

# Speculations on the Origin and Evolution of the Genus *Leishmania*

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*Recently two hypotheses have been proposed for the evolution of Leishmania involving respectively a Neotropical or Palearctic origin for the species. Here an alternative proposal on the phylogeny of Leishmania based on the major divisions within the genus is presented. In this hypothesis a Neotropic origin is retained for L. (Viannia) and Paraleishmania, a recently described section within the genus Leishmania, while an African origin is proposed for L. (Leishmania) and possibly Sauroleishmania. The current distribution of Leishmania in the Neotropics is explained as the product of multiple introductions of Leishmania parasites into the New World. Problems with organismal identity in Sauroleishmania and the use of molecular sequence data in inferring phylogenies are also discussed.*

Key words: Euleishmania - Paraleishmania - *Leishmania* evolution - biogeographical associations - phylogeny

Recent molecular sequence studies have revived interest on the origin and evolution of the genus *Leishmania*. Noyes (1998) has renewed the hypothesis of a Neotropical origin for the genus using arguments mainly based on the published gene sequence phylogenies. This hypothesis has been contested by Kerr (2000) who instead proposed a Palearctic origin for *Leishmania* and suggested that the genus was only introduced into the Neotropics during the Pliocene after the formation of the Panamanian land bridge about 3 million years ago.

## MAJOR DIVISIONS IN THE GENUS *LEISHMANIA*

More recently Cupolillo et al. (2000) have proposed the separation of the genus *Leishmania* into two divisions, Euleishmania and Paraleishmania, called sections by analogy with a similar division in *Trypanosoma*. The Euleishmania is comprised of the subgenera *Leishmania* and *Viannia* as described by Lainson and Shaw (1987) and the Paraleishmania, which consists of *L. hertigi*, *L. deanei*, *L. colombiensis*, *L. equatorensis*, *L. herreri* as well as strains of *Endotrypanum*. These two sections can be clearly distinguished by a variety of molecular techniques (Cupolillo et al. 2000).

The Paraleishmania can also be further subdivided by the molecular criteria, one group formed

by parasites of histricomorph rodents (porcupines) namely *L. hertigi* and *L. deanei* and the other group formed by the remaining species which are principally parasites of sloths. Strains of *Endotrypanum* currently maintained in laboratory collections form a polyphyletic group within the Paraleishmania. The strains cannot currently be shown to infect erythrocytes nor demonstrate the characteristic trypomastigote or epimastigote forms of the genus. They are probably better considered as species of Paraleishmania from which they are currently indistinguishable and the name *Endotrypanum* reserved for the true intraerythrocytic parasite of sloths when this parasite is eventually re-isolated and can demonstrate features associated with the description of the genus.

In the Table the major groupings presently recognised in the genus *Leishmania* are listed. An examination of this Table would indicate an Old World origin for *Sauroleishmania* and a New World origin for Paraleishmania and *L. (Viannia)* as the most parsimonious explanations (without involving reverse migrations between or extinctions within either the Old World or New World) for the evolution of these groups. As the genus *Leishmania* is thought to be monophyletic (Thomaz-Soccol et al. 1993, Croan et al. 1997), the origin of the *L. (Leishmania)* subgenus which has a worldwide distribution appears then to be the key for understanding the phylogeny of the genus.

## AN AFRICAN ORIGIN FOR *L. (LEISHMANIA)*

*L. (Leishmania)* can be divided into a number of species complexes. In the Old World the principal species complexes are *L. donovani/L. infantum*, *L. tropica*, *L. major* and *L. aethiopica*. There are

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TABLE  
Distribution of principal *Leishmania* groups

Genus/sub-genus/species	Section	Vector	Distribution
Sauroleishmania	?	<i>Sergentomyia</i>	Old World
<i>L. (Leishmania)</i>	Euleishmania	<i>Phlebotomus/Lutzomyia</i>	Worldwide
<i>L. (Viannia)</i>	Euleishmania	<i>Lutzomyia</i>	New World
<i>L. hertigi, L. deanei</i> <sup>a</sup>	Paraleishmania	<i>Lutzomyia</i>	New World
<i>L. colombiensis, L. equatorensis, L. herreri</i> <sup>a</sup>	Paraleishmania	<i>Lutzomyia</i>	New World

a: includes laboratory strains currently classified as *Endotrypanum*.

strong indications that all of these complexes have an African origin.

*L. aethiopica* occurs only in the Ethiopian and Kenyan highlands, its reservoir is the rock hyrax and its vector is *P. (Larroussius)*. Due to its restricted geographical range it seems reasonable to assume an African origin for this species as well as for other *L. (Leishmania)* – hyrax systems that occur in Africa such as that found in Namibia (Lanotte et al. 1986).

Four host parasite-ecology systems have been described for *L. major* based on the principal vertebrate hosts namely *Psammomys*, *Meriones*, *Rhombomys* and *Arvicanthis* and the sand fly vectors of the genus *Phlebotomus*. Of these the *Arvicanthis/Phlebotomus* has been assumed to be the most primitive system and an evolutionary process originating with *Arvicanthis* transferring to *Meriones* and then to the other hosts has been postulated (Ashford 1986). The distribution of *Arvicanthis* is restricted to sub-Saharan Africa, therefore an African origin for this species is possible.

A number of studies have shown that the *L. donovani* and *L. infantum* complexes are monophyletic (Mauricio et al. 1999) and a common origin in East Africa has been postulated for these visceralizing species based on a cladistic analysis of isoenzymes (Moreno et al. 1986). An African origin for the visceralizing species of the *L. donovani* complex has also been argued by Ashford et al. (1992). They have suggested an ancient cluster derived from an ancestral root stock in the Sudan from which all other forms of the complex have derived. A primitive host parasite-ecology system in *Arvicanthis* has also been proposed for this species (Ashford 1986).

Traditionally the distribution of *L. tropica* was not associated with Africa (Lysenko 1971) however *L. killicki* a member of the species complex is found in this continent (Rioux et al. 1986). More recently foci of *L. tropica* have been identified in Kenya (Mebratu et al. 1992) and *L. tropica* is not known to be zoonotic anywhere else but Africa in its distribution (Sang et al. 1994). Man is believed to have originated in Africa and it is reasonable to

consider that anthroponotic parasites such as members of the *L. tropica* and (*L. donovani*) complex which have evolved with him may also have originated there.

We therefore propose that the Old World species of *Leishmania* evolved in Africa probably from an ancestral origin in East Africa. In the New World *L. (Leishmania)* contains the *L. mexicana* complex and *L. chagasi*. Many authors now consider *L. chagasi* to have originated from *L. infantum* strains brought to the New World in historical times (Killick-Kendrick et al. 1980, Moreno et al. 1986, Momen et al. 1987, Thomaz-Soccol et al. 1993, Mauricio et al. 1999). This parasite therefore also seems to be derived from the same African root stock as *L. infantum*.

*L. mexicana* shares many characteristics with *L. major* (Lainson & Shaw 1987). In particular *L. mexicana* in North America (Texas) has many similarities to *L. major* in Asia (Kerr 2000). It has been postulated that *L. (Leishmania)* could have evolved in the Palearctic and dispersed to the Nearctic together with its rodent reservoirs during the Eocene at which time the Bering land bridge was intact (Thomaz-Soccol et al. 1993, Kerr 2000). *L. (Leishmania)* could then have entered the Neotropics either by island hopping (Cox & Moore 2000) as species of the *L. mexicana* complex are found presently on a number of Caribbean islands, or after the formation of the Panamanian land bridge during the Pliocene. In South America climatic and ecological factors could have favoured further speciation giving rise to other members of the the *L. mexicana* complex such as *L. venezuelensis*, *L. amazonensis*, *L. garnhami*, *L. foratinni* and by adaptation to guinea pigs the related species *L. enrietti*.

The alternative hypothesis of a Neotropical origin for *L. (Leishmania)* and subsequent migration to the Nearctic and Palearctic raises several problems as outlined by Noyes (1998) and Kerr (2000). These problems include inconsistency with current classifications of sand flies, the necessity for multiple adaptations to murine rodents in the Neotropic, Nearctic and Palearctic or reverse migration of the parasite across the Behring straits.

## CAN WE TRUST THE TREES WE HAVE

If *L. (Leishmania)* arose in Africa, how can we reconcile this with the origin of the other major clades within the genus and the molecular phylogenetic trees. Molecular sequence data have been the basis for much of the advances in modern phylogenetics revealing new relationships between organisms that were overlooked by more traditional methods. However the vast increase in molecular data has also brought to surface increased concern about the resolution of molecular phylogenies.

These criticisms can be classified into at least three kinds. The first kind of criticism can be considered methodological and include problems such as long branch attractions (Felsenstein 1978) mutational saturation, and among site rate variation (Yang 1996). Phillippe (1998) has reviewed the numerous phylogenies that have been published for the kinetoplastids and discusses possible explanations for the observed discrepancies. He has also demonstrated the lack of molecular clock behaviour for all genes studied in the kinetoplastids. Stothard (2000) has pointed out the problem of paralogous genes producing false phylogenies when tandem repeated genes present in the genome are used to construct trees.

The second kind of criticism is directed at the conceptual basis of molecular phylogeny and can be considered epistemological. It is concerned with the idea that gene phylogeny cannot be equated with organismal phylogeny and that organisms are more than just the sum of their genes (Doolittle 1999). The third kind of criticism has arisen from the surge in genome sequence information which has demonstrated numerous cases of horizontal gene transfer (HGT) between organisms. For example 234 examples of HGT have been detected since the divergence of *Escherichia coli* and *Salmonella* comprising about 17% of the genome (Lawrence & Ochman 1998). Although most examples are from prokaryotes, eukaryotes have also been shown to possess the same capacity and similar mechanism for HGT (Cruz & Davies 2000). These authors have suggested that HGT can provide an alternative explanation for macroevolution in certain situations and thus questioning in these cases phylogenies based on fixed mutations.

These concerns have led Doolittle (1999) to conclude that the relationship between genes "is thus not a fair (at least complete) depiction of the actual evolutionary history of any lineage of real organisms".

## SAUROLEISHMANIA

Bearing Doolittle's warning in mind we can now return to our analysis of the major *Leishmania* groups. The position of the reptile *Leishmania* has

been a point of contention. The position of these parasites at the crown of the molecular trees (Croan et al. 1997) indicating a recent origin and monophyly with *L. (Leishmania)* is at odds with the opinion of most field parasitologists who consider the parasites belong to a separate genus *Sauroleishmania* based on a variety of extrinsic and intrinsic characters (Killick-Kendrick et al. 1986).

In addition to the general concerns of molecular phylogenetics, the trees involving *Sauroleishmania* have particular problems involving organismal identity. Most *Sauroleishmania* isolates have been maintained as cryostabilates and/or *in vitro* culture for several decades with all the implications for gene selection and confusion over identity. In addition the isolate of *L. adleri* used in several studies is indistinguishable from a reference strain of *L. major* (Cupolillo, unpublished results). A further example of possible misidentification in reptile *Leishmania* is given by Telford (1985). For these reasons the *Sauroleishmania* were not included in the sections proposed by Cupolillo et al. (2000).

Therefore molecular trees involving *Sauroleishmania* should be treated with particular caution until many fresh isolates from a variety of reptile hosts become available. In view of the current lack of field parasitologists working on reptile *Leishmania* we would agree with Noyes et al. (1998) that if these parasites were placed in a separate genus there is a risk that they will be ignored as an academic curiosity.

The molecular tree of Croan et al. (1997) could indicate that the *Sauroleishmania* diverged from *L. (Leishmania)* in the Palearctic prior to the migration of the latter through the Behring straits. However alternative explanations for the origin of *Sauroleishmania* which are not supported by the current molecular phylogeny have been proposed. These generally place the *Sauroleishmania* at the root of the tree due to development in the vector similar to that of primitive insect trypanosomatids and the ancient origins of lizards (Lainson & Shaw 1987). *Sauroleishmania* have been isolated from several species of lizards in East Africa so that *L. (Leishmania)* could have diverged from reptile parasites in this region under this alternative hypothesis. In this sense the report by Okot-Kotber et al. (1989) of the isolation of *L. major* from a lizard in this area is of interest.

## L. (VIANNIA) AND PARALEISHMANIA

Kerr (2000) has considered that *L. (Viannia)* originated through rapid speciation, greatly favoured by climatic and ecological factors, after the introduction of *L. (Leishmania)* in the Neotropic. However the large genetic distance

between the two subgenera and the great genetic diversity within *L. (Viannia)* (Cupolillo et al. 1995) together with the preferential association of the latter subgenus with indigenous mammals of the New World favours a more ancient origin. We prefer the hypothesis that with the separation of Gondwana in the Mesozoic, *Euleishmania* evolved into *L. (Leishmania)* in Africa and *L. (Viannia)* in South America as previously proposed by Saf'janova (1986) and Thomaz-Soccol et al. (1993).

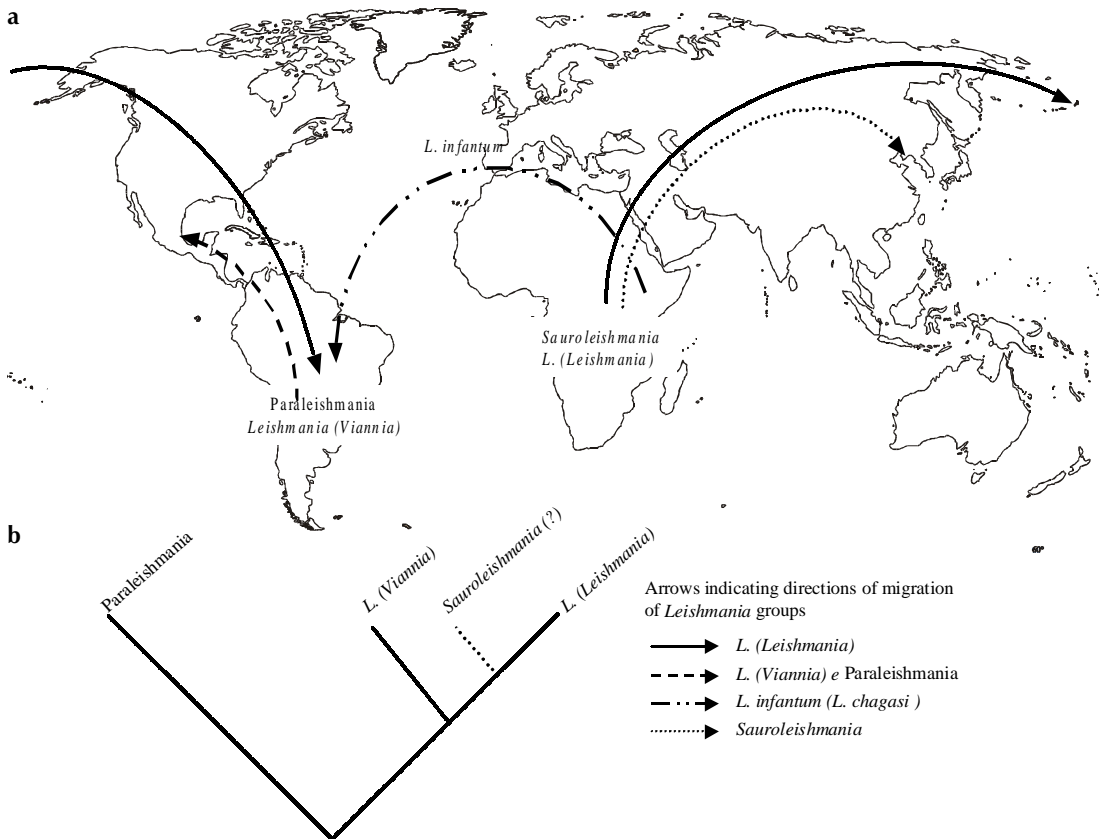
Further studies are required to understand the evolution of *Paraleishmania*. The wide difference between the molecular properties of *Euleishmania* and consequently *L. (Viannia)* on the one hand and *Paraleishmania* on the other (Cupolillo et al. 2000) would also favour an ancient divergence between these groups. However some similarities between *Paraleishmania* and *L. (Viannia)* have also been noted such as cross-hybridization with kDNA (Pacheco et al. 1990) as well as absence of GP46/M2 genes in *Paraleishmania* and *L. (Viannia)* and their presence in *L. (Leishmania)* and *Sauroleishmania* (McMahon-Pratt et al. 1992, Cupolillo, unpublished results). We can speculate that the separation of the genus into two sections occurred either

before the separation of Gondwana or the distinct *Paraleishmania* of porcupines may suggest an origin in these animals as porcupines are host to no other kind of *Leishmania*.

**MULTIPLE ORIGINS OF LEISHMANIA IN THE NEOTROPICS**

The present hypothesis on the origin of *Leishmania* better reflects the molecular systematic data available and in particular the large differences between the *Paraleishmania* and the *Euleishmania* subgenera *Viannia* and *Leishmania*. It is important to compare phylogenies based on several independent genes that display different evolutionary constraints as suggested by Phillippe (1998). He suggests the elongation factor (EF-1<sup>a</sup>), heat shock protein (HSP70) and glyceraldehyde dehydrogenase (GAPDH) genes, all single copy protein coding genes, as suitable candidates for the study of the molecular systematics of kinetoplastids.

Until further data with large sampling becomes available we would propose an African origin for *L. (Leishmania)* associated with *Sauroleishmania* and four separate events or introductions of the genus *Leishmania* into the Neotropics (Figure). *L. (Viannia)*



a: map indicating possible migration routes of major *Leishmania* groups; b: phylogram showing relationships among major *Leishmania* groups.

with the separation of Gondwana in the Mesozoic; Paraleishmania with the introduction of hystricomorph rodents in the early Cenozoic; *L. mexicana* with the formation of the Panamanian land bridge in the Pliocene and *L. chagasi* in Recent.

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