# *Schellackia* (Apicomplexa: Eimeriidae) of the Brazilian Tree-frog, *Phrynohyas venulosa* (Amphibia: Anura) from Amazonian Brazil

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*Endogenous stages of a* Schellackia *species are described in histological sections of the intestine of the tree-frog,* Phrynohyas venulosa, *from North Brazil. Most oocysts sporulate within the epithelial cells of the gut, but a few were detected in the* lamina propria.

Key words: Apicomplexa - Eimeriidae - *Schellackia* sp. - *Phrynohyas venulosa* - Amphibia - tree-frog - Brasil

Although *Schellackia* species have been reported in a number of different lizards (Lainson et al. 1976), there appears to be but a single record of this parasite from an amphibian, *Bufo marinus*, in French Guyana (Le Bail & Landau 1974). In this communication we describe a *Schellackia* sp., discovered in the tree-frog, *Phrynohyas venulosa*, from North Brazil.

## MATERIALS AND METHODS

The tree-frogs were collected in low forest in Capanema, 50 km East of Belém.

Blood films and impression smears of the liver, spleen, lungs and kidneys were air-dried, fixed in absolute methyl alcohol and stained in Giemsa (30 drops of stain to 15.0 ml of distilled water at pH 7.4) for 1 hr.

Tissues for histology were fixed in buffered, neutral formalin and embedded in glycol methacrylate medium (Agar Company, U.K.). Sections, cut at 3.0-4.0 µm on a Sorval JB4 glass-knife microtome, were either stained with Meyer's haemalum and eosin, or post-fixed in aqueous Bouin's fluid for 20 min, washed until colourless in 70.0% ethyl alcohol, stained in Giemsa for 90 min, differentiated and dehydrated by passing them rapidly through acetone:xylol mixtures of 95:5, 70:30, 30:70, cleared in pure xylol and mounted in "Permount".

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## RESULTS

Moderate and heavy *Schellackia* infections were detected in histological sections of the intestines from two of four specimens of *P. venulosa* examined. No parasites could be found in films of the peripheral blood or impression smears of the visceral organs of the same animals.

All endogenous stages developed to maturity in the epithelial cells of the small intestine. Meronts measured from 12.0-24.0 x 9.0-12.0 µm, and they divided by exogeny (Fig. 1A). The maximum number of merozoites counted in sections of mature meronts was 14 (Figs 1B, 2). During their differentiation, microgamonts increased their size from 12.0 x 7.0 to 45.0 x 10.0 µm, and it was possible to detect over 20 nuclei or a similar number of microgametes (Figs 1C-E, 3-7). Microgamonts appeared to outnumber the macrogamonts.

The macrogamonts ranged in size from 7.0 x 6.0 to 14.0 x 7.0 µm and contained a few vacuole-like amylopectin bodies (Fig. 1F). These entirely filled those zygotes or early oocysts measuring about 8.0-12.0 µm (Fig. 1G). Some of these more mature forms also exhibited a small number of cytoplasmic granules which possibly represent wall-forming bodies (Figs 1G, 8,9).

Sporulating oocysts were from 9.0-10.0 µm in diameter and lay within a parasitophorous vacuole of about 12.0 µm: the sporozoites emerged from a large residual body (Figs 1H, I) and measured, on average, 6.0 x 2.0 µm. Although most oocysts completed their sporulation in the epithelial cells of the gut (Fig. 10), a few developing oocysts were seen in the *lamina propria*, which also contained numerous free or intracellular sporozoites. The latter measured from 8.0-9.0 x 2.0-3.0 µm and possessed a distinct refractile body (Figs 1K, 5,7).

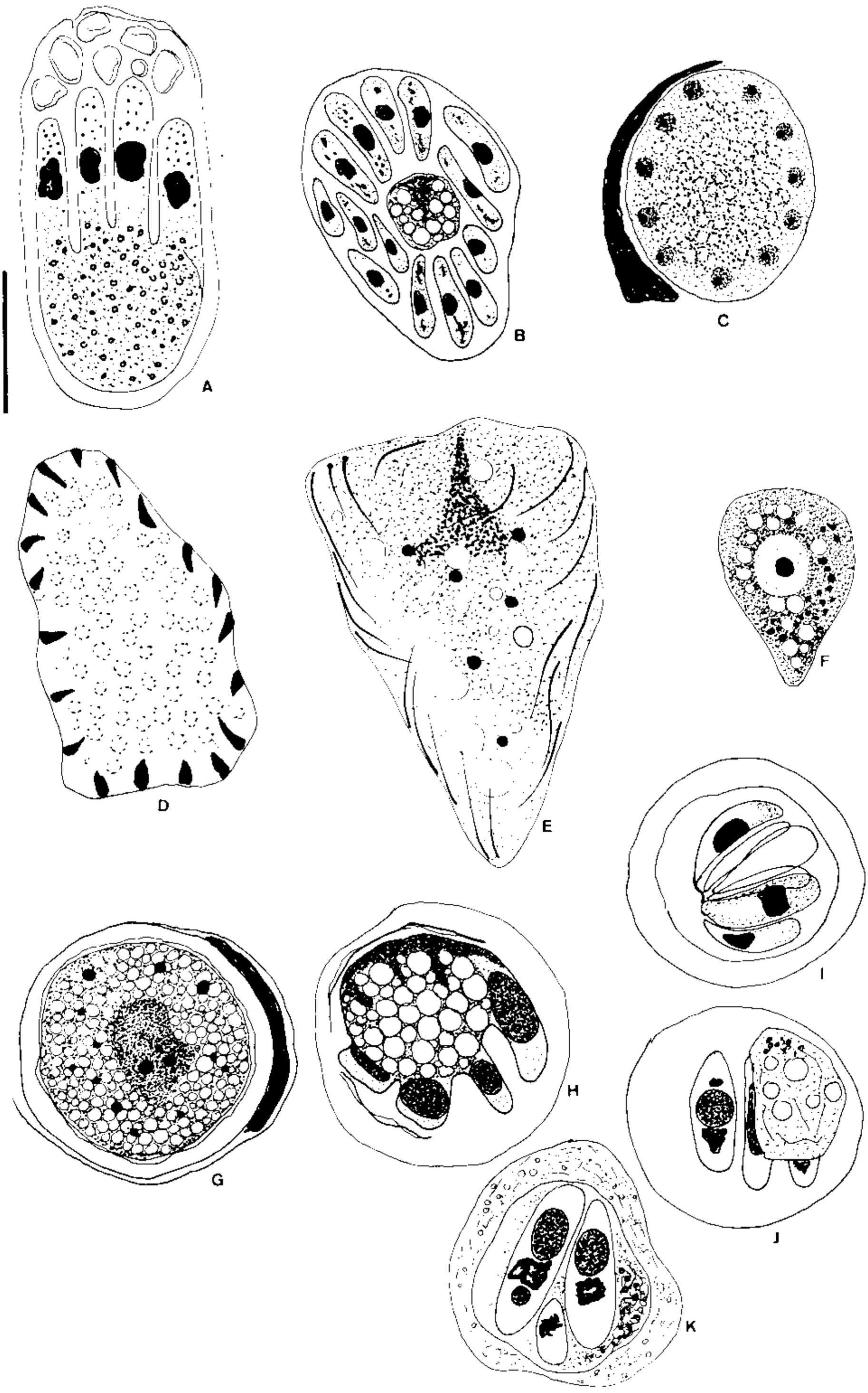
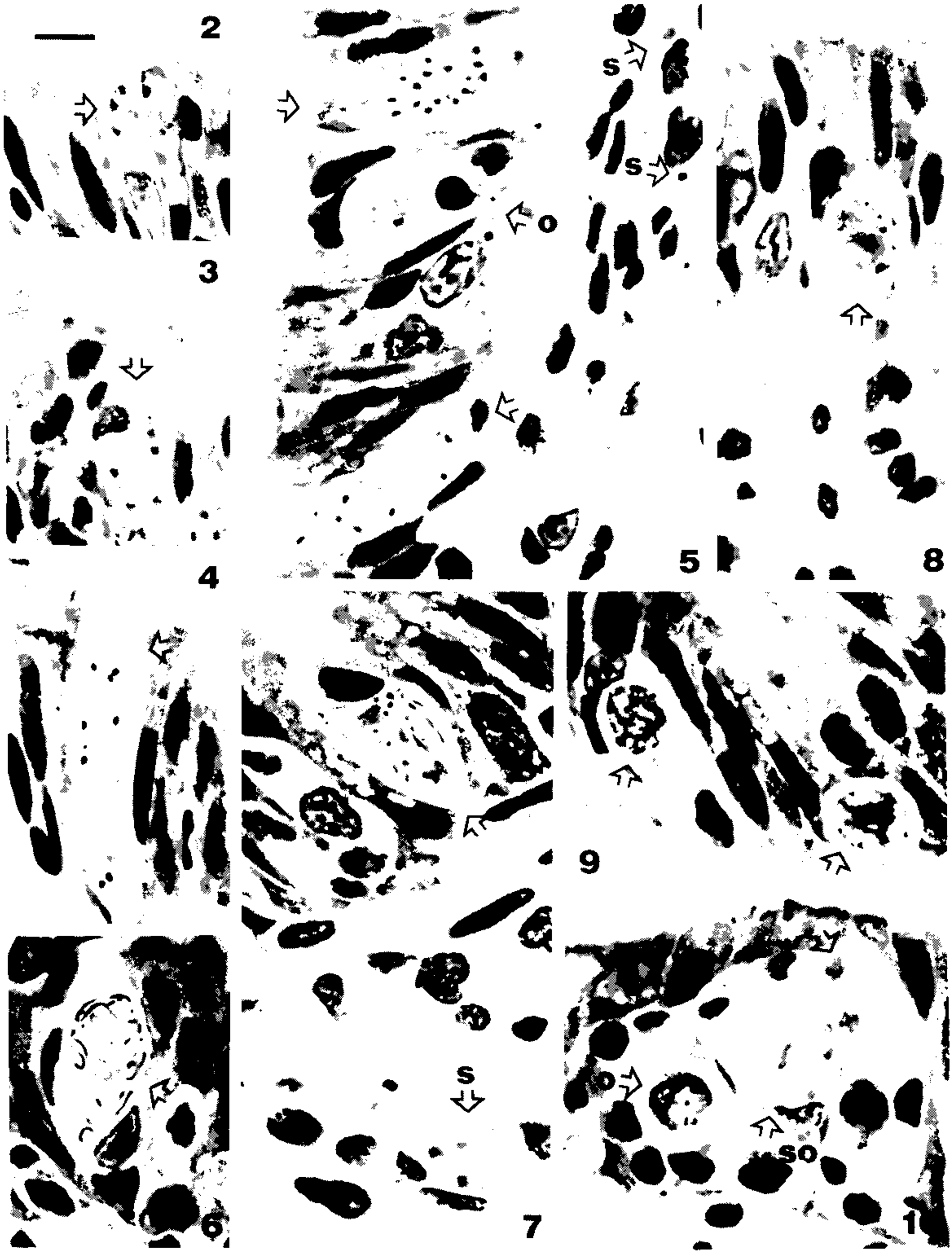



Fig.1: a *Schellackia* sp., of the tree-frog *Phrynohyas venulosa*: line-drawings of developmental stages in histological sections of the small intestine. A: dividing meront. B: mature meront with 14 merozoites and residual body. C: young microgamont. D: almost mature microgamont. E: mature microgamont. F: young macrogamont. G: zygote or early oocyst, with large numbers of vacuole-like amylopectin granules, and a smaller number of dense globules which are possibly wall-forming bodies. H: sporulating oocyst. I, J: mature oocysts. All parasites within the intestinal epithelium. K: intracellular sporozoites in the *lamina propria*. Scale: bar – 10.0 μm.




Photomicrographs of the developmental stages of a *Schellackia* sp., in the small intestine of the tree-frog *Phrynohyas venulosa* (all same scale). Fig. 2: dividing meront. Figs 3,4: developing microgamonts. Fig. 5: almost mature microgamonts (arrowed) and disintegrating oocyst (o) in the epithelium, and free sporozoites (s) in the *lamina propria*. Figs 6,7: mature microgamont with microgametes in the epithelium, and sporozoites (s) in the *lamina propria*. Figs 8, 9: zygotes or early oocysts. Fig. 10: early oocyst (o), sporulated oocyst (so) and a disintegrating oocyst (arrow) in the gut epithelium. Bar = 10.0 μm.

## DISCUSSION

From the development and morphology of the oocysts, there is no doubt that the parasite of *P. venulosa* is a species of *Schellackia*.

Le Bail and Landau (1974) described a *Schellackia*, *S. balli*, in the toad, *B. marinus*, from French Guyana. The data presented by both those authors and ourselves, however, are insufficient for us to say whether or not we are dealing with this parasite in *P. venulosa*. Morphologically the parasites from both of these amphibia are very similar, although whereas Le Bail and Landau did not detect any sporogony of *S. balli* in the *lamina propria*, we did note that a few oocysts of the *Schellackia* of *P. venulosa* escaped from the gut epithelium into the *lamina propria* prior to sporulation. In this respect the parasite more resembles *S. brygooi* Landau, 1973 of lizards, which also sporulates in both the gut epithelium and the *lamina propria*.

*Lankesterella* spp., have been found to be of common occurence in both *B. marinus* and *P. venulosa* in Amazonian Brazil (Lainson & Paperna, unp. obs.), but the extraintestinal sporulation of this parasite's asporocystic, polyzoic oocysts readily differentiates it from *Schellackia*. Finally, a previously undescribed *Eimeria* sp., of *B. marinus*, from the same locality, does undergo endogenous sporulation in the epithelial cells of the intestine, but is easily recognized by its oocysts with four sporocysts, each containing two sporozoites, typical of the genus (Paperna & Lainson, unp. obs.). It may well be that *Schellackia* species are more common in amphibians than previously supposed.

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