

Diversity of the egg tertiary membranes in some elasmobranch species and comments on the actual yolk-sac viviparous reproductive mode

Diversidad de las membranas terciarias en algunas especies de elasmobranquios y comentarios sobre el modo reproductivo vivíparo saco-vitelino actual

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Resumen. - Después de la fertilización, los huevos de los elasmobranquios (mayormente megalecitos) son encapsulados en una membrana terciaria, de diferente complejidad, en la glándula oviductal. Las características macroscópicas morfológicas de esta membrana son altamente variables, y su desarrollo más sofisticado es encontrado en las especies ovíparas. El objetivo de este trabajo fue realizar una contribución al conocimiento de las membranas terciarias en los elasmobranquios, tanto en las especies ovíparas como en las vivíparas, y discutir la relación con la protección de los embriones durante su desarrollo. Además de eso, las limitaciones de la viviparidad sacovitelina como una única categoría de modo reproductivo son comentadas, y se sugiere una reorganización de los taxa dentro de este modo y nueva terminología. Los datos sobre membranas terciarias en elasmobranquios provienen de un gran número de fuentes, desde 1977 hasta el presente. Los especímenes fueron capturados en cruceros de pesca comercial o de investigación, realizados en las siguientes grandes áreas geográficas: Atlántico Sudoriental, Atlántico Sudoccidental, Pacífico Sudoccidental, y varias áreas del Mar Mediterráneo. En total, fueron registradas membranas terciarias para diez especies de tiburones pertenecientes a cinco ordenes; Hexanchiformes, Squatiniformes, Heterodontiformes, Lamniformes, Carcharhiniformes, y ocho especies de batoides de cuatro ordenes; Rajiformes, Rhinoprístiformes, Torpediniformes y Myliobatiformes. Los modos reproductivos reportados para todas estas especies fueron lecitotróficos (viviparidad sacovitelina, oviparidad corta y simple) y matrotócos (oofagia, placentotrofia e histotrofia limitada y lipídica). Seis patrones principales de estructura y morfología fueron detectados en las membranas terciarias de los vivíparos sacovitelinos (tipo I-VI): una membrana fina y transparente, sin forma definida; velas; cápsulas ovígeras semejantes a la de los elasmobranquios ovíparos y casos en que no había ningún tipo de membrana terciaria.

Palabras clave: Cápsula ovígera, embriología, tiburones y rayas, lecitotrofia

Abstract. - After fertilization, elasmobranch eggs (mostly megalecithal) are encapsulated in a tertiary membrane of different complexity, in the oviductal gland. The gross morphological characteristics of this membrane in elasmobranchs are highly variable, attaining it most sophisticated forms within the oviparous species. The purpose of this study was to contribute to the knowledge of the elasmobranch tertiary membranes, in both viviparous and oviparous species, and to discuss the relation with their role in protecting the embryos their development. In addition, the limitations of yolk-sac viviparity as a single reproductive mode category are commented, suggesting a new arrangement of taxa within that reproductive mode and new terminology. Data on tertiary membranes in elasmobranchs come from a large number of sources ranging from 1977 to date. Specimens were captured on board of commercial and scientific research cruises, from the following some major geographic regions: South Western Atlantic, South Western Pacific, and different areas of the Mediterranean Sea. In all, our records of tertiary membranes belong to ten species of sharks of five orders; Hexanchiformes, Squatiniformes, Heterodontiformes, Lamniformes, Carcharhiniformes, and eight species of rays and skates, of four orders: Rajiformes, Rhinoprístiformes, Torpediniformes and Myliobatiformes. The reproductive modes reported for all this species were both lecithotrophic (yolk-sac viviparity, short single oviparity) and matrotrophic (ovotrophy, placentotrophy, limited and lipidic histotrophy). Six morphological and structural major patterns were detected within the yolk-sac viviparous species (type I to VI); a thin, diaphanous, shapeless membrane; candle capsules; oviparous-like tough egg capsule, and cases where no tertiary membrane was present.

Key words: Egg capsule, oviparity, embryology, shark and rays, lecithotrophy



INTRODUCTION

The occurrence of an egg shell is an attribute of animals that display: (1) internal fertilization, (2) a gland supporting the construction of the shell and (3) large yolky oocytes (magnitude order of centimetres). This description perfectly fits Chondrichthyan species, but also Sauropsida (reptiles and birds), whom sharks, rays, skates and chimaeras have a lot in common concerning reproduction, despite their obvious absence the lack of an amniotic membrane. However, the largest oocytes recorded in nature belong to elasmobranch species (Mellinger 1989). Wourms (1977) noted that the oocytes of the Nurse shark *Ginglymostoma cirratum* (Bonnaterre, 1788) and the Friller shark *Chlamydoselachus anguineus* Garman, 1884 are not only the largest oocytes found among these fishes but probably the largest known cells from any living animal. Furthermore, Lombardi (1998) noted that the 15 cm diameter oocytes of *C. anguineus* may well be the largest cells ever produced. Additionally, in a female of gulper shark *Centrophorus granulosus* (Bloch & Schneider, 1801) caught from the Tunisian coast, Capapé (1985) found a ripe oocyte ready to be ovulated which measured 14.65 cm in diameter and weighed 336.46 g.

Huettner (1946) noted that the giant female gamete produced by some vertebrates such as elasmobranchs may be in certain instances millions of times larger than the average body cell or the spermatozoon, due to the massive accumulation of yolk, that consists of granules and platelets associated with small amounts of cytoplasm (Wourms 1977). Megalecithal or macrolecithal oocytes (Dye 2012) are related to lecithotrophic mode of reproduction (Houillon 1972). In elasmobranchs, most species have this type of oocytes, although in some species telolecithal oocytes have been reported.

Lecithotrophy is a trophically unspecialized reproductive mode, where species display a substantial loss of organic matter weight during embryo development, and where embryos depend solely on yolk laid down during oogenesis (Wourms 1981, Musick & Ellis 2005). Such losses vary from 20% in oviparous to around 20-50% in viviparous elasmobranchs (Ranzi 1932, 1934). Matrotrophy occurs exclusively within viviparity, and represents the maternal contribution to the offspring, which happens during two distinct periods. A first period, during oogenesis, where the female allocates energy to the production of high energy yolk; and then, a second one, during embryonic development, when additional nutrients are supplied (Wourms 1981). Following Hamlett *et al.* (2005a), females are able to supplement yolk with uterine secretions (histotrophy), eggs (ovatrophy), siblings (adelphotrophy) or placental transfer (placentatroph). These supplemental maternal contribution varies among sharks and rays from minimal (1%) to extensive (1,200.000%), in terms of gain in weight of organic material during gestation (Ranzi 1932, Wourms 1981).

All metazoarian oocytes excepting some cnidarians, are surrounded by membranes (coats), probably having different properties and origin. The first one enclosing the oocyte is the vitelline membrane, which can be the only one in some organisms, [*e.g.*, the chorion in teleost species (Balinsky 1965)]. However, in vertebrates, additional membranes are produced. Huettner (1946) argued about the origin of the oocyte membranes. In this respect, the vitelline membrane is a primary membrane, produced by the own oocyte. Secondary membranes are produced by follicular cells, in the ovary. According to this author, the insect chorion and the cyclostomes eggs chorion are secondary membranes. Tertiary membranes, also called “shells” are derived from the oviducts or uterus and accessory glands, and are diversified in form and consistency. The chick oocyte has three tertiary membranes, the egg albumen, the shell membrane, and the hard calcareous shell. Similar membranes are found in Sauropsida and egg laying mammals. Fertilization takes place prior to the formation of the tertiary membranes (Wourms 1977).

Elasmobranchs lack primary membranes, as the vitelline envelope is produced by the follicle, and is therefore, a secondary membrane; on the other hand, the egg jelly and capsule are produced by the oviducal gland, being tertiary membranes (Hamlett & Hysell 1998, Menkhurst & Selwood 2008). These species are able to produce two types of egg shell, known as egg capsules; the latter by oviparous species, that are permanent, and deposited on or near the bottom, and those elaborated by viviparous species, only functional in utero during a short period (Wourms 1977). In some viviparous species (placentotrophic), however, the egg capsule may be retained and function as a part of the placenta (TeWinkel 1963). Structurally, egg capsules consist of a layer of albumen and orthogonally stacked layers of protein fibrils (Lombardi 1998). Egg capsules are synthesized by the oviductal gland, which also controls its deposition and morphogenesis (Dean 1906, Fitz & Daiber 1963). Egg capsules are absent in *Urolophus halleri* (Babel, 1967) and in torpedinid species (Mellinger 1989).

The current classifications of reproductive modes, do not take into account the fact that yolk-sac viviparous species have egg capsules structurally different. These egg capsules vary from the delicate, diaphanous egg capsules of *Squalus acanthias* (commonly known as “candle capsule” or simply “candle”) (see for instance Jones & Ugland 2001, Hamlett *et al.* 2005b) to the tough, oviparous-like egg capsules as that of *Rhincodon typus* (Joung *et al.* 1996). This species had even been previously considered as possibly oviparous, because of the egg capsule structural complexity (Baughman 1955).

The purpose of this paper was to contribute to the knowledge of the elasmobranch egg capsules, in both viviparous and oviparous species, and to discuss the relation with their role in protecting the embryos during the embryonic development. In addition, we discuss the limitations of yolk-sac viviparity as a single reproductive mode category. Additionally, due to the fact that some elasmobranch species display different kinds of tertiary membranes, a new arrangement of taxa is suggested within that reproductive mode and new terminology is proposed.

MATERIALS AND METHODS

Data gathered include records related to the occurrence of tertiary membranes in elasmobranchs species, in particular, those which included photographic register. In general, specimens under study represent a single occurrence belonging to a larger data set, which may have been published, in some cases not even for reproductive assessment. In any case, the observation, record and discussion of the tertiary membranes for some reason left aside in those opportunities, is presented and discussed here. Photographic records of the tertiary membranes are included in all cases. Additionally, specific cases of species without tertiary membrane were also documented. Specimens were arranged by taxon along with a brief description of the reproductive mode.

Data belong to a large number of sources that ranges from 1977 to date. Specimens were captured in both commercial cruises and scientific research cruises, from the following some major geographic regions: South Western Atlantic, South Western Pacific, and different areas of the Mediterranean Sea. A complete list of the specimens including their taxonomic context and collection information is presented in Table 1. A single egg capsule found among carcasses of several pelagic species during an artisanal fishery landing activity in Manta, Ecuador was identified according to Castro (2009, figure 11). The following preserved material deposited in the Chondrichthyes Collection of the Instituto de Ciências Biológicas, Universidade Federal do Rio Grande (FURG) was examined and photographed: CC00071 (*Myliobatis ridens*), CC00256 (*Rhinobatos percellens*), CC00276 (*Squatina guggenheim*) and CC00155 (*Mustelus fasciatus*). This material was collected by Professor Carolus Maria Vooren and belongs in all cases to the Southern Brazilian shelf, South Western Atlantic. The specimens were part of the former collection of the Laboratório de Elasmobrânquios e Aves Marinhas, Instituto de Oceanografia, of the FURG and were collected in commercial and research fishing cruises carried out in the decades of 1980 to 2000.

Nomenclature related to the different phases of the cell undergoing oogenesis and fertilization, is both confusing and commonly erroneously used in the scientific literature. In all cases, terminology regarding the concept of the egg is in agreement with Dye (2012). Throughout this manuscript, systematic classification for sharks follows Compagno (2005). For batoids, nomenclature is in agreement with Last *et al.* (2016) for skates and Naylor *et al.* (2016), for the remaining ray species. The egg capsules occurrences were organized by shark, or ray/skate order. In the specific case of *Myliobatis ridens*, this species was described after the publication of the checklist of live chondrichthyans by Compagno (2005) and the taxonomic context follows Ruocco *et al.* (2012). This is also the case for the Sarawak swellshark *Cephaloscyllium sarawakensis* Yano, Ahmad & Gambang, 2005. Reproductive modes terminology agrees with Mellinger (1989) and Musick & Ellis (2005). Remarks and nomenclature on oviparity and lecithotrophic viviparity follows the recent classification of Nakaya *et al.* (2020). Tertiary membrane terminology follows Hamlett *et al.* (2005a); which exception of the term “case” which has been substituted by “capsule”. In this way, the tertiary membrane of placental and compartmentalized uterus sharks is referred as egg envelope, and for the yolk-sac viviparous species we used the term “candle capsule”. This corresponds to the also known as polyvitelline egg capsule used to design a “candle” that bears more than one egg according to Sunye & Vooren (1997) (polyvitelline “unit”). Maternal and embryo specimens were measured to the nearest centimetre and millimetre respectively as total length (TL), when possible. For egg capsules that assume the shape of an ellipse, the anterior and posterior capsule ends were referred as “extremes”, following the geometrical nomenclature. The egg capsule width was measured in the middle of the egg capsule, corresponding to the widest point (Hubbs & Ishiyama 1958).

RESULTS

In all, our records of egg capsules belong to ten species of sharks belonging to five orders; Hexanchiformes, Squatiniformes, Heterodontiformes, Lamniformes, Carcharhiniformes, and eight species of rays and skates, corresponding to four orders; Rhinopristiformes, Torpediniformes, Rajiformes and Myliobatiformes (Table 1).

Table 1. Species of elasmobranchs with record of tertiary membrane; data on catches in terms of oceanic area [for the South Western (SW) Atlantic refers to South Brazil and Southeast Brazil specifically for *Atlantoraja platana*; Central Eastern (CE) Pacific, refers to Manta, Ecuador, and for the Mediterranean, three areas were considered: Central, Eastern and Northern]; the date (year) of capture and source of each occurrence are included / Especies con registro de membrana terciaria, y datos de las capturas en términos de área [para el Atlántico Sudoccidental (SW), se refiere al sur de Brasil y al sudeste de Brasil en el caso específico de *Atlantoraja platana*; Pacífico Centro-oriental (CE), se refiere a Manta, Ecuador, y para el Mediterráneo, tres áreas fueron consideradas: Central, Oriental y Norte]; fecha (año) y fuente de cada ocurrencia

Specimen	Area	Year	Source
Sharks			
Order Hexanchiformes			
<i>Heptanchias perlo</i> (Bonnaterre, 1788)	SW Atlantic	2001	Vooren & Oddone (2019)
<i>Hexanchus griseus</i> (Bonnaterre 1788)	Central Mediterranean	2020	Capapé <i>et al.</i> (2004), Ounfi-Ben Amor <i>et al.</i> (2017), Ben Amor <i>et al.</i> (2020)
<i>Squatina guggenheim</i> Marini, 1936	SW Atlantic		Collection ⁴
Orden Heterodontiformes			
<i>Heterodontus mexicanus</i> Taylor & Castro-Aguirre, 1972	CE Pacific	2010	Other ¹
<i>Alopias</i> sp.	CE Pacific	2010	Other ¹
<i>Cetorhinus maximus</i> (Gunnerus 1765)	Eastern Mediterranean	2012	(Ali <i>et al.</i> 2012)
Order Carcharhiniformes			
Family Triakidae Gray, 1851			
<i>Galeorhinus galeus</i> (Linnaeus, 1758)	Central Mediterranean	1977	Capapé <i>et al.</i> (2005)
<i>Mustelus fasciatus</i> (Garman, 1913)	SW Atlantic	1981	Vooren & Klippel (2005), Collection ⁴
<i>Mustelus mediterraneus</i> Quignard & Capapé, 1972	Central Mediterranean	1990	Capapé & Quignard (1977)
Family Carcharhinidae Jordan & Evermann, 1896			
<i>Prionace glauca</i> (Linnaeus, 1758)	SW Atlantic	2014	Gowert & Oddone (2018) ⁵ , Research project ²
Rays and skates			
Order Rhinopristiformes			
Family Rhinobatidae Müller & Henle, 1837			
<i>Rhinobatos percellens</i> (Walbaum, 1792)	SW Atlantic	2004	Vooren <i>et al.</i> (2005), Collection ⁴
<i>Zapteryx brevirostris</i> (Müller & Henle, 1841)	SW Atlantic	2014	Research project ³
Order Torpediniformes			
Family Torpedinidae Bonaparte, 1838			
<i>Torpedo torpedo</i> (Linnaeus, 1758)	Central Mediterranean	2013	El Kamel-Moutalibi <i>et al.</i> (2013)
Order Rajiformes			
Family Arhynchobatidae Fowler, 1934			
<i>Atlantoraja cyclophora</i> (Regan, 1903).	SW Atlantic	2001	Oddone & Vooren (2004, 2005)
<i>Atlantoraja platana</i> (Günther, 1880)	SW Atlantic	2005	Oddone (2007), Oddone & Amorim (2008)
Order Myliobatiformes			
Family Gymnuridae Fowler, 1934			
<i>Gymnura altavela</i> (Linnaeus, 1758)	Central Mediterranean	2011	Capapé <i>et al.</i> (1992), Alkusaury <i>et al.</i> (2014)
Family Myliobatidae Bonaparte, 1838			
<i>Myliobatis aquila</i> (Linnaeus, 1758)	Northern Mediterranean	2002	Capapé <i>et al.</i> (2007)
<i>Myliobatis ridens</i> Ruocco, Lucifora, Díaz de Astarloa, Mabragaña & Delpiani, 2012	SW Atlantic, South Brazil	1982	Collection ⁴

¹Photographed during the scientific workshop III Taller Interregional para la Ordenación y Conservación de Tiburones en el Océano Pacífico Oriental, 6-9 July 2010, Manta, Ecuador

²Biología, ecología e conservação das populações de tubarões (Chondrichthyes: Elasmobranchii) do extremo Sul do Brasil. Universidade Federal do Rio Grande. Registration code PROESP-814440/2014. [unpublished project outcome]

³Emplastos do Sul. Universidade Federal do Rio Grande. Registration code PROESP-086576/2011, [unpublished project outcome]

⁴Chondrichthyes Collection, Instituto de Ciências Biológicas, Universidade Federal do Rio Grande-FURG, Brazil

⁵Gowert Y & MC Oddone. 2018. Descrição da placenta e do cordão umbilical de tubarão-azul, *Prionace glauca* (Linnaeus, 1758). Livro de Resumos do VI Simpósio Acadêmico de Biologia Marinha, Osório, p. 38.

SHARK EGG CAPSULES ORDER HEXANCHIFORMES

Species and reproductive mode: Sharpnose sevengill shark *Hepranchias perlo* and Bluntnose sixgill shark *Hexanchus griseus*, yolk-sac viviparous.

Material observed and remarks:

Four 100 mm long and completely transparent monovitellin candles of *H. perlo* removed from pregnant female immediately after capture (Fig. 1, Table 1). Candles were ellipsoid and bore short robust filaments, one on each extreme. Embryos (40-50 mm total length) presented active movements and conspicuous external gills, and were attached to each yolk sac through a robust stalk with their own blood vessels visible to the naked eye.

Two females captured in the central Mediterranean Sea measured 350 cm in total length and weighed 700 kg in total body weight, they carried 120 and 118 fully-yolked eggs ready to be ovulated, respectively (Ben Amor *et al.* 2020). Each egg was covered by a fine diaphanous membrane, and included together in a single membranous capsule (Fig. 2, Table 1). Such double membrane is a mean of protection, avoiding to be spilled. Such unfavourable phenomenon unfortunately occurs during handling by fishermen (Ounifi-Ben Amor *et al.* 2017). Following Capapé *et al.* (2004), the diameter of the eggs ranged between 68-75 mm (mean= 71.71 ± 2.6) and weighed between 127-147 g (mean= 134 ± 8.4). Dozens of eggs were removed from the uteri of a female *Hexanchus griseus* (Fig. 3, Table 1). The eggs were individually encapsulated in a very thin membrane. The diameters of the eggs ranged between 68-75 mm (mean= 71.71 ± 2.6) and weighed between 127-147 g (mean= 134 ± 8.4).



Figure 1. Three monovitelline candles (from a total of four) extracted from a female of *Hepranchias perlo*. Embryos with external gills are visible through the transparent egg capsules. Scale bar= 30 mm. Photo by C. M. Vooren / Tres velas monovitelinas (de un total de cuatro) retiradas de una hembra de *Hepranchias perlo*. Los embriones con branquias externas son visibles a través de las cápsulas transparentes. Barra de escala= 30 mm. Fotografía de C. M. Vooren



Figure 2. Fully yolked oocytes removed from a female of *Hexanchus griseus*: (1) each oocyte was covered by a fine diaphanous membrane. (2) All eggs were enveloped together in one single membranous capsule. Photo by K. Ounifi-Ben Amor / Ovocitos repletos de vitelo extraídos de una hembra de *Hexanchus griseus*. (1) Cada ovocito se presenta revestido por una fina membrana transparente. (2) Todos los ovocitos se encontraban revestidos en conjunto por una cápsula simple membranosa. Foto de K. Ounifi-Ben Amor



Figure 3. Fertilized eggs removed from both uteri of the female *Hexanchus griseus* captured in the Gulf of Tunis. Photo by K. Ounifi-Ben Amor / Huevos fertilizados extraídos de ambos úteros de una hembra de *Hexanchus griseus* capturada en el Golfo de Túnez. Foto de K. Ounifi-Ben Amor

SHARK EGG CAPSULES ORDER SQUATINIFORMES

Species and reproductive mode: Hidden angelshark *Squatina guggenheim* Marini, 1936, yolk-sac viviparous (preserved material).

Material observed and remarks:

A candle capsule 200 mm long extracted in utero and preserved in 70% ethanol (Fig. 4, Table 1). The extremely thin egg capsule remained preserved. The capsule had the shape of an elongated ellipsoid and contained five eggs.

SHARK EGG CAPSULES ORDER HETERODONTIFORMES

Species and reproductive mode: Mexican hornshark *Heterodontus mexicanus* Taylor & Castro-Aguirre, 1972, short single oviparous.

Material observed and remarks:

A 75 cm TL adult female captured prior to egg laying had the egg capsules tendrils already exposed through the cloaca (Fig. 5a and b, Table 1). The dissection of the female revealed egg bearing uteri, which have assumed the term egg capsules morphology (Fig. 5c and d). Oviducal gland and distended uteri, after the extraction of the egg capsules are shown in Figure 6a, along with the ovary, with a batch of vitellogenic follicles. The egg capsules were golden light brown and measured 100 mm of length (Fig. 6b and c).

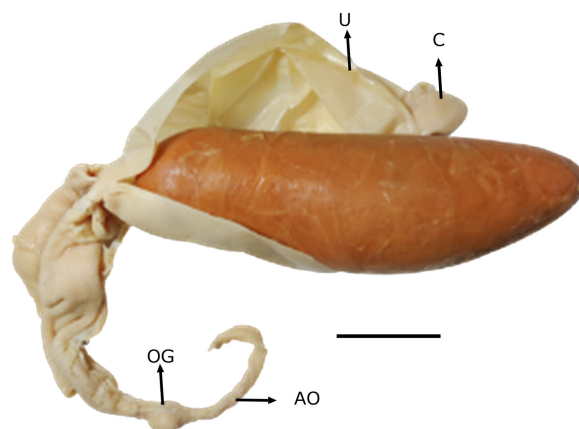


Figure 4. Reproductive duct of *Squatina guggenheim* containing a polyvitelline candle capsule from a female captured in South Brazil containing five eggs. Part of the uterus was removed to expose the candle. AO= anterior oviduct; OG= oviducal gland; U= uterus; C= cloacae. Scale bar= 50 mm. Photo by M. C. Oddone / Ducto reproductivo de *Squatina guggenheim* con una cápsula de vela en un ejemplar hembra capturada en el Sur de Brasil conteniendo cinco huevos. Parte del útero fue removida para mostrar la vela polivitelina. AO= oviducto anterior; OG= glándula oviductal; U= útero; C= cloaca. Barra de escala= 50 mm. Fotografía de M. C. Oddone

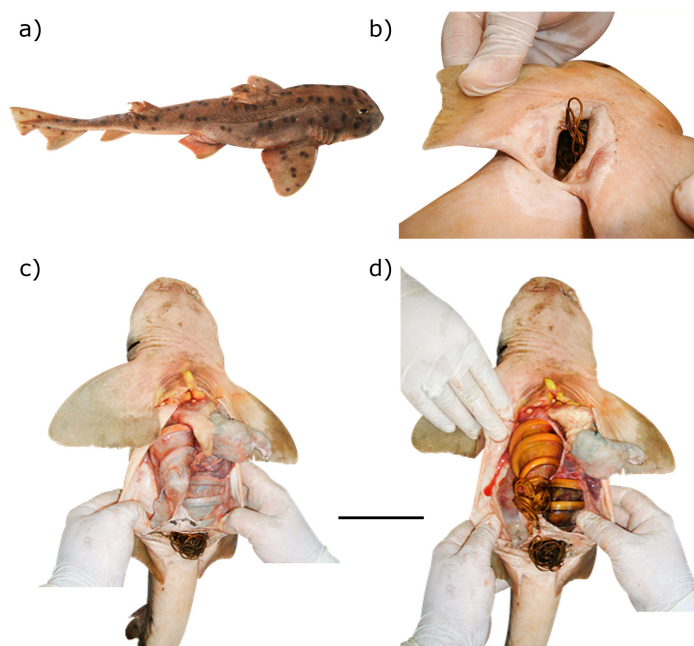


Figure 5. *Heterodontus mexicanus*. a) A 750 mm total length female of captured off Manta, Ecuador. b) The long egg capsules tendrils are already off the cloaca, prior to oviposition. c) After ventral dissection and removal of the digestive tract, the uteri are visible, and reveal the presence of egg capsules (one per uterus), after having assumed their morphology. d) A dissection of the uteri shows the term egg capsules. Scale bar= 250 mm. Photos by M. C. Oddone) / *Heterodontus mexicanus*. a) Hembra de 57 cm de longitud total capturada en Manta, Ecuador. b) Los largos zarcillos se encuentran ya en la cloaca, antes de la oviposición. c) Luego de una disección ventral y remoción del tracto digestivo, los úteros son visibles y revelan la presencia de cápsulas ovígeras (una por útero), habiendo adoptado su morfología. d) La disección de los úteros muestra un par de cápsulas a término. Barra de escala= 250 mm. Fotografías de M. C. Oddone

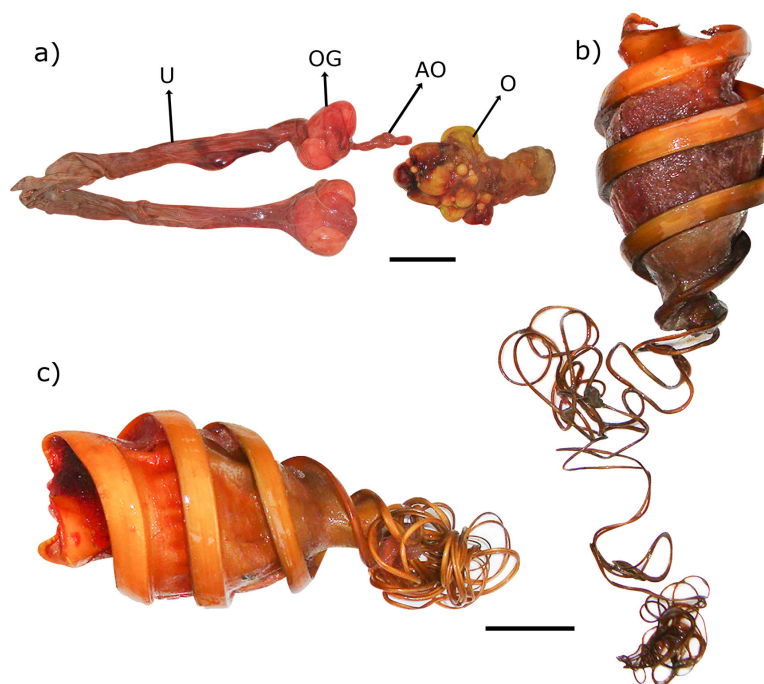


Figure 6. *Heterodontus mexicanus*. a) Distended and vascularized posterior oviducts and oviducal glands (6.0 cm width). b-c) right and left egg capsules, respectively. AO= anterior oviduct; O= ovary; OG= oviducal gland; U= uterus. Scale bar a) 30 mm; b-c) 50 mm. Photos by M. C. Oddone / *Heterodontus mexicanus*. a) Oviductos posteriores distendidos y vascularizados y glándula oviductal (6,0 cm de ancho). b-c) cápsulas derecha e izquierda, respectivamente. AO= oviducto anterior; O= ovario; OG= glándula oviductal; U= útero. Barra de escala a) 30 mm; b-c) 50 mm. Fotografías de M. C. Oddone

SHARK EGG CAPSULES ORDER LAMNIFORMES

Thresher shark, *Alopias* sp. and Basking shark *Cetorhinus maximus* (Gunnerus, 1765), viviparous matrotrophic, oophagous (see comments on *C. maximus* in Discussion section)

Material observed and remarks:

A fresh, transparent egg capsule attributed to *Alopias* sp., most probably to *A. pelagicus* (Fig. 7, Table 1), with a 10 mm TL embryo and yolk sac. This egg capsule was found on the beach shore in Manta, Ecuador, soon after the artisanal fishing landings of large pelagic sharks. The capsule had the shape of an elongated ellipse with one rounded extreme and a more flattened one (probably the anterior end).



Figure 7. An encapsulated fertilized egg of *Alopias* sp. found on the beach off Manta, Ecuador, soon after the artisanal fishing landings of large pelagic sharks. Yolk sac and a small embryo are visible through the transparent capsule. Photo by M. C. Oddone / Huevo fertilizado encapsulado de *Alopias* sp. encontrado en la playa en Manta, Ecuador, inmediatamente después del desembarque de la pesca artesanal de grandes tiburones pelágicos. Se visualiza un saco de vitelo y un pequeño embrión a través de la cápsula transparente. Fotografía de M. C. Oddone

A 690 cm TL pregnant female of *C. maximus* captured from the coast of Syria in 2012 contained 34 egg capsules (Fig. 8, Table 1). The egg capsules had 96 mm length and 46 mm width, ovoid-shaped, symmetrically convex with a highest point centrally located; longitudinally keeled, and with curved anterior margins, with hornlike short projection on each corner, followed by anterior of 87 mm long tendrils, rounded in cross section of 3 mm diameter, exhibiting two short curved expansions. The capsule body was followed by a posterior horn, sharp and triangular, measuring 68 mm in length, 26 mm in maximum width, and 12 mm in minimum width, ended by a filament measuring 90 mm in length, 8 mm in maximum width and 4 mm in minimum width. The complete egg capsule weighed 40.39 g. Two empty capsules weighed 9.49 and 9.37 g, respectively with an average of 9.43 ± 0.08 g, so the egg weighed 30.96 g approximately (Ali *et al.* 2012).

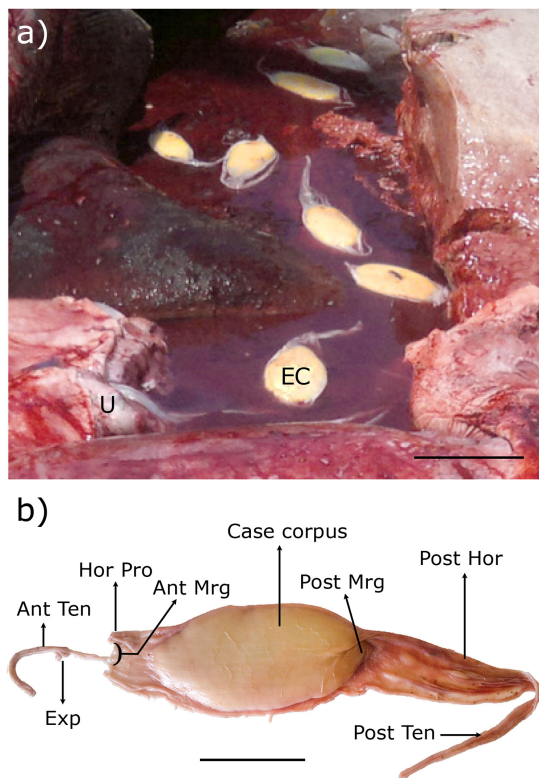


Figure 8. *Cetorhinus maximus*. a) egg capsules (EC) outside the uterus (Ut) of specimens captured off the coast of Syria. b) single egg capsule. Ant Ten= anterior filament; Ant Mrg= anterior margin; Exp= expansions; Hor Pro= horny process; Post Ten= posterior filament; Post Hor= posterior horn; Post Mrg= posterior margin. Scale bar: a) 100 mm; b) 50 mm. Photos from Ali *et al.* (2012), with permission / *Cetorhinus maximus*. a) cápsulas ovígeras (EC) fuera del útero (Ut), de un ejemplar capturado en la costa de Siria. b) cápsula ovígera. Ant Ten= zarcillo o filamento anterior; Ant Mrg= margen anterior; Exp= expansiones; Hor Pro= proceso córneo; Post Ten= zarcillo o filamento anterior; Post Hor= cuerno posterior; Post Mrg= margen posterior. Barra de escala a) 100 mm y b) 50 mm. Fotografías obtenidas de Ali *et al.* (2012), con autorización

SHARK EGG CAPSULES ORDER CARCHARHINIFORMES

Tope shark *Galeorhinus galeus* (Linnaeus, 1758), Striped smoothhound *Mustelus fasciatus* (Garman, 1913), Blackspot smoothhound *Mustelus mediterraneus* Quignard & Capapé, 1972 and Blue shark, *Prionace glauca* (Linnaeus, 1758), yolk-sac viviparous with limited histotrophy (*G. galeus*) and placentotrophic [*P. glauca*, *M. fasciatus* (preserved material) and *M. mediterraneus*].

Material observed and remarks:

Twenty early gestation individually encapsulated eggs of *G. galeus* extracted from both uteri of a pregnant female captured in the Maghreb coast, Southern Mediterranean (Fig. 9a, Table 1). Eggs were enveloped in a diaphanous ellipsoid capsule with large brownish filaments at both ends. These capsules were fragile and eggs rapidly spilled during handling. Uteri of pregnant females were compartmentalized into chambers, each one containing a single developing embryo of about 120 mm. In the Figure 9b, advanced embryos from a late pregnant female of 120 mm TL are shown. At that size,

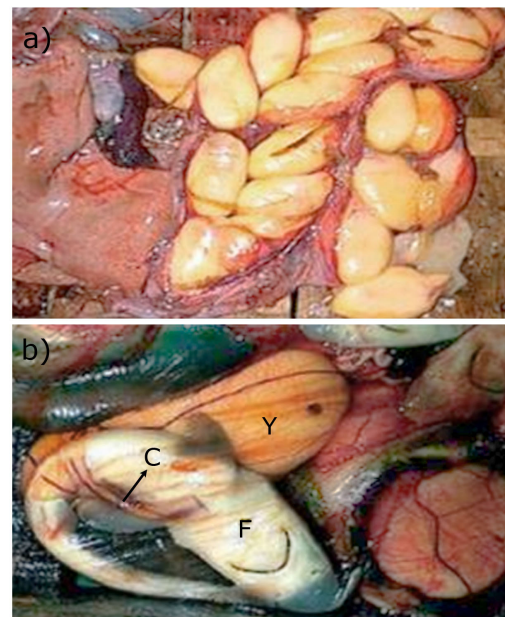


Figure 9. *Galeorhinus galeus*. a) Encapsulated eggs removed from an adult. b) Developing embryo in a chamber removed from a pregnant female. Specimens captured in the Maghreb shore, southwestern Mediterranean. C= umbilical cord. F= developing embryo. Y= yolk sac. Photos by F. Hemida, from Capapé *et al.* (2005), with permission / *Galeorhinus galeus*. a) Huevos encapsulados removidos de una hembra adulta. b) Embrión en desarrollo en una cámara extraída de una hembra gestante. Especímenes capturados en la costa de Magreb, Sur del Mediterráneo. C= cordón umbilical. F= embrión en desarrollo. Y= saco vitelino. Fotografías de F. Hemida obtenida de Capapé *et al.* (2005), con autorización

embryos had a large yolk sac and nutrients were transferred from the yolk sac to the embryo through the umbilical stalk. Five advanced embryos of *M. fasciatus* with umbilical cords and placenta were extracted from a pregnant female capsuled in South Brazil (Fig. 10, Table 1). The egg capsules were found intact around each embryo occupying a single chamber. Embryos were 120 mm in TL. An egg capsule from an early pregnant female of *M. fasciatus* was also preserved jointly. Pregnant females of *M. mediterraneus* were captured in 1977 from the Tunisians waters, central Mediterranean Sea. Egg was 2.0 cm wide and contained jelly. The egg envelope bore

long filaments at both ends of approximately 12 cm (Fig. 11a). Also a near term embryo measuring 400 mm TL with umbilical cord and placenta was reported (Fig. 11b). Rest of egg capsules were observed in a postpartum of *P. glauca* (Fig. 12a, b; Table 1). The placentae were still attached to the uterus epithelium after the landing of the female, which had a TL of 241 cm. After parturition, rests of the egg capsule that remained loose in the uterus coating the embryos during the whole gestation are visible, also in association with the umbilical cords and placentae (Fig. 12c).

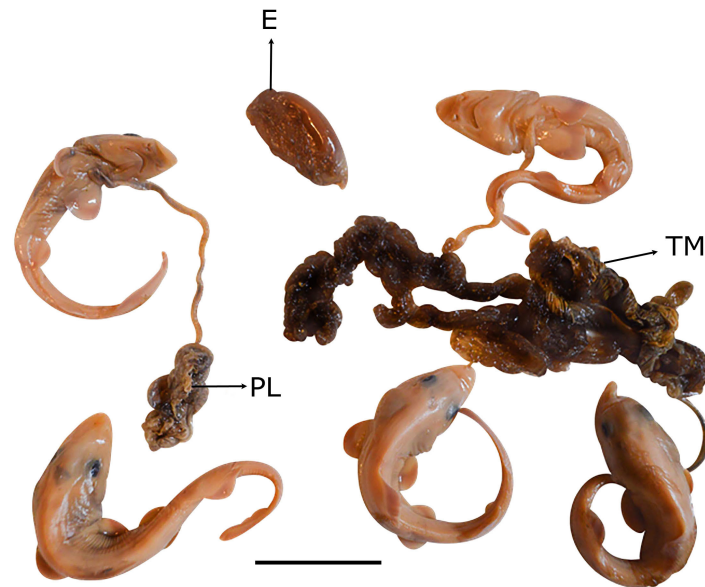


Figure 10. Five advanced embryos of *Mustelus fasciatus* with umbilical cords and placenta (in some) and a uterine encapsulated egg. Tertiary membranes remained intact during gestation. PL= placenta; E= uterine egg; TM= tertiary membrane. Scale bar= 30 mm. Photo by M. C. Oddone / Cinco embriones avanzados de *Mustelus fasciatus* con cordón umbilical y placenta (en algunos) y un huevo uterino encapsulado. Las membranas terciarias permanecieron intactas durante la gestación. PL= placenta; E= huevo uterino; TM= membrana terciaria. Barra de escala= 30 mm. Fotografía de M. C. Oddone

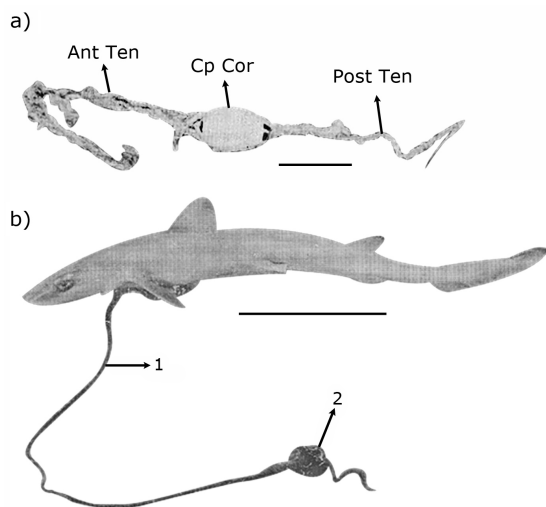


Figure 11. *Mustelus mediterraneus*. a) A 30 mm wide egg capsule extracted from a female uterus captured in 1977 from the Tunisians waters, central Mediterranean Sea. b) A 400 mm total length term embryo of the same species, with umbilical cord and placenta. Ant Ten= anterior tendrils; Post Ten= posterior tendrils; Cp Cor= capsule corpus. Scale bar= 50 mm. Photos from Capapé & Quignard (1977), with permission / *Mustelus mediterraneus*. a) Cápsula de 30 mm de ancho extraída de una hembra capturada en 1977 en aguas de Túnez, Mediterráneo Central. b) Embrión a término de 400 mm de longitud total de la misma especie, con cordón umbilical y placenta (PL). Ant Ten= zarcillos anteriores; Post Ten= zarcillos posteriores; Cp Cor= cuerpo de la cápsula. Barra de escala= 50 mm. Fotografías obtenidas de Capapé & Quignard (1977), con autorización

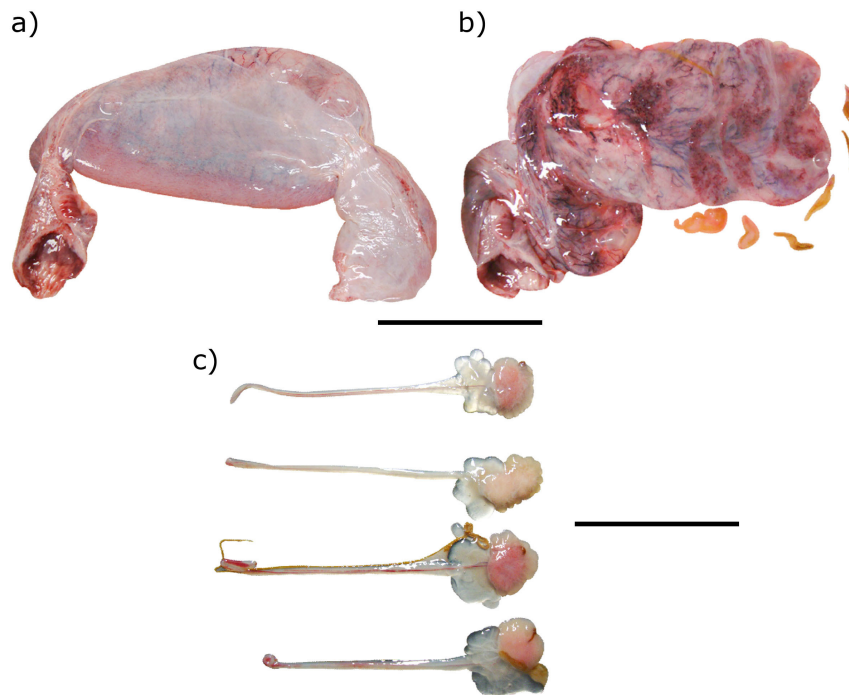


Figure 12. *Prionace glauca*. a-b) Fresh uterus of a 241.0 cm total length female landed in the port of Rio Grande (South Brazil) Brazil, in 2014, that had given birth immediately before capture. After dissection, the uterus internal lining shows several placental scars; around the uterus rests of the tertiary membrane are visible (brown fragments). c) Detail of four placentas with umbilical cords attached, the two inferior have rests of the tertiary membrane (in brown). Scale bar: a-b) 200 mm; c) 100 mm. Photos by M. C. Oddone / *Prionace glauca*. a-b) Útero fresco de una hembra de 241,0 cm de longitud total, desembarcada el 2014 en el Puerto de Rio Grande (Sur de Brasil), inmediatamente después de dar a luz. Luego de la disección, el útero muestra en su revestimiento interno varias cicatrices placentarias, alrededor del mismo, se encuentran restos de la membrana terciaria (fragmentos marrones). c) Detalle de cuatro placentas con cordón umbilical adherido, las dos inferiores poseen restos de la membrana terciaria (en marrón). Barra de escala: a-b) 200 mm; c) 100 mm. Fotografías de M. C. Oddone

RAYS EGG CAPSULES ORDER RHINOPRISTIFORMES

Southern guitarfish *Rhinobatos percellens* (Walbaum, 1792) and Shortnose guitarfish *Zapteryx brevirostris* (Müller & Henle, 1841), yolk-sac viviparous (preserved material)

Material observed and remarks:

Eight advanced embryos of *Rhinobatos percellens* extracted from a pregnant female (Fig. 13), captured off Southern Brazil in 2004, during an artisanal fish landing, in Cassino Beach, South Brazil. The embryos had conspicuous yolk sacs with diameters of about 30 mm. The tertiary membranes were also present in the uteri. The consistence of

this egg capsules was tough, and resembled the insect elytra, remaining intact in ethanol preservation. A 40.0 cm TL female of *Z. brevirostris* was captured off South Brazil in 2014 by bottom trawl commercial fisheries (Fig. 14a). Both uteri were pregnant. The dissection revealed two embryos per uterus (Fig. 14b and c). The tertiary membrane and the ovary (with ripe vitellogenesis follicles) are also recorded. The embryos were completely encapsulated and the capsule consistency was considerably different from that of *R. percellens*, being much thinner, and collapsed soon after the contact with ethanol 70%.



Figure 13. Two advanced embryos of *Rhinobatos percellens* captured off Southern Brazil in 2004, with conspicuous yolk sacs. In the centre of the picture, rests of the tertiary membranes, taken from the uterus. Scale bar= 30 mm. Photo by M. C. Oddone / Dos embriones avanzados de *Rhinobatos percellens* con sacos de vitelo conspicuos. En el centro de la figura, restos de la membrana terciaria, sacados del útero. Barra de escala= 30 mm. Fotografía de M. C. Oddone

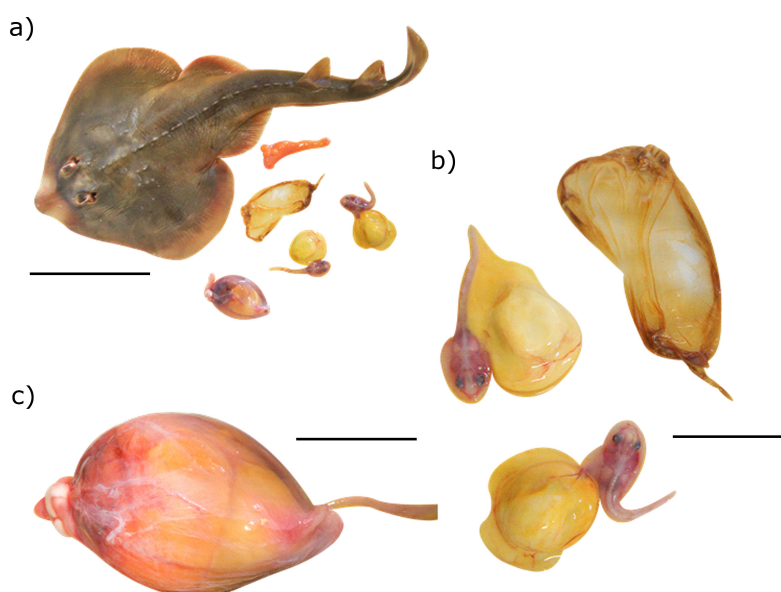


Figure 14. *Zapteryx brevirostris*. a) A 40.0 cm total length female captured off South Brazil in 2014. One of the uteri has been dissected and two embryos have been extracted. The tertiary membrane and the ovary are also visible. b) Content of left uterus, two embryos with conspicuous yolk sacs. Embryos were encapsulated, the tertiary membrane was extracted. c) The pregnant intact right uterus with oviductal gland. Scale bar: a) 150 mm; b) 50 mm; c) 40 mm. Photo by M. C. Oddone / *Zapteryx brevirostris*. a) Hembra de 40,0 cm de longitud total capturada en el litoral del sur de Brasil en el 2014. Uno de los úteros fue diseccionado y se extrajeron dos embriones. También se visualiza la membrana terciaria y el ovario. b) Contenido del útero izquierdo, dos embriones con sacos de vitelo conspicuos. Los embriones estaban encapsulados y la membrana terciaria fue extraída. c) Útero izquierdo gestante intacto con glándula oviductal. Barra de escala: a) 150 mm; b) 50 mm; c) 40 mm. Fotografía de M. C. Oddone

RAYS EGGS ORDER TORPEDIFORMES

Ocellate or common torpedo, *Torpedo torpedo* (Linnaeus, 1758), yolk-sac viviparous

Material observed and remarks:

An early pregnant of *T. torpedo* bore two eggs, one per uterus, without any kind of tertiary membrane (Fig. 15). The female was 330 mm TL, and was captured in the Lagoon of Bizerte, north-eastern Tunisia in 2013.

SKATES EGG CAPSULES ORDER RAJIFORMES

Eyespot skate *Atlantoraja cyclophora* (Regan, 1903) and La Plata skate *Atlantoraja platana* (Günther, 1880), short single oviparous

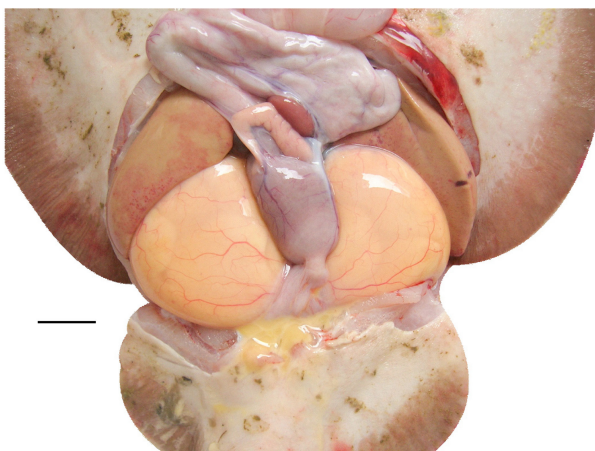


Figure 15. Ventral view of the abdominal cavity of *Torpedo torpedo* showing two eggs, one per uterus, without any kind of tertiary membrane, in a specimen of 330 mm total length captured in the Lagoon of Bizerte, northeastern Tunisia. Scale bar= 50 mm. Photo by O. El Kamel-Moutalibi) / Vista ventral de la cavidad abdominal de *Torpedo torpedo* mostrando dos huevos, uno por útero, sin ningún tipo de membrana terciaria. Espécimen de 330 mm de longitud total capturado en la Laguna de Bizerte, noreste de Túnez. Barra de escala= 50 mm. Fotografía de O. El Kamel-Moutalibi

Material observed and remarks:

During the biological sampling carried out in 2001 in the South Brazil for *A. cyclophora*, and in 2006 in the Southeast Brazil for *A. platana* (Fig. 16), instances of ovulation were recorded. In both cases, the yolk was observed in the exact moment it passed through the anterior oviduct, just before entering the oviducal gland. Furthermore, in both cases the formation of egg capsules in the posterior oviduct was observed, with approximately 50-70% of the capsule formed.

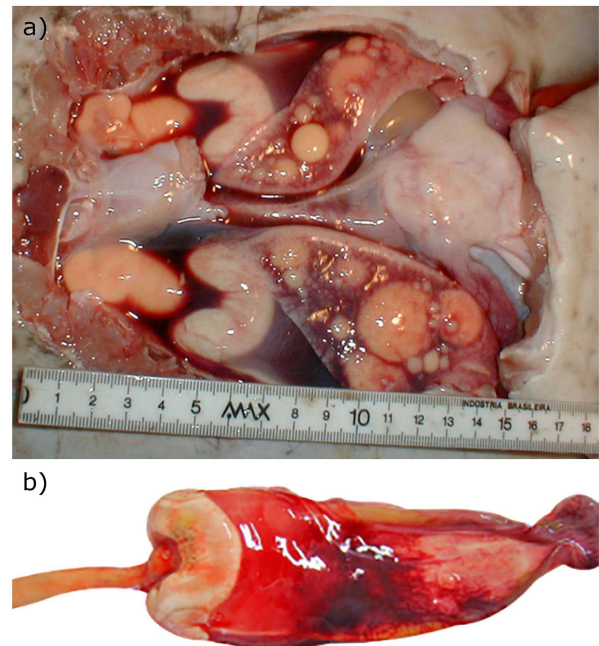


Figure 16. *Atlantoraja cyclophora*. Instances of ovulation and formation of the tertiary membranes. a) ventral view of the opened abdominal cavity. Ovulation can be observed concomitantly in both anterior oviducts, beneath the ovaries, the formation of an egg capsule can be seen in the uteri, in a female captured off South Brazil, 2001. b) the complete right oviduct was removed; the anterior oviduct is full of yolk, due to of ovulation. The posterior oviduct reveals the formation of an egg capsule with the posterior horns complete. Female captured off Southeast Brazil, in 2006. Photos by M. C. Oddone / *Atlantoraja cyclophora*. Instancias de ovulación y formación de la membrana terciaria. a) vista ventral de la cavidad abdominal abierta. La ovulación se observa en concomitancia en los dos oviductos anteriores, debajo de los ovarios, la formación de dos capsulas ovígeras se observa en ambos úteros. Hembra capturada en el sur de Brasil en el 2001. b) un oviducto completo removido, con el oviducto anterior lleno de vitelo debido a la ovulación. En el oviducto posterior se observa la formación completa de los cuernos posteriores. Hembra capturada en el sudeste de Brasil en 2006. Fotografías de M. C. Oddone

RAYS EGG CAPSULES ORDER MYLIOBATIFORMES

Spiny butterfly ray *Gymnura altavela* (Linnaeus, 1758), Common eagle ray or bullray *Myliobatis aquila* (Linnaeus, 1758) and Shortnose eagle ray *Myliobatis ridens* Ruocco, Lucifora, Díaz de Astarloa, Mabragaña & Delpiani, 2012, matrotrophic viviparous, with lipidic histotrophy.

Two uterine egg capsules of *G. altavela* were extracted from a female captured off the coast of Syria, in 2011 (Fig. 17). One of the capsules contained two eggs, and the other one, a single one. The eggs were encapsulated in a diaphanous capsule, and were filled by transparent jelly. A pair of egg capsules of *M. aquila* containing fertilized eggs was extracted from a female captured from the coast of Languedoc, southern France, northern Mediterranean Sea, in 2002. The ellipsoid capsules had very robust filaments in the extremes. A pair of egg capsules of *Myliobatis aquila* containing fertilized oocytes were removed from a female captured off the coast of Languedoc, southern France, northern Mediterranean Sea, in 2002 (Fig. 18). The capsules of these two species were ellipsoid in shape and presented tendril like horns at both extremes. Three encapsulated uterine eggs of *Myliobatis ridens* were collected off South Brazil in 1982, after a beach artisanal fishing lading. The eggs were ellipsoid shaped and bore short thin filaments at its extremes, except for one of the eggs, which have a filamentous extreme but the other

one round; unlike the two other, this egg seemed to have been wrapped by the capsule and closed by a tendril like horn at one end only, forming a bag (Fig. 19). The same was observed in the *G. altavela* eggs. The capsule was extremely thin and easily breakable to touch. The pink coloration is due preservation. In fresh, egg capsules are transparent, and yellow because of the yolk.

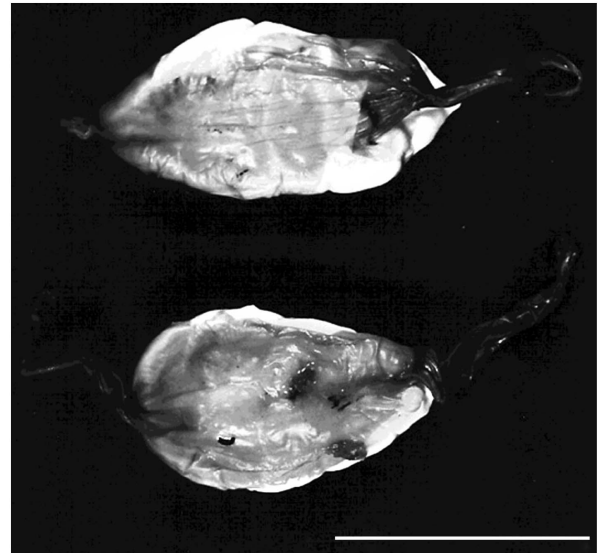


Figure 18. A pair of egg capsules of *Myliobatis aquila* containing fertilized, extracted from a female captured from the coast of Languedoc, southern France, northern Mediterranean Sea, in 2002. Scale bar= 50 mm. Photo with permission of Capapé et al. (2007) / Par de cápsulas ovígeras de *Myliobatis aquila* conteniendo huevos fertilizados, extraídos de una hembra capturada en la costa de Languedoc, sur de Francia, Norte del Mediterráneo, en el 2002. Barra de escala= 50 mm. Fotografía con autorización de Capapé et al. (2007)

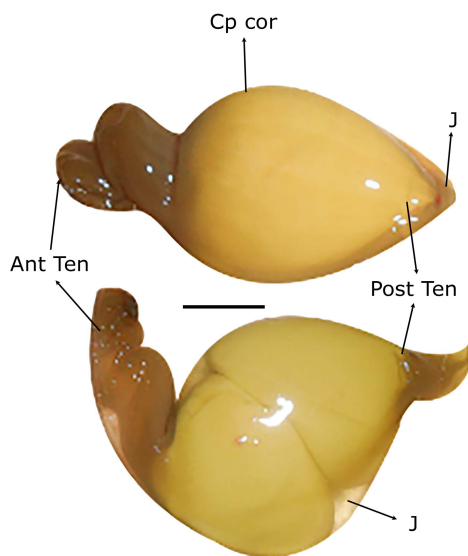


Figure 17. Two uterine egg capsules of *Gymnura altavela* extracted from a female captured off the coast of Syria, eastern Mediterranean Sea in 2011. One of the egg (on top) capsules contained a single egg and the other one, two. Photo by: H. Alkusaury. Cp cor= capsule corpus; Ant Ten= anterior tendrils; Post Ten= Posterior tendrils; J= jelly. Scale bar= 50 mm / Dos cápsulas ovígeras uterinas de *Gymnura altavela* extraídas de una hembra capturada en aguas de Siria, Mediterráneo Central, en 2011. Una de las cápsulas (imagen superior) contenía un huevo y la otra dos. Cp cor= cuerpo de la cápsula; Ant Ten= zarcillos anteriores; Post Ten= zarcillos posteriores; J= clara. Barra de escala= 50 mm. Foto de H. Alkusaury



Figure 19. Encapsulated uterine egg of *Myliobatis ridens* collected off South Brazil in 1982. Scale bar= 20 mm. Photo by M. C. Oddone / Huevos uterinos encapsulados de *Myliobatis ridens* colectados en el Sur de Brasil en 1982. Barra de escala= 20 mm. Fotografía de M. C. Oddone

DISCUSSION

In this paper, results include different instances of the egg encapsulation process and diversity of the tertiary egg envelope from early pregnant to postpartum females, in five shark orders and four skates and rays orders of from rather different areas. From oviparity to the different forms of viviparity, a wide range of tertiary membranes, in terms of morphology and complexity, was observed. This diversity reflects the oviducal gland structure; a unique structure that attains its largest size in oviparous species among elasmobranchs (Hamlett *et al.* 2005a). In viviparous species, the oviducal gland tends to be reduced or even vestigial, being capable to secrete albumin (egg jelly) and mucus in all species and egg capsule proteins in the oviparous (Wourms 1977). Secreting the tertiary egg envelope is not the only function of the oviducal gland; it is also able to store sperm; protect it from immunological attack by the mother and nourish it (Hamlett *et al.* 2005a). The description or even mention of the tertiary membrane in viviparous species, especially on those having a transient tertiary membrane, has been neglected in the literature. Most reproductive studies only focus on recording the presence or absence of uterine eggs. Of special importance are those studies that have fortunately photographically recorded the early gestation stages, which have particularly chosen over this discussion. Observations on the general morphology of the tertiary membrane types discussed have been arranged here by complexity rather than by taxon.

THE CANDLE CAPSULE TERTIARY MEMBRANE

Yolk-sac viviparous species have a wide variety of tertiary membranes, ranging from a thin and transient membrane, such as the candle capsule, to more conspicuous and tough forms (see below). The presence of transient candle capsules is a characteristic shared by a group of yolk-sac viviparous species (sharks and rays). Candle capsules can be polyvitelline or monovitelline. The former have been recorded in some yolk-sac viviparous species like Hexanchiformes, Squatiniformes and Carcharhiniformes and Pristiophoriformes. In *S. guggenheim* candles can bear five eggs (Sunye & Vooren 1997, this study). However, the presence of encapsulated eggs may be a characteristic unshared by the Squatiniformes, as were not recorded for *S. oculata* and *S. squatina* (Capapé *et al.* 1990, 2002). According to Sunye & Vooren (1997) Capapé *et al.* (1990, 2002) did not find egg capsules probably because they studied the uteri after embryos hatching.

Polyvitelline capsules may be common in genus *Squalus*, as they were recorded capsules with three to five eggs in *S. acanthias* (von Bonde 1945, Jones & Ugland 2001, Hamlett *et al.* 2005b, Oddone *et al.* 2015), one or two in *S. megalops* (Braccini *et al.* 2007) and two to five in *S. mitsukurii* (Oddone *et al.* 2010, Furumitsu *et al.* 2012, Vooren & Oddone 2019). Eggs can be also individually encapsulated, forming a monovitelline candle capsule (Orozco-Velásquez & Gómez-Delgado 2016) in *S. cubensis* and Common sawshark *Pristiophorus cirratus* (Hamlett *et al.* 2005a).

Heptranchias perlo, from the oldest shark order, dating at least back to the lower Jurassic (Musick & Ellis 2005) was observed to produce a thin, transparent monovitelline egg capsule. In *Hexanchus griseus*, however, candles were not recorded, being the eggs capsulated individually in extremely thin double membrane, that does not assume an ellipsoidal shape; because of its consistence, the shape of this membrane is the same of that of the egg.

THE EGG ENVELOPE OF SOME TRIAKIDAE AND PLACENTOTROPHIC CARCHARHINIFORMES

In *G. galeus* eggs were individually encapsulated by a tertiary membrane thinner than the candle. Also, unlike any of the abovementioned species *G. galeus* develops uterine compartments during gestation (Capapé *et al.* 2005, Walker 2005). This configuration is shared with other members of the Triakidae family (*e.g.*, *Mustelus antarcticus* and *M. schmitti* which, as *G. galeus*, are confirmed viviparous species with limited histotrophy). In such species in spite of the presence of uterine compartments as in typical placentotrophic species (Hamlett & Koob 1999), no placenta is developed (Storrie *et al.* 2009, Orlando *et al.* 2015, Vooren & Oddone 2019). According to Musick & Ellis (2005) the line between yolk-sac viviparity and limited histotrophy may be difficult to discern without data on the organic content of the eggs and term embryos. Within placentotrophic species, as *M. fasciatus* and *M. mediterraneus* (Capapé & Quignard 1977, Vooren & Klippel 2005) recorded in this study, a particular kind of tertiary membrane has been reported. It is greatly reduced in thickness, reflecting altered function of the oviducal gland, a diaphanous filmy (acellular) membrane rather than an egg capsule, according to (Hamlett & Koob 1999). The membrane is initially pleated but unfolds as the embryo grows (Hamlett *et al.* 2005a). The dimension and thickness of this membrane is shown in a photograph showed by (Fig. 3b, Vooren & Oddone 2019) of a recently in utero egg extracted from an early pregnant female of *G. galeus* captured off South Brazil. In this way, Castro & Wourms (1993) described the tertiary membrane of the Atlantic Sharpnose shark, *Rhizoprionodon terraenovae* as a “sac-like structure”. The tertiary membrane is in most cases -except for *P. glauca* recorded in this study, and genus *Scoliodon*-, retained during gestation, tightly incorporated into the closely interdigitated junction between maternal and embryonic tissues (TeWinkel 1963, Lombardi & Files 1993, Hamlett & Koob 1999). Lombardi & Files (1993) studied the configuration of the egg envelope and the transcapsular transport in *M. canis* during gestation. Initially, the envelope material is tightly folded into lateral pleats along the capsular axis forming two darkened and distinct pleated regions. Volume of the early-term intracapsular fluid is minimal (2-3 cc). Later, during terminal phases of gestation, egg capsules encompass a considerably greater volume (approximately 200-250 cc, including the volume of the embryo). Before parturition, the egg envelope accommodates distention of the intracapsular compartment by unfolding of

the lateral and anterior pleats rather than by stretching of the capsular laminae. The total surface area of an unfolded egg capsule is estimated to be between 600 and 800 cc. these authors also studied the permeability of the egg envelope in *M. canis*, finding that glucose and urea readily pass across it, while urea passes across more quickly than glucose. Studies of this kind are lacking for most of the viviparous species.

THE TERTIARY MEMBRANE IN SOME ORECTOLOBIFORMES: AN OVIPAROUS-LIKE TOUGH CAPSULE

By definition, yolk-sac viviparous species are those which undergo a certain loss of organic weight during gestation (Wourms 1981), independently of the presence and complexity of the egg capsule. However, the presence, kind and complexity of the egg capsule should not be ignored. A radical case is that of Nurse shark, *Ginglymostoma cirratum* and Whale shark *R. typus*, where embryos are enclosed in egg capsules for the first months of gestation and after in utero hatching, they continue their development to term (Joung *et al.* 1996, Castro 2000). The egg capsules complexity remains to that of the oviparous species, being completely different of the fragile and transparent candle capsule. This species had been previously considered as ovoviviparous, a term that was abandoned (see Musick & Ellis 2005). Moreover, the Whale shark, had been even previously declared to be an oviparous species, when its egg capsule was recorded for the first time (Baughman 1955).

THE VERSATILE TERTIARY MEMBRANE IN LAMNIFORMES

Within viviparous elasmobranchs, probably the widest variation in the tertiary membrane characteristics may be among Lamniformes. Matthews (1950) inferred that, according to the characteristics of the oviducal gland, the Basking shark *C. maximus* would have a tertiary membrane similar to that of *S. acanthias*, (*i.e.*, a candle capsule). However, the confirmation came only a half a century later, when Ali *et al.* (2012) recorded an early pregnant female containing egg capsules. Castro (2009) recorded uterine egg capsules of the Pelagic thresher *Alopias pelagicus*, both fertilized and unfertilized; he noted that only the first two ovulating eggs are fertilized and each one is individually encapsulated. The capsule is similar at that register in this study for *Alopias* sp. The subsequent eggs are not fertilized and are collectively encapsulated (10-20 eggs) and intended to feed the embryos. These egg capsules are transparent and very similar to the candle capsule in consistency but not in morphology. Unlike the egg capsule of *C. maximus* which has two well delimited ends and tendrils, the egg capsule of *A. pelagicus* seems like a sac with a sole opening and only at anterior (most probably) end. Morphology and characteristics of the egg capsule can also vary within the same species, as recorded for the Sand shark *Carcharias taurus* (Gilmore *et al.* 1983). During early gestation the oviducal gland of *C. taurus*

produced at least six distinct types of egg capsules (Type I to VI). Capsules varied in morphology and content during the progression of gestation. Initially, most encapsulated eggs contain blastodiscs. Afterwards, the capsule content changes to a large amount of yolky unfertilized eggs, developing a flattened tail in one end and a round head and the other one. Yolk content begins to reduce, and capsule turn gel capsules, with no yolk and full of jelly. Before turning embryo capsules, gel capsules have their size reduced and develop a short tail. Variation in the size, shape and number of encapsulated eggs seems to be common in oophagic species, and has been also recorded for other oophagous species like the Bigeye thresher shark *Alopias superciliosus* (Gilmore, 1983) and Crocodile shark *Pseudocarcharias kamoharui* (Fujita 1981).

TERTIARY MEMBRANES IN OVIPAROUS SHARKS AND SKATES: ACTUAL EGG CAPSULES

Wourms (1977) referred to the oviparous tertiary membranes as “leathery” egg capsules. This tough consistency is due to the physical structure of the egg capsule walls; which consists of an albumin layer and orthogonally stacked layers of protein fibrils and secreted by the oviducal gland (Lombardi 1998). However, there is a wide variety of egg capsules wall complexity, even totally transparent ones, as the recently described by Nakaya *et al.* (2020) for the Sarawak swellshark *Cephaloscyllium sarawakensis* Yano, Ahmad & Gambang, 2005. Egg capsules of skates are quadrangular with horn-like processes at each corner; the dorsal surface is usually arched while the ventral surface is flattened (Wourms 1977), though in some cases, both surfaces are equally convex (Oddone *et al.* 2004). The morphology of the skate’s egg capsules has been subject of taxonomic and phylogenetic studies since Ishiyama (1958). Nowadays, there are published descriptions of the egg capsules for 35% of the skates (M. C. Oddone, unpublished data), see for instance, Ishiyama (1958), Treloar *et al.* (2006), Ebert & Davis (2007), Mabragaña *et al.* (2011), Ishihara *et al.* (2012), Gordon *et al.* (2016), Cordeiro & Oddone (2019), Mancusi *et al.* (2021).

Unlike the quadrangular egg capsules of skates, sharks egg capsules, tend to be more rounded and resemble ellipsoids (Wourms 1977). Exceptions to these patterns exist, the most radical may be fascinating egg capsule of Heterodontiformes, like the one of *H. mexicanus* reported in this study. The screw-shaped egg capsules of *Heterodontus* spp., is a result of the spiral capsule flange, which form such a remarkable feature and is directly related to the oviposition behaviour (Smith 1942). Studies on the fine structure and permeability characteristics of elasmobranch egg capsules have been mostly devoted to oviparous species, by means of electron microscopy, histochemistry, chemical analyses, and X-ray diffraction studies (Lombardi & Files 1993). Recently, another source of taxonomical and biological studies has been done through manual collection of egg capsules washed ashore on the beaches (Gordon *et al.* 2016, Cordeiro & Oddone 2019).

In this study, two instances of ovulation in skates are recorded. To our knowledge, such photographic records of ovulation are unprecedented. Templeman (1982) described several instances of ovulation in the Thorny skate *Amblyraja radiata* (Donovan, 1808), during the formation of the egg capsule. The egg enters the capsule when it is at least 70% formed. For Koob *et al.* (1986), the egg capsule formation begins prior to ovulation, and in the oviducal gland, 50% of the egg capsule has been formed. Our observations demonstrate that in *A. platana* and *A. cyclophora* about 40-50% of the egg capsule had been secreted when the yolk has reached the oviducal gland. Though the time length of these processes are extremely difficult to access, ovulation and passage of the egg into the capsule may be a rapid processes, and therefore difficult to observe.

THE EGG CAPSULES OF GUITARFISHES

Reports of the tertiary membrane in rhinobatids are scarce. Most studies mention the presence of uterine eggs and or its size with no further description (*e.g.*, Torres-Huerta *et al.* 2019). Lessa *et al.* (1986) reported information on the egg capsule of the Brazilian guitarfish *Pseudobatos horkelii* (Müller & Henle, 1841), where hatched egg capsules were found in utero, like in the two rhinobatids recorded in this study. Photographic record of fresh egg capsules with diapause eggs was also reported. In *P. horkelii*, Romo-Curiel *et al.* (2017) reported photographic record of the egg capsule of the Shovelnose guitarfish *Pseudobatos productus* (Girard, 1854), referring to them as candles. Each egg capsule is able to bear six eggs in that species. For *Z. brevirostris*, Colonello *et al.* (2011) mentions that “Females with uterine eggs contained within a brown-coloured proteinaceous membrane were collected from winter to spring months”. In the present study, egg capsules of *P. percellens* and *Z. brevirostris* are reported. In spite of the fact that in both cases material observed was preserved, the actual structure of the tertiary membrane seems to be tougher of that composing the candles. Embryonic diapause has been documented in at least six rhinobatids (Wyffels 2009), which may be related with the more complex egg capsule.

THE ABSENCE OF A TERTIARY MEMBRANE IN SOME BATOIDS

To our knowledge, apart from the case of *T. torpedo* reported here, the lack of tertiary membrane are restricted to some batoids (except for the *Squatina* spp. abovementioned) and have been recorded for *Urobatis halleri* and *U. jamaicensis* (Babel 1967, Hamlett *et al.* 2005a) and for the genus *Narcine*, where also the oviducal gland does not exist (Prasad 1945, Burgos-Vázquez *et al.* 2017).

THE TERTIARY MEMBRANE OF STINGRAYS

Of all the described tertiary membranes those of the Myliobatiformes has been the most neglected. After fertilization, eggs are encapsulated along with a small amount of egg jelly and fluid within a soft and pliable tertiary egg envelope (Wyffels 2009). The egg envelope of *M. ridens* recorded in this study was extremely thin and easily torn while handling for photographing. Araújo *et al.* (2016) recorded the presence of egg capsules in Southern eagle ray *Myliobatis goodei* Garman, 1885 and in *M. ridens*. The fresh egg capsules of these species are extremely fragile to touch, being extremely difficult to extract them intact from the uteri (P. Araújo, pers. comm.¹). In the species recorded so far, egg capsules share the shape of an ellipsoid, somewhat compressed by its extremes. Hamlett *et al.* (2005b) recorded this pattern in the Yellow stingray *Urobatis jamaicensis* (Cuvier, 1817). Also, the egg capsules of this group are small, in proportion to the female size, when comparing with egg capsules of other rays like rhinobatids. This may be related to the lipid histotrophic reproductive mode (Hamlett *et al.* 2005b).

FINAL CONSIDERATIONS AND CONCLUSIONS

Prasad (1951) stated that little attention seems to have been paid to the study of the egg capsules of viviparous elasmobranchs. Four decades later, little or no information was available on the composition and organization of greatly thinned tertiary membrane that occur in many viviparous matrotrophic species (Lombardi & Files 1993). For these authors, the general configuration of the egg capsule in viviparous species is a “contiguous ellipsoid sac which surrounds the entire embryo and serves to contain the perimbryonic (=intracapsular) fluid”. Their observations on the egg envelope of *M. canis* indicate that the basic structural organization is comparable to that reported in oviparous species. However, these characteristic does not fit all the tertiary membranes reported so far, given the actual wide variety. According to Musick & Ellis (2005), the term “aplacental viviparity” obfuscates the true diversity of elasmobranch reproductive modes. Something similar probably happens with the actual term “yolk-sac viviparous”, because is obfuscate the true diversity of egg tertiary envelopes. Table 2 summarizes our reports on the egg tertiary membrane in elasmobranchs, for yolk-sac viviparous. There are six major patterns, which have been named Type I to VI. In this way, species with such contrasting characteristics in early embryonic development, such as *S. acanthias*, and *R. typus*, may be referred as yolks-sac viviparous type III and IV, respectively. In this paper, the focus was not on the fine structure of the elasmobranch tertiary membrane. This could be subject of future studies, to elucidate the similarities and differences among taxa.

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Table 2. Proposal of new terminology for the yolk-sac viviparous elasmobranch reproductive mode (Type I to VI) based on the gross morphology of the seven tertiary membrane patterns discussed in this study. The column 'taxa' includes shark and rays orders, but within some of them, several reproductive modes may occur. A single species is given as an example, from this study. The main characteristics of the tertiary membrane are provided / Propuesta de nueva terminología para el modo vivíparo saco vitelino en elasmobranchios (Tipo I a VI), con base en la morfología de los siete patrones de membranas terciaria discutidos en este estudio. La columna 'taxa' incluye órdenes de tiburones y rayas, pero dentro de estos, varios modos reproductivos pueden existir. Una especie de este estudio se indica como ejemplo para cada caso. Se presentan las principales características de la membrana terciaria

Tertiary membrane	Name	Taxa	Example	Characteristics	Reference
1. Thin membrane	Type I	Hexanchiformes	<i>H. griseus</i>	Same as egg (round), double, transparent	Ben Amor <i>et al.</i> (2020) This study
2. Candle capsule (monovitelline)	Type II	Pristiophoriformes Hexanchiformes Carcharhiniformes	<i>H. perlo</i>	Short ellipsoid, transparent Extremes short, robust	This study
3. Candle capsule (polyvitelline)	Type III	Squatiniiformes, Carcharhiniformes	<i>S. guggenheim</i>	Elongated ellipsoid-cylinder Extremes short, robust	This study Jones & Uglund (2001) Oddone <i>et al.</i> (2015)
4. Oviparous-like tough egg capsule	Type IV	Orectolobiformes	<i>R. typus</i>	Quadrangular, dorsoventrally flattened, conspicuous	Baughman (1955) Joung <i>et al.</i> (1996)
5. Guitarfishes candle	Type V	Rhinopristiformes	<i>P. percellens</i>	Short ellipsoid, brownish, tougher than the shark candle	This study
6. No tertiary membrane	Type VI	Torpediniformes	<i>T. torpedo</i>	-	El Kamel-Moutalibi <i>et al.</i> (2013) This study

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