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Note préliminaire.

MONOPHYLETIC ORIGIN OF THE GENUS *LEISHMANIA* ROSS, 1903

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In 1987 Lainson and Shaw published an improved version of their classification of the genus *Leishmania* [3]. This was, however, mainly based on extrinsic, eco-epidemiological criteria and a Linnean type of taxonomy which did not lend itself fully to the question of the mono- or polyphyletic origin of the genus. By integrating Hennig's concepts [1] and using biochemical techniques and a cladistic approach, it is now possible to provide an unequivocal solution to this problem.

MATERIALS AND METHODS

The method is based on the use of enzyme proteins as characters, isoenzymes as « character states » and zymodemes as operational taxonomic units (OTU's). The phylogenetic analysis is based on the concepts of Hennig (monophylogeny, sharing of ancestral and derived characters) with the aid of cladistic constructions (parsimony, homology).

This study was based on 1031 stocks from 52 countries of the Old World (770 stocks) and the New World (261 stocks), the stocks originating from man (712), mammalian reservoirs (238) and phlebotomine sandflies (81).

Thirsteen enzymes were used: EC 1.1.1.40: ME; EC 1.1.1.42: ICD; EC 1.1.1.44: PGD; EC 1.1.1.49: G6PD; EC 1.6.2.2: DIA; EC 2.4.2.1: NP1; EC 2.4.2.*: NP2; EC 2.6.1.1: GOT1; EC 2.6.1.1: GOT2; EC 2.7.5.1: PGM; EC 4.2.1.2: FH; EC 5.3.1.8: MPI; EC 5.3.1.9: GPI.

The isoenzymes were demonstrated with the aid of two techniques : 1) as reference technique, thick starch gel electrophoresis; 2) as control technique, isofocussing on PH gradients (PH 4-6.5 and 5-8).

In the absence of an external group, the phylogenetic construction was carried out in two steps using the MIX program (from PHYLIP, versions 2.7 and 3.2), first determining the shortest network, then the shortest tree or cladogram. The common ancestor was determined by comparison between several hypothetical taxonomic units (HTU's) selected at the centre of the network. In order to do this, 40 Old World zymodemes (from a total of 65) containing 91 isoenzymes and 40 New World zymodemes (out of 62) totalling 125 isoenzymes were retained after eliminating as far as possible those units which carry autapomorphic characters, while conserving the different phenetic and/or phyletic complexes as previously determined [3, 4, 5, 7]. The congruence of these various constructions with the complete « trees » was then tested. After that, 300 networks and 350 trees were built. Note that all the Old World complexes belong to the subgenus *Leishmania,* while those of the New World belong both to the subgenera *Leishmania* and *Viannia* [3].

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RESULTS AND DISCUSSION

Among the 201 different isoenzymes forming 80 zymodemes, 15 (7 %) are common to the Old and New Worlds. They are to be found in the systems ME, ICD, NP2, GOT1, GOT2, PGM, FH and MPI. Among the remaining isoenzymes, 76 are specific to the Old and 110 to the New World. Three zymodemes (MON* 1, MON* 30 and MON* 111) are common to both, all belonging to the *L. infantum* complex [4, 5].

From the minimal tree constructed from 80 OTU's, the following conclusions are drawn :

— No major disturbance is created in the structure by combining the *Leishmania* of both Worlds [7]. The subgenus *Viannia* (i. e. the complexes *L. braziliensis* containing *L. peruviana*; *L. guyanensis* including *L. panamensis* and *L. shawi*; *L. lainsoni*; *L. naiffi*) remains separate and forms one of the two main branches of the tree. The other branch contains all the complexes of the subgenus *Leishmania* of the Old World (*L. aethiopica*; *L. arabica*; *L. donovani*; *L. gerbilli*; *L. infantum*; *L. major* including *L. turanica*; *L. tropica* including *L. killicki*) as well as the New World (*L. amazonensis* including *L. aristidesi*; *L. enriettii*; *L. hertigi* including *L. deanei*; *L. mexicana*);

— the *L. hertigi* complex merits special attention. Two equally parsimonious trees place this complex near the root but one together with the subgenus *Viannia* and the other with the subgenus *Leishmania,* recalling the difficulty already reported by Lainson and Shaw. Nevertheless, in the New World cladogram this complex shows a strong synapomorphy of the isoenzyme FH^{95} with the complexes of the subgenus *Leishmania* which speaks in favour of its association with this subgenus;

— in the subgenus *Leishmania,* the Old World complexes and the small New World branch which includes the *L. amazonensis, L. enriettii* and *L. mexicana* complexes constitue two sister groups.

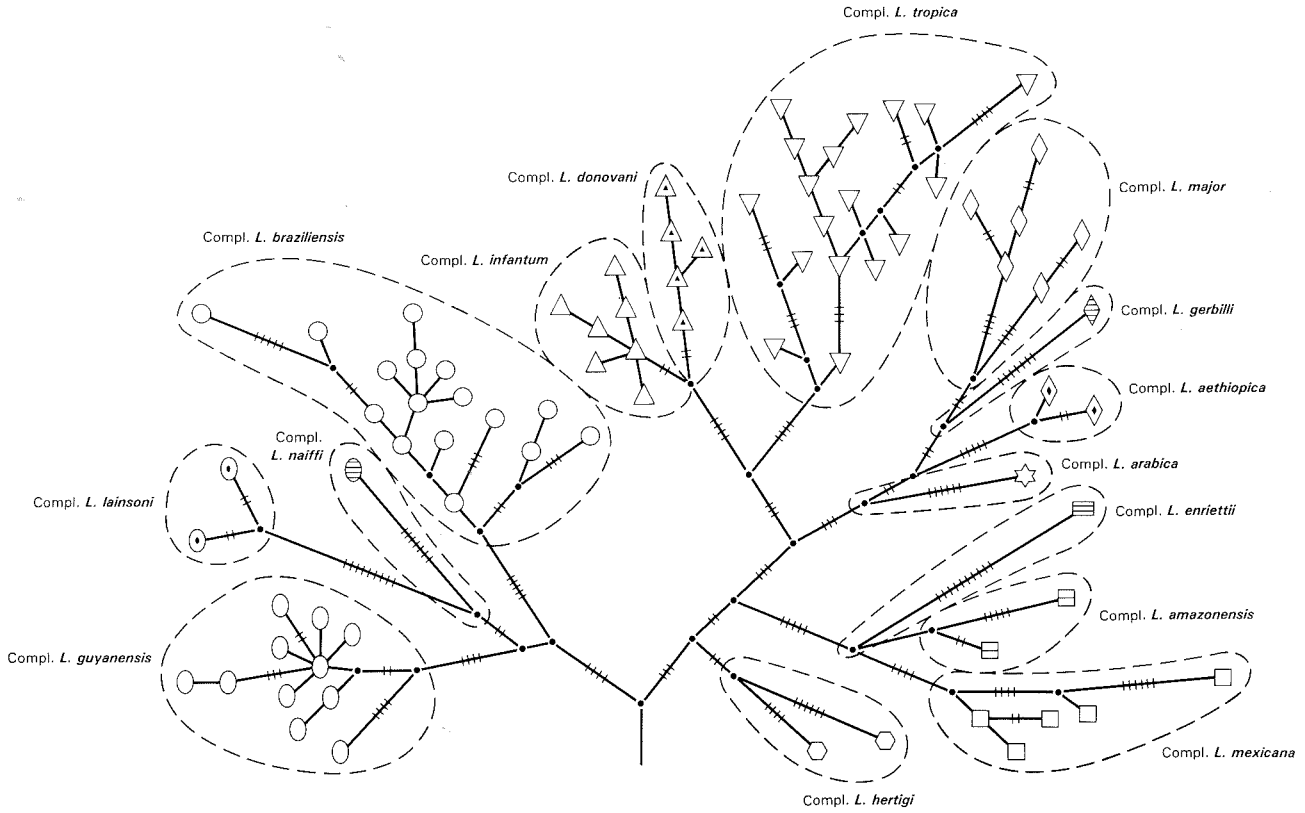

FIG. 1. — Cladogram of the genus *Leishmania* Ross, 1903, constructed on the basis of 80 zymodemes, 13 enzymes and 201 isoenzymes. *Leishmania* of the Old World remain grouped in the same clade and share a common ancestral origin with their homologues of the New World in the subgenus *Leishmania*. The complexes of the subgenus *Viannia* are grouped together on the left hand branch.

In conclusion, the results obtained largely confirm the 1987 classification of Lainson and Shaw and allow the hypothesis that the genus *Leishmania* has a monophyletic origin to be unreservedly accepted. Moreover the phylogenetic hierarchy of the complexes in this genus has been defined.

These conclusions place the origin of the genus in the Mezozoïc era prior to the separation of Gondwanaland, an hypothesis previously hold by Saf'janova [6]. After the separation of the South American and African continents, the two subgenera *Viannia* and *Leishmania* became separated, then diversified together with the diversification of the sandflies of the New World *(Lutzomyia)* and Old World *(Phlebotomus)*. The New World representatives of the subgenus *Leishmania* became separated from their sister species of the Old World at a later date, in the early Cenozoïc era, by the movement from East to West in rodent reservoirs, perhaps Phiomorph ancestors of the Caviomorphs. As regards *L. infantum* (syn. *L. chagasi*), this may have arrived in the New World much later, in the Pliocene-Pleistocene era, possibly during historic times in infected canids [2].

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