

WHAT IS OUR CURRENT KNOWLEDGE OF ACARINE EMBRYOLOGY?

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INTRODUCTION

The title of this paper is put into an interrogative form in order, of course, to receive an answer! Consequently, this might be the shortest contribution to this Congress, because the answer to the question 'What do we know about the embryology of acarines today?', could be: 'Nothing really new during the past 25 years!'

This does not mean that no recent research has been carried out, and that no papers have been published. If anyone needs to be convinced, he has only to consult the literature summarized by van der Hammen (1972), Anderson (1973), Krantz (1978), and the original articles recently published by El Kammah *et al.* (1982) and Kamel *et al.* (1982). However, the embryonic development of mites still remains poorly studied. Ticks are better known because of their larger eggs. Furthermore, the white colour of the tick embryo, contrasting with the reddish dark brown colour of the yolk (a haemo-lipo-glyco-protein complex) allows favourable microscopical observation.

In fact, the external features of tick development (in some cases, mite development) are more or less satisfactorily described. However, our knowledge of their internal development is superficial, because of histological and other technical difficulties. The eggs of acarines, with some exceptions, possess a centrolecithal structure. They are not at all easy to fix or to cut into sections for either light or electron microscopy.

As an example of acarine embryology, we propose to summarize and illustrate with some original scanning electronmicrographs (SEM) the development of the argasid tick *Ornithodoros moubata* and to emphasize the main points of controversy.

This paper was also enhanced by the presentation of a colour film.

MATERIAL AND METHODS

The SEM illustrations were produced from embryos taken out of the *O. moubata* colony maintained in our institute (origin: Ifakara, Tanzania). Each egg was violently sucked into a pipette, whose roughened opening was slightly smaller than the maximum egg diameter, thus assuring the chorion of each egg was broken. This procedure was carried out in Medium 199 (with Hanks salts and 0.35 g/l NaHCO₃). The embryos were then rinsed in this solution and subsequently fixed in glutaraldehyde (Sabatini *et al.* 1963) containing 3% sucrose, post-fixed with osmium tetroxide (OsO₄) (Palade 1952), dehydrated in acetone, critical point dried in CO₂, mounted, and carbon and gold coated before they were observed in a Philips 500 SEM. For other technicalities see Aeschlimann (1958).

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RESULTS

General egg structure (Aeschlimann 1958)

The structure of almost all acarine eggs is of a centrolecithal type, with a central nucleus, surrounded by cytoplasm. A cytoplasmic reticulum connects this centropoplasm to the periplasm at the surface. The yolk spherules are included in the meshes of the cytoplasmic reticulum. A chorion and a vitelline membrane are present. All the Ixodid and Mesostigmatid eggs are similar in their basic structure. The anatomy of the eggs of other mites requires further study, but one can, nevertheless, state that there is great unity of structure among acarine eggs. The recorded differences seem to be of secondary importance. However, a yolk reduction has been observed in the Trombidiformes and Sarcoptiformes which does influence the method of cleavage.

Cleavage

The nuclear divisions are intralecithal. Segmentation is never complete and the yolk never presents a defined and symmetrical arrangement present in the yolk pyramids of some spiders. The nuclear divisions take place during the migration of the nuclei towards the periplasm and occur in synchronous waves. Each cleavage 'crisis' is followed by a short migration of the nuclei. Between the cleavages, a resting period is seen. During its migration each nucleus remains surrounded by a cytoplasmic area without a cell membrane. The nuclei are connected to each other by reticuloplasmic strands which also seem to move towards the surface with the migrating nuclei. One can observe filipods which probably play a role in the migrating process of the nuclei. Finally, the nuclei with, their cytoplasmic halos reach the periplasm where they form a uniform monocellular blastoderm layer on the surface, surrounding a mass of yolk spherules now free of cytoplasmic components. The embryo has reached the stage of a periblastula with a blastocoele obliterated by an important central yolk mass. The fate of the vitelline membrane is not known. It is thought that perhaps it takes part in the formation of the membrane of the blastoderm cells. Some of the cleavage nuclei remain between the yolk spherules, as vitellophages in the blastocoele. It was supposed (Aeschlimann 1958) that such vitellophages do not disintegrate during the embryonic development, but take part in the formation of the digestive epithelium during the organogenesis. The future of these cells needs further investigation.

Some other acarines, with less of a yolk mass show, in a preliminary phase, a total cleavage preceding blastoderm formation (Tetranychidae) but, as Anderson wrote (1973), 'The final result, a uniform low, cuboidal blastoderm around a unitary yolk mass, is common to all Acarines'.

Gastrulation and metamerization

The ventral side of the future larva is characterized by the appearance on its surface of a germ disc (the future telson). This disc is an aggregation of blastoderm cells which proliferate in the yolk as a funnel-shaped body. The single germ disc (cumulus primitivus) migrates backwards, thus establishing the definitive axis of bilateral symmetry. The funnel-shaped aggregation of cells, anchored in the yolk, represents the endo-mesodermal layer. The blastoderm cells surrounding the surface of the embryo represent the ectoderm. If the vitellophages really take part in the formation of the mid-gut, they must be considered as a primary endoderm, and the internal proliferating large cells of the germ disc as a secondary endoderm. The appearance of the germ disc may be considered as a gastrulation. Mesodermal cells evolve from the germ disc. The latter can be considered as the primitive centre of endo-mesodermal cell production and differentiation.

The germ band is built up anterior to the germ disc (telson) without exhibiting metamerization, until both ends, acron (cephalic lobe) and telson, reach the dorsal side of the embryo. The intensely proliferating cells of the germ band are smaller than the ectodermal cells from the extra-embryonic area (Fig. 1).

Metamerization starts with the appearance of the first three ambulatory metameres (Fig. 2). This is followed by the pedipalpal and the fourth ambulatory metameres, and, within a short time, the cheliceral metamere. Thus, the prosoma is composed of the acron, plus six metameres. The abdominal part of the germ band divides later into five opisthosomal segments with a terminal telson (Fig. 3). The telson is merely a primary germinal disc acting perhaps as a growth zone for opisthosomal segments.

In *O. moubata* the separation of the germ band into two bilateral parts is well marked by a neural groove (sulcus ventralis) as soon as each successive pair of ventral ganglions develops into each metamere, thus shaping the classical ventral nervous cord of any arthropod. This organization of the embryo is also observed in other tick species as well as in mites. In the latter, one may notice the secondary loss of external opisthosomal segmentation. The six pairs of appendage buds (Fig. 4) appear in the same succession as the metameres. It is difficult to check if small and fugacious buds appear or not on the first opisthosomal segments (Fig. 3).

Blastokinesis (contraction of the germ band)

The next step in the development concerns the longitudinal contraction of the germ band. This is an important morphogenetic phenomenon, termed 'blastokinesis', which allows the future larva to take its definitive form according to the already fixed polarity and symmetrical axes. This contraction eliminates the ventral sulcus. The germ band becomes shorter and is finally concentrated on the ventral surface of the yolk mass (Fig. 5). The contraction occurs under strong pulsations of the whole embryo. A further important result of the blastokinesis in ticks is the total disappearance of the external primitive metamerization.

Another general feature common to all the studied species is the regression of the fourth pair of legs. These never develop further than a bud (in some Prostigmata the fourth pair fail to develop externally). In *O. moubata*, as in other ticks (*Hyalomma*, *Amblyomma*), the buds of the fourth pair of legs retract within the body during late embryogenesis. The fully developed fourth legs will reappear during the moult of the hexapod larva into an octopod nymph (Vogel 1975).

At the end of blastokinesis, chelicerae, pedipalps, and the first three pairs of legs grow towards the centre of the ventral surface while their coxae take a more lateral position (Fig. 6). Prosoma and opisthosoma form now a morphological unit, the idiosoma, while the anterior metameres together with the acron form the gnathosoma (Figs 5 and 6).

Differentiation of the gnathosoma

The formation of the gnathosoma is particularly interesting. Again it has been more carefully studied in ticks than in other acarines. On the acron, a medial unpaired labral bud arises anterior to the stomodeal invagination. Chelicerae and pedipalps are initially situated posterior to the stomodaeum (Fig. 7). Two post-stomodeal lips are present which neither Aeschlimann (1958) nor Anderson (1973) had previously observed (Fig. 7). The chelicerae migrate from the post-oral position forward to a pre-oral position. Finally, after a 90° rotation, they are situated in front of the stomodaeum. The pedipalps migrate to a position lateral of the stomodaeum (Figs 7 and 8).

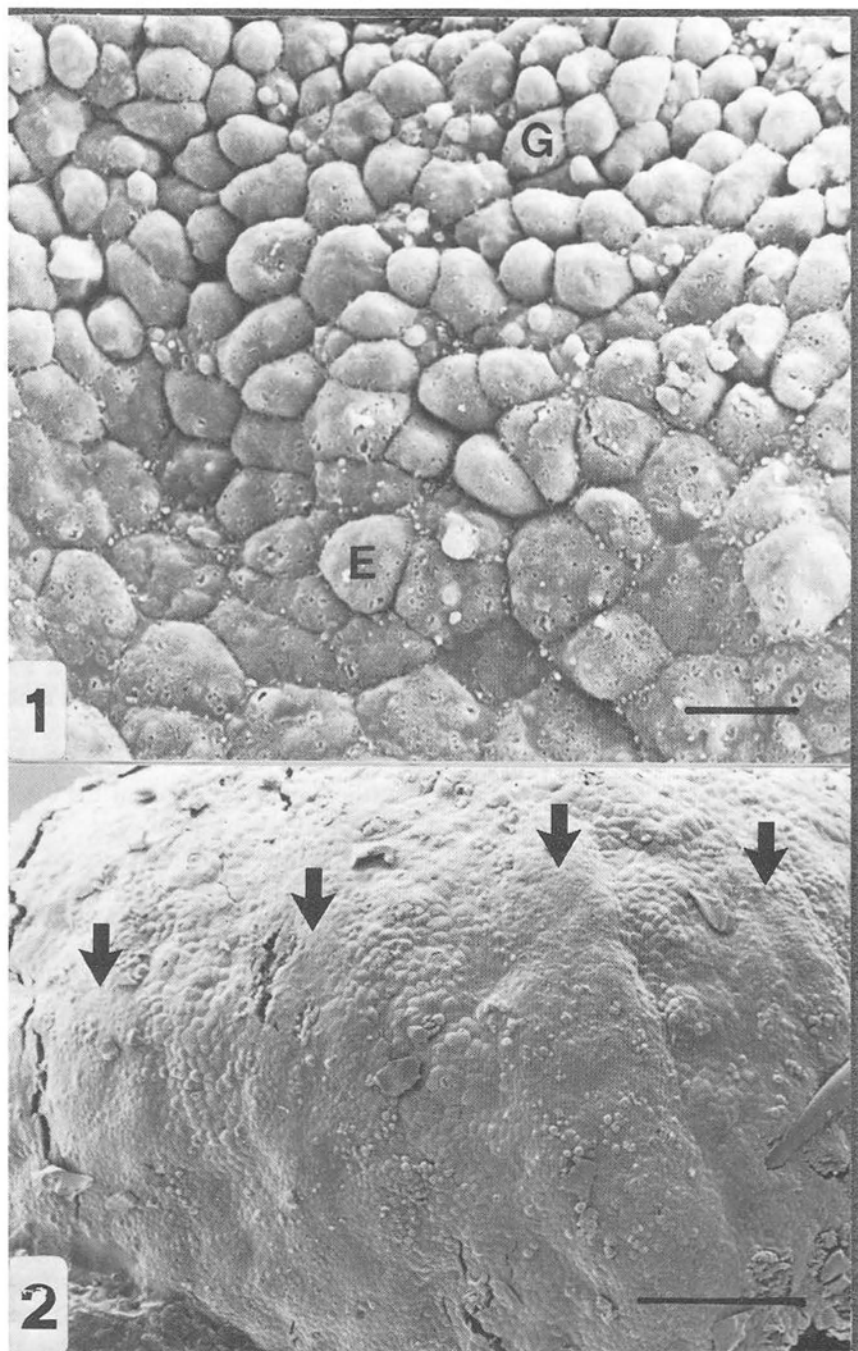


Fig. 1 – Germ band showing small cells (G) and extra-embryonic area with large blastoderm cells (E). Notice different osmotic behaviour (artefacts) of the two cell types. Bar = 10 μm .

Fig. 2 – Germ band during early metamerization. The metameres (arrows) are separated by bands of larger ectodermal cells. Bar = 100 μm .

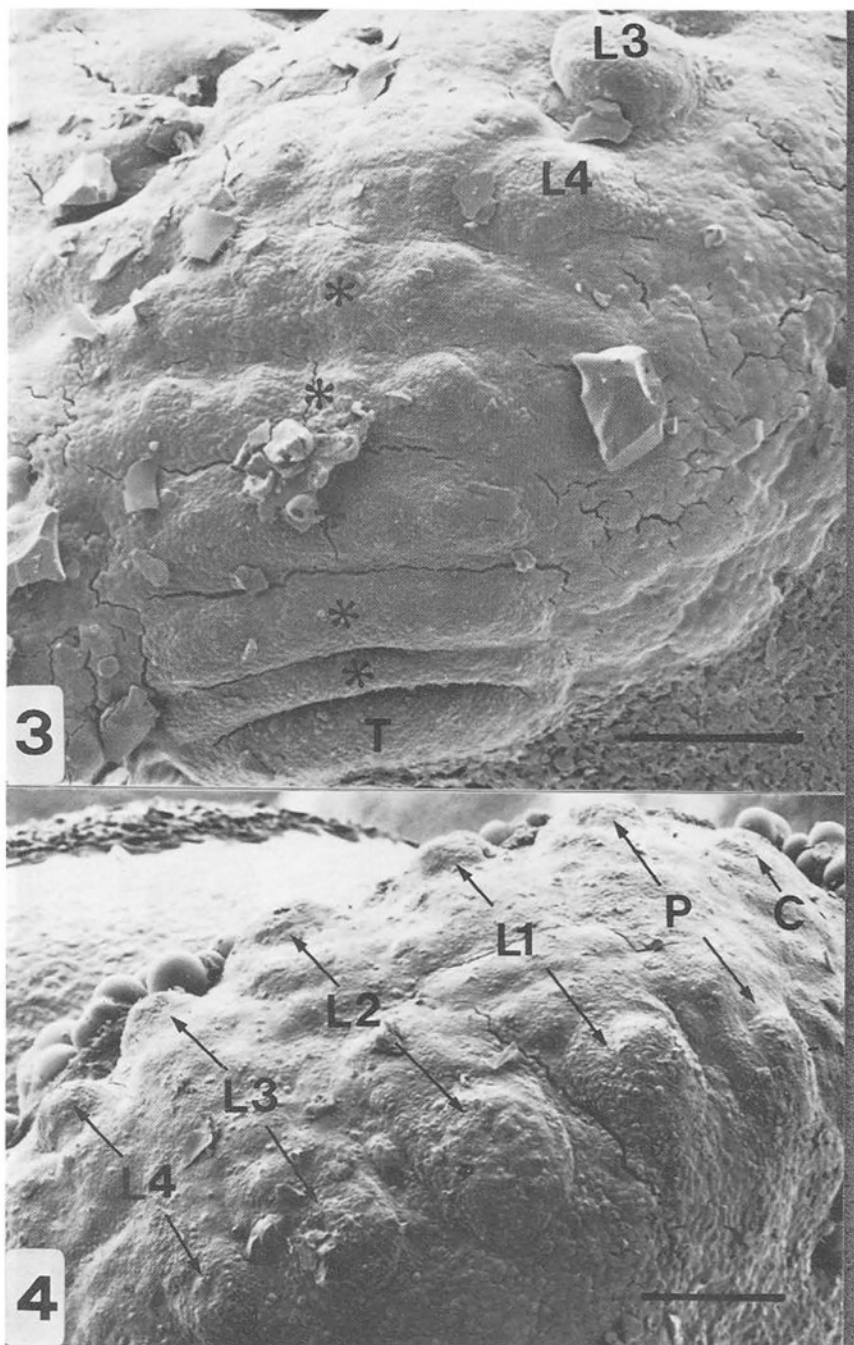


Fig. 3 – Germ band during late metamerization. Buds of the 3rd and 4th legs (L3, L4) appear on the ambulatory metamerites. The opisthosoma is subdivided into five metamerites (asterisks) and the telson (T). Bar = 100 μ m.

Fig. 4 – Germ band during late metamerization. Buds of legs (L1–L4) of the pedipalps (P), and of the chelicerae (C) appear on the corresponding metamerites. Bar = 100 μ m.

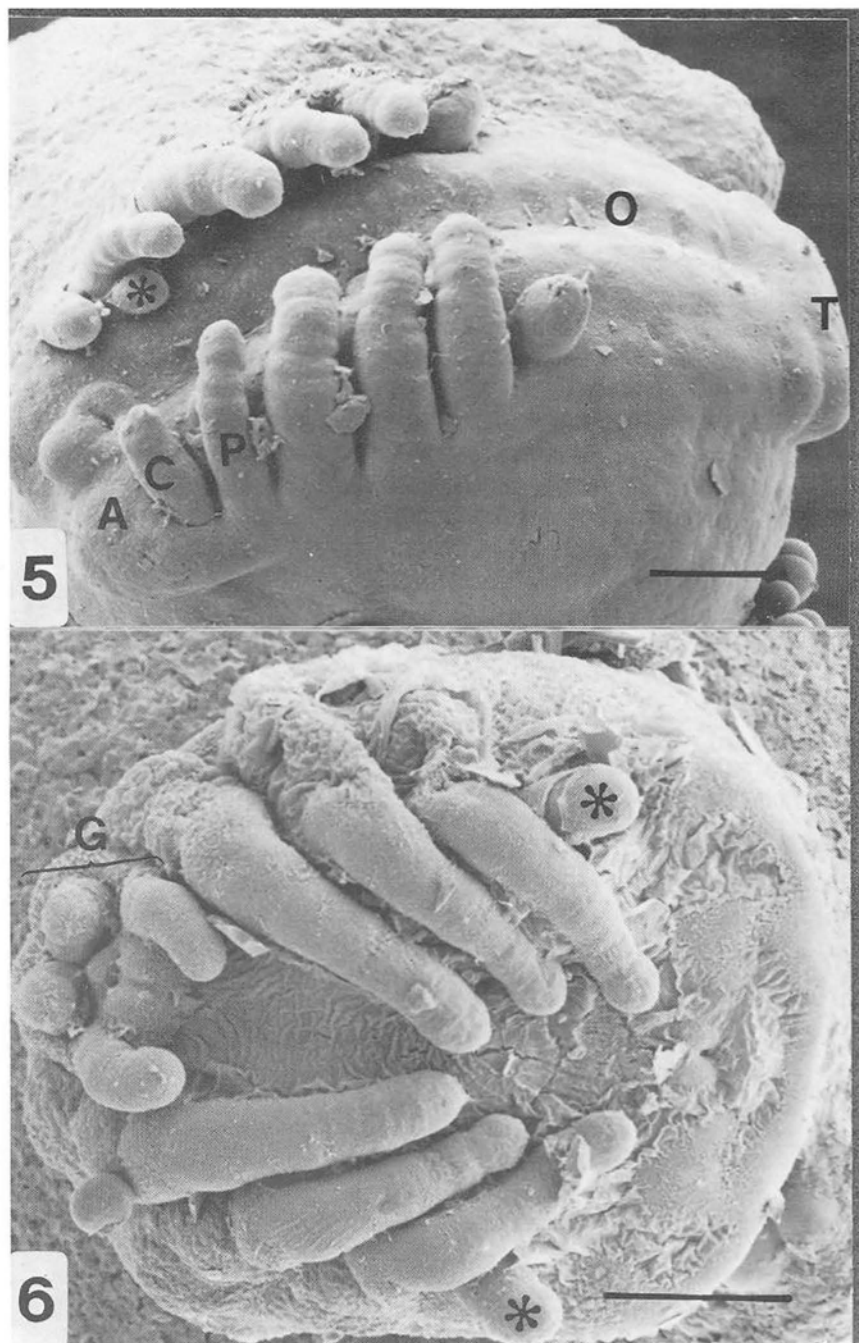


Fig. 5 - Embryo during blastokinesis. Acron (A), cheliceral (C), and pedipalpal (P) metameres begin to form a gnathosomal unit. O: opisthosoma; T: Telson; Asterisk: pedipalpal coxal lobe. Bar = 100 μ m.

Fig. 6 - Embryo after blastokinesis, exhibiting roughly larval morphology. The legs have a lateral position (asterisk: bud of 4th pair), and the gnathosoma differentiates (G). Notice the envelope surrounding the embryo. Bar = 100 μ m.

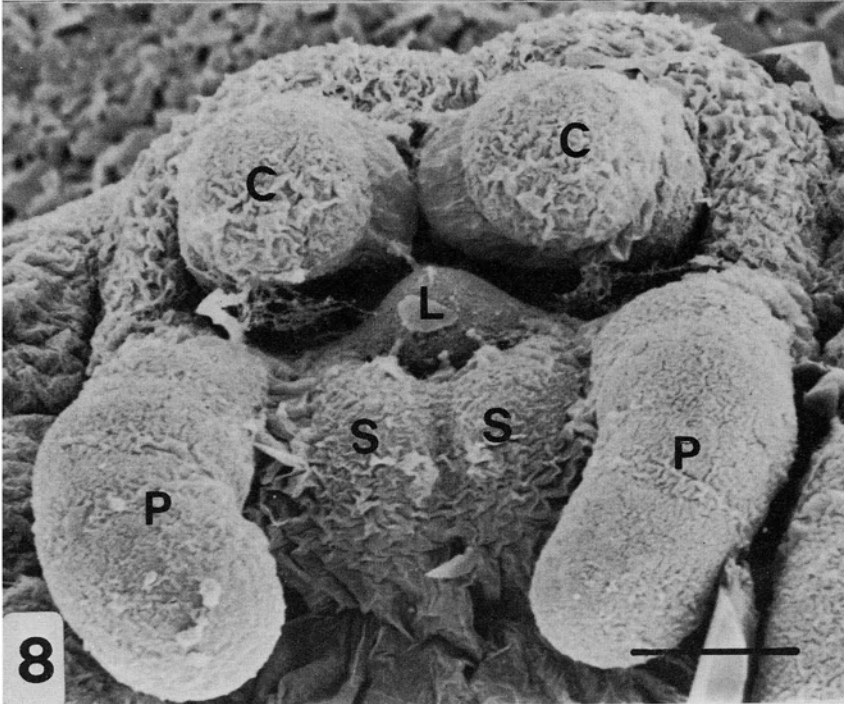
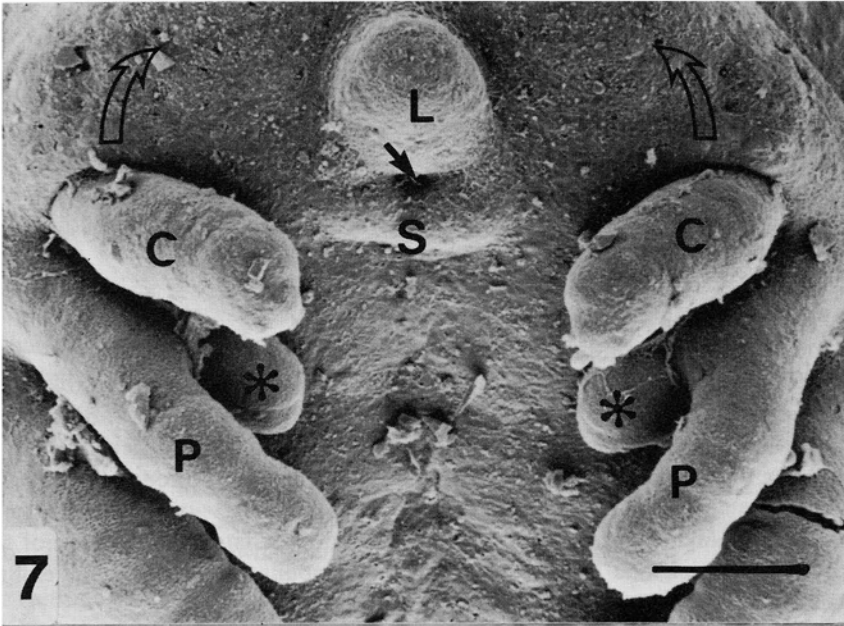


Fig. 7 - Appearance of the labral bud (L), the stomodeal invagination (single arrow), and the post-stomodeal lips (S). Notice the posterior position of the chelicerae (C) and pedipalps (P) with regard to the stomodeum. Asterisks: coxal lobes of pedipalps; pair of arrows: direction of migration of the chelicerae (C) and the pedipalps (P). Bar = 50 μ m.

Fig. 8 - Gnathosoma after the migration of the chelicerae (C) and pedipalps (P). Have the coxal lobes of the pedipalps fused with the post-stomodeal lips (S)? L: labrum. Bar = 25 μ m.

Another interesting event concerns the medially directed coxal expansions. These appear after the contraction of the germ band on each pedipalp (Figs 5 and 7). Many authors have supposed that these coxal lobes of the pedipalps form the hypostome (Aeschlimann 1958, Anderson 1973). However, after studying the situation using the SEM, we consider that the true fate of these pedipalpal expansions remains unresolved and that the ventral lips probably also take part in the morphogenesis of the hypostome. We conclude that the formation of the gnathosoma needs further research to be fully understood.

Additional remarks

Two facts are important concerning the internal organogenesis of the embryo:

- (a) The straightened cord of the ventral ganglia contracts, as a result of blastokinesis, into a synganglion situated finally on the antero-ventral section of the germ band. During this 'cephalization', all the symmetric ganglia are arranged into an homogeneous nervous mass surrounding the oesophagus, thus, the oesophagus finally passes through the 'brain'.
- (b) The rectal sac develops from the invagination of the proctodaeum into the telson (Fig. 9), and the paired Malpighian tubules originate as a double lateral evagination of the rectal sac. Both the Malpighian tubules and the rectal ampulla are active in the embryo since crystals of brilliant white guanine excrements become apparent.

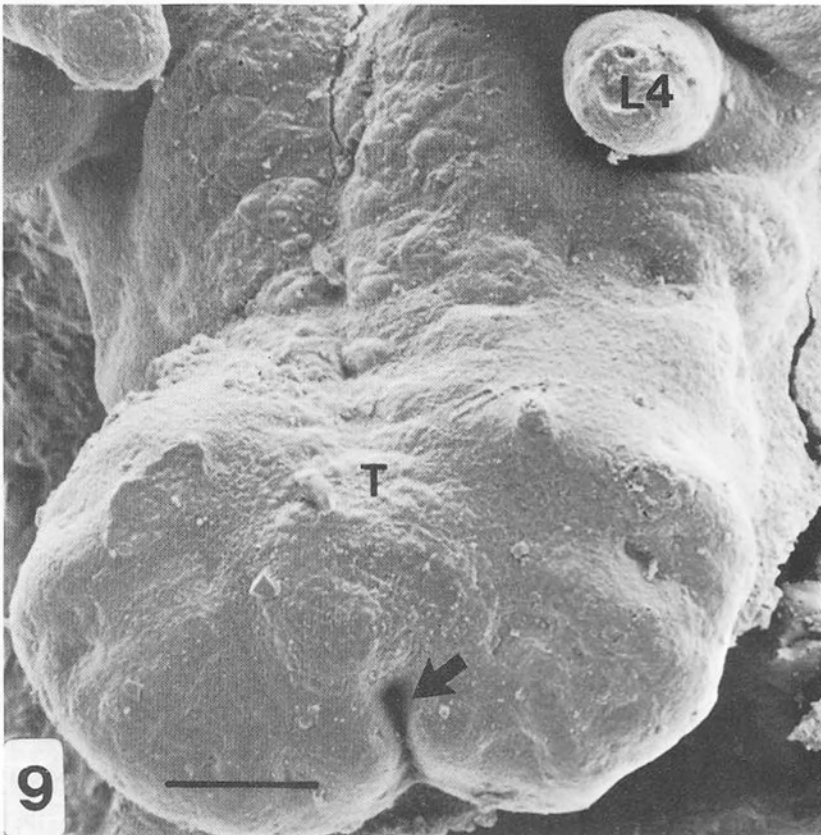


Fig. 9 – Proctodeal invagination (arrow) situated on the telson (T). L4: Bud of 4th leg. Bar = 50 μ m.

CONCLUSIONS

It is not easy to compare the embryonic development of ticks with the development of mites, because of the lack of research on the latter. However, we can be sure, without being very audacious, that what has been observed in some tick species may be representative for more or less all acarines. Their embryology presents a remarkable uniformity in its basic patterns. Three points may be highlighted:

- there is no shift of yolk observed during segmentation
- the germ disc represents in fact the telson: its appearance determining the dorsoventral and cephalocaudal symmetry of the embryo
- the bilateral formation of the germ band and its blastokinesis is of a simple mode: no inversion of the germinative band, as it appears in spiders, is observed.

The external development of acarines shows common features with the Opilionids, while the gastrulation seems to be similar to that of the spiders. It is too early to express conclusions about the organogenesis in acarines because of the lack of precise knowledge.

In our opinion, the following subjects need further investigation:

- (1) The role and evolution of vitellophage cells.
- (2) Establishment of a comparative diagram of the metamerization of the germinative band (number of metameres, homology, existence of a precheliceral metamere, evolution of each metamere). Such research has to be done to compare the various families of acarines, and to compare the various orders in the class Chelicerata.
- (3) The mechanisms of the blastokinesis are not known. How can we explain the independence of this phenomenon with regard to normal development of the germ band?
- (4) Interpretation of the SEM illustrations of the gnathosoma in this paper suggests that published explanations, by Aeschlimann and others, are far from satisfactory.
- (5) Information on organogenesis is scarce.
- (6) Another interesting and quite 'up-to-date' problem concerns the embryonic envelopes. What do we find under the eggshell? Does the vitelline membrane persist? Do amnios and serosa exist as in insects?
- (7) What about intrachorionic moults? New observations made in our laboratory seem to confirm that the tick larva moults under its eggshell at least once, if not twice, before hatching. Are intrachorionic moult(s) related to the fact that ecdysteroids are present in the freshly laid eggs of *O. moubata* and *A. variegatum*? (Diehl, pers. comm.). These hormones persist during embryogenesis. What is their function? Do they control the secretion of embryonic cuticle and the intrachorionic ecdysis?

All these points are new fields of research in acarine development.

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