

## The early Embryonic Development of the Argasid tick *Ornithodoros moubata* (Acarina: Ixodoidea: Argasidae)

François Fagotto\*, Ernst Hess & André Aeschlimann

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The mode of cleavage of the centrolecithal tick eggs has so far been considered to be superficial. Evidence could be obtained from an electron-microscopic study of *Ornithodoros moubata* Murray 1877 that cleavage of this species is total. The early development leads to a diploblastic stage composed of a superficial layer of yolk-free micromeres (ecto-mesoderm) and a core of yolk-rich macromeres (primary entoderm).

Key words: embryonic development, cleavage, *Ornithodoros moubata*, ticks.

### 1 Introduction

Arthropod eggs commonly have a centrolecithal structure and a superficial cleavage pattern. This particular pattern, unknown from the other phyla, has been well studied in pterygot insects, where we find the very classical examples such as *Drosophila*, *Smittia* [Sander et al 1985]. Too often, however, superficial cleavage is considered to be the rule in Arthropoda, whereas actually many primitive species of Insecta, Myriapoda and Chelicerata exhibit total cleavage [Anderson 1973]. Blastulation of spider and crustacean eggs does not proceed in an orthodox manner either, even though the final result, a blastoderm coating an acellular yolk mass, is considered by many authors to be identical to an insect blastula. In Arachnida and Crustacea, eggs divide totally during the migration of the cleavage nuclei, and intralecithal partitions seem to be aborted only secondarily. Such a mode of cleavage has been called "mixed" [Dawydoff 1928, Fioroni 1970]. One generally admits that superficial and mixed cleavage patterns are characteristic of the more evolved groups of Arthropoda, and that they are related to the high yolk content of their eggs. Studies on the embryology of Acarina are very scarce [Aeschlimann 1958, Anderson 1973, Krantz 1978, Aeschlimann & Hess 1984]. They chiefly deal with general morphology, lacking, however, ultrastructural or experimental approaches. The accurate description of the embryonic development of *Ornithodoros moubata* Murray 1877, an African soft tick, by Aeschlimann [1958], is still considered as the reference example for embryogenesis of Acarina.

The *O. moubata* egg is centrolecithal, with a large amount of brown-colored yolk embedded in a thin cytoplasmic layer. According to Aeschlimann's description [1958], cleavage is superficial, the egg preserving a syncytial organisation as the dividing nuclei migrate towards the yolk surface. After 8 mitotic cycles the cleavage nuclei reach the periphery and subsequently cellulation occurs, leading to the formation of the superficial blastoderm. Some nuclei, called

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\* Part of PhD thesis of the first author

vitellophages, leave this layer and penetrate the yolk mass. This description is based on low magnification observations of living embryos, and on light microscopic histological studies of eggs which are less than 1 mm Ø. Under the initiative of Prof. Aeschlimann, the early development of *O. moubata* is reinvestigated at the ultrastructural level in order to verify the cleavage pattern and to study the vitellophages in a more detailed manner.

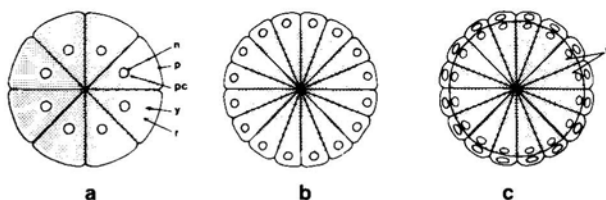
## 2 Material and Methods

The ticks used for this study were reared by membrane feeding on swine blood. The fed and mated ♀♀ were maintained at 27 °C. A patch of eggs was fixed every 12 hours, the precise stage of each embryo being determined later according to histological characteristics, mainly from the position of the cleavage nuclei. For fixation, the eggs were immersed in glutaraldehyde [Sabatini et al 1963] containing 4–5% sucrose, and the egg shell was pierced using a tungsten needle. The hypertonic solution compensates the high internal hydrostatic pressure of the embryos and thus prevents their explosion. Immediately after their perforation, the embryos were transferred to fixative at normal osmolarity (2% sucrose), and kept there for 24 h at 4 °C, then rinsed in phosphate buffer and postfixed for 2 h with osmium tetroxide [Palade 1952]. After dehydration, the specimens were embedded in Spurr's resin [Spurr 1969]. Thin sections stained with uranyl acetate and lead citrate were observed on a Philips EM210 Transmission Electron Microscope [TEM]. Semi-fine sections were stained with toluidine blue. In order to isolate macromeres from more than 2 d old embryos for Scanning Electron Microscopic [SEM] studies, the following method was used: an egg was repeatedly and violently sucked towards the broken opening of a pipette of which the Ø was slightly smaller than the egg. Thus the shell was slit open and could easily be removed. The embryos were then dissociated on glass slides in 0.2 h phosphate buffer containing 5% sucrose and 40 mg/100 ml EDTA<sup>H2</sup>. After 30 min, this solution was replaced by an equivalent buffer without EDTA, containing traces of CaCl<sub>2</sub>. After 1 h, were most of the isolated cells adhered to the glass where they were fixed as for TEM. Acetone dehydrated specimens were critical-point dried with CO<sub>2</sub> and coated with carbon and gold before being observed in a Philips PSEM 500.

## 3 Results

### 3.1 Progress of cleavage

From the 8-cell stage on (beginning of the observations) the blastomeres are arranged radially. They are completely separated from each other. After each division the cells become narrower, but their length does not change. Thus, each blastomere has a pyramidal shape and extends from the center to the



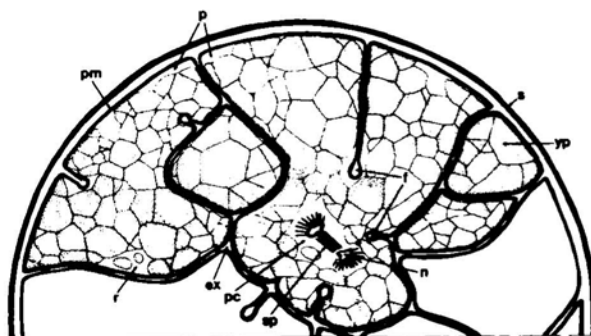
**Fig 1:** *Ornithodoros moubata* Murray 1877 [Ixodoidea: Argasidae], schematic representation of the total cleavage pattern. — (a) Stage 18 h. The embryo is composed of pyramidal blastomeres, of which the nuclei migrate towards the periphery. (b) Stage 25 h (blastula). The cleavage nuclei have reached the periplasm. (c) Stage 35 h (diploblastic stage). Each pyramidal blastomere has divided into an outer micromere and an inner macromere, resulting in a superficial ectomesoblast surrounding the central primary entoderm. n nucleus, p periplasm, pc perinuclear cytoplasm, r reticuloplasm, v "vitellophages", y yolk.

periphery (Fig 1 a). During this early phase, the nuclei migrate synchronously toward the surface of the egg reaching the limit of the periplasm after 7–8 mitotic cycles (stage 24 h). At this stage which is defined as the blastula, the pyramidal blastomeres are still arranged radially (Fig 1 b). During the following division (8th or 9th mitotic cycle), each blastomere divides into an outer micromere and an inner macromere, most of the periplasm being separated from the yolk (Fig 1 c). Thus, after 30–35 h of development, the egg is composed of 2 sheets: the central sphere of macromeres containing all the yolk platelets, which is considered as the primary entoderm, and a thin external layer of yolk-free micromeres. Cleavage ends with this diploblastic stage. From this moment on, each embryonic sheet follows its own fate.

Some of the macromeres are supposed to build up the embryonic midgut epithelium or secondary entoderm, which appears at day 6, while most of them probably divide only once more and then remain unchanged until hatching. Their fate has not yet been studied in detail.

### 3.2 Morphology and cytology of blastomeres

The observation of serial sections in LM and TEM reveals that the early embryo is composed of individual cells. These seem to have a simple pyramidal shape under low magnification, but semi-fine sections and TEM observations show that their morphology is highly complicated, characterized by numerous lobes (Fig 2).



**Fig 2:** Semi-schematic transverse section of an *Ornithodoros moubata* Murray 1877 [Ixodoidea: Argasidae], embryo at stage 16 h during late mitosis. One of the 8 dividing blastomeres is drawn in detail. It is composed of numerous lobes. **ex** extracellular space, **f** furrow, **n** nucleus, **p** periplasm, **pc** perinuclear cytoplasm, **pm** plasma membrane, **r** reticuloplasm, **s** egg shell, **sp** spindle, **yp** yolk platelet surrounded by a unit membrane.

The blastomeres are filled with dense and homogenous yolk platelets (20–50  $\mu\text{m}$   $\varnothing$ ) grouped in clusters of various sizes. The yolk mass is so compact and so condensed that the platelets, which are spherical during oogenesis, are now polygonal, touching each other tightly (Fig 2, 4). Yolk-free cytoplasm is restricted to a very small volume. It comprises the perinuclear cytoplasm, the periplasm and the reticuloplasm (Fig 2). The perinuclear cytoplasm represents the major part, and it can be compared with the insect energid. The periplasm has a maximum thickness of 5  $\mu\text{m}$  in the early stages. Subsequently it thickens and raises up to 20  $\mu\text{m}$  at the blastula. Some yolk-free cytoplasm is found  $\pm$  randomly distributed between the yolk platelets, forming the reticuloplasm. The yolk-free cytoplasm strongly resembles the cytoplasm of the mature ovocyte [Aeschlimann & Hecker 1969], since mainly high quantities of glycogen, lipid droplets, ribosomes and mitochondria are found (Fig 3). It is divided into large transparent zones, where glycogen is concentrated and electron denser cytoplasm containing the organelles. It is characterized by numerous microtubules and endoplasmic reticulum. The network of microtubules is continuous with the mitotic apparatuses (spindles and asters). The latter are striking because of their large size (spindles are up to 50  $\mu\text{m}$  long), and because they are still present during early

interphase. The nuclei have a  $\varnothing \approx 15 \mu\text{m}$ , and are of a very irregular shape. They are euchromatic and no nucleoli occur earlier than at the blastula stage. Nuclear pores are extremely numerous, especially at the end of cleavage. The plasma membrane presents numerous, sometimes deep invaginations the number of which decreases during development. These pass through the reticuloplasm (Fig 3), but also directly between the yolk platelets (Fig 5). The invaginations are either simple folds separating the lobes of the blastomeres, or they are so called furrows and present a characteristic terminal structure. The furrows always originate from cell-boarding cytoplasm (periplasm or reticuloplasm) (Fig 6), and then penetrate between the yolk platelets in the deeper regions. They show a terminal bud (the base) surrounded by vesiculated cytoplasm (Fig 7). Behind the base, the furrow is closed so that 2 plasma membranes are in intimate contact with each other. Cellular junctions have not been observed, even at high magnification, but in their periplasmic region, the blastomeres are attached each other by push-button-like structures (Fig 3). In the diploblastic embryo, the macromeres are the only cells containing yolk. They are of irregular shape and have a  $\varnothing 100\text{--}300 \mu\text{m}$  (Fig 9). Their polymorphous nucleus of  $10 \mu\text{m} \varnothing$  is embedded in a small area of yolk-free cytoplasm representing the so called vitellophages. Most of the cell volume is occupied by the yolk platelets; glycogen and lipids droplets are, however, more abundant in these cells than in the early polygonal blastomeres, and various inclusions such as dense-cored vesicles appear. The micromeres are completely yolk-free, and are cytologically undifferentiated at this stage.

#### 4 Discussion

Cleavage of tick eggs has so far been considered to follow the superficial pattern. The present observations of the early embryo show, however, that the 8 first blastomeres are clearly separated from each other. From this stage on, cleavage of the *O. moubata* egg is unequivocally total. Perhaps, it is total from the beginning on. (The eggs prior to the 8 cell stage could not be observed, because they did not withstand the dissection process.) The first plane of mitosis probably corresponds to the bilateral plane of the animal, as concluded from the descriptions of perfect longitudinal gynandromorphous ticks [Stampfli 1985, Kostrzewski et al 1986]. An unmistakable sign of the total cleavage pattern can be seen at low magnification during the migration of the nuclei: the "polygonal fields" described by Aeschlimann [1958] represent the periplasmic areas of the pyramidal blastomeres, their margins indicating the cellular limits. This is in contrast to insect eggs following the superficial cleavage pattern. In those the division of the periplasm only occurs when the nuclei have reached the periphery. Aeschlimann [1958] was very close to guess the total cleavage when he wrote: "when the nuclei reach the periphery (of the 'polygonal fields') the 'blastema' (the periplasm) seems to be prepared to receive them".

The reason why previous authors did not recognize the total cleavage pattern, is the complicated structure of the cells and the extreme compactness of the egg. In the LM, it is therefore impossible to identify the cellular limits and the numerous invaginations of the plasmalemma. Even in the TEM, high magnification has to be used to distinguish the plasma membranes and the unit membranes of the yolk platelets (Fig 4, 5). Dissection of the egg results in the partial dissociation of the egg making it possible to distinguish the individual blastomeres, and chemical dissociation is successful in separating the macromeres from the micromeres in diploblastic stages.

The numerous invaginations of the plasma membrane of blastomeres represent either the limits of cellular lobes or furrows with a characteristic terminal structure typical for cleavage furrows [Arnold 1976]. Early blastomeres have several furrows the number of which decreases during development. This indicates that these invaginations represent preformed cleavage furrows.

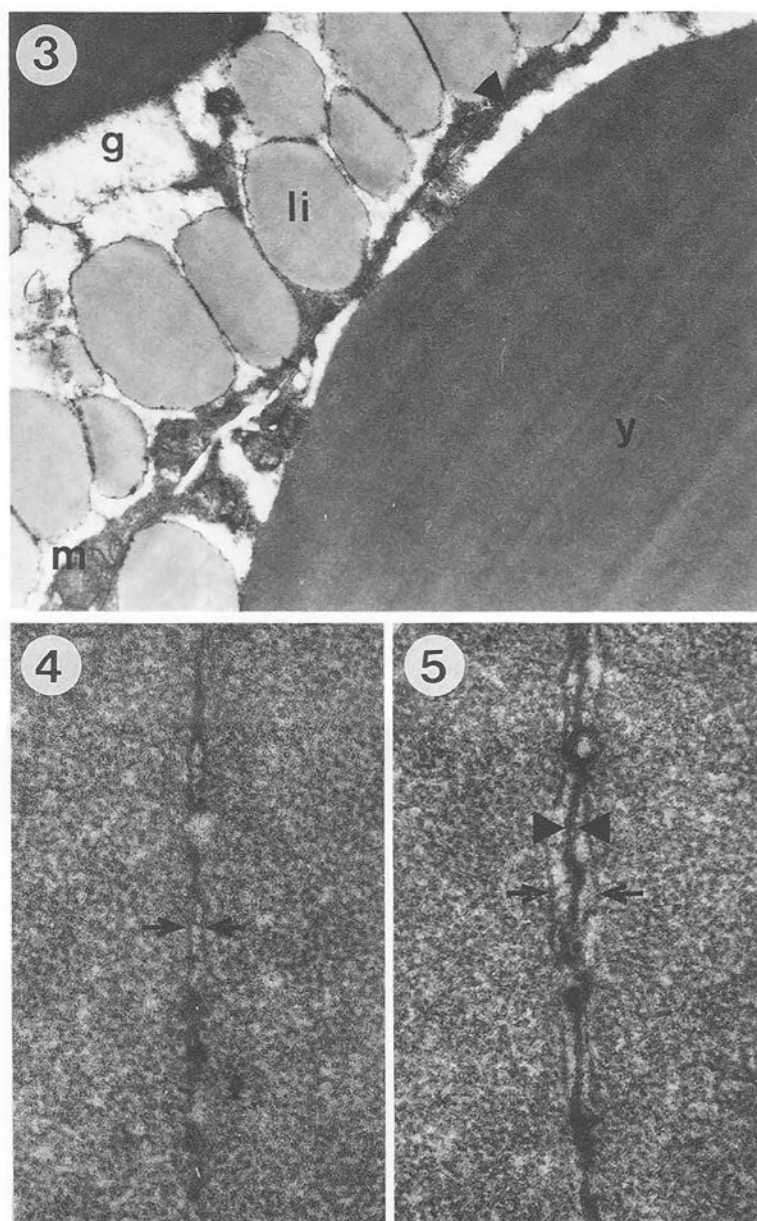


Fig 3—5: 16 h old *Ornithodoros moubata* Murray 1877 [Ixodoidea: Argasidae] embryos. — 3 The reticuloplasm is composed of small areas of yolk-free cytoplasm divided in 2 lobes on this picture (arrowhead). g glycogen, li lipid droplet, m mitochondrion, y yolk (10 200 x). 4 The yolk platelets, which are surrounded by a unit membrane (arrows), are generally tightly packed. It was not possible to preserve the integrity of the unit membranes, which are disrupted in several places. Dark particles are artifacts due to staining (83 000 x). 5 Though the high compactness of the yolk, cell membranes (arrowheads) are found in the yolk mass; arrows: unit membrane of the yolk platelet. Dark particles are artifacts (83 000 x).

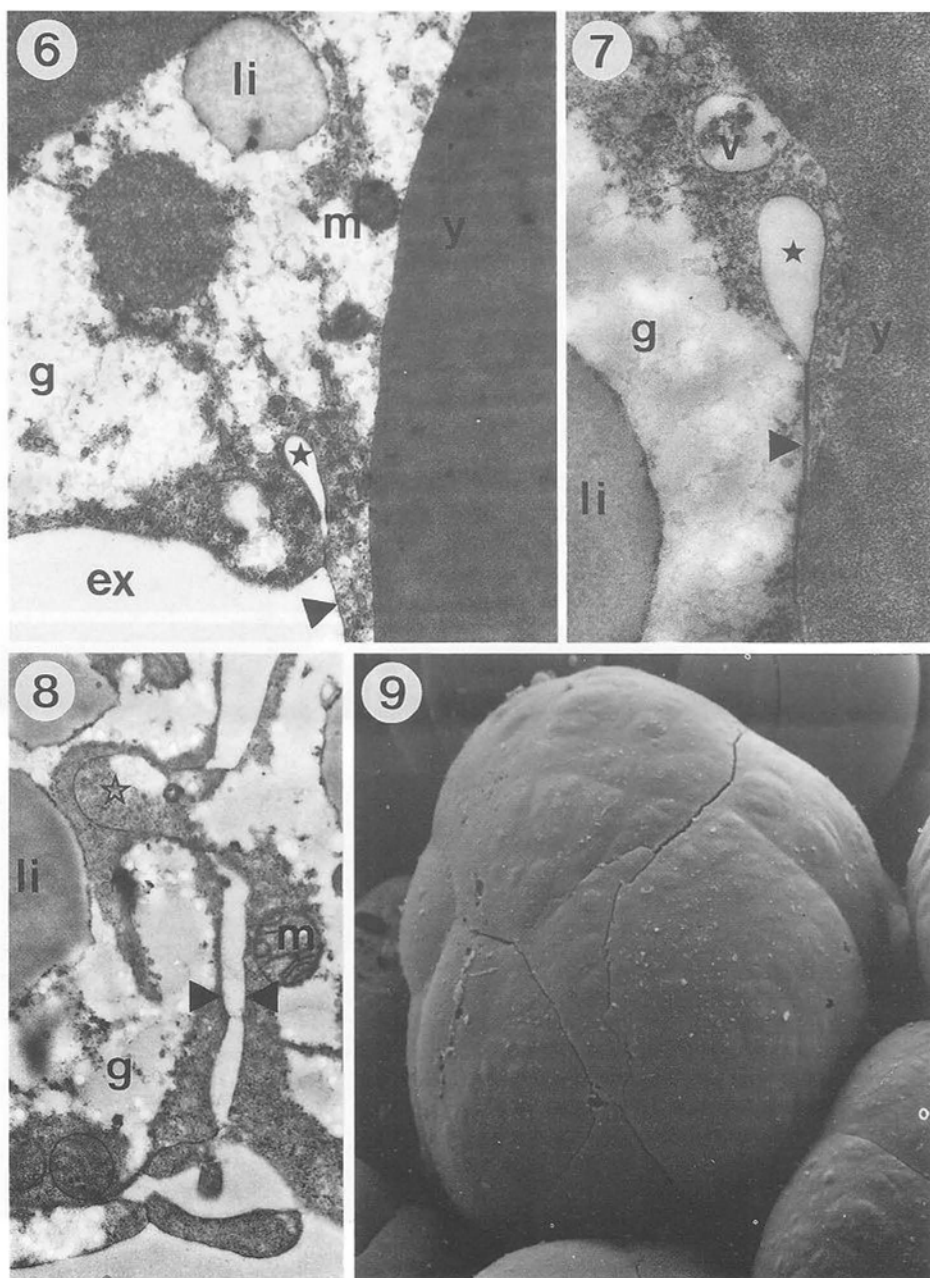


Fig 6–8: 16 h old *Ornithodoros moubata* Murray 1877 [Ixodoidea: Argasidae], embryos. — 6 Small invagination of the plasmalemma (arrowhead) with a terminal bud (\*) which probably represents the beginning of a furrow. The enlarged extracellular space (ex) represents an artifact due to dissection.

The cytoplasm of the early embryo blastomeres is similar to the cytoplasm of the ovocyte [Aeschlimann & Hecker 1969]. Many features such as euchromatic nuclei, which lack a nucleolus during cleavage, large amounts of glycogen, lipid droplets and ribosomes, are common to the early embryos of insects [Zissler & Sander 1982]. Concerning cell division, the *O. moubata* egg represents an exception. In fact, it is generally admitted that cytokinesis does not progress through dense cytoplasm, as observed in all telolecithal and centrolecithal eggs, where the furrows are arrested as soon as they reach the yolk [Arnold 1969]. In the tick, however, the cleavage furrows pass through the yolk mass in spite of its density which is much higher than in *Drosophila* eggs, for example.

The yolk-rich blastomeres develop into yolk-free micromeres and macromeres which contain all the yolk. No such a cell type with a yolk content comparable with the tick blastomeres has been described before. The yolk-free area of these cells, including the nucleus, most of the RER and the lysosomal system, is confined to a very small space compared to the total cell volume. Therefore, previous authors have considered the perinuclear cytoplasm as an autonomous entity, i.e. a small cell "swimming" in the yolk and digesting it [Aeschlimann 1958]. This was in good agreement with what was called the "vitellophages", supposed to occur in many other yolk-rich embryos. Recently, the interpretation of "vitellophages" has greatly evolved. In insects, the yolk is comprised in a syncytium, which is sometimes secondarily divided into "yolk cells" (the latter term is now generally preferred to the "vitellophages"), and the yolk cells contain several "yolk nuclei" and a certain amount of yolk platelets [Mori 1983]. A comparable syncytium is also found in fishes [Kimmel et al 1984]. Thus, the idea of small pseudopod-forming cells, the vitellophages, living between the yolk platelets progressively disappears. This study demonstrates that vitellophages do not exist in *O. moubata* either.

The macromeres of *O. moubata* form an autonomous embryonic sheath, representing a primary entoderm, of which the function is to store reserves including yolk, lipids, and glycogen, and to provide the embryo with nutrients and energy. Related activities such as yolk digestion and lysosomal activity have clearly been observed at the periphery of the entoderm. Preliminary histological and cytological observations show that cells of the primary entoderm probably build up the midgut epithelium, which is responsible of the final yolk digestion during larval life [Aeschlimann 1958, Diehl unpubl]. In conclusion, we state that the development of *O. moubata*, which was considered as the typical example of acarine embryology, does not present a superficial, but a total cleavage pattern. This pattern seems to be the rule among the Ixodoidea, as indicated by preliminary observations on eggs of *Amblyomma hebraeum*, *Amblyomma variegatum* and *Boophilus microplus* [Dotson: personal communication]. What about Araneida and Crustacea, which have eggs comparable to those of ticks? Comparisons might be useless as long as no TEM studies have been undertaken on the early embryos of these groups. High magnification analysis of fine sections is essential for the identification of the mode of development of small, yolk-rich eggs.

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**g** glycogen, **li** lipid droplet, **m** mitochondrion, **y** yolk (14 600 x). **7** Terminal bud (\*) of a deep furrow surrounded by vesicular cytoplasm. Near the terminal bud, the plasma membranes are in close apposition (arrowheads). **g** glycogen, **li** lipid droplet, **v** vesicule, **y** yolk (32 000 x). **8** Periplasmic area with the cell limits (arrowhead) showing a typical press-button-like structure (\*). **g** glycogen, **li** lipid droplet, **m** mitochondrion (14 500 x).

**Fig 9:** 48 h old *Ornithodoros moubata* Murray 1877 [Ixodoidea: Argasidae] embryo. SEM micrograph of a macromere filled with yolk platelets visibles through the plasmalemma (780 x).

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**Authors' address — Anschrift der Verfasser:** Dr François Fagotto; Dr Ernst Heß & Professor Dr André Aeschlimann, Institut de Zoologie, Université de Neuchâtel, Chantemerle 22, CH-2000 Neuchâtel; Suisse — Schweiz — Switzerland.

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Bunk, B., & Tausch, J.: **Moderne Biologie im Unterricht**. Bakteriologie mit einfachen Mitteln. [251 Seiten, 46 Abb., 120 x 175 mm]. — Verlag: Georg Westermann, Braunschweig; ISBN: 3-14-167121-4. — — — [EGR-Nr 687].

Das Buch enthält allgemeine Anweisungen zur Einrichtung eines mikrobiologischen Arbeitsplatzes, detaillierte Versuchsanweisungen und exemplarische Unterrichtsentwürfe. Die wichtigsten Inhalte der Mikrobiologie sind erfaßt und methodisch gründlich aufgearbeitet. Hinweise auf modernere Hilfsmittel und aktuelle Probleme fehlen. Manfred Noll (Dortmund)