



Molecular Systematics of the Long-Snouted Deep Water Dogfish (Centrophoridae, *Deania*) With Implications for Identification, Taxonomy, and Conservation

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According to the most recent taxonomical revision, the deep-sea dogfish genus *Deania* encompasses four species. Three of them, *D. calcea*, *D. profundorum*, and *D. hystricosa*, occur in the North Atlantic. Whilst *D. profundorum* can be identified by the presence of a subcaudal keel, the other two species are not easily visually distinguished. Uncertainties over identification raises concerns over stock units and whether management plans are adequate. In this study we compared onboard visual identification of *Deania* specimens, with morphological inspection of skin denticles under stereo microscope and with independent molecular taxonomical assignment using two molecular markers. Particular emphasis was paid to specimens identified as *D. calcea* and *D. hystricosa* in the NE Atlantic where these species potentially occur sympatrically and may be easily confused. In the past the species have been discriminated on the basis of the size of skin denticles (skin roughness), but our study showed that the crown length of skin denticles covaries with size (and sex), irrespective of species, and therefore this is not a reliable morphological character and should not be used to discriminate between the two species. Phylogenetic analyses did not indicate that *D. hystricosa* to be a distinct lineage from *D. calcea*. Interestingly, however four individuals (specimens from: UK, Azores Is., Madeira Is. and Seine seamount) formed a well-defined sub-clade nested within the *D. calcea* clade, possibly a signature of a past vicariance event or a result of coalescent stochasticity.

Keywords: deep-sea sharks, fishery by-catch, phylogeny, Atlantic Ocean, deep-sea dogfish, skin denticles

INTRODUCTION

The vulnerability of sharks to commercial fishing pressure has been well-documented (Baum et al., 2003; Myers and Worm, 2003) and it is widely acknowledged that deep-sea sharks (>200 m depth) are some of the most vulnerable species to fishing pressure (Garcia et al., 2008). Deep-sea sharks have slow growth rates, long life span with late maturation, very low fecundity, and long

gestation periods (up to 2 years). Hence deep-sea sharks have population doubling times that can be twice as long as their shallower counterparts (Stevens et al., 2000) and populations can take decades to recover from fishing impacts (Garcia et al., 2008; Simpfendorfer and Kyne, 2009). An International Plan of Action for the Conservation and Management of Sharks adopted in 1999 stipulated that shark-fishing countries should produce national programmes for the conservation and management of sharks. However, only a few countries are currently providing detailed information on deep-sea bycatch, with many lumping species together as “deep-sea sharks” when they report their catches (Ebert and Mostarda, 2015). Furthermore, when species names can be traced back from the original datasets, frequent misidentification and/or taxonomic uncertainties make it difficult to estimate the true impact the fishery is having on deep-sea shark species (e.g., Iglésias et al., 2010; Veríssimo et al., 2014).

Despite their ecological importance and the fact that many are red-listed by the International Union for Conservation of Nature (IUCN, 2020), phylogenetic relationships within deep-sea sharks are still poorly understood and taxonomy is still a challenge (e.g., Veríssimo et al., 2014; White et al., 2017). In this study, we focus on the four nominal species of the deep-sea squaloid sharks belonging to the genus *Deania* (Jordan and Snyder, 1902) from the family Centrophoridae. The genus *Deania* includes species commonly caught by deep-sea commercial fisheries. The conservation and extinction risk assessed by the IUCN reports that *D. calcea* is categorized as “least concern,” same for all other *Deania* spp. with the exception of *Deania hystricosa* reported as “data deficient” (Nieto et al., 2015; Millar and Dickey-Collas, 2018; IUCN, 2020). *Deania* spp. are bathydemersal species inhabiting continental slopes and seamounts usually between 200 and 2,000 m depth (Bass et al., 1976; Yano, 1991; Compagno, 2005; Weigmann, 2016) depending on the latitude. In terms of distribution, the long snouted deepwater dogfish *Deania calcea* (Lowe, 1839) and the arrowhead dogfish *Deania profundorum* Smith and Radcliffe, 1912 have been described as present worldwide; while the rough longnose dogfish *Deania hystricosa* Garman, 1906 and the longsnout dogfish *Deania quadrispinosa* McCulloch, 1915 have patchy records (Froese and Pauly, 2019).

Identification keys are effective to distinguish *Deania* species from other deep-water sharks, but within the genus it is problematic to discern among the species. Whilst *D. profundorum* can be identified by the presence of a subcaudal keel on the lower surface of the caudal peduncle (Ebert and Stehmann, 2013), the other species lack this feature and are not easily distinguished by visual examination. *D. quadrispinosa* can be distinguished from either *D. calcea* or *D. hystricosa* by comparing the distance from exposed origin of first dorsal-fin spine to first dorsal fin rear tip, and the distance from free rear tip to second dorsal-fin spine (Ebert and Mostarda, 2015). According to the literature, separation between *D. calcea* and *D. hystricosa* is based on the skin denticle size (Compagno, 2005; Ebert and Stehmann, 2013). Moderately large lateral trunk denticles (crown length of about 0.5 mm) correspond to *D. calcea*, while very large denticles (crown length of about 1 mm) correspond to *D. hystricosa* (e.g., Ebert and Stehmann, 2013). Due

to these putative differences, some simplified guides for onboard practical identification indicate *D. hystricosa* as the species with very rough skin (Ebert and Fowler, 2015) or “harsh to the touch” as reported in the original description by Garman (1906). Measurement of denticle size while onboard is difficult and impractical, and using the level of “skin roughness” is a subjective criterion. Furthermore, the relationship between body size and skin roughness (denticle size and density) has not yet been investigated. Uncertainties on assigning the correct identification while onboard has raised concern for the correct management of local as well as global fisheries (Ebert and Stehmann, 2013).

In this study we compared onboard visual identification of *Deania* specimens with laboratory inspection of skin denticles under stereo microscope and two molecular markers for an independent taxonomical assignment. Particular emphasis was paid to *D. calcea* and *D. hystricosa* in the NE Atlantic and, by performing a detailed examination of skin denticles on representative specimens belonging to these two putative species, we evaluated the diagnostic performance of skin denticle characters for species discrimination.

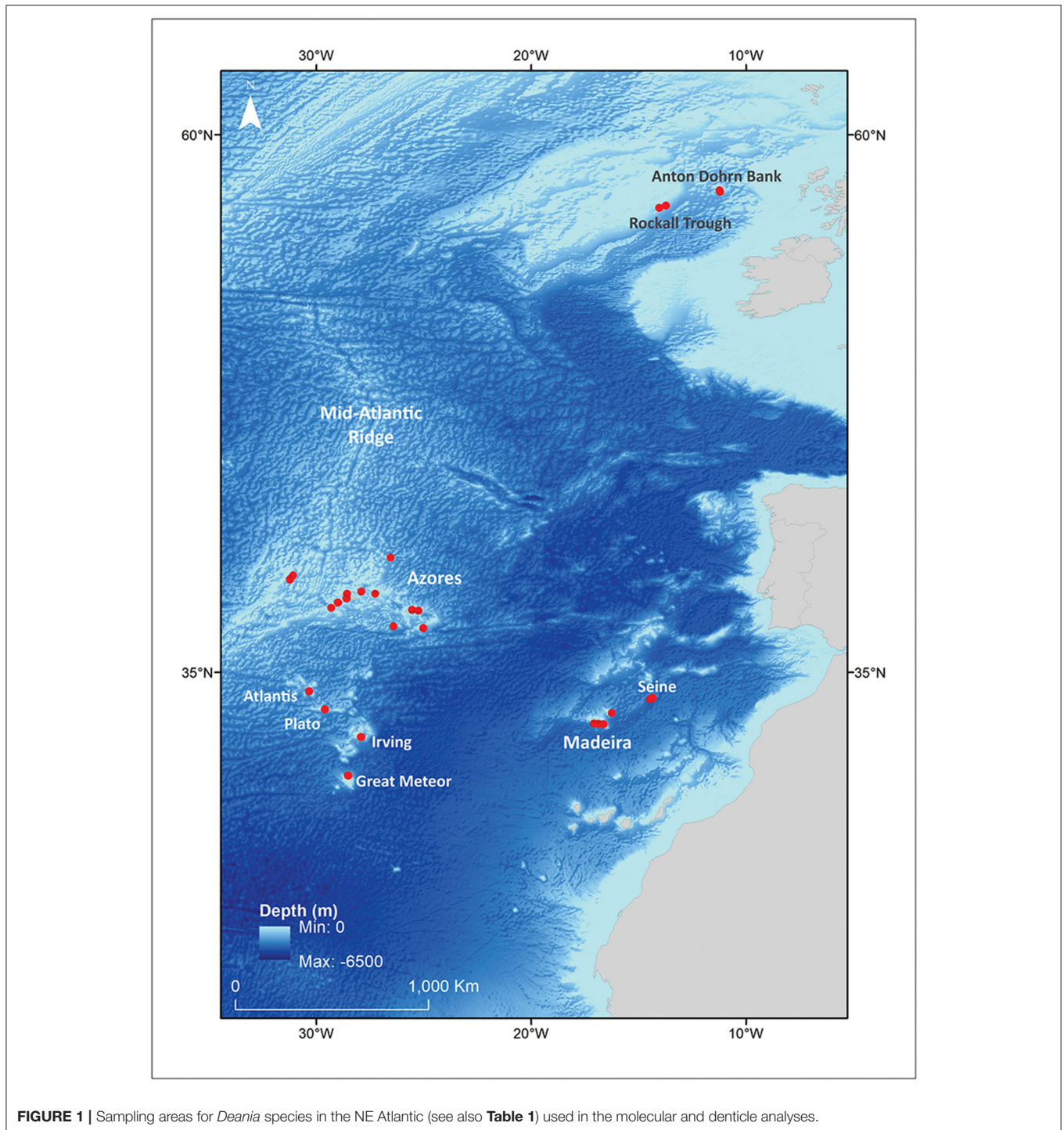
MATERIALS AND METHODS

Sample Collection

A total of 271 *Deania* samples were collected between 2003 and 2010 from various scientific surveys (Figure 1). All specimens were measured, sexed, and identified onboard according to key morphological characters (e.g., Ebert and Stehmann, 2013). Preliminary identification of the specimens was: 76 *D. calcea*, 42 in doubt between *D. calcea* and *D. hystricosa*, 63 *D. hystricosa*, and 90 *D. profundorum*. A subset of 117 representative samples was selected for further molecular screening (Table 1, Supplementary Table 1). Samples of white muscle tissue were stored in 95% ethanol and maintained at -20°C until processed for molecular analysis.

Molecular Analysis

Total genomic DNA was extracted from a small portion of muscle tissue using the E.Z.N.A.[®] Mag-Bind Tissue DNA Isolation Kit (Omega Bio-tek) following the manufacturer’s protocol and using the autonomous extractor KingFisher mL (Thermo—Electron Corporation, USA). A fragment of ~680 bp of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified in 102 individuals by polymerase chain reaction (PCR) using FishF1 and FishR1 primers (Ward et al., 2005). A ~1,100 bp fragment encompassing the entire mtDNA control region was amplified by PCR in 108 individuals using primers Pro-L and 12S282, which are complementary to the flanking proline tRNA and 12S rRNA genes, (Keeney et al., 2003), respectively. PCR reaction mixtures were similar for both markers and included ~20 ng of DNA template, 0.2 μM of each primer, 10 μL of 2x PCR Master Mix (Promega) containing the reaction buffer, dNTPs, MgCl₂, and *Taq* polymerase, and ultra-pure water to a final volume of 20 μL. The PCR thermal profile was 2 min at 94°C, 35 cycles of 30 s at 94°C, 1 min at 50°C (for COI) or 57°C (for the control region) and 1.5 min at 72°C, and a final extension step of 7 min at 72°C. A no template control



reaction was included in all PCR reactions. PCR products were either purified with ExoSAP-IT (GE Healthcare) or separated by electrophoresis on a 1% agarose gel and excised using the illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare). Sequencing of both strands of each PCR product was performed at BMR Genomics commercial facility (www.bmr-genomics.it) based in Padua, Italy, using the same set of primers.

Sequences were checked using Aliview v. 1.24 (Larsson, 2014) and aligned by eye or using MAFFT v. 7 (Kato and Standley, 2013). Fifty additional *Deania* COI sequences available in Genbank and BOLD System (**Table 1**, **Supplementary Table 1**) were added along with sequences of *Centrophorus squamosus* (COI: JF493080) and *Squalus acanthias* (NC_002012 for both CR and COI) to be used as outgroups. Missing or ambiguous

TABLE 1 | List of *Deania* specimens collected for this study and mtCOI and/or mtCR sequences available in literature or public databases.

Ocean	Area	Location	Year	N	Visual ID	Sequencing	Depth (m)	Sex	References	
Atlantic	UK	Anton Dohrn Seam.	2006	3	<i>D. calcea</i>	2CR; 1COI+CR	650–675	2M; 1F	In this study	
		Rockall Through Seam.	2006	7	<i>D. calcea</i>	3COI; 4COI+CR	650–850	5M; 2F	In this study	
		Ireland	2006	1	<i>D. calcea</i>	COI	?	?	Veríssimo et al., 2014	
	MAR-middle	Azores bank		2007	1	<i>D. calcea/D. hystricosa</i>	COI+CR	975	F	In this study
					4	<i>D. profundorum</i>	CR	775–975	2M; 2F	In this study
		Princess Alice bank	2003	1	<i>D. profundorum</i>	COI	751–800	M	In this study	
		Faial/Pico Is.		2007	5	<i>D. calcea/D. hystricosa</i>	1CR; 4COI+CR	975	3M; 2F	In this study
					8	<i>D. profundorum</i>	7CR; 1COI+CR	725–775	8F	In this study
		southern Pico Is.	2009	1	<i>D. hystricosa</i>	COI+CR	1,120	F	In this study	
		Flores/Corvo Is.		2007	6	<i>D. calcea/D. hystricosa</i>	1CR; 5COI+CR	575–1,175	5M; 1F	In this study
					1	<i>D. profundorum</i>	CR	725	1F	In this study
		Flores Is.	2003	2	<i>D. profundorum</i>	COI+2CR	775	1M; 1F	In this study	
		Terceira Is.	2007	1	<i>D. profundorum</i>	CR	625	F	In this study	
		S. Miguel Is.	2007	12	<i>D. profundorum</i>	1COI; 9CR; 2COI+CR	675	6M; 2F; 4?	In this study	
		S. Maria Is.	2007	7	<i>D. profundorum</i>	CR	625–775	2M; 5F	In this study	
		Sedlo Seam.	2003	1	<i>D. calcea</i>	COI+CR	901–950	M	In this study	
		Mid-Atlantic Ridge	?	3	<i>D. hystricosa</i>	COI	?	?	EU148140-1 ^a ; MAECO250-06 ^b	
	MAR-southern	Atlantis Seam.		2007	1	<i>D. calcea</i>	COI	975	M	In this study
					5	<i>D. calcea/D. hystricosa</i>	2CR; 3COI+CR	825–975	2M; 3F	In this study
					2	<i>D. profundorum</i>	COI	625–775	2F	In this study
		Great Meteor Seam.		2007	1	<i>D. calcea</i>	COI+CR	825	F	In this study
					5	<i>D. calcea/D. hystricosa</i>	COI+CR	1,275	M	In this study
				5	<i>D. profundorum</i>	1COI; 4COI+CR	825–975	1M; 4F	In this study	
Atlantic	MAR-southern	Irving Seam.	2007	9	<i>D. profundorum</i>	4CR; 5COI+CR	725–825	3M; 5F; 1?	In this study	
		Plato Seam.	2007	3	<i>D. calcea/D. hystricosa</i>	CR	925	3M	In this study	
Atlantic	Madeira Is.	Seine Seam.	2004	4	<i>D. calcea</i>	2COI; 2COI+CR	850–1,250	3M; 1F	In this study	
				2	<i>D. profundorum</i>	COI	700–850	2F	In this study	
			2	<i>D. hystricosa</i>	COI+CR	1,000	2M	In this study		
	Bay of Funchal		2004	13	<i>D. hystricosa</i>	2COI; 11COI+CR	1,000	7M; 6F	In this study	
		Porto Santo	2005	1	<i>D. hystricosa</i>	COI+CR	1,500	F	In this study	
		Desertas Is.	2005	1	<i>D. hystricosa</i>	COI+CR	1,000	F	In this study	
	Cabo Girão		2011	1	<i>D. hystricosa</i>	COI	1,200	F	In this study	
			?	3	<i>D. calcea</i>	COI	?	?	KJ083233-5 ^a	
	Iberian Peninsula	Portugal			2	<i>D. profundorum</i>	COI	?	?	KJ083236-7 ^c
				2006	4	<i>D. profundorum</i>	COI	735	?	Costa et al., 2012
Spain		?	2	<i>D. calcea</i>	COI	?	?	JN161152-3 ^a		
				1	<i>D. profundorum</i>	COI	?	JN161154 ^a		
USA	Gulf of Mexico	2009	2	<i>D. calcea</i>	COI	?	2F	GMSHK078-9 ^{b,c}		
Pacific	Australia	Tasman Sea	2010	9	<i>D. calcea</i>	CR	?	4M; 5F	Genetics in this study; Samples from Rochowski et al. (2015)	
		Tasman Sea	1998	4	<i>D. calcea</i>	COI	950–1,121	?	Ward et al., 2005	
		New S. Wales	?	1	<i>D. quadrispinosa</i>	COI	?	M	IRREK406-08 ^b	
	Chile	?	2007	8	<i>D. calcea</i>	COI	?	?	KU737827-34 ^a	
		Huinay	2007	1	<i>D. calcea</i>	COI	?	?	Straube et al., 2010	
	Taiwan	?	2005	1	<i>D. quadrispinosa</i>	COI	?	F	FOAG104-07 ^b	
		?	2014	6	<i>D. quadrispinosa</i>	COI	?	?	Chuang et al., 2016	
		Taitung County	2001/3	1	<i>Deania</i> sp.	COI	?	?	Liu et al., 2013	
	Indian	India	?	?	5	<i>D. profundorum</i>	COI	?	?	KF899378-82 ^a
		south Indian O.	?	?	1	<i>D. profundorum</i>	COI	?	?	AB639850 ^a

M = male; F = female; ? = missing information.

^aGenbank.

^bBOLD.

^cSequences wrongly assigned to *Deania*, therefore not used in our analysis.

end regions were trimmed to the same length so that all COI sequences in the data set had the same final length of 619 bp, while the length of CR sequences varied from 1,008 to 1,013 bp. Summary statistics (number and frequency of haplotypes, number of polymorphic sites, transitions, transversions, and nucleotide composition), haplotype diversity (h) and nucleotide diversity (π) were computed using DnaSP 5.10 (Librado and Rozas, 2009).

We used jmodeltest (Guindon and Gascuel, 2003; Darriba et al., 2012) for the nucleotide substitution model selection under AICc (Akaike, 1974), and MrBayes v. 3.2.6 (Ronquist et al., 2012) and PhyML v 3.0 (Guindon and Gascuel, 2003) for phylogeny reconstruction (Bayesian inference, BI, and Maximum likelihood, ML, respectively). We have reconstructed phylogenies for both markers and for the concatenated data set. We ran PhyML online on the ATCG bioinformatics platform (<http://www.atcg-montpellier.fr/>) using the proposed model and assessed node supports with 1,000 bootstrap replicates. We have run MrBayes on the CIPRES Science Gateway portal v. 3.3 (Miller et al., 2010) using the BEAGLE library (Ayres et al., 2012) in two independent runs of 10 million generations sampled every 1,000 generations for each of the data sets. After each run we verified adequate sampling (ESS >> 200) and convergence with Tracer (Rambaut et al., 2014) and applied a 25% burn-in.

To resolve the phylogenetic relationships between the main *Deania* clades we compared the marginal likelihoods of different topological hypothesis. We estimated those using the Bayes factors (BF) and the stepping-stone (SS) approaches as proposed by Xie et al. (2011). For the marginal likelihood calculations we selected a subset of COI sequences composed of two sequences from each clade and sequences belonging to the outgroups. We used pairwise distances estimated with Maximum Composite Likelihood in MEGA X v. 10.1 (Stecher et al., 2020) to select the two most distant sequences from each clade. We tested the topological hypothesis in MrBayes by placing a hard constrain to force the sister relationships between all the combinations of the three *Deania* clades and compared their marginal likelihoods. For the BF approach we estimated marginal likelihoods using harmonic means of the likelihood values of the MCMC sample by running two independent runs of 10 million generations sampled every 1,000 generations for each of the three tested hypotheses. Finally, we plotted the distribution of pairwise genetic distances using uncorrected p-distances and applied two species delimitation models for testing the presence of a barcoding gap and partitioning the data into candidate species. We applied the sequence-based ABGD (Automatic Barcode Gap Discovery; Puillandre et al., 2012) and the phylogeny-based mPTP (Multi-rate Poisson tree processes; Kapli et al., 2017) models to both mtDNA markers. ABGD analysis was performed using the default setting with both pairwise uncorrected p-distances and by applying the K2P nucleotide substitution model; while the mPTP analyses were fed with the BI tree performing four independent MCMC chains each lasting 10 million iterations with samples saved every 50,000 generations.

The relationships between the COI and CR haplotypes were also estimated and visualized as haplotype networks constructed using the Integer neighbor-joining (IntNJ)

technique approach available in the PopART software v. 1.7 (Leigh and Bryant, 2015).

Dermal Denticle Analysis

A portion of skin was collected from the area just below the first dorsal fin from specimens caught in the Azores and Madeira archipelagos. A total of 149 *Deania* species skin were sampled from individuals of different sizes and sexes, collected between 575 and 1,500 meters depth. Skins were then inspected under a Leica MZ 16FA stereomicroscope, using a 13.5x zoom, and measurements of the denticles were taken using tpsDig 2.31 software (Rohlf, 2017). *D. profundorum* were checked for comparison purposes only, as emphasis was addressed on those specimens visually identified as either *D. calcea*, *D. hystricosa* or of uncertain taxonomic status (*D. calcea*/*D. hystricosa*).

Images were taken from the top and the side to reveal details on shapes and sizes of the denticles, and the crown lengths were used to perform a multiple regression analysis using linear models. The mean of the measurement of three dermal denticles belonging to each specimen was used to investigate any correlation between biological variables (total length, sex, and capture depth) and denticle size and the interaction effect between variables. The best model fit was chosen according to the lowest AIC value, and in case the difference in AIC between two models were <2 units, the model with less estimated parameters was selected for statistical inference (Burnham and Anderson, 2002). Statistical analyses were performed in R v. 3.6.3 (R Core Team, 2020).

RESULTS

Genetic Diversity Among *Deania* Species

The concatenated sequence alignment was 1,663 bp long (1,629 bp without outgroups): 619 bp (for COI) and 1,012 bp (1,044 bp without outgroups; for CR), respectively. All sequences were deposited in Genbank (**Supplementaries Table S2, S3**). All samples visually identified as *D. profundorum* and *D. quadrispinosa* grouped according to their predicted taxonomy, except for two *D. calcea* sequences downloaded from BOLD that were wrongly identified as *D. quadrispinosa* (FOAN841-11.COI-5P, FOAN842-11.COI-5P). On the other hand, the specimens initially identified as *D. calcea* or *D. hystricosa* grouped together in a single clade except for four specimens that clustered in a separate well-defined subclade. The large clade was named *D. calcea* while the subclade (housing haplotypes from the UK, Azores Is., Madeira Is., and Seine seamount) was named *D. calcea2* (**Figure 2**). While for two of the four *D. calcea2* specimens both markers were sequenced (shared haplotype for CR “CR_DH-SH01,” and unique haplotypes for COI “COI_DH-MD102” and “COI_DH-MAD115”) for the other two only the CR (haplotypes “CR_DH-UK135” and “CR_DH-AZ102”) was sequenced (**Figure 2**).

Overall, the analysis of COI sequences of 144 and CR sequences of 111 *Deania* individuals, identified 30 COI and 26 CR distinct sequences, respectively. Most of these sequences belonged to the *D. calcea* clade: 83 COI (19 haplotypes) and 60 CR (20 haplotypes). The *D. profundorum* clade had a smaller

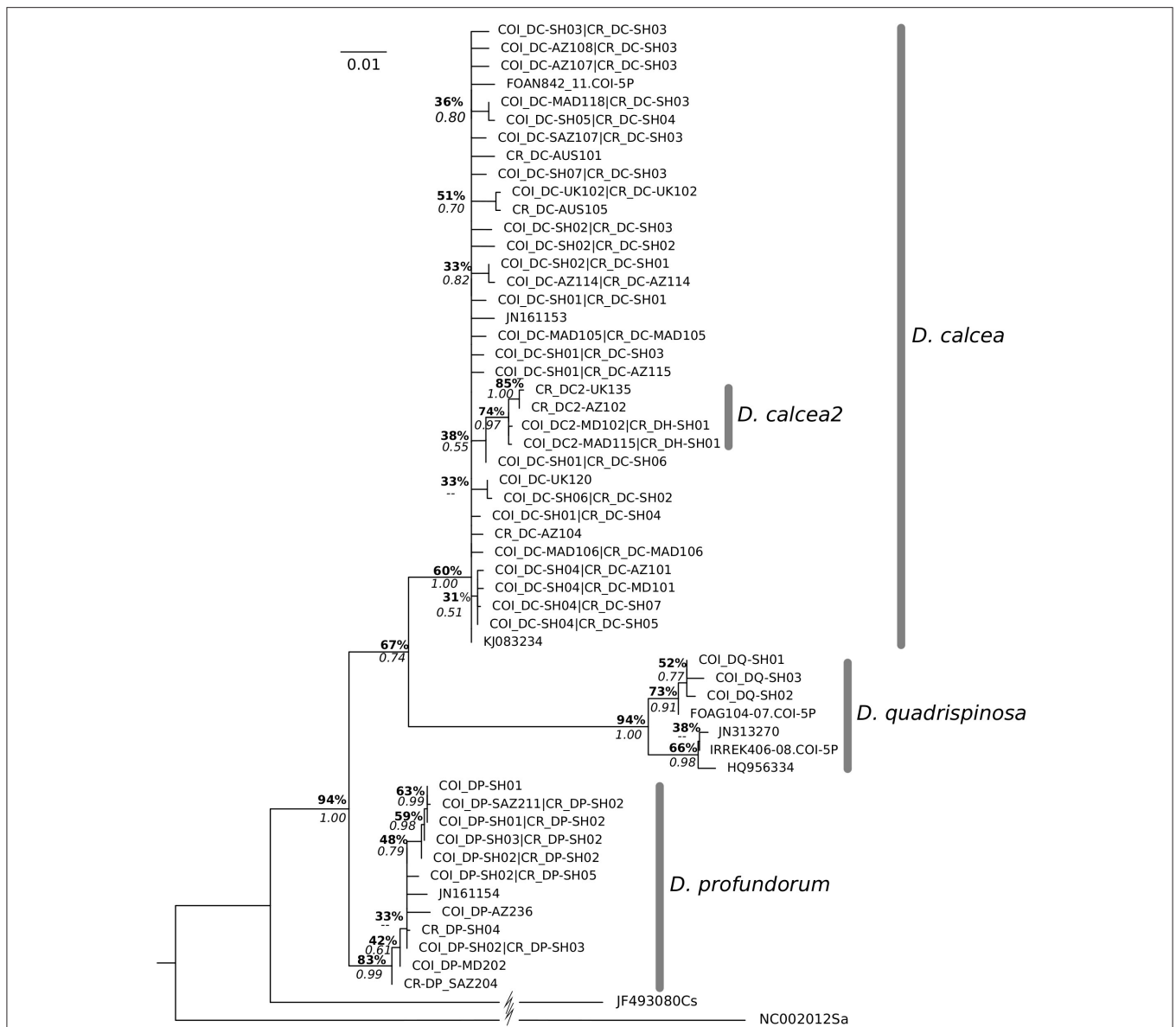


FIGURE 2 | Bayesian phylogenetic tree of *Deania* species obtained using concatenated sequences (COI and CR). The scale bar indicates the number of substitutions per site and values at branches represent Bayesian posterior probabilities and bootstrap values obtained for the Maximum Likelihood tree. Nodes where Bayesian posterior probability was under 0.50 are not reported.

number of sequences: 55 COI (eight haplotypes) and 48 CR (six haplotypes). The *D. quadrispinosa* clade had the lowest number of sequences: 12 COI (seven haplotypes). The *D. calcea* clade hosted the highest levels of haplotype diversity in CR ($Hd = 0.889$), while *D. quadrispinosa* showed the highest in COI ($Hd = 0.872$), and *D. profundorum* had the lowest values for both mtDNA genes ($Hd = 0.654$ and 0.671 , in CR and COI respectively) (Table 2). *D. quadrispinosa* hosted by far the highest levels of nucleotide diversity in COI ($\pi = 0.0086$), while values for *D. profundorum* were among the lowest for both genes ($\pi = 0.0020$ and 0.0025 , in CR and COI, respectively) (Table 2).

The best nucleotide substitution model for COI and CR was HKY+G+I (Hasegawa et al., 1985; Yang, 1993). The phylogenetic

inference based on the concatenated, as well as separated mitochondrial DNA markers (Supplementary Figures 1, 2), retrieved four distinct clades with *D. quadrispinosa* being the sister taxon to the clade *D. calcea* and *D. profundorum* represented the basal split within *Deania* (Figure 2). Both tree topologies placed *D. calcea2* subclade nested within the *D. calcea* grouping (Figure 2). Bootstrap support and posterior probability values of the *D. calcea2* subclade nested within the *D. calcea* grouping are not very high (0.74 and 0.97, respectively; Figure 2), but there is consistency in recovering the same topology also from phylogenetic inference using individual genes. Marginal likelihood of this tree topology has been compared with the other two alternatives by placing a hard constrain to force different

TABLE 2 | Summary statistics for COI and CR.

	N	H _n	H _u	S	M	ti/tv	H _d	π
COI								
<i>Deania</i>	150	36	22	60	69	5.65	0.882	0.0253
<i>D. calcea</i>	83	19	15	19	21	3.83	0.760	0.0025
<i>D. calcea</i> (no <i>D. calcea2</i>)	81	17	10	15	16	5.19	0.748	0.0017
<i>D. calcea2</i>	2	2	2	1	1	–	0.500	0.0016
<i>D. profundorum</i>	55	8	4	8	8	7.01	0.654	0.0020
<i>D. quadrispinosa</i>	12	7	4	16	16	10.10	0.872	0.0086
CR								
<i>Deania</i>	111	26	13	49	49	2.64	0.904	0.0186
<i>D. calcea</i>	63	20	12	17	17	2.87	0.889	0.0023
<i>D. calcea</i> (no <i>D. calcea2</i>)	59	17	10	13	13	2.62	0.874	0.0020
<i>D. calcea2</i>	4	3	2	3	3	2.00	0.833	0.0018
<i>D. profundorum</i>	48	6	1	9	9	3.51	0.671	0.0025

N = number of individuals; H_n = number of haplotypes; H_u = number of unique haplotypes; S = number of polymorphic (segregating) sites; M = total number of mutations; ti/tv = transition to transversion ratio; H_d = haplotype diversity; π = nucleotide diversity.

TABLE 3 | Marginal likelihood calculations for the three tree topologies using subset of COI sequences (see text for details) by placing a hard constrain to force different sister relationships within the *Deania* clade.

Topology	Marginal likelihood (SS)	Marginal likelihood (BF)
(DC,DP)DQ	–1617.94 (–1617.93 to –1617.96)	–1592.90 (–1591.88 to –1593.39)
(DC,DQ)DP	–1616.43 (–1616.40 to –1616.40)	–1591.40 (–1589.13 to –1591.96)
(DP,DQ)DC	–1618.63 (–1618.59 to –1618.69)	–1593.47 (–1591.08 to –1594.11)

BF = Bayes factors approach; SS = stepping-stone approach; DC = *D. calcea*; DP = *D. profundorum*; DQ = *D. quadrispinosa*. Selected topologies according to SS and BF are in bold.

sister relationships within the *Deania* clade (Table 3). Both approaches support as the most probable phylogenetic inference, the topology where *D. profundorum* is basal in the *Deania* genus and *D. calcea2* is nested within *D. calcea* clade (SS = –1616.43 and BF = –1591.40, respectively) (Table 3).

The ABGD analysis does not provide any support for *D. calcea2* subclade to be considered as a distinct species, either using K2P or uncorrected p-distances, for COI and CR. Nevertheless, applying the test on the COI matrix of distances calculated with K2P model, ABGD detects recursive partitioning within the *D. quadrispinosa* clade (Figure 3).

The tree topologies estimated for the mPTP analyses reflected the ABGD output providing no support for *D. calcea2* as distinct species for both mtDNA markers, but indicating the two putative species within *D. quadrispinosa* clade (Supplementary Figure 3).

The intraspecific relationship between *D. calcea* and *D. calcea2* was further explored using a haplotype gene genealogy reconstruction analysis. The IntNJ network positions the

D. calcea2 in a peripheral position of *D. calcea* haplotypes, for both mtDNA markers (Figure 4). There are a minimum of 3 (in CR) or 4 (in COI) nucleotide substitutions from the nearest *D. calcea* haplotype, one (in CR) or two (in COI) mutations more than among the *D. calcea* haplotypes (Figure 4A). The nucleotide substitutions that unambiguously distinguish *D. calcea* from *D. calcea2* are: two transitions (C–T at position 721 and G–A at position 738) in CR, and two transversions (T–A at position 49, A–T at position 235) and two transitions (T–C at position 379 and 475) in COI (Figure 4B).

Denticle Shape and Size Among *Deania* Species of the NE Atlantic

From the 149 *D. calcea*/*D. hystricosa* skin samples analyzed, 52 belonged to females measuring between 80 and 111 cm TL (mean = 96.2 cm TL), while 92 belonged to males measuring between 75 and 90 cm TL (mean = 84.3 cm TL). Skin denticle height varied between 1.080–2.102 and 0.753–1.908 mm (for females and males, respectively); while crown length varied between 0.544–1.200 and 0.380–1.013 mm (for females and males, respectively).

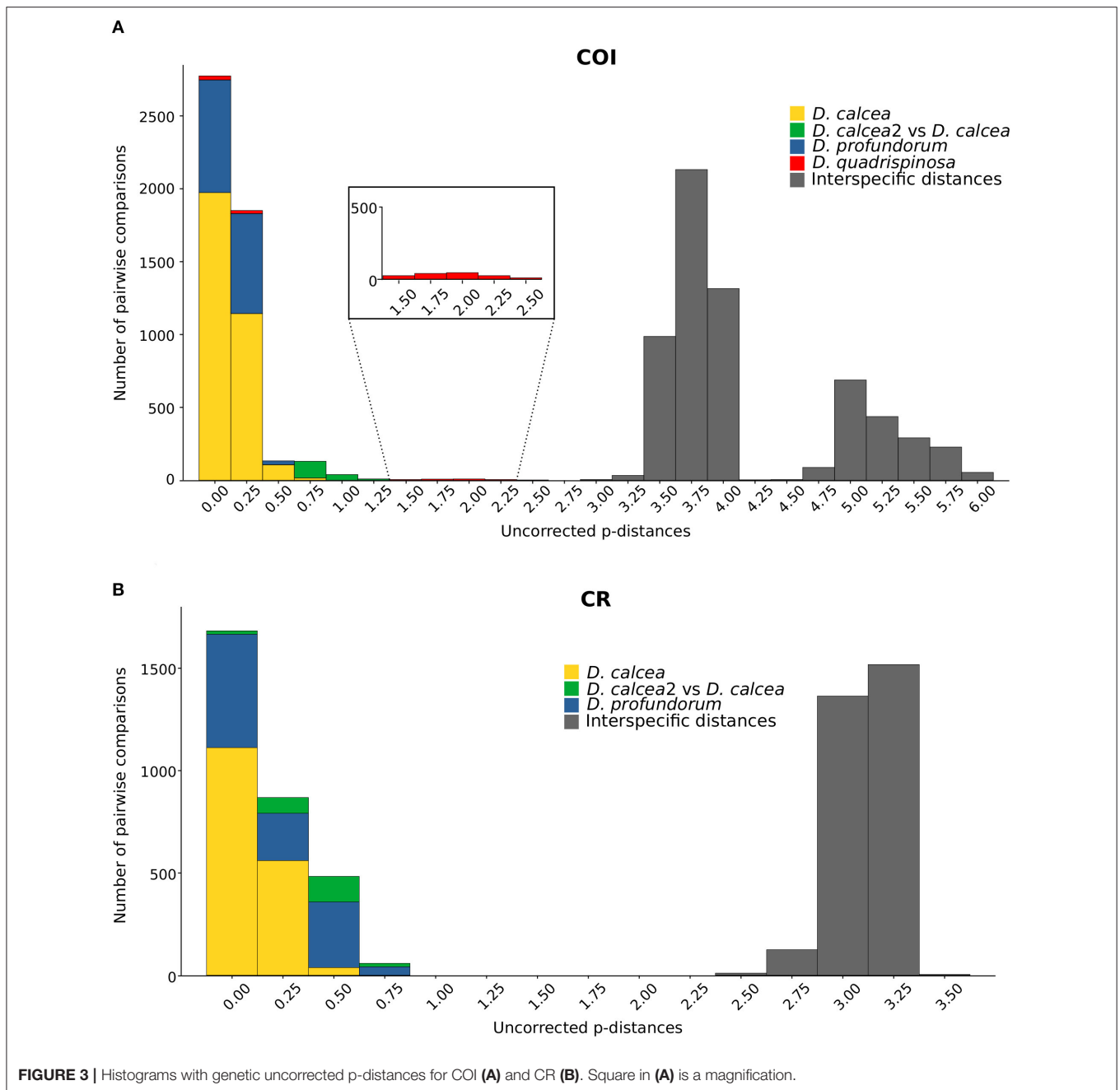
Dermal denticles in *Deania* are pitchfork-shaped (Figure 5), formed by a medial cusp and two lateral cusps, one on each side. From the visual inspection, differences among individuals are noticeable in the shape (e.g., slender or wide) and relative size (elongated or short) of the three cusps. Some denticles also showed extra spines along the cusps or at the anterior edge of the crown (e.g., Figure 5D).

Among the 4 specimens whose sequences clustered as *D. calcea2*, dermal denticles were only available for specimen MAD115 (Figure 5B) and they presented short and wide cusps. In *D. calcea* specimens, the majority had elongated and slender denticles (Figure 5C), however a few individuals (e.g., MAD111; Figure 5F) had shorter denticles with wide cusps.

The analyses of denticle data indicated that crown length increases proportionally with specimen body size and it is also related to sex. The best supported model was the one where denticle size is explained by both TL and sex (linear model: $\beta = 0.20$, SE = 0.18: $p < 0.0001$, adjusted $R^2 = 0.48$; AIC: –220.86, df = 4), but still, size alone is a good predictor of denticle crown length ($p < 0.001$). On the other hand, depth did not show any statistical correlation in predicting denticle size, although large females were captured at shallower depths than males (Supplementary Figure 4).

DISCUSSION

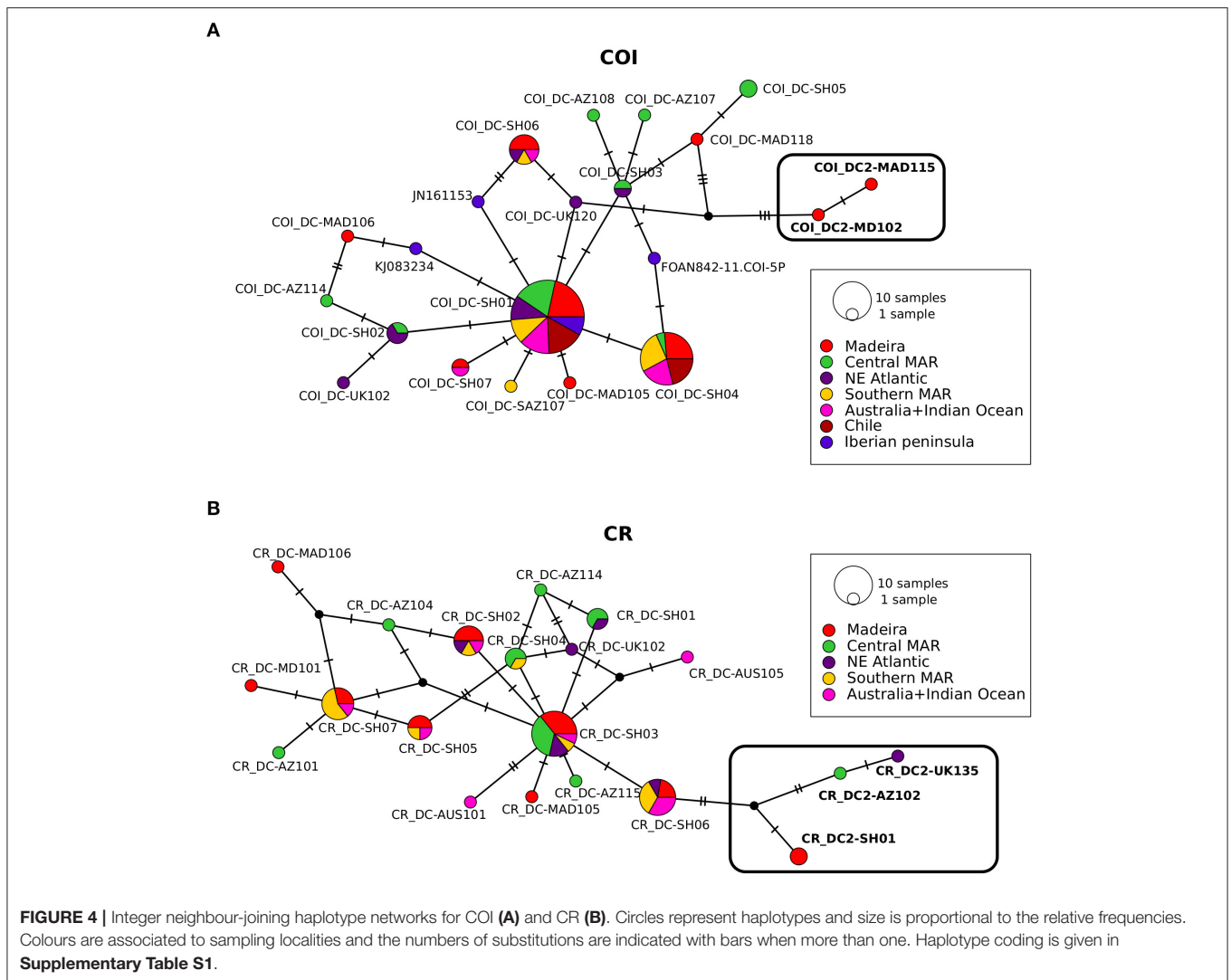
According to the literature accompanied by dichotomous keys for the identification of three *Deania* species for the North Atlantic (e.g., Cadenat and Banche, 1981; Compagno, 1984; Castro, 2011; Ebert and Stehmann, 2013), the character that uniquely identifies *D. profundorum* is the presence of a subcaudal keel, which is absent in the other species. Our molecular findings based on the two mtDNA genes (COI and CR) supported the visual identification for *D. profundorum*, but leave uncertainty for the separation between *D. calcea* and *D. hystricosa*. Morphological identification relying on the skin roughness assessment was not



supported by the genetic analysis, since all sequences of the specimens classified as *D. hystricosa* or as *D. calcea*/*D. hystricosa* clustered together as *D. calcea*. Such findings are also supported by the recent study by Rodríguez-Cabello et al. (2020) indicating the difficulties of visually inspecting of *Deania* specimens from northern Spain.

Phylogenetic inferences suggest *D. profundorum* to be the basal split in *Deania* and *D. quadrispinosa* as the sister taxon of the clade *D. calcea* (Figure 2). Although, four of our individuals showed distinct haplotypes that formed the only well-defined subclade *D. calcea2* nested within the *D. calcea* clade (Figure 2), more focused analyses shows that the supports on the *D. calcea2*

node are not very high, regardless of the inference approach used. In both ABGD and mPTP analyses the subclade *D. calcea2* was never delimited as a distinct species, but always tightly clustered within the *D. calcea* clade. The only situation in which the two analyses reveal any further clustering on putative species is in *D. quadrispinosa*. *D. quadrispinosa* is the clade with the lowest number of COI sequences but hosted by far the highest levels of nucleotide diversity. However, *D. quadrispinosa* was not actively sampled in our research and all sequences derived from publicly accessible databases (GenBank and BOLD). Therefore, interpretation of this results should be taken with caution as nucleotide diversity may be overestimated.



D. calcea2 uniquely differs genetically from *D. calcea* sequences for two transitions in CR and two transversions + two transitions in COI. This level of genetic differentiation is comparable to the one reported for the intraspecific variability between *Centroscyrnus coelolepis*, another deep water shark, from the Atlantic and the Mediterranean Sea (Catarino et al., 2015). Here the Strait of Gibraltar may have acted as geographical barrier and the authors invoked cryptic speciation by genetic drift (Catarino et al., 2015), but for *D. calcea*, the distribution of the main clade and the subclade overlaps as they live in sympatry (Weigmann, 2016; Froese and Pauly, 2019). For this reason, we might consider two plausible hypotheses to explain the relationship between these two units: incipient speciation or introgression. Nevertheless, the fact that *D. calcea2* sub-clade is not supported by morphology nor phylogeography, coalescent stochasticity could also be accounted for the formation of a separate sub-clade as coalescence is a random process (Knowles and Maddison, 2002; Hudson and Turelli, 2003).

No inference can be made on the basis of the molecular markers used in this study and validations of the most plausible

hypothesis, either biological or merely a result of stochastic forces, can only be made exploring genomic data. Only fine resolution analysis of nuclear markers might provide further insights on the speciation processes associated to these two species. A recent comparative study applying ddRAD sequencing to identify inter-gene pool SNPs in *Deania calcea* from the Atlantic and the Pacific, reported effective panmixia across sampling sites (Keggins, 2017). However, this work does not seem to have included specimens whose COI sequences match with any those reported for *D. calcea2* specimens, that for us remains an open question. The lack of hybrids between *D. calcea* and *D. calcea2* would support the hypothesis of incipient speciation, while evidence of hybridization between the two would favor the hypothesis of an historical vicariance event. An example of vicariance or lineage sorting in a large panmictic population of deep-sea sharks is reported for *Centroscyrnus crepidater* (Cunha et al., 2012). This species presents a marked divergence between the two clades (24 mutational steps in CR), and the trigger for the separation was attributed to the oceanic cooling observed during the Miocene (Cunha et al., 2012).

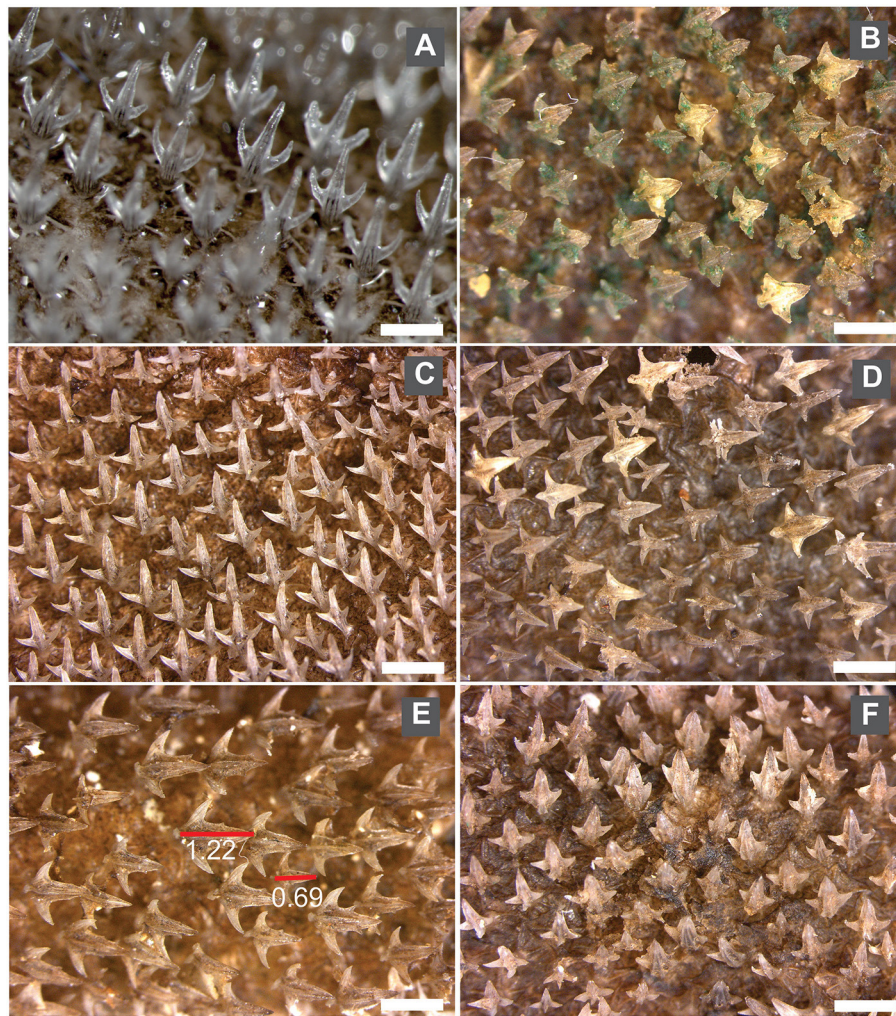
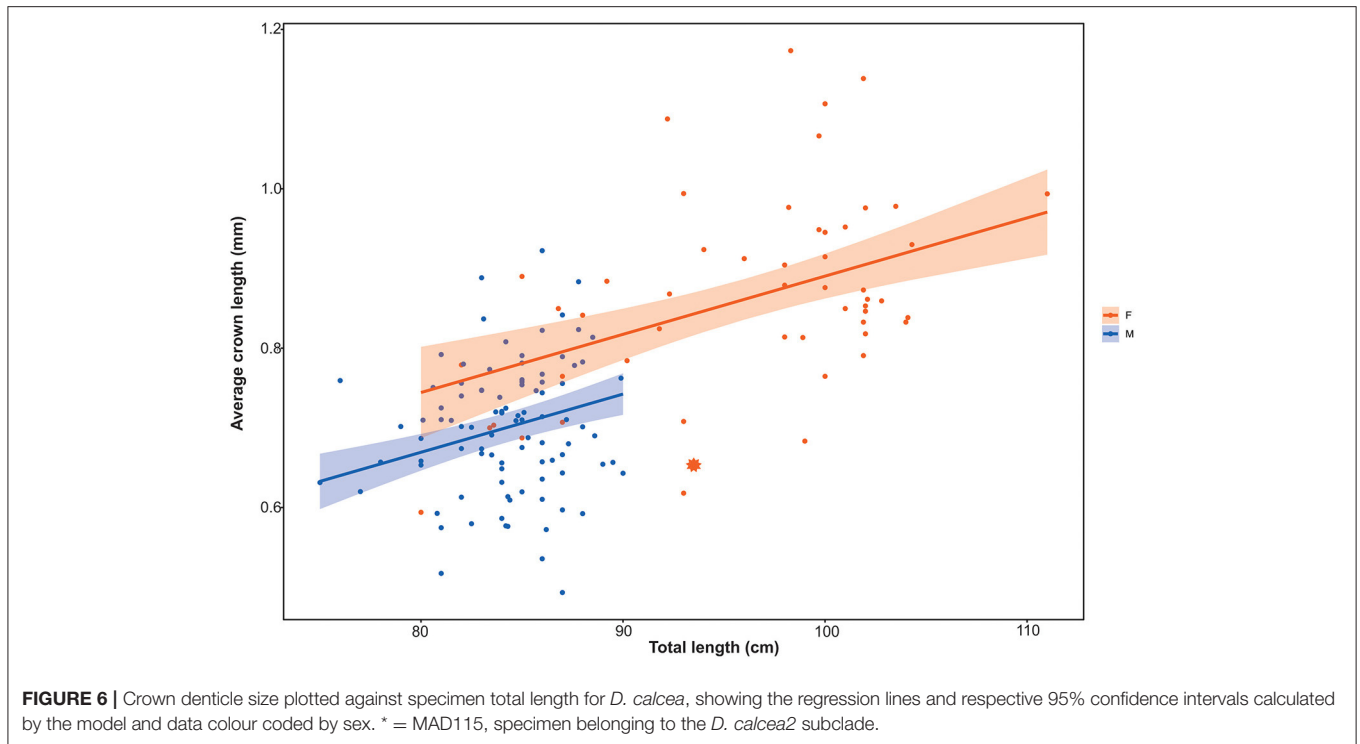


FIGURE 5 | Images of dermal denticles belonging to different *Deania* species from the NE Atlantic **(A)** Dermal denticles from *D. profundorum*; **(B)** *D. calcea2* MAD115 (see **Figure 2**), a female with TL = 93.5 cm; **(C)** *D. calcea* MAD117, a male with TL = 83.7 cm; **(D)** *D. calcea* MAD110, a female with TL = 83.6. The white mark in each image corresponds to 1 mm.

The original description of *D. hystricosa*, refers to the type specimen that was a female captured off Japan (Garman, 1906). Half a century later, with the revision of several species belonging to the suborder Squaloidea, Bigelow and Schroeder (1957) revised the classification of *Deania* species based on the shape of their teeth. The *D. hystricosa* type specimen was lost, but from the detailed original description the authors did not find any support to maintain the status of distinct species for *D. hystricosa*, considering it a synonym of *D. calcea*. A few decades later, Compagno (1984) resurrected the species name after revising the description of *D. mauii* (Cadenat and Banche, 1981) from Madeira Island. This newly reported species presented large denticles compared to those of *D. calcea*, but very similar to the ones reported for *D. hystricosa* (Garman, 1906). Therefore, *D. mauii* was synonymised with *D. hystricosa*, and no further revision on this genus has been made since, adopting as discriminant character between *D. calcea* and *D. hystricosa* the size of their dermal denticles (e.g., Ebert and Stehmann, 2013).

In our study the specimens genetically identified as *D. calcea* exhibited skin denticles with a large variety of patterns (e.g., slender/wide, extra spines, etc), including similar to the ones reported in Cadenat and Banche (1981) described as *D. calcea* and *D. mauii*. We did not find distinctive patterns or size ranges to be attributed to either *D. calcea* or *D. hystricosa*. Moreover, the only denticles available for *D. calcea2* (MAD115, **Figure 5B**) were among the smallest compared to those measured in *D. calcea* specimens of similar total length (**Figure 6**). According to our findings, denticle crown length increased accordingly to the size of the specimen (**Figure 6**) strengthening the doubt on the validity of considering *D. hystricosa* as a nominal species. In this respect, our findings are in agreement with Rodríguez-Cabello et al. (2020) although they scrutinized a limited number of specimens. Our results also suggested that crown length was marginally correlated with sex, contrarily to the findings of Rodríguez-Cabello et al. (2020). Although in *D. calcea*, as in many squaloid sharks, females attain bigger sizes than males (Irvine



et al., 2012; Rochowski et al., 2015), the results shown by the regression lines, mean that for specimens of the same size, in general females had slightly bigger crown lengths compared to males, suggesting some degree of sexual dimorphism in denticle crown length. While positive correlation between denticle and body size in sharks is commonly reported (e.g., in deep-sea sharks: Weigmann et al., 2015), evidence of sexual dimorphism in denticle morphology is rarely described (e.g., in *Scyliorhinus canicula*: Crooks et al., 2013). Sexual dimorphism associated with larger denticles in females has been suggested to be an adaptation to male biting during mating (Crooks et al., 2013) that in extreme situations may cause severe wounds on the female partner (Ritter and Amin, 2019).

According to the crown length diagnosis, most of the females were identified as *D. hystricosa*, while the majority of males were identified as *D. calcea*. In our dataset ($n = 149$) we found that those specimens with an average crown length ≥ 0.85 mm (as a proximity to the definition of “very large—around 1 mm” and therefore attributable to the classification as *D. hystricosa*), resulted to be mainly females with only few males (58 vs. 4%, respectively). We found that crown length could not be used as a diagnostic character (either 0.5 or 1.0 mm), since it was impossible to draw a boundary to distinguish either one or the other species. Moreover, denticle size varies within individuals according to region of the body (Rodríguez-Cabello et al., 2020), leading to the conclusion that this is not a good identification character for discriminating between *D. calcea* and *D. hystricosa*. Although shape and size of dermal denticles are useful to distinguish between families or genera (e.g., *Centrophorus* vs. *Deania*), there are cases in which dermal denticles go through marked ontogenic changes from juveniles to adults that could

resemble denticles belonging to distinct taxa (e.g., White et al., 2013; Veríssimo et al., 2014; *Centrophorus* spp.).

Targeted fishing of deep-water sharks is prohibited in European waters, although by-catch is still allowed in certain circumstances and limits (quota; Reg. UE 2018/2025) and is likely to be a threat for this long lived species. Biological and catch information are fundamental to assess species vulnerability and for a sustainable fisheries management, and at the base of this information is an accurate species identification. A better knowledge of the species diagnostic features and molecular taxonomy, will reduce the level of taxonomic uncertainties and misidentification, improving fisheries management and promoting species conservation. In European waters, the long snouted deepwater dogfish is captured as a by-catch of deep-water fisheries that started to be exploited during the early 1960's (Gordon, 2001), when the traditional fisheries on the continental shelf declined (Koslow et al., 2000).

Current conservation assessments and fisheries management consider *D. calcea* and *D. hystricosa* as separate species, at least in the NE Atlantic. According to our analyses, past datasets reporting them separately, should now be merged and any time series or stock assessments adjusted accordingly. The landings of *D. calcea* in Portugal were around 120 tons/year in the last decade (DGPA, 2010). Similarly, the IUCN red list reports *D. calcea* as a species of least concerned (LC) and *D. hystricosa* as data deficient (DD) species. The IUCN red list assessment should likewise be revised to reflect the fact that these are the same species in the NE Atlantic. Lumping of species is likely to result in a more favorable assessment of conservation or stock status, but it should be born in mind that this species, like most deep-water sharks, is still highly vulnerable to exploitation. Despite protection in the form

of landings prohibition and an EU wide trawling ban at depths >800 m, bycatch by longline and trawler vessels remains a threat.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

All specimens were caught during scientific surveys authorized by: the local Government of the Azores and the local Government of Madeira following the protocols accepted by the Institute for Marine Research (IMAR) and the University of the Azores, Department of Oceanography and Fisheries (UAz-DOP) in accordance with the Portuguese law and the Scottish Fishery Research annual surveys.

AUTHOR CONTRIBUTIONS

SS, GM, and FN conceived and designed the study. SS, DC, PR, GM, FN, and MF carried out the sampling. SS, DC, PR, and DS performed all the analysis. SS, DC, and DS drafted the manuscript. All authors contributed to reach the final version, gave final approval for publication, and agree to be held accountable for the work performed therein.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2020.588192/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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