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ORIGINAL PAPER

New Haplotypes of *Trypanosoma evansi* Identified in Dromedary Camels from Algeria

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Abstract

Purpose Surra is a zoonotic disease caused by *Trypanosoma evansi* (*Trypanozoon*), a salivary trypanosome native to Africa which affects a wide range of mammals worldwide and causes mortality and significant economic loss. The present study was devoted to the molecular characterization of *T. evansi* derived from naturally infected dromedary camels in Algeria.

Methods A total of 148 blood samples were collected from mixed age camels living in one of four geographic regions (Ouargla, El Oued, Biskra and Ghardaia) of Algeria. Samples underwent PCR amplification and sequencing of the internal transcribed spacer 1 (ITS1) complete sequence.

Results DNA of *Trypanosoma* spp. was found in 19 camels (12.84%). *Trypanosoma* spp. molecular positivity was not affected by sex ($p=0.50$), age ($p=0.08$), or geographic location ($p=0.12$). Based on multiple sequence alignment of the obtained DNA sequences with representative *T. evansi ITS1* sequences available globally, the Algerian sequences were grouped within four different haplotypes including two which were original.

Conclusion Results of this study provide preliminary data on which future studies of genetic diversity and molecular epidemiology of *T. evansi* can be based.

Keywords Trypanosoma evansi · Camels · Algeria · Molecular characterization · ITS1 · Haplotypes

Introduction

Trypanosoma evansi is a single-celled eukaryotic parasite which is closely related to *T. brucei*, the causative agent of sleeping sickness and nagana disease in human and animals, respectively [1]. *T. evansi* is the etiological agent of "Surra", or mal de cadeiras, characterized by anemia, immunosuppression and damage to the central nervous system causing ocular and reproductive disorders [2]. It affects a wide range of domestic and wild mammalian species [2], and it is one of the most pathogenic trypanosomes transmitted mechanically by different blood-sucking fly species such as *Tabanus* spp. and *Stomoxys* spp. [3] as well as by vampire bats *Desmodus rotundus* [4]. Due to the high mortality and morbidity rates affecting livestock productivity, this disease is responsible for disastrous economic losses estimated at $404,630 USD per year [5, 6]. Although some authors have suggested that *T. evansi* originated and mainly affected camels in Africa [7], all domestic mammals studied to date are susceptible [8]. The disease results in a high rate of mortality in horses, camels, and dogs [2, 8]. Outbreaks of animal surra have been reported in North Africa, the Middle East, South America, Asia, and recently Europe [9]. *T. evansi* infection in humans was not only reported initially in India [10] but also confirmed recently in Vietnam [11]. Other probable cases in humans have been reported worldwide, but molecular parasite detection was not performed [2].

Many diagnostic methods (with varying degrees of sensitivity and specificity) are available to detect *T. evansi* infections including parasitological, serological, and molecular assays [12, 13]. Some methods detect trypanosome infection by microscopical examination of fresh or stained bloodsmears [14], whereas others identify different *T. evansi*

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strains, being classified as Type A or Type B, according to their Kinetoplast DNA minicircle sequences [15] and the presence of the *RoTat1.2* variant surface glycoprotein (VSG) gene [16, 17] or antigen [18]. Other molecular-based methods target sequences within ribosomal genes of *Trypanosoma* spp., such as the small sub-unit ribosomal gene (18S) [19] or the internal transcribed spacers (ITS) [20, 21], and have been used for species identification.

In Algeria, a huge number of dromedary camels (*Camelus dromedarius*) is concentrated in the dry desert of the Sahara which occupies 87% of the total area of Algeria (2,381,741 km^2). In this region, camels constitute the major source of animal protein and transport for nomads; however, there is a paucity of information regarding camel diseases—especially those concerning hemoparasites [22]. A variety of diagnostic tests has been used to assist in the detection of *T. evansi* infections in Algeria, and these tests have demonstrated species differences in infection susceptibility among camels, dogs, horses, and donkeys [23–26]. However, those previous studies did not extensively study the genetic structure and/or the genetic diversity of *T. evansi,* particularity in camels from Algeria. Therefore, the present study was devoted to the molecular characterization of *T. evansi* derived from naturally infected camels in eastern Algeria and comparison between isolates from different parts of the world in order to describe distinct *T. evansi* haplotypes.

Materials and Methods

Study Area

This study was conducted in four provinces (Ouargla, El Oued, Biskra and Ghardaia) located at 2°04′–7°35′ E and 28°32′–34°56′ N (Fig. 2). These areas are known for extreme aridity and extreme heat; they are among the hottest places on Earth during the height of summer. These regions comprise one of the most significant camel rearing areas in Algeria and play a key role in food security and transportation of Saharan and steppe community people [22].

Camel Blood Sampling

Between July 2018 and June 2019, a total of 12 camel herds ranging in size from 10 to 70 head were randomly selected for this study. Blood samples ($n=148$) were collected from a random set of animals which included both sexes (20 males and 128 females) and animals in various age groups: <1 year ($n=7$), 1–3 years ($n=33$), 4–9 years ($n=53$), 10–15 years ($n=45$), and >15 years ($n=10$) based on dental wear and owner information. Whole blood was collected from each camel via jugular venipuncture into a 5-mL Vacutainer®tube containing the anticoagulant ethylene diamine tetra acetic acid (EDTA). Samples were stored (in the blood collection tubes) at −20 °C until DNA extraction was performed.

Molecular Characterization of *T. evansi*

DNA Extraction from Camel Blood

Total genomic DNA was extracted from 200 µL of EDTA-preserved whole blood of each of the 148 camel blood samples using the DNeasy Blood and Tissue Kit (Qiagen®, Hilden, Germany), according to the manufacturer's instructions. DNA was eluted in a final volume of 200 µL and stored at −20 °C until used for molecular characterization.

DNA Amplification and Sequencing of *ITS* Ribosomal Spacer

Genomic DNA samples were subjected to a standard conventional PCR assay to amplify DNA. In order to obtain the *ITS1* complete sequence, it was necessary to incorporate the partial 18S and 5.8S sequences so that they could act as anchors for the *ITS1* sequence using the two previously described primers *ITS1 CF* (5′CCG-GAA-GTT-CAC-CGA-TAT-TG3′) and *ITS1 BR* (5′TTG-CTG-CGT-TCT-TCA-ACG-AA3′) amplifying African Trypanosomal species [21]. Standard PCR reaction was performed in a final volume of 25 µL containing 3.5 mM of $MgCl_2$, 0.2 mM of each deoxyribonucleoside triphosphate (dNTPs) mixture, 1 × PCR buffer, 1 U of Taq DNA Polymerase (Roche, Germany), 0.2 µM of each primer (Eurogentec, Belgium), and 5 µL of genomic DNA. DNA-free distilled water was used as negative control. The PCR reactions were performed in an automated DNA thermal cycler (Biometra TRIO Thermoblock Heat Cycler, Germany) using the following reaction conditions: 95 °C for 10 min for initial denaturation, 35 cycles at 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min, and a final extension at 72 °C for 5 min. The PCR products were subject to electrophoresis in a 1.5% agarose gel stained with ethidium bromide and then visualized using UV light. Six positive PCR product samples were subsequently purified using QIAquick PCR Purification Kit (QIAGEN, Germany) according to manufacturer's recommendation. Purified PCR products were sequenced in both directions with the same primers used in the PCR amplification. Sequencing reactions were performed by an ABI 3100 Genetic Analyzer automated sequencer (Applied Biosystems, USA) using an ABI PRISM BigDye™ terminator cycle sequencing kits (Applied Biosystems, Foster City, USA).

Phylogenetic and Phylogeographic Analyses

The ChromasPro software (Technelysium PTY, Australia) was used to analyze, assembl and edit the DNA sequences

obtained in this study. A nucleotide BLAST was made to compare the identity of the sequences with the NCBI database. Multiple sequence alignment was performed based on *ITS1* complete sequences after ligation of the amplicons products using ClustalW. In order to determine the potential novelty of haplotypes identified in our experimental animals, an exhaustive search on GenBank was performed to obtain all sequences of *T. evansi* for which the *ITS1* region was complete. Altogether 96 available sequences were included in the analysis: 69 from Asia, 21 from Africa, and six from South America. A ClustalW alignment was performed on sequences belonging to the same country, and representative haplotypes from different countries, including haplotypes of the present study, were subsequently aligned. According to previous studies on gene diversity of different parasites, each sequence containing an original nucleotide signature is considered a new haplotype [27–29].

The phylogeny reconstruction was performed with the DNA sequences of our 6 *T. evansi* isolates and 39 other representative haplotypes of *T. evansi* (previously identified in Africa, Asia and South America and deposited in GenBank). The tree was inferred with complete deletion using the maximum likelihood (ML) method, with 500 bootstrap replicates, using Molecular and Evolution Genetic Analysis (MEGA v5 software) [30]. For the latter, the best model was the Tamura 3-parameter with five rate categories and assuming that a certain fraction of sites are evolutionarily invariable (+I). Genetic distances [Kimura 3-parameter distance] were estimated with MEGA v5 software [30]. All codon positions were used, and all positions containing gaps or missing data were eliminated. Haplotype diversity <Hd> and nucleotide diversity (π) were calculated using DNA Sequence Polymorphism software (DNASP 5.10.01) [31].

Nucleotide Sequence Accession Numbers

The *ITS 1* complete sequences generated from this study were deposited in GenBank through accession numbers MT539996 to MT540001.

Results

Descriptive Epidemiology

Of 148 camel blood samples, 19 (12.84%) were PCR-positive for *Trypanosoma* species. All *ITS1* PCR products were approximately 480 bp [specific size of all members of the subgenus *Trypanozoon* [21]]. The result revealed a non-significant difference between localities ($\chi^2=5.83$, $df=3$, $P=0.12$); the prevalence rate was highest in Ghardaia (40.0%), followed by 17.1% in El Oued, 10.0% in Biskra, and 0% in Ouargla (Table 1). Similarly, there was no association of sex ($\chi^2=0.45$, $df=1$, $P=0.50$) or age ($\chi^2=8.33$, $df=4$, $P=0.08$) with recorded prevalence rate. Prevalence of trypanosome detection was higher in females (13.3%) than in males (10.0%) and higher in camels between 4 and 9 years of age (18.9%) than in other age groups (Table 1).

Table 1 Molecular prevalence rates of *Trypanosoma* spp. among dromedary camels in eastern Algeria stratified by locality, animal sex and age class

Risk factors	Category	Camel's number	*Trypanosoma* sp. positive (%)	*P*
Locality	Ouargla	23	0 (0)	0.12*
	El-Oued	70	12 (17.14)	
	Biskra	50	5 (10)	
	Ghadaia	5	2 (40)	
Sex	Male	20	2 (10)	0.5*
	Female	128	17 (13.28)	
Age class	1 (< 1 year)	7	1 (14.29)	0.08*
	2 (1–3 years)	33	6 (18.18)	
	3 (4–9 years)	53	10 (18.87)	
	4 (10–15 years)	45	1 (2.22)	
	5 (> 15 years)	10	1 (10)	
Total		148	19 (12,84)	

(%) percentage, *P* prevalence rate

*Test was not statistically significant ($P>0.05$)

Molecular Characterization and Phylogenetic Analysis

Sequencing of the rDNA complete internal transcribed spacer (*ITS 1*) region, including the partial sequences of 18S and 5.8S, generated six sequences corresponding to *Trypanosoma evansi* with BLAST. Multiple sequence alignment revealed limited heterogeneity among the six sequences (nucleotide diversity $\pi=0.00550$), in the form of five polymorphic or segregating sites and two alignment gaps or missing data, leading to the exhibition of four different haplotypes with a rate of gene diversity Hd corresponding to 0.800. This heterogeneity was localized at the 3′ region of the fragment corresponding to *ITS1* (336 bp). Overall, there is one Singleton variable site at position 207 and four Parsimony informative sites at positions 212, 232, 233 and 341(Fig. 1).

The first haplotype is represented by isolate 23 from Biskra. The second haplotype is represented by isolate 74 from El Oued. Three identical sequences corresponding to isolates 81, 99 and 119 from El Oued represent the third haplotype, and isolate 139 from Ghardaia represents the fourth haplotype (Fig. 1).

To determine the phylogeographic relationships of *T. evansi* isolates in camels from Algeria, *ITS1* complete sequences of different haplotypes previously reported in

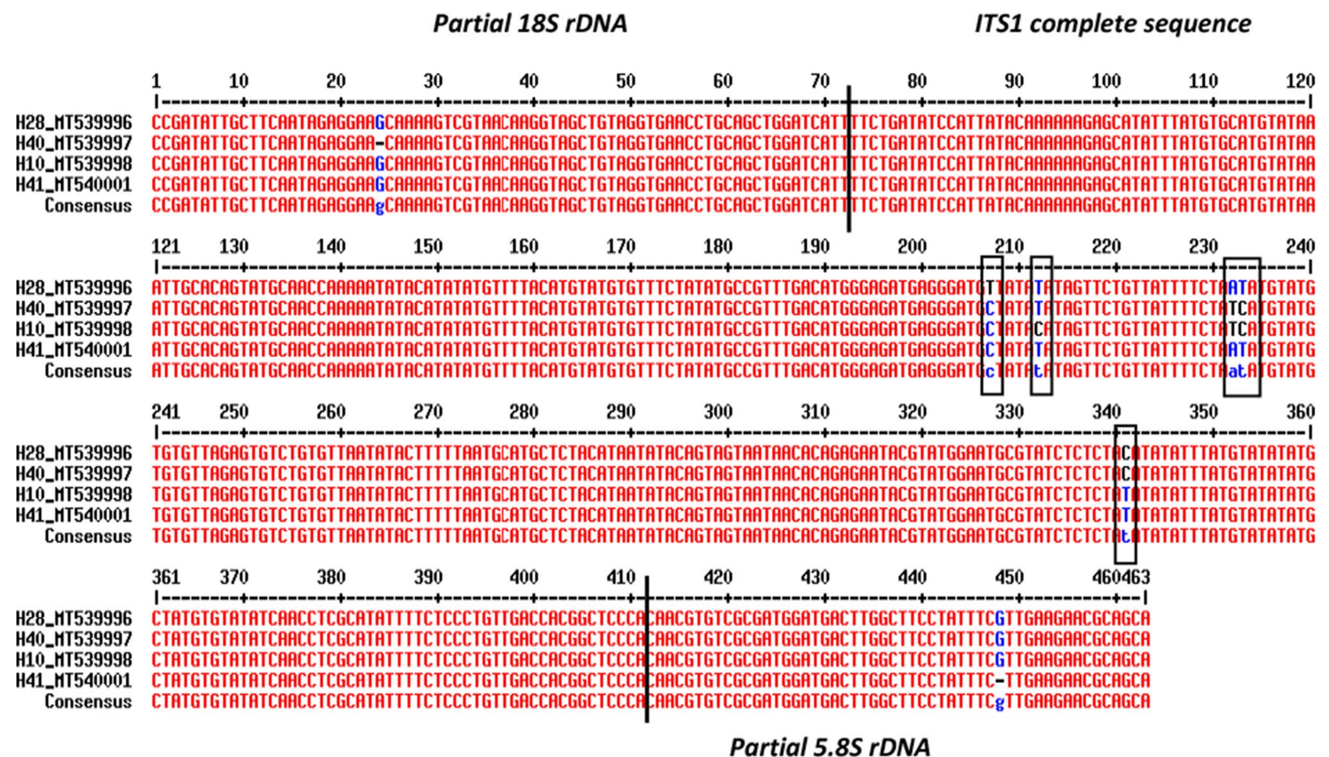

Fig. 1 Alignment of the four *Trypanosoma evansi ITS1* sequence haplotypes. The single nucleotides polymorphisms are marked with colors in the frame.

Africa, Asia and South America were used. The resulting alignment of the complete *ITS1* region exhibited at least 41 different haplotypes confirmed by DNASP (Table 2), with a high rate of gene diversity Hd corresponding to 0.993. The first 30 haplotypes (H1-H30) were found in Asia, two haplotypes (H32 and H33) were found in South America and the remainder of the haplotypes (H34 to H41) originated in Africa. Some haplotypes are shared across continents; for example, H3 was found in China and Kenya, H8 was found in China, Thailand and Egypt, and H10 which has a worldwide distribution. No host specificity was observed.

The new Algerian sequences of *T. evansi* from camel blood samples are grouped into four different haplotypes: H28 is identical to the isolate found in Saudi Arabia (Genbank N°MN625864; unknown source); H10 is identical to a haplotype found in a wide range of domestic and wild mammalian species and having a worldwide distribution; H40 and H41 are unique and do not match any known haplotypes. Pairwise genetic distances (computed using the Tamura 3-parameter model) between our new haplotypes and other representative haplotypes of the dataset showed a genetic divergence between 0.3 and 14% for H40 and between 0.3 and 15% for H41 (Table 3). Phylogenic analyses were performed with all different representative haplotypes, including our Algerian sequences. The unrooted tree topology shows that each haplotype clustered separately, including the two new haplotypes from Algeria (Fig. 2).

Discussion

In the present study, *Trypanosoma* spp. DNA was isolated from 12.84% of camel blood samples collected in eastern Algeria. Six sequences based on *ITS1* complete sequence amplification were obtained and identified as *T. evansi*. Two previous studies concerning *T. evansi* in camels using molecular tools have been reported in Algeria. Overall molecular prevalence of *T. evansi* in 1056 camels from four provinces of southern Algeria (El Bayadh, Bechar, Ouargla, Tamanrasset) was 11.2% with *RoTat 1.2 PCR* [26], and rates of 13% were recorded with the *18S qPCR* and 6.2% with the *ITS1 PCR* in 161 camels from Ghardaia [24], However, those PCR positivity rates were lower compared with results from other countries. For example, *T. evansi* was detected in 42% of camels from Sudan [32], 32% of camels in Egypt,

Table 2 Species and geographical source of *Trypanosoma evansi ITS1* sequences characterized in the phylogeographic analysis

Haplotype	Total	Accession Number	Algeria	Moroco	Tunisia	Sudan	Egypt	South Africa	Kenya	Saudi Arabia	Iraq	Iran	Philippines	India	Taiwan	Indonesia	Thailand	China	Venezuela	Brazil	Colombia	Others	Host
H1	1	FJ712714																1					
H2	1	FJ712712																1					
H3	4	KU552351							1									3					
H4	1	FJ712716																1					
H5	1	KU552350																1					
H6	1	KU552344																1					
H7	1	FJ712711																1					
H8	3	FJ712713					1										1	1					
H9	1	AY912278															1						
H10	18	EF547550	3*	1			3		3			1		1	1	1	1			1	1	1	
H11	1	AY912276															1						
H12	1	AY912270															1						
H13	18	AY912271											2	10		1	5						
H14	1	MN121257															1						
H15	1	AY912274															1						
H16	2	MN121259															2						
H17	4	KX898423										3		1									
H18	1	KR858271												1									
H19	1	KR858269												1									
H20	1	KR858267												1									
H21	1	KR858272												1									
H22	1	MT233332												1									
H23	1	MN097905												1									
H24	5	HQ593645											5										
H25	3	HQ593638											3										
H26	1	JN896754										1											
H27	4	KX898421									2	2											
H28	2	MN625864	1‡							1													
H29	1	MN611173								1													NR
H30	2	MN625863								2													NR
H31	1	MN611174								1													NR
H32	1	KR055671																	1				
H33	2	KY014245																			2		
H34	1	FJ712717							1														
H35	6	MH247176							6														
H36	1	KX870082						1															
H37	1	AB551920					1																
H38	2	LC492115				2																	
H39	1	KJ741365		1																			
H40	1	**MT539997 §**	**1**																				
H41	1	**MT540001 ¥**	**1**																				

NR: not reported; Six accession numbers generated in this study: (*) MT539998 to MT540000, (‡) MT539996, (§) and (¥)

: Camel; : Horse; : Mule; : Buffalo; : Cattle; : Donkey; : Dogs; : Mouse; : Deer; : Leopard; : Capybaras; : Fly

: Africa; : Asia; : America

31% of camels in Pakistan, 26% of camels in Kenya and 25% of camels in Saudi Arabia [8], as well as in 16.8% of camels in Iran [33]. The lower PCR positivity rate in our study may reflect the fact that the majority of our Algerian samples were collected from apparently healthy camels intended for slaughter (and not from animals showing clinical signs or symptoms of disease). There was a huge difference of sampling among males and females and, independent of previous studies, this difference made analysis concerning the influence of sex on *T. evansi* infection in the present study difficult. Moreover, the lack of sex or age effects on the prevalence of infection is in agreement with previous studies which revealed that sex and age groups are equally exposed to trypanosomiasis in Pakistan [13] and in Algeria [34].

There are ample reports of characterization studies on trypanosomes in general and *T. evansi* in particular, among which, DNA targets were used to distinguish *T. evansi* interspecifically and intraspecifically including *18S rDNA*, kinetoplast DNA, microsatellite sequences and the expression-site-associated genes (ESAGs) [35, 36]. Amongst those studies, the molecular targets of variable surface glycoprotein (VSG) *RoTat 1.2* gene of *T. evansi* are widely employed [37] because it is an integral part of the parasite's surface coat and is known to be expressed in early, middle and late stages of infection [38]. The heterogeneity of *T. evansi* cannot be demonstrated using those targets; however, studies of *ITS* regions are more efficient for differentiation and may reflect geographical and/or host range effects [5, 35].

To increase knowledge of genetic diversity of *T. evansi* in Algeria, and to infer phylogenies and relatedness of the Algerian isolates, molecular analysis based on *ITS1* complete sequences was performed on naturally infected dromedary camels. The obtained sequences revealed limited nucleotide variability leading to identification of four different

Table 3 Estimate of evolutionary divergence between *Trypanosoma evansi ITS1* sequences

	Sequences	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
1	H3 KU552351 Mule China																																												
2	H33 KY014244 Dog Colombia	0,9																																											
3	H5 KU552350 Camel China	0,3	0,6																																										
4	H6 KU552344 Water Buffalo China	2,1	1,2	1,8																																									
5	H18 KR858271 Donkey India	2,1	1,2	1,8	0,6																																								
6	H13 AY912271 Buffalo Thailand	1,8	0,9	1,5	0,3	0,3																																							
7	H19 KR858269 Donkey India	2,4	1,5	2,1	0,9	0,9	0,6																																						
8	H15 AY912274 Buffalo Thailand	5,9	5	5,6	4	4,7	4,3	5																																					
9	H20 KR858267 Horse India	5,6	4,7	5,3	3,7	4,3	4	4,7	0,9																																				
10	H1 FJ712714 Horse China	5,3	4,3	5	3,4	4	3,7	4,3	0,6	0,3																																			
11	H34 FJ712717 Mouse Kenya	5	4	4,7	3,1	3,7	3,4	4	2,8	2,4	2,1																																		
12	H35 MH247176 Fly Kenya	3,7	2,8	3,4	1,8	2,4	2,1	2,8	2,1	1,8	1,5	1,2																																	
13	H9 AY912278 Deer Thailand	4	3,1	3,7	2,1	2,8	2,5	3,1	2,4	2,1	1,8	2,1	1,5																																
14	H28 MN625864 Saudi Arabia	3,4	2,4	3,1	1,5	2,1	1,8	2,5	3,1	2,8	2,4	2,1	0,9	0,6																															
15	**H28 MT539996 Camel Algeria 23**	3,4	2,4	3,1	1,5	2,1	1,8	2,5	3,1	2,8	2,4	2,1	0,9	0,6	0																														
16	H17 KX898423 Camel Iran	3,1	2,1	2,8	1,2	1,8	1,5	2,1	2,8	2,4	2,1	1,8	0,6	0,9	0,3	0,3																													
17	H27 KX898421 Camel Iran	2,8	1,8	2,4	0,9	1,5	1,2	1,8	3,1	2,8	2,4	2,1	0,9	1,2	0,6	0,6	0,3																												
18	H2 FJ712712 Mule China	3,1	2,1	2,8	1,2	1,8	1,5	2,2	3,4	3,1	2,8	2,4	1,2	1,5	0,9	0,9	0,6	0,3																											
19	H16 MN121259 Buffalo Thailand	2,8	1,8	2,4	0,9	1,5	1,2	1,8	3,7	3,4	3,1	2,8	1,5	1,8	1,2	1,2	0,9	0,6	0,3																										
20	**H41 MT540001 Camel Algeria 139**	2,8	1,8	2,4	0,9	1,5	1,2	1,8	3,1	2,8	2,4	2,1	0,9	1,2	0,6	0,6	0,3	0,6	0,9	0,6																									
21	H32 KR055671 Cattle Venezuela	2,4	1,5	2,1	1,2	1,2	0,9	1,5	3,4	3,1	2,8	2,4	1,2	1,5	0,9	0,9	0,6	0,3	0,6	0,9	0,9																								
22	H39 KJ741365 Dog Tunisia	2,4	1,5	2,1	1,2	1,2	0,9	1,5	4	3,7	3,4	3,1	1,8	2,1	1,5	1,5	1,2	0,9	0,6	0,3	0,9	0,6																							
23	H30 MN625863 Saudi Arabia	2,1	1,2	1,8	0,9	0,9	0,6	1,2	3,7	3,4	3,1	2,8	1,5	1,8	1,2	1,2	0,9	0,6	0,9	0,6	0,6	0,3	0,3																						
24	**H40 MT539997 Camel Algeria 74**	2,4	1,5	2,1	1,2	1,2	0,9	1,5	4	3,7	3,4	3,1	1,8	1,5	0,9	0,9	1,2	0,9	1,2	0,9	0,9	0,6	0,6	0,3																					
25	H29 MN611173 Saudi Arabia	2,4	1,5	2,1	1,2	1,2	0,9	1,5	4,7	4,3	4	3,7	2,4	2,1	1,5	1,5	1,8	1,5	1,8	1,5	1,5	1,2	1,2	0,9	0,6																				
26	H31 MN611174 Saudi Arabia	2,4	2,1	2,8	1,8	1,8	1,5	2,1	5,3	5	4,7	4,3	3,1	2,8	2,1	2,1	2,5	2,1	2,5	2,1	2,1	1,8	1,8	1,5	1,2	0,6																			
27	H23 MN097905 Buffalo India	2,8	1,8	2,4	1,5	1,5	1,2	1,8	5	4,7	4,3	4	2,8	2,4	1,8	1,8	2,1	1,8	2,1	1,8	1,8	1,5	1,5	1,2	0,9	0,9	1,5																		
28	H4 FJ712716 Camel China	2,4	1,5	2,1	1,2	1,2	0,9	1,5	4,7	4,3	4	3,7	2,4	2,1	1,5	1,5	1,8	1,5	1,8	1,5	1,5	1,2	1,2	0,9	0,6	0,6	1,2	0,9																	
29	H7 FJ712711 Buffalo China	3,4	2,4	3,1	2,1	2,1	1,8	2,5	4,3	4	3,7	4	3,4	1,8	2,4	2,4	2,8	2,5	2,8	2,5	2,4	2,1	2,1	1,8	1,5	1,5	2,1	1,8	0,9																
30	H8 FJ712713 Buffalo China	2,1	1,2	1,8	0,9	0,9	0,6	1,2	4,3	4	3,7	3,4	2,1	2,4	1,8	1,8	1,5	1,2	1,5	1,2	1,2	0,9	0,9	0,6	0,9	0,9	1,5	1,2	0,3	1,2															
31	H11 AY912276 Cattle Thailand	2,4	1,5	2,1	1,2	1,2	0,9	0,9	4,7	4,3	4	3,7	2,4	2,8	2,1	2,1	1,8	1,5	1,8	1,5	1,5	1,2	1,2	0,9	1,2	1,2	1,8	1,5	0,6	1,5	0,3														
32	H12 AY912270 Buffalo Thailand	1,5	1,2	1,8	0,9	0,9	0,6	1,2	4,3	4	3,7	3,4	2,1	2,4	1,8	1,8	1,5	1,2	1,5	1,2	1,2	0,9	0,9	0,6	0,9	0,9	0,9	1,2	0,9	1,8	0,6	0,9													
33	H10 EF547550 Camel Morocco	1,8	0,9	1,5	0,6	0,6	0,3	0,9	4	3,7	3,4	3,1	1,8	2,1	1,5	1,5	1,2	0,9	1,2	0,9	0,9	0,6	0,6	0,3	0,6	0,6	1,2	0,9	0,6	1,5	0,3	0,6	0,3												
34	H38 LC492115 Donkey Sudan	2,1	1,2	1,8	0,9	0,9	0,6	1,2	4,3	4	3,7	3,4	2,1	2,5	1,8	1,8	1,5	1,2	1,5	1,2	1,2	0,9	0,9	0,6	0,9	0,9	1,5	1,2	0,9	1,8	0,6	0,9	0,6	0,3											
35	**H10 540000 Camel Algeria 119**	1,8	0,9	1,5	0,6	0,6	0,3	0,9	4	3,7	3,4	3,1	1,8	2,1	1,5	1,5	1,2	0,9	1,2	0,9	0,9	0,6	0,6	0,3	0,6	0,6	1,2	0,9	0,6	1,5	0,3	0,6	0,3	0	0,3										
36	**H10 MT539998 Camel Algeria 81**	1,8	0,9	1,5	0,6	0,6	0,3	0,9	4	3,7	3,4	3,1	1,8	2,1	1,5	1,5	1,2	0,9	1,2	0,9	0,9	0,6	0,6	0,3	0,6	0,6	1,2	0,9	0,6	1,5	0,3	0,6	0,3	0	0,3	0									
37	**H10 MT539999 Camel Algeria 99**	1,8	0,9	1,5	0,6	0,6	0,3	0,9	4	3,7	3,4	3,1	1,8	2,1	1,5	1,5	1,2	0,9	1,2	0,9	0,9	0,6	0,6	0,3	0,6	0,6	1,2	0,9	0,6	1,5	0,3	0,6	0,3	0	0,3	0	0								
38	H37 AB551920 Camel Egypt	2,1	1,2	1,8	0,9	0,9	0,6	1,2	4,3	4	3,7	3,4	2,1	2,5	1,8	1,8	1,5	1,2	1,5	1,2	1,2	0,9	0,9	0,6	0,9	0,9	1,5	1,2	0,9	1,8	0,6	0,9	0,6	0,3	0,6	0,3	0,3	0,3							
39	H26 JN896754 Camel Iran	2,1	1,2	1,8	0,9	0,9	0,6	1,2	4,3	4	3,7	3,4	2,1	2,4	1,8	1,8	1,5	1,2	1,5	1,2	1,2	0,9	0,9	0,6	0,9	0,9	1,5	1,2	0,9	1,8	0,6	0,9	0,6	0,3	0,6	0,3	0,3	0,3	0,6						
40	H25 HQ593638 Water Buffalo Philippines	2,1	1,2	1,8	0,9	0,9	0,6	1,2	4,3	4	3,7	3,4	2,1	2,4	1,8	1,8	1,5	1,2	1,5	1,2	1,2	0,9	0,9	0,6	0,9	0,9	1,5	1,2	0,9	1,8	0,6	0,9	0,6	0,3	0,6	0,3	0,3	0,3	0,6	0,6					
41	H14 MN121257 Deer Thailand	1,5	0,6	1,2	0,6	0,6	0,3	0,9	4,3	4	3,7	3,4	2,1	2,4	1,8	1,8	1,5	1,2	1,5	1,2	1,2	0,9	0,9	0,6	0,9	0,9	1,5	1,2	0,9	1,8	0,6	0,9	0,6	0,3	0,6	0,3	0,3	0,3	0,6	0,6	0,6				
42	H24 HQ593645 Watter Buffalo Philippines	2,1	1,2	1,8	0,9	0,9	0,6	1,2	4,3	4	3,7	3,4	2,1	2,5	1,8	1,8	1,5	1,2	1,5	1,2	1,2	0,9	0,9	0,6	0,9	0,9	1,5	1,2	0,9	1,8	0,6	0,9	0,6	0,3	0,6	0,3	0,3	0,3	0,6	0,6	0,6	0,6			
43	H22 MT233332 Leopard India	2,8	1,8	2,4	1,2	1,2	0,9	1,5	5,3	5	4,7	4,3	3,1	3,4	2,8	2,8	2,4	2,1	2,5	2,1	2,1	1,8	1,8	1,5	1,8	1,8	2,4	2,1	1,8	2,8	1,5	1,8	1,5	1,2	1,5	1,2	1,2	1,2	1,5	1,5	1,5	1,2	1,5		
44	H36 KX870082 Fly South Africa	6,3	5,3	6	5	5	4,7	5,3	8,3	7,6	7,6	7,6	6,3	6,3	5,9	5,9	5,6	5,3	5,6	5,3	5,3	5	5	4,7	5	5	5,6	5,3	5	5,6	4,7	5	4,7	4,3	4,7	4,3	4,3	4,3	4,7	4,7	4	4,7	4	4,3	
45	H21 KR858272 Horse India	15	14	15	13	13	13	14	17	16	16	16	16	15	15	15	15	15	15	15	15	14	14	14	14	14	14	15	14	15	14	14	14	13	14	13	13	13	14	13	14	13	13	14	17

There were a total of 333 positions in the final dataset. Analyses were conducted using the Tamura 3-parameter model. Distances are given as percentages

haplotypes. However, in a recent study, identical sequences of *T. evansi* were reported from partial *ITS1* belonging to different dogs in northeastern Algeria [39]. The phylogenetic analysis of our isolates, including representative sequences of the *ITS1* complete sequences from Africa, Asia and South America, showed that Algerian sequences isolated from camels belong to four different haplotypes: (i) H28, identical to a sequence isolated from Saudi Arabia;(ii) H10, present worldwide in a different host and (iii) two novel sequences representing new haplotypes H40 and H41.

Phylogenetic analyses of reported *T. evansi* sequences from various species revealed neither host nor geographic specificity. However, due to lack of nucleotide sequences from previous cases of *T. evansi* in Algeria, history of these haplotypes could not be determined. For this reason, future population and phylogeographic analyses involving a larger number of samples from different Algerian regions and North African countries are needed to evaluate this evolutionary process.

In conclusion, genetic diversity of *T. evansi* among dromedary camels from Algeria has been demonstrated and two new haplotypes have been identified. These novel findings can serve as the basis for future studies of *T.evansi* genetic diversity. Nevertheless, additional studies including more samples from different hosts and geographic areas are needed to fully understand the extent of distribution of *T.evansi* haplotypes in Algeria.

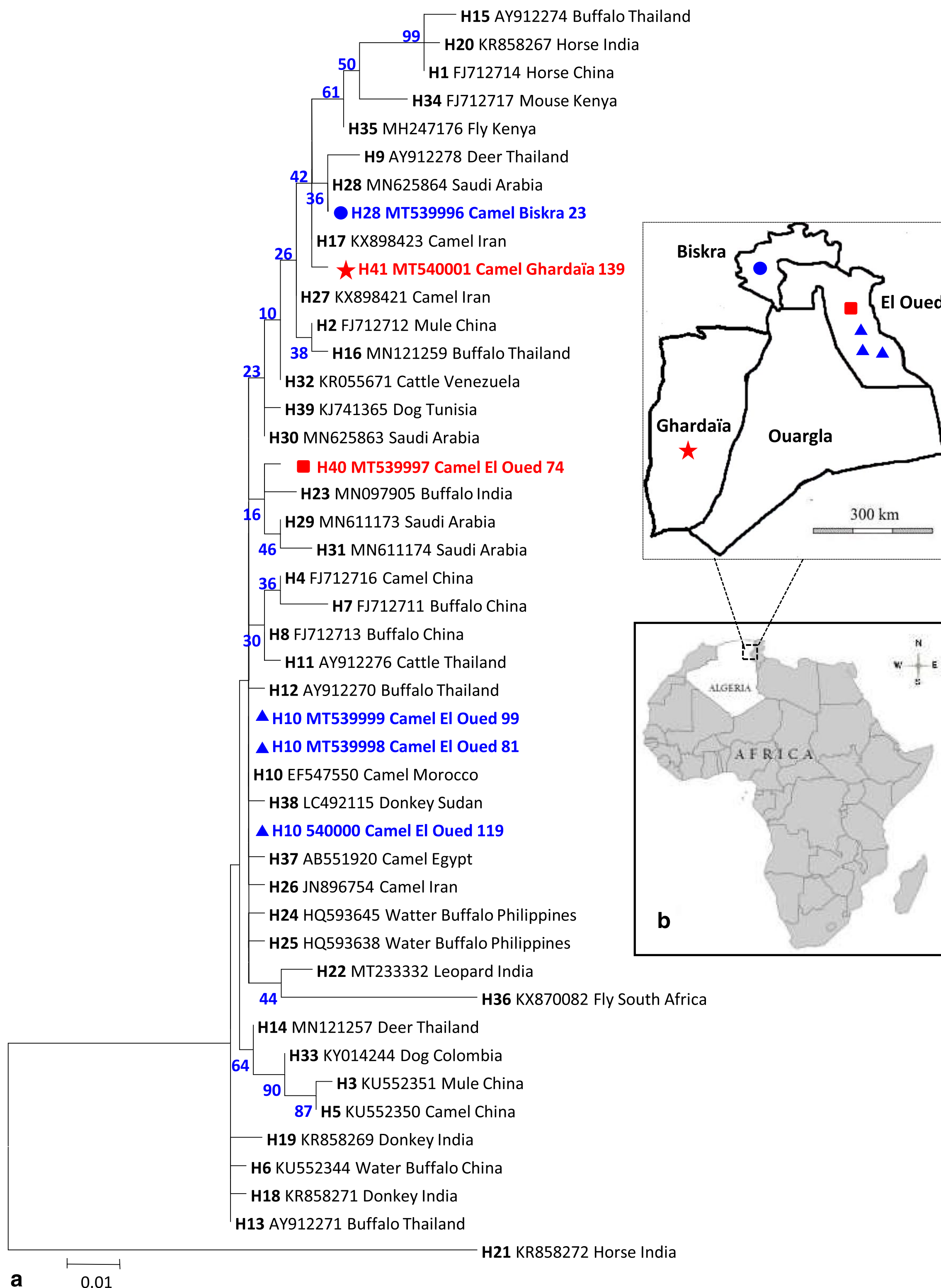

Fig. 2 Phylogenetic (**a**) and geographic (**b**) distribution of *Trypanosoma evansi* haplotypes identified in dromedary camel populations in eastern Algeria in relation to *T. evansi* haplotypes reported worldwide

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Compliance with Ethical Standards

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

Ethics Approval This study with camels was supervised by the division of biological resources of the Scientific and Technical Research Centre for Arid Areas (CRSTRA). It was conducted in accordance with the World Animal Health Organization (OIE) guiding principles on animal welfare included in the OIE Terrestrial Animal Health Code [40].Verbal consent of farm owners involved in this investigation was obtained prior to the collection of blood samples from their animals.

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