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

by

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**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 description 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.

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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)

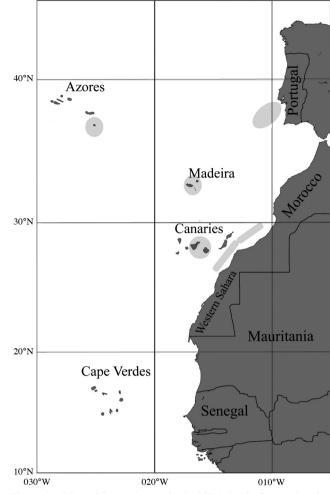
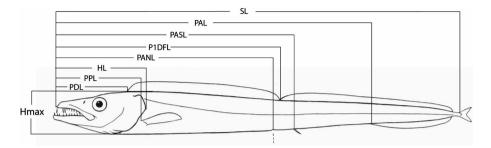


Figure 1. - Map of the study area in the NE Atlantic Ocean showing the locations where specimens of *A. carbo* and *A. intermedius* were collected (shaded areas).

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* 



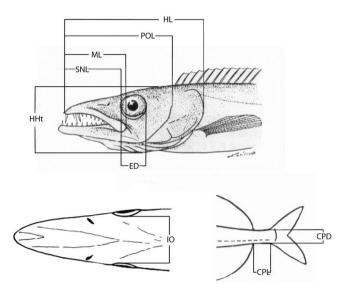


Figure 3. - Schematic drawing of head and tail of a trichiurid showing measurements used in the present study (adapted from Nakamura and Parin, 1993). See table I for abbreviations.

*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 "species-length" interactions were significant (p < 0.05, samples with unequal slopes) were not included in any further

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.

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 ( $\varkappa$ ) 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

		Ap	hanopus carbo	Aphanopus	s 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)

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).

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, ShS130 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

			]	Dorsal-f	in spine	s									
	38	39	40		42	43	44	n							
A. carbo	5	18	31		_	-	-	66							
A. intermedius	-	1	13		8	2	_	41							
					Do	orsal-fin	rays					]			
	51	52	53		55	56	57	58	59	60	n	1			
A. carbo	2	12	19		10	4	1	-	-	-	66				
A. intermedius	-	1	-		13	9	6	3	1	1	41				
						Ι	Dorsal-fi	n elem	ents (tota	1)					
	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-ca	udal ve	rtebrae				]					
	40	41	42		44	45	46	47	n						
A. carbo	11	22	15		_	-	_	_	55						
A. intermedius	-	_	-		9	11	17	2	46						
				Caudal v	vertebra	e			7						
	55	56	57		59	60	61	n							
A. carbo	3	4	25		5	1	-	55	1						
A. intermedius	-	10	11		4	8	1	46							
						Tot	al verte	orae						]	
	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		
		Р	osition of	anal fin	spines	in relation	on to do	rsal-fin	spines a	nd soft r	ays				
over	Last sp	ine	1 <sup>st</sup> ray	2 <sup>nd</sup> ra	ıy 🤅	3 <sup>rd</sup> ray	4 <sup>th</sup> ra	у	5 <sup>th</sup> ray	6 <sup>th</sup> ra	y	n			
A. carbo			5	25		12	11			1		54			
A. intermedius	2		1	7		6	13		8	1		38			
				Positio	n of anı	ıs in rela	tion to d	orsal-f	ìn spines						
over	Penu	ltimate	e spine	1 <sup>st</sup> ra	y 2	2 <sup>nd</sup> ray	3 <sup>rd</sup> ra	y	4 <sup>th</sup> ray	5 <sup>th</sup> ra	y	n			
A. carbo		2		6		33	4					54			

Table II Comparison of meristic c	characters of Aphanopus carbo and A. i	intermedius, based on genetic identification.
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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 C = 22.9%, T = 31.1%, A = 31.6% and 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-

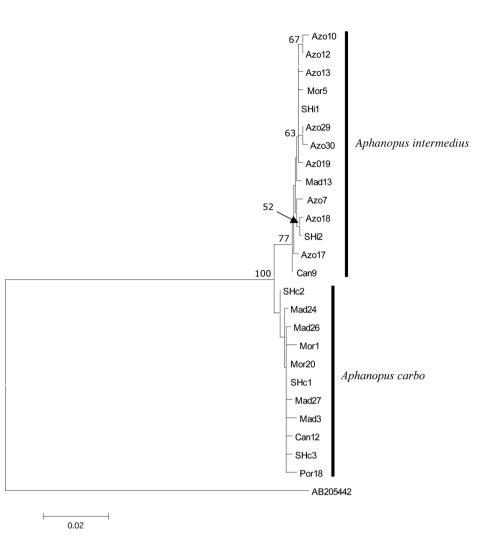


Figure 4. - Neighbour-joining tree constructed from sequences of the COI using PAUP (Swofford, 1999) software and implementing the HKY (Hasegawa *et al.*, 1985) nucleotide substitution model with no invariable sites and equal rate. Numbers above internal branches indicate bootstrap values out of 1000 replicates (only if greater than 50%). AB205442 = *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 C = 23.1%, T = 30.6%, A = 31.4% and 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 C = 29.9%, T = 28.7%, A = 22.5% and G = 18.9% and the transition/transvertion ratio of 1.4. The 10 haplotypes described an overall haplotypic diversity of  $0.4527\pm0.4054$  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 C = 29.5%, T = 28.9%, A = 22.6% and 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 (p < 0.05) values of  $\Phi_{\text{ST}}$  (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	Le	ngth	b
Variables	F	р	F	Significant	
Pre-anal length (PAL) <sup>1</sup>	0.040	0.842	1203.891	0.000	0.624
Pre-first anal spine length (PASL) <sup>1</sup>	0.188	0.665	2238.472	0.000	0.601
Pre-anus length (PANL) <sup>1</sup>	0.070	0.791	1022.289	0.000	0.559
Pre-pectoral length (PPL) <sup>1</sup>	2.757	0.101	533.955	0.000	0.194
Pre-dorsal length (PDL) <sup>1</sup>	1.219	0.273	306.477	0.000	0.168
Pre-first dorsal soft ray length (P1DFL) <sup>1</sup>	2.138	0.147	548.496	0.000	0.564
Maximum body depth (Hmax) <sup>1</sup>	0.381	0.539	66.352	0.000	0.138
Depth of body at level of first anal spine (H1SFA) <sup>1</sup>	2.902	0.092	150.996	0.000	0.064
Caudal peduncle length (CPL) <sup>1</sup>	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) <sup>2</sup>	1.601	0.209	275.794	0.000	0.387
Eye diameter (ED) <sup>2</sup>	0.035	0.852	95.897	0.000	0.259
Inter-orbital width (IO) <sup>2</sup>	8.145	0.005	-	-	-
Maxillary length (ML) <sup>2</sup>	6.777	0.011	-	-	-
Head height (HHt) <sup>2</sup>	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<sup>1</sup> or head length<sup>2</sup>.

Variables	Wilks' lambda	F	df1	df2	Р
Pre-anal length (PAL)	0.924	7.144	1	87	0.009
	0.924	3.817	1	87	0.009
Pre-first anal spine length (PASL)			-		
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 soft rays (ASDF)	0.891	10.620	1	87	0.002
Pre-caudal vertebrae (PCV)	0.237	280.098	1	87	< 0.001
Caudal vertebrae (CV)	0.984	1.418	1	87	0.237
Total vertebrae (TV)	0.136	553.803	1	87	< 0.001

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 an	ong mc	rphom	letric a	nd meı	istic va	id meristic variables to select variables with less relation (<0.400)	o selec	t variab	les witl	h less r	elation	(< 0.4(	.(00								
Variables	PAL	PASL	PANL	ЪРL	PDL	PIDFL	Hmax	H1SFA	CPL	POL	SNL	ED	DS	DF	DT	ANDF	AF A	ASDF	PCV	CV	TV
Pre-anal length (PAL)	1.000																				
Pre-first anal spine length (PASL)	0.767 1.000	1.000																			
Pre-anus length (PANL)	0.511	0.511 0.587 1.000	1.000																		
Pre-pectoral length (PPL)	0.189	0.397 0.358	0.358	1.000																	
Pre-dorsal length (PDL)	0.390	0.390 0.553 0.386	0.386	0.424 1.000	1.000																
Pre-first dorsal soft ray length (P1DFL)	0.243	0.343 0.249	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	0.011 0.133 -0.014	-0.014		-0.028 0.281	0.094	0.216	1.000													
Caudal peduncle length (CPL)	-0.104	-0.104 -0.147 -0.021	-0.021	-0.130	-0.130 -0.191	0.029	-0.114	0.124	1.000												
Pre-opercular length (POL)	-0.041	-0.041 -0.005 0.117	0.117	-0.103	0.150	-0.045	-0.007	0.322	0.032	1.000											
Snout length (SNL)	0.101	0.101 0.027 0.063	0.063	-0.178	0.183	-0.049	-0.062	0.250	0.275	0.269	1.000										
Eye diameter (ED)	-0.089	-0.089 0.001 0.029	0.029	0.271	0.271 -0.028	0.044	0.104	0.023	-0.088 -0.078 -0.289 1.000	-0.078	0.289	1.000									
Dorsal-fin spines (DS)	0.256	0.256 0.294 0.203	0.203	-0.104	0.104 0.101	0.225	0.083	0.036	-0.034 -0.031 0.006 -0.105 1.000	-0.031	0.006 -	-0.105	1.000								
Dorsal-fin rays (DF)	-0.366	-0.366 -0.319 -0.402	-0.402	0.055	0.055 -0.340	-0.234	-0.173	-0.211 -0.228 -0.034 -0.280 0.048 -0.237 1.000	-0.228	-0.034	0.280	0.048	-0.237	1.000							
Dorsal-fin elements (total) (DT)	-0.193	-0.193 -0.126 -0.259	-0.259		-0.026 -0.263		-0.110	-0.092 -0.110 -0.176 -0.237 -0.040 -0.259 -0.028 0.372	-0.237	-0.040	0.259 -	-0.028		0.811 1.000	1.000						
Anus in relation to dorsal fin spines 0.262 0.334 0.051 (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	-0.052	0.033	-0.058	0.401	0.179		1.000					
Anal-fin rays (AF)	-0.154	-0.154 -0.051 0.031	0.031	0.294	0.294 -0.164	-0.050	-0.122	-0.131	-0.217	0.067 -0.269 0.170 -0.126 0.282	0.269	0.170	-0.126		0.190	-0.093 1.000	1.000				
Anal-fin spines in relation to dor- sal-fin spines and soft rays (ASDF)	-0.123	-0.123 -0.113 0.059	0.059	0.163	0.163 -0.009	-0.169	0.022	-0.354 -0.005 0.017 -0.124 0.074 -0.092 0.104	-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	0.043 0.116 0.161	0.161	-0.055	0.055 -0.184	-0.018	-0.077	-0.172	0.140 -0.014 0.050 -0.023 0.193	-0.014	0.050	-0.023		0.005 (	0.121	-0.139 -0.126 0.228	0.126 (		1.000		
Caudal vertebrae (CV)	-0.130	-0.130 -0.185 -0.196	-0.196		0.103 0.035	-0.065 -0.065		$0.126  \left  \begin{array}{c} -0.114 \\ 0.047 \\ \end{array} \right  \begin{array}{c} 0.024 \\ 0.024 \\ \end{array} \right  \begin{array}{c} 0.098 \\ -0.127 \\ \end{array} \right  \begin{array}{c} 0.067 \\ -0.008 \\ \end{array} \right  \begin{array}{c} 0.024 \\ 0.024 \\ \end{array}$	-0.114	0.047	0.024	. 860.0	-0.127	0.067	0.008		0.136 -0.287 -0.760 1.000	0.287	0.760	1.000	
Total vertebrae (TV)	-0.142	-0.142 -0.130 -0.090	060.0-		-0.229 -0.186	-0.122	-0.198	-0.032	0.008	0.053	0.101 0	0.120	0.058	0.107 0	0.144	-0.144 (	).044	0.141 (		0.536	1.000
Total vertebrae (TV)	-0.142	-0.130	-0.090		-0.186							0.120			0.144	-0.14	4	4 0.044	-0.144 0.044 -0.141 (	0.141	4 0.044 -0.141 0.141 0.536 1.000

Stop	Variables introduced	Wilk	s' lai	nbda			Fer	kactly	/
Step	variables introduced	Statistic	df1	df2	df3	Statistic	df1	df2	Significant
	Morphor	netric and	l mer	istic	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
	М	orphomet	ric da	ata					
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.

A stual group	Predicted gro	oup membership	Correct identification	Misidentification	Cohen's
Actual group	A. carbo	A. intermedius	(%)	(%)	kappa
Morphometric and meristic data			100.0	0.0	1.000
A. carbo	53 (100%)	0 (0%)			
A. intermedius	0 (0%)	36 (100%)			
Morphometric data			84.3	15.7	0.680
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  $\varkappa$  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°N, off the Western Sahara coast. This southern limit

of distribution of *A. carbo* was until present set with certitude to about 30°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  $\Phi_{ST}$  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

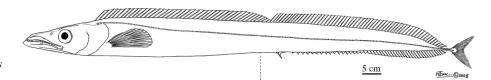


Figure 5. - Aphanopus intermedius Parin, 1983 (MMF 39099).

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 32.3-42.3.

*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; pre-first anal spine length 15.2-60.0; pre-anus length 52.7-64.0; pre-pectoral length 18.6-20.8; maximum body depth 6.1-12.7; 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.

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**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.

				Aphano	pus carbo		
	ID Code	Sex	SL	Locality	Method of collection	HCR	H COI
	AZO02	Μ	1150	Azores	Drifting mid-water longline	Azo2	SHc1
	AZO11	F	1134	Azores	Drifting mid-water longline	SHc1	SHc1
	AZO21	Μ	1047	Azores	Drifting mid-water longline	SHc2	SHc2
*	AphCar-142-CI-a	Μ	1046	Canary Is.	Drifting mid-water longline	SHc1	SHc1
*	AphCar-143-CI-a	Μ	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	Μ	1100	Canary Is.	Drifting mid-water longline	SHc1	SHc1
*	AphCar-146-CI-a	Μ	1065	Canary Is.	Drifting mid-water longline	SHc2	SHc3
*	AphCar-147-CI-a	Μ	1115	Canary Is.	Drifting mid-water longline	Can6	SHc1
*	AphCar-148-CI-a	М	1087	Canary Is.	Drifting mid-water longline	Can7	SHc1
*	AphCar-151-CI-a	Μ	1122	Canary Is.	Drifting mid-water longline	Can10	SHc1
*	AphCar-152-CI-a	Μ	1070	Canary Is.	Drifting mid-water longline	Can11	SHc1

				Aphano	pus carbo		
	ID Code	Sex	SL	Locality	Method of collection	HCR	H COI
*	AphCar-153-CI-a	М	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.a.	n.a.	Canary Is.	Drifting mid-water longline	_	SHc1
*	AphCar-164-CI-a	n.a.	n.a.	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	М	1093	Canary Is.	Drifting mid-water longline	SHc1	SHc1
	AphCar-167-CI-a	М	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	М	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	М	1084	Madeira	Drifting mid-water longline	SHc1	SHc1
	MAD02	М	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	М	1115	Madeira	Drifting mid-water longline	Mad6	SHc1
*	MAD7	n.a.	n.a.	Madeira	Drifting mid-water longline	SHc1	SHc1
	MAD08	Μ	1002	Madeira	Drifting mid-water longline	SHc5	SHc1
	MAD10	Μ	1028	Madeira	Drifting mid-water longline	Mad10	SHc3
*	MAD12	F	1293	Madeira	Drifting mid-water longline	SHc4	SHc1
	MAD15	М	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	М	1092	Madeira	Drifting mid-water longline	Mad21	SHc1
	MAD22	М	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	Μ	1073	Madeira	Drifting mid-water longline	Mad28	SHc1
	MAD30	Μ	1085	Madeira	Drifting mid-water longline	_	SHc1
	MAD33	F	1152	Madeira	Drifting mid-water longline	_	-
	MAD35	F	1072	Madeira	Drifting mid-water longline	_	-
	MAD40	М	913	Madeira	Drifting mid-water longline	-	-
*	AphCar-35-MAR-a	M	921	W-Sahara	Bottom trawl	SHc1	SHc1
*	AphCar-39-MAR-a	М	1012	Morocco	Bottom trawl	Mor17	Mor17
*	AphCar-42-MAR-a	М	990	Morocco	Bottom trawl	Mor20	Mor20
*	AphCar-43-MAR-a	М	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	SHc1	SHc1
	PORT02	M	1107	Sesimbra	Drifting mid-water longline	Por2	SHc1
	PORT03	F	997 027	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

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

				Aphanopus	s intermedius		
	ID Code	Sex	SL	Locality	Method of collection	H CR	COI
	AZO01	М	1020	Azores	Drifting mid-water longline	SHi1	SHi1
	AZO03	F	1210	Azores	Drifting mid-water longline	Azo3	SHi1
	AZO04	Μ	1140	Azores	Drifting mid-water longline	SHi 1	SHi1
	AZO05	F	1054	Azores	Drifting mid-water longline	SHi 2	SHi1
	AZO06	Μ	970	Azores	Drifting mid-water longline	Azo6	SHi1
	AZO07	Μ	963	Azores	Drifting mid-water longline	SHi1	Azo7
	AZO08	Ind	1020	Azores	Drifting mid-water longline	SHi2	SHi1
	AZO09	Μ	1150	Azores	Drifting mid-water longline	SHi1	SHi1
	AZO10	F	1090	Azores	Drifting mid-water longline	SHi1	Azo10
	AZO12	F	1010	Azores	Drifting mid-water longline	SHi2	Azo12
	AZO13	Μ	870	Azores	Drifting mid-water longline	Azo13	Azo13
	AZO14	Μ	1020	Azores	Drifting mid-water longline	Azo14	SHi1
	AZO15	Μ	927	Azores	Drifting mid-water longline	SHi2	SHi1
	AZO16	Μ	969	Azores	Drifting mid-water longline	Azo16	SHi1
	AZO17	F	930	Azores	Drifting mid-water longline	SHi3	Azo17
	AZO18	Μ	852	Azores	Drifting mid-water longline	SHi1	Azo18
	AZO19	F	890	Azores	Drifting mid-water longline	SHi3	Azo19
	AZO20	F	1026	Azores	Drifting mid-water longline	Azo20	SHi1
	AZO22	Μ	913	Azores	Drifting mid-water longline	SHi1	SHi1
	AZO23	Μ	872	Azores	Drifting mid-water longline	Azo23	SHi1
	AZO24	Ind	912	Azores	Drifting mid-water longline	SHi1	SHi1
*	AZO25	F	950	Azores	Drifting mid-water longline	SHi1	SHi1
	AZO26	F	1015	Azores	Drifting mid-water longline	SHi1	SHi1
*	AZO27	Μ	957	Azores	Drifting mid-water longline	SHi4	SHi1
	AZO28	F	905	Azores	Drifting mid-water longline	SHi2	SHi1
	AZO29	Μ	960	Azores	Drifting mid-water longline	SHi1	Azo29
	AZO30	Μ	896	Azores	Drifting mid-water longline	Azo30	Azo30
*	AphCar-149-CI-a	М	n.a.	Canary Is.	Drifting mid-water longline	Can8	SHi1

	Aphanopus intermedius						
	ID Code	Sex	SL	Locality	Method of collection	H CR	COI
*	AphCar-150-CI-a	М	1117	Canary Is.	Drifting mid-water longline	_	Can9
*	AphCar-154-CI-a	M	1122	Canary Is.	Drifting mid-water longline	SHi1	SHi1
*	AphCar-155-CI-a	F	1208	Canary Is.	Drifting mid-water longline	SHi4	SHi1
*	AphCar-157-CI-a	F	n.a.	Canary Is.	Drifting mid-water longline	SHi6	SHi1
*	AphCar-158-CI-a	n.a.	n.a.	Canary Is.	6 6	Can17	SHi1
*	AphCar-159-CI-a	n.a.	n.a.	Canary Is.	6 6	SHi1	SHi1
*	AphCar-160-CI-a	n.a.	n.a.	Canary Is.	6 6	Can19	SHi1
*	AphCar-161-CI-a	n.a.	n.a.	Canary Is.	Drifting mid-water longline	SHi1	SHi1
*	AphCar-163-CI-a	n.a.	n.a.	Canary Is.	Drifting mid-water longline	SHi2	SHi1
	MAD9	Μ	1026	Madeira	Drifting mid-water longline	Mad9	SHi1
	MAD11	F	1233	Madeira	Drifting mid-water longline	SHi1	SHi1
*	MAD13	F	1274	Madeira	Drifting mid-water longline	Mad13	Mad13
*	MAD14	n.a.	n.a.	Madeira	Drifting mid-water longline	SHi6	SHi1
	MAD16	M	1116	Madeira	Drifting mid-water longline		SHi1
*	MAD19	М	1188	Madeira Madeira	Drifting mid-water longline	SHi3 SHi1	SHi1
	MAD23 MAD25	n.a. M	n.a. 1072	Madeira	Drifting mid-water longline Drifting mid-water longline	Mad25	SHi2 SHi1
	MAD23 MAD29	F	1072	Madeira	Drifting mid-water longline	SHi6	SHi1
	MAD29 MAD31	г F	1265	Madeira	Drifting mid-water longline	5110	SHII
*	MAD32	M	1060	Madeira	Drifting mid-water longline		
	MAD32 MAD37	M	887	Madeira	Drifting mid-water longline		
*	AphCar-23-MAR-a	F	817	W-Sahara	Bottom trawl	SHi1	SHi1
	AphCar-24-MAR-a	F	872	W-Sahara	Bottom trawl	SHi1	SHi1
*	AphCar-25-MAR-a	F	805	W-Sahara	Bottom trawl	SHi1	SHi1
*	AphCar-26-MAR-a	Ind	638	W-Sahara	Bottom trawl	SHi1	SHi1
*	AphCar-27-MAR-a	Ind	681	W-Sahara	Bottom trawl	SHi1	Mor5
*	AphCar-28-MAR-a	Ind	682	W-Sahara	Bottom trawl	SHi1	SHi1
*	AphCar-29-MAR-a	Ind	831	W-Sahara	Bottom trawl	SHi1	SHi1
*	AphCar-30-MAR-a	М	622	W-Sahara	Bottom trawl	SHi1	SHi1
*	AphCar-31-MAR-a	Μ	1050	W-Sahara	Bottom trawl	Mor9	SHi1
*	AphCar-32-MAR-a	F	807	W-Sahara	Bottom trawl	SHi1	SHi1
*	AphCar-33-MAR-a		807	W-Sahara	Bottom trawl	SHi1	SHi2
*	AphCar-34-MAR-a		795	W-Sahara	Bottom trawl	Mor12	SHi1
*	AphCar-36-MAR-a		730	W-Sahara	Bottom trawl	SHi6	SHi1
*	AphCar-37-MAR-a		782	Morocco	Bottom trawl	Mor15	SHi1
*	AphCar-38-MAR-a	Μ	775	W-Sahara	Bottom trawl	SHi4	SHi1
*	AphCar-40-MAR-a	М	645	W-Sahara	Bottom trawl	Mor18	SHi2
*	AphCar-41-MAR-a	М	816	W-Sahara	Bottom trawl	Mor19	SHi1
*	AphCar-44-MAR-a	М	867	W-Sahara	Bottom trawl	Mor22	SHi1
*	AphCar-45-MAR-a	M	800	W-Sahara	Bottom trawl	SHi3	SHi1
*	AphCar-46-MAR-a	Ind	680	W-Sahara	Bottom trawl	Mor24	SHi1
*	AphCar-47-MAR-a	M	920	W-Sahara	Bottom trawl	SHi1	SHi2
*	AphCar-55-MAR-a		910	Morocco	Bottom trawl	SHi1	SHi1
* *	AphCar-57-MAR-a		647 752	W-Sahara	Bottom trawl	SHi1	SHi1
*	AphCar-58-MAR-a	Ind	753	Morocco	Bottom trawl	SHi1	SHi1