

TRYPANOSOMES OF THE SUBGENUS *MEGATRYPANUM* FROM ARMADILLOS (XENARTHRA: DASYPODIDAE)

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A new species of trypanosome, Trypanosoma (Megatrypanum) peba, is described from the peripheral blood of the armadillo Euphractus sexcinctus setosus from Bahia State, Brazil. Ten out of 29 specimens of the armadillo Dasypus novemcinctus from Pará State were found to have trypanosomes, including epimastigote forms, in impression smears of subcutaneous lymph nodes. The trypanosomes from D. novemcinctus are illustrated and were identified as belonging to the subgenus Megatrypanum on the basis of their general appearance, although they failed to multiply in blood-agar culture medium and no bloodstream forms were seen. This is the first published record of trypanosomes of this subgenus from armadillos and the first demonstration of epimastigote trypanosomes in the mammalian host other than in the bloodstream, or in the anal glands of opossums.

Key words: *Trypanosoma (Megatrypanum) peba* – new species – *Euphractus sexcinctus* *Dasypus novemcinctus* – lymph nodes – trypanosome development

Although the order Xenarthra (= Edentata) contains a diverse group of 29 living species (Wetzel, 1982), the only described trypanosome of the subgenus *Megatrypanum* from these mammals is *Trypanosoma legeri* Mesnil & Brimont 1910, a common parasite of *Tamandua* spp. (Myrmecophagidae) (Shaw, 1985). Very little is known about the mode of reproduction of *Trypanosoma (Megatrypanum)* species in the mammalian host (Hoare, 1972). Barrett (1979) illustrated a bloodstream trypomastigote belonging to this subgenus, from the yellow armadillo *Euphractus sexcinctus*, and more recently we have observed what appear to be reproductive stages of a similar trypanosome in the lymphatic system of the common long-nosed armadillo *Dasypus novemcinctus*. The purpose of the present communication is to describe and name the parasite from *E. sexcinctus* and to report what is known of these trypanosomes in armadillos.

MATERIALS AND METHODS

A juvenile female yellow armadillo, *E.*

sexcinctus, captured near the town of Castro Alves, Bahia State, Brazil, was examined for haemoparasites during a survey of wild hosts of *T. cruzi* in 1976. A single large trypanosome was observed in a preparation of fresh blood, but no parasites were seen in 25 Giemsa-stained blood smears made at the same time. One of four cultures, made by inoculating a drop of tail blood onto a fortified NNN blood-agar slant with F29 medium as overlay (Pan, 1968) became positive within 10 days. Overlay from the positive culture was inoculated intraperitoneally into 4 white laboratory mice and intraperitoneally and intradermally into a second *E. sexcinctus* purchased in the district of Oliveira dos Breijinhos, in Central Bahia.

The present description is based on the morphology of the blood parasites from the second armadillo, which developed a patent parasitaemia 4 days after inoculation. Measurements refer to mean and range in micrometers of 15 organisms on slides fixed with methanol and stained with Giemsa.

Twenty *Rhodnius prolixus* and 20 *Triatoma infestans* fifth-instar nymphs were fed on each of the armadillos, and were found to be negative for flagellates when dissected after 25-30-days.

Fresh blood examination and xenodiagnosis with triatomines on the 4 mice was also nega-

Supported in part by the Research Agreement ELETRONORTE/INPA.

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Received 26 June 1990.

Accepted 20 July 1990.

tive. The second armadillo died 10 days after inoculation, and tissue samples were preserved in 10% formol saline for histological examination. The first armadillo remained in good health until killed 6 months later for necropsy.

In September 1987, 29 *D. novemcinctus* and 1 *D. kappleri* were captured in the district of Repartimento, on the Transamazonian Highway near the Tucuruí hydroelectric project in Pará State, Brazil. The animals were subjected to haemoculture with NNN Difco blood-agar medium with condensation fluid as overlay, xenodiagnosis with 5-10 *R. prolixus* (Tucuruí colony), examination of fresh blood, and examination of fresh and stained impression smears of subcutaneous lymph nodes. Whenever trypanosomes were seen in fresh impression smears, a piece of the lymph node was inoculated into a tube of the culture medium.

RESULTS AND DESCRIPTION

Trypanosoma peba n. sp. (Figs 1-5)

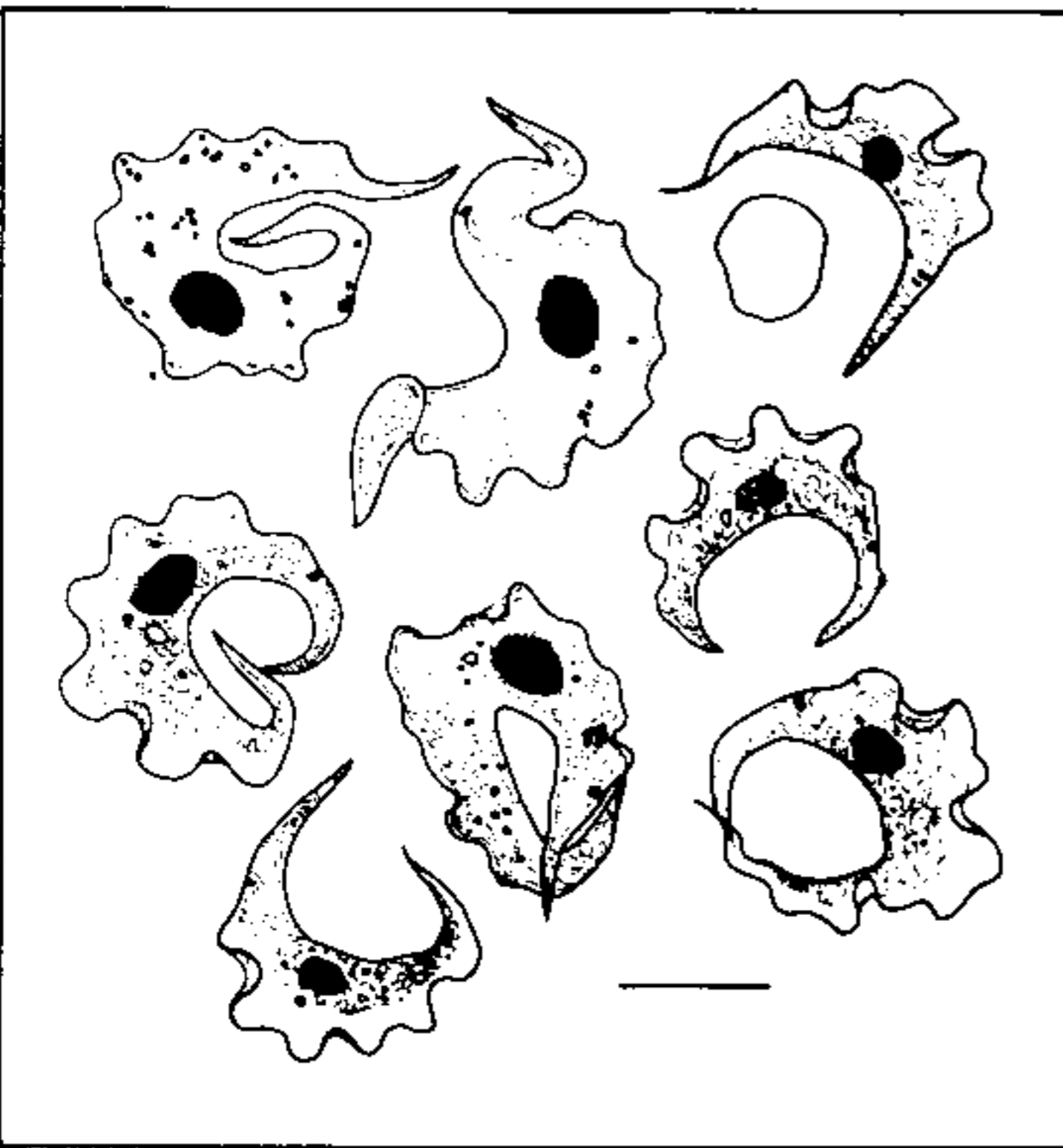


Fig. 1: *Trypanosoma peba*, n. sp., bloodstream trypomastigotes in *Euphractus sexcinctus*. From Giemsa-stained smears. Bar = 10 μ m.

Trypanosoma (Megatrypanum) sp. Barrett, 1979, p. 321. Total length including free flagellum when present 44.1 (32.5-53.5). Posterior end to kinetoplast 11.4 (7.5-16.0). Kinetoplast to centre of nucleus 9.9 (6.5-11.8). Posterior end to centre of nucleus 21.3 (16.5-

27.0). Centre of nucleus to anterior end 22.5 (14.0-29.5). Length of free flagellum 0.4 (0.0-2.3). Nuclear index 1.0 (0.7-1.4). Kinetoplastic index 2.2 (1.8-2.9). Kinetoplast oval and marginal, heavily stained in some organisms but difficult to discern in those with a distinct free flagellum. Nucleus usually oval and longitudinal to the long axis of the trypanosome. Cytoplasm and occasionally nucleus may contain numerous vacuoles. Undulating membrane highly developed.

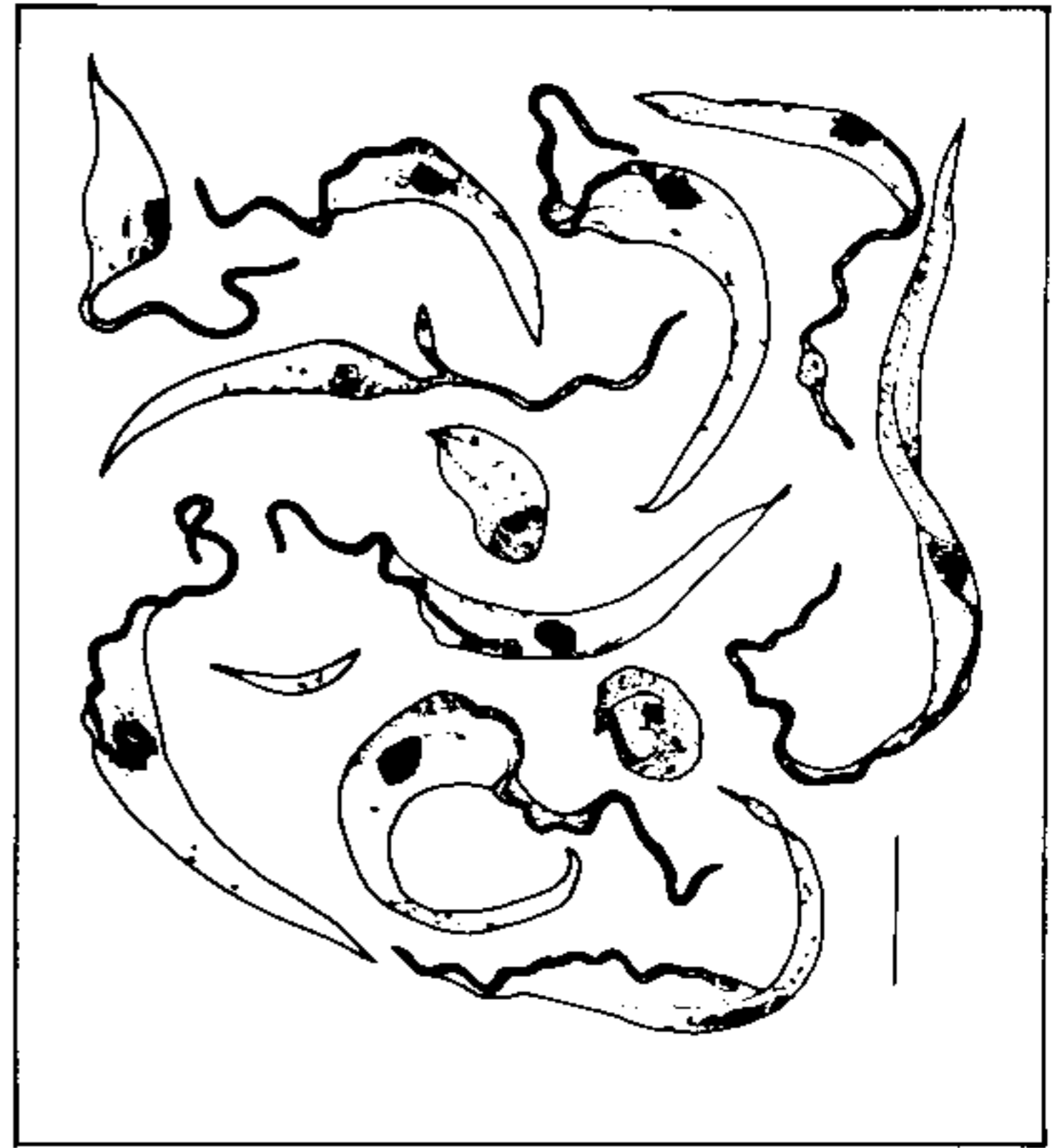


Fig. 2: *Trypanosoma peba*, n. sp., forms in culture. From Giemsa-stained smears. Bar = 10 μ m.

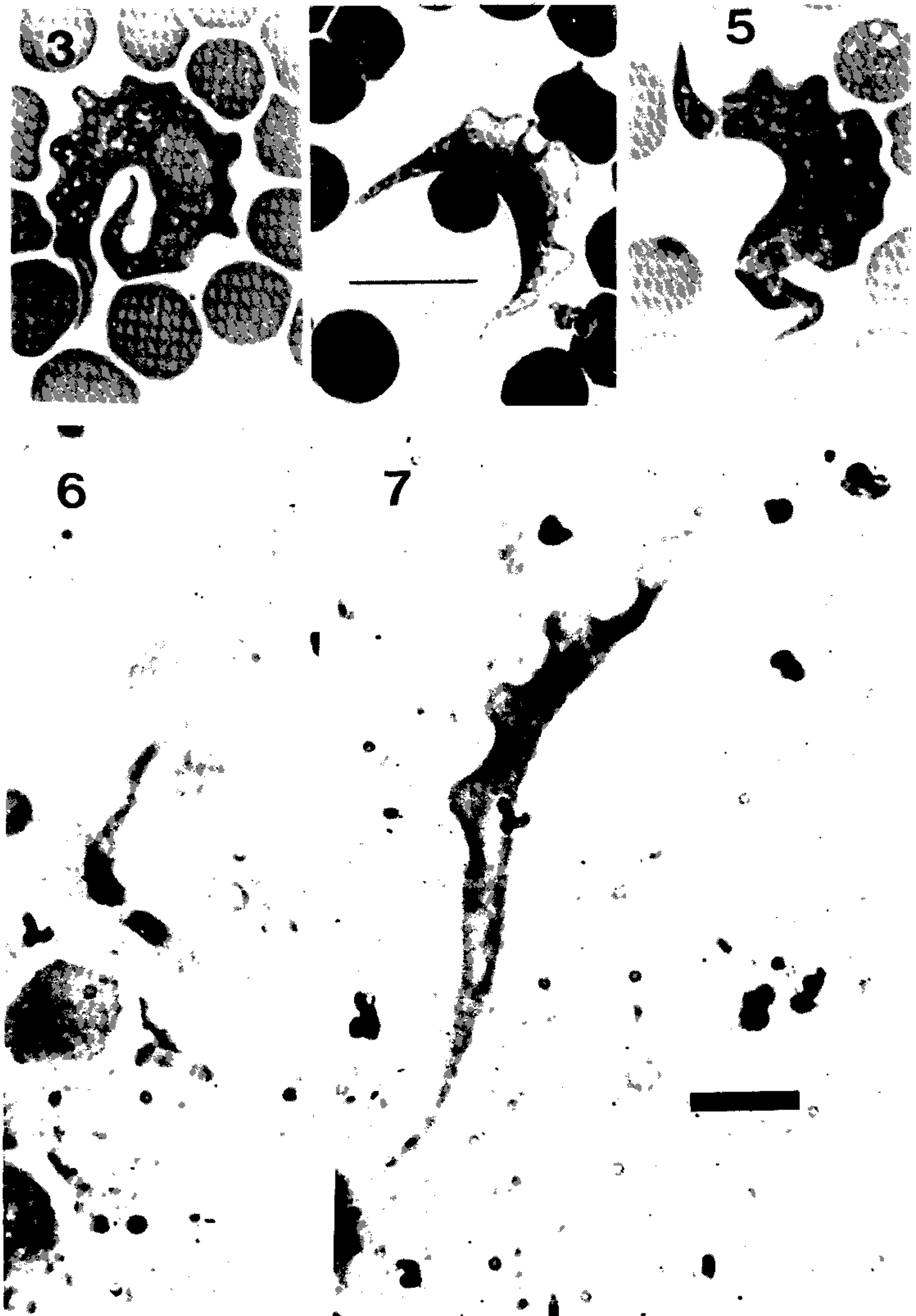
Growth in NNN culture with F29 medium as overlay luxuriant.

Type host: *Euphractus sexcinctus setosus* (Wied, 1826).

Invertebrate host: unknown

Culture cryopreserved over liquid nitrogen as stabilate number LUMP 1307 at the London School of Hygiene and Tropical Medicine. Hapantotype slides at the Departamento de Ciências da Saúde, Instituto Nacional de Pesquisas da Amazônia.

The specific name refers to the broad, flattened aspect of the bloodstream trypomastigotes in fresh preparations, and is derived



Figs 3-5: *Trypanosoma peba*, n. sp., in the blood of *Euphractus sexcinctus*. Giemsa. To same scale. Bar in Fig. 4 = 10 μ m. Figs 6-7: Trypanosomes in impression smears of subcutaneous lymph nodes of *Dasypus novemcinctus*. Giemsa. To same scale. Bar in Fig. 7 = 5 μ m.

from the Tupi *pewa*, flat, as in *tatupeba* (flat armadillo), the Brazilian vernacular name for the type host.

Infectivity to triatomines and laboratory mice was not demonstrable, and no tissue forms or lesions attributable to trypanosome infection were found in either armadillo.

Trypanosoma (Megatrypanum) sp. from
Dasyus novemcinctus (Figs 6, 7).

Large trypanosomes including epimastigote forms were seen in fresh and stained preparations of subcutaneous lymph nodes from 10 out of 29 *D. novemcinctus* (34%). The only trypanosome species isolated by haemoculture or xenodiagnosis was *T. cruzi*, from 9 *D. novemcinctus*. Cultures of material from infected lymph nodes were all negative for trypanosomes. The trypanosomes in the lymph nodes were ascribed to the subgenus *Megatrypanum* on the basis of their size and general similarity to culture forms of *T. peba*.

DISCUSSION

The only trypanosome hitherto known from *E. sexcinctus* is *T. (Schizotrypanum) cruzi* Chagas, 1909. The large size of the bloodstream forms of *T. peba* and the position of the kinetoplast far from the posterior end of the body are characteristic of the subgenus *Megatrypanum* Hoare, 1964. The only other trypanosome of this subgenus known from Xenarthra is *T. legeri* Mesnil & Brimont, 1910, from anteaters of the genus *Tamandua*. Characters which distinguish *T. peba* from *T. legeri* include the position of the nucleus (anterior in *T. legeri*, nuclear index ca. 1.6) and the length of the free flagellum (3.8-13.0 μm and apparently always present in *T. legeri*).

As far as we are aware this is the first report of epimastigote forms of a trypanosome in a mammal outside the bloodstream or tissue capillaries (Cf. Deane, 1969; Hoare, 1972), except for the special case of opossum anal scent glands (Deane & Jansen, 1986).

In early-passage cultures of *T. peba* the peculiar satellite bodies on the flagella of some organisms (Fig. 2) were noted in fresh and stained preparations. Whether or not they bear any relation to the flagellar cysts or satellites of the monoxenous insect flagellates *Lepto-*

monas and *Blastocrithidia* is unclear. Both the order Xenarthra, with a fossil record of some 60 million years, and the mammalian trypanosome subgenus *Megatrypanum*, which has affinities with some corresponding parasites of reptiles and amphibians (Hoare, 1972), are considered to be phylogenetically primitive. This makes *T. peba* and the parasite from *D. novemcinctus* (should the latter prove to be a distinct species of trypanosome) suitable subjects for further research at a time of renewed interest in the evolution of mammalian haemoflagellates (Shaw, 1985; Deane & Jansen, 1988).

ACKNOWLEDGEMENTS

The work in Bahia was carried out during a collaborative research programme financed by the Wellcome Trust and involving the Núcleo de Pesquisas da Bahia of the Fundação Oswaldo Cruz, the Federal University of Bahia, the Pan American Health Organization, the Department of Tropical Public Health of the Harvard School of Public Health, and the London School of Hygiene and Tropical Medicine. We are particularly grateful to Drs Zilton Andrade and Moyses Sadigursky of the Department of Pathology, Faculty of Medicine, Federal University of Bahia for performing post-mortem examinations on the two *E. sexcinctus*, and to Dr Rodney Hoff and Vera Mascarenhas for maintaining the trypanosome isolates in culture.

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