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Discriminant analysis of geographic variation in hard structures of *Todarodes sagittatus* from the North Atlantic

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Discriminant function analysis was applied to the morphometrics of the ommastrephid squid *Todarodes sagittatus* (Lamarck 1798) in an attempt (1) to discriminate possible differences in the morphometric characteristics of groups from different geographic locations; (2) to elucidate the possible use of cephalopod hard structure morphometrics to identify populations; and (3) to determine which of the morphometric characteristics are the most significant in this respect. A total of 27 morphometric characters were measured using the bodies, arms, gladii, beaks, and statoliths of specimens caught off northern Norway, Scotland and Portugal. Statistically significant differences were shown to exist among the three geographic areas, indicating the possible presence of different populations of *T. sagittatus* in the Northeast Atlantic. The results demonstrate the usefulness of hard structure morphometrics as a tool for analysing variation within species.

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Introduction

Todarodes sagittatus (Lamarck, 1798) is a squid species which occurs over the entire Northeast Atlantic and in the Mediterranean Sea. The vast area of distribution, from the Arctic Ocean to 13° South (Roper *et al.*, 1984), makes the question of population integrity a natural one.

Intraspecific groups of organisms have been described by such terms as race, tribe, population, stock, and subspecies; terms intended to reflect the magnitude of differences among such subdivisions. A population may be defined as a group of organisms with unimpeded gene flow (Amaratunga, 1987). This means that the organisms belonging to one species are localized into a unit within a space and time matrix which permits interbreeding.

Population definition from a fisheries management point of view takes a different form. Ricker (1975) defines a stock as "part of a (fish) population which is under consideration from a point of view of actual or potential utilization". Cushing (1981) shows that a species or a subspecies can comprise a single or several stocks, but that a convenient description of a unit population would be "a group of organisms that could be treated as a homogeneous unit in a management area".

Several methods have been used to identify and discriminate between intraspecific groups of organisms. Ihssen *et al.* (1981a) consider seven types of materials and methods for distinguishing stocks of fish: population parameters, markings, physiological and behavioural characters, morphometric and meristic characters, calcareous characters, cytogenic characters, and biochemical characters.

Morphometric and meristic characters are described as being "potentially powerful" for measuring discreteness and relationships among populations. Individual populations can be accurately characterized on the basis of large volumes of multivariate data (Ihssen *et al.*, 1981b).

Multivariate statistical analysis deals with observations on more than one variable where there is some inherent interdependence among the variables. It deals with data containing observations on two or more variables each measured on a set of objects (Mardia *et al.*, 1979).

Multivariate analysis has been widely used in studies of the geographic variation, racial differentiation, and population systematics of many species. However, few authors have used multivariate techniques on data obtained from cephalopods.

Discriminant function analysis is one of the multivar-

Table 1. Results for computations of the discriminant analysis of geographic variation in *Todarodes sagittatus*. Univariate F-tests (columns) and multivariate tests (last rows). All data log-transformed. No. 1 = hard structure morphometrics; no. 2 = body and arm morphometrics; no. 3 = all morphometrics; no. 4 = the same as no. 3, except the non-significant variables and lnHW. (NS = not significant; *, **, ***, + = significant at the 5%, 2.5%, 1% and 0.1% level, respectively; – not included in the computation).

	Computation							
	No. 1		No. 2		No. 3		No. 4	
VAR (ln)	F2/128	P	F2/130	P	F2/123	P	F2/129	P
ML	–	–	3.69	*	3.76	*	3.82	**
TW	–	–	3.23	*	3.36	*	3.47	*
MW	–	–	0.13	NS	0.25	NS	–	–
FL	–	–	7.61	+	7.56	+	7.87	+
FW	–	–	3.02	*	3.20	*	3.32	*
HL	–	–	3.75	*	3.78	**	3.95	**
HW	–	–	20.10	+	19.76	+	–	–
IRA	–	–	3.58	*	3.66	*	3.83	**
IIRA	–	–	3.99	**	4.10	**	4.23	**
IIIRA	–	–	4.36	**	4.61	**	4.69	***
IVRA	–	–	4.47	**	4.72	**	4.77	***
PL	3.16	*	–	–	2.94	NS	–	–
PW	2.00	NS	–	–	1.57	NS	–	–
RL	4.75	***	–	–	4.42	**	4.51	***
RW	3.84	**	–	–	3.32	*	3.48	*
URL	3.36	*	–	–	3.06	*	3.20	*
UHL	4.84	***	–	–	4.39	**	4.55	***
UCL	4.89	***	–	–	4.44	**	4.56	***
UWL	3.80	**	–	–	3.48	*	3.54	*
URW	7.27	+	–	–	6.55	***	6.74	***
LRL	3.79	**	–	–	3.41	*	3.56	*
LHL	2.16	NS	–	–	1.98	NS	–	–
LCL	2.38	NS	–	–	2.19	NS	–	–
LWL	3.26	*	–	–	2.96	NS	–	–
LRW	8.13	+	–	–	7.24	+	7.40	+
StL	2.67	NS	–	–	2.00	NS	–	–
StW	1.83	NS	–	–	1.61	NS	–	–
Wilks'	0.130		0.087		0.027		0.095	
F	12.53	+	25.98	+	18.35	+	13.97	+
DF	32/226		22/240		54/194		36/224	

iate techniques most widely used to investigate patterns of variation. The idea behind discriminant analysis is to find combinations of the components which maximize differences between already known groups (Andersen, 1966). It maximizes variation between populations in relation to the local variation (Thorpe, 1983).

Clarke *et al.* (1980) used discriminant function analysis on the morphometric characteristics of statoliths to evaluate differences between closely related species. Mercer *et al.* (1980) showed that discriminant function analysis can be employed usefully in identifying the sex of *Illex illecebrosus* from either upper or lower beak morphometrics. Kristensen (1982) used the same technique to analyse geographic variation in *Gonatus fabricii* to the lowest geographic level.

None of the above studies, however, attempted to use the hard structures – gladius, upper beak, lower beak, and statolith morphometrics – as a means of differentiating populations.

In the present work, discriminant function analysis was also chosen as the most appropriate multivariate method. It was applied to *T. sagittatus* morphometrics with the following objectives: (1) to discriminate possible differences in the morphometric characteristics of groups from different geographic locations; (2) to elucidate the possible use of cephalopod hard and soft structure morphometrics to identify populations; (3) to determine which morphometric characteristics were the most significant in this respect.

Materials and methods

This study is based on the measurements of 20 samples collected from the commercial fisheries in waters off

Table 2. Table of actual group (rows) and predicted group (columns) for each computation. Frequency and percentages.

Predicted		Actual group Norway	Scotland	Portugal	Total
Hard structures					
	Norway	55	3	1	59
		(93.2)	(5.1)	(1.7)	(100.0)
	Scotland	1	56	3	60
		(1.7)	(93.3)	(5.0)	(100.0)
	Portugal	0	0	12	12
		(0.0)	(0.0)	(100.0)	(100.0)
	Total	56	59	16	131
Body and arm					
	Norway	58	1	1	60
		(96.7)	(1.7)	(1.7)	(100.0)
	Scotland	1	59	2	62
		(1.6)	(95.2)	(3.2)	(100.0)
	Portugal	1	0	10	11
		(9.1)	(0.0)	(90.9)	(100.0)
	Total	60	60	13	133
All morphometrics					
	Norway	55	0	0	55
		(100.0)	(0.0)	(0.0)	(100.0)
	Scotland	1	58	1	60
		(1.7)	(96.7)	(1.7)	(100.0)
	Portugal	0	0	11	11
		(0.0)	(0.0)	(100.0)	(100.0)
	Total	56	58	12	126
All morphometrics, except NS and lnHW					
	Norway	57	4	0	60
		(93.4)	(6.6)	(0.0)	(100.0)
	Scotland	0	58	2	60
		(0.0)	(96.7)	(3.3)	(100.0)
	Portugal	0	1	10	11
		(0.0)	(9.1)	(90.9)	(100.0)
	Total	57	63	12	132

northern Norway and Scotland (Aberdeen Bay, Shetland, and Rockall) between September 1985 and November 1988. A single sample was collected off southern Portugal during a research cruise in continental Portuguese waters in August 1987.

A total of 135 gladii were measured for pen length (PL), pen width (PW), rachis length (RL), and rachis width (RW).

A total of 154 upper beaks and 155 lower beaks were measured using vernier calipers. Five dimensions were measured on each beak (upper and lower), using the method of Clarke (1962): rostrum length (URL and LRL), hood length (UHL and LHL), crest length (UCL and LCL), wing length (UWL and LWL), and rostral gap (URW and LRW).

A total of 162 pairs of statoliths were measured, under a dissecting microscope, for length (StL) and width (StW). The mean lengths of the left and right statoliths were tested at the 5% level of significance, and in agreement with Lipinski (1980) no significant difference in length was found. The statoliths were therefore chosen randomly with no preference for left or right statolith.

All measurements were recorded in hundredths of a millimeter, and data from both sexes were pooled.

In addition to the hard structure morphometrics (gladius, beaks, and statoliths), the following body and arm morphometrics ("soft structures") were also used: mantle length (ML), total wet weight (TW, in grams), mid-mantle width (MW), fin length (FL), greatest width of fins (FW), head length (HL), greatest width at level of eyes (HW), length of right arms I to IV (IRA to IVRA), length of right tentacle (TentL). Normally the right arm and tentacle were measured, but when they were damaged or missing the left arm and tentacle were measured.

Ratios were completely avoided, since they are only acceptable for comparative purposes if the relationship between numerator and denominator is linear, and

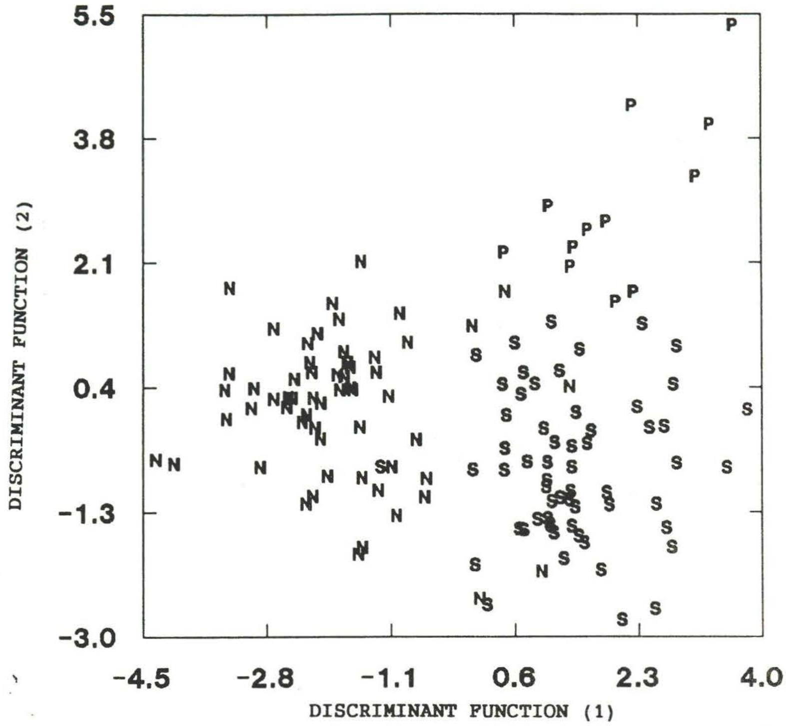

Figure 1. Scatterplot obtained by discriminant function analysis of the hard structure morphometrics of *Todarodes sagittatus* from Norway (N), Scotland (S), and Portugal (P).

passes through the origin (Thorpe, 1983). In this case, none of the dimensions could fulfil these two requirements. It was therefore decided not to use any ratios. However, all data used in this study were log transformed.

Discriminant function analyses were performed using an Ericsson portable personal computer and the software packages STATGRAPHICS STATISTICAL GRAPHICS SYSTEM and SYSTAT.

Four computations were first performed:

1. All hard structure morphometrics were used to elucidate the possible use of cephalopod hard structures to identify populations and to determine which morphometric characteristics were the most significant (16 variables).

2. All soft structure morphometrics (body and arms) were used (11 variables), except measurement of the tentacle (TentL), because of the damage done by the congelation to the muscle fibres (these could be more or less extended depending on the damage).

3. All hard and soft structure morphometrics used in the two previous computations (27 variables) were combined.

4. Morphometrics used in computation no. 3, with the exception of the variables which were not significant (NS) and the head width (lnHW), were used. The reason for the exclusion of lnHW was that the width of the head is measured at the level of the eyes and sometimes the eyes of the specimens studied had burst. This measurement was, therefore, probably not a reliable measurement, because of the possibility of specimens from one area being in better condition than others.

Since racial differentiation includes not only differentiation between spatially segregated populations but also differentiation between temporally segregated populations (Thorpe, 1983), two further computations were performed with selected material so as to overcome the problem with temporally separated groups.

5. To compare *T. sagittatus* from Norway and from Portugal, 9 specimens caught in September 1987 in Norway and 12 specimens caught in August 1987 in Portugal were used. For this specific study, only the morphometric characteristics of the upper and lower beaks were available (7 variables).

6. To compare *T. sagittatus* from Norway and from Scotland, 21 specimens from Norway and 31 from Scotland, collected in September and October 1988, were used. For this comparison all significant morphometrics were considered, with the exception of the lnHW (18 variables).

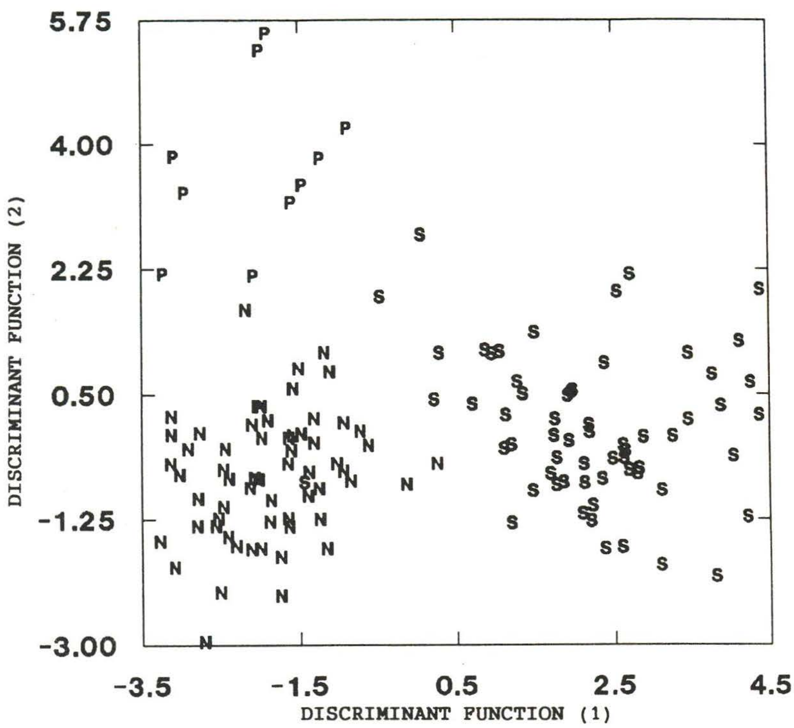

Figure 2. Scatterplot obtained by discriminant function analysis of the body and arm morphometrics (soft structures) of *Todarodes sagittatus* from Norway (N), Scotland (S), and Portugal (P).

For each computation, the actual observations were later classified according to whether or not they fell within the statistically predicted variability of the group to which they belonged.

The test statistic usually presented in statistical packages is a function of the product of the eigenvalues (Mardia *et al.*, 1979) and is called Wilks' Lambda. In the present study each variable was also tested with an F-statistic test to examine its contribution to the discrimination of the groups.

Results

The analyses for morphometric variation due to geographic origin demonstrate highly significant ($p < 0.001$) differences in all computations (Table 1).

1. Hard structure morphometrics

Discriminant analysis based on the hard structure morphometrics shows that the two statolith measurements are not significant ($p > 0.05$) for the separation of the groups. For the beaks, all measurements of the upper beak are significant, while two of the lower beaks are not: the lower hood length (lnLHL) and the lower crest length (lnLCL). The gladius measurements are also significant, with the exception of the pen width (lnPW).

The most important measurements for separating the groups are first the lower rostral gap (lnLRW) and then the upper rostral gap (lnURW).

When the actual data are classified to show individual affinity to the statistically predicted group (Table 2), the percentage of individuals correctly classified is between 93% and 100%. The discrimination based on the hard structures gladius, beaks, and statoliths of Norwegian, Scottish, and Portuguese *T. sagittatus* groups is shown in Figure 1.

2. Soft structure morphometrics

In the discriminant analysis based on the body and arm morphometrics, only one measurement is not significant for the separation of the groups: mantle width (lnMW).

The measurement most important for separating the groups is head width (lnHW), followed by fin length (lnFL).

Classification of the results of this discriminant analysis shows that the proportion of the individuals correctly classified is between 91% and 97%. Discrimination based on the soft structure morphometry of body and arms of Norwegian, Scottish, and Portuguese *T. sagittatus* groups is shown in Figure 2.

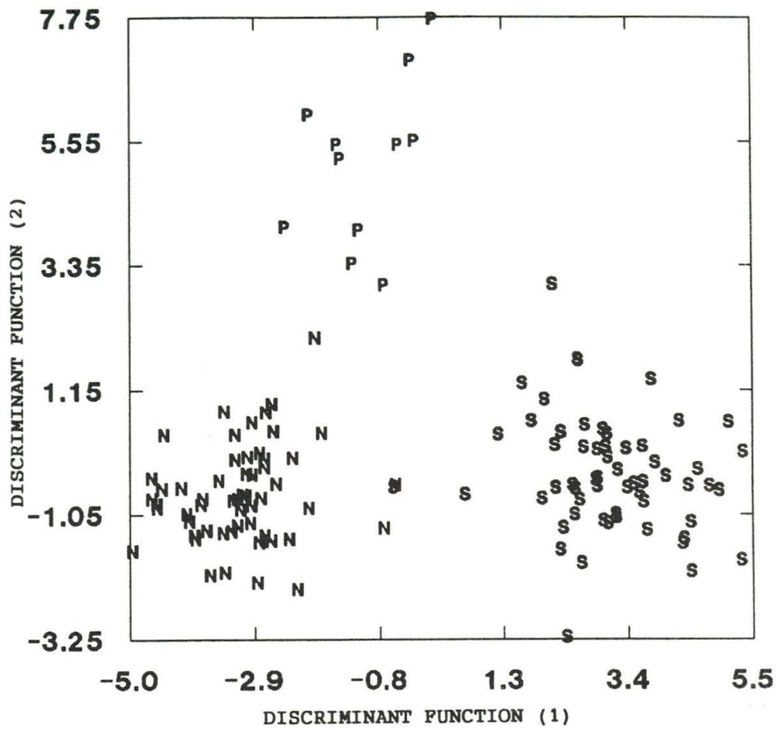

Figure 3. Scatterplot obtained by discriminant function analysis of all morphometrics (hard and soft structures) of *Todarodes sagittatus* from Norway (N), Scotland (S), and Portugal (P).

3. All morphometrics

When the hard and soft structures are combined, the discriminant analysis shows the same results as previously, with only two additional measurements not significant for the separation of the groups: pen length (lnPL) and lower wing length (lnLWL).

The most important measurements for the separation of the groups are also the same as previously: head width (lnHW), fin length (lnFL), lower rostral gap (lnLRW), and upper rostral gap (lnURW).

The proportion of individuals correctly classified in this discriminant analysis is 97–100%. Discrimination based on the hard structure morphometry of gladius, beaks and statoliths, and soft structure morphometry of body and arms of Norwegian, Scottish, and Portuguese *T. sagittatus* groups is shown in Figure 3.

4. All morphometrics, except NS and lnHW

In this discriminant analysis all measurements are significant for the separation of the groups, with half of them highly significant ($p < 0.01$).

The most important measurements for separation of the groups are fin length (lnFL), lower rostral gap (lnLRW) and upper rostral gap (lnURW).

The proportion of individuals correctly classified is between 91% and 97%. Discrimination based on the 18 significant measurements of the head and soft structures (except the lnHW) of Norwegian, Scottish, and Portuguese *T. sagittatus* groups is shown in Figure 4.

5 and 6. Selected material

The analysis of morphometric variation between the Norwegian group (basic group) and specimens from any of the other two areas (Scotland and Portugal), showed very highly statistically significant differences ($p < 0.001$).

For the two computations, the separation occurs along one axis, since only two groups are compared.

The Wilks' Lambda values for the multivariate test are 0.027, with a chi square of 55.9, for the Norway/Portugal discrimination, and 0.270, with a chi square of 51.0, for the Norway/Scotland discrimination.

Classification of the actual results in relation to the predicted group shows that 100% of the individuals in the Norwegian versus Portuguese analysis were correctly classified. For the Norwegian versus Scottish analysis, 95% and 100% of the individuals were correctly classified.

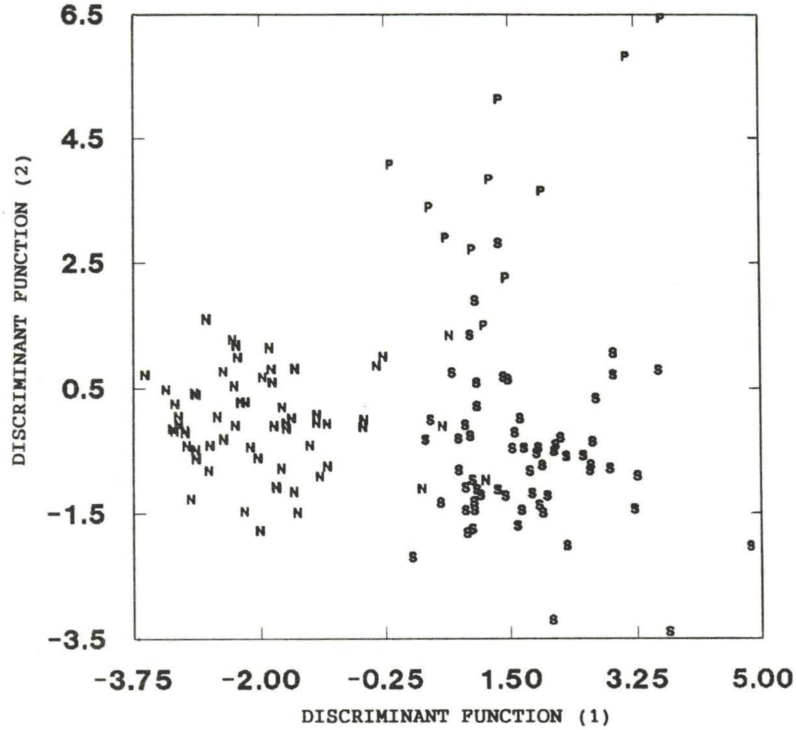

Figure 4. Scatterplot obtained by discriminant function analysis of the hard and soft structure morphometrics of *Todarodes sagittatus*, excluding the dimensions not significant in previous analysis (see text). (N = Norway; S = Scotland; P = Portugal).

Discussion

The results of the discriminant analysis study demonstrate that the individuals which provided the data studied can be separated into three distinct groups corresponding to the area of collection.

The reason for the observed variation in the morphometric characteristics of *T. sagittatus* is not known. It may be due to environmental factors or to genetic differences. Unfortunately, not many studies have been carried out on either environmental or genetic effects on the morphology of cephalopods.

Using discriminant analysis, Kristensen (1982) found morphologically distinct populations of *Gonatus fabricii*, even in groups living in areas close to each other. This author assumed that the reason for this distinction was a genetic difference, since differences in times of reproduction in this species were found.

The hypothesis proposed by Rosenberg *et al.* (1980) and discussed by Borges (1990) of there being both spring and autumn spawning populations contributing to the northward migration, would tend to support the idea of genetically founded differences, assuming that the spawnings were sufficiently separated and not just weak peaks during a protracted spawning season.

The second objective of this study was to elucidate the possible role of cephalopod hard and soft structure morphometrics to separate populations. The results demonstrate that these may be used successfully to separate and identify populations.

Statoliths, however, did not give significant results. It is important to point out that, since the statoliths are so small, the measurement unit was probably not an appropriate one. Measurements to the nearest micron, rather than hundredth of a millimeter, would have been more suitable.

The third objective was to determine which of the morphometric characteristics were the most significant for discrimination. The soft structure morphometrics – body and arm – gave better results than the hard structures, although the differences were small (see Table 1 and Figs. 1 and 2). However, the hard and soft structure morphometrics combined gave the best discrimination of *T. sagittatus* groups (Fig. 3). Even when the greater contributor for the discrimination is excluded as unreliable (lnHW) the discrimination of the groups is still very good (Fig. 4).

Of the hard structures, the best contribution for discrimination of the groups was given by the beaks, especially the upper beak. It is important to point out that both beaks were well-defined morphologically (10 measurements), which gave a good definition of their form. The same was not true of the gladius and the statoliths, where only four and two measurements, respectively, were taken.

It seems, therefore, that the 18 measurements used in computation no. 4 are the best morphometric characters to use in the discrimination of this species (Table 1). The head width is undoubtedly also very important. It was excluded from the last computation only because of the insecurity felt over the method of measurement in this specific study.

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