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TAXONOMY AND IDENTIFICATION OF LEISHMANIA

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The need for establishing a global taxonomy of Leishmania as a keystone for a research programme on the leishmaniases was recognized by the Scientific Working Group on the Leishmaniases, and included as a priority research goal during the first meeting of this group. Accurate taxonomic knowledge is not merely an interesting academic pursuit, but has great practical importance, particularly in the case of organisms which cause human disease, such as Leishmania. Knowledge of the parasite and the disease can be expanded only when new facts can be related to organisms which are adequately characterized and named. Taxonomic understanding of the causative parasites is relevant to solving the problems which will bring about the eventual control or eradication of the various leishmaniases.

Taxonomy in the simplest terms is the recognition of types or kinds of organisms by their inherent characteristics and the classification of different forms into an orderly system which recognizes naturally related groups of similar origin. These groups are arranged in a hierarchical order according to a perceived degree of relationship, and each rank in the hierarchy is called a taxon. A species, which is the basic unit of taxonomy, is a taxon, and a genus, which is usually comprised of a group of related species, is also a taxon. The application of names to taxa in the Animal Kingdom is governed by an internationally accepted codified set of rules, the International Rules of Zoological Nomenclature. Taxonomy is thus concerned with two separate processes, classification and nomenclature. The former is a subjective, philosophical pursuit, and the latter a purely legalistic exercise.

A third process, identification, concerns recognition of an organism as belonging to any existing taxon. Identification is often confused with taxonomy, but strictly speaking it is not a function of taxonomy since the classification and assigning of names has already been accomplished.

The early taxonomic development of the genus reflects the fact that there are no significant morphological differences between different leishmanias in either the amastigote or promastigote stage, and separation of the various forms recognized was based primarily upon differences in the form of clinical disease produced.

It is well known that at the time when the genus Leishmania was erected by Lühe in 1906 only two species were included, L. donovani from India, and L. tropica from the Middle East, and that they were differentiated only on the basis that the former caused visceral disease and the latter cutaneous ulcers. By the time that Vianna had recognized that the parasite present in Brazil which caused mucocutaneous disease was different from both of these, and proposed that it be called Leishmania braziliensis (his spelling), the pattern for differentiating leishmanial species was well established. What does not seem to be as well appreciated, is that there was another parameter considered, although it was more implied than emphasized. The basis of recognition was actually twofold, the clinical disease, i..e. nosological entities, and the geographic distribution. The earliest species then, were defined as noso-geographical entities, and three gained universal acceptance.

Old World visceral disease = L. donovani
Old World cutaneous ulcers = L. tropica
New World mucocutaneous disease = L. braziliensis

This extreme oversimplification which was current some 50 years ago, had the advantage of convenience and remained surprisingly persistent in medical and biological texts, and is even occasionally encountered today.

There has been a strong tendency to resist changes or additions to these species, but the obvious inadequacy of this classification has brought about an acceptance of other names put forward by astute observers who recognized variations within those broad clinical categories accompanied by epidemiological differences, or by those who recognized differences in biological characters of the parasites themselves. However, with few exceptions, the criteria applied have resulted in a quite conservative classification which, for parasites of humans, remains largely medically oriented and very utilitarian. An excellent detailed review of the history of the developments of the taxonomy of the genus Leishmania is that of Gardener (1977).

Although there is still no universally accepted classification for the Leishmania, especially in regard to criteria for species, general agreement seems to prevail on a scheme for a working classification. The position of the genus Leishmania in the Order Kinetoplastida, Suborder Trypanosomatina and Family Trypanosomatidae is not controversial and the relationship of the higher categories has been accepted from a practical point of view (de Raadt, 1973). The definition of the genus is not without some apparent contradictions, but these are avoided if only the forms infecting mammals are considered (Hommel, 1978). The most widely accepted species and subspecies of mammalian Leishmania are shown in the diagram in Figure 1.

Groupings at the level of subgenus have gained quite a wide acceptance since the demonstration by Lainson and Shaw (1972) that New World non-visceral leishmanias fall into two natural groups, which they termed "complexes", and named the braziliensis and mexicana-complexes after the species names, although they did not assign taxonomic rank to the grouping. Other authors then followed this lead and grouped the parasites causing visceral disease into the donovani-complex and the Old World cutaneous parasites into the tropica-complex (Zuckerman & Lainson, 1977). Subsequently, Lainson and Shaw utilized another grouping at the subgeneric level, called the Hypopylaria, Suprapylaria, and Peripylaria, according to the site of development in the sandfly. These groups, which include all known species, were termed "Sections", a term which, unfortunately, has been used for taxa at at least two different levels in the taxonomic hierarchy. The practice of grouping into the complexes shows signs of becoming generally accepted, but complexes must be defined, as well as named, to constitute valid taxa.

The greatest inconsistency and lack of agreement concerns the assignment of specific or subspecific rank to the recognized entities, i.e. use of binomials or trinomials. The choice of usage appears to be regarded as a

matter of preference or convenience, as much as conviction about their relative rank, since there have been few published reports setting forth the criteria for either specific or subspecific rank. A notable exception is a recent paper on application of numerical taxonomy from the Montpellier group (Lanotte et al., 1981). However, since the noso-geographical entities are generally recognized, the question of specific or subspecific rank is of little practical consequence, and is a question which will no doubt be settled by concensus in due time.

One persistent cause of confusion, over many years, has been the inconsistent use of the names L. tropica tropica and L. tropica minor, (or L. minor) for the same parasite. This problem arose and has persisted because it was not always appreciated that scientific names must be applied in conformance with the International Rules of Zoological Nomenclature, a codified set of rules which has been internationally accepted since 1889, and much modified and amended over the years. A name which is designated in contravention of these rules is invalid and must be suppressed. This confusion began when L. tropica major was recognized and named to distinguish it from the smaller form which was then given the very logical opposite (but taxonomically non-permissible) sobriquet, minor. The use of a trinomial automatically indicates subspecific rank, so L. tropica was thus divided into two subspecies. Article 12 of the Rules states that "A specific name becomes a subspecific name when the species so named becomes a subspecies, and vice versa". Thus the subspecies derived from L. tropica must be L. tropica tropica and L. t. minor is counter to the rules and has no validity. The reasoning behind Article 12 becomes clear when the two subspecies are raised to specific rank, as has been proposed by some workers. If the invalid name, L. t. minor is used, one would have L. minor and L. major raised to specific rank, and L. tropica would disappear. Conformance with the Rules must be observed in nomenclatural procedure, and the names L.tropica minor and L. minor must be suppressed.

The difficulties involved with a taxonomy based upon clinical manifestations, geographical distribution and epidemiological features are obvious, particularly in a group of diseases in which there is a wide range of host responses which can mask the parasite characteristics. The efforts to find more suitable taxonomic characters have occupied a prominent position in research on Leishmania for many years, and studies of the parasite, as distinct from the study of the disease, have provided a basic understanding of taxonomic relationships. The early serological studies of Noguchi (1926) and Adler (1963), the excretory factor typing (Schnur et al., 1972) and biological characterization (Lainson and Shaw, 1972) are examples of this approach.

However, more recently the methodology of molecular level biology has been applied to the study of Leishmania with the object of revealing parasite characteristics which are not modified or obscured by host or environmental factors. These so-called "intrinsic" characters are a direct expression of the genome, as contrasted with "extrinsic" characters, which are only indirect expressions of parasite characteristics, such as behaviour in cultures or the invertebrate host, or pattern of pathogenesis in man or experimental animals.

A variety of methods have been used to characterize the macro-molecular make-up of leishmanias. These include the nucleotide composition of nuclear and kinetoplast DNA, protein structure (particularly as reflected by isoenzymes), metabolic pathways demonstrated by radio-respirometry and immunological character (through identification of single macromolecular antigen components by monoclonal antibodies). By far the most widely adopted and employed method for Leishmania characterization at the present time is analysis of isoenzymes by electrophoresis. However, it is too early to say which of the methods will prove the most useful. Newer and simpler techniques are being devised, and many characters are currently being examined for criteria which will be of taxonomic value. The literature is extensive and an in-depth review of these developments is beyond the scope of this discussion.

However, it is important to emphasize that although these techniques provide extremely valuable tools, their role in taxonomy of the leishmanias is still limited. For the most part, studies of such techniques have been limited to comparing characters of "known" species or subspecies, and the value of a system is uniformly judged by the ability to show characters for the successful differentiation of species or subspecies based on the "classical" nosogeographical and biological characters. The fact that these taxa have been so often and amply confirmed by the newer techniques of molecular biology is in itself a vindication of the present classification scheme and a tribute to the pioneer taxonomists who devised it.

The role of these techniques has not yet been extended to developing a system of classification, and their great utility is in the process of identification. For example, the classification of L. mexicana was based on the observation that there is no mucosal disease in the area in which it is endemic. This was based upon tens of thousands of cases over a period of years, and, if an individual patient in the Yucatan with an abnormal immune response develops espundia, it does not invalidate the basis for classification of this species. However, it does create a special problem to determine that the parasite is not different from others in the area. In this situation, or in the case of an isolate for which the geographic origin is unknown, identification is difficult or impossible without a means of verifying intrinsic characters, and the "modern" tools are the only means by which this can be done.

Any one of several methods can be used to demonstrate characters which will serve to identify the isolate. However, it does not necessarily follow that classification should be based on these characters, although there is little doubt it could be. From a philosophical viewpoint, there is no one "correct" classification and a system which makes new groupings based on presence or absence of certain enzymes, or on the variation of DNA nucleotide sequences, could be more "logical" than the present scheme. However, from a practical point of view, the present classification evolved pragmatically in response to rather specific needs. To replace the current disease orientation would serve no useful purpose, and would be likely to cause great confusion, foster disagreement and non-uniform terminology. This could significantly hamper the development of knowledge of the leishmanias and delay eventual control of the leishmaniases. However useful the methods of molecular biology might be, the "Biochemical Taxonomy" or "New Taxonomy" of Leishmania proclaimed by some authors is not yet a reality, in the sense of having provided a new basis for classification to replace the old.

Recognition of the great value of these identification procedures has created an urgent demand for typing of isolates on the few institutions currently offering this service. At a recent meeting of the Scientific Working Group on the Leishmaniases it was noted that a vast backlog of untyped isolates has accumulated in cryobanks in several parts of the world, and is still mushrooming. More capacity for central identification facilities is needed to provide timely and accurate identification of isolates, since demand far exceeds the current capabilities of identification centres. Identification centres should not be confused with reference centres since these include other institutions in addition to thecryobank reference centres which provide reference strains and materials as well as identification services. Identification is costly and few centres can provide service without some degree of external support.

Another problem associated with the identification of leishmanial isolates by molecular biology methods is the need for standard reference material. In all such methods the unknown isolate is compared against known reference strains, which have sometimes been referred to as marker strains or "markers". These reference strains are of critical importance, since they serve to define the taxon and are the equivalents of the type specimens of metazoan taxonomists. Their selection must be made with great care. Naturally, there are no strains existing of the original parasites described for any of the

human pathogenic forms, so it is a matter of conjecture as to what they really were. However, strains from patients with similar disease, from the same area are assumed to be representative, and can be selected to serve as "neotypes" or "topotypes". Unfortunately, these criteria have not always been observed by investigators and reference strains are sometimes used which are not authenticated, or which are "second generation" markers, which have been identified only by comparison with other strains by the same system. Even more questionable is selection of marker strains because they give a "good" or "strong" expression of a character. To avoid confusion, standard reference material should be used. A list of recommended reference strains is given as Appendix III in the report "Biochemical Characterization of Leishmania" (1982) and is reproduced here as Table 1. These strains are available to requestors through TDR cryobank centres.

Great advances have been made in the taxonomic knowledge of the genus in the past few years, and newer methodology and identification techniques promise to promote even greater progress in the near future. However, enthusiasm for new tools should not be allowed to obscure or obliterate the basic features of the existing disease-oriented classification scheme, which is both useful and accepted.

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FIG. 1

TAXONOMY OF SOME IMPORTANT MAMMALIAN LEISHMANIAS

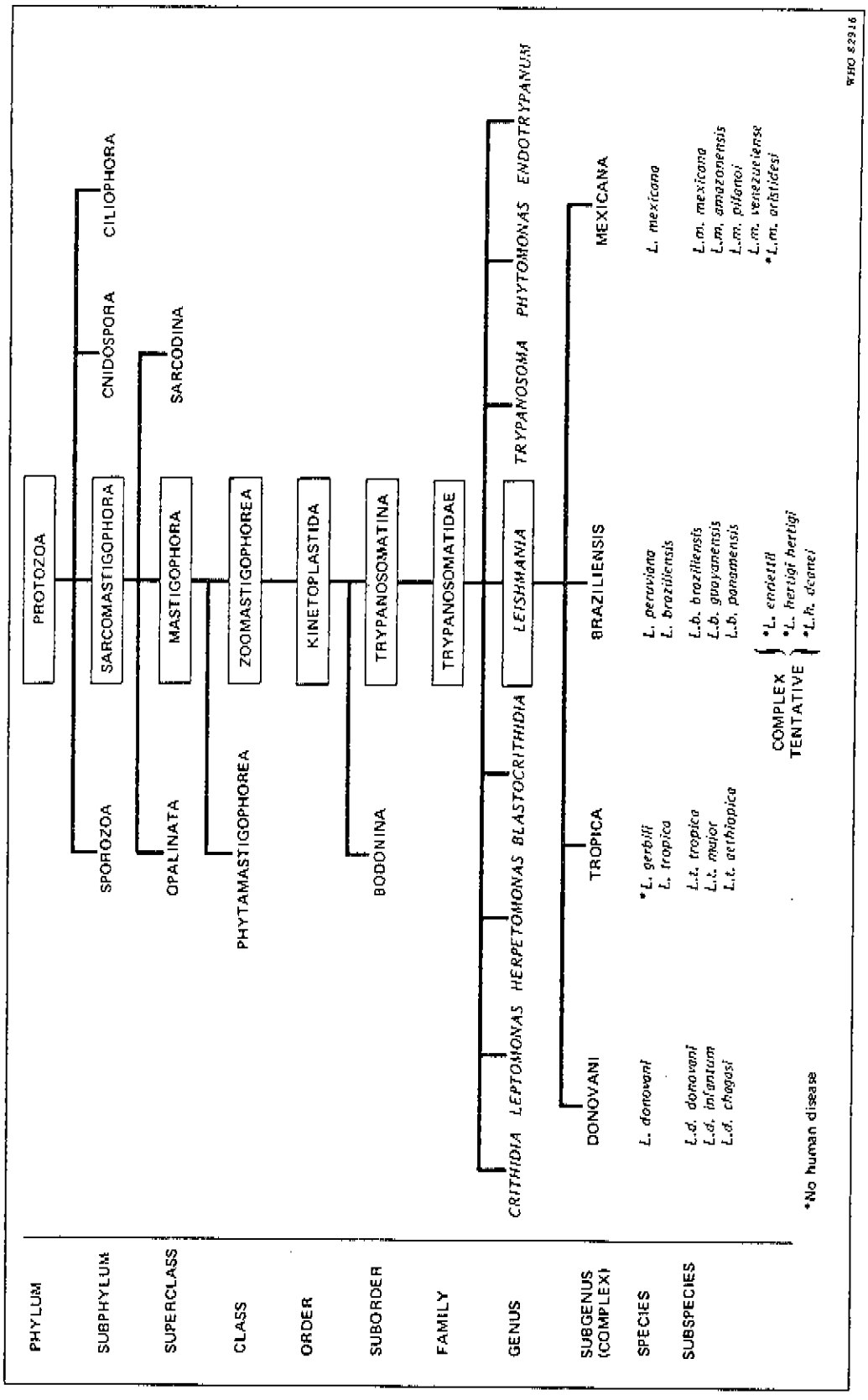

TABLE 1. *LEISHMANIA* REFERENCE STRAINS

Species	Where isolated	Source	Isolate No. or Donating Lab. No.	Reference laboratory numbers			Clinical manifestation	Infective to laboratory animals
L. major	Israel	Man	Jericho 11	LRC-L137	LV561		CL	Yes
	USSR, Uzbekistan	Rhombomys	P strain	LRC-L227	LV39	R/SU/59/Neal P		Yes
	Sudan, Darfur	Man			LV305		CL	
	Senegal	Man	DK 1		LV550		CL	
L. tropica	USSR, Turkestan	Man		LRC-L39	LV357	Man/SU/60/LRC-L39	CL	
	Iraq (Origin)	Man	Avraham, Sinai	LRC-L32	LV142		Recidivans	
	Iraq	Man	IR 3, L75	LRC-L36	LV556	Man/IQ/65/L75	CL	
L. aethiopica	Ethiopia	Man	LL00	LRC-L147	LV24	Man/ET/72/LL00	CL	
	Kenya, Mt. Elgon	Man	KPS H2	LRC-L167	LV266	Man/KE/71/KP5H2	CL	
L. donovani (sensu stricto)	India, Bihar	Man	DD8			Man/IN/80/DD8	VISC	
L. donovani (sensu lato)	Ethiopia, Humera	Man	HU3, L82	LRC-L133	LV9	Man/ET/67/L82	VISC	Yes
	France	Man	KA ♂	LRC-L229	LV114		VISC	
	Brazil	Man	M2682		LV474	Man/BR/ /M2682	VISC	Yes
L. mexicana mexicana	Belize	*Nyctomys sumichrasti*	M379, L11	LRC-L252	LV4	R/BZ/62/M379		Yes
L. m. amazonensis	Brazil, Para	*Lutzomyia flaviscutellata*	PH8		LV10	Vec/BR/67/PH8		Yes
L. m. aristedesi	Panama	*Proechimys semispinosus*	1235	LRC-L183	LV40			
L. m. pifanoi	Venezuela	Man	LL1	LRC-L90	LV96	Man/VE/57/LL1	DCL	
L. braziliensis braziliensis	Brazil, Para	Man	M2903		LV436	Man/BR/75/M2903	CL	Yes
L. b. guyanensis	Brazil, Para	Man	M4147		LV676	Man/BR/75/M4147	CL	Yes
L. b. panamensis	Panama	Man	LS94		LV44	Man/PA/71/LS94	CL	
L. peruviana	Peru	Man	SL2		LV605		CL	
L. hertigi hertigi	Panama	*Coendou rothschildi*	C8		LV42	WA/PA/65/C8		
L. h. deanei	Brazil, Para	*Coendou prehensilis*	M4042		LV438			
L. enriettii	Brazil	*Cavia porcellus*			LV90			Yes

The reference laboratory numbers refer to those laboratories which hold some or all of these strains:

LRC Leishmaniasis Reference Collection, Jerusalem, Israel

LV Liverpool School of Tropical Medicine, United Kingdom

The four element code according to recommendation 5.1:

London School of Hygiene and Tropical Medicine, United Kingdom

Reproduced from:

Biochemical Characterization of *Leishmania* (1982)