

DNA MICROSATELLITE MARKERS IN SERVICE OF SWORDFISH STOCK-STRUCTURE ANALYSIS IN THE ATLANTIC AND MEDITERRANEAN

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SUMMARY

Analysis of mtDNA variation has been successfully used to describe swordfish stock structure around the globe. Despite the great usefulness of this marker, we still need a lot of research to understand stock structure and migration of swordfish. To deal with complications like mixed fisheries or eventual hybridization between stocks, suitable markers alone do not suffice. Suitable sampling and continual population monitoring is necessary as well. We have been using nuclear markers, namely microsatellites on swordfish samples from the Atlantic and the Mediterranean to address questions related with the population structure and migration patterns. Here we present some preliminary results.

RÉSUMÉ

L'analyse de la variation de l'ADNmt a été utilisée avec succès pour décrire la structure du stock d'espadon autour du globe. Malgré la grande utilité de ce marqueur, nous avons encore besoin de beaucoup de recherche pour comprendre la structure du stock et la migration de l'espadon. Pour résoudre les complexités, telles que les pêcheries mixtes ou l'éventuelle hybridation entre les stocks, des marqueurs appropriés ne sont pas à eux seuls suffisants. Il faut également un échantillonnage adéquat et un suivi continu de la population. Nous avons utilisé des marqueurs nucléaires, à savoir des microsatellites sur des échantillons d'espadon de l'Atlantique et de la Méditerranée, pour répondre aux questions liées à la structure de la population et aux schémas de migration. Nous en présentons les résultats préliminaires.

RESUMEN.

El análisis de la variación del ADNmt ha sido fructífero a la hora de describir la estructura del stock de pez espada en el mundo. A pesar de la gran utilidad de este marcador, es necesario desarrollar una gran investigación para comprender la estructura del stock y la migración del pez espada. Los marcadores adecuados no resultan suficientes para solucionar complicaciones como las pesquerías mezcladas o la eventual hibridización entre stocks. También es necesario llevar a cabo muestreos adecuados y un seguimiento continuo de la población. Hemos utilizado marcadores nucleares, microsatélites, en muestras de pez espada del Atlántico y Mediterráneo para abordar las cuestiones relacionadas con la estructura de la población y los patrones de migración. Presentamos en este documento algunos resultados preliminares.

KEYWORDS

Swordfish, Population genetics

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1. INTRODUCTION

Molecular markers have allowed getting some useful insights in the population structure of swordfish. Mitochondrial DNA (mtDNA) analyses by different authors (Kotoulas *et al.*, 1995, Alvarado Bremer *et al.*, 1996, Rosel and Block, 1996, Chow *et al.*, 1997, Chow and Takeyama, 2000) have revealed at least four breeding units (stocks) of the swordfish: Mediterranean, North Atlantic, South Atlantic and Indo-Pacific. The once, though unique Indo-Pacific stock, revealed subtle genetic differentiation corresponding to original migratory roots (Reeb *et al.*, 2000).

In the case of within Atlantic differentiation, however, variation is not as high as to allow identification of the origin of individual fish. This leads to the rather uncomfortable situation, where samples are defined on the sole bases of geographic origin of captured fish, and may be misleading in the case, for example, of co-occurrence of two stocks in the same feeding ground. This is why nuclear markers are necessary, since a high number of them may be obtained, and they are highly polymorphic, thus allowing individual-level assignment to stocks, even for relatively low degree of differentiation between stocks. In the case that both nuclear and mitochondrial markers are analyzed for the same individuals, sex-specific differences in migration and breeding patterns may be revealed. Another issue of importance is the density of samples over space and time. Simple snapshots of the gene pool may be misleading, as different stocks may migrate at different moments. To get a clear idea about swordfish migration, any sample or single individual will be useful and should be added in order to construct a dynamic puzzle, as close to the biological reality as possible.

Here we have analyzed samples from North Atlantic (USA coast), Gulf of Guinea, South Atlantic, East North Atlantic, and the Aegean Sea. The objective has been to use nuclear markers to unravel heterogeneity in sample collections of the same locality and, when possible, to analyze temporal sampling within region in order to better assess the migration behavior of different stocks.

2. MATERIALS AND METHODS

In the present study swordfish samples were scored for two microsatellite (XgA and Xg195). The data presented here comprise 453 specimens. Sample origin and size are given in **Figure 1**. Samples have been obtained by longliners with observers on board or at ports. Samples of North West Atlantic (NWA90 and NWA93) have been presented in previous studies of mtDNA analysis (Alvarado-Bremer *et al.*, 1996) or with mtDNA and nuclear markers of low mutation rate and therefore of low variability (Chow and Takeyama 2000).

The data have been analyzed mainly by Factorial Correspondence Analysis as in She *et al.*, 1987, by use of the software GENETIX (Belkhir K, *et al.*, 1996). Theta values of Weir and Cockerham, 1984 were estimated using the same software. In one case theta values have been estimated on groups of individuals, which were suggested by the Factorial Correspondence Analysis.

3. RESULTS

The total number of alleles revealed for loci XgA and Xg195 was 54 and 65 respectively, which is very high in general, but also in comparison with an average of 2 alleles per locus for allozymes (Pujolar *et al.*, 2002). The data are mainly analyzed by a series Factorial Correspondence Analysis, whereby individuals are projected in a three-dimensional graph, based on their combined genotype for the two markers.

In the first analysis (**Fig. 2**), Mediterranean samples are clearly distinguished from all others. This differentiation gives highly significant theta values (heterogeneity test, software GENEPOP). (**Table 1**), Fish of Atlantic origin, seem to form two main groups. Among them, Group I represent 16% of the individuals of Northwest Atlantic samples (used in the literature as “reference” samples for the North Atlantic stock, Alvarado-Bremer *et al.*, 1996 & Chow *et al.* 1997) and also comprises some individuals from the Gulf of Guinea (7% of them) but is absent from South Atlantic. Group II is the major

constituent of any sample collection in the Atlantic and the exclusive one in the South Atlantic. Samples from South Atlantic and from the Mediterranean appear as homogeneous gene pools, and helped in understanding the origin of observed genetic heterogeneity. This *mixed stock situation* had made difficult the estimation of genetic divergence between North and South Atlantic in previous works, which have been contradictory (Alvarado-Bremer *et al.*, 1995, vs. Alvarado-Bremer *et al.*, 1996). We advance the hypothesis that these contradictory results may not be due to small sample sizes as suggested by Chow *et al.*, 1997, but rather to sample timing as related to change in stock composition. Interestingly, the theta values (**Table 1**) between Group I and Group II within the very same locality in Northwest Atlantic, is among the highest ones.

Figure 3 presents results of the same Factorial Correspondence Analysis, where samples from the Aegean are analyzed together with samples from the northeastern Atlantic (+36 lat, -20 Long). The differentiation between Atlantic and Mediterranean is quite efficient, even with only two microsatellites (**Figure 3A**). In the northeastern Atlantic sample we have temporal sampling extending from the beginning of August to the end of March. As we can see by comparing **Figure 3B** to **Figure 3D**, a higher percentage of Mediterranean origin fish is captured in the northeastern Atlantic in winter than in early autumn. This mixed stock situation that is changing over time, is also suggested by high heterozygosity deficiencies for these samples (data not shown).

Finally **Figure 4** presents the Northeast Atlantic sample in relation to the Groups revealed by the first analysis (**Figure 2**). This sample consists mainly of fish of Group II, of Mediterranean origin, while a small percentage seems to be of Group I.

4. DISCUSSION

The results obtained in the present work revealed high intra sample heterogeneity in Northwest Atlantic. We have found that samples having been used as “reference” samples for North Atlantic cannot be actually characterized as such, since they have been shown to constitute a collection of at least two divergent gene pools. These gene pools are highly divergent, as evidenced by the high theta values. Within the sampling availability of this study, the South Atlantic sample seems to be homogeneous, and this is also the case of the Mediterranean sample. On the contrary, North Atlantic seems to be heterogeneous. One component of this heterogeneity is common with the South Atlantic, while the other is present only in North Atlantic. Finally, temporal sampling seems to be a necessity in order to understand migratory behavior of the swordfish. In northeastern Atlantic a considerable percentage of fish seems to be of Mediterranean origin. The percentage of these fish change in different periods of the year and may also vary from day to day, although higher sample sizes are needed to follow the phenomenon in detail.

The results of the present study should be considered as preliminary, as the analysis of 3.240 specimens, including 0⁺ class fish from the Mediterranean, is currently under way. All specimens will be analyzed for 10—15 microsatellite loci, while a subset of them will be sequenced at the control region of mtDNA. The combined information from the two types of markers will allow to detect sex specific differences in migration behavior, inter-stock hybridization and to obtain unambiguous assignment of individuals to stocks.

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LITERATURE

- ALAVARADO-BREMER, J.R., Mejuto, J., Greig, T.W. & Ely, B. 1996. Global population structure of the swordfish (*Xiphias gladius* L.) as revealed by analysis of the mitochondrial DNA control region. *J.Mar.Biol.Ecol.*, 197; pp. 295-310.
- BELKHIR, K, P. Borsa, J. Goudet, L. Chikhi, F. Bonhomme. 1996-1998. GENETIX, logiciel sous Windows™ pour la génétique des populations. Laboratoire Génome et Populations, CNRS UPR 9060, Université de Montpellier II, Montpellier (France).
- CHOW, S. and H. Takeyama. 2000. Nuclear and mitochondrial DNA analyses reveal four genetically separated breeding units of the swordfish *J. Fish Biology*, 56; pp. 1087-1098.
- KOTOULAS, G., A. Magoulas, N. Tsimenides, and E. Zouros. 1995. Marked mitochondrial DNA differences between Mediterranean and Atlantic populations of the swordfish, *Xiphias gladius*. *Molecular Ecology*, 4; pp. 473-481.
- SHE, J.X., M. Autem, G. Kotoulas, N. Pasteur, and F. Bonhomme. 1987. Multivariate analysis of genetic exchanges between *Solea aegyptiaca* and *Solea senegalensis* (Teleosts, Soleidae). *Biological Journal of Linnean Society*, 32; pp. 357-371.
- PUJOLAR, J.M., M.I. Roldan and C. Pla. 2002. A genetic assessment of the population structure of swordfish (*Xiphias gladius*) in the Mediterranean Sea. *J. Expr. Mar. Biol. Ecol.*, 276; pp. 19-29.
- RAYMOND, M. and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Heredity*, 86; pp. 248-249.
- REEB, C.A., L. Arcangeli, and B.A. Block. 2000. Structure and migration corridors in Pacific populations of the Swordfish (*Xiphias gladius*), as inferred through analyses of mitochondrial DNA. *Marine Biology*, 136; pp. 1123-1131.
- ROSEL, P. and B. A. Block. 1996. Mitochondrial control region variability and global population structure in the swordfish, *Xiphias gladius*. *Marine Biology*, 125; pp. 11-22.
- WEIR, B.S. and C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38; pp. 1358-1370.

Table 1: Estimation of theta values (Weir & Cockerham 1984), by the software GENETIX (Belkhir K *et al.*, 1996)

Theta	Group-II	Guinea	S-Atl	Aegean
<i>Group-I</i>	0.05216	0.03753	0.04997	0.14534
<i>Group-II</i>		0.00635	0.00830	0.07451
<i>Guinea</i>			-0.00155	0.07335
<i>S-Atl</i>				0.07183
Theta	Guinea	S-Atl	Aegean	
NWA90&93	0.00596	0.00925	0.07844	
<i>Guinea</i>		-0.00132	0.07402	
<i>S-Atl</i>			0.07183	
Theta	NWA93	Guinea	S-Atl	Aegean
NWA90	0.00693	0.00615	0.01460	0.08711
NWA93		0.00943	0.00919	0.07981
<i>Guinea</i>			-0.00132	0.07402
<i>S-Atl</i>				0.07183

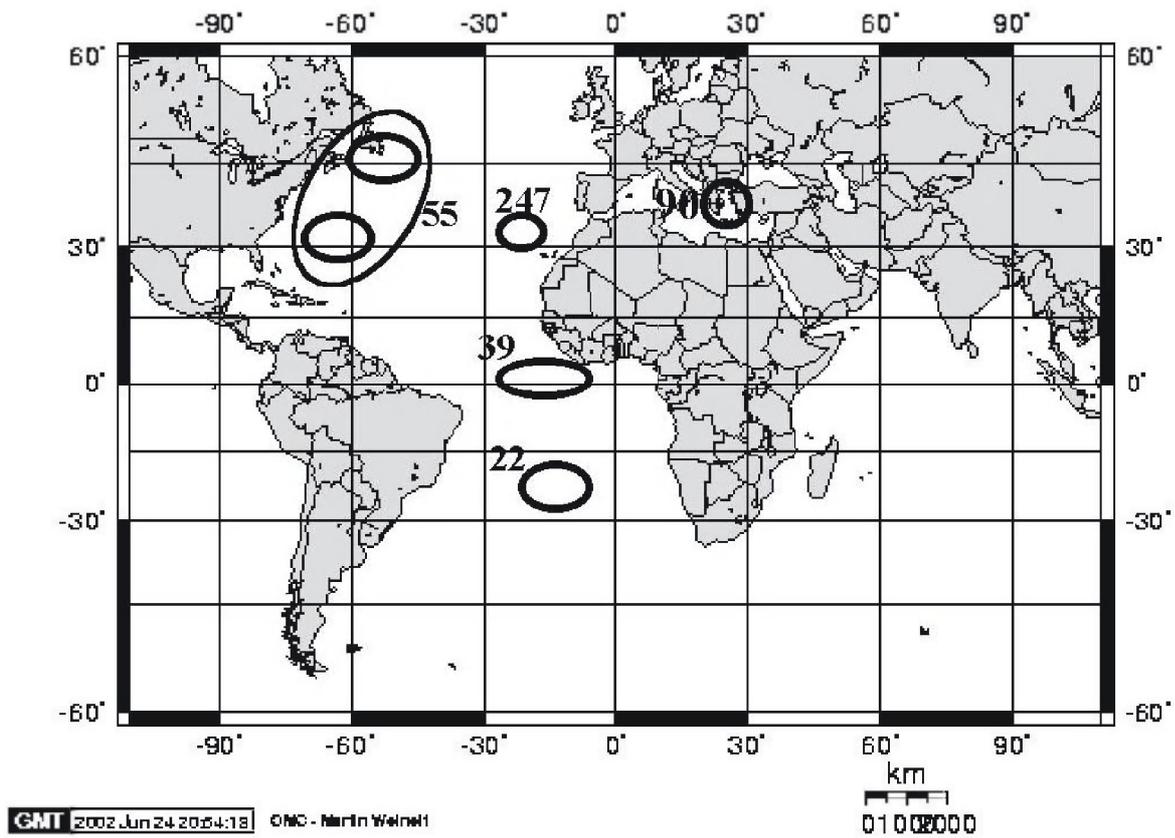


Figure 1. Analyzed sample sizes of swordfish: numbers correspond to the sample size.

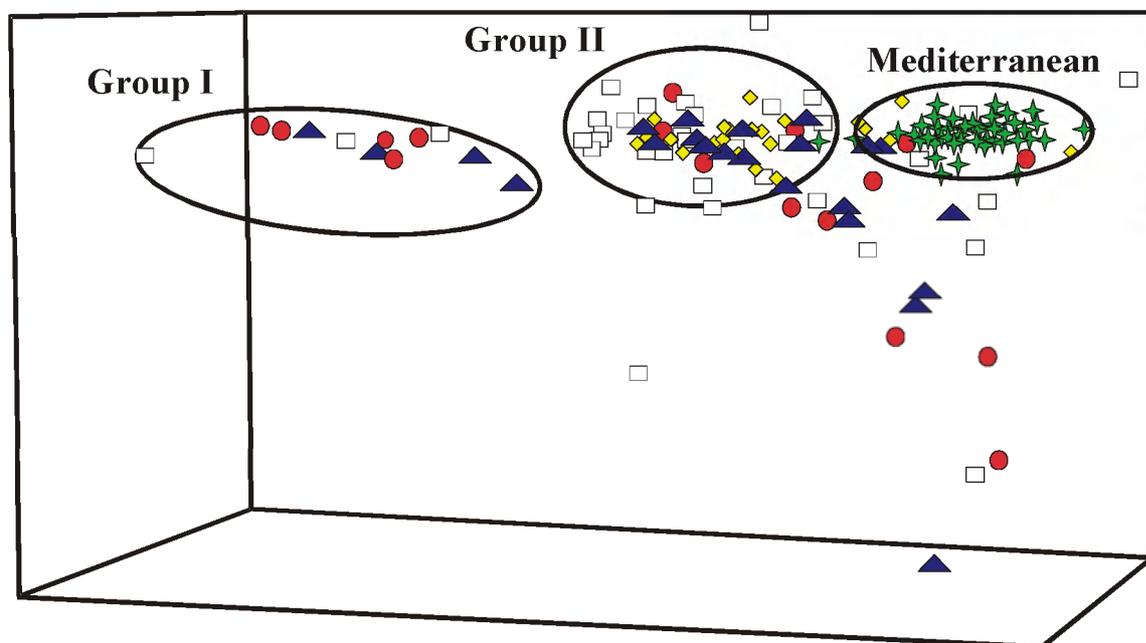


Figure 2. Three dimensional presentation of a Factorial Correspondence Analysis of swordfish samples based on two microsatellite markers (XgA, Xg195), explaining 8.5% of the total variability. Each symbol represents an individual fish.

- ▲ : Northwest Atlantic "reference" sample, □ Guinea Gulf (tropical) sample,
- ◆ : South Atlantic sample and ✦ Mediterranean sample.

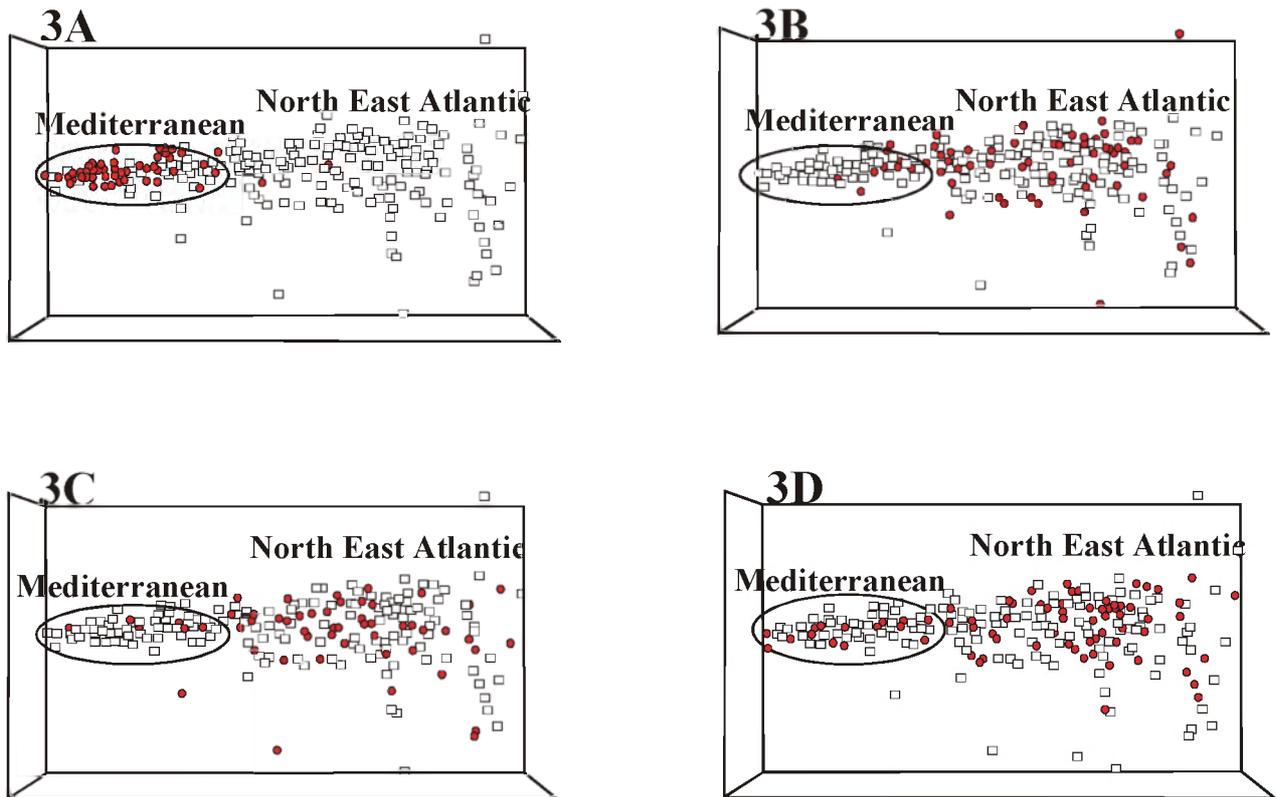


Figure 3. Factorial Correspondence Analysis opposing samples of the Aegean to samples of North-East Atlantic [+35 Lat, -20 Long].

In each of the plots, one sample is highlighted (dark circles):

A: Mediterranean fish;

B: North-East Atlantic fish, captured from 1/8/2000 to 9/11/2000;

C: North-East Atlantic fish, captured from 13/11/2000 to 3/12/2000 and

D: North-East Atlantic fish, captured from 4/12/2000 to 20/03/2001

Sample sizes are similar in the tree fishing periods (88, 77, 82 respectively).

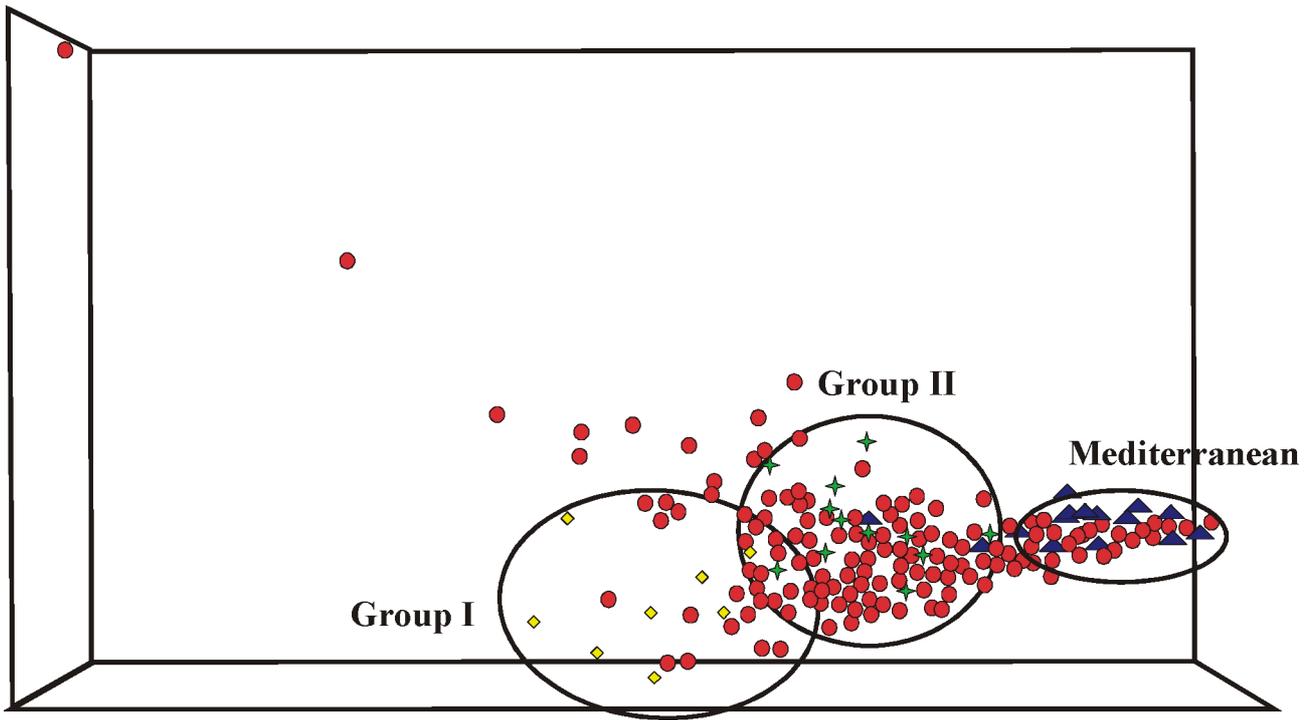


Figure 4. Factorial Correspondence Analysis presenting North-East Atlantic sample in relation to the groups revealed by FCA analysis in Figure 2.

- ◇ : specimens of Group I.
- ★ : specimens of Group II.
- ▲ : specimens of the Mediterranean (Aegean) and
- : specimens of North-East Atlantic.