Parasitology Research (2019) 118:97–109
https://doi.org/10.1007/s00436-018-6120-3

ORIGINAL PAPER

Integrative taxonomic approach of trypanosomes in the blood of rodents and soricids in Asian countries, with the description of three new species

Eliakunda Mafie[1] · **Atsuko Saito-Ito**[2,3] · **Masatoshi Kasai**[2] · **Mochammad Hatta**[4] · **Pilarita T. Rivera**[5] · **Xiao-Hang Ma**[6] · **Eng-Rin Chen**[7] · **Hiroshi Sato**[1,8] · **Nobuhiro Takada**[9]

Received: 22 June 2018 / Accepted: 15 October 2018 /

Abstract

Trypanosoma lewisi (Kinetoplastea: Trypanosomatida: Trypanosomatidae) with a cosmopolitan distribution is the type species of the subgenus *Herpetosoma*, which includes ca. 50 nominal species isolated mainly from rodents. Since members of *Herpetosoma* in different host species have an almost identical morphology of bloodstream forms, these trypanosomes are referred to as '*T. lewisi*-like', and the molecular genetic characterization of each species is necessary to verify their taxonomy. In the present study, we collected blood samples from 89 murid rodents of 15 species and 11 soricids of four species in Indonesia, Philippines, Vietnam, Taiwan, and mainland China for the detection of hemoprotozoan infection. *T. lewisi* and *T. lewisi*-like trypanosomes were found in the blood smears of 10 murid animals, which included *Bandicota indica* (two rats), *Rattus argentiventer* (one rat), and *Rattus tiomanicus* (two rats) in Indonesia; *Rattus rattus* (one rat) in the Philippines; and *Niviventer confucianus* (four rats) in mainland China. Furthermore, large- or medium-sized non-*T. lewisi*-like trypanosomes were detected in two soricids, *Crocidura dracula* in Vietnam and *Anourosorex yamashinai* in Taiwan, respectively. Molecular genetic characterization of the small subunit (SSU) ribosomal RNA gene (rDNA) and glycosomal glyceraldehyde-3-phosphate dehydrogenase (gGAPDH) gene indicated that the trypanosomes from all the murid hosts had identical SSU rDNA or gGAPDH gene nucleotide sequences except for those in *N. confucianus* in mainland China. These *N. confucianus*-infecting trypanosomes also showed several unique morphological features such as smaller bodies, anteriorly positioned nuclei, and larger rod-shaped kinetoplasts when compared with *T. lewisi* trypomastigotes. *Trypanosoma* (*Herpetosoma*) *niviventerae* n. sp. is erected for this new species. Similarly, based on morphological and molecular genetic characterization, *Trypanosoma sapaensis* n. sp. and *Trypanosoma anourosoricis* n. sp. are proposed for the trypanosomes in *C. dracula* in Vietnam and *A. yamashinai* in Taiwan, respectively. More effort directed toward the

---

Eliakunda Mafie and Atsuko Saito-Ito contributed equally to this work.

Section Editor: Marta Teixeira

✉ Hiroshi Sato
sato7dp4@yamaguchi-u.ac.jp

[1] Laboratory of Parasitology, United Graduate School of Veterinary Science, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, Japan

[2] Section of Parasitology, Division of Microbiology, Kobe University Graduate School of Medicine, Kobe, Hyogo 650-0017, Japan

[3] Section of Microbiology, School of Pharmacy, Hyogo University of Health Sciences, 1-3-6 Minatojima, Chuo-ku, Kobe, Hyogo 650-8530, Japan

[4] Department of Medical Microbiology, Molecular Biology and Immunology Laboratory, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi, Indonesia

[5] Department of Parasitology, College of Public Health, University of the Philippines Manila, 625 Pedro Gil Street, 1000 Manila, Philippines

[6] College of Life Sciences, Zhejiang University, Hangzhou 310058, People's Republic of China

[7] Department of Parasitology, Kaohsiung Medical University, No. 100, Shih-Chuan 1st Road, Kaohsiung, Taiwan

[8] Faculty of Veterinary Medicine, Airlangga University, Campus C, Mulyorejo, Surabaya 60115, Indonesia

[9] Faculty of Medical Sciences, University of Fukui, Fukui 910-1193, Japan

morphological and molecular genetic characterization of the trypanosomes of rodents and soricids is required to fully understand the real biodiversity of their hemoflagellates.

Keywords *Trypanosoma lewisi* · *Trypanosoma niviventerae* n. sp. · *Trypanosoma sapaensis* n. sp. · *Trypanosoma anourosoricis* n. sp. · Rodent · Soricid · rDNA · gGAPDH · Asia

Introduction

Hemoflagellates of the genus *Trypanosoma* (Kinetoplastea: Trypanosomatida: Trypanosomatidae) take a wide spectrum of hosts such as fish, amphibians, reptiles, birds, and mammals and are transmitted by blood-feeding invertebrates (Hoare 1972; Bardsley and Harmsen 1973; Simpson et al. 2006; Cooper et al. 2017). The subgenus *Herpetosoma* with the type species *Trypanosoma lewisi* in rats (*Rattus* spp.) is given mainly to rodent trypanosomes with *T. lewisi*-like morphology (Hoare 1972) and includes ca. 50 nominal species. Members of the subgenus, *T. lewisi* and '*T. lewisi*-like' species, were believed to have a rigid host specificity irrespective of their almost identical morphology of bloodstream forms (Hoare 1972). Over the last two decades, however, several sporadic cases of human infection with *T. lewisi* have been recorded in Gambia, Thailand, and India under special circumstances where close contact was found between human residents and rat fleas with metacyclic *T. lewisi* (reviewed by Truc et al. 2013). Lun et al. (2015) demonstrated that *T. lewisi* is resistant to normal human serum, i.e., trypanolysis induced by human serum protein apolipoprotein L-1, while other animal trypanosomes are susceptible to this protein and cannot survive in human blood (Vanhamme et al. 2003; Pérez-Morga et al. 2005). Due to this recently recognized zoonotic potential as a cause of atypical human trypanosomiasis, the taxonomy and biological characters of trypanosomes of *Herpetosoma* are now a major priority for the specific diagnosis of the disease and also for the basic taxonomic concerns relating to morphologically similar trypomastigotes in diverse rodent species (Mafie et al. 2016).

Prior to the stimulus of the recent recognition of zoonotic infection with *T. lewisi*, the molecular genetic characterization of *Herpetosoma* trypanosomes has been attempted over the last two decades using the ribosomal RNA gene (rDNA) and protein-coding genes such as the glycosomal glyceraldehyde-3-phosphate dehydrogenase (gGAPDH) gene (Haag et al. 1998; Stevens et al. 1998; Noyes et al. 1999, 2002; Sato et al. 2003, 2005, 2007; Hamilton et al. 2004, 2005, 2007). These basic data contributed to the specific diagnosis of *T. lewisi* infection in humans and nonhuman primates, as well as molecular epidemiological surveys of the species in wild rodents (Maia da Silva et al. 2010; Pumhom et al. 2014). An additional polymerase chain reaction (PCR)-based specific differentiation of *T. lewisi* and its related species using a different genetic marker has recently been attempted (Hong et al. 2017; Ortiz et al. 2018). At present, the available nucleotide sequences of *Herpetosoma* species are limited, making the taxonomic discussion on special DNA sequences with a few or several nucleotide substitutions compared with those of *T. lewisi* rather difficult. For example, *T. lewisi* in rats and *Trypanosoma musculi* in the house mouse (*Mus musculus*), showing distinct biological characters in their vertebrate hosts and unsuccessful cross infection between these two murid hosts, have only one nucleotide substitution in the small subunit (SSU) rDNA over a 2187-bp length (e.g., DDBJ/EMBL/GenBank accession nos. AJ223566 and AJ223568), whereas *Trypanosoma blanchardi* in the garden dormouse (*Eliomys quercinus*) has an identical SSU rDNA nucleotide sequence (AY491764) to that of *T. musculi*. On the contrary, different isolates of *Trypanosoma grosi* in field mice (*Apodemus* spp.) show occasional nucleotide substitutions ranging from two to eight over a 2159-bp length (AB175622–AB175624 and FJ694763; Sato et al. 2005; Guan et al. 2011). The collection of more isolates for nucleotide sequencing and biological characterization is necessary in order to clarify the taxonomic significance of such molecular genetic uniqueness of different *Herpetosoma* isolates.

In the present study, we collected blood samples from 89 murid rodents and 11 soricids in Indonesia, the Philippines, Vietnam, Taiwan, and mainland China for the detection of hemoprotozoan infection. Trypanosomes isolated from 10 murids and two soricids were morphologically and molecular-genetically characterized, thus providing additional data for the taxonomic discussion of trypanosomes of rodents and soricids.

Materials and methods

Parasitological survey

Between 2002 and 2005, cooperative surveys of zoonotic hemoprotozoan agents such as *Babesia microti* and related species in murid rodents were conducted at three localities in Indonesia, one locality in the Philippines, one locality in Vietnam, four localities in Taiwan, and two localities in mainland China (Table 1). In total, 89 murids of 15 species and 11 soricids of four species were trapped alive using Sherman traps. Species identification of the collected murids and soricids in Indonesia and the Philippines was conducted by mammalogists in the Faculty of Medicine, Hasanuddin University, Indonesia, and the College of Veterinary Science and Medicine, Central Luzon State University, Philippines, respectively. The species identification of trapped animals in Vietnam, Taiwan, and

Table 1 Blood smears examined in the present study and detected trypanosomes

Locality and collected hosts	No. of animals	Date of collection	No. of positive animals[a]	Trypanosomes detected	SSU rDNA[b]	gGAPDH[b]
Indonesia (Sulawesi): Toraja and Makassar	(28 in total)	March 3, 2003				
Bandicota indica	15		2 (IN6; IN26)	*T. lewisi*	AB242273	–
Rattus argentiventer	4		1 (IN8)	*T. lewisi*	AB242273	–
Rattus exulans	3		0			
Rattus tiomanicus	5		2 (IN28; IN29)	*T. lewisi*	AB242273	LC369597
Mus caroli	1		0			
Indonesia (Sulawesi): Palopo	(6 in total)	March 17, 2003				
Bandicota indica	1		0			
Rattus argentiventer	2		0			
Rattus exulans	1		0			
Rattus tiomanicus	2		0			
Philippines (Luzon): Nueva Ecija	(7 in total)	March 19–21, 2003				
Rattus rattus	7		1 (Ph7)	*T. lewisi*	AB242273	LC369597
Vietnam (Lào Cai Province): Sapa	(6 in total)	December 17 and 18, 2003				
Rattus flavipectus	2		0			
Apodemus draco	2		0			
Crocidura dracula	1		1 (Vietnam5)	*T. sapaensis* n. sp.	AB242822	–
Crocidura horsfieldii	1		0			
Taiwan: Taipei	(3 in total)	September 6, 2004				
Rattus losea	2		0			
Apodemus agrarius	1		0			
Taiwan (Nantou): Renai, Guoshing, and Puli	(23 in total)	December 22, 2002–December 23, 2004				
Mus caroli	2		0			
Apodemus semotus	15		0			
Anourosorex yamashinai	2		1 (Tw II-1)	*T. anourosoricis* n. sp.	AB242823	–
Crocidura horsfieldii	2		0			
Suncus murinus	2		0			
Taiwan (Chiayi): Minshiung	(3 in total)	December 23, 2002				
Suncus murinus	3		0			
Taiwan (Kaohsigung): Touyuan and Linyuan	(8 in total)	December 23, 2002–December 23, 2004				
Bandicota indica	7		0			
Rattus coxingi	1		0			
China (Shaanxi Province): Qinling Mountains	(8 in total)	October 31–November 3, 2003				
Apodemus chevrieri	2		0			
Apodemus peninsulae	6		0			
China (Zhejiang Province): Hangzhou	(8 in total)	February 11, 2005				
Niviventer confucianus	7		4 (Ch II-24; Ch II-27; Ch II-31; Ch II-32)	*T. niviventerae* n. sp.	AB242274	LC369598
Apodemus agrarius	1		0			

[a] Number of positive animals is followed by the name of isolates in parentheses

[b] DDBJ/EMBL/GenBank accession number

mainland China was carried out following the principles of Ma et al. (1987) and Zhang et al. (1997).

Blood was collected by cardiac puncture from each animal under diethyl ether anesthesia and immediately mixed in plastic tubes with a drop of heparin sodium (Mochida Pharmaceutical, Tokyo, Japan). Thin blood films were prepared individually, fixed in absolute methanol, then stained in Giemsa's solution. Blood films were rigorously examined at ×200 magnification under a light microscope.

The ethics of animal trapping and sample collection adhered to in this study followed the guidelines outlined by each survey participant's university, but at that time, no individual permission numbers of sample collection were provided to us from these organizations.

Morphological examination

Trypanosome-positive blood films were further examined under oil immersion at ×1000 magnification. Undistorted, well-stained trypanosomes were photographed at ×1000 magnification, transformed into photographs with Adobe® Photoshop® v. 5.0, then printed at a higher magnification. Measurements were conducted on printed photographs, and a digital curvimeter type S (Uchida Yoko, Tokyo, Japan) was used when necessary. The measurements (expressed in μm) included total length (TL), maximum width with undulating membrane (MW), maximum width without undulating membrane (BW), distance between the posterior end and the middle of the kinetoplast (PK), distance between the middle of the kinetoplast and the middle of the nucleus (KN), distance between the middle of the nucleus and the anterior end (NA), length of the free flagellum (FF), and lengths and widths of the kinetoplast (KL and KW) and nucleus (NL and NW). The nuclear index (NI) and kinetoplast index (KI) were calculated as follows: NI = (PK + KN)/NA and KI = (PK + KN)/KN, following Hoare (1972).

Blood films on glass slides examined in this study were deposited in the Meguro Parasitological Museum, Tokyo, Japan, under the collection numbers 21386–21394.

DNA extraction, PCR, and nucleotide sequencing

Parasite DNA was extracted from 0.2 ml of each blood sample from 13 trypanosome-positive animals using a nucleic acid purification kit, MagExtractor™-Genome- (Toyobo, Osaka, Japan), according to the instructions of the manufacturer.

PCR amplification of three overlapping fragments of the SSU rDNA was conducted using the primer pairs shown in Table 2 with the following protocol: 5 min at 95 °C followed by 40 cycles of 20 s at 94 °C, 40 s at 55 °C, and 60 s at 72 °C, then a final extension at 72 °C for 5 min. The gGAPDH gene was amplified using degenerate primers G3 (5′-TTYGCCGYATYGGYCGCATGG-3′) and G5 (5′-ACMAGRTCCACCACRCGGTG-3′) with the following protocol: 3 min at 95 °C followed by 35 cycles of 60 s at 95 °C, 30 s at 55 °C, and 60 s at 72 °C, then a final extension at 72 °C for 7 min, according to Hamilton et al. (2004). Next, a nested PCR using a primer pair of G1 (5′-CGCGGATCCASGGYCTYMTCGGBAMKGAGAT-3′) and G4a (5′-GTTYTGCAGSGTCGCCTTGG-3′), or that of G1 and G4b (5′-CCAMGASACVAYCTTGAAGAA-3′) in the second round, was conducted according to Hamilton et al. (2004). The PCR protocol for the second round was similar to that of the first-round PCR detailed above. As sequencing primers, G6 (5′-GYGGTKTCSVTSAAGGACTG-3′) and G7 (5′-CSCCTGTBGTGCTBGGTATG-3′) were used (Hamilton et al. 2004).

Phylogenetic analysis

For phylogenetic analyses, nucleotide sequences of the SSU rDNA and gGAPDH gene were used. The newly obtained SSU rDNA sequences of trypanosomes isolated in the present study (AB242273, AB242274, AB242822, and AB242823) and those of related trypanosome species retrieved from the DDBJ/EMBL/GenBank databases were aligned using the CLUSTAL W multiple alignment program (Thompson et al. 1994), with subsequent manual adjustment. Trypanosomes of the '*Trypanosoma cruzi* clade' (such as *T. cruzi*, *Trypanosoma dionisii*, and *Trypanosoma erneyi*) were used as an outgroup due to their close phylogenetic relationships with trypanosomes of the *Herpetosoma*/soricid trypanosome (*Trypanosoma talpae*) clade (Sato et al. 2008; Paparini et al. 2011; Mafie et al. 2018). The accession numbers of the sequences analyzed in the present study are given in the figures showing phylogenetic trees. Regions judged to be poorly aligned and characters with a gap in any sequence were excluded from subsequent analyses; 1710 characters, of which 125 were variable, remained for subsequent analyses. Similarly, the newly obtained nucleotide sequences of the gGAPDH gene of trypanosomes isolated in the present study (LC369597 and LC369598) and those of related trypanosome species retrieved from the aforementioned databases were aligned and processed as described above; 804 characters, of which 340 were variable, remained for subsequent analyses. As an outgroup, trypanosomes of the *T. cruzi* clade and marsupial mammals were again used due to their close phylogenetic relationships. Maximum likelihood (ML) analysis was performed with the program PhyML (Guindon and Gascuel 2003; Dereeper et al. 2008) provided on the 'phylogeny.fr' website (http://www.phylogeny.fr/). The probability of inferred branches was assessed by the approximate likelihood ratio test (aLRT), an alternative to the nonparametric bootstrap estimation of branch support (Anisimova and Gascuel 2006).

Results

Incidence of trypanosomes in the blood films

As shown in Table 1, trypanosomes were found in 12 blood films prepared from the cardiac blood of 10 murids (*Bandicota indica* (two animals), *Rattus argentiventer* (one animal), and *Rattus tiomanicus* (two animals) in Indonesia; *Rattus rattus* (one animal) in the Philippines; and *Niviventer confucianus* (four animals) in mainland China) and two soricids (*Crocidura dracula* (one animal) in Vietnam and *Anourosorex yamashinai* (one animal) in Taiwan). The levels of parasitemia were variable but generally low in all blood films, particularly those prepared from soricids in which only a few trypomastigotes were detected.

Table 2 Primers used for amplification of SSU rDNA nucleotide fragments in the present study

Segment no.	Primer for PCR amplification	Primer for sequencing	Direction[a]	Sequence	Position of 5′-end[b]	Reference
1	SSU/U1m		F	5′-TTGATTCTGCCAGTAGTCATA-3′	− 21	Haag et al. (1998), modified
	TRY2R		R	5′-AACCAACAAAAGCCGAAACG-3′	929	Present study
		TRY4R	R	5′-CTATTTCTTTTCGCTGCCTC-3′	505	Present study
2	TRY1F		F	5′-CTACCACTTCTACGGAGGG-3′	427	Present study
	TRY927mR		R	5′-CTACTGGGCAGCTTGGATC-3′	1688	Noyes et al. (1999), modified
		SSU561mF	F	5′-TGGGATAACAAAGGAGCAT-3′	879	Noyes et al. (1999), modified
		SSU561mR	R	5′-TGAGACTGTAACCTCAAAGC-3′	1446	Noyes et al. (1999), modified
3	TRY3F		F	5′-ATGACACCCATGAATTGGGG-3′	1261	Present study
	SSU/L1		R	5′-CTACAGCTACCTTGTTACGA-3′	2167	Haag et al. (1998)
		TRY5F	F	5′-TCGGTGGAGTGATTTGTTTG-3′	1629	Present study

[a] F: forward and R: reverse

[b] Relative position of the 5′-end of each primer in an rDNA sequence of *Trypanosoma lewisi* from a *Bandicota indica* in Indonesia (DDBJ/EMBL/GenBank accession no. AB242273)

Morphological and molecular genetic characterization of trypanosomes in the blood

The trypomastigotes in the blood of *B. indica* (two animals) and the three *Rattus* spp. (four animals) collected in Indonesia and the Philippines were typical *T. lewisi* bloodstream forms (Figs. 1a–c and 2a and Table 3). Molecular genetic characterization using the SSU rDNA nucleotide sequences supported this specific identification of *T. lewisi*; the nucleotide sequences of all isolates of trypanosomes from these animals (IN6, IN8, IN26, IN28, IN29, and Ph7), 2147 bp in length, were identical to each other (AB242273) and to SSU rDNA reference sequences of *T. lewisi*, i.e., Molteno B3 and ATCC 30085 isolates (AJ009156 and AJ223566).

The trypomastigotes in the blood of *N. confucianus* exhibited a somewhat unique *T. lewisi*-like appearance and were consistently smaller in size (Figs. 1d–f and 2b and Table 3) in all blood smears prepared from different host individuals. The SSU rDNA nucleotide sequences of all successfully characterized isolates (Ch II-24, Ch II-27, and Ch II-32) collected at Hangzhou, Zhejiang Province, 2147 bp in length, were identical to each other (AB242274) and showed the highest identity with those of *T. blanchardi* and *T. musculi* (AY491764 and AJ223568) with a single nucleotide substitution site over the 2147-bp length, followed by the aforementioned *T. lewisi* sequences with two nucleotide substitution sites over a similar length. The gGAPDH nucleotide sequences of trypanosomes from *N. confucianus*, 842 bp in length, were identical to each other (LC369598) and showed the highest identity with those of *T. lewisi* isolates collected from *Rattus* spp. in Indonesia and the Philippines (IN28, IN29, and Ph7; LC369597) with two nucleotide substitution sites over the 842-bp length or that of a *T. lewisi* isolate collected from *R. rattus* in the UK (AJ620272) with three nucleotide substitution sites, followed by those of *T. grosi* isolates (AB362557 and AB362558) with seven or eight nucleotide substitution sites over the same length.

The trypanosomes from *C. dracula* in Vietnam and those from *A. yamashinai* in Taiwan showed a rigid appearance of bodies with granular structures in the central cytoplasm and the kinetoplast localized rather near the nucleus, features distinct from *T. lewisi* and other species in the subgenus *Herpetosoma* (Figs. 1g–i and 2c–d and Table 4). The SSU rDNA nucleotide sequences of these two isolates from different soricid species in Vietnam (Vietnam5) and Taiwan (Tw II-1) were successfully obtained: 2158-bp long (AB242822) and 2166-bp long (AB242823), respectively. These sequences showed the highest affinities with the SSU rDNA sequence of *T. talpae* from the European mole (*Talpa europaea*) in the UK (1828-bp long; AJ620545), the only deposited SSU rDNA sequence of soricid trypanosomes. The sequence of trypanosomes from *C. dracula* had the higher nucleotide identity (99.56%; 1813/1821) with that of *T. talpae* but contained 19 insertion/deletion sites (indels) and that of trypanosomes from *A. yamashinai* had 98.93% (2132/2155) identity with 14 indels. The Vietnam5 and Tw II-1 isolates showed 99.56% (1813/1821) identity with 28 indels.

Based on the morphological uniqueness of trypomastigotes in the blood as well as their molecular genetic characters, three new species, *Trypanosoma* (*Herpetosoma*) *niviventerae* n. sp., *Trypanosoma sapaensis* n. sp., and *Trypanosoma anourosoricis* n. sp., are erected for the three morphotypes in *N. confucianus*, *C. dracula*, and *A. yamashinai*, respectively.

Description

Trypanosoma (*Herpetosoma*) *niviventerae* n. sp. (Kinetoplastea: Trypanosomitida: Trypanosomatidae) (Figs. 1d–f and 2b and Table 3)

The body of the trypomastigotes in the cardiac blood ($n = 6$), which measured 19.7–30.0 (24.0) × 1.6–2.4 (2.0), had a curved

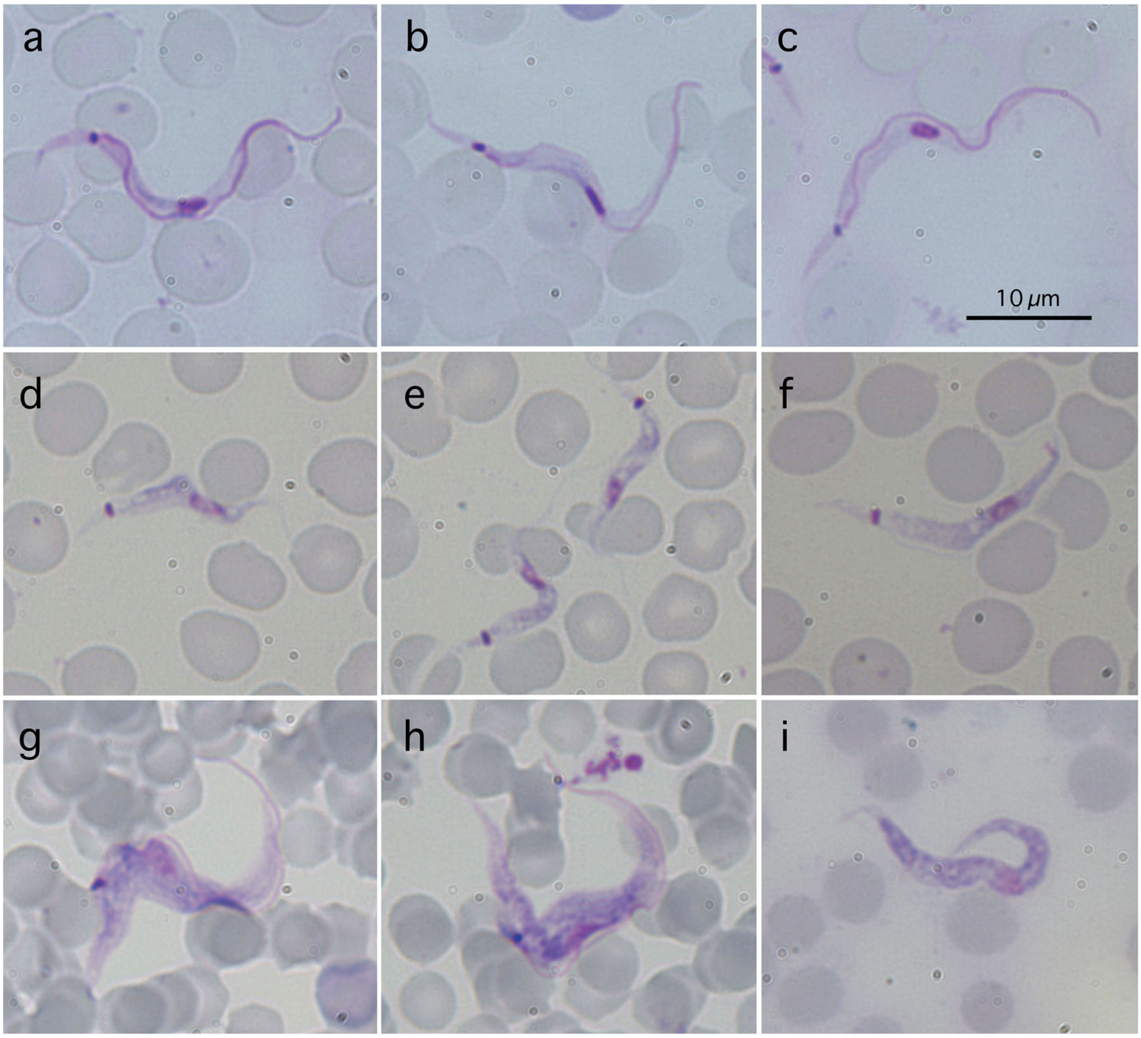

Fig. 1 Photographs of trypomastigotes of *Trypanosoma* (*Herpetosoma*) *lewisi* in the blood of *Rattus tiomanicus* in Indonesia (**a**–**c**); *Trypanosoma* (*Herpetosoma*) *niviventerae* n. sp. in the blood of *Niviventer confucianus* in China (**d**–**f**); *Trypanosoma sapaensis* n. sp. in the blood of *Crocidura dracula* in Vietnam (**g**, **h**); and *Trypanosoma anourosoricis* n. sp. in the blood of *Anourosorex yamashinai* in Taiwan (**i**). All photographs are at the same magnification and the scale bar is shown in **c**

anterior end and a spindle-shaped posterior end, with the free flagellum measuring 3.1–5.6 (3.9) in length. The undulating membrane was apparently narrow, adhering closely to the body. The oval nucleus, which measured 2.4–3.4 (2.7) × 1.0–2.1 (1.4), was situated near the 1/3 point of the body from the anterior end, with NI 1.2–2.2 (1.9). The nucleus occupied the complete width of the body. The rod-shaped, relatively large kinetoplast, which measured 0.9–1.3 (1.1) × 0.5–0.7 (0.6), was situated at the posterior 1/3 point between the nucleus and posterior end, with KI 1.3–1.5 (1.4). Additional measurements of this new species in *N. confucianus* (Ch II-27) are given in Table 3, along with the measurements of *T. lewisi* in *B. indica* (IN6) and *R. argentiventer* (IN8). The SSU rDNA and gGAPDH nucleotide sequences of this new species are deposited in and available from the DDBJ/EMBL/GenBank databases under the accession numbers AB242274 and LC369598, respectively.

Taxonomic summary

Host: *Niviventer confucianus* (Milne-Edwards, 1871), Chinese white-bellied rat (Mammalia: Rodentia: Muridae).

Locality: Hangzhou, Zhejiang, China.

Materials deposited: Hapantotype no. 21393 (Ch II-27), Meguro Parasitological Museum, Tokyo, Japan. Paratype nos. 21394b (Ch II-24, Ch II-31, and Ch II-32) in the same museum.

Prevalence: Four of seven rats at Hangzhou, Zhejiang. Relatively high intensity as the level of parasitemia.

Etymology: The species is named after the name of the host genus.

Remarks

This new *T. lewisi*-like species was found in four rats at different levels of parasitemia. The observed trypomastigotes were

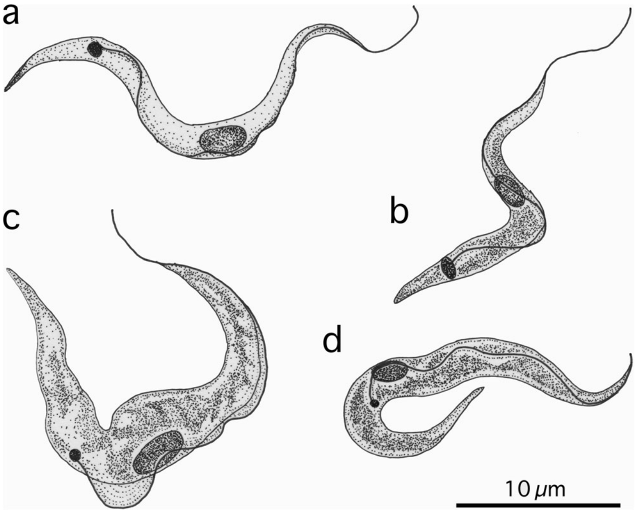

Fig. 2 Stylized drawings of the four *Trypanosoma* spp. shown in Fig. 1: *T.* (*H.*) *lewisi* (**a**), *T.* (*H.*) *niviventerae* n. sp. (**b**), *T. sapaensis* n. sp. (**c**), and *T. anourosoricis* n. sp. (**d**). All drawings are at the same magnification

consistently smaller in size when compared with *T. lewisi* (Table 3). The cytoplasm often assumed a granular or mottled appearance due to heterogeneous basophilic deposition. As discussed above, the molecular genetic characterization based on the SSU rDNA and gGAPDH gene supports the morphological differentiation of *T. niviventerae* n. sp. from *T. lewisi* or *T. lewisi*-like trypanosomes such as *T. musculi*.

Description

Trypanosoma sapaensis n. sp. (Kinetoplastea: Trypanosomitida: Trypanosomatidae) (Figs. 1g–h and 2c and Table 4)

The body of the trypomastigotes in the blood ($n = 3$), which measured 36.8–38.8 (37.9) × 3.7–5.4 (4.8), had a unique morphological appearance: broad body with posteriorly situated kinetoplasts near the nucleus rather than the posterior end, granular or mottled cytoplasm, and elongated sharpened ends. The free flagellum measured 5.8–7.8 (6.7) in length. The undulating membrane was distinct, measuring 1.0 maximal width. The oval nucleus, which measured 3.1–3.7 (3.3) × 1.7–2.0 (1.8), was situated almost at the midpoint between both ends, with NI 0.8–1.1 (1.0). The nucleus was located close to the side of the undulating membrane and the kinetoplast. The small oval kinetoplast, which measured 0.7–0.9 (0.8) × 0.6–0.7 (0.6), was situated rather near the nucleus, with KI 3.2–3.8 (3.4). Additional measurements of this new species in *C. dracula* (Vietnam5) are given in Table 4, along with the measurements of *T. talpae* and other *Trypanosoma* spp. recorded from the Soricidae. The SSU rDNA nucleotide sequence of this new species is deposited in and available from the DDBJ/EMBL/GenBank databases under the accession number AB242822.

Table 3 Morphometric comparison of *Herpetosoma* trypanosomes from murid hosts

Species	*T. lewisi*			*T. musculi*	*T. niviventerae* n. sp.
Isolate	IN6	IN8	lab T3	lab LGT	CHN II-27
Host	*Bandicota indica*	*Rattus argentiventer*	*Rattus norvegicus*	*Mus musculus*	*Niviventer confucianus*
Reference	Present study	Present study	Davis (1952)	Davis (1952)	Present study
	$n = 15$	$n = 41$	$n = 50$	$n = 50$	$n = 6$
TL	34.3 ± 0.8 (33.2–35.9)	34.7 ± 1.1 (32.2–37.0)	29.4 ± 1.1	29.2 ± 0.8	24.0 ± 4.6 (19.7–30.0)
PK	4.2 ± 0.5 (3.6–5.3)	5.3 ± 0.5 (3.7–6.6)	2.6 ± 0.4	5.3 ± 0.5	3.5 ± 0.8 (2.7–4.9)
KN	10.8 ± 0.5 (11.2–12.9)	11.5 ± 0.6 (10.2–12.5)	10.9 ± 0.7	8.5 ± 0.4	9.4 ± 1.4 (8.1–11.5)
NA	10.8 ± 0.8 (8.8–12.5)	11.1 ± 1.0 (8.8–14.4)	8.6 ± 0.9	9.7 ± 1.0	7.2 ± 2.3 (5.1–11.2)
FF	7.4 ± 0.8 (6.6–9.3)	6.8 ± 0.9 (4.8–9.7)	7.2 ± 1.0	5.8 ± 0.9	3.9 ± 0.9 (3.1–5.6)
BW	2.2 ± 0.3 (1.5–2.7)	1.8 ± 0.4 (1.2–2.7)	1.8	1.8	2.0 ± 0.3 (1.6–2.4)
MW	2.6 ± 0.4 (2.0–3.4)	2.3 ± 0.3 (1.7–2.9)	–[a]	–[a]	2.0 ± 0.3 (1.6–2.4)
NL	3.0 ± 0.3 (2.4–3.6)	2.5 ± 0.2 (1.9–2.8)	2.3	3.2	2.7 ± 0.4 (2.4–3.4)
NW	1.2 ± 0.2 (0.8–1.4)	1.2 ± 0.2 (0.9–1.5)	1.2	1.3	1.4 ± 0.4 (1.0–2.1)
KL	0.8 ± 0.1 (0.7–1.0)	0.8 ± 0.1 (0.6–1.0)	1.0	1.1	1.1 ± 0.2 (0.9–1.3)
KW	0.6 ± 0.1 (0.5–0.7)	0.5 ± 0.1 (0.4–0.7)	0.6	0.5	0.6 ± 0.1 (0.5–0.7)
NI	1.5 ± 0.2 (1.3–1.9)	1.5 ± 0.2 (1.2–1.9)	1.6	1.4	1.9 ± 0.4 (1.2–2.2)
KI	1.4 ± 0.1 (1.3–1.5)	1.5 ± 0.1 (1.3–1.6)	1.2	1.6	1.4 ± 0.1 (1.3–1.5)

All measurements are in micrometers and expressed as mean ± standard deviation with range in parentheses

BW, maximum body width without undulating membrane; *FF*, free flagellum; *KI*, kinetoplast index; *KL*, length of kinetoplast; *KN*, distance between kinetoplast and the middle of nucleus; *KW*, width of kinetoplast; *MW*, maximum width including undulating membrane; *NA*, distance between the middle of nucleus and anterior end; *NI*, nuclear index; *NL*, length of nucleus; *NW*, width of nucleus; *PK*, distance between posterior end and kinetoplast; *TL*, total length

[a] No available data

Table 4 Morphometric comparison of non-*T. lewisi*-like trypanosomes from soricid hosts

Species	*T. sapaensis* n. sp.	*T. talpae*	*T. hoarei*	*T. anourosoricis* n. sp.	*T. ornata*
Isolate	Vietnam5			Tw II-1	
Host	*Crocidura dracula*	Talpa spp.	Sorex spp.	*Anourosorex yamashinai*	*Neomys fodiens*
Reference	Present study	Hoare (1972)	Davis (1969)	Present study	Karbowiak et al. (2005)
	n = 3			*n* = 6	*n* = 100
TL	37.9 ± 1.0 (36.8–38.8)	ca. 21–38	34 (24.7–44.3)	28.7 ± 6.2 (22.9–38.3)	29.4 ± 4.1 (20.5–40.9)
PK	10.7 ± 1.4 (9.5–12.2)	6–20	–[a]	6.2 ± 1.2 (4.8–8.1)	4.3 ± 1.5 (2.6–10.7)
KN	4.5 ± 0.2 (4.4–4.8)	3–4.7	–	5.6 ± 2.3 (3.9–8.6)	6.5 ± 1.4 (2.5–14.4)
NA	15.9 ± 1.9 (13.7–17.5)	–[a]	–	12.3 ± 3.4 (9.2–18.1)	15.9 ± 2.3 (11.0–21.0)
FF	6.7 ± 1.0 (5.8–7.8)	3.5–6.3	Max. ca. 3	4.7 ± 0.8 (3.6–5.4)	3.1 ± 1.3 (1.0–9.2)
BW	4.1 ± 1.0 (3.1–4.9)	–	–	2.6 ± 0.2 (2.4–2.9)	–[a]
MW	4.8 ± 0.9 (3.7–5.4)	3.5–6.5	5.8 (2.8–10.3)	2.9 ± 0.4 (2.4–3.6)	3.1 ± 0.4 (1.9–4.1)
NL	3.3 ± 0.4 (3.1–3.7)	–	–	2.8 ± 0.1 (2.6–3.0)	3.8 ± 0.9 (1.2–6.9)
NW	1.8 ± 0.2 (1.7–2.0)	–	–	1.5 ± 0.2 (1.4–1.9)	1.2 ± 0.4 (0.7–3.0)
KL	0.8 ± 0.1 (0.7–0.9)	–	–	0.6 ± 0.2 (0.4–0.9)	1.2
KW	0.6 ± 0.1 (0.6–0.7)	–	–	0.5 ± 0.1 (0.3–0.6)	0.6
NI	1.0 ± 0.2 (0.8–1.1)	–	ca. 0.6	1.0 ± 0.1 (0.8–1.1)	0.7 ± 0.2 (0.3–1.2)
KI	3.4 ± 0.3 (3.2–3.8)	ca. 3	ca. 2	2.2 ± 0.3 (1.8–2.7)	1.7 ± 0.2 (1.3–2.5)

Abbreviations similar to Table 3

[a] No available data

Taxonomic summary

Host: *Crocidura dracula* (Thomas, 1912), large white-toothed shrew (Mammalia: Eulipotyphla: Soricidae), based on Abramov et al. (2012).

Locality: Sapa, Lào Cai Province, Vietnam.

Materials deposited: Hapantotype no. 21391 (Vietnam5), Meguro Parasitological Museum, Tokyo, Japan.

Prevalence: One of one shrew examined. Extremely low intensity as the level of parasitemia.

Etymology: The species is named after the name of the location where the host was trapped.

Remarks

Hoare (1972) listed two trypanosome species with non-*T. lewisi*-like morphology from soricides, i.e., *T. talpae* in European moles and *Trypanosoma hoarei* in North American shrews of the *Sorex* genus (*Sorex trowbridgii*, *Sorex vagrans*, *Sorex ornatus*, and *Sorex palustris*). The present new species is differentiated from *T. talpae* in having distinct undulating membranes and a different location of the nucleus (near the convex side vs. close to the side opposite to the undulating membrane and kinetoplast). Similarly, the new species is differentiated from *T. hoarei* in having a different location of the nucleus (approximately at the midpoint of the body vs. posterior localization, reflected in different average NI values, 1.0 vs. 0.6), a long tapering posterior end, and the presence of a distinct free flagellum (5.8–7.8 vs. 0–3). As discussed above, the molecular genetic characterization based on the SSU rDNA supports the morphological differentiation of *T.* (*M.*) *sapaensis* n. sp. from *T. talpae*. No gGAPDH nucleotide sequence has been obtained for this isolate probably due to an extremely low parasitemia.

Description

Trypanosoma anourosoricis n. sp. (Kinetoplastea: Trypanosomitida: Trypanosomatidae) (Figs. 1i and 2d and Table 4)

The body of the trypomastigotes in the blood (*n* = 6), which measured 22.9–38.3 (28.7) × 2.4–3.6 (2.9), had granular or mottled cytoplasm and elongated sharpened ends. The free flagellum measured 3.6–5.4 (4.7) in length. The undulating membrane was indistinct, measuring 0–0.7 (0.3) in width. The ovoid nucleus, which measured 2.6–3.0 (2.8) × 1.4–1.9 (1.5), was situated almost at the midpoint between both ends, with NI 0.8–1.1 (1.0). The tiny indistinct kinetoplast, which measured 0.4–0.9 (0.6) × 0.3–0.6 (0.5), was situated midway between the nucleus and posterior end, with KI 1.8–2.7 (2.2). Additional measurements of this new species in *A. yamashinai* (Tw II-1) are given in Table 4, along with the measurements of other trypanosomes recorded from the family Soricidae. The SSU rDNA nucleotide sequence of this species is deposited in and available from the DDBJ/EMBL/GenBank databases under the accession number AB242823.

Taxonomic summary

Host: *Anourosorex yamashinai* (Kuroda, 1935), Taiwanese mole shrew (Mammalia: Eulipotyphla: Soricidae).

Locality: Renai, Nantou County, Taiwan.

Materials deposited: Hapantotype no. 21392 (Tw II-1), Meguro Parasitological Museum, Tokyo, Japan.

Prevalence: One of two mole shrews examined. Extremely low intensity as the level of parasitemia.

Etymology: The species is named after the genus name of the host from which this species was detected.

Remarks

The present species, *T. anourosoricis* n. sp., showed a rigid, narrow but elongated spindle-shaped body with an indistinguishable undulating membrane. These morphological characters are distinct from *T. sapaensis* n. sp., *T. talpae*, and *T. hoarei*, all of which have undulatory broad bodies. As discussed above, the molecular genetic characterization based on the SSU rDNA supports the morphological differentiation of the present specimens from *T. sapaensis* n. sp. and *T. talpae*, although these species have close phylogenetic relationships with the present specimens (discussed below). Karbowiak et al. (2005) described a new species, *Trypanosoma ornata*, from the Eurasian water shrew, *Neomys fodiens* (Pennant, 1771), in Poland solely based on the morphology of trypomastigotes. Since morphological differentiation between the present specimens and *T. ornata* is difficult, the molecular genetic characterization of *T. ornata* is important. The collection and intensive morphological examination of additional trypomastigote of *T. anourosoricis* n. sp. is also necessary. At the same time, genetic characterization of the gGAPDH nucleotide sequence of the species is important, since it has not been obtained in this study probably due to an extremely low parasitemia.

Phylogenetic relationships of newly isolated trypanosomes with known species

Trypanosomes of the subgenus *Herptetosoma* formed a well-supported clade in the ML phylogenetic tree based on the SSU rDNA (Fig. 3), which consisted of four major subgroups: (1) *Trypanosoma microti* and related species, (2) sciurid trypanosomes such as *Trypanosoma otospermophili* and *Trypanosoma kuseli*, (3) *T. grosi* isolates, and (4) *T. lewisi* and related species. The available SSU rDNA nucleotide sequences of *T. lewisi* were divided into two groups: a group of European and Asian isolates from *Rattus* spp. and *B. indica* (AJ009156, AJ223566, and AB242273) and another of Brazilian isolates from various hosts such as *Rattus* spp. and nonhuman primates (GU252209–GU252215). This division was based on a single nucleotide substitution at the 1013th base relative to the 5′-end of the deposited reference sequence of *T. lewisi* (ATCC 30085 isolate; AJ223566). In the same way, *T. niviventerae* n. sp., *T. musculi*, *T. blanchardi*, and *T. rabinowitschae* formed a clade having consistently and minimally a single nucleotide substitution at the 1313th base.

The SSU rDNA nucleotide sequences of trypanosomes with morphology of ‘the subgenus *Megatrypanum*’ (Hoare 1972) from soricids, *T. talpae*, *T. sapaensis* n. sp., and *T. anourosoricis* n. sp., formed a distinct clade from that for *Herpetosoma* trypanosomes, but a close relationship clearly existed (Fig. 3). In an earlier study from our laboratory, *Megatrypanum* trypanosomes of ruminants such as *Trypanosoma theileri* and those of primates such as *Trypanosoma minasense* were found to locate distantly from a clade of these soricid trypanosome species (see Fig. 3 of Sato et al. 2008).

The available gGAPDH nucleotide sequences of *Herpetosoma* trypanosomes were limited to eight taxa of six species (Fig. 4). The phylogenetic topology of *Herpetosoma* species to other groups of trypanosomes was almost identical to the relationships observed using the SSU rDNA sequences.

There were no available gGAPDH nucleotide sequences of soricid trypanosomes. Indeed, we were unable to obtain gGAPDH nucleotide sequences from the two trypanosome isolates from soricids in this study.

Discussion

Hoare (1972) devoted the subgenus *Herpetosoma* to *T. lewisi* and its related species recorded from the blood of rodents, ca. 50 in number, which included unnamed species. Although the recent occurrence of sporadic human cases of *T. lewisi* infection in Thailand and India (reviewed by Truc et al. 2013) as well as *T. lewisi* infection in Brazilian monkeys in captivity (Maia da Silva et al. 2010) has promoted the molecular genetic characterization of rodent trypanosomes, there are still only a limited number of deposited nucleotide sequences, thus making a proper discussion on the taxonomy of *Herpetosoma* trypanosomes difficult. The latest status of research on the molecular genetic characterization of *Herpetosoma* trypanosomes has been reported in a recent study from our laboratory (Mafie et al. 2016). Briefly, *T. lewisi* with a cosmopolitan distribution was found to have a fixed SSU rDNA nucleotide sequence, which is represented by the deposited reference sequences AJ009156 (Molteno B3 isolate) and AJ223566 (ATCC 30085 isolate) in the DDBJ/EMBL/GenBank databases. Brazilian isolates of *T. lewisi* from *R. rattus*, *R. norvegicus*, *Alouatta fusca*, *Aotus* sp., and *Callithrix jacchus* were discovered to be the exception to this finding, consistently having a single nucleotide substitution at base position 1013 relative to the 5′-terminus of AJ223566 (aforementioned deposited reference sequence of *T. lewisi*). Contrary to *T. lewisi* isolates, *T. musculi*, *T. blanchardi*, and *T. rabinowitschae*, as well as

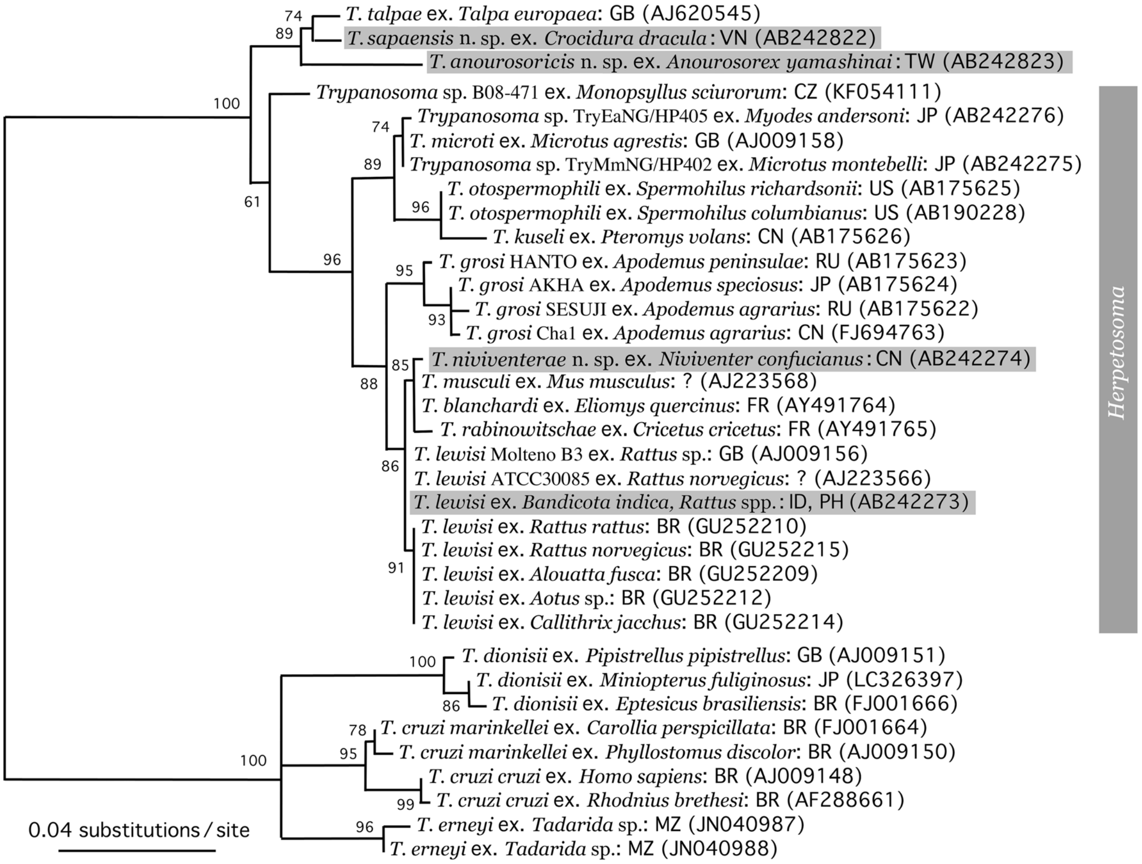

Fig. 3 ML phylogenetic tree based on the SSU rDNA nucleotide sequence. Species names are followed by host names and localities (DDBJ/EMBL/GenBank accession numbers in parentheses). The newly sequenced isolates are indicated with a gray background. Brazil (BR), China (CN), Czech Republic (CZ), France (FR), United Kingdom (GB), Indonesia (ID), Japan (JP), Mozambique (MZ), Philippines (PH), Russian Federation (RU), Taiwan (TW), United States of America (US), and Vietnam (VN)

T. niviventerae n. sp. in the present study, had at least a single nucleotide substitution at base position 1313, forming a separate subclade (Fig. 3). Zhang et al. (2015) reported a partial SSU rDNA nucleotide sequence (KP098535), 682 bp in length, of *Herpetosoma* trypanosomes in *Rattus losea*, in addition to a partial but typical *T. lewisi* SSU rDNA sequence (KP098536)

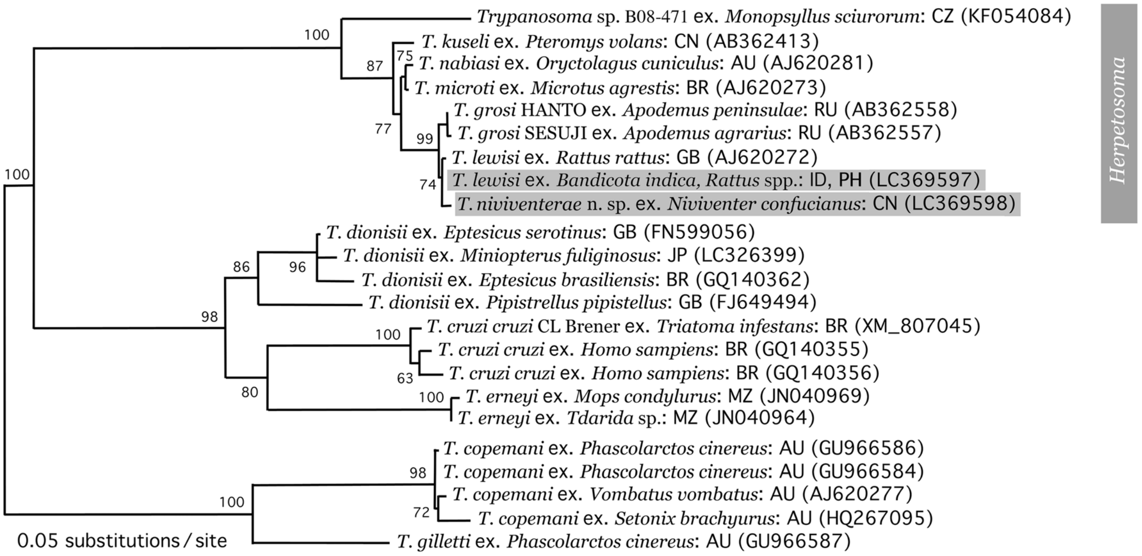

Fig. 4 ML phylogenetic tree based on the gGAPDH nucleotide sequence. See Fig. 3 legend for details. Australia (AU)

of trypanosomes from *Rattus flavipectus*. The former SSU rDNA sequence (KP098535) is identical to that of *T. niviventerae* n. sp. rather than that of *T. lewisi*. Furthermore, the SSU rDNA of *T. niviventerae* n. sp. has an additional nucleotide substitution at base position 1759 compared with *T. lewisi*, *T. musculi*, *T. blanchardi*, and *T. rabinowitschae* (see Table 2 in Mafie et al. 2016); however, no information is available on the trypanosomes in the blood of *R. losea* mentioned above. In order to clarify the taxonomic status of the *Herpetosoma* trypanosomes in *R. losea* reported by Zhang et al. (2015), i.e., identical to or distinct from *T. niviventerae* n. sp. reported here, a longer SSU rDNA sequence including parts close to both the 5′- and 3′-termini of the gene needs to be obtained. Although molecular genetic differences are few in the SSU rDNA sequences among *Herpetosoma* trypanosomes of murid hosts, the morphological uniqueness of *T. niviventerae* n. sp., different from *T. lewisi*, justifies our erection of the new species for trypanosomes isolated from *N. confucianus* in China. Due to a dearth of morphological differences among *T. musculi*, *T. blanchardi*, *T. rabinowitschae*, and *T. lewisi* (Hoare 1972), the new species is also differentiated from the three former species. Surprisingly, the two genetic groups of *T. lewisi* based on a single nucleotide substitution in the SSU rDNA sequence are not consistent with two genetic groups based on different profiles of nucleotide sequences of the internal transcribed spacer (ITS) 1 region (see Table 3 and Fig. 2 of Mafie et al. 2016). Therefore, it is vital to collect more data on not only the SSU rDNA but also other regions of the gene in order to fully discern the nucleotide changes critical for specific differentiation.

In the present study, two trypanosome isolates of the so-called subgenus *Megatrypanum*, not of the subgenus *Herpetosoma*, were obtained from soricids (*C. dracula* in Vietnam and *A. yamashinai* in Taiwan). The level of parasitemia was extremely low in both hosts, a similar finding to the majority of previous studies investigating the so-called *Megatrypanum* trypanosomes in the peripheral blood of hosts (Hoare 1972). Long SSU rDNA nucleotide sequences were successfully obtained for these two soricid trypanosome isolates, making them the second and third deposited nucleotide sequences of soricid '*Megatrypanum*' trypanosomes after that of *T. talpae* from the European mole (AJ620545). Intriguingly, these three nucleotide sequences were found to have frequent indels, 14, 19, or 28 in number against each other, with eight or 23 nucleotide substitutions over a 1821- or 2155-bp length, respectively. Hoare (1972) considered members of the subgenus *Megatrypanum* to be primitive forms of trypanosomes, although species of this subgenus from different mammalian hosts are dispersed in the phylogenetic tree and do not form a special subgenus clade. Indeed, *Megatrypanum* trypanosomes from soricids form a well-supported clade comprised of only themselves, which has a sister relationship with a clade of *Herpetosoma* trypanosomes (Fig. 3), but is distant from *T. theileri*, the type species of the *Megatrypanum* subgenus.

The taxonomic relationship of soricid trypanosomes in *A. yamashinai* from Taiwan with *T. ornata* from the Eurasian water shrew (*N. fodiens*) in Poland is uncertain, although they appear to be morphologically closer to each other. Molecular genetic characterization of *T. ornata* could determine the precise taxonomic relationship between these two species. It is well recognized that the morphological differentiation of trypanosomes in the blood of rodents and soricids is difficult due to very similar morphotypes, i.e., *T. lewisi*-like and soricid '*Megatrypanum*' morphology, respectively. Indeed, sometimes only biological characters such as host specificity and reproductive manner enable species to be separated, as in the case of *T. lewisi* and *T. musculi*.

Which target genes should be considered when undertaking molecular taxonomy to differentiate *Herpetosoma* or soricid '*Megatrypanum*' trypanosomes? As detailed here and in a recent study from our laboratory (Mafie et al. 2016), there is only a single or just a few nucleotide substitution(s) among 2140-bp long SSU rDNA sequences of apparently distinct species (e.g., *T. lewisi* vs. *T. musculi*). Furthermore, a Brazilian population of *T. lewisi* isolates showed a single nucleotide substitution when compared with European and Asian isolates of *T. lewisi*. Contrarily, *T. grosi* showed more greater intraspecific variation of the SSU rDNA sequence, two to four nucleotide substitutions over a 2150-bp length. All the current data suggest a low resolution of molecular analyses using the SSU rDNA sequences of *Herpetosoma* species. In contrast, based on just three available sequences at present, interspecific nucleotide variation is high in the SSU rDNA sequences of soricid trypanosome species. Similar to the SSU rDNA nucleotide sequences, those of the gGAPDH gene of *Herpetosoma* trypanosomes are also well conserved with only a few nucleotide substitutions between different species. Regarding the ITS region, direct sequencing may be hampered by the presence of repeats of nucleotide units, making sequencing after gene cloning likely to be necessary, which increases the amount of labor and cost. Additionally, genetic groups based on the SSU rDNA and ITS nucleotide sequences do not tend to show consistency.

We are still a long way from establishing an ideal target gene(s) for the molecular taxonomic purpose of separating a wide range of *Herpetosoma* trypanosomes. At present, the molecular genetic characterization of almost the entire length of SSU rDNA sequences of isolated trypanosomes from various sources must be a basic minimum requirement to support the morphological characterization of trypanosomes. Our future research efforts will be directed toward resolving the issue of defining an ideal target gene(s) to sequence.

Acknowledgments We are indebted to the mammalogists of Hasanuddin University, Indonesia, and Central Luzon State University, Philippines, for their species identification of the collected murids and soricids.

Compliance with ethical standards

The ethics of animal trapping and sample collection adhered to in this study followed the guidelines outlined by each survey participant's university, but at that time, no individual permission numbers of sample collection were provided to us from these organizations.

Conflict of interest The authors declare that they have no conflict of interest.

References

Abramov AV, Bannikova AA, Rozhnov VV (2012) White-toothed shrews (Mammalia, Soricomorpha, Crocidura) of coastal islands of Vietnam. ZooKeys 2007:37–47

Anisimova M, Gascuel O (2006) Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. Syst Biol 55:539–552

Bardsley JE, Harmsen R (1973) The trypanosomes of anura. Adv Parasitol 11:1–73

Cooper C, Clode PL, Peacock C, Thompson RCA (2017) Host–parasite relationships and life histories of trypanosomes in Australia. Adv Parasitol 97:47–109

Davis BS (1952) Studies on the trypanosomes of some California mammals. University of California Publications in Zoology 57:145–249

Davis BS (1969) *Trypanosoma (Megatrypanum) hoarei* sp. nov. (Prozoa: Trypanosomatidae) from shrews of the genus *Sorex* in California. Parasitology 59:469–491

Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM, Gascuel O (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res 36:465–469

Guan G, Niu Q, Yang J, Li Y, Gao J, Luo J, Yin H (2011) *Trypanosoma (Herpetosoma) grosi*: first isolation from Chinese striped field mouse (*Apodemus agrarius*). Parasitol Int 60:101–104

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52:696–704

Haag J, O'hUigin C, Overath P (1998) The molecular phylogeny of trypanosomes: evidence for an early divergence of the Salivaria. Mol Biochem Parasitol 91:37–49

Hamilton PB, Stevens JR, Gaunt MW Gidley J, Gibson WC (2004) Trypanosomes are monophyletic: evidence from genes for glyceraldehyde phosphate dehydrogenase and small subunit ribosomal RNA. Int J Parasitol 34:1393–1404

Hamilton PB, Stevens JR, Holz P, Boag B, Cooke B, Gibson WC (2005) The inadvertent introduction into Australia of *Trypanosoma nabiasi*, the trypanosome of the European rabbit (*Oryctolagus cuniculus*), and its potential for biocontrol. Mol Ecol 14:3167–3175

Hamilton PB, Gibson WC, Stevens JR (2007) Patterns of co-evolution between trypanosomes and their hosts deduced from ribosomal RNA and protein-coding gene phylogenies. Mol Phylogenet Evol 43:15–25

Hoare CA (1972) The trypanosomes of mammals. Blackwell Scientific, Oxford, p 749

Hong XK, Zhang X, Fusco OA, Lan YG, Lun ZR (2017) PCR-based identification of *Trypanosoma lewisi* and *Trypanosoma musculi* using maxicircle kinetoplast DNA. Acta Trop 171:207–212

Karbowiak G, Wita I, Rychlik L (2005) *Trypanosoma (Megatrypanum) ornata* sp. n., a parasite of the Eurasian water shrew *Neomys fodiens* (Pennant, 1771). Acta Protozool 44:363–367

Lun ZR, Wen YZ, Uzureau P, Lecordier L, Lai DH, Lan YG, Desquesnes M, Geng GQ, Yang TB, Zhou WL, Jannin JG, Simarro PP, Truc P, Vincendeau P, Pays E (2015) Resistance to normal human serum reveals *Trypanosoma lewisi* as an underestimated human pathogen. Mol Biochem Parasitol 199:58–61

Ma Y, Wang F, Jin S, Li S (1987) Glires (rodents and lagomorphs) of northern Xinjiang and their zoogeographical distribution. Science Press, Beijing, p 274 (in Chinese)

Mafie E, Rupa FH, Setsuda A, Saito-Ito A, Sato H (2016) Brief review on atypical trypanosomiasis of *Trypanosoma lewisi*. Jpn J Vet Parasitol 15:24–33

Mafie E, Rupa FH, Takano A, Suzuki K, Maeda K, Sato H (2018) First record of *Trypanosoma dionisii* of the *T. cruzi* clade from the eastern bent-winged bat (*Miniopterus fuliginosus*). Parasitol Res 117:673–680

Maia da Silva F, Marcili A, Ortiz PA, Epiphanio S, Campaner M, Catão-Dias JL, Shaw JJ, Camargo EP, Teixeira MMG (2010) Phylogenetic, morphological and behavioural analyses support host switching of *Trypanosoma (Herpetosoma) lewisi* from domestic rats to primates. Inf Genet Evol 10:522–529

Noyes HA, Stevens JR, Teixeira M, Phelan J, Holz P (1999) A nested PCR for the ssrRNA gene detects *Trypanosoma binneyi* in the platypus and *Trypanosoma* sp. in wombats and kangaroos in Australia. Int J Prassitol 29:311–339 (Corrigendum: International Journal for Parasitology 30: 228 [2000])

Noyes HA, Ambrose P, Barker F, Begon M, Bennet M, Bown KJ, Kemp SJ (2002) Host specificity of *Trypanosoma (Herpetosoma)* species: evidence that bank voles (*Clethrionomys glareolus*) carry only one *T. (H.) evotomys* 18S rRNA genotype but wood mice (*Apodemus sylvaticus*) carry at least two polyphyletic parasites. Parasitology 124:185–190

Ortiz PA, Garcia HA, Lima L, Maia da Silva FM, Campaner M, Pereira CL, Jittapalapong S, Neves L, Desquesnes M, Camargo EP, Teixeira MMG (2018) Diagnosis and genetic analysis of the worldwide distributed *Rattus*-borne *Trypanosoma (Herpetosoma) lewisi* and its allied species in blood and fleas of rodents. Infect Genet Evol 63: 380–390

Paparini A, Irwin PJ, Warren K, McInnes LM, de Tores P, Ryan UM (2011) Identification of novel trypanosome genotypes in native Australian marsupials. Vet Parasitol 183:21–30

Pérez-Morga D, Vanhollebeke B, Paturiaux-Hanocq F, Derek P, Nolan DP, Lins L, Homblé F, Vanhamme L, Tebabi P, Pays A, Poelvoorde P, Jacquet A, Brasseur R, Pays E (2005) Apolipoprotein L-I promotes trypanosome lysis by forming pores in lysosomal membranes. Science 309:469–472

Pumhom P, Pognon D, Yangtara S, Thaprathorn N, Milocco C, Douangboupha B, Herder S, Chaval Y, Morad S, Jittapalapong S, Desquesnes M (2014) Molecular prevalence of *Trypanosoma* spp. in wild rodents of Soureast Asia: influence of human settlement habitat. Epidemiol Infect 142:1221–1230

Sato H, Ishita K, Matsuo K, Inaba T, Kamiya H, Ito M (2003) Persistent infection of Mongolian jirds with a non-pathogenic trypanosome, *Trypanosoma (Herpetosoma) grosi*. Parasitology 127:357–363

Sato H, Osanai A, Kamiya H, Obara Y, Jiang W, Zhen Q, Chai J, Une Y, Ito M (2005) Characterization of SSU and LSU rRNA genes of three *Trypanosoma (Herpetosoma) grosi* isolates maintained in Mongolian jirds. Parasitology 130:157–167

Sato H, Al-Adhami BH, Une Y, Kamiya H (2007) *Trypanosoma (Herpetosoma) kuseli* sp. n. (Protozoa: Kinetoplastida) in Siberian flying squirrels (*Pteromys Volans*). Parasitol Res 101:453–461

Sato H, Leo N, Katakai Y, Takano J, Akari H, Nakamura S, Une Y (2008) Prevalence and molecular phylogenetic characterization of *Trypanosoma (Megatrypanum) minasense* in the peripheral blood of small Neotropical primates after a quarantine period. J Parasitol 94:1128–1138

Simpson AGB, Stevens JR, Lukeš J (2006) The evolution and diversity of kinetoplastid flagellates. Trends Parasitol 22:168–174

Stevens J, Noyes H, Gibson W (1998) The evolution of trypanosomes infecting humans and primates. Mem Inst Oswald Cruz 93:669–676

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Truc P, Büscher P, Cuny G, Gonzatti MI, Jannin J, Joshi P, Juyal P, Lun Z-R, Mattioli R, Pays E, Simarro PP, Teixeira MMG, Touatier L, Vincendeau P, Desquesnes M (2013) Atypical human infections by animal trypanosomes. PLoS Negl Trop Dis 7:e2256. https://doi.org/10.1371/journal.pntd.0002256

Vanhamme L, Paturiaux-Hanocq F, Poelvoorde P, Nolan DP, Lins L, Van Den Abbeele J, Pays A, Tebabi P, Van Xong H, Jacquet A, Moguilevsky N, Dieu M, Kane JP, De Baetseller P, Brasseur R, Pays E (2003) Apolipoprotein L-I is the trypanosome lytic factor of human serum. Nature 422:83–87

Zhang Y, Jin S, Quan G, Li S, Ye Z, Wang F, Zhang M (1997) Distribution of mammalian species in China. China Forestry Publishing House, Beijing, p 280

Zhang LL, Ni QX, Yao LN, Zhang XH, Chen HL, Feng Y, Zhang X, Yu KG, Ruan W (2015) [Identification of *Trypanosoma* species in *Rattus flavipectus* and *R. losea* using microscopy and PCR method]. Zhongguo Ji Sheng Chong Xye Yu Ji Sheng Chong Bing Za Zi (Chin J Parasitol Parasit Dis) 33:277–280