

Primary Research Paper

Morphological differentiation between geographically separated populations of *Neomysis integer* and *Mesopodopsis slabberi* (Crustacea, Mysida)

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Abstract

Morphological variation was examined in *Neomysis integer* and *Mesopodopsis slabberi*, two abundant, low dispersal mysid species (Crustacea, Mysida) along the European coasts. Both species dominate the hyperbenthic communities in the northeast Atlantic, and *M. slabberi* is also widely distributed in the Mediterranean and Black Sea. Three populations of these species were sampled throughout their distribution range; samples of *N. integer* were collected in the northeast Atlantic Eems-Dollard, Gironde and Guadalquivir estuaries; in the case of *M. slabberi*, mysids were sampled in two northeast Atlantic estuaries (Eems-Dollard and Guadalquivir) and one Mediterranean site (Ebro Delta). A total of 12 morphometric and 2 meristic characters were measured from 30–64 mysids per sample. Multivariate analysis showed clear morphometric differences between populations of both species. The morphological differentiation within *M. slabberi* was highly concordant with the available genetic data from mitochondrial loci, pointing to a large divergence between the Atlantic and Mediterranean populations. However, due to some overlap of individuals between the different populations, the present morphometric analysis does not suffice to assign the different populations to a separate (sub)species status. In the case of *N. integer*, the morphometric patterns showed a divergence of the Gironde population. Differentiation of populations within this mysid, as in *M. slabberi*, were mainly related to eye and telson morphology. Potential interactions of the mysid morphology and environmental conditions are discussed.

Introduction

Multivariate analysis of a set of morphometric and meristic characters has been widely used in stock identification of freshwater and marine fish species (Mamuris et al., 1998; Cadrin, 2000; Murta, 2000; Pakkasmaa & Piironen, 2001; Cabral et al., 2003), and to a lesser extent in marine invertebrates (e.g., Henderson et al., 1990; Kassahn et al., 2003). This method is also regarded as more appropriate than the use of single morphological characters for investigating taxonomic problems in determining relationships between

populations or closely related (cryptic) species (e.g., Scapini et al., 1999; Clark et al., 2001; De Grave & Diaz, 2001; Debusse et al., 2001; Doadrio et al., 2002; Lee & Frost, 2002). Moreover, morphometric analyses can also be a tool in assessing habitat-specific differentiation of populations, such as differentiation related to predation pressures, salinity, temperature, food availability, etc. (e.g., Gee, 1988; Scapini et al., 1999; Maltagliati et al., 2003). Differences in morphometric and meristic characters among populations of a species are thought to be the result of genetic differences or environmental

factors, or their interactions (Lindsey, 1988; Scheiner & Callahan, 1993; Hoffman & Merilä, 1999). Strong genetic differentiation of populations, accompanied with reproductive isolation, can lead to local adaptation. On the other hand, changing environmental conditions can produce phenotypic plasticity in genetically similar populations (Thompson, 1991). Hence, the comparison of the degree of variation in molecular markers with that of morphological characters may be important in assessing the degree of phenotypic plasticity shown by a species (O'Reilly & Horn, 2004).

Neomysis integer and *Mesopodopsis slabberi* are two of the most common mysid species in European coastal (*M. slabberi*) and brackish (*M. slabberi* and *N. integer*) habitats, where they are believed to play a key role (Mees et al., 1995b; Azeiteiro et al., 1999; Hostens & Mees, 1999). Both species are euryhaline and eurythermic, and have a wide distribution: *N. integer* occurs along the NE Atlantic from the Baltic Sea to the North African coasts of Morocco (Tattersall & Tattersall, 1951) and *M. slabberi* is distributed from the western Baltic, the NE Atlantic, up to the entire Mediterranean, Marmara, Black and Azov Seas (30–59° N, 10° W–1° E) (Wittmann, 1992). This wide distribution of both species spanning different biogeographical regions (Subarctic, Celtic, Lusitanian & Mediterranean region, cfr Adey & Steneck, 2001) with varying environmental conditions, combined with the limited dispersal capacities of these mysids (brooding behavior and lack of free-living larvae), may be expected to produce differences in both molecular and morphological traits among populations (Planes, 1998; O'Reilly & Horn, 2004).

The taxonomy of the genus *Mesopodopsis*, and in particular of the species *M. slabberi* has been a matter of controversy, mainly due to the limited phylogenetic resolution of the morphological characters used to describe and diagnose different species within this genus. Based on a study by Wittmann (1992) on the morphogeographic variations within the genus *Mesopodopsis*, the cosmopolitan *M. slabberi* was split into four species: *M. slabberi* (NE Atlantic, Mediterranean, Black Sea), *M. aegyptia* (Mediterranean), *M. tropicalis* (equatorial W.Africa) and *M. wooldridgei* (South Africa). Morphological differences between Atlantic,

Mediterranean and Black Sea populations of *M. slabberi* were reported by Wittmann (1992). However, the observed variation was small and statistically overlapping, without any consistent pattern related to environment or geography. It must be noted that this study did not use a multivariate statistical analysis of morphometric characters to elucidate variation between populations. On the other hand, morphological variation within *N. integer* is considered to be small (Tattersall & Tattersall, 1951; Parker & West, 1979), but has not been studied in detail. A number of 'forms' or varieties within the species *N. integer* were introduced by Czerniavsky (1882), but since these varietal divisions were based on trivial differences, they have been largely ignored in subsequent descriptions (Tattersall & Tattersall, 1951). However, given the slight taxonomic differences observed between populations of the North American congeneric *N. americana* (Williams et al., 1974), morphometric variation between populations of *N. integer* may be expected.

Studies on genetic variation between populations of *N. integer* and *M. slabberi*, based on several mitochondrial loci, have shown significant heterogeneity within both species (Remerie et al. submitted a, b). Analysis of Atlantic and Mediterranean populations of *M. slabberi* showed a clear differentiation between both basins, with very high genetic distances, probably pointing to the existence of different cryptic species. Phylogeographic analyses of *N. integer* identified a large genetic break at the southern distribution range (= divergent Guadalquivir population) and showed a genetic isolation of the populations south of the English Channel, including the Irish population. In this respect, a morphometric analysis within both species could lead to a better understanding of the intraspecific evolutionary and systematic diversity and its biological significance.

The aims of this study were to (i) examine the pattern and the extent of morphometric variation in populations of the mysids *N. integer* and *M. slabberi*, and (ii) compare these results with the available genetic data. For this purpose, three population samples of each species, covering, at least for *N. integer*, most of its geographical distribution range, were examined morphologically and analysed using multivariate methods.

Materials and methods

Sampling

Samples of *Neomysis integer* were collected in three NE Atlantic estuaries covering most of the species' distribution range. *Mesopodopsis slabberi* was collected in two NE Atlantic and one Mediterranean estuary (see Fig. 1). Most samples were collected with a hyperbenthic sledge, with exception of the sample of the Ebro delta, which was collected with a hand net (mesh size 1 mm). All sampling was done during the summer months between 1991 and 2001. The samples were either fixed and preserved in 7% formaldehyde, diluted with ambient water, (all *N. integer* samples and *M. slabberi* from the Eems-Dollard estuary) or in 70% ethanol, diluted with distilled water (only *M. slabberi* samples from the Guadalquivir and Ebro estuaries). In the case of *M. slabberi*, the different fixation and preservation liquids used could result in different levels of material shrinkage, producing a bias in the different measurements compared between populations. However, due to the lack of sufficient material preserved in different liquids from a single *M. slabberi* population, the shrinkage



Figure 1. Sampling locations (*N* = *Neomysis integer*, *M* = *Mesopodopsis slabberi*), sampling site abbreviations: ED = Eems-Dollard, GI = Gironde, GU = Guadalquivir, EB = Ebro.

effects caused by the different liquids could not be taken into account in the statistical analysis.

Measurements and statistical analyses

From each sample a random number of about 50 adults, and mostly gravid, females were examined morphologically. A total of 12 metric and 2 meristic characters were measured (Fig. 2). The metric measurements were related to the shape of the telson, antennale scale, eyes and uropods. The meristic counts included the number of spines on the lateral margin of the telson (only for *M. slabberi*) and on the inner margin of the uropod endopodite. Standard length was measured from whole animals under a binocular microscope. Other characters were measured from slide mounts of the appendages under a microscope and recorded with a digitizer.

All statistical analyses were performed using the STATISTICA 6.0 software package (STATSOFT 2001). The most conspicuous outliers were excluded when suspecting measurement error, and missing data were case-wise deleted in the statistical analyses. To minimize size effects in all analyses, the continuous variables were divided by standard length followed by an arc-sin transformation. Univariate analysis of variance (ANOVA) was performed, in case of homogeneity of the variances, to test whether the different populations showed significant differences in morphometric measurements and meristic characters. In cases where homogeneity of variances was violated, even after transformations of the raw data, a non-parametric test was used (Kruskal-Wallis and Mann-Whitney). The data set (only metric measurements with exclusion of the standard length) was subjected to a backward stepwise discriminant function analysis (DFA). DFA finds linear combinations of variables (roots), that maximize differences among *a priori* defined groups (in this case populations). The resultant discriminant functions were used to classify individuals into samples. The classification success rate (cross-validation test) was evaluated based on the percentage of individuals correctly classified in the original sample. Alternatively, a principal components analysis (PCA) was performed and in order to eliminate the size effect, the first principal

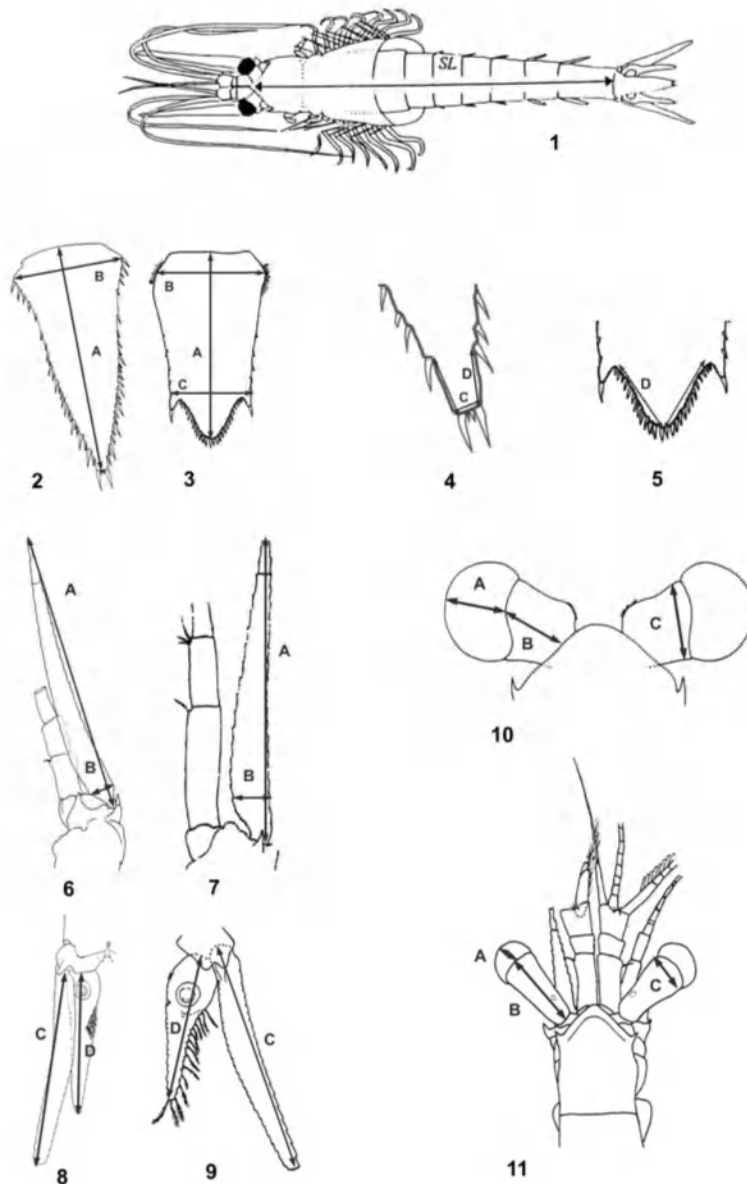


Figure 2. Morphometric measurements: **1**: Standard length (SL); **2, 3, 4 & 5**: Telson of *Neomysis integer* (**2 & 4**) and *Mesopodopsis slabberi* (**3 & 5**). A = telson length (TELL), B = distal telson width (TELDW), C = caudal telson width (TELCW), D = caudal telson length (TELCL); **6 & 7**: Antennule scale of *Neomysis integer* (**6**) and *Mesopodopsis slabberi* (**7**). A = length of antennule scale (ANTL), B = width of antennule scale (ANTW); **8 & 9**: Uropode of *Neomysis integer* (**8**) and *Mesopodopsis slabberi* (**9**). C = exopodite length (EXOL), D = endopodite length (ENDOL); **10 & 11**: Eye of *Neomysis integer* (**10**) and *Mesopodopsis slabberi* (**11**). A = cornea length (CORNEA), B = length of eyestalk (EYESTL), C = width of eyestalk (EYESTW).

component (PC1) was eliminated. Subsequently the other PC scores (PC2-n) were subjected to a canonical variate analysis (see Väinölä et al., 2002). However, since a similar pattern was obtained as with the DFA, the results of the PCA-method are not presented.

Results

Neomysis integer

The mean standard length of *Neomysis integer* across all populations amounted to 10.29 mm (SD

1.61). A significant difference in standard length was observed between the three populations (Kruskal–Wallis test: $H(2, n = 168) = 8.55$; $p = 0.0139$), with the mysids of the Eems-Dollard population having the largest length (mean = 10.60 mm; SD 1.33) and those of the Guadalquivir being the smallest (mean = 10.03 mm; SD 2.19) (see Table 1).

All morphometric characters could be used in the discriminant analysis since no multicollinearity was registered between the variables (for all correlations: $R < 0.7$). The backward stepwise DFA, using geographical origin of each population as separator factor, revealed that four of the 12 morphometric characters contributed significantly to the multivariate discrimination between the three *N. integer* populations (Table 2). Wilks' lambda amounted to 0.1304 and was highly significant (approx. $F_{8,278} = 61.489$; $p < 0.001$). The morphometric characters showed a low degree of overlap (maximal 57.22% in case of the eyestalk width (EYESTW), see Table 2). Squared Mahalanobis distances (D^2) between populations (i.e., a distance measure between the group centroids) are listed in Table 3. All distances were significant ($p < 0.001$) and the largest distance was observed between the Eems-Dollard (ED) and Gironde (GI)

populations, while the distance between the Eems-Dollard and Guadalquivir (GU) populations seemed to be smaller. A scatterplot of the individual canonical scores is presented in Figure 3. The relative importance of Root 1 in distinguishing the three populations was up to 3 times higher than Root 2 (Eigenvalue of Root 1 = 2.8666, Eigenvalue of Root 2 = 0.9836), and the first discriminant function accounted for 74.5% of the explained variance. A clear separation of the Gironde population could be observed along Root 1. In contrast, Root 2 separated the Eems-Dollard (ED) and Guadalquivir (GU) populations, although some overlap existed between both populations. The segregation along Root 1 was mainly caused by differences in the variables eyestalk width (EYESTW) and cornea length (CORNEA) (Gironde < Eems-Dollard & Guadalquivir mysids), as evidenced by the high correlation of these morphometric characters and the canonical Root (Table 4). The differences along Root 2 were almost exclusively related to the variable caudal telson length (TELCL) (Guadalquivir < Gironde < Eems-Dollard mysids). The cross-validation test using the discriminant functions derived from the morphometric characters showed that overall 87.34% of the *a priori* grouped cases were

Table 1. Mean and standard deviation (in parenthesis) of the different metric and meristic characters

	<i>Neomysis integer</i>				<i>Mesopodopsis slabberi</i>			
	OVERALL	ED (n = 50)	GI (n = 54)	GU (n = 64)	OVERALL	ED (n = 50)	GU (n = 52)	EB (n = 30)
STD L	10.29 (1.61)	10.60 (1.33)	10.30 (0.82)	10.03 (2.19)	8.45 (0.94)	8.49 (0.85)	8.87 (0.89)	7.64 (0.63)
EYESTW	0.46 (0.07)	0.53 (0.05)	0.39 (0.02)	0.46 (0.05)	0.31 (0.03)	0.33 (0.3)	0.28 (0.03)	0.30 (0.02)
CORNEA	0.26 (0.05)	0.31 (0.05)	0.21 (0.02)	0.26 (0.03)	0.21 (0.03)	0.23 (0.03)	0.21 (0.03)	0.19 (0.02)
EYESTL	0.43 (0.06)	0.48 (0.06)	0.41 (0.04)	0.42 (0.05)	0.72 (0.06)	0.73 (0.05)	0.74 (0.06)	0.68 (0.04)
TELL	1.64 (0.23)	1.74 (0.21)	1.66 (0.12)	1.53 (0.29)	0.78 (0.14)	0.88 (0.08)	0.81 (0.08)	0.58 (0.05)
TELDW	0.75 (0.08)	0.78 (0.08)	0.74 (0.05)	0.73 (0.10)	0.54 (0.06)	0.56 (0.04)	0.57 (0.05)	0.46 (0.04)
TELCW	0.10 (0.02)	0.10 (0.02)	0.10 (0.03)	0.9 (0.02)	0.37 (0.04)	0.39 (0.03)	0.38 (0.03)	0.32 (0.02)
TELCL	0.15 (0.04)	0.18 (0.04)	0.16 (0.03)	0.11 (0.02)	0.24 (0.05)	0.27 (0.03)	0.24 (0.03)	0.18 (0.02)
ANTW	0.30 (0.04)	0.33 (0.03)	0.29 (0.02)	0.28 (0.05)	0.20 (0.02)	0.20 (0.01)	0.21 (0.01)	0.16 (0.02)
ANTL	2.73 (0.39)	3.02 (0.34)	2.66 (0.20)	2.51 (0.38)	1.25 (0.17)	1.29 (0.10)	1.29 (0.21)	1.23 (0.09)
EXOL	2.18 (0.33)	2.39 (0.25)	2.17 (0.17)	2.02 (0.39)	1.69 (0.20)	1.75 (0.12)	1.80 (0.16)	1.45 (0.12)
ENDOL	1.52 (0.20)	1.64 (0.15)	1.51 (0.14)	1.43 (0.22)	1.12 (0.11)	1.17 (0.07)	1.16 (0.08)	0.96 (0.06)
#SPENDO	28.55 (4.07)	28.36 (4.82)	28.93 (5.05)	28.38 (1.89)	20.97 (1.24)	20.66 (1.68)	21.65 (0.48)	20.40 (0.56)
#SPTL	–	–	–	–	6.56 (0.81)	6.96 (0.20)	7.00 (0.34)	5.57 (1.07)

Metric values are in mm. For the sampling site and metric measurement abbreviations see Figures 1 and 2. Meristic character abbreviations: #SPENDO = number of spines on the inner margin of the uropod endopodite, #SPTL = number of spines on the lateral margin of the telson.

Table 2. Summary of the discriminant function analysis on the morphometric characters of *Neomysis integer*

	Wilks' Lambda	Partial Lambda	F-remove (2.139)	p-level	Toler.	1-Toler. (R^2)
EYESTW	0.2190	0.5951	47.2443	<0.0001	0.4278	0.5722
CORNEA	0.1496	0.8715	10.2433	<0.0001	0.7302	0.2698
TELDW	0.1837	0.7095	28.4490	<0.0001	0.4599	0.5401
TELCL	0.2691	0.4845	739.581	<0.0001	0.9495	0.0505

Table 3. Squared Mahalanobis Distances between the *Neomysis integer* populations

	ED	GI	GU
ED	–	***	***
GI	14.3579	–	***
GU	6.0109	14.0135	–

correctly classified, with the within-group correct classifications ranging from 78.18 (GU) to 96.23% (GI) (see Table 5).

Analyses of the meristic characters (spines on the inner margin of the uropod endopodite) revealed no significant differences between the three populations (Kruskal–Wallis test: $H(2, n = 163) = 5.0697$ $p = 0.0793$). In addition, a total of 12 aberrant telsons were recorded (ED = 5, GI = 3, GU = 4); the morphology of these telsons were similar to those described in Mees et al. (1995a).

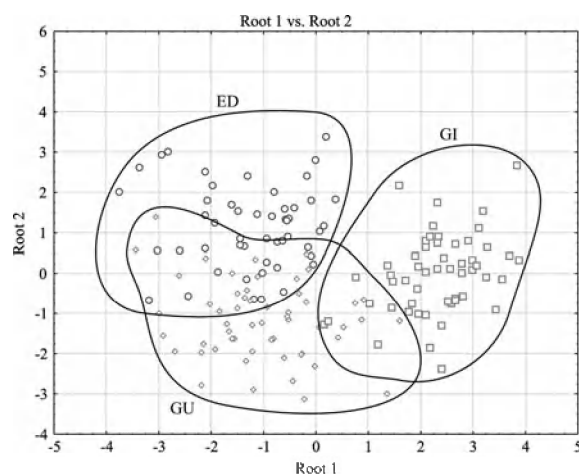


Figure 3. Scatterplot of the DFA scores along the first and second root for the morphometric characters in *Neomysis integer*. For sampling site abbreviations see Figure 1.

Mesopodopsis slabberi

Mean standard length of *Mesopodopsis slabberi* across all populations amounted to 8.45 mm (SD 0.94). A significant difference in standard length was observed between the three populations (ANOVA: $F_{2,193} = 23.91$; $p < 0.001$), with the mysids of the Mediterranean Ebro population having the lowest standard length (mean = 7.64 mm; SD 0.63) (see Table 1).

Again, no multicollinearity was registered between the variables and consequently all morphometric characters could be used in the discriminant analysis. The backward stepwise DFA revealed that only 3 out of the 12 morphometric characters contributed significantly to the multivariate discrimination between the 3 *M. slabberi* populations (Table 6). The largest Mahalanobis (D^2) distances were observed between the Mediterranean Ebro population and both Atlantic populations (Table 7). The Atlantic–Mediterranean divergence between populations of *M. slabberi* was supported independent of the liquid used for fixation and preservation (7% formalin or 70% ethanol). Hence, possible differences in the level of material shrinkage between both liquids are thought to have a minimal effect on the general patterns observed in *M. slabberi*. The canonical analysis showed that most of the observed variance between the populations (83%) was observed

Table 4. Structure matrix of discriminant loadings for each of morphometric variable of *Neomysis integer* selected by the backward stepwise discriminant function analysis (DFA)

	Root 1	Root 2
EYESTW	–0.6785	0.1669
CORNEA	–0.6112	0.1503
TELDW	–0.1200	–0.0583
TELCL	0.2151	0.9306

Table 5. Results of the discriminant analysis classification on the *Neomysis integer* specimens, showing the numbers and percentage of specimens classified in each group (Rows: observed classifications, Columns: predicted classifications)

	% Correct	ED	GI	GU
ED	88	44	0	6
GI	96.23	0	51	2
GU	78.18	8	4	43
TOTAL	87.34	52	55	51

along Root 1 (Eigenvalue = 3.44 vs. Eigenvalue Root 2 = 0.70), with a clear distinction between the Ebro (EB) and Eems-Dollard (ED) populations (Fig. 4). The differentiation along Root 1 mainly correlated with the variables telson length (TELL) and caudal telson length (TELCL) (ED > GU > EB mysids), while the differences along Root 2 were related to the variable eye-stalk width (EYESTW) (ED > EB > GU mysids) (Table 8). The morphometric discriminant analysis correctly classified, on average, 83.85% of the individuals (Table 9). The highest classification success rate was obtained for the Ebro mysids with 93.33%, while a lower amount of individuals (74%) were correctly classified in case of the Guadalquivir mysids.

Analysis of the meristic characters showed a significant difference in the number of spines on the inner margin of the uropod endopodite (#SPENDO) and on the lateral margin of the telson (#SPTL) between the different populations (Kruskal–Wallis test for #SPENDO: H (2, $n = 128$) = 36.013 $p < 0.001$; #SPTL: H (2, $n = 132$) = 75.82 $p < 0.001$). Mysids of the Mediterranean Ebro populations had, on average, less spines on the lateral margin of the telson, while those of the Guadalquivir population possessed, on average, more spines on the inner margin of the uropod endopodite (Table 1). Contrary to *N. integer*, no aberrant telsons were observed in the samples of *M. slabberi*.

Table 6. Summary of the discriminant function analysis on the morphometric characters of *Mesopodopsis slabberi*

$n = 114$	Wilks' Lambda	Partial Lambda	F-remove (2,139)	p-level	Toler.	1-toler. (R^2)
EYESTW	0.2458	0.5370	46.9902	< 0.0001	0.8585	0.1415
TELDW	0.2310	0.5713	40.9025	< 0.0001	0.7854	0.2146
TELCL	0.1629	0.8102	12.7675	< 0.0001	0.9068	0.0932

Table 7. Squared Mahalanobis Distances between populations of *Mesopodopsis slabberi*

	ED	GU	EB
ED	–	***	***
GU	5.1972	–	***
EB	21.0172	13.5901	–

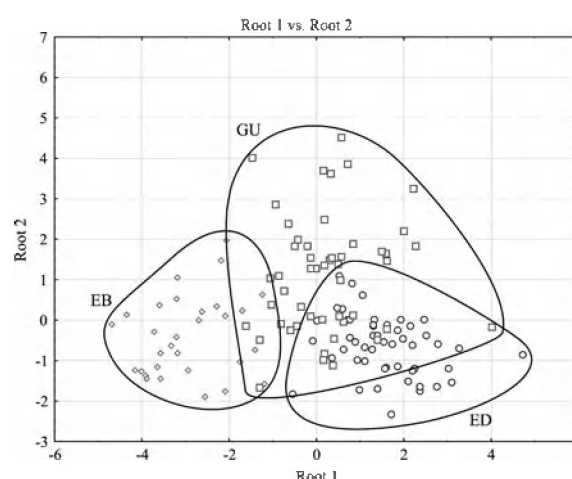


Figure 4. Scatterplot of the DFA scores along the first and second root for the morphometric characters in *Mesopodopsis slabberi*. For sampling site abbreviations see Figure 1.

Discussion

The multivariate analyses of morphometric characters revealed a significant differentiation between populations of both *Neomysis integer* and *Mesopodopsis slabberi* throughout their distribution range. Very often, such differences are to a large extent related to sexual dimorphism, allometric growth and/or different cohort size (Thorpe, 1976; Mamuris et al., 1998; De Grave & Diaz, 2001). In order to minimize the variances caused by these parameters, the present study used only adult, (mostly gravid) female specimens from the summer generation. In addition, all measurements were size-standardized and transformed prior to statis-

Table 8. Structure matrix of discriminant loadings for each of morphometric variable of *Mesopodopsis slabberi* selected by the backward stepwise discriminant function analysis (DFA)

	Root 1	Root 2
EYESTW	-0.0733	-0.9403
TELDW	0.8057	-0.2767
TELCL	0.6674	-0.3742

Table 9. Results of the discriminant analysis classification, showing the numbers and percentage of specimens of *Mesopodopsis slabberi* classified in each group (Rows: observed classifications, Columns: predicted classifications)

	% Correct	ED	GU	EB
ED	88	44	6	0
GU	74	10	37	3
EB	93.33	0	2	28
TOTAL	83.85	54	45	31

tical analysis. The method used here to correct the measurements for size proved to be effective, since all correlation coefficients which were close to 1 decreased to lower values after data transformation. Moreover, the second method used to eliminate the size effect gave similar results (i.e. performing a PCA and subsequently performing a canonical variate analysis on the individual PC scores (PC2–12) with elimination of the first principal component, see Materials and methods).

Both species showed significant latitudinal differences in standard length. In the case of *N. integer*, the mysids of the southern Guadalquivir population had, on average, a shorter length. For *M. slabberi*, the Mediterranean mysids were smaller than those of the Atlantic populations. Considerable variations in life history characteristics (e.g. length, growth rate, number of cohorts, brood size) of mysid species at different latitudes, including *N. integer* and *M. slabberi*, have been reported (Pezzack & Corey, 1979; Mauchline, 1980; Sorbe, 1984; Morgan, 1985; Greenwood et al., 1989; San Vicente & Sorbe, 1995; Delgado et al., 1997). Water temperature, light cycle and food conditions seem to be the principal environmental factors influencing the growth and reproductive cycle of crustaceans (Pezzack & Corey, 1979; Winkler & Greve, 2002). In general, there

is a tendency towards an extended reproductive season with decreasing latitude in shallow-water mysid species (Delgado et al., 1997). In the case of *M. slabberi*, the Atlantic reproductive cycle with three generations (spring, summer and winter generation) shifts to a more or less continuous breeding throughout the whole year in Mediterranean populations (Delgado et al., 1997; Azeiteiro et al., 1999; Uppabullung, 1999). Hence, the present results corroborate the general observations of lower cohort-size in populations with an extended breeding season.

Phenotypic variation in populations of Neomysis integer and Mesopodopsis slabberi

Extensive variation in morphometric characters was apparent between all three populations of *N. integer* and *M. slabberi*. This was not only supported by the DFA scores along the first two roots, but also by the significant, large Mahalanobis distances between the populations of both mysids (see Tables 3 & 7) and the high percentage of correctly re-classified specimens in the original groups (populations) (see Tables 5 & 9). For *N. integer*, the variables of primary importance in separating the populations along Root 1 were related to eye morphology: eyestalk width (EYESTW) and cornea length (CORNEA). While the morphometric variable related to the caudal telson morphology, caudal telson length (TELCL), had the largest discriminatory power along Root 2.

In the case of *M. slabberi*, the DFA showed that again the morphometric variables related to telson (TELL: telson length, TELCL: caudal telson length) and eye morphology (EYESTW: eyestalk width) were the most important variables in differentiating the populations. Contrary to *N. integer*, a significant difference in meristic characters was observed between the Atlantic and Mediterranean populations. According to Mauchline (1980) the number of spines in the margins of telsons and both endopod and exopod of the uropods is correlated to the overall body size of several mysid species. However, in the present study the size effect on spine numbers between populations is thought to be minimal since we tried to uniform our samples by selecting only adult (gravid) females of the summer generation.

The assumption that meristic characters are independent of mysid size was further confirmed by the absence of correlations between the meristic characters and standard length or uropod endopodite/telson length.

Causes of the phenotypic variation

The causes of morphological differences between populations are often quite difficult to explain. In general, changes in morphology are under the control of environmental conditions or genetic background, or (most often) a combination of both. However, separating the effects of environmental induction from those under genetic control can be one of the most intricate problems in the analysis of geographic variation (Thorpe, 1976). Genetic differences and reproductive isolation between populations can lead to local adaptation, which is reflected in morphology, behaviour, physiology and/or life history traits (Taylor, 1991). The alternative possibility is that morphological variation may result from phenotypic plasticity in response to varying environmental conditions (e.g. temperature, salinity, food availability, flow regime, predator/prey interactions, etc.) within different geographical areas (Scheiner & Callahan, 1993).

Extensive genetic surveys of different mitochondrial loci revealed a significant differentiation of populations of both *N. integer* and *M. slabberi* (Remerie et al. submitted a, b). Although not yet supported with nuclear genetic markers, a large phylogeographic break was observed between Atlantic and Mediterranean populations of *M. slabberi*, indicating the possible existence of different cryptic species. On the other hand, the observed genetic distances between populations throughout the whole distribution range of *N. integer* were smaller. Still, an isolation of the Gironde population and a well-supported break at the southern distribution range (i.e. of the Guadalquivir population) could be observed. Concordance between the molecular data and the present morphometric analyses could be observed for *M. slabberi*, where the largest molecular and morphometric distances were found between the Mediterranean and Atlantic populations. Hence, the combination of the genetic differentiation (with possible reproductive isolation) and the adapta-

tions to environmental conditions may have played a role in the Atlantic–Mediterranean separation and the morphological variability (mainly related to telson morphology) between both regions. In contrast, the patterns of genetic differentiation within *N. integer* do not correspond fully with the present morphometric results. Largest squared Mahalanobis distances were observed for the Gironde populations (Table 3), while the largest genetic divergence was found for the Guadalquivir and not the Gironde population (Remerie et al. submitted a). However, it must be noted that the patterns of genetic differentiation within *N. integer* were only based on a single mitochondrial marker and hence need further validation of other (unlinked) molecular markers in order to fully correlate them with the present morphometric results.

One of the morphometric characters of primary importance in separating the populations, both in *N. integer* and *M. slabberi*, was related to the eye morphology. It is not unlikely that this morphological character can vary in association with environmental conditions. Mysids have well-developed compound eyes, and are known to use vision in various situations, e.g. schooling behaviour and choice of specific habitats, diurnal migrations, feeding and predator avoidance behaviour (Fulton, 1982; Nilsson & Modlin, 1994; Lindström, 2000; Lindén et al., 2003). A study on the eye function of mysids has shown that there may be functional intraspecific differences in the visual systems of mysids living in different photic environments (Lindström, 2000). Another study has shown differences in predator avoidance behaviour of mysids, and more specifically in the way of predator detection (chemical or visual signals) related to habitat characteristics (light vs. darker water) (Lindén et al., 2003). Hence, it is not unlikely that the higher turbidity of the water in the Gironde estuary (Castel, 1993) could lead to a slightly reduced development of the eye in the case of *N. integer* (e.g. narrow eyestalks and reduced cornea size). However, at this moment this hypothesis remains very speculative and additional morphological analyses, as well as breeding experiments under different environmental conditions could be useful to further elucidate these patterns and to disentangle the functional relationships.

Implications for species status and general conclusions

The final question which arises is whether the morphologically differentiated populations of *N. integer* and *M. slabberi* deserve a separate subspecies or species status. Although the discriminant analysis showed that the classification rate of individuals to correct populations was high (87.34% and 83.85% in case of *N. integer* and *M. slabberi*, respectively), there is still some overlap of individual mysids. Thus, no individual mysid can be assigned unambiguously to a particular geographical area ('population') on the basis of these linear measurements alone. In addition, the observed variation in meristic characters (e.g., number of spines on the lateral margin of the telson of *M. slabberi*), which generally is thought to be a variable with more operational taxonomic utility than morphometric measurements (Spotte, 1997; De Grave & Diaz, 2001), did overlap between the populations despite the significant differences detected between their averages.

Intraspecific geographical variation within other mysids has been observed, such as variation in the numbers of spines on the lateral margins of the telson between populations of *Praunus flexuosus* and *P. neglectus* (Mauchline, 1971), geographical differences in the proportions of the antennal scale of *N. americana* (Williams et al., 1974), and differences in the numbers of ommatidia in Atlantic and Mediterranean populations of *Eucopia hanseni* (Casanova, 1977). However, these variations are considered to be of a minor nature and could be consistent with the normal patterns of variation expected within species (Mauchline, 1980). In his review of the genus *Mesopodopsis*, Wittmann (1992) also reported (minor) morphological differences between Atlantic, Mediterranean and Black Sea populations of *M. slabberi*. However, the residual differences were found to be small and statistically overlapping and hence Wittmann (1992) noted that a reintroduction of the Czerniavsky's (1882) species (*goesi* and *cornuta*) and varieties (*major* and *minor*) was not appropriate.

In conclusion we can state that despite the limited number of populations analysed within both species and the selection of only adult female specimens which lowers the value of the present analyses in terms of general conclusions for both

species, clear morphometric differences were observed between populations of *N. integer* and *M. slabberi*. These results corroborate the expectations for a species inhabiting a wide geographic range and possessing limited dispersal capacities. However, the present morphometric analysis in itself does not allow us to conclude that the present species status of both mysids is in need of a revision. Hence, the observed morphological variation can be rather interpreted as geographical variation. On the other hand, the strong concordance of the morphometric results with the mitochondrial DNA data in the case of the Atlantic–Mediterranean separation of *M. slabberi* probably indicates that these populations are approaching the subspecies stage in the evolutionary continuum of speciation. This aspect definitely deserves more attention. Consequently, future research should focus on a larger number of populations and morphological characters, preferably using geometric morphometric techniques since these 'new' morphometric techniques are regarded as more powerful in analysing the external morphology and shape differences among organisms (Rohlf & Marcus, 1993; O'Reilly & Horn, 2004).

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