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Comparative embryonic development patterns in three deep-water skates from the southwest Atlantic

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## 1 **Comparative embryonic development patterns in three deep-water skates from the** 2 **southwest Atlantic**

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### 11 Abstract

12 Embryonic development is a vulnerable and key period during the life cycle of an oviparous  
13 elasmobranch. Captivity studies are difficult to carry out on non-coastal species; hence,  
14 embryonic development can be analysed from samples collected from the seabed. Here,  
15 embryonic development of three shelf and deep-water skates from the southwest Atlantic Ocean,  
16 *Bathyraja brachyurops*, *B. macloviana* and *Amblyraja doellojuradoi* was studied. Egg cases  
17 containing embryos in different stages were collected from 84 to 1006 m depth in the northern  
18 part of the Argentinean continental shelf and continental slope (36°S-41°S), including the Mar  
19 del Plata Canyon (38° S). Common development patterns were observed among the three species  
20 and also with other skate species previously studied. Anatomical structures and embryo features  
21 were similar among species in initial, early and middle stages. Advanced and pre-hatching  
22 embryos showed species-specific differences regarding pigmentation and spinulation. This study  
23 sheds light on the identification of neonates of shelf and deep-water species, which could be  
24 useful to recognize specific nursery areas in the deep ocean. In addition, this embryological  
25 comparative study expands the existing database on the different chondrichthyan lineages, thus  
26 making a step forward towards understanding of their phylogenetic relationships.  
27

### 28 Keywords

29 *Amblyraja*, *Bathyraja*, Deep-sea, Embryonic stages, Nursery areas, Pre-hatching embryos.  
30

### 31 1. Introduction

32

33 Skates comprise a diverse order (Rajiformes) of benthic and demersal chondrichthyans.  
34 They are oviparous, a reproductive mode also found in three families of sharks (Heterodontidae,  
35 Scyliorhinidae, and Hemiscylliidae) and holocephalans (Hamlett, 2005). Eggs are fertilized and  
36 encapsulated in a structurally complex capsule and then laid onto the sea floor, where the embryo  
37 develops by feeding on the yolk until hatching. Skates play important ecological roles in marine  
38 ecosystems, acting as energetic linkers between different habitats and as bioturbators by  
39 removing the sediments when feeding (Martins et al., 2018). In addition, they play key trophic  
40 roles in benthic communities since they consume a wide range of prey items, but also are preyed  
41 on by several organisms (Ebert and Bizarro, 2007; Barbini et al, 2018; Martins et al., 2018).  
42 Among members of this order, genera *Bathyraja* and *Amblyraja* (Families Arhynchobatidae and  
43 Rajidae, respectively) are distributed worldwide and are by far the most common skates in the  
44 deep-sea ocean (Last and Yearsley, 2002), occurring on continental shelf and slope, reaching  
45 depths of more than 3100 m (Last et al., 2016; Weigmann, 2016).

46 Even though deep-water marine environments are difficult to access, much progress has  
47 been made into the field of taxonomy and biology of deep-water skates (Ebert, 2015; 2016; Last  
48 et al., 2016). Even more, valuable information in deep-water skates has recently been obtained  
49 by means of *in situ* observations using remotely operated and autonomous underwater vehicles  
50 (ROVs and AUV's) (Hoff, 2010; Amsler et al., 2015; Salinas-de-León et al., 2018; Kuhnz et al.,  
51 2019). However, the available information regarding some aspects of life history is still  
52 fragmentary. Embryonic development, for instance, is almost unknown for most shelf and deep-  
53 sea skates.

54 Embryonic development is a vulnerable and key period during the life cycle of an  
55 organism that determines survivability of species. Skate egg cases have anti-microbial and  
56 antifouling properties (Kormanik, 1993; Thomason et al., 1996) and also provide mechanical  
57 protection to the developing embryo. However, some predators such as gastropods, feed on egg  
58 cases, which is the most widely reported mortality source in skate embryos (Cox and Koob,  
59 1993; Lucifora and García, 2004). Early embryonic stages are more susceptible to predators than  
60 later stage embryos, given the fact that a more voluminous and nutritive yolk is present at the  
61 beginning of development. Early embryos have a large external yolk sac and are embedded in a  
62 jelly matrix, whereas later stage embryos are bigger and the yolk sac dramatically shrinks in size.  
63 Some *Bathyraja* embryos absorb yolk slowly during early development, and much more rapidly

64 at later stages (Hoff, 2009). Predators could indeed prefer freshly deposited egg cases over those  
65 in advanced stages since these have thinner walls and, therefore, are easier to bore into. Mortality  
66 caused by predation is likely to differ among skate species as predation rates do (Lucifora &  
67 García, 2004). Understanding embryonic development as a whole, including internal processes  
68 and external threats can help shed light on early-life mortality, which is relevant to fish  
69 recruitment. Also, a clear understanding of all chondrichthyan life stages can lead to implement  
70 better and more successful management and conservation strategies.

71 Most skate development studies have been carried out by keeping individuals in captivity  
72 (Luer and Gilbert, 1985; Koop, 2005; Harahush et al., 2007; Jañez and Sueiro, 2007; Mabrugaña  
73 et al., 2015). In these conditions, detailed monitoring of embryonic development is possible from  
74 the day of laying until hatching. Species that live at great depths (beyond 200 m) are especially  
75 adapted to low temperatures, low light intensity and high hydrostatic pressure (Marshall, 1979;  
76 Herring, 2000), conditions that cannot be easily achieved in experimental environments.  
77 Information on embryonic development in these species is sparse and comes from studying egg  
78 cases collected from the seabed (Stehmann and Merrett, 2001; Scenna, 2011; Henry et al. 2016).  
79 First studies focused on marine life inhabiting the depths of the Argentine sea began only a few  
80 years ago, with 4 cruises sampling up to 3500 m depth (Flores et al., 2019; Penchaszadeh et al.,  
81 2019). During these expeditions egg case nursery sites were identified for several oviparous  
82 chondrichthyans, including those of genera *Bathyraja* and *Amblyraja* (Vazquez et al., 2016).  
83 However, embryonic stages from these egg cases were not analysed.

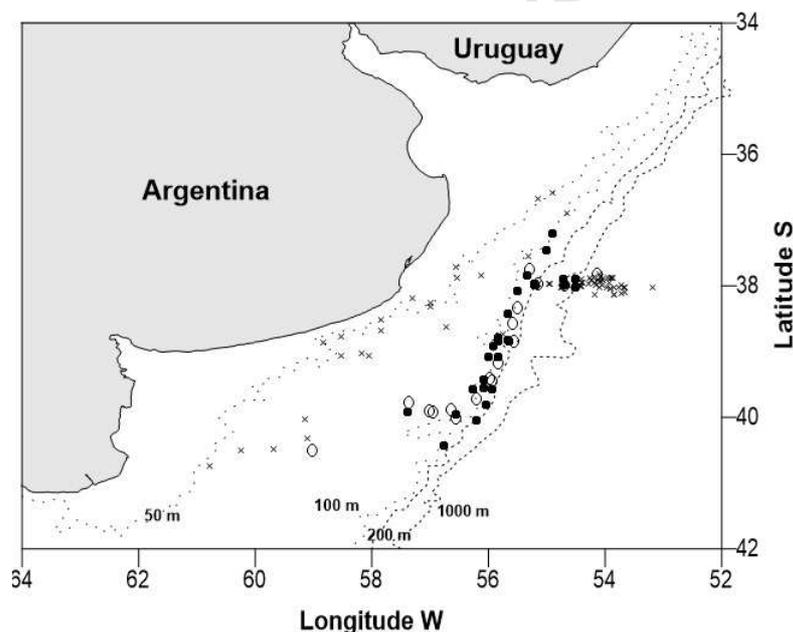
84 Here, as part of a major study delving deeper into the southwest Atlantic (SWA) shelf and  
85 slope habitat, stages of embryonic development of the broadnose skate *B. brachyurops*, the  
86 Patagonian skate *B. macloviana* and the southern thorny skate *A. doellojuradoi* are described,  
87 focusing on similarities and differences between initial, early, middle, advanced and pre-hatching  
88 development stages. This study sheds light on the identification of neonates of shelf and deep-  
89 water species, which can be useful to recognize specific nursery areas in the deep ocean. This  
90 paper can help to elucidate phylogenetic relationships through the comparison of the  
91 morphological changes that occur throughout embryonic development in different  
92 chondrichthyan lineages.

93

## 94 2. Materials and methods

## 95 2.1 Sample collection

96 Sampling was carried out in the northern part of the Argentinean continental shelf and  
 97 continental slope (36°S-41°S), including the Mar del Plata Canyon (38° S). Depth range  
 98 surveyed was from 50 to 3447 m (Figure 1). Samples came from eight research cruises carried  
 99 out on board of the Argentinean O/V Puerto Deseado (OVPD) and commercial vessels between  
 100 2009 and 2014. Samples from research cruises were collected using two bottom trawls, a shrimp  
 101 net (50 mm mesh in the wings, and 20 mm in the cod end; vertical height 1 m, horizontal  
 102 opening 4 m) and a bottom trawl net (135 mm mesh in the wings, and 60 mm in the cod end;  
 103 vertical height 3.7 m, horizontal opening 10 m). Two dredges (horizontal openings 0.6 and 0.8  
 104 m) were also used. Those from commercial vessels were collected using a bottom trawl net  
 105 (mesh size 120 mm, horizontal opening 22 m).



106 **Figure 1.** Location of samples collected in the northern part of the Atlantic continental shelf and  
 107 continental slope (36°S-41°S), including the Mar del Plata Canyon (38°S). Black circles: sites  
 108 with egg cases with embryos; empty circles: sites with empty egg cases; crosses: sites with no  
 109 catches.  
 110

## 111 112 2.2 Egg cases identification and description of development stages

113 Most samples came from research cruises and were frozen on board. Those egg cases  
 114 from commercial vessels were fixed in formalin 4% since it was not possible to freeze them on  
 115 board. Egg cases were examined in the laboratory. They were identified following Mabragaña et

116 al. (2011). Egg case length without horns (ECL) and maximum width (MAW) were recorded to  
117 the nearest 0.5 mm. Egg cases were opened and the following measurements and features were  
118 recorded for the embryos: embryo position relative to the egg case, embryo total length (TL) and  
119 disc width (DW), external and internal (if present) yolk-sac diameter, and sex. A relative  
120 development stage was assigned to each embryo following Ballard et al. (1993) and Caldeira  
121 Brant (2003), who proposed a total of 34 stages (in sharks) and 35 (in skates), respectively.  
122 These authors consider the first 17 stages as microscopic. In addition, a recent embryo scale of  
123 five stages (I to V) proposed by Luer et al. (2007) was considered. Morphological features such  
124 as pharyngeal arches, external gill filaments, development of pectoral and pelvic fins, formation  
125 of eyes and spiracles, pigmentation and spinulation patterns were recorded for each species. All  
126 these features allowed assignment of the embryonic development stage. Each embryo was  
127 photographed using a digital camera or microscope camera (Biotraza) when needed. Embryonic  
128 stages were grouped into initial (< 17), early (18 to 24, I to III), middle or intermediate (25 to 29,  
129 III to IV), advanced (30 to 34, IV to V) and pre-hatching (35, V) for subsequent comparison  
130 between species. Egg cases and embryos were fixed in formalin 4% and stored in alcohol 70% in  
131 the Fish Collection of Instituto de Investigaciones Marinas y Costeras (IIMyC), Mar del Plata,  
132 Argentina (IIMyC-CEGAR 12, 13, 16, 21, 41, 71-74, 89, 105-110, 112, 114, 117, 160-163, 166-  
133 169, 172, 174-184, 186, 187, 190-192, 195-197, 212-215 and 221). Given the difficulty of  
134 obtaining deep-water samples, a complete collection of all stages of embryonic development of  
135 each deep-water skate species was not possible. Descriptions of development were realized by  
136 comparing similar stages, and only the best representative photos of them are presented.

137         Given that egg cases of *Amblyraja doellojuradoi* were collected over a wide depth range  
138 (95 to 1006 m), small pieces of tissue sample were taken in some embryos in order to check that  
139 they corresponded to the same species. Samples were preserved in 96% ethanol at -20° C for  
140 genetic analysis. DNA extraction, polymerase chain reaction (PCR), and sequencing of the 5´  
141 region of the COI gene were performed following standard DNA barcoding protocols (Ivanova  
142 et al., 2006) coupled with primers and primer cocktails developed for fishes (Ward et al., 2005;  
143 Ivanova et al., 2007). DNA extraction and amplification were performed at the Argentine  
144 International Barcode of Life Laboratory reference (IIMyC, CONICET, Mar del Plata,  
145 Argentina). Sequencing was performed in Advanced Analysis Centre's Genomics Facility  
146 (College of Biological Sciences, University of Guelph, Ontario Canada) and the Canadian Centre

147 for DNA Barcoding (CCDB) at the Biodiversity Institute of Ontario, (University of Guelph,  
 148 Ontario, Canada). Using the library of sequences available on BOLD (Barcode of Life Data  
 149 Systems), the closest matches to our DNA sequences were obtained.

150

### 151 3. Results

#### 152 *3.1. Collected samples*

153 Fifty two egg cases containing embryos were collected from 84 to 1006 m depth: 19 of  
 154 *Bathyraja brachyurops* (ECL range: 76.9-107.4 mm, MAW range: 51.7-64.7 mm), 18 of  
 155 *Bathyraja macloviana* (ECL range: 68.2-95.1 mm, MAW range: 40.5-50.1 mm) and 15 of  
 156 *Amblyraja doellojuradoi* (ECL range: 60.9-81.6 mm, MAW range: 36.1-52.9 mm). When  
 157 compared to the BOLD “Species Level Barcode Records” database, all deep-water *Amblyraja*-  
 158 like samples (IIMyC-CEGAR 160, 162 and 163) matched *A. doellojuradoi* with a similarity  
 159 range of 99.85-100% supporting that all samples belonged to this skate species. Information  
 160 about development stage of each embryo, number of embryos analysed and depth range is  
 161 summarized in Table I.

162

163

Species	Proposed staging	TL (mm)	DW (mm)	Caldeira Brant (2003)	Luer et al. (2007)	Total	Depth (m)
<i>Bathyraja brachyurops</i>	Initial	-	-	<17	I	7	95-136
	Early	21.4	<5	23	III	1	98
	Middle	23	<5	24/25	III	1	104
	Middle	30.5	<5	25	III	1	104
	Middle	58.3	11.4	28/29	IV	1	95
	Middle	59.8-62.9	11.5-12.3	29	IV	2	98
	Advanced	70.3	20.4	30	IV	1	98
	Advanced	90.2-92.9	26.2-26.8	32/33	IV	3	95-98
	Advanced	108.4	39.5	33/34	IV	1	136
Pre-hatching	155.5	82.5	35	V	1	251	
<i>Bathyraja macloviana</i>	Initial	-	-	<17	I	6	97-110
	Early	9.23	<5	19	III	1	117
	Middle	34.3-35.7	4.2-5.3	26	IV	2	98-108
	Middle	40	5	27	IV	1	96
	Middle	65.9	15.5	29	IV	1	84

	Advanced	95	33.3	32/33	IV	1	105
	Advanced	101	42	33	V	1	97
	Advanced	100.8-128.7	38.9-54.8	33/34	V	2	92-105
	Advanced	122.1-131.1	55.8-65.3	34/35	V	2	84-105
	Pre-hatching	135	71.6	35	V	1	105
	Initial	-	-	<17	I	8	95-852
	Early	17	<5	19/20	III	1	998
	Middle	39.6	4.4	25/26	III/IV	1	998
<i>Amblyraja doellojuradoi</i>	Middle	63	11.8	29/30	IV	1	110
	Advanced	74.3-76.5	22.9	31	IV	2	852-1006
	Advanced	86.6	31.3	32	IV/V	1	998
	Pre-hatching	97.3	44.4	34/35	V	1	780

164 **Table 1.** Embryonic stages found for *Bathyrāja brachyurops*, *Bathyrāja macloviana* and  
 165 *Amblyraja doellojuradoi* considering Caldeira Brant (2003) and Luer et al. (2007) staging table.  
 166 TL: Embryo total length and DW: disc width. “Total” indicates the number of embryos analysed.  
 167 “Depth” shows the depth range where egg cases with embryos were collected, in meters.

168

### 169 3.2. Initial embryonic stages

170 Initial stages are characterized by the presence of a large external yolk sac. The full  
 171 content is embedded in a jelly matrix, which has a protective and supportive function. This  
 172 matrix also blocks the opening of the respiratory canals, which are located along the lateral edge  
 173 of each horn, towards the external environment (Figure 2a).

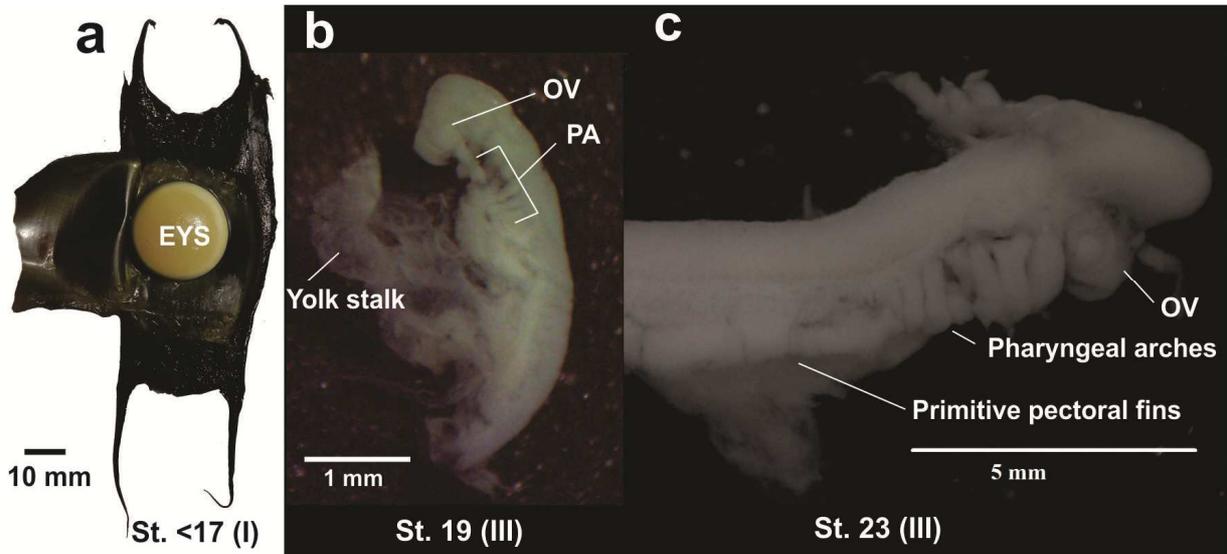
174

### 175 3.3. Early embryonic stages

176 Besides the aforementioned features a small embryo is visible. An anterior curvature in  
 177 the trunk is present forming the head region, where optic vesicles and six pairs of pharyngeal  
 178 arches are already developed. These arches are opened on their ventral side so that the mouth is  
 179 not yet formed. The embryo is attached to the yolk sac through a long yolk stalk. Neither fins nor  
 180 pigmentation are observed (Figure 2b).

181 In later stages (by stage 23), pharyngeal arches start to close ventrally delimiting the  
 182 primitive mouth and behind them pectoral fins begin to emerge as small flaps on either side of  
 183 the trunk (Figure 2c).

184



185

186 **Figure 2.** Egg case of *Amblyraja doellojuradoi* initial stage with a remarkable external yolk sac  
 187 (a); early stages of *Bathyraja macloviana* (b) and *B. brachyurops* (c). EYS: external yolk sac,  
 188 OV: optic vesicles, PA: pharyngeal arches. Embryonic stage (St.) corresponds to Caldeira Brant  
 189 2003 (arabic numbers) and Luer et al. 2007 (roman numbers in brackets).

190

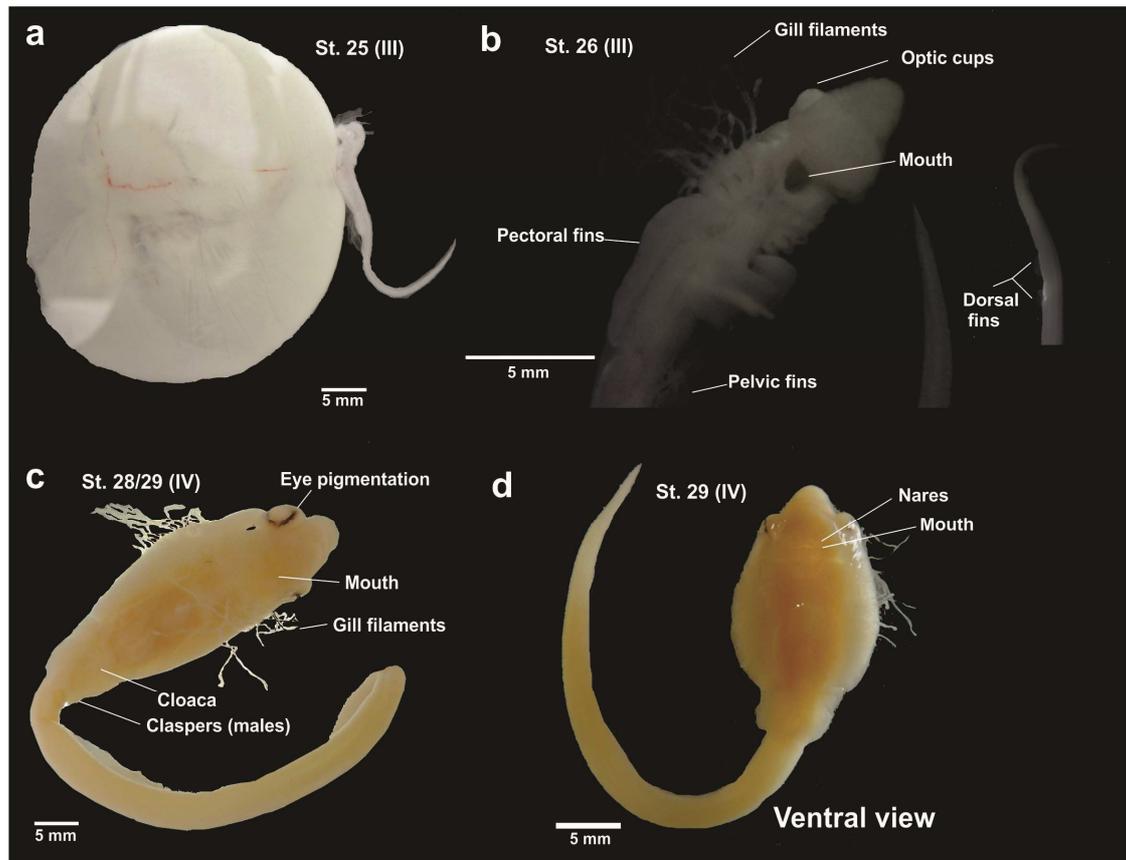
### 191 3.4. Middle embryonic stages

192 Both rounded-shaped pectoral and pelvic fins are observed and the external yolk sac is  
 193 slightly vascularised (Figure 3a). Buds of external gill filaments are visible on all arches and start  
 194 to gradually grow except in the first arch, where spiracles are differentiated. The tail is  
 195 dorsoventrally covered by a veil or membrane from which the two dorsal fins develop  
 196 posteriorly, and the cloaca can be observed. In the head region, the mouth becomes circular and  
 197 optic cups are visible (Figure 3b).

198 Later stages show a lateral expansion of the pectoral fins. The anterior end of these fins is  
 199 not welded to the main axis initially but gradually begins to expand to form a complete disc.  
 200 Pelvic fins have an initial middle notch and then acquire a triangular shape differentiating the  
 201 anterior and posterior lobes. The first outlines of claspers can be observed in those posterior  
 202 lobes in males. At this stage, nares are present, mouth becomes oval and then triangular and a  
 203 ring of pigment is visible around the eyes. Gill filaments reach their maximum length and start to  
 204 be absorbed (Figure 3c).

205 More advanced stages (older than stage 28) show mouth and nares in the neonatal  
 206 position (Figure 3d).

207



208

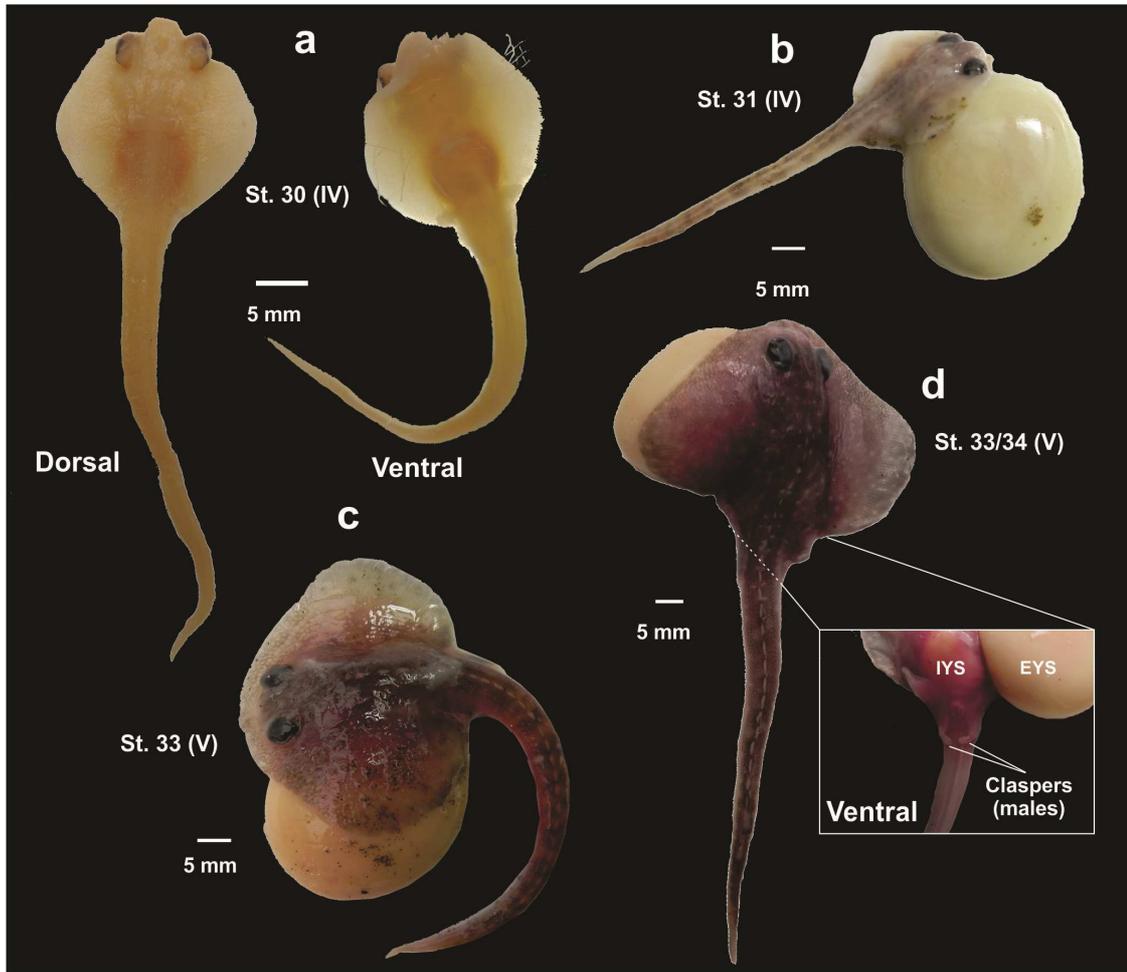
209 **Figure 3.** Middle embryonic stages of *Bathyraja brachyurops* (a, c and d) and *B. macloviana* (b).  
 210 Embryonic stage (St.) corresponds to Caldeira Brant 2003 (arabic numbers) and Luer et al. 2007  
 211 (roman numbers in brackets).  
 212

212

### 213 3.5. Pre-hatching and advanced embryonic stages

214

215 Advanced stages of embryonic development start with the formation of a complete disc  
 216 (Figure 4a). Pelvic fins increase in size and gill filaments begin to reabsorb until they disappear.  
 217 Eyes are completely pigmented (Figure 4b). A small internal yolk sac in the abdominal cavity is  
 218 observed and the external yolk sac is greatly reduced (Figure 4c), with a little remaining external  
 219 yolk at the time of hatching. Males can be easily recognized by the presence of claspers which  
 are absent in females (Figure 4d).



220

221 **Figure 4.** Advanced embryonic stages of *Bathyraja brachyurops* (a), *B. macloviana* (c and d)  
 222 and *Amblyraja doellojuradoi* (b). EYS: external yolk sac, IYS: internal yolk sac. Embryonic  
 223 stage (St.) corresponds to Caldeira Brant 2003 (arabic numbers) and Luer et al. 2007 (roman  
 224 numbers in brackets).

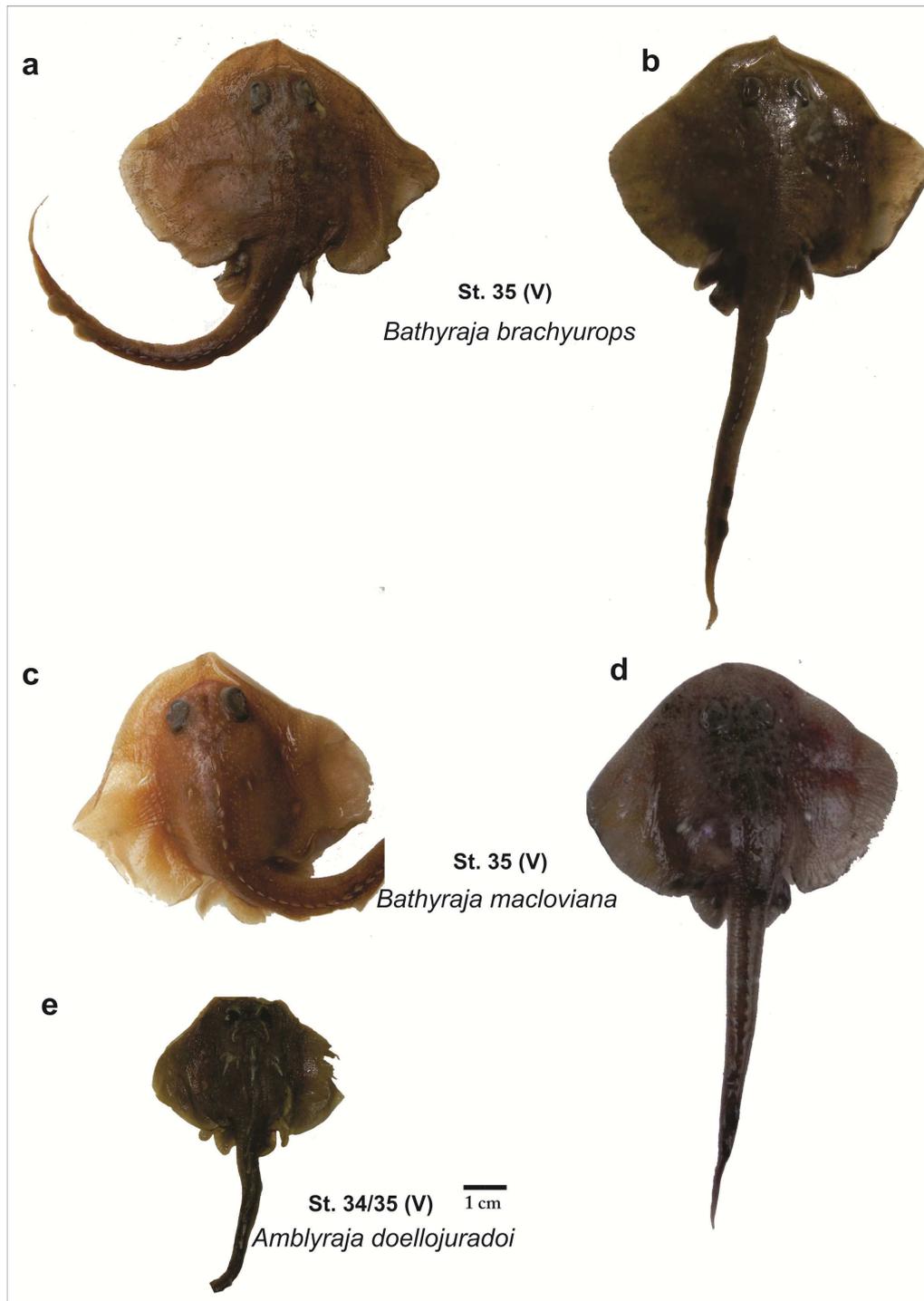
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226

227 Spinulation and body pigmentation patterns are set, and remarkable differences between  
 228 the three species are found. In *Bathyraja brachyurops*, primordia of 17 caudal thorns can be  
 229 observed along the tail (from the middle of the pelvic fins) with an interdorsal primordium,  
 230 which then hardens. Two nuchal thorns subsequently emerge in the midline. The whole embryo  
 231 dorsal surface is covered with small dermal denticles, which are less abundant in the posterior  
 232 margin of the disc. There is a gap between the nuchal and the midline thorns covered with  
 233 denticles (Figure 5a). The first signs of pigmentation appear as small dark dots across the medial  
 234 disc zone. Pigmentation progressively becomes more intense in the central area until it reaches a  
 bright dark brown, with several small white circular spots scattered all over the disc (Figure 5b).

235           The spinulation pattern in *Bathyraja macloviana* starts with 29 primordia in the tail (from  
236 the nuchal region), followed by one or two interdorsal primordia. Subsequently, two ocular  
237 primordia appear in the inner margin of each eye and one primordium of the scapular thorn on  
238 each side of the midline of disc. Dermal denticles are present covering all the dorsal side of the  
239 disc (Figure 5c and d). Body pigmentation begins with brown spots in the centre, then becomes  
240 more intense and covers the entire embryo. Several white spots are distributed on the disc, of  
241 which the two biggest occur in the posterior third of the disc, near the pelvic fin notch (Figure  
242 5d).

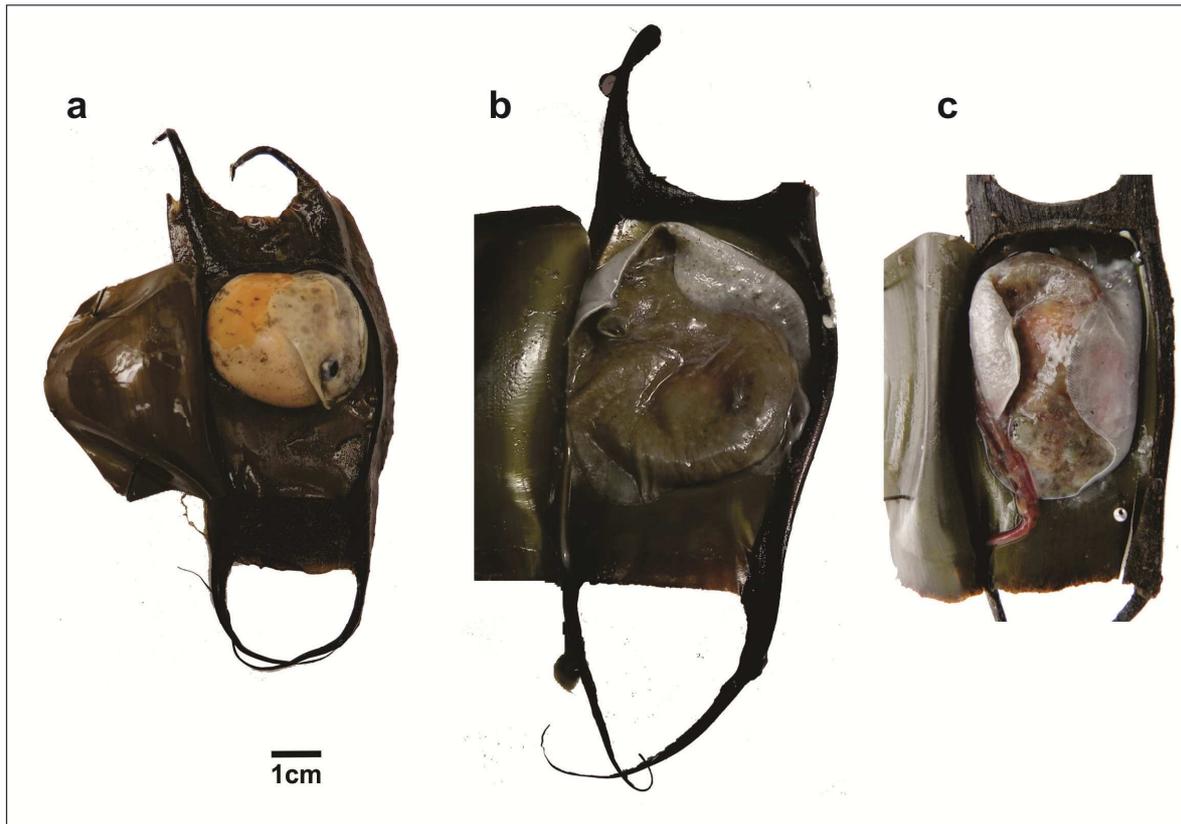
243           Fourteen primordia of thorns can be seen along the tail in *Amblyraja doellojuradoi*, from  
244 the nuchal region to the first dorsal fin (two nuchal plus twelve midline thorns, Figure 4b). Three  
245 primordia of ocular thorns appear in the inner margin of each eye and finally three pairs of  
246 primordia of scapular thorns are arranged on each side of the midline. All of these primordia will  
247 harden to form strong thorns. Dorsal body surface is completely covered with small dermal  
248 denticles. First signs of pigmentation appear as dark irregular spots scattered in the dorsal  
249 surface, except at the edges of the pectoral fins. These spots gradually become more abundant  
250 and fill the entire dorsal surface, thus forming a uniform dark brown colouration pattern. Ventral  
251 face is white (Figure 5e).



252

253 **Figure 5.** Pre-hatching embryonic stages of *Bathyraja brachyurops*, showing the spinulation  
 254 pattern (a) and colouration (b); *B. macloviana*, showing spinulation (c) and colouration (d); and  
 255 *Amblyraja doellojuradoi*, showing both patterns (e). Embryonic stage (St.) corresponds to  
 256 Caldeira Brant 2003 (arabic numbers) and Luer et al. 2007 (roman numbers in brackets).  
 257

258 Embryos can freely move throughout development since they have enough space within  
 259 the egg case (Figure 6a). In pre-hatching embryos space becomes limiting so they fold their  
 260 pectoral fins dorsally over the body and place the long tail over the edge, with a tail filament  
 261 (whip-like extension) next to the respiratory region (Figure 6b). Shortly before hatching, the  
 262 embryo faces its rostrum towards the posterior end of the egg case preparing to hatch (Figure  
 263 6c).



264  
 265 **Figure 6.** Embryo arrangement inside the egg case. *Amblyraja doellojuradoi*, embryo with  
 266 enough space to freely move inside the egg case (a); *Bathyraja brachyurops*, space becomes  
 267 limiting so embryo folds its pectoral fins dorsally over the body and place the long tail over the  
 268 edge (b); and *B. macloviana*, the embryo faces its rostrum towards the posterior end of the egg  
 269 case and is ready to hatch (c).

270

#### 271 4. Discussion

272 Early stages of the life cycle of oviparous chondrichthyans (skates, holocephalans and  
 273 some sharks) include the release of leathery egg cases on the seafloor, where the embryo fully  
 274 develops until hatching. Embryonic development time (also called incubation period) may take  
 275 from several months to years, depending on the species and environment conditions

276 (Berestovskii, 1994; Hoff, 2007; Jañez and Sueiro, 2007; Mabragaña et al., 2015). Even though  
277 complete sets of embryological data are difficult to obtain in non-coastal chondrichthyans, a  
278 comparative description of different embryo stages of three shelf and deep-water skates from the  
279 SWA was performed here.

280 ECL and MAW showed a relatively wide range within each of the species under study.  
281 Previous studies have correlated egg case size in skates with female's length, showing that the  
282 larger the female, the bigger the egg case laid (Templeman, 1982; Scenna, 2011). As the size at  
283 maturity within each species studied here is wide (Paesch and Oddone, 2008; Delpiani, 2016), it  
284 is likely that females of different length lay egg cases of different sizes.

285 Early development features observed here are similar to those reported previously for  
286 other skate species. Early embryos show the typical curvature of the trunk present in the  
287 ontogeny of all vertebrates, and there are no marked differences with embryos of different  
288 chondrichthyan lineages (Ballard et al., 1993; Didier et al., 1998; Caldeira Brant, 2003; Luer et  
289 al., 2007; Maxwell et al., 2008; Onimaru et al., 2018). Development of sharks and skates can be  
290 differentiated at the time when pectoral fins start to emerge as little flaps in the lateral sides,  
291 forming the typical batoid disc. In *Raja eglanteria* (= *Rostroraja eglanteria*) the pectoral fins and  
292 mouth appear first than gill filaments (Luer et al., 2007), the opposite of what was observed for  
293 the three skate species studied here, in which the gill filaments develop first. However, the period  
294 of time between these two events seems to be very short and insignificant, since in *Sympterygia*  
295 *acuta* mouth appears shortly before pectoral fins and gill filaments (Caldeira Brant, 2003).

296 Middle stages do not exhibit major differences compared with those observed by  
297 Maxwell et al. (2008) for *Leucoraja ocellata*, Luer et al. (2007) for *R. eglanteria* and Caldeira  
298 Brant (2003) for *S. acuta*. In these skate species gill filaments appear for the first time from  
299 stages 24-25. This is also seen in shark and chimaera embryos (Ballard et al., 1993; Didier et al.,  
300 1998; Onimaru et al., 2018). Dorsal fins can be distinguishable at late stage 26 and early stage 27  
301 as also happens in other skate species (Caldeira Brant 2003; Maxwell et al., 2008) and the shark  
302 *Chiloscyllium punctatum* (Onimaru et al., 2018). In other shark species such as *Scyliorhinus*  
303 *canicula* (Ballard et al., 1993) and holocephalans (Didier et al., 1998), dorsal fin development  
304 seems to be delayed, appearing at stage 30 and 28, respectively. Copulatory organs or claspers in  
305 males appear at the same stage 29-30 in skates (Caldeira Brant, 2003; Maxwell et al., 2008;  
306 present work) and in *C. punctatum* (Onimaru et al., 2018) whereas in *S. canicula* (Ballard et al.,

307 1993) claspers are recognized from stage 31. A delay in claspers development is already  
308 observed in chimaeras (Didier et al., 1998) appearing from stage 35. When all features are  
309 compared, it seems to be a high concordance in early and middle development stages of several  
310 skates (*L. ocellata*, *R. eglanteria*, *S. acuta* and species studied here), and also in some shark  
311 species (*C. punctatum*). The Chimaera lineage shares many early developmental events with  
312 elasmobranchs, but start to diverge when reaching late middle stages (Ballard et al., 1993; Didier  
313 et al., 1998; Caldeira Brant, 2003, Maxwell et al., 2008).

314 Advanced stages start in a similar way in the three skate species studied here. From stage  
315 30, pectoral fins begin to expand fusing to the head to form a whole disc, pelvic fins become  
316 triangular-shaped and a small yolk sac appears in the abdominal cavity of the embryo. These  
317 events were also reported, but in later stages, in *L. ocellata* (stage 31, Maxwell et al., 2008) and  
318 *S. acuta* (stage 32, Caldeira Brant, 2003). First signs of epidermis pigmentation are visible from  
319 stage 31 in *A. doellojuradoi* (present work) and *S. acuta* (Caldeira Brant, 2003), whereas in *L.*  
320 *ocellata* (Maxwell et al., 2008), *S. canicula* (Ballard et al., 1993), *B. brachyurops* and *B.*  
321 *macloviana* (present work) they begin to be noticeable from stage 32. However, in *C. punctatum*  
322 the typical stripe pigmentation starts from stage 34 (Onimaru et al., 2018). Gill filaments  
323 completely disappear from stages 32 and 33 in the present work as was also reported in *L.*  
324 *ocellata* (Maxwell et al., 2008) and *S. canicula* (Ballard et al., 1993), whereas in *S. acuta* it  
325 happens from stage 34 (Caldeira Brant, 2003).

326 More remarkable differences between the three species became evident in the advanced  
327 stages. Colour and spinulation patterns resembled those of juveniles and adults of *B.*  
328 *brachyurops* reported by Cousseau et al. (2007). The advanced embryos do not have irregular  
329 brown spots on the ventral side of the tail (present work), as does occurs in juveniles and adults.  
330 Spinulation and colouration patterns in *B. macloviana* advanced embryos agreed with those  
331 described for juvenile and adults of this species (Cousseau et al., 2007). Pre-hatching embryos of  
332 *B. macloviana* and *B. brachyurops* can be easily distinguished by the high number of midline  
333 thorns and the presence of scapular and ocular thorns in the former. In addition, *B. brachyurops*  
334 pre-hatching embryos were larger (in both TL and DW) than those of *B. macloviana*, as also  
335 happens in adults of these species (Cousseau et al., 2007). Spinulation and colouration patterns in  
336 *A. doellojuradoi* embryos corresponded with those reported by Cousseau et al. (2007) for  
337 juvenile and adult specimens of this species. Dorsal surface of disc was dull dark brown without

338 the characteristic white spots ahead of the eyes and posterior ends of the pectoral fins. Pre-  
339 hatching embryos of *A. doellojuradoi* were shorter (in both TL and DW) than those of *B.*  
340 *brachyurops* and *B. macloviana*. This is consistent with maximum sizes reported for adults  
341 (Cousseau et al., 2007; Delpiani, 2016).

342 As observed in skate species studied here, similar behaviours of pre-hatching embryos  
343 inside the egg case have been reported by Luer and Gilbert (1985) for *Raja eglanteria*. The  
344 proximity of the tail filament to the gill region seems to be related to more efficiently circulate  
345 seawater through the respiratory canals to facilitate the embryo respiration, as also suggested for  
346 *R. eglanteria*. After hatching, the remaining external yolk sac will be absorbed in the first weeks.

347 Unlike coastal areas, deep water habitats are relatively constant environments, with little  
348 variability in physical conditions such as temperature, salinity and oxygen content. Embryonic  
349 development is mainly dependent on physical conditions of water, and temperature plays a key  
350 role in duration of the incubation period. Coastal skate species are exposed to higher  
351 temperatures than those of deeper waters, and incubation periods tend to be shorter (few months,  
352 Luer et al., 2007; Mabragaña et al., 2015). Conversely, shelf and deep-water skates are exposed  
353 to colder temperatures and have longer incubation periods. Neonates of some species of  
354 *Bathyraja* and *Amblyraja* can take up to 3.5 years to hatch at temperatures of 4.4 °C in the deep  
355 ocean (Berestovskii, 1994; Hoff, 2008). If incubation temperature increases only 0.5°C,  
356 incubation period can be reduced by 16% (six months) in *Bathyraja parmifera* (Hoff, 2008).  
357 Temperature also affects embryonic development itself since metabolism and rate of embryo  
358 development increases markedly with increasing temperature (Hume, 2019). However, no  
359 differences between coastal and shelf and deep-water species were noticed in relation to the  
360 order of appearance of morphological structures throughout development.

361 Skates lay their egg cases in areas known as egg case nurseries, where embryonic  
362 development and neonate hatching take place (Hitz, 1964; Hoff, 2007; 2016). Also, nursery areas  
363 are associated with the presence and occurrence of juveniles (Martins et al., 2018). Egg-laying  
364 sites for the species studied in this paper have recently been discovered in the SWA (Vazquez et  
365 al., 2016). However, areas where neonate and young-of-the-year skates concentrate are still  
366 unknown, possibly due to the difficulty in distinguishing neonates among these species.  
367 Characterization of colouration and spinulation patterns in pre-hatching or advanced embryos is  
368 crucial to unambiguously identify neonate and young skates. McEachran (1983) observed that

369 some species of the South American skate genus *Psammobatis* have conspicuous differences in  
370 spinulation and colour patterns when juveniles and adults are compared. Neonates of  
371 *Psammobatis normani* and *Psammobatis rudis* are different from juveniles and adults regarding  
372 colouration and spinulation patterns. This makes it difficult to recognize nursery areas for these  
373 species (Mabragaña, pers. com.). Identification of areas supporting critical life stages is of great  
374 importance in terms of conservation of these vulnerable species.

375 This work represents the first attempt to characterize embryonic development stages in  
376 three skate species occurring in the continental shelf and slope of the SWA. Furthermore, it  
377 constitutes a contribution to the knowledge of deep-water elasmobranchs since it is the deepest  
378 record of *Amblyraja* embryos worldwide. Other congeneric species of *Amblyraja* possibly co-  
379 occur with *A. doellojuradoi* in the slope. *Amblyraja frerichsi* (Krefft, 1968) was registered in the  
380 SWA from 600-2600 m (Last et al., 2016), *A. georgiana* (Norman, 1938) was recorded in  
381 Malvinas Islands up to 600 m (Agnew et al., 1999), and *A. taaf* (Meisner, 1987) was reported  
382 from northern Burdwood Bank at 1000 m depth (Coggan et al., 1996). Even though all these  
383 other species are larger than *A. doellojuradoi*, their egg cases are unknown. Based on molecular  
384 data, we could confirm that all *Amblyraja*-samples correspond to the same species supporting the  
385 wide depth range of egg-laying recognized by Vazquez et al. (2016) for *A. doellojuradoi*.

386 Chondrichthyan embryos have been used as models in the study of the origin of paired  
387 appendages in vertebrates (Cole and Currie, 2007). As a basally positioned gnathostome clade  
388 possessing paired fins, chondrichthyans are important in terms of vertebrate evolution.  
389 Comparative embryological studies help to expand the available database on the different  
390 chondrichthyan lineages, and allow the ontogeny to be useful in determining phylogenetic  
391 relationships; these studies can also be used to explore homology and convergence issues (Zusi  
392 and Livezey, 2000). In this sense, the present paper contributes some steps towards better  
393 understanding of phylogenetic relationships among skates, the most diverse group of batoids.

394

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402

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#### 407 7. References

408 Agnew DJ, Nolan CP, Pompert J. 1999. Management of the Falkland Islands skate and ray  
409 fishery. *FAO Fisheries Technical Paper*, 378/1: 20 pages

410 Amsler MO, Smith KE, McClintock JB, Singh H, Thatje S, Vos SC, Brothers CJ, Brown A, Ellis  
411 D, Anderson J, Aronson RB. 2015. *In situ* observations of a possible skate nursery off the  
412 western Antarctic Peninsula. *Journal of Fish Biology* 86(6): 1867–1872. DOI:10.1111/jfb.12679

413 Ballard WW, Mellinger J, Lechenault H. 1993. A Series of Normal Stages for Development of  
414 *Scyliorhinus canicula*, the Lesser Spotted Dogfish (Chondrichthyes: Scyliorhinidae). *The*  
415 *Journal of Experimental Zoology* 267: 318-336. DOI: 10.1002/jez.1402670309

416 Barbini SA, Sabadin DE, Lucifora LO. 2018. Comparative analysis of feeding habits and dietary  
417 niche breadth in skates: the importance of body size, snout length, and depth. *Reviews in Fish*  
418 *Biology and Fisheries* 28(3): 625–636. DOI: 10.1007/s11160-018-9522-5

419 Berestovskii EG. 1994. Reproductive biology of skates of the family Rajidae in the seas of the  
420 far north. *Journal of Ichthyology* 34: 26–37.

421 Caldeira Brant F. 2003. Morfologia e Biometria do desenvolvimento embrionário da raia  
422 *Sympterygia acuta* Garman, 1877 (Elasmobranchii, Rajidae). MSc thesis, Universidad Federal de  
423 Río Grande, Brasil, 86 pages.

424 Coggan RA, Nolan CP, George MJA. 1996. Exploratory deep-sea fishing in the Falkland Islands,  
425 south-western Atlantic. *Journal of Fish Biology* 49 (Suppl. A): 298-310. DOI: 10.1111/j.1095-  
426 8649.1996.tb06083.x

427 Cole NJ, Currie PD. 2007. Insights from sharks: evolutionary and developmental models of fin  
428 development. *Developmental Dynamics* 236: 2421-2431. DOI: 10.1002/dvdy.21268.

429 Cousseau MB, Figueroa DE, Díaz de Astarloa JM, Mabrugaña E, Lucifora LO. 2007. Rayas,  
430 Chuchos y Otros Batoideos del Atlántico Sudoccidental (34°–55°S). *Publicaciones Especiales*  
431 *INIDEP*, Mar del Plata, Argentina. 102 pages.

432 Cox DL, Koob TJ. 1993. Predation on elasmobranch eggs. *Environmental Biology of Fishes* 38:  
433 117–125. DOI: 10.1007/BF00842908

- 434 Delpiani GE. 2016. Reproductive biology of the southern thorny skate *Amblyraja*  
435 *doellojuradoi* (Chondrichthyes, Rajidae). *Journal of Fish Biology* 88(4): 1413-1429. DOI:  
436 10.1111/jfb.12917
- 437 Didier DA, Leclair EE, Vanbuskirk, DR. 1998. Embryonic staging and external features of  
438 development of the Chimaeroid fish, *Callorhinchus milii* (Holocephali, Callorhinchidae).  
439 *Journal of Morphology* 236: 25-47. DOI: 10.1002/(SICI)1097-4687(199804)236:1<25 ::AID-  
440 JMOR2>3.0.CO;2-N.
- 441 Ebert DA. 2015. Deep-sea cartilaginous fishes of the southeastern Atlantic Ocean. FAO Species  
442 Catalogue for Fishery Purposes No. 9, 264 p.
- 443 Ebert DA. 2016. Deep-sea cartilaginous fishes of the southeastern Pacific Ocean. FAO Species  
444 Catalogue for Fishery Purposes No. 10, 241 pp.
- 445 Ebert DA, Bizzarro JJ. 2007. Standardized diet compositions and trophic levels of skates  
446 (Chondrichthyes: Rajiformes: Rajoidei). *Environmental Biology of Fishes* 80: 221–237. DOI:  
447 10.1007/s10641-007-9227-4
- 448 Flores JN, Brogger MI, Penchaszadeh PE. 2019. Reproduction and development of the brooding  
449 sea urchin *Austrocidaris canaliculata* from deep-sea off Argentina. *Deep-Sea Research Part I*  
450 143: 35–42. DOI: 10.1016/j.dsr.2018.11.012
- 451 Hamlett WC. 2005. Reproductive Biology and Phylogeny of Chondrichthyes: Sharks, Batoids  
452 and Chimaeras. Enfield, NH: Science Publishers, Inc, 562 pages.
- 453 Harahush BK, Fischer ABP, Collin SP. 2007: Captive breeding and embryonic development of  
454 *Chiloscyllium punctatum* Müller & Henle, 1838 (Elasmobranchii: Hemiscyllidae). *Journal of*  
455 *Fish Biology* 71(4): 1007-1022. DOI: 10.1111/j.1095-8649.2007.01569.x
- 456 Henry LA, Stehmann MFW, De Clippele L, Findlay HS, Golding N, Roberts JM. 2016.  
457 Seamount egg-laying grounds of the deep-water skate *Bathyraja richardsoni*. *Journal of Fish*  
458 *Biology* 89(2): 1473-1481. DOI: 10.1111/jfb.13041
- 459 Herring PJ. 2000. Species abundance, sexual encounter and bioluminescent signalling in the  
460 deep sea. *Philosophical Transactions of the Royal Society B* 355: 1273-1276.
- 461 Hitz CR. 1964. Observations on egg cases of the big skate (*Raja binoculata* Girard) found in  
462 Oregon coastal waters. *Journal of the Fisheries Research Board of Canada* 21: 851–854. DOI:  
463 10.1139/f64-076
- 464 Hoff GR. 2007. Reproductive Biology of the Alaska Skate *Bathyraja parmifera*, with Regard to  
465 Nursery Sites, Embryo Development and Predation. PhD Thesis. University of Washington,  
466 Seattle, WA. 161 pages.
- 467 Hoff GR. 2008. A nursery site of the Alaska skate (*Bathyraja parmifera*) in the eastern Bering  
468 Sea. *Fishery Bulletin* 106: 233–244.

- 469 Hoff GR. 2009. Embryo developmental events and the egg case of the Aleutian skate *Bathyraja*  
470 *aleutica* (Gilbert) and the Alaska skate *Bathyraja parmifera* (Bean). *Journal of Fish Biology* 74:  
471 483–501. DOI: 10.1111/j.1095-8649.2008.02138.x.
- 472 Hoff GR. 2010. Identification of skate nursery habitat in the eastern Bering Sea. *Marine Ecology*  
473 *Progress Series* 403: 243–54. DOI: 10.3354/meps08424
- 474 Hoff GR. 2016. Identification of multiple nursery habitats of skates in the eastern Bering Sea.  
475 *Journal of Fish Biology* 88: 1746–1757. DOI: 10.1111/jfb.12939
- 476 Hume JB. 2019. Higher temperatures increase developmental rate & reduce body size at  
477 hatching in the small-eyed skate *Raja microocellata*: implications for exploitation of an  
478 elasmobranch in warming seas. *Journal of Fish Biology* 95: 655–658. DOI: 10.1111/jfb.13997
- 479 Ivanova NV, deWaard JR, Hebert PDN. 2006. An inexpensive, automation-friendly protocol for  
480 recovering high-quality DNA. *Molecular Ecology Notes* 6: 998–1002. DOI: 10.1111/j.1471-  
481 8286.2006.01428.x.
- 482 Ivanova NV, Zemlak TS, Hanner RH, Hebert PDN. 2007. Universal primer cocktails for fish  
483 DNA barcoding, *Molecular Ecology Notes* 7: 544–548. DOI: 10.1111/j.1471-  
484 8286.2007.01748.x.
- 485 Jañez JA, Sueiro MC. 2007. Size at hatching and incubation period of *Sympterygia bonapartii*  
486 (Müller & Henle, 1841) (Chondrichthyes, Rajidae) bred in captivity at the Temaiken Aquarium.  
487 *Journal of Fish Biology* 70: 648–650. DOI:10.1111/j.1095-8649.2007.01332.x
- 488 Koop JH. 2005. Reproduction of captive *Raja* spp. in the Dolfinarium Harderwijk. *Journal of the*  
489 *Marine Biological Association of the United Kingdom* 85: 1201–1202.
- 490 Kormanik GA. 1993. Ionic and osmotic environment of developing elasmobranch embryos.  
491 *Environmental Biology of Fishes* 38: 223–240. DOI: 10.1007/BF00842919
- 492 Kuhnz LA, Bizzarro JJ, Ebert DA. 2019. *In situ* observations of deep-living skates in the eastern  
493 North Pacific. *Deep-Sea Research Part I* 152: 103104. DOI: 10.1016/j.dsr.2019.103104
- 494 Last PR, White WT, de Carvalho MR, Séret B, Stehmann MFW, Naylor GJP. 2016. Rays of the  
495 World. CSIRO Publishing, Locked Bag 10, Clayton South VIC 3169, Australia.
- 496 Last PR, Yearsley GK. 2002. Zoogeography and relationships of Australasian skates  
497 (Chondrichthyes: Rajidae). *Journal of Biogeography* 29(12): 1627–1641. DOI: 10.1046/j.1365-  
498 2699.2002.00793.x
- 499 Lucifora LO, García VB. 2004. Gastropod predation on egg cases of skates (Chondrichthyes,  
500 Rajidae) in the southwestern Atlantic: quantification and life history implications. *Marine*  
501 *Biology* 145: 917–922. DOI: 10.1007/s00227-004-1377-8
- 502 Luer CA, Gilbert PW. 1985. Mating behavior, egg deposition, incubation period, and hatching in  
503 the clearnose skate, *Raja eglanteria*. *Environmental Biology of Fishes* 13(3): 161–171.

- 504 Luer CA, Walsh CJ, Bodine AB, Wyffels JT. 2007. Normal embryonic development in the  
505 clearnose skate, *Raja eglanteria*, with experimental observations on artificial insemination.  
506 *Environmental Biology of Fishes* 80: 239–255.
- 507 Mabragaña E, Figueroa DE, Scenna LB, Díaz de Astarloa JM, Colonello JH, Delpiani G. 2011.  
508 Chondrichthyan egg cases from the south-west Atlantic Ocean. *Journal of Fish Biology* 79:  
509 1261–1290.
- 510  
511 Mabragaña E, Lucifora LO, Corbo MdeL, Díaz de Astarloa JM. 2015. Seasonal reproductive  
512 biology of the bignose fanskate *Sympterygia acuta* (Chondrichthyes, Rajidae). *Estuaries and*  
513 *Coasts* 38: 1466–76. DOI: 10.1007/s12237-014-9888-0
- 514 Marshall NB. 1979. Developments in Deep-Sea Biology. *Blandford Press*, Poole, Dorset, 566  
515 pages.
- 516 Martins APB, Heupel MR, Chin A, Simpfendorfer CA. 2018. Batoid nurseries: definition, use  
517 and importance. *Marine Ecology Progress Series* 595: 253-267. DOI: 10.3354/meps12545
- 518 Maxwell EE, Fröbisch NB, Heppleston AC. 2008. Variability and Conservation in Late  
519 Chondrichthyan Development: Ontogeny of the Winter Skate (*Leucoraja ocellata*). *The*  
520 *Anatomical Record* 291(9): 1079-1087. DOI: 10.1002/ar.20719
- 521 McEachran JD. 1983. Results of the research cruises of FRV “Walter Herwig” to South America  
522 LXI. Revision of the South American skate genus *Psammobatis* Günther, 1870 (Elasmobranchii:  
523 Rajiformes, Rajidae). *Archiv für Fischereiwissenschaft* 34(1): 23-80.
- 524 Onimaru K, Motone F, Kiyatake I, Nishida K, Kuraku S. 2018. A staging table for the embryonic  
525 development of the brownbanded bamboo shark (*Chiloscyllium punctatum*): bamboo shark  
526 development. *Developmental Dynamics* 247(5): 712-723. DOI: 10.1002/dvdy.24623
- 527 Paesch L, Oddone MC. 2008. Size at maturity and egg capsules of the softnose skates *Bathyraja*  
528 *brachyurops* (Fowler, 1910) and *Bathyraja macloviana* (Norman, 1937) (Elasmobranchii:  
529 Rajidae) in the SW Atlantic (37°00–39°30S). *Journal of Applied Ichthyology* 25 (1): 66-71. DOI:  
530 10.1111/j.1439-0426.2008.01114.x
- 531 Penchaszadeh PE, Pastorino G, Martinez MI, Miloslavich P. 2019. Spawn and development of  
532 the gastropod *Americominella longisetosa* (Castellanos and Fernández, 1972) (Mollusca:  
533 Buccinidae) from the Southwestern Atlantic deep sea. *Deep-Sea Research Part I* 143: 43–49.  
534 DOI: 10.1016/j.dsr.2018.11.011
- 535 Salinas-de-León P, Phillips B, Ebert D, Shivji M, Cerutti-Pereyra F, Ruck C, Fisher CR, Marsh  
536 L. 2018. Deep-sea hydrothermal vents as natural egg-case incubators at the Galapagos Rift.  
537 *Scientific Reports* 8(1): 1-7. DOI: 10.1038/s41598-018-20046-4
- 538 Scenna LB. 2011. Biología y Ecología Reproductiva de las Especies del Género *Bathyraja*  
539 (Elasmobranchii, Rajidae) en la Plataforma Continental Argentina. PhD Thesis. Universidad  
540 Nacional de Mar del Plata, Mar del Plata, Argentina. 176 pages.

- 541 Stehmann MFW, Merrett NR. 2001. First records of advanced embryos and egg capsules of  
542 *Bathyraja* skates from the deep north-eastern Atlantic. *Journal of Fish Biology* 59: 338–349.
- 543 Templeman W. 1982. Development, occurrence and characteristics of egg capsules of the thorny  
544 skate, *Raja radiata*, in the Northwest Atlantic. *Journal of Northwest Atlantic Fisheries Science* 3:  
545 47-56.
- 546 Thomason JC, Marrs SJ, Davenport J. 1996. Antibacterial and antisettlement activity of the  
547 dogfish (*Scyliorhinus canicula*) eggcase. *Journal of the Marine Biological Association of the*  
548 *United Kingdom* 76: 777-792. DOI: 10.1017/S0025315400031453
- 549 Vazquez DM, Mabragaña E, Gabbanelli V, Díaz de Astarloa JM. 2016. Exploring nursery sites  
550 for oviparous chondrichthyans in the Southwest Atlantic (36°S–41°S). *Marine Biology Research*  
551 12(7): 715-725. DOI: 10.1080/17451000.2016.1203948.
- 552 Ward RD, Zemplak TS, Innes BH, Last PR, Hebert PDN. 2005. DNA barcoding Australia's fish  
553 species. *Philosophical Transactions of the Royal Society B* 360: 1847-1857. DOI:  
554 10.1098/rstb.2005.1716
- 555 Weigmann S. 2016. Annotated checklist of the living sharks, batoids and chimaeras  
556 (Chondrichthyes) of the world, with a focus on biogeographical diversity. *Journal of Fish*  
557 *Biology* 88: 837–1037.
- 558 Zusi RL, Livezey BC. 2000. Homology and phylogenetic implications of some enigmatic cranial  
559 features in galliform and anseriform birds. *Annals of Carnegie Museum* 69: 157–193.

- The first record of skate egg cases with embryos in Southwest Atlantic deep waters is reported
- The embryo features were similar among species in initial, early and middle stages
- Species-specific differences between advanced and pre-hatching embryos were identified
- Spinulation in pre-hatching embryos is coincident with that recorded for adults

Journal Pre-proof

**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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