

SHELLACKIA CALOTESI N. SP. FROM AGAMID LIZARDS OF THE GENUS *CALOTES* IN THAILAND

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Summary :

Schellackia calotesi n. sp. is described from the Thai agamids *Calotes mystaceus* and *C. versicolor*. *Schellackia*-type sporozoites were recovered from blood and liver of one *C. versicolor* from Kon Kaen North-East Thailand and two *C. mystaceus* from Chiang Mai, North Thailand. Specimens of both species were fed on sporozoite infected blood, of these only one *C. mystaceus* developed endogenous infection in the anterior intestine. Description, from histological material includes early and dividing meronts, micro and macrogamonts and non sporulated oocysts.

KEY-WORDS : *Schellackia calotesi*, meronts, microgamonts, macrogamonts, oocysts sporozoites, gut, liver, blood, *Calotes mystaceus*, *Calotes versicolor*, Thailand.

Résumé : *SHELLACKIA CALOTESI* n. sp. CHEZ LE LÉZARD DU GENRE *CALOTES* EN THAÏLANDE

Schellackia calotesi n. sp. est décrit chez les lézards thaïlandais *Calotes mystaceus* et *C. versicolor*. Des sporozoïtes de type *Schellackia* ont été retrouvés dans le sang et le foie d'un *C. versicolor* de la région de Kon Kaen, au nord-est de la Thaïlande, et de deux *C. mystaceus* de Chiang Mai, en Thaïlande du nord. Des spécimens de chacune de ces deux espèces nourris de sang infesté de sporozoïtes, seul un *C. mystaceus* a développé une infection au niveau de l'intestin antérieur. La description à partir de coupes histologiques concerne des mérontes jeunes et en cours de division, des micro- et macrogamontes et des oocystes non sporulés.

MOTS CLÉS : *Schellackia calotesi*, mérontes, microgamontes, macrogamontes, oocystes, sporozoïtes, intestin, foie, sang, *Calotes mystaceus*, *Calotes versicolor*, Thaïlande.

INTRODUCTION

The genus *Schellackia* comprises nine heterogeneous species of eimerian coccidia, parasites of either saurian reptiles or anurans transmitted by hematophagous Acarina or insects (Klein *et al.*, 1988; Bristovetzi & Paperna, 1990). The few known species have nonetheless been reported so far from all continents except Australia. The presently described new species is from a Thai agamid lizard.

MATERIALS AND METHODS

Three *Calotes versicolor* and one *C. mystaceus* were captured in Thailand near Kon Kaen (Northeastern region) and 12 *C. versicolor* and 34 *C. mystaceus* from Chiang Mai (Northern region) (12 and 34, respectively). Films were prepared from blood obtained by clipping the tip of the lizard's tail. Smears were prepared from the gut and liver. Prepared films and smears were air-dried fixed in absolute methanol

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and stained in Giemsa (1/10 diluted in phosphate buffer pH 7.4) for one hour. Two apparently uninfected specimens of each species were fed on blood and liver of a sacrificed infected *C. versicolor* from Kon Kaen and sacrificed after 12 days. The gut and the liver were fixed in neutral buffered Formalin for histology. Fixed tissue, after dehydration in graded ethanols, was embedded in glycol-methacrylate medium (GMA of Agar Comp., UK). Sections, 3-4 µm were cut with a glass knife in a Sorval JB4 microtome and stained with Meyer's haemalum-eosin (MH-E). Descriptions of all developmental stages were made from histological sections and refer to cross sections. All measurements in micrometers (µm).

RESULTS

Of all examined lizards sporozoites were recovered only in one *C. versicolor* from Kon Kaen, and in two *C. mystaceus* from Chiang Mai. In the first, sacrificed for histology, sporozoites occurred in the liver as well as in the blood, but no stages were found in the intestine. Of the two *C. mystaceus* and two *C. versicolor* fed on infected blood and liver, only one specimen of the first species developed an endogenous infection.

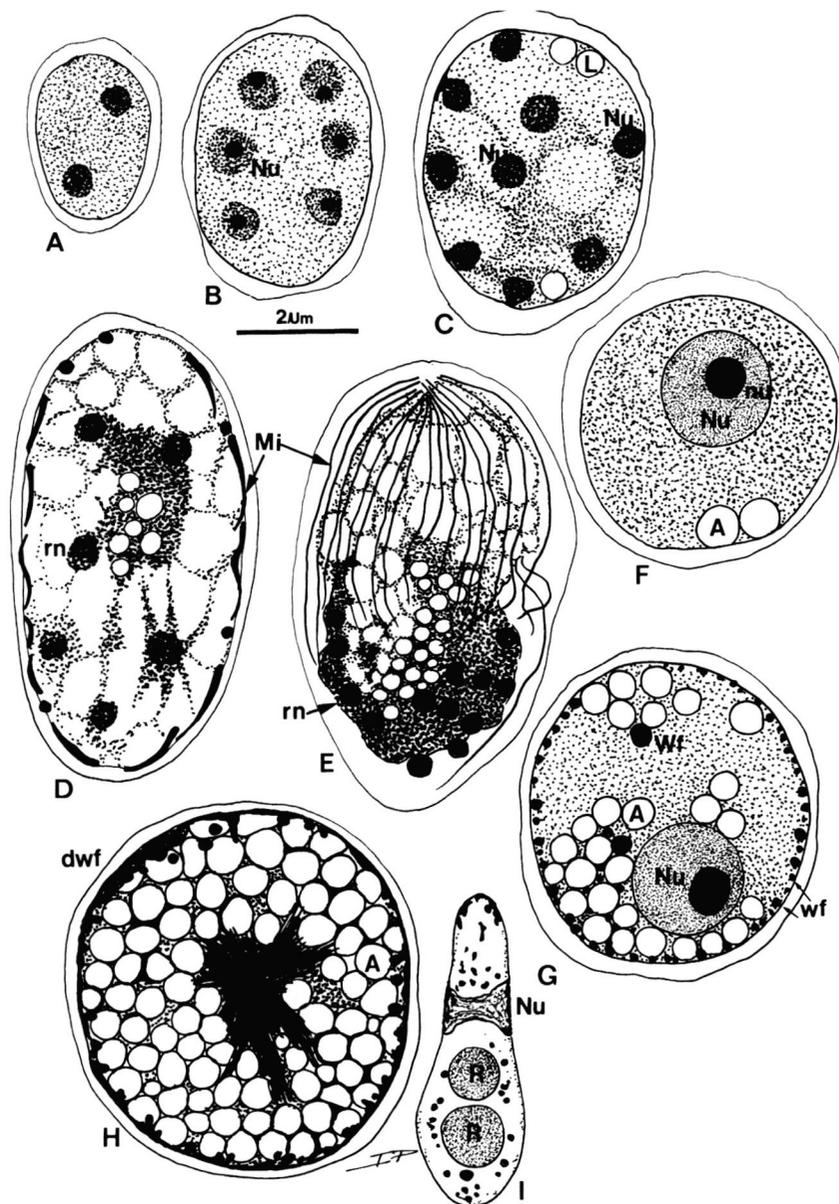


Fig. 1. – A, B: Young meronts. C: Young microgamont. D: Premature microgamont. E: Mature microgamont. F: Young macrogamont. G: Mature macrogamont. H: Zygote/young oocyst. I: Free sporozoite.

Abbreviations: A: amylopectin granules; dwf: disaggregating wall-forming bodies; L: lipid vacuole; Mi: microgametes; Nu: nuclei; nu: nucleolus; R: refractile body; rn: residual nuclei; wf: type 1 wall-forming bodies; Wf: type 2 wall-forming bodies.

SHELLACKIA CALOTESI N. SP.

Type host: *Calotes mystaceus*.

Locality: Chiang Mai, Thailand.

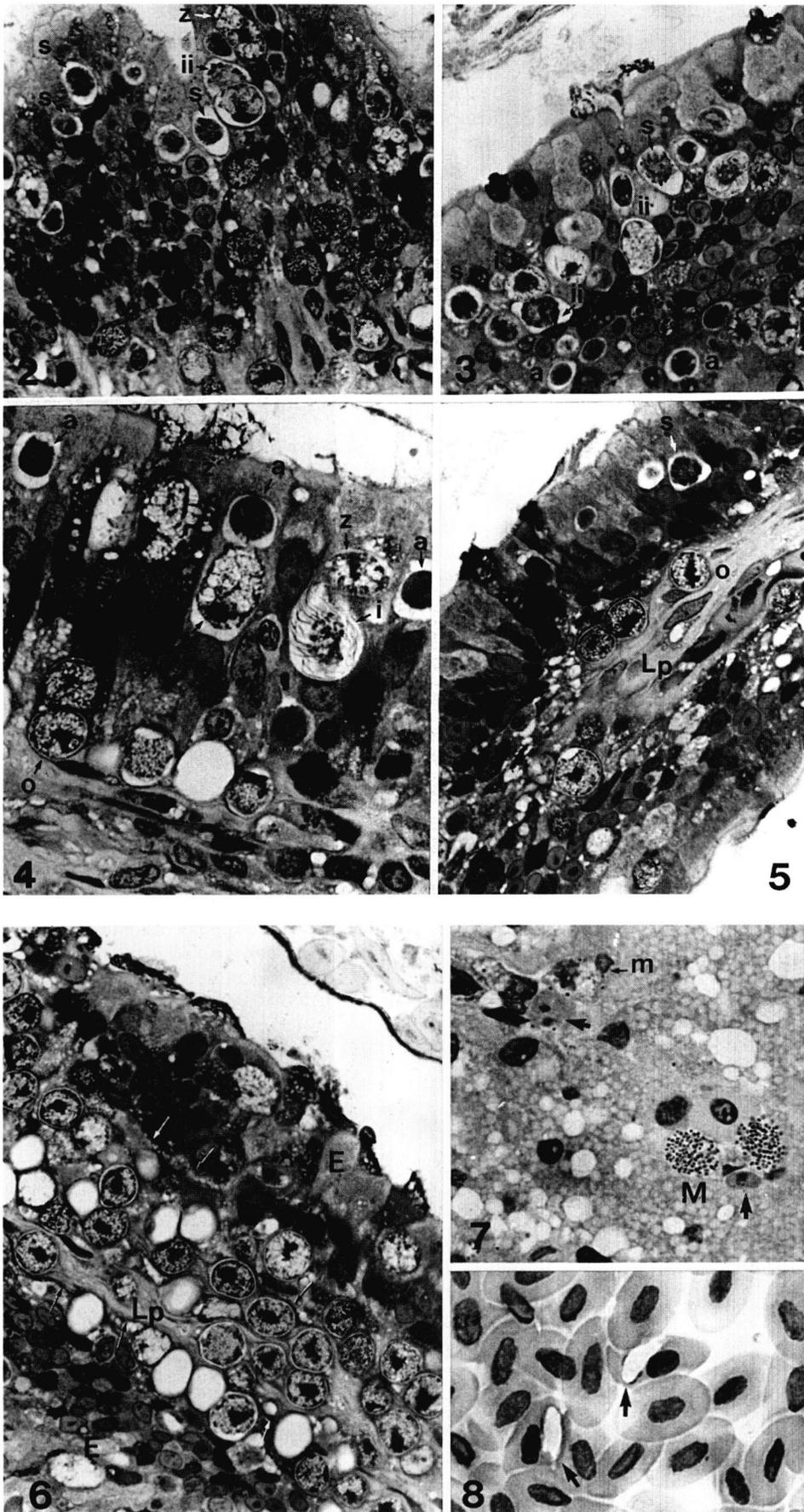
Other hosts and occurrence: Sporozoites were traced in the blood of two *C. mystaceus* from Chiang Mai and one *C. versicolor* from Kon Kaen, Thailand.

DESCRIPTION

Endogenous stages (Fig. 1) occurred in the mucosal epithelial layer of the anterior gut (Figs. 2-6). All stages except mature oocysts were lodged in an expanded parasitophorous vacuole (PV). Although most infected cells hosted one parasite, a few however contained two (Figs. 2, 4) or even three macrogamonts or both macrogamonts and microgamonts. Meronts had a dense

cytoplasm with very few, or no inclusions or vacuoles (Fig. 1A). Nuclei contained a very distinct nucleolus. Meronts ranged from 5.6×2.8 (with two nuclei, Fig. 1A) to 8.4×8.4 (with six nuclei, Fig. 1B), their PVs sizes ranged from 7×4.2 to 11.2×7.7 . Premature meronts were seen with up to eight nuclei per section and dividing meronts showed up to ten emerging merozoites (Figs. 2, 3, 5).

Young microgamonts with eight to nine nuclei were $7-9 \times 6.2-8.4$ in size, and were located in a $9.8-12.6 \times 8.4-11.2$ PV (Figs. 1C). The cytoplasm was lucent to foamy and the nuclei had a homogeneous consistency (Figs. 2, 3). Premature and mature microgamonts, $11.2-14.0 \times 6.2-8.0$ in size (Figs. 1D, E), were lodged in a $16.8-18.2 \times 9.8-14.0$ PV. Toward the completion of differentiation the microgamont cytoplasm became increasingly vacuolated.



Figs. 2 to 6. – View of histological cross sections in the anterior portion of the intestine ; double infections in host cells shown in Fig. 2 ii & z, and Fig. 4 a & z and o & o ; arrows denote the limits of the basal membrane (Fig. 6) ; GMA-embedded, glass knife/ JB4-sectioned, MH-E stained (Figs. 2, 3, 5, 6×650 ; 4×830).

Figs. 7-8. – Fig. 7. Sporozoites in the liver, adjacent to macrophages (m) and melanomacrophages (M), smear, Giemsa stained (× 500). Fig. 8. Sporozoites in the blood erythrocytes (arrows), Giemsa-stained blood film (× 1100).

Abbreviations: a: young macrogamont ; E: epithelium ; i: mature microgamont ; ii: premature microgamont ; Lp: lamina propria ; o: oocysts ; s: premature meronts ; z: zygote.

Parts of the cytoplasm seemingly disaggregated, while retaining a dense cytoplasm where a few residual or non-differentiated nuclei remained. Microgametes flagella were conspicuously long (Figs. 3, 4).

Macrogamonts had a characteristically large vesicular nucleus with a large, deep-staining nucleolus (Fig. 4). Young macrogamonts, $7.0-8.4 \times 7.0-8.0$ in size (Fig. 1F), had a homogenous cytoplasm, with large vacuoles (apparently amylopectin granules) appearing gradually with maturation (Figs. 1F, 3, 4). In apparently mature $7.0-9.8 \times 8.4-9.0$ macrogamonts more than half of the cytoplasm volume was filled with amylopectin granules and wall-forming bodies were visible (Fig. 1G). Both young and mature forms were lodged in $9.8-10.5 \times 8.4-9.8$ PVs.

Zygotes or early oocysts reached 15.4×9.8 in size, and were lodged in a PV reaching up to 19.6×11.2 in size (Figs. 1H, 2, 4). They were densely filled with amylopectin granules and in their nuclei, the nucleolus became less conspicuous or disappeared. Wall-forming bodies, conspicuous (type 2) and minute (type 1) occurred in the cytoplasm, the first concentrated mainly along the oocyst wall (Fig. 1G).

Mature oocysts were almost round ($9.8-10.5 \times 9.0-9.8$), lodged in $11.2-11.8 \times 9.8-10.5$ PVs, and densely filled with amylopectin granules. The centrally located nucleus had an irregular perimeter and was homogeneously dense. Wall-forming bodies, or their disaggregated product, were concentrated beneath the rims of the oocyst wall (Figs. 1H, 4, 5). Mature oocysts occurred in both the epithelial layer (Figs. 2, 4), and lamina propria (Figs. 5, 6); some appeared to be pressing the basal membrane into the lamina propria layer (Figs. 4, 5). None of these oocysts were sporulating, and there were no free sporozoites. The latter were found in another, naturally infected specimen, in the liver, next or inside melanomacrophages (Fig. 7). In the same host, all oocysts had disappeared from the intestine, and in the blood only, erythrocytes were found infected (Fig. 8). Sporozoites were $8.4-10.5 \times 2.6-5$; their nucleus was in a somewhat anterior position and the two refractile bodies were located in the post-nuclear zone (Fig. 1I).

DISCUSSION

Species of *Schellackia* demonstrate limited inter-specific morphological diversity, and the variability in each stage's dimensions (added to the inevitable variation due to processing) is sufficiently large to result in overlapping size ranges among the same stages of different species, even of walled oocysts prior to sporulation or waiting intra-erythrocytic sporozoites. However, species may be otherwise distinguished according to the particular site at which

macrogamont fertilization and sporozoites formation occurs, e.g. either in the lamina propria or in the gut epithelial cells, and the sporozoites location in cells and tissues (Lainson *et al.*, 1976; Klein *et al.*, 1988; Paperna & Finkelman, 1996). Discrimination according to these criteria, restricts the number of species similar to the presently described one to the two species infecting agamid lizards, namely *S. agamae* (by Rogier, 1974) and *S. cf. agamae* (by Bristovetzki & Paperna, 1990). High host specificity among *Schellackia* spp. has been suggested (Lainson *et al.*, 1976) and recently has been experimentally also demonstrated, at least at the family level for *S. occidentalis*, *S. golvani* (Klein *et al.*, 1988) and *S. cf. agamae* (Bristovetzki & Paperna, 1990). PVs of all developing stages of presently described species, except mature oocysts, were relatively larger than the PVs of other species. The flagella of the microgametes were particularly long. Unlike in the other species from agamids, sporozoites were not found in circulatory leucocytes. On the other hand, sizes of all stages of *Schellackia* from *Agama* spp. (Rogier, 1977; Bristovetzki & Paperna, 1990; Paperna, 1992) and of *S. calotesi* overlap. Nevertheless, difference in the host genus (*Agama* vs. *Calotes*) and geographical origin (Africa and Near East vs. Thailand) seems to be satisfactory arguments for specific distinction.

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