Porcu *et al. Helgol Mar Res (2017) 71:10*
DOI 10.1186/s10152-017-0490-2

Helgoland Marine Research

ORIGINAL ARTICLE

Morphological descriptions of the eggcases of skates (Rajidae) from the central-western Mediterranean, with notes on their distribution

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Abstract

Eggcases of eight rajiform skates (*Dipturus nidarosiensis*, *D. oxyrinchus*, *Leucoraja melitensis*, *Raja asterias*, *R. brachyura*, *R. clavata*, *R. miraletus* and *R. polystigma*) present in the central-western Mediterranean are described, based on specimens obtained from fishery surveys. Eggcase features such as dimensions, horns and apron lengths, and presence/absence of lateral keels were crucial to discriminate the eggcases of the various species. Morphological and morphometric data, confirmed by the multivariate analysis, indicated that the eggcase of *R. miraletus* and *L. melitensis* were distinct from those of the other species for being unkeeled. Within the species having keeled eggcases, those of the genus Dipturus and *R. brachyura* were discriminated from the remaining group by having the largest dimensions and aprons. Sandy bottoms (<100–150 m depth) were identified as egg-laying sites (i.e. sites with females bearing eggcases in *uteri*) for many species belonging to genus Raja *Raja asterias*, *R. brachyura*, *R. miraletus* and *R. polystigma*). The finding of *R. asterias* and *R. miraletus* carrying eggcases yearly on the same sites, seems to confirm the theory that many rajid species demonstrate site fidelity, returning to the same depositional area on an annual basis. Some remarks on reproductive biology of these skates are also provided. The eggcase identification key reported here represents the first for the Mediterranean and may be useful, in the future, to identify egg-laying grounds of skates with a nonlethal method.

Keywords: Rajidae, Mediterranean Sea, Eggcases, Identification key, Distribution

Background

The Rajidae family represents the most species-rich group among cartilaginous fish, having 30 genera and 245 valid species [1]. These demersal elasmobranchs often have limited, well-defined distributions, presumed low natural mortality [2] and are an important component of marine biodiversity. Many species live close to shore, generally at depths <100 m [3]. Some species are known to undertake seasonal migrations towards egg-laying grounds which maybe located close to shore [4], or over continental slopes, and usually discovered during exploratory surveys [5].

Among elasmobranchs, skates, together with the Scyliorhinidae family and the Heterodontiformes order, are the only strictly oviparous group [6] producing a tough eggcase that preserves the embryo development, ranging from months to years depending on the species [7, 8]. Most rajids show single oviparity (i.e., one embryo per eggcase), with pairs of eggcases (one from each oviduct) deposited during the spawning season [9]. The few exceptions include *Raja pulchra* [10] and *R. binoculata* [11], which can produce multiple embryos in each eggcase.

One of the greatest issues characterizing this family is historically linked to their taxonomy, often problematical,

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due to phenotypic similarity between some taxa and individual variability in others, and are responsible for their misidentification, related probably to biological and environmental characteristics. Another tool to distinguish skate species from each other, after examining their external morphology, is looking at their eggcases morphology. This feature is unique to these species and can be used for their identification [10, 12–17]. Moreover, the finding of sites where demersal eggcases are laid on the bottom gives information concerning distribution and reproductive ecology [16, 18], such as the spawning habitats.

Four genera of Rajidae live in the Mediterranean basin (*Dipturus*, *Leucoraja*, *Raja* and *Rostroraja*) with 16 possible valid species [19]. In spite of the fact that chondrichthyan reported landings in the Mediterranean Sea have considerably increased in recent years [20], many aspects of their reproductive biology are still unknown. Eggcases have been described for some skates in the Mediterranean basin [15, 22, 23], but there are few published data on their lengths and widths. Furthermore, regional identification keys based on eggcase morphology and comparative studies are lacking.

The aim of this work is to provide a detailed morphometric and morphological description of eight Mediterranean skate species eggcases, providing also comparisons with previously published data. In particular, seven of these were caught around Sardinian waters, central-western Mediterranean (*Dipturus nidarosiensis* recently reported for the Mediterranean [24, 25], *D. oxyrinchus*, *Raja asterias*, *R. brachyura*, *R. clavata*, *R. miraletus* and *R. polystigma*) and only one (*Leucoraja melitensis*) was found in the Sentinelle Bank (Sardinian Channel, off the Sardinian waters). Moreover, a specific key that could be useful in the identification of eggcases found on the sea bed, information on the distribution of active females per species and a brief description of the bottom biocenosis, useful to identify possible nursery sites for these vulnerable organisms, were provided.

Furthermore, given the common pattern for which an intraspecific latitudinal cline in elasmobranch sizes between Atlantic and Mediterranean species (e.g. [26]) exists, it could be hypothesized that the same dynamic could be reflected in the eggcase dimensions [27, 28]. For this reason, we investigated in this way, considering also the interspecific variability of eggcases, that may represent an adaptation to several kind of bottoms in order to minimize the competition for nursery grounds.

Methods

Active females (i.e. with eggcases in the *uteri* or extruding from the cloaca; henceforth referred to as active) of seven rajids present in Sardinian waters (central-western Mediterranean Sea), were collected between 2005 and 2016 during seasonal experimental surveys and commercial hauls (trammels and trawlings) at depths from 28 to 1700 m. Additional active individuals belonging to *L. melitensis* were caught in 2017 (February and March) during commercial trawlings in the "Sentinelle Bank" at 200 m of depth from a vessel registered in a Sardinian district (Cagliari).

For each individual, the total length (TL) was recorded in millimeters (mm).

For an accurate identification, eggcases were removed directly from the *uteri* of active females, except those of *D. nidarosiensis* specimens, which were collected from the sea bed. Eggcases were photographed using a Canon EOS 1100D, preserved in 80% ethanol and deposited in the collection at the Department of Life and Environmental Sciences, University of Cagliari, Italy. To assure a proper identification of a fully formed embryo found within an eggcase on the sea floor, presumably belonging to *D. nidarosiensis*, a piece of muscle was collected from the embryo and stored in absolute ethanol at −20 °C in order to proceed to the genetic identification. The COI-3 primers cocktail and PCR conditions from Ivanova et al. [29] were used to amplify the cytochrome oxidase I gene (COI).

As reported by Gordon et al. [30], the term "anterior" used in this work is considered to refer to the part that forms first in the oviducal gland. Ten morphometric measures (Fig. 1) were recorded in millimeters using a caliper following Concha et al. [31]: eggcase length (ECL, measured longitudinally between the anterior and posterior apron borders); eggcase width (ECW, the transverse width of the eggcase in its lateral plane included the keels); anterior and posterior apron length (AAL and PAL, the distance from the central body eggcase to the apron border); left and right keel width (LKW and RKW, the transverse width of the case from the eggcase keel junction to the keel edge); left and right anterior horn length (LAHL and RAHL, the distance from the anterior apron border to the horn tips); left and right posterior horn length (LPHL and RPHL, the distance from the posterior apron border to the horn tips). All measurements were then expressed in % of ECL and ECW in order to easily compare them to the others. The measurements in millimeters were also reported to contrast with those present in literature. In addition, the general morphology (shape and other features), fresh color (using the Pantone Matching System, PMS, Carlstadt, NJ, USA), texture and presence and position of adhesion fibres were recorded.

Statistical analysis

The PRIMER (v6) package [32] was used to analyze the morphometric measures matrix. The outcomes were

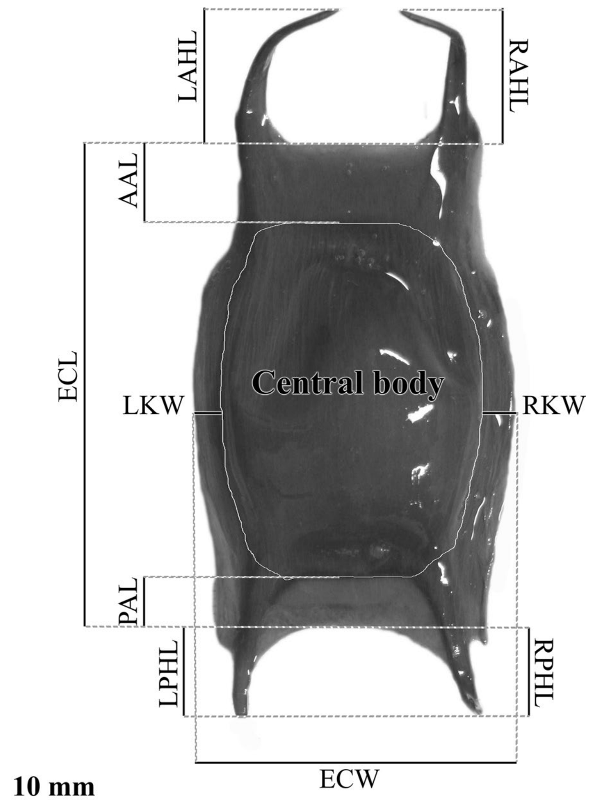

Fig. 1 Morphometric measurements performed in the eggcases. *AAL* anterior apron length, *ECL* eggcase length, *ECW* eggcase width, *LAHL* left anterior horn length, *LKW* left keel width, *LPHL* left posterior horn length, *PAL* posterior apron length, *RAHL* right anterior horn length, *RKW* right keel width, *RPHL* right posterior horn length

subjected to a Principal Component Analysis (PCA). The components that mostly contributed to the variance were identified. In addition, the SIMPER procedure (SIMilarity PERcentage Analysis) was used to identify those measures responsible for discriminating between groups. The observed differences between groups were tested using an analysis of Similarity Randomization Test (ANOSIM) [33]. Moreover, for each species, the geographical coordinates where females carried eggcases were visualized were provided using Mapsource software version 6.16.3 (Garmin). During fishing activities, the benthic community, associated with the bottom at each site, found in the net, was recorded. For each haul, all taxa were identified following the taxonomic guide for the Mediterranean [34] and in addition number and weight of every species (if possible) were registered in order to determine their abundance.

Results

A total of 177 eggcases of eight rajid species were examined and measured.

Eggcase description

Genus: Dipturus

***Dipturus nidarosiensis* (Storm, 1881) (n = 5)**

During the sampling period, only one female carrying eggcases (not yet fully formed with only anterior horns and the apron visible) was caught in September 2015 at a depth of 991 m (Table 1). However, one eggcase containing a well-developed embryo and four empty eggcases were trawled at depths of 974–1212 m.

The embryo was a male (TL 193 mm) showing the typical features of this species: medium brown to grey-brownish dorsal surface and dark brown ventral surface, darker than the dorsal surface. The embryo's sequence (658 bp long) was compared to 38 COI sequences of the genus Dipturus from Cariani et al. [35] and resulted identical to the specimens of *D. nidarosiensis* from the Mediterranean Sea (GenBank accession id: KT307210).

The eggcase appeared very large (Fig. 2a) reaching a maximum of 177 mm ECL (Table 2). The width was 43.9% of the length, giving a long rectangular shape (Table 3). Anterior and posterior aprons were well developed (25.1

Table 1 Females carrying eggcases analyzed from 2005 to 2017, represented by species, total length (TL) range, depth-range and mean (mean ± SD) and sampling time

Species	TL range (mm)	Depth range (m)	Sampling time											
			J	F	M	A	M	J	J	A	S	O	N	D
Dipturus nidarosiensis	1376	991									*1*			
Dipturus oxyrinchus	1010–1070	100–620 (500 ± 144)	*5*	*2*	*4*		*4*	*4*	*1*	*1*		*4*	*3*	*3*
Leucoraja melitensis	335–382	200		*1*	*3*									
Raja asterias	517–725	28–92 (44 ± 13)					*1*	*37*	*34*	*7*				
Raja brachyura	730–1060	30–62 (47 ± 9)					*3*	*7*	*6*	*3*				
Raja clavata	610–871	42–473 (144 ± 118)	*3*	*1*				*8*	*4*	*3*				
Raja miraletus	346–481	32–158 (69 ± 24)	*3*				*2*	*39*	*13*	*17*				
Raja polystigma	512–595	38–126 (56 ± 24)						*7*	*4*	*1*				

In italics, the number of females carrying eggcases per month and 20.2% of ECL, respectively): the first was straight, on the contrary, the latter was rounded and slightly shorter (Table 3). Both anterior and posterior horns were short (presumably damaged) and lateral keels were present and well developed (9.0% of ECW) (Table 3). It possessed adhesion fibrils attached to the keels, and in the dorsal and ventral surfaces.

Dipturus oxyrinchus ***(Linnaeus, 1758) (n = 37)***
Active females were found during all months of the year, except in April and September, at a mean depth of 500 m (Table 1).

This species (Fig. 2b) had a smaller eggcase than its congeneric *D. nidarosiensis* with a maximum of 116.2 mm ECL (Table 2). The eggcases had a rectangular shape (Table 3), since the ECW was 54.9% of ECL. Anterior and posterior aprons were well developed (25.1 and 14.2% of ECL, respectively), and the anterior one was longer and straighter in contrast to the posterior, which was more rounded and shorter (Table 3). Horns were short with anteriors slightly longer and tapered then the posterior ones (33.7 and 31.3% of ECL, respectively). Lateral keels were pronounced (9.6% of ECW) and had adhesive fibres. The fresh color varied from brown shades (PMS 4485, 1535) to greenish ones (PMS 3975) (Fig. 2b; Table 3).

Genus: Leucoraja

Leucoraja melitensis ***(Clarke, 1926) (n = 6)***
Females with eggcases were caught in the Sentinelle Bank (N38° 03′ 314″ E9° 41′ 998″) in February and March at a depth of 200 m (Table 1). *L. melitensis* had small eggcases (45.0 mm ECL maximum, Table 2), rectangular in shape (ECW 54.0% of ECL). Anterior apron (13.5% of ECL) was longer then the posterior one (1.7% of ECL). The posterior horns were moderate and thin, differently from the anteriors, very long, thin and intersecting (103.9% of ECL). No lateral keels were observed and the surface was smooth and semi-transparent with no external fibres covering the surface (Fig. 2c). The fresh color was yellowish (PMS 110) with brown shades (PMS 125) (Table 3).

Genus: Raja

Raja asterias ***Delaroche, 1809 (n = 59)***
Active females were found in late spring and summer (May to August) exclusively on the continental shelf (28–92 m depth) (Table 1). The eggcases (Fig. 2d) were

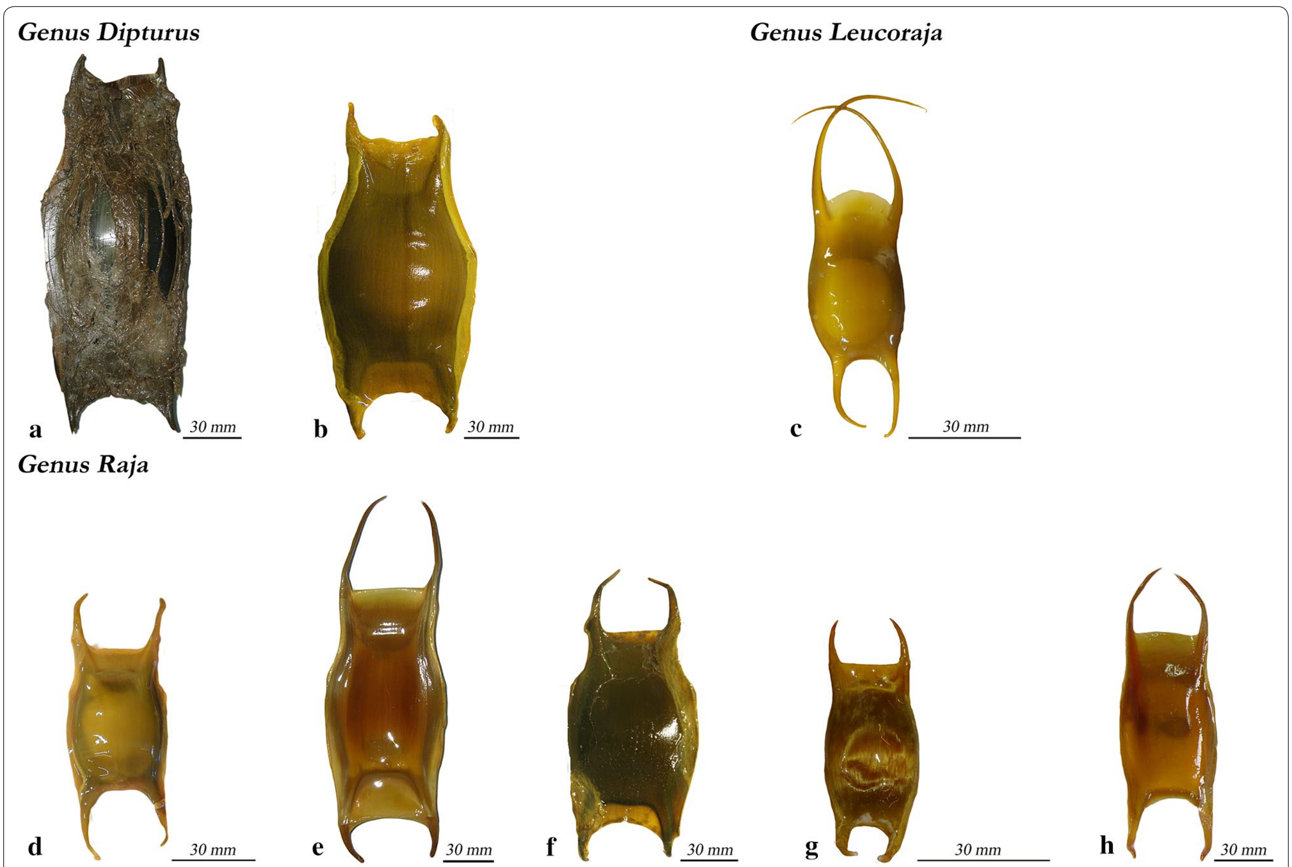

Fig. 2 Eggcases belonging to the eight rajid species present in central-western Mediterranean. **a** *D. nidarosiensis*; **b** *D. oxyrinchus*; **c** *L. melitensis*; **d** *R. asterias*; **e** *R. brachyura*; **f** *R. clavata*; **g** *R. miraletus*; **h** *R. polystigma*

Table 2 Eggcase morphometric measurements of the eight rajid species analyzed

Species	N		ECL	ECW	AAL	PAL	RKW	LKW	RAHL	LAHL	RPHL	LPHL
D. nidarosiensis	5	Range	153–177	61.8–80.5	38.2–49.7	26.2–40.9	6.5–7.7	6.9–7.8	9–14.6	9–12.4	18.1–25	18.1–35.0
		Mean ± SD	170.2 ± 9.8	74.8 ± 7.5	42.6 ± 4.4	34.3 ± 5.4	7.2 ± 0.5	7.3 ± 0.4	10.9 ± 2.1	10.5 ± 1.2	21.2 ± 2.5	23.2 ± 6.7
D. oxyrinchus	37	Range	91.6–116.2	48.9–67.5	20.3–31.7	7.7–20.8	3.2–8	3.2–8	27.1–47.4	27.1–47.4	26.1–40.4	26.1–40.4
		Mean ± SD	103.8 ± 5.3	57.0 ± 4.0	26.0 ± 2.5	14.9 ± 3.0	5.4 ± 1.1	5.4 ± 1.1	35.0 ± 6.1	35.0 ± 6.1	32.5 ± 5.1	32.5 ± 5.1
L. melitensis	6	Range	42.3–45	22.4–26	5.6–6.4	0.4–1.3	–	–	42.5–52.5	38.9–52.0	16.5–20.8	13.1–20.2
		Mean ± SD	44.1 ± 1.0	23.8 ± 1.4	6.0 ± 0.4	0.7 ± 0.4	–	–	47.0 ± 4.0	44.7 ± 4.8	18.2 ± 1.9	16.6 ± 2.6
R. asterias	59	Range	39.7–55.4	22.4–40.5	4.5–8.9	1–3.8	1.5–4.3	1.5–4.3	18.8–30.5	18.8–30.5	12.1–27.3	12.1–27.3
		Mean ± SD	48.3 ± 3.0	33.7 ± 2.6	6.9 ± 1.0	2.1 ± 0.7	2.4 ± 0.7	2.4 ± 0.7	24 ± 3.3	24 ± 3.3	22.3 ± 3.1	22.3 ± 3.1
R. brachyura	12	Range	113–133	64.7–72.2	18.5–25.7	17.5–26.6	3.5–7.4	3.5–7.4	55.6–80.7	55.2–80.7	30.4–59.2	30.4–59.2
		Mean ± SD	119.3 ± 4.9	69.5 ± 2.3	21.9 ± 2.5	21.7 ± 2.6	5.5 ± 1.4	5.7 ± 1.4	69.7 ± 10.0	69.5 ± 10.3	43.7 ± 11.0	44. ± 10.7
R. clavata	10	Range	62.5–67.5	41.7–47.4	9.5–15.9	6–8.4	3.1–4.8	3.1–4.8	25–40.6	25–40.6	19.2–24.5	19.2–24.5
		Mean ± SD	64.1 ± 1.9	43.8 ± 2.0	11.4 ± 1.8	6.8 ± 0.9	3.8 ± 0.6	3.8 ± 0.6	32.5 ± 5.0	32.5 ± 5.0	22.7 ± 1.6	22.7 ± 1.6
R. miraletus	34	Range	41.8–56.5	22.8–29.2	3–9.9	1.3–4.2	–	–	8.8–27.9	8.8–27.9	10.2–21.7	10.2–21.7
		Mean ± SD	47.4 ± 4.0	24.7 ± 1.7	7.2 ± 1.7	2.7 ± 0.8			18.1 ± 4.7	18.1 ± 4.7	16.3 ± 3.3	16.3 ± 3.3
R. polystigma	14	Range	56.6–69.2	33.9–44.3	4.7–13.4	1–2.8	0.8–2.7	0.8–2.3	18.9–36.0	18.4–35.2	12–26.5	12–26.6
		Mean ± SD	62.3 ± 4.3	37.9 ± 2.9	9.4 ± 2.4	2.0 ± 0.6	1.7 ± 0.6	1.6 ± 0.5	27.5 ± 5.2	27.3 ± 5.1	22.4 ± 4.8	22.3 ± 4.8

Table 3 General description of rajid eggcases

Species	Shape	Anterior horns	Posterior horns	Anterior apron	Posterior apron	Keels	Fresh color
D. nidarosiensis	Long rectangular	Short	Short and sturdy	Straight and well developed	Rounded and well developed	Well evident	–
D. oxyrinchus	Rectangular	Short	Short and sturdy	Straight and well developed	Rounded and well developed	Well evident	Shades of brown (PMS 4485, 1535), and greenish (PMS 3975)
L. melitensis	Rectangular	Long, thin and intersecting	Moderate and thin	Rounded and moderate	Rounded and narrow	Absent	Yellowish (PMS 110) with brown shades (PMS 125)
R. asterias	Rectangular	Moderate and thin	Moderate and thin	Straight	Rounded and narrow	Evident	Yellowish (PMS 110) with brown shades (PMS 125)
R. brachyura	Rectangular	Long and thin	Short and sturdy	Straight and well developed	Rounded and well developed	Well evident	Reddish brown (PMS 469) and greenish (PMS 399)
R. clavata	Rectangular almost square	Moderate and thin	Short and sturdy	Straight and moderate	Rounded and moderate	Well evident	Reddish brown (PMS 1395, 139), yellowish brown (PMS 119)
R. miraletus	Rectangular	Short and thin	Short and sturdy	Straight	Rounded and narrow	Absent	Brown (PMS 161) and amber (PMS 138)
R. polystigma	Rectangular	Moderate and thin	Moderate and sturdy	Straight and moderate	Rounded and narrow	Narrow	Brown (PMS 133, 161) and yellowish (PMS 126)

small (55.4 mm ECL maximum, Table 2) with a rectangular shape (ECW 70.0% of ECL). Aprons were different to each other: the posterior was rounded and narrow (4.3% of ECL), and the anterior was straight and long (14.2% of ECL) (Table 3). Anterior and posterior horns were moderate in length and thin (49.6 and 46.0% of ECL, respectively). Lateral keels were pronounced (2.4% of ECW) with adhesive fibres (Table 3). The fresh color was yellowish (PMS 110) with brown shades (PMS 125) (Fig. 2d; Table 3).

Raja brachyura **LaFont, 1873 (n = 12)**

Active females were caught from May to August in shallow waters (30–62 m depth) (Table 1). The eggcases had a rectangular shape (ECW about 58.3% of ECL) and were large in size (maximum ECL = 133 mm) (Fig. 2e; Tables 2, 3). Posterior apron was rounded and pronounced and the anterior one was slightly longer and straighter (Table 3). The posterior horns were short and sturdy, while the anterior ones were thin and long (18.2% of ECL). Keels were developed (8.3% of ECW) and had attachment fibres. The fresh color was reddish brown in the eggcase body (PMS 469) and greenish (PMS 399) in the edges along the keels (Fig. 2e; Table 3).

Raja clavata **(Linnaeus, 1758) (n = 10)**

Active females were sampled during the summer (from June to August) and winter months (January and February) displaying a broad bathymetric distribution (42–473 m depth) (Table 1).

This skate had a medium size eggcase (maximum ECL = 67.5 mm, Table 2) with a rectangular shape (ECW 68.3% of ECL) (Fig. 2f). The eggcases had a rounded anterior apron and a straight posterior one (Table 3); the former was longer than the posterior one (17.8 and 10.6% of ECL respectively). Posterior horns were short and sturdy (35.3% of ECL), instead the anterior ones were thin and moderately long (50.4% of ECL). Lateral keels were pronounced (8.7% of ECW) and presented adhesive fibres (Table 3). To the naked eye, the eggcase surface appeared covered by several fibres; the fresh color varied from reddish brown (PMS 1395, 139) in the eggcase body, to yellowish in the edges (PMS 119) (Fig. 2f; Table 3).

Raja miraletus **Linnaeus, 1758 (n = 34)**

Active females were caught mostly in late spring and summer (May to August) and only a few times in winter (January), exclusively in the continental shelf (32–158 m depth) (Table 1).

The eggcases were small (Fig. 2g) with a maximum of 56.5 mm ECL (Table 2) and rectangular in shape (ECW 52.3% of ECL). The posterior apron was rounded and short (5.5% of ECL), and the anterior one was straight and three times the length of the anterior (15.2% of ECL). Posterior horns were short (34.5% of ECL) and sturdy, while the anteriors were thinner and slightly longer (38.3% of ECL) (Tables 2, 3). Lateral keels were totally absent and the adhesion fibres were attached to the horns (Table 3). The eggcase surface was covered with visible fibres; the fresh color varied from brown (PMS 161) to amber (PMS 138) (Fig. 2g; Table 3).

Raja polystigma **Regan, 1923 (n = 14)**

As with *R. asterias* and *R. brachyura*, active females were found only during the summer season (June to August) on the continental shelf (38–126 m depth) (Table 1). This skate presented a medium size eggcase (maximum ECL = 69.2 mm, Table 2) with rectangular shape (ECW 60.9% of ECL) (Fig. 2h). The eggcases had a rounded and narrow posterior apron and a moderately developed straight anterior one (3.2 and 15.0% of ECL, respectively) (Table 2). Posterior horns were moderate and sturdy (35.8% of ECL), instead the anterior ones were thin and had a moderate length (43.9% of ECL). Lateral keels were narrow (4.4% of ECW) and had adhesion fibres (Table 3). The fresh color had shades of brown (PMS 133, 161) in the eggcase body, and yellowish in the edges (PMS 126) (Fig. 2h; Table 3).

Statistical analysis

Eggcase measurements of *D. nidarosiensis* (all found on the sea bed) were excluded from this analysis because they could have been damaged during the recovery in fishing operations (especially the horns) and the measurements could have been different with respect to those found in *uteri*.

The results of the PCA (Fig. 3) highlighted the existence of the four groups with high dissimilarity values. In particular, group A (composed of eggcases of *D. oxyrinchus* and *R. brachyura*) was different from groups B (composed of eggcases of *R. asterias*, *R. clavata* and *R. polystigma*) principally in the eggcase dimension (ECL) and the anterior and posterior aprons length (AAL and PAL) and from group D (*L. melitensis*) in ECL and PAL. Group C (composed by eggcases of *R. miraletus*) was dissimilar to group B essentially for the lack of the keels (RKW and LKW) and to the other group (A) for eggcase length (ECL), width (ECW) and anterior horn length RAHL and LAHL). Finally, group D has discriminated from groups B and C especially for the anterior horn lengths (RAHL and LAHL) as confirmed also by the SIMPER routine (Table 4).

Based on the main eggcase characteristics of each species and their differences with the other ones and considering statistical analysis, we provided a species identification key based on the eggcase descriptions (Table 5).

Eggcase distribution

Females carrying eggcases of *R. asterias* and *R. brachyura* (Fig. 4) were caught mainly around the west coast in shallow waters (within a depth of 100 m) characterized by sandy bottoms in association with the seagrass *Posidonia*

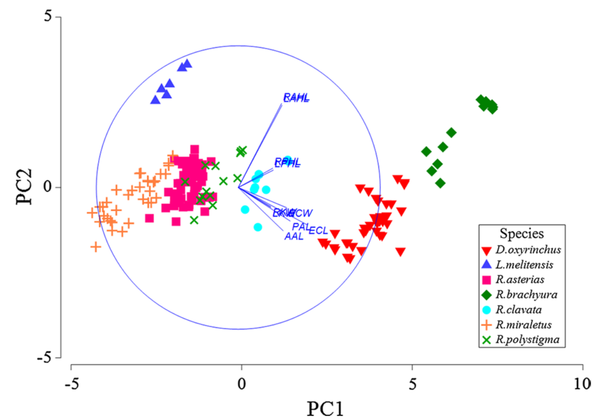

Fig. 3 Principal components analysis (PCA) conducted of eggcase morphometric measurements

oceanica (Linnaeus) Delile 1813, the sea star *Astropecten aranciacus* (Linnaeus, 1758) and the seaweed *Codium bursa* Agardh, 1817.

Raja miraletus active females (Fig. 4) were distributed all around Sardinia with the exception of the south-eastern part. The specimens preferred sandy substrata with low algal cover populated mainly by the irregular sea-urchins *Spatangus purpureus* (Müller, 1776) and the holothuroid *Parastichopus regalis* (Cuvier, 1817). The highest occurrence of females carrying eggcases was observed in the central western side, with more specimens caught on the same sites (N40° 11′131″ E8° 23′155″ at a depth of 80 m) over several years of sampling. Also *R. polystigma* females were sampled in sandy bottoms within the continental shelf (38–126 m), but given the few specimens collected (Fig. 4), it was difficult to establish if this species had a geographical preference. The same pattern was observed for *R. clavata* females (Fig. 4), poorly sampled over the years and at a wide depth range (42–473 m). *D. oxyrinchus* females carrying eggcases were observed around all the Sardinian Island (Fig. 4) at a mean depth of 500 m where the bottoms were mainly muddy and constituted by the bathyal biocoenosis as *Axinella cannabina* (Esper, 1794), *Echinus melo* Lamarck, 1816 and *Gryphus vitreus* (Born, 1778). Also the congeneric *D. nidarosiensis* seemed to prefer muddy substrata; the eggcases and the only one female carrying developing eggcases were found in deep-waters (>750 m depth) in the south-eastern part of the Island (Fig. 4).

No data about the benthic community associated to *L. melitensis* active were available, but preliminary information on their distribution was given (Fig. 4).

Table 4 Results of the SIMPER analysis routine considering the morphometric measures in the different groups

Morphometric measures	Average squared distance	Squared distance/SD	Contribution %
Group D versus A	60.61		
ECL	14.00	4.51	23.16
PAL	10.50	3.02	17.36
ECW	8.33	3.28	13.74
Group D versus B	15.45		
RAHL	3.56	1.89	23.02
LAHL	3.06	1.62	19.78
RKW	2.47	2.64	15.97
Group A versus B	38.32		
ECL	10.20	2.59	26.71
PAL	6.57	2.04	17.14
AAL	5.12	2.55	13.37
Group D versus C	15.26		
RAHL	7.21	2.28	47.26
LAHL	6.46	2.00	42.34
Group A versus C	64.75		
ECL	12.40	3.56	19.12
ECW	7.91	3.12	12.21
LAHL	6.81	0.94	10.52
Group B versus C	10.88		
RKW	2.47	2.64	22.69
LKW	2.46	2.89	22.60
ECW	1.17	0.82	10.79

Only highest contributing measures are shown

Group A, *D. oxyrinchus* and *R. brachyura*; Group B, *R. asterias*, *R. clavata*, *R. polystigma*; Group C, *R. miraletus*; Group D, *L. melitensis*; SD, standard deviation

Discussion

Eggcase reports and information on the egg-laying rate for the overwhelming majority of rajids have received little attention in the Mediterranean context. In this regard, the present study provides updated eggcase descriptions and quantitative data on their sizes for developing regional and specific identification keys, useful in the recognition of eggcases found over sea beds, providing useful information on the distribution and reproductive biology of skates living in the central-western Mediterranean. We do report for the first time information on eggcases of the endemic *L. melitensis*, considered Critically Endangered in the Mediterranean [36].

The eggcases from the eight analyzed species exhibited peculiar morphologies between genera and even species. Despite some overlap in size between species, features such as horn and apron lengths, and presence/absence of lateral keels were crucial to discriminate the eggcase at species level. In particular, from morphological and morphometric analysis, actually corroborated by multivariate analyses, *R. miraletus* and *L. melitensis* stood out from all the others for having eggcases without keels. Moreover, within the species having keeled eggcases, those belonging to *D. oxyrinchus* and *R. brachyura* were discriminated to the remaining group for having the longest eggcases and aprons (ECL and AAL-PAL).

Considering the ECL (without horns), females of *D. nidarosiensis* possessed, overall, the biggest eggcases (153–177 mm ECL). Our results represent the first available qualitative and quantitative characterization for eggcases from this species in the Mediterranean basin.

Table 5 Identification key for rajid eggcases of central-western Mediterranean

1a	Eggcase large >90 mm ECL, with well-developed aprons	2
1b	Eggcase small-medium in length 40 < ECL < 70 mm, with moderate or narrow aprons	3
2a	Eggcase rectangular: ECL approximately two times the ECW (ECL range = 153–177 mm; ECW range = 61.8–80.5 mm) with well-developed aprons (AAL range = 38.2–49.7 mm; PAL range = 26.2–40.9 mm)	*D. nidarosiensis*
2b	Eggcase rectangular: ECL approximately two times the width (ECL range = 91.6–116.2 mm; ECW range = 48.9–67.5 mm). Developed aprons (AAL range = 20.3–31.7 mm PAL; range = 7.7–20.8 mm). Short anterior and posterior horns	*D. oxyrinchus*
2c	Eggcase rectangular: ECL approximately two times the ECW (ECL range = 113–133 mm; ECW range = 64.7–72.2 mm). Developed aprons (AAL range = 18.5–25.7 mm; PAL range = 17.5–26.6 mm). Long and thin horns, particularly the anterior (about 50% of ECL)	*R. brachyura*
3a	Eggcase small-medium in length 40 < ECL < 70 mm with evident or narrow keels	4
3b	Eggcase small and rectangular (ECL range = 41.8–56.5 mm) without keels	5
4a	Eggcase moderate in size (ECL range = 62.5–67.5 mm), rectangular/almost square in shape (ECL approximately 1.4 times the ECW). Aprons and keels developed. Anterior horns moderate and thin, posterior horns short and sturdy	*R. clavata*
4b	Eggcase small (ECL range = 39.7–55.4 mm) rectangular/almost square in shape (ECL approximately 1.4 times the ECW). Evident keels and both anterior and posterior horns moderate and thin	*R. asterias*
4c	Eggcase moderate in size (ECL range = 56.6–69.2 mm) with rectangular shape (ECL approximately two times the ECW). Narrow keels. Anterior horns moderate and thin, posterior horns moderate and sturdy	*R. polystigma*
5a	Anterior and posterior horns short	*R. miraletus*
5b	Moderate posterior horns and very long, thin and curved inwards	*L. melitensis*

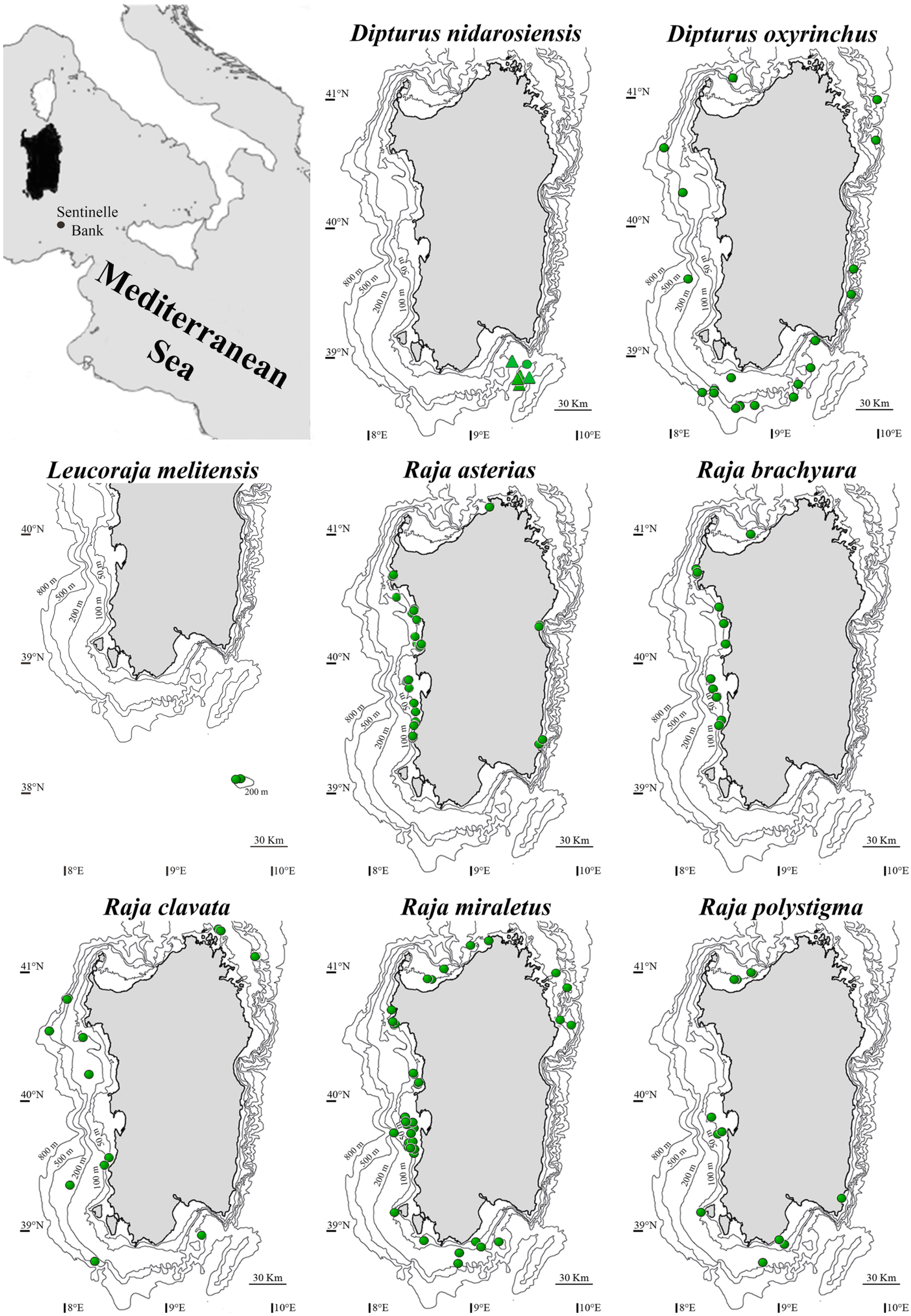

Fig. 4 Map of the study area where active females (*filled circle*) of the eight investigated species and eggcases (*filled triangle*) laid on the bottom were indicated

In addition, considering the two distinct eggcase forms described in literature for the genus Dipturus [17], on the basis of our observations, it was possible to insert *D. nidarosiensis* and *D. oxyrinchus* in the D-II phylogenetic type. The latter, matching with other Dipturus species (e.g. *D. gigas* [13], *D. trachiderma* [16], *D. batis* [17]) was characterized by well-developed aprons and lateral keel united with the main portion rectangular in shape, differently from the D–I type showing close resemblance from other genera of the family Rajidae [17].

Generally, the length of the horns shows rather large intraspecific variations with the anterior horns longer than the posteriors [12, 37, 38]. In our specific case, eggcases of *L. melitensis* presented the longest anterior horns (intertwining) with a ratio anterior/posterior horns of 2.6, followed by *R. brachyura* egg cases with a ratio of 1.6, that enable them to be fixed to various structures as seagrasses, algae or debris. In other studies, similar, or even higher horn proportions were found in other species belonging to the family Rajidae as *Atlantoraja cyclophora* and *A. platana* [18], *Leucoraja naevus* [38], *Rioraja agassizi* [39] and *Psammobatis scobina* [37].

Almost all the analyzed eggcases were covered by adhesive fibers providing their camouflage and anchoring to marine debris, plants, mud and rock and may function as a barrier to predation [40]. As a general rule, eggcases covered by fibers were produced by mixed gland tubules (mucous and serous) situated deep in the terminal zone of the oviducal gland as reported for many Rajidae species (e.g. *D. oxyrinchus*, *R. montagui*, *R. brachyura*, *R. clavata* and *R. undulata*) by Maia et al. [38] and Marongiu et al. [41]. *L. melitensis* differed from the remaining species, because its eggcases were smooth, devoid of fibres on their surface. This feature seems to be common to other species belonging to the genus Leucoraja, as *L. naevus*, marked by the mucous gland tubules secretions (suphated acid mucins) of the terminal zone that could work as an important chemical defense against predation and pathogens [42, 43].

Furthermore, *D. nidarosiensis, D. oxyrinchus* and *R. brachyura* eggcases were the most robust. This feature was certainly related to their oviducal gland microarchitecture, characterized by a higher number of lamellae of the baffle zone [38, 41], if compared with the eggcases from *R. miraletus* characterized by a lower number of lamellae [41]. From an ecological perspective, these features, probably due to the length of the developmental period, may represent an advantage against predation and water turbulence [38] considering that their big dimensions did not allow a proper camouflage.

Sandy bottoms (<100–150 m depth) were identified as egg-laying sites (i.e. sites with females bearing eggcases in *uteri*) of many species belonging to genus Raja (e.g. *R. asterias*, *R. brachyura*, *R. miraletus* and *R. polystigma*). A similar behavior was observed for *R. brachyura* females in Portugal [38] and around the British Isles [44] as well as for *R. miraletus* in the south west of India where eggcases were found on the soft sea bed at a depth range of 112–123 m [45].

With the exception of *R. polystigma*, all other species showed a clear distribution in the western part of Sardinia, which is characterized by a wide continental shelf (tens of kilometres) and a continental margin dwelling between a depth of 150 and 200 m depth, contrarily to other sides (especially the eastern side), where the shelf is narrower and steeper [46]. Regarding *R. clavata*, it presented a high diversity of egg-laying habitats with a wide bathymetric range, which may differ in terms of bottom topography or sediment composition, as reported also by other authors in the Atlantic Ocean [38, 44]. Finally, *D. oxyrinchus* active females showed a clear preference for deep muddy bottoms (mean depth of 500 m) around Sardinia, confirming their bathyal habits as reported for the same waters by Mulas et al. [47]. The occurrence of *D. nidarosiensis*, exclusively in the south eastern part, was related to the deep trawl-surveys performed only in that area.

Throughout the analyzed period (12 years), *R. asterias* and *R. miraletus* active skates were found on the same sites every year, located in the central western coast of Sardinia, confirming the theory that many rajid species demonstrate site fidelity, returning to the same depositional area or nursery ground on an annual basis as reported for *Bathyraja aleutica*, *B. interrupta* and *B. parmifera* in eastern Bearing Sea [48]. Moreover, this finding could also suggest the existence of possible nursery areas, as recently observed for oviparous species as *R. clavata* and *Galeus melastomus* [49] and *Scyliorhinus canicula* [50] around Sardinian waters.

Because of their inherent low fecundity and slow growth rates, skates may reproduce with distinct seasonal pulses, over protracted periods, or in some cases continuously throughout the year [51, 52]. In this regard, our results suggested a continuous reproduction throughout the year for the deeper *D. oxyrinchus* confirmed by the presence of spawning capable females during all seasons with a predominance of active individuals in autumn and winter months (late September to late March) [53]. A reproduction restricted mainly to summertime was described, instead, for the other species (except for *L. melitensis* for which no sufficient reproductive data was available), as also confirmed by other studies in Italian waters [54, 55]. Year-round reproduction may be a strategy to compensate the high maternal energy investment and late maturity typical to the elasmobranchs. Given the generalized high biological productivity in the summer period, a distinct pulse of egg deposition during this period could represent an advantage for those species with shallower habits in order to maximize the fitness.

Table 6 Eggcase dimensions reported in several geographical areas by latitudinal clines

Species	Eggcase length (mm)	Eggcase width (mm)	Area	References
D. nidarosiensis	182–260	95–120	Atlantic (Norwegian Sea)	[56]
	182–260	92–113	Atlantic (North Sea)	[57]
	153–177 (170.2 ± 9.8)	61.8–80.5 (74.8 ± 7.5)	Mediterranean (Sardinia)	Present study
D. oxyrinchus	133	79.5	Atlantic (British waters)	[58]
	128–133	74–101	Atlantic (British waters)	[59]
	120–235	58–120	Atlantic (North Sea)	Bor [57]
	102–126 (112 ± 10.4)	60–66 (63.8 ± 2.6)	Atlantic (British waters)	[30]
	100–150	–	Mediterranean/Atlantic	[3]
	91.6–116.2 (103.8 ± 5.3)	48.9–67.5 (57 ± 4.0)	Mediterranean (Sardinia)	Present study
	35–46 (38.4 ± 0.7)	17–28 (24.2 ± 0.2)	Mediterranean (Tunisia)	[23]
	140	–	Mediterranean (Naples)	[21]
L. melitensis	42.3–45 (44.1 ± 1.0)	22.4–26.0 (23.9 ± 1.4)	Mediterranean (Sentinelle Bank)	Present study
R. asterias	30–45	–	Mediterranean	[3]
	39.7–55.4 (48.3 ± 3.0)	22.4–40.5 (33.7 ± 2.6)	Mediterranean (Sardinia)	Present study
	45	–	Mediterranean (Naples)	[21]
R. brachyura	122 ± 5	68 ± 4	Atlantic (Portuguese waters)	[38]
	136	76	Atlantic (British waters)	[60]
	128.4	78.5	Atlantic (British waters)	[58]
	115–143	72–90	Atlantic (British waters)	[59]
	82–132.2 (108.6 ± 10.1)	32.7–86.4 (65.4 ± 11.7)	Atlantic (British waters)	[30]
	121	79	Atlantic (North Sea)	[61]
	115	70	Atlantic (North Sea)	[62]
	120	–	Mediterranean/Atlantic	[3]
	115–143	–	Mediterranean	[15]
	113–133 (119.3 ± 4.9)	64.7–72.2 (69.5 ± 2.3)	Mediterranean (Sardinia)	Present study
	105	–	Mediterranean (Naples)	[21]
R. clavata	65 ± 5	48 ± 5	Atlantic (Portuguese waters)	[38]
	80	50	Atlantic (France)	[63]
	63–90 (74.9)	49–68.5 (67.1)	Atlantic (British waters)	[58]
	60–90	50–70	Atlantic (British waters)	[59]
	32.7–83.3 (67.2 ± 10)	32.7–69 (37 ± 8.2)	Atlantic (British waters)	[30]
	70	50	Atlantic (North Sea)	[62]
	60–90	–	Mediterranean/Atlantic	[3]
	61–66	–	Mediterranean (France)	[22]
	62.5–67.5 (64.1 ± 1.9)	41.7–47.4 (43.8 ± 2.0)	Mediterranean (Sardinia)	Present study
	70–78 (75)	50–54 (52)	Mediterranean (Tunisia)	[64]
	60	–	Mediterranean (Naples)	[21]
R. miraletus	48–52	–	Atlantic (Senegal)	[65]
	46 ± 3	26 ± 2	Atlantic (Portuguese waters)	[38]
	<50	–	Atlantic (South Africa)	[66]
	45–52	–	Mediterranean/Atlantic	[3]
	41.8–56.5 (47.4 ± 4.0)	22.8–29.2 (24.7 ± 1.7)	Mediterranean (Sardinia)	Present study
	42–47	–	Mediterranean (Tunisia)	[67]
	59	–	Indian Ocean	[45]
R. polystigma	35–46	–	Mediterranean	[3]
	56.6–69.2 (62.3 ± 4.3)	33.9–44.3 (37.9 ± 2.9)	Mediterranean (Sardinia)	Present study

We also observed that eggcase dimensions seem to change in relation to geographical area. From a comparison with the available literature from the Mediterranean (Table 6), our samples were similar in sizes (ECL and ECW) to those observed in other areas of the basin. Instead, eggcases analysed here (excluding the endemic species) seemed to be smaller than those described in the Atlantic studies (Table 6). The only exception was represented by *R. brachyura*, showing eggcases slightly larger than the Atlantic ones, probably due to similar body dimensions between the two areas [55]. This pattern was reported also for other chondrichthyan eggcases (e.g. *G. melastomus*, [28]) and it could probably be due to the intraspecific latitudinal cline in elasmobranchs size in which Atlantic specimens reach a bigger body size than Mediterranean ones [26, 39, 68].

In conclusion, given the absence of Mediterranean eggcase descriptions, the present study should shed light on the taxonomy, distribution and reproductive habits of many Mediterranean skates. Indeed, this identification key could act as useful tool for non-invasive identification of eggcases through image analysis (i.e., ROV imaging), which have proven to be an efficient tool for identifying nursery ground and delineate ecological traits of species. In addition, since knowledge of the location of nursery grounds for elasmobranchs is practically nonexistent [50] and given the most recent policy approaches to the protection of marine ecosystems (e.g. the Marine Strategy Framework Directive, MSFD; 2008/56/EC currently in force in European seas), the identification of essential fishing habitats such as nursery grounds of sensitive species like skates represent an indispensable component to protect and manage.

Abbreviations

n: number of eggcase analyzed; TL: total length; ECL: eggcase length; ECW: eggcase width; AAL: anterior apron length; PAL: posterior apron length; LKW: left keel width; RKW: right keel width; LAHL: left anterior horn length; RAHL: right anterior horn length; LPHL: left posterior horn length; RPHL: right posterior horn length; PCA: Principal Component Analysis; SIMPER procedure: SIMilarity PERcentage Analysis; ANOSIM: Similarity Randomization Test.

Authors' contributions

CP, MFM and MCF conceived the study; CP wrote the manuscript with significant input of MFM and MCF. MFM and CP analyzed, described and measured all specimens' samples and wrote the morphometric and morphological part of the manuscript. AC performed statistical analysis; RM performed molecular analysis to genetic identification; CP, MFM, MCF, AB, AM, RC, LV and GS performed sampling and all the analyses and data handling. All authors contributed to data interpretation. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

Funding

This study was financed by Autonomous Region of Sardinia within the frame of the research project 'Approccio multidisciplinare per la conservazione e gestione della selacofauna del Mediterraneo' (LR7 CRP-25321) and carried out within the Data Collection Regulation and Framework—module trawl surveys MEDITS (Mediterranean International Trawl Surveys).

Received: 8 November 2016 Accepted: 15 June 2017
Published online: 24 June 2017

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