Morphological and molecular characteristics of a Trypanosoma sp. from breeding Triatomines (Triatoma rubrofasciata) in China

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Abstract

Background

Triatomines (kissing bugs) are natural trypanosome vectors that transmit trypanosome species, including *Trypanosoma cruzi*, *Trypanosoma conorhini* and *Trypanosoma rangeli.* Here we reported the first solid record of *Trypansoma conorhini* from *Triatomia rubrofaciata* in China.

Methods

The intestinal contents of the *Triatomine rubrofasciata* were collected to prepare smears and examined by microscopy. The morphological indices of trypanosome species were measured and calculated. The genomic DNA fragment of trypanosomes was extracted, and the genes of 18S rRNA gene, HSP70 and glycosomal glyceraldehyde-3-phosphate dehydrogenase genes (gGAPDH) were PCR amplified and sequenced. The obtained sequences were subjected to a BLAST search in NCBI and followed by phylogenetic analysis with other homologous trypanosomes. To investigate the prevalence of this trypanosome, 135 *T. rubrofasciata* samples from different regions of Guangxi were collected and assayed. Moreover, laboratory infection was conducted to test the possible transmission between *Tr. rubrofasciata* and rats (or mice) though *Tr. Rubrofasciata* bite.

Results

The parasite found in the intestinal contents of *Tr. rubrofaciata*, which were collected in the Guangxi region of southern China, exhibits the typical characteristics of epimastigotes, such as the presence of a nucleus, a free flagellum and a kinetoplast. The body length ranged from 6.3–33.9 μm, and flagellum length ranged from 8.7–29.8 μm; the nucleus index was 0.6, and the kinetoplast index was − 4.6. The BLAST analysis showed that the 18S rRNA, HSP70, and gGAPDH sequences of the *Trypanosoma* sp. exhibit the highest degree of similarity with *T. conorhini* (99.7%, 99.0%, 99.0%), and formed a well-supported clade close to *T. conorhini* and *T. vespertilionis*, while exhibiting a significant distance from *T. rangeli* and *T. cruzi*. Our investigation uncovered a high prevalence of *Trypanosoma* sp. infection in *Tr. rubrofaciata* up to 36.3% in the field. The laboratory experiments showed that both rats and mice could get *Trypansoma* sp. infected through bites from wild *Tr. rubrofaciata*, and lab-feeding *Tr. rubrofaciat* can get *Trypansoma* sp. infected through bloodmeals of infected mice.

Conclusion

Trypanosoma conorhini was found in *Tr. rubrofasciata* in China, as judged by morphology and molecular markers. This parasite had a high prevalence in *T. rubrofasciata*, and lab experiment showed

Trypanosoma conorhini in China could be transmitted between *Tr. rubrofasciata* and rat(mice) through *Tr. rubrofasciata* bites; whether this parasite is pathogenic to human need to be future research.

Background

The *Trypanosoma* genus of mammalian parasites comprises flagellate species of seven subgenera, three for stercorarians and four for salivarians. These blood parasites are widespread in globally and can infect human and diverse animal species through bloodborne and be transmitted by hematophagous vectors [1–5]. The infection may cause economically and socially significant diseases, i.e. sleeping sickness and Chagas disease [6]. The pathogen of Chagas disease, *Trypanosome cruzi* is prevalent throughout the Latin Americas. It is spread by *Triatominae* insects, commonly called “kissing bugs”. Chagas disease can lead to high morbidity and mortality rates among adults in endemic countries; annual deaths are at least 10,000 [7]. *Trypanosome brucei* is responsible for sleeping sickness, most found in equatorial Africa. Two subspecies can infect humans: *Trypanosoma brucei gambiense* causes a chronic form of human trypanosomiasis in West and Central Africa. At the same time, *Trypanosoma brucei rhodesiense* is the pathogenic agent for the acute form of the disease in Eastern Africa [8–9]. Tsetse flies can transmit both species.

The species of trypanosomes (Euglenozoa: Kinetoplastea: Trypanosomatidae) is enormous, and the host is vast. Moreover, the new *Trypanosoma* species and genotypes were constantly reported from specific hosts [10–13]. Vertebrates and invertebrates got infected mainly through various animal bloodsucking like bats, triatomines, tsetse flies and rats—the blooding triatomines harbour of *T. cruzi, T. rangeli* and *T. conorhini. T. cruzi* is a significant human disease which can cause about 6 million infections in the Americas [14]. Contrary to *T. cruzi, T. rangeli* and *T. conorhini* infection has not been associated with symptomatic pathologies or diseases. The geographical distribution of *T. rangeli* often overlaps with that of *T. cruzi*, the same vertebrate and invertebrate hosts being infected, which could mislead the correct diagnosis of *T. cruzi* infection [15]. *T. conorhini* is spread to hosts in the faeces of its vector, the *Triatoma rubrofasciata* (*Tr. rubrofasciata*), the only worldwide spread bloodsucking triatomine. And this parasite is reported to have a restricted host range in rats, mice and non-human primates [16–18]. In Asia, some countries have reported a possible infection of *T. conorhini* in *Tr. rubrofaciata* [19–20], and in Vietnam, sometimes even co-infected with *T. lewisi* [20]. However, as the data solo based on morphological data, it would be too arbitrary to claim *Tr. rubrofasciata* carries *T. conorhini.*

Previous studies revealed that *Tr. Rubrofaciata* was widely distributed in south China, like Guangxi, Guangdong and Hannan provinces and people bitten were commented on literatures [21–24]. In the present study, flagellates were found in the captured *Tr. rubrofaciata*, and its morphological, molecular characteristics, prevalence and animal infection experiment were analyzed.

Material and methods

Parasite sample collection and identification of triatomine

Triatomine specimens were collected from June to November 2020-2022 in Chongzuo city and Beihai city in Guangxi, China, including Jiangzhou district (22.40520083N,107.35192533E), Hepu county (21.8213004237N, 109.4140264191E). Triatomines were collected in the woodpile and transferred to the laboratory to feed. Triatomine species were identified based on morphologically according to the descriptions by Xiao et al. [25], and mitochondrial DNA genes 16S rRNA and cytochrome-b (Cytb) gene were amplified and analyzed [21].

Smear and microscopy were observed.

The trypanosome was identified by microscopic observation of fresh smears and stained blood smears by Giemsa staining and observed by optical microscopy. Apply the Microscope Graticules to calculate the morphological indices of trypanosomes. Identification was based on the morphological features of the parasites described by Hoare [3].

DNA extraction and polymerase chain reaction (PCR)

According to the manufacturer's recommendations, total DNA of 200 µl gut content from each triatomine was extracted using the QIAamp DNA Mini Kit (Qiagen, German). The genes of the 18S rRNA, heat shock protein 70 (HSP70) and glyceraldehyde-3-phosphate dehydrogenase (gGAPDH) were PCR amplified using the primers listed in Table 1. PCR reactions were conducted in a final volume of 25 µl, consisting of 30 ng of DNA template, 1 × Taq PCR Master Mix (Takara, China), and 0.4 µM of each primer. The fragments were amplified with the following thermal cycling conditions: 95°C for 3 min; 35 cycles of 94°C for 30 s, 48-58°C for 60 s, and 72 °C for 90 s; and 72 °C for 10 min.

The amplicons were purified using the MiniBEST Agarose Gel DNA Extraction Kit (Takara), according to the manufacturer’s recommendations, and subsequently conjugated with PMD20-T vector (Takara). Positive clones were selected by universal primers M13, M13-47 (5’-3’ CGCCAGGGTTTCCCAGTCACGAC) and primer RV-M (5’-3’ CAGGAAACAGCTATGAC) and the amplified primers. Two positive clones were sent to Sangon Biotech (Shanghai, China) for both strands sequencing. The sequencing reactions were performed using the ABI Prism® BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems), with the rimersM13 in ABI 3730 sequencers.

Molecular analysis and phylogenetic analysis

The obtained sequences were compared with those available in the GenBank database by Basic Local Alignment Search Tool (BLASTn http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify the homological sequences and download sequences from different species of trypanosomes. A multiple-sequence alignment containing homological trypanosomes such as *T. conorhini*, *T. vespertilionis, T. cruzi*, *T. rangeli, T. wauwu*, *T. dionisii*, *T. erneyi*, and *T. livingstonei* from different hosts or geographic origin were downloaded from GenBank and conducted alignment using the ClustlW (https://www.genome.jp/tools-bin/clustalw). The maximum-likelihood and neighbour-joining models were used to construct analysis trees with MEGA11 [26]. Bootstrap support for 1000 replicates was performed.

Investigation on nature infection and experimental infection

To investigate the prevalence of trypanosomes in wild *Tr. rubrofasciata*, 135 triatomine specimens were collected from 2020 to 2022 from different areas of Guangxi. Trypanosomes were observed via intestinal content examinations with optical microscopy. In addition, 16 *Rattus norvegicus* were collected nearby where the triatomine was found, and the blood was collected by tail cut and blood wet mounts were checked.

To identify whether *Tr. rubrofacsiata* can transmit the trypanosomes, The fresh fecal triatomine was mixed with one drop of PBS to detect the trypanosome directly under the microscopy. Lab animals like Kunming mice, C57BL/6J, BALB/cA-nu and Sprague-Dawley rats were stung and blood-sucking by the wild-infected *Tr. rubrofacsiata*. Mice and rat blood were taken from the tail to detect the trypanosomes every 12 h post-infection. Then the infected mice and rats were bitten and blood-sucking by the lab hatch and feed *Tr. rubrofacsiata*, checking the fresh fecal and detecting the trypanosomes as previously described.

Transovarian transmission experiment

Six infected female *T. rubrofacsiata* were used in this experiment. After laying eggs, all the eggs were collected in a new container. After egg hatching, the blood of the healthy Kunming mice was fed from the first-stage nymphs to the five-stage nymphs and adults. The suspension drop method was used to detect trypanosome in fresh feces twice every week.

Results

Triatomine specimen identification

The collected 135 triatomines from the two collecting sites were all identified morphologically as *Tr. rubrofasciata* by the characteristics as previously reported [21]. the *T. rubrofasciata* 16S rRNA and cytb PCR, yielding 499 bp and 667 bp fragments, are all identical and 100% matched to the *Tr. rubrofasciata* refences in the GenBank (accession no. MH236905 and MH368021).

Microscopic observations

The flagellates were found in the content of the triatomine and observed to possess the characteristic of *Trypanosoma* species and have motility in the *Tr. rubrofacsiata* faeces (Supplementary Movie S1); the parasites appeared as classical epimastigotes (Fig 1a-d) and a few promastigotes (Fig 1e). A nucleus, an undulating membrane, a flagellum and a kinetoplast are apparent. The nucleus is anterior; the kinetoplast is far from the posterior extremity; the nucleus is oval; the kinetoplast is a heavy dot; the nucleus and the kinetoplast are close. The body length of observed epimastigotes ranges from 6.3–33.9 μm, and the free flagellum length range from 2.3–19.0 μm (Table 2). According to morphology, the trypanosomes could be divided into three primary forms; the first is slender epimastigote with a fine posterior end (Fig 1a); the second form is stumpy epimastigote with a finger-shape or fine posterior end (Fig 1b,1c); the third form is

rare epimastigote with a round posterior end. The promastigote is also rare and in a short leaf shape (Fig 1d, Table 2). The trypanosomes in mice and rat by experimental infection mentioned later, were found as typical trypomastigotes with well-developed undulating membranes body and clear flagellar pocket after Giemsa staining. The trypomastigotes' body length was 36.7–52.5 μm, and the free flagellum length ranged from 4.8–10.5 μm (Fig 2a, Table 2).

We manage to compare the morphological characteristics with closely related trypanosomes (Table 2 and Fig 2b-d), based on the molecular markers below. It is not surprise that the morphological data are split into two major groups by kinetoplast index (KI, equal to PN/KN), trypomastigotes and the rest (epimastigotes and promastigotes). The distribution of these two major groups is stretched nearly horizontally into patches, attribute to total length of cell (TL, equal to BL plus FF) and flagellar index (FI, equal to FF/BL). A mix of our data points with *T. conorhini* points from literuature highly suggest their close relationship.

Phylogenies based on 18S rRNA, gGAPDH and HSP70 sequences

After cloning and sequencing, we obtained 2018 bp, 922 bp and 769 bp fragments of the 18S rRNA (GenBank accession no. in submitting), gGAPDH (GenBank accession no. MZ043866) and HSP70 (GenBank accession no. MZ043865) sequences, respectively. BLAST analysis identified that the closest 18S rRNA sequence has the highest similarity (99.7%) with *T. conorhini* strain Tco025E (GenBank acc. MKKU01000460), which was isolated from *Rattus rattus* in Brazil. BLAST analysis of gGAPDH hit with *T. conorhini* Tco025E_10018 (98.x%, GenBank accession no. XM_029376810). HSP70 hit with *T. conorhini* Tco025E_09744 (GenBank accession no. XM_029376553), which isolated from *Rattus rattus* in Brazil and TCC2156 (GenBank accession no. MF144909), which isolated from *Tr. rubrofasciata* in the USA.

The obtained 18S rRNA, HSP70 and gGAPDH sequences were subjected to phylogenetic trees constructioin with representative species of all major trypanosome clades (Fig 3-5). The new triatomine trypanosomes formed a well-supported clade close to *T. conorhini* and *T. vespertilionis* in all phylogenetic trees. In 18S rRNA phylogenetic trees, the newly isolated cluster with four isolates of *T. conorhini* TCC1452, TCC2156, USP and Tco025E, which were from rat or *T. rubrofasciata,* and Three *T. vespertilionis* G1, G2 and P14 which from bats and bugs. The new isolated area is far from *T. rangeli* and *T. cruzi* (Fig 3). In the gGAPDH and HSP70 phylogenetic trees, the newly isolated clade with two isolates of *T. conorhini* (TCC1452 and TCC2156) and have a closer distance with *Trypanosoma sp* Hoch-like G3 and HochNdi1 from bat and monkey and *T. vespertilionis* G1, G2, EU and P14 than *T. rangeli* and *T. cruzi* (Figs 4,5).

An additional sequencing of ITS1 (652 bp) from the isolated trypanosome also reveal an 98.8% similar with *T. conorhini* reference strain Tco025E (GenBank acc. MKKU01000460). Putting together, the newly isolated trypanosomes are actually the first solid Chinese record of *T. conorhini*. By applying possible divergence time of 100 Million years ago (Ma) between *Trypanosoma cruzi* and *Trypanosoma brucei brucei* [27], we estimate a possible divergence time of 1 Ma between Chinese *T. conorhini* and South American strains (Fig 6).

Trypanosoma sp. investigation and experimental infection

We collected 135 triatomine specimens, including 61 adults and 74 larvae from different areas of Guangxi from 2020 to 2022. Forty-nine specimens were observed motility trypanosomes with optical microscopy, the infection ratio was 36.30%. And the adult and larva infection ratioes were 39.34% (24/61) and 33.78% (25/74), respectively. Among the collected 16 *Rattus norvegicus*, trypanosomes in the fresh blood was not detected neither by PCR nor smear.

The experimental infection of the Chinese *T. conorhini* showed that the Kunming mice, C57BL/6J, BALB/C, BALB/cA-nu and Sprague-Dawley rats could be infected through wild *Tr. rubrofasciata* bites and bloodsucking. The trypanosomes can be detected after two- or three days of blood-sucking, and the parasitemia could presist for more than one month. However, the number dropped quickly and kept a very low parasitaemia (less than 1×10^2 parasites per 1ml blood) which detected by direct smear; the infected rats and mice had no symptoms like anaemia and depression. The lab-feeding *Tr. rubrofasciata* got infected with *T. conorhini* after blood-sucking the infected rats or mice, the trypanosomes were detected in the feces after about one week.

Transovarian transmission experiment

This experiment lasted for 20 weeks until all the nymphs became adults. No trypanosome was detected in the fresh feces by all the time.

Discussion

In the present study, *Trypanosoma conorhini* was isolated from the *Tr. rubfrofasciata*, the first reported trypanosomes isolated from triatomines in China. Based on the morphology observed and the molecular analysis, we confirmed this identity. *T. conorhini* is one of the four trypanosomes (*T. cruzi, T. raneli, T. conorhini* and *T. lewisi*) that reported can be transmitted by blood-sucking *Tr. rubfrofasciata*. *T. conorhini* is transmitted to a restricted host range in rats, where it causes a mild and transient infection, and it is thought to be nonpathogenic to humans. However, the laboratory experiment showed that non-human primates can get infected with *T. conorhini* [16–18]; whether this trypanosome contribute to the cutaneous symptoms in human bite case [21] needs more investigation.

A large portion of trypanosomes species have been reported worldwide. However, some new species or genotypes of trypanosomes were still identified recently [10–13]. The species identification of trypanosome by morphology is difficult, and in contrast, molecular analysis is an effective method. Phylogenies based on 18s rRNA, HSP70 and gGAPDH have been used for evolutionary and taxonomic studies of trypanosomatids, and it has been recommended that all new trypanosome species are phylogenetically validated using at least these two genes [28–31]. This study employed 18srRNA, gGAPDH and HSP70 to analyze the trypanosome species isolated from *Tr. rubrofasciata*. The 18S rRNA, HSP70 and gGAPDH sequences and phylogenetic trees showed that the new Chinese triatomine trypanosomes are actually *T. conorhini* with limited difference to the Brazil one.

In China, some trypanosomes species have been identified from different hosts, like *T. evansi* from cattle and buffalo [32], *T. dionisii*, *T. brucei, T. wauwau* and *Trypanosoma* sp. from bat [33–34], *T. lewisi* from rat and mice [35], *T. carassii* from fish [36], *Trypanosoma (Megatrypanum) bubalisi* sp. *nov* from leech [10]. In the present study, we isolate a trypanosome which closer to *T. conorhini* from *Tr. rubrofasciata*, *Tr. rubrofasciata* is the only global distribution triatomine, and it has been reported in several Asian countries and has a significant increase tend [37–38]. Moreover, our previous investigation showed that people bit by *Tr. rubrofasciata* is common in some regions of China. It is becoming a public health problem, producing severe anaphylactic reactions [21]. The triatomine trypanosomes *T. cruzi* and *T. rangeli* can get human infection, and the animal trypanosomes *T. lewisi*, which also detected in *Tr. rubrofasciata* can cause atypical human infections [39], the *T. conorhini* can infect the primate [16]. Therefore, whether the Chinese *Trypanosoma conorhini* can infect primates including humans still need to future more study.

Rat is the common host of *T. conorhini* and *T. lewis*. To identify the origin of the newly isolated trypanosome, we collected 16 *Rattus norvegicus* where the *Tr. rubrofasciata*, like woodpiles or old houses, were feeding chicken, cattle or pigs nearby to detect trypanosome. However, no direct evidence could confirm a natural infection of trypanosome in *Rattus norvegicus*. That may be related to the limited sample and the common infection of *Rattus norvegicus.* In our study, the the Chinese *T. conorhini* couldn't transovarian transmission by *Tr. Rubrofasciata*, the high prevalence of the parasite indicate the presence of other suitable natural host, which still needs larger scale investigation. The experimental condition indicated that rats and mice could be infected with this trypanosome through the nature-collected *Tr. rubrofasciata* bit, and the lab-feeding *Tr. rubrofasciata* can be got infected with a bit the infected rat and mice indicated that the *Tr. rubrofasciata* as a vector transmitted this trypanosome between rats or mice. As many *Tr. Rubrofasciata* were found near chicken coops [21], a highly possible of chicken as nature host is proposed.

Anyway, molecular clock analysis suggests a potential of 1 Ma divergency time between Chinese and South American *T. conorhini*, which is likely corresponding to the period of hominid dominate the Asia. However, whether the prevalence of *Tr. rubrofasciata* and *T. conorhini* in Asia was associate with hominid activities or other mammals or birds remain uncertained.

Conclusions

In the present study, we identified a *Trypanosoma* sp. from *T. rubrofasciata* in China; the morphology and molecular indicated that this trypanosome is *T. conorhini*, which couldn't be transovarial transmission by *Tr. rubrofasciata*. And it can establish an infection from infected *Tr. rubrofasciata* to laboratory mice/rats. A high prevalence (> 36%) of Chinese *T. conorhini* in *Tr. rubrofasciata* collected in the field, but not the mice/rats in same area, suggesting a more suitable natural host, whether this parasite is pathogenic to human need to be future research.

Abbreviations

Trypanosoma conorhini
T. conorhini
Traitomina rubrofasciata
Tr. rubrofasciata
NJ
neighbour-joining
Heat shock protein 70
HSP70
Glycosomal glyceraldehyde-3-phosphate dehydrogenase
gGAPDH.

Declarations

Ethics approval and consent to participate

The Guangxi Medical University Ethics Committee approved the study under licence of Grant No.82260413.

Consent for publication

Not Applicable.

Availability of data and materials

The data from this study are available upon request from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YLS, QL, DHL and YWL developed the study protocol. YLS, DYL, XYF, YYL, PCD, LLT, XQL and SSH performed the field work and contributed to the data analysis. YLS, QL, DHL and YWL performed the final

analysis. YLS, QL and DHL wrote the first manuscript draft. All authors have read and approved the final version of the manuscript.

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Tables

Table1.The primers information

Target gene	Sequence 5'-3'	Annealing temperature	Amplicon size	References
18s RNA	zc-18-f:5-CATATGCTTGTTTCAAGGAC-3 zc-18-r:5-GACTTTTGCTTCCTCTATTG-3	45℃	~2200bp	Maslov, et al.1996
HSP70	H70F:5-TGATGCAGCTGGTGTCGGACTT-3 H70R:5-CTGGTACATCTTCGTCATGATG-3	58℃	~800	Espinosa-Álvarez, 2018
GAPDH	GAF:5-GGBCGCATGGTSTTCCAG-3 GAR:5-CCCCACTCGTTRTCRTACC-3	55℃	~800	Borghesan. 2013
ITS	TRYP1S:5-GGAAGCCAAGTCATCCATCG-3 TRYP1R:5-CGTCCCTGCCATTTGTACACAC-3	55℃	652	Jittapalapong S, 2008

Table 2 Morphometrics of isolated trypanosomes compared with *Trypanosoma conorhini*.

Cell Form[1]		KN[2]	PK	PN	NA	BW	BL	FF
E	RT	-1.4 ± 0.4	6.1± 1.9	5.1 ± 1.7	10.2 ± 5.6	2.1 ± 0.7	15.5 ± 6.7	8.9 ± 4.3
		-2.7–-0.6	2.2–10.1	2.6–9.1	3.4–25.3	1.1–4.0	6.3–33.9	2.3–19.0
E	28°C[3]	-3.4–-1.5	4.6–9.7	3.6–10.0	5.0–17.7	1.0–4.1	8.5–24.6	2.1–15.7
E	37°C[3]	-2.4–-1.1	2.9–14.3	2.5–14.0	4.7–22.7	2.0–5.0	7.3–36.8	4.9–13.5
P	RT	-1.0	4.4–5.4	3.6–4.5	3.4–6.2	1.3–2.2	6.9–11.0	5.8–9.7
P	37°C[3]	-1.4	4.5	3.0	4.9	3.0	7.8	3.2
T	RT	2.0–3.5	0.3–0.5	2.4–3.8	6.8–9.6	1.1–1.7	9.9–13.8	2.8–4.5
T	28°C[3]	1.7–5.0	0.4–11.3	2.6–13.9	2.3–12.4	1.6–4.0	5.8–24.7	1.7–14.7
T	37°C	5.4 ± 0.8	10.0 ± 1.3	15.0 ± 1.7	26.7 ± 3.5	2.6 ± 0.4	42.0 ± 5.0	6.5 ± 1.6
		4.4–7.0	7.9–12.2	12.7–17.9	22.9–34.5	1.9–3.2	36.7–52.5	4.8–10.5
T	37°C[3]	1.6–6.5	0.4–16.7	1.7–23.0	2.4–24.7	1.6–4.9	6.9–42.3	1.2–15.1

Table 2. Morphometrics of isolated trypanosomes compared with *Trypanosoma conorhini* (continued).

Cell Form[1]		TL	NL	NW	FL	NI	KI[2]	FI	n
E	RT	23.7 ± 6.8	1.9 ± 0.5	1.2 ± 0.3	18.5 ± 5.3	0.6 ± 0.4	-4.6 ± 1.3	0.7 ± 0.5	81
		6.3–37.7	1.2–3.8	0.7–2.5	8.7–29.8	0.2–1.6	-7.6– -1.4	0.1–1.9	
E	28°C[3]	15.1– 38.2	1.6–2.7	1.1–2.5	10.1– 32.2	0.2–0.7	-4.1– -2.2	0.1–0.8	17
E	37°C[3]	12.2– 48.5	2.0–2.6	1.2–2.5	8.9–50.0	0.4–1.1	-7.3– -2.5	0.3–0.7	8
P	RT	16.6– 16.7	1.2–1.7	1.1–1.3	11.1-13.2	0.7–1.1	-5.6– -4.4	0.5–1.4	2
P	37°C[3]	11.0	2.0	1.8	6.7	0.6	-3.1	0.4	1
T	RT	12.7– 18.3	2.8–2.9	0.8–0.9	12.5– 19.2	0.4–0.4	0.1–0.2	0.3–0.3	2
T	28°C[3]	8.0–39.4	1.5–2.9	0.8–2.4	9.8–32.0	0.3–1.6	0.1–3.8	0.3–1.1	23
T	37°C	48.6 ± 5.8	2.7 ± 0.5	1.9 ± 0.3	47.8 ± 5.5	0.6 ± 0.1	1.9 ± 0.3	0.2 ± 0.0	10
		43.0– 58.4	2.1–3.6	1.6–2.4	41.1– 58.7	0.5–0.7	1.4–2.4	0.1–0.2	
T	37°C[3]	11.6– 56.4	1.6–2.5	0.6–2.5	12.6– 50.9	0.2–1.5	0.2–5.6	0.1–0.9	40

Biometric data (center to center distances across the cell axis) in µm are provided as mean± SD and ranges: KN, kinetoplast to nucleus; PK, posterior end to kinetoplast; PN, posterior end to nucleus; NA, nucleus to anterior end; BL, body length; FF, free flagellum; L, total length; NL, nucleus length; NW, nucleus width; BW, body width; NI, nucleus index (PN/NA); KI, kinetoplast index (PN/KN); FI, flagellum index (FF/BL). RT, room temperature.

1, E for epimastigote, P for promastigote, and T for trypomastigotes; 2, minus value is applied for KN and KI values of epimastigotes and promastigote, as kinetoplast position is prior to nucleus position. 3, Measurement of *Trypanosoma conorhini* drawings from Deane and Deane, 1961, therefore mean and SD are not calculated to avoid bias interpretation.

Figures

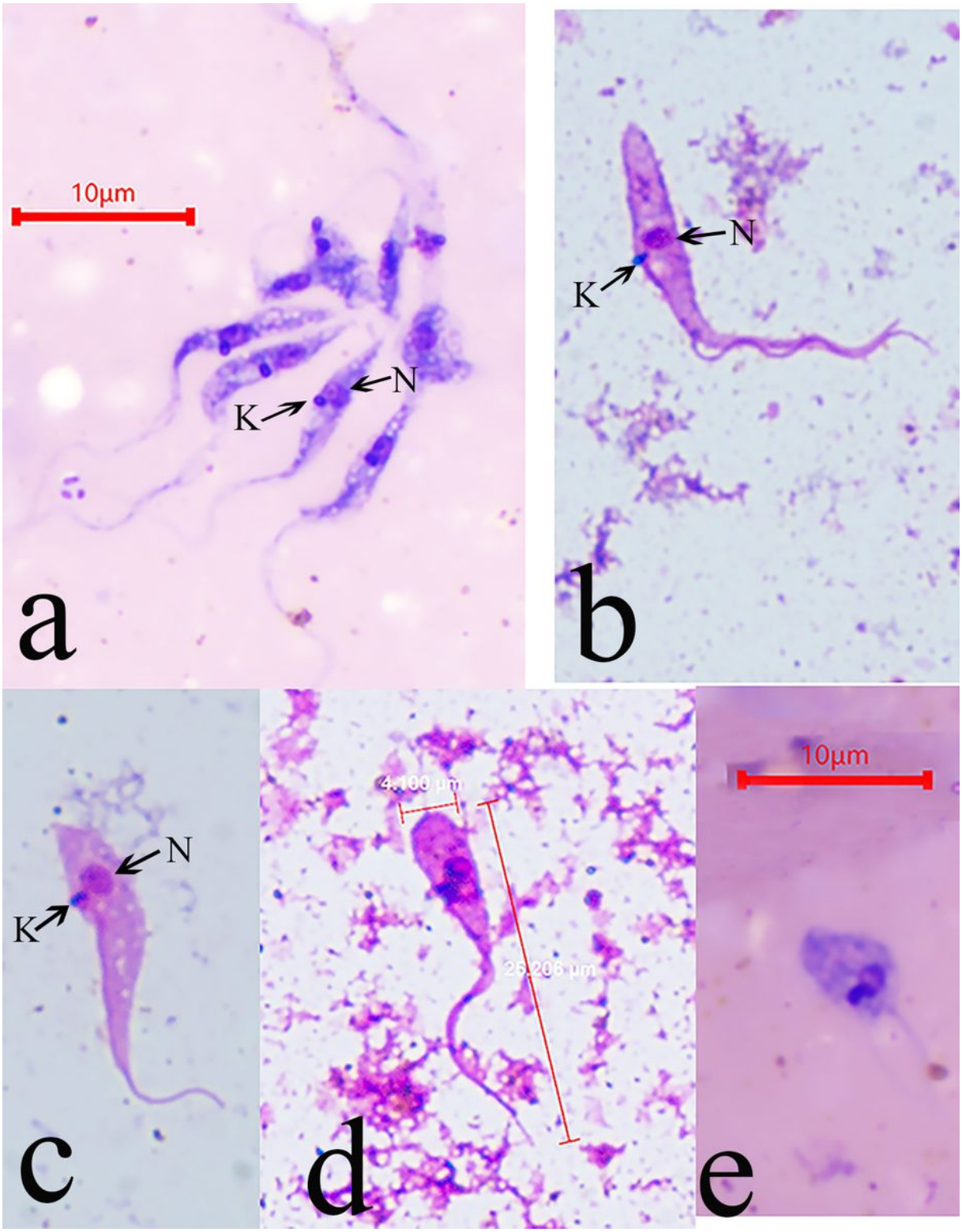

Figure 1

The morphology of *Trypanosoma* sp. in *Triatoma rubrofasciata*.

a) slender epimastigote form; b and c) stumpy epimastigote form; d) epimastigote form with round posterior end; e) promastigote form.

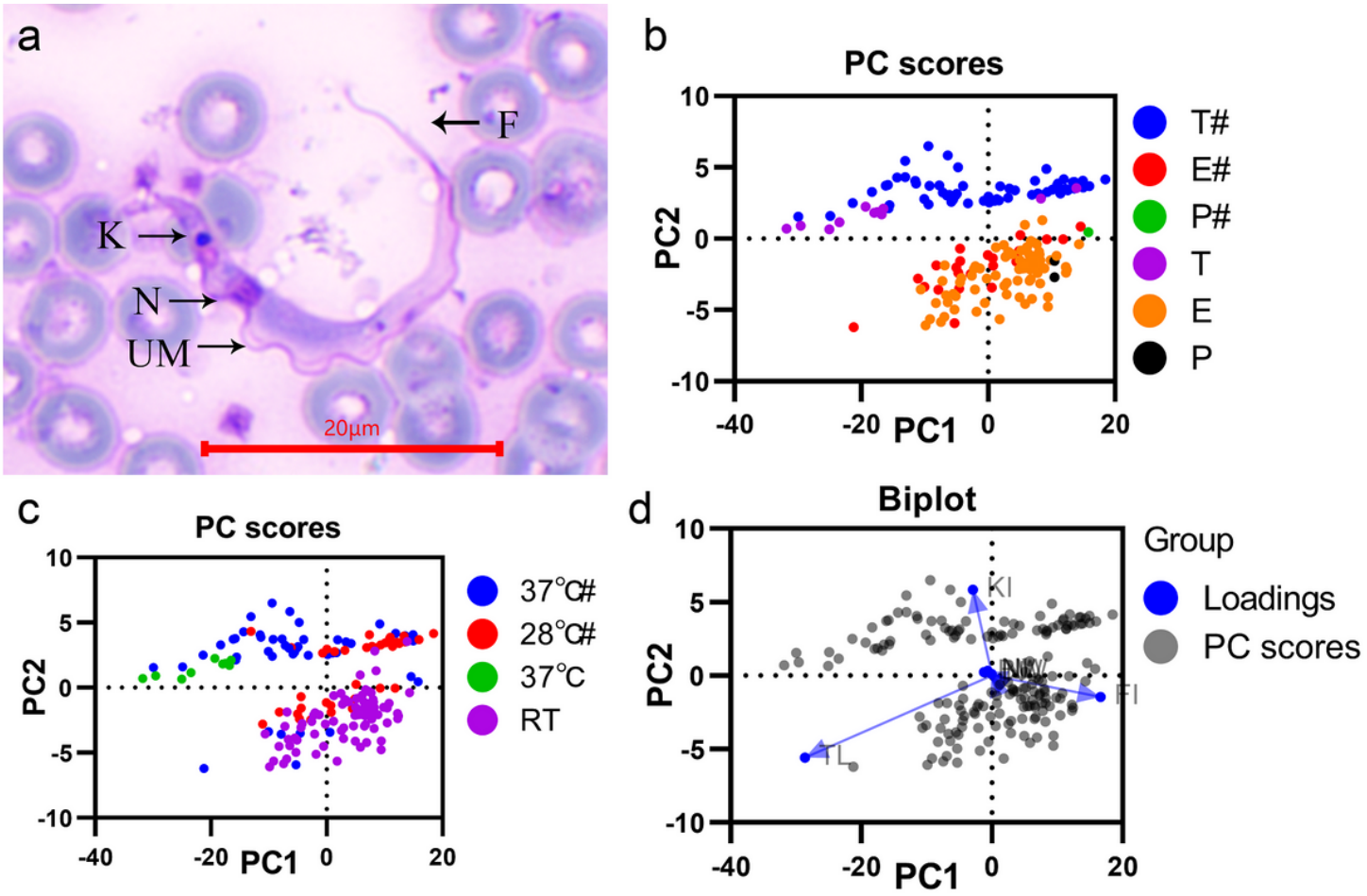

Figure 2

The morphology of *Trypanosoma* sp.in lab infected rat and comparison with the ones in *Triatoma rubrofasciata* and in literature.

a) ***Trypanosoma* sp. in lab infected rat**; b-d) Principal component analysis on morphological data (Table xx, TL, BW, NL, NW, NI, KI and FI) of our isolated trypanosomes with literuature data. Grouping by cell forms (b) or by living temperature (c). The loading of parameters were indicted in blue (d). # data from *Trypanosoma conorhini* drawings (Deane and Deane, 1961).

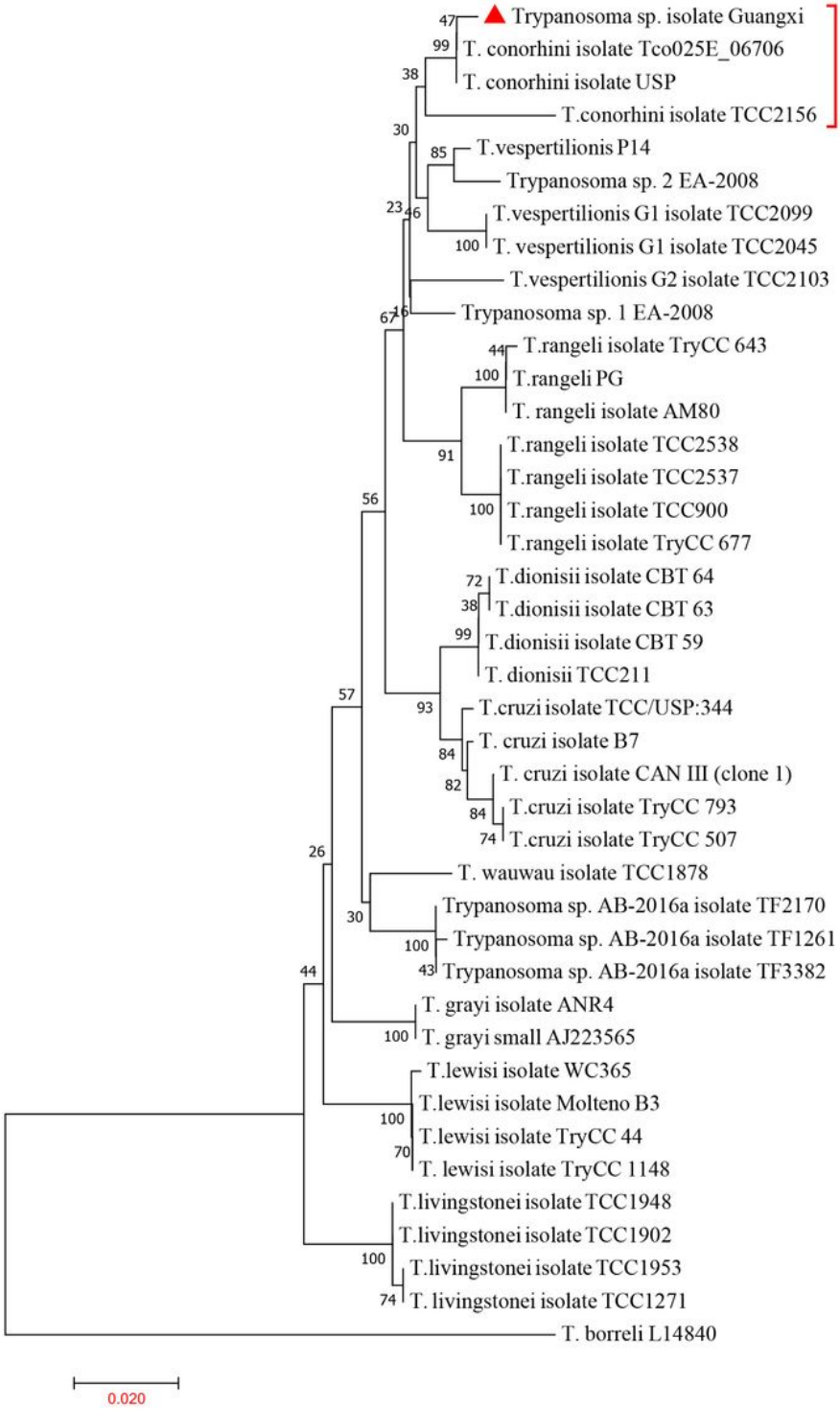

Figure 3

The Phylogenetic tree is based on the 18S rRNA gene sequences from newly isolated *Trypanosoma* sp. and other related species. The phylogenetic tree was constructed by MEGA using the neighbour-joining (NJ) method with 1000 bootstrap replications.

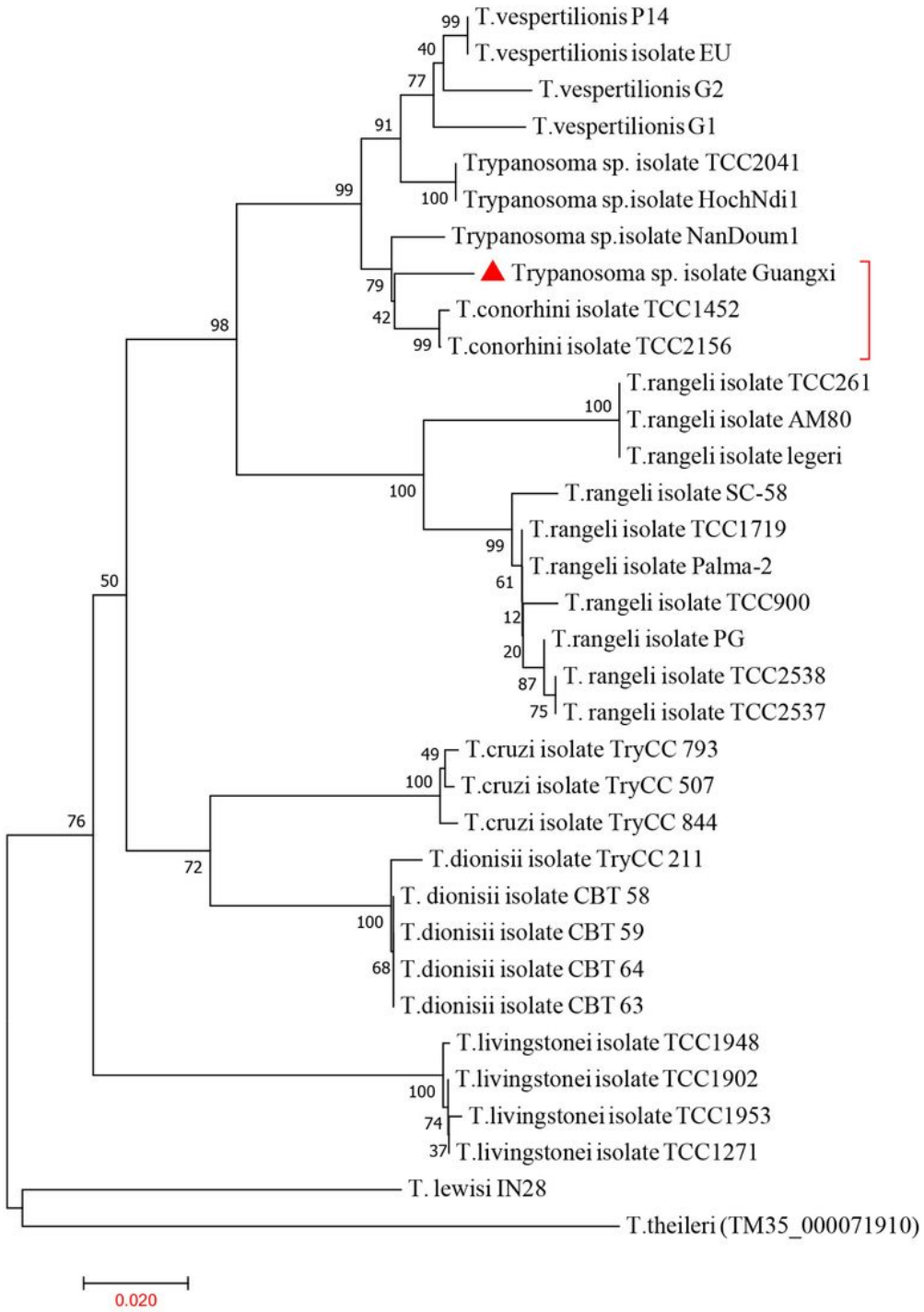

Figure 4

The Phylogenetic tree is based on the heat shock protein 70 sequences from newly isolated *Trypanosoma* sp. and other related species. The phylogenetic tree was constructed by MEGA using the neighbour-joining (NJ) method with 1000 bootstrap replications.

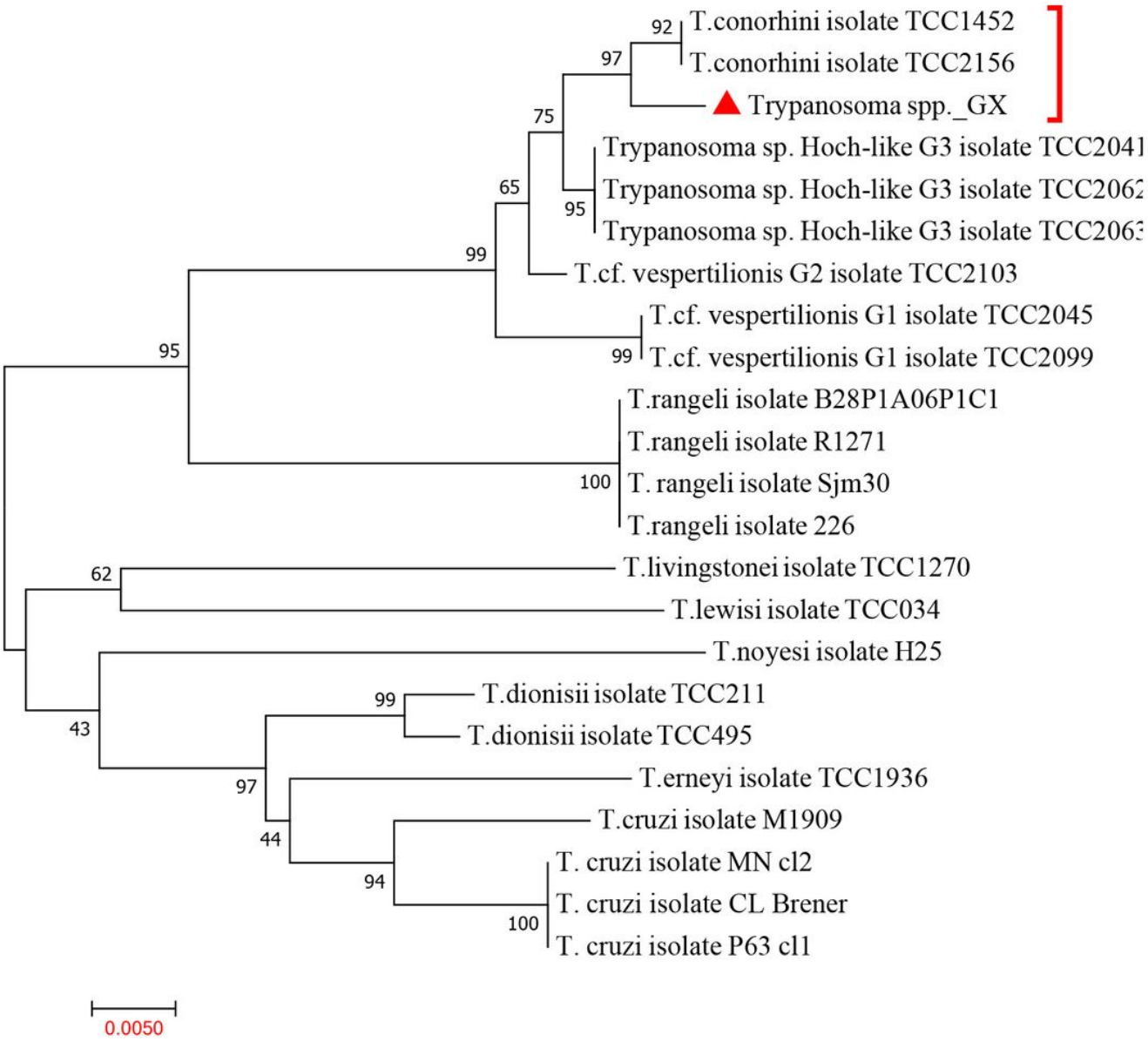

Figure 5

The Phylogenetic tree is based on the glycosomal glyceraldehyde-3-phosphate dehydrogenase sequences from newly isolated *Trypanosoma* sp. and other related species. The phylogenetic tree was constructed by MEGA using the neighbour-joining (NJ) method with 1000 bootstrap replications.

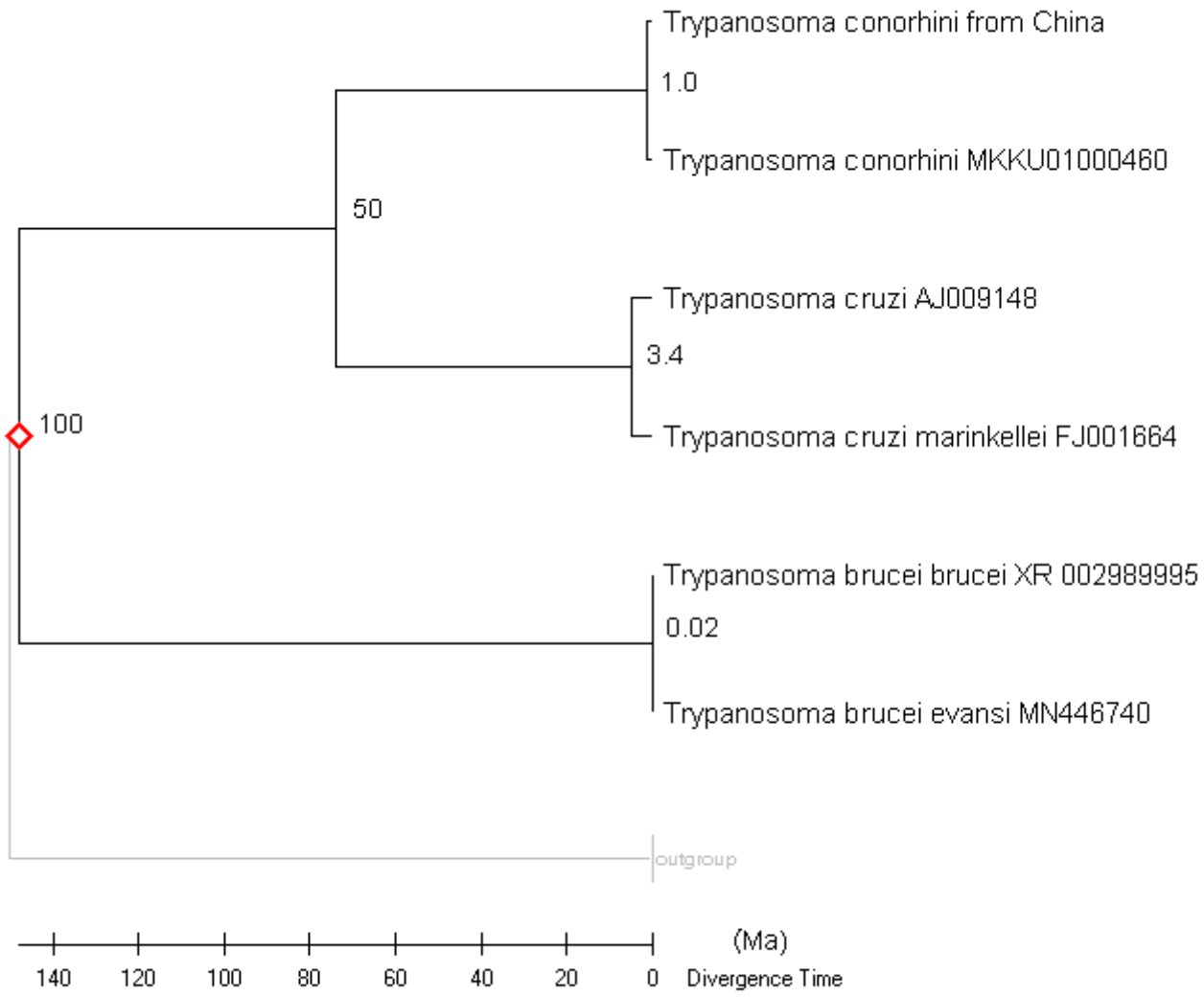

Figure 6

Divergence time calculation of Chinese *Trypanosoma conorhini* and South American strains

Supplementary Files

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