

The Status of *Leishmania tarentolae*/*Trypanosoma platydictyli*

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Taxonomic studies and classification of Leishmania species have developed rapidly in recent years, but controversy still surrounds the relationships between those species infecting lizards and those infecting mammals. Some authorities maintain that the leishmanias of lizards form a sufficiently distinct group to be ranked as a separate genus – Sauroleishmania^{1,2} – while Wallbanks et al. have gone further to suggest that such species might be classified within the genus Trypanosoma³. This suggestion followed from work showing that promastigote forms of Trypanosoma platydictyli from a gecko, had similar isoenzyme profiles to Leishmania tarentolae, a well-known species from lizards. In this article however, Larry Simpson and George Holz Jr discuss conflicting evidence, concluding from recent studies of DNA and lipid composition that the lizard leishmanias are more closely related to mammalian leishmanias than to trypanosomes.

The genus *Leishmania* encompasses a diverse group of digenetic parasitic kinetoplastids, which have in common the assumption of the promastigote morphology in the lumen of the invertebrate sandfly host, and the amastigote form in the lysosomal compartment of the vertebrate macrophage. Mammalian *Leishmania* species include both New World and Old World parasites causing visceral, mucocutaneous and cutaneous leishmaniasis in humans and zoonotic infections in animal reservoirs. Classification of these species has progressed rapidly, and it is clear – especially for the New World forms – that each species represents a complex of strains with different biological and molecular properties^{1,2,4,5}.

In contrast to the mammalian *Leishmania* species, the taxonomic status of the leishmanias of lizards is uncertain, mainly because the presence of intracellular amastigotes within the lizard host has never been well established. In lizards, the

parasites live predominantly as promastigotes in the lumen of the doaca and intestine, or in the bloodstream⁶. Amastigotes, either free or inside monocytes, are seen only rarely^{6–8}. Most promastigote lines from lizards have been recovered by culture of blood from infected animals. Hence the relationship of the *Leishmania* species from lizards to the *Leishmania* species from mammals is unclear.

Most recently, the status of lizard leishmanias has been called into question by the report of Wallbanks et al.³ that a double clone of a trypomastigote form of *Trypanosoma platydictyli* from the blood of a gecko (*Tarentolae mauritanica*) from the south of France, differentiated in culture into a stable promastigote form with an identical isoenzyme profile to that of stock cultures of *L. tarentolae*. On the basis of this evidence, Wallbanks et al.³ proposed that *L. tarentolae* be synonymized with *T. platydictyli* and that the validity of other *Leishmania* parasites of lizards also be questioned. This proposal has been

accepted by several workers such that several recent reviews state that lizard *Leishmania* actually belong to the genus *Trypanosoma*⁹. We would like to review the evidence that, on the contrary, lizard leishmanias do belong in the genus *Leishmania*¹⁰. In addition to the general importance of understanding the phylogenetic relationships of the kinetoplastid protozoa, this question is specifically important since there is a great deal of information about the molecular biology of the mitochondrial and kinetoplast DNA in *L. tarentolae*^{11,12} and it is necessary to establish the relevance of this information for the mammalian parasites (Box 1).

We examined eight strains of *L. tarentolae*, three of which (T, K and UC) were originally derived from an Algerian gecko isolate, and four of which (LEM 115, 124, 125 and 306) were isolated from geckos in the Banyuls region of southern France by Rioux and collaborators. One strain (LEM 87) was isolated from an infected sandfly in the same region. For comparison, we also examined the putative promastigote clonal strain of *T. platydictyli* (TPCL2) of Wallbanks et al.³. This was reported to have been isolated from an infected gecko in the same vicinity of southern France that the five LEM strains were isolated. We also examined four other supposed lizard *Leishmania* strains (*L. hoogstrali*, *L. adleri*, *L. agamae*, and *Leishmania* sp. LizS). Strains of *L. major*, *L. tropica* and *L. donovani* were used as references for comparison with mammalian *Leishmania*, and four trypano-

Box 1. Identification of Kinetoplastid Protozoa

Classification of parasitic kinetoplastids by means of vertebrate host specificity, the type of disease produced and morphology under the light microscope are the classical procedures for the establishment of genera and species. However, identification of kinetoplastid protozoa by morphology under the light microscope alone is often unreliable because morphological differences between species or strains are not dramatic, and cells differ in morphology depending on culture conditions, stage of growth and stage in the life cycle. In addition, the monogenetic kinetoplastids (with a single invertebrate host) often resemble the insect stages of the digenetic parasites. More reliable identification methods include isoenzyme analysis, the use of monoclonal antibodies, analysis of chromosome patterns in OFAGE (orthogonal field agarose gel electrophoresis) gels, analysis of restriction enzyme digests of kinetoplast DNA minicircles in acrylamide gels, hybridization with total kinetoplast DNA or with cloned kDNA minicircles or minicircle fragments, analysis of nuclear DNA restriction site polymorphisms, and analysis of membrane lipids. The term 'zymodeme' is used to indicate strains with related isoenzyme patterns, and the term 'schizodeme' is used to indicate strains with related kDNA minicircle restriction profiles or sequences.

An additional problem in kinetoplastid classification and identification is that most strains represent uncloned stocks and frequently consist of mixtures of different strains in different proportions, depending on culture conditions. For example the WHO-TDR *Leishmania* reference strains (see *Parasitology Today* 1, 172-173) are uncloned, and significant heterogeneity has been observed among clonal lines derived from several of the reference stocks.

Another problem is that changes in strains, resulting either from selection of subpopulations in a nonclonal line, or due to fixation of mutations and selection by culture conditions, or even as a result of cross-contamination of strains, does occur in laboratories maintaining stocks of these protozoa. This can be eliminated by preservation of frozen stabilates and frequent revival of original stocks, but these protozoa often recover only slowly from the insult of freezing and must be readapted to culture conditions.

It should be stressed that any new kinetoplastid strain entering the laboratory from whatever source should be checked by several criteria for authenticity and a sample should be frozen for stabilate preservation.

some species (*T. conorhini*, *T. cruzi*, *T. cyclops* and *T. lewisi*) were used as stercorarian trypanosome references. Most of these strains were compared in terms of kinetoplast DNA minicircle and maxicircle sequences, chromosome patterns in OFAGE (orthogonal field acrylamide gel electrophoresis) gels, and membrane lipids.

Two Groups of *L. tarentolae* Strains

The presence of two schizodemes within the eight *L. tarentolae* strains was detected by analysis of digestion products of kDNA on acrylamide gradient gels (Figs 1, 2). The group A schizodeme included the UC, T and K strains from Algeria, and the group B schizodeme included the four LEM strains from France. Hybridization of Southern blots of digested kDNA from the other putative lizard *Leishmania* strains and the mammalian *Leishmania* strains with labelled minicircle DNA, both native and cloned, from the UC strain of *L. tarentolae* showed sequence similarities between the group A *L. tarentolae* strains, the group B *L. tarentolae* strains, the Mongolian lizard *Leishmania* LizS strain and the Sudanese *L. hoogstrali* strain, but no similarities to *L. major* kDNA or to the *L. adleri* strain (presumably isolated from a Kenyan lacertid) and

the *L. agamae* strain (presumably isolated from an Israeli agamid). The latter two strains gave identical kDNA profiles, implying that these ATCC (30816, 30815) stocks represent the same strain. Moreover, it may be that this *L. adleri*/*L. agamae* strain is not even a lizard *Leishmania*, although lipid analysis (see below) does confirm that it belongs to the genus *Leishmania*. The *T. platydictyli* TPCL2 strain clearly falls into the *L. tarentolae* group A in terms of kDNA minicircle profiles, and is most closely related to the UC strain. This is inconsistent with the prediction from Wallbanks *et al.*³ that the TPCL2 strain, which was derived from an infected gecko in southern France, would be closely related to the LEM *L. tarentolae* strains, derived from the same infected lizards.

Conserved Maxicircle Sequences

Blots of agarose gels of digested kDNA were hybridized with gene-specific probes from the maxicircle DNA of *L. tarentolae* (UC strain). The *ORF4*, *COIII* and *ORF12* maxicircle genes, which are absent from the maxicircle DNA of

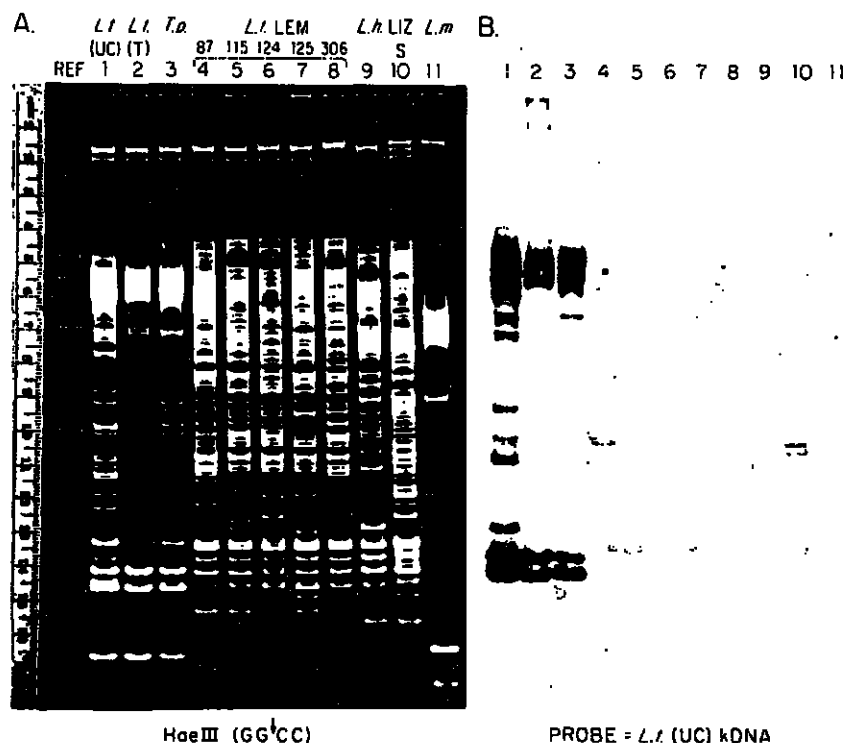


Fig. 1. Comparison of minicircle fragment profiles in acrylamide, of several strains of *Leishmania* and the TPCL2 strain of *T. platydictyli*. The kDNAs were digested with *HaeIII* and the fragments separated on a 4.0-10% acrylamide gradient gel. The gel was stained with ethidium bromide and blotted onto a nylon filter. The blot was hybridized with nick-translated kDNA from the UC strain of *L. tarentolae*. The origin of the kDNA is given above each lane. L.t. = *L. tarentolae*, T.p. = *Trypanosoma platydictyli*, L.h. = *L. hoogstrali*, LIZ = *Leishmania LizS*, L.m. = *L. major*. REF = a mixture of lambda DNA digested with *HindIII* and OX174 DNA digested with *HaeIII*. (Reprinted from Ref. 8, by permission.)

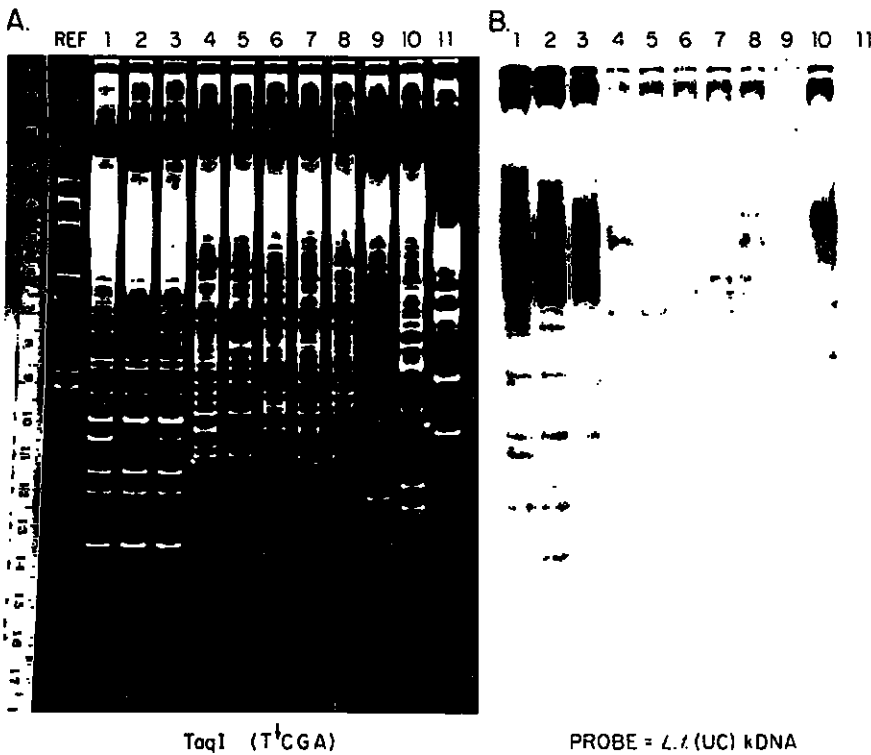


Fig. 2. Comparison of minicircle fragment profiles in acrylamide of same strains as in Fig. 1. The kDNAs were digested with TaqI. The kDNAs are given in the legend to Fig. 1. The blot was hybridized with the same kDNA probe as in Fig. 1. (Reprinted from Ref. 8, by permission.)

the African trypanosome, *T. brucei*, are present in all eight *L. tarentolae* strains and also in all other *Leishmania* strains examined – although at different levels of sequence conservation. It is not known if these genes are also conserved in maxicircle genomes of stercorarian trypanosomes as these were not examined, but it is clear that the TPCL2 strain is not closely related to the African salivarian trypanosomes which exhibit this deletion event in the mitochondrial genome.

Hybridization of a cloned fragment from the divergent region of the *L. tarentolae* (UC) maxicircle also distinguished the eight *L. tarentolae* strains from the other lizard *Leishmania* and from the mammalian *Leishmania* strains (Fig. 3). Our data indicate that divergent region sequences may provide a species-specific marker, whereas the kDNA minicircle sequences appear to provide a marker for lizard *Leishmania* species as a group.

Chromosome Profile in OFAGE gels

Approximately 23 chromosome bands can be visualized by the OFAGE method using agarose-embedded cells of the *L. tarentolae* UC strain. The chromosome profile of the *T. platydictyli* TPCL2 cells is identical to that of the UC strain and shows at least three large

chromosome bands of different mobility compared with the T strain of *L. tarentolae* (Fig. 4). The chromosome profiles of the five LEM strains differ somewhat from those of the Group A strains, but

the other lizard *Leishmania* strains exhibit very different chromosome profiles. This provides additional evidence that the TPCL2 strain is closely related to the *L. tarentolae* UC strain and not to the LEM strains of *L. tarentolae*.

Lipid Analysis

Lipid analysis showed that the composition of sterol and phospholipid fatty acyl groups of the *T. platydictyli* TPCL2 strain resembles that of various *Leishmania* species but differs from that of stercorarian trypanosomes. 5-Dehydroepisterol, α -linolenic acid and dihydrosterculic acid have been found to be useful in identifying kinetoplastid flagellates. They coexist as major lipids in *Leishmania* species but not in stercorarian or salivarian trypanosomes¹³⁻¹⁵. We¹⁰ found that the free sterol fractions of the *T. platydictyli* TPCL2 strain, the *L. tarentolae* Group A strains, the *L. tarentolae* Group B strains, the two other lizard *Leishmania* strains (*Leishmania* LizS, *L. hoogstrali*), and the mammalian reference *Leishmania* strains (*L. major*, *L. tropica*, *L. donovani*) all contained 5-dehydroepisterol as the major sterol. The phospholipid fractions contained a high proportion of α -linolenic acid and variable amounts of dihydrosterculic acid. The sterol and the

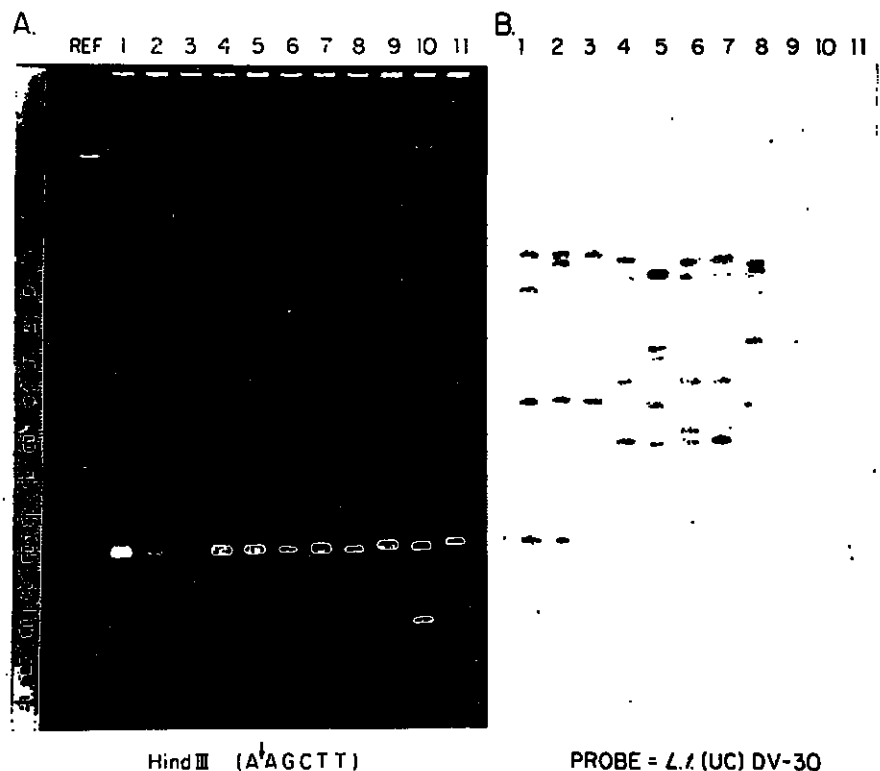


Fig. 3. Sequence variation within the maxicircle divergent region of several lizard *Leishmania* strains and species. The kDNAs were digested with HindIII. The kDNAs are given in the legend to Fig. 1. Lane 12 contains a mixture of digested *L. t. UC* kDNA and LEM125 kDNA. The probe was a cloned fragment (DV-30) from the divergent region of the UC strain of *L. tarentolae*. (Reprinted from Ref. 8, by permission.)

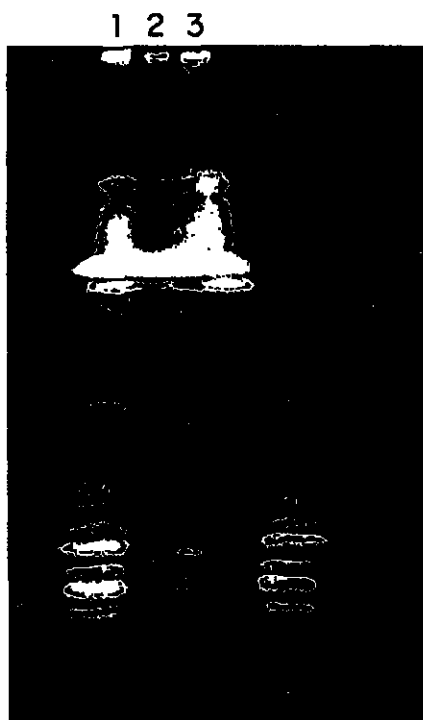


Fig. 4. Comparison of OFAGE separation of chromosomes from the UC strain (lane 1), the T strain (lane 2) and the TPCL2 strain (lane 3). Conditions: 1.5% agarose, 210 V, 48 h, 15°C, 90 s switching time. (Reprinted from Ref. 8, by permission.)

two fatty acids were not major lipids of *T. cyclops*, *T. conorhini*, *T. cruzi* and *T. lewisi*. These results support the conclusions reached by the examination of genomic properties.

We conclude therefore, that there is no evidence from DNA or lipid composition to support the interpretation of Wallbanks *et al.*³ that *L. tarentolae* strains are derived from *T. platydactyli*, a trypanosome parasite of the gecko. It is obvious that additional basic biological studies are required to clarify the status of the parasite within the lizard host, but it is also clear that the saurian *Leishmania* almost certainly represent a subgroup of the genus *Leishmania* and not a type of *Trypanosoma*, and that research on the molecular biology of *L. tarentolae* should yield results which can be extrapolated to other members of this genus and, hopefully, to other kinetoplastid protozoa.

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