On the presence of *Dipturus nidarosiensis* (Storm, 1881) in the Central Mediterranean area

Pierluigi Carbonara Corresp., 1 , **Rita Cannas** 2 , **Marilena Donnaloia** 1 , **Riccardo Melis** 2 , **Cristina Porcu** 2 , **Maria Teresa Spedicato** 1 , **Walter Zupa** 1 , **Maria Cristina Follesa** 2

1 COISPA Tecnologia & Ricerca, Stazione Sperimentale per lo Studio delle Risorse del Mare, Bari, Italy
2 Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy

Corresponding Author: Pierluigi Carbonara
Email address: carbonara@coispa.it

The Norwegian skate *Dipturus nidarosiensis* (Storm, 1881) has only recently been recorded in the western Mediterranean Sea (along the coast of southern Sardinia, off Algeria and the Alboran Sea). The present study confirms the presence of the species in the Central Mediterranean Sea by means of morphometric, morphological features and molecular markers. Biological sampling was conducted on 8 specimens collected through commercial landings, offshore observer programme and scientific surveys, in Adriatic and Ionian waters at depths between 320 and 720 m, from 2010 to 2016 . Specimens (juveniles and adults) had total lengths ranging from 268 to 1422 mm and body weights from 44.5 to 12,540 g. They showed morphometric features corresponding to those of Norwegian skates from the Northeast Atlantic and the Western Mediterranean. As previously analysed molecular data were obtained by mitochondrial COI sequences. The haplotype network showed the occurrence of a common haplotype (Hap_1) shared by the individuals from North Atlantic, Sardinian, Algerian and Spanish areas except for South Africa The occurrence of individuals in different stages of life (juveniles, sub-adults, adults) and sexual development (immatures and matures) suggested the presence of a species with a permanent reproductive allocation in the deep waters of Mediterranean water, exposed to a low level of fishing exploitation. Indeed, the deep depth distribution of the species could be the source of the data absence in onshore or offshore fishery data collection programmes and scientific surveys.

On the presence of *Dipturus nidarosiensis* (Storm, 1881) in the Central Mediterranean area

Pierluigi Carbonara[1], Rita Cannas[2], Marilena Donnaloia[1], Riccardo Melis[2], Cristina Porcu[2], Maria Teresa Spedicato[1], Walter Zupa[1] and Maria Cristina Follesa[2]

[1] COISPA Tecnologia & Ricerca, Stazione Sperimentale per lo Studio delle Risorse del Mare, via dei Trulli 18-20, Bari, (BA), 70126, Italy.

[2] Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy, Via T. Fiorelli 1, Cagliari, (CA), 09126, Italy.

Corresponding author:
Pierluigi Carbonara[1]
via dei Trulli 18–20, Bari, (BA), 70126, Italy
Email address: carbonara@coispa.it

ABSTRACT

The Norwegian skate *Dipturus nidarosiensis* (Storm, 1881) has only recently been recorded in the western Mediterranean Sea (along the coast of southern Sardinia, off Algeria and the Alboran Sea). The present study confirms the presence of the species in the Central Mediterranean Sea by means of morphometric, morphological features and molecular markers.

Biological sampling was conducted on 8 specimens collected through commercial landings, offshore observer programme and scientific surveys, in Adriatic and Ionian waters at depths between 320 and 720 m, from 2010 to 2016 . Specimens (juveniles and adults) had total lengths ranging from 268 to 1422 mm and body weights from 44.5 to 12,540 g. They showed morphometric features corresponding to those of Norwegian skates from the Northeast Atlantic and the Western Mediterranean. As previously analysed molecular data were obtained by mitochondrial COI sequences. The haplotype network showed the occurrence of a common haplotype (Hap_1) shared by the individuals from North Atlantic, Sardinian, Algerian and Spanish areas except for South Africa

The occurrence of individuals in different stages of life (juveniles, sub-adults, adults) and sexual development (immatures and matures) suggested the presence of a species with a permanent reproductive allocation in the deep waters of Mediterranean water, exposed to a low level of fishing exploitation. Indeed, the deep depth distribution of the species could be the source of the data absence in onshore or offshore fishery data collection programmes and scientific surveys.

INTRODUCTION

Species discrimination is a challenging issue largely because it is based only on morphological diagnoses. Indeed, species classifications errors are frequent due to several factors, such as phenotypic plasticity, the presence of cryptic taxa, morphological changes linked to life stage and the high level of expertise requested for this kind of analysis (Herbert *et al.*, 2003a).

The misclassification of skates is common (e.g. Serena, 2005; Frodella *et al.*, 2016; Iglesias *et al.*, 2010). It has been estimated that about 50 known species are still only partially described or completely undescribed (Coulson *et al.*, 2011). Consequently, in the last decades, DNA-based species discrimination has been applied (Herbert *et al.*, 2003b) in an increasing number of cases (Spouge, 2016). The use of molecular markers and specifically the cytochrome *c* oxidase I gene (COI) has proven to be very helpful on several occasions for the identification of ray and skate species in the Mediterranean, Atlantic and worldwide (Ball *et al.*, 2016; Bineesh *et al.*, 2017; Cannas *et al.*, 2010; Cariani *et al.*, 2017; Cerutti-Pereyra *et al.*, 2012; Coulson *et al.*, 2011; Frodella *et al.*, 2016; Griffiths *et al.*, 2013; Iglesias, Toulhoat & Sellos, 2010; Landi *et al.*, 2014; Lynghammar *et al.*, 2014; Mabragana *et al.*, 2011; Ordines *et al.*, 2017; Ramirez-Amaro *et al.*, 2017; Serra-Pereira *et al.*, 2011; Spies *et al.*, 2006; Vargas-Caro *et al.*, 2016; Vella, Vella & Schembri, 2017; Ward *et al.*, 2008; Ward *et al.*, 2005).

In this context, Rajidae display conservative morphological traits both among and between alive and fossil species (Valsecchi *et al.*, 2005). Hence, particularly for this taxonomic group, DNA barcoding has been used to describe new species (e.g. Last & Séret, 2016), extirpation (e.g. Ball *et al.*, 2016) as well as well as species' expansion range (e.g. Ordines *et al.*, 2017).

In particular, taking into consideration the genus *Dipturu*s, the misclassification problem is particularly crucial (Griffiths *et al.*, 2010; Iglésias *et al.*, 2010). In eastern Atlantic Ocean and in the Mediterranean basin, it includes *Dipturus batis* (Linnaeus, 1758), *Dipturus oxyrinchus* (Linnaeus, 1758) and *Dipturus nidarosiensis* (Storm, 1881) with a high percentage of misclassification in the fishery data as reported for the French coast (Iglésias *et al.*, 2010). mainly among *D. oxyrinchus*, *D. batis*, *D. nidarosiensis* and *Rostroraja alba* (Lacepede, 1803). These findings could have led to underestimation of the risk of a dramatic decline and collapse of the spawning stock (Griffiths *et al.*, 2010; Iglésias *et al.*, 2010). Hence, the risk of extinction of these species might be higher than previously calculated (Iglésias *et al.*, 2010).

D. nidarosiensis is one of the species that is often misclassified in both the Atlantic and the Mediterranean Basin (Cannas *et al.*, 2010; Cariani *et al.*, 2017; Ramírez-Amaro *et al.*, 2017). The geographical distribution of the Norwegian skate *D. nidarosiensis* is known to occur along the northeast Atlantic, from Iceland, Faroe and northern Norway to Madeira and northern Mauritania (Stehmann, 1990). This species is considered endemic in the northeast Atlantic and the Bay of Biscay (Priede *et al.*, 2010; Stehmann & Bürkel, 1984; Rodríguez-Cabello *et al.*, 2013). However, more recently, it has been recorded in the western Mediterranean Sea, along the southern Sardinia coast (Cannas *et al.*, 2010; Follesa *et al.*, 2012, Cariani *et al.*, 2017, Marongiu *et al.*, 2017; Porcu *et al.*, 2017), off Algeria (Cariani *et al.*, 2017), in the Alboran Sea (Ramírez-Amaro *et al.*, 2017) and in the southern Adriatic and Ionian seas (Cariani *et al.*, 2017). The first catch of Norwegian skate specimens in the western Mediterranean waters (2005) was reported to occur in Sardinia

(Cannas *et al.*, 2010), while the records from the Alboran Sea are more recent (2012–2016; Ramírez-Amaro *et al.*, 2017).

The abovementioned authors hypothesised an ancient presence of the Norwegian skate, which was not detected due to a misclassification between *D. nidarosiensis* and *D. batis* (Cannas *et al.*, 2010).

Following the IUCN Red List, the Norwegian skate *D. nidarosiensis* is listed as near threatened (Stehmann *et al.*, 2015), and in both the northeast Atlantic and the Mediterranean areas, this species is rare (Williams *et al.*, 2008; Cannas *et al.*, 2010). Moreover, the Norwegian skate has some similar biological characteristics to other elasmobranchs, such as low fecundity, slow growth and high length at first maturity (Follesa *et al.*, 2012), making it particularly vulnerable to environmental and anthropogenic pressures. Until now, information on the life history of the Norwegian skate in the Mediterranean Basin has been limited and based only on specimens caught around Sardinia Island (central-western Mediterranean) (Follesa *et al.*, 2012).

For the first time, the present study describes and characterises, both from morphological and genetic point of view, the Norwegian skate specimens caught in the southern Adriatic Sea and northwestern Ionian Sea (Central Mediterranean Basin), where this species had never been reported (Marano *et al.*, 2002; Sion *et al.*, 2003; Sion *et al.*, 2004; Zupa *et al.*, 2010, Relini *et al.*, 2000, 2010) until very recently (Cariani *et al.*, 2017). This study could further inform on geographical distribution and life-history traits of this species. Morphological comparisons with specimens from Sardinian waters and the Alboran Sea were made in order to provide a correct classification of this species in the Mediterranean Basin (Cannas *et al.*, 2010; Follesa *et al.*, 2012; Ramírez-Amaro *et al.*, 2017).

METHODS

Sampling

Samples of *D. nidarosiensis* were collected onshore (e.g. market sampling), along the port of Apulia region, from offshore observers (discard monitoring) for a fishery sampling programme (Data Collection Framework – DCF; EU Reg. 1543/2000, 1639/2001, 1581/2004, 199/2008 and 1004/2017) and from fisheries/independent scientific surveys (Mediterranean International Trawl Survey, MEDITS) (AAVV, 2017) (Fig. 1).

In total, eight specimens were morphologically analysed and preserved in the deep freezer (-20 °C) of COISPA Institute Laboratory. Morphology and colour analysis as well as morphometric measurements (nearest 1 mm) (Fig. 2), weights (nearest 1 g) and meristic counts were performed, following the previous descriptions of skate species (Ebert & Stehmann, 2013; Serena, 2005). All measurements and meristic counts are listed in Figure 2 and Table 1.

Sex and maturity were determined macroscopically, and the gonad weights were recorded to the nearest 0.1 g. Gonadal maturity was classified using the MEDITS maturity scale (AAVV, 2017; Follesa and Carbonara, 2019), and the results are reported in Table 2 as follows: Stage 1 (virgin immature); Stage 2 (maturing); Stage 3A (mature); Stage 3B (mature/extruding active); Stage 4A (resting); Stage 4B (regenerating).

Genetic analyses

Eight specimens were used for the genetic analysis, with four previously analysed by Cariani *et al.* (2017) (codes 1–4 – Table 1) while the remaining four specimens (codes 5–8 – Table 1) analysed for the first time here.

Total genomic DNA was extracted from tissues samples using a salting-out protocol (Miller *et al.*, 1988), these samples (skin, white muscle) were collected from the ventral side of the pectoral fin (ca. 0.5 cm^3 in size). The tissue samples were preserved in 80% ethanol at 4°C.

The primers for the amplifications of the mitochondrial COI gene (COI-3 primer cocktail) were obtained from Ivanova *et al.* (2007). The PCR reactions were set up in a 25µl reaction volume containing 2.5µl of 10X Taq Buffer (Thermo Scientific), 2.5µl of 2mM dNTPs, 2.5µl of 25mM of MgCl2, 0.1µl of each (forward and reverse) 20mM primers, 0.16µl of Taq polymerase (Dream Taq® Thermo Scientific) and 1µl of DNA (50–100ng). The amplification was based on the following cyclic parameters: 5 min at 94°C for the initial denaturation, followed by 40 cycles of 30 sec at 94°C, 40 sec at 53°C for the annealing of primers, 50 sec at 72°C for extension and then 7 min at 72°C for the final extension. PCR products were outsourced to Macrogen Europe (Netherlands) for sequencing. The sequences were obtained in both directions and submitted to GenBank under accession number MG699904-07. The sequences were edited and aligned in MEGA v. 6 (Tamura *et al.*, 2013) and translated into aminoacidic sequences using the vertebrate genetic code to exclude the occurrence of codon stop and nuclear pseudogenes.

The number of haplotypes, haplotype diversity [hd], average number of nucleotide differences [k] and nucleotide diversity [π] were retrieved using DnaSP v. 5.1 (Librado & Rozas, 2009).

Graphically, the haplotypes were arranged in a network with PopART (http://popart.otago.ac.nz) using the Median-Joining method (Bandelt, Forster & Röhl, 1999).

The sequences obtained in this study were compared to COI sequences published for the species from Mediterranean and eastern Atlantic Ocean individuals of the genus *Dipturus* (*D. nidarosiensis, D. oxyrinchus, D. batis, D. cf flossada, D. cf intermedia*) available in GenBank (https://www.ncbi.nlm.nih.gov/genbank) and BOLD (http://www.boldsystems.org/). *Rostroraja alba* was used as the outgroup. The list and details of the sequences used in the analyses are provided as a supplementary table (Table S1). At least three sequences per species were used for species such as *D. intermediate* and *D. flossada*, up to a maximum of 42 sequences for *D. oxyrinchus*.

The relationships among haplotypes were investigated using two approaches: the Bayesian method using MrBayes v. 3.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) and the Neighbour-Joining method in MEGA using Kimura 2-parameter genetic distances (K2P; Kimura 1980) and a bootstrap test with 1000 replicates. In MrBayes, the analyses were performed using two parallel runs of two million generations each, using four chains, sampling every 100 generations, burn-in of 0.25 and saving branch lengths. The performance of the analyses was evaluated using Tracer v. 1.6 (Rambaut *et al.*, 2014). The trees were visualised with MEGA.

Ethical compliance

All Norwegian skate (*Dipturus nidarosiensis*) analysed in this study from commercial fishing activity (DCF; EU Reg. 1004/2017), do not fit under the European Commission recommendations (Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010) or Italian National Law (Decree Law n. 26 of 4 March 2014) regarding the protection of animals used for scientific experiments. Moreover following the welfare guidelines under the Decree Law n. 26 of 4 March 2014 (Italian Ministry of Health) and the protocol approved by the Committee on the Ethics of Animal Experiments of COISPA (Italian Ministry of Health 15/2015-UT), the specimens brought on-board alive, during the scientific survey or the monitoring on the commercial fishing vessel, should be killed with an overdose of anaesthetic. However all the animals sampled in this study were already dead at the time of sampling both the specimens sampled at the landing and on board (discard monitoring and scientific survey when the specimens brought on-board).

RESULTS

The Norwegian skate specimens were captured around the pit of Bari and in the northwest part of the Ionian Sea (Fig. 2) at depths ranging between 320 and 720 m.

All measurements, meristic counts, sex and maturity are presented in Table 1. All specimens caught showed, uniformly dark ventral sides, including the ampullary pores (Fig. 3). The disc was wider (WD 64.0-74.0% of TL) than long (LD 52.5-59.7% of TL), covered uniformly by darkish mucous on both side (Fig. 3). The teeth were pointed in parallel rows in males and molar-like sets in pavement in females (Fig. 4), with a number of tooth rows on the upper and lower jaw between 42–45 and 39–43, respectively. It seemed that the number of thorns on the tail increased with the total length of the specimens as shown in Table 1. Moreover, both dorsal and ventral sides were prickly in the area of snout (Fig. 5). A morphometric variability between juveniles and adults has been detected for the relationship between the Length Tail (Ltail)/ Total Length and Distance interorbital (Dorb)/Total Length (Table 1). The Ltail is longer in terms of percentage on TL in juveniles about half of the TL, and one-third of the TL in sub-adults and adults (Fig. 2). On the contrary, the Dorb increased with TL. Moreover, juveniles did not showed spines on the median line of the disc and on both lateral sides of the tail, present in adults (Table 1).

Juveniles (maturity stage 1) were caught in shallow waters between 320 and 377 m, while sub-adult (maturity stage 2) and adult specimens (maturity stage 3A and 3B) in deeper bottoms ranging between 550 and 720 m..

Furthermore, all samples caught during the MEDITS trawl survey were associated with a biocenosis typical of compact bathyal muds. In particular, juveniles (specimens 5, 7, 8, Table 1), caught at upper depths, were found in association with a biocoenosis characterised by tall sea pen *Funiculina quadrangularis* facies.

Genetic analyses

A 633 bp fragment of the COI gene was obtained from eight individuals from the South Adriatic and Ionian seas.

The combination of these data with those available for *D. nidarosiensis* from other areas within the Mediterranean and adjacent Atlantic Ocean, provided with a final alignment of 38 sequences and seven haplotypes (555 bp; Hd: 0.515; k: 0.586; π: 0.00106). The haplotype network (Fig. 6) showed the occurrence of a common haplotype (Hap_1) shared by 26 individuals from all investigated areas except for South Africa. It also showed six additional haplotypes, with four exclusive to the Mediterranean (Hap_2-Hap_5) and two exclusive to the Atlantic (Hap_6-Hap_7). The phylogenetic analyses (Fig. 7) further confirmed the identification of the Ionian–Adriatic specimens as *D. nidarosiensis*. They clustered in a strongly supported clade, clearly distinguishable from the other species of *Dipturus* found in the Mediterranean and eastern Atlantic Ocean. The most similar sequences to *D. nidarosiensis* were those of *D. cf flossada* (95% base pair similarity), and the least similar were those of *R. alba* (87.82% base pair similarity).

DISCUSSION

The present study confirms the presence of the Norwegian skate *Dipturus nidarosiensis* in the Central Mediterranean Sea by means of morphometric and morphological features and molecular markers. The morphological parameters described are in line with the data collected in Sardinian waters (Cannas *et al.*, 2010) and the Alboran Sea (Ramírez-Amaro *et al.*, 2017). In particular, the relationship between the morphometric measurements and total length is similar to the data reported in the Alboran Sea (Ramírez-Amaro *et al.*, 2017). Specifically, the measurements of the juveniles from the Spanish area (Ramírez-Amaro *et al.*, 2017) are in accordance with those of the Adriatic basin. Furthermore, the high variability in Length Tail during the different stages of life, reported in the paper, seems to be common to other Rajidae species as well, including *R. clavata*, *D. oxyrinchus* and *Amblyraja radiata* (Mnasri *et al.*, 2009; Capapé *et al.*, 2015; Templeman, 1987) Genetic analysis of the mitochondrial gene COI made possible to confirm the morphological identification of the Adriatic–Ionian samples as *D. nidarosiensis*. In particular, in this study, molecular data highlighted the fact that Mediterranean specimens of *D. nidarosiensis* (from Sardinia, Algeria and Alboran Sea) share COI mitochondrial haplotypes with Atlantic specimens (see Table S1 for details). However, additional analyses based on more powerful markers (i.e., microsatellites and SNPs) and larger numbers of individuals are needed to investigate possible intraspecific differences and the genetic connectivity among the different areas within the Mediterranean and between the Mediterranean and the Atlantic populations (Griffiths *et al.*, 2011). The captures of *D. nidarosiensis,* reported in this paper, are mostly located around the Bari Pit (maximum depth of 1233 m) in an area between the western Italian coast and the eastern Montenegrin and Albanian coasts. This area is connected with the northern part of the Ionian Sea through the Otranto Channel, and it is part of a system of currents that exchanges and mixes deep water coming from the eastern part of the Mediterranean Sea and the northern part of the Adriatic Sea (Vilibic & Orlic, 2002). Although the capture locations of Norwegian skates are dispersed over 200 miles, all these areas are connected by deep-water currents (Artegiani *et al.*, 1997). The southern Adriatic and Ionian bathyal system show the absence of barriers to gene flow for deep species as well as *Aristeus antennatus* (Marra *et al.*, 2015). In the case of *A. antennatus*, it was hypothesised that the presence of the population could be structured into two layers: a deeper stock, not affected by fishing, which preserves genetic homogeneity, and an upper stock living on the fishing ground. The larval dispersal, adult migration and continuous movements of individuals from deeper grounds to upper areas could explain their genetic homogeneity throughout the Adriatic and Ionian system (Marra *et al.*, 2015). In the same way, the partial overlap between the Norwegian skate's habitat and fishing grounds in the Adriatic and Ionian Seas suggests that the Adriatic and Ionian *D. nidarosiensis* specimens are part of one population.

The occurrence of the Norwegian skate in the southern Adriatic Sea and northern Ionian Sea sheds new light on the dispersion of this species in the Mediterranean basin. Through interviews with fishermen in the southern Adriatic, it emerged that the occurrence of this species, called with the local name 'black monk', is not unusual.

However, the unnoticed presence of this species in the Mediterranean up to 2010 (Cannas *et al.*, 2010) can be attributed to two main reasons. Firstly, it has been misclassified with others species of the *Dipturus* genus (Cannas *et al.*, 2010) as reported for the French coast (Iglésias *et al.*, 2010). Indeed, the presence of *Dipturus* species is not recent (Griffits *et al.*, 2011): in the southern Adriatic, catches of *D. oxyrinchus* were reported in 1994–1995 (Relini *et al.*, 2000; Marano *et al.*, 2003), in the Western Ionian, catches of *D. batis* and *D. oxyrinchus* were recorded in 1992 and 2000–2001, respectively (Relini *et al.*, 2000; Sion *et al.*, 2003) while in Central Western part of the basin, *D. oxyrinchus* has been caught since the first surveys funded by the DCF in the middle of 1980 (Relini *et al.*, 2000, 2010). Secondly, considering that in the southern Adriatic the bottom trawl fleet fishing grounds are concentrated mostly on the shelf and the upper part of slope (Sabatella & Maninni, 2013) and in the western Ionian, as in the other part of the basin, they are located mainly on the slope until 800 m depth (Russo *et al.*, 2017), the catches of *D. nidarosiensis* characterized by a wider depth distribution (between 300 and 1700 m) (Cannas *et al.*, 2010; Follesa *et al.*, 2012; Ramírez-Amaro *et al.*, 2017) could be unrecorded. Hence, in the studied areas, the depth of the fishing activity only partially overlaps with the deep distribution of the Norwegian skate. In addition, in the Mediterranean sites where *D. nidarosiensis* were found, the seabed presents similar characteristics (e.g. canyons and slopes with high depths), favourable to this species but unfavourable to trawling activity for both commercial (Sabatella & Maninni, 2013) and research vessels (AAVV, 2017).

The presence of maturing and mature males and females in early autumn (end of September/beginning of October) and summer, respectively, could support the preliminary observations on the reproductive biology of the species in Sardinian waters (Follesa *et al.*, 2012). Furthermore, the collection of empty egg cases on the sea floor as well as of individuals in different life stages (embryos within egg case, juveniles, sub-adults and adults) of both sexes suggests the presence of a species with a permanent reproductive allocation in the Mediterranean Sea (Cannas *et al.*, 2010; Follesa *et al.*, 2012; Cariani *et al.*, 2017, Porcu *et al.*, 2017, Ramírez-Amaro *et al.*, 2017).

From the data recorded, despite the few specimens analysed, the Norwegian skate shows a bathymetric segregation with juveniles, only occurred on the upper part of the slope, and sub-adults and adults in lower depth as reported for Sardinian waters (Follesa et al., 2012). This deep segregation by ontogenic phase has been observed in other species of skates as well, including *Raja clavata*, *Raja asterias* and *Rhinoptera bonasus* (Rouset, 1990; Ferrà *et al.*, 2016; Fisher *et al.*, 2013).

In addition, the finding of Ionian and Adriatic *D. nidarosiensis* specimens on compact mud bottoms confirmed as the species shows preference for this type of sea bed as observed also by Follesa *et al.* (2012) off Sardinia. Moreover, for the first time, the presence of juveniles in association with a biocoenosis characterised by bathyal muds with tall sea pen *Funiculina quadrangularis* facies, is recorded.

Several aspects of *D. nidarosiensis* are still poor investigated. For this reason, it will be useful to continue the monitoring and sampling on this species in order to collect additional data to clarify aspects on its biology (e.g. reproduction and growth) and ecology (e.g. trophic relationship and geographical distribution).

CONCLUSION

The data reported in this study confirms the presence of *D. nidarosiensis* in the Central Mediterranean Sea (southern Adriatic and northwest Ionian Seas), contribute to inform on its geographical distribution and life-history traits a in the Mediterranean basin and improve the understanding of its morphological traits. Indeed, misclassification has occurred frequently with the Mediterranean skate species (Iglésias *et al.*, 2010), resulting in the misinterpretation of several biological characteristics. The morphological data are similar with data reported in Sardinia and Alboran Sea (Cannas *et al.*, 2010; Ramirez-Amaro *et al.,* 2017). Moreover, the morphological differences between juveniles and adults were highlighted in term of morphological and meristic characteristics.

Genetic analysis carried out through the mitochondrial gene COI was useful (Spouge, 2016) and made it possible to confirm the morphological identification of the Adriatic–Ionian samples as *D. nidarosiensis*.

ACKNOWLEDGEMENT

The authors are grateful to the Academic Editor Dr. Antonina Dos Santos and three anonymous reviewers for the constructive comments and suggestions that greatly helped to improve the manuscript.

REFERENCES

AAVV. 2017. MEDITS-Handbook. Version n.9, MEDITS Working Group : 106 pp. Available at: *http://www.sibm.it/MEDITS%202011/principaledownload.htm* (accessed 26 January 2018).

Artegiani A, Bregant D, Paschini E, Pinardi N, Raicich F, Russo A. 1997. The Adriatic Sea general circulation. Part I: air-sea interaction and water mass structure. *Journal of Physical Oceanography* 27:1492-1514. DOI: 10.1175/1520-0485(1997)027<1492:TASGCP>2.0.CO;2

Ball RE, Serra-Pereira B, Ellis J, Genner MJ, Iglésias S, Johnson AF, Jones CS, Leslie R, Lewis J, Mariani S, Menezes G, Neat F, Noble L R, Sims D W, Griffiths AM. 2016. Resolving taxonomic uncertainty in vulnerable elasmobranchs: are the Madeira skate (*Raja maderensis*) and the thornback ray (*Raja clavata*) distinct species? *Conservation Genetics* 17(3):565-576. DOI: 10.1007/s10592-015-0806-1.

Bandelt H-J, Forster P, Röhl A. 1999. Median-Joining Networks for Inferring Intraspecific Phylogenies. *Molecular Biology and Evolution* 6(1):37–48. DOI: 10.1093/oxfordjournals.molbev.a026036

Bineesh KK, Gopalakrishnan A, Akhilesh KV, Sajeela K A, Abdussamad EM, Pillai NGK, Basheer V S, Jena J K, Ward R D. 2017. DNA barcoding reveals species composition of sharks and rays in the Indian commercial fishery. *Mitochondrial DNA Part A DNA Mapping, Sequencing, and Analysis* 28(4):458-472. DOI: 10.3109/19401736.2015.1137900.

Cannas R, Follesa M C, Cabiddu S, Porcu C, Salvadori S, Iglesias S P, Deiana AM, Cau A. 2010. Molecular and morphological evidence of the occurrence of the Norwegian skate *Dipturus nidarosiensis* (Storm, 1881) in the Mediterranean Sea. *Marine Biology Research* 6(4):341-350. DOI: 10.1080/17451000903428496.

Capapé C, Ali M, Saad A, Alkusairy H, Reynaud C. 2015. Atypical characteristics in the longnosed skate Dipturus oxyrinchus (Linnaeus, 1758) from the coast of Syria (Eastern Mediterranean). *Thalassia Salentina* 37: 71-80. DOI 10.1285/i15910725v37p71

Cariani A, Messinetti S, Ferrari A, Arculeo M, Bonello J J, Bonnici L, Cannas R, Carbonara P, Cau A, Charilaou C, El Ouamari N, Fiorentino F, Follesa MC, Garofalo G, Golani D, Guarniero I, Hanner R, Hemida F, Kada O, Lo Brutto S, Mancusi C, Morey G, Schembri P J, Serena F, Sion L, Stagioni M, Tursi A, Vrgoc N, Steinke D, Tinti F. 2017. Improving the Conservation of Mediterranean Chondrichthyans: The ELASMOMED DNA Barcode Reference Library. *PLoS One* 12(1):e0170244. DOI:10.1371/journal.pone.0170244.

Cerutti-Pereyra F, Meekan MG, Wei N W, O'Shea O, Bradshaw CJ, Austin CM. 2012. Identification of rays through DNA barcoding: an application for ecologists. *PLoS One* 7(6):e36479. DOI:10.1371/journal.pone.0036479.

Coulson M, Denti W, Van Guelpen L, Miri C, Kenchington E, Bentzen P. 2011. DNA barcoding of Canada's skates. *Molecular Ecology Resources* 11(6):968-978. DOI: 10.1111/j.1755-0998.2011.03034.x.

Ebert DA, Stehmann. MFW. 2013. Sharks, batoids, and chimaeras of the North Atlantic FAO Species Catalogue for Fishery Purposes. No. 7. Rome, FAO. 523 pp.

Ellis JR, Cruz-Martinez A, Rackham BD, Rogers SI. 2005. The distribution of chondrichthyan fishes around the British Isles and implications for conservation. *Journal of Northwest Atlantic Fishery Science*, 35:

Fisher RA, Call GC, Grubbs RD. 2013. Age, Growth, and Reproductive Biology of Cownose Rays in Chesapeake Bay. *Marine and Coastal Fisheries*, 5 (1): 224-235. DOI: 10.1080/19425120.2013.812587

Follesa MC, Cannas R, Cabiddu S, Cau Al, Mulas A, Porcu C, Cau A. 2012. Preliminary observations of the reproductive biology and diet for the Norwegian skate *Dipturus nidarosiensis* (Rajidae) from the Central Western Mediterranean Sea. *Cybium* 36(3): 473-477.

Follesa MC, Carbonara P. 2019. Atlas of the maturity stages of Mediterranean fishery resources. Studies and Reviews n. 99. Rome, FAO. 268 pp. Available at www.fao.org/3/CA2740EN/ca2740en.pdf

Frodella N, Cannas R, Velonà A, Carbonara P, Farrell E D, Fiorentino F, Follesa M C, Garofalo G, Hemida F, Mancusi C, Stagioni M, Ungaro N, Serena F, Tinti F, Cariani A. 2016. Population connectivity and phylogeography of the Mediterranean endemic skate Raja polystigma and evidence of its hybridization with the parapatric sibling R. montagui. *Marine Ecology Progress Series* 554:99-113. DOI: 10.3354/meps11799

Griffiths AM, Sims DW, Cotterell SP, El Nagar A, Ellis JR, Lynghammar A, McHugh M, Neat FC, Pade NG, Queiroz1 N, Serra-Pereira B, Rapp T, Wearmouth VJ, Genner MJ. 2010. Molecular markers reveal spatially segregated cryptic species in a critically endangered fish, the common skate (*Dipturus batis*) *Proceeding of the Royal Socety B* 277: 1497–1503. DOI: 10.1098/rspb.2009.2111

Griffiths AM, Sims DW, Johnson A, Lynghammar A, McHugh M, Bakken T, Genner MJ. 2011. Levels of connectivity between longnose skate (*Dipturus oxyrinchus*) in the Mediterranean Sea and the north-eastern Atlantic Ocean. *Conservation Genetics* 12:577-582. DOI: 10.1007/s10592-010-0127-3

Griffiths AM, Miller DD, Egan A, Fox J, Greenfield A, Mariani S. 2013. DNA barcoding unveils skate (Chondrichthyes: Rajidae) species diversity in 'ray' products sold across Ireland and the UK. *PeerJ* 1:e129. DOI:10.7717/peerj.129.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17:754-755. DOI: 10.1093/bioinformatics/17.8.754

Iglesias SP, Toulhoat L, Sellos DY. 2010. Taxonomic confusion and market mislabelling of threatened skates: important consequences for their conservation status. *Aquatic Conservation-Marine and Freshwater Ecosystems* 20(3):319-333. DOI: 10.1002/aqc.1083.

Kimura M. 1980. A simple method for estimating evolutionary ratesof base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16:111–120.

Landi M, Dimech M, Arculeo M, Biondo G, Martins R, Carneiro M, Carvalho G R, Brutto S L, Costa F O. 2014. DNA Barcoding for Species Assignment: The Case of Mediterranean Marine Fishes. *PLoS One* 9(9):e106135. DOI:10.1371/journal.pone.0106135.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data Bioinformatics, 25, (11): 1451–1452. DOI: 10.1093/bioinformatics/btp187

Lynghammar A, Christiansen J S, Griffiths A M, Fevolden S-E, Hop H, Bakken T. 2014. DNA barcoding of the northern Northeast Atlantic skates (Chondrichthyes, Rajiformes), with remarks on the widely distributed starry ray. *Zoologica Scripta* 43(5):485-495. DOI: 10.1111/zsc.12064.

Mabragana E, Martin Diaz de Astarloa J, Hanner R, Zhang J, Gonzalez Castro M. 2011. DNA Barcoding Identifies Argentine Fishes from Marine and Brackish Waters. *PLoS One* 6(12). DOI: 10.1371/journal.pone.0028655.

Marano CA, Manfrin Piccinetti G, Pasolini P, Tinti F, Ungaro N. 2002. Annotated checklist of the skates (Chondrichthyes, Rajidae) in the Adriatic Sea. *Biologia Marina Mediterranea* 10 (2): 856–862.

Marongiu MF., Porcu C, Bellodi A, Cannas R, Cau A, Cuccu D, Mulas A, Follesa M C. 2017. Temporal dynamics of demersal chondrichthyan species in the central western Mediterranean Sea: The case study in Sardinia Island. *Fisheries Research* 193:81–94. DOI: 10.1016/j.fishres.2017.04.001

Martin Diaz De Astarloa J, Mabragana E, Hanner R, Figueroa D E. 2008. Morphological and molecular evidence for a new species of longnose skate (Rajiformes: Rajidae: Dipturus) from Argentinean waters based on DNA barcoding. *Zootaxa* 1921:35-46. DOI: 10.5281/zenodo.184713.

Marra A, Mona S, Sà RM, D'Onghia G, Maiorano P. 2015. Population Genetic History of Aristeus antennatus (Crustacea: Decapoda) in the Western and Central Mediterranean Sea. *PLoS One* 10(5): e0128609. DOI: 10.1371/journal.pone.0128609

Mnasri N, Boumaïza M, Ben Amor MM, Capapé C. 2009. Polychromatism in the thornback ray, Raja clavata (Chondrichthyes: Rajidae) off northern Tunisian coast (central Mediterranean). *Pan-American Journal of Aquatic Sciences* 4(4): 572-579.

Ordines F, Baro J, Ramírez-Amaro S, Serena F, Sobrino I. 2017. First substantiated record of Raja asterias Delaroche, 1809 (Elasmobranchii: Rajiformes: Rajidae) in the Gulf of Cádiz, North-eastern Atlantic. *Acta Ichthyologica et Piscatoria* 47(1):101-106. DOI: 10.3750/aiep/02161.

Porcu C, Marongiu MF, Bellodi A, Cannas R, Cau A, Melis R, Mulas A, Soldovilla G, Vacca L, Follesa MC. 2017. Morphological descriptions of the eggcases of skates (Rajidae) from the central-western Mediterranean, with notes on their distribution. *Helgoland Marine Research* 71:10.. DOI:10.1186/s10152-017-0490-2.

Priede I, Godbold JA, King NJ, Collins MA, Baile Y DM, Gordon JDM. 2010. Deep-sea demersal fish species richness in the Porcupine Seabight, NE Atlantic Ocean: global and regional patterns. *Marine Ecology* 31: 247-260. DOI: 10.1111/j.1439-0485.2009.00330.x

Rambaut A, Suchard MA, Xie D, Drummond AJ. 2014. Tracer v1.6, Available at *http://tree.bio.ed.ac.uk/software/tracer/* (accessed 26 January 2018).

Ramírez-Amaro S, Ordines F, Puerto M Á, García C, Ramon C, Terrasa B, Massutí E. 2017. New morphological and molecular evidence confirm the presence of the Norwegian skate Dipturus nidarosiensis (Storm, 1881) in the Mediterranean Sea and extend its distribution to the western basin. *Mediterranean Marine Science* 18(2):251-259. DOI: 10.12681/mms.1950.

Relini G, Biagi F, Serena F, Belluscio A, Spedicato M T, Rinelli P, Follesa MC, Piccinetti C, Ungaro N, Sion L, Levi D. 2000. I selaci pescati con lo strascico nei mari italiani. *Biologia Marina Mediterranea* 7 (1): 347-384.

Relini G, Mannini A, De Ranieri S, Bitetto I, Follesa MC, Gancitano V, Manfredi C, Casciaro L, Sion L. 2010. Chondrichthyes caught during the Medits surveys in Italian waters. *Biologia Marina Mediterranea* 17(1): 186-204.

Rodríguez-Cabello C, Pérez M, Sánchez F. 2013. New records of chondrichthyans species caught in the Cantabrian Sea (southern Bay of Biscay). *Journal of the Marine Biological Association of the United Kingdom* 93(07):1929-1939. DOI:10.1017/s0025315413000271.

Ronquist, F. and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572-1574. DOI: 10.1093/bioinformatics/btg180

Rousset J. 1990. Population structure of Thornback Rays Raja clavata and their movements in the Bay of Douarnenez. *Journal of the Marine Biological Association of the United Kingdom* 70:261–268.

Russo T, Bitetto I, Carbonara P, Carlucci R, D'Andrea L, Facchini MT, Lembo G, Maiorano P, Sion L, Spedicato MT, Tursi A, Cataudella S. 2017. A Holistic Approach to Fishery Management: Evidence and Insights from a Central Mediterranean Case Study (Western Ionian Sea). *Frontiers in Marine Science* 4:193. DOI: 10.3389/fmars.2017.00193

Serena F. 2005. Field identification guide to the sharks and rays of the Mediterranean and Black Sea - FAO Species Identification Guide for Fishery Purposes. Rome: FAO 97p. Available at *http://www.fao.org/docrep/009/y5945e/y5945e00.htm* (accessed 26 January 2018).

Serra-Pereira B, Moura T, Griffiths A M, Serrano Gordo L, Figueiredo I. 2011. Molecular barcoding of skates (Chondrichthyes: Rajidae) from the southern Northeast Atlantic. *Zoologica Scripta* 40(1):76-84. DOI: 10.1111/j.1463-6409.2010.00461.x.

Sion L, Bozzano A, D'Onghia G, Capezzuto F, Panza M. 2004. Chondrichthyes species in deep waters of the Mediterranean Sea. *Scientia Marina* 68 (Suppl 3): 153–162.

Sion L, D'Onghia G, Tursi A, Matarrese A. 2003. Annotated check list of the skates (Chondrichthyes, Rajidae) in the North-Western Ionian Sea. *Biologia Marina Mediterranea*, 10 (2): 935–940.

Spies I B, Gaichas S, Stevenson D E, Orr J W, Canino M F. 2006. DNA-based identification of Alaska skates (Amblyraja, Bathyraja and Raja: Rajidae) using cytochrome c oxidase subunit I (coI) variation. *Journal of Fish Biology* 69:283-292. DOI:10.1111/j.1095-8649.2006.01286.x.

Spouge J.L. 2016 Measurement of a Barcode's Accuracy in Identifying Species. In: Trivedi S., Ansari A., Ghosh S., Rehman H. (eds) DNA Barcoding in Marine Perspectives. Springer, Cham

Stehmann M, Bürkel DL. 1984. Rajidae. In: Whitehead PJ., Bauchot ML, Hureau JC, Tortonese E, ed. *Fishes of the Northeastern Atlantic and Mediterranean*. Paris: Unesco, 163-196.

Stehmann, MFW, Ellis J, Walls R, Lynghammar A. 2015.*Dipturus nidarosiensis* The IUCN Red List of Threatened Species 2015: e.T161729A48927468. DOI: *http://dx.doi.org/10.2305/IUCN.UK.2015-1.RLTS.T161729A48927468.en*. (accessed 25 February 2018)

Stehmann, M. 1990. Rajidae. In J.C. Quero, J.C. Hureau, C. Karrer, A. Post and L. Saldanha (eds.) Check-list of the fishes of the eastern tropical Atlantic. Junta Nacional de Investigaçao Cientifica e Tecnológica, Lisbon, Portugal. Vol. 1: 29-50

Templeman W. 1987. Length-weigth relationship, Morphometric characteristics and Thorniness of Thorny skate (Raja radiate) from Northwest Atlantic. *Journal of Northwest Atlantic Fishery Science* 7:89-98.

Vargas-Caro C, Bustamante C, Lamilla J, Bennett M B, Ovenden J R. 2016. The phylogenetic position of the roughskin skate Dipturus trachyderma (Krefft & Stehmann, 1975) (Rajiformes, Rajidae) inferred from the mitochondrial genome. *Mitochondrial DNA Part A DNA Mapping, Sequencing, and Analysis* 27(4):2965-2966. DOI:10.3109/19401736.2015.1060462.

Vella A, Vella N, Schembri S. 2017. A molecular approach towards taxonomic identification of elasmobranch species from Maltese fisheries landings. *Marine Genomics* 36:17-23. DOI: 10.1016/j.margen.2017.08.008.

Vilibic I, Orlic M. 2002. Adriatic water masses, their rates of formation and transport through the Otranto Strait. *Deep Sea Research Part I: Oceanographic Research Papers* 16: 171-178. DOI: 10.1016/S0967-0637(02)00028-6

Ward R D, Holmes B H, White W T, Last P R. 2008. DNA barcoding Australasian chondrichthyans: results and potential uses in conservation. *Marine and Freshwater Research* 59(1):57-71. DOI: 10.1071/MF07148.

Ward R D, Zemlak T S, Innes B H, Last P R, Hebert P D. 2005. DNA barcoding Australia's fish species. *Philosophical transactions of the Royal Society of London Series B, Biological sciences* 360(1462):1847-57. DOI:10.1098/rstb.2005.1716.

Williams T, Helle K, Aschan M. 2008. The distribution of chondrichthyans along the northern coast of Norway. *ICES Journal of Marine Science* 65: 1161-1174. DOI: 10.1093/icesjms/fsn103

Zupa W, Donnaloia M, Gaudio P, Intini S, Carbonara P. 2010. Occurence of Leucoraja fullonica (Linnaeus, 1758) in the south Adriatic sea. *Biologia Marina Mediterranea* 17 (1): 260-261

Figure 1

The geographical locations of the *D. nidarosiensis* catches are shown in figure 1

Figure 1. Spatial distribution of Norwegian skate *Dipturus nidarosiensis* caught during 2010–2016. The numbers correspond to the specimen codes reported in Table 1.

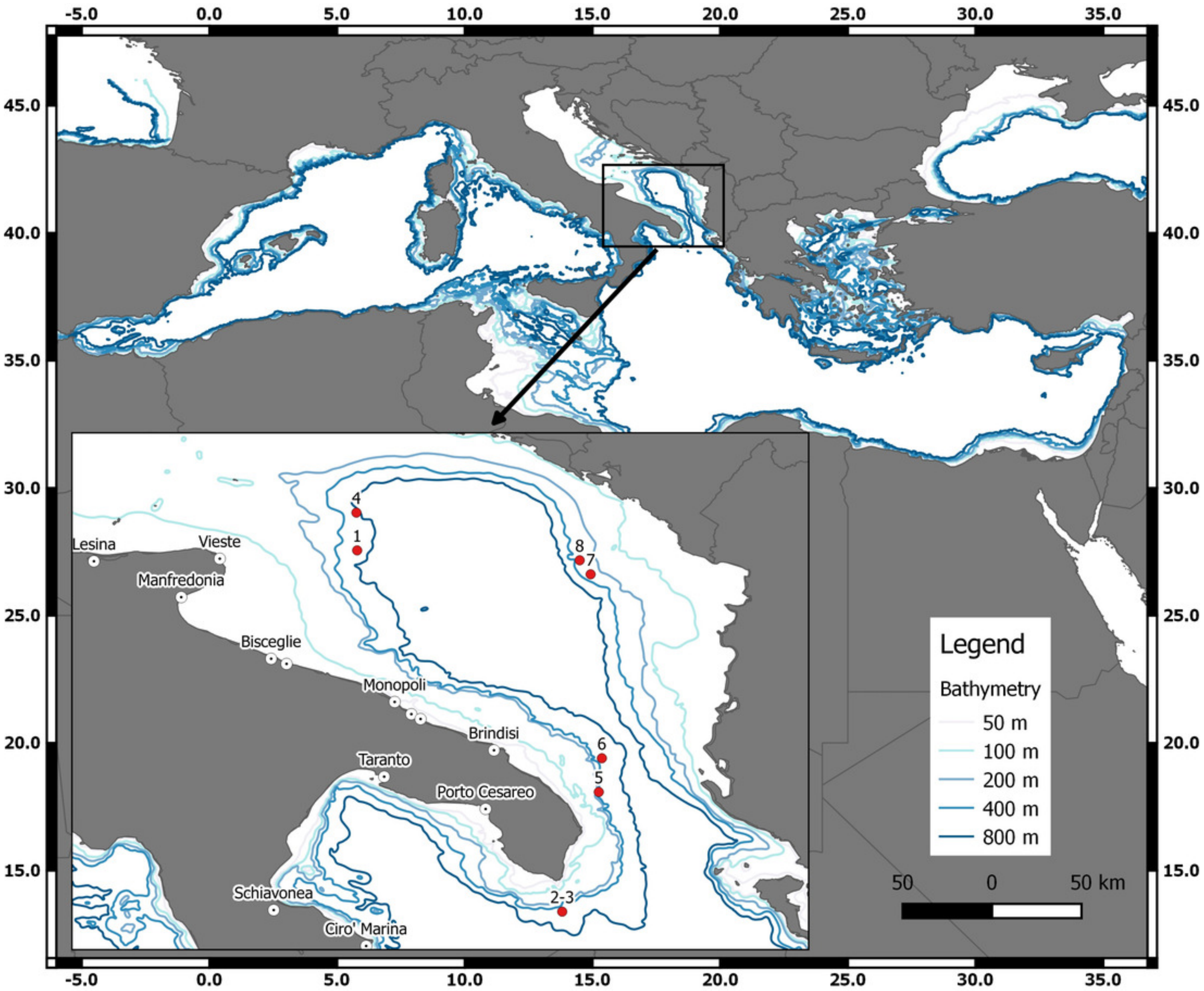

Figure 2

The morphometric measurements recorded for *D. nidarosiensis* specimens are represented in figure 2

Figure 2. Schematic representation of the morphometric measurements recorded for *Dipturus nidarosiensis* specimens caught in the central Mediterranean Sea. Dorsal view at left (A) and ventral view at right (B). Total Length (TL); Disc Length (DL); Pre 1° dorsal fin Length (L1P); Pre 2° dorsal fin Length (L2P); Preorbital or snout length (Sporb); Preoral Length (SP); Interorbital Length (Dorb); Internostril Length (Dnos), Disc Width (DW); Tail Length (Ltail).

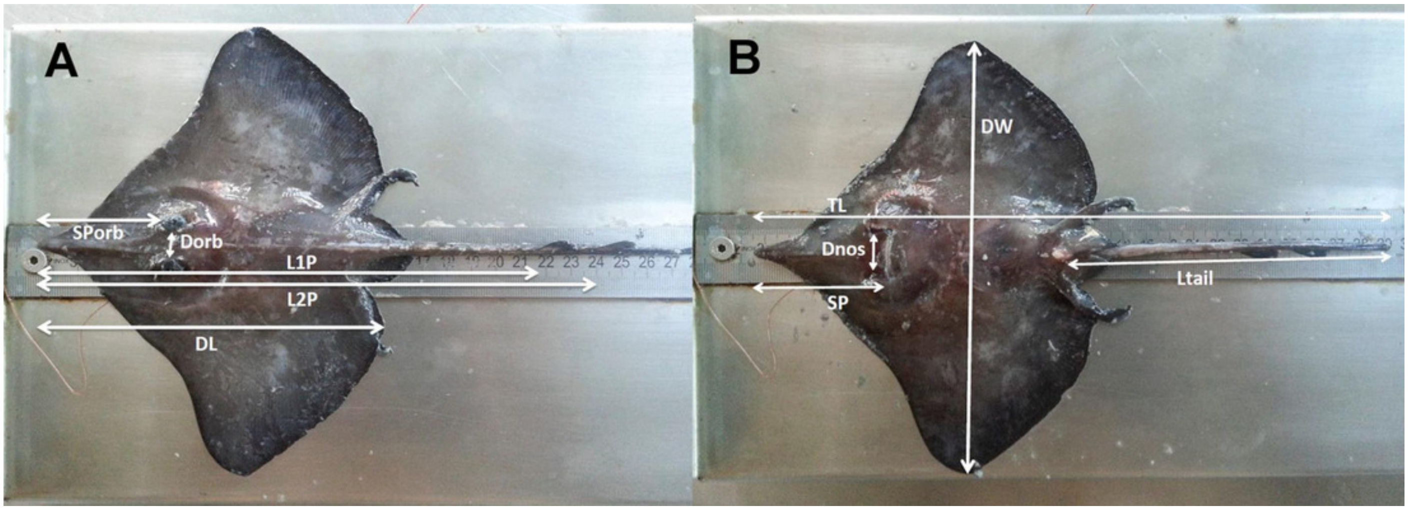

Figure 3

Juvenile and adult specimens (dorsal-ventral view) of *D. nidarosiensis* are shown in figure 3.

Figure 3. Juvenile (A: dorsal view; B: ventral view) and adult female (C: dorsal view; D: ventral view) of *Dipturus nidarosiensis*. Photo: Pierluigi Carbonara.

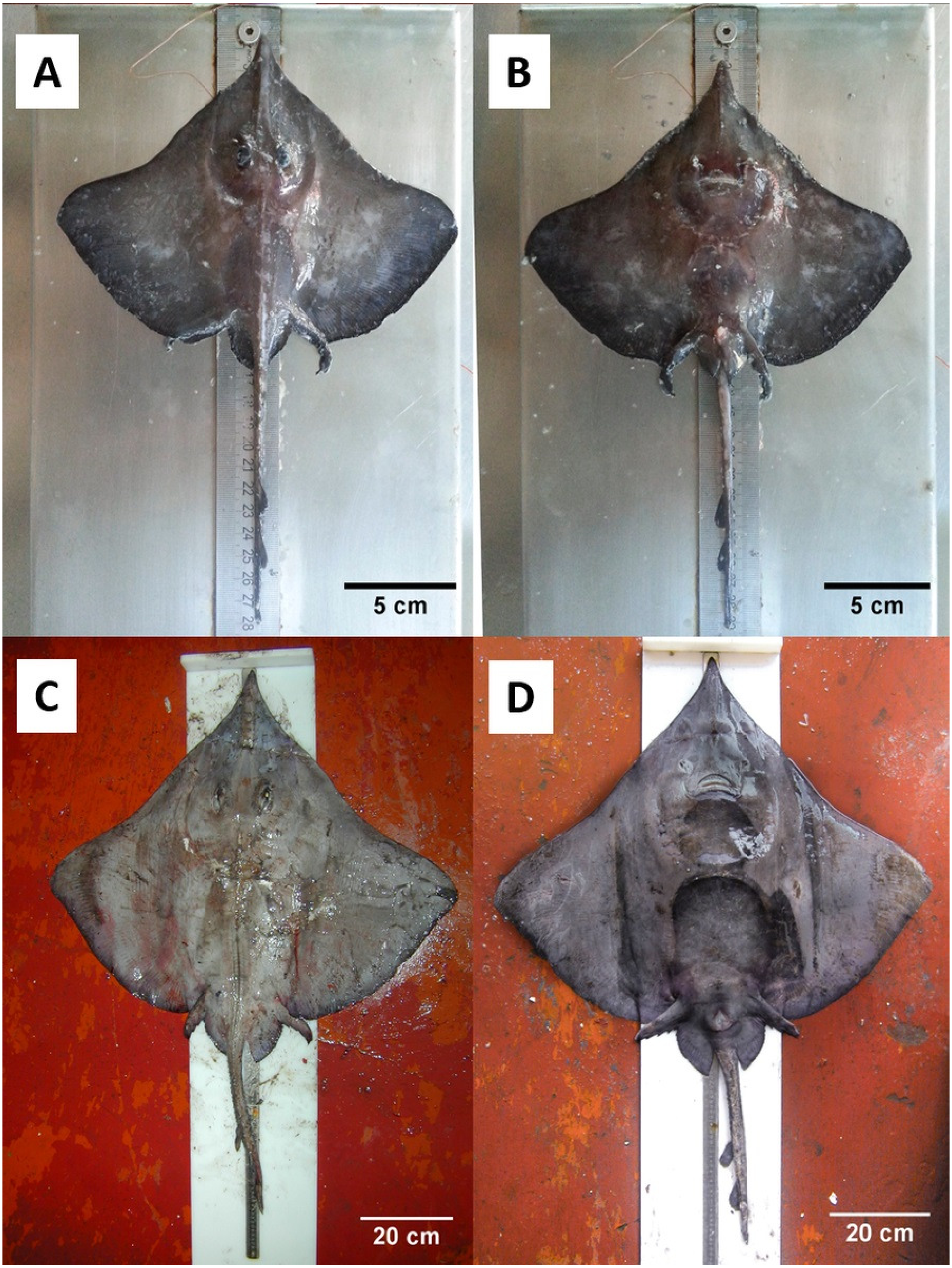
A
B
C
D
5 cm
5 cm
20 cm
20 cm

Figure 4

The mouth of female and male *D. nidarosiensis* specimens are shown in figure 4

Figure 4. Detail of the mouth of (A) female with molar-like teeth and (B) male with pointed teeth. Photo: Pierluigi Carbonara.

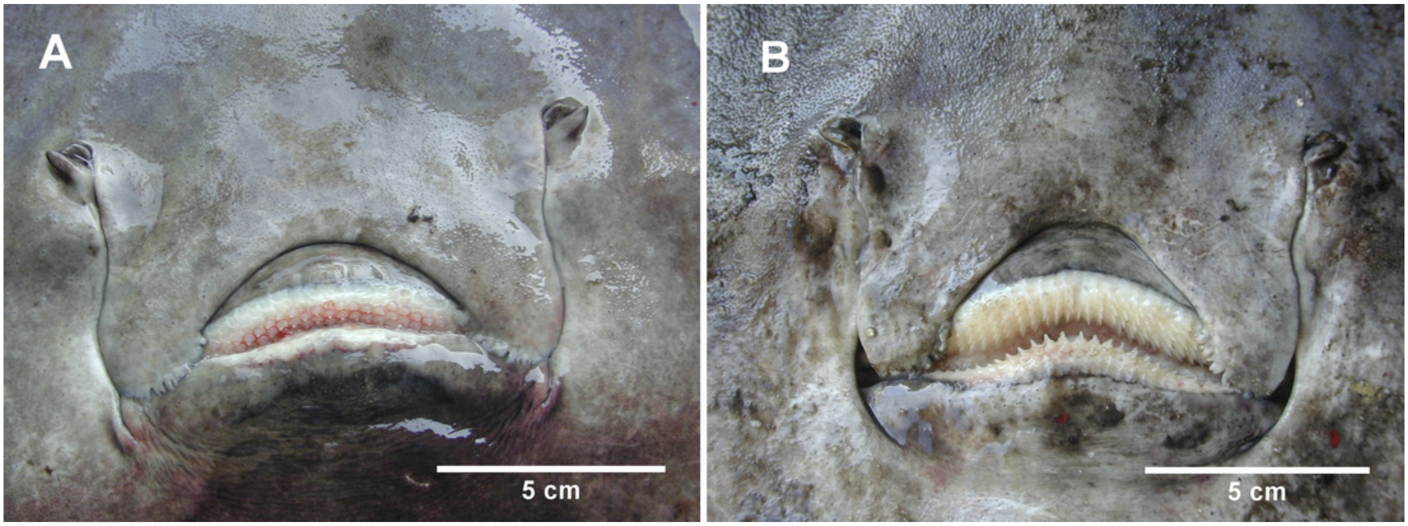

Figure 5

The snout of *D. nidarosiensis* is shown in figure 5

Figure 5. Detail of the snout (A) dorsal and (B) ventral side. Photo: Pierluigi Carbonara.

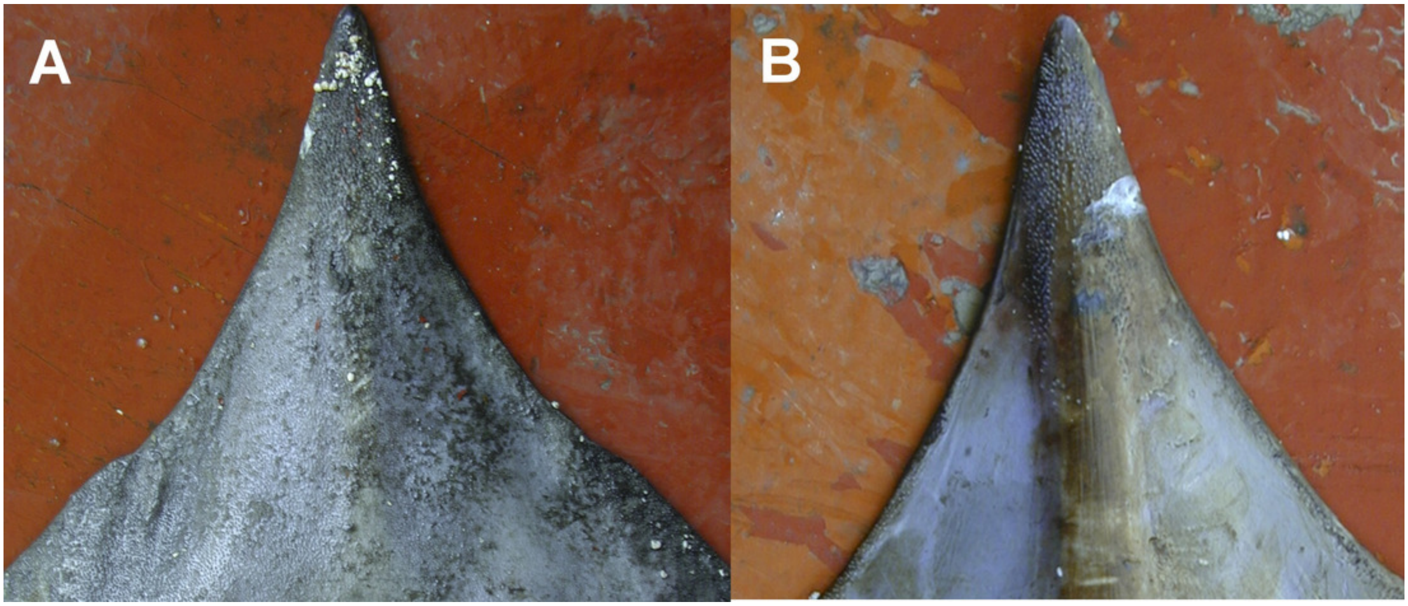

Figure 6

Median-Joining network of the COI haplotypes.is shown in figure 6

Figure 6. Median-Joining network of the COI haplotypes. Each circle represents a haplotype, and the area of the circle is proportional to the haplotype frequency. All mutational steps are equal to 1 and are represented with a vertical line. Colours correspond to different areas of origin of the sequence (SAF = South Africa; ATL: Northeast Atlantic; ALB = Alboran Sea; ALG = Algeria; SAR = Sardinia; IAD = Ionian + Adriatic seas). Haplotype code correspondence is shown in Table S1.

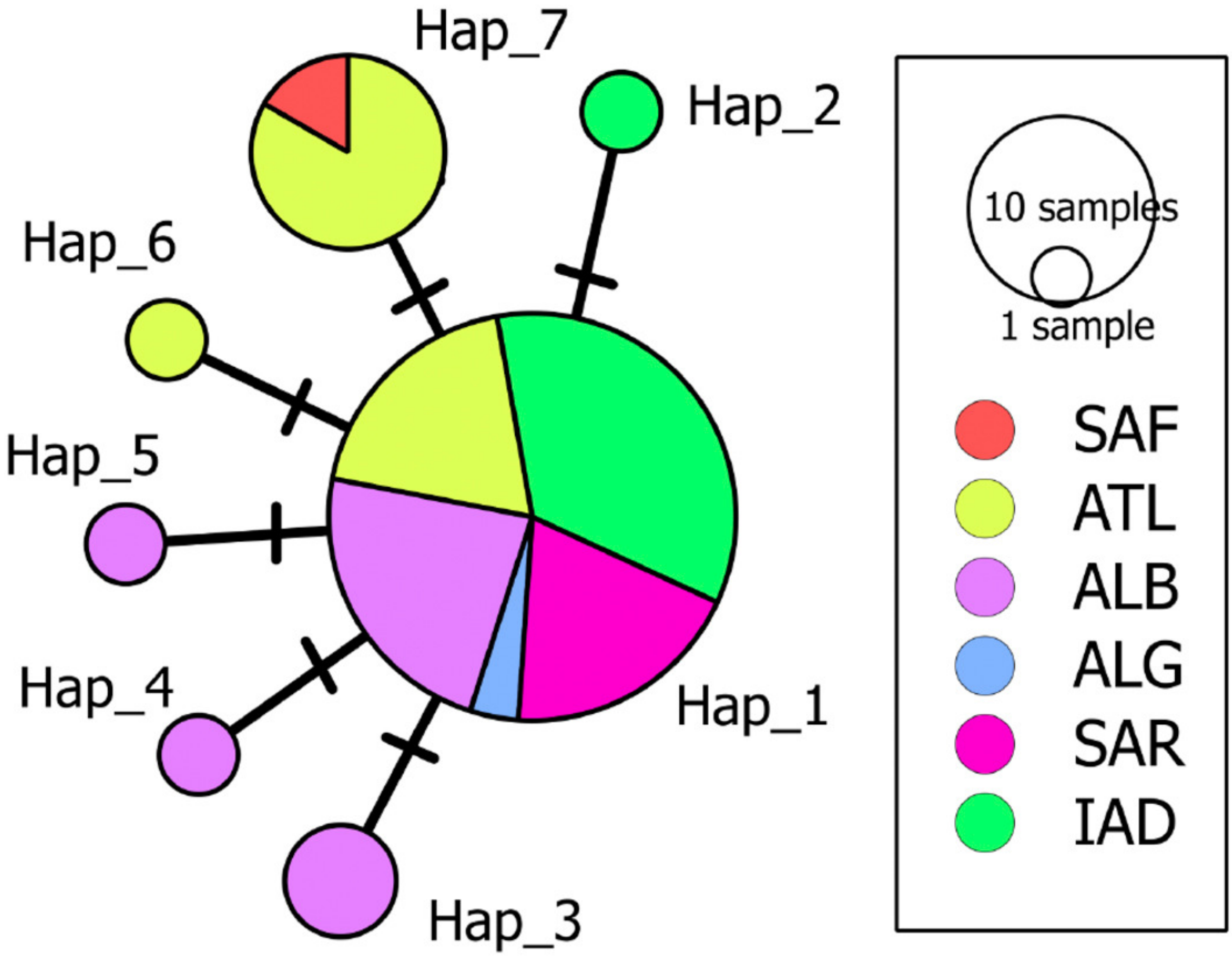
Hap_7
Hap_2
Hap_6
Hap_5
Hap_4
Hap_3
Hap_1
10 samples
1 sample
SAF
ATL
ALB
ALG
SAR
IAD

Figure 7

Phylogenetic tree is shown in figure 7

Figure 7. Phylogenetic tree based on mitochondrial COI sequences. Bootstrap/posterior probability values >70 are shown next to the nodes for the Neighbour Joining and Bayesian methods, respectively. In red the clade were the Ionian-Adriatic sequences clustered.

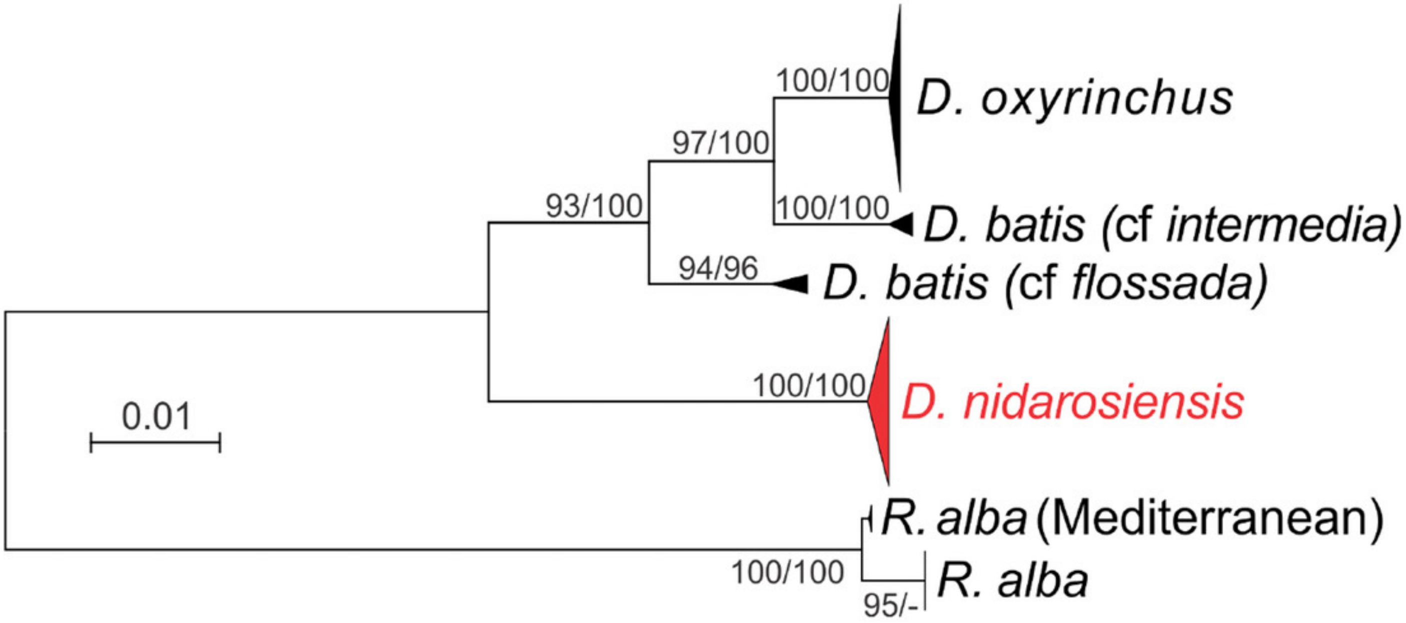

Table 1(on next page)

The morphometrics, meristics, sexes, maturity, depth, dates of capture and origins data are reported in the Table 1

Table 1. Summary of data collected from commercial landings and scientific surveys, NA: not available.

Code specimens	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Date	29/09/2010	31/07/2011	31/07/2011	06/10/2011	13/08/2014	13/08/2014	02/08/2015	01/08/2016
Source	Landing (Market)	Landing (Discards)	Landing (Discards)	Landing (Market)	MEDITS	MEDITS	MEDITS	MEDITS
Depth (m)	610	600	600	720	377	550	373	320
Morphometric measurements (mm) – Percentage of total length (%)								
Total Length (TL)	1235	705	912	1194	269	1422	356	305
Width Disc (WD)	855 - 69.2	511 – 72.5	653 – 71.6	884 – 74.0	186 – 69.1	1003 – 70.5	228 – 64.0	215 – 70.5
Length Disc (LD)	720 - 58.3	415 – 58.9	524 – 57.5	713 – 59.7	146 – 54.3	842 – 59.2	187 – 52.5	172 – 56.4
Length 1° dorsal fin (L1P)	1020 - 82.6	560 – 79.3	723 – 79.3	977 – 81.8	206 – 76.6	1162 – 81.7	252 – 70.8	237 – 77.7
Length 2° dorsal fin (L2P)	1087 - 88	622 – 88.2	802 87.9	1049 – 87.9	229 – 85.1	1259 – 88.5	282 – 79.2	266 – 87.2
Distance interorbital (Dorb)	63 - 5.1	34 – 4.8	46 – 5	59 – 4.9	12 – 4.5	75 – 5.3	15 – 4.2	14 – 4.6
Distance internostril (Dnos)	99 - 8	55 – 7.8	74 – 8.1	98 – 8.2	19 – 7.1	114 – 8	25 – 7	20 – 6.6
Snout preorbital Sporb (Sporb)	246 - 19.9	141 – 20	181 – 19.8	242 – 20.3	51 – 18.9	286 – 20.1	67 – 18.8	63 – 20.7
Snout preoral (SP)	228 - 18.5	133 – 18.9	173 – 19	215 – 18	56 – 20.8	314 – 22.1	69 – 19.4	68 – 22.3
Length Tail (Ltail)	416 - 33.7	240 - 34	309 – 33.9	412 – 34.5	136 – 50.6	568 – 39.9	160 – 44.9	157 – 51.5
Meristic counts (N°)								
Thorns on median row disc	20	12	12	15	0	19	0	0
Median thorns on tail	32	18	23	33	9	35	10	11
Lateral torn on tail	35	30	34	37	0	33	0	0
Rows of teeth upper	44	45	43	44	44	45	42	42

jaw								
Rows of teeth lower jaw	42	43	41	42	42	43	39	39
Gill slits	5	5	5	5	5	5	5	5
Weight (g)								
Total Weight	10043	1138	2596	9200	45	12540	105	75
Gonad weight	60	1.9	6.9	400	NA	58	NA	NA
Liver weight	560	44.4	133.6	495.7	1.4	62.7	1.8	1.6
Gutted weight	8140	1023.1	2250.4	8580	39.6	11090	71.8	62.2
Sex and Maturity								
Sex	Male	Male	Male	Female	Female	Female	Male	Female
Maturity	3B	3A	3A	3A	1	2	1	1

Table 2(on next page)

The description of the maturity scale, used in this study, is reported in the table 2.

Table 2. Maturity scale for the Elasmobranch oviparous species (AAVV, 2017; Follesa and Carbonara, 2019)

STAGE	**MATURATION STATE**	**REPRODUCTIVE APPARATUS ASPECT**	
		FEMALES	**MALES**
1	IMMATURE/VIRGIN	Ovaries barely discernible with small isodiametric follicles. Distal part of oviducts thick-walled and whitish. Oviducal glands not clearly visible.	Small and flaccid claspers not reaching the posterior edge of the pelvic fins. Spermducts not differentiated. Small and narrow testes..
2	MATURING	Whitish and/or few yellow maturing follicles visible in the ovary. Distal part of oviducts (uteri) well developed but empty. Developing oviducal glands.	Clasper extending past the posterior edge of the pelvic fins. Spermducts well developed, and starting to meander.
3A	MATURE	Ovaries with yellow follicles (large yolk follicles). Enlarged oviducal glands and distended oviducts.	Hard and ossified claspers extendings well beyond the posterior edge of the pelvic fin. with internal structure is generally. Testes greatly enlarged. Spermducts meandering over almost their entire length.
3B	MATURE/EXTRUDING/ ACTIVE	Ovaries walls transparent. White or yellow follicles. Large oviducal glands. Egg-cases more or less formed in the oviducts (Extruding Stage).	Clasper longer than tips of posterior pelvic fin lobes, skeleton hardened with axial cartilages hardened and pointed. Spermducts largely. Sperm flowing on pressure from cloaca (Active Stage).
4A	RESTING	Ovaries walls transparent. White or yellow follicles. Enlarged oviducts, collapsed and empty. Reduced oviducal glands diameter.	Clasper longer than tips of posterior pelvic fin lobes, skeleton hardened withaxial cartilages still hardened. Spermducts empty and flaccid.
4B	REGENERATING	Ovaries full of small follicles similar to stage 2, enlarged oviducal glands and uteri	Clasper longer than tips of posterior pelvic fin lobes, skeleton hardened withaxial cartilages still hardened. Spermducts empty and flaccid.