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Acarine embryology: Inconsistencies, artificial results and misinterpretations

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Abstract

In this paper, we discuss how views of early stages in acarine embryology – from the first cleavage to the blastula – have changed over time, starting with historical works of the 19th century and ending with electron microscopic analyses in the 21st century. Our goal is to identify errors and inconsistencies in both observations and the interpretation of information throughout this time span, and to show how they have related to technical improvements. Surprisingly, the questions about cleavage pattern and its implications for acarine classification have not changed, despite the advent of electron microscopy and molecular biology.

In the last century authors attempted to develop a general concept of cleavage types and their distribution among the major subgroups of the Acari. Based on available data, all of which was from light microscopy, the type of cleavage for both the Anactinotrichida and Actinotrichida was considered to be interlecithal, with the exception that some actinotrichid mites show mixed/combination cleavage. Newer data obtained by transmission electron microscopy and molecular biology point to a very different generalization: early acarine cleavage seems to be a special type of total cleavage.

Keywords: total cleavage, superficial cleavage, Acari, macromere, micromere, Oribatida, *Archegozetes longisetosus*, transmission electron microscopy (TEM)

1. Introduction

As embryology was established and began to develop as an integral part of biology in the 19th century, studies of acarine embryology were rare – and unfortunately remain so. Information from the pioneering studies was limited by the transparency of eggs and the resolution of microscope lenses, but by the end of the 19th century Wagner (1894) had distinguished three different types of cleavage in mites and ticks: (1) total (holoblastic) cleavage, (2) interlecithal cleavage (a form of superficial cleavage) and (3) superficial cleavage. All studied species of mites belonged to the actinotrichid suborders Astigmata or Prostigmata, or the anactinotrichid suborder Ixodida (ticks). But the distribution of cleavage type among them formed no distinct phylogenetic pattern (Tab. 1).

Tab. 1 Compilation of some suggestions of cleavage modi (interlecithal cleavage, superficial cleavage and total cleavage) for different species of the Acari by various authors in the 19th century. All analyses were done by LM. This list is not exhaustive.

	Interlecithal	Superficial	Total
Actinotrichida			
Astigmata			
Tyroglyphus domesticus			Salensky 1869 (cited in: Reuter 1909a)
Tyroglyphus viviparus			Salensky 1869 (cited in: Reuter 1909a)
Pterolichus falciger			Robin & Megnin 1877
Pterolichus delibatus			Robin & Megnin 1877
Acarus plumiger		Leydig 1848	
Dermanyssus avium			Leydig 1848
Sarcoptes cati			Leydig 1848
Sarcoptes hominis	Bourguignon 1854		
	Fürstenberg 1861		
Prostigmata			
Trombidium fuliginosum		Henking 1882	
Tetranychus urticae		Claparéde 1868	
Tetranychus telarius		Claparéde 1868	
		Donnadieu 1875	
Cheyletus eruditus			Kramer 1881
Hydrachna cruenta	Salensky 1869 (cited in: Reuter 1909a)		
Anactinotrichida			
Ixodida			
Ixodes calcaratus	Wagner 1893		
	Wagner 1894		
Boophilus microplus	Wagner 1893		

The techniques for staining, sectioning and magnifying biological specimens had reached a high art by the end of the 19th century, and many of the currently-used histological methods were already developed. Instrumentation was greatly improved by the inventions of the microtome and corrected microscope lenses for light microscopy (LM). The first serial sections of acarine eggs were made at the beginning of the 20th century, and with these came the first inconsistencies in acarine embryology. In 1909a Reuter postulated a mechanism of cleavage for the tarsonemid mite *Pediculopsis graminum*, in which early divisions are total but those of later stages switch to a superficial cleavage. This mode of cleavage (as interlecithal cleavage and superficial cleavage) results in a superficial sheet of cytoplasm containing the cleavage nuclei surrounding a central mass of yolk, thereby forming a syncytial blastoderm. Reuter also postulated that in some earlier studies this mode of cleavage was present but not recognised. Later such a mode of cleavage has been termed 'mixed cleavage' (Dawydoff 1928, Fioroni 1970) or 'combination cleavage' (Johannsen & Butt 1941).

In 1943 Vitzthum debated the inconsistencies mentioned by Reuter and reviewed the acarine cleavage types established by Wagner in the 19th century. In his book he still distinguished between interlecithal and superficial cleavage, but assumed mixed/combined cleavage for all taxa previously thought to exhibit total cleavage. At this time most embryological analyses established with the novel techniques postulated or assumed mixed/combination cleavage to be dominant among the actinotrichid (acariform) suborders Prostigmata and Astigmata, but again without a phylogenetically congruent distribution pattern (Vitzthum 1943, Gasser 1951, Sokolov 1952, Türk & Türk 1957, Edwards 1958, Dittrich 1968, Prasse 1968; Tab. 2).

In his classic book, Hughes (1959) again summarised cleavage types and concluded that interlecithal cleavage might be the usual mode of cleavage in the Acari, possibly because it seemed the most widespread. This became the consensus view (Anderson 1973, Aeschlimann & Hess 1984, Evans 1992): early mite cleavage is interlecithal, except in the small, relatively yolk-poor eggs of some Actinotrichida where mixed/combination cleavage prevails. When put in a phylogenetic context, interlecithal cleavage would be the plesiotypic process in the Acari, with mixed/combination cleavage being secondarily evolved (Anderson 1973).

When transmission electron microscopy (TEM) was applied to studies of acarine embryology a different picture emerged. Examined mites had a specialised total cleavage in early embryology, and represented species from both major acarine orders, the Anactinotrichida (Parasitiformes s.l.) (Fagotto et al. 1988) and the Actinotrichida (Acariformes) (Walzl et al. 2004, Laumann et al. 2010). In analysed species blastomeres segregate into two cell types: yolk-free micromeres and macromeres containing all the yolk. In the astigmatid *Sancassania berlesei* and the oribatid *Archegozetes longisetosus*, micromeres are scattered between macromeres on the embryonic surface, building the preblastula, and divide further tangentially until they cover the whole surface of the embryo, building the blastula. In the anactinotrichid argasid *Ornithodorus moubata* the micromeres cover the whole surface of the embryo after division of the blastomeres in micromeres and macromeres, thereby surrounding the yolky macromeres building the blastula. These novel, but consistent, TEM data strongly conflict with previous data for *O. moubata* and for Astigmata. As discussed below, in combination with further novel data these cast strong doubt on the generality that early embryology of mites include a syncytial phase. Moreover, the form of cleavage revealed by TEM exhibits a specialised total cleavage, which might shed new light on the traditional interpretations of chelicerate development.

Tab. 2 Compilation of some suggestions of cleavage modi (interlecithal cleavage, superficial cleavage, total cleavage and mixed/combination cleavage) for different species of the Acari by various authors in the 20th century. All analyses were done by LM. This list is not exhaustive.

	Interlecithal	Superficial	Total	Mixed/combination
Actinotrichida				
Astigmata				
Knemidocoptes mutans		Langenscheidt 1958		
Tyrophagus infestans				Prasse 1968
Tyrophagus noxius				Sokolov 1952
Acarus farinae				Hughes 1950
Acarus siro				Prasse 1968
Caloglyphus berlesei				Prasse 1968
Caloglyphus michaeli				Prasse 1968
Acaridae				Türk & Türk 1957
Prostigmata				
Acarapis woodi		Klumpp 1954		
Tetranychus urticae				Dittrich 1968
Tetranychus telarius				Dittrich 1965
Cheyletus eruditus		Hafiz 1935		Edwards 1958
Pediculopsis gramium				Reuter 1909a
Pediculoides ventricosus			Brucker 1900	Patau 1936
Panonychus ulmi				Gasser 1951
Panonychus citri				Fukuda & Shinkaji 1954
Anactinotrichida				
Ixodida				
Ornithodorus moubata	Aeschlimann 1958	Geigy & Wagner 1957		
Hyalomma aegypticum	Bonnet 1907			
Hyalomma rufipes		Rahman 1983		
Gamasida				
Spinturnix vespertilionis			Akimov & Yastrebtsov 1990	
Macrocheles matrius		Yastrebtsov 1992		
Phytoseiulus persimilis		Yastrebtsov 1992		
Eulaelaps stabularis		Yastrebtsov 1992		
Ichoronyssus flavus		Yastrebtsov 1992		
Dermanyssus gallinae		Warren 1940		
Cosmolaelaps cuneifer		Warren 1941		
Cosmolaelaps claviger		Warren 1941		
Cyrtolaelaps nemorosis		Warren 1941		

Our general goal is to re-evaluate the cleavage patterns of mite embryogenesis. We identify errors and inconsistencies in both observations and the interpretation of information throughout the time span of acarine embryology, and show how they have related to technical improvements. One constraint in analysing historical data is that LM results were usually presented as schematic drawings rather than as micrographs; as a result, most publications lack information that could be used to detect artifacts introduced by various preparation techniques or to identify misinterpretations of samples. For this reason we used different fixation techniques for LM and TEM, with various staining procedures for the semi-thin sections for LM, to check for artifacts and misinterpretations of earlier analyses of the acarine cleavage pattern. Results of these studies are integrated below into the historical perspective.

2. Materials and methods

Origin and culture of specimens

For our analyses we used the parthenogenetic oribatid mite *A. longisetosus* Aoki (Trhypochthoniidae), specifically the strain *A. longisetosus* ran (Heethoff et al. 2007), a genetic lineage established in 1993 and used in subsequent studies on a variety of topics (e.g. Sakata & Norton 2003, Alberti et al. 2003, Smrž & Norton 2004, Köhler et al. 2005, Seniczak 2006, Heethoff et al. 2007, Bergmann et al. 2008, Laumann et al. 2008, 2010). Our stock-cultures were kept in constant conditions (darkness, 23 °C, relative humidity 90 %) in plastic boxes on a plaster-of-Paris/charcoal substrate. Cultures were fed twice each week with unicellular green algae (mainly *Protococcus* sp.) growing on bark of various trees. Gravid adult females were removed from the culture to obtain eggs in different cleavage stages; eggs were easily seen through the translucent cuticle.

Preparation of eggs

Eggs were removed from gravid females by dissection in saline buffer containing 4 % sucrose, using micro-needles. Because of its impermeability to aqueous fixatives, the egg-shell was pierced for fixation or the eggs were placed for 120 s in 2.8 % sodium hypochloride solution (DanKlorix), which rendered the egg-shell permeable. Afterwards the eggs were washed in pre-chilled 0.1 M cacodylate buffer containing 4 % sucrose.

Chemical fixation of eggs for LM and TEM

Chemical fixation was performed at 4 °C unless otherwise noted: primary fixation of prepared eggs was done in half-strength Karnovsky`s fixative in 0.1 M cacodylate buffer containing 4 % sucrose for 60 min. Samples were washed in pre-chilled 0.1 M cacodylate buffer. Secondary fixation was done in 2 % osmium tetroxide in 0.1 M cacodylate buffer for 60 min. Samples were washed in pre-chilled 0.1 M cacodylate buffer and passed through graded concentrations of 30 %, 50 % and 70 % ethanol. Staining with uranyl acetate was done in 70 % ethanol for 60 min. Dehydration was continued in 10 % steps; finally the absolute ethanol was replaced by propylene oxide for 15 min. Samples were gradually infiltrated in Epon 812 (Serva, Germany) at room temperature, with propylene oxide – resin mixture in ratios of 3:1, 2:1, 1:1, 1:2, 1:5 and pure resin for 1 h each, then with fresh pure resin overnight on a rotary shaker and finally embedded in epoxy resin in BEEM-capsules. Polymerisation

was allowed to occur at 45 °C for 12 h followed by 60 °C for 48 h. Semi-thin and ultra-thin sections were cut using a Reichert Ultracut microtome (Leica, Austria).

Cryofixation of eggs for LM and TEM

To improve the preservation of cellular ultrastructure, prepared eggs were transferred to aluminium planchettes (cavity 150 µm) filled with 1-hexadecene. High pressure freezing was done in a HPM 010 (Bal-Tec, Liechtenstein). Samples were freed from 1-hexadecene below –100 °C and transferred in cryotubes into a Leica FS unit (Leica, Germany) at –90 °C, filled with acetone containing 2–4 % water, 0.1 % osmium tetroxide, 0.5–2.5 % methanol and 0.1–0.2 % uranyl acetate. After 60 h at –90 °C samples were kept at –60 °C for 8 h with 0.5 % glutaraldehyde in acetone added. After 10 h at –35 °C samples were washed with acetone, further brought to 0 °C for 60 min and washed with acetone while further warming to room temperature. Samples were embedded in resin as described above. Semi-thin and ultra-thin sections were cut using a Reichert Ultracut microtome (Reichert, Austria).

Staining techniques for LM and TEM

Semi-thin sections were stained with iron-haematoxylin and Safranin O or with methylene blue and alkaline fuchsin and then embedded in Entellan (Merck, Germany). Sections were surveyed with a Zeiss Axioplan light microscope and documented with a Zeiss MrC5 digital camera (Zeiss, Germany). Ultra-thin sections were stained with aqueous lead citrate for 3 min and viewed in a Philips Tecnai 10 transmission electron microscope (FEI, Netherlands) or a LEO 906 transmission electron microscope (Zeiss, Germany) at 80 kV.

Scanning electron microscopy

For scanning electron microscopy (SEM) dissected eggs remained untreated or were rendered permeable with sodium hypochloride solution as described above, fixed in a mixture of ethanol (80 %), formaldehyde (4 %) and acetic acid in the ratio 6:3:1 for 60 minutes and dehydrated in a graded ethanol series of 70 %, 90 % and 100 %, each concentration twice with incubation time of 5 minutes. Samples were critical-point dried in CO_2 in a Polaron CPD (Polaron, England) and sputter-coated with a 20 nm thick layer of a gold-palladium mixture in a Balzers SCD030 (Balzers, Swiss). Micrographs were produced on a Cambridge Stereoscan 250 Mk2 scanning electron microscope at 20 kV (Cambridge Scientific Instruments, England).

3. Results

The advent of acarine embryology

The transparent egg-shell of acarine eggs provided in the beginning a significant advantage for detection of the first cleavages. But with subsequent cell divisions the blastomeres become gradually smaller and their cell membranes become more difficult to be observed in conventional LM (Fig. 1A).

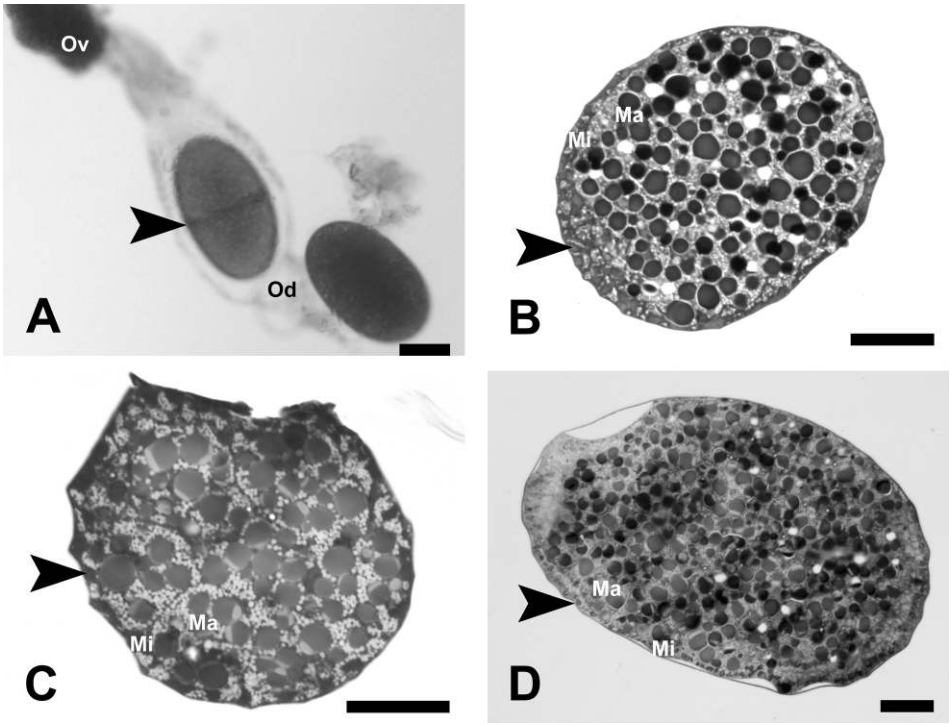

Fig. 1 A: Dissected female genital tract of *Archegozetes longisetosus* showing a part of the ovary, the proximal region of the oviduct, the first cleavage and later cleavage stage of an egg (arrowhead indicating cleavage furrow; LM, saline buffer). While cleavage continues detection of blastomeres is increasingly difficult. Scale bar = 50 µm. B–D: Semi-thin sections of blastulae of *A. longisetosus* viewed with light microscopy. Arrowheads indicate the nuclei of micromeres, as was verified by TEM (for Fig. 1A and 1B refer to Fig. 3B, for Fig. 1C refer to Fig. 3D). B: Cross-section stained with Heidenhain´s haematoxylin and eosin (chemical fixation). Scale bar = 20 µm. C: Cross-section stained with methylene blue and alkaline fuchsin (chemical fixation) of the same egg. Due to artifactual shrinking of the macromeres their cell membranes are visible. Neither Heidenhain´s haematoxylin/eosin nor methylene blue/alkaline fuchsin allow detection of the cell membranes of micromeres. Scale bar = 20 µm. D: Longitudinal section stained with methylene blue and alkaline fuchsin (cryofixation). Cryofixation does not result in cell membranes being visible in light microscopy. Scale bar = 20 µm. Abbreviations: Od: oviduct; Ov: ovary; Ma: macromere; Mi: micromere.

Acarine embryology in the 20th century

We analysed the type of cleavage in *Archegozetes longisetosus* using semi-thin sectioning and traditional staining techniques for LM to simulate data of this time. In the semi-sections the nuclei of the micromeres were clearly visible, but the delicate cell membranes were not (Fig. 1B). For comparison with the traditional haematoxylin/eosin staining method, some

semi-sections were stained with methylene blue and alkaline fuchsin. With this staining method the cell membranes of the macromeres could be identified, but those of the micromeres were not detectable (Fig. 1C). Cryofixation followed by freeze-substitution leads to an improved preservation of ultrastructure (Ripper et al. 2008), but even with this sophisticated method the cell membranes of the micromeres were not visible in LM (Fig. 1D). Thus, even today a dataset based only on LM would be misleading, since the delicate cell membranes of the micromeres can not be seen.

Acarine embryology in the 21th century

Before the 21st century acarologists pierced acarine eggs with needles prior to fixation. But this technique has some major limitations: (1) because of the high intraoval pressure the eggs often burst even when hypotonic fixatives are used; (2) in order not to destroy regions of interest piercing must be closely controlled, which is very difficult or even impossible; and (3) sufficient infiltration of the eggs for downstream applications is limited to the area of piercing, even with the use of low viscosity resins. A new technique established a few years ago by Walzl & Gutweniger (2002) renders the egg-shell permeable by maceration with sodium hypochloride. An experimental test of this maceration method results in the introduction of micro holes and fissures in the egg-shell which seems to permit the fixative to penetrate the hydrophobic vitelline-membrane consistently over the whole surface of the egg (Fig. 2).

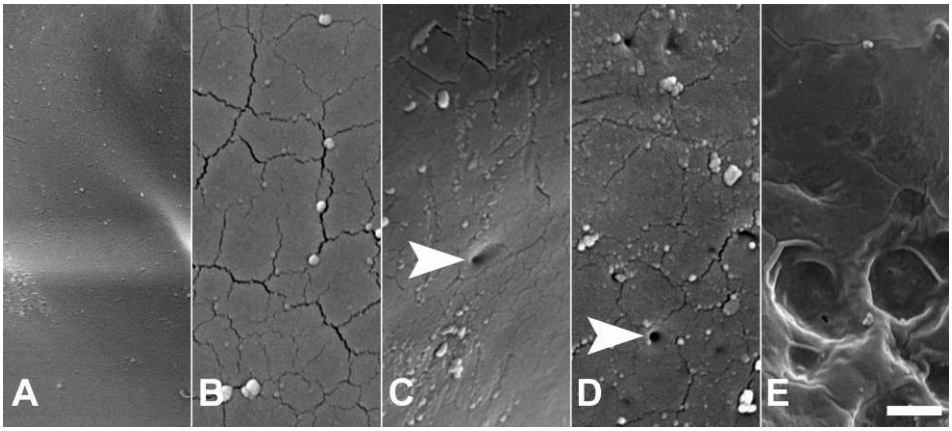

Fig. 2 Detail of the surface of an egg of *Archegozetes longisetosus* without and after maceration with sodium hypochloride, viewed with scanning electron microscopy. All micrographs are taken with the same magnification. A: Surface of an egg without sodium hypochloride treatment. The egg-shell does not show any fissures or micro holes. B–D: Details of different eggs after identical maceration with sodium hypochloride. The micrographs demonstrate fissures and micro holes in the surface of the egg-shell. Whereas fissures can be observed over the whole surface, the micro holes (indicated by arrowheads) are scattered over the surface of the eggs: B: region without micro holes, C: region with scarce micro holes, D: region with clustered micro holes. E: Detail of the surface of an egg after excessive sodium hypochloride treatment. The micro holes changed to crater-like structures up to 2 μm wide. Scale bar = 2 μm.

Our studies show that within pierced eggs the preservation of tissue is not assured, regardless of the mode of fixation (Fig. 3A and C). Fixation caused disruption of the macromeres and a decrease of their cytoplasmic density. Cellular organelles, such as the endoplasmatic reticulum (ER), the Golgi apparatus and the mitochondria could not be recognised in their normal state. The nuclei were less affected but showed an enlarged *spatium perinucleare* in the chemical fixation (Fig. 3A), whereas with cryofixation the nuclear membrane was not readily identifiable (Fig. 3C). The nucleoplasm showed a non-homogeneous precipitation of colloidal complexes with both fixation methods. The yolk-platelets and the lipid droplets were always normal in their appearance, but cell membranes were not recognisable at all.

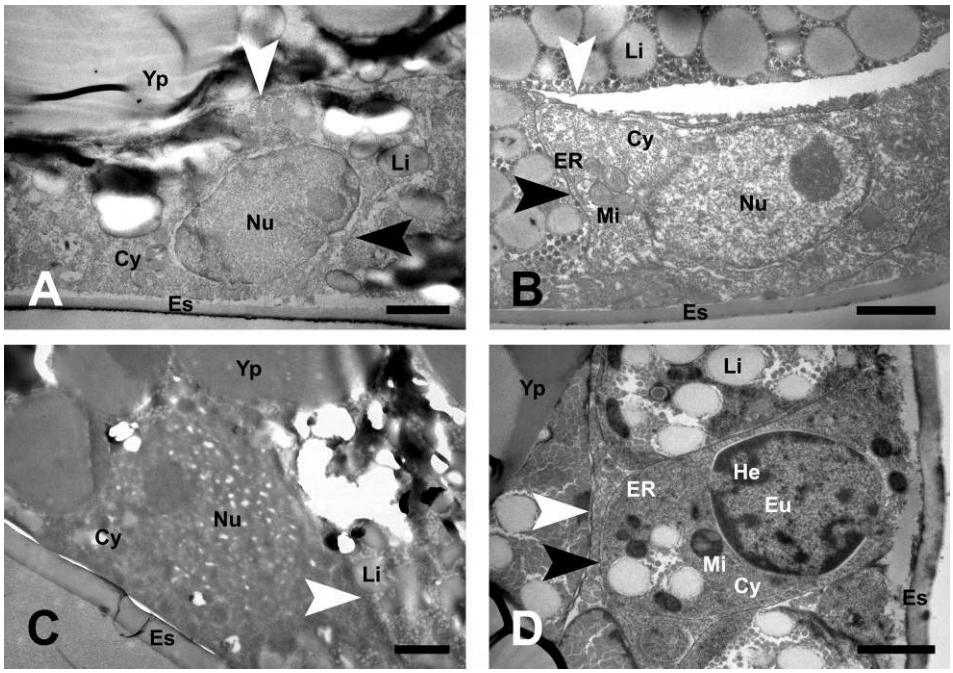

Fig. 3 Transmission electron micrographs of blastulae of *Archegozetes longisetosus*. The black arrowheads are indicating the cell membranes of micromeres; the white arrowheads indicate the cell membranes of macromeres. A: Pierced egg, chemical fixation. Scale bar = 1 µm. B: Permeable rendered egg-shell, chemical fixation. Chemical fixation of this specimen and the specimen shown in Fig. 1B were identical procedures. Scale bar = 1 µm. C: Pierced egg, cryofixation. Scale bar = 1 µm. D: Permeable rendered egg-shell, cryofixation. This is a micrograph of the specimen shown in Fig. 1C. Tissue preservation is superior compared to chemical fixation. Scale bar = 1 µm. Abbreviations: Cy: cytoplasm; ER: endoplasmatic reticulum; Es: egg-shell; Eu: euchromatin; He: heterochromatin; Li: lipid droplet; Mi: mitochondrion; Nu: nucleus; Yp: yolk platelet.

By contrast, pretreatment with sodium hypochloride resulted in a reliable preservation of tissue (Fig. 3B and D). The cell membranes of macromeres and micromeres were intact and displayed a bilayer structure, cellular organelles were identified without deformities and clearing of the cytoplasm did not occur. Despite the high osmolarity during chemical fixation, shrinkage did not result in artifacts that could be misinterpreted (Fig. 3B). Compared to the chemically fixated samples, the cryofixated samples exhibited tissue with smoother cell membranes and cellular components (Fig. 3D). Also, fixation differentially affected the cytoplasm and nucleoplasm: the cytoplasm appeared to form non-homogeneous precipitations in the chemically fixated samples, but was equally dispersed and appeared to vary in electron density in cryofixated samples (Fig. 3B and D). In chemical fixation the heterochromatin was agglomerated, whereas in the cryofixation heterochromatin and euchromatin were evenly distributed in the nucleus, indicating a considerably improved preservation of the ultrastructure.

4. Discussion

The pioneers in acarine embryology

Embryology as a modern science began in the 1820s, but during the first half of the 19th century histological technique and observation were both at a primitive stage. Each worker generally constructed his own microscope and other equipment, thus the knowledge of the histological structure of tissue was severely limited by the quality of instrumentation (Rabdrury 1979).

In the earliest work, the choice of particular mite species with which to investigate embryology was strongly influenced by the ease of study. A transparent egg-shell provided a significant advantage, because such eggs could be examined by whole mount preparations (Claparède 1868). Since the eggs of mites were diminutive, difficult to obtain and presented many technical problems in processing, sectioning and orientation, every analysis done in this period faced massive problems (Oudemans 1885). This may be reflected in some of the shortened descriptions of acarine cleavage modes of this time, characterising only the first cleavages until the two-cell or the four-cell stage (Reuter 1909a). Stains were rarely used and sections, if done at all, were cut by hand and mounted in aqueous media (Haseloff 2003). Up to this time only the study by Wagner (1894) of an anactinotrichid mite, the tick *Ixodes calcaratus*, included sectioning of eggs. The bulk of the pioneering work was done with whole-mount preparations of gravid females or eggs in watery media, a technique not adequate for resolving the cleavage mechanism of mites in detail. Thereto Leydig stated in 1848: ‘Water darkens the oily bodies [of the eggs] yellowish brown and induces a granular precipitate’. Cleavage was thought to be total if the egg consisted of molecular elemental bodies, if the egg also contained fatty bodies, cleavage was thought to be superficial (Leydig 1848).

The end of the 19th century saw the first of two periods of spectacular technical developments. The microtome and microscopic lenses corrected for chromatic and spherical aberration were developed in the 1870s. Aniline dyes were invented for the textile industry around 1875 and rapidly adopted by microscopists as cytological stains (Haseloff 2003). Also, by the end of the 19th century, many new fixative solutions, such as those of Carnoy and Bouin, and new staining techniques, such as Heidenhain´s haematoxylin/eosin had been developed (Rabdrury 1979).

20th century developments and the advent of TEM

The novel techniques established by the end of the 19th century were used by acarologists throughout the 20th century. In particular, Bouin´s fixative (Reuter 1909b, Hafiz 1935, Warren 1940, Prasse 1968) and Carnoy´s fixative (Reuter 1909b, Klumpp 1954, Geigy & Wagner 1957, Langenscheidt 1958, Prasse 1968, Caspersson et al. 1986) became the standard preservatives for acarine eggs and Heidenhain´s haematoxylin/eosin was the typical stain for semi-thin sections (Reuter 1909b, Hafiz 1935, Klumpp 1954, Prasse 1968). Of the two fixatives, Carnoy´s gave superior tissue preservation in acarine eggs (Reuter 1909b). Again, there was a strong bias in the choice of mite species to study, but during this period the decision seems to have focused on monetary, rather than technical, factors. Most attention was given to important pests or parasites among the actinotrichid suborders Prostigmata (Hafiz 1935, Klumpp 1954, Edwards 1958, Dittrich 1968, Casperson et al. 1986) and Astigmata (Langenscheidt 1958, Prasse 1968) and among the anactinotrichid suborders Ixodida (Geigy & Wagner 1957, Aeschlimann 1958, Rahman 1983) and Gamasida (Warren 1940).

With the exception of Caspersson et al. (1986) and Fagotto et al. (1988), described below, all investigations of acarine embryology done in the 20th century relied on LM techniques. With LM techniques the cleavages of blastomeres can be observed, thus, if the fixation was successful, these techniques are helpful to differentiate between an interlecithal or superficial cleavage and a mixed/combination cleavage. But, as our results indicated, no information can be gained concerning the cell membranes after differentiation of blastomeres into micromeres and macromeres, as in the case of a total cleavage. Thus even semi-thin sections of a cryofixation of a total cleavage after the division of blastomeres into micromeres and macromeres analysed by LM alone would be misleading and wrongly support a mode of mixed/combination cleavage. The main reason why previous authors did not recognise the total cleavage pattern even with successful tissue preservation is the impossibility to identify the cellular limits especially of micromeres in LM (Fagotto et al. 1988, Walzl et al. 2004).

Also the inability to demonstrate the cell membranes of micromeres and macromeres in LM potentially resulted in the diversity of the previously considered cleavage types. For example, Hafiz (1935) supposed superficial cleavage after fixation with Bouin´s, sectioning and haematoxylin/eosin staining for the prostigmatan mite *Cheyletus eruditus*. In 1958 Edwards published micrographs of eggs of this species *in vivo*, which clearly demonstrated the first cleavages to be total, indicating that fixation artifacts in Hafiz analysis lead to a misinterpretation of the cleavage type. Different fixation artifacts therefore may result in at least misleading cleavage types, which would simulate non-existant variety of cleavage types. Unfortunately, nearly all studies of acarine embryology of the 20th century show schematic drawings rather than micrographs, which makes an analysis of these studies regarding artifacts almost impossible.

In the 1950s and 1960s the invention and development of electron microscopy represented the second major technological advance in embryology. The transmission electron microscope broke through the limits of resolution and depth of focus that constrained the light microscope and the ultra-microtome had overcome the constraints of section thickness (Rabdrury 1979). The first TEM analysis of acarine early embryology was done in 1986 by Caspersson et al. for a prostigmatan, the spider mite *Tetranychus urticae*. They analysed the first cleavages in whole-mount preparations and the following cleavages in histological LM sections, and concluded that the first two cleavages were total with a following switch to a

superficial cleavage mode. Unfortunately they do not show figures of these stages. Their first figures are TEM-micrographs of blastulae, an embryological stage in which cell membranes of blastomeres or micromeres are certainly visible, regardless of the mode of cleavage, since cellularisation starts at this embryological stage. Their LM-based conclusions about early cleavage were later shown to be incorrect, as noted below.

The second TEM-analysis of acarine embryology, by Fagotto et al. (1988), was more significant, as it included early embryonic stages. They postulated that early cleavage in an anactiotrichid species, the argasid tick *O. moubata*, was total.

Advances in acarine embryology in the 21st century

The main reason for our poor knowledge of acarine embryology has been the impermeability of the vitelline membrane to aqueous fixatives (Beament 1951, Yastrebtsov 1992, Walzl & Gutweniger 2002). A common technique of molecular biologists was the treatment of embryos with bleach in combination with a heptane-formaldehyde fixation, which was routinely used for dechorionation of *Drosophila*-embryos (Zalokar & Erk 1977, Patel 1994). This procedure was suitable for fluorescence-LM and SEM studies, but its use in TEM preparations led to unsatisfactory results (Wieschaus & Nüsslein-Volhard 1986).

To be effective, fixative penetration must be fast, and this is a particular problem with eggs protected by shells (Ripper et al. 2008). In 2002 Walzl & Gutweniger published a preparation technique using a modified sodium-hypochloride treatment of acarine eggs for TEM analyses. Micro-holes and fissures are formed in the egg-shell which seems to allow the fixative to penetrate across the entire surface of the egg-shell. Since fixation by immersion is a diffusion-limited process, and therefore relatively slow, this technique is superior to piercing, as discussed above. Studies using this technique showed that the cleavage pattern of the astigmatid mite *S. berlesei* and the oribatid mite *A. longisetosus* both follow a specialised total cleavage mode (Walzl et al. 2004, Laumann et al. 2010).

Even if fast, conventional chemical fixation can be a source of problems. The fixative is selective for reactive molecules, and pH-related and osmotic changes caused by the fixative solution are not entirely predictable for all compartments in a cell or in all tissues. In contrast, cryofixation is non-selective, in that there is complete arrest of all cellular processes, and problems caused by inappropriate pH or osmotic shock do not occur (Ripper et al. 2008). We demonstrated that this technique results in considerably improved preservation of the ultrastructure of the acarine embryos and is superior to conventional chemical fixation.

LM techniques remain important for the study of the cleavage mode of the Acari, but TEM analyses are indispensable for clarifying details and in the future must take center stage. In 1987 White et al. demonstrated that scanning confocal microscopes give an improved visibility of sections and resolution. With the application of fluorescent dyes and clearing techniques high-resolution sections can be collected into samples to distances of 200 µm (Haseloff 2003). These documented picture series of samples at different distances allow conversion into 3D images. Modelling techniques then allow the construction of a precise geometric description of the cellular architecture. This technique is more and more in the focus of embryologists for revealing the cleavage modes of small-sized eggs (e.g. Alwes & Scholtz 2004, Wennberg et al. 2008, Ungerer & Scholtz 2009), and this may become an efficient technique for studying the early embryology of Acari.

Reconsideration of early cleavage in acarines

Few species have been studied using modern methods, but the two orders of mites – Actinotrichida and Anactinotrichida – seem to present a remarkable uniformity in their basic cleavage pattern, as present studies show: they exhibit a total cleavage pattern with differentiation of blastomeres into micro- and macromeres. Yet, it is not easy to compare their embryonic development.

In Actinotrichida the blastomeres of the astigmatid mite *S. berlesei* and the oribatid mite *A. longisetosus* are not radially arranged extending from the center to the periphery, but are oriented along the minor and major axis of the ellipsoid egg. Because of asynchronous cell divisions it is not yet known exactly when their blastomeres divide into yolk-free micromeres and yolky macromeres. In both species the micromeres divide further tangentially until they cover the surface of the embryo, whereas the macromeres no longer divide (Walzl et al. 2004, Laumann et al. 2010; Fig. 4). These results are further consistent with those of Dearden et al. (2002), who analysed the early cleavages of the prostigmatid spider mite *T. urticae* and showed with dextran injections and fluorescent microscopy that – in contrast to Caspersson et al. (1986) – the embryos did not have an early syncytial phase; instead, total cleavage divisions result in the formation of a blastoderm surrounding a yolky interior. In a further study using fluorescent staining, Dearden et al. (2003) showed, that cells internal to the blastoderm surface are larger and less numerous than blastoderm cells. While focusing on other topics their results – total cleavage resulting in many small cells surrounding few large yolky cells – strongly indicate a similar type of cleavage including blastomere differentiation as was suggested for both *A. longisetosus* and *S. berlesei*.

Regarding Endeostigmata, to our knowledge there is only one study postulating equal cleavage with separation of blastomeres (Lange & Tolstikov 1999, citing Lange 1996 in cyrillic).

These consistent results of modern studies comprising Prostigmata, Endeostigmata, Oribatida and Astigmata strongly question the previous idea of a syncytial phase as normal feature in actinotrichid embryogenesis. Instead, a specialised form of total cleavage – involving unequal yolk distribution into micromeres and macromeres – may be the general mode of cleavage in the Actinotrichida.

Because of the lack of data any generalisation concerning the mode of cleavage in Anactinotrichida is inordinately more difficult. For the anactinotrichid Holothyrida and Opilioacarida to our knowledge no data is available concerning early embryogenesis. Early cleavage in the Gamasida is thought to be superficial (Warren 1940, 1941, Yastrebtsov 1992), with the exception of the Spinturnicidae, with a total cleavage (Akimov & Yastrebtsov 1990, Yastrebtsov 1992). Cited data established with methods shown in this study are prone to misunderstandings; also some of the data at hand are inconsistent. Unfortunately no ultrastructural study of early embryogenesis has been made for this major group. A re-investigation with sophisticated methods would be helpful to shed light on the cleavage pattern of the Gamasida.

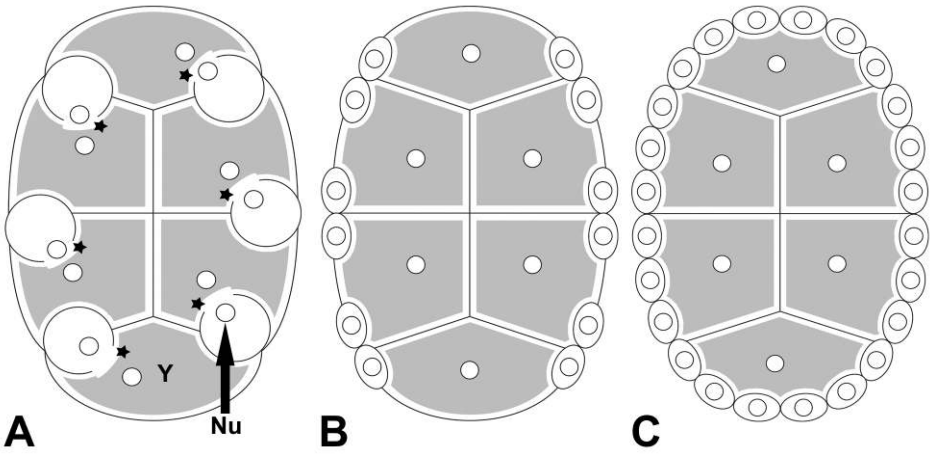

Fig. 4 Schematic drawing of the supposed total cleavage pattern in the oribatid mite *A. longisetosus* (after Laumann et al. 2010) and the astigmatid mite *S. berlesei* (after Walzl et al. 2004). Note that the egg-shell surrounding the embryo is not included in the scheme. A: After the early cleavages the blastomeres differentiate into an outer micromere and an inner macromere containing all the yolk. Asterisks indicate the differentiation events. Please note that the differentiation need not occur simultaneously in all blastomeres as indicated in the schematic drawing. B: The micromeres do not form a continuous layer, but are scattered on the embryonic surface embedded between macromeres. Their cleavages proceed tangentially on the embryonic surface. The yolky macromeres do not subsequently divide or do so only sporadically. C: The macromeres are covered successively by the micromeres. Abbreviations: Nu: nucleus; Y: yolk.

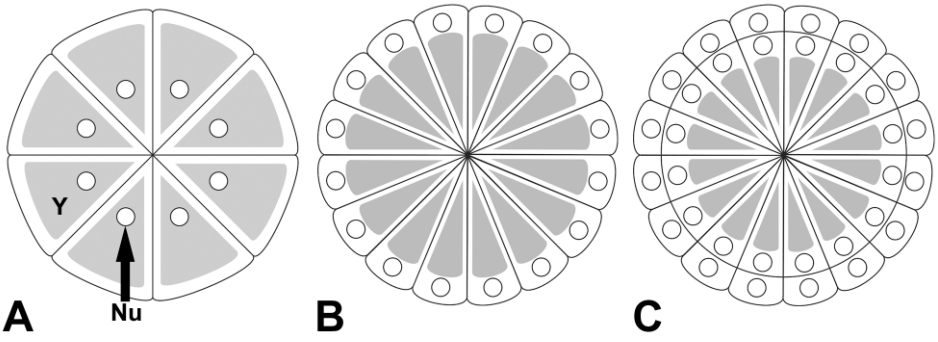

Fig. 5 Schematic drawing of the progress of the total cleavage pattern in the argasid tick *Ornithodorus moubata*. Note that the egg-shell surrounding the embryo is not included in the scheme. A: In the eight-cell stage the blastomeres are arranged radially and completely separated from each other. The nuclei migrate towards the periphery. B: After each division the cells become narrower, but their length does not change. The nuclei have reached the periphery of the blastomeres. C: Each blastomere has differentiated into an outer micromere and an inner macromere containing all the yolk (modified from Fagotto et al. 1988). Abbreviations: Nu: nucleus; Y: yolk.

The description of the embryonic development of the argasid tick *O. moubata* by Aeschlimann (1958) is considered as the reference example for embryogenesis of Acari, but was revised some years ago: in the eight-cell stage (beginning of observations) the blastomeres are arranged radially and extend from the centre to the periphery. In the 8th or 9th mitotic cycle each of the blastomeres divides into an outer yolk-free micromere and an inner macromere containing all the yolk, together building the blastula. Whereas the micromeres divide further, most of the macromeres probably divide only once more and then remain unchanged (Fagotto et al. 1988; Fig 5). Furthermore they generalise this cleavage pattern: 'This pattern seems to be the rule among the Ixodoidea, as indicated by preliminary observations on eggs of *Ambylomma hebraeum*, *Amblyomma variegatum* and *Boophilus microplus*.'

The data at hand consider total cleavage to be the rule among the Ixodoidea, which is mainly based on one ultrastructural study of *O. moubata*. However, this taxon was considered in the last years as the typical example for acarine embryogenesis (Anderson 1973, Aeschlimann & Hess 1984, Fagotto et al. 1988, Evans 1992). If considering this still holds true, then the early cleavage of Anactinotrichida may present a total cleavage pattern.

None of the modern ultrastructural and molecular studies have shown evidence for the existence of a superficial part in cleavage of any acarine species, even in those earlier thought to exhibit superficial or mixed/combined cleavage. Hence, total cleavage with blastomere differentiation may represent the cleavage ground-plan for Actinotrichida and probably for Anactinotrichida.

Further investigations on early embryology of Acari promise to provide more evidence for a reconstruction of the ancestral cleavage pattern of this group and might contribute to a reexamination of the traditional interpretations of chelicerate development.

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6. References

Aeschlimann, A. (1958): Developpement embryonnaire d´*Ornithodorus moubata* (Murray) et transmission transovarienne de *Borellia duttoni*. – Acta Tropica **15**: 15–62.

Aeschlimann, A. & E. Hess (1984): What is our current knowledge of acarine embryology? – In: Griffiths, D. A. & C. E. Bowman (eds): Acarology 6, Volume 1. – Ellis Horwood, Chichester: 90–99.

Akimov, I. A. & V. Yastrebtsov (1990): Embryonic development of the mite *Spinturnix vespertiliones* (Parasitiformes: Spinturnicidae). – Acarologia **31**: 3–12.

Alberti, G., A. Seniczak & S. Seniczak (2003): The digestive system and fat body of an early-derivative oribatid mite, *Archegozetes longisetosus* Aoki (Acari: Oribatida, Thrypochthoniidae). – Acarologia **43**: 149–219.

Alwes, F. & G. Scholtz (2004): Cleavage and gastrulation of the euphausiacean *Meganyctiphanes norvegica* (Crustacea, Malacostraca). – Zoomorphology **123**: 125–137.

Anderson, D. T. (1973): Embryology and Phylogeny in Annelids and Arthropods. – Pergamon Press, Oxford: 492 pp.

Beament, J. W. L. (1951): The structure and formation of the egg of the fruit tree red spider mite, *Metatetranychus ulmi* Koch. – Annals of Applied Biology **38**: 1–24.

Bergmann, P., M. Laumann, P. Cloetens & M. Heethoff (2008): Morphology of the internal reproductive organs of *Archegozetes longisetosus* Aoki (Acari, Oribatida). – Soil Organisms **80**: 171–195.

Bonnet, A. (1907): Recherches sur l´anatomie comparee et le developpement des Ixodides. – Annales de l´Universite de Lyon, Nouv. ser. 1. Sciences, medicine, Lyon: 180 pp.

Bourguignon, H. (1854): Traite entomologique et pathologique de la gale de l´homme. – Memoire Couronne par l`Academie des Sciences. Acad. des Sciences, Paris: 168 pp.

Brucker, E. A. (1900): Monographie de *Pediculoides ventricosus* Newport et theorie des pieces buccales des Acariens. – Bulletin Scientifique de la France et de la Belgique **35**: 355–442.

Casperson, G., U. Stark, D. Otto, & H. B. Schmidt (1986): Licht- und Elektronenmikroskopische Untersuchungen der Eischale und der Embryogenese der Gemeinen Spinnmilbe (*Tetranychus urticae* Koch, Acari, Tetranychidae). – Zoologische Jahrbücher, Abteilung für Anatomie **114**: 235–253.

Claparéde, E. (1868): Studien an Acariden. – Zeitschrift für wissenschaftliche Zoologie **18**: 445–546.

Dawydoff, C. (1928): Traite d`embryologie comparee des Invertebres. – Masson, Paris: 930 pp.

Dearden, P. K., C. Donly & M. Grbic (2002): Expression of pair-rule gene homologues in a chelicerate: early patterning of the two-spotted spider mite *Tetranychus urticae*. – Development **129**: 5461–5472.

Dittrich, V. (1965): Embryonic development of Tetranychids. 5th European Mite Symposioum, Milano 1965. – Bollettino di Zoologia Agraria e di Bachicoltura, Serie II, **7**: 101–104.

Dittrich, V. (1968): Die Embryonalentwicklung von *Tetranychus urticae* Koch in der Auflichtmikroskopie. – Zeischrift für angewandte Entomologie **61**: 142–153.

Donnadieu, A. L. (1875): Recherches pour servir a l´histoire des Tetranyques. – Annales de la Societe Linnéenne de Lyon, Ser. 2, **25**: 153–155.

Edwards, A. R. (1958): Cleavage in *Cheyletus eruditus* (Acarina). – Nature **181**: 1409–1410.

Evans, G. O. (1992): Principles of Acarology. – CAB International, Wallingford: 563 pp.

Fagotto, F., E. Hess & A. Aeschlimann (1988): The early embryonic development of the argasid tick *Ornithodorus moubata* (Acarina: Ixodoidea: Argasidae). – Entomologia Generalis **13**: 1–8.

Fioroni, P. (1970): Die organogenetische und transitorische Rolle der Vitellophagen in der Darmentwicklung von *Galathea* (Crustacea, Anomoura). – Zeitschrift für Morphologie der Tiere **67**: 263–306.

Fukuda, J. & N. Shinkaji (1954): Experimental studies on the influence of temperature and relative humidity upon the development of the Citrus Red Mite (*Metatetranychus citri* McGregor). Part 1: On the influence of temperature and relative humidity upon the development of the eggs. – Bulletin of Tokai-kinki Agriculture Experiment Station (Horticulture) **2**: 160–171.

Fürstenberg, M. H. F. (1861): Die Krätzmilben der Menschen und Tiere. – Verlag von Wilhelm Engelmann, Leipzig: 240 pp.

Gasser, R. (1951): Zur Kenntnis der gemeinen Spinnmilbe *Tetranychus urticae* Koch. – Mitteilungen der Schweizerischen Entomologischen Gesellschaft **14**: 217–262.

Geigy, R. & O. Wagner (1957): Ovogenese und Chromosomenverhältnisse bei *Ornithodorus moubata*. – Acta Tropica **14**: 88–91.

Hafiz, H. A. (1935): The embryological development of *Cheyletus eruditus*. – Proceedings of the Royal Society of London Series B **117**: 174–201.

Haseloff, J. (2003): Old botanical techniques for new microscopes. – Bio Techniques **34**: 1174–1182.

Heethoff, M., M. Laumann & P. Bergmann (2007): Adding to the reproductive biology of the parthenogenetic oribatid mite, *Archegozetes longisetosus* (Acari, Oribatida, Trhypochtoniidae). – Turkish Journal of Zoology **31**: 151–159.

Henking, H. (1882): Beiträge zur Anatomie, Entwicklungsgeschichte und Biologie von *Trombidium fuliginosum* Hermann. – Zeitschrift für wissenschaftliche Zoologie **34**: 553–663.

Hughes, T. E. (1950): The embryonic development of *Tyroglyphus farinae* Linnaeus 1758. – Proceedings of the Zoological Society of London **119**: 873–886

Hughes, T. E. (1959): Mites or the Acari. – The Athlone Press, London: 225 pp.

Johannsen, O. A. & F. H. Butt (1941): Embryology of Insects and Myriapods. – McGraw-Hill, New York: 462 pp.

Klumpp, W. (1954): Embryologie und Histologie der Bienenmilbe *Acarapis woodi* Rennie 1921. – Zeitschrift für Parasitenkunde **16**: 407–442.

Köhler, H. R., G. Alberti, S. Seniczak & A. Seniczak (2005): Lead-induced hsp70 and hsp60 pattern transformation and leg malfunction during postembryonic development in the oribatid mite, *Archegozetes longisetosus* Aoki. – Comparative Biochemistry and Physiology C **141**: 398–405.

Kramer, P. (1881): Ueber Milben. – Zeitschrift für die gesammten Naturwissenschaften, 3. Folge, Band 6: 417–452.

Lange, A. B. & A. V. Tolstikov (1999): Ovoviviparity, prelarva and the peculiarities of eclosion in freshwater oribatid mites *Thrypochthoniellus setosus* and *Hydrozetes lemnae*. – Acarina **7**: 13–21.

Langenscheidt, M. (1958): Embryologische, morphologische und histologische Untersuchungen an *Knemidocoptes mutans* (Robin et Lanquetin). – Zeitschrift für Parasitenkunde **18**: 349–385.

Laumann, M., P. Bergmann & M. Heethoff (2008): Some remarks on the cytogenetics of oribatid mites. – Soil Organisms **80**: 223–232.

Laumann, M., P. Bergmann, R. A. Norton & M. Heethoff (2010): First cleavages, preblastula and blastula in the parthenogenetic mite *Archegozetes longisetosus* (Acari, Oribatida) indicate holoblastic rather than superficial cleavage. – Arthropod Structure and Development, in press, doi:10.1016/j.asd.2010.02.003.

Leydig, F. (1848). Die Dotterfurchung nach ihrem Vorkommen in der Thierwelt und nach ihrer Bedeutung. – Oken´s Isis **H3**: 161–193.

Oudemans, A. C. (1885): Die gegenseitige Verwandtschaft, Abstammung und Classification der sogenannten Arthropoden. – Tijdschrift der Nederlandsche Dierkundige Vereeniging **1**: 37–56.

Patau, K. (1936): Cytologische Untersuchungen an der haploid-parthenogenetischen Milbe *Pediculoides ventricosus*. – Berliner Zoologische Jahrbücher Abteilung Allgemeine Zoologie und Physiologie der Tiere **56**: 277–322.

Patel, N. H. (1994): Imaging neuronal subsets and other cell types on whole-mount *Drosophila* embryos and larvae using antibody probes. – In: Goldstein, L. S. B. & E. A. Fyrberg (eds): Methods in Cell Biology, Volume 44, *Drosophila melanogaster*: Practical Uses in Cell and Molecular Biology. – Academic Press, San Diego: 445–487.

Prasse, J. (1968): Untersuchungen über Oogenese, Befruchtung, Eifurchung und Spermatogenese bei *Caloglyphus berlesei* Michael 1903 und *Caloglyphus michaeli* Oudemans 1924 (Acari, Acaridae). – Biologisches Zentralblatt **6**: 757–775.

Rabdrury, D. M. (1979): The golden ages of histological technique. – Malaysian Journal of Pathodology **2**: 33–37.

Rahman, H. (1983): Embryogenesis in *Hyalomma rufipes* Koch, 1844 (Ixodoidea: Ixodidae). – Bangladesh Journal of Zoology **11**: 25–38.

Reuter, E. (1909a): Zur Morphologie und Ontogenie der Acariden. – Acta Societatis Scientiarum Fennicae **36**: 1–288.

Reuter, E. (1909b): Merokinesis, ein neuer Kernteilungsmodus. – Acta Societatis Scientiarum Fennicae **34**: 3–56.

Ripper, D., H. Schwarz & Y. D. Stierhof (2008): Cryo-section immunolabelling of difficult to preserve specimens: advantages of cryofixation, freeze-substitution and rehydration. – Biology of the Cell **100**: 109–123.

Robin C & P. Mégnin 1877. Mémoire sur les Sarcoptides plumicoles. – Journal de L'anatomie et de la Physiologie Normales et Pathologiques de L'homme et des Animaux, Paris, **13:** 209–248, 391–429, 498–520, 629–656.

Sakata, T. & R. A. Norton (2003): Opisthonotal gland chemistry of a middle-derivative oribatid mite, *Archegozetes longisetosus* (Acari: Trhypochthoniidae). – International Journal of Acarology **29**: 345–350.

Seniczak, A. (2006): The effect of density on life-history parameters and morphology of *Archegozetes longisetosus* Aoki, 1965 (Acari: Oribatida) in laboratory conditions. – Biological Letters **43**: 209–213.

Smrž, J. & R. A. Norton (2004): Food selection and internal processing in *Archegozetes longisetosus* (Acari: Oribatida). – Pedobiologia **48**: 111–120.

Sokolov, I. I. (1952): Observations on the embryonic development of the granary mites. I. Construction of the egg and segmentation. – Trudy Leningrad, Society of Naturalists **71**: 245–260.

Türk, E. & F. Türk (1957): Systematik und Ökologie der Tyroglyphiden Mitteleuropas. – In: Stammer, H. J. (ed.): Beiträge zur Systematik und Ökologie der mitteleuropäischen Acarina, Teil 1, Abschnitt 1. – Akademische Verlagsgesellschaft, Leipzig: 1–231.

Ungerer, P. & G. Scholtz (2009): Cleavage and gastrulation in *Pycnogonum litorale* (Arthropoda, Pycnogonida): morphological support fort he Ecdysozoa? – Zoomorphology **128**: 263–274.

Vitzthum, H. (1943): Acarina. – In: Bronns, H. G. (ed.): Klassen und Ordnungen des Tierreiches 5 (4. Abteilung, 5. Buch), 1.-7. Lieferung. – Geest & Portig, Leipzig: 1011 pp.

Wagner, J. (1893): On the embryology of the mites: Segmentation of the ovum, origin of the germinal layers, and development of the appendages in *Ixodes*. – Annals and Magazine of Natural History **11**: 220–224.

Wagner, J. (1894): Die Embryonalentwicklung von *Ixodes calcaratus*. – Travaux de la Societe des Naturalistes de St. Petersbourg, Section Zoologie et Physiologie **24**: 214–246.

Walzl, M. G. & A. Gutweniger (2002): A simple preparation technique for transmission electron microscopic investigations of acarine eggs. – Abhandlungen und Berichte des Naturkundemuseums Görlitz **74**: 3–7.

Walzl, M. G., Gutweniger, A. & P. Wernsdorf (2004): Embryology of mites: new techniques yield new findings. – Phytophaga **14**: 163–181.

Warren, E. (1940): On the genital system of *Dermanyssus gallinae* (de Geer) and several other Gamasidae. – Annals of the Natal Museum **9**: 409–459.

Warren, E. (1941): On the genital system and the modes of reproduction and dispersal in certain gamasid mites. – Annals of the Natal Museum **10**: 95–127.

Wennberg, S. A., Janssen, R. & G. E. Budd (2008): Early development of the priapulid worm *Priapulus caudatus*. – Evolution & Development **10**: 326–338.

White, J. G., Amos, W. B. & M. Fordham (1987): An evaluation of confocal versus conventional imaging of biological structures by fluorescence light microscopy. – The Journal of Cell Biology **105**: 41–48.

Wieschaus, E. & C. Nüsslein-Volhard (1986): Looking at embryos. – In: Roberts, D. B. (ed.): *Drosophila*: a practical approach. – IRL Press, Oxford: 199–228.

Yastrebtsov, A., 1992: Embryonic development of gamasid mites (Parasitiformes: Gamasida). – International Journal of Acarology **18**: 121–141.

Zalokar, M. & I. Erk (1977): Phase-partition fixation and staining of *Drosophila* eggs. – Stain Technology **52**: 89–95.

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