FINE STRUCTURE OF THE EPICYTOPLASMIC EIMERID COCCIDIUM *ACROEIMERIA PINTOI* LAINSON & PAPERNA, 1999, A GUT PARASITE OF THE LIZARD *AMEIVA AMEIVA* IN NORTH BRAZIL

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Summary :

An account is given of the fine structure of *Acroeimeria pintoi*, an epicytoplasmic coccidium infecting the small intestine of the teiid lizard *Ameiva ameiva* in north Brazil. The merozoite becomes encircled by outgrowths of the host-cell wall which then merge to form a parasitophorous sack in which the parasite continues to develop when this bulges out above the epithelium surface. The account includes a description of merozoites, young meronts and young and mature macrogamonts. The parasitophorous vacuole has a complex tubular system connected to its junction with the host-cell. The parasites are coated with a droplet-like glycocalyx and covered by a fine filamentous layer.

KEY WORDS : fine structure, epicytoplasmic development, small intestine, *Acroeimeria pintoi*, Coccidia, *Ameiva ameiva*, Brazil.

Résumé : ÉTUDE ULTRASTRUCTURALE DE *ACROEIMERIA PINTOI* LAINSON & PAPERNA, 1999, COCCIDIE EIMERIIDE ÉPICYTOPLASMIQUE, PARASITE INTESTINAL DU LÉZARD *AMEIVA AMEIVA* AU NORD DU BRÉSIL

L'étude ultrastructurale montre que les mérozoïtes sont encerclés par des expansions des parois de la cellule-hôte qui font saillie pour former un sac parasitophore dans lequel le parasite effectue sa croissance. Étude des mérozoïtes, des jeunes mérontes et des macrogamétocytes jeunes et mûrs. La vacuole parasitophore présente un système complexe de tubules relié à sa jonction avec la cellule-hôte. Les parasites sont revêtus d'un glycocalix en forme de gouttelette et couverts par une fine membrane filamenteuse.

MOTS CLÉS : *ultrastructure, développement épicytoplasmique, petit intestin,* Acroeimeria pintoi, *Coccidie,* Ameiva ameiva, *Brésil.*

Sporocysts of most named *Eimeria* species from reptilian hosts differ from those of the species found in birds and mammals in their lack of Stieda and substieda bodies (Cannon, 1967, Vetterling & Widmer, 1968). This includes the "epicytoplasmic" species which, after becoming enclosed by extensions of the host cell wall, continue to develop within the resulting parasitophorous "sack" which bulges out above the surface of the intestinal mucosa. Paperna & Landsberg (1989*a*) proposed *Acroeimeria* as a new generic name to contain the latter species. Although a similar development is common among piscine coccidia (Paperna, 1995) it is, so far, unknown among coccidia infecting birds and mammals. Recently (Lainson & Paperna, 1999) we described the oocysts and endogenous stages of a new species of the genus *A. pintoi* in the small intestine of the teiid lizard *Ameiva ameiva* from north Brazil. In this communication we present further evidence of unique fine structural characters of this parasite to justify separation of the genera *Acroeimeria* and *Eimeria* s.l. The only other ultrastructural account of an epicytoplasmic coccidium developing in a reptilian host is by Paperna (1989) of *Acroeimeria lineri* (MacAllister, Upton & Freed, 1988) Paperna & Landsberg, 1989.

MATERIALS AND METHODS

The specimens of *Ameiva ameiva* (Linn) were from Capanema, Pará. They were the same lizards used in our previous description of the oocysts and endogenous stages of *A. pintoi* by light microscopy. Intestinal infection was verified by demonstration of oocysts in the faeces or in the contents of the rectum of the sacrificed lizards. The intestine of the positive lizards were cut lengthwise: one half was used to locate abundant endogenous stages by the microscopic examination of the gut pressed between a slide and a cover slip, and when these were found, corresponding segments of the other half of the intestine were fixed for transmission electron microscopic (TEM).

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The material was placed in 2.5 % glutaraldehyde in cacodylate buffer (0.1 M, pH 7.4) for 24 hrs at 4 °C, rinsed repeatedly in the same buffer, post-fixed in 1.0 % osmium tetroxide in the same buffer for one hour and, after rinsing in the buffer, dehydrated in graded ethyl alcohols and embedded in Agar 100 medium (Agar Scientific, Ltd., UK). Thin sections, cut on a Reichert Ultracut microtome with a diamond knife, were stained on grids with uranyl acetate and lead citrate, and examined with a Jeol 100CX TEM.

RESULTS

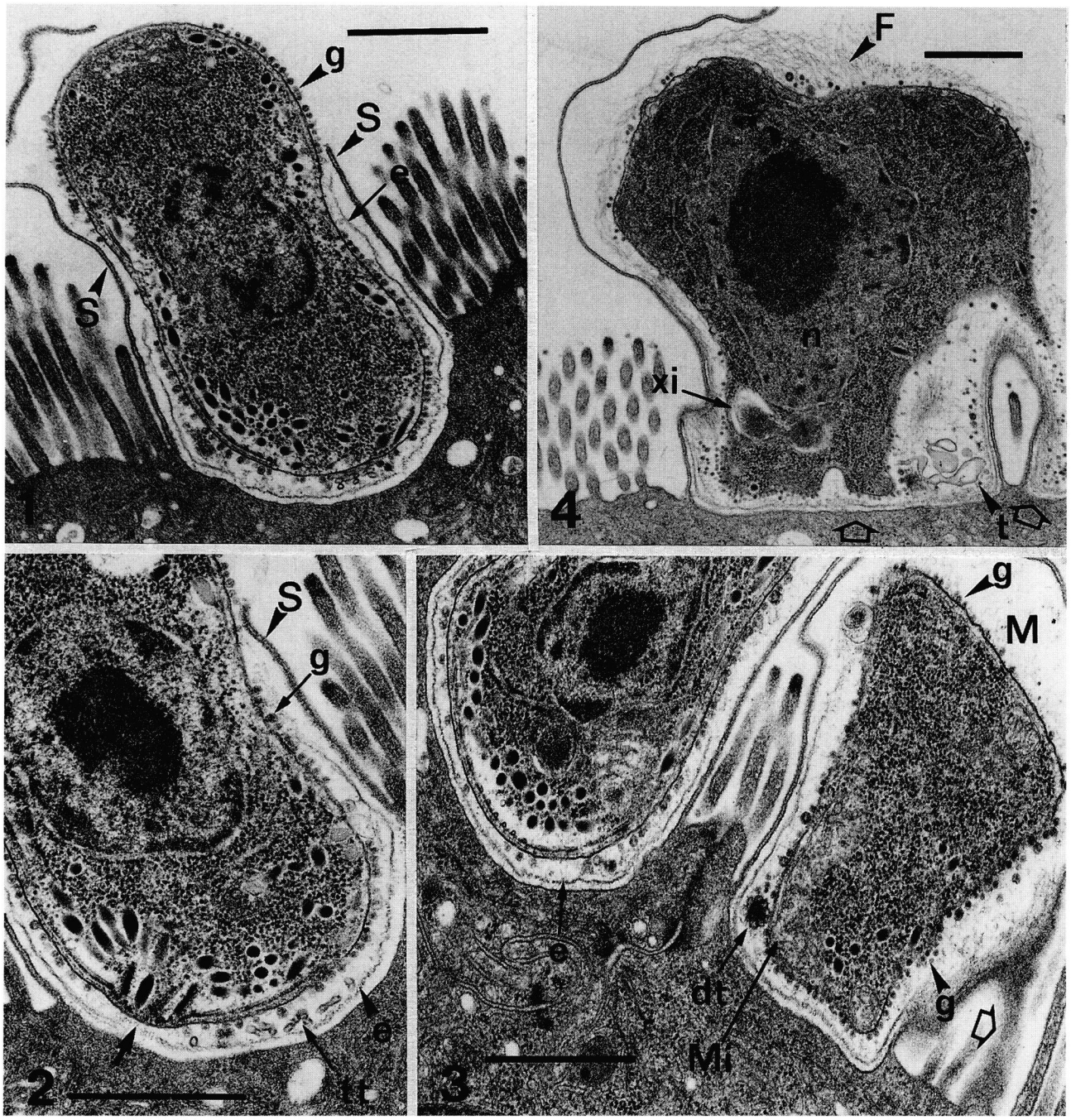
The merozoite enters a gap formed in the brush-border of its host-cell and becomes enclosed by extensions of the host cell wall (Figs. 1, 2). The resulting "parasitophorous sack" bulges out above the epithelial surface. In Fig. 1 the break at the distal part of this sack is clearly caused by processing damage. A second, interior membrane is formed beneath the wall of the parasitophorous vacuole (PV) and is either confined to the proximal part of the PV, or ruptured distally: the membrane is retained throughout the growth and differentiation of the parasite. The space between the parasite and this envelope contains some walled structures of round or oblong shape, and the surface of the parasite is densely covered by a glycocalyx of small electron-dense droplets. The young newly established parasites show all the features of typical merozoites i.e. apical complex, sub-pellicular tubules and micronemes (Figs. 1-3).

With further development the merozoites either transform into meronts (Fig. 3) or young gamonts (Fig. 4). The young meront is bound by a single membrane, densely covered by a glycocalyx that is seemingly composed of fine droplets, and its cytoplasm contains large mitochondria and a few micronemes (Fig. 3). A section of an apparently intravacuolar tubular organelle may be seen in the PV lumen. A whole range of developing macrogamonts was identified up to late zygote stage (Figs. 4-11), but microgamonts were not found in the presently studied TEM material.

Young macrogamonts are recognized by their large, vesicular nucleus which contains a conspicuous central nucleolus: their cytoplasm does not exhibit any peculiar features other than a bilobed medium-density granular inclusion of unknown designation. The parasites are bound by a single membrane that is enveloped by a thick filamentous substance: the PV lumen contains a bunch of intravacuolar tubules. The expanding PV-host cytoplasm junction zone of on-growing meronts and macrogamonts becomes folded (invaginated). In cross section it assumes a convoluted appearance (Figs. 3, 4).

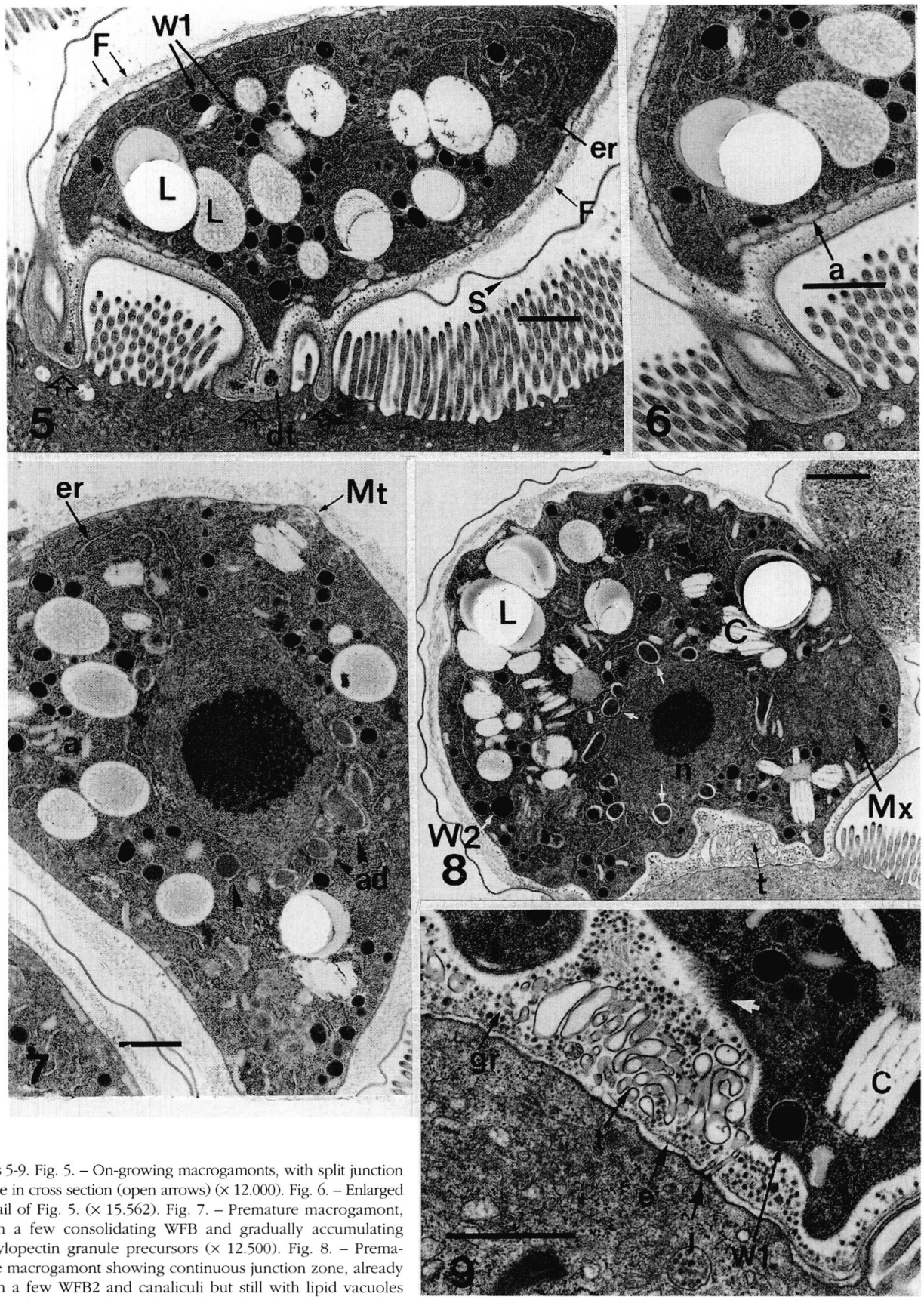
Within their PV, macrogamonts are covered by a discrete and usually continuous layer of dense, finely fibrillar substance, and the outlines of the inner PV membrane are sometimes lost. Possibly, this occurs when processing the material for TEM (Figs. 5-9). The space between the fibrillar layer and the parasite wall is filled with electron-dense granules, and at times there can be seen sections of tubular organelles ((Figs. 5, 6). The extremely invaginated PV junction zone of the on-growing and mature macrogamonts is seen in some cross sections to be split into narrow stilt-like connections (Figs. 5, 6); in others it is seen to retains its broad base (Fig. 8). At the junction zone with the host-cell cytoplasm there are intravacuolar tubules, occurring either in compound clumps or an assemby of winding tubes. At one point they appear to be connected via narrow tubules to a hemi-desmosomal-like junction on the PV wall (Fig. 9).

The macrogamonts are bound by a single unit membrane, coated by a variable amount of glycocalyx which in oblique sections assumes the texture of parallel filaments (Fig. 9). The macrogamont cytoplasm is densely packed with ribosomes and also shows a dense endoplasmic reticulum (ER, Figs. 5-7) and numerous mitochondria (Figs. 7, 10), which may form a sizable aggregate (Fig. 8). Mitochondrial contents tend to increase in density in the late stage macrogamonts (Fig. 11), and the nucleus of the parasite is fringed by numerous adnuclear bodies (Figs. 7, 8). The abundant cytoplasmic lipid vacuoles (Fig. 5) are partly retained up to a late stages of maturation, when the cytoplasm then becomes filled with amylopectin granules (Figs. 7-11). In premature macrogamonts these first appear as elongated, flat bodies which are concentrated mainly beneath the cell wall (Figs. 5, 6). The canaliculi increase in size and number as the macrogamonts mature (Figs. 8, 10). Wall forming bodies (WFB) of the premature macrogamonts all have the same appearance: namely, electron-dense spheres of similar dimensions surrounded by a distinct halo, which is clearly not a cisterna (Figs. 5 -7). With maturation, two sizes of WFB are apparent. First to appear are the smaller Type 1 bodies (WFB1), (Figs. 5, 7), which are usually bound in a conspicuous shell (Fig. 9). The larger Type 2 wall forming bodies (WFB2) appear only in fully mature macrogamonts or zygotes: they are also uniformly electron-dense, but are lodged within a distinct cisterna (Figs. 10-11), and are often accompanied by an aggregate of small globules, of variable size, embedded in a fine granular matrix (Fig. 10). These seemingly disintegrating WFB2 were more common in the less developed (Fig. 10) than the more mature macrogamonts (Fig. 11). Young oocysts with the oocyst wall in formation were not found in the material examined.

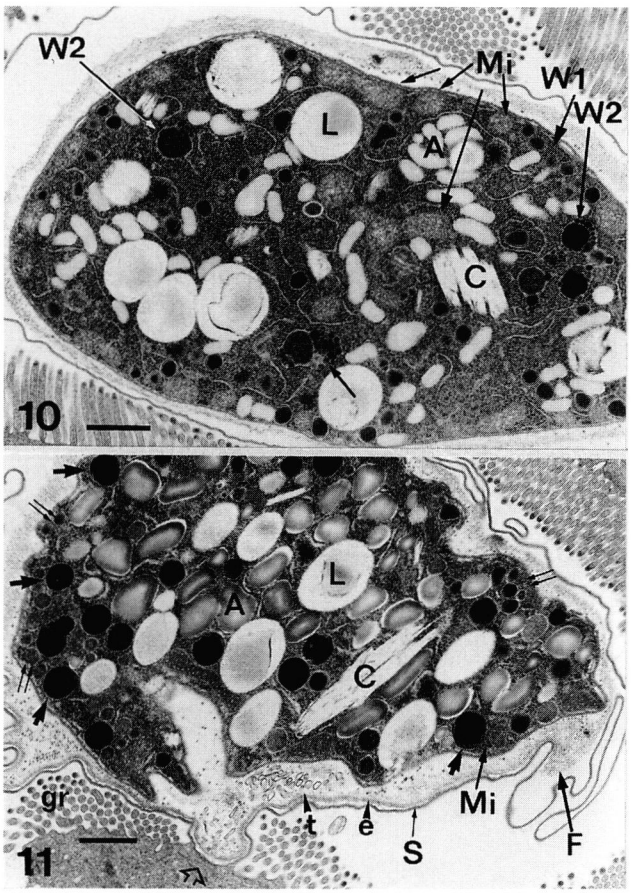


Figs 1-4. Fig. 1. – A merozoite attached to the brush-border zone of the mid-gut epithelium (× 21.400). Fig. 2. – Enlargement of an establishing merozoite showing an apical complex (bold arrow) (× 28.000). Fig. 3. – An established merozoite (left) and a young meront (M). Open arrow points at the faint outlines of the opposite end of the invaginated edges of the PV-host-cell junction (× 24.000). Fig. 4. – Young macrogamont in cross section showing a split junction zone (open arrow) (× 15.000).

Abbreviations: a. anlagen of amylopectin granules; ad. adnuclear bodies; C. canaliculi; dt. intravacuolar tubular organelle; e. interior PV membrane; er. endoplasmic reticulum; F. fine filament coat; g. glycocalyx droplet coat; gr. intravacuolar granules; j. hemidesmosal junction with the intravacuolar tubular complex; M. meront; L. lipid vacuoles; Mi. mitochondrion; Mx. mitochondrial assemlage; n. nucleus; S. parasitophorous "sack"; t. intravacuolar tubular complex. xi. bilobed inclusion; w. consolidating WFB; W1. Type 1 wall-forming bodies; W2. Type 2 wall forming bodies. Scale bars: 1 µm.



Figs 5-9. Fig. 5. – On-growing macrogamonts, with split junction zone in cross section (open arrows) (× 12.000). Fig. 6. – Enlarged detail of Fig. 5. (× 15.562). Fig. 7. – Premature macrogamont, with a few consolidating WFB and gradually accumulating amylopectin granule precursors (× 12.500). Fig. 8. – Premature macrogamont showing continuous junction zone, already with a few WFB2 and canaliculi but still with lipid vacuoles outnumbering amylopectin granules. Adnuclear bodies marked by white arrows, (× 11.000). Fig. 9. – Enlarged sector of Fig. 8, at the PV-host cell cytoplasm junction; note the striated texture of the obliquely cut glycocalyx coat (bold white arrow) (× 23.000).



Figs 10-11. Fig. 10. – A presumed zygote, with WFB2s, some disintegrating (arrow), and lipid granules coexisting with amylopectin granules (× 12.500). Fig. 11. – A zygote loaded with amylopectin granules: WFB1 marked by a double fine arrows, WFB2 by bold arrows. Mitochondria are filled with dense matrix (× 11.500).

DISCUSSION

A pintoi, from the neotropical teiid lizard (*Ameiva ameiva)*, and *A. lineri*, from Levantine and South African species of geckoes (*Hemidactylus turcicus* and *H. mabouia*) clearly represent distinctly different species on conventional taxonomic criteria. They are, however, very similar at TEM level, and their fine structure (present communication & Paperna, 1989) is quite distinct from that of the reptilian intracytoplasmic intestinal eimerian *Eimeria boveroi* (Paperna & Lainson, 1999*a*) or the gall-bladder inhabiting species of the genus *Choleoeimeria* (Paperna & Landsberg, 1989*b*, Paperna & Lainson, unpublished observations). It is our opinion that these differences provide a sound argument supporting validity of the genera *Acroeimeria* and *Choleoeimeria*, established by Paperna & Landsberg (1989*a*).

In the paper on the ultrastructure of *A. lineri* (Paperna, 1989) it was suggested that eimeriid epicytoplasmic development, common to piscine and reptilian hosts and lacking in the more evolved vertebrates, might be considered a more primitive form of host-parasite association. However, wall formation processes and the organelles involved in *Acroeimeria* are the same as in the other reptilian coccidia, and uniformly different from the piscine coccidia (Paperna, 1989, 1995).

It has been suggested (Levine, 1984) that the epicytoplasmic coccidians are related to *Cryptosporidium*. There are, however, many structural and developmental features including the absence of flagella in the microgametes and the desmosomal type of junction with the host cytoplasm (Ostrovska & Paperna, 1990) which separate *Cryptosporidium* from all eimeriid coccidia.

Some of the ultrastructural features seen in *Acroeimeria* appear to be very characteristic and are seemingly an expression of a specialized adaptation for epicytoplasmic ralationship with the host cell. Such features include the fine fibrillar coat covering the parasite and the tubular complexes present in the PV lumen and connected to the PV/host-cytoplasm junction zone. The granules filling the PV lumen seem to be eroded from the glycocalyx coat. Also noteworthy is the disintegration of the WFB 2 in zygotes which do not yet show signs of oocyst wall formation. This is very common among *Isospora* species of reptilian hosts (Paperna & Finkelman, 1998, Paperna & Lainson, 1999*b*), but exceptional in the eimeriines of reptiles (Paperna & Lainson, 1999*a*).

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