

## ***Leishmania (Sauroleishmania)* and other trypanosomatids characterization (*hsp70* sequencing and MLEE) of isolates from reptiles and sand flies**

Blanca Gurrea-Arocas<sup>1</sup>, Alba Abras<sup>2</sup>, Albert Arnau<sup>1,2</sup>, Silvia Tebar<sup>1</sup>, Teresa Llovet<sup>3</sup>, Francine Pratlong<sup>4</sup>, Carme Muñoz<sup>3,5,6</sup>, Montserrat Gállego<sup>1,7,8</sup>, Cristina Ballart<sup>1,7\*</sup>, Anna Fernández-Arévalo<sup>1\*</sup>

1. Universitat de Barcelona, Barcelona, Spain. 2. Universitat de Girona, Girona, Spain. 3. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain. 4. Centre National de Référence des Leishmanioses, Montpellier, France. 5. Institut de Recerca Biomèdica Sant Pau, Barcelona, Spain. 6. Universitat Autònoma de Barcelona, Bellaterra, Spain. 7. Institut de Salut Global de Barcelona (ISGlobal), Barcelona, Spain. 8. Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC), Spain.

\*[afernandezare@ub.edu](mailto:afernandezare@ub.edu); [cristinaballartferrer@ub.edu](mailto:cristinaballartferrer@ub.edu)

### **Background**

*Sauroleishmania* is a subgenus of kinetoplastida protozoa within the genus *Leishmania*. It is transmitted by *Sergentomyia* sand flies and infects mainly reptiles, although cases have been described in mammals, including humans [1]. As classically it is not considered pathogenic, this group of leishmanias has been less studied and neither their epidemiological role nor their genetic diversity have been deeply explored. In order to provide data in this regard, the aim of this study was to characterize a collection of suspected *Sauroleishmania* strains isolated from reptiles and *Sergentomyia* spp. and stored in the trypanosomatids cryobanks of the Universities of Montpellier and Barcelona.

### **Materials and methods**

Thirty-six strains isolated from three reptile species and six *Sergentomyia* species, from diverse geographical origins within the Old World, were characterized by sequencing a 1245pb fragment of the *hsp70* gene and by a preliminary multilocus enzyme electrophoresis (MLEE) analysis. The genetic variability between and within the strains was studied in comparison with reference sequences available in GenBank. In addition, phylogenetic relationships between the strains were analysed by the construction of a neighbour-joining tree. For MLEE, only marker strains representing the two *Sauroleishmania* zymodemes described until now (MON-95 and MON-96) and zymodeme MON-1 were used.

### **Results**

Most of the strains (26/36) were characterized as *L. (S.) tarentolae* by *hsp70* sequencing. Twenty-one of these were confirmed by MLEE as MON-95 (n=19) and MON-96 (n=2), and five showed slight variations of these electromorphic profiles. Seven additional strains were characterized as *Sauroleishmania* by *hsp70*, but it was not possible to assign them a specific species. On the phylogenetic analysis, these strains formed three independent clusters (named *Sauroleishmania* II (n=5), III (n=1) and IV (n=1)), separated from *L. tarentolae*, but within the *Sauroleishmania* branch. By MLEE, strains within *Sauroleishmania* group II were characterized as MON-95 (n=2) or variants (n=3). Groups III and IV presented a major different profile. The remaining three strains included in this study showed completely different mobilities in the MLEE analysis, which did not correspond to *Sauroleishmania*. By *hsp70*, two of them were identified as human pathogenic *Leishmania* species: one *L. tropica* isolated from a reptile of unknown geographical origin and one *L. donovani* complex strain from a *Sergentomyia* sand fly from Portugal. The third strain, a *Sergentomyia* isolate from Spain, was characterized as *Trypanosoma* sp. (Table 1). Further research on *Sauroleishmania* would not only contribute to a better understanding of this neglected subgroup but would also improve the knowledge of pathogenic species [2].

**Table 1.** Summary of characterization results and information about isolation hosts and countries.

Nº of strains	<i>hsp70</i> identification	MLEE	Hosts	Country
26	<i>L. (S.) tarentolae</i> (group I)	MON-95 (n=19)	<i>Tarentola mauritanica</i>	Italy, Spain
			<i>Cyrtodactylus kotschyi</i>	Italy
		MON-96 (n=2)	<i>Sergentomyia minuta</i>	France, Spain
			<i>T. mauritanica</i>	Algeria
5	<i>L. (Sauroleishmania)</i> (group II)	Variant (n=3)	<i>S. minuta</i>	Spain
			<i>T. mauritanica</i>	Italy, Spain
			<i>Sergentomyia fallax</i>	Morocco
1	<i>L. (Sauroleishmania)</i> (group III)	Different	<i>S. minuta</i>	Morocco
5	<i>L. (Sauroleishmania)</i> (group II)	MON-95 (n=2)	<i>Sergentomyia antennata</i>	Algeria
Variant (n=3)		<i>Gymnodactylus</i> sp.	Sudan	
		<i>Sergentomyia antennata</i>	Algeria	
1	<i>L. (Sauroleishmania)</i> (group IV)	Different	<i>Sergentomyia clydei</i>	Algeria
1	<i>L. (L.) tropica</i>	Different	<i>Sergentomyia baghdadis</i>	Algeria
1	<i>L. (L.) donovani</i> complex	Different	<i>Sergentomyia schwetzi</i>	Sudan
1	<i>Trypanosoma</i> sp.	NA	<i>T. mauritanica</i>	Unknown
1	<i>L. (L.) donovani</i> complex	Different	<i>S. minuta</i>	Portugal
1	<i>Trypanosoma</i> sp.	NA	<i>S. minuta</i>	Spain

### Conclusions

Four different *Sauroleishmania* clusters and three other species of trypanosomatids were found in reptiles and *Sergentomyia* sandflies by *hsp70*. MLEE analysis revealed the existence of new variants in addition to the two zymodemes of *Sauroleishmania* described. On the one hand, these results show a great genetic diversity still poorly explored within the subgenus *Sauroleishmania*, and how the limited number of studies and characterised reference strains hampered the definitive identification of some strains. On the other hand, this work highlights the presence of unexpected *Leishmania* species (*L. tropica* and *L. donovani* complex) in the hosts studied, as already reported in the literature.

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### References

- Mendoza-Roldan JA, Votýpka J, Bandi C, Epis S, Modrý D, Tichá L, et al. *Leishmania tarentolae*: A new frontier in the epidemiology and control of the leishmaniases. *Transbound Emerg Dis.* 2022;69:e1326–37.
- Campino L, Cortes S, Dionísio L, Neto L, Afonso MO, Maia C. The first detection of *Leishmania major* in naturally infected *Sergentomyia minuta* in Portugal. *Mem Inst Oswaldo Cruz.* 2013;108:516–8.