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Taxonomic research on *Deania calcea* and *Deania profundorum* (Family: Centrophoridae) in the Cantabrian Sea (Northeast Atlantic) with comments on *Deania hystricosa*

Cristina Rodríguez-Cabello [a,*], Montse Pérez [b], Irene Grasa [a]

[a] Instituto Español de Oceanografía, Promontorio San Martin s/n. 39004 Santander, Spain
[b] Instituto Español de Oceanografía, Subida a Radio Faro 50, 36390 Vigo, Spain

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ABSTRACT

Three elasmobranch species of the genus *Deania* are currently reported in NE Atlantic waters: *D. calcea*, *D. hystricosa* and *D. profundorum*; however, in north Spanish waters (NE Atlantic), only *D. calcea* and *D. profundorum* have been caught. Among the criteria used to discriminate *Deania* species one is dermal denticle length and body colour. In this study the authors explore the feasibility of these criteria and examine other morphological characters to investigate if sexual or ontogenic features had relevance for the distinction of the species particularly those sampled in this study, *D. calcea* and *D. profundorum*. Molecular analyses were conducted to validate these results.

In *D. calcea*, dermal denticle length ranged from 340 μm to 1400 μm (763.2 μm ± 180.4 s.d.); in *D. profundorum*, dermal denticle length ranged from 195 μm to 650 μm (372.3 μm ± 111.7 s.d.). In both *Deania* species, a significant positive correlation was found between shark total length and dermal denticle length. Dermal denticles varied in size and shape along the shark body. These differences were significant both intra- and inter-specifically.

A multivariate analysis based on morphological characters was used to test differences between *D. calcea* and *D. profundorum*. The hierarchical analysis clearly identified three groups; two groups corresponded to each species, and a third group discriminated between small and large individuals of *D. profundorum*. The morphometric characters that contributed most to the divergence between both species were mainly related to the size of the dorsal fins, the inter-dorsal distance and the distances from the snout to the origin of each dorsal fin. Based on morphological characters, no sexual dimorphism was observed.

The phylogenetic tree was reconstructed with molecular COI sequences available on BOLD and those obtained in this study. The output tree discriminate *D. calcea* from *D. profundorum*, however could not separate molecularly *D. calcea* and *D. hystricosa.*

1. Introduction

1.1. General introduction

The genus *Deania* (Jordan and Snyder, 1902) comprises medium-size (up to 162 cm, total length, (Weigmann, 2016) deep-water sharks characterised by an extremely long and broad snout and bladelike upper and lower teeth. Their bodies are cylindrical and compressed with rough skin covered by dermal denticles with pitchfork shape. In addition, *Deania* species possess two dorsal fins with strong grooved spines, no anal fin and a caudal fin with a strong sub terminal notch. Currently four *Deania* species are recognised, three occur in the North Atlantic: *D. calcea* (Lowe, 1839), *D. hystricosa* (Garman, 1906) and *D. profundorum* (Smith and Radcliffe, 1912). The main differences among these species are the presence of a subcaudal keel on the lower surface of caudal peduncle which characterises *D. profundorum* and the size of the lateral trunk dermal denticles, moderately large in *D. calcea* (0.5 mm) and very large in *D. hystricosa* (>1 mm) (Compagno, 1984; Ebert and Stehmann, 2013).

Deania calcea has a wide distribution in the Atlantic, from Iceland to southern Africa (Compagno, 1984; Ebert and Stehmann, 2013; Moura et al., 2014; Weigmann, 2016); *D. profundorum* and *D. hystricosa* are more patchily distributed (Compagno, 1984; Ebert and Stehmann, 2013; Ebert et al., 2009a,b; Iglésias, 2014; Weigmann, 2016). For more information on *Deania* species distributions see, (e.g. Weigmann, 2016).

* Corresponding author.
E-mail address: cristina.cabello@ieo.es (C. Rodríguez-Cabello).

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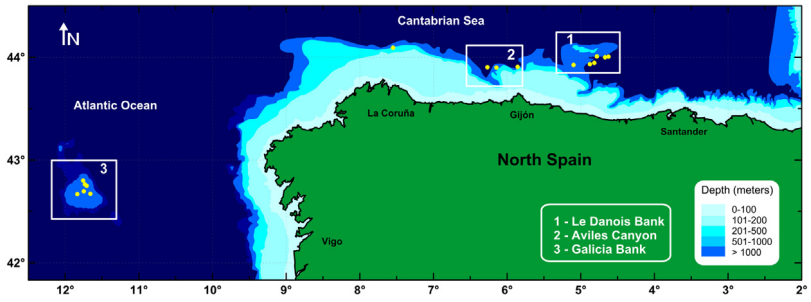

Fig. 1. Study area showing the sampling locations of *Deania calcea* and *D. profundorum*.

Fig. 2. Location of skin samples taken for the measurement of dermal denticle size.

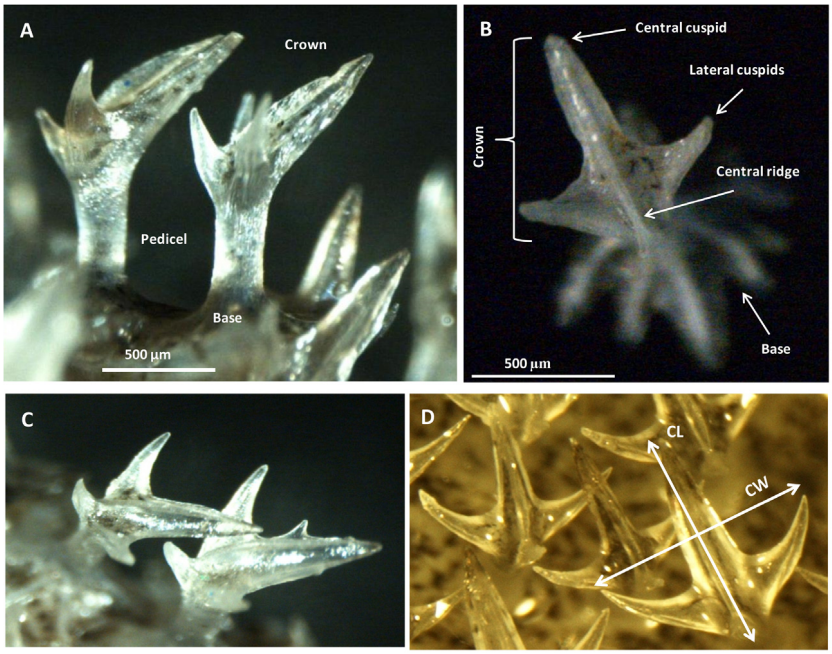

Fig. 3. Illustration of *Deania* dermal denticles from different views (a) frontal (b) upper and (c) lateral. Description of the structure (base, pedicel, crown, cuspids) and (d) measurements taken in this study. CL refers to crown length and CW to crown width.

In Spanish Atlantic jurisdictional waters, these three *Deania* species have been reported (Báez et al., 2019; Bañón et al., 2010, 2016; Brito et al., 2002). However *D. calcea* and *D. profundorum* are regularly caught in bottom trawl surveys carried out annually in the north of Spain (Sánchez et al., 1995, 2002), whereas *D. hystricosa* has not been identified neither in these surveys or other multidisciplinary surveys conducted in the study area (www.ecomarg.com). The only published records of *D. hystricosa* in Spanish jurisdictional waters are from the Canary Islands (Brito et al., 1998, 2002; González et al., 2011) and one record reported in 1980's from the Galician Bank (Bañón et al., 2010, 2016).

Due to its wide geographic distribution and the fact that it is a relatively common deep-water shark, many studies have been performed on *D. calcea* in different regions. Studies on *D. calcea* include reproductive biological parameters (Clarke et al., 2002; Irvine et al., 2012; Paiva et al., 2011; Parker and Francis, 2012; Rochowski et al., 2015), growth (Clarke et al., 2002; Francis and Maolagain, 2004; Irvine et al., 2012; Parker and Francis, 2012)

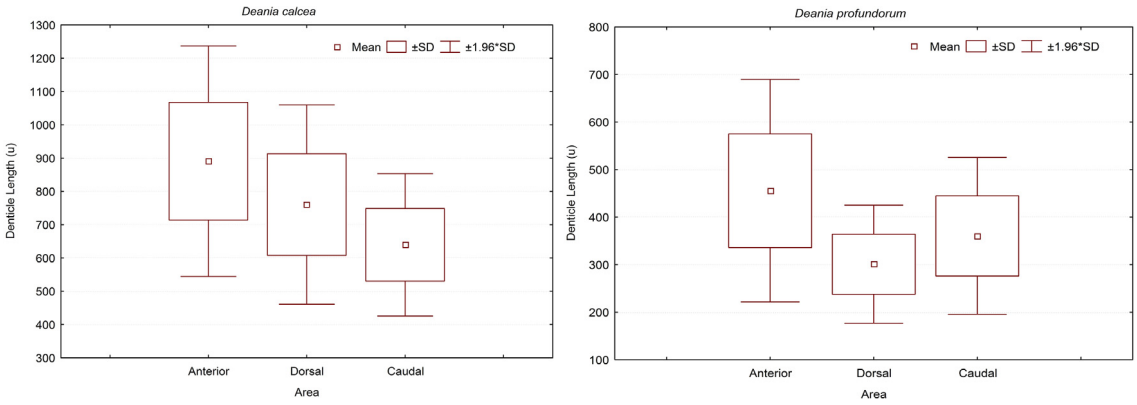

Fig. 4. Box-plot of dermal denticle length (μm) according to its location in the shark body (a) *Deania calcea* and (b) *Deania profundorum*.

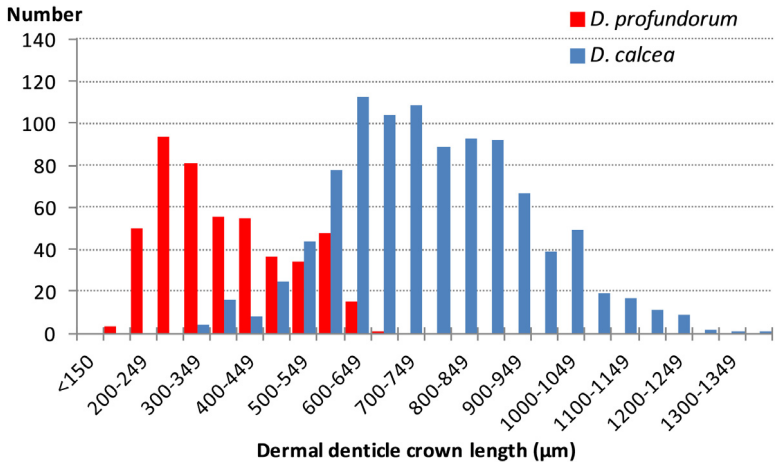

Fig. 5. Length frequency distribution of dermal denticle measurements (μm) recorded for each Deania species.

and diet (Dunn et al., 2013; Ebert et al., 1991; MacPherson and Roel, 1987; Preciado et al., 2009; Yano, 1991). Fewer studies have been published on *D. profundorum* (Hernández-Pérez et al., 1997; Palm and Schröderc, 2001; Sanjuán et al., 2012) and *D. hystricosa*, (Biscoito et al., 2018; Delgado et al., 2017; Fossen et al., 2008; Pajuelo et al., 2016). Particularly, for *D. hystricosa* many of these studies are checklists or occurrences.

Dermal denticle structure is a taxonomic criterion used in *Deania* identification, but these morphological characters have not been sufficiently described and some doubts persist (see Section 1.2).

Dermal denticles or placoid scales are a characteristic of the skin of elasmobranchs. They are composed of dentina and enameloid crown attached to a basal plate, which is anchored to the skin by collagen fibres (Applegate, 1967; Deynat, 1998). They cover the shark body and display morphologies that differ significantly among species; this feature has conventionally been used in the identification of elasmobranch species (Ankhelyi et al., 2018; Deynat, 2000; Dillon et al., 2017; Ferrón and Botella, 2017; Gravendeel et al., 2002; Raschi and Musick, 1984; Reif, 1985). Placoid scales are described by some authors as non-growing structures because their span of growth is limited (Helfman et al., 1997; Kemp, 1999). Nevertheless, during the life of an individual, scales grow to a definitive size, but it is dependent on the size of the animal. Thereafter, scales are rejected and replaced by new scales of larger size; this process is repeated continually but without a synchronous pattern (Kemp, 1999).

The size of dermal denticle is not an easy diagnostic criterion to recognise species particularly with the naked eye on board commercial vessels. The size range to discriminate between *D. calcea* and *D. hystricosa* is also vague, about 0.5 mm or about 1 mm. Due to these issues with the dermal denticles, misidentifications likely could have occurred. The objectives of this study were: (a) to check the consistency of dermal denticle size character used is *Deania* identification (b) to examine other morphological characters that could be significant for the identification of *Deania* species, (c) to construct a phylogenetic tree based on molecular cytochrome c-oxidase subunit I (COI) sequences and (d) to collect and revise data from original *Deania* descriptions.

1.2. Taxonomical history of Deania species caught in Atlantic waters

Deania calcea was first described as *Acanthidium calceus* by (Lowe, 1839) from waters of Madeira Island, Portugal. Jordan and Snyder (1902) described *Deania eglantina* from waters of Hondo Island, Japan, which was included in a new genus, *Deania* (Jordan and Snyder, 1902). *Deania eglantina* was later synonymised to *D. calcea*. Garman (1906, 1913) described three *Acanthidium* species collected from Japanese waters: *Acanthidium rostratum, A. aciculatum* and *A. hystricosum*. *Acanthidium rostratum* and *A. aciculatum* were later accepted as *Deania calcea*; *A. hystricosum* was later recognised as *Deania hystricosa*. According to Garman's description (Garman, 1906), the main characters that distinguished *D. hystricosa* from *D. calcea* were the colour, the position of the first dorsal fin and the size of the dermal denticles. Body colour dark brown and black inside of the mouth, gill openings, nostrils and edges of fins in *D. hystricosa vs* ashy or grey-brown colour in *D. calcea*. First dorsal spine closer to caudal fin than the end of the

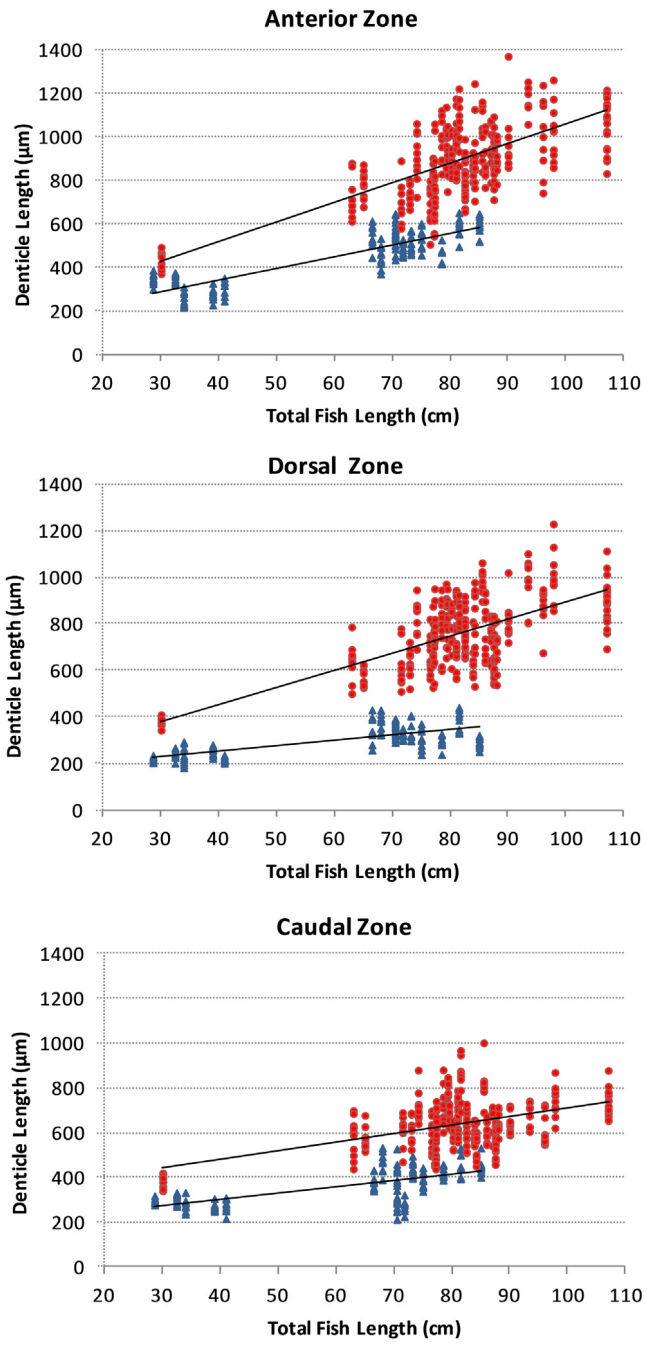

Fig. 6. Relationship between total fish length (TL) and dermal denticle length (CL) in (a) *Deania calcea* and (b) *Deania profundorum*. All sampled specimens are shown.

snout in *D. hystricosa vs* first dorsal spine equidistant from the end of the snout and caudal fin in *D. calcea*. Finally, length of the crown of the dermal denticles much larger in *D. hystricosa*. Bigelow and Schroeder (1957) and Garrick (1960) synonymised *D. hystricosa* with *D. calcea*. Cadenat (1960) described *D. cremouxi*, a new *Deania* species, from the coast of Senegal that was much more common at depths between 350–600 m; this was later synonymised with *D. profundorum*. Cadenat and Blache (1981) described a new species, *D. mauli* from Madeira Island waters; the main character that discriminated this species from *D. calcea* was the exceptional size of its dermal denticles. Compagno (1984) tentatively synonymised *D. mauli* from the Atlantic with *D. hystricosa* and recognised *D. hystricosa* as valid species noting the differences in denticle size and colour (Garman, 1906) that distinguish it from *D. calcea*.

Deania profundorum was firstly described by Smith and Radcliffe (1912) during an expedition around the Philippine archipelago; it was described as *Nasisqualus profundorum*, which they also referred as a new genus. The main feature that characterised this species was that the snout was very broad and flat. Additionally, the dorsal fins were nearly equal in length; the second dorsal fin was higher than first one and began behind the base of ventral fins. The base of the second dorsal fin was also longer than that of first dorsal fin. Furthermore, the skin was described as velvety and densely scaled; each denticle consisted of three slender spines in form of a trident. *Nasisqualus profundorum* was later synonymised to *D. profundorum* (Smith and Radcliffe, 1912) along with *Acanthidium natalense* (Gilchrist, 1922), *Deania elegans* (Springer, 1959) and *Deania cremouxi* (Cadenat, 1960).

2. Material and methods

2.1. Samples

Deania calcea were collected in several multidisciplinary surveys carried out between 2011 and 2015 in the Cantabrian Sea, the southern region of the Bay of Biscay, in the northeast Atlantic Ocean. The two sample sites in the Cantabrian Sea corresponded to a system of three deep canyons, Aviles Canyon System, declared site of Community Importance (SCI) of Nature 2000 network, and El Cachucho Marine Protected Area (MPA). This MPA includes Le Danois Bank seamount and the intraslope basin between this Bank and the Cantabrian Sea continental shelf (Fig. 1). *Deania profundorum* were mainly collected in the Galicia Bank seamount during 2009–2011 surveys. Specimens were frozen at −20 °C until they were used. Prior to sampling, sharks were completely thawed in the refrigerator overnight at 4 °C. A sample of muscle was extracted and preserved in 99% ethanol at 4 °C for further genetic analysis. Detailed information of surveys, gear and locations is summarised in Table 1 (more information on these areas can be found in the link: www.ecomarg.com).

To compare molecularly the samples of this study with original samples, a request was done to the National Museum of Natural History (MNHN) in Paris (France) were specimens of D. *hystricosa* (*D. mauli*) and D. *profundorum* (*D. cremouxi*) are preserved in the collection of Ichthyology (IC). Three samples were analysed, two paratypes of *Deania mauli* (code numbers 1969-0299 and 1969-0300 respectively) and one sintype of *Deania cremouxi* (code number 1969-0298).

2.2. Dermal denticles

Dermal denticles were examined from 33 specimens of *D. calcea* (30.11–07.0 cm) and 15 specimens of *D. profundorum* (28.0–86.4 cm). Skin samples of approximately 1 cm^2 were removed with a scalpel from 3 locations across the body: close to cephalic zone (above pectoral fin base), dorsal zone (below first dorsal fin) and caudal zone (Fig. 2). The underlying dermal tissue was removed using a fine scalpel blade and, after cleaning with water, skin samples were placed on filter paper to remove excess water. Measurements of the length and width of 10 dermal denticles from each skin sample were taken. Broken or not wholly visible dermal denticles were excluded from any measurement in order to eliminate size bias. A Nikon SMZ1500 stereomicroscope and a NILS Image Analysis tool were used to visualise and record measurements (in µm) of dermal denticles. Only measurements of the crown length (CL) and width (CW) were taken (Fig. 3).

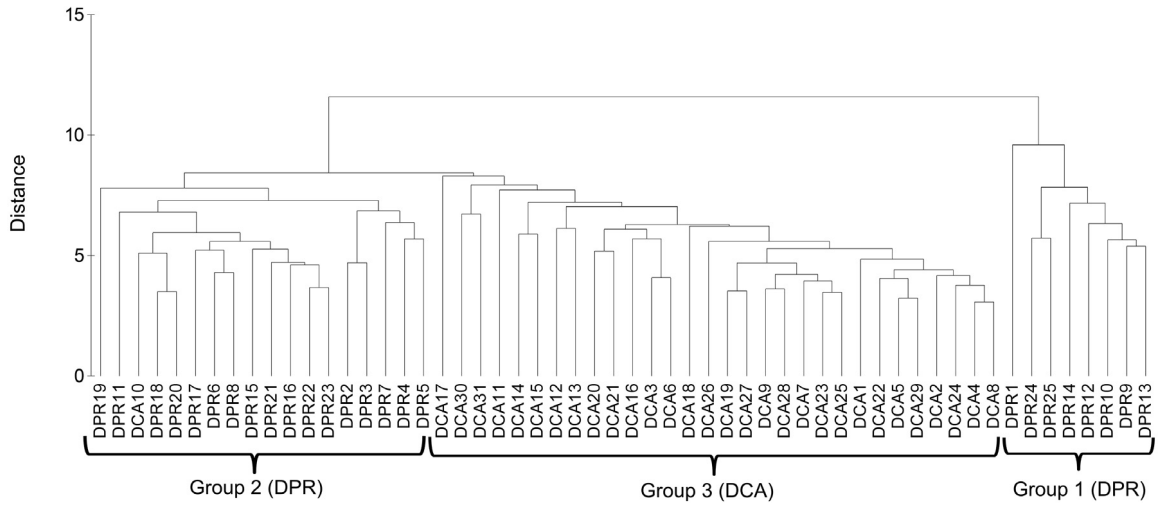

Fig. 7. Cluster of similarity based on morphometric characters values obtained from *Deania calcea* (DCA) and *Deania profundorum* (DPR) samples.

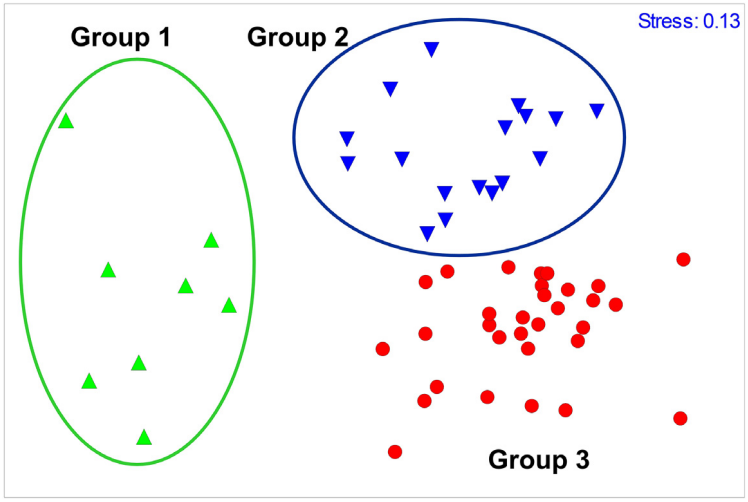

Fig. 8. Non-metric multidimensional scaling (MDS) of morphometric differences between both *Deania* species: *D. calcea* (red circles) and *D. profundorum* (triangles). Green triangle refers to small specimens <42 cm TL and blue triangles for specimens >55 cm TL.

2.3. Morphological measures

A total of 34 morphometric measurements (Table 2) covering all body areas (head, trunk, tail and fins) were taken on 31 specimens (19 ♂ and 12 ♀) of *D. calcea* (length range of 63.0–107.0 cm) and 25 specimens (13 ♂ and 12 ♀) of *D. profundorum* (length range of 28.0–86.4 cm) following procedures outlined in Compagno (1984). Additionally, seven measures were taken at the insertion of dorsal fin spines following White et al. (2013). Measurements larger than 100 mm were made with an ictiometer or metric belt (1 mm precision); measurements smaller than 100 mm were made with a calliper (0.1 mm precision). All measurements were done directly (point to point). Measurements of paired structures such as pectoral and pelvic fins were done only on the left side of the specimen. For analysis, all measurements were standardised to the total fish length (TL) and data were expressed as minimum and maximum percentages of TL. Two male specimens of *D. calcea* (TL = 30.1 cm and 81.5 cm) were excluded from the analysis since not all measurements could be recorded.

2.4. Historical morphological data

This study also used morphological data collected from several *Deania* specimens previously described and reported in the literature as: *A. aciculatum, A. rostratum, A. natalense, D. calcea, D. cremouxi, D. mauli* or *D. quadrispinosus* (Table 3). Original descriptions and data can be found in their respective published studies and in Bigelow and Schroeder (1957), Cadenat (1960), Cadenat and Blache (1981) and Garrick (1960). The description of the morphometric characters used in these studies and its equivalence to those described in Compagno (1984) is detailed (Table 3). Nevertheless, some measurements were unclear and not all measurements were recorded in all specimens. This hampered the comparison of all morphological characters among the species and thus, only individuals containing measurements on similar morphological characters were used for comparative purposes; all others were excluded from the analysis (see note in Table 3).

2.5. Molecular data

Genomic DNA was extracted using the FENOSALT method (Pérez and Presa, 2011). The mitochondrial gene selected to accurately identify the species was the Cytochrome c-oxidase subunit I (COI). Amplification and sequencing of a fragment of the COI gene was carried out with the pair of primers FishF2-FishR2 described by Ward et al. (2005).

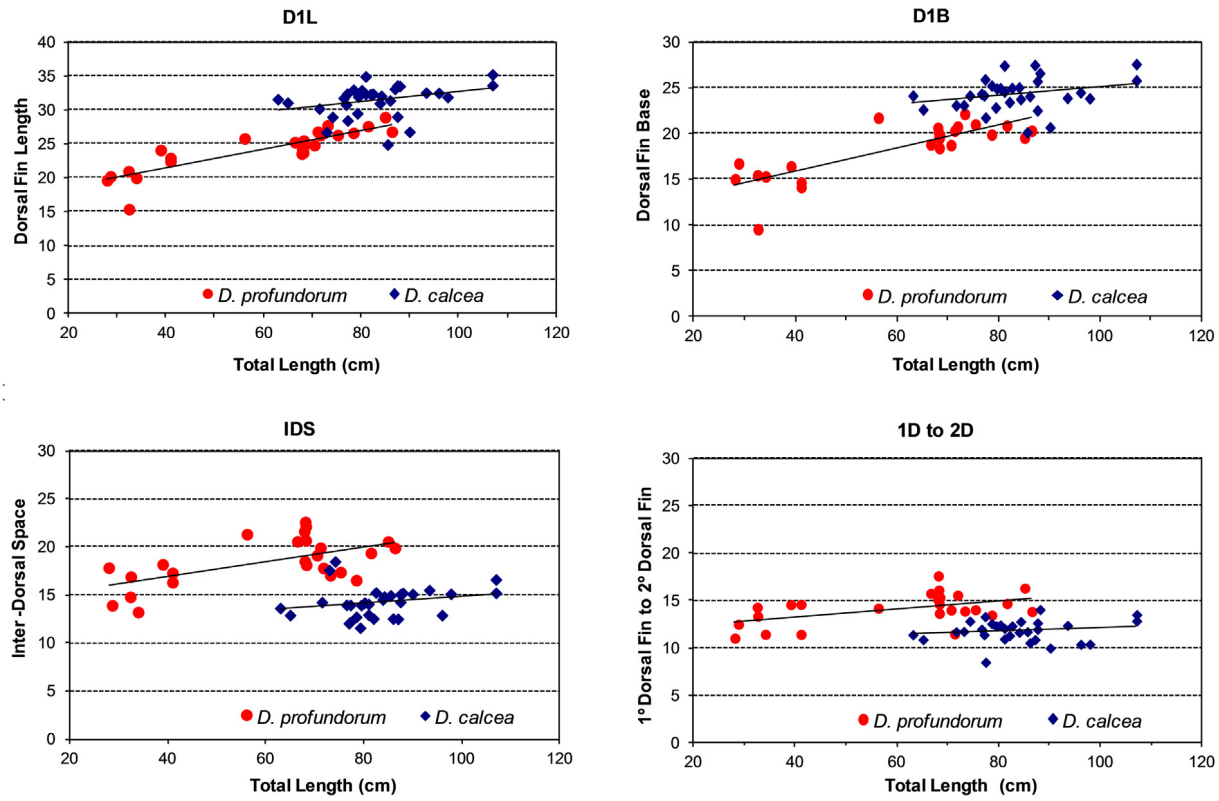

Fig. 9. Comparison of morphological measurements of *D. calcea* and *D. profundorum* expressed in percentage of TL. Above relation of first dorsal fin length (D1L) and base (D1B) to total length. Below inter-dorsal space (IDS) and distance from first dorsal fin rear tip and second dorsal fin spine (1D to 2D).

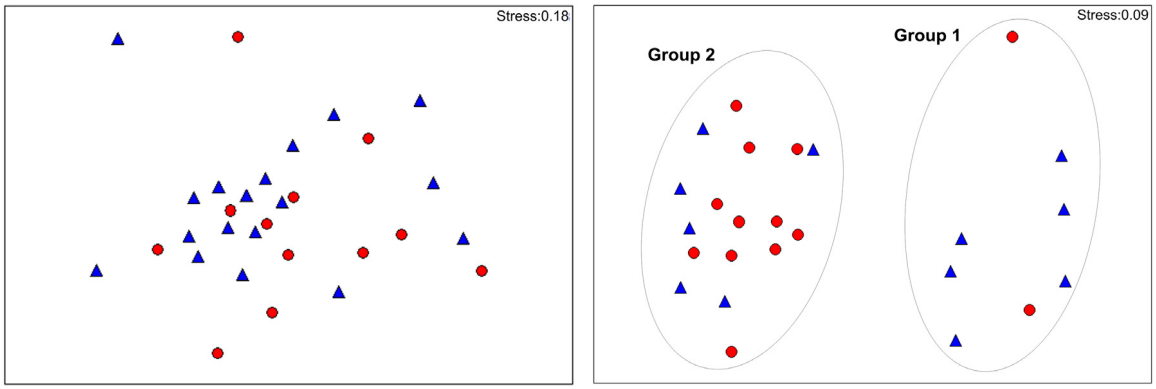

Fig. 10. Non-metric multidimensional scaling (MDS) of morphometric differences between males and females in (a) *Deania calcea* (left) and (b) *Deania profundorum* (right). Red circles refer to males and blue triangles to females. Group 1 refers to specimens <42 cm TL and group 2 to specimens >55 cm TL.

Amplifications were performed in a SureCycler 8800 thermal cycler. The reaction mixture contained 25–50 ng of purified DNA, 2 μl of 10X reaction buffer (Bioline), 10 pmol of each primer, 10 mM of dNTPs (Nzytech), 1.5 mM of MgCl2 (Bioline) and 1U BioTaq DNA Polymerase (Bioline) for a final volume of 20 μl. The PCR program consisted of 5 min at 95 °C, followed by 35 cycles at 95 °C for 45 s, 55 °C for 1 min, 72 °C for 1 min and a final extension of 10 min at 72 °C. The obtained amplicons were purified with a mixture of 10 U of Exonuclease I and 1 U of Alkaline Phosphatase (Thermo Scientific FastAP Thermosensitive Alkaline Phosphatase) following the manufacturer's protocol (Thermo Scientific). Sequencing was performed by CACTI on an ABI Prism 3100 genetic analyser using the BigDye Terminator Cycle sequencing kit (Applied Biosystems™). The electropherograms were visualised and edited with Chromas software (Technelysium, Tewantin, Australia). The dataset was completed with available *Deania* spp. COI sequences for a total of 44 sequences.

Since samples obtained from the Museum had been preserved in formalin, DNA isolation was carried out following a specific protocol for formalin-fixed material (Campos and Gilbert, 2012). Briefly, the formaldehyde-driven protein-DNA cross-links were reversed by treatment in a heated alkaline buffer (120 °C for 25 min in a 0.1 M NaOH, 1% SDS solution, pH = 12). The hot alkali treatment was followed by a phenol-chloroform extraction. DNA was resuspended in a final volume of 50 μL. PCR amplification was done using the primer pair FishF2 (Ward et al., 2005) in first instance and secondly using primers Uni-MinibarF/Uni-MinibarR.

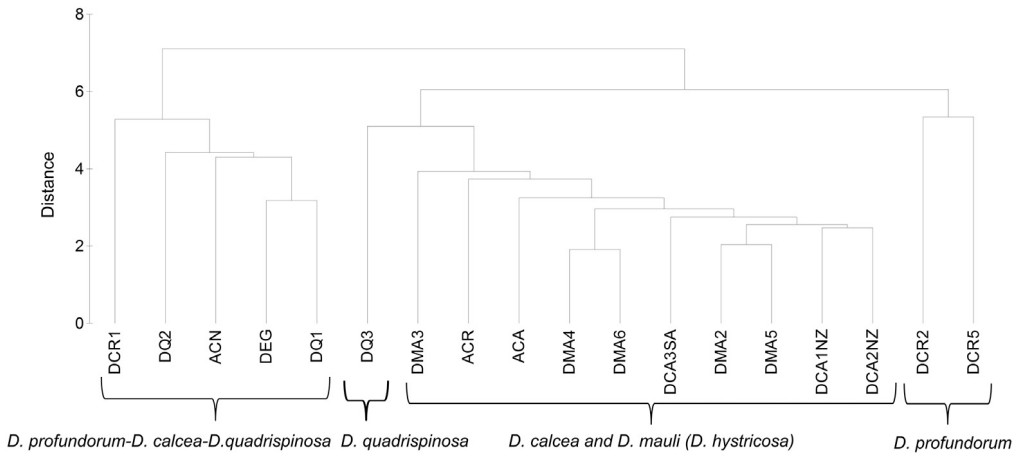

Fig. 11. Cluster of similarity based on morphometric characters values obtained from original descriptions of *Deania* species (see Table 3).

2.6. Data analysis

A multivariate analysis of the morphological characteristics was carried out with PRIMER 5 (Clarke and Warwick, 2001). Morphological measurements were standardised by total fish length (TL) and used to calculate a similarity matrix using Euclidean normalised distance. Based on this similarity matrix, a cluster analysis was carried out to obtain sample dendrograms. This matrix was also used to perform a non-metric multidimensional scaling (MDS) analysis to evaluate differences between the two species and sexes.

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

Data of (TL) and (CL) were checked for normality using a Shapiro–Wilk test. Since the assumptions of normality and homoscedasticity were not met, nonparametric tests were carried out. A Kruskal–Wallis test was performed to check if there were differences in the length of the dermal denticles according to the zone of the body. A post-hoc analysis, using Dunn test, was done to contrast differences between body sampling zones. The Mann–Whitney nonparametric test was performed to compare dermal denticle length between *Deania* species for each sampling zone.

To verify if there was a relationship between the CL and TL, a linear regression analysis and the Spearman correlation coefficient was used. The software used to perform these statistical analyses was SPSS Statistics 17.0. To determine if there were differences between sexes regarding CL, but excluding the TL effect, a linear mixed-effects model was applied. This analysis was performed using R (RCore Team, 2013) and the package Lme4. The function can be expressed as:

$$\text{Lmer} = \text{CL} \sim 1 + \text{TL} + \text{Sex} + \text{Zone} + (1|\text{code}) \text{ where,} \quad (1)$$

total fish length (TL), Sex and body Zone are the explanatory variables, dermal denticle length (CL) is the response variable and code acts as a random effect and refers to each of the measurements taken (10 per zone, total 30 per sample).

The 11 COI sequences obtained in this work were aligned with 33 sequences of *Deania* spp. available in the BOLD database using the Bioedit software version 7.0.5 (Hall, 1999). *Centrophorus squamosus* was used as outgroup. The phylogenetic analysis involved 44 nucleotide sequences with a total of 611 positions in the final dataset. The evolutionary analysis was carried out in MEGA (Kumar et al., 2018). The most appropriate nucleotide substitution model was selected according to the corrected Akaike Information Criterion (AICc). The phylogenetic tree was inferred by using the Maximum Likelihood method based on the Tamura–Nei model (Tamura and Nei, 1993). A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.1322)). In addition, the sequences obtained (from Section 2.5) were compared with the GenBank database using the BLAST local type sequence alignment software (Zhang et al., 2000).

3. Results

3.1. Dermal denticles

The comparison found significant differences of dermal denticle length among body zones; this highlights the importance of defining the body zone where the measurements are taken for comparative analysis among species. In *D. calcea* and *D. profundorum*, dermal denticles from the anterior zone were larger than in the rest of the sampled body zones. In *D. calcea*, those from the caudal region were the smallest (Fig. 4a); in *D. profundorum*, the smallest dermal denticles corresponded to the dorsal zone (Fig. 4b). The Kruskal–Wallis test showed that the differences were significant both for *D. calcea* ($K = 341.58$, $df = 2$, p-value < 0.001) and for *D. profundorum* ($K = 127.920$, $df = 2$, p-value = 0.002). Results of mean denticle length are shown in Table 4. Comparison of mean CL among body zones, using the post-hoc Dunn test, showed that in any pairwise contrast, differences were statistically significant (Table 5). In *D. calcea*, CL ranged from 340 μm to 1400 μm; in *D. profundorum*, CL ranged from 195 μm to 650 μm (Fig. 5). Comparison of CL measurements between *D. calcea* and *D. profundorum* using Mann–Whitney test showed that for each zone differences were significant: anterior zone ($n_1 = 330$, $n_2 = 150$, $Z = -16.750$, p-value < 0.001), dorsal zone ($n_1 = 330$, $n_2 = 150$, $Z = -17.409$, p-value < 0.001) and caudal zone ($n_1 = 330$, $n_2 = 150$, $Z = -16.764$, p-value < 0.001).

A positive correlation was found between dermal denticle length and total fish length in both Deania species (Fig. 6). In particularly, in *D. calcea* the Spearman test showed that the relation was moderately strong $r^2 = 0.451$ and $r^2 = 0.462$ for scales of the anterior and dorsal zone, respectively. In *D. profundorum*, the Spearman coefficient for scales of the anterior and dorsal zones was $r^2 = 0.7398$ and $r^2 = 0.7667$, respectively. In both species, the relationship was weaker for scales of the caudal region. Sexual differences were not observed in dermal denticle length on *D. calcea* or *D. profundorum*. However, in *D. calcea* dermal denticles

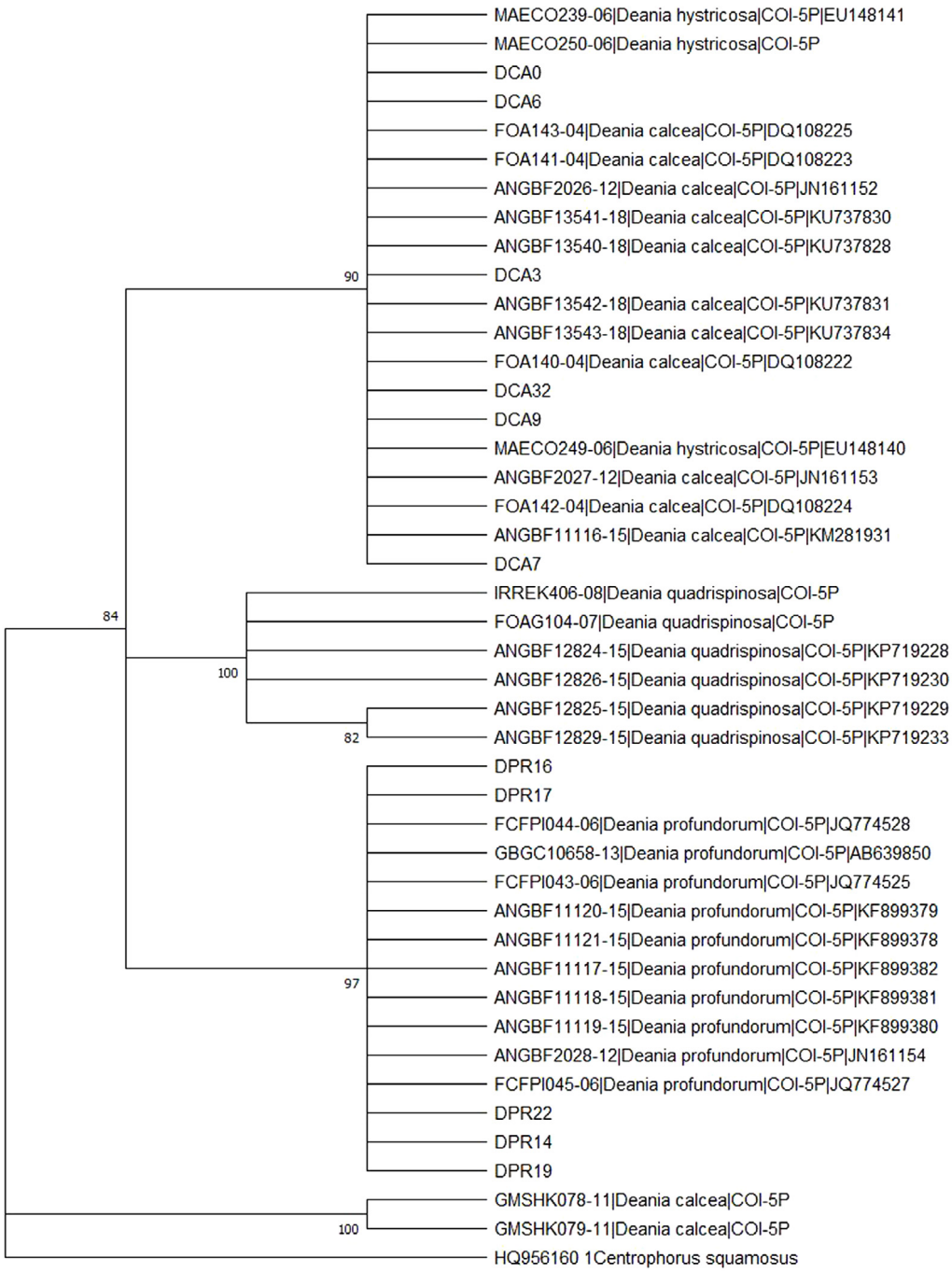

Fig. 12. Phylogenetic tree inferred using ML method (LnL −1228.9716). Bootstrap values greater than 70 shown near the respective branches. *Centrophorus squamosus* (GenBank accession number HQ956160) has been used as an out-group.

were larger in females, but this was due to fish length, as the larger individuals were all females. This effect was checked using the linear mixed model performed in R. The results obtained from the analysis of variance clearly showed an effect of shark total length and an effect of the body zone on dermal denticle length; however, no effect of sex was observed once the total length was accounted for (Table 6).

3.2. Morphological characters

The hierarchical analysis clearly identified three groups with distance values close to 10% (Fig. 7). Two groups corresponded to *D. profundorum* (DPR) individuals and the other group corresponded to *D. calcea* (DCA) individuals. In the case of *D. profundorum*, one group corresponded to small individuals, newborns and those less than 42 cm (28–41 cm) total length; the other *D. profundorum* group corresponded to larger specimens (56–86 cm). The MSD ordination analysis also produced similar results by clearly identifying the same three groups (ANOSIM R = 0.866, p-value = 0.001, Fig. 8).

The analysis performed with SIMPER showed the morphometric characters that contributed most to the divergence between the three groups, that is, between both *Deania* species and between *D. profundorum* size classes. With respect to the main differences between both *Deania* species (similar size range), the

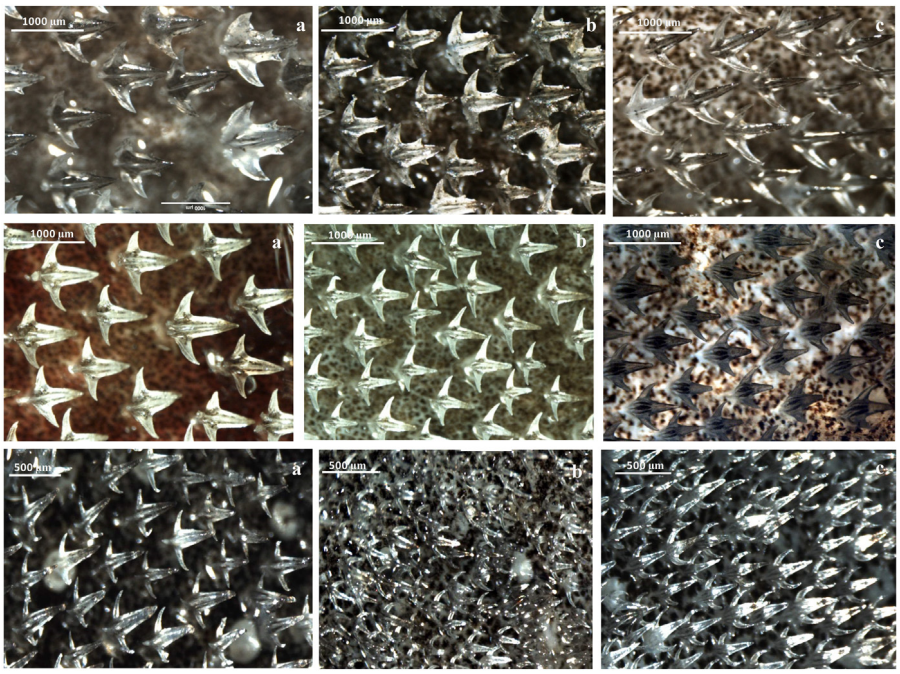

Fig. 13. Illustration of *Deania* dermal denticles taken from different body zones: (a) anterior, close to cephalic zone (b) dorsal zone and (c) caudal zone. Upper and middle images correspond to *D. calcea* male specimens (TL = 85.0 cm and TL = 87.2 cm respectively). The lower images correspond to a *D. profundorum* female (TL = 73.2 cm).

morphological characters that contributed most to the dissimilarities were those related to the first dorsal fin, distance between dorsal fins (D1L, IDS, D1B, 1Dto2D, IDS*) and the distances from the snout to the origin of dorsal and pelvic fins (PD1, PD2, PP2). Results are shown on Table 7. The ontogenic differences that contributed most to dissimilarity were also those related with the size of the first dorsal fin (D1L, D1B), distance to the origin of dorsal, pelvic and caudal fins (PD1, PP1, PD2*, CL), anterior margin of caudal fin (CDM) and distance to brachial, mouth or eye (PG1, POR, POB). Results are shown on Table 8.

According to the results, *D. profundorum* has a shorter first dorsal fin and both dorsal fins are much more separated than in *D. calcea*. The distances from the snout to origin of first dorsal fin spine and pelvic fin are slightly shorter in *D. profundorum* (Fig. 9). Small *D. profundorum* individuals have longer snout, smaller first dorsal fin, larger caudal fin, and shorter distance between dorsal fins compared to large *D. profundorum* individuals.

The MDS classification analysis based on the morphological characters of *D. calcea* and *D. profundorum* did not show significant differences between males and females (Fig. 10). In *D. profundorum*, the ontogenic differences were also evident in the MDS analysis (Fig. 10b); however, no sexual differences were observed. The visual analysis was statistically checked with the ANOSIM test and no significant differences were detected (ANOSIM R = 0.098, *p*-value = 0.231 and R = 0.098, p-value = 0.062) for *D. calcea* and *D. profundorum*, respectively.

3.3. Historical morphological data

The hierarchical analysis obtained with previously described *Deania* species (Section 2.4; Fig. 11) showed that there were mainly two distinct groups. One small group comprised by five species, one *D. eglantina* (DEG), two specimens of *D. quadrispinosa*, (DQ1, DQ2), one *A. natalense* (ACN) and one *D. cremouxi* (DCR1). DEG was later accepted as *D. calcea*; ACN and DCR were later accepted as *D. profundorum* as explained before (see Section 1.2). The larger hierarchical analysis group (thirteen species) with high similarity values included all specimens of *Deania calcea* (DCA1, DCA2, DCA3), all specimens of *D. mauli* (DMA2, DMA3, DMA4, DMA5, DMA6) and two more samples, *A. aciculatum* (ACN) and *A. rostratum* (ACR). These last two species ACN and ACR were both later accepted as *D. calcea* and *D. mauli* was later synonymised to *D. hystricosa*. Two *D. cremouxi* (DCR2, DCR5) and one *D. quadrispinosa* (DQ3) were also in this group, but more separated from the rest.

With respect to the first small group, the ACN and DCR1 samples, later *D. profundorum*, corresponded to individuals of 32.5 cm and 37.0 cm TL, respectively. The other *D. profundorum* (*D. cremouxi*) samples found in the large group, (DCR2 and DCR5) were individuals of 62 cm and 74.5 cm TL respectively.

3.4. DNA barcoding

A total of 32 sequences of *Deania* species available on BOLD database were used to compare *D. calcea* and *D. profundorum* haplotypes obtained in this study. *D. calcea* samples available on BOLD were collected from Australian waters (Ward et al., 2005). *D. hystricosa* samples were collected from the northern mid-Atlantic ridge during a barcoding campaign on deep-sea fishes. *Deania profundorum* samples available on BOLD database were collected in the Indian Ocean (Akhilesh et al., 2014) and in waters around southern Portugal coast (Costa et al., 2012).

The phylogenetic tree (Fig. 12) clustered the six sequences of *D. calcea* obtained in this study with three sequences of *D. hystricosa* and eleven sequences of *D. calcea* obtained from BOLD data. This clustering held with a bootstrap support of 90%. All *D. profundorum* sequences (five sequences from this study and ten sequences from BOLD data) were in the same cluster with a bootstrap support of 97%. Six sequences of *Deania quadrispinosa* from BOLD were in a different cluster but with 100% bootstrap support (see Fig. 12).

Two sequences of *D. calcea* were left out of these groupings. When checking these sequences and contrasting them using BLAST, two sequences (GMSHK078-11 and GMSHK079-11)

showed 99% identity with a COI sequence from *Etmopterus bigelowi* obtained by Straube et al. (2010); The eleven samples sequenced in this study were unambiguously clustered with those expected based on the morphological analyses.

Despite the specialised protocol used for formalin preserved samples obtained from *Deania* specimens kept in the museum collection of Paris (MHNH) results were unsuccessful.

4. Discussion

The family Centrophoridae (Bleeker, 1860) includes two genera, *Centrophorus* and *Deania*. Centrophoridae is one of the most taxonomically complex and confusing taxa of species currently under review (White et al., 2013, 2017). The high morphological similarity among some species, the scarcity of records and few taxonomical analyses contribute to the uncertainty concerning the taxonomy of some species.

The three *Deania* species found in NE Atlantic waters are morphologically very similar. Several authors have remarked that the characters that distinguish *D. calcea* from *D. hystricosa* need to be examined in more detail (Ebert and Stehmann, 2013; Menezes et al., 2012). Most of the literature regarding *Deania* in the northeast Atlantic showed that in very few cases the three species are caught or reported at the same location. Some of these references belong to Canary Islands (Brito et al., 1998; González et al., 2011), Galicia Bank (Bañón et al., 2010, 2016), Madeira Island (Biscoito et al., 2018) and at the Great Meteor seamount in the central East Atlantic (Palm and Schröderc, 2001).

Dermal denticles and teeth have traditionally been used as identification criteria to discriminate among elasmobranch species (Applegate, 1967; Deynat, 1998, 2000; Reif, 1982, 1985; Tanaka et al., 2002). Dermal denticles of *Deania* species showed a common morphological pattern with one main central cuspid and two lateral cuspids (Garrick, 1960; Cadenat and Blache, 1981). The size of lateral trunk dermal denticles is one of the characters used to distinguish these species, particularly *D. calcea* and *D. hystricosa* (Compagno, 1984; Ebert and Stehmann, 2013; Iglésias, 2014).

A number of studies on other elasmobranchs have demonstrated that dermal denticles vary in shape and size interspecifically and along the body of individual sharks (Deynat, 2000; Díez et al., 2015; Motta et al., 2012; Raschi and Tabit, 1992; Reif, 1985; Sullivan and Regan, 2011). Results obtained in this study show that dermal denticles of *D. calcea* were larger than those of *D. profundorum* in all the body zones examined. In both species denticles from the anterior zone (close to the head) were larger than those from other body zones (Fig. 13). In *D. calcea*, a progressive reduction of dermal denticles was found towards the caudal fin; in *D. profundorum*, dermal denticles on the dorsal zone were slightly smaller than on the caudal zone. A progressive reduction in denticle size from the cephalic to the caudal region was also observed and reported in *Scyliorhinus canicula* (Sullivan and Regan, 2011). Similar results were found on *Isurus oxyrinchus*; the smallest denticles were found in the caudal keel, which the authors linked to its hydrodynamic behaviour (Díez et al., 2015). Similarly, Dillon et al. (2017) reported that denticle morphology was highly variable across the body of an individual shark and between taxa, preventing species- or genus-level identification based on isolated denticles. So far, they found that denticle morphology is strongly correlated with shark ecology or functional aspects, similar to which was reported by Ankhelyi et al. (2018).

The relationship between the size of placoid scales (length and width) and the total length of the shark has been reported for several pelagic sharks (Raschi and Musick, 1984) and deepwater sharks such as *Centroscymnus owstonii* and *Centroscymnus coelolepis* (Weigmann et al., 2015), *Bythaelurus* spp. (Weigmann et al., 2018) and *Bythaelurus bachi* (Weigmann et al., 2016). The results obtained in this study showed a positive correlation between denticle length and total shark length for both *Deania* species. This relationship was evident in all body regions but particularly in the head and dorsal zone. Studies on New Zealand elasmobranchs made by Garrick (1960) reported that denticle changes with growth were much more common amongst squaloid sharks than was previously believed. Garrick (1960) also indicated the danger of using denticle characters as diagnostic criteria unless denticles changes are known.

No sexual dimorphism was observed in dermal denticle length in *D. calcea* and *D. profundorum*. In *D. calcea* dermal denticles were larger in females than in males; however, this result was due to the larger size of female specimens, and once the effect of length was removed, there were no significant differences for sex. Sexual dimorphism has been described in the dermal denticles of *S. canicula* (Crooks et al., 2013). These authors reported that the length, width and density of the dermal denticles of mature male and female were sexually dimorphic in pectoral fin, area posterior to the pectoral fin, caudal fin and pelvic girdle. This sexual dimorphism has been suggested to occur as a response to male biting during mating; males have been observed to bite and wrap themselves around females (Crooks et al., 2013). In the present study, only dermal denticles from three body zones were examined. These zones did not include pectoral or other fins; thus to adequately compare results, a thorough inspection on different structures should be conducted. No published studies regarding this issue or about reproductive mating behaviour on *Deania* have been found.

Regarding inter-species comparison of dermal denticle length the results of this study indicate that differences between *Deania* species were statistically significant. Thus, a priori this character could be used to discriminate *D. calcea* from *D. profundorum*. Nevertheless, for comparative analysis total fish length and the sampling zone should be considered as well, due to the relations discussed above. Little overlap exists in the denticle length range between these two *Deania* species. However, since dorsal denticle length in *D. calcea* can ranged from 300 μm to 1200 μm caution should be taken when compare to other species such as *D. hystricosa*.

Following Compagno (1984), the features which distinguish *D. profundorum* from the others in the genus *Deania*, were the presence of a subcaudal keel on the underside of the caudal peduncle and the distance from the origin of the first dorsal spine to the first dorsal fin free rear tip; in the latter, the distance in *D. profundorum* is only slightly greater than the distance from the free rear tip to the second dorsal spine. The results obtained in this study confirmed these criteria and indicated that the morphological characters that contributed most to the differences found between *D. calcea* and *D. profundorum* were mainly related to the size of the dorsal fins, the distance among dorsal fins and the distances from the snout to the origin of each dorsal fin.

Previous studies also refer to the position of the first dorsal fin (PD1*) as a significant character useful in the identification of *Deania* species (Garman, 1913). According to the classification proposed by Garman (1913), in *D. hystricosa* (*Acanthidium hystricosum*) the first dorsal fin spine would be closer to caudal fin than the end of the snout; in *D. profundorum* (*Acanthidium profundorum*), the first dorsal fin spine is nearer to the end of the snout than to caudal; in *D. calceus* (*Acanthidium calceus*) first dorsal spine is equidistant. The key suggested by Bigelow and Schroeder (1957) and adapted by Cadenat and Blache (1981) gave more significance to the position of the edge of the pectoral fin when laid back to the body with respect to the vertical of the spine of the first dorsal fin. In this key, if the edge of pectorals reached the perpendicular of the first dorsal spine, this would discriminate *D. profundorum* and *D. natalense* (*D. calcea*)

Table 1
Data summary of sampling locations, date, gear and specimens collected.

	Year	Survey area	Date	Gear	Haul code	Latitude	Longitude	Mean Depth (m)	N°indiv
Deania calcea	2011	Le Danois Bank	23/07/2011	Longline	L2	44°00.01 N	4°40.21 W	1098	5
			10/12/2012		L3	43°55.94 N	4°52.53 W	1024	1
			13/06/2013		L1	44°00.07 N	4°40.26 W	110	2
	2012		01/07/2013		L2	44°03.89 N	4°31.24 W	950	1
	2013		20/09/2013		L3	43°56.71 N	4°48.92 W	1100	2
	2014		01/06/2014		L1	44°00.51 N	4°46.89 W	1150	2
	2015		08/07/2015		L1	44°00.38 N	4°37.40 W	1150	4
			13/10/2015	Trawl	G100	43°55.53 N	5°05.88 W	815	2
	2010	Aviles Canyon	22/07/2010	Trawl	G4	43°54.21 N	6°16.09 W	980	5
			27/07/2010		G9	43°54.41 N	5°52.56 W	1137	6
	2012		06/06/2012	Longline	L3	43°54.03 N	6°08.71 W	1000	3
Deania profundorum	2009	Galicia Bank	22/07/2009	Trawl	G4	42°40.28 N	11°39.53 W	750	4
	2010		20/08/2010		G13	42°41.83 N	11°44.86 W	770	5
			24/08/2010		G10	42°44.99 N	11°42.48 W	782	5
			30/07/2011		G3	42°40.13 N	11°50.14 W	772	2
	2011		04/08/2011		G7	42°48.01 N	11°45.38 W	850	6
			09/08/2011		G11	42°45.86 N	11°44.04 W	780	1
	2018	Cantabrian shelf	05/10/2018	Trawl	G67	44°05.57 N	7°32.95 W	581	2

Table 2
Morphometric characters used in this study following Compagno (1984) and White et al. (2013) for those marked with an asterisk (*). Values represent the mean and range (in brackets). All values are expressed as percentage of total length (TL) except TL given in cm.

Morphometric measurements			*Deania calcea*				*Deania profundorum*			
Area	Description	Abbrev	Males (n = 19)		Females (n = 12)		Males (n = 13)		Females (n = 12)	
Body Length	Total length	**TL**	80.5	(63.0–88.0)	87.5	(65.0–107.0)	52.5	(28.7–71.8)	68.2	(28-86.4)
	Fork length	**FL**	89.0	(87.9–90.4)	89.6	(87.9–92.5)	88.4	(85.4–91.3)	88.0	(81.2–90.2)
	Precaudal fin length	**PCL**	80.9	(78.3–83.3)	81.4	(78.3–83.2)	80.0	(76.5–83.7)	79.7	(74.0–82.9)
	Pre-second dorsal fin length	**PD2**	64.7	(61.7–69.1)	65.9	(62.9–70.1)	64.6	(61.8–68.4)	65.6	(63.1–67.4)
	Pre-second dorsal fin length to the spine	**PD2***	70.1	(68.2–71.6)	70.9	(68.9–73.8)	69.6	(66.2–83.4)	69.4	(64.3–72.9)
	Pre-first dorsal fin length	**PD1**	28.1	(24.2–39.0)	28.7	(25.2–33.7)	31.7	(27.8–38.5)	29.3	(27.0–34.6)
	Pre-first dorsal fin length to the spine	**PD1***	41.0	(38.0–42.7)	42.2	(40.0–49.2)	39.9	(37.5–41.5)	39.6	(34.5–40.7)
	Interdorsal space	**IDS**	14.1	(12.2–17.6)	14.4	(11.6–18.5)	18.2	(13.2–22.6)	18.7	(14.8–22.1)
	Interdorsal space to the spine	**IDS***	19.4	(16.4–21.2)	19.3	(17.0–21.1)	21.2	(17.6–23.5)	20.7	(15.7–25.3)
	1ªdorsal fin rear tip to 2ªdorsal spine	**1Dto2D**	11.8	(10.9–13.3)	11.7	(10.0–13.5)	14.5	(11.5–17.6)	13.7	(11.1–16.3)
	Prepectoral fin length	**PP1**	24.3	(22.8–27.5)	24.8	(23.0–27.3)	24.8	(22.3–28.2)	24.4	(22.5–29.3)
	Prepelvic fin length	**PP2**	62.8	(61.0–64.2)	63.6	(62.3–65.3)	59.9	(58.0–63.0)	60.6	(56.4–63.7)
	Head length	**HDL**	24.2	(22.8–27.1)	24.7	(23.0–27.3)	25.1	(22.3–28.2)	24.1	(21.4–29.3)
	Prebranchial length	**PG1**	20.4	(19.3–22.2)	20.9	(19.3–23.5)	20.9	(17.4–24.4)	20.4	(18.3–23.8)
	Preoral length	**POB**	12.9	(11.6–15.1)	13.7	(11.8–15.4)	10.0	(7.7–12.3)	9.8	(8.6–11.4)
	Preorbital length	**POR**	9.6	(8.2–11.4)	10.3	(8.8–11.4)	13.9	(11.6–16.2)	13.3	(11.5–15.7)
	Prenarial length	**PRN**	4.7	(4.1–5.4)	5.1	(4.6–5.6)	4.9	(3.8–6.3)	4.7	(3.9–5.9)
	Prespiracular length	**PSP**	15.2	(14.4–16.6)	16.2	(14.4–18.5)	16.5	(14.3–19.5)	15.8	(15.1–18.8)
Head	Eye length	**EYL**	5.1	(4.4–5.4)	5.0	(4.2–5.6)	5.7	(4.5–7.4)	4.9	(4.2–6.5)
	Interorbital space	**INO**	4.2	(3.8–4.9)	4.5	(3.9–4.9)	5.5	(4.3–8.1)	5.1	(4.3–10.0)
	Internal narial space	**ENS**	7.1	(6.3–8.1)	7.5	(6.9–8.3)	3.9	(3.2–4.9)	4.1	(3.6–4.6)
	External narial space	**INW**	3.5	(3.2–4.3)	3.9	(3.5–4.3)	8.0	(6.8–10.1)	8.0	(7.3–9.6)
	Mouth width	**MOW**	7.0	(5.2–7.7)	7.0	(6.4–7.5)	7.4	(6.6–8.4)	6.9	(6.3–7.4)
Pectoral Fin	Pectoral fin length	**P1L**	11.7	(10.1–12.7)	11.5	(10.5–12.5)	13.2	(10.9–14.9)	13.1	(11.3–14.3)
	Pectoral fin base	**P1B**	3.5	(3.0–3.9)	3.4	(2.5–3.8)	4.6	(3.6–5.9)	4.9	(3.4–6.3)
	Pectoral fin height	**P1H**	6.6	(5.7–7.1)	6.4	(5.7–6.7)	7.8	(6.8–8.8)	7.6	(6.7–8.4)
Dorsal Fins	First dorsal fin length	**D1L**	31.3	(24.9–34.9)	31.5	(26.8–35.2)	23.1	(15.4–26.3)	25.4	(19.6–28.9)
	First dorsal fin length from the spine	**D1L***	17.8	(16.0–19.6)	18.3	(17.0–19.2)	14.8	(12.6–17.1)	15.8	(13.2–17.0)
	First dorsal fin base	**D1B**	24.4	(20.1–27.5)	24.1	(20.7–27.6)	17.5	(9.5–21.7)	19.3	(15.0–22.1)
	First dorsal fin base from the spine	**D1B***	11.2	(9.5–15.9)	10.8	(9.6–12.3)	8.7	(5.6–11.1)	9.1	(5.6–10.4)
	First dorsal fin height	**D1H**	4.2	(3.7–4.9)	4.3	(3.6–4.8)	5.0	(4.4–6.1)	5.3	(3.5–6.8)
	Second dorsal fin length	**D2L**	18.1	(16.2–19.6)	17.7	(16.4–20.1)	16.5	(14.2–18.4)	16.8	(15.4–18.0)
	Second dorsal fin length from the spine	**D2L***	13.1	(12.4–14.4)	13.0	(11.9–14.7)	13.0	(11.8–14.4)	12.9	(11.4–13.9)
	Second dorsal fin base	**D2B**	13.4	(11.1–15.5)	13.1	(11.8–14.7)	13.0	(10.5–15.3)	13.0	(11.2–14.6)
	Second dorsal fin base from the spine	**D2B***	8.9	(7.2–9.7)	8.7	(7.4–10.6)	9.6	(7.7–10.9)	9.6	(7.6–10.7)
	Second dorsal fin height	**D2H**	6.1	(5.1–6.5)	6.3	(5.6–7.4)	6.4	(5.6–7.5)	6.6	(5.9–7.4)
Pelvic Fin	Pelvic fin length	**P2L**	10.7	(6.5–13.0)	10.4	(9.6–12.1)	10.7	(8.3–12.0)	10.4	(8.9–11.3)
	Pelvic fin base	**P2B**	2.8	(2.0–3.2)	2.8	(2.3–3.8)	3.7	(2.3–5.4)	4.0	(2.7–6.0)
	Pelvic fin height	**P2H**	4.5	(3.6–4.7)	4.7	(4.0–7.2)	4.7	(3.8–6.7)	4.9	(3.7–6.4)
Caudal Fin	Dorsal caudal fin margin	**CDM**	18.7	(16.4–19.7)	18.2	(16.2–20.3)	19.9	(17.3–26.2)	18.8	(17.0–22.5)
	Preventral caudal margin	**CPV**	10.5	(9.0–11.6)	10.4	(9.5–11.2)	10.9	(9.6–12.4)	11.1	(10.3–11.7)
	Caudal fin length	**CL**	21.9	(16.1–22.7)	21.1	(17.0–23.3)	23.4	(21.1–26.5)	22.7	(21.2–26.2)
	Caudal fin fork length	**CFL**	10.4	(4.8–11.8)	10.6	(5.7–12.2)	10.6	(7.5–12.2)	11.0	(9.8–13.3)
	Caudal fin peduncle height	**CPH**	3.3	(3.0–3.4)	3.3	(3.0–3.5)	3.4	(3.1-3,8)	3.5	(3.1–3.8)

Table 3
Morphometric characters of several *Denia* species recorded in previous studies. All values are expressed as percentage of total length (TL). Collection type refers to: Holotype, Paratype or unknown. Sex refers to M=male and F= Female. Cross above table(X) indicates specimen not used in the cluster analysis. Symbol # indicates the morphological characters used in the similarity matrix. Asterisk * refers to those measurements linked to the spine. First column shows the original description and next the equivalence (abrev.) to Ebert and Stehmann (2013). A dotted line indicates no data recorded. In brackets and italic those values estimated.

						X				X	X								X						X	X	X
Included on reference			Bigelow and Schroeder (1957)			Springer (1959)	Penrith (1969)	Cadenat (1960)					Karrer (1973)		Penrith (1969)	Garrick (1960)		Penrith (1969)	Cadenat and Blache (1981)						Cadenat and Blache (1981)		
Collection type			Holo-type	Holotype	Holotype	Holotype	Holotype	Sintype											Holotype Sintypes								
Sample Location			Japan	Japan	Japan	NW Atlantic	South Africa	Senegal					Namibia		Sudafrica	New Zeland		South Africa	Madeira Islands						Madeira Islands		
Original species name			*Acan-thidium rostra-tum*	*Acanthidium aciculatum*	*Deania eglantina*	*Deania elegans*	*Acanthidium natalense*	*Deania cremouxi*					*Deania quadrispinosus*		*D.quadrispinosus*	*Deania calcea*		*Deania calcea*	*Deania mauli*						*Deania calcea*		
Author(s) species name and year			Garman (1906)	Garman (1906)	(Jordan and Snyder, 1902)	Springer (1959)	Gilschrist, 1922	Cadenat (1960)					McCulloch, 1915		McCulloch, 1915	Garman (1906)		Garman (1906)	Cadenat and Blache (1981)						Garman (1906)		
Accepted as			*D. calcea*	*D.calcea*	D. calcea	*D. profundorum*	*D. profundorum*	*D. profundorum*					*D. quadrispinosa*		*D. quadrispinosa*	*D. calcea*		*D. calcea*	*D. hystricosa*						*D. calcea*		
Code reference used in this study			ACR	ACA	DEG	DEL	ACN	DCR1	DCR2	DCR3	DCR4	DCR5	DQ1	DQ2	DQ3	DCA1NZ	DCA2NZ	DCA3SA	DMA1	DMA2	DMA3	DMA4	DMA5	DMA6	DCA4MD	DCA5MD	DCA6MD
Total Length (mm)			873	890	300	315	325	370	620	645	680	745	412	580	900	823	890	865	835	838	802	965	1057	1094	925	945	1000
Sex			F	M	F	M	M	F	M	M	F	F	?	M	F	M	F	M	M	M	F	F	F	F	F	F	F
Original description of morphological characters		Abrev. Adapted																									
Distance from snout to upper caudal	#	PCL	84.0	83.2	80.0	77.7	78.4	75.6	79.8	78.2	78.6	79.1	80.1	78.6	82.5	81.8	82.0	82.8	83.5	83.5	83.2	82.6	83.4	83.6	83.3	82.3	84.0
Distance from origin pectoral to origin of pelvics	#	(PP2-PP1)	40.8	39.3	33.3	–	32.0	32.4	37.9	39.5	37.5	38.9	33.0	36.6	37.4	39.5	39.7	40.4	*40.1*	*39.7*	*38.6*	*41.6*	*41.2*	*40.8*	*38.5*	*40.2*	*40.3*
Distance from origin pelvics and origin of caudal	#		13.4	14.4	13.3	–	16.0	17.5	18.5	17.0	18.3	18.1	15.3	16.9	19.3	16.1	14.6	16.2	16.1	15.5	16.0	14.7	15.6	16.1	–	–	–
Distance from snout to 1st dorsal spine	#	PD1*	39.9	40.2	42.3	41.3	40.0	41.8	39.5	–	–	37.5	44.7	41.5	41.5	41.2	42.0	40.3	38.9	40.3	40.6	39.7	41.8	39.3	41.0	38.2	38.5
Distance from snout to 2st dorsal spine	#	PD2*	70.5	70.5	68.2	–	66.8	67.5	71.7	–	–	71.8	69.8	66.0	71.4	69.5	72.0	70.6	69.7	71.1	70.0	70.6	71.4	71.9	71.8	66.8	71
Distance from snout to pectoral	#	PP1	24.0	23.2	28.7	27.6	25.2	25.3	23.3	21.7	22.7	22.1	30.8	26.2	28.2	23.8	24.6	25.2	23.9	24.7	24.9	23.0	23.6	23.1	23.6	22.2	21.9
Distance from snout to pelvics	#	PP2	64.7	62.6	61.6	–	58.2	58.1	61.2	61.2	60.2	61.0	63.1	58.6	63.0	62.3	64.0	62.6	64.0	64.4	63.5	64.6	64.8	63.9	62.1	62.4	62.2
Snout in front of mouth opening	#	POR	13.7	12.1	15.3	18.1	16.0	14.8	12.8	12.4	13.2	12.0	16.6	14.1	13.6	12.7	14.3	12.7	11.9	13.2	15.5	13.3	13.0	12.7	13.1	12	12.3
Distance from snout to eye		POB	–	–	–	–	12.3	10.8	9.0	8.5	8.8	9.3	13.5	10.9	11.1	9.5	10.6	9.8	–	9.5	11.2	9.3	10.9	9.4	9.8	–	8.1
Snout in front of outer nostrils	#	PRN	5.3	4.0	5.3	5.1	6.3	5.4	3.8	4.6	4.4	6.7	5.8	4.8	6.8	4.2	5.4	5.3	4.3	5.2	6.2	4.8	5.1	5.0	4.5	3.8	4.5
Eye horizontal diameter	#	EYL	3.6	4.7	5.3	6.3	6.0	5.4	5.0	5.4	4.4	3.4	5.5	4.9	4.3	4.6	4.8	4.6	–	4.7	4.4	4.6	4.3	4.1	4.7	4.3	3.8
Mouth breadth	#	MOW	5.6	6.4	7.7	6.7	6.8	7.3	5.9	6.9	5.5	6.7	8.3	7.8	6.0	5.9	6.1	6.1	6.8	7.0	7.5	6.9	6.9	6.8	6.4	6.3	6.5
1st dorsal fin length of base from spine origin	#	D1B*	9.7	11.8	9.5	–	8.0	6.7	10.4	–	–	10.0	10.0	10.0	8.4	10.5	11.7	12.3	13.7	11.2	12.2	12.0	10.8	13.1	9.2	10	8.2
1st dorsal fin vertical height	#	D1H	3.5	2.9	3.8	–	4.0	5.6	5.1	5.4	4.4	5.3	3.9	4.0	3.7	3.3	3.6	3.2	3.3	4.2	3.9	3.1	4.0	3.5	4.9	4.2	4.8
2nd dorsal fin length of base from spine origin	#	D2B*	10.0	10.0	9.2	–	8.0	6.7	8.0	–	–	6.7	8.6	9.0	8.4	8.9	8.9	9.4	9.5	9.2	8.8	8.6	9.0	8.3	8.4	7.9	7.8
2nd dorsal fin vertical height	#	D2H	5.5	4.8	4.7	–	6.0	6.2	7.4	6.2	6.6	6.7	6.3	5.7	5.8	5.2	5.5	6.2	5.5	5.9	5.9	4.9	5.3	5.0	6.5	6.4	6.2
Caudal fin upper margin	#	CDM	16.0	16.8	20.0	22.2	20.9	24.3	17.0	21.7	–	20.8	19.9	22.9	16.9	18.4	18.0	18.7	17.5	16.5	16.8	17.4	16.6	16.4	–	–	–
Caudal fin lower anterior margin		CPV	10.8	9.8	11.9	11.7	11.0	–	–	–	–	–	10.4	10.2	9.1	10.8	10.6	9.7	–	11.2	10.9	9.3	10.1	9.3	–	–	–
Distance from snout to 1st branchial		PG1	–	–	–	–	24.3	–	–	–	–	–	26.9	22.7	21.6	20.5	21.1	20.0	20.0	20.2	21.5	19.6	19.8	21.8	–	–	–
Pectoral fin width		P1L	6.1	6.1	6.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pectoral fin length of anterior margin		P1A	9.0	8.9	9.3	–	11.4	–	–	–	–	–	11.3	10.7	10.0	11.3	10.6	11.3	–	11.3	10.8	10.3	11.9	10.5	12.7	13.2	14.1
Nostrils distance between inner ends		INW	3.4	3.4	4.5	–	–	–	–	–	–	–	–	–	–	–	–	–	3.3	6.2	4.7	4.0	4.0	4.2	–	–	–
Interspace between origin of 1st and 2nd dorsal spine		IDS*	30.5	30.7	24.6	–	28.0	–	–	–	–	–	15.8	16.2	21.4	17.8	17.8	19.0	30.8	30.8	29.4	30.9	29.6	32.6	–	–	–
Interspace between 2nd dorsal and caudal		DCS	3.2	3.4	3.8	–	4.3	3.5	4.0	–	4.1	4.6	2.2	2.6	4.8	3.6	2.7	4.6	–	–	–	–	–	–	–	–	–
Interspace between pelvics and caudal		PCA	10.3	10.1	8.9	–	13.2	–	–	–	–	–	10.2	11.5	15.1	10.5	10.3	11.4	–	11.6	12.6	11.0	11.4	11.8	–	–	–
Trunk at origin of pectoral height		TRH	8.0	6.7	7.7	–	6.8	–	–	–	–	–	9.2	8.8	7.3	8.5	8.3	9.1	–	–	–	–	–	–	–	–	–

from the other reported species at that time, *D. calceus (D. calcea), D quadrispinosus, D. cremouxi* (*D. profundorum*) and *D. mauli* (*D. hystricosa*). This character was not recorded in most of the previously described *Deania* species, but a comparison of PD1* values against a rough estimation of the distance from snout to pectoral (PP1) plus pectoral fin length (P1A) showed that in all the species the distance from snout to pectoral fin edge was shorter than PD1*. Other morphological characters suggested by Bigelow and Schroeder (1957) and Cadenat and Blache (1981) as potential identification keys referred to the comparison of length and height of both dorsal fins.

According to Bigelow and Schroeder (1957), the height of first dorsal fin (D1H) in *D. profundorum* is about 30% lower than the height of second dorsal fin (D2H). The results obtained in the present study agree with Bigelow and Schroeder (1957); however, similar height comparisons between dorsal fins have also been found in *D. calcea* (Table 2). As it has been previously reported, the most significant criteria to discriminate between *D. profundorum* and *D. calcea* is the length of the first dorsal fin (D1L and D1B) and interdorsal space. D1L and D1B are much larger in *D. calcea* than in *D. profundorum* and both dorsal fins are more separated in *D. profundorum* than in *D. calcea*.

Similar results were described by Veríssimo et al. (2014) in congeners of Genus *Centrophorus* (Fam. Centrophoridae). The taxonomic key suggested to identify species of *Centrophorus* was mainly based on differences of the dorsal and pectoral fin shape (height, length and base), inter-dorsal space and distances from the snout to the mouth or nostrils. The results from the cluster analysis in the present study highlighted the significance of ontogenic changes in morphology that could be a major issue in the identification of deep-water sharks, particularly in Centrophoridae. Ontogenic morphology has been recently pointed out by several authors on taxonomic revision of squaliformes (White and Last, 2013; White et al., 2013, 2017; Viana and De Carvalho, 2018). Based on the results of the present study, small individuals of *D. profundorum* would have a longer snout, smaller dorsal fins and large caudal fins compared to larger individuals. There were no small individuals of *D. calcea* sampled in this study, so ontogenic differences were not checked in *D. calcea*.

According to morphometric results, no sexual dimorphism was observed in *D. calcea* and *D. profundorum*. Similar studies performed on *Etmopterus* species, have found no sexual differences in *Etmopterus spinax* or *E. pusillus* (Coelho and Erzini, 2008). However sexual morphometric differences have been reported in the lesser-spotted dogfish, *Scyliorhinus canicula* (Ellis and Shackley, 1995; Filiz and Taşkavak, 2006) and in several deep-water skates; *Amblyraja jenseni*, *Bathyraja pallida*, *Bathyraja richardsoni*, *Rajella bigelowi* and *Rajella kukujevi* (Orlov and Cotton, 2011). Regarding *Deania* species not any published information on sexual dimorphism has been found except sexual dimorphism expressed in body size (Clarke and Warwick, 2001; Irvine et al., 2012; Parker and Francis, 2012; Rochowski et al., 2015). In most chondrichthyans (viviparous and ovoviviparous), females tend to be larger than males (Ford, 1921; Clarke et al., 2002). The results obtained in this study demonstrate that despite females attaining larger size than males, the body proportions are maintained and no sexual dimorphism is observed.

Despite the variability of individual sampling (size, sex, location, collector, etc.) and of the procedures in recording the different morphological measurements, the results suggest that there were not large differences among species using historical morphological data. According to the morphological characters available and used in the analysis, *D. calcea* and *D. mauli* were grouped together. *Deania cremouxi* and *D. quadrispinosa* appeared more separated than the rest of their congeners but were very close to each other. *Deania cremouxi* was divided into two separated groups, (DCR1, ACN) and (DCR2, DCR5). The size of *D. cremouxi* specimens, belonging to the first group was 37.0 and 32.0 cm respectively, and those comprised in the second group were 62.0 and 74.5 cm respectively. This may reflect ontogenetic changes in agreement with ontogenetic morphological differences observed in this study in *D. profundorum* samples. The discrepancy found among the three *D. quadrispinosa* samples are not clearly elucidated. However, differences in total length were also observed. The specimens (DQ1, DQ2) included in the first group (41.2 and 58.0 cm respectively) were smaller than DQ3 (90.0 cm). In this analysis, only 17 morphological characters could be used; nevertheless, the most significant characters, those related with dorsal fin lengths and distance, were included.

Previous genetic studies demonstrated that there was a high similarity in the COI sequences of *Deania* species, which suggested their extremely close relationship (Sanjuán et al., 2012). Similar results were obtained in this study. The phylogenetic tree reconstructed with the Cytochrome c-oxidase subunit I fragment was unable to molecularly discriminate *D. hystricosa* from *D. calcea,* as previously observed by Ward (2009). As in the present study, no samples identified as *D. hystricosa* were collected for morphological analysis; no other molecular markers of *D. hystricosa* could be used to decipher the relationship between *D. calcea* and *D. hystricosa*. Samples obtained from the museum specimens had been preserved in formalin for long time, which is known to cause DNA fragmentation, degradation, and cross-linking to proteins (e.g., Tokuda et al., 1990; Srinivasan et al., 2002). Regrettably it was not possible to extract and amplified DNA from these samples and thus compare with other available samples. Currently, published records on *Deania* species and the molecular data available indicate that *D. calcea* and D. *hystricosa* can be considered synonymous. More morphological and molecular analysis focusing on both species would be necessary to corroborate this hypothesis.

Two *D. calcea* sequences appeared clearly separated from the D. *calcea-hystricosa* cluster and from the other *Deania* species. These *D. calcea* specimens are probably misidentified species since, according to BLAST, corresponded to *Etmopterus bigelowi* (Straube et al., 2010). A revision of the sequences deposited in the database corresponding to their morphological characteristics would be necessary.

It is likely that *D. calcea* and *D. hystricosa* could have been morphologically misidentified. A new primer pair based design on the COI reference sequences for the genus *Deania* should be recommended for molecular analysis of museum samples. This could provide better amplification efficiency than universal primers and, at the same time, prevent the amplification of non-target, contaminant DNA commonly found in museum samples.

More genetic studies, including other mitochondrial or nuclear DNA analysis, will be required to determine the extent of reproductive isolation between these two species (Menezes et al., 2012; Sanjuán et al., 2012). It is also possible that these are different phenotypes of the same species. A molecular study based on the identification of two smooth-hound sharks, *Mustelus mustelus and M. punctulatus*, using mitochondrial cytochrome c- oxidase subunit I (COI), microsatellites and Internal Transcribed Spacer 2 (ITS2) revealed that only the microsatellite-based method was successful at identifying 100% of the specimens whereas tests based on COI and ITS2 produced ambiguous results in 6.6% and 14.4% of the cases, respectively (Marino et al., 2015, 2017).

Reliable and consistent discrimination of *Deania* is essential for conservation and sustainable management of these deep-water elasmobranchs, which are regularly by-catch of deep-water fisheries. A better knowledge of the biology and ecology of these species is crucial for predicting their response to increased anthropogenic effects (Rochowski et al., 2015).

The available information for *D. calcea* has enabled assessment of this species as endangered in European waters according to

Table 4
Mean and standard deviation of dermal denticle length (μm) in each body sampling zone for both *Deania* species and results of Kruskal–Wallis test.

Species/Zone	Mean denticle size (μm)			Std. Deviation			N	Kruskal–Wallis test	
	Anterior	Dorsal	Caudal	Anterior	Dorsal	Caudal			
D. calcea	890.7	760.5	639.5	176.6	152.7	109.2	990	H = 341.580	p = 0.000
D. profundorum	455.5	300.9	360.4	119.4	63.4	84.1	450	H = 127.920	p = 0.002

Table 5
Comparison of dermal denticle length between body sampling zones for each *Deania* species. Results of non-parametric Dunn test, significance value and confidence interval.

Body zones	*Deania calcea*					*Deania profundorum*				
	Estimate	Std. Error	Sig.	95% Conf. Interv.		Estimate	Std. Error	Sig.	95% Conf. Interv.	
				Lower	Upper				Lower	Upper
Anterior - Dorsal	129.621	12.836	0.000	98.89	160.35	154.565	11.036	0.000	128.02	181.10
Anterior - Caudal	250.913	11.412	0.000	223.58	278.24	95.093	11.926	0.000	66.44	123.74
Dorsal - Caudal	121.293	10.333	0.000	96.55	146.03	−59.467	8,600	0.000	−80.12	−38.81

Table 6
Anova results obtained after applying the linear mixed model.

		Chi-square	Df	Prob(>Chisq)	Sig.
D. calcea	TL	36.43	1	1.59E−09	0.000
	Sex	2.12	1	0.145	
	Zone	1157.27	2	< 2.2e−16	0.000
D. profundorum	TL	37.88	1	7.53E−10	0.000
	Sex	0.32	1	0.5722	
	Zone	577.55	2	< 2.2e−16	0.000

Table 7
Dissimilarity index between *Deania calcea and D. profundorum* (TL> 55 cm) and the morphometric characters that contributed most to this discrepancy. The results are expressed as percentage of total length (TL).

Morphometric character	Average value (cm)		Average dissimilarity	Dissimilarity/SD	Percentage	
	D. profundorum	*D. calcea*			Contribution	Cumulative
D1L	25.83	31.37	0.34	2.25	8.93	8.93
IDS	19.62	14.23	0.33	2.37	8.56	17.49
D1B	20.11	24.29	0.26	2.18	6.65	24.14
PD1	29.45	28.32	0.19	1.15	5.00	29.13
1Dto2D	14.73	11.84	0.18	1.83	4.70	33.83
IDS*	21.52	19.36	0.15	1.43	3.84	37.67
PP2	61.03	63.13	0.15	1.66	3.81	41.48
PD1*	39.40	41.45	0.14	1.11	3.66	45.14
DIL*	16.04	18.04	0.13	1.69	3.39	48.53
PD2*	70.69	70.41	0.13	0.71	3.27	51.79
PD2	65.94	65.12	0.12	1.43	3.16	54.95
P1L	13.07	11.59	0.10	1.69	2.63	57.58
D1B*	9.54	11.04	0.10	1.17	2.58	60.16
P1B	4.91	3.48	0.09	1.59	2.27	62.43
P2B	4.15	2.79	0.09	1.45	2.24	64.67
PCL	81.17	81.09	0.08	1.32	2.14	66.81
PP1	23.56	24.46	0.08	1.19	2.10	68.91
PG1	19.60	20.56	0.08	1.23	2.02	70.93
D2B*	9.89	8.82	0.08	1.64	2.01	72.94
CL	22.18	21.57	0.08	0.9	1.95	74.90
P1H	7.62	6.52	0.07	1.69	1.79	76.69
D2L	17.05	17.92	0.07	1.39	1.79	78.48
P2L	11.12	10.59	0.07	1.17	1.71	80.19
POR	12.77	13.17	0.07	1.35	1.70	81.89
CDM	18.17	18.52	0.06	1.42	1.68	83.57
FL	89.47	89.21	0.06	1.3	1.66	85.23
POB	9.19	9.83	0.06	1.37	1.66	86.89
D1H	5.20	4.25	0.06	1.75	1.65	88.54
D2B	13.28	13.31	0.06	1.34	1.61	90.15

the IUCN Red List (Dureuil, 2015); however, *D. profundorum* and *D. hystricosa* are listed as data deficient (DD) due to the lack of reliable catch data, landings data and life history information available for these species with which to infer population trends (Ebert et al., 2009a,b).

Deania species, and particularly *D. calcea*, are relatively abundant in the Marine Protected Area of El Cachucho and have been used to characterise one of the main habitats where this species lives, the *Pheronema–Deania* community (EUNIS habitat type A6.621). This faunal assemblage with the largest distribution area in the MPA occupies the deeper and muddy flat sedimentary grounds (800–1050 m) of the inner basin (Sánchez et al., 2008). Therefore, improving the knowledge and accurate identification of these species is crucial for understanding their role

Table 8
Dissimilarity index between *Deania profundorum* size classes (TL < 55 cm and TL > 55 cm) and the morphometric characters that contributed most to this discrepancy. The results are expressed as percentage of total length (TL).

Morphometric character	Average value (cm)		Average dissimilarity	Dissimilarity/SD	Percentage	
	TL < 55 cm	TL > 55 cm			Contribution	Cumulative
D1B	14.65	20.11	0.33	2.33	6.46	6.46
D1L	20.71	25.83	0.31	1.77	6.06	12.52
PD1	33.84	29.45	0.30	1.75	5.77	18.28
CDM	22.14	18.17	0.24	2.17	4.68	22.97
PD2*	67.02	70.69	0.22	1.02	4.34	27.31
IDS	16.08	19.62	0.22	1.58	4.31	31.62
PP1	26.96	23.56	0.22	2.17	4.17	35.79
PG1	23.04	19.60	0.21	2.54	4.06	39.85
PCL	77.95	81.17	0.20	1.95	3.87	43.72
FL	86.45	89.47	0.18	2.38	3.57	47.29
POR	15.66	12.77	0.18	3.44	3.42	50.71
CL	24.94	22.18	0.17	2.19	3.25	53.96
PD2	63.49	65.94	0.15	1.71	2.97	56.93
IDS	19.76	21.52	0.15	1.26	2.96	59.88
PP2	58.67	61.03	0.15	1.43	2.88	62.76
POB	11.46	9.19	0.14	2.37	2.68	65.45
D1L*	13.90	16.04	0.14	1.86	2.64	68.09
1Dto2D	12.94	14.73	0.13	1.39	2.54	70.63
D1B*	7.63	9.54	0.12	1.41	2.41	73.04
P2L	9.46	11.12	0.10	1.88	2.00	75.04
D2B	12.34	13.28	0.10	1.59	1.94	76.99
EYL	6.44	4.83	0.10	2.88	1.91	78.89
ENS	8.99	7.52	0.09	1.84	1.78	80.68
D2L	15.73	17.05	0.09	1.71	1.77	82.44
INO	5.97	5.01	0.09	0.96	1.72	84.16
PD1*	40.55	39.40	0.09	0.97	1.65	85.81
P2B	3.25	4.15	0.08	1.44	1.57	87.38
PlL	13.56	13.07	0.07	1.27	1.39	88.77
D2L*	12.29	13.39	0.07	1.80	1.31	90.08

in deep-water ecosystems. This will help to improve management measurements such as characterisation of Marine Protected Areas.

5. Conclusions

This study focused on the two *Deania* species most frequently caught in Galicia and Cantabrian Sea of the northeast Atlantic Ocean, *Deania calcea* and *D. profundorum*. This study found that:

- Length of dermal denticles is positively correlated with total fish length in both species; therefore, shark length should be considered for comparative purposes.
- In both species, size and shape of dermal denticles varied in relation to their location on the shark body. Dermal denticles were larger in the anterior zone and progressively smaller towards the caudal zone. For species identification purposes, the location where dermal denticles are examined should be clearly defined.
- Dermal denticles of *D. profundorum* were significant smaller than those of *D. calcea;* therefore, dermal denticle length can be considered a valid taxonomic criteria for the identification of these *Deania* but considering the two previous statements.
- The morphometric characters that contributed most to the differentiation between the two *Deanias* were those related to first dorsal fin, interdorsal distance and distance between the snout and origin of dorsal fins.
- Historical morphological data separated *D. calcea* from large *D. profundorum* specimens, but small specimens were grouped with other *Deania* species. *D calcea* and *D. hystricosa (D. mauli)* were grouped together.
- *Molecular an*alysis based on cytochrome c-oxidase subunit I (COI) unambiguously discriminated *D. calcea* and *D. profundorum*.
- COI sequences available in BOLD database and those obtained from this study molecularly discriminate *D. calcea* from *D. profundorum*. However, *D. calcea* sequences were grouped together with those of D. *hystricosa.* This suggests that it is likely *D. calcea* and *D. hystricosa* are different clades of the same species.

CRediT authorship contribution statement

Cristina Rodríguez-Cabello: Conceptualization, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing - original draft, Writing - review & editing. **Montse Pérez:** Formal analysis, Investigation, Visualization, Writing - original draft. **Irene Grasa:** Investigation, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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