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MORPHOLOGICAL DIFFERENTIATION OF THE GENUS *NIPHARGUS* (AMPHIPODA) ON CORSICA — A NUMERICAL APPROACH

by

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SUMMARY

A short introduction is provided on the taxonomic status of the genus *Niphargus*, especially on the species related to *N. longicaudatus corsicanus*. Previous findings and descriptions are mentioned. An attempt is made to clarify the relationships between Corsican *Niphargus* populations by means of a cluster analysis and a principal components analysis combined with a cluster analysis. Special attention has been paid to the size-dependent variability of most of the characters. The results of both methods of analysis are compared with each other and evaluated. The morphological differentiation between populations is, on the average, greater than within populations. This, along with the large amount of character variability, makes it very difficult to fit populations into, or to distinguish them from, any of the — often poorly described — taxa of *Niphargus*.

RÉSUMÉ

Une brève introduction est dédiée au statut taxonomique du genre *Niphargus* et spécialement des espèces apparentées à *N. longicaudatus corsicanus*. On mentionne les captures et les descriptions précédentes concernant les *Niphargus* de Corse. On a essayé de tirer au clair les relations entre les populations corses de *Niphargus* en utilisant une classification automatique („cluster analyse") et une analyse des components principaux combinée avec une classification automatique. Une attention spéciale a été dédiée à la variabilité de la plupart des caractères, dépendant de la croissance. Les résultats des deux méthodes mentionnées d'analyse sont comparés et évalués. La différenciation morphologique interpopulationnelle est généralement plus importante que celle intrapopulationnelle. Compte tenu de ceci, ainsi que de l'importante variabilité des caractères, il est fort difficile d'attribuer les populations corses à l'un des taxa, parfois mal décrits, du genre *Niphargus*, ou bien de les distinguer de ceux-ci.

INTRODUCTION

The taxonomic status of many species within the genus *Niphargus* Schiödte, 1849, is not very clear. This is partly due to the fact that most species are very similar and show great variability, but also because up to the present little is known about the phylogeny of the genus. Cross-breeding tests and population research have not been performed, so interspecific sterility has not yet been applied as a criterion to distinguish the species.

In 1981 we sampled *Niphargus* in 55 localities in Corsica (France), in caves, springs, wells and interstitia; also we studied some material (from 1 locality) collected by Prof. J. Giudicelli. We decided to investigate to what extent morphological differentiation had taken place between the individuals of several populations and regions.

The study of the genus *Niphargus* has been performed in an amazingly unsystematical way ever since its discovery in 1836. Many descriptions are incomplete, character variability is almost unknown and species are described on the basis of very few specimens.

Especially in the years 1930-1940 the number of taxa increased steadily, thanks to the work of Schellenberg, Ruffo and S. Karaman. Unfortunately, as there was little agreement about the characters which should be used, it is very difficult, and often impossible, to compare the described taxa with each other.

Nowadays, hundreds of species and subspecies have been described, which are divided into 14 species groups (Straškraba, 1972). The relationships between these groups are sometimes very obscure (for instance between the *stygius-puteanus* group and the *longicaudatus* group) and between the species within each group probably even more.

Since the mid fifties numerical taxonomic methods have been applied in various groups of animals, particularly since the rapid increase of the use of computers (Sneath & Sokal, 1973). It is not the objective of this paper to discuss the merits and disadvantages of these methods; it seems to us that every method which could bring any order in some of the problems posed by the genus *Niphargus* is worth trying.

By means of some statistical and pattern detection methods we have tried to bring some clarity in the systematics of a limited group of populations of the genus, viz. those from Corsica. Our primary question was if by means of these techniques a division could be made into groups which would come up to expectations based on geographical factors, i.e. a division in agreement with a grouping into populations or otherwise separated regions. In our case these regions were separated by the most important watersheds. A secondary question was whether different methods would give the same results, and if not, which one was the better.

The specimens on which the present study is based have been deposited in the collections of the Zoölogisch Museum, Amsterdam. The complete set of data used for the analyses is not published in this paper, but has been deposited in the Artis Library of the University of Amsterdam, where it can be consulted.

THE GENUS *NIPHARGUS* ON CORSICA

The presence of the genus *Niphargus* on Corsica was established for the first time by a "Breslauer Lehrexkursion" in 1914 (Schellenberg, 1950). These specimens have neither been preserved nor described. In 1948 *Niphargus* was sampled again by Rémy (1950). These specimens were described by Schellenberg (1950) as *Niphargus corsicanus*. Ruffo (1960) mentions *N. corsicanus* and considered it to be closely related to *N. longicaudatus* Costa, 1851, which he treats as a subspecies of *N. stygius* Schiödte, 1847. In 1968 *Niphargus* was found for the first time in a corsican cave (Beron, 1972). These specimens were not described, but Beron remarks: "Il diffère nettement de *N. corsicanus* Schellenberg, 1950".

In 1969, Vigna Taglianti (1972), Morand-Chevat (1972) and Straškraba (1972) consider *N. corsicanus* closely related to *N. longicaudatus*, still with the very brief description of Schellenberg as the sole basis. By this time *N. stygius* and *N. longicaudatus* are placed in different species groups by Vigna Taglianti (1972), Morand-Chevat (1972) and Straškraba (1972), but an argumented revision of the genus *Niphargus* has never been written.

In 1970 and 1971 *Niphargus* was sampled in several localities by Stock (1972). He distinguished two species. One of them he considered identical with the species described by Schellenberg, which he named *N. longicaudatus corsicanus*. The other species (3 specimens from 2 localities) he considered related to the *tatrensis* group, but no definite taxonomic status was assigned (*N.* aff. *tatrensis* Wresniowski, 1890). On account of our results we reexamined Stock's material.

DISPERSION PATTERN

The dispersion pattern found by us, of *Niphargus* on Corsica (fig. 1) is not only influenced by the actual occurrence of *Niphargus*, but also greatly by the possibilities of catching them. Water in caves is very rare, wells are only found in the region of Bonifacio and in the alluvial plains of the east and the north, so the main possibilities are restricted to interstitia and springs. Most of the populations were found in artificially constructed springs, where it is often possible to follow the course of the water 1 or 2 meters inside the rock with a brush, tied to a flexible stick. The more "natural" springs often consisted of muddy pools, or of water seeping through small crevices in the rock, yielding only few *Niphargus* specimens.

This resulted in the fact that most populations have been found in regions with many man-made springs, which are usually the most populated and cultivated parts of the island. These last two factors however, are closely cor-

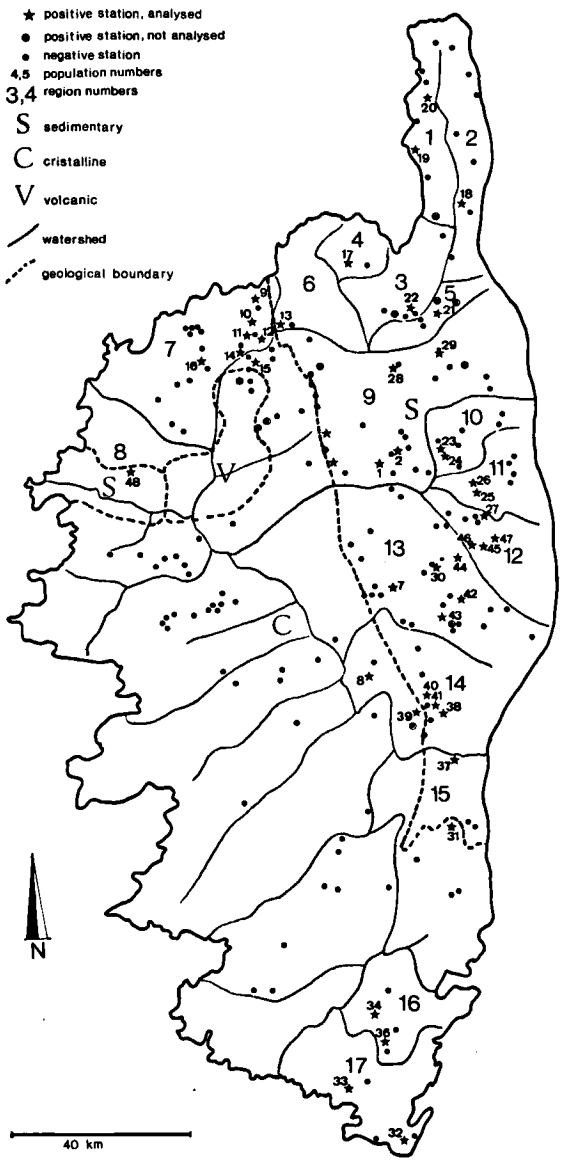

Fig. 1. Map of Corsica, indicating the sampling stations and regional subdivisions.

related with the composition of the soil and therefore with the underlying material. Sedimentary rock yields a fairly rich soil and so the sedimentary regions, including the alluvial plains, are more cultivated than cristalline regions, with their poor and acid soils. In this way historical and cultural factors are at least partly responsible for the fact that most of the populations have been found in sedimentary regions.

METHODS OF ANALYSIS

The material collected contained ca. 560 specimens of the genus *Niphargus* varying in length from approx. 1 to 14 mm. From as many localities as possible, the least damaged specimens, longer than 4 mm, have been dissected and mounted on microscope slides. With a microscope and eyepiece micrometer 107 characters have been measured or counted (see appendix). An important criterion for choosing the characters was easy and accurate measurability. Apart from that, the characters were chosen arbitrarily, since little or nothing is known about there adaptive values.

Since the specimens differ greatly in size, we had to investigate to what extent the values of the characters measured are dependent on body size, so the dimension of each character was plotted against the length of the propodus of pereiopod VI.

From fig. 2, in which the sum of the lenghts of all body somites, except the last urosomite, is plotted against the length of the propodus of pereiopod VI, it emerges that both parameters are linearly correlated. Pereiopod VI was chosen as a relative measure of body length because this character was present in nearly all

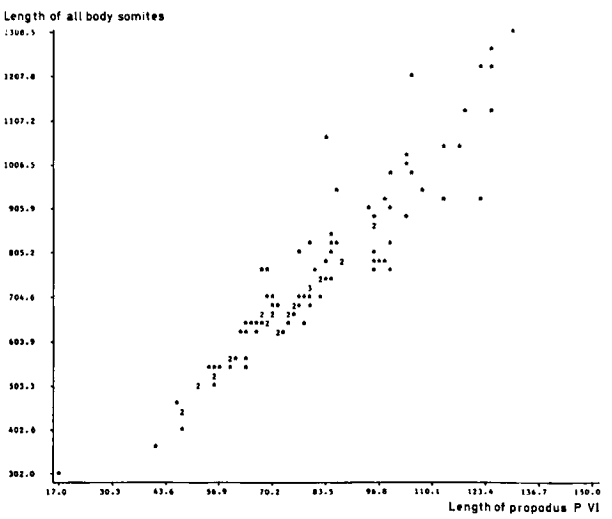

Fig. 2. The sum of the length of all body somites, but for urosomite III, plotted against the length of the propodus of P VI. Scale expressed in mm × 10^{-3}.

specimens. So this value can be used directly as a measure of body size.

Other plots showed that nearly all characters are dependent on size. Of all characters only one (the number of unidentate spines on the outer lobe of the first maxilla) is not correlated with size.

Such a size dependence is awkward from a taxonomic point of view, especially because some of the correlation coefficients are rather low. Although the correlations are statistically significant, the relationship between the variables is weak. There are no discontinuities in the plots which could indicate that several species are involved, of which the character dimensions are differently related to size. Discontinuities in the plots could have been caused by different moulting stages as well. Growth, and consequently the size at moulting, can be dependent of, among other things, the availability of food, temperature, current velocity, and possibly the Calcium-ion content of the water (Dickson, 1977). Very probably these factors will appear in all possible combinations at different places, so it is not surprising that such discontinuities have not been found in the plots. This absence of discontinuities made us choose for a size-independent method of classification.

Secondary sexual dimorphism within the *N. longicaudatus* group is expressed in the first and third uropods (figs. 3 & 4). For this reason these characters are better not used for classification purposes. By mistake the lengths of the rami of uropod 1 were not excluded, but this seems to have been of little or no consequence for the results.

Three methods were used to obtain a size-independent classification:

(a) A cluster analysis of the largest specimens using characters showing a low correlation with size. This method did not contradict the other results, but will not be discussed here any further.

(b) A cluster analysis using a size-independent similarity measure (Pearson's product-moment correlation coefficient). This method will be referred to as the PMCC clustering.

(c) A cluster analysis following a principal components analysis (PCA clustering). A principal components analysis transforms a set of data in such a way that a new set of axes is formed. The total amount of variance remains the same, and the first axis or component represents the greatest

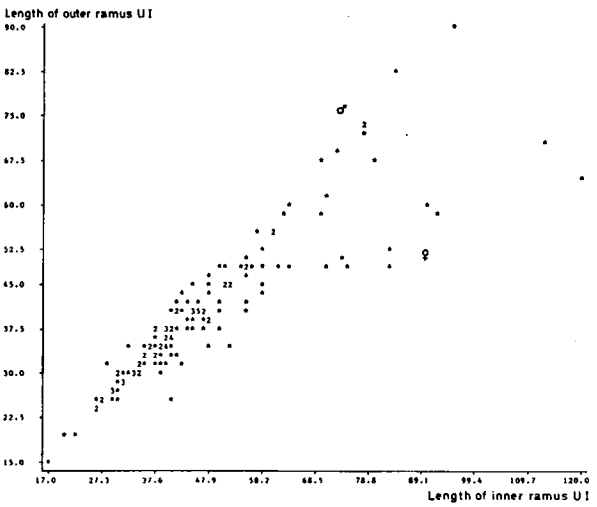

Fig. 3. The length of the outer ramus of U I plotted against the length of the inner ramus of U I. Scale expressed in mm × 10^{-3}.

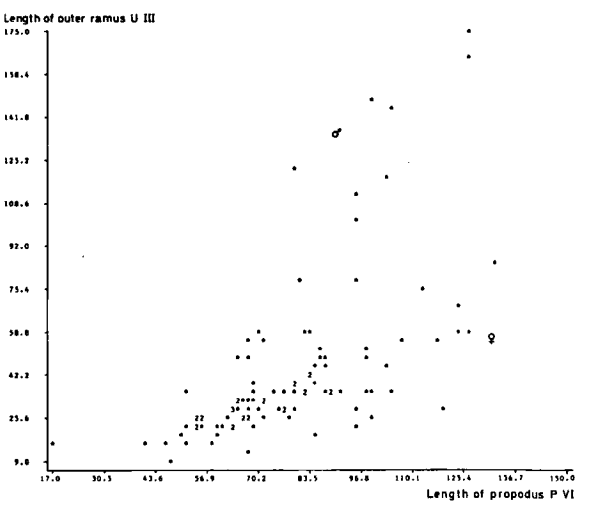

Fig. 4. The length of the outer ramus of U III plotted against the length of the propodus of P VI. Scale expressed in mm × 10^{-3}.

part of the variance. If, as in our case, it can be shown that the greatest part of the variance is caused by differences in body size, a size-independent classification can be obtained by performing a cluster analysis using the coordinates on the remaining axes.

Only 95 of the original 107 characters were used in the analyses. Ten characters were excluded because they were missing in too many cases. One was excluded because of secondary sexual dimorphism. Objects missing too many characters were excluded as well.

The analyses (b and c) have been performed by the computer program BIOPAT (written by P. Hogeweg and B. Hesper).

TABLE I

Resemblance between samples and clusters. N is the number of times a combination between a sample and a cluster is found. The group size is the number of individuals of a sample which is found together in a cluster.

PMCC clustering			PCA clustering		
Group size	χ^2 contrib.	N	Group size	χ^2 contrib.	N
≥ 1	329	72	≥ 1	309	71
≥ 2	281	33	≥ 2	242	26
≥ 3	139	12	≥ 3	100	8
≥ 4	37	5	≥ 4	81	4
≥ 5	37	5	≥ 5	81	4
≥ 6	21	3	≥ 6	66	3
$\chi^2 = 408$ ($df = 225$)			$\chi^2 = 379$ ($df = 234$)		

RESULTS AND DISCUSSION

The results of the cluster analyses (see dendrograms, figs. 5 & 6) have been compared regarding the following characteristics:
(1) The resemblance between clusters and populations.
(2) The resemblance between clusters and regions.
(3) The size dependence of the clusters.
(4) The characters which have been important in forming the clusters.

In order to investigate the resemblance between the populations and the clusters, a contingency table was constructed for all samples containing 2 or more individuals, and 10 clusters.

The χ^2 value of this table yields a good estimate of the total resemblance between clusters and samples, but it does not give any indication whether this is due to numerous small groups of individuals of one sample in a cluster or to a few larger groups. For this reason the contribution to the χ^2 value is calculated for different group sizes, i.e. 2 or more, 3 or more, etc. up to 6 or more individuals of a sample in a cluster (table I).

As can be seen from the χ^2 values, the resemblance between samples and clusters is statistically significant in both clusterings. The contributions to the χ^2 values indicate that in the PMCC clustering more often small groups (4 or less) of individuals are joined together in one cluster, and in the PCA clustering more often groups of 5, 6 or more individuals.

The χ^2 values do not take into account the dissimilarity level at which the clusters are joined. All clusters are regarded separately, so the relations with other clusters are not included in the calculation.

For this reason we thought it important to examine in what way the significance of the χ^2 values would change when the χ^2 values are calculated for a smaller number of clusters. It is obvious that when the significance of the χ^2 values increases more rapidly (or decreases less rapidly) in one of the clusterings, this clustering is in a way better than the other one.

The χ^2 values have been calculated to a level of 7 clusters (table II), and are quite similar for both clusterings. This makes them just as good (or as bad), regarding the dissimilarity level at which the clusters are joined.

For the calculations of the resemblance between clusters and geographic regions not exactly the same method is used. It is very well possible that specimens of a region are often found within a cluster just because of the

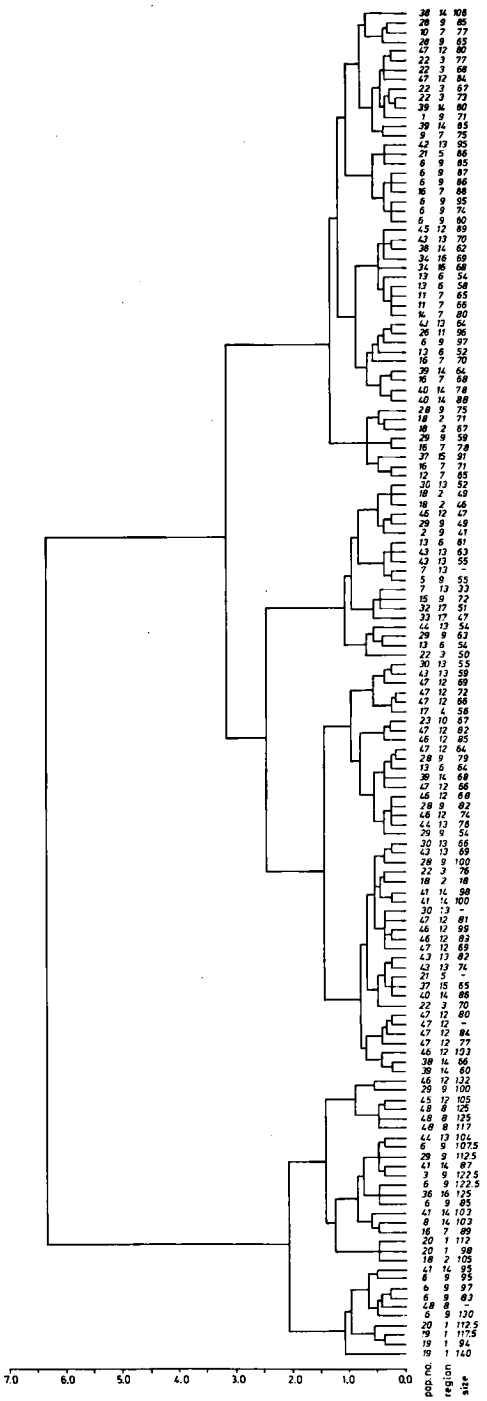

Fig. 5. Dendrogram PMCC clustering. Cluster strategy: Ward's criterion; similarity measure: cityblock distance. Pop. no. = population number; region = region number; size = length of the propodus of pereiopod VI (in mm × 10^{-3}).

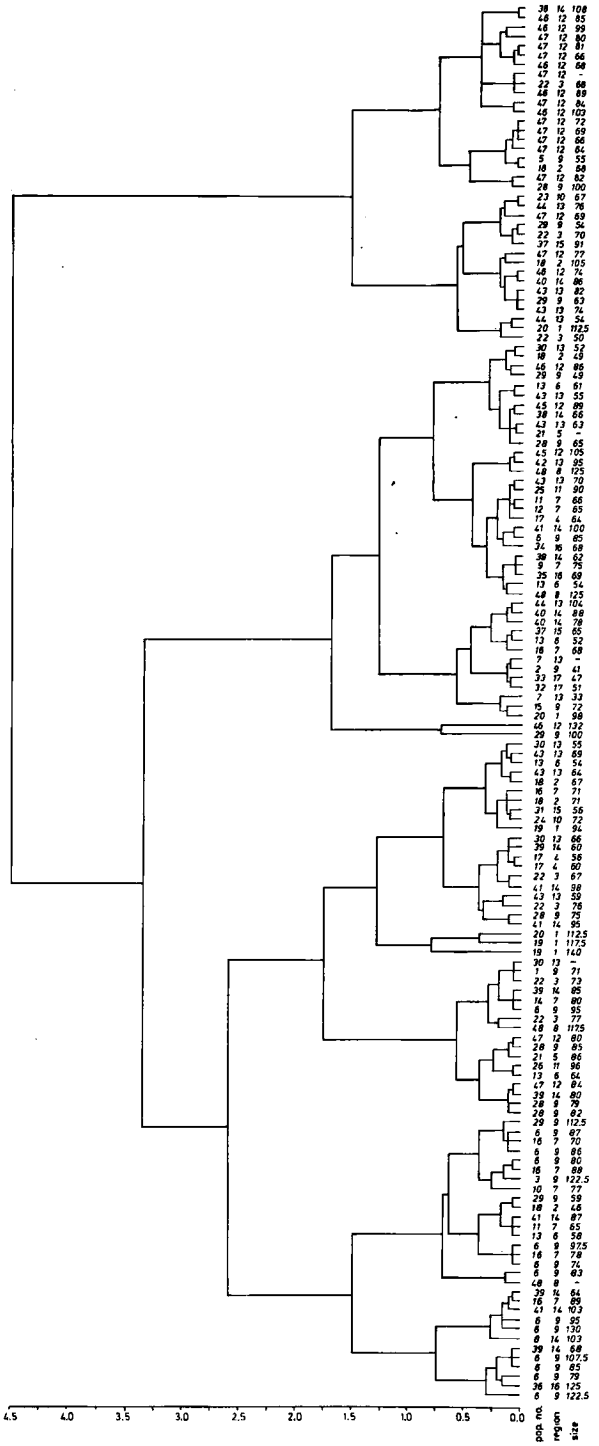

Fig. 6. Dendrogram PCA clustering. Cluster strategy: Ward's criterion; similarity measure: cityblock distance. Pop. no. = population number; region = region number; size = length of the propodus of pereiopod VI (in mm × 10^{-3}).

TABLE II

χ^2 values of the resemblance between samples and clusters on different similarity levels.

No. of clusters	PMCC clustering χ^2	PMCC clustering *df*	PCA clustering χ^2	PCA clustering *df*
10	408	225	389	234
9	343	200	340	208
8	295	175	271	182
7	269	150	260	156

TABLE III

Resemblance between regions and clusters. *N* is the number of times a combination between a region and a cluster is found. The group size is the number of times a region is found in a cluster.

PMCC clustering Group size	PMCC clustering χ^2 contrib.	PMCC clustering *N*	PCA clustering Group size	PCA clustering χ^2 contrib.	PCA clustering *N*
≥ 1	75	35	≥ 1	90	47
≥ 2	51	19	≥ 2	65	18
≥ 3	13	8	≥ 3	12	7

similarity of individuals of only one sample of that region, but what we want to know is how often several samples from one region are joined in that cluster. So we corrected for the number of individuals within samples, by subtracting from the size of a cluster the number of times a region is found more than once in a cluster, when this was due to individuals of the same sample. A corrected χ^2 value was calculated for all regions containing at least 2 samples, and 10 clusters, and also the contribution to the χ^2 value for the co-occurrence of 2 or more, or 3 or more samples of one region in a cluster (table III).

As can be seen from the χ^2 values in table III, the resemblance between regions and clusters is significant for the PMCC clustering, but not for the PCA clustering.

TABLE IV

Mean sizes ($\bar{x}$) and standard deviations (SD) of the individuals in a cluster (*N* is the number of individuals in a cluster).

cluster	PMCC clustering $\bar{x}$	PMCC clustering SD	PMCC clustering *N*	PCA clustering $\bar{x}$	PCA clustering SD	PCA clustering *N*
1	81.3	10.0	23	79.3	10.0	23
2	71.5	13.0	19	75.3	15.1	16
3	72.0	9.6	8	75.1	21.3	26
4	52.3	8.9	18	66.4	22.6	12
5	68.7	9.2	19	116.0	22.0	2
6	80.0	13.3	22	69.2	13.3	20
7	117.3	12.5	6	123.0	14.6	3
8	105.5	11.0	11	80.6	19.1	17
9	105.0	3.0	3	80.6	19.1	17
10	107.0	19.0	9	99.1	20.9	12

To estimate which clustering is the better one regarding the size dependence, we calculated for both clusterings the mean sizes and standard deviations of the specimens in 10 clusters. The data (table IV) show that the mean sizes of the clusters are somewhat more homogeneous in the PCA clustering. The joining of 2, 3 or 4 individuals of the same sample in a cluster, which was more important in the PMCC clustering than in the PCA clustering, may have been partly due to the division of samples into groups of 2, 3, or 4 individuals of about the same size. Within most samples body sizes differ greatly, and when the clustering is size dependent, it will be easier to divide a sample in small groups of individuals of the same size, than to divide the samples into a few large groups of individuals.

Size dependence of a clustering may also strongly influence the results when the mean sizes within samples or regions are heterogeneous. The heterogeneity of the means was calculated for the samples and the regions (Bartlett's approximate test of equality of means) and in both cases turned out to be significant. However, the inequality of means is much more significant for the regions than for the samples, and this, in combination with the size dependence, may also have caused the fact

that the resemblance between regions and clusters is much better for the PMCC clustering, while this is not the case for the resemblances between samples and clusters.

As far as the characters are concerned which have been important in forming the clusters, it seems that in both clusterings a complex of characters has been responsible for the main divisions. Among these characters there are several to which formerly hardly any attention has been paid in the systematics of the genus, like groups of setae on the gnathopods and pereiopods, probably because a great deal of the variability of these characters is dependent on size. Still it seems that the remaining part of the variability is not distributed randomly among the populations.

CONCLUSIONS

Morphological differentiation between the samples is more pronounced than between regions. Spatial and genetical separation between populations therefore seems to be more important than between regions.

A cluster analysis following a principal components analysis seems to be a better method to exclude size-dependent variability than a cluster analysis using Pearson's product-moment correlation coefficient as a size-independent similarity measure.

The size-dependent variability, even within populations, is such that we did not think it useful to compare our material with species of which the variability is not sufficiently described.

The variability found in most of the characters made us wonder if the taxonomic status of the two previously described taxa from Corsica (Stock, 1972) is still valid. The differences used by Stock (1972) to distinguish the two taxa were: the number of setae on the palp of maxilla I, the number of spines on the first lobe of the maxilliped, the shape of the retinacula, the presence of a spine on the posteroanterior corner of the epimeral plates, and the shape of the basis of pereiopod V.

The first three characters turned out to be variable within populations and showed none or little dependence on body size. The spine on the posteroanterior corner of the epimeral plates is present on all epimeral plates in all populations examined, including those of Stock, except two, and one of these two populations was used by Stock to redescribe *N. longicaudatus corsicanus*. In the other population (station 48, sampled by Prof. J. Giudicelli) a spine is present in about half of the material. The two populations are located close to each other and differ in one other aspect from all other populations: there is a very high frequency of pereiopods which have two, or sometimes three small teeth on the dactylus, instead of one. We also reexamined Stock's material of *N.* aff. *tatrensis* concerning the basis of pereiopod V, but we did not find any noteworthy differences with other populations. On account of our results we consider the form *N.* aff. *tatrensis* identical with *N. longicaudatus corsicanus*.

Finally, we strongly recommend the use of statistical parameters in describing and distinguishing samples or species. It would be useful to know if statistical parameters could be used successfully as characters in cluster analyses using samples as operational units.

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REFERENCES

BERON, P. C., 1972. Aperçu sur la faune cavernicole de Corse. Série Documents Publication **3**. Laboratoire Souterrain du C.N.R.S., Moulis (Ariège), France: 1-8.

DICKSON, G. W., 1977. Variation among populations of the troglobitic amphipod crustacean Crangonyx antennatus Packard, living in different habitats. I. Morphology. Int. J. Speleol., **9**: 43-58.

MORAND-CHEVAT, C., 1972. Bilan actuel du genre Ni-

phargus en France et en Espagne. Actes du 1er Colloque international sur le genre Niphargus. Memorie fuori Ser. Mus. civ. Storia nat. Verona, **5**: 25-31.

RÉMY, P., 1950. Grottes de Corse. Annls. Spéléol., **5**: 3-47.

RUFFO, S., 1960. Contributo alla conoscenza dei crostacei anfipodi delle acque sotterranee della Sardegna e delle Baleari. Atti Ist. veneto Sci., (Cl. Sci. mat. nat.) **118**: 169-180.

SCHELLENBERG, A., 1950. Subterrane Amphipoden korsikanischer Biotope. Arch. Hydrobiol., **44** "1951": 325-332.

SNEATH, P. H. A. & R. R. SOKAL, 1973. Numerical taxonomy. The principles and practice of numerical classification: i-xv, 1-573 (W. H. Freeman & Co., San Francisco).

STOCK, J. H., 1972. Les Gammaridae (Crustacés Amphipodes) des eaux douces et saumâtres de Corse. Bull. zoöl. Mus. Univ. Amsterdam, **2** (19): 197-220.

STRAŠKRABA, M., 1972. Les groupements des espèces du genre Niphargus (sensu lato). Actes du 1er Colloque international sur le genre Niphargus. Memorie fuori Ser. Mus. civ. Storia nat. Verona, **5**: 85-90.

VIGNA TAGLIANTI, A., 1972. Le attuali conoscenze sul genere Niphargus in Italia (Crustacea, Amphipoda). Actes du 1er Colloque international sur le genre Niphargus. Memorie fuori Ser. Mus. civ. Storia nat. Verona, **5**: 11-23.

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APPENDIX

List of characters used

Length of:
- Gn I, segments 2-5
- Gn II, segment 5
- Propodus of P III-VI
- inner and outer ramus of U I
- telson
- head
- pereionites I-VII
- pleonites I-III
- urosomite I
- diagonals of carpus Gn I-II

Width of:
- segments 1-2 and 4-5 Gn I
- segment 5 Gn II
- telson

Number of setae:
- in several groups (15) on the carpus of Gn I
- on anterior and posterior margin dactylus Gn I
- on posterior and anterior margin propodus P III-VI
- on base of dactylus P III-VI
- on posterodistal corner of segments 1-2 Gn I
- on distal and lateral margins of segment 3 Gn I
- on posteroproximal and posterodistal margins of segment 4 Gn I
- on anterodistal corner segment 4 Gn I
- on proximolateral side of mandibular palp
- on distolateral side of mandibular palp
- on proximal margin of mandibular palp
- on segment 2 of palpus Mx I
- on distal margin of inner lobe Mxp

Number of groups of setae:
- on posterior margin propodus Gn I
- on anterior margin propodus P III-VI
- on posterior margin propodus P III-VI
- on lateral side mandibular palp

Number of spines:
- on telson (greatest number when not symmetrical):
  - basal
  - distal at one lobe
  - on inner margin of one lobe
  - on outer margin of one lobe
  - dorsally on one lobe
- distal on inner lobe of Mxpd
- on outer lobe of Mx I
- on inner lobe of Mx I
- on mandibular palp
- Number of unidentate spines on outer lobe Mx I

Number of segments:
- of main flagellum A I
- of flagellum A II