THE APPLICATION OF A NUMERICAL METHOD FOR TAXONOMY FROM ISOENZYMATICAL RESULTS : COEFFICIENT OF PARENTAL CORRELATION BETWEEN *SCHISTOSOMA* SPECIES

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ABSTRACT

Using enzyme characters by starch gel electrophoresis, we have applied the method of Numerical Taxonomy to the Schistosomes isolate/stocks.

Seven isoenzymes (Phosphoglucomutase = PGM; Phospho glucose isomerase = PGI; Hexokinase = HK; Mannose phosphate isomerase = MPI; Alcaline phosphatase = ALP; Malic enzyme = ME and Malate deshydrogenase = MDH) of 15 isolats/stocks are examined.

Twenty six electromorphs, corresponding to equivalent number of isoenzymes, are identified by this method, and then grouped into 8 zymodemes. These zymodemes were used as Operational Taxonomy Unit (OTU) and compared pairwise, using these indice and it forms the basis for the taxonomic scheme elaborated. The final relationships are exhibited in the agglomerative dendrogram, constructed using complete linkage.

The separation into phenons is confirmed by correspondence analysis. It is concluded that the original lines fall into four groups correspondings to the complexes Schistosoma mansoni, S. bovis, S. curasonni and S. haematobium. The phenons are recognised by Numerical Taxonomy, can be equated with the taxa of traditional systematics.

INTRODUCTION

Although *Schistosoma* taxonomy was classically identified by morphological characteristics of adult worms, eggs and also its intermediate host, it is quite difficult to identify because some species of *Schistosoma* have the same intermediate hosts and their eggs are very similar.

Recently, other taxonomic criterias have been used. Among others these include the enzyme system of individual parasites using the electrophoresis method. By this method, each species of the parasite can be identified by its enzyme profile. A zymogram from seven different enzyme systems was constructed.

All isolates/stocks, had relatively the same mobility in each isoenzyme from all using enzyme systems studied and were grouped in the same zymodeme. Using both the classification systems of ADANSON and the coefficient of similarity of JACCARD, the coefficient of parental correlation between *Schistosoma* can be obtained.

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MATERIALS AND METHODS

Application of numerical methods of taxonomy consists of choice and codage of characters, use of operational taxonomic units (OTU) estimation of the affinity degree between different zymodemes, individualization of groups and elaboration of numerical taxonomy :

1. Choice and codage of characters

In these studies, the unitary characters obtained from electromorphs consisting of 7 enzymes systems are : PGM (Phosphoglucomutase); PGI (Phospho glucose isomerase); HK (Hexokinase); MPI (Mannose phosphate isomerase); ALP (Alkaline phosphatase); ME (Malic Enzyme) and MDH (Malate deshydrogenase).

Individialisation of one electromorph was achieved by comparison of the relative counting of electrophoretic mobility of every enzyme system from each isolate stock with the reference sample (*Schistosoma mansoni* from Brazil and which has been adapted for over 20 years in the laboratory). For these calculations μSmB represents the electrophoretic mobility of enzymes from the reference sample.

μSmB = 100 (distance value from cathode to anode)

For all enzymes/isoenzymes (X) from the same system, the relative value of the electrophoretic mobility is :

$$\mu X = \frac{d_X}{d_{SMB}} \times 100$$

μ = Relative electrophoretic mobility

d = The band distance (mobility) from the first point of electrophoresis[2].

In the monomeric system, the distance of two bands was measured from the start point of electrophoresis. In the multimeric systems only the pheripheral bands were measured (the fastest band and the lowest band).

2. O T U (Operational Taxonomic Units)

Based on the same character (the same mobility) from all enzyme systems studied the definition of OTU corresponds to that of zymodeme.

3. Estimation of the affinity degree between different zymodemes (OTU)

In this study the coefficient of similarity (S) was calculated using the Jaccard[3] formula:

$$S = \frac{a}{a + b + c}$$

a = the same quantity of characters of 2 zymodemes compared (OTU)

b = the original character quantity of the first zymodeme

c = the original character quantity of the second zymodeme.

4. Individualisation of groups and elaboration of numerical taxonomy

The complete linkage integration was used : a zymodeme was assigned to zymodemes groups that were formed early in the integration with the lowest affinity degree of these zymodemes, allowing homogeneity between each group and relatively net distinctions between the different groups. These results are shown in a dendrogram graph : the different zymodemes are placed in the absis and the coefficient of similarity (S) is in the ordinate.

INTERPRETATION AND DISCUSSION

Twenty-six unitary characters analogous with the electromorph were obtained in this study. These characters include : PGM^{50}, PGM^{65}, PGM^{100}, PGM^{113}, PGI^{100}, PGI^{160}, PGI^{180}, HK^{50}, HK^{65}, HK^{70}, HK^{80}, HK^{90}, HK^{100}, MPI^{86}, MPI^{92}, MPI^{100}, ME^{60}, ME^{100}, ALP^{80}, ALP^{100}, ALP^{180}, ALP^{190}, ALP^{200}, $MDH\text{-}2^{-83}$, $MDH\text{-}2^{-100}$.

Data obtained from counting relative electrophoretic mobility compared to the marker (SmB) from 7 enzymes systems can be grouped in 8 zymodemes (Table I).

Table 1. Matrix of the Basic data

\E Z\	PGM	PGI	HK	MPI	ALP	ME	MDH
Z_1	100	100	100	100	100	100 60	- 100
Z_2	113	100	100	100	N.D.	100 60	- 100
Z_3	100 65	180	65	86	180 80	100 60	- 83
Z_4	100 65	180	80	86	190 80	100 60	- 83
Z_5	100 65	180	80	92	190 80	N.D.	N.D.
Z_6	100 50	180	90	100	200 80	100 60	- 83
Z_7	100 65 50	180	70	100	N.D.	N.D.	- 83
Z_8	100 65	160	50	100	180 90	100	- 83

E = Enzyme
Z = Zymodeme

N.D. = Not done

This table has 7 columns and 8 lines in which the unitary characters were manifested by the enzyme systems (column) and the zymodemes (line).

Individualisation of zymodemes :

Z_1 : From a murine stock from naturally infestation of *Holochilus braziliensis*, origin Maranhao (Brazil) that was adapted in *H.braziliensis, Rattus rattus* and mice. This stock is identical with the marker.

Z_2 : From a human stock, origin Central Africa.

Z_3 : From 3 isolates that were obtained from human urine, origin Senthiou Malem (Senegal).

Z_4 : From an isolate that was obtained from human urine, origin Kerkerat (Mauritania).

Z_5 : From an isolate that was obtained from a natural infestation in mollusca *(Bulinus jousseaumei)*, origin Sare Sara (Senegal).

Z_6 : From a stock that was obtained from a natural infestation in mollusca *(B.umbilicatus)*, origin Senthiou Malem (Senegal).

Z_7 : From 2 isolates that were obtained from a natural infestations in *B.umbilicatus*, origin Sory (Senegal).

Z_8 : From a stock that was obtained from zebu liver that was infected by Schistosoma, origin Saint Louis (Senegal), and adapted in *B.forskalii, B.guernei*, and B.*truncatus*, mice, hamster, mastomys and sheep.

For the counting of the affinity index (coefficient of similarity) by comparison of 2 by 2 from 8 zymodemes, the correlation between zymodemes can be obtained as seen in table 2.

Table 2. Matrix of similarity from the counting of the affinity index of JACCARD by the comparison of 2 by 2 from 8 zymodemes for all the enzymatic system study

Zymodeme	1	2	3	4	5	6	7	8
2	0,46	-						
3	0,23	0,15	-					
4	0,21	0,14	0,35	-				
5	0,14	0	0,23	0,29	-			
6	0,27	0,20	0,29	0,27	0,20	-		
7	0,22	0,11	0,23	0,21	0,18	0,33	-	
8	0,27	0,23	0,29	0,24	0,14	0,24	0,23	-

After using the complete linkage integration, we can obtain a dendrogram graph (Figure 1) from the electrophoretic mobility data. The dendrogram was formed by the groups according to hierarchial linkage (correlation).

Four groups of Schistosoma that were obtained from the dendrogram are comparable :

- *S.haematobium* : consists of 3 zymodemes (Z_3, Z_4 and Z_5).
- *S.curassoni* : consists of 2 zymodemes (Z_6 and Z_7).
- *S.bovis* : consists of one zymodeme (Z_8).
- *S.mansoni* : consists of 2 zymodemes (Z_1 and Z_2).

According to the coefficient of similarity, the nearest degree of parental correlation exists between *S.haematobium* and *S.curassoni* groups and the fastest exists between *S.haematobium* and *S.mansoni* groups. From this study, we know that there exists a complex in Schistosoma species according to zymodeme.

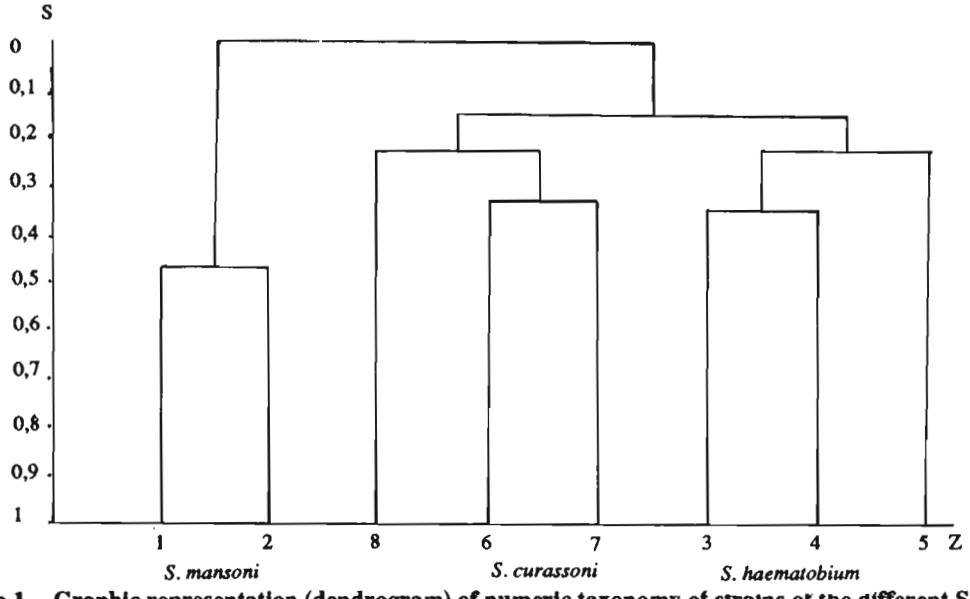

Figure 1. Graphic representation (dendrogram) of numeric taxonomy of strains of the different Schistosoma species analysed, aid by 7 enzymes systems with complete linkage integration.

In a view of a mutual, but independent, interest in animal and human Schistosomiasis from Senegal and the redescription of *S.curassoni*[4]. This species has been defined in two zymodemes (Z_6 and Z_7).

CONCLUSION

The application of a numerical method for the taxonomy from isoenzymatical result can be individualised to 8 zymodemes differents from 7 enzyme systems.

Construction of the dendrogram can differentiate clearly between schistosoma zymodemes to 4 groups that is in accordance with the classical taxonomy.

The validity and the legimity of this method to solve taxonomic problems in determining sub-specific degree and differentiating inter-specific hybrids is possible with isoenzyme analysis.

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