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COLOR PATTERNS OF THE THORNBACK SKATE, *RAJA CLAVATA* LINNAEUS, 1758, FROM THE SEA OF MARMARA SUGGESTING POSSIBLE MISIDENTIFICATIONS OF SEVERAL RAJIDS IN THE REGION

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Trawl surveys conducted in shelf waters of the northeastern Sea of Marmara revealed for the first time the occurrence of atypically colored thornback skates, *Raja clavata* Linnaeus, 1758 (Rajiformes: Rajidae), in the region. Since atypical coloring may lead to confusion and misidentification of *R. clavata*, an integrated approach of conventional alpha taxonomy and genetic studies is needed to resolve the taxonomic status of *Raja* species occurring in the Sea of Marmara. Accurate taxonomic resolution is the first step to properly differentiate the populations of the aforementioned species *prior* to performing further study and effective conservation.

Keywords: Rajiformes, polychromatism, aberrations, taxonomy, description

The skates (Chondrichthyes: Rajiformes) currently comprise nearly 304 species representing four families: Arhynchobatidae, or softnose skates; Rajidae, or hardnose skates; Anacanthobatidae, or legskates; and Gurgesiellidae, or pygmy skates [Weigmann, Reinecke, 2023]. Members of the family Rajidae which includes approximately 160 validly described species from 16 genera vary in size from small to very large (total length, TL, ranges 33 to 264 cm as adults) and occur in mainly oceans and seas, from shallow waters down to depth of 4,156 m [Last et al., 2016; Weigmann, Reinecke, 2023]. In the Mediterranean Sea, the family is represented by 4 genera (*Dipturus, Leucoraja, Raja*, and *Rostroraja*) and 16 species, one of which is the thornback skate, *Raja clavata* Linnaeus, 1758 [Barone et al., 2022]. The thornback skate is one of the first described and best-known members of the family Rajidae which is widespread in the eastern Atlantic and southwestern Indian Ocean, from Iceland to Madagascar, including the Mediterranean Sea, Sea of Marmara, and Black Sea [Last et al., 2016].

The thornback skate is distinguished from other skates in its range by the presence of strong thorny tubercles (bucklers) on both dorsal and ventral surfaces of large specimens and lateral rows of strong hooked thorns along the edge of the tail having dark and light crossbars even in large individuals [Barone et al., 2022; Last et al., 2016]. However, the coloring pattern on the dorsal surface can be remarkably variable which does not correspond to the generally accepted description

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of *R. clavata* leading to misidentifications with several other Mediterranean species, such as the spotted skate, *Raja montagui* Fowler, 1910, and the speckled skate, *Raja polystigma* Regan, 1923 [Capapé et al., 2018; Chatzispyrou et al., 2019; Mnasri et al., 2009]. Discrimination of these species which are morphologically very similar but have different life cycles [Ebert, Stehmann, 2013; Last et al., 2016] is due to an unavoidable and unmet need for accurate fisheries management and conservation efforts.

In the present article, the authors report on the occurrence of polychromatic specimens of *R. clavata* in the Sea of Marmara and possible implications of unusual color patterns of the thornback skate on accurate identification.

MATERIAL AND METHODS

During recent scientific bottom trawling surveys in the Sea of Marmara (Fig. 1), several thornback skates with atypical dorsal color patterns (Fig. 2) were caught. On 19 February, 2024, a male specimen (hereinafter referred to as RC1) was captured on a muddy-sandy bottom at depths ranging 85.2 to 87.6 m (trawl positions: start, 40°86.343'N, 29°00.997'E; end, 40°85.515'N, 29°04.568'E). Two more males (hereinafter referred to as RC2 and RC3, respectively) were caught on 22 February, 2024, on a similar bottom type at depths ranging 35 to 50 m (trawl positions: start, 40°95.250'N, 28°98.405'E; end, 40°92.498'N, 28°97.550'E). Morphometric measurements of the captured thornback skates presented as percentages of total length (TL) or disc width (DW) of the mean ± standard deviation (SD) (Table 1) were recorded following the procedure outlined by Hubbs and Ishiyama [1968]: either with a measuring type for distances ≥ 10 cm to the nearest 0.5 mm or with a Vernier caliper for distances < 10 cm to the nearest 0.05 mm. TL is the distance from the tip of the snout to the tip of the tail, and DW is the distance between the outermost tips of the pectoral fins [Barone et al., 2022]. The angle of the snout in front of the level of the spiracles was measured according to Ebert and Stehmann [2013]. Maturity stages of the examined specimens were determined in accordance with MEDITS (the international bottom trawl survey in the Mediterranean) maturity scale for oviparous elasmobranchs [Atlas, 2019]. Studied skates are preserved in a deep-freezer (-20 °C) at the Istanbul University, Faculty of Aquatic Sciences, Department of Fisheries Technology and Management laboratories without providing catalogue numbers. Raw data, photographs, and frozen specimens are available upon request for further examination.

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The thornback skates were examined within the scope of the ongoing Stock Identification of Demersal Fishes in the Eastern Sea of Marmara project; ethics committee approval granted by the Local Ethics Committee of Istanbul University Animal Experiments covers the present study as well (project ID: 2714; approval granted on 17.04.2015). Before dissection, the thornback skates were kept in well-aerated seawater tanks. Then, each thornback skate was anesthetized by keeping it in seawater with a sufficient dose of MS-222 for at least an hour, and if it did not show signs of life, the specimen was measured and dissected.

RESULTS

The following morphometric and morphological characteristics were registered in the examined specimens. The angle of the snout in front of the level of the spiracles was 114° (RC1), 112° (RC2), and 116° (RC3). Tail length ranged 52.42 to 55.68% of TL (mean was 53.74% of TL). In the specimens studied, mean interorbital length was 2.34 ± 0.28 times the prenarial length (range of 2.03 to 2.72); mean eye length was 1.25 ± 0.25 times the prenarial length (range of 0.98 to 1.59). Measured morphometric data are provided in Table 1. Buckler thorns were present only on the dorsal surface of RC1 (Fig. 3); however, the dorsal surfaces of all three specimens were entirely prickly and not smooth (Fig. 3). The number of thorns along the midline from nape to the origin of first dorsal

fin and interdorsal thorns were counted as 34/2, 32/1, and 32/2, in specimens RC1, RC2, and RC3, respectively. Widely spaced minute lateral buckler thorns were also observed along the tail, but these were not as prominent as those on large juveniles and adults. The dorsal coloring of the examined specimens was yellowish brown with numerous dark spots of various size, denser in the central parts of the disc and widely spaced at the margins; there were dark and light crossbars on the tail (Fig. 2). Dark crossbars were also observed on the dorsal surface of tails of all three specimens (Fig. 3). On the dorsal surface of RC1, two prominent dark colored eye-spots surrounded by cream-colored small spots were present along the line connecting the tips of the disc, and the eye-spots were closer to the midline extending from the snout to the tip of the tail (Fig. 2). Two small but prominent cream-colored spots irregularly surrounded by small dark spots were also present on the dorsal surfaces of specimens RC2 and RC3, which were also in line with the axis connecting the tips of the disc and were closer to the snout-tail tip axis (Fig. 2). The ventral surface was whitish.

The claspers of three specimens were short not reaching the tips of the pelvic fins, not visible from the dorsal view (Fig. 2), and very soft. Therefore, they were considered immature or at stage 1.

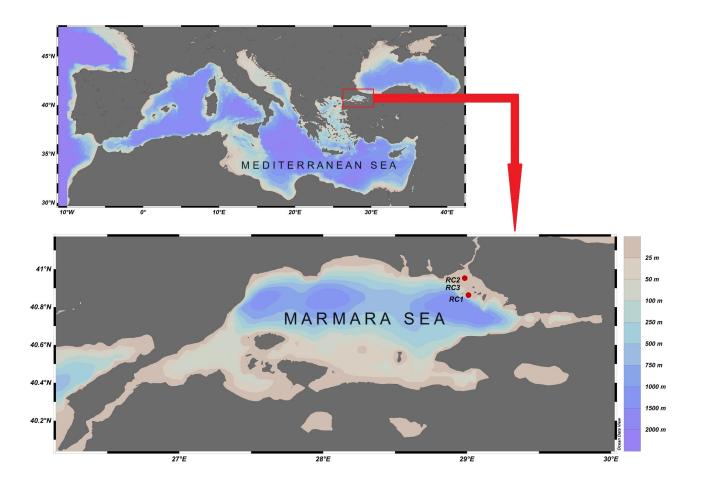


Fig. 1. Small map on the top panel depicts the locality (red rectangle) of the Sea of Marmara in the Mediterranean ecosystem. On the lower panel, approximate localities (red circles) of captures of the examined thornback skates are depicted

Рис. 1. Маленькая карта на верхней панели показывает местоположение (красный прямоугольник) Мраморного моря в средиземноморской экосистеме. На нижней панели отмечены примерные места (красные круги) поимки исследованных морских лисиц

Table 1. Morphometric measurements and meristic counts of the examined *Raja clavata* specimens (N/A denotes missing measurement due to dorsal fin and tail tip aberrations)

Таблица 1. Морфометрические измерения и меристические подсчёты исследованных экземпляров
<i>Raja clavata</i> (N/A — отсутствие измерения из-за аберраций спинного плавника и кончика хвоста)

	RC1, ♂	RC2, ♂	RC3, ♂	RC1	RC2	RC3	07 of TI	±SD
Measurement, mm	TL	TL	TL	DW	DW	DW	% of TL of the	of the
wiedsureinent, mm	480 mm	308.9 mm	307.1 mm	310 mm	190 mm	210 mm	mean	mean %
		% of TL			% of DW		mean	of TL
Total length	100	100	100	154.84	162.58	146.24	100	0
Disc width	64.58	61.51	68.38	100.00	100.00	100.00	64.82	2.81
Disc length	47.29	48.24	54.05	73.23	78.42	79.05	49.86	2.99
Trunk length	42.08	43.38	46.08	65.16	70.53	67.38	43.85	1.66
Precaudal length	93.75	51.47	N/A	145.16	83.68	N/A	48.41	38.33
Tail length	53.33	55.68	52.43	82.58	90.53	76.67	53.81	1.37
Predorsal-tail length	33.54	3.12	N/A	51.94	5.08	N/A	12.22	15.13
D1 origin to tail tip	19.78	16.75	N/A	30.63	27.24	N/A	12.18	8.7
D1 basal length	5.39	5.55	5.6	8.34	9.03	8.19	5.51	0.09
D1 height	3.31	2.87	3.45	5.13	4.66	5.05	3.21	0.25
D2 basal length	5.88	5.28	3.94	9.1	8.58	5.76	5.03	0.81
D2 height	2.51	2.95	1.79	3.89	4.79	2.62	2.42	0.48
Distance D1/D2	2.46	2.99	2.74	3.81	4.87	4.00	2.73	0.22
Postdorsal length	1.39	3.82	N/A	2.15	6.21	N/A	1.74	1.58
Head length	26.75	28.63	29.18	41.42	46.55	42.67	28.19	1.04
Preocular length	12.41	12.79	14.65	19.21	20.79	21.43	13.28	0.98
Preoral length	10.61	12.35	13.90	16.44	20.08	20.33	12.29	1.34
Prenarial length	9.41	10.84	11.41	14.56	17.63	16.69	10.55	0.84
Internarial length	7.45	6.38	6.51	11.53	10.37	9.52	6.78	0.48
Nasal-curtain length	4.74	4.13	4.33	7.34	6.71	6.33	4.4	0.25
Nasal-curtain width	3.50	3.93	5.06	5.42	6.39	7.40	4.17	0.66
Mouth width	9.56	10.13	10.86	14.81	16.47	15.88	10.18	0.53
Eyeball length	2.91	4.06	4.25	4.5	6.61	6.21	3.74	0.59
Interorbital width	4.63	3.98	5.03	7.16	6.47	7.36	4.55	0.43
Spiracle length	3.43	2.56	2.96	5.31	4.16	4.33	2.98	0.36
Interspiracular width	6.05	6.98	7.57	9.37	11.34	11.07	6.87	0.62
Orbit + spiracle	5.02	5.97	5.88	7.77	9.71	8.6	5.62	0.43
1 st gill slit length	1.55	1.96	1.81	2.4	3.18	2.64	1.77	0.17
3 rd gill slit length	1.75	1.78	1.99	2.71	2.89	2.90	1.84	0.1
5 th gill slit length	1.72	1.89	1.82	2.66	3.08	2.67	1.81	0.07
1 st interbranchial width	15.73	14.96	15.65	24.35	24.32	22.88	15.44	0.35
3 rd interbranchial width	13.10	12.72	13.04	20.29	20.68	19.07	12.96	0.17
5 th interbranchial width	8.41	9.57	9.65	13.02	15.55	14.12	9.21	0.57
Eye-spot length	4.40	1.93	2.31	6.81	3.13	3.38	2.88	1.08
Eye-spot width	4.81	2.12	1.99	7.45	3.45	2.9	2.97	1.3
Between eye-spots	18.43	12.43	14.1	28.53	20.21	20.62	14.99	2.53
Clasper length	5.21	3.56	5.42	8.06	5.79	7.93	4.73	0.83
Clasper width	1.09	0.86	1.12	1.69	1.39	1.64	1.03	0.12
Weight, g	550	139.5	153.9		1	1	1	
Number of thorns in midline	34	32	32					
Number of interdorsal thorns	2	1	2					
Number of orbital thorns	2	2	3					

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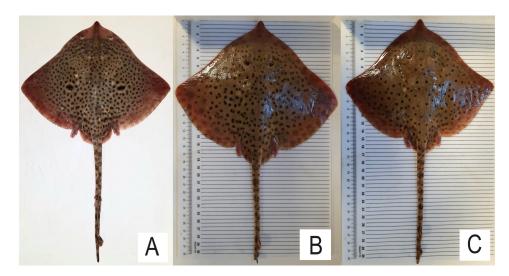


Fig. 2. Examined specimens of *Raja clavata*; A, specimen RC1 (TL 480 mm); B, RC2 (TL 308.9 mm); C, RC3 (TL 307.1 mm)

Рис. 2. Исследованные экземпляры *Raja clavata*; А — экземпляр RC1 (TL 480 мм); В — RC2 (TL 308,9 мм); С — RC3 (TL 307,1 мм)

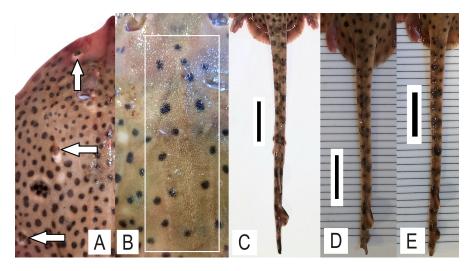


Fig. 3. Squamation and color patterns observed on the examined specimens of *Raja clavata*. A, arrows depict the buckler thorns of specimen RC1; B, rectangle depicts the prickles on dorsal disc of RC2; C, D, and E, dark crossbars on dorsal tails of RC1, RC2, and RC3, respectively. Scale bars are 30 mm

Рис. 3. Чешуйчатость и цветовые узоры исследованных экземпляров *Raja clavata*. А — стрелками обозначены шипы щитка экземпляра RC1; В — прямоугольником выделены шипы на дорсальном диске RC2; С, D и Е — тёмные перекладины на дорсальных хвостах RC1, RC2 и RC3 соответственно. Масштабные линейки — 30 мм

DISCUSSION

Ebert and Stehmann [2013] define *R. clavata* as a "chameleon" among its congeners, mainly due to its dorsal ground color and remarkable variety of patterns. The ground color of this species ranges from brown to grey in light to dark shades, variegated or marbled with dark and light spots or blotches, and it may show a pattern, such as eye-spots; however, single-colored thornback skates have also been recorded [Ebert, Stehmann, 2013; Last et al., 2016]. Based on dorsal coloration and patterns, Mnasri *et al.* [2009] defined 7 types of polychromatism in *R. clavata* specimens caught off the Tunisian coast (the central Mediterranean Sea). Furthermore, 5 out of these 7 types of polychromatism have also been described in thornback skates captured in the eastern Ionian Sea [Chatzispyrou et al., 2019].

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Recently, Capapé *et al.* [2018] reported one of the atypical coloring patterns (type 7, vermiculated, *sensu* [Mnasri et al., 2009]) on the dorsal surface of the thornback skate captured off Izmir coast (the eastern Aegean Sea). Although the dilemma of polychromatism or atypical coloring which makes it difficult to distinguish *R. clavata* from *R. montagui*, *R. maderensis*, or *R. polystigma* [Capapé et al., 2018; Chatzispyrou et al., 2019; Mnasri et al., 2009] has been very well documented for the Mediterranean Sea, the present study is the first to report atypically colored thornback skates from the Sea of Marmara.

The types of polychromatism observed in the examined specimens are consistent with type 2 (ocellated) and type 4 (spotted) described by Mnasri *et al.* [2009]. At first sight, the ocellated dorsal pattern of specimen RC1 could be confused with that of *R. polystigma* which has not been recorded in the Sea of Marmara. Furthermore, in contrast to the smooth dorsal surface of *R. polystigma* [Last et al., 2016], the dorsal surface of RC1 was completely prickly, and very few large buckler thorns were also present. On the other hand, the spotted dorsal pattern of specimens RC2 and RC3 resembled the dorsal coloration of *R. montagui*, and this species has been reported from the Sea of Marmara by Bilecenoğlu *et al.* [2014]. Nevertheless, dorsal surfaces of RC2 and RC3 were observed to be completely prickly, and those are completely smooth in young specimens of *R. montagui*. Therefore, one of the main characteristics that allows distinguishing atypically colored *R. clavata* from its congeners *R. montagui* and *R. polystigma* is the presence (*R. clavata*) or absence (*R. montagui* and *R. polystigma*) of prickles.

According to Last et al. [2016], the maximum TLs of R. clavata, R. montagui, and R. polystigma are ~130, ~80, and ~71 cm, respectively. Furthermore, males of R. clavata, R. montagui, and R. polystigma reach maturity at 60-77-cm TL, ~40-cm TL, and ~53-cm TL, respectively. As for TL of the examined skates which ranged 307.1 to 480 cm (Table 1), these are clearly juvenile specimens with respect to the above sizes at which males of R. clavata and R. polystigma reach maturity. However, as males of R. montagui reach maturity at around 40-cm TL, the observed TLs of the studied specimens suggest that they are mature or subadult R. montagui specimens. According to Last et al. [2016], one of the key descriptive characteristics of R. montagui and R. polystigma is the dorsal disc of these two species which is largely smooth due to the absence of prickles. However, the dorsal discs of the examined specimens were completely prickly and not smooth (Fig. 3). Furthermore, the presence of dark crossbars on the dorsal surface of the tails of studied skates which is another important descriptive characteristic of the thornback skate [Last et al., 2016] also confirms that the examined specimens are R. clavata. Although the presence of large buckler thorns is considered as an essential characteristic for positive identification of *R. clavata* [Barone et al., 2022; Last et al., 2016], they are confined to the snout of juvenile and adult males [Last et al., 2016]; those are seen on the snout area of specimen RC1. Moreover, occasional specimens without thorns but with a spinulose dorsal surface have been reported as well [Ebert, Stehmann, 2013]; those are seen on dorsal discs of specimens RC2 and RC3. Therefore, TLs of the studied specimens and the observed squamation patterns were consistent with those reported for R. clavata juveniles [Ebert, Stehmann, 2013; Last et al., 2016].

Ratios of interorbital length to prenarial length (IL/PL) and eye length to prenarial length (EL/PL) can also distinguish *R. clavata* (IL/PL ~2 and EL/PL ~1.4) from its congeners *R. montagui* and *R. polystigma* [Last et al., 2016]. Although the mentioned ratios of the examined thornback skates (IL/PL ~2.3 and EL/PL ~1.25) separate them from the ratios of *R. polystigma* (IL/PL ~2 and EL/PL ~2.1 [Last et al., 2016]), they were found to be closer to the ratios of *R. montagui* (IL/PL ~2.5 and EL/PL ~1.2 [Last et al., 2016]) than *R. polystigma*. However, the presence of buckler thorns (RC1) and fully prickly dorsal surfaces (RC2 and RC3) allowed the studied specimens to be positively identified as *R. clavata* rather than *R. montagui* or *R. polystigma*.

Although *R. montagui* has been previously registered in the Sea of Marmara and in the Black Sea (only 1 record; the specimen caught at 41°10′N, 39°36′E) [Bilecenoğlu et al., 2014; Turan, 2008], this species did not occur in the following years in the mentioned marine areas. Therefore,

there is a reasonable uncertainty that: 1) R. montagui was noted as a result of the vagrant occurrence of the species in the Sea of Marmara and Black Sea and, accordingly, was not found repeatedly in the following years; or 2) atypically colored R. clavata individuals could have been misidentified as R. montagui. Although the record of *R. montagui* in the Black Sea is based on genetic material (GenBank accession No. EU476889 [Turan, 2008]), the fact that it has not been registered in the region since 2008 [Karadurmus, Sari, 2024] strengthens the possibility of vagrant occurrence or misidentification. According to Bilecenoğlu et al. [2014], 13 species of the family Rajidae have been described in Turkish marine waters to date, and 7 out of 13 rajids also occur in the Sea of Marmara. Due to the above-mentioned taxonomic confusion in congeneric Raja species, taxonomic issues of the rajids occurring in the Sea of Marmara need to be resolved before conducting future studies on their populations in the region. As highlighted by Pyšek et al. [2013], conventional alpha taxonomy integrated with contemporary genetic taxonomic procedures will certainly improve the accuracy of species identification and further refine the taxonomic classification at the population level of rajids occurring in the Sea of Marmara. Combining the observations of Capapé et al. [2018] who reported on the occurrence of vermiculated pattern on the thornback skate caught off Izmir coast (the eastern Aegean Sea) with our observations, it is clear that types 2, 4, and 7 of atypically colored R. clavata (sensu [Mnasri et al., 2009]) occur in Turkish marine waters as well. Atypical coloring in Raja species can also be exhibited by several other types [Gajić et al., 2023; Quigley et al., 2018], such as the single unilateral ocellus noted in R. miraletus or the xanthochromism observed in R. montagui which makes it difficult to correctly identify the species.

Conclusion. Atypical coloring may lead to confusion and misidentification of *Raja clavata*. Therefore, an integrated approach of conventional alpha taxonomy and genetic investigation is needed to resolve the taxonomic status of *Raja* species occurring in the Sea of Marmara. Accurate taxonomic resolution is the first step to properly differentiate populations of the aforementioned species before conducting further studies and effective conservation of them. The Sea of Marmara is considered as an ecological gateway from the Mediterranean to the Black Sea ecosystem; accordingly, accurate identification of the fish fauna of the Sea of Marmara is a clear requirement for a better understanding of the northerly distribution of fish species. Taxonomic issues of the rajids occurring in the Sea of Marmara need to be resolved before conducting studies on their populations in the region.

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ЦВЕТОВОЙ УЗОР МОРСКОЙ ЛИСИЦЫ *RAJA CLAVATA* LINNAEUS, 1758 ИЗ МРАМОРНОГО МОРЯ, ИЗ-ЗА КОТОРОГО ВОЗМОЖНЫ ОШИБКИ ИДЕНТИФИКАЦИИ НЕСКОЛЬКИХ СКАТОВ В РЕГИОНЕ

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Траловые исследования в шельфовых водах северо-восточной части Мраморного моря впервые выявили в этом регионе нетипично окрашенных скатов — морскую лисицу *Raja clavata* Linnaeus, 1758 (Rajiformes: Rajidae). Поскольку нетипичная окраска может привести к путанице и неправильной идентификации *R. clavata*, необходим комплексный подход, сочетающий методы традиционной таксономии и генетических исследований. Точное таксономическое определение скатов рода *Raja* — первый шаг к корректной дифференциации их популяций перед их изучением и разработкой мер эффективной охраны.

Ключевые слова: Rajiformes, полихроматизм, аберрации, таксономия, описание