







Communication

Basic Intersexuality (Abnormal Hermaphroditism) in the Blackmouth Catshark, *Galeus melastomus*, (Rafinesque, 1810), from the Southern Tyrrhenian Sea (Central Mediterranean Sea)

Claudio D'Iglio ^{1,2,†}, Marco Albano ^{1,†}, Sergio Famulari ¹, Nunziacarla Spanò ^{2,3}, Paola Rinelli ²,
Serena Savoca ^{2,3,*} and Gioele Capillo ^{2,4}

- ¹ Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale F. Stagno d'Alcontres 31, 98166 Messina, Italy; cladiglio@unime.it (C.D.); malbano@unime.it (M.A.); serfamulari@unime.it (S.F.)
- ² Institute for Marine Biological Resources and Biotechnology (IRBIM), National Research Council (CNR), Section of Messina, Spianata San Raineri 86, 98122 Messina, Italy; spano@unime.it (N.S.); paola.rinelli@irbim.cnr.it (P.R.)
- ³ Department of Biomedical, Dental and Morphological and Functional Imaging, University of Messina, Via Consolare Valeria 1, 98125 Messina, Italy
- ⁴ Department of Veterinary Sciences, University of Messina, 98168 Messina, Italy; gcapillo@unime.it
- * Correspondence: ssavoca@unime.it
- † These authors contributed equally to this work.



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Abstract: The present paper represents the first description of abnormal hermaphroditism in *Galeus melastomus* (Rafinesque, 1810). The black mouth cat shark specimen, collected in summer from southern Tyrrhenian Sea at the entrance of Salerno gulf, showed a basic intersexuality characterized by the presence of male secondary and female primary sexual characters. The reproductive system has been described with an accurate morphological analysis, also including a histological assessment of oocytes. Results showed the presence of only right clasper, not exceeding the pelvic fin in length, with the absence of internal males' sexual organs. Concerning the female primary characters, the analysis of histological assessment showed the structure of mature oocyte, confirming the maturity stage of a mature female in active extruding stage, as highlighted by the macroscopic morphological analysis. Despite in many marine organisms' hermaphroditism is a physiological condition showed by specimens during their lifecycle, in Elasmobranchs this is considered an abnormal condition, with some exceptions. The lack of information on hermaphroditism in elasmobranchs from the Mediterranean Sea requires an increase of scientific community's attention, improving the knowledge on the reproductive biology of this sensitive taxon. This is essential to enhance the conservation of elasmobranchs populations in the entire Mediterranean basin.

Keywords: elasmobranch; morphological abnormalities; sexual dimorphism; life cycle; reproductive system

1. Introduction

The congenital morphological variations are widely reported in elasmobranchs worldwide. According to literature, these abnormalities mainly involve the skeleton morphology [1–7], the reproductive system [8–14], and the skin color [15–19]. The most severe morphological malformations are those concerning the skeleton (e.g., deformities in skeleton structure, missing or additional fins, anomalies on cephalic horns, alterations in the cranium), which are mainly observed in embryos, being often fatal beyond birth, as confirmed by the relatively few observations on free swimming individuals [20]. The alterations on skin color (e.g., albinism, leucism and irregular skin pigmentation) or on reproductive system (e.g., abnormal hermaphroditism and anomalies on claspers structure) are considered less severe, having been widely observed in several free-swimming adult specimens [20].

According to previous literature [8], hermaphroditism is defined as the presence in the same individual of male and female primary or secondary sexual characters. Despite in many taxa (teleost fishes included) hermaphroditism is a physiological condition shared by individuals of a species during their ontogeny [21–24]. In Chondrichthyans this condition is usually defined as “abnormal”, with few exceptions, such as records of normal hermaphroditism in *Apristurus longicephalus*, Nakaya, 1975, *Notorynchus cepedianus*, Péron, 1807, *Hexanchus griseus*, Bonnaterre, 1788, *Etmopterus granulosus*, Günther, 1880, *Centroscyminus coelolepis*, Barbosa du Bocage & de Brito Capello, 1864, *Heterodontus portusjacksoni*, Meyer, 1793, *Prionace glauca*, Linnaeus, 1758, *Scyliorhinus canicula*, Linnaeus, 1758, and *Scyliorhinus stellaris*, Linnaeus, 1758 [25–32]. Difference between normal and abnormal hermaphroditism lies in the uniform presence of both male and female functional gonads in many of all individuals of a species at a given point of their lifetime [33,34]. In abnormal hermaphroditism, also called intersexes, the presence of both sexes characters is not uniform in the individuals of a species and one or both sexes are unfunctional, despite the presence of primary and/or secondary sexual morphological features of either.

Even if hermaphroditism is considered an adaptative way to increase the reproductive yield in teleost fishes [33], intersexuality in Chondrichthyans has usually been considered as an “un-adaptative characteristic” [8]. Indeed, all the cartilaginous fishes are considered gonochoristic species, i.e., the individuals’ sex is genetically determined, with very evident secondary sexual characters, without sexual inversions during their entire life cycle and with a wide range of reproductive strategies (oviparity, aplacental and placental viviparity) [35,36]. Despite its un-functionality, according to literature, hermaphroditism was recorded worldwide in many families of sharks, such as Triakidae, Squalidae, Somniosidae, Carcharhinidae, Etmopteridae, Centrophoridae, Heterodontidae, Hexanchidae and Scyliorhinidae [14,27,29,31,32,34,37–39].

Concerning the Pentanchidae family, no reports are available from literature regarding intersexuality. The blackmouth catshark, *Galeus melastomus*, (Rafinesque, 1810), is the most common species in Mediterranean Sea belonging to this family. It is ubiquitous in the entire basin [40,41], and it is also widely reported on the Eastern Coast of the Atlantic Ocean [42,43]. It is a demersal generalist opportunist meso-predator [44–46] which inhabits the benthic environments with a wide bathymetric range (from 55 to 1400 m) [47]. In its entire distribution area, it is mainly found at a depth range from 300 to 800 m, while in the Tyrrhenian Sea it is mainly distributed from 500 to 800 m [41,48]. It is among the most common Elasmobranch species reported in trawl fisheries by-catch [49,50], but thanks to its bathymetric distribution (extending in depth less exploited by trawling) and to its peculiar life cycle, common among Pentanchidae and Scyliorhinidae species, populations of *G. melastomus* have maintained their structure despite the high fishing efforts all over the Mediterranean Sea [51–53]. *G. melastomus* is an oviparous species, with a continuous reproductive cycle in almost all the distribution areas and a lifecycle characterized by an early sexual maturation, a shorter generation time and a faster population increment than other Chondrichthyans families.

The aim of this study was to report the first case of abnormal hermaphroditism in *G. melastomus* from the Southern Tyrrhenian Sea (Central Mediterranean Sea), with an accurate morphological description of the primary and secondary sexual characters.

2. Materials and Methods

Specimens of *G. melastomus* were regularly collected using trawl nets during the annual scientific trawl-survey MEDITS (International Trawl Survey in the Mediterranean) [54,55]. The blackmouth catshark pool, to which the hermaphrodite specimen belonged, came from the southern Tyrrhenian Sea (Geographical Subarea, GSA-10) (Figure 1a,b). The GSA-10 extends from the coastal area of Campania (41°14'38.7" N 13°37'04.2" E) to San Vito lo Capo (38°11'41.0" N 12°44'29.8" E), including the entire Sicilian north coast [56].

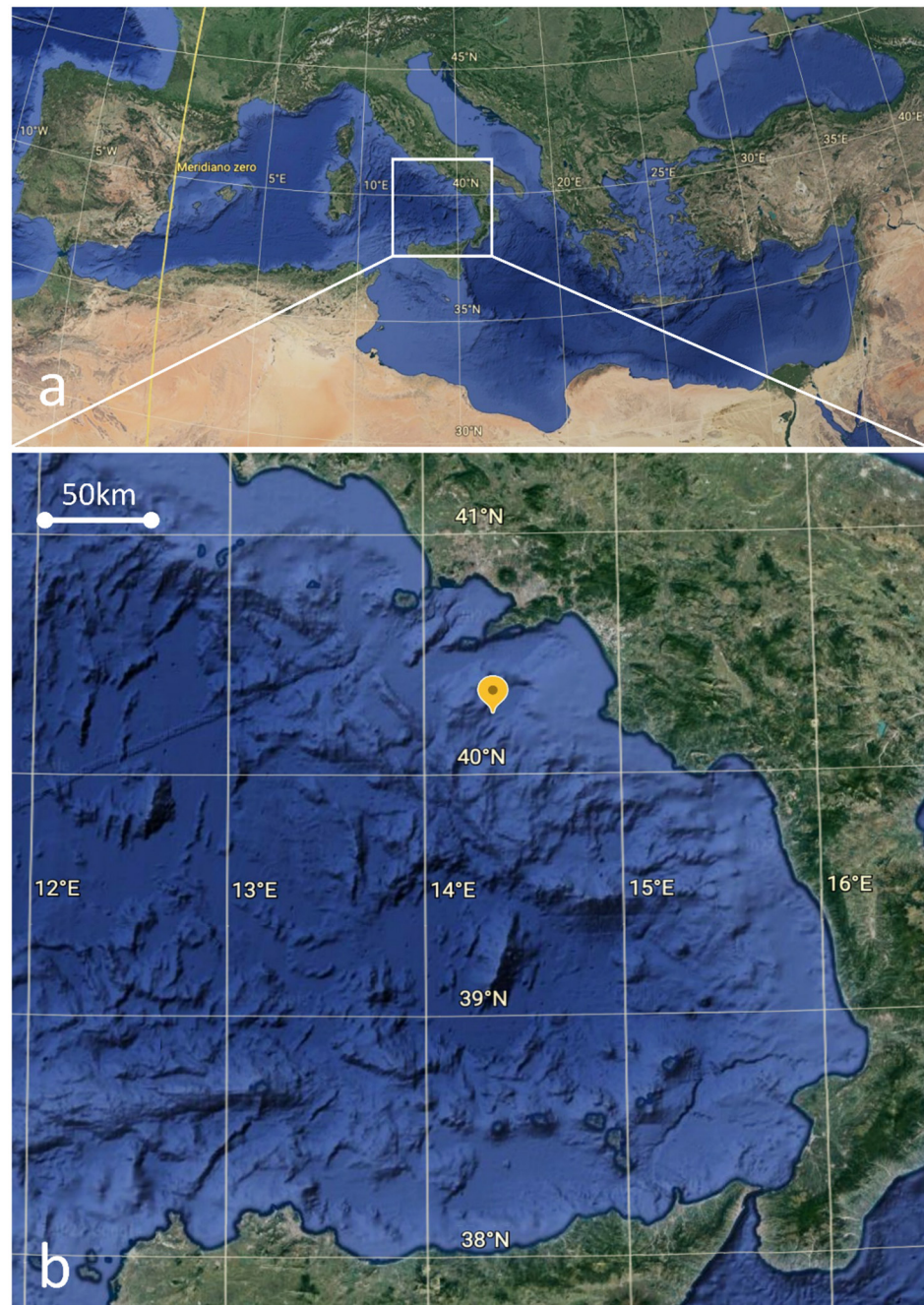


Figure 1. General view of the Mediterranean basin (a), with a particular of the studied area (GSA 10) (b). The yellow mark indicates the sampling point with coordinates ($40^{\circ}24.087''$ N $14^{\circ}35.396''$ E).

Samples collected during autumn 2021 at 316 m of depth were frozen on board to preserve the integrity of biological tissues and were immediately transferred to the laboratory after landing. During the gross necropsy performed in laboratory, each specimen was measured (total length in mm) weighted (weight in g) and sex maturation degree was determined according to Follesa, and Carbonara [57]. Concerning the hermaphrodite specimen, the maturation degree of both expressed sexes was evaluated. Measurements of the reproductive system were also performed: the Inner clasper length (i.e., distance in mm between the distal clasper tip to the insertion point at the cloaca), the Outer clasper length (distance in mm between the clasper tip and the outside insertion point in the pelvic fin), the Uteri (left and right) length (mm), maximum oviducal gland (left and right) diameter (mm) and weight (g), length (mm), width (mm) and weight (g) of ovaries and oocytes

diameter (mm). Measurements were taken with an analog caliper (0.01 mm precision) and weight with an analytical balance (accuracy of 0.01 g).

Histological Assessment

Presumed mature eggs were immediately fixed 4% paraformaldehyde in phosphate buffered saline (PBS) 0.1 mol/L (pH 7.4) for 4 h, dehydrated in graded ethanol, cleared in xylene, embedded in Paraplast[®] (McCormick Scientific, St. Louis, MO, USA) and cut into 5 µm serial sections. The sections were stained with Masson trichrome, and AB pH 2.5 staining followed by PAS (AB/PAS) [58]. Sections were examined under a Leica DM6B microscope equipped with a Leica DFC7000T.

3. Results

Galeus melastomus hermaphrodite specimen was caught in September 2021 at the entrance of Salerno Gulf (40°24.087'' N 14°35.396'' E) (Figure 1b), in a total catch of 15 specimens, ranging in size from 101 to 440 mm. Specimen's total length and weight were 440 mm and 260 g, respectively (Figure 2a). It showed only the right clasper, which did not exceed the pelvic fin in length (Figure 2b), characterized by a skeleton still flexible, an Inner length of 22.5 mm and an Outer length of 11.6 mm. According to literature [57], the maturation stage of the secondary male sexual character, represented in the studied individual by the single clasper, (in males elasmobranchs claspers normally are two and they are peculiar appendices on the pelvic fins, used during the copulation to transfer sperms inside females' reproductive system), was attributable to a maturing specimen (Stage 2: presence of robust but still flexible claspers, as long or longer than pelvic fins). Moreover, males' primary sexual characters (e.g., epididymis, testis, spermiducts) were totally absent.

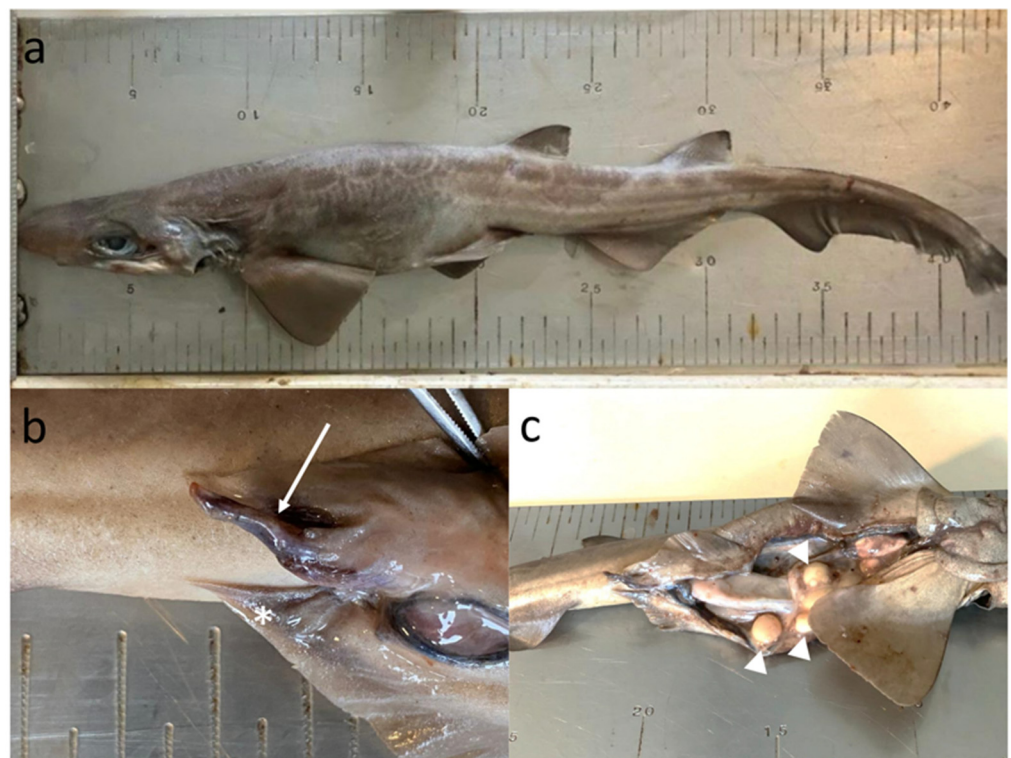


Figure 2. External lateral view (a) of the *Galeus melastomus* specimen caught in southern Tyrrhenian Sea, with a particular view of secondary male sexual organs (b) and primary female sexual organs (c). Arrow indicates the external right clasper; asterisk indicates the location of the absent right clasper and arrow heads indicate the large yellow oocytes.

Concerning the females' sexual characters, according to Follesa and Carbonara [57] the maturation stage was of a mature female in active extruding stage (Stage 3b). As showed in Figure 2c, the ovary was transparent with a length of 55 mm and a width of 33 mm. Inside the ovary, the 12 oocytes detected were yellow and different in size (with a diameter from 3 to 17 mm and a weight from 0.13 to 0.46 g). The oviducts, 120 mm in length, showed the presence of large oviducal glands (with a length of 25 and 32.8 mm, and a weight of 0.52 and 1.18 g, respectively) in the second portions and eggs well formed, ready for the emission, in the last portion nearest the cloaca.

The analysis of histological assessment showed the structure of mature oocyte with four different layers from inside to outside as follow: 1-pellucid zone, 2-granular layer, 3-basal surface, 4-theca cells (Figure 3a,b).

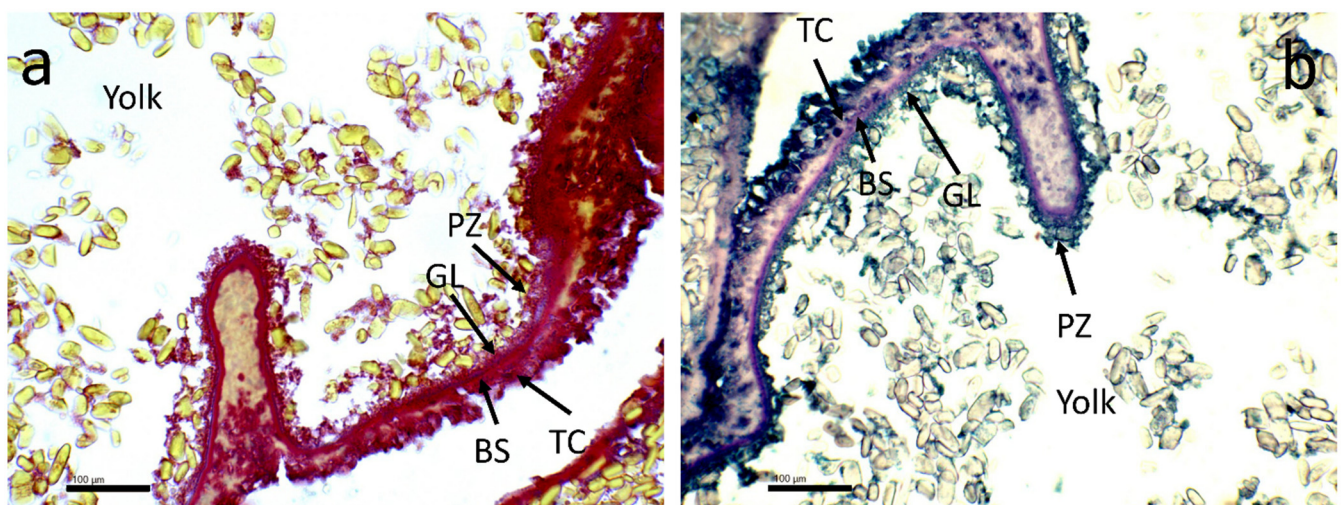


Figure 3. (a,b) Representative figures of mature oocyte showing four different layers: PZ = pellucid 149 zone, GL = granular layer, BS = basal surface, TC = theca cells, highlighted using Masson trichrome (a) and AB/PAS (b). Scale bars: 100 mm.

4. Discussion

According to the terminology present in [59] and the classification introduced by [34] the elasmobranchs intersexuality can be categorized as follow: (i) basic intersexuality, (ii) incomplete intersexuality and (iii) complete intersexuality. The basic intersexuality is the most reported among Elasmobranchs, resulting in the presence of external claspers and female gonads internally [11,13,39,60,61] or no clasper externally and internal male gonads occurrence [62,63]. Incomplete intersexuality is reported as the category of intersexuality showing the most frequent association of both sexual characters (e.g., the contemporary presence of ovaries, oviduct, testes and claspers; the presence of clasper and testis in gravid females; the presence of both male and female tissue in a gonad, called “ovotestis”; the presence in the mesovarium of a rudimentary testis in females individuals; the absence of claspers with the contemporary presence of ovary, oviduct and a testis) [64–70]. Finally, in the complete intersexuality, the individuals exhibit both sexes with the most developmental degree and a very frequent improper maturation/development resulting in a no functionality of one or both sexes (e.g., a peritoneal cavity exhibiting both male and female tract in left and right sides; presence of ovotestes; peritoneal cavity exhibiting both male gonad and tract, and female gonad and tract) [29,31,32,37,71].

The specimen of *G. melastomus* analyzed in present paper showed a basic intersexuality, characterized externally by the presence of the left clasper, while internally by the presence of a well-developed female reproductive tract and the totally absence of any male reproductive organs, such as epididymis or testis. The female reproductive tract was fully functional, also showing the presence of mature big and yellow oocytes and well-formed

eggs ready to be deposited. Also, histological analyses highlighted the maturity of oocytes, with a well-developed follicular epithelium differentiated by four layers [72,73].

On our best knowledge, this represents the first description of hermaphroditism in *G. melastomus*. According to literature, there were other reports of abnormalities only at morphological level in this species. Previous literature [74] have reported a male specimen from the Algerian coast with an abnormal morphology of claspers and pelvic fins (the right pelvic fin smaller than the left, with an inconspicuous right clasper, smaller than the left one). While in 2020 [18] it was reported the first case of a *G. melastomus* specimen without skin-related structures.

Concerning other elasmobranchs species, several cases of basic intersexuality have been recorded worldwide [34, and references therein]. In the Mediterranean Sea basic intersexuality has been reported in a specimen of *Squalus blainville*, Risso, 1827, from eastern basin [62], in *Torpedo torpedo*, Linnaeus, 1758, [68] and in Rayformes species, such as *Raja asterias*, Delaroche, 1809, [75]. Despite the high occurrence of reported hermaphroditism cases in *S. canicula*, from the Atlantic Ocean [37,76–78], and its high distribution in the Mediterranean basin, only one case has been recorded in Mediterranean population, with no occurrence of basic intersexuality [31]. According to literature reported below, the highest occurrence of hermaphrodites *S. canicula* in the Atlantic Ocean was recorded in the English Channel, during the first decades of 1900; while, regarding the Mediterranean Sea, the only reported case of hermaphroditism in this species was recorded in Tunisia. Further analysis and monitoring are required to study the occurrence of hermaphroditism in Scyliorhinidae family, especially in Mediterranean Sea.

5. Conclusions

Although the constant monitoring of Mediterranean Sea fisheries and stocks, and the growing interest on elasmobranchs biology and conservation, the lack of knowledge about the occurrence of hermaphroditism in this taxon especially in the Mediterranean basin, requires an increase in effort and attention of the entire marine scientific community. Increase the knowledge about intersexuality in cartilaginous fish is essential to fully understand their population dynamics, biology, and life history traits. These are essential information to improve their conservation through better fisheries management policies. Moreover, focusing the attention on these peculiar cases, deepening the knowledge with accurate histological assessments and timely reports and descriptions, can clarify the possible causes and ecological dynamics underlying them, improving monitoring and evaluation about their condition in a heavily exploited environments such as the Mediterranean Sea. Further analysis on elasmobranchs populations and stocks are required to understand and monitoring the occurrence of hermaphroditism and other “abnormalities” or modification on the reproductive biology in this sensitive and ecologically essential taxon.

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Conflicts of Interest: The authors declare no conflict of interest.

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