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Principal component analysis (PCA) of morphometric data taken from the opisthaptoral hard parts of *Dactylogyrus vastator* Nybelin, 1924 (Monogenea) reared at three experimental temperature

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Abstract

Dactylogyrus vastator Nybelin, 1924 is a common economically significant pathogenic gill parasite of *Cyprinus carpio. D. vastator* attaches to gills by means of an attachment organ, the opisthaptor, which carries two large hamuli, a connecting bar and fourteen marginal hooks. Principal component analysis (PCA) is a multivariate analysis using morphometric data used to separate morphologically close species on the basis of considering all the measured variables simultaneously and loading for differences between the specimens. Principal component analysis was used to investigate the effects of temperature on sclerite measurements. This study was carried out to stress the morphological variation or discrimination from the measured variables of *D. vastator* reared at three different water temperatures using PCA. The measured variables such as spike length of the hamuli, outer root length of the right hamuli, and internal root length are major factors which clusters more than 75.0% within the eclipse by which the population of the parasite reared at different temperature regimes will be discriminated. The length measurements clusters and fall within the eclipse, the eclipse of different temperature will be separated off very clearly for the 12°C reared specimens than those reared at 14°C and 19°C.

key words : *Cyprinus carpio*, *Dactylogyrus vastator*, Hamuli, Marginal hooks, Opisthaptor, Principal component analysis, Sclerite.

1. Introduction

Principal component analysis (PCA) is a multivariate analysis using morphometric data used to separate morphologically close species on the basis of considering all the measured variables simultaneously and loading for differences between the specimens [1]. Seasonal variation or local environmental changes may influence the parameters of the sclerotized portions of the opisthaptor in monogeneans [2,3,4,5]. Since opisthaptoral measurements are very important in the specific identification of dactylogyrids it is obviously important that any intra–specific variation should be identified, in order to avoid possible mis-identifications. In view of this a method to compare the measurements of sclerotised parts of the opisthaptor from worms grown at different temperautures would be useful.

Mathematical modelling applied to morphometric data, is a way to separate or discriminate one pattern of measurements from another. Bray and des Clers [6] used principal component analysis in a series of stepwise linear discriminate analysis to show the existence of five species of Lepocreadiidae in gadiform fish using 22 measured variables. This present study was carried out to indicate the morphological variation of the hamuli or discrimination of hamuli from measured variables of *D. vastator* reared at three different water temperatures using Principal Component Analysis (PCA).

2. Materials and Methods

Dactylogyrus vastator infected *Cyprinus carpio* were maintained in the aquarium at the defined temperature in the auto temperature control rooms. Flat preparations of *D. vastator* were prepared and mounted in ammonium picrate glycerine [7] for light microscope studies. Specimens from the 12°C , 14°C and 19°C were selected for the analysis.

Specimens were measured under x 40 magnification using an Olympus BH2 binocular microscope with an eye piece graticule (100 x 0.01 mm divisions). A series of measurements were taken on the opisthaptoral sclerites (Figure 1).

All data were included in the first Principal Component Aanalysis (PCA) test. Principal component analysis explains the relationship between the measured variables. Each selected axis of the PCA plot is arranged by the amount of variation which they explain [1]. A cluster analysis was performed on the PCA plots to detect natural groupings within the data set. The way in which these groups are produced is by calculating some measure of dissimilarity between the specimens. Pearson's correlation can be used as the basis of dissimilarity. The cluster analysis is interactive, and is instructed to look for 2 clusters, then 3 clusters, 4,5, etc. up to 10 clusters (in this data set) [6]. The number of groupings within the data by PCA given

by the F ratio and to a lesser degree by the value of probability in the summary statistics for the number of clusters pulled out by the analysis. The clusters indicated by the analysis, were cross referenced back to the specimens and re-examined.

3 Results

The first component has the longest axis, the second is the next largest and is perpendicular to the first, and the third is the next largest, in the measured array, and arises perpendicular to the first two components. To calculate the principal component, all variables are made equal. When the components with the above variables (co-efficients) have been calculated, the total variance on the components is the same as the total variance on the original variables. The component loadings are the covariances of the original variables. If each of the loadings is squared and added up for each component then this will be the variances accounted for by each component. The eigenvector values calculated by the analysis explain how much each character contributes to each axis of the PCA and also explains how much each axis contrib utes to overall variation.

Histograms were produced for each of the measured structures to determine whether it has a normal distribution. It was found that the data did not show a normal distribution and was therefore transformed to a logarithmic value to make the histograms more symmetrical.

Variation of PCA is shown in Figure 2. Table 1a,b gives the variance explained by the components and percentage of the total variance explained. The first three factors here account for 55.15% of the total variance explained. The cluster analysis suggested that three natural groups existed within the specimens run in the first PCA. Two specimens deviated widely from the majority of the clustering specimen points, one having a large internal root length of the hamuli and the other having a large total length of the hamuli. These outliers were re moved prior to running the next PCA.

Table 1b: Principal component analysis of the correlation between the 12 variables.

Eigen values and proportion of the variance explained by the first four components				
Eigenvalue	3.057	2.106	1.456	1.389
Proportion	0.254	0.175	0.121	0.115
Cumulative	0.254	0.429	0.550	0.665

Coefficient of each variable on the first four principal component				
Variable	PC1	PC2	PC3	PC4
Spike length right hamuli	0.148	0.008	0.375	-0.018
Outer root length of right hamuli	0.177	-0.224	-0.155	0.047
Marginal spike length	0.053	0.022	0.139	0.549
Length of the worm	0.086	0.326	0.021	0.535
Internal root length of left hamuli	0.067	-0.356	-0.221	0.225
Basic length of right hamuli	0.253	0.192	-0.148	-0.117
Spike length of left hamuli	0.211	0.039	0.302	0.146
Overall length of left hamuli	0.210	0.082	-0.347	-0.321
Outer root length of left of hamuli	0.160	-0.322	0.046	0.273
Length of marginal blade	0.152	-0.151	0.313	-0.321
Dorsal bar length	0.089	0.050	0.252	-0.389
Basic length of left hamuli	0.223	0.132	-0.238	0.047

The results of the second PCA plot are shown in Fig 3. The component loadings for the PCA are given in Table 2a,b which also gives the variance explained by shows the components and percentage of the total variance explained. The first three factors here account for 50.72% of the cumulative variance. The cluster analysis again suggested three natural groups within the data. The data for each cluster were then examined carefully. Factor 1, factor 2 and factor 3 were analyzed separately to ascertain the extent to which each of these factors was responsible for the cluster produced, and more importantly, which point to point measurements contributed to the separation of the measured individuals. It was found that the length of the basal portion of the hamuli explained the most variation and was the key measurement in separating the specimens.

Table 2b: Principal component analysis of the correlation between the 12 variables.

Eigen values and proportion of the variance explained by the first four principal components				
Eigenvalue	2.646	1.833	1.607	1.362
Proportion	0.220	0.153	0.134	0.113
Cumulative	0.220	0.373	0.507	0.620

Coefficient of each variable on the first four principal component				
Variable	**PC1**	**PC2**	**PC3**	**PC4**
Spike length right hamuli	0.103	0.391	-0.132	0.238
Outer root length of right hamuli	0.231	- 0.071	-0.285	0.153
Marginal spike length	0.054	0.026	0.026	-0.540
Length of the worm	0.034	0.093	0.093	0.151
Internal root length of left hamuli	0.119	- 0.233	-0.233	-0.083
Basic length of right hamuli	0.237	- 0.067	-0.067	0.337
Spike length of left hamuli	0.165	0.338	0.338	0.089
Overall length of left hamuli	0.204	- 0.352	-0.352	0.009
Outer root length of left of hamuli	0.188	0.094	0.094	-0.149
Length of marginal blade	0.224	0.181	0.181	-0.251
Dorsal bar length	0.159	0.099	0.099	-0.348
Basic length of left hamuli	0.245	- 0.173	-0.173	0.070

The variables operating in F_1, F_2, F_3 and F_4 were calculated from the component loadings. From the factor loading plots of the component loadings it can be calculated that factor 1 explains the basic length of the left hamulus acting along the x axis, factor 2 is principally explained by the spike length of the right hamulus acting principally along the y- axis and in factor 3 it is the total length of the left hamulus which acts along the z-axis.

4 Discusssion

Logarithmic transformation of the data was necessary as the data was not normally distributed and this makes the variance independent of the mean and the frequency distributions more symmetrical. In particular, some structures exhibited a degree of bimodality, such as marginal spike. The length of the marginal spike represents one of the smallest structures measured in the haptoral complement, with a small range in the size measurements. The length of the marginal spike exhibited a high degree of variability as shown by the histogram and therefore did not have a normal distribution. A possible reason for that it is a small structure (3.12 – 4.68 mm). When measuring with the light microscope, its accurate resolution is

approximately 0.5 mm, the number of measurements in "score" classes is small and any errors that may happen will therefore significant. The major result of this analysis was that in the opisthaptoral complement any structure below 10 mm in size is likely to produce a confusing picture when making a comparison of the armature at different temperatures. When the preparation is not completely flat, particularly the opisthaptor armature, this can introduce an error in point to point measurements. In addition some of the variation may be caused by the fixative, eg: the hamulus root is a region of unconsolidated hook material and changes in shape may occur as a result of the fixative.

The distribution of specimens within the temperature cluster was carefully analyzed. The out liers were removed to get a specific PCA plot, these structures influencing the separation of the specimens affected as a result of different temperatures on the opisthaptoral armature. It was found that the basic length of the left hamulus was the key measurement acting along factor 1 separating the specimens whilst the spike length of the right hamulus acted through factor 2 and the total length of the left hamulus acted through factor 3.

From Fig 2 it can be clearly seen that temperature has a marked effect on the size of the opisthaptor armature and that the hooks measured at 12°C and 14°C can be separated from those measured at 19°C. Measurements of the sclerotised parts of monogeneans taken from populations at different temperature may therefore be significantly different and this could be significant in the identification of species or the establishment of new taxa.

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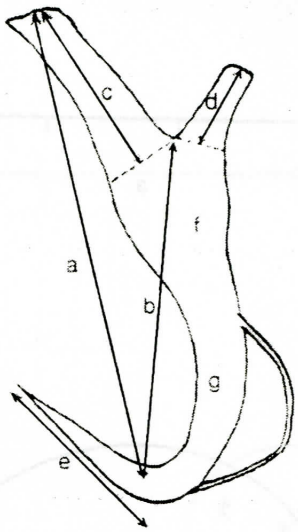
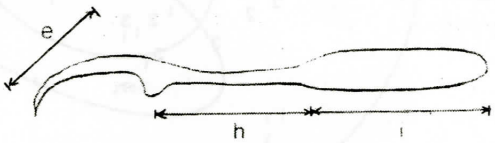


Hamulus



Marginal hook

a total length of hook
b length of basic portion or base
c length of outer root or external process
d length of inner root or internal process
e length of blade or spike
f expanded section of hamulus shaft
g narrow section of hamulus
h blade of marginal hook or keel
i handle of marginal hook

Figure 1: Diagram showing the details of the measurements taken from the hamulus and marginal sclerite

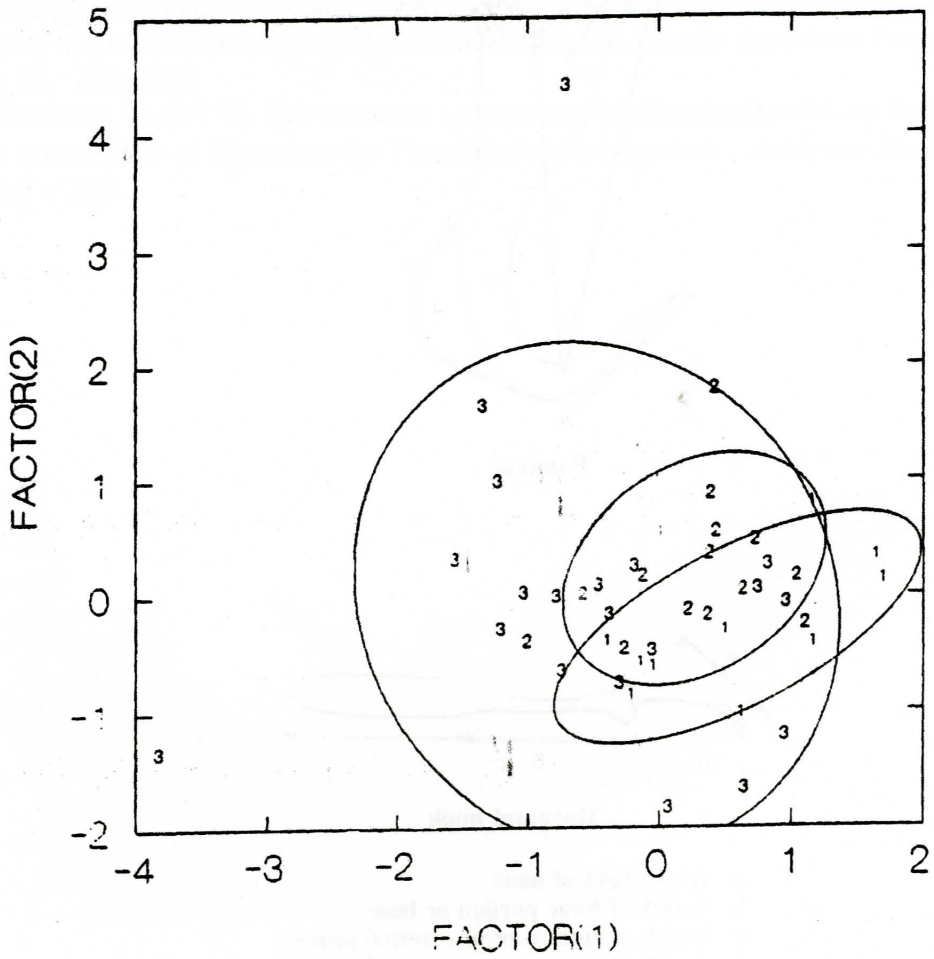


Figure 2: The PAC plot of logarithmic hamuli measurements for the temperature regimes

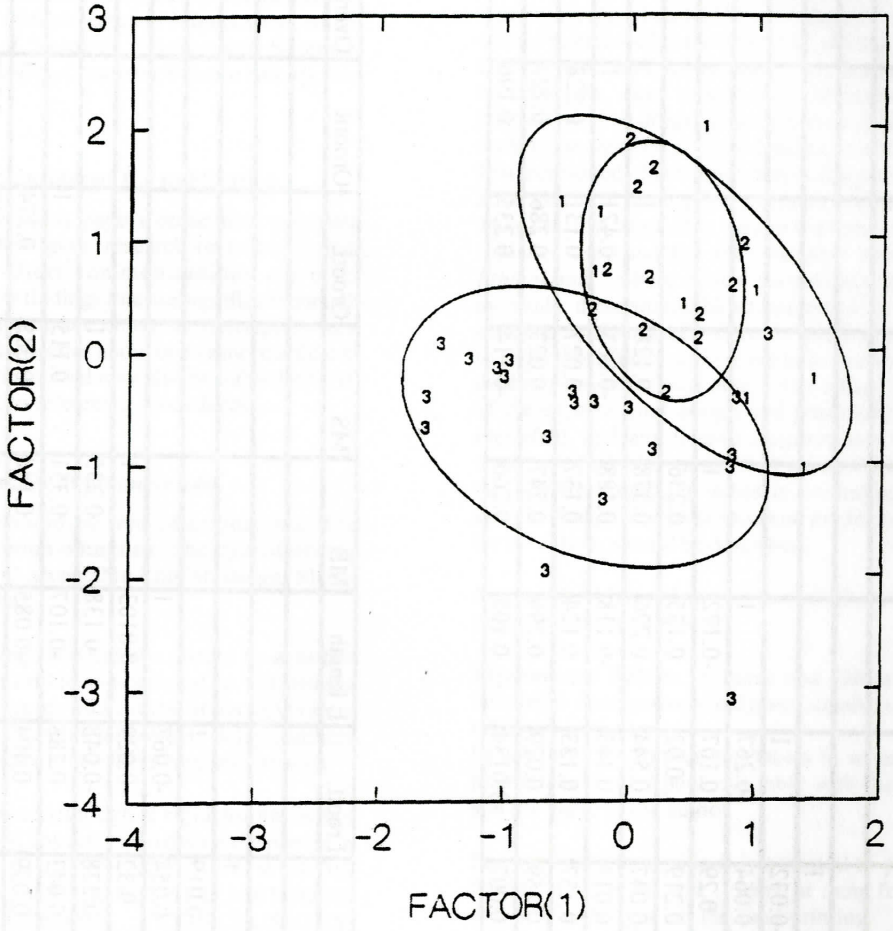


Figure 3: The PAC plot of logarithmic hamuli measurements for the temperature regimes after removal of out liers.

Table 1 a : Correlation matrix

	Basic L	Basic R	DB	I rootL	L length	MB	MS	OrootL	OrootR	Overall	SpikeL	SpikeR
Basic L	1											
Basic R	0.606	1										
DB	0.162	0.035	1									
Iroot L	0.038	-0.154	-0.032	1								
Llength	0.282	0.386	0.064	-0.267	1							
MB	0.15	0.176	0.29	0.107	-0.172	1						
MS	0.187	-0.008	0.219	-0.02	0.135	0.236	1					
Oroot R	0.091	0.138	-0.047	0.544	-0.226	0.378	0.128	1				
Oroot L	0.301	0.231	0.013	0.443	-0.118	0.188	-0.063	0.421	1			
Overall	0.52	0.669	0.137	0.133	0.124	0.137	0.027	0.131	0.228	1		
Spike L	0.213	0.4	0.259	0.029	0.299	0.247	0.023	0.286	0.227	0.175	1	
Spike R	0.113	0.3	0.007	-0.0134	0.105	0.266	-0.114	0.235	0.196	-0.035	0.492	1

Table 2 a : Correlation matrix

	Basic L	Basic R	DB	I rootL	L length	MB	MS	OrootL	OrootR	Overall	SpikeL	SpikeR
Basic L	1											
Basic R	0.511	1										
DB	0.183	0.068	1									
Iroot L	0.162	-0.132	0.019	1								
Llength	0.15	0.249	0.054	-0.067	1							
MB	0.15	0.249	0.32	-0.029	-0.0103	1						
MS	0.173	-0.073	0.228	-0.048	0.138	0.235	1					
Oroot R	0.119	0.04	0.043	0.288	-0.107	0.351	0.115	1				
Oroot L	0.321	0.233	0.039	0.404	-0.085	0.146	-0.085	0.374	1			
Overall	0.398	0.503	0.189	0.303	-0.116	0.2	0.027	0.047	0.232	1		
Spike L	0.059	0.185	0.328	0.009	0.23	0.272	-0.027	0.169	0.186	-0.12	1	
Spike R	-0.01	0.144	0.022	-0.15	0	0.293	-0.152	0.24	0.177	-0.284	0.408	1