Taxonomy of *Trypanosoma cruzi:* a Commentary on Characterization and Nomenclature

Hooman Momen

Departamento de Bioquímica e Biologia Molecular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

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Early in the history of Chagas disease it became apparent that there was considerable variation in the incidence and severity of infections with parasites classified as being *Trypanosoma cruzi* (see Pessoa 1960 for a review of early findings by scientists such as Carlos Chagas and Emmanuel Dias). A variety of typing schemes were developed as a means of finding the basis of this variation and more finely, classifying the organisms within the species. Here instead of reviewing the literature on this topic a critical perspective on the typing of *T. cruzi* is presented.

Early attempts at typing strains included the immunological types of Nussensweig et al. (1963) however it was the pioneering work of Andrade (1974) who first correlated specific arrays of morphobiological and behavioural characters to particular types within *T. cruzi*. The molecular typing of *T. cruzi* strains was pioneered with isoenzymes (Toye 1974) and Miles used the technique to classify isolates of this parasite into strain-groups (Miles et al. 1977) and types (Miles et al. 1978). The term zymodeme was later introduced (Barrett et al. 1980) to refer to "trypanosome populations that possess like forms of specified enzymes". Ready and Miles (1980) suggested that the *T. cruzi* zymodemes indicated distinct taxa, however, Miles et al. (1981a, b) were reluctant to give the taxa subspecific status. This reluctance was followed by nearly all subsequent authors, eventhough the basic zymodeme divisions were confirmed by many subsequent studies using a variety of techniques at both the protein and DNA level (Table) and a strong correlation between the intrinsic and extrinsic characters (Lumsden 1977) of *T. cruzi* types was convincingly demonstrated (Andrade et al. 1983, Andrade 1985).

Fax: +55-21-590.3495.
E-mail: hmomen@gene.dbbm.fiocruz.br
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RELUCTANCE TO NAME FORMAL TAXA

This contrast between the eagerness to sub-divide *T. cruzi* and the reluctance to name formal taxa is curious in the light of the comparison with the related trypanosomatid genus *Leishmania*. For example the phylogenetic diversity in *T. cruzi* is comparable to that observed in the whole of the genus *Leishmania* (Tibayrenc 1998a), which is currently divided into nearly 50 species. Even if the comparison is limited to the same geographical area and a single order of reservoir, there are still about twenty mammalian species of New world *Leishmania* as compared to a single *T. cruzi* species. Although there is some criticism of the excess number of species in *Leishmania*, with the level of phylogenetic divergence between some species of *Leishmania* comparable to lower clades of *T. cruzi* (Tibayrenc 1998a), the benefit of the named species in clarifying the ecoepidemiology and causes of the diverse clinical manifestations of the leishmaniases is undoubted. Furthermore the studies of Andrade (1974) provided a similar basis for *T. cruzi* to that of *Leishmania* for the description of new taxa.

Several reasons can be put forward to explain this reluctance for describing named taxa for *T. cruzi*. At the time the principal zymodeme divisions were proposed and in the period afterwards several other studies raised questions about the divisions. For example, Brenner (1977) proposed two polar types (Y and CL). These strains were shown later to posses a number of fundamental differences such as differences in the course of infection in a variety of hosts including morphology of blood forms at peak of parasitemia which occurred at different times and differences in infectivity to mouse peritoneal macrophages, tissue culture cells and *in vivo* infections. These fundamentally different types appeared to belong to the same zymodeme. The zymodemes themselves appeared not to be stable (Romanha et al. 1979) a finding reinforced by apparent instability of isoenzyme profiles in other parasites (Mirelman et al. 1986). The principal zymodemes also appeared to

TABLE

Correlation among the different sub-divisions proposed for *Trypanosoma cruzi*

(See Annex to this supplement)	*T. cruzi* I	*T. cruzi* II	
Andrade (1974)	Type III	Type II	Type I
Miles et al. (1977)	Strain-group 1	Strain-group 2	
Miles et al. (1978)	Type 1	Type 2	
Barret et al. (1980)	Zymodeme 1	Zymodeme 2	
Romanha (1979)		Zymodeme A	Zymodeme B
Ebert (1982)	Group 1	Group 2	
Schottelius (1982)	Type 2 (PNA)	Type 1 (WGA)	
Tibayrenc & Miles (1983)	Braz ZI	Braz Z2	Bol Z2
Zillman & Ebert (1983)	Group A	Group B	
Tibayrenc et al. (1984)	Isoenzyme strain (IS) 1	IS 2e	IS 2
Miles et al. (1984)	Chilean Z1	Chilean Z2a	Chilean Z2b
Tibayrenc & Ayala (1988)	Zymodeme 17	Zymodeme 30	Zymodeme 39[a]
Muhlpfordt & Berger (1990)	DNA group 1	DNA group 2	
Clark & Pung (1994)	Ribodeme II	Ribodeme I	
Tibayrenc (1995)	Group I	Group II	Genotype 39
Souto et al. (1996)	Lineage II	Lineage I	Group 1/2
Andrade & Magalhaes (1997)	Biodeme III	Biodeme II	Biodeme I
Nunes et al. (1997)	Group II	Group I	
Tibayrenc (1998a)	First Major Clade[b]	Second Major Clade[b]	Lower Clade 39[b]

The Table presents the principal correlations among the many typing schemes proposed by various authors for classifying *T. cruzi* strains. As the techniques were applied on different collections of strains not all strains or isolates within each subdivision may exactly correspond in all of the studies.
a: also referred to as major clone (Tibayrenc & Breniere 1988) or clonet (Tibayrenc & Ayala 1991); *b*: also referred to as Discrete Typing Unit (DTU) (Tibayrenc 1998b).

have geographical variations and could be divided into a number of isoenzyme strains (Tibayrenc & Ayala 1988). At the same time the technique of schizodeme analysis (Morel et al. 1980) showed an extensive heterogeneity within *T. cruzi*, which could not be readily classified into types. These results were supported by many further DNA studies using a variety of techniques demonstrating the genetic variability of *T. cruzi* (Macedo & Pena 1998).

Morever the use of these techniques indicated the possibility of heterogeneity within the *T. cruzi* strains, with particular strains or isolates being mixtures of at least two populations (Morel et al. 1980) and the probability of selective isolation of clones or strains (Deane et al. 1984, Macedo & Pena 1998). These and other reasons favoured the view of *T. cruzi* as a single polytypic species and against a formal subdivsion, as well as illustrating the difficulty of correlating strains with patient morbidity. However the possibility of a strain or even clone having more than one population of parasites was in fact the explanation for the observed instability of the isoenzyme characters and apparent similarity between the enzyme profile of the polar types (Goldberg & Perreira 1983, Gomes et al. 1991, Clark & Diamond 1993).

PRIMARY PHYLOGENETIC DIVISIONS

Lumsden (1977) defined three classes of nomenclature, (i) operational, without any indication of characterization, which included terms such as population, sample, isolate, clone, stock and (ii) Linnean, including genus, species and subspecies. The third class he called "a new nomenclature to designate the manifold new subspecific categories which are being discovered by new methods of characterization – the multiplicity of functionally different populations which exist within the same morphological species". Although he did not formally name this class we can refer to it as infraspecific, however as pointed out by Lumsden for many microrganisms, non-contentious recognition is more often at the level of genus and subgenus. This third class has proved very popular in molecular studies of *T. cruzi* as the profusion of names in the Table demonstrates.

Attention has again been recently focused on two primary phylogenetic divisions within *T. cruzi* (Tibayrenc 1995, Souto et al. 1996, Nunes et al. 1997). While there are differences of opinion about the significance of this division (Brisse et al. 1998, Souto et al. 1998, Macedo & Pena 1998) the basis for the division is well supported (Table). The discovery that microbial lineages maintain their ge-

netic integrity over long time intervals and over great distances, that is, their genomes are not rapidly broken down or reshuffled by recurrent mutation and recombination is known as the clone concept (Orskov & Orskov 1983). Tibayrenc et al. (1986) have proposed this model as the main population genetic structure for *T. cruzi*. The application of this model with the presence of a primary infraspecific division in *T. cruzi* means that Chagas disease can no longer be considered as a single disease entity. At least two diseases corresponding to the two divisions must be considered with obvious implications for clinical and experimental studies as well as control of the disease. Results of many investigations need now to be reinterpreted based on the classification of the strains used. This may also be an explanation for differences in observations among researchers in many studies such as the use of diagnostic techniques reported in the literature.

FINAL COMMENTS

In the history of Chagas disease, the wheel has been reinvented many times (Dvorak 1984). A sound taxonomy may often have avoided much wasted time and effort. The third class of nomenclature as proposed by Lumsden (1977) has been usefully applied to *T. cruzi* (as shown in Table) however it may be time to consider the use of formal Linnean designations for the divisions within this parasite. Among the arguments used against the naming of *T. cruzi* taxa have been the presence of putative hybrids between the two main lineages of *T. cruzi* (major clone 39 and its equivalents); the need for further studies on the population structure as there is evidence of genetic recombination (Bogliolo et al. 1996, Carrasco et al. 1996); the difficulty of correlating strains with patient morbidity and the genetic variability of *T. cruzi* clones. The arguments against the formal naming of *T. cruzi* taxa though valid are disputed and in any case are not particular to this parasite and have not impeded the naming of taxa in other organisms.

The present situation is similar to the early 80´s where the work of Miles et al. (1977, 1978) and Andrade (1974) had laid the basis for the formal naming of *T. cruzi* taxa. Again the strong correlations between major phylogenetic divisions in *T. cruzi* and biological characters (Andrade & Magalhaes 1997, Revollo et al. 1998) are being emphasized. The naming of species for the principal divisions and subspecies for the lower divisions would clearly aid in the comprehension of studies on this parasite. As pointed out by Steel (1962) "nomenclature should be our servant and not our master".

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