

1 **Host competence of African rodents *Arvicanthis neumanni*,**  
2 ***A. niloticus* and *Mastomys natalensis* for *Leishmania major*.**

3 Jovana Sadlova<sup>a\*</sup>, Barbora Vojtkova<sup>a</sup>, Katerina Hrnairova<sup>a</sup>, Tereza  
4 Lestinova<sup>a</sup>, Tatiana Spitzova<sup>a</sup>, Tomas Becvar<sup>a</sup>, Jan Votypka , Paul  
5 Bates<sup>b</sup> and Petr Volf<sup>a</sup>

6  
7 <sup>a</sup> Department of Parasitology, Faculty of Science, Charles University,  
8 Prague, Czech Republic

9  
10 <sup>b</sup> Division of Biomedical and Life Sciences, Faculty of Health and  
11 Medicine, Lancaster University, Lancaster, United Kingdom

12  
13  
14 \* corresponding author, [sadlovaj@natur.cuni.cz](mailto:sadlovaj@natur.cuni.cz)

15

## 16 **Abstract**

17 Cutaneous leishmaniasis caused by *Leishmania major* is a typical zoonosis circulating in  
18 rodents. In Sub-Saharan Africa the reservoirs remain to be identified, although *L. major* has  
19 been detected in several rodent species including members of the genera *Arvicanthis* and  
20 *Mastomys*. However, differentiation of true reservoir hosts from incidental hosts requires in-  
21 depth studies both in the field and in the laboratory, with the best method for testing the  
22 infectiousness of hosts to biting vectors being xenodiagnosis.

23 Here we studied experimental infections of three *L. major* strains in *Arvicanthis*  
24 *neumanni*, *A. niloticus* and *Mastomys natalensis*; the infections were initiated either with  
25 sand fly-derived or with culture-derived *Leishmania*. Inoculated rodents were monitored for  
26 several months and tested by xenodiagnoses for their infectiousness to *Phlebotomus*  
27 *duboscqi*, the natural vector of *L. major* in Sub-Saharan Africa. The distribution and load of  
28 parasites were determined *post mortem* using qPCR from the blood, skin and viscera  
29 samples. The attractiveness of *Arvicanthis* and *Mastomys* to *P. duboscqi* was tested by pair-  
30 wise comparisons.

31 Three different *L. major* strains used significantly differed in infectivity: the Middle  
32 Eastern strain Friedlin infected a low proportion of rodents, while two Sub-Saharan isolates  
33 from Senegal (LV109, LV110) infected a high percentage of animals and LV 110 also  
34 produced higher parasite loads in all host species. All three rodent species maintained  
35 parasites of the LV109 strain for 20-25 weeks and were able to infect *P. duboscqi* without  
36 apparent health complications: infected animals showed only temporary swellings or  
37 changes of pigmentation on the site of inoculation. However, the higher infection rates,  
38 more generalized distribution of parasites and longer infectiousness period to sand flies in

39 *M. natalensis* suggest that this species plays the more important reservoir role in the life  
40 cycle of *L. major* in Sub-Saharan Africa. *Arvicanthis* species may serve as potential reservoirs  
41 in seasons/periods of low abundance of *Mastomys*.

42 **Key words:** wild reservoir, xenodiagnosis, Grass Rats, Multimammate Mice,  
43 leishmaniasis, *Arvicanthis*, *Mastomys*

## 44 **1. Introduction**

45 *Leishmania* (Kinetoplastida: Trypanosomatidae) are parasites with a digenetic life cycle,  
46 alternating between blood feeding insects - sand flies (Diptera: Psychodidae) and  
47 mammalian hosts including humans. *Leishmania major* is a causative agent of human  
48 cutaneous leishmaniasis (CL) affecting millions of people in the Old World. It is transmitted  
49 by sand flies of the genus *Phlebotomus*. Proven vectors are *P. papatasi*, a species with wide  
50 distribution from North Africa and Southern Europe to India, and *P. duboscqi*, a species  
51 occurring in a wide belt through the Sub-Saharan Africa ranging from Senegal and  
52 Mauritania in the west to Ethiopia and Kenya in the east (Maroli 2013).

53 CL caused by *L. major* is a typical zoonosis maintained in reservoir rodent hosts.  
54 Humans are infected incidentally; lesions appear at the site of insect bite and cure without  
55 treatment after about three months. The short duration of the disease precludes survival of  
56 the parasite in humans through any non-transmission season (Ashford 2000). Proven  
57 reservoir hosts are the Fat Sand-Rat *Psammomys obesus* and gerbils of the genus *Meriones*  
58 in North Africa and the Middle East, and the Great Gerbil *Rhombomys opimus* in Central  
59 Asia. On the other hand, reservoir rodent species in Sub-Saharan Africa remains to be  
60 confirmed. *Leishmania major* has been isolated from several rodent species in this region;  
61 most isolates have been made from Grass Rats *Arvicanthis* spp. and Multimammate Mice  
62 *Mastomys* spp. which live in immediate vicinity of humans, and are the most dominant  
63 rodents in many Sub-Saharan African endemic localities of CL (reviewed by Ashford 1996,  
64 Ashford 2000, Desjeux 1996). *Arvicanthis* and *Mastomys* belong to the same large subfamily  
65 Murinae, but are separated in different tribes – Arvicanthini and Praomyini, respectively  
66 (Lecompte et al. 2008). The origin of both tribes was estimated to about 10. 2 Mya. Recently,

67 the genus *Arvicanthis* was reported to include seven species and the genus *Mastomys* eight  
68 species (Granjon and Ducroz 2013, Leirs 2013).

69 Identification of reservoir hosts is essential for the control of zoonoses. However, it  
70 requires longitudinal in-depth studies both in the field and in the laboratory. True reservoir  
71 hosts must satisfy many parameters - the most important being longevity sufficient to  
72 provide a habitat for the parasite during a non-transmission season, high population density  
73 of the host, and the location of the parasite within the host suitable for transmission. In  
74 addition, the infection is likely to be too benign (or too infrequent) to have any regulatory  
75 effect on host population (Ashford 1997, 2000). Finding PCR positive animals does not  
76 necessarily mean they serve as parasite reservoirs for biting sand flies (Silva et al. 2005).  
77 Indeed, such animals may simply serve as parasite sinks, i.e. animals upon which infected  
78 sand flies feed but do not contribute to vector infection and transmission to the next host  
79 (Chaves et al. 2007). The best method for testing the infectiousness of hosts to biting vectors  
80 is by xenodiagnosis, i.e., feeding of laboratory reared insects on the infected host with  
81 subsequent examination of the insects for presence of parasites.

82 The main aim of this laboratory study was to contribute to analysis of the host  
83 competence of the African rodents *Arvicanthis neumanni* (Neumann's Grass Rat), *A. niloticus*  
84 (Nile Grass Rat) and *Mastomys natalensis* (Natal Multimammate Mouse) for *L. major*.  
85 *Arvicanthis neumanni* is the smallest *Arvicanthis* species, ranging from Ethiopia to Kenya; *A.*  
86 *niloticus* is widespread from the Nile Delta to Kenya and West Africa and *Mastomys*  
87 *natalensis* widely distributed in almost all Sub-Saharan Africa throughout many biotic zones  
88 (Granjon and Ducroz 2013, Leirs 2013). Their response to the infection and ability to present  
89 the parasites to feeding sand flies were tested using experimental infections and

90 xenodiagnoses. Feeding rates of *P. duboscqi* on these rodents were tested by host-choice  
91 experiments.

92

## 93 **2. Material and Methods**

### 94 **2.1. Sand flies, parasites and rodents.**

95 The colony of *P. duboscqi* was maintained in the insectary of the Department of Parasitology,  
96 Charles University in Prague, under standard conditions (26°C on 50 % sucrose and 14 h  
97 light/10 h dark photoperiod) as described previously (Volf and Volfova 2011).

98         Three *L. major* strains were used: MHOM/IL/81/Friedlin, a human isolate from  
99 Israel, and two strains isolated in Senegal by Ranque - MARV/SN/XX/RV24;LV109 and  
100 MHOM/SN/XX/BO-DK;LV110. The identity of the Senegalese strains was confirmed by  
101 sequencing of the RPL23a intergenic sequence (Dougall et al., 2013). Promastigotes were  
102 cultured in M199 medium (Sigma) containing 10% heat-inactivated fetal bovine calf serum  
103 (FBS, Gibco) supplemented with 1% BME vitamins (Basal Medium Eagle, Sigma), 2% sterile  
104 urine and 250 µg/mL amikacin (Amikin, Bristol-Myers Squibb).

105         Breeding colonies of *A. neumanni* and *A. niloticus* (originating from Prague Zoo and  
106 Pilsen Zoo, respectively) and *M. natalensis* (originating from a commercial source, Karel  
107 Kapral s.r.o.) were established in the animal facility of the Department of Parasitology.  
108 BALB/c mice originated from AnLab s.r.o. Animals were maintained in T IV breeding  
109 containers (Velaz) equipped with bedding German Horse Span (Pferde), breeding material  
110 (Woodwool) and hay (Krmne smesi Kvidera), provided with a standard feed mixture ST-1  
111 (Velaz) and water ad libitum, with a 12 h light/12 h dark photoperiod, temperature 22-25°C  
112 and humidity 40-60%.

113

## 114 **2.2. Experimental infection of sand flies.**

115 Promastigotes from log-phase cultures (day 3-4 post inoculation) were washed twice in  
116 saline and resuspended in heat-inactivated rabbit blood (LabMediaServis) at a concentration  
117 of  $5 \times 10^6$  promastigotes/ml. Sand fly females (5-9 days old) were infected by feeding  
118 through a chick-skin membrane (BIOPHARM) on the promastigote-containing suspension.  
119 Engorged sand flies were maintained under the same conditions as the colony.

120

## 121 **2.3. Infections of rodents**

122 Two methods of rodent infections were used – infections initiated with sand fly-derived  
123 *Leishmania* according to Sadlova et al. (2015) and infections initiated with culture-derived  
124 promastigotes. For the first method, *P. duboscqi* females experimentally infected with *L.*  
125 *major* (for details see above) were dissected on day 10 or 12 post bloodmeal (PBM); their  
126 midguts were checked microscopically for the presence of promastigotes, and thoracic  
127 midguts (the site of accumulation of metacyclic forms) with a good density of parasites were  
128 pooled in sterile saline. Pools of 100 freshly dissected thoracic midguts were homogenized in  
129 50  $\mu$ l of saline.

130 For inoculation of rodents with culture-derived promastigotes, stationary-phase  
131 promastigotes (day 7 post inoculation) were washed twice in saline and counted using a  
132 Burker apparatus. Pools of  $10^8$  promastigotes were resuspended in 50  $\mu$ l of saline.  
133 Dissected salivary glands of *P. duboscqi* females (SG) were pooled in sterile saline (10 glands  
134 per 5  $\mu$ l of saline) and stored at -20°C. Prior to mice inoculation, SG were disintegrated by 3  
135 successive immersions into liquid nitrogen and added to the parasite suspension.

136 Rodents anaesthetized with ketamin/xylazine (33 mg and 13 mg/kg in *A. neumanni*,  
137 62 mg and 7 mg/kg in *A. niloticus*, 50 mg and 20 mg/kg in *M. natalensis*, 62 mg and 25  
138 mg/kg in mice, respectively) were injected with 5.5 µl of the mixed parasite and SG  
139 suspension intradermally into the ear pinnae. Therefore, the inoculum dose per one animal  
140 with culture-derived promastigotes comprised  $10^7$  parasites. Exact numbers of sand fly –  
141 derived parasites stages were calculated using a Burker apparatus, and the proportions of  
142 metacyclic forms were identified on Giemsa stained smears based on morphological criteria  
143 described previously (Sadlova et al. 2010). The inoculum dose in sand fly-derived parasites  
144 was  $3.6 \times 10^4$  with LV 110 strain (35% of metacyclic forms) and ranged between  $3.5 \times 10^4$  -  $7 \times$   
145  $10^4$  parasites/rodent with FVI strain (23-69% of metacyclic forms) and  $4.1 \times 10^4$  -  $5.4 \times 10^4$  with  
146 LV109 strain (43 – 68% of metacyclic forms). Animals were checked weekly for external signs  
147 of the disease until week 20-35 post infection (p.i.) when they were sacrificed.

148

## 149 **2.4. Xenodiagnosis**

150 Five to seven-day-old *P. duboscqi* females were allowed to feed on the site of inoculation of  
151 *L. major* (ear pinnae) of anaesthetized rodents (using ketamin/xylazine) between weeks 2  
152 and 25 p.i. Smaller size rodents *M. natalensis* and *A. neumanni* were covered with the cotton  
153 bag, so that only the left ear pinnae were accessible to sand flies, placed into a small cage  
154 (20 x 20 x 20 cm) and 40-70 sand fly females were allowed to feed for one hour. In the larger  
155 sized *A. niloticus*, the xenodiagnoses were made using small plastic tubes with 30 sand fly  
156 females covered with fine mesh. The tubes were held on the ear of the anaesthetized animal  
157 for one hour (Fig 1A). Fed sand fly females were separated and maintained at 26°C on 50%  
158 sucrose. On day 7-10 PBM, females were dissected and their guts examined under the light

159 microscope. Intensities and locations of infections were evaluated as described previously  
160 (Sadlova et al. 2010).

161

## 162 **2.5. Tissue sampling and quantitative PCR**

163 Rodents were sacrificed at different weeks p.i by injecting them with an overdose of  
164 ketamin/xylazine anesthesia. Both ears (inoculated and contralateral), both ear-draining  
165 lymph nodes, spleen, liver, paws and tail were stored at -20°C for qPCR. Extraction of total  
166 DNA from rodent tissues and sand flies was performed using a DNA tissue isolation kit  
167 (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's instructions.

168 Quantitative PCR (Q-PCR) for detection and quantification of *Leishmania* parasites was  
169 performed in a Bio-Rad iCycler & iQ Real-Time PCR Systems using the SYBR Green detection  
170 method (iQ SYBR Green Supermix, Bio-Rad, Hercules, CA) as described (Sadlova et al. 2010).

171

## 172 **2.6. Host choice experiments and assessment of mortality and**

### 173 **fecundity of sand flies fed on different hosts.**

174 Pair-wise comparisons between two types of host were performed using a row of three  
175 connected small cages (20 x 20 cm). *P. duboscqi* females (200 specimens) were placed into  
176 the central cage and left for habituation for 20 minutes. Anaesthetized animals were placed  
177 in each of the lateral cages and partitions with the central cage were opened. After one  
178 hour, the cages were separated and closed, host animals removed and the numbers of  
179 blood-fed sand flies in each host cage was counted. *Arvicanthis neumanni* and *M. natalensis*  
180 are species of comparable size (60-80 g) and therefore one animal each was placed in cages.  
181 For comparison between mice and *Arvicanthis* or *Mastomys*, two mice were used against

182 one *Arvicanthis* or *Mastomys* to counterbalance weight differences between these host  
183 types. Each pair of different hosts was tested four times, with the hosts alternated between  
184 lateral cages in each repeat. Experiments were conducted in darkness at 24-26°C.

185 Fed females were maintained in the same conditions as the colony and their  
186 mortality was recorded for 4 days post-feeding. Then, females were introduced individually  
187 into small glass vials equipped with wet filter papers, closed with fine gauze and allowed to  
188 oviposit (Killick-Kendrick and Killick-Kendrick 1991). Small pieces of cotton wool soaked in  
189 sugar solution (50% sucrose) were placed on the mesh and changed every second day. All  
190 vials were placed into a single plastic box with its base filled with the wet filter paper to  
191 ensure a uniform microclimate. The humidity was checked and numbers of laid eggs were  
192 recorded daily.

193

## 194 **2.8. Animal experimentation guidelines**

195 Animals were maintained and handled in the animal facility of Charles University in Prague in  
196 accordance with institutional guidelines and Czech legislation (Act No. 246/1992 and  
197 359/2012 coll. on Protection of Animals against Cruelty in present statutes at large), which  
198 complies with all relevant European Union and international guidelines for experimental  
199 animals. All the experiments were approved by the Committee on the Ethics of Laboratory  
200 Experiments of the Charles University in Prague and were performed under permission no.  
201 MSMT-10270/2015-5 of the Ministry of the Environment of the Czech Republic. Investigators  
202 are certificated for experimentation with animals by the Ministry of Agriculture of the Czech  
203 Republic.

204

## 205 **3. Results**

### 206 **3.1. Experimental infections and xenodiagnosis with *A. neumanni*.**

207 In total, 33 females of *A. neumanni* were infected by three different *L. major* strains, most of  
208 them (30) using sand fly-derived *Leishmania*. The strain Friedlin originating from the Middle  
209 East showed only very weak infectivity for *A. neumanni* (Table 1). None of 12 female *A.*  
210 *neumanni* inoculated with sand fly-derived *Leishmania* developed lesions. Q-PCR revealed  
211 presence of *Leishmania* in 1 specimen only, with parasites localized in the inoculated ear  
212 pinnae in very low numbers (less than 100). All 532 *P. duboscqi* females used in various time  
213 intervals p.i. for xenodiagnoses were negative (Table 2).

214 The Sub-Saharan strain LV110 originating from Senegal infected all six female *A.*  
215 *neumanni* inoculated with sand fly-derived *Leishmania* (Table 1), but animals did not show  
216 any external signs of the disease throughout the entire experiment. Q-PCR revealed the  
217 presence of parasites in left ear pinnae (site of inoculation) in all the six animals, however,  
218 the numbers of parasites were very low and all 442 females *P. duboscqi* used for  
219 xenodiagnoses were negative (Table 2).

220 The second Sub-Saharan strain LV109 originating from Senegal was inoculated into  
221 15 *A. neumanni* (Table 1); 12 with sand fly-derived *Leishmania* (experimental groups A and  
222 B) and 3 with culture-derived promastigotes (experimental group C). Wet skin lesions did not  
223 develop, but hyper-pigmentations of left ear pinnae (site of inoculation) were observed in 3  
224 animals, two from the group A and one from the group C (Fig 1B). PCR showed presence of  
225 parasites in 7 from 15 animals. They were localized mostly in the left ear (site of inoculation)  
226 and once in the blood. Interestingly, the numbers of detected *Leishmania* were higher  
227 (hundreds to thousands) in 3 animals, two of which also showed hyper-pigmentation of the

228 ear. All three animals with hyperpigmentation were infective to sand flies, two by week 5  
229 and the third by week 10 p.i. In total, 0.4 % of 748 *P. duboscqi* females tested were positive  
230 (Table 2).

231

### 232 **3.2. Experimental infections and xenodiagnosis with *A. niloticus*.**

233 Twelve *A. niloticus* of both sexes were inoculated with the strain LV109 originating from  
234 Senegal. Six *A. niloticus* (3 males and 3 females) were infected with sand fly-derived  
235 *Leishmania* (experimental group A) and the same numbers of animals were infected with  
236 culture-derived promastigotes (experimental group C), but one animal from group C died  
237 early during the experiment and thus was not evaluated. In both groups, the first external  
238 signs of the disease appeared on inoculated ear pinnae on week 6 p.i. The affected area was  
239 characterized by mild flaking of the skin and hyper-pigmentation (Fig 1C). The pigmentation  
240 was lost in the centre while the borders remained hyper-pigmented in a part of animals (Fig  
241 1D, Tab. 3). These dry lesions increased to 3-4 mm by weeks 12-14 p.i; then, in 3 animals the  
242 lesion size remained constant until the end of the experiment by week 25 p.i., while in the  
243 others lesions decreased or completely disappeared (Tab. 3).

244 PCR confirmed the presence of *Leishmania* in 4 of 11 animals, with localization in  
245 ears, forepaws, hindpaws and tail (Table 1). The numbers of detected parasites were higher  
246 (hundreds to thousands) in the animal killed by week 12 p.i., while no parasites or only low  
247 numbers (around one hundred) were present in organs dissected by week 25p.i. (by the end  
248 of the experiment). This fact corresponds with results of xenodiagnoses: similarly to *A.*  
249 *neumanni*, the period of infectiousness of *A. niloticus* to *P. duboscqi* was restricted to weeks  
250 5 and 10 p.i. (4.1 % and 10.0 % of sand fly females became infected, respectively) while no  
251 females developed *Leishmania* infection in feeding experiments in weeks 15-25 p.i.(Table 2).

252

### 253 **3.3. Experimental infections and xenodiagnosis with *M. natalensis*.**

254 In total, 23 *M. natalensis* were inoculated with two *L. major* strains. Thirteen *M. natalensis*  
255 were all inoculated with sand fly–derived promastigotes of the Israeli strain Friedlin. Q-PCR  
256 revealed presence of *L. major* in 46% of animals (Table 1). However, none of the 13 *M.*  
257 *natalensis* tested developed lesions or other external signs of the disease. *Leishmania* were  
258 localized mostly in the inoculated ear pinnae (4 animals), less often in the contralateral ear  
259 pinnae (3 animals) and exceptionally also in forepaw (1 animal) and liver (1 animal).  
260 However, parasites were present in very low numbers (less than 100) in all the tissues.  
261 Therefore, animals were not infectious to sand flies (Table 1, 2).

262 Ten *M. natalensis* were experimentally infected with the LV109 strain (Table 1), 5  
263 with sand fly-derived *Leishmania* (experimental group A) and 5 with culture-derived  
264 promastigotes (experimental group C). Skin swellings developed at the site of inoculation  
265 (left ear pinnae) in animals of both experimental groups approximately 10 weeks p. i. (Table  
266 4, Fig 1E)). Prior to the swelling the affected site usually reddened, which was observed more  
267 often in specimens of the group C. The size of the swelling increased gradually to 6-8 mm,  
268 then decreased and finally disappeared. Hyper-pigmentation often accompanied healing of  
269 the swellings (Table 4) and it mostly persisted until the end of the experiments.

270 Parasites were detected by Q-PCR in all tested animals and they disseminated to  
271 draining lymph nodes, forepaws, hindpaws and tail in several animals and also to the spleen  
272 in one specimen (Table 1). Infectiousness to sand flies was tested at weeks 15 and 25 p.i.:  
273 0.7% of females from group A became infected after feeding at week 15 p.i., while 3.3% and

274 4.1% of females from the group A and C, respectively, became *Leishmania* positive at week  
275 25 p.i. (Table 2).

276

### 277 **3.4. Host choice experiments with *P. duboscqi*.**

278 Two host types were offered to *P. duboscqi* females in each pair-wise comparison.

279 Preliminary experiments showed that *P. duboscqi* did not distinguish between males and  
280 females of *A. neumanni* and both species of the genus *Arvicanthis* (smaller *A. neumanni* and  
281 bigger *A. niloticus*). Then, different host genera (represented by *A. neumanni*, *M. natalensis*  
282 and BALB/c mice) were compared: each host combination was tested twice with hosts  
283 alternated between lateral cages. Sand fly females showed a high feeding rate on all tested  
284 rodents: 40.5 - 80.5 % of females took bloodmeals during experiments (Table 5). The only  
285 significant preference was observed when *Arvicanthis* was compared with BALB/c mice -  
286 *Arvicanthis* was significantly preferred over mice. On the other hand, no difference was  
287 observed between *Mastomys* and *Arvicanthis* or *Mastomys* and BALB/c mice.

288 Engorged females were further followed for comparison of mortality and fecundity  
289 of females which took bloodmeals on different hosts. Mortality was assessed until day 4 post  
290 bloodmeal and ranged between 5 % and 27%, but was not significantly influenced by host  
291 types (Table 5). Four days PBM, females were allowed to oviposit in small glasses where they  
292 were kept individually. Blood source did not influence significantly either fecundity of fed *P.*  
293 *duboscqi* females (Table 5) or numbers of eggs laid by individually kept females (Table 6).

294

## 295 **5. Discussion**

296 The present study is, to our knowledge, the first one assessing the importance of Sub-  
297 Saharan rodents as hosts of *L. major* based on experimental infections of animals and testing  
298 of their infectiousness to sand flies.

299           Rodents of the genera *Arvicanthis* and *Mastomys* have been frequently found  
300 infected with *Leishmania major*: infections of *A. niloticus* have been reported from the NW  
301 and SW of Ethiopia, from Kenya, Senegal and Sudan, infections of *M. natalensis* from Kenya  
302 and *M. erythroleucus* from Senegal (reviewed by Desjeux 1996). The fact that only *A. niloticus*  
303 (and no other species of the genus *Arvicanthis*) have been mentioned could be explained by  
304 the poorly understood taxonomy of the genus. Only recently have investigations using  
305 cytogenetic and molecular data revealed the presence of at least three sibling species in  
306 western and central Africa where the single species *A. niloticus* was previously reported  
307 (Granjon and Ducroz 2013). In Ethiopia, which is situated in the center of *A. niloticus* origin  
308 (Dobigny et al. 2013), even four species of the genus are now recognized, including *A.*  
309 *niloticus* and *A. neumanni* (Granjon and Ducroz 2013).

310           Frequent field findings of *L. major* in *Arvicanthis* and *Mastomys* have been reported,  
311 and the eco-etiological and physiological characteristics of these rodents match the  
312 requirements essential for reservoirs: they live in colonies with high population numbers in  
313 the vicinity of humans in endemic localities, and they have sufficient longevity. These  
314 characteristics encouraged us to perform laboratory experiments which can help to confirm  
315 or exclude their reservoir role. The results revealed the importance of the *L. major* strain  
316 used for the experiments. Substantial differences were observed in the infectivity of *L. major*  
317 strains isolated from the Middle East and Sub-Saharan Africa. The Sub-Saharan strain LV109  
318 persisted in all three tested rodent species for several months and, importantly, the  
319 parasites were accessible and infective to *P. duboscqi* females. On the other hand, the

320 Middle Eastern strain FV1 produced only poor infections in *A. neumanni* and *M. natalensis*,  
321 parasites were present in low numbers and the animals were not infectious to sand flies.  
322 These differences correspond with results of the study of Elfari et al. (2005) testing cross-  
323 infectivity of three *L. major* strains differing in geographical origin in three rodent species –  
324 *Psammomys obesus*, *Rhombomys opimus* and *Meriones libycus*. No infections were detected  
325 in *R. opimus* when infected with the African or Middle Eastern strains and no signs of disease  
326 were seen in any *P. obesus* infected with a Central Asian strain (Elfari et al. 2005).

327           Important methodological points influencing results of experimental infections are  
328 the size and nature of the inocula and the infection route (reviewed by Loria-Cervera and  
329 Andrade-Narvaez 2014). It has been shown repeatedly that the number of parasites  
330 transmitted by sand flies to the host is highly variable but it does not exceeded  $10^5$  parasites  
331 inoculated per bite (Kimblin et al. 2008, Maia et al. 2011, Secundino et al. 2012). Here we  
332 used an intradermal route of inoculation which is close to the natural mode of transmission,  
333 since parasites are exposed to the localized immune responses in the skin (Belkaid et al.  
334 1998, 2002). Infections were initiated with either  $3-7 \times 10^4$  of sand fly-derived parasites or  
335 with  $10^7$  of parasites derived from stationary-phase promastigote cultures. The former  
336 inocula comprised mainly metacyclic stages present in thoracic regions of sand fly midguts  
337 during the late stage infections. Rodent infections initiated in our study by natural numbers  
338 of sand fly derived *Leishmania* showed the same outcome as those initiated with an  
339 unnaturally large inoculum from the culture. Dissemination of parasites in the host's body as  
340 well as infectiousness to sand flies was very similar with both types of infection.

341           Infection rates, the percentage of sand flies that became infected while biting on  
342 experimental animals, ranged between 0 -1.2% in *A. neumanni*, 0 - 10% in *A. niloticus* and 0 –  
343 4.1% in *M. natalensis*. Similarly low infection rates were detected previously: 0 – 7% in *P.*

344 *sergenti* feeding on rats (*Rattus rattus*) experimentally infected with *L. tropica* (Svobodová et  
345 al. 2013), 0 – 5% in *Lu. youngi* feeding on *Proechimys semispinosus* experimentally infected  
346 with *L. panamensis* (Travi et al. 2002) or 0 – 11% in *P. perniciosus* feeding on hares (*Lepus*  
347 *granatensis*) naturally infected with *L. infantum* (Molina et al. 2012). Higher infection rates  
348 have been reported more rarely, for example 19% of *P. orientalis* feeding on BALB/c mice  
349 experimentally infected with *L. donovani* (Sadlova et al. 2015) or up to 27- 28 % of *L.*  
350 *longipalpis* feeding on symptomatic dogs infected with *L. infantum* in Brazil (Michalsky et al.  
351 2007, Courtenay et al. 2002).

352 External clinical manifestations of *L. major* observed in ears of infected rodents in  
353 this laboratory study (changes in pigmentation in *Arvicanthis* and swellings, redness and  
354 hyper-pigmentation in *Mastomys*) appeared 6 and 10 weeks post infection, respectively.  
355 They generally resembled natural manifestation of *L. major* infections in *Psammomys obesus*  
356 and *Meriones shawi* described from Sidi Bouzid in Tunisia: hyper-pigmentation, depilation,  
357 ignition and edema of the ears were found frequently in both these North African reservoir  
358 hosts (Ghawar et al. 2011). Changes in pigmentation and swellings were often accompanied  
359 by the presence of high numbers of parasites in our experiments. This is important as only  
360 animals with high numbers of parasites in the site where sand flies fed were able to infect  
361 the vector. It was also pointed out by Courtenay et al. (2017) that among dogs infected with  
362 *L. infantum*, only some were “super-spreaders”, while others contributed little to  
363 transmission (15% to 44% of dogs were responsible for > 80% of all sand fly infections).  
364 Based on the model proposed by Miller et al. (2014) only 3.2% of the people infected by *L.*  
365 *donovani* in Ethiopia were responsible for of 53 - 79% of infections in the sand fly  
366 population.

367 One of the important prerequisites of the involvement of any rodent species in the  
368 life-cycle of *Leishmania* parasites is its attractiveness to sand flies. It is also known from  
369 laboratory colonies that some sand fly species are opportunistic and readily feed on mice,  
370 while the others, like species in the subgenera *Larroussius* and *Adlerius* , prefer hamsters or  
371 rabbits (Volf and Volfova 2011). Since the blood of vertebrate species varies in several  
372 properties influencing its nutritive value (Harrington et al. 2001), host choice affects the  
373 fitness of fed females as was repeatedly demonstrated in mosquitoes (Lyimo and Ferguson  
374 2009). In the neotropical sand fly *Lutzomyia longipalpis* significant differences in the  
375 numbers of eggs laid among flies fed on various hosts were reported (Macedo - Silva 2014),  
376 and in fleas significant differences in the energetic cost of blood digestion was found even at  
377 the level of two rodent species from the same family (Sarfati et al. 2005). On the other hand,  
378 studies on the Old World sand fly species *P. papatasi* and *P. halezensis* revealed no  
379 appreciable differences between the fecundity of females fed on human blood and different  
380 animal blood sources (Hare et al. 2001, Sadlova et al. 2003). In our experiments, *P. duboscqi*  
381 females manifested as opportunistic feeders which were ready to feed on all offered rodent  
382 species, although they preferred *Arvicanthis* over laboratory mice. Mortality and fecundity of  
383 *P. duboscqi* females was comparable post feeding on all rodents tested. This is in accordance  
384 with a study from Kenya where *P. duboscqi* also showed opportunistic behavior, being  
385 attracted to wild rats, chickens, mongooses, dogs and goats (Mutinga et al. 1985).

386 The definition of reservoir hosts in leishmaniasis has changed in recent years.  
387 Ashford (1996, 1997) originally distinguished primary reservoirs (species ensuring long-term  
388 persistence of the parasite) and secondary reservoir hosts (species acting as liaison between  
389 primary reservoirs and incidental hosts), but this division was assessed to be arbitrary by  
390 Chaves et al. (2007), as hosts may vary locally and seasonally with the dynamics of

391 transmission. According to the widely accepted ecological concept of Pulliam (1988),  
392 populations generally exhibit source – sink dynamics, where sources sustain exponential  
393 growth and are characterized by emigration while sinks operating under worse conditions  
394 demonstrate positive immigration. Chaves et al. (2007) applied this concept on reservoirs  
395 for leishmaniasis and proposed to recognize reservoirs (sources) as species which have a  
396 dynamic feedback to the hosts through pathogen transmission by the vector. Incidental  
397 hosts (sinks) lack such a dynamic feedback and cannot transmit the pathogen to new hosts.  
398 In this light, our results suggest that both *Mastomys* and *Arvicanthis* can be assessed as  
399 promising reservoirs (sources of the parasite) as both are able to maintain parasites for  
400 several months and infect the vector without apparent health complications. However, the  
401 higher infection rates, more generalized distribution of parasites and longer infectiousness  
402 period to sand flies in *M. natalensis* suggest that this species plays the more important  
403 reservoir role in the life cycle of this parasite in Sub-Saharan Africa. *Arvicanthis* species may  
404 serve as potential reservoirs in seasons/periods of low abundance of *Mastomys*.

405           Both *Arvicanthis* and *Mastomys* are known to undergo enormous abundance  
406 fluctuations: they are able to breed very rapidly and their population numbers may become  
407 very large when environmental conditions are favorable but with deteriorating conditions  
408 the numbers decline very rapidly (Granjon and Ducroz 2013, Leirs 2013). In the same locality,  
409 the Paloich district in Sudan, numbers of *Arvicanthis* and *Mastomys* alternated in two  
410 consecutive years (Hoogstraal and Dietlein 1964). Therefore, the scenario that these species  
411 maintain the parasite alternatively is highly likely: in localities/seasons with a low abundance  
412 of *Mastomys* then *Arvicanthis* could serve as source of the parasite and vice versa. A similar  
413 scenario, alteration of *L. major* between two host species *P. obesus* and *M. shawi*, was  
414 proposed in Central Tunisia (Ghawar et al. 2011). Involvement of another rodent species in

415 maintenance of *L. major* in Sub-Saharan region is also not excluded - it was suggested in  
416 Kenya where *Tatera robusta* possessed higher infection rates of *L. major* than *A. niloticus*  
417 and *M. natalensis* (Githure et al. 1996). Moreover, a high prevalence of *L. major* in invasive  
418 *Rattus rattus* was recently described in the southern part of Senegal (Cassan et al. 2018).

419 In conclusion, the results of this laboratory study support the field findings and give  
420 further support to the involvement of *Arvicanthis* and *Mastomys* spp. in the life cycle of *L.*  
421 *major* in Sub-Saharan Africa. This information is essential for any proposed control efforts  
422 against the human infection. However, more studies concerning other rodent species are  
423 needed to reveal the whole complexity and diversity of the epidemiology of *L. major* in this  
424 region.

425

## 426 **6. Acknowledgements**

427 This study was funded by Czech Science Foundation GACR (grant number 17-01911S), GA UK  
428 (grant number 288217) and ERD Funds; project CePaViP (16\_019/0000759).

## 429 7. References

- 430 Ashford, R.W. 2000. The leishmaniasis as emerging and reemerging zoonoses. *Int. J.*  
431 *Parasitol.* 30, 1269-1281.
- 432 Ashford, R.W. 1996. Leishmaniasis reservoirs and their significance in control. *Clin. Dermatol.*  
433 14: 523-532.
- 434 Ashford, R.W. 1997. What it takes to be a reservoir host. *Belg. J. Zool.* 127, 85-90.
- 435 Belkaid, Y., Kamhawi, S., Modi, G., Valenzuela, J., Noben-Trauth, N., Rowton, E., Ribeiro, J., Sacks, D.  
436 1998. Development of a Natural Model of Cutaneous Leishmaniasis: Powerful  
437 Effects of Vector Saliva and Saliva Preexposure on the Long-Term Outcome of  
438 *Leishmania major* Infection in the Mouse Ear Dermis. *J. Exp. Med.* 188(10): 1941-  
439 1953.
- 440 Belkaid, Y., Von Stebut, E., Mendez, S., Lira, R., Caler, E., Bertholet, S., Udey, M.C., Sacks, D. 2002.  
441 CD8<sup>+</sup> T Cells Are Required for Primary Immunity in C57BL/6 Mice Following Low-  
442 Dose, Intradermal Challenge with *Leishmania major* *J. Immunol.* 168: 3992-4000.
- 443 Chaves, L.F., Hernandez, M.-J., Dobson, A.P., Pascual, M. 2007. Sources and sinks: revisiting  
444 the criteria for identifying reservoirs for American cutaneous leishmaniasis.  
445 *Trends Parasitol.* 23(7), 311-316.
- 446 Cassan, C., Diagne C.A., Tatard, C., Gauthier, P., Dalecky, A., Ba, K., Kane, M., Niang, Y., Diallo,  
447 M., Sow, A., Brouat, C., Bañuls, A-L. 2018. *Leishmania major* and *Trypanosoma*  
448 *lewisii* infection in invasive and native rodents in Senegal. *PLoS Negl. Trop. Dis.*  
449 12 (6), e0006615.

450 Courtenay, O., Quinnell, R.J., Garcez, L.M., Shaw, J.J., Dye, C. Infectiousness in a cohort of  
451 Brazilian dogs: why culling fails to control visceral leishmaniasis in areas of  
452 high transmission. *J. Infect. Dis.* 186, 1314–1320.

453 Courtenay, O., Peters, N.C., Rogers, M.E., Bern, C. 2017. Combining epidemiology with basic  
454 biology of sand flies, parasites, and hosts to inform leishmaniasis transmission  
455 dynamics and control. *PLoS Pathog.* 13(10) e1006571

456 Desjeux, P. 1996. Information on the epidemiology and control of the leishmaniasis by  
457 country and territory. WHO/LEISH/91.30. Geneva: World Health Organisation,  
458 1991, 47.

459 Dobigny, G., Tatard, C., Gauthier, P., Ba, K., Duplantier, J.M., Granjon, L., Kergoat, G.J. 2013.  
460 Mitochondrial and Nuclear Genes-Based Phylogeography of *Arvicanthus*  
461 *niloticus* (Murinae) and Sub-Saharan Open Habitats Pleistocene History. *PLoS*  
462 *ONE* 8(12): 10.1371/annotation/a34daea8-8922-4eb0-8b4e-b0f9dbfd28ca.

463 Dougall, A.M., Alexander, B., Holt, D.C., Harris, T., Sultan, A.H., Bates, P.A., et al. 2011  
464 Evidence incriminating midges (Diptera: Ceratopogonidae) as potential  
465 vectors of *Leishmania* in Australia. *Int J Parasitol.* 41:571–9.

466 Elfari, M., Schnur, L.F., Strelkova, M.V., Eisenberger, C.L., Jacobson, R.L., Greenblatt, C.L.,  
467 Presber, W., Schönian, G. 2005. Genetic and biological diversity among  
468 populations of *Leishmania major* from Central Asia, the Middle East and  
469 Africa. *Microbes Infect.* 7, 93-103.

470 Ghawar, W., Toumi, A., Snoussi, M.-A., Chlif, S., Zaatour, A., Boukthir, A., Hamida, N.B.H.,  
471 Chemkhi, J., Diouani, M.F., Ben-Salah, A. 2011. *Leishmania major* infection  
472 among *Psammomys obesus* and *Meriones shawi*: reservoirs of zoonotic

473 cutaneous leishmaniasis in Sidi Bouzid (Central Tunisia). Vector-Borne  
474 Zoonot.11(12), 1561-1568.

475 Githure, J.I., Ngumbi, P.M., Anjili, C.O., Lugalia, R., Mwanyumba, P.M., Kinoti, G.K., Koech,  
476 D.K. 1996. Animal reservoirs of leishmaniasis in Marigat, Baringo district,  
477 Kenya. E. Afr. Med. J. 73(1), 44-47.

478 Granjon, L., Ducroz, J.-F. 2013. Genus *Arvicanthis* Grass Rats; pp. 379-380 in Happold D.C.D.  
479 (ed) 2013. Mammals of Africa: Volume III. Bloomsbury Publishing, London.

480 Hare, J.G., Dorsey, K.M., Armstrong, K.L., Burge, J.R., Kinnamon, K.E. 2001. Comparative  
481 fecundity and survival rates of *Phlebotomus papatasi* sandflies membrane fed  
482 on blood from eight mammal species. J. Med. Entomol. 15(2): 189-196.

483 Haile, T.T., Lemma, A. 1977. Isolation of parasites from *Arvicanthis* in Ethiopia. T. Roy. Soc.  
484 Trop. Med. Hyg. 71, 180-181.

485 Harrington, L.C., Edman, J.D., Scott, T.W. 2001. Why do female *Aedes aegypti* (Diptera :  
486 Culicidae) feed preferentially and frequently on human blood? J. Med.  
487 Entomol. 38, 411-422.

488 Hoogstraal, H., Dietlein, D.R. 1964. Leishmaniasis in the Sudan Republic: Recent results. Bull.  
489 World Health Organ. 31, 137-43.

490 Killick-Kendrick, M., Killick-Kendrick, R. 1991. The initial establishment of sand fly colonies.  
491 Parasitologia 33 (Suppl. 1), 313-320.

492 Kimblin, N., Peters, N., Debrabant, A., Secundino, N., Egen, J., Lawyer, P., Fay, M.P.,  
493 Kamhawi, S., Sacks, D. 2008. Quantification of the infectious dose of  
494 *Leishmania major* transmitted to the skin by single sand flies. Proc. Natl. Acad.  
495 Sci. U. S. A. 105, 10125–30

496 Lecompte, E., Aplin, K., Denys, Ch., Catzefflis, F., Chades, M., Chevret, P. 2008. Phylogeny and  
497 biogeography of African Murinae based on mitochondrial and nuclear gene  
498 sequences, with a new tribal classification of the subfamily. BMC Evol. Biol. 8:  
499 199.

500 Leirs, H. 2013. Genus *Mastomys* Multimammate Mice, pp 460-471 in Happold, D.C.D. (ed.)  
501 2013. Mammals of Africa: Volume III. Bloomsbury Publishing, London.

502 Loría-Cervera, E.N., Andrade-Narváez, F.J. 2014. Animal models for the study of leishmaniasis  
503 imunology. Rev. Inst. Med. Trop. Sao Paulo 56(1), 1-11.

504 Lyimo, I.N., Ferguson, H.M. 2009. Ecological and evolutionary determinants of host species  
505 choice in mosquito vectors. Trends Parasitol. 25, 189-196.

506 Maia, C., Seblova, V., Sadlova, J., Votypka, J., Volf, P. 2011. Experimental transmission of  
507 *Leishmania infantum* by two major vectors: a comparison between a  
508 viscerotropic and a dermatropic strain. PLoS Negl. Trop. Dis. 5e1181. 35.

509 Maroli, M., Feliciangeli, M.D., Bichaud, L., Charrel, R.N., Gradoni, L. 2013. Phlebotomine sand  
510 flies and the spreading of leishmaniasis and other diseases of public health  
511 concern. Med. Vet. Entomol. 27(2), 123-147.

512 Michalsky, E.M., Rocha, M.F., da Rocha Lima, A.C., Franca-Silva, J.C., Pires, M.Q., Oliveira,  
513 F.S., Pacheco, R.S., dos Santos, S. L., Barata, R.A., Romanha, A.J., Fortes-Dias,  
514 C.L., Dias, E.S. 2007. Infectivity of seropositive dogs, showing different clinical  
515 forms of leishmaniasis, to *Lutzomyia longipalpis* phlebotomine sand flies. Vet.  
516 Parasitol. 147, 67–76.

517 Miller, E., Warburg, A., Novikov, I., Hailu, A., Volf, P., Seblova, V., Huppert, A. 2014.  
518 Quantifying the Contribution of Hosts with Different Parasites Concentrations

519 to the transmission of Visceral Leishmaniasis in Ethiopia. PLoS Negl. Trop. Dis.  
520 8(10), e3288.

521 Molina, R., Jiménez, M.I., Cruz, I., Iriso, A., Martín-Martín, I., Sevillano, O., Melero, S., Bernal,  
522 J. 2012. The hare (*Lepus granatensis*) as potential sylvatic reservoir of  
523 *Leishmania infantum* in Spain. Vet. Parasitol. 190, 268–271.

524 Myskova, J., Votypka, J., Volf, P. 2008. *Leishmania* in sand flies: Comparison of quantitative  
525 polymerase chain reaction with other techniques to determine the intensity  
526 of infection. J. Med. Entomol. 45, 133-138.

527 Mutinga, M.J., Kyai, F.M., Kamau, C., Omogo, D.M. 1986. Epidemiology of Leishmaniasis in  
528 Kenya. 3. Host Preference Studies Using Various Types of Animal Baits at  
529 Animal Burrows in Marigat, Baringo District. Insect Sci. Appl. 7: 191-197.

530 Pulliam, H.R. 1988. Sources, sinks, and population regulation. Am. Nat. 132, 652-661.

531 Sadlova, J., Price, H.P., Smith, B.A., Votypka, J., Volf, P., Smith, D.F. 2010. The stage-regulated  
532 HASPB and SHERP proteins are essential for differentiation of the protozoan  
533 parasite *Leishmania major* in its sand fly vector, *Phlebotomus papatasi*. Cell.  
534 Microbiol. 12, 1765-1779. CMI1507 [pii];10.1111/j.1462-5822.2010.01507.x  
535 [doi].

536 Sadlova, J., Hajmova, M., Volf, P. 2003. *Phlebotomus (Adlerius) halepensis* vector  
537 competence for *Leishmania major* and *Le. tropica*. Med. Vet. Entomol. 17: 1-7

538 Sadlova, J., Seblova, V., Votypka, J., Warburg, A., Volf, P. 2015. Xenodiagnosis of *Leishmania*  
539 *donovani* in BALB/c mice using *Phlebotomus orientalis*: a new laboratory  
540 model. Parasite Vector 8 doi: 10.1186/s13071-015-0765-x

541 Sarfati, M., Krasnov, B.R., Ghazaryan, L., Khokhlova, I.S., Fielden, L.J., Degen, A.A. 2005.  
542 Energy costs of blood digestion in a host-specific haematophagous parasite. J.  
543 Exp. Biol. 208: 2489-2496.

544 Secundino, N.F.C., de Freitas, V.C., Monteiro, C.C., Pires, A.C.A.M., David, B.A., Pimenta,  
545 P.F.P. 2012. The transmission of *Leishmania infantum chagasi* by the bite of  
546 the *Lutzomyia longipalpis* to two different vertebrates. Parasit Vectors. 5, 20.

547 Silva, E.S., Gontijo, C.M.F., Melo, M.N. 2005. Contribution of molecular techniques to the  
548 epidemiology of neotropical *Leishmania* species. Trends Parasitol. 21(12),  
549 550-552.

550 Svobodova, M., Votýpka, J., Nicolas, L., Volf, P. 2013. *Leishmania tropica* in the black rat  
551 (*Rattus rattus*): persistence and transmission from asymptomatic host to sand  
552 fly vector *Phlebotomus sergenti*. Microbes Infect. 5(5), 361-364.

553 Travi, B.L., Arteaga, L.T., León, A.P., Adler, G.H. 2002. Susceptibility of Spiny Rats (*Proechimys*  
554 *semispinosus*) to *Leishmania (Viannia) panamensis* and *Leishmania*  
555 (*Leishmania*) *chagasi*. Mem. Inst. Oswaldo Cruz 97 (6), 887-892.

556 Volf, P., Volfova, V. 2011. Establishment and maintenance of sand fly colonies. J. Vector Ecol.  
557 36 Suppl 1, S1-S9.

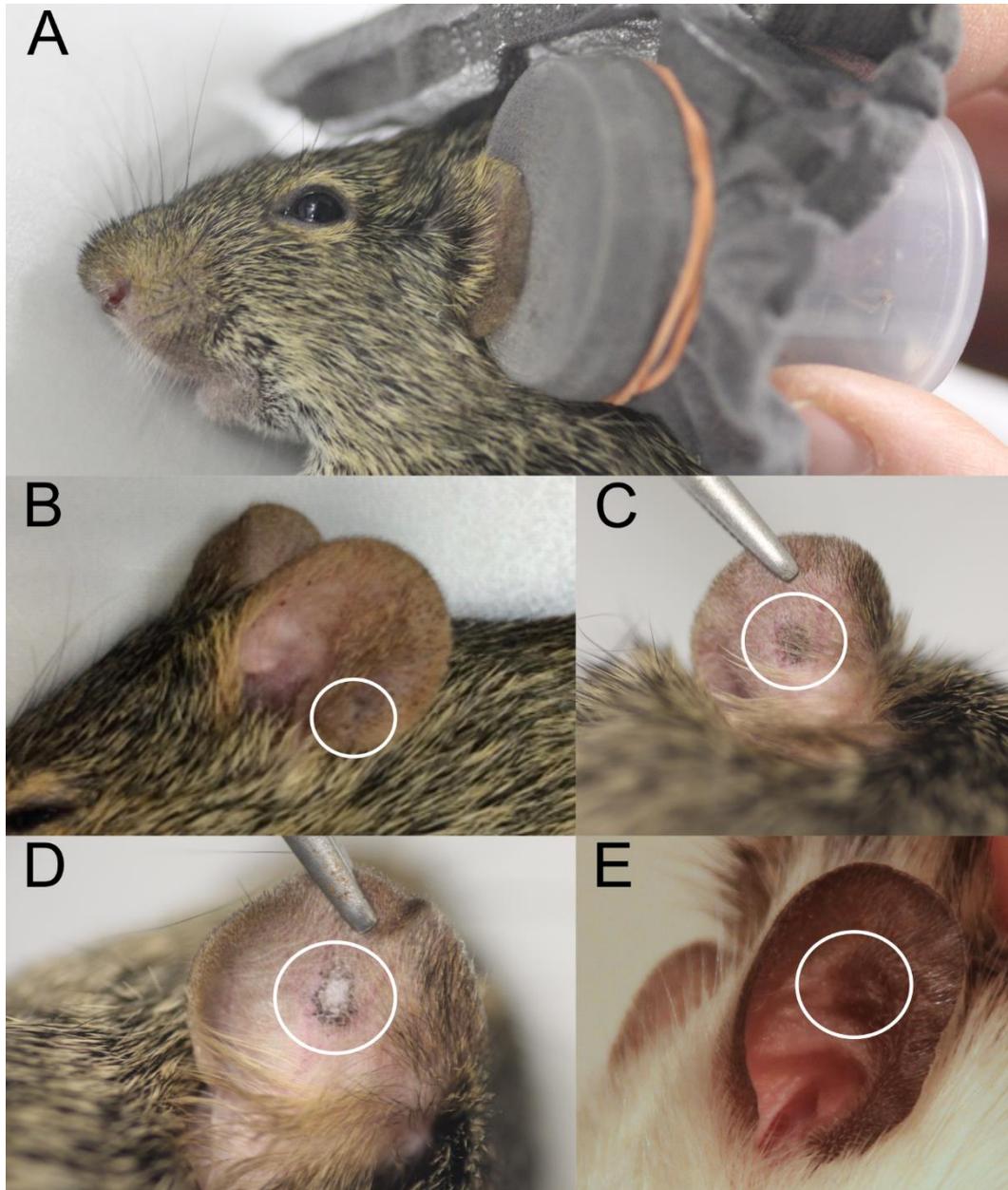
558

## 559 LEGENDS TO FIGURES:

560 **Figure 1. Xenodiagnosis and external manifestation of *L. major* in rodents.** Direct  
561 xenodiagnosis with *P. duboscqi* in plastic tubes covered with fine mesh held on the ear of the  
562 anaesthetized *A. niloticus* (A) and external manifestation of *L. major* LV109 in ear pinnae

563 (site of inoculation) of *A. neumanni* by week 10 p.i., (B); *A. niloticus* by week 30 p.i. (C, D) and  
564 *M. natalensis* by week 19 p.i. (E).

565



566

567 **TABLES:**

Rodent species	<i>L. major</i> strain	Experimental group	Week p.i.	No of animals tested	No of PCR positive animals (%)	Location (No) of parasites determined by qPCR in individual animals	External signs of the disease (on the inoculated ear)	No of animals infective for sand flies
<i>A. neumanni</i>	Friedlin	Group A	20	6	1	IE*	No	0
		Group B	10	2	0	-	No	not tested
			15	2	0	-	No	not tested
			20	2	0	-	No	not tested
	<b>Total</b>			<b>12</b>	<b>1 (8.3 %)</b>			<b>0</b>
	LV110	Group A	5	1	1	IE*	No	0
			10	2	2	IE*	No	0
						IE*	No	0
			15	1	1	IE*	No	0
			20	2	2	IE*	No	0
						IE*	No	0
	<b>Total</b>			<b>6</b>	<b>6 (100%)</b>			<b>0</b>
	LV109	Group A	20	6	4	IE*	No	0
						IE**	H-Pi	1
						IE**	H-Pi	1
					IE**	No	0	
Group B		10	2	0	-	No	not tested	
		15	2	1	IE*	No	not tested	
		20	2	1	B*	No	not tested	
Group C		15	3	1	IE*	H-Pi	1	
<b>Total</b>			<b>15</b>	<b>7 (47 %)</b>			<b>3 (33%)<sup>a</sup></b>	

<i>A. niloticus</i>	LV109	Group A	25	6	1	A5:FP*	H-Pi	1
		Group C	12	1	1	C1: IE***, CE**, HP*	H-Pi	not tested
			25	4	2	C2: IE**	H-Pi	1
						C4: CE**, T*, HP*	H-Pi	0
		<b>Total</b>		<b>11</b>	<b>4 (37%)</b>			<b>2 (20%)<sup>b</sup></b>
<i>M. natalensis</i>	Friedlin	Group A	35	6	3	IE*	No	0
						CE* and L*	No	0
						FP*	No	0
		Group B	10	2	0	-	No	not tested
			15	2	2	IE*	No	not tested
	LV109	Group A	20	2	1	IE*, CE*	No	not tested
			35	1	0	IE*, CE*	No	not tested
						-	No	not tested
			<b>Total</b>		<b>13</b>	<b>6 (46%)</b>		
	LV109	Group A	20	5	5	A1: IE**	Swelling, H-Pi	1
					A2: IE**, DN-CE**, HP***	Swelling	0	
					A3: IE*, S**	Swelling, H-Pi	0	
					A5: IE**, FP***, HP****, T****	Swelling, H-Pi	0	
					A4: IE***, DN-IE*	Swelling	0	
Group C		15	3	3	C1: IE**, T***	Swelling	0	
					C4: IE**	Swelling	0	
					C5: IE*	Swelling	0	
		25	2	2	C2: IE***, FP***, HP**	Swelling	1	

			C3: IE**	Swelling, H-Pi	0
	<b>Total</b>	<b>10</b>	<b>10 (100%)</b>		<b>2 (20 %)</b>

568

569 Table 1. Presence of *L. major* DNA in *A. neumanni*, *A. niloticus* and *M. natalensis* and their infectiousness to *P. duboscqi*. Group A, rodent  
570 infections initiated with sand fly-derived *Leishmania* and animals exposed to sand flies; Group B, rodent infections initiated with sand fly-  
571 derived *Leishmania* and animals not exposed to sand flies; Group C, rodent infections initiated with culture-derived promastigotes and animals  
572 exposed to sand flies. IE, inoculated ear; CE, contralateral ear; DN-IE, draining lymph nodes of the inoculated ear; DN-CE, draining lymph nodes  
573 of the contralateral ear; FP, forepaws; HP, hindpaws; T, tail; L, liver; S, spleen; B, blood; \*, <100 parasites; \*\*, 100 – 1000 parasites; \*\*\*, > 1000  
574 parasites; H-Pi, hyper-pigmentation. A1-A6 and C1-C5 - individual marks of animals referring to tables 3 and 4. <sup>a</sup>9 tested animals, <sup>b</sup> 10 tested  
575 animals.

Rodent species	<i>L. major</i> strain	Experimental group	Week p.i.	No of animals exposed	No of dissected sand flies	No and (%) of positive sand flies	
<i>A. neumanni</i>	Friedlin	Group A	2	6	124	0	
			5	6	179	0	
			10	6	95	0	
			15	5	54	0	
			20	5	80	0	
				<b>Total</b>		<b>532</b>	<b>0</b>
	LV110	Group A	5	6	143	0	
			10	5	177	0	
			15	3	105	0	
			20	2	17	0	
						<b>Total</b>	
	LV109	Group A	5	6	85	1 (1,2)	
			10	6	287	1 (0,3)	
			15	5	78	0	
			20	5	148	0	
		Group C	5	3	98	1 (1,0)	
			15	3	52	0	
						<b>Total</b>	
	<i>A. niloticus</i>	LV109	Group A	5	2	30	3 (10.0)
				10	2	33	2 (6.1)
15				2	63	0	
20				2	31	0	
25				6	108	0	
Group C			5	3	49	2 (4.1)	
			10	2	18	1 (5.6)	

			15	3	66	0
			20	2	31	0
			25	4	47	0
		<b>Total</b>			<b>476</b>	<b>5 (1.1)</b>
<i>M. natalensis</i>	Friedlin	Group A	2	6	126	0
			5	6	130	0
			10	6	166	0
			15	6	150	0
			20	6	66	0
		<b>Total</b>			<b>638</b>	<b>0</b>
	LV109	Group A	15	5	145	1 (0.7)
			25	4	61	2 (3.3)
		Group C	15	5	136	0
			25	2	24	1 (4.1)
		<b>Total</b>			<b>366</b>	<b>4 (1.1)</b>

576

577 Table 2. Direct xenodiagnosis of *L. major* in *A. neumanni*, *A. niloticus* and *M. natalensis*: feeding of *P. duboscqi* on inoculated ears.

578 Group A, rodent infections initiated with sand fly-derived *Leishmania*; Group C, rodent infections initiated with culture-derived promastigotes.

Animals	Weeks post infection												
	2	4	6	8	10	12	14	16	18	20	22	24	25
C1*			2	3	4	X	X	X	X	X	X	X	X
C2					1	2.5	3.5	3.5	4	4	4	4	4
C3					1	2.5	3.5	3.5	4	4	4	4	4
C4			1	1	1	1	3.5	3.5	4	4	4	3	3
C5				1	1	2.5	3.5	4	4	4	4	3	3
A1					1	2	2	2.5	3	3	3	3	3
A2			1	1	2	4	4	3	3	3	3	3	3
A3				1	1	4	4	2	1	1	1	1	1
A4					3	3	2	2	2	2	1	1	1
A5			1	1	2.5	2.5	3	1.5	1.5	1.5	1	1	1
A6			1	2	2.5	3	3	1.5	1				

579

580 Table 3. Time-course of the external manifestation of *L. major* LV109 in ear pinnae (site of inoculation) of *A. niloticus*. Animals C1-C5 were  
581 infected with culture-derived promastigotes (Group C), animals A1-A5 were infected with sand fly-derived *Leishmania* (Group A). Black colour –  
582 hyper-pigmentation, grey colour – depigmentation in the centre surrounded with hyper-pigmented borders. The numbers are the length of the  
583 affected area in mm. \*, animal died by week 10 p.i.

Animals	Weeks post infection																		
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
C1*				1	1	1	1	1	5.8	X	X	X	X	X	X	X	X	X	X
C2			1	1.7	1.7	4	4.9	5.3	6.8	7	7	7	7	5	5	5	5	1	
C3				1	1	1	1	1	2.8	3.4	5.2	5.4	5.6	2					
C4*				1	2.9	3	4.7	5	5	X	X	X	X	X	X	X	X	X	X
C5*				1	1	2	3.3	3.8	4	X	X	X	X	X	X	X	X	X	X
A1				2	2	2	3	4.6	5.2	6	6	6	6	3.7					
A2				1	1	1	2	2	4	5.2	5.2	5.2	5.2	5.2	5.2	5.2	3	3	1.6
A3								3	4.2	5.9	5.9	5.9							
A4				1	3	3.5	4.7	6.3	6.3	6.3	6.8	7	8	5.2	2.4	1			
A5		1	1	1	1	1	1	5	6.3	7	6.5	5.8	5	1					

584

585 Table 4. Time-course of the external manifestation of *L. major* LV109 in ear pinnae (site of inoculation) of *M. natalensis*. Animals C1-C5 were  
586 infected with culture-derived promastigotes (Group C), animals A1-A5 were infected with sand fly-derived *Leishmania* (Group A). Light grey  
587 colour – red macula, dark grey colour – swelling, black colour – hyper-pigmentation of the site where swelling had healed. The numbers are the  
588 length of the swelling area in mm. \*, animals killed by week 15 p.i.

Host combination	Host	N (%) of fed sand flies	Significance of between-species differences	Mortality post feeding: N dying/N (%)	Significance of between-species differences	Fecundity N lying eggs/N (%)	Significance of between-species differences
<i>Arvicanthis</i> vs. BALB/c mouse	<i>Arvicanthis</i>	161 (80.5%)	$\chi^2 = 17.015,$	12/161 (7.4%)	$\chi^2 = 0.118,$	26/76 (34.2%)	$\chi^2 = 0.119,$
	BALB/c mouse	95 (47.5%)	$P < 0.0001$	6/95 (6.3%)	$P = 0.472$	24/76 (31.6%)	$P = 0.432$
<i>Arvicanthis</i> vs. <i>Mastomys</i>	<i>Arvicanthis</i>	94 (47.0%)	$\chi^2 = 0.129,$	25/94 (26.6%)	$\chi^2 = 0.007,$	20/28 (71.4%)	$\chi^2 = 0.012,$
	<i>Mastomys</i>	81 (40.5%)	$P = 0.719$	22/81 (27.2%)	$P = 0.534$	14/20 (70.0%)	$P = 0.582$
<i>Mastomys</i> vs. BALB/c mouse	<i>Mastomys</i>	134 (67.0%)	$\chi^2 = 0.055,$	6/100 (6.0 %)	$\chi^2 = 0.787,$	18/20 (90.0 %)	$\chi^2 = 0.784 ,$
	BALB/c mouse	135 (67.5%)	$P = 0.808$	10/200 (5.0 %)	$P = 0.132$	16/20(80.0 %)	$P = 0.661$

589 Table 5. Feeding preferences, mortality and fecundity of *P. duboscqi* females fed on different host species. The between-species differences were tested by  
590 the Chi-squared test.

Host combination	Host	Number of eggs		Significance of between-species differences in distribution and means
		N	Median (Min, Max)	
<i>Arvicanthis</i> vs. BALB/c mouse	<i>Arvicanthis</i>	26	21 (2, 75)	P = 0.426, P = 0.777
	BALB/c mouse	24	13 (1, 54)	
<i>Arvicanthis</i> vs. <i>Mastomys</i>	<i>Arvicanthis</i>	20	45 (15, 75)	P = 0.290, P = 0.727
	<i>Mastomys</i>	14	40 (3, 70)	
<i>Mastomys</i> vs. BALB/c mouse	<i>Mastomys</i>	33	20 (4, 81)	P = 0.379, P = 0.190
	BALB/c mouse	13	31 (5, 72)	

591

592 Table 6. Numbers of eggs laid by *P. duboscqi* females fed on different hosts. The differences were tested by the nonparametric Mann Whitney U test.