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ORIGINAL ARTICLE

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Cleavage and gastrulation of the euphausiacean *Meganyctiphanes norvegica* (Crustacea, Malacostraca)

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Abstract According to the 'Articulata' hypothesis the cleavage of arthropods must be derived from spiral cleavage. However, arthropods show a great variety of cleavage modes with a widespread occurrence of superficial cleavage. In the Malacostraca, holoblastic cleavage occurs in some taxa such as Amphipoda, Euphausiacea and Dendrobranchiata. In particular, the cleavage of euphausiaceans has been proposed to be a modified spiral cleavage. The cell lineage of early stages up to blastoderm formation of the euphausiacean *Meganyctiphanes norvegica* is reconstructed using recent methods of fluorescent staining. Only the oblique angle of the mitotic spindles during the transition from the 2- to the 4-cell stage resembles the spiral cleavage mode. At the 8-cell stage, four cells each form a pattern of two interlocking bands which is preserved until the 122-cell stage. One blastomere is delayed in division and shows an oblique division from the fourth cleavage on. It is the precursor cell of two enlarged and cleavage-arrested cells at the 32-cell stage. At the 62-cell stage, these two cells are surrounded by eight cells following a specific cell division pattern during the subsequent division cycles. The cleavage pattern of *M. norvegica* occurs in two mirror images. A comparative approach reveals distinct similarities between the early cleavage patterns of Euphausiacea and Dendrobranchiata which are suggested to be homologous. Furthermore, the relationships to non-malacostracan cleavage patterns are discussed. It is shown that the early cleavage pattern of *M. norvegica* does not offer an example of a spiral cleavage within arthropods.

Keywords Cell lineage · Arthropods · Spiral cleavage · Articulata · Ecdysozoa

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Introduction

Arthropods display a large variety of cleavage modes ranging from superficial cleavage to holoblastic development with a spectrum of intermediate modes (Anderson 1973; Scholtz 1997). Hence, the comparison of arthropod cleavage patterns still leads to a controversial interpretation of the ancestral condition ranging from superficial to total cleavage and from radial to spiral cleavage (see, for example, Siewing 1969; Anderson 1973; Weygoldt 1986; Scholtz 1997; Nielsen 2001). The recent discussion of whether the closest relatives of arthropods are found among the Cycloneuralia ('Ecdysozoa' hypothesis) or Annelida ('Articulata' hypothesis) (Aguinaldo et al. 1997; Schmidt-Rhaesa 1998; Wägele et al. 1999; Giribet et al. 2000; Wägele and Misof 2001; Scholtz 2002) revived interest in arthropods with total cleavage because they might reveal hints that point either to the spiral cleavage of annelids or to the bilateral cleavage of cycloneuralians.

Most cases of total cleavage among arthropods are found in the Crustacea, and total cleavage is claimed to be the plesiomorphic feature of this group (Anderson 1973; Dohle and Scholtz 1997). However, the mode of the plesiomorphic crustacean cleavage pattern is still not clear. Many researchers have interpreted the early development of crustaceans as a modified spiral cleavage (see, for example, Taube 1909, 1915; Shiino 1968; Anderson 1973; Nielsen 2001). This suggestion was disputed by several authors (see, for example, Kühn 1912; Siewing 1969, 1979; Zilch 1978, 1979; Dohle 1979, 1989; Hertzler and Clark 1992; Scholtz 1997; Wolff and Scholtz 2002). The controversial interpretations of the early crustacean cleavage mode are mainly due to the insufficient knowledge of the exact cell lineages and the origin of germ layers in most species investigated.

The use of modern methods in embryology, such as lineage tracing with vital markers, fluorescent dyes and the use of laser scanning confocal microscopy, allows a more detailed view of early developmental processes in crustaceans. Recent examples include the cleavage of some malacostracan crustaceans, a dendrobranchiate de-

capod (Hertzler and Clark 1992; Hertzler et al. 1994; Hertzler 2002) and two amphipod species (Gerberding et al. 2002; Scholtz and Wolff 2002; Wolff and Scholtz 2002). These reports reveal that the cleavage modes of dendrobranchiate decapods and amphipods are very different in terms of cell division patterns and cell lineage. However, none of these cleavage modes was interpreted as spiral by the authors. In contrast, the studies of Taube (1909, 1915) claim a spiral cleavage for Euphausiacea and suggest some similarities between euphausiacean and dendrobranchiate cleavages.

Here we describe the early cleavage pattern of the euphausiacean *Meganyctiphanes norvegica* (M. Sars, 1857) from the zygote to gastrulation. We use DNA-selective fluorescent dyes as an easy and distinct method to reconstruct development by comparing the position and the orientation of dividing nuclei by which cleavage patterns are defined (Freeman 1983; Wang et al. 1997). We compare the cleavage pattern of euphausiaceans with that of other malacostracans, namely dendrobranchiate decapods, and non-malacostracans with total cleavage in order to reveal similarities that can be used for crustacean phylogenetic systematics. This study also addresses the question of whether the cleavage of euphausiaceans shows characteristics of spiral cleavage.

Materials and methods

Animals and embryos

Specimens of *M. norvegica* were collected in the Gullmarsfjord off Fiskebäckskil in Sweden during the summer-to-fall reproductive season 2001. Animals were maintained in a 1-m^3 flow-through seawater aquarium according to the recommended conditions by Komaki (1966). Gravid females were isolated for spawning in 0.5-l jars and eggs were collected after passing through a grid at the bottom of the jars. Live embryos were also directly observed over time using an inverted microscope and the progress of development was documented by sketches. For further processing the eggs were fixed at 30-min intervals for 10–15 min in 3.7% formaldehyde in PBS (8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4, pH 7.4). Subsequently the eggs were washed in PBS where embryonic envelopes were removed manually. The eggs were kept at −8°C in absolute methanol.

Nuclear staining

For staining with the DNA-selective fluorescent dye Hoechst (Bisbenzimide, H33258), the preserved and dissected eggs were washed in PBS several times (3×5 min and 4×30 min). Then they were transferred into Hoechst solution (0.9 μg Hoechst in 1 ml PBS). Unbound dye was removed by washing in PBS, as described. After embedding in DABCO–glycerol [2.5 mg/ml DABCO (1,4-diazobicyclo[2.2.2.]octane; Merck) in 90% glycerol–PBS], the eggs were observed under a fluorescence microscope (Zeiss Axiophot 1; excitation 352 nm, emission 461 nm). Additionally, eggs were stained with the fluorescent dye $Sytox_{green}$ (Molecular Probes) which has a high affinity to nucleic acid in general. The staining procedure followed that of the Hoechst staining but, instead of PBS, the embryos were washed in TBS (TRIS-buffered saline: 0.9% NaCl, 10 mM TRIS, pH 7.5) and incubation was done in Sytox solution [2.5 μl 5 mM Sytox–DMSO diluted in 1 ml TE buffer (1 mM TRIS-HCl, 0.1 mM EDTA, pH 7.5)].

Relevant stages were documented by drawings and with a Nikon D1 digital camera. Brightness and contrast of the digital images was adjusted using Photoshop 6.0.

Histology

Relevant Hoechst-stained stages were returned to PBS and washed several times. Following dehydration in a graded ethanol series the embryos were transferred to methacrylate embedding medium (Technovit) following standard protocols. The eggs were oriented in the optimal position under a fluorescence microscope (Zeiss Axiophot 1). Serial semithin sections (3–5 μm) were obtained by using a Zeiss microtome (HM 355). Sections were dried on slides and stained with 0.1% toluidine blue following standard protocols.

Nomenclature

The nomenclature is based on the four capital letters **A**, **B**, **C** and **D** which designate the blastomeres of the 4-cell stage (Fig. 1). The blastomeres of the 2-cell stage are designated **AB** and **CD** (Fig. 1). The smaller cell **AB** produces the daughter cells **A** and **B** and the larger one divides into **C** and **D**. Their daughter cells are each designated with the indices I and II such as $\mathbf{A}_{I}$ and $\mathbf{A}_{II}$ (Fig. 1), while index I marks the cells at the so-called ‘Brechungsfurche’ (‘breaking furrow’; Zilch 1979), the ‘cross-furrow’ in the English literature, and index II marks the cells at the end of each ring (see Fig. 2G, H).

The descendants of these cells are marked by addition of the indices a (anterior), p (posterior), v (ventral) and d (dorsal), such as $\mathbf{A}_{I,a}$ and $\mathbf{A}_{I,p}$, according to the relative position traced back from later stages. With the establishment of the dorsoventral body axis during fifth cleavage we refer to the position of the cells from the 32-cell stage on by addition of the indices a, p, v and d, and due to bilaterality, additionally r (right) and l (left). There are two mirror-imaged cleavage types (type I and type II, see below). We used type I as the standard for our nomenclature. In the mirror image of cleavage type I, cleavage type II, the capital letters are additionally marked by a stripe (for example, ‘**D**). Note that those cells in cleavage type II designated by the indices r and l due to their relative position to the body axis have different cell lineages from cells with the corresponding index in type I (for example, ‘$\mathbf{D}_{II,v,l,v}$ in the cleavage type II has a different cell lineage from $\mathbf{D}_{II,v,l,v}$ in the cleavage type I, but it has the same cell lineage as $\mathbf{D}_{II,v,r,v}$ in type I). To make descriptions more transparent, ‘important’ cells are successively labelled by single capital letters during the description of the advanced cleavage and differentiation processes.

Results

The fertilized egg has a spherical shape of about 350 μm in diameter. After spawning it is first closely surrounded by two thin transparent envelopes each temporarily associated with one polar body. The polar bodies disappear during the initial cleavages. No spatial relationship of the polar bodies and the first division plane of the egg could be recognized. A short time after spawning the envelopes expand and the distance to the egg is about 150 μm, forming a large perivitelline space around the egg (Fig. 2A). Throughout further development the dividing egg is suspended within these embryonic envelopes until the nauplius hatches after about 1 day.

In general, early cleavage of *M. norvegica* is holoblastic and not influenced by the yolk. The yolk is distributed evenly in granules. It is veined with thin cytoplasmic threads connecting a central mass of cyto-

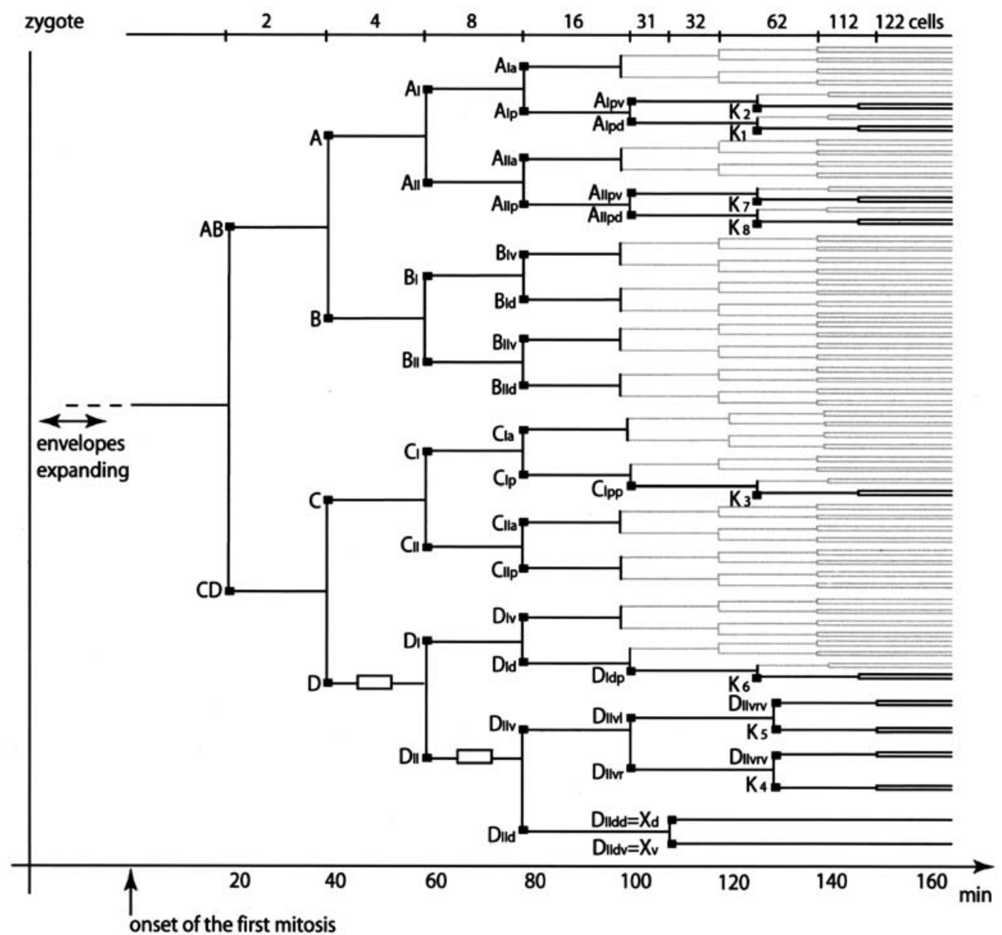


Fig. 1 Schematic overview of the cell lineage of *Meganyctiphanes norvegica* in relation to the timing of the cleavage events (symbolized as *vertical lines*). The cell lineages of X_d and X_v, the eight Kranzzellen (K_1 to K_8) and of the cells $D_{II,v,l,v}$ and $D_{II,v,r,v}$ are emphasized from the fifth mitosis on (*black lines*), cell lineages that are not analysed in detail are represented as *grey lines*. The duration of the cell cycles in minutes are approximated data. The designations here only refer to the important cells of the lineage of cleavage type I. The *white boxes* symbolize the delay of mitosis of D and D_{II} during anaphase

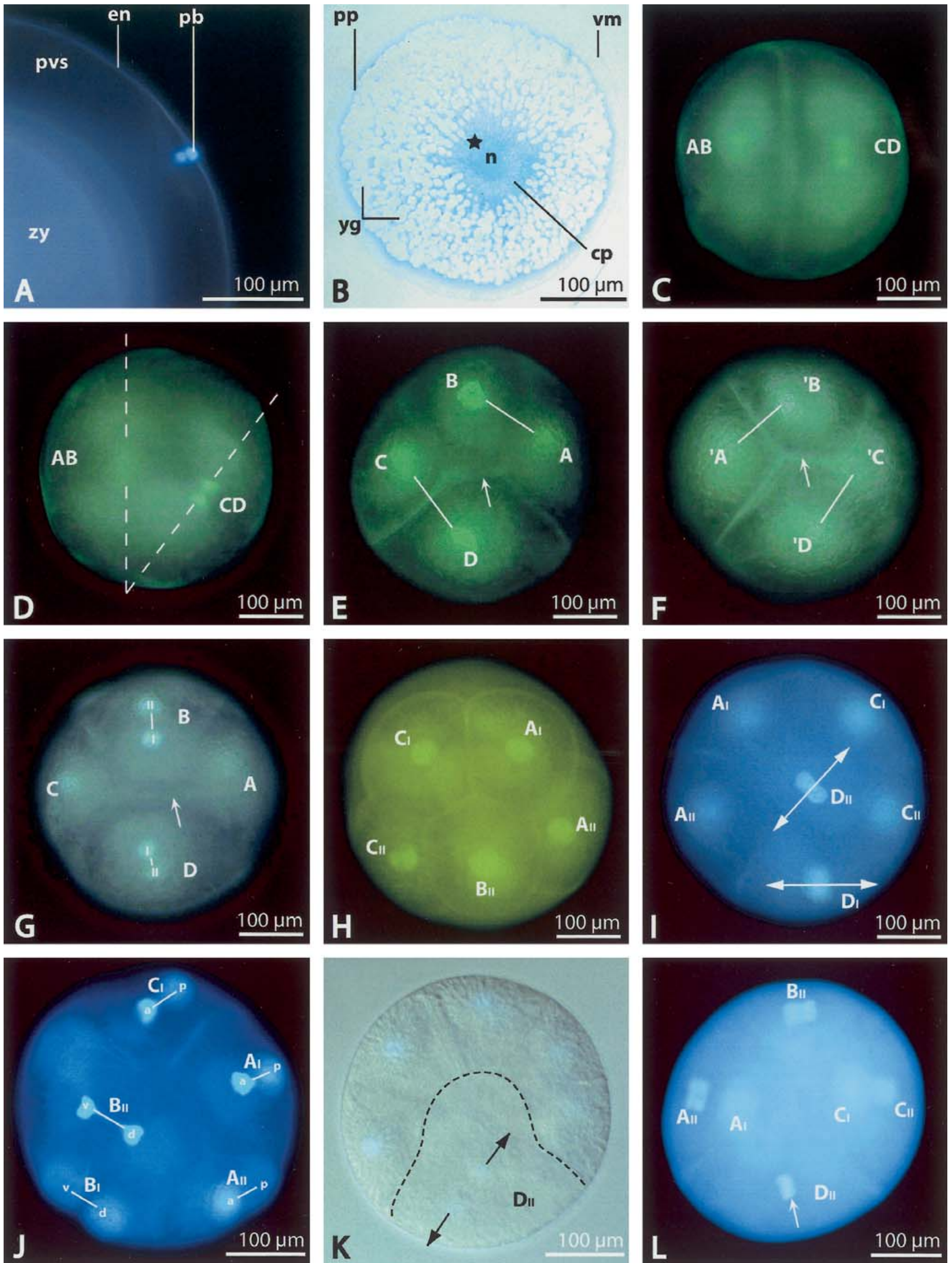
plasm around the nucleus with a thin layer of periplasm in the periphery of the egg (Fig. 2B). With the onset of the first mitosis a rapid series of cleavage cycles of about 20 min follows until gastrulation *sensu stricto* takes place after about 3 h. Up to gastrulation, the blastomeres are packed closely without forming a blastocoel. No increase in embryonic size was observed until late gastrulation when the embryo starts growing at last filling out the whole volume of the envelopes as a nauplius.

The first two cleavages: formation of the 4-cell stage

At metaphase of the first mitotic cycle the nucleus is slightly off-centre (Fig. 2B). The first cleavage divides the zygote into two slightly unequal blastomeres (Fig. 2C). The smaller cell is designated **AB** and the larger one **CD**. After separation of the two blastomeres, the mitosis of the following cleavage is already initiated without having formed nuclei at interphase. The second division occurs synchronously. At anaphase the spindles form an angle of about nearly 45° (Fig. 2D). As a result, the subsequent cleavage leads to a spatial arrangement in which two cells each form an additional plane of contact, which appears as a cross-furrow (Fig. 2E). The four blastomeres differ slightly in size. One blastomere is detectable as the smallest cell **A**, one as the largest cell **D** and the two middle and equally sized cells are designated **B** and **C** (Fig. 2E). Two blastomeres that are each derived from different precursor cells form the cross-furrow and these two cross-furrows lie at right angles to each other. The smaller cell **A** from **AB** contacts with **C**, and the larger cell **D** from **CD** contacts with **B** (Fig. 2E). As shown in Fig. 2E, F, the arrangement of the four blastomeres appears in two stereotyped mirror images designated type I (Fig. 2E) and type II (Fig. 2F), which become more obvious at later stages (see fifth cleavage and following). In the following description it will be mainly referred to the cleavage type I.

The third cleavage: formation of the 8-cell stage

The initiation of the third cleavage is synchronous in all blastomeres. During the course of the third mitosis cell **D** is temporarily delayed in early anaphase. The spindles of **A** and **C** and of **B** and **D** each orient end-to-end resulting in two interlocking semicircles of four cells each (Fig. 2G). One semicircle designated **AC** is formed by $\mathbf{A_{II}}$, $\mathbf{A_{I}}$, $\mathbf{C_{I}}$ and $\mathbf{C_{II}}$ (Fig. 2H), and the other semicircle designated **BD** by $\mathbf{B_{II}}$, $\mathbf{B_{I}}$, $\mathbf{D_{I}}$ and $\mathbf{D_{II}}$ (Fig. 2G). A similar arrangement at the 8-cell stage in the dendrobranchiate



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Sicyonia ingentis (Burkenroad, 1938) is compared with interlocking 'horseshoe-like sheets' (Hertzler and Clark 1992).

At the completed 8-cell stage the blastomeres are approximately equal in size, except cell $\mathbf{D_{II}}$ which is slightly larger than all other cells. $\mathbf{D_{II}}$ expands deeper into the blastula and marks the region of the vegetal pole (defined by the future gastrulation area). During further development the blastopore and later the anus in the posterior of the nauplius larva is formed in this area. The opposite pole marks the prospective anterior part of the nauplius.

The fourth cleavage: formation of the 16-cell stage

At the beginning of the fourth mitosis the pattern of the two interlocking semicircles is still preserved (Fig. 2I, J). During this cleavage the nuclei of all cells move more apically. The spindle orientation of each cell is perpendicular to that of the preceding mitosis. Consequently, the spindle orientations of all mitoses in each semicircle are parallel to each other (Fig. 2J), except in $\mathbf{D_{II}}$ at the end of the semicircle **BD** which is oblique relative to that of all other mitoses (Fig. 2I). The spindle in $\mathbf{D_{II}}$ projects obliquely into the blastula (Fig. 2K). The division of $\mathbf{D_{II}}$ is slightly unequal, resulting in the smaller cell $\mathbf{D_{II,v}}$ and the larger cell $\mathbf{D_{II,d}}$. This division is temporarily delayed during early anaphase (Fig. 2L). All other cells divide synchronously (Fig. 2L). As a result the semicircles widen to interlocking cell bands which are composed of two rows of four cells each. One cell band now contains the cells $\mathbf{A_{II,a}}$ $\mathbf{A_{II,p}}$ $\mathbf{A_{I,a}}$ $\mathbf{A_{I,p}}$ $\mathbf{C_{I,a}}$ $\mathbf{C_{I,p}}$ $\mathbf{C_{II,a}}$ and $\mathbf{C_{II,p}}$ and the other band $\mathbf{B_{II,v}}$ $\mathbf{B_{II,d}}$ $\mathbf{B_{I,v}}$ $\mathbf{B_{I,d}}$ $\mathbf{D_{I,v}}$ $\mathbf{D_{I,d}}$ $\mathbf{D_{II,v}}$ and $\mathbf{D_{II,d}}$. Due to the size and position of cell $\mathbf{D_{II,d}}$ the pattern of the interlocking cell bands is altered at the end of the cell band **BD** (vegetal pole).

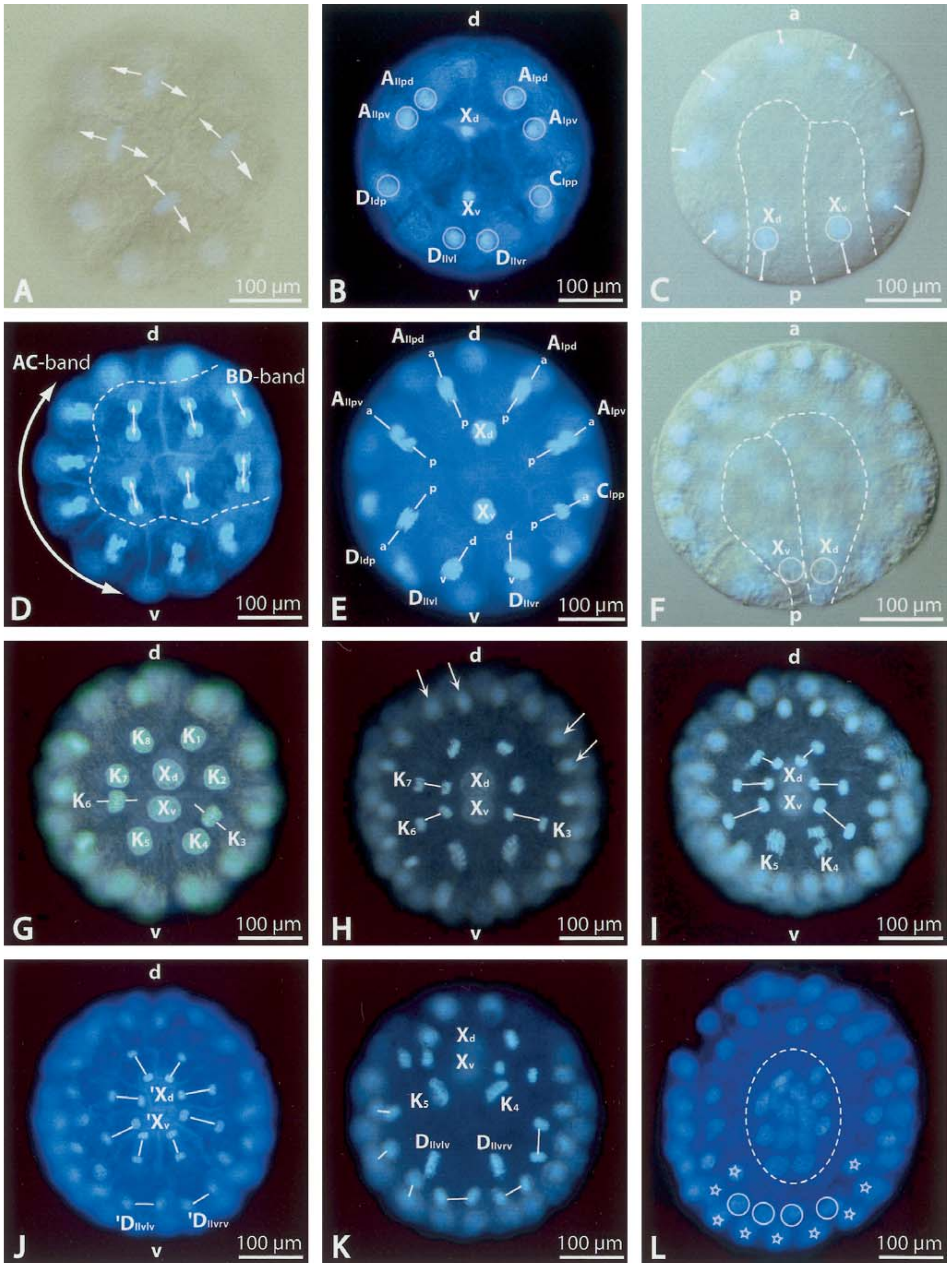
Fig. 2A–L Early cleavage stages until the fourth cleavage stained with fluorescent dyes (Hoechst: **A**, **I–L**; Sytox: **C–H**), except panel **B** which is a semithin section stained with toluidine blue. **A** The zygote (*zg*) is surrounded by two envelopes (*en*), each associated with one polar body (*pb*). Between the envelopes and the zygote a perivitelline space (*pvs*) is formed. **B** Section of a zygote at the beginning of mitosis. The nucleus (*n*) is adjacent to the immediate centre of the egg (*star*). The cytoplasm (*cp*) is accumulated around the nucleus and is connected by thin cytoplasmic threads to the peripheral periplasm (*pp*). *yg* Yolk granules, *vm* vitelline membrane. **C** The 2-cell stage with the nuclei at anaphase of the following mitosis. Cell AB is slightly smaller than cell CD. **D** The same egg as in **C**. The directions of the spindles of the second mitosis form an angle of nearly 45° (*broken line*). **E** The 4-cell stage of type I after second cleavage. The previous spindle orientations are marked by the *lines*. The largest cell D forms with cell B the cross-furrow BD (*arrow*). The smallest cell A forms with C the cross-furrow AC. The cross-furrows of AC and BD are at right angles to each other (not shown here). **F** A 4-cell stage that represents the mirror image type II of the type I in **E**. As in cleavage type I cross-furrow 'AC is formed by cell 'A and 'C and cross-furrow 'BD is formed by cell 'B and 'D, but due to the different previous spindle orientation (*lines*) A lies left and C lies right from cross-furrow 'BD (*arrow*). **G** View of the cross-furrow BD (*white arrow*) of a stage during third cleavage. During this cleavage the spindle of B and D as well as A and C orientate end-to-end. **H** The end-to-end orientation during the third cleavage leads to two interlocking cell half-rings of four cells each. Those cells that are at the end of one ring are designated with the index II and those at the former cross-furrow are designated with the index I. **I** The 8-cell stage in which the following mitosis is just initiated. View of blastomere D_{II} in focus. The nuclei of the cell band AC are positioned in one plane, not in focus here. Their spindles orient at right angles to this plane. The *white arrows* indicate the spindle direction of the following mitosis. **J** The fourth cleavage cycle. The cells at late anaphase show that the spindle orientation (*lines*) of each cell band are parallel. **K** Superimposed photograph with fluorescence and interference focused on the centre of the blastula of an 8- to 16-cell stage. D_{II} expands deeper into the blastula (*broken line*). The spindle orientation of D_{II} indicated by the *black arrows* is oblique into the egg. **L** The retardation of the cell division of D_{II} at early anaphase (*white arrow*) during the fourth cleavage. The nuclei (for example, see A_{II} and B_{II}) are at a later stage of anaphase

The fifth cleavage: formation of the 32-cell stage

The fifth cleavage results in a 32-cell stage. Spindles are oriented perpendicularly to those of the previous division extending the cell bands to two rows of eight cells each (Fig. 3A). The pattern of the two interlocking cell bands is best preserved at the animal pole. At the vegetal pole the pattern is less obvious due to the deviant behaviour of cell $\mathbf{D_{II,d}}$ (Figs. 3B, 4A). Whilst all other cells have just completed mitosis, cell $\mathbf{D_{II,d}}$ is still in late anaphase leading to a transient stage of 31 cells (Fig. 3B). The orientation of the spindle of $\mathbf{D_{II,d}}$ is oblique relative to that of all other cells. Due to this spindle orientation and due to the enlarged size of $\mathbf{D_{II,d}}$, cell $\mathbf{D_{II,v}}$ is shifted more ventrally and the derivatives of $\mathbf{C_{II}}$ are moved more anteroventrally (see Fig. 4A). Tracing back from later stages shows that the division of $\mathbf{D_{II,d}}$ establishes the dorsoventral body axis with $\mathbf{D_{II,d,d}}$ ($\mathbf{X_d}$) in a dorsal and $\mathbf{D_{II,d,v}}$ ($\mathbf{X_v}$) in a ventral position (Fig. 3B). In contrast to all other cells, $\mathbf{X_d}$ and $\mathbf{X_v}$ do not participate in cell division for at least two cycles. $\mathbf{X_d}$ and $\mathbf{X_v}$ can be distinguished from each other by their shape from the late 32-cell stage on. $\mathbf{X_d}$ exhibits a slightly elongated shape whereas $\mathbf{X_v}$ is broader (Fig. 3C). $\mathbf{X_d}$ and $\mathbf{X_v}$ basally fill the whole blastula at the end of the 32-cell stage. As in other cells, the nuclei of $\mathbf{X_d}$ and $\mathbf{X_v}$ are located at the apical region but in contrast to the other cells they lie slightly deeper inside the blastula (Fig. 3C).

The dorsoventral axis defined by $\mathbf{X_d}$ and $\mathbf{X_v}$ divides the population of the surrounding cells in a bilateral pattern reflecting the two mirror images already found in the arrangements of the blastomeres at the 4-cell stage (compare Figs. 3B, 4A, B). As shown in Fig. 4A, B in type I the cell band **AC** surrounds $\mathbf{X_d}$ and $\mathbf{X_v}$ in a right-handed pattern while cell band **BD** extends from vegetal pole to animal pole on the left. Type II represents the exact mirror image arranged with cell band **'AC** in a left-handed pattern and cell band **'BD** on the right.

At the beginning of the fifth cycle, five cells ($\mathbf{A_{II,p}}$ $\mathbf{A_{I,p}}$ $\mathbf{C_{I,p}}$ $\mathbf{D_{II,v}}$ and $\mathbf{D_{I,d}}$) adjoin $\mathbf{D_{II,d}}$. Due to their spindle orientation both daughter cells of each of the three cells



A
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d
A_{IIpd}
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A_{IIpv}
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A_{Ipv}
D_{Idp}
C_{Ipp}
X_v
D_{IIvl}
D_{IIvr}
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$\mathbf{A}_{II,p}$, $\mathbf{A}_{I,p}$ and $\mathbf{D}_{II,v}$ remain adjacent to $\mathbf{D}_{II,d}$, and only one daughter cell of $\mathbf{C}_{I,p}$ and $\mathbf{D}_{I,p}$ each (that is $\mathbf{C}_{I,p,p}$ and $\mathbf{D}_{I,d,p}$) still contact $\mathbf{D}_{II,d}$, so that at the 32-cell stage $\mathbf{X}_d$ and $\mathbf{X}_v$ are eventually surrounded by eight cells ($\mathbf{A}_{II,p,v}$, $\mathbf{A}_{II,p,d}$, $\mathbf{A}_{I,p,d}$, $\mathbf{A}_{I,p,v}$, $\mathbf{C}_{I,p,p}$, $\mathbf{D}_{II,v,r}$, $\mathbf{D}_{II,v,l}$ and $\mathbf{D}_{I,d,p}$; Figs. 3B, 4A).

The sixth cleavage: formation of the 62-cell stage

The sixth cleavage begins at the animal pole. Again, the spindle orientation is perpendicular to that of the preceding mitosis. The cleavage pattern of the two interlocking cell bands is still maintained at the animal pole but the rows are widened to four rows of eight cells each (Fig. 3D). At the vegetal pole the two cells $\mathbf{X}_d$ and $\mathbf{X}_v$ are arrested at the interphase for at least two cycles so that the sixth cleavage results in a 62-cell stage (Fig. 3E). $\mathbf{X}_d$ and $\mathbf{X}_v$ become more and more bottle-shaped with their yolk concentrated in the basal region and the nuclei located apically (Fig. 3F). Due to the tapering of the external parts of these cells during the course of the next two division cycles, the nuclei become closely apposed (compare Fig. 3E, G and H).

The spindles of the eight surrounding cells ($\mathbf{A}_{II,p,v}$, $\mathbf{A}_{II,p,d}$, $\mathbf{A}_{I,p,d}$, $\mathbf{A}_{I,p,v}$, $\mathbf{C}_{I,p,p}$, $\mathbf{D}_{II,v,r}$, $\mathbf{D}_{II,v,l}$ and $\mathbf{D}_{I,d,p}$) all point towards $\mathbf{X}_d$ and $\mathbf{X}_v$. This spindle arrangement interrupts the pattern of the interlocking cell bands at the vegetal pole but each of these cells still divides perpendicular to the previous division (Figs. 3E, 4C, D). Compared to the cells at the animal pole these eight cells ($\mathbf{A}_{II,p,v}$, $\mathbf{A}_{II,p,d}$, $\mathbf{A}_{I,p,d}$, $\mathbf{A}_{I,p,v}$, $\mathbf{C}_{I,p,p}$, $\mathbf{D}_{II,v,r}$, $\mathbf{D}_{II,v,l}$ and $\mathbf{D}_{I,d,p}$) are delayed in division. Two of these, $\mathbf{D}_{II,v,l}$ and $\mathbf{D}_{II,v,r}$ located ventrally of $\mathbf{X}_d$ and $\mathbf{X}_v$, are even more delayed resulting in four daughter cells ($\mathbf{D}_{II,v,l,d}$, $\mathbf{D}_{II,v,r,d}$, $\mathbf{D}_{II,v,l,v}$ and $\mathbf{D}_{II,v,r,v}$) forming a striking quartet of cells at the late 62-cell stage (Fig. 4E, F). Even during the following cycle these cells remain delayed in division.

The eight daughter cells of $\mathbf{A}_{II,p,v}$, $\mathbf{A}_{II,p,d}$, $\mathbf{A}_{I,p,d}$, $\mathbf{A}_{I,p,v}$, $\mathbf{C}_{I,p,p}$, $\mathbf{D}_{II,v,r}$, $\mathbf{D}_{II,v,l}$ and $\mathbf{D}_{I,d,p}$ form a ring of cells around $\mathbf{X}_d$ and $\mathbf{X}_v$ (Fig. 3G). They differ from all other cells by their arrangement and their conical shape and are called 'Kranzzellen' (wreath cells). During the seventh cycle their spindles become oriented towards $\mathbf{X}_d$ and $\mathbf{X}_v$ and from this stage on they do not follow the rule of perpendicularity any longer (see below; Fig. 4G, H).

Due to the distinctive features of these Kranzzellen they will be referred from here on by the capitals $\mathbf{K}_1$ to $\mathbf{K}_8$ with $\mathbf{K}_1$=$\mathbf{A}_{I,p,d,p}$, $\mathbf{K}_2$=$\mathbf{A}_{I,p,v,p}$, $\mathbf{K}_3$=$\mathbf{C}_{I,p,p,p}$, $\mathbf{K}_4$=$\mathbf{D}_{II,v,r,d}$, $\mathbf{K}_5$=$\mathbf{D}_{II,v,l,d}$, $\mathbf{K}_6$=$\mathbf{D}_{I,d,p,p}$, $\mathbf{K}_7$=$\mathbf{A}_{II,p,v,p}$ and $\mathbf{K}_8$=$\mathbf{A}_{II,p,d,p}$ in the cleavage type I (Fig. 3G). In the cleavage type II we refer to the clonal situation compared to cleavage type I: '$\mathbf{K}_1$='$\mathbf{A}_{I,p,d,p}$, '$\mathbf{K}_2$='$\mathbf{A}_{I,p,v,p}$, '$\mathbf{K}_3$='$\mathbf{C}_{I,p,p,p}$, '$\mathbf{K}_4$='$\mathbf{D}_{II,v,l,d}$, '$\mathbf{K}_5$='$\mathbf{D}_{II,v,r,d}$, '$\mathbf{K}_6$='$\mathbf{D}_{I,d,p,p}$, '$\mathbf{K}_7$='$\mathbf{A}_{II,p,v,p}$ and '$\mathbf{K}_8$='$\mathbf{A}_{II,p,d,p}$ (compare Fig. 4E and F). The numbering of the Kranzzellen $\mathbf{K}_1$ to $\mathbf{K}_8$ differs from that of Taube (1909) to emphasize the origin and position of these cells in relation to the dorsoventral axis.

Fig. 3A–L Early cleavage stages from the fifth cleavage until the 122-cell stage stained with fluorescent dyes (Hoechst: **A–F**, **H–L**; Sytox: **G**). **A**, **C**, **F** Superimposed photographs (fluorescence and interference). **A** View of the animal pole of a 16- to 32-cell stage. *Arrows* indicate the extension of the BD cell band to two rows of eight cells. **B** View of the vegetal pole of a 31-cell stage. The division of $D_{II,d}$ into $D_{II,d,d}$ (X_d) and $D_{II,d,v}$ (X_v) is not finished in this embryo. The nuclei of the surrounding cells are stressed by *circles*. The derivatives of C_{II} are concealed by descendents $D_{II,v}$ (see also Fig. 4A). **C** The two enlarged cells X_d and X_v (surrounded by the *broken white lines*) at the late 32-cell stage. The cells at the animal pole have already initiated mitosis. The nuclei of X_d and X_v lie slightly deeper inside the blastula compared to all other cells (compare *white stripes* marking the distance between nuclei and embryo surface). View from the right part of the embryo. **D** View of the animal pole of a 32- to 62-cell stage. The two interlocking cell bands become widened to four rows of eight cells each (except at the vegetal end of the BD band due to the cleavage arrest of X_d and X_v), indicated by *arrows* for the BD band. **E** View of the vegetal pole of a 32- to 62-cell stage. X_d and X_v do not participate in cell division. The directions of the cell divisions of the surrounding cells are indicated by the *white lines*. Their daughter cells oriented to the posterior are the eight Kranzzellen. **F** View from the left part of the embryo on the vegetal pole of a 62-cell stage focused on the bottle-shaped cells X_d and X_v which fill out the inner of the blastula. **G** Posterior view of a 62-cell stage. K_3 and K_6 are the first Kranzzellen that initiate mitosis of the seventh cleavage cycle. K_3 lies closer to K_4 and K_6 closer to K_7. **H** Posterior view of a 62- to 122-cell stage. While K_7, K_8, K_1 and K_2 are at early anaphase of mitosis, those cells lying nearer to the animal pole are already at telophase (*arrows*). **I** Posterior view of a 62- to 122-cell stage slightly later than in **H**. K_4 and K_5 ventral to X_d and X_v are still at anaphase while all other Kranzzellen have already finished anaphase. **J** Stage of 112 cells of cleavage type II in which 10 cells have still not finished mitosis. The eight Kranzzellen and the ventral cells '$D_{II,v,l,v}$ and '$D_{II,v,r,v}$ are still in the course of mitosis leading to a brief cell stage of 112 cells. **K** Ventral view of the vegetal pole of a 62- to 122-cell stage. K_4, K_5, $D_{II,v,l,v}$ and $D_{II,v,r,v}$ form a quartet of retarded cells surrounded by six cells at late anaphase (direct daughter nuclei connected by *lines*). The pattern of their spindle orientation is a result of the cleavage behaviour of the two interlocking cell bands (compare also the two mirror images in Fig. 4E, F). **L** Late 122-cell stage. X_d and X_v sink deeper into the blastula followed by the 16 daughter cells of the Kranzzellen forming the gastrulation centre (*broken line*). The nuclei of $D_{II,v,l,v,l}$, $D_{II,v,l,v,r}$, $D_{II,v,r,v,l}$ and $D_{II,v,r,v,r}$ (*circles*) ventrally form a row of four cells and they are surrounded lateroventrally by nine surrounding cells (*stars*) composed of derivatives of cells D and C from the 4-cell stage. *d* Dorsal, *v* ventral, *a* anterior, *p* posterior

The seventh cleavage: formation of the 122-cell stage

During the seventh cleavage the gradient of cell division delays becomes more distinct. The cells at the animal pole are clearly ahead in division compared to those at the vegetal pole (Fig. 3H–L). During this cell division cycle the pattern of the two cell bands at the animal pole disappears. At the vegetal pole the Kranzzellen follow a distinct division pattern with the spindles again oriented towards $\mathbf{X}_d$ and $\mathbf{X}_v$. Divisions at the vegetal pole begin in $\mathbf{K}_3$ and $\mathbf{K}_6$ (Fig. 3G). Due to their position it is still possible to distinguish between the two mirror images. $\mathbf{K}_3$ is closer to $\mathbf{K}_4$ and $\mathbf{K}_6$ closer to $\mathbf{K}_7$. The anaphase of $\mathbf{K}_3$ and $\mathbf{K}_6$ is followed by the mitosis of $\mathbf{K}_7$, $\mathbf{K}_8$ and $\mathbf{K}_1$, $\mathbf{K}_2$ (Fig. 3H, I). The ventral cells $\mathbf{K}_4$ and $\mathbf{K}_5$ as well as $\mathbf{D}_{II,v,l,v}$ and $\mathbf{D}_{II,v,r,v}$ just enter mitosis when all other Kranzzellen are already in late anaphase (Fig. 3I, J). This ventral

quartet of delayed cells is lateroventrally surrounded by nine cells in a distinct arrangement which is the result of the division of three cells of each of the two cell bands (Figs. 3K, 4G, H).

Due to this delay a stage of 112 cells is temporarily formed. It comprises 100 ectodermal cells at the animal pole, the two arrested cells $\mathbf{X}_d$ and $\mathbf{X}_v$, the eight Kranzzellen $\mathbf{K}_1$ to $\mathbf{K}_8$ which are still in mitosis and the two also delayed cells $\mathbf{D}_{II,v,l,v}$ and $\mathbf{D}_{II,v,r,v}$ ventral to $\mathbf{K}_4$ and $\mathbf{K}_5$ (Fig. 4G, H). During the 122-cell stage $\mathbf{X}_d$ and $\mathbf{X}_v$ sink more and more into the blastula. Subsequently the 16 daughter cells of the Kranzzellen (Fig. 3L) initiate the gastrulation *sensu stricto*.

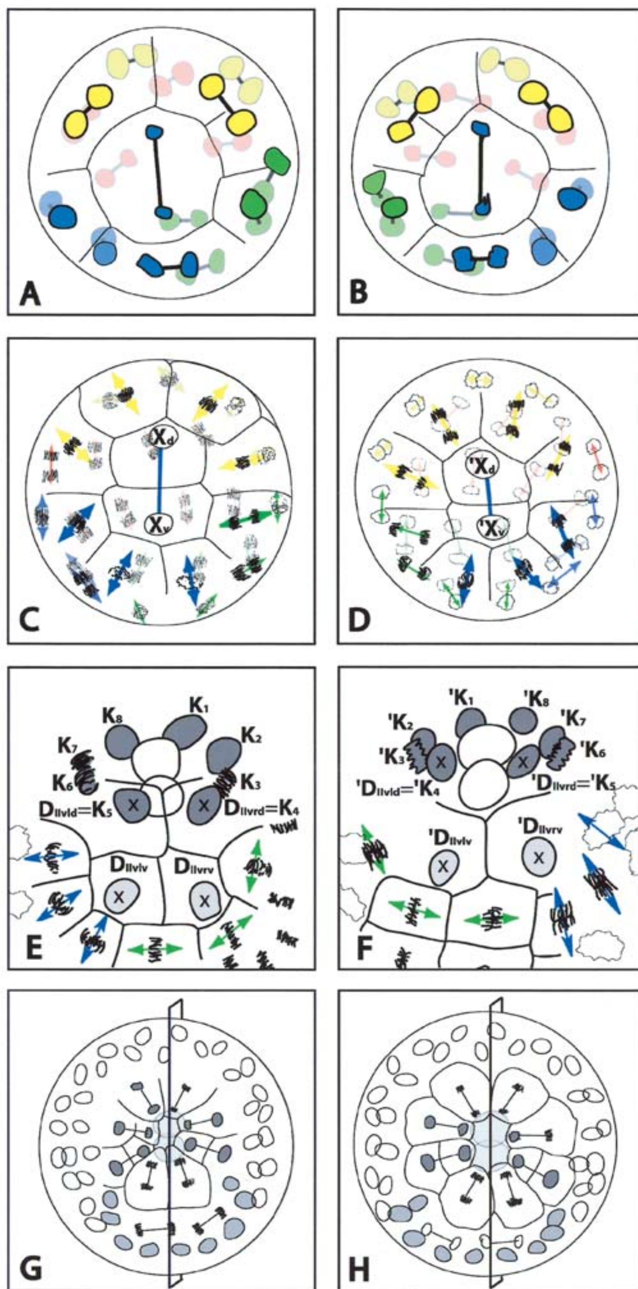

Fig. 4A–H Schematic drawings of the cells at the vegetal pole during the fifth up to the seventh cleavage cycle. Not all cell membranes considered. **A**, **C**, **E** and **G** resemble cleavage stages of type I, and **B**, **D**, **F** and **H** the equivalent mirror image type II. *Strokes* mark the direct sister cells, *arrows* indicate the spindle orientation of the following mitosis. **A**, **B** Vegetal pole of the two cleavage types at 31-cell stage. The nuclei are coloured according to the cell lineage of the corresponding blastomeres at 4-cell stage: A *yellow*, B *red*, C *green* and D *blue*. In cleavage type I the AC cell band surrounds X_d and X_v on the right (**A**) and in cleavage type II it surrounds ‘X_d and ‘X_v on the left (**B**). **C**, **D** Sixth cleavage of cleavage type I (**C**) and type II (**D**). Except in the arrested cells X_d and X_v, the direction of mitosis (*arrows* coloured the same as in **A** and **B**) is still perpendicular to the preceding direction (compare **A** with **C**, **B** with **D**). **E**, **F** Ventral view of the vegetal pole of the late 62-cell stage in which the cleavage cycle to the 122-cell stage is just initiated. The nuclei of the Kranzzellen are coloured *dark grey*. The pattern of cell division (*coloured arrows*) that surrounds the ventral cell quartet (*crosses*) is a result of the two interlocking bands occurring in two mirror images type I (**E**) and type II (**F**). **G**, **H** Vegetal pole of an early 122-cell stage in which cell division is not completed in all cells leading to the brief stage of 112 cells. The spindle orientation of all Kranzzellen (*dark grey*) points into the site of the future gastrulation centre, not following the rule of perpendicularity anymore. At this stage the two cleavage types I (**G**) and II (**H**) can be distinguished on the vegetal view [compare the position of the ventral, posterior nuclei in relation to the dorsoventral body axis (*vertical line*) in both panels]

Discussion

The early cleavage of *Meganyctiphanes norvegica* shows an invariant cell division pattern

The early development of *M. norvegica* was reconstructed by comparing the position of the nuclei and orientation of the mitotic spindles of different developmental stages using contemporary methods of fluorescent staining. At the 4-cell stage the two cells that are not immediate sister cells form the cross-furrow. This arrangement results in two interlocking cell bands which is recognized by the same spindle orientation of the cells in each ring and can be followed until the seventh cleavage. One blastomere ($\mathbf{D}_{II,d}$) is delayed in division (see Fig. 1) and its spindle is oriented obliquely during the fifth cleavage from the 16- to the 32-cell stage establishing the dorsoventral body axis through $\mathbf{X}_d$ and $\mathbf{X}_v$ at the vegetal pole. The surrounding cell formation of the Kranzzellen shows a distinct cell division pattern during the seventh cleavage. A mirror image of the position of blastomeres is evident from the 4-cell stage on. These results reveal that early development of *M. norvegica*, occurring in two mirror images, follows an invariant cell division pattern, which is a prerequisite for a detailed comparison with cleavage patterns of other crustaceans or other animals.

Some former investigations on early euphausiacean development exist (Sars 1898; Taube 1909, 1915; George and Strömberg 1985). The only work focusing on cell lineage is that of Taube (1909, 1915). Taube (1909) was not able to identify the species that were investigated, and thus he combined observations from several species. However, the general results found for *M. norvegica* in the present study correspond to those of Taube (1909).

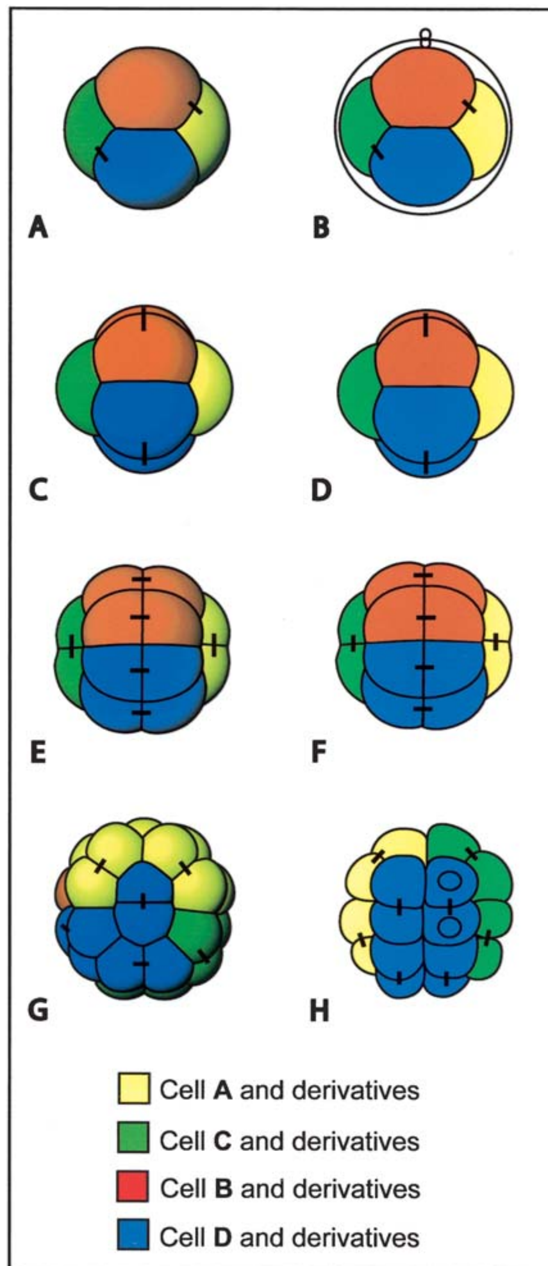

Fig. 5A–H Schematic overview of the early cleavage pattern of *M. norvegica* (**A**, **C**, **E**, **G**) and *Sicyonia ingentis* (after Hertzler and Clark 1992) (**B**, **D**, **F**, **H**) until 32-cell stage. Colours are assigned according to the cell lineage of the corresponding blastomeres at 4-cell stage: A *yellow*, B *red*, C *green* and D *blue*. Only cleavage type I is considered here for *M. norvegica*. The *strokes* mark the direct sister cells. **A**, **B** 4-cell stage, **C**, **D** 8-cell stage, **E**, **F** 16-cell stage, **G**, **H** 32-cell stage

Taube (1909) distinguished two slightly larger and two smaller cells at the 4-cell stage forming a similar arrangement described here for *M. norvegica*, but it remains unclear which of the four blastomeres are sister cells. Regardless, a pattern of two interlocking semicircles of four cells each is recognized at the 8-cell stage, but no pattern of interlocking cell bands is observed during the subsequent development (Taube 1909). The higher resolution of the methods used in the present investigation could demonstrate that this pattern is manifested in the same mitotic spindle orientation in each cell band in *M. norvegica* and is only disrupted by the aberrant cell formation at the vegetal pole. It could also be shown that in *M. norvegica* the circular orientation of the spindles of the eight cells $\mathbf{A}_{II,p,v}$, $\mathbf{A}_{II,p,d}$, $\mathbf{A}_{I,p,d}$, $\mathbf{A}_{I,p,v}$, $\mathbf{C}_{I,p,p}$, $\mathbf{D}_{II,v,r}$, $\mathbf{D}_{II,v,l}$ and $\mathbf{D}_{I,d,p}$ corresponds to their position in the cell bands and follows the rule of perpendicularity up to the sixth cleavage. During the seventh cleavage the spindles of their daughter cells, adjacent to $\mathbf{X}_d$ and $\mathbf{X}_v$, again become oriented towards the site of invagination not following the rule of perpendicularity anymore (Kranzzellen). Their specific sequence of division could not be resolved by Taube (1909), but his description implies a similar process. The correspondences to Taube's investigation, the observations of studies of other representatives of euphausiaceans (George and Strömberg 1985) and the pattern found for *M. norvegica* indicate that the results presented in the present study might lead to a general view of the early cleavage pattern of euphausiaceans.

According to Taube (1909, 1915) the cells $\mathbf{X}_d$ and $\mathbf{X}_v$ probably generate mainly endoderm and the primordial germ cells, the cells $\mathbf{D}_{II,v,l,v}$ and $\mathbf{D}_{II,v,r,v}$ are mesoteloblast precursor cells and the Kranzzellen form the mesoderm. However, to confirm the true fate of these cells the techniques of cell lineage tracing with vital markers (see Wolff and Scholtz 2002) and/or 4D microscopy (see Schnabel et al. 1997) have to be applied.

Indications of a homologous early cleavage pattern of euphausiaceans and dendrobranchiate decapods

Comparing the cleavage pattern of *M. norvegica* with that of other malacostracans reveals striking similarities to the early cleavage pattern of the dendrobranchiate *S. ingentis* (Hertzler and Clark 1992; Hertzler et al. 1994; Fig. 5). In *S. ingentis*, the same arrangement of the blastomeres at the 4-cell stage results in two interlocking cell bands as in *M. norvegica*. Two enlarged cells arrested in division and denoted as mesendoderm cells are formed in *S. ingentis* during sixth cleavage at the vegetal region, i.e. at the prospective posterior end of the nauplius (Hertzler and Clark 1992). Such a cleavage-arrest of two invaginating cells, though with a different timing, has also been shown for other dendrobranchiates such as *Lucifer* sp. Thompson, 1829 at the 32-cell stage (Brooks 1882), *Penaeus japonicus* Bate, 1888 at the 64-cell stage (Kajishima 1951) and *Penaeus trisulcatus* Leach, 1815 at the 16-cell stage (Zilch 1978).

The surrounding arrangement of cells (Kranzzellen) at the vegetal pole with a radial orientation of the mitotic spindles which are also delayed in division compared to all other cells, is also found in dendrobranchiates and euphausiaceans (Kajishima 1951; Zilch 1978; Hertzler and Clark 1992; present study). As in *M. norvegica*, the

number of Kranzzellen is eight in *P. japonicus* and in *P. trisulcatus* (Kajishima 1951; Zilch 1978), while in *S. ingentis* there are nine Kranzzellen surrounding the two mesendoderm cells (Hertzler and Clark 1992). According to Hertzler and Clark (1992) this may be due to the asymmetrical position of the two mesendoderm cells in the corresponding cell band. This suggestion is not supported by our study, since the position of the two enlarged cells $\mathbf{X}_d$ and $\mathbf{X}_v$ in *M. norvegica* is also asymmetrical in the cell band **BD**. However, it can be shown that the final arrangement within the neighbouring cells is a result of the oblique spindle orientation of cell $\mathbf{D}_{II,d}$ during its division into $\mathbf{X}_d$ and $\mathbf{X}_v$ establishing the dorsoventral axis. Such an oblique spindle orientation in the precursor cell of the two cleavage-arrested cells is not observed in *S. ingentis*. This difference may cause a different number of Kranzzellen. It was beyond the scope of Hertzler and Clark (1992) to resolve the sequence of divisions of the Kranzzellen in *S. ingentis* and to analyse the spindle orientation in detail, but it may be worthwhile to investigate this aspect in more detail in the Dendrobranchiata.

In *S. ingentis* there are two different cleavage types related to the lineage of the two cleavage-arrested cells (Hertzler and Clark 1992). In the most frequent type the Kranzzellen are composed of the derivatives of cells **C** and **D** at the 4-cell stage. In the other type, called 'ventral type', derivatives of **B** are also involved. In *M. norvegica* the Kranzzellen are composed of derivatives of cells **A**, **C** and **D** (see Fig. 1). Derivatives of **B** form exclusively the anterior part of the embryo. No cleavage type comparable to the 'ventral type' in *S. ingentis* was found in *M. norvegica*. The differences in the origin of the Kranzzellen are perhaps based on differences in the establishment of the body axis with respect to the cell genealogy in *S. ingentis* and in *M. norvegica*. In *M. norvegica* we have found the occurrence of two cleavage types (type I and II) which form mirror images. Apart from a mirror image arrangement, these two types do not lead to a different clonal composition of the Kranzzellen.

Another difference is the arrangement of the cell bands with respect to the body axes. In the most common cleavage type of *S. ingentis* the dorsoventral axis at the 32-cell stage dorsally extends through the initial cross-furrow formed by **A** and **C**, so that cell band **AC** curves dorsally from left to right and cell band **BD** curves ventrally from posterior to anterior (Fig. 5H). In contrast, in *M. norvegica* the dorsoventral axis dorsally extends through derivatives of **A** (Fig. 5G). Beginning with derivatives of **A**, the **AC** cell band curves posterior dorsally around $\mathbf{X}_d$ and $\mathbf{X}_v$ reaching **C** derivatives at the ventral site. Cell band **BD** curves from $\mathbf{X}_d$ and $\mathbf{X}_v$ laterally to the anterior end occurring in the two mirror images (compare Fig. 4A and B). Hence, the pattern of interlocking cell bands, the formation of the two cleavage-arrested enlarged cells and the number of the surrounding cells are invariant patterns both in *S. ingentis* and in *M. norvegica*, but their cell lineage and the position of the dorsoventral axis are different.

Comparing the early cleavage pattern found in dendrobranchiates and euphausiaceans with existing studies of other total cleaving malacostracans reveals only few similarities. For the yolk-rich eggs of amphipods, holoblastic cleavage is described up to the formation of a germ disc and the separation of yolk (Scholtz and Wolff 2002). First cleavages result in an 8-cell stage with four micromeres and four macromeres with a characteristic arrangement of the blastomeres for amphipods in general (Gerberding 2002; Scholtz and Wolff 2002; Wolff and Scholtz 2002). The anteroposterior and dorsoventral body axes are already cell-genealogically determined at the 4- and 8-cell stages (Gerberding et al. 2002; Wolff and Scholtz 2002). From the 16-cell stage on derivatives of the smallest quadrant **A** are delayed in their mitotic division (Scholtz and Wolff 2002). In contrast to all other malacostracans, gastrulation occurs in the anterior part of the embryo (Scholtz and Wolff 2002). The process of gastrulation involves 4–8 cells sinking into the yolk and proliferating the main part of the mesendoderm. This cleavage pattern has no equivalent in euphausiaceans and dendrobranchiates or in other malacostracans, and it is suggested that the developmental pattern of amphipods represents an additional apomorphic feature for the Amphipoda (Scholtz and Wolff 2002).

The early development of the anaspidacean *Anaspides tasmaniae* Thomson, 1892 is characterized by a total and equal cleavage up to the 8-cell stage (Hickmann 1937). At the 16-cell stage one enlarged cell invaginates into the blastocoel and then divides without distinctive pattern. According to Hickmann (1937) this is the origin of the mesoderm, whilst the endoderm is formed later by a cell invagination in the same region where the mesoderm cell invaginated before. In *A. tasmaniae* no equivalent of the interlocking cell bands or a cell formation corresponding to the Kranzzellen is described. Further examples of total cleaving malacostracan eggs only occur secondarily among the highly derived parasitic Epicarida (Strömberg 1965, 1971) within the isopods that generally undergo superficial cleavage (McMurrich 1895). Examples of a mixed cleavage type, in which superficial cleavage takes over total cleavage or vice versa are found in the Thermosbaenacea (Zilch 1974, 1975) and among representatives of the Caridea within the decapods (Weldon 1892; Gorham 1895; Weygoldt 1963).

Many malacostracans pass exclusively through superficial cleavage, as it is described for the basal groups Leptostraca (Butschinsky 1897; Manton 1934) and Stomatopoda (Shiino 1942), but also in mysids (Manton 1928) and in the Cumacea (Dohle 1970). Caridea and Reptantia (both Decapoda) show a variety of superficial and mixed cleavage modes (reviewed by Anderson 1973). The different mode of cleavage makes it difficult to compare patterns found in superficial development with those found in total cleaving development (see below).

Compared to other malacostracans the similarities of the early cleavage patterns of dendrobranchiate and euphausiacean shrimps are obvious and several authors previously were encouraged to stress these similarities

(Taube 1909; Shiino 1968; Anderson 1973; Zilch 1978; Hertzler and Clark 1992). It appears that these matching data are distinct and complex enough, as is required for the methodical test of homology (Dohle 1989; Scholtz 2002), to claim homology of the cleavage and gastrulation patterns of Dendrobranchiata and Euphausiacea.

The fact that the corresponding cleavage pattern of these two taxa has a different cell lineage does not necessarily rule out homology. For example, a nearly identical differentiation of the grid-like pattern of cells in the post-naupliar germ bands of Malacostraca is not determined by their origin. However, these grid-like post-naupliar germ band patterns in malacostracans are doubtlessly homologous (Dohle and Scholtz 1988; Scholtz and Dohle 1996). In this respect the early cleavage pattern may provide one more example of how cell genealogy does not necessarily play a causative role for a specific cell pattern during malacostracan development.

There are two alternative views concerning the phylogenetic position of Euphausiacea within the Malacostraca. One scenario favours a clade Eucarida comprising Euphausiacea and Decapoda (Siewing 1963; Schram and Hof 1998; Wills 1998; Watling 1999), the other interprets Euphausiacea as close relatives of the Peracarida (and Pancarida) and thus disputes a sister group relationship of Euphausiacea and Decapoda (Jarman et al. 2000; Richter and Scholtz 2001). According to both reconstructions of malacostracan phylogeny a superficial cleavage seems plesiomorphic for the Malacostraca (Richter and Scholtz 2001; Wolff and Scholtz 2002). If the first view is correct, the similarities in the cleavage pattern of euphausiaceans and dendrobranchiates could be interpreted as an apomorphy for the Eucarida with a secondary loss of total cleavage within the Decapoda. The second view, however, would at first sight imply that the cleavage pattern found in dendrobranchiate decapods and euphausiaceans would be apomorphic for the Caridoida, a clade comprising Decapoda, Syncarida, Euphausiacea, Pancarida and Peracarida (see Richter and Scholtz 2001) with secondary losses of total cleavage in most groups and different patterns of total cleavage in anaspidaceans and amphipods (see above). Alternatively, the assumption of homology might be wrong and the cleavage patterns of euphausiaceans and dendrobranchiates evolved convergently, a solution that is hard to accept with respect to the detailed similarities described above. Interestingly enough, there are some resemblances to the euphausiid/dendrobranchiate cleavage pattern in the cumacean *Diastylis rahtkei* (Krøyer, 1841), although this species undergoes a superficial cleavage (Dohle 1970). Gastrulation in *D. rathkei* is initiated by the delayed mitoses of two energids at the vegetal pole at the 64-energid stage (Dohle 1970). These two energids become surrounded by 4–6 energids during the subsequent stages. The mitotic spindles of these surrounding energids are oriented towards the two immigrating energids showing a delay in division compared to energids at the animal pole (Dohle 1970). This correspondence might hint at the possibility that the position and lineage of cleavage products can be similar irrespective of whether it is a total or a superficial cleavage, in other words, these are independent phenomena. Accordingly, just the aspect of *total* cleavage might be convergent between euphausiaceans and dendrobranchiates (for the related problem concerning the evolution of a free nauplius see Scholtz 2000). However, to solve the intriguing problem of cleavage pattern and cleavage mode evolution among malacostracans more detailed studies of species with superficial cleavage are needed.

Comparison to non-malacostracan crustaceans

In contrast to that of most malacostracans, the cleavage pattern of some non-malacostracan crustaceans reveal some interesting overlapping features. In the copepod *Megacyclops viridis* (Jurine, 1820) each two of four nearly equal blastomeres also form a cross-furrow at the 4-cell stage (Fuchs 1914). Although the pattern of two interlocking bands of cells with the same spindle orientation in each cell band is not explicitly described, a similar pattern is apparent until the 16-cell stage from the plates (see Fuchs 1914). In this interpretation a precursor cell (designated as 'Stammzelle') of two cleavage-delayed cells which later invaginate is also situated at the end of one cell band. According to Fuchs (1914) one derivative of this cell migrates between the cross-furrow of the other cell band with the fifth cleavage, so that during a short 31-cell stage it is surrounded by five cells. By the late 32-cell stage the 'Stammzelle' has divided into two cells, one of them giving rise to the endoderm and the other to the presumptive germ cell (Fuchs 1914). During the following cleavage both cells become surrounded by 6–8 cells with their spindles pointing towards the presumptive endoderm and germ cell (Fuchs 1914). In the branchiopod waterflea *Polyphemus pediculus* L., 1761 we find a similar arrangement of eight cells surrounding two germ cells (according to Kühn's interpretation) at the 62-cell stage (Kühn 1912). In addition, for both groups the occurrence of mirror image cleavage types has been reported (Kühn 1912; Fuchs 1914). Comparative studies have repeatedly stressed the similarities in early development of total cleaving malacostracans and non-malacostracan taxa (Shiino 1968; Anderson 1973). However, the question whether there is evidence for a developmental ground pattern of Crustacea still remains open and requires more indications and further resumption of studying early development using contemporary methods.

Meganyctiphanes norvegica as a representative of a total cleaving arthropod

Many authors interpreted the early cleavage of crustaceans as being a modified spiral cleavage. This is based on the idea that arthropods are closely related to annelids and therefore must have derived from an ancestor with spiral cleavage (Taube 1909, 1915; Shiino 1968; Ander-

son 1973; Nielsen 2001). A well-known example is the detailed study of the cirripede *Tetraclita rosea* (Krauss, 1848) (Anderson 1969). This interpretation has been generally disputed by other researchers (Kühn 1912; Siewing 1969; Dohle 1979, 1989; Scholtz 1997) and, in particular, for malacostracan dendrobranchiates by Zilch (1978, 1979) and Hertzler and Clark (1992) and for amphipods by Wolff and Scholtz (2002). The arguments of Taube (1909, 1915) in favour of spiral cleavage in euphausiaceans are not convincing. In his description of the early cleavage he suggests an alternation of leio- and dexiotropic divisions from 4- to 32-cell stages (Taube 1915). However, in our study on the cleavage of *M. norvegica* we were unable to confirm this. *M. norvegica* shows a stereotyped cleavage pattern but no alternating oblique spindle directions after the 4-cell stage. Furthermore, the two interlocking cell bands with parallel-oriented spindles in each band are characterized by a widening of the 'cross-furrow' with every second division. For instance, the region of the 'cross-furrow' between the derivatives of the **A** and **C** quadrants is already two cells wide at the 16-cell stage and four cells wide at the 62-cell stage (compare Figs. 3D and 5). In contrast, the cross-furrow at the animal and vegetal poles in spiral cleavage is always one cell wide during early development and this is true for equally and unequally cleaving spiralians (Anderson 1973; Groepler 1986; Luetjens and Dorresteijn 1995). In addition, presence, origin and division pattern of the Kranzzellen find no correspondence in spiral cleavage.

The controversial discussion about the 'Articulata' versus 'Ecdysozoa' hypothesis offers an opportunity to rethink traditional interpretations of arthropod development (Scholtz 2002). Further investigations on early development tracing cell lineage of total cleaving crustaceans promise to provide more evidence for a reconstruction of the ancestral cleavage pattern of arthropods and might contribute to the present discussion of whether arthropods are more related to the bilateral cleaving nematodes or to the spiralian annelids.

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