

A new species of *Trypanosoma* (Kinetoplastida: Trypanosomatidae) infecting catsharks from South Africa

E.M. Yeld* and N.J. Smit^{†‡}

*Marine Biology Research Institute, Department of Zoology, University of Cape Town, Rondebosch, 7701, South Africa.

[†]Department of Zoology, University of Johannesburg, PO Box 524, Auckland Park, 2006, South Africa.

[‡]Corresponding author, e-mail: njs@rau.ac.za

During a study on the parasite assemblages of two endemic catshark species, the dark shyshark (*Haploblepharus pictus*) and the puffadder shyshark (*Haploblepharus edwardsii*), from the west and south coasts of South Africa, a trypanosome new to science was observed in the blood of all sharks examined. This trypanosome species, *Trypanosoma haploblephari* sp. nov., is characterized by its large size, particularly its width, the shape and orientation of the cell nucleus, and its geographical location. *Trypanosoma haploblephari* is the first species of elasmobranch trypanosome to be described from southern Africa and represents the first description of a blood parasite from South African sharks.

INTRODUCTION

Parasite community studies on two species of catshark (Elasmobranchii: Scyliorhinidae), the dark shyshark *Haploblepharus pictus* (Müller & Henle, 1838) and the puffadder shyshark *Haploblepharus edwardsii* (Voight, 1832), revealed a trypanosome infecting the peripheral blood of all sharks examined. *Haploblepharus pictus* ranges from Cape Agulhas to the mouth of the Orange River, and *H. edwardsii* from Cape Point to northern KwaZulu-Natal. Both species live in shallow, coastal waters (Branch et al., 1995), are small species of shark, reaching a maximum length of only 60 cm and 70 cm respectively, and are endemic to South Africa.

At least ten species of trypanosomes have been described from elasmobranch species world-wide (Morillas et al., 1987), with more species known from skates and rays than from sharks (Table 1). As far as can be determined, there has been no previous report of trypanosomes from elasmobranchs from the southern African coast. This paper reports on the first trypanosome species from elasmobranchs in this region and provides information on its prevalence in *H. pictus* and *H. edwardsii*.

MATERIALS AND METHODS

Specimens of *Haploblepharus pictus* and *H. edwardsii* were collected from February 2004 to February 2005, at the De Hoop Nature Reserve, Miller's Point and Cape Point on the south and at Granger Bay on the west coasts of South Africa, by SCUBA divers and rod and line fishing. Both species were collected from Miller's Point and Cape Point and only *Haploblepharus pictus* from Granger Bay and *H. edwardsii* from De Hoop Nature Reserve. The sharks were returned live to a marine aquarium at the University of Cape Town where they were measured, sexed, identified with the aid of Smith & Heemstra (1986), anaesthetized with ice and clove oil and sacrificed by pithing. Four

dragged blood smears were made per shark, fixed in absolute methanol, stained with phosphate-buffered Giemsa following standard procedures (Smit & Davies, 2001, 2005), and screened for blood parasites with a Zeiss Axioplan compound microscope. Photomicrographs were obtained with a Zeiss photomicroscopic digital camera system and 55 trypanosomes constituting 15 separate infections were measured with the aid of Zeiss Axioplan Software, Version 4.1. Total body length was taken along the midline of the animal. Posterior end to kinetoplast centre (PK), kinetoplast centre to nucleus centre (KN), nucleus centre to start of free flagellum at anterior end (NA), cell width at nucleus (BWN) and nucleus dimensions (nuclear length=NuL and nuclear width=NuW) were also recorded following Karlsbakk et al. (2005). Measurements are presented as mean \pm standard deviation (minimum–maximum). Blood was fixed in 100% molecular grade ethanol and deposited in the South African Museum for DNA analysis. All sharks collected were also screened for leeches, which, when found, were removed and either preserved or kept alive in marine aquaria for transmission experiments.

RESULTS

The identity, numbers and lengths of sharks captured, and the trypanosome prevalence in each species, are recorded in Table 2. A total of 143 sharks, of two species (*Haploblepharus pictus* and *H. edwardsii*), was infected with trypanosomes, with infections ranging from 11 to 72 flagellates per blood film. All sharks examined were also infected with an unknown species of haemogregarine which will be described elsewhere. Some sharks were found to bear ectoparasitic haematophagous leeches that might act as vectors of these infections. Studies are underway in order to identify the leech species and to determine its role as possible vector.

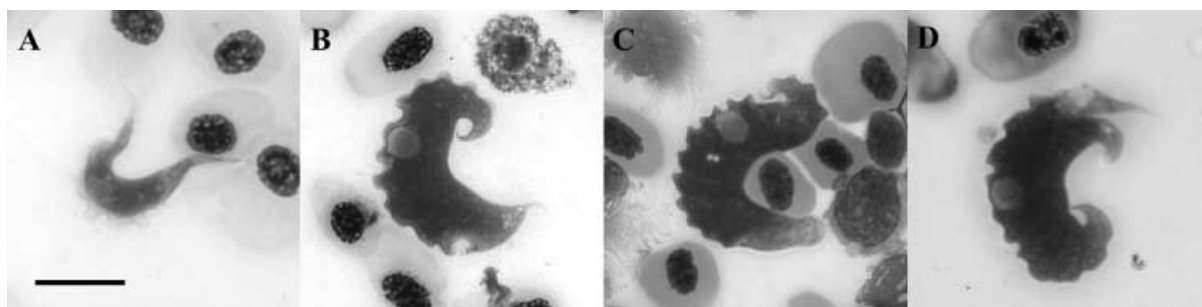


Figure 1. Light micrograph pictures of *Trypanosoma haploblephari* sp. nov. from the type host *Haploblepharus pictus* (Slide SAM A25081): (A) small stage with short free flagellum; (B) large stage with distinct kinetoplast and undulating membrane; (C) large stage with rounded, blunt posterior end; and (D) possible division in peripheral blood. Scale bar: 20 μm .

SYSTEMATICS

Class MASTIGOPHORA Diesing, 1866
 Order KINETOPLASTIDA Honigberg, 1963
 Suborder TRYPANOSOMATINA Kent, 1880
 Family TRYPANOSOMATIDAE Doflein, 1911
 Genus *Trypanosoma* Gruby, 1843
Trypanosoma haploblephari sp. nov.
 (Figures 1 & 2)

Type material

Syntype: one blood film with 72 trypanosomes (South African Museum, Cape Town; SAM A25081; Granger Bay, Western Cape, South Africa; coordinates: 33°52'S 18°24'E). Collected by E.M. Yeld, 20 January 2005.

Twenty-five additional slides with trypanosomes in the collection of N.J. Smit in the Department of Zoology, University of Johannesburg.

Blood with trypanosomes from *Haploblepharus pictus* and *Haploblepharus edwardsii* in 100% molecular grade ethanol (South African Museum, Cape Town; SAM A25081 and SAM A25081; Miller's Point, False Bay, Western Cape, South Africa; coordinates: 34°14'S 18°29'E). Collected by E.M. Yeld, 27 January 2006.

Type host

Haploblepharus pictus (Müller & Henle, 1838).

Other host: *Haploblepharus edwardsii* (Voight, 1832).

Vector

Unknown, but possibly the unidentified hirudinean present on the sampled hosts.

Diagnosis

Large deep blue staining trypanosome (53.7–99.4 μm body length) with short flagellum (6.9 μm). Body wide (12.6–24.3 μm), distinct undulating membrane with 8–16 undulations. Kinetoplast prominent, situated on average 16.8 μm from posterior. Nucleus circular with prominent karyosome and in anterior half of animal. Longitudinal striations visible on larger specimens.

Description

Trypanosomes found in the blood films taken from *Haploblepharus pictus* and *H. edwardsii* are morphometrically and morphologically indistinguishable. All the trypanosomes collected from *H. edwardsii* fall within the size-ranges of those from *H. pictus* (see below). The cytoplasm,

kinetoplast and nucleus are basophilic and stain deep blue with Giemsa, with numerous chromatic granules visible in the cytoplasm. The karyosome is prominent within the nucleus (Figures 1B–D & 2). The undulating membrane is richly spiralling, with numerous undulations (8–16), and is well developed and easily stained (Figure 1B). The width of the undulating membrane fluctuates between 1 and 4 μm . A distinct, very short free flagellum is observed, but not easily stained or measured, even when over stained. A distinct kinetoplast (Figures 1B & 2), approximately 2 μm in diameter, is found nearly halfway to the nucleus from the posterior end of the body, PK=16.8 $\mu\text{m} \pm 4.5$ or 23.9% of body length (range 6.9–45.6 μm) for *H. pictus* parasites and PK=15.5 $\mu\text{m} \pm 4.2$ or 23.3% of body length (range 8.3–40.3 μm) for *H. edwardsii* parasites. The posterior end in smaller stages tends to be slender, elongated and pointed (Figure 1A), whereas the larger stages have a more variable posterior end shape, often tending to be blunt and rounded (Figure 1C). Body length 70.4 $\mu\text{m} \pm 9.4$ (range 53.7–99.4 μm) (N=55); body width (including the undulating membrane) BWN=17.4 $\mu\text{m} \pm 2.6$ (12.6–24.3 μm) (N=55) for *H. pictus* trypanosomes and 66.9 $\mu\text{m} \pm 10.6$ (range 59.7–91.2 μm) (N=55); body width (including the undulating membrane) BWN=15.2 $\mu\text{m} \pm 2.2$ (11.3–21.4 μm) (N=55) for *H. edwardsii* parasites. Nucleus approximately circular,

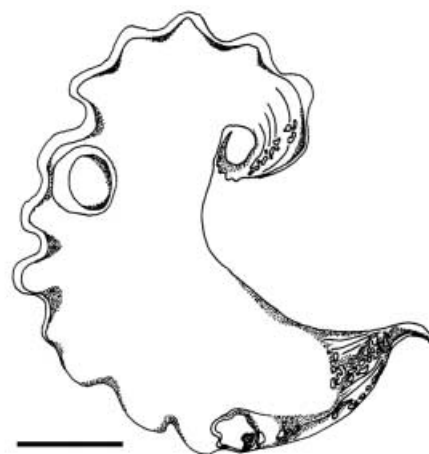


Figure 2. Line drawing of *Trypanosoma haploblephari* sp. nov. from the type host *Haploblepharus pictus* (Slide SAM A25081). Scale bar: 10 μm .

Table 1. Trypanosome species described from elasmobranchs, detailing host species, location, original description reference and morphometrics. All measurements given in micrometres (μm); mean \pm standard deviation (minimum–maximum) where appropriate.

Trypanosome species	Host species	Location	Reference	Body length	Flagellum length	Width	Nucleus length	Nucleus width
<i>Trypanosoma gargantua</i>	<i>Raja nasuta</i>	South Pacific	Laird, 1951	66.7–131.1	absent	4.6–13.7	3.9–9.3	3.3–6.7
<i>T. taeniurae</i>	<i>Taeniura lymna</i>	Heron Island, Australia	Burreson, 1989	55	9	4	3.5	4.5
<i>T. torpedinis</i>	<i>Torpedo marmorata</i>	South-west France	Sabrazes & Muratet, 1908	n/a	n/a	n/a	n/a	n/a
<i>T. rajae</i>	<i>Raja</i> spp.	Northern Atlantic	Laveran & Mesnil, 1902	55–60	20	6	3	2.2
<i>T. marplatensis</i>	<i>Psammotus microps</i>	Atlantic, off Argentina	Bacigalupo & de la Plaza, 1948	60–65	up to 15	10–12	7.2	4.2
<i>T. giganteum</i>	<i>Raja oxyrhynchus</i>	Mediterranean Sea	Neumann, 1909	100	25–30	8	3–5	2.5–4
<i>T. carcharias</i>	<i>Odontaspis</i> sp.	South Pacific	Laveran, 1908 (in Wenyon, 1926)	35–40	25–30	n/a	n/a	n/a
<i>T. scylli</i>	<i>Scyliorhinus stellaris</i>	Roscoff, France	Laveran & Mesnil, 1902	54–61	14	5–6	n/a	n/a
<i>T. mackerrasi</i>	<i>Hemiscyllium ocellatum</i>	Heron Island, Australia	Burreson, 1989	125	absent	15	7	10
<i>T. boissoni</i>	<i>Zanobatus schoeleini</i>	Cape Verde, Senegal	Ranque, 1973	60.1 (45–67)	1.3 (0–7)	4.6 (3.3–7)	n/a	n/a
<i>T. humboldti</i>	<i>Schroederichthys chilensis</i>	Pacific coast, Chile	Morillas et al., 1987	87.0 \pm 3.8 (78–93)*		7.4 \pm 1.5 (4–10)		5.3 \pm 0.4 (5–6)
<i>T. haploblephari</i>	<i>Haploblepharus pictus</i>	Atlantic, off South Africa	This paper	70.4 \pm 9.4 (53.7–99.4)	n/a	17.4 \pm 2.6 (12.6–24.3)	6.5 \pm 0.8 (5.2–8.8)	6.5 \pm 1.1 (4.7–8.8)

n/a, not available; *, includes free flagellum length.

Table 2. Identity, number and length of sharks captured from the west and south coasts of South Africa; and prevalence of trypanosome blood parasites in these sharks.

Species	Sharks		Trypanosomes
	N	ML \pm SD (range) in mm	Prevalence (%)
<i>Haploblepharus pictus</i>	90	411.6 \pm 89.5 (256–672)	90/90 (100)
<i>Haploblepharus edwardsii</i>	53	547.9 \pm 105.9 (309–662)	53/53 (100)

N, number; ML, mean length; SD, standard deviation.

NuL=6.5 μ m \pm 0.8 (5.2–8.8 μ m) and NuW=6.5 μ m \pm 1.1 (4.7–8.8 μ m) (N=55) in *H. pictus* parasites and NuL=6.1 μ m \pm 0.7 (5.2–8.1 μ m) and NuW=6.0 μ m \pm 0.9 (4.6–8.0 μ m) (N=55) in *H. edwardsii* trypanosomes. The location of the nucleus NA=34.7 μ m \pm 4.2 (25.7–45.6 μ m) from the anterior end, or 49.3% of the body length, thus lies just anterior to the midpoint of the body for the majority of specimens examined. Kinetoplast to nucleus KN=18.9 μ m or 26.9% body length. Longitudinal striations sometimes visible on the more deeply stained, larger specimens, particularly over the nuclear area (Figure 1C).

Remarks

Given the reported morphometrics of this trypanosome, the only possible overlap would be with the largest reported trypanosome species, such as *Trypanosoma giganteum* Neumann, 1909 and *T. gargantua* Laird, 1951. *Trypanosoma haploblephari* is markedly different from both these trypanosomes, since *T. giganteum* has a length of up to 95–100 μ m, a width of only 8 μ m, a free flagellum of up to 30 μ m, and a wider undulating membrane of up to 6 μ m; whereas *T. gargantua* measures 66.7–131.1 \times 4.6–13.7 μ m. Since *T. haploblephari* measures 70.4 μ m \pm 9.4 (53.7–99.4 μ m) in length with a body width (including the undulating membrane) of 17.4 μ m \pm 2.6 (12.6–24.3 μ m), it clearly differs from the aforementioned two, most strikingly in body width. The shape and size of the nucleus also separates *T. haploblephari* from the other trypanosome species, being very large and circular. *Trypanosoma mackerrasi* Burreson, 1989, although overlapping in some measurements, differs clearly in that it has a much longer body, a larger and distinctly oval nucleus, and no free flagellum (Burreson, 1989). Although measurements for the free flagellum of our species were difficult to obtain, it is present and visible (Figure 1A). *Trypanosoma haploblephari*, unusually, does not display any notable pleomorphism in the specimens examined. Interestingly, dividing forms have been found in the peripheral blood in this study (Figure 1D), whereas other studies found no dividing forms in peripheral blood, kidney or spleen (Pulsford, 1984). Other reported species of trypanosome do not overlap with the morphometrics of *T. haploblephari* (Table 1), and given the features described, its geographical location and the endemism of the host shark species, we propose it as a new species.

Etymology

The species name is derived from the genus of host shark.

DISCUSSION

A thorough comparison of the morphometrics from elasmobranch trypanosomes is difficult due to the paucity of valid data available, and, since there are only minor morphological differences among many trypanosome species, they may still prove to be synonymous with biochemical characterization (Pulsford, 1984). When considering factors influencing similarity between species this is further complicated, since geographical proximity, which is usually given priority, does not have any significance in this case where the type location is so far removed from any prior elasmobranch descriptions.

The most marked pleomorphism examples can be drawn from marine species, for example *Trypanosoma gargantua*, *T. giganteum*, *T. rajae* Laveran & Mesnil, 1902 and *T. murmanensis* Nikitin, 1927. *Trypanosoma rajae* is the best-known example of extreme polymorphism, which prompted Minchin & Woodcock (1910) to propose *T. variabile* Neumann, 1909 as a junior synonym of it (Laird & Bullock, 1969). There are a few fish trypanosomes which seem to be virtually monomorphic with variability displayed only in size and/or width to length ratio.

This infection of *T. haploblephari* is unique in that sharks from all size and age categories were infected, unlike previous studies where only rays (family Rajidae) less than 500 g were infected with *T. giganteum* (Aragort et al., 2005), or only the larger classes of dogfish *Scyliorhinus canicula* infected with *T. scylli* (Pulsford, 1984). Also, unlike previous studies, parasitaemia is relatively high, with a mean of 11 trypanosomes per blood smear (although infections of up to 72 per smear have been observed) as opposed to infection rates of 0–2 (Aragort et al., 2005) or 1–4 (Pulsford, 1984).

Piscicolid leeches are the only known vectors of marine fish trypanosomes, although the vectors of most described trypanosome species have not been identified (Molyneux, 1977; Jones & Woo, 1991). However, not all leeches transmit trypanosome infections (Khan et al., 1980) and thorough transmission experiments need to be carried out before the vector can be conclusively determined. Life near the bottom (more benthic-oriented fish, such as the shark hosts *Haploblepharus pictus* and *H. edwardsii*) increases exposure to leeches, and therefore to the transmission of trypanosomes and other blood parasites. Khan et al. (1980) showed that there was a higher prevalence of trypanosome infection observed in cold-water, benthic, sedentary fish. In other words, both ecological and behavioural factors of the vertebrate and invertebrate hosts have an effect on marine fish blood infections.

An unidentified leech species has been noted on sharks infected with *T. haploblephari*. Although this has not been conclusively shown to be a vector, possibly it can transmit *T. haploblephari*, since leeches are known to transmit haematozoans. A study is currently underway to test this hypothesis. There is no apparent correlation between the prevalence of the trypanosome infection (100%) and the leech infestation levels in the sharks examined, but this

can possibly be attributed to the behaviour of leeches, which detach from their hosts after feeding (Morillas et al., 1987).

According to Lom & Dykova (1992) sharks and rays are known to host some of the largest trypanosomes known. Our discovery of *T. haploblephari* in the blood of South African catsharks further emphasizes this phenomenon.

The authors thank the Research Diving Unit and the Zoology Department (University of Cape Town), and Dr C.G. Attwood and the De Hoop Tag & Release angling team, for assistance in data collection. This work was funded through the Sea and Coast Programme of the National Research Foundation and the University of Johannesburg's SASOL Research Fund.

REFERENCES

- Aragort, W., Alvarez, M.F., Leiro, J.L. & Sanmartin, M.L., 2005. Blood protozoans in elasmobranchs of the family Rajidae from Galicia (NW Spain). *Diseases of Aquatic Organisms*, **65**, 63–68.
- Bacigalupo, J. & Plaza, N. de la, 1948. Presencia de tripanosomas en las rayas de Mar del Plata. *Revista de la Sociedad Argentina de Biología*, **24**, 269–274.
- Branch, G.M., Griffiths, C.L., Branch, M.L. & Beckley, L.E., 1995. *Two oceans: a guide to the marine life of Southern Africa*. Claremont, South Africa: David Philip Publishers.
- Burreson, E.M., 1989. Haematozoa of fishes from Heron I., Australia, with the description of two new species of *Trypanosoma*. *Australian Journal of Zoology*, **37**, 15–23.
- Jones, S.R.M. & Woo, P.T.K., 1991. Development and infectivity of *Trypanosoma phaleri* in leech and fish hosts. *Canadian Journal of Zoology*, **69**, 1522–1529.
- Karlsbakk, E., Haugen, E. & Nylund, A., 2005. Morphology and aspects of growth of a trypanosome transmitted by the marine leech *Johanssonia arctica* (Piscicolidae) from Northern Norway. *Folia Parasitologica*, **52**, 2092–3015.
- Khan, R.A., Barrett, M. & Murphy, J., 1980. Blood parasites of fish from the northwestern Atlantic Ocean. *Canadian Journal of Zoology*, **58**, 770–781.
- Laird, M., 1951. Studies on the trypanosomes of New Zealand fish. *Proceedings of the Zoological Society of London*, **121**, 2853–2909.
- Laird, M. & Bullock, W., 1969. Marine fish haematozoa from New Brunswick and New England. *Journal of the Fisheries Research Board of Canada*, **26**, 1075–1102.
- Laveran, A., 1908. Sur une haemogregarine, un trypanosome et un spirille trouves dans le sang d'un requin. *Bulletin de la Société de Pathologie Exotique*, **1**, 148–150.
- Laveran, A. & Mesnil, F., 1902. Des trypanosomes des poisons. *Archiv für Protistenkunde*, **1**, 496–498.
- Lom, J. & Dykova, I., 1992. *Protozoan parasites of fish*. Amsterdam: Elsevier. [*Developments in Aquaculture and Fisheries Science*, vol. 26.]
- Minchin, E.A. & Woodcock, H.M., 1910. Observations on certain blood parasites of fish occurring at Rovigno. *Quarterly Journal of Microscopical Science*, **55**, 113–154.
- Molyneux, D.H., 1977. Vector relationships in the Trypanosomatidae. *Advances in Parasitology*, **15**, 1–82.
- Morillas, J., George-Nascimento, M., Valeria, H. & Khan, R.A., 1987. *Trypanosoma humboldti* n. sp. from the Chilean catshark, *Schroederichthys chilensis* (Guichenot, 1848). *Journal of Protozoology*, **34**, 342–344.
- Neumann, R.D., 1909. Studien über protozoische Parasiten im Blut von Meeresfischen. *Zeitschrift für Hygiene und Infektionskrankheiten*, **63**, 1–112.
- Pulsford, A., 1984. Preliminary studies on trypanosomes from the dogfish, *Scyliorhinus canicula* L. *Journal of Fish Biology*, **24**, 671–682.
- Ranque, P., 1973. *Etudes morphologique et biologique de quelques trypanosomes recoltés au Senegal*. PhD thesis, University of Marseille, France.
- Smit, N.J. & Davies, A.J., 2001. An encapsulated haemogregarine from the evileye pufferfish in South Africa. *Journal of the Marine Biological Association of the United Kingdom*, **81**, 751–754.
- Smit, N.J. & Davies, A.J., 2005. Intraerythrocytic merogony in the development of *Haemogregarina koppiensis* (Apicomplexa: Adeleorina: Haemogregarinidae). *Folia Parasitologica*, **52**, 277–278.
- Smith, M.M. & Heemstra, P.C., 1986. *Smith's sea fishes*. Cape Town, South Africa: Struik Publishers.
- Wenyon, C.M., 1926. *Protozoology: a manual for medical men, veterinarians and zoologists*, vol. 1. London: Baillière, Tindall and Cox.

Submitted 21 October 2005. Accepted 9 March 2006.