

Supplementary Table S1. Primers used in this study to amplify the species of leech, trypanosomes and leech crop contents.

Gene	Primer sequence (5'→3')	Annealing temperatures	PCR product (bp)	Reference
eukaryotic 18S rRNA	F: AACCTGGTTGATCCTGCCAGT R: TGATCCTTCCGCAGGTTACCT	47°C	600	Medlin et al., 1988
Tryp. 18S rRNA	S762: GACTTTTGCTTCCTCTATTG S763: CATATGCTTGTTTCAAGGAC	52°C	2000	Malsov et al., 1996
Tryp. <i>gGAPDH</i> (First PCR)	G3: TTYGCCGYATYGGYCGCATGG G5: ACMAGRTCCACCACRCGGTG	55°C	-	Hamilton et al., 2007
Tryp. <i>gGAPDH</i> (nest PCR)	G1: CGCGGATCCASGGYCTYMTCGGBAMKGAGAT G4a: GTTYTGCAGSGTCGCCTTGG	55°C	900	
mammalian <i>Cyt b</i>	Cytbb1F: CCATCMAACATYTCADCATGATGAAA Cytbb2R: GCHCCTCAGAATGAYATTTGKCCTCA	55°C	360	Rádrová et al., 2013
vertebrate mt16S rRNA	F: TGTTTACCAAAAACATCACCTCCA R: AGTTAAAGCTCCATAGGGTCT	58°C	200	Bo et al., 2016

Tryp., trypanosomatidae. IUPAC/IUB base codes: Y= C or T; M= A or C; K= G or T; B= C, G or T.

References

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- Maslov, D.A., Lukeš, J., Jirku, M., Simpson, L., 1996. Phylogeny of trypanosomes as inferred from the small and large subunit rRNAs: implications for

the evolution of parasitism in the Trypanosomatid protozoa. *Mol. Biochem. Parasitol.* 75, 197-205.

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Rádrová, J., Šeblová, V., Votýpka, J., 2013. Feeding behavior and spatial distribution of *Culex* mosquitoes (Diptera: Culicidae) in wetland areas of the Czech Republic. *J. Med. Entomol.* 50, 1097-1104.

Supplementary Table S2. The accession numbers of *gGAPDH* and 18S rRNA gene sequences obtained from GenBank.

Genus	Species	GenBank Accession numbers	
		<i>gGAPDH</i>	18S rRNA
<i>Trypanosoma</i>	<i>T. avium</i>	AJ620263	AJ009140
	<i>T. binneyi</i>	AJ620266	
	<i>T. boissoni</i>		U39580
	<i>T. brucei brucei</i>		M12676
	<i>T. brucei gambiense</i>	AF047499	AJ009141
	<i>T. cervi</i>	KJ195884	
	<i>T. cobitis</i>		AJ009143
	<i>T. congolense</i>		AJ009144
	<i>T. cruzi</i>		AJ009147
	<i>T. cruzi</i> c8	AJ620268	
	<i>T. cyclops</i>	FJ649493	AJ131958
	<i>T. dionisii</i>		AJ009151
	<i>T. epinepheli</i> n. sp.		
	<i>T. equiperdum</i>		AJ223564
	<i>T. evansi</i> ,		AJ009145
	<i>T. godfreyi</i>		AJ009155
	<i>T. grayi</i> ANR4	AJ620257	
	<i>T. granulosum</i>		AJ620551
	<i>T. lewisi</i> L32		AJ009156
	<i>T. mega</i>		AJ223567
	<i>T. melophagium</i>		FN666409
	<i>T. murmanensis</i>		DQ016616
	<i>T. neveulemairei</i>		AF119809
	<i>T. pleuronectidium</i>		DQ016613
	<i>T. rangeli</i>		AJ009160
	<i>T. rotatorium</i>		AJ009161
	<i>T. siniperca</i>		DQ494415
	<i>Trypanosoma</i> sp.		AJ009167
	<i>Trypanosoma</i> sp. carpio		EF375882
	<i>Trypanosoma</i> sp. CLAR		AJ620555
	<i>Trypanosoma</i> sp.		EF375883
	<i>Trypanosoma</i> sp. KrSI7		MK156792

<i>Trypanosoma</i> sp. TL. AQ.	AJ620280	AJ620574
<i>T. trinaperronei</i>	MN752212	
<i>T. theileri</i> TthbV15	HQ664791	
<i>T. theileri</i> Tth KM		AB007814
<i>Trypanosoma</i> sp. KrS14		MK156792
<i>T. theileri</i> Tthb10	HQ664784	
<i>T. theileri</i> Tthb14	HQ664785	
<i>T. theileri</i> Tthb16	HQ664786	
<i>T. theileri</i> Tthb17	HQ664787	
<i>T. theileri</i> Tthb19	HQ664788	
<i>T. theileri</i> Tthb20	HQ664789	
<i>T. theileri</i> Tthb22	HQ664790	
<i>T. theileri</i> Tthc1	HQ664792	
<i>T. theileri</i> Tthc3	HQ664793	
<i>T. theileri</i> Tthc29	HQ664799	
<i>T. theileri</i> Tthc30	HQ664801	
<i>T. theileri</i> Tthc32	HQ664802	
<i>T. theileri</i> Tthc37	HQ664803	
<i>T. theileri</i> Tthc39	HQ664794	
<i>T. theileri</i> Tthc40	HQ664795	
<i>T. theileri</i> Tthc41	HQ664796	
<i>T. theileri</i> TthcV4	HQ664797	
<i>T. theileri</i> TthcV5	HQ664798	
<i>T. theileri</i> K127	AJ620282	
<i>T. theileri</i> TREU124	HQ664800	
<i>T. theileri</i> CepCamp4	HQ664804	
<i>T. theileri</i> CepCamp5	HQ664805	
<i>T. theileri</i> SitaBip1	FM164792	
<i>Trypanosoma</i> sp. TspD30	HQ664806	AJ009165
<i>T. therezieni</i>		AJ223571
<i>T. triglae</i>		U39584
Non-<i>Trypanosoma</i>	<i>Trypanoplasma borreli</i>	X74535
		L14840

Supplementary Table S3. Alignment of mitochondrial 16S rRNA gene sequences from leech crop contents with those of close-related sequences from GenBank.

Specimens	Polymorphic positions
	Between sites 848 and 906
Water buffalo	TGGCCATTCATACGAGTCCCTATTTAGAGAACAAGTGATTATGCTACCTTTGCACGGT
SLL1.2 ^a	-----
SLL2.5 ^b	-----A--
XTL5.1 ^c	-----
Cattle	C-----A-----T-----A-----G--
SLL1.1 ^d	C-----A-----T-----A-----G--
	Between sites 907 and 965
Water buffalo	CAGGATACCGCGGCCGTTTAAACGGTTGTCCTGGGCAGGCAGTGCCTCCAATACTAGGG
SLL1.2 ^a	-----
SLL2.5 ^b	-----
XTL5.1 ^c	-----A
Cattle	-----A--A-----A-----G--A
SLL1.1 ^d	-----A--A-----A-----G--A

The sequences of water buffalo (*Bubalus bubalis*, MT186740) and cattle (*Bos taurus*, MN714218) were cited from GenBank. Samples beginning SLL/XTL/NLL originate TA cloned sequences from the Shalang/Xintong/Nanlang leech crop contents. -, identical to water buffalo.

^aSeventy-three identical sequences (GenBank accession no. MZ520977), including SLL1.2-10, SLL2.1-4, SLL2.6-10, SLL3.1-10, XTL4.1-10, XTL 5.2-4, XTL 5.6-10, XTL6.1-10, NLL8.1-10, NLL9.1-5 and NLL9.7-10.

^bSLL2.5, GenBank accession no. MZ520980.

^cTwo identical sequences (GenBank accession no. MZ520978), including XTL5.1 and XTL5.5;

^dFour identical sequences (GenBank accession no. MZ520979), including SLL1.1, XTL5.2, XTL5.4 and NLL9.6.