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Comparison of measurements and number of annuli in *Caloosia paradoxa* (Nemata : Hemicycliophorinae)

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Summary – Statistical analyses were made on some data from six samples of *Caloosia paradoxa* and one sample of *Hemicycliophora arenaria* for comparing two methods for measuring the dimensions of organs or the distance between landmarks. The two methods are direct measurement in micrometers and count of the number of body annuli along the organs or between the landmarks. Length in micrometer is more correlated with the width of one annulus than with the corresponding number of annuli. It is wrong to estimate the number of annuli by dividing a length by the width of one annulus and it is imperative to count the actual number of annuli when this type of character is needed. It is more accurate to count the number of annuli along the stylet and the oesophagus than to measure these organs, but it is more accurate to measure total body length and distance from labial disc to excretory pore than to count the annuli. The opposite is true when we compare methods that give the less variable results. Measurement of stylet and oesophagus are less variable than the corresponding number of annuli whereas total number of body annuli and number of annuli between labial disc and excretory pore are less variable than the corresponding measurements. For discriminative purposes, the measurements in micrometers do a better job than the counts of annuli for discriminating between different species while keeping together different samples of the same species. It is concluded that measurements are to be preferred for discriminating purposes, but that the complete description of a species should rely on both measurements and counts of annuli.

Résumé – ***Comparaison entre mesure et comptage du nombre d'anneaux chez*** **Caloosia paradoxa** ***(Nemata : Hemicycliophorinae)*** – Des analyses statistiques ont été faites sur des données décrivant six échantillons de *Caloosia paradoxa* et un échantillon de *Hemicycliophora arenaria* pour comparer deux méthodes pour la mesure des dimensions d'un organe ou la distance entre deux points remarquables du corps. Ces deux méthodes sont la mesure directe en micromètres et le comptage du nombre d'anneaux du corps le long de l'organe ou entre les deux points. La longueur en micromètres est plus fortement corrélée à la largeur d'un anneau qu'au nombre d'anneaux correspondant. Il est erroné d'estimer un nombre d'anneaux en divisant une longueur par la largeur d'un anneau et il est impératif de compter le nombre exact d'anneaux quand ce type de caractère doit être utilisé. Il est plus exact de compter le nombre d'anneaux le long du stylet et de l'œsophage que de mesurer ces organes, mais il est plus exact de mesurer la longueur totale du corps et la distance de l'extrémité antérieure au pore excréteur que de compter les anneaux. L'inverse est vrai lorsque l'on compare les deux méthodes pour trouver celle qui donne les résultats les moins variables. La mesure de l'œsophage et celle du stylet sont moins variables que les nombres d'anneaux correspondants, tandis que le nombre total d'anneaux du corps et le nombre d'anneaux entre l'extrémité antérieure et le pore excréteur sont moins variables que les mesures correspondantes. Lorsque l'on veut séparer des espèces, les mesures en micromètres sont mieux capables que les nombres d'anneaux de séparer les espèces différentes tout en regroupant les échantillons appartenant à la même espèce. Il faut conclure que les mesures doivent être préférées aux nombres d'anneaux quand il s'agit de séparer des espèces, mais que la description complète d'une espèce doit s'appuyer sur les deux types de caractères.

Key-words : Nematodes, *Caloosia*, biometry, systematics.

The body annuli of most nematodes in the family Criconematidae are extremely well marked and few in numbers (as little as 40 to 50 annuli in some *Criconema* and *Ogma*, 24 annuli in *Criconema pauperum*). It has long been a practice of nematode taxonomists working with this group to count and report the exact number of body annuli and to give some morphological dimensions, not as measurements in micrometers, but as a number of annuli. For example, the length of the stylet is given as the number of body annuli between the tip and the base of this organ. The position of some morphological features, such as vulva, excretory pore, anus, stylet end, and oesophageal glands end, also are measured by the number of annuli between the feature and a landmark (i.e., labial disc and posterior end). De Grisse (1965) proposed using the following codes : R (total number of body annuli), Rst (number of annuli from labial disc to stylet end), Roes (number of annuli from labial disc to oesophagus end), Rhem (number of annuli from labial disc to the first annulus after the hemizonid), Rex (number of annuli from labial disc to the first annulus after the excretory pore), Rv (number of annuli from posterior end to vulva), Ran (number of annuli from posterior end to anus), and Rvan (number of annuli between vulva and anus).

The hemicycliophorids have more body annuli than typical criconematids. For example, *Caloosia paradoxa* has 256-263 body annuli. Counting several hundred annuli in a representative number of specimens is a lengthy process, certainly taking more time than direct

Table 1. Origin of the specimens studied.

Sample number	Locality	Host	n	Remarks
1	Adiopodoumé, Ivory Coast	Millet	11	Type specimens
2	Adiopodoumé, Ivory Coast	Millet	17	From greenhouse culture, 1976
3	Bouaké, Ivory Coast	Tobacco	9	
4	Lamto, Ivory Coast	Savanna	11	
5	Burkina Faso	Peanut	11	
6	Adiopodoumé, Ivory Coast	Millet	17	Same as 2, remeasured in 1987
7	Mecca, California	*Citrus limonia*	7	*H. arenaria*

measurement of corresponding lengths in micrometers. The question of whether it is " better " to count body annuli than to take direct measurements has never been studied. Here, " better " can be defined in three different ways : **more accurate,** with the smallest measurement or counting error, **more descriptive,** with the smallest variation within a sample, and **more discriminative,** best able to separate samples belonging to different species, while not differentiating samples from the same species.

The present note proposes an answer to this question for the species *C. paradoxa*. A sample of *H. arenaria* was included in the study for comparison purposes.

Material and methods

Five geographically distinct samples of *C. paradoxa* were measured in 1976. The same observations were made a second time in 1987 on one of the samples. *Hemicycliophora arenaria* was also measured in 1987 as a control. Table 1 describes the samples studied.

Thirteen variables were measured (Table 2). All observations were made with the same type of microscope

Table 2. List of measurements and annulus counts.

Variable code	Variable name
L	Body length
R	Number of body annuli
Hvu	Distance head to vulva
Rhv	Number annuli head-vulva
Sty	Stylet length
Rst	Number annuli along stylet
Oes	Oesophagus length
Roes	Number annuli along oesophagus
Expo	Distance head to excretory pore
Rex	Number annuli head-excretory pore
Tail	Tail length
Ran	Number tail annuli
w	Width of one body annulus

(Ortholux II). Magnification was 400 × for total length and 1000 × (oil immersion objective) for the other characters. In 1976, total number of annuli were counted from photographs of the specimens. Other counts were made directly under the microscope. The observations made in 1987 were all made under the microscope. Statistics were calculated using the statistical package SAS PC (Anon., 1985*a, b*).

Some of the traditional characters are linked by a direct relationship and were not included in the analysis. Distance vulva to tail end is equal to L – Hvu (Table 2), the number of annuli between vulva and posterior end, Rv, is equal to R – Rhv. Distance vulva to anus is equal to L – Hvu – Tail, and Rvan, the number of annuli between vulva and anus is equal to Rv – Rvan – 1.

Results

Preliminary investigations

The values (means and standard deviations) of all the variables are given in Table 3 for the seven samples studied. The normality of the data was checked using the procedure UNIVARIABLE of SAS PC. Table 4 shows that all variables (including counts of annuli) are normally distributed within a reasonable approximation.

Some authors do not bother counting annuli, but they estimate annulus counts by dividing the corresponding measurement by the width of one annulus. If w is the width of one annulus, m the measurement in micrometers of a feature ($m = L, Hvu...$), and n the number of annuli of the same feature ($n = R, Rhv,...$), this assumes that $m = n.w$ and $n = m/w$.

Each measurement and each annulus count was compared to its estimate based on w (e.g., L was compared to $R.w$, R was compared to L/w, etc.). The comparisons were made by first calculating the difference between the actual count or measurement and the corresponding estimate (e.g., $LD = (R.w) - L$; $RD = (L/w) - R$; etc). The null hypothesis that the mean of the difference (*LD*, *RD*, etc.) is equal to zero was tested using the procedure MEANS of the SAS PC package.

Table 5 shows that the null hypotheses for all variables are rejected in all cases (with probability always better

Table 3. Mean and standard deviation of the variables (see list in Table 2) measured in the seven samples studied (see Table 1).

Variable	Sample 1		Sample 2		Sample 3		Sample 4	
L	632.3	23.2	639.0	43.8	626.9	45.9	600.5	23.5
R	234.7	7.1	229.9	7.2	230.4	11.6	237.2	10.9
Hvu	542.0	21.6	551.8	41.1	537.2	38.8	512.2	21.2
Rhv	205.2	8.0	201.6	5.8	200.6	8.2	208.3	8.3
St	62.3	1.5	60.4	2.2	61.7	2.4	57.0	0.9
Rst	25.9	1.6	24.0	1.6	25.2	1.2	24.7	1.2
Oes	116.2	5.1	113.1	5.2	116.0	4.4	110.0	2.4
Roes	45.8	2.9	42.9	3.2	44.4	2.5	46.4	2.3
Expo	114.2	3.9	117.2	9.6	113.1	6.5	109.8	3.9
Rex	45.5	1.8	44.9	2.6	43.7	1.8	46.8	1.0
Tail	65.5	3.2	61.6	5.8	65.3	5.8	62.7	5.3
Ran	29.5	2.4	28.4	3.0	28.8	2.2	28.9	3.2
w	2.6	0.2	2.7	0.2	2.7	0.2	2.4	0.2

Variable	Sample 5		Sample 6		Sample 7	
L	666.3	36.6	645.1	39.7	799.6	30.3
R	273.3	7.3	238.3	7.3	178.8	5.7
Hvu	567.3	32.4	557.9	36.8	723.6	30.0
Rhv	239.4	5.8	202.0	7.3	157.1	4.4
St	64.3	3.8	60.9	2.4	89.0	2.2
Rst	30.7	1.8	23.9	1.4	20.7	0.9
Oes	117.4	6.1	115.3	5.4	165.4	3.8
Roes	52.7	2.9	42.8	3.1	37.6	1.6
Expo	112.5	6.2	117.9	10.0	169.3	7.2
Rex	50.8	2.2	44.1	2.4	38.3	1.0
Tail	72.3	4.6	62.6	5.3	41.2	9.1
Ran	33.9	3.1	29.1	2.2	11.8	2.6
w	2.3	0.1	2.7	0.3	4.6	0.2

Table 4. Test of the null hypothesis that the data values are a random sample from a normal distribution (small values of W would lead to reject the null hypothesis). Samples : see Table 1; Variables : see Table 2.

Variables	Sa. 1	Sa. 2	Sa. 3	Sa. 4	Sa. 5	Sa. 6	Sa. 7
L	0.930288	0.950783	0.90432	0.954759	0.87936	0.947555	0.90436
R	0.93401	0.985493	0.912378	0.970366	0.917845	0.964499	0.951257
Hvu	0.897741	0.941367	0.902623	0.979274	0.896007	0.947614	0.891348
Rhv	0.893547	0.926354	0.953617	0.952395	0.937098	0.962954	0.684124
Sty	0.854861	0.941058	0.841635	0.801891	0.742357	0.930927	0.950426
Rst	0.922825	0.94793	0.939614	0.898034	0.938475	0.912668	0.870415
Oes	0.978404	0.842741	0.947574	0.894186	0.909366	0.770537	0.926579
Roes	0.867235	0.965471	0.842219	0.96167	0.900225	0.956449	0.961532
Expo	0.96264	0.968088	0.934803	0.943069	0.911672	0.984464	0.850891
Rex	0.839393	0.894493	0.883872	0.805876	0.941577	0.867271	0.917811
Tail	0.939868	0.974606	0.976339	0.913494	0.923942	0.968521	0.92123
Ran	0.891262	0.87515	0.962707	0.938155	0.956862	0.909085	0.939325
w	0.916391	0.95793	0.816853	0.928252	0.885478	0.942877	0.969685

than 99 %, often as high as 99.99 %). Actual measurements or annulus counts are not equal to their estimates based on annulus width. The number of annuli must be counted when this character is needed, and it would be wrong to estimates counts by dividing measurements by the width of an annulus.

A second preliminary question of interest is to decide whether an increase of length (for example, longer body) is due to an increase in the width of body annuli or to an increase in the total number of annuli. This was studied for total body length *(L)* and total number of body annuli *(R)* in the six populations of *C. caloosia.* Coefficients of correlations were computed between *L*, *R*, and *w*, the width of a body annulus (Table 6).

The total number of body annuli *(R)* is poorly correlated with both body length *(L)* and width of one body annulus *(w)*. On the other hand, *L* and *w* are always highly correlated. The width of body annuli increases when the body length increases, which would indicate that the number of body annuli remains a constant.

Table 5. Difference between actual measurements or counts and estimates based on *w*, the width of a body annulus (n = 86).

Variable	Mean	Std Error	T	Prob > |T|
LD	- 12.731	3.5912248	- 3.5451402	0.0006
RD	6.0842	1.3031721	4.6688017	0.0001
HvuD	- 7.7639	2.5104340	- 3.0926738	0.0027
RhvD	3.4549	0.9370452	3.6870588	0.0004
StD	4.8290	0.5511829	8.7612829	0.0001
RstD	- 1.6647	0.1994058	- 8.3486696	0.0001
OesD	2.9604	0.8466499	3.4966815	0.0008
RoesD	- 0.8718	0.3077774	- 2.8327776	0.0058
ExpoD	3.9534	0.8280723	4.7743277	0.0001
RexD	- 1.2606	0.2989612	- 4.2166684	0.0001
TailD	12.0255	0.7301241	16.4705986	0.0001
RanD	- 4.2911	0.2515276	- 17.0601941	0.0001

Table 6. Coefficients of correlations and their significance between body length *(L)*, number of body annuli *(R)*, and width of one body annulus *(w)* in sample 1 to 5 (see Table 1).

	L/R	*L/w*	*R/w*
1	- 0.43590	0.84931	- 0.62301
	0.1802	0.0009	0.0406
2	- 0.03229	0.77812	- 0.47076
	0.9021	0.0002	0.0565
3	0.16053	0.65178	- 0.29305
	0.6799	0.0572	0.4441
4	0.28757	0.63438	- 0.27429
	0.3912	0.0360	0.4144
5	0.15827	0.63256	- 0.24123
	0.6421	0.0367	0.4749

ACCURACY

When " best " means " most accurate ", the best method is the one that gives a true result. As the true distance or the true number of annuli is not known, the most accurate method is taken to be the one that changes least when the measurement or the count is taken twice from the same specimens. Changes may be caused by observation errors or by the fact specimens deteriorate slowly in storage. Two series of observations were made at a ten-year interval, first in 1976 (sample 2 in Table 1) then in 1987 (sample 6, Table 1) on specimens from millet grown in the greenhouse at Adiopodoumé (Ivory Coast).

The values of each variable measured in samples 2 and 6 were compared by a test t (T TEST procedure of SAS) to test the hypothesis that the true means are the same. An F' (folded) statistic verified that the variances of the two samples were equal.

Table 7 shows that the results are not consistent between the various couplets of measurements. It is definitely better to measure the body length *(L)* than the number of annuli *R*, because the probability that the mean length *L* is the same in the two series of measurements is much higher than the probability for *R*. The same is true for the distance from the labial disc to the excretory pore *(Expo)* compared to the number of annuli *Rex*. On the other hand, it is better to estimate the size of the stylet in number of annuli *(Rst)*, and to count the annuli between labial disc and end of the oesophagus *(Roes)* than to take direct measurements. The two methods are about equal in accuracy with a slight advantage to the measurements method for the position of the vulva *(Hvu)* and the tail length *(Tail)*.

It was to be expected that measuring the total body length and the distance from head to vulva would be more accurate than annulus counts because of the high likelihood of error in counting several hundred annuli. It was also to be expected that counting a few (21 to 27) annuli along the stylet would be more accurate than measuring this organ, because the stylet tip may be difficult to see. Posterior end annuli are not as well marked as those in the anterior end, which makes it difficult to count the tail annuli. This may explain why it is better to make a direct measurement for tail length. It is difficult

Table 7. Test of the difference between measurements and counts (Table 2) taken twice on the same specimens.

L	=	0.6701	>	R	=	0.0009
Expo	=	0.8322	>	Rex	=	0.3381
Tail	=	0.6729	>	Ran	=	0.3405
Hvu	=	0.6236	>	Rhv	=	0.6037
Oes	=	0.2616	<	Roes	=	0.9583
St	=	0.4686	<	Rst	=	1.000

to explain why it is better to measure the position of the excretory pore while the length of the oesophagus is best given as a number of annuli.

VARIABILITY

When " best " means " less variable ", the best method is the one that exhibits the smallest coefficient of variability.

Coefficients of variability were compared for each couplet of variables in the various samples. In Table 8, the lowest coefficient is printed in bold character. The coefficients of variability of *R* and *Rex* are almost always smaller than in the corresponding measurements (respectively body length and distance from labial disc to excretory pore). The opposite is true for oesophagus and stylet lengths. There is no clear conclusion for tail length and distance head to vulva. For each couplet of character, the method that best reduces the variability is the one that does poorly for increasing the accuracy. This may be because it is more difficult to hit a narrow target.

The results leave us with no clear instruction as to what method should be used, as the " best " method differs from character to character and differs depending on whether we want to increase the accuracy or decrease the variability. We are left with the third meaning of the term, what is the best method for discriminating species.

DISCRIMINATION

Several discriminant function analyses (DFA) between the various samples of *C. paradoxa* and one sample of *H. arenaria*, a quite different species, were made to decide which kind of variables (measurements or number of annuli) best keeps together all the *C. paradoxa* samples while separating these samples from *H. arenaria*. The CANDISC Procedure of SAS PC was used.

A first DFA was made with only the six samples of *C. paradoxa*. All the variables (counts and measurements) were included. Table 9 gives the total canonical structure, that is the bivariate correlations between each variable and the discriminant function, independently of any correlations between variables.

Obviously, axis 1 is correlated more with the annuli counts than with the measurements. This axis pulls apart sample 5 from the others. Axis 2 is correlated more with the measurements (except for *Rex*). It somewhat separates sample 4 from samples 1, 2, and 3. Graphs A and B of Figure 1 show the results of two other DFA made on the same six samples with only the annulus counts (graph A) or with only the measurements (graph B). It is obvious that the six samples are best kept together in graph B with only measurements.

In a DFA with all samples (including sample 7, *H. arenaria*) the measurements do as good a job, or a better job, than the counts for separating sample 7 from the other samples along axis 1. Counts are more important along axes 2 and 3, that pull apart the different samples of *C. paradoxa* (Table 10). The other two graphs in Figure 1 show the results of two DFA, one with only annulus counts (graph C) the other with only measurements (graph D). Measurement variables best separate *H. arenaria*, while keeping together the *C. paradoxa* samples.

This result was unexpected. I have shown above (Table 6) that in each of the five samples of *C. paradoxa* studied here, the number of annuli *(R)* is rather independant from the general size, as measured by the body length *(L)*. It could have been assumed that this was caused by a high dependence of measurements to ex-

Table 9. Discriminant function analysis with measurements and counts (Table 2) on samples 1 to 6 (Table 1). Total canonical structure for the first three axes.

	CAN 1	CAN 2	CAN 3
L	0.043385	0.425419	0.117352
R	0.534495	0.740089	– 0.346421
Hvu	– 0.040317	0.390386	0.128377
Rhv	0.699398	0.611198	– 0.304223
Sty	0.205977	0.584296	0.457473
Rst	0.701646	0.597536	– 0.009468
Oes	0.025186	0.398611	0.311745
Roes	0.672342	0.423374	– 0.188503
Expo	– 0.263601	0.070402	0.116697
Rex	0.660811	0.334788	– 0.347204
Tail	0.424144	0.449740	0.067362
Ran	0.387933	0.480584	– 0.109997

Table 8. Coefficients of variability of couplets of characters (Table 2) in the seven samples studied (Table 1).

Sample	1	2	3	4	5	6	7
Length - R	4-**3**	7-**3**	7-**5**	**4**-5	5-**3**	6-**3**	4-**3**
Excret. pore/Rex	**3**-4	8-**6**	6-**4**	4-**2**	6-**4**	9-**5**	4-**3**
Tail/Ran	**5**-8	**9**-11	9-**8**	**8**-11	**6**-9	8-**7**	22-22
Head-vulva/Rhv	4-4	7-**3**	7-**4**	4-4	8-**2**	7-**4**	4-**3**
Oesophagus/Roes	**4**-6	**5**-8	**4**-6	**2**-5	5-5	**5**-7	**2**-4
Stylet/Rst	**2**-6	**4**-7	**4**-5	**2**-5	6-6	**4**-6	**3**-4

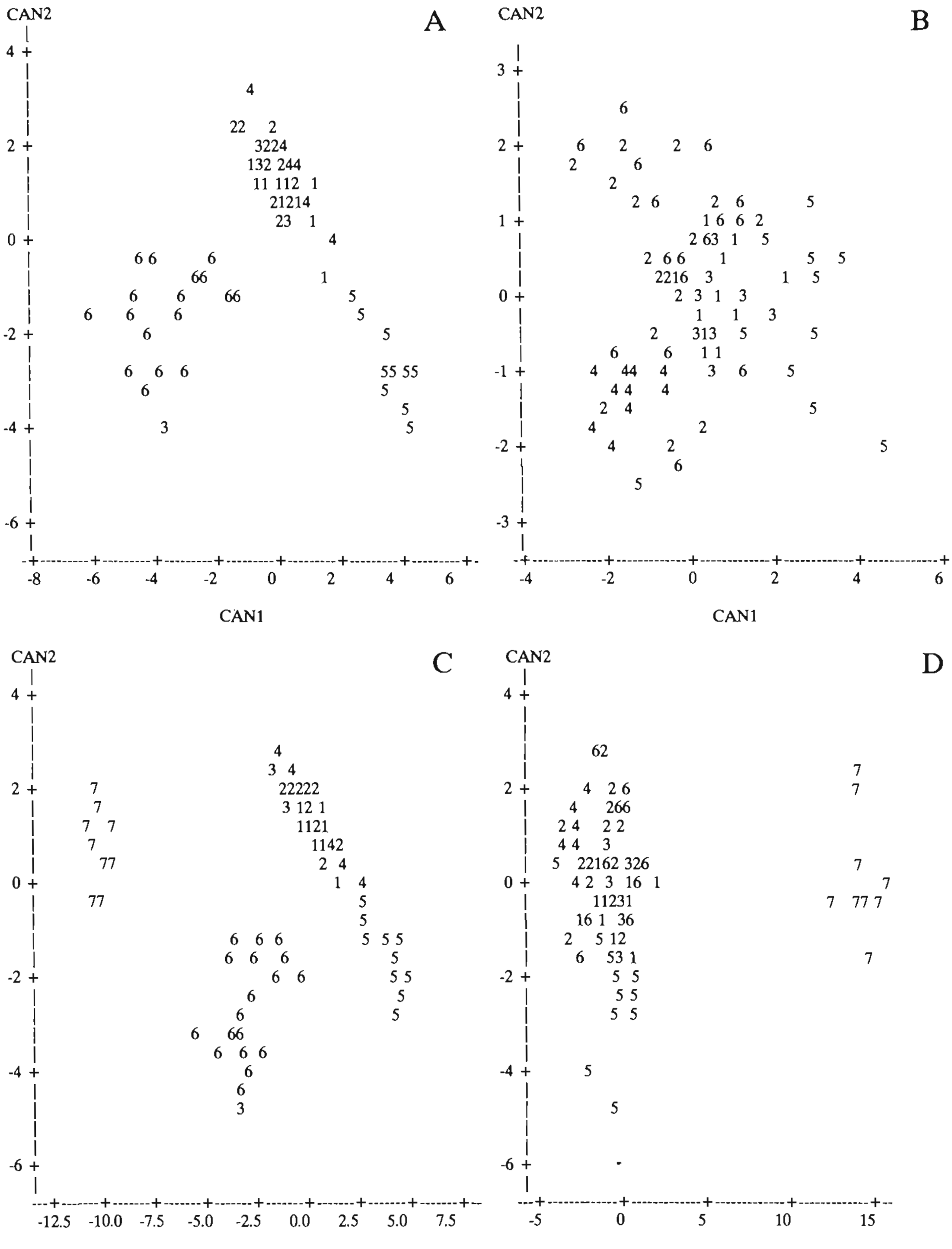

Figure 1. Results of four discriminant function analyses on seven samples of *Caloosia paradoxa* and *Hemicycliophora arenaria* (see Table 1) using either measurements in micrometers or counts of body annuli (see Table 2). A : *C. paradoxa,* annulus counts; B : *C. paradoxa,* measurements; C : *C. paradoxa* and *H. arenaria,* annulus counts; D : *C. paradoxa* and *H. arenaria,* measurements.

Table 10. Discriminant function analysis with measurements and counts (Table 2) on samples 1 to 7 (Table 1). total canonical structure for the first three axes.

	CAN 1	CAN 2	CAN 3
L	0.779571	0.234665	0.212040
R	− 0.829605	0.178746	0.499965
Hvu	0.834729	0.192525	0.167283
Rhv	− 0.834204	0.298235	0.420656
Sty	0.929326	0.309023	0.121579
Rst	− 0.603806	0.537902	0.483590
Oes	0.938792	0.234749	0.069410
Roes	− 0.616557	0.459935	0.355116
Expo	0.915285	0.097104	− 0.005541
Rex	− 0.723541	0.350354	0.279684
Tail	− 0.774820	0.131228	0.272652
Ran	− 0.887888	0.017810	0.256364

ternal factors while number of annuli was more of an internal factor, more genetically determined. In this case, it would have been expected to see the annulus counts provide a better species discrimination than the measurements. The results from the DFA show the opposite. A possible explanation would be that the number of annuli are more linked to the genetic composition of each population sampled, rather than to the species as a whole.

Another possibility would be that the number of annuli changes with the age of the specimen. Samples of the same species that happen to be composed of specimens of different ages would have different numbers of annuli.

Conclusions

The statistical analyses made on six couplets of variables measured on six samples of *C. paradoxa* show that measurements are better than annulus counts for discriminating purposes. However, annulus counts are not highly correlated with corresponding measurements, which means that they measure an original characteristic, one that cannot be inferred directly from measurements. For descriptive purposes, it would be best to give both types of characters.

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