Bull. zool. Surv. India, 6 (1-3) : 151-157, 1984

A NEW SPECIES OF TRYPANOSOME FROM AN INDIAN MICROHYLID FROG, *KALOULA PULCHRA TAPROBANICA* PARKER

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ABSTRACT

A new trypanosome, *Trypanosoma taprobanica* sp. nov. is described from an Indian microhylid frog, *Kaloula pulchra taprobanica* Parker. It is monomorphic and attenuated at both the ends, measuring 24.9 μm in total length. It's affinities with related species have been discussed.

Introduction

During the last 5 years (1976-1980) 204 microhylid frogs, *Kaloula pulchra taprobanica* Parker were collected from different districts of West Bengal, India. Blood examination of the frogs revealed the presence of a trypanosome in only 14 of them (6.8%) which seemed to be different from any of the described species from the Anura.

In India, relatively few trypanosomes have been described from the anuran amphibians. It was Dr. N. Berestneff (1903) who initiated the study by reporting a *Trypanosoma* from *Rana tigrina* and *Rana limnocharis*. Patton (1908) also recorded a new species of trypanosome in *Rana tigrina* from India and named it as *T. hendersoni*. Scott (1926,1927) reported the occurrence of *Trypanosoma* in the blood of the same frogs which were brought from India to the Zoological Gardens, London. Afterwards, Pujati (1953), Ray (1979, 1980) and Ray and Choudhury (1981) studied the anuran trypanosomes in details from India.

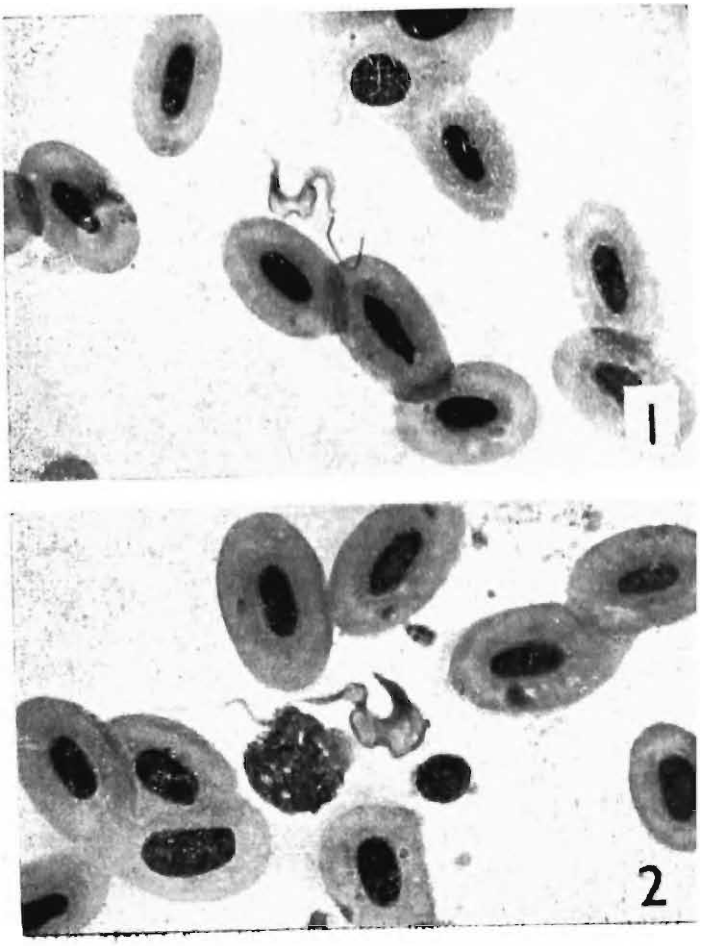
The present communication deals with a new species of trypanosome from *Kaloula pulchra taprobanica*, a small frog belonging to the family Microhylidae.

Material and Methods

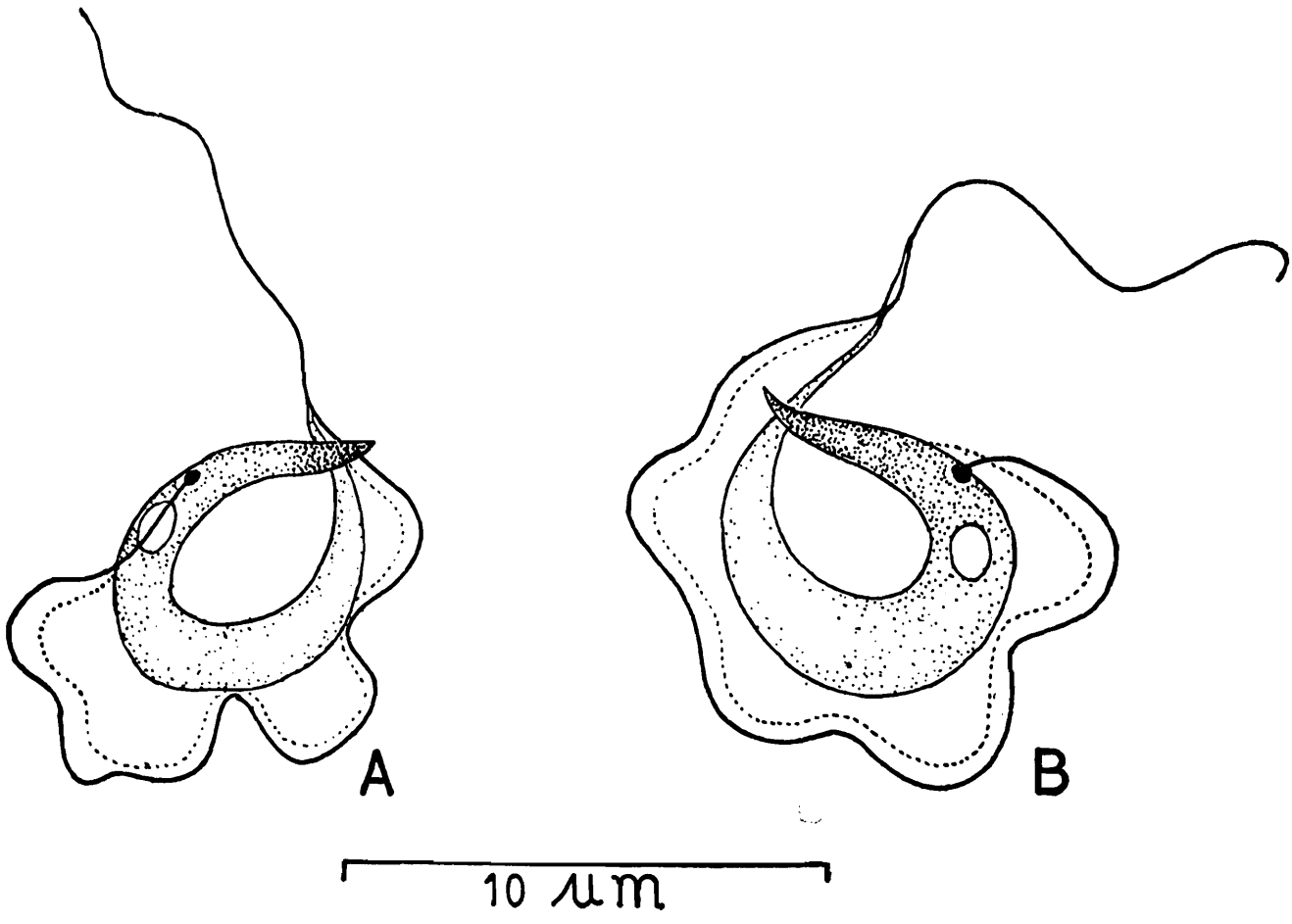
A total of 204 microhylid frogs, *Kaloula pulchra taprobanica* Parker were collected from different districts of West Bengal, India. Of which 14 (6.8%) were found to be infected with trypanosomes. Five frogs were sacrificed and two blood films were drawn from each frog. Blood was obtained directly from the heart and blood films were prepared on clean grease-free slides. Imprint smears of different organs *viz.*, liver, lungs, heart, kidney etc. were also made. All the smears were fixed in 100% methanol and stained with Romanowsky type of stains.

Camera-lucida drawings of 20 trypanosomes (N=20), 4 from each frog, were drawn on drawing paper at a magnification of 1000×. *T. loricatum* and *T. rotatorium* were also obtained from *Rana limnocharis* and *Rana*

RAY & CHOUDHURY PLATE VI



Figs. 1 and 2. Photomicrographs of *Trypanosoma taprobanica* sp. nov. in *Kaloula pulchra taprobanica*. X 1235, 1500.



Figs. A and B. Camera-lucida drawings of *Trypanosoma taprobanica* sp. nov. in *Kaloula pulchra taprobanica*.

tigrina respectively from the same localities of West Bengal. Ten (N=10) trypanosomes were drawn and measurements were taken after Hoare (1972) for both the species to establish their specific identity. Trypanosomes were measured from the camera-lucida drawings by means of a fine divider, the points of which were separated at a small unit measurements. Standard deviation (SD) was calculated for each parameter and all the data were compared statistically for significance test by using 'Student test' method. Photomicrographs were taken with the help of an 'Ergavel Carlzeis' microscope using PM6 'Olympus' attachment camera.

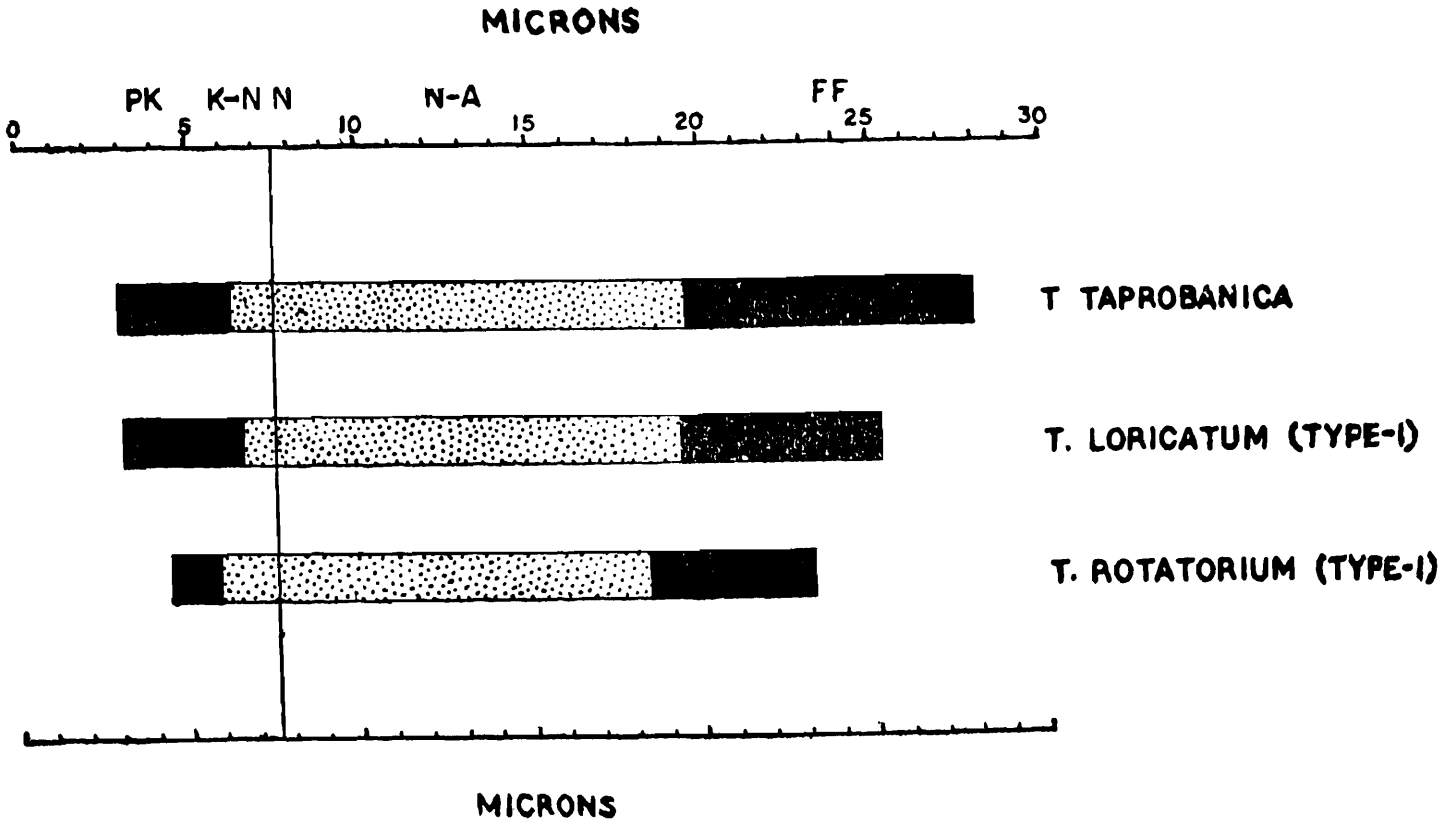
Observation

Trypanosoma taprobanica sp. nov.

(Plate VI, figs. 1, 2 & Figs. A, B)

Description : The trypanosomes are monomorphic (Table-1), very small, 'C' or 'S' shaped in configuration. Body curved, elongated with both ends attenuated and pointed. The trypanosome measures 16.62 µm (1.54) in length (excluding free flagellum) and 1.82 µm (0.20) in width.

Cytoplasm : The cytoplasm is finely granular, homogeneous, stained light blue in Leishman. Body costae or striations were not found.



GRAPH-I

Graph 1. Comparison of overall morphometric measurements of 3 species of trypanosomes *viz.*, *Trypanosoma taprobanica* sp. nov., *T. loricatum* (Type I) and *T. rotatorium* (Type I).

PK—Posterior end to the kinetoplast ;
K-N—Kinetoplast to the centre of the nucleus ;
N —Centre of the nucleus ;
NA—Centre of the nucleus to the anterior end ;
FF—Free flagellum.

Nucleus : The nucleus is small, rounded to oval, measuring 1.0×0.57 μm ; stained red with Leishman and always situated on the posterior part of the body parallel to the body axis and close to the kinetoplast (Nuclear Index (NI)=0.36).

Kinetoplast : The kinetoplast is a dot-like structure measuring 0.45×0.45 μm ; stained blue-black with Leishman and situated on the posterior part of the body very close to the nucleus (kinetoplastic index (KI)=3.65).

Flagellum and undulating membrane : The flagellum emerges from the posterior end of the kinetoplast and traverses the whole body producing 4-6 prominent folds of the undulating membrane and finally leaves the body as a long free flagellum. The free flagellum is 8.3 μm in length and is about half the length of the body (FF/PA=0.49).

The method and site of reproduction in the vertebrate host has not been encountered in numerous samples of peripheral and heart

blood and stained impression smears of heart, lungs, liver and kidney examined.

Type-host : *Kaloula pulchra taprobanica* Parker.

Site of infection : Blood.

Locality : Santaldi, Purulia District, West Bengal, India.

Vector and life-cycle : Unknown.

Registration Nos. : Holotype—Z. S. I. Pt. 1971.

Paratype—on the same slide.

Diagnosis of *Trypanosoma taprobanica* sp. nov.

The trypanosome is monomorphic, measuring 24.9 μm in total length. Cytoplasm is finely granular and homogeneous. Nucleus is small, rounded or oval situated on the posterior part of the body. Kinetoplast is dot-like and situated on the posterior part of the body very close to the nucleus. Undulating membrane is very prominent with 4-6 folds. The free flagellum is about half the length of the body.

Discussion

Numerous workers (Woodcock, 1914, Bennett, 1961) indicated that the relation of the kinetoplast to the nucleus and to the posterior end may be used as a specific criteria for the determination of trypanosome species, as they stained well in Romanowsky type of stains and were subjected to little distorsion. Mackerras and Mackerras (1961) separated on this basis three species of reptilian trypanosomes of about the same size and form from closely related hosts.

The anuran trypanosomes (except *T. rotatorium*) exhibit a restriction in occurrence at their host family level as shown by most of the blood parasites of avian families. A perusal of literature on anuran trypanosomes revealed that there is no trypanosome reported so far from the family Microhylidae to which the frog *Kaloula pulchra taprobanica* belongs.

However, the species under discussion is monomorphic (Table-I) and did not correspond to any known anuran trypanosomes described so far. It has some resemblances to the slender form of *T. loricatum* (Mayer, 1843) and the Juvenile form of *T. rotatorium* (Mayer, 1843) but differs from them by being monomorphic. Moreover, the cytoplasm of the present species is less granular and non-striated in contrast to the coarsely granular and thickly striated cytoplasm of *T. loricatum* and *T. rotatorium*. A comparison of the statistical analysis of the morphometric parameters of the present species with that of *T. loricatum* and *T. rotatorium* is presented in Table-2 and Graph-I. There are significant differences (at 5% level) in some of the important morphological features *viz.*, the length of free flagellum (FF), the body width (BW), the distance between kinetoplast and nucleus (KN) and the kinetoplastic index (KI).

The proposed new species can also be separated from *T. gaumontis* and *T. lavalia* (Fantham *et al.*, 1942) of *Bufo americanus* by having a long free flagellum which is absent in the latter forms.

Therefore, a new name *Trypanosoma taprobanica* sp. nov. is proposed after its host's name to mark its specific identity.

TABLE 1. Morphological measurements of *Trypanosoma taprobanica* sp. nov. from *Kaloula pulchra taprobanica*. Measurements in micrometers taking four from each of the 5 host specimens (SAN 5, PUR 10, SAN 11, PUR 25 & SAN 40) have been represented to show the monomorphic species.

	SAN 5				PUR 10				SAN 11				PUR 25				SAN 40			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
PK	3.0	3.2	3.5	3.1	3.0	3.5	3.5	3.1	3.0	3.5	3.5	3.0	3.5	3.2	3.1	3.0	3.2	3.4	3.0	3.5
KN	1.0	2.0	1.0	1.0	1.5	1.2	1.4	2.0	2.0	1.0	1.0	1.2	1.0	1.5	1.2	2.0	1.0	1.6	1.5	2.0
PN	4.0	5.5	4.5	4.0	4.0	4.5	5.0	4.5	5.5	4.0	4.5	4.0	4.0	4.5	5.0	4.5	4.5	5.0	4.5	4.0
NA	12.5	13.0	10.5	10.5	13.0	12.5	13.5	10.5	12.0	13.5	13.5	13.5	13.5	10.5	13.5	12.0	13.5	13.0	12.5	10.5
PA	15.0	12.5	13.0	15.0	15.0	13.0	13.5	15.5	15.0	13.5	13.0	15.0	15.5	13.5	13.0	15.0	13.5	15.5	15.0	16.4
FF	8.5	8.0	8.2	8.5	8.2	8.0	8.5	8.5	8.0	8.2	8.5	8.5	8.5	8.0	8.5	8.2	8.5	8.0	8.2	8.5
BW	1.5	2.0	2.0	1.8	1.8	2.0	2 0	1.5	2.0	2.0	1.8	1.5	1.8	1.5	2.0	2.0	1.5	2.0	2.0	1.8
Kinetoplast																				
L	0.4	0.5	0.5	0.4	0.5	0.4	0.5	0.4	0.5	0.4	0.5	0.4	0.5	0.4	0.5	0.4	0.5	0.5	0.5	0.4
W	0.4	0.5	0.5	0.4	0.5	0.4	0.5	0.4	0.5	0.4	0.5	0.4	0.5	0.4	0.5	0.4	0.5	0.5	0.5	0.4
KI	2.7	4.0	4.0	3.9	4.0	2.7	3.9	4.0	4.0	3.9	2.7	4.0	2.7	4.0	4.0	3.9	3.9	4.0	4.0	2.7
Nucleus																				
L	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
W	0.5	0.8	0.5	0.5	0.5	0.5	0.5	0.5	0.8	0.5	0.8	0.5	0.5	0.8	0.5	0.8	0.5	0.5	0.5	0.5
NI	0.32	0.42	0.35	0.35	0.42	0.32	0.35	0.35	0.42	0.35	0.32	0.35	0.42	0.32	0.35	0.35	0.42	0.32	0.35	0.35
No. of undulations	4	4	5	6	4	6	5	4	5	6	4	4	4	5	4	6	4	4	5	6

Abbreviations : PK, posterior end to the kinetoplast ; KN, Kinetoplast to the centre of the nucleus ; PN, Posterior end to the centre of the nucleus ; NA, Centre of the nucleus to the anterior end ; PA, posterior end to the anterior end ; FF, length of free flagellum ; BW, Body width, KI, Kinetoplastic index ; NI, Nuclear index.

TABLE 2. Comparative morphological measurements of *T. taprobanica* sp. nov. with slender form of *T. loricatum* and juvenile form of *T. rotatorium*. All measurements in micrometers. Standard deviations in parentheses.

	T. taprobanica (N=20)		*T. loricatum* (N=10)		*T. rotatorium* (N=10)	
	Range	Mean	Range	Mean	Range	Mean
PK	3-3.5	3.2 (0.22)	2.5-4.5	3.5 (0.65)	1-2	1.33 (0.47)
*KN	1-2	1.3 (0.40)	0.5-1	0.9 (0.17)	1.5-2	1.66 (0.23)
PN	4-5.5	4.5 (0.47)	3-5.5	4.4 (0.77)	2.5-4	3.0 (0.70)
NA	10.5-13.5	12.3 (1.18)	10-14	11.85 (1.21)	9-14	10.83 (1.69)
PA	15-18.5	16.6 (1.54)	15.3-17	16.25 (0.62)	11.5-18.0	13.83 (2.19)
*FF	8-8.5	8.3 (0.21)	3-10	5.85 (2.51)	3-7	4.91 (1.30)
*BW	1.5-2	1.82 (0.20)	1.6-2.8	2.34 (0.35)	2-2.5	2.26 (0.23)
Kinetoplast						
Length	0.4-0.5	0.45 (0.07)	0.4-0.5	0.41 (0.03)	0.50-0.75	0.66 (0.11)
Width	0.4-0.5	0.45 (0.07)	0.4-0.5	0.42 (0.03)	0.5-0.6	0.53 (0.04)
*KI	2.75-4	3.65 (0.55)	4-6	4.97 (0.69)	1.66-2.0	1.77 (0.16)
Nucleus						
Length	—	1.0 —	0.9-1.5	1.01 (0.02)	1.8-2.0	1.90 (0.10)
Width	0.5-0.8	0.56 (0.15)	—	5.0 —	1-1.2	1.03 (0.07)
NI	0.32-0.42	0.36 (0.03)	0.21-0.55	0.37 (0.10)	0.20-0.38	0.27 (0.05)

*Significant at 5% level.

Acknowledgements

The authors are very much thankful to Dr. B. K. Tikader, Director, Zoological Survey of India for providing laboratory facilities to one of them (R. R.). Thanks are also due to Dr. A. K. Mandal, Superintending Zoologist and Dr. N. C. Nandi, Zoologist, Zoological Survey of India, for their valuable suggestions during the works.

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