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Morphological and mitochondrial DNA divergence validates blackmouth, *Galeus melastomus*, and Atlantic sawtail catsharks, *Galeus atlanticus*, as separate species

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A total of 60 morphometric traits and nucleotide sequences of the entire mtDNA NADH dehydrogenase subunit 2 (ND2) gene [1047 base pair (bp)] in 23 individuals of blackmouth, *Galeus melastomus*, and 13 individuals of sawtail catsharks, *Galeus atlanticus*, caught in Southern Portugal, were examined to test the validity of these two taxa. These sharks closely resemble each other, have overlapping geographical ranges and are difficult to identify by morphological characters. Non-metric multidimensional scaling of morphometric variables indicates a clear separation between the two species, with 10 characters each contributing 2·12–2·45% of the total variability between species. Maximum likelihood, parsimony and neighbour-joining trees revealed two major mtDNA haplotype clades, corresponding to the two species, with an average corrected sequence divergence between them of 3·39 ± 0·56%. Within species divergences between haplotypes averaged 0·27 ± 0·18% in *G. melastomus* and 0·12 ± 0·08% in *G. atlanticus*. A total of 35 diagnostic nucleotide site differences and four restriction fragment length polymorphism recognition sites in the ND2 gene can be used to distinguish the two species.

Key words: catsharks; delimiting species; elasmobranch; mtDNA; ND2 gene.

INTRODUCTION

The family Scyliorhinidae is the most speciose family of Elasmobranchii, with at least 15 genera (*Apristurus*, *Halaelurus*, *Scyliorhinus* and *Galeus* among the most representative) and over 100 species (Compagno & Niem, 1999). The genus *Galeus* is widely distributed and currently includes 17 described species (Compagno *et al.*, 2005). The earliest fossil record for this genus dates to the early Miocene of France (Musick *et al.*, 2004). The blackmouth, *Galeus melastomus* Rafinesque 1810, and Atlantic sawtail, *Galeus atlanticus* Vaillant 1888, catsharks

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closely resemble each other and occur sympatrically over parts of their ranges. Their morphological similarity and geographic overlap have made species identifications difficult, and some authors have considered synonymizing *G. atlanticus* under *G. melastomus* (Compagno, 1984). Muñoz-Chapuli & Ortega (1985), however, resurrected *G. atlanticus* as a valid species, and Rey *et al.* (2006) redescribed *G. atlanticus* on the basis of field markings. The blackmouth catshark is widely distributed over the North-eastern Atlantic from the Faeroe Islands and Trondheim, Norway to Senegal and Mediterranean Sea, while the Atlantic sawtail catshark appears to have a more restricted distribution in the Mediterranean Spanish coasts, Atlantic waters off Portugal and Morocco.

Taxonomic confusion between the two species distorts the by-catch fishery statistics, as blackmouth and Atlantic sawtail catshark catches are often recorded only as blackmouth catshark (Erzini *et al.*, 2002; Coelho *et al.*, 2005; Pawson & Ellis, 2005). The blackmouth catshark is managed as a single population, in which 'pseudo/metapopulation segments can be distinguished and treated as management units' (Pawson & Ellis, 2005). Local aggregations in these species are also typical of other Scyliorhinid species (Musick *et al.*, 2004). Like other elasmobranchs, these fishes are particularly susceptible to population declines because slow growth rates, late maturity, low fecundity and longevities of several years make them vulnerable to direct or indirect fishing pressure. Depleted populations may take several decades to recover (Stevens *et al.*, 2000). In Portuguese waters, for instance, a major impediment to implementing species-specific conservation and management has been the difficulty in accurately identifying individuals to species, a problem common to morphologically similar sharks exploited in multispecies fisheries (Abercrombie *et al.*, 2005).

In addition to the traditional use of morphology to distinguish species, molecular markers can be used to estimate the extent of evolutionary divergence between taxa. These markers have been used to resolve phylogenetic relationships among species in several shark groups and include allozymes (Smith & Benson, 2001), restriction fragment length polymorphisms (RFLPs) (Heist & Gold, 1999; Pank *et al.*, 2001) and multiplex polymerase chain reaction (PCR), including species-specific primer assays (Shivji *et al.*, 2002, 2005). Species identifications by DNA sequence analysis are also widely used to discriminate between species. Recently, Greig *et al.* (2005) and Ward *et al.* (2006) were able to successfully distinguish several shark species with a 1400 base pair (bp) mtDNA sequence spanning the 12S rDNA, 16S rDNA, tRNA-val and cox1 (655 bp) genes. MtDNA sequences have also been useful for resolving higher level relationships among the Carcharhiniformes and Lamniformes, among Sphyrnidae and Triakidae (Greig *et al.*, 2005) and Batoidea (Douady *et al.*, 2003).

In the present study, sequences of the entire NADH dehydrogenase subunit 2 (ND2) gene (1047 bp) and 60 morphometric measurements were examined in the two catshark species to test their validity. The ND2 gene has been used for phylogenetic analysis in several groups of organisms, including sharks (Naylor *et al.*, 1997). The results here provide evidence for two genetically well-differentiated catshark species. Single nucleotide polymorphisms (SNPs) within the ND2 gene provide several species markers for the unambiguous identification of individuals.

MATERIALS AND METHODS

SAMPLING

Individuals were captured in Autumn 2003 by the commercial fishing vessel 'Branca de Sagres' with deep-water longlines targeting the wreckfish, *Polyprion americanus* (Bloch & Schneider, 1801). Specimens of catsharks were caught southwest of the Cape São Vicente (36°50′ N; 9°05′ W), off the southwest tip of the Iberian Peninsula, at 550–590 m. Specimens of *G. melastomus* ranged in total length (L_T) from 345 to 527 mm, and specimens of *G. atlanticus* ranged from 336 to 440 mm. Samples of both species included both sexes.

MORPHOLOGICAL CHARACTERIZATION

Sixty morphological measurements were made on 33 specimens (20 *G. melastomus* and 13 *G. atlanticus*), following measurements in Compagno (2001). Measurements larger than 100 mm were made with 1 mm precision, and measurements smaller than 100 mm were made with 0·01 mm precision using an electronic dial calliper. Paired structures, such as pectoral and pelvic fins, were measured on only the left side of a specimen. Measurements were standardized by the L_T of a specimen, and morphological traits were expressed as minimum and maximum percentages of L_T.

MULTIVARIATE ANALYSIS

A multivariate analysis of the morphological characteristics was carried out with PRIMER 6 (Clarke & Warwick, 2001). Morphological measurements were standardized by L_T and used to calculate a matrix of Euclidean distances (Clarke & Warwick, 2001). Non-metric multidimensional scaling (MDS) of distances in this matrix was used to evaluate differences between the two species.

Analysis of similarities (ANOSIM) tests were used to determine whether the differences in the MDS plots were significant (Clarke & Warwick, 2001). This test was also used to search for sexual dimorphism in each species. A similarity of percentages analysis was used to calculate the percentage contribution of each morphological measurement to the overall difference between species (Clarke & Warwick, 2001).

MOLECULAR METHODS

Taking into account the multivariate morphometric results, the authors have randomly chosen 10 individuals from each of the two putative species found to sequence the protein-coding ND2 gene. Additionally, two specimens of *G. atlanticus* collected from the Alboran Sea during the Mediterranean International Trawl Survey cruise in April 2003 identified by field markings by Rey *et al.* (2006) were used in the present work as 'voucher' specimens: these specimens were carefully identified by experts. Also a single individual from Mauritania (where only *G. melastomus* has been described) caught in 2005 was included in the work. Samples were preserved in 96% ethanol solution and stored at −20° C. Total genomic DNA was extracted from white muscle using an Invitrogen Micro-tissue Extraction Kit®. A 1047 bp segment of the entire ND2 mitochondrial gene was sequenced. PCR amplifications were performed in a total 25 μl reaction volume of 2·5 μl 10× buffer, 4 mM $MgCl_2$, 0·2 μM deoxynucleotide triphosphates, 1·5 units *Taq* DNA polymerase and 0·3 μM of each primer ND2-Ile 5′-CCGGATCACTTTGATAGAGT-3′ (Naylor *et al.*, 1997) and ND2-Asn 5′-CGCGTTTAGCTGTTAACTAA-3′ (Kocher *et al.*, 1995). PCRs consisted of 40 cycles of 1 min at 94° C, 1 min at 45° C and 45 s at 72° C. Negative controls were included in each set of reactions. Amplifications were checked by electrophoresis in a 1% agarose gel and sequenced by Macrogen Inc, Seoul. Each individual was sequenced in both directions with the above primers.

STATISTICAL ANALYSIS

Sequences were aligned with Geneious (Drummond *et al.*, 2006) and checked manually. DnaSP 4 (Rozas *et al.*, 2003) was used to estimate nucleotide diversity and genetic distances. The monophyly of each species was assessed in phylogenetic trees constructed with maximum parsimony (MP) with PAUP* 4.0b10 (Swofford, 2002) using a heuristic search with random taxon addition and TBR branch swapping. Maximum likelihood (ML) trees were constructed with PHYML (Guindon & Gascuel, 2003) and with the HKY85 model of mutation (Hasegawa *et al.*, 1985) selected by Modeltest 3.7 (Posada & Crandall, 1998). The significances of nodes in trees were assessed with 1000 bootstrap resamplings (Felsenstein, 1985). Haplotype genealogies were constructed with a statistical parsimony network using TCS 1.21 (Clement *et al.*, 2000).

EnzymeX 3 (Griekspoor & Groothuis, 2006) was used to locate polymorphic nucleotide sites in RFLPs that were present in only one species for use in species identification.

RESULTS

MORPHOLOGICAL CHARACTERIZATION

Minimum and maximum standardized values of 60 morphological characters displayed some overlap between the two species (Table available from last author upon request). Prepelvic-fin length showed the least amount of overlap between species, ranged from 36·3 to 38·6% of L_T in *G. atlanticus* and from 38·3 to 41·9% of L_T in *G. melastomus.* Other characters, including nostril width, exhibited almost complete overlap ranging from 1·8 to 2·4% of L_T in *G. atlanticus* and from 1·6 to 2·5% of L_T in *G. melastomus*.

MULTIVARIATE ANALYSIS

A multivariate analysis of morphological differences between individuals showed clear separation between *G. melastomus* and *G. atlanticus* (ANOSIM, $P < 0{\cdot}05$) (Fig. 1). The 10 most informative morphological characters

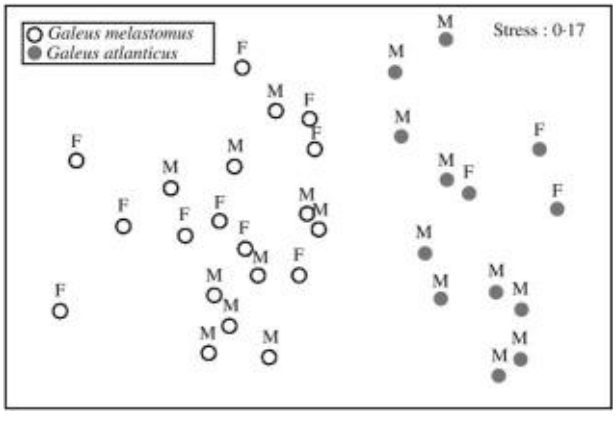

FIG. 1. Non-metric multidimensional scaling (MDS) of *Galeus melastomus* and *Galeus atlanticus* based on 60 morphologic measurements. In both species, M refers to males and F to females. The stress value (0·17) reflects the error created when multidimensional data are plotted in two axes.

accounted for 23·04% of the total variation, with individual traits contributing 2·12 to 2·45% to the total (Table I). Head and snout characters standardized by total length contributed most to differences between species. These characters included head length, preorbital length, prebranchial length, prenarial length and preoral length, all of which were larger in *G. melastomus*. Prepelvic-fin and preanal fins length also tend to be greater in *G. melastomus*. The position of the second dorsal fin and the length of the caudal peduncle (dorsal caudal-fin space) were generally larger in *G. atlanticus*.

MOLECULAR GENETICS

A total of 35 transitions and 17 transversions defined 13 ND2 haplotypes in 23 individuals of *Galeus* (GenBank accession numbers DQ902834 to DQ902846) (Table II). These sequences were aligned to homologous ND2 sequences from *Scyliorhinus canicula* (Linnaeus) (GenBank accession number NC001950) and contained no indels or stop codons. The 1047 bp *Galeus* sequences included 51 (4·9%) polymorphic sites, of which 38 (3·6%) were parsimony informative. Six haplotypes, defined by three transitions and two transversions at five polymorphic sites, appeared in 12 individuals of *G. atlanticus*. In *G. melastomus*, seven haplotypes were defined by seven transitions and five transversions at 12 polymorphic sites. A total of 35 (3·3%) fixed nucleotide substitutions appeared between species, and 16 (1·5%) of these substitutions represent amino acid replacements (Table II).

The two voucher individuals of *G. atlanticus* grouped both in haplotype 1, whereas the individual caught in Mauritania constitutes haplotype 2.

Hierarchical likelihood tests identified the HKY model of mutation (Hasegawa *et al.*, 1985) as the best fit to the data. The transition/transversion ratio for

TABLE I. Cumulative list of the 10 morphologic characters contributing most to the differences found between *Galeus melastomus* and *Galeus atlanticus*. Average value refers to each character value as a percentage of total length (L_T) and contribution differences refer to the contribution, in percentage, that each morphologic character is giving to the overall differences and the cumulative values to the successive sum of values

	Average value (%L_T)		Differences (%)	
Measurement	*G. atlanticus*	*G. melastomus*	Contribution	Cumulative
Prepelvic-fin length	37·20	39·75	2·45	2·45
Head length	19·43	21·30	2·42	4·87
Preorbital length	6·91	7·90	2·41	7·28
Internarial space	2·26	2·75	2·36	9·64
Dorsal caudal-fin space	5·20	3·74	2·35	11·99
Prebranchial length	15·08	17·03	2·26	14·25
Prenarial length	4·38	5·19	2·24	16·49
Preoral length	7·44	8·37	2·24	18·73
Preanal-fin length	51·42	54·28	2·19	20·91
Spiracle length	1·10	0·82	2·12	23·04

TABLE II. Variable sites in the 13 haplotypes identified, diagnostic characters (#) and changes in nucleotides that imply change of coding amino acid (*), frequencies of haplotypes of *Galeus atlanticus* (Ga) and *Galeus melastomus* (Gm)

Nucleotide positions																																																						
Diagnostic characters					#	#	#	#	#	#	#	#	#	#	#	#		#		#	#		#	#		#	#	#	#	#	#		#	#	#			#	#	#			#			#	#	#	#	#				
Change amino acid	*	*	*		*						*									*			*				*		*								*	*								*	*	*		*	*			
Haplotypes	66	68	74	81	144	225	231	237	256	267	268	321	330	366	369	382	423	438	441	452	460	465	490	504	523	534	589	600	618	669	717	756	759	765	768	780	809	820	837	861	864	888	933	936	948	955	956	961	970	1027	1028	Ga	Gm	GenBank accession number
H1	T	G	A	C	C	G	T	A	T	G	C	T	A	A	C	T	T	T	C	C	C	A	A	A	T	C	T	T	A	C	C	T	G	T	C	T	T	A	A	C	A	T	G	T	A	A	C	G	C	A	T	3	—	DQ902846
H2	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	1	—	DQ902842
H3	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	1	—	DQ902845
H4	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	1	—	DQ902844
H5	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	5	—	DQ902843
H6	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	1	—	DQ902841
H7	·	·	·	·	A	A	C	G	C	A	A	C	C	G	T	C	C	C	T	A	T	G	G	G	C	T	G	C	T	T	T	·	T	A	T	·	·	G	T	T	·	·	A	·	·	T	T	A	T	G	·	—	1	DQ902834
H8	·	·	·	·	A	A	C	G	C	A	A	C	C	G	T	C	C	C	·	A	T	·	G	G	C	T	G	C	T	T	T	·	T	A	T	·	·	G	T	T	·	·	A	·	·	T	T	A	T	G	·	—	3	DQ902837
H9	·	·	·	·	A	A	C	G	C	A	A	C	C	G	T	C	C	C	·	A	T	·	G	G	C	T	G	C	C	T	T	·	T	A	T	·	·	G	T	T	·	·	A	·	·	T	T	A	T	G	·	—	1	DQ902839
H10	·	·	·	·	A	A	C	G	C	A	A	C	C	G	T	C	C	C	·	A	T	·	G	G	·	T	G	C	C	T	T	·	T	A	T	·	·	G	T	T	·	·	A	·	·	T	T	A	T	G	·	—	1	DQ902840
H11	·	·	·	·	A	A	C	G	C	A	A	C	C	G	T	C	C	C	·	A	T	·	G	G	C	T	G	C	T	T	T	·	T	A	T	·	·	G	T	T	T	·	A	C	·	T	T	A	T	G	·	—	1	DQ902835
H12	·	·	G	·	A	A	C	G	C	A	A	C	C	G	T	C	C	C	·	A	T	·	G	G	C	T	G	C	T	T	T	·	T	A	T	·	G	G	T	T	·	G	A	·	G	T	T	A	T	G	G	—	1	DQ902838
H13	A	·	·	·	A	A	C	G	C	A	A	C	C	G	T	C	C	C	·	A	T	·	G	G	C	T	G	C	T	T	T	·	T	A	T	·	·	G	T	T	·	·	A	·	·	T	T	A	T	G	·	—	1	DQ902836

these sequences was 2·085, with equal substitution rates for all sites. Base frequencies were estimated to be A = 0·3150, C = 0·2509, G = 0·1040 and T = 0·3301. Corrected sequence divergences between haplotypes in *G. atlanticus* averaged 0·12 ± 0·08% but was 0·27 ± 0·18% in *G. melastomus*. The level of divergence between species was one order of magnitude higher at 3·39 ± 0·56%.

The topology of the MP tree (Fig. 2) was the same as the topology of the ML tree (not shown). In these trees, haplotypes were grouped into two clades corresponding to the two species. The bootstrap value between the branches leading to the two species was 100%, strongly supporting the monophyly of haplotypes from each species.

Haplotype networks for each species (Fig. 3) displayed a star-like shape pattern in which several low frequency haplotypes were located a few mutation steps from a common central haplotype. However, the network for *G. melastomus* included several hypothesized but unsampled intermediate haplotypes (six unobserved for seven observed). The network for *G. atlanticus* contained no intermediate unobserved haplotypes.

The identification of the two well-supported clades provides an opportunity to estimate the time of their divergence. However, there are no mutation rate estimations for ND2 in sharks to pursue this matter further.

Among the 35 diagnostic polymorphisms distinguishing these species from each other, are four recognition sites within restriction enzyme cleavage sites.

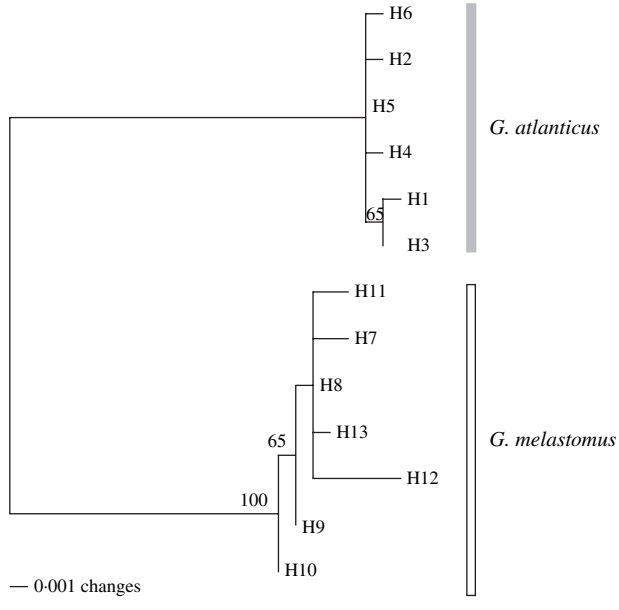

FIG. 2. Phylogenetic relationships inferred from a maximum likelihood tree of the ND2 gene sequences (1047 bp) using a HKY model of evolution (transition/transversion ratio of 2·085, proportion of invariable sites, $I = 0$ and equal rates for all sites). Data were constructed by using PAUP* (MP tree) and PHYML (ML tree), bootstrapped with 1000 replicates. Only bootstrap values over 50% are shown.

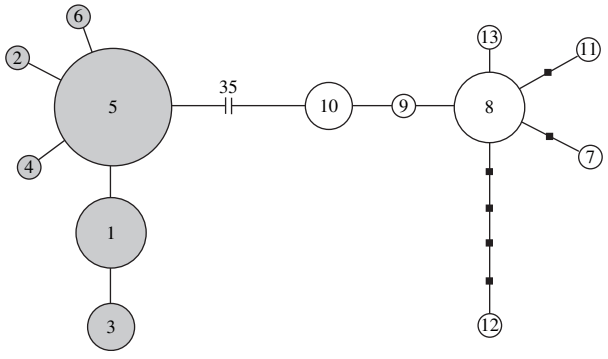

FIG. 3. Statistical parsimony network for ND2 haplotype of *Galeus atlanticus* (grey circles) and *Galeus melastomus* (white circles). Each haplotype is defined by its corresponding number in Table III. Unobserved intermediate haplotypes are indicated as black squares. The size of circles is proportional to the haplotype frequency.

Recognition sites in only *G. atlanticus* included variable positions at sites 225 (enzyme *Nmu* CI – GTGAC), 256 (*Asu* HPI – GGTGAGTGAAGTT) and 759 (*Bst* EII – GGTTACC). Sequences for *G. melastomus* had one restriction enzyme recognition site at position 768 (*Eco* NI – CCTCTCTCAGG).

DISCUSSION

Numerous studies of molecular markers in fishes have demonstrated the presence of previously unrecognized distinct species in groups of taxa that are often morphologically cryptic (Carreras-Carbonell *et al.*, 2005; Gharrett *et al.*, 2006). *Galeus atlanticus* has had a long uncertain taxonomic history, after it was originally described by Vaillant (1888). The name was considered a synonym of *G. melastomus*, because of its morphological similarity (Compagno, 1984), but resurrected again based on small differences in morphological ratios and meristic values (Muñoz-Chapuli & Ortega, 1985). *Galeus atlanticus* was recently redescribed by Rey *et al.* (2006) based on field markings. Genetic data is now presented completely validating the separation of *G. atlanticus* from *G. melastomus*.

The analyses of morphological and genetic data in the present study provide evidence for the taxonomic separation of *G. atlanticus* and *G. melastomus* species and for the occurrence of *G. atlanticus* in Portuguese as well as in Mauritanian waters.

Although none of the individual morphological characters exhibit significant differences between species, the analysis of 10 characters shows a clear separation between species. Vaillant (1888 in Compagno & Niem 1999) previously expressed a similar conclusion in the original description of *G. atlanticus* and recognized the need to use several characters to discriminate between these two catshark species. The result here, however, shows that *G. melastomus* tends to have a longer head and wider snout than *G. atlanticus*. The use of multivariate analysis is ideal for combining the small effects of individual characters into an overall measure of morphological divergence.

Despite the low level of morphological differentiation, a substantial level of mtDNA ND2 divergence (3·39%) indicates a large amount of evolutionary divergence between these species of catshark. The level of divergence between these taxa far exceeds the level of sequence divergence between intraspecific haplotypes and provides a strong rationale for separating these species. These two well-supported clades correspond to the cladistic haplotype aggregation species definition (Brower, 1999; Sites & Marshall, 2003), which assumes that monophyly of haplotypes at one locus can be used to define a species.

The level of molecular divergence between species also provides 35 diagnostic SNP species markers suitable for the species identification of individual fish. Although the number of fixed substitutions is likely to decrease with larger samples, the ND2 gene still provides a substantial number of species markers. In practice, this means that consistent and robust identifications of both species can be made with DNA sequences or with SNP assays, such as PCR-RFLP. Research vessels and fisheries organizations operating in the North-eastern Atlantic area where blackmouth and Atlantic catsharks are present should make provisions to sample tissue from by-catch individuals, as SNP scoring is considerably cheaper when compared with other molecular techniques.

Corrected sequence divergences between haplotypes within *G. melastomus* were more than double the haplotype divergences in *G. atlanticus*. It is uncertain whether this reveals an underlying difference in the level of diversity within each species or whether the difference is due to sample error from relatively small sample sizes. However, sample sizes were similar for both species, as was the number of observed haplotypes. Moreover, both haplotype diversities in *G. atlanticus* ($h = 0{\cdot}812$) and *G. melastomus* ($h = 0{\cdot}911$) and nucleotide diversities ($\theta_\pi = 0{\cdot}12$ and 0·27%, respectively) are similar to those in other species of shark (Table III). Haplotype diversities among the seven species listed in the Table III range from 0·717 to 0·956, and nucleotide diversities range from 0·12 to 2·2%. Among the species listed in Table III, the white shark (*Carcharodon carcharias*) (Linnaeus) and scalloped hammerheads (*Sphyrna lewini*) (Griffith and Smith) have a within-species nucleotide diversity that is an order of magnitude larger than other species and similar to the values for both species of *Galeus* combined. The white and the scalloped hammerhead sharks data (Pardini *et al.*, 2001; Duncan *et al.*, 2006) were estimated on the basis of the evolutionary and geographically deep lineages from worldwide samples, and this fact may well explain the greater nucleotide diversities observed. The difference in diversity between the two *Galeus* species, if confirmed by subsequent sampling, may be due to differences in mutation rate between species or due to a longer evolutionary age or greater geographic subdivision in *G. melastomus*. A likelihood ratio test did not reject the hypothesis of homogeneous evolutionary rates among branches of the trees for both species, indicating that different mutation rates are unlikely to have produced the difference in diversity. An alternative explanation is that the *G. melastomus* lineage is older than the lineage leading to *G. atlanticus*. However, the differences in diversity may also be due to the indirect effects of ecology and biogeography on genetic diversity. *Galeus melastomus* is geographically more wide spread and populations of this species may be more fragmented than those of *G. atlanticus*. This difference could lead to greater levels of geographic diversity and hence greater diversity

TABLE III. Summary genetic statistics for several shark species

Species	Geographical range sampled	Marker	Haplotype diversity	Nucleotide diversity	Reference
Cetorhinus maximus	Worldwide	Control region	0·720 ± 0·028	0·0013 ± 0·0009	Hoelzel *et al.* (2006)
Carcharhinus limbatus	Northwestern Atlantic Ocean, Gulf of Mexico and Caribbean Sea	Control region	0·805 ± 0·018	0·0021 ± 0·0013	Keeney *et al.* (2005)
Carcharias taurus	South Africa	Control region	0·717 ± 0·010	0·0030 ± 0·0001	Stow *et al.* (2006)
Carcharodon carcharias	South Africa, Australia and New Zealand	Control region	0·956 ± 0·028*	0·022 ± 0·001*	Pardini *et al.* (2001)
Sphyrna lewini	Worldwide	Control region	0·820 ± 0·080	0·0130 ± 0·0068	Duncan *et al.* (2006)
Galeus atlanticus	South Iberia + Mauritania	ND2	0·812 ± 0·082	0·0012 ± 0·0009	Present study
Galeus melastomus	South Portugal	ND2	0·911 ± 0·077	0·0027 ± 0·0018	Present study
All *Galeus*	South Iberia + Mauritania	ND2	0·929 ± 0·033	0·0198 ± 0·001	Present study

*Calculated from sequences GenBank Accession numbers: AY026196–AY026224.

in the sample of *G. melastomus* examined in this study. Ecological or behavioural differences between species may also indirectly influence genetic diversity. These hypotheses are difficult to test with the limited set of data available.

What ecological or palaeoclimate events may have led to the separation of ancestral populations? One important feature of these species is the inclusion of *G. atlanticus* within the geographical range of *G. melastomus*. Sympatry can occur after allopatric isolation and subsequent migration after the removal of a barrier. Another possibility is that one of these species is more closely related to a species located elsewhere, perhaps in the South-eastern Atlantic. The lack of a close outgroup taxon within the genus *Galeus* precludes a firm assessment of sister-species status of the two species examined in this study. Other species in the genus *Galeus* (*Galeus polli* [Cadenat, 1959] and *Galeus murinus* [Collett, 1904]) with an eastern Atlantic distributions, which do not include the present study area, are morphologically well differentiated from both blackmouth and Atlantic catsharks (Compagno, 1984). It is therefore logical to assume that the two species are sister taxa, and the sympatry of *G. atlanticus* within the range of *G. melastomus* may reflect some form of ecological adaptive speciation where similar environmental adaptive pressures have limited morphological differentiation despite relatively large genetic distance between them.

Evolutionary isolation through ecological divergence has been implicated in some instances, for example in Brazilian reef fishes (Rocha *et al.*, 2005), and may also be relevant to the origins of these catshark species.

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