

Research Article

Zoobank number for Article:
urn:lsid:zoobank.org:pub:B907C84C-5221-
4E0B-9D63-7FF1F7C54F6C

Cite this article: Le Roux C, Cook CA, Netherlands EC, Truter M, Smit NJ (2025) Molecular and morphological characterization of one known and three new species of fish parasitic *Trypanosoma* Gruby, 1972 from the south coast of South Africa. *Parasitology* **152**, 531–550. <https://doi.org/10.1017/S0031182025000496>



Received: 31 October 2024
Revised: 15 February 2025
Accepted: 17 March 2025
First published online: 8 April 2025

Keywords:

blood parasites; life below water; marine fish; molecular; morphology; trypanosomiasis

Corresponding author: Nico J. Smit;
Email: nico.smit@nwu.ac.za

Molecular and morphological characterization of one known and three new species of fish parasitic *Trypanosoma* Gruby, 1972 from the south coast of South Africa

Chandra Le Roux¹, Courtney A. Cook¹, Edward C. Netherlands^{1,2} ,
Marliese Truter^{1,3} and Nico J. Smit^{1,3} 

¹Water Research Group, Unit for Environmental Sciences and Management, North-West University – Potchefstroom Campus, Potchefstroom, South Africa; ²Department of Zoology and Entomology, University of the Free State, Bloemfontein, South Africa and ³NRF-South African Institute for Aquatic Biodiversity, Makhanda, South Africa

Abstract

The genus *Trypanosoma* Gruby, 1843 encompasses unique, flagellate haemoparasites infecting all vertebrate classes globally (excluding Antarctica). While trypanosomes in terrestrial mammals are well-studied due to their medical and veterinary significance, those in fishes remain largely unexplored, with limited data on their life cycles and ecological roles. Furthermore, the phylogenetic relationships of numerous aquatic species are unresolved. This gap is notable in South Africa, a region with high marine fish biodiversity, yet only 2 documented marine trypanosome species, 1 in teleosts and 1 in elasmobranchs are known. Our research aims to bridge this knowledge gap for marine fish trypanosomes along South Africa's southern coast. Blood samples were collected from 246 fishes spanning 23 species at Chintsa East, Tsitsikamma (Garden Route National Park), Boknes, Kariega River Estuary and Groot River West Estuary from 2020 to 2023. Giemsa-stained blood smears were screened for trypanosomes, which were morphologically characterized. Molecular analyses targeting the 18S rRNA gene region were conducted on blood samples positive for trypanosomes. Combined morphological and molecular evidence identified 4 *Trypanosoma* species: 1 known species, *Trypanosoma nudigobii* from the klipfish (*Clinus superciliosus*) and 3 new species: *Trypanosoma* sp. A from the prison goby (*Caffrogobius gilchristi*), *Trypanosoma bakana* n. sp. from the white steenbras (*Lithognathus lithognathus*) and *Trypanosoma bokkom* n. sp. from 5 mullet species [the grooved mullet (*Chelon dumerili*), South African mullet (*Chelon richardsonii*), striped mullet (*Chelon tricuspidens*), flathead grey mullet (*Mugil cephalus*) and the freshwater mullet (*Pseudomyxus capensis*)].

Introduction

Marine trypanosomes are single-celled eukaryotic organisms belonging to the phylum Euglenozoa (Kinetoplastida). Often overshadowed by their terrestrial counterparts, such as the well-known *Trypanosoma brucei* (Plimmer and Bradford, 1899), which causes human sleeping sickness, marine trypanosomes have recently emerged as an intriguing topic of scientific inquiry (Hoare, 1972; Karlsbakk et al., 2005; Hayes et al., 2006, 2014; Yeld and Smit, 2006; Su et al., 2014; Pretorius et al., 2021). These parasites have been documented in various marine environments worldwide, ranging from coastal regions to the open ocean (Bureson, 2007; Hayes et al., 2014). Understanding their ecological roles and potential impact on marine ecosystems is essential to advance our knowledge of marine parasitology and ecosystem health. Although most trypanosome life cycles are unknown, leeches have been identified as the invertebrate hosts or vectors for some marine and freshwater fish trypanosomes (Hayes et al., 2014; Smit et al., 2020). Leal et al. (2009) proposed that the dissonance in the characterization of species of *Trypanosoma* Gruby, 1843 is due to the extreme polymorphism that may be found in a single species, as well as individual species being able to infect numerous vertebrate and invertebrate host species. Additionally, by restudying trypanosomes using fresh material from type hosts and utilizing modern methods such as molecular data, additional inferences on species identification can be obtained. This approach may help infer likely synonymies of species described in different hosts or geographical regions (Leal et al., 2009; Hayes et al., 2014). The inclusion of non-human trypanosome molecular data in phylogenetic analyses is important for better understanding of evolutionary and ecological relationships of all the host species infected with trypanosomes. The most significant division in the phylogenetic relationships of non-human trypanosomes is between the species infecting terrestrial and aquatic

© The Author(s), 2025. Published by Cambridge University Press. This is an Open Access article, distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives licence (<http://creativecommons.org/licenses/by-nc-nd/4.0>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided that no alterations are made and the original article is properly cited. The written permission of Cambridge University Press must be obtained prior to any commercial use and/or adaptation of the article.

vertebrates. Based on phylogenetic evidence of 18S rRNA, amphibian and fish trypanosomes are the origin from which all other trypanosomes are derived (Hamilton et al., 2007; Fermino et al., 2019). Despite the value of the division of the clades, the utilization of subgenera (see Kostygov et al., 2021) has been largely disregarded, possibly due to the expectation of conflicts between morphology- and phylogeny-based systems that did not complement each other. The subgenus *Haematomonas* (Mitrophanow, 1883 emend. Votýpka and Kostygov, 2021) comprises leech-transmitted parasites that infect aquatic vertebrates, and its classification is based on phylogenetic analyses using the 18S rRNA gene region. Mitrophanow (1883) was the first to describe 2 monoflagellates from freshwater fish, placing them in the genus *Haematomonas* (*Haematomonas cobitis* Mitrofanov, and *Haematomonas carassii* Mitrofanov, 1972), but Doflein (1901) later reassigned them to *Trypanosoma*. Following Mitrophanow (1883)'s description of the first fish trypanosomes, more than 190 trypanosome species have been described from both marine and freshwater aquatic hosts including amphibians, teleost, elasmobranchs, crocodilians, turtles and the platypus (Woo, 2006). Of these, 29 are known from marine teleost fish and 12 from elasmobranchs, of which only 2 have been reported from the coast of South Africa. The first record of marine trypanosomes from South Africa was by Fantham (1919), who described 2 new species: *Trypanosoma capigobii* (Fantham, 1919) and *Trypanosoma nudigobii* (Fantham, 1919), from the heart blood of a barehead goby, *Caffrogobius nudiceps* (Valenciennes) at Kalk Bay on the southern coast. Later, *Trypanosoma blenniclini* (Fantham, 1930) was recorded from the blennioid, *Parablennius cornutus* (Linnaeus) and the clinid *Blennophis anguillaris* (Valenciennes). Fantham (1930) also reported larger forms of *T. capigobii* from *Ca. nudiceps* collected close to Kalk Bay, at St James. However, following a redescription and molecular analysis of the trypanosomes of the Blenniidae and Clinidae, Hayes et al. (2014) proposed that the 3 species described by Fantham (1919, 1930) are a single pleomorphic species, retaining the name *T. nudigobii* and considering *T. capigobii* and *T. blenniclini* as junior synonyms. The second species of marine trypanosome from South Africa is *Trypanosoma haploblephari* (Yeld and Smit, 2006), originally described from the dark shyshark, *Haploblepharus pictus* (Müller and Henle) and the puffadder shyshark, *Haploblepharus edwardsii* (Schinz) from False Bay and Granger Bay, and more recently molecularly characterized from *H. pictus* and the leopard catshark, *Poroderma pantherinum* (Smith), also from Granger Bay (Yeld and Smit, 2006; Pretorius et al., 2021). South Africa is known for hosting more than 1900 marine fishes, including many endemic species (Smit and Hadfield, 2015). Surprisingly, as mentioned above, only 2 trypanosomes have been described across this diverse range of fish hosts. Thus, the current study aimed to increase the known diversity of marine fish trypanosomes by collecting and screening blood from different species of teleost hosts from selected locations along the south coast of South Africa. All trypanosomes found were described using morphological and morphometrical characterization and molecular analyses.

Materials and methods

Collection of research specimens

Nearshore fish species, mainly of the family Sparidae, were collected using rod and line fishing; intertidal species of the Clinidae and Gobiidae were caught using baited traps; and estuarine

species of the Gobiidae and Mugilidae were collected using sein and cast nets. Fish collections took place on the south coast of South Africa at the coastal towns of Chintsa East (32°50'12''S, 28°7'1''E), Kenton-on-Sea (Kariega River Estuary) (33°36'32''S, 26°39'16''E) and Boknes (33°43'32''S, 26°34'60''E) (Figure 1, Table 1). Sampling was also done at 2 sites in the Garden Route National Park, Tsitsikamma Storms River Mouth (34°1'15''S, 23°52'43''E) and in the Groot River West Estuary, Natures Valley (33°58'49''S, 23°34'2''E) (Figure 1, Table 1). Following collection, fishes were identified using the keys in Smith's Sea Fishes (Smith and Heemstra, 2003). As mullet species are particularly difficult to identify on morphology alone, DNA from fin clips of each sampled specimen was barcoded using the Cytochrome c oxidase subunit I marker following Thieme et al. (2022), and resulting sequences were compared to available sequences in GenBank using the Basic Local Alignment Search Tool (BLAST, <https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The fish used in this study formed part of a larger project on the parasites of South African marine fishes (see Vermaak et al., 2023), wherein fish were humanely killed using the Standard Operating Procedure (SOP no. NWU-00267-17-A5) of blunt force trauma followed by cervical transection. A maximum of 0.4 ml of blood was drawn from the caudal vein using a hypodermic needle (Ethics nos. NWU-00372-16-A5, NWU-01265-23-A9). Thin blood smears were prepared. Each blood smear was air-dried, then fixed with absolute methanol and allowed to air-dry and subsequently stained for 20 min in a 10% dilution of Giemsa stain (Sigma-Aldrich, Steinheim, Germany). For genetic analysis, the remainder of the blood drawn was preserved in a 2-mL microtube containing 96% molecular-grade ethanol (Pretorius et al., 2021).

Morphological characterization

Giemsa-stained blood smears were screened for blood parasites using a Nikon Eclipse Ni microscope (Nikon, Amsterdam, Netherlands) at 100–400× magnification and immersion oil was added for photography and detailed study at 1000× magnification. When trypanosomes were observed, they were examined, photographed and measured with a fixed digital camera (DS-Fi3) using the NIS-Elements BR Ver. 4.60 camera analysis software (Nikon, Tokyo, Japan). Measurements included the distance from the mid-nucleus to the posterior tip of the flagellate (MP), the distance from the mid-nucleus to the anterior end (MA), the nuclear length (NL) and the distance from the posterior tip of the flagellate to the kinetoplast (PK) following Hayes et al. (2014) and Pretorius et al. (2021) (Table 2). Additionally, the nuclear index (NI) was calculated by dividing MP by MA (Dias and de Freitas, 1943). Body width, with the undulating membrane [BW(UM)] and at the nucleus [BW(N)], flagellum length (FL) and total body length (TBL) were measured following Pretorius et al. (2021). Measurements are presented in micrometres (µm) as range (mean ± standard deviation). The aim was to measure at least 20 trypanosome specimens per host species, however, if less than 20 in total were on the slides, all of them were measured.

A non-metric multidimensional scaling (nMDS) biplot (PRIMER v7) was constructed to illustrate the correlation of trypanosome measurements across different species. The data matrix was compiled, with rows representing species and columns representing quantitative measurements. The stress value indicates whether the data were compressed or manipulated to show a specific result. Stress values close to 1 suggest a reduction in plotting accuracy, while values closer to 0 demonstrate that the dataset was not compressed or manipulated. A distance matrix

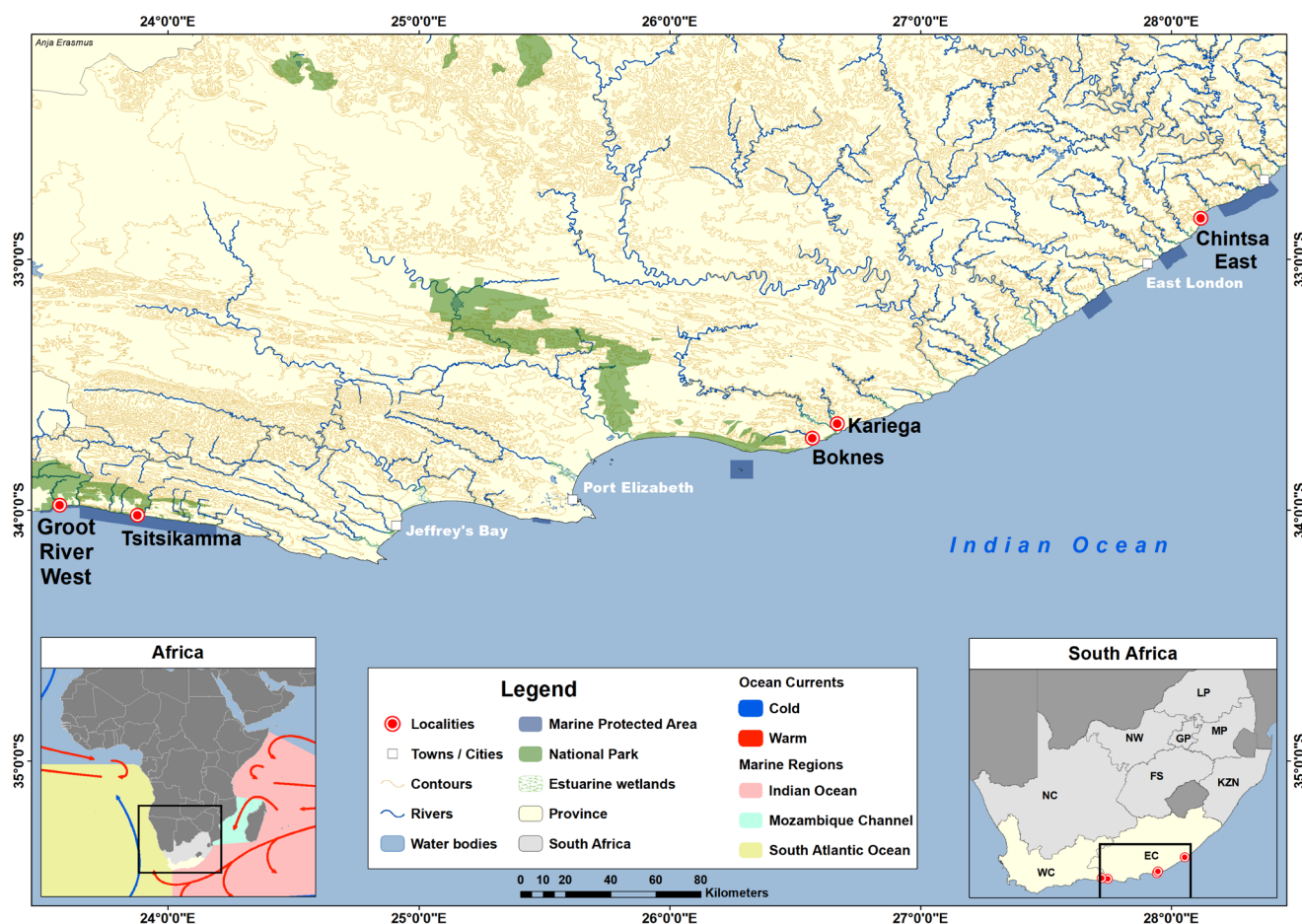


Figure 1. Map showing the sampling locations. Chintsa East, Tsitsikamma Storms River Mouth, Boknes, Kariega River Estuary and Groot River West Estuary.

was calculated from this data using Euclidean metric to quantify dissimilarities. The nMDS analysis utilized the distance matrix to reduce dimensionality, typically to 2 dimensions for visualization. The coordinates from the nMDS output were extracted and plotted on a scatter plot to generate the biplot. This visualization showed the spatial relationships between species based on their measurements, allowing for the assessment of similarities and differences. Additionally, features or vectors were highlighted to indicate the correlation of specific measurements with the dimensions of the nMDS plot, and the stress value was evaluated to ensure the adequacy of the dimensional reduction. A stress value below 0.10 is ideal, showing a reliable representation, while values between 0.10 and 0.20 are acceptable but may have some distortion. Values above 0.20 suggest a poor fit, indicating that the nMDS may not adequately capture the data's relationships.

DNA extraction, amplification and sequencing

Whole blood of hosts with trypanosome-positive blood smears were used for DNA extraction using the KAPA Express Extract Kit (Kapa Biosystems, Cape Town, South Africa) following the manufacturer's instructions specific for animal blood. The 18S rRNA gene was selected as the target because it is widely used for classifying ectotherm trypanosomes and has the highest number of reference sequences currently available of species in the genus *Trypanosoma* (Jordaan et al., 2023). For the polymerase

chain reaction (PCR), the resulting supernatant obtained from the DNA extraction served as the template. PCRs were carried out in final volumes of 25 μ L. The reaction mixture consisted of 12.5 μ L of Thermo Scientific DreamTaq PCR master mix (2 \times) (2 \times DreamTaq buffer, 0.4 mM of each dNTP, 4 mM $MgCl_2$), 1.25 μ L of each primer (10 μ M) and at least 25 ng of DNA. PCR-grade nuclease-free water was used to adjust the final reaction volume. A nested PCR was followed, which utilized primary PCR primers SLF (5'-GCTTGTTC AAGGACTTAGC-3') and S762.2 (5'-GACTTTTGCTTCCTCTAATG-3') and for the secondary PCR, 2 rounds were conducted using different primer sets, namely B (5'-CGAACAAC TGCCCTATCAGC-3') and I (5'-GACTACAATGGTCTCTAATC-3'), and S825 (5'-ACCGTTTCGGCTTTTGTG-3') and SLIR (5'-ACATTGTAGTGCGCGTGTC-3'). The thermal cycling conditions were as follows: for the primary PCR, an initial single cycle (\times 1) of 95°C denaturation for 5 min, 50°C annealing for 2 min, 72°C extension for 4 min; followed by 35 cycles of 94°C denaturation for 30 sec, 52°C annealing for 30 sec, 72°C extension for 2 min 20 sec, followed by a final 72°C extension for 7 min. For the secondary PCR (B & I), the same thermal profile was followed, but with an annealing temperature of 60°C instead of 52°C. Finally with the last set of primers (S825 & SLIR) an initial single cycle (\times 1) of 95°C annealing for 3 min; followed by 35 cycles of 95°C denaturation for 30 sec, 57°C annealing for 30 sec, 72°C extension for 1 min, followed by a final 72°C extension for 7 min. To verify

the presence of DNA amplicons, visualization was done using a 1% agarose gel electrophoresis. Positive PCR products were sent to Inqaba Biotechnical Industries (Pty) Ltd for purification and sequencing.

Phylogenetic analysis

Sequence data for each isolate were assembled, and chromatogram-based contigs were generated and trimmed (1278–1506 bp) using Geneious Prime® 2024.0.7 (Kearse et al., 2012). To identify the closest congeners to the sequences obtained in the present study, the sequences were subjected to a BLAST search (Altschul et al., 1990) followed by an alignment with congeners using the MUSCLE (Edgar 2004) alignment tool in Geneious Prime®. Relevant information regarding the country in which each *Trypanosoma* has been found, the host it was recorded in and an accession number for each trypanosome, respectively are provided in Table 3. The uncorrected *p*-distance and number of base differences per site between sequences were calculated under the 'pairwise deletion' option using MEGA11 (Supplementary Table 1) (Tamura et al., 2021). This analysis included 83 unique nucleotide sequences and the most suitable nucleotide substitution model (GTR + I + G) was determined using jModelTest (Guindon and Gascuel, 2003; Darriba et al., 2012). A Bayesian Inference (BI) analysis of the alignment (2564 bp including gaps) was done using MrBayes 3.2.7 (Ronquist et al., 2012) through the online computational resource CIPRES (Miller et al., 2010) with parameters with 4 category Gamma distribution. Markov Chain Monte Carlo chains were run for 10 000 000 generations, sampling every 100 generations. The 'burn-in' parameter was set at 25% discarded. A Maximum Likelihood (ML) analysis was implemented using PhyML 3.0 (Guindon et al., 2010), and 1000 rapid bootstrap inferences were run on the ATGC Montpellier Bioinformatics Platform (available from <http://www.atgc-montpellier.fr/phyml/>, Guindon et al., 2010). Phylogenetic trees obtained from the respective analyses were visualized using FigTree v. 1.4.4 (Rambaut, 2012).

Results

General observations of trypanosomes in the fish blood

A total of 246 fishes belonging to 23 species in 9 families were collected and screened for trypanosomes. Individuals of 8 of the 23 species were infected with trypanosomes (Table 1). The infected species were the klipfish [*Clinus superciliosus* (Linnaeus)], prison goby [*Caffrogobius gilchristi* (Boulenger)], white steenbras [*Lithognathus lithognathus* (Cuvier)], South African mullet [*Chelon richardsonii* (Smith)], grooved mullet [*Chelon dumerili* (Steindachner)], striped mullet [*Chelon tricuspidens* (Smith)], flat-head grey mullet [*Mugil cephalus* Linnaeus] and freshwater mullet [*Pseudomyxus capensis* (Valenciennes)]. All 8 infected species had unidentified leeches in their mouth or on their gills that could potentially serve as vectors of these trypanosomes.

Description and diagnosis of stages found in the fish blood

Diagnosis

Phylum: Euglenozoa Cavalier-Smith, 1981
Class: Kinetoplastea Honigberg, 1963, emend. Vickerman, 1976
Subclass: Metakinetoplastina Vickerman, 2004
Order: Trypanosomatida Kent, 1880
Family: Trypanosomatidae Doflein, 1951

Genus: *Trypanosoma* Gruby, 1843

Trypanosoma nudigobii Fantham 1919

Host from the present study: *Clinus superciliosus* (Linnaeus)

Other hosts: *Caffrogobius nudiceps* (type host), *Blennophis anguillaris*, *Clinus agilis*, *Clinus cottoides*, *Clinus taurus*, *Parablennius cornutus* (Hayes et al., 2014).

Localities from the present study: Chintsa East, Eastern Cape, South Africa (32°50'12''S, 28°7'1''E) and Tsitsikamma Storms River Mouth, Eastern Cape, South Africa (34°1'15''S, 23°52'43''E).

Other localities: Kalk Bay (type locality), St. James (Fantham, 1919), Tsitsikamma and Koppie Alleen (Hayes et al., 2014).

Site of infection: Peripheral blood

Vector: The leech, *Zeylanicobdella arugamensis* Silva (see Hayes et al., 2006, 2014)

Present study

Voucher material: Three peripheral blood smears deposited in the parasite collection of the National Museum, Bloemfontein, South Africa, under accession numbers NMB P 1085, NMB P 1086 and NMB P 1087.

Representative DNA sequences: The sequence data specifically associated with *T. nudigobii* have been submitted to GenBank: nuclear 18S rRNA (nu 18S) seven partial sequences PV344731 to PV344737.

Morphology: Twenty-eight trypanosomes were measured: total body length 57.2 ± 11.6 (29.2–69.0); body width 5.8 ± 1.9 (2.0–8.7); nucleus length 3.5 ± 0.9 (1.6–5.2); nucleus width 1.4 ± 0.4 (1.0–2.2); mid-nucleus-to-anterior-body-end distance 32.8 ± 8.6 (13.8–47.7); mid-nucleus-to-posterior-body-end distance 25.6 ± 6.4 (16.8–34.3); undulating membrane width 2.2 ± 0.8 (1.2–4.5); number of undulations 15.7 ± 2.1 (13–18) and kinetoplast width 0.9 ± 0.1 (0.7–1.2). Free flagellum length 7.3 ± 1.6 (4.5–9.7). Body index 10.4 ± 2.1 (7.4–14.3); nucleus index 0.8 ± 0.2 and nucleus position as a percentage 56.7 ± 7.1 (47.3–69.2%) (Table 2). The body stains bluish purple in colour with uniform density (Figure 2A–F). The undulating membrane stains lighter purple with a colourless and transparent outer edge. The nucleus stains light pink and is oval-shaped, extending the width of the body. It is positioned parallel to the body and present in the posterior half. The kinetoplast is distinct, stains dark purple in colour and is typically positioned close to the posterior end, on the edge of the body and posterior to the undulating membrane. The flagellum is visible in most of the individuals. The individuals are mostly positioned in a curly manner with the posterior and anterior ends bent.

Remarks

Consequently, and due to the identical nucleotide sequences at both locations, we are clearly dealing with the same species of trypanosome from both these localities and the same host species. The present trypanosome from *Cl. superciliosus* at both sites agrees with the original morphological description of *T. nudigobii* by Fantham (1930) as well as the redescription provided by Hayes et al. (2014). The latter is expected as it also includes specimens from *Cl. superciliosus* collected from Tsitsikamma. In general, it shares common features such as a long, slender body, a free flagellum and an undulating membrane. Although the *T. nudigobii* described by Fantham (1930) is larger in size, our specimens are proportionality relative to the dimensions presented in the redescription of this species by Hayes et al. (2014) (73.3 ± 11.0 vs. 57.2 ± 11.6). Our trypanosomes are the same length as the small trypanosomes

Table 1. Study sites on the south coast of South Africa with year of collection, details of the fish species samples, number and size of collected fishes, as well as the prevalence of trypanosome infections

Site and date	Fishes			Prevalence
	Species	No.	TL \pm s.d. (range) in cm	
Chintsa East (2020; 2022)	<i>Acroteriobatus annulatus</i>	2	51.5 \pm 7.1 (47–57)	0/2 (0%)
Chintsa East (2020; 2022)	<i>Amblyrhynchote honckenii</i>	15	10.6 \pm 4.5 (9–28)	0/15 (0%)
Chintsa East (2020)	<i>Arothron immaculatus</i>	1	28	0/1 (0%)
Chintsa East (2020)	<i>Boopsoidea inornata</i>	1	13.3	0/1 (0%)
Nature's Valley (2023)	<i>Caffrogobius gilchristi</i>	8	10.6 \pm 1.6 (9.5–13)	5/8 (63%)
Tsitsikamma (2024)	<i>Caffrogobius nudiceps</i>	5	12.6 \pm 1.8 (9.6–17.8)	0/5 (0%)
Chintsa East and Kariega river (2020; 2023)	<i>Chelon dumerilli</i>	14	18.6 \pm 9.8 (11.9–36.5)	11/14 (79%)
Chintsa East and Tsitsikamma (2020; 2022; 2023)	<i>Chelon richardsonii</i>	50	23.6 \pm 12.5 (8–40.5)	44/50 (88%)
Boknes (2023)	<i>Chelon tricuspidens</i>	1	37	1/1 (100%)
Tsitsikamma and Chintsa East (2020; 2022; 2023)	<i>Clinus superciliosus</i>	37	14.4 \pm 1.8 (11–18)	30/37 (81%)
Chintsa East; Tsitsikamma and Boknes (2020; 2021; 2022; 2023)	<i>Diplodus capensis</i>	40	25.7 \pm 6.6 (12–34)	0/40 (0%)
Chintsa (2020; 2022)	<i>Diplodus hottentotus</i>	6	19.6 \pm 2.4 (15.5–22)	0/6 (0%)
Chintsa (2022)	<i>Galeichthys feliceps</i>	1	21.5	0/1 (0%)
Boknes (2023)	<i>Glossogobius callidus</i>	12	9.4 \pm 1.8 (5.2–11.7)	0/12 (0%)
Chintsa East and Boknes (2022; 2023)	<i>Lithognathus lithognathus</i>	9	25 \pm 9.5 (13.5–36)	5/9 (56%)
Tsitsikamma (2020; 2021; 2022)	<i>Lithognathus mormyrus</i>	4	24 \pm 6.4 (14.5–28.5)	0/4 (0%)
Tsitsikamma and Boknes (2020; 2023)	<i>Monodactylus falciformis</i>	4	13.6 \pm 3.7 (9.5–17)	0/4 (0%)
Boknes (2023)	<i>Mugil cephalus</i>	3	48	1/3 (33%)
Chintsa East (2022)	<i>Pachymetopon grande</i>	2	33.5 \pm 7.8 (28–39)	0/2 (0%)
Chintsa East (2022)	<i>Pavoclinus (Clinus spp.) mentalis</i>	1	23	0/1 (0%)
Chintsa East (2020)	<i>Pomadasys olivaceus</i>	8	10.3 \pm 2.3 (7.3–14)	0/8 (0%)
Boknes (2023)	<i>Pseudomyxus capensis</i>	18	28.3 \pm 2.5 (25–34)	10/18 (56%)
Boknes (2022; 2023)	<i>Sparodon durbanensis</i>	4	37 \pm 21.9 (17.5–68)	0/4 (0%)
TOTAL		246		107/246 (44%)

No., number of fishes sampled; TL, total length; s.d., standard deviation.

(25.2–46.3) redescribed from *Clinus cottoides* Valenciennes, but not as large as the big trypanosomes (55.1–97.7) from *Cl. superciliosus* reported in Hayes et al. (2014) (Figure 2A and D). Free flagella, especially in large form, were challenging to stain, with some trypanosomes, especially the large ones, appearing to be without a free flagellum (Figure 2A). However, when comparing the body width and nucleus length and orientation, it's the same as the description by Hayes et al. (2014), the posterior region appeared thicker than the anterior, and both extremities were attenuated, often reflexed, or curled. The kinetoplast, referred to as the parabasal body by Fantham (1930), is consistently positioned away from the posterior end with long PK regions (6–9 μ m for *T. blenniini*, and 10 μ m for *T. capigobii*). This is one of the differences between the originally described *T. nudigobii* and the current redescription by Hayes et al. (2014). Additionally, the posterior ends of

the trypanosomes did not taper and were rounded. The undulating membrane was generally well developed (Figure 2B and C), with larger trypanosomes exhibiting 8–10 waves close to the body. The nucleus of normal trypomastigote forms of *T. nudigobii*, as described by Fantham (1930), is rounded or oval. Parasitaemia in the blood of *Cl. superciliosus* was relatively high, a similar finding to that of Hayes et al. (2014), with 5–6 trypanosome individuals per slide.

Molecular characterization

DNA was extracted from 10 samples of infected fish. Following PCR, 7 samples were positive with the correct amplicons, yielding a success rate of 70%. All the trypanosome sequences were from the host *Cl. superciliosus*, on which the morphological characterization

Table 2. Morphometrics of all the trypanosomes collected from various host fishes at the study sites

Number measured and morphometrics of fish trypanosomes										
Fish hosts by species	No	MA	MP	MK	PK	NL	BW	TBL	KI	NI
<i>Trypanosoma nudigobii</i> (Clinus superciliosus)	28 ^a	32.8 ± 8.6 (13.8–47.7)	25.6 ± 6.4 (16.8–34.3)	21.7 ± 4.2 (10.2–25.9)	3.3 ± 0.7 (2.8–4.4)	3.5 ± 0.9 (1.6–5.2)	5.8 ± 1.9 (2.0–8.7)	57.2 ± 11.6 (29.2–69.0)	1.2	0.8
<i>Trypanosoma</i> sp. A (Caffrogobius gilchristi)	18	30.8 ± 4.8 (22.2–36.9)	34.4 ± 2.4 (31.5–38.7)	29.4 ± 5.2 (28.3–34.0)	4.7 ± 1.3 (2.6–5.4)	3.0 ± 0.6 (2.4–4.0)	6.2 ± 1.0 (5.2–7.8)	63.1 ± 6.3 (52.2–70.8)	1.2	1.1
<i>Trypanosoma bakana</i> n. sp. (Lithognathus lithognathus)	26	19.1 ± 4.8 (10.9–25.6)	20.1 ± 5.8 (11.0–26.2)	19.4 ± 3.2 (10.8–25.2)	0.7 ± 1.3 (0.5–1.3)	3.1 ± 0.7 (2.4–4.9)	2.8 ± 0.4 (2.1–3.3)	38.6 ± 4.1 (31.8–44.7)	1.0	1.1
<i>Trypanosoma bokkom</i> n. sp. (Chelon richardsonii)	30	15.1 ± 3.7 (10.0–23.3)	28.7 ± 3.7 (17.2–35.4)	27.9 ± 5.0 (15.5–34.3)	0.9 ± 0.4 (0.4–1.5)	3.4 ± 0.6 (2.1–4.4)	2.5 ± 0.4 (1.9–3.3)	43.1 ± 5.0 (29.2–52.3)	1.0	1.9
<i>Trypanosoma bokkom</i> n. sp. (Chelon dumerilli)	12	14.6 ± 1.7 (12.0–17.5)	30.2 ± 4.0 (23.0–36.7)	28.9 ± 2.7 (22.2–35.3)	0.8 ± 0.6 (0.3–1.6)	3.7 ± 0.8 (2.6–5.6)	2.8 ± 0.5 (2.2–3.5)	46.5 ± 4.8 (34.0–51.6)	1.0	2.2
<i>Trypanosoma bokkom</i> n. sp. (Chelon tricuspidens)	11	15.2 ± 2.5 (10.4–24.2)	27.3 ± 3.5 (17.4–32.2)	25.3 ± 5.1 (16.9–31.6)	1.6 ± 0.4 (0.9–1.8)	3.5 ± 0.7 (2.0–4.1)	2.6 ± 0.3 (1.8–3.0)	44.2 ± 5.1 (29.0–51.2)	1.1	1.9
<i>Trypanosoma bokkom</i> n. sp. (Mugil cephalus)	10	14.8 ± 2.8 (12.3–18.1)	27.4 ± 3.8 (20.4–33.2)	26.1 ± 3.4 (18.1–32.8)	1.5 ± 0.9 (0.9–1.7)	3.3 ± 0.5 (2.3–5.3)	2.7 ± 0.6 (2.3–3.2)	43.3 ± 5.2 (34.1–50.0)	1.0	2.0
<i>Trypanosoma bokkom</i> n. sp. (Pseudomyxus capensis)	12	15.0 ± 3.4 (13.3–17.7)	28.4 ± 4.1 (25.2–35.9)	27.5 ± 2.9 (24.4–35.1)	1.0 ± 1.2 (0.6–1.3)	3.4 ± 0.6 (2.4–5.5)	2.5 ± 0.4 (2.0–3.5)	44.1 ± 4.9 (34.3–49.8)	1.0	2.1

^aInclude specimens from fish collected at Tsitsikamma and Chintsa East.

No., number measured; MA, mid-nucleus to anterior region; MP, mid-nucleus to posterior region; MK, mid-nucleus to kinetoplast; PK, posterior region to kinetoplast; BW, body width; TBL, total body length; nuclear index (NI) = MP/MA; kinetoplast index (KI) = MP/MK.

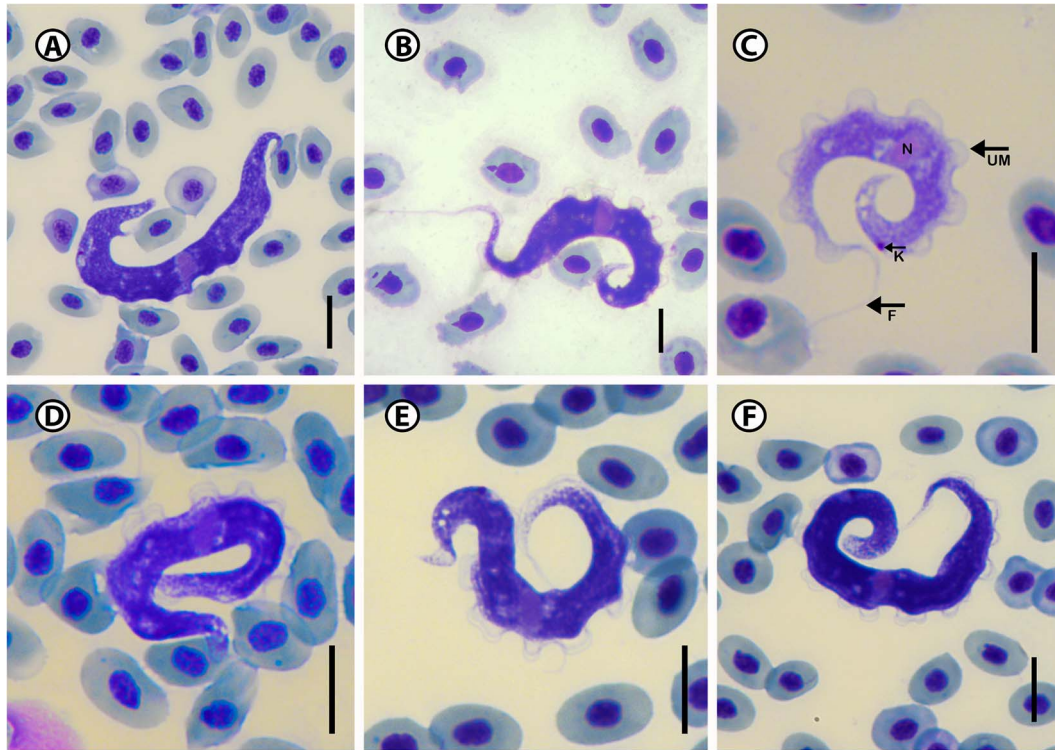


Figure 2. Photomicrographs (A–F) of *Trypanosoma nudigobii* (Fantham, 1919) from *Clinus superciliosus* (Linnaeus). Scale bar = 10 μ m. N, nucleus; UM, undulating membrane; K, kinetoplast; F, free flagellum.

was based. The sequences generated from this host were 99–100% identical to each other. According to GenBank, the best match for these sequences was *Trypanosoma pleuronectidium* (Karlsbakk and Nylund, 2006) with a similarity of 97.97%, and *Trypanosoma rajae* (Kefil and Grellier, 2018) with a similarity of 97.95%.

Description of *Trypanosoma* sp. A

Host: *Caffrogobius gilchristi* (Boulenger) (syn. *Gobius gilchristi*)

Voucher material: Three peripheral blood smear deposited in the parasite collection of the National Museum, Bloemfontein, South Africa, under accession number NMB P 1088, NMB P 1089 and NMB P 1990.

Locality: Groot River West Estuary, Natures Valley, Eastern Cape, South Africa (34°1'15''S, 23°52'43''E).

Site of infection: Peripheral blood

Vector: Unknown

Representative DNA sequences: The sequence data associated with *Trypanosoma* sp. A have been submitted to GenBank: nuclear 18S rRNA (nu 18S) partial sequence PV344729 and PV344730.

Description

Eighteen trypanosomes were measured, yielding the following results: total body length 63.1 ± 6.3 (52.2–70.8); body width 6.2 ± 1.0 (7.8–5.2); nucleus length 3.0 ± 0.6 (2.4–4.0); nucleus width 2.2 ± 1.4 (1.2–2.1); mid-nucleus-to-anterior-body-end distance 30.8 ± 4.8 (22.2–36.9) and mid-nucleus-to-posterior-body-end distance 34.4 ± 2.4 (31.5–38.7) and kinetoplast width 1.1 ± 0.5 (0.8–2.3). Body index 10.4 ± 2.3 (8.0–13.1); nucleus index 1.1 ± 0.2 and nucleus position as a percentage 48.6 ± 3.8 (42.4–53.7%) (Table 2). The body stains purple in colour with striae in the cytoplasm of varying density (Figure 3A–F). The undulating membrane stains lighter purple with a colourless and transparent outer edge, which

is difficult to visualize in some of the individuals. The nucleus stains light pink and is narrow and elongated. It is typically oriented with its long axis transversely, running across the body of the organism and present in the middle of the body. The kinetoplast has a round form and stains dark purple in colour and is positioned on the posterior end of the body with a kinetoplast index of 1.3 and PK distance of 3.3 ± 0.7 (2.8–4.4). The free flagellum is a short, thin extension of the anterior end and may be absent in some of the specimens.

Remarks

Trypanosoma sp. A is characterized by having a half-moon shape body. Morphologically the closest trypanosome described in relation to *Trypanosoma* sp. A is the species previously reported and described from the *Caffrogobius* species in South Africa, namely *T. nudigobii* and *T. capigobii* (Table 4). Fantham (1919) described *T. nudigobii* from *Caffrogobius nudiceps*. This species is 60–85 μ m long and has a body width of 6.6–7.5 μ m. This is similar to *Trypanosoma* sp. A, with a total body length ranging from 52.2 to 70.8 μ m and a body width ranging from 5.2 to 7.8 μ m. *Trypanosoma capigobii* is smaller than *Trypanosoma* sp. A, with a body length ranging from 42 to 60 μ m and body width of 2–4.4 μ m. Both *Trypanosoma* sp. A and *T. nudigobii* have short flagellums, their membrane is close to their body and the nuclei are oval. However, this species is not similar to *T. nudigobii* redescribed by Hayes et al. (2014). Individuals of *Trypanosoma* sp. A differ from those of *T. nudigobii* [redescribed by Hayes et al. (2014)] in their overall elongated body with a small and oval-sized nucleus positioned centrally. *Trypanosoma* sp. A can also be differentiated from other described marine species by being overall stretched out and half-moon shaped (Figure 3A and E) compared to the curled shape of *T. nudigobii* (Figure 2C). The undulating membrane is

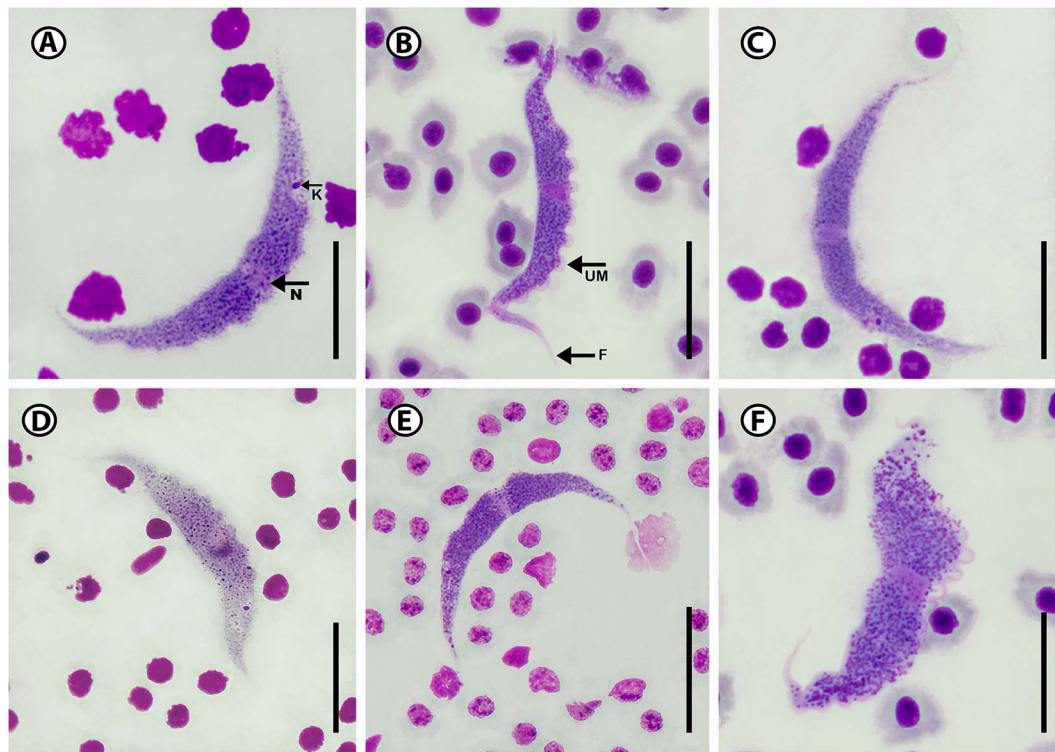


Figure 3. Photomicrographs of *Trypanosoma* sp. A (A–F) from *Caffrogobius gilchristi* (Boulenger). Scale bar = 10 µm. N, nucleus; UM, undulating membrane; K, kinetoplast; F, free flagellum.

not clearly visible which makes counting of the waves challenging. Further, *Trypanosoma* sp. A has an overall larger body size than *T. nudigobii* (63.1 ± 6.3 (52.2–70.8) vs. 57.2 ± 11.6 (29.2–69.0)). No discernible difference in stain colour or density between the anterior and posterior regions was observed. Based on the above morphological comparison, it now seems quite possible that this species (*Trypanosoma* sp. A) represents the true *T. nudigobii*, and that the synonymizing of *T. nudigobii*, *T. capigobii* and *T. blenni-clini* by Hayes *et al.* (2014) was erroneous. However, in order to make a conclusive recommendation regarding the synonymy proposed by Hayes *et al.* (2014), molecular data from the type hosts and type localities of all the 3 original species described by Fantham (1919) are needed. In order not to create more confusion regarding these 3 synonymized species by introducing a fourth name, we refrain from naming the species infecting *Ca. gilchristi* here until such genetic data from these trypanosomes become available. Parasitaemia in the blood of *Ca. gilchristi* was relatively low, with 3–4 trypanosome individuals per slide.

Molecular characterization

Molecular data were obtained from DNA extracted from 5 samples of infected fish. Of these, 4 samples were positive following PCR for the correct amplicons, resulting in a success rate of 80%. All trypanosome sequences were derived from the host *Ca. gilchristi*, which was the basis for the morphological characterization. The sequences generated from 2 infected *Ca. gilchristi* were 100% identical. According to GenBank, the closest match for these sequences was *Trypanosoma pleuronectidium* (Karlsbakk and Nylund, 2006), with a similarity of 97.09%, and *Trypanosoma rajae* (Kefil and Grellier, 2018), with a similarity of 96.94%.

Description of *Trypanosoma bakana* n. sp.

Type host: *Lithognathus lithognathus* (Cuvier) (syn. *Pagrus lithognathus*)

Type material: Hapantotype, 1× peripheral blood smear deposited in the parasite collection of the National Museum, Bloemfontein, South Africa, under accession number NMB P 1079. Other voucher material, 2× peripheral blood smears NMB P 1080 and NMB P 1081. Under Article 73.3.2 of the ICZN.

Type locality: Boknes, Eastern Cape, South Africa (33°43′32″S, 26°34′60″E).

Site of infection: Peripheral blood

Vector: Unknown

Representative DNA sequences: The sequence data specifically associated with *T. bakana* n. sp. have been submitted to GenBank: nuclear 18S rRNA (nu 18S) partial sequence PV344721.

ZooBank registration: n. sp.:
urn:lsid:zoobank.org:act:0808EDA4-84A4-474 F-9AE6-297E66CA9BC5

Etymology: The name ‘Bakana’ is derived from the Khoekhoen language indigenous to the area and means ‘father’s river’. The Bakana river flows into Boknes where the hosts of this trypanosome were collected.

Description

Twenty-six trypanosome specimens were measured: total body length 38.6 ± 4.1 (31.8–44.7); body width 2.8 ± 0.4 (2.1–3.3); nucleus length 3.1 ± 0.7 (2.4–4.9); nucleus width 2.3 ± 1.5 (1.2–2.3); mid-nucleus-to-anterior-body-end distance 19.1 ± 4.8 (10.9–25.6) and mid-nucleus-to-posterior-body-end distance 20.1 ± 5.8 (11.0–26.2); undulating membrane width 2.4 ± 0.6 (1.2–2.8); number of undulations 2.5 ± 0.6 (1–3) and kinetoplast width 1.6 ± 0.9 (0.7–2.5). Free flagellum length 3.7 ± 0.4 (2.1–3.3).

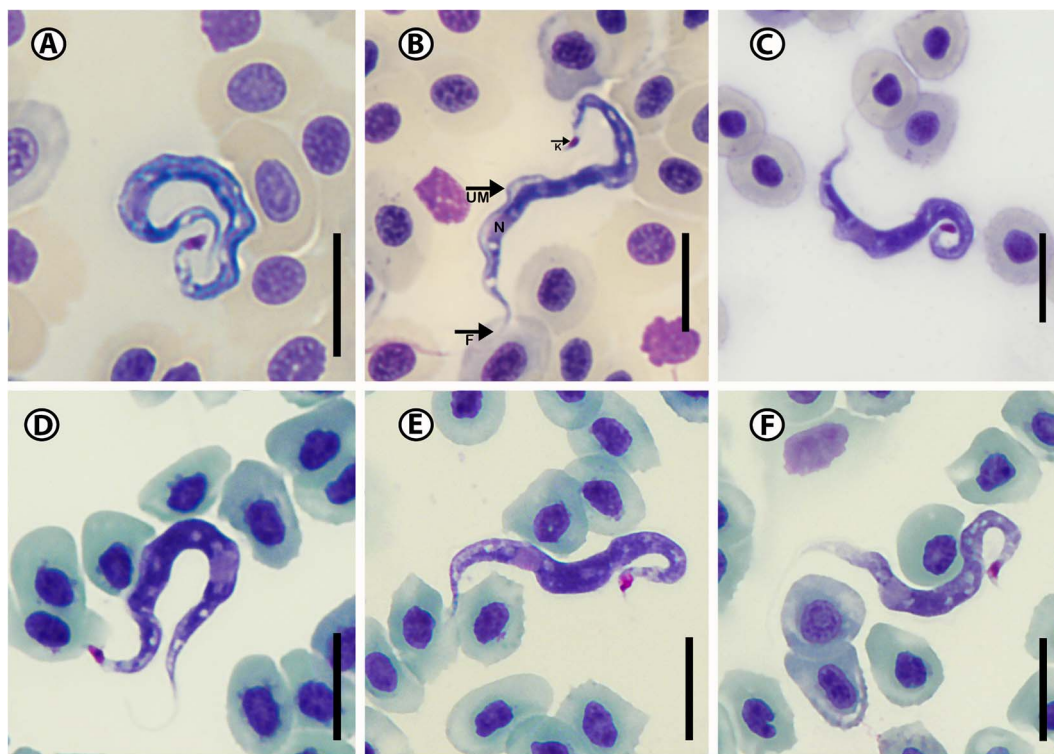


Figure 4. Photomicrographs of *Trypanosoma bakana* n. sp. (A–F) from *Lithognathus lithognathus* (Cuvier). Scale bar = 10 µm. N, nucleus; UM, undulating membrane; K, kinetoplast; F, free flagellum.

Body index 14.2 ± 3.1 (10.8–21.6); nucleus index 1.1 ± 0.6 and nucleus position as a percentage 50.0 ± 13.8 (30.9–69.3%) (Table 2). The body is stained purple in colour with uniform density and has white dots (Figure 4A–F). The undulating membrane stains lighter purple with a darker purple outer edge. The nucleus is oval or round and stains light pink. It is positioned parallel to the body and present in the anterior half. The kinetoplast is distinct, big, stains deep pink in colour and is typically positioned on the posterior end (Figure 4E). The flagellum is short and visible in most of the individuals.

Remarks

Trypanosoma bakana n. sp. is elongated with an S-shape body (Figure 4B), has a large round or oval nucleus that is positioned towards the anterior end (Figure 4A) and the kinetoplast is located at the tip of the posterior region (Figure 4E). *Trypanosoma bakana* n. sp. is large and clear in all the images (Figure 4A–F). The posterior region is more likely to present curled while the anterior region does not curl, flagellum is curved (Figure 4D). The undulating membrane composed of 3–4 waves from the anterior region to just past the nucleus, is not as clear as in *T. nudigobii* but stains light purple and is more visible when compared to that of *Trypanosoma* sp. A. The posterior region of *T. bakana* n. sp. is thicker and darker than its anterior region. According to our knowledge, this is the first marine teleost trypanosome described from a host of the Sparidae; however, Fantham (1919) reported and described a *Herpetomonas denticis* (Fantham, 1919) from *Argyrozona argyrozona* (Valenciennes) in South Africa, which could potentially be an early form of a trypanosome. When com-

pared to South African species *Trypanosoma bakana* n. sp. is overall smaller than both *Trypanosoma* sp. A and *T. nudigobii* with a total length of 38.6 ± 4.1 (31.8–44.7). The closest non-South African marine teleost trypanosome species to *T. bakana* n. sp., based on measurements and an average body length of 39 µm, is *Trypanosoma yakimovi* (Yakimov, 1911), which was described from the greater pipefish *Syngnathus acus* Linnaeus in the Gulf of Naples, Italy (Table 4). *Trypanosoma yakimovi* has a similar nucleus length (2.8 µm) but is smaller in body length (31.2–35.5 µm) compared to *T. bakana* n. sp. Additionally, *T. yakimovi* exhibits a curly body shape with a long flagellum (4.3–9.9 µm) and a distinctive undulating membrane. Furthermore, *T. yakimovi* has a distinctive kinetoplast positioned further away from the posterior end (KI = 1.28) than the kinetoplast of *T. bakana* n. sp. (see Karlsbakk and Nylund, 2006) and has a more centrally positioned nucleus. In contrast, *T. bakana* n. sp. has a long, S-shaped body with a less distinctive undulating membrane, while its nucleus is located toward the anterior end of the body, and the kinetoplast is large, distinctive and situated at the posterior end (Figure 4). The thickened flagellar end makes *T. yakimovi* unique among marine fish trypanosomes, but its size and proportions are otherwise similar to *T. platessae* (Karlsbakk and Nylund, 2006). *Trypanosoma pulchra* (Mackerras and Mackerras, 1925), described in Sydney, Australia, from the white-ear scalyfin *Parma microlepis* Günther, is larger in size (40.8–57.1 µm) and has a long free flagellum (7.3 µm), but has a similar body width (3.5 µm) compared to *T. bakana* n. sp. The nucleus positions differ, and the undulating membrane is more visible than in *T. bakana* n. sp. Parasitaemia in the blood of *L. lithognathus* was relatively low, with 5–6 trypanosome individuals per slide.

Molecular characterization

DNA was extracted from the whole blood of 4 infected fish for PCR analysis. Of these, 2 samples tested positive for the correct amplicons, resulting in a success rate of 50%. All the trypanosome sequences originated from the same host specimens, which was used for the morphological characterisation. These sequences showed 97.8% identity with those of *Trypanosoma* sp. A. The closest matches in GenBank were *Trypanosoma pleuronectidium* (Karlsbakk and Nylund, 2006), with a 97.55% identity, and *Trypanosoma murmanense* (Karlsbakk and Nylund, 2006), with a 97.35% identity.

Description of *Trypanosoma bokkom* n. sp.

Type host: *Chelon richardsonii* (Smith) (syn. *Mugil richardsonii*)

Other hosts: *Chelon dumerili* (Steindachner), *Chelon tricuspidens* (Smith), *Mugil cephalus* Linnaeus and *Pseudomyxus capensis* (Valenciennes)

Type material: Hapantotype, 1× peripheral blood smear deposited in the parasite collection of the National Museum, Bloemfontein, South Africa, under accession number NMB P 1082. Other voucher material, 2× peripheral blood smears NMB P 1083 and NMB P 1084. Under Article 73.3.2 of the ICZN.

Type locality: Tsitsikamma Storms River Mouth, Eastern Cape, South Africa (34°1'15''S, 23°52'43''E)

Other localities: Chintsa East, Eastern Cape, South Africa (32°50'12''S, 28°7'1''E), Boknes, Eastern Cape, South Africa (33°43'32''S, 26°34'60''E) and Kariega River, Eastern Cape, South Africa (33°36'32''S, 26°39'16''E).

Site of infection: Peripheral blood

Vector: No vector data

DNA sequences: The nuclear 18S rRNA (nu 18S) partial sequence data from all the different hosts and localities of *T. bokkom* n. sp. have been submitted to GenBank: Sequences from trypanosomes from: *Ch. richardsonii* (PV344723; PV344724), *Ch. dumerili* (PV344722), *Ch. tricuspidens* (PV344726), *M. cephalus* (PV344727) and *P. capensis* (PV344728).

ZooBank registration: n. sp.:
urn:lsid:zoobank.org:act:BDF2BA09-E6AC-4794-91B0-4F85FD22D97C

Etymology: The name 'bokkom' refers to whole, salted and dried mullet, a well-known delicacy from South Africa.

Description

In total, 75 specimens were measured from all 5 hosts including 45 specimens infecting the type host. Measurements of specimens from the type host: Total body length 43.1 ± 5.0 (29.2–52.3); body width 2.5 ± 0.4 (1.9–3.3); nucleus length 3.4 ± 0.6 (2.1–4.4); nucleus width 2.5 ± 1.6 (1.2–3.1); mid-nucleus-to-anterior-body-end distance 15.1 ± 3.7 (10.0–23.3) and mid-nucleus-to-posterior-body-end distance 28.7 ± 3.7 (17.2–35.4); undulating membrane width 1.3 ± 0.5 (0.7–1.9); number of undulations 1.8 ± 0.4 (1–10) and kinetoplast width 1.0 ± 0.5 (0.9–1.7). Free flagellum length 3.4 ± 0.6 (2.4–4.8). Body index 17.5 ± 3.2 (13.2–27.7); nucleus index 1.9 ± 0.5 and nucleus position as a percentage 33.9 ± 6.0 (23.9–48.9%) (Table 2). The body stains purple or blue in colour with uniform density (Figure 5A–L). The undulating membrane stains light purple with a colourless and transparent outer edge. The nucleus stains light pink and is stretched. It is positioned parallel to the body and present in the anterior half. The kinetoplast is prominent, large, stains deep pink in colour and is positioned on the posterior end. The flagellum is long (Figure 5A, C, K).

Remarks

Consequently, and due to the identical nucleotide sequences at all the locations, the morphometrics of specimens from these areas were amalgamated in Table 2 as all the trypanosomes of the different mullet species are here considered to be of the same species. *Trypanosoma bokkom* n. sp. is characterized by having a long, thin and wavy body shape where the anterior and posterior regions tend to curl more than the middle part of the body (Figure 5D). The undulating membrane is not clear and almost absent (Figure 5A and C). The posterior and anterior regions stained lighter with a prominent pink kinetoplast at the very tip of the posterior region. The nucleus is oval or sometimes elongated more in the anterior region and the flagellum is mostly invisible (Figure 5K and L). *Trypanosoma bokkom* n. sp. is easily distinguishable from *T. bakana* n. sp., *Trypanosoma* sp. A and *T. nudigobii* in the following morphological features. Overall, *T. bokkom* n. sp. is longer in total body length compared to *T. bakana* n. sp.; further, *T. bokkom* n. sp. has a wavy body shape, compared to the half-moon shape of *Trypanosoma* sp. A. The undulating membrane of *T. bokkom* n. sp. is not as visible as seen in *T. nudigobii*. To our knowledge, 3 trypanosome species have been described from mullets of the genus *Mugil*: *Trypanosoma froesi* (Lima, 1976); *Trypanosoma mugilicola* (Becker and Overstreet, 1979); and *Trypanosoma platanusi* (Ribeiro et al., 1996). *Trypanosoma froesi* was first recorded by Lima (1976) in *Mugil platanus* (later changed to *Mugil liza* Valenciennes) in Brazil. A morphologically similar parasite, *T. mugilicola*, was described from *M. cephalus* captured in the Gulf of Mexico by Becker and Overstreet (1979), and *T. platanusi*, which also resembles *T. froesi* morphologically, was recorded from *M. liza* (reported as *M. platanus*) by Ribeiro et al. (1996) in Brazil. The latter authors were either not aware or did not accept the proposal of Eiras et al. (1995), who indicated that *T. mugilicola* and *T. platanusi*, based on their morphological similarity and with reference to Conroy and Conroy (1984), are the same species as *T. froesi*. Revisiting the morphology and morphometrics of these 3 species, we agree with Eiras et al. (1995) and consider *T. mugilicola* and *T. platanusi* junior synonyms of *Trypanosoma froesi*. *Trypanosoma bokkom* n. sp. can be distinguished from *T. froesi* in that the latter has a long free flagellum (10.0–12.0 µm) (Table 4), while *T. bokkom* n. sp. has a shorter flagellum (2.4–4.8 µm), which is more similar in size to the flagellum of *T. platanusi* (mean 4.87 µm). Furthermore, *Trypanosoma bokkom* n. sp. differs from *T. froesi* (reported as *T. mugilicola*) in the position of the kinetoplast that is at the posterior end in *T. bokkom* n. sp. and the nucleus that is closer to the anterior end. Other measurements that differ from those of *T. bokkom* n. sp. include the following from *T. froesi* (reported as *T. platanusi*) total body length (27.0–43.5 µm); free flagellum length (2.0–11.8 µm); mid-nucleus to posterior end (17.0–25.0 µm); kinetoplast diameter (0.5–1.2 µm); and kinetoplast to posterior end (1.1–3.0 µm) (see Ribeiro et al., 1996). Parasitaemia in the blood of all the parasitized mullet hosts was notably high, with 10–15 trypanosome individuals per slide.

Molecular characterization

DNA was extracted from 30 samples of infected fish for PCR analysis as part of the molecular data collection. Of these, 23 samples were positive for the correct amplicons, yielding a success rate of 77%. To avoid repetition and ensure cost-effectiveness, representatives of each sample from the same host and locality were sent for sequencing. All 6 trypanosome sequences were derived from

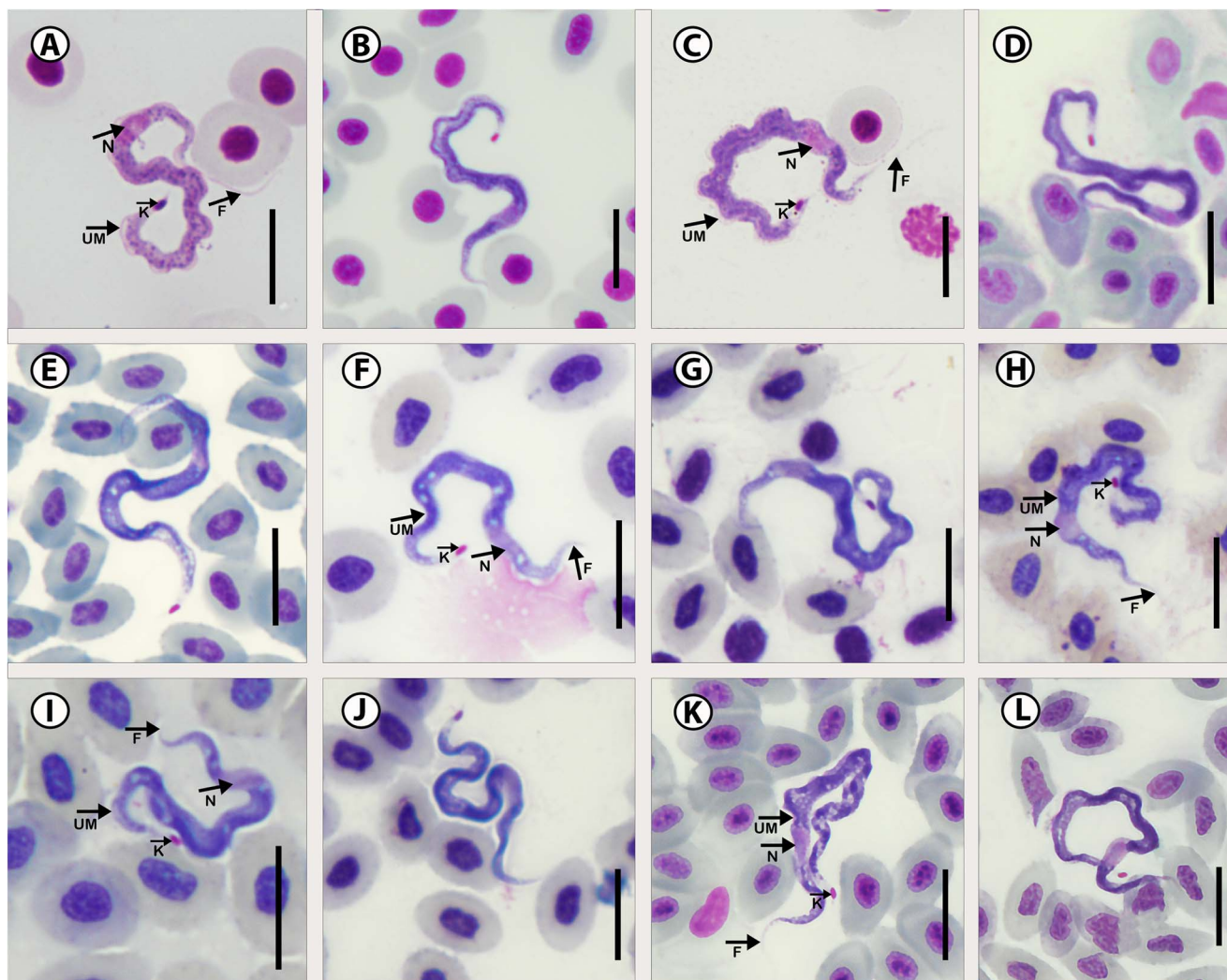


Figure 5. Photomicrographs of *Trypanosoma bokkom* n. sp. From 5 mullet species. (A–C) *Chelon richardsonii* (Smith); (D–F) *Chelon dumerili* (Steindachner); (G,H) *Chelon tricuspidens* (Smith); (I,J) *Mugil cephalus* Linnaeus; (K,L) *Pseudomyxus capensis* (Valenciennes). Scale bar = 10 μ m. N, nucleus; UM, undulating membrane; K, kinetoplast; F, free flagellum.

the same host specimens that served as the basis for the morphological characterization. The sequences obtained from these hosts showed 99.9–100% similarity. The closest matches in GenBank were *Trypanosoma pleuronectidium* (Karlsbakk and Nylund, 2006), with a similarity of 97.08%, and *Trypanosoma rajae* (Kefil and Grellier, 2018), with a similarity of 96.94% identity.

Swimming behaviour

Another new observation in the description of these above trypanosomes is their swimming behaviour. This behaviour was observed during the screening of a live thick blood smear. The swimming behaviour observed for *T. nudigobii* during the present study is considered to fall within the intermediate swimming behaviour category as described in Doro et al. (2019), showing a combination of directional and non-directional movements (see Supplementary Video 1). The swimming behaviour of *Trypanosoma* sp. A can be defined as being persistent, where parasites travel in a straight line over significant distances (Doro et al., 2019). This represents another clear difference between

Trypanosoma sp. A and *T. nudigobii*, an intermediate swimmer (see Supplementary Video 2). *Trypanosoma bakana* n. sp. is categorized as a tumbler, with non-directional movement or swimming, travelling no further than their body length (Doro et al., 2019) (see Supplementary Video 3). This swimming behaviour represents an additional clear difference between *T. nudigobii*, an intermediate swimmer, and *Trypanosoma* sp. A, a persistent swimmer. According to Doro et al. (2019), this swimming behaviour is classified as tumbling. Tumbling behaviour in trypanosomes, as detailed by Doro et al. (2019), is characterized by erratic, non-directional movement where the parasites move only short distances, generally not exceeding their body length (see Supplementary Video 4). This highlights a distinct difference between *T. nudigobii*, an intermediate swimmer, and *Trypanosoma* sp. A, which displays persistent swimming behaviour but shares the same tumbling behaviour as *T. bakana* n. sp. These live appearances of trypanosomes may vary between host individuals. This may potentially also depend on host species, host immune status, nutrients in the blood and stage of trypanosomes and on inapparent plesiomorphy.

Table 3. List of marine *Trypanosoma* species used in the phylogenetic analyses of this study, with associated host, country, references and GenBank accession numbers

<i>Trypanosoma</i> species	Host	Location	Reference	Accession number
<i>Trypanosoma bakana</i> n. sp.	<i>Lithognathus lithognathus</i>	South Africa	Present study	PV344721
<i>Trypanosoma bolissoni</i>	<i>Zanobatus atlanticus</i>	Senegal	Maslov <i>et al.</i> 1996	U39580
<i>Trypanosoma bokkom</i> n. sp.	<i>Chelon dumerili</i>	South Africa	Present study	PV344722
<i>Trypanosoma bokkom</i> n. sp.	<i>Chelon richardsonii</i>	South Africa	Present study	PV344723
<i>Trypanosoma bokkom</i> n. sp.	<i>Chelon richardsonii</i>	South Africa	Present study	PV344724
<i>Trypanosoma bokkom</i> n. sp.	<i>Chelon tricuspidens</i>	South Africa	Present study	PV344726
<i>Trypanosoma bokkom</i> n. sp.	<i>Mugil cephalus</i>	South Africa	Present study	PV344727
<i>Trypanosoma bokkom</i> n. sp.	<i>Pseudomyxus capensis</i>	South Africa	Present study	PV344728
<i>Trypanosoma epinepheli</i>	<i>Epinephelus fuscoguttatus</i>	China	Su <i>et al.</i> (2014)	JQ999962
<i>Trypanosoma haploblephari</i>	<i>Paroderma pantherinum</i>	South Africa	Pretorius <i>et al.</i> (2021)	MZ061641
<i>Trypanosoma</i> sp. A	<i>Caffrogobius gilchristi</i>	South Africa	Present study	PV344729
<i>Trypanosoma</i> sp. A	<i>Caffrogobius gilchristi</i>	South Africa	Present study	PV344730
<i>Trypanosoma murmanense</i>	<i>Gadus morhua</i>	Norway	Karlsbakk and Nylund (2006)	DQ016616
<i>Trypanosoma nudigobii</i>	<i>Clinus superciliosus/Zeylanicobdella arugamensis</i>	South Africa	Hayes <i>et al.</i> (2014), Present study	KF871790, PV344731–PV344737
<i>Trypanosoma pleuronectidium</i>	<i>Gadus morhua</i>	Norway	Karlsbakk and Nylund (2006)	DQ016613
<i>Trypanosoma rajae</i>	<i>Raja</i> spp.	Algeria	Unpublished (Kefil and Grellier, 2018)	MG878995
<i>Trypanosoma rajae</i>	<i>Raja</i> spp.	Algeria	Unpublished	MG878996
<i>Trypanosoma triglae</i>	<i>Chelidonichthys lastoviza</i>	USA	Maslov <i>et al.</i> (1996)	U39584

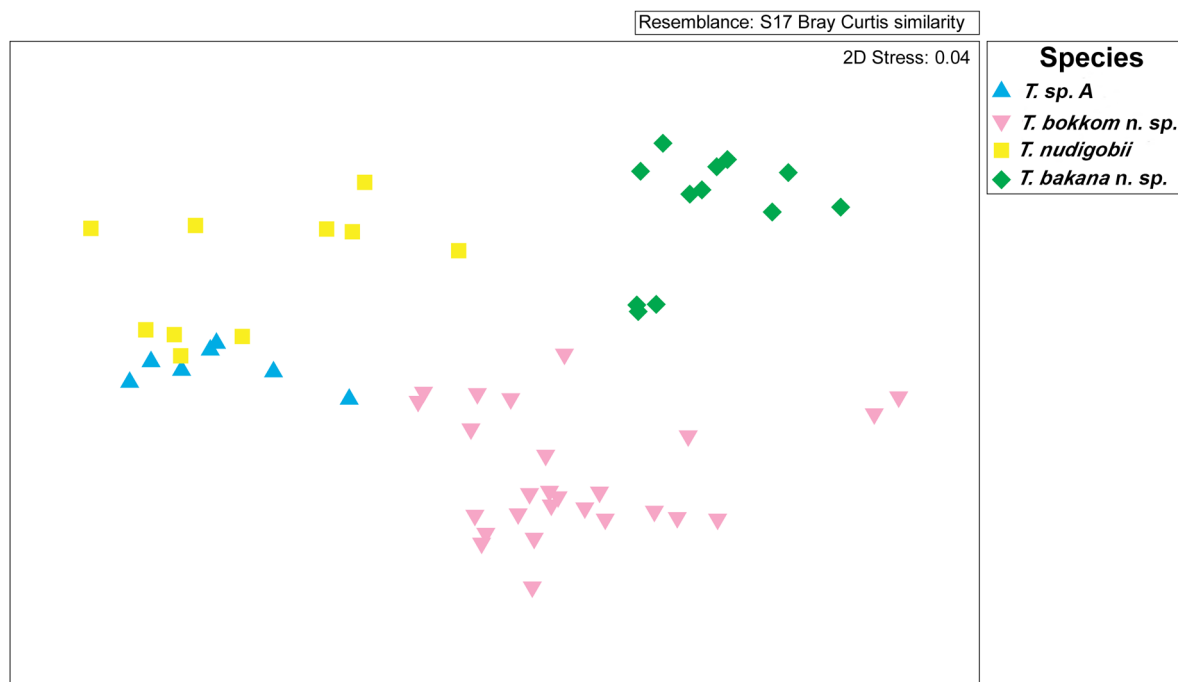


Figure 6. The nMDS biplot indicates the differences in trypanosome measurements across different species.

Phylogenetic analysis

A final alignment of the 18S rRNA spanning 2581 bp (including gaps) contained 83 unique sequences of various trypanosome species from a range of vertebrate hosts including amphibians, reptiles and freshwater fishes (Table 3). The BI and ML trees obtained during the analyses have similar topologies. Trypanosome species from marine hosts were recovered as sister to all trypanosome species isolated from clawed frogs, freshwater turtles and terrapins from South Africa, a platypus from Australia and freshwater turtles from sub-Saharan Africa. All representative sequences of *T. nudigobii* form a well-supported clade. The isolate *T. nudigobii* [KF871790], previously reported by Hayes et al. (2014) from *Cl. superciliosus* and *Z. arugamensis* from Tsitsikamma National Park, nested between 2 subclades from isolates in the current study. The intraspecific genetic divergence calculated as uncorrected p-distances among *T. nudigobii* isolates ranged from 0.0% to 0.9% and 0–13 bp differences (1316 bp compared; Figure 7; Supplementary Table 1). The 2 sequences obtained of *Trypanosoma* sp. A were identical and formed a sister taxon to *T. bakana* n. sp., which is its closest relative. The genetic divergence between *Trypanosoma* sp. A and *T. bakana* n. sp. was 1.6%, corresponding to a 21 bp difference (1303 bp compared; see Supplementary Table 1). The relatedness of these 2 species could be due to *L. lithognathus* and *Ca. gilchristi* utilizing estuaries as nursery grounds, where the hosts are exposed to similar vectors (Figure 7). This could potentially suggest past host switching, which may explain the close genetic similarity observed between the species. The interspecific divergence between *T. nudigobii* and *T. bakana* n. sp. ranged from 2.4% to 3.7%, with 48–52 bp differences (1314 bp compared; see Supplementary Table 1). There were 44–47 bp differences between *T. bokkom* n. sp. and *T. bakana* n. sp., with an uncorrected p-distance ranging from 3.1% to 3.5% (1498 bp compared). All 6 isolates of *T. bokkom* n. sp. formed a sister clade to *T. bakana* n. sp. and *Trypanosoma* sp. A (Figure 7). Isolates 1 and 3–6 were identical to each other (see Supplementary Table

1), but isolates 3–5 were not identical to isolate 2 sequenced from *Ch. richardsonii* from Kariega River, with an uncorrected p-distance of 0.07% and 1 base pair difference. All these mullets occur in the same geographical regions and habitat types, suggesting that they are exposed to similar vectors, explaining the monophyletic clade. *Trypanosoma bokkom* n. sp. forms part of the marine clade within the aquatic clade and forms a sister clade with *T. bakana* n. sp. and *Trypanosoma* sp. A. The sister group to this clade consists of trypanosomes parasitizing marine fish from the Eastern Atlantic [U39580; U39584] and Indo-Pacific [JQ999962] regions.

Discussion

The current study enriched knowledge of marine fish trypanosomes by proposing 4 distinct species from 8 host species of which 3 are new to science (all illustrated in Figure 8). According to Hayes et al. (2014), *T. nudigobii* infects a wide range of intertidal fishes (Fantham, 1930; Hayes et al., 2014), with the type host being *Caffarogobius nudiceps*. In the present study, the *Cl. superciliosus* was collected inside and outside intertidal zones, from 2 localities and their morphological and molecular characterization revealed that the species parasitizing *Cl. superciliosus* was morphologically and genetically similar to *T. nudigobii*. Interestingly *Ca. nudiceps* (the type host of *T. nudigobii*) were collected from intertidal pools in Tsitsikamma, but none were found to be infected (see Table 1). This was unexpected, as *T. nudigobii* was previously reported to infect fish collected by Hayes et al. (2014) from Tsitsikamma National Park. Furthermore, a congeneric host, *Ca. gilchristi* were found to be infected with *Trypanosoma* sp. A, from an estuary, the Groot River West Estuary, which leads us to believe that despite belonging to the same host genus, the difference in habitat exposes hosts to different vectors and consequently to distinct trypanosome species. The third species reported here is *T. bakana* n. sp. (ex.

Table 4. Comprehensive list of all 32 known marine fish *Trypanosoma* species described location, reference, vectors and measurements where available

<i>Trypanosoma</i> species	Host species	Location	Reference	Vector	BL	FL	BW	NL	NW
<i>Trypanosoma aulopi</i> Mackerras and Mackerras, 1925	<i>Latropiscis purpurisatus</i> (Richardson)	Sydney, N.S.W.	Mackerras and Mackerras, 1925	Not known	29.1–57.1	N/A	N/A	N/A	N/A
<i>Trypanosoma bakana</i> n. sp.	<i>Lithognathus lithognathus</i> (Cuvier)	Boknes, Eastern Cape, South Africa	Present study	Not known	31.8–44.7	2.1–3.3	2.1–3.3	2.4–4.9	1.2–2.3
<i>Trypanosoma balistes</i> Saunders, 1959	<i>Balistes capricornis</i> Gmelin	Waters around Key Largo, Florida	Saunders, 1959	Not known	55.5	12.0	3.0	1.2	0.6
<i>Trypanosoma bokkom</i> n. sp.	<i>Chelon richardsonii</i> (Smith)	Tsitsikamma, Eastern Cape, South Africa	Present study	Not known	29.2–52.3	2.4–4.8	1.9–3.3	2.1–4.4	1.2–3.1
<i>Trypanosoma callionymi</i> Brumpt and Lebaillly, 1904	<i>Callionymus lyra</i> Linnaeus	Luc-sur-Mer, France	Karlsbakk and Nylund, 2006	Not known	65–70	N/A	5	3–3.5	N/A
<i>Trypanosoma capigobii</i> Fantham, 1919	<i>Caffrogobius nudiceps</i> (Valenciennes)	Kalk Bay	Fantham, 1919	Not known	42–60	N/A	2–4.4	N/A	N/A
<i>Trypanosoma caulopsettae</i> Laird and Porter, 1951	<i>Arnoglossus scapha</i> (Forster) and <i>Rhombosolea plebeia</i> (Richardson)	Cape Campbell, Cook Strait	Laird and Porter, 1951	Not known	26.3–94.8	21.7	1.1–8.4	1.7–2.3	0.8–1.1
<i>Trypanosoma cephalacanthi</i> Ranque, 1973	<i>Dactylopterus volitans</i> (Linnaeus)	Not known	Fantham, 1919	Not known	N/A	N/A	N/A	N/A	N/A
<i>Trypanosoma coelorhynchi</i> Laird and Porter, 1951	<i>Coelorhynchus australis</i> (Richardson) and <i>Pseudophycis bachus</i> (Forster)	Cape Campbell, Cook Strait	Laird and Porter, 1951	Not known	61.5–70.1	9.4–13.0	2.0–4.2	2.7–4.0	1.9–3.0
<i>Trypanosoma congiopodi</i> Laird and Porter, 1951	<i>Congiopodus leucopaeilius</i> (Richardson)	Cape Campbell, Cook Strait	Laird and Porter, 1951	Not known	41.9–45.8	N/A	4.3–4.7	N/A	N/A
<i>Trypanosoma cotti</i> Brumpt and Lebaillly, 1904	<i>Taurulus bubalis</i> (Euphrasen)	Luc-sur-Mer, France	Karlsbakk and Nylund, 2006	<i>Calliobdella punctata</i> Beneden and Hesse, 1863	48.7 ± 5.0	8.3 ± 1.3	3.6 ± 0.6	4.5	3.5

(Continued)

Table 4. (Continued.)

<i>Trypanosoma</i> species	Host species	Location	Reference	Vector	BL	FL	BW	NL	NW
<i>Trypanosoma delagei</i> Brumpt and Lebaill, 1904	<i>Lipophrys pholis</i> (Linnaeus)	Roscoff, France	Karlsbakk and Nylund, 2006	Not known	21	12	2.5	N/A	N/A
<i>Trypanosoma dohrni</i> Yakimov, 1911	<i>Monochirus hispidus</i> Rafinesque	Gulf of Naples, Italy	Karlsbakk and Nylund, 2006	Not known	36.9	4.3	4.3	N/A	N/A
<i>Trypanosoma epinepheli</i>	<i>Epinephelus fuscoguttatus</i> (Forskål)	Xincun Bay, province of Hainan, South China Sea	Su et al., 2014	Not known	17.6–25.9	7.4–13.3	1.3–2.0	2.2–4.1	1.0–1.6
<i>Trypanosoma fraesi</i> Lima, 1976 (syn. <i>Trypanosoma mugilicolum</i> Becker and Overstreet, 1979; <i>Trypanosoma platanusi</i> Ribeiro et al., 1996)	<i>Mugil liza</i> Valenciennes	Brazil; Mississippi Sound, Gulf of Mexico; Estuarine-lagunar region of Cananéia, State of São Paulo, Brazil	Eiras et al., 1995; Becker and Overstreet, 1979; Ribeiro et al., 1996	Not known	31.3–37.5	10.0–12.0	3.5	2.8–3.2	N/A
<i>Trypanosoma gobic</i> Brumpt and Lebaill, 1904	<i>Gobius niger</i> Linnaeus	Luc-sur-Mer, France	Karlsbakk and Nylund, 2006	Not known	28–36	14–20	N/A	2.5–3.6	1.1–2.6
	<i>Microstomus pacificus</i> (Lockington)	Pacific Ocean, 10 km off Newport, Oregon	Burreson, 2007	Not known	30.8	0–4.9	1.9	2.6	1.5
<i>Trypanosoma laternae</i> Lebaill, 1904	<i>Arnoglossus laterna</i> (Walbaum)	Luc-sur-Mer, France	Karlsbakk and Nylund, 2006	Not known	44	11	4–5	N/A	3.5–4 × 3
<i>Trypanosoma murmanense</i> Nikitin, 1927 (syn. <i>Trypanosoma myoxocephali</i> Fantham, Porter and Richardson, 1942)	<i>Gadus morhua</i> Linnaeus	Aleksandrovsk (Polyarny), Kola Fjord, Russia	Karlsbakk and Nylund, 2006	<i>Johannsson arctica</i> (Johannsson, 1898)	59.5–75	6–13.5	2–3.5	2–5	2–3
<i>Trypanosoma nudigobii</i> Fantham, 1919	<i>Coffragobius nudiceps</i> (Valenciennes)	Kalk Bay	Hayes et al., 2014	<i>Zeylanicobdella arugamensis</i> Silva, 1963	60–85	N/A	6.6–7.5	N/A	N/A
<i>Trypanosoma pacifica</i> Burreson and Pratt, 1972	<i>Parophrys vetulus</i> Girard	Pacific Ocean, 5–10 km off Newport, Oregon	Burreson and Pratt, 1972	Not known	N/A	N/A	N/A	N/A	N/A
<i>Trypanosoma paraperis</i> Laird and Porter, 1951	<i>Paraperis colias</i> (Forster)	Cannibal Cove, Marlborough Sounds	Laird and Porter, 1951	Not known	50.8–53.8	N/A	2.2–2.3	3.3–3.6	2.3–2.5

(Continued)

Table 4. (Continued.)

<i>Trypanosoma</i> species	Host species	Location	Reference	Vector	BL	FL	BW	NL	NW
<i>Trypanosoma platense</i> Lebailly, 1904 (syn. <i>Trypanosoma bothi</i> Lebailly, 1905; <i>Trypanosoma flesi</i> Lebailly, 1904; <i>Trypanosoma limandae</i> Brumpt and Lebailly, 1904)	<i>Pleuronectes platessa</i> Linnaeus	Luc-sur-Mer, France	Karlsbakk and Nylund, 2006	Not known	35.8–38.4	10.0–15.2	3–3.5	1.6–2.2	2.0–3.2
<i>Trypanosoma pleuronectidium</i> Robertson	<i>Pleuronectes platessa</i> Linnaeus	Millport, Scotland	Karlsbakk and Nylund, 2006	<i>Calliobdella nodulifera</i> (Malm, 1863)	40–74	0–14.5	2.5–7	2.5–7.5	2–6
<i>Trypanosoma pulchra</i> Mackerras and Mackerras, 1925	<i>Parma microlepis</i> Günther	Sydney and Broken Bay, N.S.W.	Mackerras and Mackerras, 1925	Not known	40.8–57.1	7.3	3.5	N/A	N/A
<i>Trypanosoma scorpaenae</i> Neumann, 1909	<i>Scorpaena notata</i> Rafinesque	Gulf of Naples, Italy	Karlsbakk and Nylund, 2006	Not known	58–65	5–7	2–2.5	N/A	4 × 2.5
<i>Trypanosoma senegalense</i> Ranque, 1973 (syn. <i>Trypanosoma triglae senegalensis</i> , Ranque, 1973)	<i>Chelidonichthys lucerna</i> (Linnaeus)	Senegal	Su et al., 2014/Karlsbakk and Nylund, 2006	Not known	39	11.6	2.2	2.1	1.5
<i>Trypanosoma soleae</i> Laveran and Mesnil, 1901	<i>Solea solea</i> (Linnaeus)	St. Martin, French Channel Area	Karlsbakk and Nylund, 2006	<i>Hemibdella soleae</i> (van Beneden and Hesse, 1863)	32–35	4–5	N/A	N/A	N/A
<i>Trypanosoma</i> sp. A	<i>Coffragobius gilchristi</i> (Boulenger)	Groot River West Estuary, Natures Valley, Eastern Cape, South Africa	Present study	Not known	52.2–70.8	N/A	7.8–5.2	2.4–4.0	1.2–2.1
<i>Trypanosoma triglae</i> R.O. Neumann, 1909	<i>Chelidonichthys lucerna</i> (Linnaeus)	Gulf of Naples, Italy	Karlsbakk and Nylund, 2006	Not known	45	c.15	8	N/A	5 × 4
<i>Trypanosoma tripterygium</i> Laird and Porter, 1951	<i>Forsterygion varium</i> (Forster) and <i>Bellapiscis medius</i> (Günther)	Poisoning rock pools at, Island Bay, Wellington	Laird and Porter, 1951	Not known	36.3–58.1	3.3–16.8	1.3–7.4	2.4–2.4	1.1–1.7
<i>Trypanosoma yakimovi</i> Yakimov, 1911	<i>Syngnathus acus</i> Linnaeus	Gulf of Naples, Italy	Karlsbakk and Nylund, 2006	Not known	31.2–35.5	4.3–9.9	2.8	2.8	N/A

BL, body length; FL, flagellum length; BW, body width; NL, nucleus length; NW, nucleus width.

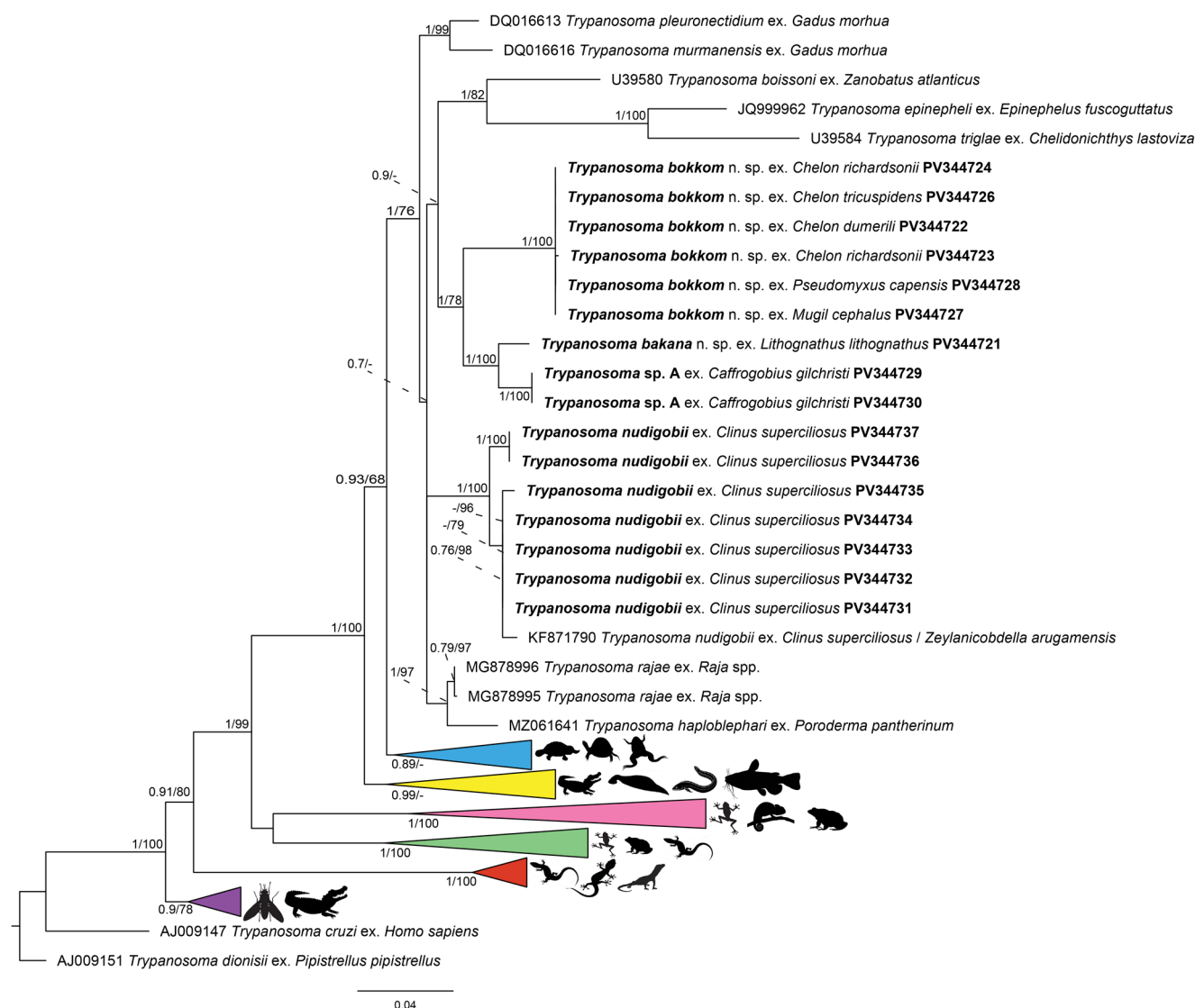


Figure 7. Phylogenetic position of fish trypanosomes from Chintsa East, Tsitsikamma Storms River Mouth, Boknes and Kariega River inferred from the partial 18S rRNA gene region. The outgroups used are *Trypanosoma cruzi* ex. *Homo sapiens* and *Trypanosoma dionisii* ex. *Pipistrellus pipistrellus*. Pink: Trypanosome sequences from frogs and toads. Green: Trypanosome sequences from frogs, lizards and toads. Black: trypanosome sequences from marine fish. Blue: Trypanosome sequences from clawed frogs, freshwater turtles, platypus and terrapins. Yellow: Trypanosome sequences from caiman, freshwater fish and a freshwater leech. Red: Trypanosome sequences from geckos, lizards and monitors. Purple: Trypanosome sequences from caiman and a tsetse fly.

L. lithognathus) collected from Boknes, and the fourth species, *T. bokkom* n. sp. collected from 5 mullet species. Morphological and morphometric analyses revealed differences among the 4 trypanosome species, as illustrated in Figure 6. Notably, the trypanosomes in *Ca. gilchristi* (*Trypanosoma* sp. A) was the largest. These findings advance our understanding of these parasites, particularly when considering early 20th-century research, which followed a strict host-specificity paradigm. This earlier view suggested that each newly infected host species represented a distinct haemoflagellate species (Lom, 1979; Karlsbakk and Nylund, 2006). Recent studies, particularly by Karlsbakk and Nylund (2006), using leech vectors, have shown that a single haemoflagellate species can infect multiple fish species. Furthermore, the concept of host specificity among fish trypanosomes has been further challenged by pairwise genetic distance analyses of 12S rRNA gene sequences from freshwater fish trypanosomes in Europe (Figueroa et al., 1999), which have failed to support the classification of distinct

species for trypanosomes isolated from various host species. The comprehensive review by Karlsbakk and Nylund (2006) on marine fish trypanosomes in the North Atlantic has led to the synonymizing of several species based on consistent size measurements. In some cases, various trypanosome species were delineated within a single host species based solely on minor size variations (Lom, 1979). Karlsbakk and Nylund (2006) emphasize the importance of focusing on individual infections across multiple host species, a practice applied in the case of the 3 novel species described in this study. The nMDS is usually used to show similarity between individuals; however, here differences are shown between the morphometric data of the trypanosome species reported from various hosts. The plot depicted in Figure 6 strongly supports the similarity in morphometrics between individual trypanosomes within a specific host family, but the dissimilarity of trypanosomes between host families reinforces the morphological evidence of 3 distinct species (Dexter et al., 2018).

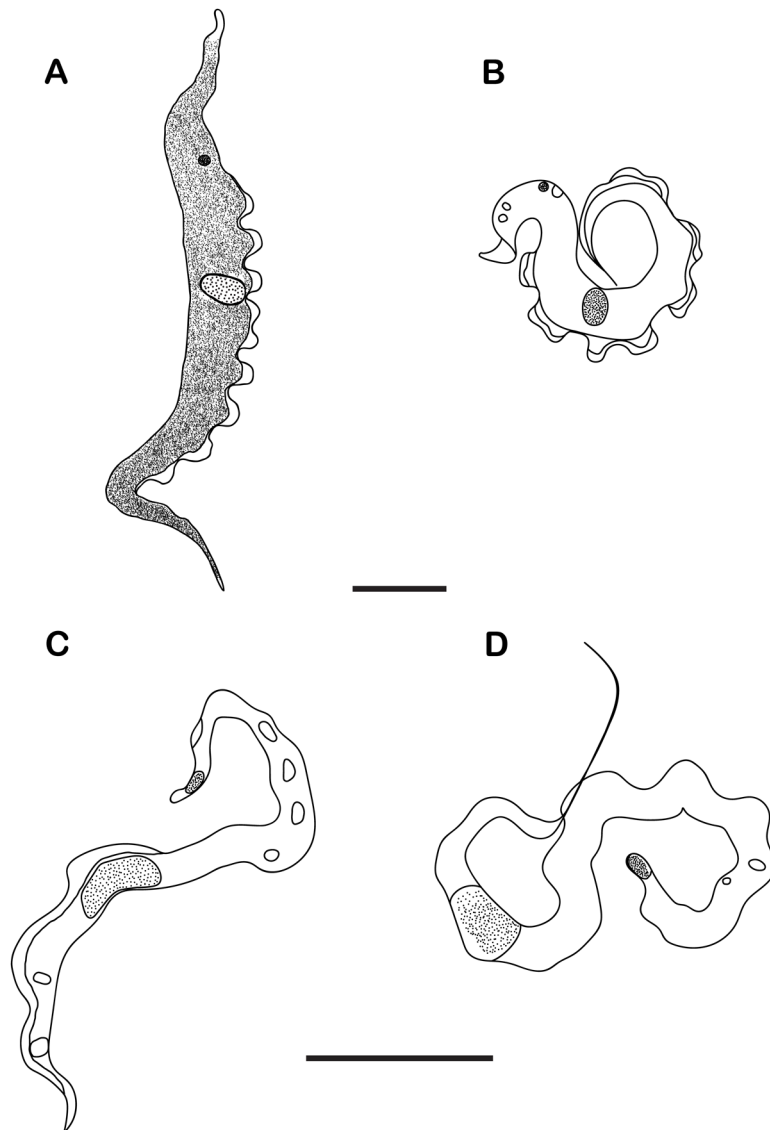


Figure 8. Line drawings of (A) *Trypanosoma* sp. A from *Caffrogobius gilchristi* (Boulenger); (B) *Trypanosoma nudigobii* from *Clinus superciliosus* (Linnaeus); (C) *Trypanosoma bakana* n. sp. From *Lithognathus lithognathus* (Cuvier); (D) *Trypanosoma bokkom* n. sp. From *Chelon richardsonii* (Smith). Scale bar = 10µm.

It is essential to recognize the challenges associated with earlier descriptions, as historical accounts were often brief. However, current knowledge regarding inter- and intraspecific morphological variation among trypanosomes remains incomplete. Variations in fixation, staining, microscopy and measurement tools further complicate these challenges. The morphological description of distinct trypanosome species, or the claim of novel species, remains difficult due to the extensive descriptions based on morphological characterizations across different species and pleomorphism (Kunz, 2002; Hayes *et al.*, 2014; Lemos *et al.*, 2015; Zhang *et al.*, 2023). Consequently, researchers have increasingly turned to DNA sequencing to clarify species designations and confidently differentiate between species. Both ML and BI phylogenies define marine fish trypanosomes as a distinct clade (Figure 7). While the overall structure of the ML and BI phylogenetic trees shows some variation, the mullet species are grouped closely in both analyses. All sequences from the present study are part of the marine fish, shark and ray clade. Additionally, the sequences from *Trypanosoma* sp. A from *Ca. gilchristi* and *T. bakana* n. sp. from *L. lithognathus* were recovered as sister taxa. Each species

– *Trypanosoma bakana* n. sp., *Trypanosoma bokkom* n. sp. and *Trypanosoma* sp. A – isolated from *L. lithognathus*, *Ca. gilchristi*, and all mullet hosts, respectively, formed their own monophyletic clade. These hosts inhabit estuaries as adults or at some point in their life cycle (Whitfield, 2023). In contrast, *Cl. superciliosus*, infected with *T. nudigobii*, does not inhabit estuaries, suggesting that the vector for this trypanosome species may differ. The majority of sequences from *Cl. superciliosus* (*T. nudigobii*) cluster together, with one *T. nudigobii* sequence from Tsitsikamma and another from Chintsa East clustering below the known sequence of *T. nudigobii*, showing a bootstrap value of 84%, indicative of high genetic diversity within the host species. However, these sequences are sister to *T. nudigobii* [GenBank: KF871790], isolated from *Cl. superciliosus* and the leech *Zeylanicobdella arugamensis* Silva, 1963. It is important to note that both ML and BI trees exhibit similar branch lengths, with only a few nodes having bootstrap values below 50. This highlights the close relationships among putative trypanosome species infecting marine fish, as evidenced by the 18S rRNA gene region. The combination of morphological and molecular evidence supports the identification of 3

new trypanosome species, indicating a relationship to previously known marine trypanosomes in southern Africa. These findings enhance the knowledge of marine trypanosome diversity within the region.

Understanding the 3 different swimming behaviours described by Doro et al. (2019) – intermediate swimmers, persistent swimmers and tumblers – adds value to comprehending the dynamics of newly described trypanosome species. While limitations exist, such as the influence of thick blood smears and the timing of screenings, information was still obtained to distinguish between species and warrants further investigation. Modern molecular techniques facilitate the differentiation of closely related or morphologically similar species, and these techniques have also been employed to assess the high levels of pleomorphism found within the same trypanosome species (Pretorius et al., 2021).

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0031182025000496>.

Acknowledgements. The authors are grateful for the assistance of members of the Water Research Group for access to their facilities, as well as to Prof Alan Whitfield, South African Institute for Aquatic Biodiversity, for confirming the identification of the collected goby species. This paper was submitted as part of C le Roux's MSc degree at the North-West University (supervisor N. J. Smit and co-supervisors C. A. Cook and M. Truter).

Author contributions. C. le Roux and N. J. Smit conceived and designed the study. C. le Roux, N. J. Smit, C. A. Cook and M. Truter conducted data gathering and analyses thereof. C. le Roux, N. J. Smit, C. A. Cook, M. Truter and E. C. Netherlands wrote the article.

Financial support. This work is based on the research supported in part by the National Research Foundation (NRF) of South Africa (PMD522060819989). Opinions expressed and conclusions arrived at are those of the authors and are not necessarily those of the NRF. MT acknowledges funding from the North-West University (NWU) Postdoctoral fellowship programme and the NRF-Department of Science and Innovation (DSI) Postdoctoral Professional Development Programme (Grant UID 144831).

Competing interests. None.

Ethical standards. This study forms part of an approved North-West University (NWU) Faculty of Natural and Agricultural Research Ethic Committee (FNASREC) project entitled: Biodiversity, systematics and life cycles of freshwater and marine fish trypanosomes in southern Africa (NWU-01265-23-A9) and a NWU Animal Care, Health and Safety in Research Ethics Committee (NWU-AnimCareREC) approved project entitled: Ecology, systematics and evolutionary biology of ectotherm blood parasites (NWU-00372-16-A5). Permit numbers for the collection of materials: SANParks, SMIT-NJ/2023; EDEDEAT, HO/RSH/16/2022; DFE NWU 0-55505; DFE RES2023/26; SANParks, SMIT-NJ/2024 and DAFF, Greyling-RES2022/44, 01/01/2022-31/12/2022.

References

- Altschul SE, Gish W, Miller W, Myers EW and Lipman DJ (1990) Basic local alignment search tool. *Journal of Molecular Biology* **215**(3), 403–410. doi:10.1016/S0022-2836(05)80360-2.
- Becker CD and Overstreet RM (1979) *Haematozoa* of marine fishes from the northern Gulf of Mexico. *Journal of Fish Diseases* **2**, 469–479. doi:10.1111/j.1365-2761.1979.tb00408.x.
- Brumpt E and Lebaillly C (1904) Description de quelques nouvelles espèces de trypanosomes et d'hémogregarines parasites des téléostéens marins. *Comptes Rendus de L'Académie Des Sciences, Paris* **139**, 613–615.
- Burreson EM (2007) Hemoflagellates of Oregon marine fishes with the description of new species of *Trypanosoma* and *Trypanoplasma*. *Journal of Parasitology* **93**(6), 1442–1451. doi:10.1645/GE-1220.1.
- Burreson EM and Pratt I (1972) *Trypanosoma pacifica* sp. n. from the English sole *Parophrys vetulus* Girard from Oregon. *The Journal of Protozoology* **19**(4), 555–556. doi:10.1111/j.1550-7408.1972.tb03528.x.
- Conroy G and Conroy DA (1984) Diseases and parasites detected in grey mullets (Mugilidae) from coastal waters of São Paulo State, Brazil. I. Adult silver mullet (*Mugil curema* Val., 1836). *Rivista Italiana Di Piscicoltura E Ittiopatologia* **19**, 14–33.
- Darriba D, Taboada GL, Doallo R and Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* **9**(8), 772. doi:10.1038/nmeth.2109.
- Dexter E, Rollwagen-Bollens G and Bollens SM (2018) The trouble with stress: A flexible method for the evaluation of nonmetric multidimensional scaling. *Limnology and Oceanography: Methods* **16**(7), 434–443. doi:10.1002/lom3.10257.
- Dias E and de Freitas L (1943) Introdução ao estudo biométrico dos hemoflagelados do gênero *Schizotrypanum*: 1. Introdução, material e técnica, problema e métodos estatísticos. *Memórias do Instituto Oswaldo Cruz* **38**, 427–436.
- Doflein F (1901) Die Protozoen als Parasiten und Krankheitserreger nach Biologischen Gesichtspunkten Dargestellt. Jena, Germany: Fischer Pantham HB. 1919. Some parasitic protozoa found in South Africa - II. *South African Journal Science* **16**, 185–191.
- Doro E, Jacobs SH, Hammond FR, Schipper H, Pieters RP, Carrington M, Wiegertjes GF and Forlenza M (2019) Visualizing trypanosomes in a vertebrate host reveals novel swimming behaviours, adaptations and attachment mechanisms. *Elife* **8**, e48388. doi:10.7554/eLife.48388.
- Eiras JC, Ranzani-Paiva MJT and Davies AJ (1995) Observations on *Haemogregarina mugili* (Apicomplexa) and *Trypanosoma froesi* (Sarcomastigophora) from the blood of *Mugil platanus* Gunther, 1880 (Pisces: Mugilidae) in Brazil. *Research and Reviews in Parasitology* **55**, 173–176.
- Fantham HB (1919) Some parasitic protozoa found in South Africa. *South African Journal of Science* **16**, 195–191.
- Fantham HB (1930) Some parasitic protozoa found in South Africa. *South African Journal of Science* **27**(11), 376–390.
- Fermimo BR, Paiva F, Viola LB, Rodrigues CM, Garcia HA, Campaner M, Takata CS, Sheferaw D, Kisakyee JJ, Kato A and Jared CA (2019) Shared species of crocodilian trypanosomes carried by tabanid flies in Africa and South America, including the description of a new species from caimans. *Trypanosoma Kaiowa* N. Sp. *Parasites & Vectors* **12**, 225. doi:10.1186/s13071-019-3463-2.
- Figuerola F, Mayer WE, Lom J, Dykova I, Weller M, Peckova H and Klein J (1999) Fish trypanosomes: Their position in kinetoplastid phylogeny and variability as determined from 12S rRNA kinetoplast sequences. *Journal of Eukaryotic Microbiology* **46**, 473–481. doi:10.1111/j.1550-7408.1999.tb06064.x.
- Guindon S, Dufayard JE, Lefort V, Anisimova M, Hordijk W and Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology* **59**(3), 307–321. doi:10.1093/sysbio/syq010.
- Guindon S and Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52**(5), 696–704. doi:10.1080/10635150390235520.
- Hamilton PB, Gibson WC and Stevens JR (2007) Patterns of co-evolution between trypanosomes and their hosts deduced from ribosomal RNA and protein-coding gene phylogenies. *Molecular Phylogenetics & Evolution* **44**(1), 15–25. doi:10.1016/j.ympev.2007.03.023.
- Hayes P, Lawton S, Smit N, Gibson W and Davies A (2014) Morphological and molecular characterization of a marine fish trypanosome from South Africa, including its development in a leech vector. *Parasites and Vectors* **7**, 50. doi:10.1186/1756-3305-7-50.
- Hayes PM, Smit NJ, Seddon AM, Wertheim DF and Davies AJ (2006) A new fish haemogregarine from South Africa and its suspected dual transmission with trypanosomes by a marine leech. *Folia Parasitologica* **53**, 241–248. doi:10.14411/fp.2006.031.
- Hoare CA (1972) *The Trypanosomes of Mammals. A Zoological Monograph*. Oxford, UK: Blackwell Scientific Publications.

- Jordaan BJ, du Preez LH and Netherlands EC (2023) Taxonomic re-evaluation of African anuran trypanosomes with the redescription and molecular diagnosis of *Trypanosoma (Trypanosoma) nelspruitense* Laveran, 1904 and *Trypanosoma (Haematomonas) grandicolor* Pienaar, 1962. *Parasitology* **150**, 477–487. doi:10.1017/S0031182023000203.
- Karlsbakk E, Haugen E and 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**(3), 209.
- Karlsbakk E and Nylund A (2006) Trypanosomes infecting cod *Gadus morhua* L. in the north Atlantic: A resurrection of *Trypanosoma pleuronectidium* Robertson, 1906 and delimitation of *T. murmanense* Nikitin, 1927 (emend.), with a review of other trypanosomes from north Atlantic and Mediterranean teleosts. *Systematic Parasitology* **65**, 175–203. doi:10.1007/s11230-006-9049-3.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P and Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**(12), 1647–1649. doi:10.1093/bioinformatics/bts199.
- Kostygov AY, Karnkowska A, Votýpka J, Tashyreva D, Maciszewski K, Yurchenko V and Lukeš J (2021) Euglenozoa: Taxonomy, diversity and ecology, symbioses and viruses. *Open Biology* **11**(3), 200407. doi:10.1098/rsob.200407.
- Kunz W (2002) When is a parasite species a species? *Trends in Parasitology* **18**, 121–124. doi:10.1016/S1471-4922(01)02210-3.
- Laird M and Porter A (1951) Studies on the Trypanosomes of New Zealand Fish. *Proceedings of the Zoological Society of London* **121**, 285–309. doi:10.1111/j.1096-3642.1951.tb00797.x.
- Leal DD, O'dwyer LH, Ribeiro VC, Silva RJ, Ferreira VL and Rodrigues RB (2009) Hemoparasites of the genus *Trypanosoma* (Kinetoplastida: Trypanosomatidae) and hemogregarines in anurans of the São Paulo and Mato Grosso do Sul States-Brazil. *Anais da Academia Brasileira de Ciências* **81**, 199–206. doi:10.1590/S0001-37652009000200006.
- Lemos M, Fermínio BR, Simas-Rodrigues C, Hoffmann L, Silva R, Camargo EP, Teixeira MM and Souto-Padrón T (2015) Phylogenetic and morphological characterization of trypanosomes from Brazilian armoured catfishes and leeches reveal high species diversity, mixed infections and a new fish trypanosome species. *Parasites and Vectors* **8**, 1–17. doi:10.1186/s13071-015-1193-7.
- Lima DF (1976) Une espèce nova de *Trypanosoma* na tianha (*Mugil brasiliensis* Agassiz, 1829) (Pisces, Mugilidae). *Revista Brasileira de Biologia* **36**, 167–169.
- Lom J (1979) Biology of the trypanosomes and trypanoplasms of fish. In Lumsden WHR and Evans DA (eds.), *Biology of the Kinetoplastida*, Vol. 2. London, UK: Academic Press, 269–337.
- Mackerras IM and Mackerras MJ (1925) The haematozoa of Australian marine teleostei. *Proceedings of the Linnean Society of New South Wales* **50**, 359–366.
- Maslov D A, Lukeš J, Jirků M and Simpson L. (1996). Phylogeny of trypanosomes as inferred from the small and large subunit rRNAs: implications for the evolution of parasitism in the trypanosomatid protozoa. *Molecular and Biochemical Parasitology*, **75**(2), 197–205. doi: 10.1016/0166-6851(95)02526-X
- Miller MA, Pfeiffer W and Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In 2010 gateway computing environments workshop (GCE) 1–8. doi: 10.1109/GCE.2010.5676129.
- Mitrophanow P (1883) Beiträge zur Kenntnis der Haematozoen. *Biologisches Zentralblatt* **3**, 35–44.
- Pretorius C, Smit N, Schaeffner B and Cook C (2021) The neglected diversity: Description and molecular characterisation of *Trypanosoma haplophari* (Yeld and Smit, 2006) from endemic catsharks (Scyliorhinidae) in South Africa, the first trypanosome sequence data from sharks globally. *International Journal for Parasitology: Parasites and Wildlife* **15**, 143–152. doi:10.1016/j.ijppaw.2021.04.008.
- Rambaut A (2012) FigTree, Version 1.4. <http://tree.bio.ed.ac.uk/software/figtree> (accessed 09 September 2024)
- Ribeiro RD, Ranzani-Paiva MJT, Ishikawa CM, Lopes RA, Satake T, Albuquerque SD and Carraro AA (1996) Trypanosomes of Brazilian fishes. XVI. *Trypanosoma platanusi* sp. n. from the mullet *Mugil platanus* Günther, 1880 (Pisces, Mugilidae), from the estuarine-lagoon region of Cananéia, São Paulo State, Brazil. *Brazilian Journal of Biology* **56**(2), 263–267.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA and Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**(3), 539–542. doi:10.1093/sysbio/sys029.
- Saunders DC (1959) *Trypanosoma balistes* n. sp. from *Balistes capriscus* Gmelin, the Common Triggerfish, from the Florida Keys. *The Journal of Parasitology* **45**(6), 623–626. doi:10.2307/3274813.
- Smit NJ and Hadfield K (2015) Marine fish parasitology in South Africa: History of discovery and future direction. *African Zoology* **50**(2), 79–92. doi:10.1080/15627020.2015.1043644.
- Smit NJ, Joubert A, Lawton SP, Hayes PM and Cook CA (2020) Morphological and molecular characterization of an African freshwater fish trypanosome, including its development in a leech vector. *International Journal for Parasitology* **50**(10–11), 921–929. doi:10.1016/j.ijpara.2020.06.004.
- Smith MM, and Heemstra PC (Eds) (2003) *Smiths' Sea Fishes*. Cape Town: Struik Publishers, 1047.
- Su Y, Feng J, Jiang J, Guo Z, Liu G and Xu L (2014) *Trypanosoma epinepheli* n. sp. (Kinetoplastida) from a farmed marine fish in China, the brown-marbled grouper (*Epinephelus fuscoguttatus*). *Parasitology Research* **113**, 11–18. doi:10.1007/s00436-013-3626-6.
- Tamura K, Stecher G and Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution* **38**(7), 3022–3027. doi:10.1093/molbev/msab120.
- Thieme P, Bogorodsky SV, Alpermann TJ, Whitfield AK, Freitas R and Durand JD (2022) Contributions to the taxonomy of the mugilid genus *Chelon Artedi* (Teleostei: Mugilidae), with a major review of the status of *C. persicus* Senou, Randall & Okiyama, 1995. *Zootaxa* **5188**(1), 1–42. doi:10.11646/zootaxa.5188.1.1.
- Vermaak A, Kudlai O, Yong RQ and Smit NJ (2023) Novel insights into the genetics, morphology, distribution, and hosts of the global fish parasitic digenean *Proctoeces maculatus* (Looss, 1901) (Digenea: Fellodistomidae). *Parasitology* **150**(13), 1242–1253. doi:10.1017/S0031182023001026.
- Whitfield AK (2023) Life cycles and challenges for fish species that breed in South African estuaries. *Journal of Fish Biology* **104**(3), 508–516. doi:10.1111/jfb.15618.
- Woo PTK (2006) *Fish Diseases & Disorders, Volume 1: Protozoan and Metazoan Infections*, 2nd edn. Wallingford: CABI Published.
- Yeld E and Smit N (2006) A new species of *Trypanosoma* (Kinetoplastida: Trypanosomatidae) infecting catsharks from South Africa. *Journal of the Marine Biological Association of the United Kingdom* **86**(4), 829–833. doi:10.1017/S0025315406013750.
- Zhang P, Liu J, Yin XM, Zhou JY, Lukeš J, Lun ZR and Lai DH (2023) Towards disentangling the classification of freshwater fish trypanosomes. *Marine Life Science & Technology* **5**(4), 551–563. doi:10.1007/s42995-023-00191-0.