CHAPTER VII

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 of 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 each 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 two 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 overlap between populations, the morphometric analysis does not suffice to assign the populations to a separate species status. In the case of *N. integer*, the morphometric patterns showed a divergence of the Gironde population. 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). The method is regarded 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; De Grave & Diaz, 2001; Clark *et al*, 2001; Debuse *et al*, 2001; Doadrio *et al*, 2002; Lee & Frost, 2002). Moreover, morphometric analyses can 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, 1993; Hoffman & Merilä, 1999). Strong genetic differentiation of populations, accompanied with reproductive isolation, may lead to local adaptation. On the other hand, changing environmental conditions may produce phenotypic plasticity in genetically similar populations (Thompson, 1991). Hence, the comparison of the degree of variation in molecular markers with 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*, 1995; 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 – 41°E) (Wittmann, 1992). This wide distribution of both species spanning different biogeographical regions (Subarctic, Celtic, Lusitanian and 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.

Previous studies on genetic variation between populations of *N. integer* and *M. slabberi*, based on several mitochondrial loci, have shown significant heterogeneity within both species (see Chapters 3, 4 & 5). 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 (see Chapter 5). Phylogeographic analyses of *N. integer* identified a large genetic break at the southern distribution range (= divergent Guadalquivir population) and showed a genetic isolation of each population south of the English Channel, including the Irish population (see Chapters 3 & 4). 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.

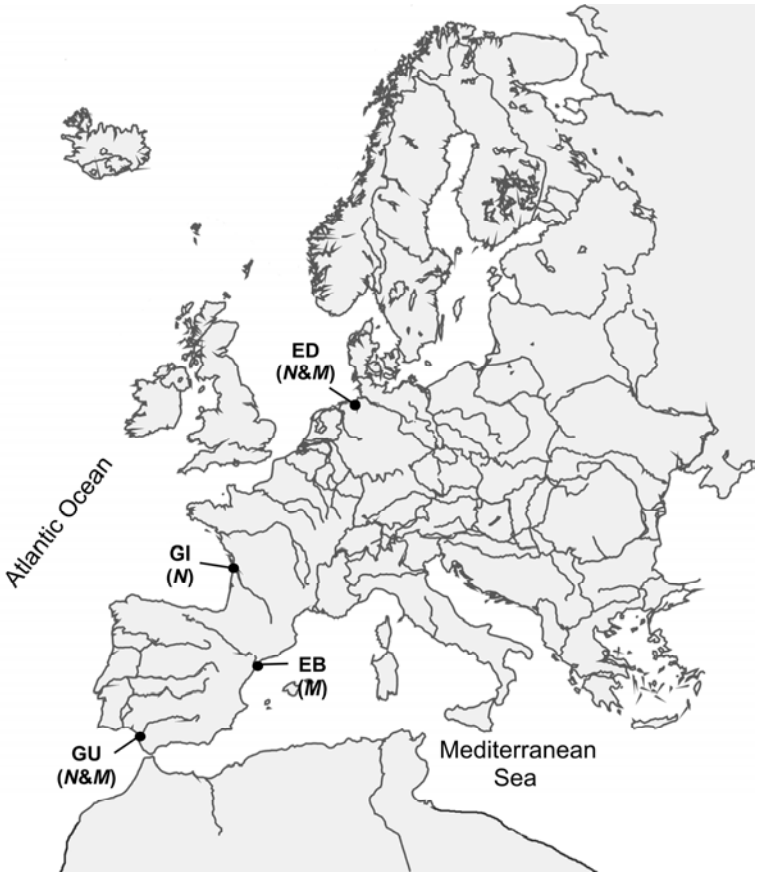

Fig. 7.1: Sampling locations (*N* = *Neomysis integer*, *M* = *Mesopodopsis slabberi*), sampling site abbreviations: ED = Eems-Dollard, GI = Gironde, GU = Guadalquivir, EB = Ebro

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. 7.1). 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 stored in 7% formaldehyde (all *N. integer* samples and *M. slabberi* from the Eems - Dollard) or in 70% ethanol (*M. slabberi* samples from the Guadalquivir and Ebro). The ethanol-preserved samples of *M. slabberi* were also used for molecular analyses (see Chapter 5).

Measurements and statistical analyses

From each sample a random number of about 50 adult, and mostly gravid, females were examined morphologically. A total of 12 metric (Fig. 7.2) and two meristic characters. 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 arcsin 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 those cases where homogeneity of variances was violated, even after transformations of the raw data, a non-parametric test was used (Kruskall-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

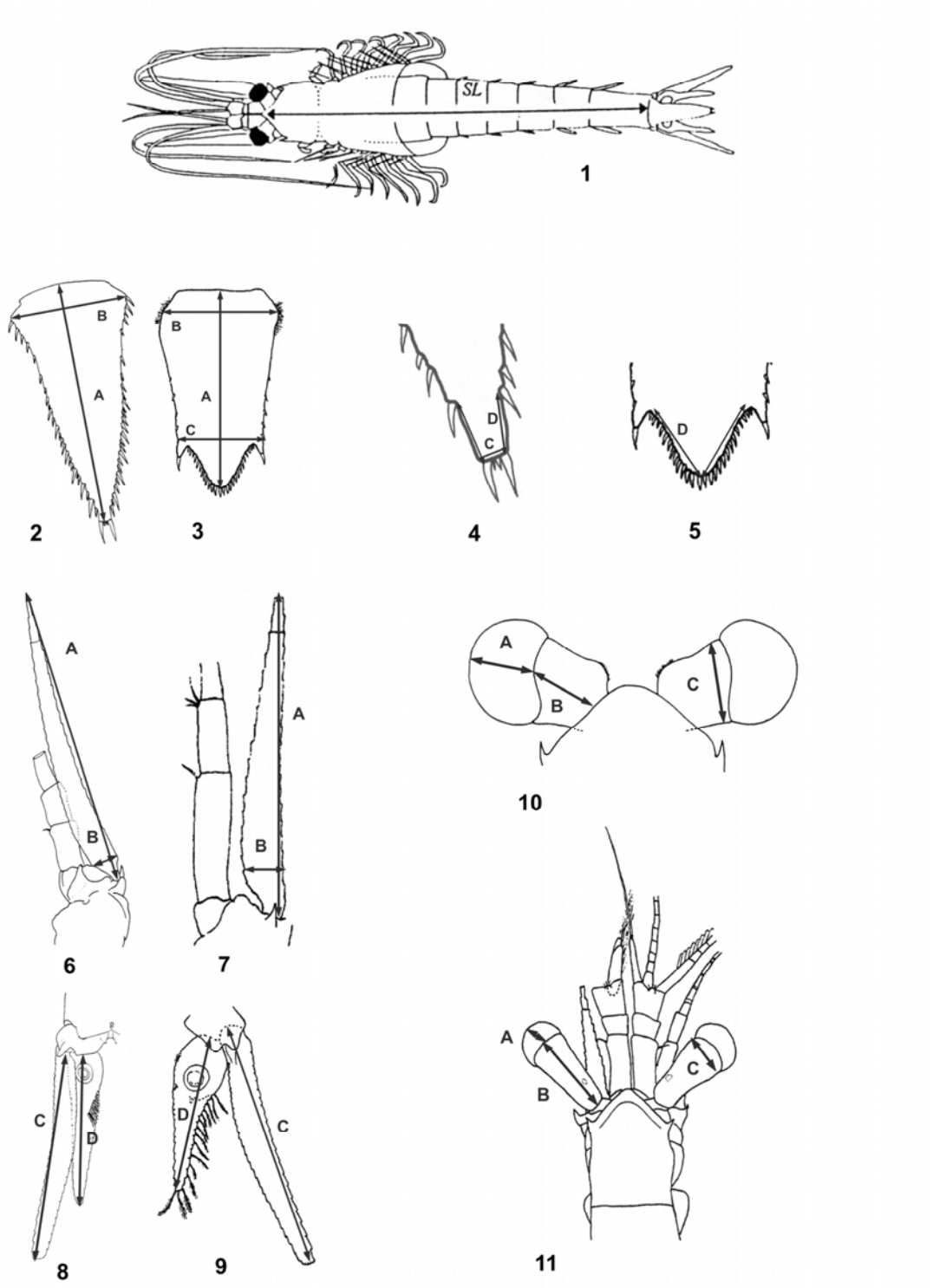

Fig. 7.2: Morphometric measurements: **1**: Standard length (SL); **2, 3, 4 & 5**: Telson of *Neomysis integer* (**2&4**) and *Mesopodopsis slabberi* (**3&5**), A = telson length (TELL), B = distal telson width (TELDW), C = caudal telson width (TELCW), D = caudal telson length (TELCL); **6 & 7**: Antennale scale of *Neomysis integer* (**6**) and *Mesopodopsis slabberi* (**7**), A= length of antennale scale (ANTL), B = width of antennale 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).

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 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.

Table 7.1: Mean and standard deviation (in parenthesis) of the different metric and meristic characters. Metric values are in mm. For the sampling site and metric measurement abbreviations see Figs. 7.1 and 7.2. Meristic character abbreviations: #SPENDO = number of spines on the inner margin of the uropod endopodite, #SPTEL = number of spines on the lateral margin of the telson.

	Neomysis integer				*Mesopodopsis slabberi*			
	OVERALL	**ED (N = 50)**	**GI (N = 54)**	**GU (N = 64)**	**OVERALL**	**ED (N = 50)**	**GU (N = 52)**	**EB (N = 30)**
STDL	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 (0.03)	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)	1.73 (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)	0.09 (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)
#SPTEL	-	-	-	-	6.56 (0.81)	6.96 (0.20)	7.00 (0.34)	5.57 (1.07)

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 7.1).

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 Discriminant Function Analysis (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 7.2).

Table 7.2: Summary of the Discriminant Function Analysis.

	Wilks' Lambda	Partial Lambda	F-remove (2,139)	*P* -level	Toler.	1-Toler. (R-Sqr.)
EYESTW	0.2190	0.5953	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

Wilks' lambda amounted to 0.1304 and was highly significant (appox. $F_{8,278} = 61.489$; $P < 0.001$). The morphometric characters showed a low degree of overlap (maximal 57.22% in case of the eyestalk width (EYESTW), see Table 7.2). Squared Mahalonobis distances (D^2) between populations (i.e. a distance measure between the group centroids) are listed in Table 7.3. 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-

Table 7.3: Squared Mahalanobis Distances

	ED	GI	GU
ED	-	***	***
GI	14.3579	-	***
GU	6.0109	14.0135	-

Dollard and Guadalquivir (GU) populations seemed to be smaller. A scatterplot of the individual canonical scores is presented in Fig. 7.3. 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 7.4). 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 correctly classified, with the within-group correct classifications ranging from 78.18 (GU) to 96.23% (GI) (see Table 7.5).

Table 7.4: Structure matrix of discriminant loadings for each of morphometric variable 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 7.5: Results of the discriminant analysis classification, 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

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* (1995).

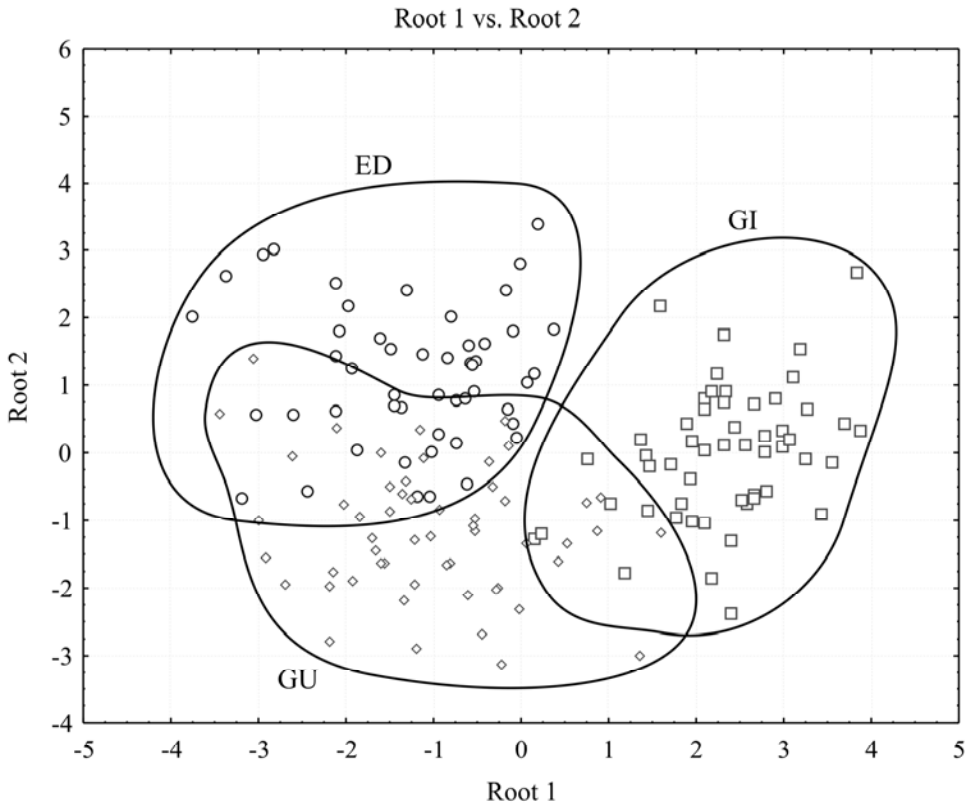

Fig. 7.3: *Neomysis integer*: Scatterplot of the DFA scores along the first and second root. For sampling site abbreviations see Fig. 7.1.

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 7.1).

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 three out of the 12 morphometric

characters contributed significantly to the multivariate discrimination between the three *M. slabberi* populations (Table 7.6). The largest Mahalanobis (D^2) distances were observed between the Mediterranean Ebro population and both Atlantic populations (Table 7.7). The canonical analysis showed that most of the observed variance between the populations (83%) was observed 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. 7.4). 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 eyestalk width (EYESTW) (ED > EB > GU mysids) (Table 7.8). The morphometric discriminant analysis correctly classified, on average, 83.85% of the individuals (Table 7.9). 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.

Table 7.6: Summary of the Discriminant Function Analysis.

N=114	Wilks' Lambda	Partial Lambda	F-remove (2,109)	*P*-level	Toler.	1-Toler. (R-Sqr.)
EYESTW	0.2458	0.5370	46.9902	< 0.0001	0.8585	0.1415
TELL	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 7.7: Squared Mahalanobis Distances between populations.

	ED	GU	EB
ED	-	***	***
GU	5.1972	-	***
EB	21.0172	13.5901	-

Table 7.8: Structure matrix of discriminant loadings for each of morphometric variable selected by the backward stepwise Discriminant Function Analysis (DFA).

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

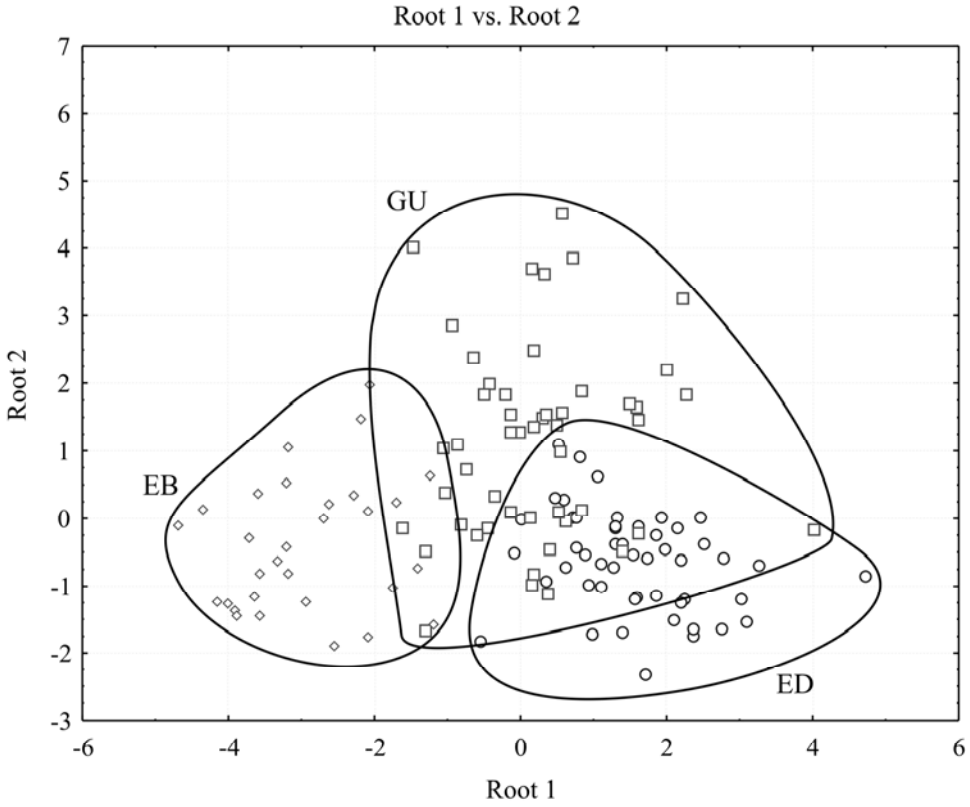

Fig. 7.4: *Mesopodopsis slabberi*: Scatterplot of the DFA scores along the first and second root. For sampling site abbreviations see Fig. 7.1.

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 (#SPTEL) between the different populations (Kruskal-Wallis test for #SPENDO: H (2, N = 128) =36.013 $P < 0.001$; #SPTEL: 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 7.1). Contrary to *N. integer*, no aberrant telsons were observed in the samples of *M. slabberi*.

	% 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

Table 7.9: Results of the discriminant analysis classification, showing the numbers and percentage of specimens classified in each group (Rows: Observed classifications, Columns: Predicted classifications).

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 statistical 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 & 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; San Vicente, 1996; 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 7.3 and 7.7) and the high percentage of correctly reclassified specimens in the original groups (populations) (see Tables 7.5 and 7.9). 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 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, 1993).

Extensive genetic surveys of different mitochondrial loci revealed a significant differentiation of populations of both *N. integer* and *M. slabberi* (see chapters 3, 4 and 5). Although not yet supported with nuclear markers, a large phylogeographic break was observed between Atlantic and Mediterranean populations of *M. slabberi*, indicating the possible existence of 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 were noticed 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 adaptations 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 7.3), while the largest genetic divergence was found for the Guadalquivir and not the Gironde population (see Chapters 3 & 4). 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 morphological overlap of individual mysids. Thus, no individual mysid can be assigned unambiguously to a particular geographical area ('population') on the basis of linear measurements. 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 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, 1971b), 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* (Cassanova, 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 should be 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 species 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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CHAPTER VIII

General conclusions and perspectives

1. Phylogeography of the brackish water mysid Neomysis integer: *restricted gene flow, multiple glacial refugia and complex postglacial recolonisation.*

Because of its typical life history characteristics (brooder and absence of free-living larvae) and the particular habitat preferences (brackish part of estuaries, brackish lagoons), the mysid *Neomysis integer* was selected as a potential model organism for inferring the impact of the Pleistocene glaciations on low dispersal marine taxa along the northeast Atlantic coast. The results of the mitochondrial DNA analyses (of both the cytochrome *b*, cyt *b*, and cytochrome *c* oxidase 1, COI, genes) clearly corroborated these expectations (see Chapters 3 & 4). A clear phylogeographic structure was observed with a very high proportion of population-specific haplotypes (up to 88% in the case of COI). The cyt *b* gene turned out to be more conserved, since one dominant haplotype was distributed throughout the whole distribution range with exception of the Gironde and Guadalquivir populations.

These results were interpreted in relation to historical patterns and processes using paleoclimatic and paleobiogeographic knowledge. This lead to some striking patterns which contradicted the general expectations according to the current paleoclimatological models:

→ **no trend of declining haplotype diversity was detected at higher latitudes**, the levels of genetic diversity were relatively uniform throughout the whole distribution range, even in glaciated areas, with exception of a decline at the northern and southern edge of the natural distribution range.

→ **the Iberian Peninsula did not act as a single glacial refugium for *N. integer,*** and according to the COI data these **southern refugial populations did not participate in the most recent range expansion** after the last glacial maximum (LGM).

→ there are **multiple northern refugia**, probably located in the southern North Sea or English Channel, around the British Isles, and an additional refugium in the Bay of Biscay, leading to **a complicated recolonisation history.**

These observations are supported by several facts, such as the relatively high heterogeneity of populations in glaciated areas, the apparent lack of a (postglacial) demographic expansion of the populations in these areas, levels of divergence

between northern mitochondrial lineages pointing to a pre-LGM differentiation, the detection of an isolation-by-distance pattern in glaciated areas and the lack of any southern haplotype in these areas. Moreover, similar patterns (e.g. northern refugia) have been observed in other marine vertebrates, as well as invertebrates (see references in Chapters 3 & 4).

However, due to the lack of mysid fossil data along the NE Atlantic, the absence of a species-specific molecular clock and the use of a single (mitochondrial) locus in the present research, alternative scenarios cannot be discarded. Hence, **future research** should focus on the use of several (unlinked) molecular markers combined with a more intensive sampling on the Iberian Peninsula, the Bay of Biscay and the coasts of Bretagne. In addition, new studies on the phylogeographic patterns within mysid species should also consider **the impact of Holocene warming** on the genetic composition of the southern populations (see Dahlgren *et al*, 2000; Consuegra *et al*, 2002; Coyer *et al*, 2003). At this moment it still remains unclear whether the observed **divergence of the southern Guadalquivir population** and the **genetic diversity decline** at these latitudes is linked to enhanced selective pressure at the distribution edge of *N. integer* related to increased Holocene temperatures. Extensive geographic sampling within the Gulf of Cadiz (and north African coasts) combined with detailed molecular analysis might generate complementary information. It would also provide insights in the **sustainability of these southern *N. integer* populations**.

2. Phylogeography of the mysid Mesopodopsis slabberi: *strong genetic divergence between Atlantic and Mediterranean populations with complex patterns of cryptic speciation.*

The mitochondrial DNA analyses of both the COI and 16S rRNA genes of the mysid *Mesopodopsis slabberi* revealed an **extraordinary degree of phylogeographic structuring** throughout its distribution range. **Four monophyletic clades** were apparent in the COI and 16S phylogenies: a large Atlantic clade, two Mediterranean clades corresponding to the haplotypes observed in the Ebro and the Alicante samples, and a fourth clade comprising a subset of the haplotypes of the Atlantic Mondego sample. In general, the levels of divergence between the different clades obtained from the 16S fragment were lower than those from the COI fragment, probably

caused by the higher conservation, and slower evolution of the mitochondrial 16S rRNA gene (Simon *et al*, 1994).

As mentioned in chapter 5, unravelling the evolutionary history that lead to the contemporary distribution of the different mitochondrial lineages in the populations of *M. slabberi* remains challenging. When putting the observed **divergences between the Atlantic and Mediterranean populations** (**16%**) in a broader perspective, they seemed to be **amongst the largest** thus far reported for Atlanto-Mediterranean marine taxa (see Table 4.7). The estimates of divergence time date back to the late Miocene/ early Pliocene (9.8 - 6.3 Mya), pointing to a **vicariant event during the Messinian salinity crisis** when sea-level dropped 115-120 m below the present-day level (Nilsson, 1982; Maldonado, 1985). The **two divergent mitochondrial clades** within the Atlantic **Mondego** estuary further complicate the phylogeographic patterns within *M. slabberi*. However, the lower genetic distances, at least for the 16S fragment, between this clade and the haplotypes of the Ebro population suggest **a Mediterranean origin** of this divergent Mondego clade. **Ship's ballast water transport** may have played a role in the transportation of these mysids to Atlantic waters. Analysis of the major ship routes from Mediterranean to Portuguese ports, as well as a more detailed sampling within the Mediterranean Sea (in potential 'source regions') are needed to resolve the identity and evolutionary origin of these haplotypes. Moreover, detailed analysis of Mediterranean *M. slabberi* populations inhabiting different habitats (estuaries, brackish lagoons, coasts) will also clarify the underlying evolution of the disjunct Mediterranean populations of *M. slabberi* (allopatric, parapatric divergence or ecological diversification between populations in marine and brackish environments).

Finally, the question remains whether the **different mitochondrial clades** should be considered **cryptic species?** The answer largely depends on the species concept that is favoured. If Cracraft's (1989) **phylogenetic species concept** (i.e. species are defined as minimum diagnosable units) is used, the answer is yes, since a high number of fixed differences is present between the different mitochondrial clades. However, purely applying this species concept could lead to the recognition of trivially divergent taxa at the species level. In addition, it is also greatly dependent on the polymorphic level (variability) of the selected marker system (Knowlton, 2000; Müller, 2000). According to Avise & Wollenberg (1997), a better criterion for recognizing species boundaries would be the existence of multiple concordant

differences at several (unlinked) loci. This approach also resembles that of the **biological species concept** (i.e. a species can be defined as a group of actually or potentially interbreeding individuals, with boundaries between species defined by intrinsic barriers to gene flow that have a genetic basis; Mayr, 1963), because reproductive barriers will emerge during the long-lasting geographic isolation that is required for many (unlinked) loci to acquire fixed (diagnostic) differences (Avise & Ball, 1990; Avise & Wollenberg, 1997).

The difficulty in defining species boundaries is further evidenced by the results of the morphometric analyses (Chapter 7). Although multivariate analyses clearly separated the Atlantic and Mediterranean populations based on telson and eye morphology and meristic characters, some (small) overlap existed between both populations. Hence, no individual mysid could be assigned unambiguously to a particular geographical area ('population') on the basis of these linear measurements alone. Moreover, phenotype-environment interactions ('phenotypic plasticity') could further confound the species division based purely on morphometric grounds.

In conclusion, our results (and especially the mitochondrial data) largely suggest the existence of different cryptic species within *M. slabberi*, but **further evidence from unlinked genetic markers (e.g. nuclear genes) are needed to confirm these patterns.** Future research should preferably make use of an integrative approach, using molecular (joint analysis of mitochondrial and nuclear loci), extended morphometrical (using geometric morphometric techniques) and environmental information (e.g. Rocha-Olivares *et al*, 2001; Pfenninger *et al*, 2003).

3. Are the differences in molecular diversity and genetic population structure between Neomysis integer and Mesopodopsis slabberi related to species-specific characteristics?

Both *Neomysis integer* and *Mesopodopsis slabberi* lack free-living larvae resulting in a low dispersal potential, which is reflected by a high phylogeographic structuring. But on the other hand, both species show some marked differences in their habitat preferences and physiological tolerance. ***N. integer*** is a true brackish water species, occurring in relatively discrete ('natural fragmented') habitats such as estuaries and brackish lagoons (= **'closed' populations**). In contrast, ***M. slabberi*** lives in marine (coastal, surfzone) and estuarine habitats, and hence may have a more

continuous distribution (= **'open' populations**). The geographical distribution of both species along the European coasts shows some differences, *N. integer* is restricted to Atlantic waters, while *M. slabberi* is also distributed throughout the whole Mediterranean and Black Sea. Along the NE Atlantic the distribution of both species largely overlaps, but *N. integer* seems to occur far further north (whole Baltic Sea, and even the White Sea, although recent observations are lacking) than *M. slabberi*. The evolutionary history of both genera, as well as the temperature tolerance (*N. integer* restricted by higher temperatures, *M. slabberi* restricted by colder temperatures) may have largely affected the contemporary distribution of both species.

A comparison of the genetic diversity patterns in both species may be useful for recognizing the effects of intrinsic (= biological, ecological, physiological or behavioural) differences on phylogenetic and phylogeographical patterns. Several studies in various marine taxa have shown that relatively small difference in species-specific intrinsic factors may result in the development of quite disparate patterns of population genetic structure and phylogeography for sympatric species (e.g. Wilke & Davis, 2000; Dawson *et al*, 2002; Bargelloni *et al*, 2003; McMillen-Jackson & Bert, 2003).

The standard diversity values (number of haplotypes, haplotype and nucleotide diversity) showed large differences between *N. integer* and *M. slabberi* (Table 8.1). **Haplotype diversity of almost all *M. slabberi* populations was more than twice the values for *N. integer*.** In addition, the levels of nucleotide diversity were much higher in the *M. slabberi* populations. The AMOVA's in both species further corroborate these patterns: in *N. integer* the highest percentage of variance was observed among populations while for *M. slabberi* the within population variance component was the largest (Table 8.2). These discrepancies in genetic diversity levels between both species may not be surprising. **High levels of within population haplotype diversity** have been considered a typical phenomenon of many **marine species**, as evidenced for both vertebrates and invertebrates (Baldwin *et al*, 1998; Grant & Waples, 2000; Benzie *et al*, 2002; McMillen-Jackson & Bert 2003; Karaiskou *et al*, 2004), while **low within-population variability** is a common characteristic for **brackish-water species** (Maltagliati 1999; Cognetti & Maltagliati, 2000;Bilton *et al*, 2002; Maltagliati, 2002). A common explanation for the high haplotype diversity and for the large numbers of low frequency haplotypes may lie in the enormous population sizes of marine organisms, which could cause a retention of

numerous haplotypes and result in an undersampling of the populations (Bucklin & Wiebe, 1998). However, given the sometimes astonishing densities of *N. integer* in the Westerschelde estuary (peaks of 100s of thousands mysids per 1000 m² and yearly averages up to 6500 mysids per 1000 m², Mees *et al*, 1993a, 1995; see also census population size estimations of *N. integer* in chapter 6), other ecological and evolutionary processes may have been involved in the reduction of genetic diversity (e.g. environmental interactions, natural selection, a population bottleneck, different age of both species, small or historical variable effective population sizes; see Bucklin & Wiebe, 1998 & discussion in Chapter 5).

Table 8.1: Standard diversity values for the overlapping sampling locations of *Neomysis integer* and *Mesopodopsis slabberi*. N_h = number of haplotypes, *h* = haplotype diversity, π = nucleotide diversity. Standard deviations of *h* and π are indicated between brackets. All values were calculated from the mitochondrial COI data presented in Chapters 4 & 5.

Sampling location	Sample Size	N_h	*h* (SD)	π (SD)
Neomysis integer				
Westerschelde	60	6	0.4689 (0.0652)	0.00335 (0.00227)
Seine	48	4	0.4193 (0.0810)	0.00329 (0.00225)
Ria de Aveiro	30	5	0.6115 (0.0510)	0.00272 (0.00198)
Guadalquivir	40	5	0.2359 (0.0880)	0.00128 (0.00118)
Mesopodopsis slabberi				
Westerschelde	25	21	0.9667 (0.0292)	0.010888 (0.006104)
Seine	19	16	0.9766 (0.0267)	0.010483 (0.005985)
Ria de Aveiro	16	11	0,9500 (0,0364)	0,008461 (0,005022)
Guadalquivir	18	18	1.0000 (0.0185)	0.019993 (0.010789)

Table 8.2: Comparison between the results of the hierarchical analysis of molecular variance (AMOVA). **Top:** *Neomysis integer*, AMOVA on all the Atlantic samples and a separate AMOVA excluding the Guadalquivir samples (GU); **Below:** *Mesopodopsis slabberi*, AMOVA on all the Atlantic samples, with exclusion of the divergent haplotypes in the Mondego sample (MO-B, see Chapter 5).

	Source of variation	% Total variance	Fixation indices	*P*
Neomysis integer				
All samples	Among populations	78.67	Φ_{ST} = 0.7867	< 0.001
	Within populations	21.33		
without GU sample	Among populations	71.39	Φ_{ST} = 0.7139	< 0.001
	Within populations	28.61		
Mesopodopsis slabberi				
Atlantic samples	Among populations	40.08	Φ_{ST} = 0.4001	< 0.001
	Within populations	59.92		

A comparison of the pairwise genetic distances between populations of both species revealed a **clear difference of the genetic structure at a meso-geographic scale** (i.e. between the Westerschelde and Seine populations). In the case of *N. integer* both populations were significantly differentiated, while for *M. slabberi* no differentiation was observed. This could imply high levels of contemporary gene flow between these *M. slabberi* populations, or recent common ancestry (which seems not unlikely for populations inhabiting areas that have been severely affected by glaciations) (Avise *et al*, 1987). **At a macro-geographic scale (> 500 km) both species showed similar trends**, with exception of the higher differentiaton of the *N. integer* population in the Guadalquivir estuary. The peculiar pattern of the *M. slabberi* Guadalquivir population (= higher similarity with the northern Westerschelde & Seine populations than with the geographically closer Ria de Aveiro populations) remains unexplained and will need further examination.

Table 8.3: Pairwise genetic distances (Tamura & Nei, 1993) based on the mitochondrial COI data. *Above diagonal*: genetic distances of *Mesopodopsis slabberi*. *Below diagonal*: genetic distances of *Neomysis integer*. ^{ns} = value not significant at the 95% level. Population abbreviations: WS, Westerschelde; SEI, Seine; Rda, Ria de Aveiro; GU, Guadalquivir. All data compiled from Chapters 3 and 4.

	WS	**SEI**	**Rda**	**GU**
WS	-	0^{ns}	0.590	0.219
SEI	0.624	-	0.604	0.215
Rda	0.641	0.551	-	0.471
GU	0.901	0.879	0.911	-

Since both species occur sympatrically along the NE Atlantic, it can be assumed that they must have been subjected to the same paleoclimatological events (e.g. Pleistocene glaciations). Hence, a comparison of the phylogeographic patterns along the NE Atlantic within both species could reveal something about the species-specific responses to these historical climate events. However, the lopsided sampling regime in both studies (461 mysids from 11 sampling sites for *N. integer*, 78 mysids from 5 Atlantic sampling sites for *M. slabberi*) might hamper a clear comparison of the phylogeographic patterns, and hence, the conclusions for *M. slabberi* must be considered provisional. Nevertheless, some remarkable differences were apparent between both species, probably pointing to **a different response of *M. slabberi* to changing climatological conditions.**

When comparing the mismatch distributions of different geographical samples for both species (Fig. 8.1) the situation for the **northern populations** (North Sea &

English Channel) was clearly different. The distribution was unimodal for ***M. slabberi***, which is consistent with a model of **rapid population growth** from a small number of mysids, while a fit to the sudden expansion model was significantly rejected for ***N. integer***, pointing to **a more stable population structure** (see also mismatch distribution parameters in Chapters 4 & 5). These differences are also visible in the haplotype networks, with a star-like network for *M. slabberi* (see Chapters 4 & 5). The **mismatch distributions for the Portuguese samples seemed concordant for both species**; a fit to the sudden expansion model was rejected. Compression of the distribution range of *M. slabberi* to southern Europe (in the Bay of Biscay or maybe the northern Iberian Peninsula) during glacial periods caused by **lower temperatures and absence of suitable habitats**, followed by a postglacial range expansion to northern Europe, which is a common pattern in many European biota (see Hewitt, 1996, 2000), could have produced the unimodal mismatch distribution of the northern populations. In contrast, *N. integer* seemed to be able to withstand the glacial conditions in northern Europe and could have survived in isolated northern refugia (see previous discussions).

In conclusion, the present phylogeographic study of *M. slabberi* has **opened some interesting research perspectives**. Especially the large phylogeographic breaks (signals of cryptic speciation?) between *M. slabberi* populations and the disparate phylogeographic patterns of the sympatric mysids *N. integer* and *M. slabberi*, probably triggered by differences in eco-physiological tolerances, deserve detailed future research.

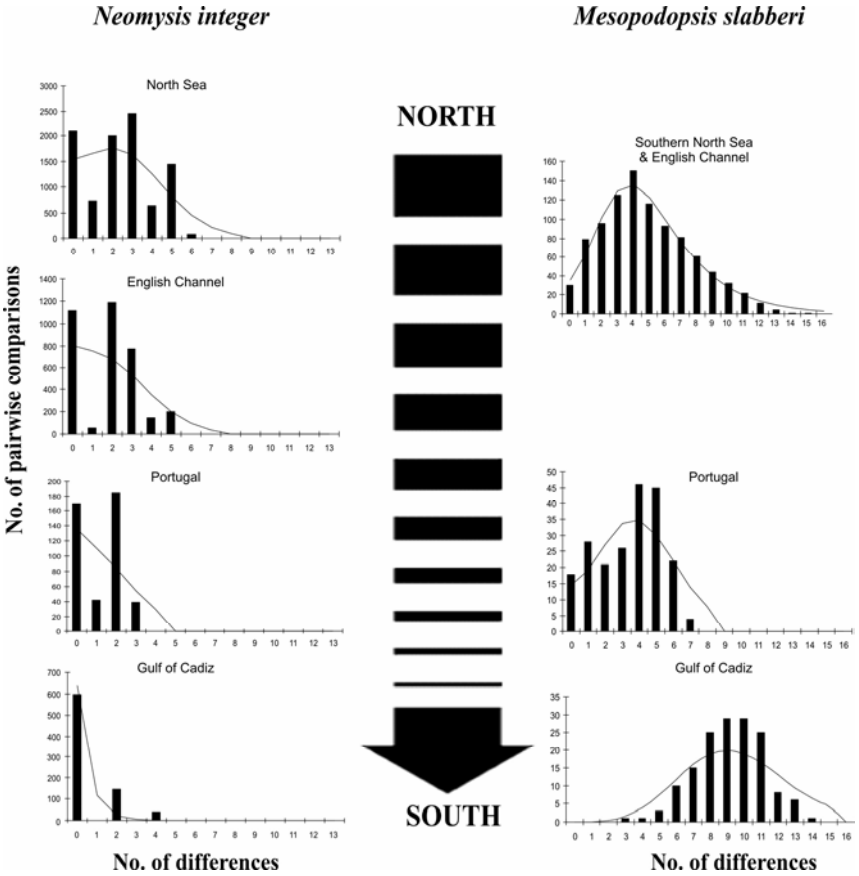

Fig. 8.1: Comparison of the mismatch distributions of different geographical samples for *Neomysis integer* and *Mesopodopsis slabberi*. In each case the bar represents the observed frequency of the pairwise differences among haplotypes, while the solid line shows the distribution expected under the model of a sudden demographic expansion (Rogers, 1995).

4. Small-scale and temporal patterns of genetic differentiation within Neomysis integer.

Most studies on the genetic structuring within species, including the present study of both mysid species, have focused on geographical patterns of genetic variation **regardless of the temporal variation**. However, the assessment of both spatial and temporal components of the genetic structure of a species is necessary to thoroughly understand the microevolutionary processes that influence the genetic variability and relationships among its populations (Maltagliati & Camilli, 2000). Therefore we conducted a temporal, as well as a fine-scale (intra-estuarine) genetic study of the Westerschelde population of *N. integer*. Although **(small) intra-estuarine differentiation** was detected within two of the three analysed years, and there seemed to be **no evidence for temporal stability** of this structure, the (single locus) molecular marker used in this study has **several limitations in terms of interpretation** of these contemporary genetic patterns (Allendorf & Seeb, 2000; Nevo, 2001; Wan *et al*, 2004). Hence, future research on the temporal and small-scale variation within mysids should preferably make use of a multilocus approach (e.g. microsatellites). In addition, the observations that *N. integer* has migrated further upstream, to more polluted sites, within the Westerschelde during the last decade warrants future investigation by continuing genetic monitoring. Bearing in mind the strong genetic differentiation of populations of *N. integer* (linked to the natural fragmented habitat and low dispersal capacities), the **very low (female) effective population size** estimations (see chapter 6) and the high potential of bioaccumulating endocrine disrupters and other toxicant compounds (Roast *et al*, 1999, 2000, 2002; Verslycke, 2003), these populations may be especially prone to rapid loss of genetic diversity.

5. The family Mysidae is in need for a taxonomical revision, as evidenced by the 18S rRNA phylogeny.

A phylogenetic study of the Mysidae, the largest family within the order Mysida, based on 18S rRNA sequences was conducted in order to test the morphology-based classification within this family. The molecular analysis did not support the monophyly of two of the three subfamilies included in the study. The

subfamily Gastrosaccinae was clearly resolved in two groups: "*Gastrosaccus*-group" and "*Anchialina*-group", which was further supported by morphological evidence. The paraphyly of the large subfamily Mysinae (comprising 91% of the genera and 80% of all species classified within the family Mysidae) highlights the problematic division into tribes, once introduced to permit an 'easier' structuring of this large subfamily. Hence, a revision of the tribes within this subfamily is suggested in order to tune taxonomy to phylogenetic relationships based on morphological and molecular data. In addition, representatives of the subfamilies Boreomysinae, Rhopalophtalmidae and Mysidellinae, which were not analysed in the present study, should be included in future research to evaluate the taxonomical rigidity of the whole Mysidae family.