# Morphological identification of two sympatric species of Trichiuridae, Aphanopus carbo and A. intermedius, in NE Atlantic 

by<br>Manuel BISCOITO* (1), João DELGADO (2), José A. GONZÁLEZ (3), Sérgio STEFANNI (4), Víctor M. TUSET (3), Eduardo ISIDRO (4), Antonio GARCÍA-MEDEROS (3) \& Dalila CARVALHO (2)


#### Abstract

The black scabbardfish has been subjected to a commercial fishery in the waters of the archipelago of Madeira for more than 150 years, which is probably the oldest deep-sea commercial fishery in the world. Over this period the presence of two sympatric species (Aphanopus carbo Lowe, 1839 and A. intermedius Parin, 1983) in the area has been ignored, mainly due to the difficulty in separating the two species using external morphological characters. The need for a more accurate management of this highly important resource, reinforced by an emergent fishery in Portugal mainland and elsewhere in the North Atlantic, justified a new effort for morphological characterization of the two species, based on the largest genetically validated sample obtained to date. The results presented in this paper demonstrate that it is possible to discriminate the two species on the bases of meristic and morphometric data using discriminant analysis. The outcome of this analysis is supported by the genetic identification based on CR and COI sequences. In addition, a redescription of A. intermedius incorporating the new character ranges found is presented.


RÉSUMÉ. - Identification morphologique de deux espèces sympatriques de Trichiuridae, Aphanopus carbo et A. intermedius de l'Atlantique du Nord-Est.

Le sabre noir a fait l'objet de la plus ancienne pêche commerciale en eaux profondes. Son histoire, longue de 150 ans dans les eaux de l'archipel de Madère, n'a cependant pas permis de déceler la présence de deux espèces sympatriques : Aphanopus carbo Lowe, 1839 et A. intermedius Parin, 1983. L'explication de ce hiatus tient à la difficulté de séparer les deux espèces sur la base des seuls caractères morphologiques externes. Or, l'émergence des activités de pêche tant dans les eaux continentales portugaises qu'en d'autres points de l'Atlantique Nord implique une gestion fine de cette ressource halieutique. Cela inclut la caractérisation des deux espèces grâce à l'analyse d'un grand nombre de spécimens dont l'identification est systématiquement validée au niveau génétique. La présente étude démontre ainsi qu'une analyse discriminante appliquée à l'ensemble des données méristiques et morphométriques permet de séparer les deux espèces. D'autre part, cette même étude montre que ces résultats sont validés au niveau génétique par les séquences issues du gène COI. Les travaux réalisés dans le cadre de cet article permettent d'envisager en outre, l'actualisation des caractères descriptifs des deux espèces A. intermedius et A. carbo.

Key words. - Trichiuridae - Aphanopus - Black scabbardfish - ANE - Discriminant analysis - Distribution - mtDNA - Taxonomy.

The black scabbardfish Aphanopus carbo (Lowe, 1839), has been subjected to a commercial fishery in the waters of the archipelago of Madeira for more than 150 years, in which is probably the oldest deep-sea commercial fishery in the world (Maul, 1950). This long-line fishery has yielded in the last seven years an average of 3450 tons per year (data obtained from Direcção Regional de Pesca, Madeira), which is mainly consumed on the island where it is used as emblematic dish.

Until recently, A. carbo was the only recognized species in this genus, although several other junior synonyms were described, including one from Madeira, A. acus Maul, 1948 (Parin, 1983; Nakamura and Parin, 1993). In 1983, a descrip-
tion of A.intermedius, partially sympatric with A. carbo, was published and now the genus Aphanopus comprises seven species distributed throughout all oceans except in the polar regions and the Mediterranean Sea (Parin, 1983, 1995).

For fisheries purposes these two species have been treated as one (A. carbo) in Madeira and consequently landing statistics and fisheries research refer to a mixture of these two similar species. It is worthwhile pointing out that the main differences by which A. carbo may be separated from A. intermedius (dorsal fin and vertebral counts) (Nakamura and Parin, 1993; Parin, 1995) are not easy to use in the field and are totally unsuitable for large scale fisheries-purpose identification, on board or at landing sites.

[^0]Several projects and initiatives aimed for the search of new deep-sea species of potential commercial interest in the waters of the Macaronesian archipelagos of the Azores, Madeira and the Canaries have been carried out along the last decade. This gave the opportunity to acquire specimens of Aphanopus in the three regions involved, either from the commercial fishery or from experimental fishing. The need for a more accurate management of this highly important resource, reinforced by an emergent fishery in Portugal mainland (Figueiredo et al., 2003), the Azores and elsewhere in the North Atlantic (Lorance and Dupouy, 2001), also including the Canary region and nearby seamounts, and the findings by Stefanni and Knutsen (2007), raised again the question of the species identification. However, the issue of discriminating the $A$. carbo from $A$. intermedius based on their morphology was not resolved yet. With this contribution the authors aim to characterize morphologically a large sample of specimens of Aphanopus collected in different fishing grounds of the Northeast Atlantic. To have comparable data to previous work, molecular sequences of two mtDNA regions (Control Region and COI) were amplified in these specimens, thus allowing a correct identification of A. carbo and A. intermedius.

## MATERIAL AND METHODS

A series of 145 specimens of Aphanopus spp. were collected in the waters off Sesimbra (mainland Portugal), the islands of the Azores, Madeira, and Canaries; off the coasts of Morocco and Western Sahara (Appendix I, Fig. 1). The specimens from Sesimbra and the Azores were taken randomly from the commercial fishery. In the other localities, specimens were obtained from experimental fishing.

All specimens were measured, weighed and dissected for determination of sex, maturity stage and vertebral counts. A tissue sample was also extracted and preserved in $70 \%$ ethanol. Eighteen measurements were made point to point to the nearest millimetre and follow Nakamura and Parin (1993) with modifications introduced in the present paper (Figs 2, 3 ) and nine counts were made directly. Due to damage to the dorsal fin, it was not always possible to count spines and soft rays separately, although it was possible to count the total number of dorsal fin elements in those specimens. Vertebral counts were divided in total, pre-caudal and caudal vertebrae. Fused vertebrae forming the hypural plate were counted as one. The position of anus and first anal spine in relation to dorsal-fin elements was also noted.

## Methodology used for genetic analysis

All specimens were screened for two mtDNA genes to assign to each specimen the correct identification, using the available sequences of the mtDNA Control Region (CR)


Figure 1. - Map of the study area in the NE Atlantic Ocean showing the locations where specimens of A. carbo and A. intermedius were collected (shaded areas).
from Stefanni and Knutsen (2007) and Cytochrome Oxidase subunit I COI from Stefanni et al. (2009). A total of 138 sequences were aligned for the complete Control Region (CR) (GenBank Accession Nos. EU853865-EU854002) and 144 for the partial Cytochrome Oxidase subunit I (COI) (GenBank Accession Nos. EU854003-EU854146). Although the majority of the specimens had amplified and produced good quality sequences for both genes, in some individuals (7 for the CR and 1 for the COI, see Appendix 1) the PCR amplification was very weak and of poor quality. However, as the individual that did not amplify for the COI was not one of the 7 that did not amplify for the CR, all 145 fish were screened for correct identification.

The thermal cycling profile for the fragment including the CR followed Stefanni and Knutsen (2007), while for COI it followed Stefanni et al. (2009).

All sequences were aligned using Seaview (Galtier et al., 1996) and levels of genetic diversity as well as genetic signatures were estimated using Arlequin 3.0 (Excoffier et


Figure 2. - Schematic drawing of a trichiurid showing body measurements used in the present study (adapted from Nakamura and Parin, 1993). See table I for abbreviations.


Figure 3. - Schematic drawing of head and tail of a trichiurid showing measurements used in the present study (adapted from Nakamura and Parin, 1993). See table I for abbreviations.
al., 2005) implementing the same parameters estimated in Stefanni and Knutsen (2007). All sequences from the CR were aligned with the ones reported by Stefanni and Knutsen (2007) for detection of shared haplotypes and to assign the correct species identification to the specimens used for the morphological work.

## Methodology used for discriminant analysis

Only in 53 specimens of A. carbo (SL 905-1188 mm) and 36 specimens of A. intermedius (SL 852-1345 mm) it was possible to obtain the whole set of measurements and counts (Tab. I), therefore only 89 out of 145 specimens were used for discriminant analyses.

All morphometric variables were first examined for normality (Kolmogorov-Smirnov test) and homogeneity of variances (Levene test), and were log-transformed to statistical analysis if these criteria were not satisfied. Analysis of covariance (ANCOVA) was then used to determine the effect of length (standard or head length) on the magnitude of each shape variable. "Species" was treated as the main factor and length was the covariate. Variables for which "spe-cies-length" interactions were significant ( $\mathrm{p}<0.05$, samples with unequal slopes) were not included in any further
analysis because they could be corrected for length. Those variables found to have samples with equal slopes were corrected using their respective common within-group slope (b) (Bolles and Begg, 2000; Begg et al., 2001; De Vries et al., 2002). The length of reference for body variables was the standard length, whereas for cephalic variables the head length.

Multivariate analysis of variance (MANOVA) was used to test the hypothesis of no difference in morphometric and meristic variables among species. This procedure was explored using a canonical discriminate analysis (CDA). This technique allows to evaluate the differences between groups using several discriminant variables and to predict the ownership to a group. The first step was to carry out a single factor ANOVA to find out which variables discriminate between species, using the $F$ statistics to rank the potential predictors. To avoid multicolinearity, a matrix correlation was obtained and eliminated of CDA analysis those variables with a high correlation and small F-score from ANOVA. Stepwise linear discriminant analysis was used to guide selection of variable sets used in each function. This procedure chooses variables to enter or leave the model on the basis of the significance level of an F-test by ANOVA. Homogeneity of the within-group covariance matrices was tested and either a linear (matrices are homogenous and the pooled matrix is used) or a quadratic (matrices are not homogenous and individual within-group matrices are used) discriminant function was computed (Friedland et al., 1994). Classification efficiency (percent correctly classified) estimates were cross-validated according to the methods of Lachenbruch and Mickey (1968). To establish the bias of the analysis Cohen's kappa ( $火$ ) statistic was used, which estimates the improvement over chance of the percent correct classification rates (Titus et al., 1984). The prior probability of classification was equal for both groups. Junquera and Pérez-Gándaras (1993) and Camacho (1995) indicated that if the number of individuals minus the number of variables is greater than 30 , then the sample can be considered adequate for analysis and it is only necessary to construct one discriminant function. The misclassification rate was assessed by classifying the same number of fish used to form the discriminant analysis database and summing the number of misclassified fish (Reddin et al., 1988). Two CDA analyses were

Table I. - Morphometric and meristic characters of Aphanopus carbo and A. intermedius. PESCPROF specimens were genetically identified. Relationships marked with an asterisk mean data from holotype and three paratypes (from Parin, 1993).

|  |  | Aphanopus carbo |  | Aphanopus intermedius |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | PESCPROF | Nakamura \& Parin (1993) | PESCPROF | Parin (1995) |
|  | Standard length (SL, mm) | 905-1293 (70) | - | 622-1345 (63) | 515-1010 (17) |
|  | Head length (HL, mm) | 174-250 (73) | - | 123-270 (70) | 95.3-212.1 (17) |
| \%SL | Pre-anal length (PAL) | 58.6-64.4 (55) | - | 57.0-63.8 (46) | - |
|  | Pre-first anal spine length (PASL) | 55.6-60.5 (55) | - | 55.2-60.0 (46) | 56.1-58.0* |
|  | Pre-anus length (PANL) | 54.2-59.2 (70) | 55.6-58.8 | 52.7-64.0 (63) | 52.8-55.0* |
|  | Pre-pectoral length (PPL) | 18.3-20.9 (55) | - | 18.6-20.8 (46) | - |
|  | Pre-dorsal length (PDL) | 15.3-18.8 (55) | - | 14.9-18.5 (46) | 16.2-17.8* |
|  | Pre-first dorsal soft ray length (P1DFL) | 50.3-60.2 (54) | - | 50.4-59.2 (40) | 54.8-56.9* |
|  | Maximum body depth (Hmax) | 7.8-13.2 (55) | 7.5-9.3 | 6.9-12.7 (46) | 6.1-8.6 (17) |
|  | Depth of body at level of first anal spine (H1SFA) | 6.0-14.2 (70) | - | 6.0-10.5 (63) | - |
|  | Least depth of caudal peduncle (CPD) | 0.4-0.5 (55) | - | 0.3-0.5 (46) | 0.3-0.4* |
|  | Caudal peduncle length (CPL) | 1.2-2.9 (55) | - | 2.0-4.2 (46) | - |
|  | Head length (HL) | 18.4-22.1 (70) | 19.2-21.3 | 17.9-22.5 (63) | 18.5-21.0 (17) |
| \%HL | Pre-opercular length (POL) | 77.7-82.8 (55) | - | $77.0-83.9$ (46) | - |
|  | Snout length (SNL) | 37.4-49.8 (73) | 40.0-43.5 | 36.7-50.4 (70) | 40.4-43.2 (17) |
|  | Eye diameter (ED) | 16.5-26.8 (73) | 17.2-20.4 | 13.8-24.8 (70) | 17.8-20.1 (17) |
|  | Inter-orbital width (IO) | 13.6-19.2 (73) | - | 11.6-21.7 (70) | 12.3-15.6 (17) |
|  | Maxillary length (ML) | 43.8-51.0 (54) | 45.5-47.6 | 45.6-49.8 (45) | 46.9-49.4 (17) |
|  | Head height (HHt) | 32.3-42.3 (55) | - | 31.4-42.1 (46) | 34.5-35.6* |
|  | Meristic characters |  |  |  |  |
|  | Dorsal-fin spines (DS) | 38-41 (66) | 38-41 | 39-43 (41) | 40-44 (55) |
|  | Dorsal-fin soft rays (DR) | 51-57 (66) | 52-56 | 52-60 (41) | 54-59 (55) |
|  | Total dorsal-fin elements (DT) | 89-96 (70) | 90-96 | 92-102 (60) | 96-101 (55) |
|  | Anal-fin rays (without spines) (AF) | 42-48 (66) | 43-48 | 45-50 (59) | 46-50 (55) |
|  | Pre-caudal vertebrae (PCV) | 40-43 (55) | 40-44 | 43-47 (46) | 44-47 (55) |
|  | Caudal vertebrae (CV) | 55-60 (55) | 55-60 | 56-61 (46) | 57-61 (55) |
|  | Total vertebrae (TV) | 98-101 (55) | 97-100 | 101-105 (46) | 102-107 (55) |

constructed, one using morphometric data only and the other using morphometric and meristic variables together.

## RESULTS

## Morphology and meristics

The morphometric relationships and meristic characters used for identification of the specimens of the two species of Aphanopus studied, which were previously separated based on the genetic results, are given in table I. A comparison with data from the bibliography (Parin, 1983, 1995; Nakamura and Parin, 1993) is also made (Tab. I). Frequency distributions of the nine meristic characters used are also given in table II, in order to show comparatively both range and mode of the different counts. Previously known ranges of most of the characters measured are enlarged for both species. In addition, an overlap of all measurements and counts in both species was found.

## Genetics

On the basis of the sequences of two mtDNA genes, 74 A. carbo and 71 A. intermedius were identified. The complete sequences of the CR were 733 bp long in $A$. carbo and 732 bp long in A. intermedius. The partial sequences of the COI were 668 bp long for both species. From the alignment with the CR dataset from Stefanni and Knutsen (2007) several common haplotypes were found, either as A.carbo or A. intermedius. Within A. carbo group, all new sequences coded as SHc1, SHc3, SHc4, SHc5, Mad15, Can10, Mor17, SHc7, SHc8 and SHc9 (see column H CR in Appendix 1) correspond to the sequences ShP345, Az22, SN7, FD1, ShM790, ShA374, ShA380, ShP190, ShS 130 and SN8 obtained by Stefanni and Knutsen (2007). On the other hand, within A. intermedius group, the common haplotypes between the two datasets are all new sequences coded as SHi1, SHi2, SHi3, Azo23, SHi4, SHi6 and Mad25 (see column H CR in Appendix 1) and correspond to the sequences of the dataset published by Stefanni and Knutsen (2007) as

Table II. - Comparison of meristic characters of Aphanopus carbo and A. intermedius, based on genetic identification.

|  | Dorsal-fin spines |  |  |  |  |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 38 | 39 | 40 |  | 42 | 43 | 44 | n |
| A. carbo | 5 | 18 | 31 |  | - | - | - | 66 |
| A.intermedius | - | 1 | 13 |  | 8 | 2 | - | 41 |


|  | Dorsal-fin rays |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 51 | 52 | 53 | 55 | 56 | 57 | 58 | 59 | 60 | n |
| A. carbo | 2 | 12 | 19 | 10 | 4 | 1 | - | - | - | 66 |
| A. intermedius | - | 1 | - | 13 | 9 | 6 | 3 | 1 | 1 | 41 |


|  | Dorsal-fin elements (total) |  |  |  |  |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 89 | 90 | 91 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 | 101 | 102 | n |
| A. carbo | 1 | 2 | 2 | 22 | 16 | 12 | 3 | - | - | - | - | - | - | 70 |
| A. intermedius | - | - | - | - | - | 11 | 14 | 14 | 11 | 3 | 4 | 1 | 1 | 60 |


|  | Anal-fin rays |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 42 | 43 | 44 | 46 | 47 | 48 | 49 | 50 | n |
| A. carbo | 1 | 6 | 11 | 10 | 7 | 7 | - | - | 66 |
| A. intermedius | - | - | - | 12 | 18 | 13 | 11 | 3 | 59 |


|  | Pre-caudal vertebrae |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 40 | 41 | 42 | 44 | 45 | 46 | 47 | n |
| A. carbo | 11 | 22 | 15 | - | - | - | - | 55 |
| A. intermedius | - | - | - | 9 | 11 | 17 | 2 | 46 |


|  | Caudal vertebrae |  |  |  |  |  |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 55 | 56 | 57 |  | 59 | 60 | 61 | n |  |
| A. carbo | 3 | 4 | 25 |  | 5 | 1 | - | 55 |  |
| A. intermedius | - | 10 | 11 |  | 4 | 8 | 1 | 46 |  |


|  | Total vertebrae |  |  |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 97 | 98 | 99 | 101 | 102 | 103 | 104 | 105 | 106 | 107 | 108 | n |
| A. carbo | - | 29 | 15 | 1 | - | - | - | - | - | - | - | 55 |
| A. intermedius | - | - | - | 1 | 18 | 18 | 8 | 1 | - | - | - | 46 |


|  | Position of anal fin spines in relation to dorsal-fin spines and soft rays |  |  |  |  |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| over | Last spine | $1^{\text {st }}$ ray | $2^{\text {nd }}$ ray | $3^{\text {rd }}$ ray | $4^{\text {th }}$ ray | $5^{\text {th }}$ ray | $6^{\text {th }}$ ray | n |
| A.carbo |  | 5 | 25 | 12 | 11 |  | 1 | 54 |
| A.intermedius | 2 | 1 | 7 | 6 | 13 | 8 | 1 | 38 |


|  |  | Position of anus in relation to dorsal-fin spines |  |  |  |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |  |  |  |
|  | over | Penultimate spine | $1^{\text {st }}$ ray | $2^{\text {nd }}$ ray | $3^{\text {rd }}$ ray | $4^{\text {th }}$ ray | $5^{\text {th }}$ ray |  |
| n |  |  |  |  |  |  |  |  |
| A.carbo | 2 | 6 | 33 | 4 |  |  | 54 |  |

ShA999, ShA412, Az21, Az6, Az75, ShA129 and ShA334, respectively.

The 70 CR sequences of $A$. carbo defined 46 haplotypes, 37 of which were represented by a single specimen while the remaining sequences were shared among 9 haplotypes
(Appendix 1). The nucleotide composition was estimated to be $\mathrm{C}=22.9 \%, \mathrm{~T}=31.1 \%, \mathrm{~A}=31.6 \%$ and $\mathrm{G}=14.4 \%$ and the transition/transvertion ratio of 2.23 . The 46 haplotypes described an overall haplotypic diversity of $4.7850 \pm 2.3662$ and nucleotide diversity of $0.0065 \pm 0.0036$, and they con-

Figure 4. - Neighbour-joining tree constructed from sequences of the COI using PAUP (Swofford, 1999) software and implementing the HKY (Hasegawa et al., 1985) nucleotide substitution model with no invariable sites and equal rate. Numbers above internal branches indicate bootstrap values out of 1000 replicates (only if greater than $50 \%$ ). $\mathrm{AB} 205442=$ Cubiceps paradoxus sequence used as outgroup. Codes for OTU's are described in appendix 1 .

tained 41 polymorphic sites. On the other hand, the 68 CR sequences of $A$. intermedius defined 28 haplotypes, 22 of which were unique and the other 6 were shared with the other 36 specimens (Appendix 1). The nucleotide composition was estimated to be $\mathrm{C}=23.1 \%, \mathrm{~T}=30.6 \%, \mathrm{~A}=31.4 \%$ and $\mathrm{G}=14.9 \%$ and the transition/transvertion ratio of 7 . The 28 haplotypes described an overall haplotypic diversity of $1.5812 \pm 0.9526$ and nucleotide diversity of $0.0022 \pm 0.0014$, and they contained 23 the polymorphic sites.

The more conservative and shorter fragment of the COI identified 10 haplotypes in $A$. carbo, 7 of which were represented by a single fish and 3 were shared with the other specimens (Appendix 1).

The nucleotide composition was estimated to be $\mathrm{C}=29.9 \%, \mathrm{~T}=28.7 \%, \mathrm{~A}=22.5 \%$ and $\mathrm{G}=18.9 \%$ and the transition/transvertion ratio of 1.4. The 10 haplotypes described an overall haplotypic diversity of $0.4527 \pm 0.4054$ and nucleotide diversity of $0.0007 \pm 0.0007$, and they contained 12 the polymorphic sites.

In A. intermedius, this fragment was characterized by 14
haplotypes, 12 of which were uniquely represented and 2 shared with the other specimens (Appendix 1).

The nucleotide composition was estimated to be $\mathrm{C}=29.5 \%, \mathrm{~T}=28.9 \%, \mathrm{~A}=22.6 \%$ and $\mathrm{G}=19.0 \%$ and the transition/transvertion ratio of 1.6 . The 14 haplotypes described an overall haplotypic diversity of $0.5614 \pm 0.4641$ and nucleotide diversity of $0.0008 \pm 0.0008$, and they contained 12 the polymorphic sites.

The corrected sequence divergence, the algorithm that compensates for the average number of pairwise differences between and within the two groups, one represented by A. carbo and the other by A. intermedius, was estimated to be $23.40 \%$ for CR and $6.86 \%$ for COI. Regarding the sequence divergence within each group, the values for CR were $4.78 \%$ in A. carbo and $1.58 \%$ in A. intermedius, while for the partial COI were $0.45 \%$ in A. carbo and $0.56 \%$ in A. intermedius. Highly significant ( $\mathrm{p}<0.05$ ) values of $\Phi_{\mathrm{ST}}(0.8795$ for CR and 0.9314 for COI) put in evidence a strong genetic partitioning between the species. A phylogenetic tree for the COI sequences is shown in figure 4 .

| Variables | Length x species |  | Length |  | $b$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | $F$ | p | $F$ | Significant |  |
| Pre-anal length (PAL) ${ }^{1}$ | 0.040 | 0.842 | 1203.891 | 0.000 | 0.624 |
| Pre-first anal spine length (PASL) ${ }^{1}$ | 0.188 | 0.665 | 2238.472 | 0.000 | 0.601 |
| Pre-anus length (PANL) ${ }^{1}$ | 0.070 | 0.791 | 1022.289 | 0.000 | 0.559 |
| Pre-pectoral length (PPL) ${ }^{1}$ | 2.757 | 0.101 | 533.955 | 0.000 | 0.194 |
| Pre-dorsal length (PDL) ${ }^{1}$ | 1.219 | 0.273 | 306.477 | 0.000 | 0.168 |
| Pre-first dorsal soft ray length (P1DFL) ${ }^{1}$ | 2.138 | 0.147 | 548.496 | 0.000 | 0.564 |
| Maximum body depth (Hmax) ${ }^{1}$ | 0.381 | 0.539 | 66.352 | 0.000 | 0.138 |
| Depth of body at level of first anal spine (H1SFA) ${ }^{1}$ | 2.902 | 0.092 | 150.996 | 0.000 | 0.064 |
| Caudal peduncle length (CPL) ${ }^{1}$ | 0.338 | 0.563 | 15.361 | 0.000 | 0.020 |
| Pre-opercular length (POL) ${ }^{2}$ | 2.044 | 0.157 | 2016.868 | 0.000 | 0.780 |
| Snout length (SNL) ${ }^{2}$ | 1.601 | 0.209 | 275.794 | 0.000 | 0.387 |
| Eye diameter (ED) ${ }^{2}$ | 0.035 | 0.852 | 95.897 | 0.000 | 0.259 |
| Inter-orbital width (IO) ${ }^{2}$ | 8.145 | 0.005 | - | - | - |
| Maxillary length (ML) ${ }^{2}$ | 6.777 | 0.011 | - | - | - |
| Head height (HHt) ${ }^{2}$ | 10.400 | 0.002 | - | - | - |

Table III. - Morphometric variables significantly correlated with length, and the corresponding regression coefficients (b) required to standardizing the variables for length. Standardized with respect to standard length ${ }^{1}$ or head length ${ }^{2}$.

| Variables | Wilks' lambda | $F$ | df1 | df2 | P |
| :--- | :---: | ---: | ---: | ---: | ---: |
| Pre-anal length (PAL) | 0.924 | 7.144 | 1 | 87 | 0.009 |
| Pre-first anal spine length (PASL) | 0.958 | 3.817 | 1 | 87 | 0.054 |
| Pre-anus length (PANL) | 0.975 | 2.185 | 1 | 87 | 0.143 |
| Pre-pectoral length (PPL) | 1.000 | 0.024 | 1 | 87 | 0.877 |
| Pre-dorsal length (PDL) | 0.981 | 1.674 | 1 | 87 | 0.199 |
| Pre-first dorsal soft ray length (P1DFL) | 0.945 | 5.103 | 1 | 87 | 0.026 |
| Maximum body depth (Hmax) | 0.990 | 0.856 | 1 | 87 | 0.357 |
| Depth of body at level of first anal spine (H1SFA) | 0.767 | 26.470 | 1 | 87 | $<0.001$ |
| Caudal peduncle length (CPL) | 0.925 | 7.047 | 1 | 87 | 0.009 |
| Pre-opercular length (POL) | 0.999 | 0.115 | 1 | 87 | 0.735 |
| Snout length (SNL) | 0.996 | 0.324 | 1 | 87 | 0.570 |
| Eye diameter (ED) | 0.907 | 8.946 | 1 | 87 | 0.004 |
| Inter-orbital width (IO) | 0.982 | 1.551 | 1 | 87 | 0.216 |
| Maxillary length (ML) | 1.000 | 0.043 | 1 | 87 | 0.837 |
| Head height (HHt) | 0.966 | 3.032 | 1 | 87 | 0.085 |
| Dorsal-fin spines (DS) | 0.713 | 34.966 | 1 | 87 | $<0.001$ |
| Dorsal-fin rays (DR) | 0.662 | 44.403 | 1 | 87 | $<0.001$ |
| Dorsal-fin elements (total) (DT) | 0.468 | 98.957 | 1 | 87 | $<0.001$ |
| Anus in relation to dorsal fin spines (ANDF) | 0.946 | 4.952 | 1 | 87 | 0.029 |
| Anal-fin rays (AF) | 0.578 | 63.525 | 1 | 87 | $<0.001$ |
| Anal fin spines in relation to dorsal fin spines and | 0.891 | 10.620 | 1 | 87 | 0.002 |
| soft rays (ASDF) | 0.237 | 280.098 | 1 | 87 | $<0.001$ |
| Pre-caudal vertebrae (PCV) | 1.418 | 1 | 87 | 0.237 |  |
| Caudal vertebrae (CV) | 0.984 | 553.803 | 1 | 87 | $<0.001$ |
| Total vertebrae (TV) | 0.136 |  |  |  |  |

Table IV. - Results of ANOVA to test morphometric and meristic relationships between species to show variables with highest $F$ statistics.

## Discriminant analyses

The CPL variable was log-transformed to correct nonnormality, whereas CPD was eliminated of the study due to the impossibility to change the variance heterogeneity.

ANCOVA detected significant "species-length" interac-
tions for IOD, SML and HCL being eliminated from posterior analysis. All the remaining variables were significantly correlated with length and therefore were corrected for variable length with their respective common within-group slope (Tab. III). Morphometric and meristic variables, with
Table V. - Correlation matrix among morphometric and meristic variables to select variables with less relation (<0.400).

| Variables | PAL | PASL | PANL | PPL | PDL | P1DFL | Hmax | H1SFA | CPL | POL | SNL | ED | DS | DF | DT | ANDF | AF | ASDF | PCV | CV | TV |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Pre-anal length (PAL) | 1.000 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Pre-first anal spine length (PASL) | 0.767 | 1.000 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Pre-anus length (PANL) | 0.511 | 0.587 | 1.000 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Pre-pectoral length (PPL) | 0.189 | 0.397 | 0.358 | 1.000 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Pre-dorsal length (PDL) | 0.390 | 0.553 | 0.386 | 0.424 | 1.000 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Pre-first dorsal soft ray length (P1DFL) | 0.243 | 0.343 | 0.249 | 0.112 | 0.250 | 1.000 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Maximum body depth (Hmax) | 0.274 | 0.335 | 0.360 | 0.311 | 0.379 | 0.219 | 1.000 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Depth of body at level of first anal spine (H1SFA) | 0.011 | 0.133 | -0.014 | -0.028 | 0.281 | 0.094 | 0.216 | 1.000 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Caudal peduncle length (CPL) | -0.104 | -0.147 | -0.021 | -0.130 | -0.191 | 0.029 | -0.114 | 0.124 | 1.000 |  |  |  |  |  |  |  |  |  |  |  |  |
| Pre-opercular length (POL) | -0.041 | -0.005 | 0.117 | -0.103 | 0.150 | -0.045 | -0.007 | 0.322 | 0.032 | 1.000 |  |  |  |  |  |  |  |  |  |  |  |
| Snout length (SNL) | 0.101 | 0.027 | 0.063 | -0.178 | 0.183 | -0.049 | -0.062 | 0.250 | 0.275 | 0.269 | 1.000 |  |  |  |  |  |  |  |  |  |  |
| Eye diameter (ED) | -0.089 | 0.001 | 0.029 | 0.271 | -0.028 | 0.044 | 0.104 | 0.023 | -0.088 | -0.078 | -0.289 | 1.000 |  |  |  |  |  |  |  |  |  |
| Dorsal-fin spines (DS) | 0.256 | 0.294 | 0.203 | -0.104 | 0.101 | 0.225 | 0.083 | 0.036 | -0.034 | -0.031 | 0.006 | -0.105 | 1.000 |  |  |  |  |  |  |  |  |
| Dorsal-fin rays (DF) | -0.366 | -0.319 | -0.402 | 0.055 | -0.340 | -0.234 | -0.173 | -0.211 | -0.228 | -0.034 | -0.280 | 0.048 | -0.237 | 1.000 |  |  |  |  |  |  |  |
| Dorsal-fin elements (total) (DT) | -0.193 | -0.126 | -0.259 | -0.026 | -0.263 | -0.092 | -0.110 | -0.176 | -0.237 | -0.040 | -0.259 | -0.028 | 0.372 | 0.811 | 1.000 |  |  |  |  |  |  |
| Anus in relation to dorsal fin spines (ANDF) | 0.262 | 0.334 | 0.051 | 0.029 | 0.186 | 0.134 | 0.210 | 0.198 | -0.173 | -0.052 | -0.033 | -0.058 | 0.401 | -0.179 | 0.078 | 1.000 |  |  |  |  |  |
| Anal-fin rays (AF) | -0.154 | -0.051 | 0.031 | 0.294 | -0.164 | -0.050 | -0.122 | -0.131 | -0.217 | 0.067 | -0.269 | 0.170 | -0.126 | 0.282 | 0.190 | -0.093 | 1.000 |  |  |  |  |
| Anal-fin spines in relation to dor-sal-fin spines and soft rays (ASDF) | -0.123 | -0.113 | 0.059 | 0.163 | -0.009 | -0.169 | 0.022 | -0.354 | -0.005 | 0.017 | -0.124 | 0.074 | -0.092 | 0.104 | 0.036 | -0.450 | 0.037 | 1.000 |  |  |  |
| Pre-caudal vertebrae (PCV) | 0.043 | 0.116 | 0.161 | -0.055 | -0.184 | -0.018 | -0.077 | -0.172 | 0.140 | -0.014 | 0.050 | -0.023 | 0.193 | 0.005 | 0.121 | -0.139 | -0.126 | 0.228 | 1.000 |  |  |
| Caudal vertebrae (CV) | -0.130 | -0.185 | -0.196 | -0.103 | 0.035 | -0.065 | -0.065 | 0.126 | -0.114 | 0.047 | 0.024 | 0.098 | -0.127 | 0.067 | -0.008 | 0.024 | 0.136 | -0.287 | -0.760 | 1.000 |  |
| Total vertebrae (TV) | -0.142 | -0.130 | -0.090 | -0.229 | -0.186 | -0.122 | -0.198 | -0.032 | 0.008 | 0.053 | 0.101 | 0.120 | 0.058 | 0.107 | 0.144 | -0.144 | 0.044 | -0.141 | 0.141 | 0.536 | 1.000 |


| Step | Variables introduced | Wilks' lambda |  |  |  | $F$ exactly |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Statistic | df1 | df2 | df3 | Statistic | df1 | df2 | Significant |
| Morphometric and meristic data |  |  |  |  |  |  |  |  |  |
| 1 | Total vertebrae (TV) | 0.136 | 1 | 1 | 87 | 553.8031 | 1 | 87 | 0.000 |
| 2 | Pre-caudal vertebrae (PCV) | 0.106 | 2 | 1 | 87 | 364.4042 | 2 | 86 | 0.000 |
| 3 | Anal-fin rays (AF) | 0.096 | 3 | 1 | 87 | 265.5407 | 3 | 85 | 0.000 |
| 4 | Eye diameter (ED) | 0.092 | 4 | 1 | 87 | 208.0681 | 4 | 84 | 0.000 |
| Morphometric data |  |  |  |  |  |  |  |  |  |
| 1 | Depth of body at level of first anal spine (H1SFA) | 0.767 | 1 | 1 | 87 | 26.4696 | 1 | 87 | 0.000 |
| 2 | Caudal peduncle length (CPL) | 0.699 | 2 | 1 | 87 | 18.5286 | 2 | 86 | 0.000 |
| 3 | Eye diameter (ED) | 0.663 | 3 | 1 | 87 | 14.3782 | 3 | 85 | 0.000 |
| 4 | Pre-anal length (PAL) | 0.632 | 4 | 1 | 87 | 12.2245 | 4 | 84 | 0.000 |

Table VI. - Order of variables and value of Wilks' statistics obtained during the stepwise procedure of discriminant analysis.

Table VII. - Results of discriminant functions for identifying species.

| Actual group | Predicted group membership |  | Correct identification | Misidentification <br> $(\%)$ | Cohen's <br> kappa |
| :--- | :---: | :---: | :---: | :---: | :---: |
| Morphometric and meristic data |  |  | 100.0 | 0.0 | 1.000 |
| A. carbo | $53(100 \%)$ | $0(0 \%)$ |  |  |  |
| A. intermedius | $0(0 \%)$ | $36(100 \%)$ |  | 15.7 | 0.680 |
| Morphometric data |  |  | 84.3 |  |  |
| A. carbo | $46(86.8 \%)$ | $7(13.2 \%)$ |  |  |  |
| A. intermedius | $7(19.4 \%)$ | $29(80.6 \%)$ |  |  |  |

the exception of DF and ANDF, presented highest F-score (Tab. IV) and the lowest correlation (Tab. V) among them.

The first canonical discriminant analysis (CDA) was constructed with morphometric and meristic data, the latter being the most important. Total number of vertebrae was selected as first meristic variable and among morphometric variables only eye diameter was included in the function (Tab. VI). Canonical correlation index was 0.953 with $100 \%$ classification success (Tab. VII). The second CDA was calculated using only morphometric variables. Depth of body at level of first anal spine was the variable showing highest differences between species (Tab. VI). The canonical correlation index obtained was 0.607 with $86.4 \%$ classification success and Cohen's $x$ indicated a classification efficiency of $68 \%$ (Tab. VII).

## DISCUSSION AND CONCLUSIONS

The genetic structure obtained from the two mtDNA markers supports the findings reported by Stefanni and Knutsen (2007) therefore confirming the validity of both species (A. carbo and A. intermedius). The current work also provides more details on the geographical distribution of the two species. It is confirmed that the only species that reaches mainland Europe is A. carbo and extending southwards to at least $27^{\circ} \mathrm{N}$, off the Western Sahara coast. This southern limit
of distribution of A. carbo was until present set with certitude to about $30^{\circ} \mathrm{N}$ (Nakamura and Parin, 1993). Concerning A. intermedius, it has been found living in sympatry in the islands of the Azores, Madeira and the Canaries and off the coasts of Morocco and Western Sahara, therefore contributing to the clarification of the northern limit of its distribution, as already proposed by Nakamura and Parin (1993).

The values of genetic diversity (at intra- and inter-specific levels) are of similar order of magnitude as reported in Stefanni and Knutsen (2007). Pairwise values of $\Phi_{\text {ST }}$ for the two species indicate high level of divergence and phylogenetic trees constructed from the sequence alignment of the two mtDNA markers propose only two monophyletic clades. Bootstrap supports are very strong between the two phylogroups but very weak within either of the two (Fig. 4), suggesting the presence of single populations in the NE Atlantic for both species. The calculation of the divergence time between the two species based on the COI sequences are supporting a recent speciation event between A. carbo and A.intermedius as reported in Stefanni and Knutsen (2007).

For most morphometric relationships and meristic characters, data obtained have enlarged their previously published ranges for each species (Tab. I). These new ranges contributed in most cases to increase the overlap between the two species and therefore reinforcing their closeness. This enlargement may be due to the size of the sample studied (74 A. carbo and 71 A. intermedius), apparently the largest used

Figure 5. - Aphanopus intermedius Parin, 1983 (MMF 39099).

to date for a comparative taxonomical study of these species. This overlap might be related to the recent evolutionary split of the two species, Stefanni and Knutsen (2007) estimated a divergence time of 400 KY , which has not fully determined marked phenotypic differences between them. Another equally valuable explanation might be due to the fact that genetic differences were determined based on mtDNA genes and, as it is commonly known, mitochondrial DNA is transferred to the following generations by mothers, therefore if hybrids are present (fish with "intermediate" features) they are not genetically detected. Detection of presence of interbreeding between the two species is an undergoing work for which specific microsatellites have been recently designed (Knutsen et al., 2009) and the screening is under process.

It was not possible to find a single meristic or morphometric character allowing de per se the separation of A. carbo from A. intermedius, as it was already found by Parin (1983, 1995). The present study also reveals that all body proportions and counts show a more or less extensive overlap, this being the smallest in total vertebrae ( 2 specimens) and pre-caudal vertebrae (14) counts. Due to these overlaps 19 specimens of $A$. carbo ( $26.1 \%$ ) and 25 specimens of $A$. intermedius ( $35.7 \%$ ) could not be correctly identified in a classical taxonomical way, using morphometric and meristic characters only. A full separation of the two species could only be obtained using the combination of these characters in a numerical taxonomic approach (Tab. VII). These morphological variations might be correlated to the genotypic expression of the genes passed on by both parents.

The differences found between meristic and morphometric data obtained in the present study and those previously published (Parin, 1983, 1995; Nakamura and Parin, 1993) make worthwhile giving a new characterization of the two species:

Aphanopus carbo is characterized by the following characters: dorsal-fin spines 38-41; dorsal-fin soft rays 51-57; total dorsal-fin elements 89-96; anal-fin rays II+42-48; precaudal vertebrae 40-44; caudal vertebrae 55-60; total vertebrae 97-101. In percentage of SL: head length 18.4-22.1; pre-dorsal length 15.3-18.8; pre-first dorsal soft ray length 50.3-60.2; pre-anal length 58.6-64.4; pre-first anal spine length 55.6-60.5; pre-anus length 54.2-59.2; pre-pectoral length 18.3-20.9; maximum body depth 7.5-13.2; depth of body at level of first anal spine 6.0-14.2; least depth of caudal peduncle 0.4-0.5; length of caudal peduncle 1.2-2.9. In percentage of HL: snout length: 37.4-49.8; eye diameter 16.5-26.8; interorbital width 13.6-19.2; upper jaw length
43.8-51.0; pre-opercular length 77.7-82.8; head height 32.342.3.

Aphanopus intermedius (Fig. 5) is characterized by the following characters: dorsal-fin spines 39-44; dorsal-fin soft rays 52-60; total dorsal-fin elements 92-102; anal-fin rays II+45-50; pre-caudal vertebrae 43-47; caudal vertebrae 56-61; total vertebrae 101-107. In percentage of SL: head length 17.9-22.5; pre-dorsal length 14.9-18.5; pre-first dorsal soft ray length 50.4-59.2; pre-anal length $57.0-63.8$; prefirst anal spine length 55.2-60.0; pre-anus length 52.7-64.0; pre-pectoral length 18.6-20.8; maximum body depth 6.112.7; depth of body at level of first anal spine 6.0-10.5; least depth of caudal peduncle 0.3-0.5; length of caudal peduncle 2.0-4.2. In percentage of HL: snout length: 36.7-50.4; eye diameter 13.8-24.8; interorbital width 11.6-21.7; upper jaw length 45.6-49.8; pre-opercular length 77.0-83.9; head height 31.4-42.1.

Acknowledgements. - This study was done in the framework of the research project PESCPROF 3 (ref.: 05/MAC/4.2/M11, cofinanced by the EU Interreg III-B programme) and pursued in collaboration with the projects $D E E C O N$ (European Science Foundation, under the EUROCORES programme, proposal No 06-Euro-DEEP-FP-008) and MarBEF (Network of Excellence: "Marine Biodiversity and Ecosystem Functioning" - contract nr. GOCE-CT-2003-505446). S. S. is a researcher contracted by IMAR/DOP under the "Ciência 2007" recruitment funded by FCT (Foundation for Science and Technology, Portugal) with co-funding of POCI 2010 (Portugal) and E. S. Fund (EU). IMAR/DOP is funded through the FCT pluri-annual and programmatic funding scheme as research unit \#531 and associate laboratory \#9. Moroccan and Western Saharan specimens were caught in the framework of two Spanish pilot actions of experimental fishing off the northwest Africa (projects RAI-AP-36/2005 and RAI-AP-37/2005) co-funded by the EU. The authors are indebted to Helena Encarnação, from the Museu Municipal do Funchal (História Natural), for the accurate scientific illustration of $A$. intermedius presented in this paper.

## REFERENCES

BEGG G.A., OVERHOLTZ W.J. \& MUNROE N.J., 2001. - The use of internal otolith morphometrics for identification of haddock (Melanogrammus aeglefinus) stocks on Georges Bank. Fish. Bull., 99: 1-14.
BOLLES K.L. \& BEGG G.A., 2000. - Distinction between silver hake (Merluccius bilinearis) stocks in U.S. waters of the northwest Atlantic based on whole otolith morphometrics. Fish. Bull., 98: 451-462.
CAMACHO J., 1995. - Análisis multivariado con SPSS/PC+. 348 p. Barcelona: Ediciones Universitarias de Barcelona.

## Biscoito et al.

DE VRIES D.A., GRIMES C.B. \& PRAGER M.H., 2002. - Using otolith shape analysis to distinguish eastern Gulf of Mexico and Atlantic Ocean stocks of king mackerel. Fish. Res., 57: 51-62.
EXCOFFIER L., LAVAL G. \& SCHNEIDER S., 2005. - Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinf. Online, 1: 47-50.
FIGUEIREDO I., BORDALO-MACHADO P., REIS S., SENACARVALHO D., BLASDALE T., NEWTON A. \& GORDO L.S., 2003. - Observations on the reproductive cycle of the black scabbardfish (Aphanopus carbo Lowe, 1839) in the NE Atlantic. ICES J. Mar. Sci., 60: 774-779.
FRIEDLAND K.D., ESTEVES C., HANSEN L.P. \& LUND R.A., 1994. - Discrimination of Norwegian farmed, ranched and wild-origin Atlantic salmon, Salmo salar L., by image processing. Fish. Manag. Ecol., 1: 117-128.
GALTIER N., GOUY M. \& GAUTIER C., 1996. - SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny. Comp. Appl. Biosci., 12: 543-548.
HASEGAWA M., KISHINO H. \& YANO T.A., 1985. - Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol., 22: 160-174.
JUNQUERA S. \& PEREZ-GÁNDARAS G., 1993. - Population diversity in Bay of Biscay anchovy (Engraulis encrasicolus, L. 1758) as revealed by multivariate analysis of morphometric and meristic characters. ICES J. Mar. Sci., 50: 383-391.
KNUTSEN H., CATARINO D., SANNÆS H. \& STEFANNI S., 2009. - Development of eleven microsatellite loci in the deepsea black scabbardfish (Aphanopus carbo). Conserv. Genet. Resour., 1: 89-92.
LACHENBRUCH P. \& MICKEY R.M., 1968. - Estimation of error rates in discriminant analysis. Technometrics, 10: 1-11.
LORANCE P. \& DUPOUY H., 2001. - CPUE abundance indices of the main target species of the French deep-water fishery in ICES Sub-areas V-VII. Fish. Res., 51: 137-149.
MAUL G.E., 1950. - A espada preta. Publ. Liga Prot. Nat., Lisboa, 4: 1-10.

NAKAMURA I. \& PARIN N.V., 1993. - FAO Species Catalogue. Vol. 15. Snake mackerels and cutlassfishes of the world (Families Gempylidae and Trichiuridae). An annotated and illustrated catalogue of the snake mackerels, snoeks, escolars, gemfishes, sackfishes, domine, oilfish, cutlassfishes, scabbardfishes, hairtails, and frostfishes known to date. FAO Fish. Synop., No. 125, Vol. 15, 136 p., 200 figs. Rome.
PARIN N.V., 1983. - Aphanopus mikhailini sp. n. and A. intermedius sp. n. (Trichiuridae, Perciformes) two new scabbardfishes from the temperate waters of the southern hemisphere and the tropical Atlantic. J. Ichthyol., 23(3): 1-12.
PARIN N.V., 1995. - Three new species and new records of cutlass fishes of the genus Aphanopus (Trichiuridae). J. Ichthyol., 35(2): 128-138.
REDDIN D.G., STANSBURY D.E. \& SHORT P.B., 1988. - Continent of origin of Atlantic salmon (Salmo salar L.) at West Greenland. J. Cons. Int. Expl.Mer, 44: 180-188.
STEFANNI S. \& KNUTSEN H., 2007. - Phylogeography and demographic history of the deep-sea fish Aphanopus carbo (Lowe, 1839) in the NE Atlantic: Vicariance followed by secondary contact or speciation? Mol. Phyl. Evol., 42, 38-46.
STEFANNI S., BETTENCOURT R., KNUTSEN H. \& MENEZES G., 2009. - Rapid polymerase chain reaction-restriction fragment length polymorphism method for discrimination of the two Atlantic cryptic deep-sea species of scabbardfish. Mol. Ecol.Res., 9: 528-530.
SWOFFORD D.L., 1999. - PAUP*: Phylogenetic Analysis Using Parsimony (and Other Methods), version 4.0b. Sunderland, Massachusetts: Sinauer Associates.
TITUS U., MOSHER J.A. \& WILLIAMS B.K., 1984. - Chancecorrected classification for use in discriminant analysis: ecological applications. Am. Mid. Nat., 111: 1-7.

Reçu le 25 février 2010.
Accepté pour publication le 3 février 2011.

Appendix I. - List of specimens of Aphanopus spp. genetically identified and used in the present study. H CR and H COI represent the codes adopted for unique and shared (SH) haplotypes for control region and Cytochrome Oxidase subunit I, respectively. Note: Specimens marked with an asterisk were not used in the statistical analyses due to lack of measurement of one or more meristic or morphometric characteristics.

| Aphanopus carbo |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | ID Code | Sex | SL | Locality | Method of collection | HCR | H COI |
|  | AZO02 | M | 1150 | Azores | Drifting mid-water longline | Azo2 | SHc1 |
|  | AZO11 | F | 1134 | Azores | Drifting mid-water longline | SHc1 | SHc1 |
|  | AZO21 | M | 1047 | Azores | Drifting mid-water longline | SHc2 | SHc2 |
| * | AphCar-142-CI-a | M | 1046 | Canary Is. | Drifting mid-water longline | SHc1 | SHc1 |
| * | AphCar-143-CI-a | M | 1008 | Canary Is. | Drifting mid-water longline | Can2 | SHc1 |
| * | AphCar-144-CI-a | F | 1162 | Canary Is. | Drifting mid-water longline | SHc6 | SHc1 |
| * | AphCar-145-CI-a | M | 1100 | Canary Is. | Drifting mid-water longline | SHc1 | SHc1 |
| * | AphCar-146-CI-a | M | 1065 | Canary Is. | Drifting mid-water longline | SHc2 | SHc3 |
| * | AphCar-147-CI-a | M | 1115 | Canary Is. | Drifting mid-water longline | Can6 | SHc1 |
| * | AphCar-148-CI-a | M | 1087 | Canary Is. | Drifting mid-water longline | Can7 | SHc 1 |
| * | AphCar-151-CI-a | M | 1122 | Canary Is. | Drifting mid-water longline | Can10 | SHc1 |
| * | AphCar-152-CI-a | M | 1070 | Canary Is. | Drifting mid-water longline | Can11 | SHc1 |


|  | Aphanopus carbo |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | ID Code | Sex | SL | Locality | Method of collection | HCR | H COI |
| * | AphCar-153-CI-a | M | 1081 | Canary Is. | Drifting mid-water longline | Can12 | Can12 |
| * | AphCar-156-CI-a | F | 1194 | Canary Is. | Drifting mid-water longline | SHc1 | SHc1 |
|  | AphCar-162-CI-a | n. | n.a. | Canary Is. | Drifting mid-water longline | - | SHc1 |
|  | AphCar-164-CI-a | n.a. | n. | Canary Is. | Drifting mid-water longline | Can23 | SHc1 |
|  | AphCar-165-CI-a | n.a. | n.a. | Canary Is. | Drifting mid-water longline | Can24 | SHc2 |
|  | AphCar-166-CI-a | M | 1093 | Canary Is. | Drifting mid-water longline | SHc1 | SHc1 |
|  | AphCar-167-CI-a | M | 1041 | Canary Is. | Drifting mid-water longline | SHc1 | SHc1 |
|  | AphCar-168-CI-a | F | 1081 | Canary Is. | Drifting mid-water longline | Can27 | SHc1 |
|  | AphCar-169-CI-a | F | 1023 | Canary Is. | Drifting mid-water longline | Can28 | SHc1 |
|  | AphCar-170-CI-a | M | 1100 | Canary Is. | Drifting mid-water longline | SHc1 | SHc1 |
| * | AphCar-171-CI-a | n.a. | 1072 | Canary Is. | Drifting mid-water longline | Can30 | SHc1 |
|  | MAD01 | M | 1084 | Madeira | Drifting mid-water longline | SHc 1 | SHc1 |
|  | MAD02 | M | 1005 | Madeira | Drifting mid-water longline | Mad2 | SHc1 |
|  | MAD03 | F | 1071 | Madeira | Drifting mid-water longline | SHc3 | Mad3 |
| * | MAD04 | n.a. | n.a. | Madeira | Drifting mid-water longline | Mad4 | SHc1 |
|  | MAD05 | F | 1156 | Madeira | Drifting mid-water longline | SHc4 | SHc1 |
|  | MAD06 | M | 1115 | Madeira | Drifting mid-water longline | Mad6 | SHc1 |
| * | MAD7 | .a. | n.a. | Madeira | Drifting mid-water longline | SHc1 | SHc1 |
|  | MAD08 | M | 1002 | Madeira | Drifting mid-water longline | SHc5 | SHc1 |
|  | MAD10 | M | 1028 | Madeira | Drifting mid-water longline | Mad10 | SHc3 |
| * | MAD12 | F | 1293 | Madeira | Drifting mid-water longline | SHc4 | SHc1 |
|  | MAD15 | M | 1029 | Madeira | Drifting mid-water longline | Mad15 | SHc1 |
|  | MAD17 | F | 1296 | Madeira | Drifting mid-water longline | Mad17 | SHc1 |
|  | MAD20 | F | 1345 | Madeira | Drifting mid-water longline | Mad20 | SHc1 |
|  | MAD21 | M | 1092 | Madeira | Drifting mid-water longline | Mad21 | SHc1 |
|  | MAD22 | M | 1080 | Madeira | Drifting mid-water longline | Mad22 | SHc1 |
|  | MAD24 | F | 1289 | Madeira | Drifting mid-water longline |  | Mad24 |
| * | MAD26 | n.a. | n.a. | Madeira | Drifting mid-water longline | Mad26 | Mad26 |
|  | MAD27 | F | 1055 | Madeira | Drifting mid-water longline | Mad27 | Mad27 |
|  | MAD28 | M | 1073 | Madeira | Drifting mid-water longline | Mad28 | SHc1 |
|  | MAD30 | M | 1085 | Madeira | Drifting mid-water longline | - | SHc1 |
|  | MAD33 | F | 1152 | Madeira | Drifting mid-water longline | - | - |
|  | MAD35 | F | 1072 | Madeira | Drifting mid-water longline | - | - |
|  | MAD40 | M | 913 | Madeira | Drifting mid-water longline | - | - |
| * | AphCar-35-MAR-a | M | 921 | W-Sahara | Bottom trawl | SHc1 | SHc1 |
| * | AphCar-39-MAR-a | M | 1012 | Morocco | Bottom trawl | Mor17 | Mor17 |
| * | AphCar-42-MAR-a | M | 990 | Morocco | Bottom trawl | Mor20 | Mor20 |
| * | AphCar-43-MAR-a | M | 1042 | Morocco | Bottom trawl | Mor21 | SHc1 |
|  | AphCar-56-MAR-a | F | 1057 | Morocco | Bottom trawl | SHc7 | SHc1 |
| * | AphCar-59-MAR-a | F | 1025 | Morocco | Bottom trawl | SHc8 | SHc1 |
|  | PORT01 | M | 905 | Sesimbra | Drifting mid-water longline | SHc 1 | SHc1 |
|  | PORT02 | M | 1107 | Sesimbra | Drifting mid-water longline | Por2 | SHc1 |
|  | PORT03 | F | 997 | Sesimbra | Drifting mid-water longline | - | SHc1 |
|  | PORT04 | M | 927 | Sesimbra | Drifting mid-water longline | SHc4 | SHc1 |
|  | PORT05 | F | 1115 | Sesimbra | Drifting mid-water longline | Por5 | SHc1 |
|  | PORT06 | F | 1045 | Sesimbra | Drifting mid-water longline | SHc9 | SHc1 |
|  | PORT07 | F | 965 | Sesimbra | Drifting mid-water longline | SHc6 | SHc2 |
|  | PORT08 | F | 982 | Sesimbra | Drifting mid-water longline | Por8 | SHc1 |


| Aphanopus carbo |  |  |  |  |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| ID Code | Sex | SL | Locality | Method of collection | HCR | H COI |  |
| PORT09 | F | 1053 | Sesimbra | Drifting mid-water longline | SHc3 | SHc1 |  |
| PORT10 | M | 947 | Sesimbra | Drifting mid-water longline | SHc7 | SHc1 |  |
| PORT11 | M | 984 | Sesimbra | Drifting mid-water longline | Por11 | SHc2 |  |
| PORT12 | M | 1011 | Sesimbra | Drifting mid-water longline | Por12 | SHc1 |  |
| PORT13 | M | 1050 | Sesimbra | Drifting mid-water longline | Por13 | SHc1 |  |
| PORT14 | M | 938 | Sesimbra | Drifting mid-water longline | SHc1 | SHc1 |  |
| PORT15 | M | 978 | Sesimbra | Drifting mid-water longline | SHc1 | SHc1 |  |
| PORT16 | F | 967 | Sesimbra | Drifting mid-water longline | SHc7 | SHc1 |  |
| PORT17 | M | 970 | Sesimbra | Drifting mid-water longline | SHc1 | SHc1 |  |
| PORT18 | M | 938 | Sesimbra | Drifting mid-water longline | SHc5 | Por18 |  |
| PORT19 | M | 1022 | Sesimbra | Drifting mid-water longline | Por19 | SHc1 |  |
| PORT20 | M | 1030 | Sesimbra | Drifting mid-water longline | Por20 | SHc1 |  |
| PORT21 | M | 1005 | Sesimbra | Drifting mid-water longline | SHc1 | SHc1 |  |
| PORT22 | M | 941 | Sesimbra | Drifting mid-water longline | Por22 | SHc1 |  |
| PORT23 | M | 946 | Sesimbra | Drifting mid-water longline | SHc8 | SHc1 |  |
| PORT24 | M | 929 | Sesimbra | Drifting mid-water longline | Por24 | SHc2 |  |
| PORT25 | M | 942 | Sesimbra | Drifting mid-water longline | SHc9 | SHc1 |  |


|  | Aphanopus intermedius |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | ID Code | Sex | SL | Locality | Method of collection | H CR | COI |
|  | AZO01 | M | 1020 | Azores | Drifting mid-water longline | SHi1 | SHi1 |
|  | AZO03 | F | 1210 | Azores | Drifting mid-water longline | Azo3 | SHi1 |
|  | AZO04 | M | 1140 | Azores | Drifting mid-water longline | SHi 1 | SHi1 |
|  | AZO05 | F | 1054 | Azores | Drifting mid-water longline | SHi 2 | SHi1 |
|  | AZO06 | M | 970 | Azores | Drifting mid-water longline | Azo6 | SHil |
|  | AZO07 | M | 963 | Azores | Drifting mid-water longline | SHi1 | Azo7 |
|  | AZO08 | Ind | 1020 | Azores | Drifting mid-water longline | SHi2 | SHi1 |
|  | AZO09 | M | 1150 | Azores | Drifting mid-water longline | SHi1 | SHil |
|  | AZO10 | F | 1090 | Azores | Drifting mid-water longline | SHil | Azol0 |
|  | AZO12 | F | 1010 | Azores | Drifting mid-water longline | SHi2 | Azo12 |
|  | AZO13 | M | 870 | Azores | Drifting mid-water longline | Azol3 | Azol3 |
|  | AZO14 | M | 1020 | Azores | Drifting mid-water longline | Azol4 | SHi1 |
|  | AZO15 | M | 927 | Azores | Drifting mid-water longline | SHi2 | SHil |
|  | AZO16 | M | 969 | Azores | Drifting mid-water longline | Azol6 | SHil |
|  | AZO17 | F | 930 | Azores | Drifting mid-water longline | SHi3 | Azol7 |
|  | AZO18 | M | 852 | Azores | Drifting mid-water longline | SHi1 | Azol8 |
|  | AZO19 | F | 890 | Azores | Drifting mid-water longline | SHi3 | Azol9 |
|  | AZO20 | F | 1026 | Azores | Drifting mid-water longline | Azo20 | SHil |
|  | AZO22 | M | 913 | Azores | Drifting mid-water longline | SHil | SHil |
|  | AZO23 | M | 872 | Azores | Drifting mid-water longline | Azo23 | SHi1 |
|  | AZO24 | Ind | 912 | Azores | Drifting mid-water longline | SHil | SHi1 |
| * | AZO25 | F | 950 | Azores | Drifting mid-water longline | SHi1 | SHi1 |
|  | AZO26 | F | 1015 | Azores | Drifting mid-water longline | SHil | SHil |
| * | AZO27 | M | 957 | Azores | Drifting mid-water longline | SHi4 | SHil |
|  | AZO28 | F | 905 | Azore | Drifting mid-water longline | SHi2 | SHil |
|  | AZO29 | M | 960 | Azores | Drifting mid-water longline | SHi1 | Azo29 |
|  | AZO30 | M | 896 | Azores | Drifting mid-water longline | Azo30 | Azo30 |
| * | AphCar-149-CI-a | M | n.a. | Canary Is. | Drifting mid-water longline | Can8 | SHil |


|  | Aphanopus intermedius |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | ID Code | Sex | SL | Locality | Method of collection | H CR | COI |
| * | AphCar-150-CI-a | M | 1117 | Canary Is. | Drifting mid-water longline | - | Can9 |
| * | AphCar-154-CI-a | M | 1122 | Canary Is. | Drifting mid-water longline | SHil | SHil |
| * | AphCar-155-CI-a | F | 1208 | Canary Is. | Drifting mid-water longline | SHi4 | SHil |
| * | AphCar-157-CI-a | F | n.a. | Canary Is. | Drifting mid-water longline | SHi6 | SHil |
| * | AphCar-158-CI-a | n.a. | n.a. | Canary Is. | Drifting mid-water longline | Can17 | SHil |
| * | AphCar-159-CI-a | n.a. | n.a. | Canary Is. | Drifting mid-water longline | SHi1 | SHil |
| * | AphCar-160-CI-a | n.a. | n.a. | Canary Is. | Drifting mid-water longline | Can19 | SHil |
| * | AphCar-161-CI-a | n.a. | n.a. | Canary Is. | Drifting mid-water longline | SHi1 | SHil |
| * | AphCar-163-CI-a | n.a. | n.a. | Canary Is. | Drifting mid-water longline | SHi2 | SHil |
|  | MAD9 | M | 1026 | Madeira | Drifting mid-water longline | Mad9 | SHil |
|  | MAD11 | F | 1233 | Madeira | Drifting mid-water longline | SHil | SHil |
|  | MAD13 | F | 1274 | Madeira | Drifting mid-water longline | Mad13 | Mad13 |
| * | MAD14 | n.a. | n.a. | Madeira | Drifting mid-water longline | SHi6 | SHil |
|  | MAD16 | M | 1116 | Madeira | Drifting mid-water longline | - | SHi1 |
|  | MAD19 | M | 1188 | Madeira | Drifting mid-water longline | SHi3 | SHi1 |
| * | MAD23 | n.a. | n.a. | Madeira | Drifting mid-water longline | SHil | SHi2 |
|  | MAD25 | M | 1072 | Madeira | Drifting mid-water longline | Mad25 | SHil |
|  | MAD29 | F | 1044 | Madeira | Drifting mid-water longline | SHi6 | SHil |
|  | MAD31 | F | 1265 | Madeira | Drifting mid-water longline | - | - |
| * | MAD32 | M | 1060 | Madeira | Drifting mid-water longline | - | - |
|  | MAD37 | M | 887 | Madeira | Drifting mid-water longline | - | - |
| * | AphCar-23-MAR-a | F | 817 | W-Sahara | Bottom trawl | SHil | SHil |
|  | AphCar-24-MAR-a | F | 872 | W-Sahara | Bottom trawl | SHil | SHil |
| * | AphCar-25-MAR-a | F | 805 | W-Sahara | Bottom trawl | SHil | SHil |
| * | AphCar-26-MAR-a | Ind | 638 | W-Sahara | Bottom trawl | SHil | SHi1 |
| * | AphCar-27-MAR-a | Ind | 681 | W-Sahara | Bottom trawl | SHil | Mor5 |
| * | AphCar-28-MAR-a | Ind | 682 | W-Sahara | Bottom trawl | SHil | SHi1 |
| * | AphCar-29-MAR-a | Ind | 831 | W-Sahara | Bottom trawl | SHil | SHil |
| * | AphCar-30-MAR-a | M | 622 | W-Sahara | Bottom trawl | SHi1 | SHil |
| * | AphCar-31-MAR-a | M | 1050 | W-Sahara | Bottom trawl | Mor9 | SHil |
| * | AphCar-32-MAR-a | F | 807 | W-Sahara | Bottom trawl | SHil | SHil |
| * | AphCar-33-MAR-a | M | 807 | W-Sahara | Bottom trawl | SHi1 | SHi2 |
| * | AphCar-34-MAR-a | M | 795 | W-Sahara | Bottom trawl | Mor12 | SHil |
| * | AphCar-36-MAR-a | M | 730 | W-Sahara | Bottom trawl | SHi6 | SHil |
| * | AphCar-37-MAR-a | Ind | 782 | Morocco | Bottom trawl | Mor15 | SHil |
| * | AphCar-38-MAR-a | M | 775 | W-Sahara | Bottom trawl | SHi4 | SHil |
| * | AphCar-40-MAR-a | M | 645 | W-Sahara | Bottom trawl | Mor18 | SHi2 |
| * | AphCar-41-MAR-a | M | 816 | W-Sahara | Bottom trawl | Mor19 | SHil |
| * | AphCar-44-MAR-a | M | 867 | W-Sahara | Bottom trawl | Mor22 | SHi1 |
| * | AphCar-45-MAR-a | M | 800 | W-Sahara | Bottom trawl | SHi3 | SHil |
| * | AphCar-46-MAR-a | Ind | 680 | W-Sahara | Bottom trawl | Mor24 | SHil |
| * | AphCar-47-MAR-a | M | 920 | W-Sahara | Bottom trawl | SHil | SHi2 |
|  | AphCar-55-MAR-a | Ind | 910 | Morocco | Bottom trawl | SHil | SHil |
| * | AphCar-57-MAR-a | Ind | 647 | W-Sahara | Bottom trawl | SHi1 | SHil |
| * | AphCar-58-MAR-a | Ind | 753 | Morocco | Bottom trawl | SHil | SHil |


[^0]:    (1) Museu Municipal do Funchal (História Natural), Rua da Mouraria, 31, 9004-546 Funchal, Madeira, PORTUGAL.
    (2) Direçção de Serviços de Investigação das Pescas, Estrada da Pontinha, 9004-562 Funchal, Madeira, PORTUGAL. [joaodelgado.sra@gov-madeira.pt] [dalilacarvalho.sra@gov-madeira.pt]
    (3) Instituto Canario de Ciencias Marinas, Agencia Canaria de Investigación, Innovación y Sociedad de la Información, 35214 Telde, Las Palmas, Canary Islands, SPAIN. [solea@iccm.rcanaria.es] [vtuset@icm.csic.es] [antoniogm@iccm.rcanaria.es]
    (4) IMAR/DOP, University of the Azores, Cais Sta. Cruz, 9901-862 Horta, Azores, PORTUGAL. [sstefanni@uac.pt] [eduardo@uac.pt]

    * Corresponding author [manuel.biscoito@cm-funchal.pt]

