1   **Molecular characterization of pathogenic African trypanosomes in biting flies and camels**

2   **in surra-endemic areas outside the tsetse fly belt in Kenya**

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Abstract

Background: African animal trypanosomosis is becoming prevalent beyond its traditionally defined geographical boundaries and is a threat to animals beyond the tsetse belts in and outside Africa. However, knowledge of infections with clinically important trypanosome species and their diversity among field-collected hematophagous biting flies and domestic animals is limited mainly to tsetse and their mammalian hosts in tsetse-infested areas. This study aimed to examine the presence of trypanosomes in both biting flies and domestic animals outside the tsetse belt in northern Kenya, potential mechanical vector species, and their host-feeding profiles.

Methods: We screened for pathogenic African trypanosomes in blood samples from domestic animals and field-trapped flies by microscopy and sequencing of internal transcribed spacer (ITS1) gene PCR products. We sequenced kinetoplast maxicircle genes to confirm *Trypanosoma brucei* detection and the RoTat 1.2 and kinetoplast minicircle genes to differentiate type-A and type-B *Trypanosoma evansi*, respectively. Further, we identified the hosts that field-trapped flies fed on by PCR-HRM and sequencing of 16S rRNA genes.

Results: *Hippobosca camelina*, *Stomoxys calcitrans, Tabanus* spp., and *Pangonia rueppellii* are potential vectors of trypanosomes outside the tsetse belt in Marsabit County, northern Kenya. We identified *Trypanosoma spp*., including *Trypanosoma vivax*, *T. evansi*, *T. brucei*, and *T. congolense* in these biting flies as well as in camels (*Camelus dromedarius*). Trypanosomes detected varied from single up to three trypanosome species in *H. camelina* and camels in areas where no tsetse flies were trapped. Similar trypanosomes were detected in *Glossina pallidipes* collected from a tsetse-infested area in Shimba Hills, coastal Kenya, showing the wide geographic distribution of trypanosomes. Furthermore, we show that these biting flies acquired blood meals from camels, cattle, goats, and sheep. Phylogenetic analysis revealed diverse *Trypanosoma* spp. associated with variations in virulence and epidemiology in camels, which suggests that camel trypanosomosis may be due to mixed trypanosome infections rather than only surra (*T. evansi*), as previously thought.

Key words: Surra, camel, non-tsetse transmitted trypanosomes, biting flies, tsetse belt, Kenya.

1. Introduction

Trypanosoma evansi is one of the most important *Trypanosoma* spp. infecting livestock globally. Its wide geographic distribution [1–3], mode of transmission [4, 5], zoonotic potential [6, 7], pathogenicity to several domestic animals [8], high genetic diversity, and variation in virulence [5, 9] makes it an important parasite. Animal trypanosomosis caused by *T. evansi* is called surra in camels and is the most lethal disease of camels worldwide [4, 10, 11]. In addition to causing camel mortality, *T. evansi* infections reduce production of milk, an important staple food and the primary source of protein for pastoralists. Furthermore, *T. evansi* is an important pathogen in cattle and buffalo that results in morbidity and mortality and induces higher rates of abortion in domestic animals in Asia [11, 12].

Basic knowledge of the epidemiology and diversity of clinically important trypanosomes (such as *Trypanosoma vivax*, *T. congolense*, and *T. evansi*) in non-tsetse hematophagous flies and domestic animals from tsetse-free areas is significantly outweighed by that of tsetse and trypanosomes studied in tsetse-infested areas. However, several laboratory and semi-field experiments have demonstrated that different *Trypanosoma* spp., including *T. congolense* [13], *T. vivax* [14], and *T. evansi* [15], are potentially transmitted to domestic animals by various biting flies, such as *Stomoxys* spp. and *Tabanus* spp.

To evaluate the presence of various *Trypanosoma* spp. and the potential role of non-tsetse biting flies in their transmission within a tsetse-free area of northern Kenya, we investigated: (i) the diversity of hematophagous biting flies that could be involved in the mechanical transmission of trypanosomes; (ii) the identity, and diversity of economically and clinically important pathogenic trypanosomes within randomly collected and sampled flies and domestic animals, and (iii) on what vertebrates these biting flies feeding.

2. Materials and Methods

2.1. Study sites

Among three study sites, two sites in Marsabit County, northern Kenya, were in Ngurunit (N01°.74', E 037.29') at the edge of the tsetse distribution map and Shurr, a tsetse free area (N02°.08', E038°.27') (Fig. 1A-B). The third site was in Nanyuki in central Kenya, which is also tsetse free area (N00°.41', E036°.90') (Fig. 1A). All the three study sites are characterized by arid and semi-arid climatic conditions. The main means of livelihood is animal husbandry. The area has suitable biomass, especially for browsers such as goats and camels. Even though

90   Ngurunit falls within the tsetse distribution map, a previous study reported no tsetse flies in the

91   area [15] and therefore ideal sites to study non-tsetse transmitted trypanosomes. Furthermore,

92   Marsabit County, where the two sampling sites are located have high number of camels, 1.3 –

93   1.9 camels/km^2 [16], making it suitable for camel trypanosomosis studies. Tsetse flies (*G.*

94   *pallidipes*) were collected from Shimba Hills in Kwale County, coastal Kenya for *Trypanosoma*
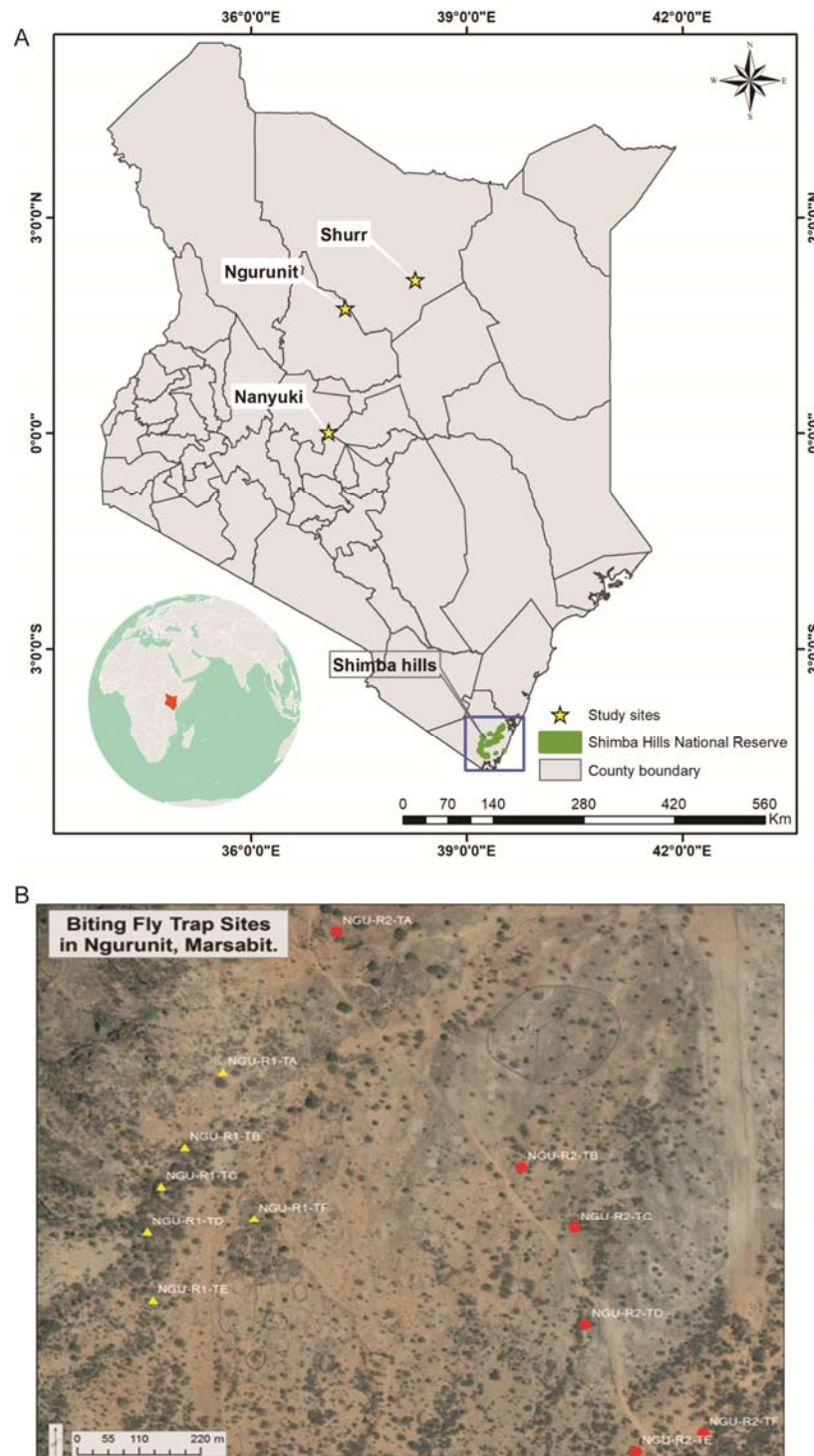
95   spp comparisons.

Fig. 1. Map of study sites. (A) Map showing the study sites (B) Map of biting fly trap sites in Ngurunit village in Marsabit County. Yellow triangles represent the first replication of fly trapping in Ngurunit, which was in a forested area, and red circles represent the second replication, which was in an area with sparse vegetation.

1.2. Fly trapping

Flies were trapped using monoconical traps [17], placed ~150 m apart. Camel urine odour dispensed from plastic bottles (release rate was not quantified) was used as an attractant. Biting flies feeding on camels were collected using sweep nets and preserved in absolute ethanol and identified using appropriate taxonomic keys [18]. Camels, from which biting flies were collected, were randomly chosen regardless of their sex, age, or health status of the animal. The flies were trapped during both dry and rainy seasons using 25 monoconical traps per site for five days at each season and emptied every 24 hr. The fly trapping sites included thick bushland, *Acacia* spp. woodlands, watering points, animal enclosures, and open grassland areas (Fig.1B).

2.3. **Fly density per camel**

Hippobosca camelina and *Stomoxys calcitrans* counts were made by approaching ten randomly selected camels slowly from the side. An estimate number of *S. calcitrans* and *H. camelina* was made by counting the total number of flies on the legs and belly of the camel. Counts were made from a distance of 0.5 to 1 m. Two experienced technicians with expertise in distinguishing between the target flies performed all the counting. Stable flies could be differentiated from morphologically-similar house flies by their distinctive feeding posture as they feed parallel to the camel's body [19], whilst house flies display a more random position when resting on the camel.

2.4. Blood sampling and microscopy

Approximately 5-10 mL of blood was drawn from the jugular vein of camels, goats, sheep, donkeys, and cattle into vacutainer tubes containing disodium salt of ethylene diamine tetra-acetate (EDTA) (Plymouth PLG, UK). An aliquot from each vacutainer tube was transferred into heparinized capillary tubes (75×1.5 mm) and spun in a micro-haematocrit centrifuge at 12,000 rpm for 5 minutes to separate the red and white blood cells and plasma, hence concentrating the trypanosomes [20]. Packed cell volume (PCV), an indicator of the animal's anaemic status, was measured using Haematocrit Reader (Hawksley & Sons Limited, England) and expressed as a percentage of PCV to total blood volume. The buffy coat plasma interface was placed onto a microscope glass slide and examined under a microscope for the presence of moving trypanosomes. The trypanosome species were provisionally identified based on cell motility and morphology using wet blood film examination [20, 21]. Furthermore, thin blood smears were

132   prepared from the samples, fixed with methanol, and stained with 10% Giemsa [20]. The stain

133   was flushed with running tap water and allowed to dry for 35 minutes. Slides were then

134   examined under the 100× oil immersion objective for trypanosomes and positive cases recorded.

135   The rest of the well-mixed blood contents were appropriately labelled and stored in liquid

136   nitrogen for transportation to Nairobi-based *icipe* laboratories for further screening. Domestic

137   animals (including camels), were randomly sampled from various herds, for up to a maximum of

138   10% of the herd population to accommodate sampling of more herds. Camels in the herd were

139   assigned a reference number and numbers were selected randomly for blood sampling. The mean

140   number of camels per household varied from four to 35 on average in Ngurunit and Shurr sites,

141   respectively. However, in Nanyuki, with relatively smaller population of camels, only one herd

142   was sampled. All the identified infected camels were treated with triquin (Vetoquinol®) at a dose

143   of 5 mg/kg body weight [10].

144

145   **2.5 DNA extraction from blood and biting flies**

146   Total genomic DNA was extracted from all collected blood samples using DNeasy Blood &

147   Tissue Kits (Cat No./ID: 69504, Qiagen, Hilden, Germany), as follows: 100 µL of each blood

148   sample was pipetted into 1.5-mL Eppendorf® tubes and mixed with 20 µL proteinase K, and the

149   volume adjusted to 220 µL with PBS at pH 7.4. Subsequently, 200 µL Buffer AL were added to

150   the reaction mix, thoroughly mixed and incubated for 10 minutes at 56°C. After the 10-minute

151   incubation, 200 µL of absolute ethanol were separately pipetted into each tube and vortexed

152   thoroughly before pipetting the mixture into the DNeasy Mini spin column placed in a 2-mL

153   collection tube for centrifugation at 8000 rpm for 1 minute. The DNA samples on the spin

154   columns were separately washed with 500 µL of Buffer AW1 Buffer AW2 preceding elution

155   into clean 1.5-mL microcentrifuge tubes, with100 µL Buffer AE. The freshly eluted DNA

156   samples were stored at -20°C until PCR analysis. Similarly, the total DNA from the crushed guts

157   of biting flies were extracted and purified after brief surface sterilisation with ethanol and

158   cleaning with distilled water. A negative extraction control was also performed for

159   contamination assessment.

160

161 **2.6 Identification of trypanosomes in the blood and fly samples**

162 To check the presence of trypanosomes, a random subset of the flies from diverse biting flies

163 collected were analysed from the whole fly using PCR. To check trypanosomes in blood we

164 combined light microscopy with more sensitive molecular techniques, primarily using DNA-

165 based markers [22] that enabled differentiation of trypanosome species and their subgroups. We

166 employed PCRs targeting the internal transcribed spacer (ITS-1) gene fragment, which is a

167 conserved gene across all African trypanosomes [23]. The diagnostic PCR assays were carried

168 out in 10-µL reaction mixtures containing 5 µL 2× DreamTaq mix, 3 µL PCR water, 0.5 µL ITS-

169 1 primers (F: 5'-CCGGAAGTTCACCGATATTG-3'; R: 5'-TTGCTGCGTTCTTCAACGAA-

170 3') [24] and 1 µL DNA template. PCR amplification conditions were programmed as follows:

171 95°C denaturation step for 1 minute, 35 cycles of 95°C for 30 seconds, 61°C for 30 seconds,

172 72°C for 1 minute and final extension of 72°C for 10 minutes. Additionally, primers designed to

173 amplify kinetoplast 9S ribosomal RNA subunit (kDNA 12 (modified): 5′-

174 TTAATGCTATTAGATGGGTGTGG-3′; kDNA 13: 5′-

175 CTCTCTGGTTCTCTGGGAAATCAA-3′) [25] and CO1 (Tb_kDNA_COI_Max1: 5'-

176 CCCTACAACAGCACCAAGT-3'; Tb_kDNA_COI_Max2: 5'-

177 TTCACATGGGTTGATTATGG-3') [26] genes were used to differentiate *T. brucei* from *T.*

178 *evansi* as previously described. To separate *T. evansi* subtypes A and B, we used type A-specific

179 primers targeting the VDG RoTat 1.2 gene (F: 5′-GCGGGGTGTTTAAAGCAATA-3′; R: 5′-

180 ATTAGTGCTGCGTGTGTTCG-3′) and type B-specific primers targeting the minicircle gene

181 (EVAB-1: 5′-ACAGTCCGAGAGATAGAG-3′; EVAB-2: 5′-CTGTACTCTACATCTACCTC-

182 3′) [27, 28]. For each PCR, a negative PCR control (non-template control) was set up alongside

183 samples (SF1). This enabled detection of contamination during PCR set up.

184 The PCR amplicons were purified using Quickclean II gel extraction kit (GeneScript USA Inc.,

185 Piscataway, USA) according to the manufacturer's instructions. Briefly, DNA bands of interest

186 were excised from the agarose gel with a sharp, clean scalpel (replaced for each sample) into

187 sterile 1.5-mL Eppendorf tubes and three volumes of binding buffer II added to each sample. The

188 reaction tubes were then incubated at 55°C for 10 minutes with occasional vortexing until all

189 gels melted and a pale-yellow mixture was observed. The samples were then transferred into spin

190 columns and centrifuged at 6000 ×g for 1 minute. Subsequently, the samples were washed with

191 650 µL wash buffer and centrifuged at 12000 ×g for 1 minute. The DNA samples were then

8

192 eluted from the columns with 50 µL elution buffer into sterile 1.5-mL Eppendorf tubes and

193 stored at -20°C. Confirmation of the purified DNA was performed by gel electrophoresis and

194 sent for sequencing at Macrogen (Netherlands).

195 All nucleotide sequences were edited and aligned using the MAFFT plugin in Geneious software

196 version 11.1.4 [29]. Sequence identities were revealed by querying in the GenBank nr database

197 using the Basic Local Alignment Search Tool (www.ncbi.nlm.nih.gov/BLAST/). The aligned

198 ITS-1 sequences were used to construct a maximum likelihood phylogenetic tree using PHYML

199 v. 3.0 [30]. The phylogeny employed the Akaike information criterion [31] for automatic model

200 selection and tree topologies were estimated using nearest neighbor interchange (NNI)

201 improvements over 1,000 bootstrap replicates. The phylogenetic tree was visualized using

202 FigTree v1.4.2 [32].

203

204 **2.7. Bloodmeal source identification in biting flies by PCR – high-resolution melting (HRM)**

205 **analysis**

206 Genomic DNA from blood-fed *H. camelina* and *S. calcitrans* and known vertebrate whole blood

207 samples as positive controls were isolated using DNeasy Blood and Tissue kit (Cat

208 No./ID: 69504, Qiagen, Hilden, Germany). Fed flies were selected by observing engorged

209 abdomens and then the guts of blood-fed flies were crushed and homogenized individually using

210 ten 2-mm yttria-stabilised zirconia beads in 400 μL of cold homogenization phosphate buffered

211 saline (PBS) to each tube on ice, then agitating for 10 seconds in a Mini-BeadBeater-16

212 (BioSpec, Bartlesville, OK, USA). The homogenates were then centrifuged for 10 seconds in a

213 bench top centrifuge (Eppendorf, USA) at 1500 relative centrifugal force at 4˚C. Aliquots of 210

214 μL of each homogenate were used for nucleic acid extraction.

215 High-resolution melt profiles from PCR amplicons of different vertebrate 16S rRNA DNA in

216 100 *H. camelina* were analysed in Applied Biosystems QuantStudio 3 real-time PCR system

217 (Thermo Scientific, USA) and used to identify the various vertebrate bloodmeal sources in fed

218 flies as previously described [33, 34]. Blood-meal profiles of vertebrates were specifically

219 matched to selected domestic and wild animal positive controls. Control vertebrate host samples,

220 including human, cow (*Bos taurus*), sheep (*Ovis aries*), warthog (*Phacochoerus africanus*),

221 African buffalo (*Syncerus caffer*), goat (*Capra aegagrus hircus*), elephant (*Loxodonta africana*),

222   Sprague Dawley rat (*Rattus norvegicus*), and camel (*Camelus dromedarius*) served as reference

223   controls. The 10-µL PCRs reaction consisted of 1 µL DNA template, 6 µL of PCR water, and 2

224   µL of 5× HOT FIREpol EvaGreen HRM Mix (Solis BioDyne, Tartu, Estonia) and 0.5 µM

225   concentrations of each primer (see [33, 34] for details). The PCR thermal cycling conditions for

226   cytochrome b were set as follows: initial denaturation 95°C for 15 minutes, 35 cycles of

227   denaturation at 95°C for 30 seconds, annealing 58°C for 20 seconds, extension 72°C for 30

228   seconds and final extension of 72°C for 7 minutes with final PCR products kept at 4°C. The

229   annealing temperature of the 16S rRNA DNA marker was 56°C. Following PCR amplification,

230   HRM analysis was performed within normalised temperature regions of between 65°C - 78°C

231   and 88°C - 95°C. The different melt curve profiles of the samples were compared to the

232   reference standards, and representative samples under each peak were selected for gene

233   sequencing.

2.8 Data analysis

235   Biting fly densities on camels were compared using the Mann-Whitney test as the data was not

236   normal following normality according to Levene's test of homogeneity of variance. We used the

237   following formula developed by [35]: $n = \ln(\alpha)/\ln(1-p)$ to determine the minimum number of

238   camels and biting flies to be sampled for trypanosomes detection . We used sensitive molecular

239   tools for pathogen detection, and we assumed that 3% of field collected flies and camels were

240   infected at 95% confidence limit, thus, $\alpha = 0.05$, $P = 0.03$ (probability of detecting infected biting

241   flies, camel). Sample size $n = \ln(\alpha)/\ln(1-p)$ therefore $n = -2.99/-0.03 = 99.6$, which suggested that

242   a minimum of 100 randomly sampled camels and biting flies were needed [35]. However, when

243   biting flies were few, we analyzed 50 flies.

244   Chi-squared tests were used to compare differences in the number of trypanosomes detected in

245   biting fly species, and among different domestic animals. The independent t-test was used to

246   compare PCV values between infected and non-infected camels. Blood-meal sources were also

247   compared using the chi-squared test. All analyses were performed using GraphPad software

248   (GraphPad Software, Inc, USA). The Shannon diversity index (H) was used to characterise the

249   diversity of pathogens in biting flies using percent prevalence data and the diversity index of

250   biting flies between sites was analyzed using number of individuals per trap calculated using

251   PAST 3.11 (www.folk.uio.no/ohammer/past/) [36]. The relative feeding index of *H. camelina*

252   and *S. calcitrans* was calculated according to [36, 37] as follows; $W_i = O_i/P_i$, where, W_i =

253   feeding ratio for livestock i, O_i = percentage of livestock, I, in the blood meals, P_i = proportion

254   or percentage of livestock i available in the environment.

255

256

3. RESULTS

3.1. Diverse biting flies were identified as potential trypanosome vectors in the tsetse-free area

We collected a variety of biting flies of the Order Diptera from the study areas using monoconical traps (Table 1). Similar hematophagous flies were also observed feeding on camels, including *H. camelina* (Leach), *S. calcitrans* (L), *Pangonia rueppellii* (Jaenn), *Haematopota pluvialis*, and *Tabanus* spp. (Fig. 2A–F). *Hippobosca camelina* and *S. calcitrans* were present all year-round and observed feeding together on the same camel. The abundance of biting flies on a given camel varied between biting fly species. For instance, more *H. camelina* flies per camel (n = 10) were recorded as compared to *S. calcitrans* ($P < 0.005$ Mann-Whitney Test) (Fig. 2G). However, the number of other biting flies on camels (*P. rueppellii, Tabanus* spp., and *Hae. pluvialis*) were too low for meaningful comparisons.
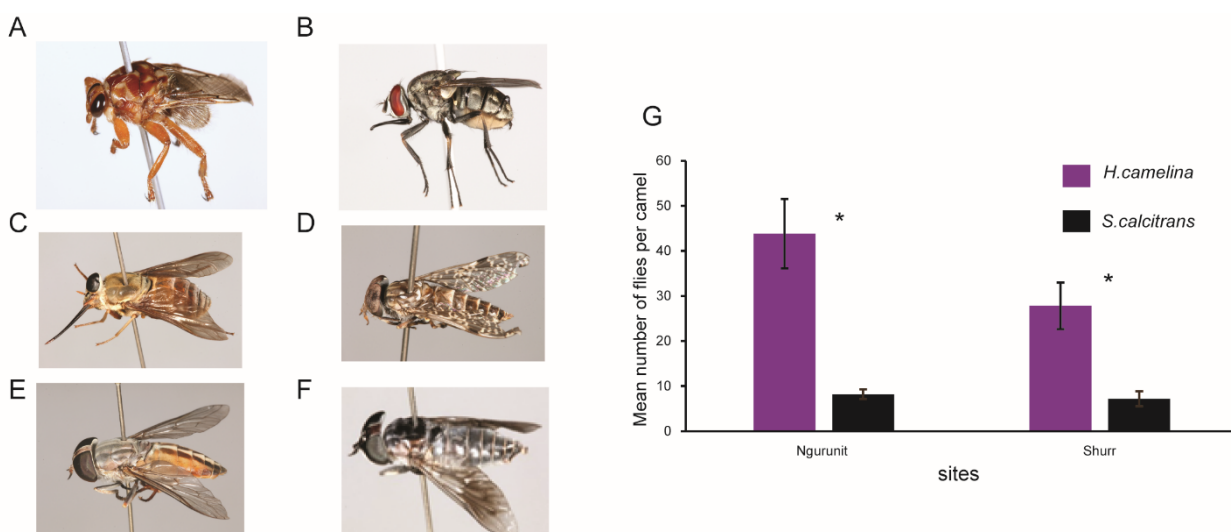

Fig. 2. **Diversity and abundance of biting flies** (A) *H. camelina*, (B) *S. calcitrans*, (C) *P. rueppellii*, (D) *Hae. pluvialis*, (E - F) *Tabanus* spp., (G) Mean number of *H. camelina* and *S. calcitrans* per camel at Ngurunit and Shurr sites, bars represent standard error of the mean, *depicts significant differences in fly density, $P < 0.05$, N = 10.

The diversity of biting flies varied from place to place; Ngurunit had diverse species of biting flies (Shannon index, $H = 1.3$), including *H. camelina, P. rueppellii, Hae. pluvialis, S. calcitrans* and *Tabanus*. Shurr (Shannon index, $H = 0.6$) had all the species of biting flies, except *P. rueppellii*, while Nanyuki (Shannon index, $H = 0$) had only *S. calcitrans*.

12

280 **Table 1. Diversity of biting flies at three sites**

Site	*H. camelina*	*S. calcitrans*	*Tabanus* spp.	*P. rueppellii*	*Hae. pluvialis*	Shannon index (H)
Ngurunit	+	+	+	+	+	1.3
Shurr	+	+	+	-	+	0.6
Nanyuki	-	+	-	-	-	0

281 (+) indicates the specific biting fly was detected; (-) indicates not detected.

282

283 **3.2. *Trypanosoma* spp. identified in biting flies from the tsetse-free area detected using**

284 **molecular technique**

285 **3.2.1. Trypanosomes in *H. camelina***

286 Out of the 150 *H. camelina* (Fig. 2A) flies analysed, 3% had detectable *T. congolense* savannah

287 PCR amplicons (700 bp), 39% had *T. vivax* amplicons (250 bp), 9% had *Trypanozoon* amplicons

288 (500 bp), 6% had mixed *T. vivax* and *Trypanozoon* DNA, 3% had mixed *T. congolense* and

289 *Trypanozoon* DNA, and 1% had DNA of all three species (*T. congolense*, *Trypanozoon*, and *T.*

290 *vivax*) (Fig. 3A & B). *Trypanosoma vivax* was more common compared to *Trypanozoon* ($\chi^2 =$

291 36.883, df = 1, *P* < 0.001) and *Trypanozoon* was more common than *T. congolense* ($\chi^2 = 4.771$,

292 *P* = 0.0289). Occurrence of mixed DNA was low (Fig. 3A–B). Trypanosomes were detected

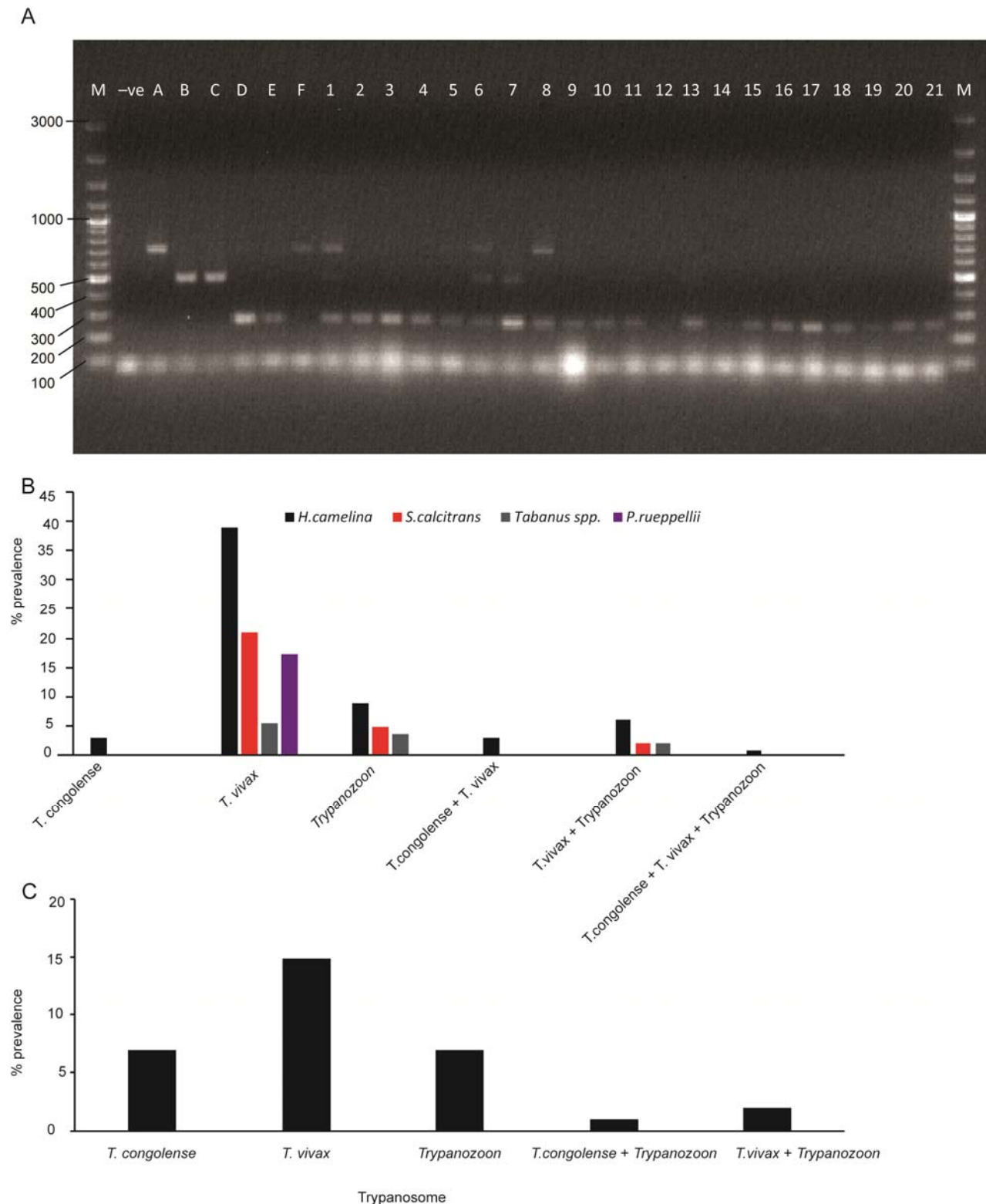293 using total DNA isolated from individual flies by PCR targeting the trypanosomal ITS1 gene

294

Fig. 3. **Prevalence and diversity of trypanosomes in biting flies.** (A) PCR products were resolved through 1% ethidium-bromide stained agarose gel at 80V for 1.5 hrs**.** Lane M 100-bp

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299 marker (Thermo Scientific, USA); Lane '-ve': negative control; Lane A – D contain positive

300 controls for Lane A: *T. congolense* savannah (IL3000); Lane B: *T. brucei* ILTat 1.4; Lane C: *T.*

301 *evansi* KETRI 2479; Lane D: *T. vivax* IL 2136; Lanes E and F: Trypanosome-infected camel

302 blood from the same site as that of the flies; Lanes 1 – 21: some of selected *H. camelina* samples

303 (**B**) The percentage prevalence of trypanosome species in biting flies. (**C**) The prevalence and

304 diversity of trypanosomes detected from field collected *G. pallidipes* (n = 101).

305 **3.2.1.1 Diversity of trypanosomes in *H. camelina* from two sampling sites**

306 The prevalence of trypanosomes in *H. camelina* collected from two sampling sites in northern

307 Kenya, namely Ngurunit and Shurr, was variable. Out of the 150 flies analysed (75 flies from

308 each site), 33% of flies from Ngurunit carried *T. vivax* (25/75), *Trypanozoon* was detected in

309 2.7% of flies (2/75), whereas about 5% of flies contained *T. congolense* savannah (4/75).

310 However, 45% of *H. camelina* from Shurr were positive with *T. vivax* (34/75), whereas 14.7% of

311 flies contained *Trypanozoon* (11/75). Thus, *H. camelina* collected from Shurr had significantly

312 higher trypanosome-positive flies than those from Ngurunit ($\chi^2 = 6.23$, $P = 0.013$). However, *T.*

313 *congolense* was not detected in flies sampled from Shurr flies. No *H. camelina* flies were found

314 in Nanyuki (Table 1).

315 **3.2.2. Trypanosomes detected in *S. calcitrans***

316 Field-trapped *S. calcitrans* (n = 100), 50 from each of the two sampling sites in Marsabit (Fig.

317 2B) were analysed for the presence of trypanosomes; one fly from Ngurunit and four flies from

318 Shurr were positive for *Trypanozoon* in total (5%) and in 21 flies *T. vivax* DNA was detected,

319 eight from Ngurunit and 13 from Shurr site, in total, in 21% of flies. Similarly, more *S.*

320 *calcitrans* were positive with *T. vivax* as compared to *Trypanozoon* (P < 0.001, χ^2 test).

321 Approximately 2% of the *S. calcitrans* from Shurr were positive for mixed *Trypanozoon* and *T.*

322 *vivax* DNA (Figs. 3B and 4).

323

15

324 ### 3.2.3. Trypanosomes detected in *Tabanus* spp.

325 Field trapped *Tabanus* spp. (Fig. 2E and F) contained *T. vivax* DNA (5.4%; n = 50 flies) and

326 *Trypanozoon* (3.6%; n = 50 flies)*,* trypanosomes DNA detected relatively lower in *Tabanus spp.*

327 than in *H. camelina* and *S. calcitrans* (Figs. 3B and 4), and no significant difference was

328 observed between the number of *T. vivax* and *Trypanozoon* DNA detected in the analysed

329 *Tabanus spp.* flies (χ^2, $P > 0.05$).

330 ### 3.2.4. Trypanosomes detected in *P. rueppellii*

331 We also identified another potential trypanosome vector, *P. rueppellii* (Fig. 2C) with *T. vivax*

332 DNA in 17.4% of flies (n = 50), no other species of trypanosome was detected in *P. rueppellii*

333 (Figs. 3B and 4). However, no trypanosomes were identified in 50 *Hae. pluvialis,* Fig. 2D.

334 Among all biting flies analysed (except *Tabanus* spp.), *T. vivax* was the most abundant

335 trypanosome species, followed by *Trypanozoon* (Fig. 3B).

336
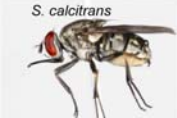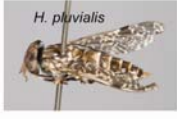
Biting flies	Trypanozoon	T. vivax	T. congolense	Mixed DNA	Shannon diversity index	sample size
H. camelina	9%	39%	3%	10%	1.1	150
S. calcitrans	5%	21%	-	2%	0.7	100
P. rueppellii	-	17.4%	-	-	0.0	50
Tabanus sp.	3.6%	5.4%	-	2%	0.7	50
H. pluvialis	-	-	-	-	0.0	50
G. pallidipes	7%	15%	7%	2%	1.4	101

337

338 **Fig. 4. Diversity and percent of trypanosome species in biting flies.** (-) indicates negative for

339 that specific trypanosome species.

16

340 **3.2.5. Trypanosomes detected in *G. pallidipes***

341 The trypanosomes diversity and prevalence were compared with freshly field-trapped

342 trypanosome biological vector, *G. pallidipes*, from Shimba Hills in coastal Kenya (Fig. 1A).

343 From 101 field-trapped *G. pallidipes* analysed, DNA of various trypanosomes were detected in

344 32%, 7.14% of which were *T. congolense* savannah (700 bp), 14.8% were *T. vivax* (250 bp), and

345 6.9% were *Trypanozoon* (500 bp). However, no significant differences in prevalence were

346 recorded for the three trypanosome species, namely *T. congolense*, *T. vivax*, and *Trypanozoon*

347 (either *T. brucei* or *T. evansi*) ($P = 0.08$). Similarly a low rates of mixed trypanosome DNA

348 (*T.congolense* and *Trypaanozoon*) in 1% and in 2 % of *G. pallidipes*. *T. vivax* and *Trypanozoon*

349 detected (Fig. 3C). Our results show that diverse non-tsetse hematophagous biting flies from the

350 tsetse-free area harbour similar trypanosomes detected in *G. pallidipes* (Fig. 3C). The diversity

351 of trypanosomes indicated by Shannon Index H varies between the different biting flies, *H.*

352 *camelina* and *G. pallidipes* harboured more diverse trypanosomes DNA (Figure 4).

353

354 **3.3. Trypanosome prevalence and diversity in camels**

355 **3.3.1. Parasitological examination using light microscopy**

356 Direct thin blood smears from the jugular vein showed an active infection of *Typanozoon* in 16

357 camels ($7.2 \pm 3.4\%$, 95% confidence intervals) of the sampled camels (n = 222). Trypanosomes

358 were morphologically identified as belonging to *Trypanozoon* subgenus based on their

359 morphology and motility. *T. vivax* is known to move fast but is less curved, whereas *T. brucei*

360 moves fast but straight across a microscope field [21]. Trypanosome infections in camels varied

361 in the parasite load from lowest parasitemia estimated at 10^6 to highest of 5.10^8

362 trypanosomes/mL blood (Figs 5A–B).

363 For further confirmation of the morphological identification, molecular analysis of

364 microscopically positive and negative samples was performed. Figure 5C combines selected

365 samples that were microscopically positive and those that were negative microscopically.

366 Morphologically, *Trypanozoon*-positive samples 25 (GenBank accession MH247163), 31

367 (GenBank accession MH247168), 42 (GenBank accession MH247174), and 47 (GenBank

368 accession MH247157) were also *Trypanozoon* positive by PCR and sequencing (Fig 5C) (for

369 details, see section 3.3.2). In Fig 5C, six of the microscopically negative samples (26, 32, 37, 38,

370 41, and 45) remained negative with PCR. However, 13 samples (27-30; 33, 34-36; 39, 40, 43, 44,

17

371   46) that were negative microscopically, were found to be *Trypanozoon* positive with PCR,

372   showing the sensitivity of PCR technique. A clear difference was observed in those

373   microscopically positive and negative samples, relatively sharp and intense PCR band in those

374   samples that were microscopically positive as compared to those microscopically negative (Fig

375   5C +). For further confirmation six microscopically *Trypanozoon* positive (GenBank accessions

376   MH247157, MH247162, MH247163, MH247168, MH247170, and MH247174) and 10

377   microscopically negative, but *Trypanozoon* positive by PCR (GenBank accession MH247155,

378   MH247158, MH247159, MH247160, MH247161, MH247166, MH247167, MH247169,

18

379   MH247173 and MH247177) were sequenced (for details, see section 3.3.2).

380
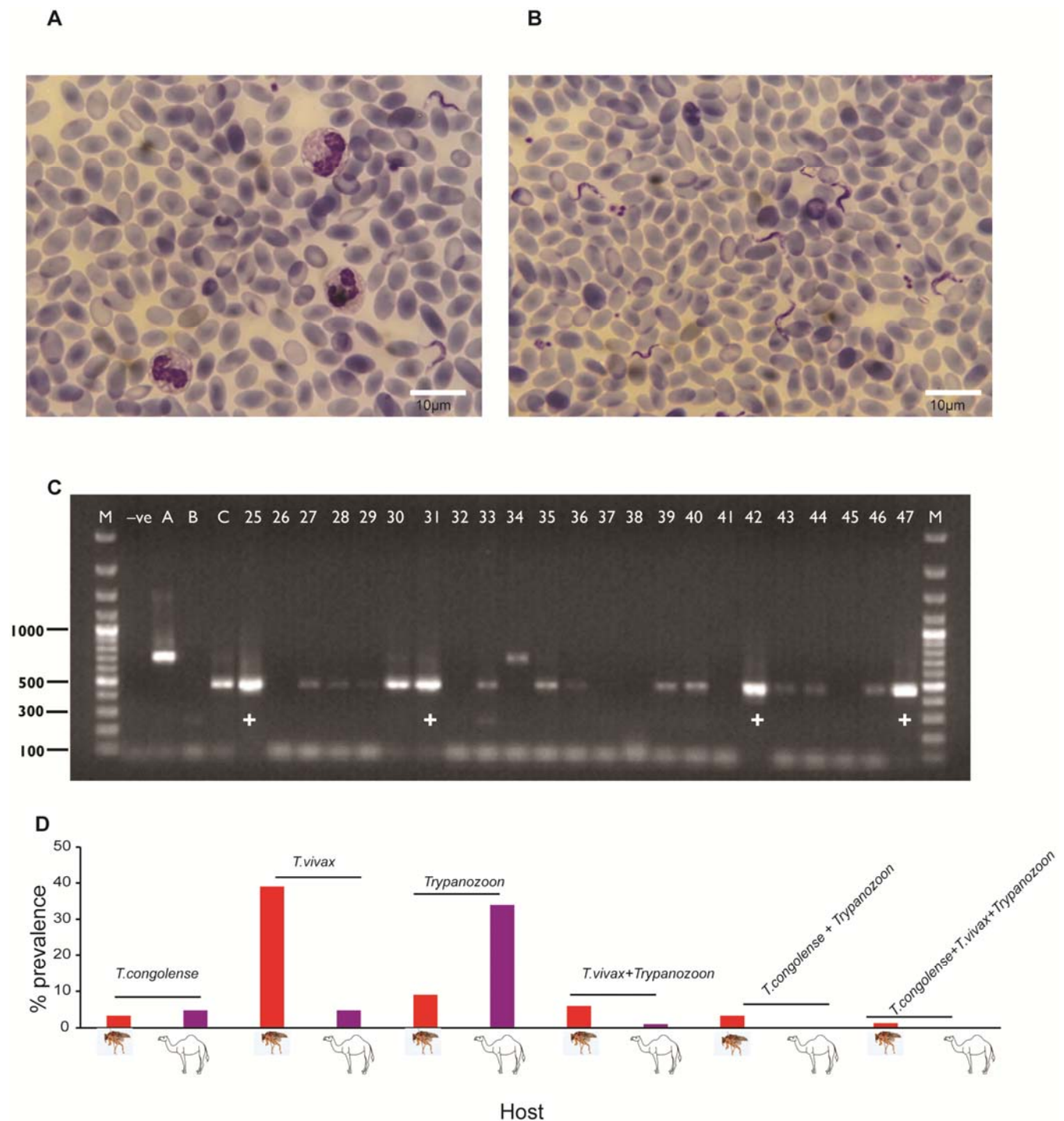
Fig. 5. Detection of trypanosomes in camels and *H. camelina*. (**A - B**) Representative light

micrographs of Giemsa-stained camel blood sample smears from two different camels showing

varying parasitemia (A) low and (B) high parasitemia, *T. evansi* (GenBank accessions

384   MH247174) with a small sub terminal kinetoplast at the pointed posterior end, a long free

385   flagellum and a well-developed undulating membrane. (C) Agarose gel electrophoresis (1.2 %)

386   performed on ITS1 PCR amplicons. Lane M: 100 bp marker; Lane '-ve': negative control (non-

387   template control); Lane A: *T. congolense* savannah IL3000; Lanes B: *T. vivax* IL2136; Lane C:

388   *T. evansi* KETRI 2479; Lanes 25-47: selected camel blood samples, + shows camel that was

389   trypanosome-positive by microscopy (D). Prevalence of trypanosome species in *H. camelina* and

390   camels.

391

3.3.2. PCR-based trypanosome detection in camels

393   Trypanosomal ITS-1 PCR amplification showed 34% (75/222) of the camels were infected with

394   *Trypanozoon*, which was more common than *T. vivax* infections (4.8%), with 250-bp PCR

395   products ($\chi^2 = 14.4$, $P < 0.001$), and *T. congolense* savannah infections (4.8%), identified by 700-

396   bp PCR amplicons. Mixed infections of *T. vivax* and *Trypanozoon* (*T. evansi* or/and *brucei*)

397   accounted for 1.4% of the 222 camel samples analysed from the three sites. For further

398   confirmation of size-based identification, we randomly selected 32 samples from PCR amplicons

399   from each trypanosome species, those with good quality DNA and required concentration, for

400   sequencing and those trypanosomes with known clinical symptoms. We sequenced 32 selected

401   samples from all trypanosome species for further confirmation. The PCR amplicons of 32

402   samples were successfully sequenced and obtained sequences that clustered with known *T. vivax*

403   or *T. evansi* and *T. brucei* isolates (Fig. 6). Five camels (GenBank accessions MH247140,

404   MH247142, MH247145, MH247147, MH247149) and two *H. camelina* (GenBank accession

405   MH247150, MH247152) were infected with *T. vivax* (100% nucleotide identity to GenBank

406   accession MK880189). Two camels, one *H. camelina*, and one *S. calcitrans* were infected with

407   trypanosomes (GenBank accessions MH247172, MH247174, MH247175, MH247177) sharing

408   >99% ITS-1 nucleotide sequence identity with *T. evansi* sequences. Twenty-four sequences

409   (GenBank accessions MH247139, MH247141, MH247146, MH247148, MH247151,

410   MH247153-MH247163, MH247165-MH247170, MH247173, MH247176) clustered with *T.*

411   *brucei*, sharing 99.3% identity with *T. brucei* (GenBank accession X05682), but also < 98%

412   identity with *T. evansi* reference sequences. Further attempts to amplify and sequence *T. brucei*-

413   specific kinetoplast maxicircle sequences, which are absent in *T. evansi*, confirmed four of the

414   samples, one from *G. pallidipes* and three camel samples, as harbouring *T. brucei* (GenBank

20

415   accessions MH247139, MH247159, MH247166, MH247167). We were unable to determine

416   conclusively whether the other 17 samples with trypanosome ITS-1 sequences clustering among

417   *T. brucei*, were *T. brucei* or *T. evansi* (Fig. 6).

418   Further characterization of the samples using the *T. evansi* type A-specific primers differentiated

419   the *T. evansi* in the *S. calcitrans* (GenBank accession MH247175) as *T. evansi* type A. Three of

420   the camel samples with ITS-1 sequences (GenBank accessions MH247157; MH247160 and

421   MH247169) that clustered among *T. brucei* also amplified using the *T. evansi* type A-specific

422   primers, indicating either that *T. brucei* and *T. evansi* can share identical ITS-1 sequences or

423   could be indicative of mixed infections with *T. brucei* and *T. evansi*. We failed to amplify *T.*

424   *evansi* type B from *Trypanozoon* positive samples, though we cannot rule out the complete

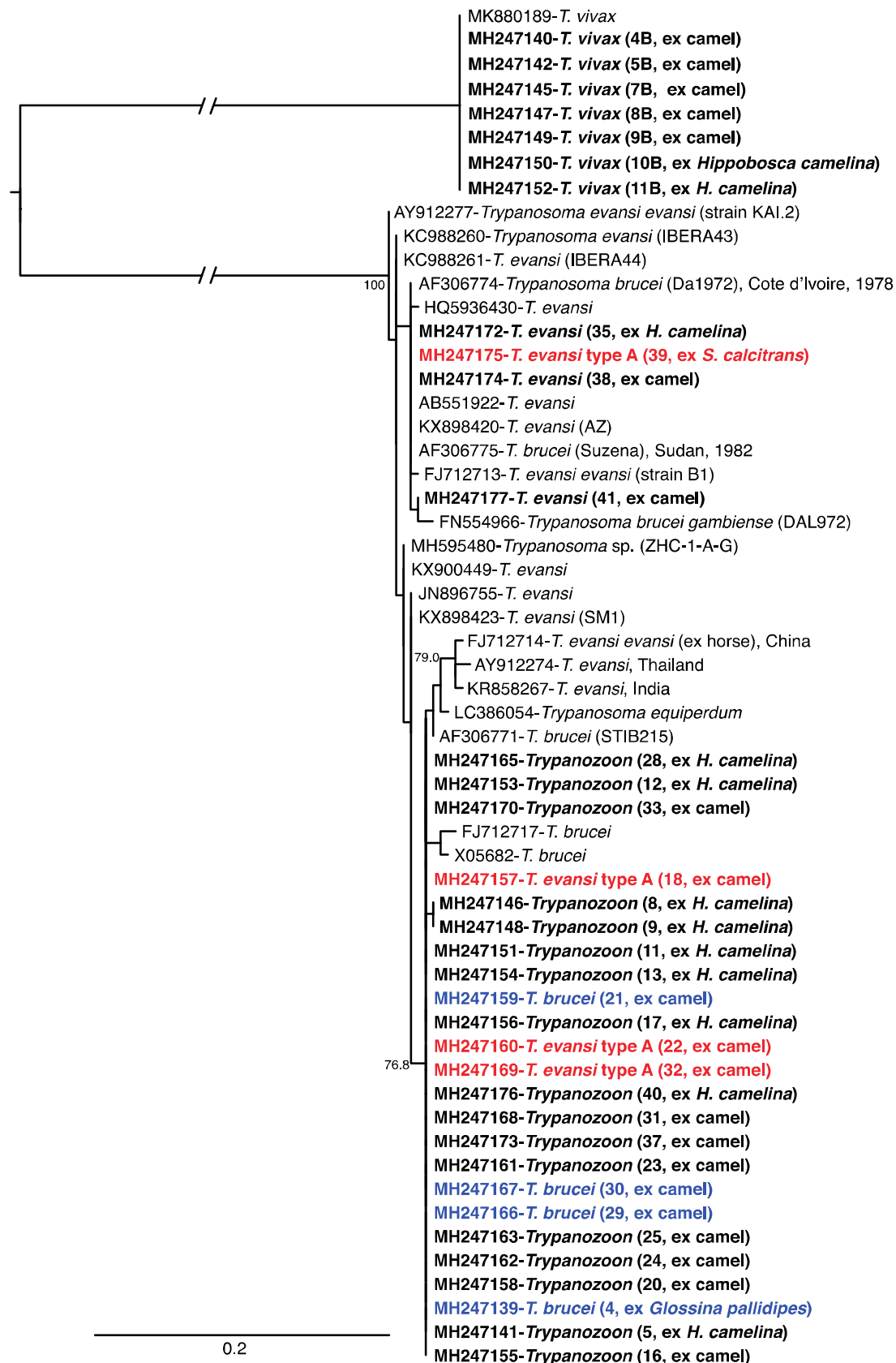425   absence of Type B as we only analysed a subset of the samples.

MK880189-*T. vivax*
MH247140-*T. vivax* (4B, ex camel)
MH247142-*T. vivax* (5B, ex camel)
MH247145-*T. vivax* (7B, ex camel)
MH247147-*T. vivax* (8B, ex camel)
MH247149-*T. vivax* (9B, ex camel)
MH247150-*T. vivax* (10B, ex *Hippobosca camelina*)
MH247152-*T. vivax* (11B, ex *H. camelina*)
AY912277-*Trypanosoma evansi evansi* (strain KAI.2)
KC988260-*Trypanosoma evansi* (IBERA43)
KC988261-*T. evansi* (IBERA44)
AF306774-*Trypanosoma brucei* (Da1972), Cote d'Ivoire, 1978
HQ5936430-*T. evansi*
MH247172-*T. evansi* (35, ex *H. camelina*)
MH247175-*T. evansi* type A (39, ex *S. calcitrans*)
MH247174-*T. evansi* (38, ex camel)
AB551922-*T. evansi*
KX898420-*T. evansi* (AZ)
AF306775-*T. brucei* (Suzena), Sudan, 1982
FJ712713-*T. evansi evansi* (strain B1)
MH247177-*T. evansi* (41, ex camel)
FN554966-*Trypanosoma brucei gambiense* (DAL972)
MH595480-*Trypanosoma* sp. (ZHC-1-A-G)
KX900449-*T. evansi*
JN896755-*T. evansi*
KX898423-*T. evansi* (SM1)
FJ712714-*T. evansi evansi* (ex horse), China
AY912274-*T. evansi*, Thailand
KR858267-*T. evansi*, India
LC386054-*Trypanosoma equiperdum*
AF306771-*T. brucei* (STIB215)
MH247165-*Trypanozoon* (28, ex *H. camelina*)
MH247153-*Trypanozoon* (12, ex *H. camelina*)
MH247170-*Trypanozoon* (33, ex camel)
FJ712717-*T. brucei*
X05682-*T. brucei*
MH247157-*T. evansi* type A (18, ex camel)
MH247146-*Trypanozoon* (8, ex *H. camelina*)
MH247148-*Trypanozoon* (9, ex *H. camelina*)
MH247151-*Trypanozoon* (11, ex *H. camelina*)
MH247154-*Trypanozoon* (13, ex *H. camelina*)
MH247159-*T. brucei* (21, ex camel)
MH247156-*Trypanozoon* (17, ex *H. camelina*)
MH247160-*T. evansi* type A (22, ex camel)
MH247169-*T. evansi* type A (32, ex camel)
MH247176-*Trypanozoon* (40, ex *H. camelina*)
MH247168-*Trypanozoon* (31, ex camel)
MH247173-*Trypanozoon* (37, ex camel)
MH247161-*Trypanozoon* (23, ex camel)
MH247167-*T. brucei* (30, ex camel)
MH247166-*T. brucei* (29, ex camel)
MH247163-*Trypanozoon* (25, ex camel)
MH247162-*Trypanozoon* (24, ex camel)
MH247158-*Trypanozoon* (20, ex camel)
MH247139-*T. brucei* (4, ex *Glossina pallidipes*)
MH247141-*Trypanozoon* (5, ex *H. camelina*)
MH247155-*Trypanozoon* (16, ex camel)

100

79.0

76.8

0.2

426

22

427 **Figure 6: Maximum likelihood phylogeny of trypanosome ITS-1 nucleotide sequences.**

428 GenBank accession numbers and isolation sources are indicated. Sequences from this study are

429 indicated in bold; trypanosomes were isolated from *G. pallidipes*, *S. calcitrans*, *H. camelina*, and

430 camels. Sequences associated with samples confirmed to harbour *T. evansi* type A by

431 amplification of the VDG RoTat 1.2 gene are highlighted in red. Sequences associated with

432 samples confirmed to harbour *T. brucei* by maxicircle kDNA amplification are highlighted in

433 blue. Bootstrap values at the major nodes represent agreement among 1000 replicates. The

434 branch length scale represents substitutions per site. Branch gaps in the mid-point root branches

435 represent 2.7 substitutions per site.

436 **3.3.4 Anaemia associated with *Trypanozoon* infection**

437 To determine the association between *Trypanozoon,* active infection and PCV, a measure of

438 anaemia, which is one of the consequences of trypanosome infection [20, 39] and a symptom of

439 surra, we compared the mean PCVs between camels with active *Trypanozoon* infection and those

440 that were negative by microscopy. *Trypanozoon* infection affected the PCV values significantly;

441 camels that had active *Trypanozoon* infection were anaemic with the mean PCV value of 24.64 ±

442 6; however, microscopically negative camels had a higher PCV value of 30.14 ± 6, t = 3.303, *P* =

443 0.002, n = 222).

444 **3.4. Diversity of trypanosomes in other domestic animals co-herded with camels**

445 We further analysed the prevalence of trypanosomes in other domestic animals such as goats,

446 sheep, donkeys, and cattle that are co-herded with camels using PCR technique. *Trypanozoon*

447 DNA was detected in small ruminants (goats and sheep), but not in cattle (Fig. 7). *Trypanosoma*

448 *vivax* was more prevalent in sheep as compared to *Trypanozoon* (χ^2 = 13.146, *P* = 0.0003, df =

449 1). In goats, *T. vivax* infections accounted for 8.2%, while the infection rate by *Trypanozoon* was

450 10.2% showing no significant difference (χ^2, *P* > 0.05). Cattle were infected with *T. vivax* (9.5%)

451 and *T. congolense* (2.4%) with no significant difference (Fig. 7). Furthermore, *T. vivax* was

452 detected in 14% of donkeys, but no other trypanosomes species were detected (Fig. 7). However,

453 all these livestock were negative microscopically.
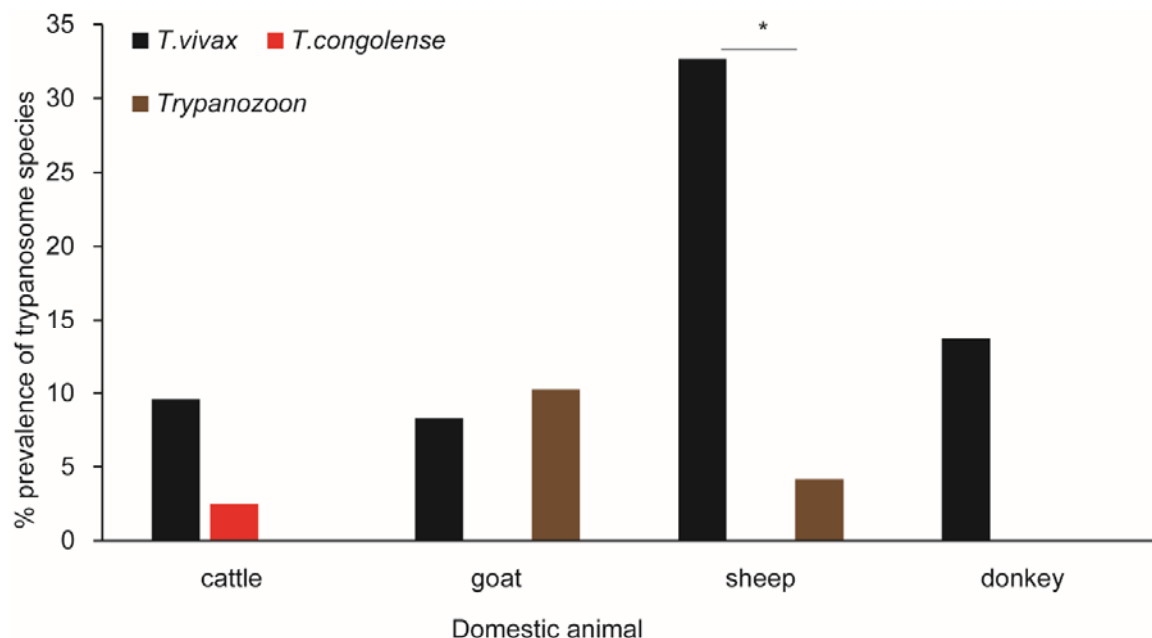
454

455

Fig. 7. Prevalence and diversity of trypanosomes in domestic animals co-herded with camels. Percent prevalence of the three trypanosomes species in four domestic animals that co-herded with camels based on PCR. Significant difference in prevalence between trypanosomes species are depicted by an asterix. An equal number of blood samples were collected from Ngurunit and Shurr for cattle, goats, and sheep, while donkeys were exclusively sampled from Shurr.

3.5. Identification of vertebrate hosts from bloodmeal analysis of *H. camelina* and *S. calcitrans*

3.5.1. Proportion of blood-meals taken by *H. camelina* from different hosts

The majority of *H. camelina* collected using traps had fed on domestic animals (95%, $\chi^2 =$, $P < 0.0001$). The camel host was the primary source of bloodmeals for 60% of the fed flies ($\chi^2 = 7.96$, $P = 0.005$), followed by goats (15%), sheep (14 %), and rats (4%) (Fig. 8A–B). Some of the flies fed on multiple vertebrate hosts; for instance, 1% of *H. camelina* fed on both human and sheep, 3% on camel and sheep, and 3% on goats and sheep. Each household kept diverse domestic animals (Fig. 8D). Sheep and goats were the most abundant, followed by camels, and relatively few cattle. The relative feeding index of *H. camelina* was calculated according to [37][38] as follow Wi = Oi/Pi, where, Wi = feeding ratio for livestock i, Oi = percentage of livestock i, in the blood meals, Pi = proportion or percentage of species i available in the

24

475   environment. We calculated the relative feeding preference of *H. camelina* to camel against

476   sheep, the most abundant livestock per household in Shurr. The average camel population per

477   household was 35, while the average sheep population per household was 223. The camel: sheep

478   abundance ratio was 35/223 = 0.157. 60% of *H. camelina* fed on camels, 15% fed on sheep, thus

479   the observed feeding rate was 60/15 = 4. Therefore, the feeding index was 25.5 (4/0.157) as

480   obtained from blood meal analysis of *H. camelina* shows a higher feeding preference on camels

481   than sheep.

482

483   **3.5.2. Proportion of blood meals taken by *S. calcitrans* from vertebrate hosts**

484   The bloodmeals of *S. calcitrans* included mainly cattle, camels, buffaloes, and goats (Fig. 8C).

485   The relative feeding index of *S. calictrans* was calculated using the formula $W_i = O_i/P_i$ described

486   earlier in section 3.5.1. About 15% of *S. calictrans* fed on camel, 7% flies fed on sheep, observed

487   feeding ratio 15/7= 2. Therefore, the feeding index of 12.7 (2/0.157) as obtained from blood

488   meal analysis of *S. calcitrans* shows higher feeding preference to camel rather than sheep.

489   However, *S. calcitrans* preferred to feed on cattle than camel. The camel: cattle abundance ratio

490   was: 5 (35/7), and, the ratio of the number of feeds by *S. calcitrans* on camel and cattle, 0.428

491   (15/35). Thus, the feeding index for camel was 0.428/5 = 0.09, showing the high preference to
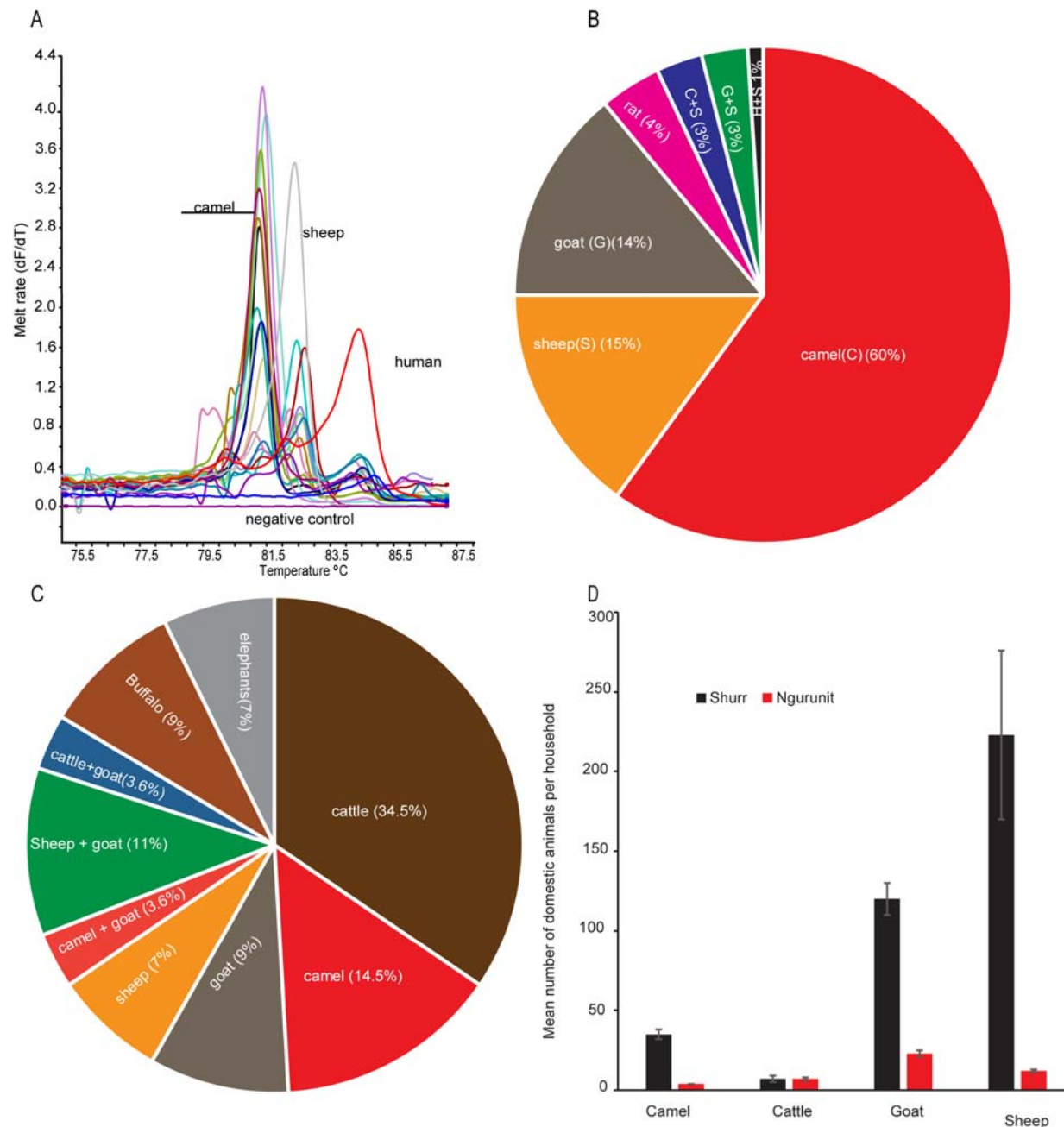
492   feed on cattle than camel.

493

Fig. 8. **Bloodmeal analysis in *H. camelina* and *S. calcitrans* to determine vertebrate hosts.**
(**A**) Representative HRM profiles of selected *H. camelina* bloodmeal sources analyzed using 16S rRNA gene target. Positive controls were included for comparison. (**B**) Percent of bloodmeals for *H. camelina* identified using the 16S rRNA primer (n = 100). (**C**) Percent of blood meals from various vertebrate in *S. calcitrans* identified using16S rRNA primer (n = 40). (**D**) Diversity and abundance of domestic animals in Shurr and Ngurunit. Error bar indicates standard error, n = 96 and 92 households from Shurr and Ngurunit, respectively.

26

4. Discussion

502 **4. Discussion**

503 Animal trypanosomosis is caused by several trypanosome species of genus *Trypanosoma* that are

504 transmitted cyclically by tsetse flies and mechanically by other hematophagous flies. Since there

505 were no tsetse flies in the study sites, other blood-sucking insects identified, such as *H. camelina*

506 and stable flies, could serve as mechanical vectors that maintain trypanosomes in circulation

507 among livestock. One of the factors that influences the mechanical transmission of trypanosomes

508 by biting flies is the survival of the pathogen in the proboscis and midgut. Previous reports

509 indicate that *T. evansi* can survive in the midgut of *H. camelina* for 75–90 minutes [15]. With our

510 parasite establishment experiment *T. congolense* survived for 3 hrs and trypanozoon for 5 hr in

511 the mid gut of *S. calcitrans*. Similarly, Sumba et al., 1998 [40] demonstrated that *T. congolense*

512 survived for 3 and half hours and *T. evansi* for 8 hours in the gut of *Stomoxys niger* and *S.*

513 *taeniatus*, showing their might be vectoral competence variation between *Stomoxys* species that

514 need to be investigated**.** To determine how long it takes for *H. camelina* to find the next camel

515 host when it is interrupted from feeding, we studied its mobility using a mark–release–recapture

516 method by displacing them from the hosts. These flies were able to re-locate camels in ~60

517 minutes when translocated up to 1.5 Km away, which is within the time range of the parasite

518 survival (SF2). Similarly, various *Stomoxys species,* which can transmit the pathogen at different

519 time intervals and have long-distance mobility [13, 41].

520 The high number of *H. camelina* and *S. calcitrans,* their occurrence throughout the year,

521 and the diversity of trypanosomes detected makes these two biting flies the most important

522 potential vectors of trypanosome in tsetse-free ecologies. Furthermore, these two biting flies fed

523 preferably on camels, but also on other hosts such as cattle and goats. However, the role of other

524 biting flies identified, such as *Tabanus* spp. and *Pangonia* spp.*,* should not be underestimated

525 [13, 40, 42, 43]. These biting flies harbour similar trypanosome species as *G. pallidipes,* one of

526 the major tsetse species in sub-Saharan Africa, due to its wider geographical distribution and

527 economic importance [44–46]. The detection of diverse trypanosomes in non-tsetse infested

528 areas shows that trypanosome diversity is conserved across a broader biogeography that includes

529 tsetse-free regions.

530 The low percentage of the camels identified with active infection might be due to the low

531 sensitivity of the microscopic technique [39, 47]. However, the molecular data showed that a

532 significant number of camels were infected by trypanosomes, indicating that they might have

27

533   subclinical infections. Thus, there is a possibility that sub-clinically infected camels can serve as

534   a reservoir of trypanosomes. We also documented infected camels presenting with high

535   parasitemia (Fig. 5B GenBank accession MH247157), which is required for mechanical

536   transmission by biting flies [48]. Another reason for not detecting trypanosomes in blood might

537   be due to trypanosomes moving to other tissues; for example, the skin is an anatomical reservoir

538   of parasites of arthropod-borne diseases, such as trypanosomes [49]. Thus, we checked the

539   lymph node aspirate (LNA) from a few sick camels with clear clinical signs of trypanosomosis,

540   but with negative results during blood examination. We found that camel blood samples could

541   test negative for trypanosome infection through microscopy and PCR but be positive in LNA

542   (SF3 A-B). This finding agrees with previous reports that showed the presence of trypanosomes

543   in LNA, but absence in the blood of cattle and sheep [50].

544   Camels that showed active infection microscopically were anaemic with average PCVs

545   below 25, as compared to those microscopically negative camels with an average PCV of 30.

546   With regards to different clinical outcomes, we have seen severely anaemic haemorrhagic camels

547   that were infected by *T. vivax* (GenBank accessions MH247149) (PCV 9%) or by *T. evansi* type

548   A (GenBank accessions MH247169) (PCV 11%). However, other camels with high *T. evansi*

549   parasitaemia (millions of trypanosomes/mL of blood) were feeding well and in good body

550   condition (PCV 21%) (GenBank accessions MH247174), which indicates that there could be

551   different *T. evansi* populations as reflected in varying degrees of virulence to camels, that need

552   further investigation. However, virulence also depends on the immune status of the host, past and

553   recent infection by complex pathogens, as well as the genetic makeup of individual hosts.

554   Similarly, *T. vivax*-infected camels with as low as 9% PCV have been reported previously[39]

555   We sequenced the trypanosome ITS-1 gene, which is the preferred target for species-

556   specific molecular diagnostics of trypanosomes [23, 47]. The ITS-1 sequences produced distinct

557   clusters for *T. vivax,* and *Trypanozoon* (Fig. 6). *Trypanosoma vivax* was the most prevalent

558   trypanosome followed by *Trypanozoon*, both in flies and domestic animals, but not in camels, in

559   which *Trypanozoon* was most common. We further detected maxicircle kDNA [25] in our

560   *Trypanozoon* samples, confirming the presence of *T. brucei* in *G. pallidipes* and three camel

561   samples. Additionally, we characterized *T. evansi* type A by the presence of the type A-specific

562   marker targeting the RoTat 1.2 VSG gene, but we failed to detect *T. evansi* type B. The absence

563   of *T. evansi* type B could be due to diagnostic limitations or the small sample size analysed, but

28

564   these findings are congruent with previous findings of low occurrence of the *T. evansi* B subtype

565   in camels [27, 28, 51]. However, for 20 of the *Trypanozoon* ITS-1 sequences obtained, we were

566   not able to determine conclusively whether they were of *T. brucei* or *T. evansi,* showing that the

567   ITS-1 gene cannot be used to effectively differentiate between these species. This challenge of

568   *Trypanozoon* subgenus identification and the need for more specific and sensitive diagnostics is

569   discussed by [52].

570   The detection of *T. brucei* and *T. congolense* both in biting flies and camel in the tsetse-free

571   areas of northern Kenya might suggest the long-distance travelling of camels and other domestic

572   animals between tsetse-free and the tsetse-infested areas, as some of the neighbouring counties

573   are infested with tsetse flies. Similarly, previous studies detected *T. brucei* from camel in tsetse

574   free area in northern Kenya [53][54]. To support this claim, a study done in North Eastern Kenya

575   showed that animals move more than 120 Km from their homestead for better pasture and water,

576   and infection of Rift Valley fever increases in herds that move than in those that remain at the

577   homestead [55]. The other possibility is the presence of unidentified biological vectors, which

578   will require further investigation, combined with vectorial capacity study of the identified biting

579   flies and population genetics of trypanosomes.

580   **5. Conclusions**

581   The detection of diverse trypanosomes species/strains both in various biting flies and in camels

582   suggests that trypanosomosis in camels is not only due to surra (*T. evansi* infection), but also

583   nagana (*T. brucei*, *T. congolense*, and *T. vivax* infections). Such knowledge helps in drug

584   administration because one could tailor the treatment to each trypanosome species [56].

585   Furthermore, our analysis shows that other domestic animals could serve as a reservoir of

586   different trypanosomes, such as *T. vivax* and *T. congolense*, which are more deadly to camels [5,

587   9, 57]. Finally, the similarity of pathogens found in biting flies and their domestic animal blood-

588   meal hosts demonstrates that these hematophagous flies could be used for xenomonitoring to

589   track trypanosomes circulating in domestic animals as an early detection method.

590   **List of abbreviations**

591   HRM: High-resolution melting; *icipe*: International Centre of Insect Physiology and Ecology;

592   ITS1: Intergenic transcribed spacer gene 1; LNA: Lymph node aspirate; PCV: Packed Cell

593   Volume; PCR: Polymerase chain reaction

594   **Declarations**

595   **Acknowledgements**

596   We thank Dr Robert Copeland for helping to identify the biting flies and taking the biting flies

597   photo; Tiberius Marete, David Mbuvi, Peter Muasa and Irene Onyango for technical help;

598   Kimathi, Emily and Barbara Kagima, for the study site map; Kelvin Muteru for his help in the

599   bioinformatics; James Kabii for his technical help in the molecular studies, Collins Kigen,

600   JohnMark, and Tawich K. Simon for technical help in the parasite survival experiments in *S.*

601   *calcitrans* and Caroline Muya for handling the administrative issues. Special thanks go to Daud

602   Tamasot (MCA), Malkash Lolkitarakino and Huka Guyo Qutte, Hussein Haji Abdulahi who

603   facilitated our research at Shurr, Nanyuki and Ngurunit area. We thank Dr Mario Younan for his

604   valuable technical advice on camels. We are grateful for the camel owners of Ngurunit, Nanyuki,

605   and Shurr for their cooperation.

606   **Funding**

621

Availability of data and materials

All data generated or analysed in this study are included in the article and as additional files. The newly generated sequences were deposited in the NCBI Nucleotide database under the accession numbers listed in Supplementary table.

Authors' contributions

MNG, BT, DM, SR conceptualized and designed the experiments. MNG, JLB, AO, POA, JN, JMM generated experimental data, JV contributed in the molecular part of the study MNG analysed the data and wrote the manuscript. All authors reviewed, edited and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests

Ethics approval and consent to participate

We collected blood samples within the framework of epidemiological surveillance activities, in accordance to the International Centre of Insect Physiology and Ecology*'s* Institutional Animal Care and Use Committee (IACUC) guidelines as performed during prophylaxis or diagnostic campaigns. Local authorities did not require ethical statements for the research studies. We did the blood sampling of domestic animals with the authorisation of the owner. Herdsmen/women gave their consent for their animal sampling after explaining the objectives of the study. No samples other than those for routine screening and diagnostic procedures were collected. All animals sampled and found positive with trypanosomes were treated using trypanocides.

Consent for publication

Not applicable.

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792   **Additional file**

793   Additional file 1: Figure S1. PCR products were resolved 1% ethidium-bromide stained agarose

794   gel (8V for 1.5 hrs) to check for any contamination. The DNA isolated from whole fly was

795   amplified targeting trypanosomal ITS1 gene. Lane: M 10- bp marker, Bf (reaction buffer), wt

796   (PCR water), TB (*T. brucei* ILTat 1.4) TV (*T. vivax* IL 2136), TC (*T. congolense* savannah

797   (IL3000)), and TE (*T. evansi* KETRI 2479), F1- F10 DNA sample from *H. camelina* flies. The

798   absence of PCR product under Bf, and wt show no contamination from extraction buffer.

799   Additional file 2: Figure S2, Number of *H.camelina* recaptured at the specified distance from

800   pint of release. Number in parentesis shows percentage of flies recaptured.

801   Additional file 3: Figure S3. (A) PCR products were resolved 1% ethidium-bromide stained

802   agarose gel (8V for 1.5 hrs) to check for trypanosomes in blood and lymph node aspirate. The

803   DNA isolated from blood and lymph node aspirate was amplified targeting trypanosomal ITS1

804   gene. Lane: M 10- bp marker, -Ve (reaction buffer), TE (*T. evansi* KETRI 2479) TV (*T. vivax* IL

805   2136), TC (*T. congolense* savannah (IL3000)), and LN_C1, LN_C2, DNA sample from two

806   camels lymph node aspirate, B_C1 and B_C2 DNA from corresponding blood samples from the

807   same camel. The result shows both samples of the lymph node aspirate were positive, while

808   blood samples were negative from the same camel. (B) Five camels blood and lymph node

809   aspirate were analysed, only camel five lymph node aspirate was positive for *T.vivax* but blood

810   sample from the same camel was negative.

811   Additional File 2. Supplementary Table 1. Trypanosomes identified based on ITS1 gene

812   sequence from different host included in Fig.6.