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Cirripede Cleavage Patterns and the Origin of the Rhizocephala (Crustacea: Thecostraca)

Gerhard Scholtz*, Ekaterina Ponomarenko & Carsten Wolff

Humboldt-Universität zu Berlin, Institut für Biologie, Vergleichende Zoologie,
Philippstr. 13, 10115 Berlin, Germany
[gerhard.scholtz@rz.hu-berlin]

* Corresponding author

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> Abstract

Several aspects of phylogenetic relationships among barnacles (Cirripedia) are still unresolved. One of the contentious issues is the position of the parasitic Rhizocephala. In most molecular phylogenies Rhizocephala are resolved as sister group to a monophyletic Thoracica. However, since Rhizocephala are morphologically highly derived there is not a single morphological character supporting this view. Here we present data on the early cleavage patterns and the gastrulation of two rhizocephalan species. Based on our results and data from the literature we suggest that early cleavage and gastrulation indicate a monophyletic group comprising the thoracican Iblomorpha and the Rhizocephala. This renders thoracicans paraphyletic with respect to Rhizocephala. Based on this, we develop a new hypothesis for the origin of parasitism in the rhizocephalan stem lineage starting with parasite-like dwarf males of an iblomorph-like ancestor which already had the ability to penetrate the surface of a host animal – originally conspecific females, but later the decapod, probably a pagurid, host.

> Key words

Cell division, gastrulation, phylogeny, evolution of development, parasitism.

Introduction

Cirripedia is a strange group of animals that most laypersons would not identify as Crustacea. Adults are either sessile filter feeders, like the well visible and common goose and acorn barnacles, or parasites. These lifestyles correlate with dramatic alterations of body organization compared to other crustaceans and arthropods in general (Anderson 1994; Deutsch et al. 2004; Høeg et al. 2004). This makes cirripedes highly interesting for evolutionary considerations and they have even been classified as putative "hopeful monsters" (Géant et al. 2006). The parasitic forms, in particular the endoparasitic Rhizocephala, are even more derived and sometimes hardly recognizable as animals (e.g. Høeg 1992; Gruner 1993). Adults are not much more than a root-like network of tissue in the body cavities of the hosts, the interna, and an outer sack, the externa, containing the reproductive organs and the early life stages (Glenner & Høeg 2002). Only since Thompson (1836) detected the nauplius and cypris larvae of rhizocephalans was it clear that they are arthropods, and more specifically a group of crustaceans closely related to filter feeding barnacles (see Winsor 1969; Scholtz 2008). Nevertheless, the pathway from a sessile, filter feeding barnacle-like animal to an, in many aspects derived, parasitic rhizocephalan remains largely obscure and is subject to speculations based on analogies (Newman et al. 1969; Glenner & Høeg 2002). To resolve the problems of the evolution of parasitism of Rhizocephala and their correlated idiosyncratic body organization, the phylogenetic position and, in particular, the sister-group relationship of Rhizocephala has to be reconstructed. However, the recognition of the rhizocephalan's phylogenetic position is hampered for the above mentioned reason of a

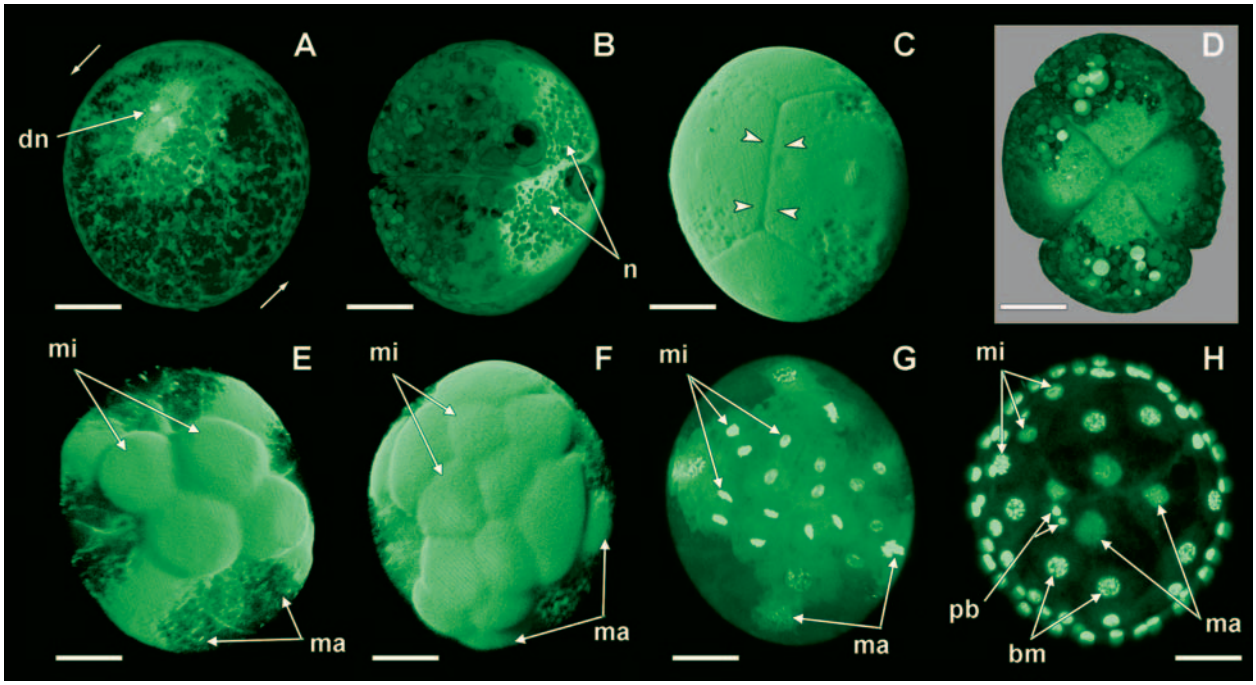

Fig. 1. Cleavage and gastrulation of *Sacculina pilosella*. **A**: First cleavage showing the dividing nucleus (dn) and the yolk free cytoplasm at one of the longitudinal ends of the egg. The arrows indicate the rotation that happens during the further process of the first cleavage resulting in an arrangement of the two blastomeres as seen in B. Blend mode of surpass view in Imaris. **B**: The 2-cell stage with the nuclei (n) and the yolk free cytoplasm at the short axis of the embryo indicating the future animal pole, lateral view. Blend mode of surpass view in Imaris. **C**: The 4-cell stage with the long cross furrow (arrowheads) at the animal pole connecting two cells that are not sister cells. The nuclei are in early metaphase indicating the beginning third cleavage to the 8-cell stage. Blend mode (with "light enabled") of surpass view in Imaris. **D**: The 4-cell stage with a different arrangement of blastomeres lacking a cross furrow. Blend mode of surpass view in Imaris. **E**: The 8-cell stage showing four micromeres (mi) and four macromeres (ma), which contain all the yolk. Blend mode (with "light enabled") of surpass view in Imaris. **F**: The 16-cell stage, animal view. The eight micromeres are of equal size (mi). The macromeres divide asymmetrically resulting in four large macromeres (ma) and four smaller cells forming additional micromeres. Blend mode (with "light enabled") of surpass view in Imaris. **G**: 8- to 16-cell stages, view on the animal pole. The micromeres (mi) forming a cap of smaller cells; the four macromeres (ma) contribute by their unequal divisions to this cap. The smaller macromere derivatives divide symmetrically. MIP mode of surpass view in Imaris. **H**: Epibolic gastrulation, seen from the vegetal pole, which is marked by four large macromeres (ma) containing most of the yolk and forming a cross furrow. They are surrounded and overgrown by medium sized blastopore cells (bm) and the micromere cap (mi). Note the polar bodies in the region of the blastopore, the vegetal pole. MIP mode of surpass view in Imaris. (Scale bars: 50 μm)

highly derived adult anatomy and accordingly, there is only a preliminary notion of their affinities based on morphology. In contrast to this, several molecular studies resolve the Rhizocephala as sister group to the whole group of barnacles, the Thoracica (e.g. Spears et al. 1994; Billoud et al. 2000; Pérez-Losada et al. 2002, 2004, 2008, 2009). To overcome this lack of morphological data for reconstructing rhizocephalan affinities we chose development as a potential source for resolving phylogenetic relationships. In particular for crustaceans, this has been shown to be a promising approach (e.g. Siewing 1979; Anderson 1973; Scholtz & Wolff 2002; Scholtz et al. 2009).

Here we present data on the early cleavage and gastrulation of two rhizocephalan species, *Sacculina pilosella* and *Pelterogasterella gracilis*. Our results in combination with a comparison of early developmental patterns amongst Cirripedia suggest close affinities between Rhizocephala and the thoracican subtaxon Iblomorpha. This would render Thoracica paraphyletic. The implications of our results on the view on evolution of parasitism and evolutionary transformation of adult morphology in the stem lineage of Rhizocephala are discussed.

Materials and methods

Material for the study was collected at the Biostation Vostok (Sea of Japan, Far East of Russia) in June and July 2006 and in August 2007. *Pugettia quadridens* (De Haan, 1839) crabs infested with *Sacculina pilosella* Van Kampen & Boschma, 1925 were captured at a depth of 0.5–2 m. Hermit crabs *Pagurus brachiomastus* (Thallwitz, 1891) with multiple externae of *Peltogasterella gracilis* (Boschma, 1927) were collected at 2–3 m depths. Hosts with parasites were kept in the laboratory at 18°C in aerated marine water.

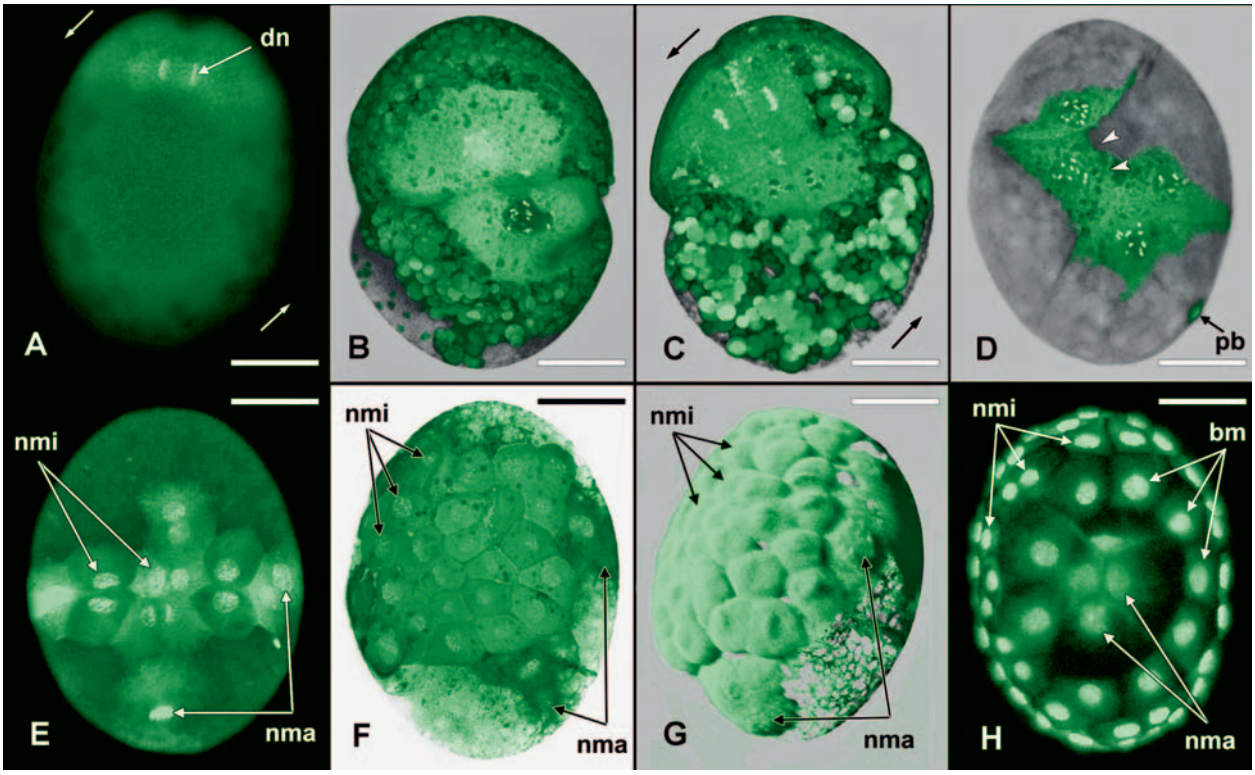

Fig. 2. Cleavage and gastrulation of *Peltogasterella gracilis*. **A**: First cleavage showing the dividing nucleus (dn) and the yolk free cytoplasm at one of the longitudinal ends of the egg. The arrows indicate the rotation that happens during the further process of the first cleavage resulting in an arrangement of the two blastomeres as seen in B. View in epifluorescent microscope. **B**: The 2-cell stage with the nuclei and the yolk free cytoplasm at the short axis of the embryo indicating the future animal pole. Blend mode of surpass view in Imaris. **C**: The second cleavage leading to the 4-cell stage. Again the blastomeres undergo a rotation as is indicated by the arrows. The chromosomes of two cells are seen in metaphase. Blend mode of surpass view in Imaris. **D**: The 4-cell stage with the long cross furrow (arrowheads) at the animal pole connecting two cells that are not sister cells. The nuclei are in prophase indicating the beginning of the third cleavage to the 8-cell stage. The polar bodies (pb) are recognisable. They are not situated at the animal pole. Blend mode of surpass view in Imaris. **E**: The transition between the 8- and 16-cell stages. All eight micromeres (mi) are present, whereas the divisions of the macromeres (ma) are somewhat delayed. MIP mode of surpass view in Imaris. **F**: The micromeres (mi) forming a cap of smaller cells the four macromeres (ma) contribute by their unequal divisions to this cap. Blend mode of surpass view in Imaris. **G**: A similar stage as in F from a more lateral perspective. Blend mode (with "light enabled") of surpass view in Imaris. **H**: Epibolic gastrulation, seen from the vegetal pole which is marked by four large macromeres (ma) containing most of the yolk. They are surrounded and overgrown by medium-sized blastopore cells (bm) and the micromere cap (mi). MIP mode of surpass view in Imaris. (Scale bars: 50 µm)

Embryos of *P. gracilis* were preserved together with the externae, since this species is colonial and has many small, elongated reproductive bodies. In each of these, the embryos are almost at the same developmental stage. In the case of *S. pilosella* we had to take developing embryos directly out of the mantle cavity of the living externa at certain periods of time.

A developmental sequence of embryos was preserved with 4% PFA in PBS (Phosphate Buffered Saline) for light microscopy, fluorescence microscopy, and confocal-laser-scanning-microscopy (CLSM). Fixed specimens were observed with a light microscope (Zeiss Axiophot 2plus) with Nomarski optics. Pictures were obtained by means of a digital camera (Axiocam HRc).

The dye applied was nucleic acid specific Sytox Green (S-7020; Molecular Probes). In addition to staining nuclei it allows the visualization of the cytoplasm of stained cells and thus the cell shape which is crucial for detailed reconstructions. Preserved specimens were washed in Tris-buffer (TBS) several times and transferred to Sytox solution in TBS (1:1000). Incubation time was 3 h. Embryos were then washed again in TBS and mounted on microscopic slides in an anti-bleaching medium (DABCO-Glycerol).

Samples were scanned under the confocal-laser-scanning-microscope (Leica SP2) and the stacks of images were processed with the 3D-reconstruction software "Imaris 5.5.3" (Bitplane). The computer-aided three-dimensional reconstructions allow not only the visualization of the embryos in total, but also the evaluation of the relative size of cells, the detection of the position of nuclei and their spatial relation to one another.

Results

The early development of *Peltogasterella gracilis* and *Sacculina pilosella* is very similar. Accordingly, we describe the cleavage of both species together. The eggs are more or less oval, with those of *Sacculina pilosella* being slightly rounder (Figs. 1, 2). The polar bodies show a variable position and the animal-vegetal axis of the eggs cannot be determined in the early stages (Figs. 1, 2). Nevertheless, the zygotes are already polarized with the nuclei and some yolk-free cytoplasm situated at one end of the longitudinal egg axis (Figs. 1A, 2A). The spindle of the first division is almost perpendicular to the long egg axis. During division, the embryo rotates to 90° within the eggshells, and from now on the axis between the yolky and the yolk free cytoplasmatic areas is oriented transversally along the short egg axis (Figs. 1A,B, 2A,B). The first division is adequal, and the yolk is almost evenly distributed between two blastomeres. The second cleavage is adequal and synchronous in the two blastomeres (Figs. 1C, 2C,D). Only sometimes the division of one blastomere is slightly retarded. Spindles are parallel to each other and perpendicular to the long axis of the egg. During the second division the embryo rotates 45° with respect to the long axis. Accordingly, the four blastomeres show a specific arrangement (Figs. 1C,D, 2C,D). In most cases, two of the cells (non-sister-cells) form a contact zone, the cross-furrow, at the yolk-free pole, which is oriented along the long egg axis. The other two cells form a contact zone (cross-furrow) at the yolky pole. In this case the cross-furrow is perpendicular to the long egg axis. Since the yolky area marks the region of the future blastopore, the yolk-free/yolky axis corresponds to the animal-vegetal axis.

The third cleavage is equatorial and highly unequal (Fig. 1E). It leads to a tier of four small, almost yolk-free cells (micromeres) at the animal pole and four large yolk containing cells (macromeres) at the vegetal pole. The arrangement of the micro- and macromeres in relation to each other can vary. The micromeres are placed directly upon their sister macromeres, when the spindles of the third cleavage are parallel to the animal-vegetal axis of the embryo. Sometimes the spindles are slightly oblique to this axis, and the resulting micromere positions resemble to some degree a "spiral pattern." Also, the arrangement of the micromeres with respect to the cross-furrows varies. In most cases they show a cross-furrow that is parallel to the long egg axis, but sometimes the cross furrow is perpendicular to the long axis.

The fourth cleavage is asynchronous and starts in the micromeres (Figs. 1F, 2E). These begin their divisions synchronously or with slight retardation of one or two blastomeres. It is not clear whether this delay always occurs in the same cells. The spindle orientations in the micromeres vary. The two micromeres that are not forming the cross-furrow show parallel spindles in most of the embryos studied. Although there is no common pattern of the spindles in the micromeres forming the cross-furrow, it is still possible to distinguish at least three common positions: (1) spindles are parallel to each other but perpendicular to those of the two non-contacting micromeres; (2) spindles are perpendicular to each other, one being parallel to the spindles of the non-contacting two micromeres; (3) spindles are almost parallel to each other and oblique or almost parallel to the other pair of micromere spindles (Figs. 1F, 2E). The macromeres divide again unequally after the cleavage round of the micromeres has been completed. In some cases, the division of macromeres is delayed by only one phase of mitosis (Figs. 1F, 2E). Again, the spindles are oriented along the animal-vegetal axis and parallel to each other. Thus, after the fourth cleavage, the embryo consists of sixteen cells, twelve micromeres and four macromeres. Eight of the micromeres have a micromere origin and four are the product of the macromeres. As a consequence of the variations described above, the 16-cell-stage varies with respect to the position of micromeres. Therefore, from this stage on it is almost impossible to definitely establish sister relationships between cells in the preserved embryos.

The following divisions of the micromeres are almost synchronous with slight retardation of those originating from the macromeres (Figs. 1G, 2F,G). In contrast to this, the macromeres continue with their unequal divisions giving rise to quartets of micromeres. Step by step the micromeres cover the yolk-rich macromeres, thus performing an epibolic gastrulation. In the meantime the four nuclei of the macromeres migrate towards the vegetal pole of the embryo (Figs. 1H, 2H). These macromere nuclei maintain their positions at the surface until they are surrounded by micromeres forming the margin of the blastopore (Figs. 1H, 2H). Embryos at this stage are composed of approximately 128 cells. After epiboly has been completed, the macromeres divide more or less equally.

Discussion

The current view of cirripede phylogenetics

In contrast to older views (e.g. Newman et al. 1969), there is now a general agreement based on morphological and molecular studies that Rhizocephala is a monophyletic group (see Gruner 1993; Høeg et al. 2004; Glenner & Hebsgaard 2006; Pérez-Losada et

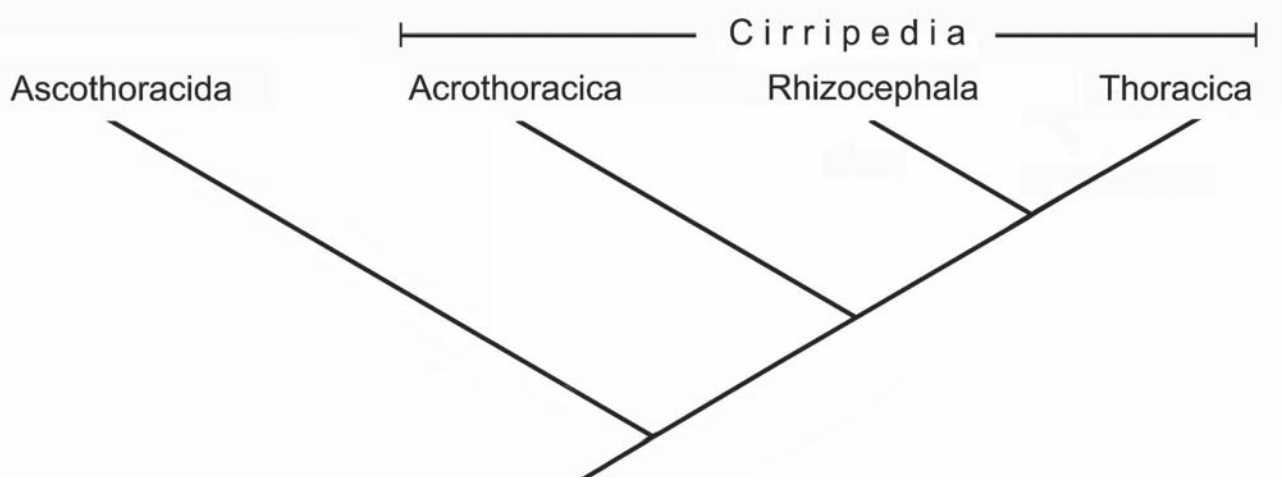

Fig. 3. The current consensus of the phylogenetic relationships of the Cirripedia based on morphological and molecular analyses.

al. 2009). According to these analyses it is also obvious that Rhizocephala is part of the Cirripedia, together with Acrothoracica and Thoracica. Furthermore, most recent studies resolve Ascothoracida as sister taxon to Cirripedia and within Cirripedia, the Acrothoracica as sister taxon to a group formed by Thoracica and Rhizocephala (Pérez-Losada et al. 2002, 2009; Høeg et al. 2004) (Fig. 3). The monophyly of Cirripedia is supported by a number of different data sources including larval morphology, spermatology, molecular and genetic studies (e.g. Healy & Anderson 1990; Spears et al. 1994; Glenner et al. 1995; Mouchel-Vielh et al. 1998; Billoud et al. 2000; Høeg & Kolbasov 2002; Pérez-Losada et al. 2002, 2009; Høeg et al. 2004). The close affinities between the thoracican barnacles and rhizocephalans are evident based on larval characters such as the type of lattice organs and molecular data sets (Spears et al. 1994; Glenner et al. 1995; Pérez-Losada et al. 2002, 2009; Høeg et al. 2004). The crucial question, however, is whether Rhizocephala is the sister-group to a monophyletic Thoracica (Fig. 3) or to a subgroup of the latter, which would render thoracicans paraphyletic (Schram & Høeg 1995). Adult and larval morphologies do not resolve the question because the morphology of Rhizocephala is so highly derived compared to thoracican barnacles that there are hardly any morphological similarities to be found. Molecular studies so far suggest a sister group relationship between the whole Thoracica and the Rhizocephala (Spears et al.1994; Mizrahi et al. 1998; Harris et al. 2000; Perl-Treves et al. 2000; Pérez-Losada et al. 2002, 2004, 2008, 2009) (Fig. 3). Interestingly enough, some of the molecular trees presented in the article by Glenner & Hebsgaard (2006: figs. 2, 4, 5) show the Thoracica as paraphyletic with respect to Rhizocephala.

The internal relationships of Thoracica are also contentious. This concerns, in particular, the position of the Iblomorpha (see Glenner et al. 1995; Høeg et al. 1999). Based on several morphological features such as a capitulum with a chitinous cover, the poorly pronounced border between the capitulum and peduncle, the absent carina, and the primitive cirri construction, the classical view interprets Iblomorpha as the sister group to all other Recent Thoracica (e.g. Klepal 1985; Newman 1987; Buckeridge & Newman 2006). This suggestion is supported by a number of phylogenetic analyses using morphology (Høeg et al. 1999) and molecular data sets including the most recent multiple gene approaches (Harris et al. 2000; Glenner & Heebsgaard 2006; Pérez-Losada et al. 2004, 2008, 2009). In contrast, a morphological cladistic and several earlier molecular analyses reveal Iblomorpha as nested in different places within the Thoracica (Glenner et al. 1995; Mizrahi et al. 1998; Perl-Treves et al. 2000). Glenner et al. (1995) discuss their own result critically and suggest that there is also a high likelihood for the iblomorphs being the sister group to the rest of the thoracicans. In a later study including fossils and using a different character treatment these authors achieved exactly this result (Høeg et al. 1999). Discussing the phylogenetic position of Iblomorpha, Høeg et al. (2009) found no morphological support for a sister group relationship between *Ibla* and either the Acrothoracica or Rhizocephala. However, based on their analysis of cypris morphology and other data, these authors restrict the possible field of iblomorph phylogenetic positions to three putative sister-group relationships, namely to a clade comprising Rhizocephala and the other Thoracica, to the Rhizocephala, or to the remaining Thoracica.

In summary, leaving Rhizocephala aside, it seems a relatively well supported view to place Iblomorpha as the sister group to all other thoracican barnacles. In contrast to this, the exact relationship of the rhizocephalans to thoracicans needs better resolution based on additional data. We think that early developmental patterns may contribute to this issue.

Early development suggests a sister group relationship between Iblomorpha and Rhizocephala

The early development of Rhizocephala

The early development of the embryos of *Peltogasterella gracilis* and *Sacculina pilosella* studied by us corresponds to that of other rhizocephalan barnacles: *Peltogasterella sulcata* (Liljeborg, 1859), *Sacculina carcini* Thompson, 1836, and *Chtamalophilus delagei* Bocquet-Védrine, 1957 (SHIRASE & YANAGIMACHI 1957; BOCQUET-VÉDRINE 1961, 1964). It can be generalized as follows. The first two divisions are total and almost equal. During this time the embryo rotates twice. The first rotation results in the position of the animal and vegetal poles at the lateral sides of the egg. The third cleavage is unequal and forms quartets of yolky macromeres and yolk-free micromeres. Subsequently, macromeres continue to divide unequally giving rise to additional micromeres. Thus, the number of four macromeres does not change until late gastrulation. After epiboly has been completed, the four yolk-containing macromeres divide almost equally resulting in eight internal cells covered entirely by the blastoderm formed by the micromeres.

The number of studies on the early development of Rhizocephala is limited, and the embryology of some akentrogonid taxa such as the Clistosaccidae, Thompsoniidae, or Duplorbidae has not been studied at all. Nevertheless, if the occurrence of the corresponding cleavage pattern is mapped onto the phylogeny of Rhizocephala (GLENNER & HEBSGAARD 2006) it is reasonable to assume that this type of early development is plesiomorphic for the whole group.

Developmental patterns in Ascothoracida, Acrothoracica, and Thoracica

The development of Ascothoracida (LACAZE-DUTHIERS 1883; KNIPOWITSCH 1892; WAGIN 1949), Acrothoracica (TURQUIER 1967) and most Thoracica (GROOM 1894; BIGELOW 1902; DELSMAN 1917; KRÜGER 1922; BATHAM 1946; ANDERSON 1969), as far as is known, follows a uniform general pattern. It starts with a total and highly unequal division resulting in a macromere containing almost all the yolk and a micromere which is more or less yolk-free. Subsequent divisions of the macromere are also highly unequal and the yolk remains in one large cell. Because of the retardation in division related to the yolk, the cleavage of the blastomeres is highly asynchronous. The blastopore forms at the lower lateral side in the macromere region of the egg indicating an oblique animal vegetal axis with respect to the long axis of the egg. The gastrulating embryos consist of about 300 cells in Ascothoracida (WAGIN 1949) and of 250 cells in Acrothoracica (TURQUIER 1967). In contrast to this, thoracican embryos are made up of only 32–62 cells during gastrulation (e.g. GROOM 1894; BIGELOW 1902; DELSMAN 1917; ANDERSON 1969). This is due to a less pronounced asynchrony in cell division between the macromere and the micromeres. Therefore, one can see stages of 4, 8, 16, and 32 cells, which are typical for synchronous cleavages (e.g. GROOM 1894; BIGELOW 1902; DELSMAN 1917; ANDERSON 1969). The development of *Lepas* is slightly different, in that, due to low general yolk content, the size difference between the macromere and the micromeres is less pronounced than in other thecostracans (GROOM 1894; BIGELOW 1902). However, the general pattern of blastomere arrangement and division corresponds to that in other thoracican species.

This distribution of these particular cleavage and gastrulation modes within the Cirripedia and the Ascothoracida clearly indicates that they are at least characteristic for the ground pattern found in the last common ancestor of these sister taxa. Furthermore, it is evident that the same patterns have been conserved in the lineage leading to the Thoracica (Fig. 5).

The development of Iblomorpha

In contrast to this ancestral pattern, the embryos of the thoracican subtaxon Iblomorpha show a different type of early cleavage, as is exemplified in *Ibla quadrivalvis* Cuvier, 1817 (ANDERSON 1965) (Fig. 4). The first two cleavages are meridional and divide the embryo into four similarly sized blastomeres. The third division is unequal and perpendicular to the previous ones (equatorial). It leads to a quartet of yolk-free micromeres and a quartet of yolky macromeres. During the first divisions the embryo rotates in such a way that the animal-vegetal axis is shifted to the lateral sides of the egg and that the blastomeres show a specific arrangement in the 4-cell stage. With further development, the macromeres continue their unequal divisions producing more micromeres. Only at later stages they show equal cleavages (ANDERSON 1965). The micromeres apparently undergo only equal divisions. During gastrulation the micromeres overgrow the larger yolk containing macromere descendants via epiboly (Fig. 4).

A comparison of iblomorph and rhizocephalan development

In summary, the iblomorph type of development is different and derived in several aspects compared to

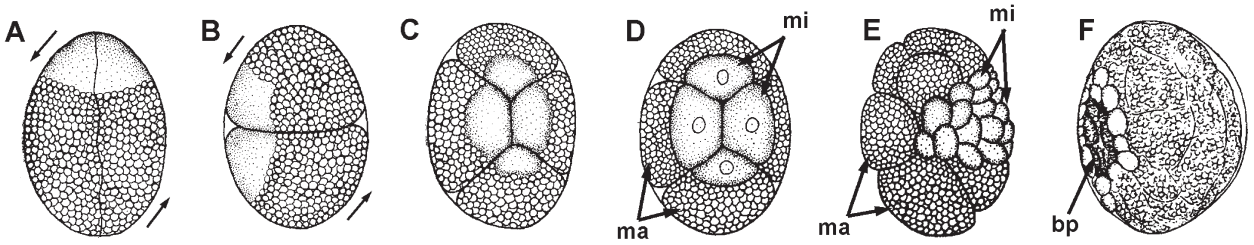

Fig. 4. Cleavage and gastrulation of *Ibla quadrivalvis* (modified after Anderson 1965). **A**: Beginning first cleavage along the longitudinal axis of the egg. The arrows mark the direction of the rotation. **B**: 2-cell stage after the rotation. **C**: Early 8-cell stage with four micromeres and four macromeres containing all the yolk, animal view. **D**: Late 8-cell stage with the four micromeres (mi) showing the nuclei, the nuclei of macromeres (ma) are hidden in the yolk. **E**: Advanced stage, lateral view, showing the activity of the micromeres (mi) forming a cap on the macromeres (ma). Micro- and macromeres divide symmetrically. **F**: Epibolic gastrulation. The yolk containing macromeres at the blastopore (bp) marking the vegetal pole are overgrown by micromeres (compare with Figs. 1, 2).

that of all other thoracican representatives and that of Acrothoracica and Ascothoracida. In contrast to this, it looks very much like the development described for Rhizocephala. Several shared characteristics of the early development of these two groups can be pointed out. First of all, the two first meridional cleavages result in four almost equal-sized yolky cells and the third equatorial cleavage produces the quartets of the micro- and macromeres. This leads to a different distribution and arrangement of the cells in the micromeres between the remaining cirripedes and ascothoracidans, on the one hand, and the iblomorphs and rhizocephalans, on the other hand. In addition, the embryos of Iblomorpha and Rhizocephala undergo the same rotation during the first two divisions (90° and 45° successively). These rotations are independent characters and not necessarily a mechanical constraint due to the oval egg shape as is exemplified by other crustaceans with oval eggs which show a great variability of the arrangement of the blastomeres at the 4-cell stage (see Scholtz & Wolff 2002). As a consequence of the first rotation, the animal and vegetal poles of the embryo are placed laterally in the egg. Hence, the animal-vegetal axis is not oblique as in the other cirripedes and ascothoracidans, but perpendicular to the long egg axis. The only difference between the cleavages in iblomorph and rhizocephalan embryos is related to the division of macromeres. Those in Rhizocephala divide unequally and remain four in number until epiboly is completed (Figs. 1, 2), and only then do they start to divide equally (Bocquet-Védrine 1964). In addition to these unequal divisions which contribute to the formation of micromeres, the macromeres of *I. quadrivalvis* show some equal divisions before epiboly is completed (Anderson 1965) (Fig. 4). Despite this difference, the general developmental similarity suggests the homology of the above mentioned characteristics in iblomorph and rhizocephalan cleavage and gastrulation. Recent studies on crustacean early development imply that the older views that the cleavage pattern of *Ibla* might be plesiomorphic among crustaceans and thus cirripedes (e.g. Anderson 1994; Scholtz 1997) are no longer tenable. There is no substantial correspondence to cleavage patterns in other crustaceans such as Malacostraca and Branchiopoda (e.g. Scholtz & Wolff 2002; Alwes 2008; Scholtz et al. 2009). Moreover, based on the overall tree topology and the distribution of the early developmental characters shared by the other cirripedes and ascothoracidans (Figs. 3, 5), we consider the cleavage and gastrulation patterns of Iblomorpha and Rhizocephala as being synapomorphies indicating that these two taxa may form a clade (Fig. 5). This would render thoracicans paraphyletic and parasitic rhizocephalans originating within thoracicans.

A scenario for the evolution of rhizocephalan parasitism

The evolution of parasitism in the rhizocephalan lineage is not well understood; although some ideas exist that suggest reasonable scenarios based on analogies to other parasitic thecostracans (Newman et al. 1969; Newman 1987; Glenner & Høeg 2002). If our view of an iblomorph/rhizocephalan clade is correct, then this would allow a new perspective on the origin of parasitism in the lineage leading to rhizocephalans. This new scenario is not only based on analogy, but on more direct evidence such as character transformation during the habitual change from a sessile stalked filter feeding organism to an endoparasite as represented by iblomorphs and rhizocephalans, respectively. Glenner & Hebsgaard (2006) make a good case for the assumption that the original hosts for rhizocephalans are representatives of anomalan decapods, namely hermit crabs. This makes sense because the rigid cuticle found in most reptant decapods creates a problem

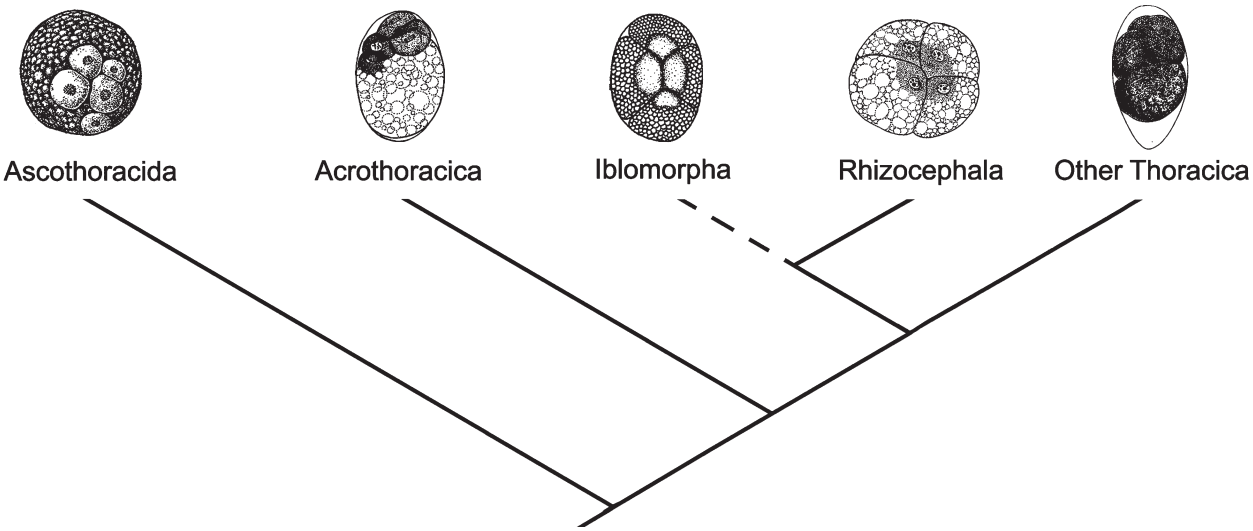

Fig. 5. The new hypothesis based on cleavage and gastrulation patterns in which Rhizocephala originate within the "Thoracica" forming a clade with Iblomorpha. According to this idea, "Thoracica" would be paraphyletic. However, the questions of whether Iblomorpha are monophyletic with respect to Rhizocephala and where to put the Iblomorpha/Rhizocephala clade are unresolved (indicated by the dotted line). Characteristic features of the early cleavages are depicted for every taxon (see text). After various authors.

for the parasites to enter the host and in most cases they use the less calcified intersegmental areas of the leg joints, the arthrodial membranes (GLENNER & HØEG 2002). Nevertheless, these authors suggest that the ancestral settling site of rhizocephalans was the branchial chamber of the decapod host. Opposed to the view of GLENNER & HØEG (2002), however, and based on the phylogeny of GLENNER & HEBSGAARD (2006), the distribution of the attachment site of rhizocephalan cyprids allows the conclusion that ancestrally rhizocephalans entered the hosts via the arthrodial membranes, i.e. through the outer cuticle. Pagurids have a cuticle lacking rigid calcification over large parts of their body, making it much easier for the infectious stages of parasites to enter the host. This is reflected, for instance, by the fact that representatives of pelterogastrids often infest hermit crab individuals in high numbers. This soft body of the hermit crabs is correlated with the use of gastropod shells for protection. Iblomorpha mostly settle either epizoically on other cirripedes or on a hard substrate, often on shelly grounds (KLEPAL 1985). This could lead to the situation that shells used by hermit crabs are colonized by iblomorph individuals. Iblomorpha possess dwarf males which are attached to the females in the mantle cavity (DARWIN 1852; KLEPAL 1985). DARWIN (1852) discussed the morphology of these dwarf males in some detail showing that they are not parasites, but are indeed the reduced males of iblomorphs. Their epizoic lifestyle, however, implies that these miniaturized males penetrate the cuticle of the female "host" in order to anchor themselves in the tissue (DARWIN 1852; KLEPAL 1985). The males possess a functional digestive tract, thus it is unlikely that they also gain nutrients from the female's body liquid, although this cannot be excluded. In any case, this anatomical precondition of the dwarf males, which is part of the iblomorph genomic constitution, in combination with the colonization of shelly substrates might indeed be the starting point for using soft hermit crabs and later more and more calcified decapod species as hosts.

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References

ALWES, F. 2008. Cell lineage studies in Crustacea – Aspects of the early development and germ layer formation in *Meganyctiphanes norvegica* (Malacostraca, Euphausiacea) and *Bythotrephes longimanus* (Cladocera, Branchiopoda). – Doctoral Thesis, Humboldt-Universität zu Berlin. 108 pp.

ANDERSON, D.T. 1965. Embryonic and larval development and segment formation in *Ibla quadrivalvis* Cuv. (Cirripedia). – Australian Journal of Zoology **13**: 1–15.

ANDERSON, D.T. 1969. On the embryology of the cirripede crustaceans *Tetraclita rosea* (Krauss), *Tetraclita pur-*

purascens (Wood), *Chthamalus antennatus* (Darwin) and *Chamaesipho columna* (Spengler) and some considerations of crustacean phylogenetic relationships. – Philosophical Transactions of the Royal Society of London B **256**: 183–235.

Anderson, D.T. 1973. Embryology and Phylogeny in Annelids and Arthropods. – Pergamon Press, Oxford. 495 pp.

Anderson, D.T. 1994. Barnacles – Structure, Function, Development and Evolution. – Chapman & Hall, London. 357 pp.

Batham, E.J. 1946. *Pollicipes spinosus* Quoy and Gaimard. II. Embryonic and larval development. – Transactions of the Royal Society of New Zealand **75**: 405–418.

Bigelow, M.A. 1902. The early development of *Lepas*. A study of cell lineage and germ layers. – Bulletin of the Museum of Comparative Zoology Harvard **40**: 61–144.

Billoud, B., M.-A. Guerrucci, M. Masselot & J.S. Deutsch 2000. Cirripede phylogeny using a novel approach: Molecular morphometrics. – Molecular Biology and Evolution **17**: 1435–1445.

Bocquet-Védrine, J. 1961. Monographie de *Chtamalophilus delagei* J. Bocquet-Védrine, rhizocéphalan parasite de *Chtamalus stellatus* (Poli). – Cahiers de Biologie Marine **2**: 455–593.

Bocquet-Védrine, J. 1964. Embryologie precoce de *Sacculina carcini* Thompson. – Zoologische Mededelingen **39**: 1–11.

Buckeridge, J.S. & W.A. Newman 2006. A revision of the Iblidae and the stalked barnacles (Crustacea: Cirripedia: Thoracica), including new ordinal, familial and generic taxa, and two new species from New Zealand and Tasmanian waters. – Zootaxa **1136**: 1–38.

Darwin, C.R. (1851 [=1852]). A monograph of the sub-class Cirripedia, with figures of all the species. The Lepadidæ or pedunculated cirripedes. – The Ray Society, London. 400 pp., 10 pls.

Delsman, H.C. 1917. Die Embryonalentwicklung von *Balanus balanoides* L. – Tijdschrift der Nederlandsche Dierkundige Vereeniging (Ser. 2) **15**: 419–520.

Deutsch, J.S., E. Mouchel-Vielh, È. Quéinnec & J.-M. Gibert 2004. Genes, segments, and tagmata in cirripedes. Pp. 19–42 *in*: G. Scholtz (ed.), Evolutionary Developmental Biology of Crustacea; Crustacean Issues 15. – Balkema, Lisse.

Géant, É, E. Mouchel-Vielh, J.-P. Coutanceau, C. Ozouf-Costaz & J.S. Deutsch 2006. Are Cirripedia hopeful monsters? Cytogenetic approach and evidence for a Hox gene cluster in the cirripede crustacean *Sacculina carcini*. – Development Genes and Evolution **216**: 443–449.

Glenner, H. & J.T. Høeg 2002. A scenario for the evolution of the Rhizocephala. Pp. 301–310 *in*: F. Alvarez & E. Escobar-Briones (eds.), Modern Approaches to the Study of Crustacea. – Kluwer Academic/Plenum Publishers, New York.

Glenner, H.M. & M.B. Hebsgaard 2006. Phylogeny and evolution of life history strategies of the parasitic barnacles (Crustacea, Cirripedia, Rhizocephala). – Molecular Phylogenetics and Evolution **41**: 528–538.

Glenner, H.M., M.J. Grygier, J.T. Høeg, P.G. Jensen & F.R. Schram 1995. Cladistic analysis of the Cirripedia Thoracica (Crustacea: Thecostraca). – Zoological Journal of the Linnean Society **114**: 365–404.

Groom, T.T. 1894. On the early development of Cirripedia. – Philosophical Transactions of the Royal Society of London B **185**: 119–232.

Gruner, H.-E. 1993. Crustacea. Pp. 448–1030 *in*: H.-E. Gruner (ed.), Arthropoda (ohne Insecta); Lehrbuch der Speziellen Zoologie, Bd. I, 4. Teil. – Gustav Fischer Verlag, Jena.

Harris, D.J., L.S. Maxson, L.F. Braithwaite & K.A. Crandall 2000. Phylogeny of the thoracican barnacles based on 18S rDNA sequences. – Journal of Crustacean Biology **20**: 393–398.

Healy, J.M. & D.T. Anderson 1990. Sperm ultrastructure in the Cirripedia and its phylogenetic significance. – Records of the Australian Museum **42**: 1–26.

Høeg, J.T. 1992. The phylogenetic position of the Rhizocephala: are they truly barnacles? – Acta Zoologica (Stockholm) **73**: 323–326.

Høeg, J.T. & G.A. Kolbasov 2002. Lattice organs in y-cyprids of the Facetotecta and their significance in the phylogeny of the Crustacea Thecostraca. – Acta Zoologica **83**: 67–79.

Høeg, J.T., M.A. Whyte, H. Glenner & F.R. Schram 1999. New evidence on the basic phylogeny of the Cirripedia Thoracica. Pp. 101–114 *in*: F.R. Schram & J.C. von Vaupel Klein (eds.), Crustaceans and the Biodiversity Crisis; Proceedings of the Fourth International Crustacean Congress. – Brill, Leiden.

Høeg, J.T., N.C. Lagersson & H. Glenner 2004. The complete cypris larva and its significance in thecostracan phylogeny. Pp. 197–215 *in*: G. Scholtz (ed.), Evolutionary Developmental Biology of Crustacea; Crustacean Issues 15. – Balkema, Lisse.

Høeg, J.T., Y. Achituv, B.K.K. Chan, K. Chan, P.G. Jensen & M. Pérez-Losada 2009. Cypris morphology in the barnacles *Ibla* and *Paralepas* (Crustacea: Cirripedia Thoracica) implications for cirripede evolution. – Journal of Morphology **270**: 241–255.

Klepal, W. 1985. *Ibla cumingi* (Crustacea; Cirripedia) – a gonochoristic species (anatomy, dwarfing and systematic implications). – Marine Ecology **6**: 47–119.

Krüger, P. 1922. Die Embryonalentwicklung von *Scalpellum scalpellum* L. I. Furchung und Anlage der Keimblätter. – Archiv für Mikroskopische Anatomie **96**: 355–386.

Knipowitsch, N. 1892. Materialy k poznaniyu gruppy Ascothoracida (Beiträge zur Kenntniss der Gruppe Ascothoracida). – Trudy Obshchestva Estestvoispytatelei **23**: 1–155.

Lacaze-Duthiers, H. 1883. Histoire de la *Laura gerardiae* type nouveau de Crustacé parasite. – Mémoires de l'Académie des Sciences de l'Institut de France (Ser. 2) **42**: 1–160.

Mizrahi, L., Y. Achituv, D.J. Katcoff & R. Perl-Treves 1998. Phylogenetic position of *Ibla* (Cirripedia: Thoracica) based on 18S rDNA sequence analysis. – Journal of Crustacean Biology **18**: 363–368.

Mouchel-Vielh, E., C. Rigolot, J.-M. Gibert & J.S. Deutsch 1998. Molecules and the body plan: the Hox genes of cirripedes (Crustacea). – Molecular Phylogenetics and Evolution **9**: 382–389.

NEWMAN, W.A. 1987. Evolution of cirripedes and their major groups. Pp. 3–42 *in*: A.J. SOUTHWARD (ed.), Barnacle Biology; Crustacean Issues 5. – A.A. Balkema Press, Rotterdam.

NEWMAN, W.A., V.A. ZULLO & T.H. WITHERS 1969. Cirripedia. Pp. 206–295 *in*: R.C. MOORE (ed.), Treatise on Invertebrate Paleontology, Part R, Arthropoda 4, Vol. 1. – University of Kansas and the Geological Society of America, Boulder.

PÉREZ-LOSADA, M., J.T. HØEG, G.A. KOLBASOV & K.A. CRANDALL 2002. Reanalysis of the relationships among the Cirripedia and the Ascothoracida and the phylogenetic position of the Facetotecta (Maxillopoda: Thecostraca) using 18S rDNA sequences. – Journal of Crustacean Biology **22**: 661–669.

PÉREZ-LOSADA, M., J.T. HØEG & K.A. CRANDALL 2004. Unravelling the evolutionary radiation of the thoracican barnacles using molecular and morphological evidence. – Systematic Biology **53**: 244–254.

PÉREZ-LOSADA, M., M. HARP, J.T. HØEG, Y. ACHITUV, D. JONES, H. WATANABE & K.A. CRANDALL 2008. The tempo and mode of barnacle evolution. – Molecular Phylogenetics and Evolution **46**: 328–346.

PÉREZ-LOSADA, M., J.T. HØEG & K.A. CRANDALL 2009. Remarkable convergent evolution in specialized parasitic Thecostraca (Crustacea). – BMC Biology **7**: 15.

PERL-TREVES, R., L. MIZRAHI, D.J. KATCOFF & Y. ACHITUV 2000. Elucidation of the phylogenetic relationships of three thecostracans, *Verruca*, *Paralepas*, and *Dendrogaster* based on 18S rDNA sequences. – Journal of Crustacean Biology **20**: 385–392.

SCHOLTZ, G. 1997. Cleavage, germ band formation and head segmentation: the ground pattern of the Euarthropoda. Pp. 317–332 *in*: R.A. FORTEY & R.H. THOMAS (eds.), Arthropod Relationships. – Chapman & Hall, London.

SCHOLTZ, G. 2008. Zoological detective stories – the case of the facetotectan life cycle. – Journal of Biology **7**: 16.

SCHOLTZ, G. & C. WOLFF 2002. Cleavage pattern, gastrulation, and germ disc formation of the amphipod crustacean *Orchestia cavimana*. – Contributions to Zoology **71**: 9–28.

SCHOLTZ, G., A. ABZHANOV, F. ALWES, C. BIFFIS & J. PINT 2009. Development, genes, and decapod evolution. Pp. 31–46 *in*: J.W. MARTIN, K.A. CRANDALL & D.L. FELDER (eds.), Decapod Crustacean Phylogenetics; Crustacean Issues 18. – Taylor & Francis, CRC Press, Boca Raton.

SCHRAM, F.R. & J.T. HØEG 1995. New frontiers in barnacle evolution. Pp. 297–312 *in*: J.T. HØEG & F.R. SCHRAM (eds.), New Frontiers in Barnacle Evolution; Crustacean Issues 10. – Balkema Press, Rotterdam.

SHIRASE, S. & R. YANAGIMACHI 1957. The early development of *Peltogasterella socialis* Kruger (a rhizocephalan). – Doubutsugaku Zasshi **66**: 253–257.

SIEWING, R. 1979. Homology of cleavage types? – Fortschritte in der Zoologischen Systematik und Evolutionsforschung **1**: 7–18.

SPEARS, T., L.G. ABELE & M.A. APPLEGATE 1994. A phylogenetic study of cirripeds and their relatives (Crustacea, Thecostraca). – Journal of Crustacean Biology **14**: 641–656.

THOMPSON, J.V. 1836. Natural history and metamorphosis of an anomalous crustaceous parasite of *Carcinus maenas*, the *Sacculina carcini*. – Entomological Magazine London **3**: 456.

TURQUIER, Y. 1967. L'embryogenése de *Trypetesa nassarioides* Turquier (Cirripède Acrothoracique). Ses rapports avec celle des autres Cirripèdes. – Archives de Zoologie Expérimentale et Générale: Histoire Naturelle, Morphologie, Histologie, Évolution des Animaux **108**: 111–137.

WAGIN, V.L. 1949. On development in Ascothoracida Wagin (Crustacea, Entomostraca) and original types of cleavage in Arthropoda (O Droblenii u Ascothoracida Wagin (Crustacea, Entomostraca) i ishodnyh tipah drobleniya Arthropoda). – Ucenye Zapiski Leningradskogo Ordena Lenina Gosudarstvennogo Universiteta Imeni A.A. Zdanova, Serija Biologiceskich Nauk, **113**: 143–180.

WINSOR, M.P. 1969. Barnacle larvae in the nineteenth century. – Journal of the History of Medicine and Allied Sciences **24**: 294–309.