Experimental infections of sand flies and geckos with *Leishmania* (*Sauroleishmania*) *adleri* and *Leishmania* (*S.*) *hoogstraali*

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**Abstract**

**Background:** Species belonging to the subgenus *Sauroleishmania* are parasites of reptiles, and traditionally considered to be non-pathogenic to mammals. Knowledge of the development of these parasites in sand flies and their mechanism of transmission is currently lacking. The main aim of this study was to test the susceptibility of various sand fly species to infection by two *Sauroleishmania* species, focusing on the localization of parasites in the sand fly intestinal tract.

**Methods:** The development of *Leishmania* (*Sauroleishmania* [*S.*]) *adleri* and *Leishmania* (*S.*) *hoogstraali* was studied in six sand fly species (*Phlebotomus orientalis*, *P. argentipes*, *P. sergenti*, *P. papatasi*, *P. duboscqi*, *Sergentomyia schwetzi*). Sand flies were fed through a chick-skin membrane on blood containing *Sauroleishmania* promastigotes, and they were dissected at various time intervals post blood meal (PBM). Guts were examined microscopically for the presence of parasites, and the intensity and localizations of infections were recorded. Morphological forms of both *Sauroleishmania* species developing in *P. orientalis* were analyzed. Experimental infections of geckos using sand fly-derived promastigotes were also performed, and the reptiles were repeatedly examined for *Sauroleishmania* infection by xenodiagnosis and PCR analysis.

**Results:** High infection rates for both *Sauroleishmania* species were observed in *P. orientalis* and *P. argentipes*, with the parasites migrating anteriorly and undergoing a peripylarian type of development, including colonization of the stomodeal valve. Conversely, the development of *L. (S.)* *adleri* in *P. sergenti*, *P. papatasi* and *Se. schwetzi* was restricted to the sand fly hindgut (hypopylarian type of development). Five morphological forms were distinguished for both *Sauroleishmania* species developing in *P. orientalis*. All experimentally infected geckos scored negative for *Sauroleishmania* based on xenodiagnosis and molecular analysis.

**Conclusions:** The results showed that *Sauroleishmania* promastigotes can undergo either a peripylarian or hypopylarian type of development in the sand fly intestinal tract, depending on the sand fly species infected. We demonstrated that *P. argentipes* and *P. orientalis*, two sand fly species known as permissive vectors for mammalian parasites of subgenus *Leishmania*, are also highly susceptible to *Sauroleishmania* as the parasites developed mature late-stage infections, including colonization of the sand fly stomodeal valve. Thus, the role of *Phlebotomus* sand flies in transmission of *Sauroleishmania* should be reconsidered and further investigated.

**Keywords:** *Sauroleishmania*, Sand flies, *Phlebotomus*, *Sergentomyia*, Geckos, Leishmaniasis

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Background
Protozoa of the genus Leishmania (Kinetoplastida: Trypanosomatidae) are causative agents of leishmaniasis. They are transmitted to vertebrate hosts by phlebotomine sand flies (Diptera: Psychodidae), with one exception, namely members of the subgenus Mundinia, for which biting midges (Diptera: Ceratopogonidae) are the main vectors [1]. The genus Leishmania is currently divided into four subgenera, of which three: Leishmania, Viannia and Mundinia include species infecting mammals, while the subgenus Sauroleishmania comprises of reptilian parasites [2, 3].

Various Sauroleishmania species have been found in reptiles of five families (Agamidae, Gekkonidae, Lacertidae, Scincidae and Varanidae) [4, 5], where they occur in two different forms: as free-living promastigotes and/or intracellular amastigotes [6]. Amastigote forms have been observed in different blood cells, mainly in monocytes or macrophages, but also in thrombocytes and erythrocytes [7–10].

The mechanism of Sauroleishmania transmission from sand flies to reptilian hosts is still being debated, as it has never been demonstrated under laboratory conditions [11]. Two possible modes of transmission are considered, either via sand fly bites and/or by ingestion of infected sand flies [6]. Sand flies of the genus Sergentomyia, which feed preferentially on cold-blooded animals, are generally accepted as the vectors of Sauroleishmania [12]. However, it has been reported that some Sauroleishmania species can cause late-stage infections in Phlebotomus sand flies; consequently, their possible involvement in Sauroleishmania transmission should also be considered [13, 14].

In the sand fly intestinal tract, the parasites initially multiply as promastigotes within the blood meal surrounded by a peritrophic matrix. Once the peritrophic matrix is broken, promastigotes migrate into various parts of the sand fly gut [14, 15] and, according to the description of Lainson and Shaw [16], undergo either hypopylarian, peripylarian or suprapyliarian types of development. Hypopylarian development is confined to the hindgut (pylorus and rectum) and is considered typical of Leishmania (Sauroleishmania [S.]) species; peripylarian development includes a phase of development in the pylorus region of the hindgut followed by midgut and foregut colonization and is typical of Leishmania (Viannia [V.]) species, such as L. braziliensis; suprapyliarian development occurs in the midgut and foregut only and is typical of Leishmania (Leishmania [L.]) species, such as L. donovani.

Leishmania (S.) adleri and Leishmania (S.) hoogstraali are two Sauroleishmania species distributed in sub-Saharan Africa. They were first isolated from the lacertid lizard Latastia longicaudata [17] and from the gecko Hemidactylus turcicus [18], respectively, but their vectors are unknown. The main aim of our study was to test the susceptibility of various sand fly species to Sauroleishmania infections. We investigated the development of L. (S.) adleri and L. (S.) hoogstraali in six sand fly species differing in susceptibility to Leishmania, with the focus on localization of parasites in the sand fly intestinal tract. We also performed experimental infections of geckos using sand fly-derived parasites.

Methods
Parasites, sand flies and geckos
Two Sauroleishmania species, Leishmania (S.) adleri (RLAT/KE/LV30) isolated from a lizard (Latastia sp.) in Kenya and Leishmania (S.) hoogstraali (RHEM/SD/LV31) isolated from a gecko (Hemidactylus sp.) in Sudan were used in this study. Cryopreserved parasites were shipped from Lancaster to Prague, and low-passage parasites (< 5) were used for the experimental infections of sand flies. Promastigotes were cultivated at 23 °C in Medium 199 (Sigma-Aldrich, Prague, Czech Republic) supplemented with 20% heat-inactivated fetal calf serum (Gibco, Prague, Czech Republic), 1% Basal Medium Eagle vitamins (Sigma-Aldrich, Prague, Czech Republic), 2% sterile human urine and 250 µl amikacin (Medipharm, Pozorice, Czech Republic).

In the first series of experiments, three sand fly species, each with a different susceptibility to various Leishmania species, were selected: Sergentomyia schwetzii (refractory to all Leishmania species tested so far [19]; colony originating from Ethiopia), and two Phlebotomus species: Phlebotomus papatasi (natural vector of Leishmania major [20], colony originating from Turkey) and Phlebotomus argentipes (natural vector of Leishmania donovani [21], colony originating from India). In the second series of experiments, we tested the susceptibility of three Phlebotomus species sharing an overlapping geographical distribution with the parasites used in the study: Phlebotomus duboscqi (colony originating from Senegal), Phlebotomus sergenti (colony originating from Turkey) and Phlebotomus orientalis (colony originating from Ethiopia). All sand fly colonies were maintained under standard conditions (26 °C, 14/10-h light/dark photoperiod, 50% sucrose), as described previously [22].

Twelve specimens of the gecko Hemidactylus turcicus were used for experimental infections with Sauroleishmania parasites. They were maintained individually in plastic boxes (32.5 × 22 × 21 cm) equipped with sand substrate, shelters and water dish, under a 12/12-h light/dark photoperiod and constant temperature maintained by heating pads and cables. Geckos were provided with water ad libitum, and twice a week they were fed with...
crickets (Gryllus assimilis) or mealworms (Tenebrio molitor) dusted in vitamins (Roboran, Univit, Czech Republic) to satiety.

**Experimental infections of sand flies**
Female sand flies (5–9 days old) were experimentally infected by feeding through a chick-skin membrane on heat-inactivated blood containing 5 × 10⁶ promastigotes per milliliter. Engorged sand flies were then separated out, kept at 26 °C under standard conditions [22] and dissected at different time intervals post blood meal (PBM). The intensity of infections and localizations of parasites in the sand fly gut were examined under a light microscope. The intensity of infections was categorized according to Myskova et al. [23] as light/weak (<100 parasites/gut), moderate (100–1000 parasites/gut) or heavy (>1000 parasites per gut). All experiments were repeated at least twice for each Sauroleishmania-sand fly combination. Differences in infection rates were evaluated statistically by the Chi-square (χ²) tests using the SPSS version 27 statistical software package (SPSS IBM Corp., Armonk, NY, USA).

**Morphometry of parasites from gut smears**
Sauroleishmania morphological forms were studied in Phlebotomus orientalis as this sand fly species was shown to be susceptible to both L. (S.) adleri and L. (S.) hoogstraali. Sand fly females were dissected on days 5, 7 and 9 PBM and their guts were used for analysis of morphological forms. Smears of sand fly guts positive for Sauroleishmania were fixed with methanol, stained with Giemsa staining solution and observed under a light microscope using an oil-immersion objective; promastigotes were photographed with an Olympus D70 camera (Olympus Corp., Tokyo, Japan). Body length, body width and flagella length of at least 140 randomly selected promastigotes from a minimum of three female sand flies were measured using ImageJ software and evaluated.

Morphological stages of the parasites were determined as described previously for members of the subgenus Sauroleishmania [14]: (i) long nectomonad promastigotes (body length ≥14 μm); (ii) short nectomonad promastigotes (body length <14 μm and flagella length <2-fold the body length); (iii) metacyclic-like promastigotes (body length <14 μm and flagella length ≥2-fold body length); (iv) amastigote-like forms; and (v) haptomonad promastigotes. Differences in number of metacyclic-like stages were tested by the Chi-square (χ²) tests using SPSS software version 27 (SPSS IBM Corp.).

**Experimental infections of geckos**
Geckos were infected with sand fly-derived parasites according to the methodology described for mammal-infecting Leishmania species [24] with a single modification: as the localization of metacyclic and reptile-infecting stages of Sauroleishmania have not been described yet, whole dissected sand fly guts (not only thoracic midguts) were used for the experimental infections. Briefly, two parasite-vector combinations displaying the highest infection rates and intensities of infections were chosen: P. orientalis for L. (S.) adleri and P. argentinae for L. (S.) hoogstraali. Sand fly females were infected by feeding through a chick-skin membrane with 10⁷ promastigotes per milliliter, as described in preceding text, and maintained under standard conditions until day 7 PBM. Engorged sand flies were then dissected and their guts examined for the presence of parasites under a light microscope. Sand fly guts with high parasite loads were pooled and homogenized in sterile saline solution. Each gecko was infected with 10 μl of homogenate, which corresponds to 10 sand fly guts.

Twelve geckos were separated into two groups of six specimens each for experiments with L. (S.) adleri and L. (S.) hoogstraali, respectively. Three geckos from each group were infected intraperitoneally by insulin syringe and the remaining three in each group were infected via the oral route using pipette tips. For each gecko infected via the oral route, 90 μl of saline solution was added to the sand fly gut homogenate. The infection doses (calculated using a Bürker chamber) were determined as 3.39 × 10⁵ for L. (S.) adleri and 7.5 × 10⁴ for L. (S.) hoogstraali.

Infected geckos were monitored weekly for the external signs of the infection, and they were examined for the presence of parasites at different time intervals post-infection (p.i.) using xenodiagnosis. At the end of the experiments (21 weeks p.i.) they were sacrificed and dissected. Samples from the liver, skin, tail, feet and blood were stored at −20 °C for subsequent molecular analysis. Other parts of these tissues were cultivated on SNB-9 blood agar [25] with M199 medium as an overlay supplemented with 20% heat-inactivated fetal calf serum (Gibco), 1% Basal Medium Eagle vitamins (Sigma-Aldrich), 2% sterile human urine, 250 μl amikacin (Medopharm) and 1.5 μg/ml of fluorocytosin (Sigma-Aldrich). Cultures were checked microscopically for the presence of parasites once a week for a total of 5 weeks.

**Xenodiagnosis of geckos**
Xenodiagnostic experiments were performed on weeks 3, 7, 12 and 18 p.i. using a laboratory-reared colony of Se. schwetzi. This was the only sand fly species which regularly fed on geckos in our laboratory. However, it does not support late-stage development of Sauroleishmania and
parasites could be found in its midgut only before defe-
cation. Each gecko was placed in separated net containing
30–50 sand fly females (5–7 days old) that were allowed
to feed on the gecko for a maximum of 2 h. Engorged
sand flies were separated out, maintained in the nets
for 2 days and then (before defecation) placed individu-
ally into microcentrifuge tubes with Tissue Lysis Buffer
(Roche, Prague, Czech Republic) and stored at −20 °C
for subsequent DNA extraction and analysis by PCR.

PCR assay
Extraction of total DNA from sand flies and tissues of the
gechos was performed using the High Pure PCR Tem-
plate Preparation Kit (Roche Diagnostic, Mannheim,
Germany) according to the manufacturer’s instructions.
Extracted DNA was used as a template for PCR amplifi-
cation targeting a region of the ribosomal internal tran-
scribed spacer 1 (ITS1; approx. 300 bp) using the forward
primer LITSR (5′-CTGGATCATTTTCCGATG-3′) and reverse primer L5.8S (5′-TGATACCATTATCGCAC
TT-3′) as described previously [26]. Reactions were per-
duced with EmeraldAmp® GT PCR Master Mix at the
following cycling conditions: denaturation at 95 °C for
3 min; 35 amplification cycles of 95 °C for 20 s, 53 °C for
30 s, 72 °C for 40 s; and a final cycle at 72 °C for 6 min.
The PCR products were analyzed using a SYBR Green
fluorescent probe on 1% agarose gels. DNA extracted
from the cultures of Sauroleishmania spp. and Leishma-
nia major were used as positive controls (in the prelimi-
nary experiment, we tested these primers with various
Leishmania species: Leishmania (L.) major, Leishmania
(L.) amazonensis and Leishmania (S.) spp.; all of them
gave positive results and, therefore, for the main experi-
ment we chose only L. major). Additionally, serial dilu-
tions were performed to confirm the detection of a
minimum of 10^2 parasites per sample.

Results
Experimental infections of sand flies I.
Development of L. (S.) adleri and L. (S.) hoogstraali was
studied in Se. schwetzi, P. papatasi and P. argentipes at
two time points: day 1 PBM (before defecation) and day
7 PBM (late-stage infection). Altogether, 506 female sand
flies were dissected and examined for the presence of
parasites. Statistically significant differences in infection
rates were observed between the two Sauroleishmania
species (Table 1).

On day 1 PBM, in all three sand fly species tested, vari-
able but relatively high infection rates (57–100%) were
observed for L. (S.) adleri and very high infection rates
(79–96%) for L. (S.) hoogstraali (Fig. 1). All parasites
were present in the ingested blood meal within the peritrophic
matrix. Statistically significant differences in infection
rates were observed on day 1 PBM among sand fly spe-
cies tested for L. (S.) adleri (χ^2 = 10.084, df = 2, P = 0.006),
but not significant among sand fly species tested for L.
(S.) hoogstraali (χ^2 = 4.350, df = 2, P = 0.114).

On day 7 PBM, after the defecation of blood meal
remains, infection rates of both Sauroleishmania
species were significantly reduced in Se. schwetzi and P.
papatasi. Leishmania (S.) adleri was found in 20% of
Se. schwetzi and 23% of P. papatasi females in which

![](image)

**Table 1** Comparison of infection rates of Leishmania (Sauroleishmania [S]) adleri and Leishmania (S) hoogstraali in three sand fly species

| Sand fly species       | Day 1 PBM | Day 7 PBM |
|------------------------|-----------|-----------|
| Sergentomyia schwetzi  | χ^2 = 2.162, df = 1, P = 0.129 | χ^2 = 8.086, df = 1, P = 0.004 |
| Phlebotomus papatasi   | χ^2 = 0.975, df = 1, P = 0.513 | χ^2 = 6.909, df = 1, P = 0.008 |
| Phlebotomus argentipes | χ^2 = 5.399, df = 1, P = 0.020 | χ^2 = 14.415, df = 1, P = < 0.001 |

Statistical analysis was performed using the Chi-square (χ^2) test.
PBM: Post blood meal.
infections of weak intensity prevailed. Parasites occupied the hindgut (mainly pylorus and ileum) (Fig. 2), where attached haptomonad promastigotes were the prevailing forms, but in a few females, long free-swimming flagellates were also present. Infection rates of \( L. (S.) \) hoogstraali and \( L. (S.) \) schwetzi and \( P. \) papatasi were negligible, reaching 4% and 7%, respectively.

In contrast, higher infection rates were observed in \( P. \) argentipes on day 7 PBM when 32% of dissected sand flies were positive for \( L. (S.) \) adleri and 66% for \( L. (S.) \) hoogstraali. \textit{Leishmania} (\( S. \)) adleri developed in the hindgut, but also migrated anteriorly into the \( P. \) argentipes midgut (82% of infected sand flies). In two sand fly females, promastigotes reached the cardia (i.e. part of the midgut immediately behind the stomodeal valve), and in a single female the stomodeal valve was successfully colonized.

Infections of \( L. (S.) \) hoogstraali in \( P. \) argentipes were the most successful, with the presence of promastigotes detected in 66% of dissected sand flies on day 7 PBM. In most cases, parasites developed heavy-intensity infections and underwent the peripylarian type of development. In addition to the hindgut, promastigotes were observed in the abdominal and thoracic midgut (41% and 26%, respectively), reaching the cardia and colonizing the stomodeal valve in 13% and 21% of infected females, respectively.

Infection rates between sand fly species on day 7 PBM were not significantly different for \( L. (S.) \) adleri \((\chi^2 = 2.782, df = 2, P = 0.249)\), but they were significantly different for \( L. (S.) \) hoogstraali \((\chi^2 = 79.850, df = 2, P \leq 0.001)\).

**Experimental infections of sand flies II.**

Development of \( L. (S.) \) adleri and \( L. (S.) \) hoogstraali was studied in \( P. \) duboscqi, \( P. \) sergenti and \( P. \) orientalis at various time intervals, namely on days 1, 5, 7 and 9 PBM. In total, 783 sand flies were examined for the presence of parasites.

**Development of \( L. (S.) \) adleri**

Promastigotes of \( L. (S.) \) adleri multiplied abundantly in the ingested blood meal on day 1 PBM, and infection rates reached 84–100% in all three sand fly species tested, with statistically significant differences among the three sand fly species \((\chi^2 = 9.848, df = 2, P = 0.007; \text{Fig. } 3)\). Infections of heavy intensity prevailed in \( P. \) duboscqi and \( P. \) sergenti, while the intensity of infection in \( P. \) orientalis was slightly lower. In all tested sand flies, \( L. (S.) \) adleri successfully survived defecation and developed late-stage infections. Significant differences were found in infection rates among sand fly species on day 7 PBM \((\chi^2 = 19.418, df = 2, P \leq 0.001)\), while the differences were not significant on day 5 PBM \((\chi^2 = 5.074, df = 2, P = 0.079)\) and day 9 PBM \((\chi^2 = 3.852, df = 2, P = 0.146)\).
In *P. duboscqi* the infection rate was almost 70% on day 5 PBM, then dropped to < 40% on days 7 and 9 PBM, with the majority of infections being of moderate and weak intensity. Promastigotes were localized in the hindgut and migrated to abdominal and thoracic midgut (peripylarian type of development) (Fig. 4). In the hindgut, haptomonad promastigotes were the most abundant forms, but free flagellates were also observed to a lesser extent.

Conversely, the hypopylarian type of development prevailed in *P. sergenti*. Relatively high infection rates were recorded at all designated time intervals (> 60%), and the intensity of most infections was moderate and weak/light. Parasites mainly occupied the hindgut (pylorus and ileum), with haptomonad promastigotes as the prevailing forms, while the presence of flagellates in the abdominal midgut was detected in only two *P. sergenti* females (5%).

In *P. orientalis*, heavy late-stage infections were observed on days 5 to 9 PBM, with > 50% positive sand flies, in which the peripylarian type of development prevailed. Promastigotes multiplied and migrated rapidly as they were present in thoracic midgut (50%) and cardia (20%) on day 5 PBM, and colonization of stomodeal valve had occurred in 21% of infected sand flies on day 7 PBM. Similar dynamics of the infections then persisted until day 9 PBM.

**Development of *L. (S.) hoogstraali***

On day 1 PBM, high infection rates (94–100%) were reported in all three sand fly species, with no significant differences ($\chi^2 = 2.231$, $df = 2$, $P = 0.328$; Fig. 5). The intensities of infections were mostly weak/light or moderate, and parasites were present in the blood meal enclosed by peritrophic matrix (endoperitrophic space). After defecation, however, significant differences in infection rates were observed between sand fly species at all designated time intervals: day 5 PBM ($\chi^2 = 8.564$, $df = 2$, $P = 0.014$), day 7 PBM ($\chi^2 = 46.269$, $df = 2$, $P \leq 0.001$) and day 9 PBM ($\chi^2 = 35.113$, $df = 2$, $P \leq 0.001$).

In *P. duboscqi* and *P. sergenti* females, *L. (S.) hoogstraali* was not able to survive defecation. The number of positive sand fly females decreased over time, and only...
infections of weak intensity were observed. *Leishmania (S.) hoogstraali* migrated anteriorly in *P. duboscqi*: parasites colonized mainly the hindgut but were also present in the abdominal (57%) and thoracic (7%) midgut on day 5 PBM (Fig. 6). In contrast, *L. (S.) hoogstraali* development in *P. sergenti* was restricted to the hindgut (Table 2), and promastigotes were observed in the abdominal midgut only when the remnants of ingested blood were still present.

*Leishmania (S.) hoogstraali* successfully survived defecation and developed late-stage infections in *P. orientalis*, with infection rates reaching > than 50% at all designated time intervals. Both attached haptomonad promastigotes and free-swimming flagellates were observed in the hindgut, but *L. (S.) hoogstraali* more tended to acquire an anterior position in this sand fly species: promastigotes reached the cardia (5%) and colonized the stomodeal valve (10%) on day 7 PBM. A similar tendency was observed on day 9 PBM, when colonization of the stomodeal valve had occurred in 14% of dissected females.

**Morphological transformations**

Five morphological forms were observed in both *Sauroleishmania* species tested (Fig. 7). Long nectomonad promastigotes and short nectomonad promastigotes were the most abundant forms, while haptomonad promastigotes, metacyclic-like promastigotes and amastigote-like forms were presented to a lesser extent (Additional file 3: Table S5; Additional file 3: Table S7). Long nectomonad promastigotes prevailed in *L. (S.) adleri* (75%), whereas short nectomonad promastigotes were more frequent in *L. (S.) hoogstraali* (62%) (Fig. 8). Both long and short nectomonad promastigotes were present also in a variation with significantly shortened flagella (approx. 4 μm).

Metacyclic-like promastigotes were recorded at all designated time intervals, and these stages were

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**Table 2** Differences in *L. (S.) adleri* and *L. (S.) hoogstraali* development in various sand fly species

| Leishmania speciesa | Sand fly speciesb |
|---------------------|------------------|
|                     | SCHW | PAP | ARG | DUB | SER | ORI |
| ADL                 | Hypopylarian    | Hypopylarian | Peripylarian | Peripylarian | Hypopylarian | Peripylarian |
| HOOG                | Hypopylarian    | Peripylarian | Peripylarian | Peripylarian | Hypopylarian | Peripylarian |

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a ADL Leishmania (S.) adleri, HOOG Leishmania (S.) hoogstraali
b SCHW Sergentomyia schwetzi, PAP Phlebotomus papatasi, ARG Phlebotomus argentipes, DUB Phlebotomus duboscqi, SER Phlebotomus sergenti, ORI Phlebotomus orientalis

**Fig. 7** Sauroleishmania morphological forms in sand flies. Morphological analysis was performed on *L. (S.) adleri* and *L. (S.) hoogstraali* developing in *P. orientalis* on days 5, 7 and 9 PBM. **A** Long nectomonad promastigote, **B** short nectomonad promastigote, **C** long nectomonad promastigote with shortened flagella, **D** long slender metacyclic-like promastigote, **E** haptomonad promastigote, **F** amastigote-like form (stained by Giemsa, 1000× magnification, scale bars: 10 μm)
morphologically highly variable in terms of body length and width. We distinguished three cell types: short rounded, short slender and long slender metacyclic promastigotes. Moreover, some of these forms had remarkably elongated flagella (up to 50 μm). Statistical analysis showed that the number of metacyclic-like promastigotes was significantly different on day 7 PBM ($\chi^2 = 10.381$, $df = 1$, $P \leq 0.001$), but not on days 5 PBM ($\chi^2 = 0.045$, $df = 1$, $P = 0.494$) and 9 PBM ($\chi^2 = 1.204$, $df = 1$, $P = 0.279$).

Haptomonad promastigotes with typically leaf-shaped flagella were harder to detect as they are strongly attached to the cuticular lining of the sand fly gut and, therefore, their number is significantly underestimated. Rounded (amastigote-like) forms with very short or no flagella were also reported. Detailed measurements of individual morphological forms are summarized in Additional file 3: Table S6, Table S8.

**Experimental infections of geckos and xenodiagnoses**

No external signs of the infections were observed in any geckos. Xenodiagnostic experiments (Fig. 9) were performed on weeks 3, 7, 12 and 18 p.i., and among the 604 Se. schwetzi females tested, none were found to be positive (for more details see Additional file 4: Table S9). The experiment was terminated 21 weeks p.i. when geckos were sacrificed and dissected for tissue sampling. Nevertheless, *Sauroleishmania* DNA was not detected in any of the samples tested (i.e. liver, skin, tail, feet, and blood) and no parasites were observed in tissue cultures.

**Discussion**

In the present study we demonstrated that the ability to undergo different types of development in sand flies is typical for parasites of the subgenus *Sauroleishmania* and that this variability is influenced by sand fly vectors. It is interesting to note that none of the parasite-vector combinations tested showed suprapylarian development and that there was always hindgut involvement to varying degrees, indicating this may be a fundamental property of *L. (Sauroleishmania)* species.

Although it is generally accepted that *Sauroleishmania* parasites are transmitted by reptile-biting sand flies of the genus *Sergentomyia*, the role of other sand flies in *Sauroleishmania* transmission should be reconsidered. The susceptibility of *Phlebotomus* species to *Sauroleishmania* infections has been experimentally demonstrated by several authors [13, 14] and now confirmed in the present study. Some *Phlebotomus* species were reported to feed on reptiles [4, 13, 27], and recent molecular detection of *Leishmania* (*Sauroleishmania*) *tarentolae* in *Phlebotomus* spp. [28–30] further supports the hypothesis that these sand flies are alternative vectors of *Sauroleishmania* [14].

It has been assumed that *Sauroleishmania* development in sand flies is restricted to the hindgut and described as hypopylarian [16]. Therefore, infection per the oral route was considered as one of the possible modes of *Sauroleishmania* transmission to reptiles [6]. Conversely, some older studies reported *Sauroleishmania* promastigotes in the anterior midgut [11, 13, 31]. The tendency to obtain an anterior position in the sand fly gut suggests that members of this group may be transmitted via sand fly bites, in a manner similar to mammal-infecting *Leishmania* species [15]. Nonetheless, a recent study showed that *L. (S.) tarentolae* underwent both hypopyllarian or peripylarian type of development depending on the sand fly species infected [14]; consequently, the mechanism of *Sauroleishmania* transmission from sand flies to reptilian hosts remains unclear.

Despite the proven role of members of the genus *Sergentomyia* as vectors of *Sauroleishmania*, the involvement of *Se. schwetzi* in the transmission of *L. (S.) adleri* is
unlikely as only 20% of females displayed the presence of parasites on day 7 PBM, with majority of infections being of weak/light intensity. It was also shown that Se. schwetzi is refractory to mammalian Leishmania spp. due to its delayed degradation of peritrophic matrix until the time of defecation, which does not provide sufficient time for promastigotes to escape the endoperitrophic space and attach to the midgut epithelium [19, 32].

Attachment of promastigotes to the sand fly gut is a key part of the Leishmania life-cycle as it prevents the expulsion of parasites during defecation [33]. The successful development of L. (S.) adleri in the hindgut of P. papatasi and P. sergenti may be due to the parasite’s ability to attach to the cuticular lining of the hindgut but its inability to bind to the sand fly midgut. Phlebotomus sergenti is known to be a specific vector of Leishmania tropica [34], while P. papatasi is specific for L. major [20] and Leishmania turanica [35]. In specific vectors, the attachment of promastigotes to the midgut epithelium is mediated by species-specific surface lipophosphoglycan (LPG) [36]. Nevertheless, the role of LPG in the Sauroleishmania life-cycle is understudied and it has been reported that some Sauroleishmania spp. appear to lack LPG or certain enzymes involved in LPG modification [37, 38].

Conversely, P. argenteipes and P. orientalis are known to be permissive vectors susceptible to multiple Leishmania spp. under laboratory conditions [33] in which promastigotes attach via a different, glycan-mediated, mechanism [39]. In both of these sand fly species, the highest infection rates and highest intensities of infections were recorded for L. (S.) adleri and L. (S.) hoogstraali, suggesting that some species of Sauroleishmania may non-specifically attach to the midgut of permissive vectors in a manner similar to mammalian Leishmania.

As Sauroleishmania transmission from sand flies to reptilian hosts has never been demonstrated under laboratory conditions, stages infectious for the reptiles are not known [15]. Only a few studies have described Sauroleishmania morphological forms produced in vectors [13, 14], assuming they do not differ from those described for mammalian Leishmania. In this study, we demonstrated the presence of stages morphologically identical to metacyclic promastigotes. Nevertheless, the metacyclogenesis of Sauroleishmania has not been studied and thus the potential infectiousness of these forms is unclear.

Although sand fly-derived parasites were used for the experimental infections of geckos, Sauroleishmania infection was not detected in any of the H. turcicus tested. Selection of the wrong host species is unlikely, as L. (S.) hoogstraali was primarily isolated from H. turcicus geckos and this species has also been shown to be susceptible to L. (S.) adleri [18]. Therefore, we assumed that one of the possible explanations of unsuccessful transmission may be the loss of infectivity of both Sauroleishmania strains. Most Sauroleishmania isolates were obtained decades ago and have since been passed for long periods in media without the opportunity to undergo a complete life cycle. It has been shown that prolonged cultivation results in genetic drift and noticeable changes in the mitochondrial genome [40] and, therefore, we consider it necessary to acquire new isolates for future research work.

Conclusions
This study provides experimental evidence that Sauroleishmania development in vectors is variable and significantly affected by sand fly species. Some Phlebotomus species, particularly P. orientalis and P. argenteipes, are highly susceptible to Sauroleishmania infections and, therefore, the role of these sand flies in Sauroleishmania circulation should be reconsidered and further investigated. We also demonstrated the anterior migration of Sauroleishmania in their intestinal tract and confirmed the peripylarian type of development reported by several old studies.

Abbreviations
ITS1: Internal transcribed spacer 1; LPG: Lipophosphoglycan; PBM: Post-blood meal; p.i.: Post-infection.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s13071-022-05417-1.

Additional file 1: Table S1. Localization of Leishmania (Sauroleishmania) adleri promastigotes in three sand fly species differing in vector competence to Leishmania. Table S2. Localization of Leishmania (Sauroleishmania) hoogstraali promastigotes in three sand fly species differing in vector competence to Leishmania.

Additional file 2: Table S3. Development of Leishmania (Sauroleishmania) adleri in three sand fly species sharing an overlapping geographical distribution. Table S4. Development of Leishmania (Sauroleishmania) hoogstraali in three sand fly species sharing an overlapping geographical distribution.

Additional file 3: Table S5. Representation of individual morphological forms of Leishmania (Sauroleishmania) adleri developing in Phlebotomus orientalis on days 5 to 9 post blood meal. Table S6. Detailed measurements of individual forms of Leishmania (Sauroleishmania) adleri developing in Phlebotomus orientalis on days 5, 7 and 9 post blood meal. Table S7. Representation of individual morphological forms of Leishmania (Sauroleishmania) hoogstraali developing in Phlebotomus orientalis on days 5 to 9 post blood meal. Table S8. Detailed measurements of individual forms of Leishmania (Sauroleishmania) hoogstraali developing in Phlebotomus orientalis on days 5, 7 and 9 post blood meal.

Additional file 4: Table S9. Xenodiagnoses of Hemidactylus turcicus geckos experimentally infected with Leishmania (Sauroleishmania) adleri and Leishmania (Sauroleishmania) hoogstraali.
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Author contributions
LT carried out the experimental infections of sand flies and geckos, dissections of sand flies, morphometry of parasites, xenodiagnoses, and molecular analysis. JS contributed to dissections of sand flies, experimental infections of geckos and carried out the statistical analysis. Parasites were provided by PB, PV and JS participated in the design on the study and supervision. Article was drafted by LT and PV. JS and PB contributed with the revision of the manuscript. All authors read and approved the final version of the manuscript.

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Availability of data and materials
All the data are included within the article and its additional files.

Declarations

Ethics approval and consent to participate
Animals were maintained and handled in the animal facility of Charles University in Prague in accordance with institutional guidelines and Czech legislation (Act No. 246/1992 and 359/2012 coll. on the Protection of Animals against Cruelty in present statutes at large), which complies with all relevant European Union and international guidelines for experimental animals. All experiments were approved by the Committee on the Ethics of Laboratory Experiments of various Leishmania (Sauroleishmania) tarentolae strains in three Phlebotomus species. Microorganisms. 2021;9:2326.

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