A TECHNIQUE FOR IDENTIFICATION OF CERCARIAE OF *SCHISTOSOMA HAEMATOBIUM, S. CURASSONI, S. BOVIS* AND *S. INTERCALATUM*

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SUMMARY

The chaetotaxy of 84 samples or isolates of *Schistosoma* spp. from western or central Africa has been studied. Three indices were calculated for cercariae of each sample; their average value, the skewness and kurtosis of each indice was established. Each species *(S. haematobium, S. curassoni, S. bovis* and *S. intercalatum)* was discriminated with nine variables. The present work gives information to assess, specific diagnosis with simple calculations easily achieved on a small computer.

RÉSUMÉ : **Technique pour la diagnose des cercaires de** *Schistosoma haematobium, S. curassoni, S. bovis* et *S. intercalatum.*

La chétotaxie de 84 échantillons ou isolats appartenant à *Schistosoma haematobium, S. curassoni, S. bovis* et *S. intercalatum* d'Afrique occidentale et centrale a été étudiée. Trois indices ont été calculés; pour chacun d'eux, la valeur moyenne, la dissymétrie et l'aplatissement ont été calculés. Les neuf variables de la chétotaxie des cercaires des 4 espèces ont dont été soumis à une analyse discriminante. Une technique simple de diagnose réalisable sur des micro-ordinateurs est proposée.

The specific identification of *Schistosoma* cercariae shed by their intermediate host *i. e. Bulinus* spp, is of the highest importance for the epidemiologist. The chaetotaxy of cercariae is of great help to assess specific diagnosis either in natural or experimental conditions (Richard, 1971 ; Bayssade-Dufour *et al.,* 1989; Ngendahayo, 1989). After impregnation with silver nitrate, three indices are established as presented in *figure 1.* The use of discriminant analysis has been of a great help for the diagnosis of three species of *Schistosoma* as recorded in Bayssade-Dufour *et al.,* 1989, or four species of *Schistosoma* (Ngendahayo, 1989). The latter work was done on 102 samples or isolates originating from Africa; they were well discriminated in 93 % of the cases.

The calculations are often time consuming and require a fairly sophisticated hardware and statistical package. The aim of the present work is to propose an easy technique for the diagnosis of the four species of *Schistosoma* above mentioned.
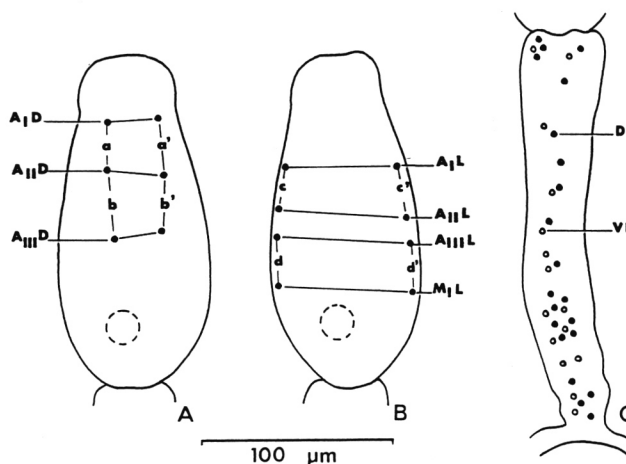
FIG. 1. — Papillae used for calculation of cercarial indices.

A: body, dorsal view. *a, a'*: distances between dorsal papillae A_ID and $A_{II}D$. *b, b'*: distances between dorsal papillae $A_{II}D$ and $A_{III}D$. B: body, dorsal view. *c, c'*: distances between lateral papillae A_IL and $A_{II}L$. *d, d'*: distances between dorsal papillae $A_{III}L$ and M_IL. C: tail stem, dorsal view. DP: dorsal papillae. VP: ventral papillae.

$$AD = \frac{a + a'}{b + b'} \qquad AL = \frac{c + c'}{d + d'}$$

U = total number of caudal papillae

MATERIAL AND METHODS

— SAMPLES OR ISOLATES

84 were studied: they originated from Africa. 20 were of *S. bovis*, 11 of *S. curassoni*, 10 of *S. intercalatum* and 43 of *S. haematobium*. Their description is in Ngendahayo, 1989, and can be obtained on request (File T 11028).

— MEASUREMENTS

On each cercariae, when feasible, the indices AD, AL and U were obtained *(Fig. 1)*. These incides fluctuated within the cercariae emitted by one mollusc, and thus for each sample the following characteristics of distribution were calculated:
— Pearson coefficient of skewness (equal to zero for normal distribution),
— Pearson coefficient of kurtosis (equal to three for normal distribution). These coefficients were established for 30 cercariae and should not be established for fewer than 15 cercariae of an isolate.

— ANALYSIS OF DATA

Discriminant analysis was performed on nine variables (average value, skewness and kurtosis of the three indices). They were coded as: ALX (average of AL), ADX (average of AD), UX (average of U), ALS, ADS, US (skewness of AD, AL and U) and ALA, ADA, UA (kurtosis of AD, AL and U). The computations were done with a statistical package (Stat-Itcf, 1987).
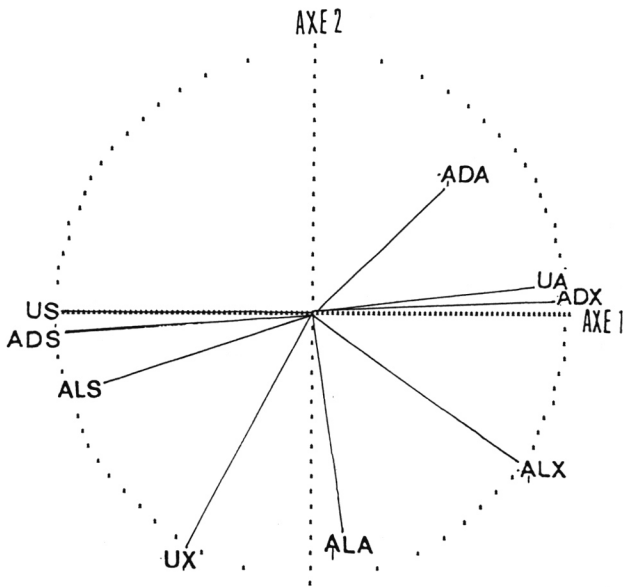

FIG. 2. — Discriminant analysis of cercarial indices of the four species of *Schistosoma*. Correlation circle of parameters ADX, ALX, UX (average indices), ADA, ALA, UA (Pearson kurtosis coefficient) and ADS, ALS, US (Pearson skewness coefficient).

RESULTS

— SPECIFIC IDENTIFICATION OF OUR DATA

The inertia of the first and second axis were respectively of 73.8 and 24.7. They were both significant (P < 0.01)
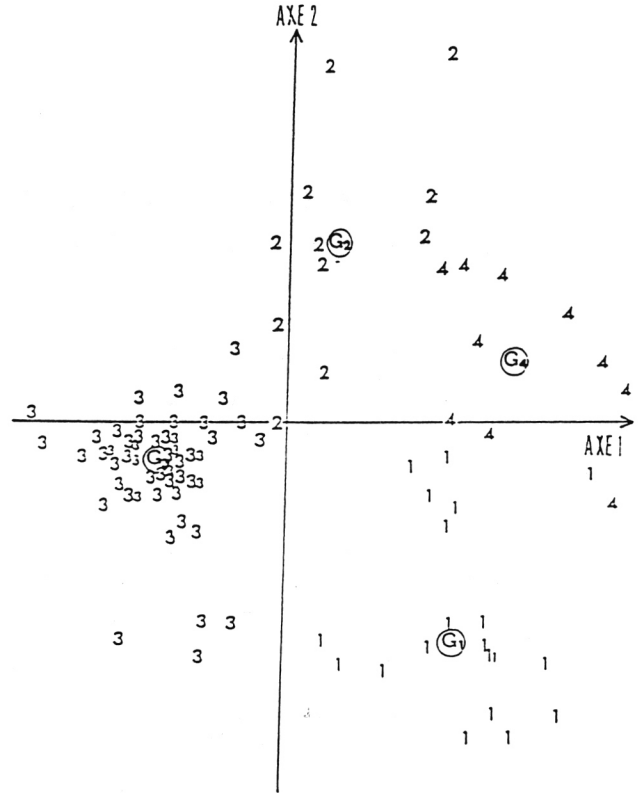

FIG. 3. — Discriminant analysis of cercarial indices of the four species of *Schistosoma*. Location of the samples. 1 = *S. bovis*. 2 = *S. curassoni*. 3 = *S. S. haematobium*. 4 = *S. intercalatum*. G₁, G₂, G₃, G₄: gravity centers of each species.

and most of the needed information was on the plane constructed with axis one and two.

All the variables, taken separately played a significant role in the identification of species except for two of them (ADA and UA). The value of pseudo F (all the variables are included) are much higher than any F observed for any variable taken separately; it may be concluded that all the variables are important. This is seen on the circle of correlation in *figure 2*: all the variables are near the circle, that means that their discriminating value is high.

All the samples or isolates are arranged on the plane constructed with axis one and two *(Fig. 3)*. The gravity centers of the four species (G1 to G4) are well separated: the D2 of Mahalanobis extended from 2.00, *S. bovis* and *S. intercalatum* to 2.82 *S. bovis* and *S. curassoni*). 78 samples or isolates were well classified (93 %). The misclassified samples were:

one *S. bovis* missclassified as *S. intercalatum*,

one *S. curassoni* as *S. haematobium*,

one *S. haematobium* as *S. curassoni*, two *S. intercalatum* as *S. bovis*.

The misclassifications did not occur in natural infections

but they occurred in experimental infections when the Trematode and the Mollusc were allopatric.

— IDENTIFICATION OF NEW ISOLATES

A graphical method is used. The coordinates of gravity centers were:

G1 = *S. bovis:* 0.99 on axis 1 and 1.00 on axis 2,
G2 = *S. curassoni:* respectively 0.28 and 1.72,
G3 = *S. haematobium:* respectively − 0.86 and − 0.13,
G4 = *S. intercalatum:* respectively 1.43 and 0.67.

The values of indices and their distribution are calculated with the program in annex 1. They may be calculated with another program but the values of ADX and ALX are multiplied by 100 and UX by 10; the coefficients of skewness and kurtosis are insensitive to the units of data.

The coordinates ($y1$ on axis one and $y2$ on axis 2) of the new sample or isolate are computed from the following equations:

$y1 = 0{,}01533 \, ADX + 0.00021 \, ADA - 0.00072 \, ADS + 0.00937 \, ALX - 0.00042 \, ALS - 0.00015 \, ALA - 0.00079 \, UX - 0.00011 \, US + 0.0052 \, UA - 3.87,$

$y2 = - 0.000264 \, ADX + 0.000333 \, ADA - 0.000493 \, ADS - 0.0111 \, ALX - 0.000249 \, ALS - 0.00134 \, ALA - 0.0386 \, UX + 0.00039 \, US + 0.000359 \, UA + 16.02.$

The examination of the coordinates allows to assess specific identification of the new sample of *Schistosoma*.

CONCLUSION

The technique we propose for identification of four species of african *Schistosoma* is relatively easy to perform:

— measure of chaetotaxic indices after silver nitrate impregnation of cercariae,
— calculations of the average, skewness and kurtosis coefficients for the three indices for the sample to be identified,
— calculations of the coordinates of the sample and comparison to those of the gravity center.

In most cases this technique will allow specific identification; in dubious samples the origin and snail host from which the cercariae were obtained may be supplementary help.

REFERENCES

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ANNEX 1

Programme in gwbasic for calculation of mean, skewness and kurtosis of cercarian chaetotaxic indices.

```
05 REM calculations of mean, skewness, kurtosis
10 REM of cercarian chaetotaxic indices of African Schistosomas
11 REM (AD: dorsal, AL: lateral, U: no. of tail stem papillae)
12 REM see Bayssade-Dufour et al., 1989
13 REM in International Journal de Parasitology
14 REM -- freeware in gwbasic: skewkur --
15 REM in case of any problem contact: J. Cabaret
16 REM INRA Patho. aviaire and parasitol., 37380 Monnaie
   (France)
17 REM (tel.: 47-42-77-68 at working hours)
18 rem You need only a PC compatible computer and Gwbasic
19 rem to run the programme, type at dos prompt: gwbasic
   skewkur
20 INPUT Number of cercariae of Schistosoma for the indice;
   N
25 X1 = 0 : X2 = 0 : X4 = 0
30 FOR I = 1 to N
40 PRINT"x("; I; ") = ";
50 INPUT" ";XI
60 X1 = X1 + XI
70 X2 = X2 + XI*XI
80 X3 = X3 + XI^3
90 X4 = X4 + XI^4
100 NEXT I
110 M1 = X1/N
120 PRINT"M1 = MEAN = "; M1
130 M2 = (X2/N) − M1*M1
140 PRINT"M2 = VARIANCE, no. of degree of freedom"; M2
150 M3 = (X3/N) − (3*M1*X2/N) + 2*M1^3
160 PRINT"M3 = "; M3
170 M4 = X4/N(4*M1*X3/N)
180 M4 = M4 + 6*M1*M1*X2/N − 3*M1^4
190 PRINT"M4 = "; M4
200 G1 = (M3*M3)/(M2^3)
210 PRINT"SKEWNESS: coefficient of Pearson > 0 left skew-
    ness = "; G1
220 G2 = M4/(M2*M2)
230 PRINT"KURTOSIS: coefficient of Pearson > 3 sharp = ";
    G2
240 INPUT" Enter 0 for a new calculation, CTRL and C to quit
    "; K
245 REM Press at the same time on CTRL and C to quit
250 IF K = 0 then goto 10
260 CLS: end
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