Notes on the occurrence of *Trypanosoma* sp. (Kinetoplastida: Trypanosomatidae) in freshwater fishes from South Africa

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A total of 257 fishes from four families, Clariidae, Cichlidae, Cyprinidae and Schilbeidae were collected from three localities: the Sand River Dam, Swaziland; the Nylsvlei Nature Reserve, South Africa and the Vaal Dam and Vaal River Barrage, South Africa. Only fishes (n = 154) from Clariidae and Cichlidae were found to be infected with trypanosomes. A total of 221 *Clarias gariepinus* (Burchell 1822) were collected from the Vaal Dam and Vaal Barrage area, South Africa. Of these, 74% (89/121) were infected with trypanosomes from the Vaal Dam and 63% (63/100) from the Vaal River Barrage, with no seasonal infection pattern. A prevalence of 25% (1/4) was found in *C. gariepinus* from the Sand River Dam, Swaziland, and a 50% (1/2) prevalence was found in *Tilapia sparrmanii* from the Nylsvlei Nature Reserve, South Africa. Standard measurements conformed closely to the morphometric and morphological descriptions of *Trypanosoma mukasai*. This article provides new locality records for *T. mukasai* from the Vaal Dam, Vaal River Barrage and Nylsvlei Nature Reserve (South Africa) and the Sand River Dam (Swaziland). *Tilapia sparrmanii* collected in the Sand River Dam in Swaziland is also noted as a new host record.

Communication

Since the first piscine trypanosome was discovered from the blood of *Salmo fario* by Valentin in 1841, more than 190 trypanosome species have been recorded from around the world (Woo 1998). Several authors have recorded piscine trypanosomes from many of the major freshwater systems of Africa (Baker 1960, 1961), however, only three known species are noted from Africa: *Trypanosoma toddi* Bouet, 1909; *Trypanosoma mukasai* Hoare, 1932 and *Trypanosoma tobeyi* Dias, 1952. Baker (1960) noted that nuclear position might be an important factor in separating the three African freshwater fish trypanosome species. It was noted that small morphological differences separate *T. toddi* from *T. tobeyi* and that *T. mukasai* and *T. tobeyi* might prove to be a synonym of the same trypanosome (Baker 1960). In 1962, Pienaar reported *Trypanosoma clariense* from *Clarias gariepinus* (Burchell, 1822) in the North West Province (Pienaar 1962). This species was first described by Fantham (1919) from the same host. It was suggested by Smit, Van As and Davies (2004) that *T. clariense* might be a synonym of *T. mukasai*, owing to the apparent common staining properties and morphometrics. *Trypanosoma mukasai* was originally described from four species of cichlids (Hoare 1932) and has since been known to infect a broad range of fish hosts across Africa (Negm-Eldin 1997, 1998). Some of the most recent records are those of Davies and Van As (2000), Smit, Van As and Davies (2004), Smit, Moll and Van der Bank (2008) and Hussein *et al.* (2010). According to Smit, Van As and Davies (2004), morphometrically variable haemoflagellates were found within a single host from the Okavango Delta, Botswana. However, despite these findings, they concluded that all Okavango trypanosomes examined might be *T. mukasai* due to their nuclear indices NI > 1. Since these findings, Davies *et al.* (2005) reported that two genotypic groups of trypanosomes (even though morphologically similar) are present in the fish from the Okavango. A great deal of molecular research has been done in recent years regarding the phylogenetic relationships of trypanosomes (Davies *et al.* 2005; Gibson *et al.* 2005; Gu *et al.* 2007, 2010; Jakes, O'Donoghue & Adlard 2001; Karlsbakk, Haugen & Nylund 2006; Martin *et al.* 2002; Seghal, Jones & Smith 2001; Votypka *et al.* 2002; Votypka, Lukes & Obornik 2004). The aim of this article is to increase the distribution and host data of a species of trypanosome preliminarily identified as *T. mukasai.*

Fishes were collected using fishing rods from the Sand River Dam (25° 59′ 16″ S, 31° 42′ 16″ E) (channelled from the Inkomati River), Swaziland (February 2012) and with gills nets from the Nylsvlei Nature Reserve (24° 39′ 17.28″ S, 28° 41′ 27.6″ E), Limpopo Province, South Africa (May 2012); Vaal Dam (26° 53′ 40.99″ S, 28° 8′ 43.98″ E) and Vaal River Barrage (26° 45′ 53.28″ S, 27° 41′ 30.12″ E) from the Vaal River System, South Africa (November 1998 to February 2000 and February 2012). Fish were identified (see Table 1) and blood films were prepared, air dried, fixed with methanol and stained with phosphate-buffered Giemsa solution prior to screening (Davies

et al. 2005). Microscopic images of the blood protozoans were captured and the organisms were measured with a 100x oil immersion objective on a Zeiss Axioplan 2 photomicroscope. Standard measurements of the haemoflagellates were taken according to the method of Karlsbakk (2006). No possible leech vectors were observed.

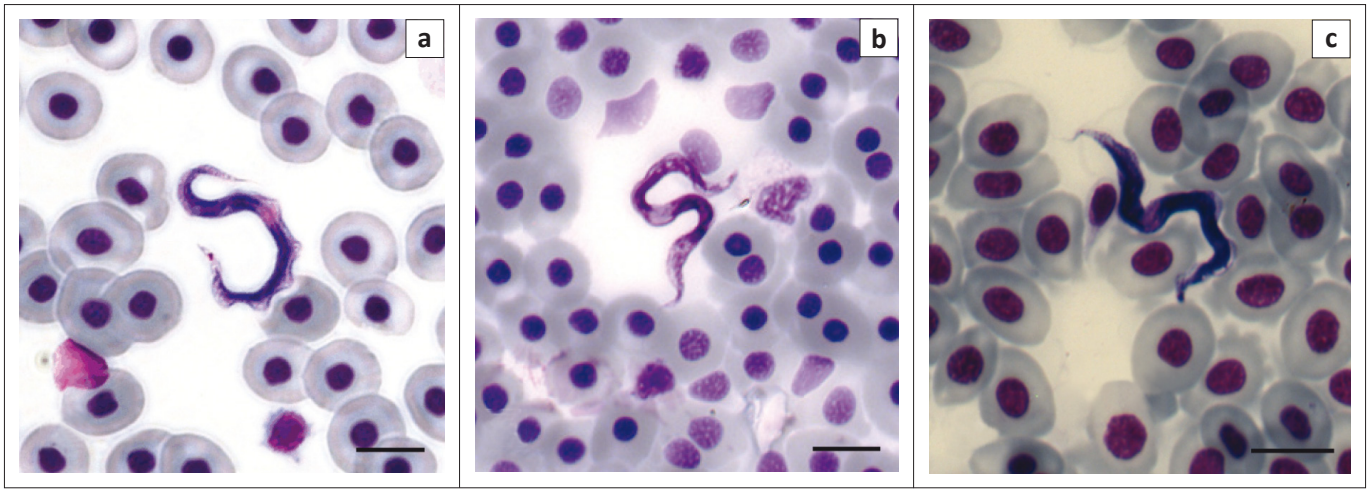
Of the 257 fishes collected, positive infections were observed in two families, Clariidae and Cichlidae. Fish numbers and prevalence are recorded in Table 1. No seasonal pattern was notable. Out of the *Clarias gariepinus* collected from the Vaal Dam, 74% (89/121) were infected and from the Vaal Barrage 63% (63/100) were infected (Table 1). A total of 427 parasites were counted in the Vaal Dam over four months, with 262 parasites counted in the Vaal River Barrage. Parasite intensities were not calculated due to the variation in blood volume used for blood film preparation. There was no seasonal pattern observed with regard to prevalence. All 60 *Labeo capensis*, 57 *Labeo umbratus* and 35 *Cyprinus carpio* collected during the same sample trip were uninfected.

Of the 26 fishes collected from the four different piscine families in the Sand River Dam, Swaziland, 25% (1/4) of *C. gariepinus* were infected with trypanosomes (Table 1).

TABLE 1: Fish species collected, total number of fish, prevalence and total number of fish hosts infected with trypanosomes from the Sand River Dam, Swaziland, Nylsvlei Nature Reserve, South Africa, Vaal Dam and Vaal Barrage, South Africa.

Locality	Fish family	Fish species collected	*n*	Prevalence		Total number of parasites
				Number infected	%	
Sand River Dam, Swaziland	Cichlidae	*Oreochromis macrochir* (Boulenger, 1912)	3	0/3	0	-
		Oreochromis mossambicus (Peters, 1852)	4	0/4	0	-
		Serranochromis robustus (Günther, 1864)	3	0/3	0	-
		Tilapia rendalli (Boulenger, 1896)	3	0/3	0	-
		Tilapia sparrmanii (Smith, 1840)	1	0/1	0	-
	Clariidae	*Clarias gariepinus* (Burchell, 1822)	4	1/4	25	1
	Cyprinidae	*Barbus trimaculatus* (Peters, 1852)	4	0/4	0	-
	Schilbeidae	*Schilbe intermedius* (Rüppell, 1832)	4	0/4	0	-
		Total	**26**	**1/26**	**4**	**1**
Nylsvlei Nature Reserve, South Africa	Cichlidae	*Tilapia sparrmanii* (Smith, 1840)	2	1/2	50	2
		Oreochromis mossambicus (Peters, 1852)	2	0/4	0	-
	Clariidae	*Clarias gariepinus* (Burchell, 1822)	4	0/4	0	-
		Total	**10**	**1/10**	**10**	**2**
Vaal Dam, South Africa	Clarridae	*Clarias gariepinus* (Burchell, 1822)	121	89/121	74	427
	Cyprinidae	*Labeo capensis* (Smith, 1841)	20	0/20	0	-
		Labeo umbratus (Smith, 1841)	36	0/36	0	-
		Cyprinus carpio (Linnaeus, 1758)	10	0/10	0	-
		Total	**187**	**89/187**	**48**	**427**
Vaal River Barrage, South Africa	Clariiidae	*Clarias gariepinus* (Burchell, 1822)	100	63/100	63	262
	Cyprinidae	*Labeo capensis* (Smith, 1841)	40	0/36	0	-
		Labeo umbratus (Smith, 1841)	21	0/21	0	-
		Cyprinus carpio (Linnaeus, 1758)	25	0/25	0	-
		Total	**186**	**63/186**	**34**	**262**

n, number of fish caugh.



Scale bars, 10 µm.

FIGURE 1: Light micrographs of Giemsa stained *Trypanosoma* sp. (a) Trypomastigote stage in *Clarias gariepinus* Burchell, 1822 blood film from the Vaal Dam, South Africa. (b) Trypomastigote stage in *Clarias gariepinus* blood film from the Sand River Dam, Swaziland. (c) Trypomastigote stage in *Tilapia sparrmanii* Smith, 1840 blood film from the Nylsvlei Nature Reserve, South Africa.

TABLE 2: Morphometrics of trypanosomes collected at three sampling sites (Sand River Dam, Swaziland, Nylsvlei Nature Reserve, South Africa and Vaal Dam/Vaal Barrage, South Africa).

Site	Fish Family	Fish host	*n*	BL		FL		OL		BW		NL		NW		MA		MP		Mean ± SD (NI)	Range (FI)
				Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range		
Nylsvlei Nature Reserve, South Africa	Cichlidae	*Tilapia sparmanii*	2	39.1 ± 5.5	35.2–43.1	7.0 ± 4.3	4–10.1	46.2 ± 1.2	45.3–47	2.8 ± 0.4	2.5–3.1	2.7 ± 0.6	2.3–3.1	2.7 ± 0.4	2.4–3	16.2 ± 4.5	13–19.4	23.1 ± 1.9	21.7–24.4	1.5	7.8
Vaal Dam/ Vaal Barrage, South Africa	Clariidae	*Clarias gariepinus*	25	40.1 ± 5.5	25.4–50.4	5.2 ± 2.3	0.9–11.1	45.3 ± 6.8	27.1–54.5	1.6 ± 0.5	0.8–3.1	2.8 ± 0.6	1.9–4.1	1.6 ± 0.5	0.8–3.1	20.7 ± 2.9	13.4–27.6	20.3 ± 2.6	16.1–25.1	1	9.4
Sand River Dam, Swaziland	Clariidae	*Clarias gariepinus*	1	42.6		1.6		44.2		1.8		3.3		2.8		21.2		21.1		1	14

n, number of trypanosomes measured; BL, body length; SD, standard deviation; FL, flagellum length; OL, overall body length; BW, body width; NL, nucleus length; NW, nucleus width; MA, mid nucleus to anterior; MP, mid nucleus to posterior; NI, nucleus index; FI, flagellum index.

Ten fish from two families were collected at Nylsvlei Nature Reserve, South Africa. A 50% (1/2) prevalence was found in *Tilapia sparrmanii*, with two parasites present (Table 1). Trypomastigote sizes varied between the three sites (see Table 2). The cytoplasm is stained dark blue with a pale section anterior to the kinetoplast, the nucleus is stained light pink and the flagellum (undulating membrane) originating near the kinetoplast is trailing anteriorly beyond the body as a free flagellum. The tail is short and almost absent (see Figure 1a–c). Nuclear indices (NI values) (Becker & Overstreet 1979) for trypanosomes measured at all three sites were NI > 1, indicating an anteriorly positioned nucleus (Table 2).

As reported by Hoare 1932, *T. mukasai* measured 40 µm – 55 µm in length, with the 'nucleus near the middle of the body and the kinetoplast at the posterior end. The undulating membrane was strongly developed and a short free flagellum is present' (see Hoare 1932). Trypomastigotes measured in the current study have similar body lengths, a nucleus just in front of the midline, posterior kinetoplast and prominent undulating membranes. From this, it can be concluded that the *Trypanosoma* sp. in the current study resembles and possibly is *T. mukasai*, which can be confirmed by molecular sequencing. Future research should include molecular assessment of trypanosomes from all three sampling sites. It is evident that a phylogenetic analysis of all freshwater fish trypanosomes of Africa is long overdue, especially to resolve the confusion regarding the synonymy of species. Sand River Dam, Swaziland, Nylsvlei Nature Reserve, South Africa, Vaal Dam and Vaal River Barrage, South Africa serve as new locality records for *T. mukasai*, and *T. sparmanii* as a new host record.

Ethical considerations

The parasites were obtained from fish sampled for the approved projects by Mr Beric Gilbert and Mr Ebi Hussain. Ethical clearance was obtained from the University of Johannesburg Faculty Ethics Committee in 2011 for both these projects. Fish were sampled according to the guidelines of a permit obtained from the Gauteng Nature Conservation for Annemariè Avenant-Oldewage and from the Limpopo Nature Conservation for R. Greenfield. The fish from Swaziland were caught during a fishing competition. Potential health and safety hazards were disclosed to the Ethics Committee. Staff and students were covered by the universitity's 3rd party insurance. All researchers participated voluntarily.

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Author's contributions

M.L.F. (University of Johannesburg) studied slides from all localities, stained slides from all localities apart from the Vaal River and provided the first draft of the manuscript. A.A.O. (University of Johannesburg), provided all of the Vaal River data and slides, and collaborated on various drafts of the manuscript.

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