth of invagination of the body wall directed to the intestine anlage to form ectodermal esophagus. There is no doubt that the esophagus anlage grew the forming hydrocoel ring, which was not necessarily completely closed in adult blastozoan echinoderms. The hydrocoel ring gave rise to the radial ambulacral canals, which pushed out the ectoderm surrounding the mouth to form the primary ambulacral tentacles around it. These tentacles proba-



bly initially developed as three lobes, two of which, along with the ambulacral canals enclosed in them, were soon divided into two to form a characteristic pentaradial symmetry of the 2-1-2 type, in which the ambulacra BC and DE are morphogenetically connected with each other (Rozhnov, 2002, 2012b, 2014). Such order of the appearance of ambulacra probably determined the symmetry and arrangement of plates in the oral region, because the initial ambulacra and probably simultaneously with them, the oral plates and initial oral covering plates (according to the terminology of Sumrall (2010)) appeared in accordance with the symmetry; first, there were three large plates and, then, two slightly smaller ones. In the generalized variant of the oral region (Kammer et al., 2015; Sumrall, 2010), the oral plate CD is divided into two by a suture passing through the madreporite.

Because of the absence of the vestibulum in blastozoan echinoderms, the primary ambulacral tentacles probably appeared external to the thecal skeleton (Fig. 4b). According to this approach, the main difference between crinozoan and blastozoan echinoderms is the presence/absence in ontogeny of the closed vestibulum. Consequently, the vestibulum could have become isolated gradually due to the appearance and growth of folds bearing oral plates. This assumption is apparently supported by the appearance of a special peristomal cavity in some paracrinoideans (Parsley and Mintz, 1975), which resembles the tegmen of *Rhipidocystis*, judging from its widely open peristomal part and features of brachiole deviation, and also, for example, well-detached tegmen in the eocrinoid *Ridersia*.

As the cavity and vault of the vestibulum are formed, the primary ambulacral tentacles developed under the thecal skeleton. Approaching at early developmental stages of the skeleton the proximal plates of the theca, the primary ambulacral tentacles enclosing the radial canal probably could induce for support the serial growth of such plates, along with adjacent structures of soft tissues and coeloms. This could have been the pattern of the appearance of crinoid arms. Simultaneously, the formation of plates covering the ambulacrum bottom could continue, as in Early Ordovician Protocrinida, or additional lateral series of plates at the edges of the main single-row series, as in the unusual diploporid cystoidean *Eumorphocystis* from the Ordovician of North America. This is evidence of an opportunity of parallel appearance of arms and armlike food-gathering appendages in several groups of blastozoan echinoderms. A decrease in the inductive role of the radial canal sometimes results in transformation of double-row brachioles into single-row ones, as in *Rhipidocystis*.

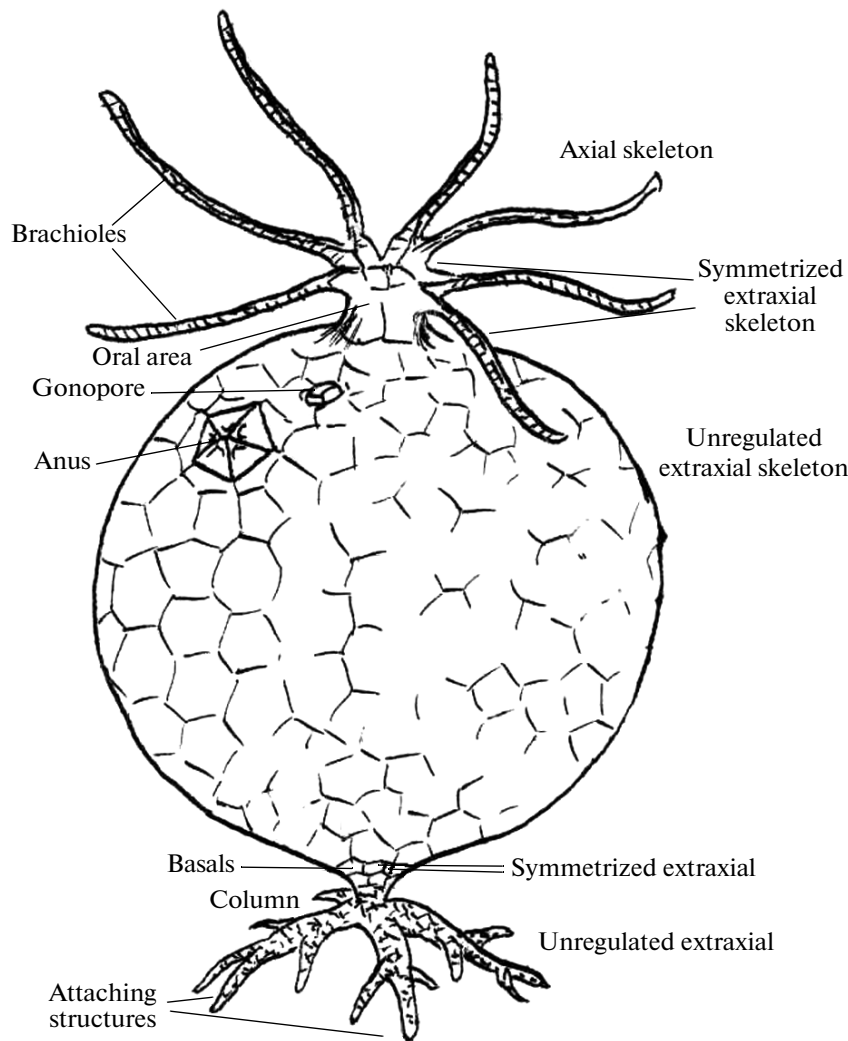
All modifications of the food-gathering ambulacral system of pelmatozoan echinoderms are restricted by prohibition of direct disturbance of serial monopodial branching of the axial skeleton, according to which

one metamere can have only one branch. This is particularly prominently manifested in pinnulation, i.e., formation of small nonbranching branchlets on the main branch. This process is uniform in all pelmatozoan echinoderms.

It is possible that, in phylogeny, the anlage initially passes a phase of dependent differentiation, at which it depends on inductors and other conditions external relative to the anlage. Therefore, Early Paleozoic crinoids display a similar developmental phase in the skeleton of the food-gathering ambulacral system. This is especially distinctly pronounced in the diversity of the arm structure in Ordovician hybocrinid crinoids. In the genus *Hybocystites*, the food-gathering groove deviates directly from the mouth, passes on the tegmen surface between oral plates, reaches the radial plates, where it comes in contact with the radial ambulacral canal, which enters it through a special opening. Further fate of the united food-gathering system differs in the triad and diad of radii; in the radii A, C, and D, the food-gathering canals pass onto the skeleton supporting simple single-row arms, extended on their oral side, and sometimes come onto the adoral side. In the radii B and E, they bend, passing on the external surface of the calyx, spreading on it, and sometimes continuing on the column. The genus *Tripatocrinus* lacks arms and, in the radii A, C, and D, food-gathering grooves spread over the external surface of the theca and, in two radii, they are absent. In the genus *Cornucrinus*, metameric skeleton of arms of three radii is replaced by long appendages of radial plates supporting the food-gathering grooves. In *Hybocrinus* and *Hoplocrinus*, simple nonbranching arms are present in all of five radii.

It is noteworthy that, in hybocrinids, the food-gathering grooves extending on the tegmen have double-row covering plates, although the radial ambulacral canal did not pass directly under them (Sprinkle, 1973). This suggests that arms of hybocrinids could have developed on the basis of an ancestral brachiole system, in which the ambulacral canal and food-gathering groove met directly at the mouth, rather than were new formations. Possibly, they appeared in parallel with arms of other crinoids. If this is the case, hybocrinids should be ranked subclass (Rozhnov, 1985a, 1985b) or even higher.

This approach gives concrete expression to relationships between the axial skeleton and hydrocoel in the extraxial theory developed by David and Mooi for homology of the echinoderm skeleton; the axial skeleton includes all skeletal elements, the arrangement of which is controlled by induction influence of the radial ambulacral canals. Other skeletal elements compose the extraxial skeleton. Let us consider this in pelmatozoan echinoderms in more detail.



**Fig. 5.** Scheme of relations of the axial, symmetrized extraxial, and nonsymmetrized extraxial skeletons exemplified by the rhombiferan *Echinospaerites* (figured by S. Terent'ev).

#### ROLE OF HYDROCOEL IN THE DEVELOPMENT OF THE SKELETON PATTERN AND THE EXTRAXIAL THEORY BY DAVID AND MOOI

Above we considered presumable organizing role of the radial ambulacral tentacles, which deviate from the hydrocoel, in phylogenetically initial development of the arm and brachiole skeletons. However, the organizing role of the hydrocoel is not restricted to the influence of its radial appendages on the axial skeletal structures. It also concerns other skeletal parts of pelmatozoan echinoderms, determining, for example, their pentaradial symmetry caused by pentamery of the ambulacral ring. These conclusions can be used for concretization of the extraxial theory proposed by David and Mooi (1998; Mooi and David, 1997, 2008).

They divided the echinoderm skeleton into the axial (which is closely connected in its development with the left middle coelom, the hydrocoel) and

extraxial parts (more closely connected with the right and left somatocoels, which form almost entirely the remaining body part). This division of the skeleton was primarily based on different growth patterns of these skeleton parts. In the axial skeleton, elements are added terminally and, according to the rule of the ocular plates, near the proximal margin of a special plate named the ocular in echinoids and the plate terminal in starfishes. The authors of the extraxial theory believe that these plates are of great importance and the zone corresponding to these plates is present in all echinoderms, but not always calcified. Actually, a similar zone is present in all echinoderms in the shape of a growth zone around the distal end of the radial ambulacral canal, that is, the blastema. The terminal plates, if present, perform a different function, i.e., restrict the growth of the serial ambulacral skeleton by the test skeleton in echinoids, body of starfishes, or theca of other echinoderms. Therefore, the terminal plates are only present in those echinoderms,

the ambulacra of which do not extend beyond the theca or shell. In various appendages of the ambulacral system, such as arms of crinoids or brachioles of blastozoan echinoderms, terminal plates are absent, providing unlimited apical growth and distal branching.

The extraxialaxillary theory strictly opposes the axial and extraxial skeletons and does not take into account the role in morphogenesis of the organizing role of the hydrocoel, which concerns not only axial skeleton, but also extraxial skeleton. Its organizing role is distinctly shown in ontogeny and phylogeny. Therefore, the hydrocoel and its derivatives, primarily the radial ambulacral canals, can be regarded as organizers, successively inducing a number of alternating events, including the development of the general skeleton pattern. This inductive events are analogous in some sense to the dorsal lip of the blastopore and its derivatives, notochord and prechordal mesoderm of vertebrates.

According to Mooi and David (1997), the extraxial skeleton is divided in echinoderms into perforated and nonperforated ones. The perforated part includes the regions with openings, i.e., the anus, madreporite, gonopore, and sutural pores. The nonperforated part is remaining extraxial skeleton. The authors of this theory believe that the perforated skeleton is more closely connected with the left somatocoel and the nonperforated skeleton, with the right somatocoel. The criteria for this division are uncertain, because the character of relationships between particular elements of the skeleton and coeloms. This also concerns relationships of the axial skeleton with the hydrocoel. I agree with the division of the echinoderm skeleton into axial and extraxial parts and propose a criterion for this division. The axial skeleton is defined here as a part of the echinoderm skeleton that is initially formed under the organizing influence of the radial ambulacral canal. From this point of view, the entire arm skeleton of crinoids (oral and adoral) is axial. The axial skeleton is characterized by the terminal growth similar in the growth model to the radial ambulacral canal. The remaining skeleton is extraxial. It is divided into two parts (Fig. 5). In one part, the arrangement of skeletal elements is under direct or indirect organizing influence of the hydrocoel, which primarily determines the symmetry of skeletal elements of the extraxial skeleton. This part of the extraxial skeleton can be termed symmetrized. The other part of the extraxial skeleton is unregulated.

In the skeleton of pelmatozoan echinoderms, this division can be concretized by the following examples:

**Axial skeleton:** oral and aboral skeletons of arms of crinoids, ambulacra on the tegmen of crinoids, fixed arms of camerate crinoids, brachioles and, if present, skeleton of brachiole-bearing ambulacra of blastozoan echinoderms.

The symmetrized extraxial skeleton includes the oral plates on the tegmen of crinoids and their aboral skeleton, the theca of the majority of cystoideans,

eocrinoids, and paracrinoids with the regularly arranged plates, etc.

The unregulated extraxial skeleton is located between the radials and basals of camerate acrocrinoids, the thecal skeleton of primitive eocrinoids, thecal skeleton between the orals and basals of some blastozoan echinoderms, for example, *Achradocystites* and *Echinosphaerites*.

## CONCLUSIONS

The assumption of the initial organizing role of the radial ambulacral canals of the skeleton surrounding them explains the similarity in the structural model of the skeleton of the brachiole food-gathering system of Blastozoa and the arm systems of Crinozoa, which is based on the apical growth of radial appendages of the hydrocoel with the enantiomorphously shifted branches of the triads of ambulacral tentacles. The difference in the positions of brachioles and arms relative to the theca (exothecal and endothecal) is connected with the formation of the primary ambulacral tentacles directly on the body surface of the majority of Blastozoa and in the special closed vestibular cavity formed by the fusion of folds around the forming mouth in crinoids. Therefore, the ambulacra with brachioles of Blastozoa initially appeared on the body surface of the developing larva and, then, expanded directly onto the external surface of the skeleton forming around them. The skeleton supporting brachioles developed as a branch of the plates covering the ambulacrum bottom, if they were present, or formed similarly as a new formation outside the theca. The skeleton supporting arms, brachials, was formed as the serial growth of the radially positioned plates at the boundary of the aboral skeleton and tegmen formed due to the appearance of the vestibulum. Among paracrinoids, there are taxa with an intermediate, incompletely closed vestibulum, which is reconstructed in the skeleton based an additional cavity in the skeleton around the mouth.

The role of the hydrocoel and its radial ambulacral appendages as the organizer of arrangement of skeletal elements of echinoderms enables concretization of the principle of skeleton division into the axial and extraxial parts proposed by David and Mooi. The axial skeleton is that formed under the organizing influence of the radial ambulacral canals. The remaining skeleton is extraxial. It can be subdivided into the symmetrized extraxial skeleton arranged under direct or mediated organizing effect of the hydrocoel and unregulated, nonsymmetrized, which is not initially connected with the influence of the hydrocoel. This division of the skeleton has a morphological manifestation and can be extended on all echinoderms.

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