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2.1 Introduction

Taxonomy stems from the human need for order, as it is much easier for the human brain to deal with things that can be put away neatly in boxes. *Leishmania* taxonomists have long been known to create and use many boxes, based on different and often inconsistent criteria, in an attempt to organize the huge clinical and geographical diversity observed in this genus. In the last three decades, with the use of molecular biology and ever more reliable phylogenetic tree building methods, as well as the findings of new variants, researchers have reorganized the various *Leishmania* boxes.

This chapter will present and discuss the most current consensus classification, the identity of recently described species and the added complexity of hybrid or recombinant lineages, in the context of the methods and markers used in taxonomic studies.

2.2 The Genus *Leishmania*

The genus *Leishmania* Ross, 1903, is considered to belong to:

Empire Eukaryota Cavalier-Smith, 1998
Kingdom Protozoa Cavalier-Smith, 2002
Infrakingdom Excavata Cavalier-Smith, 2003
Phylum Euglenozoa Cavalier-Smith, 1993

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Class Kinetoplastida Honigberg, 1963 (or the synonymous Kinetoplastea Cavalier-Smith, 1981), although Kinetoplastida is also often used for order
Order Trypanosomatida Kent, 1880
Family Trypanosomatidae Doflein, 1901

The genus *Leishmania* has previously been proposed to be divided into section Paraleishmania and section Euleishmania (Cupolillo et al. 2000), which is comprised of three subgenera: *L. (Leishmania)*, *L. (Viannia)* and *L. (Sauroleishmania)*. At least 39 described species of *Leishmania* can be found in the literature, with additional, yet unnamed or informally named, parasites. Many of these described species have, since, been shown to be synonymous, and taxonomy simplification has been argued for (Fraga et al. 2010; Schönian et al. 2010). It is, thus, worth to briefly look into what a species is.

2.3 Concepts of Species

The biological concept of species (Mayr, 1942) is based on reproductive isolation between populations. Briefly, if individuals from different groups are not able to produce fertile offspring, then the two groups can be considered separate species. The biological concept of species has not been and cannot be applied for *Leishmania* taxonomy, because sexual reproduction is difficult to detect and its importance in nature is still controversial (Ramírez and Llewellyn 2014; Rougeron et al. 2017).

Initial definitions of species in *Leishmania* followed relatively ad hoc principles, in a mixture of ecological, morphological, phenetic and clinical concepts. Thus, many species definitions and their names reflect this heritage. One such example is *L. major*, which was once considered a sub-species of *L. tropica*—*L. tropica major*—based on morphology, and in relation to the also since abandoned sub-species *L. tropica minor*, which is now the species *L. tropica* which corresponded to smaller forms (Safjanova and Aliev 1973). *L. infantum* was based on epidemiological data, as it is considered to be more prevalent in infants, whereas species such as *L. guyanensis*, *L. mexicana*, *L. braziliensis*, etc. were named, as well as defined, based on their geographical distribution or region of first description. Some of the current issues in *Leishmania* taxonomy and classification are heirs to such criteria.

Other concepts that have been applied to microorganisms, particularly those without recognizable sexual reproduction, such as bacteria, include the phylogenetic concept of species, which is based on common descent. Briefly, a species will be defined as a group of individuals with a common ancestor and that are closely related or share certain traits. It has been proposed that the phylogenetic criteria should form the basis of a Universal Species Concept (Staley 2009). However, species thus defined can be somewhat arbitrary, because it depends on the level of distance chosen as cut-off point.

The phylogenetic concept of species has gained prominence in *Leishmania*. However, for the species or groups to be useful they should make biological and clinical sense. In *Leishmania*, systematic revisions have re-evaluated several groups

of species, based on phylogenetic analyses of a range of markers, from the most polymorphic, such as microsatellite loci, which perform well in population studies, to the most conserved, such as RNA polymerases and the ribosomal small subunit (SSU), which perform well in the study of deeper phylogenetic relationships. Unfortunately, to date, no single marker has been applied to all described taxa within the genus *Leishmania*.

2.4 Organizing *Leishmania*

Organisms in the genus *Leishmania* cause a wide range of clinical manifestations, from visceral (VL) to cutaneous leishmaniasis (CL) (see Chap. 6). They can infect and be transmitted by a wide range of hosts and vectors, respectively (see Chaps. 3 and 4).

Recent taxonomic revisions have shown that several species designations correspond to low diversity genetic groups, effectively indistinguishable from another species, which often includes other similar sized genetic groups. Examples include *L. shawi* within *L. guyanensis*, *L. peruviana* within *L. braziliensis*, *L. killicki* within *L. tropica* and even *L. infantum* within *L. donovani*. The main question is whether *Leishmania* research requires more or less boxes. Should we recognize the intricate and complex genetic diversity within a larger group and assign species names to all groups? Or should we work with a much reduced number of species? Increasing the number of species would, undoubtedly, increase confusion among researchers and would introduce greater uncertainty, as the boundaries between species would be much more difficult to define. On the other hand, reducing the number of species could remove information about variants that can have clinical and epidemiological implications. A good example of the dilemma faced by taxonomists is *L. donovani* or the *L. donovani* complex. By eliminating species names such as *L. infantum*, the information that a group of isolates or lineages has dogs as reservoir is lost. But recognizing that subgroups with geographical associations, such as in Sudan/Ethiopia and in India have quite different vectors and clinical traits, would increase dramatically the number of species, possibly to the point of being unusable.

One option is to apply the concept of sub-species, even if not in the strict sense of a geographically isolated group within a species. In that sense, *L. infantum* would become a sub-species of *L. donovani*, so *L. donovani infantum*. But, what to make of the South American variants of *L. donovani infantum*, which are effectively isolated geographically? Or of the two groups of *L. infantum*, as defined by microsatellite analyses, that have become known as (zymodeme Montpellier) “(MON)-1” and “non-MON-1” and that preferentially cause visceral or cutaneous disease, respectively? Should these also be given a taxonomic status or not?

It might be useful to apply the concept of superspecies and infra-species in this context (Mallet 2007). This concept was initially introduced by Mayr and Rensch and a notation proposed by Amadon (1966), in which the designation for the superspecies would appear in brackets. An example would be *Leishmania (Leishmania) [donovani] donovani* and *Leishmania (Leishmania) [donovani] infantum*.

Ultimately, taxonomic revisions should take into account the experience and needs of clinicians and laboratory scientists, and there should be greater communication between the two approaches. Taxonomy influences the choice of samples when studying parasite–host relationships, drug testing and, crucially, diagnostics. Are the samples representative of the species or of its diversity? Could some samples be too closely related that results obtained are too similar and not possible to extrapolate to the entire species?

As pointed out by Kuhn and Jahrling (2010), for the recent classification of viruses, most people, including researchers, are not fully equipped to deal with the intricacies of what constitutes different species, and many do not regularly read taxonomic revisions. As such, incorrect or outdated designations often persist in the literature. This issue is further confounded and maintained by taxonomic uncertainties. The next sections will review the consensus taxonomy for specific groups and the points of current debate.

2.4.1 A Visceral Question: *Leishmania donovani*

Leishmania donovani includes the aetiological agents of VL, a form that can be fatal without treatment. It has one of the widest geographical ranges of *Leishmania*, which only excludes Oceania and Antarctica, and it is the main species of *Leishmania* present in Europe. As such, it is perhaps not surprising that, despite its low intraspecific diversity, it is one of the most intensively sampled and genotyped taxa of the genus *Leishmania*. In spite, or because, of this, it has suffered extensive splitting into different species by some authors, although recognized to be close and considered by some authors as the *L. donovani* complex of species. Four species have been described: *L. donovani* (also often divided into *sensu stricto*, in India, and *sensu lato*, in East Africa), *L. infantum*, *L. archibaldi* and *L. chagasi* (Table 2.1). The initial support for such divisions was based on limited markers, particularly the description of *L. archibaldi* (Rioux et al. 1990) or a small number of strains, as reviewed previously regarding the *L. infantum*/*L. chagasi* debate (Mauricio et al. 2000). Indeed, in the past two decades, it has been consistently shown that it is not possible to distinguish populations from Europe and South America of, respectively, *L. infantum* and *L. chagasi*, using several techniques and targets, such as multilocus microsatellite typing (MLMT) (Kuhls et al. 2007) and multilocus sequence typing (MLST) (Zemanova et al. 2007; Mauricio et al. 2006), ribosomal internal transcribed spacer (ITS) and mini-exon PCR-RFLP (Mauricio et al. 2004) or random amplification of polymorphic DNA (RAPD) (Mauricio et al. 1999). These studies, which have included several strains from a wide geographical range, have supported synonymy. Considering that the epidemiology in the two regions is very similar, there are no molecular, clinical or epidemiological reasons that warrant separation into two species. However some researchers still claim they can be distinguished (e.g. Marcili et al. 2014), despite using restricted and unrepresentative sampling. In fact, an MLMT extensive study of 450 strains of the *L. donovani* complex had already shown that South American strains were most similar to those from Portugal and Spain and supported a recent introduction (in the past 500 years) consistent with the European colonization of South America (Leblois et al. 2011).

Table 2.1 Simplified nomenclature of the genus *Leishmania* (adapted from Fraga et al. 2010; Schönian et al. 2010)

Subgenus	Species	Other associated species names	Notes
<i>L. (Leishmania)</i>	<i>L. donovani</i>	<i>L. archibaldi</i>	
		<i>L. chagasi</i>	
		<i>L. infantum</i>	
	<i>L. major</i>	<i>L. arabica</i>	
		<i>L. gerbilli</i>	
		<i>L. turanica</i>	
	<i>L. tropica</i>	<i>L. aethiopica</i>	
		<i>L. killicki</i>	
	<i>L. mexicana</i>	<i>L. amazonensis</i>	
		<i>L. aristidesi</i>	
		<i>L. garnhami</i>	
		<i>L. forattinii</i>	
		<i>L. pifanoi</i>	
<i>L. venezuelensis</i>			
	<i>L. waltoni</i>		
<i>L. (Sauroleishmania)</i> ^a	<i>L. tarentolae</i>		Telford (2009)
	<i>L. adleri</i>		
	<i>L. gymnodactyli</i>		
	<i>L. hoogstraali</i>		
	<i>L. guliki</i> ^b		
	<i>L. zuckermani</i> ^c		
<i>L. platycephala</i> ^c	Telford (2009)		
<i>L. (Viannia)</i>	<i>L. braziliensis</i>	<i>L. peruviana</i>	
	<i>L. guyanensis</i>	<i>L. panamensis</i>	
	<i>L. lindenbergi</i>	<i>L. shawi</i>	
	<i>L. utingensis</i>		
	<i>L. lainsoni</i>		
	<i>L. naiffi</i>		
Unnamed	<i>L. enrietti</i>		Harkins et al. (2016) and
	<i>L. sp. (Ghana)</i> ^d		Kwakye-Nuako et al. (2015)
	" <i>L. siamensis</i> "		No formal description
	<i>L. martiniquensis</i>		
	<i>L. sp. (AM-2004)</i>		
<i>L. (Endotrypanum)</i> ^e	<i>L. hertigi</i>	<i>L. deanei</i>	
	<i>E. monterogei</i> ^a	<i>E. schaudinni</i>	
		<i>E. sp</i>	
		<i>L. colombiensis</i>	
		<i>L. equatoriensis</i>	

^aSubject to confirmation^bSpecies description and one mitochondrial sequence available, with greatest similarity to *L. tarentolae*^cSpecies description, no molecular data^dPossible revision as *L. enrietti*^eSuggestion of subgenus, in alternative to section Paraleishmania or genus *Endotrypanum* for all species

Another taxonomic problem was the description of a separate species from *L. donovani*, *L. archibaldi* (Rioux et al. 1990), which has been shown not to be valid in its original definition based on multilocus enzyme electrophoresis (MLEE) analysis, but instead aspartate aminotransferase (ASAT) heterozygotes of *L. donovani*, and which thus led to misclassification of some *L. donovani* samples from East Africa as *L. infantum*, both demonstrated by further studies using markers such as ITS and the mini-exon (Mauricio et al. 2004), MLST (Mauricio et al. 2006; Zemanova et al. 2007) and MLMT (Kuhls et al. 2007) and, finally, in a joint analysis with a large number and variety of markers (Lukes et al. 2007).

It is of note that, although members of the *L. donovani* complex are agents of VL, a group within *L. infantum* (and referred to as non-MON-1) has been identified, through MLMT, that is mostly associated with CL in individuals without immune depressions (Kuhls et al. 2007). It would be more clinically useful to attribute to this group a sub-species status than to all South American *L. infantum*.

The genetic diversity within *L. donovani* can also have implications for control and diagnostics, such as it happened with rk39, which, despite successful implementation elsewhere, was found to have very low sensitivity when applied in Sudanese populations (Ritmeijer et al. 2006).

Despite some evidence for genetic groups and specific characteristics in some groups, genetic recombination has been shown to have occurred between *L. donovani* populations, including with *L. infantum* (Mauricio et al. 2006). As a result, it is very difficult to find consistent phylogenetic or diagnostic markers for any species, or subgroups, within the complex, and it is likely that it will become more difficult as more variants are found, such as in Sri Lanka and Cyprus (Alam et al. 2009). So, for diagnostic purposes and epidemiological studies, it seems more useful to recognize the existence of a single species, *L. donovani*, and analyse it as a single entity (Table 2.1), although research should take into account its full genetic diversity range.

2.4.2 A Major Issue: Parasite Species of Old World Rodents

L. major has been a non-controversial species, with limited genetic and clinical diversity, although subpopulations have been identified by MLMT with strong geographical associations (Al-Jawabreh et al. 2008). However, in the Old World, some *Leishmania* isolates from gerbils, the reservoir host of *L. major*, and so far not found to infect humans, had been found to be sufficiently distinct to be classified into different species: *L. turanica*, *L. arabica* and *L. gerbilli* (Table 2.1). Phylogenetic analyses that have included these species have shown that they form a monophyletic group with *L. major*, but that they are each sufficiently distant from *L. major*, by comparison with other species and complex of species, to warrant separate species status: a gp63 gene that included *L. turanica* and *L. arabica* (Mauricio et al. 2007), cytochrome b that included *L. turanica* and *L. arabica* (Asato et al. 2009), MLST of seven loci that included *L. turanica* and *L. gerbilli* (Auwera et al. 2014) or all three species (Baidouri et al. 2013). Such results, and considering the common association with gerbils,

would support definition of a *L. major* species complex to include these four species to reflect a common origin and ecological similarities (Table 2.1).

2.4.3 Not a Minor Issue: The Case of *Leishmania tropica* and Hyraxes

Phylogenetics can be used to generate hypothesis regarding newly isolated species, for example, in terms of the reservoir hosts, vectors, possible clinical presentations, drug response, etc.

One such case is of *L. tropica* and *L. aethiopica*. *L. tropica*, once named as *L. tropica minor*, is considered to have an anthroponotic life cycle for most of its geographical range, whereas *L. aethiopica* has hyraxes as reservoirs, although also capable of infecting humans. Phylogenetic studies had showed that *L. aethiopica* and *L. tropica* were closely related species, suggesting that *L. tropica* could have evolved from a parasite of hyraxes and that it could still infect this species, a close relative of elephants. Indeed, *L. tropica* isolates were eventually found in hyraxes (Jacobson et al. 2003; Jaffe et al. 2004) leading to the hypothesis that differentiation between the *L. major* and the *L. tropica/L. aethiopica* lineages was driven by host associations, in particular by successful colonization of hyraxes by parasites originally associated with rodents (Mauricio et al. 2007).

A third related species, *L. killicki*, has been shown to be a small subgroup of *L. tropica* and should, thus, be considered synonymous (Baidouri et al. 2013; Chaara et al. 2015). It is not so consensual how to classify *L. aethiopica*, as it can appear to be very close to *L. tropica* (Fraga et al. 2010; Krayter et al. 2015) or to form a separated group from *L. tropica* in analyses of several strains (Asato et al. 2009; Baidouri et al. 2013; Auwera et al. 2014), although in an analysis of *hsp20*, they did not form a monophyletic cluster (Fraga et al. 2013). However, by comparison of distances between species, and for consistency and simplicity, it has been proposed that the entire complex is considered as a single species, *L. tropica* (Schönian et al. 2010) (Table 2.1).

2.4.4 From Mexico to the Amazon: Parasite Species of New World Rodents

Leishmania parasites of small rodents in the New World have been classified into the species *L. mexicana* (synonymous with *L. pifanoi*), *L. amazonensis* (synonymous with *L. garnhami*) (Asato et al. 2009) and *L. forattinii*, *L. venezuelensis* and *L. aristidesi* (Lainson 1997; Schönian et al. 2010) (Table 2.1). *Leishmania forattinii* was found to be closely related to *L. aristidesi* by Cupolillo et al. (1994). The ITS study by Berzunza-Cruz et al. (2002) found *L. venezuelensis* to be more closely related to *L. major*. Recently, the description of a new species (*L. waltoni*) has been published for a subset of strains within *L. mexicana* (Shaw et al. 2015) that is reportedly associated with diffuse CL in the Dominican Republic. The authors reported a single-nucleotide polymorphism among the five studied strains in 2.5 kbp of

concatenated single gene sequences, which is very low, and only 37 polymorphic sites for the entire complex, which represents less than 1.5% genetic diversity across. It is more likely that this group of strains represents a clonal expansion or a geographically restricted group, and this new species name should, in fact, be considered synonymous with *L. mexicana*.

The overall phylogeny of this group has been less extensively studied than for other *Leishmania*, and *L. forattinii*, *L. venezuelensis* and *L. aristidesi* are seldom represented. The *hsp70* analysis by Fraga et al. (2010) failed to resolve between the species and respective strains of New World *Leishmania* parasites of rodents, thus concluding that it should be considered a single species. Other authors were able to separate the two main species within the group (*L. mexicana* and *L. amazonensis*) based on ITS (Berzunza-Cruz et al. 2002; Davila and Momen 2000) as well as by MLST and mini-exon (Auwera et al. 2014). In phylogenetic trees based on other markers, the genetic diversity within the group was low and comparable to that found within other species (Asato et al. 2009; Fraga et al. 2013; Kwakye-Nuako et al. 2015), including a recent genome-based tree (Harkins et al. 2016) (Fig. 2.1). Increased sampling of the least represented members of this group and more detailed analyses of all members with multilocus markers would further elucidate relationships and species status within it, although a conservative approach would group all species under *L. mexicana* (Table 2.1).

2.4.5 The *Viannia* Group

A group of *Leishmania* parasites that was found to colonize the hindgut of the sand fly vector was placed in the subgenus *L. (Viannia)*, in contrast with the other known *Leishmania* that were only detected in the foregut of the vector (Lainson and Shaw 1987). Species in this subgenus have only been found in South America (Lainson and Shaw 1987). The most common species and the main agent of mucocutaneous leishmaniasis (MCL) is *L. braziliensis*, which is distributed throughout the endemic range of MCL in South America, with species with a more restricted geographical range, such as *L. peruviana* (in the Andes) and *L. guyanensis* (mostly in the tropical forest). Most strains cluster into two main groups that include, first, *L. braziliensis* and *L. peruviana* and, second, *L. guyanensis*, *L. panamensis* and *L. shawi*, according to multilocus analyses (Boité et al. 2012; Auwera et al. 2014). Other species, with fewer isolates obtained so far, are also placed in the subgenus *L. (Viannia)* (Table 2.1): *L. lindenbergi* and *L. utingensis*, which are phylogenetically closer to both *L. braziliensis* (Boité et al. 2012) and *L. guyanensis*, and *L. lainsoni* and *L. naiffi* (Fraga et al. 2010; Boité et al. 2012; Fraga et al. 2013; Auwera et al. 2014). Phylogenetic relationships within the subgenus *Leishmania (Viannia)*, however, are not as well resolved as within the subgenus *Leishmania (Leishmania)*, with lower bootstrap values in general and lack of resolution for cytochrome b-based phylogeny (Asato et al. 2009). An MLST network analysis suggests some level of recombination within and between groups (Boité et al. 2012), thus complicating phylogenetic inference.

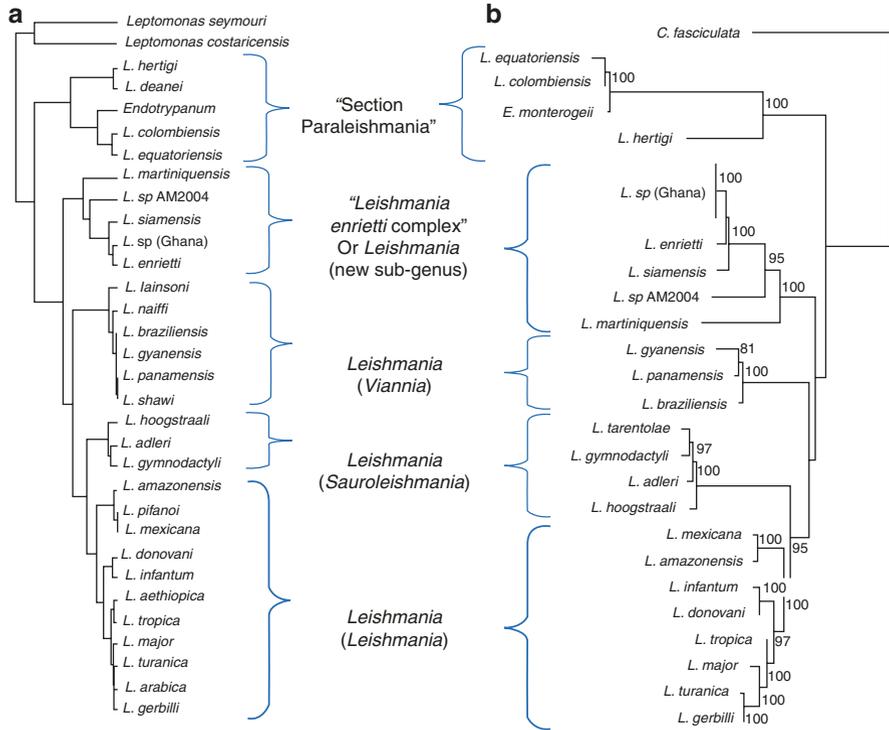


Fig. 2.1 Comparison of *Leishmania* phylogenies and corresponding taxa. **(a)** Phylogeny constructed in BEAST v1.8.2 using the loci available for isolate *L. sp. AM-2004*: 18S, ITS1/5.8S, RNA polymerase II large subunit partial sequences. Two species of *Leptomonas* were used as outgroup. Adapted from: Harkins et al. (2016) <https://doi.org/10.1016/j.meegid.2015.11.030>. **(b)** Maximum likelihood tree based on RNA polymerase II large subunit (RNAPolII) gene sequences, using *Chritidia fasciculata* as an outgroup. Bootstrap values above 80% are shown. Adapted from Kwakye-Nuako et al. (2015) <https://doi.org/10.1016/j.ijpara.2015.05.001>

2.4.6 A New Subgenus? The End of Solitude for *Leishmania enrietti*

While research on well-sampled taxa has been mostly concerned with clumping and reducing taxa diversity and complexity, better sampling and increased awareness of leishmaniasis has not only identified previously unknown *Leishmania* endemic regions, as it has uncovered new variants that are sufficiently genetically distant from known species to be awarded a separate species status. The most striking example has been the *L. enrietti* branch, for which four new groups of isolates, at genetic distances comparable to those between species in other groups, have been described recently. Once considered an “enigmatic” species (Lainson 1997), and neglected among the neglected, *L. enrietti* once stood isolated in its long branch in all *Leishmania* trees. At present, the group comprises at least five species-level groups, with a level of diversity and genetic distances suggestive of a much larger

number of species. The story of this group represents a triumph of active case detection, molecular detection methods and increased awareness of leishmaniasis in previously unknown endemic regions.

In the island of Martinique, in 1995, the parasite responsible for locally acquired CL cases was reported from an HIV patient and presumed to be a “monoxenous ‘lower’ trypanosomatid” based on isoenzyme analysis (Dedet et al. 1995). In 2002 this isolate was identified as a divergent member of the genus *Leishmania* (Noyes et al. 2002), based on a combination of markers (DNA polymerase alpha catalytic subunit and RNA polymerase II largest subunit), but the closest to a *L. enrietti* isolate. The species *L. martiniquensis* was formally described in 2014 (Desbois et al. 2014). Meanwhile, in Thailand, recent isolates have been found to contain RNA polymerase II sequences (Pothirat et al. 2014) or ITS sequences (Siriyasatien et al. 2016) indistinguishable from *L. martiniquensis*, suggesting that this species has a much wider distribution than initially thought. Other Thai isolates were found to possess RNA polymerase II sequences more closely related to *L. enrietti* and informally given the designation of “*L. siamensis*,” which has not yet been formally described as a new species. Pathogenicity in humans by species in this group seems to be associated with immune depression, such as that associated with HIV infection, steroid therapy (Noppakun et al. 2014), although restricted to cutaneous manifestations. Only very recently, an isolate identified as *L. martiniquensis* has also been obtained from a case of VL, which developed in an individual HIV+ (Liautaud et al. 2015), and a case of VL in a child, who was seronegative for HIV, has also been found to be caused by “*L. siamensis*” (Osatakul et al. 2014). Although not compared with *L. martiniquensis* sequences, a multilocus analysis of *Leishmania* samples from Thailand suggests that two distinct species of parasites circulate in that country (Leelayoova et al. 2013): one is “*L. siamensis*” and the other is likely to be *L. martiniquensis*. Similarly to *L. martiniquensis*, “*L. siamensis*” seems to have a wide global distribution, with closely related sequences isolated from Florida, USA (Reuss et al. 2012), from a horse, and in Central Europe, from a cow (Lobsiger et al. 2010) and from horses (Müller et al. 2009). The presence of these parasites in non-human hosts is highly suggestive of a zoonotic parasite, with occasional development in humans, particularly those with compromised or immature immune systems.

Furthermore, a third species (still unnamed) has been proposed for parasites isolated from Ghana (Kwakye-Nuako et al. 2015), also from human cutaneous cases.

All three of these species have the capacity to cause disease in humans. However, a related, although so far unnamed, group of isolates was obtained from kangaroos in Australia (Rose et al. 2004). Considering that other species in this group have been found capable of infecting humans, it can be considered that these parasites pose a risk for the human population in Australia, particularly if immunocompromised.

At the moment, based on ribosomal protein L23a intergenic region and RNA polymerase II large subunit gene sequences, it has been proposed (Kwakye-Nuako et al. 2015) that this group warrants a subgenus status, alongside the subgenera *Leishmania* (*Leishmania*), *Leishmania* (*Sauroleishmania*) and *Leishmania* (*Viannia*)

(Fig. 2.1). Such position of the *L. enrietti* branch at subgenus level is supported by a genome-based phylogeny (Harkins et al. 2016) (Fig. 2.1). However, the entire group is often referred to as “*L. enrietti* complex,” which should be more accurately applied only to the group that includes *L. enrietti*, “I” and the Ghanaian samples, upon comparison of genetic distances with other *Leishmania* species (Fig. 2.1 and Table 2.1).

2.4.7 One Genus or Two Genera?

At the beginning of the twentieth century, an intraerythrocytic parasite resembling *Leishmania* was observed in sloths, and the genus *Endotrypanum* was created to accommodate these isolates (Mesnil and Brimont 1908). To date, two species have been described in this genus, *E. monterogeii* and *E. schaudinni*, with several isolates not assigned to a species (*Endotrypanum* sp). However, intracellular forms have not been observed in experimental infections, and as the isolated parasites grow well in standard *Leishmania* medium, it is possible that the isolates do not correspond to the originally observed forms, but to other parasites present in the host (Cupolillo et al. 2000). Indeed, phylogenetic analyses place these isolates in the same cluster as the *Leishmania* species *L. colombiensis*, *L. equatoriensis*, *L. hertigi*, *L. herreri* and *L. deanei* (Croan et al. 1997; Croan and Ellis 1996; Noyes et al. 1996, 1997; Cupolillo et al. 2000; Harkins et al. 2016; Kwakye-Nuako et al. 2015; Asato et al. 2009) (Fig. 2.1). This group is, genetically, quite distinct from other *Leishmania*, leading Cupolillo et al. (2000) to propose a division of the genus in two sections: section Paraleishmania to include these species and section Euleishmania the remainder.

Taxonomy of this group is still not agreed upon, although it can be argued that it should be revised to avoid polyphyly¹ of the genus *Endotrypanum* and paraphyly² of the genus *Leishmania*. In 2000, Cupolillo et al. proposed maintenance of the genus *Endotrypanum* for the existing isolates and pending revision of the genus with fresh isolates from sloths. However, more recently, Marcili et al. (2014) proposed that *L. hertigi* and *L. equatoriensis* (and presumably the other species in the same clade) should be renamed to become genus *Endotrypanum*, which would effectively form a sister genus to *Leishmania*. However, *L. colombiensis* and *L. equatoriensis* (Delgado et al. 1993; Rodriguez-Bonfante et al. 2003; Kreutzer et al. 1991; Ramírez et al. 2016) have reportedly been isolated from human CL and VL cases. Furthermore, no new descriptions of the elusive intraerythrocytic parasites of sloths have emerged. Finally, it has been reported that species of the subgenus *L. (Sauroleishmania)* can develop inside erythrocytes (Telford 2009). It is, thus, possible that either the first observations of intraerythrocytic parasites in sloths corresponded to non-isolated *L. (Sauroleishmania)* or that the two groups share this capacity. In any case, it is not a unique character within the genus *Leishmania*, and, therefore, it does not justify a

¹Polyphyly: a group of organisms whose last common ancestor is not a member of the group.

²Paraphyly: a group of organisms that includes the last common ancestor, but not all of its descendants.

separate genus per se. Instead, this group could become a subgenus within the genus *Leishmania* to keep consistency across the genus *Leishmania*, to reflect the identity and the history of this group and to recognize the capacity of at least some species for causing pathology in humans. One possibility would be to name the subgenus as *L. (Paraleishmania)*. Alternatively, a subgenus *Leishmania (Endotrypanum)* could be proposed, considering priority, to keep the connection with a formal taxon name (*Endotrypanum*) (Table 2.1) and because it would replicate the process undergone for *Sauroleishmania*.

2.5 Perspectives

Several typing methodologies are available to researchers trying to unravel the taxonomy of *Leishmania*, many of which have been mentioned in this chapter, but mostly based on sequencing of conserved DNA regions, such as heat-shock protein 70 (Fraga et al. 2010, 2013), DNA and RNA polymerases (Croan et al. 1997), the ribosomal internal transcribed spacer (Berzunza-Cruz et al. 2002; Davila and Momen 2000; Mauricio et al. 2004), the mini-exon (Mauricio et al. 2004), the small subunit rDNA (Berzunza-Cruz et al. 2002; Marcili et al. 2014), GAPDH (Marcili et al. 2014), glycoprotein 63 (Mauricio et al. 2007), cytochrome b (Asato et al. 2009), multilocus sequences (Baidouri et al. 2013; Leelayoova et al. 2013; Mauricio et al. 2006; Zemanova et al. 2007) and entire genomes (Harkins et al. 2016), although other methods have been useful to resolve relationships between closely related species, such as multilocus microsatellite analysis (Al-Jawabreh et al. 2008; Alam et al. 2009; Kuhls et al. 2007).

Ideally, *Leishmania* taxonomy should be based on genomic data for all species. However, a case can be put forward for simplification and quick identification, such as the use of barcoding methods (Hebert et al. 2016). Barcoding is based on a small number of markers to identify species, typically mitochondrial targets, such as COI, or nuclear markers such as ribosomal RNA or spacer regions. However, *Leishmania* species can cross in nature to produce hybrids (Rougeron et al. 2015), and barcoding using only mitochondrial targets would not detect species hybrids due to uniparental transmission (Romano et al. 2014). As such, any barcoding system for *Leishmania* should be based on or include at least one nuclear region that could detect both parental sequences.

Phylogenetic inference methods have become quite sophisticated, and increased computational power allows application of complex and computer-intensive methods, such as maximum likelihood and Bayesian analyses, to larger number of samples and large volumes of genotyping data (Yang and Rannala 2012). However, good phylogenetic trees can only be obtained from adequate data, which should include neutral markers or markers shown to be good representatives of *Leishmania* genome evolution, from which robust alignments can be produced. Importantly, such trees should be based on sufficiently wide sampling of the biological and genetic diversity of the genus, which should include all known species of the genus *Leishmania*, as well as intraspecific diversity. Good sample representativity is

crucial, as it has been quite rightly pointed out that “any sound taxonomy should take into account the full biological diversity of the group under study” (Auwera et al. 2011). From studies of well-represented species, such as *L. donovani*, *L. tropica* or *L. braziliensis*, it has become clear that small numbers of isolates can lead to misleading divisions within those species. Most initial phylogenetic analyses of the genus *Leishmania* were based on a small number of samples, from a reduced number of locations and from markers with limitations, such as MLEE (as reviewed in the previous section). Such phylogenies have introduced taxonomic problems, such as the description of *L. archibaldi* as a separate species from *L. donovani* (Rioux et al. 1990). Efforts should, thus, be made to look for and study isolates related to new or poorly represented species or groups, even if from uncultured samples, and with a wide selection of markers, ideally, whole genome sequences.

Conclusions

Leishmania taxonomy remains complex and challenging. Some factors that have made a consensus difficult to reach by *Leishmania* taxonomists include lack of classic sexual recombination that precludes application of the biological concept of species, occasional recombination, including between different species, that blurs the boundaries between phylogenetic groups, the large number of described species that are now considered to be synonymous, lack of homologous genotyping data for all species as well as recent discoveries of new species or variants. However, as reviewed in this chapter, there is a clear case for taxonomic simplification at species level, as well as for a revision at genus and subgenus levels to reflect the now overwhelming molecular and phylogenetic data.

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