DESCRIPTION AND ULTRASTRUCTURE OF *LEISHMANIA ZUCKERMANI* N. SP. AMASTIGOTES DETECTED WITHIN THE ERYTHROCYTES OF THE SOUTH AFRICAN GECKO *PACHYDACTYLUS TURNERI* GRAY, 1864

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Summary :

In erythrocytes recovered from blood of geckoes of the species *Pachydactylus turneri* collected in Gauteng Province, Republic of South Africa, *Leishmania zuckermani* n. sp. were detected. Giemsa stained erythrocytes contained amastigotes, either single or numerous, in loose assemblies or in a compact rounded aggregates which may condense to become a round basophilic bodies with a central hollow. This new species of *Leishmania* differs from all previously described species in being almost exclusively parasitic in circulating erythrocytes. Three to seven amastigotes lodged all within one, or divided between several parasitophorous vacuoles were detected at the EM level. The amastigotes demonstrated essentially all the cytological components characteristic of leishmania species known to parasitize mammals. A point which emphasizes an already suggested close affiliation between mammalian and lizard *Leishmania*.

KEY WORDS : *Leishmania zuckermani* n. sp., amastigotes, intraerythrocytic, *Pachydactylus turneri*, South Africa, ultrastructure.

Résumé : DESCRIPTION ET ULTRASTRUCTURE D'AMASTIGOTES DE *LEISHMANIA ZUCKERMANI* N. SP. DANS LES ÉRYTHROCYTES DU GECKO D'AFRIQUE DU SUD *PACHYDACTYLUS TURNERI* GRAY, 1864

Les érythrocytes de geckos Pachydactylus turneri *capturés dans la province de Gauteng, République d'Afrique du Sud, hébergent* Leishmania zuckermani *n. sp. Après coloration au Giemsa, on note la présence d'un ou de plusieurs amastigotes disposés en un ensemble lâche ou en un aggrégat rond et compact qui peut se condenser pour former un corps basophile arrondi présentant une cavité centrale. Cette nouvelle espèce de* Leishmania *diffère de celles déjà décrites par sa localisation presque exclusive dans les érythrocytes circulants. L'étude en microscopie électronique montre des érythrocytes infectés par trois à sept amastigotes, localisés dans une ou plusieurs vacuoles parasitophores. Les stades amastigotes de* L. zuckermani *possèdent les organites cellulaires caractéristiques des leishmanies parasites de mammifères. Ceci renforce l'idée, déjà émise, d'une proche filiation entre les leishmanies de mammifères et celles de lézards.*

MOTS CLÉS : Leishmania zuckermani *n. sp., amastigotes, érythrocyte,* Pachydactylus turneri, *Afrique du Sud, ultrastructure.*

In squamate reptiles *Leishmania* amastigotes occur in blood cells, promastigotes in both blood and cloaca, and were cultured as promastigotes from the blood and the viscera (Telford, 1984). Their relationship to mammalian *Leishmania* is still debated, they have been regarded as member of a separate genus - *Sauroleishmania* Ranque 1973 (Killick-Kendrick *et al.*, 1986). Isoenzyme (Okot-Kotber *et al.*, 1989), as well as molecular genetic studies (Croan *et al.*, 1977; Brewster & Barker, 1999), however, showed close affiliation between mammalian and lizard *Leishmania* and suggest that the lizard parasites might be best retained in the genus *Leishmania*.

In this communication we provide a fine structural description of *Leishmania* amastigotes infecting the erythrocytes of the South African gecko *Pachydactylus turneri*.

MATERIALS AND METHODS

The geckoes were collected in Gauteng Province (Northern Pretoria area – 25° 36' 51" S – 28° 00' 06" E, Republic of South Africa. Blood films for light microscopic (LM) examination were prepared by clipping the geckoes' toe, the prepared slides were air dried and stained in Giemsa's solution (10 % in buffer pH 7.4) for one hour.

A male *P. turneri*, with high parasitaemia verified from Giemsa-stained blood films, was sacrificed to obtain material for the fine structural study. Segments of liver, spleen, kidney and bone marrow from the femur were fixed in 2.8 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for one hour at room temperature, rinsed and postfixed in 1 % osmium tetroxide in the same buffer for one hour and rinsed in 0.1 M cacodylate buffer. After dehydration in graded ethanols, tis-

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sues were embedded in Epon 812 mixed with Araldite.

Thin sections were cut on a Reichert "Ultracut" with a diamond knife, stained on a grid with uranyl acetate and lead citrate and examined in a Philips EM 201, at the Centre interuniversitaire de Microscopie électronique, CIME Jussieu, Paris VI-VII, France. Terminology of ultrastructural details is according to Zuckerman & Lainson (1977).

RESULTS

In LM Giemsa-stained blood films, levels of parasitaemia in the infected geckoes ranged from 2.7 to 11 ‰. Amastigotes infecting erythrocytes occurred singly, but more commonly were seen to form either loose assemblies or compact rounded aggregates. Some of the round aggregates condensed to a round basophilic bodies with a central hollow (Fig. 6). Loose assemblies of amastigotes comprised of four-eight parasites (Figs 2, 5), while some aggregates could contain over 25 parasites (Fig. 4) suggesting that multiple invasion processes do occur. Erythrocytes may contain several assemblies, and in addition several single flagellates. In one erythrocyte 50 amastigotes were counted (Fig. 4). The single amastigotes were oblong (2 × 1 µm, in size, n = 10) basophilic, with an apical "vacuole" (the flagellum site). Aggregates could reach 4 to 5 µm and the compact ones to 3-4 µm. Exceptionally, single amastigotes were found in monocytic leucocytes only in the spleen. Infection by *Leishmania* coincided in the same hosts with an infection by *Haemoproteus* sp. and a hemogregarine (Fig. 5).

Examining by TEM, ultrathin section of erythrocytes, it was possible to establish that these host cells contain three to seven amastigotes (2.0-2.2 × 1.0-1.5 µm, n = 6) all within one (Figs 7, 8) or partitioned between two parasitophorous vacuoles (PV) (Fig. 9). Amastigotes were bound in a double periplast, and encased beneath by a layer of subpellicular microtubules (Figs 8, 9); the cytoplasm was densely loaded with ribosomes with centrally positioned nucleus. In this nucleus, the chromatin concentrated on the periphery and the single, electron dense nucleolus was in the center (Figs 7, 8). The flagellum was located within a flagellar pocket, its outline was emphasized by a dense thick sheath, enclosing peripheral and central sets of microtubules (Figs 7, 9, 10). Section plane of the basal body obscured details ((Figs 8, 9). The kinetoplast was positioned perpendicularly to the flagellum, with the fibrilar DNA band and the cristae (Figs 7-9). Golgi complex were vaguely tracable, while mitochondria were conspicuous, positioned alongside and behind the nucleus (Figs 8, 9).

DISCUSSION

All available accounts emphasise the presence of saurian *Leishmania* in blood cells other than erythrocytic: thrombocytes, granulocytes and monocytes (Telford, 1979, 1984). In the presently reported infection in *P. turneri,* infection seemingly occurs entirely in erythrocytes. The exoerythrocytic amastigotes exceptionally seen in the spleen, were phagocytized individuals.

The amastigotes' loose, dense and compact aggregations within the erythrocytes apparently represent a developmental process. The compact aggregates seem to represent aged stages. These assemblies appear to comprise a single progeny, evolved from a single invading flagellate. Erythrocytes, however, sustained multiple infections. It appears that following division, the amastigote progeny remains within their parent PV. To our best knowledge, ultrastructure of amastigote stages of reptile *Leishmania* was thus far not described. The amastigotes demonstrated essentially all the components earlier described in mammalian species (see Zuckerman & Lainson, 1977) , thus providing a further support to the recent biochemical and molecular studies suggesting close relationship between reptile and mammal infecting species (Okot-Kotber *et al.*, 1989; Brewster & Barker, 1999). Comparison of DNA and RNA polymerase gene sequences (Croan *et al.*, 1977) also provided, contrary to expected, strong support for the hypothesis that *Leishmania* infecting reptiles have evolved from mammalian *Leishmania.* Most of the 17 described species from reptile hosts (listed by Ovezmukhammedov & Safyanova, 1989), were from promastigotes obtained from cultured host's heart blood, and were subjected to either isoenzyme or serological analysis or DNA sequencing (Safyanova *et al.*, 1991). Fewer species (about six) were described from blood smears (Telford, 1979, 1984), sometimes without mentioning the type of host cells (Edeson & Himo, 1973). As blood from the gecko under study in this analysis was not cultured, we do not have promastigote material for biochemical or molecular typing.

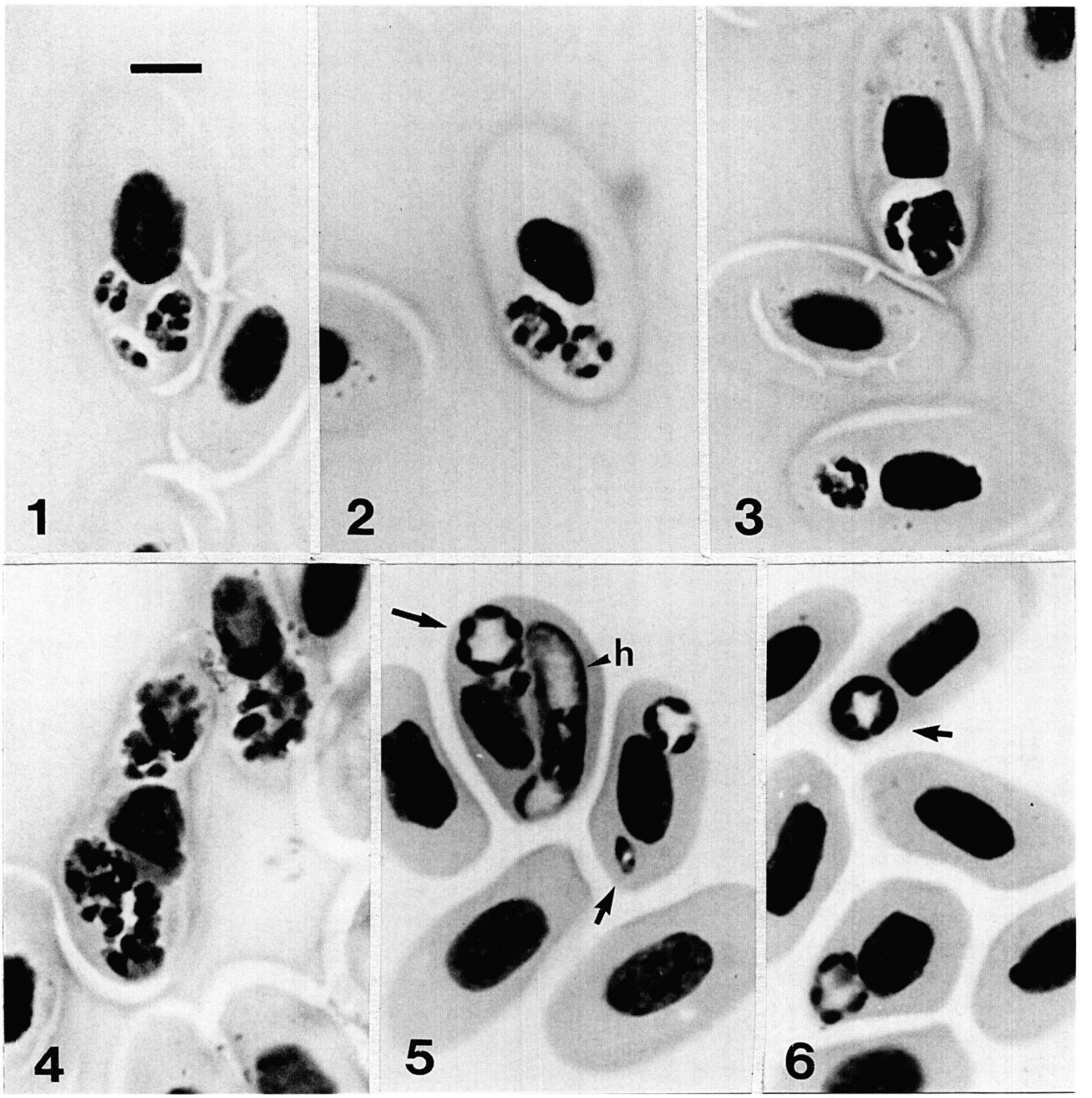
Diagnosis: *Leishmania zuckermani* n. sp.

Type host: *Pachydactylus turneri* Gray, 1864.

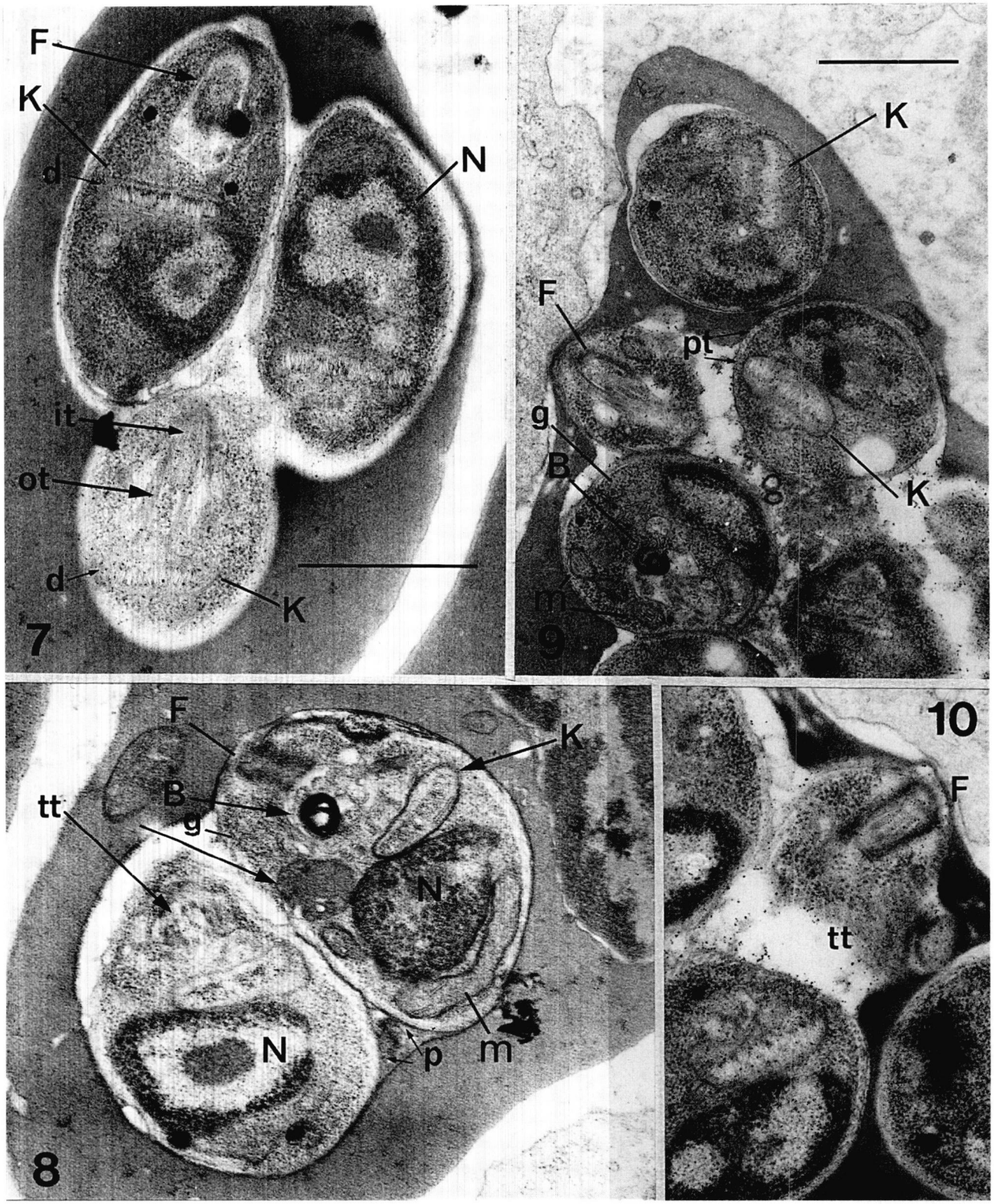
Type locality: Gatueng Province, Republic of South Africa.

Type specimen: Giemsa stained blood film N°. 225 PZ (Co-type: 222 PZ) in the collection of the Muséum National d'Histoire Naturelle, Paris, France.

The presently described parasite is unique in its affinity to erythrocytes, "appearance" and its mode of division in the blood cells. Its host species and its geographical location are new records for *Leishmania* from reptilian hosts (see Telford, 1984). In our opinion the presently available data are sufficient to demons-



Figs 1-6. – *Leishmania zuckermani*-infected erythrocytes of *Pachydactylus turneri* in Giemsa-stained blood film. Scale = 5 µm, same for Figs 1-6. Fig. 1. Single, double and assembly of four amastigotes. Figs 2, 3. Loose and round amastigote aggregates. Fig. 4. Single and double infection with large amastigote aggregates. Fig. 5. Round aggregates (large arrow), and single amastigote (small arrow), and a hemogregarine (h). Fig. 6. Round and condensed (arrow) amastigote aggregates.



Figs 7-10. – Electron microscopic view of amastigotes infecting *P. turneri* erythrocytes. Fig. 7. PV containing three promastigotes. Fig. 8. PV with two amastigotes. Fig. 9. PV with a single amastigote and another with at least 5. Fig. 10. Details of amastigotes flagellar zone (scale bar = 1 µm, same for figs 7, 8, 10).

B, Basal body. d, fibrillar DNA band. F, Flagellum within its pocket. it, Peripheral microtubules of the flagellum. ot, central paired microtubules of the flagellum. g, Golgi complex. K, Kinetoplast. m, Mitochondria. N, nucleus. p, Periplast. pt, periplast and the underlying subpellicular microtubules. tt, subpellicular microtubules exposed in section.

trate a unique characteristic, which together with the specific host and geographic range can justify the consideration of the presently described *Leishmania* as a new species. Our present TEM data, however, reconfirm previous conclusions (see Zuckerman & Lainson, 1977) that species of *Leishmania* are indistinguishable by ultrastructural details.

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