Results

We tested *Phlebotomus (Adlerius) halepensis* Theodor (Jordan strain) for vector competence, compared with three standard vectors of cutaneous leishmaniases: *Phlebotomus (Phlebotomus) duboscqi, Phlebotomus (Paraphlebotomus) sergenti* and *Lutzomyia*

longipalpis. *Phlebotomus halepensis* showed high susceptibility to both, *Leishmania major* and *L. tropica*, supporting typical suprapylarian parasite development similar to the other vectors. Development of infections was relatively fast, colonizing the thoracic midgut by 6 days post-bloodmeal in every case and reaching the stomodeal valve in >80% of flies. Host choice experiments in the laboratory showed that *P. halepensis* females fed readily on rat or rabbit and preferred the human forearm. In view of its vector competence and partial anthropophily, we infer that *P. halepensis* is a potential vector of cutaneous as well as visceral leishmaniases.

The zinc protease (gp63) of promastigotes was found to play a role in the sand fly part of the *Leishmania* life cycle. *Lutzomyia longipalpis* females were fed with promastigotes of a *Leishmania amazonensis* clone whose gp63 was up- and down-regulated by directional cloning into P6.5 for sense- and anti-sense transcription. Early development was found to differ significantly between the sense- and anti-sense transfectants 2 days post-feeding. The sense transfectants overexpressing gp63 were found similar to those with the vector alone: both developed in the gut at high rates of ~90–100% and at a high density with moderate to heavy parasite loads in >70% of the infected females. In contrast, the anti-sense transfectants with gp63 down-regulated developed at a lower rate (~70%) and, significantly, at a very low density, with moderate to heavy parasite loads only in ~30% of the infected females. On day 9 post-feeding, all three groups of transfectants developed at a similar rate of ~50% with comparable parasite loads. Thus, gp63 plays a role at the early stage of *L. amazonensis* establishment in *L. longipalpis*.

The regurgitation of metacyclic stages from the sand fly cardia is thought to be the prevailing mechanism of *Leishmania* transmission. This regurgitation may result through damage of the stomodeal valve and its mechanical block by the parasites. We found this phenomenon in three sand fly–*Leishmania* models and also in avian trypanosomes transmitted by *Culex* mosquitoes. *Phlebotomus duboscqi, Phlebotomus papatasi, Lutzomyia longipalpis,* and *Culex pipiens* were membrane-fed on blood containing *Leishmania major, Leishmania chagasi* (syn. *infantum*)

and an unidentified avian *Trypanosoma* from *Trypanosoma corvi* clade, respectively. Females with the late-stage infections were processed for the optical and transmission electron microscopy. Localization of the parasites and changes to the stomodeal valve were in some aspects similar in all vector–parasite pairs studied: (i) a large plug of flagellates was observed in cardia region, (ii) parasites were attached to the chitin lining of the stomodeal valve by the formation of zonal hemidesmosome-like plaques. *Leishmania* promastigotes were found both attached to the valve as well as unattached in the lumen of midgut. The stomodeal valve of infected sand flies was opened, its chitin lining was destroyed and the unique filamentous structures on the apical end of cylindrical cells were degraded. In the *Culex– Trypanosoma* model, the whole population of epimastigotes was found in close contact with the chitin lining, and degenerative changes of the valve were less pronounced. We suggest that the phenomenon involving a blocked valve facilitating the regurgitation of parasites into the vertebrate host may occur generally in heteroxenous trypanosomatids transmitted by the bite of nematoceran Diptera.