THE TICK-TRANSMITTED HAEMOGREGARINID OF THE AUSTRALIAN SLEEPY LIZARD *TILIQUA RUGOSA* BELONGS TO THE GENUS *HEMOLIVIA*

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Summary :

Nymphae of *Amblyomma limbatum* Neumann, engorged on the Australian sleepy lizard *Tiliqua rugosa* Gray infected with haemogregarine blood-stage gametocytes, were studied by light and electron microscope 3-57 days after detachment. Examined ticks demonstrated haemogregarine oocysts and sporogonic stages. Oocysts found in the intestinal epithelium, were three to five arm star-shaped, and divided into numerous sporokinetes. The sporokinetes spread to various tissues and, after transforming into hard-walled sporocysts, divided into sporozoites. This course of development in the vector is characteristic of the genus *Hemolivia* Petit, Landau, Baccam & Lainson, 1990 reported earlier from toads and ticks. The reported haemogregarinid is therefore described as *Hemolivia mariae* n. sp.

KEY WORDS : *Hemolivia mariae* n. sp., haemogregarine, *Amblyomma limbatum*, *Tiliqua rugosa*, Australia, development, ultrastructure.

Résumé : L'HÉMOGRÉGARINE TRANSMISE PAR LA TIQUE DU LÉZARD AUSTRALIEN *TILIQUA RUGOSA* APPARTIENT AU GENRE *HEMOLIVIA*

*Des nymphes d'*Amblyomma limbatum *Neumann, gorgées sur le lézard australien* Tiliqua rugosa *Gray infecté avec des gamétocytes d'hémogrégarine au stade sanguin, sont étudiés en microscopie optique et électronique 3 à 57 jours après leur détachement. On retrouve chez les tiques examinées des oocystes d'hémogrégarine et des stades sporogoniques. Les oocystes trouvés dans l'épithélium intestinal ont la forme d'étoiles à trois à cinq branches, et se divisent en de nombreux sporokinètes. Les sporokinètes gagnent différents tissus et, après leur transformation en sporocystes à paroi épaisse, se divisent en sporozoïtes. Cette séquence de développement au sein du vecteur est caractéristique du genre* Hemolivia *Petit, Landau, Baccam & Lainson, 1990, précédemment rapporté chez les crapauds et les tiques. L'hémogrégarine rapportée est donc décrite sous le nom de* Hemolivia mariae *n. sp.*

MOTS CLÉS : Hemolivia mariae *n. sp., hémogrégarine,* Amblyomma limbatum, Tiliqua rugosa, *Australie, développement, ultrastructure.*

Sleepy lizards *Tiliqua rugosa* Gray (Scincidae) from Mt Mary, South Australia were found infected with haemogregarinids occurring in the circulating erythrocytes. The population is commonly infested with the tick *Amblyomma limbatum* Neumann. Nymphae of *A. limbatum* were allowed to engorge on haemogregarine infected lizards and engorged ticks were dissected 3-57 days after spontaneous detachment. The course of development in engorged nymphae and newly moulted adult ticks was established from examination of Giemsa stained smears from internal organs by light microscope and from a study by transmission electron microscopy (TEM). Tick viscera were fixed in 2.5 % glutaraldehyde in cacodylate buffer (0.1M, pH 7.4) for 24 h at 4.0 °C, rinsed repeatedly in the same buffer, post-fixed in 1.0 % osmium tetroxide in the same buffer for 1 h and, after rinsing in the same buffer, dehydrated in graded alcohols and embedded in Agar 100® resin (Agar Co. UK). Thin sections, cut on a Reichert « Ultracut » with a diamond knife, were stained on-grid with uranyl acetate and lead citrate and examined in a Jeol 100CX TEM.

Haemogregarine oocyst development and sporogony in the tick takes place in the gut epithelial cells. Young oblong oocysts 7-8 × 3 µm in size, lodged in a parasitophorous vacuole (PV) filled with dense material, contained nuclei of homogenous granular nucleoplasm with a large conspicuous nucleolus, few electron-dense rhoptries and a few micronemes (Fig. 1). Older oocysts, reaching 18 × 7 µm in size were lodged in an expanded PV with an undulated and folded wall (Fig. 2). Cytoplasmic contents in such oocysts became loaded with ribosomes and aggregates of a granular substance, intersected by endoplasmic reticulum, and also with lipid vacuoles and amylopectin granules. At this stage, oocysts still retained a nucleus with large nucleolus and a fair number of micronemes (Fig. 2). Smears from tick gut, examined by light microscope (LM) demonstrated three-arm stellate oocysts (Fig. 3) typical to the toad *(Bufo marinus)* haemogregarine *H. stellata* which develops in the tick *Amblyomma rotondatum* (Petit *et al.*, 1990). In addition to the com-

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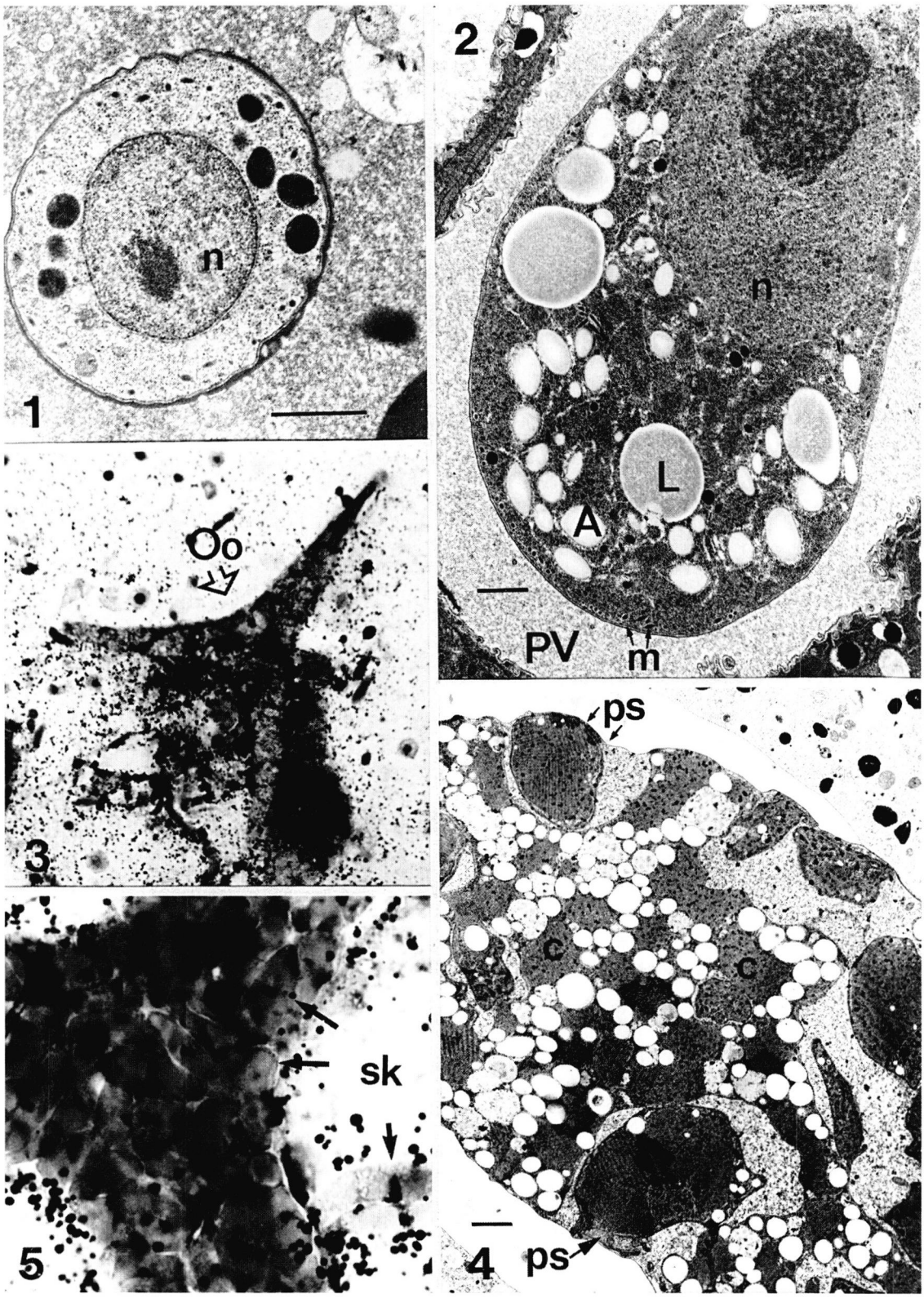

Fig. 1. Ultra-thin crosss ection through early stage oocyst, scale 1 µm, TEM × 15,100. Fig. 2. Young oocysts within an expanded PV, scale 1 µm, TEM × 9,500. Fig. 3. Stellate oocyst (Oo) Giemsa stained smear, × 235. Fig. 4. Differentiating stellate oocysts filled with crystalline bodies, around some of which sporokinete primordia (ps) start consolidating, scale 2 µm, TEM × 2,700. Fig. 5. Stellate oocyst, ripe filled with sporokinetes (sk), Giemsa stained smear, × 1,533.

Abbreviations to figures 1-10 : A, amylopectin granule; a, apical complex; c, crystalline body; hT, Tick's gut epithelial cell; L, lipid vacuole; m, micronemes; mt, mitochondrion; n, nucleus; Oo, oocyst; PV, parasitophorous vacuole; r, rhoptry; rl, rhoptry-like organelle; S, sporokinete; z, sporozoite.

monly occurring three-arm oocysts there were some with four or five arms, the length of each arm measuring from 142 to 201 µm. The granular aggregates seen in the younger oocysts could have been the analgen for the crystalline bodies which fill the cytoplasm of the older (stellate) oocysts; around these bodies were consolidating the division progeny of these oocysts (Fig. 4). The end-product of the oocyst endogenous division (Fig. 5) were oblong motile stages, 15-16 × 5-6 µm in size, which resembled the sporokinetes which are formed by the *H. stellata* oocysts. Newly formed sporokinetes had an apical complex accompanied by rhoptry-like organelles consisting of fine granular substance with a superficial layer of electron dense material. A crystalline substance filled almost the entire sporokinete body, on both sides of the homogenous nucleus. Older sporokinetes found scattered throughout the tick intestine as well as in extra-intestinal organs were of variable sizes (from 15-18 × 5-11 µm to 19-24 × 8-14 µm, apparently prior encapsulation) and altogether differed from the early ones in the texture and contents of their cytoplasm; they also contained many minute micronemes, and had their crystalline mass subdividing. All sporokinetes were intracellular, lodged within either an expanded PV with wavy wall or a narrow space bound by a single lamella (Fig. 7). Differentiation of sporokinetes into sporocysts lead to the thickening of their walls which resulted in their increased resistance to fixation and infiltration (Figs. 8 and 9). Division in these hard-walled, 29-33 × 14-18 µm sporocysts yielded numerous (8-20) sporozoites (Fig. 10).

The life cycle in the tick has been found to be very similar to the development cycle of the toad parasite *H. stellata* described from the tick *A. rotundatum* (Petit *et al.*, 1990), with the formation inside the gut epithelial cells of stellate oocysts. These oocysts likewise formed, through endogenous division, mobile sporokinetes which spread into other gut cells before transforming into sporocysts containing sporozoites. In the presently described species, sporokinetes were found spreading throughout the tick tissue also into extra-intestinal locations. This cycle differs from many haemogregarines of the genus *Hepatozoon* developing in mosquitoes' haemocoel (Mackerras, 1962; Ball & Oda, 1971; Bashtar *et al.*, 1984; Wozniak & Telford, 1991). In *Hepatozoon* the progeny of sporocysts, dividing into sporozoites remained often located within the parent oocyst wall (Bashtar *et al.*, 1984; Lowichik *et al.*, 1993). *Hemolivia* sporokinetes, unlike the sporocysts formed in *Hepatozoon* retain a distinct apical complex, fine structural features of mobile stages (such as the ookinete of haemosporidians, Paterson & Desser, 1989). Development of oocysts in the vector's gut cells have been also reported in *Hepatozoon mauritanicus* of the land tortoise transmitted via the tick *Hyalomma aegyptium* (Brumpt, 1938; Michel, 1973) and a skink's *Hepatozoon lygosomarum* transmitted via mites (Allison & Desser, 1981), stellate oocysts in these species, however, were not found. Their taxonomic status is presently revised (see Landau & Paperna, same issue). Sporogonic development of *Hepatozoon* octosporei from skinks, in mites was shown, however, to occur in the haemocoel (Ramanadan Shanavas & Ramachandran, 1990). Sporogonic development in the haemocoel has been also reported in mammalia-host *Hepatozoon: H. canis* in the tick *Haemophysalis longicornis* (Murata *et al.*, 1995), and *H. balfouri* in the mite *Haemolaelaps aegyptius* (Hoogstraal, 1961). Worth consideration also is the similarity between *Hemolivia* and *Karyolysus*. Oogenesis and sporulation of the latter haemogregarine takes place in the lumen or the epithelium of the gut of dermanyssid mites, and similarly leads to the formation of sporokinetes. These, unlike those of *Hemolivia*, penetrate mite's eggs and are transovarially transmitted to the vector's offspring (Reichenow, 1921; Svahn, 1975).

Taxonomic status

Hemolivia mariae n. sp.

Hosts: *Tiliqua rugosa* Gray and *Amblyomma limbatum* Neumann

Geographic locality: Mount Mary, South Australia.

Etymology: Named by its type locality.

Type material: Deposited in the South Australian Museum.

Paratypes: Deposited in Muséum National d'Histoire Naturelle, Paris.

Description

In Giemsa stained blood smears, mature, encapsulated gametocytes reach 18 × 5 µm. Oocysts developing in the ticks gut epithelium are stellate, with three to five arms, each 142-201 (mean 177) µm in size. The latter divide into mobile sporokinetes (15-23.8 × 5.6-14 µm in size) which spread throughout the tick tissues where they become established as hard-walled oval sporocysts (29-34 × 14-18 µm in size), divided into 8-20 sporozoites (12-14 × 1.4-2.1 µm in size).

Differential diagnosis

Conspecificity with *H. stellata* is unlikely on accounts of differences in host taxa (skink *vs.* toad), and geographic location. *B. marinus* (cane toads) the natural hosts of *H. stellata* have been introduced into Australia, but, thus far none have been found infected with either *H. stellata* nor ticks (Speare, 1990), and an attempt to experimentally infect cane toads failed

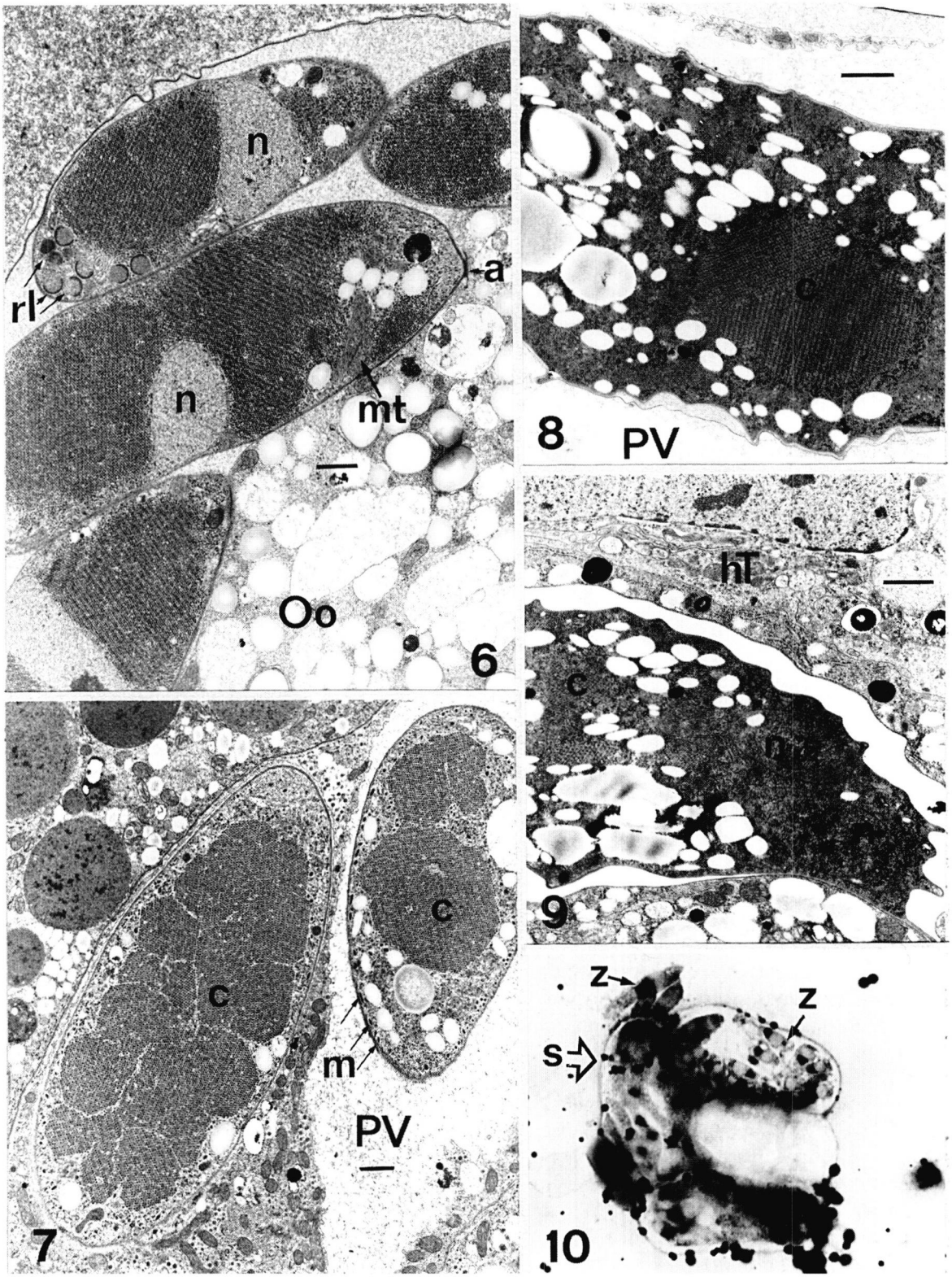

Fig. 6. Newly formed sporokinetes inside the oocyst, scale 1 µm, TEM × 6,200. Fig. 7. Variable size sporokinetes inside gut epithelial cell, filled with fragmented crystalline body (c), scale µm, TEM × 5,775. Figs. 8 and 9. Hard-walled sporocyst before division to sporozoites, scales 1 µm, TEM × 8,700 and × 8,800. Fig. 10. Sporocysts (S) containing sporozoites (z), Giemsa stained smear × 1,000.

Abbreviations to figures 1-10 : A, amylopectin granule; a, apical complex; c, crystalline body; hT, Tick's gut epithelial cell; L, lipid vacuole; m, micronemes; mt, mitochondrion; n, nucleus; Oo, oocyst; PV, parasitophorous vacuole; r, rhoptry; rl, rhoptry-like organelle; S, sporokinete; z, sporozoite.

(Smallridge, unpublished). Infection with both organisms in native toads in Brazil is highly prevalent (Lainson & Paperna, unpublished).

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