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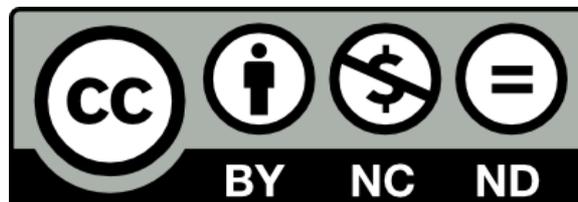
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## Accepted Manuscript

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***Trypanosoma teixeirae*: a new species belonging to the *T. cruzi* clade causing trypanosomosis in an Australian little red flying fox (*Pteropus scapulatus*)**

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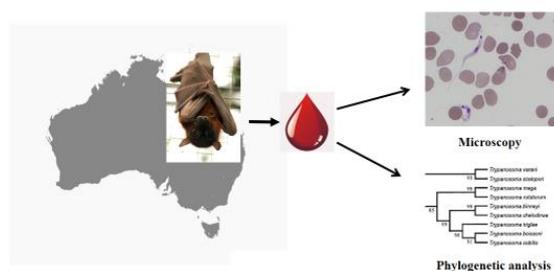
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Graphical abstract

*Trypanosoma teixeirae*: a new species belonging to the *T. cruzi* clade causing trypanosomosis in an Australian little red flying fox (*Pteropus scapulatus*)



## Highlights

- Characterization of *Trypanosoma teixeirae* sp. n. from a little red flying fox
- First trypanosome species associated with clinical disease in an Australian bat
- Morphological and molecular analyses
- *T. teixeirae* sp. n. clustered within the *T. cruzi* clade
- Evolutionary implications discussed

## Abstract

Little is known about the genetic diversity and pathogenicity of trypanosomes in Australian bats. Recently a novel trypanosome species was identified in an adult female little red flying fox (*Pteropus scapulatus*) with clinical and pathological evidence of trypanosomosis. The present study used morphology and molecular methods to demonstrate that this trypanosome is a distinct species and we propose the name *Trypanosoma teixeirae* sp. n. Morphological comparison showed that its circulating trypomastigotes were significantly different from those of *Trypanosoma pteropi* and *Trypanosoma hipposideri*, two species previously described from Australian bats. Genetic information was not available for *T. pteropi* and *T. hipposideri* but phylogenetic analyses at the 18S ribosomal RNA (rRNA) and glycosomal glyceraldehyde phosphate dehydrogenase (gGAPDH) loci indicated that *T. teixeirae* sp. n. was genetically distinct and clustered with other bat-derived trypanosome species within the *Trypanosoma cruzi* clade.

Keywords: *Trypanosoma teixeirae* sp. n.; little red flying fox (*Pteropus scapulatus*); morphology; PCR, 18S ribosomal RNA (rRNA); glycosomal glyceraldehyde phosphate dehydrogenase (gGAPDH); phylogeny

## 1.0 Introduction

Bats (order Chiroptera) are reservoirs of numerous zoonotic pathogens including rabies, Australian bat lyssavirus, severe acute respiratory syndrome (SARS), Hendra virus, Nipah virus and Ebola virus (Wood et al., 2012). Trypanosomes are blood-borne flagellate protozoan parasites that can infect a wide range of vertebrate hosts including humans. Numerous trypanosome species have been identified in bats in Asia, Africa, South America and Europe (Hoare, 1972; Baker, 1973; Marinkelle, 1976, 1979; Gardner and Molyneux, 1988a, b; Hamanaka and Pinto Ada, 1993; Steindel et al., 1998; Barnabe et al., 2003; Grisard et al., 2003; Lisboa et al., 2008; Cottontail et al., 2009; Maia da Silva et al., 2009; Cavazzana et al., 2010; Garcia et al., 2012; Hamilton et al., 2012; Lima et al., 2012; Lima et al., 2013; Marcili et al., 2013; Silva-Iturriza et al., 2013; Cottontail et al., 2014; Ramirez et al., 2014).

In Australia, three *Trypanosoma* spp. have been described in bats to date: *Trypanosoma pteropi* from the black flying fox (*Pteropus gouldii*) (Breinl, 1913; Mackerras, 1959), *Trypanosoma hipposideri* from the dusky horseshoe bat (*Hipposideros bicolor albanensis*) and *Trypanosoma vegrandis*, in pteropid bats (Yangochiroptera) and microbats (Yinpterochiroptera) (Austen et al., 2015). None of these have been associated with clinical disease. In addition, Mackie et al. (2015) recently described the first case of trypanosomosis in a little red flying fox (*Pteropus scapulatus* - suborder Yinpterochiroptera) from eastern Australia, caused by an apparently novel trypanosome species.

Molecular and phylogenetic studies have suggested that bat trypanosomes are implicated in the evolutionary history of the *T. cruzi* clade and may potentially be the precursor of trypanosomes from Australian marsupials and several African terrestrial mammals (Hamilton et al., 2012; Lima et al., 2013). There is however very limited knowledge about the genetic diversity of Australian bat trypanosomes, where only 9 of 76 indigenous bat species have been screened for this parasite (Thompson et al., 2014).

In the present study, we describe the morphological and genetic characterisation of the novel trypanosome in the little red flying fox (Mackie et al., 2015), for which we proposed the name *Trypanosoma teixeirae* sp. n.

## **2.0 Material and Methods**

### *2.1 Sample collection*

A venous blood sample was collected from the cephalic vein of an adult female little red flying fox that presented to the Australia Zoo Wildlife Hospital (AZWH) in April, 2014. The flying fox had been rescued from the ground at Redcliffe in south-eastern Queensland, Australia and was moribund with anaemia and icterus. Clinical and pathological evidence of disease consistent with trypanosomosis in this flying fox was described by Mackie et al. (2015).

### *2.2 Morphological analyses*

Thin blood smears were made from a drop of fresh blood and stained with Diff Quick (Siemens, Germany). After air-drying, the slides were then cover-slipped using DePeX

mounting medium Gurr (Merck Pty. Limited, Kilsyth, Victoria, Australia). Stained films were systematically examined using a BX50 microscope (Olympus, Japan) with screen views generated by a DP Controller (version 3.2.1.276, Olympus, Japan). Digital light micrograph images of any trypomastigotes observed were taken at x1000 magnification.

Digital images of the organisms identified in the blood films were used to measure key morphological features such as total length (TL), width (W), posterior to kinetoplast (PK), kinetoplast to nucleus (KN), nucleus to anterior (NA) and free flagellum (FF), according to parameters described by Hoare (1972) and Mackerras (1959). Means and standard errors were calculated. The morphological measurements were taken using the software Image J (Abramoff et al., 2004).

As two trypanosome species have previously been described in Australian bats based on morphological analysis only (Breinl, 1913; Mackerras, 1959), morphometrics of the novel trypanosome was compared statistically with available measurements for *T. pteropi* and *T. hipposideri*. Mean values for each morphological feature were calculated for *T. teixeirae* sp. n. whilst median values of reported ranges were used as input data for *T. pteropi* and *T. hipposideri*, as means were not available in the bibliographical references. Statistical analyses were conducted using the one sample t-test, in the software PAST 1.43 (Hammer et al., 2001).

### 2.3 DNA Extraction

Genomic DNA was extracted from 200 µl of whole blood, using the MasterPure Purification Kit (Epicentre Biotechnologies, USA). A DNA extraction blank (with sterile molecular-grade water instead of blood) was included in the extraction to exclude the contamination of reagents and consumables with DNA.

#### 2.4 18S rRNA and GAPDH amplification and sequencing

A nested PCR protocol using generic *Trypanosoma* sp. primers SLF, S762R, S823F and S662R (Maslov et al., 1996; McInnes et al., 2009) was performed to amplify an approximately 900bp fragment of the 18S rRNA gene, as previously described by McInnes et al. (2009). The DNA sample was also amplified at the glycosomal glyceraldehyde phosphate dehydrogenase (gGAPDH) gene using a heminested PCR protocol (McInnes et al., 2009).

PCR products were run on a 2% agarose gel containing SYBR Safe Gel Stain (Invitrogen, USA), and visualized with a dark reader trans-illuminator (Clare Chemical Research, USA). The gel bands were purified using an in-house filter tip method as previously described (Yang et al., 2013). All controls (positive, negative and DNA extraction blank) produced appropriate PCR results.

The purified PCR products were sequenced using the corresponding internal reverse primers diluted at 3.2 picomoles with an ABI Prism™ Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, California, USA) on an Applied Biosystem 3730 DNA Analyzer.

#### 2.5 Phylogenetic analysis

Nucleotide sequences obtained at both 18S rRNA and GAPDH loci were aligned with additional trypanosome sequences retrieved from GenBank (Table 1) by MUSCLE (Edgar, 2004) using the default settings. Ambiguous regions containing gaps or poorly aligned were removed by Gblocks (Castresana, 2000), available on the Phylogeny.fr platform (Dereeper et al., 2008), using low stringency parameters. The curated alignments were imported into

MEGA 6 (Tamura et al., 2013) and the most appropriate nucleotide substitution model was selected using the dedicated function.

The evolutionary histories at both 18S rRNA and GAPDH genes were inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). The gamma shape parameter was estimated directly from the data. Reliability for internal branch was assessed using the bootstrapping method (500 bootstrap replicates) and support values (>60%) indicated at the left of each node. The phylogenetic trees were drawn to scale, with branch lengths measured in the number of substitutions per site.

Estimates of genetic divergence between sequences were generated in MEGA 6 based on the Tamura-Nei algorithm, using uniform rates and a partial deletion of 95%.

### **3.0 Results**

#### *3.1 Microscopy and morphometric analysis of T. teixeirae sp. n.*

A total of nine organisms morphologically consistent with a trypanosome were detected by light microscopy in blood films from the little red flying fox. The extracellular organisms were slender with tapered ends, with long free flagellums and either an undeveloped or absent undulating membrane. A nearly central nucleus and a terminal small round deeply staining internal structure consistent with a kinetoplast were also observed (Fig. 1 a, b, c). The trypomastigotes varied in length from 20.4 to 30.8  $\mu\text{m}$  (average 25.9  $\mu\text{m}$ ) and in width from 1.3 to 2.3  $\mu\text{m}$  (average 1.9  $\mu\text{m}$ ) (Table 2).

Among the nine long slender organisms observed, two were not true trypomastigotes as their kinetoplast was located at the very end of the posterior, what made it impossible to calculate the PK distance. In another instance, the trypomastigote's free flagellum was

apparently under a red blood cell, hence any measurements taken of FF or TL would have been inaccurate. We have therefore only measured what was feasible, which explains the divergence in the number of organisms measured for each morphological feature (Table 2).

Three flagellate round forms with a flagellum running round the organism about 90 degrees were also observed (Fig. 1d). Their body shape was consistent with a sphaeromastigote or a round epimastigote if their flagellar position was considered.

Morphometric analysis revealed that although the reported length and width ranges for the *T. teixeirae* sp. n. and *T. pteropi* overlap, the former was significantly longer and thinner than the latter ( $p < 0.01$ ) (Table 3). There was no significant difference between KN, NA and FF dimensions between *T. teixeirae* sp. n. and *T. pteropi*. In addition, *T. hipposideri* was significantly smaller than *T. teixeirae* sp. n. for TL and FF dimensions ( $p < 0.01$ ) (Table 3). No significant difference was observed for B, PK and KN between *T. teixeirae* sp. n. and *T. hipposideri*.

### 3.2 Sequence and phylogenetic analysis

Maximum Likelihood analysis at both the 18S rDNA and GAPDH loci (Fig. 2 and 3, respectively) produced concordant tree topologies and revealed that *T. teixeirae* sp. n. grouped with other trypanosomes belonging to the *T. cruzi* clade, including 7 bat-derived isolates (*T. cruzi* Tcbat, *T. cruzi* marinkellei, *T. erneyi*, *T. dionisii*, *T. rangeli*, *T. vespertilionis* and *T. sp.* AJ012418/ GQ140365) and three isolates from Australian marsupials (*T. sp.* H25 from a kangaroo- AJ009168/ AJ620276; *T. sp.* AB-2003-G8 from a woylie- KC753537/ KC812988; and *T. sp.* AP-2011-64 from a brush-tailed possum – JN315383/ AJ620276). The phylogenetic trees also corroborated the evolutionary relationships among all major trypanosome clades described in previous broader analyses. Nucleotide sequences were

obtained at both loci for *T. teixeirae* sp. n. were submitted to GenBank under the following accession numbers: KT907061 and KT907062.

Estimates of evolutionary divergence between nucleotide sequences revealed that *T. teixeirae* sp. n. was genetically distinct but most closely related to *T. minasense* and *T. rangeli* (genetic distances of 1% at the 18S rDNA locus and 14%-15% at the gGAPDH, respectively) (Table 4).

### Species Description

Species Name: *Trypanosoma teixeirae* sp. n. (Fig. 1)

Type host: Little red flying fox (*Pteropus scapulatus*).

Other hosts: Unknown

Type Locality: Redcliffe peninsula, Queensland, Australia.

Prevalence: Unknown

Morphology: *T. teixeirae* sp. n. trypomastigotes are on average 25.9 µm long and 1.9 µm wide, exhibiting a slender shape with tapered ends, a nearly central nucleus and a small terminal kinetoplast. Undulating membrane either absent or under-developed.

Etymology: The species is named *T. teixeirae* sp. n. in honour of Prof. Marta Maria Geraldine Teixeira, from the University of Sao Paulo, who has greatly contributed to the biology and phylogeny of trypanosome species.

### 4.0 Discussion

In the present study, we have described *T. teixeirae* sp. n., the causative agent of trypanosomiasis in an Australian little red flying fox, using morphological and molecular

techniques. This is the fourth trypanosome species to be reported in indigenous Australian bats and the first one associated with clinical disease.

Unfortunately no genetic data was available for two of the previously reported bat-derived trypanosomes in Australia (*T. pteropi* and *T. hipposideri*). *Trypanosoma pteropi* was described as having a slender body (total length 18-22  $\mu\text{m}$ ; width 2-4  $\mu\text{m}$ ), an under-developed undulating membrane and a long free flagellum whereas *T. hipposideri* is very small and slender (total length 10.5-13  $\mu\text{m}$ ; width 1.5-2  $\mu\text{m}$ ), with a delicate short free flagellum at the anterior end (Breinl, 1913; Mackerras, 1959). Statistical analysis however, revealed that *T. teixeirae* sp. n. was significantly larger than both *T. pteropi* and *T. hipposideri*, even though they had several other overlapping morphometric features. However, morphology alone is not a reliable tool to delimit trypanosome species due to the interspecific similarities and intraspecific variability (Dunn et al., 1963; Marinkelle, 1966; Dunn, 1968).

Besides the typical trypomastigotes, two round forms (sphaeromastigotes or round epimastigotes) were also observed. The term 'sphaeromastigote' (Brack, 1968) refers to the parasite body shape only and has been applied without reference to the flagellar development. However, as these forms may occur within different stages of the parasite's development, it is more appropriate to characterise the round organisms observed in the present study as round epimastigotes, considering both their body form and flagellar features (Elliott et al., 1974). This stage normally occurs in the interior of the cell, in vessels or in the insect gut.

Evolutionary reconstructions at both 18S rDNA and gGAPDH revealed that *T. teixeirae* sp. n. was genetically distinct from all known trypanosomes. The use of these two genes is recommended for taxonomic analysis of trypanosomatids and validation of new trypanosome species (Hamilton et al., 2004; Viola et al., 2009; Teixeira et al., 2011; Lima et al., 2012; Borghesan et al., 2013; Lima et al., 2013).

Phylogenetic analyses at both 18S rRNA and gGAPDH loci revealed that *T. teixeirae* sp. n. clustered within the *T. cruzi* clade together with all other bat-derived trypanosome species described to date, except *T. livingstonei* (which was positioned basal to the *T. cruzi* clade), *T. evansi* (which belongs to the *T. brucei* clade) and *T. vegrandis* (which forms a separate group associated with other marsupial-derived trypanosomes found in Australia) (Hamilton et al., 2007; Botero et al., 2013; Lima et al., 2013; Austen et al., 2015; Carnes et al., 2015). At the gGAPDH locus, *T. teixeirae* sp. n. was closest to *T. minasense* and *T. rangeli* and exhibited 14% and 15% genetic distance from these two species respectively. *T. minasense* has been found in neotropical non-human primates from South America (Ziccardi and Lourenco-de-Oliveira, 1999) whilst *T. rangeli* has been reported in a range of mammalian hosts including Brazilian bats (Maia da Silva et al., 2009). Although *T. teixeirae* sp. n. exhibited a relatively low (1%) genetic distance from its closest related species at the 18S rRNA locus, a similar pattern was observed when comparing other previously described species among each other. For instance, genetic distances between *T. minasense* and *T. vespertilionis* were 1% and 12% at the 18S rRNA and gGAPDH loci respectively. Trypanosomes have few morphological features detectable using light microscopy which can adequately delimit species (Gibson, 2009). Previous studies have reported that a genetic distance of 3.75% at the GAPDH gene is sufficient to delimit a new trypanosome species (McInnes et al., 2011). By this criterion, *T. teixeirae* sp. n. is clearly a separate species.

Bat trypanosomes have been implicated in the evolutionary origin of *T. cruzi*, the causative agent of Chagas disease, one of the most important public health issues in South America (Hamilton et al., 2012; Bonney, 2014). The ‘bat-seeding’ theory suggests that *T. cruzi* evolved from within a broad clade of bat-derived species, which have made the switch into terrestrial mammals (Hamilton et al., 2012; Lima et al., 2013). The theory also implies that these arboreal trypanosomes species could potentially be evolutionary precursors for the

terrestrial trypanosome lineage within Australian mammals (Hamilton et al., 2012; Lima et al., 2013; Thompson et al., 2014).

It is therefore possible that *T. teixeirae* sp. n. could be the precursor of three marsupial-derived trypanosomes belonging to the *T. cruzi* clade: *T. sp.* H25 (Averis et al., 2009), *T. sp.* AP-2011-64 (Paparini et al., 2011) and *T. sp.* AB-2013-G8 (Botero et al., 2013). As most native bat species remain unsampled (Thompson et al., 2014), future studies are required to provide more evidence to support the ‘bat-seeding’ theory in Australia and elucidate evolutionary relationships between trypanosomes.

Similarly to most bat trypanosomes described worldwide, the prevalence, distribution, vectors, life cycle and zoonotic potential of *T. teixeirae* sp. n. remain unclear. Therefore, more studies comprising a larger sample size are required to better understand the prevalence and clinical impacts of *T. teixeirae* sp. n. on bat populations, taking into account ecological and stress factors that could play a role in the expression of clinical disease.

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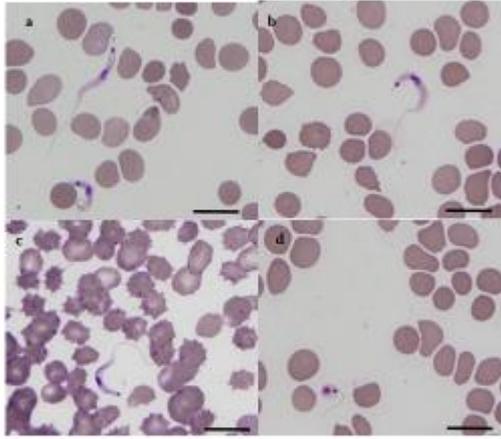
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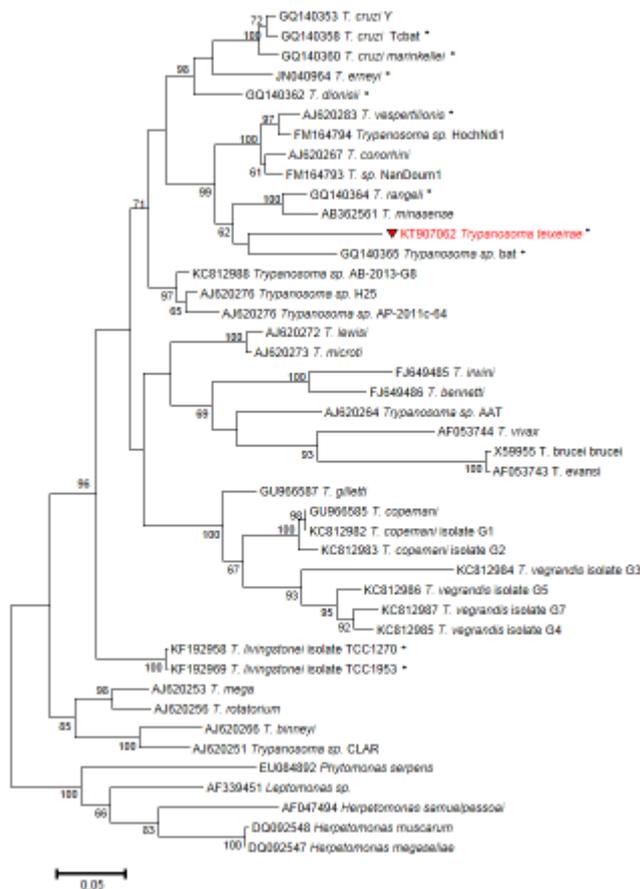
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**Fig. 1** Light photomicrographs of Diff Quick stained blood film showing *Trypanosoma teixeirae* sp. n. trypomastigotes in the blood of a red flying fox (*Pteropus scapulatus*) (a, b, c) and (d) Round epimastigote form. Scale bars represent 10  $\mu\text{m}$





**Fig. 3** Phylogenetic relationships of *Trypanosoma teixeirae* sp. n. with other trypanosomes, based on gGAPDH partial sequences (~775bp). Evolutionary relationships were determined by Maximum Likelihood, based on the Tamura-Nei model (Tamura et al. 2013). Bootstrap values (>60%) based on 500 replicates are indicated at the left of each supported node. The scale bar is the proportion of base substitutions per site. Trypanosome species from bats are shown with an asterisk

Table 1 GenBank accession numbers and sources (where known) of trypanosome isolates included in the phylogenetic analyses

Trypanosome species	Host origin	Geographic origin	GenBank accession numbers	
			18S rDNA	gGAPDH
<i>T. teixeirae</i> sp. n.	Bat ( <i>Pteropus scapulatus</i> )	Australia	KT907061	KT907062
<i>T. rangeli</i>	Bat ( <i>Platyrrhinus lineatus</i> )	Brazil	FJ900242	GQ140364
<i>T. minasense</i>	Tamarin ( <i>Saguinus midas</i> )	Japan	AJ012413	AB362561
<i>T. dionisii</i>	Bat ( <i>Eptesicus brasiliensis</i> )	Brazil	FJ001666	GQ140362
<i>T. erneyi</i>	Bat ( <i>Tadarida</i> sp.)	Mozambique	JN040987	JN040964
<i>T. vesperilionis</i>	Bat ( <i>Pipistrellus pipistrellus</i> )	England	AJ009166	AJ620283
<i>T. sp. bat</i>	Bat ( <i>Rousettus aegyptiacus</i> )	Gabon	AJ012418	GQ140365
<i>T. livingstonei</i> TCC1270	Bat ( <i>Rhinolophus landeri</i> )	Mozambique	KF192979	KF192958
<i>T. livingstonei</i> TCC1953	Bat ( <i>Hipposideros caffer</i> )	Mozambique	-----	KF192969
<i>T. cruzi</i> Tcbat	Bat ( <i>Myotis levis</i> )	Brazil	FJ900241	GQ140358
<i>T. cruzi marinkellei</i>	Bat ( <i>Carollia perspicillata</i> )	Brazil	FJ001664	GQ140360
<i>T. cruzi</i> Y	Human ( <i>Homo sapiens</i> )	Brazil	AF301912	GQ140353
<i>T. conorhini</i>	Rat ( <i>Rattus rattus</i> )	Brazil	AJ012411	AJ620267
<i>T. sp. NanDoum1</i>	Palm civet ( <i>Nandinia binotata</i> )	Cameroon	FM202492	FM164793
<i>T. sp. HochNdi1</i>	Monkey ( <i>Cercopithecus nictitans</i> )	Cameroon	FM202493	FM164794
<i>T. sp. H25</i>	Kangaroo ( <i>Macropus giganteus</i> )	Australia	AJ009168	AJ620276
<i>T. sp. AP-2011-64</i>	Possum ( <i>Trichosurus vulpecular</i> )	Australia	JN315383	AJ620276
<i>T. sp. AB-2013-G8</i>	Woylie ( <i>Bettongia penicillata</i> )	Australia	KC753537	KC812988
<i>T. avium</i>	Eagle ( <i>Aquila pomarina</i> )	Slovakia	AF416559	-----
<i>T. sp. AAT</i>	Currawong ( <i>Strepera</i> sp.)	Australia	AJ620557	AJ620264
<i>T. bennetti</i>	American kestrel ( <i>Falco sparverius</i> )	Germany	AJ223562	FJ649486
<i>T. irwini</i>	Koala ( <i>Phascolarctos cinereus</i> )	Australia	FJ649479	FJ649485
<i>T. lewisi</i>	Rat ( <i>Rattus rattus</i> )	England	AJ009156	AJ620272
<i>T. microti</i>	Vole ( <i>Microtis agrestis</i> )	England	AJ009158	AJ620273
<i>T. vivax</i>	Cattle		EU477537	AF053744
<i>T. brucei brucei</i>			-----	X59955
<i>T. brucei rhodesiense</i>	Human ( <i>Homo sapiens</i> )	Uganda	AJ009142	-----
<i>T. brucei gambiense</i>	Human ( <i>Homo sapiens</i> )	Nigeria	AJ009141	-----

<i>T. evansi</i>	Capibara ( <i>H. hydrochaeris</i> )	Brazil	AJ009154	AF053743
<i>T. copemani</i> Charlton	Koala ( <i>Phascolarctos cinereus</i> )	Australia	GU966588	-----
<i>T. copemani</i> Mika	Koala ( <i>Phascolarctos cinereus</i> )	Australia	-----	GU966585
<i>T. copemani</i> G1	Woylie ( <i>Bettongia penicillata</i> )	Australia	KC753530	KC812982
<i>T. copemani</i> G2	Woylie ( <i>Bettongia penicillata</i> )	Australia	KC753531	KC812983
<i>T. gilletti</i>	Koala ( <i>Phascolarctos cinereus</i> )	Australia	GU966589	GU966587
<i>T. vegrandis</i> G3	Woylie ( <i>Bettongia penicillata</i> )	Australia	KC753533	KC812984
<i>T. vegrandis</i> G4	Woylie ( <i>Bettongia penicillata</i> )	Australia	KC753532	KC812985
<i>T. vegrandis</i> G5	Woylie ( <i>Bettongia penicillata</i> )	Australia	KC753534	KC812986
<i>T. vegrandis</i> G6	Woylie ( <i>Bettongia penicillata</i> )	Australia	KC753535	-----
<i>T. vegrandis</i> G7	Woylie ( <i>Bettongia penicillata</i> )	Australia	KC753536	KC812987
<i>T. mega</i>	African toad ( <i>Bufo regularis</i> )	Africa	AJ009157	AJ620253
<i>T. rotatorium</i>	Bullfrog ( <i>Rana catesbeiana</i> )	Canada	AJ009161	AJ620256
<i>T. binneyi</i>	Platypus ( <i>Ornithorhynchus anatinus</i> )	Australia	AJ132351	AJ620266
<i>T. granulorum</i>	Eel ( <i>Anguilla anguilla</i> )	Portugal	AJ620552	-----
<i>T.sp.</i> CLAR	Catfish ( <i>Clarias angolensis</i> )	Africa	AJ620555	AJ620251

Table 2 Mean dimensions and standard errors (S.E.) of morphological features of *Trypanosoma teixeirae* sp.n. isolated from a little red flying fox's blood

<b>Feature*</b>	<b>No. of organisms measured</b>	<b>Observed range (<math>\mu\text{m}</math>)</b>	<b>Mean <math>\pm</math> S.E. (<math>\mu\text{m}</math>)</b>
Total length	8	20.4 - 30.8	25.9 $\pm$ 1.2
Width	8	1.3 - 2.3	1.9 $\pm$ 0.1
PK	7	1.5 - 2.4	2 $\pm$ 0.15
KN	9	3.3 - 6.2	4.9 $\pm$ 0.3
NA	9	5.1 - 9.8	7.8 $\pm$ 0.5
FF	8	10.0 - 12.9	11.3 $\pm$ 0.4

\*Total length: total body length measured along mid-line including free-flagellum

Width: maximum width measured at nucleus level (undulating membrane included)

PK: distance between the posterior end and the kinetoplast

KN: distance between the kinetoplast and posterior edge of the nucleus

NA: distance between the anterior edge of the nucleus and the anterior end of the body

FF: length of the free flagellum

Table 3 Comparison between morphological dimensions of blood trypomastigotes of *Trypanosoma teixeirae* sp. n. with *Trypanosoma pteropi* and *Trypanosoma hipposideri*

	Morphological Feature ( $\mu\text{m}$ )					
	Total length	Width	PK	KN	NA	FF
<i>Trypanosoma teixeirae</i> sp.n.	20.4-30.8 25.9	1.3-2.3 1.9	1.5-2.4 2.0	3.3-6.2 4.9	5.1 - 9.8 7.8	10.0-12.9 11.3
<i>Trypanosoma pteropi</i>	18.0-22.0 20.0*	2.0-4.0 3.0*	1.5-4.0 2.75*	4.0-5.0 4.5	8.0-10.0 9.0	8.0-12.0 10.0
<i>Trypanosoma hipposideri</i>	10.5-13.0 11.65*	1.5-2.0 1.75	1.0-2.5 1.75	4.0-6.0 5.0	1.5-5.0 3.25*	4.0-8.0 6.0*

Total length: total body length measured along mid-line including free-flagellum; Width: maximum width measured at nucleus level (undulating membrane included); PK: distance between the posterior end and the kinetoplast; KN: distance between the kinetoplast and posterior edge of the nucleus; NA: distance between the anterior edge of the nucleus and the anterior end of the body; FF: length of the free flagellum. Ranges given with mean for *Trypanosoma teixeirae* sp. n. As the mean dimensions were not available for *Trypanosoma pteropi* or *Trypanosoma hipposideri* the median value of the range is presented in the table and was used for statistical analysis. For each column, values followed by an asterisk are significantly different to the *T. teixeirae* sp.n. value ( $p < 0.01$ ).

Table 4 Genetic distances between *Trypanosoma teixeirae* sp. n. and other trypanosome species at 18S rRNA and gGAPDH loci.

Trypanosome species/isolate	Genetic distances (%)	
	18S rDNA	gGAPDH
<i>T. minasense</i> (AJ012413/ AB362561)	1%	14%
<i>T. rangeli</i> (FJ900242/ GQ140364)	1%	15%
<i>T. sp. bat</i> (AJ012418/ GQ140365)	1%	16%
<i>T. conorhini</i> (AJ012411/ AJ620267)	1%	16%
<i>T. sp. NanDoum1</i> (FM202492/ FM164793)	1%	17%
<i>T. vespertilionis</i> (AJ009166/ AJ620283)	1%	17%
<i>T. sp. HochNdi1</i> (FM202493/ FM164794)	1%	17%
<i>T. sp. H25</i> (AJ009168/ AJ620276)	2%	17%
<i>T. sp. AB-2013-G8</i> (KC753537/ KC812988)	1%	18%

Genetic distances were calculated in MEGA 6 (Tamura et al. 2013) using the Tamura-Nei model (Tamura and Nei 1993).