Brachiopod development in light of Nielsen's "folding hypothesis"

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ABSTRACT: Brachiopoda is a phylum of marine invertebrates with a fossil record dating back to the Early Cambrian. Despite their extensive paleontological history, the evolution of their body plan remains debated. Claus Nielsen (1991) suggested the "folding hypothesis" based on data on development of the craniiform brachiopod Novocrania anomala. During metamorphosis, the larva N. anomala folds ventrally, with both juvenile shell valves derived from the dorsal side. It has been hypothesized that this process may reflect the evolutionary origin of the brachiopod body plan: brachiopods evolved from a creeping ancestor that curled ventrally. This study revisits the "brachiopod fold hypothesis" by analyzing data on development of three brachiopod subphyla: Craniiformea, Rhynchonelliformea, and Linguliformea. Analysis of rhynchonelliform development reveals that "folding" occurs during embryonic and larval stages, influencing the arrangement of larval structures. In rhynchonelliform larva (cephalula), dorsal and lateral setal pairs are positioned at a right angle relative to the anterior-posterior axis of the larva, while in craniid larva (chaetotrocha), setae are arranged sequentially along the dorsal side. In early cephalula, the mantle rudiment forms obliquely to the anterior-posterior axis, with the dorsal side closer to the larval apical lobe. The dorsal pair of setal bundles is also located closer to the apical lobe than the lateral pair. As the cephalula grows, the dorsal sector of the mantle expands, causing the setal bundles to align. We propose that this dorsal expansion reflects the folding process, with the early cephalula corresponding to the chaetotrocha stage during folding at metamorphosis, and the competent cephalula corresponding to the folded juvenile stage observed in craniids. In discinid and lingulid brachiopods, development suggests a trend toward earlier, more embryonized "folding processes". Data on fate map construction of discinids reveals dorsal expansion in the embryo, contributing to both dorsal and ventral valve formation in juveniles. In lingulids, both shell valves develop from the dorsal side of the embryo. Our findings suggest a progressive embryonization of the folding process in brachiopods: in craniids, "folding" occurs during metamorphosis; in rhynchonelliforms, during embryonic and larval development; and in linguliforms, at early embryonic stages. How to cite this article: Kuzmina T.V., Malakhov V.V. 2025. Brachiopod development in light of Nielsen's "folding hypothesis" // Invert. Zool. Vol.22. No.1. P.68-80. doi: 10.15298/ invertzool.22.1.06

KEY WORDS: brachiopoda, development, ontogeny, evolution, folding hypothesis.

Devoted to memory of Claus Nielsen.

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РЕЗЮМЕ: Брахиоподы — тип морских беспозвоночных известных с раннего кембрия. Несмотря на обширную палеонтологическую историю, эволюция плана строения брахиопод остается предметом дискуссий. Клаус Нильсен (1991) на основе данных по развитию краниформной брахиоподы Novocrania anomala предложил «гипотезу складывания». Во время метаморфоза личинка N. anomala сгибается на брюшную сторону, а обе створки раковины ювенильной стадии формируются на дорсальной стороне. Было предположено, что этот процесс может отражать эволюционное происхождение плана строения брахиопод: ползающий предок свернулся на вентральную сторону. В настоящей работе проведен анализ данных по развитию представителей трех подтипов брахиопод: Craniiformea, Rhynchonelliformea и Linguliformea с точки зрения «гипотезы складывания». Анализ данных по развитию ринхонеллиформных брахиопод показал, что «складывание» происходит на эмбриональных и личиночных стадиях, что отражено в расположении личиночных пучков щетинок. У личинки ринхонеллиформных брахиопод (цефалулы) дорсальные и латеральные пары щетинок расположены под прямым углом к передне-задней оси личинки, в то время как у личинки краниид (хетотрохи) щетинки расположены последовательно вдоль дорсальной стороны. У ранней цефалулы зачаток мантии формируется косо по отношению к передне-задней оси, при этом дорсальная сторона ближе к апикальной лопасти личинки. Кроме того, у ранней цефалулы дорсальная пара пучков щетинок расположена ближе к апикальной лопасти, чем латеральная пара. По мере роста цефалулы дорсальный сектор мантии расширяется, что приводит к тому, что щетинки становятся расположенными на одном уровне. Мы предполагаем, что дорсальный рост мантии цефалулы отражает «процесс складывания», причем ранняя цефалула соответствует хетотрохе во время складывания при метаморфозе, а зрелая цефалула соответствует сложенной ювенильной стадии у краниид. У дисцинид и лингулид в онтогенезе «процесс складывания» наблюдается на более ранних этапах развития. Данные по построению карт презумптивных зачатков у дисцинид показывают разрастание дорсального сектора эмбриона, который в дальнейшем даст начало как дорсальной, так и вентральной створкам раковины у ювенильных особей. У лингулид обе створки раковины также развиваются на дорсальной стороне эмбриона. Таким образом, наши результаты предполагают прогрессивную эмбрионизацию процесса складывания у брахиопод: у краниид складывание происходит во время метаморфоза; у ринхонеллиформных брахиопод — на эмбриональных и личиночных стадиях; а у лингулиформных брахиопод — на ранних эмбриональных стадиях онтогенеза. Как цитировать эту работу: Kuzmina T.V., Malakhov V.V. 2025. Brachiopod development in light of Nielsen's "folding hypothesis" // Invert. Zool. Vol.22. No.1. P.68-80. doi: 10.15298/invertzool.22.1.06

КЛЮЧЕВЫЕ СЛОВА: брахиопода, развитие, онтогенез, эволюция, гипотеза складывания.

Introduction

Brachiopoda is a phylum of marine invertebrates with a rich paleontological history. This group first appeared in the Early Cambrian, reached its peak during the Paleozoic, but after the mass extinction at the end of the Permian, it was unable to regain its previous taxonomic diversity. The paleontological record of brachiopods is well-documented due to their mineralized bivalve shells, which are well-preserved as fossils and have been extensively studied by paleontologists. Brachiopods are divided into three subphyla: Linguliformea, Craniiformea, and Rhynchonelliformea, which differ in shell structure and composition, internal anatomy, and developmental processes (Williams et al., 1996). There is still no consensus on the origin and development of their body plan.

The investigation of the anatomy and development of extant brachiopods has historically lagged behind that of paleontological specimens. For an extended period, the field relied on a limited number of frequently cited anatomical studies, the schemes from which were often reproduced in textbooks (Hyman, 1959; Ivanov et al., 1981; Ruppert et al., 2004). Research on brachiopod development has primarily concentrated on rhynchonelliforms, with foundational studies by Kovalevsky (1874, 1883) significantly contributing to our understanding of rhynchonelliform development. In contrast, research on the development of lingulids is predominantly represented by a single detailed study by Yatsu (1902), while craniids have remained largely unexamined for a considerable period.

In 1991, Claus Nielsen's article on the embryonic and larval development of *Novocrania anomala* (Nielsen, 1991) was groundbreaking. This study revolutionized the understanding of brachiopod evolution, significantly influenced further research and laid the foundation for the "folding hypothesis" regarding the development of the brachiopod body plan.

According to Nielsen's work (Nielsen, 1991), during settlement, the *N. anomala* larva folds ventrally, with both juvenile shell valves being of dorsal origin. It was suggested that the metamorphosis of extant craniids reflects the evolutionary origin of the brachiopod body plan: brachiopods likely evolved from a creeping ancestor that curled ventrally. In the 20th century,

the "folding hypothesis" was widely accepted by both zoologists and paleontologists (Malakhov, 1995; Cohen *et al.*, 2003; Malakhov, Kuzmina, 2006). However, the evolutionary scenario proposed by Claus Nielsen (1991) is now the subject of active debate. The scientific community has essentially split into two camps: one that supports the "folding hypothesis" (Kuzmina *et al.*, 2019; Malakhov *et al.*, 2021; Plandin, Temereva, 2023), and another that criticizes it (Altenburger *et al.*, 2013, 2017).

This study presents an opinion paper, in which we analyze brachiopod developmental data from the perspective of Claus Nielsen's "folding hypothesis".

Brachiopod Development

Brachiopods are characterized by a pelagobenthic life cycle, which includes a sessile or benthic adult stage and a planktonic dispersal stage (Williams *et al.*, 1997; Malakhov *et al.*, 2021). Most brachiopods exhibit external fertilization, radial cleavage, and an enterocoelic mode of coelom formation (Nielsen, 1991; Percival, 1940, 1960; Kuzmina, Temereva, 2024). These traits align this group with deuterostomes. However, according to current data, brachiopods belong to the clade Lophotrochozoa, which includes lophophorates and trochozoans (Halanych *et al.*, 1995). The ontogenetic development varies among representatives of the three brachiopod subphyla.

Craniiform development

In Craniiformea, ontogeny has been thoroughly studied in one species, Novocrania anomala, thanks to the work of Claus Nielsen (1991). Furthermore, the development of craniids has been studied using blastomere-marking techniques (Freeman, 2000). Additionally, the metamorphosis of craniids has been investigated independently (Altenburger et al., 2013). A spherical, ciliated blastula emerges from the egg envelope and begins to swim in the water column (Fig. 1A). Gastrulation then occurs through invagination (Fig. 1B-C). At the gastrula stage, the mesoderm begins to form as two layers of cells separate from the endodermal cells (Fig. 1D), which then divide into four plates (Fig. 1E). The gastrula elongates, with the blastopore at the



Fig. 1. Development of craniiforms. A — blastula; B — gastrula; C–E — schemes of frontal sections of: C — early gastrula; D — late gastrula; E — early larva; F — early larva, ventral view, arrow indicates the direction of the blastopore closure; G — scheme of frontal section of chaetotrocha; H — scheme of sagittal section of chaetotrocha; I — chaetotrocha, lateral view; J — chaetotrocha, dorsal view; K — metamorphosis, lateral view; L — juvenile, lateral view. A, B, F, J — based on Nielsen, 1991. C–E, G. H — modified after Nielsen, 1991.

Abbreviations: acb — apical lobe ciliary band; al — apical lobe; ar — archenteron; bl — blastopore; bo — body lobe; ces — coelomic sacs; dv — dorsal valve; ec — ectoderm; en — endoderm; me — mesoderm; mr — rudiment of mesoderm; rpr — rudiment of protegulum (juvenile dorsal valve); se — setae; vcz — ventral ciliary zone.

posterior end of the larva, gradually closing from front to back (Fig. 1F). The endoderm forms the primary gut, or archenteron (Fig. 1E). Four pairs of coelomic sacs develop from the mesodermal plates (Fig. 1G–H), a process referred to as modified enterocoely.

The larva develops two lobes: an apical (or head) lobe, surrounded by a ciliated band, and a body (or mantle) lobe (Fig. 1I–J). Three pairs of setae bundles form on the dorsal side of the body lobe. A ciliated zone is located on the ventral side of the body lobe. The first pair of coelomic sacs is located in the apical lobe. The remaining three pairs of coelomic sacs are found in the body lobe; their coelomic epithelium forms a muscular sheath that surrounds the bases of the setae bundles. On the dorsal side of the larva's body lobe, there is a protein plate - the rudiment of the periostracum of the first region of the juvenile shell, known as the protegulum. This type of larva, bearing a ciliated band and larval setae, is referred to as a chaetotrocha (Malakhov et al., 2021). The chaetotrocha is a lecithotrophic larva. All development lasts from four to ten days, after which the chaetotrocha settles to the substrate and undergoes metamorphosis (Fig. 1K-L). According to Nielsen (1991), upon settling, the larva folds onto its ventral side. The rudiment of the dorsal valve is formed before metamorphosis, while the ventral valve, which cements to the substrate, is secreted after metamorphosis on the surface of the bent portion of the body lobe. Based on this information, Nielsen considered both valves to be dorsal.

During larval settlement, the third posterior pair of larval setae bundles is shed, while the first and second pairs fall off a few days after settlement. In the apical lobe of the settled larva, the mouth opens, and simultaneously, three pairs of tentacles begin to form around the periphery of the apical lobe. The anus appears between the dorsal and ventral valves. Nielsen described that the second pair of coelomic sacs gives rise to the coelom of the lophophore, while the third pair forms the perivisceral coelom. The fate of the first and fourth pairs of coelomic sacs has not been traced (Nielsen, 1991).

Altenburger and coauthors (2013) describe the metamorphosis of craniid larvae as follows: "Larvae ready for metamorphosis appear to be slightly curled, as they turn the anterior lobe and the posterior tip of the posterior lobe ventrally." They interpret the origin of the shell valves differently than Nielsen (1991). According to Altenburger and coauthors, the ventral valve of craniids, which cements to the substrate, develops at the posterior end of the larva and corresponds to the portion of the pedicle in rhynchonelliforms that attaches to the substrate. Consequently, craniids lack a ventral valve homologous to the ventral valve of rhynchonelliforms and linguliforms. If craniids are considered as an ancestral group of brachiopods, this suggests that brachiopods originally possessed only one dorsal valve, with the second (ventral) valve emerging as a synapomorphy of the clade uniting linguliforms and rhynchonelliforms. Alternatively, if brachiopods originally had two valves, then rhynchonelliforms and linguliforms retain the plesiomorphic condition, while craniids represent a derived group, characterized by the reduction of the ventral valve as an apomorphy. However, it is important to note that craniid ontogeny provides no substantial evidence to support their classification as a derived group. Craniids exhibit more pronounced segmentation during development compared to other brachiopods. Their larvae possess three pairs of seta bundles arranged sequentially along the anterior-posterior axis, whereas rhynchonelliform brachiopods have two pairs of seta bundles, which are oriented perpendicular to the larval anterior-posterior axis.

Rhynchonelliform development

The development of the rhynchonelliforms has been studied in the most details (see for examples, Kovalevsky, 1874, 1883; Percival, 1944, 1960; Malakhov, 1976, 1983; Chuang, 1996; Freeman, 1993, 2003; Lüter, 2001; Kuzmina et al., 2019; Kuzmina, 2021; Kuzmina, Temereva, 2024). Hatching from the egg envelope occurs at the stage of a late blastula or early gastrula (Fig. 2A-C). Gastrulation proceeds by invagination, forming the archenteron, which is connected to the external environment through a rounded blastopore (Fig. 2B-C). Then, the blastopore at the vegetal pole of the gastrula elongates, forming a bilaterally symmetrical late gastrula, with the plane of symmetry coinciding with the plane of the elongated blastopore (Fig. 2D). At this stage, the anterior and posterior coelomic mesoderm form via enterocoely (Fig. 2E). The anterior and posterior coelomic sacs have a



Fig. 2. Development of rhynchonelliforms. A — late blastula; B — gastrulation; C — early gastrula, lateral view; D — late gastrula, lateral view; E — mesoderm formation; F — bilobed larva, ventral view, arrow indicates the direction of the blastopore closure; G — early three-lobed larva, ventral view; H — early three-lobed larva, lateral view, red line circles the lateral setal bundle, blue line circles the dorsal setal bundle; I — coeloms in early three-lobed larva; J — full grown cephalula, ventral view; K — full grown cephalula, lateral view; L — eversion of the mantle lobe after settlement. A–B, F — modified after Long, Stricker, 1991. C, D, G, H — modified after Kuzmina *et al.*, 2019. E, I — based on Kuzmina, Temereva, 2024. K — modified after Malakhov *et al.*, 2021. C, D, G, H, J, L — scanning electron microscopy.

Abbreviation: acb — apical lobe ciliary band; al — apical lobe; am — anterior mesoderm; ao — apical organ; ar — archenteron; bl — blastopore; bo — body lobe; dse — dorsal setal bundle; ec — ectoderm; es — eye spots; en — endoderm; lse — lateral setal bundle; ml — mantle lobe; pl — pedicle lobe; pm — posterior mesoderm; rml — rudiment of mantle lobe; vb — vesicular bodies; vcb — ventral ciliary band.

pair of lateral outgrowths that are positioned on either side of the archenteron. In some species of rhynchonelliform brachiopods, the coelomic rudiments represent a group of closely arranged cells surrounded by a basal lamina (Lüter, 2000). Next, a bilobed larva is formed, consisting of an apical lobe and a body lobe (Fig. 2F). The apical (anterior) lobe contains an apical organ with a tuft of cilia. The anterior mesoderm gives rise to a coelomic sac located in the apical lobe, while the posterior mesoderm gives rise to two pairs of coelomic sacs located in the body lobe of the larva (Fig. 2I). Then the coelomic pouches start fusing to form a common coelomic rudiment, which is partly divided into three pairs of coelomic cavities. After some time, the body lobe differentiates into the mantle and pedicle regions. Thus, an early trilobed larva is formed (Fig. 2G). At this stage, the blastopore assumes a teardrop shape and closes from the posterior end toward the anterior. It is important to note that in the early trilobed larva, the rudiment of the mantle lobe is positioned obliquely relative to the anterior-posterior axis: on the dorsal side, it is closer to the apical lobe, and on the ventral side, it is closer to the pedicle lobe (Fig. 2H). Two pairs of setae bundles begin to form on the mantle lobe: lateral and dorsal, with the dorsal pair located closer to the anterior end of the larva.

Larval growth is accompanied by the expansion of the dorsal sector of the mantle lobe, so in a competent (ready-to-settle) larva, the dorsal pair of setae bundles shifts to the posterior end of the larva and is aligned with the lateral pair (Fig. 2J–K). The apical lobe is covered with cilia, but along its edge, the cilia are longer and form a ciliated band. Eye spots are located on the apical lobe. Small spherical structures, vesicular bodies, are situated at the base of the apical lobe. On the ventral side of the mantle lobe, there is a ciliated band resembling the neurotroch of trochophore larvae. This type of larva is called a cephalula (Beecher, 1892; Malakhov et al., 2021). The full grown cephalula swims in the water column and, when threatened, curls onto its ventral side, spreading its setae in different directions. In each bundle, the bases of the setae are surrounded by a muscular sheath formed by the coelomic epithelium of the second and third pairs of coelomic sacs. The mantle lobe takes the form of a fold that encircles the pedicle lobe like a skirt. The epithelium of the pedicle lobe and the inner epithelium of the mantle fold secrete a protein rudiment of the periostracum (the outer layer of the shell). After settlement, the larva attaches to the substrate by the posterior end of the pedicle and inverts the mantle lobe so that it covers the apical lobe (Fig. 2L). The mouth opens on the apical lobe, and the lophophore tentacles begin to form. The mantle starts to secrete the first region of the shell — protegulum. The larval setae are shed, and adult setae begin to form along the mantle's edge.

Recent study has shown that in some representatives of Rhynchonellida, an order of rhynchonelliform brachiopods, early stages of ontogeny — such as gastrulation, blastopore closure, and cephalula formation — occur within the egg envelope, with hatching taking place at the competent cephalula stage (Kuzmina, 2021).

Linguliform development

The development of linguliform is characterized by embryonization: cleavage, gastrulation, mesoderm formation, blastopore closure, and larval formation, all of which occur within the egg envelope (Figs 3, 4). Linguliformea comprise only two extant families, Discinidae and Lingulidae, whose development differ from each other.

In Discinids, embryogenesis and the early stages of development have been extensively documented in the study by Freeman (1999). Within the egg envelope, cleavage occurs, leading to the formation of a ciliated blastula (Fig. 3A). Subsequently, the cells of the vegetal plate undergo invagination, transforming the blastula into a radial symmetrical early gastrula (Fig. 3B). Following this, the blastopore elongates and begins to close from the posterior end, while the apical pole shifts anteriorly, resulting in the elongation of the embryo and the formation of a bilateral late gastrula (Fig. 3C). Hatching occurs at the stage of a lecithotrophic bilobed larva, consisting of an apical lobe and a mantle lobe (Fig. 3A-D; Chuang, 1977; Freeman, 1999; Lüter, 2001). The apical lobe has an apical tuft and is surrounded by a ciliated band. On the ventral side of the mantle lobe, there is a ciliated field, and on the sides of the mantle lobe, there are three long setae. It has been suggested that the lecithotrophic larva of Disciniidae is correspond to the trilobed larva of rhynchonelliform brachiopods and can also be referred to as a cephalula (Malakhov et al., 2021). However, the discinid cephalula does not settle on the bottom; instead, in the water column, it develops a definitive mouth, a through gut, and a lophophore rudiment surrounding the mouth. This gives rise to the next planktonic stage, which bears a lophophore and retains larval setae — called the chaetolopha (Fig. 3E; Malakhov et al., 2021). The chaetolopha is actually a juvenile (Nielsen, 2005; Lüter, 2007). The lophophore of the chaetolopha includes a



Fig. 3. Development of discinids. A — blastula; B — early gastrula; C — late gastrula, arrow indicates the direction of the blastopore closure; D — cephalula; E — chaetolopha; F — tegulolopha. A–C — modified after Freeman, 1999. D–F — modified after Malakhov *et al.*, 2021.

Abbreviations: al — apical lobe; an — anus; ao — apical organ; bs — brefic shell; cse — curved setae; ec — ectoderm; en — endoderm; env — egg envelope; lo — lophophore; lse — larval setae; m — mouth; ml — mantle lobe; mt — median tentacle; pr — protegulum.

medial tentacle containing a nerve center, and one tentacle on each side of the medial one. The chaetolopha swims and feeds, with new tentacles added on both sides of the medial tentacle. In the water column, the first sections of the shell are secreted, starting with the protegulum, followed by the brephic shell. This gives rise to the next planktonic stage, which bears a small shell and a lophophore — the tegulolopha (Fig. 3F; Malakhov *et al.*, 2021). At this stage, two



Fig. 4. Development of lingulids. A — blastula; B — gastrula; C — embryo with lophophore rudiment; D — scheme of sagittal section of tegulolopha; E — tegulolopha immediately after hatching; F — tegulolopha with six pairs of tentacles. A–D — modified after Yatsu, 1902. E–F — modified after Malakhov *et al.*, 2021. Abbreviations: an — anus; bs — brefic shell; dv — dorsal valve; ec — ectoderm; en — endoderm; env — egg envelope; lo — lophophore; lr — lophophore rudiment; m — mouth; mt — median tentacle; pr — protegulum; vv — ventral valve.

types of juvenile setae are formed: curved and flexible (Chuang, 1977). The tegulolopha swims for 2–3 weeks and settles on the bottom at the stage when it has six pairs of tentacles. While in the water column, the tegulolopha sheds its larval setae, and after settling, it loses its curved setae, although the flexible bristles may persist in adult individuals. In lingulids, cleavage occurs within the egg membranes, resulting in the formation of a ciliated blastula (Yatsu, 1902; Fig. 4A). Gastrulation proceeds via invagination (Yatsu, 1902; Freeman, 1995; Fig. 4B). According to Yatsu's illustration (fig. 13 in Yatsu, 1902), in lingulid gastrulae, the blastopore, which later becomes the mouth, is displaced anteriorly, while the developing gut shifts



Fig. 5. Formation of the body plan of brachiopods (modified after Malakhov, Kuzmina, 2006). A — creeping ancestor; B — the same after folding.

Abbreviations: av — anterior valve; dv — dorsal valve; g — gut; lo — lophophore; pv — posterior valve; se — setae; vv — ventral valve.

posteriorly. Subsequently, both the dorsal and ventral shell valves form on the enlarged dorsal side of the embryo, and an ectodermal thickening in front of the mouth marks the development of the lophophore (Fig. 4C-D). It is important to note that, initially, the mantle secretes a small, circular embryonic shell, which subsequently bends along the transverse midline to form the dorsal and ventral valves of the shell (Yatsu, 1902). At this stage, the embryo hatches from the egg membranes. The first planktotrophic stage of lingulids, called the tegulolopha (Malakhov et al., 2021), has a rudimentary shell (protegulum) and a developing lophophore with five tentacles (Fig. 4D-E; Yatsu, 1902; Freeman, 1995). The tegulolopha swims in the water for several weeks (Yatsu, 1902; Paine, 1963; Freeman, 1995; Collin et al., 2019). During this time, the lophophore elongates, the brephic shell is secreted, and the internal organs, including the pedicle and adult setae, begin to form within the soft body (Fig. 4F). Settlement occurs at the stage of 10–15 pairs of tentacles. After settlement, the pedicle emerges from beneath the shell valves, and the juvenile begins to burrow into the substrate.

The evolution of "folding process" in brachiopod ontogeny

Nielsen (1991) proposed that the developmental data of extant craniids may represent a plesiomorphic form of brachiopod development. It was suggested that the metamorphosis of extant craniids reflects the evolutionary origin of the brachiopod body plan: brachiopods likely evolved from a worm-like ancestor possessing two dorsal valves. When attacked by predators, the brachiopod ancestor would curl ventrally (Fig. 5). Over time, this folded state became permanent, as the shell in this position protects the entire soft body (Nielsen, 1991; Malakhov, 1995; Kuzmina *et al.*, 2019).

It is important to note that signs of folding have also been observed in the ontogeny of other brachiopod groups. Nielsen (1991) highlighted that in rhynchonelliform cephalulae, larval setae are positioned along the edge of the mantle lobe at an angle to the anterior-posterior axis, whereas in craniid chaetotrocha, they are arranged sequentially along the dorsal side. Nielsen suggested that the position of setae in rhynchonelliform cephalulae reflects their displacement due to folding, with the folding itself occurring at an earlier developmental stage. This hypothesis was supported by data on the development of the rhynchonelliform brachiopod Coptothyris gravi (Kuzmina et al., 2019). It was shown that the formation of the competent cephalula in this brachiopod is accompanied by the growth of the dorsal sector of the mantle lobe. It was proposed that the early cephalula corresponds to the chaetotrocha during folding at metamorphosis, while the later competent cephalula corresponds to the folded juvenile stage of craniids (Fig. 6).

Interestingly, Freeman's (1999) work on constructing fate maps for development also supports the "folding hypothesis". In discinids, at the four-cell embryo stage, Freeman marked a



Fig. 6. The evolution of "folding process" in brachiopod ontogeny. A1 — craniiform gastrula; A2 — rhynchonelliform gastrula; A3 — discinid gastrula; A4 — lingulid gastrula; B — craniid larva (chaetotrocha); C1 — craniid semi-folding juvenile; C2 — rhynchonelliform early larva; C3 — early discinid larva; C4 lingulid late gastrula; D1 — craniid folded juvenile; D2 — rhynchonelliform full-grown larva (cephalula); D3 — discinid planktonic stage (tegulolopha); D4 — lingulid planktonic stage (tegulolopha). A1, B, C1, D1 — modified after Nielsen, 1991; A2, C2, D2 — modified after Kuzmina *et al.*, 2019; A3, C3, D3 — modified after Freeman, 1999. A4, C4, D4 — modified after Freeman, 1995.

Abbreviations: green — dorsal valve; blue — ventral valve; purple — a mark on the discinid development stages; lilac — ectoderm; rose — endoderm; light green — lophophore rudiment; arrow indicates anterior-posterior axis.

region at an equatorial level along the first cleavage plane (Freeman, 1999; Fig. 6). In the gastrula, this marked region is located in the dorsal sector of the embryo (Fig. 6, A3). Subsequently, the marked area expands to cover both part of the dorsal and part of the ventral side of the larva (Fig. 6, C3, D3). In our opinion, this process corresponds to the folding of the larva, observed in craniids during metamorphosis. The region of the discinid embryo, initially located only on the dorsal side (Fig. 6, A3), later extends to also cover part of the ventral side (Fig. 6, C3, D3).

The development of lingulids is highly embryonized, making comparisons between their ontogenetic stages and those of other brachiopod groups difficult. Nonetheless, both Yatsu (1902) and Freeman (1995) depict a radially symmetrical blastula. During gastrulation, the blastopore shifts anteriorly, indicating significant growth of the dorsal side. The formation of both dorsal and ventral valves on the dorsal side of the embryo, along with the development of the lophophore in front of the blastopore, suggests that in lingulids, the folding process occurs during gastrulation. Consequently, both valves are of dorsal origin (Fig. 6).

Thus, in the evolution of brachiopod ontogeny, there is a progressive embryonization of the "folding" process. In craniids, "folding" occurs during metamorphosis. In rhynchonelliforms, part of the "folding process" takes place during embryonic development, with the remainder occurring during the larval stage. In contrast, in discinids and lingulids, "folding" occurs at the early stages of embryonic development, and the structure of the pelagic stage corresponds to the folded juvenile stage of craniids.

Compliance with ethical standards

CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

Acknowledgements. The study is supported by Russian Science Foundation (#23-14-00020). The study was conducted under the state assignment of Lomonosov Moscow State University.

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Responsible editor E.N. Temereva